

Impact of light availability on benthic algal
assemblages and invertebrate species composition

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This study addresses the influence of light availability on benthic ecosystems. An observational study done in Copper Creek, located at the Rocky Mountain Biological Laboratory in Colorado, examined the PAR reaching the water at full sun sites and those that were shaded. In addition, invertebrate samples were taken and analyzed for differences between shaded and open reaches (n.s.). An experiment using 24 small artificial channels was also done to more directly examine the effect of light on algal biomass. Three different species of grazers (*Baetis bicaudatus*, *Cinygmula spp.*, and *Epeorus spp.*) were added to the channels in different abundances, while maintaining biomass, to look for any interaction between the invertebrates and light availability. The grazer treatment had a highly significant effect as shown by a 2-way ANOVA ($p = 0.0004$), and the presence of shade was also significant, though not as much ($p = 0.01$). Paired t-tests within each species treatment between open and shaded data showed a significant difference only with 1 or 2 species present (1 species $p = 0.01$, 2 species $p = 0.005$). The data suggests that there is a photo-inhibiting effect on the algae from too much light, but that when all three species are present the richness had more of an effect than the light, and the same amount of algae was removed.

Introduction

It is widely known that the impacts of human land use changes are pervasive and persistent. However, many people are likely to consider mountainous regions relatively unaffected. A review by Wohl (2006) has shown that very few mountains have streams not at least moderately affected by land use. In fact, land use changes are the most common impact facing mountain streams around the world. Even in regions without any regulation of flow or direct alteration of channels, there is a history of changes in vegetation, and therefore habitat, associated with clearing forests, planting crops, and grazing domestic animals (Wohl 2006). Such changes can have dramatic influences on stream communities.

Both direct and indirect human changes to land use that influence riparian structure, and therefore light availability, can have dramatic impacts on the stream community structure (Hauer and Hill 1996). Direct changes include the introduction of non-native riparian species and riparian grazing (Wohl 2006). While exotic species are not a problem in the Copper Creek system, many of the streams in the East River Valley are subjected to riparian grazing by cattle. Overall, this reduces the amount of vegetation present in the riparian zone, thereby reducing the amount of shaded streambed present. Similarly, deforestation and a shift to agricultural or urbanized land use can indirectly change the composition of riparian vegetation species as a result of increased

sedimentation and nutrient loading (Wohl 2006). The US Rocky Mountains as a whole have been the location of locally intensive mining and extensive deforestation in the past, a land use change that also occurred in the East River Valley, as seen by the town of Gothic itself. Today, there is continued deforestation, mining, and intensive grazing occurring throughout the range, which is again a trend that continues within this system (Wohl 2006).

Light concentrations in streams are important for a variety of different reasons, both direct and indirectly. The influence of light on water temperatures is important because it directly regulates oxygen concentrations as well as organism metabolic rates. This can affect the fitness of organisms and thereby the distribution of species in both space and time. (Baron et al. 2002) However, the Copper Creek is a very fast moving stream, and therefore it is unlikely that there are large enough differences in water temperature between shaded and open reaches that could affect the life processes of invertebrates. The more direct influence of light availability on benthic algal assemblages is more likely to have an impact in biotic populations.

Resource availability, particularly nutrients and light, are two of the factors that influence the benthic algal assemblages, and therefore indirectly affect stream invertebrate populations (Stevenson 1997). As algal density increases on substrata, this creates a limiting effect on the peak algal biomass as a result of the density-dependent decreases in nutrient and light availability (Stevenson 1997). However, when the resource availability is limited by external factors such as riparian shading, different factors affect algal growth rates and biomass. When peak biomass is too low for herbivores to use, resource stress is the dominant determinant of algal growth, however, with higher resource availability, herbivory rates are the dominant determinant of peak biomass (Stevenson 1997). For most diatoms, this threshold between resource limitation and herbivory control can be reached with light intensities less than $50 \mu\text{mole quanta m}^{-2} \text{s}^{-1}$ (Stevenson 1997). At that point, algae can accumulate to densities that grazers can regulate, though the abundance of those herbivores is dependent on the peak biomass, because they are also resource limited. With the further increase of resource availability, algal species composition may change toward an increase of filamentous green forms, which would certainly have an effect on benthic invertebrate species composition.

However, the sections sampled in Copper Creek do not have any filamentous forms, so any differences seen in biota richness or abundance is presumed to be the result of algae biomass.

This study attempts to answer a few questions around the issue of benthic response to light availability. The first aspect to determine is whether light is a limiting factor on the algae growth in Copper Creek and the streamside channels. This includes the possibility of both a positive and negative effect on the algal population as a result of being shaded, as a result of too much or not enough light, respectively. The difference in light availability may have little effect on algal biomass accumulation if herbivores are regulating algal biomass, despite increases in algal reproduction rates (Stevenson 1997).

In addition, does a difference in algal biomass or assemblage relate to a difference in benthic invertebrate abundance or richness? The algal production, biomass, and species composition in a stream has direct effects on the habitat structure for benthic macro-invertebrates (Hart 1985, Holomuzki and Short 1988, McIntire et al. 1996; in Stevenson 1997). If changes in algal species composition are seen, we can expect differences in herbivore composition and habits. In addition, if there is an increase in algal production, this will enhance herbivore production. This will be examined with the use of the species *Baetis bicaudatus*, *Cinygmula spp.*, and *Epeorus spp.*, since they are the grazers used in the experimental aspect of this study. By holding the biomass of herbivores constant, the algal biomass may be able to accumulate above the herbivory threshold, especially in those exposed to full light. With light availability greater than the herbivory threshold, peak algal biomass should be regulated by exploitive competition for nutrients and light (Stevenson 1997)

Methods

This study was done in two parts, the first in Copper Creek, in a section within the townsite of Gothic. Measurements were taken at four sites, which were matched into pairs, two shaded and two receiving full sun exposure. The sites were labeled A and B, with the pairs referred to as light and shade. The sites at A and B were paired based on similar substrate size, water depth, and flow velocity. Therefore, the water depth and velocity at each sample location was taken in order to keep these aspects as similar as possible.

At each site, three Surber samples were taken in order to collect the invertebrates present in a standard area of substrate, for a total of twelve samples. All of the invertebrates were removed from these samples and counted for overall abundance and species distribution, then preserved in ethyl alcohol. Species richness was calculated, including all of the species, as well as the richness of invertebrate nymphs excluding the chironomid and diptera individuals found. Using the total number of individuals, counted in the same two ways, the Shannon diversity index was calculated. In order to test the effect of light and shade on richness and diversity, I analyzed the data using t-tests. Light availability was measured at each site using a Li-Cor Li-1400 data logger with underwater, spherical quantum radiation sensors for three days. These sensors detect photosynthetically active radiation (PAR) in units of $\mu\text{moles quanta s}^{-1} \text{ m}^{-2}$ from every direction. The data logger recorded the PAR every fifteen minutes at both a shaded and full sun site.

In addition to this observational study, an experiment was conducted to test the difference in algal assemblage and growth between full sun and shaded sections. Twenty-four small tanks were set up with about 10 rocks that could be sampled for algae in each. This number varies based on the size of the rocks, but represents enough to cover the bottom of the tank. Flow was controlled through the tanks via PVC tubing and a set volume of water. Every other channel was covered in mesh cloth to reduce the amount of light able to reach the rock substrate, as shown in Table 1 by the shade column. The channels were separated into three different treatments and a control, based on the number of invertebrate grazers added. Three species were used based on their abundance in Copper Creek: *Baetis bicaudatus*, *Cinygmula spp.*, and *Epeorus spp.* The number of individuals added was based on the biomass found in the average Surber sample. In order to maintain the same biomass of herbivores across all of the tanks, fewer *Baetis* individuals were used since they have a higher average mass than the other two. The three treatments are the number of species present in each tank; 1, 2, or all 3, as shown in Table 1. All three combinations of two species were each tested twice, as were each single species. All three species were tested in 6 tanks, and there were 6 control tests. Light availability was also measured in one shaded and one open tank for twenty-four hours using the same Li-Cor sensors and data logger as used in Copper Creek.

Again, data was recorded every fifteen minutes. After five days in the tanks, three rocks were removed from each and sampled for algal biomass. This involved removing all of the algae from each rock except for a standard area, created by the lid of a 4oz. Nalgene bottle, and then using a Dremel tool to remove the remaining algae into a liquid sample. This sample was then filtered onto a 47mm glass fiber filter, which was placed into a 4oz. Nalgene bottle without light exposure, and frozen for future chlorophyll α analysis. After removing the samples from the freezer, 20mL buffered COH was added to each sample bottle to begin the chlorophyll extraction. This had to sit overnight, but for less than 24 hours before the liquid was again filtered. 10mL of the remaining liquid was added to a glass vial and placed into a fluorometer, which measured the amount of chlorophyll α ($\mu\text{g/L}$) in the sample. Both filters were saved in aluminum foil for future ash free dry mass testing in order to get a better idea of algal biomass. Chlorophyll data was analyzed using a 2-way ANOVA between shade and grazer treatment. In addition, paired t-tests were run on each treatment using shade or open as the independent variable.

Results

The Copper Creek study illustrated that there is a clear difference in light availability between shaded and open sections (see Figures 1 and 2). Site A light had a mean of $255.15 \mu\text{moles s}^{-1} \text{m}^{-2}$; A shade was 27.92; B light was 341.89; and B shade was 73.13. At site A, the average PAR reading was 9.1 times higher in the full sun section, and 4.7 times higher at site B. This data was not tested for significance. Invertebrate species richness was not significantly different in the shaded and open sections of Copper Creek (n.s.).

Light data taken from the channel experiment showed similar results to Copper Creek, though there was less of a difference between the open and shaded data (see Figure 3). The mean PAR measured here were $344.61 \mu\text{moles s}^{-1} \text{m}^{-2}$ in the light and 105.89 in shade, with a difference of 3.2 times higher in the light. Again, this data was not tested for significance. A 2-way ANOVA of the chlorophyll data for variance with treatment and shade was highly significant. For the treatment effects alone there is clearly a large interaction ($\text{DF} = 3$, $p = 0.0004$), though shade alone also has a strong correlation ($\text{DF} = 1$, $p = 0.01$). In addition, the interaction of the two variables is also significant ($\text{DF} = 3$, $p = 0.01$). Paired t-tests within each of the treatments illustrated

significant differences between light and shade for 1 and 2 species additions (1 species $p = 0.01$, 2 species $p = 0.005$). For both of these treatments, the shaded channels showed much higher concentrations of chlorophyll. While there was no significant difference between light and shade for the other two treatments, they showed the expected response, with the control having the highest overall concentrations, and 3 species having the lowest (see Figure 4).

Discussion

The light data from Copper Creek suggests that there is an important difference between the amount of useable radiation reaching stream reaches in the sun and those that are shaded. Both of shaded sites do reach above $50 \mu\text{moles s}^{-1} \text{m}^{-2}$ during the day, and though not for the whole day, they do reach it for about two hours each day. It is possible that this enough PAR to allow the algae to grow without light limitation. However, it has been suggested that the limiting value for an herbivory threshold is actually closer to $200 \mu\text{moles s}^{-1} \text{m}^{-2}$ for the streams around the Rocky Mountain Biological Laboratory (Brad Taylor). The shaded sites rarely reach values that high, and when they do, only remain there for an hour at most. Over the course of a day, it is unlikely that that is a sufficient amount of energy to make any difference. This suggests that the algae in shaded sections of Copper Creek are in fact light limited because they are not reaching this threshold at $200 \mu\text{moles s}^{-1} \text{m}^{-2}$. This is the more likely explanation because even if the limiting value is lower, that much radiation is only reaching the water for a few hours each day, if that. Even if this is true, it does not necessarily mean that photo-inhibition from too much radiation is not also occurring. If the algae species found in Copper Creek grow well in shaded reaches, even if it is below the herbivory threshold, they may do better there than when exposed to full sun. Since the algal biomass was not tested in Copper Creek, this question cannot be answered without further investigation, however the invertebrate diversity at each site should be a good indication.

It is unfortunate that no difference was seen in the species richness or diversity between light and shade; however, this does not mean that it should be ruled out. With a larger sample size, differences might be seen between shade and open sites. Including more sites up and downstream, as well as taking samples over the course of the season could do this. By examining the temporal variation, it is possible that light availability

would relate to differences in emergence dates. This could help to suggest any differences in algal biomass as well as interactions between grazer species and the algal environment.

The light data collected in the channels was similar to the stream sites, but showed less difference between the light and shade. In addition, the mean value of the shaded channel was $105.89 \mu\text{moles s}^{-1} \text{ m}^{-2}$, well above a $50 \mu\text{moles s}^{-1} \text{ m}^{-2}$ threshold. Therefore, any differences seen in the chlorophyll results are likely to be the result of photo-inhibition, since neither the shade nor open channels were light limited. This is assuming that the herbivory threshold is closer to $50 \mu\text{moles s}^{-1} \text{ m}^{-2}$, and that there will be an effect separate from the grazer treatment.

While the invertebrates did have a significant effect on the overall chlorophyll values ($p = 0.0004$), the shade cloth did as well ($p = 0.01$). The channels that were shaded had more chlorophyll overall, suggesting a higher biomass of algae. This was not what was initially hypothesized, but follows the conclusion that the algae are photo-inhibited when exposed to full sun. If the algae in the shaded channels had higher reproduction rates than the open channels, it explains why it would be higher in channels with 1 and 2 species of grazers. However, this difference was not seen in the control channels, which suggests that there is a species interaction effect occurring as well.

When all three species are present together, there may be competition between the grazers such that they remove the algae at a faster rate than its production rate. While the biomass of invertebrates was maintained throughout the channels, the grazers' habits and nutrient requirements are different and so could lead to a difference in feeding pattern (source?). This difference in habits would not be seen in the 1 and 2 species treatments because they represent the averages of all three species individually and in different combinations. There were only two replicates of each of these types (see Table 1), and so any species effects would be cancelled out. In addition, it may only be in the presence of the other species that the grazers change their habits, and so these competition behaviors would not be seen with only 1 species.

There are some potential errors with the experiment, which can be remedied with more time and replicates. The first would be simply to run the experiment more times to increase the sample size, especially for the 1 and 2 species treatments. Secondly, with

more data, it would be possible to look for differences in algal growth between the presences of each species. Though chlorophyll analysis was the quickest way to estimate algal biomass, it is not necessarily a great measure, and so an ash free dry mass (AFDM) calculation should be made from this experiment. One potential problem is that shade cloths have been shown to increase chlorophyll production in different species of photosynthesizing plants, which may partially lead to the increase seen here. The AFDM will be done in the fall, and will hopefully support the findings of this study.

The fact that the algae species here are photo-inhibited suggests that the removal of riparian vegetation by human or other methods could have negative effects on the stream community. This means that it is even more important to protect from invasive species incursion and cattle grazing. Such factors have the ability to greatly alter the riparian landscape, and thereby influence the stream health. The physical and ecological importance of mountain streams like the Copper is disproportionately greater than the extent of these streams within the river basins of the world (Wohl 2006).

References

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Figures and Tables

Channel	Shade	Treatment	Number/Type of Grazers
1	Open	1 species	77 Ep
2	Shade	2 species	39 Cin, 39 Ep
3	Open	All 3	16 Ba, 26 Cin, 26 Ep
4	Shade	2 species	24 Ba, 39 Ep
5	Open	2 species	24 Ba, 39 Cin
6	Shade	1 species	77 Cin
7	Open	All 3	16 Ba, 26 Cin, 26 Ep
8	Shade	All 3	16 Ba, 26 Cin, 26 Ep
9	Open	Control	
10	Shade	1 species	48 Ba
11	Open	2 species	39 Cin, 39 Ep
12	Shade	Control	
13	Open	Control	
14	Shade	2 species	24 Ba, 39 Cin
15	Open	2 species	24 Ba, 39 Ep
16	Shade	1 species	77 Ep
17	Open	1 species	48 Ba
18	Shade	Control	
19	Open	All 3	16 Ba, 26 Cin, 26 Ep
20	Shade	All 3	16 Ba, 26 Cin, 26 Ep
21	Open	Control	
22	Shade	Control	
23	Open	1 species	77 Cin
24	Shade	All 3	16 Ba, 26 Cin, 26 Ep

Table 1. Experimental Design. Ep = *Epeorus spp*; Cin = *Cinygmula spp.*; Ba = *Baetis bicaudatus*

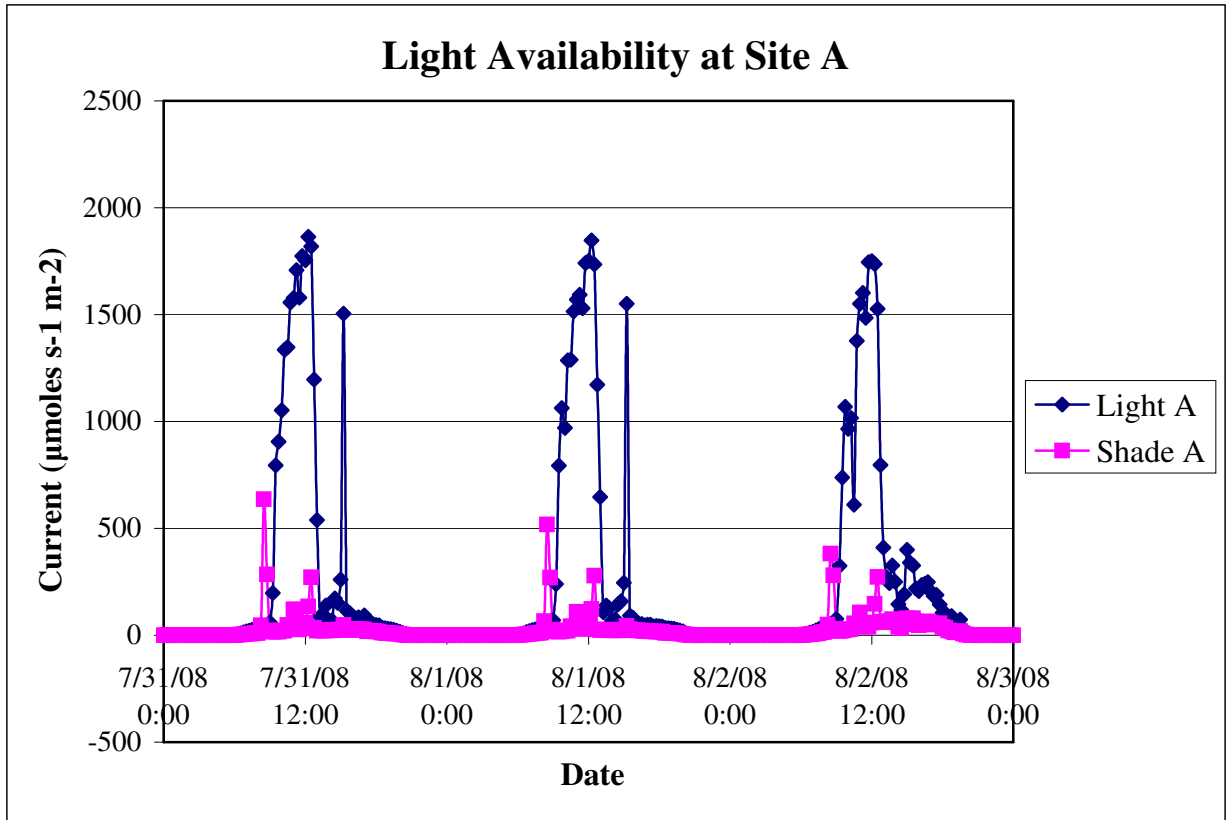


Figure 1. Light availability measured as PAR at site A, shaded and light.

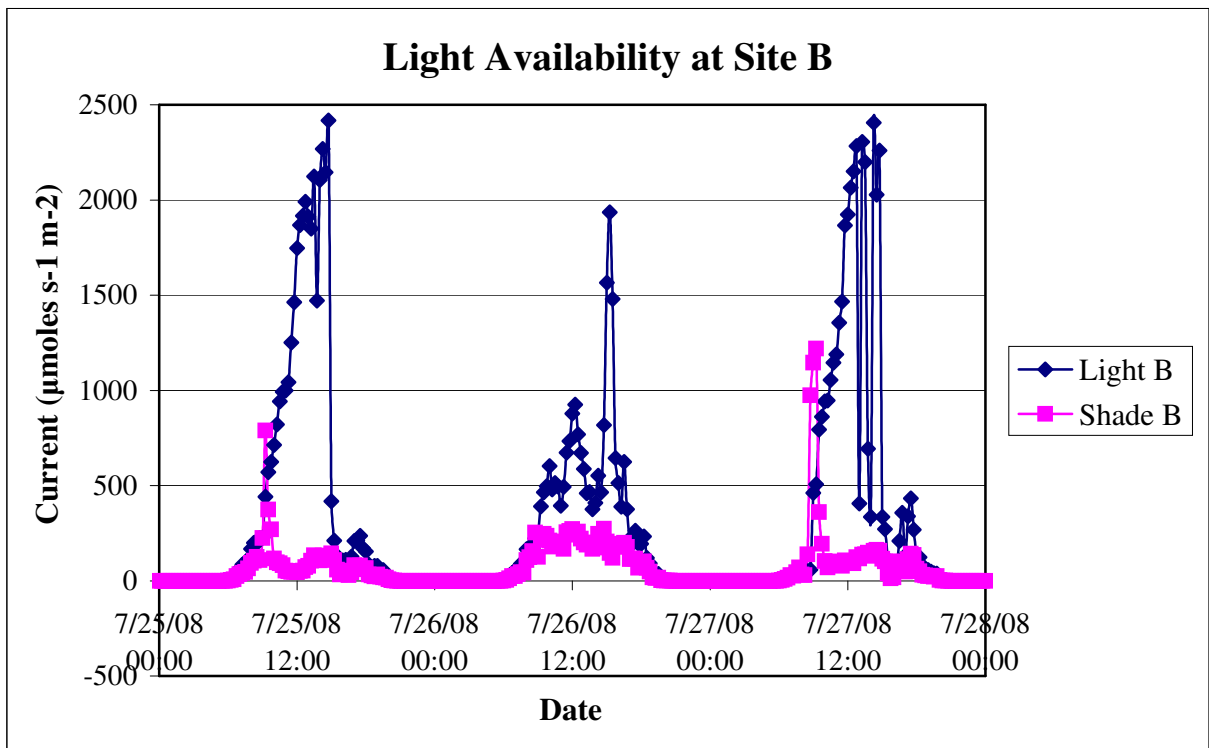


Figure 2. Light availability measured as PAR at site B, shaded and light.

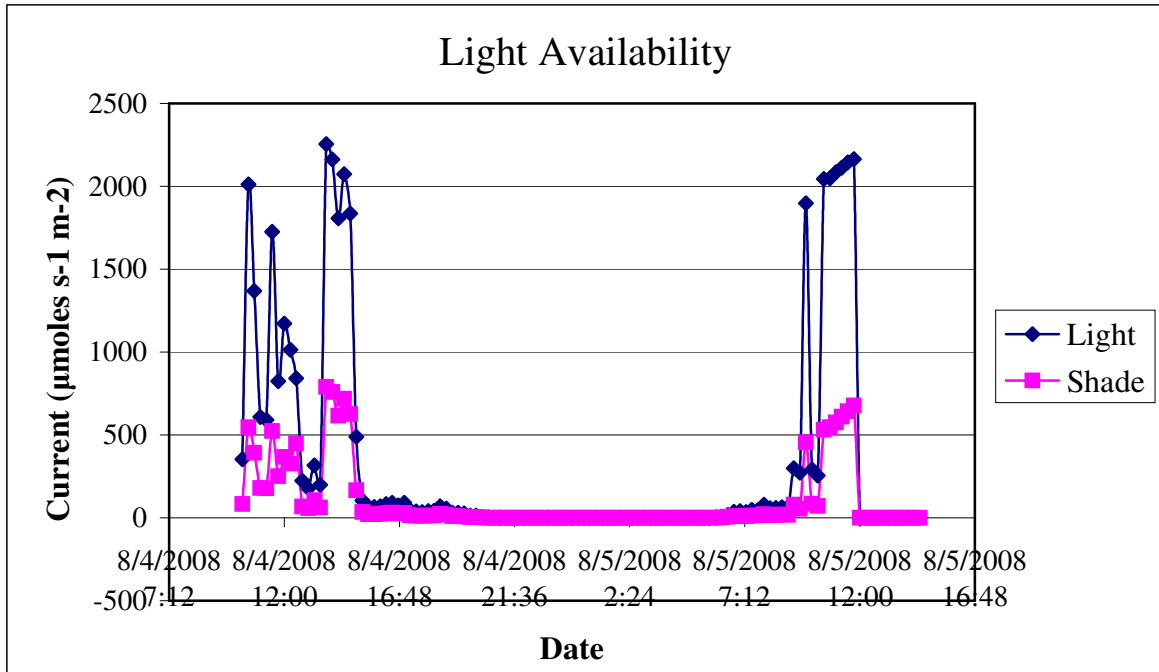


Figure 3. Light availability measured as PAR in the experimental channels, shaded and light.

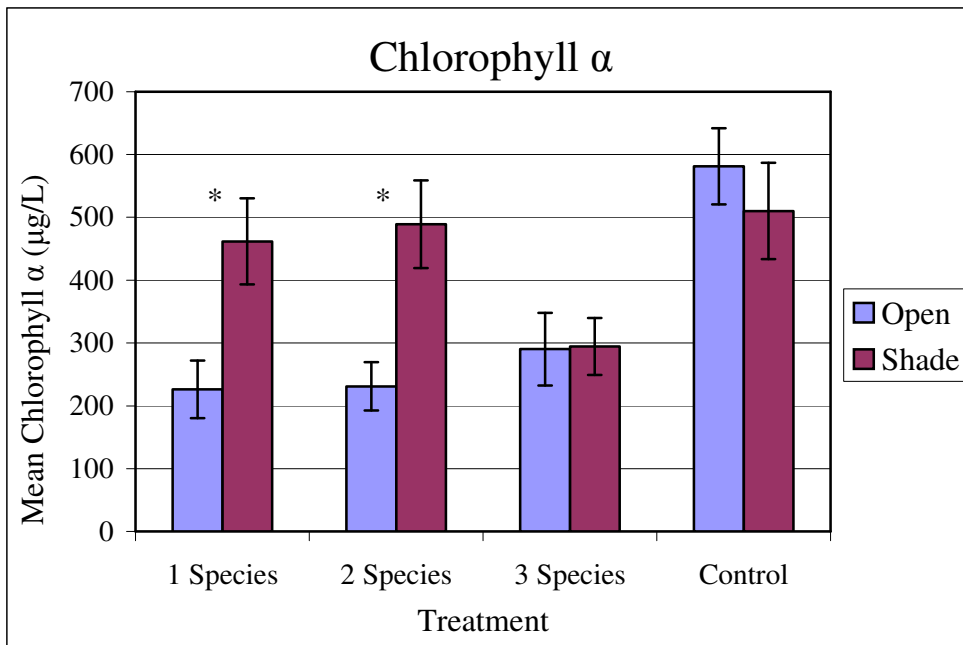


Figure 4. Algae accumulation measured as chlorophyll α concentrations from open and shaded channels for each treatment.