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Variation in benthic colonization on tiles and in leaf degradation – results from a flume experiment in Norway

Vibeke Hoff
General Ecology

Abstract

Clean water support economic and recreational activities as well as provide a healthy habitat for flora and fauna. Gradual pollution of water in Europa over the past decades have resulted in a water management across country borders and increased the need for research in running waters. Freshwater research has gained important knowledge through experiments in artificial streams. Results from artificial streams may not be comparable with processes in nature, but replication and flume design improve data strength. In this thesis, eight artificial streams were constructed of stainless steel with bottom substrate of coarse gravel. Water was circulated separately for each flume. Tiles and leaves were exposed for six weeks at different longitudinal positions in each flume. It was tested if periphyton colonized the tiles differently between the flumes or between the positions in each flume, and if leaf litter degraded differently between the flumes or between the positions in each flume. Two different sampling methods were used. There were no differences between the sampling methods, which indicate that removal of random tiles and leaf litter bags did not affect the remaining tiles. There were no differences in leaf litter degradation between the flumes or between the positions in a flume. Similarly, there were no differences in colonization of algae on tiles between the flumes or positions in each flume, so neither the location of the flume nor the longitudinal position within the flume affected litter degradation or algal growth. However, algal density in Flume 1 differed from that of the other flumes. Flume 1 received groundwater while the other flumes were filled with surface water. Different water quality may be the reason why Flume 1 had different algal assemblage as well as less leaf litter degradation, than the other flumes. It was concluded that the artificial streams at Solbergstrand were suited for future experiments and they may well contribute to our further knowledge on how to keep freshwater healthy and clean.

Preface

This thesis is a completion of my Master's degree in General Ecology at the Norwegian University of Life science (NMBU), Department of Ecology and Natural Resource Management (INA). The project was founded by Norwegian Institute for Water Research (NIVA) and most of the research took place at NIVA's field station at Solbergstrand, Drøbak, in late spring and summer of 2014.

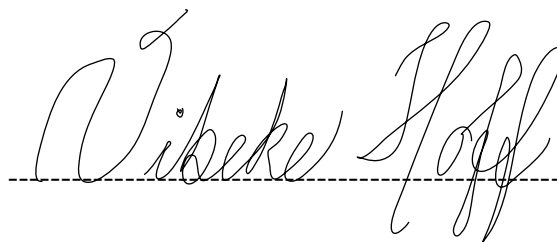
Jon Frank, associate professor at INA shared of himself and got me in contact with my head supervisor. I therefore want to thank him for leading me to this project. My supervisor was Susanne Claudia Schneider, professor at INA and senior research scientist at NIVA, and my co-supervisor was Nikolai Friberg, research director for biodiversity at NIVA, and I would like to give my thanks for their input and guidance throughout the practical and written work behind this paper.

I would like to thank Semonna Issa and Jens Thaulow for their valuable contribution to the flume construction. My thanks also go to the staff at Solbergstrand, for all their hospitality and friendly help.

I would offer my thanks to the many enthusiastic lecturers at NMBU that have touched my life and inspired me. I would further give my gratitude to Dinalva Almeida Oliveira for her support, encouragement and laughs along the way. Finally, I would like to thank my partner, Pål Reinhardtson†. Although he left me last year, I am grateful that he always had faith in me and gently steered me in the direction of university.

Norwegian University of Life Science

May 2017

A handwritten signature in cursive script, reading "Vibeke Hoff", written over a horizontal dashed line.

Vibeke Hoff

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1 Introduction

Clean waters ensure safe drinking water, protect human health, support recreational activities and provide healthy habitats for flora and fauna. However freshwater has faced serious contamination in response to mankind's technological development. Water degradation has been a problem in several countries for decades, particularly due to pollution from urban waste and intensive agriculture. Sewage treatment methods and agriculture practices have been controlled separately by each country, when, at the end of the millennium, the public in Europe demanded an improved and comprehensive water management. This resulted in the EU's Water Framework Directive (WFD), implemented in the EU in 2000 (European Commission, 2000), which has been effective in Norway since January, 2007 (Vannportalen, 2015).

Implementation of WFD required a restructured water management so that it included whole watersheds, even where rivers crossed country borders. Further, WFD called for an assessment system to be used across Europe that could compare rivers and streams water quality based on several biological elements. Although the importance of each element and the best overall method still are discussed (Furse, Hering, Brabec, Buffagni, Sardin and Verdonschot, 2006; Friberg, Sandin and Pedersen, 2009, 2011, Friberg, 2014), biological elements (biota) are found most reliable to assess water ecosystems, compared to solely use of chemical qualities. A chemical measurement can only capture water quality at that point in time when the sample was collected. Biota on the other hand, reflects water quality over longer time periods of weeks to years. WFD's classification guide in Norway, explains how to assess stream water quality by the biota: periphyton and macroinvertebrates (Veileder 01:2009, 2015), but their specific relevance to a broader spectrum of stressors needs to be identified through further research.

Water researchers through the last five decades, have built and used a variety of artificial streams (McIntire, 1993). Although artificial streams may inadequately reconstruct a natural ecosystem, they have the advantage that parameters like for example flow velocity, heterogeneity and water chemistry may be controlled and manipulated. These parameters do change naturally, or due to human activity, and for stream organisms, the change require adaptation in order to survive, called organisms' stressor response. The intentional change of

parameters, increase our knowledge on stream organism's response to stressors (Lamberti and Steinman, 1993). Velocity is one stressor that was found to influence periphyton architecture of epiphytes, as well as the algae community assemblage (Bergey, Boettiger and Resh, 1995). The stress on filamentous algae at high velocity resulted in less branching and shorter filaments than what was found on algae that grew in slower velocity (Bergey et al., 1995; Briggs, Goring and Nikora, 1998). Periphyton and algae community are also affected by different macroinvertebrate's interactions. Wellnitz and Poff (2012) found less filamentous algae at low and medium velocity where a caddisfly larva and a mayfly nymph grazed together than where each grazed alone. Macroinvertebrates were affected by stream substrata. Tiny micro pores in the substrate facilitated macroinvertebrates establishment, growth and possibly grazing abilities in freshwater streams (Dudley and D'Antonio, 1991). Water velocity, together with heterogenic substrate size, created hydraulic microhabitats, which affected macroinvertebrate assemblages (Brooks, Haeusler, Reinfelds and Williams, 2005), and algae heterogeneity (Stevenson, 1997). Natural stream environment in artificial streams would aid the researcher to produce ecological relevant results, and its successfulness depends on flume design.

Artificial streams have been designed in different ways depending on research focus and economy: often as small as possible, round or straight, indoor or outdoor, with or without substrate, where water circulate or flow through. However, to produce ecological relevant data, some flume design details are recommended: surface water is best for use in stream research (Craig, 1993; Elbrecht et al., 2016) and stream bed heterogeneity is important (Hart and Finelli, 1999, Nowell and Jumars, 1984). Lamberti and Steinman (1993) found that some stream research were not replicated enough and their recommendation is a minimum of 6 replicates to produce statistically sound data. Stanzer et al., (1988) claim that reconstruction of exact hydrology, measured as Reynolds number and Froude number, will increase the replicability in studies, while Ceola et al., (2013) argue that water flow is the key parameter that structure and control ecosystems. Although the paper was produced in 1993, Lamberti and Steinman (1993)'s conclusion may still be valid: no best design of artificial stream exists, however, if designed with care and results are validated in nature, artificial streams may give valuable insight in the future.

In the future, impacts on water ecosystems may be different than in the past. In the past, sewage has negatively affected freshwater ecosystems by its high organic matter content. Indicators of increased organic matter may not be sensitive to other stressors. Both the

Millennium Ecosystem Assessment (MEA 2005) and Intergovernmental Panel on Climate Change (IPCC 2007) name climate- and land-use-change as the most important threat to our future freshwaters. Climate change is predicted to produce heavier and more frequent rainfall with altered run-off patterns and, together with alterations in the watershed and increased temperature, will influence stream biota in ways not yet understood. Other biological indicators may reflect these changes better than the ones currently in use. Friberg et al., (2011) claim some indicators might be out-dated, because they are built on past problems, and that old indicators possibly disregard more relevant influence. Friberg (2014) highlights the need for a broader set of indicators and suggest, among other things, the use of leaf degradation.

Leaves are an important source of carbon for aquatic lifeforms and leaf degradation is an important ecosystem process (Wallace, Eggert, Meyer and Webster, 1997). Leaves are degraded by microorganisms and macroinvertebrates and the degradation times can easily be measured. Several recent studies include leaf decay in their flume experiments (Elbrecht et al., 2016, Briggs et al., 1998, Ceola et al., 2013, Colas et al., 2017), but the indicator is yet to be standardized in water management. Lamberti and Steinman (1993) pointed out the possible lack of strength in past results from artificial streams and suggested caution in design and validation of data produced in a man-made environment.

The aim of this study was to analyze colonization of tiles by benthic algae, and leaf degradation over a period of six weeks in eight newly built flumes. Data was analyzed across flumes in order to detect possible differences among flumes, as well as between upstream and downstream end of each flume. In addition, two different sampling methods were analyzed. If colonization is similar across flumes, then this will indicate that the flumes may be used as true replicates for future studies; analyzing different sampling methods is important to document how sensitive results are to a particular sampling method.

The hypotheses were:

- 1) There are no differences in periphyton biomass and species composition, as well as leaf litter degradation among the flumes.
- 2) There are no differences in periphyton biomass and species composition, as well as leaf litter degradation between upstream and downstream positions in the flumes

3) The sample method does not affect periphyton biomass, species composition or leaf degradation.

2 Material and Methods

2.1 Study site



Figure 1: Map over Solbergbekken area. Solbergbekkens outlet and NIVA's research station is marked with a red arrow. Solbergbekken is creating the border between Frogn and Vestby and receives run-off from Frogn, Vestby and Ås district. Map is found at Kartverket, seeiendom.no, 06.02.2017.

This study took place at the Norwegian Institute of Water Research (NIVA)'s station, Drøbak, where Solbergbekken runs out in the Oslo fjord. Solbergbekkens ecological status is estimated to be moderate, based on measured benthic fauna and periphyton (vann-nett.no, 12.02.2017). The water type is medium calcium and humus rich; the stream receives little agricultural runoff and medium runoff from spread household sewage (vann-nett.no, 12.02.2017). The area's mean rainfall during summer 2015 was 5,1 mm and mean temperature was 15,3° C (yr.no, 12.02.2017). Solbergbekken runs 42 km through grain fields and

forest patches, with catchment mostly in marine clay, but glacial till is visible both in the upper end and as bottom substrate in the lower end (own observation). The stream are meandering and banks are frequently caved in, causing suspended material in the water. The most common trees along the stream are alder (*Alnus incana*), birch (*Betula pubescens*), willow (*Salix caprea*), bird cherry (*Prunus padus*) and Norway spruce (*Picea abies*) (own observation).

2.2 Flume design

Flume construction started in May 2015. Four frames were built, each held 2 flumes à 10 meter long, 35 cm high and 50 cm wide. The flume bottom received a generous layer of

mixed river gravel, sizes from 20 – 160 mm, to provide bottom heterogeneity and natural near-bed water flow conditions.

The water flow was tried out visually in flume 1 at an early stage, before water supply from Solbergbekken was in place, so this flume was filled with ground water. For the remaining flumes, water from Solbergbekken was used, entering the flumes via a 5000 L reservoir tank. The tank ensured constant water supply, which is important as water level in Solbergbekken varies with temperature and precipitation. As suspended mineral particles are normal in Solbergbekken after heavy rainfall, and not desirable in the flumes, the tank worked conveniently as a settling zone. From the reservoir tank, water was gravity fed into the flumes. Evaporation for this area in normal to high temperature is 2 – 6 mm (Agropub,2017) and loss was calculated to 10-30 L /day/flume:

$$2 \text{ mm or } 6 \text{ mm} \times 10 \text{ m} \times 0,50 \text{ m} = 10\,000 \text{ m}^3 \text{ or } 30\,000 \text{ m}^3 = 10 \text{ or } 30 \text{ L} \quad (1)$$

Evaporation and small leakages made water level fall below critical pump level within a day or two without continuous water replacement. Water loss was replaced with gravity fed water from a 25 mm pipe connected to the reservoir tank. The pipe ran across all the flumes and replaced 3 L/h through a 2 mm hole, illustrated in figure 2. This hole got blocked by debris

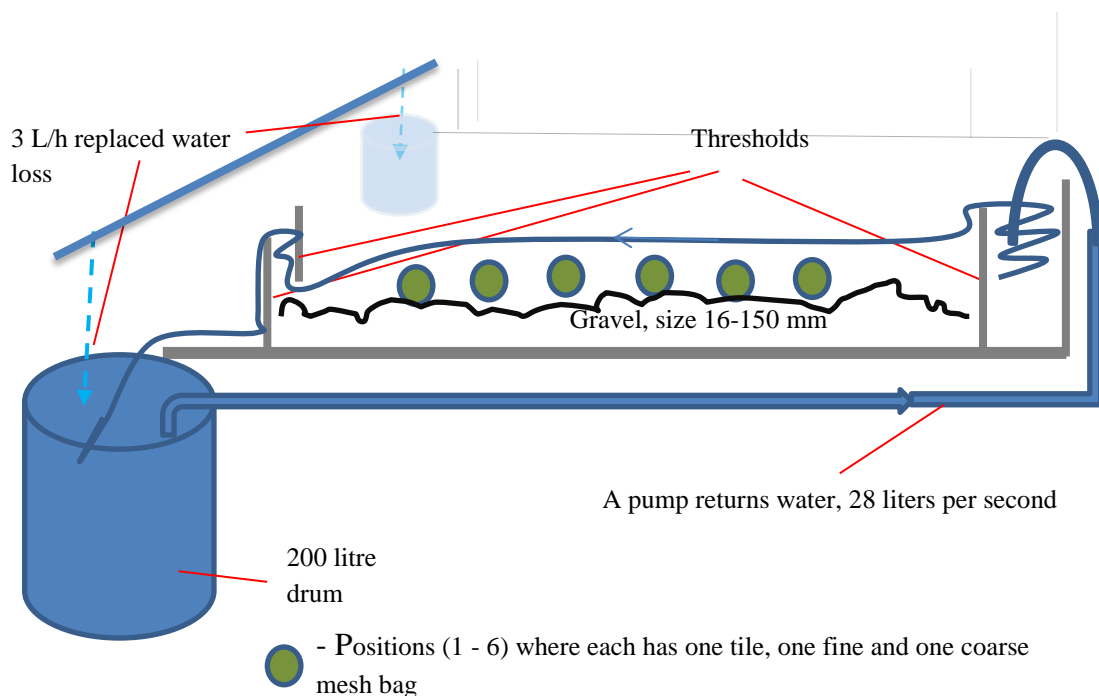


Figure 2: A sketch of the flume design at Solbergstrand. 8 flumes were assembled like this: with independent, circulated water, six positions rested on coarse river gravel with threshold controlled current and water level.

repeatedly and was therefore checked daily.

The amount of water in the system was regulated by two factors, drum capacity in the outlet end of each flume and thresholds fitted in the flume end. The drum held about 120 L water and housed a submersible pump (Grundfos, Unilift AP35B) that circulated the water through a 50 mm pipe at a constant flow of 28 L/s. Thresholds at the start and end of each flume ensured a constant water depth and flow. The inlet threshold, shown in Fig. 3, distributed flow along the width of the flume, while the last of the two thresholds at the outlet determined water level. The last threshold had a 100 mm deep, centered v-notch and blocked the channel, so water flowed through the v-notch or over the top. This design concentrated water flow in the center and top of the water column, so another threshold was fitted 100 mm upstream. This second threshold blocked the channel only in the top 150 mm, and forced water to flow underneath it before it flowed through the v-notch.

Benthic organisms were sampled from Solbergbekken as described in the AQEM manual (AQEM Consortium, 2002), by the use of a surber sampler. From the thoroughly mixed sample, an equal amount (0,5 L of sample containing macroinvertebrate) was added to each flume.

Granite tiles were used as substrate for periphyton colonization in this experiment. The unglazed and unpolished granite tiles (150 mm x 150 mm) were scrubbed thoroughly before use. In each flume, six tiles were positioned one meter apart; the most upstream tile was situated one meter downstream of the inlet threshold. All tiles were positioned about 20 cm below water surface and just upstream of the mesh bags with alder leaves.

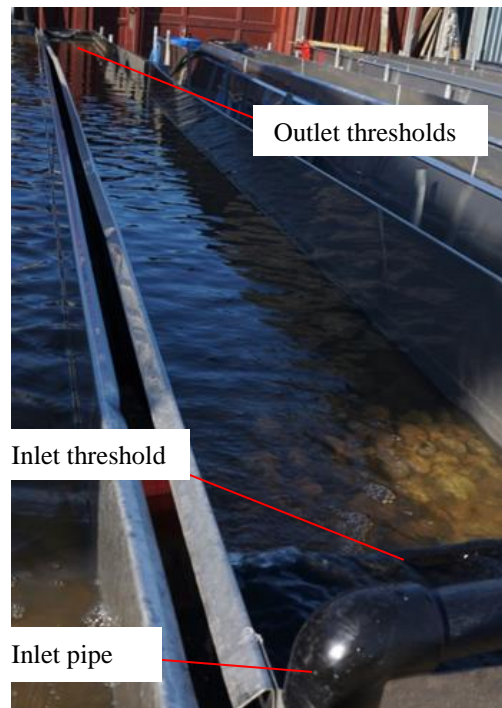


Figure 3: Picture of flume 4 before the start of experiment. Water came into a chamber through the inlet pipe and flowed over the inlet threshold towards the outlet threshold. The thresholds were designed to even out the current in width and depth, but the outlet threshold was also a hindrance that created backflow near the end of the flume. The bottom gravel was covered with about 20 cm free flowing water where the murkiness, seen in this picture, settled before the trial started. Photo by Vibeke Hoff, 4th of May, 2015

Mesh bags were filled with dry alder leaves. Alder leaves were stripped from branches of nearby trees and dried in a drying oven (type Termaks, TS 11). Dry leaves were weighed (scale: Ohaus pioneertm, PA2102) in 2 gram lots, filled in mesh bags, size 150 mm x 250 mm, before plastic strips were used to close each bag. The mesh used in the bags was of two sizes, coarse mesh with holes ≥ 2 mm x 2 mm and fine with holes 0.5 mm x 0.5 mm. Filled mesh bags were soaked in tap water for 24 hours to condition the leaves for microbial and invertebrate consumption. Once soaked, one bag of each mesh size was placed side by side, just downstream of each tile and similar in all flumes.

The flumes were divided into two lots, Lot 1 and Lot 2. In Lot 1, alga on all the tiles were measured weekly but otherwise left undisturbed. In Lot 2, in each flume, one tile and the two mesh bags beside it were randomly sampled per week (detailed list of positions sampled in Appendix A). In Lot 2, algal biomass was measured immediately prior to removal of the tile.

Before experiment started seagulls took an interest in our study. To shield the channels from birds and to add the shade that is natural to a stream, all the flumes were covered with black shade cloth (available at garden supply stores). This cloth was stretched over the top on each frame or pair of flumes, so that it stayed above the water and did not touch the water surface. However, rain and wind brought the cloth occasionally in contact with the water, so at the end of the experiment, most of the shade cloth had algae on it. During the experiment some mesh bag were found afloat. These bags were promptly put back in their position.

The experiment started on 1st of July, 2015 when tiles and mesh bags were placed in the flumes. Within 14 days flume 5, from Lot 2, suffered severe water loss and was taken out of the experiment. Measurements were done weekly over a 42 days period and the experiment was concluded the 12th of August, 2015.

2.3 Method/Collecting Data

2.3.1 Chlorophyll a

The chlorophyll a (chl a) on the tiles was measured in situ with a BenthosTorch, i.e. a Pulse Amplitude Modulated (PAM) fluorimeter developed by BBE Moldaenke GmbH. Four measure points were taken on each tile and their mean value used in later analysis. The Benthos Torch gives separate readings for diatoms, cyanobacteria and green algae; total chl a is calculated as the sum of diatoms, cyanobacteria and green algae.

2.3.2 Leaf degradation

Mesh bags were lifted carefully, while a sieve held underneath caught the macroinvertebrates that tried to escape the bags. Getting the leaves out of the bag while wet, was difficult without loss of biomass. Therefore the leaves were left in their bags and dried at 100 degrees Celsius (Termaks, TS 11) for 4 hours before they were weighed (Ohaus pioneertm, PA2102). In Lot 2 the mesh bags were dried and weighed as their position was removed. In Lot 1 all bags were removed and dried the last day of the experiment, and leaf mass weighed and recorded.

Leaf biomass from fine meshed bags was subtracted from the leaf biomass in coarse mesh bags to calculate leaf biomass loss caused by macroinvertebrates. Leaf decay may be described with a single exponential function as suggested by Bärlocher (2005) and Wieder (1982), and leaf weight was used to calculate decay rate (k).

$$M_t = M_0 \times e^{-kt} \quad (1)$$

solved with:

$$\ln[M_t] = \ln[M_0] - k \times t \quad (2)$$

Where M_t is leaf rest at time t , M_0 the initial leaf weight.

2.3.3 Algal species

In week 6, after the final BenthosTorch measurement, all the tiles in Lot 1 were collected and algae sampled as suggested for hard, removable substrates by Barbour, Gerritsen, Snyder and Stribling (1999). When investigated under microscope (SM-LUX, Leitz) two drops of the

greenest flask content were put under an object glass and observed under 40 x magnifications. The sample were analyzed systematically from left to right, from one edge to the other and from right to left until all area was covered. Green algae and cyanobacteria were identified to species level where possible. Density of each taxon was estimated according to a five point scale, as used by Schaumburg et al. (2004).

2.4 Statistical analysis

All data was analyzed and graphically illustrated in R studio, version 3.2.3. Differences between the various parameters and the significant level were found with analysis of variance (ANOVA) and analysis of linear mixed effects. Formulas and calculations in R were taken from the packages: tidyverse, magittr, lme4, Matrix and dplyr. Plots were made with package ggplot2 and significance was tested at $p < 0.05$.

3 Results

Filamentous green algae increased visibly as weeks passed. During the last two weeks, the tiles at every position were hard to locate and green algae afloat had to be pushed aside to position the BenthosTorch correctly. Algae on the tiles itself did not visibly appear to increase during the last 3 weeks. Flume 1, which was filled with ground water, had clearer waters than the other flumes during the first 2 weeks, but by week 32 the visible impression was the same for all the flumes. Also noticeable in the flumes were the different water level at the upstream and downstream end. The end threshold seemed to hold up the water, so that depth increased gradually from the most downstream position toward the outlet.

3.1 Chlorophyll a

Chl a was affected by time, and increased by an average of $0.80 \mu\text{g chl a /cm}^2$ per week. The chl a for each of the algae taxons is shown in Fig. 4. Mean values across all weeks and flumes were $0.26 \mu\text{g/cm}^2$ for cyanobacteria, $0.57 \mu\text{g/cm}^2$ for diatoms and $1.59 \mu\text{g/cm}^2$ for green algae. There were no significant difference in total chl a between Lot 1 and Lot 2 ($p=0.94$). Total chl a, calculated as the sum of chl a from cyanobacteria, diatoms and green algae, across all the weeks and flumes, is shown in Fig 5. Similarly, there were no significant difference in chl a across all the weeks and flumes for each of the algae types; cyanobacteria ($p=0.82$), diatoms ($p=0.63$), or green algae ($p = 0.61$, Fig. 6).

In Lot 1, there were no differences in total chl a among the positions ($p=0.96$, Fig. 7), but when algal groups were analyzed separately, as in Fig. 8, patterns were different across algal groups. Green algal chl a was higher than the other algal groups already in week 29, except in position 1, flume 2. Green algae grew fast and reached a peak by week

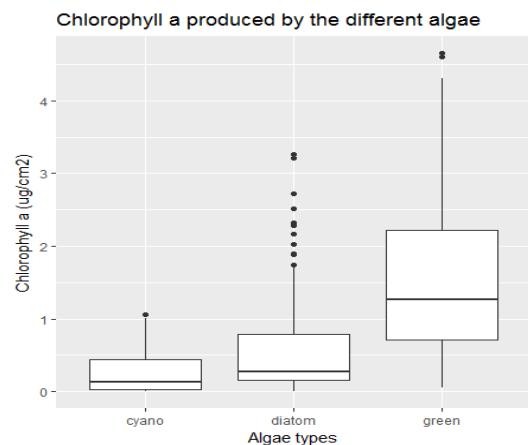


Figure 4: Boxplot of chl a in cyanobacteria, diatoms and green algae with mean values of $0.26 \mu\text{g/cm}^2$, $0.57 \mu\text{g/cm}^2$ and $1.59 \mu\text{g/cm}^2$ respectively.

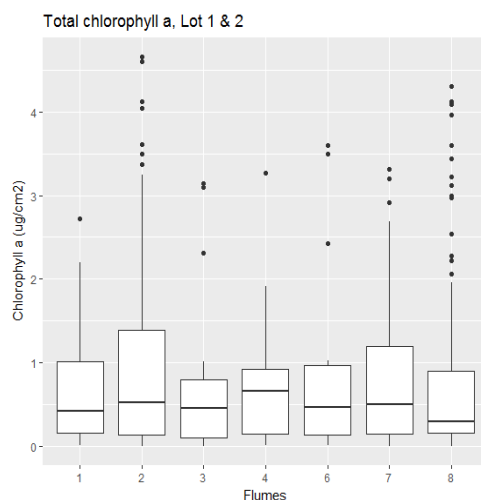


Figure 5: Total chl a across all the flumes are similar ($p= 0.92$).

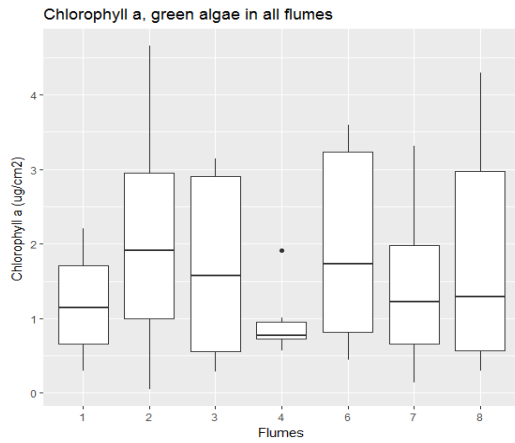


Figure 6: Chl a for green algae show similar variation across the flumes ($p=0.61$).

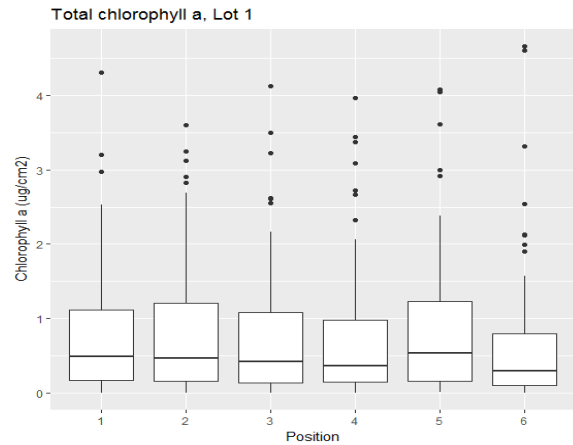


Figure 7: In Lot 1, the total chl a across the positions were similar, ($p=0.96$).

33, before it leveled out or decreased in week 34. Only position 6 in flume 1 demonstrated a slight increase in green algae chl a in week 34. Diatoms started to grow slower than green algae, and exhibited exponential growth near the end. Between week 33 and 34, diatoms surpassed green algae at four of the positions in flume 1 and 7. In flume 2 and 8, diatoms exceeded green algae only in position 1. Cyanobacteria also increased near the end, but to a less so than diatoms, and cyanobacteria did not exceed any of the other algae groups.

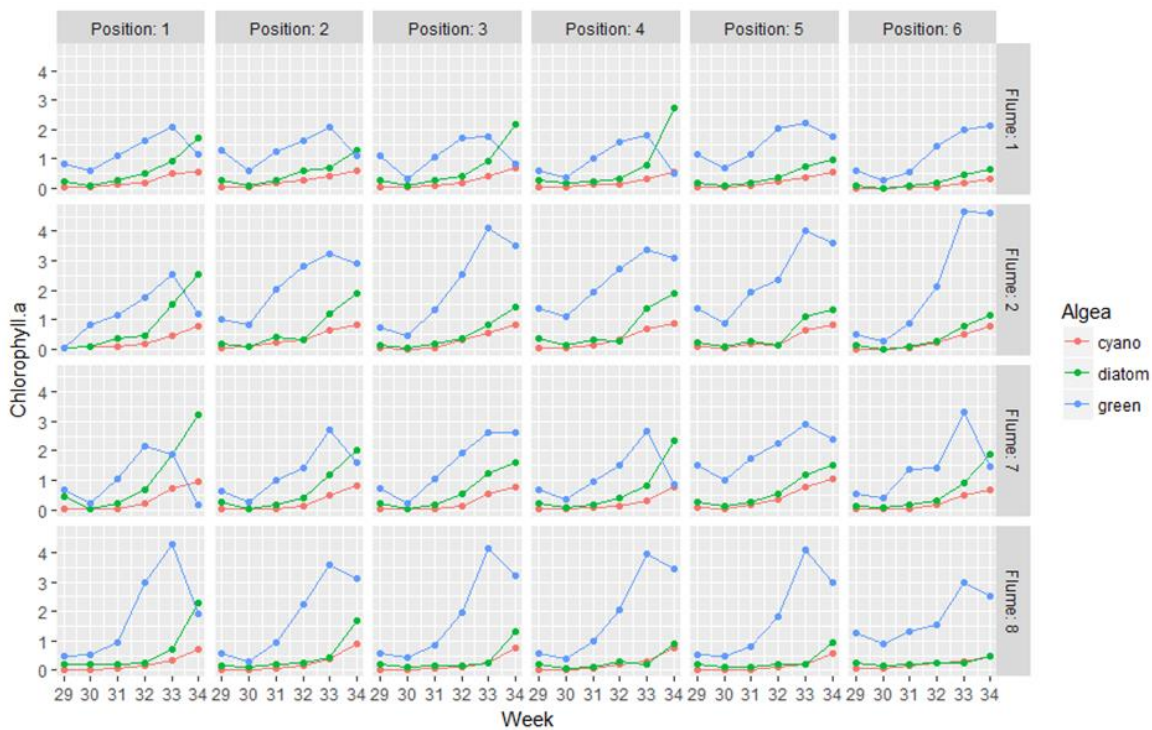


Figure 8: Chl a ($\mu\text{g}/\text{cm}^2$) in Lot 1 presented graphically. Green algae (blue, top line) halted in many positions around week 33. Diatom algae (green, middle line) had exponential growth and passed green algae in several positions in week 33 or 34. Cyanobacteria (red, bottom line) also increased toward the end, but not as much as diatoms.

3.2 Leaf degradation

Biomass degradation across flumes did not differ significantly (Fig.10, $p=0.41$), although flume 3 and 4 included negative values and therefore showed a lower average leaf loss than the other flumes. When the Lots were analyzed separately, there were no significant difference in Lot 1 across the flumes ($p= 0.85$, Appendix A), but more difference across flumes in Lot 2 ($p= 0.09$). In Lot 2 leaf loss in week 29 and 34 had negative mean value, but the total degraded biomass for each week showed no significant difference ($p=0.93$, Fig. 11).

When Lot 1's leaf biomass was analyzed in relation to position, p-value indicated same longitudinal degradation ($p= 0.85$, Fig.12), but decay rate differed significantly across the

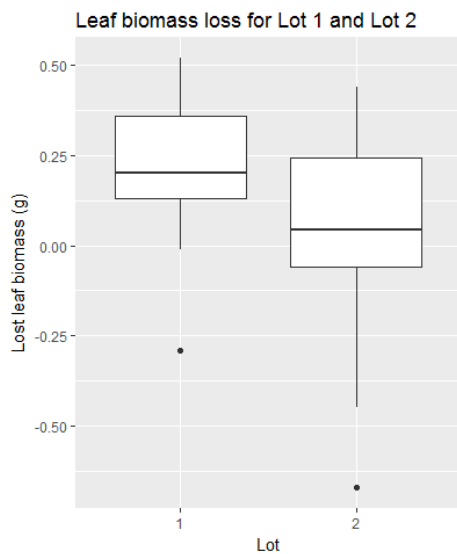


Figure 9: Leaf biomass loss were significantly different in the two Lots ($p=0.01$).

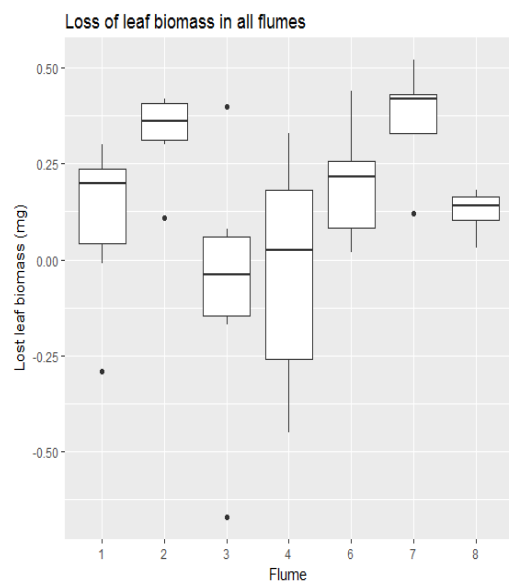


Figure 10: There were no significant differences in leaf degradation between the flumes, ($p= 0.41$).

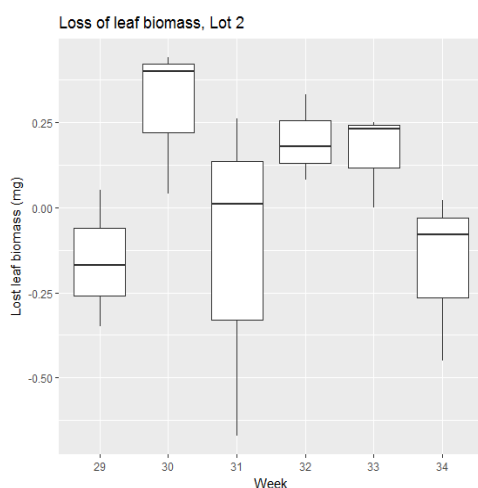


Figure 11: There were no significant differences in degraded leaf biomass in Lot 2 across the weeks ($p=0.93$).

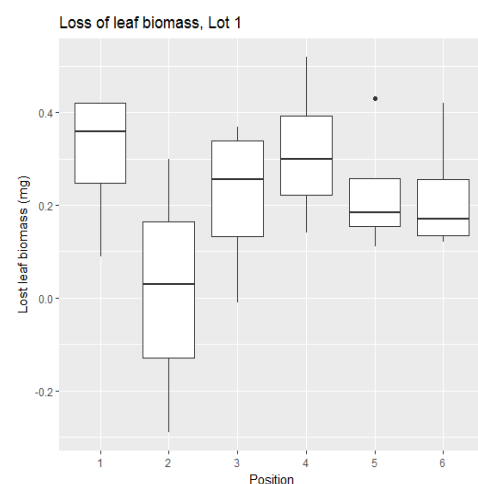


Figure 12: There were no significant differences in degraded leaf biomass in Lot 1 for positions ($p=0.85$).

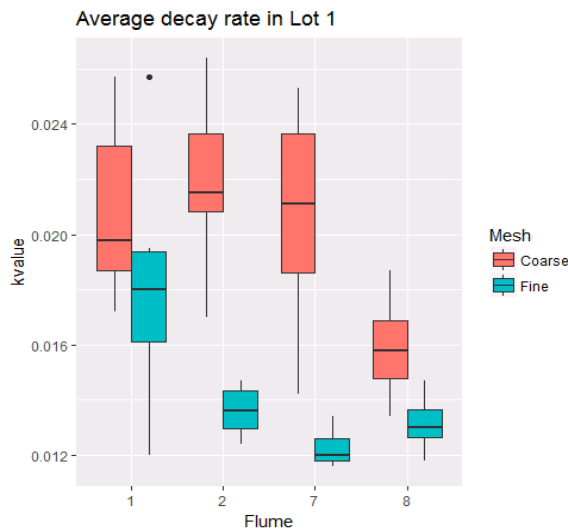


Figure 13: Decay rate across the flumes in Lot 1. The total decay rate in Flume 1 is higher than in the other flumes.

flumes ($p=0.01$, Fig. 13). Flume 1 had a higher decay rate in fine mesh bags than in the other flumes. Also in Flume 1, the difference between fine and coarse mesh bags were less pronounced than in Flume 2, 7 and 8. Average decay rate were 0.01 for fine mesh bags and 0.02 for coarse mesh bags. Flume 8 had the lowest average decay rate in the coarse mesh. (For full list of decay rates, see appendix C, and for calculated decay averages, see appendix D).

3.3 Macroinvertebrates

Live macroinvertebrates were not visible on tiles at any inspected time in the experiment, or at the time of removal of tiles and mesh bags. Occasionally dead adult insects were seen afloat.

3.4 Alga species

An average of 19 algal species was observed on each tile, ranging from 14 to 22. The number of species not statistically different across the flumes ($p=0.86$, Fig. 14) and positions ($p=0.77$, Fig. 15), but Flume 8's position 5 and 6 had the highest number of species. The flumes had much the same species, but in Flume 1, 7 species occurred in different densities than in the other flumes; *Scenedesmus*, *Cosmarium*, *Ulothrix* and *Mougeotia* were less dense and *Palmella*, *Coenocystis* and *Tetraspora* were denser in Flume 1, compared to the other flumes (Table 2, Complete results in Appendix E).

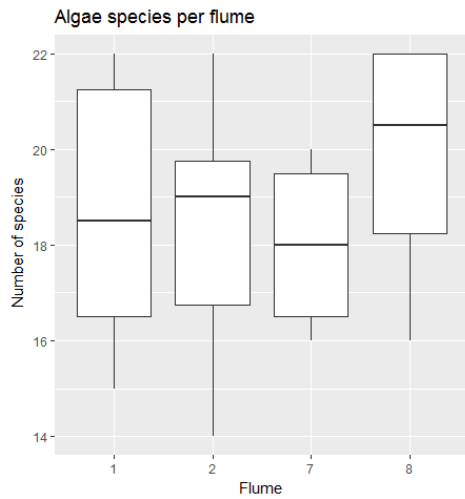


Figure 14: Number of algae species across the flumes, lot 1 only (p=0.86).

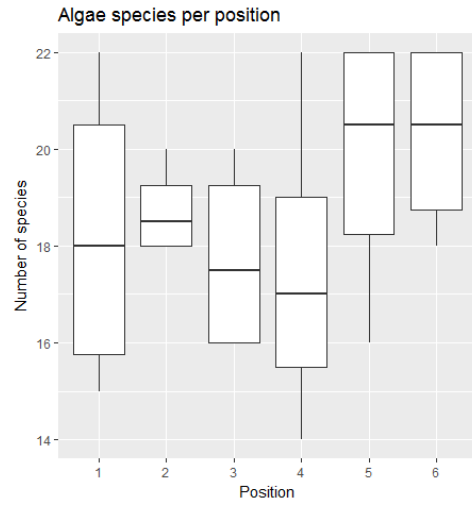


Figure 15: Number of algae species for each of the positions in lot 1 and lot 2

Table 3: The algae species whose density differed in Flume 1 from that in the other flumes. The first four were less abundant in Flume 1 and the three last species were more abundant in Flume 1 than in the remaining flumes.

Algae species	Flume 1	Flume 2	Flume 7	Flume 8
	Results/ position 1 (2) 3 (4) 5 (6)	Results/ position 1 (2) 3 (4) 5 (6)	Results/ position 1 (2) 3 (4) 5 (6)	Results/ position 1 (2) 3 (4) 5 (6)
<i>Scenedesmus</i>	5(4)4(4)4(5)	5(5)5(5)5(5)	5(5)5(5)5(5)	5(5)5(5)5(5)
<i>Cosmarium</i>	3(3)3(3)3(4)	4(4)3(3)4(4)	4(4)4(4)4(4)	4(3)3(4)4(3)
<i>Ulothrix</i>	0(1)0(0)1(1)	0(1)2(0)2(2)	1(2)2(1)2(2)	3(1)0(1)1(1)
<i>Mougeotia</i>	4(3)3(4)3(4)	4(4)5(4)3(4)	4(4)5(4)4(3)	5(3)5(4)3(4)
<i>Palmella</i>	4(5)5(5)5(5)	3(3)5(1)2(4)	2(3)4(4)3(3)	3(2)4(1)2(4)
<i>Coenocystis</i>	3(0)3(4)3(3)	0(2)1(1)1(0)	1(1)0(2)0(3)	1(1)2(3)2(3)
<i>Tetraspora</i>	0(1)1(0)2(2)	1(0)0(0)0(0)	0(0)0(0)0(0)	1(0)0(1)0(1)

4 Discussion

In this thesis, the variation of algal colonization and leaf degradation in newly constructed flumes was studied. Similarity across the flumes was expected. Algal colonization did develop similar in all the flumes and at all the positions, so neither the location of the flume nor the longitudinal position within the flume affected algal growth. No differences in chl *a* were measured between sampling methods in Lot 1 and Lot 2, which indicates that random removal of tiles and mesh bags may not affect the algae on the remaining tiles. Green algae dominated at the start of the experiment, but the algal community switched to a more diatom-dominated assemblage at the end of the experiment. Other studies have shown that green algae detach from substrate when drag forces exceed tensional cell strength (Asaeda and Son, 2000), and green algal detachment may have caused the switch. Tensional cell strength is adapted to water velocity but is also affected by algal size. Filamentous green algae grow large, with an open matrix, influenced by drag force, until it detaches and algal communities switch from green algae to diatom dominance (Asaeda and Son, 2000, Briggs et al., 1998, Horner et al., 1990). Horner et al (1990) also recorded that while *Phormidium* dominated in higher velocity channels, *Mougeotia* were the most abundant filamentous algae at low velocity (< 0,2 m/s), a velocity similar to the one used in this study. In addition, in this study, *Mougeotia* was an abundant taxon in all the flumes, possibly the one that detached in large quanta and was seen afloat. When green algae detach, the community change to be diatom dominant for a few days before green algae recover its loss (Briggs et al., 1998). Green algae in different stages of re-growth illustrate best the wide range of green algae values in figure 8. Herbivores may have influenced green algae growth patterns, but this experiment lack conclusive evidence on that part.

There was a significant difference in leaf mass loss between Lot1 and Lot2. This can be explained by the different sampling strategy. In Lot 1 all mesh bags were removed at the end of the experiment while the bags in Lot 2 were removed successively, and therefore the leaves in Lot1 were on average more degraded than in Lot 2. Leaf litter decomposition in Lot 2 per week, were not significantly different when analyzed on leaf biomass loss. Data in week 29, 31 and 34 held negative values, which means that weight of leaves increased relative to the initial weight. This can happen when macroinvertebrates have been included in the leaves weight, something they are sure to be, according to Hauer (1991). In addition benthic algae colonize the leaves and may also add to the leaves weight. Results in the remaining three weeks, 30, 32 and 33, indicate a decreased leaf loss over time, similar to results found by

Cummins et al., (1980). The initial leaf loss is high due to easy degradable parts in the leaves, and followed by a slower decay for the less degradable parts (Cummins et. al, 1980). Leaf biomass loss is also analyzed as decay rate.

When transformed to decay rate, leaf degradation from this study followed the exponential pattern predicted by Bärlocher (2005). The decay rate in Flume 1, were high in both fine and coarse mesh bags, unlike the decay rate in the other flumes. This may possibly be because of Flume 1's ground water content, but the exact explanation is not clear. Groundwater may be expected to have lower levels of microbes, than surface water from Solbergbekken but this would result in low decay rate. However, in the present study the average decay rate was 0, 02 and 0, 01 for coarse and fine mesh bags respectively, which corresponds to Leucercf (2016)'s results. Leucercf (2016) found that alder leaves degradation rate differs significantly between leaves of different quality, but leaves used at Solbergstrand were harvested from the same side of the same tree, so different leaf quality cannot explain the difference observed between fine and coarse mesh bags and between the flumes, as seen in Fig. 13.

The groundwater content in Flume 1 did not significantly affect algal chl a. Investigation of algae species density, however, revealed differences; the species community in Flume 1 showed a decreased density of four taxa and increased density of three taxa. *Scenedesmus*, *Cosmarium*, *Ulothrix* and *Mougeotia* grew less dense in flume 1, *Palmella*, *Coenocystis* and *Tetraspora* grew denser. A greater difference was expected between Flume 1 and the other flumes, but the difference was possibly leveled out by the constant refill of 3 liters of water from Solbergbekken per hour. Water quality is reflected in algal species composition. Schneider and Lindstøm, (2011) have developed a way to identify water quality based on periphyton, named PIT index. The PIT index may differ between the flumes but to work it out, the width of the green algal filament is needed, a parameter not possible to measure with the microscope available at NMBU.

The flumes at Solbergstrand, have the complexity and length recommended by Swift, Troelstrup, Detenbeck and Foley (1993) to study biological responses to physical, chemical and biological stressors as well as processes over longer time periods. However, further adjustments may easily be done to create a more natural stream environment and longer experimental channel. The backflow observed in the flumes are one hydrological aspect that can adversely affect both periphyton and invertebrates (Ceola et al., 2013, Stanzer et al., 1988), and which shortened the replicable length in each flume. The back flow may be

eliminated if the outlet threshold is replaced by a louvered gate as suggested by Nowell and Jumars (1987).

The macroinvertebrate community may be difficult to replicate across flumes as well as between experiments, the way it is described in this study. Solbergbekken has a great variability of benthic habitats and macroinvertebrates collected may not be suitable for the relatively homogenous environment in the flumes. To ease invertebrate's shift from stream to flume, Craig (1993) and Statzner et al (1988) suggest identifying Froude number and Reynolds number in the stream and reconstruct similar value in the flume. Ceola et al (2013) and Wellnitz (2001) isolated insect preferences with near-bed water velocity. Froude and Reynolds number as well as near bed water velocity can be measured to more accurately fit a natural macroinvertebrate community into these flumes and thus improve replicability. Elbrecht et al. (2016) skipped these parameters but rather recorded exact macroinvertebrates in each artificial stream environment and the number of insects that escaped. Either way may provide better replicable macroinvertebrate community, than accomplished in this study.

5 Conclusion

We have studied variation of algae colonization in artificial streams, constructed at Solbergstrand, Drøbak, and found them to be true replicates where water quality was kept the same. No differences in algal biomass, composition and leaf litter degradation were detected among upstream – downstream positions in the flumes.

All positions and flumes experienced similar algae growth both in total volume and weekly development. The decline in algae biomass was possibly caused by aging of the assemblages, and similar patterns were observed in most positions and flumes. Leaf decay rate were different in Flume 1 than in the other flumes. Flume 1's ground water content may have caused both different leaf degradation and algae species distribution; three algae species increased and four species decreased in density in Flume 1 compared with the other flumes.

As long as same water quality is used, these flumes will give satisfactory replicates and be able to produce valuable data on processes in running water. Results from this set up may, however, be strengthened by comparison of algae colonization on tiles and leaf degradation in Solbergbekken. A measure on how well the flumes ecosystem processes fit natural processes, would display the flumes ability to imitate a natural environment. And a natural environment in the flumes may offer foundation for future research of more complexity. It is suggested that

a knowledge gap exist on stream-community's responses to hydro-morphological change (Friberg, 2014), something that may be possible to study in these flumes. Their complexity and size make them suitable for experiments over longer time periods, experiments that is needed to pin point present and future activities that negatively affect water quality.

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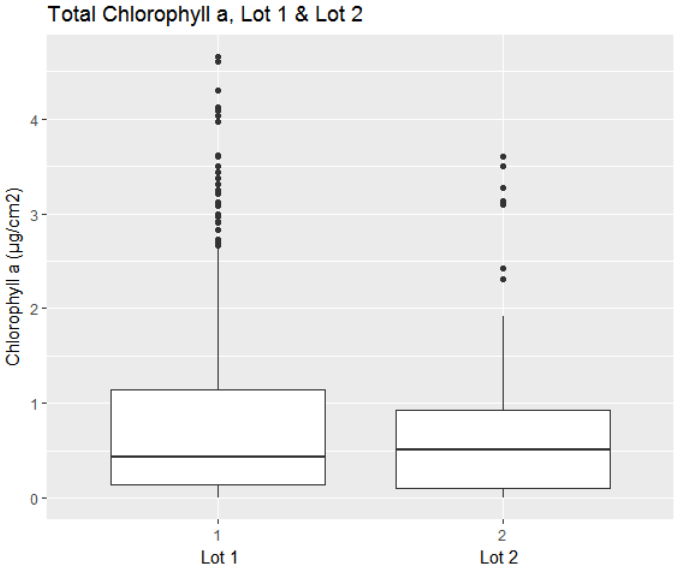
Appendix

Appendix A

In Lot 2, Flume 3, 4 and 6, one tile and two mesh bags were randomly removed from the flumes, and the table show which positions were taken out in each week.

	Flume			
		3	4	6
Week				
29		1	1	5
30		4	4	3
31		6	2	4
32		2	3	1
33		3	6	6
34		5	5	2

Appendix B



Visualization of chlorophyll a in the two lots.

Appendix C

Remaining leaf mass in coarse (CM) and fine (FM) mesh bags and their k-value.

Flume	Position	Remaining CM(g)	Remaining CM(%)	k-value CM	Remaining FM(g)	Remaining FM(%)	k-value FM
1	1	0.91	0.455	0.0187	1.21	0.605	0.012
1	2	0.97	0.485	0.0172	0.68	0.34	0.0257
1	3	0.91	0.455	0.0187	0.9	0.45	0.019
1	4	0.73	0.365	0.024	0.98	0.49	0.017
1	5	0.83	0.415	0.209	1.03	0.515	0.0158
1	6	0.68	0.34	0.0257	0.88	0.44	0.0195
2	1	0.72	0.36	0.0243	1.14	0.57	0.0134
2	2	0.82	0.41	0.0212	1.12	0.56	0.0138
2	3	0.8	0.4	0.0218	1.17	0.585	0.0128
2	4	0.84	0.42	0.0207	1.19	0.595	0.0124
2	5	0.98	0.49	0.017	1.09	0.545	0.0145
2	6	0.66	0.33	0.0264	1.08	0.54	0.0147
7	1	0.72	0.36	0.0243	1.14	0.57	0.0134
7	2	0.94	0.47	0.018			
7	3	0.85	0.425	0.0204	1.18	0.59	0.0126
7	4	0.69	0.345	0.0253	1.21	0.605	0.012
7	5	0.8	0.4	0.0218	1.23	0.615	0.0116
7	6	1.1	0.55	0.0142	1.22	0.61	0.0118
8	1	1.06	0.53	0.0151	1.15	0.575	0.0132
8	2	1.14	0.57	0.0134	1.17	0.585	0.0128
8	3	1	0.5	0.0165	1.18	0.59	0.0126
8	4	0.98	0.49	0.017	1.12	0.56	0.0138
8	5	0.91	0.455	0.0187	1.08	0.54	0.0147
8	6	1.08	0.54	0.0147	1.22	0.61	0.0118

Appendix D

Table show the calculated average of remaining leaf biomass and the average K-value in Lot 1

Flume	Mesh	Avg Remain	Avg K-value
1	Coarse	0.84	0.02
1	Fine	0.95	0.02
2	Coarse	0.80	0.02
2	Fine	1.13	0.01
7	Coarse	0.85	0.02
7	Fine	1.20	0.01
8	Coarse	1.03	0.02
8	Fine	1.15	0.01

Appendix E

All the green algae and cyanobacteria species identified on tiles in Lot 1, and their density on a scale from 1 - 5. Values found for each position are shown in the order described in the header. The list starts with non-filamentous green algae, and two cyanobacteria (lightest shade). The darkest shades show filamentous algae, six cyanobacteria species first and six green algae species last.

Algae species	Flume 1 Position 1(2)3(4)5(6)	Flume 2 Position 1(2)3(4)5(6)	Flume 7 Position 1(2)3(4)5(6)	Flume 8 Position 1(2)3(4)5(6)
<i>Scenedecmus</i>	5(4)4(4)4(5)	5(5)5(5)5(5)	5(5)5(5)5(5)	5(5)5(5)5(5)
<i>Cosmarium</i>	3(3)3(3)3(4)	4(4)3(3)4(4)	4(4)4(4)4(4)	4(3)3(4)4(3)
<i>Staurasmus</i>	2(3)2(2)2(3)	3(3)2(3)4(3)	3(3)3(4)3(3)	3(3)3(3)3(3)
<i>Ankistrodesmus</i>	3(4)5(3)4(4)	3(2)3(4)3(3)	4(4)3(3)3(3)	4(3)3(3)3(2)
<i>Coelastrum</i>	5(4)3(4)3(4)	4(4)3(4)4(4)	4(3)2(3)3(4)	3(5)4(4)3(3)
<i>Palmella</i>	4(5)5(5)5(5)	3(3)5(1)2(4)	2(3)4(4)3(3)	3(2)4(1)2(4)
<i>Pidiastrum</i>	1(3)1(1)1(1)	1(1)0(0)2(1)	1(1)0(1)1(1)	1(0)0(1)1(1)
<i>Coenocystis</i>	3(0)3(4)3(3)	0(2)1(1)1(0)	1(1)0(2)0(3)	1(1)2(3)2(3)
<i>Tetraedron</i>	1(0)0(1)1(1)	2(1)1(1)1(1)	1(1)0(1)1(2)	1(1)1(1)1(1)
<i>Clostrium</i>	1(0)1(1)1(1)	1(1)1(2)1(2)	1(2)1(2)1(2)	1(0)0(0)1(1)
<i>Gloeocystis</i>	0(1)0(0)2(2)	2(1)0(2)1(1)	0(1)1(0)2(0)	3(1)2(1)2(1)
<i>Treboxia</i>	0(1)0(0)1(1)	0(0)0(0)1(0)	0(0)0(0)0(1)	0(1)0(0)1(0)
<i>Asterococcus</i>	0(0)0(0)0(0)	0(0)0(1)1(0)	0(0)0(0)0(0)	1(0)0(0)1(0)
<i>Chlorococcum</i>	0(0)1(0)1(1)	0(0)0(0)0(0)	1(0)0(0)0(0)	0(0)0(1)0(0)
<i>Dictoshearium</i>	0(0)0(0)0(0)	0(0)0(0)0(1)	0(1)0(0)0(0)	0(0)0(0)0(0)
<i>Planktonoshearua</i>	0(0)1(0)0(0)	0(0)0(0)1(0)	0(1)0(0)0(1)	0(0)0(0)0(0)
<i>Tetraspora</i>	0(1)1(0)2(2)	1(0)0(0)0(0)	0(0)0(0)0(0)	1(0)0(1)0(1)
<i>Sphaerellopsis</i>	0(0)0(0)0(1)	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(0)
<i>Tetracycstis</i>	1(0)0(0)0(2)	0(0)1(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(1)
<i>Schizochlamydeella</i>	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(1)
<i>Merismopidia</i>	3(2)1(1)1(1)	0(1)1(0)2(2)	1(2)1(1)1(2)	1(2)1(2)0(1)
<i>Microcystis</i>	2(1)1(1)2(0)	2(0)1(1)1(1)	1(1)1(1)2(2)	1(1)2(1)2(2)
<i>Pseudoanabena</i>	5(1)3(3)3(3)	3(3)3(3)0(3)	3(3)3(3)3(3)	1(0)1(0)0(2)
<i>Oscillatoria</i>	0(1)1(0)1(1)	0(2)0(0)1(1)	1(0)2(0)0(0)	2(1)1(2)2(0)
<i>Phormidium</i>	2(1)2(1)2(1)	1(1)2(2)0(0)	2(1)1(0)1(0)	2(3)0(1)3(1)
<i>Leptolyngbya</i>	0(3)2(0)2(1)	0(1)1(0)3(3)	1(3)0(1)0(0)	2(3)0(1)3(1)
<i>Dactylococcopsis</i>	0(0)0(0)0(0)	0(0)0(0)0(1)	0(0)0(0)0(0)	0(0)0(0)0(0)
<i>Planktothrix</i>	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(0)
<i>Stigeclonium</i>	0(0)0(0)0(0)	1(1)1(0)1(0)	0(0)0(0)0(0)	0(0)0(0)0(0)
<i>Oedogonium</i>	0(0)0(1)0(0)	0(0)2(0)5(0)	3(0)1(1)0(1)	5(5)2(3)2(2)
<i>Cladophora</i>	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(1)0(0)
<i>Ulothrix</i>	0(1)0(0)1(1)	0(1)2(0)2(2)	1(2)2(1)2(2)	3(1)0(1)1(1)
<i>Moegotia</i>	4(3)3(4)3(4)	4(4)5(4)3(4)	4(4)5(4)4(3)	5(3)5(4)3(4)
<i>Klepsomidium</i>	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(0)0(0)	0(0)0(1)0(0)



Norges miljø- og biovitenskapelig universitet
Noregs miljø- og biovitenskapelige universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway