Grazer control on benthic algae in the Berg River, Western Cape, South Africa

Dunja Basic

Botany Department, University of Cape Town, Private Bag, Rondebosch 7701, South Africa.

2011

Supervisor: Ms Justine Ewart-Smith
Co-supervisor: Associate Professor Jenny Day

Grazer control on benthic algae in the Berg River, Western Cape, South Africa

Dunja Basic

Botany Department, University of Cape Town, Private Bag, Rondebosch 7701, South Africa.

Abstract

Benthic environments in streams are areas of high biological activity where primary producers and consumers interact with each other and the environment. Various factors such as light, nutrient availability and temperature affect algal biomass accumulation however disturbance factors and grazing lead to biomass losses as well as changes in the species composition and densities of algae. There are little, if any, studies done on the effects of grazers on benthic algae in South African rivers and as such, this experiment was done in the Berg River, in the Western Cape of South Africa. The aim was to ascertain the effects of herbivores on benthic algae within the river and it was hypothesized that periphyton biomass would be higher where grazer densities were lower and that the differences observed in biomass of periphyton between treatments would increase over time. It was also hypothesized that there would be a lower diversity of algae where there were lower herbivore densities. A randomized block design experiment was set up in the Berg River and treatments of 'excluded herbivores' and 'included herbivores' were set up, with two pairs of each treatment spread across four replicates. Stones from the stream were randomly selected and placed in the boxes. After five days half of the boxes were sampled and after eight days, the remaining half was sampled. Stones were scrubbed to remove periphyton from the stones and a sub-sample of 50ml was removed from each for later algal species identification. Invertebrates were removed from the boxes, preserved in ethanol and later identified to family level. Chl-a and AFDW (mg/m^2) were determined from the samples as were invertebrate and algal densities. The results showed that there were no herbivore density effects on biomass most likely due to the season of sampling and the length of the experiment. However the effect on algal densities was more pronounced as there was a higher variety of algal forms present where herbivore densities were lower. To get a better idea of how herbivores affect periphyton other factors need to be taken into account like experimental time period, season and different environmental conditions.

The [illegible] grazing [illegible]
[illegible] the [illegible] control [illegible]
disturbance regime [illegible]
[illegible] have [illegible]
[illegible] following [illegible]

Introduction

Benthic environments in streams are areas of high biological activity where primary producers and their consumers co-occur and interact with each other (Hauer and Lamberti 2007). In most streams, benthic algae (periphyton) are the dominant primary producers and can grow on practically any surface which is submerged (Hauer and Lamberti 2007, Stevenson *et al.* 1996). Stream invertebrates, although small, have a huge impact on the biological, chemical and physical components of their environments (Jacoby 1987). Their importance is illustrated by the fact that structural and functional characteristics of stream periphyton are determined by grazing of benthic herbivores (Jacoby 1987, Holomuzki *et al.* 2010, Holomuzki *et al.* 2006). Physico-chemical factors of the in-stream environment determines periphyton biomass, however, grazing will reduce this level in proportion to the grazer densities (Jacoby 1987). Within streams, grazing effects vary both spatially and temporally (Jacoby 1987, Holomuzki *et al.* 2010) due to grazer density, mobility and life history as well as the biotic attributes of the periphyton such as the strength of competitive hierarchies (Holomuzki *et al.* 2010, Holomuzki *et al.* 2006). In some low-productivity lakes nutrient limitation on periphyton might override the grazing pressure therefore resulting in a selection for algal nutrient competitors that are grazer-vulnerable (Holomuzki *et al.* 2010). In a similar fashion, grazers may be unable to reduce periphyton biomass when nutrients are not limiting to their growth (Holomuzki *et al.* 2010). Periodic disturbances may also alter the taxonomic composition and reduce densities of lotic filter-feeders and nullify their effect on algal productivity (Holomuzki *et al.* 2010, Stevenson *et al.* 1996). The feeding modality of grazers also has a huge impact on which algal species and physiognomies are consumable. Different feeding modalities include; rasping, gathering or brushing, cutting and scraping. Stalked, erect and filamentous forms of algae are often vulnerable to most herbivores while prostrate forms are often vulnerable only to raspers and scrapers (Holomuzki *et al.* 2010). Raspers and scrapers generally have a greater effect on periphyton biomass and assemblage structure than do other herbivores due to their feeding modes and the fact that they dislodge overstorey forms when they maneuver through biofilms (Holomuzki *et al.* 2006, Holomuzki *et al.* 2010). This thinning of overstory forms by herbivores allows light to penetrate to the understory levels and therefore enables the growth of microalgae and accelerates patch recovery (Holomuzki *et al.* 2006). In addition to their ability to generate patch structure, after leaving a patch of grazed algae, different herbivore types leave behind different residual effects which may affect the microalgal successional patterns as well as time of recovery (Holomuzki *et al.* 2006).

International literature has shown that periphyton accrual and loss in streams is regulated by many factors working together. Periphyton accrual is affected mainly by nutrient supply (Dodds 2003, Anderson *et al.* 1999) and light availability (DeNicola et al. 1992) as well as temperature which interacts with both of these factors to influence the metabolic rates and growth of algae (Stevenson *et al.* 1996). Periphyton loss is influenced mainly by hydrological events (floods), desiccation and grazing by herbivores (Jacoby 1987, Holomuzki *et al.* 2010, Stevenson *et al.* 1996). The significance of grazing by invertebrates (Jacoby 1987, Holomuzki *et al.* 2010, Holomuzki *et al.* 2006) and fish (Power 1992) is usually associated with physically stable or undisturbed environments (Steinman *et al.* 1991). Work done by Ewart-Smith (2011) on rivers in the Western Cape has shown that despite these key controllers, (nutrients, light and disturbances, grazing by invertebrates may act as a 'top-down' control of periphyton biomass during certain times of the year as well as have possible effects on algal species compositions.

The present study was done in the south-western Cape of South Africa which has a Mediterranean type climate characterized by winter rainfall with heavy rainfall over the mountain catchment (Linder 2003). Winter is therefore a period of intense and frequent flood disturbances which is followed by an extended period of flow stability in the summer months. Studies are sorely lacking within south-African streams and rivers and hence this study was focused on the upper foothill reaches of a perennial river, the Berg River (Figure 1).

The aim of this study was to determine the effects of invertebrate herbivory on the benthic algae of the Berg River. The hypotheses addressed were as follows:

Hypothesis 1: Periphyton biomass as well as live algal biomass will be significantly higher under conditions of reduced herbivore densities, relative to the natural densities found in the Berg River.

Hypothesis 2: Differences in the periphyton biomass between treatments of different herbivore densities will be enhanced with time.

Hypothesis 3: There will be a lower diversity of algal species in treatments with reduced herbivore densities relative to the stream and treatments with increased herbivore densities because grazing maintains the diversity of algal species by preventing the domination of one or two taxa.

Methods and Materials

Study Site

The Berg River is naturally acidic with low nutrient concentrations (River Health Programme 2004) and dissolved solids due partly because of the humic compounds that are leached from the surrounding fynbos and partly because of the geology of the region itself which is characterized by the quartzitic sandstones of the Table Mountain Group (Davies and Day 1998). The Berg River originates in the Franschhoek and Drakenstein Mountains and flows northwards and then westwards past Velddrif where it drains into St. Helena Bay on the West Coast of South Africa, 285km away (River Health Programme 2004). Historically, the upper catchment of the Berg River was dominated by commercial pine plantations that were then clear felled by the Working For Water programme in 1998 (River Health Programme 2004). The natural vegetation in the environment, mostly sandstone fynbos, has managed to recover over the past 14 years and therefore the catchment area upstream of the dam has a natural flow regime and is largely ~~not~~ unimpacted by human activities (River Health Programme 2004).

The study site was dominated by boulders and cobble stones; however gravel and sand also occurred. On the left was a steep mountainous bank and on the right was a bar of deposited cobble stones. The river has a natural flow regime characterized by a series of riffles, separated by runs with slackwaters along the boundaries (Figure 2).

Experimental Design

A field experiment was designed to assess the relationship between benthic periphyton biomass and community composition under different invertebrate herbivore densities. The experiment was set up on the 25th March 2011 with four replicates positioned within four hydraulically separate run biotopes in a randomized block design (Figure 3). Each replicate consisted of four boxes representing conditions with and without grazers (as far as possible) (Figure 3). After five days, one pair of each treatment per replicate was sampled and after a further eight days the remaining pair in each replicate was sampled therefore providing two independent temporal samples. The boxes were plastic, to ensure control of herbivore densities, and had an area of $0.0495m^2$. They were positioned in such a way that continuity with the stream was ensured. This was done by replacing the two shorter sides with an 80µm mesh net which allowed water to flow through without letting invertebrates leave or enter the box. Each box was then layered with gravel and placed into the river (Figure 4). Considering the potential influence of stone size on periphyton biomass, three replicate stones of similar size were randomly selected for each box therefore reducing experimental error (Figure 4). Collection of stones for the treatment where invertebrates were included involved placing an 80µm mesh net downstream of the stone in the run biotope and lifting it into the net therefore ensuring that all the invertebrates that were on or underneath the stone were washed into the net. Predators were removed from the samples as far as possible and the stones and invertebrates were placed in the boxes. Collection of stones for the treatment where invertebrates were excluded involved choosing random stones from the run biotope and removing all the invertebrates from them by picking them off gently with forceps to ensure that algal loss was minimal. These stones were then also placed in their respective boxes which were then covered with Perspex lids held on with metal clips (Figure 4).

Sampling Procedures

Sampling consisted of taking each stone out the box, carefully picking off any invertebrates found on it, placing the stone in a sampling tray and removing the periphyton by scrubbing the stone until the rinsing water had no colour change. A 50ml sub-sample was removed from each sample and preserved in Lugol's solution for further identification of algal species. The remainder of the slurry was placed in a dark container on ice for transport back to the laboratory where samples were frozen within 10hours of collection. The dimensions of each stone were then measured as the longest axis (x), the longest horizontal axis perpendicular to x (y) and the longest vertical axis of the stone (z) (Dudley *et al.* 2001). The percentage of the stone embedded was estimated from observation and hence the % of the stone covered with algae was determined. The box was then removed from the stream and the remaining gravel sifted through for invertebrates which were placed in jars with 70% ethanol. This procedure was repeated for each box within each replicate over the two sampling days as well as for eight stream stone samples taken *in situ*.

The surface area of each stone was calculated using the following regression equation for stone area (Ewart-Smith 2011):

$$\mathrm{y\ (surface\ area\ in\ m^2)} = \frac{0.014\ (\mathrm{xy} + \mathrm{xz} + \mathrm{yz}) + 33.819}{10000}$$

Stone surface area not embedded was determined by dividing the stone surface area with the percentage algal cover.

Determination of AFDW and Chlorophyll-a of Periphyton

Twenty-four hours prior to analysis, the samples were left to defrost in the dark. Once defrosted, each sample was briefly mixed to homogenize it and then it was divided into two portions for the measurement of ash free dry weight (AFDW) and chlorophyll a.

The total dry weight of each sample was measured by filtering the sample portion through a Whatmann 45µm GF/F filter paper which was then dried for at 60°C for 2hrs after which the samples were ashed at 400°C for 4hrs. The organic component (AFDW) of the periphyton was determined by ~~getting~~ calculating the difference between the dry weight and the weight ashed. The other portion of the sample was used for Chl-a determination which is used as a measure of live algal biomass. Chl-a was extracted using methanol AR and boiled for three minutes at 70°C to increase the extraction efficiency and to fix the chlorophyll by destroying the enzymes. Absorbance was measured at a wavelength of 665nm with a spectrophotometer (Spectroquant Pharo 100) and background absorbance was measured at 750nm to account for scattering of light (Thompson *et al.* 1999). As algal communities age and die, chlorophyll degrades naturally therefore resulting in products called phaeopigments which interfere with spectrophotometric measurements of live chl-a (Marker *et al.* 1980, Wasmund *et al.* 2006). It was therefore necessary to acidify the samples with 0.1M hydrochloric acid to correct for the phaeopigments in the sample measurements. The samples were then neutralised with 0.1M sodium hydroxide and absorbances were re-read at both 665nm and 750nm following both acidification and neutralization steps. The measurements were then corrected for the presence of phaeopigments and light scattering. The chl-a concentrations were then determined by multiplying the values by 36.95 which is the absorption coefficient of methanol.

Chl-a and AFDW (mg/m^2) not embedded was then determined by dividing the chl-a and AFDW values by the respective stones' surface area not embedded.

Algal Taxonomic Analysis

Each 50ml sub-sample was decanted into a container and homogenized for 10s using a hand blender to ensure that clumps of filamentous algae were broken up (Biggs 1987). This ensures that further sampling is representative of the whole sample therefore reducing variability and increasing precision with further sampling. Thereafter, a 5ml sub-sample was extracted and centrifuged at 3000rpm for 10min to concentrate the algal cells. The supernatant (4.5ml) was discarded and the remaining cells in the pellet (0.5ml) were re-suspended. All cells were then identified and enumerated using a haemocytometer under 400x magnification. Following identification and enumeration, algal density per sample was quantified as the number per m^2 of stone surface area. A weighting factor was calculated as the fraction of the longest axis of a single cell (averaged over ten individuals) relative to that of the largest individual in the data set. This compensates for the large difference in scale between micro and macro algae represented in the samples. Algal density was then weighted, using the weighting factor relative to each species, to standardise the data.

Herbivore Density Determination

Consumption of periphyton by invertebrates could be an important factor to account for when addressing patterns in periphyton community structure and biomass, it was necessary to quantify the biomass of invertebrates that are considered algal feeders. Algal feeders were identified as those that fed solely or partly on algal material. Invertebrates were identified to family level using a dissecting microscope and were separated according to functional feeding groups that feed on algae; deposit feeders, grazers and scrapers (Table 1).

Statistical Analysis

Chl-a and AFDW results were log transformed and analysed for significant differences between time and treatment using a two-way nested analysis of variance (ANOVA) with factors 'time' (two levels: time 1 and time 2) and 'treatment' (three levels: excluded, included, stream) at $p<0.05$ (Statistica Version 10; Tulsa, USA). The same two-way ANOVA model was used to test for differences in algal species using PERMANOVA (Anderson 2005), with a Bray Curtis similarity matrix calculated from 4th root transformed data. Dominant algal species were determined using SIMPER analysis in Primer version 6. The effects of the different herbivore densities on AFDW and chl-a were analysed using General Linear Models (GLM) in Statistica version 10.

Results

Determination of AFDW and Chlorophyll-a of Periphyton

Two-way ANOVA analysis of chl-a (Table 2) showed that there were no significant differences between the two time periods (MS=0.00026, df=1, p=0.925) or between the treatments themselves (MS=0.02786, df=2, p=0.389). There were also no significant differences between time periods and treatments together (MS=0.00471, df=2, p=0.846). Analysis of AFDW data (Table 2) yielded similar results where there were no significant differences between the two time periods (MS=0.0008, df=1, p=0.842) or between the treatments themselves (MS=0.0082, df=2, p=0.655). There were also no significant differences between time periods and treatments together (MS=0.02, df=2, p=0.366).

Algal Taxonomic Analysis

PERMANOVA was used to test for differences in algal composition over time and between treatments. Results show that there were no significant differences between time 1 and time 2 (MS= 845.62, df= 1, p=0.215) nor where there significant differences between treatments and time together (MS= 657.49, df=2, p=0.331). There was however a significant difference between treatments (MS= 2320.3, df=2, p=0.0011). A further pair-wise test was done to determine between which treatment pairs there was significance. Results indicated that there was no significant difference between stream samples and excluded samples (t=1.519, df=11, p=0.0592) but there were significant differences between stream samples and included samples (t=1.838, df=11, p=0.0476) as well between excluded and included samples (t=2.659, df=12, p=0.0004). This is illustrated in figure 5 where it is clear to see that algae numbers in the stream samples straddle both of the treatments. To get a better understanding of the relationship between the excluded and included treatments, stream samples were omitted from further analyses. Figure 6 shows a clear separation between excluded and included samples. The sample 'T2 Included Replicate 4' was found to have predators in the box and had a very low grazer density therefore for the analyses it was treated as an 'excluded' sample. In figure 7 it can be distinctly seen that there are three groupings that arise. The included samples make up one of these groupings (Cluster B) showing that they were very similar to each other. The other two groupings are of excluded samples (Cluster A and Cluster C), indicating that they were less similar to the included samples. Table 3 shows that included samples were more similar to each other than stream and excluded samples were. SIMPER analysis showed the average abundances of the algal species that most affected the differences between the treatments excluded and included (Table 4). In the excluded treatment, there was a higher abundance of *Eunotia rhomboidae, Mougeotia spp, Desmococcus spp, Actinotaenium spp* and *Stigeoclonium spp* than in the included treatments, while in the included treatments there was a higher abundance of *Chlorococcum spp* and *Chamaesiphon spp* (Table 4).

The algae were then divided based upon form and division to ascertain which of those were dominant within treatments and time. When the algae were split according to their form, variations could be seen across treatments and times (Figure 8). The main forms were; branched filaments (Chlorophyta), unbranched filaments (Chlorophyta), unbranched filaments/single cells (Bacillariophyta), colonial (Chlorophyta), single cells (Cyanophyta) and single cells desmid (Chlorophyta). Single celled algae dominate for both time 1 and time 2 followed by colonial and unbranched algae for time 1 and vice versa for time 2. In stream samples, there were no single celled green algae found nor were there any branched filaments found, however, single cells dominated (Cyanophytes) along with colonial algae, unbranched filaments/single cells and unbranched filaments. For the included samples there were only single cells (Cyanophyta), colonial and unbranched filaments/single cells found (Figure 8). In the excluded treatments, all the forms were found in various proportions however single celled algae (Cyanophyta) dominated along with unbranched filaments (Figure 8). When the algae were divided into the three main divisions found in the samples, Chlorophyta, Cyanophyta and Bacillariophyta, a pattern was seen (Figure 9). There were more Cyanophytes at time 1 and more Chlorophytes in time 2. Within the treatments, there were more Cyanophytes in the stream samples and included treatments whereas Chlorophytes dominated in excluded treatments. There were hardly any Bacillariophytes in the included treatments and a lot more in the excluded treatments (Figure 9).

Herbivore Density Affects

The ANOVA GLM results for Chl-a data and herbivore densities indicated that none of the functional feeding types; deposit feeders, grazers or scrapes, had any significant effects on Chl-a levels across any of the treatments (Table 5). The same results were found with the AFDW results and deposit feeder, grazer and scraper densities (Table 5).

Discussion

The aim of this experiment was to determine the effects that grazers have on benthic algae within a stream system in the Western Cape, South Africa.

The first two hypotheses dealt with periphyton biomass between treatments and time. It was hypothesized that periphyton biomass as well as live algal biomass would be significantly higher in treatments where there were reduced herbivore densities relative to treatments with increased herbivore densities and natural densities found in the Berg River. Effects of herbivores on periphyton has been observed on fine (Power *et al.* 1988), intermediate (Feminella *et al.* 1989) and large (Caraco *et al.* 1997) spatial scales however even within these scales, the effects on periphyton biomass is often patchy (Holomuzki *et al.* 2010). The results of this experiment indicate that there are no significant differences between periphyton biomass within the different treatments therefore indicating that different grazer densities did not affect the periphyton biomass. Similar results were obtained in a study by Holomuzki *et al.* (2006) where short term grazing by mostly smaller invertebrate species failed to significantly change the algal biomass. Another study by Vaughn *et al.* (1993) as well as a study by Feminella *et al.* (1989) also found that ambient densities grazers did not have any effect on algal biomass however in a number of studies done on grazer effects on periphyton (Bergey 1995, Lamberti *et al.* 1987, Steinman *et al.* 1987, Feminella *et al.* 1989, Anderson *et al.* 1999, Colletti *et al.* 1987) it was shown that grazers had a significant effect on reducing algal biomass. Effects on algae by herbivores are generally tested on small scales and therefore could underestimate the impact herbivores have on algae (Feminella and Hawkins 1995). These experiments tend to show that grazers can significantly reduce the biomass of algae over short time scales (days to weeks) (DeNicola *et al.* 1990) but there are exceptions to this (Vaughn *et al.* 1993, Holomuzki and Biggs 2006). There are many studies that state that periphyton biomass reduction due to grazers is due directly to grazer densities (Hill and Knight 1987, Steinman *et al.* 1987, Rosemond *et al.* 1993) however exceptions do occur (McCormick 1994).

The lack of response of algal biomass in this experiment could be explained by the fact that flow was impeded in the box set up and therefore algae proliferated due to there being no hydraulic hindrance (Biggs and Stokseth 1996). Another reason could be that there was a higher abundance of smaller invertebrates, such as mayflies, relative to larger invertebrates, for example caddisfly larva, present in the stream. Larger invertebrates are generally more effective at grazing (Jacoby 1987, Steinman *et al.* 1987, Feminella *et al.* 1989, DeNicola *et al.* 1990) than are smaller invertebrates due to smaller invertebrates having smaller mouthparts therefore rendering them physically incapable of handling larger filaments (Jacoby 1987, Hill and Knight 1988, Holomuzki *et al.* 2006) and suppressing algal growth (DeNicola *et al.* 1990). Selectivity of grazing is linked intimately with the mechanical efficiency of invertebrate mouthparts as well as their feeding behavior therefore they can have different effects on algal biomass and taxonomic compositions in algal communities, where they generally remove large or stalked diatoms and leave behind the small prostrate algal forms (Jacoby 1987, Steinman *et al.* 1987, DeNicola *et al.* 1990). The second hypothesis stated that the differences in periphyton biomass between the treatments of different herbivore densities will be enhanced over time. The results however showed that there were no significant differences of periphyton biomass between the time periods. This can be explained by a quantitative analysis of past experiments by Feminella and Hawkins (1995) where it was shown that short-term experiments had lesser results than did long-term experiments. They hypothesized that this could be due to the fact that in long-term experiments animals in 'grazed treatments' had a longer time to forage and therefore reduce the periphyton which was initially in the experiment and/or it could be due to periphyton in 'ungrazed treatments' having a longer time to accumulate biomass.

The third hypothesis dealt with algal taxa that dominated between the different treatments. It was hypothesized that in treatments with reduced herbivore densities there would be a lower diversity of algal species due to the proliferation of one or two dominant algal taxa according to the predation hypothesis by Paine (1966). The predation hypothesis by Paine (1966) predicts that when there is low or no grazing pressure, dominant competitors will exclude subordinate taxa and therefore reduce diversity whereas higher grazing intensity results in dominance of more taxa that are able to tolerate grazing. The results of this experiment showed that this was not the case. Species numbers did not vary much between treatments but the species densities and the variety and dominance of algal forms did. Algal densities were similar in treatments with higher densities of herbivores and patchier in treatments where herbivore densities were lower. There was a higher density of *chlorococcum spp* (colonial chlorophytes) in treatments where herbivore densities were higher possibly due to the fact that colonies allow the algae to increase their overall size and therefore enable them to exclude certain herbivores (Lurling and Beekman 1999, Power *et al.* 1988). *Chamaesiphon spp* (single celled cyanophytes) were also in higher abundance in treatments where herbivore densities were higher likely due to the fact that cyanophytes produce certain microsystins or saxitoxins which cause them to be unpalatable to certain herbivores (Holomuzki *et* *al.* 2010). Both species did also appear in treatments where grazer density was lower but in lower abundances.

In the treatments where herbivore densities were lower there was a variety of algal forms present, ranging from single cells, colonial cells, unbranched filaments and branched filaments. *Mougeotia spp*, *Actinotaenium spp* and *Stigeoclonium spp* were all completely absent from treatments where herbivore densities were higher but occurred in treatments where herbivore densities were lower not indicating that the filaments were easily grazed by the herbivores as they were more accessible. A similar result was found in Colletti *et al.* (1987) where large, overstorey species were probably more accessible than smaller, prostrate forms and therefore more frequently removed. Adnate species are also more resistant to grazing than are upright species (McCormick 1994). In an experiment by Denicola *et al.* (1990) it was found that with increasing grazer intensity there were subsequent increases in heterogeneity of algal communities. In another study by Dudley and D'Antonio (1990) it was observed that grazing had a strong effect in spring, when grazer densities were high, and in autumn when grazer densities were low therefore suggesting that productivity was correspondingly low where nutrient levels were low in autumn. This could explain the patterns seen in this experiment as it was conducted at the end of summer/beginning of autumn where grazer densities were lower and where algal growth was at a maximum (Clark and Ractliffe 2007). Grazers are able to cause significant changes in the abundance and composition of algae in lotic systems (Lamberti *et al.* 1987, Steinman *et al.* 1987) but have variable effects and can decrease (Colletti *et al.* 1987), increase (Bergey 1995) or have no effect (Kehde and Wilhm 1972) on algal diversity which could be explained due to different grazer types and densities as well as the spatial and temporal scales used during sampling (DeNicola *et al.* 1990, Lange *et al.* 2011).

Periphyton communities in undisturbed streams are generally distributed as mosaic patches at different successional stages (DeNicola *et al.* 1990). Hillebrand and Cardinale (2004), in their meta-analysis of an extensive range of systems and aquatic grazers, show that with increasing algal diversity the effect of grazers are reduced which suggests that algal community diversity influences unpalatability. There are also factors that can delay or even nullify herbivore effects. These factors are low grazer bioenergetics, low ambient light, low nutrient conditions, the availability of alternative food sources and the rate of algal recruitment (Feminella *et al.* 1989, Holomuzki and Biggs 2006). Grazers may not only influence the creation of algal patches but may in turn be influenced by the biomass, physiognomy and composition of the algal patch itself (DeNicola *et al.*

1990). For example it has been shown that caddisflies selectively graze areas where algal abundance is high (Lamberti and Resh 1983).

In conclusion herbivore densities had no effect on periphyton biomass most likely due to the season at which the experiment was done and the short-term experimental period. The effect on the algal densities was more pronounced though as was visible in the results where there was a higher variety of forms present in treatments with lower herbivore densities. These herbivore-periphyton interactions arise mainly due to algal composition as well as herbivore type. However, to get a better idea of how herbivores affect periphyton, other factors should be taken into account. For instance, experiments should be done over longer time periods and look at other affects under various environmental conditions.

Acknowledgements

I would like to thank Gareth Matokazi for help in the field and a huge thank you to Liesl Phigeland for all the help and advice in the lab analyses. A special thank you to Pashni Pillay for algal species identification and counting and to Justine Ewart-Smith who helped in the field and with comments and advice through-out the project.

References

Anderson EL, EB Welch, JM Jacoby, GM Schimek and RR Horner. 1999. *The North American Benthological Society*, 41: 633-651.

Anderson MJ. 2005. PERMANOVA. Permutational multivariate analysis of variance. Dept of Statistics, Univ. of Auckland, Auckland. Available at: /<www.stat.auckland.ac.nz/~mja/programs.htm/>.

Biggs BJF. 1987. Effects of sample storage and mechanical blending on quantitative analysis of river periphyton. *Freshwater Biology*, 18: 197-203.

Biggs BJF and S Stokseth. 1996. Hydraulic Habitat suitability for Periphyton in Rivers. *Regulated Rivers: Research and Management*, 12: 251-261.

Bergey EA. 1995. Local effects of a sedentary grazer on stream algae. *Freshwater Biology*, 33: 401 409.

Caraco NF, JJ Cole, PA Raymond, DL Strayer, ML Pace, SEG Findlay and DT Fischer. 1997. ZEBRA MUSSEL INVASION IN A LARGE, TURBID RIVER: PHYTOPLANKTON RESPONSE TO INCREASED GRAZING. *Ecology*, 78(**2**): 588-602.

Clark B and G Ractliffe. (eds). 2007.Berg River Baseline Monitoring Programme. Final Report Volume 5: Synthesis. DWAF Report No. P WMA 19/G10/00/2107.

Colletti PJ, W Blinn, A Pickart and VT Wagner. 1987. Influence of Different Densities of the Mayfly Grazer Heptagenia criddlei on Lotic Diatom Communities. *Journal of the North American Benthological Society*, 6(**4**): 270-280.

Davies BR and JA Day. 1998. *Vanishing waters*. University of Cape Town Press, Rondebosch, ZA.

DeNicola M, CD McIntire, GA Lamberti, SV Gregory and LR Ashkenas. 1990. Temporal patterns of grazer-periphyton interactions in laboratory streams. *Freshwater Biology*, 23: 475-489.

DeNicola M, KD Hoagland and SC Roemer. 1992. Influences of Canopy Cover on Spectral Irradiance and Periphyton Assemblages in a Prairie Stream. *Journal of the North American Benthological Society*, 11(**4**): 391-404.

Dodds WK. 2003. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. *The North American Benthological Society*, 22(**2**): 171-181.

Dudley TL and CM D'Antonio. 1991. The Effects of Substrate Texture, Grazing, and Disturbance on Macroalgal Establishment in Streams. *Ecology*, 72(**1**): 297-309.

Dudley JL, W Arthurs and TJ Hall. 2001. A Comparison of Methods Used to Estimate River Rock Surface Areas. *Journal of Freshwater Ecology*, 16(**2**): 257-261.

Ewart-Smith J. 2011. The relationship between periphyton, flow and nutrient status in south western Cape foothill rivers. *Literature Review, report to the Water Research Commission (WRC) as part of project K5/1676*. South Africa.

Feminella JW, ME Power and VH Resh. 1989. Periphyton responses to invertebrate grazing and riparian canopy in three northern California coastal streams. *Freshwater Biology*, 22: 445 457

Feminella JW and CP Hawkins. 1995. Interactions between Stream Herbivores and Periphyton: A Quantitative Analysis of past Experiments. *Journal of the North American Benthological Society*, 14(**4**): 465-509.

Hauer FR and GA Lamberti. (eds). 2007. *Methods in Stream Ecology*. Burlington: Academic Press.

Hill WR and AW Knight. 1987. Experimental Analysis of the Grazing Interaction Between a Mayfly and Stream Algae. *Ecology*, 68(**6**): 1955-1965.

Hillebrand H and BJ Cardinale. 2004. Consumer effects decline with prey diversity. *Ecology Letters*, 7: 192-201.

Holomuzki JR and BJF Biggs. 2006. Food limitation affects algivory and grazer performance for New Zealand stream macroinvertebrates. *Hydrobiologia*, 561: 83-94.

Holomuzki JR, RL Lowe and JA Ress. 2006. Comparing herbivory effects of stream macroinvertebrates on microalgal patch structure and recovery. *New Zealand Journal of Marine and Freshwater Research*, 40: 357-367.

Holomuzki JR, JW Feminella and ME Power. 2010. Biotic interactions in freshwater benthic habitats. *Journal of the North American Benthological Society*, 29(**1**): 220-244.

Jacoby JM. 1987. Alterations in periphyton characteristics due to grazing in a Cascade foothill stream. *Freshwater Biology*, 18: 495-508.

Linder HP. 2003. The radiation of the Cape flora, southern Africa. *Biological Review*, 78: 597-638.

Kehde PM and JL Wilhm. 1972. The Effects of Grazing by Snails on Community Structure of Periphyton in Laboratory Streams. *American Midland Naturalist*, 87(**1**): 8-24.

Lamberti GA and VH Resh. 1983. Stream Periphyton and Insect Herbivores: An Experimental Study of Grazing by a Caddisfly Population. *Ecology*, 64(**5**): 1124-1135.

Lamberti GA, LR Ashkenas, SV Gregory and AD Steinman. 1987. Effects of Three Herbivores on Periphyton Communities in Laboratory Streams. *Journal of the North American Benthological Society*, 6(**2**): 92-104.

Lange K, A Liess, JJ Piggott, CR Townsend and CD Matthaei. 2011. Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. *Freshwater Biology*, 56: 264-278.

Lurling M and W Beekman. 1999. Grazer-induced defenses in Scenedesmus (Chlorococcales; Chlorophyceae): coenobium and spine formation. *Phycologia*, 38(**5**): 368-376.

Marker AF, CA Crowther and RJM Gunn. 1980. Methanol and acetone as solvents for estimating chlorophyll a and phaeopigments by spectrophotometry. *Ergeb-nisse der Limnologie*, 14:52 69.

McCormick PV. 1994. Evaluating the multiple mechanisms underlying herbivore-algal interactions in streams. *Hydrobiologia*, 291: 47-59.

Paine RT. 1966. Food Web Complexity and Species Diversity. *The American Naturalist*, 100(**910**): 65-75.

Power ME, AJ Stewart, WJ Matthews. 1988. Grazer Control of Algae in an Ozark Mountain Stream: Effects of Short-Term Exclusion. *Ecology*, 69(**6**): 1894-1898

Power ME. 1992. Top-Down and Bottom-Up Forces in Food Webs: Do Plants Have Primacy. *Ecology*, 73(**3**): 733-746.

River Health Programme (2004). *State-of-Rivers Report: Berg River System*. Department of Water Affairs and Forestry. Pretoria. ISBN No: 0-620-32075-3.

Rosemond AD, PJ Mulholland and JW Elwood. 1993. Top-Down and Bottom-Up Control of Stream Periphyton: Effects of Nutrients and Herbivores. *Ecology*, 74(**4**): 1264-1280.

Schael D. (2005) Distributions of physical habitats and benthic macroinvertebrates in western Cape headwater streams at multiple spatial and temporal scales, *PhD thesis, UCT*. 239pp.

StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com.

Steinman AD, CD McIntire, SV Gregory, GA Lamberti and LR Ashkenas. 1987. Effects of Herbivore Type and Density on Taxonomic Structure and Physiognomy of Algal Assemblages in Laboratory Streams. *Journal of the North American Benthological Society*, 6(**3**): 175-188.

Steinman AD, JP Mulholland and D Kirschtel. 1991. Interactive effects of nutrient reduction and herbivory on biomass, taxonomic structure and P uptake in lotic periphyton communities. *Canadian Journal of Fisheries and Aquatic Sciences*, 48: 1951-1959.

Stevenson RJ, ML Bothwell and RL Lowe. (eds). 1996. *Algal ecology: Freshwater Benthic Ecosystems*. United Kingdom: Academic Press Inc.

Thompson RC, ML Tobin, SJ Hawkins and TA Norton. 1999. Problems in extraction and spectrophotometric determination of chlorophyll from epilithic microbial biofilms: towards a standard method. *Journal of the Marine Biological Association of the United Kingdom*, 79: 551-558.

Vaughn CC, FP Gelwick and WJ Matthews. 1993. Effects of Algivorous Minnows on Production of Grazing Stream Invertebrates. *Oikos*, 66(**1**): 119-128.

Wasmund N, I Topp and D Schories. 2006. Optimising the storage and extraction of chlorophyll samples. *Oceanologia*, 48(**1**): 125-144.

Figures and Tables

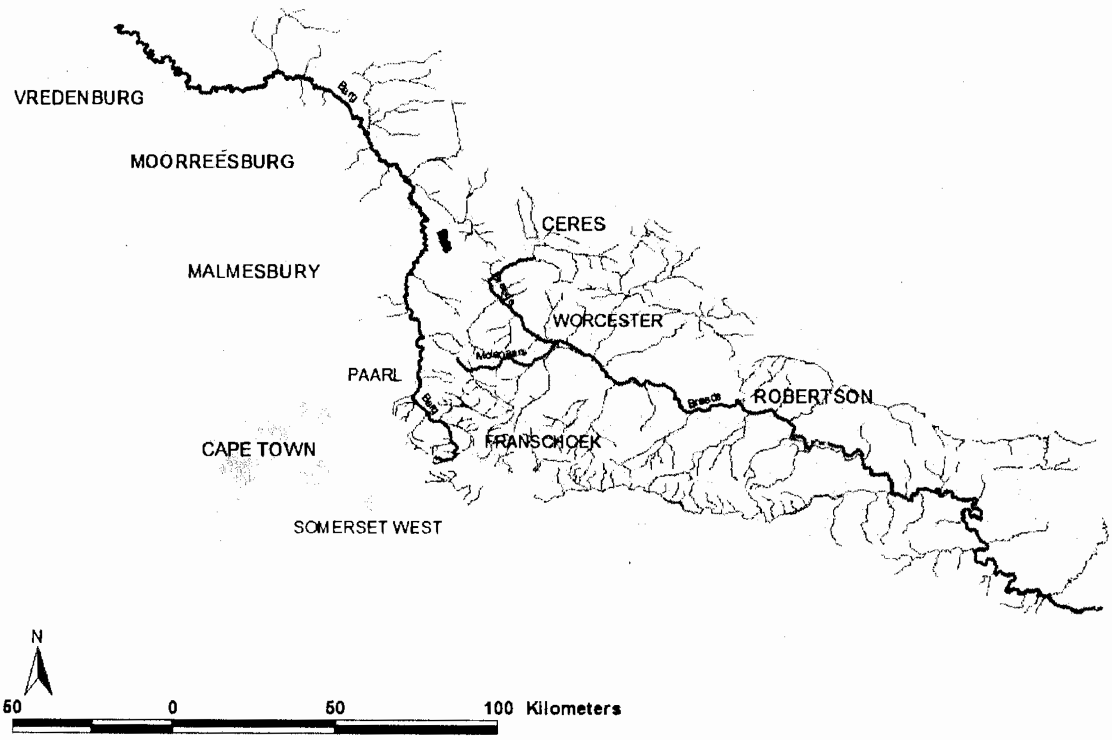

Figure 1: The study area showing the location of the Berg River within the South Western Cape region. Where is the study site indicated ?

Figure 2: Study site on the Berg River in March-April 2011

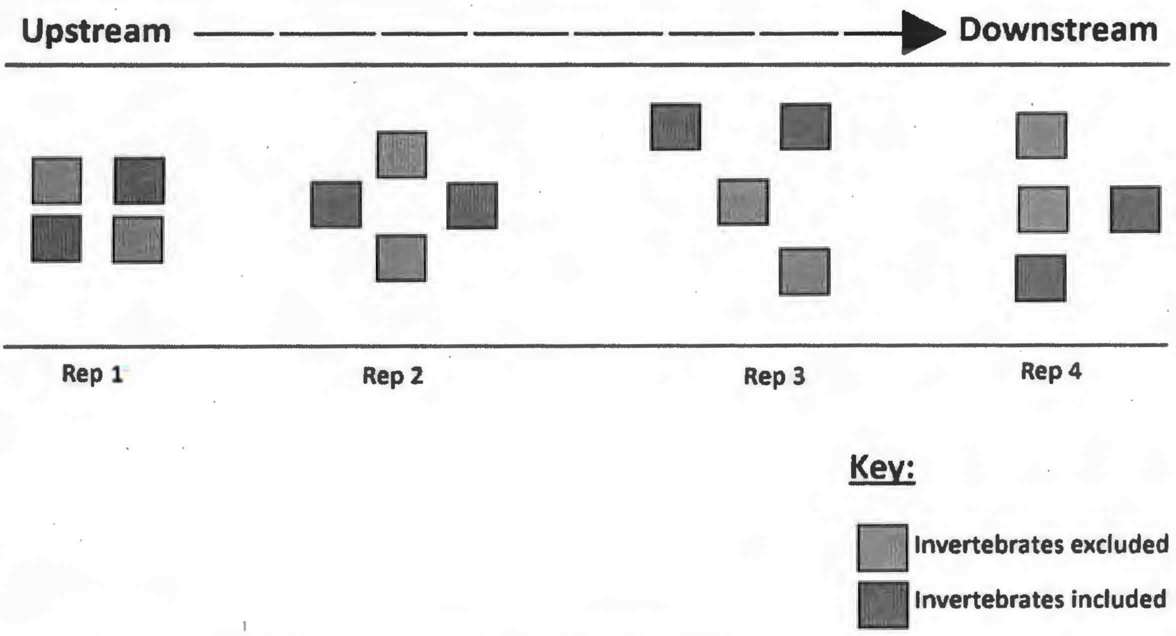

Figure 3: Schematic of replicate placement in the Berg River. Replicates were approximately 3m apart and set up in a randomised block design.

Figure 4: Experimental set up showing a pair of plastic boxes representing a single replicate with and without grazers. The mesh that permits through flow was placed at the upstream and downstream ends. Each box was layered with gravel and three stones from the run biotopes were placed in each box. Boxes were covered with Perspex lids held down with metal clips. The dimensions for the boxes were 0.3m and 0.165m for the length and width respectively.

Table 1: Summary table showing key food types of each functional feeding group modified from Schael (2005).

FFG	**Feeding Mode**	**Dominant Food Type**
Deposit feeder 2		Algae and detritus in comparative amounts dependent on availability.
Scraper 1	Scraper – scrapes **thin film of micro-organisms** off substrata	Algae
Scraper 2		Algae and detritus
Grazer 1	Grazer – feeds on **whole living plants, leaves and stems. Algal mats** can be included where scraping is not employed as mechanism for collection.	Vegetation and algae
Grazer 2		Algae

Table 2: Averages (± std. error) for chl-a (mg/m^2) and AFDW (mg/m^2) for the two treatments and stream stone samples for the two time periods.

	Log (x+1) Chl a (mg/m^2)		**Log (x+1) AFDW (mg/m^2)**	
	Time 1	Time 2	Time 1	Time 2
Stream stone	0.896 ± 0.082	0.902 ± 0.074	3.346 ± 0.077	3.44 ± 0.086
Included stone	0.969 ± 0.056	1.004 ± 0.067	3.400 ± 0.044	3.367 ± 0.076
Excluded stone	0.904 ± 0.042	0.844 ± 0.143	3.493 ± 0.050	3.394 ± 0.0685

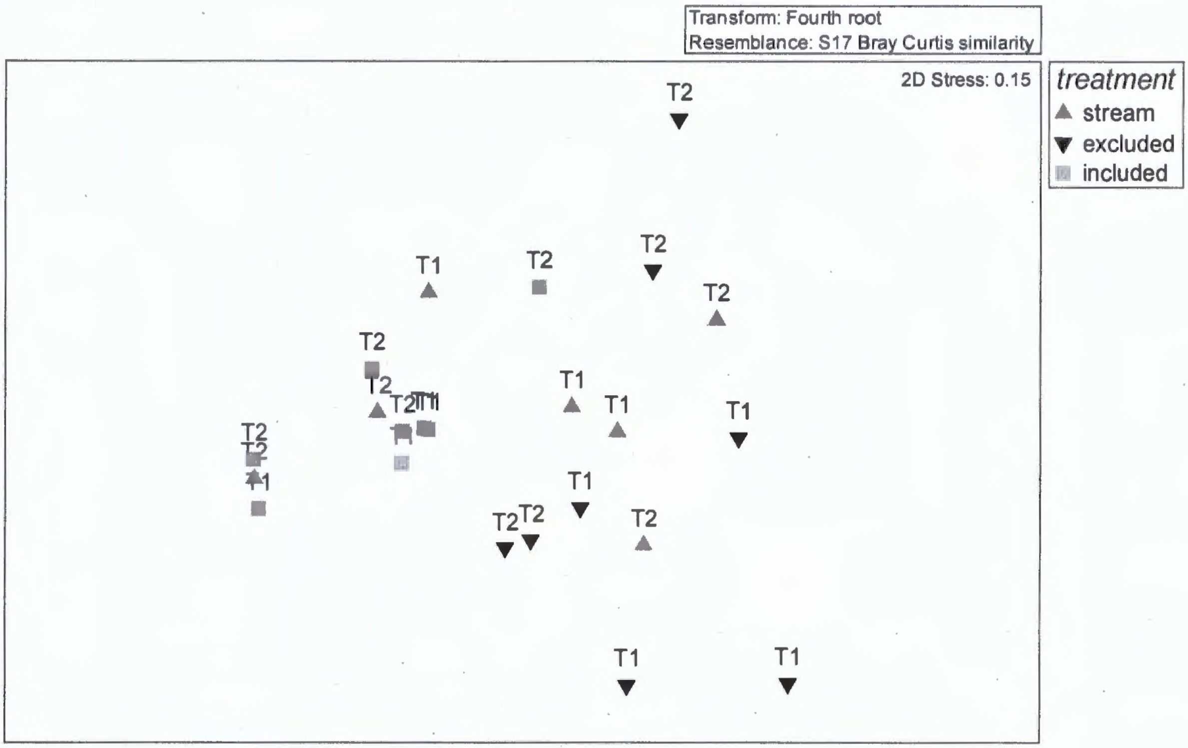

Figure 5: A MDS (multi-dimensional scaling) plot showing the degree of similarity of algal species numbers in the treatments, excluded and included, as well as the stream samples.

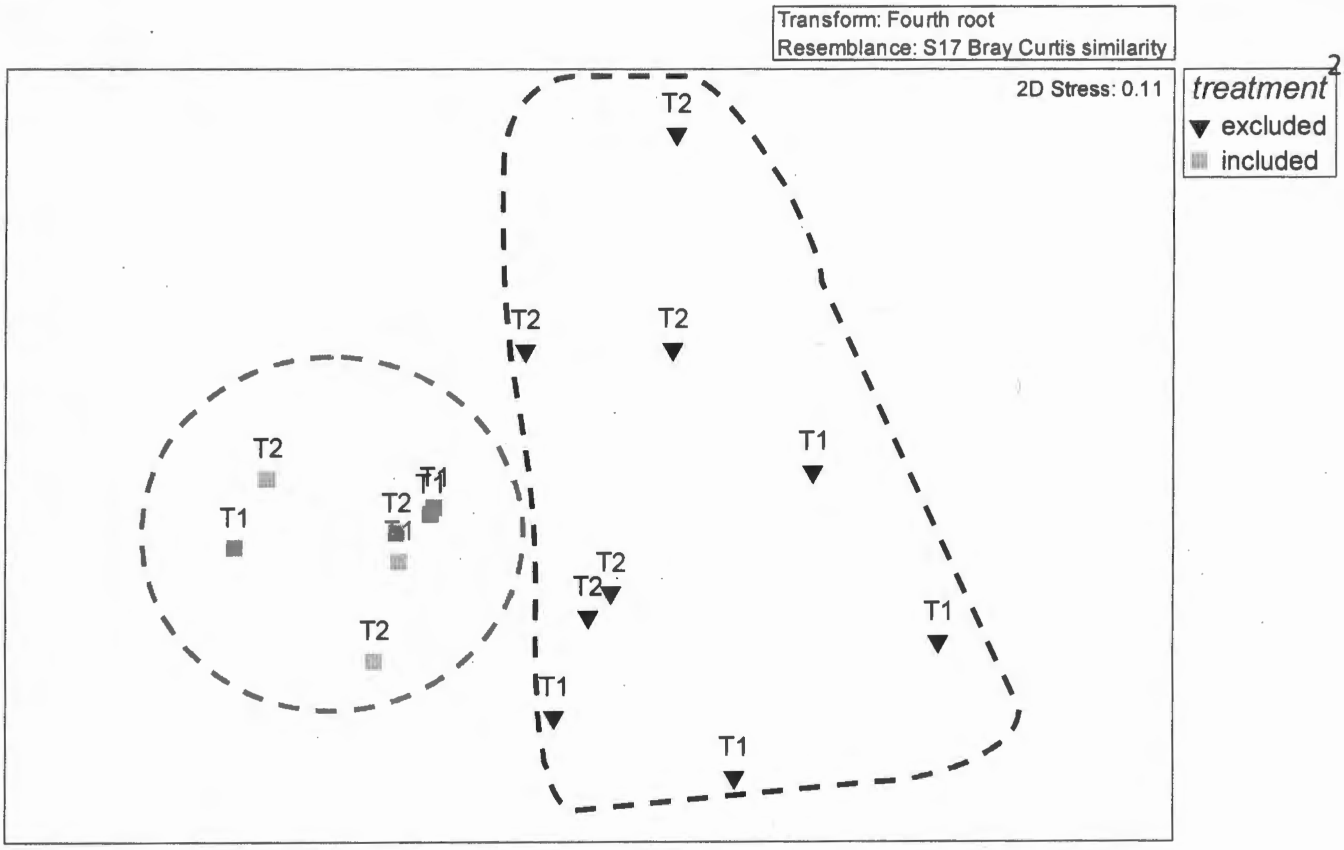

Figure 6: A MDS (multi-dimensional scaling) plot showing the degree of similarity of algal species numbers between the two treatments, excluded and included, only.

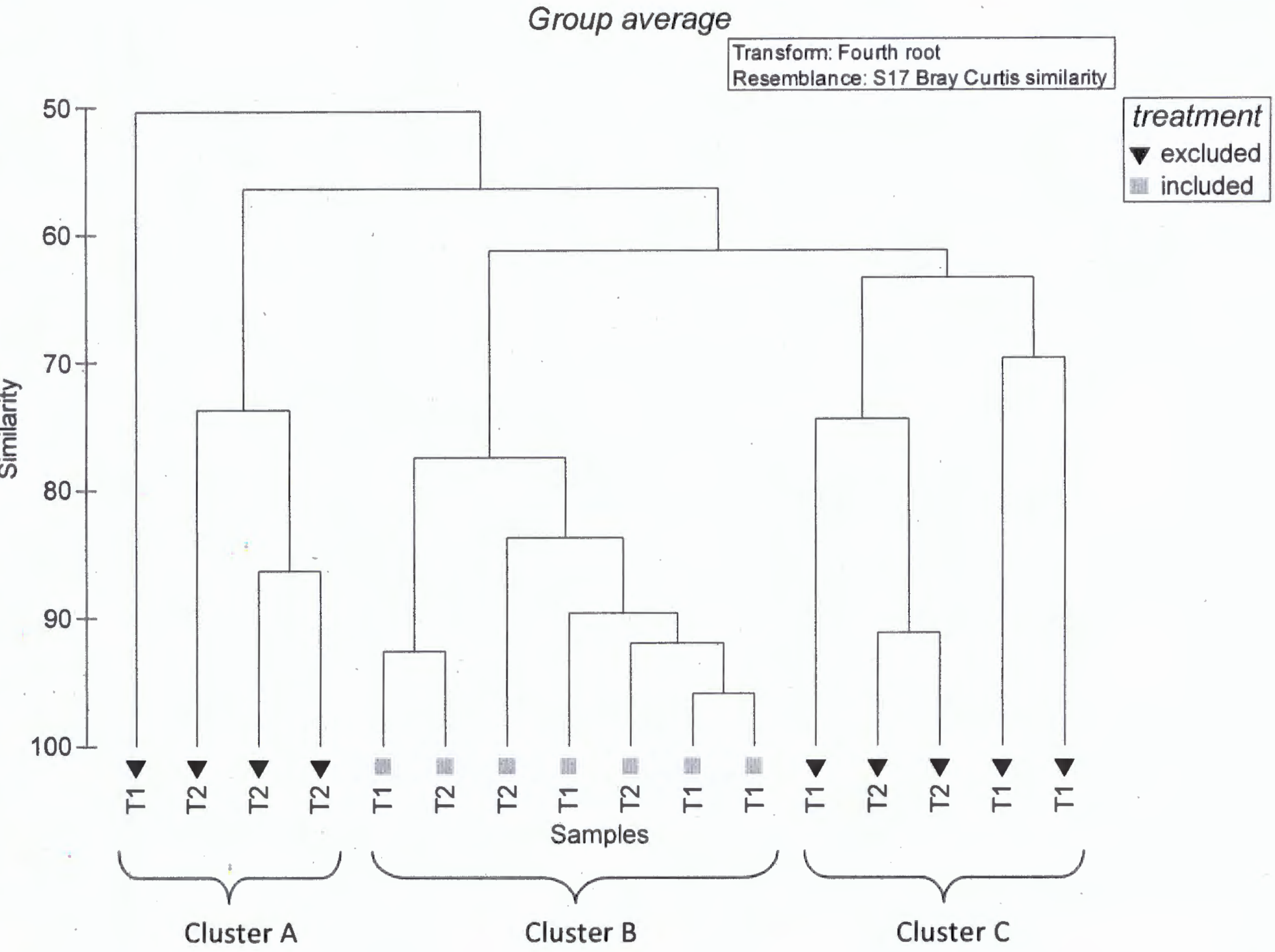

Figure 7: Cluster dendogram showing similarity of algal species between the treatments, excluded and included.

Table 3: Average similarity (%) between and within the groups.

	Stream	**Excluded**	**Included**
Stream	66.4		
Excluded	60.3	60.9	
Included	68.2	57.8	78.8

Table 4: Algal species that influenced the differences between the two treatments, excluded and included.

Species	**Excluded** **Average abundance**	**Included** **Average abundance**
Eunotia rhomboidae	33.06	4.24
Mougeotia spp 2	21.86	9.05
Mougeotia spp 1	21.90	0.00
Chlorococcum spp	20.34	40.64
Desmococcus spp	32.32	29.71
Chamaesiphon spp	66.28	78.59
Actinotaenium spp	12.51	0.00
Stigeoclonium spp	7.96	0.00

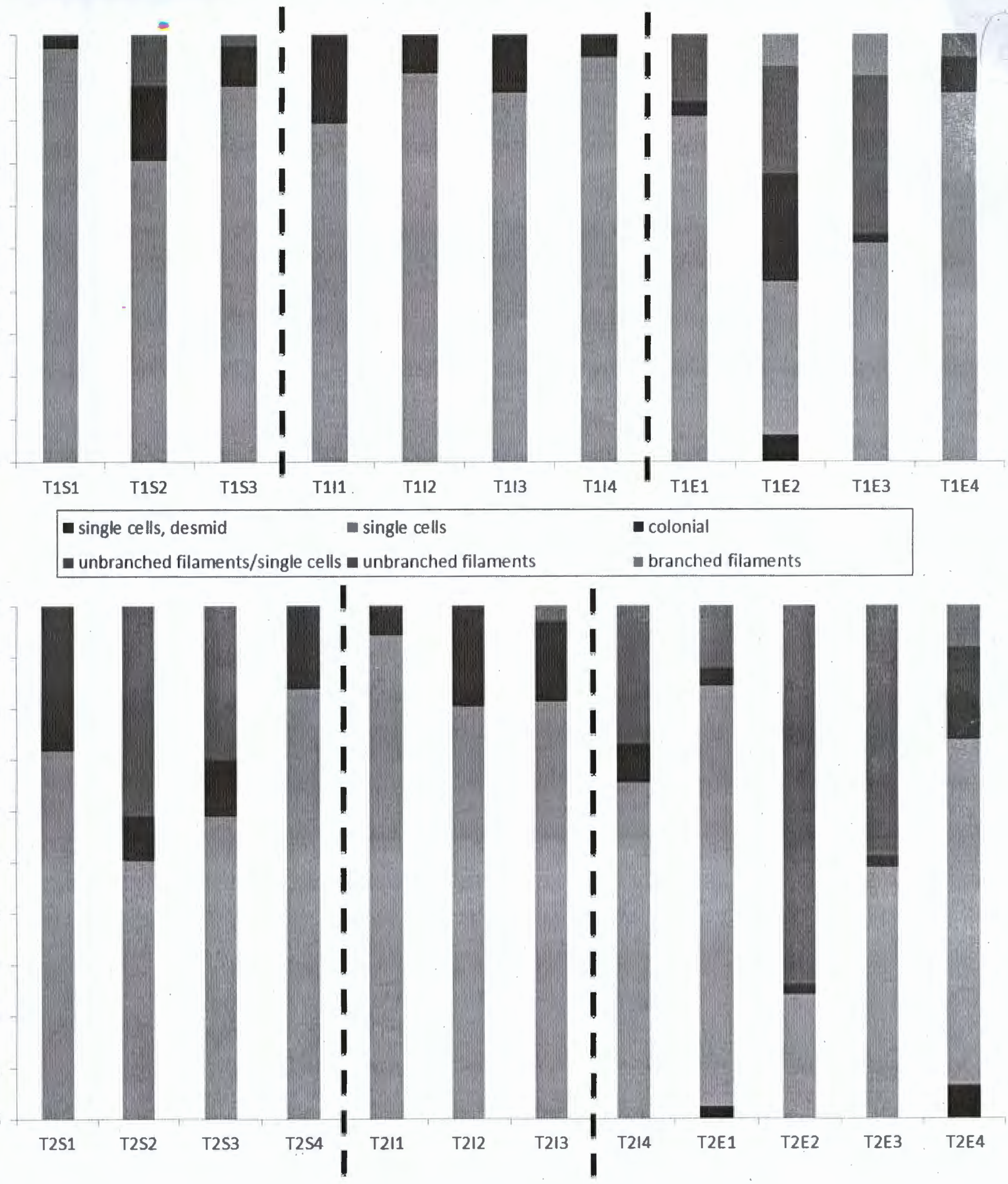

Figure 8: Proportions of the various forms of algae found in each sample for time 1 and time 2.

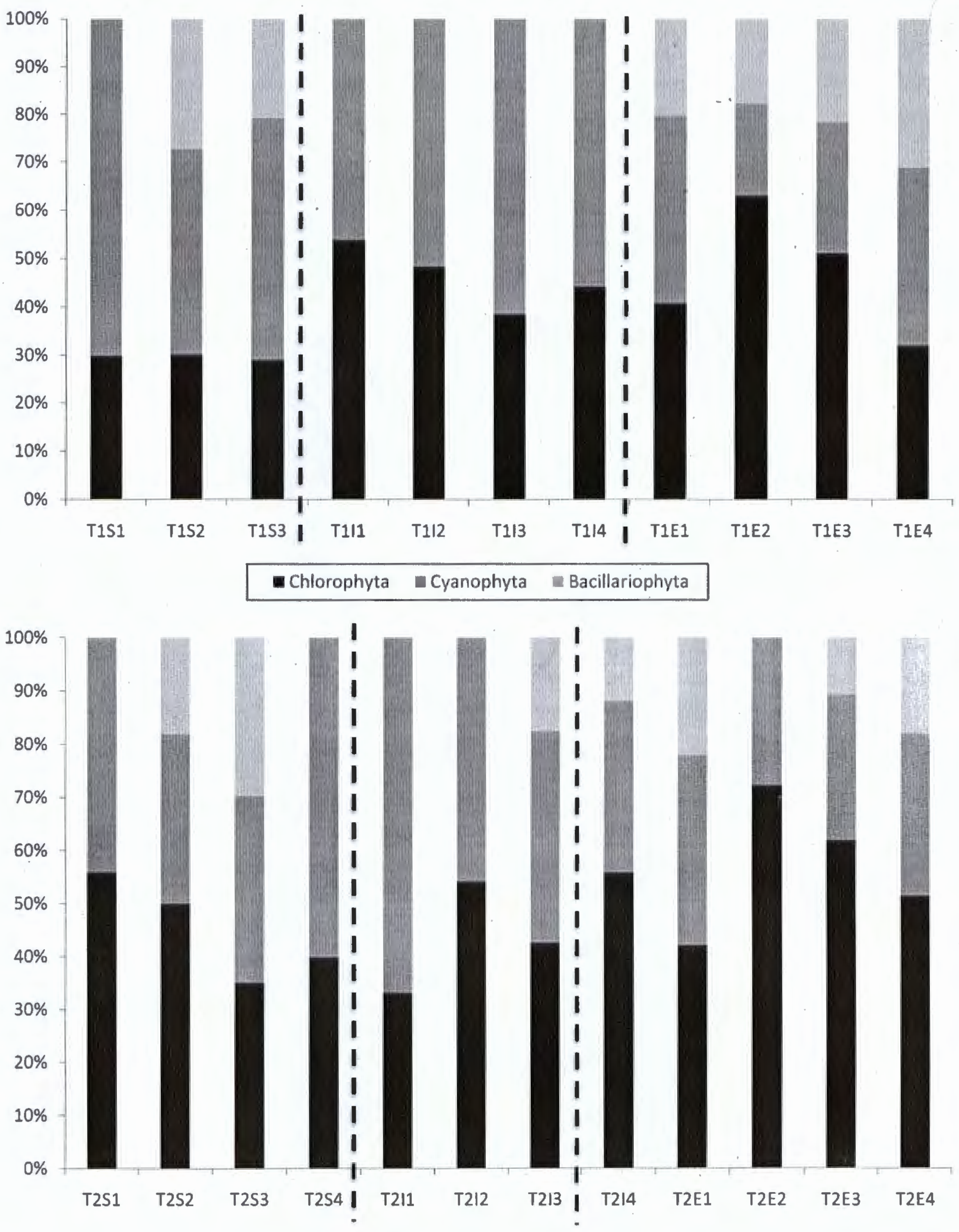

Figure 9: Proportions of the three divisions found in each sample for time 1 and time 2.

Table 5: GLM results for Chl-a (mg/m^2) and AFDW (mg/m^2) and their interactions with deposit feeder, grazer and scraper densities (per m^2)

	Chl-a parameter	Chl-a Standard Error	Chl-a p value	-95% Confidence Interval	+95% Confidence Interval	AFDW parameter	AFDW Standard Error	AFDW p value	-95% Confidence Interval	+95% Confidence Interval
Deposit Feeders	-0.0589	0.0957	0.5458	-0.2601	0.1422	0.1292	0.0791	0.1199	-0.0371	0.2954
Grazers	-0.0280	0.0995	0.7820	-0.2370	0.1811	-0.1390	0.0822	0.1082	-0.3118	0.0338
Scrapers	0.0955	0.0828	0.2638	-0.0785	0.2695	0.0559	0.0685	0.4248	-0.0879	0.1997

Appendix

Table 1: Densities of invertebrate functional feeding groups identified in samples (per m^2) of rock surface areas.

Time	Treatment	Deposit Feeder	Filter Feeder	Grazer	Predator	Scraper	Shredder
T1	Stream 1	138.0846735	0	61.37096601	11.50705613	0	30.685483
T1	Stream 2	82.52993615	0	88.87839277	1.587114157	4.232304418	0
T1	Stream 3	58.26053754	0	113.5333552	4.481579811	11.9508795	0
T1	Stream 4	131.4589046	0	78.87534278	3.286472616	17.52785395	0
T1	Excluded 1	0	0	7.504346893	0	7.504346893	0
T1	Excluded 2	0	156.3739124	52.12463745	0	0	0
T1	Excluded 3	36.54212137	1461.684855	97.44565699	0	0	0
T1	Excluded 4	9.640356847	115.6842822	19.28071369	0	0	0
T1	Included 1	221.09817	1031.79146	524.0845512	3.070807917	163.7764223	0
T1	Included 2	86.71141747	115.6152233	231.2304466	5.780761164	92.49217863	0
T1	Included 3	237.6371769	120.4920897	209.7454895	1.673501246	31.23868992	0
T1	Included 4	149.0598053	24.84330088	173.9031062	6.21082522	132.4976047	0
T2	Stream 1	212.7675742	0	222.8993635	7.598841936	25.32947312	0
T2	Stream 2	52.41570899	0	227.1347389	9.827945435	43.67975749	26.20785449
T2	Stream 3	191.0813552	0	34.42907301	5.164360952	41.31488761	0
T2	Stream 4	262.1256518	0	312.0543474	0	12.4821739	0
T2	Excluded 1	4.493986148	17.97594459	11.98396306	0	0	0
T2	Excluded 2	0	68.98724426	22.99574809	0	0	0
T2	Excluded 3	28.67953677	0	12.74646079	0	0	0
T2	Excluded 4	0	0	0	0	0	0
T2	Included 1	150.940966	161.003697	221.3800834	10.06273107	73.79336115	20.12546213
T2	Included 2	382.3836818	137.2659371	267.9954009	17.15824213	477.1625431	0
T2	Included 3	308.5822031	127.6891875	85.12612499	23.94172265	113.5015	42.5630625
T2	Included 4	37.08907949	84.77503884	56.51669256	5.298439927	49.45210599	0