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Colonisation and Growth of Benthic Algae in a Recently Deculverted Urban Stream

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Abstract

Stream restoration by the process of deculverting is a comprehensive form of restoration which aims to improve morphological, chemical and ecological condition through the re-creation of a naturalized stream from a piped channel. Norway has adopted the process, and the municipalities are increasingly attempting to restore culverted streams wherever possible, particularly in the city of Oslo. It is, however, largely unknown how aquatic ecosystems will respond to the substantial changes associated with deculverting. This study examines the colonisation and growth of benthic algae assemblages a period after deculverting in a newly restored reach of the stream Hovinbekken in Oslo. Samples were collected monthly from May to November 2016 and in May and June the following year. Accumulation of benthic algae and concentration of nutrients were investigated to determine the stream's ability to self-clean downstream the restored reach. Colonisation and composition of benthic algae taxa were investigated to determine whether the newly established ecosystem showed signs of being normal functioning. The ecological status of the restored reach was determined using benthic algae as indicators according to the periphyton index of trophic status (PIT). Additionally, this study assesses the performance of the BenthoTorch, an in-situ tool that provides estimations of algal biomass measured as Chlorophyll-a.

Results from the samplings showed no clear evidence of an overall decline in concentrations of nutrients along the restored reach. Neither was the differences in nutrient concentration along the restored reach significantly related to algal biomass measured as estimated percent cover. High levels of nutrients were conceivably a contributing factor for the massive growth of the cyanobacterium *Oscillatoria sancta* and the filamentous green algae *Spirogyra majuscula* which occurred during both seasons of sampling. Additionally, the estimated percent cover of benthic algae was generally high in the restored reach, which after a period of drought rapidly accumulated at the sites that had been affected by these disturbances. Furthermore, according to PIT, the classification for all sites in the restored reach ranged between moderate and very poor ecological status. And, there were no significant differences in PIT downstream along the restored reach. Thus, the results of this study imply that nutrient was not a limiting factor for algal growth in the restored reach and that levels of nutrients were too high for algae to noticeable control the nutrient dynamics in the stream. Conceivable, without reducing levels of nutrients contaminating the water from upstream sources, it is likely that the benthic algae growing in the restored reach would only be limited by substrate space on which to colonize.

Results from the samplings showed that the algal community in the restored reach had a significantly larger richness of benthic algal taxa in 2017 compared to the previous season 2016. An interesting observation was also that new taxa were establishing in the restored reach in 2017. And, a temporal pattern of seasonal succession in the benthic algae community occurred during both seasons, a seasonal shift which happened earlier in 2017 than in 2016. Thus, these findings indicate that the dynamics of the benthic algal ecosystem were showing signs of functioning normally. Findings also suggest an ongoing colonisation process among benthic algal communities in the restored reach.

Results from assessing the BenthoTorch showed that μg chlorophyll-a/cm^2 measured by the BenthoTorch was significantly related to benthic algal volume estimated as percent cover. However, there was a large degree of deviation from the regression line. Thus, results of this study demonstrate that the BenthoTorch should be used with extreme care as there is a risk that the BenthoTorch will greatly under or overestimate benthic algal biomass.

Preface

This is a Master's thesis in the study program Environmental and Natural Resources within the specialisation Limnology and Water Resources, at the Norwegian institute of Life Science. This study is conducted in collaboration with the Norwegian Institute for Water research - NIVA.

My supervisors Susanne Schneider and Therese Fosholt Moe are gratefully acknowledged for help during field work, supervision the laboratory and critical feedbacks during the process of writing. Thanks also to Susanna Burgess and David Arnott for assistance and cooperation during sample collection.

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Contents

1 Introduction

Background

Streams and rivers in urban watersheds are frequently routed into culverts and buried during the process of urbanisation. Contaminated water is transported away through pipes, releasing new areas for industrial and residential development (Walsh et al., 2005, Elmore and Kaushal, 2008). Indeed, this is something resembles Oslo's development. During the 20th century, the city of Oslo grew larger, which led to more industrialisation and increased population density. Urban streams were considered as an obstacle for development and construction, and consequently, several streams were buried and routed through culverts. The whole watershed of Oslo has been affected by this practice and today, approximately two-thirds of the water ways in Oslo are closed and buried in favour of roads and buildings (Oslo kommune, 2015, Moland, 2017).

Although little empirical research has been undertaken on the topic, there are consistent symptoms showing that the practice of culverting streams has several environmental costs. Routing the water through a pipe fundamentally changes the structure, the morphology and the hydrology of a stream. For example, in a concreted pipe the streams natural contact with the groundwater and surrounding catchment area disappears and the natural elevations and fluctuations of the stream are replaced by a straight channel (Walsh et al., 2005, Meyer et al., 2005). The available studies on environmental effects of culverted streams have also shown that the practice has several negative consequences for the function of aquatic ecosystems. Hope et al. (2014) and Beaulieu et al. (2014) have reported that decreased light availability in culverts negatively affects the retention of nutrients and the rate of primary production. Kreuger (1998) found that culverts contribute to an increased transport of pesticides and contaminants due to a lack of dilution by groundwater. Others have reported that culverting leads to a decreased richness in macroinvertebrate taxa and downstream habitat degradation (Nealea and Moffett, 2016, Meyer et al., 2005).

In the late 1990s however, after inspiration from abroad, the trend of burying streams was reversed in Norway and the government has begun to restore urban streams by a process referred to as deculverting (Oslo kommune, 2015). This deculverting trend accelerated when the Water Framework Directive (WFD) was introduced to national legislation in 2006 (Lovdata, 2007). WFD has been a driving force behind the allocation of resources to deculverting projects in urban areas, and in Oslo, several reaches of the main streams Akerselva, Alnaelva and Hovinbekken have been deculverted since the trend was adopted (Oslo kommune, 2015). Several reasons explain why deculverting projects have become important priorities. For instance, global warming is predicted to lead to more rapid, heavier rain falls. Oslo, along with many other cities with its paved areas, needs better storm-water management. If the city has more open waterways, the capacity to convey floodwaters will increase (NCCS, 2015). And, according to WFD, deculverting streams can contribute to ensuring a good ecological and chemical status of water bodies (EC, 2000). Increasing the amount of urban streams also makes a positive contribution to the urban environment for the people living in the city. People are attracted to water environments, and urban streams can also provide recreational areas.

Deculverting is considered as one of the most extensive forms of stream restoration because the practice means establishing a new naturalized stream (Elmore and Kaushal, 2008). Norwegian deculverting projects, along with deculverting projects worldwide, are carried out with a vision of re-establishing the ordinary aquatic ecosystem with a rich biodiversity and increased water quality (EEA, 2016, Oslo kommune, 2015). In theory, such environmental benefits are achieved by opening up the channel to sunlight and restoring the natural stream structure so that flora and fauna will start to colonise it (Meyer et al., 2005). Even so, few empirical studies focusing on whether or not such ecological benefits are actually achieved in these projects, and there is a lack of long term monitoring on the effects arising from deculverting (Nealea and Moffett, 2016, Pander and Geist, 2013). The municipality of Oslo has decided to give the water back its place in the cityscape (Oslo kommune, 2015). This represents a perfect opportunity to explore the environmental and ecological effects of deculverting in practice, an assessment which can be of broader significance to other cities and communes who might be considering whether and how to deculvert.

Benthic algae

Biological indicators are useful for monitoring the effect of changing environmental conditions in streams (Friberg et al., 2011). One such indicator commonly used for monitoring is Benthic algae. These are algae, including cyanobacteria, that attach to substrata in the benthic zone of a stream (Allan and Castillo, 2007). The reason why they make good indicators is that benthic algae are sessile, which means they cannot avoid contaminated water by migration. Each taxa of benthic algae have its own preferences and tolerance according to nutrient concentration, substrate, access to light and temperature, and respond rapidly to changes in these conditions (Lowe and Yangdong, 1996). Assessing the abundance and taxon composition of the benthic algal assemblages growing in a stream therefore can provide an integrated evaluation of the stream's health over time (Schneider and Lindstrøm, 2011, 2009). By investigating the development of benthic algae during a period after deculverting, it is possible to evaluate the development of the new ecosystem, and the ecological conditions of the stream after such restoration (Pander and Geist, 2013).

Urban streams tend to be contaminated by high levels of nutrients and chemical pollutants (Walsh et al., 2005). Such conditions might alter benthic algae assemblages and structure, possibly leading to the dominance and massive growth of a restricted range of tolerant taxa (Scrimgeour and Chambers, 2000, Sabater et al., 2000). Urban streams usually flow through populated areas, and deculverted streams are often constructed with the intention of being recreational areas for the people living in the city. Massive growth of benthic algae may be associated with water quality problems and give the impression of residing in an unhealthy environment (Sabater et al., 2000). Hence, knowledge of how the biotic responds to the changes associated with deculverting will be useful for the municipality to be able to provide information to the public of what expectations to have regarding water quality and visible algal growth. Additionally, such assessments will be helpful to reveal upstream sources of contamination, and possibly to develop guidelines for the planning and construction of future deculverting projects.

Aim of study and hypothesis

This study examines development of benthic algae assemblages during a period after deculverting in a newly restored stream (Hovinbekken) in Oslo. Hovinbekken is a highly deculverted stream, and parts of it still runs through culverts upstream of the restored reach this study investigates (Moland, 2017). Sampling of benthic algae was undertaken during the period between May and November in 2016 and in May and June 2017. The establishment of the new ecosystem was evaluated by investigating the abundance and composition of benthic algae taxa. Benthic algae were also used as indicator organisms to determine the ecological status according to the periphyton index of eutrophic status (PIT) in the restored reach. The restored reach is designed to be a self-purification facility.

The system's ability to purify the water was evaluated by examining the growth of benthic algae and the concentration of nutrients measured along the restored reach.

Monitoring algae growth is a comprehensive procedure, which requires considerable expertise to be carried out. Given the lack of empirical studies on the ecological benefits and biotic responses to deculverting, there is a need for a more accessible procedure of monitoring algal growth. A relatively new, simpler method of measuring algal biomass by using the in-situ tool BenthoTorch (bbe Moldaenke, 2013) is being tested in this study, in addition to the conventional methods performed. This is used to evaluate whether the BenthoTorch gives results of algal biomass consistent with the results of the conventional methods.

In my thesis, I tested the following hypothesis:

1. Self purification will improve the water quality downstream the restored reach. This will be reflected by downstream differences in abundance and composition of benthic algae, declining values of periphyton index of trophic status (PIT), and decreased concentrations of ammonium, nitrate and phosphate.

2. The algal communities found will be dominated by benthic algae assemblages which are either associated with pollution or are tolerant to eutrophic conditions.

3. The restored reach will show signs of ecosystem stabilization throughout the sampling period in terms of establishing a diverse benthic algae community. This will be reflected in a more taxon rich algal community in 2017 than in 2016.

4. There will be a correlation between estimated percent cover of benthic algae and total chlorophyll-a measured by the BenthoTorch.

2 Materials and methods

2.1 Site description

The field work of this study was carried out in a recently de-culverted reach of the stream Hovinbekken, situated north east in the city of Oslo (Fig. 2.1). The de-culverted section is in this study referred to as the restored reach.

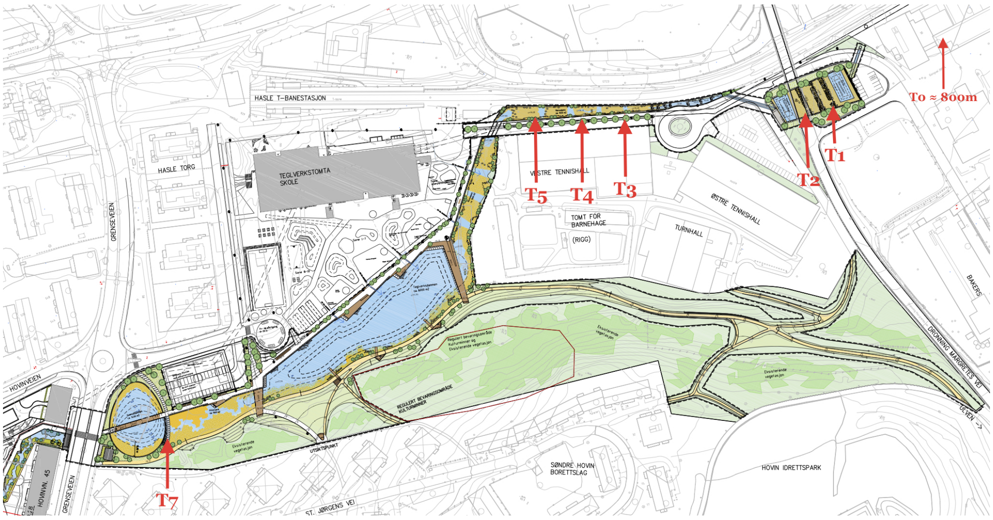

Figure 2.1: Map of sampling sites T1-T7 located in the restored reach. In addition, a reference site is located approximately 800m upstream. (Map retrieved from Oslo Vann og Avløp)

The restored reach is designed as an approximately 650 meter nature-based purification facility, consisting of three sedimentation ponds with wetland filters, permeable thresholds and risle zones in between (Oslo kommune, 2013). To take samples of benthic algae and measure additional parameters for water quality, six sampling sites were selected along the water course. Site T1 and T2 were located close to the facility inlet, in between upper and lower part of the first cleansing pond "Tennisdammen". The three following sites T3, T4 and T5 were located along the stream leading towards the main pond "Teglverksdammen". The last site T7 was located downstream "Teglverksdammen", near the outlet of the restored reach. In addition, a reference site was situated approximately 850 meters upstream the facility at Risløkka, not far from a culvert leading the water underground the highway Ring 3, towards the restored reach.

The restored reach was officially opened on 20th of August 2015. This means that the samples taken within this study were taken during the first and the second vegetative growing season of the newly established ecosystem.

2.2 Sampling

Table 2.1: Overview of dates of sampling and sampling sites during the seasons 2016 and 2017.

Date of sampling	Sampling sites	Days since last sampling
24th of May 2016	T0,T1,T2,T3,T4,T5,T7	-
15th of June 2016	T0,T1,T2,T3,T4,T5,T7	22
14th of July 2016	T0,T1,T2,T3,T4,T5,T7	29
17th of August 2016	T0,T1,T2,T7	34
14th of September 2016	T0,T1,T2,T3,T4,T5	28
12th of October 2016	T0,T1,T2,T3,T4,T5,T7	28
16th of November 2016	T0,T1,T2,T3,T4,T5,T7	35
30th of May 2017	T0,T1,T2,T3,T4,T5,T7	195
29th of June 2017	T0,T1,T2,T3,T4,T5,T7	30

Samples were taken once a month from May to November 2016, a total of seven samples covering the season in 2016 (Table 2.1) The following year, samples were collected in May and June to have a basis of comparison and to reveal if algae growth, taxa composition and concentration of nutrients followed a pattern similar to the first season.

Water quality was measured at each sampling site. This was done prior to the algae sampling, to avoid disturbance of sediments that might influence the result. A sample of 1 litre was collected in a rinsed plastic container on each sampling site. Water samples were delivered to the laboratory of Oslo Vann og Avløp few hours after sampling, for chemical analysis of phosphate, nitrate and ammonium. Water conductivity (μS/cm) and temperature ($^\circ$C) were measured in situ using the digital multi parameter "WTW Multi 3420 Set C".

Benthic algae were observed and collected in a range between 5 and 10m^3 at each sampling site. Samples were taken of all macroscopically visible, seemingly different algae and stored separately in containers of 20ml. Microscopic algae were collected by brushing the top surface area of five rocks using a toothbrush. This was done in a container of approximately 1 litre of water retrieved from the sampling site. The brushed material was mixed well, and a 20ml sub sample was taken. All samples of algae were preserved with a few drops of formaldehyde and stored for further species determination. The percent cover of each collected growth form of benthic algae was estimated at each site, if necessary by using an aquascope (a plastic bucket with a transparent bottom).

BenthoTorch measurements were conducted at each sample site. The BenthoTorch is an instrument that enables real time measurements of benthic algae concentrations. Being directed towards a wet substrate, at a given surface 0,78cm^2 per measurement, it measures the intensity of chlorophyll fluorescence of algal cells. A displayed result is given after each measurement. The result is given as separate values of chlorophyll-a (in μg chl-a/cm^2) for cyanobacteria, green algae and diatoms (bbe Moldaenke, 2013). On each sample site, six randomly picked rock surfaces were analysed with the BenthoTorch, trying carefully to avoid disturbance of the biofilm to get an accurate result. One measurement was done on each rock.

2.3 Sample analysis

Oslo Vann og Avløp contributed with a set of data including daily values of water discharge in the restored reach. The dataset contained values from June 2015 until November 2016.

Analysis of benthic algae was carried out during the period from November 2016 to July 2017. The preserved samples were examined under a Leica 2000 microscope (200-600X magnification) following a procedure in line with CEN standard for sampling and processing of benthic algae (NS-EN 15708:2009, 2010). All non-diatom benthic algae were determined to species level wherever possible. For certain algae, it can be difficult to determine the species when they are not in reproduction mode. For these taxa, categories based on filament width, number and spiraling density of chloroplast and cell length/width ratio were used. Categories of such algae were retrieved from the list of taxa developed by

Schneider and Lindstrøm (2011). Identification of species was done using identification keys according to Gutowski and Foerster (2009), Komarek (2008) and Rueness et al. (2011). All taxa found in each sample were noted, and their abundance in the sample was estimated. For the main taxa found in a sample, the estimate of percent cover done in the field was used. The abundance of all other taxa found in the microscope was estimated and translated into percent cover as rare=0,001 % cover, common=0,01% cover and abundant=0,1% cover. In samples where the in-situ observation was noted as $< 1\%$, the percent cover used for further data analysis was 0,1%. When diatoms were the main algae present in a sample, its abundance was estimated following the procedure as described, but no determination of family or species was done.

2.4 Data treatment and statistics

A list of taxa was created on the basis of the analysis of benthic algae. This list was used to examine the abundance and composition of benthic algae taxa. The total number of taxa was calculated and divided into red algae, green algae, yellow-green algae and cyanobacteria to explore richness on each site throughout the period of sampling. There were three areas within the sample sites that were particularly interesting: T0-T1, T1-T5 and T5-T7. The reference site T0 and site T1 were separated by an approximately 850m culverted water course situated underground the highway Ring 3. Comparing T0 and T1 would indicate the influence of this culvert. The sites T1-T5 were located along the stream leading the water towards the main pond "Teglverksdammen". Comparing T1 with T5 would indicate the purification capacity of this part of the restored reach. The site T5 and the site T7 were separated by the main pond "Teglverksdammen. Comparing these sites would indicate the effect of the main sedimentation pond. In addition, the difference between benthic algae samples conducted in 2016 and 2017 was compared to investigate the development of the new ecosystem.

The total cover of benthic algae was calculated as the sum of cover of all taxa on each sample site. As certain taxa periodically stood out as the main benthic cover, the abundance of these was closely examined. These were the abundance filamentous green and yellow-green algae in comparison with total cover of diatoms. Also the occurrence and cover of the cyanobacterium *Oscillatoria sancta* was examined closely, as it was observed in large amounts in the restored reach during both periods of sampling.

The eutrophication index PIT ("Periphyton Index of Trophic status"; Schneider and Lindstrøm (2011)) was calculated for all sites. PIT is related to stream phosphorus supply, thus it provides a link to eutrophication and ecological condition of the stream. Based on indicator values of non-diatom benthic algae found, PIT was calculated as follows:

$$PIT = \frac{\sum_{i=1}^{n} IV_i}{n}$$

Where IV_i is the indicator value of taxa i and n is the number of indicator taxa. PIT is uncertain if less then 2 indicator taxa occures at a site.

Chlorophyll-a (μg/cm^2) was measured by the BenthoTorch. Total chlorophyll-a was calculated as a sum of the values for cyanobacteria, diatoms and green algae given by the BenthoTorch (Kahlert and Mckie, 2014). 6 measurements were done on each sampling site and calculated as total chlorophyll-a. The mean value of these measurements was used for further analyses.

Statistical analyses were all carried out at a 0.05 level of significance. All the statistical analyses were done using Minitab statistical software (version 16) All figures were made using Microsoft Excel and Minitab. Paired t-test was carried out to determine significant differences between sample sites in terms of 1)total number of taxa and 2)PIT index values calculated. Pearsons correlation analysis was carried out to determine significant correlations between 1)The change in water chemistry concentrations and total estimated percentage cover of benthic algae along the sites T1-T5 and 2)Estimated percent cover of benthic algae and BenthoTorch measurements of chlorophyll-a.

3 Results

3.1 Water discharge

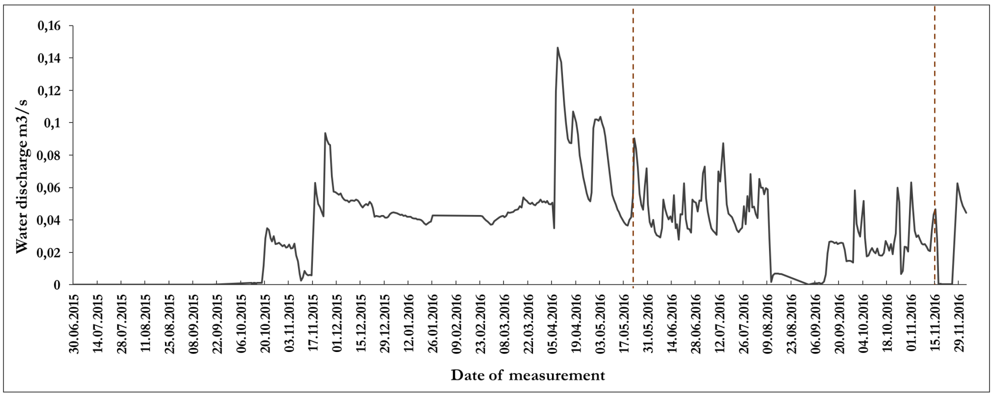

Figure 3.1: Daily values of water discharge in the restored reach during the first year after opening. Data retrieved from Oslo Vann og Avløp. Discharge is measured at the inlet of the restored reach. The dotted lines mark the first period of sampling 2016.

The restored reach was filled with water for the first time in June 2015, but the system did not receive running water until October 2015 (Fig. 3.1). The sampling of benthic algae started on 24th of May 2016, meaning that the system had been active for seven months at the time when the first sample was taken.

The flow through the restored reach was relatively stable throughout the winter period of 2015/2016. The discharge reached its highest value of 0,14m^3/s in the beginning of April 2016. Calculated mean discharge was 0.04m^3/s from April to beginning of August 2016, when water was shut of due to maintenance work on 11th of August. The shut-down affected sampling at site T3, T4 and T5 in August, as water level was too low to collect algae or to use the BenthoTorch at these sites. In September, these sites received running water, and sampling was possible. However, in September the discharge had been low for four weeks, and site T7 was dry. Therefore, no samples were collected at T7 in September.

The restored reach received normal flow of water from mid September, and the samples in October and November were taken without any disturbance regarding discharge. However, it is worth mentioning that in October the water was contaminated with a large amount of clay particles. This was probably caused by construction work upstream. This led to poor visibility, which might have affected the accuracy of sampling and estimate of percent cover.

3.2 Water quality measurements

Water temperature

Table 3.1: Water temperature (°C) for the sampling period 2016 and 2017 at seven sites along the restored reach.

Site	May 2016	June 2016	July 2016	Aug 2016	Sept 2016	Oct 2016	Nov 2016	May 2017	June 2017
T0	9.3	12.8	14.0	12.3	13.6	7.5	5.0	10.6	11.8
T1	9.5	12.3	14.1	15.1	14.4	8.3	5.3	10.6	11.0
T2	9.5	12.4	14.2	15.1	14.7	8.1	5.2	11.0	10.8
T3	9.7	12.5	14.2	17.1	15.0	7.9	5.2	11.0	11.1
T4	9.7	12.6	14.3	17.2	15.5	7.7	5.1	11.0	11.4
T5	9.8	12.7	14.4	17.9	17.7	7.4	5.1	11.0	11.5
T7	11.7	12.7	16.8	17.4	NA	6.3	3.6	13.0	14.8
Average	**9.9**	**12.6**	**14.6**	**16.0**	**15.2**	**6.3**	**5.0**	**11.2**	**11.8**

Average water temperature throughout the first sampling season ranged from 9.9°C in May to 16.0°C mid summer, after which it declined down to 5.0°C in November (Table 3.1). In 2017, the average temperature in May was 1.3°C warmer than in 2016. The average temperature in June 2017 was 0.8°C lower than in June 2016.

For all months, with the exception of June 2016, temperature increased from sampling site T0 to T1. This was most noticeable during August and September, the months with minimal water flow. These were also the months with the warmest water temperatures. In the period from May to September 2016, the temperature increased slightly from site T1 to T7. This pattern also occurred in 2017. In October and November 2016, the temperature decreased from T1 to T7.

Conductivity

Table 3.2: Conductivity for the sampling period 2016 and 2017 at seven sites along the restored reach.

Site	May 2016	June 2016	July 2016	Aug 2016	Sept 2016	Oct 2016	Nov 2016	May 2017	June 2017
T0	303	466	346	340	373	370	372	224	278
T1	382	520	409	374	414	349	440	254	323
T2	381	521	409	374	417	350	441	254	323
T3	378	516	398	359	419	393	453	358	324
T4	377	513	395	359	418	411	451	257	323
T5	376	512	392	361	420	427	445	259	323
T7	344	506	448	310	NA	406	582	267	323
Average	**363**	**508**	**400**	**354**	**410**	**387**	**455**	**268**	**317**

Average water conductivity was highly variable between months, and no seasonal pattern was apparent (Table 3.2). Conductivity increased between T0 and T1 every month of sampling during both season with the exception of October 2016. From May to September 2016, and in June 2017 conductivity declined downstream the system between T1 to T7. This with the exception of July where there was an increase between site T5 and T7. In October and November 2016 and in May 2017, the conductivity increased between T1 and T7.

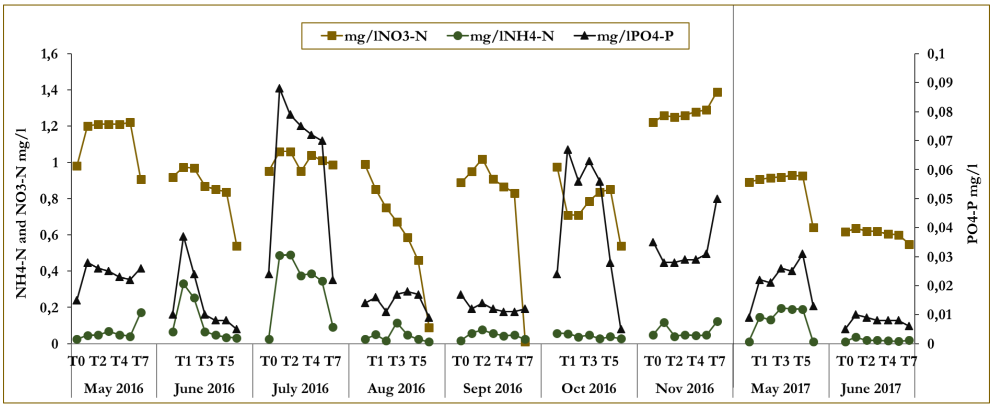

Figure 3.2: Concentrations of nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4) for the sampling period 2016 and 2017, measured at seven sites along the restored reach. A vertical line marks the transition to the next year.

Concentrations of phosphate varied between months with maximum values of 0.09mg/l in July and 0.07mg/l in October and with minimum values of 0.01mg/l in September 2016 and June 2017. (Fig. 3.2). Between T0 and T1, concentrations of phosphate were increasing in 7 out of 9 months, although the difference between the sites was not significant (Table 3.3). Concentrations of phosphate were slightly declining between T1 and T5 and between T5 and T7, but no significant difference in concentrations of phosphate was found at these sites (Fig. 3.2, Table 3.3).

Table 3.3: Paired t-tests of concentrations of nutrients at sampling sites along the restored reach during sampling seasons 2016 and 2017.

Nutrient	**Sites**	**T-value**	**P-value**
PO_4	T0 ; T1	-2.19	0.060
PO_4	T1 ; T5	1.70	0.128
PO_4	T5 ; T7	1.36	0.212
NH_4^+	T0 ; T1	-2.25	0.054
NH_4^+	T1 ; T5	1.77	0.115
NH_4^+	T5 ; T7	0.78	0.458
NO_3^-	T0 ; T1	-0.26	0.799
NO_3^-	T1 ; T5	1.15	0.283
NO_3^-	T5 ; T7	2.98	0.018

Concentrations of ammonium were of relatively stable values of in the range of 0.02 - 0.04mg/l throughout the sampling period, with the exception of a peak in July 2016 and May 2017 (Fig. 3.2). Between T0 and T1, concentrations of ammonium increased each month of sampling except from October 2016. The difference between T0 and T1 was significant (Table 3.3). Between T1 and T5 and between T5 and T7, ammonium followed a similar pattern as phosphate regarding declining and increasing in concentrations, with the exception of October and November 2016. For ammonium, no significant difference was found neither between T1 and T5, nor between T5 and T7 (Table 3.3).

Concentrations of Nitrate varied between months with a peak in concentrations in May and November 2016 with maximum values of 1.2mg/l (Fig. 3.2). There was no significant difference between T0 and T1 or between T1 and T5. However, concentrations of nitrate declined between T5 and T7, with the exception of November. The difference between T5 and T7 was significant (Fig. 3.2, Table 3.3).

Concentrations of phosphorus, nitrate and ammonium all increased between T0 and T1 the first three months of sampling in 2016, and the following season 2017 (Fig. 3.2). No common pattern was observed between T1 and T5. Concentrations of phosphorus, nitrate and ammonium all declined between T5 and T7 throughout the sampling period except in May and November the first season. The decrease of nutrients in the restored reach was most noticeable during the months of summer.

3.3 Number of taxa

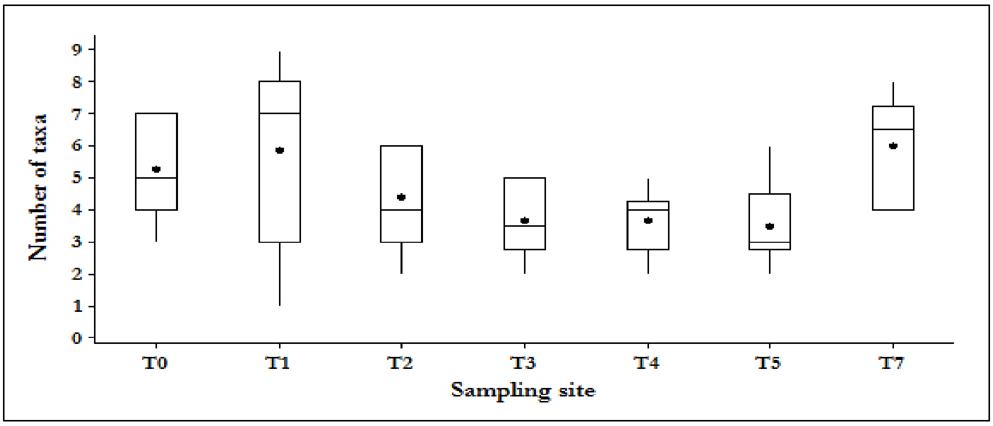

Figure 3.3: Boxplot of total number of taxa for the first sampling period 2016 at seven sites along the restored reach. 2017 is removed from the dataset to avoid a skewed basis of comparison in number of taxa between sites. The box represents the middle 50% within all observations. The line inside the box represents the median value of all observations. A round symbol marks the mean number of taxa fount at the site.

A total of 26 different taxa of non-diatom benthic algae were observed during the sample seasons 2016 and 2017. In 2016, the total number of taxa did not change from the reference site T0 to the inlet of the restored reach T1 (Fig. 3.3, Table 3.4). Between T1 to T5, the sites along the stream in the restored reach, taxon number slightly decreased although the difference between T1 and T5 was not significant. Total number of taxa slightly increased between the inlet of the main pond "Teglverksdammen" T5 and the outlet of the restored reach T7. However, the difference between sites was not significant (Fig. 3.3, Table 3.4).

Table 3.4: Paired t-tests of total number of taxa found along the restored reach during the first season of sampling 2016. 2017 is removed from the dataset to avoid a skewed basis of comparison in number of taxa between sites.

Site	T-value	P-value
T0 ; T1	-0.42	0.689
T1 ; T5	1.52	0.189
T5 ; T7	-1.91	0.129

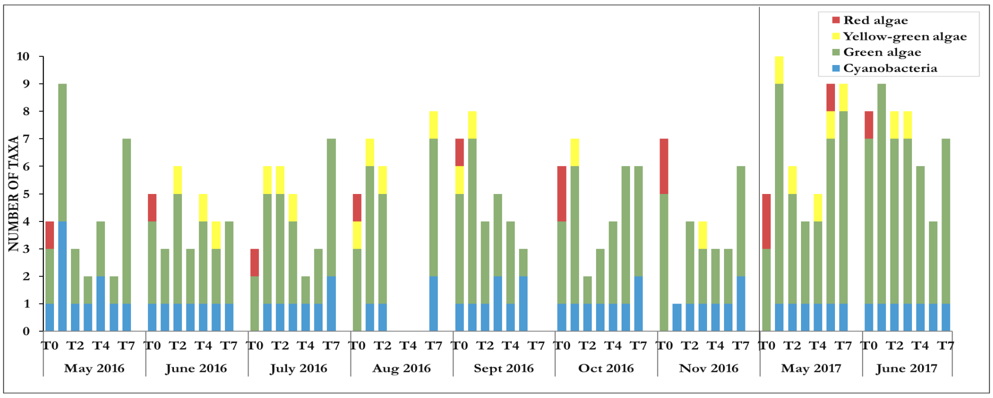

Figure 3.4: Total number of taxa found during the sampling periods 2016 and 2017 at seven sites along the restored reach. A vertical line marks the transition to the next year.

The first season 2016 started with mostly microscopical findings in the restored reach with a peak in number of taxa at T1 and T7 (Fig. 3.4). In June, yellow-green algae were observed microscopically at site T2, T4 and T5. In July, yellow green and green algae were present in larger amounts between T1 and T5, where the green algae *Ulothrix Zonata* and the yellow-green algae *Vaucheria sp.* were noticeably dominant. At T7, the green algae *Spirogyra majuscula* occurred (see complete taxon list in Appendix A). In August, the number of green algae taxa increased at all sites, and the green algae *Oedogonium e* was dominating along the restored reach. August and September were very similar as to which taxa were present and dominating between T1 to T5, but the number of taxa found between T2 and T5 was low compared to T1. In October the number of taxa between T1 and T5 declined compared to the previous sampling, and the sites were still dominated by the green algae *Oedogonium e*. In November, the composition of algae was similar to May, and the findings were mostly microscopical, indicating the end of the growing season. The cyanobacterium *Oscillatoria sancta* was the only benthic algae that, with few exceptions, was present at all sites throughout the sampling period. There were microscopical findings of the filamentous bacterium *Sphaerotilus natans* along the restored reach throughout the sampling period, but as it is non-autotrophic and per definition not a benthic algae, it was excluded from the counting of total number of taxa found in the restored reach.

At the reference site T0, there was a difference in taxa found compared to the remaining sites. Red algae occurred only at the T0 in 2016. The red algae *Audionella chalybea* was possible to identify to species level. Others were either too small, or they did not have reproduction organs developed, which are necessary to determine species of red algae. During the main part of the growing season, the dominating green algae at T0 was *Cladophora glomerata*, which only occurred microscopically or in small amounts in the restored reach.

At site T7, downstream "Teglverksdammen", there was a difference in taxa found compared to the remaining sites. The green algae *Spirogyra majuscula* only occurred at T7 in 2016, and was dominating the site in July and August. It was also observed in large amounts close to the site in September, but was not conducted as the site was dried out at this point. *Spirogyra majuscula* did occur upstream T7 in 2017. The green algae *Stigeoclonium tenue* was found exclusively at T7 during both sampling seasons.

There were some noticeable differences between the two years of sampling. In May 2017, red algae were observed for the first time in the restored reach. The previous season, red algae only occurred at the reference site T0. Also, yellow-green algae were present at several sites in May 2017. The first season, yellow-green algae did not appear until June. The green algae *Spirogyra a* was observed at several sites in May and June 2017. This taxon did not appear in the findings from the first season. There were significantly higher richness of benthic algae taxa in May 2017 than May 2016 and in June 2017 than in June 2016 (Table 3.5).

Table 3.5: Paired t-tests of total number of taxa found in May and June at seven sites along the restored reach during 2016 and 2017

Months of Sampling	T-value	P-value
May 2016 ; May 2017	-2.99	0.024
June 2016 ; June 2017	-3.57	0.012

3.4 Cover of benthic algae

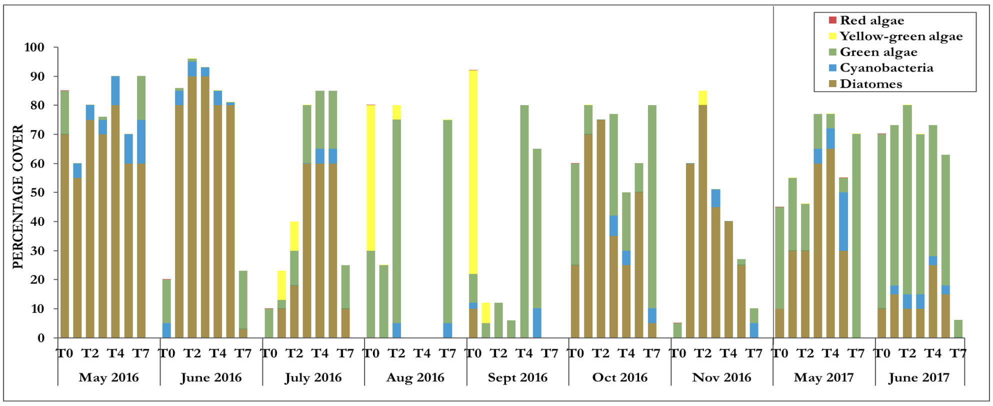

Figure 3.5: Estimated percentage cover of all benthic algae during the seasons 2016 and 2017, at seven sites along the restored reach. A vertical line marks the transition to the next year.

The percent benthic cover of all taxa observed at each sample site (Fig. 3.5) ranged from 5 to 96% in the sampling seasons 2016 and 2017. Low percent cover was observed only on few sites during the period. Low percent cover occurred at sampling sites T3, T4 and T5 in September, right after the drying of these sites.

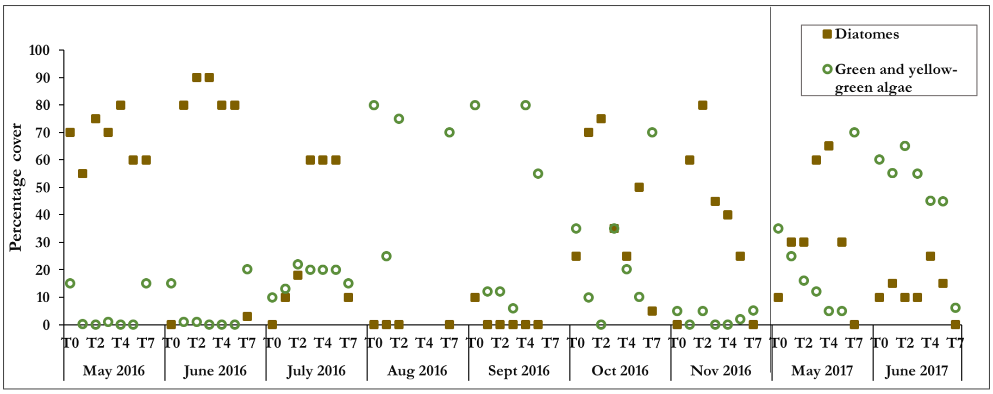

Figure 3.6: Total cover of green and yellow-green algae in comparison with diatoms, at seven sites along the restored reach. A vertical line marks the transition to the next year.

Diatoms dominated in May, June and July, and again in October and November

2016. Green and yellow-green algae dominated in August and September (Fig. 3.6). In 2017, diatoms dominated in May, while green algae dominated in June. In 2017 the domination of green algae occurred earlier than in 2016.

The benthic cyanobacterium *Oscillatoria sancta* occurred only in small amounts at the reference site T0, but was highly visible in the restored reach throughout the sampling period (Fig. 3.7). The abundance peaked in May and June. From July to November, it appeared occasionally in the stagnant part of the stream between T3 and T5. At site T7 it only occurred microscopically during the summer months, but covered 5 % of the site in October and November. In May and June 2017, the abundance was similar to May and June 2016. Benthic cyanobacteria developed primarily in littoral areas of the stream or directly downstream the ponds in the restored reach, where waters were shallow and slow-moving.

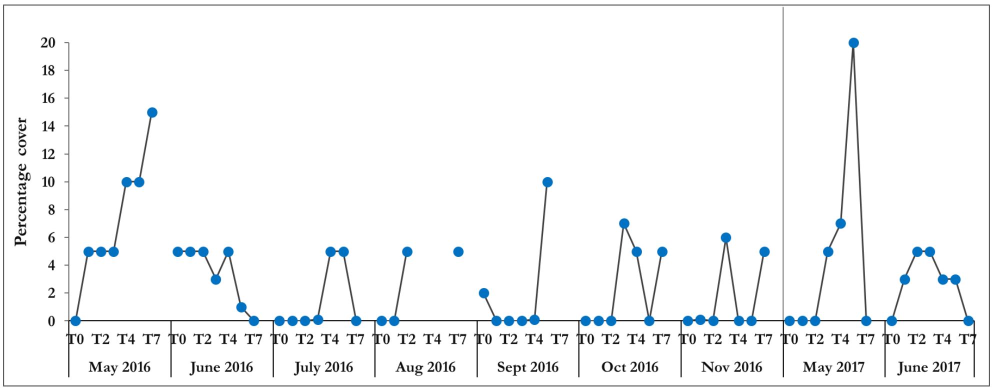

Figure 3.7: Abundance of the cyanobacteria species *Oscillatoria sancta* during the seasons 2016 and 2017 at seven sites along the restored reach. A vertical line marks the transition to the next year

In order to investigate whether the the biomass of benthic algae estimated as % cover correlated with the change in nutrients along the restored reach, a Pearson correlation test was performed. There were no significant correlations between % cover of benthic algae and the changes in concentrations of nutrients (Table 3.6).

Table 3.6: Pearson correlation test between percent cover of benthic algae and change in nutrient concentration between sampling sites.

% cover ; Δ concentration of nutrient between sites	Correlation (r)	P-value
% cover T1; Δ PO_4 T1-T2	-0.392	0.297
% cover T2; Δ PO_4 T2-T3	0.068	0.873
% cover T3; Δ PO_4 T3-T4	-0.326	0.431
% cover T4; Δ PO_4 T4-T5	0.419	0.301
% cover T1; Δ NH_4^+ T1-T2	-0.283	0.461
% cover T2; Δ NH_4^+ T2-T3	0.201	0.604
% cover T3; Δ NH_4^+ T3-T4	-0.408	0.316
% cover T4; Δ NH_4^+ T4-T5	0.126	0.767
% cover T1; Δ NO_3^- T1-T2	-0.063	0.873
% cover T2; Δ NO_3^- T2-T3	0.382	0.311
% cover T3; Δ NO_3^- T3-T4	0.453	0.260
% cover T4; Δ NO_3^- T4-T5	-0.542	0.165

3.5 The periphyton index of trophic status

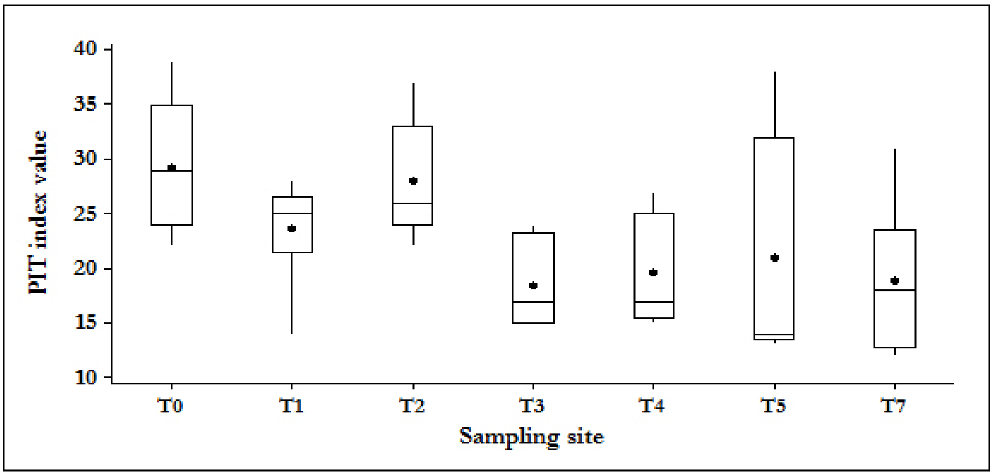

Figure 3.8: Boxplot of PIT values of the season 2016 at seven sites along the restored reach. 2017 is removed from the dataset to avoid a skewed basis of comparison in PIT between sites. The box represents the middle 50% within all observations. The line inside the box represents the median value of all observations. A round symbol marks the mean number of taxa fount at the site.

The periphyton index of trophic status (PIT) was calculated for all sites where there occurred at least two indicator species (see a full list of PIT in Appendix B). Overall, PIT index of all months was significantly higher at T0 than at T1 (Fig. 3.8, Table 3.7). No significant difference in PIT was observed between site T1 and T5 or between site T5 and T7.

All sites ranged between the classifications moderate, poor and very poor ecological status (Fig. 3.9).

Table 3.7: Paired t-tests of PIT index at seven sites along the restored reach during the first sampling season 2016.2017 is removed from the dataset to avoid a skewed basis of comparison in PIT between sites.

Site	T-value	P-value
T0 ; T1	3.40	0.019
T1 ; T5	0.33	0.762
T5 ; T7	0.74	0.512

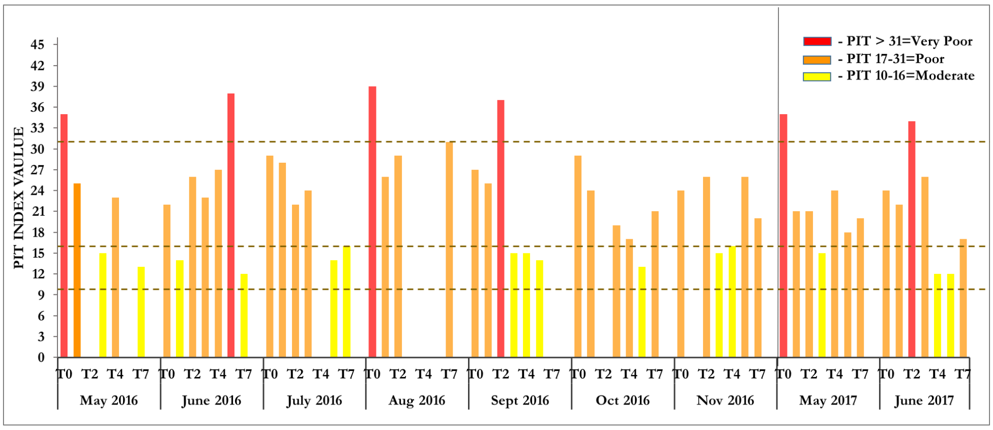

Figure 3.9: PIT values for seven sampling sites along the restored reach throughout the first sampling period. Sites with bars missing are inconclusive (there were fewer than two indicator species found at the site). Horizontal lines mark the boundaries between ecological status: Very poor, poor and moderate.

3.6 BenthoTorch

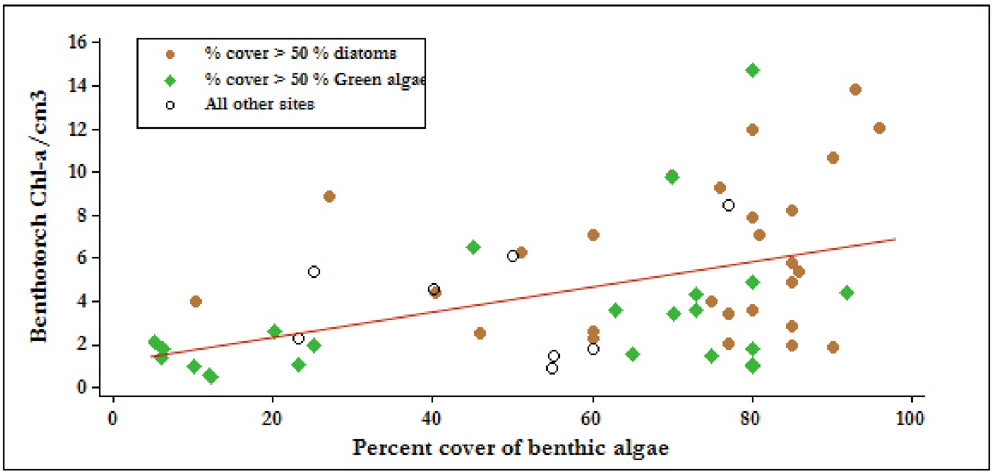

Figure 3.10: Correlation between estimated % cover of benthic algae and Chlorophyll-a chlorophyll-a ($\mu g/cm^2$) measured with BenthoTorch at seven sites along the restored reach. Correlation was done on all sites. Sites are marked afterwords according to whether the % cover is dominated by diatoms, green (and yellow-green) algae or an even distribution of these (all other sites).

There was a significant correlation between % cover of benthic algae and chlorophyll-a ($\mu g/cm^2$) measured by BenthoTorch (Fig. 3.10, Table 3.8). However, when the dataset was to divided into sites with less than 60% cover and sites with more than 60% cover, the correlation was not significant (Table 3.8).

Table 3.8: Correlation between percent cover of benthic algae and chlorophyll-a measured with BenthoTorch.

% cover ; BenthoTorch	**Correlation (r-value)**	**P-value**
% cover ; BenthoTorch	0.418	0.001
% cover < 60 ; BenthoTorch	0.314	0.126
% cover > 60 ; BenthoTorch	0.284	0.103

The sites measured with the BenthoTorch were marked as of which was dominated by diatomes and wich was dominated by green and yellow green algae. This was done to reveal if diatoms would deviate more from the correlation than filamentous green and yellow green algae. No clear pattern was observed regarding sites deviating from the correlation (Fig. 3.10).

4 Discussion

Conductivity and water chemistry

Large variations were observed between months in the concentration of nutrients and conductivity measured in the restored reach (Table 3.2, Fig. 3.2). This is likely due to the large degree of culverting of Hovinbekken upstream the restored reach. Culverts have been shown to contribute to a direct transport of nutrients and chemical compounds to urban streams, mainly due to the lack of dilution by the groundwater and leakages into the culvert from nearby sources (Hatt et al., 2004, Kreuger, 1998). Additionally, the lack of sunlight and oxygen input in culverted sections of a stream have been shown to contribute to decreased stream metabolism and reduced retention of nutrients (Elmore and Kaushal, 2008, Beaulieu et al., 2014, Pennino et al., 2014). The impacts of such upstream events might explain the large variation between the months in concentration of nutrients and conductivity measured in the restored reach. The differences measured between the reference site T0 and the inlet of the restored reach T1 also suggest a direct leakage from nearby sewage or draining pipes into the culvert situated between these sites.

Experimental work with filamentous benthic algal species has shown that the in situ concentrations of phosphate which growth of benthic algae is saturated varies between 0.007-0.050mg/l (Bothwell, 1985, 1988). To my knowledge, studies of benthic algal access to nitrogen to determine maximum growth rate, similar to those conducted on phosphorus, have not been undertaken. However, Grimm and Fisher (1986) and Lohman et al. (1991) have reported benthic algal nitrogen limitation when concentrations of nitrate were between 0.055 and 0.100mg/l. Concentrations of phosphate measured at the inlet of the restored reach T1 ranged between 0.010 and 0.088mg/l, while concentrations of nitrate at T1 ranged between 0.637-1.260mg/l, both of which must be regarded as high values. For several of the months, concentration of nutrients decreased slightly between the inlet of the restored reach T1 and sampling site T5, although no significant difference was found between these sites. This trend also occurred between T5 and sampling site T7, although nitrate was the only nutrient that significantly decreased between these sites during the sampling period. The decline in concentrations was most noticeable during summer, between June and September. Summer is considered as the main growing season for plants which are taking up and removing nutrients from the water as they grow (Butturini et al., 2000, Biggs, 1996). In colder periods, the metabolism of flora declines and nutrients are released to the water as plant material is degraded (Wetzel, 2001). Hence, because plant growth theoretically to a certain extent controls nutrient dynamics in a stream, the observed decline in nutrient concentrations in the period between June and September was expected. Nevertheless, the lack of an overall significant decline in concentrations suggests that there were several elements beyond algae that affected the retention of nutrients along the restored reach. Although the degree of self-purification seemed to have a more positive effect during summer months, no clear evidence was found regarding an overall decline of nutrients along the restored reach.

Percent cover of benthic algae

The impacts of nutrient availability on benthic algae development and growth have been well studied (e.g. Rosemond et al. (2000), Dodds et al. (2002), Marcus (1980)). Overall, high availability of phosphorus and nitrogen have been shown to increase benthic algal density and thickness, especially downstream nutrient point sources in urban environments (Scrimgeour and Chambers, 2000). The estimated percent cover of benthic algae was generally high in the restored reach (Fig. 3.5), probably reflecting the high level of nutrients, which provides optimal conditions for development and growth. Under such conditions, a relationship between accumulated biomass and differences in nutrient concentrations would be expected, as the algae take up nutrients from the water as they grow (Butturini et al., 2000). Nevertheless, statistical analyses showed no significant correlation between differences in nutrient concentrations and percentage cover of benthic algae along the restored reach. Such findings are comparable with previous research on interactions between benthic algal growth and the concentration of nutrients in urban streams that are highly contaminated by nutrients (Murdock et al., 2004). The lack of correlation between growth and nutrients in the restored reach implies that the current nutrient load in the restored reach was not a limiting source for algal growth, nor was the algal growth capable of noticeably reducing the concentrations of nutrients.

Benthic algae development and composition

Massive growth of the cyanobacterium *Oscillatoria sancta* occurred along the restored reach during spring and autumn 2016 and again in spring 2017 (Fig 3.7). Additionally, large amounts of the green algae *Spirogyra majuscula* were growing in the area around T7 in July and August during 2016 and at T0 and T7 in 2017. Massive growth of benthic algae has frequently been observed in eutrophic waters, where benthic algae assemblages are commonly dominated either by rapidly growing filamentous green algae, or cyanobacteria (Sabater et al., 2003, Biggs, 1996). Growth of cyanobacteria is usually favoured by low water discharge and excessive nutrient loads (Paerl, 1996). Massive blooms of cyanobacteria are most unwanted because large assemblages in many occurrences have been associated with unpleasant odours and flavours in the water (Izaguirre and Taylor, 1995, Sabater et al., 2003). The benthic variety that was growing in the restored reach were developing as large mats covering the benthic substrate, and large colonies were detached from the substrate as it was growing, which drifted with the water downstream (Sabater et al., 2003). To a large extent, this reduced the aesthetic appeal of the restored reach. Some species of cyanobacteria are capable of producing toxins (cyanotoxins), which might represent health and ecological risks (Bláha et al., 2009, Codd et al., 1999) Whether or not the cyanobacterium *Oscillatoria sancta*, which were growing in the restored reach, produces toxins is still being tested. It was recently revealed that the species tested negative for the toxin microcystin (Information retrieved from the Norwegian Institute for Water research, (NIVA)). The filamentous green algae *Spirogyra majuscula* was growing at T7, a part of the restored reach were the water was of low velocity due to the sedimentation ponds right upstream this sampling site. Such conditions are optimal for growth of benthic *Spirogyra* (Graham et al., 2009). Accumulations of *Spirogyra majuscula* growing at T7 covered large parts of the water surface shaped as long, green filaments with a slimy texture. Just like *Oscillatoria sancta*, the growth of *Spirogyra majuscula* was highly visible and had a noticeable effect on the aesthetic quality of the restored reach. The excessive growth of cyanobacteria and filamentous green algae in the restored reach implies that the structure and function of the reach over time will be highly influenced by massive growth of benthic algal communities. Thus, with the current situation where neither the rate of biomass production nor the amount of biomass produced seems to be limited by access to nutrients, it is likely that benthic algal assemblages will only be limited by substrate space on which to colonise and grow in the restored reach.

Despite the high accumulation of benthic algae observed in the restored reach, the composition of taxa support the current theory of normal succession and colonisation of benthic algal assemblages. In the restored reach, the seasonal shift with diatoms dominating in spring, taken over by green algae during the warm summer period, followed by diatoms dominating in the end of the growing season (Fig. 3.6) is comparable with studies of seasonal cycles of benthic algae (Biggs, 1996, Cattaneo and Kalff, 1978, Meulemans and Roos, 1985). Such patterns have been shown to occur as a response to varying water temperatures. Temperature generally increased between T0 and T1 supporting the indications of an inside leakage which warms up the water in the culvert situated between T0 and T1. From May to September, the temperature increased between T1 to T5 in the restored reach, a normal warming of the water as the stream was exposed to sunlight. In general, temperature affects growth and respiration of benthic algae, both of which are mechanisms that determine which taxa succeed in dominating the benthic algal community. Thus, as the temperature increases and declines during the growing season, the composition of taxa will change (Wetzel, 2001), as observed in the restored reach. In addition to the temporal pattern of a seasonal cycle, the frequent accumulation of diatoms during the first season suggests a typical starting phase of the benthic algal assemblages. Diatoms are referred to as pioneer organisms with a high growth rate, which are typically the first to establish in benthic algal communities (Biggs, 1996, Allan and Castillo, 2007). This assumption is supported by the findings during the second season 2017, where the seasonal cycle occurred noticeable earlier than in 2016. These patterns of community development and seasonal cycle of the benthic algae observed in the restored reach fits well with typical succession theory of nutrient rich waters, implying that the dynamics of the recently established ecosystem are showing signs of functioning normally.

The restored reach had been active for seven months when sampling of benthic algae started. From a colonisation point of view, this is long enough time to establish a layer of biomass on the benthic surfaces, but the succession among the species is believed to be an ongoing process over time (Hoagland et al., 1982, Kralj et al., 2006). The ecosystem naturally needs time to develop important ecosystem functions to result in a sustainable composition of benthic algal taxa (Pander and Geist, 2013), and the significantly larger richness in taxa found in 2017 than the previous season suggests an ongoing colonisation process in the restored reach. During 2016, red algae occurred rapidly in samples from the reference site T0, but were not found in the restored reach during that season. In 2017 red algae occurred microscopically at T5, suggesting that red algae species have a longer colonisation time than other varieties of benthic algae, but are in an ongoing process of becoming established in the restored reach. And, there were no findings of *Spirogyra a* in 2016, but in 2017 the taxon occurred in the restored reach and at the reference site T0. This suggests that *Spirogyra a* was a newly established taxon in the stream ecosystem. Thus, the establishing of red algae and findings of *Spirogyra a* in 2017 supports the indications of an ongoing colonisation process in the restored reach.

The drought period at sampling sites T3, T4 and T5 in August made it possible to observe the dynamics and recolonisation of the benthic algal community after a short term disturbance. In September, the water discharge in the restored reach was still low, and the sites were noticeably affected by the recent drought with a low estimated algae cover of $< 12\%$ (Fig. 3.5). The ongoing re-establishment of benthic algae assemblages indicates that there was a remnant community of algae at the sites that could be regenerated when the water flow was stabilised. However, in September the composition of taxa at T3, T4 and T5 was dominated by the green algae *Oedogonium e*. This was the dominating taxa at the upstream sites T1 and T2 in August and September, which indicates a colonisation due to drifting cells from these upstream sites. Re-establishment of algal communities after disturbances such as the drought in the restored reach is generally determined by the abundance of cells emigrating from upstream algal communities (Biggs, 1996, Peterson and Stevenson, 1990). Hence, it is likely that the recolonisation at the sites was due to a combination of a growth of drifting cells and remaining biomass. With access to nutrients and light, algal communities are able to re-establish rapidly, and a peak algal biomass might be reached within a period of two weeks (Biggs, 1996). Such accumulation was observed during the next sampling in October, where the estimated percent cover at these sites was $> 50\%$, being dominated by *Oedogonium e* and diatoms. In September, T7 was dry due to low discharge in the restored reach. During July and August, before the drought, this site had been dominated by massive growth of the green algae *Spirogyra majuscula*. In October, when the site received running water, the composition of taxa had markedly changed to be dominated by *Oedogonium a*. This indicates that the algal community was set back in an early successional stage after being dry, giving other algae the opportunity to establish. Such development is comparable with previous studies of benthic algal recolonisation (Biggs, 1996, Peterson and Stevenson, 1990). On the other hand, benthic algae of Spirogyra taxa have been shown to have certain acquirements of water flow and temperature for growing, thus unstable discharge and declining temperature can limit their growth (Hynes, 1970, Graham et al., 2009). The change in taxa composition at T7 in October might therefore be a natural development due to the disturbance of varying discharge and change in season. Overall, the recolonisation after periods of drought observed in the restored reach shows that the established algal community is developed enough to rapidly reassemble after such disturbance.

Periphyton Index of Trophic status

According to Periphyton Index of Trophic status (PIT), the classification for all sites in the restored reach ranged between moderate and very poor ecological status (Fig. 3.9). PIT is related to stream phosphorus supply (Schneider and Lindstrøm, 2011), which means that the results can be interpreted as reflecting the high levels of nutrients measured in the restored reach.

Overall, PIT was significantly higher at the reference site T0 than at T1. This was not expected because the concentrations of nutrients generally increased between T0 to T1. The composition of taxa which mainly originated the high PIT values at T0 was a combination of the red algae *Audionella chalybea* (index value=49.42), *Cladophora glomerata* (index value=47) and the yellow-green algae *Vaucheria sp.* (index value=42,15), which only occurred occasionally or separately at T1. Red algae did not occur at T1. Additionally, there were several species with relatively low index values found at T1, contributing to a lower PIT at this site. Concentrations of phosphorus measured at T0 ranged between 0.005 and 0.035 mg/l compared to sampling site T1 where values ranged between 0.010 and 0.088mg/l, both of which must be regarded as high values. This implies that the levels of nutrients at the reference site also were too high to limit algal growth. The differences in indicator taxa which occurred at T1 compared to those found at T0 suggest that differences in PIT were a result of a more established benthic algal community at the reference site T0.

According to the water management regulation, sampling for PIT calculation should be conducted respectively between August and September (Veileder, 2013), but during this period PIT was inconclusive for several sampling sites due to drought. However, PIT was with no exceptions between moderate and very poor ecological status throughout the sampling period, meaning that the restored reach was unlikely to reach good ecological status even with a full calculation in August and September.

There was no significant difference in PIT between site T1 and T5 or between T5 and T7 in the restored reach. Theoretically, the water should be purified along the restored reach, which would be reflected by a downstream decline in PIT. The high PIT values obtained indicates that the water in the restored reach was eutrophic, meaning that nutrient concentrations were too high. Eutrophic conditions have been shown to consequently leading towards a benthic algae community consisting of taxa tolerant to such environment (Scrimgeour and Chambers, 2000, Sabater et al., 2000). In the restored reach several taxa with these traits were found, such as *Tribonema vulgare* (Index value=68,91), *Vaucheria sp.*(Index value=42,15), *Ulothrix tenerrima* (Index value=20,14), all of which are being tolerant to eutrophic conditions. A non-existent decline in PIT downstream between T1 and T5 and between T5 and T7 supports the indications that the level of nutrients was too high and suggests that, regarding the current nutrient loads, the length of the restored reach might not be long enough to ensure a significant improvement of the water quality.

BenthoTorch

There was a correlation between benthic algal volume estimated as percent cover and μg chlorophyll-a/cm^2 measured by the BenthoTorch in the restored reach (Fig. 3.10), a result that confirms hypothesis 4 in this thesis. Theoretically, the BenthoTorch measurements should account for algal biomass and thus correlate with percent cover of benthic algae, and thus the correlation obtained is a satisfying result. Nevertheless, the correlation is though significant, but not satisfying, as there is a lot of deviation from the regression line.

In evaluating the results obtained by using the BenthoTorch with those from the estimated percent cover, it is necessary to consider the conditions of the sampling sites regarding availability for BenthoTorch measurements. In the restored reach, particularly between sampling site T3 and T5, large parts of the benthic algae were growing as algal mats on soft sediments, a substrate which is not suitable for BenthoTorch measurements. This led to fewer surfaces to choose from when using the BenthoTorch at these sites. This indicates that the BenthoTorch should be used with great caution in streams where large parts of the benthic zone are covered with mud, as the BenthoTorch needs a hard substrate to give results. Additionally, the algae cover in the restored reach were overall of high density and thickness. Previous studies have shown that the BenthoTorch efficiency is strongly influenced by the thickness of the algal layer and the composition of algal taxa (Echenique-Subiabre et al., 2016, Kahlert and Mckie, 2014). When using the BenthoTorch on a thick algae mat, the reflection of light between the multiple layers of growth might interfere an accurate result (Kahlert and Mckie, 2014). However, plots deviating from the correlation also occurred at low percent cover, indicating that other elements than the thickness of algae layer affected the measurements, such as environmental conditions at the sampling sites or pigment content within the algal cells at the area measured.

When using the BenthoTorch in this study, the values used in the correlation analysis was the total calculation of μg chlorophyll-a/cm^2 from each measurement. This was done based on current knowledge about which result to read from the BenthoTorch showing that the BenthoTorch rarely gives accurate results for the composition of benthic algae which is a lack when considering it as a possible replacement of conventional methods for monitoring streams (Echenique-Subiabre et al., 2016, Kahlert and Mckie, 2014).

When dividing the dataset into $<$ and $>$ 60 % cover, the correlation between benthic algal volume estimated as percent cover and μg chlorophyll-a/cm^2 measured by the BenthoTorch was no longer there. The lack of such correlation revealed that the Benthotorch can not confidently differentiate between e.g. 10% and 50% cover, neither can it confidently differentiate between 60 and 100% cover. The results of this study demonstrate that the Benthotorch should be used with extreme care as there is a risk that the BenthoTorch will greatly under or overestimate benthic algal biomass.

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Appendices

A Full list of taxon included estimated percent cover

TAXON	PIT	24.05.16							15.06.16							14.07.16						
		T0	T1	T2	T3	T4	T5	T7	T0	T1	T2	T3	T4	T5	T7	T0	T1	T2	T3	T4	T5	T7
CYANOBACTERIA																						
Cyanophyceae																						
Homoeothrix janthina	*12,53*																					0,001
Oscillatoria sancta		0,001	5	5	5	10	10	15	5	5	5	3	5	1	0,01		0,01	0,001	0,1	5	5	0,001
Phormidium sp.			0,001			0,001																
Phormidium automnale			0,01																			
Psaudoanabena sp.			0,001																			
GREEN ALGAE																						
Chlorophyceae																						
C.f. Klebsormidium sp.			0,001																			
Cladophora glomerata	*47*	5	0,001						15		1					10	3					
Cladophora rivularis	*47*																					
Closterium sp.								0,001														0,001
Microspora abraviata	*37,63*					0,001						0,001										
Microspora amoena	*11,58*																					
Oedogonium a	*5,84*							0,001			0,001				0,001							
Oedogonium b	*7,73*							0,001					0,001	0,001								
Oedogonium c	*9,09*								0,001													
Oedogonium d	*10,87*								0,001							0,001			20		0,001	
Oedogonium e	*16,05*																0,1	8				
Spirogyra a	*8,38*																					
Spirogyra majuscula																						15
Stigeoclonium tenue	*21,64*							0,1														0,01
Ulothrix zonata	*8,39*	10	0,01	0,001	1	0,1	0,001	0,001		1	0,001	0,001	0,001		0,1		0,001	4		20	20	0,01
Ulothrix tenerrima	*20,14*		0,001					15		0,01					20			0,001	0,001			0,01
Ulothrix tenuissima			0,1	0,01																		
YELOW-GREEN ALGAE																						
Xanthophyceae																						
Tribonema vulgare	*68,91*												0,001	0,001								
Vaucheria sp.	*42,15*										0,001						10	10	0,001			
RED ALGAE																						
Rhodophyceae																						
Audionella chalybea	*49,42*	0,01																				
Unidentified red algae									0,001							0,001						
DIATOMES																						
Unidentified diatomes		70	55	75	70	80	60	60	0,01	80	90	90	80	80	3	0,01	10	18	60	60	60	10
HETEROTROPH GROWTH																						
Sphaerotilus natans	*22,28*		0,01		0	0,01						0,1	0,001					0,001			0,001	
TOTAL % COVER		85,01	60,13	80,01	76,00	90,10	70,00	90,10	20,01	86,01	96,00	93,00	85,00	81,00	23,11	10,01	23,11	40,00	80,10	85,00	85,00	25,03

TAXON	PIT	17.08.16							14.09.16							12.10.16						
		T0	T1	T2	T3	T4	T5	T7	T0	T1	T2	T3	T4	T5	T7	T0	T1	T2	T3	T4	T5	T7
CYANOBACTERIA																						
Cyanophyceae																						
Homoeothrix janthina	*12,53*							0,001						0,001								0,1
Oscillatoria sancta			0,01	5				5	2		0,001	0,001	0,1	10		0,001	0,001	0,001	7	5	0,001	5
Phormidium sp.										0,001		0,001										
Phormidium automnale																						
Psaudoanabena sp.																						
GREEN ALGAE																						
Chlorophyceae																						
C.f. Klebsormidium sp.																						
Cladophora glomerata	*47*	30	5	15				0,001	0,001	0,001	2					35	5				10	0,001
Cladophora rivularis	*47*										0,1											
Closterium																						
Microspora abraviata	*37,63*																					
Microspora amoena	*11,58*									0,001												
Oedogonium a	*5,84*		0,001					0,001									0,001					70
Oedogonium b	*7,73*									0,001		0,001										
Oedogonium c	*9,09*												0,01				0,001				0,001	
Oedogonium d	*10,87*			0,001					0,001							0,001				0,001	0,001	
Oedogonium e	*16,05*	0,001	20	55						5	10	6	80	55		0,001		0,001	35	20	0,1	
Spirogyra a	*8,38*																					
Spirogyra majuscula				0,001				70														0,001
Stigeoclonium tenue	*21,64*							0,01														
Ulothrix zonata	*8,39*								10													
Ulothrix tenerrima	*20,14*		0,01										0,001				5		0,001	0,1	0,001	0,01
Ulothrix tenuissima										0,001		0,001										
YELOW-GREEN ALGAE																						
Xanthophyceae																						
Tribonema vulgare	*68,91*							0,001														
Vaucheria sp.	*42,15*	50	0,001	5					70	7							0,001					
RED ALGAE																						
Rhodophyceae																						
Audionella chalybea	*49,42*	0,001														0,001						
Unidentified red algae									0,001							0,1						
DIATOMES																						
Unidentified diatomes		0,001	0,001	0,1				0,001	10	0,01	0,001	0,001	0,01	0,1		25	70	75	35	25	50	5
HETEROTROPH GROWTH																						
Sphaerotilus natans	*22,28*									0,001		0,001				0,01	0,01		0,001	0,001		
Total % cover		80,00	25,02	80,10				75,02	92,00	12,02	12,10	6,01	80,12	65,10		60,11	80,01	75,00	77,00	50,10	60,10	80,11

TAXON	PIT	16.11.16							30.05.17							29.06.17						
		T0	T1	T2	T3	T4	T5	T7	T0	T1	T2	T3	T4	T5	T7	T0	T1	T2	T3	T4	T5	T7
CYANOBACTERIA																						
Cyanophyceae																						
Homoeothrix janthina	*12,53*																					
Oscillatoria sancta			0,1	0,001	6	0,01	0,001	5		0,01	0,001	5	7	20	0,001	0,001	3	5	5	3	3	0,001
Phormidium sp.																						
Phormidium automnale																						
Psaudoanabena sp.																						
GREEN ALGAE																						
Chlorophyceae																						
C.f. Klebsormidium sp.																						
Cladophora glomerata	*47*	5					2	0,001	25	5						20	25	5				3
Cladophora rivularis	*47*																					
Closterium															0,001							
Microspora abraviata	*37,63*																0,01	0,001				
Microspora amoena	*11,58*																					
Oedogonium a	*5,84*							0,1							60							1
Oedogonium b	*7,73*	0,01			0		0,001	5		0,001						0,001				0,001		0,001
Oedogonium c	*9,09*					0,01																
Oedogonium d	*10,87*	0,01		0,001						0,01	0,001			0,01			0,001					
Oedogonium e	*16,05*													0,001							0,001	
Spirogyra a	*8,38*									0,001				0,001		0,1	2		0,01	0,001		0,1
Spirogyra majuscula									10	0,001		0,01			10	40	0,1	0,001	0,01	0,1		2
Stigeoclonium tenue	*21,64*														0,1	0,01	0,001		0,01			
Ulothrix zonata	*8,39*	0,001							0,001	20	10	10	5	0,01	0,001	0,01	28	60	55	45	45	
Ulothrix tenerrima	*20,14*																					
Ulothrix tenuissima		0,001		0,001	0,01	0,1				0,001	6	2	0,001	5	0,001		0,001	0,001	0,01	0,001	0,001	0,001
YELOW-GREEN ALGAE																						
Xanthophyceae																						
Tribonema vulgare	*68,91*																		0,001			
Vaucheria sp.	*42,15*			5						0,001	0,001		0,001	0,001	0,001			0,001				
RED ALGAE																						
Rhodophyceae																						
Audionella chalybea	*49,42*	0,1							0,01							0,01						
Unidentified red algae		0,01							0,01					0,001								
DIATOMES																						
Unidentified diatomes		0,01	60	80	45	40	25	0,1	10	30	30	60	65	30	0,01	10	15	10	10	25	15	0,1
HETEROTROPH GROWTH																						
Sphaerotilus natans	*22,28*	0,001	0,01	0,1	0,1	0,1	0,01			0,1	0,01	0,001	0,001	0,001			0,001		0,001	0,001		
Total % cover		5,14	60,10	85,00	51,01	40,12	27,00	10,20	45,02	55,03	46,00	77,01	77,00	55,02	70,12	70,13	73,11	80,00	70,04	73,10	63,00	6,20

B Full list of PIT

Sampling date	Site	Total number of taxa	Total number of indicator taxa	Indicator value	Index value	PIT classification
24.05.16	T0	4	3	104.8	35	Very poor
24.05.16	T1	10	4	97.8	25	Poor
24.05.16	T2	3	1	8.4	8.4	Inconclusive
24.05.16	T3	3	2	30.7	15	Moderate
24.05.16	T4	5	3	68.3	23	Poor
24.05.16	T5	2	1	8.4	8.4	Inconclusive
24.05.16	T7	7	5	63.7	13	Moderate
15.06.16	T0	5	3	67.0	22	Poor
15.06.16	T1	3	2	28.5	14	Moderate
15.06.16	T2	6	4	103.4	26	Poor
15.06.16	T3	4	3	68.3	23	Poor
15.06.16	T4	6	4	107.3	27	Poor
15.06.16	T5	4	2	76.6	38	Very poor
15.06.16	T7	4	3	34.4	12	Moderate
14.07.16	T0	3	2	57.9	29	Poor
14.07.16	T1	6	4	113.6	28	Poor
14.07.16	T2	7	5	109.0	22	Poor
14.07.16	T3	5	3	73.2	24	Poor
14.07.16	T4	2	1	8.4	8.4	Inconclusive
14.07.16	T5	4	3	41.5	14	Moderate
14.07.16	T7	7	4	62.7	16	Moderate
17.08.16	T0	5	4	154.6	39	Very poor
17.08.16	T1	7	5	131.2	26	Poor
17.08.16	T2	6	4	116.1	29	Poor
17.08.16	T7	8	5	156.0	31	Poor
14.09.16	T0	7	4	108.4	27	Poor
14.09.16	T1	9	6	146.8	25	Poor
14.09.16	T2	4	3	110.1	37	Very poor
14.09.16	T3	6	3	46.1	15	Moderate
14.09.16	T4	4	3	45.28	15	Moderate
14.09.16	T5	3	2	28.58	14	Moderate
12.10.16	T0	7	5	145.6	29	Poor
12.10.16	T1	8	6	146.5	24	Poor
12.10.16	T2	2	1	16.1	16.1	Inconclusive
12.10.16	T3	4	3	58.3	19	Poor
12.10.16	T4	5	4	69.3	17	Poor
12.10.16	T5	6	5	66.2	13	Moderate
12.10.16	T7	6	4	85.51	21	Poor
16.11.16	T0	8	6	145.7	24	Poor
16.11.16	T1	2	1	22.3	22	Inconclusive
16.11.16	T2	5	3	75.3	26	Poor
16.11.16	T3	5	2	30.0	15	Moderate
16.11.16	T4	4	2	31.4	16	Moderate
16.11.16	T5	4	3	77.0	26	Poor
16.11.16	T7	4	3	60.6	20	Poor
30.05.17	T0	5	3	104.8	35	Very poor
30.05.17	T1	11	7	146.8	21	Poor
30.05.17	T2	7	4	83.7	21	Poor
30.05.17	T3	5	2	30.7	15	Moderate
30.05.17	T4	6	3	72.8	24	Poor
30.05.17	T5	10	6	108.1	18	Poor
30.05.17	T7	9	4	78.0	20	Poor
29.06.17	T0	8	6	142.6	24	Poor
29.06.17	T1	10	7	156.2	22	Poor
29.06.17	T2	8	4	135.2	34	Very poor
29.06.17	T3	9	5	129.6	26	Poor
29.06.17	T4	7	4	46.78	12	Moderate
29.06.17	T5	4	2	24.44	12	Moderate
29.06.17	T7	7	4	69.0	17	Poor

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