

Water Quality in Kvernabekken and Grimstadpollen, Bergen



Group 7

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1 INTRODUCTION

The coastline is an interface between water and land, and developed seacoasts represent key regions where the sustainability of natural ecosystems may be compromised (Mallin et al., 2000). We come in contact with the seawater through different means, including recreational purposes like swimming, boat traffic and fishing. In developed areas along the coast of Norway the water we come in contact with will be affected by the increasing coastal population. Long-term observations of such areas have shown that the cumulative impact of land use change result in an increase of direct runoff (Davies et al., 2008, Haase, 2009) which causes floral and faunal changes and physical alternation of the environment. Runoff can come from point sources of pollution, including outlet points from wastewater treatment plants (WWTP) (Lovdata, 2012), as well as non-point runoff from the environment (Vinten et al., 2004). Wastewater from urban and suburban areas contains pathogenic organisms of human and animal origin, and if wastewater remains untreated or receives poor treatment, people may ingest pathogens directly or the pathogens may infect marine and aquatic species utilized for human consumption (Liberti & Notarnicola, 2003). Development can therefore pose as a human health risk through the contact we have with these water bodies. To protect the marine and aquatic ecosystems as well as human health, water quality standards are established to regulate the quality of the water. Water quality of different water bodies is classified depending on the intended use, ecosystem like recreational use or drinking water, in addition to be an indicator of the state of the ecosystem (EPA, 2011). Local authorities in Norway have the responsibility to monitor the water quality for recreational use (Folkehelseintituttet, 2000). How sensitive an area is to pollution and eutrophication for instance is categorized by Norwegian law as sensitive, less sensitive and normal. In Norway, the coastline between the southern Swedish border and Lindesnes, and Grimstadjorden are areas considered sensitive (Lovdata, 2012).

Grimstadjorden is located in Bergen Municipality, where the Agency of Water and Sewage Works (a sub-division of Bergen Municipality) is responsible for monitoring and regulation of the water quality in the region (Bergen Kommune, n.d; Sørfohn, 2014). BIO300-students from the University of Bergen take part in an on-going project to gather data and perform analysis for the Municipality, regarding the quality of local water bodies. Grimstadpollen is a coastal watershed connected to Grimstadjorden, located in an area surrounded by settlement.

Wastewater in Norway is usually connected to public sewage pipes, which leads to larger treatment plants. In rural or geologically demanding areas without a public sewage system, households have to

use alternative methods for disposal. In this case the households are connected to private mini WWTPs to clean effluent water before it is directly released into the environment through an outlet point. The company Goodtech delivers around 90 % of the mini WWTPs in Norway, and their most common product is called Biovac® mini WWTP, which is used to clean wastewater for up to a maximum of 7 households (35 pe) (Goodtech, 2011). This WWTP utilize a biological and chemical procedure which Goodtech (2011) call the “active sludge method” steps in the refinement of the wastewater to remove phosphorus and biological material before it is released (Avløpsgruppen, 2011). These plants are on SINTEF’s (Stiftelsen for Industriell og Teknisk Forskning ved NTH) list of approved mini WWTPs, and the efficiency of the plants is well within the government regulation stating that the level of phosphorus and biological material should be reduced by minimum 90 percent. There are no regulations on the amount of bacteria released, but mini treatment plants are reducing bacteria levels with around 50% (A. Totland at Bergen Municipality, Pers. Comm. 26.09.14).

The households of Grimstad utilize the Biovac® mini WWTP to clean wastewater before it is released into local streams, such as Høgstølbekken and Kvernabekken, and the bay of Grimstadpollen (see Figure A.1, Appendix A). These locations have been monitored previously (2007, 2008, 2010, 2012) to determine the water quality of both the stream (Liland et al., 2010; Hitchcock et al., 2012) and the bay (Austad et al., 2007; Bernardes et al., 2008; Aryal et al., 2010) to see if the water in this area is safe for recreational use. All previous studies have focused on phosphorus and coliform bacteria levels in estimating the water quality, as well as the faunal composition visible through biodiversity indices. Other abiotic factors are also included, like pH, temperature and salinity.

Changes in pH and temperature due to anthropogenic activities, could negatively influence survival growth of plants and animals (Bronmark & Hansson, 2005), and should therefore be monitored. With lower temperatures, more O₂ will be dissolved in the water and the density of the water increase. The pH levels are affected by temperature, due to more H₂O-molecules being formed instead of existing as free H⁺ and OH⁻ ions with rising temperature (Kemker, 2014). High pH levels are also an indication of high photosynthetic activities, which may occur due to algae blooms which are usually a consequence of runoff of nutrients such as phosphorus (Andersen et al., 1997). Phosphorus is the building block of cell membranes and nucleotides, and is incredibly important for the formation of plankton. It is a macro nutrient, and is very important for the maintenance of aquatic ecosystems (Thingstad, 2013). Increased phosphorus concentration increases the biological activity, which in turn increases the O₂ consumption. Higher concentration of phosphorus in the water causes an increase in algae production – especially diatoms (Thingstad, 2013). Phosphorus is used as a parameter for

water quality mainly because it may reflect risk of increased production of problem algae that can cause odour/taste problems and possible toxic production in lakes (Andersen et al., 2007). Effluent water from households leads to this increased phosphorus concentration, and when these species increase in numbers, the availability of oxygen needed for formation of cell material decreases. This will lead to an increased competition between organisms, and could reduce biodiversity.

In suburban areas such as Grimstad, agriculture and urban activities are major sources of phosphorus contamination in aquatic ecosystems, as well as microorganisms (Carpenter et al., 1998). Runoff from e.g. chemical and biological (manure) fertilizers can become nonpoint sources of pollution during rainfall events (Vinten et al., 2004). Nonpoint inputs, compared to discharges from point sources, are difficult to monitor because the sources are often dispersed over large areas, as well as being more prone to irregular events such as change in agricultural activities and heavy precipitation (Carpenter et al., 1998). High turbidity, due to both rainfall and/or tidal influx and outflux, can cause resuspension of bottom sediments which may contribute to increased levels of microorganisms (Kistemann et al., 2002; Francy et al., 2003) and most likely phosphorus concentrations. Water polluted by microorganisms from animal wastes has a high impact on marine ecosystems especially in coastal areas close to urban settlements (Kärnä, 2010). This affects the public health through e.g. recreational activities and indirect ingestions of contaminated food (Okeke et al., 2011; Liu and Huang, 2012; Kärnä et al., 2010). Typically the microorganisms are of faecal origin (living in the intestines of warm blooded animals), which contaminates the water through different routes like e.g. untreated- or partly treated wastewater from domestic, agricultural and industrial runoff (Okeke et al., 2011). Since microorganisms are relatively difficult to identify, faecal thermotolerant coliform bacteria (TCB), which is a Gram-negative rod-shaped bacteria (Haller et al., 2009), are often used as an indicator organisms to monitor water quality (Okeke et al., 2011; Liu & Huang, 2012). This is consistent with the parameter selection in the EU directive for the quality of bathing water (Folkehelseinstituttet, 2000).

Biodiversity is another good indicator of the health and functioning of ecosystems. Low biodiversity in aquatic environments often correlates with bad water quality (Houlahan and Findlay, 2003). A bottom sediment sample in an aquatic ecosystem provides information about water quality, simply by looking at species composition and their taxonomic levels. Different taxa have different tolerances of water quality. Some species are highly tolerant of bad water quality (worms and snails), while others are sensitive (stoneflies and dragonflies), and their presence could therefore indicate good water quality. Three different indices will be used in this study: BMWP (biological quality), ASPT

(water quality) and the Shannon index (biodiversity), as described in biodiversity lab notes. They will be used to give an overview over the general biodiversity, biological pollutant tolerance and the water quality in term of the taxa. It is important to note that Grimstadpollen is part of the critical transition zone (CTZ) between landmasses and full strength seawater, and should therefore be associated with low biodiversity (Levin *et al.* 2001). The increase in salinity from the stream to the sea contributes to lowering the biodiversity (Miller, 2013). Runoff of toxins from urban activities, including airports such as Flesland which is located close to the area in question, might also have an effect on biodiversity (Corsi *et al.*, 2008). These factors were not taken into account in this study.

1.1 AIMS OF THIS STUDY

Both point and nonpoint sources of pollution can cause problems in aquatic ecosystems. This can be eutrophication due to increased levels of phosphorus, loss of oxygen, increased levels of fecal microorganisms and loss of biodiversity (Carpenter *et al.*, 1998). Consequently, there is a need to monitor and protect natural resources of water to prevent the spread of pathogenic agents that may form, to maintain public health and protect the marine ecosystems (Sidhu & Toze, 2009).

The purpose of this study is to determine the effect of Wastewater treatment plants (WWTP) on the water quality in Grimstadpollen and Kvernabekken. Concentrations of *E. coli* and phosphorus were measured, as well as temperature, oxygen and pH. Finally, sediment samples were taken in order to determine biological quality and biodiversity. All of these factors, as well as results from previous studies, are needed to answer the questions “Is the water quality in Grimstadpollen and the adjacent stream impacted by effluent water from mini-WWTPs?”, “Has the quality changed over time?” and “Is the water quality good enough for bathing and recreation?”

2 MATERIALS AND METHODS

2.1 SAMPLING SITES

Grimstadpollen is a small brackish bay just north of Flesland Airport in Bergen. This is part of an area that is classified as sensitive in “forurensningsforskriften” (Lovdata, 2012) which in short is due to the level of eutrophication (see Figure 2.1). The location is surrounded by buildings and woodland on all sides, and there are motor boats moored in the deeper end of the bay. A small stream with two sewage outlets drain into the bay. From personal communication with Bergen Municipality (Bergen Municipality, Pers. Comm. 26.09.14) it was clarified that there are in total 36 households connected to seven outlet points most relevant to the total output of sewage in Grimstadpollen (figure 2.2, [Appendix A](#)). These outlets are connected to biological/chemical mini wastewater treatment plants (WWTPs) made by Biovac® (Biovac®, year unknown). The exact number of WWTPs in the area is not surveyed, but in Figure 2.2, [Appendix A](#) the municipality account for 9 WWTPs. A horse paddock and some greenhouses are also in the area, which could possibly influence the stream. The inner part of Grimstadpollen drains almost completely at low tide, with only the stream feeding into it.

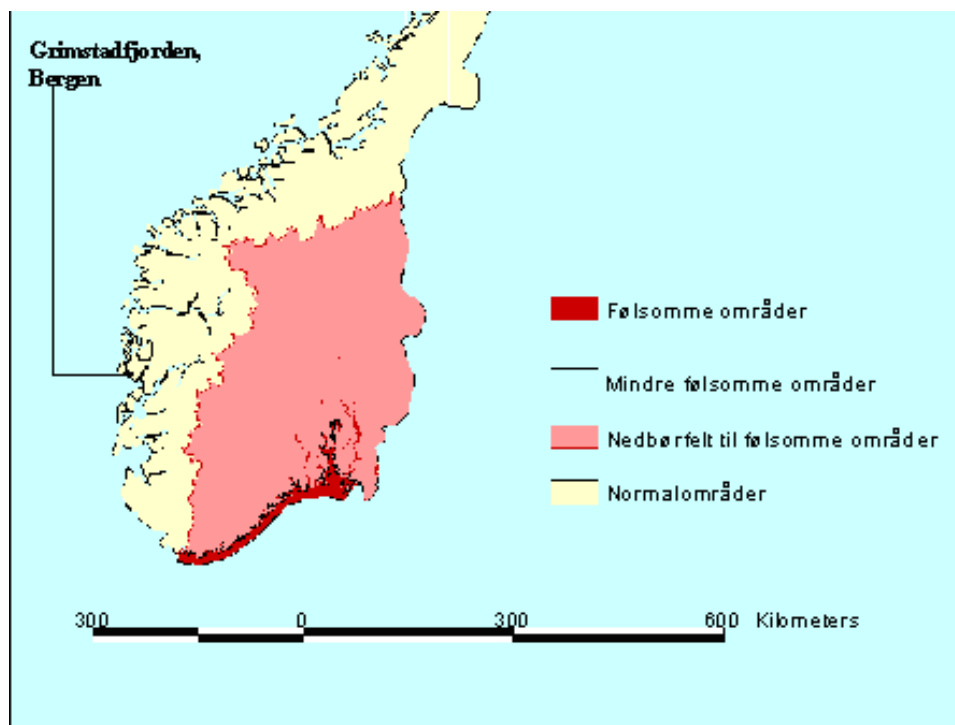


Figure 2.1 – Map over the geographic distributions of the different classification of environments in the south of Norway. Red is the sensitive areas, while pale yellow colour represents normal areas (Lovdata, 2012).

2.1.1 SITE DESCRIPTION

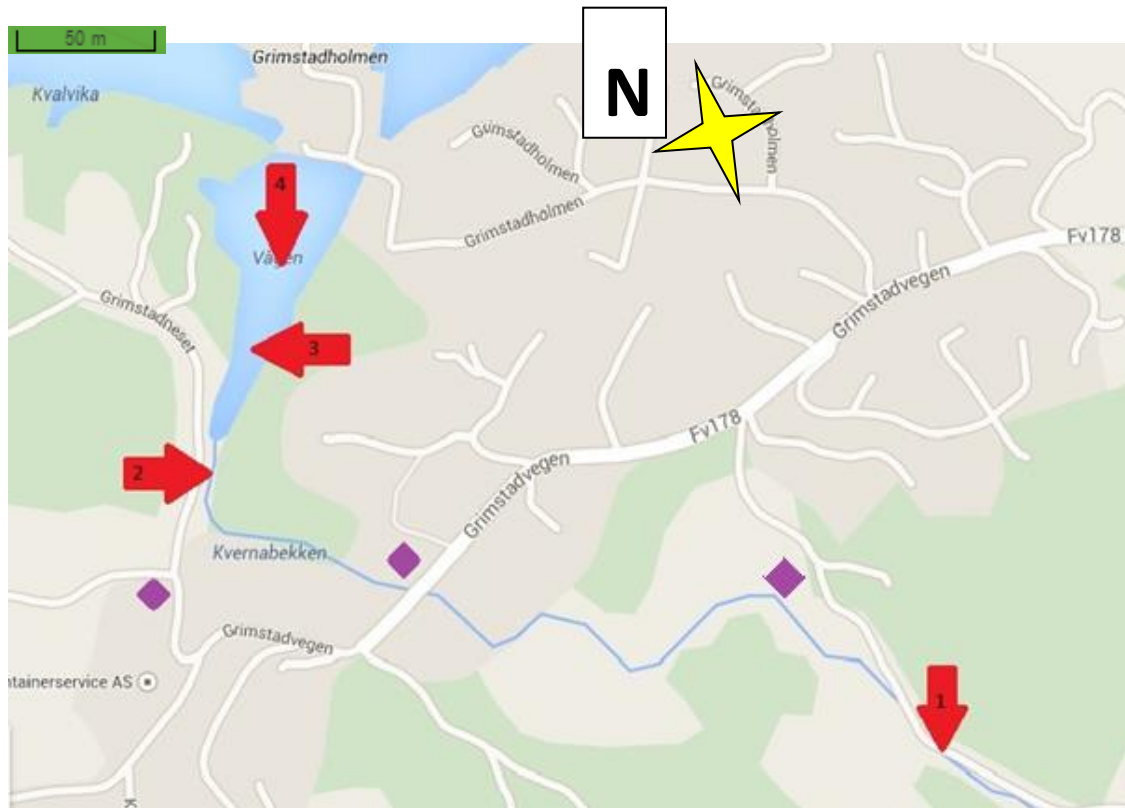


Figure 2.2 - Map of Grimstadpollen and the surrounding area. Red arrows mark sampling sites, labelled 1-4, and purple diamonds mark the wastewater treatment plants draining into the stream. The map is a print screen from Google maps, and the markers were added manually using Paint.

SITE 1

The first sample site was located upstream from the WWTP (see Figure 2.2). This was a slow flowing part of the stream, nestled in the middle of a wooded area next to a gravelled road with construction traffic on a semi-regular basis. The sediment was muddy, and the banks were slightly rocky over the about 1 meter wide stream.

SITE 2

The second site sampled was near the mouth of the creek, downstream from both WWTPs. The streambed was rocky, but there were small areas with softer sediment. The banks were slightly rocky and surrounded by trees. A paved road lay adjacent to the stream, leading to a small cluster of houses. A horse paddock as well as some greenhouses was located upstream of Site 2 and might also have influenced the water quality.

SITE 3

The third site sampled was in the inner part of Grimstadpollen. The seabed was sloping very gradually, and there was a high level of muddy sediments. The east bank was steep, with trees and a few houses. The paved road from Site 2 continued past Site 3.

SITE 4

The fourth site sampled was in the outer part of Grimstadpollen. The border between the two last areas (site 3 and 4) was made up of two small islands, and we sampled north of the largest one. The seabed at this site was also muddy, and the banks were bare rock followed by woods. There were houses on all three remaining sides of the islands, and motorized boats were moored about 20m from the sample site.

2.2 SAMPLES AND MEASUREMENTS

2.2.1 GENERAL OVERVIEW

The sampling was conducted on September 10th 2014, between 9 a.m. and 11:15 a.m. The weather was sunny, but the air was slightly hazy, and the sites were in the shade prior to and during sampling. Precipitation was low in the week leading up to sampling (Figure 2.4). The air temperature was 12 °C. On the day of sampling, there was a lot of sewage-like material floating on the surface of the bay (see Figure 2.3).



Figure 2.3 - Sewage-like material floating on the surface of Grimstadpollen (photo by Yong Lao).

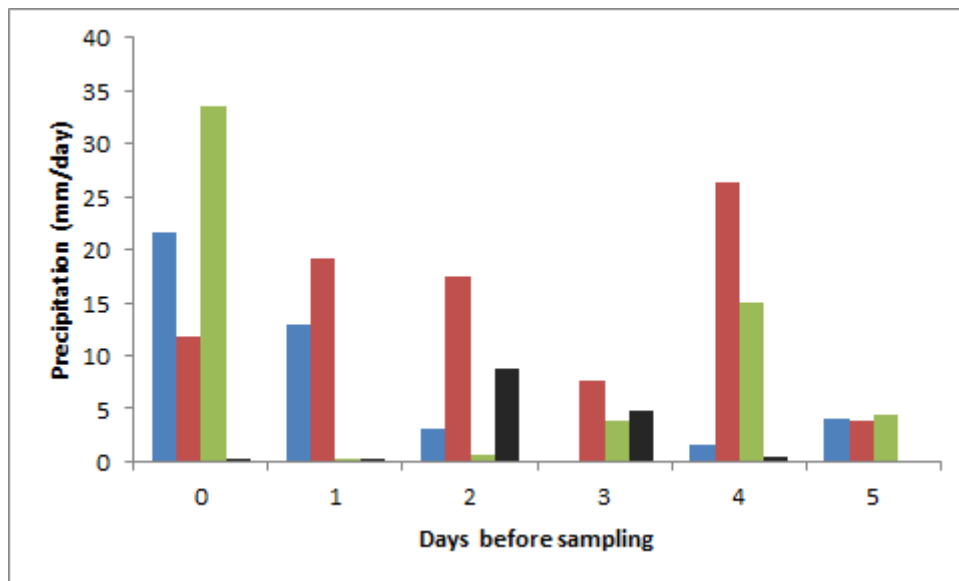


Figure 2.4 – Precipitation (mm/day) in the Grimstadpollen area in the week of sampling. Day 0 represents the day of sampling, while days 1-5 are the days leading up to it. Precipitation in previous studies is compared with this year's study. The blue bars show precipitation in 2008, the red in 2010, the green in 2012 and the black in 2014. Data was collected from <http://m.yr.no/sted/Norge/Hordaland/Bergen/Bergen/almanakk.html>, with measurements from Florida measuring station in the centre of Bergen. This is the measuring station located closest to Grimstadpollen.

Physical and chemical factors as well as biodiversity and TCB, were measured at each site. At Site 1 and 2 stream depth, width and water velocity was measured to calculate stream flow. At Site 3 and 4 water samples were collected at two different depths (upper part and middle part of the water column).

2.2.2 TEMPERATURE, OXYGEN, CONDUCTIVITY AND PH

Temperature and pH was measured in the field using a portable pH-meter (WTW pH 3110 SET 2, 2AA 112), while oxygen saturation levels (%) were measured with a portable oxygen meter. Conductivity was not measured in the field, due to a malfunction conductivity meter. A water sample from Site 4 was instead brought back to the lab in a 50mL Falcon tube, and analysed there. Salinity levels from previous studies will be used to present in the result for explanatory reasons.

At each site the instrument was held at a 10-20cm depth until the instrument was stabilized. The instruments were then rinsed with tap water before it was put to use again.

2.2.3 THERMOTOLERANT COLIFORM BACTERIA

Water samples were collected for total coliform bacteria (TCB) analysis from all four sites in 250mL sterile bottles, with five replicates at each site. At Site 1 and 2, five samples were taken 0.1m below the water surface. They were collected where the velocity of the water was slowest. At Site 4, two samples were taken at 0.1m depth, while three were taken at 0.5m depth. Five samples were also collected from Site 3, but here three of them were from 0.1m depth and two from approximately 0.5m depth. The samples taken at 0.5m were collected using an extension rod. After sampling the bottles were immediately placed in a cooler and delivered to Bergen Vann KF (komunalt foretak).

The samples were analysed by the membrane filter method (NS ISO 8199, January 2002), and the value of TCB is counts of colony forming units (CFU). The water quality based on density of coliform bacteria is classified according to Table 2.1.

Table 2.1 – Classification of condition for coliform bacteria (Andersen et al., 1997; Folkehelseinstituttet, 2000).

Classification for Coliform bacteria	Interval (col/100ml)	Description for recreational use
I(/II)	<100	Suited
III	100-300	Less suited
IV	>1000	Not suited

2.2.4 PHOSPHORUS

Water samples for phosphorus concentration were sampled in 50mL sterile Falcon tubes. Two replicates were taken at Site 1 and 2, while three samples were taken at Site 3 and 4. At Site 3 two replicates were taken at 0.10m depth, while one sample was taken at 0.5m depth. At Site 4 two were collected at about 0.5m depth, while one was collected at 0.1m depth. The samples were collected in the same manner as the coliform bacteria samples, and kept cold until delivered to Eurofins Laboratory. The water quality based on phosphorus levels are classified according to Table 2.2.

Eurofins processes the samples by the Ammonium molybdate spectrometric Method (ISO 6878:2004), and orthophosphate and total phosphorus contents are determined by flow analysis (FIA and CFA) (ISO 15681-2:2003) (Eurofins, Pers. Comm. 17.09.14).

Table 2.2 – Classification of condition for phosphate (Andersen et al., 1997; Folkehelseinstituttet, 2000).

Classification for phosphate	Interval ($\mu\text{g P/l}$)	Description for recreational use
I	<7	Well suited
II	7-11	Suited
III	11-20	Less suited
IV	>20	Not suited

2.2.5 BIODIVERSITY

To determine the fauna in Grimstadpollen, sediment samples were taken by a grab sampler on Site 1, 3 and 4 with one to tree grabs within a 1 Square meter area, in addition to a combination of a shovel sample and a kick sample at Site 2. The grab samples were sieved in a fine meshed net (200 μm) to remove most of the water and some fine particles. The samples of the sediments were kept in 250ml bottles, and later identified in the lab. The grab sampler which was used had the potential to reach 11 cm into the sediments, and had a surface area of 56 cm^2 . It was dropped from approximately 10 cm above the sediments before the grabbing mechanism was activated by dropping a metal soldering on the top of the grab sampler.

Site 1: One grab sample was taken from the soft bottom sediments.

Site 2: One kick sample and one shovel sample was taken due to the hard and rocky bottom. The kick sample was taken in the middle of the stream, where five kicks were used to stir up the sediments within a period of about 40 seconds. A bucket with a 1mm mesh was placed 0,4m downstream from the kicking, and was held in the stream for 30 seconds to collect sediments. The shovel sample were taken by sticking a small garden shovel into the sediments and bend it up, so the whole shovel was

full of sediments. For comparative purposes, four shovels were estimated to equal two grabs (11.2dm³).

Site 3-4: Three grab samples were taken at Site 3, and two in Site 4. All samples were taken within a 1m² area, and then put in a bucket before the samples were then sieved and kept in 250ml bottles.

In the laboratory the samples were washed in the sink through a sieve, to get rid of the finer sediments. The remaining animals and sediments were then put in a tray, and a staining powder (Rose Bengal) was added. Subsequently, stereo microscopes (Wild Heerbrugg, mag 6.4-40×) were used to find organisms in the samples, which were determined to the lowest possible taxonomical level. All of the sediments in the samples were examined, except for Site 3. There, a subsample was used and divided the sediments into eight squares, of which two were examined.

After the organisms were determined to the lowest taxonomical level, the taxonomical values which were found in a table were used to calculate a numeric diversity value with the Shannon index (Equation 1). A high H in the Shannon index is a sign of high species diversity.

$$H' = -\sum_{i=1}^R p_i \ln p_i \quad (\text{Equation 1})$$

p_i = relative abundance in each species (n/N).

In addition, BMWP score (Biological monitoring working party score) and ASPT (average score per taxon) was used to determine the water quality. BMWP score use the macro invertebrates in the water as a biological indicator of water quality. Each family of macro invertebrates has a tolerance score. (Appendix Table A.6) The combined scores of the present family groups is the BWMP score. A high BWMP score is a sign of high diversity which indicates good water quality. A low score is a sign of bad water quality. A disadvantage with the BMWP score is that a small sample size is likely to miss species and give a different score. (Lab handout) The ASPT is a biological indicator which takes the number of present species into account. (Lab handout) It is calculated with the BMWP score divided with the number of present species. (Lab handout)

2.3 STATISTICAL METHODS

In this study we are looking at what effect site and depth (categorical explanatory variables) have on TCB and phosphorus levels; this is an example of a casual relationship. For both the phosphorus and TCB the samples are assumed to be independent. Since phosphorus concentration (µg P/l) is a continuous response variable a normal distribution is assumed. A one-Way ANOVA was utilised to

analyse the variance in the effect of the two parameters. The distribution of the response variable TCB is assumed to be Poisson since TCB levels are counts of CFU, therefore general linear model was used. A Tukey test was further run to see where the largest difference between the sites was for both phosphorus and TCB. The significant level was set to a p-value > 0.05.

All the results compared with previous results are compared by quantitative methods. Quantitative description was also used to analyse the difference in phosphorus concentrations at the different depths, due to the lack of replicates.

3 RESULTS

3.1 TEMPERATURE, OXYGEN, CONDUCTIVITY AND PH

Temperature and pH levels were constant within both the stream and bay areas (Table 3.1). There is however slightly higher values of temperature and pH in the bay area sites compared to the stream sites. Oxygen levels were highest in the fast-flowing part of the stream, and decreased towards the bay. Temperatures in the bay areas were 14.8°C and 15.3°C in sites 3 and 4, respectively, and 10.2 °C and 11.0 °C in sites 1 and 2, respectively. In sites 3 and 4 the pH was 8.2 and 8.1 respectively, while both site 1 and 2 had a pH of 7.4. Oxygen saturation levels were 88.0 %, 103.0 %, 94.0 % and 85.0 % for sites 1, 2, 3 and 4, respectively.. Due to a malfunctioning conductivity meter, salinity was only measured in site 4. The conductivity was 42.6 mS/cm and salinity 27.1 ppt.

Table 3.1 - Overview of pH, oxygen levels and temperature at site 1-4 in Grimstadpollen, taken at approximately 10-20 cm depth, compared with previous years. (Bernardes et al. 2008; Liland et al. 2010; Aryal et al. 2010; Hitchcock et al. 2012). Since there were two studies conducted in 2010, Liland et al. are represented by 2010a while Aryal et al. is given as 2010b. Sites 1 and 2 are freshwater, while sites 3 and 4 are in the brackish water in the bay of Grimstadpollen.

Site	Year	pH	Oxygen level (%)	Temperature (°C)	Measured Salinity (ppt)
1	2010a	7.5	-	10.6	-
	2012	6.4	-	10.8	-
	2014	7.4	88.0	10.2	-
2	2008	7.5	93	8.9	0.3
	2010a	7.4	-	10.9	-
	2014	7.4	103	11.0	-
3	2008	7.4	92	8.9	0.1
	2010b	7.1	-	11.8	0.8
	2014	8.2	94	14.8	-
4	2008	-	85	8.7	-
	2010b	8.0	-	11.3	1.8
	2014	8.1	85	15.3	27.1

3.2 COLIFORM BACTERIA

The results show an increase in thermotolerant coliform bacteria (TCB) at the surface at both Site 3 and 4 (Figure 3.1), whereas site 3 showed 75CFU/100mL for bottom and 93CFU/100mL for surface, while site 4 showed 63 for bottom and 140 for surface. However, this increase is not significant (p-value = 0.74 for both, GLM Quasipoisson). As expected, the largest difference in TCB concentrations is between the control and Site 4 (p-value 0.0740, TukeyHSD), while there is close to no difference between Site 3 and 4 (p-value 0.9899, TukeyHSD) (see Table A.3, Appendix B) which are both located in the brackish water.

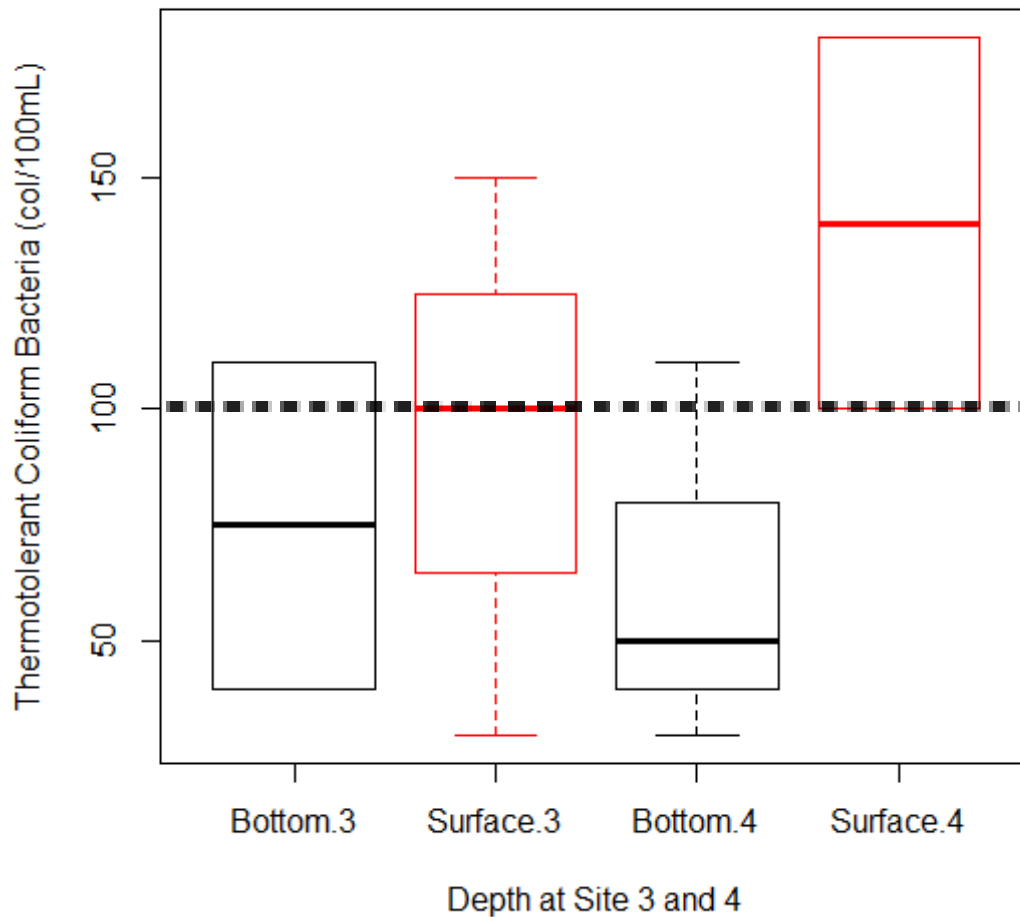


Figure 3.1 – Boxplot displaying the variance in the TCB values between the two depths; Bottom (0.5m) and Surface (0.1m) at Site 3 and 4 in Grimstadpollen. Each box represents the distribution of TCB counts at each site and depth ($n = 2$ for Bottom.3, $n = 3$ for Bottom.4, $n = 3$ for Surface.3, $n = 2$ for Surface.4). The digit 3 and 4 on the x-axis labels indicates what site the box represents. The median for each site is illustrated by the thick horizontal lines, and the variability of the values is presented by the whiskers extending from the boxes, whereas it can be seen that the mean and standard deviation for Bottom is 75 CFU/100mL and 49.5 respectively, and the mean and standard deviation for Surface is 93 CFU/100mL and 60.3 respectively. The black dotted line represents the upper limit of 100 TCB counts 100 mL^{-1} at surface for bathing and recreation, utilized by Bergen Municipality (Folkehelseinstituttet, 2000; Andersen et al., 1997; Jacobsen et al., 2007). The mean and standard deviation for Bottom is 75 CFU/100mL and 49.5 respectively. Mean and standard deviation for Surface is 93 CFU/100mL and 60.3 respectively.

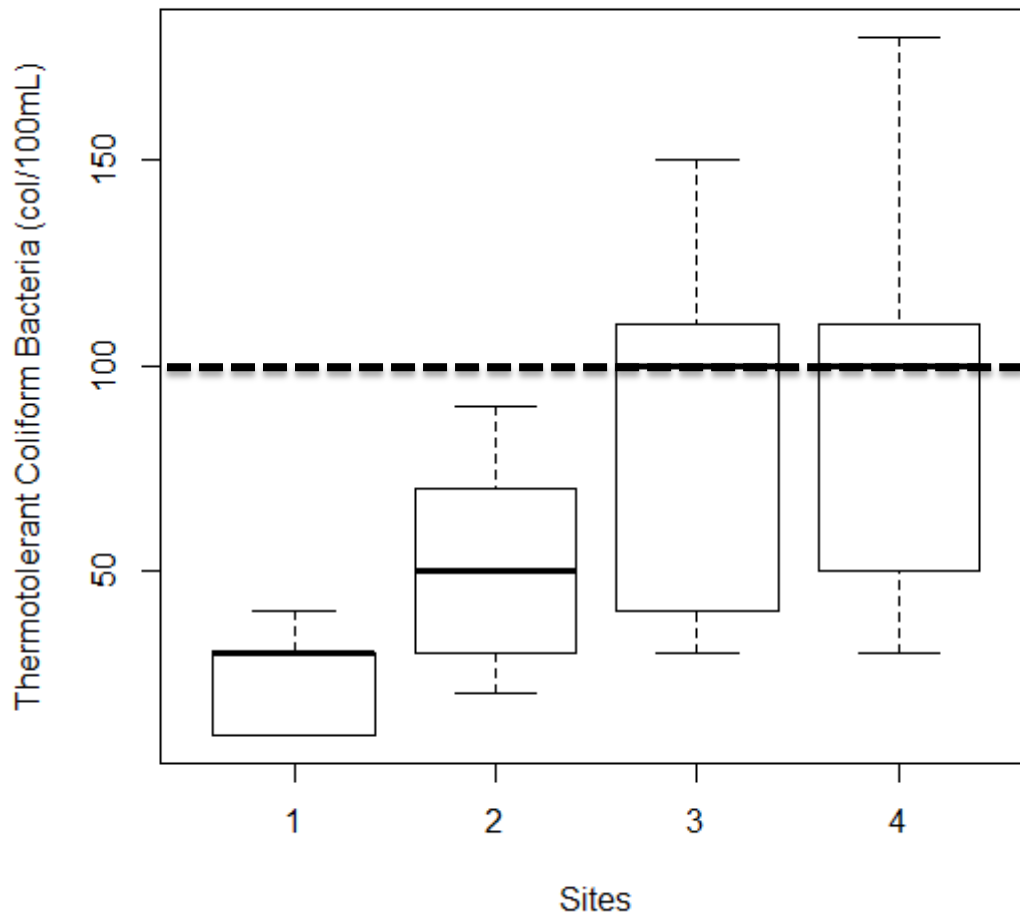


Figure 3.2 – Variance in the TCB counts between each site in the freshwater stream – sites 1 and 2 - and in Grimstadpollen ($n = 20$), in the lake saltwater – sites 3 and 4. The median for each site is illustrated by the thick horizontal lines, and the variability of the values is presented by the whiskers extending from the boxes. The black dotted line represents the upper limit of 100 col/100 mL at surface for bathing and recreation, utilized by Bergen Municipality (Folkehelseinstituttet, 2000; Andersen et al., 1997; Jacobsen et al., 2007). Mean and standard deviation for the different sites is presented in Table A.7, Appendix B. P -value < 0.05 .

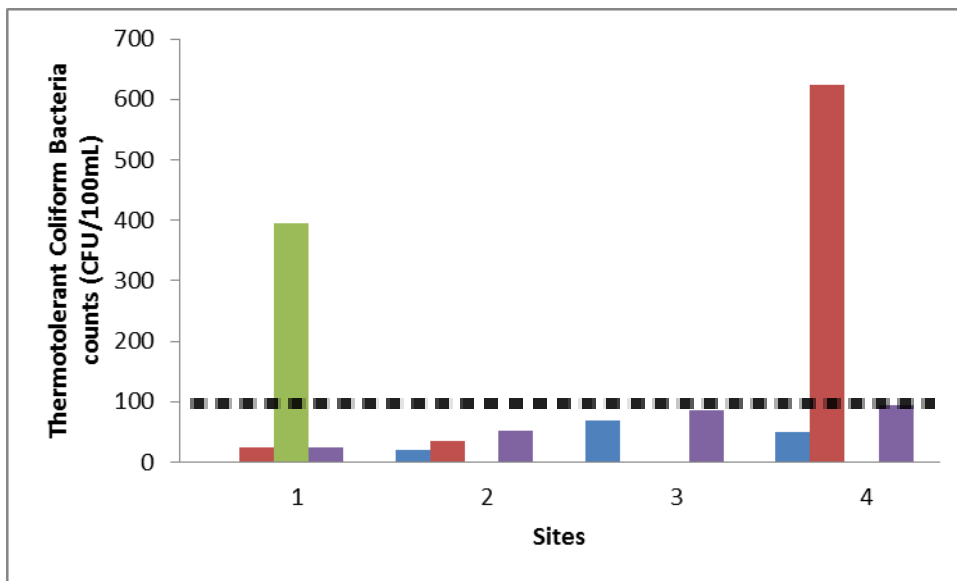


Figure 3.3 – Coliform bacteria counts from previous studies done at Grimstadpollen to this year’s study. Sites from previous years are labelled as the relevant site from this year study. Blue bar is counts from 2008, red from study done in the stream in Grimstadpollen bay in 2010, green 2012 and purple 2014 (Bernardes et al., 2008; Liland et al., 2010; Hitchcock et al, 2012. See Table A.2, Appendix B for relationship between sites. The black dotted line represents the upper limit of 100 col/100 mL at surface for bathing and recreation, utilized by Bergen Municipality (Folkehelseinstituttet, 2000; Andersen et al., 1997; Jacobsen et al., 2007).

3.4 PHOSPHORUS

Phosphorus concentrations measured – as can be seen by Table A.4, Appendix B – were given by 5.00 µg/L, 11.8 µg/L, 12 µg/L and 11 µg/L for sites 1, 2, 3 and 4, respectively. The descriptive analysis of phosphorus concentration from the two depths at site 3 and 4 shows no significant difference between depth (Table A.4, Appendix B). The two depths are therefor considered the same in the further analysis. There are also no significant (p -value=0.06019) difference in the variation in phosphorus concentration (Figure 3.4). The largest difference in phosphorus concentration is between Site 1 and Site 3 (p -value = 0.05, TukeyHSD), and the difference is smallest between the sites in the brackish water (Site 3 and 4) (see Table A.3, Appendix B for p -values extracted from the Tukey test).

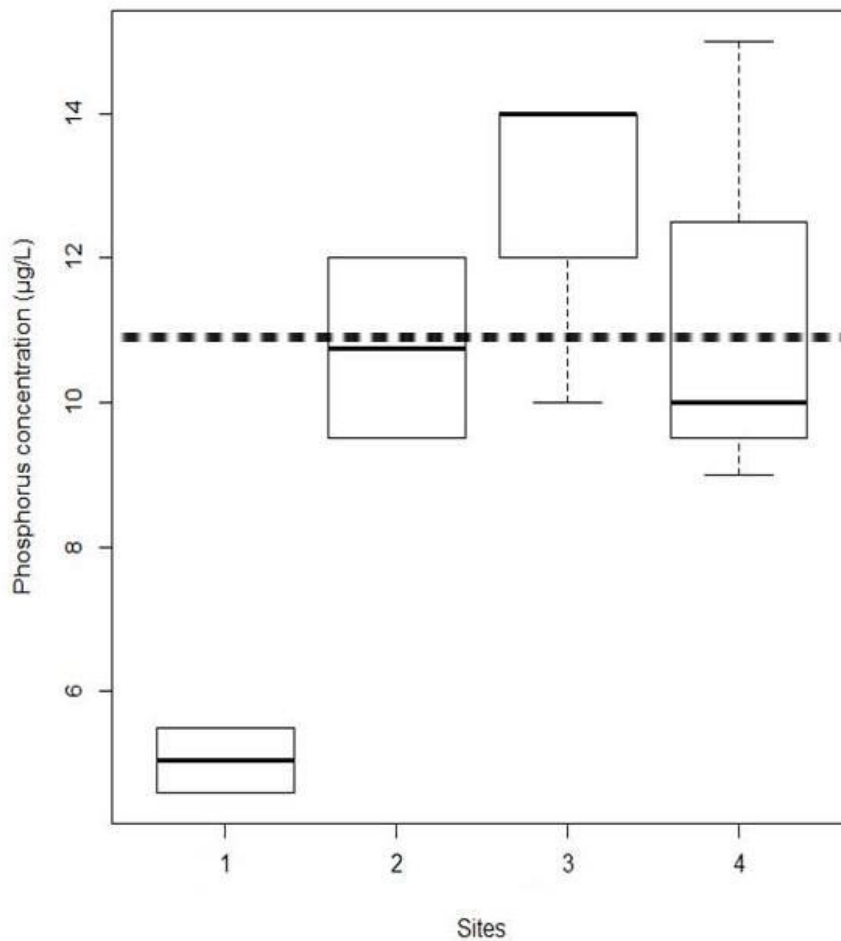


Figure 3.4 – Phosphorus concentration in $\mu\text{g/L}$ in 2014 for site 1-4 in Grimstadpollen. The median for each site is illustrated by the thick horizontal lines, and the standard deviation of the values is presented by lines extending from the boxes. The black dotted line represents the maximum Norwegian standard phosphorus levels of public use of water (e.g. bathing), $11 \mu\text{g/L}$, given by Bergen Municipality (Jacobsen et al., 2014). Mean and standard deviation is presented in Table A.7, Appendix B.

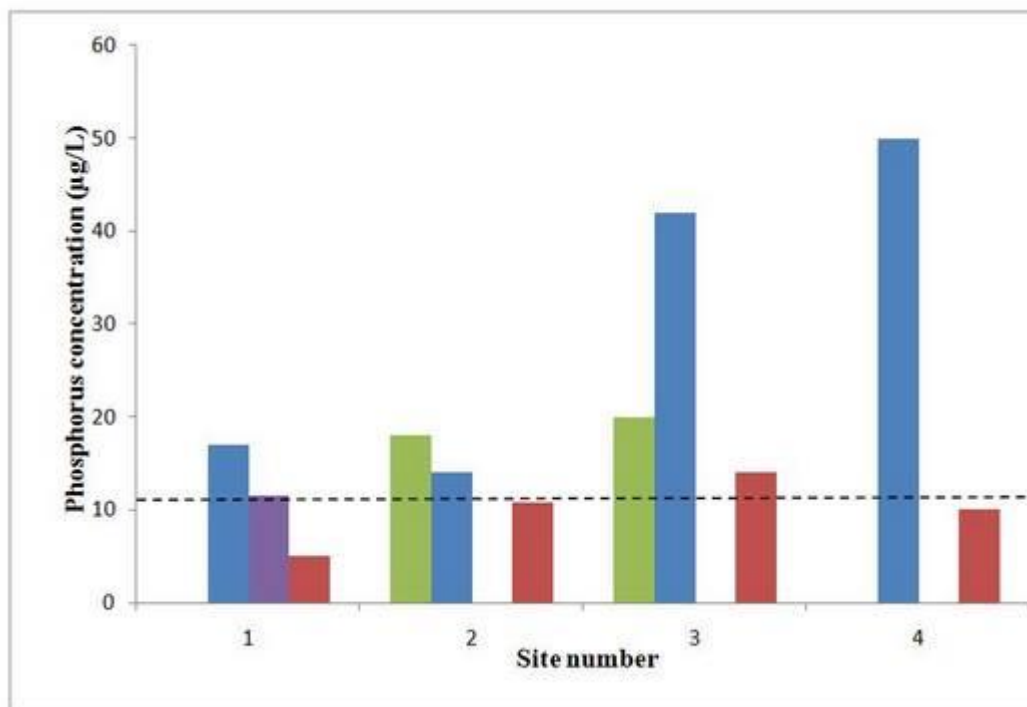


Figure 3.5 – Average phosphorus concentration in µg/L at site 1-4 in Grimstadpollen, independent of depth, compared with previous studies. Green=2008, blue=2010a and b, purple=2012, and red=2014 (Table A.5, Appendix B). The black dotted line represents the Norwegian standard phosphorus levels of public use of water (e.g. bathing) at 11 µg/L given by Bergen Municipality (Jacobsen et al., 2014) (Bernardes et al., 2008; Aryal et al., 2010; Liland et al., 2010; Hitchcock et al., 2012).

3.5 BIODIVERSITY

A total of 13 different species and 75 individuals were found. Site 2 had the highest diversity with the mean value of 16 species mainly concentrated in Dysticidae and Oligocheata. The Shannon-index in the stream was 1.08 ± 0.28 , and in the bay it was 0.52 ± 0.16 . Both biological- and water quality seems to be low in the entire Grimstadpollen area (Table 3.2, see requirements for each quality level in Table 2.3). A complete species list with BMWP-scores is listed in Table A.6,. Figure 3.6 shows the species composition within each site.

Table 3.2 - Biological quality (BMWP) and water quality (ASPT) in Grimstadpollen. Quality evaluations are taken from BIO300 Biodiversity Lab Notes, 2014.

Site	BWMP score	Quality	ASPT score	Quality
1	13.8	Poor	4.6	Poor
2	39.2	poor	5.6	Fair
3	3.5	Very poor	3.5	Poor
4	0	Insufficient data	0	Insufficient data

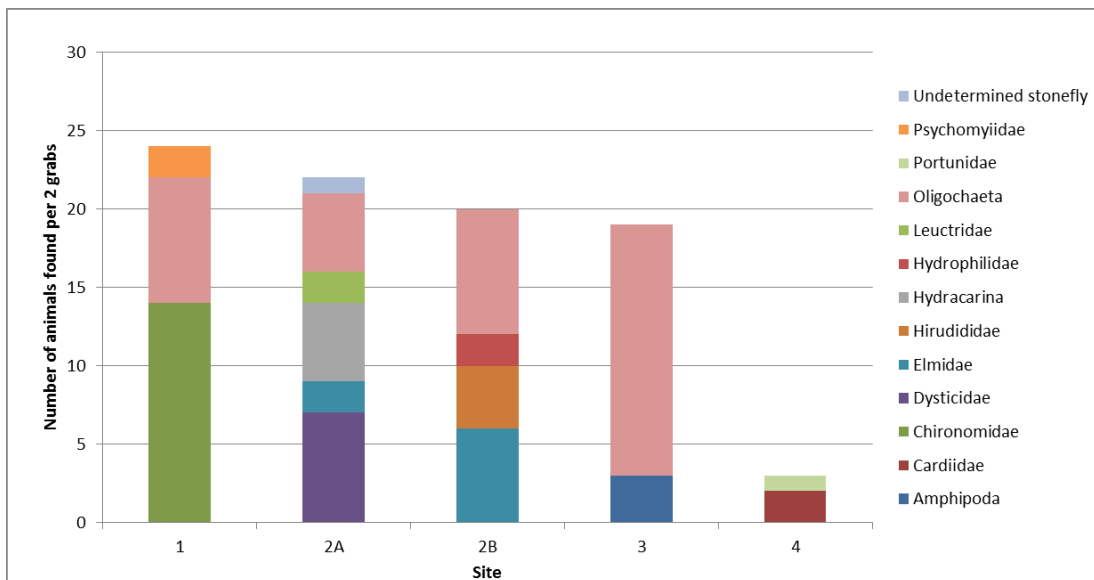


Figure 3.6 – Composition of individuals from Grimstadpollen within each site, given in number of animals found of each individuals within an 11,2dm² area (except site 2A which is a kick sample). Site 2B is the shovel sample which corresponds to around 2 grab samples and means 11,2dm².

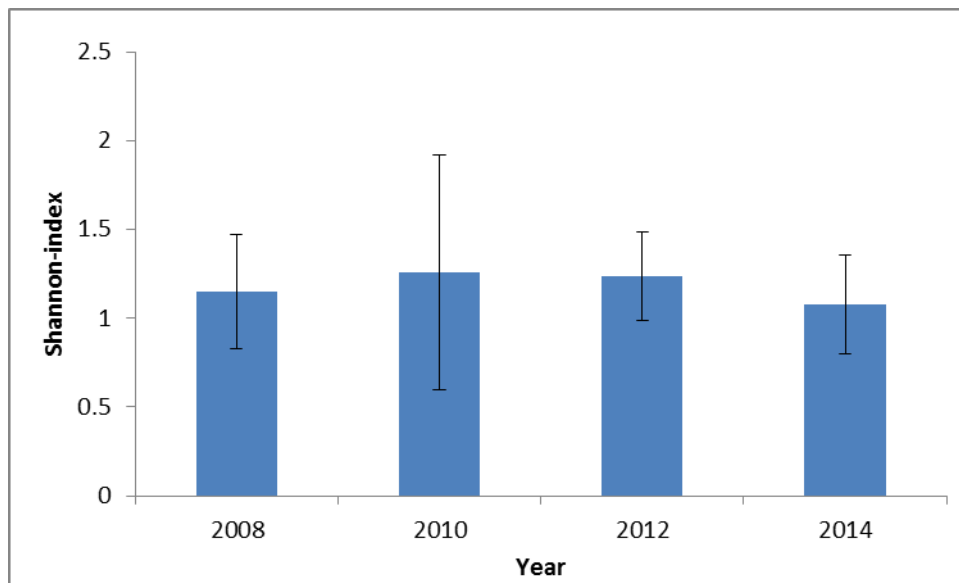


Figure 3.7 – Comparison of Shannon-indexes in fresh water – sites 1 and 2 – in the Grimstadpollen area for different years. Reports from previous studies do not include brackish water. For comparative purposes, samples from the saltier water were not included in the 2014 calculation. A higher Shannon-index represents increased biodiversity.

4 DISCUSSION

TCB and phosphorus levels obtained at each site are all lower than the upper limits at surface for bathing and recreation (Folkehelseinstituttet, 2000; Andersen et al., 1997, Jacobsen et al., 2007), indicating good water quality. Biodiversity, however, shows poor water quality in the entire survey area. The taxa composition varied between sites and sampling method.

The sampling methodology at each site was not consistent. Three different biodiversity sampling methods were used in this study: grab sampling, shovel sampling and kick sampling. The number of grab samples varied between all sites. However, both grab- and shovel samples can be standardised to a certain sampled area (in our case two grabs equal four shovels). Standardising the kick-sample to the same sampled area is nearly impossible (Hines & Comptois, 1985), causing this sample to be excluded when comparing between sites. It is worth noting that the kick sample, while not used in the index calculations, contained higher diversity, as well as a higher number of individuals than both the grab samples and the shovel sample. This indicates that sampling method influences the apparent quality of the water. Another potentially important factor is the use of subsampling, which could cause an erroneous observation of species when averaging for the entire sample (Ramsey and Suggs, 2001). Since subsampling was only used for one of the biodiversity samples, it is not fully

comparable to the rest. The differences in number of species and individuals found at each site shows that choice of sampling method is important. Species composition between grab- and shovel samples also deviate from one another. Only one type of animal (Oligochaeta) was found at both stream sites. Potential reasons for this could be sediment type (Rees *et al.* 1999) and sediment depth (Hines & Comptois, 1985), as well as oxygen availability (Levin *et al.* 2001). The biodiversity indices were used across all sites, even though they are only supposed to be used for limnic (freshwater) environments (BIO300, 2014). This might lead to inaccurate estimations in the brackish water. Inconsistent methods also occurred in the phosphorous and TCB sampling. An uneven number of replicates were taken at each site, as well as at the different depths. This was in part due to the limited sample containers available, and in part because the group wanted to get the most out of the depth measurements. All samples were taken at a maximum of 0.5m. According to Folkehelseinstituttet (2000), all such samples must be taken in water that is at least 1m deep, or the bottom sediments might influence them.

This study can be separated into two categories; a freshwater survey and a brackish water survey. Each category contains two sites. Sites 1 and 2 are freshwater, and sites 3 and 4 are brackish water. Comparable sites from previous studies were used to determine changes (Table A.1, Appendix A).

The water quality at Site 1 was good in terms of TCB and phosphorous, while biodiversity indicates poor water quality. This site was above the WWTPs, so there was no point-source pollution influencing the results at this site. Site 1 is therefore considered a control. Previous studies indicated less good water quality in terms of both TCB and phosphorous. Biodiversity in previous studies was higher than in this year's study, although it was not great (Liland *et al.*, 2010; Hitchcock *et al.*, 2012). The lower biodiversity in the current study could be due to the fact that only one grab sample was taken at this site.

Site 2 was different from all the other sites, in the sense that the sediment particles were larger, and the water was faster flowing. This was the only site where grab samples were not used. Instead, both kick- and shovel sampling were used here. TCB and phosphorous levels were within the recommended limit. Biodiversity indices were the best out of all the sites. While biological quality was poor, the water quality was fair.

TCB and phosphorus results indicate a good and less good quality respectively in the water at Site 3. TCB was in the higher region of what is deemed acceptable, while phosphorous levels were classified as grade 3 concentrations, in accordance with Folkehelseinstituttet (2000). Biodiversity scores

revealed very poor biological quality and poor water quality (Table 3.2). Only two taxa were found in the sediments. Upon arrival in Grimstadpollen, the tide was low. Site 3 was completely drained of water, with the exception of the small amount of freshwater being supplied from the stream. Because of this, sampling was carried out during rising tide. Increased turbidity due to re-suspension of bottom sediments has been stated to increase the concentration of parameters such as TCB since increased turbidity of water can stimulate bacterial growth by e.g. increasing the nutrients available for growth (Rincòn & Pulgarin, 2003; Francy *et al.*, 2003). Biodiversity might also be affected by the long periods during low tide where the sediments are exposed to air rather than water (Levin *et al.*, 2001). At this site there was an increase in pH from 2010 (Aryal *et al.*, 2010). This rise in pH could be explained by the higher temperature observed at this site. Since more H₂O-molecules are being formed instead of existing as H⁺ and OH⁻ ions when the temperature rises, water will become more alkaline with increased temperatures (Kemker, 2014). The higher temperature witnessed is most likely correlated with the low precipitation levels and warm weather experienced this year, which is markedly different from the previous years this study was conducted.

Water quality at Site 4 was borderline good in regards to both phosphorous and TCB. Both levels were in the higher region of what constitutes healthy water (Folkehelseinstituttet, 2000). This, in combination with the high levels in Site 3, indicate that Grimstadpollen might be at risk of eutrophication. Eutrophication may cause algal blooms, and phosphorus concentrations are therefore often used as a parameter for the possible presence of algal toxins (Dolah, 2000). There is, however, a visible decrease in phosphorous concentration in this year's result compared to the results obtained in 2010. Current phosphorous levels have dropped to only one fifth of the concentration present in 2010 (Aryal *et al.*, 2010) (Figure 3.5). This could be due to the low precipitation in the day leading up to the sampling (Bjørklund 1998a; Bjørklund 1998b). The biodiversity data were inconclusive at this site, due to only three animals being found. It is hard to say of this result was to be expected based on previous studies, due to the units being different. Bernardes *et al.* (2008) used volume sampled while we used area, and Aryal *et al.* (2010) did not standardise their samples. However, Levin *et al.* (2001) states that low biodiversity is associated with the extreme fluctuations in living conditions found in CTZs such as Grimstadpollen. Another factor potentially lowering the biodiversity is the runoff from the nearby airport. Studies show that areas close to airports might have elevated levels of metals, herbicides, hydrocarbons and de-icing agents (used to remove ice) due to runoff from the airport (Ellis & Llewellyn, 2007; Corsi *et al.*, 2008). Airfield-pavement de-icer materials (PDM) can be a source of toxic runoff, which can be detected by measuring ammonium levels in the water. Chlorine is also a possible parameter, which can be the

result of road-salt from the airport as well as the surrounding urban influence. Such runoff will have consequence on marine life, through lethal and sub-lethal toxic effects that affects fitness (Corsi et al., 2008; Levin et al. 2001).

There was a significant increase in TCB levels as we moved downstream from the control site (Site 1). This is consistent with previous years (Hitchcock et al., 2012). TCB levels at all sites in 2008 were similar to this year's levels (Bernardes et al., 2008), while levels from 2010 and 2012 were much higher (Liland et al., 2010; Aryal et al., 2010; Hitchcock et al., 2012). This is most likely due to agricultural runoff, such as horse faeces and fertiliser from the greenhouses. Studies show that agriculture can serve as a major source of pollution (Carpenter et al., 1998) of e.g. animal faces, in addition to runoff from fertilizers (Vinten et al., 2004). According to Bjørklund (1998a and 1998b) runoff from nonpoint sources increase during periods of high precipitation. In 2008, precipitation was low in the days prior to sampling (Bernardes et al., 2008), while in 2010 and 2012 the sampling days followed a period of high precipitation (Liland et al., 2010; Aryal et al., 2010; Hitchcock et al., 2012) (Figure 2.4). The mentioned increase in TCB and phosphorous downstream from Site 1 indicates that the output from the WWTPs do have an effect on the microorganisms living in the water, but since the values from all sites are mostly within the limits, the WWTPS seem to be functioning properly. Given the higher levels in previous years, (Liland et al., 2010; Aryal et al., 2010; Hitchcock et al., 2012), it is clear that some of the pollution comes from nonpoint sources. In addition, Bergen Municipality do not have complete control of all the WWTPs in the area (Bergen Municipality, Pers. Comm. 26.09.14). In case of higher levels of pollution, it is difficult to know whether this originates from nonpoint sources, or if some of the WWTPs are malfunctioning.

4.1 CONCLUSION

Effluent water from the wastewater treatment plants in the Grimstadpollen area seem to be affecting the concentration of both TCB and phosphorous to some degree, though the levels are still acceptable (Bergen Municipality, Pers. Comm. 26.09.14; Lovdata, 2012).

There is reason to believe that non-point sources of pollution leaking into Grimstadpollen has an amplifying effect on the TCB and phosphorus concentrations, due to the higher levels observed at site 3 and 4, as well as results from previous years (Liland et al., 2010; Aryal et al., 2010; Hitchcock et al., 2012). Current levels are still within the levels classifying Grimstadpollen as suited for bathing and recreation (see Table 2.1 and 2.2 for classification), which should mean that Grimstadpollen would be e.g. safe to swim in. The biodiversity scores do, however, indicate poor water quality. This raises the

concern that there might be other parameters having a negative effect on the water quality than the ones tested for in this study.

4.2 RECOMMENDATIONS

For future studies, we would recommend that additional parameters, such as ammonium and chlorine level should be included in the study, because it would be interesting to see if these factors might be a plausible cause for the poor biodiversity results. The study should also focus on *either* the stream or the bay, in order to avoid the erroneous values obtained for the biodiversity indices in brackish water. We also recommend using kick sampling as standard sampling method in the river. Standardization of number of samples conducted at different sites is also recommended. The phosphorus and TCB samples should be taken further from the bottom substrate in order to avoid contamination, and preferably at sites with greater depths. This could be accounted for by obtaining better knowledge on time of high and low tide prior to the field excursion.

We would also recommend Bergen Municipality to obtain better control of how many WWTP there are in the area. This would enhance the knowledge on how many households are connected to WWTP, whether or not every household is connected and if they all function to set standards.

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APPENDIX A

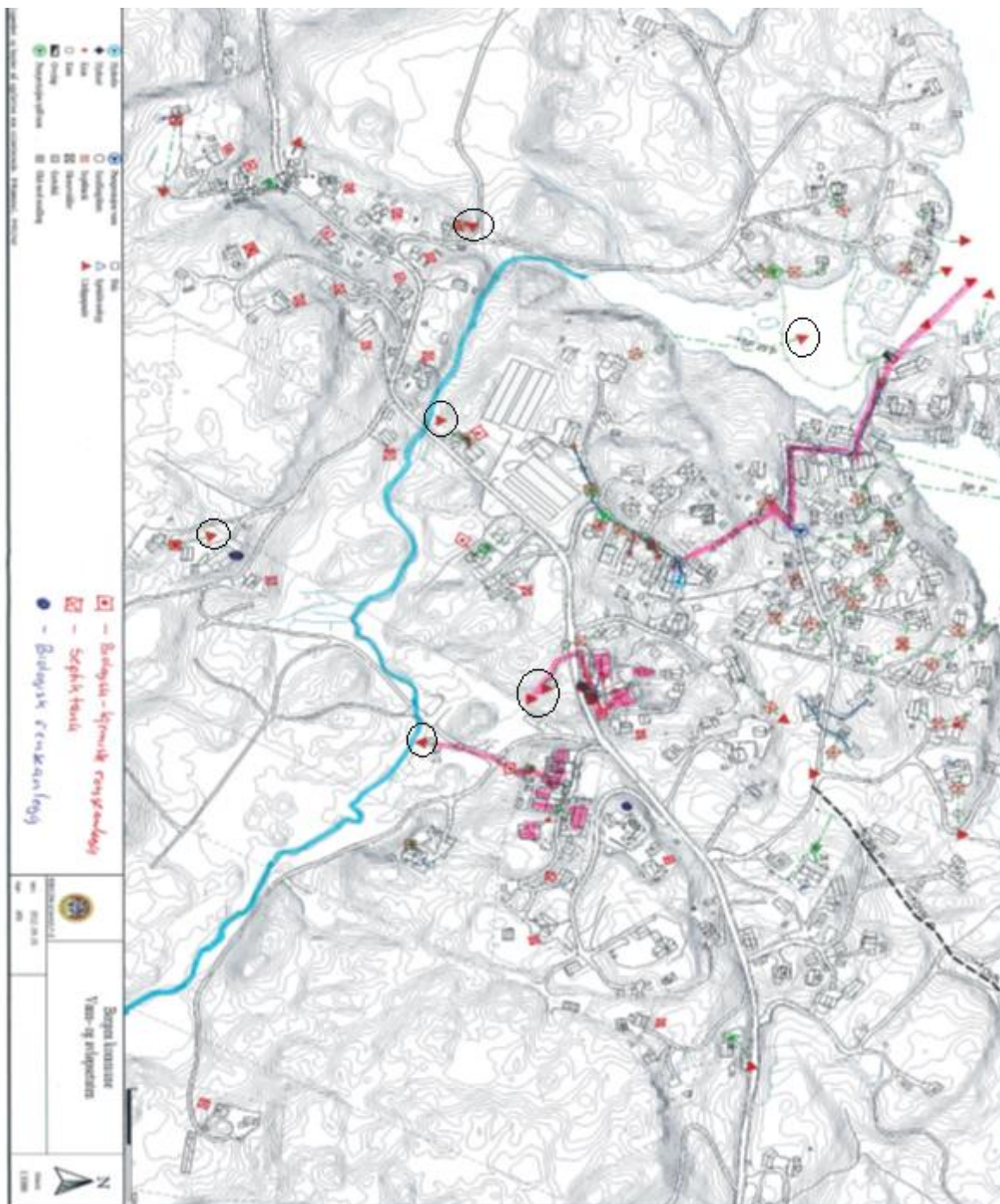


Figure A.1 - A geographical map of Grimstad supplied by Bergen Municipality. The blue line represents Høgestølsbekken and Kvernabekken, and the red triangles show output points from WWTPs. The most relevant output points are circled.

Table A.1 - Overview of this year's sites from Grimstadpollen, with comparable sites from previous studies (*Bernardes et al. 2008; Liland et al. 2010; Aryal et al. 2010; Hitchcock et al. 2012*). Since there were two studies conducted in 2010, *Liland et al.* are represented by 2010a while *Aryal et al.* is given as 2010b

Site (2014)	2008	2010a	2010b	2012
1	-	1	-	1
2	2	7	-	-
3	3	-	4	-
4	7	-	6	-

APPENDIX B

Table A.2 - TCB levels at relevant sites from previous studies done at Grimstadpollen, compared to TCB levels from relevant sites from this year's study. (Bernardes et al. 2008; Liland et al. 2010; Aryal et al. 2010; Hitchcock et al. 2012). Since there were two studies conducted in 2010, Liland et al. are represented by 2010a while Aryal et al. is given as 2010b

Year	Relevant sites	This year's sites	Mean TCB levels (CFU/100mL)	Our mean TCB levels (CFU/100mL)
2008	2	2	20	52
	3	3	70	86
	7	4	50	94
2010a	1	1	25	24
	7	2	35	52
2010b	6	4	625	94
2012	1	1	395	52

Table A.3 - Presentation of p-values for variation in TCB counts (col/100mL) and phosphorus concentration (μL) between each site in Grimstadpollen (TukeyHSD). The p-value shows no significance for the difference in TCB counts, but a larger difference in values between Site 1 and Site 4. The only p-value close to significant for phosphorus is between sites 1 and 3.

Compared sites	P-value TCB	P-value phosphorus
2-1	0.7169	0.185
3-1	0.1279	0.05
4-1	0.0740	0.1023
3-2	0.5826	0.8197
4-2	0.4104	0.9928
4-3	0.9899	0.902

Table A.4 – Phosphorus concentrations ($\mu\text{g/L}$) at site 1-4 in Grimstadpollen. Both depths and average is also presented.

Site	Depth (m)	Phosphorus ($\mu\text{g/L}$)	Average ($\mu\text{g/L}$)
1	0.1	5.4	5.00
	0.1	4.6	
2	0.1	9.5	10.8
	0.1	12.0	
3	0.5	10.0	10.0
	0.1	14.0	
	0.1	14.0	
4	0.1	10.0	10.0
	0.5	15.0	
	0.5	9.0	

Table A.5 - A comparison between years of phosphorus concentrations at site 1-4 in Grimstadpollen. Not all sites in the current study were sampled in previous years. (Bernardes et al. 2008; Liland et al. 2010; Aryal et al. 2010; Hitchcock et al. 2012). Since there were two studies conducted in 2010, Liland et al. are represented by 2010a while Aryal et al. is given as 2010b

Site Number	2008	2010	2012	2014
1		17	11.5	5
2	18	14		10.8
3	20	42		14
4		50		10

Table A.6 - Taxonomic composition at each site in Grimstadpollen, given as number of individuals found/11dm³.

Animal	Site 1	Site 2 A	Site 2 B	Site 3	Site 4	BMWP-score
Amphipoda				4		
Cardiidae					2	
Chironomidae	7					3.7
Dysticidae		7				3.5
Elmidae		2	3			6.4
Hirudididae			2			0
Hydracarina		5				
Hydrophilidae			1			5.1
Leuctridae		2				9.9
Oligochaeta	4	5	4	24		3.5
Portunidae					1	
Psychomyiidae	1					6.6
Undetermined stonefly		1				10.8

Table A.7 - Table presenting the mean for each site in Grimstadpollen and the standard deviation of the difference in TCB counts (CFU/100mL) and phosphorus concentrations (µg/L) between Site1-4.

Site	Mean TCB ±SD	Mean phosphorus ± SD
1	24 ± 13.42	5 ± 0.49
2	52 ± 28.64	10.75 ± 1.02
3	84 ± 50.30	12.68 ± 1.89
4	102 ± 58.57	11.3 ± 2.62