Filamentous Sulphur Bacteria in Lake Markermeer

Spatial and temporal influences on primary production and resuspension

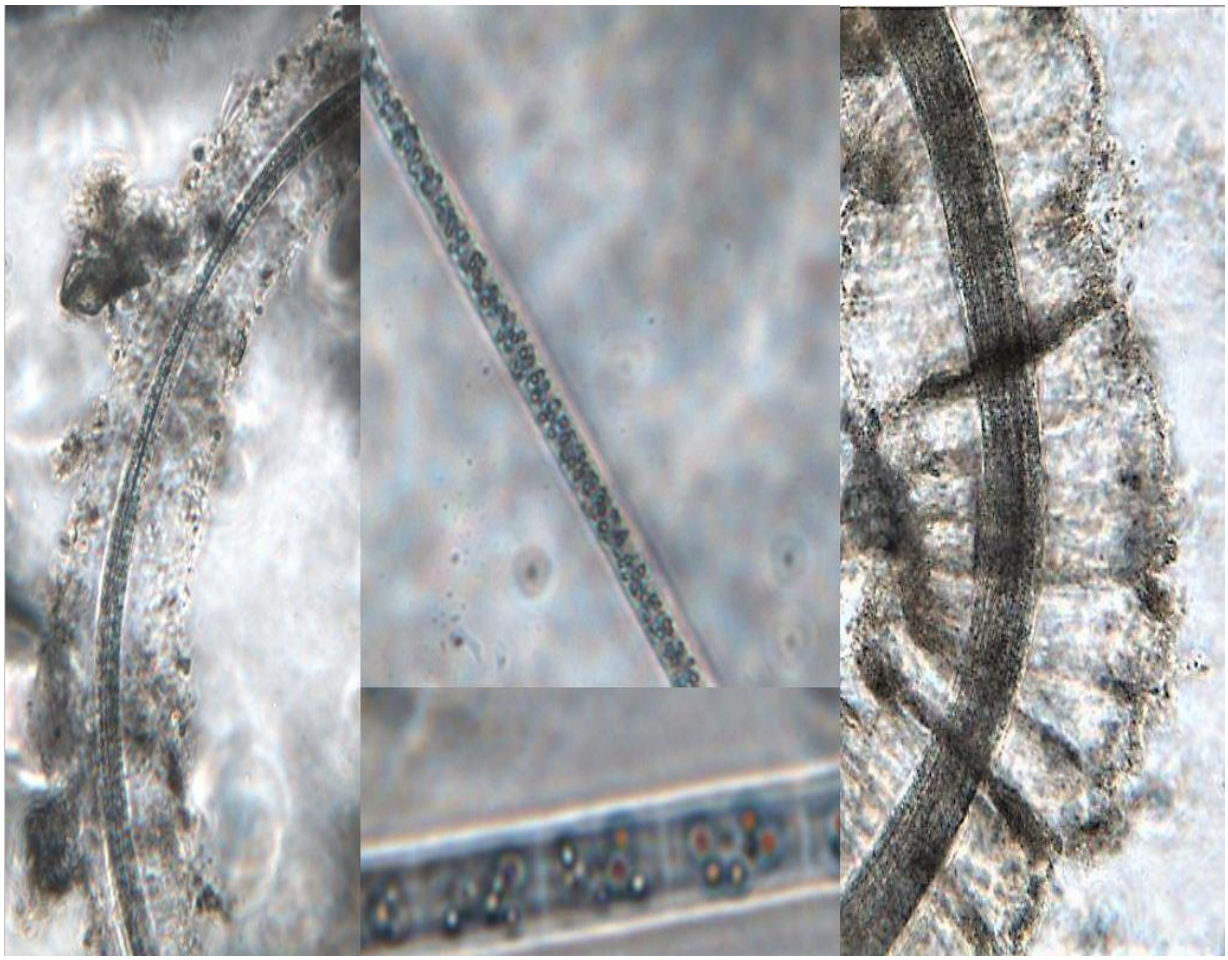

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Illustration cover page: Beggiatoa spp. and Thioploca spp. (own picture)

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Preface

Presented before you lies the final thesis: *"(Filamentous) Sulphur bacteria in Lake Markermeer"*, in which the effects of (filamentous) sulphur bacteria are investigated in the sediment of Lake Markermeer. This thesis has been written as part of my graduation of the study program Water Management (Aquatic Eco-Technology) at the HZ University of Applied Sciences. This research has been written from September 2019 until January 2020. During this research and the writing of my thesis, Ruurd Noordhuis (supervisor Deltares), Gerlinde Roskam (lab supervisor Deltares) and Alco Nijssen (supervisor HZ) have assisted me in gaining vision into the topic, finding new insights, and advising me on the process. Their critical view and intensive supervision have made this research one of the most pleasant experiences of this study in which I have learned more than I could have imagined about myself and the work I would like to do in the future. I would also like to thank the University of Amsterdam, and in particular Harm van der Geest for their cooperation during the fieldwork and their assistance. A special thanks is also given to the department of Freshwater Ecology and Water Quality of Deltares.

Luc Kauhl

January 2020

Abstract

The turbidity in Lake Markermeer limits the primary production by phototrophic algae and the growth of plants. In turn, this limits the amount of fish and other organisms that live in and around Lake Markermeer in its food sources. To determine if there are organisms that limit the resuspension, a survey was conducted in which (filamentous) sulphur bacteria were found. These bacteria were assumed to have a positive impact on the resuspension of the sediments in Lake Markermeer. To determine if this was true, multiple samples were taken and tested to determine the spatial distribution, the effects on sediments, the impacts on nutrients and the contribution to the primary production. Beforehand however, a literature study was conducted to determine possible problems and solutions these bacteria could have. This showed that the bacteria would most likely positively impact amount of sediment that was resuspended but could also negatively influence the system as the bacteria are not available as a food source. The bacteria are also able to store large amounts of nitrate and elemental sulphur in their vacuoles which could influence the nutrient balance in Lake Markermeer. In addition, literature stated that there could be an effect of the amount of oxygen that is present in the substrate by a form of microstratification. After analysing the samples, it became clear that the bacteria do have significant impacts on the resuspension of sediments, especially when there are a lot of bacteria present. It was also found that the effects on the nutrient balance in Lake Markermeer where not of significant impact. Relations where found between the nutrient concentrations in the soil and surface water, but the values were not different than expected. The availability as food source has not been fully tested. Initial results of research done by Witteveen&Bos however, shows that the bacteria do not serve as a food source available for some of the more common fish in Lake Markermeer. This becomes of greater importance as the bacteria form a significant amount of biomass in the lake. This biomass has been found to be higher than other benthic species such as mussels. Furthermore, no evidence has been found in this research of microstratification caused by the bacteria. It has therefor been recommended that further research should be conducted to determine the exact workings of these bacteria within Lake Markermeer. In addition to this, it should be investigated if these bacteria can be cultivated and if they are used as a food source for other organisms.

Introduction

History

Lake Markermeer did not exist before the 20th century. Before being closed off from the North Sea and later lake IJsselmeer, it was a low dynamic part of the Southern Sea and formed a depository for small particles such as clay and silt (De Lucas Pardo, 2014). The Northern and Southern part of the Southern Sea were split by a dam in 1932 and formed the Wadden Sea and Lake IJsselmeer. Due to the closing of Lake IJsselmeer, the water became fresh (Noordhuis, Ecosysteem IJsselmeergebied: nog altijd in ontwikkeling, 2010). A part of the original plan for the newly formed Lake IJsselmeer was to form new polders, namely the Markerwaard and the Flevopolder. In 1976 the first step for the creation of the Markerwaard was made, the Houtribdijk was built between Enkhuizen and Lelystad, separating Lake Markermeer from Lake IJsselmeer. During this period, however, some doubts about the creation of the Markerwaard arose. One of the main reasons for the creation of the Markerwaard was to increase the amount of agricultural land in the Netherlands as it was expected that in the future the food production would not be enough to feed the population. This problem had become redundant due to improved cultivation techniques and increased international trading. In contrast to this, Lake Markermeer had become an important feeding ground for aquatic birds. In addition to this the discussion arose if this lake could not serve as a freshwater reservoir for drinking water supply. Because the advantages of building the Markerwaard became less important and the disadvantages only increased the government decided in 2003 that the Markerwaard would not be made (Hans, 2018).

Present day

After Lake Markermeer had been closed off, the system changed a lot. It has been categorized as a large and shallow water body (De Rozari, 2009). The residence time of the lake is approximately 1.5 years, it has a mean water depth of 3.6 m and slopes down from west to east (De Lucas Pardo, 2014) (De Rozari, 2009). The seasonal water level variations are small as they are imposed to fulfil the lakes function. The summer level is -0.20 m NAP to ensure the water supply and the winter level is -0.40 m NAP for drainage of surrounding polders. The yearly total inflow and precipitation add up to $2.0*10^9$ m^3 which is 80% of the total storage volume (van Duin, 1992).

Problem statement

The long residence time and the composition of the lake bottom cause some problems in Lake Markermeer. The turbidity of the lake is very high due to the resuspension of sediment. This high turbidity causes multiple problems, light cannot reach the water plants in the lake decreasing their growth and the high amounts of particles in the water causes flocculation of the algae. Because of this, fish and birds have less resources to feed on and benthic communities such as filtering organisms cannot filter the algae from the water column due to the flocculation. This affects the nature value of Lake Markermeer greatly, as it has an important function within Europe for migratory birds as feeding and resting ground. The area has also been assigned as a Natura 2000 area. As a result of this, the law states that the nature in the area must be protected and cannot deteriorate (Rijkswaterstaat, 2019).

Resulting from the artificial nature and low habitat diversity of this lake the resilience of the lake is low. Pressures such as climate change, water quality changes and invading species can have great impacts on the system. As part of strengthening Lake Markermeer to increase its resilience and habitat diversity Natuurmonumenten set up a plan to build islands and shallows named Marker Wadden. These islands are meant to improve resting, breeding and feeding grounds for birds and decrease the amount of suspended material in the lake.

To predict the effects of different projects on suspended sediment concentrations, a silt model was made by Deltares. The model was also used to predict the effects of Marker Wadden. The model is, however, still missing the interaction between biological processes and the behaviour of sediment. To increase knowledge of this interaction and improve modelling, a project was set up under KIMA, the Knowledge and Information Programme Marker Wadden, in which several organisations cooperate. One of these processes is the lake bottom conditions in relation to benthic life. Considering this, sediment samples were taken around Marker Wadden to check for benthic life forms that are capable of changing resuspension and sedimentation processes. When analysing the samples, the sulphurous bacteria *Beggiatoa* spp. and *Thioploca* spp. where found (Roskam, 2019). These bacteria form mats that use nitrate and sulphur to convert energy. They live in the oxic-anoxic boundary layer in the bottom of lakes (Schutte, et al., 2018). These mats have a structure that could be of great influence on the resuspension of the mud (see Figure 1).

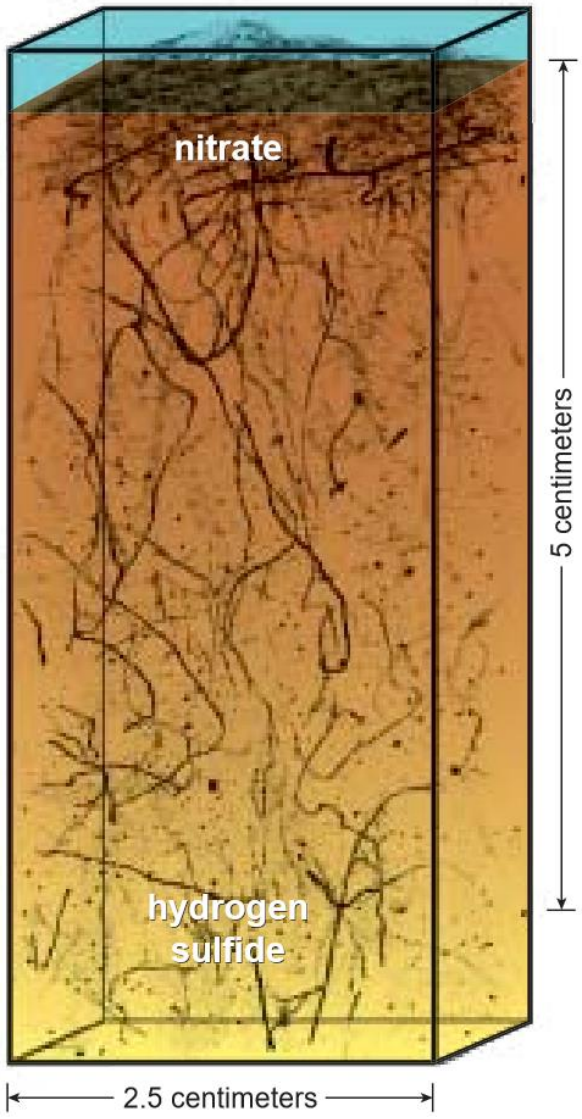

Figure 1: The structure of a sulphurous bacteria mat, the top layer has a more horizontal orientation and the bottom layer is vertically oriented (Source: Levin 2002)

Literature on these sulphurous bacteria also states that there is a large influence on the other benthic communities. In another water body in The Netherlands, lake Grevelingen, the locations where these bacteria where found showed no other benthic life (Lengkeek, Bouma, & van den Boogaard, 2010).

This raises the question if this effect is also present in Lake Markermeer. To find the effects caused by sulphurous bacteria in Lake Markermeer, a part of the project involved the determination of the effects on nutrients and benthic life. This was done in cooperation with the University of Amsterdam as part of a separate assignment from the Werkgemeenschap Levend Markermeer (WVL) which is set up by Rijkswaterstaat. In addition to this there are no known species that feed on these bacteria, it is therefore also of interest how much of the primary production is taken up by this species.

Research questions

The purpose of the research reported here is to extend the current knowledge of the ecosystem and mud resuspension in Lake Markermeer. It was carried out in cooperation with the University of Amsterdam. The client for the research is RWS-WVL (Rijkswaterstaat - Water, Verkeer en Leefomgeving; Ministry of Infrastructure and Water Management); which is the RWS department that forms the link between the regional RWS water managers (and their research questions) and the knowledge partners. Rijkswaterstaat is also involved in KIMA, the Knowledge and Information Programme Marker Wadden, under which monitoring and research is carried out. KIMA is a research platform of several universities and knowledge institutes looking to improve current knowledge of the effects of Marker Wadden. The research question posed by KIMA is:

- *What is the influence of (filamentous) sulphur bacteria on the behaviour (resuspension) of sediment?*

With as sub question:

- *What is the spatial distribution of (filamentous) sulphur bacteria in Lake Markermeer?*

The research question posed by the RWS-WVL is:

- *What is the contribution of (filamentous) sulphur bacteria in to the food web in Lake Markermeer?*

With as sub question:

- *What is the relation of (filamentous) sulphur bacteria to the availability of nutrients, organic matter and oxygen (stratification)?*

To answer these questions, field and lab work was conducted and desk research was done in order to link the collected data to the system.

Hypothesis

It is hypothesized that there will be a large influence of the bacteria on sediment resuspension due to the structure of the mat. This mat will keep the sediment captured. Because of the forming of mats, it is also expected that there is a significant contribution to the primary production in the lake and that the availability for the food web will be low due to the toxic nature of by products such as H_2S that are often found in close proximity to these mats. Literature also states that large amounts on NO_3^- are taken up by these bacteria, and that there is a form of microstratification present. It is therefore expected that nutrient concentrations will also be influenced by the sulphur bacteria.

An Eco-Technological solution

As part of this research an Eco-Technological solution was investigated. Namely, can *Thioploca* spp. and *Beggiatoa* spp. be used to limit resuspension of the Lake Markermeer? Experiments will be done to determine the effects on the resuspension of these bacterial species. In addition to this, a literature study was done to determine if there are possibilities for the cultivation of the target species. This

solution will also be linked to the findings of the other parts of this research and a determination will be made to assess the impacts on other factors in Lake Markermeer.

Structure of the study

This report was written according to the following structure. A literature study was conducted, laying a solid knowledge foundation to build the research on. This literature study has formed the theoretical framework that can be found in this report. From this literature study, it was determined that field work should be conducted at two separate moments. The surveys were conducted in two rounds. the first measurement round was conducted on the 10th and 12th of September and on the second round on the 5th of November 2019. The samples were then analysed in the lab to determine the spatial distribution, the biomass, the nutrient concentrations, physical parameters, the species present, and the effects on the resuspension of sediment of the sulphur bacteria in Lake Markermeer. Additionally, the presence of organic matter and $CaCO_3$, and the production of other species was determined. This was done to answer the questions posed by RWS-WVL and KIMA. The lab work was fitted to the questions in order to provide the answers needed for gaining the knowledge.

Theoretical framework

Thioploca spp. and *Beggiatoa* spp.

The species that have the main focus in this research are *Thioploca* spp. and *Beggiatoa* spp. They are sulphur bacteria that convert sulphurous compounds to elemental sulphur or sulphate while mostly using nitrate. Both species can form mats and live in the substrate on the oxic-anoxic boundary layer. They are among the largest bacteria known and store large amounts of nitrate in their vacuoles and sulphur in their cell walls. These liquid vacuoles occupy more than 80% of the cell volume (Fossing, et al., 1995). There are however also some significant differences between the genera. *Thioploca* spp. form bundles of filaments enclosed in sheaths. These sheaths are most likely formed of polysaccharides and there are up to a hundred filaments of *Thioploca* spp. per sheath. *Beggiatoa* spp. on the other hand occurs in single filaments without a sheath, that often lives in close proximity to other *Beggiatoa* spp. or *Thioploca* spp. Both genera move using gliding motility. *Beggiatoa* spp. moves separately from the other bacteria present. *Thioploca* spp. is also capable of movement separately of the other filaments. The difference is however, that the *Thioploca* spp. filaments move within the sheath. The movement is not linked to the other filaments in the same sheath. Additionally, the sheaths are a lot longer and thicker than the filaments of *Beggiatoa* spp. which makes the formation of mass more likely for *Thioploca* spp. Another important difference is that *Beggiatoa spp.* appears to be more tolerant to oxygen. Lab experiments show a phobic reaction of both species to increasing oxygen concentration, but this is more obvious in *Thioploca* spp. (Huettel, Forster, Kloser, & Fossing, 1996). Moreover, *Beggiatoa* spp. can use oxygen as an electron acceptor for the reaction with sulphate whereas *Thioploca* spp. cannot.

Thioploca spp. and *Beggiatoa* spp. are both species that can live in fresh, brackish and salt water sediments. There are some differences described in literature about the way they behave in the different environments. In salt water the growth of these bacteria always occurs in the microoxic niche where they grow chemolithoautotrophic. The species that grow in fresh water are often located in well aerated environments and form tight aggregates. In this aggregate they form a microoxic niche for themselves to optimise conditions (Nelson, Revsbech, & Jorgensen, 1986).

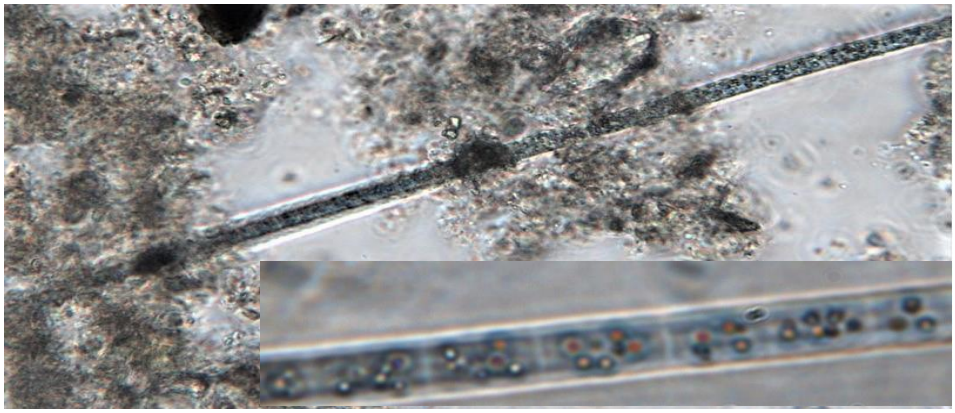

Figure 2: Beggiatoa spp. A single filament with an enlargement where elemental sulphur can be seen as orange specs (Noordhuis, 2019)

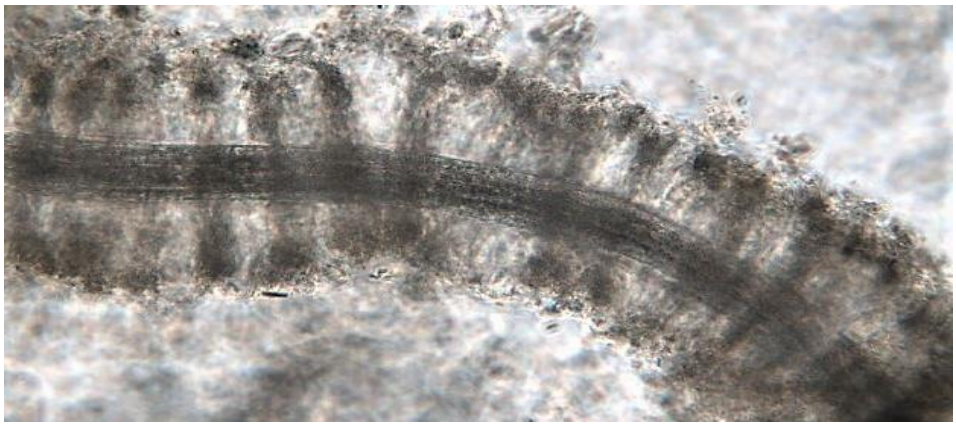

Figure 3: Thioploca spp. A sheath with multiple filaments in the middle (Noordhuis, 2019)

Mat formation

As stated before, the sulphurous bacteria *Thioploca* spp. and *Beggiatoa* spp. form mats. The effect of the mats on resuspension depends on the structure of the mats. As shown in Figure 4, the presence of only *Thioploca* spp. or a combination of *Thioploca* spp. and *Beggiatoa* spp. results in different structures. There is also a structural difference between conditions where the oxic-anoxic boundary layer and the sulphate layer are close too or far apart from each other. When there is more distance between the two layers, mats will not form in such a dense layer compared to when the boundary layer and the sulphur layer are in close proximity (Dunker, Roy, Kamp, & Barker Jorgensen, 2011). The mats can form both in- or on top of the surface; the location depends on the amount of oxygen in the water column. When there is no oxygen present in the water column, the mats will most likely form a (bigger) mat on top of the sediment that continues into the sediment towards the sulphur layer. In presence of oxygen, the mats mostly form in the sediment itself and will rarely form a visible mat on top of the sediment. This is due to the phobic reaction to oxygen described before. The main period when these mats are formed is shortly after the water column was in an anoxic state. This anoxic state causes other benthic life to die and this leaves a niche for the sulphurous bacteria that they can easily colonise (Nolte & Lagendijk, 2016).

When applying this knowledge to the current situation it is most likely that the oxic-anoxic layer and the sulphur layer are very close to each other as it is a marine deposit, which is very rich in sulphur and is not dependent on nutrient-upwelling. This would suggest that a relatively dense mat is formed in Lake Markermeer. In addition to this, the first sample taken during the measurements around Marker Wadden showed a mixed colony of *Thioploca* spp. and *Beggiatoa* spp. (Roskam, 2019). The combined mat also has a denser structure as is shown by Figure 4.

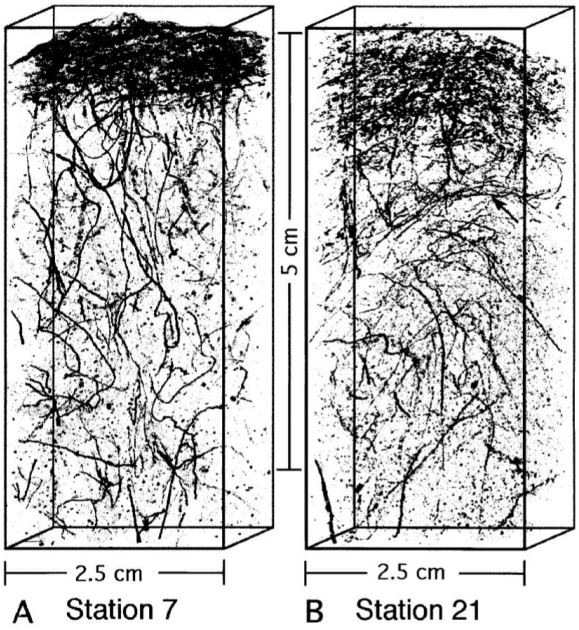

Figure 4: Mat structure, picture a shows A mat consisting of Thioploca spp. and Beggiatoa spp. combined and B shows a mat only consisting of Thioploca spp. (Schultz, B.B., Fossing, & Ramsing, 1996)

Not much information can be found about the effects of these mats on the substrate resuspension. The resuspension experiments in this research will provide insight on this topic. There is, however, literature on the effects it has on the benthic life and reaeration. Studies in Lake Grevelingen have shown that when coverage of the substrate is ten percent or more, other benthic life can no longer be found in those areas most often caused by microstratification (Lengkeek, Bouma, & van den Boogaard, 2010).

Nutrients and oxygen

Sulphurous bacteria convert sulphur compounds or elemental sulphur in order to produce energy. A lot of research has been conducted on which electron acceptor is used for the conversion of sulphur. The most common answers to this are either oxygen or nitrate. It appears that there is a difference in what the correct answer is depending on external factors. There is also a notable difference in older or newer literature.

Old vs new literature

Older literature (pre-1995) often states that the mats that are formed by the sulphurous bacteria only consume oxygen as an electron acceptor for the sulphur reaction (Bernard & Fenshel, 1995). It was thought that these bacteria consumed a large portion of the available oxygen in the system causing other species to die due to oxygen depletion. In 1995 however, it was proposed that the sulphur bacteria store nitrate in their vacuoles and transport it to the sulphurous layer where it is reduced to oxidize hydrogen sulphide (Fossing, et al., 1995). Since then, new discoveries have shown that the sulphur bacteria are very versatile and can use different compounds to convert sulphur. It has been shown that both oxygen and nitrate can be used but that there is a preference to using nitrate in most systems (Schutte, et al., 2018). There are also indications that phosphor can be used and stored by the sulphur bacteria adding to the versatility of these species (Brock, 2011).

Impacts on nutrients

Because these bacteria can be very abundant in a system, and due to their nature of using nutrients as main component for the conversion of sulphur, there could be a big effect on the nutrient

availability. It has been shown that *Beggiatoa* spp. can store up to 500 mM of nitrate in their vacuoles. This concentration exceeds the concentration in the water column by a factor up to 20.000 (Fossing, et al., 1995). This would correlate well with the nutrient values found in Lake Markermeer. A relatively sudden drop in nitrate concentrations was seen in the tributaries and adjacent lakes of Lake Markermeer starting in 2003. This was because of a decline in nutrient loading by effective regulations on the use of fertilizers in this period, strengthened by the decline of rivers discharge. Lake Markermeer however, showed a more extreme decrease of nutrients. The levels in Lake Markermeer dropped to the levels of Lake Grevelingen where concentrations were always relatively low compared to the coastal waters with which Lake Grevelingen was connected. The lakes and tributaries around lake Grevelingen all show higher amounts of nutrients then Lake Grevelingen itself. The main similarities of these two lakes is that both have long retention times and the presence of sulphurous bacteria. It is also noteworthy that they are both closed off sea arms. However, there are also many differences. Lake Grevelingen is a brackish water body and is a lot deeper, on average 5.4 metre with the deepest part being 48 metres deep (ScubaXP, 2019). Due to the salt concentrations and the depth of the lake, stratification is more common.

When comparing the values of ammonium and nitrate found in these lakes (see figure 5 and 6), there are a lot of similarities. At present, they are lower in Lake Markermeer and Lake Grevelingen then they are in other lakes or in the Rijn. This is also the case when looking at orthophosphate, which would indicate that this is also a factor in both lakes in relation to the sulphurous bacteria. This could be confirmed by a research done in the Gotland Basin in the Baltic Sea where phosphate and Dissolved Inorganic Nitrogen (DIN) releases from the sediment where estimated to be 25-30% lower respectively at a location where mats of sulphur bacteria covered 51% of the bottom as compared to the reference situations in the same fjord. (Noffke, Sommer, Dale, Hall, & Pfannkuche, 2016).

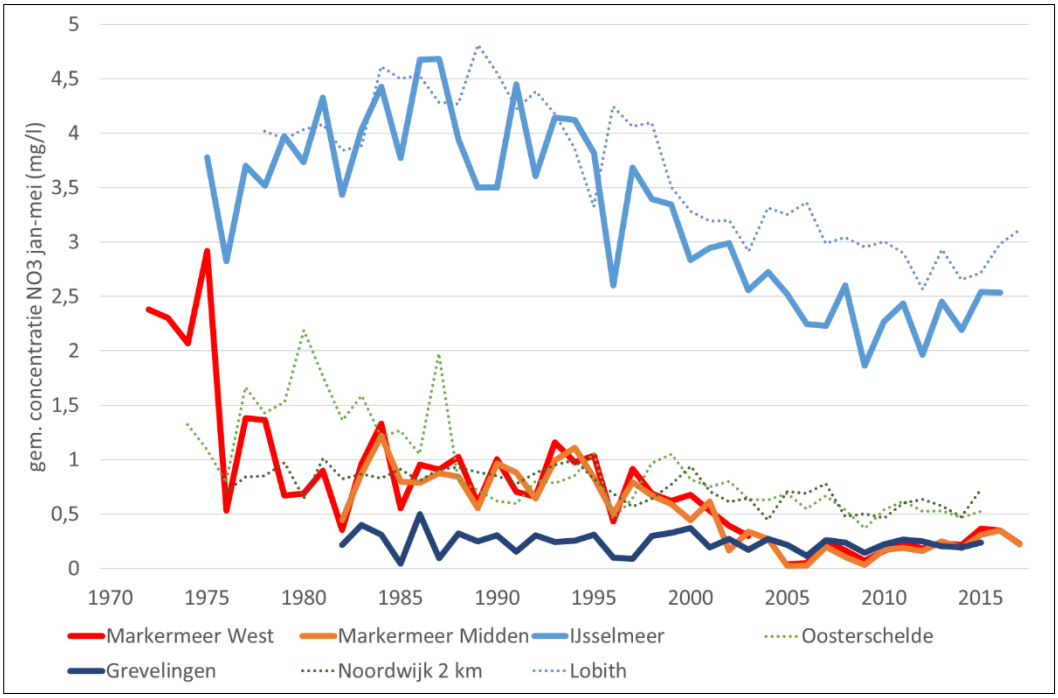

Figure 5: NO3 concentrations of Lake Markermeer compared to the Rijn and the Scheldt area (Noordhuis, 2019)

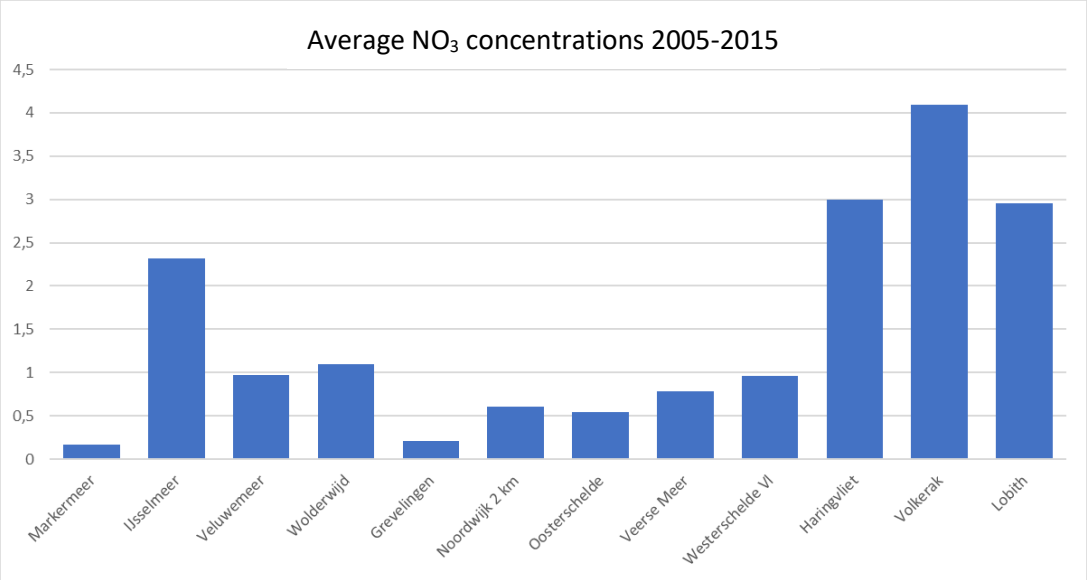

Figure 6: NO3 concentrations of different water body's in The Netherlands (Noordhuis, 2019)

This would also suggest that nitrate is mainly converted to N_2 by the sulphur bacteria in Lake Markermeer. Furthermore, a decline in ammonium has also been present starting in 2004. This would suggest that ammonium is not a main product of the sulphur bacteria. As can be seen in the reaction formulas below, there are many different ways the bacteria convert sulphur, but it has been shown that the main product depends on the environment. This would indicate that in Lake Markermeer indeed mainly N_2 will be produced (Schutte, et al., 2018).

$S^0 + 1.5\ O_2 + H_2O \rightarrow SO_4^{2-} + 4\ H^+$

$S^0 + 0.75\ NO_3^- + 1.75\ H_2O \rightarrow SO_4^{2-} + 0.75\ NH_4^+ + 0.5\ H^+$

$S^0 + 1.2\ NO_3^- + 0.4\ H_2O \rightarrow SO_4^{2-} + 0.6\ N_2 + 0.8\ H^+$

$HS^- + 0.25\ NO_3^- + 1.5\ H^+ \rightarrow S^0 + 0.25\ NH_4^+ + 0.75\ H_2O$

$HS^- + NO_3^- + H^+ + H_2O \rightarrow SO_4^{2-} + NH_4^+$

$HS^- + 0.4\ NO_3^- + 1.4\ H^+ \rightarrow S^0 + 0.2\ N_2 + 1.2\ H_2O$

$HS^- + 1.6\ NO_3^- + 0.6\ H^+ \rightarrow SO_4^{2-} + 0.8\ N_2 + 0.8\ H_2O$

Equations by Schutte, et al., 2018

Relation to oxygen

The relation between oxygen and sulphurous bacteria is complicated. As mentioned before, oxygen can be used by the bacteria to convert sulphur. However, lab experiments show a phobic reaction to oxygen. When the concentration of oxygen is increased, the filaments of the bacteria moved away from the source of the oxygen. It has also been shown that the bottom 90% of the bacterial mat is anaerobic. The oxygen will not go into this layer even when oxygen concentrations in the water column are high due to a lowered exchange rate of oxygen and sediment by the bacteria (Moller, Nielsen, & Jorgensen, 1985) (Nelson, Revsbech, & Jorgensen, 1986).

In some cases, it has also been observed that the water layer directly above the mat is kept in an anoxic state (Lengkeek, Bouma, & van den Boogaard, 2010).

Lake Markermeer is a relatively shallow lake which is greatly influenced by wind. This means that the lake rarely becomes stratified. To achieve anoxic conditions caused by stratification, the temperature needs to be high and the windspeed needs to be low. This combination then also needs to last for a longer time (at least three days). There is day stratification in Lake Markermeer, but this will not have enough effect to make the bottom layer anoxic.

Primary production

Very little information can be found on organisms that feed on the sulphurous bacteria. Some research has been conducted to determine what species do feed on them, but there is only very few and those that do only feed on bacteria smaller than 7 µm (Kiyashko, Narita, & Wada, 2001). There are only a few species of *Beggiatoa* spp. and *Thioploca* spp. that are smaller than this 7 µm, most are larger, and there are thus no species known eating them.

Due to the high biomass production of these bacteria in the form of mats, it could be the case that a large part of the primary production in this system is not available. This could lead to a further decrease of the biodiversity and health of the system caused by the fact that these bacteria are a large nutrient sink.

Opportunities and challenges

This research offers multiple challenges and opportunities. Firstly, by finding out what kind of benthic life there is in Lake Markermeer, a more detailed vision of the system can be formed. This could be used to find better ways of improving the biodiversity and reduce the amount of sediment that is resuspended. By investigating if the sulphur bacteria are present in Lake Markermeer and to what extent they influence the system, an advice can be given on what steps can be taken to decrease the negative effects of these bacteria. The challenge on the other hand could be to find concrete measures to prevent or stimulate the mat forming. The main way for these mats to get broken down is by degradation which will release the nutrients stored to the water column possibly creating new problems.

Methodology

The methodology has been written to answer the question that have been posed in this report. An overview of the samples that have been collected and what they were used for can be found in the overview below.

Samples collected on the 10th and 12th of September:

- A total number of 87 cores were collected (three per location, 29 locations in total). These cores where divided for the different analyses as follows:
  - 50 cores where used for subsampling (TGA and microscopy) and determination of the biomass. On 11 of these cores, pore water was extracted prior to the subsampling and biomass determination. These pore water samples were used to determine the anion and cation concentrations (IC and ICP-OES)
  - 8 cores where used for resuspension experiments
  - The remaining 29 cores were used for the production measurements and DNA analysis
- Surface water measurements were taken on all locations (29) to determine the physical parameters O_2, pH and water temperature
- Surface water samples were collected on all locations (29) to determine the standard anion and cation concentrations (IC and ICP-OES)

Samples collected on the 5th of November:

- A total number of 20 cores were collected (two per location, ten locations in total). These cores where divided for the different analyses as follows:
  - 15 cores where used for subsampling (TGA and microscopy) and determination of the biomass. On 5 of these cores, pore water was extracted prior to the subsampling and biomass determination. These pore water samples are used to determine the anion and cation concentrations (IC and ICP-OES)
  - 5 cores were used for resuspension experiments
- Surface water measurements were taken on all locations (13) to determine the physical parameters O_2, pH and water temperature
- Surface water samples were collected on all locations (13) to determine the standard anion and cation concentrations (IC and ICP-OES)

Sample collection

The samples were gathered on the mussel collection grid taken from the measurement campaign to determine the spatial distribution of the mussel species present in Lake Markermeer. This grid is used as it offers the possibility of comparison with other data sets (Bij de Vaate & Jansen, 2016). A spatial survey was carried out to determine the distribution of the bacteria. During the first sampling round, 29 locations were sampled along four east-west transects to encompass the wind and turbidity gradient present in Lake Markermeer (see figure 8). At each location, two cores were taken for analysis at the lab of Deltares and one additional core was taken for the production measurements and DNA analysis at the University of Amsterdam. Due to time limitations, a selection was made of 10 locations which contained sulphur bacteria for the second round to determine the changes in biomass in time which are shown with the black circles in figure 8. The samples were taken using an Uwitec core sampler (see figure 7). PVC tubes with a diameter of 6 cm and a length of 60 cm were used (for a detailed explanation of the use of an Uwitec see appendix II). When the corer reaches sand or a layer of shells, it will not penetrate the sediment any deeper. If the result was a core with sediment a layer

of 15 cm or less, a new core was taken. In several locations there was not enough sediment to extract any longer cores. The cores were closed at the bottom with a rubber plug and filled with water from the area if not completely full. After this they were capped with a water tight cap and labelled. The sediment cores were stored at 4°C upon arrival at the lab at the end of the day.

The water samples were taken from the surface water by use of an on-board pump. The pump was flushed for 30 seconds before the sample was taken. The water samples were cooled on board of the vessel. The next day, the surface water samples were filtered (0.45 µm), subsampled for analysis by ion chromatography (for nutrient concentrations) and analysis by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy). The ICP-OES sample was acidified by adding 1% of concentrated nitric acid. The analysis was performed by the University of Utrecht, partner of Deltares within Utrecht Castel.

In addition, pH, EC and temperature were measured directly in the water surface. At three locations (47, 59 and 64), a depth transect was measured to check for stratification. The collected data, time of measurement, coordinates, measurements and remarks were noted down on a field form.

Figure 7: The Uwitec core sampler

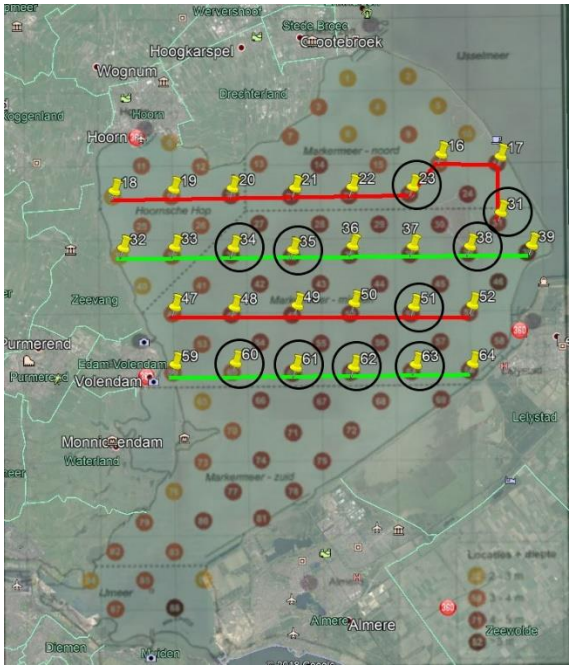

Figure 8: The sampling locations on the mussel collection grid. In green and red are the envisioned routes for the first campaign. The black circles indicate the locations that were sampled during the second round

Lab work

Two sets of cores were brought to the lab in Utrecht; 2x29 cores from the first sampling round, 2x10 cores from the second round. The cores were described (length of the core, presence of a fluffy layer, thickness of the different layers, presence of shells, presence of plants and plant material, presence of other organisms). A total of 13 cores (8 from the first sampling round, 5 from the second) were selected for the resuspension experiments. Cores shorter than 15 cm could not be used in the resuspension experiment, because the rotor would not be close enough to the sediment. These cores were stored at 4°C until the experiments were carried out. A total of 16 cores (11 from the first sampling round, 5 from the second) were selected to extract porewater. In some cores the top layers were disturbed; these cores were not selected for the resuspension experiments or porewater

extraction. Furthermore, the selection was based on spatial distribution, expected presence of sulphur bacteria and variety in soil type.

Porewater analysis

The porewater was extracted from the samples at a depth of were taken at 1, 2.5, 5 and 10 cm from the sediment surface to create a transect in depth. The porewater is extracted by a rhizon (pore size 0.1 µm) by applying a vacuum. Sub samples were taken for the Ion Chromatography (IC) and put in the fridge as soon as possible to limit nutrient change. If possible, these samples were analysed within 24 hours of collection to further limit changes in nutrient concentrations. The nutrients that are of interest are nitrite, nitrate, sulphate, phosphate and ammonium. Additionally, subsamples were taken for elemental analysis by ICP-OES. The ICP-OES samples are of interest to assess the concentrations of P and S. After sample conservation, the samples were taken to the analysts that measured the samples according to procedure.

Slicing

To determine the biomass and organic matter in the samples at different depths, the cores were sliced. The slicing was done according to the expected depth at which the bacteria would be found based on the literature study. The sampled layers are 0-1.5 cm, 1.5-3 cm, 3-5 cm, 5-7.5 cm, 7.5-10 cm and the remaining layers at 5 cm up to a total depth of 30 centimetres. This was done to get a detailed view of the top layer and to ensure the bacteria were not found at greater depth than initially expected.

The total mass in each layer was weighed, to determine the density of the sediment. Next, the sediment was homogenised and subsamples were taken for microscopy and TGA (Thermogravimetric Analysis). The left-over material was weighed again, and subsequently used for the determination of the amount of sulphur bacteria biomass.

TGA

The TGA subsample was taken at a total weight of ten grams wet weight. This was done to ensure three grams of dry weight sample could be analysed. The samples were dried at 105 degrees Celsius before analysing them in the TGA. The dry weights of the samples were noted down in order to calculate the amount of water present in the sample. After taking the dry weight, the samples were crushed with a mortar to homogenise the sample. The TGA was programmed to increase the temperature at one degree per minute until threshold temperatures of 105, 450, 550, 800 and 1000 degrees Celsius. At these threshold values the apparatus remained at the temperature until no further weight loss was measured and only then continued to increase the temperature. This analysis was done for all locations on the top four layers. This was done to take the layers (1, 2 and 3) in which sulphur bacteria where found and a reference layer (4) in which they were not found.

Microscopy

Subsamples for microscopy were taken to determine the density of the different organisms in the samples. The samples were put into an Eppendorfcup (1.5 ml) with water and put in the fridge for storage until they could be analysed. During the analysis the strain of bacteria were counted and categorised to give an approximation of the amount of bacteria found. The categories were set at 0-10, 10-50, 50-100 and >100 strains. When possible the exact number of strains was counted to increase the accuracy. Other organisms that were found where also listed. The counting was done at a 250x PHACO magnification.

Resuspension experiments

The resuspension experiments were done using the Vane, this apparatus uses a rotor blade that can be set at different speeds very accurately. The method for this experiment is taken from a research by

Penning, W.E. et all. in order to compare the data. The surface water above the core is continually refreshed, and the amount of suspended material is analysed in the removed water based on light scattering (OSLIM, https://www.deltares.nl/app/uploads/2016/04/OSLIM-optical-silt-measuring-instrument.pdf). The rotor speed is gradually increased at 100 second intervals until the suspended matter concentration is out of limits for the OSLIM. The maximum shear stress that was applied is 3.6 Pa, which corresponds to the conditions at the water bottom at an approximate wind speed of 5 at the Beaufort scale (Penning, Genseberger, Uittenbogaard, & Cornelisse, 2012). A detailed observation log was kept, which was used to compare the samples to each other. Whenever possible, pictures and videos were taken to show the different events. After the experiment a determination of the biomass in the top 10 centimetres was made to link the measurements to the amount of bacteria present. The set-up of the experiment can be seen in figure 9.

Figure 7: The experimental set-up of the resuspension experiment

Biomass determination

Determination of the biomass was done for all locations. The different layers were watered down and sieved over a 500 micrometres sieve. This was done until no further bacteria were found in the layer that was being analysed. It was assumed that below this layer, no more bacteria would be found. The material that remained on the sieve, was put in a jar with water and put in a fridge. The samples were then visually assessed on the amount of bacteria present. The choice was made to divide the samples in four categories of increasing amounts to be able to compare to the microscopy samples. After the categories were determined, samples that were not fouled by shell material or other particles were weighed to get an approximation of the weight of the bacteria per layer and category. The samples were measured for both wet and dry weight.

Statistical analysis

After all the results have been gathered and formatted, statistical analysis will be done to test the statistical relations between the data. This will be primarily done by the correlation test build into Excel which shows the correlation coefficient and by calculating the Spearman's Rho. These analyses will be done on each data set separately and on relations between the different data sets. The data will then be assessed, and the significant relations will be discussed.

Results

The samples that were collected during the measurement rounds in September and November were analysed in the lab according to the methodology described above. An overview of the gathered results can be found in this chapter.

Microscopy

The data of the first measurement round shows distribution of the sulphur bacteria that concentrates in the south-eastern part of Lake Markermeer (Figure 10). *Beggiatoa* spp. is found at all locations in Lake Markermeer and *Thioploca* spp. at almost all locations. The concentration area is less visible in the *Beggiatoa* spp. map. For *Thioploca* spp. the concentration area is more visible. The concentration area of *Thioploca* spp. is more important than that of the *Beggiatoa* spp. This is because the same category of *Thioploca* spp. represents a more significant biomass then the same category of *Beggiatoa* spp. The number of sheaths is counted for the *Thioploca* spp. map. Each sheath contains multiple filaments, which can form a relatively high biomass compared to the individually counted *Beggiatoa* spp. filaments. These individual filaments have also been found to be shorter then the *Thioploca* spp. sheaths.

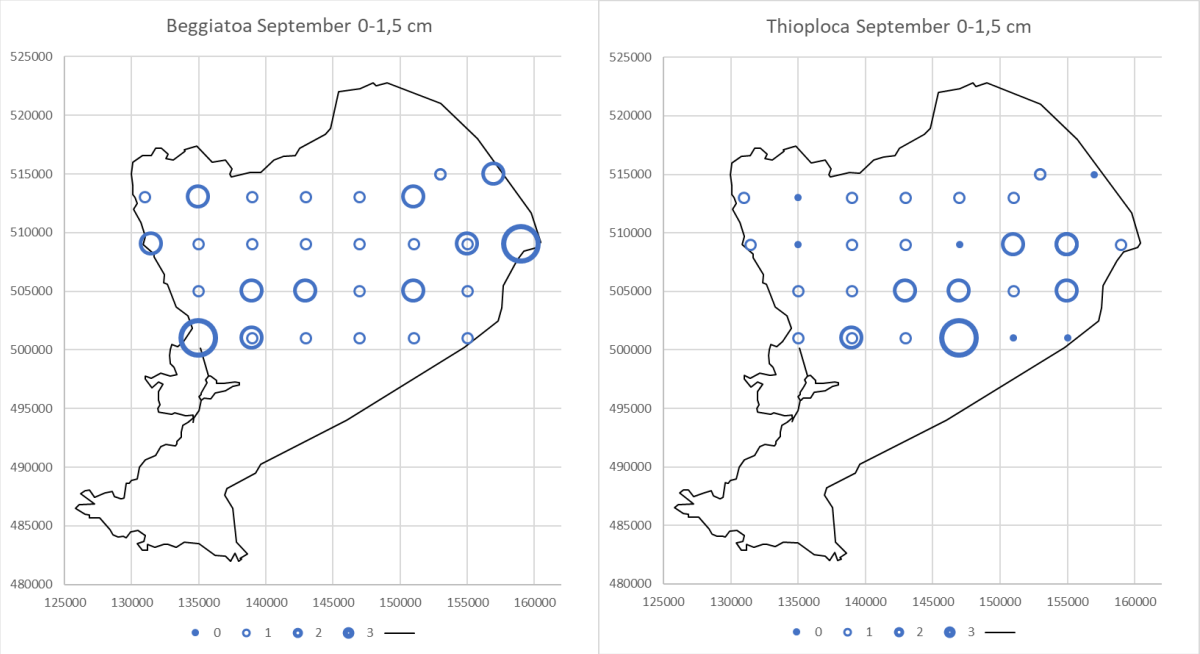

Figure 80: The microscopy categories for each measured location of the first measurement round

The second measurement round also shows a similar distribution of the bacteria (Figure 11). In this case, both *Thioploca* spp. and *Beggiatoa* spp. show the concentration area in the south-eastern side of the lake.

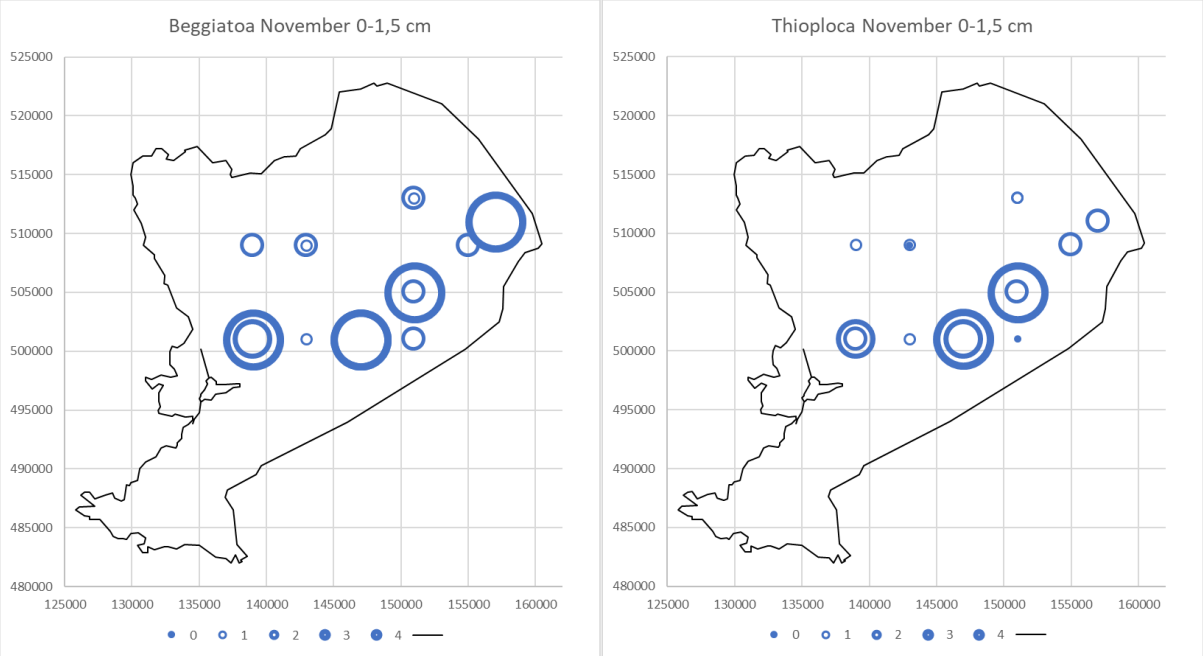

Figure 91: The microscopy categories for each measured location of the second measurement round

When looking at the depth profile, a clear decline in depth can be seen (Figure 12). In the top layer, the highest number of bacteria were counted for both *Beggiatoa* spp. and *Thioploca* spp. In the second layer a strong decrease in the number of bacteria can be seen; this decrease is most apparent for *Beggiatoa* spp. The third layer contains a very low number of bacteria. In the fourth layer, no bacteria were found.

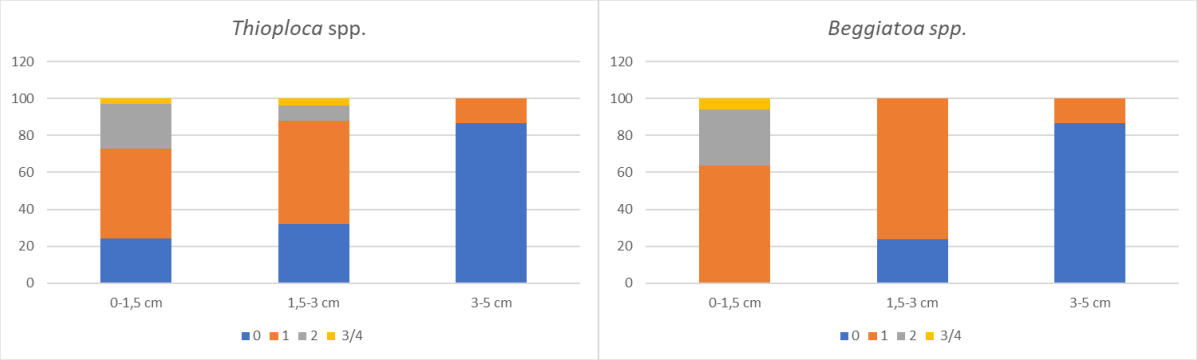

Figure 102: The depth division of bacteria of the first measurement round

The second round shows a similar decline in depth (Figure 13). However, the number of strains found are significantly higher. For a good comparison the compared locations can be seen in Figure 14. Both *Thioploca* spp. and *Beggiatoa* spp. show an increase on strains in the top layer, this is most significant for *Beggiatoa* spp. In the second layer, the samples taken in November do not contain a class 4 sample whereas the samples from September does. As a whole however, the samples from November show higher amounts of bacteria at more locations. Moreover, a higher percentage of samples contains bacteria compared to the first sampling round.

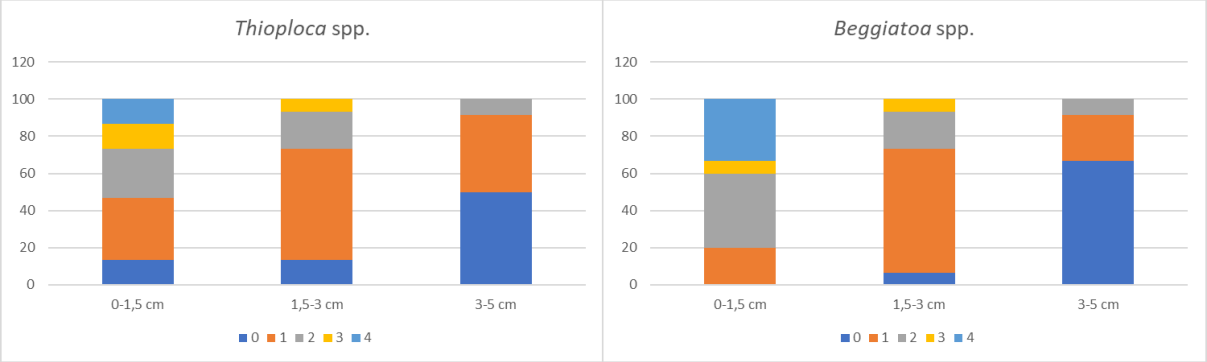

Figure 113: The depth division of bacteria of the second measurement round

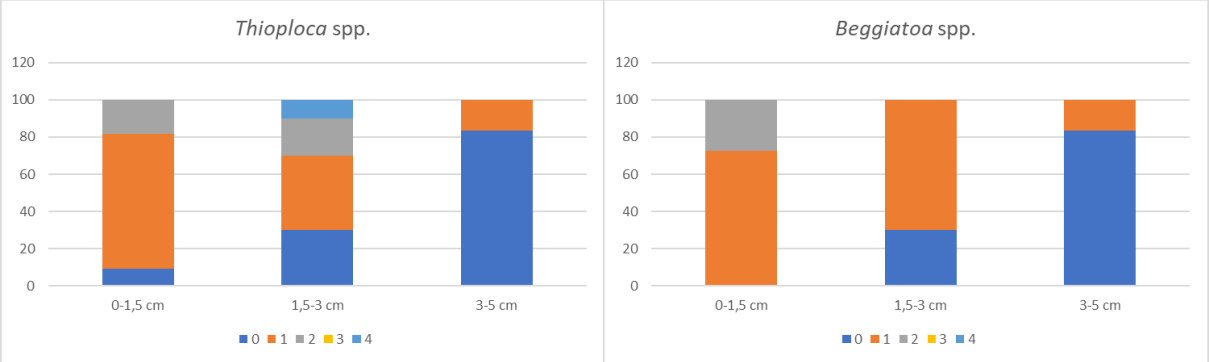

Figure 124: The depth division of bacteria in September, only showing the locations of the second measurement round for comparison

Biomass

The first round of biomass sampling shows a clear pattern with a relatively high biomass in a zone starting from Marker Wadden and turning down clockwise (see figure 15). This is also seen in the microscopy samples of *Thioploca* spp. It is also seen that at almost all locations, an amount of bacteria was visible by eye on the washed-out samples. This indicates that there is a significant biomass present in almost the whole of Lake Markermeer. When looking at the depth at which the bacteria where found, it shows that below the 3-5 cm layer no bacteria where found. The maximum depth at which the bacteria were found can also be linked to the amount of bacteria found in the top layers. When the biomass in the top layer is higher, the bacteria are found at greater depth.

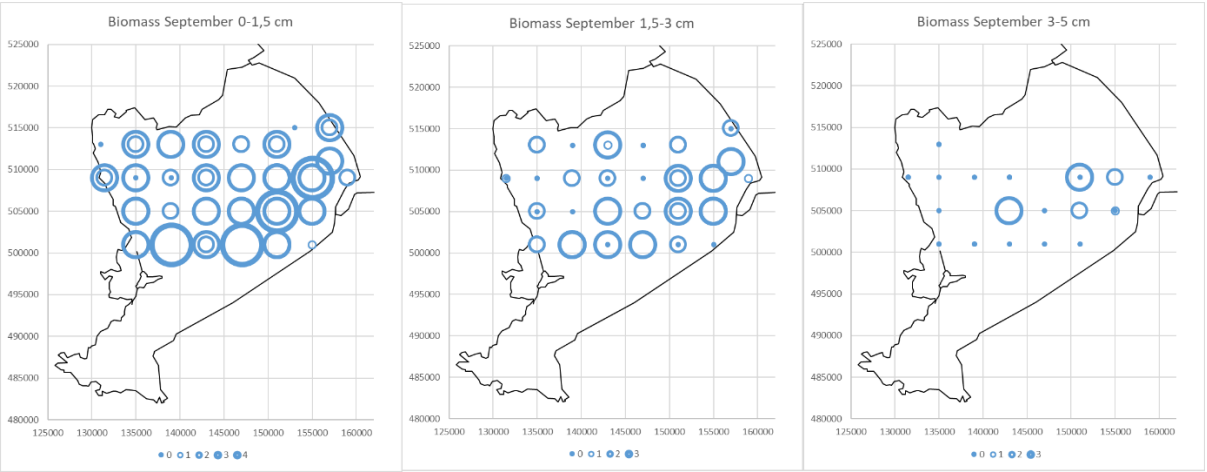

Figure 135: The found biomass classes and spatial distribution of the sampling round in September in increasing depth

During the second round of sampling, less locations were sampled. The total biomass at the resampled locations increased. This increase was mostly visible in the concentration area in the south-eastern gradient, although not at all locations. In most other parts of Lake Markermeer, a slight decrease in biomass can be seen. Noteworthy is that the decrease in depth is lower. The amount of biomass in the second and third layer on the sampled locations is higher than during the first round. Below the 3-5 cm layer no bacteria were found, in accordance with the first sampling round (Figure 16).

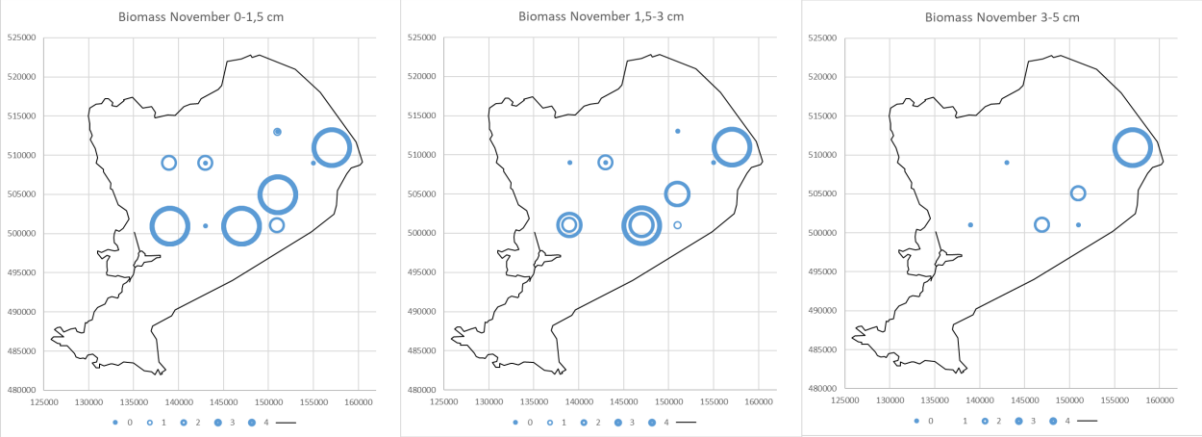

Figure 146: The found biomass classes and spatial distribution of the sampling round in November in increasing depth

In Table 1 the average weight of each category is shown. This weight was calculated by averaging the weight of several unfouled samples. Based on these weights an average weight per metre squared was calculated to give an indication of the total biomass. The biomasses per location were calculated from these average weights corrected for the subsampling. The biomasses that are calculated are higher than those of the coast of South Amerika. The measured biomasses are more than 70 times as high as the fish biomass found in Lake Markermeer (Noordhuis, 2019). Calculating the biomass of the same locations for the two measurement rounds, the biomass increases significantly. The average increases from 284 g/m^2 in September to 475 g/m^2 in November.

Category	Average weight (g)	Weight per m2 (g)
1	Below measurement limit	-
2	0,07	26,48
3	0,25	89,26
4	1,39	491,45
Highest biomass found for an entire tube (measured)	5,47	1934,62

Table 1: The found biomass weights per category

When looking at the specific locations, the increase of biomass is a lot different (Figure 17). At some locations there is a decrease to a disappearance of the bacteria, while at other locations there is a significant increase. As mentioned before, the locations where the biomass increased are in the concentration area whereas the locations with a decrease of biomass are found in the other parts of the lake. In this concentration area there are multiple samples which had a weight of around 700 gm^{-2}. This suggests that in Lake Markermeer, the optimum biomass of *Thioploca* spp. lies around this value.

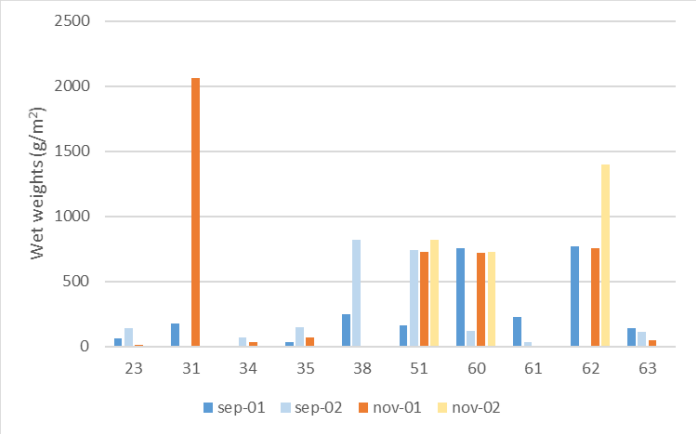

Figure 157: The biomass weights per location for both measurement rounds

Pore and surface water

The porewater concentrations of the different elements and nutrients show either increasing or decreasing concentration in depth depending on the compound being monitored. In the graphs below, the amount of elemental S can be seen for both measurement rounds. There is not much difference in the porewater concentrations between both periods. In the two top layers, the concentrations lie around 20-30 mg/l for most locations. There is a steep drop in concentration on the 5 cm depth samples. This could explain the lack of bacteria in the deeper sediment layers of Lake Markermeer. This decrease in concentration is also visible in the sulphate layer, although the values show higher concentrations.

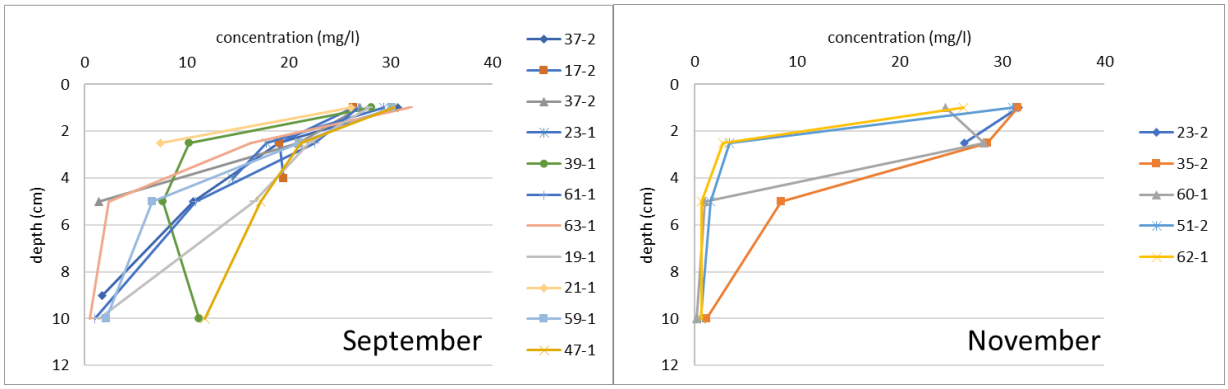

Figure 16: Pore water concentrations of elemental S after filtration

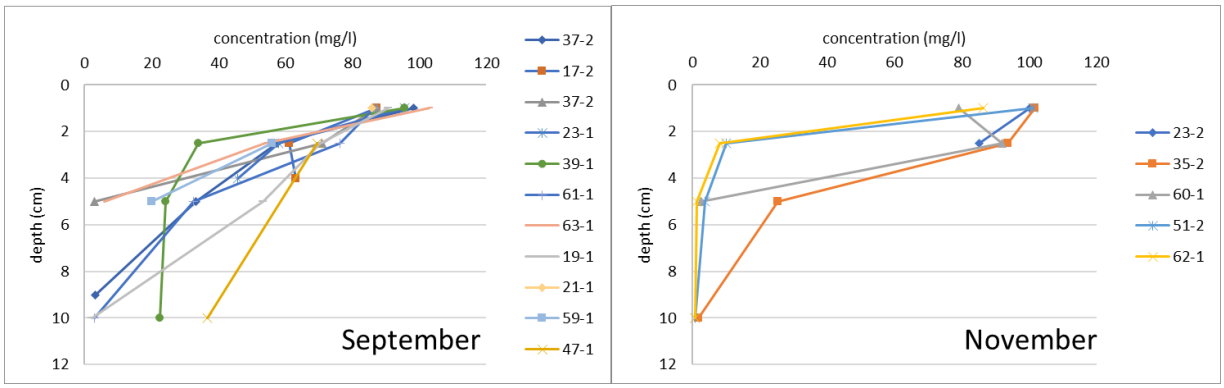

Figure 17: Pore water concentrations of sulphate after filtration

The concentration of elemental P shows an increase in depth. There is not a clear pattern that can be linked to the presence of sulphur bacteria. However, there is a relation between S and P. At higher concentrations of S, lower concentrations of P are expected due to redox reactions of S and the insensitivity to redox of P. This is also the case in these measurements, thus partly validating the data. The concentrations for phosphate do not show clear results, most of the samples where below measurement detection limit (0.93 mg/l) or not analysed and are therefore not shown. The values shown in figure 20 should be considered to be dissolved orthophosphate as the sample has been filtrated and measurements were taken from the pore water.

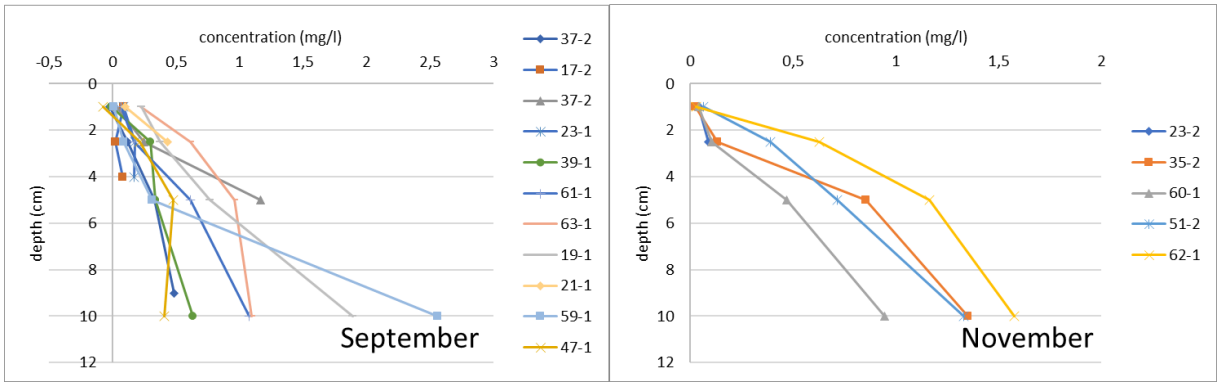

Figure 18: Pore water concentrations of elemental P after filtration

Nitrate and Nitrate porewater measurements did not show clear results. Most of the data was below measurement detection limit (0.0084 mg/l and 0.4654 mg/l respectively) or not analysed. Ammonium however, shows an increase in depth. This could indicate that the sulphur bacteria do not produce ammonium in large amounts. At location 62-1 from the November measurements, very high amounts of ammonium were measured at a depth of 10 cm.

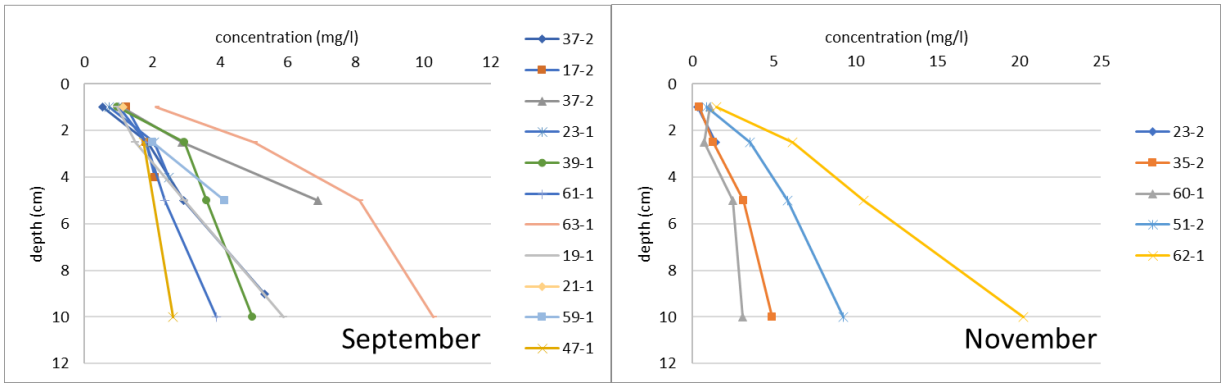

Figure 19: Pore water concentrations of ammonium after filtration

When looking at the surface water (Figure 22), there is a concentration area of nutrients in the south-eastern part of the lake which resembles the concentration area of the bacteria (Figure 10). Both phosphor and sulphur concentrations are high in these areas. This could be related to the building activities at Marker Wadden or be part of the natural cycling of nutrients in the clockwise pattern that is the main flow. The concentration part is very similar to the presence of sulphur bacteria but cannot be directly linked to their presence.

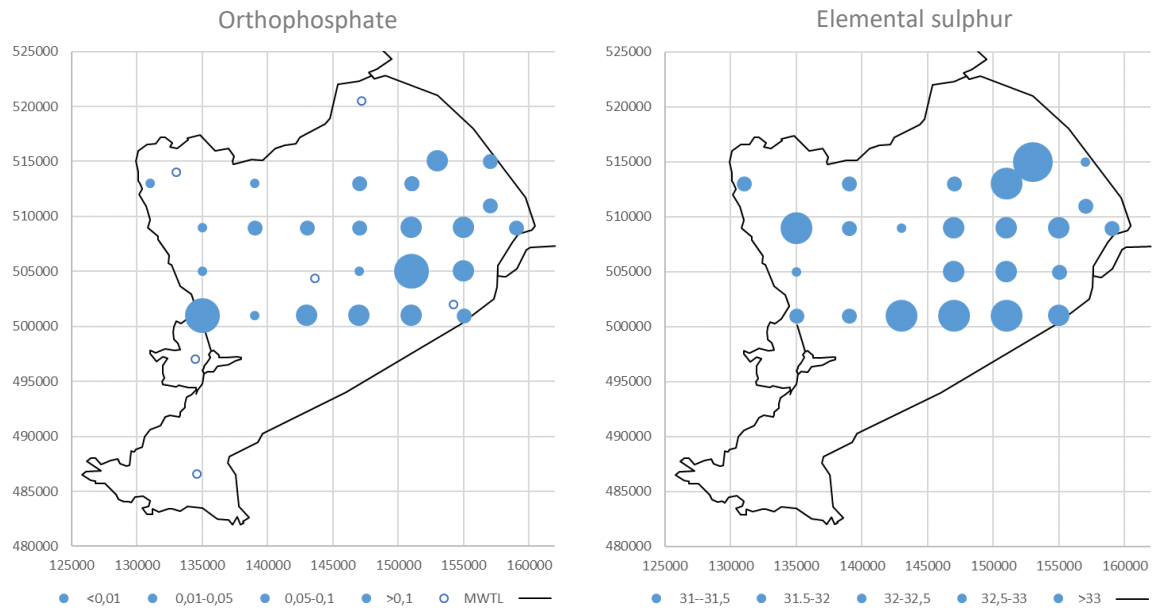

Figure 20: Spatial distribution of orthophosphate and elemental sulphur in the water column

Thermogravimetric analysis

Below a graph (Figure 23) can be found on the weight loss of multiple samples analysed in the TGA. This graph shows the temperature at which the weight loss is measured. These measurements were done for all locations. From this, the percentage of organic matter (OM) and chalk ($CaCO_3$) can be calculated for each location.

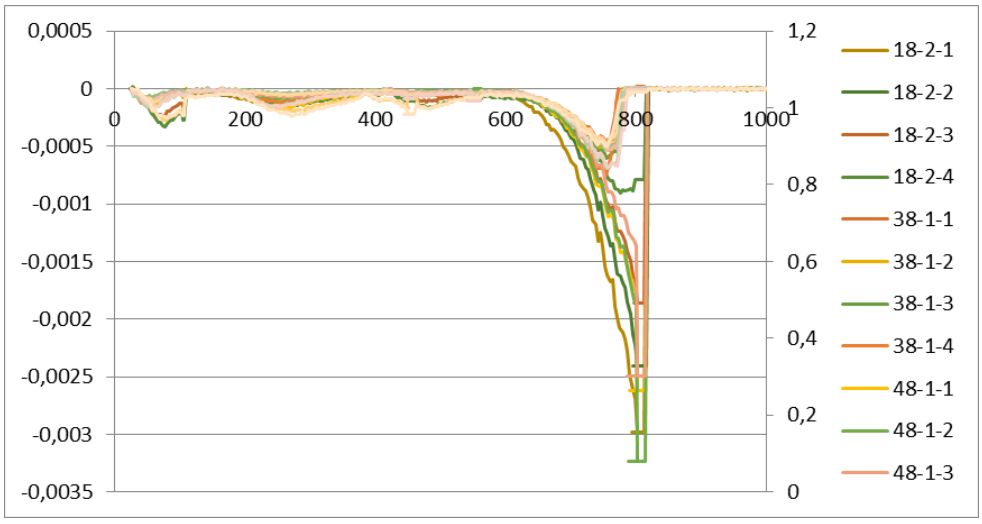

Figure 21: An example graph of the weight loss per location from the TGA

The calculated values can be found in the figure 24. At all locations, $CaCO_3$ was found. This indicates the presence of shell material. Some locations show a significantly higher amount of $CaCO_3$. The percentage of organic matter found, is relatively low. The low amounts of organic matter show that the sulphur bacteria do not need large amounts of organic matter to grow. At all locations, organic matter and $CaCO_3$ was found.

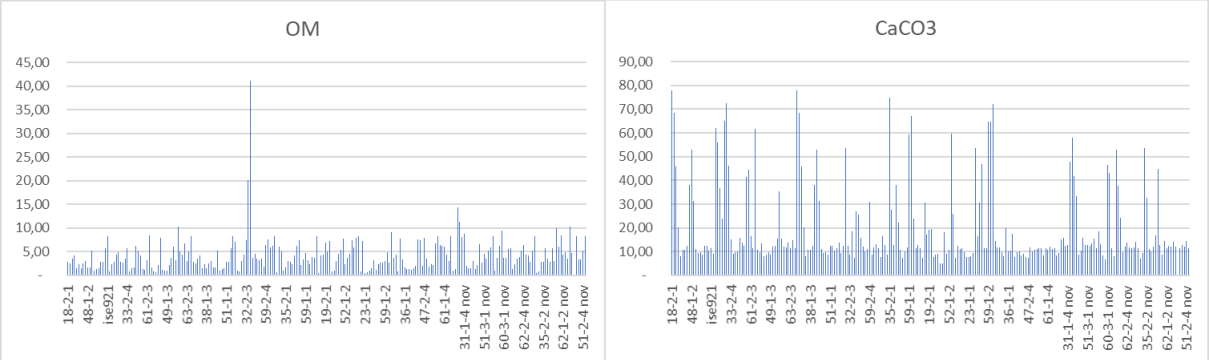

Figure 22: The graphs show the amount of OM and $CaCO_3$ per location

The organic matter is highest in the western part of the lake. This is as can be expected as most of the water plants grow in this area. The amount of organic matter in the concentration area of the sulphur bacteria is not high.

Production measurements

Production measurements were conducted by the University of Amsterdam. The Sediment Oxygen Demand (SOD) was measured to approximate the benthic production (Figure 25, middle picture). This is relevant to determine if, as stated in literature, the sulphur bacteria prevent other benthic species from living in the same environment. These measurements were taken from the samples taken in September. For this period, high production is expected as it is the growing season. In Lake Markermeer however, the SOD is low compared to other lakes and coastal areas. Although the found SOD values are low compared to other areas, the most activity was measured in the area in which the most bacteria are present. This suggests that the primary production is not inhibited by the presence of the sulphur bacteria.

The O_2 production is less clear (Figure 25, right picture). There are some locations which show higher production, but these cannot be directly translated into benthic production. The samples for the production measurements were kept in a light environment, while the natural situation is dark as light cannot reach the sediment. This means that the value could be higher due to the settled algae from the water column. The chance that there are settled algae on the lake bottom is very high due to the turbid nature of Lake Markermeer. The sediments flocculate often with the suspended algae causing them to settle. Both these graphs have no relation with the production of sulphur bacteria, assuming the bacteria are not using oxygen as electron acceptor. The spatial distribution of the production follows the same south-eastern gradient as the sulphur bacteria do.

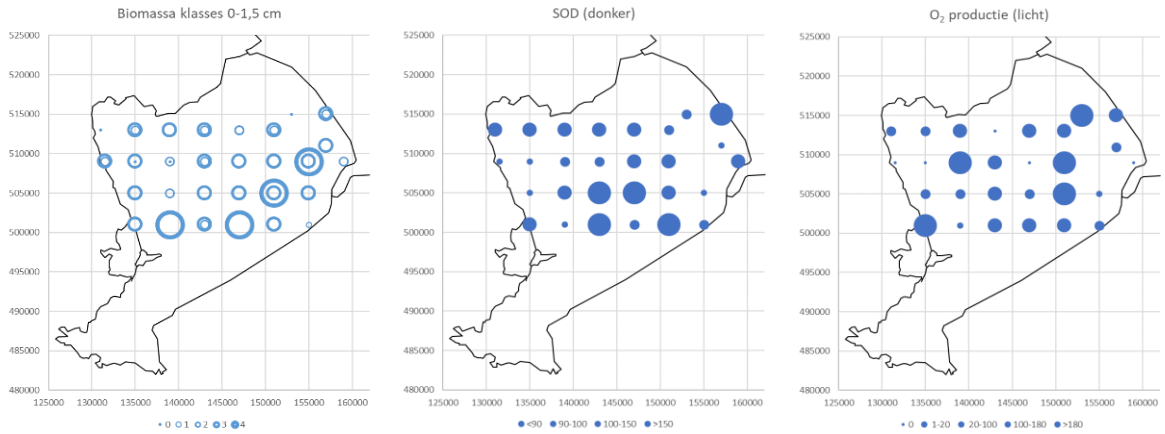

Figure 23: Spatial distribution of the biomass in the top layer of the sediment (left; see Figure 15), compared with the spatial distribution of Sediment Oxygen Demand (O_2/m^2/hour) in dark conditions (middle) and the O_2 production (O_2/m^2/hour) in light conditions (right). Data gathered from the first sampling round

Resuspension experiment

Figure 26 shows the results of the resuspension experiments per biomass category. From these graphs it becomes apparent that there is an effect of the presence of sulphur bacteria on the resuspension sensitivity of the Lake Markermeer sediment. The figures show the response of the suspended matter concentration to a stepwise increase of turbulence. In category 0 (no visible bacteria) the resuspension of the sediment starts earlier in comparison to the higher categories. The slope of the graph is steeper, this indicates a more abrupt resuspension of the sediment. The samples from category 2 start resuspending later, but with a similar slope to the category 0 samples. One sample does not follow the same slope and fits more in the category 3 graph. In category 3 the resuspension starts marginally later then the category 2 samples. The slope however, is a lot less steep. This indicates that the sediment is being held in place by the bacteria and more force is needed to suspend the same amount of sediment. Category 4 shows a less clear pattern. Three samples show the trend as seen in the other categories, the resuspension of these samples start later. In contrast to the other samples, these show clear peaks at which moment the mat was resuspended and hit by the rotor. The other two samples in this category start relatively early and do not follow the expected slope. For one of those samples it was noted down that a hole was developed in the top layer which most likely did not contain any bacteria. This influences the measured data but does not explain the earlier peak.

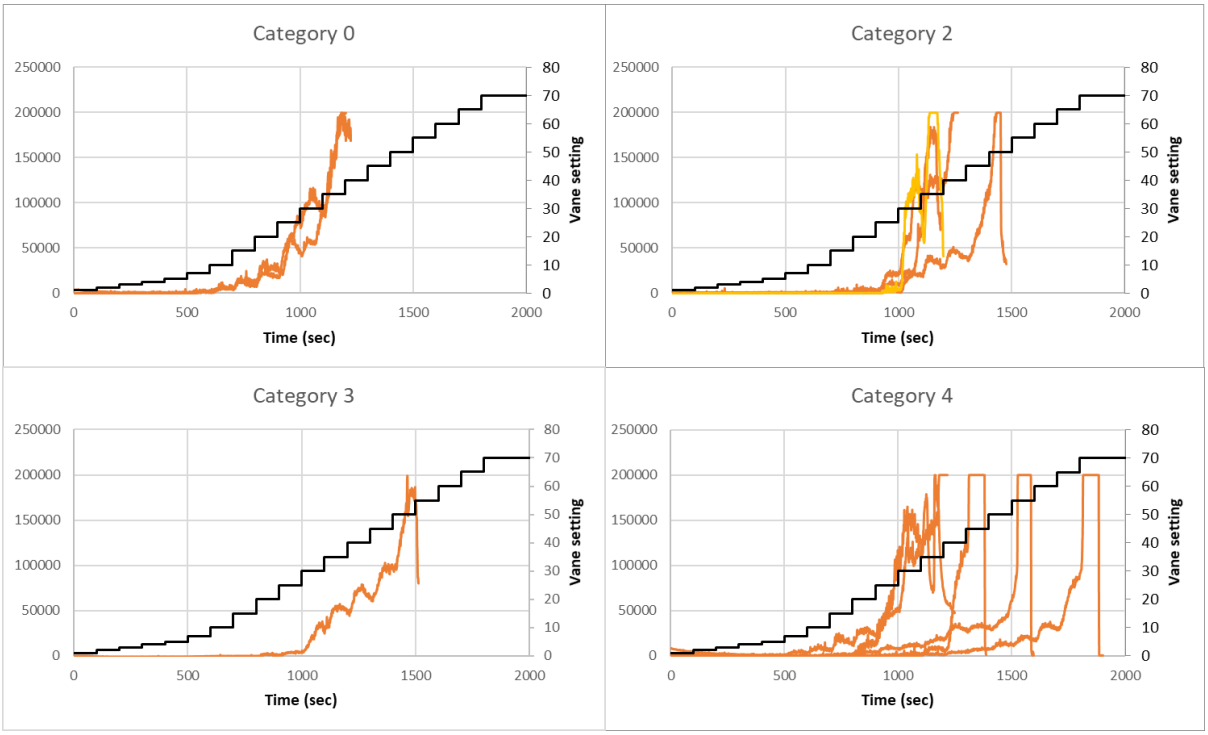

Figure 24: Shown above is the resuspended sediment in time. In black, the Vane setting is shown which can be related to wind speed

When looking at the critical shear stress, the first moment of resuspension, a relation between the amount of bacteria present and the moment at which the sediment starts resuspending can be seen (Table 2). By combining this table and the amount of bacteria found in these samples a relation can be found. When the bacteria are present in higher amounts, the critical shear stress is calculated to be higher as well in most cases.

Critical shear stress (Pa)	31-2	20-1	64-2	39-2	62-2	18-1	48-2	63-1 nov	34-3 nov	31-3 nov	61-1 nov	38-3 nov
oslim1	0.404	0.101	0.101	0.101	0.050	0.050	0.101	0.632	0.050	0.404	0.101	0.050
oslim2	0.404	0.404	0.632	0.101	0.404	0.101	0.025	0.632	0.050	0.404	0.101	0.050

Table 2: The critical shear stress (Pa) is shown for the two OSLIM measurements

Statistical analysis

Statistical analysis (Excel correlation coefficient and Spearman's Rho) was done to determine the relations between the nutrients and the presence of sulphur bacteria on the different locations (Table 3 and 4). For each of the nutrient concentration data sets, correlations were tested on significance. The porewater nutrient concentrations show multiple significant relations. Only phosphate and sulphate are not significant in the first measurement and for the second measurement only nitrate is considered not significant. This shows that the nutrient concentrations are closely linked to the presence of sulphur bacteria.

Correlation coefficient	r^2	p(2-tailed)	Element/ nutrient	Significant
0,03439	0,73333	0,00010	S	yes
0,08644	0,70115	0,00028	P	yes
-0,04351	0,68890	0,00039	Ammonium	yes
-0,42578	0,68890	0,00039	Phosphate	yes
-0,20605	0,54879	0,00905	Sulphate	yes
0,07591	0,22364	0,31708	Nitrate	no
0,13530	0,53345	0,01057	Nitrite	yes

Table 3: Statistical correlations of pore water nutrient concentrations and the number of bacteria present for the first measurement round

Correlation coefficient	r^2	p(2-tailed)	Element/ nutrient	Significant
-0,88287	0,67273	0,03304	S	yes
0,68271	0,7842	0,00725	P	yes
0,79732	0,93939	5,00E-05	Ammonium	yes
-0,48216	0,16387	0,65101	Phosphate	no
-0,87835	0,56364	0,08972	Sulphate	no
-0,38174	0,66262	0,03681	Nitrate	yes
-0,62055	0,81278	0,002426	Nitrite	yes

Table 4: Statistical correlations of pore water nutrient concentrations and the number of bacteria present for the second measurement round

The surface water correlations show similar relations to the pore water (Table 5 and 6). Most of the tested nutrients show a significant relation to the presence of sulphur bacteria. In this case only in the first round there are non-significant relations present. This is for S and nitrate. For both the pore water and the surface water correlations however, the correlation shows some discrepancies. For example, the correlation of ammonium is positive in the first round and negative in the second round. This shows that the correlations, even though they are considered statistically significant, do not give a clear result.

Correlation coefficient	r2	p(2-tailed)	Element/ nutrient	Significant
0,15041	0,6274	0	S	yes
0,43725	0,58319	1,00E-05	P	yes
0,25672	0,57667	1E-0,5	Ammonium	yes
-0,26833	0,4914	0,00029	Sulphate	yes
-0,09874	0,65426	0	Nitrite	yes

Table 5: Statistical correlations of surface water nutrient concentrations and the number of bacteria present for the first measurement round

Correlation coefficient	r^2	p(2-tailed)	Element/ nutrient	Significant
0,02923	0,42445	0,06214	S	No
0,05975	0,49854	0,02526	P	yes
-0,49266	0,51548	0,02001	Ammonium	yes
-0,02409	0,662	0,00147	sulphate	yes
-0,44432	0,32142	0,16701	Nitrate	no
0,56793	0,4574	0,04259	Nitrite	yes

Table 6: Statistical correlations of surface water nutrient concentrations and the number of bacteria present for the second measurement round

The other statistical analyses are not shown in this chapter. It is worth noting that there is also a statistically significant relation between the amount of *Thioploca* spp. and *Beggiatoa* spp. present for both measurement rounds. This relation is of importance as the structure of the mat changes when both bacteria are present.

Discussion

The wet weight biomass values in Lake Markermeer are higher than initially expected. A reference location of the coast of South-Amerika has a biomass that was calculated to be a lot lower over multiple years. The highest average wet weight biomass value found over these years of measurement is around 130-140 gm^{-2} (Montecino, et al., 2006). The average wet weight of the September measurement is 284 gm^{-2}. This is double the amount found in the year with the highest biomass for the comparison situation. During this research, the reason for this difference is not found. Schultz et al. (1996) have shown that of the coast of Chile a maximum value of up to 800 gm^{-2} was found. This value corresponds better with the highest value found in this research It should be noted that the method of determining the biomass is based on the amount of *Thioploca* spp. present. The *Beggiatoa* spp. strains are too small to filter out of the sediment and are flushed away for the most part. The remaining *Beggiatoa* spp. is also not taken for the categorization as they are too small to see with the naked eye. The remaining amount is taken in the weight calculation but contributes very little due to the small mass of a single strain. In unpublished results of a macroinvertebrate survey conducted in 2016, it becomes apparent that the sulphur bacteria biomass values are also higher than the researched macroinvertebrates species such as mussels and worms. The highest biomass found in this research is 167.8 gm^{-2} for the *Dreissena* mussel (Verdonschot, 2019).

The microscopy samples show a very similar result as the biomass indication. The spatial patterns are similar for the most part. There are some discrepancies between the results that should be addressed. The classes of filament abundance are different from the biomass samples. Part of this can be explained by the aforementioned method from the biomass determination in which only the *Thioploca* spp. strains are taken as biomass. However, the amount of *Beggiatoa* spp. strains does correspond well with the found biomass measurements even though they are not fully measured in the biomass determination because of their relatively low weight compared to *Thioploca* spp. This indicates a relation between the amount of *Beggiatoa* spp. and *Thioploca* spp. This relation is considered statistically significant ($p = <0.05$) for both measurement rounds. Within the sampling locations, there are some differences as well. Some samples show a class difference at the same measurement location at the same sample moment. This could point to a large spatial difference. The differences are not big enough to change the main observations of the concentration area (Figure 13 and 14). The increased depth at which the bacteria were found was not expected. Due to more frequent storm events in the autumn season, it was expected that the mats would be disturbed. The reason for this increase in bacteria is unknown and should be further investigated as in other areas a seasonal pattern was found (Schultz, B.B., Fossing, & Ramsing, 1996).

The pore water nutrient concentrations found show most differences in the top 5 cm of the sediment. This fits with the depth at which the sulphur bacteria were found. For most nutrients a statistically significant relation was found with the presence of nutrients. The nutrients shown in the result chapter show the nutrient values increasing or decreasing as expected in a natural system. For part of the interest nutrients however, the measurements of the IC were not useful. The measurements showed a below measurement limit or where not analysed. This can be partly explained by the exposure to air by the extraction and the filtration. This can change the nutrient composition very quickly, even though the samples where conserved as best possible. In the surface water, the measurements show a clear pattern that fits to the concentration area of the sulphur bacteria very well. After correlation calculations however, it is shown that the values found do not show a statistically significant relation to the presence of bacteria. This shows that the nutrient levels in the surface water are of less importance to the sulphur bacteria then the pore water nutrient concentrations. There are however, some contradictions in the statistical analyses that were conducted. The example provided is

ammonium, which correlates positively during the first measurement round while the correlation becomes negative in the second measurement round. These results should therefor be used with care and further research should be conducted to determine if this was a measurement fault or if the bacteria change their conversion over time.

There might be a relation to the heightened nutrient concentrations caused by the constructions taking place at Marker Wadden. Prior to the construction activities on and around Marker Wadden there are no measurements of nutrient concentrations that show, in detail, the distribution of the nutrients. There are some MWTL measurements at set locations that show the nutrient concentrations over time, but these are not located in the main plume that can be seen in the measurements that are taken during this research (Figure 22). It is therefore not clear if the Marker Wadden are the cause of the heightened nutrient concentrations in this area, or if this is a phenomenon that has been present for a longer period of time.

The thermogravimetric analysis shows a very similar pattern for all locations, there is no difference between the two measurement rounds. The organic matter values are not found to influence the presence of the bacteria in Lake Markermeer. This could indicate that organic matter is not a limiting factor for the growth of sulphur bacteria in Lake Markermeer. The presence of $CaCO_3$ is also not correlated to the presence of the sulphur bacteria.

The production measurements that have been done approximate the production at each location. The measurements are based on the oxygen production and consumption in light and dark conditions. This means that the measurements do not show the production of the sulphur bacteria as they do not use or produce significant amounts of oxygen. The data that is produced by these measurements is therefore a measure of the production and consumption of all other organisms in these samples. During the sampling, the samples have been exposed to light which might have influenced the measurements. In addition, the oxygen production measurements were done in light conditions. During normal conditions, the lake bottom is not exposed to light which could influence the accuracy of the data. In Lake Markermeer, there is an additional factor that influences the measurements. The floating algae flocculate, which results in the sedimentation of these algae. This means that the suspended algae on the lake bottom produce oxygen in light conditions and consume oxygen during dark conditions. This could also have influenced the measurements. These measurements should therefor only be used as an indication of the total production and sediment oxygen demand.

The resuspension experiments show a clearer result. The effects of the sulphur bacteria on the resuspension are clearly visible. When more bacteria are present, there is a decrease in the amount of sediment that gets resuspended by turbulence. Part of the measurements do not fit into the trend perfectly. This could be influenced by difference in mat structure due to the ratio of *Beggiatoa* spp. and *Thioploca* spp., but this cannot be confirmed. There could also have been a difference in the sediment composition. For sample 64-2 a fault was found in the categorization of the sample (yellow line in Figure 26). This sample had been categorized as a category 0 sample (no bacteria present), whereas the experiment notes and pictures show that there were bacteria present in this sample. For this reason, the sample has been treated as being category 2. This is also confirmed by the experiment results, as the critical shear stress (Table 2) and sediment resuspension graph (Figure 26) match this category very well. Looking at the category 4 samples, there are three samples that show a very sudden increase in turbidity. During the experiments, it was seen that these samples formed a dense mat that was resuspended as a whole that was broken down after only after hitting the rotor. In natural situations, these mats are likely to resuspend even later as they form a bigger mat that will have more weight and a larger surface area. In addition to this, it is very likely that the PVC tube will have influence these samples to some extent as well. When sampling, part of the bacteria on the sides

have been sliced which negatively influence the mat structure. Furthermore, the smooth surface of the PVC pipe might have allowed the sides to be eroded more easily. There are some other limitations of this experiment; the vane rotation speed is set in steps, this means that there is a sudden increase in rotor speed which might influence the experiment. In addition, the rotor hangs above the sediment. The effect of the flow of the rotor compared to the flow caused by wind and current is different. In natural situations, the flow is not gyrating but more perpendicular to the wind speed. The rotor may also have sped up the increase in turbidity by hitting larger sediment particles that deposit rather quickly again in natural situations into smaller particles that stay suspended and cause higher turbidity. For the critical shear stress calculations, there are some additional limitations. The critical shear stress measurement calculates the first moment of resuspension. In Lake Markermeer, there is a layer of sediment on top of the bacteria which is not (significantly) influenced by the bacteria present in the sediment. This sediment will be resuspended at normal rates. This means that the influence of the bacteria is not accurately pictured in the critical shear stress calculation. This could explain that some of the samples are calculated to have a lower critical shear stress while containing more bacteria. To see the effects of the bacteria more accurately, it could be better to determine the point at which a significant increase in turbidity is measured in the graph. By doing this, the layer on top of the bacteria is not taken into consideration. When doing this, there are still two samples which do not fit the expected order. This could be explained by the above-mentioned difference in mat structure.

Conclusion

At the start of this research, RWS-WVL and KIMA posed research questions that were meant to increase the knowledge on (filamentous) sulphur bacteria and their effects on Lake Markermeer and to further investigate the working of the system. In this chapter these research questions are answered to fill this knowledge gap.

The determination of the spatial distribution of (filamentous) sulphur bacteria in Lake Markermeer shows that the bacteria are present at all sampling locations. There is a spatial pattern in which there are more bacteria present in the south-eastern part of Lake Markermeer. This pattern is similar to the pattern found for nutrient concentrations and primary production. Both measurement rounds show the same pattern, and in both cases the bacteria where present at all locations. The pattern was found to be more present in the second sampling round. This is a strong indication that the bacteria can be found everywhere in Lake Markermeer. Finding these bacteria at all locations makes the effects these bacteria have more significant.

The influence of (filamentous) sulphur bacteria on the behaviour (resuspension) of sediment has been investigated. There is a relation between the amount of bacteria present and the resuspension rate of the sediment. The tested samples with lower amounts of bacteria showed a higher sensitivity to turbulence. The samples with the high amounts of bacteria showed a mat formation which held the sediment together until being hit by the rotor blade. The lowered effects of turbulence could positively influence the growth of water plants and algae in the eastern part of the lake. If these plants get the opportunity to settle in these areas, there will be many positive effects for Lake Markermeer. The fish population would most likely increase, and the nutrient balance would become more natural. In the current situation, the effects of the bacteria on the total sediment resuspension rate are not clear, but by implementing the shear stress values that have been found into the silt model, the effects can be assessed. It has been shown that on the tested locations, the resuspension is positively influence by the sulphur bacteria.

The sulphur bacteria have been found in very high amounts, the average wet weights that have been measured are significantly higher than those found in other places and of other genera in Lake Markermeer. This indicates a high contribution to the primary production in Lake Markermeer. During the research it was found that there is no apparent negative effect on the growth of other organisms. Multiple other organisms where found in the samples that contained sulphur bacteria. The primary production of other organisms based on the oxygen production and consumption, as measured by the University of Amsterdam, confirms that there are no negative effects of sulphur bacteria on other organisms as the highest production measurements are at the same locations as the highest bacteria concentrations. During research conducted by Witteveen&Bos it has become apparent that the sulphur bacteria are not being consumed by four fish species of whom the stomach contents have been investigated. This indicates that the bacteria are not available as a food source for other species. If this is the case, then a very significant part of the primary production of Lake Markermeer is not available for other organisms. This could be complemented by the fact that the feeding of the fish species is limited as they cannot feed on mats. The algae and other organisms that are living intertwined within these mats could therefore be unreachable for the fish and become unavailable to the food web.

When looking at the relation between nutrients and sulphur bacteria, there are statistically significant relations. However, the nutrient concentrations found are not different than expected compare to samples that do not contain sulphur bacteria. This shows that there are no significant impacts on the nutrient availability. Although the bacteria store large amounts of nitrate and elemental sulphur, this

does not appear to influence the surface water or pore water nutrient concentrations. This could be due to the fact that the highest amounts of bacteria are found in the concentration area of nutrients that has been found. As it is unclear when the bacteria first settled in this area, the current nutrient values cannot be compared to a reference situation. Measurements on the nutrients that have been stored in the vacuoles were not possible during this research. When looking at the organic matter contents of the samples, no relation was found. The concentration area of the organic matter is also not connected to the concentration area of the sulphur bacteria. The main masses of organic matter were found in the western part of the lake. In these locations, the plant growth is most significant and can explain the high percentage of organic matter found. It could not be shown that the sulphur bacteria have any effects on the presence of oxygen or stratification. Measurements on the top layer of the sediments were not possible and thus no microstratification could be determined. In the surface water stratification was not measured.

In conclusion, there are significant effects on the resuspension of sediments caused by sulphur bacteria. This effect is present in the whole of Lake Markermeer as the bacteria are present everywhere although in different densities. The presence and abundance of the sulphur bacteria at all locations also means that a significant part of the primary production is taken up by the sulphur bacteria. Furthermore, there are relations between nutrients and the presence of sulphur bacteria, but these do not influence the availability for other species in a significant way. It has also been concluded that there are no clear relations between the presence of organic matter or the concentrations of oxygen to the sulphur bacteria.

An Eco-Technological solution

As part of this research it has been investigated if the sulphur bacteria can be used to limit resuspension in natural systems. As mentioned before, there are significant effects on resuspension caused by these bacteria. This shows that when large amounts of bacteria are present, the effects of turbulence are mitigated by the bacteria. The resuspension of a lake bottom could be significantly decreased if the amount of bacteria is high enough. As of this moment, it has not yet been possible to cultivate these bacteria. Because of this they cannot be used as a solution for the resuspension of sediment in locations that do not contain these bacteria yet. It is also unclear what the exact needs of the bacteria are, meaning it cannot be determined if the target area is appropriate for this approach. The solution is therefore not yet implementable but shows promising possibilities.

Recommendations

It is strongly recommended that more research is conducted on the processes that are occurring during the conversion of sulphur. This could be done by r-DNA analysis to determine the active enzymes. By researching these processes, it can become more clear what requirements there are for the cultivation of these bacteria. Additionally, the effects on nutrients can be better measured as the active enzymes are known. This will make it possible to determine which nutrients are being used by the used strain of bacteria. It then becomes possible to determine if an area is suitable for the growth of sulphur bacteria.

There is also need for more research on the availability of the bacteria for the food web. Since it has been determined that a large part of the primary production is taking place in the form of sulphur bacteria, it is important to determine if the bacteria have negative effects on the food web. This can be done by extending the number of species of which the stomach contents are determined. It will then become clear if the sulphur bacteria are being eaten.

The cultivation of these bacteria should also be researched. These bacteria could be used in certain situation to lower the turbidity in a highly dynamic (anoxic) lakes or possibly even rivers. As it is not yet clear what these bacteria need for survival and growth, this would be the first step for the cultivation process.

More research should also be conducted on the relation between the heightened nutrient concentrations in the area below Marker Wadden. This to determine if the constructions on and around Marker Wadden influence the nutrient concentrations or if this is a natural process. There are however, no detailed measurements from before construction on and around the islands started. This makes it difficult to determine if this process is only present due to the building activities or if this is a process that has been present for a longer period of time. It is also unclear how this has influenced the presence of bacteria in this area. To further the understanding of Lake Markermeer and the sulphur bacteria, determining the effects of this disturbance could be key to understanding the system. To accomplish this, new measurements should be taken when the effects of the construction are not present anymore.

To better understand the effects of the sulphur bacteria on the resuspension of sediments, additional experiments should be conducted. The method that has been used in this experiment is considered accurate but with large limitations. In this experiment the Vane uses a rotor to get the water in motion, this motion is not as would be in a natural situation. Changing the apparatus could therefor increase the accuracy. In addition, it is recommended that the tube width is increased. This will ensure a less disturbed sample. Furthermore, the effect of the PVC tube will be lower. This will ensure a more accurate result can be implemented in the model and be used for further research in using the bacteria as a measure against resuspension.

It is also recommended that more knowledge is gathered on the seasonal pattern of the growth of these bacteria. The results samples were collected late summer and early autumn, it is also of importance to determine the presence of the bacteria during spring changes due to winter storms and temperature differences. This could provide evidence that the seasonal pattern caused by winter storms and lower water temperatures is also present in Lake Markermeer. Additionally, the long-term presence of the bacteria should be further investigated. The bacteria were found in lower amounts sporadically in the past. While at this moment they are present in significant amounts across the whole of Lake Markermeer. To determine if the found effects of these bacteria are long term of short term, a monitoring program should be set up. To gain the best results, it is proposed that the monitoring

will be done once every year at five locations spatially distributed across Lake Markermeer to determine the increases or decreases of biomass over the years.

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Appendices

Appendix I: Measurement plan

The following measurement plan was written for the field and lab work. This is based on the proposed samples that are to be taken and in cooperation with the client.

During field days:

Measurements O2, pH and temperature in vertical gradient.

Samples with the Uwitec:

- On mussel collection grid 59-64 (6), 39-32 (8), 47-52 (6), 31, 16, 17, 23-18 (9). Total locations 29.
- 3 cores per location
    - Core 1: Biomass (all locations), part in layers, pore water and TGA (all locations) and flora fauna determination (all locations)
    - Core 2: Resuspension experiment (25% of samples) and biomass (75% of samples)
    - Core 3: Production and DNA analysis, can most likely be put in jars

Water samples:

- Take a water sample at every location
    - Standard anions and cations

Lab work (in order of what will be done first):

Core 1:

Take porewater samples (10 cores, 4 per core)

- Must be done within 24 hours
- Samples at 1, 2.5, 5 and 10 cm
- Timeframe: Half a day
- Deliver samples for ion chromatography analysis + subsample (acidified) for ICP-OES (total element concentrations)

Core 2:

- Split the cores for biomass (2B) and resuspension experiment (2R) (75/25%)

Core 1 and 2B:

Divide (part of) the samples in thinner layers (1.5 cm, 1.5 cm, 2cm, 2.5 cm, 2.5 cm and the other layers 5 cm)

- Timeframe: 1 day
- Weigh total sample,

- take subsample for TGA and place in stove to dry.
- Subsample for determination of biomass.

Core 1 and 2B:

Determination of biomass

- Wash them
- Weigh them
- Timeframe: 2 or 3 days

Characterizing benthic flora and fauna

- Determine different species
- Determine the dominant species
- Timeframe: 1 or 2 days depending on amounts of species found

Core 2R:

Resuspension tests

- Perform the test
- Timeframe: 4 days
- Determination of biomass of 2R samples
- Wash them
- Weigh them
- Timeframe: 5 days

Core 1:

Analyse porewater samples and TGA

- Timeframe: + - 2 weeks

Analyse ICP-OES

- Timeframe: + - 2 weeks

Core 3:

Production measurements

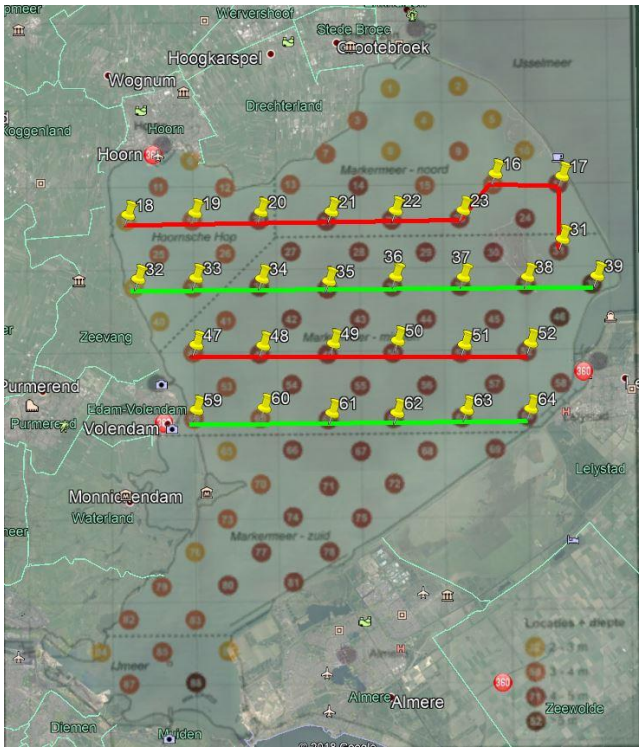
- Timeframe: 2 weeks

DNA analysis

- Timeframe: 2 weeks

Water sample analysis

- Timeframe: + - 2 weeks



Day 1 (green):

Raai 1: 59-64 (6)

Raai 2: 39-32 (8)

Day 2 (red):

Raai 1: 47-52 (6)

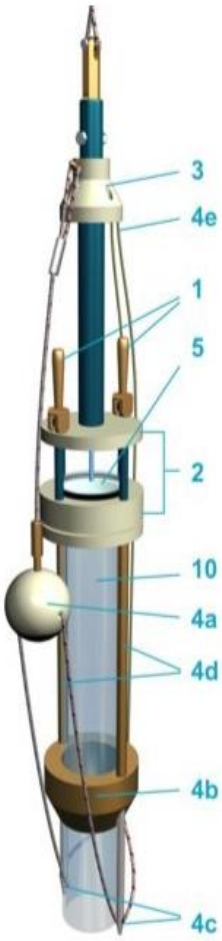
Raai 2: 31, 17, 16 ,23-18 (9)

Appendix II: Manual for the Uwitec

CORER

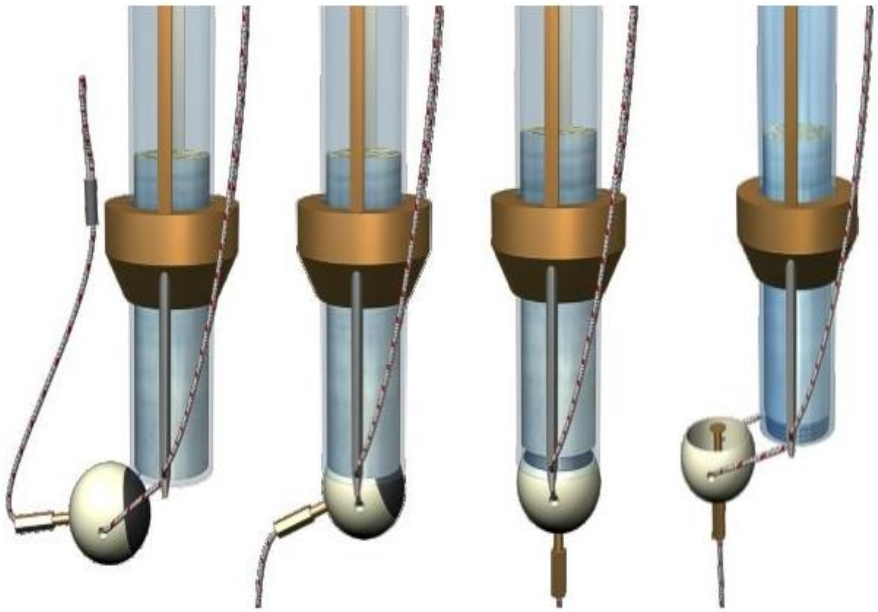
Uwitec Corer

- Equipment for easy sediment sampling in deep and shallow water.
- Special core catcher (ball), works automatic without faulty messenger.
- Easy changing of the sampling tubes by quick fastener.
- Modular construction allows an easy adaption to different sediment types and waters.
- All parts are made from high quality and non corrosive materials.



1	*Tighten levers*
2	*Head of corer with quick fastener by tighten levers*
3	*Suspension with automatic release of the core catcher*
4	***Automatic core catcher with basic weight***
4a	*Ball with release line*
4b	*Basic weight*
4c	*Tips with eyes for the release string*
4d	*Distance bar*
4e	*Rubber loop*
5	*Plexiglas valve flap*
6	*Steel pipe guide to be used with extra long core tubes*
7	*Telescop bar (3 aluminium tubes) To push the corer into the sediment by hand (max. 5 m water depth)*
10	*PVC tube crystal clear 59,5 mm or 86,0 mm inside diameter, length 60 cm or 120 cm*

See Video here



HAMMER ACTION

Aditional weights with two ropes connected to the whinsch cable makes possible to "hammer" the Corer into the sediment by raising and dropping! Arbitrarily long standard linertubes up to 3m length. Up to 6 aditional weights(a'4kg) and/or 30kg total weight.

See the Video here

Uwitec grafity corer with hydraulic core catcher (Grafity corer)

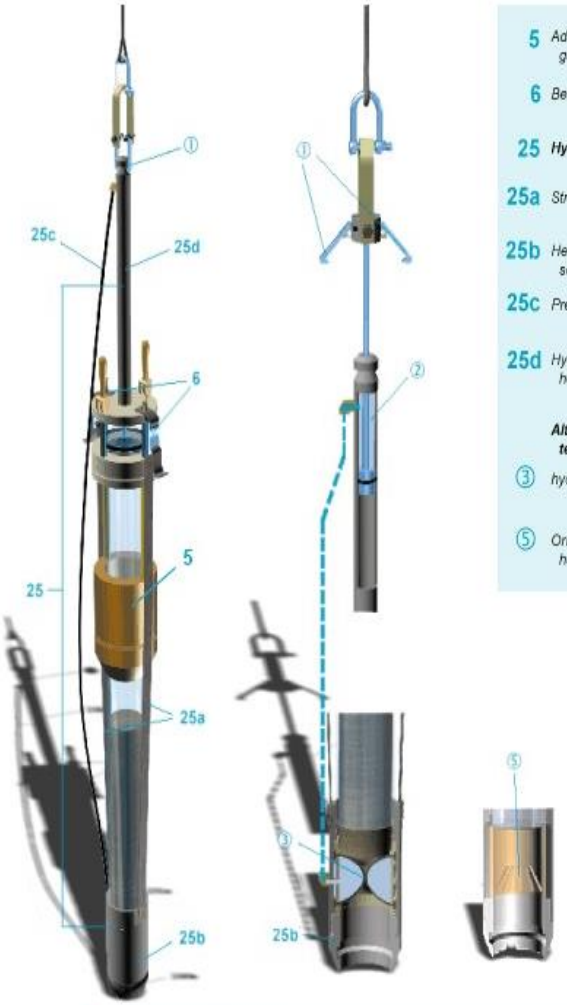
- The core catcher with rubber sleeve holds all sediment types (especially verry soft and watery sediments) reliable in the sampling tube!
- Automatic release without faulty messenger!
- Complete free cross section makes total undisturb sampling possible
- it is also possible to use orange peel core catcher for hard sediment.

Hydraulic core catcher function

① *After reach of the max. penetration depth and tractive reliev from the rope the release hooks opens.*

② *By pulling up the corer the piston press the water out from the cylinder into the rubber sleeve.*

③ *The rubber sleeve from the hydraulic core catcher close the sampling tube.*



5 *Addition weight 4 kg steel galvanized (max. 6 pieces)*

6 *Belt tighten buckle*

25 ***Hydraulic Core Catcher***

25a *Stretch belt*

25b *Head of core catcher with screwed in tempered core cutter*

25c *Pressure Tube*

25d *Hydraulik Zylinder with release hooks*

Alternatively screwed in with tempered core cutter:

③ *hydraulic rubber sleeve*

⑤ *Orange peel core catcher for hard sediment*

TECHNICAL DATAS FOR CORER		
Weight	kg	5 - 8
PVC-liner inside diameter	mm	60 / 86
Tube lenght	cm	60 / 120 Up to 2 m with telescopic tube or hydraulic core catcher
Water depth	m	0 - ∞
Addition weight	kg	4 kg Ø 60 mm 7 kg Ø 86 mm