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Outlet of Nutrients from the Fish Laboratory at Norwegian University of Life Sciences (NMBU)

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OUTLET OF NUTRIENTS
FROM THE FISHLABORATORY
AT NORWEGIAN UNIVERSITY OF LIFE SCIENCES (NMBU)

Master Thesis

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Table of contents

Table of contents i

List of figures ii

List of tables iii

Acknowledgement 1

Abstract 3

1. Introduction 5

2. Materials and Methods 11

2.1 Construction and management of the system 11

2.2 Methods for sampling and analysis 15

3. Results and Discussion 21

3.1 Variation between samples 21

3.2 Difference between sampling in Fish lab and external lab 23

3.3 Variation through the day 26

3.4 Variation from week to week 28

4. Conclusions and future perspectives 32

References 35

List of figures

Figure 1: Map of Årungen drainage basin (Borch et al., 2007).....9

Figure 2: A sketch of the RAS in Fish laboratory.....11

Figure 3: Step by step to take the samples 16

Figure 4: Test kits used to quantifying COD, TP, TN and PO_4-P 17

Figure 5: Photometer NOVA 60 (a) and Thermoreactor CR3200 (b).....17

Figure 6: Pipettes and test-tube racks.....18

Figure 7: Determination of orthophosphate 18

Figure 8: Determination of chemical oxygen demand (COD).....19

Figure 9: Determination of total phosphorus 19

Figure 10: Determination of total Nitrogen.....20

Figure 11: Variation of water quality through a day.....26

Figure 12: Variation of water quality during four weeks.....28

List of tables

Table 1: Characterization of streams draining into the lake Årungen, 2002-2007 ((Borch et al., 2007) 10

Table 2 : Feed composition (Skretting 2012).....12

Table 3: Quantify amount of make-up water, filter back flush and flushing of fish tanks, e.g.., per day 13

Table 4: Standard accuracy of a measurement for the four water parameters.....22

Table 5: Changings between values of parameters in the same sample ..23

Table 6: Differences between measurements in Fish lab and the external lab 24

Table 7: Changing the mean and standard deviation values in a day 26

Table 8: Quantity of water parameters in outlet water per day.....29

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Abstract

Recirculating aquaculture system (RAS) has been more and more applied in aquaculture farms in over the world because of its effectiveness. Like others, the RAS in fish laboratory at Norwegian University of Life Sciences (NMBU) has worked well with 94% of the degree of recirculation, which has economically meaning to the lab. However, the fish lab shall be moved to other location in the near future so it is important to document the amount of nutrients the RAS overflow water contains during a period of normal operation.

Therefore, this study was carried out andconcentrated on measurements of total Nitrogen (TN), total Phosphorus (TP), Orthophosphate (PO_4-P) and Chemical Oxygen Demand (COD) in the overflow of the system.

During one month of experiment at fish lab, these parameters were measured carefully to see how they varied in one sample, between samples, through the day and from week to week. The results shown that the concentrations in the outlet of nutrient were always quite stable with the average values around 114.75 gram total nitrogen, 13.18gram total phosphorus, 10.46 gram orthophosphate and 350.54 gram oxygen chemical demand in 34 m^3 of overflow water per day.

Of these values, the amounts of TP, PO_4-P, TNand COD per kg feed were 3.27g, 2.6g, 28.69g and 88.3g, respectively. In addition, 1 kg of biomass discharged 0.04 g TN, 0.03 g PO_4-P, 4.28g TN and 0.98g COD to the outlet water.

However, the load of total phosphorus needs to be reduced to 0.05mg/l when releasing into the small stream “Brønnerudbekken”, so limiting TP in feed with an acceptable value by using phosphate low containing ingredients and phytase enzyme can be carefully considered.

1. Introduction

Aquaculture effluents may contain a variety of constituents that could cause negative impacts when released into the environment (Sharrer et al.,2009; Sindilariu, 2007). These constituents include dissolved or particulate organics, nutrients and specific organic or inorganic compounds (Piedrahitaet al., 2003;Sugiura et al., 2006; Crab et al., 2007). The current concerns about the amount of residue generated from fish rearing suggests that it will be a decisive factor in the sustainability of fish farming in the coming years (Lazzari et al., 2008) and Recirculation Aquaculture System (RAS) have been considered.

In fact, RAS have been used successfully in many specific areas in aquaculture for the past 20 years, and are now increasingly used in shrimp maturation, hatcheries, nurseries, and ornamental fish breeding (Dunning et al, 1998; Isla, 2007). In 2009-2010, a total of 109 Norwegian salmon smolt companies and 214 licenses/smolt farms were in operation andonly some 10% of these farms turned into full RAS technology, but six to eight new intensively run RAS farms are expected each year (Drengstig et al, 2011). In addition, the high rate of reconstruction of traditional flow-through systems into re-use systems will continue.

RASs offer advantages in terms of reduced water consumption (Verdegem et al., 2006), improved opportunities for waste management and nutrient recycling (Piedrahita, 2003), better hygienic and disease management (Summerfelt et al., 2009; Tal et al., 2009) and biological pollution control (Zohar et al., 2005, Shang et al., 2011).

However, the RAS also have disadvantages, the most important is the deterioration of the water quality if the water treatment process within the system are not controlled properly, and it can cause negative effects on fish growth, increase the appear risk of infectious disease, increase fish stress, and other problems associated with water quality that resulting in deterioration of fish health and consequently loss of production (Timmons et al. 2002).

The water quality in RAS depends on different factors such as the source, the level of recirculation, the species have been cultured and the waste water treatment process within the system, to mention the most important ones (Sanni and Forsberg 1996; Losordoet al. 1999).A key to successful RAS is the use of cost-effective water treatment system components (Isla, 2007). Water treatment components must be designed to eliminate the adverse effects of waste products (Losordoet al. 1998).

Even though most water treatment methods that have been used in intensive or recirculating aquaculture systems result in a relocation of nutrients and organic matter and not in an overall reduction in discharges, this relocation makes it possible to reduce potential environmental impacts by facilitating effluent treatment.

Exceptions are denitrification, in which Nitrogen (N) is lost from the system as N_2 gas, and the decomposition of solids and the associated organic matter (Piedrahita, 2003). Nitrogen changes in a system may be related to nitrification and de nitrification processes or to the decomposition of organic matter. Nitrite is the intermediate product in

the process of nitrification of ammonia to nitrate and it is toxic for the fish because it affects the blood haemoglobin's ability to carry oxygen oxidized the iron in the haemoglobin molecule from the ferrous state to ferric state. The resulting product is called methemoblobin, which has a characteristics brown colour, hence the common name "brown colour disease" (Timmons *et al*. 2002).Nitrate (NO3-N) is the end product of nitrification process. In recirculating systems, NO3-N levels are controlled by daily water exchanges, but in some systems with low water flow rate this parameter has become increasingly important and its concentration levels should be lower than 10 mg NO3-N L-1 (Isla, 2008).

Besides, phosphorus (P) is generally considered as the limiting factor for algal reproduction in eutrophic waters (Zhang et al., 2010), and an excess of phosphorus will lead to algal blooms that are also detrimental to aquaculture. The organic and inorganic particulate and soluble forms of phosphorus undergo continuous transformations.

The dissolved phosphorus (usually as orthophosphate) is assimilated by phytoplankton and altered to organic phosphorus. The phytoplankton is then ingested by detritivores or zooplankton. Over half of the organic phosphorus taken up by zooplankton is excreted as inorganic P. Continuing to the cycle, the inorganic P is rapidly assimilated by phytoplankton (Smith, 1990; Holtan et al., 1988).Orthophosphate is sometimes referred to as "reactive phosphorus."

Nitrogen (N) and phosphorus (P) are the main end-products of fish loading, and can affect not only the rearing water, but also the environment as a whole(Lazzari et al, 2008). That is, if all phosphorus is used, plant growth will cease, no matter how much nitrogen is available. The natural background levels of total phosphorus are generally less than 0.03 mg/l. The natural levels of orthophosphate usually range from 0.005 to 0.05 mg/l (Dunne and Leopold, 1978).

A study that was carried out by Wang and colleagues (2012) indicated that 38% of total feed Nitrogen was incorporated and harvested as fish biomass, 45% was lost as dissolved inorganic N (DIN) and 15% was released as particulate organic N (PON). Approximately 3% of the total feed N was re-suspended into the water as dissolved organic N (DON) from particles, thus adding to the DON pool. Of the total feed P, 44% of the input was released as particulate organic P (POP), 30% was retained in fish, and 18% was lost as dissolved inorganic P (DIP). Approximately 8% of the total feed used was re-suspended from particles to form dissolved organic P (DOP).

The Fish laboratory at Norwegian University of Life Sciences (NMBU) shall be moved to another location very soon. In the new fish lab it will be possible to separate the effluent water. The continuously overflow from RAS which is normally very clean water and sludge water from flushing of fish tanks and back-flush water from the filters. The overflow from RAS counts for more than 80% of the total effluent from the fish lab.

Today, 100% of the effluent has gone to municipal waste water treatment plant. This is expensive and means a lot for the fish labs economy. The new Fish lab will apply for permission to release this 80% of "clean" water directly into the small stream "Brønnerudbekken" as Figure 1 below. The environmental objective of Ås municipality is to reduce the load of total P in this stream to 0.05 mg/l (Borch, et al, 2007).

Figure 1: Map of Årungen drainage basin (Borch et al., 2007)

From the data reported in table 1, every year Vollebekken discharged 1 million of water into the lake Årungen, account for 4% of the distribution, with 437 kg of TP.

Table 1: Characterization of streams draining into the lake Årungen, 2002-2007 ((Borch et al., 2007)

Locality	**Discharge (mill m3/year)**	**Average TP (µg/l)**	**Kg P/year**
Bølstadbekken	12,2	115	1403
Storgrava	4	138	552
Smedbølbekken	3,5	85	298
Vollebekken	1	437	437
Norderåsbekken	1,3	158	205
Brønnerudbekken	0,4	65	26
Others	2	100	200
Sum	**24,4**		**3121**

Therefore it is important to document the amount of nutrients the RAS overflow water contains during a period of normal operation. This study focuses on measurements of total Nitrogen, total Phosphorus, orthophosphate and Chemical Oxygen Demand (COD), a standard method for indirect measurement of the amount of pollution.

This report will hopefully be used as background material for a new outlet application from the Fish laboratory and may be for the wastewater treatment department as well.

2. Materials and Methods

2.1 Construction and management of the system

The study site was located in the Fish laboratory at Norwegian University of Life Sciences (NMBU), andconcentrated on one of the cold water RAS they have there. The systemconsisted of a header tank, a drum filter anda bio-filter connected to five square tanks (1m diameter) and two big round tanks (3m diameter) as Figure 2.

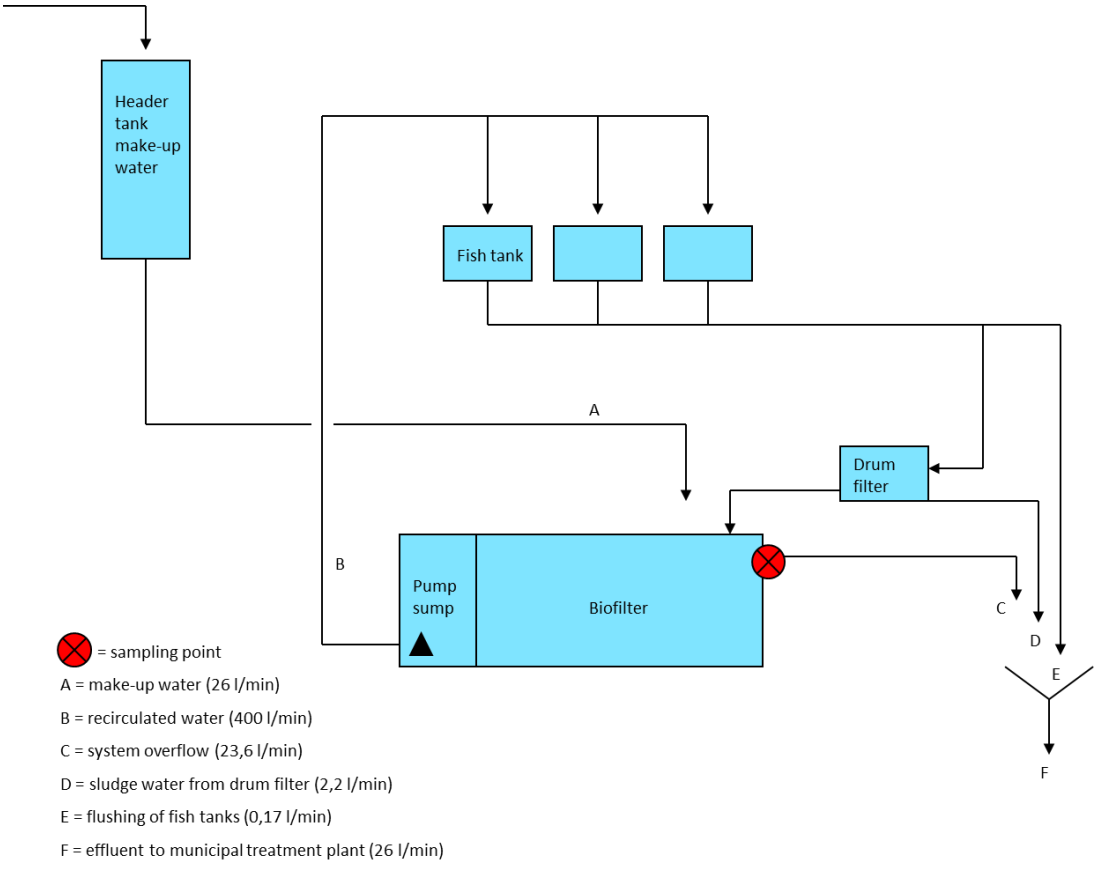

Figure 2:A sketch of the RAS in Fish laboratory

The header tank was a big municipal water container placed at the outside of the building at high attitude. It steadily supplied 26 liters of water per minute into the system.

Regard of the drum filter, it was an axial rotating screen that functioned as a mechanical filter separating particles from water in a screen with a mesh size of 60µ.

Generally, in a biological filter, ammoniumwas transformed to nitrite by the bacteria Nitrosomonas and continued to nitrate by bacteria called Nitrobacter. But in this system, no denitrification unit was installed and the nitrate concentration wascontrolled by water exchange. These bacteria were growing in the biofilm that was established on the filter media.

About the tanks, they were regularly cleaned by brushing and flushing out 50 liters of water per day on a small tank and 375 liters per week on a big one directly to the channel connected to the municipal water treatment plant (Table 2).

The total biomass was mostly stable with about 15kg of salmon and trout (size <100gram/fish), 250kg (size 100-500gram/fish) and 50kg (size >500gram/fish). The feed used were Nutra Pan (3-4mm) and VitalisRøye (7mm) from Skretting, with the composition as below (Table 1).

Table 2: Feed composition (Skretting 2012)

Protein	Oil	Ash	Moisture	Gross Energy
39 - 42%	30 - 32%	5 - 7%	7.5%	24.3 MJ/kg

Percentages of phosphorous and nitrogen by weight were 1.2% and 6.5%, respectively.

Besides, the light and feeding regime were 24 hours and the feeders ran 50 seconds after every 30 minutes.New water was added to the tanks only to make up for flushing out, evaporating and cleaning up waste materials.

Table 3: Quantify amount of make-up water, filter back flush and flushing of fish tanks, e.g.., per day

Date	Biomass (kg)	Feed (kg)	Make-up water (l/min)	Make up water/kg fish (l/day/kg)	Make up water/kg feed (l/day/kg)	Back flush drum filter (l/min)	Flushing of fish tanks (l/day)	Temp	Remarks
10/3	355	4,5	26	105.46	8320	2,2	250	9,0	
11		4,5	26		8320	2,2	250	9,0	
12		4,5	26		8320	2,2	250	9,0	
13		4,5	26		8320	2,2	250	9,0	
14		4,5	26		8320	2,2	1000	9,0	
15		4,5	26		8320	2,2	250	9,0	
16	365	4,5	26	102.58	8320	2,2	250	9,0	
17		4,5	26		8320	2,2	250	9,0	
18		3,0	26		8320	2,2	250	8,9	Starvation , grading
19		3,5	26		8320	2,5	250	8,9	Starvation , grading
20	330	4,3	26	113.45	8320	2,5	250	8,9	- 40 kg
21		4,3	26		8320	2,5	1000	8,9	
22		4,3	26		8320	2,5	250	9,0	
23		4,3	26		8320	2,5	250	9,0	
24		4,3	26		8320	2,5	250	9,1	
25		4,3	26		8320	2,5	250	9,1	
26		4,3	26		8320	2,5	250	9,1	
27	350	4,5	26	106.97	8320	2,5	250	9,2	

28		4,5	26		8320	2,5	250	9,2	
29		4,5	26		8320	2,5	1000	9,2	
30		4,5	26		8320	2,5	250	9,2	- 10 kg
31		4,5	26		8320	2,5	250	9,0	
01.04		4,5	26		8320	2,5	250	9,0	
02		4,5	26		8320	2,5	250	9,0	
03	360	4,6	26	104.00	8320	2,5	250	9,0	
04		4,6	26		8320	2,5	250	9,1	
05		4,6	26		8320	2,5	250	9,1	
06		4,6	26		8320	2,5	1000	9,1	
07		4,6	26		8320	2,5	250	9,1	
08		4,6	26		8320	2,5	250	9,0	
09		4,6	26		8320	2,5	250	9,0	
10	370	4,8	26	101.19	8320	2,5	250	9,0	- 30 kg
11		3,0	26		8320	2,5	250	9,0	Starvation, moving
12		3,0	26		8320	2,5	250	9,0	Starvation, moving
13		3,5	26		8320	2,5	250	9,0	Starvation, moving
14		3.5	26		8320	2,5	250	9,0	
15	350	4,6	26	106.97	8320	2,5	250	9,0	

The total volume of the RAS is 35m^3, in which the volumes of fish tanks, bio-filter and pump sump are 23m^3, 7m^3 and 5m^3, respectively.

In addition, during the experimental period, total water flow was 400 l/min or 0.4m^3/min and the volume of make-up water added per 24 hours was 37.4m^3. As a result, the recirculation degree during the experimental period was:

$$(Q_{tot} - Q_{make\text{-}up}) / Q_{tot} = (400\text{-}26) / 400 = 0.94 \text{ or } 94\%$$

Hydraulic retention time during the experimental period:

Total system volume/ Total water flow = 35/ (0.4*60) = 1.5 hours

Volume of make-up water per kg feed given in 24h was:

$37.4/4.5=8.3 m^3$/kg /day (approximately).

Volume of overflow from RAS:

Total make up water – (back flush + flushing)

$= 37.4 – (3.2 + 0.25) = 34\ m^3$/day

The composition of the make-up water was 0.04mg/l total nitrogen, 0.03 mg/l orthophosphate, 0.5 mg/l total nitrogen and 9.1mg/l chemical oxygen demand.

2.2 Methods for sampling and analysis

In this study, we quantified four water parameters such as total nitrogen (TN), total phosphorus (TP), orthophosphate (PO_4^-P) and chemical oxygen demand (COD) at the overflow water location in the system.

It was obviously understandable that using the handle (see Figure 3) madeit possible to take the sample with three replicates at the same time easily, which helped to minimize the errors in the sampling process. More importantly, to ensure the bottles clean completely, they were carefully washed with sampling water few times before taking true samples(Figure 3)

1 2 3

Take samples Clean up the bottles

4 5 6

Take samples again Close with the caps Ready for analyse

Figure 3:Step by step to take the samples

All four parameters were analyzed immediately after sampling following standard procedures (SeeFigure 7, Figure 8, Figure 9 and Figure 10) on the four cell test kits from Merck KGaA, 64271 Darmstadt, Germany.

Figure 4: Test kits used to quantifying COD, TP, TN and PO_4-P

All parameters were determined photo-metrically after having been added some chemicals inside the sampling cells according to the standard process given by the producer.Especially, analyzing of TN, TP and COD required for heating in the thermo-reactor for one hour (for TN) and 30 minutes (for TP and COD).

a) b)

Figure 5: Photometer NOVA 60 (a) and Thermoreactor CR3200 (b)

In addition to those mentioned above, other equipmentwerenecessarily used during analyzing period such as pipettes, gloves, test-tube racks, and so on… (Figure 6)

Figure 6: Pipettes and test-tube racks

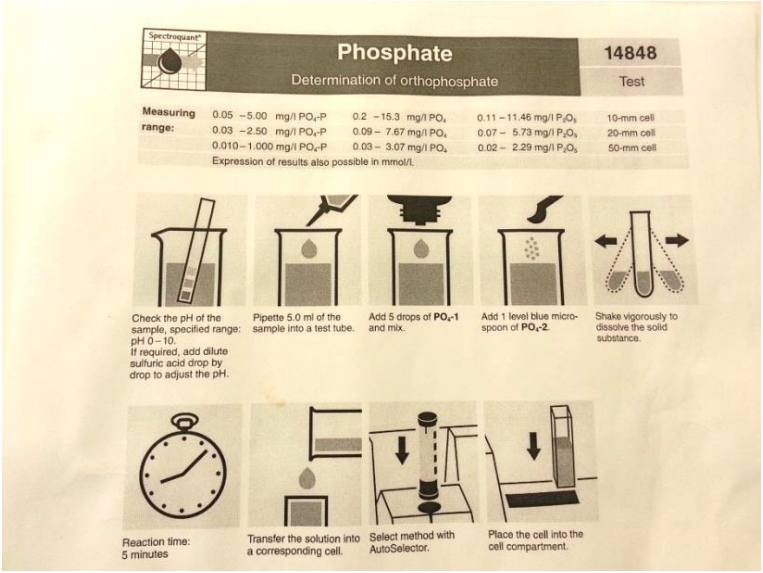

Figure 7: Determination of orthophosphate

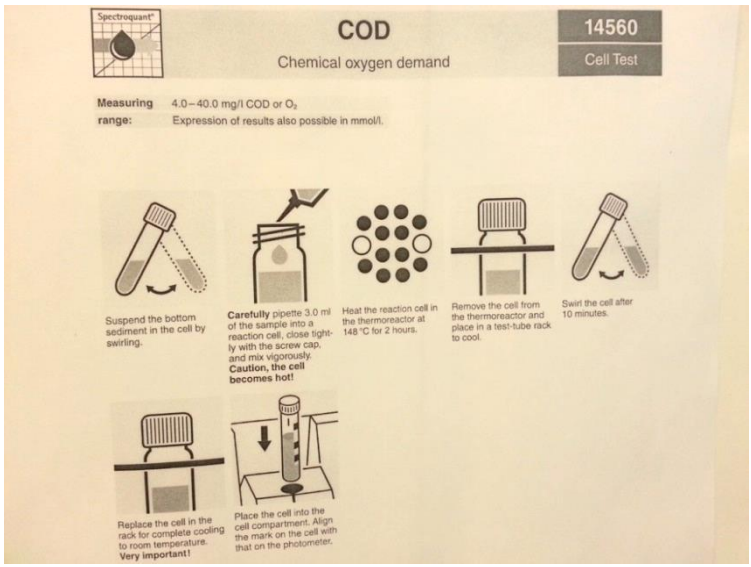

Figure 8: Determination of chemical oxygen demand (COD)

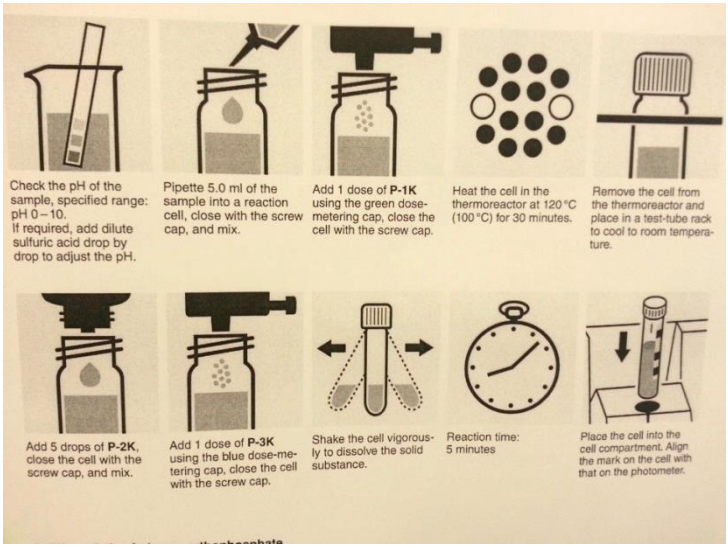

Figure 9: Determination of total phosphorus

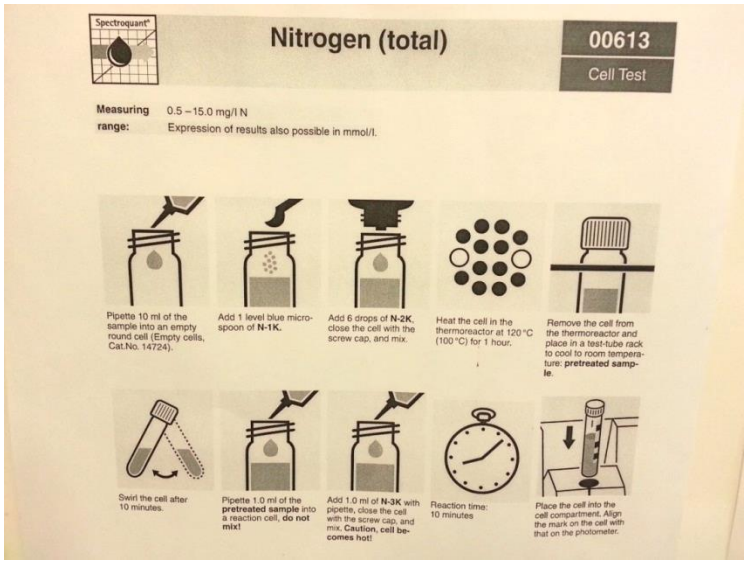

Figure 10: Determination of total Nitrogen

Sampling was conducted from March 19th to April 14th in 2014, which was divided into special sampling days shown as below:

- **Day 1st(March 19th**): Testing variation between sample, with two parallels. Register water flow and biomass.
- **Day 3rd (March 24th**): Testing variation through the day with 6 samplings from 08:00 to 18:00. Take three replications per sample. Register water flow and biomass.
- **Day 8th (March 26th):** Measure all parameters with three replications one time per day. Register water flow and biomass.
- **Day 17th (April 4th):** Measure all parameters with three replications one time per day. Register water flow and biomass.
- **Day 20th (April 7th):** Measure all parameters with three replications one time per day. Sending one parallel sample of all

TN, TP, PO_4-P and COD to analyze at external laboratory. Register water flow and biomass.

- **Day 27th (April 14th):** Measure all parameters with three replications one time per day. Register water flow and biomass.

3. Results and Discussion

In this case of experiment, the total biomass did not change too much, it ranged between 330 kg and 370 kg and weighted normally once a week. Amount of make-up water, total water flow and temperature were almost constant during the experimental period. Besides, the hygiene regime was well done regularly. Regarding feeding, there were some days with remarkably reduced feeding because of starvation prior to moving and grading. In total, this results in quite small variations for the four water parameters annalyzed.

3.1 Variation between samples

According to documents given by the test kits' producer, all measurements had error acceptable ranges that were wide or short depending on which water parameter was, which was shown as Table 3.

Table 4: Standard accuracy of a measurement for the four water parameters

Parameters' unit	mg/l TP	mg/l TN	mg/l PO_4-P	mg/l COD
Measuring range	0.05 – 5	0.50 – 15.0	0.05 – 5.	4 – 40
Accuracy of a measurement	Max. ± 0.08	Max. ± 0.50	Max. ± 0.06	Max. ± 1.5

As can be seen from the Table 4, the differences between values were in general very small, which proved that not only the measurement method was done nearly accurately, but also the amount and concentration of all parameters were well distributed in the water when taking the samples.

In both samples, the standard deviationsshowing how much variation between values were so low, which indicated that the data points tend to be very close to the mean and that was what an experiment was expected.

To be more detail, the amounts of total phosphorus in sample 1 with three replicates were 0.38 mg/l, 0.39 mg/l and 0.39 mg/l. They were almost the same with ± 0.01 mg/l of standard deviation, which also happened in sample 2 although there was very smalldifference between average value of sample 1 (with 0.39 mg/l)and sample 2 (with 0.37 mg/l). This inaccuracy was really understandable because all matters could not be completely disintegrated equally in the water, which means it also depended on how much particles includedin the solution.

Such a matter was completely true in cases of total nitrogen (TN), chemical oxygen demand (COD) and orthophosphate(PO_4^-P). The variation within a sample was very small. For instance in sample 1, these variations were ± 0.1 mg/l, ± 0.4 mg/l and ± 0.03 mg/l for TN, COD and PO_4-P, respectively. All of these values were much smaller than the standard accuracy given in Table 4 above.

Table 5: Changings between values of parameters in the same sample

Parameters	Replication	Sample 1 (mg/l)	Sample 2 (mg/l)
Total P	1	0.38	0.37
	2	0.39	0.37
	3	0.39	0.38
	Mean ± stdev	**0.39 ± 0.01**	**0.37 ± 0.01**
Total N	1	3.20	3.40
	2	3.40	3.20
	3	3.30	3.10
	Mean ± stdev	**3.30 ± 0.1**	**3.23 ± 0.15**
COD	1	10.60	9.60
	2	9.90	10.00
	3	10.60	9.90
	Mean ± stdev	**10.37 ± 0.4**	**9.83 ± 0.21**
PO_4-P	1	0.30	0.30
	2	0.35	0.29
	3	0.30	0.29
	Mean ± stdev	**0.32 ± 0.03**	**0.29 ± 0.01**

From the table value, it can be easily to see that the variations between two samples were in acceptable range.

3.2Difference between sampling in Fish lab and external lab

Other extra analyze was carried out to exam if all the methods and practices in Fish lab were worthily believable. Two samples were taken, in which, sample 1 and a part of sample 2 were kept to check in Fish laboratory and the rest was sent to external laboratory, EurofinsEnvironment Testing laboratory in Moss, Norway, and the result is presented in the Table 6.

Table 6: Differences between measurements in Fish lab and the external lab

Parameters	Replication	Sample 1 (8:30 am)	Sample 2	
			Fish lab	External lab
Total P	1	0.54		
	2	0.54		
	3	0.54		
	Mean ± stdev	**0.54± 0.00**	**0.54**	**0.50**
Total N	1	3.60		
	2	3.90		
	3	3.70		
	Mean ± stdev	**3.73± 0.15**	**4.1**	**4.3**
COD	1	10.30		
	2	11.20		
	3	12.00		
	Mean ± stdev	**11.17± 0.85**	**11.8**	**< 30**
PO4-P	1	0.47		
	2	0.47		
	3	0.47		
	Mean ± stdev	**0.47± 0.00**	**0.47**	**0.48**

The data indicates the fact that the results from both labs were very close to each other with the very low variation ± 0.02 mg/l for TP, ±

0.1 mg/l for TN, and ± 0.005 mg/l for PO_4-P, which point out thatmost measurements were well done.

Regarding COD, it measures everything that can be chemically oxidized, rather than just levels of biologically active organic matter and is easily effected on how much particle included (Boyles, 1997) so it is difficult to measure such low COD accurately.

Besides that, chloride is often the most serious source of interference in COD determination because its reaction with potassium dichromate in COD test (Boyles, 1997) follows the equation:

$$6Cl^- + Cr_2O_7^{2-} + 14H^+ \rightarrow 3Cl_2 + 2Cr^{3+} + 7H_2O$$

This is the best answer for why the amount of COD in the make-up water very high, 9.1 mg/l COD, while that parameter's value in the overflow water was 11.8 mg/l. From that, it can be seen the amount of COD released from fish very low with only 2.7 mg/l, which means there was a little organic compounds in the sampled water.

However, in comparison to other days, the values collected from this day were a little bit higher. The reason for this maybe a slightly increase of water temperature (See Table 3). In fact, many studies that have been conducted by number of scientists show that the constituent discharges will increase when the water get warmer because it promotes higher digestive physiology (Hardy, 1999; Sindilarius, 2007).

3.3 Variation through the day

With six samplings from 08:00 to 18:00 and three replications per sample, it was actually not difficult to see how the water quality changed in a day and all values were expressed in Table 7.

Table 7**:** Changing the mean and standard deviation values in a day

Time	Mean ± Standard deviation (mg/l)					
	8:00	10:00	12:00	14:00	16:00	18:00
TP	0.39 ± 0.00	0.46± 0.04	0.4 ± 0.02	0.4 ±0.02	0.42 ± 0.02	0.44 ± 0.03
TN	3.17 ± 0.12	3.8 ± 0.69	3.33 ± 0.06	3.2 ± 0.26	3.17 ± 0.31	3.00 ± 0.17
COD	10.87 ± 0.25	9.53 ± 0.35	10.03 ± 0.40	10.3 ± 0.46	10.27 ± 0.35	10.17 ± 0.75
PO4	0.37 ± 0.01	0.3 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.32 ± 0.02	0.33 ± 0.03

It can be seen from the line chart on Figure 11, the amounts of four parameters in outlet water were nearly stable from the morning to the afternoon. Despite of a little bit higher concentration in the early morning, the numbers slightly declined and stayednearly the same.

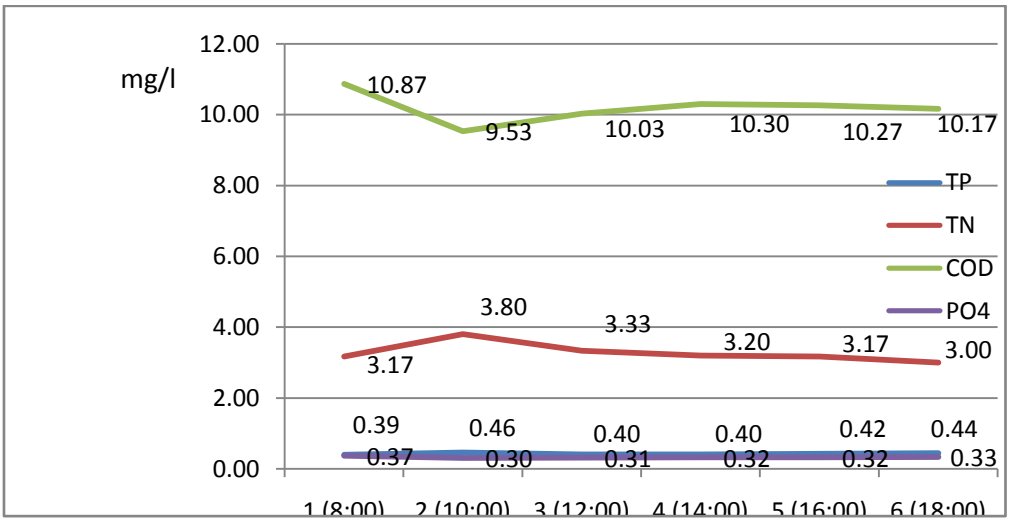

Figure 11: Variation of water quality through a day

Firstly, looking at the line illustrating for variation of COD, there was a short fall from 10.87 ± 0.25 mg/l at 8:00 am to 9.53 ± 0.35 mg/l at 10:00 ambut at which the varies were not beyond the accuracy limit of the test, +/-1.5mg/l. However, this tiny vary may be caused by 250 liters of water being flushed out of the small fish tanks directly to the channel according to daily hygiene regime at around 9:00 am. Such a change was shown most clearly on COD while other parameters seem to be nearly unchanged. Then, the concentration of COD remained constant at around 10.2 mg/l afterward.

Regarding of TN, it was approximately stable during the day except for aslightly rise at 10:00 am. In fact, at that time three values corresponded to three replications were 3.4 mg/l, 4.6 mg/l and 3.4 mg/l, which resulted in high mean value (3.8 ± 0.69 mg/l TN). The explanation for this may be because the water sample could be contained more particles than the two others, so the TN in this replication was much higher, 4.6 mg/l compared to 3.4 mg/l.

With the two remained parameters, TP and orthophosphate, there were not remarkable fluctuates between 8:00 and 18:00. The values for TP and orthophosphate were just around 0.42 mg/l and 0.32 mg/l, respectively.

Moreover, levels of N and P in fish food and the efficiency with which they are used influences the amounts of these nutrients that are excreted into the environment (Rodehutscordet al., 1995).

3.4 Variation from week to week

During nearly one month observed, the outlet water quality was not only stable through the day but also slightly changed from week to week, which can be pointed out clearly on Figure 12. Nevertheless, unusual results happened when the total biomass changed. As can be refer to Table 2, in 4th April, the total biomass was 10kg increased and the temperature was also a little bit higher, which werepossible reasons for a suddenly rise in the concentration of all parameters, especially in COD (10.87 mg/l) and TN (3.83 mg/l).

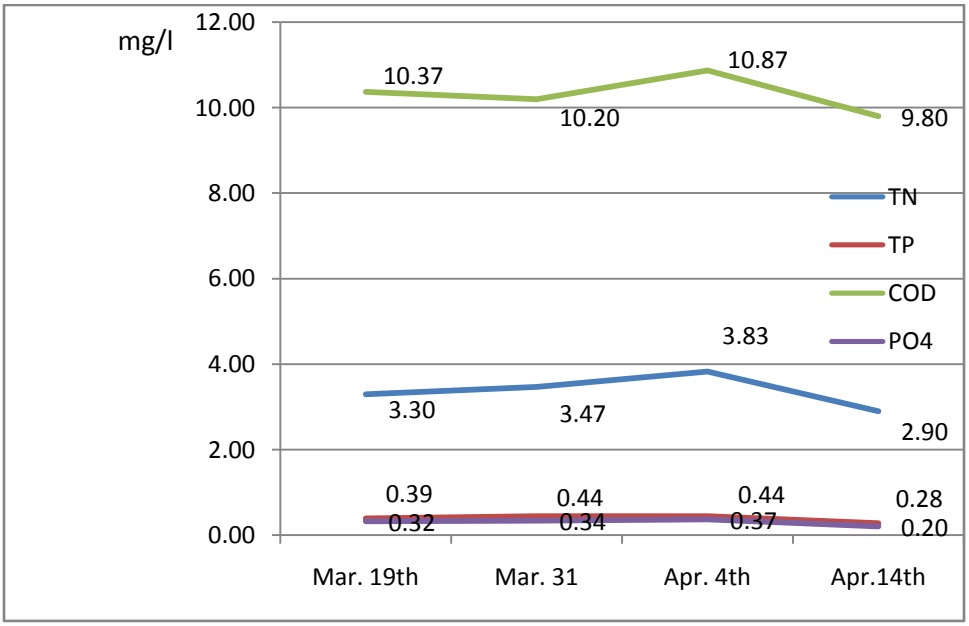

Figure 12:Variation of water quality during four weeks

However, there were dramatic dropsafter April 4th in four categories, which was resulted fromstarvation of fish for grading and moving out.

The higher biomass may cause an increased risk of negative environmental impacts due to the potential of greater production of waste materials originating from uneaten feed and metabolic waste products (Chen et al, 1993; Piedrahita, 2003).

Table 8: Quantity of water parameters in outlet water per day

Date		19-Mar	31-Mar	4-Apr	14-Apr
Total biomass (kg)		370	357	360	350
Total feed (kg)		3.5	4.5	4.6	3.5
Total N in outlet	per day (g)	**112.2**	**117.98**	**130.22**	**98.6**
	per kg feed per day(g)	32.06	26.22	28.31	28.17
	per kg biomass per day(g)	0.30	0.33	0.36	0.28
Total P in outlet	per day (g)	**13.26**	**14.96**	**14.96**	**9.52**
	per kg feed per day (g)	3.79	3.32	3.25	2.72
	per kg biomass per day (g)	0.04	0.04	0.04	0.03
Total COD in outlet	per day (g)	**352.58**	**346.80**	**369.58**	**333.20**
	per kg feed per day (g)	100.74	77.07	80.34	95.20
	per kg biomass per day (g)	0.95	0.97	1.03	0.95
Total PO_4-P in outlet	per day (g)	**10.88**	**11.56**	**12.58**	**6.80**
	per kg feed per day (g)	3.11	2.57	2.73	1.94
	per kg biomass per day (g)	0.03	0.03	0.03	0.02

As can be seen from Table 8, the amounts of all four parameters per kilogram biomass were quite stable day by daywith low concentration. According to Piedrahita (2003), partial water recirculation minimizes the waste per kilogram fish produced and improves the efficiency of the end of pipe treatment through effluent pre-conditioning. However, there were little changes in the concentration of these parameters according to how much feed given.

Those figures are very meaningful to show that it is necessary to consider in the balance of the diet and the biomass, which means the higher the biomass is, the greater the feed is given and vice versa. This will minimize uneaten feed when feeding too much compare to the biomass and avoid starvation when raising more fish but not enough feed supplied.

Based on all results, average 3.1mg/l TP and 28.7mg/l TN per kilogram feed went out as effluent while the percentages of phosphorus and nitrogen by weight in feed supplied were 1.2% and 6.5%. This means 25% TP and 44% TN were released in the effluent. These values were much less compare to the values from Wang et al. (2012) that 45% was lost as dissolved inorganic N (DIN) and 15% was released as particulate organic N (PON) and approximately 3% of the total feed N was re-suspended into the water as dissolved organic N (DON) from particles, thus adding to the DON pool; of the total feed P, 44% of the input was released as particulate organic P (POP), 30% was retained in fish, and 18% was lost as dissolved inorganic P (DIP).

Furthermore, the amount of these water parameters were also lower than that of Bergheim et al (2013) although the overflow water quantified only accounted for 80% of the total outlet water from Fish lab,average 3 mg/l TP and 30 mg/l TN in 100% effluent discharged in comparison to 1.2-6.2 mg/l TP and 9.4-80 mg/l TN in a research on other Norwegian RAS.

However, new Fish lab will apply for permission to release this 80% of "clean" water directly into the small stream "Brønnerudbekken", and the environmental objective of Ås municipality is to reduce the load of total P in this stream to 0.05 mg/l (Borch, et al, 2007). The problem is amount of TP in effluent from the RAS (average 0.4 mg/l) was much higher than the amount permited. Removal of phosphate from aquaculture effluents has been a big problematic, and the best management strategy for phosphorus is to limit the amount of phosphorus in the feeds. A key problem with phosphorus is that most of it is not available to fish (i.e. not digestible) (Mugg et al, 2003). Some feed studies suggest that addition of enzymes to feeds such as phytase can improve phosphate availability (Jahan et al, 2003; Penaflorida, 1998).

During the past decade, feed and nutrition research has shown the importance of ingredients in trout feed. By selecting grains low in phytate for the formulation of trout feeds, less phosphorus will be released by the fish. The majority of the phosphorus in plant proteins is not absorbed by trout because it is not digested in animals with only one stomach (Hardy, 1999).

Another approach, to increase the bioavailability and utilization of phosphorus in feeds, is to increase the level of phytase in the feed (Baker et al., 2001; Papatryphon, 1999; Jackson et al., 1996). This approach is more effective in warm water species. The lower water temperatures associated with trout culture reduce the impact of phytase supplementation (Rodehutscord and Pfeffer, 1995). The relationship in trout between increasing phosphorus retention and 3-phytase introut feeds was shown to be most effective with levels of phytase between 500 and 2000 FTU/kg (Baker et al., 2001).

More importantly, typical dietary phosphorus requirements in most fish and crustacean feeds are 0.3-0.8% of the dry diet (Penaflorida, 1998). But in this experimental period, the feed that was used contained 1.2% in the composition whichwas why main reason why amount of TP was much too high compared to the aim of the Ås commune on 0.05mg/l total phosphorus.

Thus, having a good management and balanced diet with low TP would be the best ways to limit the TP release in to the recipient.

4. Conclusions and future perspectives

The main conclusion that can be drawn is therefore that the recirculation aquaculture system in fish laboratory has worked effectively with 94% of recirculation degree, which helpedto save a lot of money, time and labor as well.

During the day or even from day to day, the concentration in outlet of nutrient were quite stable with the average around114.75 gram total

nitrogen, 13.18gram to total phosphorus, 10.46 gramorthophosphateand 350.54 gramoxygen chemical demand in 34 m^3 of overflow water per day. These figures were little higher if the samples containing more particles, or before flushing out of the fish tanks and when the biomass as well as the amount of fish increased.

In the light of these conclusions, I recommend that the RAS should be established with higher scale to utilize its beneficial functions with better equipment, especially the pipe line because the particles very often stick inside the pipe line and deposit as sediment. For further production expansion, the pipe technologies are needed to reach adequate effluent qualities.

Besides, in order to avoid unwanted changes in the value of all parameters when moving fish out, starving, grading and feeding, it is very important to considerably balance the diet, the biomass especially the fish size because many studies have indicated that small fish consume feed with a higher percent of their body weight per day than the larger.

In recirculating systems, good water quality must be maintained for maximum fish growth and for optimum effectiveness of bacteria in the bio-filter. Water quality factors that must be monitored and/or controlled include temperature, dissolved oxygen, carbon dioxide, pH, ammonia, nitrite and solids (Masser et al, 1999). Other water quality factors that should be considered are alkalinity, nitrate and chloride.

Future studies should beconcentrated on the water quality of the flushing out water from drum filter, and especially from the tanks that

contain more particles and uneaten feed. From that, we will have a better understanding on the whole system that makes it possible to have economically a good management in the new fish laboratory.

Although a recirculating (or water re-use) aquaculture technology is more environmentally sound than ponds or raceways because it significantly reduces the volume of water discharged, a recirculating system does produce a concentrated waste sludge that can have adverse environmental impacts (Chen et al., 1993) if not managed properly.

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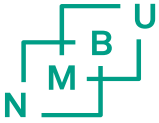

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