The Control of Water Quality and Hygienic Conditions in Aquaculture Recirculation Systems (RAS): The Use of Foam Fractionation and Ozone

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Abstract

The proper management of water quality is the key factors determining the successful operation of recirculation aquaculture systems (RAS). In this study a new engineering concept for a marine RAS was tested. The RAS involved a two-step solid separation procedure (swirl separator and ozone enhanced foam fractionation), biofiltration, and additional modules for water conditioning (pH, dissolved oxygen).

European sea bass (*Dicentrarchus labrax*) was the target fish species. Water quality within the rearing tanks was continuously monitored during the experimental period and maintained within safe limits. Water replacement in the system accounted on average about 1% per day of the total system volume. The two step solid separation techniques allowed to maintain clear water conditions. Fine solids and bacteria were efficiently removed by foam fractionation. In doing so, coastal water quality could be maintained throughout the rearing period. The data proof a good growth of European sea bass from less than 10 g to table-sized fish of 300g in approximately one year.

Key Words

Recirculation aquaculture system, ozonation, solid separation, growth, sea bass

Introduction

In view to the global decline of capture fisheries, recirculation aquaculture will gain importance not only to satisfy human nutrition but also to avoid the influence of aquaculture operations on natural ecosystems. Aquaculture production has grown continuously for years. Significant risks are connected with that. International expert commissions demand environmentally sound and sustainable technologies. The most promising technology are recirculating aquaculture systems (RAS) in which fish, shrimps, prawns, and other aquatic organisms can be grown uncoupled from the environment in a closed water cycle. The water in RAS is permanently conditioned (life support system) involving a variety of different biological, chemical and physical processes.

Recirculating aquaculture systems for marine aquaculture have been investigated by many authors. In the beginning the work was mainly descriptive and analytical (Rosenthal 1981). Presently RAS technology develops into a modern biotechnology (Timmons et al. 2001 Wheaton 1994). Nowadays studies look at overall system performance and on individual processes and components (Blancheton 2000; Kim et al. 2000; Lee et al. 2000; Olivar et al. 2000; Nijhof and Bovendeur 1990; Seo et al. 2001; Thoman et al. 2001; Borges et al. 2003; Barak et al. 2003) in order to maintain suitable conditions (Waller 2000) which are determined especially by the physiology of the species (Wedemeyer 1996).

Conventional recirculation technologies are often not suitable to satisfy the demands of the organisms under culture. Water quality is the prominent factor (Colt 2006) and requires thorough control. Therefore, water quality in conventional RAS is often maintained by means of water exchange that can reach one total replacement of water per day. Modern RAS technology is able to maintain adequate living conditions through an appropriate biotechnology that at the same time saves on energy. Many failures in RAS are caused by poor health status. Hygienic control insures welfare and survival; insufficient hygienic control may cause diseases and eventually lead to a total loss of life stock. Finally, animal behaviour is the decisive factor and supports viability. This has to be taken into account during design and operation.

A key problem in RAS relates to the load of suspended solids and in particular to very fine particles (Chen et al. 1993a; Chen et al. 1993b; Chen et al. 1994b; Han et al. 1998; Patterson and Watts 2003; Brinker and Rösch 2005; Brinker et al. 2005). Particulate waste (faeces, uneaten feed, bacteria and bacteria aggregates) in RAS impacts the water quality. Particles are subjected to microbial disintegration in RAS which consumes oxygen and increases the effort to maintain oxygen levels in the rearing tanks. Gill tissue can be damaged by particles (Bullock et al. 1994) which may lead to an insufficient internal oxygen supply besides osmoregulatory dysfunction. The brake down of organic waste increases the ammonia concentration in the RAS water affecting nitrification (Chen et al. 2006) and higher ammonia (NH_3) concentration may develop. Even small quantities of ammonia can be toxic for

epithelial tissues and disturb the elimination of excreta across the gills (Peters et al. 1984). Solids support the growth of heterotrophic bacteria within the RAS which can inhibit the nitrification process by out competing autolithotrophic bacteria strains or indirectly through an increase in dissolved carbon in the water (Ebeling et al. 2006). Suspended solids offer substrate for facultative pathogens while they try to find a final host (Bullock et al. 1994, Noble and Summerfelt 1996, Bouloux et al. 1998). The accumulation of solids can create anoxic conditions favourable for bacteria responsible for the production of geosmin and 2-methylisoborneol causing off-flavours in cultured fish (Tucker and Martin 1991). Recent findings reveal the incidence of new types of parasites in RAS like *Diplectanum sp.*, a trichodinid ciliate which normally needs good water quality (Colorni and Diamant 2005, Bouloux et al. 1998) but may use particulate matter as temporary substrate during certain stages of life. Another important aspect, especially in view to broodstock maintenance, induction of maturation, and spawning and fertilization, are pheromones which may cumulate in RAS and disturb reproduction. Ozone is very likely able to brake down pheromones as it oxidizes effectively organic molecules. It is generally accepted that ozonation increases the biodegradable organic matter, may reduce the amount of dissolved organic carbon (DOC) by mineralisation, and efficiently removes colour, smell, and taste. Unpleasant smell and aftertaste are problematic in conventional RAS.

Investigations into particulate waste focussed typically on suspended solids stemming form fish waste and/or non-consumed feed which amounts to approximately 300 g dry weight of solid waste per kg of feed (Chen et al. 1997). In view to microbial filters or reactors, which are needed in RAS to control the nutrient flow, also bacteria which had been washed off from the bio-substrate (immobilized bacteria) contribute to the very fine particle fraction. The amount can be estimated from the amount of ammonia nitrogen which is converted to nitrate during nitrification (Wheaton et al. 1997) and the amount of nitrate nitrogen which is converted to gaseous nitrogen (N_2) during anaerobic denitrification (Rheinheimer et al. 1988). In addition to this, an unknown number of heterotrophic bacteria are growing in the culture water of RAS using the dissolved organic matter which is excreted by the animals or stemming from leaching processes of feed and faeces (Lupatsch and Kissil 1998).

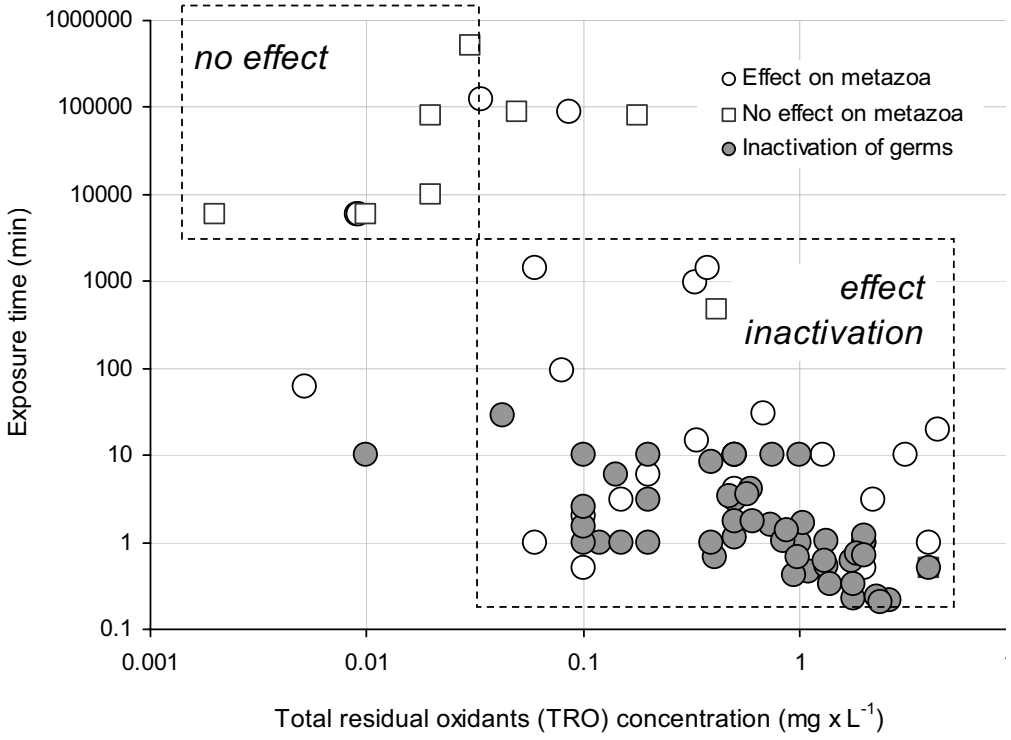

Figure 1: The effect of ozone and total residual oxidants in aqueous solution on different life history stages of fish and crustacean (egg, larvae, juveniles, and adults) and the disinfecting effect on bacteria, viruses, fungi and protozoa.

The removal of bacteria is a key process in RAS. Schlesner und Rheinheimer (1974) showed in a very early study that foam fractionation is an efficient measure to remove bacteria from recirculating aquaria systems. However, particle size in RAS ranges from some millimetres to micrometres and it appears to be necessary to employ different kind of filter systems which removes particles of different size. Larger particles can be removed by swirl separators (tubular settlers) or screen filters. Additionally, ozone may enhance the filtration process in foam fractionators, screen filters (Summerfelt 1997), or tubular settlers through aggregate formation or flocculation; the polar functional groups in oxidized organic matter enhance cross-linking between particles

In this study special attention was focussed on the ozonation of the seawater as ozone is a highly toxic gas which may impede animal welfare. Figure 1 shows effect and no effect levels for different life history stages of fish and crustacean (metazoa) of ozone versus the exposure time. However, ozone will quickly react in water and form secondary products (Herwig et al. 2006, Nicoson et al. 2002). In the following the ozone concentration is always expressed as total residual oxidants (TRO) because the secondary products interfere with the DPD measurement (Buchan et al. 2005) and

TRO measurements include the amount of ozone as well as the amount of secondary product.

Figure 1 gives a rough overview of the effect and no-effect concentrations and serves merely the orientation without the salinity, the temperature, the carbonate concentration or the pH being taken into account although these factors influence the solubility, stability and the reaction of ozone (Gottschalk et al. 2000). Ozone concentrations of more than 0.03 mg x L^{-1} are necessary to inactivate germs (Figure 1). The necessary exposure time for inactivation is likely to be longer at lower concentrations compared to high ozone concentrations (> 1 mg x L^{-1}). However, Coman et al. (2005) indicate that inactivation depends more on concentration than exposure time. In contrast, safe concentrations for different life history stages of fish and crustaceans is below 0.03 mg x L^{-1} (Figure 1, no effect on metazoan), while the effect level for metazoan is similar to the inactivation level for germs. Low concentrations will not inactivate bacteria, virus, fungi, or protozoan. Thus, inactivation of germs (disinfection) with ozone requires a separation of water treatment processes in order to avoid harmful residual oxidants in the holding tanks. In view to the tolerance of different life history stages of fish to the exposure to ozone (Figure 1) the long term doses is at least one dimension below the doses necessary for inactivation. Granulated activated carbon (GAC) filtration is a suitable method for removing toxic ozone residuals or by-products (Chang, et al. 2002; Lee and Deininger 2000; Urfer, et al. 2002; Litveld and Vogelsang 2006) from the treated water. If ozone dosage is raised to levels that bromate is formed, the removal of by-products is much more difficult (Yang 2000).

Ozone should be used in aquaculture at low doses. The application of ozone for water treatment is well known from aquaria. In these facilities metazoans as well as microbes are maintained for years (Figure 1, >> 0.5 x 10^6 min) without any adverse effects on living creatures at TRO concentrations ≤ 0.03 mg x L^{-1}.

Total residual oxidants concentrations above 0.1 mg x L^{-1} should never be reached in aquaculture situations because they are very likely caused by toxic by-products which are extremely dangerous as they are more stable in water compared to ozone. Such a situation would be inadequate in a RAS not only because of animal welfare or animal rights considerations but also in view to the economic risk of a complete failure.

Table 1: Technical specifications of both, the KIEL RAS (prototype) and the ELTZE RAS (commercial size module).

	KIEL RAS	ELTZE RAS
Total volume (m^3)	4	140
Number of fish tanks	2	1 partioned tank with 3 compartments
Tank volume	1.1 m^3	120 m^3
Production capacity	0.4 t x a^{-1}	10 t x a^{-1}
Projected stocking density	60 kg fish x m^{-3}	60 kg fish x m^{-3}
Seawater source	Baltic sea water, commercial sea salt mixture	Tap water, commercial sea salt mixture
Aeration	Air lifts, air diffuser	Air diffuser
Oxygen dosing	No	Yes
Nitrification biofilter	Moving bed	Moving bed
Denitrification biofilter	No	Moving bed
Ozone generator	4 g x h^{-1}	120 g x h^{-1}

Materials and Methods

European sea bass (*Dicentrarchus labrax*) was selected as experimental species because of its importance in European aquaculture. The fish came from a hatchery facility in northern France (Ecloserie Marine de Gravelines). During the growth trials fish were fed with a commercial pellet feed (42 % protein, 22 % fat). Pellet size was different for different size of fish.

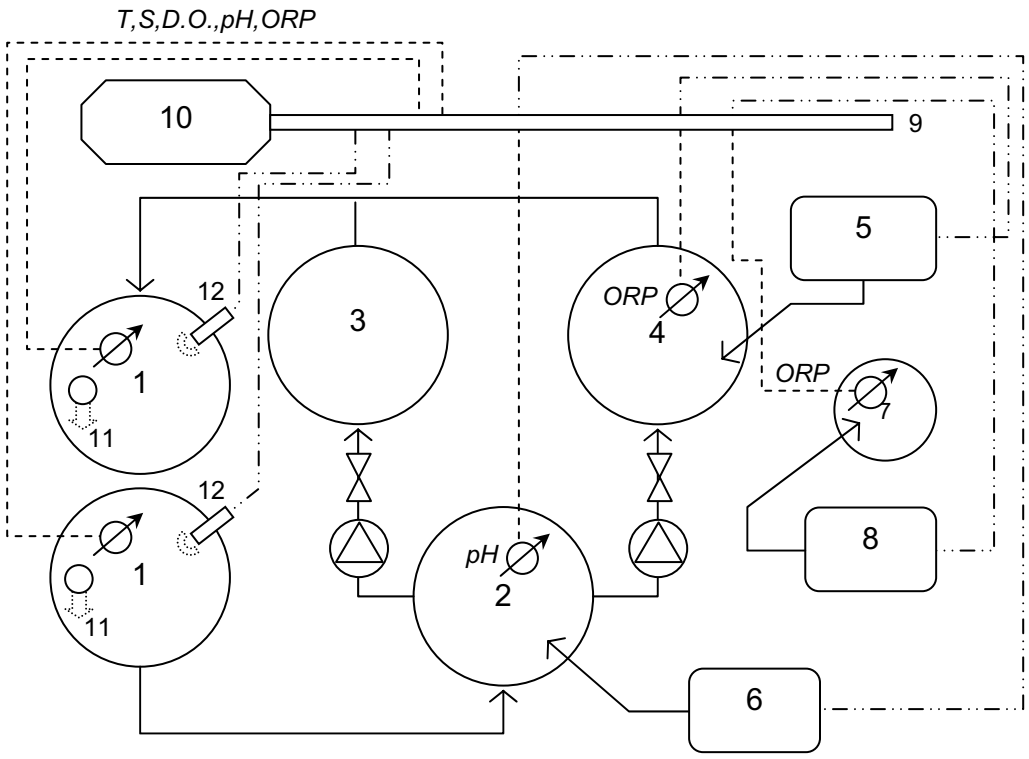

Figure 2: Principal components of the experimental recirculating aquaculture systems. 1 fish tank, 2 swirl separator, 3 nitrifying biofilter, 4 foam fractionator, 5 ozone generator, 6 buffer (CaO, $Ca(OH)_2$) metering device, 7 denitrifying biofilter, 8 methanol (CH_3OH) metering device, 9 computer bus, 10 programmable logical control (PLC, only ELTZE RAS), 11 water jet, 12 automatic feeding device. Solid lines and arrows indicate the flow of water, air, ozone, buffer solution, and methanol. Dashed lines are data input lines to the PLC. Dash-dot-dot lines are output signals to peripheral devices. Drawing is not to scale.

Experiments were conducted in two marine RAS. One prototype RAS (Waller et al. 2001) was operated at the Leibniz Institute for Marine Science in Kiel (KIEL RAS), Germany. The second system (ELTZE RAS), a commercial scale module, was operated inland in the vicinity of Hannover at Erwin Sander Elektroapparatebau GmbH in Eltze, Germany. Table 1 summarizes technical specifications of both RAS.

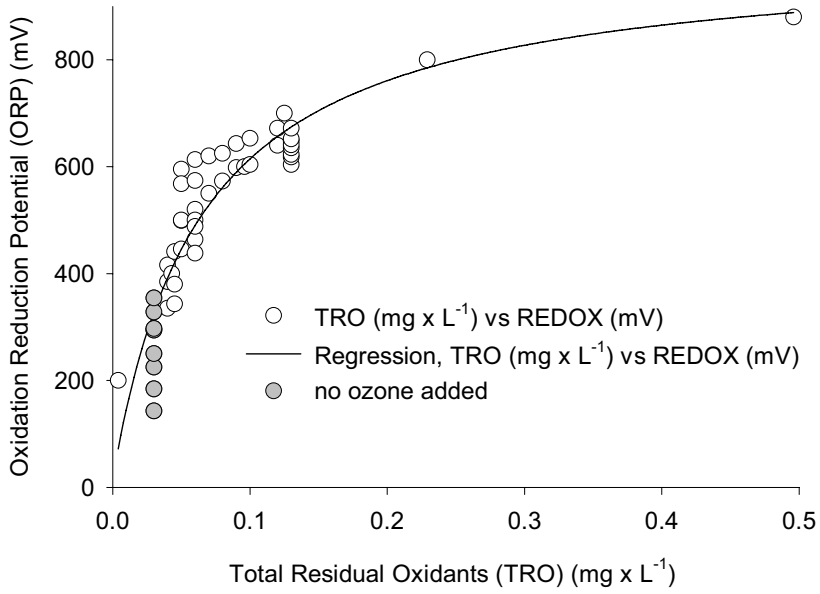

Figure 3: The concentration of total residual oxidants (TRO) in an aqueous solution versus the measured ORP. TRO concentrations were determined with the DPD (N,N-diethyl-p-phenylenediamine) colorimetric method (HACH commercial test kit No. 8177, conversion factor $Cl_2/O_3 = 0.68$).

Figure 2 shows the principal components of the RAS in Kiel and Eltze. Arrows indicate the flow (water, air, ozone, calciumhydroxid, and Methanol). The fish tanks were circular (Kiel) or rectangular (Eltze). Continuous water current was induced by air-lift jets that at the same time were supplying oxygen into the water. Automatic feeders were mounted on the tank wall evenly distributing the feed over the water surface. The water effluent to the fish tanks was initially treated in swirl separators removing the large particle fraction. In a next step, the water was passed over the nitrifying biofilter and the foam fractionator, before it flowed back into the fish tanks. The foam fractionator was operated with ozone generated in a corona discharge ozone generator. Secondary water treatment components were a buffer metering device to maintain water pH above 7. To remove nitrate (NO_3^-) from the water a denitrifying biofilter was installed as bypass system and operated with methanol (CH_3OH) as organic carbon source. All system functions of the ELTZE RAS were controlled by a programmable logic control (PLC) connected to a data bus. The KIEL RAS was controlled by decentralized analog/digital control circuits. Water replacement in the system accounted to about 1% per day of the total system volume on average, i.e. there was no regular exchange, water got lost together with the waste removed in the swirl separator and foam fractionator.

RAS design was based on growth algorithms allowing one to estimate the flow of inorganic and organic matter in the systems. The KIEL RAS was based on literature data for sea bass. The ELTZE RAS growth algorithm was based on parameters derived from both literature data and experimental results of the KIEL RAS:

[1] $w_i = 2.599 \times 10^{-3} \times t_i^2 + 3.5527 \times 10^{-15} \times t_i + 4.9386$ *sea bass growth algorithm*

where:

w_i: individual weight (g wet weight)
t_i: time (d)

The growth algorithm allowed to iteratively calculate the total biomass in the RAS based on the initial number of animals, the estimated daily mortality, the body weight of the animals, and the projected annual production. In further calculations the volume of swirl separators, biofilters, and foam fractionators was estimated in order to obtain the necessary engineering parameters.

Figure 3 shows the relationship between the concentration of total residual oxidants (TRO) and the ORP. Thus, the amount of ozone injected to the foam fractionator can be controlled by measuring the oxidation reduction potential (ORP). For that an algorithm was empirically developed, which relates the concentration of oxidants, which was determined with the DPD (N,N-diethyl-p-phenylenediamine) colorimetric method, to the ORP measured in millivolt. Because this method measures ozone and the by-products of the ozonation process as well as natural oxidants in seawater, the term TRO (total residual oxidants) is subsequently used.

[2] $ORP = 0.0047 \times^{(0.0051 \times TRO)}$

where:

ORP: Oidation reduction potential (mV)
TRO: Concentration of total residual oxidants

During RAS operation the ORP concentration in the foam fractionatior was maintained between 300 and 450 mV corresponding to a TRO < 0.05 mg x L^{-1} in the reaction chamber (Figure 3). The retention time in the reaction chamber of the foam fractionator was between 1.5 to 3.0 min. The maximum allowable ORP was set to 350 mV or < 0.04 mg TRO x L^{-1} in the fish tanks (Figure 3).

Results and Discussion

With the used technology the fish (*Dicentrarchus labrax*) grew up successfully in both RAS system. Figure 4 shows the growth of one sea bass cohort during an intense measuring period in the ELTZE RAS. The measured growth was identical to the predicted growth which is indicated in Figure 4 through the solid line. The inserted bar charts show the frequency distribution for the individual weight measured at the five sampling times emphasizing a tremendous increase in the data range and variance. This is due to the competition of fish for space and feed. The largest fish are dominant and usually control the tank area through continuous inspection. They chase subdominant individuals and drive them out of the feeding area. Under such conditions, small fish do not have full access to feed and remain smaller while the

dominant fish quickly gain weight. Future tank design has to take this into account by probably changing the type, mounting and feeding schedule of the automatic feeders.

The design parameters for the water treatment system, which had been derived from numerical modelling using the growth algorithm (equation 1), fitted optimal and water quality could always be maintained in safe limits (Table 2). As result, the growth performance compares well to that reported for the same species at similar environmental conditions (temperature, salinity) (Paspatis et al. 1999; Thetmeyer et al. 1999; Zanuy and Carrillo 1985; Waller et al. 2002; Waller et al. 2003). The feed conversion ratio (FCR) averaged 1.8 in the experiment. FCR reported from other investigations ranged from 1.2 to 1.8 for similar fish size (Ballestrazzi et al. 1998, Dosdat et al. 2003, Eroldogan et al. 2005, Montero et al. 2005, Paspatis et al. 2003, Peres and Oliva-Teles 1999). It can be concluded that sea bass attained an optimum growth in the ELTZE RAS which would allow to growth them to market size in approximately one year time.

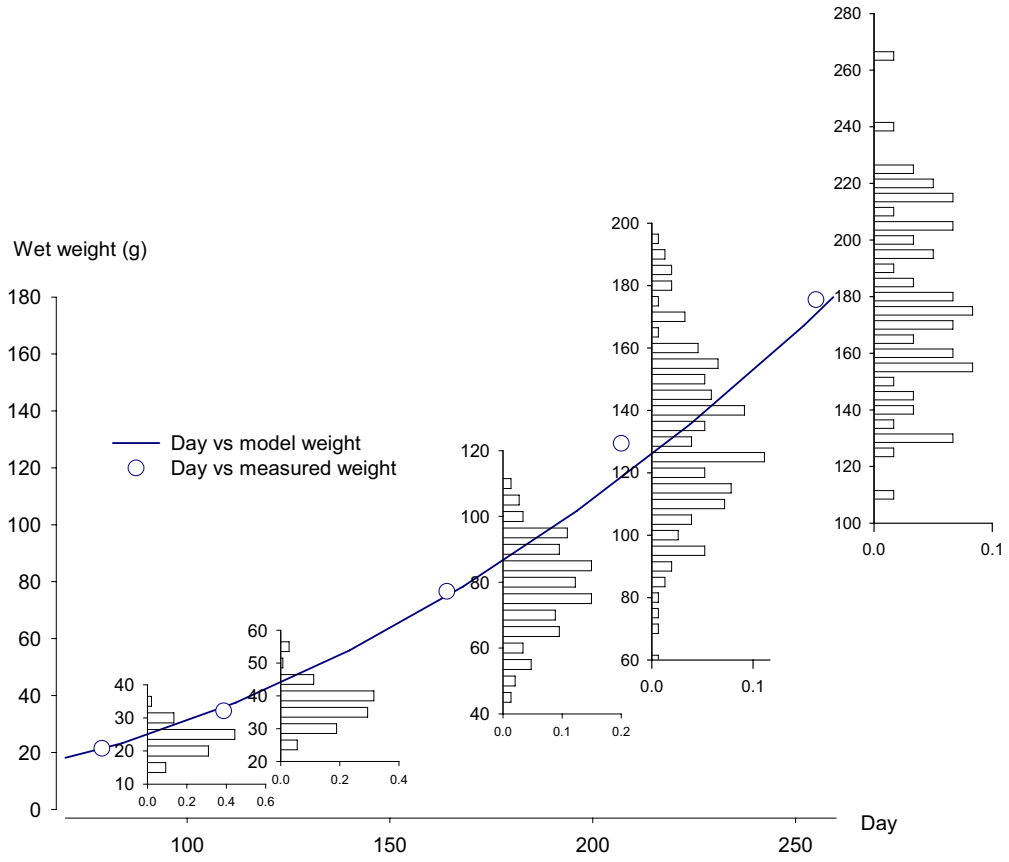

Figure 4: The growth of juvenile seabass (*Dicentrarchus labrax*) during an intense experimental period in the ELTZE RAS. Inserted bar charts show the frequency distributions for individual weight at the different sampling times. The solid line represents the projected growth using the growth algorithm (equation 1). The growth curve was shifted along the *x*-axis until the initial weight of animals matched that at the beginning of the growth curve.

Water temperature and salinity were maintained at 23.5 ± 0.2 (KIEL RAS) and 24.0 ± 1.3 (ELTZE RAS), respectively (Table 2). Sea bass (*Dicentrarchus labrax*) is a typical warm adapted marine aquaculture species with a temperature preferendum above 20 °C and a wide range of salinity (Alliot et al. 1983; Ayala et al. 2001; Claireaux and Lagardere 1999) and it can be concluded that water temperature and salinity were maintained to the need of this species. The dissolved oxygen saturation was maintained at 82 ± 7 % (Table 2) which is optimum in fish. The pH was kept at 7.7 (Table 2)

Table 2: Water quality in the ELTZE RAS at maximum stocking density of around 60 kg of fish x m^3 tank volume.

	KIEL RAS	ELTZE RAS
Water temperature (°C)	22.2 ± 1.1	23.5 ± 0.2
Salinity (psu)	22.5 ± 2.0	24.0 ± 1.3
$pH_{tank\ inlet}$	7.5 ± 0.4	7.7 ± 0.2
$pH_{tank\ outlet}$		7.7 ± 0.1
$ORP_{foam\ fractinator}$ (mV)	272 ± 89	362 ± 89
$ORP_{tank\ inlet}$ (mV)		202 ± 32
$ORP_{fish\ tanks}$ (mV)		174 ± 30
Dissolved $Oxygen_{fish\ tanks}$ (% saturation)	90 ± 8	82 ± 7
NH_3/NH_4^+ - N (mg x L^{-1})	1.01 ± 0.66	0.96 ± 0.82
NO_2^--N (mg x L^{-1})	0.22 ± 0.14	0.68 ± 0.54

Ammonia (NH_3) is a limiting factor (Tomasso 1994) in aquatic animals. In aquaculture it is desirable to keep the unionized ammonia level below 0.1 mg NH_3-N x L^{-1} because first measurable physiological changes in fish may already occur in this range. An ammonia concentration of 0.1 mg NH_3-N * L^{-1} corresponds to a total ammonia nitrogen concentration (NH_3/NH_4^+ -N) of around 2.0 mg x L^{-1} (Bower and Bidwell 1978; Whitfield 1974). During growth trials the total ammonia concentration (NH_3/NH_4^+) was maintained below 0.96 mg (Table 2). During aerobic biofiltration ammonia is converted into nitrite (NO_2^-) as intermediary product which is also toxic to fish. Nitrite-nitrogen concentrations (NO_2^--N) averaged 0.68 mg*L^{-1} (Table 2). The average values for both total ammonia and nitrite during the growth trial are safe levels in view to the reports by Blancheton (2000) and Roncarati et al. (2006).

The end product of aerobic nitrification is nitrate (NO_3^-) which cumulates in RAS. Nitrate concentrations (NO_3^--N) for marine fish should not exceed 500 mg*L^{-1} (Colt 2006). In this growth trial nitrate concentration (NO_3^--N) never exceeded 462 mg x L^{-1}. To achieve that, it is indispensable to use denitrification biofilter in RAS because of the steady supply with nitrate from the nitrification process. Another compound which is cumulating in RAS is phosphate (PO_4^{3-}) which is introduced together with the feed which contains fishmeal (bonemeal). Phosphate concentration (PO_4^{3-}-P) stayed below 16.7 mg x L^{-1} (Table 2). Phosphate was partly removed parallel with the removal of nitrate in the denitrification process as bacteria also require phosphate in their metabolism. Safe limits are not known for fish so far.

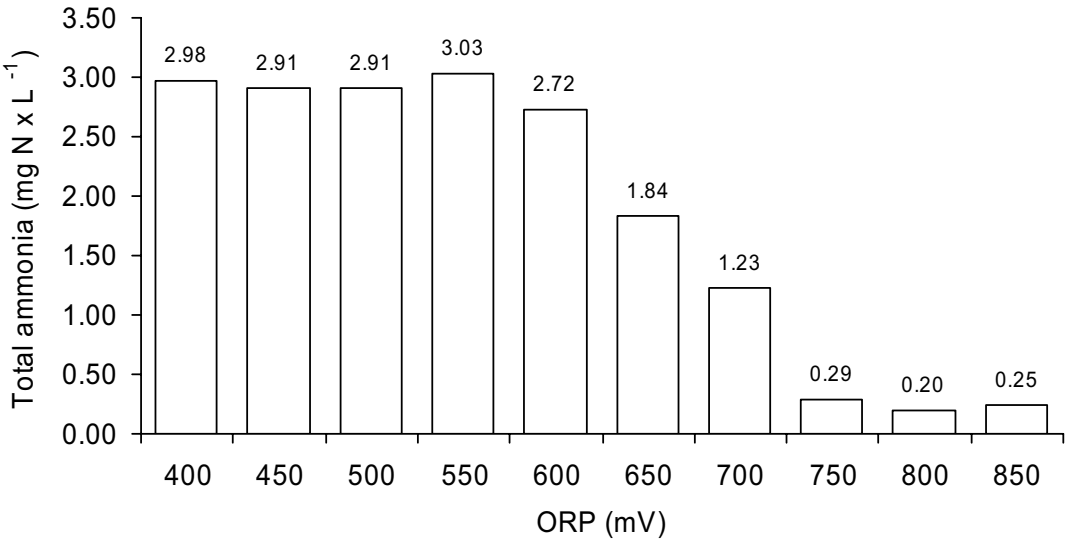

Figure 5: The oxidation of ammonia (NH_3/NH_4^+) in seawater at increasing oxidation reduction potential. The initial total ammonia concentration was adjusted by the addition of NH_4Cl salt. The ORP was stepwise increased by dosing ozone gas into the seawater. Total experimental time, i.e. the period from the start until 850 mV ORP was reached, was 19 min.

It was found during operation of both RAS that nitrite was readily oxidised during foam fractionation which was reported by Rosenthal and Wilson (1987). The comparably low nitrite concentration (0.22 mg x L^{-1}) in the KIEL RAS can possibly be attributed to the oxidation by ozone (Table 2). This, however, should not be attempted during normal operation in RAS as nitrite removal is an inherent part of the biofilter function. In the end, a stoichiometric conversion by ozone would require additional energy which is not desired in RAS. Ammonia behaves differently and cannot be oxidized at low ozone concentrations. Figure 5 shows the result of an experiment in which Ammonium was decomposed by the addition of ozone. During the first phase ammonia remained constant. Above 600 mV ORP ammonia got decomposed and concentration dropped to one tenth part of the initial value. In the experiments of this study an ORP sufficient for an oxidation of ammonia was not reached. Therefore,

ammonia and nitrite were subjected to decomposition in the nitrifying biofilter. In RAS the biofilter capacity must be sufficient to biologically detoxify ammonia and nitrite, even if ozone is used as reactive agent.

Total residual oxidants were relatively high during ozone treatment in the foam fractionator but quickly decomposed afterwards so that safe limits were never exceeded during the growth trial. While the ORP in the reaction chamber of the foam fractionator amounted to 362 mV on average (Table 2), the average values at the tank inlet and in the tank water was 202 and 174 mV, respectively. These values corresponds to TRO residuals of less than 0.03 mg x L^{-1}. Thus, a thorough control of the ozone dosage is sufficient to maintain safe levels and it appears not to be necessary to take additional measure in this type of RAS like an activated carbon treatment recommended by Litveld (2006). This may be an additional safety measure in RAS for very sensitive species or life history stages.

Another important aspect of solid separation was the removal of bacteria from the system water which had been investigated in the KIEL RAS (Figure 6). Bacteria samples were taken in the inlet of the foam fractionator, in the outlet of the foam fractionator and biofilter, and from the foam beaker where the foam condensate got collected. The data from the KIEL RAS showed that during continuous feeding the bacteria counts were reduced to one third of the initial number during foam fractionation (Figure 6, B). In the foam beaker the number of bacteria increased by two magnitudes. Another important finding was that the application of ozone reduced markedly the number of viable bacteria (Figure 6); all bacteria counted in the foam condensate were not able to build up new colonies. This is interestingly as the retention time (exposure time) to ozone and residual oxidants in the foam fractionator was comparably short and the TRO concentration comparably low (Figure 1). However, the ozone gas was leaving the reaction together with the foam so that the treatment time for bacteria attached to the foam had been much longer and it is likely that high ozone concentrations had developed at the foam/air interface.

When fish were not fed the effects were not as obvious (Figure 6, A). Bacteria counts remained stable in the inlet and the outlet of the foam fractionator. The number of bacteria removed with the foam was one third of that measured under continuous feeding. However, the inactivation of bacteria in the foam beaker is obvious (Figure 6, A, < 10 % colony forming units). A difference in bacteria counts in the inlet and outlet of foam fractionator was not detected. Reason for that was the small difference in bacteria numbers and the high water flow through the foam fractionator compared to the volume of foam condensate removed during a unit of time. However, even very small changes in bacteria counts may result in a significant decrease of bacteria in a RAS if bacteria are continuously harvested.

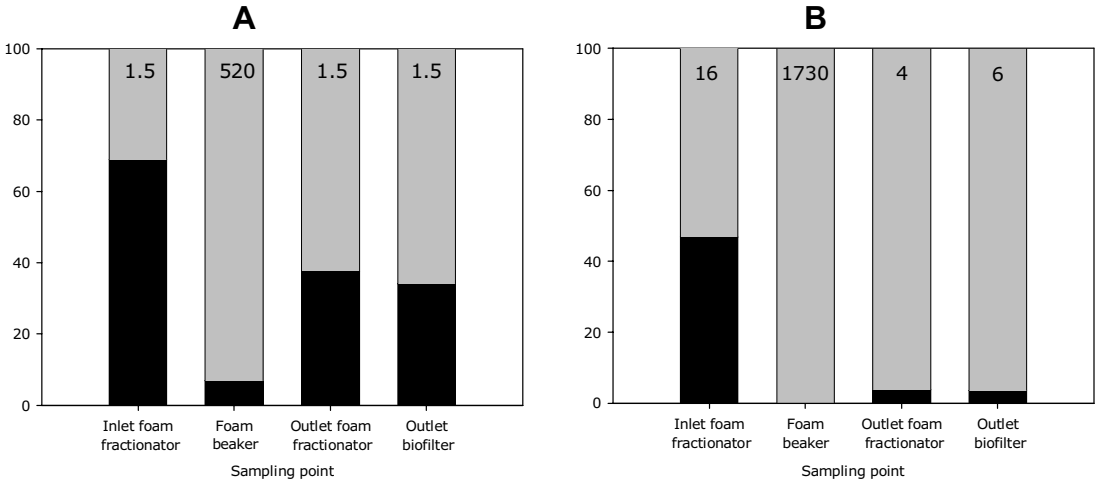

Figure 4: Total bacteria counts (% of the total), categorized in colony and non-colony forming units (CFU (black) and nCFU (grey)), collected from the inlet water, foam beaker, and outlet water of the foam fractionator. Number in the columns represent total bacteria counts in millions cells*mL^{-1}. Left: No feeding situation. Right: Continuous feeding.

Schlesner and Rheinheimer (1974) observed the elimination of bacteria through foam fractionation after the first foam fractionator had been installed in the Kiel Aquarium at the Institut fuer Meereskunde in Kiel (Sander and Rosenthal 1974). The bacteria removal by foam fractionation was between 3.6% and 35.5% in the KIEL RAS and allowed to maintain coastal water quality, i.e. 1×10^6 to 4×10^6 bacteria cells per mL. This number was higher than those reported by Leonard et al. (2000, 200 cells x ml^{-1}) and by Michaud et al. (2006) (2.3×10^5 and 7.7×10^5 cells per mL). It is assumed that the presence of particulate organic matter (POM) and the availability of dissolved organic carbon (DOC) led to a higher abundance of free living bacteria. Nevertheless, the importance of continuously removing bacteria from the system water is obvious from the results.

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