BACHELOR THESIS

Development of a method for cultivating and monitoring the growth of Skeletonema costatum

Based on the Wells-Glancy method

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Renewable Energy

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The experiment and thesis had the purpose to lay a foundation for future bachelor theses and experiments of the same sort, for students of Renewable energy at HiSF.

Abstract

The experiment lasted from the 29. Feb. to the 5. May, 2016. It was a small scale algae breeding project were the diatom *Skeletonema costatum* (*S. costatum*) was isolated and cultivated. This was done to assess the potential for *S. costatum* as a source for biomass for biofuel production. The project also aimed to lay the foundation for future bachelor theses at Høgskulen I Sogn og Fjordane (HiSF).

An open tank system with aeration at the aquaculture station at Skjær, Sogndalsfjøra (Sogndal) was used to conduct the experiment. The inoculate of algae added to the tank, was gathered from 0 – 10 m of the nearby fjord and consisted of a variety of naturally occurring algae species. The experiment was divided into three separate phases, the initial-, the growth-, and the fertilization phase. During the course of the experiment, the content of the tank was replenished on several instances, with deep water from the fjord. To try and optimize the growth conditions, fertilization was added during the later stages of the experiment.

The cultivation method used was the Wells-Glancy method, which rely on the natural bloom of algae harvested from local water sources, and added aeration.

The main task during the experiment was to collect and interpret measurements. Hydrographic measurements were conducted in tank to assess the development of the algae culture. Chlorophyll, turbidity, oxygen concentration, temperature and salinity was measured with a CTD device. Water samples were collected for counting and biomass filtration, and pH-samples were taken from the tank.

The highest production capacity found during the experiment was between the 3.- and the 4. May, with a realistic daily dry weight output of $134.7\ g\ d^{-1}$. The highest growth rate in terms of chlorophyll and dry weight was respectively 1.59- and 0.79 $double\ d^{-1}$. The organic carbon production was estimated to 6.64 $g\ d^{-1}\ m^{-2}$, and could potentially proceed the yearly organic carbon production per $m^2$of the Sogndalsfjord in 9 days.

Introduction

General introduction

The increasing greenhouse gas (GHG) emissions since the industrial revolution, has led to changes in the climate on a global scale. GHGs like CO_2 and methane re-radiates the longwave radiation back to earth, and causes global annual temperature to rise. This affects the whole climate system, leading to for instance intensified precipitation patterns, sea level rise, drought and heat waves (Stocker, 2014). In this way, climate change is expected to add additional pressure on the already anticipated future challenges such as the expected population rise, energy crisis, water crisis and catastrophic biodiversity loss. How severe the climate will change is uncertain, because in addition to limited knowledge about the climate system, it is very much dependent on future GHG emission scenarios (Stocker, 2014).

The Intergovernmental Panel on Climate Change (IPCC) states that the transport sector is responsible for approximately 23% of the CO_2 emissions coming from the energy sector in 2010 (Sims et al., 2014). This equates to 6.7 $GtCO_2eq$, and the total amount GHGs from the transport sector gives a total $GtCO_2eq$ of 7.0. These values are only calculated from the direct emissions of the vehicle usage. This phase of the emissions cycle is called tank-to-wheel (TTW). If the calculations would include emissions during the well-to-tank (WTT) phase, the well-to-wheel (WTW) CO_2eq would be much higher (Pratelli and Brebbia, 2011). Most of the world product-systems include many process steps during its life cycle, which emit GHGs. These factors, among many other factors would have increased the total CO_2eq of the products and for the transport sector as a whole. When calculating a product's WTT, TTW and WTW it is necessary to include the social and economic consequences, as well as the environmental. This makes a Life cycle assessment (LCA) of a product a demanding and comprehensive process (Jungbluth et al., 2007).

For biofuels, the TTW emissions are neutral, since it's considered to be part of a neutral carbon cycle (Hock, 2015). Biofuel's WTT emissions depends heavily on the type of feedstock and the conversion process used.

Since 1970, the transport sector has more than doubled its end-use emissions of GHGs. Even since the fourth IPCC report was published in 2007, there has been a growth in GHG emission from the transport sector (Sims et al., 2014). Despite this, the vehicle and fuel technologies have become more efficient and new policies have been adopted. According to (Jungbluth et al., 2007), 30% of the world's fossil fuel consumption were in the transport sector. (Sims et al., 2014) states that 53% of the world's oil consumption covered 94% of the worlds energy demand in the transport sector in 2010.

Biofuels covered approximately two of the remaining percentages. These numbers show the potential for climate change mitigation with low-carbon fuels.

The transport sector is heavily dependent on concentrated energy that can easily be transported, stored and used. Fossil fuels such as oil, and gas are the easiest to transport, store and apply in the current market. Microalga biofuels have the same properties and is considered the only renewable biodiesel that could potentially completely replace petroleum (Chisti, 2007). Crude oil requires millions of years in order to form harvestable deposits. The world deposits are estimated to be depleted within 35 years following the current trend (Shafiee and Topal, 2009).

The European directive (Commission, 2009) has adopted several policies when it comes to quality, amount and GHG emission reduction of biofuel in EU countries. These policies state that at least 10% of the energy consumption in each state's transport sector must come from renewable energy sources. In addition, the biofuels must give a minimum of 35% reduction in GHG emissions by 2015, which will increase to 50% in 2017 and 60% in 2018. It is also prohibited to use biofuels from land with high carbon stock and peatland or from sources high biodiversity.

In 2011, Norway produced approximately 154 million litres of biofuels. This equates to 3.75% of the total fuel used in the Norwegian transport sector the same year, as compared to 3.69% during 2010 (Ramm, 2016).

Biofuels are classified in generations depending on feedstock and conversion process. 1st generation are produced by agriculture. The use of 1st generation biofuels have been criticized since it uses land and resources which could be used for food or food production. It also has a high water demand. Examples of this are biodiesel from rape-seed oil and bioethanol from corn or sugar-cane production. 2nd generation are produced by silviculture and feedstock waste. 3rd generation biofuels are produced from aquaculture, predominantly microalgae and 4th generation will be produced by genetic engineered feedstock, which gives them specific properties to cover specific needs (Dutta et al., 2014).

Algae

The term algae are used to describe a large diverse group of photosynthetic organisms without roots and stems. Chlorophyll is the main photosynthetic pigment in these organisms, and they do not have sterile covering of cells around their reproductive cell. However, this definition is debated as it excludes similar, but not closely related organisms such as *cyanobacteria (Lee, 2008)*. Microscopic algae are called microalgae.

Most of the microalgae encountered in the following experiment are of the algae class *Bacillariophyceae,* commonly known as diatoms. Diatoms vary in size from a few µm to 1 mm and are built up by silica frustules, which make them easy to recognize. These frustules consist of two parts which fit into each other. This class is divided into two sub-classes which are called *Centricae* and *Pennatae.* The *Centricae's* frustules radiate from a center or from different points*, while Pennatae's* frustules are generally straight lines. Most diatoms do not possess the ability to move actively, and many pelagic diatoms have heavy frustules. Therefore, many diatoms have evolved in different ways to decrease their sinking rate. These characteristics include enlarged surface area, sails, reduced cell size to a few µm and reducing their specific weight by accumulating fat droplets (Newell and Newell, 1977, Sorokin, 1999).

The microalgae species encountered in the following experiment is *Chaetoceros* spp., *Thalassiosira* spp., *Melosira* spp*.* and *S. costatum*. The microalgae isolated was *S. costatum*, this is because it is a natural specie of algae in the fjord adjacent to the site in which the experiment took place and it has a high growth rate compared to other microalgae. *S. costatum* has a lens-shaped cell, with two parallel spines on each side. These spines make the cell division into straight, and sometimes long chains. In the 7-15 µm cells there can be found one or two chloroplasts. *S. costatum* don't have the ability to move, and need water currents and vertical turbulence to keep itself in the upper levels of the water column to absorb sunlight (Newell and Newell, 1977).

Algal biodiesel production

The algal biodiesel production process contains four main steps. The process starts with cultivation, which is usually done in either open pond systems or closed photo bioreactors (PHB) (Ryan et al., 2009). Open pond cultivation is typically the option with the lowest investment cost, and allows the algae to fixate CO_2 directly from of the air. Closed PHBs are more energy intensive, and need CO_2 input into the system. However, closed PHBs are much less prone to contamination, and allows for use of more productive algae strains and higher culture concentrations (Chisti, 2007).

After cultivation, the remaining biomass needs to be harvested. There are many methods for this, with varying energy demand, effectiveness and related problems.

When harvested, the biomass usually contains high percentages of water. Dehydrating and drying is normally required before the oil can be extracted. This is typically a very energy intensive step (Ryan et al., 2009), and constitutes to a negative net energy balance of algae biodiesel (Dassey et al., 2014). The process is more effective the more concentrated the algae culture is, and therefore PHBs will be the more energy efficient alternative in this step (Chisti, 2007).

For lipid extraction, the algae cell wall has to be broken down. This can be done either by exerting pressure, or by chemical methods. This step often pose a large share of the energy consumed when producing algae diesel. The lipids can be turned into biodiesel chemically via transesterification for direct use in diesel engines. It can also be turned into a variety of hydrocarbon fuels, even jet fuels, through hydro processing (Radakovits et al., 2010). The remaining residue can be burned for energy and CO_2 recycling, used as fertilizer or be turned into other energy products such as ethanol or hydrogen gas.

The goal for this bachelor thesis is to develop a method for cultivation and to monitor the growth of *Skeletonema costatum* sp. In order to lay a foundation for future bachelor theses in the same field at HiSF.

Materials and Methods

Most of the pictures presented in this thesis is taken by the authors, the exceptions are referenced in the figure text.

General description

The Wells-Glancy method was used in order to cultivate *S. costatum* (figure 9) in the initial phase. This method is originally designed to cultivate algae as feed for shellfish breeding (Anon, 1965).

The experiment was conducted at the aquaculture station at Skjær (figure 1), Sogndal in Sogn og Fjordane, Norway (figure 5), from the 29. Feb. to 5. May. The regional climate consists of warm summers and mild winters. The wind conditions vary based on the season, with winds sweeping inland from west, southwest during the summer. While during the winter, wind blows down from the mountainsides westward. The inland climate is sheltered from most of the rough weather patterns, making them less susceptible to rain and heavy wind conditions. The annual rainfall in the region is approximately 500 mm (Dannveig, 2009).

FIGURE 1 THE AQUACULTURE STATION AT SKJÆR, SOGNDAL.

The aquaculture station is located about 2.7km south of Sogndal, at the base of Stedjeåsen, and faces the fjord in the south. The station is alike most of Sogndal, surrounded by mountains and fjord. These

mountains cause the amount of sunlight during daytime hours to be reduced slightly, as the sunrise begins later in the morning, and the sunsets earlier in the evening.

A tank of 8.83 m^3 was placed at Skjær and filled with 7.74 m^3 of deep sea water pumped from a depth of a 100 m from the nearby fjord (figure 2).

FIGURE 2 TANK DURING REFILL, FOAM FROM AERATION VISIBLE ON THE SURFACE

The deep water contains higher amounts of nutrients and CO_2 than the surface water. This makes it preferable for algae growth. The tank stood on five legs to elevate it from the ground allowing for easy draining through a valve on the bottom (figure3).

FIGURE 3 TANK (BOTTOM) WITH LEGS AND DRAINING VALVE

The tanks placement at Skjær was just down by the pier to the right of the main building, and as the tank could not stand on the pier, the tank had to be placed on a location of un-even terrain. The

standardized legs on the tank would make the tank stand askew if steps were not taken to compensate for the height differences in between each leg. Several slate plates and planks were used to mend the height differences.

A sprinkler was placed at the center of the bottom of the tank. It was connected to an air pump (figure 4) through a hose. The aeration served to create necessary turbulence for the diatoms, in addition to adding CO_2 and O_2 to the water. The turbulence distributed the culture evenly, which was important for even access to sunlight.

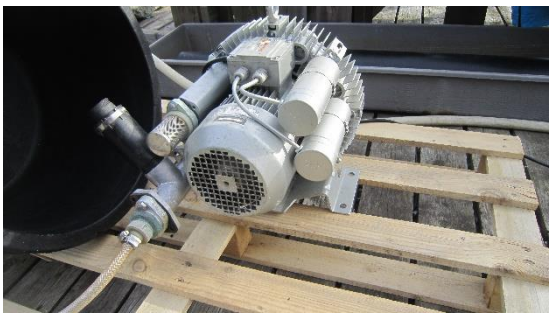

FIGURE 4 AIR PUMP

FIGURE 5 THE LOCATION OF SKJÆR AQUACULTURE STATION IN SOGNDAL MUNICIPALITY, SOGN OG FJORDANE, NORWAY.

Algae collection and inoculation

A net with 25 µm mesh (figure 6) was used to collect algae from 0 to 10 m depth in the fjord at the aquaculture station. The biomass was then filtered through a 100 µm filter (figure 6), to reduce the amount of zooplankton in the inoculate. This whole process was repeated 8 times.

FIGURE 6 THE GATHERING PROCESS OF THE INOCULATE TO THE RIGHT. 25 µM ALGAE NET IN CENTER AND 100 µM FILTER TO THE LEFT

To get an idea of the composition of the algae collected, a sample was put under a microscope (figure 7). Among the identified species were *S. costatum*, *Chaetoceros spp*., *Melosira spp*. and *Thalassiosira spp*. To ensure the concentrated algae had not been affected due to the exposure of heat by the atmosphere during the microscope examination the collection process was repeated to gather a new inoculate. Both inoculates were inoculated to the tank after it had been filled with deep water.

FIGURE 7 MICROSCOPE EXAMINATION OF INOCULATE

Phases

The experiment was divided into three different phases, the initial, growth without- and growth with-fertilization. The initial phase was the first part of the experiment. The purpose of the initial phase was to isolate *S. costatum* and to let the algae culture stabilize itself unaided. The initial phase was directly followed by the growth phase without fertilization; a phase consisting of rapid water replenishing (appendix, table 24) and algae growth cycles. The purpose of this phase was to further isolate *S. costatum*, while also locating the crisis point of the culture.

The growth phase with fertilization followed. During this phase, fertilizer was added each day with the purpose of finding out just how far the culture could be developed before the growth stagnated.

Data gathering

CTD- Measurements

Before and after the inoculate was added to the tank, a CTD-measurement was done. This gave an indication on the changes that occurred in the water due to the added algae. These measurements were collected using a CTD (SAIV 204) (figure 8).

FIGURE 8 CTD-DEVICE (SAIV 204) PICTURE ON THE RIGHT WITHOUT TURBIDITY SENSOR (KILDE T. DALE)

A CTD device can measure, as the acronym indicates, "conductivity, temperature and depth". It takes measurements every two seconds. Conductivity is measured by putting a small electric charge into the surrounding water. Temperature is measured by a thermistor. To get the depth values, the pressure is measured and calculated into meters by the device. The device then uses the conductivity, temperature and pressure to calculate the water's salinity levels. The CTD-device used in this experiment had additional sensors to measure dissolved oxygen (%), fluorescence ($\mu g\ l^{-1}$),

turbidity (FTU). The dissolved oxygen levels are measured to control the balance between photosynthesis and respiration acting in the water. Fluorescence is directly transmissible to the concentration of chlorophyll in the water, and presented by µg chlorophyll per litre by the device. This is done by emitting light and measuring the amount of light at a specific range of frequencies reflected back to the device. The turbidity sensor measures the amount of light scattered due to particles in the surrounding water.

The measurements were taken by lowering the CTD-device into the water of the tank and held at two different depths for a range of time between 30 sec. - 1 min. each. These depths were at approximately 20 cm under the surface and at the bottom of the tank. Most CTD-measurements were conducted in the afternoon, between 16 and 1700 hours, with some exceptions including the replenishing measurements which were mostly done early in the day. All the measurements were collected to a full dataset which was used to observe and compare measurements on a day to day basis (appendix, table 1). These data were processed in Excel to create the tables in the appendix. The diagrams presented was made using the data presented in the tables in the appendix. The crude segments of the thesis were written using Google docs, and Microsoft Word (2016) and End Note was used in the finishing stages of the thesis.

Counting

During the initial and the first growth phase of the experiment, water samples were collected from the tank for sedimentation and counting. The purpose of the counting process was to get an approximate composition and amount of the different algae species in the sample. The samples were collected in brown 100 ml flasks, and 1 ml of Lugol's fixative was added to preserve the algae after collection. The flasks were stored in a refrigerator at 4 ˚C until counting.

The samples were sedimented according to the method of Utermöhl (Sournia and Tangen, 1978). For the first samples 50 ml were sedimented. Since the counting process is time consuming and the algae culture thickened, 10 ml samples were used for the rest of the sedimented samples.
After the samples had sedimented, they were placed in an inverted microscope for counting after the method of (Sournia and Tangen, 1978). The species of algae counted were *S. costatum* (figure 9), *Chaetoceros* spp. (figure 10), *Melosira* spp. and *Thalassiosira* spp. (figure 10)., because these were the dominating species in the first microscope sample.
The microscope had a camera connected to a screen that made it possible for more than one person to keep count, making it less susceptible to human errors. For the first samples, half of the sedimented samples were counted, and then calculated into amount pr. litre. The following samples

were counted using a method which reduced the area that was required to be counted, from ½ to ¼, and then 1/21 (appendix, table 10).

Some samples were found to have a negative ash weight when the salt weight were subtracted. This could be due to the way the water weight of the first few samples had to be calculated based of off an average value of water weight found in samples collected in later samples. Also, another source of error is that some of the ash samples had singed to the aluminum tray they were placed on under the combustion process. This could explain some of the negative ash weight values measured as well.

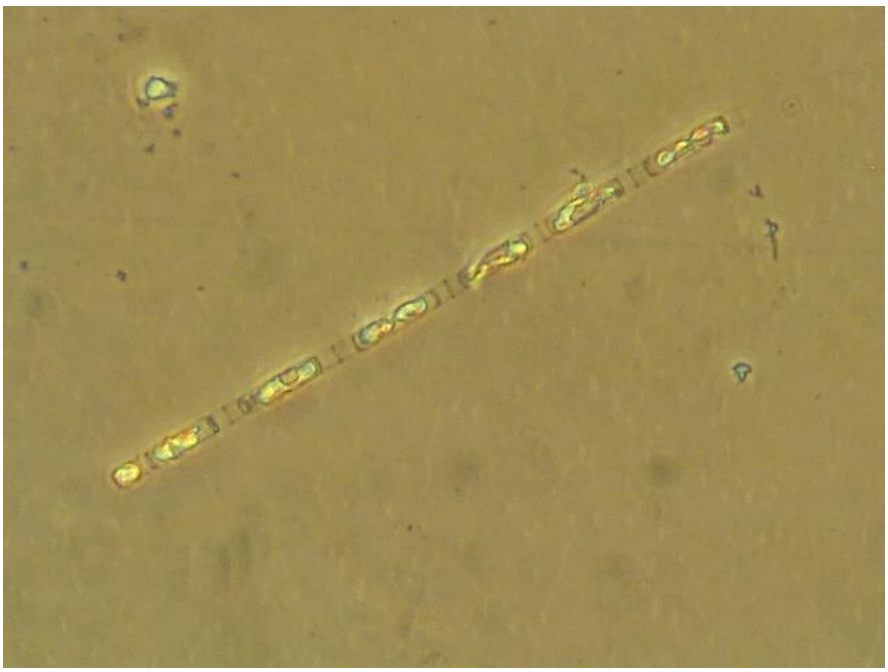

FIGURE 9 *SKELETONEMA COSTATUM*

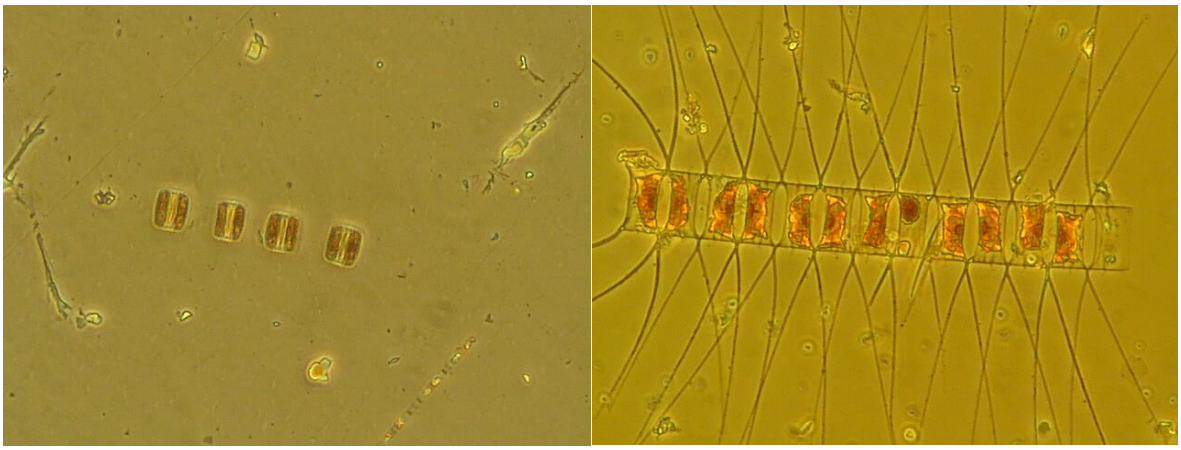

FIGURE 10 TO THE LEFT: *THALASSIOSIRA* SPP. TO THE RIGHT: *CHAETOCEROS* SPP.

The method used for counting 1/21 of the sample area consisted of placing a microscopic ruler into the reversed microscope. This was done in order to see the proportions on the sample as viewed through the screen. The proportions of the screen were 20 cm in height and 27.3 cm in width, this was found to correspond to respectively 0.52 µm in height and 0.715 µm in width as seen on the sample. Knowing this, counting each and every screen going sideways would give the row area (appendix, table 23). In each sample using the ratio, two rows were counted, giving the analyzed area, which would then be divided by the measured area (appendix, table 21 and 23).

To get an approximate amount of the individuals of different species counted, the amount of individuals per colony were counted depending on how good the first colonies represented the rest of the population in the sample. In some samples, counting the individuals of 30 colonies was enough, but for the most samples either 60 or 120 colonies was required to get a representative assortment. Then the mean number of individuals found per colony were calculated per species and then multiplied with the final amount of colonies found in the sample.

The number of algae found in the count was multiplied with the respective ratios of each sample to compensate for the area not included in the count. The resulting number provided an estimate of the amount of each algae specie per 10 ml, and could be multiplied by 100 to find the approximate amount of individuals per litre of the different strains. The 50 ml samples were as such multiplied by 20 to find the litre value.

Filtration

To calculate the amount of biomass in the tank, several water samples from the tank were filtered through a “Whatman filter” (appendix, table 13), which is a fine glass fiber filter with 45 µm meshes. The Whatman filters were burned at 500 °C for one and a half hours and weighed beforehand. This scorched away unwanted compounds such as fat from the filters, to secure an accurate filter weight after the second burning. The muffle oven used for the burning was a Heraeus mr 170 (figure 12).

The filtering process was conducted through a Millipore filtering setup (figure 11) consisting of a glass filtering flask connected to a water tap. A one-hole rubber stopper was attached at the opening of the flask. A glass funnel with a coarse filter on top was attached to the hole in the rubber stopper. The fine filter was placed on top of the coarse filter. A bottomless glass beaker was attached on top of the setup, with a clamp, to direct the water at the filter.

FIGURE 11 FILTRATION DEVICE

The water samples were poured into the beaker, and then the water tap was turned on in order to create a low pressure zone within the filtering flask. The low pressure contributed to a higher flowrate through the filter. After filtration, the wet biomass remained on top of the filter.

After the filtration process, the glass fiber filters with the attached biomass was dried at 105°C for one hour. The oven used for drying was a Termaks T1056V (figure 12).
The dried filter samples were weighed on a KERN ALJ-160-4A (figure 12), with a range from 0,1 mg-160 g, and an accuracy of 0,3mg.

FIGURE 12 FROM THE LEFT: MUSSLE OVEN (HERAEUS MR 170), DRYING OVEN (TERMAKS T1056V), WEIGHT (KERN ALJ-1604A)

After weighing, the biomass filters were burned at 500 °C for one hour, the filters were placed in the oven during warm up for half an hour before this as well. During transportation and storage, the filters were kept in a desiccator (figure 13) to keep them from rehydrating due to humidity in the air.

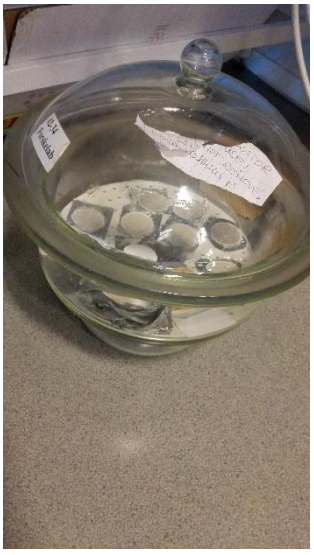

FIGURE 13 DESICCATOR USED FOR TRANSPORTATION AND STORING OF BIOMASS SAMPLES AND FILTERS

Most of the biomass samples were weighed right after filtering. When the weight of the aluminum and filter weight was subtracted, the water weight was attained. The water weight consisted of the water in the biomass and the filter combined. Water weight must not be confused with the term wet weight. The water weight made it possible to calculate the weight of salt in the filter samples by using the corresponding salinity measurements. The salt content in the filter samples was calculated by the formula:

$$Salt\ weight = \frac{water\ weight}{1000} * salinity\ (‰)$$

The salt weight was subtracted from the dry weight and the ash weight to attain dry- and ash weights.

Since the algae culture thickened throughout the experiment, only 500 ml of the water samples were filtrated. The values were then calculated into litre values by multiplying the dry weight- and ash weight- without filter by two (appendix, table 13).

Calculations

Nutrients

Nutrients had to be added to the tank to maintain the growth of the algae culture after the nutrients in the deep water was depleted (Reitan et al., 2002). Was used as the base for making a rough estimate for suitable nutrient addition. The article explains an experiment conducted to assess changes in growth and composition of scallops when nutrients were added to a landlocked bay.

During the experiment conducted by (Reitan et al., 2002), Nitrogen (N), Silica (Si) and Phosphorus (P) were added in the respective atomic ratio 16:8:1. In the year 1999, 0,8 $mg\ m^{-3}\ d^{-1}$ of P was added, and the chlorophyll level measured was 2.4 µg l^{-1}. The natural atomic ratio found in the deep water of the Sogndalsfjord was around 14:17:1 of N:Si:P respectively, between 1991 and 1993 (Dale, 1993).

The following calculations of fertilizer and waterglass dosage were provided by T. Dale:

Fertilizer

The volume of the water in the tank was roughly calculated to be 7 m^3 for the nutrient calculations. For an chlorophyll production 10 times higher than achieved in the experiment of (Reitan et al., 2002) (24 $\mu g\ ch.a.l^{-1}\ d^{-1}$) It is necessary to add:

$$0{,}8\ mg\ P\ m^{-3}\ d^{-1}\ \cdot 10 = 8\ mg\ P\ m^{-3}\ d^{-1}$$

$$8\ mg\ P\ m^{-3}\ d^{-1} \cdot 7\ m^3 = 56\ mg\ P\ d^{-1}$$

For nutrient addition, the commercial fertilizer “fullgjødsel 18-3-15” from YaraMila (table 1) was chosen. The nutrient weight composition of the fertilizer 18:3:15 = N:P:Potassium (K), and was considered fitting for the algae culture. The weight of N:P:K in percentages of the total fertilizer weight was respectively: 17.6% : 2.6% : 14.6%.

$$The\ ratio\ between\ the\ weights\ of\ nitrogen\ and\ phosphor\ was:$$

$$\frac{N}{P} = \frac{17.6}{2.6} = \frac{6.7}{1}$$

Atomic relation:

$$molar\ mass\ N\colon N = 14g\ mol^{-1}$$

$$molar\ mass\ P\colon P = 31g\ mol^{-1}$$

$$N\colon\ 17.6\ g \cdot \frac{1}{14}\ g\ mol^{-1} = 1.26\ mol$$

$$P\colon\ \ 2.6 \cdot \frac{1}{31}\ g\ mol^{-1} = 0.084\ mol$$

$$\frac{1.26\ mol}{0.084\ mol} = \frac{15\ molN}{1\ molP}$$

This shows that the atomic relation in the fertilizer was respectively N:P, 15:1 Hence, it is a bit lower than the relation of N:P 16:1 which was used I the experiment of (Reitan et al., 2002) the N:P ratio in this experiment is slightly higher than the ratio found in the Sogndalsfjord (Dale, 1993).

The total commercial fertilizer amount to be added was calculated from the amount of P required for the 7 m^3 of water:

$$daily\ amount\ of\ fertilizer = \frac{56\ mg\ P \cdot 100\%}{2.6\ \%} = 2.15\ g\ d^{-1}$$

Where 56 mg P was the total amount of P required, and 2.6 % was the weight percentage of P in the fertilizer.

This rough estimate indicates that for a chlorophyll production of 24 $\mu g\ l^{-1}\ d^{-1}$, 2.2 g of commercial fertilizer should be added on a daily basis.

TABLE 1 : THE GENERAL INFO ON THE FERTILIZER (YARAMILA FULLGJØDSEL 18-3-15) AS PROVIDED BY THE SUPPLIER.

Trademark	Designation	Reg.no.	>16% N from AN
Fertilizer 18-3-15	NPK-fertilizer with Ca, Mg, S and B	616	Yes
Declared nutrients			
N-tot.	17.6	**Ca-ws.**	0.3
NO3-	8.3	**Cl**	10.6
NH4+	9.3	**Mg-tot.**	1.5
P-cit.s.	2.6	**Mg-ws.**	1.3
P-ws.	1.9	**S**	3.8
K-ws.	14.6	**S-ws.**	3.5
Ca-tot.	1.3	**B-tot.-**	0.02

Source: (Anon, 2015)

N = nitrogen, Ca = calcium, B = boron, P = phosphorus, Cl = chloride, S = sulphur, K = potassium, Mg = magnesium, ws. = water-soluble, tot. = total content, cit.s. = citrate-soluble; dissolvable in neutral ammonium citrate.

Silicate

The sought out atomic relation was 16:8:1 of respectively N:Si:P. That means 8 mol Si per mol P.

$$8\ mg\ P\ m^{-3} \cdot \frac{1}{31} = 0.258\ mmol$$

$$The\ sought\ out\ ammount\ of\ Si\ is\ 8\ times\ that\ of\ P$$

$$8\ \cdot 0.258\ mmol\ Si = 2.064\ mmol\ Si\ m^{-3}$$

$$1\ mmol\ Si = 28\ mg\ Si$$

$$2.064\ mmol\ Si\ m^{-3} = 2.064 \cdot 28\ \mu g\ Si = 57.8\ mg\ Si\ m^{-3}$$

$$\frac{SiO2}{Si} = \frac{60}{28} = 2.14$$

$$57.8\ mg\ Si\ \cdot 2.14 = 123.7\ \mu g\ SiO2\ m^{-3}$$

$$123.7\ mg\ SiO2\ m^{-3} \cdot 7\ m^3 = 0.87 g\ SiO2$$

Water glass contains 27.5 % SiO2. The water glass required to meet the need for Si is then:

$$\frac{0.87\ g \cdot 100\ \%}{27.5\ \%} = 3.16\ g$$

The required dosage of nutrients that should be added to reach a chlorophyll production of 24 µg $l^{-1}\ d^{-1}$: 2.2 g d^{-1} commercial fertilizer and 3.13 g d^{-1} of waterglass.

The nutriants was added almost daily through the fertilization phase 1 and 2. The fertilization dosage was adjusted to higher levels as the culture got denser. The dosages were measured using a OHAUS Precision Plus weight. In the beginning, only half dosage was added in order to avoid over fertilization. The dosage added each day were adjusted as seen in (appendix, table 9)
The Si was added in such a way that it did not crystalize, this was achieved by taking the measured amount of Si and stir it out in a bucket containing tempered water. The same was done to the fertilizer as well, but with less water as it is easier to dissolve than the Si.
The buckets with dissolved fertilizer and Si were spread out in the tank evenly.

Tank volume

The volume of the tank was calculated based on measures of diameter and height given on the supplier's website (Anon, 2016) and measurements done manually at the site.

The diameter of the circular tank was 3 m and the height was 1.2 m from the bottom to the top, at the side of the tank. The calculations of the tanks volume were separated into two parts, the upper and the bottom part.

The upper volume was calculated to:

$$Diameter\ (d) = 3\ m$$

$$Radius\ (r) = 1.5\ m$$

$$A = \pi \cdot r^2 = 3.14 \cdot 2.25 = 7.07\ m^2$$

$$Upper\ volume\ (Vu) = 7.07 \cdot 1.2\ m = 8.48\ m^3$$

The bottom of the tank was conical, with a countersink in the center. The inclination of the bottom was 5.71˚ to from the side to the center. The volume of the cone shaped bottom part was calculated to:

$$Height\ botom\ (hb) = Tan(5.71) \cdot r = 0.15\ m$$

$$Bottom\ volume\ (Vub) = \frac{1}{3} \cdot hb \cdot\ \pi \cdot r^2 = 0.35\ m^3$$

The countersink was neglected due to its small volume.

$$The\ total\ tank\ volume\ (V) = Vu + Vub = 8.8m^3$$

The volume of the water when the tank was filled to 1 m was:

$$V(1m) = 7.07\ m^3 + 0.35\ m^3 = 7.4\ m^3$$

Which amounts to 7400litre.

Growth rates

Specific growth rate (SGR) is a constant that describes the amount of doubling in 1-unit time. It is used to describe how the population grows over a given time. The formulas used to calculate specific growth rate, doubling time and divisions per day, was obtained from (Jameson, 2006) and (Anning et al., 2000).

$$N(t) = N(0)e^{SGR \cdot t}$$

$$SGR =\ ln\left[\frac{N(T_2)}{N(T_1)}\right] / (T_2 - T_1)$$

Where $N(T_1)$ is the biomass at time T_1 and $N(T_2)$ is the biomass at time T_2 The time unit is given in days

The doubling time represents the number units of time per doubling, given in days ($d\ Double^{-1}$), and is calculated with the following formula:

$$d\ Double^{-1} = \frac{Ln(2)}{SGR}$$

Divisions per day was calculated with the formula:

$$Div.day^{-1} = \frac{SGR}{Ln(2)}$$

The formulas represented above has been used to calculate growth rates and doubling time using several different indicators for biomass, respectively dry weight, chlorophyll measurements, turbidity and cell count.

Conversion from algae count to dry weight

For calculating other algae count results into dry weight, the following formula has been used.

$$Nc \cdot 5.22 \cdot 10^{-11}$$

Nc is the number of cells counted per litre (cells l^{-1}) and $5.22 \cdot 10^{-11}$ is the typical weight of one cell in grams. The number representing the weight of one cell was obtained from (Lavens and Sorgeloos, 1996) and is based on literature.

Nychthemeron

Nychthemeron measurements were carried out the last two days of the experiment to map the fluctuations during a nychthemeron. The first 24 hours, CTD- and pH- measurements were taken every two hours and a biomass sample every 4 hours. The second nychthemeron, CTD- and pH-measurements were taken every four hour and two more biomass samples were taken. During the nychthemeron, the chlorophyll measurements reached the upper limit of the measuring range of the device. Samples of water from the tank had to be diluted with deep water to reach below the maximum range value. This was done by adding 4L of tank water and 8L of deep water to a bucket. The mixture was then manually blended to get an even mix of tank- and deep water. CTD-measurements were then conducted in the bucket. Since the CTD-device is too big for the bucket, only the chlorophyll sensor on the bottom of the CTD were under water. The values measured then had to be multiplied by three to get the correct values, due to the ⅓ ratio of tank water and deep water.

pH measurements

pH values were measured to check the acidity of the water in the tank. This was done by bringing water samples from the tank to the lab at the aquaculture station. The lab is equipped with a PHM 80 portable pH meter (figure 14).

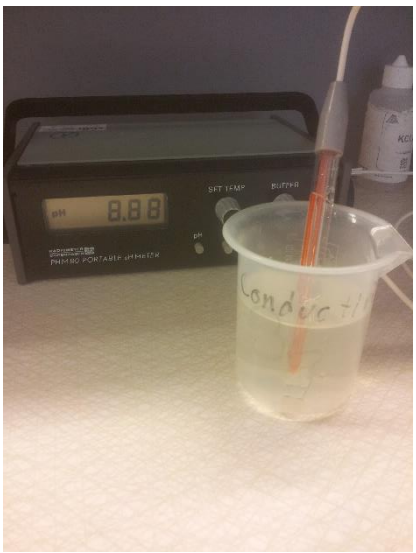

FIGURE 124 PORTABLE PH-METER (RADIOMETER: PHM 80)

Before use, the device was calibrated with a buffer of 7pH, and then checked again with a buffer with 4pH.

Results

Overview

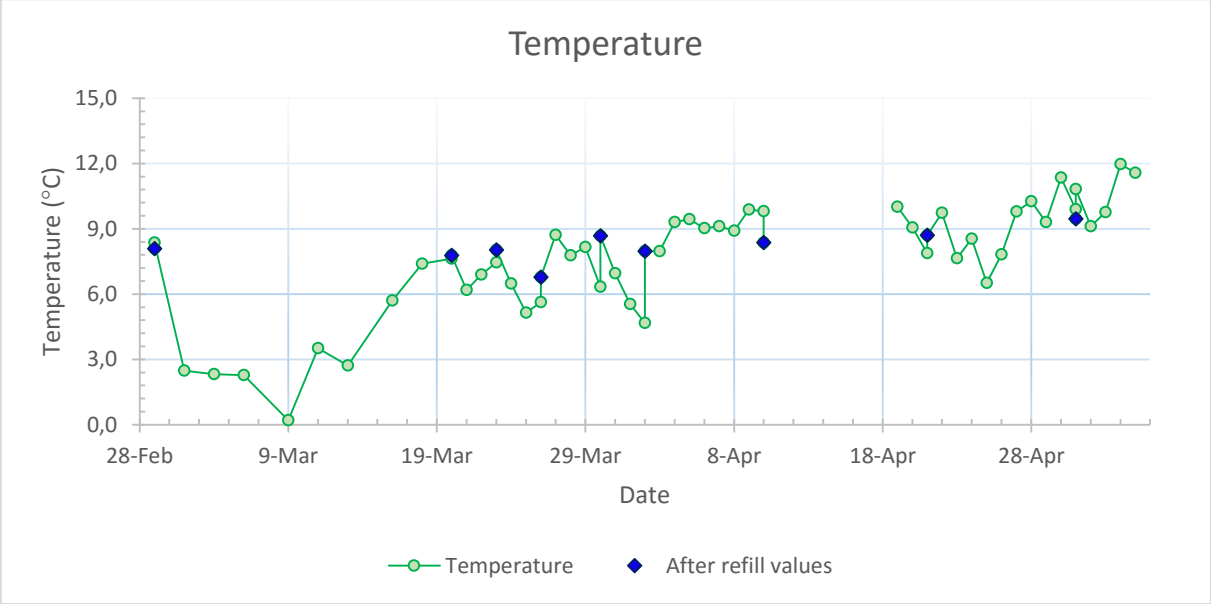

FIGURE 15 THE TEMPERATURE IN THE TANK FROM 29. FEB. TO 5. MAY

Figure 15 shows the temperature development throughout the experiment. The tank was exposed to changes in weather and, especially during the start of the isolation phase, the temperature measurements were low. The temperature was measured at 0.207°C on 9. Mar. and to keep the tank from freezing, 30 cm of water was replenished. This resulted in the temperature rise as illustrated in the graph at 11. Mar. The rising temperatures experienced throughout the experiment was due to the change in season. The measurements from 22. Mar. to 5. May averaged at 8.4°C.

The “After Refill Values” (ARV) indicates that stable temperatures at approximately 8 °C can be found at a depth of 100 m in the fjord. Variations in temperatures after refill occur mainly due to differentiating amounts of water being replenished.

From 10. Apr. to 19. Apr., the CTD device was on service, resulting in the measurement gap seen in figure 15.

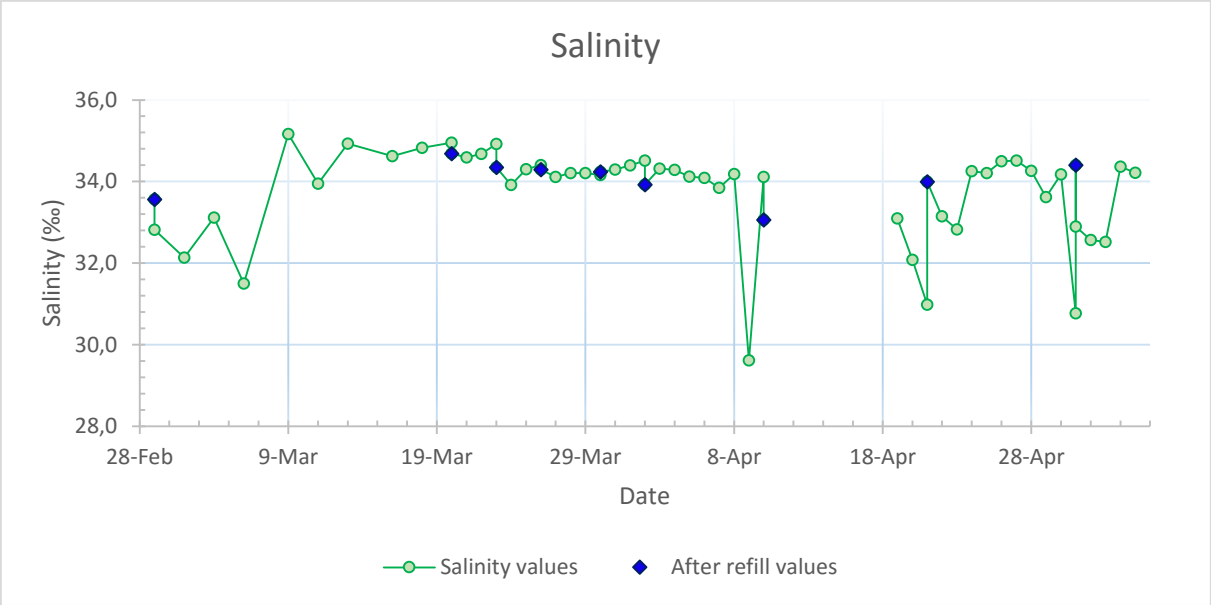

FIGURE 16 THE SALINITY IN THE TANK FROM 29. FEB TO 5. MAY

The graph in figure 16 shows the salinity values, measured in ‰. The sudden low values were likely due to several measurement errors. However, some periods do show stable values measuring around 34.5‰. This indicates that the realistic range was around this level. The highest value measured was at 9. Mar., reading 35.16 ‰.

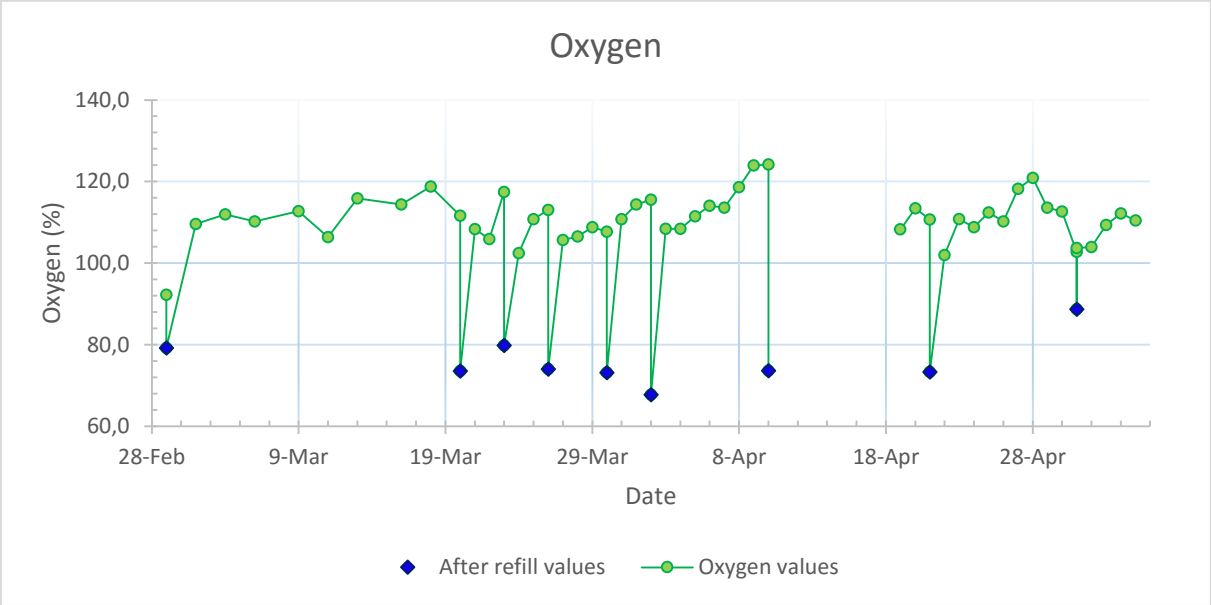

FIGURE 17 THE OXYGEN CONCENTRATION IN THE TANK FROM 29.FEB TO 5.MAY.

Figure 17 illstrates the oxygen content of the water in %, calibrated up against the atmospheric air level which is set at 101%. The ARV reads around 75%, reflecting the low values in the deep water. Without photosynthesizing organisms in the water, oxygen levels would not reach over 100%. The oxygen values, disregarding ARV, measured well above 100% throughout the daily measurements through the entire period, indicating active photosynthesis during the experiment.

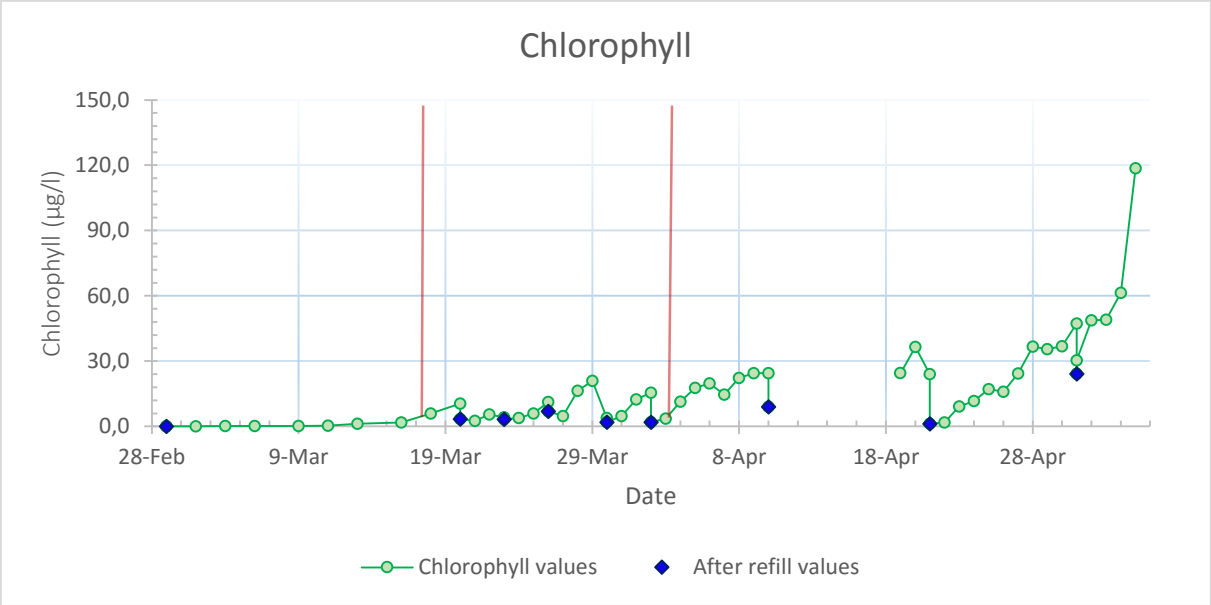

FIGURE 18 THE CHLOROPHYLL IN THE TANK FROM 29.FEB TO 5.MAY

The graph shown in figure 18 shows the chlorophyll values throughout the experiment. The experiment was divided into three phases, as illustrated in the figure with the vertical red lines. The initiation phase lasted from 29. Feb to the 16. Mar. The growth phase without fertilization lasted from 16. Mar until 2. Apr. The growth phase with fertilization lasted from 2. Apr. until the experiment ended 5. May.

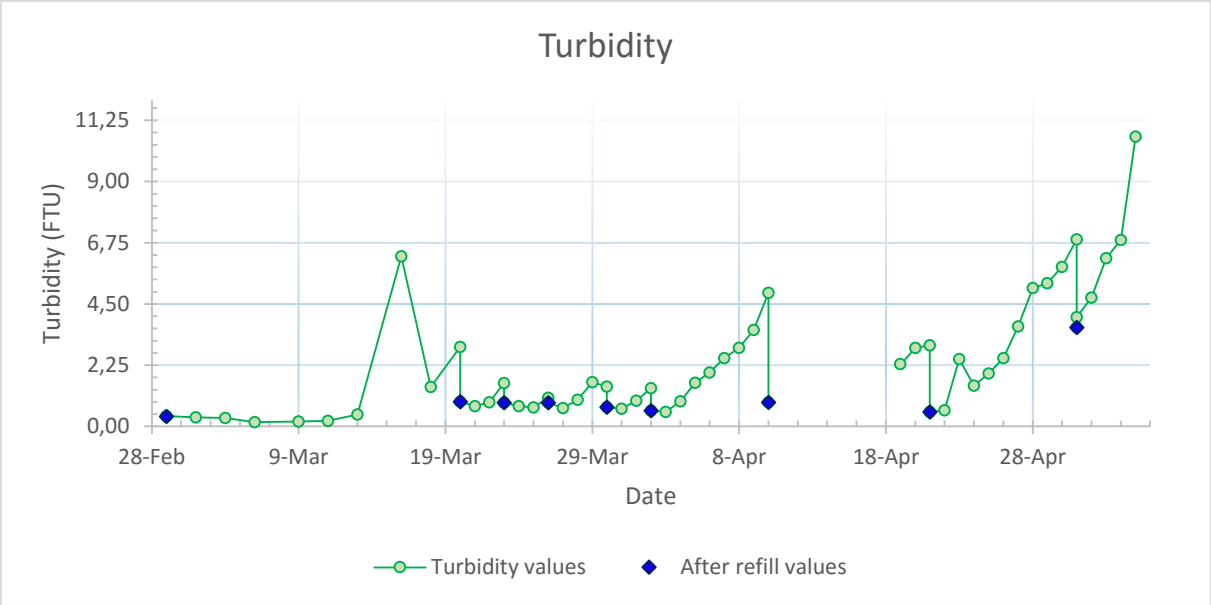

FIGURE 19 THE TURBIDITY (FTU) IN THE TANK FROM 29.FEB TO 5.MAY.

As the diagram in figure 19 illustrates the turbidity in the tank varied greatly throughout the experiment. The highest value was measured 5. May. at 10.65 FTU, and lowest at 0.163 FTU 6. Mar.

One of the highest values appeared on 16. Mar., measuring 6.25 FTU. This value doesn't correlate with the chlorophyll measurements or with the visual observation during measurements. Hence, the spike value of 16. Mar. was likely due to big particles in the water sticking to the turbidity sensor.

The ARVs shows stable values during most of the measurements, despite variations in turbidity before refill. This is due to natural turbidity caused by detritus accumulating in the deep water.

Initial phase

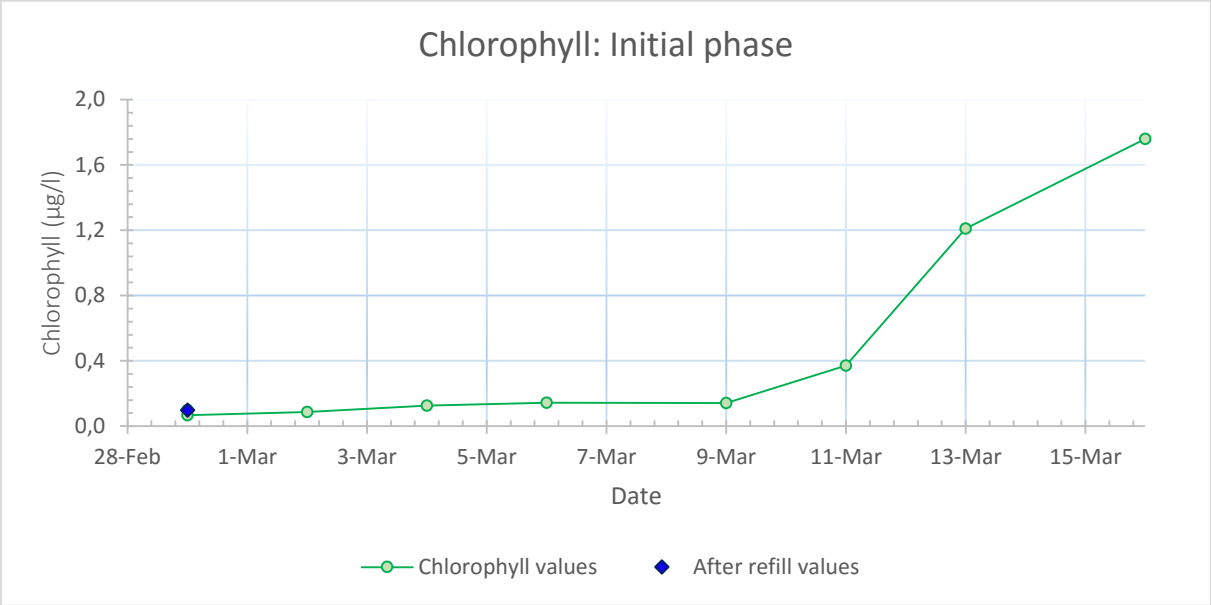

FIGURE 20 THE CHLOROPHYLL VALUES (µG CHL L^{-1}) IN THE TANK THROUGH THE INITIAL PHASE

As seen in figure 20 the chlorophyll measurements started 29. Feb. The blue dot in figure 20 presents the value measured after the addition of the algae. The increase in chlorophyll was measurable, as the value rose from 0.07 µg l^{-1} to 0.1 µg l^{-1}

Measurements remained steady below 0.15 µgl^{-1} until 9. Mar. This phenomenon was expected, and is called a lag phase. From 9. to 16. Mar. the chlorophyll content rose from 0.142 µg $l^{-1}$to 1.76 µg l^{-1}. The average growth rate between 9.- 16. Mar. was 0.36 $double\ d^{-1}$. This growth cycle was part of a longer growth cycle, which continues into the growth without fertilization phase.

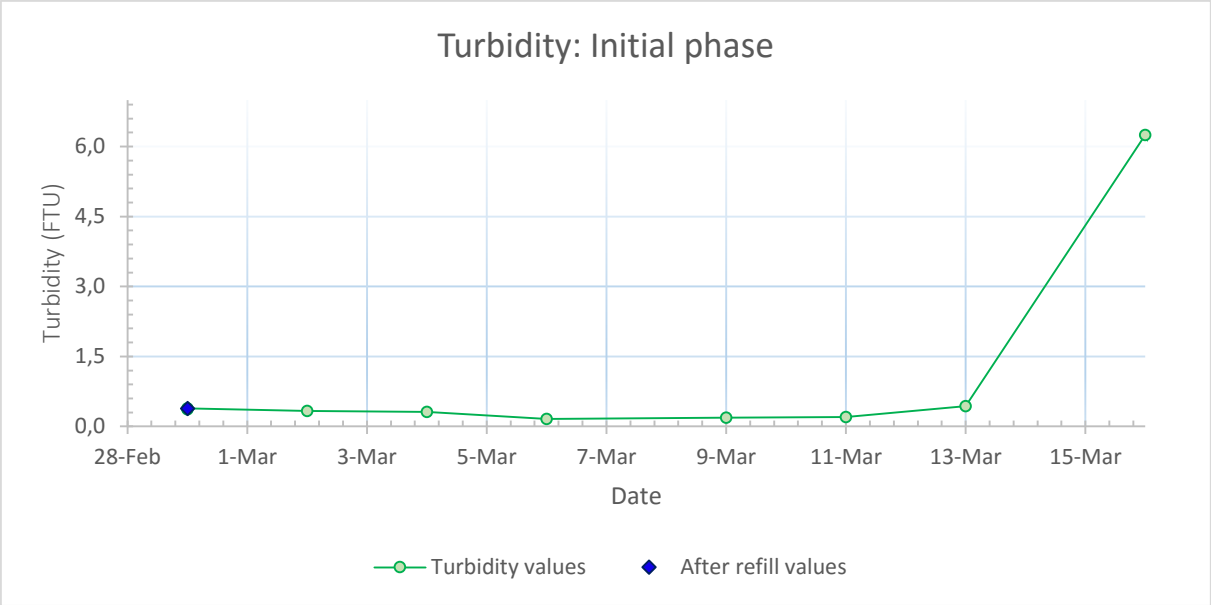

FIGURE 21 THE TURBIDITY (FTU) IN THE TANK THROUGH THE INITIAL PHASE

The graph (figure 21) shows turbidity measurements through the initial phase. The blue dot represents the measurement after addition of the algae. The difference between before and after measurements was insignificant.

The values were low in the start, from 29. Feb. to 13. Mar. all turbidity measurements was lower than 0.5 FTU. The lowest was 0.16 FTU on 6. Mar.

The peak value on 16. Mar. is most likely due to a measurement error. The data series taken this day shows many values around 10-12 FTU, and some low values as low as 0.7 FTU. Dirt in front of the lens where turbidity is measured could have caused these high values.

Algae count

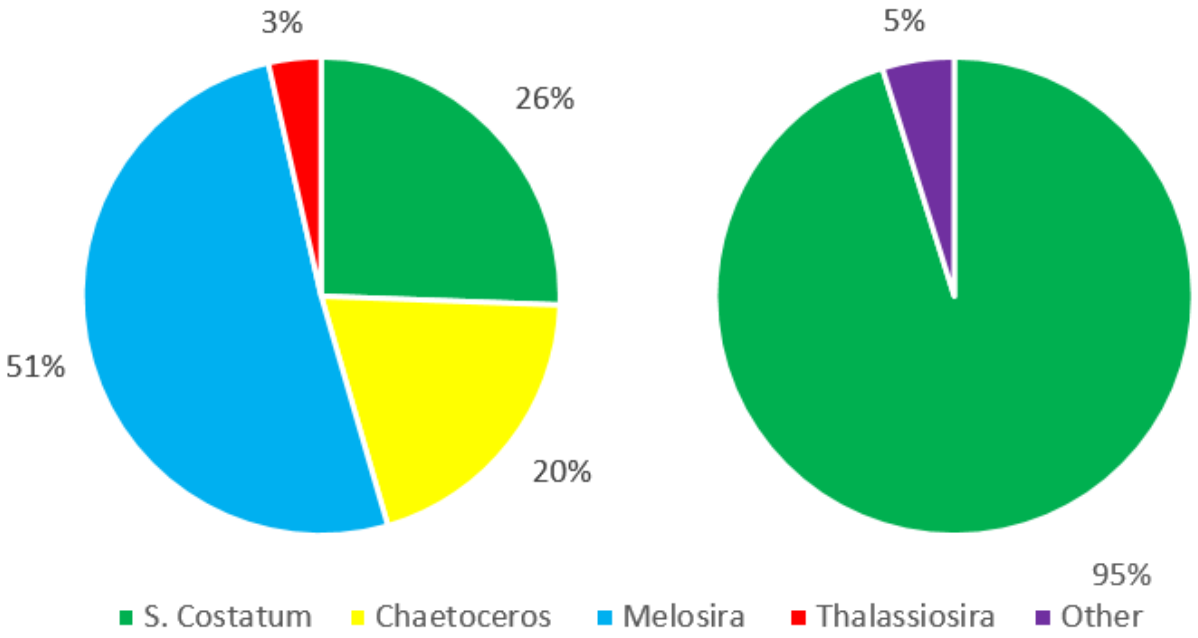

FIGURE 22 THE COMPOSITION OF THE ALGAE CULTURE ON 29. FEB. (LEFT) AND 22. MAR. (RIGHT) IN PERCENT

The diagram (figure 22) on the left shows the algae composition in the sample from 29. Feb. *S. costatum* made up 26% of the total number of algae in the tank following the inoculation. Aside from the algae represented in figure 22, several flagellates were also present, however these were not accounted for. As the sample was collected right after the algae was inoculated, this chart represents the naturally occurring composition of larger algae found in the upper 10 m water column in the fjord on the day of harvest.

The last algae count, on 22. Mar., confirmed that the environment in the tank benefitted *S. costatum* over the other strains of algae. As Figure 10 illustrates, *S. costatum* made up 95% of the number of individuals in the tank at the time of the last count. Of the remaining 5% "other" algae, *Chaetoceros* spp. made up 4% while *Thalassiosira* spp. made up 1%. The composition of number of individuals do not represent the algal biomass composition in the tank, due to differences in sizes between the individuals. While *S. costatum* holds a relatively set size, individuals of *Chaetoceros* spp. can vary greatly in size depending on the species, and *Thalassiosira* spp. individuals are generally much larger than *S. costatum*.

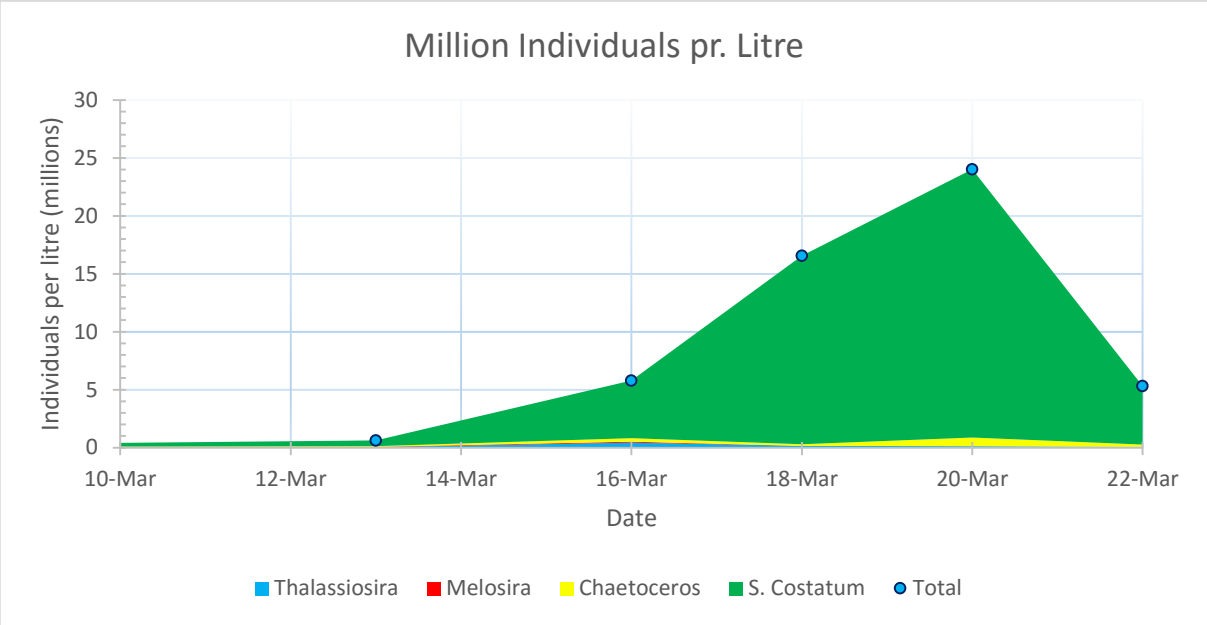

FIGURE 23 NUMBER OF GROWTH CYCLE OF ALGAE SHOWING INCREASE AND COLLAPSE, FROM 10.MAR TO 22.MAR.

S. costatum clearly outnumbered the other algae strains in the tank at the end of the counting period (figure 23). The count samples from 29. Feb., and 6. Mar. is left out of the graph. The algae count confirmed that the Wells-Glancy method was successful in promoting the growth of *S. costatum*.

No zooplankton was found in any of the samples. This indicates that the filtration had been sufficient in limiting the addition of zooplankton, that otherwise might have grazed down the microalgae.

The last sample, collected on 4. Apr., indicated a still dominating *S. costatum* population making up 97% of the total algae individuals. The "other" 3% was dominated by *Chaetoceros* spp. The count was not included in the diagrams, to accentuate the earlier samples and make the graph more readable.

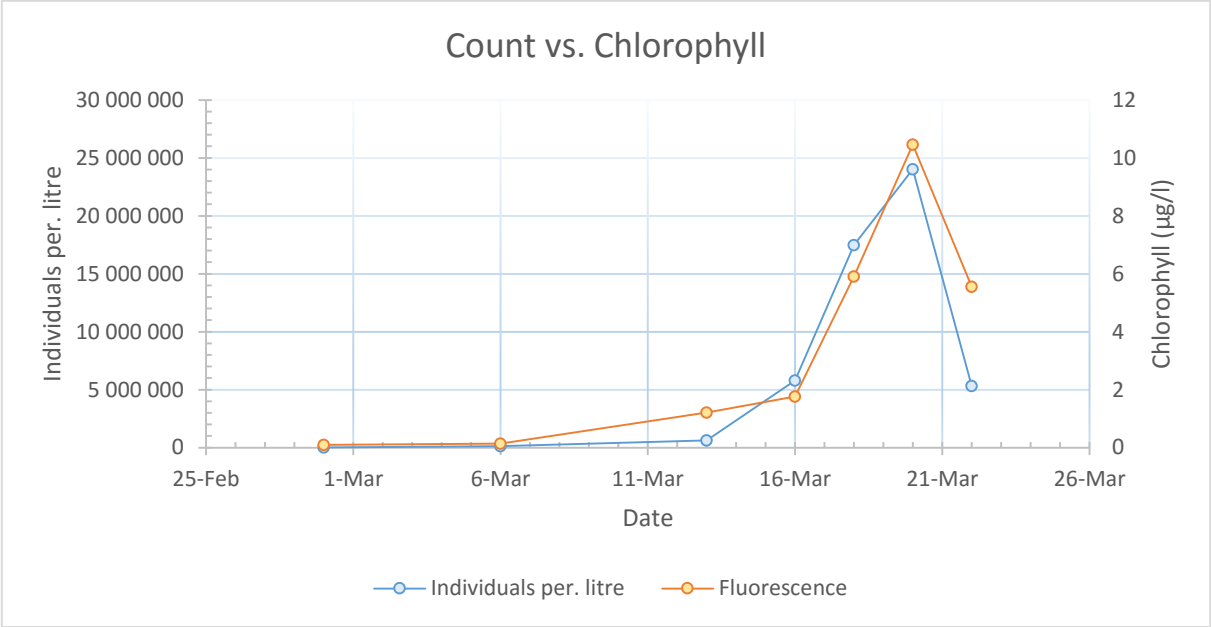

FIGURE 24 THE CORRELATION BETWEEN THE ALGAE COUNT VALUES AND CHLOROPHYLL VALUES(µGL^{-1}) FROM 29. FEB TO 22. MAR.

Figure 24 illustrates the correlation between the total number of individuals of the combined algae strains per. litre, and the chlorophyll measurements on the respective days. As expected, the measurements follow the same pattern.

The growth rate of the algae culture from 13.- 20. Mar. was 0.52 $double\ d^{-1}$ when based on the algae count. The corresponding chlorophyll growth rate during the same period was 0.31 $double\ d^{-1}$. The reason for a much higher growth rate from the algae count is due to the high "initial" chlorophyll value on 13. Mar. The differentiation on 13. Mar. could be linked to a higher number of flagellates in the earlier samples.

Growth phase without fertilization

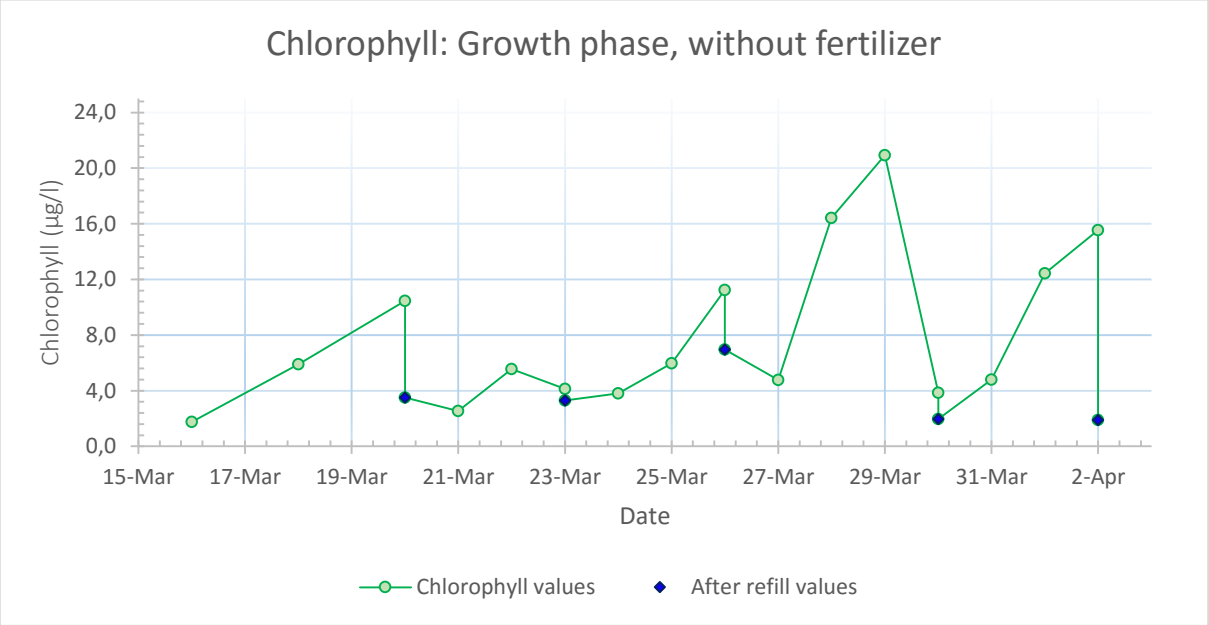

FIGURE 25 THE CHLOROPHYLL MEASUREMENTS THROUGH THE GROWTH WITHOUT FERTILIZATION PHASE

The water in the tank was drained and refilled 5 times during this phase to avoid nutrient depletion, this is shown in figure 25. The purpose of this phase was to assess the growth rates of *S. costatum in the tank* without the addition of nutrients other than what is naturally present in the deep water.

The first peak-value in the phase was found on 20. Mar. at 10.461 µgl^{-1}. The growth phase leading up to this peak lasted from 9.mar. with a growth rate of 0.39 $double\ d^{-1}$.

At 21. Mar. the chlorophyll value was at 2.52 µgl^{-1}, and rose to 5.55 µgl^{-1} at 22. Mar. The growth rate between these measurements was 0.79 $double\ d^{-1}$.

After draining to 25 cm and refilling, the measurements showed 3.29 $\mu g\ l^{-1}$ on the 23. Mar. The value had risen to 11.25 µg/l the 26. Mar. The growth rate over this time period was 0.41 $double\ d^{-1}$.

The highest value was measured 29. Mar. at 20.93 $\mu g\ l^{-1}$. The growth cycle leading up to this value lasted from 27. Mar. (at 4.76 $\mu g\ l^{-1}$.) and the growth rate during this cycle was calculated to be 0.74 $double\ d^{-1}$.

The growth phase from 30. Mar to 2. Apr. showed a growth rate at 0.52 $double\ d^{-1}$.

From 29 to 30. Mar. the culture crashed, values on 30. Mar was at 3.84 $\mu g\ l^{-1}$.

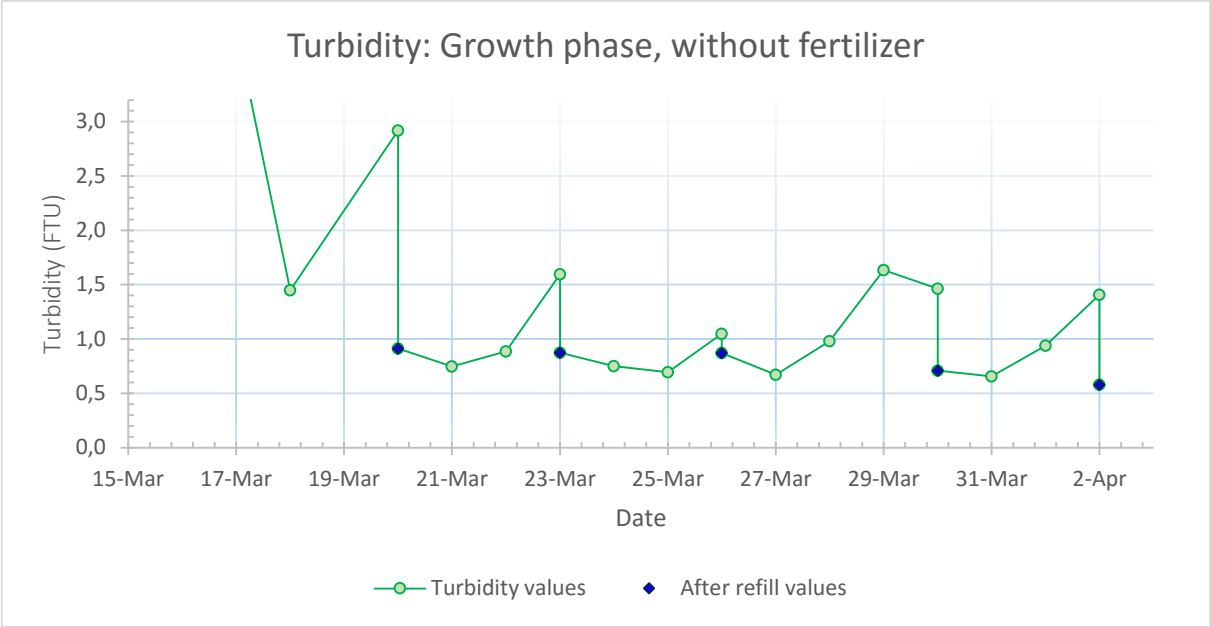

FIGURE 26 THE TURBIDITY MEASURED IN FTU THROUGH THE GROWTH PHASE WITHOUT FERTILIZATION

The range of the Y-values on the diagram (figure 26) is made shorter than what is necessary, not showing the value of 16. Mar, to accentuate the other values. The highest turbidity value was measured 29. Mar., and read 1.63 FTU. (appendix, table 20)

The ARVs show very stable values around 0.9 FTU. This might indicate higher turbidity in the deep water than during the first filling of 29. Feb.

Growth phase w/ fertilization 1

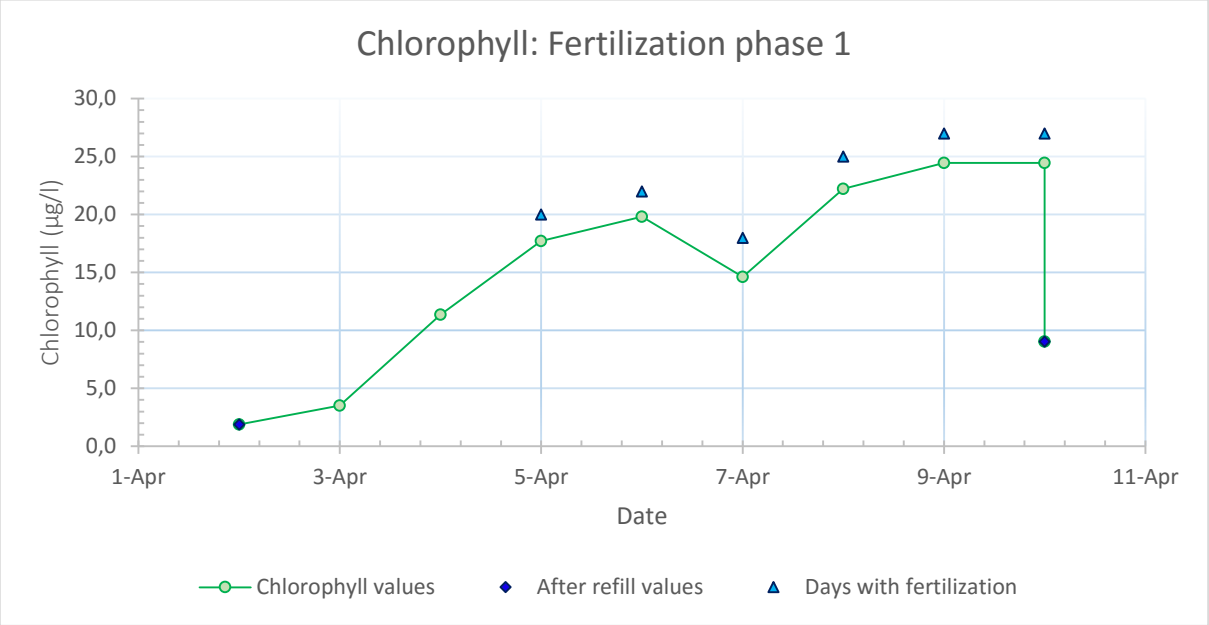

FIGURE 27 THE CHLOROPHYLL VALUES THROUGH FERTILIZATION PHASE 1

The blue triangles in the diagram mark the days where nutrients were added to the tank (figure 27). The growth period lasted from 2. Apr. to 11. Apr. Lowest value was measured in the start at 1.9 $\mu g\ l^{-1}$, and the highest at 24.5 $\mu g\ l^{-1}$ at the end of the period. The growth rate for this period is based on the values of 02.- and 9. Apr. it was calculated to be 0.37 $double\ d^{-1}$.

9.- and 10. Apr. values showed 24.5 $\mu g\ l^{-1}$. These values are not correct, because the CTD was set at a range between 0 to 24.5 $\mu g\ l^{-1}$. However, the ARV at 10. Apr. was measured to 9 $\mu g\ l^{-1}$ after replenishing around 90% of the water. This suggests that the real value could have been as high as 90 $\mu g\ l^{-1}$. This error most likely lead to a lower growth rate.

Despite daily fertilization from 5. Apr., the growth of chlorophyll decreased over time. From 6. - 7. Apr. the chlorophyll values decreased respectively from 19.8 to 14.6 $\mu g\ l^{-1}$.

The fact that the culture was maintained over an extended period of time, at higher values than earlier in the experiment, indicates that the fertilization had a clear positive effect.

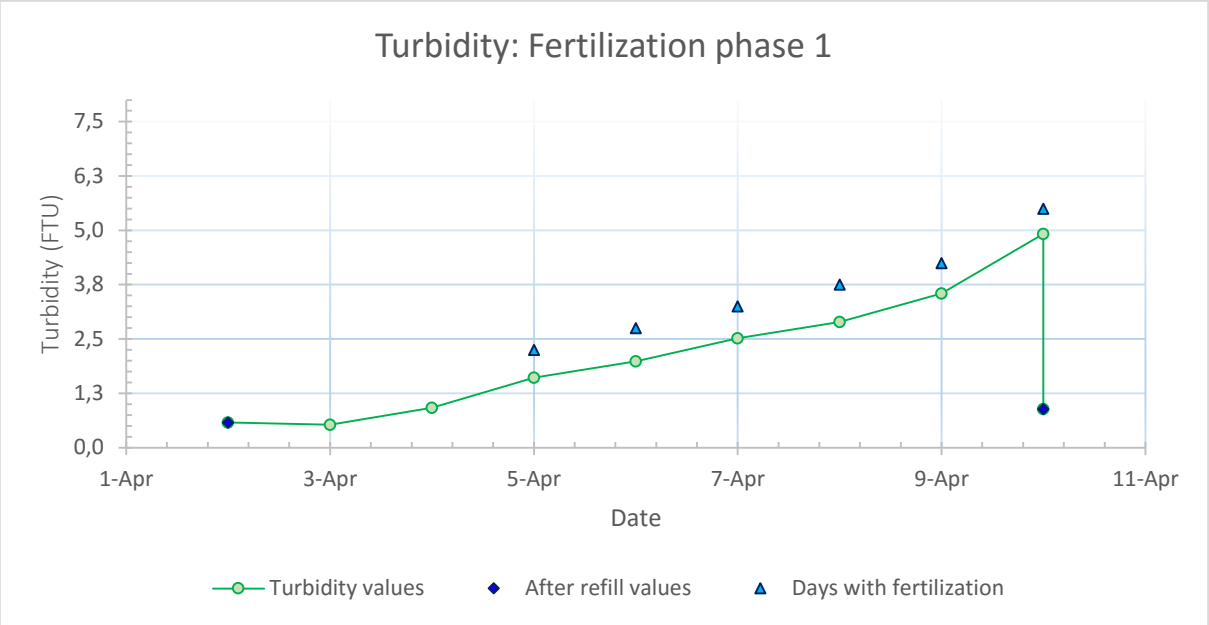

FIGURE 28 THE TURBIDITY MEASURED IN FTU THROUGH FERTILIZATION PHASE 1

The turbidity increased steadily as fertilizer was added to the tank (figure 28). This can indicate that the biomass was increasing despite variations in the chlorophyll measurements. The lowest value was at the 02. Apr, reading 0.58 FTU. The highest value was measured 10. Apr. at 4.92 FTU. The growth rate through fertilization phase 1 was 0.267 $double\ d^{-1}$

10. Apr. a sample was placed under microscope to quickly assess the algae composition in the tank. The sample confirmed that *S. costatum* was clearly the most abundant specie, but *Thalassiosira* spp. and *Chaetoceros* spp. were also spotted.

At the end of this period the CTD-device was sent to service, resulting in a gap in measuring values until it was returned 19. Apr. During this time period, nutrients were still added daily. 18. Apr. the tank was drained to 10 cm, and then refilled. Even though no measurements were taken this day, the water had visibly higher turbidity than any of the previous days, suggesting a high content of algae.

Growth phase w/ fertilization 2

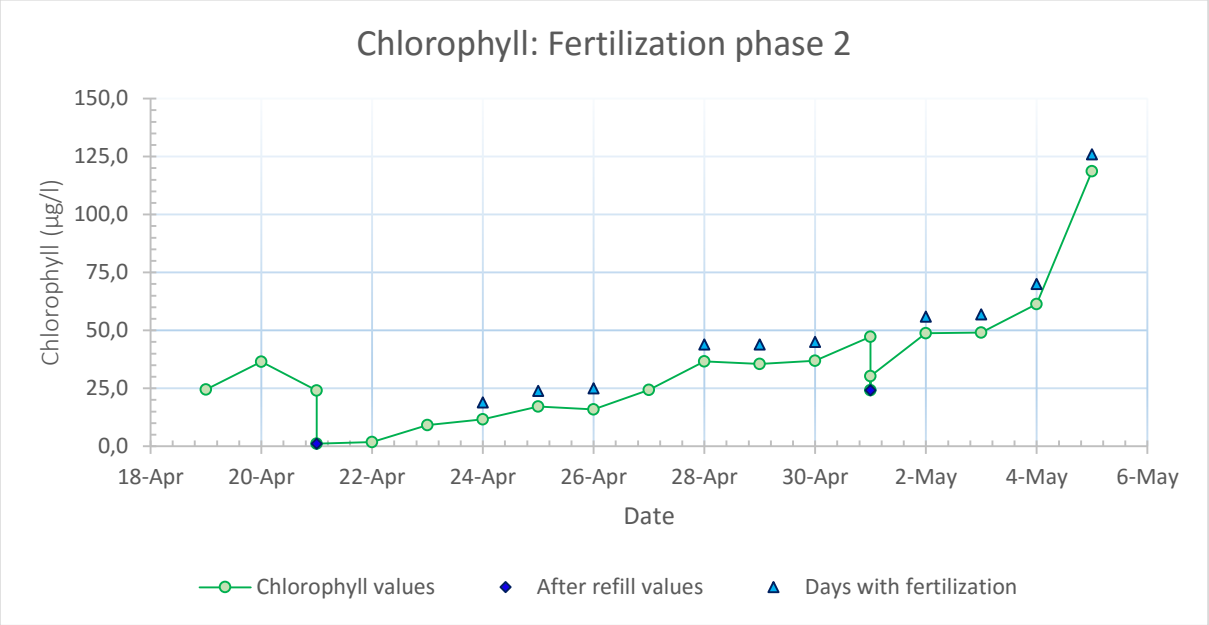

FIGURE 29 THE CHLOROPHYLL VALUES THROUGH FERTILIZATION PHASE 2

Lowest value measured through this period was the ARV 21. Apr. at 1.12 $\mu g\ l^{-1}$ (figure 29). The highest value before refill was 1. May at 47.3 and the growth rate was calculated to be 0.33 $double\ d^{-1}$ during this period.

After refill 1. May two measurements were conducted. The first one right after the refill at around 10.00 h and is represented as the ARV. The other was conducted around 17.00 h, measuring 30.33 $\mu g\ l^{-1}$. The highest chlorophyll values recorded, during the day, throughout the experiment was measured 5. May. at 118.66 $\mu g\ l^{-1}$. This measurement was done in a bucket with a 1/3 ratio of tank water. The growth rate between the two latter values is 0.4 $double\ d^{-1}$.

The values of chlorophyll measured 20. Apr. indicates a presumably high amount of algae in the tank before the refill of 18. Apr. Nutrients were not added between the refills. This could explain the following decrease in chlorophyll from 20. Apr. as algae could have depleted the nutrients in the tank faster than anticipated, thus limiting their growth.

The CTD device was recalibrated on the 19. Apr., after the measurements had been taken. This explains why the measurements still averaged at 24.46 $\mu g l^{-1}$, as the calibration of the device was still set to measure values between 0 – 25 $\mu g\ l^{-1}$.

The measurements from 21. Apr. shows an unexpectedly high difference in the chlorophyll values before and after refill. The measurements before averaged at 24.091 $\mu g l^{-1}$, the measures after

averaged at 1.188 µgl^{-1}. The expected value after the refill was approximately 2.400µg/l as the tank was drained down to 10% from the original amount.

The mean value measured on the 22. Apr. was 1.87 µg l^{-1}, it then rose to 9.19 µg l^{-1} the next day. This unexpected high growth on the 23. Apr., and the also unexpected high measured values for dissolved oxygen and turbidity on the same day, indicates that the CTD-device malfunctioned.

The lower chlorophyll values on 26. Apr. were seen as an indication of over-fertilization, resulting in the brief pause in fertilization seen in figure 29 as the lack of a blue triangle on 27. Apr. shows. On 1. May half of the water in the tank was replenished, measuring the expected decrease in fluorescent values.

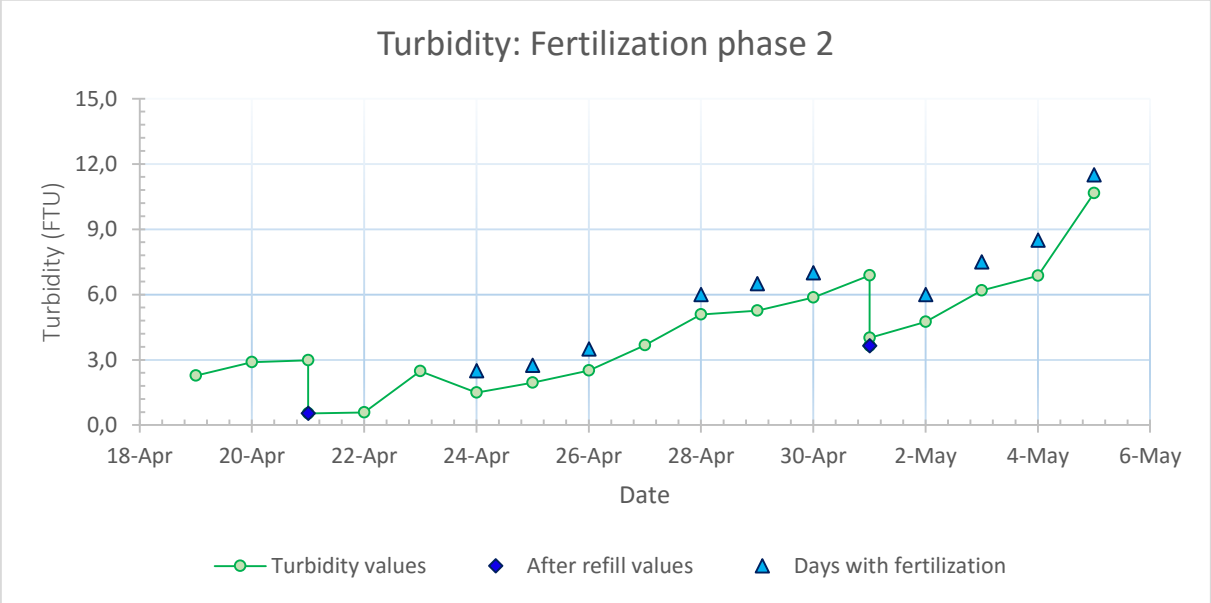

FIGURE 13 THE TURBIDITY MEASURED IN FTU THROUGH FERTILIZATION PHASE 2

The minimum mean value measured was the ARV on 21. Apr (figure 30). The maximum mean value was measured on the last day of the phase, 5. May.

An unexpected spike value occured on the 23. Apr. due to assumed malfunction of the CTD device.

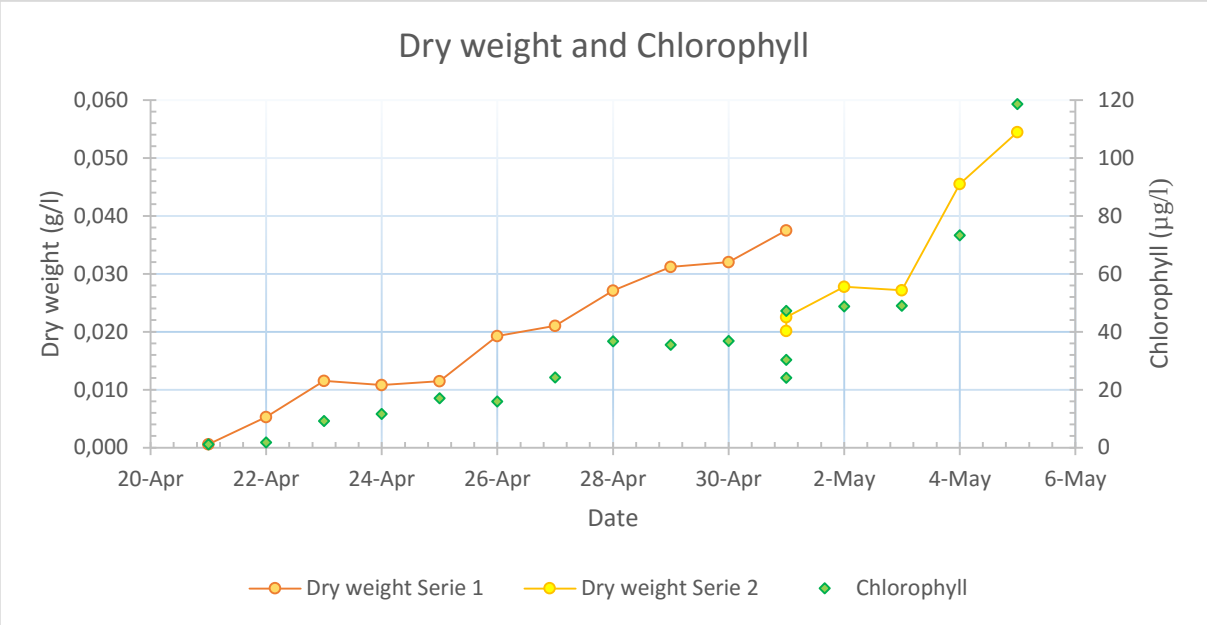

FIGURE 14 DAILY DRY WEIGHT AND CHLOROPHYLL VALUES BETWEEN 21. APR. AND 5. MAY.

The green dots in the graph represent the chlorophyll values at the same days as the biomass samples (figure 31). The correlation is strong between the two measures, though the chlorophyll values tend to vary more than the biomass values.

At the 1. May, 50 % of the water in the tank was replenished, the process finished at 10.00 h and measurements were conducted before and after. At 16.30 h new measurements were conducted. The measurements before the refill showed a dry weight content of 37.5 mg l^{-1} and chlorophyll values at 47.24 µg l^{-1}. Respectively, the ARV showed 20.1 mg l^{-1} and chlorophyll values at 24.17 µg l^{-1}. The 16.30 h measurement showed 22.5 mg l^{-1} and chlorophyll values at 30.33 µg l^{-1}.

From 2- to 3. May there was a slight decline in both measurements. This may have been due to the algae depleting the nutrients in the refilled water. On 3. May the measurements showed an increase in algae growth, this may result from the nutrients applied 2. May being fully dissolved.

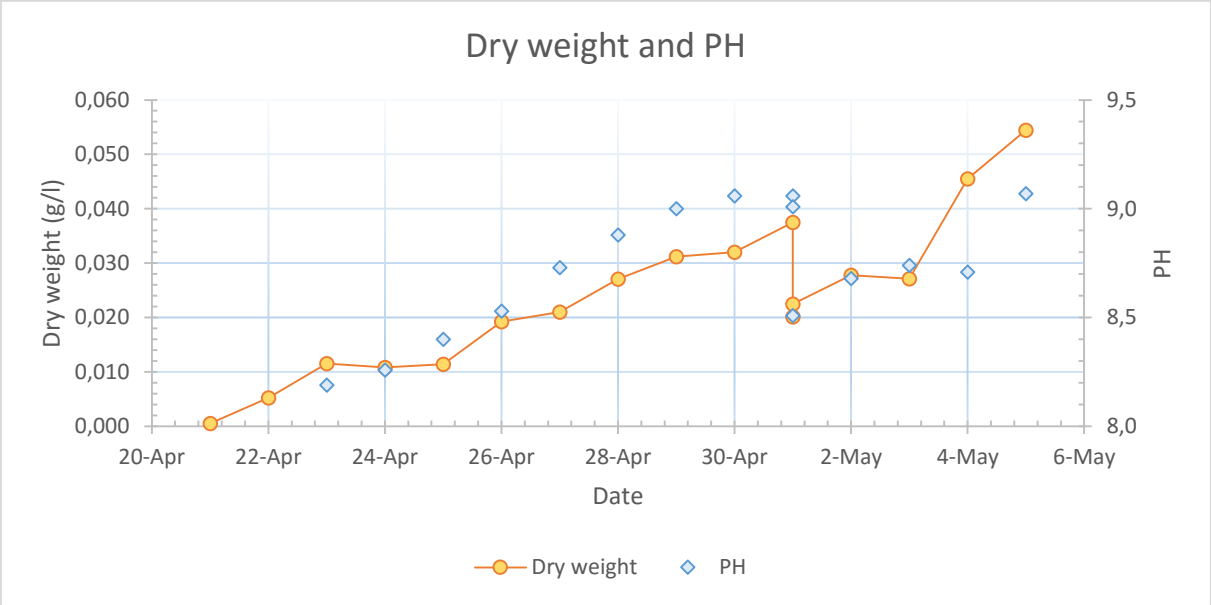

FIGURE 32 THE DRY WEIGHT (GL^{-1}) AND PH DEVELOPMENT FROM 21. APR TO 5. MAY

The pH on 22. Apr. was 7.96 (figure 32). The pH rose to 9,06 during the time to 30. Apr. The pH values measured 8.65 on 1. May. due to the refill. On 5. May. it had risen to 9.07 over a period of 4 days.

This diagram clearly shows the correlation between the amount of dry weight and PH in the water. This is a result of the photosynthesis of the algae.

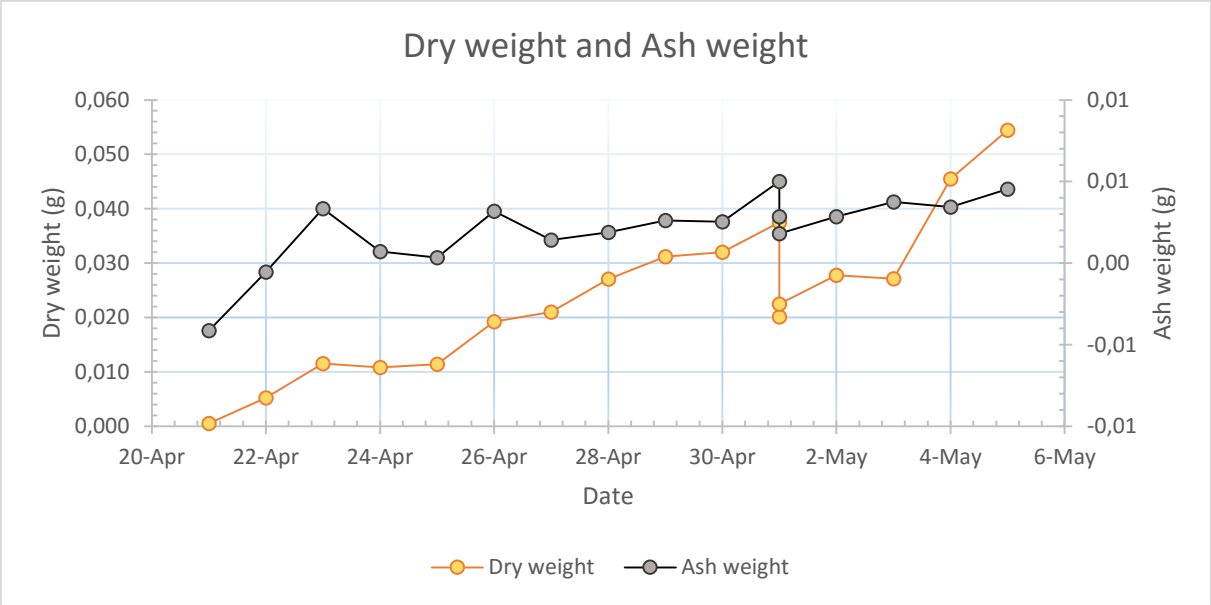

FIGURE 33 THE DRY WEIGHT AND THE ASH WEIGHT OF THE MICROALGAE FROM 21. APR. TO 5. MAY.

Figure 33 shows the dry weight and ash weight over the period. A clear correlation would be expected; The negative ash weight values in the start of the phase was most likely linked to the low margin of error due to the indirect salt weight calculations. Throughout the measurements, increasing dry weight compared to ash weight indicates either an increasing energy content in the algae or that the impact of the measurement errors is lessening.

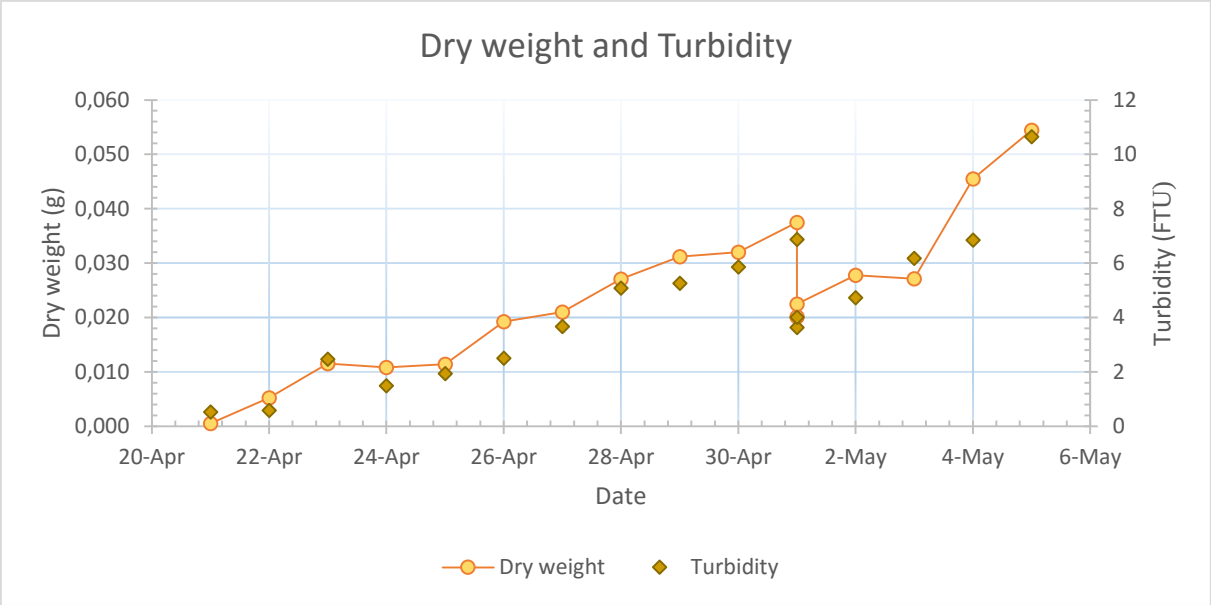

FIGURE 15 THE DRY WEIGHT (GL^{-1}) AND TURBIDITY (FTU) FROM 21. APR. TO 5. MAY.

The turbidity and dry weight share the same growth pattern throughout the period illustrated in figure 34. This indicates a correlation between dry weight and turbidity. The drop in values on 1. May is due to refill of the tank.

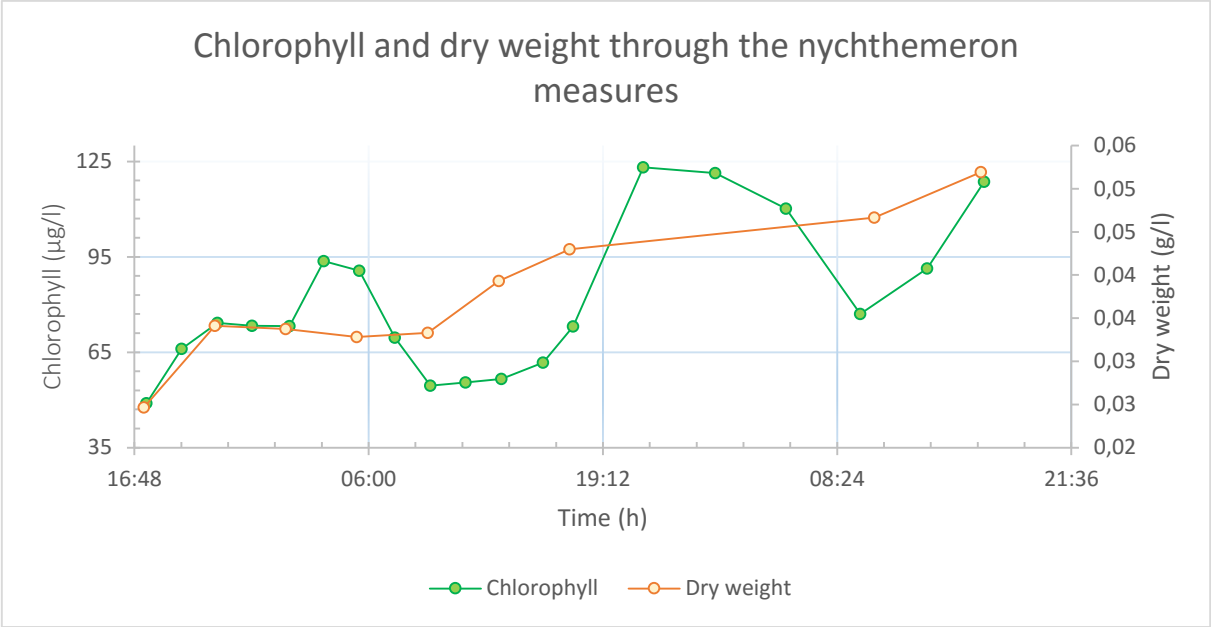

FIGURE 35 CHLOROPHYLL AND DRY WEIGHT THROUGH THE NYCHTHEMERON MEASURES

The chlorophyll in the algae fluctuates greatly through the nychthemeron measurements (figure 35). High values are observable during the night, while the chlorophyll sinks during the day. Highest chlorophyll value measured during the whole experiment was 4. May. at 21.28 h. The value was 123 $\mu g\ l^{-1}$. The measurements ended 16.30 h at 5. May. The measured chlorophyll value at this point was 118 $\mu g\ l^{-1}$. The dry weight concentrations nearly doubled during the nychthemeron measurements. From 27 $mg\ l^{-1}$ at 16.48 h at 3. May, to 54 $mg\ l^{-1}$ at 16.41 h, 5. May.

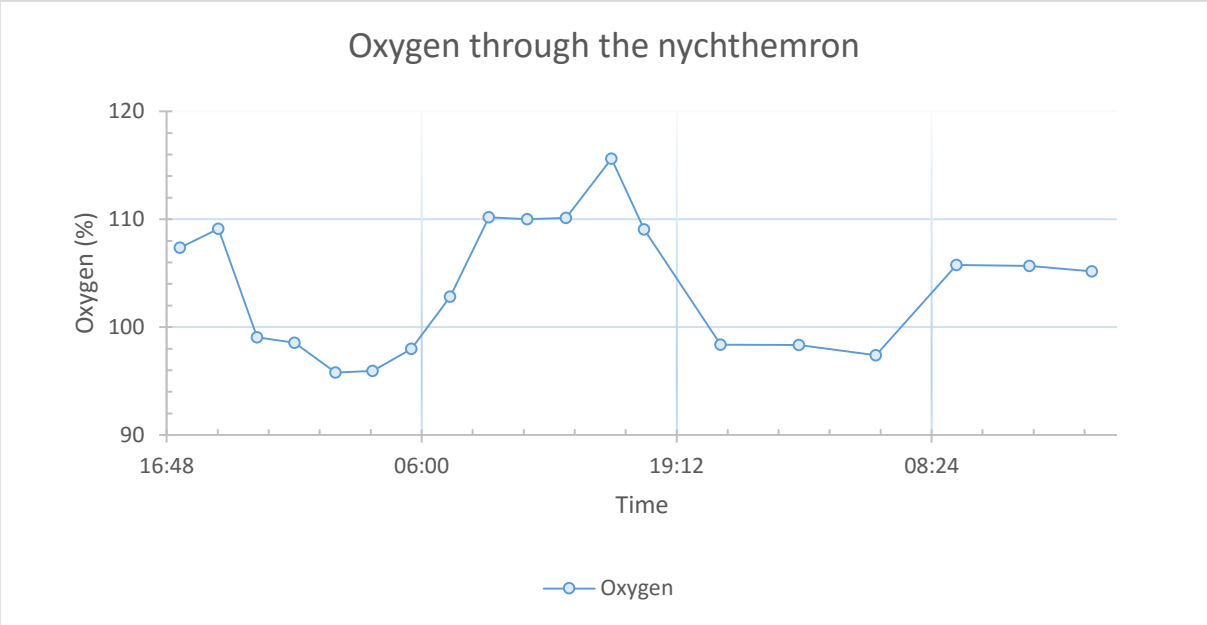

FIGURE 36 THE OXYGEN MEASUREMENTS THROUGHOUT THE NYCHTHEMERON

The oxygen concentrations in the water vary greatly throughout the period (figure 36). The variations were expected, and due to algae releasing O_2 into the water during photosynthesizing. However, at night O_2 is consumed due to respiration from microalgae and other microorganisms such as bacteria. The measurements indicate that the cultures probably were with oxygen during the nights through the experimental period.

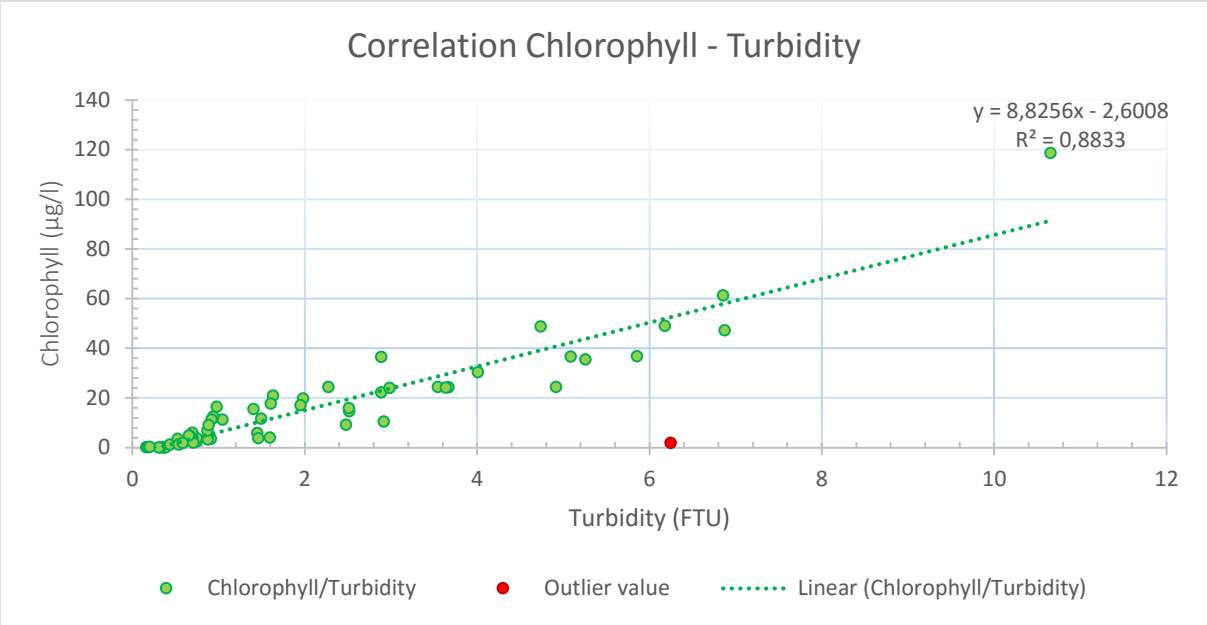

FIGURE 37 THE CORRELATION BETWEEN CHLOROPHYLL (μGL^{-1}) AND TURBIDITY (FTU) DURING THE NYCHTHEMERON

Figure 37 proves a strong linear correlation between turbidity and chlorophyll content in the tank based on 58 daily measurements taken through the experiment. The reason for strong correlation is due to algae being the major component affecting the light penetration in the tank. The outlier plot, from 16. Mar., has not been included in the linear equation in the figure above. This is because it is due to a measurement error and did not reflect the real situation in the tank. If it were to be included, it would have reduced the slope of the trend line.

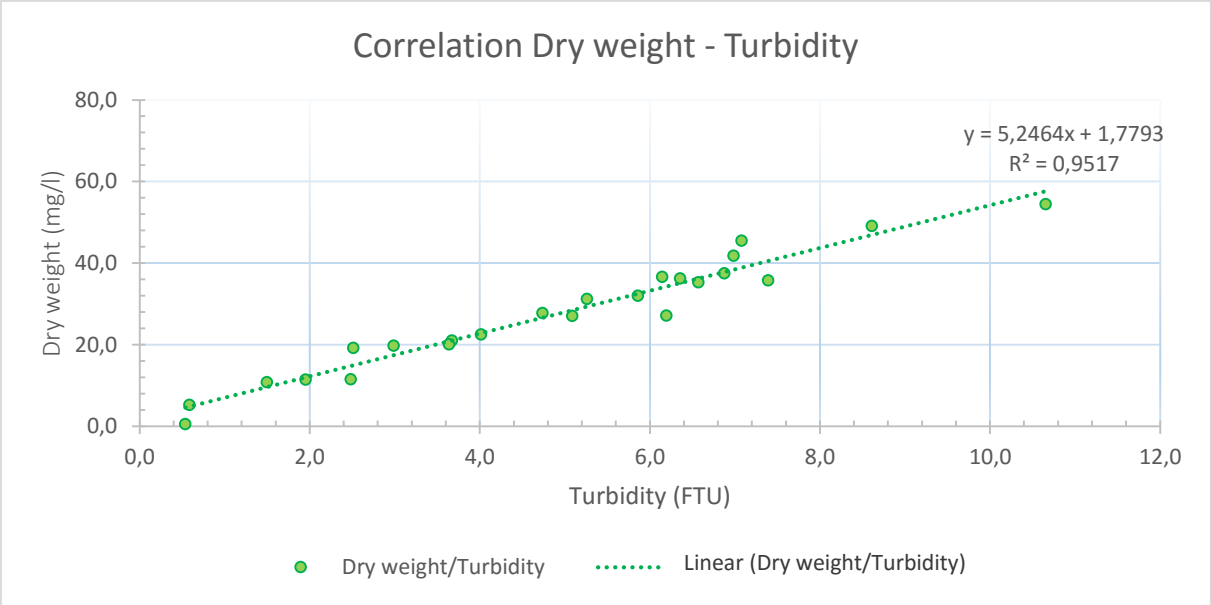

FIGURE 38 THE CORRELATION BETWEEN DRY WEIGHT (MGL^{-1}) AND TURBIDITY (FTU) VALUES FROM 21. APR. TO 5. MAY

The plots in figure 38 are based on all daily measurements from 21. Apr. to 5. May. The diagram shows the turbidity at the same time as the biomass samples were taken from the tank.

This indicates that it should be possible to determine an approximately amount of dry weight of biomass, based on the turbidity levels in the tank.

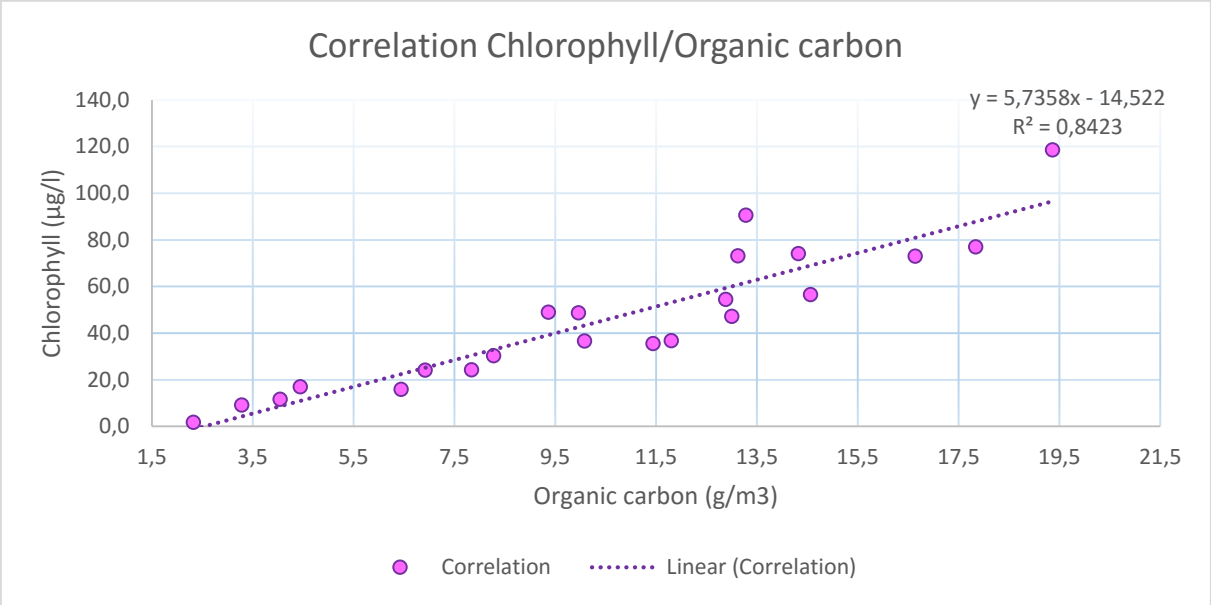

FIGURE 39 THE CORRELATION BETWEEN CHLOROPHYLL AND ORGANIC CARBON IN THE TANK

The graph is based on all daily measurements of dry weight and chlorophyll from 21. Apr. (figure 39). The correlation is apparent and similar to the correlation between dry weight and chlorophyll.

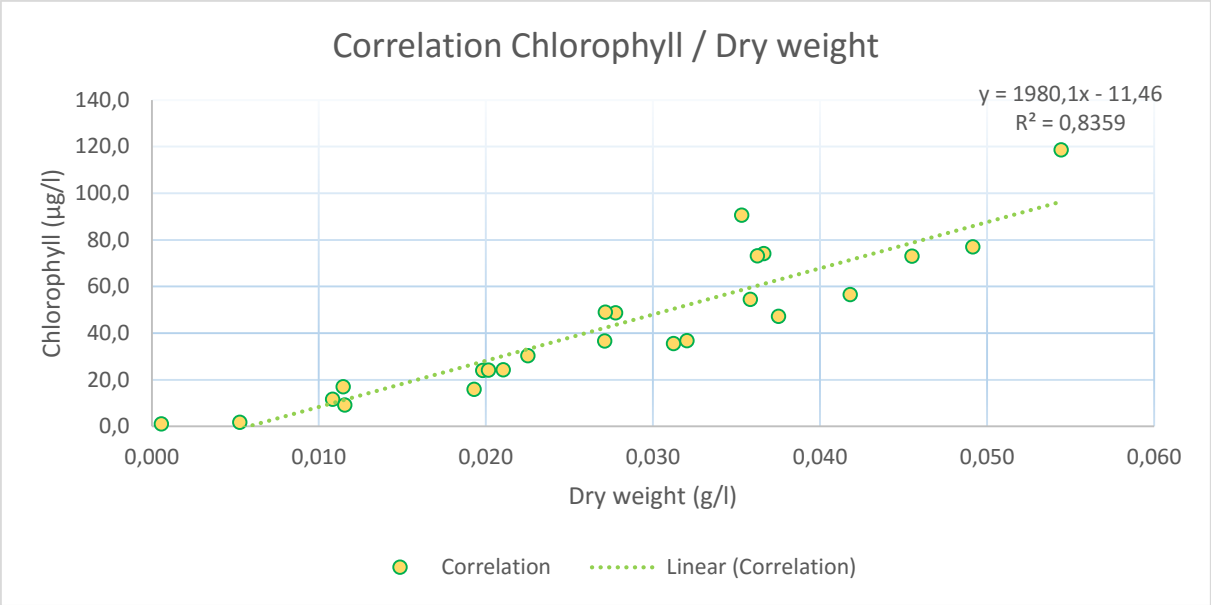

FIGURE 40 THE CORRELATION BETWEEN CHLOROPHYLL (µG l^{-1}) AND DRY WEIGHT (G l^{-1}). THE SLOPE VALUE IS ALSO INCLUDED IN THE DIAGRAM.

The plots in figure 40 shows the chlorophyll values and the dry weight of the biomass samples taken at the approximately same time of the same day.

As the figure above shows, there is a correlation between the chlorophyll values and the dry weight concentration. Most of the measured values lies beneath the trend line, indicating some spike values that increase the linear slope. This is visible in the figure above.

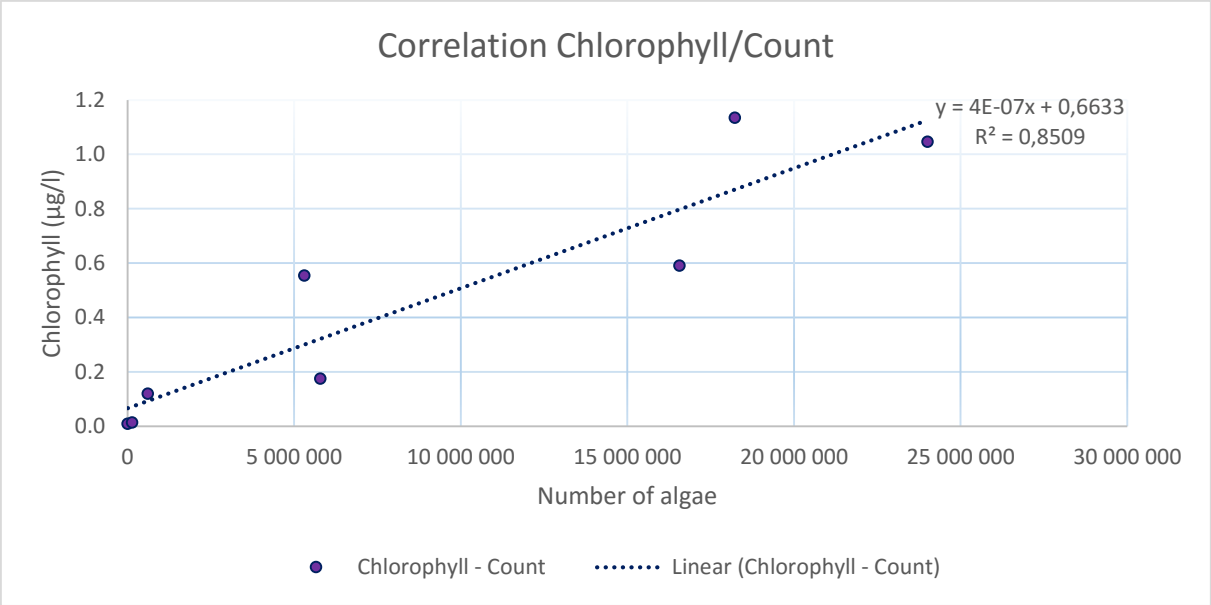

FIGURE 161 THE CORRELATION BETWEEN CHLOROPHYLL (μGL^{-1}) AND THE ALGAE COUNT

There is a weak correlation between the chlorophyll and count values (figure 41). Even so, it has been disregarded due to the low amount of available data to make a reliable graph.

Discussion

Main margins of error

Lack of competence and experience of the people involved could have led to errors during the experiment. The experiment is in a new field of bachelor theses at HiSF. Many of the problems encountered could have been avoided with better knowledge about the methods- and equipment used.

Other margins of error

On the 23. Apr., the CTD-device most likely provided too high values of chlorophyll, turbidity and dissolved oxygen in the tank. The reason for the deviation this day is uncertain.

The method used to measure the water level in the tank may have been inaccurate. The measuring tool was a homemade ruler, with accurate measures. The inaccuracy is caused by human errors during the manual measurements. Since the bottom of the tank was conical, the water level was measured at approximately the same spot, but it may have varied a couple of centimeters. This will affect the chlorophyll-, turbidity- and salinity ratio before and after replenishing. It is considered to give the results a minor margin of error of a couple %.

Chlorophyll

Chlorophyll measurements were used as the main indicator for the algae growth during the experiment. In hindsight it would have been more accurate to compare the chlorophyll values to the algae count and dry weight values from the start. This would provide more comprehensive indications about the actual biomass in the tank.

Chlorophyll levels have shown strong correlation with both dry weight and turbidity values. However, the values tend to vary more than the two latter, sometimes resulting in misleading results compared to biomass and turbidity measurements.

During the first phases of the experiment, the CTD device was calibrated to a low chlorophyll range between 0-25 $\mu g\ l^{-1}$. This resulted in lower than realistic chlorophyll values on 9.-, 10.-, and 19. Apr. When calibrated to the low range, the device provides more accurate data at lower chlorophyll levels, but is unable to measure higher values than the set range. Due to lack of knowledge about the calibration of the chlorophyll sensor, the reason for the stagnated values went unnoticed until after the measurements on 10. Apr. The CTD-device was recalibrated to measure between the range of 0-75 $\mu g\ l^{-1}$, after the measurements were conducted on the 19. Apr., which was the day the device was returned from service.

There may be several explanations for the varying chlorophyll values. Diel variations of cell chlorophyll content in *S. costatum* can cause measurements to differ throughout the nychthemeron. Varying light conditions also affect the chlorophyll cell content, as *S. Costatum* produce more chlorophyll in lower light conditions (Anning et al., 2000) to maintain the photosynthesis. Also a denser culture would cause less light to penetrate the water, leading to a higher chlorophyll content in the algae as the biomass content increases.

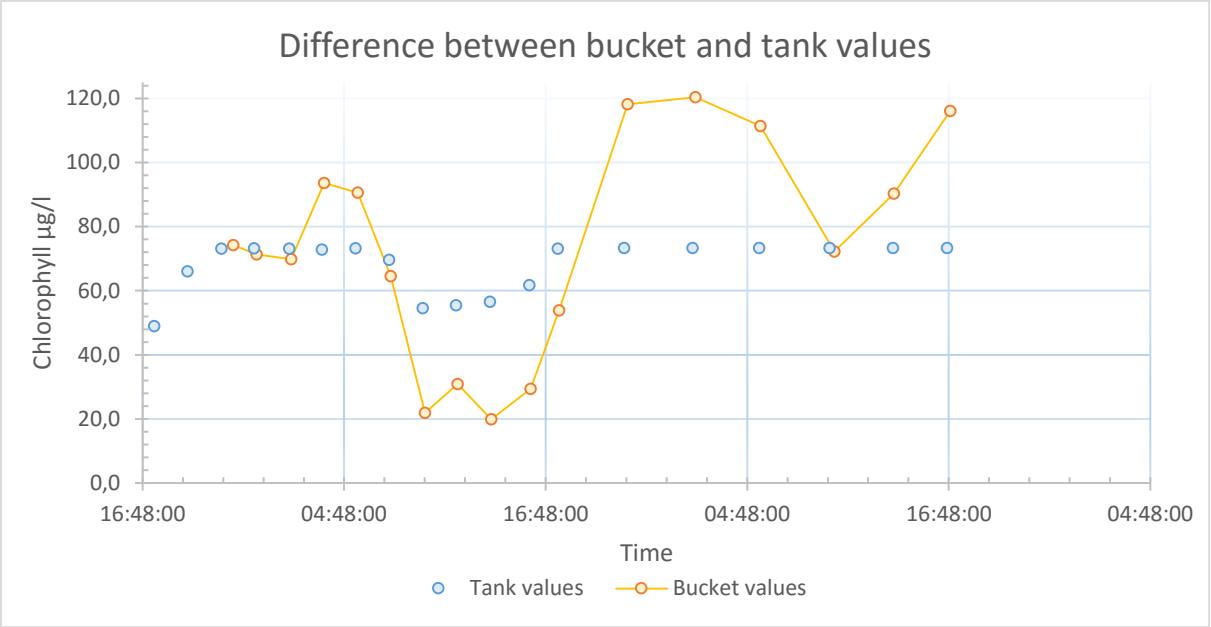

FIGURE 172 THE CHLOROPHYLL VALUES MEASURED IN THE TANK AND IN THE BUCKET THROUGH THE NYCHTHEMERON

During the nychthemeron measurement, the bucket measurements provided unrealistic low results. The chlorophyll values measured in the bucket was much lower than the corresponding values in the tank, as shown in figure 42. The tank values are represented with the blue plots, and the bucket values with the orange plots. The reason for the flat tank values at 73 µg l^{-1}, is due to the measuring range of the device reaching its max value at 75 µg l^{-1}. During the bucket measurements the values reached as low as 19.8 µg l^{-1} 13.33 h at 4. May, while the tank values measured 56.6 µg l^{-1} at approximately the same time. The difference between tank- and bucket values was not as great during the early morning and in the evening. During the day the bucket values was considerably lower than the tank values.

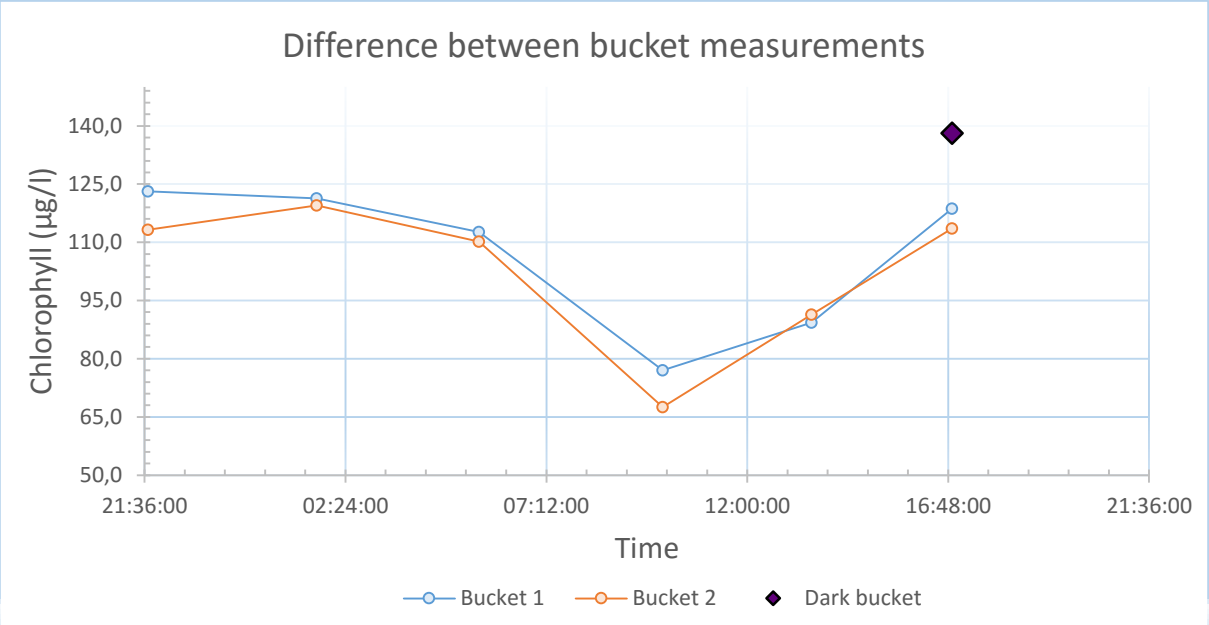

FIGURE 183 THE DIFFERENCE BETWEEN BUCKET MEASUREMENTS TAKEN DURING THE LAST NYCHTHEMERON

As the bucket measurements differed from the tank values, two bucket measurements were conducted during each tank measurement from 21.40 h on 4. Apr. to 16.50 h on 5. May (figure 43). This was done to assess if the time the algae had been exposed to the extra light in the white bucket compared to the tank could have affected their chlorophyll content. The orange plots represent the bucket which had been exposed to light for 10 minutes before measurement, the blue plots represent the measurement taken right after filling of the bucket. As seen in the graph, the short difference in time had little effect on the chlorophyll measurements.

During the measurements conducted at 16.50 h on 5. May, a third bucket measurement was conducted. The purpose of this measurement was to assess if light could affect the fluorescence sensor directly. This measurement was conducted in a black bucket, and covered, so that light could not affect the device. This measurement showed a chlorophyll value of 138.2 $\mu g\ l^{-1}$, compared to 118 $\mu g\ l^{-1}$ and 113.6 $\mu g\ l^{-1}$ from the measurements conducted in the white bucket. The bucket values during the nychthemeron measurements indicates that light might affect the fluorescence sensor, especially on days with strong sunlight, leading to underestimation of the chlorophyll concentration. This will also affect the calculation of the growth rates based on chlorophyll values.

The bucket measurement method is not accurate, since the chlorophyll values of the deep water were not taken into consideration. The reason for this is that the chlorophyll values of the deep water was very low, around 0.1 $\mu g\ l^{-1}$. At this point in the experiment, the chlorophyll levels in the tank were maxed at 75 $\mu g\ l^{-1}$, and an additional 0.1 $\mu g\ l^{-1}$ would not affect the values noticeably. It is therefore not assumed to have an impact on our data.

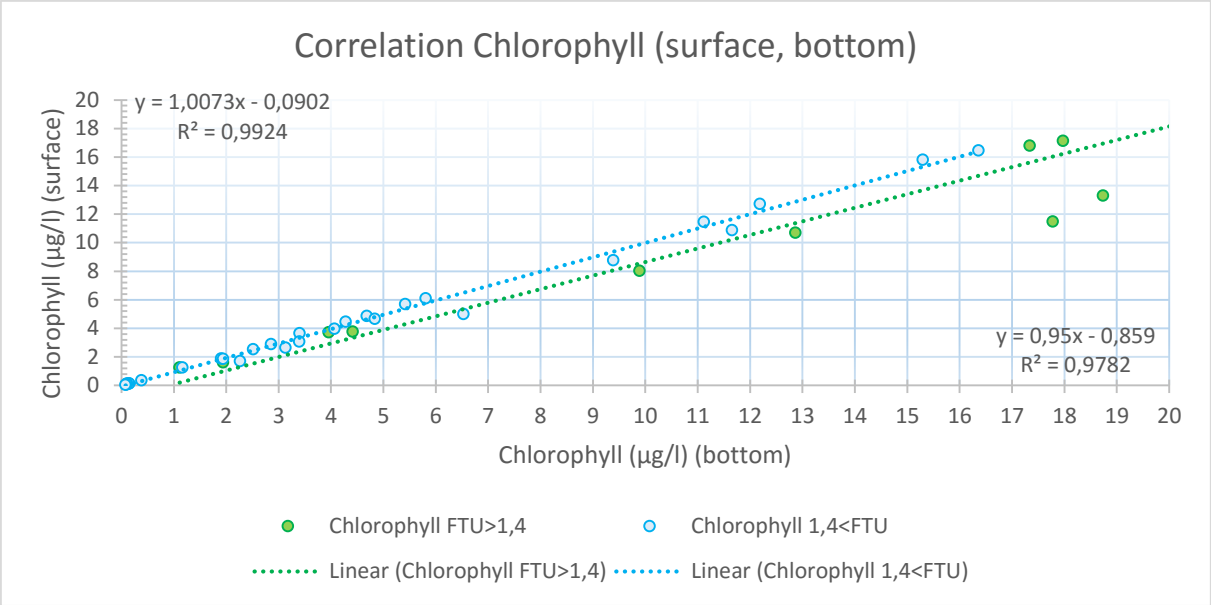

FIGURE 44 THE CORRELATION BETWEEN SURFACE AND BOTTOM CHLOROPHYLL MEASUREMENTS, AT LOW (FTU<1.4) AND HIGH (FTU>1.4) TURBIDITY LEVEL

The diagram, in figure 44, consists of the mean chlorophyll values of each surface and bottom measurement, from all daily measurements. The y axis shows the chlorophyll measurements taken at the surface, and the x axis represents the bottom values. The chlorophyll values that were higher than 20 µg l^{-1} Was included in the diagram, but is out of the visible range. The measurements were

The 58 values were divided into two categories, 29 measurements each. The ones where mean turbidity value of the combined surface and bottom measurements were below 1.4 FTU is shown in blue. The chlorophyll measurements where combined and surface bottom measurements exceeded 1.4 FTU are shown with the green plots.

The slopes in the diagram shows that the top and bottom values follows a 1:1 pattern when the turbidity < 1.4 FTU, but a slightly different 1:.095 pattern when the turbidity > 1.4 FTU. This indicates that when the turbidity is high, chlorophyll values measure higher in the bottom, then in the top of the tank. The difference is around 5 %.

The reason for the difference between top and bottom measurements with increased turbidity is not known. If light is affecting the chlorophyll sensor, it might be linked to increased differences in light between surface and bottom in the tank when the turbidity is at high levels. Also it might be linked to sedimentation of the algae during measurements.

Salinity

During the experiment, measurements showed many unrealistic salinity values. The reason for the strange values might partly have been due to low power on the CTD device, as the conductivity sensor needs to produce a current to measure the salinity levels in the water. However, when the CTD device returned from service with new batteries on 19. Apr, it still provided some strange results.

After dialogue with the supplier, it became clear that small air bubbles trapped in the glass cylinder of the measuring tube could cause measurements to show lower values than the actual salinity level (T. Dale, personal statement). This is a possible reason for some of the measurement spikes, which were always lower than the mean value. This indicates the stable majority of the measurements, at about 34.5 ‰, to be correct. The salinity content is not expected to vary much from the mean value.

A possible explanation for the higher salinity values could be linked to a combination of the aeration, evaporation of the tank water, and local wind conditions. As the bubbles pierced the water surface, small water droplets were observed to scatter in the air as the bubbles burst (T. Dale, personal statement). In case of calm wind conditions, water droplets would evaporate, while the denser salt particles would fall back into the tank water. This process could be causing some of the higher salinity values throughout the experiment. In the case of heavy wind conditions, the salt particles would be blown away, neutralizing the process. This is assumed to have a minor effect on the salinity levels, but could explain some of the values above 34.5 ‰.

As the salinity levels in the tank were used to calculate the salt weight of the biomass samples, correct salinity values were important for correct dry weight values.

Turbidity

The turbidity measurements were considered reliable through most of the experiment, except for the major spike measurement on 16. Mar. and one measurement at 21.21 h on the 4. May during the nychthemeron measurements. The spike measurements were likely caused by some bigger and smaller particles sticking to the turbidity measuring device. During the latter measurement error, all turbidity values in the dataset showed 12.45 FTU

The turbidity measurements correlated with chlorophyll values, and proved even stronger correlation with dry weight values. For this reason, turbidity could have been more actively used for *in situ* interpretation of the algae culture.

The correlation shows that the turbidity values are highly transmissible to the biomass content in the tank during the experiment, with the applied time periods between refills. If longer time periods had passed between the refills, the content in the tank would be expected to consist of a higher percentage of dead algae and heterotrophic bacteria. In this case, the correlation between turbidity and chlorophyll would be altered.

The strong correlation between dry weight and turbidity can be used to interpret some of the unexpected chlorophyll measurements during the different phases of the experiment. If chlorophyll values unexpectedly decline while the turbidity values stay stable or increases, this can indicate errors in the chlorophyll measurements, or growth of heterotrophic bacteria. This might indicate that turbidity measurements can be a good tool for assessing the growth of *S. costatum* under similar conditions.

The turbidity increased significantly during the later phases of the experiment, causing self-shading. This made the vertical turbulence more important, as the algae would have to circulate faster for better sunlight distribution. This was one of the reasons for the increased aeration from the 1. May and onwards.

Oxygen

During the experiment the oxygen levels in the tank varied greatly. On the 21. Mar. and the 20. Apr. the CTD-measurements were taken when the air pump was still on. This most likely influenced the data on these dates, providing higher percentages of dissolved oxygen than the real value in the tank. This is noticeable in figure 17.

Another reason for the varying oxygen values was due to wrong calibration of the oxygen sensor, which led to incorrect data. The sensitivity of the oxygen electrode usually decreases with time (T. Dale, personal statement) and should therefore have been recalibrated. During the experiment, the air-values sank from approximately 101% to approximately 90%. In addition to this, the air values varied a lot on a day to day basis. This was calculated into the correct measurements by subtracting the air measurements from 101% which should be the correct air value. This gave an indication of how much the calibration was off, and when the difference was added to the mean values of the measured data, it resulted in more accurate values. The dissolved oxygen values in the results are based on the manually adjusted values.

The oxygen device needed time to adjust to the different oxygen levels in the water, so the first half of the oxygen values during each measurement were incorrect. In the making of the oxygen diagrams only the last 10 oxygen values of each measurement were used to calculate the mean oxygen value.

The oxygen value before refills averaged at 105 %, while the after refill values averaged at 75.96 %. After each refill however, the oxygen quickly rose back to the normal average. This is a strong indication of photosynthesis.

Biomass samples/filtering

After burning and drying, it is recommended to let the filters cool off to room temperature in a desiccator, before they are weighed. It is also recommended to weigh filters just before use (Hauer and Lamberti, 2011). These factors have not been taken into consideration, and may influence the constant value of the filters during the experiment. This is not assumed to have a noticeable impact on our results.

In general ecology lab experiments at HiSF, and according to (Hauer and Lamberti, 2011) the drying period for the filtered biomass samples was 24 hours. During this experiment the biomass filters were put in a pre-heated oven to 105 °C for only 1 hour. This is because the filters have very low biomass attached, which make them dry faster than larger samples used in earlier lab experiments. At one time during the experiment a sample was dried for 1 hour, and then weighed. The same sample was set to dry again right after weighing, for half an hour, before being weighed once more. Both weighings provided the same value, which indicates that drying for 1 hour is enough to obtain reliable results in the experiment.

During drying and burning of the biomass filters, some of the filters singed to the aluminum trays. This affects the filter weight which was used to calculate dry weight and ash-free dry weight without filter. It is not considered to have a noticeable impact on the dry-weight, but may have a small impact on the ash-free dry weight due to the low values, which makes it susceptible to inaccurate measurements.

The water-samples from 23-28. Apr., was collected after the air pump had been turned off for 15-60 min. During this time the algae could have had time to sink in the tank. This may have affected the amount of algae in the water samples which was taken close to the surface. When compared with the turbidity values for this period, no noticeable impact on the biomass values are observed.

The last water samples had more biomass which made the filtering process go much slower compared to the first samples. This may affect the amount of biomass being stuck on the walls of the glass beaker during filtration, resulting in less biomass in the filter. This is not considered to be a noticeable factor on such a low scale experiment.

Dry weight from the biomass samples indicated unrealistic high amounts of biomass, and the dry weight between the samples did not vary according to the predictions. Because of this, different methods were applied to attain realistic biomass values.

One of the applied methods was filtering water samples from the deep water. This was done to find the particle weight in the deep water, to subtract it from the dry weight. As the particles from the deep water was added to the tank during the replenishing process, it was assumed that these particles constituted a significant part of the dry weight. However, there were too many uncertainties regarding how the particle weight would affect the biomass samples. The collected data was therefore not applied in the dry weight and ash weight calculations.

The salt weight method is not accurate, since the water in the wet Whatman filters vaporizes quickly as they are transported to the scale for weighting. This results in inaccurate values for water weight, which is used to calculate the salt weight. A Whatman filter can absorb a given amount of water which varies from filter to filter, but the water weight also includes the amount of water absorbed by the attached biomass. This might have had an impact on the ash weight measurements, but is negligible in the dry weight estimations

Another reason for the method's inaccuracy is because the measured values are very low. Small variations, due to human- and method-errors, results in a larger impact on the values and data used. The weight had an accuracy of ± 0.3 mg. This could have had an impact on the ash weight calculations, but is negligible in the dry weight estimations.

Still, this method can be used as an indication for calculating an approximate amount of biomass in the dry-weight, and therefore water weight was measured from 21. Apr. until the end of the experiment.

The dry weight number do not necessarily reflect the number of algae in the tank. The filters used had small enough mesh to filter out many types of bacteria. According to (Azam et al., 1983), bacteria can utilize 10 to 50 % of the carbon fixated by photosynthesis in the sea. The bacterial biomass is strongly linked to the algal biomass, and the dissolved organic matter (DOM) release of the algae. DOM release is strongly affected by the algae's access to nitrogen. Nitrogen depletion would lead to the algae releasing more DOM which the bacteria can thrive on (Azam et al., 1983). The concentration of the bacterial biomass in the tank has not been analyzed. However, the bacterial biomass in the tank is expected to constitute a significant part of the biomass, and hence the dry weight.

Algae count

Human errors must be taken into consideration during the counting process. One reason for this was difficulties in distinguishing *Melosira* spp. and *Thalassiosira* spp. as individuals, depending on the angle they had sedimented. It was also hard to spot *S. costatum* as individuals, and distinguish them from dirt particles. Some of the individuals and colonies may also have been counted twice. The *S. costatum* count may therefore not be entirely accurate, but since the process was completed thoroughly, the count-values are considered reliable.

The sedimentation process may not be precise due to human errors, resulting in an uneven distribution of algae in the samples. Since only parts of the sedimented samples were counted, this must be taken into consideration, even though the sedimentation process was executed thoroughly.

In the first sediment-samples, several flagellates were observed. These were included in the chlorophyll and dry weight values. The amount of flagellates decreased significantly with later sediment-samples. Some of the flagellates may have been mistaken for *Chaetoceros* spp. during the count, due to the angle they had sedimented, which made them hard to distinguish from each other.

When the count-values are compared with the chlorophyll values, it is necessary to be aware that not all algae strains in the samples were counted. This results in a lower amount of total algae compared to fluorescence with the first sedimented samples, but the margin of error will be reduced as *S. costatum* gets more and more dominating during the experiment.

pH-measurements

PH measurements were conducted from 23. Apr. This was done in order to check the acidity levels in the water. The acidity content gave an indication of how well the photosynthesis was doing in the tank. A high pH value reflects a low CO_2 content in the water, indicating high photosynthetic activity in the tank. During the experiment, the pH value increased despite the added CO_2 from the air pump, giving a strong indication of photosynthetic activity.

The nychthemeron measurements showed the pH fluctuations through the course of a diel (appendix, table 7). At 17.29 h the pH values started to decline, and reached the lowest point at 07:30, measuring 8.65 before starting to climb again, reaching peak pH value at 16.31 h. measuring 9.07. This confirms that the algae photosynthesis fluctuates throughout the day. Such high values

might be un-beneficial for the algae culture. According to (Jameson, 2006) pH values over 9 can be poisonous for many algae strains.

General info about the experiment

The method applied, Wells-Glancy, was originally developed for shellfish breeding (Anon, 1965). Hence, it was not made for the purpose of cultivating algae to be used in the production of biofuel. This method is not optimal for the cultivation of gene modified algae species, as it is difficult to contain the modified strains. Gene modification is by many considered an essential tool for increasing the efficiency of algal fuel production, and PHBs might be a better alternative for cultivating gene modified strains (Radakovits et al., 2010). Nor is the method aimed to maximize lipid content of the algae. However, the method can be of interest for low investment production, as it is cheaper than many other alternatives for cultivating algae. The data collected during the experiment can therefore be relevant for further research and potential modification of the method.

Typical phases of growth cycles

Algae cultures typically go through 5 phases. The development of an algae culture often starts with a lag phase (Jameson, 2006). During this phase the algae culture has very low growth. The same pattern occurred during this experiment, and the lag phase lasted from 29. Feb. to 9. Mar. The reason for the long lasting lag phase is likely due to a drastic shift in the environment for the algae. The initial algae culture was collected from the top layer of the water in the fjord, and brought to the tank filled with deep water. As the deep water has higher salinity, higher temperature and lower pH at this time of the year, compared to the top 10 m water, the algae need time to adjust to the new environment before the exponential growth can start. This involves physiological adaptation of the cell metabolism for growth, for example increasing the enzymes and metabolite levels that are used in carbon fixation and cell division (Sorgeloos, 1996).

The method used in this experiment probably inoculated zooplankton larvae into the tank. Low growth in the start of the initial phase compared to later in the experiment could therefore also be caused by higher amounts of zooplankton grazing down the algae before the first replenishment.

The reduction of the population for other algae species in the selection of *S. costatum* could have led to a lower chlorophyll increase in the start, as the developing *S. costatum* culture replaced other algae strains. After a while the domination of *S. costatum* was apparent, and this effect would have diminished.

The second phase of an algae culture is the exponential growth phase. When the algae culture has adapted to the new environment and all conditions for growth is met, the culture should grow exponentially (Lavens and Sorgeloos, 1996). Exponential growth was observed numerous times throughout the experiment, the longest lasting 4 days from 9.- 13 Mar., another lasting 3 days between 23.- 26 Mar. and finally 2 days during 30. Mar. – 1. Apr.

When the exponential phase has continued over a period of time, a declining growth phase follows. In this experiment a declining growth phase was observed during most growth cycles, with the exception of the growth cycle lasting from 23. - 26. Mar. During this cycle the tank was replenished before the phase could start. The reason for declining growth phases is linked to some limiting factor (Jameson, 2006). One typical example of a limiting factor which is relevant for this experiment, is depletion of micronutrients such as iron (Fe) and vitamins. Sunlight limitations due to self-shading as the culture thickens, and unbeneficial pH values due to an increased rate of photosynthesis are also relevant limiting factors. As the culture grows, so does the CO_2 requirements. CO_2 might therefore be a limiting factor as the culture grows.

The limiting factors are typically linked to increased biomass. This explains why the longest exponential growth phase was in the start of the experiment, as this phase had a lower initial biomass and took longer to reach the threshold levels for declining growth.

After the declining growth phase follows a stationary phase, and then a death phase (Jameson, 2006). Based on the dry weight diagrams, a stationary phase occurred from 23.- 25. Apr. A death phase was observed at one point during the experiment, and is apparent in the decline in both chlorophyll and turbidity from 29.- 30. Mar. The reason for this decline is believed to be linked to shortage of either N, P or Si, as the culture was maintained at higher biomass values for longer periods without a similar die off during the fertilization phases.

Nutrients

The composition and amount of the nutrient mix was not optimal for the purpose of maximizing the productivity. The nutrient mix was estimated with the intention of providing enough P, N and Si to the culture. This mix does not include all the trace metals or vitamins which is included in optimal mediums for algal growth, such as the widely used Guillard's F/2 medium (Jameson, 2006).One important missing vitamin is B12, which is a necessary component for algal growth (Croft et al., 2005).

However, the choice of nutrients in this experiment is a cheaper and easier alternative, and this would count positive in a larger scale cultivation of microalgae. (Uddin, 2007) found that the use of Guillard's F/4 medium, which is similar in composition to the F/2 medium, but only half the concentration, was significantly more effective than commercial fertilizer. However, they also found that the commercial fertilizer was more cost efficient, and concluded that commercial fertilizer was better for production efficiency and culture stability. In an experiment comparing culture density capacity in an airlift PHB and a bubble column system, the highest cell density achieved was when Si was provided in amounts of 400 % of the normal dosage. This demonstrates the importance of Si.

The nutrients were calculated for a volume of 7 m^3. The actual volume of the tank was 7.4 m^3. This led to a slightly smaller nutrient dosage than the sought out dosage. This has probably influenced the growth of the algae culture slightly.

Growth rates

The growth rates of the different indicators have been calculated throughout the experiment. The periods of the different growth rates were determined based on the apparent growth cycles in the chlorophyll, dry weight, turbidity and algae count graphs. Growth rates based on turbidity and dry weight values were also calculated for the same periods when the data was available. Predominantly, the periods were between the refills, with the exception of the lag phase at the start of the experiment. Growth rates were also determined for several daily increases, to assess the highest achieved growth rates during the experiment.

The growth rates were generally highest when based on chlorophyll values. The turbidity provided the lowest growth rates. The reason for this might be that the initial turbidity is caused by nutrient containing particles. When the particles causing this turbidity are broken down into nutrients, it will be consumed by the algae so that the biomass can increase on the expense of the turbidity values.

The following tables (Table 2, 3, 4, 5, 6 and 7) shows of the growth rates calculated for fluorescence, turbidity, dry weight and dry count found during different time periods of the experiment.

TABLE 2 THE GROWTH RATES BASED ON FLUORESCENCE FOUND IN EACH RESPECTIVE PERIOD MEASURED IN µG/L/D.

Date (S)	Date (F)	Fluor (S)	Fluor (F)	GR (fluor)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$\mu g\ l^{-1}$	$\mu g\ l^{-1}$	$double\ d^{-1}$	$d\ double^{-1}$
3/9/2016	3/20/2016	0,142	10,461	0,391	1,773
3/13/2016	3/16/2016	0,121	5,967	1,299	0,533
3/13/2016	3/20/2016	1,210	10,461	0,308	2,249
3/21/2016	3/22/2016	2,529	5,548	0,786	0,882
3/23/2016	3/26/2016	3,290	11,250	0,410	1,691
3/26/2016	3/29/2016	6,956	20,931	0,367	1,888
3/27/2016	3/29/2016	4,760	20,930	0,740	0,936
3/30/2016	4/2/2016	1,974	15,543	0,688	1,008
4/2/2016	4/9/2016	1,896	24,452	0,365	1,898
4/2/2016	4/10/2016	1,896	24,450	0,320	2,169
4/21/2016	5/1/2016	1,188	47,239	0,368	1,882
4/21/2016	5/5/2016	1,188	118,661	0,329	2,108
5/1/2016	5/5/2016	24,166	118,661	0,398	1,742

The growth rate (GR) values shown in Table 2 represents the growth rate of fluorescence found in each respective period. The highest chlorophyll growth rate was found between 13. Mar. and 16. Mar. calculated to be 1,299 $double\ d^{-1}$.

TABLE 3 GROWTH RATE BASED ON TURBIDITY FOUND IN EACH RESPECTIVE PERIOD, MEASURED IN FTU/L/D.

Date (S)	Date (F)	Turb (S)	Turb (F)	GR (Turb)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	FTU	FTU	$double\ d^{-1}$	$d\ double^{-1}$
3/9/2016	3/20/2016	0,186	2,918	0,250	2,770
3/18/2016	3/20/2016	1,449	2,918	0,350	1,980
3/23/2016	3/26/2016	0,875	1,048	0,060	11,526
3/26/2016	3/29/2016	0,871	1,633	0,210	3,308
3/30/2016	4/2/2016	0,709	1,407	0,228	3,034
4/2/2016	4/10/2016	0,579	4,916	0,267	2,592
4/3/2016	4/10/2016	0,528	4,916	0,319	2,175
4/21/2016	5/1/2016	0,538	6,874	0,255	2,721
5/1/2016	5/5/2016	3,638	10,655	0,269	2,580

Table 3 shows the growth rate of turbidity (Turb.) found in each respective period, measured in FTU Highest growth rate, as seen in table 3, occurred between 18. Mar. and 20. Mar., calculated to be 0,350 $double\ d^{-1}$.

TABLE 4 GROWTH RATE BASED ON DRY WEIGHT MEASURED IN G/L/D.

Date (S)	Date (F)	Dry weigh (S)	Dry weight (F)	GR (DW)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$g\ l^{-1}$	$g\ l^{-1}$	$double\ d^{-1}$	$d\ double^{-1}$
4/22/2016	5/1/2016	0,005	0,038	0,218	3,176
5/4/2016	5/5/2016	0,045	0,054	0,179	3,864

As seen in Table 4, the growth rate of dry weight was not measured before the very end of the experiment, and as such is a less comprehensive dataset. Highest growth rate for dry weight was found between 4. May and 5. May, calculated to be 0,179 $double\ d^{-1}$.

TABLE 5 GROWTH RATE (ALGAE COUNT)

Date (S)	Date (F)	Count (S)	Count (F)	GR (CO)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$number\ l^{-1}$	$number\ l^{-1}$	$double\ d^{-1}$	$d\ double^{-1}$
2/29/2016	3/6/2016	5626,667	133889,333	0,528	1,312
3/6/2016	3/13/2016	133889,333	611824,667	0,217	3,193
3/13/2016	3/16/2016	611824,667	5780770,812	0,749	0,926
3/16/2016	3/18/2016	5780770,812	16560620,000	0,526	1,317
3/18/2016	3/20/2016	16560620,000	24008910,603	0,186	3,733
3/20/2016	3/22/2016	24008910,603	5306613,682	-0,755	-0,918
3/22/2016	4/4/2016	5306613,682	18219277,766	0,095	7,305

Table 5 shows the growth rates of the algae count. The growth rates shown have been calculated from count to count. Highest growth rate found was between 13. Mar. and 16. Mar., calculated to be 0,749 $double\ d^{-1}$.

TABLE 6 HIGHEST GROWTH RATES OBSERVED FOR FLUORESCENCE FOUND DURING THE EXPERIMENT

Date (S)	Date (F)	Fluor (S)	Fluor (F)	GR (fluor)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$\mu g\ l^{-1}$	$\mu g\ l^{-1}$	$double\ d^{-1}$	$d\ double^{-1}$
3/27/2016	3/28/2016	4,763	16,414	1,237	0,560
3/31/2016	4/1/2016	4,786	12,442	0,955	0,726
4/3/2016	4/4/2016	3,518	11,349	1,171	0,592
4/22/2016	4/23/2016	1,867	9,187	1,593	0,435
5/4/2016	5/5/2016	61,353	118,661	0,660	1,051

Table 6 shows the days containing the highest growth rates for chlorophyll. The highest growth rate achieved throughout the experiment was found to be 1.593 $double\ d^{-1}$, between the 22. Apr. and 23. Apr., the day the CTD is assumed to have malfunctioned.

TABLE 7 HIGHEST GROWTH RATE OBSERVED FOR DRY WEIGHT FOUND DURING THE EXPERIMENT.

Date (S)	Date (F)	Dry weigh (S)	Dry weight (F)	GR (DW)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$g\ l^{-1}$	$g\ l^{-1}$	$double\ d^{-1}$	$d\ double^{-1}$
4/22/2016	4/23/2016	0,005	0,012	0,786	0,882
5/3/2016	5/4/2016	0,027	0,045	0,516	1,342
5/3/2016	5/5/2016	0,027	0,054	0,348	1,992

Table 7 shows the days containing the highest growth rates for dry weight. The highest growth rate was found between 22. Apr. and 23. Apr., calculated to be 0,786 $double\ d^{-1}$.

Production capacity

Estimated from dry weight

The period which yielded the highest algal biomass production was between 3.- 4. May. Between the two measurements the dry weight increased from 0.0271 $g\ l^{-1}$ to 0.0455 $g\ l^{-1}$ dry weight, this equals an increase of 0.0184 $g\ l^{-1}$ in one day. The growth rate for this period was 0.518 $double\ d^{-1}$. The doubling time during the period was 1.338 $d\ double^{-1}$, and divisions per day was 0.748 $div.\ d^{-1}$.

This means that with the same growth rate during a period of about 32 hours, the biomass concentration is doubled. The tank could be drained to 50% and refilled with deep water, and the cell concentration would be at approximately the initial value again.

For harvest of the algae, a semi continuous solution was considered most relevant for this experiment, because continuous harvest requires more infrastructure and batch harvest would be less productive (Sorgeloos, 1996). The dry weight concentration at 4. May. was 0.0455 g l^{-1}. To reach the initial concentration of 0.0271 g l^{-1}, a certain amount of water would have to be exchanged with deep water. The percentage of water corresponds to the percentage of increase in dry weight concentration.

$$Daily\ water\ exchange\ in\ \% = \frac{0.0184\ g\ l^{-1} \cdot 100\ \%}{0.0455\ g\ l^{-1}} = 40\ \%$$

40 % of the water in the tank, amounts to 2960 l. In theory the biomass in this water could be harvested every day, if it was possible to maintain the growth rate seen over the period. The daily production capacity of the system in grams of dry weight per day, is given by:

$$Daily\ production\ capacity = 0.0455\ g\ l^{-1}\ \cdot 2960\ l\ d^{-1}\ = 134.7\ g\ d^{-1}$$

Estimated from chlorophyll

The chlorophyll values can also be treated as an indication of production capacity. This might be a better way to measure the amount of microalgae, because a significant part of the dry weight is expected to consist of bacteria. During the experiment, algae count samples and chlorophyll measurements were conducted at the same time. Correlation between these measurements is present. However, a low number of count values, and relative low culture concentrations at the time of measurements, makes the correlation unreliable.

Many high growth rates of chlorophyll were achieved, but most of the higher growth rates was observed at an early stage of the culture, so that the total increase in chlorophyll during the periods remained low. The highest increase in chlorophyll concentration was observed during 4.- 5. May. The chlorophyll concentration rose from 61.353 to 118.661 µg l^{-1}. The growth rate for this period was 0.6596 $double\ d^{-1}$ and the total increase in chlorophyll concentration was 57.308 µg l^{-1}.

Figure 41 shows the correlation between cell count numbers and chlorophyll concentration in the tank. The trend line has the slope $y = 4 \cdot 10^{-7}x$ where y is the chlorophyll concentration given in µg, and x is the number of cells counted. This indicates that the chlorophyll content per cell averages around $4 \cdot 10^{-13}$ g $cell^{-1}$, or 0.4 $pg\ cell^{-1}$. According to (Sorgeloos, 1996), the average chlorophyll content per cell in *S. costatum* is around 0.63 pg.

A lower estimated weight of chlorophyll content per cell can be due to several reasons mentioned earlier in the thesis. This includes misleading correlation between chlorophyll and count due to the small dataset, light affecting the CTD to provide lower chlorophyll values, or that the cultivation conditions induce a lower chlorophyll content in the algae. As most of the counts were completed early in the experiment, the microalgae had adapted to a tank with low turbidity. As the chlorophyll content increases with low light conditions, higher turbidity values could have led to an increasing chlorophyll content towards the end of the experiment.

Based on the cell chlorophyll content calculated in this experiment, the number of cells per litre when the chlorophyll concentration is at 118,661 µg l^{-1}, amounts to:

$$Highest\ cell\ concentration\ chlorophyll = \frac{1.1861 \cdot 10^{-4} g\ l^{-1}}{4 \cdot 10^{-13} g\ cell^{-1}} = 2.97 \cdot 10^{8}\ cells\ l^{-1}$$

The percentage of water that should be replenished to reach the initial chlorophyll concentration of 61.353 µg l^{-1} corresponds to the percentage of increase in chlorophyll concentration between the measurements.

$$Daily\ water\ exchange\ in\ \% = \frac{57.308\ \mu g\ l^{-1} \cdot 100\ \%}{118.661\ \mu g\ l^{-1}} = 48\ \%$$

48 % of the water corresponds to 3552 l. The number of cells in 3552 l of water with the given concentration amounts to 1.1· 10^{12} $cells$. Based on the dry weight per cell (Sorgeloos, 1996), this amounts to 57.42 g of S. costatum dry weight.

$$Daily\ production\ capacity\ in\ algal\ dry\ weight =$$

$$(1.1 \cdot 10^{12}\ cell\ d^{-1}) \cdot (52.2 \cdot 10^{-12}\ g\ cell^{-1}) = 57.42\ g\ d^{-1}$$

The lower daily production capacity when calculated from chlorophyll, can be due to high amounts of bacteria in the water, which is not included in the algal dry weight estimations. The chlorophyll value which the calculations is based on, was measured in a white bucket, and may have been underestimated due to light affecting the chlorophyll sensor. As the chlorophyll content per cell was lower than expected, the dry weight is expected to be overestimated.

Compared to the fjord

Each year the fjord produces 50-60 g organic carbon m^{-2} (T. Dale Personal statement). This number includes the production far down in the water column. The production capacity of the tank was 134.7 g d^{-1} of dry weight in total. This equals 18.2 $g\ d^{-1}\ m^{-3}$ of dry weight. The average relation between dry weight and ash weight during the experiment was respectively 11.4 : 1. By applying this relation,

the ash-free dry weight of the production per m^3 was 16.6 $g\ d^{-1}\ m^{-3}$. The organic carbon produced per m^3 per day can be found by multiplying the ash free dry weight by 0.4, and equals to 6.64 $g\ d^{-1}\ m^{-3}$. As the water in the tank has a depth of 1 m, this equals the production per m^2. Hence, it would take approximately 9 days to reach the yearly production of an equally large area in the fjord.

Comparison with other experiments

In this experiment the daily production capacity was estimated to be 134.7 g d^{-1} when based on dry weight, and 57.42 g d^{-1} when based on chlorophyll measurements.

The highest culture concentration in terms of biomass was 0.0544 g l^{-1}. Even though cell size and mass can vary greatly within *S. costatum* depending on culture conditions, cell count numbers can be roughly transferred into dry weight. Cellular dry weight of *S. costatum* is 52.2 $pg\ cell^{-1}$ (Jameson, 2006). The cell number corresponding to the highest measured dry weight is then:

$$\frac{0.0544\ g\ l^{-1}}{5.22\ \cdot 10^{13}\ g\ cell^{-1}} = 1.04 \cdot 10^{9}\ cell\ l^{-1} = 1.04 \cdot 10^{6}\ cell\ ml^{-1}$$

The real number of cells is however most likely significantly lower due to bacteria constituting a part of the dry weight.

The highest estimated cell concentration based on chlorophyll was $2.97 \cdot 10^{8}\ cells\ l^{-1}$ or $2.97 \cdot 10^{5}\ cells\ ml^{-1}$.

Similar experiments to the one featured in this thesis have been conducted at other institutions. Several of these experiments have been conducted with *S. costatum* from tropical zones.

One study conducted by (Uddin, 2007) cultivated *S. costatum* using two different nutrient medias in both small scale cultures, and mass cultures in outdoor cement tanks. In the tanks, cell densities of $1.23\ \cdot 10^{6}\ cell\ ml^{-1}$ and $0.78 \cdot\ 10^{6}\ cell\ ml^{-1}$ was recorded in their respective F/4 and commercial fertilizer mediums.

One experiment conducted by (Brockmann et al., 1977) assessed the growth of *S. costatum* in plastic tanks at the size of 3-4 m^3. Based on the graphs presented in the article, the maximum cell number measured was around $7\ \cdot 10^{4}\ cell\ ml^{-1}$ after 5 days of growth.

A study was conducted by (Rekha et al., 2012) to assess the culture and biofuel production of *S. costatum* and *chlorella marina*. The maximum culture density achieved in out-door culture of *S. costatum* during this experiment was $8.1 \cdot 10^{5}\ cell\ ml^{-1}$, after 12 days of growth.

(Monkonsit et al., 2011) conducted an experiment aimed to determine the optimal design configurations and operating conditions for the growth of *S. Costatum*. In this experiment *S. costatum* was cultivated in an airlift PHB, and in a tank with bubble column. Cell densities measured in the PHB reached $4.6 \cdot 10^6\ cell\ ml^{-1}$. In the bubble column system, a cell density of $1.8 \cdot 10^6\ cell\ ml^{-1}$ was measured.

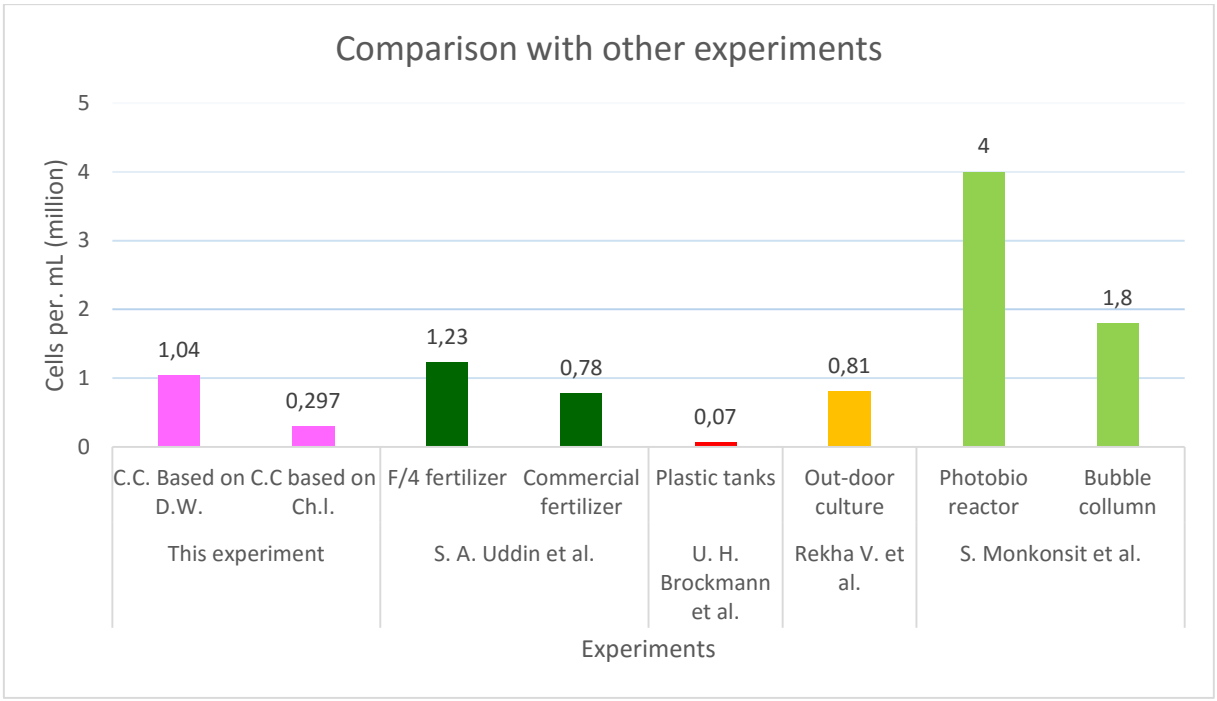

FIGURE 19: COMPARISON WITH OTHER EXPERIMENTS HIGHEST CELL CONCENTRATIONS IN G ML^{-1}

As figure 44 shows the results in this experiment is within the range of the others in terms of cell concentration when calculated from dry weight. However, a significant amount of the dry weight is believed to consist of bacteria. This would lead to an over estimation of cell concentration in this experiment based on the weight per cell. The clearly highest cell concentration was achieved in a PHB (Monkonsit et al., 2011).

Potential for improvement in the cultivation process

Several measures could be conducted to improve the production capacity of the system. Adding a better nutrient mix with addition of Fe and vitamins, for instance F/2 medium, is expected to enhance the algae growth. Making the tank area greater would increase the incoming sunlight and can have a positive effect on algae the growth, at least early in the season as the incoming sunlight is

low, and at high algal culture volumes where the sunlight might be a limiting factor due to self-shading.

The rate of nutrient addition could be improved. In this experiment the nutrient mix was added one time each day. A continually nutrient addition would likely improve the result as the algae would have a more stable nutrient access, and the optimal amount of nutrients would be easier to maintain.

Regulating the aeration based on culture density could improve the algae growth in the start since the CO_2 input can lead to lower pH values and therefore un-optimal growth conditions in the start of the cycle (Jameson, 2006). The aeration would still have to be increased throughout the growth phase, as too low CO_2 concentrations could have been a limiting factor during the experiment.

During this experiment no way of measuring the aeration was available. This made it hard to compare the aeration rate in this experiment with other similar experiments. An air flow measuring instrument would solve this issue. Also the aeration setup was very energy consuming because the air flow was adjusted with a valve and not by lowering the effect of the air pump. In biofuel production, lowering the energy requirements is essential (Dutta et al., 2014).

The initial algae culture could be cultivated in a laboratory before being transferred to the tank. This is because already exponentially growing algae cultures have shorter lag phases (Jameson, 2006).

Bacterial cultures in the tank have most likely contributed to the limited algal growth, as bacteria compete with algae for nutrients and can attack the algae directly. The way the tank was maintained during the experiment might have contributed to bacterial growth. For example, higher amounts of N addition could maybe decrease the amount of DOM release from the algae, and therefore indirectly limit the bacterial growth (Azam et al., 1983). Also a semi continuous system can deteriorate over time due to bacteria, predators and metabolites building up over time and reducing the quality of the culture (Jameson, 2006). The long period which the culture was maintained in this experiment might have limited the production capacity at the end.

Conclusion

The highest daily growth rates based on chlorophyll, dry weight and turbidity were respectively 1.59-, 0.79- and 0.59 $double\ d^{-1}$. The highest growth rate for the cell count was 0.749 $double\ d^{-1}$ The estimated daily production capacity of the 7.4 m^3 of water was $134.7\ g\ d^{-1}$ in terms of dry weight. The production capacity calculated from chlorophyll values and converted to dry weight was

$57.42\ g\ d^{-1}$. The organic carbon production was estimated to 6.64 $g\ d^{-1}\ m^{-2}$, and could potentially proceed the yearly production in a corresponding area in the Sogndalsfjord within 9 days.

The maximum *S. costatum* cell concentration was calculated directly from dry weight. The concentration was estimated to $1.04 \cdot 10^{6}\ cell\ ml^{-1}$. The real cell concentration is however most likely lower due to biomass from bacteria and other algae constituting an unknown part of the dry weight. The highest cell concentration estimation calculated from chlorophyll concentrations was $2.97 \cdot 10^{5}\ cells\ ml^{-1}$. The highest cell concentration was within the range of several other similar experiments.

During the experiment methods were improved and added to get more accurate results. If these improvements had been implemented from the beginning, the dataset and results would have been more comprehensive. Several measures could be implemented that might improve production the production capacity.

However, the method was efficient at isolating *S. costatum*, and the production capacity achieved during this experiment indicates that the fertilization had a positive effect on the productivity of the algae. This thesis is presumed to have laid a good foundation for future bachelor theses in the same field at HiSF, by illuminating the margins of error and method improvements.

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Appendix

Main data

TABLE 2 – MAIN DATA COLLECTED THROUGHOUT THE EXPERIMENT.

Date	Time	Salinity	Temperature	Fluorescence	Turbidity	Oxygen	pH
dd/mm/yyyy	hh/mm/ss	‰	°C	$\mu g\ l^{-1}$	FTU	%	
29.02.2016		33,56	8,09	0,10	0,36	92,25	
29.02.2016		32,82	8,37	0,07	0,38	79,26	
02.03.2016	14:03:59	32,14	2,49	0,09	0,33	109,62	
04.03.2016	19:16:14	33,11	2,31	0,13	0,31	111,93	
06.03.2016	18:06:31	31,50	2,27	0,14	0,16	110,24	
09.03.2016	14:02:52	35,16	0,21	0,14	0,19	112,71	
11.03.2016	18:24:31	33,95	3,51	0,37	0,20	106,35	
13.03.2016	17:43:05	34,93	2,72	1,21	0,44	115,88	
16.03.2016	14:45:42	34,62	5,70	1,76	6,25	114,38	
18.03.2016	16:23:02	34,83	7,39	5,91	1,45	118,76	
20.03.2016	17:29:25	34,95	7,64	10,46	2,92	111,65	
20.03.2016	20:43:53	34,69	7,78	3,50	0,91	73,61	
21.03.2016	13:42:04	34,59	6,18	2,53	0,75	108,39	
22.03.2016	18:37:27	34,68	6,90	5,55	0,88	105,92	
23.03.2016	17:40:10	34,92	7,45	4,13	1,60	117,44	
23.03.2016	20:32:53	34,35	8,02	3,29	0,88	79,88	
24.03.2016	19:18:25	33,92	6,49	3,81	0,75	102,45	
25.03.2016	19:34:59	34,30	5,14	5,97	0,70	110,75	
26.03.2016	17:45:43	34,40	5,63	11,25	1,05	113,09	
26.03.2016	20:21:25	34,29	6,77	6,96	0,87	74,09	
27.03.2016	18:47:34	34,12	8,71	4,76	0,67	105,72	
28.03.2016	17:41:04	34,21	7,78	16,41	0,98	106,53	
29.03.2016		34,21	8,16	20,93	1,63	108,80	
30.03.2016	14:58:51	34,16	6,33	3,84	1,46	107,68	
30.03.2016	17:37:14	34,24	8,67	1,97	0,71	73,18	
31.03.2016	20:08:21	34,29	6,95	4,79	0,66	110,77	
01.04.2016	18:28:12	34,39	5,54	12,44	0,94	114,41	

02.04.2016	17:16:59	34,51	4,67	15,54	1,41	115,54	
02.04.2016	19:57:34	33,92	7,96	1,90	0,58	67,83	
03.04.2016	18:49:22	34,32	7,97	3,52	0,53	108,40	
04.04.2016	20:49:20	34,29	9,31	11,35	0,92	108,39	
05.04.2016	18:11:38	34,12	9,44	17,72	1,61	111,46	
06.04.2016	18:30:45	34,09	9,03	19,81	1,98	114,10	
07.04.2016	17:56:28	33,85	9,11	14,63	2,52	113,62	
08.04.2016	18:32:30	34,19	8,91	22,21	2,89	118,63	
09.04.2016	18:06:54	29,62	9,88	24,45	3,54	123,94	
10.04.2016	18:22:19	34,11	9,80	24,45	4,92	124,22	
10.04.2016	21:03:34	33,06	8,37	9,05	0,89	73,68	
19.04.2016	19:20:54	33,09	10,01	24,46	2,28	108,24	
20.04.2016	18:40:33	32,08	9,06	36,52	2,89	113,47	
21.04.2016	10:13:19	30,99	7,88	24,09	2,98	110,74	
21.04.2016	13:27:55	34,00	8,70	1,19	0,54	73,34	
22.04.2016	16:08:34	33,15	9,74	1,87	0,59	101,97	
23.04.2016	15:57:02	32,83	7,64	9,19	2,48	110,77	7,960
24.04.2016	16:44:28	34,25	8,54	11,69	1,50	108,81	8,190
25.04.2016	16:23:16	34,21	6,51	17,10	1,95	112,41	8,260
26.04.2016	16:29:38	34,50	7,82	15,97	2,51	110,22	8,400
27.04.2016	17:07:20	34,51	9,79	24,30	3,67	118,22	8,530
28.04.2016	16:39:50	34,27	10,26	36,71	5,09	120,92	8,730
29.04.2016	17:09:31	33,62	9,31	35,57	5,26	113,57	8,880
30.04.2016	16:30:26	34,18	11,35	36,85	5,86	112,67	9,000
01.05.2016	08:13:33	30,77	9,90	47,24	6,87	102,76	9,060
01.05.2016	09:54:27	34,40	9,45	24,17	3,64	88,76	9,010
01.05.2016	16:51:37	32,89	10,83	30,33	4,01	103,69	8,510
02.05.2016	16:41:05	32,57	9,11	48,81	4,74	103,93	8,650
03.05.2016	17:28:29	32,52	9,77	49,02	6,18	109,38	8,680
04.05.2016	15:48:38	34,36	11,96	61,35	6,86	112,20	8,740
05.05.2016	16:41:05	34,21	11,57	73,37	10,65	110,43	9,07

The table shows the main bulk of data gathered throughout the entirety of the experiment. From the 29'th of February until the 5'th of May. Each value presented in Table 1 represents the average value

of the measurements collected for the corresponding date and time. The orange dates symbolize that these values were collected after the water in the tank had been replenished. The pink date symbolizes the value collected after the initial algae seed had been planted in the tank.

TABLE 3 - AFTER REFILL VALUES

Date	Time	Salinity	Temperature	Fluorescence	Turbidity	Oxygen	pH
dd/mm/yyyy	hh/mm/ss	‰	°C	$\mu g\ l^{-1}$	FTU	%	
29.02.2016		32,82	8,37	0,07	0,38	79,26	
20.03.2016	20:43:53	34,69	7,78	3,50	0,91	73,61	
23.03.2016	20:32:53	34,35	8,02	3,29	0,88	79,88	
26.03.2016	20:21:25	34,29	6,77	6,96	0,87	74,09	
30.03.2016	17:37:14	34,24	8,67	1,97	0,71	73,18	
02.04.2016	19:57:34	33,92	7,96	1,90	0,58	67,83	
10.04.2016	21:03:34	33,06	8,37	9,05	0,89	73,68	
21.04.2016	13:27:55	34,00	8,70	1,19	0,54	73,34	
01.05.2016	09:54:27	34,40	9,45	24,17	3,64	88,76	8,51

Table 2 shows the values collected from the tank after each refilling occurring throughout the experiment.

Surface measures

TABLE 4 : SURFACE MEASUREMENTS

Date	Time	Salinity	Temperature	Fluorescence	Turbidity	Oxygen
dd/mm/yyyy	hh/mm/ss	‰	°C	$\mu g\ l^{-1}$	FTU	%
29.02.2016		32,76	8,55	0,05	0,37	79,51
29.02.2016		33,60	8,08	0,10	0,37	89,06
02.03.2016	14:03:59	31,97	2,56	0,08	0,29	106,80
04.03.2016	19:16:14	33,19	2,32	0,15	0,19	108,54
06.03.2016	18:06:31	31,35	2,32	0,15	0,17	106,78
09.03.2016	14:02:52	35,38	0,17	0,14	0,21	113,59
11.03.2016	18:24:31	33,02	3,54	0,36	0,21	98,80
13.03.2016	17:43:05	35,14	2,77	1,25	0,36	117,64
16.03.2016	14:45:42	34,56	5,72	1,62	7,48	114,96
18.03.2016	16:23:02	34,94	7,35	5,02	1,40	120,11
20.03.2016	17:29:25	34,92	7,70	2,66	0,90	107,20
20.03.2016	20:43:53	34,45	7,91	3,97	0,89	78,60
21.03.2016	13:42:04	34,65	6,18	2,55	0,73	104,46
22.03.2016	18:37:27	34,64	6,93	5,69	0,89	105,45
23.03.2016	17:40:10	34,95	7,48	3,77	1,57	118,54
23.03.2016	20:32:53	34,38	8,04	3,09	0,85	81,42
24.03.2016	19:18:25	33,39	4,82	4,47	0,62	91,85
25.03.2016	19:34:59	34,23	5,16	6,12	0,71	104,29
26.03.2016	17:45:43	34,44	5,61	11,46	1,06	109,72
26.03.2016	20:21:25	34,20	7,86	2,90	0,70	75,26
27.03.2016	18:47:34	34,11	8,72	4,67	0,68	98,36
28.03.2016	17:41:04	34,21	7,79	16,48	1,00	96,07
29.03.2016		34,21	8,16	20,98	1,57	99,98
30.03.2016	14:58:51	34,16	6,33	3,73	1,42	101,34
30.03.2016	17:37:14	34,22	8,69	1,72	0,71	77,18
31.03.2016	20:08:21	34,33	6,93	4,88	0,65	109,40
01.04.2016	18:28:12	34,34	5,56	12,72	0,94	109,64
02.04.2016	17:16:59	34,46	4,71	15,82	1,35	108,05

02.04.2016	19:57:34	33,93	7,96	1,89	0,55	73,99
03.04.2016	18:49:22	34,36	7,96	3,67	0,53	104,44
04.04.2016	20:49:20	34,30	9,31	10,88	0,85	105,48
05.04.2016	18:11:38	34,13	9,44	17,15	1,63	103,65
06.04.2016	18:30:45	34,08	9,08	19,53	1,92	114,03
07.04.2016	17:56:28	33,87	9,21	11,48	2,37	107,09
08.04.2016	18:32:30	34,22	8,98	20,04	2,85	114,08
09.04.2016	18:06:54	29,21	9,92	24,46	3,63	117,48
10.04.2016	18:22:19	34,15	9,82	24,45	4,88	111,88
10.04.2016	21:03:34	33,09	8,37	8,78	0,87	76,99
19.04.2016	19:20:54	33,08	9,99	24,46	2,25	114,19
20.04.2016	18:40:33	30,28	9,06	37,01	2,93	117,16
21.04.2016	10:13:19	31,04	7,92	19,83	2,96	110,37
21.04.2016	13:27:55	33,99	8,69	1,27	0,64	74,11
22.04.2016	16:08:34	33,06	9,82	1,86	0,59	110,28
23.04.2016	15:57:02	32,90	7,70	8,04	2,09	114,22
24.04.2016	16:44:28	34,23	8,62	10,71	1,52	112,92
25.04.2016	16:23:16	34,17	6,57	16,80	1,96	99,77
26.04.2016	16:29:38	34,61	7,93	13,30	2,49	113,75
27.04.2016	17:07:20	34,52	9,79	23,76	3,64	111,66
28.04.2016	16:39:50	34,33	10,33	35,36	5,14	109,42
29.04.2016	17:09:31	33,64	9,36	32,35	5,32	106,38
30.04.2016	16:30:26	34,16	11,43	34,48	5,83	111,67
01.05.2016	08:13:33	30,77	9,94	47,66	6,91	98,70
01.05.2016	09:54:27	34,42	9,45	22,63	3,59	82,90
01.05.2016	16:51:37	32,93	10,84	29,02	4,10	98,35
02.05.2016	16:41:05	32,55	9,12	46,16	4,75	94,64
03.05.2016	17:28:29	30,13	9,77	47,85	5,93	103,42
04.05.2016	15:48:38	34,35	12,02	56,52	6,93	105,57
05.05.2016	16:41:05	34,20	11,58	73,37	10,69	101,12

TABLE 5 : ARV SURFACE MEASUREMENTS

Date	Time	Salinity	Temperature	Fluorescence	Turbidity	Oxygen
dd/mm/yyyy	hh/mm/ss	‰	°C	$\mu g\ l^{-1}$	FTU	%
20.03.2016	20:43:53	34,45	7,91	3,97	0,89	78,60
23.03.2016	20:32:53	34,38	8,04	3,09	0,85	81,42
26.03.2016	20:21:25	34,20	7,86	2,90	0,70	75,26
30.03.2016	17:37:14	34,22	8,69	1,72	0,71	77,18
02.04.2016	19:57:34	33,93	7,96	1,89	0,55	73,99
10.04.2016	21:03:34	33,09	8,37	8,78	0,87	76,99
21.04.2016	13:27:55	33,99	8,69	1,27	0,64	74,11
01.05.2016	09:54:27	34,42	9,45	22,63	3,59	82,90

Bottom measures

TABLE 6 : BOTTOM MEASUREMENTS

Date	Time	Salinity	Temperature	Fluorescence	Turbidity	Oxygen
dd/mm/yyyy	hh/mm/ss	‰	°C	$\mu g\ l^{-1}$	FTU	%
29.02.2016		32,85	8,26	0,08	0,39	76,82
29.02.2016		33,51	8,10	0,10	0,36	87,76
02.03.2016	14:04:34	32,28	2,42	0,09	0,36	110,45
04.03.2016	19:16:54	33,05	2,31	0,11	0,42	112,01
06.03.2016	18:07:19	31,64	2,23	0,14	0,16	108,47
09.03.2016	14:03:33	34,96	0,24	0,15	0,16	115,42
11.03.2016	18:25:01	34,66	3,49	0,38	0,20	105,19
13.03.2016	17:43:44	34,68	2,67	1,16	0,52	119,12
16.03.2016	14:46:20	34,70	5,67	1,94	4,73	118,76
18.03.2016	16:23:29	34,76	7,42	6,53	1,49	122,75
20.03.2016	17:29:56	34,98	7,59	3,13	0,93	111,92
20.03.2016	20:44:28	34,46	7,91	4,06	0,92	74,89
21.03.2016	13:42:40	34,54	6,19	2,51	0,77	109,46
22.03.2016	18:38:07	34,72	6,87	5,42	0,88	108,28
23.03.2016	17:40:48	34,90	7,42	4,41	1,62	120,05
23.03.2016	20:33:29	34,33	8,01	3,39	0,89	78,05
24.03.2016	19:18:58	33,53	4,81	4,28	0,60	95,04
25.03.2016	19:35:34	34,38	5,13	5,81	0,69	110,00
26.03.2016	17:46:21	34,38	5,64	11,11	1,04	113,21
26.03.2016	20:22:05	34,18	7,87	2,85	0,71	71,44
27.03.2016	18:48:07	34,12	8,71	4,83	0,66	101,04
28.03.2016	17:41:40	34,20	7,78	16,36	0,97	99,33
29.03.2016		34,21	8,16	20,89	1,68	103,35
30.03.2016	14:59:28	34,16	6,33	3,95	1,51	105,08
30.03.2016	17:37:48	34,26	8,66	2,26	0,71	72,08
31.03.2016	20:08:54	34,26	6,97	4,68	0,66	112,67
01.04.2016	18:28:54	34,44	5,54	12,19	0,94	114,92
02.04.2016	17:17:39	34,56	4,65	15,30	1,46	115,30

02.04.2016	19:58:23	33,92	7,96	1,90	0,59	67,63
03.04.2016	18:49:58	34,28	7,99	3,40	0,52	109,12
04.04.2016	20:49:53	34,28	9,32	11,65	0,96	108,33
05.04.2016	18:12:11	34,12	9,44	17,97	1,60	110,09
06.04.2016	18:31:23	34,11	8,98	20,09	2,04	115,07
07.04.2016	17:57:16	33,83	9,01	17,77	2,66	112,51
08.04.2016	18:33:03	34,16	8,85	23,80	2,92	116,86
09.04.2016	18:07:35	30,01	9,85	24,44	3,46	123,40
10.04.2016	18:22:36	34,08	9,79	24,45	4,95	123,46
10.04.2016	21:04:24	33,03	8,37	9,39	0,90	73,54
19.04.2016	19:21:31	33,09	10,02	24,46	2,29	112,76
20.04.2016	18:41:06	33,78	9,06	36,06	2,85	116,89
21.04.2016	10:14:18	30,95	7,86	27,22	3,00	111,18
21.04.2016	13:29:39	34,01	8,70	1,11	7,15	71,48
22.04.2016	16:09:48	33,26	9,63	1,94	0,58	105,69
23.04.2016	15:58:26	32,78	7,60	9,89	2,72	111,24
24.04.2016	16:45:43	34,29	8,46	12,87	1,47	110,39
25.04.2016	16:24:29	34,23	6,47	17,34	1,94	100,29
26.04.2016	16:31:40	34,38	7,71	18,73	2,54	109,06
27.04.2016	17:08:55	34,50	9,79	25,12	3,72	108,41
28.04.2016	16:42:22	34,24	10,23	39,38	5,09	106,92
29.04.2016	17:10:19	33,61	9,28	37,68	5,22	105,21
30.04.2016	16:31:05	34,18	11,30	38,19	5,88	109,37
01.05.2016	08:14:14	30,77	9,88	46,94	6,85	96,96
01.05.2016	09:55:12	34,39	9,44	25,10	3,67	81,94
01.05.2016	16:53:05	32,87	10,81	31,12	3,96	97,05
02.05.2016	16:42:26	32,59	9,10	52,65	4,72	94,11
03.05.2016	17:29:33	34,15	9,77	49,83	6,35	100,07
04.05.2016	15:49:58	34,37	11,92	65,51	6,79	102,74
05.05.2016	16:42:18	34,23	11,55	73,36	10,62	98,81

TABLE 7 : ARV BOTTOM MEASUREMENTS

Date	Time	Salinity	Temperature	Fluorescence	Turbidity	Oxygen
dd/mm/yyyy	hh/mm/ss	‰	°C	$\mu g\ l^{-1}$	FTU	%
20.03.2016	20:44:28	34,46	7,91	4,06	0,92	74,89
23.03.2016	20:33:29	34,33	8,01	3,39	0,89	78,05
26.03.2016	20:22:05	34,18	7,87	2,85	0,71	71,44
30.03.2016	17:37:48	34,26	8,66	2,26	0,71	72,08
02.04.2016	19:58:23	33,92	7,96	1,90	0,59	67,63
10.04.2016	21:04:24	33,03	8,37	9,39	0,90	73,54
21.04.2016	13:29:39	34,01	8,70	1,11	7,15	71,48
01.05.2016	09:55:12	34,39	9,44	25,10	3,67	81,94

Daily measure

Tank values

TABLE 8 – DAILY MEASURE : TANK VALUES

Date	Time	Salinity	Temperature	Fluorescence	Turbidity	Oxygen	pH-verdi
dd/mm/yyyy	hh/mm/ss	‰	°C	$\mu g\ l^{-1}$	FTU	%	8,74
03.05.2016	17:29:09	32,597	9,768	48,997	6,193	107,356	8,73
03.05.2016	19:28:18	34,361	9,833	66,081	6,677	109,115	8,73
03.05.2016	21:29:23	33,933	9,618	74,223	6,146	99,060	8,72
03.05.2016	23:25:48	34,336	9,497	73,265	5,984	98,564	8,71
04.05.2016	01:32:06	34,300	9,288	73,171	6,356	95,787	8,70
04.05.2016	03:28:05	34,353	9,134	93,625	6,256	95,936	8,70
04.05.2016	05:28:30	34,290	8,918	90,602	6,569	97,999	8,65
04.05.2016	07:28:37	34,294	8,757	69,627	6,599	102,835	8,76
04.05.2016	09:28:22	34,253	9,108	54,511	7,389	110,195	8,82
04.05.2016	11:27:52	34,164	9,701	55,450	6,792	109,995	8,86
04.05.2016	13:28:38	34,311	10,727	56,556	6,983	110,124	8,93
04.05.2016	15:49:24	34,366	11,964	61,747	6,855	115,610	8,99
04.05.2016	17:31:10	34,290	12,190	73,103	7,077	109,061	9,01
04.05.2016	21:28:11	34,292	11,985	123,121	12,450	98,358	8,91
05.05.2016	01:32:32	34,300	11,525	121,352	8,031	98,347	8,88
05.05.2016	05:31:08	34,265	10,927	110,159	8,889	97,382	8,91
05.05.2016	09:41:52	34,362	10,654	77,000	8,610	105,769	8,94
05.05.2016	13:28:20	34,352	11,309	91,347	9,002	105,674	9,02
05.05.2016	16:41:45	34,215	11,565	118,661	10,655	105,176	9,07

Table 7 shows the data collected from the tank throughout the entirety of the daily measure. As Table 7 should have it, fluorescence values would peak at around 73,37µg/l. In order to find the realistic fluorescence values bucket values were measured as well (Table 8).

Bucket values

TABLE 9 - DAILY MEASURE : BUCKET VALUES

Date	Time	Fluorescence
dd/mm/yyyy	hh/mm/ss	$\mu g\ l^{-1}$
03.05.2016	17:29:09	48,997
03.05.2016	19:28:18	66,081
03.05.2016	21:29:23	74,223
03.05.2016	23:25:48	73,265
04.05.2016	01:32:06	73,171
04.05.2016	03:28:05	93,625
04.05.2016	05:28:30	90,602
04.05.2016	07:28:37	69,627
04.05.2016	09:28:22	54,511
04.05.2016	11:27:52	55,450
04.05.2016	13:28:38	56,556
04.05.2016	15:49:24	61,747
04.05.2016	17:31:10	73,103
04.05.2016	21:28:11	123,121
05.05.2016	01:32:32	121,352
05.05.2016	05:31:08	110,159
05.05.2016	09:41:52	77,000
05.05.2016	13:28:20	91,347
05.05.2016	16:41:45	118,661

Table 8 shows the values found when measuring in a bucket containing a mixture of 4litres of water collected from the tank and 8litres of water taken from a depth of a 100metres in the fjord. The values shown in table 8 are all henceforth multiplied by three to show the corresponding tank value. As Table 8 shows, the realistic fluorescence values peak at around 118,66µg/l, rather than 73,37µg/l as first seen in Table 7.

Fertilization data

TABLE 10 : FERTILIZATION DATA

Date	Time	Silica	Fertilizer mix
dd/mm/yyyy	hh/mm/ss	grams	grams
05.04.2016	13:00:00	1,25	1,10
06.04.2016	13:00:00	2,50	2,20
07.04.2016	13:00:00	5,00	4,40
08.04.2016	13:00:00	10,00	8,80
09.04.2016	10:00:00	20,00	17,60
10.04.2016	10:00:00	40,00	35,00
19.04.2016	No fertilization	0	0
20.04.2016	No fertilization	0	0
21.04.2016	No fertilization	0	0
22.04.2016	No fertilization	0	0
23.04.2016	No fertilization	0	0
24.04.2016	09:00:00	3,00	2,20
25.04.2016	09:15:00	6,00	4,40
26.04.2016	09:50:00	12,00	8,80
27.04.2016	No fertilization	0	0
28.04.2016	09:30:00	1,50	1,10
29.04.2016	09:30:00	6,00	4,40
30.04.2016	09:30:00	12,00	8,80
02.05.2016	09:30:00	9,00	6,60
03.05.2016	09:27:00	15,00	11,00
04.05.2016	09:27:00	27,00	19,80
05.05.2016	09:27:00	27,00	19,80

Table 9 shows the fertilization phases throughout the experiment. It lists the date and time of fertilization as well as the amount of silica and fertilizer mix added to the tank at the respective dates and times.

TABLE 11 : FERTILIZER CONTENT

Trademark	Designation	Reg.no.	>16% N from AN
Fertilizer 18-3-15	NPK-fertilizer with Ca, Mg, S and B	616	Yes
Declared nutrients			
N-tot.	17,6	**Ca-ws.**	0,3
NO3-	8,3	**Cl**	10,6
NH4+	9,3	**Mg-tot.**	1,5
P-cit.s.	2,6	**Mg-ws.**	1,3
P-ws.	1,9	**S**	3,8
K-ws.	14,6	**S-ws.**	3,5
Ca-tot.	1,3	**B-tot.-**	0,02

The data shown in table 10 shows the content of the fertilizer applied to the tank throughout the experiments fertilization phases.

(http://www.yara.no/images/Yara%20Sortiment%202015_16%20varedeklarasjon_tcm420-219895.pdf)

Legend:

N = nitrogen

Ca = calcium

B = boron

Zn = zinc

P = phosphorus

Cl = chloride

Cu = copper

Se = selenium

K = potassium

Mg = magnesium

Mn = manganese

ws. = water-soluble

tot. = total content

cit.s. = citrate-soluble, dissolvable in neutral ammonium citrate

Algae count

TABLE 12 : ALGAE COUNT (INDIVIDUALS PER SAMPLE)

Date	Test volume	Correction	S. Costatum	Chaetoceros	Melosira	Thalassiosira
dd/mm/yyyy	mL	ratios	number	number	number	number
29.02.2016	50	1/2 count	14,400	11,200	28,667	2,000
06.03.2016	50	1/2 count	836,600	95,573	359,600	47,120
13.03.2016	50	1/2 count	4949,793	428,147	202,920	537,387
16.03.2016	10	1/21 count	49637,588	2980,083	964,695	4225,342
18.03.2016	10	1/4 count	162585,200	1491,067	71,167	1458,767
20.03.2016	10	1/21 count	231319,840	7377,868	207,671	1183,726
22.03.2016	50	1/21 count	50483,675	1817,124	83,069	682,269
04.04.2016	10	1/21 count	177287,582	4394,324	124,603	386,269

Throughout the experiment, samples were gathered of the water in the tank to be set to sedimentation and finally counted. Table 11 shows the number of algae counted in each sample. Each sample were collected in varying volumes, respectively 10- and 50mL flasks. The ratios represent the area of the water sample that was actually counted. (See table 21, 22 and 23 for further info on ratio) As such, the values seen in Table 11 are all resulting numbers from being multiplied by their ratios. Also, the numbers resulting from 50mL samples are resulting numbers from being divided by 5 in order to convert the samples into 10mL samples.

TABLE 13 : ALGAE COUNT (INDIVIDUALS PER LITRE)

Date	S. Costatum	Chaetoceros	Melosira	Thalassiosira
dd/mm/yyyy	number	number	number	number
29.02.2016	1440	1120	2866,66667	200
06.03.2016	83660	9557,333333	35960	4712
13.03.2016	494979,3333	42814,66667	20292	53738,66667
16.03.2016	4963758,792	298008,2782	96469,5369	422534,2043
18.03.2016	16258520	149106,6667	7116,66667	145876,6667
20.03.2016	23131984,04	737786,8129	20767,1274	118372,6262
22.03.2016	5048367,53	181712,3648	8306,85096	68226,9359

04.04.2016	17728758,22	439432,4159	12460,2764	38626,85697

Table 12 shows the number of individuals per litre in the tank. These values derive from the values presented in Table 5 divided by a hundred in order to convert them from 10mL into a litre.

TABLE 14 : FILTER OVER VIEW

Date	Time	Volume	Filter weight	Aluminum weight	Salt weight	Dry weight	Ash weight	Ash-free dry weight	Organic Carbon	Chlorophyll
dd/mm/yyyy	hh/mm/ss	L	grams	grams	grams	grams	grams	grams	$g\ m^{-3}$	$\mu g\ l^{-1}$
21.04.2016	10:14:00	1,0	0,0864		0,018	0,020	0,003	0,017	6,800	24,09
21.04.2016	13:27:00	1,0	0,0868		0,020	0,001	-0,004	0,005	1,880	1,19
22.04.2016	16:07:00	1,0	0,0858		0,019	0,005	-0,001	0,006	2,320	1,87
23.04.2016	15:56:00	1,0	0,0856		0,019	0,012	0,003	0,008	3,280	9,19
24.04.2016	16:43:00	1,0	0,0865		0,020	0,011	0,001	0,010	4,040	11,69
25.04.2016	16:22:00	1,0	0,0863		0,020	0,011	0,000	0,011	4,440	17,10
26.04.2016	16:28:00	1,0	0,0854		0,020	0,019	0,003	0,016	6,440	15,97
27.04.2016	17:06:00	1,0	0,086	0,155	0,022	0,021	0,001	0,020	7,840	24,30
28.04.2016	16:38:00	1,0	0,0859	0,180	0,020	0,027	0,002	0,025	10,080	36,71
29.04.2016	17:09:00	1,0	0,0864	0,154	0,020	0,031	0,003	0,029	11,440	35,57
30.04.2016	16:30:00	1,0	0,0854	0,147	0,020	0,032	0,003	0,030	11,800	36,85
01.05.2016	08:30:00	1,0	0,0847	0,144	0,017	0,038	0,005	0,033	13,000	47,24
01.05.2016	09:54:00	1,0	0,0865	0,193	0,016	0,020	0,003	0,017	6,920	24,17
01.05.2016	16:30:00	1,0	0,085	0,144	0,019	0,023	0,002	0,021	8,280	30,33
02.05.2016	16:40:00	1,0	0,0864	0,180	0,019	0,028	0,003	0,025	9,960	48,81

03.05.2016	17:20:00	1,0	0,0847	0,156	0,018	0,027	0,004	0,023	9,360	49,02
03.05.2016	21:20:00	0,5	0,086	0,194	0,019	0,037	0,001	0,036	14,320	74,223
04.05.2016	01:20:00	0,5	0,0855	0,233	0,017	0,036	0,003	0,033	13,120	73,171
04.05.2016	05:20:00	0,5	0,0872	0,147	0,017	0,035	0,002	0,033	13,280	90,602
04.05.2016	09:20:00	0,5	0,0867	0,144	0,019	0,036	0,004	0,032	12,880	54,511
04.05.2016	13:20:00	0,5	0,0874	0,180	0,018	0,042	0,005	0,036	14,560	56,556
04.05.2016	17:20:00	0,5	0,0853	0,155	0,021	0,045	0,004	0,042	16,640	73,103
05.05.2016	10:30:00	0,5	0,0859	0,194	0,022	0,049	0,005	0,045	17,840	77,000
05.05.2016	16:30:00	0,5	0,0856	0,138	0,022	0,054	0,006	0,048	19,360	118,66

Table 13 shows the date and time at which different water samples were processed throughout the experiment. Values for the original filter weight is listed as well as the values for each filters respective aluminum-, salt-, dry-, ash and ash-free dry weight. The volume of water filtered through the filters are also listed, as well as the organic carbon content and chlorophyll values found at each respective date.

Growth rates

The following tables (Table 14 - 20) shows of the growth rates for fluorescence, turbidity, dry weight and algae count found during different time periods spanning over the experiment.

TABLE 15 : GROWTH RATE (FLUORESCENCE)

Date (S)	Date (F)	Fluor (S)	Fluor (F)	GR (fluor)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$\mu g\ l^{-1}$	$\mu g\ l^{-1}$	$doubling\ d^{-1}$	$d\ doubling^{-1}$
3/9/2016	3/20/2016	0,142	10,461	0,391	1,773
3/13/2016	3/16/2016	0,121	5,967	1,299	0,533
3/13/2016	3/20/2016	1,210	10,461	0,308	2,249
3/21/2016	3/22/2016	2,529	5,548	0,786	0,882
3/23/2016	3/26/2016	3,290	11,250	0,410	1,691
3/26/2016	3/29/2016	6,956	20,931	0,367	1,888
3/27/2016	3/29/2016	4,760	20,930	0,740	0,936
3/30/2016	4/2/2016	1,974	15,543	0,688	1,008
4/2/2016	4/9/2016	1,896	24,452	0,365	1,898
4/2/2016	4/10/2016	1,896	24,450	0,320	2,169
4/21/2016	5/1/2016	1,188	47,239	0,368	1,882
4/21/2016	5/5/2016	1,188	118,661	0,329	2,108
5/1/2016	5/5/2016	24,166	118,661	0,398	1,742

The growth rate (GR) values shown in Table 14 represents the growth rate of fluorescence found in each respective period measured in $\mu g\ l^{-1}\ d^{-1}$. The highest chlorophyll growth rate was found between 3/21 and 3/22, calculated to be 0,786 $\mu g\ l^{-1}\ d^{-1}$.

TABLE 16 : GROWTH RATE (TURBIDITY)

Date (S)	Date (F)	Turb (S)	Turb (F)	GR (Turb)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$FTU\ l^{-1}$	$FTU\ l^{-1}$	$doubling\ d^{-1}$	$d\ doubling^{-1}$
3/9/2016	3/20/2016	0,186	2,918	0,250	2,770
3/18/2016	3/20/2016	1,449	2,918	0,350	1,980
3/23/2016	3/26/2016	0,875	1,048	0,060	11,526

3/26/2016	3/29/2016	0,871	1,633	0,210	3,308
3/30/2016	4/2/2016	0,709	1,407	0,228	3,034
4/2/2016	4/10/2016	0,579	4,916	0,267	2,592
4/3/2016	4/10/2016	0,528	4,916	0,319	2,175
4/21/2016	5/1/2016	0,538	6,874	0,255	2,721
5/1/2016	5/5/2016	3,638	10,655	0,269	2,580

Table 15 shows the growth rate of turbidity (Turb.) found in each respective period, measured in $FTU\ l^{-1}\ d^{-1}$. Highest growth rate, as seen in table 15, occurred between 3/18 and 3/20, calculated to be 0,350 $FTU\ l^{-1}\ d^{-1}$.

TABLE 17 : GROWTH RATE (DRY WEIGHT)

Date (S)	Date (F)	Dry weigh (S)	Dry weight (F)	GR (DW)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$g\ l^{-1}$	$g\ l^{-1}$	$doubling\ d^{-1}$	$d\ doubling^{-1}$
4/22/2016	5/1/2016	0,005	0,038	0,218	3,176
5/4/2016	5/5/2016	0,045	0,054	0,179	3,864

As seen in Table 16, the growth rate of dry weight could not be found before the very end of the experiment, and as such is a less comprehensive dataset. Highest growth rate for dry weight was found between 5/4 and 5/5/, calculated to be 0,179 $g\ l^{-1}\ d^{-1}$.

TABLE 18: GROWTH RATE (ALGAE COUNT)

Date (S)	Date (F)	Count (S)	Count (F)	GR (CO)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$amount\ l^{-1}$	$amount\ l^{-1}$	$doubling\ d^{-1}$	$d\ doubling^{-1}$
2/29/2016	3/6/2016	5626,667	133889,333	0,528	1,312
3/6/2016	3/13/2016	133889,333	611824,667	0,217	3,193
3/13/2016	3/16/2016	611824,667	5780770,812	0,749	0,926
3/16/2016	3/18/2016	5780770,812	16560620,000	0,526	1,317
3/18/2016	3/20/2016	16560620,000	24008910,603	0,186	3,733
3/20/2016	3/22/2016	24008910,603	5306613,682	-0,755	-0,918

3/22/2016	4/4/2016	5306613,682	18219277,766	0,095	7,305

Table 17 shows the growth rates of the algae count. The growth rates shown have been calculated from count to count. Highest growth rate found was between 3/13 and 3/16, calculated to be 0,749 $amount\ l^{-1}\ d^{-1}$.

Highest growth rates

TABLE 19 : FASTEST GROWTH RATES FOR FLUORESCENCE FOUND DURING THE EXPERIMENT

Date (S)	Date (F)	Fluor (S)	Fluor (F)	GR (fluor)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$\mu g\ l^{-1}$	$\mu g\ l^{-1}$	$doubling\ d^{-1}$	$d\ doubling^{-1}$
3/27/2016	3/28/2016	4,763	16,414	1,237	0,560
3/31/2016	4/1/2016	4,786	12,442	0,955	0,726
4/3/2016	4/4/2016	3,518	11,349	1,171	0,592
4/22/2016	4/23/2016	1,867	9,187	1,593	0,435
5/4/2016	5/5/2016	61,353	118,661	0,660	1,051

Table 18 shows the days containing the highest growth rates for chlorophyll. The highest growth rate achieved throughout the experiment was found to be 1,59, between the 4/22 and 4/23, calculated to be 1,593 $\mu g\ l^{-1}\ d^{-1}$.

TABLE 20 : HIGHEST GROWTH RATE FOR DRY WEIGHT FOUND DURING THE EXPERIMENT.

Date (S)	Date (F)	Dry weigh (S)	Dry weight (F)	GR (DW)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$g\ l^{-1}$	$g\ l^{-1}$	$doubling\ d^{-1}$	$d\ doubling^{-1}$
4/22/2016	4/23/2016	0,005	0,012	0,786	0,882
5/3/2016	5/4/2016	0,027	0,045	0,516	1,342
5/3/2016	5/5/2016	0,027	0,054	0,348	1,992

Table 19 shows the days containing the highest growth rates for dry weight. The highest growth rate was found between 4/22 and 4/23, calculated to be 0,786 $g\ l^{-1}\ d^{-1}$.

TABLE 21 HIGHEST GROWTH RATES FOR TURBIDITY

Date (S)	Date (F)	Turb (S)	Turb (F)	GR (Turb)	Doubling time
mm/dd/yyyy	mm/dd/yyyy	$FTU\ l^{-1}$	$FTU\ l^{-1}$	$doubling\ d^{-1}$	$d\ doubling^{-1}$
3/22/2016	3/23/2016	0,884	1,595	0,590	1,174
3/28/2016	3/29/2016	0,981	1,633	0,510	1,360

4/9/2016	4/10/2016	3,545	4,916	0,327	2,120
4/22/2016	4/23/2016	0,587	2,48	1,441	0,481
4/27/2016	4/28/2016	3,67	5,087	0,326	2,123
4/30/2016	5/1/2016	5,858	6,874	0,160	4,334
5/4/2016	5/5/2016	6,856	10,655	0,441	1,572

Table 20 lists the highest growth rates for Turbidity found throughout the experiment. Highest was found between 4/22 and 4/23, calculated to be 1,441 $FTU\ l^{-1}\ d^{-1}$.

Water sample properties

TABLE 22 : SAMPLE PROPERTIES

	Units	
Sample properties	mm	mm^2
Diameter	27,50	
Radius	13,75	
Area		593,94

Table 21 shows the properties of the water samples counted under a microscope. These measurements were used to find the ratio used when finding the approximate amount of algae in each sample. The properties shown are diameter and radius in mm and area in mm². Most important property is the area and the diameter.

TABLE 23 : SCREEN-SAMPLE CORRESPONDING PROPERTIES

Screen		**Sample**	
height	width	height	width
cm	cm	µm	µm
20	27,3	0,52	0,715

Table 22 shows the correlation between the screen height and width and what is shown through the screen. The most important property shown is the sample height.

TABLE 24 : RATIO CALCULATION

Measured area	**Row area**	**Rows**	**Analyzed area**	**Calculated ratio**
$\boldsymbol{mm^2}$	mm^2	number	mm^2	number
593,94	14,30	2	28,60	20,77

Table 23 show the measured area, the stripe area, number of rows analyzed, the analyzed area and the calculated ratio. The stripe area derives from multiplying the diameter found in table 21 by the

sample height in table 22. The stripes represent the number of times the sample were counted across, lowering the scope one screen for each row. The analyzed area would therefore be the stripe area multiplied by the number of rows counted. Finally, the calculated ratio was found by dividing the measured area by the analyzed area.

Refilling

TABLE 25 : REFILLS

Date	Height drained	Height added	Final Water height	Volume
dd/mm	cm	cm	cm	L
20.mar	50	55	105	7793,11
23.mar	25	85	110	8164,21
26.mar	20	85	105	7793,11
30.mar	20	80	100	7422,01
02.apr	10	90	100	7422,01
10.apr	10	90	100	7422,01
21.apr	10	90	100	7422,01
01.mai	50	50	100	7422,01

Table 24 shows the amounts of water replenished at each respective refill. These volume values are calculated based of off the tanks proportions.

TABLE 26 : WATER VOLUME CALCULATION

Proportion	Unit	Calculation	Value
Diameter	m	*Given by supplier*	3,00
Radius	m	*Given by supplier*	1,50
Area	m²	$\pi \cdot r^2$	7,07
Height (Upper)	m	*Given by supplier*	1,20
Volume (Upper)	m³	$7{,}07 \cdot 1{,}2\ m$	8,48
Height (Bottom)	m	$Tan(5{,}71) \cdot r$	0,15
Volume (Bottom)	m³	$\frac{1}{3} \cdot h \cdot \pi \cdot r^2$	0,35
Volume (Total)	m³	$Vu + Vub$	8,83
Water volume	m³	$7{,}07\ m^3 + 0{,}33\ m^3$	7,42
Water volume (at 100% volume)	L	*7,42*(1000*1,00)*	7422,01

Table 25 shows the water volume calculations (the volume at a 100cm water column in the tank).