The United Nations University
FISHERIES TRAINING PROGRAMME
PO Box 1390, Skulagata 4
120 Reykjavik, Iceland

Final Project 2001

Mass Balance and Water Quality in Aquaculture Tanks

Gao Guangzhi
China
Dalian Fisheries University

Supervisor:
Dr. Guðjon Atli Auðunsson,
Icelandic Fisheries Laboratories

ABSTRACT

Total nitrogen, total phosphorus and total energy balance models were established for rainbow trout in aquaculture tanks and water quality was evaluated. Weight gain, energy consumption, and fish composition were measured and their dependence on fish weight was assessed. Using known feed inputs of nitrogen, phosphorus and organic matter together with nutrient retention and energy consumption, models were established to quantify the waste discharges from rainbow trout in aquaculture tanks. The waste discharge was found to be strongly dependent on feeding ration.

TABLE OF CONTENTS

1 INTRODUCTION....4

2 MATERIALS AND METHODS....6

2.1 PROCEDURE OF THE PROJECT....6
2.2 FIELD TEST....6
2.2.1 Test facilities....6
2.2.2 Species and grouping of fish....7
2.2.3 Feeding and Sampling....8
2.3 ANALYSIS....9
2.3.1 COD....9
2.3.2 TN and TP....9
2.3.3 DO....10

3 RESULTS....11

3.1 GROWTH....11
3.1.1 Weight gain....11
3.1.2 Growth curve for each group....12
3.1.3 Growth curve for all fish....13
3.2 CORRELATION ANALYSIS, ESTIMATION OF PARAMETERS....14
3.2.1 COD & fish size....14
3.3 TN, TP AND FISH SIZE....15
3.3.1 DO consumption and fish size....16
3.3.2 FR equation....18
3.4 MASS BALANCE....19
3.4.1 Nitrogen budget....19
3.4.2 Phosphorus budget....21
3.4.3 Energy budget....22
3.4.4 Physical forms of the waste....25

4 CONCLUSION....28

ACKNOWLEDGEMENTS....29

REFERENCES....30

LIST OF FIGURES

Figure 1: Procedure of study....6
Figure 2: The experimental tanks with automatic feeding machines on top (A). The conveyer grid system collects faecal material from the tank (B)....7
Figure 3: Correlation of length and weight....7
Figure 4: Average temperature during the experiment....13
Figure 5: Correlation of the relationship between average weight and average weight gain....13
Figure 6: Growth curve of fish in the experiment....14
Figure 7: Correlation of COD and fish size....15
Figure 8: Correlation between DO consumption and fish size....16
Figure 9: Average air saturation the last 15 days....17
Figure 10: Correlation of log- log regression relationship between FR and fish size.18
Figure 11: Approach of the calculation for TN budget....19
Figure 12: Nitrogen balance budget....20
Figure 13: Phosphorus balance budget....22
Figure 14: Energy balance budget....24

LIST OF TABLES

Table 1: Size and density at the start of the experiment....8
Table 2: Sampling scheme....8
Table 3: Composition of the feed....9
Table 4: Growth rate and feeding rate....11
Table 5: Average weight of fish....12
Table 6: TN_{in} and TN_{out} of different FR....21
Table 7: TP_{input} and TP_{output} of different FR....22
Table 8: E_{in} and E_{out} of different FR....24
Table 9: TN and TP level in water....25
Table 10: Average feeding ration (g d^{-1}) on the sampling dates for water....26
Table 11: Comparison of different FR....27

1 INTRODUCTION

Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants (FAO 2000). Aquaculture has been the world's fastest growing food production system because of international demand for luxury items and regional need for food, high demand for fish products, declining wild stocks and decreased access to fisheries. Aquaculture production increased from 7.4 million tons in 1980 to more than 42 million tons in 1999. The sector's production is growing at an average rate of more than 10% per year. Asian aquaculture farmers continue to contribute about 90% of the world's aquaculture production. Projections of world fishery production in 2010 range between 107 and 144 million tons, of which about 30 million tons will probably be reduced to fish meal and oil for non-food use. Most of the increase in fish production is expected to come from aquaculture. Aquaculture has brought significant economic and employment benefits to both national economies and coastal people throughout the world (FAO 2001).

Unfortunately, existing aquaculture operations can have a negative impact on the marine environment. Aquaculture systems can produce large quantities of polluting wastes, which are often released directly into natural bodies of water. These wastes consist primarily of uneaten fish feed, faecal material and other excretory wastes. The waste is a source of nutrient pollution - carbon-based organic matter, nitrogen and phosphorous compounds. High nutrient levels can stimulate blooms of photoplankton(Goldburg 1997). When algae die in large numbers, their subsequent degradation can drastically reduce oxygen levels in water, stressing or killing fish and other organisms. However, oxygen depletion may not be the most harmful effect of nutrient-stimulated photoplankton growth (Emerson 1999). Blooms of toxic algae species can produce huge fish kills, contaminate shellfish, and potentially even pose a health hazard to humans. An example of toxic alga blooms is red tides. Preliminary evidence suggests that such blooms may be promoted by nutrient pollution from various sources.

The environmental impact of marine fish-farming depends strongly on the system, species and the culture method (Edwards 1978). Aquaculture systems can be divided into three types according to the scale of operation. Extensive system: aquaculture without no or little environmental modifications, such as abalone and sea cucumber, just put the fry into the sea. Semi-intensive system: pond aquaculture, the density is a little bit higher than the natural environment. Intensive system: usually land-based, closed system aquaculture. The systems are open (flow-through water) or closed (recirculating water). There are more than 100 species of aquatic organisms farmed in the world. The studies on the environmental impact of aquaculture production have been carried out focusing on different systems and different species.

The main species where their impact on marine environment have been studied are: shrimp (Paez-Osuna *et al.* 1998, Paez-Osuna *et al.* 1999, Trott and Alongi 2000, Paez-Osuna 2001), Gilthead seabream (*Sparus aurata*) (Tovar *et al.* 2000), Channel catfish (*Ictalurus punctatus*)(Tucker *et al.* 1996), Sea bass (*Dicentrarchus labrax*)(Pagand *et al.* 2000), salmon- mussel(Troell and Norberg 1998). salmonid fishes (Young and Bureau 1998). These studies focused on the loading of dissolved nutrients, suspended solids and organic matter and biochemical oxygen demand. From the studies mentioned above, several mass balance models have been built up from which the

total nitrogen and phosphorous discharges into receiving waters can be estimated. (Not mathematical models).

Most of these studies are conducted on open systems. The common goal of these studies is to examine the environmental impacts of aquaculture and to relax or reduce the pressure, which is put on the marine environment by pollution from fish farming. The use of recirculating water systems is an approach that is used to limit the impact of aquaculture on the environment. Although the total quantity of nutrients released is similar in flow through and recirculation systems, the small volumes of concentrated effluent from the recirculation systems are easier to deal with (Pagand *et al.* 2000). Several approaches have been taken in order to reduce pollutants discharged into the environment in flow-through systems. For example, in China, polyculture of scallops, sea cucumbers and kelp reduces eutrophication and the use of toxic antifouling compounds.

Up to now, the most commonly used quality based environmental standards are water quality standards (e.g. acceptable nitrogen, phosphorus, BOD, COD and oxygen concentration). Unfortunately, the application of the standards is sometimes limited because very little is known about the capacity of the receiving waters for pollutants. This is particularly the case in developing countries (FAO 2001). China is situated in eastern Asia, bounded by the Pacific in the east. The coastline extends well over 14,500 km with the waters of the Bohai, the Huanghai, the East China and the South China seas with the area of 103,300 square miles (NOAA 2001). China dominates global aquaculture with 63.4% of the world aquaculture production in 1995, which accounted for 70.4% of Asian production (FAO 2001). Between 1990 and 1995, Chinese fisheries production increased 16.0%. The greater part of this increase was due to aquaculture (18.5% compared to 12.7% for captures fisheries). The contribution of aquaculture to Chinese fisheries increased from 51.6% in the 1980´s to 60.5% in 1995, an increase of about 10 million tons.

Bohai Sea is the inland sea of China. The area is 2400 square nautical miles. Bohai Sea, also named "natural fishery", is a major fishery resource of China. Some traditional economic fish have disappeared in recent years. The stock of those traditional good-quality economic fishes positioned at higher levels of the food chain has been declining while the stock of those lower-quality fishes is increasing. Since the 1980´s the catch in Bohai Sea has been low. In contrast, aquaculture production has developed fast. Hence the aquaculture operations put much pressure on the marine environment. The marine biological environment is facing degradation (China. Marine Ecological Environment Protection 2001). Developing marine pastures in the Bohai Sea is one of the measures by which the marine biological environment is expected to be improved. Dalian Fisheries University is operating the project and this study will contribute to that work. It is necessary and urgent to have a good understanding of the environmental effect of aquaculture.

The aims of this study are to study the mass balance of chemical oxygen demand (COD) and nutrients (TN, TP) in aquaculture, examine the tools required for modelling material flow in aquaculture, study monitoring and analytical methods for water quality examinations, and estimate and model different physical forms of waste from aquaculture.

2 MATERIALS AND METHODS

2.1 Procedure of the project

The project consisted of mainly three parts: field-tests, laboratory experiments and data analysis. The procedure of study is shown in Figure1.

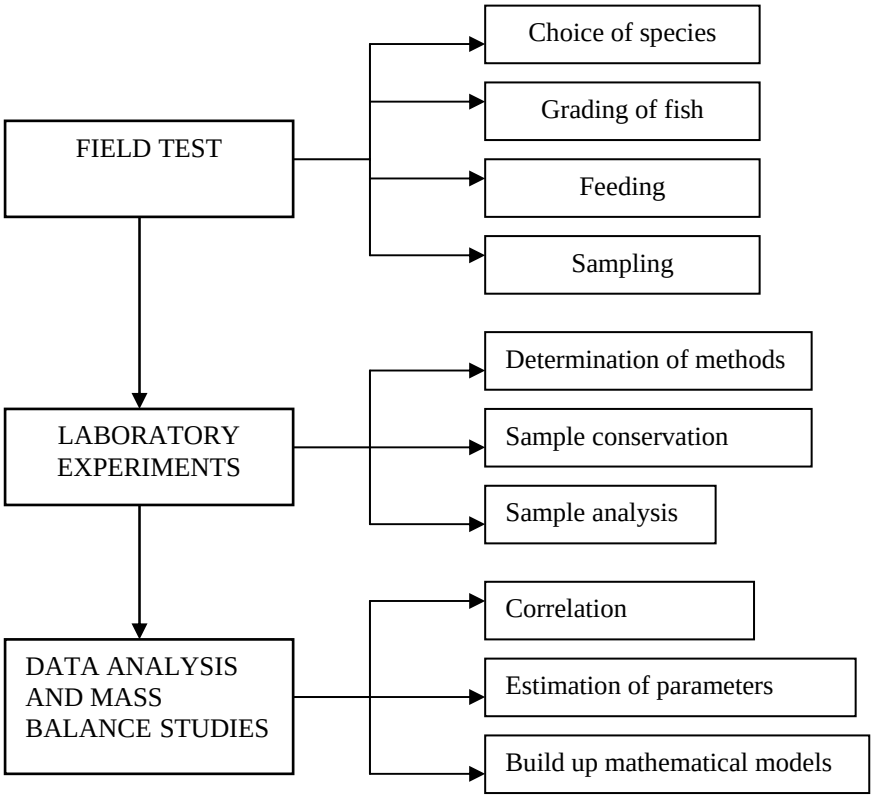

Figure 1: Procedure of study

2.2 Field test

The experiment was conducted at Icelandic Agriculture Research Institute - aquaculture station in December 2001 and January 2002 (6 weeks).

2.2.1 Test facilities

Indoor aquaculture facilities consist of eight 0.45 m^3 tanks (Figure 2). Cold water (4°C) and warm water (18°C) tubes were connected to the tanks. Faeces were collected at the outlet of each tank. In the bottom of the tank an approximately 10-cm tube was put into the water body. This made of the leftover feed accumulate in the bottom but also part of the faeces. In this way an actually separation of faeces and leftover feed was collected. Water temperature was kept about 11°C and water flow was about 0.6 m^3 h^{-1} in the tanks. The dissolved oxygen of inflow water was > 95%.

A constant 24 hour artificial ambient light was applied. The tank openings were partly covered to hinder the fish jumping out of the tanks.

A B

Figure 2: The experimental tanks with automatic feeding machines on top (A). The conveyer grid system collects faecal material from the tank (B).

2.2.2 Species and grouping of fish

Rainbow trout (*Oncorhynchus Mykiss*) was used as the experimental fish. The length and weight of the fish was measured (Figure 3) and grouped into four weight classes (Table 1). For this purpose the fish was put to sleep by adding phenoxyethanol to the water in the ratio of 1-2ml per liter. The fish was kept asleep during measurements. The fish was weighed to the nearest centigram (0.01) and total length was measured to the nearest mm.

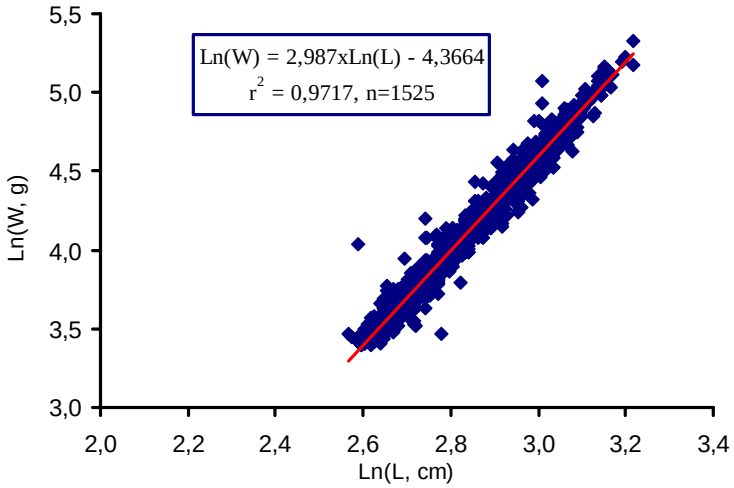

Figure 3: Correlation of length and weight.

Table 1: Size and density at the start of the experiment.

Group	Size (g)	Initial number of fish	Final number of fish	Average weight (g)	Density (kg/m^3)
1	30 -50	194	191	42	19.3
1	30-50	189	183	43	19.2
2	90-110	124	140	96	28.4
2	70 -90	152	130	81	28.5
3	90-110	127	126	81	24.5
3	90-110	133	122	78	24.6
4	>110	66	63	123	19.3
4	>110	63	64	129	19.4

2.2.3 Feeding and Sampling

The experiment started on the 8th of December and was terminated on January 15 (total of 39 days). Sampling type and sampling frequency at each sampling occasion is shown in Table 2.

Table 2: Sampling scheme.

Date	8	19	22	23	24	25	2	3	4	5	13	14	15
	December 2001						January 2002						
Weighing	♦	♦					♦						♦
Outflow of water			♦	♦				♦	♦		♦	♦	
Left over feed[1]				♦	♦				♦	♦		♦	♦
faeces				♦	♦				♦	♦		♦	♦
Tray water[2]				♦	♦				♦	♦		♦	♦
DO[3]				♦		♦	♦	♦		♦	♦	♦	♦

1. Left over feed: uneaten feed accumulated in the bottom of each tank.
2. Tray water: water of the feed and faeces were collected from this tray.
3. DO: dissolved oxygen.

Feeding: The fish was fed with commercial dry, pelleted food and the chemical compositions of the food are shown in Table 3. The composition of the feed was the same throughout the experiment. Simple feeding machines were used to feed fish automatically, twice per day. The interval was about 6 hours. The daily ration of rainbow trout depends on the water temperature, the size of fish and the type of food used (Anon. 1970). It was calculated as follows:

$$\text{Feed amount (g)} = (TW + WG) * GR * FF \quad (1)$$

Where TW is the total weight of fish in each tank, WG is the predicted weight gain, GR is the growth rate (wt % d^{-1}) and FF is the feed factor (g feed/g weight gain).

Table 3: Composition of the feed.

Constituent	Compositions
Available/digestible energy	17 MJ/kg
Total energy	22MJ/kg
Protein	50%
Fat	22%
Water	9%
Fibres	0.5%
Ash	9%
Carbohydrates	9.5%

Weighing: Total weight of the fish from each tank was obtained 3 times during the experiment (Table 2).
Water samples: Water samples were collected three times (over a period of two days each time). The samples was a mixture of 3 samples taken one hour after feeding, 6 hours after feeding and the next morning before feeding.
Solid samples (left over feed and faeces): Solid samples were collected 24 hours after water samples were taken.
Tray water: Tray water was taken in the each tray for analysing dissolved feed and faces. Volume of each tray was determined.
***Dissolved Oxygen* (*DO*)**: Samples for DO were taken from the surface water and from the bottom water of the tanks. The tanks were cleaned carefully before sampling. Oxygen was fixed at the time of sampling (Chapter 2.5.3.).

2.3 Analysis

2.3.1 COD

Analysis method of COD was based on standard methords for the examination of water and wastewater (APHA 1995). Potassiumhydrogenphthalate (KHP) was used to monitor whether the method was working properly. This was done by analysing 0.0, 1.0, 2.5, 5.0mL of the KHP-solution regularly (corresponding to 1 mg O_2/mL). 10 ml and 20 ml of outflow and inflow water samples were oxidised with 5.00 ml 0.00800M potassiumdichromate and 15 ml silversulphate-sulphuric acid. Heated to boiling. Boiling needs to start within 10 - 15 minutes and should be kept at boiling for exactly 2 hours (±5min). After cooling to room temperature, 2-3 drops of ferroin indicator were added and the surplus of dichromate is determined by titrating with 0.014M ferroammonium sulphate.
The COD method for solid samples was almost the same as for aqueous solutions, but 10 ml 0.04M dichromate and 0.07M ferroammonium sulphate were used. Left over feed and faeces and fish body (whole body) were minced to a homogenous mass and diluted 5-times with anhydrous Na_2SO_4. Feed was diluted 11-times.

2.3.2 TN and TP

Water samples (30-60 ml) were treated with a 10 ml mixture of peroxydisulphate and 3% boric acid (w/v) and autoclaved for 30 min. at 200 kP (120°C) (Valderrama 1981). TN was analysed by monitoring the absorbance of samples in 1 cm quartz cell against water at 220 nm and 275 nm, UV-lamp and 2.0 nm slit with nitrate as a standard (APHA 1995). For TP in water, samples were analysed by the molybden blue method

(APHA 1995), where colour was measured in a 1 cm glass cell against a phosphorus standard at 630 nm. The solid samples were diluted with anhydrous sodium sulphate and digested with 6 ml of concentrated sulphuric acid and 15 ml of dropping hydrogen peroxide in a Hach-apparatus (Hach *et al.* 1987). The ammonia was analysed by the phenate method (APHA 1995) and phosphorus by the molybden blue method as before.

2.3.3 DO

Analysis of dissolved oxygen was based on Hansen 1999. Samples were collected by flushing samples from the bottom of the sample flask by siphoning through plastic tubing. After pressing out the excessive water by the sample flask cap, 1 ml of the manganese solution and 1 ml of the alkaline iodide solution was added slightly below the water surface in the sampling bottles. The density of the reagents made the additions fall to the bottom. About 20 ml of the clear supernatant was removed from the sample content in the flask taking care that no precipitate is removed or stirred up. 2 ml of phosphoric acid and a magnetic stirring bar were added. The solution was stirred until all the precipitate has dissolved. As soon as possible and as fast as possible, the solution was titrated with concurrent stirring with the thiosulphate working solution from the brownish colour until faintly yellow/brownish. Five drops of the starch indicator were added and the titration was continued until the blue colour disappeared.

3 RESULTS

3.1 Growth

3.1.1 Weight gain

The initial average weight of fish (IAW), final average weight (FAW), average weight (AW), average weight gain (AWG), feeding rate (FR) and feeding factor (FF) is shown in Table 4.

Table 4: Growth rate and feeding rate.

Group	Days of feeding (DN)	Initial average weight (g) (IW)	Final average weight (g) (FW)	Average weight during the experiment (g $fish^{-1}$) (AW)	Average weight gain (g $fish^{-1}d^{-1}$) (AWG)	Feeding rate (g $fish^{-1}d^{-1}$) (FR)	Feeding factor (FF)
30-50g	39	42	81	63	1,00	1,96	1.96
30-50g	39	43	85	65	1,07	2,01	1.88
90-110g	39	96	141	114	1,42	3,24	2.85
70-90g	39	81	136	111	1,41	3,22	2.28
70-90g	39	81	152	117	1,82	3,15	1.73
70-90g	39	78	147	116	1,76	3,14	1.78
>110g	39	123	229	172	2,71	4,38	1.61
>110g	39	129	226	173	2,48	4,34	1.75

IAW is calculated as the total weight divided by the number of fish at the beginning of the experiment (Appendix 1). The calculation of FAW is done by the same way IAW. AW is the average value of the four weighings (Table 4).

$$AWG = (FAW - IAW)/DN \quad (2)$$

$$FR = \text{Total feeding amount (g } d^{-1}\text{)/ number of fish} \quad (3)$$

$$FF = FR/AWG \quad (4)$$

AWG: The average weight gain is as low as about 1.00 for small fish and the biggest weight gain is 2.71 for the largest fish. According to Austreng *et al.* (1987), the growth rate of young rainbow trout in fresh water is 2.3 -2.7 when water temperature is 10 - 12°C. The growth rate of big fish is very close to the higher level. This means the condition of this experiment was similar to normal practice of fish farming, and the fish in this group adapted to the experimental environment faster than small fish. The main reason that bigger difference of the growth rate of fish of 70 -90g is because the fish size in these tanks was not very even.

FR & ***FF***: The scientific method by which FR is calculated is based on the requirement for energy (Young and Bureau 1998). Most of methods commonly used have been based on the body length increase or live weight gain but also based on experience. For this experiment feeding amount was based on predicted weight gain and calculated as formula (1). An optimal FR should make FF equal about 0,85. That means all feed has been consumed by fish. Unfortunately the feeding factor (FF) in this experiment was higher than the values provided by most references. The reason

is that feed amount was not decreased when left over feed increased because fish was supposed to grow as fast as possible.

3.1.2 Growth curve for each group

The fish was weighed four times during the experiment and the average weight of each group were obtained from this operation (Table 5).

Table 5: Average weight of fish.

Day	Group (g)							
	30-50	30-50	70-90	70-90	90-110	90-110	>110	>110
1st	42	43	96	81	81	78	123	129
12th	59	59	89	99	107	110	161	160
26th	71	72	127	120	129	133	181	179
39th	81	85	141	136	152	147	229	226

The growth rate of each group in the first period was higher than that of the later periods. Many factors influence the growth of fish: diet, size, photoperiod and temperature. The most important reason for the fast growth in the first period is the fact that fish were starving for several days prior to the start of the experiment and food consumption was therefore much greater than usual. It can be explained using a simple growth model (From J and Rasmussen 1984):
Growth = In - Out (5)

Intake was greater than output in the first period so growth was fast. After this period the increase of weight was more or less linear. The growth rate slowed when the fish had adapted to the new environment. Another reason of slower growth in the second period is because temperature genearlly decreased. Water temperature is important factor for determining how much food fish will eat (Edwards 1978). The average water temperature in the whole run of the experiment is given in Figure 4. Water temperature fluctuated between 10 and 12°C in the whole experiment but a general decrease is seen. It was seen that food left over in the second period was much more than that of the first period and this means food consumption of fish had decreased. The third reason is decreased oxygen saturation with time, see 3.3.1.

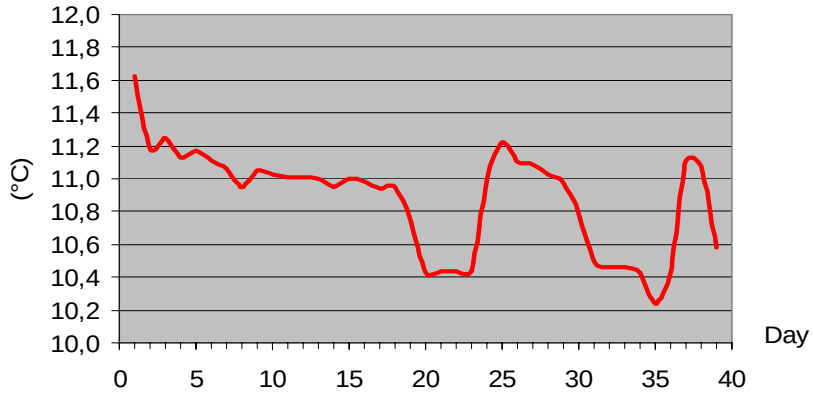

Figure 4: Average temperature during the experiment.

3.1.3 Growth curve for all fish

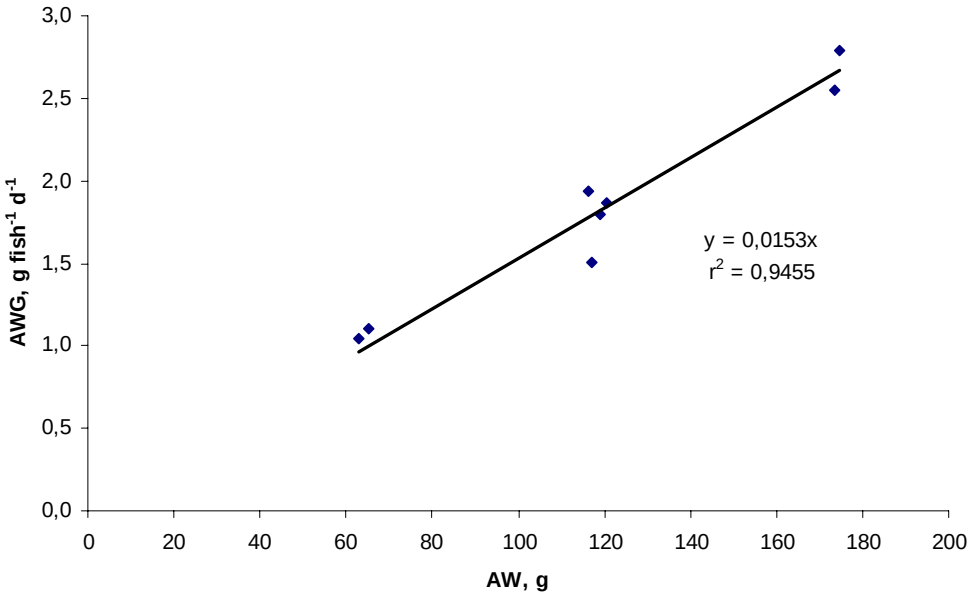

Figure 5: Correlation of the relationship between average weight and average weight gain.

The average weight of each group during the experiment was calculated (Table 5). The result of regression analysis for AW and AWG is shown in Figure 5.

The average weight gain equation for all the groups is:

$$AWG = 0{,}0153\ AW,\ r^2=0{,}94,\ n=8 \qquad (6)$$

Since AWG is the weight gain of each fish during the experiment:

$$AWG = dAW/dt = 0{,}0153\ AW$$

$$d(AW)/(AW) = 0{,}0153\ dt$$

The result of integration of this equation for weight 40 g at time 0 is as follows:

$$AW = 40\ e^{0{,}0153t} \qquad (7)$$

One might have expected the relationship between the weight gain and weight to be linear on a log-log scale. The reason for AWG to be linear with respect to weight in this experiment is probably the short time length of the experiment and limited fish sizes. Besides, the growth did not occur under normal conditions especially regarding oxygen saturation and increased density of the fish in tanks.
Corresponding to this equation the growth curve for all the fish sizes is obtained (Figure 6). It is known from the growth curve that it will take 118 days for the fish grow from 40g to 227g, *i.e.* the smallest average fish in this experiment and the largest.

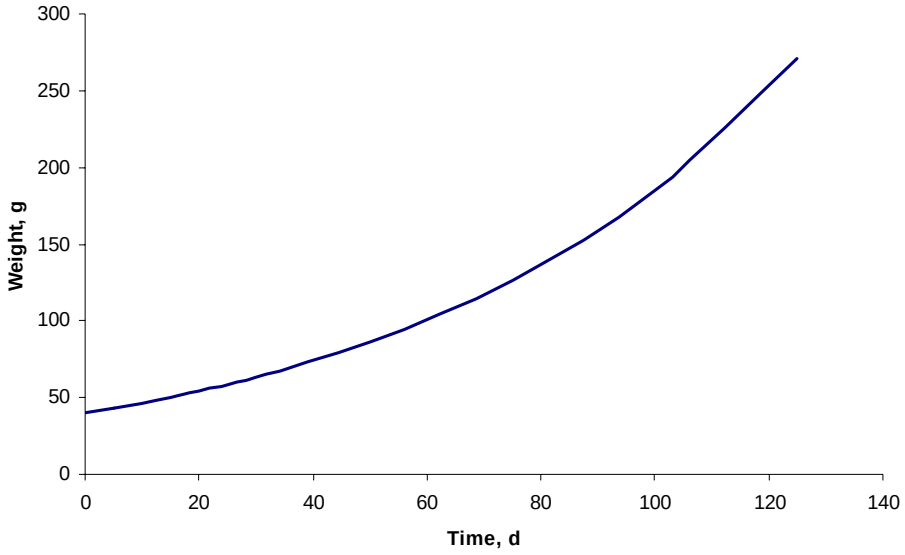

Figure 6: Growth curve of fish in the experiment.

3.2 Correlation analysis, estimation of parameters

3.2.1 COD & fish size

Ten fishes were selected randomly in each of the eight tanks and killed. The results of chemical analysis are given in Appendix 2. In order to decrease the variability, the average value of COD for each size class was applied in calculations. The correlation between COD and fish size is given in Figure 7.

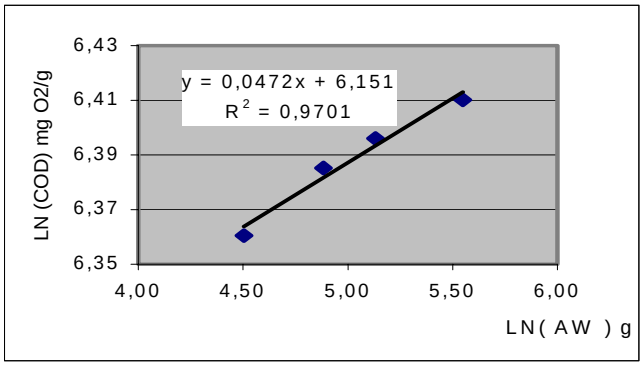

Figure 7: Correlation of COD and fish size.

A correlation of fish size and COD is shown in figure 7 and given by the expression:

$$COD\ (mg\ O_2/g\ ) = a\ *(AW)^b \quad (8)$$

with a = 6.15 and b = $e^{0.0507}$,

$$COD\ (mg\ O_2/g\ ) = 469\ *\ (AW)^{0.0472} \quad (9)$$

The result of regression analysis shows that COD increased slightly with fish size. This result is more or less in line with compositions of rainbow trout in the literature. The relation between carbon content of whole rainbow trout and weight was given by Hall *et al.* 1990):

$$(\%C) = 0.55\ *\ 17.4\ *\ W^{0.099} \quad (10)$$

$$COD\ (mg/g)\ *\ 0.375 = C\ (mg/g) \quad (11)$$

It is clear that COD increases with the weight of the fish.

3.3 TN, TP and fish size

The results of regression analysis for TP and the average weight of fish have shown that the slope of the curve is small. This indicates there is a slight trend that the total nitrogen in fish flesh decreases with fish size. The same result is obtained from the regression analysis for TP and fish size. The fish consist of water, protein, fat, ash and carbohydrate. Often carbohydrate in fish is excluded from bioenergistics as it is stated to constitute a relatively small part of the fish body (From J. and Rasmussen 1984). The amount of protein was calculated from the amount of nitrogen in the samples. The amount of ash is reflected by the amount of TP. The total energy content of fish can be determined by using energy content of protein and fat. Since nitrogen is slightly decreasing with size but carbon content is increasing fat content of fish must have increased during the experiment.

3.3.1 DO consumption and fish size

If ΔO_2 = consumption of DO, mg O_2/fish

then $\Delta O_2 = [O_2 \text{ (in inflow water)} - O_2 \text{ (in outflow water)}] * Q * 24/n$ (12)

Where Q is water flow (l hr^{-1}) and n is the number of fish.

DO of inflow and outflow is obtained by the average value of the surface and bottom water in the tanks. ΔO_2 of each group was calculated according to formula 12. The relationship between ΔO_2 and fish size is shown in Figure 8.

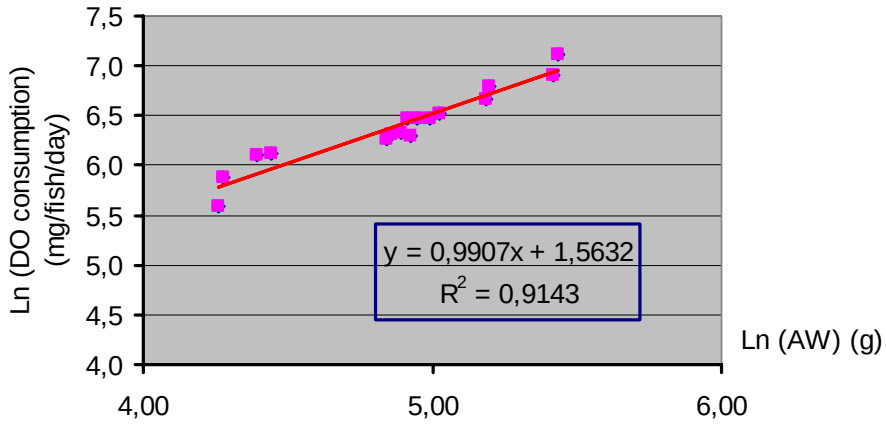

Figure 8: Correlation between DO consumption and fish size.

The relation between the amount of oxygen consumption of each fish and fish size can be expressed with the following equation where the consumption of oxygen per fish is Oc:

$$\ln(Oc) = 1.5632 + 0.9907 \ln (AW)$$

$$Oc \text{ (mg fish } ^{-1}\text{day}^{-1}) = e^{1.5632} * (AW)^{0.9907}$$

$$Oc \text{ (mg fish } ^{-1}\text{day}^{-1}) = 4.7741 * (AW)^{0.9907} \quad (14)$$

Water flow should have been increased with the growth of fish and the saturation kept >95%. However, in this experiment water flow was kept the same from beginning to the end. The air saturation was calculated for DO measurements and the results are shown in Figure 9.

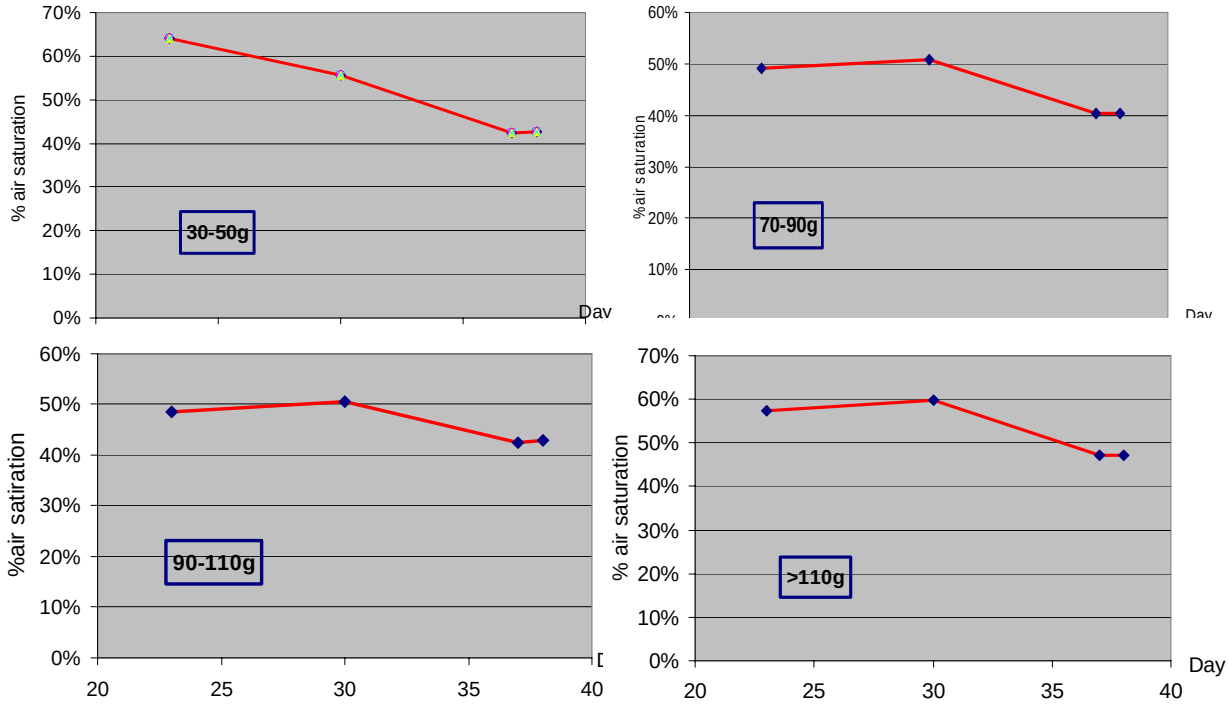

Figure 9: Average air saturation the last 15 days.

The air saturation was lower than 60% after day 23 for all groups but 30-50g. The level of air saturation was lower than the optimal value. It has been shown that limiting DO levels appeared to be around 70% air saturation for the Channel catfish (*Ictalurus punctatus*). It has also been indicted that similar results have been obtained with rainbow trout when the DO fell below 60% air saturation. The reason of air saturation decreasing was due to the increase of the density of the fish. Not keeping the same density during the experiment is a failure of the study and the lower air saturation resulted in a higher feeding factor. The reason that the growth rate decreased from second phase was decreasing air saturation. The ideal value for FF is close to one and this is always the aim in aquaculture.

3.3.2 FR equation

The regression result for AW and FR (Table 3) is given in figure 10.

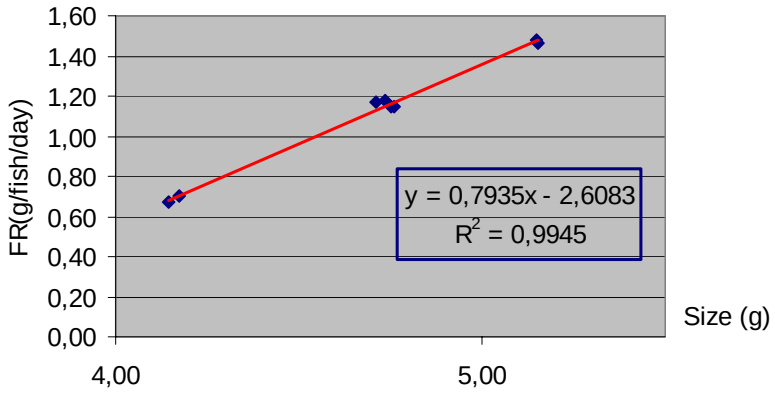

Figure 10: Correlation of log- log regression relationship between FR and fish size.

It is shown in figure 10 that the following equation fits the data:

$$\ln (FR) = 0.7935 \ln (AW) - 2.6083$$

$$FR = e^{-2.6083} * (AW)^{0.7935}$$

$$FR = a * (AW)^{b} \qquad (15)$$

$$FR = dF/dt = e^{-2.6083} * (AW)^{0.7935}$$

$$TF = e^{-2.6083} \int_{t_0} (AW)^{0.7935} dt$$

Where TF is the total feed amount that was put in during the fish grow from 40g to 227g.

It is known from formula (7) that

$$AW = 40\, e^{0,0153t}, \text{ thus}$$

$$TF = e^{-2.6083} \int_{t_0} \{40\, e^{0,0153t}\}^{0.7935} dt$$

$$TF = 113 * (e^{0,01214t} - 1) \qquad (16)$$

3.4 Mass balance

3.4.1 Nitrogen budget

Approach of calculation (Figure 11)**:**

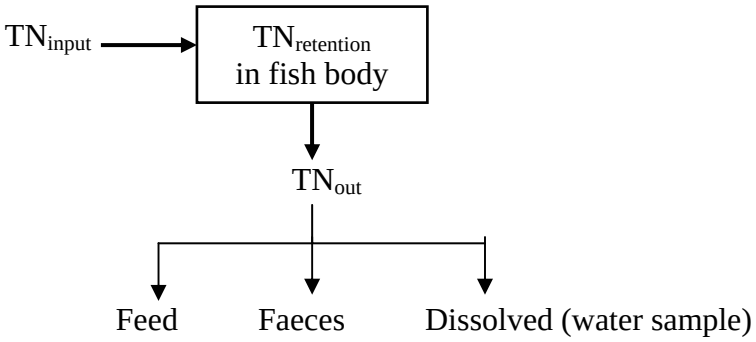

Figure 11: Approach of the calculation for TN budget.

***TN_{input}*:**

$$TN_{input} = TF * TN_{feed} \quad (17)$$

Where TN_{input} is the total nitrogen input during the experiment, TN_{feed} is the TN-content in feed (gram per gram).
It is shown by formula 16:

$$TF = 113*(e^{0,01214t}-1) \quad (18)$$

According to section 3.1.3, it should take 118 days for the fish grow from 40g to 225g.

t = 118 days.

$TF = 118*(e^{0,01214*113}-1)$

$TF = 360g\ fish^{-1}$

$TN_{feed} = 73.5(mg\ N/g) = 0.0735(g\ N\ g^{-1})$

$TN_{input} = 360\ (g\ fish^{-1})*0.0735\ (g\ N\ fish^{-1}) = 26.5(g\ N\ fish^{-1})$

***$TN_{retention}$*:**

$$TN_{retention} = \int_{w0}^{Wt} C_{TN}\, dW \quad (19)$$

For this experiment, as analysed as above, the C_{TN} can be take as constant.

$$TN_{retention} = C_{TN} \int_{w0}^{Wt} dW = C_{TN} (W_t - W_0)$$

$$TN_{retention} = C_{TN}\ (W_t - W_0) = 28.3(mg\ g^{-1})*185 = 5.24(g\ fish^{-1})$$

TN_{out}

$$TN_{out} = TN_{input} - TN_{retention} \quad (20)$$

$$TN_{out} = 26.5 - 5.2 = 21.3\ (g)$$

This result shows that 19.6 % of TN was converted into fish, the rest was discharged into water during growth of one fish from 40g to 225g in this experiment.

***Comparison of TN_{in} and TN_{out} for different FR*:**

According to formula (4)

$$FF = FR/AWG$$

$$FF = TF/\ TWG \quad (21)$$

$$\Rightarrow FF_{exp} = 360\ (g)/185(g) = 1.96$$

which is the the average FF of the FFs in table 4 as expected.

TN_{input} (FF=1)=(TF/ FF_{exp})* TN_{feed} = 360/1.96*0.0735 (g N g^{-1}) = 13.5 g N

$(TN_{input})_{dec}$ % = (26.5 - 13.5)/26.5 *100 = 49%

TN_{output} = 13.5 - 5.2 = 8.3 g

$(TN_{output})_{dec}$ % = (13.5 - 5.2)/13.5 *100 = 76%

The results are shown in Table 6 and Figure 12.

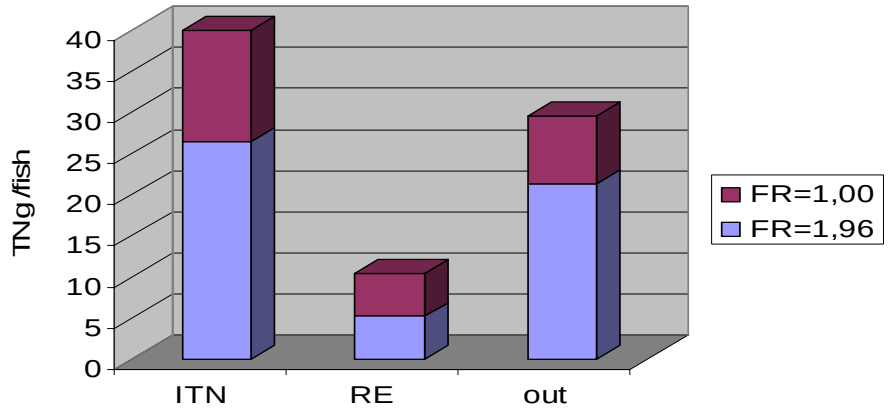

Figure 12: Nitrogen balance budget.

Table 6: TN_{in} and TN_{out} of different FR.

TN g	FR = 1.96	FR = 1.00	% TN decreased
TN_{input}	26.5	13.5	49
TN_{output}	13.5	8.3	76

This means TN_{output} depends on FR. If FR can be reduced from 1.96 to 1, TN_{output} would decrease by 76%.

3.4.2 Phosphorus budget

TP_{input}:

$$TP_{input} = TF * TP_{feed} \quad (22)$$

Where TP_{input} is the total nitrogen of input during the experiment, TP_{feed} is the TN in feed of per gram.
It is known from formula 16:

$$TF = 113*(e^{0,01214t}-1) \quad (23)$$

According to section 3.1.3, it should take 118 days for the fish grow from 40g to 225g.

t = 118 days.

$TF = 113*(e^{0,01214t}-1)$

$TF = 360g\ fish^{-1}$

$TP_{feed} = 12.37\ (mg\ P/g) = 0.0124(g\ P\ g^{-1})$

$TP_{input} = 360\ (g\ fish^{-1})*0.0124\ (g\ P\ g^{-1}) = 4.46(g\ P\ fish^{-1})$

$TP_{retention}$:

$$TP_{retantion} = \int_{W0}^{Wt} C_{TP}\, dW \quad (25)$$

For this experiment, C_{TN} can be take as constant, thus

$$TP_{retention} = C_{TP} \int_{W0}^{Wt} dW = C_{TP}(W_t - W_0)$$

$$TP_{retention} = C_{TP}\ (W_t - W_0) = 4.57(mg\ g^{-1})*185 = 0.85(g\ fish^{-1})$$

TP_{out}:

$$TP_{output} = TP_{input} - TP_{retention}$$

$$TP_{output} = 4.46 - 0.85. = 3.61g\ P$$

***Comparison of TP_{input} and TP_{output} for different FR*:**

The approach of calculation is same with that of TN. The results are shown in Table 7 and Figure 13.

Table 7: TP_{input} and TP_{output} of different FR.

TP g	FR = 1.96	FR = 1.00	% TP decreased
TP_{input}	4.46	2.28	49
TP_{output}	3.61	1.43	60

This means TP of output depends on the FR. If FR could be decreased from 1.96 to 1, TP_{output} could be decreased 60%.

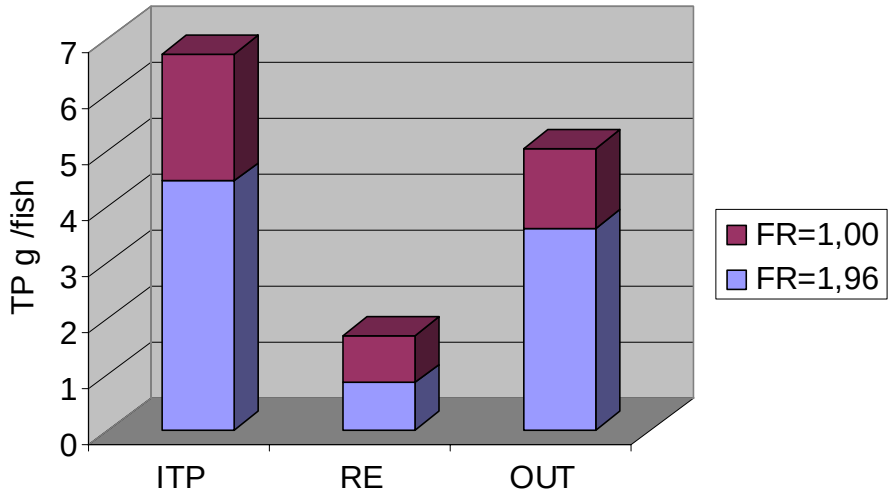

Figure 13: Phosphorus balance budget.

3.4.3 Energy budget

Approach of calculation:

$$E_{out} = E_{in} - (ME + RE) \quad (26)$$

ME Energy for respiration
RE Energy retention

***Consumption of oxygen when the fish grows from 40 to 225g*:**

The relation between the amount of oxygen consumption and fish size is expressed by formulae (13) and (14):

$$Oc\ (mg\ fish^{-1} day^{-1}) = a * (AW)^{b}$$

$$Oc\ (mg\ O_2\ fish^{-1}day^{-1}) = 4.7741 * (AW)^{0.9907}$$

This formula can be expressed as follows:

$$dOc/dt = a * (AW)^{b} \qquad (27)$$

This is the relation between oxygen consumption and growth of fish. The total amount of oxygen consumed during the fish growth from 40 to 225g can be expressed as follows:

$$Oc\ (mg\ fish^{-1}day^{-1}) = a \int_{t1}^{t2} (AW)^b\ dt = a \int_{0}^{118} (AW)^b\ dt$$

From formula (6):

$$AW = 40\ e^{0,0153t}$$

$$Oc\ (mg\ fish^{-1}day^{-1}) = 4,7741 \int_{0}^{118} (40\ e^{0,0153t})^{0,9907}\ dt$$

$$=184,5 \int_{0}^{118} e^{0,01515t}\ dt$$

$$Oc = 295.14\ (mg\ fish^{-1})$$

***Energy required when each fish grew from 40 to 225g for respiration (ME)*:**

The coefficient used to convert the oxygen consumption to energy is as follows (From and Rasmussen 1984):

1 mg (oxygen) = 3.42 cal

$$ME = 295.14 * 3.42 = 1009\ cal$$

This amount of energy was used for respiration of fish.

***Energy required when each fish grew from 40 to 225g for retention (RE)*:**

According to formula (8) for energy content of fish: $COD\ (mg\ O_2/g) = 469 * (AW)^{0.0472}$

thus d(RE)=3,42*COD * d(AW) and

$$RE = 3.42* \int_{w0}^{wt} 469 * (AW)^{0.0472}\ d(AW) \qquad (29)$$

With $W_t = 225$ and $W_0 = 40$

$$RE = 3.42*469*1/(0.0472+1)\ [\ (225)^{0.0472+1} - (40)^{0.0472+1}\ ]$$

$$RE = 372095cal \cong 372\ kcal$$

***Energy content in feed(E_{in})*:**

$$E_{in\ (cal)} = 3.42\ (cal\ mg^{-1}) * COD_{feed}\ (mg\ g^{-1}) * 360\ g \quad (31)$$

since the total amount of feed used in this experiment for one fish growing from 40 to 225g was 360g. Therefore,

$$E_{in} = 3.42*1320*360 = 1625184\ cal \cong 1625\ kcal$$

Energy amount discharged during fish growth from 40 to 225g (E_{out}):

$$E_{out} = E_{in} - (ME + RE)$$

$$E_{out} = 1625184 - (372095 + 1009) = 1252080\ cal \cong 1252\ kcal$$

Comparison of E_{in} and E_{out} for different FR (Table 8 and Figure 14)**:**

Table 8: E_{in} and E_{out} of different FR.

Energy(cal)	FR = 1.96	FR = 1.00	% energy decreased
E_{in}	1625184	829176	49
E_{out}	1251080	456072	64

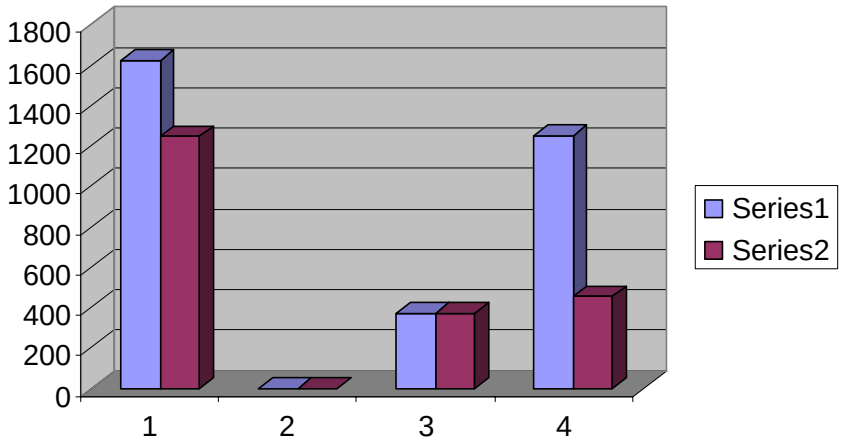

Figure 14: Energy balance budget.

3.4.4 Physical forms of the waste

TN and TP level in water

According to the results of chemical analysis the following averages are given (Table 9).

Table 9: TN and TP level in water.

Date	TN(μg L^{-1})	TP(μg L^{-1})
21/12 (01)	1019 ± 152	198±40
03/01(02)	1076 ± 236	177±49
13/01(02)	1185 ± 134	181±20
General average (GAN)	1083 ± 187	185±38
Inflow water	264	20

It is clear that there is not a significant difference of TN and TP content in water between the tanks. The reason is the high feeding ration. Much left over feed dissolved in water and reached saturation in the water. The effect of TN and TP related to growth rate was hidden by the high feeding ration and this resulted in the little change of TN and TP between the tanks.

Contribution of dissolved TN and TP to total waste

As mentioned above, there is no possibility for estimating the contribution of dissolved TN and TP related to growth rate to total waste through this study. What may be done to illustrate the amounts of dissolved nutrients in this experiment is to relate the TN and TP levels in water to the amounts of these nutrients in the feeding ration on the dates when water samples were taken. The average value of feeding ration for each tank corresponding to water sampling dates is calculated from the data in appendix 2 (Table 10).
Contribution of dissolved TN and TP to total waste is calculated as follows:
If the ratio is η:

$$\eta\ (\%) = TN_w / TN_{input} \qquad (32)$$

$$TN_w = C_{w(TN)} * Q * 24 * 10^{-6}\ (g\ d^{-1})$$

where Q is water flow (l hr^{-6}), $C_{w(TN)}$ is the concentration of dissolved TN in water (μg L^{-1}),

Table 10: Average feeding ration (**g d^{-1}**) on the sampling dates for water.

Tank	Feeding ration (21/12/2001)	Feeding ration (03/01/2002)	Feeding ration (13/01/2002)
2	390±54	350±66	445±84
3	390±54	349±66	443±84
4	410±54	400±66	498±84
5	409±54	403±66	501±84
6	428±54	361±66	442±84
7	436±54	362±66	448±84
8	299±54	236±66	288±84
9	300±54	237±66	290±84
General average	383±54	337±66	419±84
Grand average	380		

$C_{w(TN)}$ = TN(outflowing water) – TN(inflowing water)=1083- 264 = 819 (μg L^{-1}), $TN_{input} = C_{f\ eed(TN)}$* 380 (g d^{-1})

$$\eta\ (\%) = 819*600*24*10^{-6} /(0.0735*380)$$

$$\eta\ (\%) = 42\%$$

This result means that the discharge of dissolved TN is about half of the TN of the feed used.
Using the same approach the calculation for TP is given as follows:

$$\eta\ (\%) = TP_w / TP_{input} \qquad (33)$$

$$TP_w = C_{w(TP)}*Q*24*10^{-6}\ (g\ d^{-1})$$

where Q is water flow (L hr^{-1}) and $C_{w(TP)}$ is the concentration of dissolved TP in water,

$C_{w(TP)}$ = 185- 60 = 165 (μg L^{-1}) and $TP_{input} = C_{f\ eed(TP)}$* 380 (g d^{-1}) and thus

$$\eta\ (\%) = 165*600*24*10^{-6} /0.0124*380$$

$$\eta\ (\%) = 50\ \%$$

This result means that contribution of dissolved TP to total waste is about half of all the phosphorus in the feed used at the sampling date.

Comparison of different FR (Table 11)

The calculation result for different feeding ration is given in table11.

Table 11: Comparison of different FR.

FR (g d^{-1})	TN_w / TN_{input}(%)	TP_w / TP_{input}(%)
FR=1.96	42	50
FR=1.00	21	26
% decreased	50	48

The results show that the decreased feeding rate will result in a significant decrease of contribution of dissolved TN and TP to the total waste. The ratio of dissolved waste and solid waste is important when the environmental effect of waste charge from aquaculture system should be estimated. The significant difference of the environmental effect between different forms of waste has been known clearly. For this experiment the weight of leftover feed was lost by mistake and so it is impossible to estimate the ratio of different forms of the waste accurately.

4 CONCLUSION

1. Growth rate is dependent on the fish size.
2. Oxygen consumption increases with the weight of fish.
3. Energy content of fish increases slightly with fish size.
4. Total nitrogen and total phosphorus content of fish is near to constant for different fish size
5. The decrease of feeding ration will result in a significant decrease of waste discharge from the experimental aquaculture system.

ACKNOWLEDGEMENTS

The author would like to thank the supervisor of this project, Mr. Gudjon Atli Audunsson, he has given a scientific and practical direction for the project; the director of UNU - FTP, Mr. Tumi Tomasson; the vice director, Mr. Thor Asgeirsson; Ms. Þuriður Petursdottir, she has provided convenient condition for the field tests; the staff of chemical laboratory; and all of person who have helped a lot for the completion of the project. And at last, I should thank, in particular, my husband, Mr. Fuli Chen, he has provided much valuable information.

REFERENCES

Anon 1970.. Council Directive 79/923/EEC of 30 October 1979. *Official Journal of the European Communities* L281, 47.

APHA 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed.

Austreng, E., Storebakken, T. and Åsgård, T. 1987. Growth Rate Estimates for Cultured Atlantic Salmon and Rainbow Trout. *Aquaculture*, 60:157-169.

China. Marine Ecological Environment Protection.
6. Nov. 2001. < http://www.coi.gov.cn/emanager/hyg123.htm>

Edwards, D.J. 1978. *Salmon and Trout Farming in Norway.* Great Britain: Page Bros. Ltd

Emerson, C. 1999. Aquaculture Impacts on the Environment. [28/10/01] <http.//www.csa.com/hottopics/aquaculture/oview.html>

FAO 2000. *The State of World Fisheries and Aquaculture.* Rome: Food and Agriculture Organization of the United Nations .

FAO 2001. *World fisheries and aquaculture atlas.* CD-ROM. Rome: Food and Agriculture Organization of the United Nations (FAO).

From, J. and Rasmussen, J. 1984. *A growth model, gastric evacuation, and body composition in rainbow trout, Salmo gairdneri Richardson, 1836,.*

Goldburg, R. 1997. Environmental Effects of Aquaculture in the United States. The Environmental Defense Fund.

Hach, C.C., Bowden, B.K., Kopelove, A.B. and Brayton, S.V. 1987. More Powerful Peroxide Kjeldahl Digestion Method. *J. Assoc. of Anal. Chem.* 70:783-787.

Hall, P.O.J., Anderson, L.G., Holby, O., Kollberg, S. and Samuelsson, M.O. 1990. Chemical fluxes and mass balances in amarine fish cage farm. *Marine Ecol. Prog. Ser.*, 61:61 -73.

Hansen, H.P. 1999. Determination of oxygen, In Methods of Seawater Analysis, K. Grasshoff, K. Kremling and M. Ehrhrdt eds. 3rd ed. Weinheim: Wiley-VCH.

NOAA Central Library U.S. Department of Commerce. China Fishery Statistics. [23/10/01]< http://www.lib.noaa.gov/china/statistics.htm>

Páez-Osuna F., Guerrero-Galván, S. and Ruiz-Fernández, A.C. 1998. The Environmental Impact of Shrimp Aquaculture and the Coastal Pollution in Mexico. *Marine Pollution Bulletin*, 36(1):65-75.

Páez-Osuna, F., Guerrero-Galván, S. and Ruiz-Fernández, A.C. 1999. Discharge of Nutrients from Shrimp Farming to Coastal Waters of the Gulf of California, *Marine Pollution Bulletin,* 38(7):585-592.

Paez-Osuna, F. 2001. The environmental impact of shrimp aquaculture: a global perspective, *Environmental Pollution* 112: 229 - 231.

Pagand, P., Plancheton, J.P. and Casellas, C. 2000. A model for predicting the quantities of dissolved inorganic nitrogen released in effluents from a sea bass (*Dicentrarchus Labrax*) recalculating water system, *Aquaculture Engineering* 22:137-153.

Tovar, A., Moreno, C., Manuel-Vez, M.P. and Garcia-Vargas, M. 2000. Environmental Impacts Of Intensive Aquculture In Marine Waters, *Water Resources* 34: 334- 342.

Troell, M., and J. Norberg (1998). Modelling output and retention of suspended solids in an integrated salmon- mussel culture. *Ecological Modelling* 110: 65-77

Trott L. A. and Alongi D. M. 2000. Impact of Shrimp Pond Effluent on Water Quality and Phytoplankton Biomass in a Tropical Mangrove Estuary. *Marine Pollution Bulletin*, 40(11):947-951.

Tucker, C.S., Kingsbury, S.K., Pote, J.W. and Wax, C.L. 1996. Effects of water management practices on discharge of nutrients and organic matter from channel catfish (*Ictalurus punctatus*) ponds, *Aquaculture* 147(1-2):57-69.

Young C.C. and Bureau, D.P. 1998. Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture, *Aquatic Living Resources* 11(4):199-210.

Valderrama, J.C. 1981. The Simulaneous Analysis of Total Nitrogen and Total Phosphorus in Natural Waters. *Mar. Chem.* 10:109-122.