Water Quality in Lille Lungegårdsvann

BIO300 Group 2, 2014

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Introduction

Lille Lungegårdsvann (LLV; Figure 1) is a small lake located in the city centre of Bergen. It has an overall area of 27,800m^2, with a maximum depth of 6m and a total volume of 100,000m^3 (Johnsen & Brekke, 2008).

In the 19th century, Store and Lille Lungegårdsvann used to be two connected water bodies leading to Puddefjorden and the ocean beyond (Figure 1b). LLV used to be much bigger in size and has since been decreased extensively; today, it is only half of its original size (Knutsen & Huus, 2002). Due to increased urbanization of the city of Bergen in the 20th century, the strait between the two water bodies was filled up, and in 1926 LLV was completely disconnected from Store Lungegårdsvann (SLV). A channel was built as the only connection between the water bodies, with seawater being transferred to LLV through pipes (Knutsen & Huus, 2002; Johnsen & Brekke, 2008). As LLV and SLV refilled over the years, the channel also grew much longer and the water exchange between the two water bodies decreased significantly, resulting in LLV changing into a more freshwater lake (Johnsen & Brekke, 2008). Thus, LLV now remains in the centre of Bergen as an almost isolated freshwater habitat.

Due to its central location, LLV is at the heart of many cultural events in Bergen, and is a familiar landmark to the inhabitants of the city. However, the lake has had serious water quality problems in the past. In 2007, Bergen suffered an accidental leakage of municipal wastewater effluent into LLV, contaminating the water and causing major setbacks in the restoration of the lake (Bergen Kommune, 2010). Plans have been outlined by Bergen Kommune to reconnect LLV to the open sea – in order for this to happen, measures need to be taken to avoid a similar occurrence in the future. For this reason, emphasis has been

Figure 1a. Panoramic view of Bergen city centre, Norway, from the top of Mt. Fløyen. (Lille Lungegårdsvann can be seen as the green lake with the fountain in the bottom-right of the picture.) Credit to authors.
Figure 1b. Store and Lille Lungegårdsvann as connected water bodies 1882, as seen from the east. Credit: Knutsen & Huus, 2002.

placed on research into the water quality of LLV.

Water quality is a multifaceted concept that can usually be assessed using studies into bacterial content, eutrophication and nutrient content, and biodiversity within the lake. Water quality can fall under several criteria – for instance, the levels of *E.coli* in the water are important with regards to bathing; the higher the numbers, the more potential hazard it demonstrates. Ecological water quality, however, also means that important factors such as eutrophication and biodiversity of the lake are necessary inclusions to the research. Coliform bacteria are used frequently as bacterial indicators of environmental water quality. This investigation focuses mainly on one form of coliform bacteria, known as *Escherichia coli*. *E.coli* is most commonly found in the gastro-intestinal systems of endothermic animals, including birds and mammals (Singleton, 1999). However, most strains can live outside of the intestines of animals for limited periods of time, and so can be used as indicator organisms for faecal contamination of environmental water samples (Feng et al., 2002). A few persistent strains can survive for prolonged periods of time outside organisms, and can remain in the environment for much longer (Ishii & Sadowsky, 2008). *E.coli* is a fast-reproducing bacterium, taking only 20 minutes to reproduce under optimum conditions. It is also able to reproduce in anoxic conditions, which may be present in areas of stagnant water. This could pose a potential health hazard in LLV, in close proximity to the public – while most strains are harmless, some more virulent strains can cause health implications such as gastroenteritis if ingested. EU Directives (2006) dictate 'acceptable' levels of *E.coli* in freshwater lakes and ponds for uses in bathing and drinking, and these will be used in the assessment of *E.coli* numbers in LLV.

Eutrophication refers to naturally-occurring blooms of phytoplankton in response to increased levels of nutrients in water. Although this phenomenon occurs naturally in both freshwater and marine environments, these blooms can have negative environmental impacts over a certain threshold. For instance, when numbers of phytoplankton become too great, respiration of these increased numbers can result in depleted oxygen levels in the water (known as hypoxia) and depriving fish and other animals of sufficient oxygen. This problem can be worsened even after the phytoplankton die. As the autotrophs sink to the bottom of the water column, they are decomposed by various bacteria which consume even more oxygen through respiration in the process, leading to hypoxia or anoxia in poorly mixed bottom waters, such as in LLV (Correll, 1998). Stagnant water also holds more nutrients than well-circulated or well-mixed water bodies. This can indirectly impact on fish, and can be particularly detrimental, depriving them of oxygen and causing increased fish mortality and thus impacting biodiversity (Brattegard et al., 2011). It has also been shown that human health problems can occur when eutrophic conditions interfere with drinking water treatment, which can hamper attempts by municipalities to provide acceptable water quality for the public (Bartram et al., 1999). However, as human ingestion of the water from LLV is highly unlikely, this is not examined too much in our study.

Phosphorus is regarded as the most major nutrient in terms of stimulation of eutrophication, with addition of phosphorus contributing most to algal growth. Excessive concentrations have also been shown to be the most common cause of eutrophication in freshwater lakes (Correll, 1998). Although phosphorus occurs naturally in the environment, it is present in high concentrations in pollution sources, including in surface run-off and from sewage effluence. It is particularly common in lakes subjected to 'point source' pollution from sewage pipes, of which some have been previously connected to LLV (Carpenter et al., 1998).

The aim of this investigation is to examine the water quality of Lille Lungegårdsvann, and assess how this may impact both biodiversity and public health. This study is part of a collaboration between the Department of Biology at the University of Bergen and the municipality of Bergen (Bergen Kommune). In order to assess the water quality in the lake, various parameters of the water will be tested, including abiotic factors (temperature, salinity, pH, phosphorus) and biotic factors such as biodiversity and levels of *E.coli*. Examining all these factors will allow for appropriate recommendations of how to treat and improve the water quality, for the sake of both the freshwater ecosystem, for human health, and for improved public aesthetic value.

Material and Methods

The sampling at Lille Lungegårdsvann took place from 8.30am until 12.30pm on September 2nd 2014. The weather was sunny with an air temperature of 12-16 °C and a slight south-southeastern wind. For the three days previous to the study there had been a total of 12.8 mm of rain, with an estimated 11.5 mm the night before sampling the sampling took place (Table 1; YR, Meteorologisk Institutt, 2014).

Table 1. Weather information for one week prior to date of sampling at Lille Lungegardsvann (highlighted). Measurements are taken at Florida weather station.

Date	**Temperature (°C)**			**Precipitation**	**Wind (m/s)**	
Day	**Max**	**Min**	**Average**	**Accumulated precipitation (mm)**	**Max**	**Average**
August 27	20.9°	9.2°	14.6°	0.0	3.4 m/s	1.7 m/s
August 28	19.4°	10.3°	14.2°	0.0	4.3 m/s	2.1 m/s
August 29	18.9°	10.6°	14.1°	0.0	2.9 m/s	1.0 m/s
August 30	20.4°	12.7°	16.7°	6.7	9.7 m/s	5.1 m/s
August 31	23.7°	15.6°	18.9°	1.3	11.5 m/s	5.2 m/s
September 1	19.2°	13.9°	15.7°	0.0	7.2 m/s	3.6 m/s
September 2	**19.5°**	**11.5°**	**14.8°**	**11.5**	**4.7 m/s**	**1.7 m/s**

We had four sampling sites at Lille Lungegårdsvann (LLV) which is located in the centre of Bergen, surrounded by walkways and with busy traffic on two sides. Our four sample sites were equally distributed around the lake (Figure 2) according to previous studies (Aspaas et al., Birkeland et al. and Arntsen et al.), with some modifications to get evenly spread samples across the lake.

Figure 2. Satellite map of Lille Lungegårdsvann (LLV) and the surrounding area.
Image taken from kart.gulesider.no.

Site 1 is close to the channel which connects Store and Lille Lungegårdsvann. On the day of sampling, we observed scum and foam on the water surface at both Sites 1 and 3. There were also observations of large population of seagulls, pigeons and ducks in the pond that use the part of the square at Site 3 closest to the pond as a resting area.

Physical Parameters

Temperature, salinity, pH and oxygen concentration were measured at each of the four sampling sites. At each site, measurements were taken at the surface, at 0.5m and 1m depth – resulting in 12 measurements per physical parameter. Oxygen concentration in the water column was measured in percent (%) using an oxygen probe (WTW Oxi3310 IDS set 1 including a FDO 925), and temperature was measured in degrees Celsius (°C) using the same probe. Salinity was measured using a salinity probe (Cond3110 set 2 including a tetracon 325-3) in practical salinity units (p.s.u.), and pH was measured using a pH probe (WTW pH3110 set 2 including a SenTix 41). In each case, the probe was suspended at the appropriate depth for roughly a minute, in order for the reading to stabilise. This reading was then recorded. The probes were washed with sterilised water after each use.

Thermotolerant coliform bacteria (TCB) and phosphorous sampling

Water samples for TCB were collected from all four sites with one replicate at the surface and 1 m depth, and a single sample taken at 0.5 m, for a total of 20 water samples. These were collected by attaching a 250 ml sterile flask to the end of an extendable rod, which was lowered into the water. All samples were collected approximately 1 m from land, and for the samples at 0.5 m and 1 m the bottle was lowered with the opening facing down to avoid getting water from the higher water levels, then inverted at the correct depth. Depth was determined by a floating device attached to the rod by a rope of predetermined length. The samples were sent to Bergen Kommunes lab in Bergen, Vannlab, for analysis using the Termotol NS-4792 method, using number of colonies formed on agar per 100 ml of sample. As a high number of colony-forming units (CFU) were expected, we asked for a dilution of 1:100 in addition to the normal dilution of 1:10.

To measure phosphorus levels in the lake, water samples were taken at the surface and at 1 m depth at each site. At sites 1 and 3 one additional sample was taken at 0.5 m. Sampling was conducted using the same method as for the *E. coli* samples – however, this time we used 50 ml non-sterile flasks. In total, 10 phosphorous samples were sent to the Eurofins laboratory in Bergen for analysis using the NS EN ISO 15681-2 method. The phosphorus levels were measured in µg/litre.

Fish Counts

Counts of three-spined sticklebacks (*G. aculeatus*) were also taken on each site. To do this the person counting was holding up a pencil to help drawing an imagined line in the water from the shoreline and approximately 1 m from the shore. For one minute all fish passing the line were counted. This was done 5 times for each site, giving 5 pseudo-replicates for each site. Each crossing was counted as an individual fish. As three-spined sticklebacks are the only species of small fish to have been recorded previously in LLV (Rådgivende Biologer, 2011), all small fish were assumed to be three-spined sticklebacks.

Biodiversity

Plankton biodiversity was examined by collecting a 10-litre sample of water in a bucket at each site. The bucket was lowered by hand while standing on the shore, therefore the sample was from approximately the top 20 cm of the water column. The content of the bucket was sieved using a mesh (0.06 mm), and the sample was brought back to the lab for identification of the plankton. Sediment samples were taken from each site using a 250 ml bottle attached to the grabbing rod and scraping it against the bottom. The sample from site 1 contained small amounts of sediment and was taken back to the lab to look for animals living in the sediment. For identification of rotifers we used the "morpho-species" concept, using overall body shape to differentiate between different species, and then counting the number of each morpho-species.

The biodiversity samples were brought to the lab in a cooler. In the lab, we mixed the plankton samples with a pipette, and placed 10 ml subsamples in a plankton counting tray. The live organisms were then counted using a microscope. We identified the different organisms down to as low a taxonomic level as possible using the available identification literature and expertise. The Shannon Index was used to indicate biodiversity at each site,

The cyanobacteria were very small and extremely abundant. Therefore to estimate the amount of cyanobacteria per ml, we took one subsample of each of the biodiversity samples, and counted the cyanobacteria using a Fuchs Rosenthal counting chamber. For all samples we counted the bacteria in 16 cells of the counting chamber, running from the top left to the bottom right across the 16x16 grid. The count was then multiplied up using the standard formula for a Fuchs Rosenthal counting chamber, 1 cell/square = 8e^4 cells/ml (course compendium bio101, "organismebiologi 1"). Counts were then extrapolated to reflect the number of cells in unsieved water.

Turbidity

We collected water samples in non-sterile 50ml tubes from the surface at each site. The samples were then put in the cooler, and once back at the lab transferred to a fridge to reduce the rate of multiplication of the plankton in the sample. To measure turbidity in the samples we used a Shimadzu UVmini 1240 spectrophotometer set to measure absorbance at 550nm. We also made one control sample using tap water for comparison.

Statistical analysis

For the statistical analyses of data R was used (R Core Team (2014). "R: A language and environment for statistical computing.) Significance for p-values was set at 0.05, and forward selection was used. Syntax can be found in the appendix. To test for significance in variation in phosphorus between sites and with depth, a linear model was used. Linear models were also applied to look for significant changes in pH values with depth (in this case all sites were tested separately). To check for significance in yearly variations, this year's data was compared to data from earlier years (Rådgivende Biologer, 2008; Aspaas et al. 2010; Birkeland et al. 2011; Arntsen et al. 2013). For pH, phosphorus and salinity, linear models with the summary function was used, and for *E. coli* generalized linear models (GLM) were used with a poisson distribution together with the summary function, and a 95% confidence interval. This was done to look for significant changes in the values from year to year. The same statistical method was also applied to look for significant variation between all sites for fish counts. To see if there was any significant correlation between the numbers of sticklebacks and O_2 levels, the "nlme" package was used (Pinheiro et al., 2014) was used, having site as a random effect. For the cluster analyses of the biodiversity the "Vegan" package for R was used with the Bray-Curtis method for clustering (Oksanen et al. 2014).

Results

Physical parameters

Salinity and temperature seem to remain relatively constant with depth and between sites. A summary of the linear model revealed that there was no significant difference in phosphorous concentration between the sites at Lille Lungegårdsvann (P=0.06511), but there was a significant decrease in phosphorus concentrations with increasing depth (P=0.01214).

Table 2. Physical parameters measured at each site at Lille Lungegårdsvann

Site	Depth (m)	Temp (°C)	Salinity (sal)	pH	Phosphorus (µg/L)	Turbidity (A550)	Oxygen concentration (%)
1	0	16.0	0.2	8.23	310	0.100	93.3
	0.5	15.9	0.2	8.38	310		94.7
	1	15.9	0.2	8.5	290		94.6
2	0	15.9	0.2	8.44	310	0.104	99.6
	0.5	15.6	0.2	8.51	N/A		94.3
	1	15.9	0.2	8.53	280		95.5
3	0	15.9	0.2	8.23	340	0.108	86.8
	0.5	15.9	0.2	8.28	340		88.1
	1	15.9	0.2	8.32	310		86.0
4	0	16.2	0.2	8.48	370	0.090	96.5
	0.5	16.0	0.2	8.56	N/A		96.7
	1	16.0	0.2	8.59	300		98.7

Absorption measures of each site (measured at light wavelengths of 550nm) show that there are local variations in water turbidity in LLV across a relatively small spatial scale. Water is considered clear when absorption rates remain at 0.05 or below. The site with lowest absorption (and therefore the least turbidity) was Site 4, and turbidity increased from Site 1 through to Site 3. Furthermore, there is a clear trend in pH as it increases by depth. Significant differences in pH at depth were found at Site 1 and 3 (both $p<0.05$). Sites 2 and 4 show no significant difference ($p>0.05$); however, there is still a trend in pH as it clearly increases with depth (Table 2).

Colony-forming Units of Thermophilic Coliform Bacteria

All sites had values below 1000 CFU/100 ml. *E.coli* numbers varied between sites and depths at LLV, with the maximum recorded numbers at Site 3 (Figure 3). Additionally, the oxygen levels are much lower at Site 3 compared to the other sites, indicating a possible relationship between the two factors. At Sites 1, 2 and 4 where CFU numbers are much lower, high oxygen values are shown. All values found exceeded 100 CFU/100 ml and are classified as 'Less Good' according to Norwegian water quality standards (Folkehelseinstituttet, 1994).

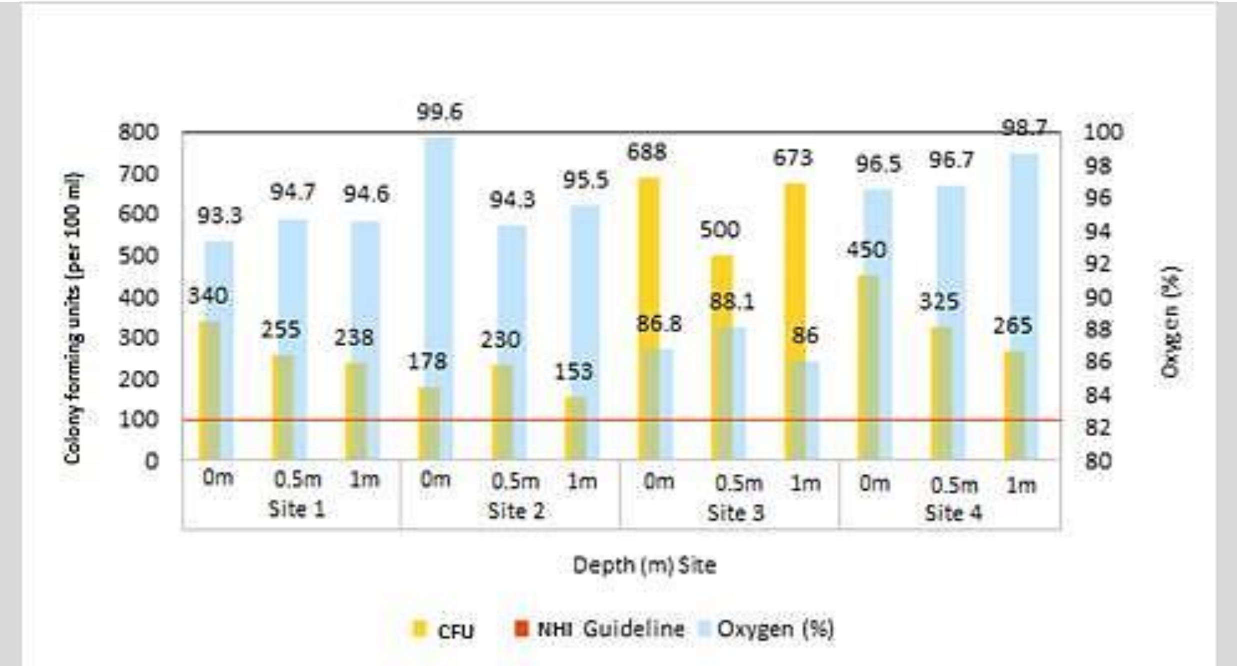

Figure 3. Bacterial colony-forming units (CFU) at each depth and Site number of Lille Lungegårdsvann, compared to the associated oxygen content of the water and the Norwegian water quality standard for 'Good' CFU numbers in freshwater for bathing areas by the Norwegian Health Institute (Folkehelseinstituttet, 1994).

There is a high variance in CFU values between the years (Figure 4). In 2010 and 2011, *E.coli* was only measured from one site, and in 2013 three sites were measured. From this year's study, *E.coli* was measured from four different sites, and Site 3 shows a significant elevation compared to Site 1, 2 and 4 ($P<0.001$). When comparing Site 3 between 2013 and 2014, there is a highly significant difference, with much higher CFU values from this year's study ($P<0.001$).

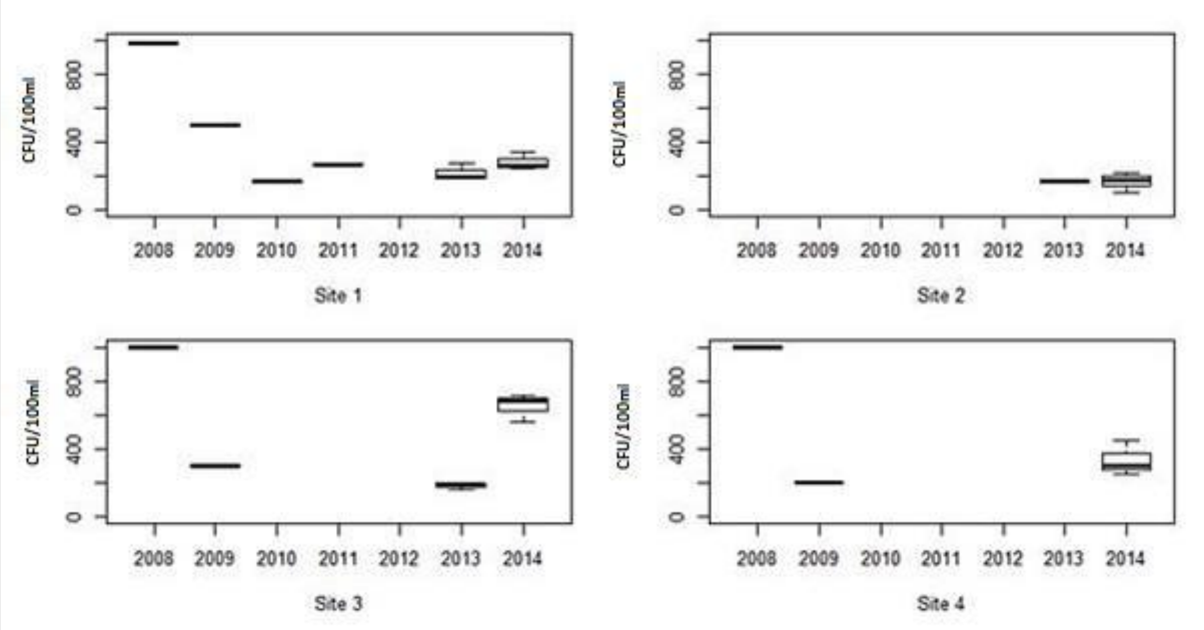

Figure 4. CFU values for available sites in Lille Lungegårdsvann from 2008 and 2009 (both from Radgivende Biologer), 2010 (Aspaas et al.), 2011 (Birkeland et al.), 2013 (Arntsen et al.) and from this year's field study.

There is a significant trend towards lower concentrations of CFU in earlier years ($P<0.001$). The reason for higher numbers than last year seems to be influenced by rainfall, 12.8 mm recorded for the sampling day and 2 days previous, compared to earlier years where they have had either very little (<1 mm) or very high amounts of rain (>60 mm). If sampling is done under similar weather conditions for the future this could give results that are more consistent.

Biodiversity

Low abundance and biodiversity of zooplankton were collected at all four sites and a total of 10 taxa were found (Figure 5). Site 4 shows the highest abundance of zooplankton with 1232 individuals in total, and Site 1 shows the lowest abundance with a total of 751 individuals. Both Sites 1 and 4 show the highest taxonomical diversity, with 9 taxa in total. Rotifers are clearly the most dominant group where Rotifer A is the most abundant species at all sites. In addition, there were highly variable numbers of cyanobacteria for all sites at LLV; 0, 900, 87 and 312 cells/mL for Sites 1-4 respectively.

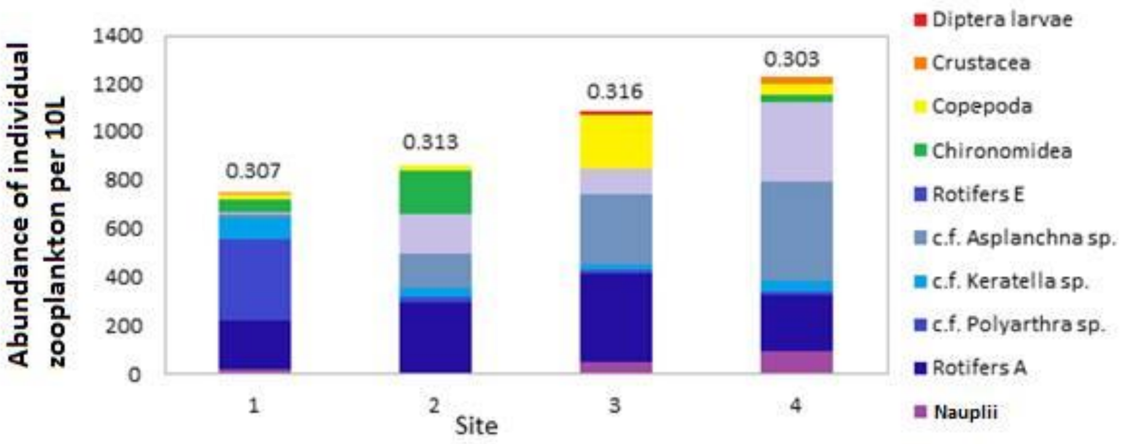

Figure 5. Abundance of zooplankton for each site at Lille Lungegårdsvann per 10 litres of water. Values above columns represent Shannon index numbers for each site. ('c.f.' - uncertainty in identification). All columns in blue represent rotifers, which are classed as indicators of pollution.

Using the Bray-Curtis similarity index on a cluster dendrogram (Figure 6), we can see that Sites 2, 3 and 4 are very close in species composition, and Site 1 differs most from the others. The species *c.f. Polyarthra* sp. and *c.f. Kertatella* sp. seem to be more abundant in Site 1, while rotifers *c.f. Asplancha* sp. and Rotifers E are more abundant in Sites 2, 3 and 4.

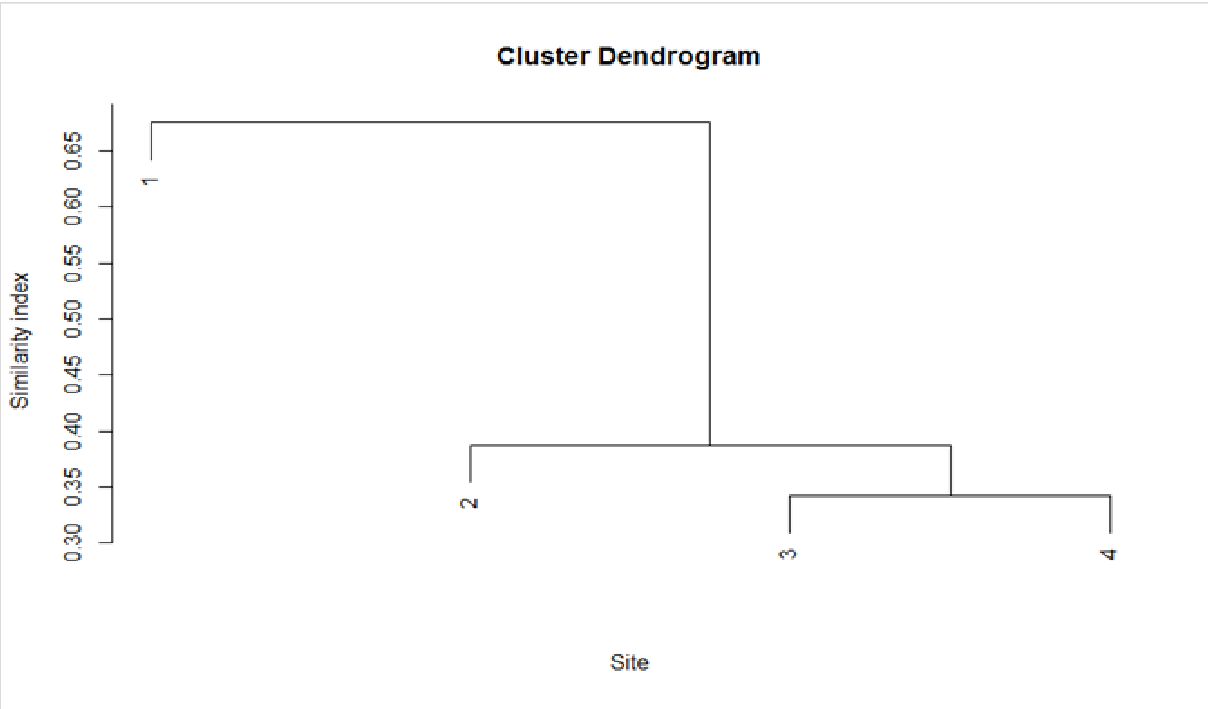

Figure 6. Cluster dendrogram showing the Bray-Curtis similarity index of zooplankton abundance between all sampling sites at Lille Lungegårdsvann.

The fish count data showed high local variation in fish numbers, with Site 3 having the lowest abundance (Figure 7). A significant difference between number of stickleback sightings was found at all sites (ANOVA; p<0.001) except for between Sites 1 and 4, where although insignificant (ANOVA; p=0.07), the p-value is close enough to the threshold of significance to not be disregarded completely.

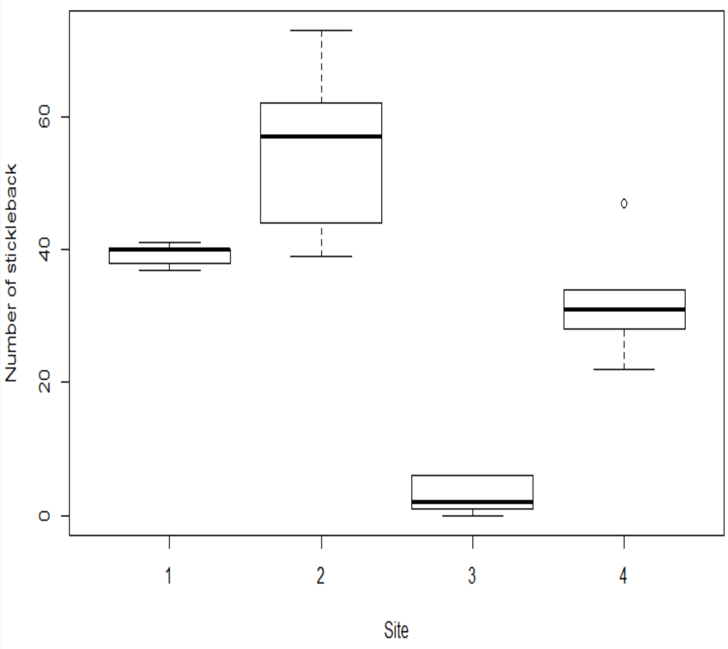

Figure 7. Number of sticklebacks observed over 1 minute at each site at Lille Lungegårdsvann (observed as 5 pseudoreplicates).

Biodiversity in the sediment was only assessed at Site 1 (due to trouble collecting sediment from the other sites), where a total of 7 species were found (Table 3). Copepoda was the most dominant taxa, with a total of 15 individuals, closely followed by Ostracoda A and B with a total of 11 individuals. In addition, 11 individuals of the ramshorn snail were found.

Table 3. Abundance of taxa found at Site 1 in the sediment sample of LLV.

Taxa	Number of individuals
Nais sp.	1
Family Chironomidae	7
Copopoda	15
Ostracoda A	11
Ostracoda B	11
Ostracoda C	5
Planorbidae (ramshorn snail)	11

Discussion

Overall, the water quality of Lille Lungegårdsvann is within reasonable standards for urban freshwater lakes. The water is well-oxygenated (all above 80% saturation), cyanobacteria numbers are low, and the CFU of TCB at Site 2 could be considered 'Good' by Norwegian standards (Folkehelseinstituttet, 2012). However, TCB was deemed 'Less Good' at the other 3 sites. With extremely high averaged phosphorus measurements and low biodiversity throughout the lake, there is room for improvement.

High numbers of small rotifer taxa such as *c.f. Polyarthra* sp., *c.f. Kertatella* sp., and c*.f. Asplancha* sp. were identified in LLV. Several zooplankton species were identified and high numbers of copepoda were also counted but there was a clear dominance of several rotifer species. It has been suggested that small rotifers are an indicator of poor water quality and tolerate low water quality and pollution (Gannon & Stemberger, 1978; Branco et al., 2002; Vandysh, 2003). Further, a Shannon index was calculated and low numbers were shown, indicating that LLV contains low biodiversity. Factors such as high levels of toxins and little or no water exchange with other water bodies may be the some of the reasons for the relatively low abundance of zooplankton found in LLV. At site 1, where the channel is located, there was a very different taxa composition compared to the other 3 sites. There may have been some errors in the classification of species as identifying zooplankton down to species level was extremely difficult. Furthermore, one possible reason for the differences in species composition could be that some zooplankton species prefer a certain pH, as similarities in pH were shown at Site 1 and 3. Both sites had a pH of 8.23 in surface water where zooplankton species were collected, whereas Sites 2 and 4 were more alkaline with a pH of 8.44 and 8.48 respectively. However, Site 1 and Site 3 did not show any similarities in species composition.

Sampling of the sediment for benthic organisms was difficult as LLV has a mostly rocky bottom along the edges; only Site 1 was successfully sampled and studied. At this site several taxa of ostracoda and one taxon of copepoda were found together with several ramshorn snails. However, this is insufficient data to state anything about the water quality and biodiversity of the benthic organisms living in LLV. For future studies, we would recommend that sediment is sampled at deeper waters, preferably by boat.

Cyanobacteria were present in samples for sites 2 and 4 in LLV (900,000 cells/L), while almost absent at sites 1 and 3. This is most likely a result of methodical errors using the cell-counting chamber, as cyanobacteria had already been observed in large numbers in the subsample used for biodiversity, and both the subsample for biodiversity and cyanobacteria came from the same sample. In reality, we would expect much higher numbers of cyanobacteria than observed in our samples. Cyanobacteria can under eutrophic conditions form algal blooms, resulting in dense surface scum and an accumulation of toxins. In previous years, much larger numbers of cyanobacteria were found (>31million cells/L) in LLV and identified as *Planktothrix cf. agardhi* and *Lyngbya* sp. (Rådgivende Biologer, 2008). Some *Lyngbya* sp. are known to cause toxic seaweed dermatitis in humans (Werner et al., 2011), while *Plantothrix* is commonly known to produce hepatoxins, which can cause liver damage (Sivonen & Jones, 1999). No toxins were evident in the sampling from 2008, but little is known about what causes some cyanobacteria to start producing toxins.

We would have expected higher counts of cyanobacteria, as the mean values of phosphorus found in LLV were extremely high (316 µg/L). This shows that LLV should be at very great risk of algal blooms under the right seasonal conditions i.e. higher temperatures. This is the highest concentration of phosphorous across all years of study in LLV except in 2011 when it was measured at an average 730 µg/L (Birkeland et al.). These levels are classified as 'unacceptable' for bathing (Heggøy et al., 2005). This also exceeds the value to be classified as poly-eutrophic by a factor of six (Heggøy et al., 2005). Even concentrations of phosphorus as low as 20 µg/L have shown to be problematic in causing eutrophication in freshwater lakes (Correll, 1999), so we know phosphorous is not a limiting factor in cyanobacteria numbers. Excessive concentrations of phosphorus are considered the main cause of eutrophication in freshwater lakes, and are likely to be the main factor for seasonal algal blooms in LLV. So why were so few cyanobacteria found in our samples?

The levels of cyanobacteria have previously been controlled successfully by maintaining a population of predatory fish in LLV. This caused a drop in the level of sticklebacks, which in turn caused the population of algae-eating zooplankton to increase. The release of predatory fish lasted from the early 1980s until 1988, and in the period from 1985 to 1992 there were no recordings of cyanobacteria blooms (Rådgivende Biologer, 2008). In April 2014, 600 brown trout were released into LLV, and the increased feeding rates of the introduced predatory fish may explain the disparity in cyanobacteria numbers found in our study (Kvamme, 2014).

No other phytoplankton was observed in any of our samples, as cyanobacteria outcompete other phytoplankton under hypereutrophic conditions as the potential for cyanobacteria dominance increases steeply when total phosphorus (TP) increase from 30 to 100 μg/l (Havens, 2008). At average phosphorus levels above 100 μg/L cyanobacteria make up over 70% of the total biomass, and there may be a steep increase up to 100% of total biomass with increasing levels of phosphorus (Watson et al., 1997). Persistent cyanobacteria blooms can also outcompete benthic plankton and vascular plants, by reducing irradiance to deeper water levels (Havens, 2008). It is also indicated that exposure to some cyanobacteria toxins can have a negative effect on growth potential in juvenile sticklebacks (Pääkkönen et al., 2008). However, an error in the counting of cyanobacteria is a strong possibility, and high numbers of cyanobacteria at all sites would seem more likely. This data is not sufficient to suggest a direct link, due to the previously mentioned problems of miscounting errors.

The pH value in LLV shows an average of 8.42 between all sites for 2014 – this is an increase of roughly 1 logarithmic unit from earlier years, making it more alkaline (Aspaas et al. 2010, Birkeland et al. 2011, Arntsen et al. 2013)). This increase in pH from earlier years could be due to the extremely warm late-summer temperatures seen in Bergen in 2014, and an increase in primary production. Some of this variation could also be explained by the yearly variation of pH in freshwater bodies, with pH maxima in the summer when primary production is the highest, and pH minima during winter (Berezina, 2001) and a more limited daily variation (Whitney R. J., 1942). This seasonal fluctuation should as a rule not exceed pH 6.5-8.5, meaning 4 out of our 12 measurements for pH are considered abnormally high for fresh water habitats (Berezina, 2001). While still within the values given by the EU as 'acceptable' for bathing quality (pH 6 to 9, as quoted in EU Directive 76/160/EEC), pH values can still influence other aquatic organisms in terms of reproductive success and survivability (Berezina, 2001). In one case, cyanobacteria blooms were found to increase the pH of a shallow water tributary to 9-10.5 for weeks (Gao et al., 2012). If a similar occurrence would happen in LLV, this could affect the abundance of Ramshorn snails that graze on dead plankton that has sunk to the floor of LLV, as mortality of these increase for pH values above 9 (Berezina, 2001), and potentially lead to even more extensive algae cover in LLV.

The salinity at all sites in LLV was measured at 0.2 ‰, meaning that at least the surface water of LLV is almost entirely freshwater. Reports from earlier years have indicated that the bottom waters are slightly more saline, due to influx of brackish water from Store Lungegårdsvann (SLV) and diffusion of ions from old marine sediment deposits from times before LLV was cut off from SLV (Rådgivende Biologer, 2011).

A surprising find was that TCB numbers decreased with depth at Sites 1 and 4 (Figure 3), but pH increased with depth at all sites (Figure 3). One suggestion could be due to the poor circulation of the water column – evidence from Avery et al. (2008) has shown that TCB shows better survival in stratified and stagnant waters such as lakes and ponds than in running or well-mixed waters such as rivers. TCB is associated with lower pH (i.e. more acidic conditions) due to the breakdown of detrital matter, so why would there be fewer CFU at depth? It provides a contradiction to research from Pachapsky & Shelton (2011), who stated that bottom sediments act as TCB "reservoirs", and resuspension of sediment may cause increases of TCB. This may, however, be explained by the poor circulation of LLV – with no flow for resuspension, CFU values remain low at depth. Instead, comparatively high numbers of CFU in surface waters may be a result of surface runoff from the surroundings of LLV. This was previously investigated by Vinten et al. (2004), showing that surface runoff was found to be a major problem in contamination of bathing water in Scotland. Immediate sources of this surface run-off could potentially include walked dogs, and the faeces of a particularly large population of birds, whose resting area was sighted near Site 3 at LLV. This is an important factor to consider if Bergen Kommune ever considers developing LLV into a public bathing area. Another considerable factor may be that not only do sediments contribute to TCB levels in urban lakes, but TCB numbers are strongly associated with rainfall (Staley et al., 2013).

Bergen is notorious for its high annual rates of rainfall – due to its situation in the Norwegian fjord network, it is subject to relief rain from the surrounding mountains. This antecedent rainfall has been shown to have a variety of effects on *E.coli* and other faecal bacteria numbers in freshwater environments. For instance, Lucas et al. (2014) found that storm events cause significant dilution of TCB in freshwater, meaning decreased numbers in the water column. However, this opposes findings from Chen & Chang (2014), who suggested that urban watersheds exhibit a stronger response of TCB to precipitation due to a 'higher degree of imperviousness'. One example of this in the case of LLV could be the area around Site 3, known in Bergen as 'Festplassen'. Concrete steps lead all the way down to the edge of the lake, and so provide an impervious surface for higher surface runoff. Similar examples have been demonstrated by previous research undertaken in Argentinian rivers that suggest that water quality (i.e. number of faecal bacteria including *E.coli*) is adversely affected by urbanization (Almeida et al., 2007). Future research could enable prediction of *E.coli* response to storm activity, and so aid effective watershed management decisions.

Another factor to consider is the seasonal nature of rainfall. Research on *E.coli* responses to seasonal rainfall in North Carolina, USA shows that numbers vary significantly by season throughout the year (Hathaway et al., 2014). It can be expected that increased numbers of *E.coli* are found in urban freshwater lakes in the summer months, as the increased temperature is more optimal to bacterial reproduction (Chen & Chang, 2014). However, a limitation with the data collection carried out for this project and in previous years is that all data has been collected in early autumn (September) each year, and so these data cannot demonstrate any seasonal variation. However, by comparing *E.coli* data from this year to previous years, it could be suggested that an increase may be a result of drier, hotter weather in summer (with much anecdotal evidence that this may be the hottest summer Bergen has experienced for many years). This hotter weather would mean less dilution of the water column in LLV, higher temperatures of surface water, and thus increase the rate of reproduction in TCB.

Overall, the highest CFU (688 CFU/100 ml) was found at Site 3, closest to Festplassen (Figure 4). This is only slightly above the maximum of 500 CFU/100 ml regulated for freshwater bathing areas (Folkehelseinstituttet, 2012). As mentioned in the Introduction, TCB (particularly *E.coli*) is found primarily in the gastro-intestinal systems of warm-blooded animals, and enters freshwater systems through faecal contamination. There are many sources of freshwater faecal contamination, including through contaminated surface runoff, entrainment of sediments (as previously discussed), from resident animal populations, and from sewage exfiltration (Guerineau et al., 2014). During data collection, many birds were seen to be living along the banks of Site 3 of LLV, and it is possible that bird excrement could be a potential source of faecal contamination (and therefore increased TCB numbers). One report found that a maximum of 2200 common gulls (*Larus canus*) had once been observed in this area, with an average of 200-300 gulls and 30-70 ducks on a regular day (Rådgivende Biologer, 2008). Different strains of TCB can be host-specific, meaning that if genetic research is carried out to assess the strain present in that environment, it is possible to find the source of the contamination. For instance, it would be possible to assess whether the *E.coli* came from a mammalian or avian source (Feng et al., 2002; Thompson, 2007).

Another possible source of TCB at Site 3 could be sewage leakage or exfiltration. In 2007, an incident involving the misconnection of sewage pipelines at this site caused the leakage of raw sewage into LLV, and prompted a strong effort from Bergen Kommune to attempt to restore the water quality of the lake. From TCB data dated from 2008 (Figure 3), a notable peak in CFU numbers can be seen, which may directly result from this sudden influx of organic material, with this enrichment followed by bacterial breakdown. Previous studies support these findings, showing that highly urbanized areas are more vulnerable to sewer exfiltration in surface water and groundwater (Guerineau et al., 2014). To investigate the potential sources of faecal contamination in LLV, future research on the genetic material of TCB could be carried out, using ‘microbial source tracking’ (Ishii & Sadowsky, 2008). As most strains of *E.coli* (a proportion of the TCB) are host-specific, a source could be easily identified as of avian or mammalian origin, and can enable management of the problem of increased *E.coli* numbers in the lake.

Szabo & Minamyer (2014) suggest that flushing and chlorination of freshwater lakes and ponds can help return a water system to a healthy state for humans. However, most common disinfectant have ‘limited effectiveness’, and this process can adversely affect fish populations (such as sticklebacks and trout) that live in LLV. An alternative solution could be to either disallow feeding of the birds or remove them completely, thus removing any further faecal contamination. Prevention of litter and dog fouling would also reduce the number of TCB breaking down organic matter in the lake system. Improvements to the infrastructure of the sewer pipelines to prevent leakage similar to the event in 2007 could also help, as well as potentially developing infrastructure to aid circulation of the water column. These solutions appear costly and time-consuming, but the long-term benefits to public health far outweigh the negatives – it is much easier to treat the source of pollution than to treat large numbers of people made ill by poor water quality.

During investigation of TCB numbers at LLV, a few problems were encountered. Firstly, the laboratory results from VannLab Bergen appear to be mismatched when comparing dilution factors – to aid comparison, these numbers were averaged in the Results. This could be a case of human error, and could potentially throw the validity of these results into question – therefore further analysis would be needed in future years. Secondly, past years have used different Sites at LLV, meaning there are some gaps in the data where there is no direct comparison. It can be suggested that in future years, the same sites should be used in order for more valid statistical analysis. Finally, all research was carried out in autumn (at the beginning of September every year), so there is no seasonal variation to show in the data. Therefore, further research is needed throughout the year in order to accurately observe trends in *E.coli* with changing temperature and rainfall.

Overall, the levels of TCB are considered 'Less Good' by FHI (Folkehelseinstituttet, 2012), phosphorus levels are abnormally high (Heggøy et al. 2005), biodiversity at all sites is low (with the presence of pollution indicator species), and pH is more alkaline than previous years. This means the water quality status of LLV is not ideal, but has the potential for improvement. Currently, the high phosphorus concentrations mean LLV is at high risk of eutrophication and the onset of toxic algal bloom events, if conditions allow. As a suggestion to improve the water quality, Bergen Kommune could put forward a public appeal to prevent littering, fouling and other potential sources of bacteria into the lake. In addition, mechanisms could be implemented to aid circulation of the water in LLV, and decrease the likelihood of eutrophication events and toxic algal blooms.

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Appendix

Syntax for pH boxplot

```
> ph.df <- read.table('clipboard', header=T)
> attach(ph.df)
> boxplot(ph.df)
> boxplot(pH~Depth)
> boxplot(pH~Depth, xlab='Depth(m)', ylab='Ph')
```

Ecoli variation by year

```
> par(mfrow=c(2,2))
> site1.df <- read.table('clipboard', header=T)
> attach(site1.df)
> boxplot(ecoli~year, xlab='Site 1', ylab='E.coli/100ml', ylim=c(0,1000))
```

Syntax for yearly variation used for e.coli, salinity, pH, and phosphorus.

```
> ecoli.df <- read.table('clipboard', header=T)
> attach(ecoli.df)
> plot(ecoli~year)
> ecoli.glm <- glm(ecoli~year, poisson)
> summary(ecoli.glm)
```

Syntax for fishcount.

```
> fish.df <- read.table('clipboard', header=T)
> attach(fish.df)
> boxplot(Stickleback.count~Site)
> fit1.glm <- glm(Stickleback.count~Site, poisson)
> anova(fit1.glm, test='Chi')
> summary(fit1.glm)
```

Summary using site 3 as baseline for comparing

```
> summary(fit1.glm)
```

Effect of 02 on fish numbers.

```
> o2fish.df <- read.table('clipboard', header=T)
> attach(o2fish.df)
> library(nlme)
> mixed.lme <- lme(Stickleback.count~o2, random=~+1|Site)
> summary(mixed.lme)
```

Syntax for dendrogram

R script for cluster dendrogram

First install vegan package for R

```
> library(vegan)
> dataframe <- read.table('clipboard', header=T)
> dist.mat <- vegdist(dataframe,method='bray')
> clust.res<-hclust(dist.mat)
> plot(clust.res, ylab='Similarity index', xlab='quadrat')
```

<u>Raw data for *E.coli* results</u>

N.B. Sample numbers 1-5 were collected from Site 1, sample numbers 6-10 were collected from Site 2, sample numbers 11-15 were collected from Site 3, and sample numbers 16-20 were collected from Site 4.

Analyserapport E. coli:

dilution sample nr	1:10	1:100
1	300	400
2	360	300
3	310	200
4	240	200
5	310	200
6	230	200
7	180	100
8	260	200
9	210	200
10	100	100
11	530	500
12	920	800
13	500	500
14	550	700
15	540	900
16	340	700
17	460	300
18	550	100
19	360	200
20	300	200