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Risk Assessment of Recirculation Systems in Salmonid Hatcheries

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Persons working for VKM, either as appointed members of the Committee or as ad hoc experts, do this by virtue of their scientific expertise, not as representatives for their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed an ad hoc group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority. The members of the *ad hoc* group are acknowledged for their valuable work on this opinion.

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Assessed by

The report from the *ad hoc* group has been evaluated and approved by Panel on Animal Health and Welfare

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Summary

Background:

To ensure good animal welfare in Norwegian salmonid hatcheries, certain water quality-related issues and various water quality parameters must fulfill the requirements set in the Regulations relating to Operation of Aquaculture establishments, and the water quality should be kept within the suggested safe levels according to these regulations issued by the authorities. When these regulations were issued, most hatcheries used flow-through systems with a continual renewal and exchange of water. In the spring of 2009 (when this risk assessment was first planned), 10-15 hatcheries recirculated their tank water. Because of an increasing interest in recirculation systems, and the fact that in the last two years other hatcheries have started with recirculation systems as well, the authorities expect that there will be a shift towards recirculation systems in the coming years.

To be able to judge whether existing legislation is adequate in safeguarding fish welfare in hatcheries where water is recirculated, the Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food Safety (VKM) to conduct an assessment of the current situation and whether, or to which degree, a risk of injury, disease or other unnecessary suffering exists.

The assessment is limited to salmon and rainbow trout in freshwater systems.

To prepare the scientific background necessary to answer the questions from the Norwegian Food Safety Authority, the VKM Panel on Animal Health and Welfare established an *ad hoc*-group consisting of 6 national and international experts. The international expert came from the Faroe Islands. The group was chaired by Dr. Brit Hjeltnes from the Panel on Animal Health and Welfare. The Panel on Animal Health and Welfare supports the conclusions from the *ad hoc* group.

Conclusions:

Based on literature data and practical experiences from recirculating aquaculture systems (RAS), possible environmental effects on fish welfare were assessed. It is clear that there is a risk that the water quality in RAS can deteriorate and cause severely compromised welfare for the fish. On the other hand, a well-managed RAS can in fact stabilize, or even improve water quality, resulting in better welfare compared with some flow-through systems. Monitoring of key water quality parameters (dissolved oxygen, pH/CO_2, Total Ammonia Nitrogen (TAN), nitrite, total gas pressure and temperature) is considered essential for safeguarding the welfare of the fish. Adequate quality assurance of the analytical methods is a prerequisite to ensure correct readings of relevant water quality parameters. Routine monitoring of fish behaviour, morphology (e.g. fins, gills and skin), production data (e.g. growth and food conversion ratio), as well as mortalities is also important.

Suggested maximum or lower limits for most relevant water quality parameters exist. The Panel is of the opinion that these limits should, however, be considered as guidelines only since the existing water quality criteria are not based on results from commercial (RAS) conditions.

A semi-quantitative risk assessment (risk = probability x consequence) was carried out. In the RAS considered here, the highest risk factors were considered to be elevated levels of nitrite

(NO_2^-) and total gas saturation, excessive feeding, and insufficient removal of particles. Proper operational routines of the biofilter are essential to provide a healthy and stable aqueous environment. In particular, RAS can be vulnerable in the initial phase after start-up of the biofilter (inadequate removal of ammonia and nitrite). Since overfeeding and faeces cause fouling of the water in the fish tanks, a quick removal of particles is important, before the particles dissolve or disintegrate. Adequate dimensioning of the RAS is a prerequisite, and the fish farmers must ensure that the biomass kept in the system shall not exceed the maximum intended level at any one time. If microorganisms (pathogens) enter RAS, there is an increased risk that they will multiply and eventually cause an adverse impact on fish health and welfare. In such cases, it may be difficult to implement adequate disinfection procedures without affecting the stability of the biofilter. With proper expertise and relevant management, it is nevertheless possible to maintain good fish health and welfare in RAS. Safe operation of RAS requires good knowledge of water chemistry and the potential hazards involved that might cause compromised fish welfare. Therefore, proper training of personnel operating RAS is required. Water chemistry in RAS can be quite different from what the fish is naturally exposed to in nature or in aquaculture flow-through systems.

Data gaps:

Some specific data gaps were recognized related to water quality parameters determined under RAS conditions. More research is needed to better understand how the water quality in RAS affects chronic stress, health and fish welfare.

Sammendrag

Bakgrunn:

For å sikre god dyrevelferd i settefiskanlegg for laks og regnbueørret er det nødvendig at ulike miljøbetingelser, inklusive grenseverdier for ulike vannkvalitetsparametre, oppfyller bestemte krav i henhold til eksisterende regelverk (’Driftsforskriften’). Da regelverket i sin tid ble utformet var det i all hovedsak vanlig i oppdrettsnæringen å benytte gjennomstrømningsanlegg (kontinuerlig utskifting av vannet i fiskekarene). Våren 2009, da dette oppdraget fra Mattilsynet først ble planlagt, var det imidlertid 10-15 settefiskanlegg i Norge som brukte resirkulert vann. For tiden er det en økende interesse for resirkuleringsanlegg, og i løpet av de siste to årene har flere kommet til. En regner med at det vil bli stadig mer utbredt med slike anlegg i de kommende årene.

For å kunne bedømme om eksisterende lovgiving fremdeles er tilstrekkelig til å sikre god fiskevelferd i settefiskanlegg, har Mattilsynet anmodet Vitenskapskomiteen for mattrygghet (VKM) om å gjennomføre en vurdering av dagens situasjon med hensyn til risiko for skader og sykdom, og om fisken påføres unødvendige lidelser når den holdes i kar med liten, eller minimal vannutskifting (resirkuleringsanlegg).

Oppdraget er begrenset til oppdrett av atlantisk laks og regnbueørret i ferskvannsfasen.

For å gjennomgå det vitenskapelige grunnlaget som var nødvendig for å besvare de spesifikke spørsmål fra Mattilsynet, etablerte VKM - Faggruppe for dyrehelse og dyrevelferd – en *ad hoc* gruppe som bestod av fem nasjonale eksperter og en internasjonal ekspert fra Færøyene. Gruppen ble ledet av dr. Brit Hjeltnes fra Faggruppe for dyrehelse og dyrevelferd. Faggruppen slutter seg til konklusjonene i rapporten fra *ad hoc*-gruppen.

Konklusjoner:

Basert på litteraturdata og gjennomgang av praktisk erfaring fra drift av resirkuleringsanlegg er det utredet hvordan ulike miljøforhold eventuelt kan påvirke dyrevelferden. Vannkvaliteten i resirkuleringsanlegg kan forringes i betydelig grad med de følger at fisken utsettes for dårlige betingelser med hensyn til stress, helse og velferd. Imidlertid, når slike oppdrettssystemer drives på en god og forsvarlig måte, kan vannkvaliteten stabiliseres, og endog forbedres. Dette kan danne grunnlag for bedre dyrevelferd enn i enkelte gjennomstrømningsanlegg. Rutinemessig overvåkning av viktige vannkvalitetsparametre (løst oksygen, pH/CO_2, TAN (NH_4^+ + NH_3), nitritt (NO_2^-), totalt gassmetning og temperatur) anses nødvendig for å trygge dyrevelferden. Analysemetodene for måling av disse vannkvalitetsparameterne må være underlagt god kvalitetssikring (hyppig kontroll av at måleinstrumentene faktisk viser riktige verdier). I tillegg må fiskens atferd, morfologi (eksempelvis finner, gjeller og skinn), produksjonsdata (eksempelvis tilvekst og fôrfaktor) og dødelighetstall vurderes fortløpende.

I litteraturen finnes foreslåtte grenseverdier (minimums- eller maksimalnivåer) for de fleste av de aktuelle vannkvalitetsparametrene. Faggruppen er av den mening at flere av disse grenseverdiene bør kun brukes som retningslinjer og ikke som absolutte grenser for hva fisken kan tolerere. Grunnen til dette er at eksisterende grenseverdier i utgangspunktet ikke er baserte på det å drive fiskeoppdrett i resirkuleringsanlegg.

Ved en semi-kvantitativ risikovurdering (risiko = sannsynlighet for at et gitt scenario inntreffer x konsekvens), ble følgende forhold forbundet med størst risiko i resirkuleringsanlegg: høye nivåer av nitritt (NO_2^-) og total gassovermetning, overfôring, og utilstrekkelig partikkelfjerning. God drift av biofilteret er av avgjørende betydning for et stabilt vannmiljø. Spesielt kan et resirkuleringsanlegg være sårbart (utilstrekkelig fjerning av ammoniakk og nitritt) i forbindelse med oppstartsfasen av biofilteret (før bakteriekulturene får stabilisert seg). Siden overfôring og feces forurenser vannet i oppdrettskarene, er det nødvendig med rask og effektiv partikkelfjerning. Riktig dimensjonering av anlegget, og at biomassen til enhver tid ikke overskrider nivået gitt av dimensjoneringskriteriene er andre viktige ting å passe på. Med god kompetanse og korrekt drift vil det være mulig å opprettholde god fiskehelse i et resirkuleringsanlegg. Imidlertid vil hensynet til et stabilt biofilter gjøre det vanskelig å gjennomføre normale desinfeksjonsrutiner. Dette gir en økt risiko for at fiskens helse og velferd påvirkes negativt dersom patogene parasitter og mikroorganismer kommer inn i et resirkuleringsanlegg. Trygg drift av resirkuleringsanlegg krever god kjennskap til vannkjemi og de mulige farene for redusert dyrevelferd som kan forekomme i delvis lukkede systemer. God opplæring av personell som skal drifte resirkuleringsanlegg er helt nødvendig. Vannkjemien i slike systemer kan være forskjellig fra det fisken normalt opplever i naturen eller i gjennomstrømningsanlegg.

Forskningsbehov:

Mer forskning under kommersielle forhold er nødvendig for å få en mer helhetlig forståelse av hvordan miljøet i resirkuleringsanlegg kan påvirke fisken med hensyn til kronisk stress, helse og velferd. I denne sammenheng er flere kunnskapshull identifiserte.

Keywords

RAS, water quality, fish welfare, fish health, salmonids, freshwater, fish physiology.

Contents

Background

To ensure good animal welfare in Norwegian fish farms certain water quality related issues and various water quality parameters must fulfil requirements set in the Regulations relating to Operation of Aquaculture establishments (*Forskrift 2008.6.17 nr. 822 om drift av akvakulturanlegg*). At the time these regulations were issued most hatcheries used a flow-through system for their tanks; that is they operated with a continual renewal and exchange of water. The recommended exchange rate is 0.25 litre of water per kilogram fish and minute. In the spring of 2009 (when this commission was first planned) 10-15 hatcheries recirculated tank water. In general, those hatcheries operated with a daily water system exchange rate as low as 5–20 percent. However cases where the renewal rate was only one percent were known. Such low renewal rates are only possible by allowing the water, after use in the culture tanks, to pass through a water treatment unit where metabolites such as carbon dioxide are removed and oxygen supplemented. As there is an increasing interest in recirculation systems, the Norwegian Food Safety Authority has experienced that during the last two years, more hatcheries have started with recirculation of tank water and that this shift towards recirculation systems will continue.

There are several accounts of cases that indicate that fish held in tanks where water is recirculated are being exposed to an environment where their welfare can be compromised. On the other hand, the Norwegian Food Safety Authority also has information indicating that animal welfare may actually improve when fish are held in recirculation systems compared to flowthrough systems.

Providing a suitable environment with an adequate supply of good quality water is a fundamental welfare principle. Even in flow-through systems there has been a tendency to use too little water. This is due either to insufficient water supply or a high cost of pumping/heating the water. For the same reasons, the fish farming industry is now showing an increasing interest in recirculating tank water. Legislation sets certain minimum requirements to ensure a good environment for the fish and to prevent injury by correct handling procedures and use of sound equipment. The *ad hoc* group is however concerned that the high rate of recirculation of water might set fish welfare at risk and that current legislation might not address the problems generated by recirculation systems.

To be able to judge whether existing legislation is adequate in safeguarding fish welfare also in hatcheries where water is recirculated, the Norwegian Food Safety Authority requested the Norwegian Scientific Committee for Food Safety (VKM) to conduct an assessment of the current situation and to assess if, or to what degree, a risk of injury, disease or other unnecessary suffering to the fish exist.

To prepare the scientific background necessary to answer the questions from the Norwegian Food Safety Authority, the VKM Panel on Animal Health and Welfare established an *ad hoc*-group consisting of 6 national and international experts. The international expert came from the Faroe Islands. The group was chaired by Dr. Brit Hjeltnes from the Panel on Animal Health and Welfare.

Terms of reference

The Norwegian Food Safety Authority requests that the following aspects be assessed in connection with water recirculation systems in land based facilities:

1) Is there a risk that methods and technical equipment commonly used in Norway for recirculating water will not allow for the provision of a suitable environment that satisfies fish's basic requirements to sufficient water of a certain quality? If so, please describe which elements of the method or component of the equipment which set fish welfare at risk. Do certain methods or types of equipment better satisfy fish needs?
2) Which risks to animal welfare exist due to faulty assembling or operation of the equipment or use of a method? What can be done to remedy this fact? Can certain operational routines or monitoring of water quality parameters compensate or prevent animal welfare being set at risk? If so, please specify which routines are necessary and which water quality parameters that need to be monitored to have sufficient control with and maintain an acceptable water quality that satisfies fishes' needs.
3) What is the risk of a fluctuating water quality environment with ever changing levels of various parameters ensuing in a recirculation system compared to a flow-through system, and which factors represent a risk to the stability of the environment provided?
4) Is there a risk of poor or inadequate water quality conditions developing due to the amount of renewal water per tank in a recirculation system? The systems total capacity to maintain a good water quality must also be taken into account in conjunction with the assessment of the water renewal rate. Will certain water renewal schemes reduce or minimize this risk? Do other factors such as feeding regimes, stocking density, etc. interact with water quality maintenance in such a manner that animal welfare is set at risk?
5) Does available knowledge on how to operate the recirculation system in accordance with the bio filter's capacity, fish density, and feeding regime, in itself represent a risk e.g. due to either inadequate or incorrect knowledge? If the operational knowledge of the system is sufficient, is it rather the farms that do not train their staff in correct management of recirculation systems thus creating an increased welfare risk?
6) Is there a greater risk of disease occurrence in recirculation systems compared to flow-through systems and is it possible to maintain a good health status for a long term perspective (years)? It should be taken into consideration that in hatcheries with a flow-through system a segregation of different life-stages and an all in all out procedure is practiced with disinfection of all equipment between different batches. If such a procedure is no longer possible in a water recirculation system, is there an increased health risk that can be attributed to retaining the bio filter between different fish groups?

Water recirculation systems are defined as: Technological solutions where more than 60 % of the tank water is reused or where biofilters convert fish waste to by-products of the nitrogen cycle. These biofilters are a prerequisite for the system as it otherwise would not be possible to maintain good water quality in the fish tanks.

This commission is limited to salmon and rainbow trout in fresh water systems.

Introduction

Recirculating Aquaculture Systems (RAS) has been developed over a period of more than thirty years by research institutions and the commercial industry. The evolving of the technology has to a large extent been driven by North American and European expertise and is now used for several fish species both in fresh water and in salt water. RAS has been used for many years for salmonid fish in the US and Canada. In Europe, the production of Atlantic smolts in the Faroe Islands has been based on RAS for more than 20-30 years. Recently, RAS has been incorporated in the production of salmonid fish in Norway and Chile where the technology to a large extent is being utilized in industrial production of fish. RAS is essentially a closed farming system with fish tanks, filtration, water treatment and limited exchange of water. In closed systems, oxygen is consumed and metabolites are excreted to the water, both by fish and bacteria. Trace compounds found in feed, and/or water is accumulated to the degree that input balances the removal rate. Consequently, such systems depend on supplementation of oxygen and water treatment. Metabolites include by-products from fish metabolism as well as compounds like hormones produced by the fish, drugs and chemicals. The concentration of the accumulated compounds without treatment will depend on the capacity of the removal systems and the chemical equilibrium of the compounds. RAS are technically more advanced than a traditional flow-through system and in general require more management skills and higher initial financial investment. In Norway, the technology is incorporated in several new hatcheries and future projects.

A proportion of the water sources in Norway have little buffering capacity and thus a poor resistance towards a drop in pH caused by acidic rain, snow melting, or intensive production. The problem is geographically distributed in a south west axis in Norway (Kristensen et al., 2009). In flow-through farms, the geological and catchment conditions in the area is of high importance, since a higher Ca^{2+} content offers better protection to pH drops. The problems caused by very soft water are likely to be found also in a proportion of the hatcheries in Scotland and Faroe Islands (pers. com. Trond Rosten), while Chile have very different raw water quality with higher pH and buffering capacity (Kristensen et al., 2009). Moreover, in certain locations in Norway, Scotland and Chile, the ground water may contain dissolved metals. In some of the cases mentioned above, it is believed that RAS can offer a more stable water quality, but generally it is our opinion that RAS and flow-through systems should not be directly compared. RAS is a very different technology, with different risks and water environment.

Assumptions for the risk assessment

Scientific data is the basic ground for our assessment, but in our work we have focused on including operational experiences from RAS systems in Norway and Faroe Islands. It is our belief that we share some of the same challenges and production forms. The activity in planning and establishing RAS in Norway is large at the moment, and Scandinavia has become a cluster for knowledge and technology for RAS for salmon. By this means we have also included dialogue with some of the largest suppliers from Denmark and Norway in our background.

Fish physiology[1] related to respiration

Fish breathe in water. Breathing rate increases with activity and temperature (Brett, 1965; Davis, 1968). Salmon breathe through active buccal movements or ram-ventilation. At high swimming speeds, the fish ventilates with its gills by swimming forward with its mouth open (ram ventilation) and buccal breathing movements cease, e.g at 15^{0}C salmon have lower buccal breathing frequency at maximum activity, than during routine activity. It is interesting to note that oxygen uptake increases 10-15 times between routine and maximum activity, whereas buccal breathing rate increases by a maximum of only threefold (Davis, 1968). There must be a large increase in volume of water pumped per breath if oxygen delivery is to match oxygen uptake during high metabolism. The forward movements of the fish through the water assist gill ventilation even when the fish is buccal breathing, and will promote a marked increase in gill water flow with each breath. One would expect the same effect when fish holds its position in a water current in e.g. a fish tank, thus this is relevant physiology for RAS systems with high waterflow. The heart pumps the blood that contains respiratory and metabolic gases from the tissues to the gills and vice versa. The oxygen cost of the cardiac pump is always less than 5 % of total oxygen consumption by the fish at maximum activity. Heart rate and stroke volume in salmonids increases with water temperature and activity (Smith et al., 1967; Davis, 1968) but a sudden fall in heart rate occurs when salmonids switch from buccal to ram ventilation (Davis, 1968). The blood transports, amongst other, oxygen from the gills to the tissues and carbon dioxide and ammonia from the tissues to the gills. Blood volumes for salmonids are typically around 5.4 – 6.2 % of body volume.

The oxygen uptake of salmonids increases with temperature, body weight and swimming speed (Davis, 1968; Grøttum and Sigholt, 1998). Lipids are the major source of energy for prolonged swimming in salmon (Krueger et al., 1968) and the respiratory quotient (RQ, molar ratio CO_2/O_2) was found to be about 0.7 in swimming salmon (van den Thillart et al., 1983). The RQ can have important consequences in RAS, since it is used in estimating e.g. CO_2 load on the removal systems. Newer data suggest that the RQ in rainbow trout is around 0.85, and that lipid dominates as the substrate for energy dissipation, although the relative contribution from the various compound classes can be affected by swimming speed.

The possibility to store oxygen is low in fish, so the oxygen uptake must equal the oxygen utilization. A limited oxygen storage place is the swim bladder. It acts as an oxygen store as well as float in salmonids, and the oxygen in the bladder is utilized during hypoxia. The swim bladder can be filled or emptied through a pneumatic duct. A common understanding is that

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[1] Modified after Randall and Wright, 1995

gases can be exchanged with blood perfusing the bladder wall rete, but resently, new research on Atlantic salmon indicates that Atlantic salmon does not have rete (Korsøren, et al., 2009). At high gas levels in the blood, gases will diffuse from the blood into the bladder. When the water is supersaturated with gas the swim bladder can become overinflated leading to a buoyancy problem. This is especially a problem in smaller fish with narrow pneumatic ducts. Salmonids appear to be able to ameliorate problems of gas super-saturation by diving to greater depths and using hydrostatic pressure to dissolve excess swim bladder gas as well as reducing buoyancy due to reduced swim bladder size caused by increased hydrostatic pressure. In intensive fish farming the tank size and depth could be relevant for offering potential for compensation of gas supersaturation. Fry which are normally produced in shallower tanks could be regarded as more vulnerable to gas supersaturation. Not all the oxygen taken up by the fish enters the blood (Randall, 1985). The skin exchanges gases directly with the water but is not involved in gas transfer between the blood and the water. Around 15 % of the resting oxygen consumption in trout comes from the skin (Kirsh and Nonnotte, 1977). The blood vessels under the skin are part of the secondary circulation. This circulation contains plasma but very low hematocrit (Vogel, 1985). It is important to know that the fish skin is very active and derives nutrients from the blood, but gases direct with the environment. Due to this only 80 % of the oxygen consumed by a resting fish is transported by the blood. The condition of the skin (mucus, fungi, sores etc) might therefore be of importance for the welfare of the fish in this context, as well as it is for osmo- and ion regulation and pathogen defence.

Oxygen is carried from the gills to the tissues bound to haemoglobin. Salmonids have multiple hemoglobins within their red blood cells (Vanstone et al., 1964; Tsuyuki and Ronald, 1970), but the numbers of haemoglobin types is reduced throughout the entire life cycle (Giles and Vanstone, 1976). Salmonid haemoglobins are of two main forms; (1) the anodal forms with high oxygen affinity, a large Bohr shift[2], and a marked Root shift[3]. The oxygen binding is sensitive to changes in temperature, organic phosphate levels, and ionic strength (Giles and Randall, 1980; Sauer and Harrington, 1988). As an opposite, the oxygen binding by the cathodal haemoglobin forms is independent of pH, organic phosphate level and temperature. Interestingly, fry have more of the first types, and their blood is more sensitive to changes in pH, organic phosphate levels and temperature than blood in adult salmon. This might be of high relevance when considering effects of water quality (e.g. high $CO_{2,}$ - low pH) on welfare. The functional significance for adult salmon of having haemoglobin less sensitive to pH and organic phosphate is that oxygen transfer can be maintained during a marked acidosis caused by e.g. burst swimming activity.

There is a linearly decrease in red blood cell (RBC) pH with plasma pH (RBC pH = 8 [0.7029 * plasma pH] – 1.94) (Randall et al., 1987). Since the hemoglobin has a Root shift caused by high CO_2, the plasma acidosis will reduce red blood cell pH and cause reduction in blood oxygen content. As compensation, a catecholamine response will stimulate Na^+ / K^+ exchange via β – adrenergic receptors in the red blood cells membrane (Heming et al., 1987) and raise RBC pH. The catecholamine response will also enhance oxygen transfer across the gills (Isaia et al., 1978), increase hematocrit due to erythrocyte release from the spleen (Perry and Kinkhead, 1989) cause several cardiovascular changes, and stimulate ventilation (Randall and

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[2] Refers to the oxygen liberating effects of H^+ on certain hemoglobins i.e.; saturation of haemoglobin occurs at higher O_2 concentration

[3] Root effects refers to the fact that some hemoglobins do not saturate even at high O_2 concentrations when in the presence of low pH

Taylor, 1991). These responses are thought to be compensatory mechanisms for acidosis caused by burst swimming (Randall et al., 1987). One of the most interesting questions relevant for welfare in intensive farming of salmonids is therefore to observere if this natural adaptive response to metabolic acidosis, are triggered by farming conditions and used for other compensatory measures (e.g. high hypercapnia, hypoxia, hyperoxia etc.).

Carbon dioxide is produced by the fish from the oxidation of carbohydrates, proteins and fats, transported by the blood and released through the gill membrane. Carbon dioxide has much higher solubility compared to oxygen, and tissue carbon dioxide stores are therefore relatively large. The respiratory exchange ratio (RQ = molar ratio $CO_{2\ excretion}/O_{2\ uptake}$) is expected to be around 0.7 – 0.8 (Randall and Wright, 1995). Molecular carbon dioxide (CO_2) dissolves in water to form carbonic acid (H_2CO_3), which again can dissociate to form hydrogen and bicarbonate ions (H^+ and HCO_3^-). The p*K*' of the CO_2:HCO_3^- reaction is 6.1 at 15 °C and the ratio of HCO_3^- to CO_2 is approximately 40:1. Molecular CO_2 can easily penetrate cell membranes, and inside the cell, CO_2 can either hydrate to form HCO_3^- or react with proteins and form carbamino compounds. The membrane permeability to HCO_3^- is low, with exception of erythrocytes, causing CO_2 to be trapped within the cell. The pH dependence of carbon dioxide distribution is the opposite of ammonia. This causes HCO_3^- to be trapped in alkaline compartments and NH_4^+ trapped in acidic compartments in the fish. However, also the transmembrane potential can influence total ammonia distribution across fish muscle membranes due to significant NH_4^+ permeability.

The gills are the main site for carbon dioxide excretion in fish. It is primarily excreted as gas (Perry et al., 1982). As blood flows to the gill, plasma HCO_3^- enters the red blood cell (RBC) through the HCO_3^-/Cl^- exchange (Cameron, 1978; Obaid et al., 1979; Heming and Randall 1982). Molecular CO_2 is formed in the RBC from dehydration of HCO_3^-, catalysed by carbonic anhydrase (CA). CO_2 diffuses across the RBC membrane and gill tissue to the water. Fish haemoglobin have a large Haldane effect[4] and this marked production of H^+ during oxygenation causes a large HCO_3^- flux through the erythrocyte. Thus we can understand that there is a strong coupling between CO_2 excretion and oxygen uptake in finfish (Steffensen et al., 1987) and that this is highly relevant for intensive fish farming of salmons. CA are absent in the plasma and the inner surface of the gill epithelium (Rahim et al., 1988, Randall and Val, 1993) and as result the bicarbonate dehydration is negligible in plasma during the rapid transit time (approx 1 sec) for blood flow through the gills. The consequence is that all HCO_3^- dehydration occurs in the erythrocytes. Since CA is absent in fish plasma a non-equilibrium state of CO_2 /HCO_3^- system in both arterial and venous blood of fish (Randall and Wright, 1995) is expected. This means that pH is changing in the blood when it flows away from the gills. There is however CA present in gill tissue (Haswell et al., 1980), but the gill epithelium is not permeable to HCO_3^- (Perry et al., 1982) and therefore gill CA was earlier considered not to play a major role in CO_2 excretion, but may play a role in ion regulation (Dimberg, 1988). Recent data indicate however that CA facilitates CO_2 excretion, acid base and ion regulation (Gilmour et al., 2009).

Another interesting aspect of fish respiration is the surface of the gills. A thin boundary layer of mucus coats the surface of the gills and CA is present is this layer (Wright et al., 1986). Molecular CO_2 excreted across the gills acidifies this boundary layer because of the catalysed formation of HCO_3^- and H^+ ions. The CO_2 and the H^+ excretion acidify the water that passes

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[4] Haldane effect, oxygenation of haemoglobin results in production of H^+

the gills. In freshwater, Wright et al. (1986) reported an inspired-to-expired water pH difference in rainbow trout of 0.7-0.9 pH units. In seawater, the much higher buffering capacity results in smaller inspired-to-expired water pH differences (Baumgarten-Schumann and Piiper, 1968). The acidification of the boundary layer is also important to ammonia excretion (Wright et al., 1988) as described later.

Ammonia is the dominant nitrogenous end-product in juvenile and adult finfish, formed from the catabolism of amino acids in the liver (Pequin and Serfaty, 1963), with some contribution from the kidneys, gills and purine nucleotide cycle in skeletal muscle (Goldstein and Forster, 1961; Walton and Cowey, 1977; Fraser et al., 1966). Ammonia is a soluble molecule and the intracellular storage is large in fish. However, although NH_3 is lipid soluble, transfer through water-filled channels are probably considerably faster. Ammonia may be reused in the tissues (Mommsen and Hochachka, 1988) or transferred to the blood until it is excreted into the water. Ammonia production can be expressed relative to oxygen consumption, as the number of moles ammonia excreted for the number of moles oxygen consumed (called the ammonia quotient). During routine activity, the ammonia quotient was 0.12 in fed sockeye salmon and 0.07 in starved fish (Brett and Zala, 1975). Forsberg (1997) showed a dramatic decrease in excretion of ammonia in non-fed versus fed Atlantic salmon.

Ammonia (NH_3) is polar substance that binds H^+ in water to form the ammonium ion (NH_4^+). The ammonium reaction in water is nearly instantaneous, since the conversion of NH_4^+ to NH_3 has a half time of less than 50 ms (Stumm and Morgan, 1981). At a normal body pH, there will be much greater concentration of NH_4^+ than NH_3, because of the p*K* value is 9.6 at 15 °C. As a consequence, at a blood pH of 7.9, 98 % of ammonia exists as NH_4^+ and 2 % as NH_3. Since NH_3 is a nonpolar gas, it diffuses rapidly across biological membranes down its partial pressure gradient at about the same rate as CO_2 (Thomas, 1974; Cameron and Heisler, 1983) but membranes are generally less permeable to NH_4^+ due to its net charge and large hydrated diameter (Jacobs, 1940). However, in fish, as demonstrated in sole and trout, NH_4^+ also have permeability in muscle cells such that distribution will reflect transmembrane potentials, not only pH gradients.

The ammonia flux over the gills in freshwater finfish increases with temperature (Guerin-Ancey, 1976), long term acid exposure (Audet et al., 1988), exercise (Sukumaran and Kutty, 1977), hypercapnia (high CO_2) (Claiborn and Heisler, 1984), feeding, and dietary amino acid composition. In contrast, exposure to high levels of ammonia in environment (Cameron, 1986) and very alkaline water (Yesaki and Iwama, 1992) results in initially reduced ammonia efflux and in increased urea efflux (Wilkie et al., 1993). Water hardness also influences ammonia excretion. Rainbow trout exposed to alkaline soft water showed a large increase in plasma ammonia concentration (Yesaki and Iwama, 1992) but this negative effect was improved when the alkalinity was increased. To be aware of this mechanism might be important when comparing risks in intensive fish farming in soft water with risk in intensive fish farming in hard water. Also removal of divalent cations as calcium or magnesium in the environment reduces ammonia excretion in cutthroat trout (*Oncorhynchus clarki*) (Randall and Wright, 1995).

The mechanisms of ammonia (and urea) excretion have been debated for decades, and only recently a picture is emerging (Wright and Wood, 2009; Zimmer et al., 2010). Traditionally, ammonia has been thought to be removed from the blood in two ways, involving only passive diffusion across the gills as NH_3 or NH_4^+, or be actively pumped through ionic exchange mechanism. Diffusion down its partial pressure gradient has been viewed a significant pathway for NH_3 excretion at the gills in *freshwater fish* (Wright and Wood, 1985), but NH_3 efflux by diffusion might not be as important for *marine fish* (Evans et al., 1989). It has been debated whether a coupled Na^+ /NH_4^+ exchange mechanism is functional both in freshwater fish (Wright and Wood, 1985) and in marine fish (Evans et al., 1989). The passive sodium influx seems to be coupled with an electrogenic H^+ pump (Randall et al., 1991). Passive movement of NH_4^+ down its electrochemical gradient by different trans- and paracellular pathways has been suggested to be more important for ammonia excretion in *marine fish* (Evans et al., 1989) than in freshwater fish (Wright and Wood, 1985).

However, recent findings of rhesus glycoprotein involvement in ammonia transport (Nakada, et al, 2007) and the existence of urea transporters in numerous ammoniotelic fish species (Walsh et al., 2001) suggest that additional nitrogen excretion mechanisms might be present in finfish nitrogen excretion. This transepithelial diffusion of NH_3 and/or NH_4^+ is facilitated by the existence of ammonia channels formed by these rhesus glycoproteins. Several studies indicate that these transporting proteins are influenced by feeding, exposure to acute high ammonia, high pH and several other environmental factors (Wright and Wood, 2009; Zimmer et al., 2010) Studies are underway regarding which genes related to ammonia and urea excretion respond to high environmental ammonia in Atlantic salmon parr (Kolarevic et al., 2011b).

There is a close relation between nitrogenous waste excretion, acid-base and osmoregulation, indicating that the mechanisms of nitrogen excretion are relevant to understand when comparing freshwater and seawater RAS.

NH_4^+ passive diffusion may be favoured in marine fish since junctions between gill cells are in general more leaky (Girard and Payan, 1980). A consequence might be that it is more difficult to remove NH_4^+ from the plasma when the concentration in the environment is high and diffusion is difficult, and the fish is in seawater (Randall and Wright, 1995). Although the latter study was done on fish of the same species (trout) acclimated to differing salinities, and that the evidence for different ammonia tolerance between seawater and freshwater species is limited (Ip et al., 2001), the fact of contrasting NH_4^+ permeability could call for different water quality criteria for TAN in marine and freshwater fish farming.

The link between CO_2 excretion and ammonia excretion is very interesting and probably not recognized as an important mechanism for the practical aquaculturist yet. Some molecular CO_2 is excreted into the gill-water boundary layer to form HCO_3^- and H^+ ions, causing acidification of the expired water when pH is above 6.0 but no CO_2 hydration occurs at lower pH levels (Randall and Wright, 1995). In contrast, excreted ammonia (NH_3) combines with H^+ and form NH_4^+ in the boundary level which raises the pH of the expired water (Randall et al., 1991). The formation of NH_4^+ keeps the levels of NH_3 next to the gill low and this might help facilitate branchial NH_3 diffusion. When CO_2 excretion is inhibited (eg. by high environmental CO_2) or water buffering capacity is increased, the expired water acidification may be reduced, causing lower ammonia excretion in freshwater (Randall and Wright, 1995). These mechanisms might be of high importance for intensive freshwater aquaculture with accumulated levels of CO_2 and TAN in the rearing water. It is however more likely that the linkage between CO_2 and NH_3 excretion is less importance in seawater due to the higher buffering capacity in the water and a down-regulated proton ATPase pump (Randall and Wright, 1995). These results points in the direction, that one must consider recirculation different in freshwater and seawater systems.

Water quality parameters and potential risk factors related to fish welfare

In RAS, the consequences of failures in the supply of electricity or loss of water in the tanks seem obvious. Depending on time before the failures are mended, a less dramatic scenario than high mortality rates can also occur where water quality is more or less impaired. Back-up systems for power-supply and for controlling water levels of the tanks are usually an integrated part of RAS. The risks related to power failures etc are discussed elsewhere in this document (page 41). In this section, the welfare risks associated with the most important water quality parameters are described. Deterioration of water quality is regarded a potential factor that can compromise welfare since fish are in intimate contact with the environment making them particularly vulnerable to poor water quality and waterborne pollutants (Huntingford et al., 2006; MacIntyre et al., 2008).

Oxygen

The dissolved oxygen (DO) level is the single most important parameter in any fish rearing system. The oxygen consumption of fish depends on body mass, temperature, feeding rate, growth rate, swimming velocity and stress level (see Thorarensen and Farrell, 2011). The solubility of DO is affected by water temperature, gas composition, salinity and total pressure (Harmon, 2009). Low oxygen (hypoxia) induces respiratory distress leading to a reduction in appetite and ultimately mortality. Symptoms include rapid gill movement, gulping, lethargy and absence of active shoaling behaviour. On a general basis, a DO level of at least 56 % saturation is recommended in aquaculture (Timmons et al., 2001). This is, however, too low for salmonids. For example, since growth performance of salmon will improve from 70-75 % to 80-85 % air saturation (Bergheim et al., 2006), and it therefore seems reasonable to suggest that at least 85 % saturation should be considered the lower DO limit in practice (Thorarensen and Farrell, 2011).

Oxygen saturation above 100 % (hyperoxia), termed gas supersaturation, can also be harmful. Supersaturation can induce emboli in tissues (gas bubble disease) and can cause even greater problems when associated with nitrogen (Noga, 2000). Notably, it has been shown that gas bubble disease, caused by exposure to high levels of oxygen *alone*, can occur in Atlantic salmon smolt farming. The first signs of the disease appeared after 14 d at a DO level of 160 %. Fish exposed to DO supersaturated water changes behaviour (swimming activity, number of turns, panic reactions) demonstrating signs of pain and discomfort (Espmark et al., 2010). On the other hand, when Atlantic salmon smolts were exposed to hyperoxic levels up to 123 % saturation, no negative effects were observed. Instead, a positive effect on growth was observed (Hosfeld et al., 2008). Where supersaturation of water occurs (oxygen or nitrogen) embolisms occur in the gills, skin and yolk sac of alevins. Hyperoxic water causes increased internal oxygen concentrations (Kristensen et al., 2010), and might cause oxidative damage (Lygren et al., 2000; Olsvik et al., 2006). Hyperoxia may also cause osmoregulation problems (Brauner, 1998), and during fish transport hyperoxia have been demonstrated to cause hypercapnia. Since oxygen saturation also affects the total gas pressure (TGP), both parameters should be considered together. For instance, when cutthroat trout (*Oncorhynchus clarki*) and rainbow trout (*O. mykiss*) were cultured in freshwater for 80 and 98 days, respectively, no differences were observed in growth, fin quality, or feed conversion when the two species were reared at average DO levels of 172 and 150 % saturation, when TGP ranged from 102-117 %, and 106-109 %, respectively. Nitrogen saturation was inversely correlated with addition of oxygen, and varied between 90-107 %. Notably, 94 % of the cutthroat trout eventually developed gas bubble disease, whereas none of the rainbow trout developed the disease, probably due to the lower rearing levels of DO and TGP for the latter species (Doulos and Kindschi, 1990). On the other hand, it should be mentioned that when rainbow trout were reared (flow-through system) at DO saturation levels of 180 % and 94 % for 125 days, no differences were observed in growth and feed conversion. Nor was mortality affected. Similar results were observed for cutthroat trout reared in 183 %, 127 % or 97 % oxygen-supersaturated water for 91 days (Edsall and Smith, 1990). Furthermore, moderate oxygen supersaturation (<140 %) did not cause harmful effects on blood chemistry and hepatic glutathione status of rainbow trout (Ritola et al., 1999).

Carbon dioxide

Normally, the concentration of carbon dioxide in water in equilibrium with air is 0.5 – 1 mg/L. In fish farming, metabolically produced carbon dioxide is excreted through the gills, and if the CO_2 is not removed, it will gradually accumulate in the system. This means the driving force for mass transfer of CO_2 between fish blood and the water will be reduced. Thus, the levels of CO_2 in the blood will increase, resulting in a decrease in the oxygen carrying capacity (Sanni and Forsberg, 1996). Note that also biofilters generate CO_2, through the microbial metabolism, and add to the total system load (Summerfelt et al., 2004). In this study, the biofilter contributed 37 % of the total CO_2 production in RAS. Excessive levels of dissolved carbon dioxide (hypercapnia) can cause stress in fish and several compensatory adaptations, such as reduced plasma Cl^-, higher plasma HCO_3^- levels, higher blood pCO_2 levels, and altered blood pH can be observed (Eddy et al. 1977, 1977; Crocker and Cech, 1996; Fivelstad et al., 2003a, b). A combination of high CO_2 levels, low pH, and high aluminium levels can be a major threat to animal welfare (Fivelstad et al., 2003 a, b) in smolt farms. Hypercapnia might also lead to calcification of kidneys and reduced growth (Fivelstad et al., 1999b). At elevated levels, rainbow trout change their normal swimming behaviour when the carbon dioxide levels exceed 35 – 60 mg/L. Equilibrium is lost at about 150 mg/L, and above 155 mg/L, narcosis is induced after 3 min at 14 °C (Clingerman et al., 2007). Atlantic salmon become lightly sedated at 70 - 80 mg/L. At 180 - 250 mg/L, narcosis is induced and if the water quality is not improved, the fish will eventually start to die as a result of cessation of respiration (Erikson, 2011).

Reduced growth rates for Atlantic salmon has been reported at carbon dioxide levels of 20 mg/L or less (Fivelstad et al., 1999b, 2003a; Hosfeld et al., 2008), and particularly at $\geq$ 30 mg/L at the parr and post-smolt stages (Fivelstad et al., 1998, 2007). Higher mortality rates occur at 19 and 32 mg CO_2/L than at 7 mg CO_2/L (Fivelstad et al., 1999a). At low DO levels, CO_2 toxicity increases (Wedemeyer, 1997). Furthermore, Atlantic salmon are more sensitive to CO_2 at low temperatures (Fivelstad et al., 2007). The recommended maximum levels of carbon dioxide, to maintain good welfare and to support maximum growth of salmonids, varies from 10 mg/L (Wedemeyer, 1996; Fivelstad et al., 1998) to 20 mg/L (Timmons et al., 2001; Portz et al., 2006). Good et al. (2010) conducted a 6-month trial where rainbow trout were exposed to 8 and 24 mg CO_2/L. Survival was high (>97 %), and no differences in growth and susceptibility to nephrocalcinosis or related pathologies were observed among groups. The results suggested that rainbow trout reared to market size in RAS can be exposed to carbon dioxide concentrations of 24 mg/L without significantly effecting health and performance. A similar study on Atlantic salmon in RAS, exposed to 10 or 20 mg/L CO_2 is currently underway.

Acidity

The acidity of the water can be altered by elevated CO_2 levels from fish metabolism. This will lead to a drop in water pH since protons are produced in the carbonic acid reaction. A good indication of accumulated CO_2 is a drop in pH from intake to outlet of the fish tank. As described above, elevated levels of CO_2 can constitute a fish welfare concern. In addition, the amount of toxic unionized ammonia (NH_3) present in the tank water is also dependent of pH, see below. Low pH (4.2 – 5.0) *per se*, is harmful for salmonids and low pH can also be lethal (Randall, 1991). Acidified water causes disturbances in the water and ion metabolism of fish (Audet and Wood, 1988), acid-base regulation (McDonald et al., 1980), transport of oxygen and excretion of carbon dioxide (Randall, 1991), and excretion of ammonia (Wright and Wood, 1985). Swimming performance of rainbow trout is also affected (Ye and Randall, 1991). Moreover, the skin surface is attacked, and the production of mucus is increased (Wendelaar Bonga and Dederen, 1986). Rainbow trout do not acclimatize to acid stress (Audet and Wood, 1988). The recommended levels of acidity are pH > 6 (Randall, 1991), and pH 6.5 – 8.5 (Timmons and Ebeling, 2007).

Ammonia and ammonium

Ammonia is a by-product of fish amino acid and to a lesser extent nucleotide metabolism (Walsh, 1997). Eventually, accumulation of NH_3 in the rearing water will increase the partial pressure of the compound and reduce the efflux of NH_3 across the gills. Thus, the plasma NH_4^+ and NH_3 will become elevated (Wright and Anderson, 2001). Other sources of ammonia are from urine, solid wastes and excess feed. In aqueous solutions ammonia is in equilibrium with the ammonium ion (NH_4^+) according to:

$$NH_4^+ \leftrightarrow NH_3 + H^+$$

The total ammonium-N of the system is described as ($TAN = NH_4\text{-}N + NH_3\text{-}N$). As we can see from the equation, the toxicity of NH_3 is dependent of the pH in the system (Suski et al., 2007). Temperature and salinity have only limited effect on TAN toxicity (Randall and Tsui, 2002). It has been suggested that oxygen levels above normal saturation might increase the ammonia tolerance of fish (Colt et al., 1991) and fish have in fact been shown to withstand higher levels of un-ionised ammonia with higher levels of oxygen (Alabaster et al., 1979). During exhaustive exercise and stress, fish increase ammonia production and are then more sensitive to external ammonia. Likewise, starved fish are more sensitive than fed fish (Randall and Tsui, 2002), possibly due to an increased expression of glutamine synthetase (GS) and thus glutamine synthesis from glutamate and ammonia (Wicks and Randall, 2002). For detailed information we refer to reviews on which factors influence ammonia and urea metabolism and production (Wood 2001; Wright and Fyhn, 2001; Wood 2004; Terjesen, 2008), and the toxicity of ammonia (Ip, et al., 2001; MacIntyre et al., 2008).

The effect of acute toxicity is mainly due to impacts on the central nervous system in vertebrates, and death may follow (Randall and Tsui, 2002). The un-ionized ammonia toxicity is believed to be due to impairment of celebral energy metabolism resulting in a depletion of high-energy compounds in the brain (see Smart, 1978), depletion of glutamate substrate for GS, or the depolarization effect of NH_4^+ on neurons (displaces K^+), eventually leading to cell death (Randall and Tsui, 2002). Chronic exposure to elevated levels of ammonia will increase metabolic rate, reduce growth rate, disease resistance and fecundity. Major symptoms of ammonia toxicity is lack of foraging, reduced swimming performance, increased gill ventilation, coughing, hyperexcitability, convulsions, coma, gulping, erratic swimming, loss of equilibrium, disruption of enzyme systems and membrane stability, gill damage and histological lesions in various internal organs, as well as osmoregulatory disturbances. Ultimately, mortality occurs (Tomasso, 1994; Ip et al., 2001; see Thorarensen and Farrell, 2011).

Although not a common strategy in salmonid farming, a fish culture system may be operated at high TAN levels at relatively low pH values although this is below optimum pH for biofilter nitrification. In such cases, pH must be kept low to ensure that the threshold value of NH_3 is not exceeded. It is then of great importance to be aware that sudden changes in water pH can lead to catastrophic consequences (Eshchar et al., 2006).

To provide good rearing conditions and adequate fish welfare, the safe limits for salmonids in aquaculture ranges from 0.012 to 0.025 mg NH_3/L (Westers, 1981, Fivelstad et al., 1995, Wedemeyer 1996, 1997; Timmons et al., 2001). For short time exposure (4 h), the recommended levels are approximately ten times higher (Wedemeyer, 1996). It is expected that safe limits will depend on salmonid species, life stage, physiological status, and other aspects of water quality. For instance, it has been shown in several experiments on rainbow trout, that low-level ammonia exposure to a partial NH_3 pressure of 23 µTorr actually promotes growth.

Considerably less information is available on the toxicity of the ammonium ion, probably since elevated levels of NH_4^+ have generally been considered unharmful (Tabata, 1962) although this view has been questioned (Tomasso, 1994; Linton et al., 1998).

Nitrite and nitrate

Metabolically produced ammonia, as well as ammonia from decomposing feed and feces, are converted by nitrifying bacteria to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-), during nitrification. If the amount of organic matter becomes too high in the recirculated water, the nitrification process becomes less effective. To ensure effective removal of both NH_3 and NO_2^-, the biofilters must be conditioned and monitored for several weeks (Timmons et al., 2001) before fish are introduced into the tanks. If not, or by biofilter malfunction, nitrite can reach toxic levels causing gill hypertrophy, hyperplasia, and lamellar separation as well as hemorrhage and necrotic lesions in the thymus (Wedemeyer and Yasutake, 1978). If nitrite enters the bloodstream, it reacts with the Fe^{2+} ion of the hemoglobin complex to form methemoglobin (Fe^{3+}) preventing blood from carrying oxygen (Jensen, 2003). This may reduce swimming performance (Brauner et al., 1993), growth, and eventually, it can become lethal (Russo et al., 1981). A visible symptom of high levels of methemoglobin is a brown colour of blood or gills. However, nitrite can also affect several other physiological systems in the fish, such as potassium balance, various enzyme systems, and endocrinology via the close relation between NO_2^- and nitric oxide. The 96 h LC_{50} for rainbow trout range from 0.19 to 12.6 mg NO_3^-/L (Russo and Thurston 1977, 1991; Russo et al., 1981; Lewis and Morris, 1986; Eddy and Williams, 1994) where toxicity is strongly affected by water pH and anion concentrations. For example, nitrite is less toxic in seawater due to the high levels of Cl^- (see Thorarensen and Farrell, 2011). Indeed, nitrite exposure studies on fish should state the level of water Cl^-, so that the data can be compared to other exposure studies. Furthermore, it appears that humic substances, accumulating in RAS, are reducing the toxicity of both ammonia and nitrite (Meinelt et al., 2010). To protect fish under most conditions, the recommended level of nitrite (as NO_2^-) in soft water is < 0.1 mg/L (Wedemeyer, 1997; Timmons et al., 2001).

Under normal conditions in aquaculture, nitrate does not reach toxic levels. The 96 h LC_{50} for salmonids is 1000 - 3000 mg/L (Colt and Armstrong, 1981). Recommended levels for nitrate range from < 1 mg/L (Wedemeyer, 1996) to 400 mg/L (Timmons et al., 2001). A mass balance for calculation of necessary biofilter size is necessary when designing RAS and a target level for maximum accumulation of nitrate (amongst other factors). The maximum nitrate concentration in a freshwater RAS for baramundi *(Lates calcarifer)* was set to 150 mg/L nitrate (North Carolina University 1998 in Hutchinson, 2004), this level seems to be used in some RAS used for salmon in Norway as well.

Total organic carbon

Little information is available on the potential effects of total organic carbon (TOC) on salmonid health in fish cultures. Davidson et al. (2009) measured 4.64 and 20.52 mg TOC/L in RAS with high and low exchange rates, respectively. Survival was high (about 99 %) in both cases.

Gas supersaturation

Supersaturation occurs when the partial pressure of one or more of the gases dissolved in the water becomes greater than the atmospheric pressure. Sudden increases in temperature, decreases in pressure, or excessive oxygenation, are all typical causes of gas supersaturation in aquaculture systems. Supersaturation of dissolved oxygen is discussed above (see ‘Oxygen’ section). External signs of gas supersaturation start to appear after several hours of exposure to gas-supersaturated water. The severity of the symptoms is closely related to percent supersaturation, O_2:N_2 ratios, and exposure time. Typical external signs are bubbles appearing on the fins, tail, opercula and head. Eventually, the eyes can be driven out from the sockets due to gas behind the eyes (‘pop-eye’). Changes in behaviour have also been observed (see Weitkamp and Katz, 1980). Ultimately, death can occur as a result of emboli, that is, bubbles are blocking the capillaries preventing normal flow of blood to various tissues (gas bubble disease). Embolisms in the heart or other vital organs normally cause death (Wedemeyer, 1996). For example, 50 % of juvenile sockeye salmon (*Oncorhynchus nerka*) exposed to 130 % total gas supersaturation at a high O_2:N_2 ratio were dead within 37 h (Nebeker et al., 1976). Total gas supersaturation should be < 110 % in intensive fish cultures (Wedemeyer, 1997). For more details on gas supersaturation and fish, refer to the comprehensive review by Weitkamp and Katz (1980).

Total suspended solids

Suspended solids are defined as particulate matter within the water with a diameter greater than 1 µm where the solids have organic and inorganic components (Chen et al., 1994). Keeping control of the levels of total suspended solids (TSS) is one of the key factors determining the success of RAS operations. This helps to stabilize and maintain good water quality with low levels of ammonia and nitrite. The particles should be removed from the tank, avoiding crushing to smaller particles in the system. Typical sources of TSS are uneaten food, faecal solids, microfauna, and particles broken off from build up material on biofilter media. Since overfeeding results in water fouling it should be avoided. Excessive amounts of feed can cause biofouling which in turn may affect the welfare of the fish by chronic stress and development of diseases. Accumulation of fine particles (5–10 µm) has been associated with lethal effects on rainbow trout (Chapman et al., 1987). Damage to fish gills can occur at

TSS levels of 44 mg/L (Magor, 1988). Indirect effects of elevated TSS levels can be increased biological oxygen demand of the culture system (and thereby reducing DO, or requiring increased oxygenation), presence of micro-organisms associated with the particles producing carbon dioxide, or presence of fish pathogens. The recommended maximum limit for TSS varies between 15 mg/L (Timmons and Ebeling, 2007), 80-100 mg/L (Wedemeyer, 1996), and 10–80 mg/L, depending on fish species (Timmons and Ebeling, 2007).

Ozone

Ozone (O_3) is a strong oxidizing agent and is commonly used as a disinfectant, or as a water quality modulating agent in RAS. The agent represents a health risk for humans and fish. In RAS, ozone is used for effective disinfection (Liltved et al., 1995) and it can be added to the tank effluent pipe to improve coagulation of fine particles for a more effective removal in the subsequent filtration step (microscreen) (Davidson et al., 2011a). The water quality can be improved by substantial reduction of total suspended solids, chemical oxygen demand (COD), dissolved organic carbon (DOC), and colour. Notably, ozone also reduces nitrite contents in the culture tanks (Davidson et al., 2011a). In such cases, it is however important to realize that this will also eventually reduce the number of bacteria in the biofilter that converts nitrite to nitrate. Thus, if the addition of ozone for some reason is interrupted, nitrite can rapidly accumulate in the system threatening fish health (Summerfelt et al., 1997). In addition to significantly improving water quality (lower levels of TSS, BOD, colour, copper, zinc, iron and heterotrophic bacteria), the use of ozone makes it possible to operate RAS with low, or near-zero water exchange rates. At low water exchange, ozone created a water quality similar to a system that was operated with 10 times greater water exchange. Better water quality led to increased growth, survival, feed conversion, and condition factor of rainbow trout (Davidson et al., 2011a). Ozonation also include large amounts of oxygen as a side effect (that can be utilized by the fish) provided that the ozone is not added before the biofilter, but instead added after the degassers (which otherwise would strip off the O_2 used in the O_3 production). Water ozonation in RAS has been demonstrated to increase growth of rainbow trout to market size without compromising fish health and welfare (Good et al., 2011a). Ozone may cause oxidative stress since the possible formation of reactive oxygen species may cause damage to certain biological molecules. Gills and blood, and later on, liver seems to be the first organs that are affected by exposure to ozone (Ritola et al., 2002). The risk of using toxic ozone in RAS is related to for example an accidental overdose where the ozone removal unit (air stripper or a hydraulic retention chamber) such that residual ozone reaches the culture tanks at toxic levels. For example, when the mean concentration of residual ozone in a rainbow trout culture tank was in the range of 3.6 to 11.2 µg/L, the ozone-induced mortalities were 3.9 to 5.0 %, respectively. During exposure to toxic levels of ozone, fish behaviour changed and the fish stopped feeding, congregated near the water surface, and ‘gasped’ for air. Erratic swimming, darting behaviour and listless behaviour gradually developed. Eventually, the fish lost equilibrium and became pale. These fish rarely survived. The gills of fish exposed to elevated levels of ozone showed excess mucus, hyperplasia, and aneurysms (Bullock et al., 1997). Ozone also destroys gill lamella epithelium which results in a rapid drop in serum osmolality (Paller and Heidinger, 1979; Wedemeyer et al., 1979). Eventually, the fish can become highly susceptible to microbial infections (Paller and Heidinger, 1979). Ozone is relatively quickly degraded. In the RAS described by Bullock et al. (1997), the longest half-lives of ozone were 15 sec. By comparison, the half-life of ozone in pure water is about 165 min at 20^{o}C (Rice et al., 1981). A safe level of residual ozone for culturing rainbow trout is reported as 2 µg/L (Wedemeyer et al., 1979) and at 8-60 µg/L, gill damage or death of rainbow trout can occur (Roselund, 1975; Wedemeyer et al., 1979). Summerfelt and

Hochheimer (1997) report an ozone 96-h LC_{50}-value of 9.3 µg/L for rainbow trout. The halogen bromine can be a challenge in RAS using ozone supplementation, due to the formation of toxic hypobromous acid (HOBr), hypobromite ion (OBr^-), or bromate (BrO_3) from bromide (Br^-) present in the make-up water (Summerfelt, 2003). Although bromide is usually present at low levels in freshwater compared to seawater (~65 mg/L), care should be exercised when commissioning O_3 systems for RAS that are using seawater supplementation during smolt production, or when using freshwater from wells containing marine sediments. In a recent study, the relationship between ORP and total residual oxidant and that UV irradiation can destroy some bromine and bromoform, but not bromate (Summerfelt et al., 2011, submitted).

Alkalinity

Alkalinity, the total concentration of alkaline substances dissolved in the water, is related to the capacity of water to neutralize hydrogen ions. Thus, water with a certain alkalinity has the potential to stabilize a water system by buffering against large and sudden pH changes. Highly alkaline waters may, however, cause problems for the fish since ammonia excretion and production can be inhibited (Wilson et al., 1998). Recommended lower and upper limits for alkalinity are >20 mg/L (to provide some buffering capacity), and <100-150 mg/L, respectively (Wedemeyer, 1996). Timmons and Ebeling (2007) recommend alkalinities (as $CaCO_3$) within the range of 50-300 mg/L. In RAS operated with minimal water exchange, Chen et al. (2006) recommended an alkalinity of 200 mg $CaCO_3$/L for optimal biofilter performance. Since the alkalinity level that is used in a salmon smolt RAS will influence running costs, dependent on the daily system water exchange, there is a need to determine the optimal alkalinity level for unit process removal rates, such as biofilter and CO_2-degassers, as well as impacts on fish welfare and physiological mechanisms.

Hardness

Hardness is defined as the total concentration, of primarily calcium (Ca^{2+}) and magnesium (Mg^{2+}), iron, and manganese ions present in the water. The concentration is expressed in terms of equivalent mg $CaCO_3$/L. Thus, hardness is also a measure of the buffering capacity of the water and is therefore important for regulation of pH in aquaculture farms. The total hardness of natural water ranges from <5 to > 10 000 mg $CaCO_3$/L. Water can be classified as soft (0-75 mg $CaCO_3$/L) up to very hard (> 300 mg $CaCO_3$/L). Recommended levels range from 20 to 300 mg $CaCO_3$/L (Timmons and Ebeling, 2007). Since fish must regulate their blood ion concentrations across the gills, water hardness will affect the amount of energy needed for the purpose according to the magnitude of the blood-water concentration gradient (Wedemeyer, 1996).

Metals/copper

Metals can be very toxic to fish (Wedemeyer, 1996). At low water exchange rates, there is a tendency that metals will accumulate in RAS (Davidson et al., 2009). However, only copper of 15 measured metals exceeded (37- 56 µg/L), the recommended safe limits given in the study by Davidson et al. (2009). Although mortality was relatively low, a linear trend between copper concentration and mortality was nevertheless observed in this study. Elevated levels of dissolved copper may be due to the possible corrosion of copper pipes and fittings in the RAS, although mass balance calculations have indicated that the major source of copper is contributed by the feed (Davidson et al., 2009). The toxicity of copper is dependent upon alkalinity and hardness in the water, with quite different recommended safe levels (0.6 µg/L Cu at alkalinity <100 mg/L and 30 µg/L at alkalinity >100 mg/L (Wedemeyer 1997; Timmons and Ebeling, 2007). Fish can tolerate higher Cu levels with increasing Ca levels in the water. When adjusting the water hardness by adding $CaCO_3$ the bicarbonate equilibrium will also increase. Added CO_3^{2-} react with Cu_2^+ to form $CuHCO_3$ and $CuCO_3$ so the hardness effect is believed to be a $CaCO_3$ effect (Di Toro, Allen et al., 2001). pH affects the toxicity of Cu in several ways. The toxicity will decrease with increasing pH as a result of the effect of pH on the chemical state and complex binding of copper. When pH increases, the proportion that exists as copper carbonate complexes increase and thus reduce the toxicity. In addition to deprotonation (release of H+) of DOC, a higher pH increase the amount of produced Cu-TOC complexes, which reduces toxicity (Di Toro et al., 2001).

Most of the literature regarding copper toxicity on salmonid fishes is based on experiments with rainbow trout. Toxicity of copper is a serious problem in Atlantic salmon smolt production (Åtland et al., 1999). Furthermore, high mortalities due to copper were observed in the start-feeding period, and it seems that salmon could be more sensitive to copper toxicity than rainbow trout. It could be questioned whether the recommended level is too high for Atlantic salmon. Furthermore, the concentration of total organic carbon is a key factor to reduce Cu-toxicity. A suggested toxic mechanism is that Cu induces failure in ammonium excretion and sodium uptake. Earlier studies have shown that fish exposed to water contaminated with copper produce high levels of ammonium in the tissues. This waterborne Cu toxicity increases with feeding (Hashemi et al., 2008, Kunwar et al., 2009). This could be of particular interest for recirculation farms since accumulation can occur (Martins et al., 2009). It should also be mentioned that dissolved copper can be significantly reduced in RAS by using ozone (Davidson et al., 2011a).

Aluminium

Aluminium (Al) is toxic to fish and the presence of the metal has caused water quality problems in Norwegian smolt farms. Even at low concentrations (0.115 -0.140 mg/L of total Al, 0.010 mg/L of labile Al), the presence of the metal can be toxic in combination with carbon dioxide and reduced pH (Fivelstad et al., 2003b).

Recommended maximum levels of labile aluminium are <0.075 mg/L (Wedemeyer, 1997) and <0.01 mg/L (Timmons and Ebeling, 2007). The maximum levels depend upon bioavailability, which is the risk of which aluminium binds to the fish gill rather than to humic acid, particles or organic materials. There is a close relationship between aluminium in the water and accumulation on the fish gills of salmon (Kroglund et al., 2001, Teien et al., 2005). In soft water, < 0.010 mg/L is accepted as a background value. At high concentrations of labile aluminum (0.300 mg/L in freshwater and 0.150 mg/L in seawater, Kroglund and Staurnes, 1999), the fish die as a consequence of failure in respiration and osmoregulation (Rosseland and Staurnes, 1994). Physiological changes of welfare interest can be seen at much lower concentrations of labile aluminum (0.100 mg/L in freshwater and 0.040 mg/L in seawater). An applied recommendation for labile aluminium toxicity for salmon smolts is 0.015 – 0.020 mg/L (Rosseland, 1999).

Temperature

In Norway, the water temperature in single-pass flow-through hatcheries varies considerably. In RAS, the possibility for control of temperature is more feasible. However, water cooling systems, or dedicated ventilation systems (Terjesen et al., 2010), may in some cases be necessary when RAS are placed inhouse, since pump and pipe friction increase RAS temperature from that of the make-up water. Whereas the optimum temperature growth of Atlantic salmon is about 15-16 ^{o}C (Weatherley and Gill, 1995; Koskela et al., 1997), the optimal physiological thermal range is 6-20 °C (see Elliott, 1981). On the other hand, studies on skeletal deformities in farmed salmon identified an increased risk of vertebral deformities in response to freshwater rearing temperatures >12°C (Baeverfjord and Wibe, 2003). Unlike some other risk factors, the temperature induced deformities may not be identifiable until later in the life cycle, and causative relation may therefore be difficult to establish under commercial rearing. In compliance with these results, Ytteborg et al. (2010) demonstrated alterations in gene transcripts in response to temperature exposure during freshwater rearing of salmon, resulting in a set of events leading to disturbances in differentiation and growth of vertebral bone and cartilage. Thus, rearing temperatures which are optimized for growth rate and (in RAS) for biofilter function, may induce skeletal deformities which can cause significant losses at harvest. In spite of this knowledge, water temperatures of 14 °C and above are not uncommon in commercial freshwater rearing. Specific studies related to temperature tolerance in RAS have so far not been done. The upper critical ranges for Atlantic salmon and rainbow trout, depending on life stage and acclimation temperature, are 20-34 °C and 19-30 °C, respectively (see Elliott, 1981). The lower lethal temperature for Atlantic salmon is reported to be around -0.7 °C (Saunders, 1986). However, Skuladottir et al. (1990) reported for Atlantic salmon (average weight 0.4 kg) that when the seawater temperature dropped gradually to -1.8 °C, mortalities started to occur at -1.4 °C.

Current water quality requirements for fish farming in Norway

In the Regulations relating to Operation of Aquaculture establishments (*Forskrift 2008.6.17 nr. 822 om drift av akvakulturanlegg* 'Driftsforskriften') issued by the Norwegian Food Safety Authority, the recommended safe levels of important water quality parameters are as shown in Table 1. Clearly, the values are basically in line with the respective recommended values shown above in this section. The validity of these values, as well as whether additional parameters should be included in this list accomodating for RAS usage, is discussed below.

Table 1. Recommended safe levels of key water quality parameters as issued by the Norwegian Food Safety Authority.

Water quality parameter	Limits
pH (inlet)	6.2 – 6.8
Dissolved oxygen	Maximum 100 % saturation (tank) and 80 % saturation (outlet)
Carbon dioxide	< 15 mg/L
TA-N ($NH_4^+ + NH_3$)	< 2 mg/L
Nitrite	< 0.1 mg/L (freshwater)
Total organic carbon (TOC)	<10 mg/L
Aluminium	< 5 µg/L (labile) and < 20 µg/g gill (gills)

Water quality criteria and commercial production of salmonids in recirculated systems

The suggested criteria for good water quality, as shown above, should be used with caution, and in many cases the maximum or minimum levels should be considered as guidelines only.

It is important to be aware of that the published criteria were derived from experiments carried out under different conditions, and often the purpose of these studies was not necessarily to focus on the intensive production of fish in cultures. One example is nitrite, which clearly has been suggested as maximum level in soft freshwater. In a RAS system, with adjusted and increased hardness, the maximum level will be higher (see text page 24). Since the toxicity of nitrite is dependent on the salt (Cl^-) concentration in the water (see page 21-22), it would be valuable for practical use if the recommended safe levels of nitrite are related to different levels of Cl^-. This is done in Canadian environmental recommended guidelines (Environment Canada, 2001). The recommended pH range of 6.2-6.8 (Table 1) is below the optimum pH for the nitrification process (Chen et al., 2006). For a more efficient removal of the toxic nitrogen compounds, higher recommeded pH values should therefore be considered.

The effect of only one water quality parameter at a time is usually reported in the literature. Although single-factor studies are valuable to improve knowledge about which specific mechanisms are affected in the fish, such results are of limited value to predict the joint impacts of several water quality parameters. Furthermore, studies that are intended to give advice about water quality criteria in RAS, should therefore be conducted in a RAS environment. In high-intensity RAS, parameters like suspended solids, refractory organics, metals, and nitrite may turn out to be of importance as limiting factors (Colt, 2006).

Possible impacts on fish welfare in RAS production of salmonids

Non-specific health effects

The health and welfare aspect of salmonid farming is a complex issue. On one side, infectious diseases can potentially cause significant losses at nearly all life stages and control of specific pathogens receive considerable attention. On the other hand, non-specific health and welfare issues may appear, that is conditions that are related to environmental conditions and production management primarily, and to a lesser extent or only secondary to infectious agents. The question remains whether the RAS environment in any way represents an additional strain on fish health, and what the critical factors may be. In particular, concern was expressed over the possible subclinical and clinical effects of a long term exposure to water quality that is less than optimal. In a commercial setting, interaction between environmentally induced effects and infectious diseases should also be expected.

In principle, RAS can create favourable conditions for growth of opportunistic microorganisms and poor water quality, or high stocking densities can cause chronic stress making the fish more susceptible to diseases. The effect of these factors related to the possible occurrence of various diseases in rainbow trout RAS have been reviewed by Noble and Summerfelt (1996). It turns out that several diseases of bacterial, parasitic, fungal or viral origin have been encountered in such cultures. It follows that good management practices to prevent the occurrence of diseases are essential for successful operation of RAS, a view which is strongly supported by the Norwegian managers which were interviewed (See section Norwegian experiences, page 49).

Fin erosion is commonly mentioned as a point of concern related to RAS, and in fact, fin erosion has been suggested to be used as an index of welfare during rearing of fish. However, successful use of high fish stocking densities were reported, as long as water quality was maintained within safe levels (please refer to section on stocking densities page 47). In such cases fish performance is high without serious deterioration of pectoral and dorsal fins.

However, the caudal fin of fish in RAS was subjected more to erosion compared with a flow-through system, irrespectively of stocking density in the range of 50-100 kg/m^3 (Roque d'Orbcastel et al., 2009b).

In interview, managers of RAS production facilities state that no specific health or welfare problems are associated with salmon smolts produced in RAS, neither during freshwater production nor following sea water transfer (See 'Norwegian experiences', page 49). On the contrary, RAS smolts perform well in seawater at a more stable level than comparable fish groups from flow-through facilities. These observations were supported by production data from several companies. Thus, a general adverse effect from the RAS environment *per se* seems unlikely.

Although RAS are widely used internationally, scientific documentation on fish health and welfare is relatively limited, in particular regarding salmonids. Some studies address the difference between RAS and flow-through systems in rainbow trout with a more general approach (Roque d'Orbcastel et al., 2009a, b), but unfortunately without any in-depth health evaluation. It was, however, noted, a differentiated response on fin condition (Roque d'Orbcastel et al., 2009a), a difference which was ascribed to differences in hydrodynamics and swimming pattern. In a study from Good et al. (2011b), fish health and welfare was compared in two groups of chinook salmon raised either in a partial reuse system of circular tanks, or in a flow-through raceway system (hence tank design was a confounding factor). However, fin condition was somewhat inferior in water reuse fish, although fin condition was generally relatively good. Some histopathological lesions were seen in both groups, but the only lesion being more consistent in reuse fish was gill epithelial hypertrophy. No effects were detected on physiological parameters. Good et al. (2009) also reported on a range of health parameters from a study on the effects of water exchange rate in rainbow trout, comparing a low daily exchange rate of 0.26 % with an exchange rate tenfold higher, 2.6 %. The results displayed significant differences in many parameters. In the low exchange groups, caudal fin quality was inferior, and there were an increased number of histopathological lesions in spleen and skin, whereas the high exchange group had a higher number of lesions in the posterior part of the kidney. There were also significant differences in plasma chloride and blood urea. Despite these differences, which were mainly subclinical, the authors concluded that there was no major treatment effect. Davidson et al. (2009) reported from the same experiment, that a significant accumulation of substances occurred in the low exchange units. The accumulation of heavy metals was noted as a point of concern, and subtoxic Cu levels observed in low exchange was associated with some mortality in the experiment, as well as with previous mortalities in similar low exchange fish groups. The effects of ozonation as a water improving measure under low exchange conditions was examined in a recently published study by Good et al. (2011a). The study demonstrated some minor differences in fin condition and blood chemistry between ozonated and non-ozonated fish groups, but the overall conclusion was that ozonation improved performance without compromising fish health and welfare. It should be noted that ozone is a potential hazard to both human and fish health if proper safety measures are not installed, such as double sets Oxygen Reduction Potential (ORP) electrodes connected to a control and alarm system. Some more specific experiments address the effects of specific water quality parameters. In a study on long term ammonia exposure to Atlantic salmon (Kolarevic et al., submitted manuscript), it was indicated that Atlantic salmon was more resilient to NH_3-N than previously suggested. Following an initial reaction in gill tissue to high levels of NH_3-N, fish adapted to and tolerated levels of 32 µg/L well, with no specific effects on a range of health and welfare parameters. Gene expression studies on these fish are in progress, and preliminary results show significant upregulation of ammonia (Rhcg1 and Rhcg2) and urea transporting (UT) genes in response to ammonia exposure (Kolarevic et al, 2011b). A controlled study on CO_2 levels to rainbow trout (Good et al., 2010) compared 8 mg/L and 24 mg/L in a 6 month trial. A similar trial is underway for Atlantic salmon. In the trout study, some differences in health parameters were observed between treatments, but the observed pathology was judged to be subclinical, and the effects went both ways, with the low CO_2 treatment giving greater gill epithelial hyperplasia. Thus, the study indicates that rainbow trout tolerance to CO_2 may be greater than previous assumptions. Similar results are indicated in a 12 week study on sub-lethal nitrite exposure at high chloride background in Atlantic salmon parr (Gutierrez et al., 2011). Average conductivity was 715 µS/cm during the study, pH 6.8-7.2, and alkalinity in the ground well water fluctuates between 5-20 mg/L as $CaCO_3$ (Terjesen et al., 2008; Zuhlke 2011). Health evaluation and molecular analyses of these fish are still in progress, but preliminary results indicate that the Atlantic salmon parr tolerated NO_2-N levels up to 9 mg/L, at a Cl:NO_2-N ratio of 23:1, without mortality or effects on growth rate when the entire experimental duration was taken into account. However, if only the first three weeks are considered, growth rate was adversely affected at the Cl: NO_2-N ratio of 23:1, but not at a ratio of 43:1 and above. In contrast, nitrite accumulated significantly in plasma also at 23:1 and 43:1, but not in fish of the 108:1 group (both early and late in the trial). Hence, this elevated plasma nitrite could have led to adverse effects at tissue level in parr of the groups below the 108:1 ratio. Further analyses and experiments in a RAS environment are expected to provide more detailed information on fish response, and possibly contribute towards more specific recommendations related to control of NO_2 toxicity by use of chloride supplementation. Thus, it may seem that studies done so far reveal only minor effects on health parameters and fish performance in long-term controlled experiments, even under some relatively extreme conditions which are exceeding current limit values for certain parameters. Although results on individual factors must be considered with caution, in combination they may seem to justify future development of RAS-specific water quality management strategies.

An important reservation when regarding the implications of these results for practical fish rearing is the complexity of RAS, and the experience that parameters will interact with each other in their effect on fish. Also, studies done specifically on health and welfare are so far relatively few, covering a limited number of topics. The studies cited are also not consistent in choice of parameters for health evaluation. It is also worth noting that although molecular biology is an integrated part in most fields of biological research, these methods are in early stages of integration in RAS-related studies. It is strongly suggested to continue studies in this field, both as regards topics and experimental models, as well as the range of health and welfare parameters. An important aspect is also the interaction between environmental impact on fish health and resistance to infectious disease, which may be difficult to model under experimental conditions.

The managers which were interviewed in connection to this evaluation reported to having observed a range of diverse health issues in practical life, demonstrating beyond discussion that health issues are an important part of RAS production. Nevertheless, none reported particular fish health problems in RAS as compared to flow-through systems. A strong interest was expressed related to obtaining more knowledge on health and welfare aspects of RAS systems, not only to the potential harmful effects of RAS environment but also the nature of any beneficial effects from these production environments.

Technology used in RAS

This chapter describes some basic principles of the recirculation system that is commonly used in Norway. The first paragraph explains some of the most commonly used definitions for recirculation degree (Anders Fjellheim, pers. comm.).

Basic definitions

There are three common ways to define recirculation in aquaculture:

1. Recirculation in percent (RD %)
2. Exchange per day in percent (ED %)
3. Exchange per day per kg feed (EDF)

1. Recirculation degree in percent (RD %)

$RD\ \% = (WF_R \text{ to fish tanks} * h^{-1} / (NW * h^{-1} + WF \text{ to fish tanks} * h^{-1})) * 100\ \%$, where

WF_R = Water Flow recirculated

NW = New Water

h = hour

2. Exchange per day in percent (ED %)

$ED\ \% = (NW * d^{-1} / V_{tot}) * 100\ \%$

NW = New Water

d = day

V_{tot} = Total water volume in the fish farm, including water treatment units

3. Exchange per day per kg feed (EDF)

$EDF = NW * d^{-1} / F * d^{-1}$

NW = New Water

d = day

F = Feed amount

Farm (A) - A recirculation farm with a water flow of recirculated water 3000 m^3 per hour (50 m^3 pr minute) and a total tank capacity of 2500 m^3, adds 20 m^3 new water per hour (333 litres per minute), and are feeding 750 kg feed per day.

Farm (B) - A single pass flow-through farm with a recirculated water flow of 0 m^3 per hour, a total tank capacity of 2500 m^3, adds 1500 m^3 new water per hour (25 000 litres per minute), and are feeding 750 kg feed per day. We can calculate the different expressions for the same recirculation operation (1) RD %, (2) ED %, (3) EDF for the two different farm concepts.

Farm (A) - Recirculation

(1) RD % = (3000 m^3 /(20 m^3 + 3000 m^3) *100 %) = 99.3 %

(2) ED % = (20 m^3 / h * 24 h/2500 m^3) * 100 % = 19.2 %

(3) EDF = (480 000 litre / 750 kg) = 640 litre * kg^{-1} feed

Farm (B) - Single pass flow-through

(1) RD % = (0 m^3 /1500 m^3) *100 %) = 0 %

(2) ED % = (36 000 m^3 / 2500 m^3) * 100 % = 1440 % (14.5 times a day)

(3) EDF = (36 000 m^3 / 750 kg) = 48 000 litre * kg^{-1} feed

The residual time (R_t) for NW in the two system are given by:

R_t = (V_{tot} / NW * h^{-1})

R_t = Residual time in hours for NW

NW = New Water

h = hour

V_{tot} = Total water volume in the fish farm

For farm (A) recirculation, the residual time of the new water added to the fish tanks (2500 m^3) is: R_t = (2500 m^3/20 m^3) = 125 hours = 5.2 days. The residual time for the recirculated water (WF_R) through the fish tanks is: R_t = (2500 m^3 / 3000 m^3) = 0.83 hours

For farm (B) single pass flow-through, the residual time for the new water added to the system is:

R_t = (2500/1500) = 1.7 hours = 0.07 days.

There are some comments necessary to take into consideration with these definitions. RD % and ED % does not take into account how much feed is added to the system. This is vital information since the feed amount to a large extent will determine the load of the system (e.g. TAN, urea and feces production). RD % expresses how quick the circulation (pumping) of water within the recirculation system is. ED % expresses the use of new water. EDF expresses of much feed that is added to the system and how much new water that are used to “process” this feed but does not say how quickly the water is circulating in the system, which is relevant for removal of metabolites. Due to this it is recommended to use the law of mass balance to calculate the different effects and concentrations. A description on how this is valid for aquaculture is given by Losordo (1994). Figure 1 describes the basic principle of a recirculation set up for aquaculture.

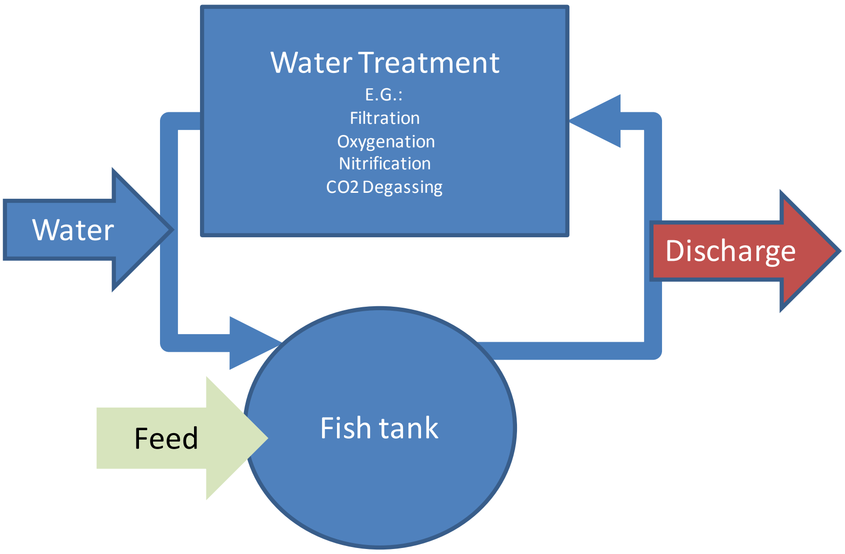

Figure 1. Simplified description of a recirculation system.

A frequently used figure describing the various degrees of complexity of recirculation systems is shown in Figure 2. Along the x-axis, the degree of recirculation is expressed along with the necessary technology needed to deal with gradually less water renewal. Most flow-through smolt farms in Norway have already applied the first two steps of this figure (oxygenation and CO_2 degassing). The *ad hoc* committee was also given a presentation by Marine Harvest, who has recently built a recirculation farm with a denitrification and a phosphate filter, which is usually introduced when a high degree of recirculation is required.

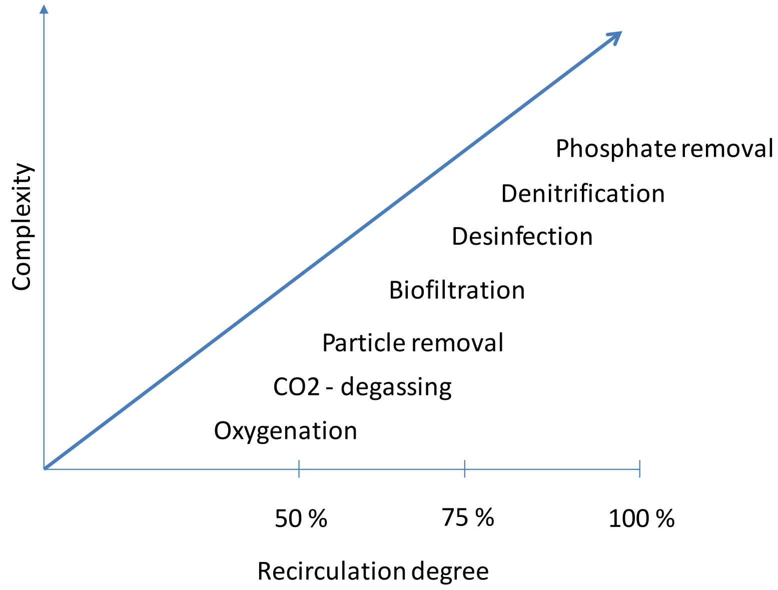

Figure 2. Illustration of the relationship between water recirculation degree and complexity of the technology needed to maintain good water quality in recirculated systems (after Muir, 1981).

Basic components in RAS

The following chapter gives a brief overview of the basic components normally included in a RAS. The description is based upon Hutchinson et al. (2004) and Timmons and Ebeling (2007), but modified to what the committee believes is representative for Norwegian conditions. The chapter covers minimum standard for design and construction of a commercial RAS. An overview of the system components is given in Figure 3 and 4.

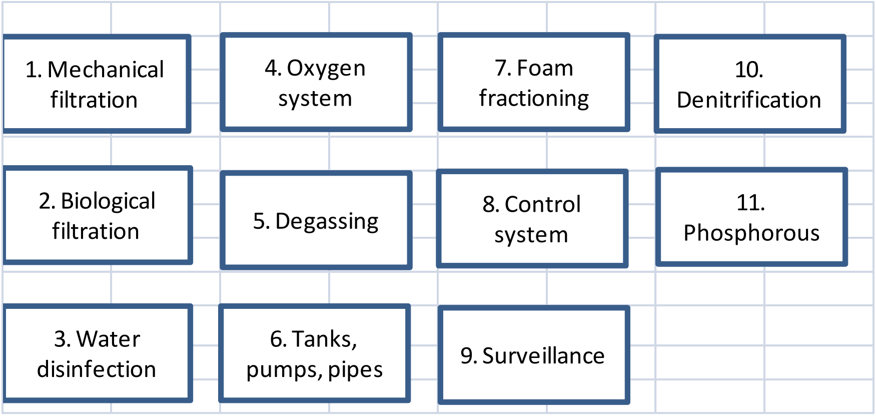

Figure 3. Basic components (1-11) of a typical RAS. Component 10 and 11 are normally not found in systems used for salmonids in Norway, and component 7 is normally used with seawater RAS only.

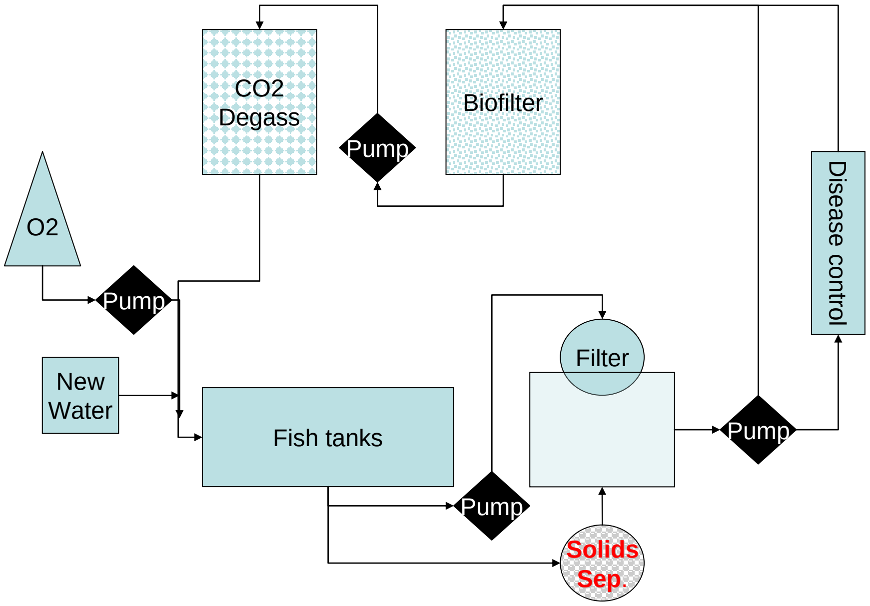

Figure 4. Example of layout for a freshwater RAS. Modified after Hutchinson et al. (2004). The following important components are not shown: (1) temperature and photoperiodic control, (2) foam fractioning (used in seawater RAS) and (3) backup power supply. 'Disease control component': UV or ozon treatment of the recirculated water. The number of pumps may vary.

Hutchinson et al. (2004) suggest three categories of components in a RAS:

1. **Essential components**: Includes water supply, mechanical filtration, biological filtration, disease control system, fish tanks, pumps, plumbing, environmental control, oxygen system, carbon dioxide and nitrogen degassing, foam fractioning (in seawater), backup power supply.
2. **Supporting infrastructure and equipment**: Includes buildings, water quality monitoring equipment, alarm systems, feeding systems, storage facilities, staff amenities, administration and workshop facilities.
3. **Additional systems to enhance production**: Includes quarantine, purging and weaning systems, auto–monitoring and process control systems

Mechanical filtration

Mechanical filtration is used to remove particle matters form a RAS. These particles are made up of fish faeces and uneaten fish food. The biological breakdown of these matters is linked to activity of heterotrophic bacteria and micro-organisms (biofilm), covering insides of pipes and other surfaces in the system. This activity consumes oxygen and will also contribute to increase ammonia loads in the system. The growth and development heterotrophic bacteria in the biofilter are normally something one want to limit since they will compete with autotrophic bacteria vital for the ammonia to nitrate transition. According to Hutchinson et al. (2004), there is no universally accepted design layout of RAS components, but mechanical filters are generally accepted as crucial to such systems, and that is should precede the biological filter and systems for disease control. It is vital to remove organic matters before they start to break down. Particles larger than 100 µm are often removed from the RAS by a settlement device (e.g. swirl separator, settlement chambers or inclined plate separator). Plumbing systems as modified sumps and double drainage points (e.g. Cornell double drain system, and Eco Trap™) are also aimed to remove coarse solids from the water at the tanks exit point, leading them further to separation and sludge removal. Suspended solids (< 100 µm) can be removed by mechanical filters using (1) depth (e.g. pressure-, sand-, cartridge-, matting filters) or (2) screen (e.g. inclined screens, rotating drum filters or conveyor belts). Rotating drum filters with a microscreen between 20-100 µm are to our impression most commonly used in RAS in Norway. The organic loading within RAS represents a problem if pressure sand filter, cartridge filter and bag filters are used. It is recommended that the entire recirculated water flow is filtered (Hutchinson et al., 2004). From empirical field data from RAS in Australia, Hutchinson et al. (2004) concluded that the filtration capacity did not match the solids load and the volume of water required in RAS. There are no such data available from RAS in Norway. Some RAS suppliers use heterotrophic bacteria filters as an additional means to remove organic matters (see Annex 2).

Biofilters

The functionality of recirculation systems depends on the balance between unit processes (e.g. degasser, mechanical filter, biofilter) and the biological components (e.g. the cultured fish, microbiota). An essential component when a higher degree of recirculation is required is the biofilter. These are unique microbial environments where microbes build up biofilms – microbial layers or mats, where bacteria contribute to stabilize the environment by removing wastes. The most important function of the biofilter is the removal of ammonia by converting it to less harmful nitrate. Inside the filter, populations of nitrifying bacteria (*Nitrosomonas*) first convert ammonia to nitrite (NO_2-N). Further *Nitrospira* sp. convert nitrite to nitrate. Both bacteria types are aerobic, requiring oxygen levels close to saturation and producing carbon dioxide.

The biofilters are created with a media that provides a very high surface area. An area of 100 – 1000 m^2/m^3 is typical. The total media area available to the nitrifying bacteria is linked to the capacity of the biofilter. The filter area is designed to achieve specified performance criteria by the use of ammonia and dissolved oxygen mass balances. There can be a lot of configurations; low density plastic media is used in trickling biofilters, where water is introduced on top. Modern biofilters often uses moving bed filter, fixed bed filters or fluidized sand filters. Biofilters requires a water flow through the filter media to offer ammonia to the bacteria population, and the necessary flow is determined based on the ammonia load, removal efficiency at the target concentration, and other aspects of the RAS. A typical RAS design specification is to allow the entire volume of water in the farming system **to pass** through the filter at least two times per hour. In some biofilters oxygen enriched air can be blown through the media to ensure oxygen for the denitrification process and allow some stripping of carbon dioxide. To maintain low levels of ammonia in RAS, biofilters with large surface areas are provided for the bacteria where ammonia is oxidized in a two-stage process to nitrate via nitrite. According to Hutchinson et al. (2004) desired concentration of TAN in RAS is often set to a maximum of 1.8- 2.0 mg/L.

Seawater RAS requires a larger biofilter capacity than freshwater RAS.

In an ideal situation – in a well functional RAS – where a stable biofilter is combined with strict control of the water quality, the microbial community of the filter is protective, stabilizing the environment. The heterotrophic bacteria population is suspected of having a positive effect against pathogenic bacteria. Several studies have described the microbial communities of biofilters (see e.g. Sugita et al., 2005; Schneider et al., 2007; Fu et al., 2010; Schreier et al., 2010). Many of the bacteria found in the biofilters may however be difficult to cultivate and thus describe properly. New tools like denaturating gradient gel electrophoresis (DGGE) or microarray-based profiling will gradually lead to a series of more detailed descriptions of the microbial communities in biofilters under different operating conditions. The composition of the microbial communities will vary from one RAS to the next. The composition will be influenced by the inocula - including the fish being introduced in the units, and the stability of the microbial community in the biofilters may be altered by the influence of a number of factors, like particulate organic carbon, oxygen levels, temperature, pH, alkalinity, salinity and turbulence (Michaud et al., 2006; Chen et al., 2006).

Although the microbial communities of biofilters may stabilize the systems, pathogenic bacteria have been detected in RAS biofilters (Schreier et al., 2010), and there are several reports of pathogens establishing and affecting fish held in recirculated rearing units. Bacterial diseases like infection with *Streptococcus iniae*, bacterial gill disease, furunculosis, bacterial kidney disease, fin rot and infestations with parasites like *Ichtyophthirius multifilis*, *Trichodina* spp., *Apiosoma* sp., *Ambiphyra* sp., *Epistylis* sp. and *Displostomum spathaceum* have been reported (Noble and Summerfelt, 1996; Bowser et al., 1998; Jørgensen et al., 2009). Pathogens may be introduced via renewal of water or with the introduction of fish, and over time, pathogens may be concentrated. The cases reported underline the importance of a strict control of the fish entering the RAS, as well as the renewal of water.

The level of organic matter is important, and a high organic load may impair the system. The C/N ratio can also affect the composition of the microbial community and nitrification rate

(Chen et al., 2006). Poor water quality may cause stressful conditions for the fish, making them more vulnerable to disease. Poorly operated RAS may in some cases favour the establishment and propagation opportunistic pathogens.

Disinfection systems (UV and ozone)

Water disinfection systems are common in RAS. The environmental conditions in RAS are favourable for growth of bacteria due to high organic load, increased pH, increased water temperature and high fish density. The methods which are commonly used are addition of ozone and ultra violet irradiation (UV). Disinfection systems are used at two separate stages, either 1) a single-pass disinfection of new water entering the system (make-up water), or 2) continuous disinfection of the recirculating flow. The new water constitutes a limited volume of water which allows for a near total destruction of bacteria and virus in inlet water, and disinfection at this stage is an important a barrier against specific fish pathogens. The corresponding treatment of the recirculating flow might be considered more as a water treatment procedure, and disinfection may be applied to a part of the flow and still be of value. Full-flow disinfection is, however, also possible if required (Summerfelt et al., 2009a), at doses which are tenfold lower than those used to disinfect inlet water in a single-pass system.

It was demonstrated that ozone was successful in reducing both specific pathogens and heterotropic bacterial count in recirculation (Bullock et al., 1997). UV-irradiation, on the other hand, was less effective (Sharrer et al., 2005). The combination of ozonation and UV-irradiation was suggested as an option for treatment of the recirculating flow (Sharrer and Summerfelt, 2007), also due to the destruction of ozone residues by subsequent low-dosage UV treatment (Summerfelt et al., 2004), if necessary.

Alternative approaches to use of ozone or UV may be suggested, e.g. use of filtration as a treatment principle. There are currently no specific filters developed for this purpose marketed, but future developments are expected.

Ultra violet irradiation

UV is typically produced by lamps that emit irradiation at wave lengths in the range 100 – 400 nm. An UV irradiation of 260 nm is regarded as the peak of disinfection effect (Lawson, 1995). The effect is due to the UV lights damaging effects of the DNA/RNA of the microorganisms (parasites, bacteria and virus). The effect is proportional to the UV radiation intensity (UV dose) and it is expressed as µmWs/cm^2. The UV dose required to kill microorganisms in RAS, range from 35 000 – 1 000 000 µWs/cm^2 (Lawson, 1995). The maximum effect when the operating temperature of UV lamps is 40 °C. Due to this the lamps are normally enclosed in a quarts glass sleeve, to prevent them from direct contact with colder water. The effectiveness of UV systems is highly influenced by the murkiness of the water (suspended solids, humic acids, organic compounds), since these components can reflect, absorb or shadow the UV light and thereby protect microorganisms. Due to this, UV systems are normally installed after mechanical filtration. UV systems come in a variety of designs, e.g. open channel or inline systems, but they all demand maintenance as removal of biofilm and replacement of weakening lamps. Normal lifetime of many UV lamps is 7 000 – 8 000 hours or approximately 12 months of continuous operation. UV light can be damaging for the human retina and health and safety precautions must be taken. Under normal conditions, UV will not cause a complete kill of all microorganisms. Very resistant virus like IPNV and

VNNV tend to survive although the number of infective particles will be reduced. UV, placed subsequent to ozone supplementation, can also act to safe-guard against O_3-overdosing.

Ozone

The disinfection ability of ozone (O_3) is linked to its strong oxidizing ability. The inactivation of fish pathogens through ozonation or UV irradiation is a well-documented procedure in aquaculture (Liltved et al., 1995, 2006). In RAS, ozone is also used as water quality improvement aiming for lower turbidity. The effects are (1) oxidation of organic compounds and reduction of BOD and water colour, (2) coagulation of particles that makes them easier to remove by mechanical filtration, (3) breakdown of large organic molecules into smaller and more biodegradable ones, 4) facilitating removal of Cu and Fe from the recirculated water, and 5) nitrite oxidation (Krumis et al., 2002). Ozone is often produced in generators were oxygen passes a high voltage created across two electrodes. It is often applied in a contact chamber, build to create the desired treatment time and allow ozone to revert back to oxygen, or into low-head oxygenators, or by using venturi injectors and on a side-stream from the full recirculated flow. The half-life of ozone in aquaculture systems with high organic loads are only a few minutes. In RAS systems typically dosages of ozone for disinfection are between 0.01 – 0.10 mg/L, with retention time for treatment between 30 sek and 20 min. In relation to use for improving water quality, dosing is done according to the RAS feed load, usually at 25 g O_3/kg feed per day. Residual ozone must be removed since it is toxic to fish and humans. That can be done by letting the ozonated water pass through an activated carbon filter or through a degasser. Alternatively, ozone residues will be efficiently removed by the biofilter, if ozone is added prior to passage through the bioreactor (Timmons and Ebeling, 2007). The dosage can be monitored through an ORP meter. Normally ozone is added automatically before the mechanical and biological filter, and is aimed to improved mechanical filtration due to decomposition of organic material (Lawson, 1995).

Oxygenating systems

Oxygen demand of fish varies with several factors (see page 18) and oxygen has to be added to the water to meet respiration requirements. The daily increase in feeding ratio and biomass has to be compensated by increasing the oxygen supply. In RAS, an often used design criteria is that for each kilo feed added, approximately 0.5 – 0.56 kg of oxygen will be consumed by the bacteria and the fish populations (see description under mechanical filtration) (Losordo et al., 1992; Parker et al., 2002). In high density RAS, it is common to use pure oxygen from either an oxygen generator system or a liquid oxygen high pressure system. The distribution of oxygen might into the water supply pipe (pressurized types as cones, u-tubes), direct through fine diffusers in the tank or a combination. Intensive RAS tend to optimize system dissolved oxygen at 100 % (Parker et al., 2002). Oxygen produced by generators can contain approximately 10 % nitrogen, so if supplied liquid oxygen is not available, the O_2 distribution devices should be limited to low-pressures types to avoid supersaturation of nitrogen.

Degassing systems

Carbon dioxide is produced by fish metabolism (see page 15 and 19) and bacteria metabolism. In intensive RAS accumulation occurs (Grace and Piedrahita, 1994) If not reduced to an acceptable level, CO_2 represent limitations to productivity and fish welfare. In RAS, CO_2 is commonly reduced by gas stripping devices provided with a very high airflow ratio to the water flow. Systems can be trickling filters dedicated cascade columns with plastic degassing media and a counter current flow of air and water, ejector based systems, or systems in which air is blow into water retention tanks, usually the biofilters. The systems can be applied in the water treatment loop and/or on each fish tank. A framework has been developed for calculating the necessary degassing media height and other parameters for cascade CO_2-degassers, in relation to the inlet and needed outlet, CO_2 concentrations (Summerfelt et al 2000). Recently, Moran (2010) showed that the removal efficiency for CO_2 when using column degassers is reduced in seawater compared to freshwater, due to the relatively slow conversion of bicarbonate back to CO_2, after the water has passed the degasser. This observation may have impact on dimensioning and/or needed water flow rate in RAS that use sea water supplementation.

Supersaturation can cause substantial morbidity and mortality of salmonids (Elston et al., 1997), but exposed salmonids will recover quite quickly when transferred to normal gas saturation (Hans et al., 1999). The trauma is called gas bubble disease and is caused by total dissolved gas supersaturation (TDG) and supersaturation of nitrogen gas (Vatsos and Angelidis, 2010). Gas supersaturation can cause exopthalmia (“pop eyes”) in juvenile Atlantic cod (*Gadus morhua* L) (Gunnarsli et al., 2008), and reduced growth in Atlantic cod larvea (Gunnarsli et al., 2009). There is a clear correlation between mortality in steelhead trout and chinook salmon, appearance of gas bubbles on lateral line, fins and gills and high total dissolved gas pressure (Mesa et al., 2000). When fish have access to depths that provide hydrostatic compensation, this can eliminates the effects of exposure to supersaturation (Weitkamp et al., 2003). In smolt farms one should be alerted by a situation where the TDG pressures are higher than 100 % and oxygen levels are lower than 100 %. Mortality can occur at supersaturation levels above 5 %. Stress reponses can occur at even lower levels. Smaller fish and fish in shallow water are more likely to be affected than larger fish and fish on greater depths in a tank (Bjerknes, 2007). Gas supersaturation can be avoided by stopping air getting pressurized and succeed into the RAS and by degassing equipment (e.g. trickling filters, air-through towers, counter-current flow of air and water). The biofilter process demands a lot of oxygen, and air is often added in the lower part of the biofilters, a process that might cause supersaturation. Hence, moving bed systems in which circulation is provided by adding air at depth, should also incorporate degassing or be followed by a degasser downstream. RAS are also highly dependent on the use of pumps, which might have leaking sealings. Due to these factors one must be aware of the risk and mitigation for gas supersaturation in RAS farms.

Buffers

The process of converting ammonia to nitrate consumes carbonate and produces carbon dioxide which causes a drop in water pH. The nitrification process has in itself a pH – optimum around 7.0 -7.8 and due to this fact, it is necessary to add a buffer to the system. This is normally done through automatic dose pumps controlled be a feedback loop. There are different buffers available, but not all types offer the wanted effect on the levels of ions and alkalinity (see Table 2).When soft water is used as a source for the make-up water, the choice of buffer can be very important to obtain adequate protection against nitrate and dissolved metals. However, it is not clear what type of buffer to use for the most effective treatment of different water qualities and various suppliers seem to use different ones. This is an area where more knowledge is needed.

Table 2. Overview of the effect of dissolving 1 mol of alternative buffers. Modified after Birnhack et al., 2011.

Dissolved buffer (1 mol)	Common name (English/Norwegian)	Na+ (ekv)	Cl^- (ekv)	C_T (mol)	Alkalinity (ekv)	Ca^{2+} (ekv)
CO_2	Carbon dioxide/ Karbondioksid	0	0	1	0	0
$NaHCO_3$	Sodium bicarbonate/ Natrium bikarbonat (natron)	1	0	1	1	0
Na_2CO_3	Sodium carbonate Natriumkarbonat (vaskesoda)	2	0	1	1	0
$Ca(OH)_2$	Calcium hydroxide/ Kalsiumhydroksid (lesket kalk)	0	0	0	2	2
$CaCl_2$	Calcium chloride/ Kalsiumklorid (kalsiumsalt)	0	2	0	0	2
NaOH	Sodium hydroxide/ Natrium hydroksid (kaustisk soda)	1	0	0	1	0

Pumps

Pumps used in RAS are normally heavy–duty 100 % industrial irrigation types, with a typical capacity of 60 m^3 water/h/kW. They need to operate continuously and are therefore critical to RAS operation. Three-phase power (380 V in Norway) is essential for efficient pump operation and longevity. Pump efficiency is particularly important to reduce the cost of pumping. Pump performance is described as capacity (e.g. L/min), head, power, pump efficiency, suction head, and specific speed (rpm). The cost is directly proportional to the head to which water is pumped (Van Gorder, 1994).

Foam fractionation

Foam fractioning is often incorporated in saltwater RAS to remove fine solids and dissolved organic matter. The process is depended upon the ability to create foam which is easier in salt water than in freshwater. RAS will accumulate dissolved organic material and fine suspended solids, size 5-10 µm. These come from proteins accumulating in the RAS from sources as decomposing feed and faeces, urine, mucous and they are not easily removed by mechanical filtration or sedimentation (Timmons, 1994). The compounds are responsible for turning the water brown or yellow in some RAS-setups. Foam fractioning is a process in which air is mixed with water to form bubbles that concentrate fine suspended solids (< 30 µm) and dissolved organics (surfactants) at the bubble surface. When the bubbles with suspended solids and surfactants rise to water surface and form foam, they can easily be removed from the RAS (Timmons, 1994) and discharged as a concentrated solution in the effluent system.

Monitoring of water quality

Correct monitoring of key water quality parameters (dissolved oxygen, pH/CO_2, TAN, nitrite, total gas pressure and temperature) is essential for a successful operation of a RAS. Therefore, adequate quality assurance of the relevant analytical methods (sensors) must be considered as a prerequisite. The bacteria population and performance in the biofilter are also dependent of these factors. Fish growth and the need of feed depended upon temperature and a stable situation needs to be established in the RAS to prevent overload of uneaten feed resulting in a too high biomass for the filters (mechanical- and biofilters). A strong emphasis on these topics was also made by all four RAS suppliers interviewed (Annex 2).

In RAS, biofilms will develop on all exposed surfaces. In a study by Munro et al. (1996) it was noted that biofilm fouling of pH electrodes may impair the function and disturb the precision of pH measurements. In a recent study, Kolarevic et al. (2011a) tested the precision of several online pH measurement systems towards manual recordings, and also the effect of automated cleaning procedures of the electrodes. The study concludes that automated probe cleaning may be feasible, or alternatively, that pH should be measured with two or more instruments regularly, to improve the precision of pH monitoring in RAS. These considerations are relevant for other instrumentation as well, besides pH probes, but further development of knowledge in this field is requested.

Backup power supply

Power failure can be a catastrophic incidence in RAS, since the response time to critical problems are very short (minutes) due to the dependence of power to run the systems. This situation calls for an obvious need for a back-up power source.

Effects of water renewal and recirculating flow rates

The water renewal rate refers to the relative amount of new water (make-up water) entering the RAS, compared to system volume, or, alternatively compared to amount of feed given. Please refer to page 30 for definitions.

Complementary to the water exchange rate in description of a recirculation system is the magnitude of the recirculating flow, relating to water volume which is recirculated through tanks and filters. A common way to describe the magnitude of the flow is by using the hydraulic retention time of the tank (HRT_{tank}, time before exchange of tank water volume). A low HRT_{tank} means that water bypass time is short, and that water will pass through filters and other water treatment devices more frequently than in a tank with a high HRT_{tank}. The necessary tank HRT can in some cases also be influenced by the unit process removal efficiencies, and the required tank water quality for the fish, such that low treatment efficiencies and a required low concentration of metabolites in the tank, will necessitate that water is passed more often across the treatment devices.

Several studies document that low water exchange rates are potentially associated with the accumulation of particles and metabolic waste, as well as trace metals. In a recent study in rainbow trout, reported by Davidson et al. (2009) and Good et al. (2009), a high water exchange rate of 2.6 % was compared with a low rate of 0.26 %, with corresponding HRTs of 0.67 days and 6.7 days, i.e. a tenfold difference in water exchange rate. Fish performance was not affected, and the differences related to health and welfare parameters were relatively small and not unambiguous. Caudal fin erosion was however clearly more pronounced in the low water exchange regime. Within the low exchange RAS, a range of water quality parameters were affected, but the authors point to TSS (total suspended solids), fine particle content and heterotropic bacteria count as the main parameters of concern. Also, there was significant accumulation of nine metals within the low exchange system, but only copper (Cu) reached a level of concern. Although below predefined acute toxic levels, association to previous unexplained mortalities in similar units was suspected. In a follow-up study, Davidson et al. (2011b) observed a relation between low and near zero exchange rates and some problems of fish health and behaviour. Rainbow trout from low exchange systems displayed a consistently higher swimming speed than controls, as well as a higher incidence of side-swimmers. Under near zero-exchange conditions, mortality was increased compared to controls, and skeletal deformities in the form of axis deviations were observed. Analyses demonstrated a possible correlation between high levels of NO_3-N (>400 mg/L in the most extreme treatment) and potassium in the rearing water. Accumulation of trace metals was observed in three RAS systems differing in exchange rate for Nile tilapia (Martins et al., 2011). Martins et al. also examined the potential accumulation of metals in liver and muscle, but concluded that accumulation in fish was absent or too low to be of risk for consumption. In a related study, Martins et al. (2009) compared the effects of ultrafiltrated waste-water from two different systems, low exchange/high accumulation vs. high exchange/low accumulation, for carp egg and larval rearing. Ultrafiltration in this study removed fine particles and suspended solids as well as microorganisms. Wastewater from the low-exchange system induced increased egg mortality and lower hatching rate, increased larval mortality and decreased larval growth rates. Water quality analyses demonstrated differences in a range of parameters, e.g. pH, conductivity and TAN, as well as minerals, although no specific conclusion was made as to which parameter or parameters were more critical.

Accumulation of hormones and xenobiotica in low exchange systems is a potential effect which so far has been little investigated. Martins et al. (2010) examined feeding behaviour in Nile tilapia (*Oreochromis niloticus*) following exposure to water from stressed fish. The authors concluded that no effects related to cortisol or other alarm cues could be detected, however, without doing any analyses of these substances. It was suggested that signal substances might be degraded, as previously demonstrated in several studies related to sewage treatment (Andersen et al., 2003, Fujii et al., 2003, Fahrback et al., 2008), or alternatively, that they were trapped onto the surface of humic acids and, although still present, thus remained undetectable for fish (Hubbard et al., 2002). In two recent conference presentations, Mota et al. (2011 a, b) demonstrated that steroids (cortisol, testosterone) were indeed removed in low-exchange RAS, in contrast to nitrate which accumulated relative to water exchange. In combination with low pH-exposure, on the other hand, cortisol increased in response to low water exchange, an effect not seen with normal pH. Whether or not the cortisol accumulation was due to reduced removal at low pH, or was mainly caused by increased production due to pH stress, remains unanswered.

It seems indicated that RAS operated at low exchange rates may represent a risk for fish, unless specific measures are taken, such as ozone treatment. The main challenges related to low exchange systems are associated with the accumulation of particles, increased heterotrophic bacteria count and heavy metals in the water. Also, accumulation of NO_3-N will require attention.

The accumulation of substances associated with low exchange RAS systems can be ameliorated through specific treatment of the recirculating flow. In a series of three controlled studies reported by Davidson et al. (2009), the effect of ozonation as a water improvement measure was examined at high (2.6 %), low (0.26 %) or near-zero water exchange rate. In all three studies, ozone contributed to an improved water quality. In particular, there was a consistent reduction in TSS, colour, biochemical oxygen demand and in Cu concentration. Ozone treatment also reduced heterotropic bacteria count, although not significantly. In two of the three studies, ozone contributed to improved fish performance. In a related study, a range of fish health and welfare parameters were examined in low water exchange system (0. 26 %) with or without ozone. (Good et al., 2011a). Survival was good (>98 %) in both RAS treatments, and growth was significantly better in ozonated units. Histopathological examination revealed a significantly higher prevalence of some specific gill and liver lesions in the ozonated RAS compared to non-ozonated, however, all were considered subclinical and of uncertain significance to health. It was concluded that ozonation of the recirculating flow in low exchange RAS restored the water quality to a level comparable to a system with a ten-fold higher flow.

Concerning other key water quality parameters, e.g. NO_2-N, TAN, CO_2, the control depends largely on dimensioning of water treatment systems and the internal flow. Thus, the water quality with regard to the most commonly cited parameters rely primarily on system dimensioning and design, and subsequently on load during operation. An additional key aspect is HRT, which indicates the frequency of passage through water treatment steps. Basic information on system dimensioning and load is available from several sources, e.g. the textbook by Timmons and Ebeling (2007) and a range of scientific publications (e.g. Wolters et al., 2009), as well as data from suppliers of RAS. Data from the construction of the RAS experimental facilities in Nofima Sunndalsøra were summarized by Terjesen et al. (2008). A key input factor into the calculation will always be expected production and the expected maximum carrying capacity of the system. The effects of any treatment step depends to a large extent on the dimensioning of the technical installation, i.e. the bioreactor, gas blowers etc. In practical life, dimensioning the water treatment system is a strong cost-driving factor. Therefore, RAS users strongly request that specific water quality requirements for RAS must be justified by documentation related to fish health and welfare (see page 49). The concern is that too strict limit values may impose restrictions on future development of RAS systems by increasing capital investment costs beyond reasonable limits. In particular, existing limit values for NO_2 were characterized as unrealistic and not justified, and similar considerations were made for several water quality parameters. Recent studies on chronic low grade ammonia exposure, for example, demonstrate absence of any adverse effects at levels exceeding the current limit values (Wood, 2004, Kolarevic et al., submitted manuscript). Thus, a re-evaluation of some of the basic water quality parameters was requested, under conditions that are relevant for RAS.

The accumulation of nitrogenous waste should consequently be prevented by biofiltration, even at low exchange, given that there is a sufficient balance between organic load, dimensioning of filtering capacity and a sufficient recirculating flow (low HRT). Nitrate-N (NO_3-N), however, is not efficiently removed by aerobic biofilters, and NO_3-accumulation is directly proportional to feeding rate and system hydraulic retention time. In contrast to other substances which accumulate in low exchange systems, NO_3 was not reduced by ozone (Davidson et al., 2011a). NO_3-N accumulation was, however, lower than that expected. Thus, some portion of NO_3-N that was produced was subsequently removed and more NO_3-N was removed as feed loading rate increased. The authors suggest that passive denitrification or other NO_3-removal processes occurred at higher NO_3-N concentrations. However, in the case of near zero-exchange systems, a specific NO_3-removal process may be necessary (Davidson et al., 2011b, van Rijn et al., 2006), requiring adaptation of denitrification stage. Denitrification is available technology and is widely used abroad, but is not so common in Norway. Whether or not denitrification will prove to be necessary in Norwegian RAS facilities will depend on future experiences and strategic choices.

A special consideration relates to water exchange rate and the potential of NO_2 accumulation, which is acutely toxic to fish. During biofilter start-up and maturation, fluctuations in water quality are expected. A typical startup curve for N-waste (presented by Timmons and Ebeling, 2007) shows a peak in ammonia concentration after 2 weeks, followed by a peak in nitrite after 4 weeks, whereas nitrate production increases from three weeks and onwards. As nitrate production takes over, ammonia and nitrite reaches a new and low steady state. Therefore, allowing for maturation of the biofilter before adding fish to the system is an important preventive measure, which is generally implemented in commercial production (See section on Norwegian experiences). Similar peaks in nitrite production may also happen during production, due to e.g. sudden appetite loss in fish or any event which causes an increase in organic load beyond the biofilter capacity. As nitrite is toxic, both in acute and sub-chronic exposure (Kroupova et al., 2008), this may cause mortalities if levels are not controlled. Thus, a functional control and contingency plan for events of nitrite accumulation is of great importance in terms of fish safety, as nitrite toxicity can be relatively easily counteracted through supplementation of NaCl (Bartlett and Neumann, 1998; Gutierrez et al., 2011). An obvious solution in such events may seem to be increasing the supply of make-up water, in order to dilute the toxic compounds. Practical experience indicates, however, that this approach may in fact delay the establishment of a new steady-state in the less critical cases (See ‘Norwegian experiences’). It was suggested that a close monitoring of NO_2 levels was preferable, to allow for the biofilter to regain sufficient removal capacity. This approach is supported by a study on RAS production in Chile (Emparanza, 2009) which pointed to variable daily water exchange as one of the main management challenges to the achievement of stable conditions.

In conclusion, the adverse effects of low exchange systems on water quality may be ameliorated through ozone treatment of the recirculating flow, either with or without complementary use of UV. Filtration or other approaches may be developed as alternative treatment principles.

Feed, feed distribution and feeding load

The production capacity of any RAS facility is closely related to the maximum feed load the system can handle. We refer to Timmons and Ebeling (2007) for a general background and practical approach for calculations of the relations between feed load and water quality. This knowledge is also the basis for commercial design and production of RAS systems done on a daily basis by technology suppliers. However, calculations of e.g. CO_2 and TAN load should use as relevant data as possible, such as nitrogen retention obtained from studies on Atlantic salmon (Helland and Grisdale-Helland, 1998; Aas et al., 2006).

When operating within the maximum limits for feed load for any given system, a number of issues of potential influence on water quality can be defined.

Variation in daily feed load and feeding rate

It is strongly recommended to keep the day-to-day variation in feed load relatively constant, as short term fluctuations will represent a challenge to the bioreactor effect. Emparanza (2009) suggests no more than 15 % increase in feed amount between days. A similar restriction was cited by one of the Norwegian producers, which implemented a day-to-day variation in feed load <10 %. The maintenance of a stable feed load is of significance to the stability of a range of water quality parameters of importance to fish health and welfare. It should be noted that any imbalance between feed given and feed consumed by fish may have detrimental effects to water quality. Such considerations apply e.g. to sudden appetite loss due to disease or change in feed type, technical failure of feeding systems or similar events. Conversely, a feeding rate which fails to satiate fish will lead to increased fin erosion (Kolarevic et al., submitted manuscript). Consequently, management of feed distribution requires continuous attention.

Feed composition

In general, feed composition with respect to the main nutrients is fairly standardized for salmonids, across fish sizes, production systems and feed producers. There is, on the other hand, a significant variation in raw materials, and consequently in the bioavailability of the various ingredients. Even so, the magnitude of these variations between the diets currently marketed in Norway is not likely to be of any particular impact for water quality. Development of commercial diet formulations designed for use in RAS systems are currently in progress. Of particular interest for future design of diets tailored for RAS would be a fine-tuning of additives, in view of the potential accumulation of minerals in low-exchange RAS, a significant proportion of Cu is expected to originate from feeds.

Feed pellet technical quality

Water solubility of feed pellet is a trait which may vary in response to process variables during feed manufacturing. A durable pellet is generally recognized as being an advantage, due to lower breakage during transport, handling and feed distribution. A pellet with low water stability given to rainbow trout resulted in separation of oil in the stomach, and is expected to contribute to the "fat belching" which is occasionally observed (Baeverfjord et al., 2006). Variation in pellet physical quality was also proven to have an effect on nutritional value of feeds to rainbow trout, as feed pellet with higher water stability displayed higher bioavailability for main nutrients and minerals (Aas et al., 2011). In RAS, it may be hypothesised that a pellet with low water stability may be particularly unfeasible, in that leakage of nutrients adds to the organic load. Such effects remain to be investigated.

Feed distribution in tanks

With increasing tank sizes in commercial production, adequate distribution of feeds is recognized as a challenge. The high water flow through tanks in RAS adds to the challenge, especially as a quick removal of excess feed is considered essential for water treatment. On the other hand, failure to provide all individuals access to sufficient amounts of feed in time and space is likely to induce feeding aggression, which may lead to fin erosion.

Feeding intervals

Theories regarding optimal feeding regimes for salmonids frequently include opinions about feeding intervals, i.e. meal sizes and meal frequencies. The main contrast is between those in favour of feeding all fish to satiation in few and well defined meals per day, as opposed to distributing the daily feed amounts in numerous small meals. With regards to RAS water quality, the latter regime would be preferable, but controlled studies are not available.

Special case: Smoltification of 0+

In production of 0+ smolts, photomanipulation is the dominant method for induction of smoltification. The procedure involves a six-week period in which the fish is subjected to photomanipulation, usually a 12 h light: 12 h darkness pattern, or similar. Commonly, fish are fed only during light hours, thereby imposing a diurnal variation in organic load, which may or may not be of significance to water quality control. Alternatively, the commercially marketed Supersmolt® method (www.supersmolt.com) is gaining popularity. The method involves no use of light manipulation, but uses feed and water additives to induce smoltification, mainly addition of Ca and Mg to induce development of seawater tolerance comparable to smoltification. These effects may be strongly influenced by the complex water quality of RAS, but so far, no documentation as to whether this method is compatible with RAS or not was presented.

Fish stocking density

Due to the relatively high capital costs of RAS, a high stocking density is more or less implied for profitable production. The tank biomass is of key importance for determination of feeding rate, water exchange rate and the required HRT_{tank}. At a given flowrate and temperature (metabolic rate), the basic factors that governs how much fish that can be put into the system are related to adequate supply of oxygen and the need to remove metabolic wastes from the fish. Excessive fish densities can cause a stress response, for example as a result of behavioural interactions (Wedemeyer, 1997; Pickering, 1998; Turnbull et al., 2005). Also, inappropriate densities were reported to give reduced growth rate, poor health condition and increased mortality (Wedemeyer, 1997; Ellis et al., 2002). Provided good water quality is maintained, North et al. (2006) concluded that it is possible to grow rainbow trout at densities up to 80 kg/m^3 without affecting growth, condition factor or mortality. At higher fish densities, however, higher incidences of fin erosion were observed. In Atlantic salmon, Hosfeld et al. (2009) reported a study in which salmon parr were reared at different stocking densities, up to a maximum of 86 kg/m^3, with continuous adjustment of critical water parameters. The authors underline the importance of maintaining a sufficient food supply at the higher densities, but otherwise, no negative effects were observed. Timmons and Ebeling (2007) presented data on rainbow trout that suggest a relation between fish size and stocking densities in RAS, ranging from 13 kg/m^3 at <1 g size to 110 kg/m^3 at ~500 g size, but underlined that such stocking densities can be used only if the water quality can be maintained at an adequate level. Ellis et al. (2002) reviewed available literature on the relation between stocking density and welfare in rainbow trout, and basically concluded that no specific limit value should be given. It is suggested to base considerations of acceptable stocking density on water quality parameters as well as effects on fish health and welfare.

The maximum allowed rearing density for post-smolts in sea-cages in Norway is presently set at 25 kg/m^3 (“Driftsforskriften”), but densities in closed systems can be much higher. Typical fish densities in Norwegian land-based farms for post-smolt salmon, reported in 1995, ranged from 10 to 100 kg/m^3 (Forsberg, 1995). Thorarensen and Farrell (2011) concluded after reviewing literature on fish density (range: 10 - 125 kg/m^3) that it appeared to be no consistent effect on the growth, survival and welfare of Atlantic salmon post-smolts in closed-contained systems up to a fish density of about 80 kg/m^3. In RAS the maximum carrying capacity of rainbow trout has been suggested to be 100 kg/m^3 at 12 ^{o}C (Roque d’Orbcastel et al., 2009b). Currently, there is little rearing of post-smolts or large rainbow trout (>150g) in land-based facilities in Norway, but this a situation which may change rapidly.

Several studies address fish stocking densities in sea bass, *Dicentrarchus labrax*. The effect of stocking densities between 10 kg/m^3 and 100 kg/m^3 was studied in a flow-through system and a RAS system in parallel, reported by Roque d’Orbcastel et al. (2010) and Sammouth et al. (2009), respectively. Some minor differences in response were observed, but in conclusion, fish reared at 70 kg/m^3 and lower performed better than those reared at 100 kg/m^3.

Thus it is indicated that there is a limit to fish stocking density in freshwater salmonids, mainly as a function of the systems carrying capacity for organic load and the resulting water quality. A relation between fish sizes and maximum stocking densities is also indicated, with small fish tolerating high stocking densities less well than larger fish. In addition, an upper limit of ~80 kg/m^3 seems indicated, based on fish performance, although further studies in this field is strongly indicated, in particular for Atlantic salmon.

Control of tank water speed

Due to the high turnover of water in tanks with low HRT_{tank}, the water current may be strong, if not controlled. To some extent, the water speed is controlled by the amount of flow, but additional control measures can be used to reduce the speed, i.e. construction of inlet and outlet. There is a certain amount of information available which substantiates that a swimming speed of 1.2-1.5 body lengths (BL) sec^{-1} is beneficial for Atlantic salmon, compared to higher and lower swimming speeds. In an older study, moderately high current speed provided exercise and gave positive effects on performance and growth, with suggested flow rates of 0.75 – 1.5 body lengths/sec (Jobling et al., 1993). Training, such as can be imposed by changing water velocity, has been studied in flow-through systems, demonstrating positive effects on growth, stress tolerance, circulatory capacity, skeletal quality and disease resistance in Atlantic salmon (Davison, 1997; Castro et al., 2011; Totland et al., 2011). Reports on effects of water velocity in RAS for Atlantic salmon, however, is to our knowledge lacking. Little evidence exists to what happens if swimming speed is higher than optimal long term, but it can be hypothesized that a chronic stress or fatigue may result if fish are forced to swim at extreme speeds for a longer period of time.

Tank hydrodynamics is also a complex feature, which has received little attention in recent years. Actual swimming speed for any fish will be strongly influenced not only by the speed of the water current, by also by position within tank. Also, presence of fish will modulate the dynamics of the water current, an effect which should be studied more extensively. At the same time, a certain speed of the water flow is necessary to efficiently remove any solids and enable self-cleansing of the tanks. The managers which were interviewed during the preparation of this document (see page 49) were uniformly in favour of maintaining a relatively high water speed in tanks, and none had reached levels of fish swimming speeds where fish lost the ability to stand against the current.

More information seems warranted as to whether or not water speed needs to be controlled specifically, and what the limits are.

Practical experiences with RAS

Published operational experiences of salmonid farms using RAS

Summerfelt et al. (2009b) concluded that partial reuse systems are an effective alternative to traditional single-pass systems for Atlantic salmon smolt production. High-quality smolts were produced, excellent water quality was maintained, and no disease outbreaks occurred. Diel variations in water quality, along with technical and biological experiences from a farm using RAS for producing Artic charr (*Salvelinus alpinus* L.), are reported by Skybakmoen et al. (2009). This also includes a description of a period with very high mortalities due to *Saprolegnia* fungal infection. In study on rainbow trout, water quality remained acceptable, although the nitrite concentration was a borderline case at 0.15 ± 0.07 mg/L (around the recommended threshold value). Moreover, chronic nitrogen supersaturation (105 %) occurred due to the depth of air injection. Nevertheless, no apparent effects on fish performance and no pathologies were observed, not even at the extreme temperatures (9 and 23 ^{o}C) (Roque d'Orbcastel et al., 2009a). A hatchery growing brook trout (*Salvelinus fontinalis*) in RAS was studied by Fischer et al. (2009). All water quality parameters were within the acceptable ranges for this species, and the fish remained healthy with no disease incidences occurring during the entire rearing period. However, some fin erosion was evident towards the end of the study. Jørgensen et al. (2009) did a 22 month study of parasites in eight Danish RAS farms culturing rainbow trout. Various types of parasites were discovered in all farms. Notably, the cause of the problem was related to the transfer of infected fish from traditional earth ponds into the recirculated systems.

The water quality in a commercial RAS farm in Norway, over 14 months is described in detail by Fjellheim (2009). We refer to the article for overview of the system criteria. The author reports that the fish health was considered apparently good, though not investigated in detail. One episode with potential mortal nitrite toxicity (peak around 2 mg/L) occurred, but mortality was avoided by adding 300 kg sea salt to the system. The biofilter efficiency was limited by pH and alkalinity, and the breakdown of the buffer dosing pump caused the nitrite episode. The water quality was kept within the recommended levels for TAN (2 mg/L) and CO_2 (15 mg/L), but 50 % of the nitrite measures were above the recommended level (0.1 mg/L). The alkalinity obtained in the system by adding sodium bicarbonate was reported to be around 20 – 40 mg/L $CaCO_3$), thus lower than the lowest recommended levels for alkalinity in biofilters (45 mg/L $CaCO_3$, in Biesterfield et al. (2003)). It is also interesting to note that this system obtained a removal degree between 60 – 97 % of the suspended solids, after mechanical filtration.

Practical unpublished experiences

There is a lack of data of water quality in operating RAS. However two sets of analytical data[5] from three RAS farms[6] producing Atlantic salmon was obtained. They were found relevant for the risk analysis performed by the Panel. We do not have any detailed description about the technical solutions and capacities used in these recirculation systems, and the data reflects a snapshot of a day in the farms. The data shown in Appendix 2, indicates higher than recommended levels of CO_2 and NO_2 (Experience I), the use up of alkalinity in the biofilters and conversion of TAN to nitrate (Experience II). This is in accordance with data presented by Gutierrez et al. (2011).

Norwegian experiences

During the preparation of this assessment, it was considered important to collect relevant practical experiences from use of RAS in Norwegian aquaculture production. Three persons with wide experience on production manager level were invited to committee meetings, to present status and experiences from their respective companies. In addition to this, a telephone survey was done.

A selection of six persons on site manager and production manager level were interviewed. A set of predefined issues were discussed, based on pre-existing knowledge and previous

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[5] Analysed by Norwegian Institute of Water Research

[6] One Norwegian and two Australian

discussions in the VKM committee. The interviews were done by phone, following a general outline but at the same time inviting personal views and comments.

The six persons represented five different companies, with experience from management and construction of 9 production sites and an additional 3 sites currently under development. The companies in question were both independent smolt producers and large integrated companies. The sites which were represented were a mixture of retrofitted flow-through systems and operations built as RAS from scratch. The oldest sites had been in production for more than ten years, whereas the newest were in production from 2008. The sites were all of a medium-sized range, with smolt production capacity of about 2 million fish per year, except one retrofit, in which the main production was broodfish and eggs, with a side production of < 1 million smolts per year.

In summary, the information given by the persons was highly diverse. The one thing they all underlined was that their smolts performed well in the sea during on-growing, with low mortalities and good growth rates. The smolts from RAS performed equal to or better than comparable fish from flow-through systems, and in particular, the fish displayed a more stable performance result than in flow-through systems.

Water exchange rate

When asked to quantify water exchange rates in their systems, none of the persons immediately wanted to identify a daily exchange rate given as a percentage of makeup water compared to total volume. The numbers given were related to daily feed ration, as litre of make-up water per kg feed per day. The parameter generally used to adjust water renewal rate was nitrate levels in outlet water, with critical values given between 100 and 150 mg/L, but with use of personal judgement in addition. Production capacities were specified as kg feed per day, and several producers commented that a common situation was to feed at 60-70 % of the theoretical total capacity. The reason for not reaching 100 % of theoretical capacity was primarily related to challenges of fish logistics, in particular catering for many fish group of different sizes and at different stages simultaneously. Accumulation of substances other than N-waste, e.g. heavy metals, was generally not analysed. When asked, some of the managers estimated the daily water exchange rates to be 2-3 %. The exception was one of the oldest retrofits, in which the system design and biofilter function itself was considered suboptimal, and water exchange rates estimated to be 10-15 % were considered necessary to achieve an acceptable water quality. The newest site in the survey was fitted with a denitrification filter and a phosphorus (P) removal treatment, thus allowing for a near zero exchange system in theory. It was, however, not considered a point to run the system so tight, and in practical use, water exchange rates >1 % was used. At this site accumulation of substances other than N-waste had been analysed, demonstrating removal of Cu, Al, Fe and TOC during denitrification and P removal, to reach levels lower than those in make-up water. Several of respondents cited plans to add denitrification filters in the future, either in existing systems or in new constructions.

Tank hydraulic retention time (HRT)

The magnitude of the recirculating flow, given as HRT-tank hydraulic retention time, was variable, with values cited between 15-20 min and 60 min, or not being able to estimate (one site). The lowest HRT was related to the period of maximum biomass just prior to smolt transfer. HRT was limited by technical installations at several sites, with tank outlets and pipes restricting the maximum flow, and consequently preventing use of a lower HRT. The typical HRT cited was 40-45 min.

Disease problems and management

Parasites

Parasites were seen occasionally at some of the sites, but none had experienced particular difficulties related to that. At one site, *Ichtyobodo* sp. (*Costia*) infections occasionally needed treatment with formalin, which was done according to standard procedures and with good efficiency. It was noted by several that parasites like *Costia* could always be found in the system if you looked for it, but without causing disease. The parasites would e.g. be found on moribund fish, but otherwise not. It was also referred to several cases where fish groups with *Costia* problems were transferred to RAS, and subsequently the clinical manifestation of the *Costia i*nfection disappeared. The conception in these cases seemed to be that the parasites were ‘engulfed’ by the microbial flora present in RAS and were not able to gain strength, or alternatively, that the increased water flow in RAS enabled the fish to withstand parasite infestation. No further documentation of such effects seems to exist.

Fungi

Most sites had occasional cases of fungus problems, but none reported to have a major or recurrent problem. Fungus was treated by different means, commonly formalin or Pyceze, or with salt. Preventive measures included a constant addition of up to 2 ppm seawater, which had proven to be effective. A tight pH control was also maintained as an important factor.

Bacteria

Only one site reported a significant bacteriological problem in the past, which was a persistent *Yersinia* infection. Attempts to combat the infection by a total separation and disinfection between year classes did not succeed; the infection persisted and analyses demonstrated that it was the same “in house-strain” of the bacteria. The strategy was changed towards implementation of a two-step vaccination program, as well as increased daily hygiene measures. The problem is currently under control on a non-significant level.

Previous problems with *Flavobacteria* were reported from one other site. The problems were brought under control by seeking advice from international scientific experts on *Flavobacteria*, and by increasing the hygienic standard. Other than that, no specific problems were noted related to bacterial pathogens.

Virus

Occasional Infectious Pancreatic Necrosis (IPN) outbreaks had been seen in most of the sites. In general, emerging outbreaks were culled by applying heat. The practice of increasing temperatures to 18-20 °C and beyond for some days seems to be widely in use, and was reported to be very effective.

Again, several examples were given in which IPN-affected fish groups were transferred from flow-through to RAS, after which the clinical disease died down. No explanation of this effect could be given, other than a general concept that the virus was "dried out" in the biofilter, and that the stable water environment and ample amounts of water enabled the fish to suppress the infection.

In general, the importance of implementing and maintaining a high hygienic standard was strongly underlined as a preventive measure against infectious disease.

Fin erosion and short operculae

The producers were challenged with a statement that fin erosion is a frequent problem with fish in RAS. In all cases, the reaction was to deny this statement. If anything, fin condition was considered better in RAS fish than in comparable groups in flow-through systems. None of the respondents were able to identify any particular risk factors for fin erosion in RAS. On the contrary, it was noted that fish with fin erosion problems introduced to RAS from flow-through seemed to heal unexpectedly fast.

The causes for fin erosion were discussed, and there is a general agreement that fin erosion problems are mainly related to feeding issues, that is underfeeding, inadequate distribution of feed in tanks or feeding system malfunction. One producer had seen an episode with fin erosion in RAS following a failure of the feeding system. An additional note was made that fin erosion may happen if fish develop territorial behaviour, e.g. in tanks with few individuals, or if fish are generally stressed for some reason.

Short operculae was mentioned as a problem that had been observed sporadically, without any further information to risk factors of relevance to RAS.

Medical and chemical treatment

In combination, the sites reported a range of medical and chemical treatments having been employed at one time or other, most commonly formalin treatment. No specific challenges had been noted, and none reported effects on biofilter integrity and function. The only precautionary note which was made was that formalin treatment needs to start carefully, and with close monitoring of system parameters.

Disinfection of water

Disinfection of make-up water

All sites had systems for disinfection of make-up water with UV and ozone. One site referred to the choice of RAS because of persistent problems with *Yersinia i*n the water source, and that the limited amount of make-up water necessary in RAS allowed for a control of this problem through a tight disinfection of new water entering the system.

Disinfection of the recirculating flow

Use of ozone and/or UV to treat the recirculating flow was highly variable. Some sites had no disinfection at this step; others had UV treatment, some ozone and UV. None of those who had disinfection at this stage treated the whole flow, only a partial flow. Use of ozone was clearly something that was associated with strong opinions. Among those who did not use it, it was clear that some considered the benefit to be mainly cosmetic, in providing a clearer tank water with less colour but with no particular other benefit. Safety issues were also mentioned, not for fish but for personnel. One manager cited ozone as a bad excuse for not optimizing function of drains and filters. In two of the most recently built systems, ozonation was installed and used with success. Here, ozone treatment was used both as a means to clear the water and to control heterotropic bacteria count. In other plants, installation of ozone systems was under consideration, both in existing and in new systems. More knowledge on the beneficial effects of ozone treatment, and also on how to use it optimally, was requested. Based on the interviews it was not possible to conclude on any specific impact on the fish either from using or not using ozone to treat the recirculating flow.

System cleaning and disinfection

There were two main strategies chosen for system disinfection, including cleaning and disinfection of the biofilters. One was to implement a yearly total shutdown of the system between year classes, with disinfection of the complete system. The second was to do a thorough cleaning and disinfection of tanks and associated structures between fish groups, but to leave the biofilter unit running. With the second strategy, it was noted that in case of acute infectious disease, a shutdown and disinfection would be done. All producers, irrespective of strategy chosen, underlined that a total biofilter shutdown requires a long restart period. A minimum of 4-5 weeks before adding fish to the system was suggested, but with the additional comment that it takes several additional months to establish optimal function. Those who leave the biofilter undisturbed were strongly opposed to the idea of regulations that require a yearly biofilter shutdown without further indication, as this would impose restrictions on their production plans, and would increase production costs beyond acceptable levels.

One of the sites that implemented yearly disinfection of biofilters referred to recurrent problems with *Yersinia.* The yearly biofilter and total system disinfection failed to remove the problems, despite extensive efforts. Control over the problems was regained through other preventive measures, i.e. vaccination and optimizing general hygienic conditions.

None of those who refrained from yearly disinfection had experienced any problems related to parasites, fungus or other specific pathogens that could be related to biofilter microbiological dynamics. At one site, an acute IPN outbreak, introduced with fish, had led to a total system disinfection, after which the problem was resolved.

Water quality control and management

Routines and available equipment for water quality control varied widely among the sites, but all had routines for monitoring of the most important parameters, including N-waste metabolites and CO_2. Some sites have extensive online monitoring supplied by regular sampling for laboratory analyses. Others had a more flexible approach, with daily measurements when starting a new production cycle, and weekly or bi-weekly analysis of some parameters once the system was stabilized. Others again maintained the importance of regularity in analyses of some key parameters.

CO_2 was regularly measured by all, as a minimum on a daily basis. The levels cited were variable. Some were well below the level of 15 mg/L which is specified by the authorities. Others were regularly at levels of 20 mg/L and beyond, some reaching levels >30 mg/L at maximum biomass. Some of the producers had strong opinions about the specified limits for CO_2, and maintained that they were unrealistic and unnecessarily low for RAS. It was suggested that CO_2 tolerance was higher as a result of the high alkalinity in RAS water. Also, use of $NaHCO_3$ to regulate pH would add to water CO_2, allegedly without affecting the fish. More research on this, done under conditions that are relevant for RAS, was requested.

TAN and NO_2 levels were measured regularly by all producers. Several comments were given about the maximum values for these parameters specified in “Driftsforskriften”. A limit of 0.1 mg/L NO_2 was considered unrealistic, and also not justified in terms of observations of fish welfare. Values cited were in the range of 0.5-3 mg/L during stable production. Many of the sites add salt on a regular basis, which will counteract the harmful effects of NO_2, and all sites had salt in stock for use if NO_2 levels became critically high. One of the sites referred to an episode of NO_2 toxicity. The episode took place during an emerging IPN outbreak, in combination with a change in feed, which in combination led to a sudden appetite loss in the fish and a subsequent organic overload of the biofilter. Once the diagnosis was made, the symptoms were relieved by addition of salt, but unfortunately with significant losses of fish. In general, however, NO_2 control was not considered as a problem per se, with the exception that the 0.1 mg/L limit is too low to be operational in RAS, and also not justified.

NO_3 was used as the main parameter for control of make-up water supplementation for most sites. Typical values were given as 100-150 mg/L, but with different approaches and action rules between companies and sites. NO_3 was measured regularly at all sites, at most sites daily or several times per week. Increasing NO_3 levels was counteracted with adding more make-up water, as this was considered the main operational indicator of unwanted accumulation of waste in the system.

Alkalinity was mentioned as a key factor related to the general water quality of RAS water, and in particular for the effects of CO_2. It was stated that CO_2 is less harmful at high alkalinities, and that some of the research done on CO_2 was just not relevant to RAS.

The key issue mentioned by all was to create a stable water quality. Feeding regime was mentioned as an important factor, likewise to avoid sudden increases in feeding rate but instead increase gradually, e.g. with no more than 10 % per day. Related to the TAN-NO_2 issue, it was also maintained strongly by several producers that fluctuations in these parameters should preferably not be counteracted by adding more make-up water. Such a strategy might easily delay the development towards a steady state.

More research on the specific water quality of RAS was requested, particularly aimed at defining specific limits for RAS for some of the key parameters. Additionally, it was noted that studies on the benefits of RAS water should be initiated, as the effects on fish health and performance seemed positive and reproducible.

pH control

Operating pH was variable between sites, from a low of pH 6.0 to a high of pH 7.3. The reason for the lowest set point was the cost of adjusting pH to a higher level than this. A set point of 6.5 at a different location was chosen as a workable compromise between fish optimum and biofilter function, according to in house-experience. Working pH levels of 7.0-7.3 were most common, with reference to optimal operation conditions for the biofilters.

The media used to control pH were diverse ($NaHCO_3$, hydrated lime, NaOH, lime slurry). It was noted good experience with hydrated lime in terms of water quality, but also that $Ca(OH)_2$ (calcium hydroxide) may be difficult to dissolve sufficiently in water, and that it may sediment in the outlet system if pipe design and flow is less than optimal. The use of $NaHCO_3$, which was the most common substance, was expected to contribute towards CO_2 in water. Some added 1-2 % seawater routinely, and one of the effects being an increased buffering capacity.

The importance of maintaining a stable pH as a key to stable conditions was strongly underlined by several of the respondents.

Gas supersaturation

Instrumentation for measurements of total gas pressure was available at all sites, but not all measured this parameter regularly. Transient episodes with N_2 supersaturation had been experienced at several sites, at one site resulting in massive mortalities. In all the cases referred to, the problems were solved by removing leakages, improving technical layout of water treatment devices and installing extra gas blowing capacities. Additional to this, several sites noted that they regularly measured a TGP which indicated a low grade N_2 supersaturation, without ever seeing any problems that could be related to these levels. It was questioned whether any special conditions related to $N_2/O_2/CO_2$ in RAS could explain these measurements, the question being whether this was not a ‘true’ N_2 supersaturation. More knowledge on the behaviour of gases in RAS was requested.

Swimming speed

None of the sites did regular measurements of water speed in tanks, and when asked, responded that such measurements were not considered necessary. In general, high water speed, and consequently high swimming speed, was considered beneficial for the fish. It was noted that presence of fish will modulate the hydrodynamic pattern in tanks anyway. The main parameter used to control fish swimming speed was behaviour, i.e. that fish were able to control positions.

Routines related to vaccination and other handling procedures

Between the sites, there were a range of different procedures in use related to vaccination and sorting and other handling procedures. Generally, the sites used the regular RAS water for all procedures without any specific precautions. One site vaccinated RAS fish in flow-through water due to practical reasons and returned to RAS afterwards. One site transferred 0+ smolts to flow-through following vaccination and kept them there until seawater transfer. No specific observations were noted related to fish health or welfare associated with the different procedures.

Technical installation as a risk factor

The biggest difference in fish welfare risk between flowthrough and RAS was identified by several managers to be failure of technical installations, the consequences of which may be more acute and more severe than in flowthrough. This applies in particular to systems which are retrofitted, and where technology is a mix of old and new. It was noted that any new system should be designed with double installation of critical technical devices, like alarm systems, emergency power and pump capacity, to maintain fish safety in case of a technical breakdown.

Training and technology transfer

The managers which were interviewed were not asked about educational background, but all had a background from smolt or other aquaculture production in flowthrough-systems. They all stated that the main source of training and transfer of operational knowledge was in house training, as well as trial and error. None of them referred to public education or technology suppliers as a significant source of knowledge. It was clear that there has been, and still is, a relatively open dialogue among the RAS producers, and that this was considered as invaluable, especially to the independent producers. Some also noted the value of international contacts, including visits to commercial sites as well as scientific groups abroad, with reference to the fact that RAS technology has a much stronger and successful history in other countries. There was a general request for a stronger contact between producers nationally, e.g. in the form of seminars or workshops, and a further development of research activities related to RAS.

General comments

During the interviews, some general comments were made. One of them related to the fact that several of the systems which are referred to in this survey are more than ten years old, and in the years between there has been a technological revolution. Thus, future RAS are likely to function better and provide better environments for the fish. There was a general optimism on behalf of RAS as a water management system in smolt production, and one producer made a guess that ten years from now, half of the smolts will be from RAS.

Faroese experiences

The following information was provided to the group by Peter Østergård.

In the Faroe Islands, there is a temperated coastal climate with a lot of precipitation. It rains on an average 300 days a year; around 100 of these days with more than 10 mm of precipitation.

Almost all freshwater sources are surface water. There is a huge number of smaller and bigger streams, but all with a waterflow strongly influenced by the amount of rain. Although it rains a lot, dry periods occur and most often in May-June. Due to this, there were in the early years of fish farming, a lot of small fish farms with limited production capacity. Originally, they produced mainly a 1-S with smolting period in late April and May. Critical situations happened frequently, and the quality of the smolts varied according to this.

In the early nineties production of 0-S began and soon this was a very important part of the production with around 50 % of the total number of salmon smolts put to sea.

In addition, different systems with aeration and also particle filtration and UV-treatment of outlet water for directly re-use, became more common. In 1994, the first farms installed biofilters, and in 2000, 14 of 18 landbased smoltfarms were operated with biofilters and a more or less intensive recirculation system. Soon, the total production of smolts was based on some kind of recirculation systems.

Smolt production capacity was the limiting factor for an increase in seafarming production, and recirculation systems was the only way to an increased numbers of smolts. The early development and implementation of RAS-technology was not originally introduced in order to produce quality smolts, but merely higher numbers. The technology has undergone continuously development, and is now considered by both fish veterinarians and fish farmers, as a safe and good way to produce high-quality smolts with very good survival and growth when transferred to seawater. The number of smoltfarms has declined and today, only eight farms are in operation, but with a higher production capacity, both in total numbers and biomass.

Initially, there were many knowledge gaps, and it was difficult to find consultants with satisfactory experience and skill. Many problems were solved locally in the single farm by trial and error. Smolts from the first period with recirculation, had neither good survial nor good growth when put to sea. Too high levels of CO_2 was considered to be the main reason for this Better systems for degassing were constructed and more intensive surveillance and knowledge on different chemical and physical water parameters were implemented. With a better understanding and control, the quality of the smolts, measured as survival and growth after seawater transfer, has improved substantially.

Many of the fish farmers still rely on knowledge gained through experience. However, the need for more in-depth knowledge of water chemistry and measurements is realized by the freshwater farmers. Furthermore, some of the farms have now started a more thoroughly monitoring of the water quality, providing a better knowledge of key water quality parameters in both inlet water and production water.

Under normal circumstances, water quality is maintained very well, and the problems noted during veterinary inspections in the recirculation farms, seems to be related to farming practices rather than to type of water supply.

Warm summers and days with a lot of sunshine could pose a risk to RAS-farms, unless the buildings are well isolated. Even in the faroes, there have been situations, where water temperature in a RAS-farm did rise to critical levels. High temperature on make-up water and high room temperature in the production facilities and especially in the aerator area just under the roof, made it impossible to reduce the water temperature. Rapid installation of cooling devices solved the acute problems. A more permanent solution to the problem has been proper insolation of the buildings. This is also very important during winter periods with outdoor temperatures below desired water temperature in the production.

A flow-through farm is very dependent on the water temperature in the inlet-water.When this is too high, serious problems may arise.Cooling down the water will be very difficult and very expensive. In the RAS-farm, availability of even a small amount of well-water can bring down the production water temperature. It is also possible only to take in make-up water during the night, where water temperatures are lowest. Under sutch circumstances a RAS-system gives better security than a flow-through system.

The smoltification is a key point for good survival and performance at sea-transfer. It has been speculated whether big deep tanks with water with low transmission of light, might be a reason for variations in the smoltification process within a group of fish. It is a practical experience that different sizes of fish are located at specific parts of the tank, which also might influence the smoltification. Fishfarmers have reported that use of submerged lights seems to have improved the smolt quality. Also size of the fish and variations within a group, might influence the results after transfer to seawater. Is the fish a real smolt or is it just big enough to survive?

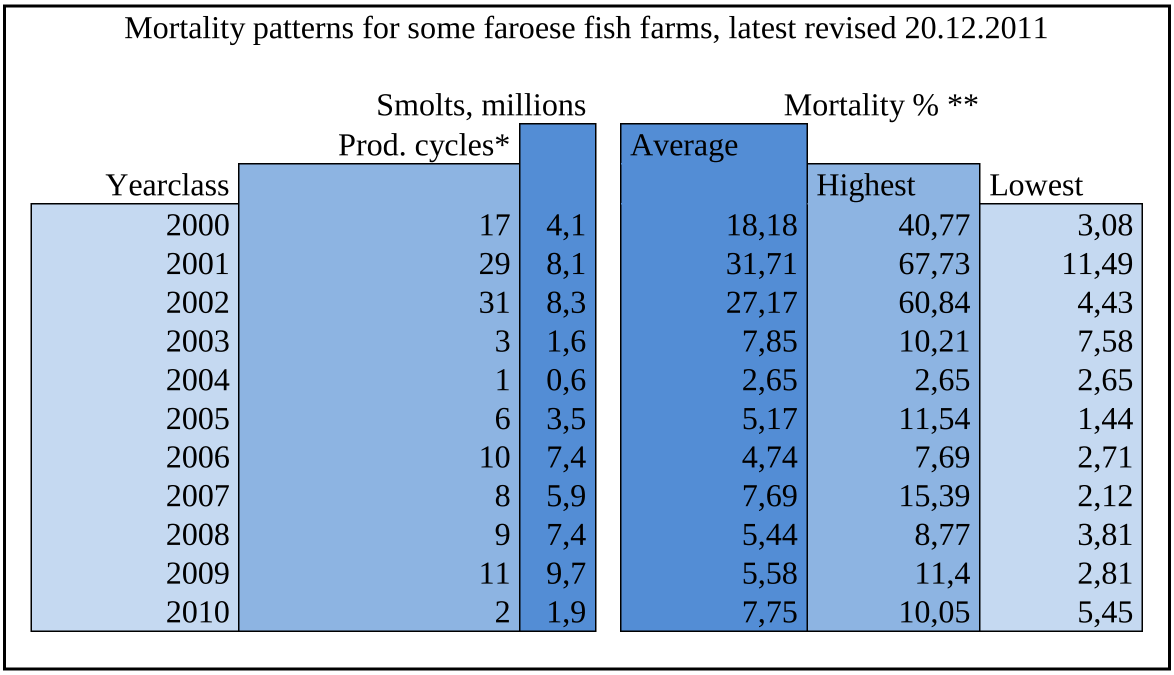
Producing smolts with fewer damages on fins is important. This seems to be very closely related to feeding and feeding systems, and to a certain degree, also dependant on stocking densities etc. Furthermore feeding the fish in a RAS-system is, with comparabel conditions, easier to control due to the much more stable waterquality and temperature. Fish with all fins in good shape, will perform better and tend to have less problems with secondary infections through damaged tissue. Furthermore and maybe even more important; at and after release to sea, the fish will have a better ability for manouvering during transport and in periods with bad weather conditions This might enable the fish to avoid contact with the netting, reducing of the change for developing classical winter ulcers or ulcerations of mouth and tail in connection with transfer to seawater, and also later in the production cycle.

The handling of infections and diseases in RAS is different from flowthrough farms. Experiences from the Faroe Islands indicate that diseases are easier to avoid getting into the farm but – maybe – a little more difficult to treat when first introduced. Furthermore, the amounts of inlet water in RAS are lower and a more thorough treatment can be applied at less costs. Fine mechanical filters followed by UV-radiation or ozone treatment are belived to provide higher security against introduction of pathogens via the water. In RAS, the nature of the biofilter must be taken into consideration if any kind of treatment of the fish is necessary. Use of antibiotics or chemicals for treatment of infections might kill or reduce the capacity of the bacterias in the biofilter.

Use of ozone for disinfection of water after the biofilters before reuse is considered. There is a lack of pratical experience with ozone treatment, but it is believed to be beneficial by reducing numbers of fungal spores and potentially harmful bacteria. In addition, the ozone-treatment might also improve the general water quality by reducing amounts of suspended solids. Several farms are installing or planning installation of ozone treatment in the near future.

The fish health authorities have introduced mandatory fallowing and disinfection of all freshwater farms between year classes and productions/batches. This will require a total kill of all the organisms in the biofilters. The restart of the biofilter is demanding and time consuming and represent a critical time periode concerning fish welfare. New methods for easy upstart of biofilters with i.e. starter cultures, the above mentioned ozone treatment of the recirculated water after the biofiltration or production units designed to meet the disinfection schemes, might solve these problems. There is also an ongoing discussion, whether ozone treatment of the water flow between the biofilters and the production units, can replace or postpone this disinfection of the biofilters.

Over all, the introduction of RAS in the Faroe Islands seems to have had a positive impact on fish welfare and smolt. Total losses in faroes salmon farming are low from time put at sea till slaughter. Average losses in the period 2000-2010 for some of the faroese farms are shown in the figure below, and mortality percents include all mortalities from day one untill slaughter. With an average loss of around 5-6% a year, the smolt quality from the RAS-farms seems to be quite good, but even better control on waterquality and smoltification will definately reduce these numbers further.



Mortality patterns for some faroese fish farms, latest revised 20.12.2011

		Smolts, millions	Mortality % **		
Yearclass	Prod. cycles*		Average	Highest	Lowest
2000	17	4,1	18,18	40,77	3,08
2001	29	8,1	31,71	67,73	11,49
2002	31	8,3	27,17	60,84	4,43
2003	3	1,6	7,85	10,21	7,58
2004	1	0,6	2,65	2,65	2,65
2005	6	3,5	5,17	11,54	1,44
2006	10	7,4	4,74	7,69	2,71
2007	8	5,9	7,69	15,39	2,12
2008	9	7,4	5,44	8,77	3,81
2009	11	9,7	5,58	11,4	2,81
2010	2	1,9	7,75	10,05	5,45

*Before "all-in/all-out" production became demanded by DO 131 23.12.2003, a production cycle could be all from one single pen to a group of pens released at the same time.

**These percent values are based on numbers of dead divided with number of smolts put at sea.

Source, Runi Dam, Avrik, runi@avrik.fo

Diseases in Faroese salmon farming

Several diseases have caused problems for the salmon farming industry in the Faroe Islands during the years. These are summerized in Table 3.

Previously, main disease problems have been IPN, furunculosis and BKD. These diseases were introduced at a time when smolts were produced in traditional single pass flow-through systems. Furunculosis was only a severe problem for a few years, as the introduction of the disease happened shortly before effective oil-adjuvanted vaccines became commercially available.

When BKD first occurenced, there were several producers of broodfish in the Faroe Islands. BKD was believed to be a covert infection in some of these farms and a program for testing of broodfish was introduced. This program was based on screening of individual broodfish and only allowing use of test-negative fish in the production. As a reaction to the importation of IPN-virus, an import ban on salmonid eggs was introduced in 1986 and only salmonid eggs from the Faroe Islands were available. From 1992, all broodfish were treated with antibiotics prior to spawning and testing for BKD.

Infections or disease signs due to BKD have not been recorded in all freshwater sites, but some of the RAS-farms did have problems with the infection. Today the disease and the infection has disappeared in both freshwater and seawater sites. Since 2005 it has only been diagnosed in one single seafarm in a very limited number of fish of icelandic origin close to slaughtering (2007 HFS).

Since the first diagnosis of IPN-virus in 1986, this disease has mainly been a problem in freshwater sites. In the Faroe Islands, this disease is primarily seen in its traditionally form, affecting frys shortly after first feeding. IPN is common in RAS and is handled in different ways. It is believed – and also shown in practice - that an elevation of water temperature can shorten the outbreak and even lead to a reduced total mortality. In addition, some farmers have experienced that a shortened outbreak seems to reduce the number of loosers after transfer to seawater. It can be speculated whether the numbers of chronically diseased fish are lower in a short outbreak compared to a prolonged period with disease. Other farms handle IPN-outbreak by changing from recirculation into a flow-through regime in the diseased tanks. These routines normally results in a reduction of water temperature, but is believed beneficial by reducing numbers of pathogens in the recirculation system.

Table 3. Diseases diagnosed in salmonid aquaculture facilities in the Faroe Islands

Type of agent / cause	**Disease**	**Year of occurrence**
VIRAL	Infectious pancreatic necrosis (IPN)	1986
	Cardiomyopathy syndrome (CMS)	1992
	Infectious salmon anaemia (ISA)	2000
BACTERIAL	Cold water vibriosis	1986
	Bacterial kidney disease (BKD)	1990
	Atypical furunculosis	1990
	Furunculosis	1991
	Yerisiniosis	2005
FUNGAL	Infections with *Saprolegnia* sp	
PARASITIC	Inf. with *Ichthyobodo* sp. (Costia)	
	Infections with *Trichodina* spp	
	Infestations with salmon lice	
	Infections with tape worms	
Production related diseases	Fin rot	
	Bacterial gill disease	
	Eye problems	

Litterature used:

Alitíðindi nr 3. 2000, Andrias Reinert, Fiskaaling, www.fiskaaling.fo

Alitíðindi nr 1. 2005, Andrias Reinert, Fiskaaling, www.fiskaaling.fo

Competence and training

Operation of recirculation systems is complicated, compared to flowthrough systems, and require a certain level of knowledge and experience. In Norway, education aimed at the aquaculture industry is offered by a number of colleges, high schools and universities.

On the college level, all counties along the coast from Rogaland to Finnmark (except from Sogn and Fjordane) offer basic fisheries and aquaculture education (see Annex 1). There is however no particular focus on RAS. One college (in Kyrksæterøra) is at present working on a plan for a special aquaculture course (Pers. com. Klemet Steen, Lerøy Midnor AS), including RAS. University colleges in Bergen and Ålesund offer courses in Aquaculture. The universities in Ås, Bergen, Trondheim and Bodø offer education on bachelor, master and doctorate levels within aquaculture related fields.

Among the educational establishments in Norway, for the time being only the University of Trondheim (NTNU) can offer a dedicated course in recirculation technology (arranged for the first time in 2011). The intended audience for the course is employees of the aquaculture industry. The contents of the course include: the structure of RAS, water chemistry, microbiology, water treatment principles (biofilter, disinfection, removal of particulate matter), hydraulics, flow and calculations based on plant type and size, and new technology for recycling.

It may be questioned which level of competence that is required to ensure a correct operation and management of recirculation systems. It seems however clear that most of the personnel working in the aquaculture industry do not have an educational background that gives sufficient knowledge and expertise to operate such systems. Additional training is therefore considered necessary. Additionally, training courses are offered by suppliers of RAS equipment to the industry, as well as private research foundations as NIVA. According to information given to us by suppliers of RAS in Norway most of them seem to offer some sort of training in technical operation of their RAS equipment in accordance with the delivery of the technology. There is however - to our understanding - no standard training program or testing of knowledge amongst these, and this training is not well pointed out by the users during our survey (see page 94). Neither is there any third party evaluation or approval of these industry-based training programs. There seems also to be quite a lot of internal training in the major aquaculture companies. This is made possible by employment of some of the very few people with background suitable for internal development of procedures and management in RAS. We also know that NIVA has carried out a few training programs in water quality in RAS. SINTEF has worked closely with a few farms introducing RAS, and finally a forum called RAS Forum North have carried out a few seminars with international and Norwegian speakers. These seminars were very well visited, which we think is mirroring the interest in the industry for knowledge about RAS.

Method - Risk Assessment

A qualitative risk assessment is carried out. The risk identification has been done on information presented in the assumptions for the risk assessment. Risk estimation is based on the probability of the event to occur as well as the magnitude of the consequences judged by the ad hoc group. A summary of this assessment is presented in Figure 5.

Definition of terms used for probabilities

High: Event would be expected to occur.

Moderate: There is less than an even chance of the event occurring.

Low: Event would be unlikely to occur.

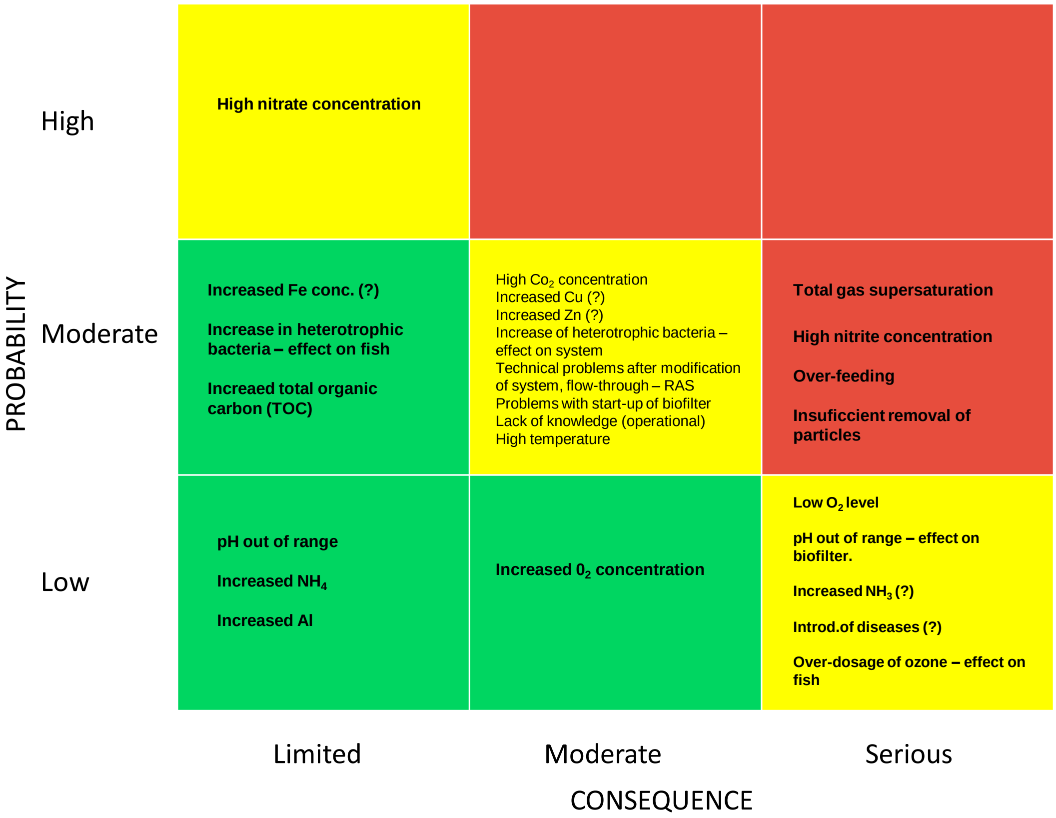
Definition of terms used for consequences

Serious: consequences for fish health and welfare (e.g. high mortality or high morbidity with significant pathological changes in affected fish) affecting a high number of fish during a longer time span

Medium: consequences associated with this event have less pronounced consequences for fish health and welfare

Limited: consequences associated with this event has mild or insignificant consequences for fish health and welfare. Easy to control.

Degree of uncertainty is expressed by questionmarks (? or ??).



PROBABILITY	Limited	Moderate	Serious
High	**High nitrate concentration**		
Moderate	**Increased Fe conc. (?)** **Increase in heterotrophic bacteria – effect on fish** **Increaed total organic carbon (TOC)**	High Co_2 concentration Increased Cu (?) Increased Zn (?) Increase of heterotrophic bacteria – effect on system Technical problems after modification of system, flow-through – RAS Problems with start-up of biofilter Lack of knowledge (operational) High temperature	**Total gas supersaturation** **High nitrite concentration** **Over-feeding** **Insuficcient removal of particles**
Low	**pH out of range** **Increased NH_4** **Increased Al**	**Increased 0_2 concentration**	**Low O_2 level** **pH out of range – effect on biofilter.** **Increased NH_3 (?)** **Introd.of diseases (?)** **Over-dosage of ozone – effect on fish**
		CONSEQUENCE	

Figure 5. A summary of the risk assessment.

Assessments

From the current review of environmental effects on fish welfare, the following conclusions can be made:

- Water quality in RAS can deteriorate and cause severely compromised welfare for the fish
- On the other hand, a well-managed RAS can in fact stabilize, or even improve water quality, resulting in better welfare compared with some flow-through systems
- Monitoring of key water quality parameters (dissolved oxygen, pH/CO_2, TAN, nitrite, total gas pressure and temperature) is essential. Adequate quality assurance of the relevant analytical methods is a prerequisite.
- Routine monitoring of fish behaviour, morphology (e.g. fins, gills and skin), production data (e.g. growth and food conversion ratio), and mortalities is also important
- Suggested maximum or lower limits for most relevant water quality parameters exist. These limits should, however, be considered as guidelines only since the existing water quality criteria are not based on results from commercial (RAS) conditions
- Proper operation of RAS requires good knowledge of water chemistry and the potential hazards involved that might cause compromised fish welfare

1) Is there a risk that methods and technical equipment commonly used in Norway for recirculating water will not allow for the provision of a suitable environment that satisfies fish's basic requirements to sufficient water of a certain quality? If so, please describe which elements of the method or component of the equipment which set fish welfare at risk. Do certain methods or types of equipment better satisfy fish needs?

RAS systems in Norway are more or less following the same principal ideas of necessary components design. Differences are linked to where components are placed and what capacity they are designed for. There are three types of biofilters in use: (1) trickling (2) moving bed and (3) fixed bed. They both serve the same purpose of establishing a bioactive filter for ammonia and nitrate removal. There are variations in capacity and how the RAS equipment is being used. For example, since the natural aim for a fish farmer is to maximize the biological output from the farming system and that this might increase the probability to exceed the capacity of the current RAS. Our assessment indicates that this is one of the areas with the largest risk and effort must be placed into obtaining a production plan with realistic and robust feeding loads. If RAS are used without sufficient conditions for the biofilters (load of suspended solids, temperature, pH, alkalinity, and substrate) there is a risk for a shift from autotrophic to heterotrophic bacteria, a condition that might cause fish welfare problems, since the water treatment cannot maintain the required water quality. It is our opinion that (a) the biofilter, (b) the systems for removal of suspended solids, (c) the system for removal of $CO_{2,}$ are the most critical components of a RAS, (d) sufficient water flow that can fulfill mass balances calculated for each case. In addition, water pipe speeds are extremely important for avoiding sedimentation and solids problems, and are often neglected. This especially concerns the low-pressure part of the loop. Water velocity within pipes should not fall below 0.6 m/s.

One of the most critical operations is the start of the autotrophic active biofilter function, e.g. (1) first time startup of new filters, or (2) start up after planned production stops. It takes a comparatively long time to establish a stable biofilter and such disturbances are regarded as negative for the autotrophic bacterial activity. There seems to be established knowledge that increased water hardness and chlorine ion concentration offer protections against toxicity of nitrite. It is recommended that water nitrite guidelines are to be associated with certain chloride concentrations. A high flow rate through the fish tanks is also brought forward to the committee as positive for fish performance and welfare. The latter might be a consequence of increased water treatment by higher flow and must be dimensioned in each case.

It is critical that RAS suppliers are asked to present, and guarantee, their technology in a conform and understandable way in bid competitions etc. A standardized presentation of the system mass balance, and unit treatment efficiency at various inlet concentrations, must (should) be provided during bid competitions. Today, this is often hard to come by. It could be an argument for Norwegian regulatory bodies to impose such requirements, and thus promote a standardized way to present the technology**.**

2)

(a) *Which risks to animal welfare exist due to faulty assembling or operation of the equipment or use of a method?*

If RAS is dimensioned or designed incorrectly, there is a high risk that the welfare of the fish can be compromised. Faulty operation can have similar consequences. Depending on the type of mistake that was done, water quality can be adversely affected in different ways. For example, if just a single water quality parameter is initially affected, this may in turn induce an imbalance in the aquatic environment. Eventually, the fish might be exposed to a number of harmful compounds. The potential adverse effects of different water quality parameters are described in: “Water quality parameters and potential risk factors related to fish welfare”, page 18-27.

(b) *What can be done to remedy this fact?*

When the RAS investment is done and the equipment is installed, the manufacturer/equipment vendor should be present during start-up to make sure the system operates satisfactory at the intended fish density. Proper training of personnel operating the RAS is essential, as is adequate emergency plans including easy access to relevant back-up systems.

(c) *Can certain operational routines or monitoring of water quality parameters compensate or prevent animal welfare being set at risk?*

To operate RAS safely, specific operational routines are essential to provide for a clean and stable environment for the fish. Accordingly, maintaining good water quality (see: “Water quality parameters and potential risk factors related to fish welfare” page 18- 27), is necessary to avoid compromised animal welfare. Systematic monitoring of certain water quality parameters is of great importance as guidance for taking relevant actions to improve water quality, when necessary. Frequent surveillance, where logged data from sensors monitoring water quality are connected to an alarm system, will make it possible in many cases to take actions before the welfare of the fish is seriously compromised.

(d) If so, please specify which routines are necessary and which water quality parameters that need to be monitored to have sufficient control with and maintain an acceptable water quality that satisfies fishes' needs.

Since RAS can be assembled in different ways, and the system may comprise various unit operations, it is not a straightforward task to devise stringent operational routines that can be used as general guidelines. Moreover, the basic environmental conditions and production plans may vary from hatchery to hatchery. However, empirical operational data from various types of RAS are available as outlined in: "Norwegian experience" (page 49-56). It is therefore recommended that adequate operational routines for each RAS should be devised based on (a) suppliers recommendation, (b) basic knowledge of the interaction between fish and environment (water quality), and (c) comparison with empirical data from other, related RAS. In addition, the importance of proper dimensioning according to production planning is strongly emphasized, using relevant and updated data for e.g. growth rate, nitrogen loss to water per kg feed, and RQ for the species and fees composition in question.

Monitoring of specific water quality parameters obviously depends on the technology available. Presently, some parameters can be monitored on-line (constantly) whereas for other parameters, occasional withdrawal of water samples is necessary. Typically, the samples are subsequently analyzed on-site using analytical 'kits' or instruments. Monitoring of the following parameters is considered necessary: (a) dissolved oxygen, (b) temperature and, (c) pH/CO_2. Since there is a relationship between the level of carbon dioxide and the pH in the water, measurement of the pH also gives an indication of the CO_2 level in the system. In fact, such electrodes are available. Alternatively, new technology has now been introduced for direct measurement of CO_2 on-line. The surfaces of all electrodes/sensors used for continuous monitoring will eventually be affected by bio-fouling. It is therefore of great importance to have good routines for periodical cleaning and calibration to ensure that the logged water quality values are indeed correct. To monitor the performance of the biofilter, (conversion of toxic ammonia and nitrite to nitrate), water samples should be routinely withdrawn for analysis of nitrite. In cases where nitrite may be expected to increase, routine determination of the chloride (Cl^-) concentration in the same water sample is recommended.

3) What is the risk of a fluctuating water quality environment with ever changing levels of various parameters ensuing in a recirculation system compared to a flow-through system, and which factors represent a risk to the stability of the environment provided?

A RAS system in balance which is run under safe limits, seem according to industry contacts to offer a more stable water quality in areas where the raw water quality is likely to have high variance. Some water sources in Norway are of this category. Stable raw water quality can be obtained through treatment of the make-up water. The RAS loop itself is changing the make-up water and can in many cases be regarded as a water treatment. The make-up water is often used to limit the nitrate levels in the RAS, and can also offer a short term solution to lower nitrite, ammonia and carbon dioxide levels. However this is a two-edged strategy if the make-up water has low alkalinity and pH (often the case in Norway). Adding more makeup water might cause a drop in the nitrification process rate and lead to toxic levels of ammonia and nitrite. The make-up water flow in RAS is normally very low compared to the recirculating water flow, offering an opportunity to develop RAS farms in places not suitable for a flow-through system. From the literature it is reported that metals can accumulate in RAS. Since the levels of substances in a RAS is following law of mass balance, the will accumulate to the levels that corresponds to the (a) introduction rate (fish, water, feed), (b) the removal rate and (c) the proportion of the make-up water. Abrupt changes in any of these factors will lead to variations. Inadequate dimensioning in any of these factors could lead to high levels of metabolites, particles or metals. It is known that metals can accumulate in biofilm. Biofilm detachment from biofilter media might also offer some possibility to remove metals. Ozonation has also been shown to remove metals. Finally, an organic compound (DOC/HA), which are generally higher in RAS, will compete with the fish gills in binding free metals and must be considered. Norwegian freshwater is normally very soft with low Ca^{2+} and alkalinity. From the literature we find that low Ca^{2+} and low alkalinity can cause aggressive water with the potential to affect metal release from biofilm, thus automatic bicarbonate or other carbon-based dosing to control pH is important. A minimum of 50 mg/L ($CaCO_3$) alkalinity must be kept. The biofilters also consumes alkalinity so buffer need to be added. Several different buffer options are in use, but not all of the offer increase in carbon, Ca^{2+} and alkalinity. The danger for exposure to toxic nitrite is higher in RAS than in a single pass flow-through system. The same counts for the probability of exposing fish to higher CO_2 levels. The probability to be exposed to high levels of total ammonium-N and ammonia may be lower with a RAS, than in a single pass flow-through system with water exchange < 0.05 L/(kg x min). However, toxic NH_3-N levels may develop also in RAS if abrupt reductions in nitrification efficiency occurs, feed loading is above specification, and malfunctions occur in pH control systems. To obtain good water quality it is necessary to establish well-functioning filtering systems (mechanical and biological). These components needs to be designed to fit together and adapted to the current capacity set by the actual biological load (feed and fish). There are several factors that might improve robustness of the water quality in a RAS. The Panel would like to draw attention to the following (1) the use of buffers for adding Ca^{2+} and alkalinity, (2) the control of heterogenic bacteria in the biofilters and circulating water, (3) the capacity to remove CO_2 and total ammonium-N from the production water (related to water flow, e.g. pumping and technical performance of the water treatment).

4) Is there a risk of poor or inadequate water quality conditions developing due to the amount of renewal water per tank in a recirculation system? The systems total capacity to maintain a good water quality must also be taken into account in conjunction with the assessment of the water renewal rate. Will certain water renewal schemes reduce or minimize this risk? Do other factors such as feeding regimes, stocking density, etc. interact with water quality maintenance in such a manner that animal welfare is set at risk?

There are two aspects of water renewal rate of relevance to water quality. The first, the daily system water exchange rate, denotes the amount of new water entering the system daily. The second aspect is the tank hydraulic retention time, which reflects the time needed to exchange one volume of the fish tank.

Maintenance of sufficient flow, both into the system and, in particular, within the system is a major critical factor for fish health and welfare.

The daily system water exchange rate (new water) will, at low rates, influence the water quality, in inducing an accumulation of heavy metals and waste metabolites and adverse effects on fish health and welfare were observed in some extreme systems under experimental conditions. The level of exchange where these effects will appear will differ between systems, depending on quality of make-up water, system removal efficiency and other factors, e.g. solubility of feeds etc. Under experimental conditions, such effects were observed at water exchange rates of 1 % or less.

Some of the adverse effects from a low water exchange rate can be overcome efficiently by ozone treatment of recirculating water flow. Ozonation will reduce the levels of total suspended solids (TSS), fine particles, heavy metals and bacteria, and will improve water colour, and have the potential of restoring these parameters to a level comparable to that of a ten-fold higher exchange rate. Ultrafiltration or other treatment principles may in future be used as an alternative to ozone, but such systems must be developed and tested before put into use. Thus, at low water exchange rates, water quality monitoring should be extended to include the relevant parameters, and adequate measures must be taken to avoid the expected reduction of water quality.

High water exchange rates may, on the other hand, challenge the stability of the system, since make-up water is different from the maturated water of the recirculated flow and must be conditioned when entering. Whether or not this represents a problem will depend on the quality of the incoming water, compared to the recirculated flow, and the robustness of the water treatment in general.

The circulation of system volume, measured as tank water residence time, or hydraulic retention time (HRT) is of key importance to the water quality, as it defines the rate at which the water is subjected to treatment in filters. Thus, continuity of a sufficient recirculation flow is more critical for water quality in terms of fish health and welfare than daily system water exchange rate *per se*. In this context, the balance between feed load and dimensioning of water treatment installations is pivotal.

During production, nitrite accumulation to toxic levels is one of the most critical factors related to fish health. Nitrite is constantly produced as the intermediary step between ammonia and nitrate, but nitrite may accumulate whenever there is an imbalance between the system organic load and the removal efficiency for nitrogenous waste. Nitrite surges are expected during biofilter start-up and maturation. Therefore, it is of great importance that any introduction of fish in a RAS is not done until a steady-state level is established. Toxic levels of nitrite may also occur during production e.g. if fish for some reason refuse to feed. Nitrite toxicity is effectively counteracted by chlorine. More studies in RAS environments are needed to determine if the often used ratio of 20:1 (Cl: NO_2-N) is sufficient to protect salmonid parr, as is questioned by preliminary data from a recent flow-through experiment. Thus, procedures for monitoring of nitrite levels, as well securing sufficient chloride supply if needed, are relevant preventive measures. Also, water exchange rates may be increased as a means of stopping nitrite accumulation. It is indicated, however, that a system undergoing a nitrite build-up should be given time to re-establish balance, given that nitrite is at subtoxic levels, and that an increase of water exchange rate may impair this process.

Feed composition, in terms of balance between main nutrients, is relatively uniform in Norwegian aquaculture. There is, however, variation in choice of feedstuffs, with an increase in amount of vegetable meals and oil being a representative trend. Some vegetable ingredients, e.g. soybean meal, may induce mild diarrhoea in the fish, which may impair faeces removal from tank water. Also, feed pellet with low water stability will disintegrate faster, and removal efficiency may be impaired. These effects are known from flow-through systems, but remain to be tested in RAS.

Feeding rates and feed load, comparable to system performance, remains a critical factor for water quality and fish welfare. The actual feed load must not exceed the corresponding removal capacity of tanks, pipes, filters and bioreactors. Dimensioning and design of system components must be based on realistic production parameters in order to provide stable and good conditions for fish. Management and production control must comply with system carrying capacity and actual fish load. At present, there seems to be a lack of consistency in information exchange related to design and construction, and the level of knowledge about how to operate RAS-systems appears unpredictable and unsatisfactory.

Irrespective of system type and production parameters, it seems generally accepted that day-to-day variation in feed load should be kept under control, and not exceed 10-15%.

Control of fish stocking density is an important factor for maintenance of good fish welfare. No specific upper limit for fish density can be specified based on existing data, which are particularly sparse for RAS systems. Existing information indicate that salmonids can tolerate stocking densities up to 80 kg m^{-3} without adverse effects in well-functioning RAS systems, but point towards a size dependent relation between fish size and maximal stocking densities.

5) Does available knowledge on how to operate the recirculation system in accordance with the biofilter's capacity, fish density and feeding regime, in itself represent a risk e.g. due to either inadequate or incorrect knowledge?

Personnel who are operating the production units usually have a background from a college offering fisheries and aquaculture courses. Their educations plans may include an introduction to recirculation systems. Teaching and training will however be on an overall design level and not thorough enough to give the students sufficient knowledge to understand and operate a recirculation unit without further training. We may therefore expect inadequate or incorrect knowledge on recirculation systems amongst a large percentage of the personnel working in traditional aquaculture production units for salmon and trout smolts and fingerlings.

If the operational knowledge of the system is sufficient, is it rather the farms that do not train their staff in correct management of recirculation systems thus creating an increased welfare risk?

Yes – probably. In order to secure correct management of the RAS, the farms must have responsible personnel that ensure that at least some of the staff has “up to date” knowledge and practical expertise. This might be handled by internal training or training offered and organized by suppliers. Large companies, with RAS units abroad will presumably be able to keep a high level of knowledge among employees, due to experiences from their production units, while smaller aquaculture companies might not have this opportunity and are more depended on training from Norwegian education system, R&D companies and RAS suppliers.

This might be recognised as common human weakness not specific only to RAS operation, but the consequence for fish welfare might be adverse.

6)

Is there a greater risk of disease occurrence in recirculation systems compared to flow-through systems and is it possible to maintain good health status for a long term perspectively (years)? It should be taken into consideration that in hatcheries with a flow-through system segregation of different life-stages and all in and all out procedure is practiced with disinfection of all equipment between different batches. If such a procedure is no longer possible in a water recirculation system, is there an increased health risk that can attribute to retaining the bio filter between different fish groups.

Introduced diseases

The possibility of introducing diseases to hatcheries is mainly associated with introduction of biologic material (eggs and fish) and to the water source. The possibility of introducing diseases will thus be reduced since less water is used. The reduction in the amount of water introduced into the system allows for a better disinfection. If a disease is introduced, the likelihood of detecting the disease is equal since both systems apply to the same mandatory requirement for fish health inspections and diagnosis. The possibility to control an introduced disease is depending on the agent. Virus like IPNV is extremely resistant and some bacteria like *Flavobacterium* form biofilms. The possibility to eradicate an introduced disease is more difficult in a recirculation system. This is linked to the function of the biofilter. The risk (possibility x consequence) associated with introduced diseases is therefore assessed to be higher in a recirculation system compared to a flow-through system, but the relative risk will depend on the infectious agent in question.

Infectious, not introduced diseases

For some water parameters, the recirculation technology offers the possibility of creating a more stable environment, which can be less stressful to the fish compared to a flow-through system with variable water quality. The environment will be different and thus creating different opportunities for fish pathogens. Since there are limited data, it is not possible to assess whether this change will represent a risk. Depending on the technology chosen, the organic load in a recirculation system will increase compared to a flow-through system. This might favour microorganisms like fungus. Problems with fungal (*Saprolegnia* sp.) infections have been reported from Norwegian RAS, but the data are too limited to conclude. So far, no increased in infectious disease problems have to our knowledge been reported. Available disease data do not allow for a direct comparison, but the disease situation in the RAS in the Faroe Islands does not seem to be worse than Norwegian flow-through systems. The risk associated with opportunistic fish pathogens is therefore juged to be comparable with flow-through system.

Non infectious diseases

A number of non-infectious fish diseases are related to unfavorable and unstable water parameters in flow-through systems. Rapid changes can have high consequences and the risk in such systems can be ranged from low to high. RAS offer a possibility to supply a far more stable environment. However, RAS also allows for the possibility to accumulate of substances that can impact fish health. This is especially true for RAS with 100 % or close to 100 % recirculation. Norwegian RAS does not currently operate in this range.

The Faroe Islands has produced Atlantic salmon smolts in RAS for more than twenty years. The overall fish health can be described as good and demonstrate that is possible to maintain good fish health status for a long term under Faroese production conditions in RAS. The recirculation systems recently built in Norway are designed for an effective production of large numbers of salmon smolts. There is so far limited data from Norwegian hatcheries with recirculation systems and no published data comparing fish health in flow-through systems to recirculation systems. There are, however, several reports from the industry that indicate a better performance and survival after transfer to sea.

Segregation of different life-stages, all in and all out procedure and disinfection of the production system

Segregation of different life-stages, an all-in and all-out procedure and disinfection of the production system are considered to be essential in fish health management. Production system where this is not possible, have increased disease risk. In RAS, the biofilter is an essential stabilizing part of the system and it will not be possible to disinfect the biofilter during a production cycle. Furthermore, the time it takes to regenerate the filter is a constraint for efficient production, as well as introducing increased risks for nitrite variations following start-up, before a robust biofilm is developed.

Based on the available experience from the industry, the ad hoc group consider it possible to maintain good health status for a long term in RAS. Disease control and management differs between RAS systems and flow-through systems mainly in relation to the time and efforts required to re-establish biofilter function after disinfection, and the challenges related to lack of a complete separation between subsequent fish groups in the system during continuous operation.

Data gaps

Based on the review of literature and compilation of practical experiences with RAS, the following issues are suggested as topics for further research:

- Safe levels for nitrite related to water chemistry
- Long-term exposure to nitrite and nitrate and effect on fish health
- Presence of organic compounds and toxicity of metals
- Diseases and pathogens in RAS
- Welfare indicators suitable for RAS
- Optimizing operational routines of biofilter during start-up
- Use of ozone – impact on water quality parameters and fish health
- Temperature tolerance limits for salmonids in freshwater RAS
- Hydrodynamics in RAS – trade-off between self-cleaning of tanks and fish swimming speed at different tank sizes and fish sizes
- Multifactorial evaluation of limit values for key water quality parameters in combinations which are relevant for RAS
- Relation between composition of microbial communities, fish health and water quality
- Optimal diet composition and technical quality of feeds in RAS
- Comparison of stability of water quality parameters in RAS and flow-through systems
- Disease management and control – preventive measures and disease management strategies
- User aspects of alternative buffers with different effects on water chemistry

Conclusions

Based on literature data and practical experiences from recirculating aquaculture systems (RAS), possible environmental effects on fish welfare were assessed. There is a risk that the water quality in RAS can deteriorate and cause severely compromised welfare for the fish. On the other hand, a well-managed RAS can in fact stabilize, or even improve water quality, resulting in better welfare compared with some flow-through locations. Monitoring of key water quality parameters (dissolved oxygen, pH/CO_2, TAN, nitrite, total gas pressure and temperature) is considered essential for safeguarding the welfare of the fish. Adequate quality assurance of the analytical methods is a prerequisite to ensure correct readings of relevant water quality parameters. Routine monitoring of fish behaviour, morphology (e.g. fins, gills and skin), production data (e.g. growth and food conversion ratio), as well as mortalities is important. Suggested maximum or lower limits for most relevant water quality parameters exist. These limits should be considered as guidelines only, since the existing water quality criteria are not based on results from commercial (RAS) conditions. Safe operation of RAS requires good knowledge of water chemistry and the potential hazards involved that might cause compromised fish welfare. Therefore, proper training of personnel operating RAS is required. Water chemistry in RAS can be quite different from what the fish is naturally exposed to in nature or in aquaculture flow-through systems. Accordingly, the need for more research under commercial RAS conditions (where several water quality parameters are considered simultaneously) was recognized. Some specific data gaps are listed.

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Annex 1

Videregående skoler som tilbyr Vg2 Akvakultur

Fylke	Skole	Utdanningstilbud
Finnmark	**Nordkapp maritime fagskole og videregående skole** Postboks 143 9755 Honningsvåg Tlf. 78 47 60 10 Fax 78 47 60 20 E-post: honningsvagvgs@ffk.no *Fare for at faget kuttes pga lave søkertall.*	1. Naturbruk Vg1 2. Akvakultur Vg2 3. Fiske og Fangst Vg2 4. Maritime fag Vg2 5. Fagskole- nautisk linje
Troms	**Skjervøy videregående skole** Postboks 250 9180 Skjervøy Telefon: 77 77 78 00 Telefaks: 77 77 78 01	1. Naturbruk Vg1 2. Akvakultur Vg2 3. Fangst og Fiske Vg2
Troms	**Senja videregående skole** *Tilbud lagt ned 2009, har nå kun Fiske og Fangst. De samarbeider nå med Skjervøy videregående skole.*	
Nordland	**Meløy videregående skole** postboks 55 8161 Glomfjord **Skolested Inndyr**: Telefon +47 75 65 26 00 Telefaks +47 75 65 26 01 E-post: meloyvgs.inndyr@nfk.no	1. Naturbruk Vg1 2. Akvakultur Vg2 3. Fiske og Fangst Vg2
Nordland	**Gravdal videregående skole** *Tilbud lagt ned 2009, har nå kun Fiske og Fangst.*	
Nord-Trøndelag	**Ytre Namdal videregående skole** Hansvikvn. 3 7900 Rørvik Tlf: 74 39 35 50 E-post: ytre-namdal.vgs@ntfk.no	1. Naturbruk Vg1 2. Akvakultur Vg2 3. Fangst og Fiske Vg2 4. Fagskole - nautisk linje

Nord-Trøndelag	**Val videregående skole AS (privat skole)** 7970 Kolvereid Tlf: 74389000 Faks: 7438900 E-post: post@val.vgs.no	1. Naturbruk Vg1 2. Akvakultur Vg2 3. Grønn profil fra Vg2 nivå
Sør-Trøndelag	**Frøya videregående skole** Postboks 44 7261 Sistranda Tlf: 73 19 51 11 Fax: 73 19 51 25 E-post: postmottak.froya@stfk.no	1. Naturbruk Vg1 2. Akvakultur Vg2 3. Fiske og Fangst Vg2
Møre og Romsdal	**Fræna videregående skole** 6440 Elnesvågen Telefon: 71 26 64 00 Fax: 71 26 64 01 E-post: frana.vgs@mrfylke.no	1. Naturbruk Vg1 2. Akvakultur Vg2 3. Fiske og fangst Vg2
Møre og Romsdal	**Kristiansund Videregående Skole** *Tilbud (Akva og TAF Marin) lagt ned 2009, har nå kun Fiske og Fangst.*	
Sogn og Fjordane	*Ingen tilbud i fylket.* *Tidligere har **Måløy videregående skole** hatt begge de blå naturbruksfagene, men har nå kun Fiske og fangst.*	
Hordaland	**Fusa videregående skole** Post boks 113 5649 Eikelandsosen Tlf:56 58 09 00 Fax: 56 58 09 01 E-post: post.fuv@post.hfk.no	1. Naturbruk Vg1 2. Akvakultur Vg2 3. TAF Marin
Hordaland	(**Austevoll videregående og maritime skole)** 5392 Storebø Tlf. 56 18 20 00 Faks 56 18 20 01 E-post: post.fia@post.hfk.no *Mistet faget pga få søkere men satser på å få startet opp igjen.*	1. Naturbruk Vg1 2. Fiske og Fangst Vg2 3. (Akvakultur Vg2) 4. Maritime fag Vg2
Rogaland	**Rygjabø videregående skole** Judaberg	1. Naturbruk Vg1 2. Akvakultur Vg2

4160 Finnøy Tlf: 51 71 43 00 Fax: 51 71 43 01 E-post: rygjabo@rogfk.no	3. Fiske og Fangst Vg2 4. Grønn profil på Vg2 nivå

Høyskoler som tilbyr akvakulturutdanning

Høyskolen i Bergen

Har spesialisering innenfor akvakulturteknikk.

Universiteter som tilbyr akvakulturutdanning

Universitetet i Bergen

Tilbyr både bachelor, master og doktorgradsstudium innenfor akvakultur. Resirkulering inngår i kurs i ”Vannkvalitet og smoltkvalitet” med innleid foreleser fra NIVA. Relativt få forelesningstimer.

Universitetet for miljø- og biovitenskap

Resirkulering inngår i grunnkurs i akvakultur. I videre nr 2-kurs er det lagt inn dimensjonering av anlegg, i nr. 3-kurs prosjektering av akvakulturanlegg, inkl. resirkulering. Dette gir en bakgrunn for drift av resirkuleringsanlegg.

Universitetet i Nordland, Bodø

Tilbyr både bachelor, master og doktorgradsstudium innenfor akvakultur. Resirkulering inngår ikke i utdanningsplanene.

Norges Fiskerihøyskole, Universitetet i Tromsø

Tilbyr både bachelor, master og doktorgradsstudium innenfor akvakultur. Resirkulering inngår i utdanningsplanene, inkludert laboratoriekurs med måling av vannkjemi etc. Tema er relativt overfladisk.

Norges teknisk naturvitenskapelige universitet (NTNU), Trondheim

Tilbyr master- og doktorgradsstudium innenfor akvakultur. Studiet er hovedsakelig biologiorientert, men med noe innslag av teknologi i enkelte fag.

Annex 2

Experiences from RAS-suppliers

Table 4. In order to introduce opportunities for interaction with important RAS suppliers, the VKM committee has contacted the following companies; AKVA Group ASA, AquaOptima AS, Aquatec Solutions A/S, Billund Aquakulturservice A/S, InterAqua (Plastsveis AS) to comment on a standard set of topics based upon their views and experience. The comments are presented in the table.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
1	Short info about company	AKVA group is the world`s largest supplier of technology to the aquaculture industry. Upon the acquisition of two Danish companies involved in RAS, AKVA group has become one of the major suppliers of technology for recirculation both for fresh- and saltwater species.	Specializing in RAS, Aqua Optima (AO) was established in 1993 and is one of the most experienced companies in Norway on recirculation systems. In case of freshwater RAS (hatcheries) in Norway, AO has up to now delivered 6 systems for Atlantic salmon and 2 systems for Arctic char, and for seawater, 1 for cod fingerlings 0-10 g, 1 for halibut grow-out 2g-5kg and 1 for halibut fingerlings	Based in Denmark with daughter company in Chile. Operating world-wide from Tasmania – Chile – Europe especially Norway, Faroe Islands and Scotland. Experience since 1983 from building more than 60 RAS systems. Specialized in RAS and various equipments for fish farming in general.	Billund Aquaculture is a Danish company located in Billund, Denmark and in Puerto Montt, Chile (Billund Aquaculture Chile S.A.). We have more than 25 years of experience in design, installations, operation and service of intensive re-circulation fish farms. Worldwide Billund Aquaculture has so far build more than 100 re-circulated systems for 24 different salt-and freshwater species in 25 different countries.	World-wide supplier of turnkey RAS with raceways or circular tanks. Since 1978 supplied >150 plants, since 1993 all including 3rd gen. Clearwater MBBR (moving bed bio-reactor) technology. Plants for fresh water and sea water fish species and shrimps. Presently main focus on supply of smolt hatcheries and landbased grow-out of salmonids, exclusive marine fish and mass production of low priced freshwater species for countries with emerging economies.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
2	Why use RAS in smoltproduction	Lack of FW of good quality has traditionally been the main reason. However, we are now seeing a trend where customers choose RAS due to the good results in existing RAS facilities. Good and stable water quality plus high and stable temperature generates good growth in the RAS and allows production of larger smolts. More importantly, the good growth and survival in the FW phase tends to continue also in the sea cages.	The main reason for selecting recirculation systems has been limitations in FW supplies and unstable water quality. Thus, by using RAS, the flow rate/water supply can be increased and water quality stable.	Allows for higher water temperatures at winter time and stable good water quality year round, with low energy usage. Allows for high production with small intake water supply. Small costs to prevent incoming pathogens in intake water.	- The water quality is fully controlled by the water treatment system (WTS). - Optimal and stable production all year round - Reduced risk of diseases - Low water requirement	- RAS technology provides increased productivity under optimal and completely controlled conditions, production cost reductions of up to 60 % under safe and bio/secure conditions at fish densities up to 100 kg/m^3.
3	RAS operating conditions	Every RAS that we design will be customized to meet the needs of the client and, more importantly, the requirements of the specie to be farmed. A smolt facility will typically be designed with approx 99 % recirculation to conserve heat. Water exchange in the tanks will, depending on life-stage of the fish, be from 30 to 50 min. Water pH in tanks from 6.8 to 7.2. Temperature most commonly 12 to 16°C.	In most cases, 10-20 % of the RAS total water volume is recirculated per day (>99 % recirculation). This will also simplify the operation of the biofilter. The retention period of the water in the tanks is 30-60 min and the pump capacity (volume/h) is dimensioned accordingly (depending on the size of the hatchery). Water pH is stabilized in a RAS. Fish density is dependent of fish size and is typically less than 50 – 70 kg/m^3. The systems are normally not desinfected after the	An AQS BASIC recirc system design allows for a raw water usage of ca. 300 l water/kg feed to dilute NO3. By using AQS Zero Water Change (ZWC) technology which takes out $NO_{3,}$ phosphor and heavy metals, the raw water usage can be reduced to ca. 30 l water/kg feed. The above 2 options relates to raw water intake per day of 20-30 % for a BASIC system and 1 – 2.5 % for a ZWC system, measured as a % of the total water volume in the system.	- Water exchange < 10 % of total water volume pr day. (400-600 L/kg feed without denitrification) - Tank flow: 1.5-2 exchanges/hr - Densities: 25-75 kg/m^3 (depends on fish size) - Temperature: 8-16 °C - pH: 6.5-7.5 - CO2: <20 mg/L - Oxygen: 80-100 % saturation in fish tanks - TAN: max 2 mg/L - Nitrite: max 0,1 mg/L	- 99.9% recirculation (exch. per circulation). Retention time in fish tanks 10 to 45 min. Depending on tank type and fish density. Water treatment incorporating in line 40 micron filtration, one step biological treatment in self cleaning Clearwater bio/reactor(s), low head water circulation with low head and(or pressure oxygenation. 10% side stream treated with fine filtration (5 micron) and UV treatment for fry and delicate fish species. Build up time of bioreactor activity from 1 week to 3 months, depending on procedure and medium. Long term stabilization in 3 to 6 months.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
			smolts are transferred to the farms. Basically the RAS are operating continuously and it takes 1-12 months to achieve a well-established biofilter. UV is not used in the RAS.	Retention time in the fish tanks depends on the required CO_2 level, but typically between 30 – 60 min. Activation of biofilters takes 4 – 6 weeks. Fish density can go up to 90 kg/m^3 if required. CO_2 is typically between 12 and 15 mg/l. Temperature range: 8 – 16 C. pH range: 6.9 – 7.5. Salinity range: 0 – 3.5 %. The RAS are typically sterilized once a year.		Limited or no use of disinfection.
4	**Comments on risks in RAS regarding**					
4 A	Raw water quality	First and foremost, the low water exchange compared to a flow-through system will dilute the effects of a fluctuating raw water quality. To reduce risk of contamination, raw water is often mechanically filtered and UV treated upon entry to the RAS. Furthermore, new water is added into biofilters where long retention time allows for potential metal	Treatment of make-up water is dependent of quality of water source. Often the water supply passes a coarse strainer, and sometimes a UV unit, before entering the RAS.	Raw water is a potential source for bringing in pathogens hence the raw water intake sterilizer system must be well equipped with sensors and back-up systems to ensure no pathogens enters the RAS	All incoming water is filtered and disinfected in addition to UV disinfection inside the farm	Ground water or sea water best sources, but any water source can be conditioned by filtration and UV or ozone treatment. Prevention

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
		polymerization to occur without risk for the fish.				
4 B	Oxygen	Oxygen is added to the water in the process of degassing. Further oxygenation is mainly done by individual oxygen cones to each tank with automatic adjustment. All tanks are equipped with emergency oxygen in case of power failure etc.	Oxygen is typically added to the water-flow, after the biofilter. A sensor placed at the tank outlet automatically determines oxygen concentration and oxygen is added to maintain the selected level of oxygen saturation (ex 80 – 100 % saturation). Heavy oxygen supersaturation is not considered a problem in salmonid hatcheries with RAS.	Lack of oxygen is the fastest way to kill the fish, hence there must be a reliable monitoring, control & alarm system and automatic emergency oxygen distribution system for each fish tank. If using LOX there should be installed pressure and level sensors on the LOX tank. If using O_2 generators, there must be O_2 purity sensors, in case there is no LOX for back-up, A back-up O_2 generator and two back-up EL generators must be available. O_2 saturation should be <100 %. Back-up pumps for oxygen cones must be present.	Oxygen is probably the most critical water quality variable, even small reductions in oxygen below the minimum desirable levels can lead to reduced growth. In order to fulfil the demand for oxygen, pure oxygen is added to a part of the water by pumping the water either going to all the tanks or the water going to each tank through an oxygen cone. The continuously monitoring and control of the oxygen in the tanks gives alarms for low or high oxygen and if it is low the emergency oxygen diffusers will turn on.	Oxygen dosed in response to demand in fish tanks individually. In raceway plants low head oxygenation is applied, in plants with circular tanks (lower flow) a combination of low head /pressure oxygenation is applied. Pressure oxygenation arranged in central manifold systems for energy optimisation and safety by mutual back up between tank supplies.
4 C	Carbon dioxide	Systems are normally designed to operate with levels lower than 15 mg/l at peak biomass. Some systems are designed to operate with lower concentrations, in particular for vulnerable species or life-stages.	Levels should be less than 15 mg CO_2/L. The gas is stripped off using aeration in sump or by packed column. Good CO_2 sensors for online use are now available.	Back-up blowers must be present as CO_2 will increase instantly in case of blower failure. More than one CO_2 degassing system lower the risk. CO_2 monitoring & alarm system must be present. Smolts produced in high levels of CO_2 will not perform as well in both RAS and later on in the sea as if they were produced in lower CO_2.	Implementation of a trickling filter (aeration) is done to reduce CO_2 levels and avoid potential problems caused by CO_2.	Central degassing in bioreactor airlifts, supplementary degassing in central cascade system (in raceway plants) or in decentralized airlifts for circular tanks. Existing documentation for 20 mg CO_2 /L as upper limit for salmon, but normally kept below 15 mg/L. Reduced digestability of commercial feeds is an increasing challenge for CO_2 control.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
				CO_2 should be lower than 15 mg/L		
4 D	Acidity	pH is mainly maintained around 7.0 by automatic addition of caustic soda. pH is an extremely important parameter, hence a double set of sensors to monitor pH is recommended.	Low pH, as a resulted of elevated levels of metabolically produced carbon dioxide or acid raw water, is determined by a pH sensor. If necessary, the pH is adjusted by automatic addition of bicarbonate. Efficient operation of the biofilter requires a pH of 6.5 – 7.0.	A good and reliable pH monitoring, control & alarm system must be present, as uncontrolled increase in pH will instantly increase the level of ammonia to dangerous levels even with relatively normal ammonium levels.	pH is monitored by the control system and adjusted to desired levels by an automatic lime dosing system. If values are below/above the desired levels an alarm will be activated. pH is measured and adjusted in front of the biofilters, so the filters are working optimal. pH should be in the range of 6.5-7.5	pH controlled at 6.8 to 7.4 (depending on fish species) by dosing of bicarbonate or sodiumhydroxide or by de/nitrification. Higher pH values favour nitrification and binds CO_2, thereby preventing inhibitory levels. Reduced digestability of feeds adds to the pool of alkalinity in the plant, reducing the need for bicarbonate.
4E	Nitrogen – containing compounds	TAN, nitrate and nitrite are routinely (each day) checked by colorimetric measurements.	The levels of metabolically produced TAN ($NH_4^+ + NH_3$) and the degraded products nitrite and nitrate, are routinely checked by manual colorimetric determination.	Special attention must be taken to ammonium and nitrite levels if a RAS is loaded with fish without activated biofilters and if chemicals are added to the system or if the system is overloaded with biomass. Ammonium and nitrite must be measured on a daily basis	TAN and nitrite is controlled and kept stable by the biological filters. Nitrate is diluted out by adding new water. Denitrification filters are added if the new water supply is limited.	TAN and nitrite maintained below 0.5 and mg)/, nitrate below 100 mg/L Daily control of parameters.
4F	Gas-(nitrogen) supersaturation	Nitrogen supersaturation can be a problem in some RAS systems. AKVA group take many measures to prevent this. Biofilter is aerated to avoid anaerobic conditions and water from biofilter is always degassed in a system with negative pressure	This is not a common problem in RAS used in salmonid hatcheries. The water is constantly aerated.	N_2-supersaturation is a potential tricker for outbreak of IPN especially for small fish. N_2 vacuum degassers are used in more systems. Special attention to if blowing air into deep water (more than 1 meter)	This is not an issue	Aeration at water depths more than 2 meters leads to significant supersaturation, which happens in MBBR, operated by simple diffusion. In the Clearwater MBBR nitrogen degassing is performed in the airlifts, counter balancing supersaturation by the simple peripheral diffusion aeration.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
		before entering fish tank.				
4G	Particles/solids	Large particles are removed by mechanical filtration. Finer particles are degraded in the first biofilter chamber and/or removed in the microparticle filter. The microparticle filter is the last of the series of chambers in the biofilter in which the water moves very slowly to facilitate sedimentation/adhesion of fine particles that can be removed from the system when needed.	Uneaten feed should be removed as soon as possible to avoid disintegration and contamination of the water. A particle trap is therefore placed at the centre bottom, of each tank. The solids are collected and removed from the system.	Important to take out particles to avoid build up of organic loads as too high levels of TSS reduces the turbidity of the water and reduces the possibilities for operators to control their fish stock. High levels of TSS increases the consumption of oxygen. Drum filters combined with fixed bed filters captures the particles	Particles/solids are effectively removed in the mechanical and biological filters.	All water from the tanks passes directly for screening in 40 micron drum filters for efficient removal of particles.
4H	Temperature	Low water exchange ensures a high and stable temperature. Some heating can be necessary in very cold winter periods. Most facilities will also need systems for cooling water in the summer period to avoid too high temperature. This is most commonly done by SW exchange systems.	In Norway, only periodical (winter) heating of the water by use of heat exchangers or heat pumps.	As the power consumed in a RAS system ends up as heat plus the heat production from biofilters and fish stock, the temperature in the RAS system can be high in the warm months, hence efficient cooling systems must be present.	Temperature is fully controlled by the PLC. Heating/chilling of the water is done by heat exchangers and/or heat pumps.	Temperature maintained constant by use of metabolic heat, heat pumps or heat exchangers.
4I	Ozone	Due to the safety risks involved (and high running costs), AKVA group does not recommend the use of ozone in our RAS. Instead, we recommend removing fine particles through a combination of fine mesh in mechanical filter, heterotrophic	Ozone is added (10 – 15 g ozone / kg feed) to facilitate microflocculation and removal of fine particulate matter. Water clarity is improved. Theoretically, ozone represents a health risk for the fish, but with its short lifetime, this is not	Ozone is an efficient oxidizer that firstly will break down organic materials and then bacteria's etc. Depending on dose it can be used to sterilize water, especially in combination with UV. A good and reliable ozone monitoring, control &	When O_3 is added precautions is taken by installing sensors in air and water. If critical levels are reached an alarm is activated.	Fine particles are digested in the bioreactors after adsorption onto the biofilm. Ozone recommended only as a tool for flocculation and elimination of excessive fine particle outbursts in connection with over feeding or grading.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
		biofilter and microparticle filters. When the other means of particle removal are designed correctly, ozone is not necessary to boost biofilter performance as one might need in e.g. a moving bed system with more particle matter in the water.	considered a risk in practice and with the dosage used.	alarm system must be present, as uncontrolled increase in ozone level will kill fish and be harm full to operators.		
4J	Bacteria and parasites	Unwanted bacteria and parasites are kept out of the system by intake water treatment and sanitary measures inside facility (sluices). Internal flow is treated with a relatively small dose of UV to stabilize bacterial dynamics.	Accumulation of bacteria in the RAS does not seem to be a problem, probably because such bacteria are outstripped by the established bacteria in the biofilter. Parasites smaller than the filter mesh size may constitute a potential health risk (although AO have not heard of this problem in their systems).	Diseases are not common in RAS if they are well designed in all areas. Bacteria's and parasites can be present in the RAS if there is insufficient sterilization of raw water or if fish are taken into the RAS from an infected facility. If disease should appear, most medicines can be used in a RAS when used correctly.	Bacteria and parasites are controlled in the system by the mechanical filter, biofilter and UV. In systems with smaller fish it is normal to UV-treat 100 % of the total water flow.	Bacteria infection is not a problem in an IAA RAS. Fungus and parasites, entering from outside, may cause a problem, which can be dealt with by treatment inside the RAS.
5	Safety and monitoring of water quality	RAS are delivered with an extensive control and alarm system that controls DO, CO_2, temperature, water levels, pH, salinity as well as motors and pumps. TAN, nitrite and nitrate are manually measured on a daily basis. Systems are equipped with emergency generators in case of power failure and emergency oxygen.	DO, pH, temperature and water level are automatically monitored. In addition, TAN and nitrite are routinely analyzed manually. In case of loss of electrical power and pump failure, all hatcheries have stand-by power units. Moreover, oxygen is automatically added to the fish tanks through diffusers in emergency cases. At the same time	Oxygen and water levels are measured in each fish tank. Automatic emergency oxygen diffuser system shall start up in case of too low oxygen levels. In the water treatment system there is monitoring, control & alarm system for pH, temperature, salinity, ozone and CO_2. Most of them have two sensors for safety matters.	The whole system is controlled by a central PLC. Selected parameters are monitored and regulated in order to insure a stable and efficient system. Regulated and alarm given parameters are oxygen, pH, temperature, carbon dioxide, salinity, water levels, pump stops, water pressure, thermal failure. Nitrite and TAN are	O_2, CO_2, pH, temperature are monitored automatically and displayed on the PC. TAN, nitrite and nitrate analyzed daily. Surveillance of all motor functions is integrated into the electrical control board and further to an alarm system. All essential functions including the power supply are duplicated. If the main and backup power supply systems fail an emergency oxygenation system will automatically switch on.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
			the alarm system is activated. In freshwater RAS, TOC, foaming and metallic ions are not considered a problem and is therefore not monitored.	All electrical motors are monitored and alarmed in case of failure, and key pumps and blowers have automatic start-up of back-up units. In case of power failure an automatic back-up electrical generator must start up. If oxygen is supplied by liquid oxygen there must be alarm for level in tank, if oxygen is supplied by O_2 generators only, the must be back-up O_2 generator and two electrical back-up generators.	checked manually.	
6	Fish health and RAS	A well designed RAS will have several potential benefits in terms of fish health compared to conventional flow-through systems. The low water exchange allows for thorough treatment of intake water hence reducing the risk of disease from intake water. Furthermore, the technology is able to provide the fish a more stable environment with fewer stressful fluctuations in water quality. The result of a good and stable environment is a fish that grows well and with low	Based on information from farming companies, in smolt farms using RAS operating in Norway, the smolts transferred to seacages have lower mortalities than their flow-through counterparts.	It is a fact that smolts from well dimensioned RAS perform as well or better than the best performing smolts from flow-through systems. In RAS with proper sterilization of intake water, dimensioned to keep good water quality during max load and only intake of healthy fish there are next to no problems with health.	The health of the fish in a RAS seems to be good due to the fact that the mortality is low, the FCR is low and the growth rate height compared to a flow-through system. In addition they have good performance when they are transferred to the cages.	All feedback from our customers clearly states that fish in RAS plants perform better than fish in flow-through systems, including better growth and lower mortality of smolt transferred from RAS.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
		mortality both in fresh- and seawater.				
7	Training of personnel	Training will always be a part of AKVA groups delivery. The training is normally split into a theoretical part followed by a practical training at the customers facility. The training will be performed by the Suppliers personnel that have several years experience with running RAS. The extent of training will depend on the size and complexity of the RAS. In addition, the Customers knowledge on the subject and previous experience will influence the need for training. Our facilities are delivered with a minimum of 14 days training, but we also have examples where we have delivered management support for up to 6 months. Whether or not the training is sufficient will be a natural decision made by the Supplier and Customer in cooperation. The Customer needs to be comfortable with running the facility and the Supplier need to be	AO offers a training package for their RAS. Furthermore, AO have the impression that the salmon producers also provides for adequate training. The training should be a combination of Theory and practice. (a) Seminar covering topics such as fish behaviour, water quality, literature (b) Practical session, start-up and operation of RAS We use AquaOptima personnel as teachers Seminar lasts for some hours. Need feedback from customer to decide whether the training course is understood and adequate. Sufficient theoretical background of the participants is necessary	We offer training in combination with the delivery of the RAS. Training takes place at the delivered RAS or other RAS of same type. All working field and processes will be thoughttraining is conducted by AQS supervicors. The length of the training period varies with competence level and what available time the customer has for training, but typical 3-6 months. We test the skills of the worker to make sure that the training is sufficient. It is common that the training is included in the sale of the RAS. The largest obstacles for a good training is limited time available due to a busy production schedule. Typical there is a 3 year hotline and support on-site visits every quartile the first year.	Billund Aquaculture will make periods of training of the personal on site and on all levels and deliver manuals consisting of: 1. Managing of a recirculated system 2. Technical description and system functionality 3. Water filtration - theoretical 4. Water parameters - theoretical 5. Service and maintenance of the system	As part of a standard package IAA provides training of personnel in context with commissioning of a plant, supplemented with on site follow up coaching 1, 3 and 6 months after commissioning. Further backup is included through telecommunication including direct PC access through the internet. Additional management supervision or full on site management support can be provided according to agreement.

Nb	Question /Theme	*AKVA Group ASA*	AquaOptima AS	Aquatec Solutions (AQS)	Billund Aquakulturservice	Interaqua
		confident that the Customer is able to run the facility in such a way that it from day 1 becomes a good reference. After the Customer has taken over the responsibility of running the facility, AKVA group will provide a ”hot-line” telephone support for a longer period of time. The facilities will also have a built-in possibility for remote control that allows the suppliers employees to advice and even take corrective measures without traveling to the site. The largest challenges in providing a good training are primarily the lack of available time for the Customer; there are always a number of other tasks that have to be done. We therefore recommend a part of the training to be undertaken “off-site”. It may also be a challenge to make the customer realize the value and need of investing in a proper training.				

Annex 3

Practical unpublished experiences

Experience I

The CO_2 levels measured in this RAS operating commercially were not in line with the recommended levels. At a fish density between 49 – 59 kg/$m^{3,}$ a water temperature of 12^0C, and a pH 6.5 – 6.7, the CO_2 levels were analysed[7] to be between 25 – 45 mg/L (see Figure 6). It should however be noticed the high CO_2 levels in the water entering the fish tanks (X, A & B), after being biofiltered, indicating that most of the CO_2 production in this system actually came from the biofilter itself.

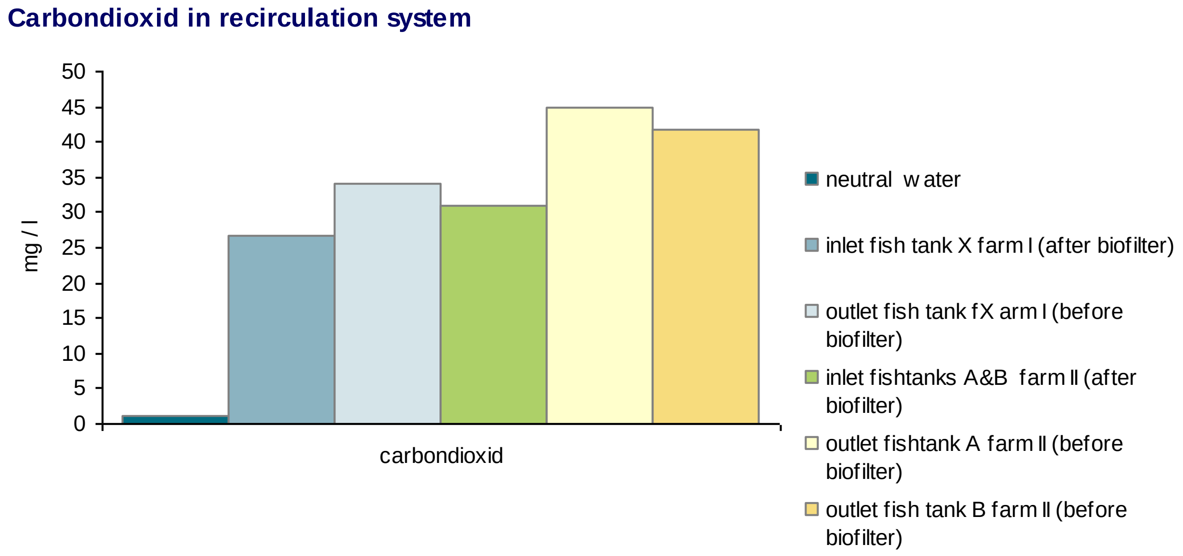

Figure 6. Carbon dioxide levels (mg/L) analysed from two fish farms using same recirculation technology compared to normal water, at the site.

From the same two farms, there are also data showing how many times the nitrite levels were above the recommended safe level for nitrite in soft water (Timmons and Ebeling, 2007). The level of nitrate was however analyzed by a kit at the farm and the accuracy of the method is uncertain. Anyhow, the data indicate that there was quite a big difference between the two farms (with the same RAS); concerning the ability to keep their levels of nitrite low (see Figure 7). We can also see that in 1999 Farm I had 97 days with too high nitrite levels, a worsening by 62 days since the year before. This indicates that the recommended value for safe levels of nitrite (in soft water) might be difficult to obtain in commercial RAS.

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[7] The CO_2 samples were conserved in glass bottles treated with mercury chloride ($HgCl_2$) and analysed by driving out CO_2 by bubbling without added acid and detection by NDIR detector (Phoenix 8000 TOC-TC) according to Standard Methods (APHA;AWWA;WEF;4500-CO_2, 4-12; 4-18).

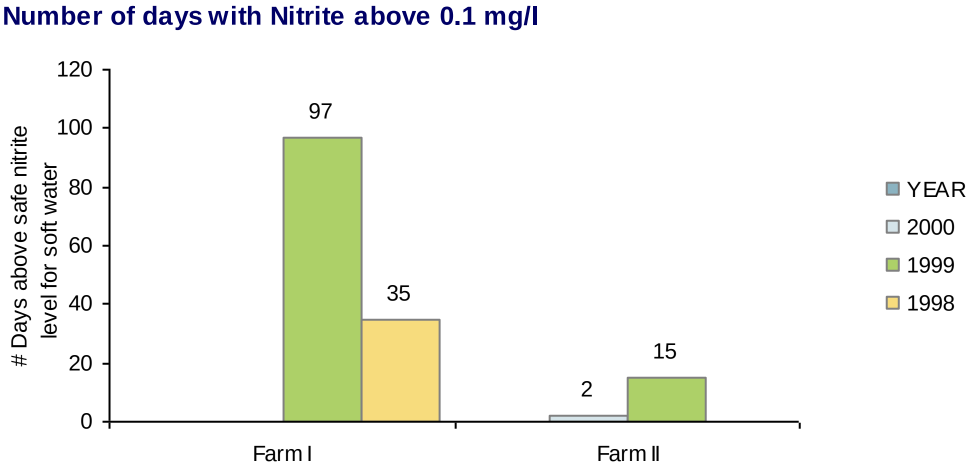

Figure 7. Number of days with nitrite levels above 0.1 mg/L in two farms using the same RAS technology.

Experience II

We have looked at some water quality data from a recirculation farm using technology from various sources. The data, which also include a single pass flow-through fish tank, illustrates some basic correlation in water quality and expected differences between single pass flow-through and recirculation systems. Figure 8 and 9 show a selection of water quality parameters of the water entering the biofilter, water leaving the biofilter, and in fish tanks. Data from one flow-through system is also given. The data can not be compared directly, but still they are rather informative as a description of operational values in a commercial farm using both technologies. The water is analysed by an accredited laboratory (NIVA) and can be trusted for good quality. It is interesting to see the higher level of total nitrogen and total nitrite-nitrate in the recirculation system compared to single pass flow-through system, and that the TAN levels were lower, or comparable to the single pass flow-through tank. We can also see indications of that the recirculation system, as expected, was "using up" alkalinity, and thus the alkalinity levels were lower than in the water passing the single pass flow-through tank. We can also see that the three different biofilters had different alkalinity and pH, which indicate a certain variation in the system. Furthermore, CO_2 was probably produced in the biofilters and therefore added extra CO_2 to the fish tanks. As for the TAN levels, around 60-70 % of the TAN flowing out of the fish tanks was converted to other nitrogen compounds. The TOC levels were quite high, but it should be pointed out that the water was very humic. Also, the single flow-through system had relatively high TOC level at 7-8 mg/L. The water-quality data illustrate the fact earlier discussed, that a recirculation system is a different production method, and cannot be directly compared to single pass flow-through systems.

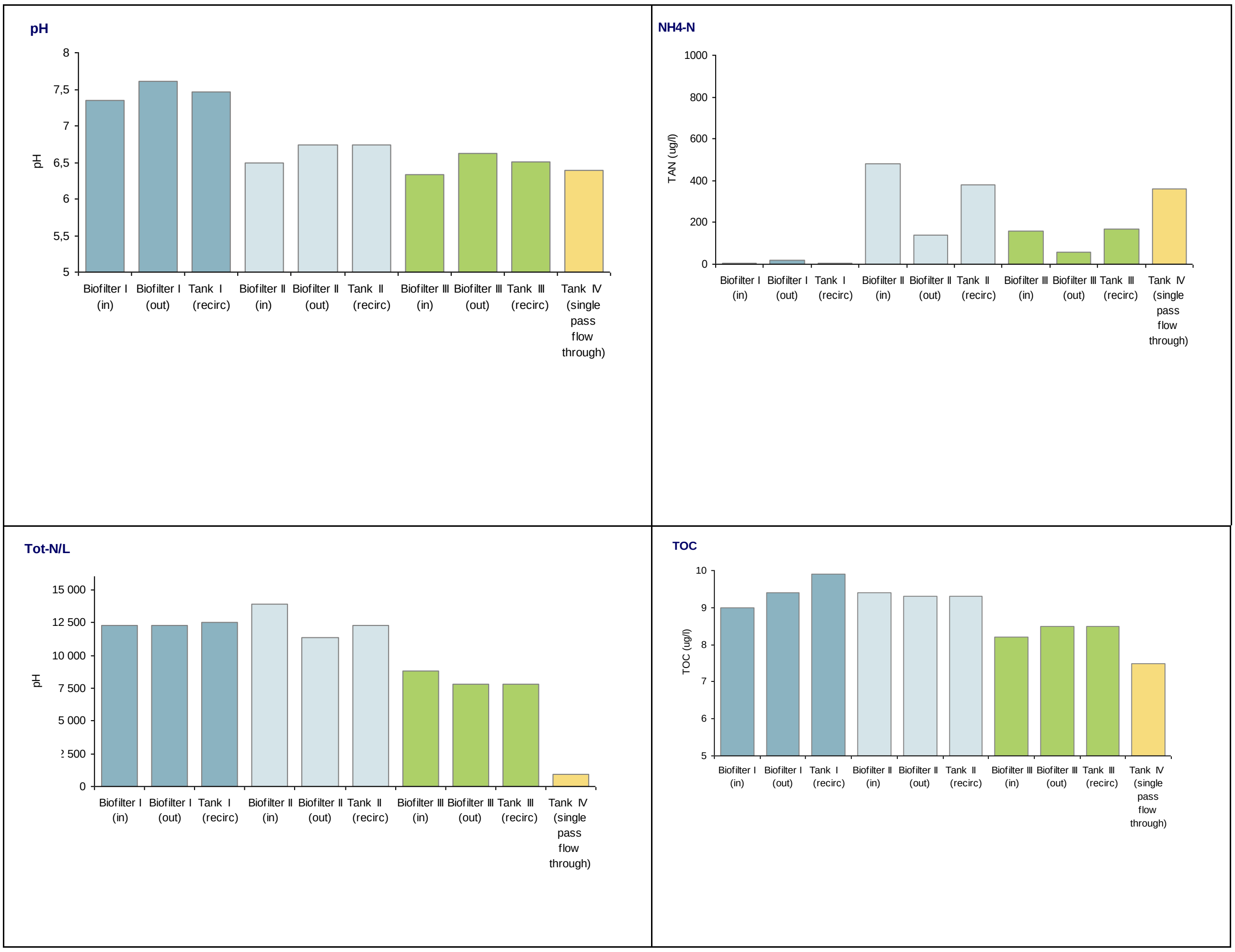

Figure 8. Water quality data from a smolt farm with both recirculation system and single pass flow-through tanks. Upper left: pH, upper right: TAN, lower left: total nitrogen, lower right: total organic carbon.

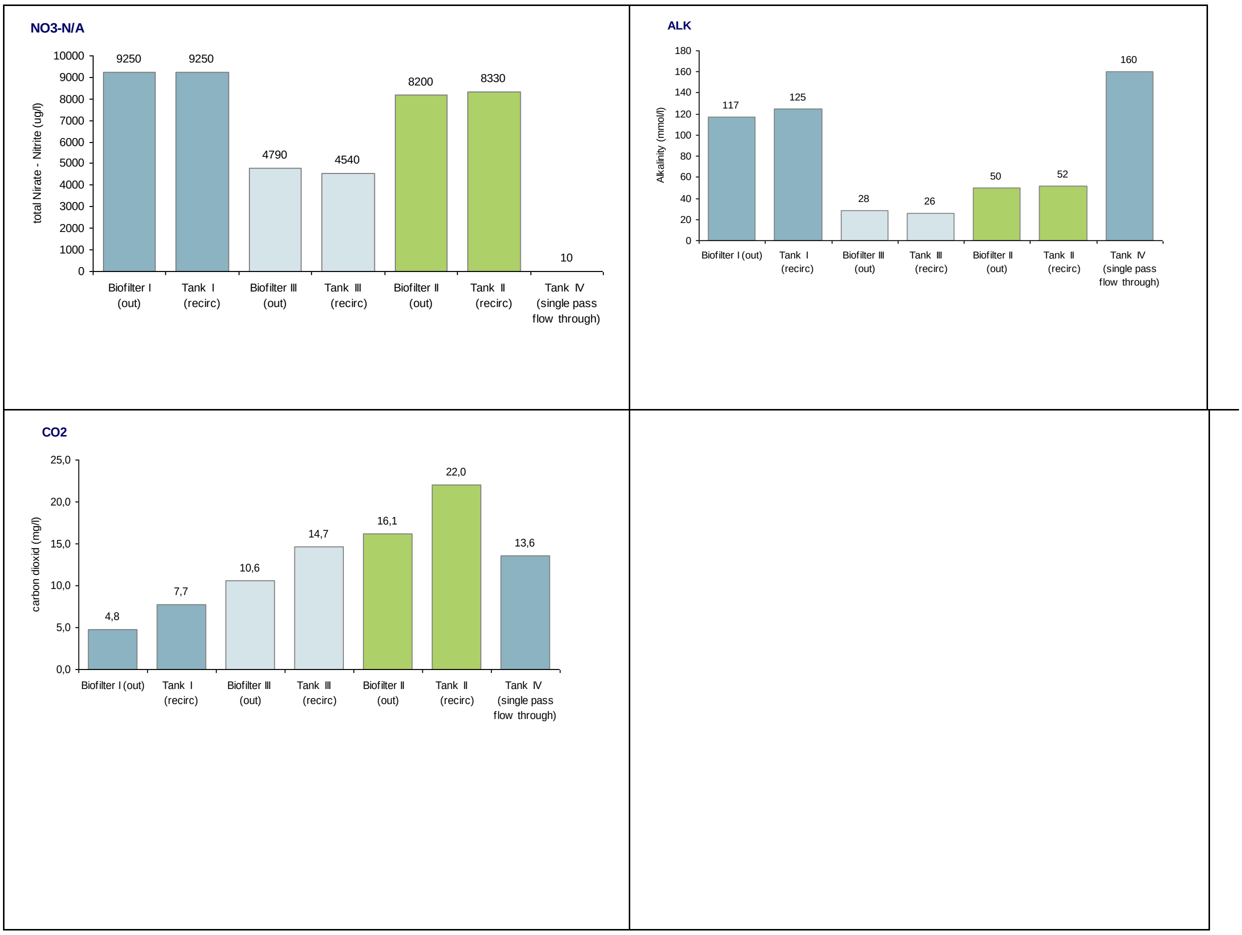

Figure 9. Water quality data from a smolt farm with both recirculation system and single pass flow-through tanks. Upper left: total nitrite-nitrate, upper right: alkalinity, lower left: carbon dioxide.