The United Nations University
FISHERIES TRAINING PROGRAMME

unuftp.is Final Project 2014

THE EFFECTS OF TEMPERATURE AND OXYGEN LEVELS ON BIOFILTER ACTIVITY

Iuliia Lysakovskaa
Institute of Fisheries of National Academy of Agrarian Science of Ukraine
135 Obukhivska str, Kyiv 03164, Ukraine
Lisakovskaa1990@gmail.com

Supervisor:
Professor Helgi Thorarensen
Holar University College
551 Saudarkrokur, Iceland
helgi@holar.is

ABSTRACT

In Ukraine, recirculating aquaculture systems (RAS) may be useful for the cultivation of high value species such as the Black Sea sturgeon (*Acipenseridae*), rainbow trout (*Oncorhynchus mykiss*) or common carp (*Cyprinus carpio*) fingerlings. Biofilters are an integral part of RAS. The primary objective of this study was to evaluate the effects of temperature and oxygen levels on biofilter activity. Furthermore, it was examined if simple test kits could be used for accurate measurements of TAN, NO_2 and NO_3. The results indicated that, when the measured in a spectrophotometer, the kits provided reliable and accurate measurements of nitrogen compounds in freshwater and sea water. A second objective of the study was to evaluate the effects of different temperatures and oxygen levels on biofilter activity. A biofilm of nitrifying bacteria was allowed to settle in the biofilters for 4 weeks at 19 ±1˚C, salinity of 12‰ and continuous aeration. Three treatments of biofilters were tested: (1) Reduced temperature, where the temperature was reduced to 8.3 ±0.3˚C with continuous aeration; (2) Reduced oxygenation, where the aeration of biofilters was suspended and (3) control group that was maintained at the same conditions as during the conditioning period. The results of this study showed that low temperature reduces the nitrification rate. Nitrification activity was also reduced in the low oxygen biofilters, but the response was probably rather due to insufficient mixing of the water when the aeration was suspended than lack of oxygen.

Keywords: Biofilter, nitrification, oxygen, temperature, recirculating aquaculture systems

This paper should be cited as:
Lysakovska, I. 2015. *The effect of temperature and oxygen levels on biofilter activity.* United Nations University Fisheries Training Programme, Iceland [final project].
http://www.unuftp.is/static/fellows/document/lysakovska14prf.pdf

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

The aquaculture sector in Ukraine expanded from 1970 to 1980 and during this time the cultivation of rainbow trout, sturgeon and catfish increased rapidly. Aquaculture production peaked in 1990 at 136,500 tones. With 110,000 tonnes of common carp (*Cyprinus carpio*), 13,000 tonnes of herbivorous fish (*Hypophthalmichthys molitrix, Hypophthalmichthys nobilis*), 1,700 tonnes of catfish (*Ictalurus punctatus*) and about 950 tonnes of rainbow trout (*Oncorhynchus mykiss*) (Bekh, 2005). After the disintegration of the Soviet Union in 1991 there was a significant decrease in the level of fish production. This was due to an economic crisis which Ukraine faced in the period of market transformation. After the 1991 fish farming was weak and was not financially sustainable. The cost of material and energy required for farming increased significantly. Purchasing power of domestic consumers also reduced drastically during this time. Without any governmental support and lack of opportunities to create sufficient working capital, many farms were forced to produce fish in an extensive way (Kovalenko, 2005). Over the last 15 years, quantity of cultured fish decreased more than 4 times. In 2012, aquaculture production was only approximately 23,000 tones (Bekh, 2005).

1.1 Current situation of aquaculture sector in Ukraine

Presently fish consumption in Ukraine is approximately 13.6 kg per capita and growing (Vlasenko, 2013). During the past 15 years, the increased demand for fish has primarily been met through increased imports of fish. Almost all of the domestic aquaculture production is consumed on the local market and not exported. From 2012 Ukraine aquaculture gradually started to rise. Still the main culture fish in Ukraine is common carp (*Cyprinus carpio*). Fish farming in Ukraine is mainly done in earthen ponds, which seasonal and depends very much on climatic conditions, the prices of aquaculture products are also subject to seasonal fluctuation (Bekh, 2005).

After annexation of Crimea peninsula by the Russian Federation in March 2014, Ukraine fisheries reduced because more than half of the nation's catch is in exclusive economic zone of the Crimea fleet (Dronik, 2014). Consequently there is increased demand on aquaculture to ensure food security for Ukraine.

New producers that enter the domestic and export markets need to meet plenty demands: lack of financial support from government, difficulties with the loan from the bank, high interest rate and modern demands for quality (Kovalenko, 2010). Today consumers are very concerned about food quality, environmental and social issues. Thus, to address all the consumer`s concern while remaining competitive, fish farmers should make a great effort (Polymeros *et al.*, 2005). Weak Ukrainian economic doesn't allow to significantly increase financing of aquaculture sector. Despite this, for the past few years interest of aquaculture investors is slowly increasing simultaneously with aquaculture.

Global aquaculture production has grown steadily in the last few decades. Meanwhile government and social communities are increasingly growing their concern about pollutions as a side effect of intensive aquaculture production. Ukraine is gradually moving towards membership in the European Union, which has many requirements for food quality and pollution restrictions. Factors such as limited access to water, restrictions on composition of water discharge, diseases, high cost of land and environmental impacts are driving the global aquaculture industry toward intensive practices (Gutierrez-Wing & Malone, 2006). As a result, producers will need to adopt more environmentally friendly and high intensive approach aquaculture production.

1.2 Pros and cons of recirculation aquaculture systems

Recirculating aquaculture systems (RAS) may offer solutions that will facilitate the growth of aquaculture in Ukraine. They require less water and have reduced discharge compared with conventional flow-through systems. RAS also offers a relatively high degree of environmental control. As long as it mainly runs indoor, therefore these systems reduce such risks of earthen ponds aquaculture as natural disaster, pollution, and disease. With RAS, fish farmers are able to maintain optimum conditions for different species all year round (Costa-Pierce & Desbonnet, 2005). Environmental control allows farmers to maintain higher growth rates and improve feed conversion. In addition, since the RAS require less water their location is more flexible than for conventional farms and, therefore, they can be located closer to markets (Ebeling *et al.*, 2007).

The main disadvantages of RAS are the high initial capital cost for installation. They also need a reliable electrical supply as well as automatic monitoring and controls systems for operational safety. Water quality and biofilter performance need to be controlled and monitored on a regular basis. The function of different components of the RAS must be checked regularly to avoid equipment malfunction and reducing in productivity (Courtland, 1999). This requires well educated staff and good management to successfully operate the farm.

1.3 Potential advantages of RAS in Ukraine

Because of the high investment cost and operational cost of RAS, they are primarily suitable for high value species or for specialized aquaculture such as fingerling production. In Ukraine, RAS may be for high value fish such as The Black Sea sturgeon (*Acipenseridae*) is endangered due to excessive fishing, poaching and destruction of spawning habitats (GFCM, 2012). It is a high value species that could be a suitable candidate for production in RAS. With the RAS it is possible to create a stable cultivation environment which may contribute to faster growth than with natural temperature fluctuation (Grytsyniak & Simon, 2014). In ponds the average age at maturation is 5-8 years. To mature again the fish may require farther 2 or even 3 years (Andryushchenko & Alimov, 2008). The extended production cycle in ponds calls for interest costs for building up the biomass. These would be reduced with a shorter production cycle in RAS and may weigh against the higher investment and operational costs for RAS systems. Furthermore, extended production cycles increase the risk of mortalities and the handling of fish in ponds in winter is also a challenge (Andryushchenko & Alimov, 2008).

Rainbow trout (*Oncorhynchus mykiss*) is other high value fish species in Ukraine. It is well received in the Ukrainian market, but high market price makes this fish inaccessible for average consumer. One of the reasons is the location of those farms. They tend to be only on the west part of Ukraine due to availability of significant amount of clean and cold water. In this case RAS can solve the location problem, improve food conversion ratio (FSR) and decline water input.

For the long time common carp (*Cyprinus carpio*) in Ukraine used to be a one of main culture species. But culture technologies were not renewed for more than twenty years. Common carp rise on the most of farms on two year basis, because of the seasonal aspects. The culture season is no more than six month long. In winter the carp does not eat, metabolic activity drops almost to zero and the fish even starts losing weight. Common carp is not a high value species, but RAS can be applied as a fingerlings station. High temperature maintained in this system can provide for farmers the fish eggs in early terms and obtain 40% higher growth of fingerlings (Zhigin, 2011). This approach will increase biomass of market fish.

1.4 The function of RAS systems

In RAS, the water exchange is low and, therefore, metabolites from the fish such as ammonia (NH_3) and carbon dioxide (CO_2) as well as faeces and uneaten pellets must be removed. The main components of the RAS are: fish tanks, mechanical filter, biological filter, water stabilization units (dagasation, aeration, alkalinity restore, temperature correction etc.) and disinfection (Figure 1). After the water leaves adjusted in the fish tanks the first step in cleaning the water is solids removal such as uneaten feed particles and fish faeces. This can be done in different types of filtration systems. The next step is to remove NH_3 which is poisonous to fish. The NH_3 is removed in biofilter where bacteria convert NH_3 to nitrite (NO_2) and then to nitrate (NO_3) which is not harmful to the fish. The fish and the bacteria in the biofilter consume oxygen and produce carbon dioxide. Therefore oxygen must be added to the water through the aeration of oxygenation and at the same time CO_2 must be removed from the water. The last stage is to restore alkalinity, which is consumed in biofilter, and disinfect the water. A number of other elements may be needed in the system, depending on the level of water exchange (Braynballe, 2010).

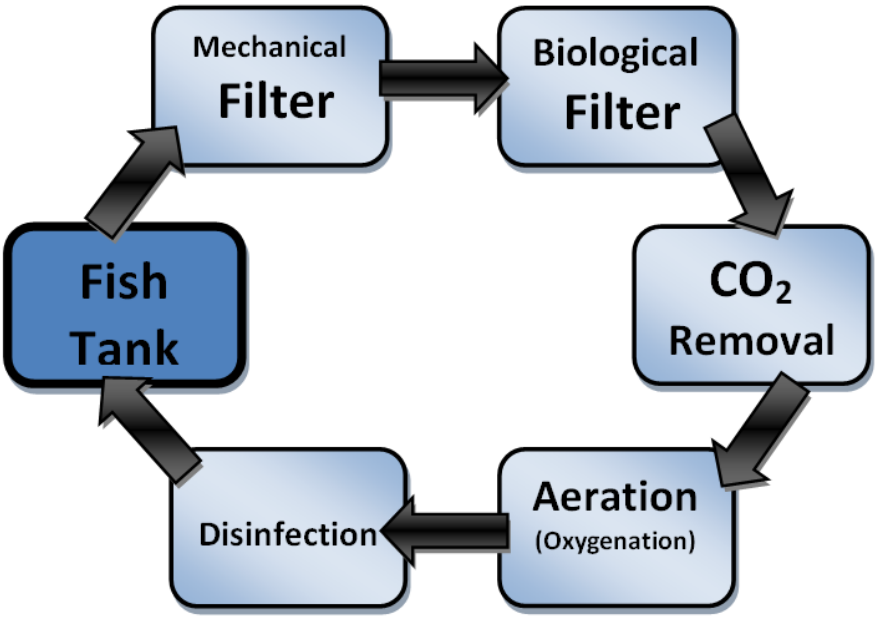

Figure 1: Schematic diagram of RAS.

1.5 The effect of ammonia on fish

Fish produce ammonia (NH_3) in the liver and excrete it into the water through the gills. Aqueous ammonia consists from two components such as NH_3 and NH_4+ (Randall & Ip, 2006). In aquaculture they usually called total ammonia nitrogen or TAN.

NH_3 is very soluble in water and diffuses easily across cell membranes. At the lower pH inside cells it is converted to NH_4^+. The rate of diffusion depends on the partial pressure of NH_3 in environment. So that it is very important to avoid accumulation of this molecule in the water. Ammonium (NH_4^+) affects fish less than ammonia (NH_3) but at high concentrations can substitute other ions transport pathways, for example Na^+ and K^+ (Evans, 1998).

Some fish habits can increase internal ammonia levels or sensitiveness. For example starved and stressed fish is more sensitive to high ammonia levels than calm and fed. Swimming fish has higher internal ammonia concentration what decrease swimming velocity (Randall & Tsui, 2002).

In spite of this some fish species has adapted to tolerate to some extent with ammonia. One way is to detoxify ammonia to glutamine which is formed from glutamate and NH_4^+. Fish also can release ammonia through the urea cycle or even volatilizes as NH_3 gas (Randall & Tsui, 2002).

The average toxicity of ammonia for freshwater fishes is 2.79 mg NH_3 L^{-1} and 1.86 mg NH_3 L^{-1} for seawater species. The tolerance of seawater fish mainly caused by environment issues either presence of salinity. Elevated levels of ammonia mainly affect central nervous system and follow with ammonia intoxication, convulsions and death (Randall & Tsui, 2002).

1.6 Biofiltration in RAS

The process of removing ammonia from the water is called nitrification, and consists of several successive stages. Ammonia in aerobic conditions oxidizes to nitrite and after to nitrate with help of autotrophic bacteria. If it is necessary to create a "zero exchange" system, denitrification stage needs to be added to the system where nitrate convert to nitrogen gas. It is an anaerobic process (Ebeling *et al.*, 2007).

A number of different designs are available for biofilters. Biofilters can be two types: suspended (heterotrophic bacteria) or fixed film (autotrophic bacteria). Fixed film biofilters divided into two groups: emergent and submerged. The main principle of first group is to ensure biofilm the large amount of atmospheric oxygen. It can be done by cascading water over the biofilter media or obtain the same effect by rotating media which is half emergent into the water. The function of the second group is to provide the highest specific surface area for biofilm to enhance the nitrification rate. This is the main limiting factor for ammonia removal. Oxygen provided by aeration of influent water.

In essence, biofilters are simple in design. Primarily, they provide a surface for the bacteria to grow. They should have high surface area to volume ratio in order to minimize the space they occupy. Various biofilter media can perform satisfy these requirements, they are simple sand, stones, polyethylene granules with plenty construction types etc. (Ebeling *et al.*, 2007).

The bacteria form a biofilm on the surface of the biofilter. The nitrifying bacteria are ubiquitous in the environment and, therefore, bacteria suspended in water will flow into the biofilter and attach to available surface area. Given favorable conditions, these bacteria will start to grow and produce the biofilm (Interdonato, 2007). The nitrification rate in the biofilter depends on abiotic factors, available surface area, nutrient concentration in water and bacterial diffusion rate into the biofilm (Ebeling *et al.*, 2007).

1.7 Bacterial communities in biofilter

The nitrification in RAS biofilter is usually performed by two groups of autotrophic bacteria (Table 1). Ammonia oxidizing bacteria that convert NH_3 to NO_2 (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus,* and *Nitrosovibrio)* which get their energy by catabolizing un-ionized ammonia to nitrite. Secondly nitrite oxidizing bacteria *Nitrobacter, Nitrococcus,*

Nitrospira, and *Nitrospina* oxidize nitrite to nitrate. Further removal of NO_3 to N_2 gas is possible through anaerobic processes (Table 1) is also possible but not employed in commercial RAS systems (Ebeling *et al.,* 2007).

Table 1: Main bacterial reactions associated with a biological filter (Ebeling *et al.,* 2007).

Process	**Reaction**	**Microorganism**	
		Freshrwater	**Marine**
Nitrification Ammonium oxidation	$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$	*Nitrosomonas oligotropha*	*Nitrosomonas* sp. *Nitrosomonas cryotolerans* *Ntrosomonas europaea* *Nitrosomonas cinnybus/nitrosa* *Nitrosococcus mobilis*
Nitrite oxidation	$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$	*Nitrospira* spp. *Nitrospira marina* *Nitrospira moscoviensis*	*Nitrospira marina* *Nitrospira moscoviensis*
Denitrification Autotrophic (sulfide-dependent)	$S2^- + 1.6NO3- + 1.6H+ \rightarrow SO_4^{2-} + 0.8N_2(g) + 0.8H_2O$		*Thiomicrosporia denitrificans* *Thiothrix disciformis* *Rhodobacter litoralis* *Hydrogenophaga* sp.
Heterotrophic	$5CH_3COO^- + 8NO_3- + 3H^+ \rightarrow 10HCO_3^- + 4H_2O$	*Pseudomonas* sp. *Comamonas* sp.	*Pseudomonas fluorescens* *Pseudomonas stutzeri* *Pseudomonas* sp. *Paracoccus denitrificans*

In recirculation water also partly present heterotrophic bacteria which take part in nitrogen removing. Nitrogen wastes bounds into a bacterial biomass. In preferable conditions heterotrophic bacteria grow significantly faster than autotrophic. With high organic load and low oxygen concentrations geterotrophs will prevail AOB and will compete with them for the space in biofilter. Therefore, it is very important in pure autotrophic system to keep it as clean as it possible by using mechanical filtration.

Although water exchange is low in RAS it plays an important role in removing NO_3 and other metabolites as well as suspended solids that build up in the system (Ebeling *et al.*, 2006). Furthermore, it can replace some of the alkalinity that is consumed in the nitrification process. Finally, refreshment of water can reduce or remove off-flavors of fish flesh (Seginer *et al.*, 2008).

1.8 Requirements of autotrophic bacteria

There are numerous physical, chemical, and biological factors that influence the rate of nitrification. These include pH, temperature, alkalinity, oxygen, and TAN concentration.

The pH affects both the rate of nitrification and in conjunction with temperature the relationships between the ionized and unionized forms of ammonia-nitrogen. High pH and temperature increase the proportion of TAN as unionized ammonia-nitrogen in water (Ebeling *et al.,* 2007). Since the nitrifying bacteria consume NH_4+, it is better to maintain pH fairly low (Timmons & Losordo, 1994). The optimum pH for autotrophic bacteria in biofilters is from 7.2 - 7.8 for *Nitrosomonas* and 7.2 – 8.2 for *Nitrobacter*. Rapid changes in pH of more than 0.5 to 1.0 units are undesirable even for a short time. It can stress bacteria and inhibit a nitrification rate (Ebeling *et al.,* 2007).

The nitrifying bacteria operate well over a wide range of temperatures (7 to 35˚C). Therefore, the operating temperature of the biofilter can be the same as that required for the culture fish. However, the nitrification rate follows an Arrhenius relationship, increasing with higher temperatures (Ebeling *et al.,* 2007). Therefore, larger biofilters with more available surface area must be designed for systems operating at low temperatures.

To convert ammonia-nitrogen to nitrate-nitrogen bacteria consume alkalinity (Table 2). This may require that alkalinity is restored if water exchange is low. Also the reduced alkalinity will result in lower pH that can be corrected with the addition of caustic soda (Ebeling *et al.,* 2007). Alkalinity should be about 150 mg/l initially to encourage bacterial growth (DeLong & Losordo, 2012).

To oxidize ammonia to nitrate oxygen is required. Therefore, oxygen must be provided as it can become the nitrification rate-limiting factor (Ebeling *et al.,* 2007). Low oxygen level decrease nitrification rate (Jianhua *et al.,* 2009). Oxygen concentration bellow 2 mg^{-1} L^{-1} decrease nitrification rate and give advantage to suspended growth of heterotrophic bacteria (Knowles *et al.,* 1964). So that is better to keep dissolved oxygen (DO) at effluent not lower than 2 mg^{-1} L^{-1}.

TAN concentration directly determine nitrification rate (Figure 2). Relationships between Nitrification rate and TAN are linear until some extent. Marginal nitrification observes at TAN level 2 - 3 mg^{-1} L^{-1}. There is some evidence that high levels of unionized ammonia-nitrogen (NH_4^+) may inhibit both, autotrophic and heterotrophic nitrification (Anthonisen & Loehr, 1976). Inhibit proportion of NH_4^+ is lower for heterotrophic bacteria. As previously was mentioned, this proportion depends on temperature and pH. Concentrations identified for *Nitrosomonas* bacteria are 10-150 mg/l and 0.1 to 1.0 mg/l for *Nitrobacter* bacteria. Nitrous acid also has inhibitory effect on biofilter bacteria concentrations of 0.22 to 218 mg/l (Anthonisen & Loehr, 1976).

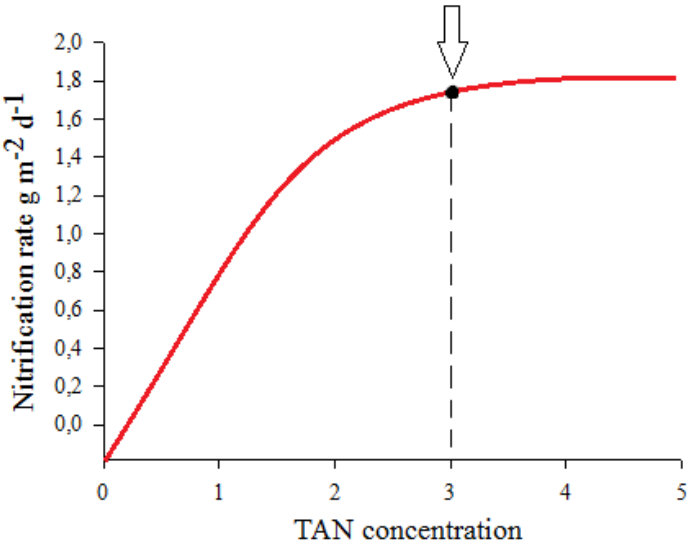

Figure 2: relationships between TAN concentration and rate of nitrification.

Table 2: Stoichiometry for autotrophic bacteria metabolism of 1.0 g NH_4^+–N (Ebeling *et al.*, 2006).

Consumables	Stoichiometry	Consumes (g)
NH_4^+–N		1.0
Alkalinity	7.05 g Alk/g N	7.05
Oxygen	4.18 g O_2/g N	4.18
Products	**Stoichiometry**	**Yields (g)**
VSS_A	0.20 g VSS_A/g N	0.20
NO_3^-–N	0.976 g NO_3^-–N/g N	0.976
CO_2	5.85 g CO_2/g N	5.85

1.9 Project objectives

The main issue of this project was to examine the development of activity in biofilters and test the effect of different temperatures and oxygen levels on the nitrification rate. Through the project, the fellow will gain experience in measuring nitrogen compounds. As a result expected to gain knowledge in starting up a biofilter, to determine what is important for the bacteria and consequently for the biofilter to function well. During the start period of biofilter will be monitored water quality removal rate of ammonia, appearance of nitrite and nitrate. Also will be studied the influence of different temperature and oxygen on nitrification rate.

The main objectives were:

- To study the management and controlling of the seeding of nitrifying bacteria cells in a biological filter.
- To define nitrification rates.
- To evaluate the effect of temperature and oxygen levels on biofilter activity.

This project mainly will monitor levels of some parameters of indicators of water quality as dissolved oxygen (DO), pH, total ammonia nitrogen (TAN), nitrite nitrogen (NO_2) and nitrate nitrogen (NO_3).

2 MATERIALS AND METHODS

2.1 Experimental design

The experiment was conducted at Verid laboratory (Aquaculture Research Facilities of Holar University College), in Saudarkrokur, Iceland. . Before the experiment started, the biofilters were given four weeks to develop bacterial cultures and commence the nitrification activity. During this period, the temperature in the biofilters was maintained at 20 °C and every day the TAN concentration was adjusted to 4 $mg \cdot L^{-1}$.All the biofilters were provided with continuous aeration during this period. After this initial period, the experiment was started. In total, 12 biofilters were subjected to three different treatments (Table 3). One group was used as a control and maintained under the same conditions as during the initial development period. The second group was exposed to lower temperature (8.3 ±0.3˚C) with continuous aerationand the third group was exposed to reduced oxygen levels by removing the aeration but kept at the same temperature as the control group.

Table 3: Schema of experiment distribution.

Day		1	2	3	4	7	8
Control	Temperature, ˚C	19.1	19.4	19.8	19.2	18.0	17.0
	Oxygensatur., %	100	100	100	100	100	100
	Salinity, ‰	12	12	12	12	12	12
	pH	7.8	-	7.7	7.7	-	7.8
Low temperature	Temperature, ˚C	19.2	9.2	9.0	9.1	8.2	8.1
	Oxygensatur., %	100	100	100	100	100	100
	Salinity, ‰	12	12	12	12	12	12
	pH	7.8	-	7.7	7.6	-	7.7
Low oxygen	Temperature, ˚C	19.1	20.3	20.7	19.5	18.7	17.7
	Oxygen satur., %	100.0	58.8	58.3	62.8	74.5	81.5
	Salinity, ‰	12	12	12	12	12	12
	pH	7.9	-	7.4	7.4	-	7.8

2.2 Experimental setup

The biofilters consisted of 2 L plastic buckets that were filled with brackish water (12‰) from fish tanks (Figure 3). Each bucket was packed with 35 aeration rings (Figure 4). The surface area of each ring was estimated to be 105 cm^2 or 236.25 m^{-2} m^3. Aeration was provided through air stones (HAILEA ACO-9610). A culture of nitrifying bacteria was introduced into the biofilters with a liquid medium for aquaria (Sera bio nitrivec) 10 ml^{-1}in 25 L^{-1} of water. During the period when the nitrification activity was developing in the biofilters, ammonium chloride was added daily to maintain TAN concentration close to 4 mg L^{-1}. Ammonium stock solution was prepared by dissolving ammonium chlorine (NH_4Cl) 2.965g in one liter of water to get a concentration 1 g L^{-1}.

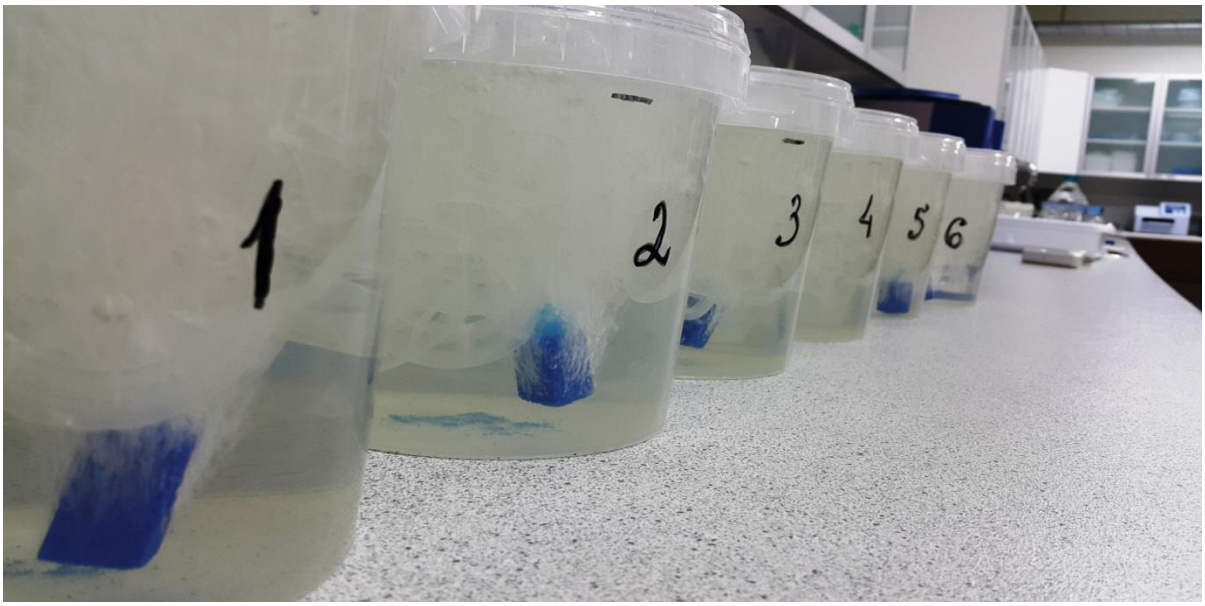

Figure 3: Biofilters.

Conditions in the biofilters while they were maturing were maintained as follows:

- pH – 7.5
- oxygen saturation – 95-98%
- temperature – 20˚C
- salinity – 12‰

To measure the conditions in the biofilter the following apparatus were used:

DO – OxyGuard Handy Polaris portable Dissolved Oxygen meter
pH – OxyGuard Handy PH
Salinity – ATAGO "Pocket" Salinity Refractometer PAL-06S
Temperature – Extech 39240 Digital Waterproof Pocket Thermometer
Spectrophotometer – Molecular Devices EMax Precision Microplate Reader.


Figure 4: Biofilter media. The plastic ring with SSA 236.25 m^{-2} m^3.

2.3 Measurements of TAN, NO_2 and NO_3

Five standards were prepared for the TAN measurements with ammonia concentration of 1,2,3,4 and 5 mg L^{-1}. The standards were made by diluting NH_4^+ 50 ppm stock solution in water. Standards for NO_2 were prepared with the same concentration. A $NaNO_2$ stock solution was prepared by diluting 74.96 mg L^{-1}. For measuring NO_3 standards were prepared from a KNO_3

stock solution by diluting 81.54 mg L^{-1}. The concentration in standards was 1,5,10,15 and 20 mg L^{-1} of NO_3.

The standards were prepared each time the nitrogen compounds were measured. A linear model was fitted to the relationship between absorption and concentration (Figure 5) and used to calculate the TAN, NO_2 and NO_3 concentration in biofilter.

For measuring TAN were prepared 5 standards with ammonia concentration 1,2,3,4 and 5 mg L^{-1}. The standards were based on dissolving NH_4^+ 50 ppm stock solution in water. Similar concentrations were done for measuring nitrite. For those standards was used $NaNO_2$ stock solution. For measuring nitrate were prepared standards with nitrate concentration 1,5,10,15 and 20 mg L^{-1}, based on KNO_3 Stock solution.

Daily was measured the absorption of standards for each parameter on spectrophotometer. Absorption was fitted to a linear model (Figure 5) and used to calculate the TAN, NO_2 and NO_3 concentration in biofilter.

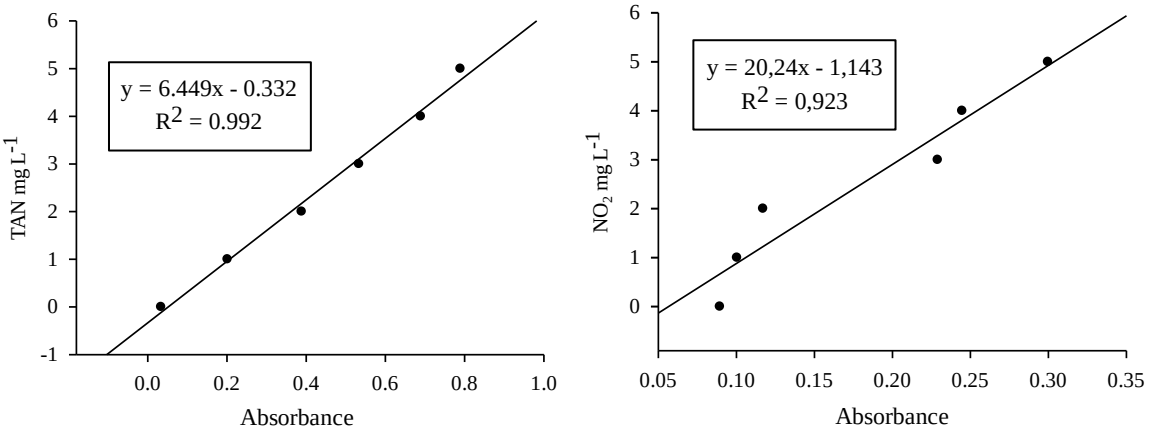

Figure 5: Examples of standard curves for TAN and NO_2.

2.4 Evaluation of the nitrification rate

During the whole experiment, including start-up period, was monitored: TAN, NO_2, NO_3, pH, O_2, and temperature. In tanks were established reliable, accurate and repeatable methods for measuring. Water samples was collected in the same proportion from each tank and analyzed promptly 5 days a week at the same time following the same procedures, using the same measuring equipment.

The TAN concentration was analyzed in 1 ml samples (Figure 6) with JBL and Sera Ammonium Test kits. Before the samples were taken from the tanks, the water was mixed and water added to restore losses from evaporation. The instructions from the JBL manufacturer called for 10 ml samples to which were added 4 drops of reagent 1 and 2 and then 5 drops of reagent 3. The weight of one drop of the reagents was determined and estimated to be 40µl. Using this information, the sample was scaled down to 1ml and added 16µl of reagents 1and 2 and 20µl of reagent 3. After that the sample was allowed 15 minutes to develop the color. After that, the samples were mixed well and 100µl pipetted into the wells of a plate and read at 650 nm in a spectrophotometer. For the Sera test kit 40µl of each reagent were used and 5 min allowed for development. The R^2 of the standard curves was between 0.993 and 0.997. Samples were taken twice each day: before and after TAN concentration was adjusted.

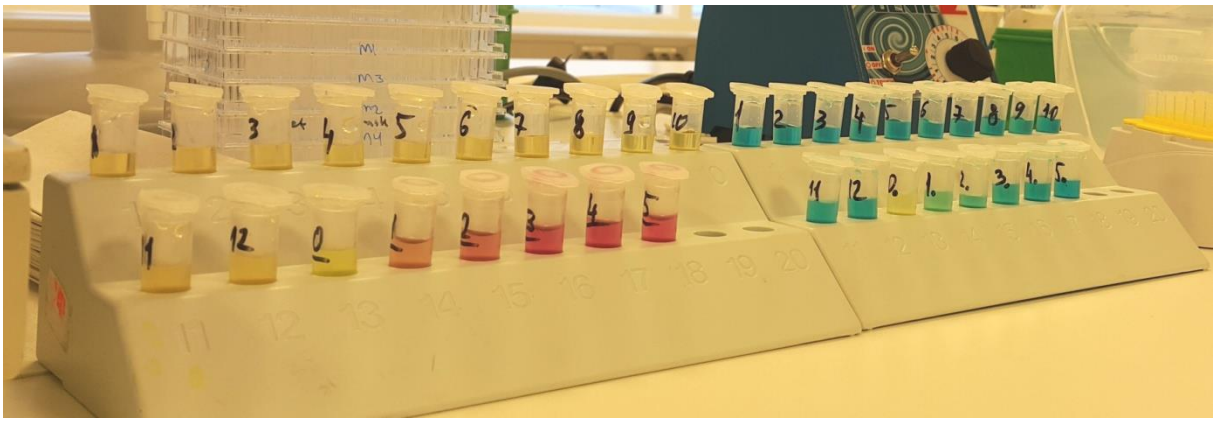

Figure 6: Samples of TAN and NO_2 with reagents prepared for reading on spectrophotometer.

The NO_2 concentration was measured with Sera and Prodac Nitrite Test kitin 1 ml samples taken at the same time as the samples for TAN. According to the directions from the Sera manufacturer the measurement should be performed on a 5 ml sample to which are added 5 drops of reagents 1 and 2 and then mixed vigorously. Similar to the TAN measurements it was determined that one drop of reagent was 45µl. Scaling the samples down to 1 ml, 45µl of reagents 1 and 2 were added to a 1 ml sample. For Prodac Nitrite Test kit30µl$ml^{-1}$of the reagents were added to each 1 ml sample. The concentration of nitrite in water was read in a spectrophotometer at *490* nanometers. The concentration was determined by fitting a line through the absorption of the standards.

The NO_3 concentration was measured with a Prodac Nitrate Test kit. The sample was taken at the same time as samples for TAN. According to the instruction from the Prodac manufacturer, the analysis was performed on a 5 ml sample with addition of reagents in drops. One drop of reagents was 35µl. The sample was scaled down to 1ml and added 28µl of reagents 1 and 2 and 1/5 of a provided measuring spoon of reagent 3. The NO_3 concentration was measured in a spectrophotometer at *490* nanometers.

2.5 Data analysis

The data was recorded in Excel but statistical analyses were performed in R statistical program. Linear models were to the standard curves. The nitrification rate and appearance of NO_2 and NO_3 were estimated by fitting a straight line to the concentration of of TAN, NO_2 and NO_3 over time during the experiment.

3 RESULTS

3.1 Standard curves

To evaluate the accuracy of standard curves what were used during the experiment they were fitted into a single linear model which explained 95% of the total variance for TAN, 73 % for nitrite and 74 % for nitrate. The same linear model, but with different intercepts explained 98% of the total variance for TAN, 75% for nitrite and 72% for nitrate. A linear model for TAN with different intercepts and slopes explained 99% of the total variance.

A test was conducted to confirm that the measuring kits, that were designed for freshwater, did work in brackish water as was used in the biofilters. The standard curve for brackish water was not significantly different from the freshwater standard curve (Figure 7). As a result, it was concluded that the test kits worked in brackish water.

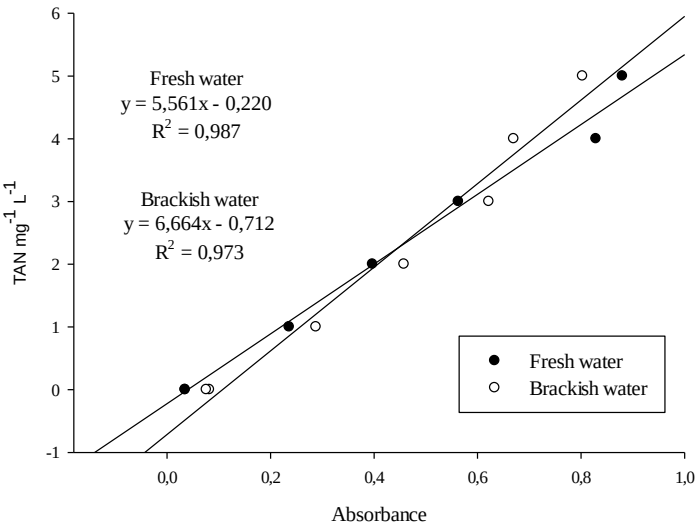

Figure 7: Comparable analysis of two TAN standard curves based on fresh and brackish water.

3.2 The effect of reduced temperature and oxygen levels on nitrification rate.

3.2.1 Dissolved oxygen (DO)

The aeration was turned off for the low oxygen group to reduce the oxygen levels. After the aeration was turned off the oxygen levels fell down up to 55–62 % saturation (Figure 8). During the next five days the oxygen saturation gradually increased up to 80%. The oxygen must have diffused from the atmosphere across the surface of the biofilter. These oxygen levels are too high to affect the biofilter activity (Chen *et al.*, 2006). In the control group the oxygen levels remained close to 100% (Figure 8).

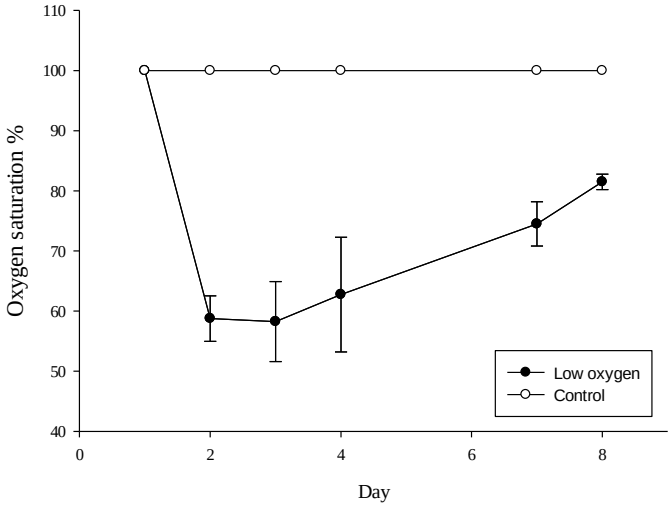

Figure 8: The oxygen levels in the control biofilters and in the biofilters where aeration was suspended (The vertical bars show standard deviation).

3.2.2 Temperature

The temperature in the biofilters that were moved to a colder room fell during the first day and remained fairly stable at 8.3 ± 0.3˚C (Figure 9). In the control group the temperature remained at 19 ± 0.5˚C.

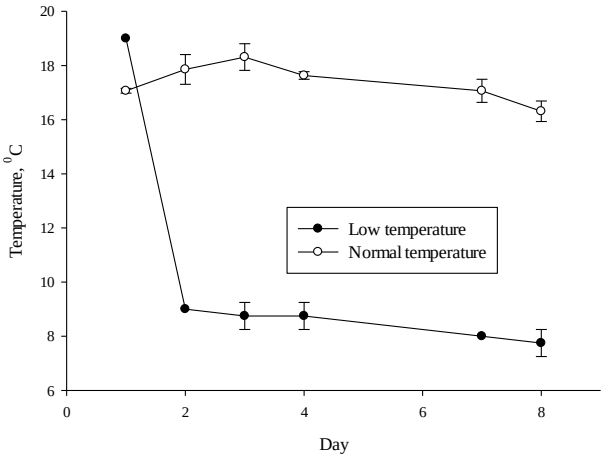

Figure 9: The temperature in the control biofilters and in the biofilters that were cooled down. The vertical bars show standard deviation.

3.2.3 pH levels

The pH didn't change significantly during the eight experiment days ranging between 7.3 and 7.7.

3.3 TAN removal

The initial TAN concentration was relatively similar in all biofilters at the beginning of the experiment (4.48 ± 0.18 mg^{-1} L^{-1}). In the control group, the TAN concentration was progressively reduced for the eight days that the experiment lasted (Figure 10). The slope of the curve was -0.16 $mg \cdot day^{-1}$. In contrast, the slopes of the curves fitted to the data for the low oxygen and low temperatures were not significantly different from zero (Table 3). The average nitrification rate per unit area of the biofilter was – 0.86 mg^{-1} m^{-2} day^{-1}, in the control biofilters (Figure 11). in The nitrification rate in the biofilters with reduced temperature and oxygen was – 0.54 mg^{-1} m^{-2} day^{-1} and 0.1 mg^{-1} m^{-2} day^{-1} respectively.

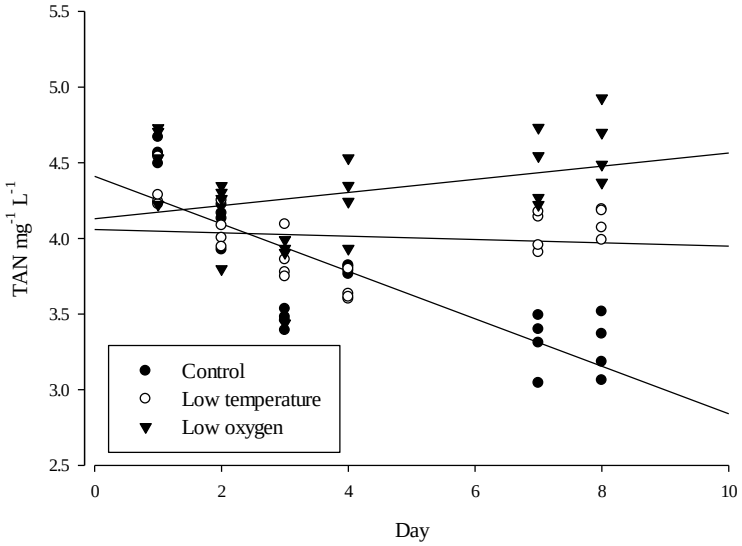

Figure 10: TAN concentration during the experiment. The lines show the linear relationship between TAN concentration and time and the parameters of the equation are shown in Table 3.

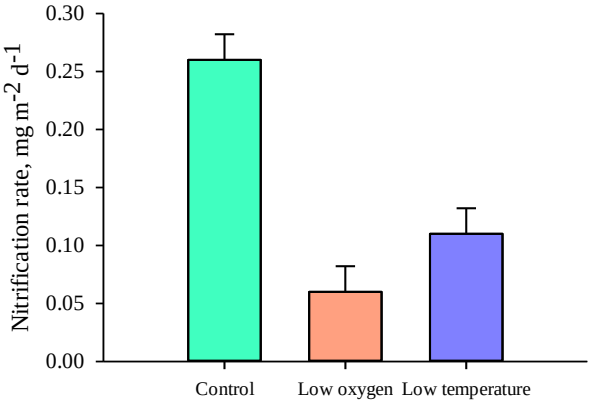

Figure 11: The average nitrification rate between the groups (The bars show standard error).

Table 4: The intercepts and slopes for the linear curves fitted to the TAN concentration over time.

	Intercept	SE	p<
Control	0	0.11879626	0
Low oxygen	0.0533	0.16800328	0.1288
Low temperature	0.6153	0.16800328	0.0658
	Slope		
Control	-0.157178	0.02205983	0
Low oxygen	0.043541	0.02205983	0.0533
Low temperature	-0.011148	0.02205983	0.6153

3.4 Nitrite (NO_2) concentration

The nitrite concentration increased significantly in the control group during the experiment (Figure 12, 13). However, neither the slopes nor the intercepts of the curves for the low oxygen and low temperature groups were significant (Table 4).

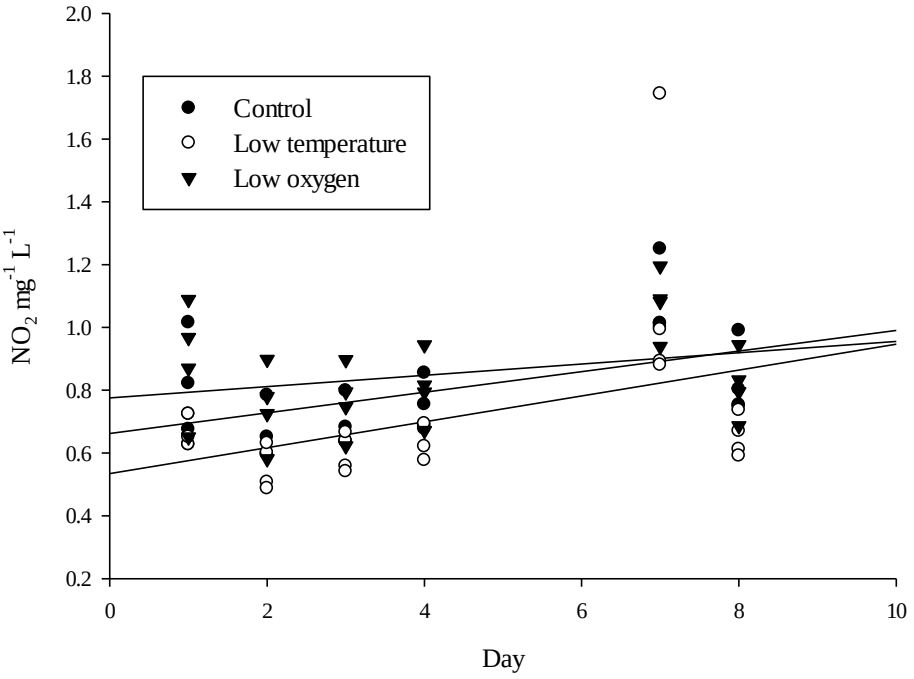

Figure 12: Nitrite concentration during the experiment. The lines show the linear relationship between nitrite concentration over time. The parameters of the equation are given in Table 4.

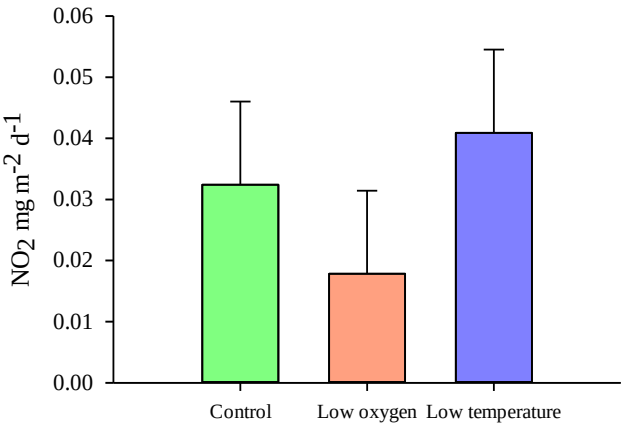

Figure 13: The average nitrite appearance between the groups (The bars show standard error).

Table 5: The intercepts and slopes for the linear curves fitted to the NO_2 concentration over time.

	Intercept	SE	p<
Control	0.663691	0.07632539	0
Low oxygen	0.7756974	0.10794041	0.3265
Low temperature	0.5357833	0.10794041	0.2664
	Slope		
Control	0.0324142	0.01360913	0.0206
Low oxygen	0.0178326	0.01360913	0.1953
Low temperature	0.040912	0.01360913	0.0039

3.5 Nitrate (NO_3) concentration

The nitrate concentration increased significantly during the experiment in the control group (Figure 14, 15). The slope of the line fitted to the concentration over time for the control group was 0.06231 and significantly higher than zero (Table 5). In contrast, the slopes and the intercepts for the curves for the low oxygen and low temperature groups were not significant.

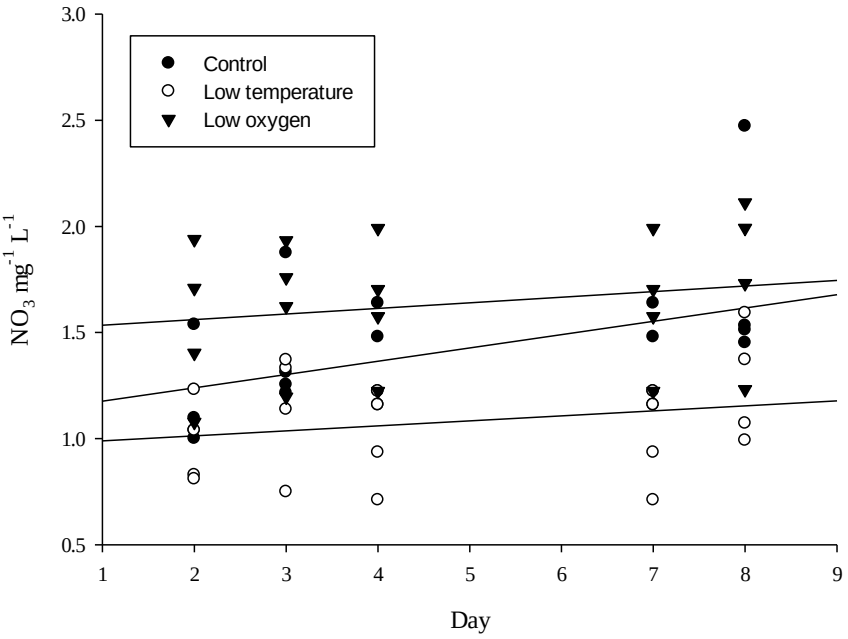

Figure 14: Nitrate concentration during the experiment. The lines show the linear relationship between nitrate concentration over time. The parameters of the equation are given in Table 5.

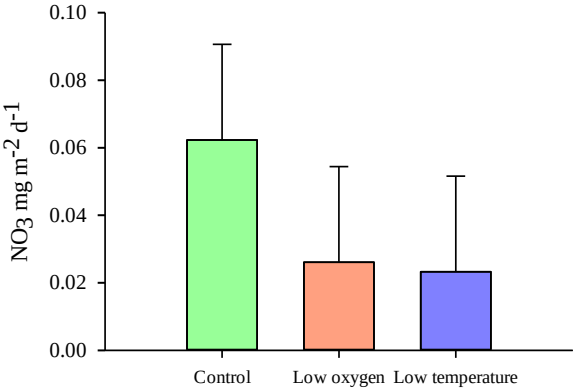

Figure 15: The average nitrate appearance between the groups (The bars show standard error).

Table 6: Analysis of nitrate concentration slopes in R statistic program

	Intercept	SE	p<
Control	1.1159	0.15105	9.68E-10
Low oxygen	1.50881	0.21362	0.0714
Low temperature	0.96724	0.21362	0.4895
	Slope		
Control	0.06231	0.02834	0.0322
Low oxygen	0.02608	0.02834	0.3616
Low temperature	0.02328	0.02834	0.415

4 DISCUSSION

4.1 Measurements

Before the start of the experiment, it was established that accurate measurements could be obtained with simple aquaria test kits. The results of the test kits could be read with more precision on a spectrophotometer than with the comparative color charts provided. The standard curves gave good fit to a straight line. The standard curves for TAN had a R^2 of 0.95 or higher (Figure 5). This suggests that TAN concentration can be read fairly good precision. The standard curves for NO_2 and NO_3 did not show quite as good fit but still with R^2 over 0.75. A curve fitted to all measurements of standard curves for TAN explained 95% of the total variation (R^2 0.95). Although there was a significant difference between days, fitting different curves for different days to the data increased R^2 only slightly for TAN and NO_2 while there was no significant difference between curves for different days for NO_3. These results suggest that the simple test kits, when adapted and read on a spectrophotometer can give reliable readings for the nitrogen compounds. The test kits were tested both in freshwater and in seawater without any significant difference. This suggest that the kits work for both types of water.

4.2 The effect of reduced temperature

The temperature in the low temperature group dropped to 8.3 degrees on the first day and remained stable after that. The nitrification rate was reduced at the lower temperature compared with the control group (Figure 11). The nitrification rate in the low temperature group was not significantly different from zero. These results are in accordance with those of Zhu & Chen (2002) how also found reduced nitrification rate at lower temperatures.

4.3 Effects of reduced oxygen.

It was attempted to reduce the oxygen levels in the biofilters the low oxygen group by switching off the aeration. The lead to a reduction of oxygen saturation down to 55%. The nitrification rate was not significantly different from zero in this group. With salinity of 12‰ and average temperature 19˚C the oxygen concentration should have been 4.7 mg O_2 L^{-1}. The recommended DO is over at 4 mg O_2 L^{-1} (Table 2). To inhibit growth of bacteria the genus *Nitrosomonas*, the DO needs to fall under 2 mg O_2 L^{-1}, while *Nitrobacter* are more sensitive and require DO levels above 4 mg O_2 L^{-1}(Chen at al. 2006). Therefore, the reduced nitrification rate may not have been due to low oxygen levels. Instead, it is suggested that the removal of aeration may have reduced the mixing of the water in the biofilter. Reduced mixing will increase the boundary layer around the filling in the biofilters and thus limit diffusion from water to the biofilm at the biofilter surface. The results of Prehn et al. (2012) show that reduced hydraulic flow biofilters reduced performance. It is unlikely that reduced nitrification rate in this group was due to in low oxygen group caused by insufficient oxygen concentration. Consequently the oxygen saturation could not affect the nitrification rate.

4.4 Biofilter performance

The average nitrification rate in the control group was 0.9 mg m^{-2} day^{-1} and comparable to levels reported by Lekang & Kleppe (2000) in trickling filters. However, the nitrification rate was very low compared with some other studies. Zhu & Chen (2002) reported a nitrification rate of 1.72 g $m^{-2}day^{-1}$at similar temperature as tested for the control group. Chen at al. (2006) report a nitrification rate of submerged biofilters at 1.5 g $m^{-2}day^{-1}$. But low TAN removal rate similar to current study was observed by Lekang & Kleppe (2000). The reasons for this discrepancy may be that too short time was given for the biofilters to develop in this study. In the study of Lekang & Kleppe (2000) the biofilters showed maximum performance after 50 days. In the present study, the biofilters were given only 35 days which may not have been enough time for the biofilm to establish on the packing material. This problem may have been exacerbated by the salinity in the biofilters. Nijhof & Bovendeur (1990) reported lower nitrification rates in sea water than in freshwater biofilters.

5 CONCLUSIONS

- It is possible to adapt simple test kits to give both accurate and precise measurements of TAN, NO_2 and NO_3.
- Low temperature reduces the activity of the biofilter.
- Reduce oxygen may also reduce biofilter activity.
- However, in the present study insufficient mixing of water in biofilters was more likely to have caused reduced activity.

ACKNOWLEDGEMENTS

I feel ingenuously very happy that life gave me the chance to take part in this wonderful program. I am very grateful for my supervisor Pr. Helgi Thorarensen for his advices, encouraging and great lectures. I want to thank Olafur Sigurgeirsson for his help and always interesting lectures. Also to his wife Sigridur Bjornsdottir, for kindness and hospitality. During the whole program I had unforgettably good and interesting lectures. All students and staff at Verid, Matis, Fisk and MRI were very king and friendly. A big thanks them for that.

I would like to thank to the director of the UNU Fisheries Training Programme, Dr. Tumi Tómasson, that he believed in me. To Deputy Director of the Programme, Thor Asgeirsson and Project Coordinator, Mary Frances for their assistance and valuable advices. To Sigriður Kr. Ingvarsdottir for being so helpful and mindful.

I also found many friends in Iceland and I am very happy to know them. Especially I want to thank my neighbors Lesvia Calderon Herrera and Le Hoang Bao Chau for their support and patience. To Ukrainian families, for having a good time with them. To Matis laboratory staff for nice talk and help.

Of course I want to thank my colleagues from Institute of Fisheries of Ukraine for the support from the distance and to the staff of Agency of Fisheries of Ukraine.

The last, but not least I want to thank my fiancé for patience, support and understanding. Also to my family for encouraging me.

LIST OF REFERENCES

Andryushchenko, A. I., & Alimov, S. I. (2008). *Pond fish farming.* Kyiv, Ukraine: NAU.

Anthonisen, A. C., & Loehr, R. C. (1976). Inhibition of nitrification ammonia and nitrous acid. *Water Pollution Control Federation* 48(5): 835-852.

Bekh, V. (2005). *Fisheries and aquaculture department*. National Aquaculture Sector Overview [December 2014] http://www.fao.org/fishery/countrysector/naso_ukraine/en

Braynballe, J. (2010). *Guidance on aquaculture in recirculating systems.* Copenhagen: Eurofish.

Chen, S., Ling, J., & Blancheton, J.-P. (2006). Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering* 34(3): 179–197.

Costa-Pierce, B., & Desbonnet, A. (2005). *Urban aquaculture.* Wallingford: CABI publishing.

Courtland, S. (1999). Recirculating system technology for shrimp maturation. *The Advocate,* 74-77.

DeLong, D. P., & Losordo, T. M. (2012). How to Start a Biofilter. *SRAC,* Publication No. 4502.

Dronik, V. (2014). *The volume of fishing and other water bioresources in 2013*. [December 2014] <http://main.golovrubvod.kiev.ua/2011-07-27-09-19-23/2011-08-03-12-04-21/3234-2013-.html>

Ebeling, J. M., Timmons, M. B., & Bisogni, J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia nitrogen in aquaculture systems. *Aquaculture* 257(1): 346–358.

Ebeling, J. M., Timmons, M. B., & Michael, B. (2007). Recirculating aquaculture systems. *Aquaculture production systems* 245--277.

Emerson, K., Russo, R., & Lund, R. a. (1975). Aqueous Ammonia Equilibrium Calculations: Effects of pH and Temperature. *Journal of the Fisheries Research Board of Canada*, 32(12): 2379-2383.

Evans, D. H. (1998). *The physiology of fishes* (2nd ed.). New York: CRS press.

GFCM. (2012). (General fisheries commission for the mediterranean) *First meeting of GFCM working group on the Black Sea, 16-18 January.* Constanta, Romania.

Grytsyniak, I., & Simon, M. (2014). History of hatchery reproduction of sturgeons (review). *Fisheries Science of Ukraine*, 37-51.

Gutierrez-Wing, M. T., & Malone, R. (2006). Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. *Aquacultural Engineering*, 34(3): 163–171.

Interdonato, F. (2007). Biofilters: focusing on bacterial communities. Messina: University of Messina.

Jianhua, G., Yongzhen, P., & Shuying, W. (2009). Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. *Bioresource Technology* 100(11): 2796–2802.

Knowles, G., Downing, A., & Barrett, M. (1964). Determination of Kinetic Constants for Nitrifying Bacteriain Mixed Culture, with the Aid of an Electronic Computer. *Journal of general microbiology* 38(2): 268-278.

Konstantinos, P., Efthimia, T., & Konstadinos, M. (2005). 95th Seminar, December 9-10, 2005, Civitavecchia, Italy. *Assessing the Competitiveness of EU Mediterranean Fisheries and Aquaculture Industries* (pp. 161-171). Civitavecchia: European Association of Agricultural Economists.

Kovalenko, V. O. (2005). Problems and prospects of fish breeding in Ukraine. *Fisheries: inter-thematic scientific collection* (64): 25-30.

Kovalenko, V. (2010). The development of aquaculture in Ukraine: problems and tasks. *Rybnik: scientific journal*, 2-4.

Lekang, O.I., & Kleppe, H. (2000). Efficiency of nitrification in trickling filters using different filter media. *Aquacultural Engineering* 21(3): 181–199.

Nijhof, M., & Bovendeur, J. (1990). Fixed Film Nitrification Characteristics in Sea-Water Recirculation Fish Culture Systems. *Aquaculture* 87(2): 133-143.

Polymeros, K., Tsakiridou, E., & Mattas, K. (2005). 95th Seminar, December 9-10, 2005, Civitavecchia, Italy. *Assessing the Competitiveness of EU Mediterranean Fisheries and Aquaculture Industries* (pp. 161-171). Civitavecchia: European Association of Agricultural Economists.

Prehn, J., Waul, C. K., Pedersen, L.-F., & Arvin, E. (2012). Impact of water boundary layer diffusion on the nitrification rate of submerged biofilter elements from a recirculating aquaculture system. *Water research* 46(11): 3516-3524.

Randall, D. J., & Ip, Y. K. (2006). Ammonia as a respiratopy gas in water and air-breathing fishes. *Elsevier* 154(1): 216-225.

Randall, D. J., & Tsui, T. (2002). Ammonia toxicity in fish. *Elsevier* 45(1): 17-23.

Seginer, I., Mozes, N., & Lahav, O. (2008). A design study on the optimal water refreshment rate in recirculating aquaculture systems. *Aquacultural Engineering* 38(3): 171–180.

Timmons, M., & Losordo, T. (1994). *Aquaculture water reuse systems: enginering design and management.* Amsterdam: Elsevier and Science Publishers.

Vlasenko, N. (2013). *Balances and consumption of basic foodstuffs by population of Ukraine.* Kyiv: State Statistics Service of Ukraine.

Zhen Hu, J. L. (2014). Influence of carbohydrate addition on nitrogen transformations. *Science of the Total Environment*, 193-200.

Zhigin, A. V. (2011). *Recirculation systems in aquaculture.* Moskov: Russian State Agrarian University - Moscow Agricultural Academy named after K.A. Timiryazev.

Zhu, S., & Chen, S. (2002). The impact of temperature on nitrification rate in fixed film biofilters. *Aquacultural Engineering*, 221-237.