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Effects of Short-Term Dewatering on Grazer Abundance and Algal Biomass in a Wastewater Treatment Algal Turf Scrubber

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Abstract:

Finding a new, clean, and sustainable energy source to replace fossil fuels is one of society's most daunting challenges. Algae biomass is a promising renewable resource for biofuel production due to its efficient conversion of solar energy into chemical energy. Algal turf scrubbers (ATS™) systems mimic natural streams, flowing wastewater over hard substrates designed to encourage attached algae growth. This study focuses on the use of replicated ATS™ systems, because they are low maintenance, are effective for removing nutrient pollutants, model natural stream ecosystems, and can produce large volumes of harvestable algal feedstock for biofuel production. A challenge that occurs in ATS™ systems are grazers. Chironomid larvae graze on the algae, dislodging large portions of the algal turf reducing the efficiency of the system. Currently, pesticides are being used to control the midge larvae populations. Limited research is published in the area of non-chemical means of midge fly control. Dewatering is the non-toxic alternative proposed in this study. Three questions were discussed in this experiment. First, is dewatering the algae an effective method of midge larvae control? It was predicted that dewatering the algae would reduce the midges in the system. Second, does dewatering alter the algal biomass of the system? It was predicted that dewatering the algae would increase the algal biomass due to reduced grazing pressures from the midges. And lastly, was there a shift in the algal taxa community composition due to dewatering? It was predicted that the algal community would shift from predominately green algae to diatoms. The methods were based upon the *Methods in Stream Ecology* (Hauer and Lamberti 2006), and the *EPA Inorganic Chemistry Unit Chlorophyll Spectrophotometric Protocol 150.1* (1991). Dewatering the algae effectively killed the midge larvae in the system as there was a 26% reduction between drying and control treatments. However, the algal biomass was negatively impacted as there was an 18% decrease, reflecting that although dewatering is an effective method of midge control, it reduces the productivity of the algae in the ATS™ system. There was not a significant shift in the algal taxa as shown by the proportions of chlorophyll b and c in the system.

Introduction:

The search for a new sustainable energy to complement or replace petroleum-based fuels is one of society's most daunting challenges; it is one linked to global stability, economic prosperity, and quality of life (Parmar et al. 2011). In recent years, awareness of the efficiency and overall benefits of the use of algae for the production of biofuels has catalyzed further research in this field (Adey et al. 2011). Algae biomass is a promising renewable resource for biofuel due to its efficient conversion of solar energy to chemical energy, and for that reason, has drawn attention to its large-scale practical uses (Show et al. 2012). Algae are by far the most productive photosynthetic organisms on the planet. These aquatic autotrophs can grow year round and are over eight times more productive than corn, their terrestrial cousin (Craggs et al. 2011). Algae and cyanobacteria can convert up to 10% of the sun's energy into biomass compared to the 1% converted by the standard crops such as corn and sugarcane (Parmar et al. 2011). Algal biomass has many uses, such as: crude bio-oil for making biodiesel, feedstock for bioethanol, biomass for animal feeds, and source of organic fertilizers (Craggs et al. 2011). Not only can algae biomass be used to generate the aforementioned products, it also plays a key role in the global carbon cycle and is responsible for removing excess carbon dioxide from the environment (Show et al. 2012). Removing excess carbon dioxide, phosphorus, and nitrogen from wastewater has been an inefficient and costly task until the integration of algae as a treatment.

Before the advent of modern wastewater treatment facilities, the untreated wastewater was often released directly into freshwater streams, and the stream ecosystems were expected to "self-purify" and remove excess nutrients and byproducts (Craggs et al. 1996b). Early wastewater treatment focused on carbon removal (i.e., primary and secondary treatment) to prevent dissolved oxygen sags and fish kills, with few limits on nutrient effluent (Cech 2010). As human populations and economies have expanded wastewater runoff has overwhelmed the capacity of freshwater ecosystem to "self-

purify" and maintain a balanced state (Craggs et al. 1996b). One promising new, non-chemical treatment technology has incorporated algae into the wastewater treatment system. Algal treatment technologies have been introduced over the last 40 years to improve wastewater treatment. Several application-scale projects have been constructed and performed successfully (Craggs et al. 1996, Craggs et al. 2001, Craggs et al. 2012). Algae thrive in secondarily treated wastewater, as it is still full of nutrients such as phosphorus and nitrogen (Adey et al. 1993). One way algae have been used to purify wastewater is the use of algal turf scrubbers (ATS™, Adey et al. 1993, Craggs et al. 1996). ATS™ systems mimic natural streams, flowing wastewater over hard substrates designed to encourage attached algae growth. This study focuses on the use of replicated ATS™ systems, because they have low maintenance requirements, are effective for removing nutrient pollutants, model of natural stream ecosystems, and can produce large volumes of harvestable algal feedstock for biofuel production.

Although ATS™ systems can improve wastewater treatment, they can also create a perfect breeding ground for chironomid pests (Craggs et al. 2001). The midge larvae build cocoons and inhabit the surface of the flow way. As the larvae consume/dislodge algae they decrease the amount of algal biomass in the system (Craggs et al. 2001). This disturbance decreases the efficiency of the ATS™ for nutrient removal and biofuel production. Since there are no predators to control midge population in the ATS™ systems, large swarms of adult flies emerge from these breeding grounds and become pests to humans (Craggs et al. 2005). In order to effectively decrease the midge fly population many wastewater treatment facilities use insecticides or other chemical treatments. Artificial insecticides are added to the water to kill midge larvae, but contaminate wastewater effluent with pesticide residual. An alternative method of controlling chironomid larvae in ATS™ systems, without the use of chemicals, is a dewatering technique suggested by Craggs et al. (2001). Chironomid larvae cannot survive in dry conditions, however, algae can endure desiccation making dewatering a possible method of midge fly control (Bay 2003, Dudley et al. 1986). The effects of drying on algal biomass need to be accounted for

to determine the practicality of dewatering for ATS™. Hunt and Denny (2008) have shown that dewatering has a direct negative effect on algal productivity, since the algae are unable to undergo photosynthesis during times of drying. Desiccation also shifts community structure toward mobile, microscopic taxa (e.g., diatoms, Ledger et al. 2008) that live entirely within the periphyton mat. The mat is a complex assemblage of organisms that includes bacteria, fungi, and microzoans held together by an interwoven matrix of polysaccharides (Hauer and Lamberti 2006). The structure of this mat may provide a refuge protecting small mobile diatoms from the detrimental effects of desiccation (Ledger et al 2008).

Because dewatering disturbances can structure algal communities (Ledger et al. 2008), it is important to understand how dewatering treatments (for midge control) affects algal biomass and algal community structure in ATS™ systems. While algal biomass and composition can be estimated using light microscopy, algal communities can also be assessed quantitatively using their photosynthetic pigments (Arar 1997, Hauer and Lamberti 2006). All algae and cyanobacteria contain chlorophyll a, but chlorophyll b and c can be found in different taxonomic groups. Thus chlorophyll a can be used to indicate the overall abundance of algae (Hauer and Lamberti 2006), and shifts in the chlorophyll a:b ratio can be used as markers to quantify shifts in the algal community composition (Fietz 2004). Determining the taxa present in an ATS™ system can provide valuable information since certain algal taxa are more efficient for the production of biofuels (e.g, cyanobacteria and green algae, Parmar et al. 2011).

The objectives of this experiment are to determine if drying is an effective method for controlling midge fly larvae populations, ascertain if this drying has an effect on the algal biomass, and lastly, determine if drying shifts the composition of the algal communities as indicated by shifts in the relative proportions of chlorophyll pigments a, b, and c. The first question was, can dewatering the algae be a useful alternative method controlling midge fly larvae in ATS™ systems? It was predicted that

dewatering the algae would reduce the number of midges. The next question posed was, will the dewatering the algae alter the biomass of algae (i.e. chlorophyll a) relative to controls? It was hypothesized that the algae treated with the dewatering protocol would have greater biomass than the control algae, because they will experience lower grazing pressure from midges. The final question for this study was, will dewatering the algae affect the taxonomic composition of the algal communities? It was predicted that there will be a shift toward communities with a greater proportion of chlorophyll a relative to chlorophyll b and c, as dewatering is better tolerated by diatoms.

Materials and Methods:

To accomplish the specific aims of this research project a replicated, recirculating Algal Turf Scrubber (ATS™) system was used at the Columbus Water Works, Inc. South Lumpkin Road wastewater treatment facility. The ATS™ had 16 flumes (white vinyl roofing gutters, 0.1m X 3m), which allows for treatments to be replicated simultaneously (Figure 1). The ATS™ maintains constant flow in the flumes using a simple gravity feed design. That constant head pressure was maintained by pumping water from the sump (570L) to two elevated plastic barrels (200L each). Barrels had overflow drains that ensure a constant water pressure feeding each of the flumes. The barrels were outfitted with eight PVC ball valves and short segments of garden hose that directed the water to each flume. These ball valves were used to regulate the flow of each flume independently.

The experiments were conducted using unchlorinated, secondarily treated effluent. Water was filtered through a course mess (~1 mm) before being pumped into the flume system. All flumes were lined with 30 unglazed clay tiles. The clay tiles provided a substrate for the periphytic algae to attach and proliferate in the flumes. The tiles mimic the concrete substrate found in most algal treatment systems. These tiles were also used to simplify algal and chironomid larvae sampling since tiles were easy to collect, harvest, remove and replace.

Research Design and Methods:

Experimental Conditions

This experiment took place at the Columbus Water Works Inc., South Facility, located on South Lumpkin Road, Columbus, GA. The study was carried out from December 21, 2012 through January 17, 2013. The average temperature during the experiment was 13°C, this was measured using an iButton device to measure the temperature every four hours.

The four hour dewatering protocol was chosen based upon a previous study done by Craggs in 2001. The ATS™ used in Craggs study was a larger scale apparatus, so in order to compensate for the size difference the time was adjusted accordingly as well. The protocols have been modified from, but were based on, *Methods in Stream Ecology* (Hauer and Lamberti 2006), and the *EPA Inorganic Chemistry Unit Chlorophyll Spectrophotometric Protocol 150.1* (1991). In this experiment there were eight replicates of each treatment (control and drying) that were randomly assigned to flumes in the replicated ATS™. For the control flumes, the secondarily treated wastewater was continuously running through the system and was never turned off throughout the duration of the experiment. For the drying treatment the water was shut off for four hour periods every nine days. There were three drying events during this experiment.

The statistical analyses varied depending on the hypothesis at hand, the results for the first hypothesis, regarding midge fly counts, was analyzed using a Chi square test. The results for the second hypothesis, regarding algal biomass, used an independent variable t-test. And finally, the results for the third hypothesis were analyzed using the same independent variable t-test.

Methods

The ATS™ recirculating system was operated continuously for two weeks to allow the algae proliferate in the system. Once the algae were well established the experiment could begin. A random number generator was used to choose the flumes that received the four hour drying treatment (four flumes per barrel, n=8). The remaining eight flumes were the controls and were not dried. Two tile samples were taken from each flume and replaced with a clean, new tile. The tiles sampled were chosen by a random number generator and could have fallen in a range from tile number one to tile number thirty in each flume. Once the numbers were generated the same two tile numbers were collected from all 16 flumes (tile 19 and tile 30 were used across all the flumes). A total of 32 tiles were collected, one tile per whirlpak bag. Each bag was labeled and the samples were frozen (-20°C) until ready to be analyzed.

Drying effects on midges

The tile was removed from the whirlpak bag and placed in a large petri dish. Any remaining algae was rinsed out of the bag using distilled water and poured onto the petri dish. All of the algae were gently brushed off of the tile using a clean nylon brush, and rinsed thoroughly with distilled water over the petri dish to collect all the algae from the sample. The petri dish was placed under a stereoscope and systematically searched for midge fly larvae under 12.5 X magnification. Midges were counted only if their head capsule is present, pieces-parts were not counted if the head was missing (Figure 2). Each sample was thoroughly examined and all midges were tallied according to the coinciding tile, flume, and barrel they came from. Once the petri dish had been scoured for midges, the algae were filtered using fiber glass filters (0.7 µm glass filters GFF). The fiber glass filters were folded in half algae-side-in and stored in individual foil pouches labeled the same as the original bag containing the tile. The foil pouches were frozen (-20°C) until ready for spectrophotometry stage. There was a method error that occurred during this stage. The freezer where the samples were being stored was unplugged for

some unknown reason for an unknown length of time. This occurrence may have affected the results of this experiment.

Drying effects on algal biomass

To prep for the spectrophotometry stage, the algae filters were to be soaked in 90% acetone + magnesium carbonate solution for a minimum of 24 hours to extract the chlorophyll. For this experiment the chlorophyll was extracted for 48 hours. This part of the experiment was done in low light conditions as the chlorophyll become vulnerable once subjected to acetone. The test tube rack that held the 15 ml conical vials was wrapped in foil to help keep the light out and the lab were kept dark throughout the process. Each sample's algae filters were placed in individual 15 ml conical vials, and labeled accordingly. The 90% acetone + $MgCO_3$ was added. Each vial had a minimum of 10ml of acetone, but for centrifuging purposes all the vials were filled up to the 13ml marker on the vial. The exact amount of acetone added for each vial was recorded to aid in calculations later. The vials were then vortexed for 15 seconds. The rack was covered in foil to reduce light exposure and was stored in the freezer for 48 hours. After the 48 hours in the 90% acetone + $MgCO_3$, the chlorophyll had been extracted and was ready for the spectrophotometer protocol. The protocol for the spectrophotometer was based on the *EPA Inorganic Chemistry Unit Chlorophyll Spectrophotometric protocol* (1991). The samples centrifuged at 2500 rpm for 20 minutes. Next diluted (1 to 10 ratio of sample to acetone mixture) and pipetted into the cuvette (2.54 cm path length). The spectrophotometer was zeroed with a 90% acetone + $MgCO_3$ sample at each of the wavelengths measured. Each cuvette with chlorophyll and 90% acetone + $MgCO_3$ was analyzed at 665, 664, 663, 647,645, 630, and 750 nm without hydrochloric acid (HCl), and again at each wavelength after adding 0.33 ml of HCl per 10ml of sample. This sequence was repeated for each centrifuged sample. Calculations were carried out to determine the chlorophyll a, b and c values. (See Appendix I for the equations).

Drying effects on the algal community composition

Using the results from the spectrophotometry analysis in the previous step, the proportions of chlorophylls b and c were compared to provide insight into the composition of the algal community in the system. Results from the calculations for the proportion of algae types present may reveal a difference in algal community composition among the treated and untreated flumes in the ATS™ system (Table 1, Figure 3).

Results:

Experimental Conditions

The experiment was carried out from December 21, 2012 to January 17, 2013 in Columbus, GA. The average temperature during the twenty-seven day period was 13°C. The lowest temperature recorded by the iButton device during this study was 0.5°C, and the highest temperature reaching 24.5° C (Figure 7).

Drying effects on midges

There were a total of 68 midge fly larvae found in the system across the 32 tiles that were sampled. The average number of midges found on the control tiles was 3, while the treatment tiles on average had two midges. Total midge fly larvae counts for the controls were 26% higher in comparison to the drying treatment (CHI SQ=3.558, df=1, P=0.007).

Drying effects on algal biomass

Chlorophyll a was 18% higher in the control compared to the drying treatment (t-test, tile 19, P=0.031). Pheophytin is an indicator of the amount of degraded chlorophyll in the system. The pheophytin was 30% higher in the control than it was in the treated tiles (t-test, tile 19, P=0.024). The

same results were mirrored in the second set of tiles that were collected showing that the algal biomass had a 25% decrease due to dewatering (t-test, tile 30, chl-a P=0.023, chl-b P=0.028, chl-c P=0.41, pheo P=0.009). In the control flumes, the algae grew on the tops, edges, and occasionally underneath the tiles. In contrast, for the flumes that underwent the drying treatment the algae tended to only grow on the edges and underside of the tile, and was very sparse on the top surface of the tile.

Drying effects on algal community composition

The proportion of chlorophyll b, in relation to the total chlorophyll of the system, present in the tile 19 samples tiles was not significant and neither was the proportion of chlorophyll c, again with respect to the total chlorophyll (t-test, P=0.599, P=0.877). The proportion of chlorophyll b and c for tile 30 was also not significant (t-test, P=0.407, P=0.885).

Discussion:

Experimental Conditions

This experiment took place in the winter time (December 21, 2012 - January 17, 2013), with average temperatures of 13°C (55.4°F), the environmental conditions for the algae were harsh to start with, and may have been a factor as to why the algae were not as resilient during the dewatering treatment (Figure 8). Had this experiment been carried out in the summer, different results may have been observed; warmer and sunnier conditions are more beneficial for algae. According to Craggs et al. (1996 b), the midge and algae populations are very high in August, so testing the dewatering technique across the seasons would be a highly beneficial future study.

Drying effects on midges

The midge fly population was reduced by 26% in the ATS™ system after dewatering treatments (Figure 3). The results from this study show that dewatering is an effective non-toxic technique for

controlling midges. In a study done by Lamberti and Resh (1983), the effects of grazing on algae using tiles placed in natural stream environments. This study found that the areas where the grazers were excluded had 10 times more algal density then those that had been grazed (Lamberti and Resh 1983). In the excluded plots the algal biomass exceeded 10μg/cm^2, while the grazed plots remained at 1μg/cm^2 (Lamberti and Resh 1983). In a study done by Ludlam and Magoulick (2010), unglazed ceramic tiles were integrated into a natural stream where season desiccation occurs. They found that in times of drying the chlorophyll a levels were 1.5 to 3 times lower due to grazing by chironomid larvae (Ludlam and Magoulick 2010).

Drying effects on algal biomass

The drying treatments had direct negative effects the algal biomass. Dewatering the algae was detrimental to the efficiency of the ATS™ system as the algae were forced to shut down photosynthesis and the frequency of the drying was too close together for the algae to recover completely (Figure 4, Figure 5). The algae in both the tile 19 and tile 30 groups responded in the same way to the dewatering treatments, with nearly identical data, showing that the two independent entities had the same outcome. In a study done by Anandarajah et al. (2011), algae biomass levels were compared at varying speeds at which the algae were dried and their findings show that the cell densities of the algae were significantly decreased (Paired t-test, $P \leq 0.05$). The chlorophyll a levels for the dried samples were 36% lower than the control samples (Anandarajah et al. 2011), which is fairly close in comparison to the 25% decrease found in the current study.

Drying effects on algal community composition

The results comparing the proportions of chlorophyll b and c in the system, in comparison to the total chlorophyll found in the system, did not show evidence of a community shift (Figure 6). In a study conducted by Ledger et al. (2008), the effects of disturbances, such as drying, in natural streams were

compared. This study was conducted over a two year period and reports that algal community structure changed noticeably during their experiment (ANOVA, F=3.12, P=0.001), specifically showing a taxonomic shift from green algae to a more diverse diatom mat in times of drying (Ledger et al. 2008).

Conclusion:

Experimental conditions

A comparison of the effects of dewatering on midge control and algal biomass needs to be carried out across the seasons, as winter may not be the ideal time to employ this method.

Drying effects on midges

Drying the algae was an effective, non-toxic, alternative method for controlling midge larvae populations in ATS™ systems. The hypothesis was that the drying treatments would reduce the number of midges in the system, and since the results showed a 26% decrease in midges (P=0.007), the hypothesis was accepted.

Drying effects on algal biomass

Drying the algae had direct negative effects on the algal biomass as indicated by the density of chlorophyll a in the system. The algae that underwent dewatering treatments had a 25% decrease in comparison to the controls, showing that dewatering was detrimental to the efficiency of the system. The hypothesis for this question was rejected, as the algal biomass did not increase due to the drying treatment.

Drying effects on the algal community composition

According to the results there was no evidence that the algal communities had shifted. It had been predicted that a shift would occur in the system as diatoms are more tolerant to drying, however this was not observed so the hypothesis was rejected.

Summary

The intention of this experiment was to create an effective method of decreasing the midge fly populations through non-chemical means without causing too much stress to the algae. Midge fly larvae need water to survive, while algae can persist in times of drying. If the midge fly larvae could be exterminated through a drying technique, the number of adult midge flies would be significantly reduced in the ATS™ system. The goal of this project was to determine the effects of drying on algal biomass as indicated by photosynthetic pigments to evaluate the method's use as a midge fly larvae exterminator. The results state that dewatering is an effective method for midge control; however, the dewatering also had direct negative effects on the algae. There was no evidence that dewatering caused an algal taxa shift. The ATS™ is a highly productive system for biomass accrual, and has many beneficial applications, such as wastewater treatment. The products collected from the ATS™ can be used to make biofuels, fertilizers, bioethanol, crude bio-oil, and animal feeds. Algae are a highly efficient renewable resource and are underutilized. Furthering research and applications for the use of algal biomass is imperative for a greener, cleaner world. Future studies related to this experiment may include: testing a broad range of drying and recovery times for the algae, analyze the midge grazing effects on algae, test dewatering effects across seasons on both the midge control and the algal biomass, identify and enumerate algal and midge taxa in the ATS™, and determine the drying effects on ATS™ efficiency.

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Table 1. Compilation of algal taxa and the major accessory pigments they possess.

Common Name	Accessory Pigment
Green Algae	Chlorophyll a, b
Diatoms	Chlorophyll a
Blue-green Algae	Chlorophyll a, b

Figure 1. The algal turf scrubbing (ATS^{TM}) recirculating system at the Columbus Waterworks Inc. (South Facility).

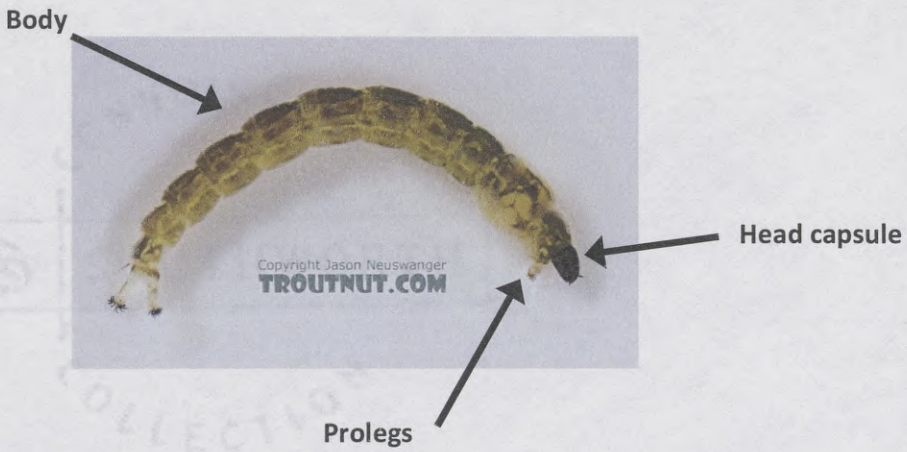

Figure 2. Chironomid midge fly larvae, body and head capsule diagram.

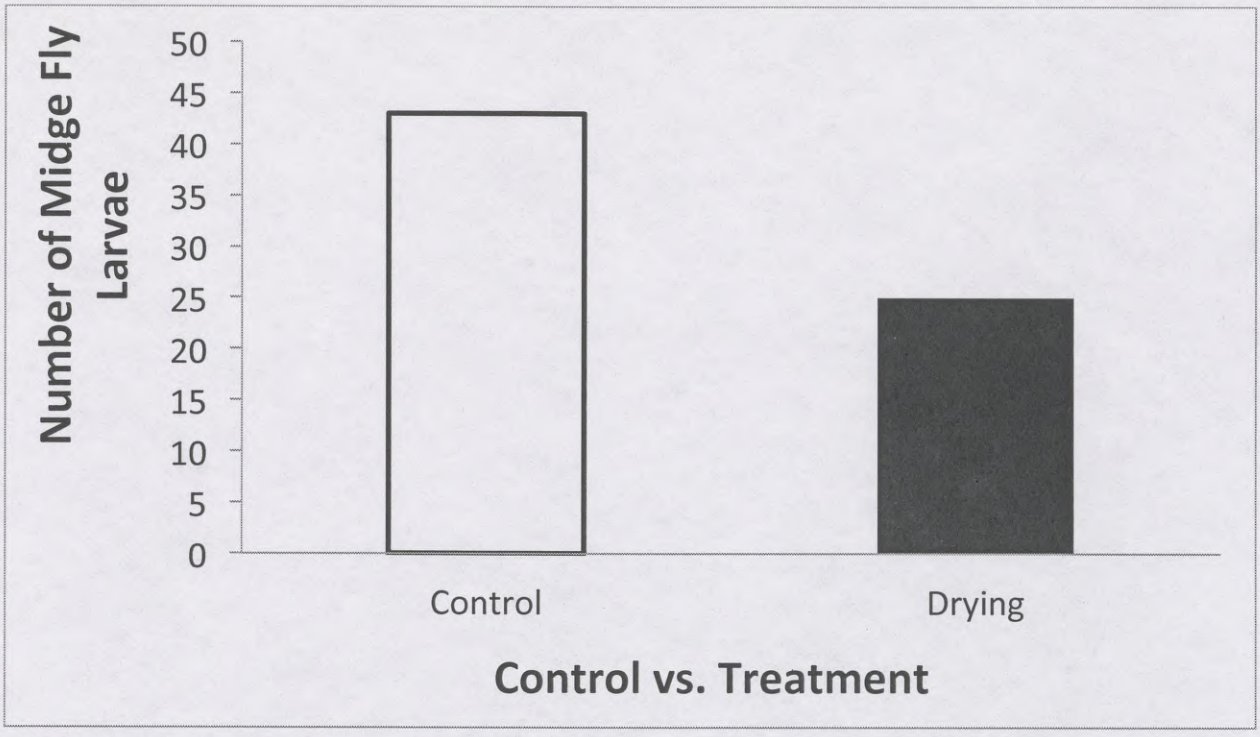

Figure 3. A comparison of the total midge fly larvae counts for control vs. drying treatments. Chi Square test reveals that midge fly totals were significantly different (Chi Sq=3.558 df=1, P=0.007).

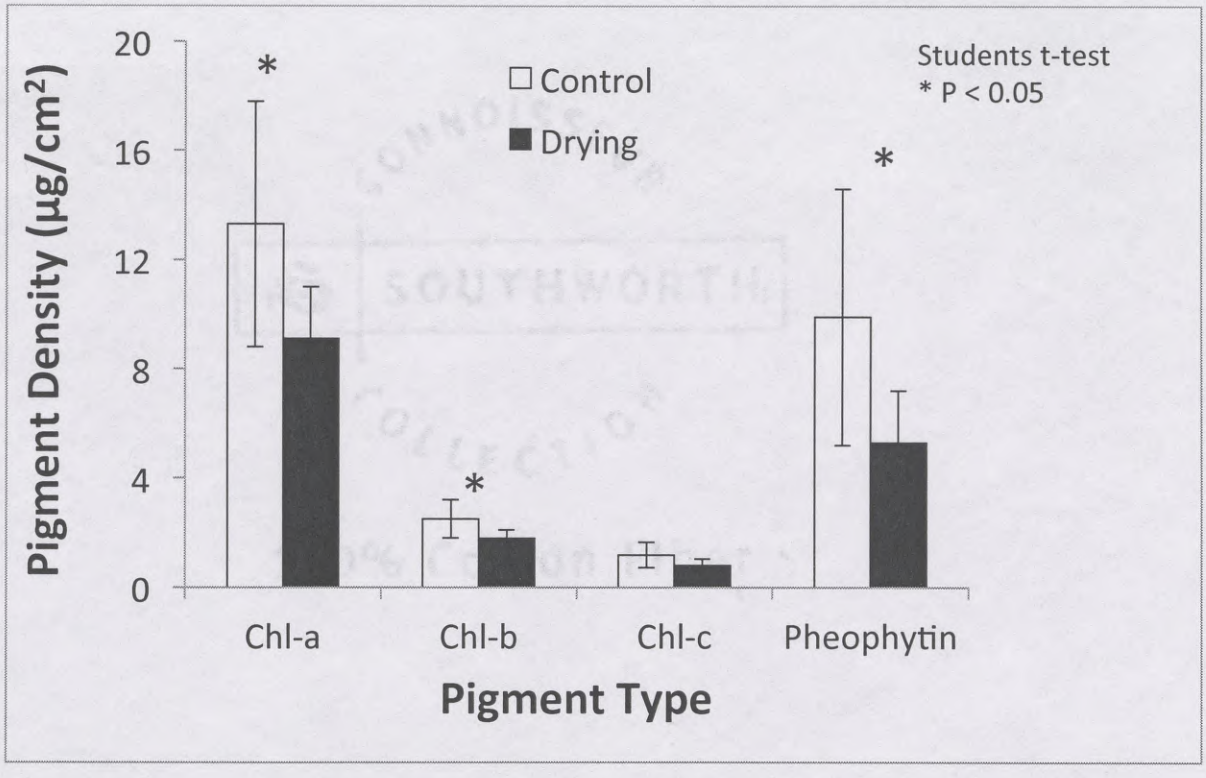

Figure 4. A comparison of the average (+/- 1 S.D.) of the pigment densities for four types of photosynthetic pigments (chlorophyll a, b, c, and pheophytin) collected from tile 19. Treatments without a (*) are not significantly different (P>0.05).

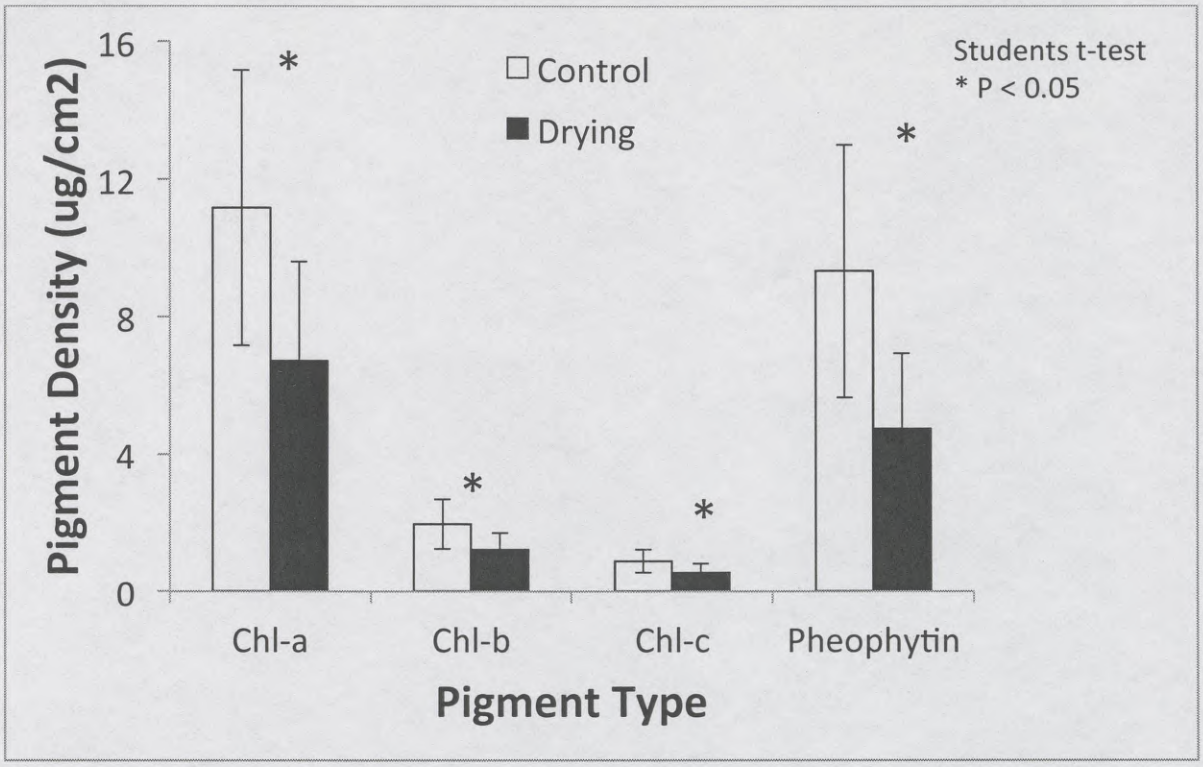

Figure 5. A comparison of the average (+/- 1 S.D.) of the pigment densities for four types of photosynthetic pigments (chlorophyll a, b, c, and pheophytin) collected from tile 30. Treatments without a (*) are not significantly different (P>0.05).

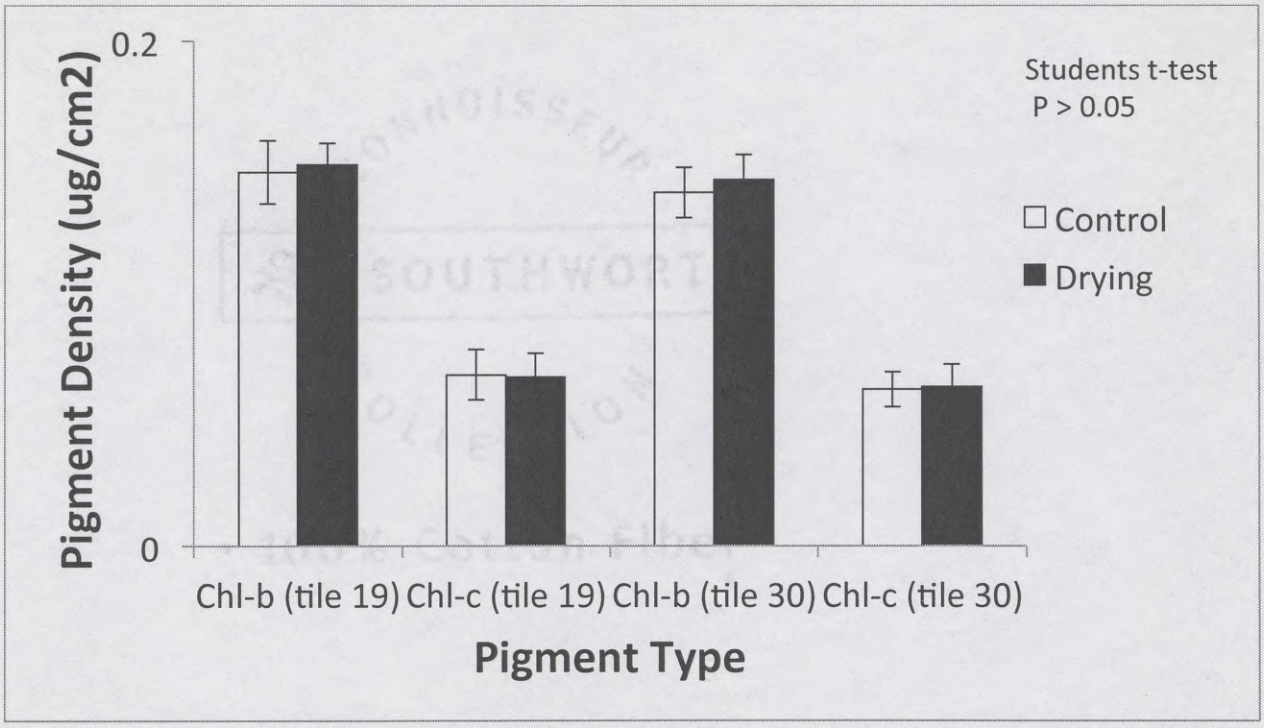

Figure 6. A comparison of the proportions of chlorophyll b and c (+/- 1 S.D.), for control and treatment in order to discern a possible taxonomic shift in the algal community. No significance shows that there was no taxonomic shift as evidenced by chlorophyll.

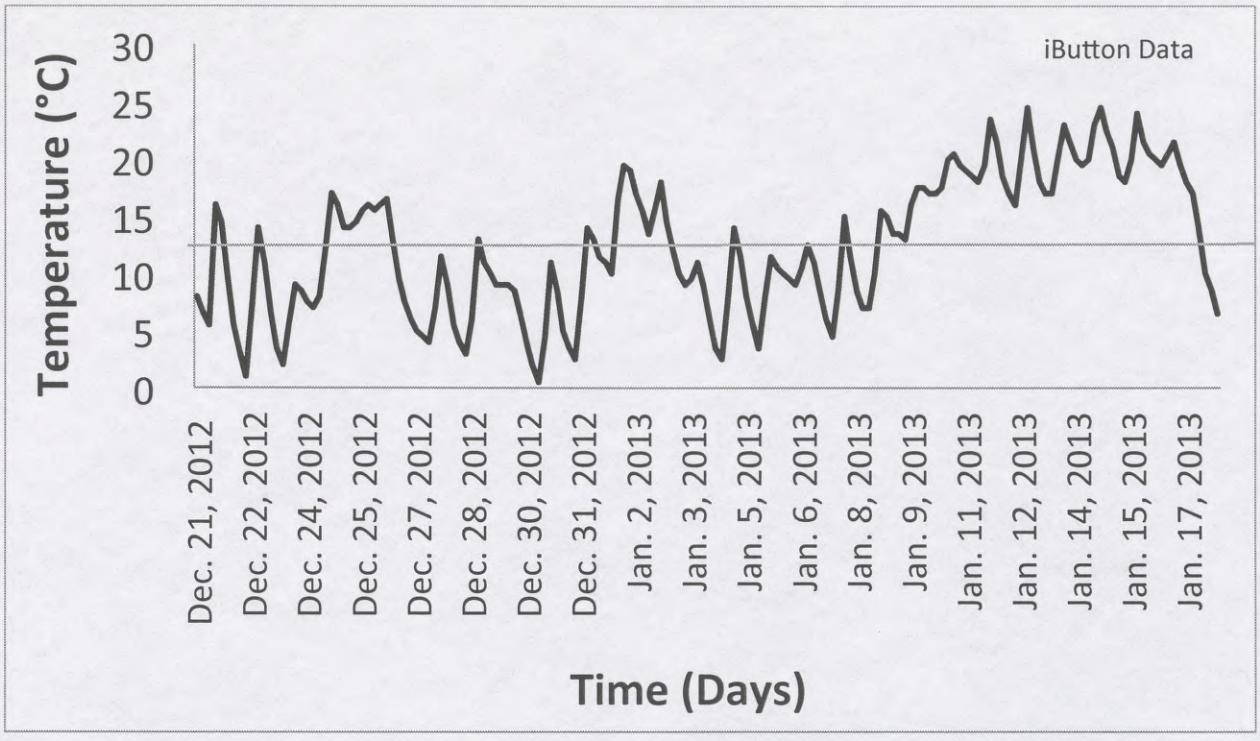

Figure 7. Temperature readings for the duration of the experiment were measured every 4 hours by the iButton. Algae dewatering treatments were conducted from mid-December (2012) to mid-January (2013). Average temperature was 13°C (55.4°F).

Appendix I. Equations to be used for calculating the values for the major photosynthetic pigments by spectrophotometry (Hauer and Lamberti 2006, Eaton et al. 2005).

Chlorophyll a (ug/cm^2) = 26.7 (E_{664b}-E_{665a}) X V_{ext}/ area of substrate (cm^2) X L

Pheophytin (ug/cm^2) = 26.7 (1.7E_{665a}-E_{664b}) X V_{ext}/ area of substrate (cm^2) X L

E_{664b}= (absorbance of sample at 664 nm – absorbance of blank at 664 nm) – (absorbance of sample at 750 nm – absorbance of blank at 750 nm) before acidification

E_{665a} = (absorbance of sample at 665 nm – absorbance of blank at 665 nm) – (absorbance of sample at 750 nm – absorbance of blank at 750 nm) after acidification

V_{ext} = volume of 90% acetone used in the extraction (mL)

L = length of the path light through cuvette (cm)

26.7 = absorbance correction (derived from absorbance coefficient for chlorophyll a at 664 nm X correction for acidification)

1.7 = maximum ratio of E_{664b} : E_{665a} in the absence of pheopigments

C_a = 11.85(OD664) – 1.54(OD647) – 0.08(OD630)

C_b = 21.03(OD647) – 5.43(OD664) – 2.66(OD630)

C_c = 24.52(OD630) – 7.60(OD647) – 1.67(OD664)

C_a, C_b, C_c = concentrations of chlorophyll a, b, and c respectively

OD664, OD647, OD630 = corrected optical densities at the respective wavelengths

After determining the concentration of pigment in the extract calculate the amount of pigment per volume:

$$\text{Chlorophyll a} = \frac{C_a \text{ X extract volume (L)}}{\text{Volume of sample}}$$