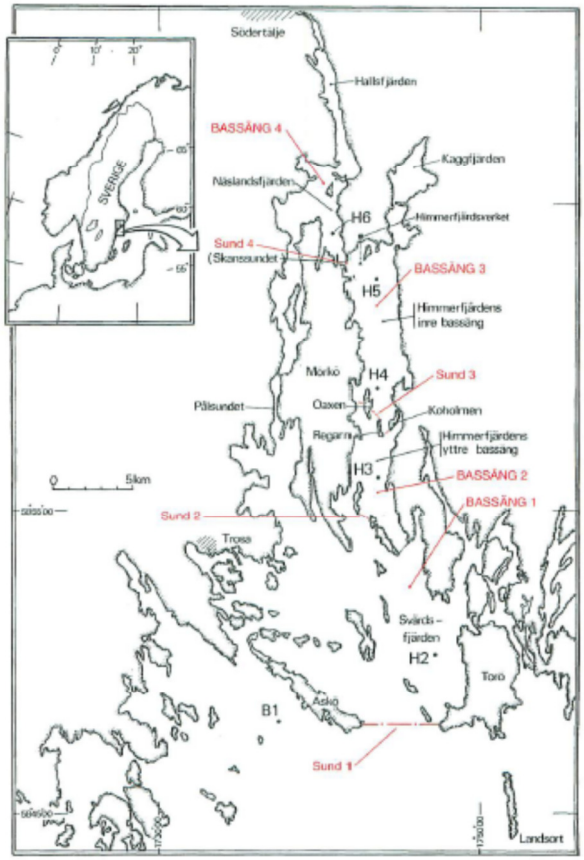
Measurement of nitrogen transformation rates in Baltic Sea sediment

Measurements of denitrification and anammox using the ^{15}N tracer method



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Abstract

Eutrophication is a contributing factor to high levels of nutrients, mainly nitrogen and phosphorus. These high levels of nutrients are mainly in case of leakage from farm land or waste water. Due to poor water mixing in the Baltic Sea, it becomes sensitive to eutrophication (Havet.nu 2011). The Baltic Sea is the world's largest brackish water area. This has created very tolerant animals which mean the animals can live in both fresh and salt water. There is a big physiological stress for these animals, they must balance their salt content by either excrete salt or collect salt to survive. This makes the Baltic ecosystem very sensitive for environmental changes. To protect the environment in the Baltic Sea, there is a convention: *The Baltic Sea Convention*, from 1992. The convention aims to, among other things, reduce emissions from anthropogenic sources. The study area in this thesis is Himmerfjärden, the archipelago outside Trosa. There is an ongoing project in Himmerfjärden since 2007, where the amount of nutrients coming from SYVAB is controlled.

The aim with this research is to determine the importance of bioturbation and different macrofauna species for the rates of nitrogen cycling in Himmerfjärden sediment; for this, a tracer method was used that uses nitrogen-15 (^{15}N-labeled nitrate and ammonium). The methods are used to determine the flux, i.e., the rates by which microbial processes catalyze the release of nitrogen from a fixed sediment area. And also determine the direction of these nutrient fluxes. Macrofauna such as the invasive Marenzelleria spp. and Macoma Baltica have been used in the bioturbation experiment, with different numbers of animals in each sample. Sediment sampling was done in Himmerfjärden and incubations of animals and nitrogen compounds at the Askö-laboratory. NH_4 concentrations were measured by a UV-spectrophotometer and fluxes for O_2, NH_4, NO_2 and NO_3 were measured with a combined mass- and gas-spectrometer.

From the results it can be seen that benthic concentrations of NH_4 vary dependent on where the sediment was taken, the monitoring stations location, compared to SYVAB, the sewage treatment plant in the inner Himmerfjärden. The closer to SYVAB, the more animals and the higher were the concentrations of bottom water NH_4. From the bioturbation samples nutrient fluxes were calculated, there was a negative flux meaning that the nutrients entered the sediment and a positive flux meant that the nutrients were transported into the water column. A good result of this is for NH_4 where the flux is higher the more animals it is in the sample. The total flux of nitrogen compounds (DIN) shows that the results follow the same trend as for NH_4.

Conclusions to be drawn from the results are that if there is a lot of nitrogen in the water column, then it is also a high amount of macrofauna in the sediment. When there is a lot of macro fauna, then there is a lot of NH_4 and then there is also an efficient degradation of organic material and the concentration of oxygen are low close to the sea floor. This is just what is proved close to the sewage plant in Himmerfjärden – A lot of NH_4, a lot of animals and a low concentration of oxygen.

Keywords: Bioturbation, Nutrient fluxes, Macrofauna, Eutrophication, Denitrification

Table of Contents

1. Introduction and background information....1
1.1. The Baltic Sea and Himmerfjärden....1
1.1.1. The Baltic Sea and its convention....1
1.1.2. Himmerfjärden and SYVAB....1
1.2. Eutrophication and sediment processes....2
1.2.1. Bioturbation....2
1.2.2. Denitrification....2
1.2.3. Anammox....3
1.3. Marenzelleria and Macoma Baltica....3
1.3.1 Marenzelleria....4
1.3.2. Macoma....4
2. Aim and research questions....5
3. Method and materials....6
3.1. Sampling methods....6
3.2. Preparation and incubation of the sediment....7
3.3. Analyzing method and materials....8
4. Results....10
4.1. Results from the UV-spectrophotometry....10
4.2. Results from the isotope pairing method....12
5. Discussion....15
5.1. Sources of error....15
5.2. Concentration changes....15
5.3. Isotope-pairing technique....16
5.4. Conclusion....18
6. References....19
6.1 Litterature....19
6.2 Internet....19
6.3 Figures....20
Appendix....21
Appendix 1 – Sampling data....21
Appendix 2 – Data from bioturbation samples....22
Appendix 3 – Reagent solution recipes....23
Appendix 4 – Different treatments for the bioturbation:....24
Appendix 5 – Concentrations for nutrients....25
Appendix 6 – Data from the bioturbation flux calculations....26

1. Introduction and background information

Eutrophication is a contributing factor to high levels of nutrients, mainly nitrogen and phosphorus. These high levels of nutrients, mainly in case of anthropogenic sources such as leakage from farm land or waste water. Due to poor water mixing in the Baltic Sea, it becomes sensitive to eutrophication (Havet.nu 2011). The study of this project is bioturbation in sediment from the Himmerfjärden in the southern Stockholm archipelago.

1.1. The Baltic Sea and Himmerfjärden

1.1.1. The Baltic Sea and its convention

The Baltic Sea is the world's largest brackish water ecosystem. The salt concentration varies from 2,0 ‰ in the Gulf of Bothnia to 30 ‰ in the Kattegat. Since the Baltic Sea consists of brackish water, a mixture of fresh and salt water, it has created a unique fauna of tolerant animals that is the most tolerant of both fresh and salt water. Which means it can live in both fresh and salt water. These animals live under a physiological stress because they must balance their salt content by either excrete salt or collect salt to survive. The animals in the Baltic Sea are also smaller than those on the West Coast. It makes the Baltic ecosystem very sensitive for environmental changes. Some of the most important factors in the water-system are the water movement, temperature, nutrients, wind, and oxygen access. The amount of dissolved oxygen is temperature-dependent and increases with decreasing temperature; respiration makes it change with depth. In deep water there is only oxygen consumption because of the degradation of organic material (Nolin 2008).

To protect the environment in the Baltic Sea, there is a convention: *The Baltic Sea Convention*, from 1992. The countries around the Baltic Sea and the European Union should prevent and eliminate pollution by harmful substances to the marine environment. Every country has the responsibility to inform and confer if there is a pollution accident, and to cooperate, report and exchange information, and to inform the public about important regulations (Mahmoudi *et al.* 2004, s. 120). One important part of the convention is that municipal sewage plants have to clean the water with biological purification and the best technique should be used (Klintberg 2011).

1.1.2. Himmerfjärden and SYVAB

The area of Himmerfjärden is 174 km^2 with an average depth of 17 m. The catchment area is 1286 km^2 and 20 % is agricultural area with conurban development. The landscape area around Himmerfjärden is an open coast landscape with a small amount, 95 km^2, of uninhabited forest area. Himmerfjärden catchment area consists of both fresh and salt water primarily from Lake Mälaren and the Baltic Sea but also runoff is important. The influx is limited by several thresholds of 18, 22 and 28 meters deep at the inlet between Askö and Torö, and the tight and shallow water inlet to Himmerfjärden obstructs the deep water exchange (Elmgren et al. 1997).

The variations of nutrients in Himmerfjärden depend mainly on the local impact, Himmerfjärdsverket and total nutrient concentrations in the open Baltic Sea. Deep water with nutrients from the Baltic Sea comes into Himmerfjärden while surface water is transported out of the bay. The difference in density for the water is the primarily driven factor for the water movement in Himmerfjärden. This difference is created by the fresh water from land and the up and down water stratification caused by the wind in the outward coastal zone. The water-circulation is controlled by the weather conditions. The weather affecting exchanges between

the coastal zone, receiving water and the deep-sea and rainfall where temperature, solar radiation and wind speed are important parameters for the current changes and the water stratification between Himmerfjärden and the Baltic Sea. In the coastal areas and in the outer part of Himmerfjärden are these most important factors for the current changes and the water stratification between Himmerfjärden and the open Baltic Sea (Elmgren *et al.* 1997).

Himmerfjärdsverket or SYVAB[1] is a sewage treatment plant in Himmerfjärden. The catchment-area for SYVAB is very large. It starts from Skärholmen in the south of Stockholm and goes down to Södertälje (www.ecology.su.se). Himmerfjärden is one of the most analyzed water areas in Sweden (Elmgren *et al.* 1997). Since 2007 the amount of nutrients (N and P) coming from SYVAB are monitored in the Himmerfjärden eutrophication project (www.ecology.su.se). The aim of the project is to see what kind of effects the nutrient discharge has on the water quality and the influence on algae blooms (Elmgren *et al.* 1997).

1.2. Eutrophication and sediment processes

Nitrogen is an important part of the eutrophication, due to the anthropogenic impact is large, where nitrogen leaches from, inter alia, agricultural, and forest land, industries and sewage treatment plants. Approximately 25% of the nitrogen and phosphorus comes from natural sources of which the rest are from anthropogenic sources (Havet.nu 2011). Especially nitrate (NO_3^-) and ammonium (NH_4^+) have a leakage from the agriculture (Diaz and Rosenberg, 2008, Science). With nitrogen leakage the primary production will increase and cause a higher sedimentation rate that in turn will cause shortage of oxygen for bacterial degradation (Nolin 2008). Previously it was thought that it was primarily phosphorus that was the cause of the toxic algal bloom, which contributed to the reduced phosphorus loading. But the amount of phosphorus and nitrogen are linked. Where high concentrations of nitrogen causes more other kinds of algae, which in turn leads to anoxic bottoms and this in turn causes the phosphorus is released (Havet.nu 2011).

1.2.1. Bioturbation

Bioturbation is defined as the mixing of sediments layers produced by the movement of macrofauna through the sediment. With this process animals mix the oxic bottom water into the surface sediment. The volume of oxygen-containing sediment will then increase. It also improves the activity of nitrification by increasing the access of particle surfaces to NH_4^+ and O_2 (Hietanen *et.al.* 2007). This biological process will mix the sediment and provide ingestion to the deeper sediment (NE *Bioturbation* 2011). Nutrients will be released from the sediment. The mixing can be done of for example by epifauna, animals living on the surface of the sediment, (Macoma) and infauna, animals that burrows into the sediment, (Marenzelleria). There will also be particle erosion through bioturbation. The microbial activity increases with increased surface area after the particle erosion (Biles *et al.* 2002).

1.2.2. Denitrification

Denitrification is one of the most important processes in the sea because it is the process that occurs where N_2 returns from the atmosphere to the sea water column. The process mainly occurs in the sediment and where the oxygen concentrations are low (Berner et.al. 1996, p. 357). This happens in sediment with abundant organic material under the oxygenated top sediment, but is disfavored by deficiency of oxygen (Nolin 2008). When the oxygen is almost gone, denitrification starts by anaerobic bacteria, although it has been shown that some denitrifiers are mixotrophic and can also respire aerobically. Denitrification is the only

[1] SYVAB – Sydvästra Stockholmsregionens va-verksaktiebolag

biological process that produces dinitrogen gas. It is strongly dependent on temperature, oxygen concentration and the availability of organic material and it occurs in coastal environments (Hensen *et.al.* 2006).

Nitrification is oxygen dependent and also denitrification, it can only occur when there is enough access of oxygen. However, microbial denitrification is an anaerobic process where nitrite and nitrate converts to dinitrogen-gas (N_2) as: $NO_x = NO_2^- + NO_3^-$. The denitrification rate increases with the presence of benthic animals (Hietanen *et.al*. 2007). Bacteria in the sea degrade organic material and ammonium creates and recreates to NO_2^- and NO_3^-. There is no nitrification on oxygen free bottoms, as a result, no NO_3^- is created and therefore no denitrification and nitrogen will be left as NH_4^+ (Nolin 2008).

1.2.3. Anammox

Eutrophication can reduce oxygen levels in the seabed and cause anoxic bottoms (Hultman 2011), in which anaerobic microbial nitrogen conversion processes take place. Anammox is a shortening of Anaerobic Ammonium Oxidation (Penton 2009). Fixed nitrogen is removed from the water ecosystem by the anammox process. This means that microbes oxidize NH_4^+ with NO_2^- to produce N_2 (Hietanen *et.al*. 2007). It is an anaerobic ammonium oxidation with nitrite (Hultman 2011): $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

In marine waters with low concentrations of oxygen or lack of oxygen, such as the summer inner waters of Himmerfjärden, the anammox reaction may be the only process for the N_2 production in the marine sediments or may be responsible for a significant fraction of the N_2 gas production. About 79 % of the N_2 production in the marine sediments is done by the anammox process (Penton 2009) but not in every system. Normally denitrification is the only significant process for converting fixed nitrogen to gaseous N_2 (Dalsgaard *et al.* 2003). Anammox and denitrification processes can be compared. The difference between these two is that anammox produces twice as much N_2 per mole of consumed nitrite than denitrification. Anammox also increases the production of N_2 in sediments where nitrification is limited (Penton 2009).

1.3. Marenzelleria and Macoma Baltica

Marenzelleria spp. and Macoma Baltica are important influencing factors to reduce the problems that exist with eutrophication. They are good indicators on the environment and will oxygenate the sediment. Macrofauna such as Marenzelleria increases the oxygen consumption (Hensen *et.al.* 2006). In figure 1 Marenzelleria and Macoma can be seen. It has been shown that macrofauna affect both nitrification and denitrification, where both Marenzelleria and Macoma are common and important for bioturbation in the Baltic Sea (Karlson *et. al*.2005).

Figure 1. To the left Marenzelleria, and to right Macoma Baltica (Marenzelleria – azote.se and Macoma – edu-net.nl).

1.3.1 Marenzelleria

Marenzelleria spp**.** are polychaetes. There are about 10 000 known species and about 500 of them living along the Swedish coasts. Marenzelleria spp. is an important part of the water ecosystem and most of them belong to the benthic fauna. All Swedish species are brackish or fully marine species. The size is normally 1-2 cm up to 10 cm (Franzén 2011). Three species of Marenzelleria are found in the Baltic Sea, Marenzelleria – neglecta, artica and virdis. In this research we consider three as Marenzelleria spp. (Olsen *et al.* 2008).

Marenzelleria has a high tolerance for low oxygen concentrations. One positive aspect of Marenzelleria is the stirring effect it has in the sediment so that the oxygen concentration in the sediment increased and degradation of organic material increases (Magnusson 2008). In ecosystems with soft bottom, the importance of Marenzelleria is expected to increase, because it can dig up to 35 cm into the sediment, which is deeper than most other soft-bottom organisms (Hietanen *et.al*. 2007). Marenzelleria exist in sandy and muddy sediments in brackish-water environments and estuaries. They can live at 0,5 m water depth and down to 286 m, but normally in soft and shallow-water sea bottoms. They feed on sediment; plankton or organic material. It is common to find a lot of Marenzelleria at same place, because it is one of the few animals in the Baltic Sea that digs such deep into the soft sediment (Magnusson 2008).

1.3.2. Macoma

Macoma Baltica is a bivalve which lives in the Northeast Atlantic and the Baltic Sea (NE *Östersjömussla* 2011), and commonly occurs in sandy sediment. They normally occur from the littoral zone down to a depth of 40 m. At the soft seabed of sand or clay, there is a lot of Macoma. The bivalve can be found in many parts of the Baltic Sea as they can handle water with low salt concentration (Jutvik 2011 p.15).

Macoma burrow into the sediment as the Marenzelleria (Jutvik 2011 p.15), but not deeper than 5 cm (Karlson *et. al.* 2005). Macoma has two siphons, which they breathe from; these are at the sediment surface and the siphons to function as filters where they get their food from being composed of plankton and detritus (Jutvik 2011 p.15).

2. Aim and research questions

In this research the aim was to determine the transformation fluxes of nitrogen between the sediment and the water column, by looking at the fluxes direction, of the Himmerfjärden. A nitrogen-15 tracer method was used to determine the rate of microbial transformation processes, denitrification and anammox, and their impact on the release of nitrogen from the sediment. The project started with two samplings weeks and experiments on Askö in Himmerfjärden and then continued with preparation and the isotopic analysis of the experiments at Stockholm University.

The sewage-plant, SYVAB, is one of the factors contributing to the high nitrogen content in Himmerfjärden. There a reason is that the water is relatively stagnant since the water exchange with the Baltic Sea is small. The hypothesis of the work is that the difference of the denitrification rate will depend both on where the monitoring station is located and the amount and type of benthic macrofauna.

The questions for the thesis are:

- Do bioturbation and the number and types of macrofauna affect the nitrogen transformation rates?
- Is the nitrogen transformation rate correlated to the absorbance of the benthic macrofauna?
- Do the types of macrofauna, Marenzelleria or Macoma, affect the nitrogen transformation flux?

3. Method and materials

Different sampling and analyzing methods were used for nitrogen compounds. The sediment sampling was done with both a van Veen sediment grab and with a multi corer instrument with big cores (d=10 cm, h= 60cm). The analyzing was done with a UV-spectrophotometer and a combined mass and gas spectrometer. The sampling and preparation were carried out in Himmerfjärden in the archipelago outside Trosa, see figure 2. The analyses were done in the geochemistry isotope laboratory at Stockholm University. The experimental setup was similar to the one described by *Hietanen et al. 2007* in the Gulf of Finland. The measurement is inspired by *Rachetti et al. 2010* done in Italy. The methods of *Hietanen* and *Rachetti* are combined. There are some modifications to the methods described in these articles to match the experiment in Himmerfjärden. Modification is done on the size of the incubation cores, in this experiment the volume of the cores (d=3,7 cm, h=25 cm) which is smaller and the amount of benthic macrofauna is the same as in the Hietanen-experiment.

Before the sampling four glass aquariums of about 20 l and two small, 5 l, plastic aquariums were placed in a cold room at 5 °C, in-situ temperature. Air-stones were prepared to be placed in the aquariums. To do the sampling and measurement as naturally as possible, only brackish water was used and bottom water from the same monitoring station as the sediment in the cores came from.

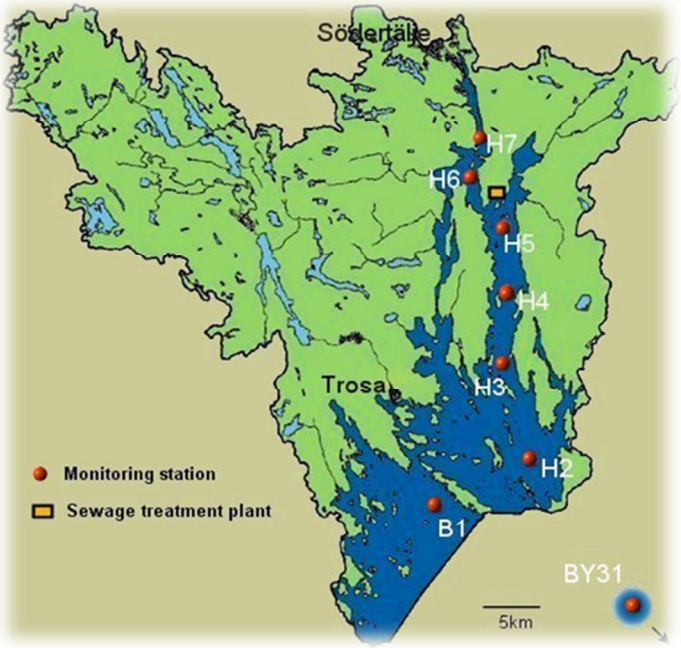

Figure 2. A map of Himmerfjärden with locations of the monitoring stations and the SYVAB sewage plant. Sampling was done at monitoring stations B1, H2, H4 and H6. The bioturbation-samples were taken from B1 (www2.ecology.su.se).

3.1. Sampling methods

The monitoring station sampling was done at four different stations; B1, H2, H4 and H6. The locations for them can be seen in figure 2, above. Data from the monitoring stations are in *appendix 1 and table A1*.

3.1.1. Mini multi-corer sampling

A mini-multi-corer (Kum Umwelt- und Meerestechnik GmbH, Kiel, Germany), containing four tubes spaced <15 cm from each other, lowered to the seabed and filled halfway with sediment. The big core was placed on a piston, it was important have vacuum in the small

cores before the big core pressed down on the piston and the sediment and water came up. From each large core were produced three small cores. Oxygen concentrations were only measured in the first four cores at each monitoring station.

The multi-corer sampling was done at the different monitoring stations. Twelve big cores were taken at every monitoring station. 20 small cores were taken from the 12 big cores. This was done at station B1, H2, H4 and H6.

3.1.2. Bottom water sampling

By doing the bottom water sampling**,** hydro-bios equipment was used, which can hold a volume of 5 l. The hydro-bios bottle was sent down in the water on a steel wire and when it was on the bottom, a trigger weight was hooked to the wire to slide down along the wire to release the closing mechanism of the water bottle at depth.

On every station the bottom water sampling was done five times to get the required volume of 25 l. This was done at station B1, H2, H4 and H6.

3.1.3. Van Veen sediment grab sampling

A van Veen sediment grab was used instead of the multi-corer for the bioturbation samples, as in the experiment by *Hietanen et al 2007*. The sediment grab could hold a sediment weight of 25 kg. The sediment grab was sent down in the water secured in a steel wire. When it hit the sediment at sea floor, it get closed and pulled up again. The grab was discharged in a big box.

The sediment grabber was used only at station B1.

3.2. Preparation and incubation of the sediment

The small cores were prepared for later use. Preparation of the solutions for the incubation cores was done and solutions of $NaNO_3$ (85,9 mg in 100 ml solution) and $ZnCl_2$ (50 mg in 100 ml solution) was used.

At station B1, a sediment sample was taken with a van Veen grab and the sediment was then sieved. The sieving started with a 0,5 mm sieve, but it was too slow and a 1 mm sieve was used instead. Only a few Marenzelleria and Macoma were found, and were placed in a small plastic aquarium with brackish bottom water from B1. The sediment was saved to be placed in the glass aquariums in the cold room. The sediment was moved to the glass aquariums of 20 l, after one more sieving in 0,5 mm sieves. The aquariums were then filled with sediment to a height of 10 cm and filled up with bottom water from B1, and placed in the cold room at 5 °C, to adapt to the cold room temperature. An older sediment core sample from H5 was sieved with a 1 mm sieve to obtain more Marenzelleria and Macoma.

After 24 hours the small cores were filled with sediments from the aquariums (20 l) in the cold room. Before they were filled, the upper layer of water above the sediment was pumped away and some organic material from the sieving was placed on the top of the sediment. The cores were 25 cm high and about half-filled (12,5 cm) with sediment. The other half was filled with brackish water from B1. The water was gently placed in the core with a 60 ml syringe without suspending the sediment.

To make the experiment similar to that *Hietanen et al. 2007* did, estimated number of animals they used for the volume of sediment to the new sediment volume that used for the present experiment. The volumes of the cores were compared to the experimental method and the amount of animals in every core. From the calculations, totally 12 Marenzelleria and 12 Macoma were used in this experiment which was about 10 % of the number of animals as in *Hietanens* experimental method.

The size of Macoma and Marenzelleria was checked, and for Marenzelleria, the colour of the polychaetes had to be as similar as possible. The species of Marenzelleria available in Sweden are difficult to distinguish. The color varies from red to light brown and to be dark green. Therefore it was decided to try to get as similar color as possible to be more likely it would be the same species (Franzen 2011). The middle size for Macoma was about 9 mm in shell size and three bigger Macomas (15 mm) were also used. Almost all Marenzelleria had the same size and colour. After the animals were placed in the sediment, it took about 10 minutes for them to dig into the sediment. A total of 16 cores were filled, with and without animals for the bioturbation (BIO) experiment: Core 1 – 3: No animals, Core 4 – 6: 1 Marenzelleria in each, Core 7 – 9: 1 Macoma in each, Core 10 – 12: 3 Marenzelleria in each, Core 13 – 15: 3 Macoma in each (two small and one big in every one) and as an extra sample Core 16: Test sample – with 2 Marenzelleria and 2 Macoma.

The incubated BIO-cores were placed in a big bucket filled with brackish water in the cold room. A magnetic stirrer was placed in the middle of the bucket and the cores were placed around it. Every core had a magnet in the water layer. This was to oxygenate the sediment and water and an air-stone was placed in the big bucket. The cores stood in the cold room for about 60 hours in darkness. Then the oxygen in the cores was measured with a Unisense oxygen microelectrode. Before the measurement, the water in the bucket and the cores was stirred by replacing the water two times from every core with a 60 ml syringe. Four 60 ml syringes were filled with brackish water and the oxygen concentration was measured.

Vials (12 ml pre-activation vials) was filled with water from the big four syringes. This type of vials were used because they are dense closed and no air enters them. The cores were incubated in four hours. The oxygen was measured with the electrode. Four replicates from time zero (t_0) – two with NH_4^+ and two with NO_x were taken and four replicates from time final (t_f) – two with NH_4^+ and two with NO_x. The samples with NH_4^+ and NO_x were filtered in glass-fiber filters before put into the vials. At the 15 N-tracer adding, the concentration added into the cores of NH_4^+ and NO_x was calculated by measuring the water and sediment height in the cores. This can be found in *Appendix 2*. At the time of the start time (t_0) was measured $^{14}NO_3$ from the box with the four replicates and after another 20 minutes was added 350 µl of 10 mM $^{15}NO_3^-$ solution to each sample and the cores were sealed. The cores were incubated in darkness. The bioturbation started about 12 hours after t_0. The sediment and water in every core was stirred. The vials were filled with stirred sediment and with 200 µl 50% $ZnCl_2$, some signs from the Marenzelleria were detected by tunnels in the sediment. Only five samples were handled at the same time with 60 ml syringes. After the incubation with t_0, t_f and bioturbation, the vials were placed in a -80 °C freezer.

One more incubation was done in the same way as above from station B1, H2, H4 and H6 but without sieving the sediment and without knowing the amount of animals. The multi-corer was used and the small cores (three from each) were filled half with sediment already on the boat. The cores were placed in a cool bag with ice to get in situ temperature on the way to the laboratory.

3.3. Analyzing method and materials

The analyses of the samples were done both by UV spectrophotometry and by a combined Mass-and Gas Spectrometry (handmade by Stefano Bonaglia).

3.3.1. UV-spectrophotometer

The UV-spectrophotometer was a Hitachi u-1100 Spectrophotometer, and the wave length that was used is 640,0 nm. The vials had to be perfectly clean to avoid contamination in the NH_4^+-solutions, because of the sensitiveness of NH_4^+. Before the preparation of the samples

the A, B and C solutions was prepared where A = Phenol-Alcohol, B = Sodium-nitroprusside and C = Hypochlorite (the oxidizing agent). Also an Ammonium-standard solution was made after instructions, see *Appendix 3* for details. And in *Appendix 4* is the treatment for bioturbation samples.

One vial, 5 cm^3, was used for every sample. Two pipettes were used, one of 500 µl and one of 1000 µl. The first sample only consisted of milli-q water as a calibration sample. All the other samples were then prepared and analyzed. 1000 µl of the standard, 7 samples, was filled in 7 vials. Standards should range from 0 to 1000 µM (1mL). 1000 µl of every sample from the different stations in Himmerfjärden was filled in one vial each. 500 µl of liquid A and B was filled in every vial, then 1000 µl of liquid C was filled in every vial. The solution was stirred with the pipette, after C was added. The vials have to "rest and react" for 90 minutes before the UV-measurement. Before the measurement the samples were moved from the vials to small plastic cuvettes.

Totally 58 samples were analyzed in two different machines and the average was used to compose a standard graph with known concentrations of NH_4^+, the absorbance on the Y-axis and concentration on the X-axis. Se figure 3 in the results.

3.3.2. Isotope ratio mass spectrometer

A combined gas chromatograph-isotope ratio mass spectrometer (GC-IRMS) was used to measure and determine the nitrogen transformation rates. The inlet system was built by Stefano Bonaglia and contained a liquid nitrogen trap and a reduction column. All samples in the vials were weighted before the analysis and were weighed one more time after the extraction and the extraction volume was calculated. The samples were sonicated in a machine for 20 minutes; air was replaced with a glass syringe (25 µl) in the vial. The air phase was replaced with water and after every 5 sample; the machine was calibrated with air.

3.3.3. Calculations of the fluxes

For the bioturbation samples from monitoring station B1 a total of 16 cores was filled with sediments, whereby three without animals and 13 with different numbers of individuals and species. The volumes of the cores were calculated by the equation:

$$V_{H2O} = A_{cross} \cdot L$$

- V_{H20} = Volume [m^3]
- A_{cross} = Cross-sectional area [m^2]
- L = Length of the core [m]

Fluxes from the bioturbation cores were calculated. When the flux is negative, it means the nutrients penetrate the sediment from the water column. When the flux is positive, it means the nutrients are transported to the water column from the sediment (Rachetti *et al.* 2010). A lower value of the flux means a higher flux into the sediment and a higher value of the flux means a higher flux into the water column of nutrients. The fluxes for NH_4, NO_2, NO_3 and O_2 were calculated by the equation:

$$F = (C_f - C_0) \cdot \frac{V_{H2O} \cdot 1000}{T \cdot 1{,}017}$$

- F = flux [µmol m^{-2} hr^{-1}]
- C_f = Final concentration [µmol cm^{-3}]
- C_0 = Start concentration [µmol cm^{-3}]
- V_{H20} = Volume [m^3]
- T = incubation time [hr]

4. Results

The results are shown in graphs and tables.

4.1. Results from the UV-spectrophotometry

From given concentrations in standard values and by measuring the absorbance for every sample, the straight-line equation, Y=K·X+M was obtained. From the equation, every samples concentration could be known. The missing part of the equation is Y (concentration), X (absorbance) is known after the UV-spectrophotometry. The R^2 has to be as close to 1 as possible, because it is the coefficient of determination, and shows how good the values are to the used model. This can be seen in figure 3.

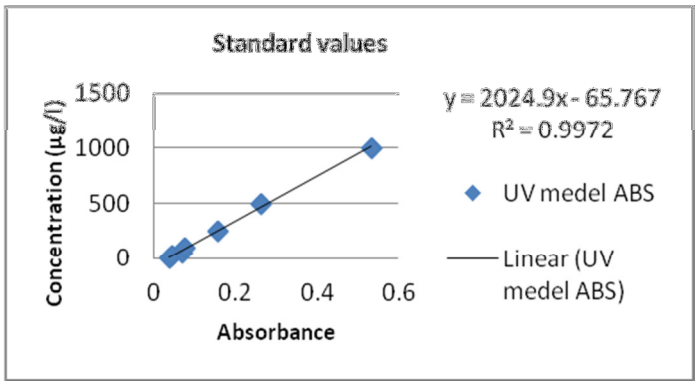

Figure 3. The standard-curve for the given concentrations of NH_4^+.

The standard values give the equation:

$$Y = 2024,9 \cdot X - 65,767$$

- Y = Concentration of the sample [μgl^{-1}]
- X = Absorbance of the sample

From the equation above with the standard values has the concentrations from the measured absorbance been calculated. And from the concentrations, initial and final, has the concentration changes for each compound been calculated before and after the incubation. From the concentrations of the samples, the fluxes have been calculated. In table 1, the concentration changes for NH_4, NO_3, NO_2 and O_2 can be seen. The result is over station B1 and for the bioturbation samples. The changes marked with red, are the changes where it has become more of the nutrients after the bioturbation, which means that the final concentration C_f, is higher than the initial concentration, C_i.

Table 1. Concentration changes for the bioturbation samples at station B1.

Core	Animals	Changes NH_4 (%)	Changes NO_3 (%)	Changes NO_2 (%)	Changes O_2 (%)
1	0 animals	0,95	0,76	0,76	0,82
2	0 animals	**1,03**	0,75	0,75	0,83
3	0 animals	0,89	0,74	0,74	0,83
4	1 marenzelleria	1,00	0,78	0,78	0,86
5	1 marenzelleria	0,93	0,74	0,74	0,84
6	1 marenzelleria	**1,02**	0,82	0,82	0,82
7	1 macoma	**1,17**	0,79	0,79	0,88
8	1 macoma	0,96	0,85	0,85	0,89
9	1 macoma	0,90	0,81	0,81	0,88
10	3 marenzelleria	**1,04**	0,77	0,77	0,87
11	3 marenzelleria	0,99	0,72	0,72	0,83
12	3 marenzelleria	**1,09**	0,81	0,81	0,85
13	3 macoma	0,90	0,82	0,82	0,84
14	3 macoma	**1,09**	0,81	0,81	0,88
15	3 macoma	**1,02**	0,80	0,80	0,91
16	2 marenzelleria 2 macoma	**1,09**	0,79	0,79	0,81

In Appendix 5 the basic data from the concentration calculation is present.

4.2. Results from the isotope pairing method

From the bioturbation-samples, monitoring station B1, the nitrogen transformation rate was determined. In Appendix 6 are the basic data present for all the graphs. The result for NH_4-fluxes with different amount of animals can be seen in figure 4. The result for NH_4 varies a lot. The lowest (most negative) flux was for samples with 3 Macoma and largest flux (most positive) for the samples with 1 Macoma. The lowest value is -64,84 µmol m^{-2} h^{-1} and highest 93,44 µmol m^{-2} h^{-1} for the NH_4-fluxes. It can be seen that the flux has changed a lot between the samples.

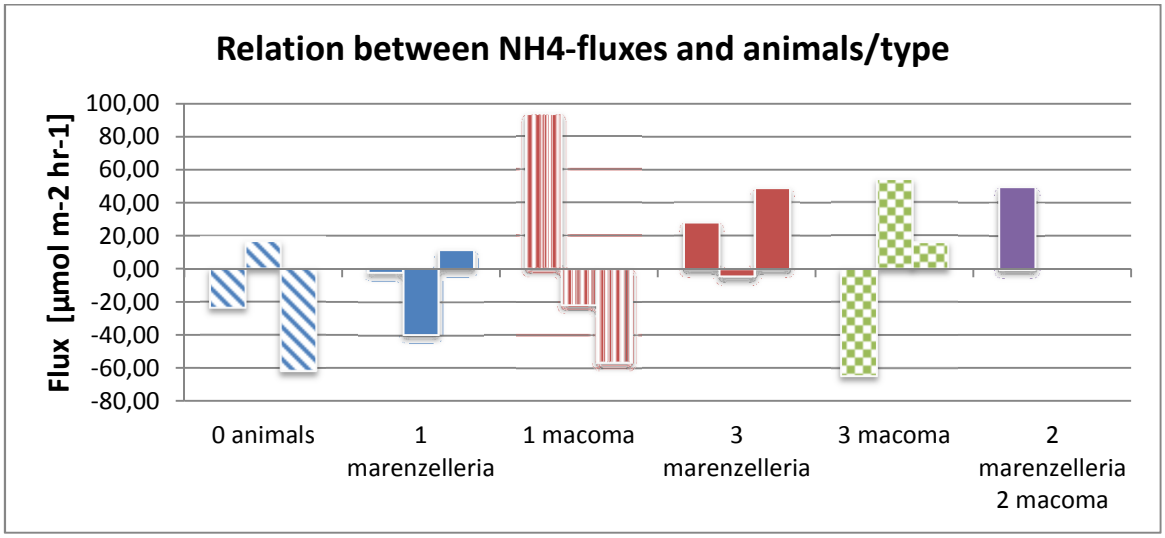

Figure 4. Fluxes for NH_4 with different amounts of macrofauna.

Fluxes have been calculated for NO_3 and NO_2. This gives the result in figure 5 and 6. There can be seen that NO_3-flux is lower, or more negative, than the NO_2-flux. The fluxes for both NO_3 and NO_2 doesn´t change a lot and the fluxes for NO_2 is about 10 times smaller than for NO_3. For NO_3 (figure 5) and NO_2 (figure 6) the lowest flux (most negative) was in the sample with 3 Marenzelleria in each sample and the highest (most positive) was with 1 Macoma in each sample. For NO_3, the lowest value is -31,78 µmol m^{-2} h^{-1} and the highest value is -16,20 µmol m^{-2} h^{-1}. For NO_2 the lowest value is -2,27 µmol m^{-2} h^{-1} and the highest value is -1,16 µmol m^{-2} h^{-1}.

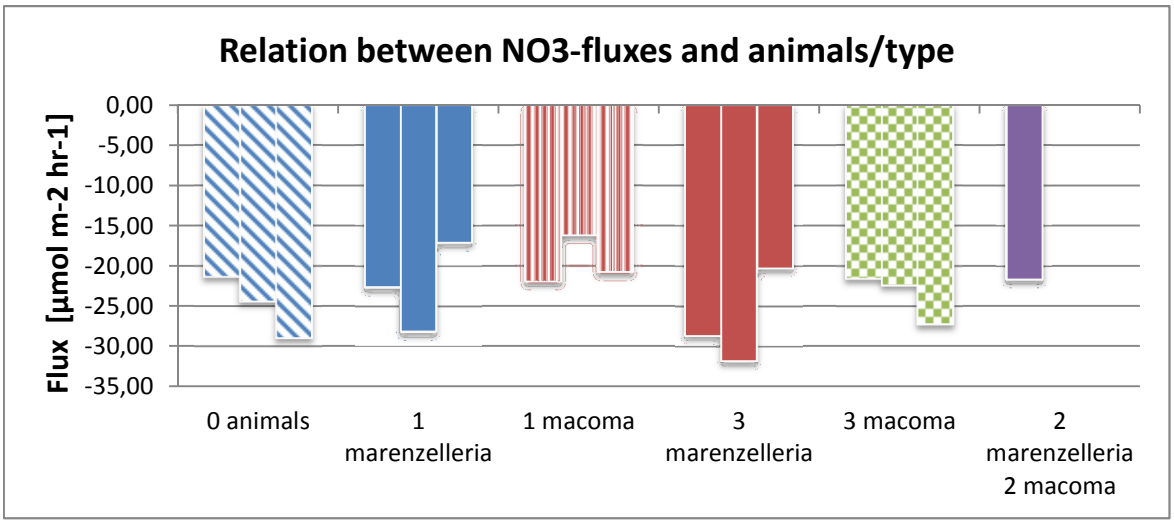

Figure 5. Fluxes for NO_3 with different amounts of macrofauna.

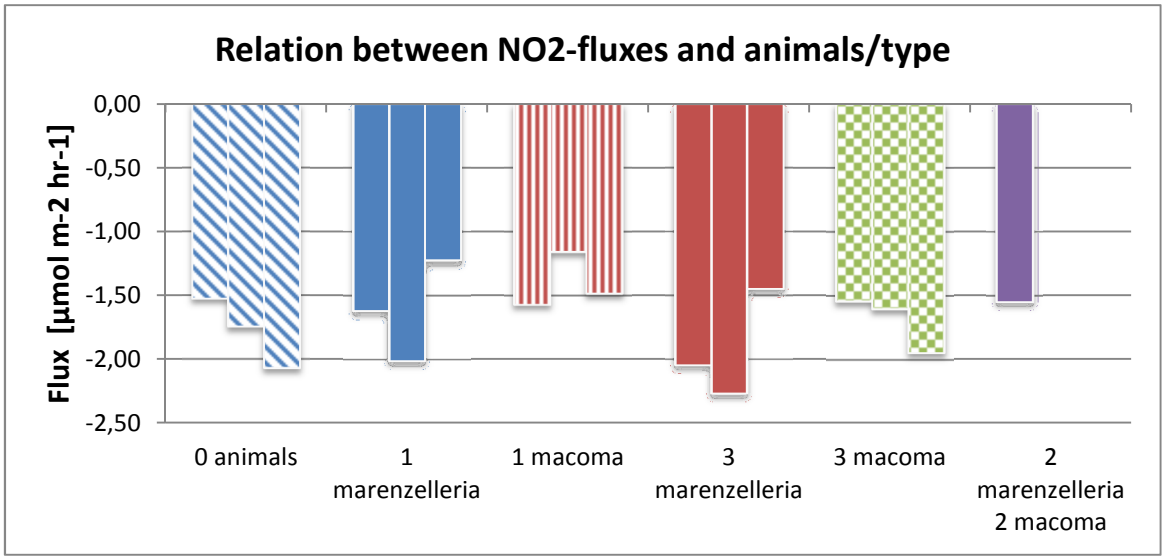

Figure 6. Fluxes for NO_2 with different amounts of macrofauna.

The total flux for NH_4, NO_3 and NO_2 is the DIN-flux in figure 7. The results of the DIN-flux, Dissolved Inorganic Nitrogen, shows that the largest negative flux of nutrients to the sediment is the sample with 0 animals, the flux size is -92,86 µmol m^{-2} h^{-1} and the largest flux (most positive) is for the sample with 1 Macoma, and the flux size is 69,88 µmol m^{-2} h^{-1}.

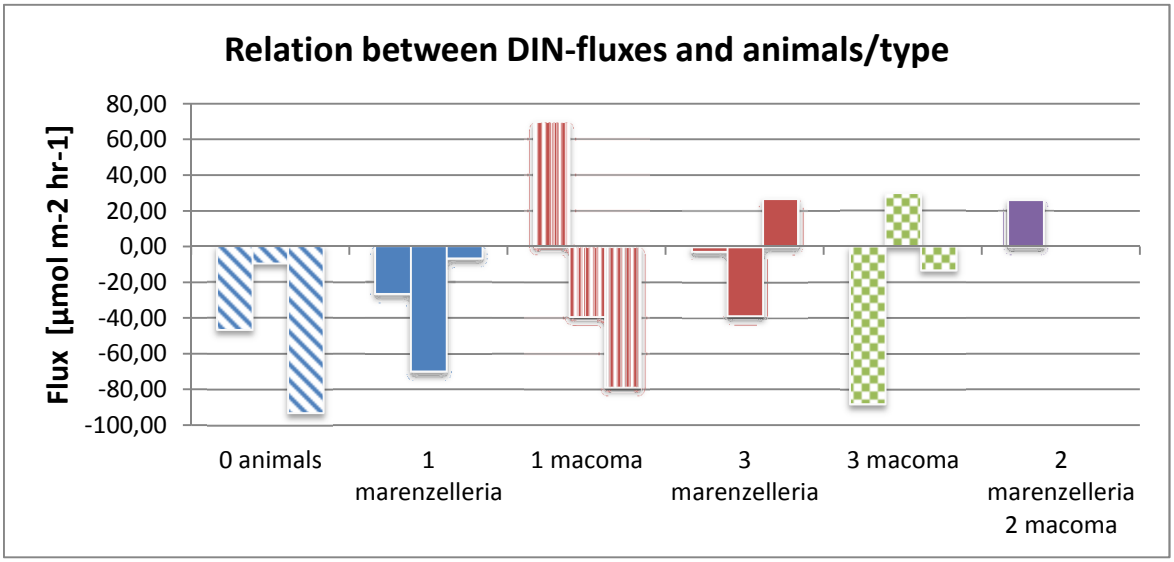

Figure 9. DIN-flux is the summary for NH_4, NO_3 and NO_2 fluxes.

The result for O_2-fluxes with different amount of animals can be seen in figure 10. The lowest (most negative) flux was for samples with 3 Macoma and largest flux (most positive) for the samples with 1 Macoma. The lowest value is -1771,33 µmol m^{-2} h^{-1} and highest -1094,12 µmol m^{-2} h^{-1} for the O_2-fluxes.

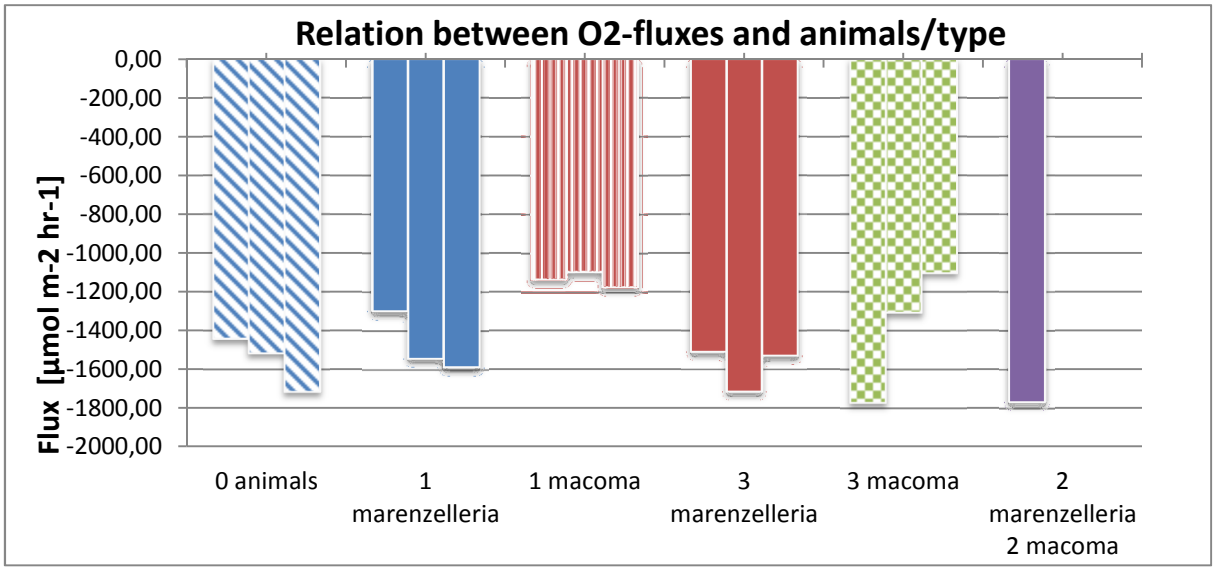

Figure 10. Fluxes for O_2 with different amounts of macrofauna.

The flux for O2 has been calculated, without bioturbation samples, for stations B1, H2, H4 and H6. This can be seen in figure 11. The lowest value (most negative) is at station B1 and the highest value (most positive) is at station H6. The lowest value is -1535,58 µmol m^{-2} h^{-1} and highest -327,16 µmol m^{-2} h^{-1} for the O_2-fluxes.

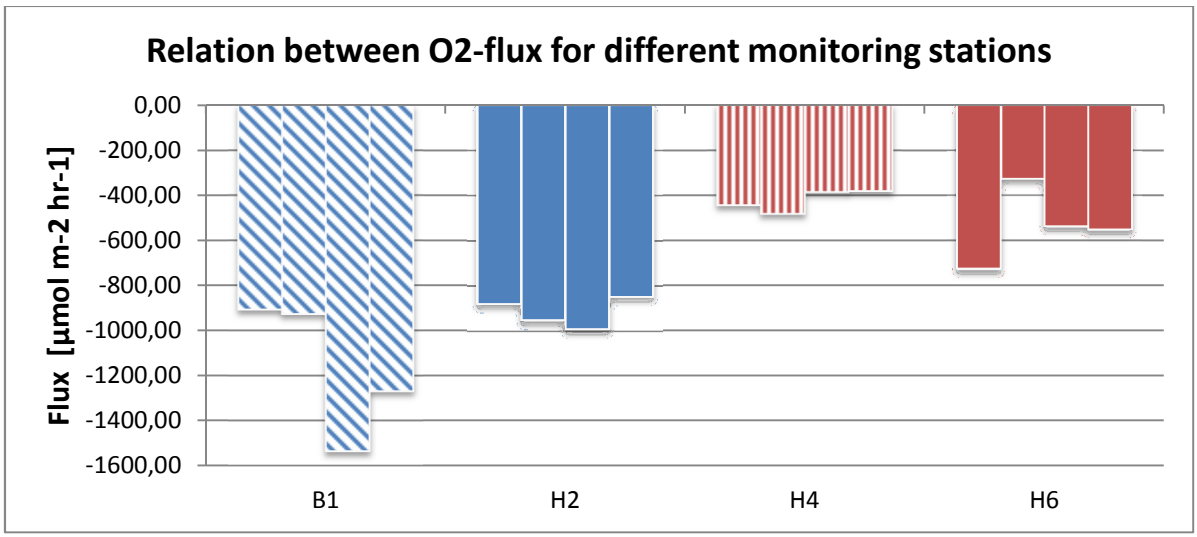

Figure 11. Fluxes for O_2 at different monitoring stations.

5. Discussion

The results generally show us that the fluxes vary with the number of animals in the samples, incubation solution, and incubation time and oxygen concentration. An important part is where the monitoring is located, both related to the distance from the sewage plant and the water inlet from the Baltic Sea to Himmerfjärden. This will influence of how good the mixing of the water is.

5.1. Sources of error

Based on the results, it can be seen that some sources of error can be recognized. One of these can be preparation of the bioturbation cores. After the sampling the sediment was not placed in the cold room to get in situ temperature because we sieved it outside in the sunlight. Brackish water was used, but not from the monitoring station B1, but instead from outside Askö laboratory – this may have caused death of Marenzelleria or Macoma. This can be one reason why there was so small numbers of animals from the sediment at B1.

When there were few animals from B1, an older sample from H5 was used and it had a lot of both Marenzelleria and Macoma. This may have been significant especially Marenzelleria was taken from H5, as sediment from B1 and H5 are from different parts of Himmerfjärden when H5 is much closer SYVAB and thus probably has higher nitrogen content.

Another source of error could be that there was too little organic material in the tubes because it gets by the sieving of the sediment for the bioturbation samples. We tried to put it back into aquariums, which samples were taken from, but it was difficult to know if there was organic material in the syringe by sucking up the sediment. This may have affected the animals' wellbeing. When the animals were added to the cores it was difficult to see if the Macoma was alive before it was released into the cores. When they disappeared into the sediment, we interpreted it as a life sign.

After the cores from B1 had been filled with sediment and would have to stand over the weekend they fell several times in the big bucket and the animals added to the cores before, this may have affected the sediment and water, especially Macoma may have fallen out from the cores. This happened when they were in the big bucket in the cold room with a magnetic stirrer and an air-stone. Another source of error could be that to small amount of oxygen was added to the cores for the animals' wellness.

When the samples were prepared before UV-spectrophotometer analyzing, the cuvettes or glass-vials could have been contaminated. It is important to use clean sample holders that have not been used for Si or PO_4 determination where ammonium molybdate is used as a reagent. This can contaminate the NH_4 determination and cause misleading results (Soloranzano et al. 1969).

5.2. Concentration changes

From the UV-spectrophotometer, the concentration of NH_4^+ was measured. By just looking at the map in figure 2, of the monitoring station that we have examined should monitoring stations H6 and H4 get a higher concentration of NH_4^+, because these monitoring stations are closer to the sewage-plant. It should also have a higher amount of animals, since *Marenzelleria prefer sediments with a high amount of organic material.* Another important factor is that H6 and H4 probably have more calm water than H2 and B1, because these two monitoring stations are located higher up in Himmerfjärden. B1 and H2 are located closer to the outlet to the Baltic Sea where it is easier for the water to get mixed.

In table 1, the change in concentrations for the bioturbation cores is present for NH_4, NO_3, NO_2 and O_2 at station B1. The only concentrations that have become higher (the final concentration of the substance is higher than the initial) are for NH_4. For the samples with no animals, the concentration change should be near to zero. But in one sample the concentration of NH_4 has risen and for two of them the concentration has dropped. For the other substances (NO_3, NO_2 and O_2) the concentration has been dropped between 9 and 29 %. The concentration of NH_4 is higher after the incubation and bioturbation in 8 of the 16 samples. In the other samples, the concentrations have just sunk some unit or are close to one. This shows that it is animals in the samples which have react and gives away NH_4. More NH_4 means more organic material and more animals. The sample with the highest increase is for the one with 1 Macoma. The rise is about 17 % from the initial concentration.

The core-samples with animals all contain a lot of NH_4, from monitoring station B1. The water here was from the water overlying the sediment core, not as the other sample, where the water was taken from the Hydrobios bottle. This could have affected the result with a higher concentration of NH_4^+. Another reason for this result is that organic carbon rich sediments have a high concentration of NH_4 (Hietanen *et al.* 2007). By comparing the results we get from our sampling at the different monitoring stations and the results from *systemekologerna* at *ecology.su.se*[2] it can be seen that in June, when we took our samples and at the depth (20-30 m) where the samples were taken, the concentration of oxygen at monitoring station B1 should be about 85 %, see appendix 1. In our result the concentration is about 81 to 91 % after the incubation. The concentration depends on the number of animals in the sample. It means, the more animals in the sample, the higher concentration because they mixing and bioturbation the sediment.

At a high concentration of NH_4 to the method we have used UV- spectrophotometry may have influenced the results. According to Solorenzano et. al. 1969, we used the method of determining small concentrations of NH4. Of the NH4 concentration was determined by UV measurement, they will not follow a constant trend, which means they have concentrations that are higher or lower at the same station. This could be a result of a smaller amount of animals in the sample or that the animals have died under the incubation.

5.3. Isotope-pairing technique

From the mass-spectrometer, fluxes of nitrogen compounds have been measured and from the flux equation it can be seen that the concentration changes and the time of incubation and water height in the core determine the flux magnitude and direction (Fig.???). But important to notice is that the flux also depends on the proportion of animals, because the animals will influence the oxygen, and thus the nitrogen content. As mentioned earlier in this text, a negative flux indicates that the nutrients go into the sediment and a positive flux indicates that the nutrients go to the water column (Rachetti *et al.* 2010).

By looking at the flux size and sign, if they are positive and negative, it can be seen that:

For the NH_4-flux, in figure 4, it can be seen that the flux-size varies with different number of animals and also depends on the animal species that is in the sample, i.e., if it is Marenzelleria or Macoma or both of them. The samples are from B1 which is quite long way from the sewage-plant and the amount of animals in it should be small. For five of the seven samples with three samples with 3 animals or more in each core, the flux is positive and nutrients go from the sediment to the water. This is because of the bioturbation in the sediment which releases nutrients such as NH_4^+, when the mixing of sediment occurs. It is a by-product from

[2] http://www2.ecology.su.se/dbhfj/h6start.htm

the animals. For NH4, the highest flux by transporting nutrient from sediment to the water column is for a sample with 1 Macoma. This indicates that it could be more animals than one Macoma in the sample or more organic material. In the sample with 2 Marenzelleria and 2 Macoma the flux is positive and the nutrient is transported from the sediment to the water. This means the nutrient concentration in the sediment is higher than for the concentration in the water.

For the nutrient fluxes with NO_3 and NO_2 in figure 5 and 6, all fluxes are negative. There are larger negative fluxes for NO_3 than NO_2. The fluxes for NO_3 are about 10 times larger. Higher negative fluxes show that the nutrients are going faster into the sediment. The most negative flux is for the sample with 3 Marenzelleria. This shows that the bioturbation has occurred and the animals have improved the activity of nitrification by increasing the particle size and the nutrients will be release from the sediment (Hietanen *et. al* 2007, Biles *et. al* 2002). This is proved in the experiment by looking at the result where the more animals in the samples, the higher the flux are from the water to the sediment.

In figure 9, with the DIN-flux, the sum of the fluxes for NH_4, NO_2 and NO_3, shows that the relation between animals and fluxes is dependent of each other. The more animals in the sediment core, the higher the flux into the water of nutrients are. This shows that the bioturbation works with mixing and release of nutrients to the water column.

For both NO_2 and NO_3 the result is relatively stable, where the proportion of animals does not affect the results significantly, nor incubation. However, NO_3, as indicated above, generally has an approximately 10-fold higher flux than NO_2. By looking at the flux of NH_4 it shifts more and depends on the number of animals in the cores. The flux will be higher the more animals there are, however, there are some remarkable values. These remarkable values will impact the result of the total nitrogen compounds flux, DIN.

By looking at the O2 flux for different stations so you can see that the measurement stations H4 and H6, these are significantly lower than for primarily B1 but also H2. This can be seen in the results in figure 11. A low oxygen flux, close to zero, means that the oxygen yield is not high and also that the oxygen enters in the sediment. There is less oxygen in the sediment than in the water. For B1 is the oxygen concentration in the water is high and oxygen entering sediment. Probably the denitrification occurs at station H4 and H6 because of the low oxygen fluxes and concentrations and also because of the higher amount of organic material (closer to the sewage plant). The result for the O_2-fluxes in figure 2 are not sieved and controlled of animals.

The cores with no animals, core 2, has a high positive NH_4-flux to the water column, 16,48 µmol m-2 hr-1, one reason of this could be that there was an animal in the sample that we did not know about. From the cores with 1 Marenzelleria, core 6, has a positive flux, which indicates of how the nutrients go into the water column, this could be a result more than one animal in the core the same is for core 7 with 1 Macoma. The sample with 2 Marenzelleria and 2 Macoma has a relatively high flux out to the water, as it should be because it is the core with the highest amount of animals. The more animals in the core, the more NH_4 are produced and the higher flux should be out to the water column.

The concentration of ammonium is an indicator of the diagenesis of organic material in the sediment. This means that in areas with efficient digestion of organic material, there are a lot of macrofauna and active bacteria's, it is an indicator of low oxygen areas (Soloranzano et al. 1969). The water quality also affects the number of animals and thus the nitrogen content. The animals accumulate nitrogen by ingestion and also add oxygen to the sediment by stirring.

In general, the animals accelerated bioturbation and thus nutrient exchange, especially Marenzelleria spp. Macoma Baltica does not have as much of an impact, probably because they do not dig as deep into the sediment and they should therefore not consume as much oxygen. In general, three animals in each core give us a more credible performance, particularly in terms of Macoma, as it has, in the individual nitrogen compounds results, given several unusual readings. This may be because, as mentioned earlier, the proportion of sediment compared to Macoma size are not compatible with each other and possibly some Macoma have died. It was difficult to ascertain whether they were alive when they were appointed in the core. I think, by looking at the cores, the Marenzelleria have dug deeper into the sediment and thus moved more because you could see the stripes down in the sediment. The area of Macoma move was less than that for Marenzelleria to bury themselves. Macoma does not have as strong bioturbation as Marenzelleria, because Marenzelleria can bury itself relatively deep into the sediment, about 5 times deeper into the sediment than Macoma.

5.4. Conclusion

The conclusion from the results and discussion that can be drawn is that there is always a concentration gradient between the sediment and the water column. When there is a higher amount of nitrogen in the water then the flux is going down to the sediment, which gives a negative flux for nitrogen. The same is true for oxygen. The other conclusion is that if there is a lot of nitrogen in the water column, then there is also a high amount of macrofauna, as in the report, Marenzelleria spp. or Macoma Balthica.

And at last, when there are a lot of animals, then there is a lot of NH_4 and then it is also an effective degradation of organic material and the concentration of oxygen is low close to the sea floor. And this is just what is proved close to the sewage plant in Himmerfjärden – A lot of NH_4, a lot of animals and a low concentration of oxygen. This is a good indicator for understanding how the sea bottom is doing environmentally, nitrogen concentration and its correlation with the number of animals and what kind of animals that live there.

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Appendix

Appendix 1 – Sampling data

Table A1. Information about the sampling stations on ASKÖ June 2011, where every data is compared with the data done from the research station "Askö-laboratoriet"[3].

Station	GPS	S-Date	Temp. [°C]	Depth [m]	[O2] [%]	Salinity [PSU]
B1	58 48.6479 N 017 36.4249 E	2011-06-15	6,3	37	85	6,8
B1	58 48.1147 N 017 37.4342 E	2011-06-21	5,3	35*	86	7
H2	58 50.5020 N 017 47.2619 E	2011-06-21	4,2	30*	77	6,4
H4	58 59.0412 N 017 43.4596 E	2011-06-22	5,7	32	66	6,5
H6	59 04.1261 N 017 40.4975 E	2011-06-22	3,1	40	46	6,5

**The depth is approximated from the bathy map at: http://www2.ecology.su.se/dbhfj/hfjstn.htm*

[3] http://www2.ecology.su.se/dbhfj/index.htm

Appendix 2 – Data from bioturbation samples

Table A2. Ground data from the bioturbation samples where the calculation has been done from.

Core	Animals	H2O (cm)	H2O (l)	Incub time (hours)	Incub time (day)
1	0 animals	10	0,10	4,67	0,19
2	0 animals	11	0,11	4,67	0,19
3	0 animals	12	0,12	4,40	0,18
4	1 marenzelleria	11,5	0,12	4,67	0,19
5	1 marenzelleria	12	0,12	4,67	0,19
6	1 marenzelleria	11	0,11	4,67	0,19
7	1 macoma	11	0,11	4,40	0,18
8	1 macoma	11	0,11	4,12	0,17
9	1 macoma	11	0,11	4,12	0,17
10	3 marenzelleria	12,5	0,13	4,12	0,17
11	3 marenzelleria	12	0,12	4,40	0,18
12	3 marenzelleria	11,5	0,12	4,40	0,18
13	3 macoma	13	0,13	4,40	0,18
14	3 macoma	11,5	0,12	4,12	0,17
15	3 macoma	13,5	0,14	4,12	0,17
16	2 marenzelleria 2 macoma	11,5	0,12	4,67	0,19

Appendix 3 – Reagent solution recipes

By Soloranzano 1969.

(A) – Phenol-alcohol solution:

- 0,8 g crystalline phenol is dissolved in 100 cm^3 of 95 % ethyl alcohol. The solution has to be daily-fresh. 1 cm^3 of liquid phenol can be used instead of crystalline phenol.

(B) – Sodium nitroprusside solution:

- 0,15 g sodium nitroprusside (sodium nitroferricyanide) is dissolved in 200 cm^3 nanopure water. The solution has to be daily-fresh.

(C) – Oxidizing solution:

- 1 cm^3 fresh sodium hypochlorite (4 % available chlorine) – chlorox will do equally well-up to 50 cm^3 alkaline solution. The solution has to be daily-fresh.

Standard – Ammonium standard:

- Ammonium chloride will be dried in oven for one night and then 5,345 g will be solved in 1 dm^3 nanopure water. Non-dried NH_4Cl can be used to determine the chloride content of the standard solution. For a feasible precision a 0,5 cm^3 aliquot should be used to obtain the same concentration for chloride as it could be in the standard sea-water from the IAPSO-reference, which is about 0,1 M.

Appendix 4 – Different treatments for the bioturbation:

- Strain the sediment with a 1 mm sieve → Find the macrofauna and organic matter
- The sediment was transferred to a tank for sedimentation
- Aquarium (20 l) were filled with water from the same station as the sediment
- Aquarium had to stand in the cold room for 24 h
- After 24 h, depleted surface water in the aquarium and the organic material was added
- Cores (l = 25 cm) filled to half with sediment using a syringe and the other half with brackish water
- Macrofauna located in the cores with different number and variety of different cores → BIO-cores
- BIO-cores are placed in a large bucket filled with brackish water in the cold room with a magnetic stirrer for 2.5 days
- Measurement of the oxygen in the bucket to the water in each cores have been absorbed twice with a syringe
- Vials are filled with water from the syringes and incubated cores now for four hours in the dark
- At the time of the start time (t_0) was measured $^{14}NO_3$ from the box with the four replicates and after another 20 minutes was added 350 µl of 10 mM $^{15}NO_3^-$ solution to each sample
- The bioturbation started about 12 hours after t_0. The sediment and water in every core was stirred
- The vials were filled with stirred sediment and with 200 µl 50% $ZnCl_2$
- After the incubation with t_0, t_f and bioturbation, the vials were placed in a -80 °C freezer

Appendix 5 – Concentrations for nutrients

Table A4. Calculations of concentration changes of nutrients.

Core	Animals	[NH4]i (µM)	[NH4]f (µM)	Changes NH4 (%)	[NO3]i (µM)	[NO3]f (µM)	Changes NO3 (%)	[NO2]i (µM)	[NO2]f (µM)	Changes NO2 (%)	[O2]i* (µM)	[O2]f (µM)	Changes O2 (%)
1	**0 animals**	**21,5**	**20,40**	**0,95**	**4,14**	**3,15**	**0,76**	**0,30**	**0,22**	**0,76**	**380**	**312,76**	**0,82**
2	**0 animals**	**21,5**	**22,20**	**1,03**	**4,14**	**3,11**	**0,75**	**0,30**	**0,22**	**0,75**	**380**	**315,68**	**0,83**
3	**0 animals**	**21,5**	**19,24**	**0,89**	**4,14**	**3,08**	**0,74**	**0,30**	**0,22**	**0,74**	**380**	**317,15**	**0,83**
4	1 marenzelleria	21,5	21,41	1,00	4,14	3,23	0,78	0,30	0,23	0,78	380	327,38	0,86
5	1 marenzelleria	21,5	19,96	0,93	4,14	3,05	0,74	0,30	0,22	0,74	380	320,07	0,84
6	1 marenzelleria	21,5	21,99	1,02	4,14	3,42	0,82	0,30	0,24	0,82	380	312,76	0,82
7	**1 macoma**	**21,5**	**25,24**	**1,17**	**4,14**	**3,27**	**0,79**	**0,30**	**0,23**	**0,79**	**380**	**334,68**	**0,88**
8	**1 macoma**	**21,5**	**20,69**	**0,96**	**4,14**	**3,54**	**0,85**	**0,30**	**0,25**	**0,85**	**380**	**339,07**	**0,89**
9	**1 macoma**	**21,5**	**19,38**	**0,90**	**4,14**	**3,37**	**0,81**	**0,30**	**0,24**	**0,81**	**380**	**336,15**	**0,88**
10	3 marenzelleria	21,5	22,42	1,04	4,14	3,20	0,77	0,30	0,23	0,77	380	330,30	0,87
11	3 marenzelleria	21,5	21,34	0,99	4,14	2,98	0,72	0,30	0,21	0,72	380	317,15	0,83
12	3 marenzelleria	21,5	23,36	1,09	4,14	3,37	0,81	0,30	0,24	0,81	380	321,53	0,85
13	**3 macoma**	**21,5**	**19,31**	**0,90**	**4,14**	**3,41**	**0,82**	**0,30**	**0,24**	**0,82**	**380**	**320,07**	**0,84**
14	**3 macoma**	**21,5**	**23,43**	**1,09**	**4,14**	**3,34**	**0,81**	**0,30**	**0,24**	**0,81**	**380**	**333,22**	**0,88**
15	**3 macoma**	**21,5**	**21,99**	**1,02**	**4,14**	**3,31**	**0,80**	**0,30**	**0,24**	**0,80**	**380**	**346,38**	**0,91**
16	2 marenzelleria 2 macoma	21,5	23,51	1,09	4,14	3,27	0,79	0,30	0,23	0,79	380	308,38	0,81

Appendix 6 – Data from the bioturbation flux calculations

Table A6a. Data for the NH_4-flux calculation

Core	Animals	H2O (cm)	H2O (l)	Incub time (hours)	Incub time (day)	[NH4]i (µM)	[NH4]i µmol	[NH4]f (µM)	[NH4]f µmol	[NH4]i - [NH4]f (µmol)	Flux µmol/10.17 cm2/day	Flux day mmol/m2/d	Flux hr µmol m-2 hr-1
1	**0 animals**	**10**	**0,10**	**4,67**	**0,19**	**21,5**	**2,2**	**20,40**	**2,08**	**0,11**	**0,58**	**0,57**	**-23,77**
2	**0 animals**	**11**	**0,11**	**4,67**	**0,19**	**21,5**	**2,4**	**22,20**	**2,48**	**-0,08**	**-0,40**	**-0,40**	**16,48**
3	**0 animals**	**12**	**0,12**	**4,40**	**0,18**	**21,5**	**2,6**	**19,24**	**2,35**	**0,28**	**1,51**	**1,48**	**-61,82**
4	1 marenzelleria	11,5	0,12	4,67	0,19	21,5	2,5	21,41	2,50	0,01	0,06	0,06	**-2,38**
5	1 marenzelleria	12	0,12	4,67	0,19	21,5	2,6	19,96	2,44	0,19	0,97	0,95	**-39,69**
6	1 marenzelleria	11	0,11	4,67	0,19	21,5	2,4	21,99	2,46	-0,05	-0,28	-0,27	**11,37**
7	**1 macoma**	**11**	**0,11**	**4,40**	**0,18**	**21,5**	**2,4**	**25,24**	**2,82**	**-0,42**	**-2,28**	**-2,24**	**93,44**
8	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**21,5**	**2,4**	**20,69**	**2,31**	**0,09**	**0,53**	**0,53**	**-21,91**
9	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**21,5**	**2,4**	**19,38**	**2,17**	**0,24**	**1,38**	**1,36**	**-56,70**
10	3 marenzelleria	12,5	0,13	4,12	0,17	21,5	2,7	22,42	2,85	-0,12	-0,68	-0,67	**27,82**
11	3 marenzelleria	12	0,12	4,40	0,18	21,5	2,6	21,34	2,60	0,02	0,11	0,11	**-4,60**
12	3 marenzelleria	11,5	0,12	4,40	0,18	21,5	2,5	23,36	2,73	-0,22	-1,18	-1,16	**48,53**
13	**3 macoma**	**13**	**0,13**	**4,40**	**0,18**	**21,5**	**2,8**	**19,31**	**2,55**	**0,29**	**1,58**	**1,56**	**-64,84**
14	**3 macoma**	**11,5**	**0,12**	**4,12**	**0,17**	**21,5**	**2,5**	**23,43**	**2,74**	**-0,23**	**-1,32**	**-1,29**	**53,89**
15	**3 macoma**	**13,5**	**0,14**	**4,12**	**0,17**	**21,5**	**3,0**	**21,99**	**3,02**	**-0,07**	**-0,39**	**-0,38**	**15,82**
16	2 marenzelleria 2 macoma	11,5	0,12	4,67	0,19	21,5	2,5	23,51	2,75	-0,23	-1,20	-1,18	**49,32**

Table A6b. Data for the NO_3-flux calculation.

Core	Animals	H2O (cm)	H2O (l)	Incub time (hours)	Incub time (day)	[NO3]i (µM)	[NO3]i (µmol)	[NO3]f (µM)	[NO3]f (µmol)	[NO3]i - [NO3]f (µmol)	Flux µmol/10.17 cm2/day	Flux day mmol/m2/d	Flux hr µmol m-2 hr-1
1	**0 animals**	**10**	**0,10**	**4,67**	**0,19**	**4,14**	**0,42**	**3,15**	**0,32**	**0,10**	**0,52**	**0,51**	**-21,35**
2	**0 animals**	**11**	**0,11**	**4,67**	**0,19**	**4,14**	**0,46**	**3,11**	**0,35**	**0,12**	**0,60**	**0,59**	**-24,43**
3	**0 animals**	**12**	**0,12**	**4,40**	**0,18**	**4,14**	**0,51**	**3,08**	**0,38**	**0,13**	**0,71**	**0,70**	**-28,97**
4	1 marenzelleria	11,5	0,12	4,67	0,19	4,14	0,48	3,23	0,38	0,11	0,55	0,54	**-22,64**
5	1 marenzelleria	12	0,12	4,67	0,19	4,14	0,51	3,05	0,37	0,13	0,69	0,68	**-28,15**
6	1 marenzelleria	11	0,11	4,67	0,19	4,14	0,46	3,42	0,38	0,08	0,42	0,41	**-17,13**
7	**1 macoma**	**11**	**0,11**	**4,40**	**0,18**	**4,14**	**0,46**	**3,27**	**0,37**	**0,10**	**0,54**	**0,53**	**-21,99**
8	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**4,14**	**0,46**	**3,54**	**0,40**	**0,07**	**0,40**	**0,39**	**-16,20**
9	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**4,14**	**0,46**	**3,37**	**0,38**	**0,09**	**0,51**	**0,50**	**-20,77**
10	3 marenzelleria	12,5	0,13	4,12	0,17	4,14	0,53	3,20	0,41	0,12	0,70	0,69	**-28,64**
11	3 marenzelleria	12	0,12	4,40	0,18	4,14	0,51	2,98	0,36	0,14	0,78	0,76	**-31,78**
12	3 marenzelleria	11,5	0,12	4,40	0,18	4,14	0,48	3,37	0,39	0,09	0,49	0,49	**-20,28**
13	**3 macoma**	**13**	**0,13**	**4,40**	**0,18**	**4,14**	**0,55**	**3,41**	**0,45**	**0,10**	**0,53**	**0,52**	**-21,59**
14	**3 macoma**	**11,5**	**0,12**	**4,12**	**0,17**	**4,14**	**0,48**	**3,34**	**0,39**	**0,09**	**0,55**	**0,54**	**-22,43**
15	**3 macoma**	**13,5**	**0,14**	**4,12**	**0,17**	**4,14**	**0,57**	**3,31**	**0,46**	**0,11**	**0,66**	**0,65**	**-27,24**
16	2 marenzelleria 2 macoma	11,5	0,12	4,67	0,19	4,14	0,48	3,27	0,38	0,10	0,53	0,52	**-21,67**

Table A6c. Data for the NO_2-flux calculation.

Core	Animals	H2O (cm)	H2O (l)	Incub time (hours)	Incub time (day)	[NO2]i (µM)	[NO2]i (µmol)	[NO2]f (µM)	[NO2]f (µmol)	[NO2]i - [NO2]f (µmol)	Flux µmol/10.17 cm2/day	Flux day mmol/m2/d	Flux hr µmol m-2 hr-1
1	**0 animals**	**10**	**0,10**	**4,67**	**0,19**	**0,30**	**0,03**	**0,22**	**0,02**	**0,01**	**0,04**	**0,04**	**-1,53**
2	**0 animals**	**11**	**0,11**	**4,67**	**0,19**	**0,30**	**0,03**	**0,22**	**0,02**	**0,01**	**0,04**	**0,04**	**-1,74**
3	**0 animals**	**12**	**0,12**	**4,40**	**0,18**	**0,30**	**0,04**	**0,22**	**0,03**	**0,01**	**0,05**	**0,05**	**-2,07**
4	1 marenzelleria	11,5	0,12	4,67	0,19	0,30	**0,03**	0,23	0,03	0,01	0,04	0,04	**-1,62**
5	1 marenzelleria	12	0,12	4,67	0,19	0,30	**0,04**	0,22	0,03	0,01	0,05	0,05	**-2,01**
6	1 marenzelleria	11	0,11	4,67	0,19	0,30	**0,03**	0,24	0,03	0,01	0,03	0,03	**-1,22**
7	**1 macoma**	**11**	**0,11**	**4,40**	**0,18**	**0,30**	**0,03**	**0,23**	**0,03**	**0,01**	**0,04**	**0,04**	**-1,57**
8	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**0,30**	**0,03**	**0,25**	**0,03**	**0,00**	**0,03**	**0,03**	**-1,16**
9	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**0,30**	**0,03**	**0,24**	**0,03**	**0,01**	**0,04**	**0,04**	**-1,48**
10	3 marenzelleria	12,5	0,13	4,12	0,17	0,30	**0,04**	0,23	0,03	0,01	0,05	0,05	**-2,05**
11	3 marenzelleria	12	0,12	4,40	0,18	0,30	**0,04**	0,21	0,03	0,01	0,06	0,05	**-2,27**
12	3 marenzelleria	11,5	0,12	4,40	0,18	0,30	**0,03**	0,24	0,03	0,01	0,04	0,03	**-1,45**
13	**3 macoma**	**13**	**0,13**	**4,40**	**0,18**	**0,30**	**0,04**	**0,24**	**0,03**	**0,01**	**0,04**	**0,04**	**-1,54**
14	**3 macoma**	**11,5**	**0,12**	**4,12**	**0,17**	**0,30**	**0,03**	**0,24**	**0,03**	**0,01**	**0,04**	**0,04**	**-1,60**
15	**3 macoma**	**13,5**	**0,14**	**4,12**	**0,17**	**0,30**	**0,04**	**0,24**	**0,03**	**0,01**	**0,05**	**0,05**	**-1,95**
16	2 marenzelleria 2 macoma	11,5	0,12	4,67	0,19	0,30	**0,03**	0,23	0,03	0,01	0,04	0,04	**-1,55**

Table A6d. Data for the DIN-flux calculation.

Core	Animals	H2O (cm)	H2O (l)	Incub time (hours)	Incub time (day)	Flux (NH4) hr µmol m-2 hr-1	Flux (NO3) hr µmol m-2 hr-1	Flux (NO2) hr µmol m-2 hr-1	Flux (DIN) hr µmol m-2 hr-1
1	**0 animals**	**10**	**0,10**	**4,67**	**0,19**	**-23,77**	**-21,35**	**-1,53**	**-46,65**
2	**0 animals**	**11**	**0,11**	**4,67**	**0,19**	**16,48**	**-24,43**	**-1,74**	**-9,69**
3	**0 animals**	**12**	**0,12**	**4,40**	**0,18**	**-61,82**	**-28,97**	**-2,07**	**-92,86**
4	1 marenzelleria	11,5	0,12	4,67	0,19	**-2,38**	**-22,64**	**-1,62**	-26,64
5	1 marenzelleria	12	0,12	4,67	0,19	**-39,69**	**-28,15**	**-2,01**	-69,84
6	1 marenzelleria	11	0,11	4,67	0,19	**11,37**	**-17,13**	**-1,22**	-6,98
7	**1 macoma**	**11**	**0,11**	**4,40**	**0,18**	**93,44**	**-21,99**	**-1,57**	**69,88**
8	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**-21,91**	**-16,20**	**-1,16**	**-39,27**
9	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**-56,70**	**-20,77**	**-1,48**	**-78,96**
10	3 marenzelleria	12,5	0,13	4,12	0,17	**27,82**	**-28,64**	**-2,05**	-2,86
11	3 marenzelleria	12	0,12	4,40	0,18	**-4,60**	**-31,78**	**-2,27**	-38,65
12	3 marenzelleria	11,5	0,12	4,40	0,18	**48,53**	**-20,28**	**-1,45**	26,81
13	**3 macoma**	**13**	**0,13**	**4,40**	**0,18**	**-64,84**	**-21,59**	**-1,54**	**-87,97**
14	**3 macoma**	**11,5**	**0,12**	**4,12**	**0,17**	**53,89**	**-22,43**	**-1,60**	**29,86**
15	**3 macoma**	**13,5**	**0,14**	**4,12**	**0,17**	**15,82**	**-27,24**	**-1,95**	**-13,36**
16	2 marenzelleria 2 macoma	11,5	0,12	4,67	0,19	**49,32**	**-21,67**	**-1,55**	26,11

Table A6e. Data for the O_2-flux calculation.

Core	Animals	H2O (cm)	H2O (l)	Incub time (hours)	Incub time (day)	[O2]i* (µM)	[O2]i* (µmol)	[O2]f (µM)	[O2]f (µmol)	[O2]i - [O2]f (µmol)	Flux µmol/10.17 cm2/day	Flux day mmol/m2/d	Flux hr µmol m-2 hr-1
1	**0 animals**	**10**	**0,10**	**4,67**	**0,19**	**380**	**38,66**	**312,76**	**31,82**	**6,84**	**35,18**	**34,59**	**-1441,35**
2	**0 animals**	**11**	**0,11**	**4,67**	**0,19**	**380**	**42,53**	**315,68**	**35,33**	**7,20**	**37,02**	**36,40**	**-1516,56**
3	**0 animals**	**12**	**0,12**	**4,40**	**0,18**	**380**	**46,39**	**317,15**	**38,72**	**7,67**	**41,86**	**41,16**	**-1714,82**
4	1 marenzelleria	11,5	0,12	4,67	0,19	380	44,46	327,38	38,30	6,16	31,66	31,13	-1297,26
5	1 marenzelleria	12	0,12	4,67	0,19	380	46,39	320,07	39,07	7,32	37,63	37,00	-1541,64
6	1 marenzelleria	11	0,11	4,67	0,19	380	42,53	312,76	35,00	7,52	38,70	38,05	-1585,48
7	**1 macoma**	**11**	**0,11**	**4,40**	**0,18**	**380**	**42,53**	**334,68**	**37,45**	**5,07**	**27,66**	**27,20**	**-1133,31**
8	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**380**	**42,53**	**339,07**	**37,94**	**4,58**	**26,71**	**26,26**	**-1094,12**
9	**1 macoma**	**11**	**0,11**	**4,12**	**0,17**	**380**	**42,53**	**336,15**	**37,62**	**4,91**	**28,61**	**28,13**	**-1172,25**
10	3 marenzelleria	12,5	0,13	4,12	0,17	380	48,32	330,30	42,00	6,32	36,85	36,23	-1509,67
11	3 marenzelleria	12	0,12	4,40	0,18	380	46,39	317,15	38,72	7,67	41,86	41,16	-1714,82
12	3 marenzelleria	11,5	0,12	4,40	0,18	380	44,46	321,53	37,62	6,84	37,31	36,69	-1528,73
13	**3 macoma**	**13**	**0,13**	**4,40**	**0,18**	**380**	**50,26**	**320,07**	**42,33**	**7,93**	**43,23**	**42,51**	**-1771,33**
14	**3 macoma**	**11,5**	**0,12**	**4,12**	**0,17**	**380**	**44,46**	**333,22**	**38,99**	**5,47**	**31,91**	**31,37**	**-1307,22**
15	**3 macoma**	**13,5**	**0,14**	**4,12**	**0,17**	**380**	**52,19**	**346,38**	**47,57**	**4,62**	**26,92**	**26,47**	**-1103,06**
16	2 marenzelleria 2 macoma	11,5	0,12	4,67	0,19	380	44,46	308,38	36,08	8,38	43,10	42,38	-1765,63