Final project report

Litus Akva AS RAS and Dynasand filter evaluation

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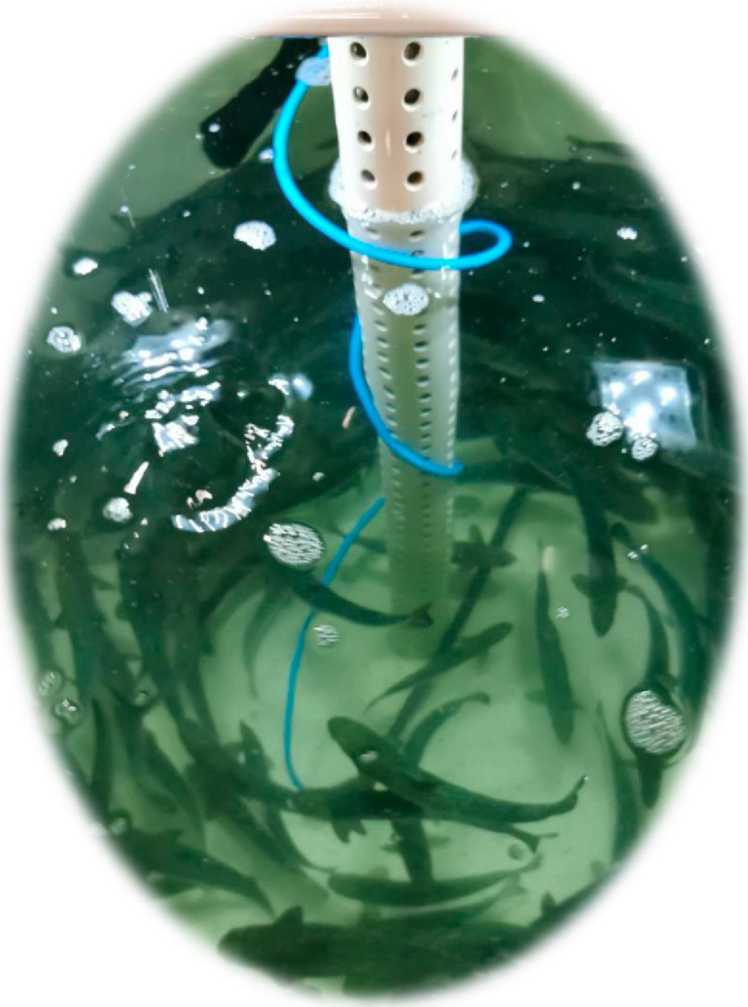

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Marineholmen RASLab

Litus

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Summary

A RAS system was set-up by Litus Akva AS located at Marineholmen RASLab AS. The system incorporated a Dynasand filter for evaluation for performance for solids and biological filtration. The system established biological filtration capabilities within 4 weeks with ammonium oxidation and nitrification occurring following use of a bacterial inoculum and supplemental use of biologically active media from RASlab to support the initial fish loading. Initial fish loading of 250 g Atlantic salmon post-smolts at a stocking density of 27 kg m^{-3}, the system was maintained until growth of the fish facilitated 80 kg m^{-3}. Water flow rates and water quality were monitored regularly through the production period. Fish were periodically sampled from the system and assessments made of gill health and fish welfare scores. Fish performance was very good with growth rates similar to those expected in most commercial RAS systems at an equivalent stocking density. Minimal impact on fish welfare was seen. System performance was excellent resulting in a high degree of water recirculation, minimal make-up water requirement and maintenance of water quality under intensive fish production. Finally, a test of the maximum nitrification capacity was made by the bulk addition of 300g NH_4Cl to the system, giving an ammonium oxidation rate of up to 0.95 mg L^{-1} h^{-1}. Overall, the system performed very well capable of maintaining and growing post-smolt Atlantic salmon to approximately 900g within 3 months to commercial stocking densities.

Keywords: Atlantic salmon; salmon RAS aquaculture; water chemistry; Dyna sand filter; fish health and welfare.

Disclaimer:

The attached report represents the evaluation of data collected by Marineholmen RASLab AS as part of the Litus Akva AS project conducted on the premises. The data are correct as of those recorded by RASLab employees for RASlab instruments or using instrumentation attached to the Litus Akva System following instruction from Litus Akva personnel. Additional material contributed by Litus Akva AS is included and discussed and indicated and duly referenced. Marineholmen RASlab AS does not warranty guarantee or endorse any data that may have been collected independently of those instructed to be collected by Litus Akva staff. All interpretations of the data presented are made in good faith and are the opinion of the author.

INTRODUCTION

Litus Akva (Litus) is a newly established company with the purpose of developing and commercializing a new filter technology for RAS facilities for juvenile Atlantic salmon (*Salmo salar*), post-smolt and food fish production on land. Litus Akva AS was founded in 2020 in Øygarden municipality (Vestland, Norway) with the owners having a long and strong foundation in hatchery production with RAS technology, both from operational operations, as engineers and as sellers of RAS technology.

In the last ten years, large investments have been made in hatcheries and post-smolt plants based on technology for Recirculating Aquaculture Systems (RAS). Despite large investments and intensive technology development, significant challenges associated with maintaining a stable and good aquatic environment for the fish remain.

The Litus Akva AS hypothesis was that the novel RAS technology would provide:

1. Reduced risk of hydrogen sulfide formation, and thus less risk of mass death.

2. In general, better water quality, and thus better fish welfare and appetite.

3. Less particles in the water, and thus better gill function for the fish.

4. Reduced costs and risk of operational interruptions in connection with maintenance and cleaning of the biofilter.

5. Eliminate the issue of microplastics from today's biofilters

The innovation - the new biofilter technology

The current state of knowledge is that sand has never been used as a medium in biofilters for RAS plants in Norway. In the food industry, drinking water systems and for final treatment of sewage, however, sand filtration is a well-established water purification method. The method has been in use for a longtime and it is thoroughly documented with regard to the removal of fine particles (fines) and organic material. Although sand filtration has been used in aquaculture culture systems elsewhere in the world, these have often been limited to relatively small-scale systems and limitations in stocking densities. The biggest challenges being that of organic accumulation in the sand filter causing a caking and channeling effect providing routes for short circuiting water flow through the filter medium. Thus, static bed filtration is not commonly used where large commercial volumes of production are required. To overcome this, the sand needs to be maintain with constant flow (kept in constant motion) where the particles become trapped within the matrix – a similar concept to that of bead filters that are popular in North America (Timmons et al. 2018).

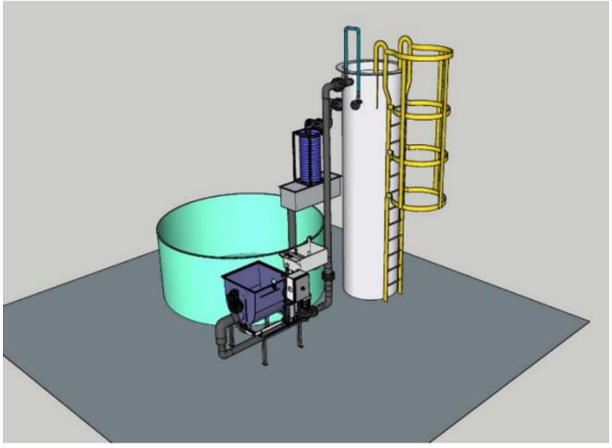
The efficiency of the biochemical processes within a biofilter biofilm depends on many factors, but temperature and biofilm area per unit volume of the biofilter media critical. Unlike plastic artificial media that is designed to have a large surface area to volume ration, sand has a large surface area per unit volume compared to synthetic biomedia used in conventional RAS facility. Thus, the use of a sand media could potentially be advantages for both solids removal as well as biofiltration and ammonia/nitrite oxidation.

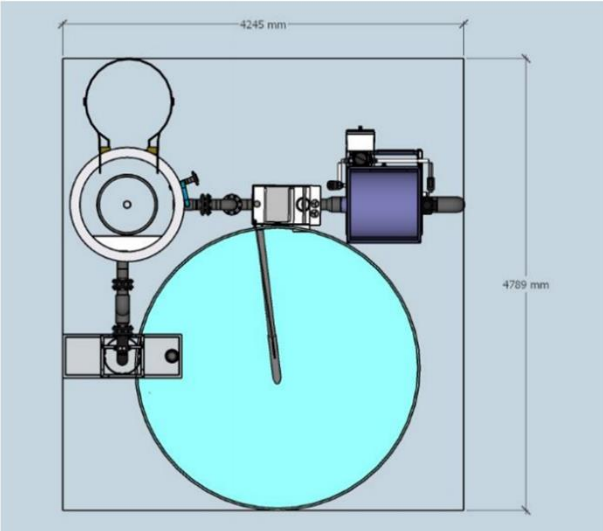
Objectives

1. Assess the ability of the RAS system using a sand filter for biological removal of nitrogenous waste as well as suspended solids filtration to maintain water quality under increasing biological load.
2. Determine the biofilter capacity development and maintenance of function under increasing biological load (growing fish)
3. Determine suspended solids removal by the sand filter and other locations within the RAS system.
4. Determine the potential ammonia oxidation rate of the sand filter biological activity under acute load stress.
5. Determine fish production characteristics and assess fish health and welfare of Atlantic salmon under post-smolt production in brackish water.

METHODS AND APPROACHES

The study was set-up following the design by Litus Akva AS where a 7m^3 round tank with a central drain and sloping floor was connected to the vertical sand filter containing 1.2 m^3 of Dynasand. High pressure (6 bar) air was used, in addition to water flow, to maintain the fluidity of the sand filter. Additionally, a degassing column located over the tank and a drum screen filter with a 40 µm screen was incorporated into the system. The system had an approximate operational volume of 8 m^3. Water circulation was maintained by a centrifugal pressure pump. Oxygenation was maintained using a high-pressure oxygen supply delivered via a ceramic diffuser rack located in the tank floor. Make-up water was added from a UV filtered and flocculated freshwater and filtered seawater (34 ppt) supply (RASLab AS) and regulated manually and adjusted daily. Alkalinity was maintained by the addition of a bicarbonate solution, titrated by pump into the filter sump controlled by a pH sensor (Oxyguard). The system was controlled and monitored using Oxyguard O_2, temperature, CO_2 and pH sensor arrays (Oxyguard commander).





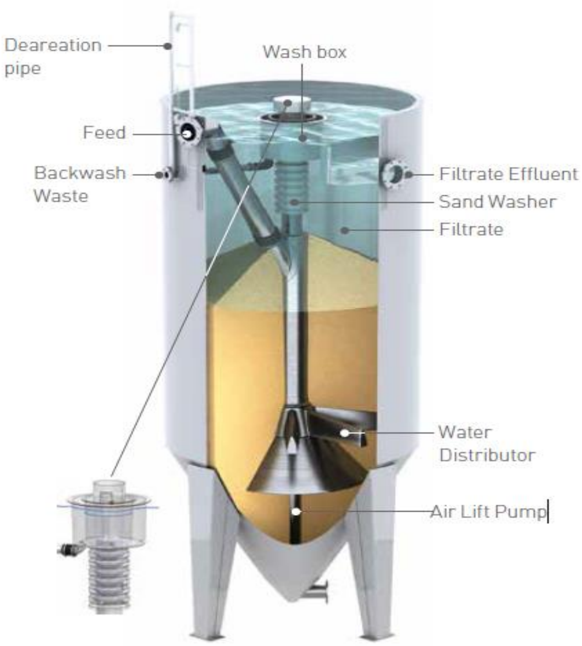


Figure1. 3D schematic and plan of the test systems and cutaway of the Dynasand filter

Figure 2. Elements of the Litus Akva AS system with a 7 m3 holding tank for fish with automatic feeding control and lights (top left), Dynasand filled biofilter sand filter tower, (top right), 40 µm screen filter (middle left), CO_2 degassing column (lower left) and main circulating pumps (lower right).

Daily measurements were made of the tank salinity, temperature, pH and O_2 as well as water consumption. The tank CO_2 concentration was measured using a CO_2 sensor (Franatech, Germany) while ammonium, nitrite and nitrate nitrogen concentrations were measured 2 times per week from the screen filter sump (system) using spectrophotometric analysis using a VWR spectrophotometer and water chemistry analysis reagent kits (Merck Norway). Additional measurements were made from the water outlets of the sand filter, in the filter sump and the tank.

Initial biofilter start up

Once constructed and water flows tested in the Litus Akva system, the Dynasand biofilter was activated. This involved the addition of 8 L of Prawnbac (Novozymes France) biofilter starter culture added directly to the Dynasand biofilter. Additionally, on day 3 of operation NH_4Cl was added at 30 g into the system (system volume estimated at 8 m^{-3}) resulting in a calculated concentration of 3.75 mg L^{-1}. Thereafter 50 or 75 g (6,25 or 9.4 mg L^{-1} respectively) added daily until maturation of the biolfilter. Ammonium, nitrite and nitrate nitrogen was measured by spectrophotometer after the first week of dosing with NH_4Cl.

Once ammonium oxidation was established and evidence of nitrification was beginning to occur with the occurrence nitrate formation, and ammonium concentrations were below 0.5 mg L^{-1}, the system was determined to be mature and ready to accept loading with fish. This occurred by day 16 of biofilter activation and operation. In order to provide additional support to the biofilter when shock loading the system with fish (tank stocking density of 27 kg m^{-3}) additional 0.7 m^3 of biomedia from an established brackish water biofilter was added in net bags in the tank to provide remedial ammonia oxidation support. After 2 weeks these were removed.

System performance test

Fish (approximate mass 250 g) stocked into the Litus Akva system to a density of approximately 27 kg m^{-3}) and were fed daily using a Skretting 4mm pellet (Skretting Nutra RC) based upon estimated feed consumption from supplier's growth table according to temperature and fish size. The feed ration was adjusted daily.

At 2 weekly intervals a sub sample of 30 fish was removed and weighed following sedation with Aqui-S (1 mL per 100 L) on 10 fish were welfare assessment made and the gills were removed and fixed in 10% neutral buffered formalin for histological assessment.

From the weight data Specific growth rate and an *estimated* feed conversion ratio were calculated:

Specific Growth Rate (SGR) was calculated between the first weigh and measure and the second event:
$(\text{Ln}\ (Mass_2 - Mass_1))/\text{days}$

Estimated Feed Conversion Ratio (FCR) was calculated:
Feed consumed/($Mass_2$-$Mass_1$)

Note: the feed conversion ratio (feed factor) is estimated on the assumption that 100% of the feed supplied to the tank was eaten since excess feed collection was not possible with the final configuration of the Litus Akva system. Feeding rate was adjusted if excess feed was observed to ensure all feed was consumed.

Welfare scoring was assessed using a hybrid of the Fishwell and SWIM scoring schemes whereby 14 external morphological parameters are assessed and scored on a 0-3 point scale. The aggregate of this average (of 10 assessed fish) represents the welfare index.

Table 1. Assessment criteria for the Fishwell/swim welfare scoring system. Each category is scored nominally 0-3.

Date:			Location:				Cage:			Recorder:				Scorer:			
SWIM SCORE																	
Fish (#)	Length (cm)	Weight (g)	ID	Skin Hemorrhage	Wounds	Scale Loss	Fin Status	Cataracts	Eye Hemorrhage	Eye Protrusion	Opercula	Gill Status	Mouth Injury	Backbone Deformity	Lower Jaw Deformity	Upper Jaw Deformity	Copepodi
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	

Histological examination of gills

At 2 weekly intervals when fish were weighed and assessments of fish welfare were undertaken. A minimum of 10 individuals were killed with an overdose of Finquel (>100 mg L^{-1}) and the second left gill arch removed and placed into 10% neutral buffered formalin for histological examination. Once fixed for a minimum of 48h, the gill tissue was embedded in paraffin wax, sectioned at 3.5 µm and stained with haematoxylin and eosin. The resultant section was then scanned with Hammamatsu digital scanner for assessment.

Biofilter Stress test

After 4 months of operation and at a maximal tank stocking density of 80 kg m^{-3} a series of biofilter stress tests were performed. These tests (2) consisted of additional bulk addition of ammonium chloride to the system in addition to the normal ammonium load from feeding. Following a baseline measurement of ammonium, nitrite and nitrate nitrogen from the system tank, three aliquots of 30g of NH_4Cl were added at 0, 3 and 6 hours. The concentration of ammonium, nitrite and nitrate was measured thereafter at 3, 4, 6, 7, 10 and 24h post-addition (Biofilter test 1). A second test was performed on the subsequent day where a single addition of 300g of NH_4Cl was made to the system and the nitrogenous species measured hourly over the first 9 hours, at 11h and finally 24h post addition (Biofilter test 2).

RESULTS AND DISCUSSION

Initial biofilter start up

Following inoculation of the Dynasand biofilter in the Litus Akva system, there was a progressive feeding of the system with NH_4Cl resulting in an increasing concentration of NH_4-N after 10 days (Fig. 3). This was concomitant with a progressive increase in both NO_2-N and NO_3-N (Fig. 3.). By 14 days post inoculation the NH_4-N concentration was significantly reduced with clearly increasing levels of NO_2-N and NO_3-N occurring (Fig. 3). At this point, the biofilter was deemed to be established and capable of oxidizing 9.4 mg NH_4 L^{-1} per day.

The establishment of ammonium oxidation and nitrification processes were relatively quick in the system. This is likely doe to the following factors. Firstly, the amount of inoculum used to establish the biofilter was quite high (approximately 1L culture per cubic meter of water) ensuring a high biomass of ammonium oxidizing bacteria. Importantly, the use of a sand-based biofiltration medium meant that there was a particularly high specific surface area of the media relative to the media volume. Thus, when coupled with a movement of the media in the biofilter tower, would potentially promote extensive biofilm establishment on the media. Secondly, the temperature within the system was relatively constant between approximately 13 and 15 ^{o}C (data not shown). At this temperature establishment of bacterial biofilms is likely to be relatively rapid. Thirdly, the pH of the system and indeed alkalinity was relatively high (albeit with decreases in pH on day 7 and 11 (Fig. 3). Since ammonia oxidising bacteria and nitrifying bacteria such as *Nitrosammonas* sp. and *Nitrospira* sp. have pH optima of approximately 8, the maintenance of a relatively stable and high pH is essential for rapid establishment of the biofilter (Holan et al. 2021). Finally, in order to ensure a safe environment for shock loading of the system with fish, additional biomedia with an established biofilm was also introduced into the system 2 weeks before completion of fish stocking. There was therefore potential for bacterial transference and the break-up of biofilm from this introduced media to seed and help promote the establishment of the Dynasand biofilter.

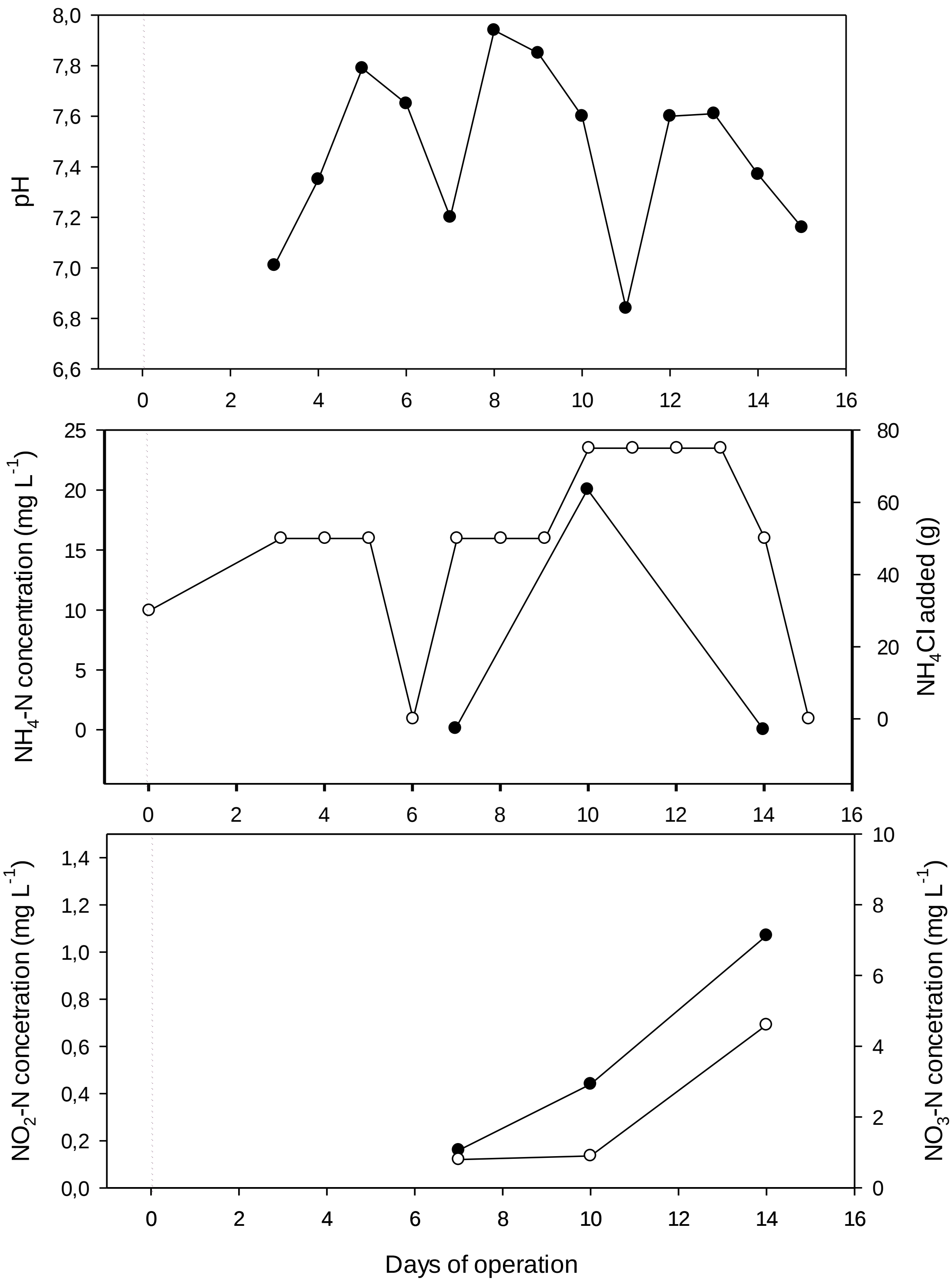


Figure 3. Litus Akva Dynasand biofilter maturation from initial inoculation (dotted line) over the subsequent 16 days prior to stocking with fish. NH_4Cl was added daily (centre panel open circles) and ammonium nitrogen concentration (open circles) and nitrite (black symbols) and nitrate nitrogen concentration (bottom panel) represented by white symbols.

System performance test

Following activation of the biofilter and stocking with fish the Litus Akva system was monitored for 3 months. Over this period several monitored parameters varied during the production as a result of either increasing biomass and feeding or due to adjustments to optimize the production system performance (Fig. 4). Many of the variables and variation in the measured values can be readily accounted for. It must also be taken into account that biofouling on the rotameter valves and flow meters may have led to significant misreporting of flow rates. Following manual measurements of flow made by Litus Akva AS personnel, the flow rates were corrected and represented here separately (Fig. 4.1). Based upon this information and recalculation, system flow rates were approximately 200-250 L min^{-1} with make up water typically <2 L min^{-1} with increased variation and make up water flow during the early adjustment phase and stabilization of the system (04/21) (Fig. 4.1). The recirculation rate was subsequently calculated at between 98.7 and 99.9% (Fig 4.1). These relative patterns are reflected in the data collected from the instrumentation albeit with consistent errors due to instrumental measurement failure (Fig 4).

There was some variation in the temperature of the system during operation. Initial set points of 15^{o}C were maintained initially although shortly followed by a marked decrease in temperature over approximately 12 days (Fig. 4). Can be accounted for by a marked increase in the total flow of make-up water into the system. Make up water was entering the system as a significantly lower temperature than that of the water within the system, thus and increase in flow would lead to a drop in the system operating temperature. A similar but smaller corresponding drop in temperature and increase in make-up water occurred in May (Fig. 4). A further consequence of the increase in total make-up water flow is reflected in the increase in water percentage of water replacement (inverse of percentage water recirculation). This also impacted and mirrored in the ratio of make-up water to system flow rate (Fig. 4). [redacted]. Subsequent fluctuations in CO_2 concentrations were most likely driven by variations in appetite and feeding depending upon the time of day that measurements were taken. Notably, increased CO_2 measurement concentrations were made towards the end of the test period, consistent with increasing system biomass.

Notably, at the point of a change of feed there were reductions in both temperature and CO_2 concentrations as well as an apparent reduction in the system flow rate. The reduction in flow probably reflected the introduction of a second CO_2 degasser at this point and thus flow through the two degasser systems reflected a split in the total flow. A reduction in feeding around this period subsequently resulted in reduced ammonium and CO_2 concentrations as well as alkalinity. This was likely due to a consequence of the high alkalinity several days before the change in diet facilitating increased ammonium oxidation and thus a relatively high nitrite concentration and the association with a change in diet is therefore potentially a result of reduced ammonium input following a change of feed (Fig. 5). As feed rate and appetite resumed following the change of feed, CO_2 concentrations increased as expected. Towards the end of the study, a second CO_2 degasser was introduced to ensure that the CO_2 concentrations measured remained below 17 mg L^{-1}

Over the duration of the performance testing period, there was an initial increase in NH_4-N concentration (Fig. 5). Reduction in high levels of NH_4-N is at the core of a RAS system, from a fish welfare perspective. Consequently, the increased make-up water occurring during the same period was a reaction to increased ammonium concentrations. Increased make-up water and thus reduced systems recirculation (water refreshment) also had a positive effect in reducing accumulating NO_2-N concentrations. Once ammonium oxidation levels were established given the increasing stocking density within the system nitrification processes steadily developed and a progressive increase in NO_3-N occurred. The turbidity and total suspended solids progressively increased an most areas of the system over the duration of the test period mirroring both NO_3-N and make up water flow rates (Figs. 4). However, total suspended solids concentrations were within normal operating values for RAS systems, despite the high stocking density. The use of the Dynasand filter would have lead to a removal of suspended particles that were perhaps not clearly reflected in the data based upon measurements made 19/4/21 and 3/5/21. Due to the nature of the sand filter, the backwashing cycle for the filter would release a small fraction of particles from the sand itself (observed as sharp-edged particles under the microscope). Over time, these smaller sand particles would be washed out of the filter system. Dynasand has an estimated erosion of 3% per year with continuous washing (Litus Akva AS, pers. comm.) so over a 4 month period of operation in the present test, this may represent as much as 1% of sand erosion from the Dynasand filter. Additionally, the filter was only washed 30% of the operational time. It is noteworthy that at the final stocking density, tank suspended solids were higher than that in the sand filter or the drum filter sump, indicating significant effects and a high degree of suspended solids filtration (Fig. 5). Many RAS systems fail to provide sufficient suspended solids removal. Despite previous recommended values from the Norwegian Food Safety Authority of being below 15 mg L^{-1}, these limits are not enforced. In fact, TSS is not often measured or monitored in many commercial RAS systems opting for the simpler measurement of turbidity (FAU units). In the present system, turbidity never exceeded 10 FAU and TSS remained under 10 mg L^{-1}, well below the recommended limits from the Norwegian Food Safety Authority (Fig. 5) even when considering the high stocking biomass and equivalent feeding at 1.3 kg feed per cubic meter of production volume (over normal dimensioned 0.7-1 kg feed m^{-3} volume, Litus Akva AS pers. comm.).

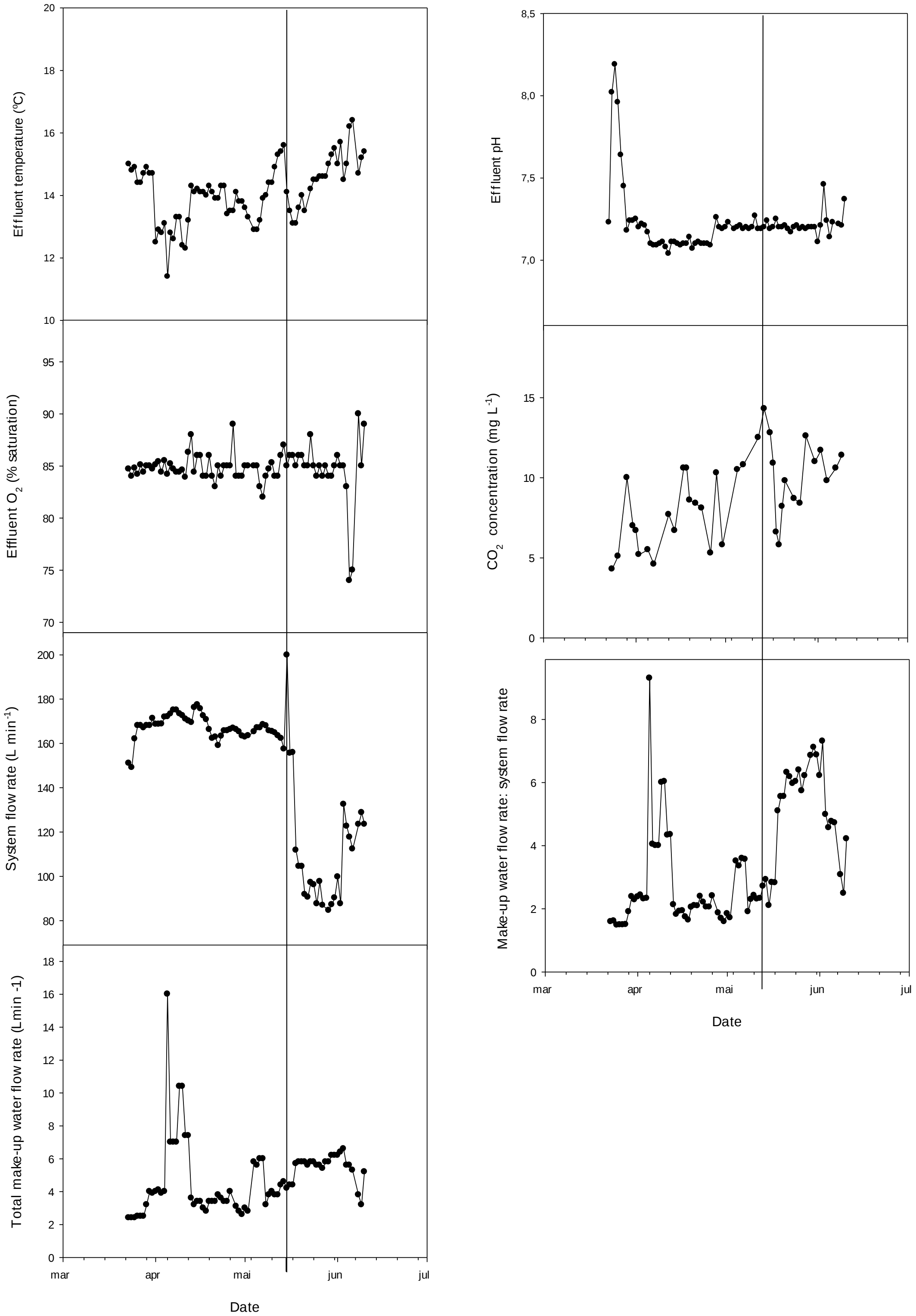


Figure 4. Litus Akva AS system performance following stocking with fish. Daily temperature, dissolved oxygen (% saturation), system flow rate, and total make-up water used in the system (left panel) and pH CO_2 concentration, ratio of make-up water relative to system flow rate (right panel) for the Litus Akva system under operation and increasing stocking density from 27 to 80 kg m^{-3}. Vertical line denotes change of feed type to from Skretting Nutra RC to Biomar.

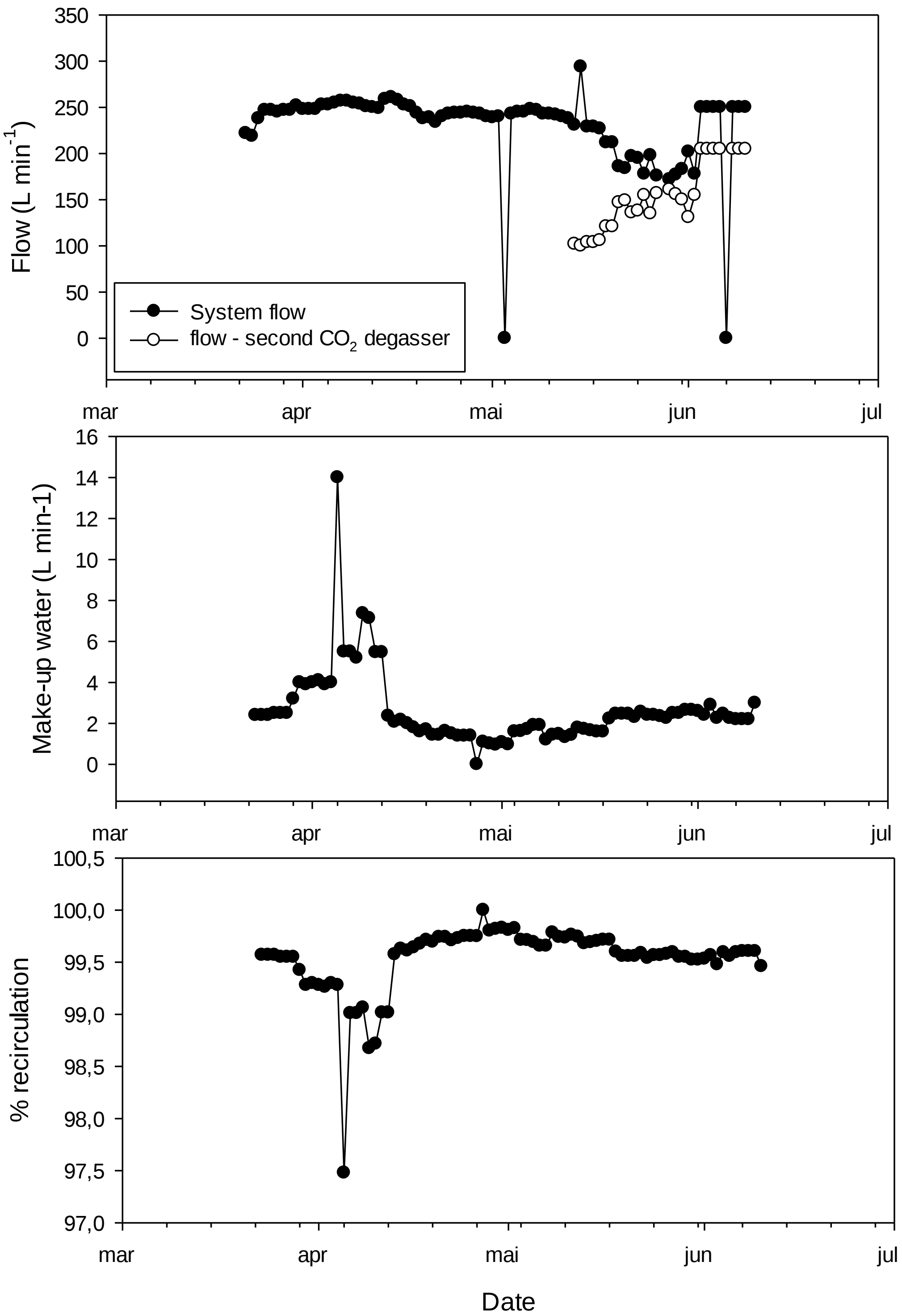


Figure 4.1. Flow rates, make-up water used and % recirculation of the Litus Akva system as calculated by manual measurement correcting the measured rates shown in Figure 4. (data supplied and calculated by Litus Akva AS).

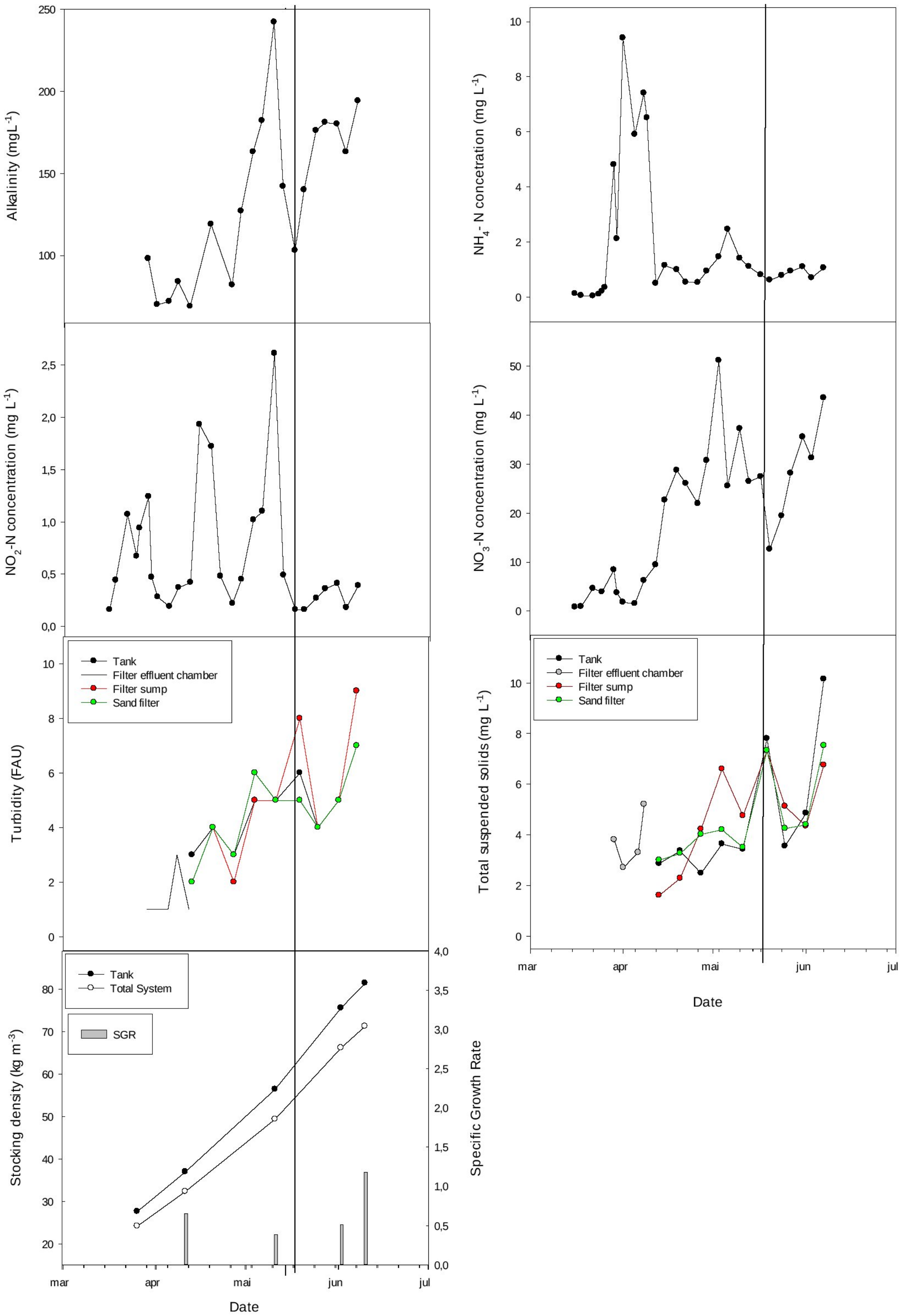


Figure 5. Alkalinity, nitrite nitrogen concentration, turbidty (FAU units) at different locations in the system and stocking density of the system and tank with related specific growth rate of the fish (left panel) and ammonium nitrogen concentration, nitrate nitrogen concentration and total suspended solids at 3 locations in the system (right panel) for the Litus Akva system under operation and increasing stocking density from 27 to 80 kg m^{-3}. Vertical line denotes change of feed type to from Skretting Nutra RC to Biomar.

Specific growth rates were within expected rages for the individual sizes of the fish and stocking density of the system (Fig. 5). Notably, however, is that growth rate appeared to decline prior to the change in feed type. This may be a consequence of several factors. Firstly, prior to this period, tank temperatures were slightly above the anticipated level (16 versus 15^{o}C; Fig. 4), thus the efficiency in use of feed may have been decreased. Secondly during this period of decreased growth rate, NH_4-N and NO_2-N concentrations were increased albeit still within acceptable limits for RAS, thus potentially stressing the fish during this period. Thirdly, the feed used (Skretting Nutra RC) is a relatively rapidly sinking feed and the change to the Biomar diet resulted in pellets that appeared to remain in suspension in the water column. Thus, it is possible that the switch to alternative diet may have increased opportunity for fish to intercept pellets during the rearing process. Certainly, after the change of diet, there was an increase in the specific growth rates and final growth rates of approximately 1.6 are well within acceptable levels even at stocking densities of 80 kg m^{-3}.

Fish welfare and gill histology

It should be noted that the fish used in the test of the Litus Akva system had been housed in small RAS systems and used in a previous study. Due to the size of the fish used in the Litus Akva test, the fish had begun to develop some cataracts and showed some signs of fin damage and scale loss due to handling (Fig. 6 time point 0). Throughout the test period, these characteristics remained consistent albeit at marginally elevated levels (from time point 0 and at subsequent time points (Fig. 6). There were mostly issues of scale loss, fin status, cataracts and opercular deformities associated with the fish during the study. These categories typically had average welfare scores of below 1 indicating minor impacts. Issues of scale loss at score 1 can represent occasional scale loss and may not necessarily reflect extensive skin damage. Similarly fin damage most typically reflects split fins rather than fin loss or open sores or bleeding fin edges. Importantly cataracts are a significant observation and characteristic of fish in RAS at relatively high temperatures (>14 ^{o}C). The reason for this is somewhat unclear although levels of histidine in the diet has been attributed to the cause. The diet used in the study (Skretting Nutra RC does have additional histadine added), and although cataract grades were uniformly low (grade 1 indicating the clouding of the lens within the eye and not obscuring of the whole cornea), the issue typical is progressive as the fish grow. Opercular deformities are a consequence of early developmental events. The population of fish used in this study were of good quality although some individuals did present with shortened operculae. Although this is characterized as a welfare issue it is primarily aesthetic with evidence suggesting that fish with such deformities along with lower jaw deformities, are not apparently compromised physiologically (Lijalad and Powell 2009).

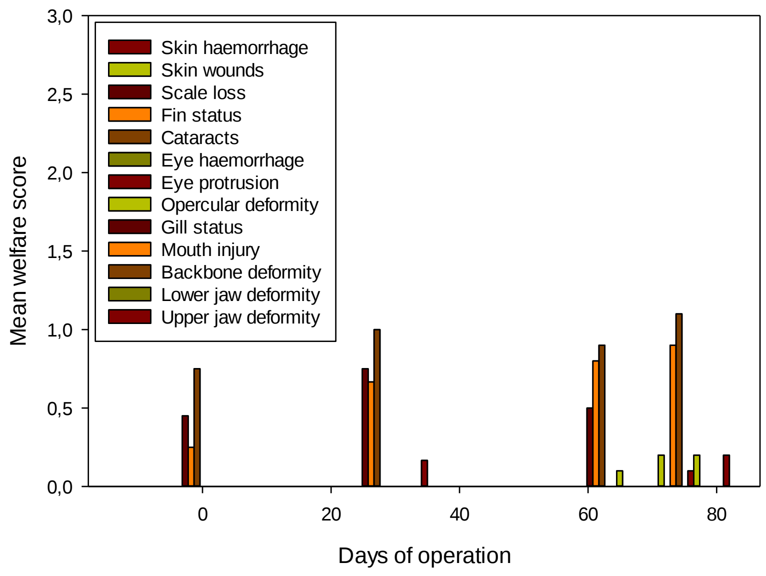


Figure 6. Mean fish welfare score over the production and test period of the Litus Akva system.

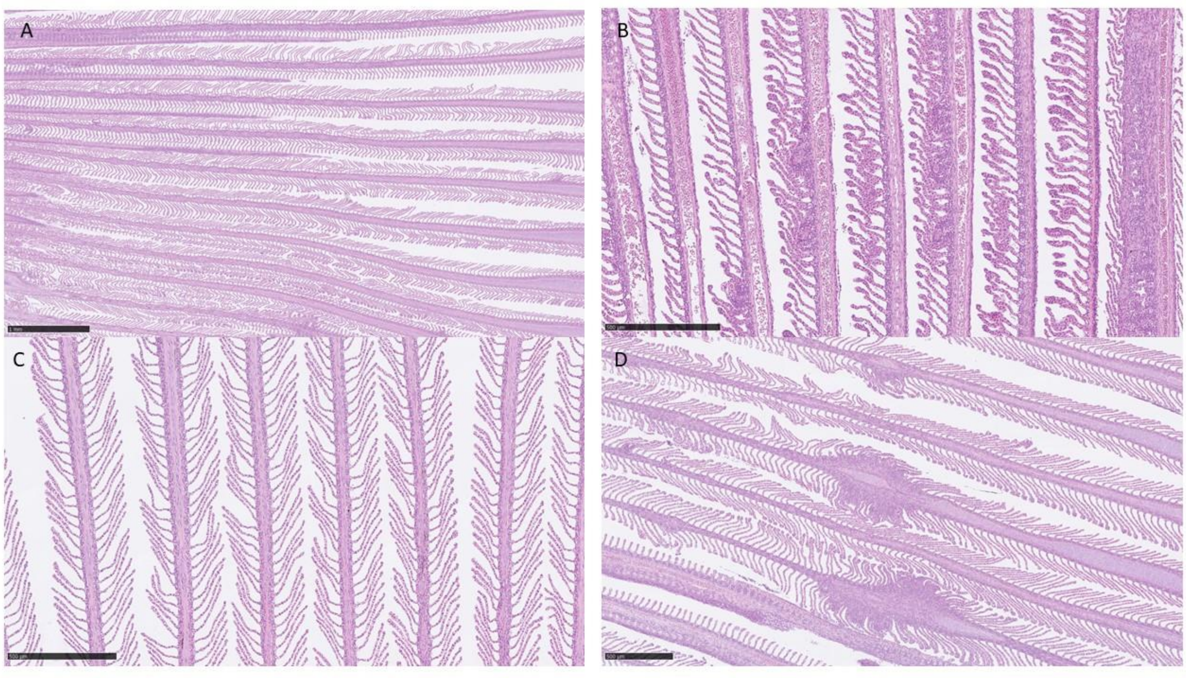


Figure 7. Representative histological images of the gills of fish sampled from the Litus Akva system. A and C are gill filaments and lamellae with no apparent injury or deformity. B represents focal inflammatory centres in the lamellae and gill filaments while C represents regions of filamental hyperplasia and inflammation.

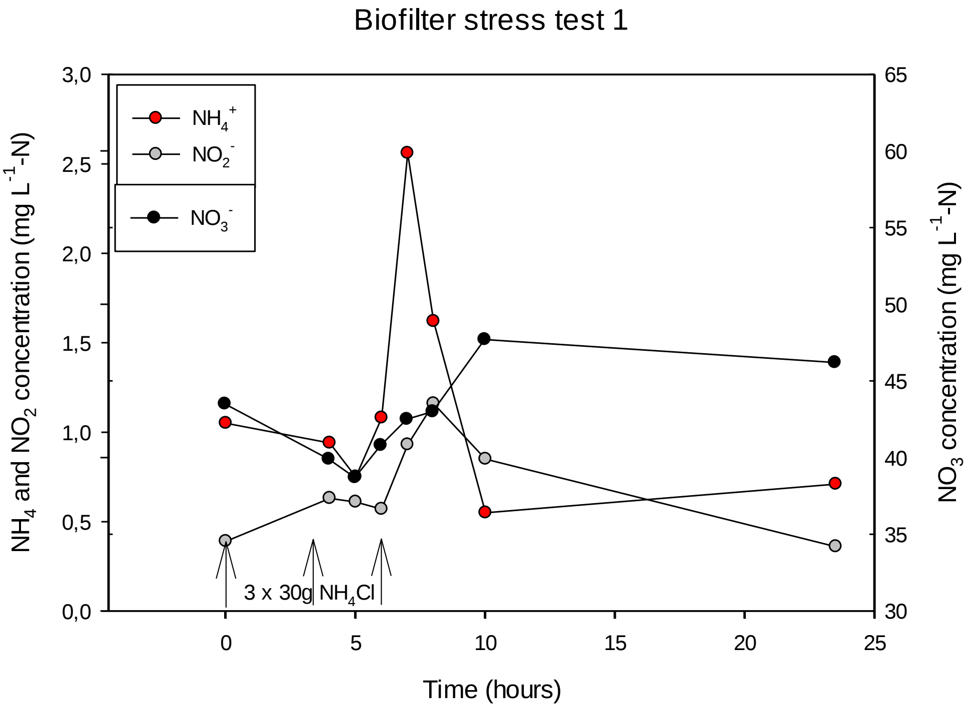
Histological examination of the gills showed there to be no apparent change in the range of appearances of the gills over the duration of the project period. Most of the gill filaments exhibited thin well-spaced lamellae with minimal extent of lamellar clubbing, telangiectasis, thrombus formation or inflammatory infiltrates, all of which have been described as progressive non-specific pathologies associated with fish in RAS (Otnes 2020) (Fig. 7A and C). However, there were some fish showing some signs of inflammatory infiltrates, multicellularity and focal hyperplasia of the filament and lamellae. In 2 individual cases, there were expanded parallel lesions on adjacent filaments associated with hyperplastic lamellae (Fig. 7B and D). The cause of inflammatory infiltrates and multicellularity associated with gills in RAS is not clear although suggestions of a correlation with increased organic load or possible increases in suspended solids load have been presented (Otnes 2020). With regard to the lesions presented in Fig. 7B and D, these may represent an earlier injury to the fill filaments and represent a fibrous response with focal hyperplasia of the filamental epithelium. The liner nature of the lesions apparat to suggest that the pathology may have occurred as a result of trauma. There was no evidence of bacterial or parasitic associated pathology in any of the gills examined. The gill status did not appear to have changed over the duration of the Litus Akva test period, despite increases in turbidity and TSS load (Fig. 5).

Biofilter stress test

Following a progressive addition of NH_4Cl to the system over a 6h period (3 x 30 g) there was a sharp increase in the measured concentration of NH_4-N. that rapidly decreased over the subsequent 4 hours to levels below the initial baseline (Fig. 8). Concomitant with the sudden rise in NH_4-N, there was a marked increase in measured NO_2-N concentrations which decreased to background levels by 24 h (Fig. 8). This represented an ammonia oxidation rate (based upon the prior total ammonium load) of 0.5 mg NH_4 L^{-1} h^{-1}.

A second biofilter stress test was performed on the following day once parameters had stabilized. In this case 300g of NH_4Cl was added as a bolus input into the system resulting in an immediate increase in measured NH_4-N concentrations to 9.5 mg L^{-1} (Fig. 8). There was a resultant and progressive decrease in measured NH_4-N concentration subsequently over the following 10 h to baseline levels. As with the previous test, there was an increase in NO_2-N concentrations and peaking at 7h post addition of NH_4Cl and subsequently decreasing to baseline levels by 24 h (Fig. 8). This represented an ammonium oxidation rate of 0.95 mg L^{-1} h^{-1}. In both cases of the biofilter stress tests, NO_3-N concentrations increased and stabilized 24 h post-test (Fig. 8).

There are very few studies where the ammonium oxidation rate of commercial RAS systems in operation have been calculated. Therefore, it is difficult to make direct comparisons between systems or benchmark if performance is good or poor. However, what can be determined from the test performed in the Litus Akva system it can be stated that the system incorporating the Dynasand biofilter tower had the capability of removing an additional 8.5 mg of NH_4 within 9 hours. Thus, in addition to a stocking density of 80 kg m^{-3} producing an estimated 480g NH_4 (TAN) daily (equivalent to 60 mg L^{-1} day^{-1}), the system had sufficient reserve capacity to potentially oxidize an additional 22.7 mg NH_4 day^{-1} i.e. a reserve capacity of approximately 38%.



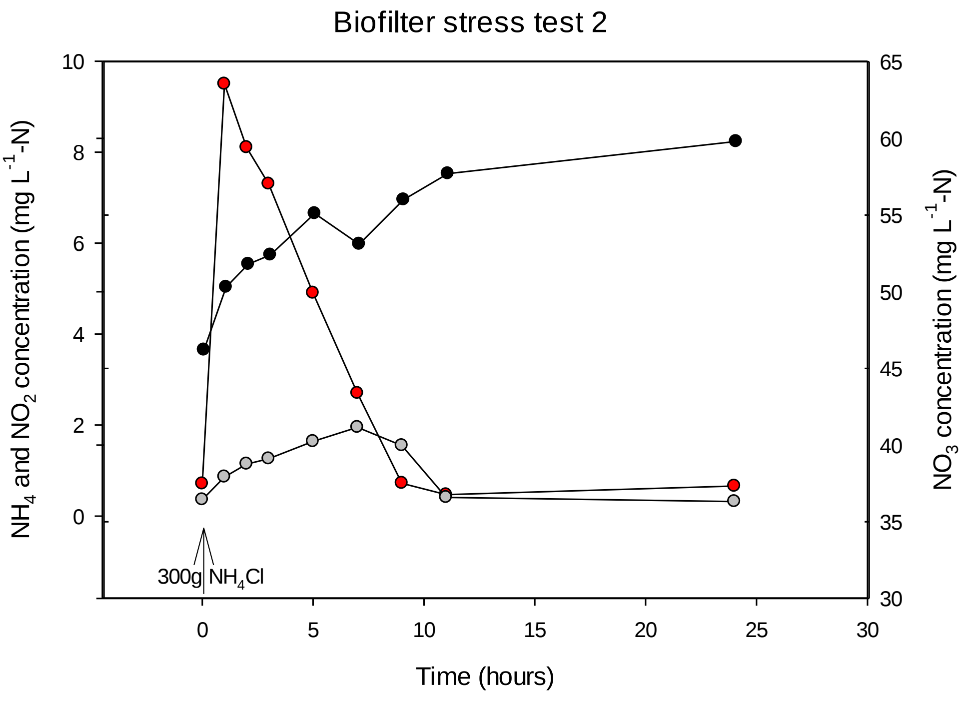


Figure 8. Ammonium and nitrite nitrogen concentrations (left axis) and nitrate nitrogen concentrations (right axis) in the Litus Akva system following addition of extra NH_4Cl (indicated) and recovery clearance of the additional nitrogen load when added in small doses (3 x 30g: biofilter test 1) and as a large bolus addition (1x 300g: biofilter test 2).

CONCLUSION AND RECOMMENDATIONS

Based upon the data presented and the interpretations made it is clear that the Litus Akva biofilter established quickly and was supported by the temporary addition of bio-active media from Marineholmen RASLab. Once established the systems was stable and performed well accommodating an increasing stocking density in the tank and input of feed above that normally associated with RAS systems (1.3 kg m^{-3} compared with 0.7-1.0 kg m^{-3}). The variations in make-up water and possible undocumented adjustments led to some degree of variation in parameters. However, the system responses in terms of water quality and performance were as one would expect. Without any doubt, the system performance was equivalent to most commercial systems currently available and exceeded the operational parameters expected from most RAS systems in terms of maintaining suspended solids loads and turbidity levels within safe limits for fish despite intensive production demands. This is no doubt aided by the use of the Dynasand filter in addition to conventional drum screen filtration. The addition of the Dynasand filter biofiltration is a distinct bonus to the system and significant advantage. Testing of biofilter performance with the biofilter stress tests indicated a high degree of potential ammonium oxidation capacity giving significant reserve capacity to the biofilter. Consequently, this indicates that the system has a high degree of reserve capacity providing approximately 33% capacity for either increasing biomass (not recommended), increased feed input or potentially tolerating a reduction in biofilter performance. The latter may occur due to fluctuations in temperature, ammonium load, pH or salinity. During the production period, the fish showed to be in good overall condition. Welfare indicators fish showed mild impacts equivalent to most RAS systems with the primary issues of minor fin damage and scale loss along with cataracts being the primary welfare considerations. The latter is a common occurrence despite the use of histidine enhanced diet indicating factors other than diet as the contributing factor. This notwithstanding, gill health was generally good. Although there were some fish with markedly more gill inflammatory infiltrates into the gills and some evidence of hyperplastic changes, most of the fish showed very mild pathologies and extensively unaffected and healthy gills.

Figure 9: Flesh appearance of produced in the Litus Akva system

There are some potential areas that can be improved upon. Firstly, the system should be managed in a careful way to reduce the large variations in input and system circulation water. This would lead to instability in the biofilter, particularly with regard to nitrification. A more reliable instrumentation for flow measurement would distinctly aid in the careful control and measurement so ensuring reduced fluctuation in water use in the system, especially during early establishment phases. In order to maintain excellent performance, this is a requirement and probably the biofilter should be established for a longer period before the addition of fish. Secondly, the suspended solids levels and turbidity levels increased to quite high levels by the end of the study albeit within the earlier recommended limits from the Norwegian Food Safety Authority. While still at acceptable levels, there was no appreciable difference between the water in the filter sump and that in coming from the sand filter albeit that these were much lower than that in the tank. A close measure and control of the Dynasand filter backwashing cycle, and wash-oout of smaller sand particiles from the Dynasand filter in the earlier stages of operation will help to ensure that the correct TSS levels are being measured. This is essential for optimal performance of the Dynasand filter ensuring both optimal nitritification and solids removal.

In conclusion, it can be suggested that:

1. The Litus Akva system using a Dynasand filter, can perform well for the production of Atlantic salmon post smolts up to stocking densities of 80 kg m^{-3} providing adequate growth rates.
2. Despite variations with water quality that can be expected in many RAS systems, the system performed well with stable ammonia oxidation occurring. Nitrification was variable in its stability and primarily appeared to reflected changes in the amount of make-up water added and feed load.
3. Water flow rates were excellent representing a high degree of water circulation and maintenance of water quality. This resulted in a high water recirculation rate exceeding 98.7 % without compromising fish growth, welfare not water quality.
4. Biofilter performance was good with a significant potential reserve capacity and high rate of ammonium oxidation.
5. Fish produced exhibited very good welfare scores with good gill quality.

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