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WATER QUALITY IN RECIRCULATING AQUACULTURE SYSTEMS FOR ARCTIC CHARR (*Salvelinus alpinus* L.) CULTURE

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ABSTRACT

Recirculating aquaculture systems (RAS) for fish culture have been used for more than three decades. The interest in RAS is due to their advantages such as greatly reduced land and water requirements in places where water resources are limited; but RAS also have disadvantages like the deterioration of the water quality if the water treatment processes within the system are not controlled properly. The water quality problems in RAS are associated with low dissolved oxygen (DO) and high fish waste metabolite levels in the culture water. The objective of this study is to compare water quality in a RAS with water quality in a limited reuse system (LRS) for Arctic charr culture taking into account the oxygen demands of the fish, the metabolites production by the fish, the removal of CO_2 by the aerators, the removal of ammonia by the biofilter and the removal of waste products in the reused water. The experiment was conducted in Verid, the Aquaculture Research Facilities of Holar University College, Iceland, during 4 weeks. The two different systems were compared during the experiment: a RAS with a biofilter and a LRS. The results of this study showed that the water quality parameters in both systems were well within the acceptable levels for Arctic charr culture and the water quality was better in the LRS than in the RAS; the important role of the biofilter unit in the RAS was demonstrated and the necessity to control all the water treatment processes within the system, especially when the RAS is using sand filters as one of the water treatment components of the system.

Keywords: Arctic charr, water quality, recirculating aquaculture systems, fish culture.

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1 INTRODUCTION

Recirculating aquaculture systems (RAS) consist of an organised set of complementary processes that allow at least a portion of the water leaving a fish culture tank to be reconditioned and then reused in the same fish culture tank or other fish culture tanks (Timmons *et al*. 2002).

Recirculating systems for holding and growing fish have been used by fisheries researchers for more than three decades. Attempts to advance these systems to commercial scale food fish production have increased dramatically in the last decade although few large systems are in operation. The renewed interest in recirculating systems is due to their perceived advantages such as greatly reduced land and water requirements; reduced production costs by retaining energy if the culture species require the maintenance of a specific water temperature, and the feasibility of locating production in close proximity to prime markets (Dunning *et al*. 1998).

However, the RAS also have disadvantages. The most important is the deterioration of the water quality if the water treatment process within the system is not controlled properly. This can cause negative effects on fish growth, increase the risk of infectious disease, increase fish stress, and other problems associated with water quality that result in the deterioration of fish health and consequently loss of production (Timmons *et al*. 2002). The water quality in RAS depends on different factors most importantly the source, the level of recirculation, the species being cultured and the waste water treatment process within the system (Sanni and Forsberg 1996, Losordo *et al*. 1999).

Most water quality problems experienced in RAS were associated with low dissolved oxygen and high fish waste metabolite concentrations in the culture water (Sanni and Forsberg 1996). Waste metabolites production of concern include total ammonia nitrogen (TAN), unionised ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N) (to a lesser extent), dissolved carbon dioxide (CO_2), suspended solids (SS), and non-biodegradable organic matter. Of these waste metabolites, fish produce roughly 1.0-1.4 mg L^{-1} TAN, 13-14 mg L^{-1} CO_2, and 10-20 mg L^{-1} TSS for every 10 mg L^{-1} of DO that they consume (Hagopian and Riley 1998). However, maintaining good water quality conditions is of primary importance in any type of aquaculture system, especially in RAS.

Prospective users of aquaculture systems need to know about the required water treatment processes to control temperature, dissolved gases (oxygen, carbon dioxide, and nitrogen), pH, pathogens, and fish metabolites such as solids (both dissolved and particulate) and dissolved nitrogen compounds (ammonia, nitrite and nitrate) levels in the culture water; the components available for each process and the technology behind each component (Losordo *et al*. 1999).

Water reuse systems generally require at least one or more of the following treatment processes, depending upon their water-use intensity and species-specific water quality requirements (Losordo *et al*. 1999):

- Sedimentation units, granular filters, or mechanical filters to remove particulate solids.

- Biological filters to remove ammonia.
- Strippers/aerators to add dissolved oxygen and decrease dissolved carbon dioxide or nitrogen gas to levels closer to atmospheric saturation.
- Oxygenation units to increase dissolved oxygen concentrations above atmospheric saturation levels.
- Advanced oxidation units (i.e. UV filters or units to add ozone) to disinfect, oxidise organic wastes and nitrite, or supplement the effectiveness of other water treatment units.
- pH controllers to add alkaline chemicals for maintaining water buffering or reducing dissolved carbon dioxide levels.
- Heaters or chillers to bring the water temperature to a desired level.

A key to successful RAS is the use of cost-effective water treatment system components. Water treatment components must be designed to eliminate the adverse effects of waste products (Losordo *et al*. 1998). In recirculating tank systems, proper water quality is maintained by pumping tank water through special filtration and aeration and/or oxygenation equipment. Each component must be designed to work in conjunction with other components of the system. To provide a suitable environment for intensive fish production, recirculating systems must maintain uniform flow rates (water and air/oxygen), fixed water levels, and uninterrupted operation (Masser *et al*. 1999).

Currently, freshwater recirculating systems are used to raise high value species or species that can be effectively niche marketed, such as Salmon smolt and ornamental fishes, as well as fingerling and food-sized tilapia, hybrid-striped bass, yellow perch, eels, rainbow trout, African catfish, Channel catfish, and Arctic charr, to name just a few. Additionally, saltwater reuse systems are being used to produce many species at both fingerling and food-size, including flounder, sea bass, turbot, and halibut; water reuse systems are also used to maintain many kinds of coldwater and warm water brood stock fish (Summerfelt *et al*. 2004a).

1.1 Cuba: current situation

Aquaculture in Cuba has been developed as commercial activity since 1976, mainly with the culture of different fresh water species such as tilapia (*Oreochromis spp.*), silver carp (*Hypophthalmichthys molitrix*), Channel catfish (*Ictalurus punctatus*) and tenca (*Tinga tinga*) in dam rivers as extensive culture. The year 1986, was the beginning of the marine species culture development with the culture of white shrimp (*Litopenaeus schmitti)* in land ponds as semi intensive culture with a total production of 27 tons that year (Cuban Statistic Annual Fisheries 2004).

Currently, white shrimp culture production in Cuba is the second line of exportation income from the Ministry of Fishing Industry to the country's economy with approximately 1700-2000 tons of total production per year, 2400 tons in 2006 after the introduction of the Pacific white shrimp (*Litopenaeus vannamei)* in 2004 to use this specie for the culture, in approximately 2300 hectares of land culture ponds (Cuban Statistic Annual Fisheries 2006). On the other side, the total fresh water aquaculture production during this decade was around 32,000-43,000 tons, and the main species were silver carps, with 12,300-25,600 tons production per year, tenca

between 13,700-15,000 tons per year and tilapia between 4500-5000 tons per year (Cuban Statistic Annual Fisheries 2006). The fresh water aquaculture production is used to supply local market demand and some tourist places on the island such as restaurants and hotels.

The Cuban marine fish culture production is low. One of the major experiments in marine fish culture in the country was conducted from 1999 until 2001 with the introduction of juveniles of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) to culture in net cages at the open sea for commercial business in four parts of the island shelf (Isla *et al*. 2006).

At present, Cuba has three experimental hatcheries for marine fish culture, one of them, the oldest one with more than ten years building, to produce mutton snapper (*Lutjanus analis*) and common snook (*Centropomus undecimales*), located in Camaguey province, at the south central part of the country; and the other two, to produce cobia (*Rachicentron canadum*), one of them located in Cienfuegos province, at the southeast part and the other in Granma province, at the southwest part of the country, with around 2 and 7 years building, respectively. At present, these hatcheries are used to maintain the brood stocks of these species in flow-through aquaculture systems.

There are no RAS in use in Cuba today, but the structure and design of the hatcheries permit installation of RAS to improve operation with a consequent reduction in the water used for the activities, mainly the fresh water use. However, the addition of RAS must be prepared carefully both in terms of design and economy. The recirculation systems are generally fairly expensive to build and require training of staff for their operation (Losordo *et al*. 1998, Masser *et al*. 1999). Nevertheless, it may be an important alternative to improve the fish culture techniques used in hatcheries for brood stock and to develop good quality future fingerling production in Cuba.

The main objectives of this study were to compare water quality in a RAS with water quality in a limited reuse system (LRS) for Arctic charr culture; mainly focusing on the changes in concentration levels of some parameters of indicators of water quality as dissolved oxygen (DO), pH, carbon dioxide (CO_2), oxygen consumption (MO_2), total ammonia nitrogen (TAN), unionised ammonia (NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and total suspended solids (TSS) of the inlet and outlet water at different points of each system to evaluate the performance of the RAS, taking into account:

- The oxygen demands of the fish.
- The production of metabolites by the fish.
- The removal of CO_2 by the aerators.
- The removal of ammonia by the biofilter.
- The removal of CO_2, TAN, NO_2-N, NO_3-N and TSS in wastewater (recirculating water).

2 LITERATURE REVIEW

Research and development in recirculating systems has been going on for nearly three decades. There are many alternative technologies for each process and operation. The selection of a particular technology depends upon the species being reared, site, infrastructure, production management expertise, and other factors (Dunning *et al*. 1998).

Noble and Summerfelt (1996) note that in aquaculture systems that reuse water, water quality should be maintained at levels sufficient for supporting healthy and fast growing fish. Operating a fish farm under limited water quality conditions can reduce the profitability of fish production, because the water quality problems can be lethal, lead to stress, and the resulting deterioration of fish health will reduce growth and increase the risk of infectious disease outbreaks and catastrophic loss of fish. The most common problems of water quality in RAS can be created by high or low water temperature, low DO levels, elevated waste metabolite concentrations, gas supersaturation, measurable dissolved ozone levels, and the presence of certain cleaning chemicals or chemotherapeutants in water (Twarowska *et al*. 1997).

2.1 Water quality in recirculation aquaculture systems (RAS)

2.1.1 Dissolved oxygen (DO) and carbon dioxide (CO_2) levels

Fish use oxygen to convert feed to energy and biomass. Depending upon species, according to Pillay and Kutty (2005), for optimum growth fish require a minimum DO concentration of approximately 5.0 mg L^{-1} (warm water species) to 7.0 mg L^{-1} (coldwater species). For salmonid species, the optimal levels of DO should be at least between 70-80% of oxygen saturation (not below 6.0 mg L^{-1} and above 9.0 mg L^{-1}), oxygen saturation below this range decreases the maximal growth rate and higher saturation levels that exceed 120-140% can compromise the welfare of the fish causing oxidative stress and increased susceptibility to diseases and mortality (Aquafarmer 2004).

CO_2 is considered a toxic compound for fishes and is a limiting factor in intensive aquaculture systems where oxygen is injected into the inlet water while the water exchange rate is reduced; an increased CO_2 concentration in the culture water will reduce the CO_2 diffusion gradient between the fish blood and inspired water, and thus result in blood acidification, leading to a reduced arterial blood oxygen carrying capacity and a reduction in oxygen uptake (Sanni and Forsberg 1996).

In general, fish ventilate CO_2 (a by-product of metabolism) through their gills as molecular CO_2 gas, when the gas reacts with water they produce carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) and the equilibrium of the reactions depends on water pH values, in an inverse exponential relationship between CO_2 partial pressure and water pH values.

$$CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{-2}$$

The interdependence of pH, carbon dioxide, bicarbonate, and carbonate is illustrated in Figure 1 (Boyd 2000). The graph shows that below about pH 5, carbon dioxide is the only significant species of inorganic carbon, above pH 5, the proportion of bicarbonate increases relative to carbon dioxide until bicarbonate becomes the only significant species at about pH 8.3. Above pH 8.3, carbonate appears and it increases in importance relative to bicarbonate if pH continues to rise.

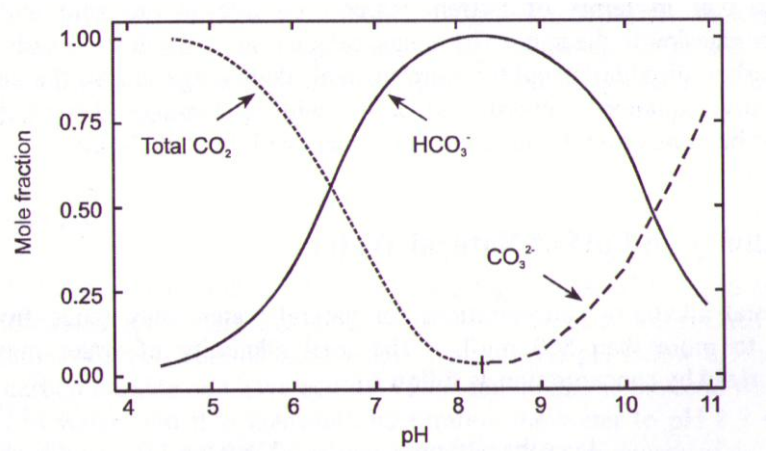

Figure 1: Effects of pH on the relative proportions of total CO_2, HCO_3^-, and CO_3^{2-}. The mole fraction of a component is its decimal fraction of all the moles present (Boyd 2000).

Some studies of CO_2 excretion rates in salmonids have been conducted (Forsberg 1997), reporting CO_2 excretion rates of 2.8-3.0 mg CO_2 kg^{-1} min^{-1} from steelhead trout (*Oncorhynchus mykiss*) and coho salmon (*O. kitsutch*) and 1-2 mg CO_2 kg^{-1} min^{-1} from rainbow trout depending on the CO_2 levels present in the culture water.

The minimum DO concentration that is safe for fish is dependent on the concentration of dissolved CO_2 present in the water, the accumulated concentration of dissolved CO_2 within the culture tank will not be limiting (with no aeration or pH control) when the cumulative DO consumption is less than 10-22 mg L^{-1}, depending upon pH, alkalinity, temperature, and the species and life stage (Summerfelt *et al*. 2000).

The minimum safe DO level should be increased by 3-4 mg L^{-1} if CO_2 concentrations are high, e.g. if dissolved CO_2 exceeds 30 mg L^{-1} for salmonids or exceeds 40-50 mg L^{-1} for certain warm water species. For example, dissolved CO_2 begins to effect salmonids at concentrations higher than 15-20 mg L^{-1} in freshwater and less than 7-10 mg L^{-1} in seawater, but many warm water species will tolerate considerably higher dissolved CO_2 levels in their environment such as cyprinids and hybrid striped bass.

Even the 20 mg L^{-1} recommended as a safe level for salmonid culture may be conservative if DO concentrations in the water are at or above saturation levels (Summerfelt *et al*. 2000, Summerfelt *et al*. 2004), although as a precautionary approach, some authors such as Fivelstad *et al*. (1998) suggest that a maximum limit of CO_2 may be as low as 10 mg L^{-1}. For these reasons, DO is usually the first water quality parameter to limit culture tank carrying capacity.

2.1.2 Oxygen consumption (MO_2)

The oxygen consumption (MO_2) of fish is variable and depends on many factors such as temperature: MO_2 increases when temperature increases. Body mass: MO_2 has an inversely exponential proportion when the body mass increases. Feeding rate: MO_2 increases when the feeding rate increases due to the digestion of food. Growth rate has a directly proportional relationship with MO_2. Swimming velocity and stress levels: increased stress levels may enhance the MO_2 of fish. The above factors are the most important that should be taken into account in any aquaculture system (Forsberg 1997, Timmons *et al*. 2002, Pillay and Kutty 2005).

The MO_2 of fish culture in tanks is calculated by the Fick equation, based on the DO concentration of the inflow and outflow water, the flow rate and the total biomass inside the tank. It is also possible to estimate oxygen requirements of fish based on feed intake.

Some authors have designed models to estimate MO_2 in salmonid species based on some factors such as body mass, temperature, water current velocity, time from feeding, water CO_2 levels and photoperiod (Fivelstad and Smith 1991, Forsberg 1994, Summerfelt *et al*. 2000). For example, Timmons *et al*. (2002) suggest, as a general rule for fish, that the ratio between MO_2 and feed intake, in units of mass, is around 0.25:1; this value is lower than values reported from studies of salmonids, where the MO_2 rate in this species fed to a maximum level is around 0.46-0.50:1 (Forsberg 1997). Timmons *et al*. (2002) also suggest, in general as respiratory quotient (the ratio of CO_2 produce when oxygen is consumed), that when 1.0 mg of oxygen per litre per minute is consumed by the fish, the fish can produce 1.3 mg of CO_2, and these values should be used for estimating expected CO_2 production in aquaculture systems; but in the case of salmonids, per 1.0 mg of DO consumed per litre they can produce 1.0 mg of CO_2 per litre (Aquafarmer 2004).

2.1.3 Nitrogen metabolites levels

2.1.3.1 Ammonia levels

The fish create and expel various nitrogenous waste products through gill diffusion, gill cation exchange, and urine and faeces excretion; in addition some nitrogenous wastes are accumulated from the organic debris of dead and dying organisms, uneaten feed, and from nitrogen gas in the atmosphere (Timmons *et al*. 2002). Ammonia exists in two forms: unionised ammonia (NH_3-N), and ionised ammonia (NH_4^+-N), the sum of these two is called total ammonia nitrogen (TAN). The relative concentration of ammonia is primarily a function of water pH, salinity and temperature (Pillay and Kutty 2005).

The excretion of TAN by the fish varies depending on the species in culture. As a general rule, when 1.0 mg of oxygen per litre per minute is consumed by the fish, the fish can produce 0.14 mg of TAN (Timmons *et al*. 2002) and specifically for salmonids species, per 1.0 mg of DO consumed per litre they can produce 0.04-0.06 mg of TAN per litre (Aquafarmer 2004).

NH_3-N is the most toxic form of ammonia, so the toxicity of TAN is dependent on the percentage of the NH_3-N form in the TAN concentration. The proportion of NH_3-N increases if the pH increases and temperature or salinity decreases (Timmons *et al*. 2002), e.g. Fivelstad *et al*. (1995) found, in a short-term experiment, that intermediate salinities reduce the ammonia toxicity to Atlantic salmon smolts. Ammonia concentration levels are not a problem in a simple flow-through system but it is a problem when using recycling and reuse systems with biofilters to remove ammonia within the system. However, the fish farmers have to take care of the biofilters' functionality to maintain the acceptable ammonia concentration levels in the culture water depending of the culture species requirements (Aquafarmer 2004).

Unfortunately, NH_3-N can kill fish when it is above certain levels depending on the species (Table 1). For salmonids, long term exposure to concentrations between 0.05 to 0.2 mg L^{-1} of NH_3-N can significantly reduce growth rate, fecundity and disease resistance and increase gill ventilation, metabolic rate, erratic and quick movements and can also cause mortality; due to the optimal conditions required for NH_3-N concentration levels in water has been less than 0.012 to 0.03 mg L^{-1} for salmonids aquaculture (Summerfelt *et al*. 2004).

Table 1: Lethal levels of NH_3-N (concentration of nitrogen bound as NH_3) for some aquaculture species.

Specie	**NH_3-N (mg L^{-1})**	**Reference**
Rainbow trout	0.32	Timmons *et al*. 2002
Arctic charr	0.03	Aquafarmer 2004
Common carp	2.2	Summerfelt *et al*. 2004
Catfish	3.10	Summerfelt *et al*. 2004

Normally, warm water fish are more tolerant to ammonia toxicity than coldwater fish, and freshwater fish are more tolerant than saltwater fish, so in general, NH_3-N concentrations should be held below 0.05 mg L^{-1} and TAN concentrations below 1.0 mg L^{-1} for long-term exposure (Timmons *et al*. 2002). For Arctic charr culture, according to Aquafarmer (2004), the NH_3-N concentrations should be less than 0.025 mg L^{-1} and TAN concentrations below 3.0 mg L^{-1}, keeping the pH levels below 8.0.

According to Forsberg (1997), the excretion of nitrogen is partitioned into two components: endogenous and post-pandrial or exogenous excretion rates. The endogenous nitrogen excretion (ENE) reflects catabolism and the turnover of body proteins, irrespective of the nutritional status of the fish. Post-pandrial excretion reflects the catabolism of proteins that originated from feeds. ENE usually ranges between 30-50 µg TAN kg^{-1} min^{-1} and 15-35 µg urea-N kg^{-1} min^{-1} for young salmonids species (Fivelstad *et al*. 1990, Forsberg 1997), these values indicate that around 80-90% of the nitrogen (TAN + urea-N) is excreted as ammonia. In the case of the post-pandrial excretion, Fivelstad *et al*. (1990), reported between 80-180 mg TAN kg^{-1} days as average daily ammonia excretion rates from post-smolt Atlantic salmon fed maximum rates, which was equivalent to 22-33% of total nitrogen supplied. They also demonstrated with this study, that post-pandrial nitrogen excretion was linearly proportional to the nitrogen intake, even in fish fed limited rations. This general

pattern in salmonid species has also been demonstrated by other authors such as Beamish and Thomas (1984) and Forsberg (1997).

2.1.3.2 Nitrite (NO_2-N) and nitrate (NO_3-N) levels

Biofilters consist of actively growing bacteria attached to some surface(s), it can fail if the bacteria die or are inhibited by natural aging, toxicity from chemicals (e.g. disease treatment), lack of oxygen, low pH, or other factors. The biofilters take around 2 or 4 weeks to start functioning property after the bacteria population is established (Figure 2).

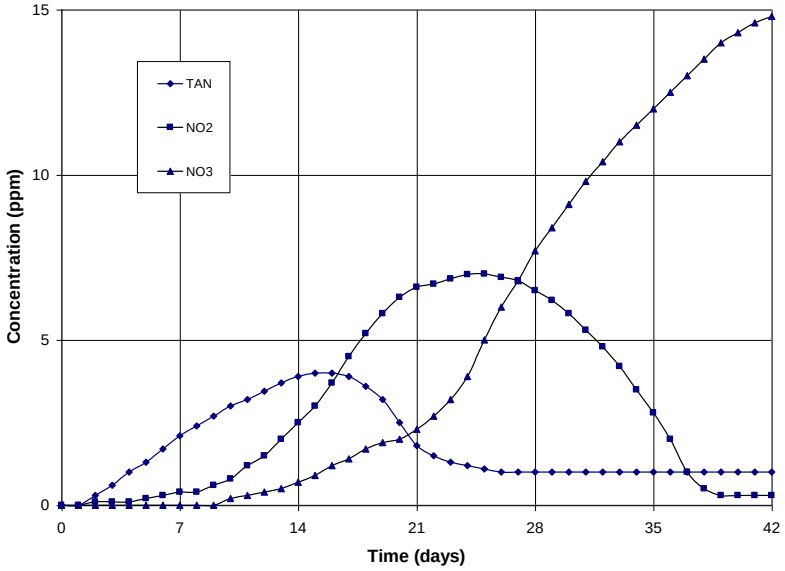

Figure 2: Typical startup curve for a biological filter showing time delays in establishing bacteria in biofilters (Timmons *et al*. 2002).

Nitrite and nitrate are produced when ammonia is oxidised by nitrifying bacteria concentrated within a biological filter, but they are also found throughout water columns and on surfaces within the recirculating system (Hagopian and Riley 1998). Non-biodegradable dissolved organic matter can also accumulate in the recirculating system water if it is degraded too slowly by the heterotrophic microorganisms in the biological filter.

According to Summerfelt and Sharrer (2004) biofilters contain both nitrifying bacteria and heterotrophic microorganisms that metabolise TAN and organic matter passing through the biofilter or trapped within the biofilter. The net results of the biofilter microbial respiration are a decrease in TAN, biodegradable organics, dissolved oxygen, alkalinity, and pH, and an increase in oxidation products of organics, as well as, NO_2-N, NO_3-N, and CO_2. Taking into account the overall stoichiometric relationship between subtracts and products produced during nitrification and nitrifier synthesis, nitrifying bacteria consume 4.6 mg L^{-1} of oxygen while producing approximately 5.9 mg L^{-1} of CO_2 for every 1.0 mg L^{-1} of TAN consumed and 1.38 mg L^{-1} of CO_2 are produced for every 1.0 mg L^{-1} of dissolved oxygen consumed, when the respiration activity of nitrifying bacteria and heterotrophic microorganisms are considered together.

Nitrite is the intermediate product in the process of nitrification of ammonia to nitrate and it is toxic for the fish because it affects the blood haemoglobin's ability to carry oxygen oxidised the iron in the haemoglobin molecule from the ferrous state to ferric state. The resulting product is called methemoblobin, which has a characteristic brown colour, hence the common name "brown colour disease" (Timmons *et al*. 2002). The amount of nitrite entering the blood depends of the ratio of nitrite to chloride (Cl) in the water, in that increased levels of Cl reduce the amount of nitrite absorption. At least a 20:1 ratio of Cl: NO_2-N is recommended for channel catfish in ponds, tilapia and rainbow trout (Timmons *et al*. 2002, Pillay and Kutty 2005), levels below than 1.0 mg NO_2-N L^{-1} are recommended for aquaculture systems (Pillay and Kutty 2005).

Nitrate (NO_3-N) is the end product of the nitrification process. As Timmons *et al*. (2002) note, NO_3-N is considered as the minimum toxic nitrogen product, with 96-h lethal concentration values more than 1000 mg NO_3-N L^{-1} for some aquaculture species. In recirculating systems, NO_3-N levels are controlled by daily water exchanges, but in some systems with low water flow rates this parameter has become increasingly important and concentration levels should be lower than 10 mg NO_3-N L^{-1} (Pillay and Kutty 2005).

2.1.4 pH levels, the relationship with nitrogen and inorganic carbon metabolites production in recirculation systems

The pH values express the intensity of the acid or basic characteristics of water. The pH scale ranges from 0 to 14, pH of 7.0 corresponding to the neutral point, while values of pH below 7.0 are acidic (the H^+ ion predominates) and above 7.0, values are basic or alkaline (the OH^- ion predominates). The pH of most ground waters and surface waters are buffered by the inorganic carbon equilibrium system and they have pH values between 5.0 and 9.0 (Timmons *et al*. 2002).

Exposure to extreme pH values can be stressful or lethal for aquatic species, but it is the indirect effects resulting from the interactions of pH with other variables that depend on the water acid-base equilibrium such as dissolved CO_2, the relationship between NH_3-N and NH_4^+-N levels and NO_2-N levels, that an increase of their concentrations depresses the pH values in water (Pillay and Kutty 2005). Low pH values increase the water solubility of some heave metals such as aluminium, copper, cadmium and zinc, their high concentrations in water cause toxic effects on fish, and also increase the toxicity of hydrogen sulphide on fish (Fivelstad *et al*. 2003). The higher toxicity levels of NH_3-N and CO_2 in water depends on the water's pH controls acid-base equilibrium; as an example, at 20^{o}C and a pH of 7.0, the mole fraction of NH_3-N is 0.004, but at a pH of 10, the NH_3-N increase to 0.8 at the same temperature (Timmons *et al*. 2002).

In general, according to Aquafarmer (2004), the changes in pH water values should be less than 0.5 and pH values should be keept in a range of 6-9 for Arctic charr culture, depending to the water salinity and temperature used.

2.1.5 Solids concentration levels

Uneaten feed, feed fines, fish faecal matter, algae, and sloughed micro-biological cell mass are all sources of solids production within recirculating systems (Chen *et al.* 1993). Solids control is one of the most critical processes that must be managed in recirculating systems, because solids decomposition can degrade water quality and thus directly and indirectly affect fish health and the performance of other unit processes within recirculating systems (Chen *et al.* 1993). Suspended solids can harbour opportunistic pathogens and speed up the growth of bacteria. They are associated with environmentally-induced disease problems, and have been reported to cause sublethal effects such as fin rot and direct gill damage (Noble and Summerfelt 1996). Suspended and settleable solids may also affect reproductive behaviour, gonad development, and the survival of the egg, embryo and larval stages of fishes (Pillay and Kutty 2005).

For example, if solids are filtered and stored in a pressurised-bead filter (a type of granular media filtration unit) between 24-hr backwash cycles, as much as 40% of the TSS generated in the recirculating system may decay (Chen *et al.* 1993). The suspended organic solids common to recirculating aquaculture systems can exert a strong oxygen demand as they degrade into smaller particulate matter and leach ammonia, phosphate, and dissolved organic matter (Cripps 1995). The fine particles and dissolved compounds produced are considerably harder to remove when broken apart and dissolved than when they were contained within the original faecal or feed pellet (Chen *et al.* 1993). This dissolution process increases the water's oxygen demand as it deteriorates the water quality within the recirculating system and in the discharged effluent.

Some authors such as Timmons *et al*. (2002) and Pillay and Kutty (2005) had considered TSS concentrations less than 80 mg L^{-1}, in general as water quality criteria for aquaculture, but in the case of sensitive species like salmonids, Aquafarmer (2004) suggests to maintain the TSS concentrations around 4.5 mg L^{-1} to keep the values on the safe side and fix as a concentration limit 15 mg L^{-1}.

Therefore, water quality should be monitored closely in a recirculating system so those problems with the water treatment units can be detected early and corrected. Water quality is also of concern if the effluent characteristics (e.g. biochemical oxygen demand, suspended solids, phosphorus, or nitrogenous compounds) of the culture facility must be controlled to meet water pollution requirements (Timmons *et al.* 2002).

2.2 Arctic charr as a farming species in Iceland

Arctic charr is a salmonid specie that can live in different environments depending on its life stage (freshwater, brackish and marine water between 30 – 70 m of depth). The Anadromous forms spend a considerable time of their lives at sea; non-migratory populations remain in lakes and rivers. The freshwater populations feed on planktonic crustaceans, amphipods, mollusks, insects and fishes and they are extremely sensitive to water pollution (cold water and oxygen oriented) in natural and captivity conditions (Aquafarmer 2004).

Around 1930 the farming of trout grew in Denmark, with farming of rainbow trout ensuing, which is now widely practised. In 1970 the growing of North Atlantic salmon took off in Norway with massive production that increases every year, as the conditions for farming salmon in sea-cages in the Norwegian fjords are excellent. Other countries and regions extensively farming North Atlantic salmon are Chile, Scotland, Ireland, the Faroe Islands, Canada, USA and Tasmania (Pillay and Kutty 2005). The farming of Arctic charr has been practised for quite some years, but never on a large scale.

Why is it desirable to develop the Arctic charr culture in Iceland? As Aquafarmer (2004) notes, Arctic charr for farming is a good choice at colder climates for various reasons:

- The access to suitable cold and clean water resources used for the culture activities.
- Arctic charr does well in cool waters because it is an indigenous species in the northern hemisphere and grows much faster at low temperatures than other salmonid species kept for farming.
- It is possible to keep Arctic charr at a greater density than many other fish species, thus making more efficient use of the farming space. Actually Arctic charr seems to grow better at 50 kg m^{-3} than at 15 kg m^{-3}.
- The Arctic charr is robust and easy to farm. It tolerates handling well and shows good resistance to many diseases. Losses are usually minor after the initial period of the embryonic stage.
- Its use of feed is good as the Arctic charr takes feed from the bottom of the tank and also eats in the dark night time.
- Arctic charr has marketable qualities such as delicate taste, attractive colour, low-fat meat and its market size is from one portion size up to two kilograms.

But there are also some disadvantages, such as:

- The charr is prone to become sexually mature already in the second year. At sexual maturity the growth rate markedly decreases and the quality deteriorates. Sexually mature fish therefore cannot be considered a marketable product.
- There is considerable variability in the growth rate depending on the season. Great size variance of fish in the same tank can create marketing problems.
- The colour of the flesh can be variable within a group. Usually the buyers want their fish strongly pink.

The commercial Arctic charr market is dominated by four producing countries: Iceland, with more than 900 tons per year is considered the major producer in Europe; Norway and Sweden, they are producing considerably less than Iceland; and Canada with less than 400 tons per year. Several other countries including Scotland, Ireland, France and Denmark are still minor producers. Including the production from the remaining countries, the total Arctic charr production is around 1800 – 1900 tons per year (Aquafarmer 2004). The main charr products for the market are either head-on frozen and gutted, or head-on chilled and gutted. At present, the price of charr is approximately ISK 380-500 for gutted fish and ISK 600-900 for fillets and in Canada prices are in the $4.50–5.0/lb range (Aquafarmer 2004).

3 MATERIALS AND METHODS

In the present study an experiment was conducted in Verid, the Aquaculture Research Facilities of Holar University College, Iceland, during 4 weeks. Two different systems were compared in the experiment: a RAS with a biofilter and a LRS. The net water used in the LRS was 0.2 L min^{-1} kg^{-1} which is similar to the water used in Icelandic charr farms. The net water used in the RAS was initially the same as the LRS (0.2 L min^{-1} kg^{-1}) and then it was gradually adjusted to 0.008 L min^{-1} kg^{-1} so that the water quality was within acceptable levels. Each system had two culture tanks (800 L), a reservoir tank, water pump, sand filter and aerator. The RAS includes a biofilter unit while the LRS does not have a biofilter (Figure 3). Arctic charr with an average body mass of around 190 g $ind.^{-1}$ were used. The initial stocking density was 157 individuals in each tank (40 kg m^{-3}), and 20 ppt of water salinity at 10°C of temperature and DO levels were kept between 100-115% of saturation (≈ 9.84-11.05 mg L^{-1}).

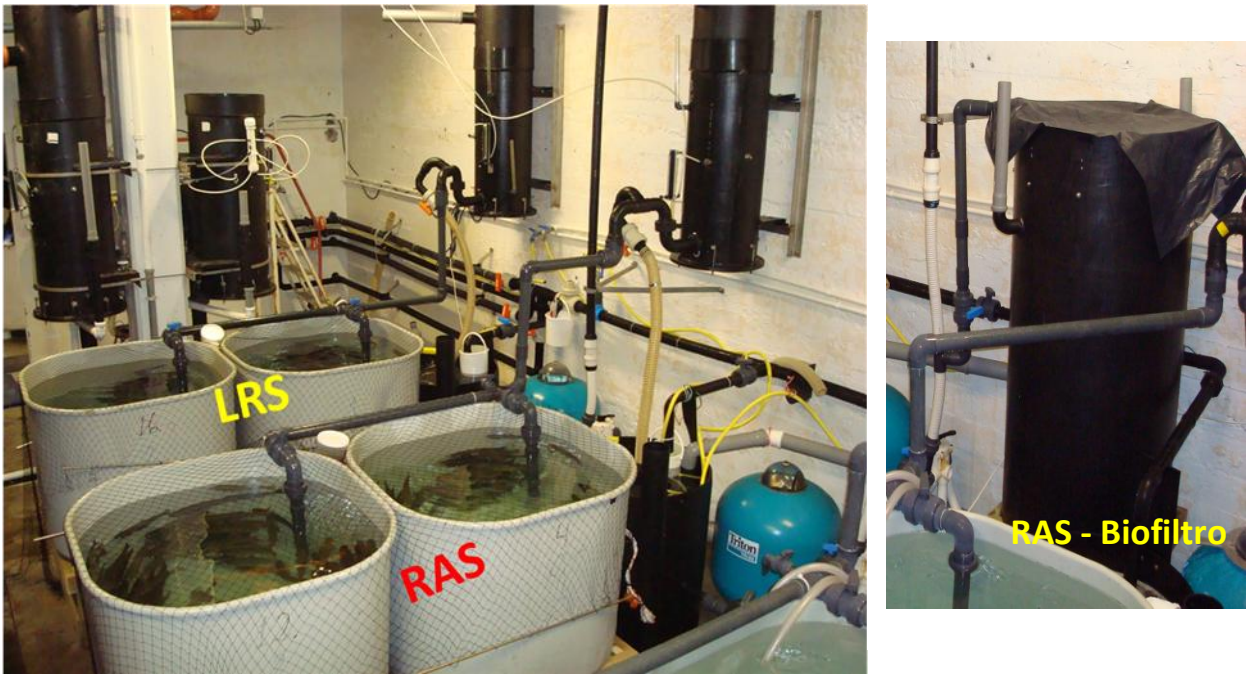

Figure 3: Aquaculture systems used for the experiment. Limited reuse system (LRS) and recirculating aquaculture system (RAS) with biofilter.

The water temperature, DO, salinity and pH were measured daily in each system in each of measurement point as show in Figure 4. The water temperature and DO water levels were measured with YIS-550A DO meter, the water salinity was measured with a PAL-06S refractometer (Atago Company) and the pH by OxyGuard pH meter. The total fish biomass of each tank in each system was measured per 2 weeks.

Water samples were collected to measure the concentrations of CO_2, TAN and TSS (3 replicas per measuring per parameter) in each system two times per week at the

measurement point as show in Figure 4, and the NO_2-N and NO_3-N concentration levels were also measured in the water samples taken from the biofilter outlet water (point 5) in the RAS two times per week.

The water samples were analysed in the laboratory of Verid to determinate CO_2, TAN and TSS concentrations according to the Standard methods for evaluation of water and wastewaters referred by Danish Standard Methods DS 224 (1975), APHA (1998) and Timmons *et al*. (2002). These methods are:

CO_2: CO_2 was measured with the single acid addition method. First, the initial temperature and salinity of the samples was measured. Then the samples were stored at 25^{o}C for at least 1 hour for the samples to reach this temperature. Finally, 100 mL of sample was measured accurately with a pipette and placed in a beaker, the temperature and pH of the sample was recorded. Then 25 ml (for samples with full salinity but only 5 to 10 ml for fresh water samples) of standanised 0.01 M HCl was added to the sample while mixing thoroughly. The resulting pH was recorded. The total inorganic carbon (TIC) and CO_2 concentrations were calculated using the programme CO_2 sys.exe program with the NBS scale option. It was assumed that the carbonic alkalinity reflected the total Alkalinity (TA) of the sample.

TSS: A well – mixed sample (? Volume) was filtered through a weighed standard glass fibre filter (Whatman GF/C). Then the filter was dried at 105^{o}C for at least one hour and the dry weight of the filter measured. The difference in the weight increase of the filter divided by the total sample volume filtered represents the total suspended solids concentration in the sample.

TAN: TAN was measured colorimetrically by indophenol blue method as describe in the Danish Standard methods DS 224 (1975). A 25 ml sample was measured into a reaction flask. Then 1.0 ml of sodium citrate solution, 1.2 mol L^{-1}, 1.0 ml of reagent A and 1.0 ml of reagent B were added in succession. The reagents should be prepared before the start of the measurements as shown in the technique DS 224. The samples were mixed well. The reaction flask was closed and left for two hours for the colour to develop in a dark place. The absorbance of the sample was measured at 630 nm in a spectrophotometer at latest 24 hours after mixing using 10 mm cuvettes. The TAN concentration was calculated using the calibration curve equation previously established.

The NO_2-N and NO_3-N concentration levels were measured using reagent test kits for Nitrite (CHEMets® Kit Nitrite K-7004) and Nitrate (CHEMets® Kit Nitrate K-6904) acquired from CHEMetrics Company, USA.

The oxygen consumption was calculated from each measurement in each system as:

$$MO_2 = (DO_{in} - DO_{out}) * Q / Bt \qquad (1)$$

where MO_2 is the oxygen consumption rate ($mgO_2\ min^{-1}\ kg^{-1}$), DO_{in} and DO_{out} are the dissolved oxygen concentrations (mg L^{-1}) in the inlet and outlet water, Q is the water flow inside the tanks (L min^{-1}) and Bt is the total fish biomass per tank (kg).

The rate of removal and addition of CO_2, TAN, NH_3 and TSS, were calculated as:

$$SX = (X_{out} - X_{in}) * Q / Bt \qquad (2)$$

where SX is the rate of either CO_2, TAN, NH_3 and TSS (mg min^{-1} kg^{-1}), X_{out} and X_{in} are the outlet and inlet concentration (mg L^{-1}) of each metabolite, Q is the water flow inside the tanks (L min^{-1}) and Bt is the total fish biomass per tank (kg).

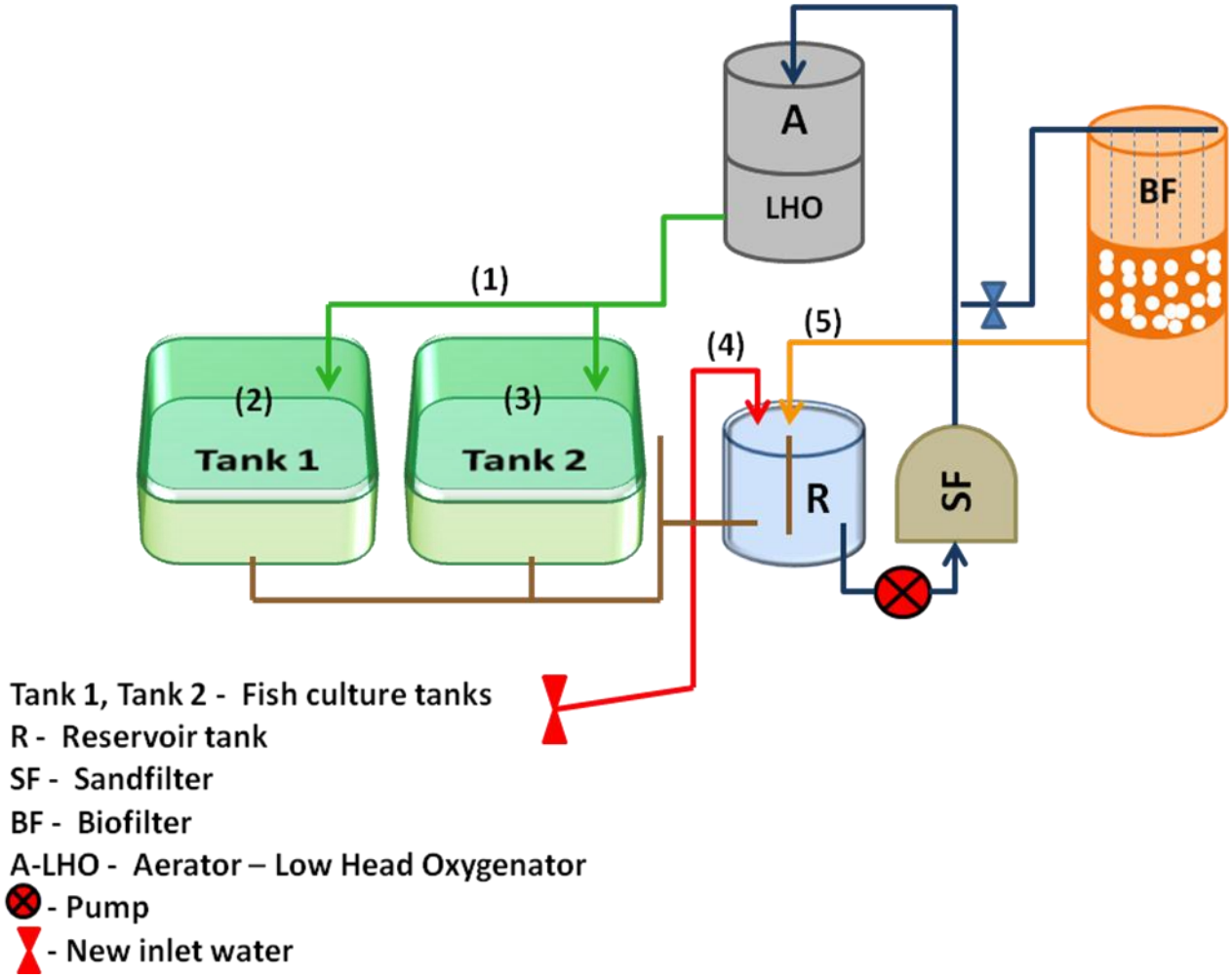

Figure 4: General diagram of the systems and measurement points. Recirculating aquaculture system (RAS) with biological filter coupling and limited reuse system (LRS) without biological filter, where (1) inlet water after total treatment, (2) fish culture tank 1, (3) fish culture tank 2, (4) inlet new water and (5) outlet water from BF.

4 RESULTS

4.1 Dissolved oxygen (DO) levels and oxygen consumption (MO_2) in the systems

The variation rates in DO concentrations and the rate of MO_2 in both systems during the experimental time are shown in Figure 5. The DO concentrations in the outlet water from the tanks in the LRS varied between 7.45-10.0 mg L^{-1}, while the inlet water tanks ranged between 8.90 and 11.89 mg L^{-1}. For the RAS, the DO concentrations ranged between 8.09 and 9.78 mg L^{-1} for the outlet water and 9.77-11.15 mg L^{-1} for the inlet water. The DO concentration was similar in both systems and higher than the recommended levels for salmonid aquaculture. The oxygen consumption (MO_2) in both systems was similar (Figure 5). The mean oxygen consumption in the LRS was 2.07 mg O_2 min^{-1} kg^{-1} ranging between 0.73 and 3.07 mg O_2 min^{-1} kg^{-1} and in the RAS the mean oxygen consumption was 1.80 mg O_2 min^{-1} kg^{-1} ranging between 0.58 and 2.62 mg O_2 min^{-1} kg^{-1}. The total body mass was 59.27 kg and 58.45 kg in the LRS and RAS respectively.

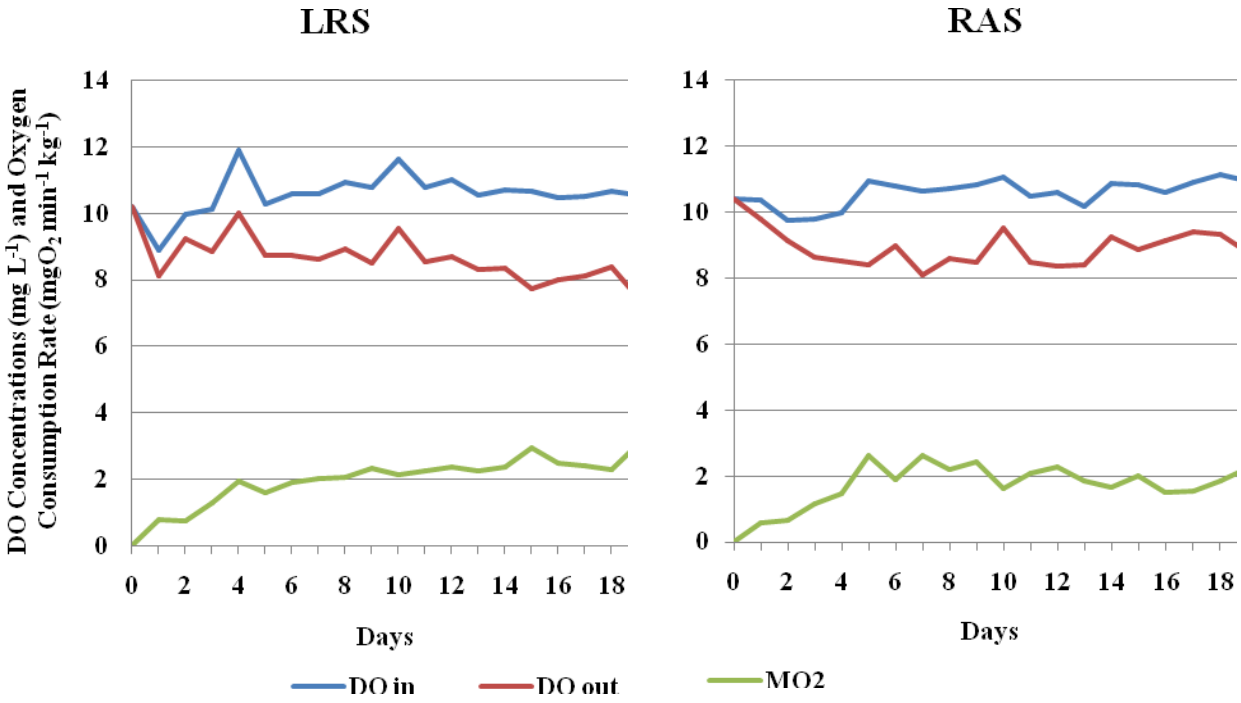

Figure 5: Dissolved oxygen (DO) concentrations (mg L^{-1}) in the water inlet tanks and in the outlet water from the tanks and the oxygen consumption rate (MO_2) of the fishes (mg O_2 min^{-1} kg^{-1}) in each system during the experimental time.

4.2 pH water levels in the systems

In both systems, the pH of the new water entering the systems and the inlet water into the tanks was similar, ranging from 7.4-7.8 and 7.7-8.0 for the LRS and RAS respectively (Figure 6). The pH for day 0 (7.98 for the LRS and 8.01 for the RAS) show values without fish in the systems. The pH in the outlet from the tanks was

lower than the pH of the inlet water ranging from 7.41-7.64 (mean 7.55) for the LRS and 7.43-7.80 (mean 7.58) for the RAS.

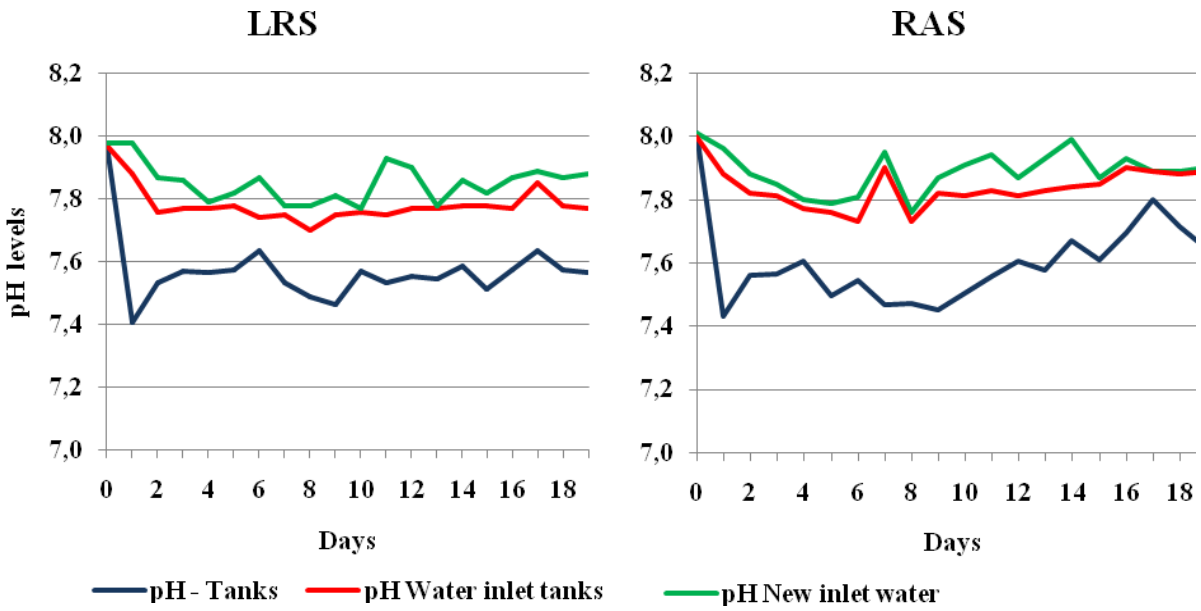

Figure 6: pH levels in the tanks water, in the water inlet tanks and in the new inlet water to the system for each system during the experimental time.

4.3 Total inorganic carbon (TIC) and carbon dioxide (CO_2) levels in the systems: removal rate of carbon dioxide (CO_2)

The concentration of TIC was similar in the inlet water to the systems and in the outlet from the tanks (Figure 7) and appears to be primarily determined by the TIC concentration in the inlet water. The TIC concentrations in all measuring points were 51.10-90.12 mg L^{-1} in the LRS and 66.70-91.89 mg L^{-1} in the RAS.

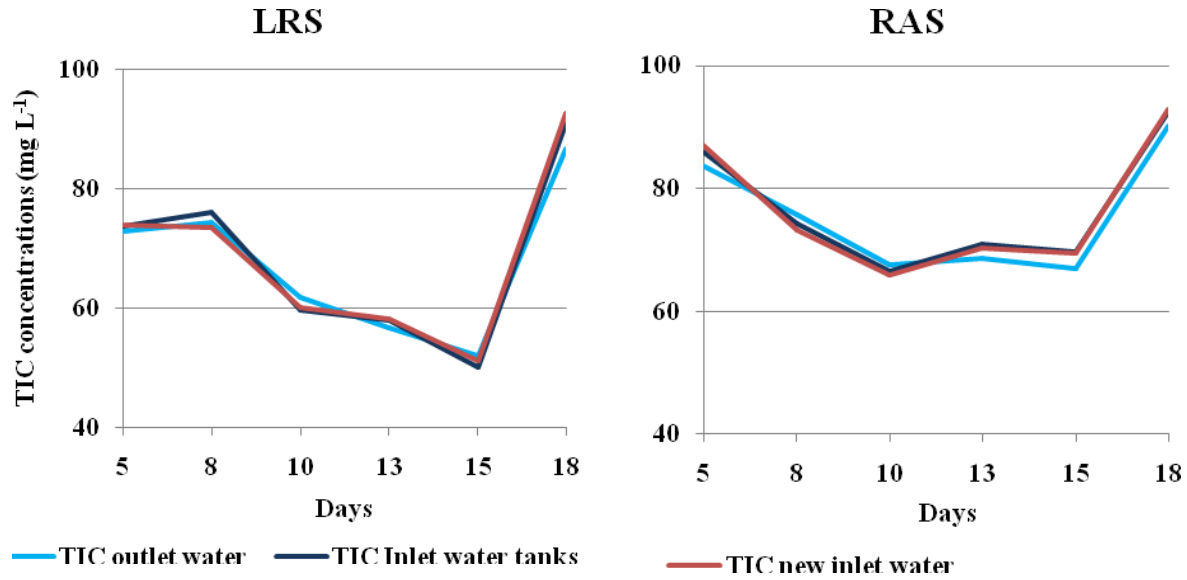

Figure 7: Total inorganic carbon (TIC) concentrations (mg L^{-1}) in the outlet and inlet water tanks and in the new inlet water to the system for each system during the experimental time.

The CO_2 concentrations were similar in the LRS and in the RAS (Figure 8). The mean CO_2 concentration in the inlets into the tanks was 2.01 mg L^{-1} in the LRS and 1.87 mg L-1 in the RAS. The CO_2 concentration in the outlet from the tanks was 1.87-4.32 mg L^{-1} in both systems and the mean values were 3.21 and 3.10 mg L^{-1} for the LRS and RAS respectively (Figure 8). During the last stage of the experiment the CO_2 concentration in the outlet from the tanks was lower in the RAS than in the LRS.

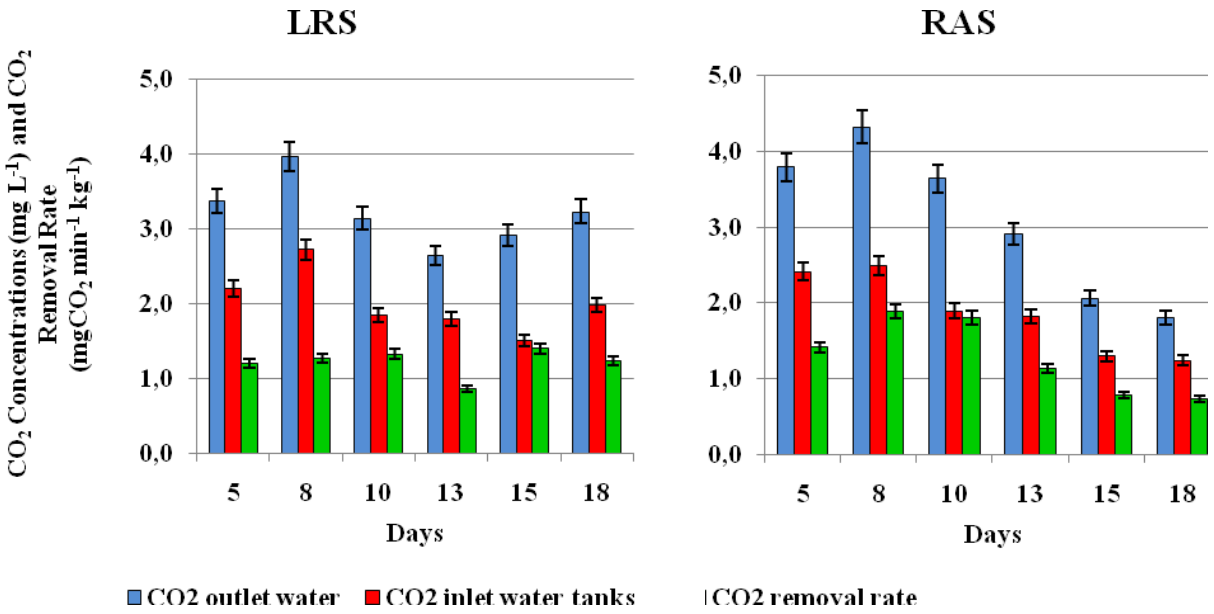

Figure 8: Carbon dioxide (CO_2) concentrations (mg L^{-1}) in the outlet water from the tanks and in the inlet water tanks and CO_2 removal rate from the system ($mgCO_2$ min^{-1} kg^{-1}) for each system during the experimental time.

4.4 Nitrogen metabolites

4.4.1 Total ammonia nitrogen (TAN) concentrations and removal rate of TAN in the systems

The TAN concentrations were higher in the RAS than in the LRS system (Figure 9). In both systems the TAN concentration increased over time albeit more in the RAS system. The TAN concentrations in the LRS were 0.163-0.482 mg L^{-1} in the outlet water from the tanks 0.149-0.447 mg L^{-1} for the water inlet to the tanks. In the RAS the TAN concentration in the outlet from the tanks was 0.251-1.520 mg L^{-1} and 0.246-1.577 mg L^{-1}.

The estimated TAN removal rate in the RAS (calculated from TAN concentration in the inlet water and outlet water to the tanks) was 0.5 to -5.7 mg TAN min^{-1} kg^{-1}. In the RAS, the TAN concentration was consistently higher in the inlet into the tanks than in the outlet resulting in negative estimates of removal rate (Figure 8). This may suggest

that TAN is also produced in other parts of the system. In fact, it was later discovered that the sand filter was not flushed adequately and that some TAN appeared to emanate from the filter. The TAN removal rate in the LRS was 0.7-1.5 mg TAN min^{-1} kg^{-1}.

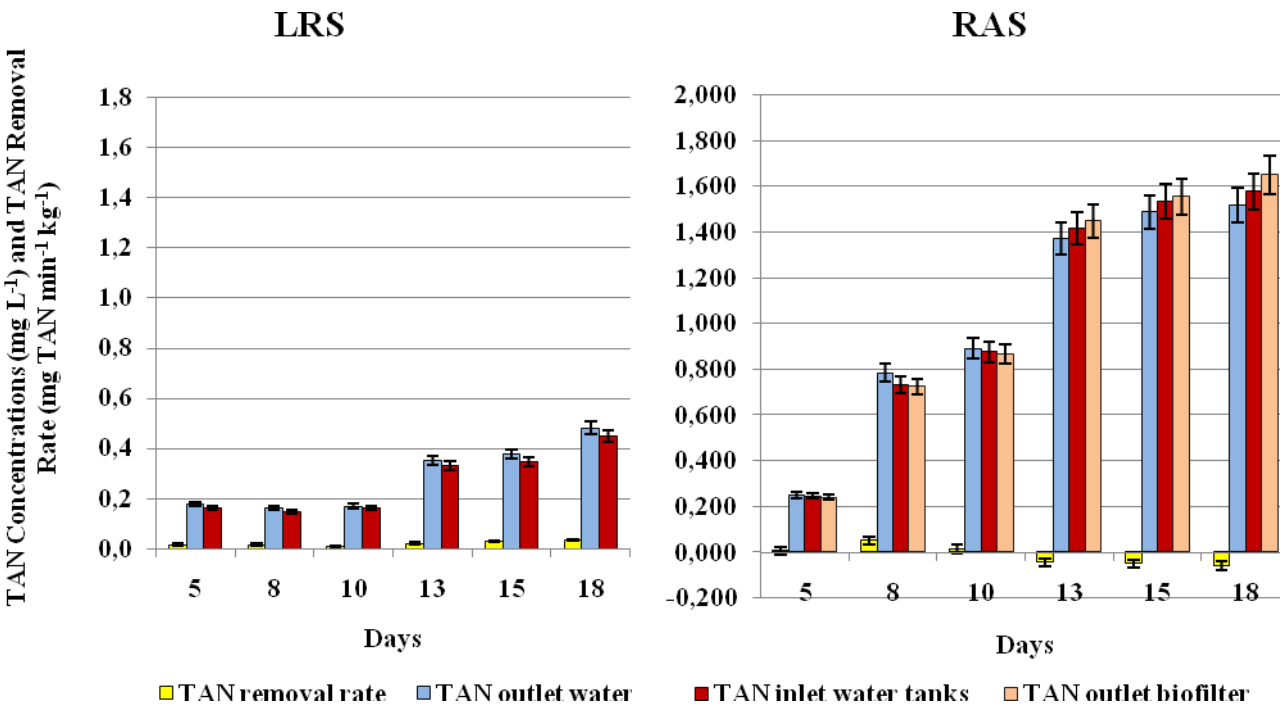

Figure 9: Total ammonia nitrogen (TAN) concentrations (mg L^{-1}) in the outlet water from the tanks and in the inlet water tanks and TAN removal rate (mg TAN min^{-1} kg^{-1}) for each system during the experimental time.

To examine the reason for the high TAN values in the inlet into the tanks, samples were taken on day 26 from the inlet into the biofilter in addition to samples from the inlet into the tanks and from the outlet (Figure 10). The outlet water from the tanks goes through a hydrocyclone and then to a reservoir and then it is pumped through a sand filter (Figure 4). From the sand filter the water goes either to the aerator or to the biofilter and then back to the reservoir. From day 0 samples were taken from the inlet to the tanks, from the outlet and from the inlet of new water to the system. On day 26, further samples were taken from the inlet into the biofilter. The TAN concentration in the water entering the biofilter was higher than in the inlet water and in the outlet of the tanks (Figure 10). This suggests that TAN is added to the water in the hydrocyclone, the reservoir or in the sand filter. After the sand filter was flushed, the TAN concentration at the inlet of the biofilter was reduced (Figure 10) suggesting that the high TAN concentration did in fact originate from the sand filter.

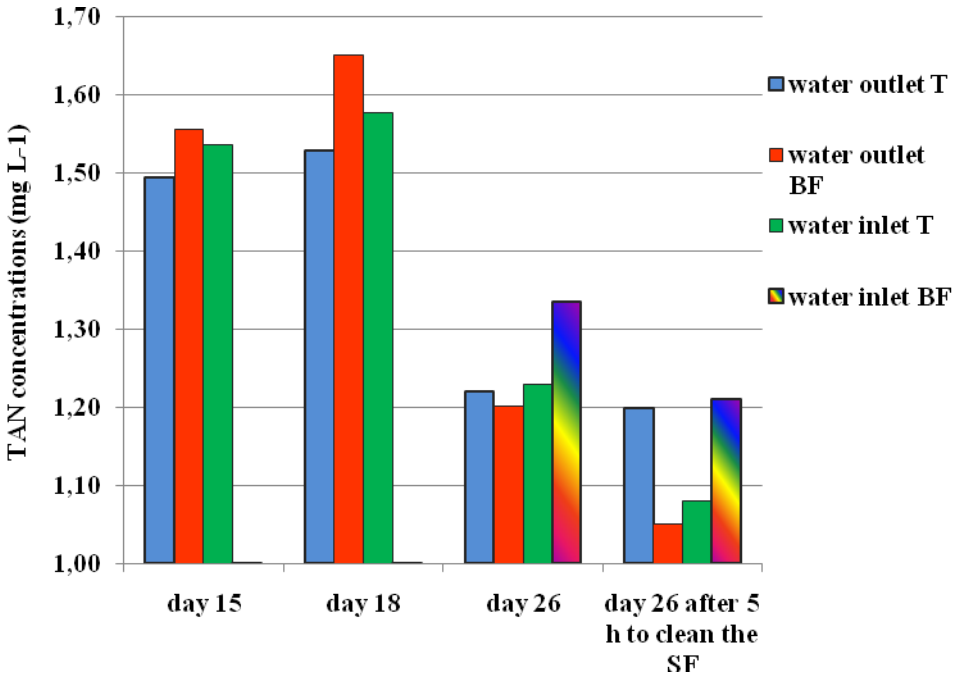

Figure 10: TAN concentration levels in different water points in the RAS at days 15 and 18 of the experimental period and at day 26, one week after the end of the experiment, before and after 5 hours to clean the sand filter.

4.4.2 Unionised ammonia (NH_3-N)

In general, the NH_3-N concentration in the systems reflected the TAN concentration, increasing during the experimental period in both systems (Figure 11). The NH_3-N concentrations were lower in the LRS than in the RAS (Figure 11). The NH_3-N concentrations in the RAS were close to 0.025 mg L^{-1}, which is the maximum recommended level for salmonid aquaculture. In the LRS, the NH_3-N concentrations were 0.001-0.003 mg L^{-1} and 0.001-0.005 mg L^{-1} in the water inlet to the tanks during all the experimental period. The NH_3-N concentrations in the RAS were 0.001-0.014 mg L^{-1} in the outlet from the tanks and 0.002-0.018 mg L^{-1} in the outlet water from the biofilter unit and 0.003-0.023 mg L^{-1} in the water inlet to the tanks.

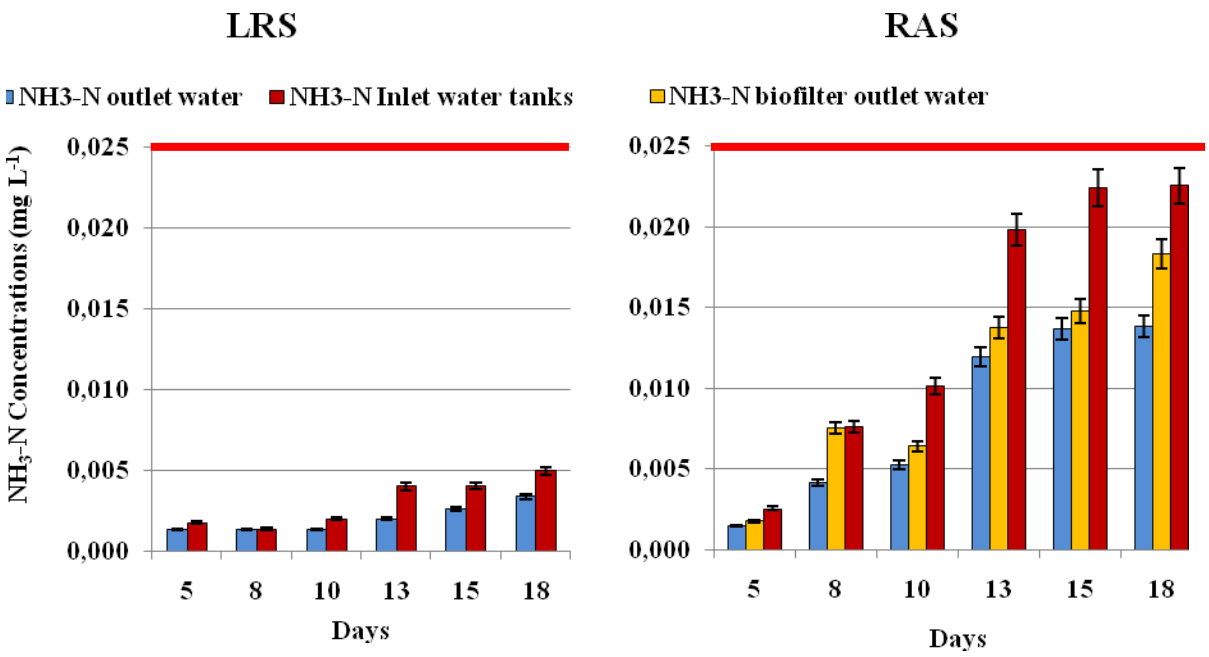

Figure 11: Unionised ammonia (NH_3-N) concentrations (mg L^{-1}) for each system in the outlet water from the tanks and in the water inlet tanks and in the outlet water from the biofilter in the RAS, during the experimental time. The red line in both charts indicates the unionised ammonia (NH_3-N) concentrations limit of water quality (mg L^{-1}) for salmonids culture.

4.4.3 Nitrogen metabolites

The nitrite concentration in the RAS increased during the experiment with a concomitant increase in nitrate concentration (Figure 12). The TAN concentration was higher than either the nitrite or nitrate concentration during the experiment. The NO_2-N and NO_3-N concentrations started to increase on day 8 and 10 ranged from 0-1.10 mg L^{-1} and 0-0.66 mg L^{-1} respectively. This pattern of increase in TAN, NO_2-N and NO_3-N suggests that the function of the biofilter was gradually increasing during the experiment.

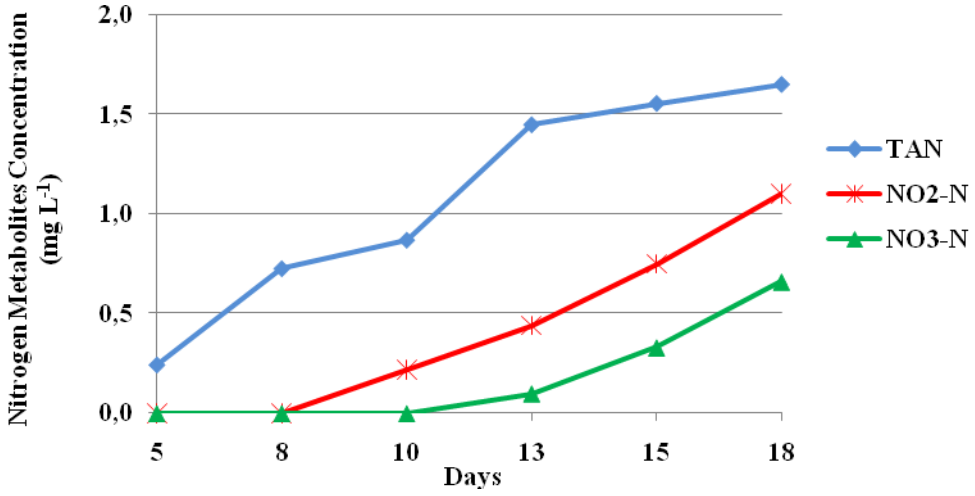

Figure 12: Nitrogen metabolites (TAN, NO_2-N and NO_3-N) concentrations (mg L^{-1}) in the outlet water from the biofilter in the RAS.

The function of the biofilter was tested by turning it off for one hour while the concentration of TAN was measured (Figure 18). Samples were taken from the water outlet from the tanks and from the water inlet to the tanks. The TAN concentrations increased after the biofilter was turned off by 0.1 mg L^{-1} and 0.2 mg L^{-1} in the outlet and inlet water respectively (Figure 18).

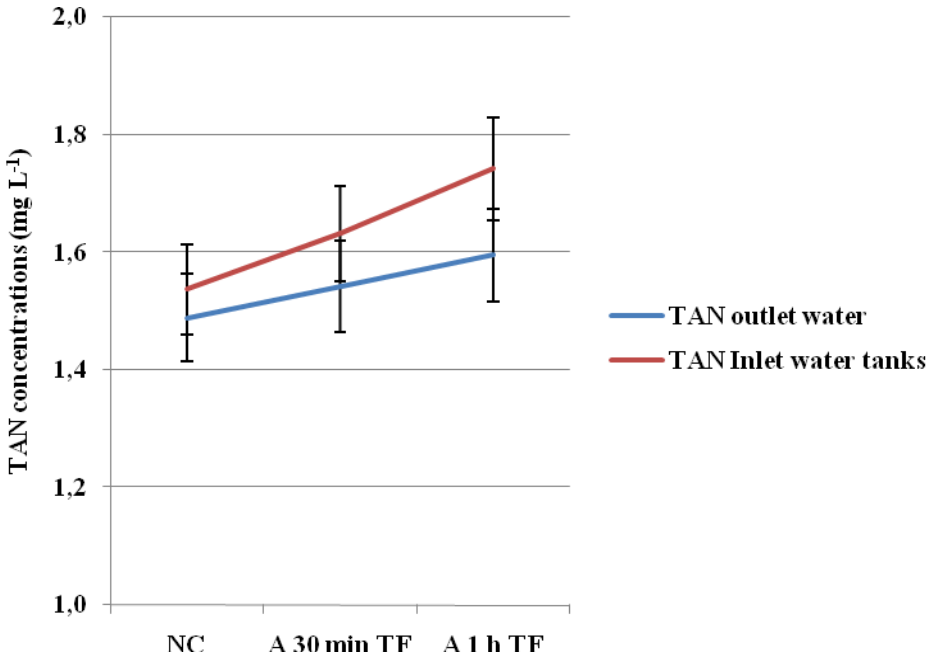

Figure 13: Total ammonia nitrogen (TAN) concentrations (mg L^{-1}) in the outlet water from the tanks and in the inlet water tanks for the RAS during three stages at the same experimental day (18), where NC (normal conditions), A 30 min TF (after 30 minutes of turn off the biofilter) and A 1 h TF (after 1 hour of turn off the biofilter).

4.5 Total suspended solids (TSS) levels and removal rate of TSS in the systems

The TSS concentrations in the outlet and inlet water performance increased during the experiment (Figure 14). The TSS concentration in the outlet water from the tanks in the LRS was 1.04-5.58 mg L^{-1} and 0.93-8.85 mg L^{-1} in the RAS. The TSS in the inlet water tanks was 0.10-4.47 and 0.70-8.75 mg L^{-1} respectively. However the TSS was higher in the RAS than in the LRS.

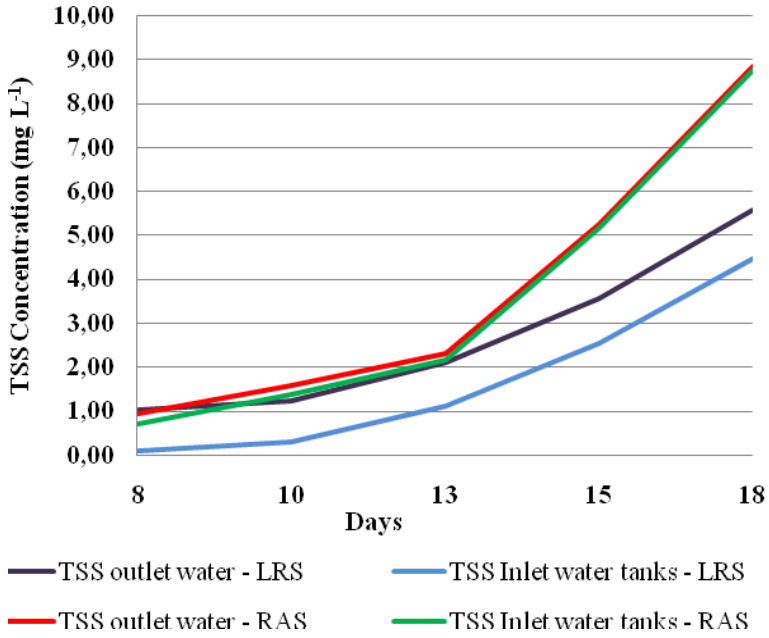

Figure 14: Total suspended solids (TSS) concentrations (mg L^{-1}) in the outlet water from the tanks and in the inlet water tanks for each system (LRS and RAS) during the experimental time.

The TSS removal rate in the systems (Figure 15) was different for each one. The LRS showed higher values of TSS removal rate than the RAS during all the experimental period with values between 96-110% and a relatively constant performance, while the RAS values were between 23-10% of TSS removal rate, however lower values were obtained at the end of the experiment than at the beginning.

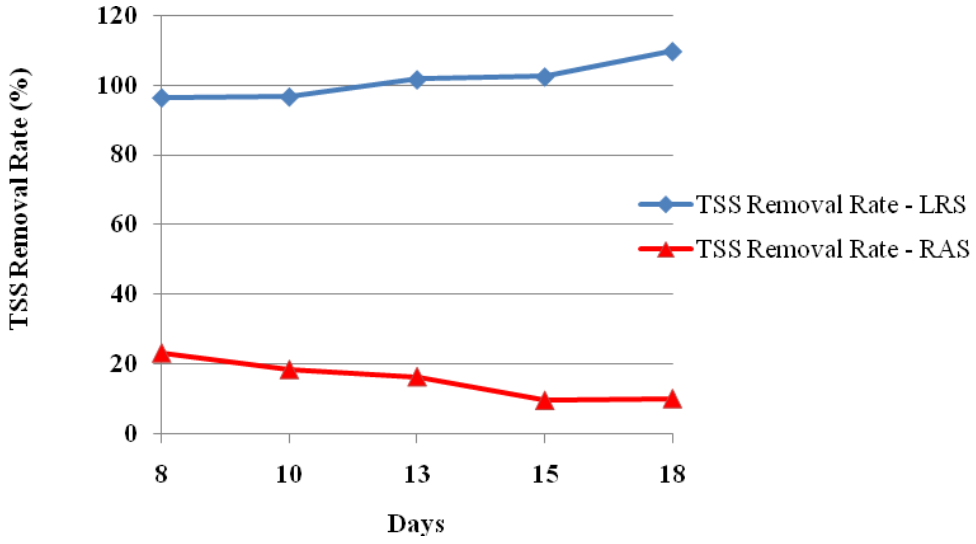

Figure 15: Total suspended solids (TSS) removal rate (%) for LRS and RAS during the experimental time.

5 DISCUSSION

5.1 Dissolved oxygen (DO) levels and oxygen consumption (MO_2) in the systems

In any aquaculture system the DO concentrations in the culture water is one of the most important parameters to maintain at safe levels to provide optimal conditions for the fish (Timmons *et al*. 2002, Pillay and Kutty 2005). The DO concentrations in the inlet water were over 8.9 mg L^{-1} and over 7.45 mg L^{-1} in the outlet water in both systems. These values are higher than the 7.0 mg L^{-1} suggested for salmonid aquaculture (Pillay and Kutty 2005).

Aquafarmer (2004) also recommends, for salmonid culture, levels of DO saturation between 70-80% for normal conditions (0 ppt of salinity and 6^{o}C of temperature) and the DO levels in both systems exceeded this value.

The fish oxygen consumption rate (MO_2) in both systems was similar, increasing during the first 4 days of the experiment while the fish adapted to new water conditions and thereafter they were fairly constant or 2.00-3.07 mg O_2 min^{-1} kg^{-1} in the LRS and 2.00-2.62 mg O_2 min^{-1} kg^{-1} in the RAS. The MO_2 was comparable to what has been reported for 200-300 g Arctic charr in other studies (Summerfelt and Sharrer 2004, Summerfelt *et al.* 2004a).

The MO_2 of the fish in the LRS was a little higher than in the RAS. The MO_2 of fish is variable and depends on many factors such as temperature, body mass, feeding rate, growth rate, stress and other factors (Forsberg 1997, Timmons *et al*. 2002, Pillay and Kutty 2005). The initial biomass in both systems was approximately 60 kg at the beginning of the experiment. During the experiment the biomass was reduced slightly because some fish died. The final biomass was slightly higher in the LRS than in the RAS.

The growth rate of the fish was also slightly higher in the LRS than in the RAS. The biomass increased by 0.110 kg day^{-1} in the LRS and only 0.035 kg day^{-1} in the RAS (see Appendix from daily measurements in the systems). Moreover, the feed intake of the fish in the RAS was lower than in the LRS.

The concentration of nitrogen metabolites was higher in the RAS and this may have contributed to higher stress levels in this system and that may have caused the fish to lose their appetite, reduce growth rates and the total body mass in the RAS system. This may also have contributed to the lower MO_2 of the fish in the RAS.

5.2 pH levels in the systems

The pH levels depend on the performance of the total inorganic carbon equilibrium in the water and which one of carbon species is predominant in the water environment as was shown in section 2, Figure 1 (Boyd 2000). The pH levels of the culture water were 7.4-8.0 in both systems during all the experimental period, values which are within the optimal rate for Arctic charr aquaculture (Aquafarmer 2004).

The pH value fluctuations observed in both systems during the period (Figure 6) depended on the total inorganic carbon (TIC) concentration of the new water in the inlet to the systems (Figure 7) and on the total CO_2 concentration in the culture water (Figure 8). The low pH levels registered in the outlet water from the culture tanks for the LRS during the whole period in comparison with the pH levels from the RAS were mainly due to the higher CO_2 concentration levels in the RAS (Fig. 8).

The pH values in the inlet water were higher than in the outlets in both systems (Figure 6) due to the function of the aerators. They removed the dissolved CO_2 from the water and, as a result, the water pH increased.

5.3 Total inorganic carbon (TIC) levels and carbon dioxide (CO_2) levels in the systems: removal rate of carbon dioxide (CO_2)

Carbon dioxide (CO_2) is a function primarily of the total amount of inorganic carbon (TIC) present in water and of pH (Summerfelt *et al*. 2000). During the experiment, the TIC concentrations measured for the outlet and inlet water from the tanks and for the new water inlet to the systems were similar and depended mainly on the TIC concentration in the new inlet water in the systems. The TIC was higher in the RAS than in the LRS during whole the period analysed. This may be related to the slightly higher temperature in the RAS system (see Appendix).

The CO_2 concentrations were similar in both systems during the entire experiment (Figure 8). The CO_2 concentration was slightly higher in the LRS (2.64-3.97 mg CO_2 L^{-1}) and than in the RAS (1.87-4.32 mg CO_2 L^{-1}). This difference may be a result of the lower metabolic rate of the fish in the RAS. The amount of CO_2 produced for each mg of oxygen consumed was about 1:1 as had been suggested by other studies in Arctic charr (Aquafarmer 2004, Forsberg 1997). The CO_2 concentrations in both systems were lower than the 10-20 mg L^{-1} which is the suggested limit for CO_2 in salmonid aquaculture (Fivelstad *et al*. 1998, Summerfelt *et al*. 2000, Summerfelt *et al*. 2004).

The low dissolved CO_2 in both systems suggests that the aerators effectively removed CO_2 from both systems (Figure 8).

5.4 Total ammonia nitrogen (TAN) and unionised ammonia (NH_3) levels in the systems: removal rate of TAN

During the study, the TAN concentrations were higher in the RAS than in the LRS (Figure 9).

The TAN concentrations in the LRS are mainly determined by water exchange. The net water inflow into the LRS was 0.2 L min^{-1} kg^{-1} during the entire experiment, it indicates that the system changes the total water volume 10 times per day. The total TAN production in the system was approximately 6.0 mg min^{-1} (12 L min^{-1} x 0.5 mg L^{-1}). This suggests that the TAN production was about 0.05 mg kg^{-1} min^{-1} which is comparable with the expected Arctic charr TAN production for each mg of oxygen consumed (0.04-0.06:1). The high water exchange rate maintained the TAN

concentration lower than 3.0 mg L^{-1} which is recommended for good water quality for Arctic charr culture (Aquafarmer 2004).
The initial water exchange in the RAS was similar to the LRS and, therefore, the initial TAN concentration was similar in both systems. Then the water exchange was reduced in the RAS up to 0.05 L min^{-1} kg^{-1} on day 6 of the experiment and then the TAN concentration increased. However, the TAN production in the RAS at this time was around 0.2 mg kg^{-1} min^{-1}, approximately 4 times higher than the LRS. During this period the major percentage of TAN was removed from the system both by water exchange and through the biofilter.

Thereafter the water exchange reduced up to 0.008 L min^{-1} kg^{-1} in the RAS on day 12 of the experiment, the TAN production in the system increased up to 1.25 mg kg^{-1} min^{-1}, around 25 times higher than the LRS and than the beginning of the experimental period for this system. During this last period, the TAN concentrations showed a difference in performance from the beginning, higher values were obtained for the water inlet to the tanks and from the water outlet biofilter than the water outlet from the tanks (Figure 9). However, the apparent TAN removal rate obtained during this time showed negative values. Its performance should be due to the influence of various factors in conjunction: the reduction of the exchange flow rate up to 40%, the possibility of ammonia production in some places between the tanks and the biofilter, and the biofilter capacity to remove ammonia.

The outlet water from the tanks goes to the reservoir tank and from there it is pumped to the sand filter. From the sand filter, part of the water goes to the biofilter and returns again to the reservoir while the remaining water goes to the aerator and then enters the tanks. During the experiment, the water coming from the sand filter was not sampled. Apparently, some TAN was produced in the sand filter thus increasing the TAN concentration in the system.

When the biofilter was turned off on day 18, the TAN concentration increased by about 0.2 mg L^{-1} $hour^{-1}$. This suggests that the biofilter was removing approximately 0.003 mg of TAN L^{-1} min^{-1} (5.49 mg of TAN min^{-1}) or about 7.32% of the TAN produced in the system.

On day 26, after the end of the experimental time, the TAN concentration was measured in the water inlet to the biofilter and in the other measurement points in the RAS both before and after the sand filter was flushed. The results showed that the water inlet to the biofilter had higher TAN concentration values than the water outlet from the tanks before flushing the sand filter; and after it was flushed, the water inlet to the tank almost had the same TAN values as the water outlet from the tanks, and TAN concentrations in the water outlet biofilter were reduced considerably. This clearly demonstrates the need for a regular back flush of the sand filter to avoid build up of heterotrophic bacteria culture which produces ammonia that can compromise the performance of the biofilter and the suitable operation of the system.

The NH_3-N concentration levels were lower than 0.025 mg L^{-1} during the whole period for both systems, in the optimal rate for NH_3-N levels recommended for Arctic charr culture (Aquafarmer 2004), although it is important to draw attention to the NH_3-N levels obtained for the RAS at the end of the experimental period, where the

NH_3-N concentrations in the inlet water tanks showed values in close proximity to 0.025 mg L^{-1} (Figure 11).

5.5 Biofilter performance in the RAS

The concentrations of nitrite and nitrate were measured during the experiment. According to Timmons *et al*. (2002), the biofilter goes through several stages while the nitrifying bacteria are multiplying and reaching full capacity. First the TAN concentrations increase but when the activity of the nitrifying bacteria increases first the NO_2-N increases. Then the NO_3-N concentrations increase while the TAN and NO_2-N begin to decrease (Fig. 2).

As shown in Figure 12, the TAN concentration in the RAS was higher than the other nitrogen metabolite concentrations, suggesting that the biofilter was still maturing. Because of the short duration of the experiment (3 weeks) it was not possible to observe the full development of the biofilter; but with the biofilter TAN removal rate values obtained during the last experiments on days 18 and 26, it was demonstrated that the biofilter was removing ammonia out the system but not in high enough amounts to keep the TAN concentrations out of the water outlet from the biofilter and the water inlet tanks lower than the TAN concentrations of the outlet water from the tanks in the system, due to the ammonia contribution from the sand filter.

5.6 Total suspended solid (TSS) levels in the systems: removal rate of TSS

Waste solids control is one of the most critical processes that must be managed in recirculating systems, it accumulates in aquaculture systems from uneaten feed, feed fines, fish faecal matter, algae, and biofilm cell mass sloughed from biofilters (Timmons *et al*. 2002). Solids decomposition can degrade water quality and thus directly and indirectly affect fish health and the performance of other unit processes within recirculating systems such as elevated organic matter in the sand filters and inhibition of the bacteria process within biofilters (Chen *et al.* 1993) because they are a major source of carbonaceous oxygen demand and nutrient input into the water (Timmons *et al*. 2002).

In this study, the TSS concentrations in the outlet and inlet water for both systems showed the same performance (Figure 14), they increase with the time from the beginning until the end of the experiment, and the concentration was lower in the LRS than the RAS during the whole period (Figure 14) as a result of the TSS removal rate in the systems (Figure 15). However, the TSS concentrations in both systems were lower than the 15 mg L^{-1} recommended for Arctic charr (Aquafarmer 2004).

Suspended solids within the fish culture tanks are very naturally difficult to remove because they do not settle out by conventional gravity settling basins and therefore a treatment process and/or high exchange flow rate is required (Timmons *et al*. 2002, Pillay and Kutty 2005). The TSS removal rate in the LRS was higher than the RAS during the entire experimental period, and for the RAS lower values were obtained at the end of the experiment. Differences between systems and within the RAS were caused by the water exchange flow rate for the system (Timmons *et al*. 2002). For the LRS the TSS removal rate values obtained showed a constant performance due to the

constant net flow rate in the system (0.2 L min^{-1} kg^{-1}) with an exchange of the total water volume out of the system 10 times per day during the whole period studied, while the RAS, at the beginning the total water volume exchange rate was the same as the LRS and thereafter decreased gradually to 0.4 times per day when the net water flow was reduced to 0.008 L min^{-1} kg^{-1}. Thus the changes in the net water flow for the RAS caused a gradual increase in the TSS concentrations within the system reducing the capacity of it to remove the TSS produced.

6 CONCLUSIONS

- The water quality parameters measured were well within the acceptable levels for Arctic charr culture.
- The water quality was better in the LRS than in the RAS during the experimental time.
- The biofilter unit in the RAS started to work around a week later than the normal performance referred to in the literature due to the lower temperatures used for the Arctic charr culture in the experiment.
- The sand filter should be cleaned regularly, 2 or 3 times per week, to avoid build up of heterotrophic bacteria culture which produces ammonia and can affect the performance of the RAS.

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APPENDIX: TABLES OF MEASUREMENTS.

Tables of Measurements for the Limited Reuse System (LRS)

Table 2: Daily measurements in the LRS tank No. 1 between days 0 – 9.

Days	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Date	**21.1.2008**	**22.1.2008**	**23.1.2008**	**24.1.2008**	**25.1.2008**	**28.1.2008**	**29.1.2008**	**30.1.2008**	**31.1.2008**	**1.2.2008**
Temperature (^oC)	9,9	9,5	9,9	9,9	9,9	10,5	10,3	10,2	10,1	10,6
pH	7,97	7,41	7,54	7,56	7,55	7,58	7,63	7,54	7,49	7,47
Salinity (ppt)	20	20	20	20	20	20	20	20	21	20
DO in (%)	104,2	88,6	99,3	102,4	120,0	101,8	106,6	106,2	109,7	108,8
DO in (mg L^{-1})	10,20	8,90	9,95	10,10	11,89	10,26	10,58	10,57	10,92	10,76
DO out (%)	104,0	81,5	91,6	88,9	100,6	88,6	88,2	86,3	90,3	86,6
DO out (mg L^{-1})	10,21	8,18	9,19	8,80	10,00	8,73	8,70	8,59	9,01	8,58
Total Biomass (kg)	0	30,02	29,79	29,79	29,79	29,31	29,42	29,53	29,64	29,75
MO_2 (mgO_2 min^{-1} kg^{-1})	0	0,72	0,77	1,31	1,90	1,57	1,92	2,01	1,93	2,20
No. Fish	0	158	158	157	157	157	155	155	155	155
Mortality (%)	0	0	0,63	0,63	0,63	1,91	1,91	1,91	1,91	1,91
No. Dead Fish	0	0	1	0	0	2	0	0	0	0
Weight Dead Fish (kg)	0	0	0,234	0	0	0,583	0	0	0	0
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0,110	0,110	0,110	0,110	0,110	0,110

Table 3: Daily measurements in the LRS tank No. 1 between days 10 – 19.

Days	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Date	**4.2.2008**	**5.2.2008**	**6.2.2008**	**7.2.2008**	**8.2.2008**	**11.2.2008**	**12.2.2008**	**13.2.2008**	**14.2.2008**	**15.2.2008**
Temperature (^oC)	10,6	10,6	10,0	10,2	10,2	10,5	10,4	10,5	10,0	10,4
pH	7,57	7,54	7,55	7,54	7,57	7,52	7,57	7,61	7,57	7,56
Salinity (ppt)	20	20	21	21	21	21	20	20	21	21
DO in (%)	117,3	108,8	109,7	105,6	107,2	107,1	105,4	105,5	106,5	106,1
DO in (mg L^{-1})	11,63	10,76	11,01	10,53	10,68	10,65	10,47	10,51	10,66	10,55
DO out (%)	96,2	85,3	86,8	81,7	81,8	76,9	81,0	80,0	82,5	74,8
DO out (mg L^{-1})	9,52	8,43	8,71	8,14	8,15	7,58	8,04	7,91	8,27	7,42
Total Biomass (kg)	29,59	29,70	29,81	29,92	30,03	30,14	30,25	30,36	30,47	30,58
MO_2 (mgO_2 min^{-1} kg^{-1})	2,14	2,35	2,31	2,40	2,53	3,06	2,41	2,57	2,35	3,07
No. Fish	155	154	154	154	154	154	154	154	154	154
Mortality (%)	2,55	2,55	2,55	2,55	2,55	2,55	2,55	2,55	2,55	2,55
No. Dead Fish	1	0	0	0	0	0	0	0	0	0
Weight Dead Fish (kg)	0,275	0	0	0	0	0	0	0	0	0
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,110	0,110	0,110	0,110	0,110	0,110	0,110	0,113	0,113	0,113

Table 4: Daily measurements in the LRS tank No. 2 between days 0 – 9.

Days	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Date	**21.1.2008**	**22.1.2008**	**23.1.2008**	**24.1.2008**	**25.1.2008**	**28.1.2008**	**29.1.2008**	**30.1.2008**	**31.1.2008**	**1.2.2008**
Temperature (^oC)	9,9	9,5	9,9	9,9	9,9	10,5	10,3	10,2	10,1	10,6
pH	7,97	7,40	7,53	7,58	7,58	7,57	7,64	7,53	7,49	7,46
Salinity (ppt)	20	20	20	20	20	20	20	20	21	20
DO in (%)	104,2	88,6	99,3	102,4	120,0	101,8	106,6	106,2	109,7	108,8
DO in (mg L^{-1})	10,20	8,90	9,95	10,10	11,89	10,26	10,58	10,57	10,92	10,76
DO out (%)	104,0	80,3	92,1	89,1	100,4	88,0	88,5	86,9	88,8	84,8
DO out (mg L^{-1})	10,21	8,07	9,27	8,87	9,98	8,70	8,79	8,63	8,85	8,38
Total Biomass (kg)	0	30,03	29,49	29,26	29,26	28,80	28,91	28,76	28,87	28,98
MO_2 (mgO_2 min^{-1} kg^{-1})	0	0,83	0,69	1,26	1,96	1,62	1,86	2,02	2,15	2,46
No. Fish	0	158	158	156	155	155	153	153	152	152
Mortality (%)	0	0	1,27	1,91	1,91	3,20	3,20	3,85	3,85	3,85
No. Dead Fish	0	0	2	1	0	2	0	1	0	0
Weight Dead Fish (kg)	0	0	0,543	0,223	0	0,571	0	0,268	0	0
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0,110	0,110	0,110	0,110	0,110	0,110

Table 5: Daily measurements in the LRS tank No. 2 between days 10 – 19.

Days	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Date	**4.2.2008**	**5.2.2008**	**6.2.2008**	**7.2.2008**	**8.2.2008**	**11.2.2008**	**12.2.2008**	**13.2.2008**	**14.2.2008**	**15.2.2008**
Temperature (^oC)	10,6	10,6	10,0	10,2	10,2	10,5	10,4	10,5	10,0	10,4
pH	7,57	7,53	7,56	7,55	7,60	7,51	7,58	7,66	7,58	7,57
Salinity (ppt)	20	20	21	21	21	21	20	20	21	21
DO in (%)	117,3	108,8	109,7	105,6	107,2	107,1	105,4	105,5	106,5	106,1
DO in (mg L^{-1})	11,63	10,76	11,01	10,53	10,68	10,65	10,47	10,51	10,66	10,55
DO out (%)	96,7	87,3	86,5	85,2	85,4	79,2	80,0	82,4	84,3	75,3
DO out (mg L^{-1})	9,56	8,64	8,67	8,49	8,52	7,86	7,94	8,28	8,46	7,47
Total Biomass (kg)	29,09	29,20	29,31	29,42	29,53	29,64	29,75	29,86	29,97	30,08
MO_2 (mgO_2 min^{-1} kg^{-1})	2,14	2,18	2,40	2,08	2,19	2,82	2,55	2,24	2,20	3,07
No. Fish	152	152	152	152	152	152	152	152	152	152
Mortality (%)	3,85	3,85	3,85	3,85	3,85	3,85	3,85	3,85	3,85	3,85
No. Dead Fish	0	0	0	0	0	0	0	0	0	0
Weight Dead Fish (kg)	0	0	0	0	0	0	0	0	0	0
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,110	0,110	0,110	0,110	0,110	0,110	0,110	0,113	0,113	0,113

Table 6: Daily measurements in the new water inlet to LRS between days 0 – 9.

Days	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Date	**21.1.2008**	**22.1.2008**	**23.1.2008**	**24.1.2008**	**25.1.2008**	**28.1.2008**	**29.1.2008**	**30.1.2008**	**31.1.2008**	**1.2.2008**
Temperature (^{o}C)	9,9	9,9	9,8	9,5	9,2	9,7	9,5	9,4	9,4	9,5
pH	7,98	7,98	7,87	7,86	7,79	7,82	7,87	7,78	7,78	7,81
Salinity (ppt)	20	20	20	20	20	20	20	20	21	20
DO (%)	107,2	106,3	108,1	99,3	96,3	89,9	94,3	98,0	98,7	105,0
DO (mg L^{-1})	10,67	10,27	10,89	9,87	9,58	9,18	9,58	10,12	10,15	10,49
Flow rate (L min^{-1})	12	12	12	12	12	12	12	12	12	12
Flow rate (L min^{-1} kg^{-1})	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2

Table 7: Daily measurements in the new water inlet to LRS between days 10 – 19.

Days	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Date	**4.2.2008**	**5.2.2008**	**6.2.2008**	**7.2.2008**	**8.2.2008**	**11.2.2008**	**12.2.2008**	**13.2.2008**	**14.2.2008**	**15.2.2008**
Temperature (^{o}C)	9,0	8,8	8,4	8,8	8,6	8,7	8,8	9,0	8,5	8,7
pH	7,77	7,93	7,90	7,78	7,86	7,82	7,87	7,89	7,87	7,88
Salinity (ppt)	20	21	21	21	21	21	20	20	21	21
DO in (%)	116,8	110,2	102,4	95,2	96,1	85,3	79,2	73,5	73,1	77,6
DO in (mg L^{-1})	11,89	11,29	10,64	9,69	9,91	8,76	8,13	7,93	7,59	8,04
Flow rate (L min^{-1})	12	12	12	12	12	12	12	12	12	12
Flow rate (L min^{-1} kg^{-1})	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2

Table 8: Values of different water quality parameters calculated in LRS tank No. 1 two times per week during the experimental time and their Removal rate values.

Items	Days					
	5	**8**	**10**	**13**	**15**	**18**
TC (mg L^{-1})	72,98	74,38	60,18	55,03	53,52	87,23
CO_2 (mg L^{-1})	3,34	3,97	2,90	2,38	2,99	3,37
Removal Rate CO_2 ($mgCO_2$ min^{-1} kg^{-1})	1,16	1,26	1,07	0,58	1,47	1,36
Removal Rate CO_2 (%)	116	126	107	58	147	136
TAN (mg L^{-1})	0,181	0,164	0,171	0,359	0,383	0,496
Removal Rate TAN (mgTAN min^{-1} kg^{-1})	0,020	0,016	0,008	0,028	0,035	0,049
Removal Rate TAN (%)	2,0	1,6	0,8	2,8	3,5	4,9
NH_3-N (mg L^{-1})	0,001	0,001	0,001	0,002	0,003	0,003
TSS (mg L^{-1})	-	1,06	1,24	2,15	3,55	5,55
Removal Rate TSS (mgTSS min^{-1} kg^{-1})	-	0,97	0,96	1,03	1,00	1,06
Removal Rate TSS (%)	-	97	96	103	100	106

Table 9: Values of different water quality parameters calculated in LRS tank No. 2 two times per week during the experimental time and their Removal rate values.

Items	Days					
	5	**8**	**10**	**13**	**15**	**18**
TC (mg L^{-1})	72,99	74,38	63,62	58,47	50,60	86,20
CO_2 (mg L^{-1})	3,39	3,97	3,38	2,91	2,83	3,09
Removal Rate CO_2 ($mgCO_2$ min^{-1} kg^{-1})	1,23	1,29	1,59	1,14	1,33	1,11
Removal Rate CO_2 (%)	123	129	159	114	133	111
TAN (mg L^{-1})	0,171	0,163	0,168	0,343	0,368	0,468
Removal Rate TAN (mgTAN min^{-1} kg^{-1})	0,011	0,014	0,005	0,012	0,021	0,021
Removal Rate TAN (%)	1,1	1,4	0,5	1,2	2,1	2,1
NH_3-N (mg L^{-1})	0,001	0,001	0,001	0,003	0,003	0,003
TSS (mg L^{-1})	-	1,02	1,23	2,10	3,59	5,60
Removal Rate TSS (mgTSS min^{-1} kg^{-1})	-	0,96	0,97	1,00	1,05	1,13
Removal Rate TSS (%)	-	96	97	100	105	113

Table 10: Values of different water quality parameters calculated in the water inlet tanks of the LRS two times per week during the experimental time and the water flow using inside the tanks in the system.

	Days					
Items	**5**	**8**	**10**	**13**	**15**	**18**
TC (mg L^{-1})	73,70	76,04	59,69	58,03	50,07	90,98
CO_2 (mg L^{-1})	2,21	2,72	1,84	1,80	1,51	1,98
TAN (mg L^{-1})	0,161	0,149	0,163	0,331	0,347	0,447
NH_3-N (mg L^{-1})	0,002	0,001	0,002	0,004	0,004	0,005
TSS (mg L^{-1})	-	0,10	0,29	1,12	2,55	4,47
Water flow (L min^{-1})	30	30	30	30	30	30

Table 11: Values of different water quality parameters calculated in the new water inlet to LRS two times per week during the experimental time and the water flow using within the system.

	Days					
Items	**5**	**8**	**10**	**13**	**15**	**18**
TC (mg L^{-1})	73,98	73,54	60,05	58,22	51,18	92,67
CO_2 (mg L^{-1})	1,91	2,16	1,29	1,32	1,39	1,96
TAN (mg L^{-1})	0,002	0	0,002	0,002	0	0
NH_3-N (mg L^{-1})	0	0	0	0	0	0
TSS (mg L^{-1})	-	0,15	0,20	0,20	0,10	0,15
Water flow (L min^{-1})	12	12	12	12	12	12

Tables of Measurements for the Recirculating Aquaculture System (RAS)

Table 12: Daily measurements in the RAS tank No. 1 between days 0 – 9.

Days	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Date	**21.1.2008**	**22.1.2008**	**23.1.2008**	**24.1.2008**	**25.1.2008**	**28.1.2008**	**29.1.2008**	**30.1.2008**	**31.1.2008**	**1.2.2008**
Temperature (^{o}C)	9,0	8,9	10,5	10,5	10,6	12,3	12,0	13,4	12,4	13,0
pH	8,01	7,43	7,56	7,57	7,60	7,49	7,55	7,45	7,46	7,45
Salinity (ppt)	20	20	19	19	19	19	22	22	20	20
DO in (%)	101,8	101,2	98,9	100,2	102,0	114,7	109,5	111,3	114,8	115,6
DO in (mg L^{-1})	10,40	10,36	9,77	9,80	9,97	10,95	10,80	10,63	10,71	10,82
DO out (%)	101,7	95,3	92,5	88,3	86,1	87,2	93,2	85,9	89,7	88,2
DO out (mg L^{-1})	10,40	9,61	9,17	8,62	8,57	8,41	8,93	8,02	8,54	8,26
Total Biomass (kg)	0	30,02	29,45	29,26	29,05	29,05	29,08	28,86	28,89	28,93
MO_2 (mgO_2 min^{-1} kg^{-1})	0	0,75	0,61	1,21	1,45	2,62	1,93	2,71	2,25	2,66
No. Fish	0	158	158	156	155	154	154	154	153	153
Mortality (%)	0	0,00	1,27	1,91	2,55	2,55	2,55	3,20	3,20	3,20
No. Dead Fish	0	0	2	1	1	0	0	1	0	0
Weight Dead Fish (kg)	0	0	0,567	0,198	0,207	0	0	0,263	0	0
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0	0,035	0,035	0,035	0,035	0,035

Table 13: Daily measurements in the RAS tank No. 1 between days 10 – 19.

Days	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Date	**4.2.2008**	**5.2.2008**	**6.2.2008**	**7.2.2008**	**8.2.2008**	**11.2.2008**	**12.2.2008**	**13.2.2008**	**14.2.2008**	**15.2.2008**
Temperature (^oC)	13,6	13,5	13,8	14,2	12,4	12,3	12,8	11,3	11,2	11,4
pH	7,50	7,55	7,61	7,58	7,67	7,61	7,70	7,80	7,71	7,64
Salinity (ppt)	19	19	19	20	20	21	20	20	21	21
DO in (%)	118,5	113,0	114,7	111,7	114,2	114,0	112,5	111,7	114,2	112,8
DO in (mg L^{-1})	11,08	10,50	10,59	10,19	10,87	10,84	10,60	10,90	11,15	10,96
DO out (%)	104,3	90,0	91,5	93,1	98,9	93,7	97,3	98,8	94,6	89,1
DO out (mg L^{-1})	9,68	8,37	8,44	8,52	9,40	8,91	9,18	9,56	9,23	8,68
Total Biomass (kg)	28,96	29,00	29,03	29,07	29,10	29,14	29,17	29,21	29,25	29,29
MO_2 (mgO_2 min^{-1} kg^{-1})	1,45	2,20	2,22	1,72	1,52	1,99	1,46	1,38	1,97	2,34
No. Fish	153	153	153	153	153	153	153	153	153	153
Mortality (%)	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20	3,20
No. Dead Fish	0	0	0	0	0	0	0	0	0	0
Weight Dead Fish (kg)	0	0	0	0	0	0	0	0	0	0
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,040	0,040	0,040

Table 14: Daily measurements in the RAS tank No. 2 between days 0 – 9.

Days	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Date	**21.1.2008**	**22.1.2008**	**23.1.2008**	**24.1.2008**	**25.1.2008**	**28.1.2008**	**29.1.2008**	**30.1.2008**	**31.1.2008**	**1.2.2008**
Temperature (^oC)	9,0	8,9	10,6	10,5	10,6	12,3	12,0	13,4	12,4	13,0
pH	8,01	7,43	7,56	7,56	7,61	7,50	7,54	7,48	7,48	7,45
Salinity (ppt)	20	20	19	19	19	19	22	22	20	20
DO in (%)	101,8	101,2	98,9	100,2	102,0	114,7	109,5	111,3	114,8	115,6
DO in (mg L^{-1})	10,40	10,36	9,77	9,80	9,97	10,95	10,80	10,63	10,71	10,82
DO out (%)	101,7	97,6	91,8	88,6	85,5	87,4	94,3	87,5	90,4	91,2
DO out (mg L^{-1})	10,40	9,95	9,11	8,67	8,49	8,42	9,02	8,16	8,62	8,68
Total Biomass (kg)	0	30,17	29,71	29,71	29,71	29,15	29,19	29,22	29,26	29,03
MO_2 (mgO_2 min^{-1} kg^{-1})	0	0,41	0,67	1,14	1,49	2,60	1,83	2,54	2,14	2,21
No. Fish	0	158	158	156	156	156	154	154	154	154
Mortality (%)	0	0	1,27	1,27	1,27	2,55	2,55	2,55	2,55	3,20
No. Dead Fish	0	0	2	0	0	2	0	0	0	1
Weight Dead Fish (kg)	0	0	0,457	0	0	0,563	0	0	0	0,260
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0	0	0	0	0	0,035	0,035	0,035	0,035	0,035

Table 15: Daily measurements in the RAS tank No. 2 between days 10 – 19.

Days / **Date**	**10** **4.2.2008**	**11** **5.2.2008**	**12** **6.2.2008**	**13** **7.2.2008**	**14** **8.2.2008**	**15** **11.2.2008**	**16** **12.2.2008**	**17** **13.2.2008**	**18** **14.2.2008**	**19** **15.2.2008**
Temperature (^oC)	13,6	13,5	13,8	14,2	12,4	12,3	12,8	11,3	11,2	11,4
pH	7,51	7,56	7,60	7,57	7,67	7,61	7,69	7,80	7,72	7,65
Salinity (ppt)	19	19	19	20	20	21	20	20	21	21
DO in (%)	118,5	113,0	114,7	111,7	114,2	114,0	112,5	111,7	114,2	112,8
DO in (mg L^{-1})	11,08	10,50	10,59	10,19	10,87	10,84	10,60	10,90	11,15	10,96
DO out (%)	101,4	92,3	90,3	90,7	96,2	93,0	96,8	95,3	96,7	92,2
DO out (mg L^{-1})	9,38	8,57	8,33	8,30	9,14	8,85	9,11	9,27	9,43	8,90
Total Biomass (kg)	29,07	29,10	29,14	29,17	29,21	29,24	29,13	29,17	29,21	29,25
MO_2 (mgO_2 min^{-1} kg^{-1})	1,75	1,99	2,33	1,94	1,78	2,04	1,53	1,68	1,77	2,11
No. Fish	153	153	153	153	153	153	153	152	152	152
Mortality (%)	3,20	3,20	3,20	3,20	3,20	3,20	3,85	3,85	3,85	3,85
No. Dead Fish	0	0	0	0	0	0	1	0	0	0
Weight Dead Fish (kg)	0	0	0	0	0	0	0,142	0	0	0
Flow rate (L min^{-1})	30	30	30	30	30	30	30	30	30	30
Daily growth rate (kg)	0,035	0,035	0,035	0,035	0,035	0,035	0,035	0,040	0,040	0,040

Table 16: Daily measurements in the new water inlet to the RAS between days 0 – 9.

Days	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Date	**21.1.2008**	**22.1.2008**	**23.1.2008**	**24.1.2008**	**25.1.2008**	**28.1.2008**	**29.1.2008**	**30.1.2008**	**31.1.2008**	**1.2.2008**
Temperature (^{o}C)	8,9	9,4	10,3	10,2	11,6	11,6	11,6	10,7	9,0	8,8
pH	8,01	7,96	7,88	7,85	7,80	7,79	7,81	7,95	7,76	7,87
Salinity (ppt)	20	20	19	19	19	19	22	22	20	20
DO (%)	109,3	108,9	108,4	106,9	96,5	101,6	101,9	102,8	108,0	99,7
DO (mg L^{-1})	11,12	11,02	10,99	10,78	9,54	9,87	10,03	10,89	11,02	10,31
Flow rate (L min^{-1})	12	12	12	12	5	5	3	3	3	3
Flow rate (L min^{-1} kg^{-1})	0.08	0.08	0.08	0.08	0,08	0,08	0,05	0,05	0,05	0,05

Table 17: Daily measurements in the new water inlet to the RAS between days 10 – 19.

Days	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Date	**4.2.2008**	**5.2.2008**	**6.2.2008**	**7.2.2008**	**8.2.2008**	**11.2.2008**	**12.2.2008**	**13.2.2008**	**14.2.2008**	**15.2.2008**
Temperature (^{o}C)	8,7	8,3	6,6	8,6	7,2	6,6	8,6	5,2	5,4	5,3
pH	7,91	7,94	7,87	7,93	7,99	7,87	8,33	7,89	7,89	7,90
Salinity (ppt)	19	19	19	20	20	21	20	20	21	21
DO (%)	119,2	109,0	102,1	99,6	95,9	92,1	93,1	74,6	73,6	81,4
DO (mg L^{-1})	12,23	11,36	11,04	10,63	10,31	10,05	9,99	8,37	8,23	9,15
Flow rate (L min^{-1})	3	3	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Flow rate (L min^{-1} kg^{-1})	0,05	0,05	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008

Table 18: Daily measurements in the outlet water from the biofilter in the RAS between days 3 – 12.

Days	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Date	**24.1.2008**	**25.1.2008**	**28.1.2008**	**29.1.2008**	**30.1.2008**	**31.1.2008**	**1.2.2008**	**4.2.2008**	**5.2.2008**	**6.2.2008**
Temperature (^{o}C)	10,5	10,8	12,3	12,0	13,0	12,3	13,0	13,5	13,5	13,8
pH	7,42	7,45	7,59	7,66	7,63	7,73	7,48	7,59	7,60	7,63
DO (%)	97,1	97,3	96,9	97,9	97,0	98,5	97,0	102,0	95,1	95,4
DO (mg L^{-1})	9,30	9,35	9,23	9,43	9,09	9,38	9,10	9,73	8,83	8,79

Table 19: Daily measurements in the outlet water from the biofilter in the RAS between days 13 – 19.

Days	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Date	**7.2.2008**	**8.2.2008**	**11.2.2008**	**12.2.2008**	**13.2.2008**	**14.2.2008**	**15.2.2008**
Temperature (^{o}C)	14,2	12,3	12,2	12,8	11,2	11,0	11,3
pH	7,66	7,73	7,71	7,73	7,80	7,78	7,80
DO (%)	94,7	96,8	97,1	96,3	96,1	96,2	95,1
DO (mg L^{-1})	8,66	9,21	9,25	9,12	9,38	9,40	9,28

Table 20: Values of different water quality parameters calculated in RAS tank No. 1 two times per week during the experimental time and their Removal rate values.

	Days					
Items	**5**	**8**	**10**	**13**	**15**	**18**
TC (mg L^{-1})	83,49	75,74	67,08	68,93	66,89	97,79
CO_2 (mg L^{-1})	3,91	4,43	3,66	2,84	2,03	1,80
Removal Rate CO_2 (mgCO_2 min^{-1} kg^{-1})	1,54	2,02	1,83	1,06	0,75	0,76
Removal Rate CO_2 (%)	154	202	183	106	75	76
TAN (mg L^{-1})	0,251	0,779	0,890	1,369	1,483	1,511
Removal Rate TAN (mgTAN min^{-1} kg^{-1})	0,006	0,047	0,013	-0,049	-0,055	-0,068
Removal Rate TAN (%)	0,6	4,7	1,3	-4,9	-5,5	-6,8
NH_3-N (mg L^{-1})	0,001	0,004	0,005	0,012	0,014	0,014
TSS (mg L^{-1})	-	0,90	1,55	2,30	5,25	8,85
Removal Rate TSS (mgTSS min^{-1} kg^{-1})	-	0,21	0,18	0,15	0,10	0,10
Removal Rate TSS (%)	-	21	18	15	10	10

Table 21: Values of different water quality parameters calculated in RAS tank No. 2 two times per week during the experimental time and their Removal rate values.

	Days					
Items	**5**	**8**	**10**	**13**	**15**	**18**
TC (mg L^{-1})	83,86	75,49	68,07	68,24	66,98	92,54
CO_2 (mg L^{-1})	3,67	4,22	3,63	2,99	2,09	1,93
Removal Rate CO_2 (mgCO_2 min^{-1} kg^{-1})	1,29	1,77	1,79	1,20	0,82	0,71
Removal Rate CO_2 (%)	129	177	179	120	82	71
TAN (mg L^{-1})	0,251	0,790	0,893	1,378	1,494	1,529
Removal Rate TAN (mgTAN min^{-1} kg^{-1})	0,005	0,058	0,016	-0,039	-0,044	-0,050
Removal Rate TAN (%)	0,5	5,8	1,6	-3,9	-4,4	-5,0
NH_3-N (mg L^{-1})	0,001	0,004	0,005	0,012	0,014	0,014
TSS (mg L^{-1})	-	0,95	1,56	2,32	5,24	8,85
Removal Rate TSS (mgTSS min^{-1} kg^{-1})	-	0,26	0,19	0,17	0,09	0,10
Removal Rate TSS (%)	-	26	19	17	9	10

Table 22: Values of different water quality parameters calculated in the water inlet tanks of the RAS two times per week during the experimental time.

Items	Days					
	5	**8**	**10**	**13**	**15**	**18**
TIC (mg L^{-1})	86,04	74,32	66,57	70,82	69,65	92,56
CO_2 (mg L^{-1})	2,42	2,49	1,90	1,82	1,30	1,24
TAN (mg L^{-1})	0,246	0,734	0,877	1,416	1,537	1,577
NH_3-N (mg L^{-1})	0,003	0,008	0,010	0,020	0,022	0,023
TSS (mg L^{-1})	-	0,70	1,38	2,15	5,15	8,75
Water Flow (L min^{-1})	30	30	30	30	30	30

Table 23: Values of different water quality parameters calculated in the new water inlet to the RAS two times per week during the experimental time.

Items	Days					
	5	**8**	**10**	**13**	**15**	**18**
TIC (mg L^{-1})	87,09	73,12	65,96	70,34	69,42	92,98
CO_2 (mg L^{-1})	1,79	1,92	1,68	1,81	1,12	1,24
TAN (mg L^{-1})	0,003	0	0,004	0,001	0,001	0
NH_3-N (mg L^{-1})	0	0	0	0	0	0
TSS (mg L^{-1})	-	0,15	0,20	0,20	0,20	0,15
Flow rate (L min^{-1})	5	3	3	0,5	0,5	0,5
Flow rate (L min^{-1} kg^{-1})	0.08	0.05	0.05	0.008	0.008	0.008

Table 24: Values of different water quality parameters calculated in the outlet water from the biofilter in the RAS two times per week during the experimental time.

Items	Days					
	5	**8**	**10**	**13**	**15**	**18**
TIC (mg L^{-1})	86,04	77,90	71,01	65,79	65,52	93,72
CO_2 (mg L^{-1})	2,42	3,54	2,94	2,67	2,42	2,87
TAN (mg L^{-1})	0,240	0,724	0,868	1,449	1,556	1,652
Removal Rate TAN (mgTAN min^{-1} kg^{-1})	0,012	0,060	0,026	-0,074	-0,066	-0,131
Removal Rate TAN (%)	1,2	6,0	2,6	-7,4	-6,6	-13,1
NH_3-N (mg L^{-1})	0,002	0,008	0,006	0,014	0,015	0,018
NO_2-N (mg L^{-1})	0	0	0,22	0,44	0,748	1,10
NO_3-N (mg L^{-1})	0	0	0	0,099	0,33	0,66
TSS (mg L^{-1})	-	0,50	0,75	1,30	4,05	8,00