Norwegian University
of Life Sciences

Master's Thesis 2017 60 ECTS
Faculty of Environmental Science and Technology, Department of
Mathematical Sciences and Technology

Documentation of water quality in a water recycling system for aquaculture (RAS)

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Master of Science in Aquaculture

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Master Thesis (60 credits)

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ABSTRACT

A water quality analysis was conducted at a recirculating aquaculture system (RAS) with Salmonbreed QTL PD smolt at Vik Settefisk AS, Bergen, Norway. The parameters oxygen (O_2), carbon dioxide (CO_2), Salinity, potential of hydrogen (pH), temperature, mortality were measured over 2 stages; Stage 1 being day 1 to day 22, and Stage 2 being from day 1 to day 122. Other parameters that measured and analysed during Stage 1 included Total ammonia nitrogen (TAN), Nitrite (NO_2-N), Nitrate (NO_3-N), Ammonia (NH_{+4}), Alkalinity and Chemical oxygen demand (COD). Recommended thresholds found in published literature were compared to these results as well as the suppliers recommended limits for their system. The system was Module 17 intensive RAS run by Vik Settefisk AS in Øygården county, Bergen, Norway. According to the results, there w ere statistically significant differences between Temperature, CO_2, Salinity and pH at Stage 1 and Stage 2 measuring data, however, the results remained within the published literature and the vendor guidelines for the Module RAS. Additionally, a blockage between the moving bed biofilm reactor (MBBR) and submerged fixed bed reactor (SBR) resulted in higher TAN, NO_2-N, NO_3-N in the SBR readings, however still within the recommended limits.

Key words: water quality, recirculating aquaculture system (RAS), MBBR, SGR, 2 stage water quality comparison.

ACKNOWLEDGEMENTS

The author would firstly like to thank Vik Settefisk AS and their employees for use of their RAS for research

Christine Pettersen from Mattilsynet for assisting with documentation

Thank you to Odd-Ivar Lekang for your feedback and guidance during this process

Lastly, thank you to Sterner AS and their employees for helping facilitate this research

ABBREVIATIONS

ATR	Areal TAN removal rate
CO_2	Carbon dioxide
COD	Chemical oxygen demand
FGR	Feed conversion rate
HRT	Hydraulic retention time
MBBR	Moving bed biofilm reactor
NH_{+4}	Ammonium
NO_2-N	Nitrite
NO_3-N	Nitrate
NVE	Norges vassdrags- og energidirektorat
O_2	Oxygen
O_3	Ozone
ORP	Oxidation reduction potential
P&ID	Piping and instrument diagram
pH	Potential of oxygen
RAS	Recirculating aquaculture systems
SBR	Submerged fixed bed reactor
SGR	Specific growth rate
TAN	Total ammonia nitrogen($NH4^+$-N) + (NH_3-N)
UV	Ultraviolet

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1. INTRODUCTION

A RAS is a closed farming system where more than 60% of the water within the tank is reused with the help of biofiltration and water treatment to preserve water quality (Hjetnes et al., 2012). Processes such as nitrification, denitrification, fish production, removal of effluent wastes, etc. are compartmentalised in other chambers from the rearing tank, ensuring production capacity (Verdegem et al., 1999). RAS had its beginnings in Japan in the 1950s and the focus of research was biofilter design and reduction of water usage (Murray, Bostock and Fletcher ,2014). Initial problems with RAS were due to the application of research from small scale projects to large industrial farms as well as the discordance of knowledge and expertise between engineers and production managers. However, with standardisation across the industry, RAS development has further contributed to the advancement of the aquaculture industry within key centres of consumption and larger distribution strongholds within Norway (Murra, et al., 2014). There are high capital costs associated with the setup and ongoing running of RAS, however, due to the high stocking density (up to 100 kg/m3), profitable production is achievable (Hjeltnes et al., 2012). Advantages of intensive RAS include production occurring in all seasons as well as an increased survival rate when transferred to sea cages (Kristensen et al., 2009; Dalsgaard et al., 2013; Terjesen et al., 2009).

The development of RAS has come with advantages including improved disease and waste management and reduced visual impact of the farm (Martins et al., 2010). Furthermore, smolt RAS development in Norway has been advantageous due to the predicted fresh water shortages to be experienced in the future. Also, increased biomass production demand, inlet water quality issues, freedom of location, accelerated fish growth and water temperature variation have all contributed positively with RAS aquaculture (Kristensen et al., 2009; Murray et al., 2014; Dalsgaard et al., 2013; Rosseland et al., 2005). To ensure fish growth and therefore economic performance, water quality analysis and maintenance is essential (Patterson, Watts & Gill, 2003). Water quality analysis is complex due to the high growth of bacterial compounds caused by high pH, temperature, organic load and fish density. Due to the reduced make-up flow rate and the environmental conditions within a RAS, a build-up of compounds and bacteria within the system is increased (Colt, 2006; Hjeltnes et al., 2012). This bacterium is controlled, and water quality is maintained via both mechanical and biological filtration methods (Schroeder et al., 2015). Initially, the water is filtered via mechanical process (pre-filter and drum filter) and then processed further by the biological filters including ozone (O_3) supplementation, MBBR and SBR (Hjeltnes et al., 2012).

A water quality comparative analysis was conducted at Vik Settefisk AS in Bergen, Norway with collaboration from Sterner Aquatech AS. The researcher conducted a water quality analysis on a full-scale intensive RAS Module 17 at the Vik Settefisk located in Øygarden, Hordaland. The report

documents 6 water quality parameters over 2 stages, Stage 1 lasting from day 1 to day 22 and Stage 2 lasting from day 1 to day 122.

The objectives of were;

- To conduct a water quality analysis of the parameters; O_2, temperature, pH, ORP, Salinity, mortality, Feed, SGR, Feed conversion rate (FCR), CO_2, $NO_{2\text{-}}N$, $NO_{3\text{-}}N$, NH_{+4}, Alkalinity, COD and TAN. Measurements are to be taken before and after the biofilter, and functioning of the filter during start-up is to be discussed. Other measurements recorded included SGR, FCR, daily mortality rate, total daily feed and biomass weight.
- To compare O_2, temperature, pH, Salinity, CO_2 and mortality taken from day 1-22 (Stage 1) and days 1-122 (Stage 2).
- To discuss how predicative the first month (Stage 1) is to the whole lifespan of RAS (start-up-final sale) (Stage 2) with a combination (established/new) biofilter.
- To discuss recommended thresholds for water quality parameters based on current evidence and government recommendations and compare these to the analysis findings.

Monitoring of water quality is an essential component of RAS (Hjeltnes et al., 2012) and the literature review in this thesis will discuss the parameters and their influence within RAS aquaculture. A system description is also provided, describing a detailed synopsis of Vik Settefisk and Module 17. The materials and methods section details all equipment and the analysis process, results and discussion of results are after that explained

2. LITERATURE REVIEW

This literature review will discuss evidence relating to RAS structure, precisely the filtration methods as well as research related to the water parameters that are discussed within the case study. Due to the range of literature relating to water quality spanning several decades, the most recent literature (last 5 years) was reviewed first with supplemental evidence from later publishing dates used to support information.

2.1 RAS structure

2.1.1 Mechanical filters

Mechanical filters quickly remove particles caused by overfeeding and faeces reducing chances of their disintegration (Hjeltnes et al., 2012). The drum removes suspended solid materials and can remove up to 60-80% of organic material (Patterson, Watts and Gill, 2003; Sterner Aquatech AS, n.d). Furthermore, drum filters are used for the filtration of large solid particles from the tank, whereas finer solids are filtered via further biological filters (Masser et al. 1999).

2.1.2 Biological filtration

The purpose of biological filtration within RAS is to reduce the toxic concentration of TAN via oxidation of ammonia through the process of nitrification (Guerdat et al., 2010; Drennan et al., 2006; Schroeder et al., 2015). TAN can negatively affect fish stock and therefore its essential to remove within RAS systems (Guerdat et al., 2010). By using a combination of MBBR and SBR filtration methods, nitrogen can be successfully removed (Masser et al. 1999). During biofilter start-up, the nitrifying bacteria can be sensitive to sudden changes within the environment and the formation of the biofilm during the start-up of a biofilter (Rusten et al., 2006).

The MBBR was developed in Norway in the late 1980s-early 1990s to reduce nitrogen discharge supporting the growth of heterotrophic bacteria (Leiknes and Ødegaard, 2007; Rusten et al., 2006). Advantages of a MBBR include low maintenance requirements, effective biochip-volume relation due to the reactor volume and surface area, insusceptible to system clogging and scheduled backwashing not required (Rusten et al., 2006). Furthermore, the MBBR allows for flexible and continuous operation as well as easy operation and maintenance (Drengstig et al., 2011). The size of the biofilter is related to the surface area (m^2/m^3 of media) or the volume of media (m^3) and the biomass, feeding rate, temperature, total volume, salinity and TAN. Acceptable TAN levels are estimated via protein content in feed, size of filter and pump as well as Hydraulic Retention Time (HRT) (Drennan et al., 2006).

O_3 is used within RAS as a support system for the biofiltration process of effectively oxidising nitrite to nitrate thus improving water quality (Schroeder et al., 2015). It allows for micro flocculation

of the organic matter which in turn improves the filtration of the suspended solids (Otte and Rosenthal, 1979). There is an automatic adjustment of O_3 which matches changes within the system, i.e. fish feeding rate which is important as it improves fish performance water quality including total suspended solids, colour, NO_2-N and UV transmittance (%) (Summerfelt, 2003; Summerfelt et al., 2009). O_3 is discussed further in section 2.2.4.

2.2 Water quality parameters

2.2.1 O_2

Monitoring of O_2 is an essential component and the single most important water quality parameter within a RAS (Summerfelt, Vinci and Piedrahita, 2000; Hjeltnes et al., 2012). Pure O_2 usage results in higher biomass production, limits water flow requirements and reduces costs due to mortality (Summerfelt, Vinci and Piedrahita, 2000; Malone and DeLosReyes Jr, 1997). Publications and researchers within RAS aquaculture state that an oxygen saturation no higher than 100% is most suitable within RAS aquaculture (FOR, 2004; Terjesen et al., 2013; Mattilsynet, 2014) with specifically inlet dissolved O_2 ranging from 90-120% (FOR, 2004) and at the outlet < 80% (Mattilsynet, 2014). The effects of oxygen saturation out of these levels has been found to contribute to reduced growth, mortality, disease resistance and gas bubble disease (Hjeltnes et al., 2012) at levels over 140-150% (Lygren, Hamre and Waagbo, 2000) and induced respiratory distress, reduction in appetite and therefore increased mortality at low levels (Hjeltnes et al., 2012; Colt, 2006).

Additionally, nitrite can accumulate within the nitrification biofilters causing toxicity with low O_2 concentration levels of <2 mg/L (Chen, Ling and Blancheton, 2006; Kolarevic and Terjesen, 2011; Picioreanu et al., 1997) with symptoms including gulping, lethargy, lack of active shoaling behaviour and rapid gill movement (Hjeltnes et al., 2012). Furthermore, O_2 diffusion needs to be evenly spread throughout the RAS to reduce the chance for fish to be exposed to eutrophication (Thorarensen and Farrell, 2011).

2.2.2 Temperature

Monitoring of temperature in RAS is the second most important water quality parameter after oxygen as feeding, growth, respiratory processes, deformities and behaviour can be directly affected (Timmons and Ebeling, 2013). The optimal range of temperature for salmonids is not higher than 8 °C in eggs and not higher than 18°C in the fry and parr stage (Mattilsynet, 2014), with a survival range between 3-18 °C for smolt (Hordaland fylkeskommune, 2009). Temperature also directly affects the bacterial activity in the biofilter and is essential to daily RAS operations (Chen et al., 2006; Lekang, 2012).

2.2.3 pH

pH refers to the relationship between water and hydrogen and as such abrupt changes in pH cause stress to the fish and destroy the bacteria within the biofilters (Pattillo, 2014). In relation to pH, appropriate levels for optimal salmonid health in a RAS are different amongst the published data, with the lowest values ranging from 6.2-6.8 (Mattilsynet, 2014; FOR, 2004; Terjesen et al., 2013) up to 7-7.8 for salmonoids within RAS (Fjellheim et al., 2010; Malone & DeLosReyes Jr, 1997; Terjesen et al., 2013; Mattilsynet, 2014). The significance of high pH within RAS is important to note as nitrification rates quadruples when the pH is above 7 (Pattillo, 2014) and at low pH the nitrifying bacteria within the biofilter can be inhibited and TAN is elevated (Fjellheim et al., 2010; Malone and DeLosReyes Jr, 1997; Eding et al., 2006).

2.2.4 ORP, O_3 and Ultraviolet (UV)

Redox, or ORP refers to ion concentrations, temperature and the electron transfer in both oxidation and reduction processes (Banhidi, 2000). A study by Li et al. (2015) on the long-term effects of ORP on sea bass found that levels over 300-320 mV decreased the feed intake and growth, however, the fish's ability to react against bacterial infection was improved. Therefore, the limits of safe ORP need to be monitored to promote disease resistance. Furthermore, a study by Terjesen et al. (2013) set an ORP limit of 270 mV with O_3 dosing for their study assessing the water quality requirements for Atlantic salmon smolt production. The level of ORP is controlled via the addition of O_3 (Terjesen et al., 2013). O_3 is an effective chemical disinfection agent that oxidises microorganisms and viruses as well as facilitating efficient biofilters (Summerfelt, 2003).

O_3 and UV are commonly used in RAS to depress bacteria and control pathogens (Drengstig et al., 2011; Bullock et al., 1997). O_3 allows for RAS to operate with minimal water exchange rates which in turn results in greater growth survival and feed conversion (Davidson et al., 2012; Hjeltnes et al., 2012). The use of O_3 in RAS needs to be controlled and its by products can be negatively impact fish health (Tango & Gagnon, 2003). The fish behaviour exhibited with toxic O_3 levels includes erratic swimming, stopping feeding and gasping for air at the surface (Hjeltnes et al., 2012). Furthermore, NO_2-N can accumulate quickly in the RAS if the O_3 dosing is interrupted (Hjeltnes et al., 2012; Summerfelt et al., 1997). UV is commonly used for new water entering the system and is used within Module 17 to inactivate and kill microorganisms (Hjeltnes et al., 2012; Liltved, 2002)

2.2.5 CO_2

CO_2 is excreted through the gills of the fish and produced in the biofilter through microbial metabolism (37% of total CO_2) (Summerfelt et al., 2013; Summerfelt and Sharrer, 2004; Summerfelt et al., 2004). With higher feed loads and a large fish production, oxygen supplementation is required which can create pockets of accumulated undissolved CO_2 if inadequate air-to-water contacting is not

ensured (Summerfelt et al., 2000). In terms of safe CO_2 levels, there have been several different sources who have published varied levels of CO_2 safety for salmonids in RAS. The most recent data (post 2009) states that CO_2 must be less than 10 mg/L (Hordaland fylkeskommune, 2009; Terjesen et al., 2013) or < 15mg/L (Mattilsynet, 2014). If these levels are breached, respiratory processes are impacted and pH decreases which in turn inhibits nitrifying bacteria. In relation to low levels of CO_2, a study by Fivelstad et al. (2003) found that salmonids exposed to CO_2 levels as low as 6 mg/L can induce nephrocalcinosis, a build-up of calcium within the kidneys.

2.2.6 Salinity

A project completed by the Nofirma Centre for Recirculation in Aquaculture called "The optimised postsmolt production experiment" found that 12‰ salinity had a greater positive effect on the growth of postsmolt compared with smolt in 22‰ and 32‰ salinity levels. CO_2 stripping and TAN removal efficiency was found to be more efficient when salinity level were 12‰ (Ytrestøyl et al., 2014). Additionally, the Hordaland fylkeskommune (2009) published a survival range of between 15-35‰ (Hordaland fylkeskommune, 2009).

2.2.7 NH+4/ NO_2-N / NO3-N

NH_{+4} is the primary metabolite excretion of fish, very toxic to marine species and therefore important to be removed from the RAS. It is for this reason that biofilter nitrification is of such importance in RAS (Schram et al., 2010). During nitrification, ammonia oxidises from NO_2-N to the less harmful NO_3-N by ammonia-oxidising bacteria, Nitrosomanas or Nitrosospira. Losordo and Westers (1994) determined that an ammonia concentration of 0,025 mg/L was an appropriate design criterion in RAS.

NO_2-N refers to the ionized form of nitrous acid (Colt, 2006). Per Hjeltnes et al. (2012), elevated nitrite levels are the highest risk factors within a RAS and can be caused by the start-up and maturation of the biofilter. Optimal levels of NO_2-N in freshwater are <0.1mg/L (Mattilsynet; 2014; Terjesen et al., 2013; Kolarevic and Terjesen, 2011) and seawater is <0.5 mg/L. At elevated levels, respiratory stress is induced as well as gill hypertrophy, lamellar separation and hyperplasia (Malone and DeLosReyes Jr, 1997; Wedemeyer and Yasutake, 1978). Toxic levels of NO_2-N are counteracted by using chlorine (Hjeltnes et al., 2012).

Compared to other water quality parameters such as NH_{+4} and NO_2-N, NO_3-N is relatively non-toxic to fish (van Rijn, Tal and Schreier, 2006). Malone & DeLosReyes (1997) states the optimal NO_3-N level as <200mg/L with a maximum of 400-500 mg/L as recorded by van Rijn, Tal and Schreier (2006). Davidson et al (2012) found that NO_3-N levels from 75-100 mg/L caused side swimming, decreased growth and decreased survival for juvenile rainbow trout. However, these maximum levels will differ for each RAS due to differences in water exchange rates and nitrification

and NO_3-N removal efficiency (van Rijn, Tal and Schreier, 2006). Nitrate is not actively removed within the biofilters and its accumulation is directly related to the feeding rate and the HRT of the RAS (Hjeltnes et al., 2012).

2.2.8 Alkalinity

Alkalinity refers to the concentration of alkaline dissolved in water and related to the naturalisation of hydrogen ions within the water and is critical for sustaining nitrification (Hjeltnes et al., 2012; Summerfelt et al., 2015). A study by Rusten et al. (2006) found a drop in the rate of nitrification within the MBBR when alkalinity dropped from 115mg/L to 57 mg/L with pH readings of 7.3 and 6.7 respectively. The lower limits for alkalinity range from >20-50 mg/L and the upper limits are >100-300mg/L (Wedemeyer, 1996; Timmons and Ebeling, 2013) with Malone and DeLos Reyes (1997) stating optimal alkalinity as >80mg/L. A study by Chen et al. (2006) found that for optimal biofilter performance, the recommended alkalinity is 200mg/L. If the water in the RAS becomes highly alkaline, the excretion and production of ammonia can be inhibited whereas low alkalinity can inhibit the nitrifying bacteria within the biofilter (Wilson et al., 1998; Malone and DeLosReyes Jr, 1997).

2.2.9 COD

A COD ranging from 3.0 to 6.0 mg/L allows for complete reduction of nitrate to nitrogen (van Rijn, Tal and Schreier, 2006). Further detailed in section 3.8.2.

2.2.10 TAN

TAN refers to the concentration of both ionised and unionised ammonia within a solution (Guerdat et al., 2010). The elimination is essential when considering biofilter design and operation within a RAS (Chen et al., 2006). The optimal level of TAN in RAS has been published by various sources as <0.7 mg/L (Terjesen et al., 2013), <1.0mg/L (Malone & DeLosReyes, 1997) and <2 mg/L (Mattilsynet, 2014). When the TAN concentration increases, the environment becomes stressful for fish within the system and if the efficiency of the biofilter is negatively impacted due to the increase of nitrite concentration (Svobodova´ et al., 2005). Furthermore, intensive production is negatively affected due to impaired growth, induced stress and increased mortality (Emparanza, 2009; Malone and DeLosReyes Jr, 1997)

TAN is linked and dependent on pH within the RAS and less so to temperature and salinity (Hjeltnes et al., 2012). With a mature biofilter and a pH of 6.9-7.3 Emparanza (2009) stated that TAN levels could be controlled easily if the feed amount is not 15% more of less from the previous day. Additionally, at low pH, the nitrification process is slowed, and ionised ammonia is increased with the

loss of unionised ammonia, therefore, is the key limiting water quality parameter (Colt, 2006; Chen, Ling and Blancheton, 2006).

The structure of a RAS and the monitoring of its water quality is involved. Therefore, understanding the relationship between these parameters and their lifespan of a RAS is central to this analysis and will be explored further in the following experiment.

3. INTRODUCTION TO VIK SETTEFISK AND STERNER AS

The following system description will discuss the Module 17 RAS where the water quality analysis was conducted.

3.1 Site location and vendor

Vik Settefisk AS is a post-smolt producer located in Vik, near main road 561 at Toftøyna, Øygarden municipality, on the western coast of Norway near Bergen. As a shareholder of Salmon Group, Vik Settefisk AS belongs to a network of locally owned fish farming producers (over 40) located throughout Norway, with a total of 115 licences for salmon and trout production. Smolt production reaches 50 million smolts per year from all stakeholders of the Salmon Group (Salmon Group, 2016). The weather that services this catchment area is Skredderdalen weather station, located 26,5 km from Øygarden county. As the station has only been operational since August 2016, there is no long-term data on temperature, wind and rainfall (yr.no, 2016). Sterner Aquatech AS is a water treatment vendor for Vik Settefisk located in Bergen. They were the primary supplier for the RAS and provided a base point for the researcher to conduct the experiment.

3.2 Freshwater source

Figure 3.2.1 shows the water source used for the RAS originates from Midtvatnet and Nordlavatnet (NVE, 2016). The freshwater catchment, Lake A 22 MASL (Midtvatnet), runs into the main water supply, Lake B 20 MASL (Nordlavatnet) as shown by the blue line in Figure 3.2.1. The water is then pumped from Lake B to the farm (C) via a 200mm PE pipeline. The amount of water available is dependent on the drainage from the surrounding mountains. The drainage area is approximately 306520 m2 (Figure 3.2.2) with a yearly average drainage of 1458 mm (Norgeskart, 2016). Water volume per minute to the farm was calculated to be 0.83m3 per minute in average during the year (Appendix B) (NVE, 2016). The yearly runoff measurements differ due to variable weather conditions (snow melting, winter/autumn storms, etc.), impacting water quality and flow to the farm.

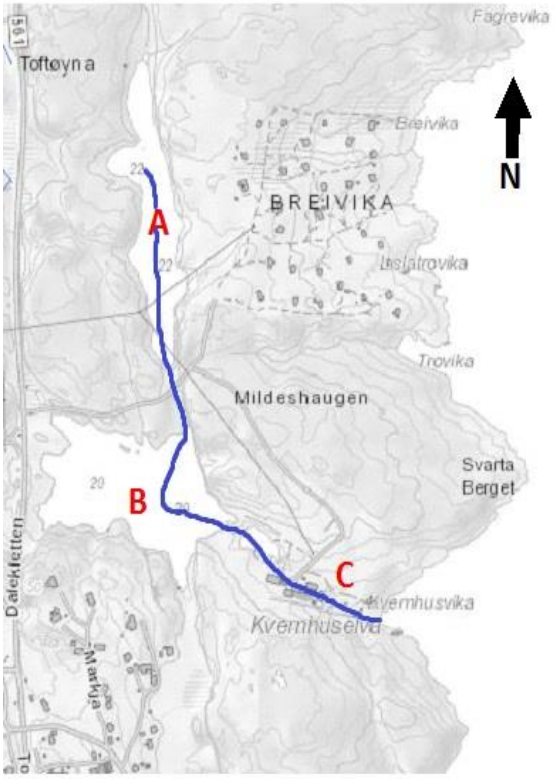


Figure 3.2.1: Map adapted from website (Norwegian Water Resources and Energy Directorate (NVE), 2016).

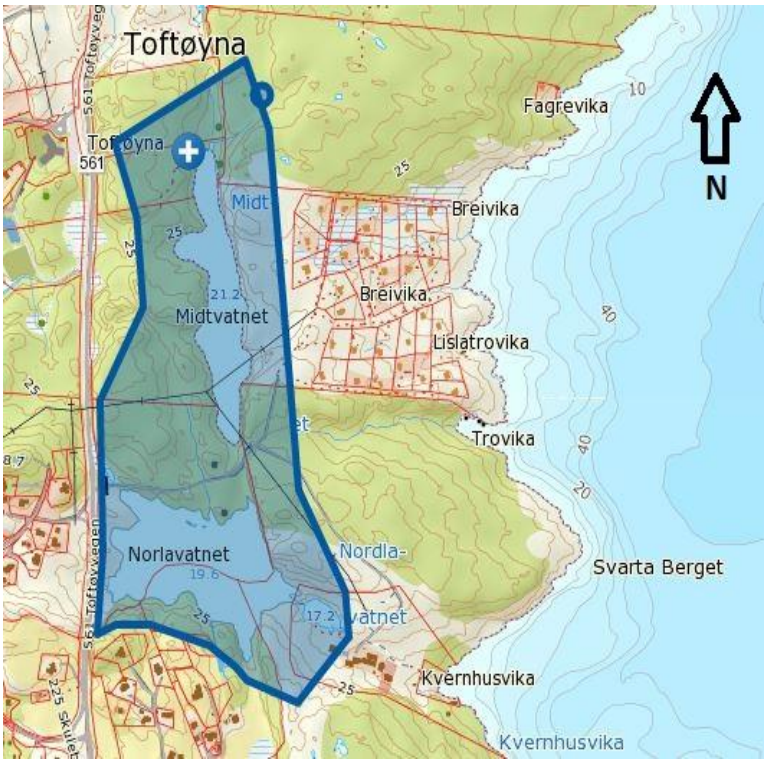


Figure 3.2.2: Drainage/runoff area (Norgeskart, 2016)

3.3 Saltwater source

Figure 3.3 displays a map of the seawater source for the RAS outside the farm. Location “a” shows the dock where unloading and loading of smolt takes place on the well boat, MS Moviestar. Location “b” is 270 meter from shore with a depth of 46 meter and marks the site of the seawater inlet source positioned downstream. Location “c” is upstream and shows the estimated position of the outlet.

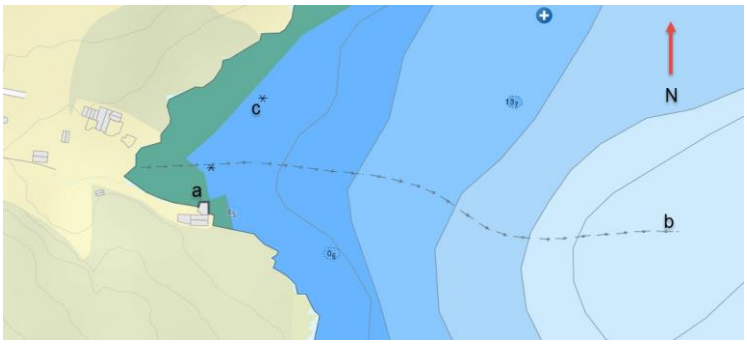


Figure 3.3: Adapted map of seawater source (Norgeskart, 2016)

3.4 RAS Module 17

The RAS installed at Vik settefisk is a modular design (LR12) so that the biomass production can be adjusted per the total available water and the client’s fish concession. Each module is a separate system, which lowers the risk of cross contamination and spreading of pathogens between the separate units. Figure 3.4.1 is a flow sheet for RAS 17 showing the separate compartments, the direction of flow and the measuring points used in this experiment to collect water samples.

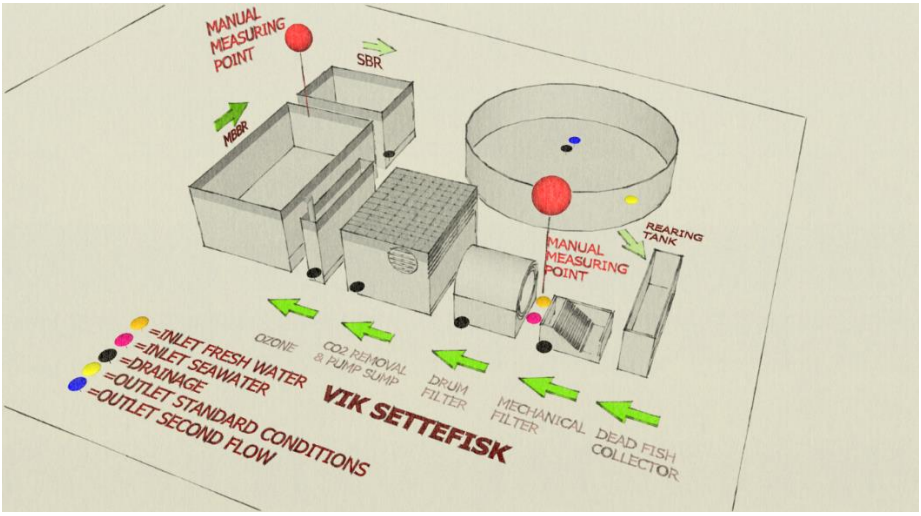


Figure 3.4.1: RAS module 17 showing the flow in the system as well as the inlet fresh/saltwater, drainage from tanks, outlet under standard operating conditions and the secondary flow used for cleaning and vaccinations. (Levik, K., 2017a)

Figures 3.4.2 and 3.4.3 show the rearing tank with and without water. The rearing tank is installed on a layer of aggregate and then a layer of sand. The 4mm galvanised steel plating of the tank is bolted together (shown in dark grey brick pattern) to form the main structure. A PE liner is then placed on top of the plating to make the tank impervious to water. The base of the tank is sloped towards the central drainage to promote a self-cleaning tank.

Figure 3.4.2: Tank without water showing central drainage (Levik, K., 2017b)

Figure 3.4.3: Rearing tank with water and jumping barrier (black ring) (Levik, K., 2017b)

Table 3.4 has been created and adapted from the LR 12 module specifications including maximum or specific values for each measurement and where the unit is measured (Sterner, n.d).

Table 3.4: Key operating figures from Sterner's LS 12 module (Sterner, n.d)

Unit	Value
Fish tank volume (m^3)	440
Maximum biomass - Salmon (kg)	33 000
Production biomass density (kg/m^3)	75
Maximum feed spent per day (kg/day)	528
Maximum flow (m^3/min)	12
New water added p/kg fish (L/min)	90
New water added p/kg feed (L/min)	0,79±0,66 (n=104)
Percentage of water recirculated (%) with max biomass and under full operating conditions	98.8
Water recirculation rate (p/min)	36,7
Temperature based on location (°C) under operating conditions	14
Power usage with maximum feed (kW)	75
Total power available (kW)	92

The researcher created a P&ID (MicroStation, 017); Appendix A) based on Sterners system description including their process description drawing 5418-P-XB-Vik RAS modul-00 and visual observations noted on site (MicroStation, 2017). This drawing doesn't show places for injection of O_3, O_2, air, electrical, power, heating, cooling medium or make-up water.

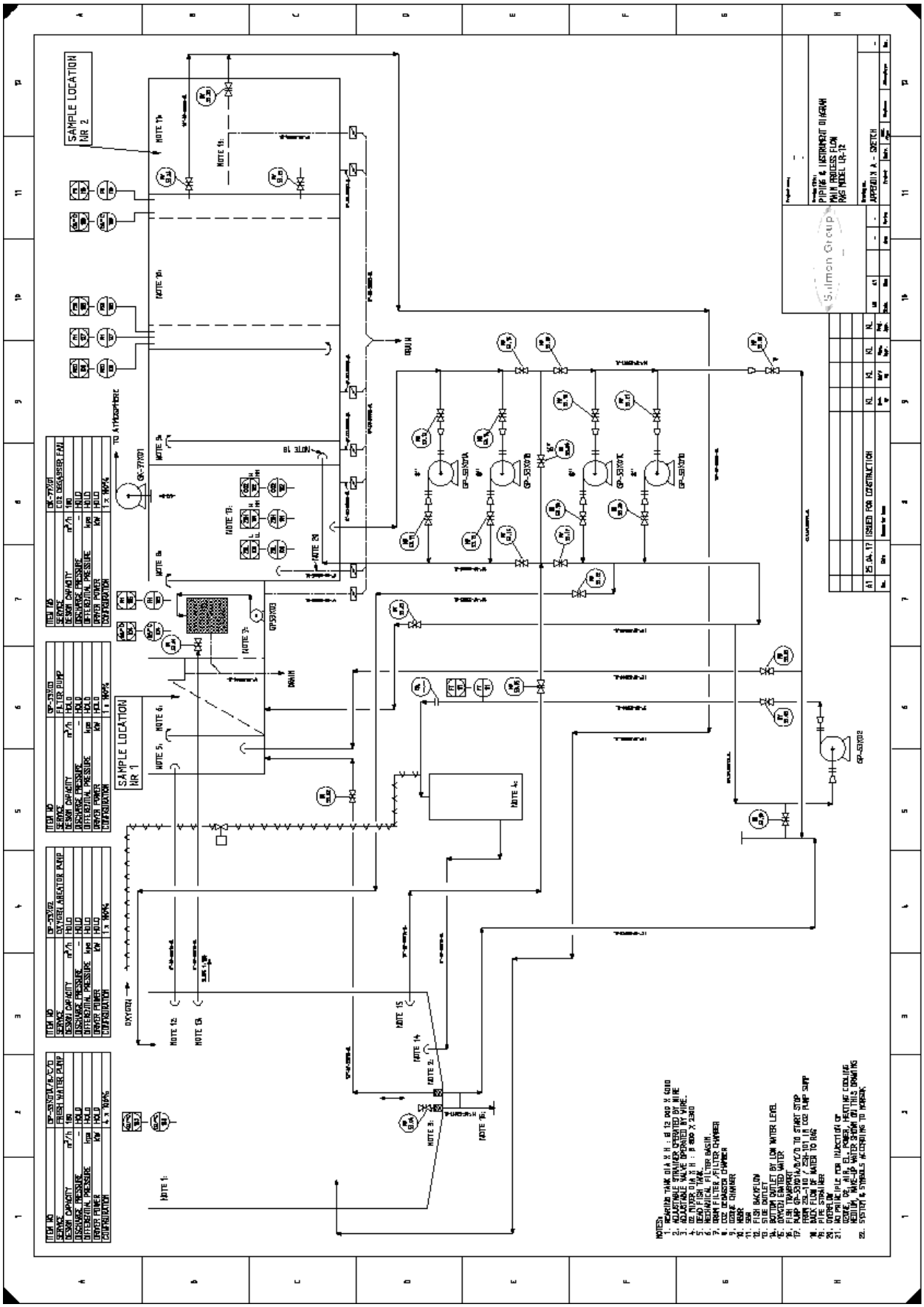


Figure 3.4.4: P&ID of Main process flow of LR 12 (Levik, K., 2017c, MicroStation, 2017).

3.5 Water flow

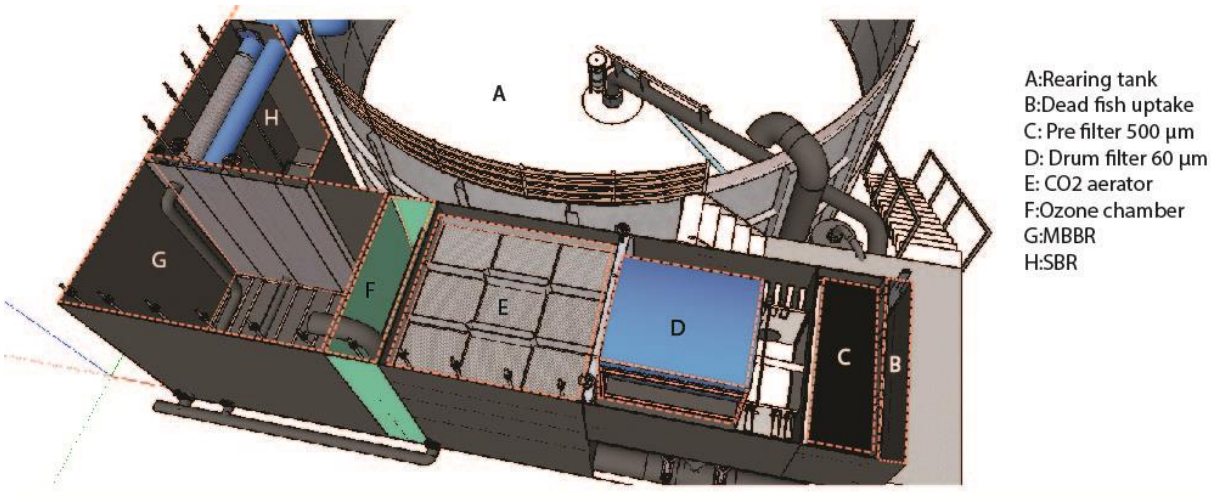


Figure 3.5.1: Modified picture of filtration units within RAS (Sterner Aquatech AS, n.d.)

Figure 3.5.1 displays the RAS module including rearing tank and filters. Under standard operating conditions, the effluent water is pumped from the rearing tank (A) to the pre-filter chamber (C), via the dead fish chamber (B). Alive fish can swim back from the dead fish holding unit back to the rearing tank. From C, the water is led to the drum filter (D) for finer filtration. Then the water travels through the CO_2 aerator and trickling filter (E) whilst being divided into 2 flows at the sump, located underneath E. It is also at the manifold after the sump that oxygenation takes place.

The first flow accounts for approximately one third of the pumped water and enters the ozone chamber (F) via pump GP-53X01A and GP-53X01B (Appendix A). Within the Ozone chamber water is mixed before entering the MBBR (G) and the SBR (H) before returning to the rearing tank. There is a bypass between the MBBR and rearing tank which gives the possibility to regulate or shut off the water flow to the SBR when performing scheduled washing operations. The second flow (two-thirds of pumped water) is filtrated via the drum filter (D) and aerated (F) before being pumped back into the rearing tank (A) via pump GP-53X01C and GP-53X01D (Appendix A).

When grading, washing and vaccination routines are being completed, there is a need to lower the water level to crowd the fish. Firstly, the water is lowered and then pumped from the rearing tank (A) to the prefilter (C) and then pumped to the drum filter (D) to be aerated. Ozone can be added (F) before the water is returned to the rearing tank via the MBBR (G) and SBR (H) (Sterner Aquatech AS, n.d.).

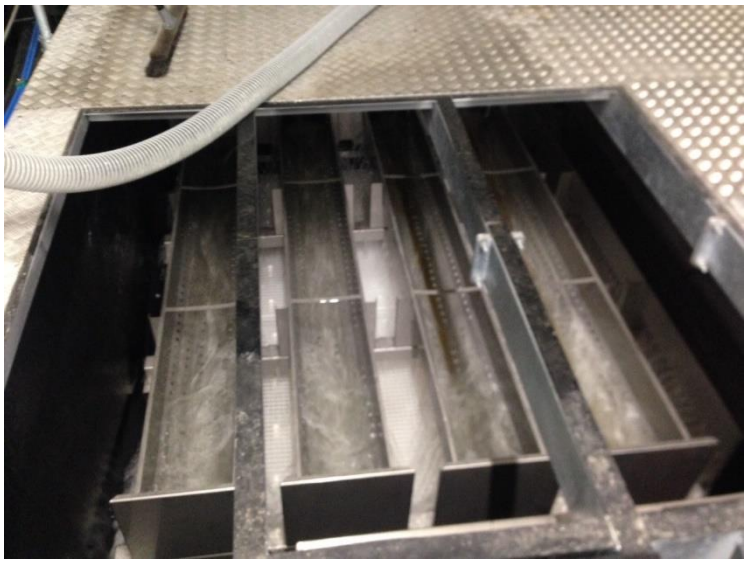


Figure 3.5.2: Photo of the trickling filter (E in Figure 3.5.1) (Levik, 2017b).

3.6 Mechanical filtration within the RAS

3.6.1 Pre-filter

The pre-filter is the initial water filtration processes from the rearing tank (Figure 3.6.1). It filters unwanted particles vertically with larger particles (500µm) being removed by a scraper continuously relocating particles to the sludge treatment catchment above the filter. The effluent water from the drum filter assists in cleaning of the residue in the catchment basin which is then removed from the system. By installing a pre-filter in front of the drum filter the load to the drum filter is reduced and the overall cleaning effect is improved.

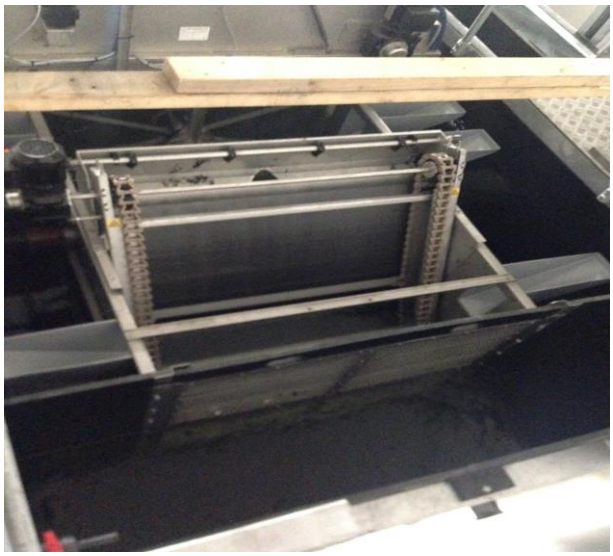


Figure 3.6.1: The pre-filter with scraper (Levik, K., 2017b)

3.6.2 Drum filter

The drum filter (Figure 3.6.2) consists of an open filter with a 60µm mesh that filters out fine category particles (1 < µm < 100) (Dolan, Murphy and O'Hehir, 2013). This type of micro-screen filtration is configured based on micro-screen rating, particle size distribution and water quality. Leftover feed, faeces and other organic material are filtrated via micro straining with effluent water. This, in turn, reduces the weight of organic material entering the MBBR and SBR (Sterner Aquatech AS, n.d). There is a rinse cycle that is triggered when the water level is low which cleans, using high pressure water, excess growth on the filter screen.

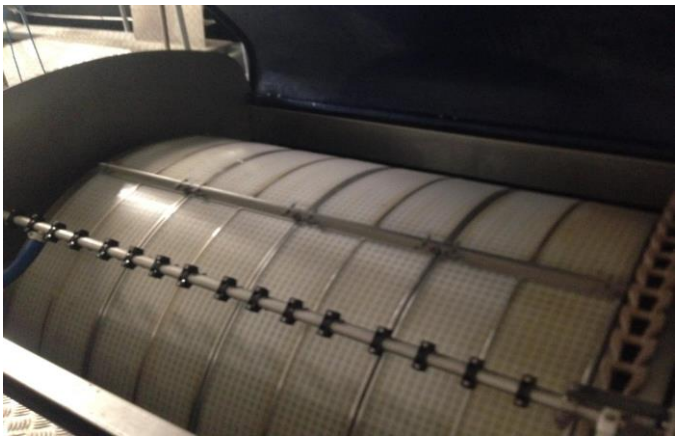


Figure 3.6.2: The rotating drum filter is partly submerged in the water and a micro polyester screen to filtrate suspended matter. (Levik, K., 2017b)

3.7 Outlets

The outlet water in the main rearing unit is divided into 3 flow patterns; central, side and dead fish collection (Sterner Aquatech AS, n.d).

3.7.1 Central outlet

In normal operating conditions, the central outlet, at the bottom of the rearing tank is the main outlet where the primary current is being drained. Approximately one third of the water that has a high particle content is being led first to the mechanical filter then via drum-filter. The primary

current, water flow and suction ability can be controlled by valves MV 53.02 and MV 53.10 shown in Figure 3.7.1 (Appendix B)

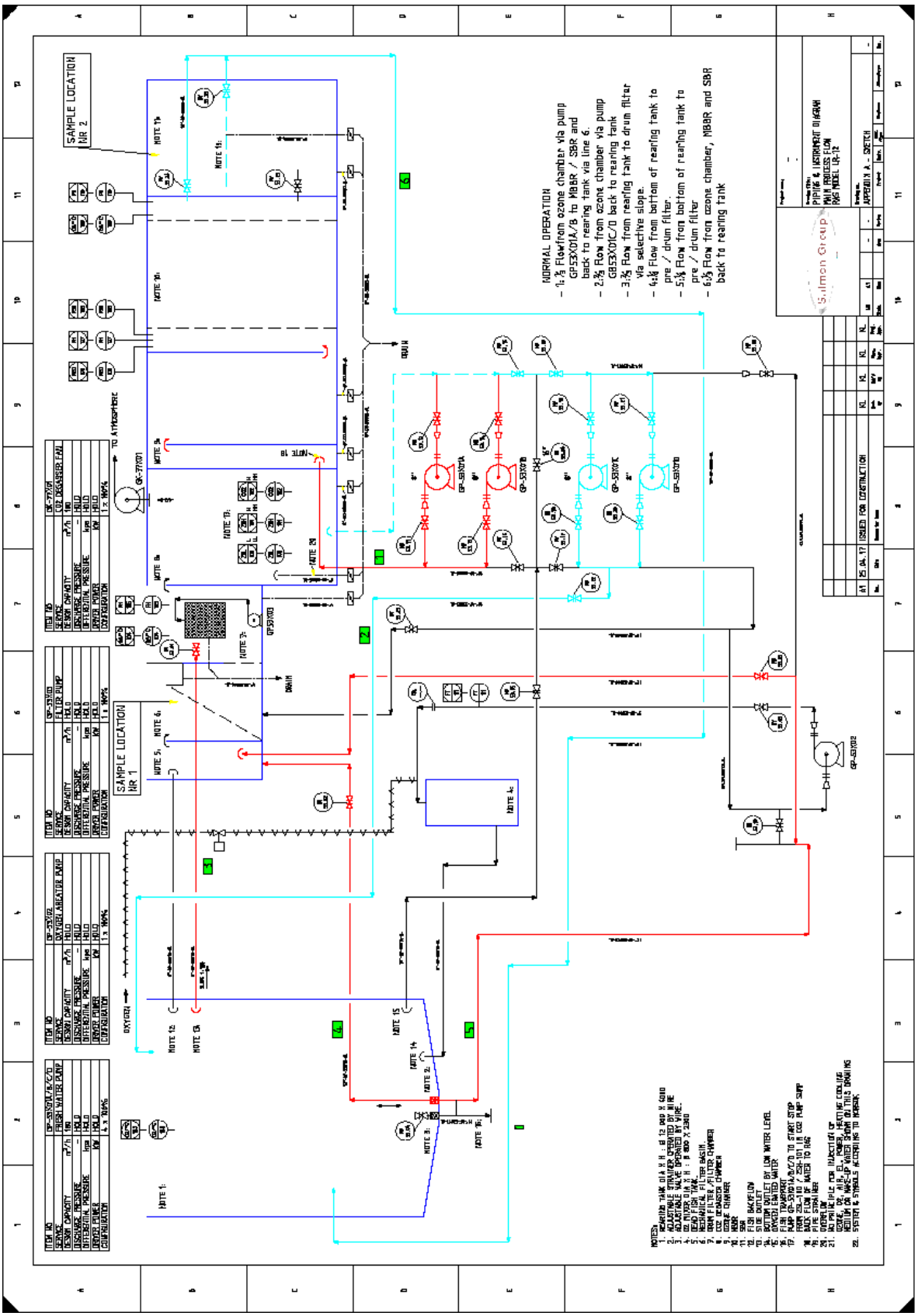


Figure 3.7.1: P&ID displaying the system under normal conditions (Levik, K., 2017c; MicroStation, 2017)

3.7.2 Side outlet

The secondary current (approximately two thirds of total water) is led directly to the drum filter by gravity drainage. The volume of water can be adjusted by valve MV 53.01 (Figure 3.7.2, Appendix C) Additionally, oxygenation can take place at this location preventing eutrophication during grading operations.

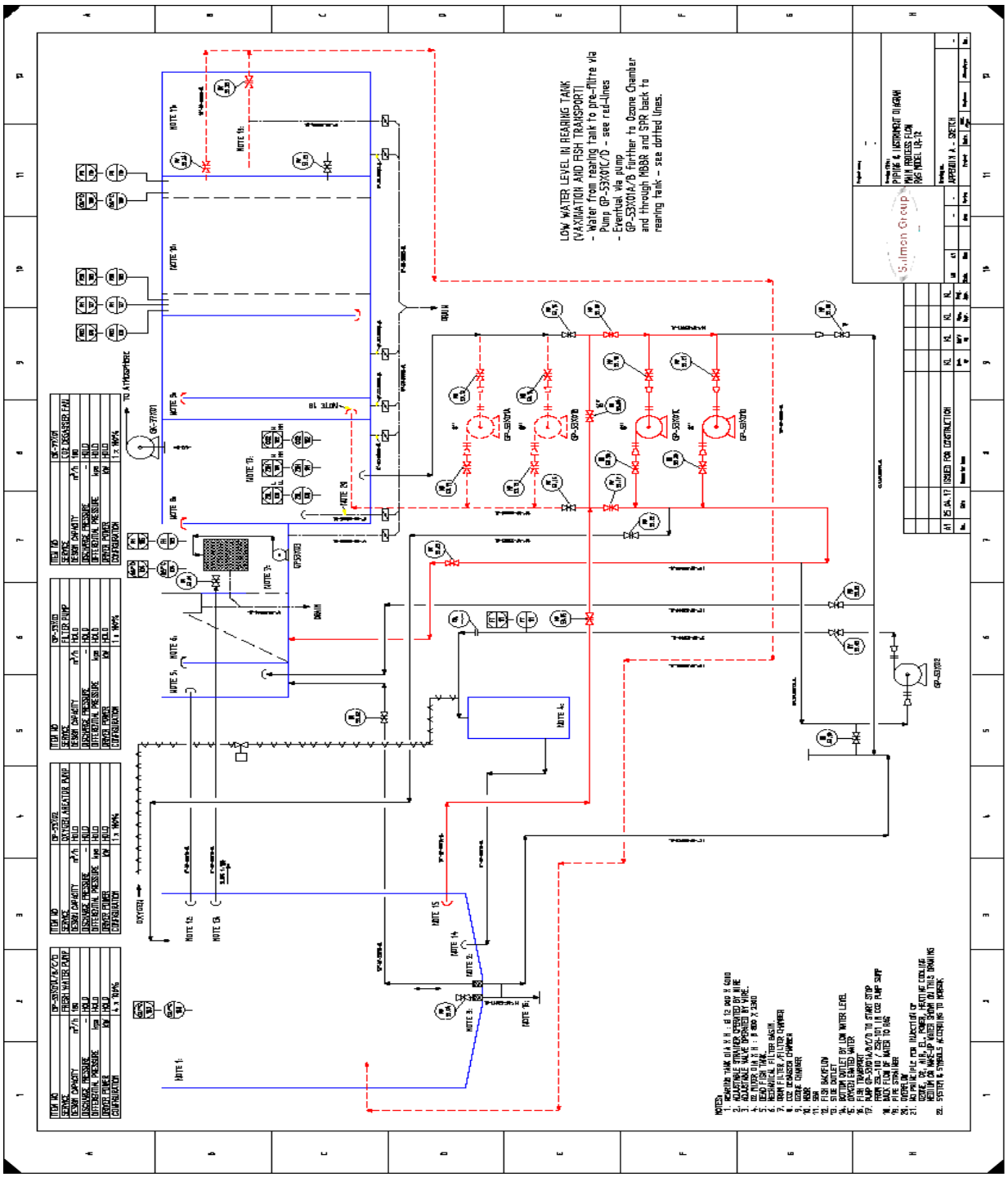


Figure 3.7.2: The RAS showing low water level during vaccination and transport. (Levik, K., 2017c; MicroStation, 2017)

3.7.3 Dead fish outlet

To collect dead fish, there is a manually operating unit and a fish transport system next to the central outlet in the rearing tank. The manually operated pulley system is operated from the top of the rearing tank (Figure 3.7.3.1) which opens a valve (Figure 3.7.3.2). Moreover, the fish is transported to the dead fish uptake to be removed, then graded (Figure 3.7.3.3).

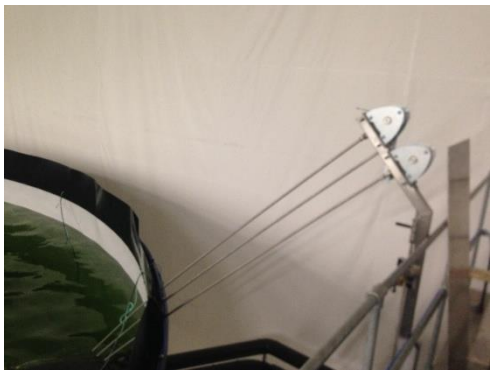


Figure 3.7.3.1: Manual operating pulley system for dead fish collection (Levik, K., 2017b)

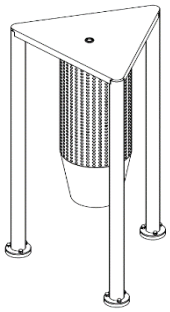


Figure 3.7.3.2: Dead fish transport valve installed centre rearing tank next to central outlet

Figure 3.7.3.3: Dead fish collector transfer system (Levik, 2017b).

3.8 Biological filtration processes

3.8.2 O_3 treatment

Figure 3.8.1 shows the O_3 generating room where the Wedeco Ozone generators are installed (one generator per tank). The O_3 processes are detailed in section 2.24

Figure 3.8.1: Wedeco Ozone generators (Levik, K., 2017b)

3.8.3 MBBR

Figure 3.8.2.1 shows the functioning of the aeration system within the MBBR, with the arrow in the middle (A) showing the flow direction within the MBBR. The white nodes (B) at the bottom of the figure supply aerated water intermittently as the water coming from the ozone chamber is super saturated (Sterner Aquatech AS, n.d). Within this aerobic process, the air that enters the system causes the biofilm carriers (D) to be agitated and then move. This coarse bubble aeration system is closely controlled to reduce the effects of excessive use of air which causes, heavy shelling of the biofilm, decreased capacity within the biofilters and the creation of fine particles that leads to fish gill irritation and inflammation (Sterner Aquatech AS, n.d).

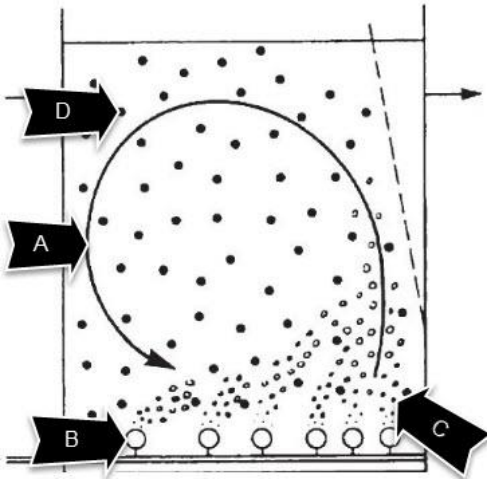


Figure 3.8.2.1: Modified figure of the principle function of an Aerobic MBBR (Ødegaard, 2006)

There were 2 different biofilter chips used within Module 17 RAS, RK BioElements (Medium) and Mutag. The RK BioElements carrier structure can be seen in greater detail in Figure 3.8.2.2 and the Mutag biochip is displayed in Figure 3.8.2.3. The RK BioElements biochips are made from high density polyethylene with a cylindrical shape, internal "cross like" structure and "fins" on the exterior (Ødegaard, 2006) whereas the Mutag biochips have a round/paraboloid structure with a detailed pore system on the surface. Further details regarding these chips has been included in Table 3.8.2.1.

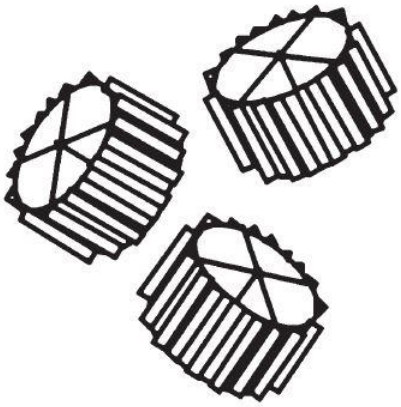


Figure 3.8.2.2: RK Bioelements (Medium) biochip carrier

Figure 3.8.2.3 Mutag biochips with surface areas 3000m^2/m^3 (Wateronline.com, 2015)

Table 3.8.2.1: Technical specifications of the RK BioElements Biochip and the Mutag Biochip

	Nominal diameter (mm)	Nominal thickness (mm)	Bulk density (kg/m^3)	Protected surface area (m^2/m^3)	Volume weight ($kg/m^{3)}$
Mutag Biochip	22	1	0,95	3000	170
RK BioElements	-	-	1	750	120

3.8.4 SBR

In the SBR is a constructed wash system which can be cleaned with a back flush. Fine particles and the residue biofilm is collected which makes the water clearer (Sterner Aquatech AS, n.d). Within Module 17, due to leaking between the MBBR and SBR, the RAS used within this case study functioned without the SBR filtration whilst the case study water analysis was being conducted (Figure 3.8.3).

Figure 3.8.3: Clogged SBR with biochips (Levik, 2017b).

3.9 Emergency oxygen

There are 12 ceramic diffusers installed in the rearing tank forming a main ring distribution cable. The main ring cable increases security as the ring shape distributes the pressure evenly ensuring even diffusion of emergency oxygen from the emergency oxygen dosage unit. The dosage is controlled by the Oxyguard Commander system via an actuated solenoid valve in fail close (FC) state (Oxyguard, 2017).

4 MATERIALS AND METHOD

4.1 Experiment conditions

On the 9th of December 2016, Vik Settefisk released 105,658 smolt into the rearing tank as a part of their commercial production. The Salmonbreed QTL PD smolt hatched in July 2015, started feeding in August 2015 and was stained and reared at Dåfjorden hatchery AS (Salmobreed, 2016; Salmongroup.no, 2016). The average individual weight was between 69g ±1.2g with an expected grow out weight of 350g. They fed on commercial feed with 40% protein, Biomar Orbit, (Biomar.com, 2016). As there was no automatic feeder installed for two weeks, a Steinsvik automatic feeder was installed as manual feeding was completed where the amount of feed was calculated per the SGR. Feeding began on day 7 of the experiment period.

The experiment period spanned over 122 days from the 9th of December 2016 until the 9th of April 2017 when the fish were sold. For the first 22 days (Stage 1) the following water quality measurements were recorded; O_2, Temperature, pH, ORP, CO_2, Salinity, NO_2-N, NO_3-N, NH_4, Alkalinity and COD. After this period; O_2, Temperature, pH, CO_2, Salinity and mortality were recorded for the duration of the set out (Stage 2).

4.1.1 Water parameter tests

Automatic tests are parameters that were automatically logged by Vik Settefisk via Aquafarmer software (Aqua Farmer, n.d.). Table 4.1.1 displays the equipment used to measure each parameter including the unit of measurement the range of measurement and the accuracy.

Table 4.1.1: Equipment used for each automatic parameter including the recording range and reference information.

Parameter	Equipment	Range	Accuracy	Frequency
O_2(mg/L and %)† Temperature (°C)†	Oxyguard OxyLog oxygen probes with temperature sensor (OxyGuard, n.d)	0-200%, 0 to + 40°C	± 0.1%,± 0.2ºC	10 min. intervals
pH†	Oxyguard pH sensors, (OxyGuard, 2016a)	pH 0-14	0 to 60 ºC	10 min. intervals
ORP (mV)	OxyGuard Redox Manta transmitter (Oxyguard, 2016a)	-	10 bar at 25ºC	10 min. intervals
CO_2(mg/L)†	OxyGuard dissolved CO_2 analyser (OxyGuard, 2016b)	0-50 mg\l	Calibration accessories 1.0 kg	10 min. intervals
Salinity (ppt)†	Meinsberg sensor (GmbH, 2017)	2 - 200 µS/cm,	C1: 0.1 KCl solution (12.9 mS/cm 25 °C); C2: 0.01 N KCl solution (1.41 mS/cm 25 °C))	10 min. intervals

†Stage 2 parameters.

Table 4.1.2 details the equipment that was used to complete the manual testing. In the equipment section, the number of test kits used is detailed including what their contents.

Table 4.1.2: Equipment required for each manual testing parameter, including vendor information. All parameters are measured in mg/ L

Parameter	No. of tests conducted	Equipment	Frequency of measurement
NO_2-N (mg/L)	18	Spectroquant® Nitrite test kits (1.14776.0001) including; 6 bottles of reagent NO_2-1 and 1 auto selector (Merck Millipore, 2013a)	3 times per week
NO_3-N (mg/L)	18	Spectroquant® Nitrate test kits (1.14773.0001) including reagent NO_3-1, reagent NO_3-2, and 1 auto selector (Merck Millipore, 2016a)	3 times per week
NH_{+4} (mg/L)	18	Spectroquant® Ammonium test kits (1.14752.0001/2) including reagent NH_4-1, reagent NH_4-2, reagent NH_4-3 and 1 auto selector (Merck Millipore, 2013b)	3 times per week
Alkalinity (mg/L)	18	Spectroquant® (1.01758.0001) including reagent AC-1, reagent AC-2 and four empty cells with bar codes (Merck Millipore, 2014)	3 times per week
COD (mg/L)	8	Spectroquant® COD test kits (1.14895.0001) including 25 reaction cells (Merck Millipore, 2016b)	Once a week

4.2 Experiment Plan

4.2.1 Measurement location

O_2, Temperature, pH, ORP, CO_2 and Salinity, were all automatically measured by probes from fixed locations (Figure 4.2.1). In the rearing tank, drum filter basin and SBR there is an oxygen and temperature probe, with an additional temperature probe in the sump. A pH probe is in the drum filter, the MBBR and the SBR. For Redox and salinity, the probe is in the MBBR and lastly the CO_2 probe is in the sump. All probes are located on Appendix A. For parameters that had more than one probe before the biofilter, one probe result was excluded from the analysis. Each probe measures the parameters every 10 minutes, 24 hours a day and the data is recorded digitally via Aquafarmer software (Merck Millipore 2016a; Aqua Farmer, n.d.).

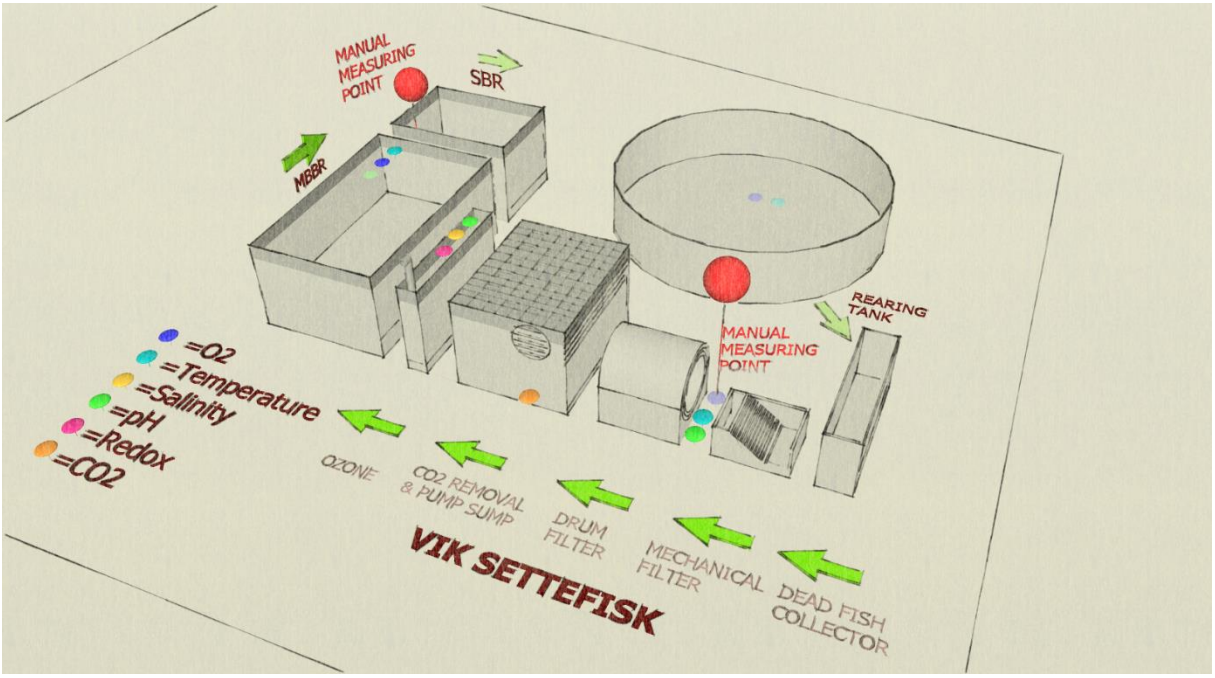


Figure 4.2.1: Sketch showing all measuring locations for the following automatic parameters; O_2, temperature, pH, Redox, Salinity, CO_2; also the manual measuring points where NO_2-N, NO_3-N, NH_{+4}, Alkalinity, COD were taken (Levik, 2017a)

For each manual measure, each Monday, Wednesday and Friday at 10:00am of the experiment period, 2 water samples were taken upstream, in the mechanical filter basin and downstream, in the SBR, (Figure 4.2.1 & Table 4.2.1). Figure 4.2.2 shows the feeding times over a 24-hour period and that the water samples for this experiment were collected after the 7:30am feeding session. Once the samples were collected, testing for Nitrite, Nitrate, Ammonia, Alkalinity, COD (only on Mondays) were completed by 12:30pm. Table 4.2.1 details the schedule for the manual sampling and testing for

each parameter. COD testing was completed in accordance with Norwegian standard ISO: 6060 (Standard Norge, 2003).

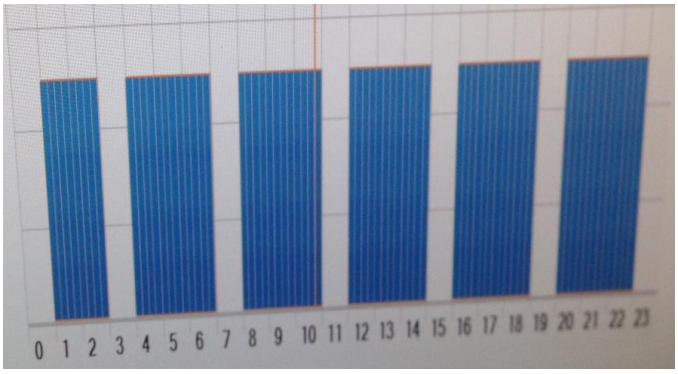


Figure 4.2.2: Feeding times shown in blue over a 24-hour period starting from 0:00 (12am) until 23:00 (11pm) (Aqua Farmer, n.d)

Table 4.2.1: Sampling and testing schedule for manual sampling

Time	Monday	Wednesday and Friday
10:00-10:20	Sample water from mechanical filter basin, SBR	Sample water from mechanical filter and SBR
10:20-12:30pm	Nitrite, Nitrate, Ammonia, Alkalinity, TAN, COD	Nitrite, Nitrate, Ammonia, Alkalinity, TAN

4.2.2 Statistical Analysis

The results for this experiment are expressed quantitatively as mean, standard deviations, correlations and test of differences. For automatic tests the mean ± SD of readings were recorded and calculated including each tenth minute reading from 10-12pm. Paired and 2 sample t tests and Pearson correlation coefficient calculations are to be completed with an assumed statistical significance of $p < 0.05$. For the Stage 1, paired t tests of differences are to be conducted for parameters, O_2, Temperature, pH, NH_4, NO_2-N, NO_3-N, Alkalinity, COD and TAN. For the comparison between Stage 1 and Stage 2, a 2-sample *t*-test will be conducted to determine statistical difference. Statistics were completed with the software program Minitab 17 (Minitab 17 Statistical Software, 2010) and Statgraphics (Statgraphics Centurion XVI, 2010).

4.3 Data registration

Between 10am - 12pm each day of the experiment period, the researcher logged onto Aquafarmer on the Vik Settefisk intranet and recorded all automatic data analysis onto an independent excel spreadsheet and the average of all measurements between 10-12pm was used for data comparison. This time was decided as it coincided with the researcher's regular meetings/physical sampling at Vik Settefisk. (Aqua Farmer, n.d.). Other parameters used in this study including SGR,

FCR, Mortality, Biomass and fish weight were all recorded at the end of Stage 1 and the final weight of the fish was recorded on final sale.

To collect the water samples for the manual water parameters, 3x 500ml large plastic containers were used, which were then taken to a laboratory within the hatchery building. The mean air temperature within the laboratory was 7.9°C, as the experiment was conducted in winter and the laboratory was insulated but had no added heating. Personal protective equipment used included, latex gloves, eyewear and alcohol based wash. Other cleaning equipment included; 1 cleaning bucket with distilled water solution, 1 glass container for effluent samples. At the end of the experiment, the effluent samples were disposed of at Fjellvar renovation station, located in Øygarden. Equipment required for the water sample analysis for each test included; 8 small test tubes (10 when testing COD), 7 pipettes and a stopwatch. The protocol followed during the water analysis is detailed in Appendix D. Tests for Nitrite, Nitrate and Ammonia were completed within 15-20 minutes, Alkalinity took 5 minutes and COD took 2.1 hours including waiting and notation time (Appendix E).

4.3.1 Manual test considerations

For Ammonia, testing of brackish water (up to 12ppt) followed the same procedure for seawater, as stipulated by vendor when contacted by email (Paulsrud, 2016) (See step 5, Appendix D). For the brackish and seawater testing, an additional bottle of NaOH was required. For salinity, within the first two weeks of production only fresh water was being used and tested. After this time, seawater was gradually added, until 7 ppt was reached and therefore testing had to be adjusted accordingly.

4.3.2 Calculations

Calculation for water drainage

Below displays the calculation used to determine the water drainage for the RAS.

$$WV = A \; x \; Ad$$

Where WV is the Water volume (p/year);
A is the Area (m^2) and
Ad is the Average drainage p/year (mm)

Calculation for the total protected surface area for Mutag biochip

The total protected surface area for the Mutag biochip was calculated via the following equation.

$$\text{TPSA} = 3000\text{m}^2\ m^3 \times V$$

Where TPSA is the total protected surface area of the Mutag biochip and V refers to the volume of the Mutag biochip.

Calculation for the total protected surface area for RK Bioelements biochip

The total protected surface area for the Bioelements biochip was calculated via the following equation.

$$\text{TPSA} = 750\text{m}^2\ m^3 \times V$$

Where TPSA is the total protected surface area of the Bioelements biochip and V refers to the volume of the Bioelements biochip.

Calculation for Areal TAN removal rate

The removal rate for TAN was calculated via the below calculation

$$ATR = K_C(TAN1 - TAN0)Q/A$$

ATR is the Areal Tan removal rate ($g/m^3/day$)

K_C is the unit conversion factor (24 hours x60 minutes/1000) =1,4

TAN1 is the SBR TAN concentration (mg/L);

TANO is the drum filter TAN concentration (mg/l);

Q is the flow rate (L/min) and

A is the Mutags total protected surface area (m2)

Calculation for Areal NO_2-N removal rate

The removal rate for NO_2-N was calculated via the below calculation. ATR or the TAN removal rate is added to the beginning of the equation as when TAN is converted, NO_2-N is produced (Malone and Beecher, 2000).

$$ANR = ATR + K_C(NO2 - N1 - NO2 - N0)Q/A$$

ANO2R	is the Areal NO_2-N removal rate (g/m^3/day)
ATR	is the Areal TAN removal rate (g/m^3/day)
K_C	is the unit conversion factor (24 hours x60 minutes/1000) =1,4
NO_2-N 1	is the SBR NO_2-N concentration (mg/L);
NO_2-N O	is the drum filter TAN concentration (mg/l);
Q	is the flow rate (L/min) and
A	is the Mutags total protected surface area (m2)

Calculation for Areal NO_3-N removal rate

The removal rates were calculated via the below calculation

$$ANO3R = K_C(TAN1 - TAN0)Q/A$$

ANO3R	is the Areal Tan removal rate (g/m^3/day)
K_C	is the unit conversion factor (24 hours x60 minutes/1000) =1,4
TAN1	is the SBR TAN concentration (mg/L);
TANO	is the drum filter TAN concentration (mg/l);
Q	is the flow rate (L/min) and
A	is the Mutags total protected surface area (m2)

Calculation of TAN

Below is the calculation used to work out TAN. The researcher tested NH_{4+} via water sampling procedures mentioned in section 4.2 and $NH3_+$ is the percent of $NH3_+$ in TAN calculated from Emerson et al. (1975) and pH and temperature (°C) data.

$$TAN = \frac{NH4+}{1 - NH3}$$

Where	TAN is the TAN concentration (mg/L)
	$NH4_+$ is the NH4 concentration (mg/L) and
	$NH3_+$ is the percent of $NH3_+$

Calculation of SGR (estimated)

$$SGR = 100(InV1 - InV0)/t$$

Where SGR is the specific growth factor (%/day)

V1 is the final fish weight

V0 is the fish weight on day 22 (g)

t is number of days

Calculation of SGR (predicted)

The below calculation was used to calculate the predicted SGR according to the Temperature and weight of the fish (Forsberg, 1995).

$$SGR = 0{,}9T^{0{,}97} \times W^{-0{,}34}$$

Where SGR is the specific growth factor (%/day)

T is the temperature in °C

W is the weight (g)

% of feed change

The percentage change between 2 consecutive days was calculated via the calculation below.

$$\frac{(F(2) - F(1))}{F(1)} \times 100$$

Where F(2) is the total feed (kg) from day 2

F(1) is the total feed (kg) from the day being calculated (the day before F(2))

Mortality (%)

To find the total percent mortality loss for Module 17 for stage 1 and 2 the below calculation was used. Total mortality refers to the total number of fish that died and final biomass refers to the final weight (kg) of fish.

$$\frac{\text{Total mortality}}{\text{Final biomass}} \times 100$$

Calculation for estimating final growth

The below equation was used to estimate using Stage 1 values what the final weight of the fish would be.

$$V1 = V0(1 + SGR)/100)^t$$

Where V1 is the expected growth (g)

VO is the weight at the end of Stage 1(g)

SGR is the mean SGR for Stage 1

t is the days

Calculation for stocking density

Below is the calculation used to determine stocking density.

$$SD = \frac{W}{V} * 1000$$

Where SD is stocking density in kg/m^3

W is total fish weight (kg)

V is tank volume (L)

5 RESULTS

5.1 Stage 1

5.1.1 Water quality parameters overview

Table 5.1.1 displays data for the water quality parameters measured before, inside and after the biofilter. O_2 in the rearing tank (91.81±5.53%) and SBR (97.58±4.03%) were within the recommended threshold and had a statistically significant difference (p=0). Temperature mean and standard deviation data in the pump sump was 7.35±0.41°C and 7.40±0.39°C in the SBR, also with a statistically significant difference (p=0,01). The maximum pH was recorded in the pump sump at 8 and a minimum recording in the SBR at 6.54. There were higher pH recordings in the pump sump compared to the drum filter and the SBR and the difference was statistically significant. The max value recorded for Redox was 254.9mV with a mean and standard deviation of 153.90±48.37mV and the mean and standard deviation for CO_2 was 6.33± 1.75mg/L. In relation to salinity (6.39 ±0.62ppt), salt water was added to the system from day 14 and this data reflects only these days as not to skew the data. Ammonia has mean and standard deviations of 0.60 ±0.38mg/L and 0.61±0.34mg/L in the mechanical filter basin and SBR respectively. The nitrite mean and standard deviations for the mechanical filter basin (0.15 ±0.05mg/L) and SBR (0.16 ± 0.06mg/L) were not significant. Furthermore, Nitrate indicated in the mechanical filter basin (2.96±1.49 mg/L) and SBR (3.51 ±1.81 mg/L), however the *t* test indicated no statistical significant difference (p=0,07). Alkalinity mean and standard deviations were 98.3±75.82 mg/L and 110.05±77.77 mg/L in the mechanical filter basin and the SBR, however the difference was not statistically significant (p=0,76). The highest COD recordings in the mechanical filter basin and SBR were 133mg/L and 137mg/L with the lowest recordings being 19mg/L and 15 mg/L contributing to means with large standard deviations, however, no statistical significance between the two measurement groups (p>0,05). TAN in the mechanical filter basin and SBR had similar means and standard deviations of 0.61± 0.38 mg/L in the mechanical filter basin and 0.61±0.33 mg/L in the SBR. The p-value for TAN indicated statistical insignificance (p>0,05).

Table 5.1.1: Mean and standard deviation calculations for each water quality parameter over Stage 1, p-value (paired t-test), recommended threshold from literature

	Pre- biofilter (Mean ± SD)		**Biofilter (Mean ± SD)**	**Post – biofilter (Mean ± SD)**	**p value**
	RT	**MFB/PS**	**MBBR**	**SBR**	
O_2 (%) *(n=528)*	91.81±5.53	-	-	97.58±4.03	0,00**
Temperature (°C) *(n=528)*	-	7.35±0.41	-	7.40±0.39	0,01**
pH *(n=528)*	-	-	7.25±0.30	6.90±0.24	0,00**
Redox (mV) *(n=264)*	-	-	153.90±48.37	-	-
CO_2 (mg/L) *(n=264)*	-	6.44± 1.78	-	-	-
Salinity (‰) *(n=108)*		-	6.35 ±0.59	-	-
NH_4 (mg/L) *(n=20)*	-	0.60 ±0.38	-	0.61±0.34	0,95
NO_2-N(mg/L) *(n=20)*	-	0.15 ±0.05	-	0.16±0.06	0,86
NO_3-N(mg/L) *(n=20)*	-	2.96±1.49	-	3.51 ±1.81	0,07
Alkalinity (mg/L) *(n=20)*	-	98.3±75.82	-	110.05±77.77	0,51
COD (mg/L) *(n=8)*	-	80.50±47.28	-	77.50±50.00	0,93
TAN (mg/L) *(n=26)*	-	0.61±0.38	-	0.61±0.33	0,95

*RT= Rearing tank, MFB= mechanical filter basin, PS= pump sump, SBR= Submerged fixed bed reactor

**p<0,05

5.1.2 O_2

Figure 5.1.2 displays the dissolved oxygen readings in the rearing tank and SBR. As stated previously (Section 2.2.1) the recommended upper limit for O_2 is 100%. There was a rise in O_2 above this limit on day 11 in the rearing tank and drum filter. The O_2 measurement in the SBR was higher than the limit on several occasions attributed to the addition of oxygen after the pump sump. The amount of O_2 added to the system per kilo of fish or feed was not calculated by Vik Settefisk nor Sterner Aquatech.

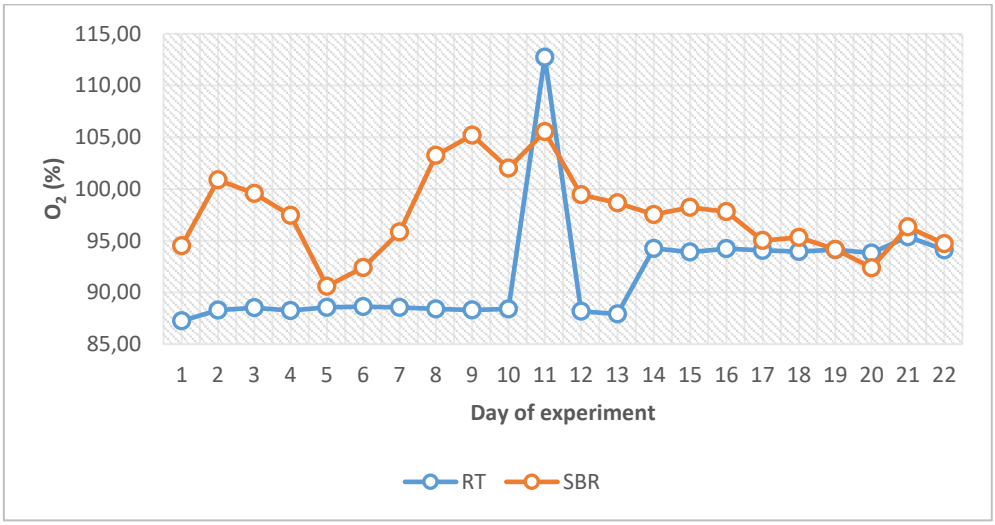


Figure 5.1.2: O_2(%) in the rearing tank and SBR during Stage 1

5.1.3 Temperature

Figure 5.1.3 shows the temperature readings for each day of Stage 1. There is a strong correlation (0,98, p=0) between temperature in the pump sump and the SBR as shown in Figure 5.1.3 with the similar fluctuations. There is a drop-in temperature on day 14 corresponding with the addition of salt water.

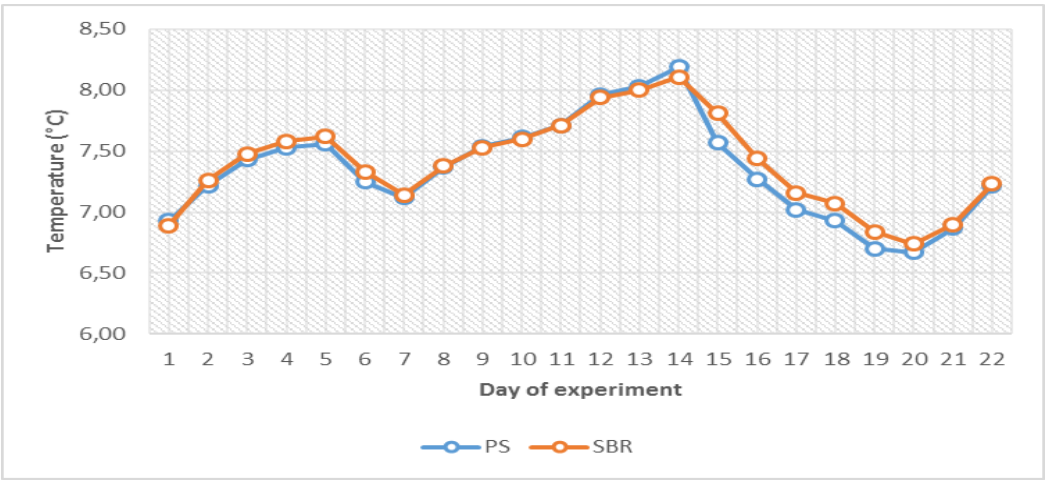


Figure 5.1.3: Stage 1 readings for Temperature (°C) in the pump sump (PS) and SBR

5.1.4 pH

Figure 5.1.4 shows the first 22 days and the pH readings. There was a spike in pH on day 11 in the pump sump and SBR and pH levels peaked over the recommended limit in the pump sump from day 1-4, 6-12 and 14-21. However, from day 14, pH levels plateaued and remained consistent for the rest of Stage. These peaks in pH have corresponded with an increase in nitrite during the nitrification process. A strong Pearson correlation coefficient of 0,78 was found between PS and SBR measurements (p=0).

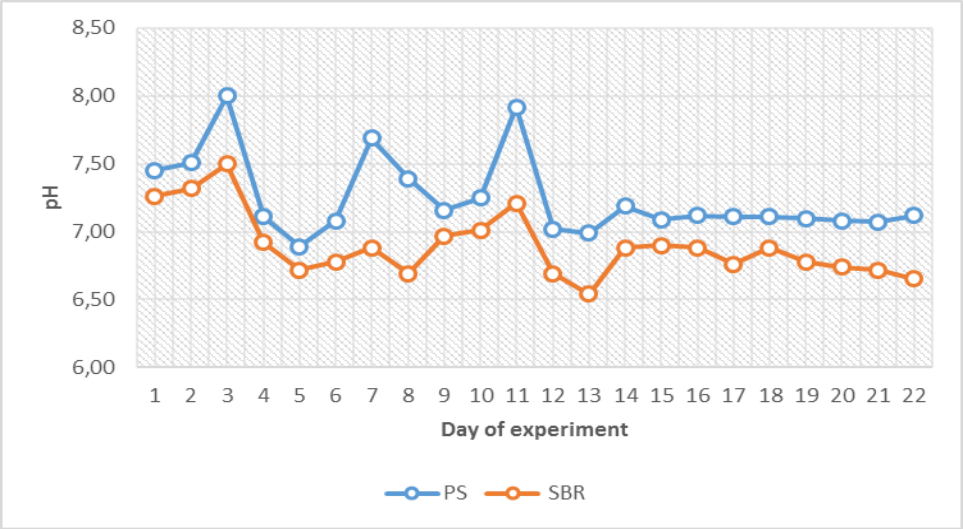


Figure 5.1.4: Stage 1 readings for pH in the PS and the SBR

5.1.5 ORP

The ORP peaked on day 4 and 9-10 to over 200mV in the PS.ORP values ranged between 91,72 and 254,59. There is a linear decline from day 10 (=236,04mV) to the lowest point at day 22 (=91,72mV) (Figure 5.1.5).

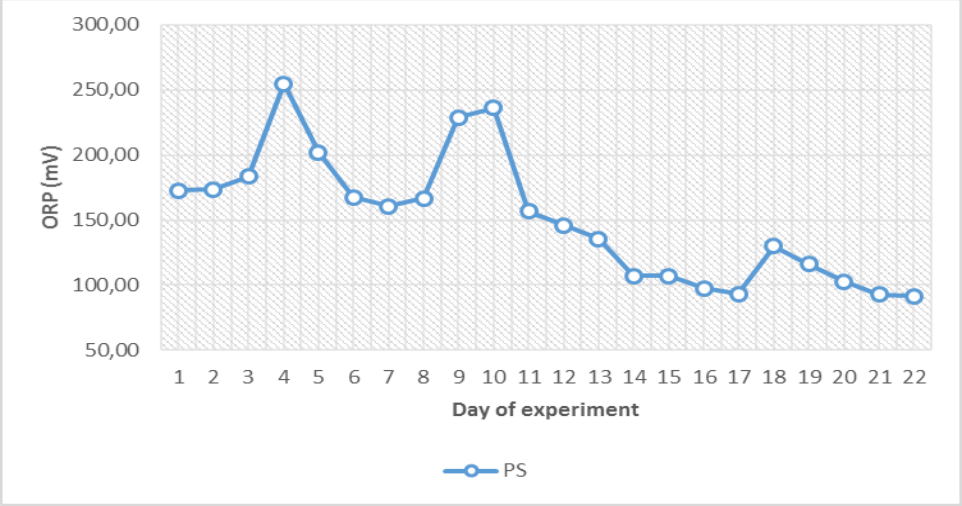


Figure 5.1.5: Stage 1 readings for ORP (mV) in the PS

5.1.6 CO_2

Figure 5.1.6 displays the CO_2 readings in the PS during the 22 days of stage 1. The graph shows fluctuations in CO_2, with the sharpest drop between day 4 (=6,87mg/L) and day 5(=3,22mg/L). CO_2 then plateaued from day 6 to day 11 before increasing on day 12 and then dropping to 5,60mg/L on day 15. The highest reading was experienced on day 21 with a CO_2 of 9,68.

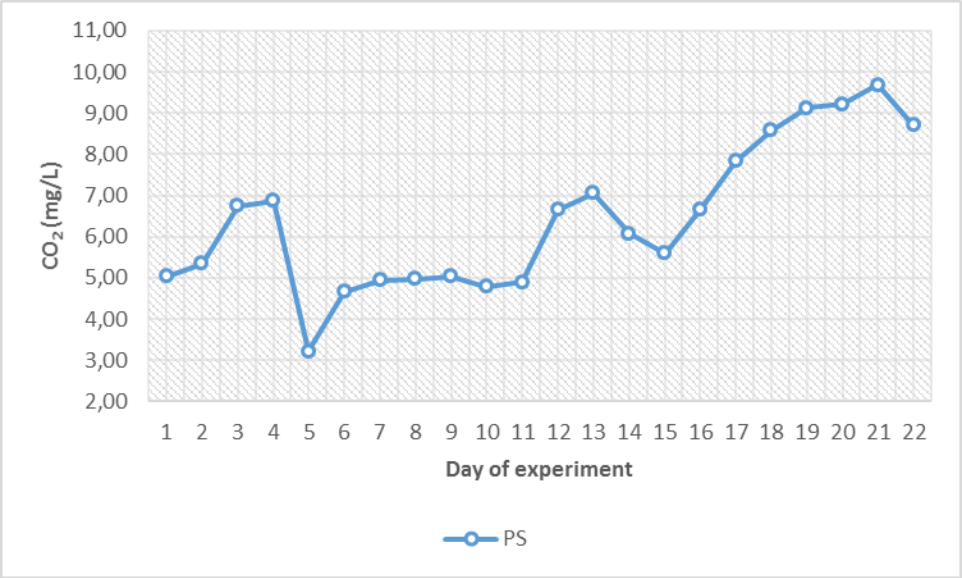


Figure 5.1.6: Stage 1 readings for CO_2 (mg/L) in the PS

5.1.7 Salinity

Figure 5.1.7 shows that on day 14 seawater was added to the system with a reading in the MBBR of 5,19ppt. The salinity then began to rise until 7,04 ppt on day 16 before levelling out to 6,01ppt on day 20.

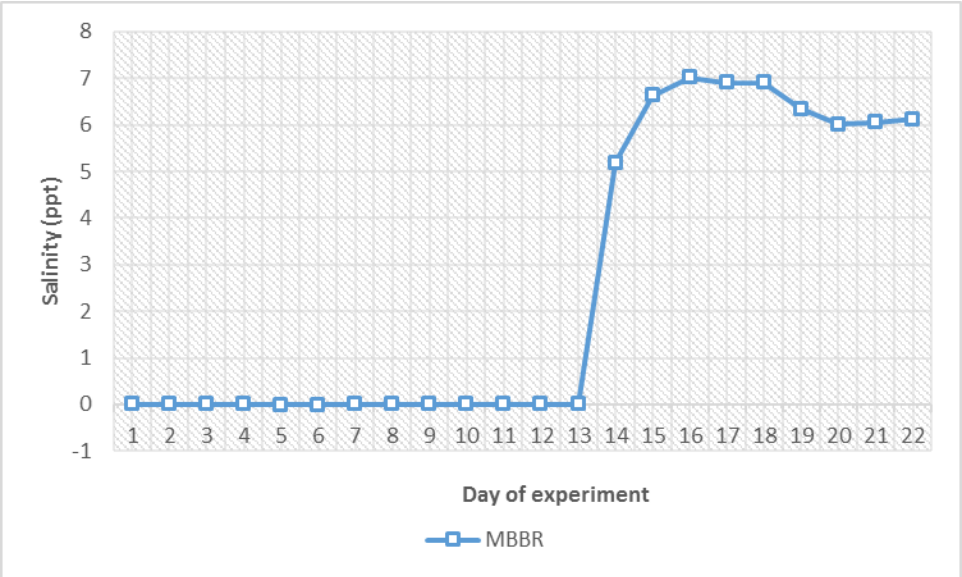


Figure 5.1.7: Stage 1 readings for Salinity (ppt) in the MBBR

5.1.8 Alkalinity

Figure 5.1.8 displays the alkalinity measurements from day 1 to day 22. The highest alkalinity measurement was taken on day 22 in the MFB (211mg/L) and the SBR (213.5mg/L). For the first 8 days of the experiment, Alkalinity measurements in the MFB and SBR were similar. From this time, there was fluctuations every 2-3 days in the MFB and SBR. A Pearson correlation coefficient was conducted, indicates a strong correlation (0,73; p=0,02).

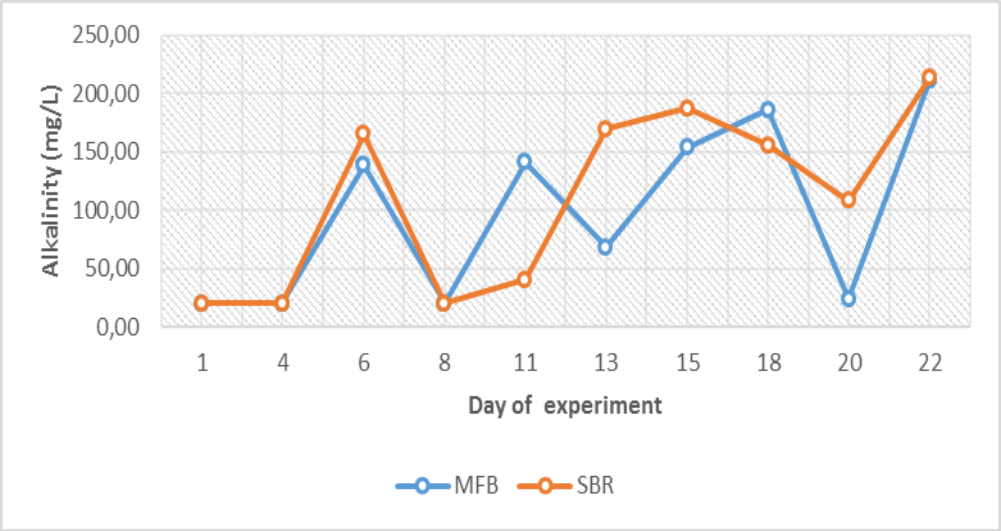


Figure 5.1.8: Stage 1 readings for Alkalinity (mg/L) in the MFB and SBR

5.1.9 COD

COD measurements were taken once a week during the first 22 days of the experiment and Figure 5.1.9 displays the 4 measurements recorded in the MFB and SBR. These recordings are higher than the recommended readings mentioned in section 2.2.9.

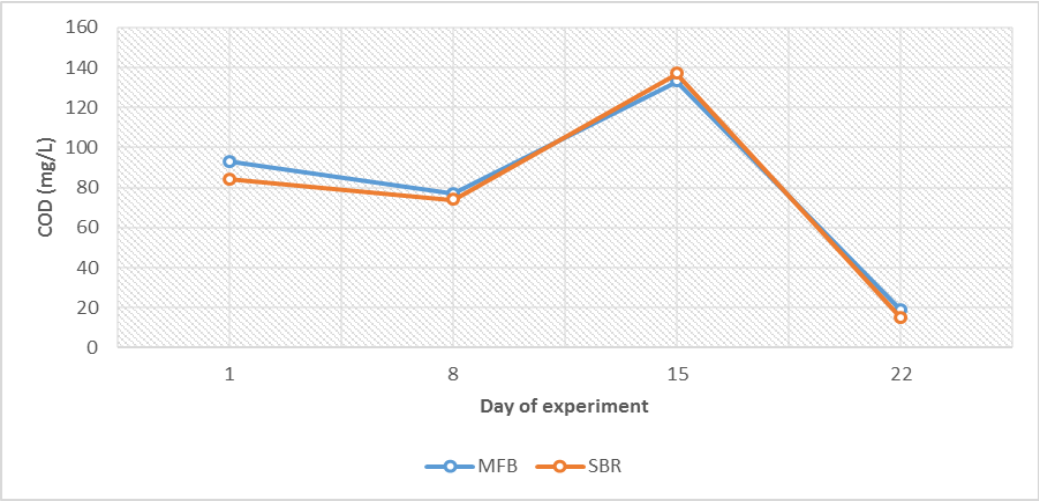
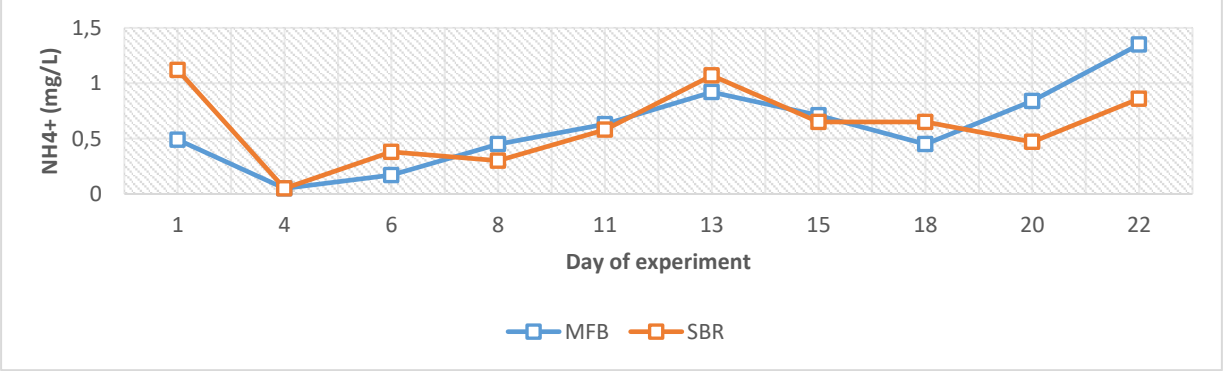


Figure 5.1.9: Stage 1 readings for COD (mg/L) in the Drum filter and SBR

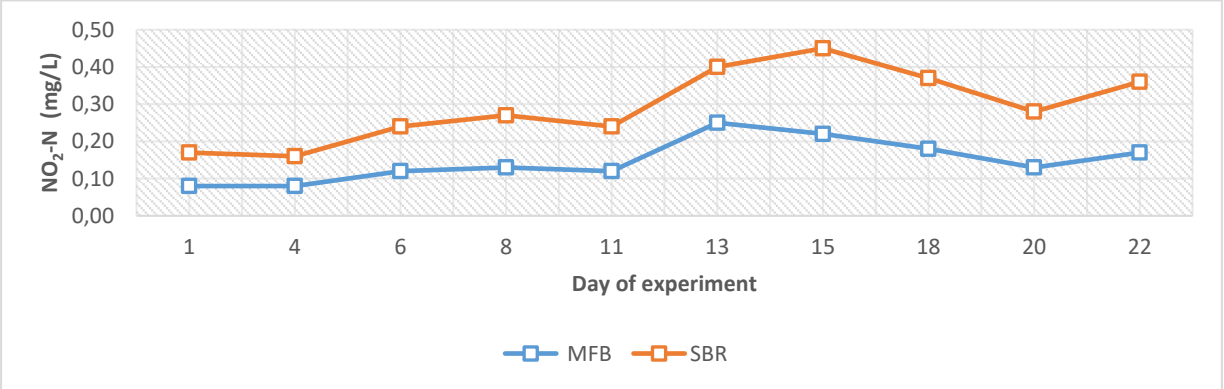
5.1.10 NH_{4+} / NO_{2}-N / NO_{3}-N/ TAN

The data in Figure 5.1.10 displays the NH_{4+}, (A), NO_2-N (B), NO_3-N (C) and TAN (D) levels before (MFB) and after the biofilter (SBR). NH_{4+} (A) was higher in the SBR than the MFB on 4 measurement times and there was no statistically significant correlation between these two measurements (0,61, p=0,06). Similarly, there is higher TAN (D) in the SBR than the MFB throughout the experiment period, however is the Pearson coefficient correlation is statistically insignificant (p= 0,06). Similarly, to NH_{4+} (A) and TAN (D), both NO_2-N and NO_3-N levels were higher post biofilter. There is a strong statistically significant correlation in the fluctuations in NO_2-N (B) (p=0,0075) and NO_3-N (C) (p=0,00) before and after the biofilter congruent with the nitrification process. There is a general increase in both TAN and NO_2-N through the experiment with a peak on day 13, corresponding with the increase of feed on this day (see figure 5.1.3). In the SBR, NO_2-N is seen to be higher than TAN, however not statistically significant (p= 0,43). This pattern suggests that the biofilter was increasing over the test period.

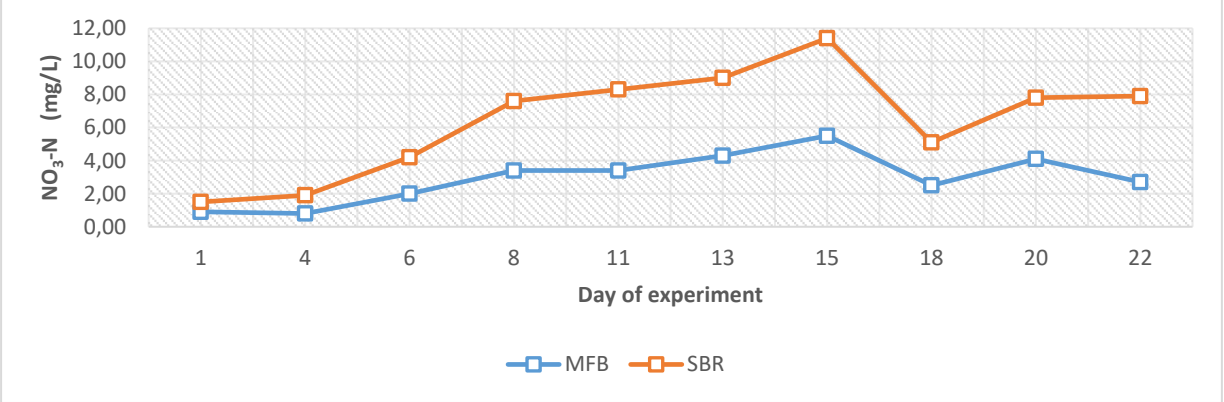
(A)



(B)



(C)



(D)

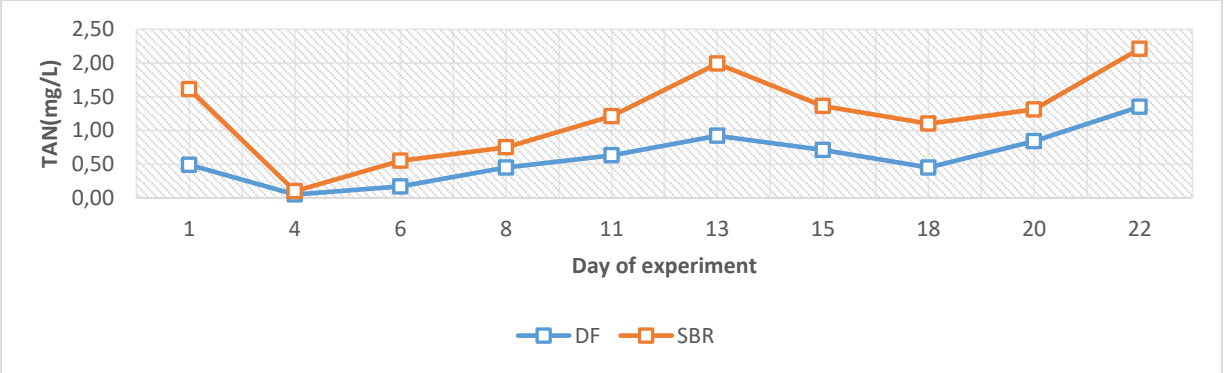


Figure 5.1.10: Comparison of NH4+(A), NO_2-N (B), NO_3-N (C) and TAN (D) in the pre-and post-filter testing.

5.1.11 TAN and mortality

Figure 5.1.11 shows a comparison of the TAN readings in the Drum filter and SBR and their relation to the daily mortality count. In day 3 there was a high mortality (400) which was the highest mortality reading for the test period due to fish transport and vaccination. This is the highest loss during the testing period as TAN levels were stable below the recommended threshold. However, on day 1 and 13, TAN was higher in the SBR and on day 22 in the Drum filter. Fluctuations in TAN (0-0.921) had little impact on mortality and were statistically insignificant (p=0,06). On day 1 there was 105658 smolt and on day 22 there was 104602 smolt resulting in 1% mortality loss. Mortality mean and standard deviation recordings ranged greatly with a mean and standard deviation of 47.57±107.80.

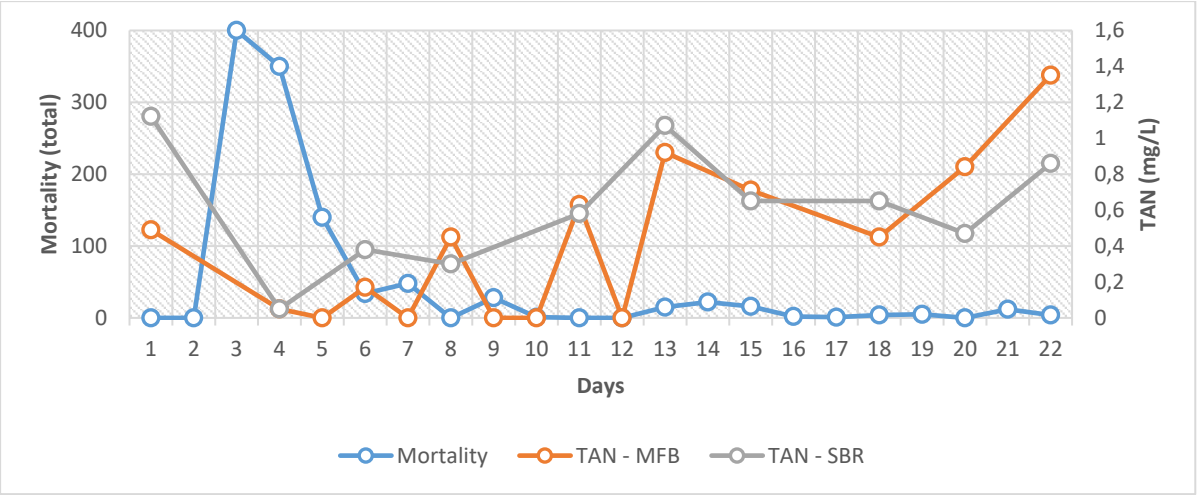


Figure 5.1.11: TAN (mg/L) in the drum filter and SBR in comparison to the daily mortality.

5.1.12 TAN and Feed

Figure 5.1.12 below shows the influence that % of feed change from each day has on TAN levels. Most % change values in feed was over 50% with the maximum increase in feed being 2595% on day 8. Feeding only began on day 7 due to fish being released into the rearing tank on day 0 and hence why there is no previous data. On day 13 when there was a TAN recording in the SBR over 1, the feed rate had been increased over 40% from the previous day. Table 5.1.12 shows the feed given in kg and then the fluctuation in feed from the previous day. The fluctuation in feed shows that when feed was given there was large fluctuations from the previous day. The mean and SD for feed for Stage 1 was 60,61±44,83 and for the % change in feed, 28,14±814,24.

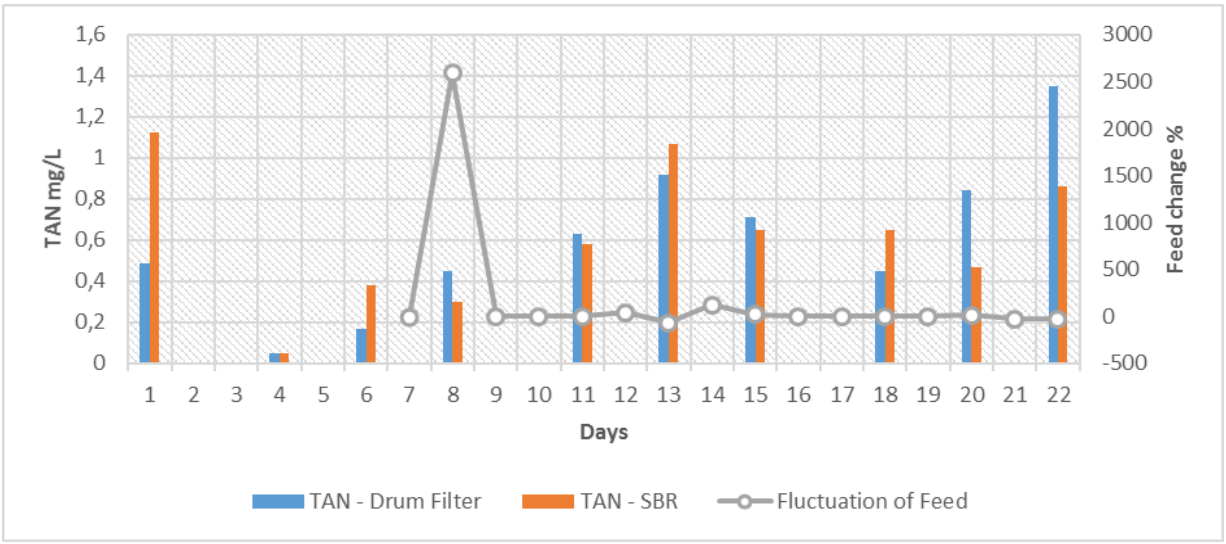


Figure 5.1.12: A comparison of TAN (mg/L) in the, drum filter and SBR. and the % of change of feed given.

Table 5.1.12: The Feed (kg) given per day and the fluctuation in feed from previous day (%)

Day of experiment	Feed (kg) (n=13)	Fluctuation of feed (%) (n=13)
7	0.61	0
8	16.44	2595
9	-	0
10	-	0
11	70.09	0
12	100.66	44
13	35.65	-65
14	81.08	127
15	101.38	25
16	102.17	1
17	100.92	-1
18	-	0
19	102.04	0
20	116.37	14
21	82.42	-29
22	-	-27

5.1.13 Removal rates

The mean and standard deviations for the Areal TAN, Areal $NO_{2\text{-}N}$, $NO_{3\text{-}N}$ removal rates are displayed in Table 5.1.13 and plotted in Figure 5.1.13. A one-way ANOVA indicated that there wasn't a statistically significant difference (p=0,07) between the groups. A Pearson correlation coefficient calculation indicated statistical significant correlations between Areal TAN and the $NO_{2\text{-}N}$ (1, p=0) and $NO_{3\text{-}N}$ (-0,63, p=0,05) removal rates. Furthermore, there was a strong negative correlation between $NO_{2\text{-}N}$ and $NO_{3\text{-}N}$ (-0,63, p=0,05) removal rates.

Table 5.1.13: Mean and standard deviations for Areal TAN, $NO_{2\text{-}N}$, $NO_{3\text{-}N}$ removal rate ($g/m^3/day$)

	Areal TAN removal rate ($gTAN\ m^3/day$)	Areal $NO_{2\text{-}N}$ removal rate ($gNO_{2\text{-}N}\ m^3/day$)	Areal $NO_{3\text{-}N}$ removal rate ($gNO_{3\text{-}N}\ m^3/day$)
M±SD	0,001 ±0,011 (n=10)	0,001±0,001 (n=10)	0,018±0,03 (n=10)

Figure 5.1.13 shows the Areal removal rates for TAN, $NO_{2\text{-}N}$ and NO3-N. $NO_{2\text{-}N}$ removal rate remained consistent with very little fluctuation. Conversely, TAN and NO3-N fluctuated and as stated previously this relationship of fluctuation was opposite and of medium correlation.

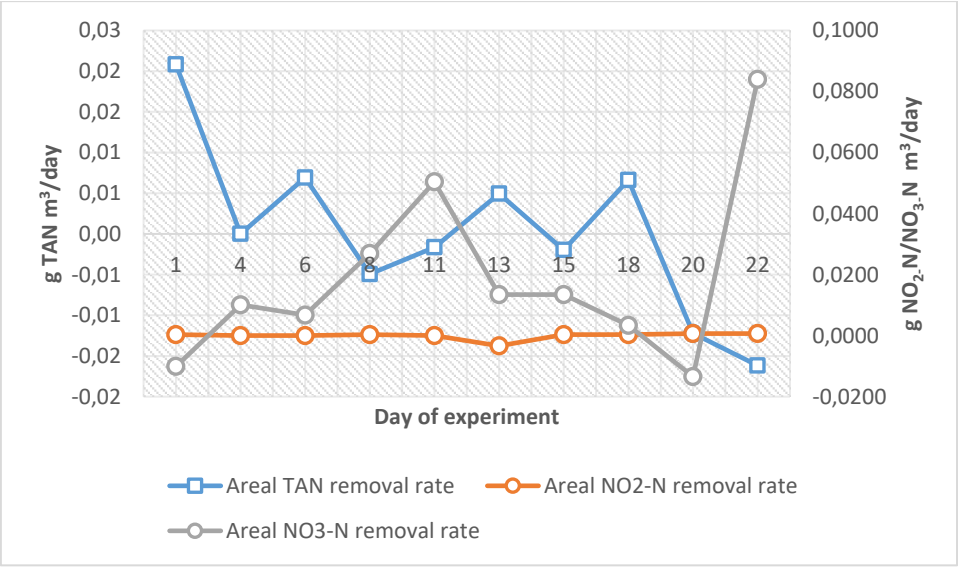


Figure 5.1.13: Areal removal rates for TAN, NO_2-N and NO_3-N.

5.1.14 Fish growth

The mean fish weight was 79.51 ± 6.47g (mean ± SD, n = 16) for Stage 1 and the finish sale weight was 307g. Mean FCR *(n=16)* was 1,375 ± 0,02 and mean SGR *(n=16)* was 0,48 ± 0,39%/day.

As the SGR data was not available for Stage 2 a manual calculation for predicted growth was completed using the Stage 1 mean fish weight and mean SGR, however and estimated SGR calculation was used to find the SGR for Stage 2 (Table 6.12). The result indicated that at the end of Stage 2, the weight of the fish would be 128g with an estimated SGR to be 0,22%/day. This is less than 178g than the final sale weight and a reduction in SGR.

Table 5.1.14 displays the Pearson Correlation coefficients between SGR and the water quality parameters for the one month analysis. Strong statistically significant results were found for Ammonia (0,22) in the MFB/PS, and TAN in the drum filter. Both results indicate a large positive correlation (0,77,0,74).

Table 5.1.14: Pearson correlation coefficient between SGR and the water quality parameters.

	Pre- biofilter C (p value)			Biofilter C (p value)	Post – biofilter C (p value)
	RT	DF	MFB/PS	MBBR	SBR
O_2	0,29 (0,17)	0,06 (0,82)	-	-	0,16 (0,54)
Temperature	0,28 (0,27)	0,28 (0,27)	0,38 (0,15)	-	0,28 (0,27)
pH	-	-0,17 (0,52)	-0,10 (0,69)	-	-0,18 (0,48)
Redox	-	-	-	-0,04 (0,87)	-
CO_2	-	-	-0,48 (0,06)	-	-
Salinity	0,02 (0,94)	-	-	-	-
Ammonia	-	-	0,77 (0,02) *	-	0,48 (0,15)
Nitrite	-	-	0,56 (0,09)	-	0,46 (0,18)
Nitrate	-	-	0.44 (0.20)	-	0,51 (0,13)
Alkalinity	-	-	-0,19 (0,59)	-	-0,22 (0,55)
COD	-	-0,17 (0,83)	-	-	-0,19 (0,81)
TAN	-	0.74 (0.01) *	-	-	0.36 (0.30)

*($p<0,05$), RT= rearing tank, DF= drum filter, MFB= mechanical filter basin, PS= pump sump

Figure 5.1.15 shows the difference between the total amount of fish on day one and the mean and standard deviations for stage 1 and stage 2.

Table 5.1.15: Day 1 Total fish on set out and mean and standard deviation readings for the total fish at Stage 1 and Stage 2

Day 1 (Total fish)	Stage 1 (M±SD) (n=22)	Stage 2 (M±SD) (n=122)
105658	104722±322,983	104174±448,34

5.2 Stage 2

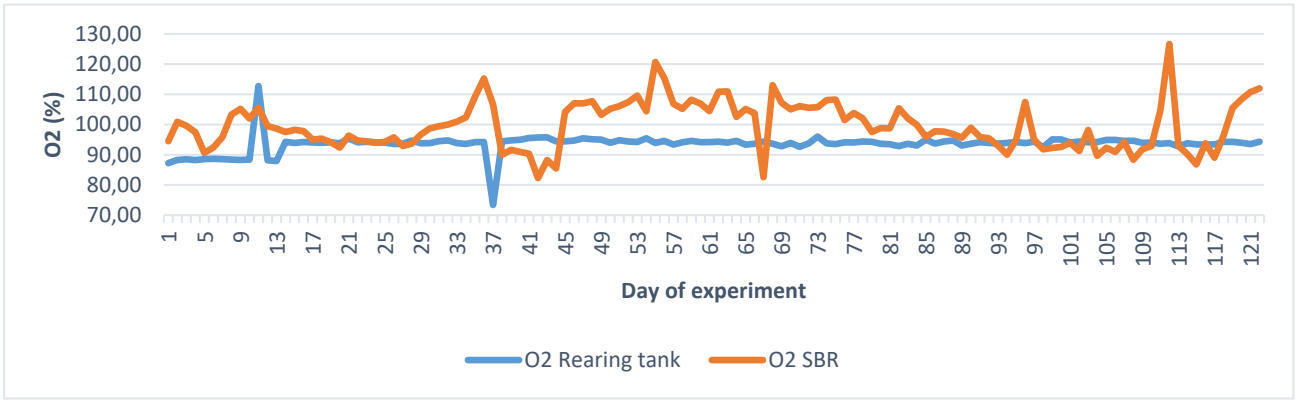
Table 5.2.1 shows the mean and standard deviations for the whole experiment for O_2, Temperature, pH, CO_2, Mortality and salinity. O_2 was higher in the SBR compared to the rearing tank and both parameters had measurements over the 100% recommendation. Mortality had a large standard deviation due to the large variance in the mortality count each day. The researcher decided to keep the outliers as they were reflective of the RAS functioning. Salinity also had a large standard deviation with a high coefficient of variation (48,08) due to salinity being added to the RAS on day 14 of the experiment (Figure 5.2.1F). Salinity began being measured on the 7th day of the experiment.

Table 5.2.1: Means and standard deviation measurements for all parameters that were measured from set out to final sale.

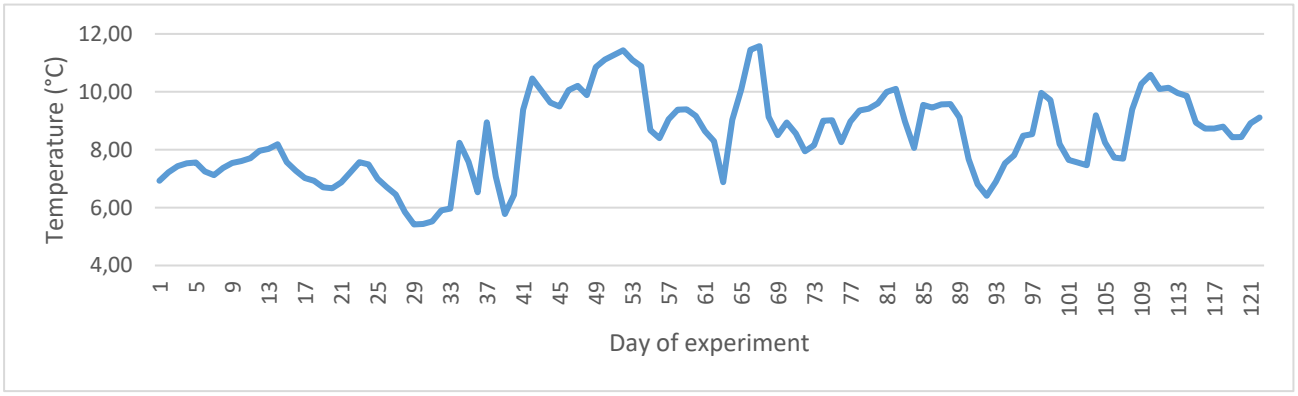
	n	M	SD	CV (%)
O_2 RT (%)	1464	93,31	3,32	3,56
O_2 SBR (%)	1464	99,14	7,57	7,63
Temperature (°C)	1464	8,40	1,44	17,22
pH PS	1464	7,44	0,42	5,68
pH SBR	1464	7,13	0,37	5,14
CO_2 PS (mg/L)	1464	8,30	3,85	46,36
Mortality	1464	25,73	67,54	262
Salinity (ppt)	1404	11,26	5,41	48,08

Figure 5.2 (A- F) shows Stage 2 data graphs for O_2, Temperature, pH, CO_2, Mortality and Salinity with Table 5.2.2 displaying the Pearson correlation coefficients and p values for this data. Graph A shows a consistent O_2 level in the rearing tank, apart from a peak on day 11 (112%) and a trough on day 37 (73%), O_2 levels remained consistent. There does not seem to be a consistent relationship between the rearing tank and SBR and the correlation coefficient indicates a weak, statistically insignificant relationship ($p>0,05$, Figure 5.2.2). The temperature lifespan is shown in Figure 5.2(B) which shows that temperature did not remain consistent throughout the set out, however, remained within the recommended threshold. Table 5.2.2 indicates that there were statistically significant fluctuations between temperature and O_2 (SBR), pH (PS & SBR), CO_2, salinity and Feed (kg). All these relationships were positive, statistically significant and low/medium in strength. Figure 5.2 (C) shows that the RAS pH fluctuated between 6,04 and as high as 8,7 and that fluctuations in pH in the pump sump were strongly correlated (0,9) to the SBR with statistical significance ($p=0$). CO_2 is shown in D to steadily increase with small fluctuations under 10mg/L until on days 43-45, 64-67, 70-73 and 97 when the CO_2 goes above 15mg/L. These readings are above the guidelines stipulated by research publications and Sterner. In terms of correlations, CO_2 had a fair positive linear relationship with salinity (Table 5.2.2). There were no further peaks in mortality after what was discussed in section 5.1.4 and mortality remained consistently below 50 per day throughout the rest of the experiment with a total mortality of 2,19% (E). There were statistically significant fluctuations between mortality and Feed rate (Table 5.2.2), however, they were weak and negative, indicating opposite changes between the parameters. Graph F shows that salinity was added at day 14 and variated slowly to highs of 19,05ppt (day 66) which is above the recommended optimal threshold of 12ppt.

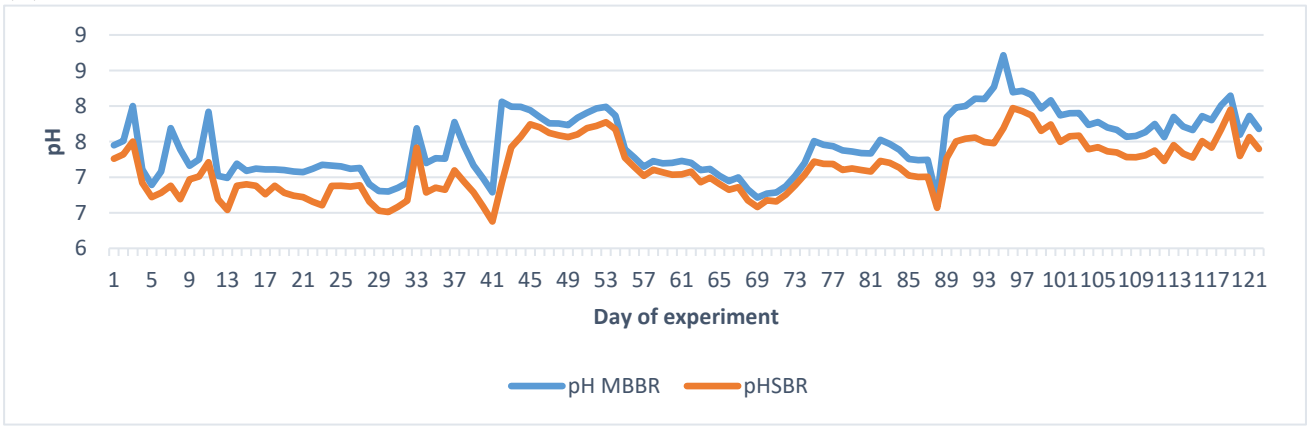
(A)


O2 (%)
130,00
120,00
110,00
100,00
90,00
80,00
70,00
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101 105 109 113 117 121
Day of experiment
O2 Rearing tank
O2 SBR

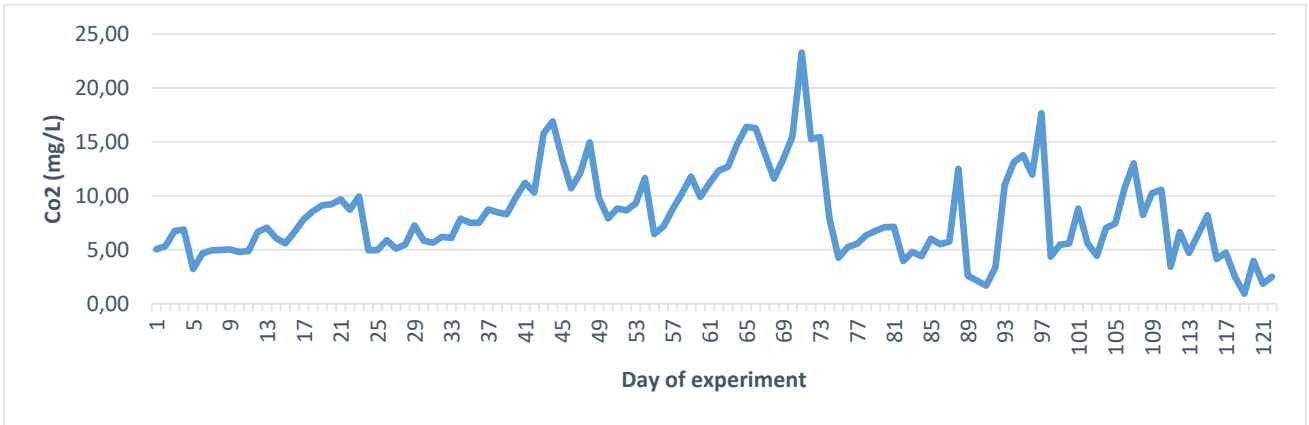
(B)


Temperature (°C)
12,00
10,00
8,00
6,00
4,00
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101 105 109 113 117 121
Day of experiment

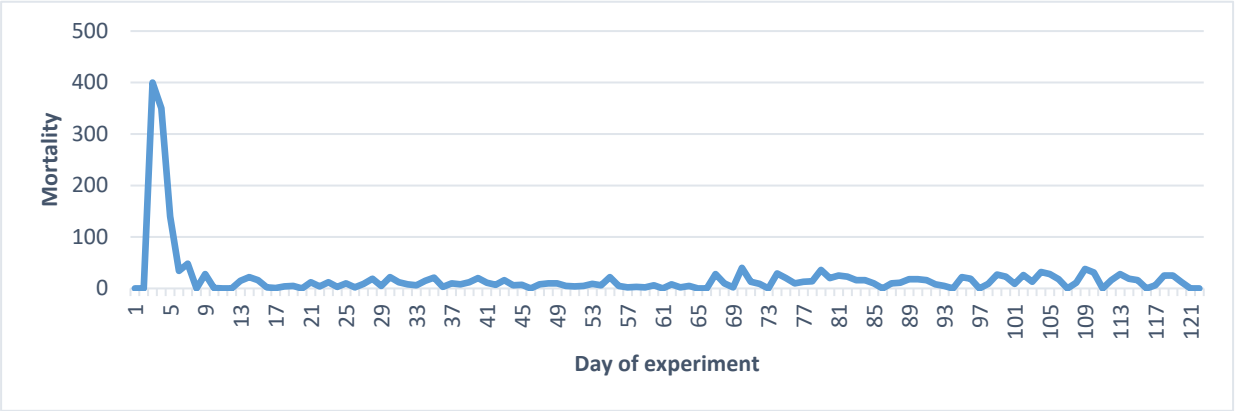
(C)


pH
9
9
8
8
7
7
6
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101105109113117121
Day of experiment
pH MBBR
pHSBR

(D)


Co2 (mg/L)
25,00
20,00
15,00
10,00
5,00
0,00
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61 65 69 73 77 81 85 89 93 97 101 105 109 113 117 121
Day of experiment

(E)



(F)

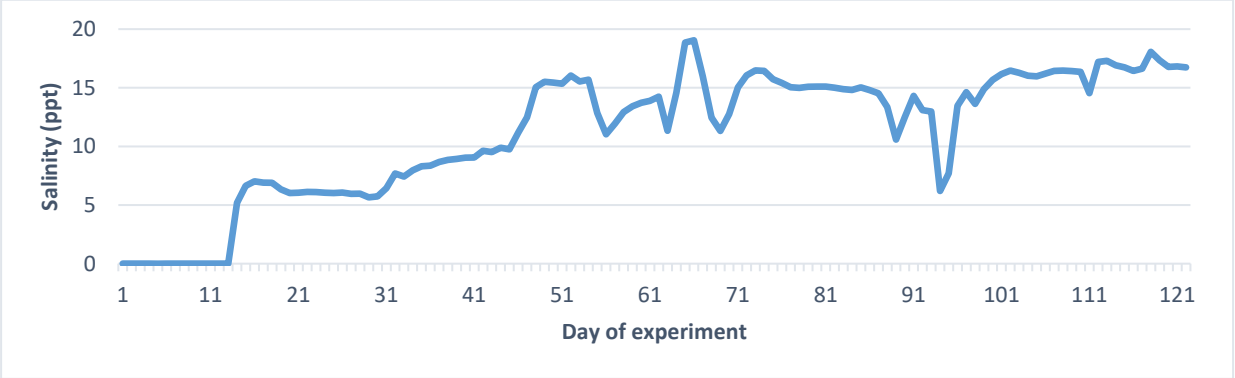


Figure 5.2: O_2 (A), Temperature (B), pH (C), CO_2 (D), Mortality (E), Salinity (F) for Stage 2(122 days).

Table 5.2.2: Pearson correlation coefficients for the parameters recorded for Stage 2. Statistically significant results (p<0,05) are marked with a *.

	Temperatu ren PS (°C)	O_2 SBR (%)	pH MBBR	pH SBR	CO_2 PS (mg/L)	Mortality	Salinity (ppt)	Feed (kg)
O_2 RT (%)	0,13 (0,14)	0,01 (0,93)	0,09 (0,30)	0,11(0,24)	0,17 (0,05)*	-0,31 (0,00)*	0,42 (0,00)*	0,21 (0,02)*
T PS (°C)		0,17 (0,05)*	0,27 (0,00)*	0,40(0,00)*	0,30 (0,00)*	-0,11 (0,21)	0,56 (0,00)*	0,23 (0,01)*
O_2 SBR (%)			-0,14 (0,12)	0,02 (0,83)	0,12 (0,21)	-0,07 (0,42)	0,13 (0,17)	-0,27 (0,00)*
pH MBBR				0,91 (0,00)*	-0,08 (0,39)	0,03 (0,75)	0,30 (0,00)*	0,39 (0,00)
pH SBR					-0,02 (0,83)	0,02 (0,87)	0,40 (0,00)*	0,37 (0,00)
CO_2 PS						-0,13 (0,14)	0,40 (0,00)*	0,08 (0,40)
Mortality							-0,41 (0,00)*	-0,21 (0,02)*
Salinity (ppt)								0,60 (0,00)*

5.3 Stage 1 and 2 comparison

Table 5.3 shows the 2 sample t tests conducted to compare the difference between Stage 1 and Stage 2 values for O_2, Temperature, pH, Salinity, CO_2 and Mortality. The results indicate statistically

significant differences between Stage 1 and 2 for Temperature, pH (MBBR and SBR), Salinity and CO_2.

Table 5.3: 2 sample *t*-test results comparing data from Stage 1 and 2. Statistically significant results are highlighted with a *

Parameter	T-value	p value
O^2 (RT)	1,44	0,16
O^2 (SBR)	1,78	0,08
Temperature (PS)	-7,01	0,00*
pH (MBBR)	-2,86	0,01*
pH (SBR)	-4,12	0,00*
Salinity (MBBR)	-14,62	0,00*
CO2 (PS)	-3,31	0,00*
Mortality	1,27	0,22

6. DISCUSSION

The research questions that guided this analysis were centred around the monitoring of water in RAS and how predicative the first month of water quality parameters is to Stage 2 of the RAS. The objectives of were to conduct a water quality analysis over 2 stages; Stage 1 being 22 days in length and Stage 2 being 122 days in length. The parameters assessed in Stage 1 were O_2, temperature, pH, ORP, Salinity, mortality, Feed, CO_2, NO_2-N, NO_3-N, NH_{+4}, Alkalinity, COD and TAN and the parameters assessed in Stage 2 were O_2, temperature, pH, Salinity, mortality and CO_2. Secondly, to compare the above analysis to water quality measurements taken for Stage 2 of the RAS (O_2, temperature, pH, Salinity, mortality and CO_2). Thirdly, to discuss how predicative Stage 1 is to Stage 2 of RAS (start-up-final sale) with a combination (established/new) biofilter. Lastly, to discuss recommended thresholds for water quality parameters based on current evidence and government recommendations and compare these to the analysis findings.

The assessment of water quality in a RAS is essential to maintaining good animal welfare and therefore apart of every successful RAS operation (Hjeltnes et al., 2012; Godoy-Olmos et al., 2016). There are water quality requirements that are regulated by governing bodies and research studies where water quality requirements are suggested (Hjeltnes et al., 2012). Table 6 shows the published data (as discussed in Chapter 2), the recommendations from Sterner AS per their system as well as the research results which includes results after 1 month and results from start-up to grow out (Sterner AS n.d). This will form the basis for the discussion below.

Table 6: A comparison of each parameter measured in the study (one month and whole set out) and the published data discussed in chapter 2. * = Results not within published data threshold † = Results not within Sterner recommendation.

	Published data	Sterner Recommendation	Stage 1 (M ± SD)	Stage 2 (M ± SD)
O_2 (%)	80- 100	85	92,05±7,31 †	(RT) 93,31 ± 3,32;(SBR) 99,14 ±7,57*†
Temperature (°C)	3-18	14	7,35±0,39 †	8,40 ± 1,44†
pH	6.2-7.8	7,5	6,98±0,05 †	(MBBR) 7,44; ± 0,42; (SBR) 7,13 ± 0,37
ORP (mV)	270-300	Na	153.90±48.37*	-
Salinity (ppt)	12	Na	6.39 ±0.62*	11,26 ± 5,41*
CO_2 (mg/L)	<10-15	15	6.33± 1.75*	8,30 ± 3,85*
NO_2-N (mg/L)	<0.1-0.5	≤ 0,5	0.15± 0,02	-
NO_3-N (mg/L)	<200-400	80	3,235±0,52* †	-
NH_{+4} (mg/L)	0.025	Na	0,60 ± 0,11	-
Alkalinity (mg/L)	>80	Na	104± 24,30	-
COD (mg/L)	3-6	Na	79 ± 24,33* †	-
TAN (mg/L)	<0.7-1	< 2	0,61± 0,11 †	-

6.1 O_2

Within a RAS, oxygen is often the first limiting parameter and therefore essential within a water quality analysis (Eding et al., 2006). Sterner states their system is designed to operate with 85% O_2 in the outlet. The results from Stage 1 shows a larger mean O_2 saturation with a statistically significant difference between pre-and post-biofilter readings (p=0). This could be due to the lower temperature (SBR, 7,35±0,39) causing less O_2 uptake than expected (Davis, 1968). Also, Figure 5.1.2 showed higher O_2 readings in the SBR due to oxygenation in the RAS. Similarly, to stage 1, the O_2 SBR mean and standard deviations (99,14±7,57, Table 5.2.1) for Stage 2 was high and over 100% for majority of the data. O^2 saturations above 100% in some studies have found to negatively affect the respiratory and ventilation process of the fish affect blood pH in the fish (Colt, 2006), however, in a study by Hosfeld et al. (2008), saturations of 123% showed no negative effect on Atlantic salmon smolt. For stage two, O_2 in the rearing tank remained constant. O_2 in the SBR fluctuated and hence why the correlation between the two measurements was statically insignificant. Due to the high stocking density (75kg/m^3) fluctuations in O_2 are common and could provide the reasoning for these fluctuations (Chadwick, Parsons and Sayavong, 2010). In comparison between Stage 1 and Stage 2 results, there was no indicating no statistical significance indicating that O^2 remained consistent between the stages.

6.2 Temperature

Besides added motion energy in the water flow, no modification to the raw water temperature from the original water source was used in the RAS and hence the reason for the lower temperature for both Stage 1 (7,35±0,39) and Stage 2 (8,40±1,44) than Sterners recommended temperature reading. For the comparison between the two stages, the t test (Table 5.3) indicated a statistical significant difference (p=0,00) and could be attributed to the change of season (winter to spring). Also as no heat pump was installed during the trial, all water temperatures with friction were naturally occurring.

6.3 pH

For stage 1, pre-and post-filter pH readings indicated a statistical significant correlation in their linear relationship of this parameter a symptom of nitrification as it is enhanced when the pH is above 7 (Pattillo, 2014). Salt water was added to the RAS from day 14 to maintain the buffering of the water (pH) and to begin to build seawater tolerance. Many of the water sources in Norway have little buffering which in turn can cause drops in pH due to snow melting, production intensity and acidic rain (Hjeltnes et al., 2012). Figure 5.1.4 shows that after saltwater was added, pH reduced and began to stabilise (Timmons and Ebeling, 2013). Nitrification is activated by a reduction in pH which can be

seen on day 11 as a decrease in pH (figure 5.1.4) and an increase in NO_2-N (figure 5.1.10 B) signifying symptoms of nitrification (US-EPA, 2002).

In relation to Stage 2, there was a strong correlation (0,9) between the fluctuations in pH in both pre (7,44±0,42) and post biofilter (7,13±0,37) readings with the post biofilter was lower than the prebiofilter reading due to the aerator. Furthermore, when there was an increase in pH from day 40 (6.99) to day 44 (8), this corresponded with a rise in CO_2 to over 10mg/L (Day 44, 16.9mg/L) this is also congruent with day 71 pH lowering to 6.65 and CO_2 increasing to 23,28mg/L (Timmons and Ebeling, 2013; Fivelstad et al., 2003, Figure 5.2 C&D). A study by Good et al. (2016) with similar pH readings studied the effects of long term CO_2 exposure on Atlantic salmon smolt and found there was no change in growth which can be seen in this trial as growth increased from the predicted growth rate discussed in section 5.1.14.

In comparison between Stages 1 and 2: For stage 1, the pH grew from a mean and standard deviation of 6,98±0,05 at Stage 1 to 7,44; ± 0,42 in the MBBR and 7,13 ± 0,37 in the SBR, however still registering below the recommended threshold of 7.8 and the limit set by Sterner of 7,5. There was also a statistically significant difference between the MBBR and SBR measurements in pH at both Stage 1 and Stage 2. pH did increase, however, remained within Sterners and published data.

6.4 ORP

The ORP readings were recorded for Stage 1 and well below the recommended thresholds for ORP (153.90±48.37). A study by Terjeson et al. (2013) set the ORP at 270mV for Atlantic salmon smolt indicating that the O^3: ORP ratio can be increased within this RAS. Furthermore, increasing ORP has be found to improve fish's ability to respond to infection however, reduced growth. It is therefore important to judge the importance of these factors when setting an ORP limit for the RAS.

6.5 CO_2

In Stage 1 as CO_2 is directly affected by temperature and pH, the mean and standard deviations for CO_2 in the one month experiment are congruent with a stable system and below the recommended threshold (Hordaland fylkeskommune, 2009; Terjesen et al., 2013; Mattilsynet, 2014). As stated in section 6.3, there were high readings for CO_2 (Figure 5.1.6) which at this level incur higher mortality, reduced growth and built up calcium in the kidneys (Fivelstad et al., 1999; Fivelstad et al., 2003).

The 2-sample t test of CO_2 readings over the 2 stages indicated a statistically significant difference. The means and standard deviations shows an increased from 6.33±1.75 (Stage 1) to 8.30±3.85 (Stage 2). The difference although significant was within the proposed guidelines from Sterner and the published data.

6.6 Salinity

For Salinity, Stage 1 shows that salt water was added on day 14 to the ppt of 5,20. Onto Stage 2, as Vik Settefisk increased salinity ready for sell out, the difference between results in stage 1 and stage 2 was large and therefore the reasoning for the statistically significant difference between Stage 1 and Stage 2. Salinity measurements for Stage 2 (Figure 5.2, F) were above the optimal conditions for post salmon smolt as studied by Ytrestøyl et al. (2014), however within the survival range (Hordaland fylkeskommune, 2009).

6.7 Alkalinity

Alkalinity was only measured in stage 1 with mean and standard deviation readings (104± 24,30) above the recommended optimal limit of 80 as stipulated by Malone & DeLos Reyes (1997). A study by Summerfelt et al., 2015 on the effects of varying alkalinity on nitrification rates found that at higher alkalinities (70 and 100mg/L), the TAN levels within the RAS were steady, which supports the results from this experiment, where TAN concentrations remained steady with a high alkalinity. Also, readings before and after the biofilter were strongly correlated (0,73; p=0,02) which could indicate issues with the nitrification/denitrification process as the alkalinity in the SBR was higher (110.05±77.77) than the pre filter reading (98.3±75.82) (Li and Irvin, 2007). This is also supported by the fact that the blockage between the MBBR and SBR affected this process.

In recent years, as aquaculture system stocking density and hydraulic retention time has increased, the relationship between pH and alkalinity has become a significant issue. This relationship requires careful monitoring and adjustment of both alkalinity and carbon dioxide levels to maintain optimum pH for both the aquatic species being grown and the biofilters. Alkalinity is easily adjusted through the addition of sodium bicarbonate (NaHCO3), common baking soda. Other materials can be used, but sodium bicarbonate is commercially available in 50 to 100 lb (23 to 45 kg) bags, safe, inexpensive, and easy to apply. It has very high water solubility and rapidly dissolves in water at ambient temperature. A general rule of thumb is that for every pound of feed, approximately 0.25 lbs (113 g) of sodium bicarbonate should be added to the water

6.8 COD

COD readings for this study indicated large variation and with only 8 in the sample, conclusions on the COD readings are limited.

6.9 NH_{4+} / NO_2-N / NO_3-N/ TAN

Ammonia, or TAN (unionised NH_3 and ionised NH_4+), NO_3-N and NO_2-N are important water quality parameters to measure in RAS due to their potentiality to become toxic and negatively affect fish health (Timmons & Ebeling, 2013). Table 6.1 shows that all three parameters were below Sterners recommendations as well as published guidelines for both Stage 1 and Stage 2. This could have been achieved because the RAS had previously been operational and $6m^3$ of the total $12m^3$ biochips were already an established culture. In Stage 1, there was a strong statistically significant correlation in the fluctuations in NO_2-N ($p=0,0075$) and NO_3-N ($p=0,0009$) before and after the biofilter, congruent with the nitrification process (Timmons & Ebeling, 2013). TAN remained constant and majority of the time below the recommended threshold most likely due to the high alkalinity as studied by Summerfelt et al. (2015), who found that increased alkalinity levels (70 or 200 mg/L) resulted in lower TAN concentrations. Interestingly all three parameters had higher data in the SBR compared to the pre-biofilter measurement (Figure 5.5.1). This difference, although not statistically significant ($p= 0,4319$), There was more TAN, NO_3-N and NO_2-N in the SBR throughout the experiment ($p>0,05$), attributed to the clogging of a filter between the MBBR and SBR.

6.10 Tan and the influence of feed and mortality

Feed management is a key issue in maintaining a stable water quality in RAS as well as growth of nitrifying bacteria and growth of the fish (Emparanza, 2009; Hjeltnes et al., 2012). Total feed given for the Stage 1 was 1 571,53kg with a mean and standard deviation (M±SD) (71,47±42,78) and over the whole set out the total feed was 20 965,51kg with a mean and standard deviation of (150,84±83,10). In the Module 17, RAS there was no automatic feeder installed for two weeks, then a Steinsvik automatic feeder was installed where the amount of feed was calculated according to the SGR. This in turn meant that each day the feed amount was different and as Table 5.1.12 shows large fluctuations (over 15%) between each day feed amount. This is important to note as Emparanza (2009) stated that TAN levels can be controlled easily if the feed amount is not 15% more of less from the previous day. Furthermore, Hjeltnes et al., (2012) discusses Norwegian producers limiting the daily variation in feed to <10% to not only control TAN levels but to control balance between feed given: feed consumed. Although TAN levels remained below recommended levels (Table 6.1), Figure 5.1.10 D shows that fluctuations in feed resulted in fluctuations in TAN and that the biofilter was a combination of established and new biofilm carriers. Although fluctuations in feed can result in toxic levels of TAN, the results from this study do not indicate that these fluctuations increased TAN levels. If high TAN levels were recorded a study by Bergheim et al. (2009) found that when TAN peaked in intensive smolt farms, there was not any operational and fish risks if the Tan concentration was

<5mg/L and the pH <7.5 (Bergheim et al. 2009). Table 5.1.11 indicated that TAN readings made little impact on mortality. The increased mortality on days 3 and 4 were attributed to initial set out and high initial TAN levels in the initial days.

6.11 Removal rates

Table 5.1.13 and Figure 5.1.13 show the relationship between the ATR, ANO2R and ANO3R for Stage 1. The NO_2-N removal rate was added to the ATR as the ANO2R efficiency was near zero due to the pre-and post filter readings being nearly identical and the conversion of nitrite to nitrate. (Malone and Beecher. 2000). The mean Areal TAN removal rate was 0,001 ±0,011, potentially due to the higher post filter recordings for TAN. As stated previously, the blockage between the MBBR and SBR caused TAN to accumulate resulting in low removal rates. TAN was for the most part below the recommended limits suggesting that TAN oxidation could be occurring by O_3 processes in the conversion to nitrogen gas (Schroeder et al., 2011)

Table 5.1.13 in Chapter 5 displays the nitrite removal rate over the test period. The study by Kinyage and Pedersen, (2016) on the effect of temperature on nitrite removal rates found a nitrite removal rate of 4 ± 1% at 6 °C. Their results for the nitrite removal rate shown in Figure 6.4.2, show that the NO_2-N removal rates is quicker at higher temperatures, highlighting the impact that temperature can have on the microbial activity within the MBBR (Chen et al., 2006; Lekang, 2012). NO_3-N removal rates were also low however showed a peak on day 11 symptoms of the nitrification/denitrification process.

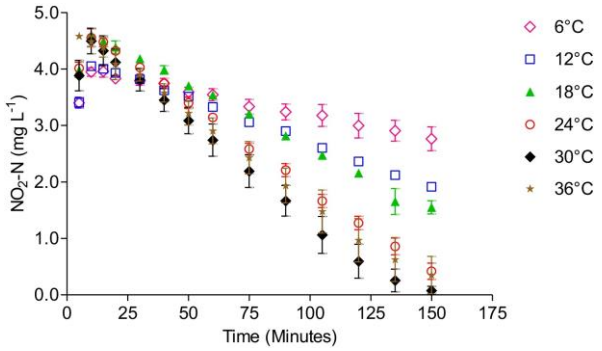


Figure 6.4.2: NO_2-N removal rates were quicker at higher temperatures (Kinyage and Pedersen, 2016)

6.12 Feed and growth

The FCR was higher than expected at 1,375± 0,02 and is generally around 0.7-0.8 in the smolt phase. This could be attributed to the feed type being used and the large fluctuations in feed

(Dalsgaard et al., 2013). Maintaining a high FCR can negatively impact the RAS' environmental impact (Roque d'Orbcastel et al., 2009).

Table 6.12.1 shows a comparison of the predicted and estimated SGRs for Stage 1 and 2. The SGR Model refers to the equation (Calculations 4.3.2; SGR (predicted)) used to determine what the SGR is based on temperature and weight. The SGR result for both Stage 1 (1,4%/day) and Stage 2 (1%p/day) was supported by the SGR research by Austreng, Storebakken and Åsgård (1987). The actual SGR for Stage 1 was largely different to the predicted SGR of 1,4. This is like the Stage 2 readings for SGR which also show a large difference between the estimated and predicted values. A decrease in SGR is related to first, the SGR and temperature increasing to an optimal growth level, and then because of higher fish weight the SGR decreases (Chadwick, Parsons and Sayavong, 2010; Handeland et al., 1998). Furthermore, the low SGR could be attributed to the high stocking density (75kg/m^3) as a study by Seymour et al. (1992) found that with systems running high stocking densities (54-124 kg/m^3), there was a decrease in growth than other Atlantic salmon grown at lower fish densities. However, good growth does not necessarily result in optimal fish welfare and therefore maintaining water quality parameters to safe levels also needs to be ensured (Chadwick, Parsons and Sayavong, 2010). Table 6.12.2 shows the increase in stocking density from day 0 to the final sale (Stage 2), the final sale density (72 kg/m^3) was below suggested value of 75 kg/m^3.

There was a strong statistical significant correlation between SGR and Ammonia and TAN(p=0,22) in the MFB/PS, and TAN in the drum filter. Both results indicate a large positive correlation. Due to the relationship between SGR and Feed given, the TAN will fluctuate (Chadwick, Parsons and Sayavong, 2010).

Table 6.12.1 Comparison of SGR (%/day) from Stage 1 to Stage 2. The Model used to estimate SGR for both Stage 1 and Stage 2 is in the calculations section (4.3.2). The SGR for Stage 1 was given by Vik Settefisk and the equation in section 4.3.2 (estimated SGR) was used to calculate the SGR in stage 2.

Stage 1		Stage 2	
SGR Model	Actual SGR (M±SD)	SGR Model	Estimated SGR
1,4	0,48±0,39	1	0,22

Table 6.12.2: Stocking density for Day 0, Stage 1 and Stage 2

Stocking Density (kg/m^3)		
Day 0	**Stage 1**	**Stage 2**
16	21	72

6.13 Limitations

Firstly, the water flow from the MBBR to the SBR (Figure 6.6.1) became clogged during the trial due to a construction error (Figure 6.6.2). The biochips migrated from the MBBR to the SBR clogging the filter, leading to higher TAN, NO2-N readings in the SBR compared to the MFB. This error was adjusted at the next set out (after the analysis period) by emptying all the biochips. Furthermore, at the beginning of the experiment the new mixed with the old media in the biofilter in Module 17 had only been running for 2-3 days due to an unplanned production stoppage. According to Hjeltnes et al., (2012) this operation is crucial in the beginning stages of bio filter function, as disturbances can negatively affect bacterial activity.

Figure 6.6.1: MBBR and SBR (yellow) Figure 6.6.2: Construction failure in grating

Another limitation of the experiment was the delay in the completion of the system. The first fish were to enter the system in early October. The fish did not enter until the 9th of December. The RAS had a combination biofilter which consisted on already established bio culture which reduced the start-up time and therefore reduced the experiment period for Stage 1 values.

Per the user manuals of the tests, the optimal air temperature should be room temperature. However, the water quality tests are being completed and stored in an insulated but unheated laboratory as well as been completed in the Norwegian autumn and winter. The temperature ranged from 6 to 12 degrees Celsius.

For the data that was collected by Vik Settefisk (Section 4.1.2), the quality and accuracy of the data cannot be confirmed, however as Vik settefisk use this data within their own operation it can be assumed that this data is accurate. Also, the Spectrometer was also used offsite on one occasion by Sterner which lead to some issues when de-calibrating the equipment on return.

7. CONCLUSION

Overall, although there were statistically significant results between Stage 1 and Stage 2, all remained within the recommended guidelines described in the literature and set out by the vendor, Sterner Aquatech indicating a well-functioning RAS.

This analysis aimed to assess how predictive Stage 1 measurements of water quality was to Stage 2 of a RAS. The parameters assessed in Stage 1 were O_2, temperature, pH, ORP, Salinity, mortality, Feed, CO_2, NO_2-N, NO_3-N, NH_{+4}, Alkalinity, COD and TAN and the parameters assessed in Stage 2 were O_2, temperature, pH, Salinity, mortality and CO_2. A comparison between the results of these stages and their relation to published literature and the recommended guidelines from the supplier, Sterner Aquatech AS was discussed. The study illustrated that changes from Stage 1 to 2, although for some water parameters the differences were statistically significant (CO_2, pH, Salinity and Temperature), the difference was still within the thresholds in the published literature and vendor specifications. Therefore, the results from the study indicate that with a well-functioning RAS for the first month, predicting ongoing stability. However, a larger study comparing various RAS would need to be conducted, this can potentially find initial problems within the RAS and be compared to similar RAS in the future. For the parameters investigated in Stage 1, the blockage in the SBR contributed to the higher TAN, NO_2-N and NO_3-N in the SBR than in the pre-biofilter. Also, this analysis also investigated literature to determine thresholds for optimal growth of salmonoids in RAS. Searches for a central document that had up to date recommendations for water quality measurements was not found. A literature review found that current research into different variables and their effect on water quality is a large area of research and therefore contributes to a possibly confusing environment for quick access to this information in a real working environment.

For further research, the researcher recommends testing water quality parameters over the $75m^3$ biomass density that was used during this study. The rearing tank can be dimensioned up or down as the biofilter can easily be adjusted to deal with added biomass. The choice of a 12x4m design and a production model of 200 000 fish at Vik was the limit for the well boat.

7.1 Recommendations

The researcher worked at Vik settefisk sporadically from August to December where performance observations where noted, both from the researcher and local staff running the system. Below are listed some technical modifications that the researcher has suggested.

7.1.1 Extension of the footbridge between mechanical filtration and dead fish collection.

The footbridge (Figure 7.1.1) between these two areas is too narrow and is hard to manoeuvre when completing work tasks. This can potentially be a future safety issue as workers can fall or be subjected to other workplace injuries as often workers carry heavy loads through this section.

Figure 7.1.1: Footbridge with approx. 1 metre distance between railing

7.1.2 Excess feed accumulation

Excess feed is accumulated along to external edge of the jump barrier (Figure 7.1.2) when hand feeding and automatic feed is in operation. This can lead to excess feed build up and potential vermin issues. It is recommended that the jump barrier be redesigned or refitted.

Figure 7.1.2: Excess feed accumulation along jump barrier

7.1.3 Dead fish collector – redesign from manual dead fish collection to semi-automatic grate skip.

Currently dead fish are collected manually which requires the operator to retrieve the dead fish via a landing net causing significant hazard, time and effort. It is recommended that a submerged grate

skip design is installed within the dead fish collection unit. The grate skip should have a sloped bottom with a hatch in the low end. The skip is to be raised/lowered by use of an overhang block/electrical winch so when emptying the skip this will be lifted to upper position “to el. end stop signal” – the hatch to open “on el. Signal” and the dead fish to be transported to a lower placed container “on ground” by a sloped ditch. By installing a window including light in the dead fish collector drum sidewall it could easily be determined when to empty the skip. This application can be installed for a reasonable cost giving the operator better working conditions and time to do other tasks.

7.1.4 Initial design

Currently the RAS (excluding the rearing tank) is designed with 25mm PE plastic sheets with a metal framework. However, it is recommended that using fully welded 2-3 mm 316 stainless steel plates including sufficient stiffening plates inside a prefabricated framework/support will improve installation, minimize leakages, look and ease future modifications. Also, windows with light would preferably be installed along the rearing tank for inspection of water quality.

7.1.5 Central Drainage in rearing tank

Currently the central drainage in the rearing tank (Figure 7.1.5) includes an angular lifting/sinking outlet which can be improved by including lifting and sinking drains from a centre block above to minimize moment in tackle.

Figure 7.1.5: Existing tubing and angular lifting/sinking design with alternative lifting procedure

7.1.6 Symbol and drawings

System drawings and symbols should be congruent with recognized standards e.g. NORSOK. A slightly modified NORSOK standard should easily cover all aspects related to P&ID`s symbols for

each system “discipline” together with system/tag numbering for common understanding of operation procedures etc.

7.1.7 General construction recommendations

Below is a list of recommendations relating to the construction of the RAS;

- Use of galvanized flanges, bolts & nuts. Should be replaced with Stainless 316 due to corrosive environment.
- Use of ball valves for air supply in tanks. To be changed to other type of valves for individual flow. “No/low flow regulation on ball valves”
- No redundancy on circulation pump for O_2.
- More individual system pictures in SAS display could be preferable but most likely ongoing. (separate picture of freshwater intake and sweater intake)
- Spare Part List / Min-max storage of operational spares and operation/maintenance manuals are essential to operate the system with minimum down time / loss.
- Fabricators should make material able to travel on flatbed trucks and not semitrailers, as there are lots of small farms with limited access

Overall, Temperature, pH, Salinity and CO_2 all increased from Stage 1 to Stage 2, however, the water quality parameters were within the acceptable limits for Salmonoids. The SBR blockage resulted in higher TAN, NO_2-N and NO_3-N in the SBR but still below recommended levels.

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8 APPENDICES

A. P&ID – Main process flow
B. P&ID: normal conditions
C. P&ID – low water level during vaccination and transport
D. Water Analysis protocol
E. Test protocols (5 documents)

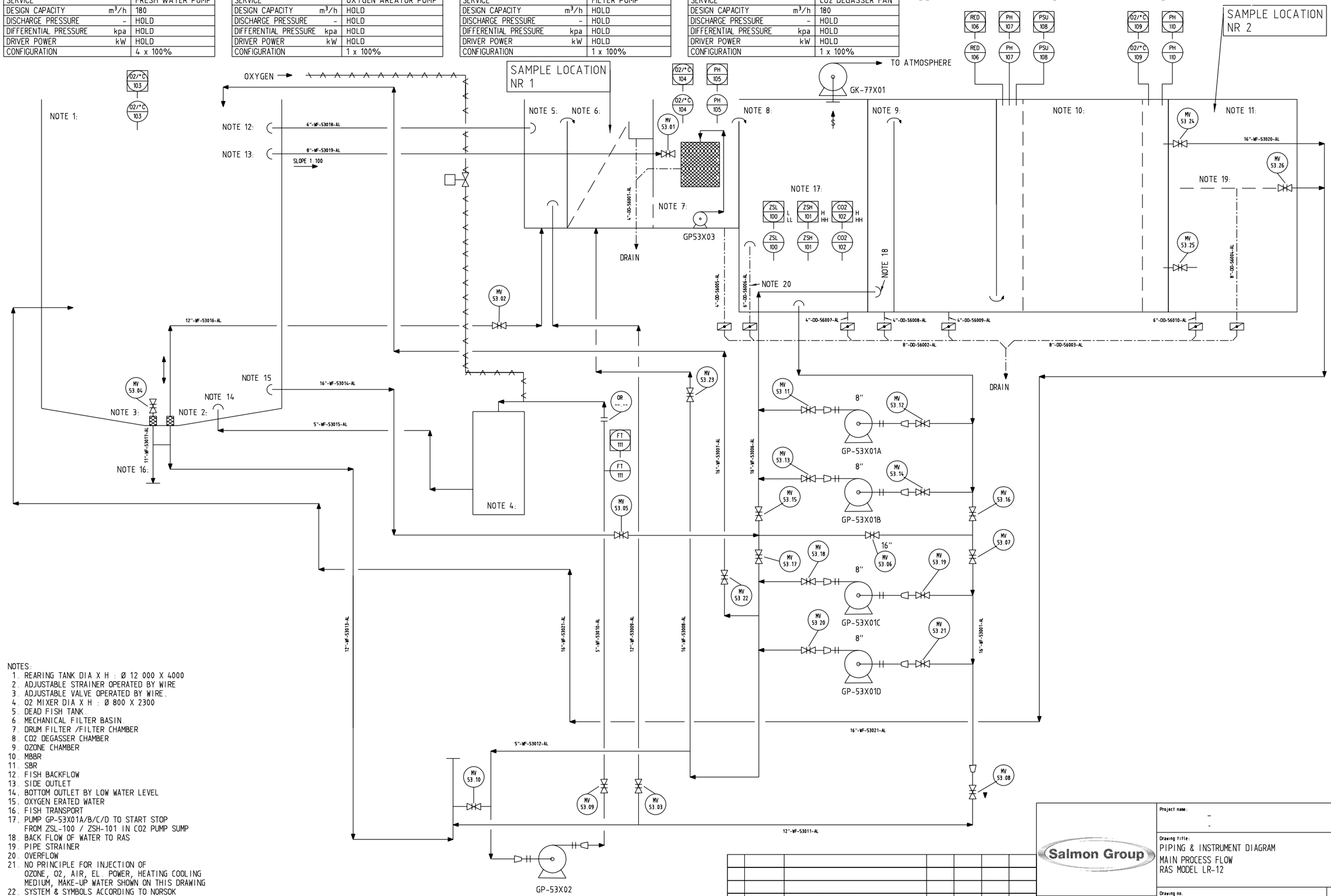
Appendix A P&ID - Main process flow & Sample location

ITEM NO		GP-53X01A/B/C/D
SERVICE		FRESH WATER PUMP
DESIGN CAPACITY	m^3/h	180
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		4 x 100%

ITEM NO		GP-53X02
SERVICE		OXYGEN AREATOR PUMP
DESIGN CAPACITY	m^3/h	HOLD
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		1 x 100%

ITEM NO		GP-53X03
SERVICE		FILTER PUMP
DESIGN CAPACITY	m^3/h	HOLD
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		1 x 100%

ITEM NO		GK-77X01
SERVICE		CO2 DEGASSER FAN
DESIGN CAPACITY	m^3/h	180
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		1 x 100%



NOTES:
1. REARING TANK DIA X H : Ø 12 000 X 4000
2. ADJUSTABLE STRAINER OPERATED BY WIRE
3. ADJUSTABLE VALVE OPERATED BY WIRE.
4. O2 MIXER DIA X H : Ø 800 X 2300
5. DEAD FISH TANK.
6. MECHANICAL FILTER BASIN.
7. DRUM FILTER /FILTER CHAMBER
8. CO2 DEGASSER CHAMBER
9. OZONE CHAMBER
10. MBBR
11. SBR
12. FISH BACKFLOW
13. SIDE OUTLET
14. BOTTOM OUTLET BY LOW WATER LEVEL
15. OXYGEN ERATED WATER
16. FISH TRANSPORT
17. PUMP GP-53X01A/B/C/D TO START STOP FROM ZSL-100 / ZSH-101 IN CO2 PUMP SUMP
18. BACK FLOW OF WATER TO RAS
19. PIPE STRAINER
20. OVERFLOW
21. NO PRINCIPLE FOR INJECTION OF OZONE, O2, AIR, EL. POWER, HEATING COOLING MEDIUM, MAKE-UP WATER SHOWN ON THIS DRAWING
22. SYSTEM & SYMBOLS ACCORDING TO NORSOK

Rev	Date	Reason for issue	Made By	Chk'd By	Disc. Appr	Proj Appr
A1	25.04.17	ISSUED FOR CONSTRUCTION	KL	KL	KL	KL

Salmon Group

Project name: -

Drawing title: PIPING & INSTRUMENT DIAGRAM
MAIN PROCESS FLOW
RAS MODEL LR-12

Drawing no. APPENDIX A - SKETCH

Scale	Size		Area	System	Project	Contr.	Disc./Type	Sequence	Subsequence	Rev.
NA	A1		-	-						-

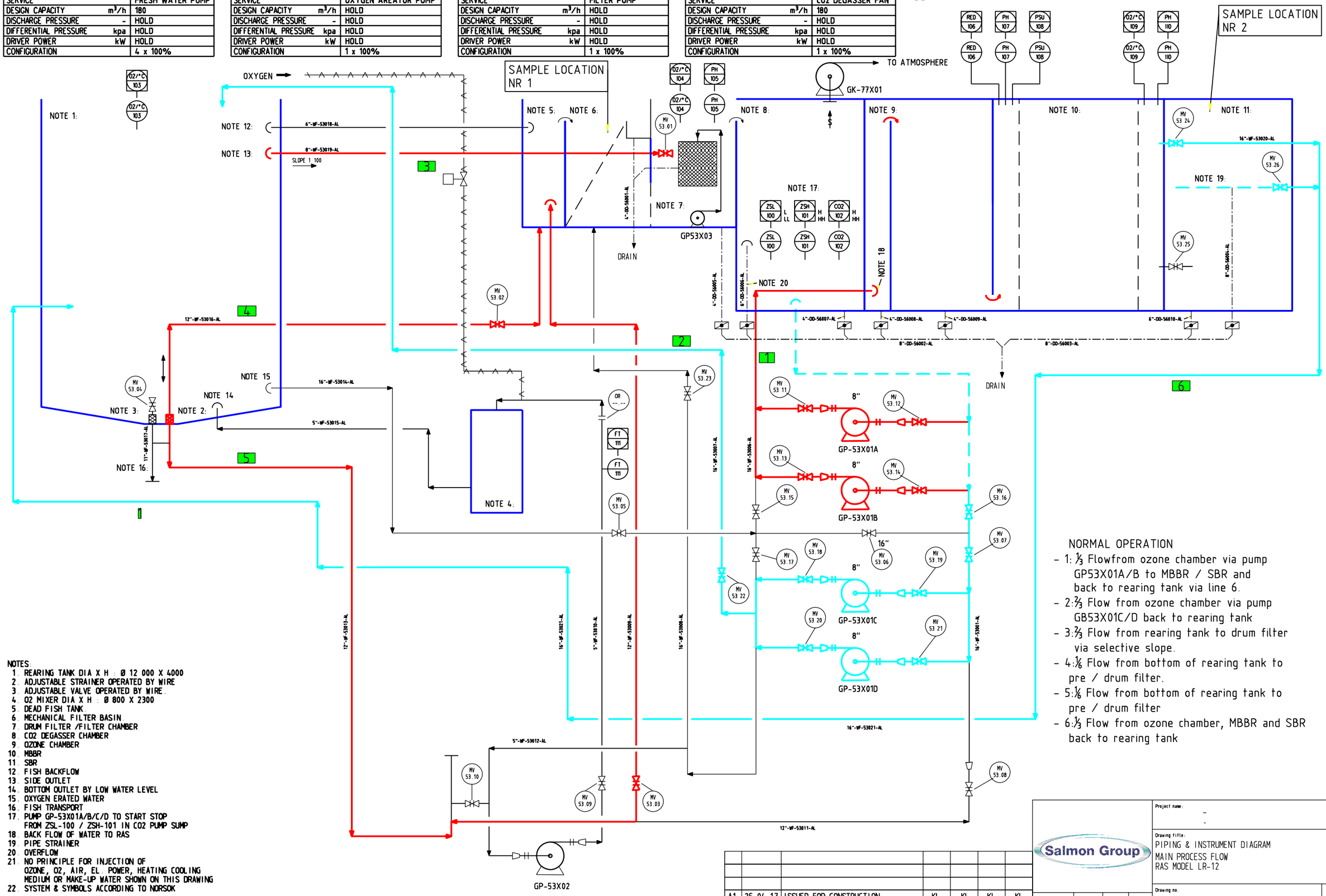
Appendix B P&ID - Normal conditions

ITEM NO		GP-53X01A/B/C/D
SERVICE		FRESH WATER PUMP
DESIGN CAPACITY	m^3/h	180
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		4 x 100%

ITEM NO		GP-53X02
SERVICE		OXYGEN AREATOR PUMP
DESIGN CAPACITY	m^3/h	HOLD
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		1 x 100%

ITEM NO		GP-53X03
SERVICE		FILTER PUMP
DESIGN CAPACITY	m^3/h	HOLD
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		1 x 100%

ITEM NO		GK-77X01
SERVICE		CO2 DEGASSER FAN
DESIGN CAPACITY	m^3/h	180
DISCHARGE PRESSURE	-	HOLD
DIFFERENTIAL PRESSURE	kpa	HOLD
DRIVER POWER	kW	HOLD
CONFIGURATION		1 x 100%



NOTES:
1. REARING TANK DIA X H : Ø 12 000 X 4000
2. ADJUSTABLE STRAINER OPERATED BY WIRE
3. ADJUSTABLE VALVE OPERATED BY WIRE.
4. O2 MIXER DIA X H : Ø 800 X 2300
5. DEAD FISH TANK.
6. MECHANICAL FILTER BASIN.
7. DRUM FILTER /FILTER CHAMBER
8. CO2 DEGASSER CHAMBER
9. OZONE CHAMBER
10. MBBR
11. SBR
12. FISH BACKFLOW
13. SIDE OUTLET
14. BOTTOM OUTLET BY LOW WATER LEVEL
15. OXYGEN ERATED WATER
16. FISH TRANSPORT
17. PUMP GP-53X01A/B/C/D TO START STOP FROM ZSL-100 / ZSH-101 IN CO2 PUMP SUMP
18. BACK FLOW OF WATER TO RAS
19. PIPE STRAINER
20. OVERFLOW
21. NO PRINCIPLE FOR INJECTION OF OZONE, O2, AIR, EL. POWER, HEATING COOLING MEDIUM OR MAKE-UP WATER SHOWN ON THIS DRAWING
22. SYSTEM & SYMBOLS ACCORDING TO NORSOK

NORMAL OPERATION
- 1: ⅓ Flowfrom ozone chamber via pump GP53X01A/B to MBBR / SBR and back to rearing tank via line 6.
- 2: ⅔ Flow from ozone chamber via pump GB53X01C/D back to rearing tank
- 3: ⅔ Flow from rearing tank to drum filter via selective slope.
- 4: ⅙ Flow from bottom of rearing tank to pre / drum filter.
- 5: ⅙ Flow from bottom of rearing tank to pre / drum filter
- 6: ⅓ Flow from ozone chamber, MBBR and SBR back to rearing tank

Rev.	Date	Reason for issue	Made By	Chk'd By	Disc. Appr.	Proj Appr
A1	25.04.17	ISSUED FOR CONSTRUCTION	KL	KL	KL	KL

Salmon Group

Project name: -

Drawing title: PIPING & INSTRUMENT DIAGRAM
MAIN PROCESS FLOW
RAS MODEL LR-12

Drawing no. APPENDIX B - SKETCH

Scale	Size		Area	System	Project	Contr.	Disc. /Type	Sequence	Subsequence	Rev.
NA	A1		-	-						-

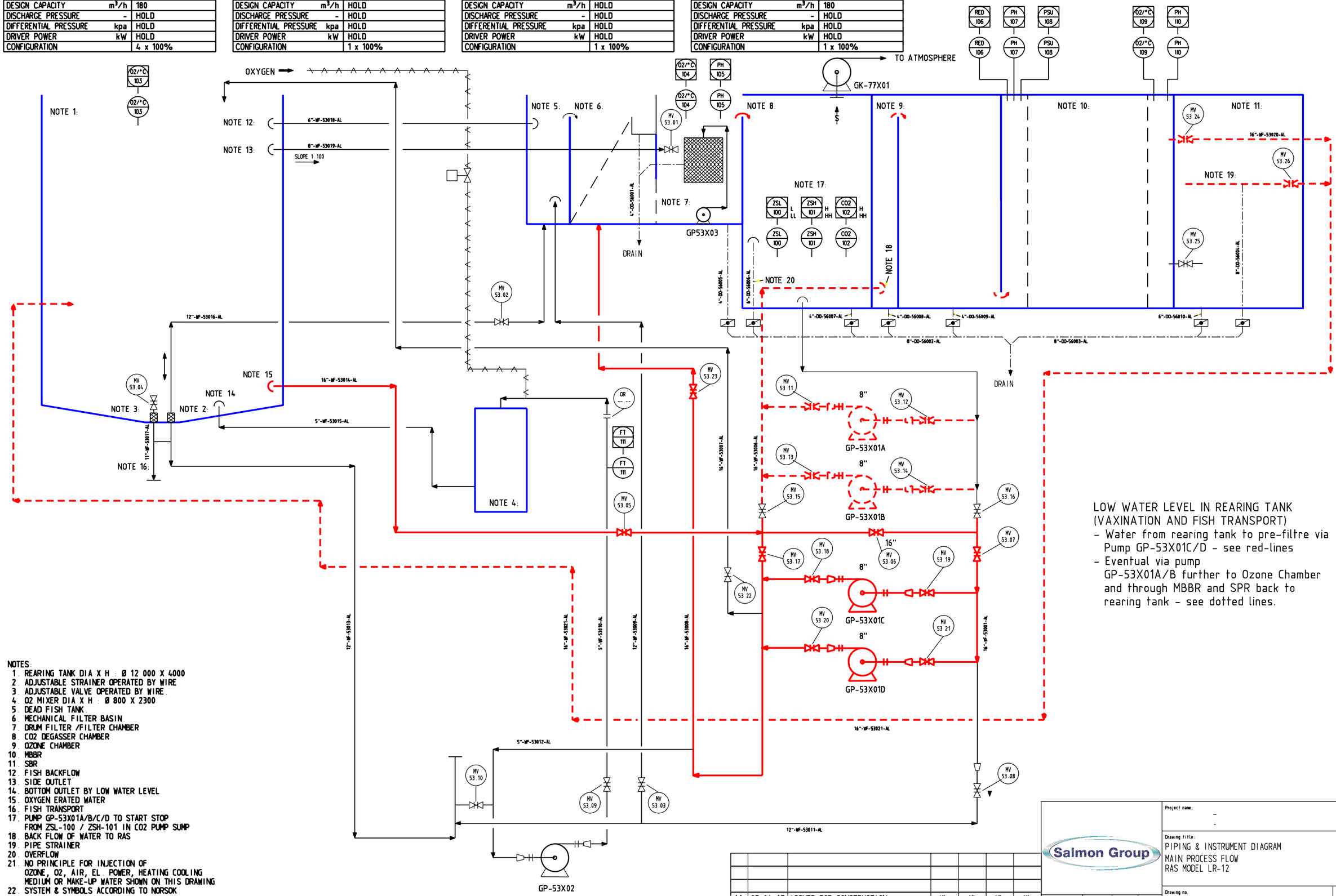
Appendix C P&ID - low water during vaccination and grading

ITEM NO	GP-53X01A/B/C/D
SERVICE	FRESH WATER PUMP
DESIGN CAPACITY m³/h	180
DISCHARGE PRESSURE -	HOLD
DIFFERENTIAL PRESSURE kpa	HOLD
DRIVER POWER kW	HOLD
CONFIGURATION	4 x 100%

ITEM NO	GP-53X02
SERVICE	OXYGEN AREATOR PUMP
DESIGN CAPACITY m³/h	HOLD
DISCHARGE PRESSURE -	HOLD
DIFFERENTIAL PRESSURE kpa	HOLD
DRIVER POWER kW	HOLD
CONFIGURATION	1 x 100%

ITEM NO	GP-53X03
SERVICE	FILTER PUMP
DESIGN CAPACITY m³/h	HOLD
DISCHARGE PRESSURE -	HOLD
DIFFERENTIAL PRESSURE kpa	HOLD
DRIVER POWER kW	HOLD
CONFIGURATION	1 x 100%

ITEM NO	GK-77X01
SERVICE	CO2 DEGASSER FAN
DESIGN CAPACITY m³/h	180
DISCHARGE PRESSURE -	HOLD
DIFFERENTIAL PRESSURE kpa	HOLD
DRIVER POWER kW	HOLD
CONFIGURATION	1 x 100%



LOW WATER LEVEL IN REARING TANK
(VAXINATION AND FISH TRANSPORT)
- Water from rearing tank to pre-filtre via Pump GP-53X01C/D - see red-lines
- Eventual via pump GP-53X01A/B further to Ozone Chamber and through MBBR and SPR back to rearing tank - see dotted lines.

NOTES:
1. REARING TANK DIA X H : Ø 12 000 X 4000
2. ADJUSTABLE STRAINER OPERATED BY WIRE
3. ADJUSTABLE VALVE OPERATED BY WIRE.
4. O2 MIXER DIA X H : Ø 800 X 2300
5. DEAD FISH TANK.
6. MECHANICAL FILTER BASIN.
7. DRUM FILTER /FILTER CHAMBER
8. CO2 DEGASSER CHAMBER
9. OZONE CHAMBER
10. MBBR
11. SBR
12. FISH BACKFLOW
13. SIDE OUTLET
14. BOTTOM OUTLET BY LOW WATER LEVEL
15. OXYGEN ERATED WATER
16. FISH TRANSPORT
17. PUMP GP-53X01A/B/C/D TO START STOP FROM ZSL-100 / ZSH-101 IN CO2 PUMP SUMP
18. BACK FLOW OF WATER TO RAS
19. PIPE STRAINER
20. OVERFLOW
21. NO PRINCIPLE FOR INJECTION OF OZONE, O2, AIR, EL. POWER, HEATING COOLING MEDIUM OR MAKE-UP WATER SHOWN ON THIS DRAWING
22. SYSTEM & SYMBOLS ACCORDING TO NORSOK

Rev	Date	Reason for issue	Made By	Chk'd By	Disc. Appr	Proj Appr
A1	25.04.17	ISSUED FOR CONSTRUCTION	KL	KL	KL	KL

Salmon Group

Project name: -

Drawing title: PIPING & INSTRUMENT DIAGRAM
MAIN PROCESS FLOW
RAS MODEL LR-12

Drawing no. APPENDIX C - SKETCH

Scale	Size		Area	System	Project	Contr.	Disc. /Type	Sequence	Subsequence	Rev.
NA	A1		-	-						-

Appendix D. Water analysis protocol

The protocol for water analysis was as follows

1. Clean test tubes with corresponding sampled water, either inlet or outlet
2. Line up each sample per test and sampling site (outlet or inlet), in the following order; COD, nitrite, nitrate, ammonia and alkalinity
3. Use pipettes to fill up each test tube with sampled water per each specific test kit.
4. Conduct each test according to test instructions
5. Add tested water samples individually to the spectrometer
6. Record data on excel spreadsheet within laboratory
7. Dispose of water sample in sealed glass container

Appendix E: Test protocol (1 of 5)

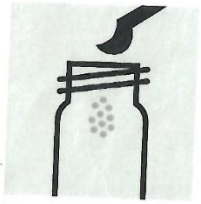
NO_3^-

Nitrate

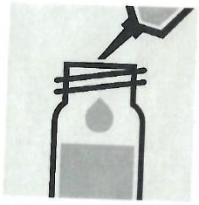
114773

Test

Measuring range:	0.5 – 20.0 mg/l NO_3-N	2.2 – 88.5 mg/l NO_3 10-mm cell
	0.2 – 10.0 mg/l NO_3-N	0.9 – 44.3 mg/l NO_3 20-mm cell
	Expression of results also possible in mmol/l.	

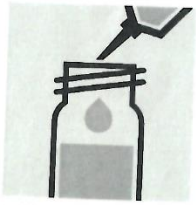


Place 1 blue micro-spoon of **NO_3-1** into a dry empty round cell (Empty cells, Cat.No. 114724).



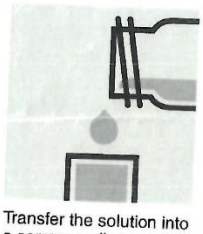
Add 5.0 ml of **NO_3-2** with pipette into the cell. Close the cell with the screw cap.

Shake vigorously for 1 minute to dissolve the solid substance.

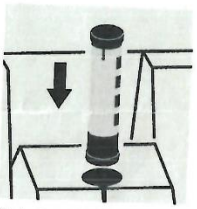


Add very slowly 1.5 ml of the sample with pi-pette, close the cell with the screw cap, and mix **briefly**.
Caution, cell becomes hot!

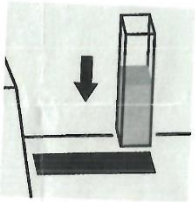
Reaction time: 10 minutes



Transfer the solution into a corresponding rectan-gular cell.



Select method with AutoSelector.



Place the cell into the cell compartment.

Note:

Empty cells with screw caps, Cat.No. 114724 are recommended for the preparation. These cells can be sealed with the screw caps, thus enabling a hazard-free mixing of the sample.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) we recommended to use Spectroquant® CombiCheck 10 and 20, Cat.No. 114676 and 114675, or the Standard solution for photometric applications, CRM, Cat.No. 125036, 125037, and 125038.

Ready-for-use nitrate standard solution Certipur®, Cat.No. 119811, concentration 1000 mg/l NO_3^-, can also be used after diluting accordingly.

To check for sample-dependent effects the use of addition solutions (e.g. in CombiCheck) is highly recommended.

NO_2^-

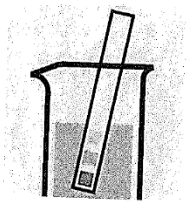
Nitrite

114776

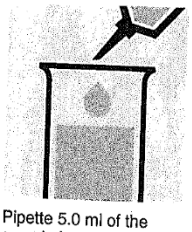
Test

Measuring range:	0.02 – 1.00 mg/l NO_2-N	0.07 – 3.28 mg/l NO_2	10-mm cell
	0.010 – 0.500 mg/l NO_2-N	0.03 – 1.64 mg/l NO_2	20-mm cell
	0.002 – 0.200 mg/l NO_2-N	0.007 – 0.657 mg/l NO_2	50-mm cell

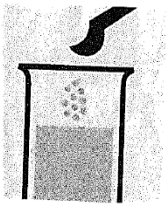
Expression of results also possible in mmol/l.



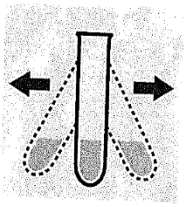
Check the pH of the sample, specified range: pH 2 – 10.
If required, add dilute sulfuric acid drop by drop to adjust the pH.



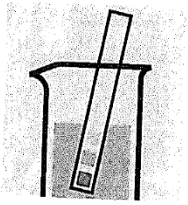
Pipette 5.0 ml of the sample into a test tube.



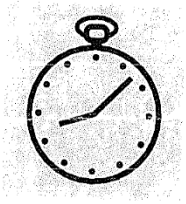
Add 1 level blue micro-spoon of **NO_2-1**.



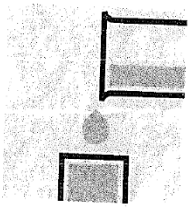
Shake vigorously to dissolve the solid substance.



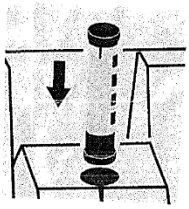
Check the pH, specified range: pH 2.0 – 2.5.
If required, add dilute sodium hydroxide solution or sulfuric acid drop by drop to adjust the pH.



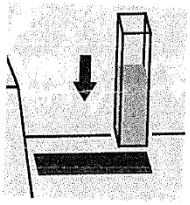
Reaction time: 10 minutes



Transfer the solution into a corresponding cell.



Select method with AutoSelector.



Place the cell into the cell compartment.

Important:

To measure in the 50-mm cell, the sample volume and the volume of the reagents have to be doubled for each. Alternatively, the semi-microcell, Cat.No. 173502, can be used.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) ready-for-use nitrite standard solution Certipur®, Cat.No. 119899, concentration 1000 mg/l NO_2^-, can be used after diluting accordingly as well as the Standard solution for photometric applications, CRM, Cat.No. 125041.

3 of 5

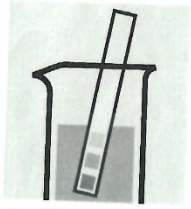
Ammonium

114752

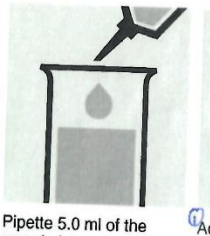
Test

Measuring range:	0.05 – 3.00 mg/l NH_4-N	0.06 – 3.86 mg/l NH_4	10-mm cell
	0.05 – 3.00 mg/l NH_3-N	0.06 – 3.65 mg/l NH_3	10-mm cell
	0.03 – 1.50 mg/l NH_4-N	0.04 – 1.93 mg/l NH_4	20-mm cell
	0.03 – 1.50 mg/l NH_3-N	0.04 – 1.82 mg/l NH_3	20-mm cell
	0.010 – 0.500 mg/l NH_4-N	0.013 – 0.644 mg/l NH_4	50-mm cell
	0.010 – 0.500 mg/l NH_3-N	0.016 – 0.608 mg/l NH_3	50-mm cell

Expression of results also possible in mmol/l.



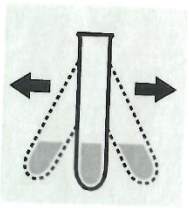
Check the pH of the sample, specified range: pH 4 – 13.
If required, add dilute sodium hydroxide solution or sulfuric acid drop by drop to adjust the pH.



Pipette 5.0 ml of the sample into a test tube.

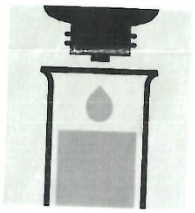
Add 0.60 ml of **NH_4-1** with pipette and mix.

Add 1 level blue microspoon of **NH_4-2.**



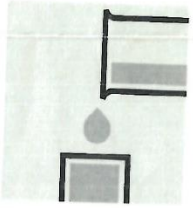
Shake vigorously to dissolve the solid substance.

Reaction time:
5 minutes

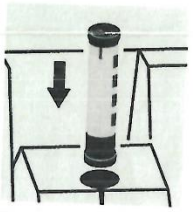


Add 4 drops of **NH_4-3** and mix.

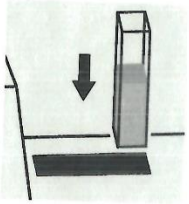
Reaction time:
5 minutes



Transfer the solution into a corresponding cell.



Select method with AutoSelector.



Place the cell into the cell compartment.

Important:

Very high ammonium concentrations in the sample produce turquoise-colored solutions (measurement solution should be yellow-green to green) and false-low readings are yielded. In such cases the sample must be diluted (plausibility check).

To measure in the 50-mm cell, the sample volume and the volume of the reagents have to be doubled for each. Alternatively, the semi-microcell, Cat.No. 173502, can be used.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) we recommended to use Spectroquant® CombiCheck 50, Cat.No. 114695, or the Standard solution for photometric applications, CRM, Cat.No. 125022, 125023, and 125024.

Ready-for-use ammonium standard solution Certipur®, Cat.No. 119812, concentration 1000 mg/l NH_4^+, can also be used after diluting accordingly.

To check for sample-dependent effects the use of addition solutions (e.g. in CombiCheck 50) is highly recommended.

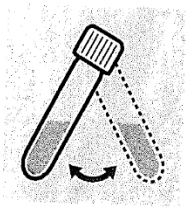
COD

Chemical Oxygen Demand

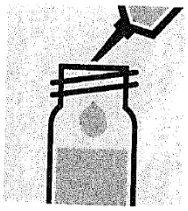
114895

Cell Test

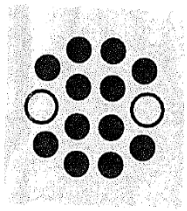
Measuring range: 15–300 mg/l COD or O_2
Expression of results also possible in mmol/l.



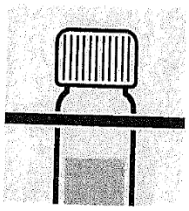
Suspend the bottom sediment in the cell by swirling.



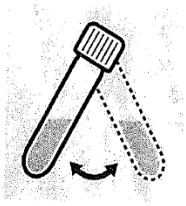
Carefully pipette 2.0 ml of the sample into a reaction cell, close tightly with the screw cap, and mix vigorously. **Caution, the cell becomes hot!**



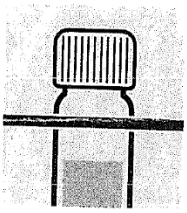
Heat the reaction cell in the thermoreactor at 148 °C for 2 hours.



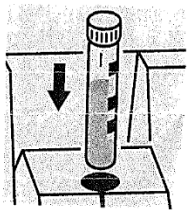
Remove the cell from the thermoreactor and place in a test-tube rack to cool.



Swirl the cell after 10 minutes.



Replace the cell in the rack for complete cooling to room temperature. **Very important!**



Place the cell into the cell compartment. Align the mark on the cell with that on the photometer.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) we recommended to use Spectroquant® CombiCheck 60, Cat.No. 114696, or the Standard solution for photometric applications, CRM, Cat.No. 125029 and 125030.

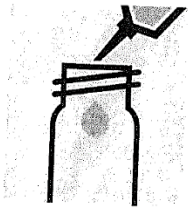
To check for sample-dependent effects the use of addition solutions (e.g. in CombiCheck 60) is highly recommended.

Acid Capacity to pH 4.3 (Total Alkalinity)

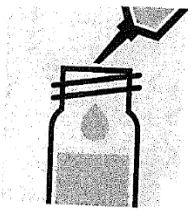
101758

Cell Test

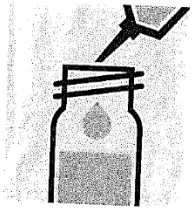
Measuring range: 0.40 – 8.00 mmol/l
20 – 400 mg/l $CaCO_3$



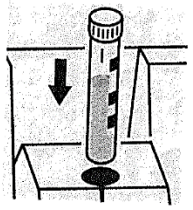
Pipette 4.0 ml of **AC-1** into a round cell.



Add 1.0 ml of the sample with pipette, close the cell with the screw cap, and mix.



Add 0.50 ml of **AC-2** with pipette, close the cell with the screw cap, and mix.



Place the cell into the cell compartment. Align the mark on the cell with that on the photometer.

Quality assurance:

To check the measurement system (test reagents, measurement device, and handling) a sodium hydroxide solution 0.1 mol/l, Cat.No. 109141, can be used after diluting accordingly (see section "Standard solutions").

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