Dimensioning of a Calcium reactor in abio filter for restitution of calcium concentrations in shellfish

Assessment of nutrient flow and algae production in the bio filter pond

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Foreword

This final report was put together by the 3rd year AET-international students Rita Lemos and Thalita Arruda as one of the products of their 3rd year minor. The report was requested by the research group of aquaculture of the Hogeschool Zeeland, together with the company Aquaculture Zeeland (costumer) and it is intended to be used as a monitoring overview of the bio filter pond of the company Aquaculture Zeeland and a first simulation (laboratory small scale experiment) of a calcium reactor for the bio filter pond.

The responsible students for this project would like to thank Kristiaan Van Rooijen and Joshi Lenferink for their help on transportation, Jorik Creemers and Jan van der Vleuten for their guidance and Wessel Bakhuizen from Aquaculture Zeeland for all the help during the project.

Dimension of Calcium reactor in a bio filter for restitution of Calcium concentration in shellfish

Assessment of nutrient flow and algae production in the bio filter pond

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Abstract

In order to optimize the usage of water in Aquaculture Zeeland by recirculating the water, an assessment of the current pond bio filter and its performance was made in this work, and a solution to the possible problem of calcium and carbonate depletion from the shellfish was researched. It was concluded that the microalgae which are not eaten by the shellfish culture and reach the bio filter are taking in a significant dose of the Total Nitrogen from the water and hence the algae themselves are proliferating. It was therefore proposed and that a good solution for this problem is the creation of a small reactor using ground oyster shells.

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1. Introduction

Zeeland Aquaculture is a pilot farm for cultivating oysters and clams on land. The farm also produces the algae that will be used to feed the shellfish. The company produces annually 5400kg of shellfish and uses a water recirculation system, which include the ponds where the algae are cultivated and the shellfish ponds. Currently the water for the algae ponds is pumped from the Eastern Scheldt, that water is filtered and three different nutrients are added with different concentrations, NH_4CL, MAP, and NA_2SiO_3. These nutrients are important for the algae growth. Afterwards the water from the algae pond is pumped into the shellfish pond so the shellfish can be fed using the provided algae (see figure 1).

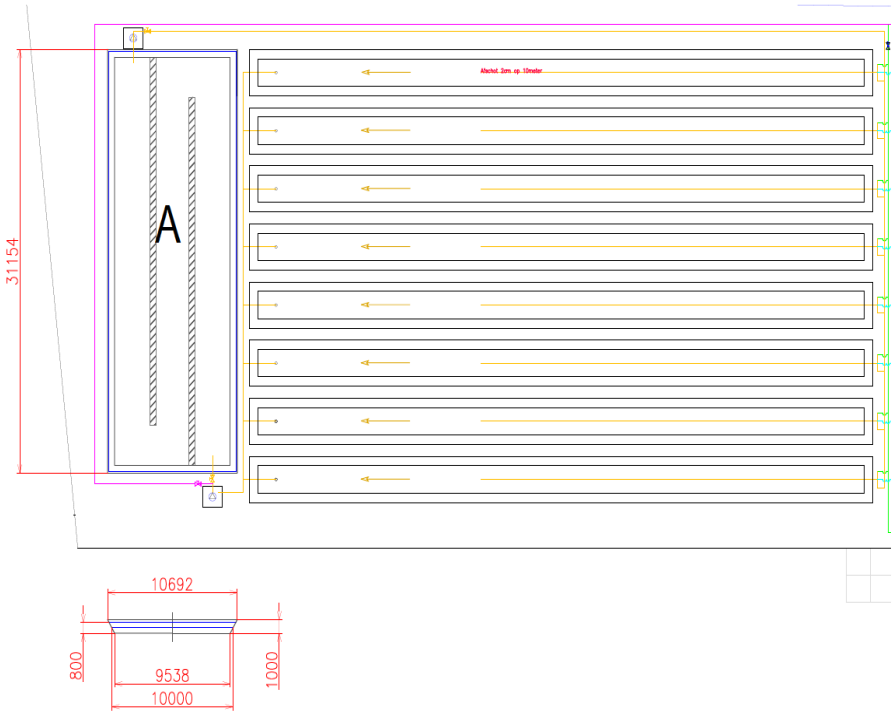


Figure 1: Schematic overview of Zeeland Aquaculture. "A" indicates the pond bio filter.

Last year the company implemented a bio-filter that was supposed to filter the water that comes out from the whole system with the aim of optimizing the re-use of that water and reduce its waste to purify the effluent before discharging it to the Eastern Scheldt. The aim was to reduce the waste load of the effluent and thereby reduce the discharge tax, but the macroalgae was not showing a good growth rate and was being eaten by resident microorganisms.

During this, a spontaneous growth of microalgae was noticed on the seaweed pond. Using the spontaneous growth of that microalgae, Zeeland Aquaculture decided to recirculate the water in the filter back on the shellfish ponds so that that same algae could be consumed by the shellfish and no additional taxes would have to be paid for the water discharge.

But to make the water of the circulation viable to go back in the shellfish ponds, some aspects must be considered. The water must be purified in order to remove some nutrients that may be poisonous for them and also the concentration of calcium carbonate in the water replenished. The calcium carbonate is important for the shellfish to build their shells. The calcium carbonate concentration in the water after it passes trough the shellfish ponds is decreased from its original concentration because the shellfish already consumed a great amount of it. A possible solution found by Aquaculture Zeeland for the restitution of the calcium carbonate is the implementation in the seaweed pond of a lime reactor made from ground shells. Due the fact that the amount of smashed shells for a project of this magnitude would be extremely high, it was decided that a simulation of the original conditions in a laboratory setting was in order. The proposed experiment consists in the introduction of a small lime reactor made of smashed shells, the same material that will be used on the seaweed filter, now called bio-filter pond, where the water will pass through. In the experiment it will be possible to monitor different conditions and combine different aspects. The experiment was run using the water from the inflow of the bio-filter pond. The main question of this research is:

Does a combination of microalgae growth and the addition of a lime reactor in the form of crushed shells treat the water in order it to contain enough calcium carbonate and a good nutrient balance for the shellfish?

Monitoring the effect of this combination in the pond will make it possible to determine if the concentration of the algae in the bio-filter pond is enough to feed the shellfish, also if the microalgae are able to purify the water in a way that can be consumed by the shellfish and finally using this experiment to determine

the calcium carbonate uptake using the lime reactor and to monitor the variables that may influence the calcium uptake ratio such as retention time and alkalinity.

2. Background

Generally, bivalve shells have three layers: the periostractum and two calcium carbonate layers ***(S.Hahn, 2011)*** therefore, shells from dead shellfish could be a good source of calcium carbonate. Under normal conditions calcium carbonate is water insoluble, having the solubility of 14 mg/L but in presence of carbon dioxide the solubility is amplified 5 times. The presence of CO_2 in the water on the monitored pond may vary according to the biological activities of the microalgae. If the CO_2 fraction rises it must be accompanied by a decrease in pH to maintain equilibrium ***(Glassman, 2010)***.

Besides the amount of the calcium carbonate on the water, other water characteristics are also important for a good maintenance of the shellfish and must be taken into account on the present project. A monitoring of the nutrients' flow becomes important, as the nutrient proportion and quantity will affect the growth of other species which will also be used to maintain the shellfish, in this case, the aforementioned species were microalgae.

All organisms have approximately the same nutrient requirements because they are all built with the same major types of molecules. The relative ratio of these nutrients to each other is called stoichiometry. The stoichiometry of carbon, nitrogen, and phosphorus at balanced growth is generally taken as 106:16:1 (C:N:P by atoms or moles) and is referred to as the Redfield ratio. The Redfield ratio is derived from nutrient contents of phytoplankton grown with excess concentrations of all nutrients at conditions optimal for maximum growth. Deviations from these ratios indicate nutrient limitation (Dodds, 2010).

The use of microalgae is a good way of taking out the excessive nutrients, which arrive from the wastewater and at the same function as an extra food source. The disadvantage in the solution is in the difficulty to control the population (bloom and crash cycles), and find a balance between the residence time of the population, as too much water exchange might wash away the

culture, and not allow it to grow to the appropriate levels for taking up all the nutrients (Troell M., 2003).

3. Material and Methods

The tasks to achieve the answer for the main question of this research were divided in:

- Sample collection
  - Collection of small amounts of water in order to analyse the nutrients removal and the algae production in the pond bio filter
- Algae concentration calculation
  - Algae cell count in order to determinate the concentration of algae in the inflow (beginning) and in the outflow (end) of the pond and to calculate the production of algae
- Calculation of SGR
  - The SGR (specific growth rate) is a number that determinate the growth rate per day that helps to understand and compare the production of algae in different collecting days
- Water Balance
  - The water balance allows the observer to know about the amount of water, which flows in and out, and how much time it takes for the water system to be fully refreshed (concept known as the residence time).
- Substance Balance
  - A measurement of each specific nutrient and the percentage of its removal in the bio-filter.
- Lime Reactor
  - An experience made in the laboratory that simulates a lime reactor using different materials like smashed shells of oyster and cockles and also limestone

3.1. Sample collection

The samples were collected directly from the bio-filter pond using sampling bottles of 100 mL. The bottles were labelled according to which function they were used for, such as for the measurement of algae, nutrients and calcium concentration. The collections occurred five times, always with double sampling. All the algae samples were fixated as soon as they are collect. For the fixation, Lugol's solution (concentrated) was used. It was added to the samples

drop by drop until it turned a light brown colour. The algae samples were collected on the inflow and the outflow of the pond.

Sample	Date	Temperature (ºC)	Weather	Time
Sample 1	16/April	8ºC	Cloudy	10:00
Sample 2	19/April	8ºC	Partly cloudy	17:00
Sample 3	24/April	16ºC	Scattered clouds	11:00
Sample 4	17/May	10ºC	Drizzle	10:00

Table 1. Sample legend with date, temperature, weather and time of collection.

3.1.1. Algae concentration

The algae concentration measurement took place in the laboratory of the university using the counting chamber.

Materials:

- Pasteur pipette
- Cell counting chamber
- Microscope
- Sample bottles

Methods:

Using the pasteur pipette, some drops were taken from the sample bottles and placed on the counting chamber very carefully using the sides of the chamber. Then the counting chamber was placed on the microscope for proper visualization. The counting starts from left to right in each chamber. All twenty-five chambers had their microalgae counted. The amount of algae found on the samples taken on the inflow was compared with the samples taken on the outflow so that the production rate could be calculated. In the counting chamber the sides of the corner squares as well as those of the middle square are each 1mm long. Therefore, the area of each corner square and of the middle square is $1mm^2$. When assembling the chamber by fixing the cover glass, a three-

dimensional space is created and the depth is 0,1mm. As a consequence, the volume to be counted for each corner square as well as the middle square is $0{,}1mm^3 = 0{,}1\mu L$. By multiplying the cells counted in one corner square by 10^4 (= 10.000) the cell number per mL is calculated as $10^4 * 0{,}1\mu L = 1mL$.

3.2 Specific Growth Rate (SGR)

To calculate the production of microalgae in the pond, the formula of Specific Growth Rate was applied. This formula allows to determine how many microalgae cells have been produced in the bio-filter pond per day.

$$SGR = \frac{(\ln Wt - \ln Wo)}{(t1 - t0)}$$

(Where *Wo* is the concentration in the inflow of the bio-filter pond and *Wt* is the concentration in the outflow. *t1* and *t0* are the different timepoints at the end and the beginning of the contemplated timeframe, respectively)

3.3 Water balance

The water balance allows the observer to know the amount of water from both the inflow and the outflow, and how much time it takes for the entire system to be fully refreshed (concept known as the residence time).
In the present case, the inflow and outflow were given by the client.

For the calculations of the retention time:

The average retention time (R) can be generally determined as follows if volume (V) and discharge (Q) from outflow/inflow are known:

Equation 1:

$$R = \frac{V}{Q}$$

with R = Residence Time [days]; V = Volume [m^3]; Q = Discharge [m^3/day].

3.4 Substance balance

A "substance balance" is an accounting of all substance accumulations that enter and leave a 3-dimensioned space over a specified period of time. Changes in internal substance storage must also be considered. Both the spatial and temporal boundaries of a substance balance must be clearly defined in order to compute and to discuss a substance balance. A complete substance balance is not limited, but includes all substances that enter and leave the spatial boundaries.

For this assignment, the substances taken into account are:

- Nitrite (mg/L NO_2^-N)
- Ammonium (mg/L NH_3-N)
- Orthophosphate (mg/L PO_4^{3-})
- Nitrate (mg/L NO_3-)
- Alkalinity (CaCO3 mg/L)

Nitrite and Orthophosphate and Ammonium were determined with the Hach kit DR/2400. The test used for this work measures the ionized ammonium plus the de-ionized ammonia. When in amounts superior of 19mg/l, the de-ionized ammonia can become toxic to shellfish. In order to analyze the toxicity of the ammonia in the water, it is necessary to calculate de percentage of NH_3 in the Total Ammonia Nitrogen (TAN). The corresponding instructions can be found in the hand guide of the Hach kit.

The methods used for the determination of alkalinity and Nitrate content are the following:

Alkalinity

For the detailed laboratory protocol, please refer to Appendix 2.

Alkalinity is expressed in the $CaCO_3^-$ concentration (mg/L) and it is calculated with the following Chemical formula:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$$

The amount mmol of HCO_3^- is calculated by the results obtained in the "P" alkalinity results of the alkalinity protocol (Appendix 2) by converting the result in mmol/L.

In this experiment HCO_3^- was always the limiting factor, so the $CaCO_3$ was given by:

$N (CaCO_3) = N (HCO_3^-)/2$ (in mmol)

And the concentration is given by the following equation: $[CaCO_3] = M (CaCO_3) * N (CaCO_3)$ (in mg/L)

3.5. The Lime reactor

The objective of the experiment was to find if the shells release enough calcium and what is the necessary shell quantity in order to release the ideal calcium amount for the shellfish. The experiment setup is, in short, a simulation of the situation in the pond.

Three lime reactors were used containing different materials: oyster shell, cockleshell and limestone. To build the lime reactors, three measuring cylinders of 1 litter were used by filling them with 600 mL of water each and then one had added to it 400 mL of oyster shell, the second one had 400 mL of cockleshell and the last one had 14 mg of limestone. The water from the inflow of the bio-filter pond reacted with the shells during 4 different retention times: 7 minutes, 14 minutes, 30 minutes and 1 hour, except for the limestone that was used just during the retention times of 7 min and 14 min because it diluted very quickly on the water. The same procedure was done 4 times for each material: twice without aeration and twice with aeration. The calcium concentration was

measured for each retention time using the Atomic Absorption Spectroscopy (AAS) method, which will be described bellow.

Figure 2: Oyster and cockles experimental setup.

Calcium

To determine the calcium the AAS method was applied. The AAS technique involves the suction of an aqueous sample into a flame where the analyte is atomized. An isolated atom absorbs light at very specific wavelengths that are unique to each element.

Materials:

- Atomic Absorption Spectroscopy
- 100mL volumetric flask

- Calibration series Ca
- Filter paper

Methods:

Samples have to be filtered.

Measure the blank: one volumetric flask filled with denim water.

After measuring the blank the volumetric flask has to be removed and replaced for the first volumetric flask with the diluted stock solution to determine the calibration curve and afterwards with the sample.

At the end the unknown concentration of Ca can be determined by the formula from the calibration series.

Knowing the concentration in each one of the inflows and the outflow, and using the value of the volume of water which comes in from each inflow and the volume of water which comes out (outflow) one calculates the load by the equation:

$$Load = Q * n$$

where Q = Discharge [L/day] and n = substance concentration [mg/L].

This value is used to have a visible mass of substance which comes inside and outside the system per day.

Using the load values found, we calculate the substance balance by applying $Load_{inflow} - Load_{outflow}$, obtaining with this the substance balance of the basin per day.

The reactor dimensions will afterwards be dimensioned using the results of the 30 minutes and 60 minutes residence time of Calcium and Carbonate, in the following equation:

Equation 3:

$$[Calcium]_{60\ minutes\ residence\ time} - [Calcium]_{30\ minutes\ residence\ time} = [Calcium]_{theoretical\ 30\ minutes\ residence\ time}$$

And then, this value is multiplied by 2 in order to obtain the calcium concentration of one hour and in this way multiply it by the amount of water which will pass by in 1 hour time (discharge). This is afterwards converted in concentration of Calcium per year.

Equation 4:

$2^{*}\ [Calcium]_{theoretical\ 30\ minutes\ residence\ time}\ ^{*}280\ m^3/h\ ^{*}\ 24h\ ^{*}365\ days$ = Calcium released in the water by the reactor in one year time.

These calculations are repeated for the 15 minutes residence time and the Carbonate calculations.

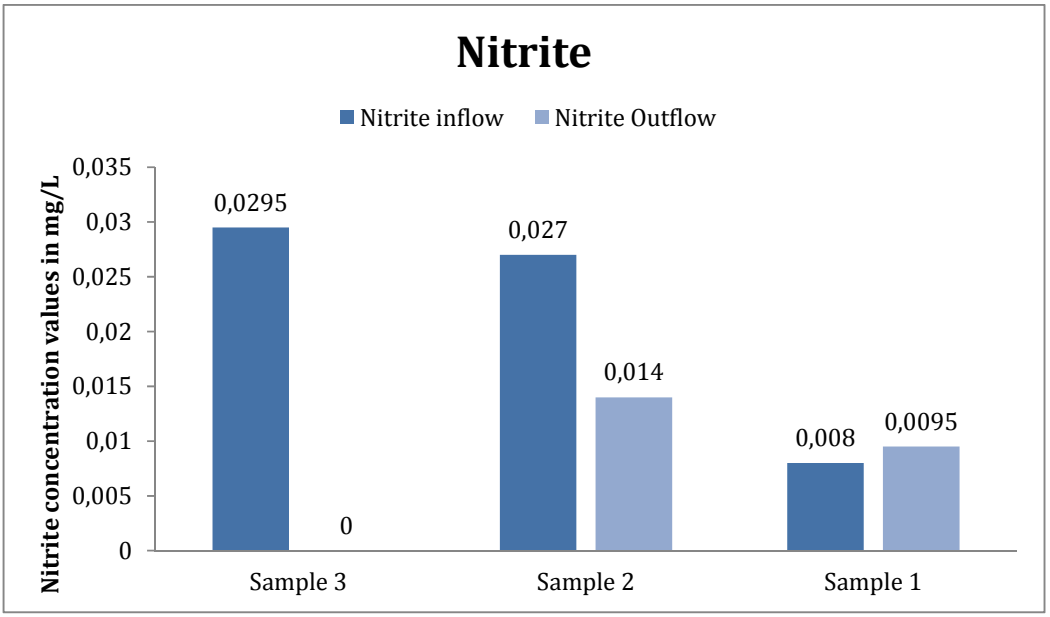
4. Results

4.1 Nutrient flow

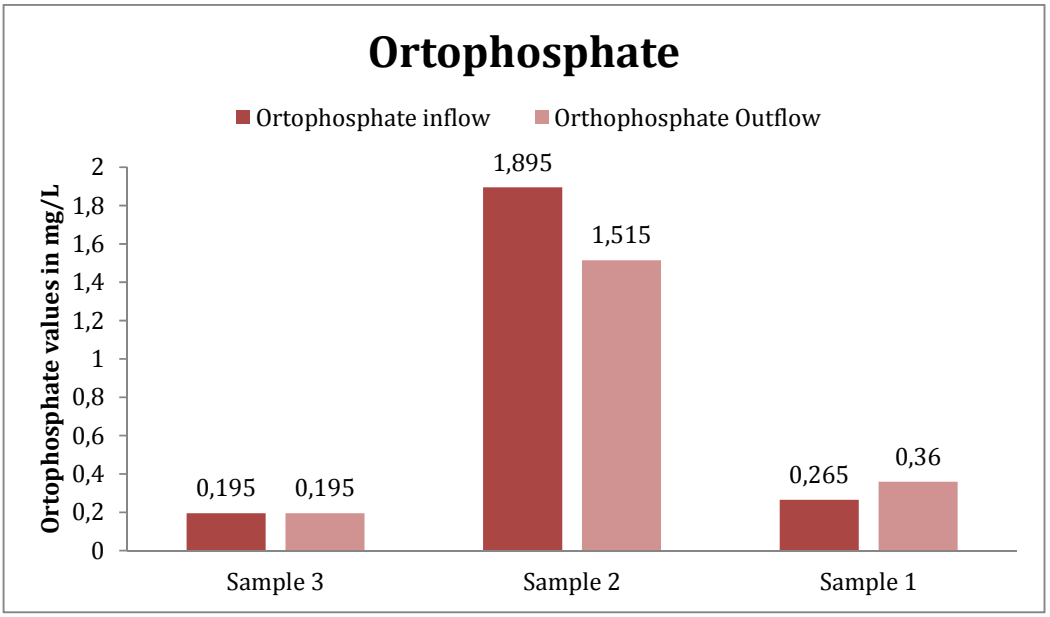
The analysis of the nutrients shows that in sample 1 and 2 the concentration in the outflow is lower than in the inflow. In sample 3, ammonium has showed a lower concentration in the outflow and nitrite is not found in the outflow while the orthophosphate shows no difference in the concentration between the two points (inflow and outflow). The three samples were collect in different days. The ammonium concentration in the three cases was high, surpassing the concentration limits covered by the Hach kit, thus they were diluted 10 times in samples 2 and 3, and 2 times in sample 1. In the ammonium chart below, the concentration is showed without the dilution. The nitrate concentration was always below 0,1 mg/L.

Sample	Date	Temperature (ºC)	Weather	Time
Sample 2	19/April	8ºC	Partly cloudy	17:00
Sample 3	24/April	16ºC	Scattered clouds	11:00
Sample 4	17/May	10ºC	Drizzle	10:00

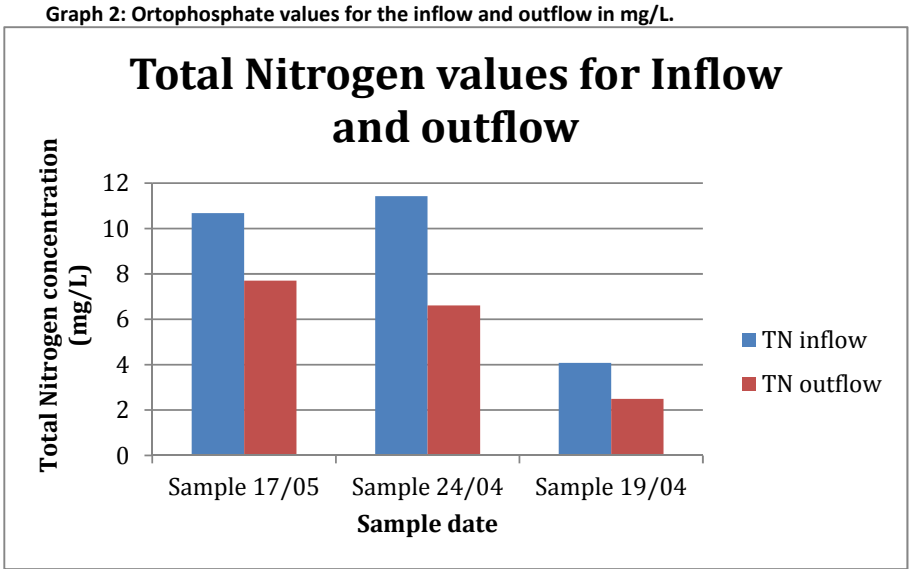
Table 2. Sample legend with date, temperature, time and weather of collection.



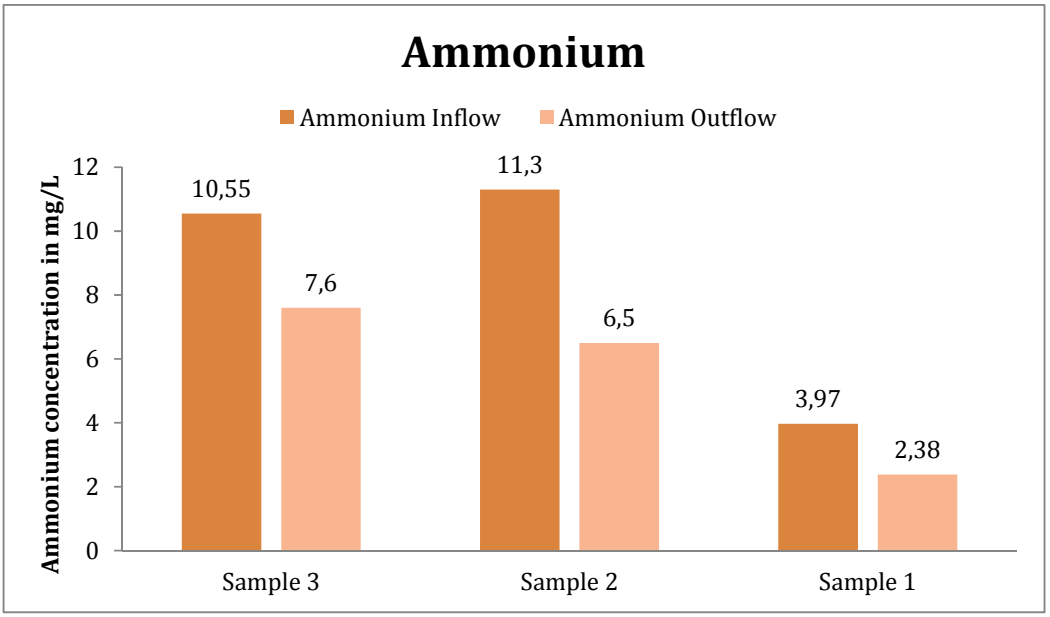
Graph 1: Nitrite values for the inflow and outflow in mg/L.



Graph 2: Ortophosphate values for the inflow and outflow in mg/L.



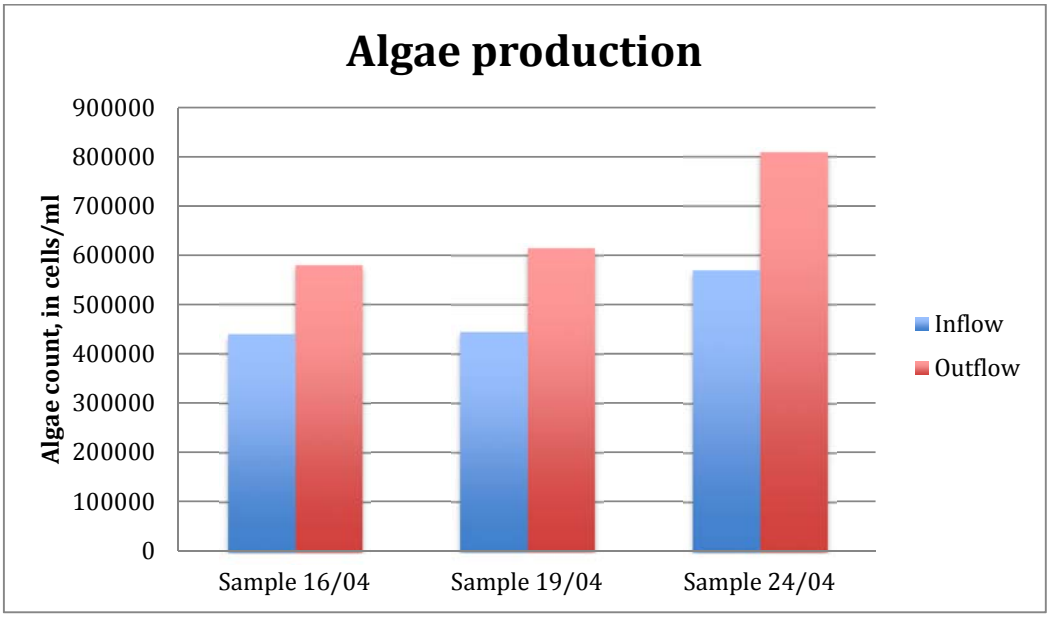
Graph 3: Total Nitrogen in the inflow and outflow for each sample day (mg/L)



Graph 4: Ammonium values for the inflow and outflow in mg/L.

4.2. Algae production

The algae production is directly affected by the weather and it has showed a different production ratio for each day of collection. The warmest sampling day (16ºC) was in the 24th of April and it has a SGR of 1,40/day while in the 19th of April the SGR was 1,29/day and in the 16th of April it was 1,10/day.

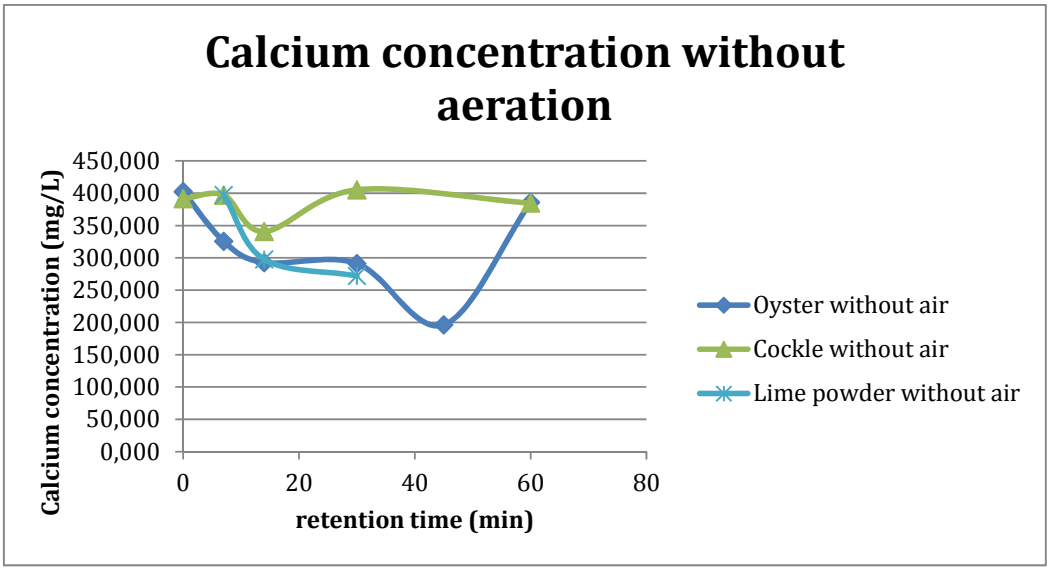


Graph 5: Algae production, in cells/ml

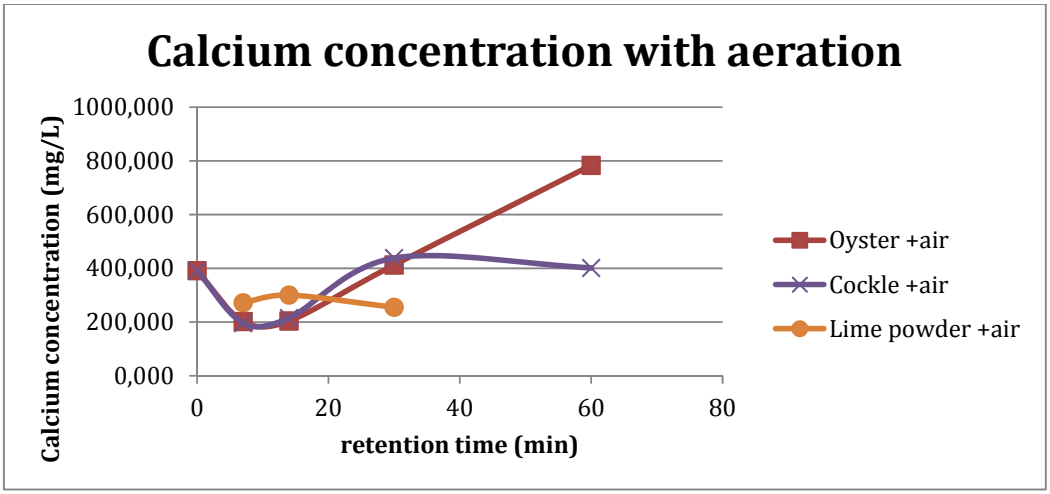
4.3. Calcium uptake:

After running the experiment in the laboratory with different materials, retention time with and without aeration, the calcium concentration was measured using the ASS machine. Looking in the graphics below it can be observed that between the timepoints of 0 and 20 minutes, all the materials tested showed a decrease in the calcium concentration. However, after this point the concentration level increases again and the different materials differ in the time they released the calcium. It is observed that the oyster shells with aeration are the material that better succeeds in the calcium release. Cockleshells with aeration have the smaller drop in the beginning and the level of the calcium concentration is kept until the final retention time. The oyster shells without the aeration after the initial drop in the first minutes have a slight increase afterwards but only until the initial concentration value of 40 mg/L is again attained. Also, the cockleshells without aeration don't rise higher than the initial concentration. Limestone also had showed a non-representative raise in the calcium concentration with or without aeration.

The following graphs were obtained by diluting of the samples (obtained during the experiment) 1000 times and the use of 3 calibration curves (one for each day in which the calcium was measured in laboratory). The 1000 dilution was made since the spectrophotometer range is from 0 mg/L to 5 mg/L.



Graph 4: Calcium concentration without aeration in the experiment, in mg/L.



Graph 5: Calcium concentration with aeration in the experiment, in mg/L.

4.4. Alkalinity

After conducting the experiments, the carbonate concentration was measured by the titration method (see in methods). However, since this method is connected to the pH and for pH values of less than 8.3 there is no alkalinity. The carbonate concentrations will have quite some variations:

The lime powder had no values for carbonate in all the residence times (with or without aeration), as such, it will not have any alkalinity values (since the limiting factor is the HCO_3^-).

Alkalinity is then expressed in the $CaCO_3^-$ concentration (mg/L) and it is calculated with the following Chemical formula:

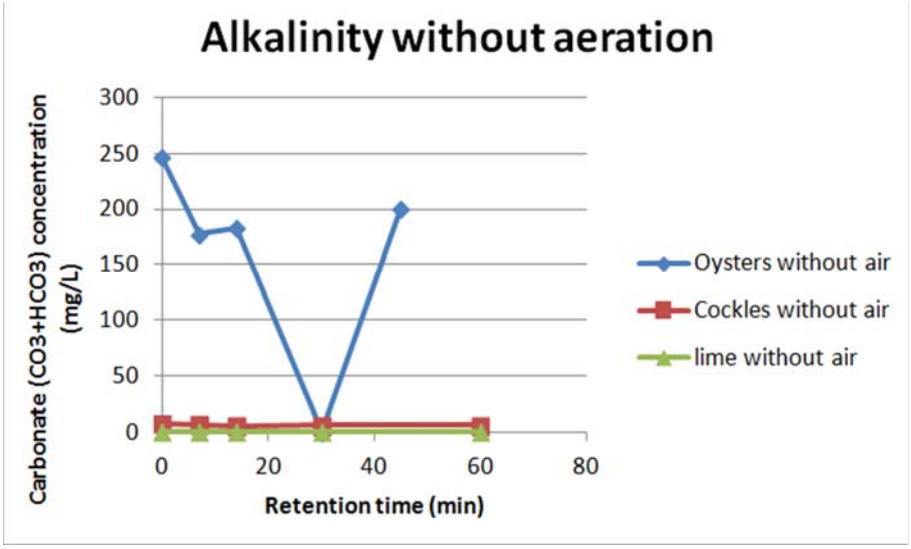
$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$

The amount (mmol) of HCO_3^- is calculated by the results obtained in the "P" alkalinity results of the alkalinity protocol (see in appendix 2) by converting the result in mmol/L.

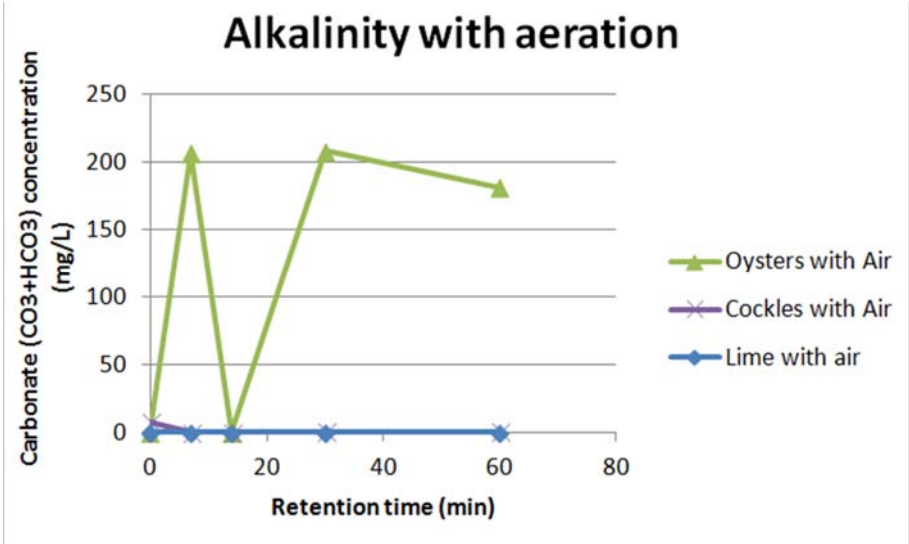
In this experiment HCO_3^- was always the limiting factor, so the $CaCO_3$ was given by:

$N(CaCO_3) = N(HCO_3^-)/2$ (in mmol)

And the concentration is given by the following equation: $[CaCO_3] = M(CaCO_3) * N(CaCO_3)$



Graph 6: Alkalinity for the experiment without air, in mg/L.



Graph 7: Alkalinity for the experiment with access to air

4.5. Shells reactor dimensioning

Considering that the 5,4 tons of shellfish produced by the company, their requirement of Calcium and Carbonate will be respectively 1.08 and 1.62 tons.

Dimensioning for oysters without aeration:

If in half an hour retention time using oysters release 189.7 mg/L of calcium in the water,

189.7*2= 379.4mg/L in one hour.

If the amount needed of Calcium for the oysters is 1,08 tons (1080kg) of calcium:

1080kg/(3.794*10^-4 kg/L in 1h) = 2849604.2 hours needed to release the 1080 kg of calcium from a 1L reactor

Which is equal to 325 years for 1 L reactor.

So the minimum dimension for the reactor is 325L reactor, or 0.325 m^3 shell volume.

Dimensioning for oysters with aeration:

If in half an hour retention time using oysters with aeration releases 370.9 mg/L of calcium in the water,

370.9*2= 741.8mg/L in one hour.

If the amount needed of Calcium for the oysters is 1,08 tons (1080kg) of calcium:

1080kg/(7.418*10^-4 kg/L in 1h) = 1455918.0 hours needed to release the 1080 kg of calcium from a 1L reactor
Which is equal to 166 years for 1 L reactor.

So the minimum dimension for the reactor is 166L shell volume, or 0.166 m^3 shell volume.

5. Discussion

5.1 Nutrient removal

The lower nutrient concentration in the outflow and then in the inflow has showed that biological activity is occurring in the pond; both algae and bacterial activity can be noticed. The nutrient removal in the bio-filter pond might be made also by macroalgae that are growing spontaneously in the pond. The bacterial activity can be easily seen since the nitrogen cycle must be completed by some bacteria species, for instance, *Nitrosomas* and *Nitrospira* that will turn NH_4 into NO_2 and NO_3, though the level of Nitrate is not significant what might be happening is due to consumption by the micro and macroalgae in the pond. The algae has great consumption of nitrogen for its biological activities and comparing the total nitrogen (graph 5) to the algae production it is possible to recognize the day that there was higher algae production (24/04) was also the same one where nitrogen had greater removal. Concentrations of phosphate can fluctuate according to different factors, the lower removal in the bio filter might have happened due to the lower algae uptake and also because phosphate can bind itself to the soil or come free from it at will. Ammonia concentration levels are not a problem in a simple flow-trough system but might become a problem when using recycling and reuse systems with bio filters to remove ammonia within the system. Though the levels of ammonia showed in the charts above showed the total ionized ammonia (NH_4-N) plus the deionized ammonia (NH_3-N), making the calculations to find out the percentage of toxic ammonia in the water was used the highest level (11,3 mg/L) registered in the pond, the outcome of this calculation was the amount of 1,38 mg/L of deionized NH_3-N in 8,3 pH. In general, NH_3-N concentrations should be held below 0.05 mg/L for long-term exposure (Timmons, 2002) but according to Li(1997), oysters are the most resistant marine organism to ammonia and can handle up 19,102 mg/L. From the charts is also possible to observe that in Sample 3 (24th of April) all the nutrients had a higher concentration then the other sampling days, this might have happened because the oysters tend to decrease their metabolic activity when the temperature drops and these collections were made at the end of the

afternoon and the temperature was around 16ºC while the other samples were collected during the morning in colder days.

5.2. Algae production

Looking up the results of the algae production it is remarkable the difference between each sampling day. As a live photosynthetic organism, its metabolism and reproduction can change according to the weather and water conditions. The highest SGR registered in this research was of 1,40/day while at this same time of the year in 2012 the algae counting showed a SGR of 4,39/day. This decrease in the algae production can be explained by the long winter and a colder summer compared to last year. The lack of light and a low temperature influence directly the algae activity making it slower.

5.3Calcium releasing and alkalinity

In several residence times of the experiment, the alkalinity values were equal to zero, which is why when it came for the pond dimensioning the results used were the Calcium results and not the alkalinity. Although it is verified that there will be a release of carbonate over time (for oysters).
In terms of the materials for the calcium reactor, the lime powder had an effect of making the water turbid (which might affect the algae production if used in the pond). It was also the material which released less calcium and carbonate into the water.
As such, the results of this material were registered, but it was not considered a good material for a reactor, and the dimensioning was not calculated for this substance.

Due to the lack of consistency in the results for the calcium carbonate (alkalinity), the dimensioning of the oyster shell did not take these values into consideration, simply taking the calcium values instead.
Regarding the results of the calcium uptake in the experiment, an initial decrease of calcium in the first 15 minutes of the experiment is observed, this might be

due to the reaction of the shells to the water, which initially might cause the calcium attaching itself to the shell. However, considering that the initial experiment occurred in a static environment, it is likely that it won't happen in a running water situation.

6. Conclusion

6.1. General

Regarding the main question "*Does a combination of microalgae growth and Calcium reactor in the form of crushed shells treats the water in order it to contain enough calcium carbonate and a good nutrient balance for a good growth rate of the shellfish?"*, one can answer that in theory, yes.

The microalgae currently in the pound do have the intended effect of diminishing the total nitrogen concentration.

The production of algae has a positive K, which means that the algae will multiply in the bio filter and produce an extra food source for the shellfish.

Finally, the results obtained in the laboratory with the small scale calcium reactor allow to dimension a realistic reactor for the bio filter in question, which in theory, will aid the shellfish to replenish their calcium needs.

6.2. Nutrient removal

Looking at the results and analyses is possible to say that the bio-filter pond is a functional bio filter in terms of making the water proper for the oyster feeding. All the nutrients had showed a non-harmful level in the outflow due to the algae purification ratio, therefore the recirculation of this water can be done straight to the oyster pond without additional treatment for nutrient removal.

6.3. Algae production

Since the oyster pond received enough feeding from the algae ponds, the production level in the bio-filter pond can be considered enough if used as a complement and not as the main source of algae for the oysters. Even without an ideal temperature and weather the algae has been shown enough nutrient removal in the water making it consumable for the oyster and a representative growth rate. In better weather conditions, the level of nutrient removal and the algae production in the pond could improve.

6.4. Calcium release and Alkalinity

In terms of materials, the oyster shells was the one option which showed the best results, the minimum volume of oysters shells required for the minimum amount of Calcium release (for one year) without air is 0,325 m^3 and the ideal amount for oysters with aeration is 0,166 m^3.

7. Recommendations

It is recommended that further studies are conducted on the bio filter and close monitoring is performed once the production goes from using fresh water to a full recycling circle.

It is advised to exceed the minimum volume of oyster shells in order to secure that enough calcium will reach the shellfish.

It is also recommended that, when applied, the calcium reactor should be monitored in the field, in order to assess if in *in situ* the results maintain. More precisely, if the calcium and carbonate release maintains itself over the course of the year.

In the case of the nutrients concentration is recommended that a constant monitoring in the pond especially during the summer time, due the fact that was registered a substantial increase in the nutrients concentration during warmer days (maximum registered temperature 16°C). This monitoring is important to be sure that the amount of algae in the bio filter would be able to filtrate the nitrogen so it wont reach toxic level for the shellfish.

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Appendix 1: Results tables

Sample Legend:

Sample name	Date DD/MM/YY	Temperature ºC
Sample 1	16/04/2013	10ºC
Sample 2	19/04/2013	8ºC
Sample 3	24/04/2013	16ºC
Sample 4	17/05/2013	10ºC

Calcium results

Oyster without air	
resilience time (min)	Calcium concentration (mg/L)
0	402.530
7	325.543
14	292.549
30	291.174
45	196.316
60	386.032
Oyster +air	
0	391.531
7	201.815
14	204.564
30	412.716
60	783.603
Cockle without air	
0	391.531
7	397.031
14	340.665
30	405.279
60	384.658
Cockle + air	
0	391.531
7	196.316
14	215.562
30	438.273
60	401.562
Lime powder	

7	397.379
14	297.527
30	272.009
Lime powder +air	
7	272.009
14	300.362
30	254.998

Table 3: Calcium Concentration in the calcium reactor experiment

	CO3	**HCO3**				
Without air	P alkalinity	M alkalinity	mmol (CO_3)	mmol (HCO_3)	mg of CO_3/L	mg of HCO_3/L
exp n2 - 16/04 water (min) cockles						
0	0.99	7.59	0.0198	0.1518	23.763168	185.244576
7	0.29	6.63	0.0058	0.1326	6.960928	161.814432
14	1.23	5.49	0.0246	0.1098	29.523936	133.991136
30	0.59	6.31	0.0118	0.1262	14.161888	154.004384
60	0.84	6.2	0.0168	0.124	20.162688	151.31968
exp n1 - 21/03 - oyster						
0	1.5	8.63	0.03	0.1726	36.0048	210.627232
7	1.54	5.77	0.0308	0.1154	36.964928	140.824928
14	0.43	7.08	0.0086	0.1416	10.321376	172.797312
30	less 8.3					
45	0.91	7.335	0.0182	0.1467	21.842912	179.020944
Lime powder						
7	less 8.3					
14	less 8.3					
30	less 8.3					

Table 4: Alkalinity results in the laboratory, without air. The water used in this experiment was inflow water.

	P alkali	**M alkali**	**mmol (co3)**	**mmol (HCO3)**	**mg of CO3/50**	**mg of HCO3/50**	**mg of CO3/L**	**mg of HCO3/**

	nity	nity			ml	ml		L
8/05 water (min) oyster								
7	0.7	7.8	0.014	0.156	0.840112	9.518496	16.80224	190.36992
14	less 8.3							
30	0.57	7.985	0.0113	0.1597	0.6780904	9.7442552	13.561808	194.885104
60	0.24	7.18	0.0048	0.1436	0.2880384	8.7618976	5.760768	175.237952
8/05 water (min) cockle								
7	less 8.3							
14	less 8.3							
30	0.54	8.23	0.0108	0.1646	0.6480864	10.0432336	12.961728	200.864672
60	0.34	7.44	0.0068	0.1488	0.4080544	9.0791808	8.161088	181.583616
Lime powder								
7	less 8.3							
14	less 8.3							
30	less 8.3							

Table 5: Alkalinity results in the laboratory, with air. The water used in this experiment was inflow water.

Oysters without air	mmol (HCO_3)	Ca (mg/L)	Ca (mmol)	$CaCO_3$ (mmol)	$CaCO_3$ (mg/L)
0	3.452	402.530	4.022	1.726	172.748
7	2.308	325.543	3.253	1.154	115.499
14	2.832	292.549	2.923	1.416	141.722
30		291.174	2.909	0.000	0.000
45	2.934	196.316	1.961	1.467	146.826
Cockles without air	**mmol (HCO_3)**	**Ca (mg/L)**	**Ca (mmol)**	**$CaCO_3$ (mmol)**	**$CaCO_3$ (mg/L)**
0	0.1518	391.531482	3.912	0.076	7.597
7	0.1326	397.03052	3.967	0.066	6.636
14	0.1098	340.665384	3.404	0.055	5.495
30	0.1262	405.279076	4.049	0.063	6.315
60	0.124	384.65768	3.843	0.062	6.205

		5			
Oysters with Air	**mmol (HCO_3)**	**Ca (mg/L)**	**Ca (mmol)**	**$CaCO_3$ (mmol)**	**$CaCO_3$ (mg/L)**
0	3.452	391.531482	3.912	1.726	172.748
7	0.156	201.814682	2.016	0.078	7.807
14		204.564201	2.044	0.000	0.000
30	0.1597	412.716118	4.124	0.080	7.992
60	0.1436	783.6029	7.829	0.072	7.186
Cockles with Air	**mmol (HCO_3)**	**Ca (mg/L)**	**Ca (mmol)**	**$CaCO_3$ (mmol)**	**$CaCO_3$ (mg/L)**
0	0.1518	391.531482	3.912	0.076	7.597
7		196.315645	1.961	0.000	0.000
14		215.562277	2.154	0.000	0.000
30	0.0108	438.273302	4.379	0.005	0.540
60	0.0068	401.561629	4.012	0.003	0.340

Table 6: Table used to calculate the Calcium Carbonate concentration.

	N-NO2		**N-NH4**		**N-NO3**			
	Nitrite inflow (mg/L)	Nitrite Outflow (mg/L)	Ammonium Inflow (mg/L)	Ammonium Outflow (mg/L)	Nitrate Inflow (mg/L)	Nitrate Outflow (mg/L)	TN inflow	TN outflow
Sample 17/05	0.0295	0	10.55	7.6	0.1	0.1	10.6795	7.7
Sample 24/04	0.027	0.014	11.3	6.5	0.1	0.1	11.427	6.614
Sample 19/04	0.008	0.0095	3.97	2.38	0.1	0.1	4.078	2.4895

Table 7: Table with the results of the Nitrite, Ammonium and Nitrate concentrations obtained in the laboratory for each sample and Total Nitrate for inflow and outflow calculated in excel.

Appendix 2: Methods protocols

To calculate the Alkalinity a titration method was utilized. The alkalinity of water refers to the total amount of substances that can shift the pH to the alkaline side of neutrality (pH values >pH 7). By titration with an acid the alkaline compounds

in a sample are neutralized. The addition of acid shifts the $CO_3^{2-} \leftrightarrows HCO_3^- \leftrightarrows CO_2$ equilibrium in the direction of CO_2. At pH 8.3 (color change of Phenolphthalein) all CO_3^{2-} ions have been converted to RCO_3^- or CO_2. CO_2 remains partly dissolved and partly disappears from the solution as gas. If further titrated to pH 4.8 (color change of Methyl red), all HCO_3^- ions have been converted to CO_2.

Materials:

- 300 ml of sample
- Hydrochloric acid 0,02M
- Beaker
- Volumetric pipette 50ml
- 250ml Erlenmeyer flask
- 50ml burette
- Brom cresol green and methyl red mixed indicator
- Phenolphthalein indicator

Methods:

- Collect approximately 300 ml of sample in a beaker.
- Use a volumetric pipette and measure 50 ml of sample.
- Put 50.00 ml of sample into a 250 ml Erlenmeyer flask.
- Five to six drops of phenolphthalein indicator will be added to the sample. In the case that no pink color develops, the pH of the sample is less than 8.3 and the "P" alkalinity is zero.
- If a pink color developed, then the pH of the sample is above 8.3 and the sample needs to be titrated with 0.02 M hydrochloride acid until the color changes to colorless to define the "P" alkalinity.
- With the following calculations the total Alkalinity can be determined:
  Concentration of HCL * nr of ml reacted = n (HCL)
  N (HCL) = N (CO_3^{2-})
  N (CO_3^{2-}) / 50ml of sample = Concentration of sample