

**The effects of temperature on diatom species richness and diversity in a streams lab facility from the Maple River of Northern Michigan**

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**Abstract**

The effects of temperature on diatoms species diversity and species richness were investigated at the University of Michigan Biological Station Streams Lab Facility located in Pellston, MI. Eighteen re-circulating closed PVC flumes were separated into three water treatments (averages of 18.4° C, 20.8° C, and 22.7° C) and water from the Maple River was pumped into each flume to ensure equivalent nutrient and chemical factors. Diatoms grew on ceramic tiles and diatom species composition was measured using species richness, relative abundance, and the Shannon-Weiner species diversity index. A T-test showed significant statistics among various weeks concerning species richness and diversity. As temperature increased both species diversity and species richness increased.

**Introduction**

Global warming is a worldwide phenomenon that has begun to intensify in recent years. An increase in the temperature of freshwater systems may have consequences concerning species diversity (Rouse et al., 1997). The effects of ecosystem warming are the result of a complex set of processes, more than just measuring the effects of CO<sub>2</sub> increases. Temperature impacts chemical and biological processes whereas CO<sub>2</sub> mainly impacts photosynthesis and other processes of the leaves in higher plants (Koch and Mooney, 1996). According to other studies, it has been difficult to understand the indirect effects of global warming (Shaver et al., 2000).

The impact of rising temperatures in inland, freshwater streams, however, has had little research attention. Attached algae are important producers in stream ecosystems and support much of the food web. Increases in stream temperature have the potential to alter the quality and quantity of benthic algal communities and these changes may have consequences for the rest of the food web. Previous studies have shown that diatom species have optimum ranges in which they prefer to grow (Patrick 1971). Each type of algae has a specific optimum range of temperature in which it grows, but the ones that prefer an intermediate temperature range are most important to study because of they grow abundantly in most places (Hickman 1974). This study focuses on the intermediate range of temperatures because of these reasons.

The question remains as to how algal community structure will be influenced with an increasing temperature gradient over a finite amount of time. In a previous study, it was found that species richness increased as temperature increased but species diversity did not along a naturally occurring thermal stream gradient (Maguire et al., 2007). Because Maguire et al.'s experiment had confounding variables due to the experiment being a naturally occurring one, this study attempted to remove all variables except for temperature through the experimental design.

Algal communities are temporally and physically compact so that within three to four weeks, mature communities will completely develop onto bare substrates. Due to their short life cycle and their ability to respond quickly to changing environmental factors, diatoms are a good choice in quantifying responses to a factor of increasing temperature (Dixit et al., 1992). With an artificial stream system, one can evaluate the effects of temperature on the development of algal community structures. The purpose of

this experiment was to focus on species richness and diversity as compared to varying temperatures simulating the potential effects of global warming.

### **Materials and Methods**

This experiment was conducted between July 10 and August 7, 2007 at the University of Michigan Biological Station streams laboratory research facility located in Emmet County, Michigan. Re-circulating closed PVC flumes (volume of thirty-four liters) with the top half cut lengthwise were filled with stream water from the Maple River (Fig. 1). Six flumes were placed into each of the three water baths for a total of eighteen flumes. This set-up was based on Steven Rier's set up from his experiment testing the effects of nitrogen and phosphorous on diatom communities (2006).

Using a Secondnature Challenger II air pump and an air stone attached to the end of plastic vinyl tubing, current was created within each flume to allow for recirculation of the stream water through the flume. Additional stream water was added at an average rate of 0.356 cm/s from the Maple River and filtered through a step-ladder of buckets to ensure that most of the unnecessary sediment was removed out of the stream water. Twenty-six meters of 0.95 cm outside diameter, 0.64 cm inside diameter, vinyl tubing was used to supply the eighteen flumes with enough stream water to ensure a constant turnover rate everyday. The addition of stream water to all flumes was necessary to guarantee that all substrates received an adequate amount of nutrients to ensure the growth of algal communities.

Temperature was manipulated by placing six flumes into one of three water baths allowing the bottom half of each flume to be immersed. The cold water bath had an average temperature of 18.4° C through the addition of cold, ground water to the bath at a

constant velocity. The ambient water bath did not receive any additional treatment and was at an average temperature of 20.8° C. The warm water bath was at an average of 22.7° C due to the addition of a hand-made water heater using a water heater piece and an air conditioner cord attached to a thermostat to regulate the temperature of the bath to a constant temperature. These water baths had a two degree difference between each to ensure proper variation.

Two ceramic tiles, each with an area of 21.16 cm<sup>2</sup>, were placed eighty centimeters apart from one another in each flume, creating a total of twelve replicates per temperature trial. Three of these tiles were randomly sampled once a week (July 17, July 24, July 31, and August 7, 2007) and prepared using the Van der Werf (1955) method. The organic components of the diatoms were cleaned by oxidizing the material in a mixture of 30% hydrogen peroxide and potassium dichromate. The supernatant was poured off in intervals of at least twelve hours, or until the sediment completely settled, and deionized water was added to the solution until the cleaned material was clear. This solution was added to two coverslips (two per tile collected), dried, and then mounted onto a slide using Naphrax<sup>®</sup>. The first 600 valves encountered were counted and identified to species level, when possible, using Krammer & Lange-Bertalot (1986-91).

Total species richness, relative abundance and the Shannon-Weiner species diversity index were calculated using Microsoft Excel for Windows 2007. A T-test between the averages of each temperature variable was calculated to determine statistical significance between the total mean species richness (number of species total).

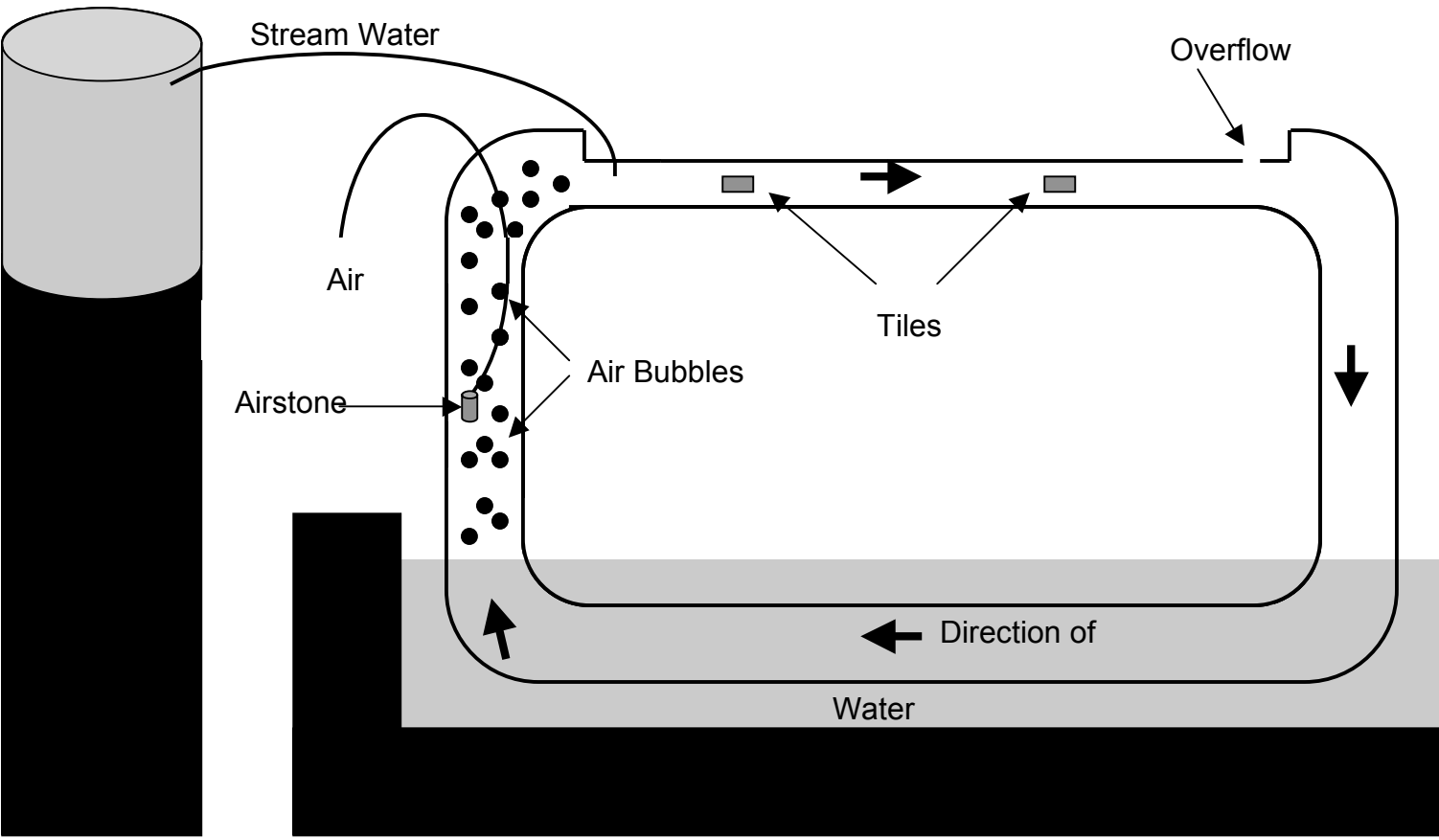


Figure 1. Diagram of a re-circulating flume used in the experiment



The set-up of the experiment!





Water flowing into the bucket and then to the flumes through vinyl tubing



Left: hand-made heater for the warm treatment; Right: ground water for cold treatment



Air pump set-up to get the water to recirculate in the flumes



Sample collecting day



Processing Samples

## Results

The average temperature between the three water treatments was about a two degree difference with a four degree difference between the coldest and warmest treatments. The average temperature of the cold treatment was 18.4° C, for the ambient temperature it was 20.8° C and the warm was 22.7° C. The daily variation is graphed in Figure 2 using the average temperature of all six flumes in a given treatment.

Concerning species diversity, the warm was consistently higher than the ambient which was higher than the cold; however, the species diversity for the cold treatment was slightly higher than that of the ambient in week one (Figure 3). In the subsequent two weeks the data followed the trend that the warm treatment had the highest species diversity. From T-test results calculated in Microsoft Excel, some significant statistics were found when comparing species diversity between temperature groups in the same week as well as between the same temperature group for different weeks. In week 2, both the cold to ambient ( $p=0.023$ ) and cold to warm ( $p=0.049$ ) were found to be statistically significant (with  $p < 0.5$ ). In week 3, the cold to ambient ( $p=0.032$ ) and cold to warm ( $p=0.034$ ) were similarly found to be significant. Between weeks one and three, the ambient groups in those two weeks were found to be significantly different ( $p=0.0208$ ) (Figure 4).

With regards to species richness, the same general trend as species diversity was seen (Figure 5). The warm treatment started with the lowest species richness and then became the treatment with the highest species richness in the following weeks. In week two between cold and ambient as well as between cold and warm the differences were significant ( $p=0.0076$  and  $p=0.045$  respectively). In week three these same temperature



treatments were found to have significant differences. Between the cold and warm treatments there was a difference ( $p=0.00094$ ) and between the ambient and warm ( $p=0.037$ ). When comparing weeks one and three the warm treatment was also significantly different ( $p=0.0283$ ) (Figure 6).

There are also trends between temperature treatments of individual species that should be noted among as well as trends that can be seen between weeks. Between all three weeks *Nitzschia palea* was found to be most dominant species in the warm temperature and found less so in the ambient and the fewest number found in the cold treatment. This same trend held true for *Gomphonema angustatum*, *Navicula gastrum*, and *Planothidium lanceolata*. A curious find was *Aulocoseira sp.* that was abundant in many of the slides. *Eunotia sp.* preferred the warmer treatment and was found to have the same trend as *Nitzschia palea* and tended to be most abundant in the warmer treatment.

As the dominant species in all three weeks tended to be the araphid diatoms of *Fragilaria sp.* and *Martyana olsenii* as well as the majority of the monoraphids being *Achnanthes minutissima*, *Achnanthes bioretii* and *Planothidium dubium* (in the later weeks) they were lumped into the top five percent of the count and disregarded for the remaining analyses. The following information concerns the remaining 95% of the identifiable species. All nine groupings of diatoms, Rhopaloidiod, Nitzschiod, Eunotiod, Monoraphid, Naviculoid, Cymbelloid, Gomphonemoid, Centric, and Araphid, were present after taking out the dominant five percent species.

A consistent finding within the three weeks was that the Naviculoid diatoms made up approximately 18-19% of the remaining group. In any given week less than one

percent of the diatoms consisted of Rhopaloidiod diatoms but, out of these few numbers, it was found to prefer the ambient treatment. A trend that was seen in all three weeks was that the Gomphonemoid diatoms were seen in highest proportion in the warm treatment than in the ambient and found least in the cold water temperature. This was also seen with the Nitzschiod diatoms in week one but the opposite trend occurred in weeks two and three. In weeks one and two the Eunotiod diatoms were found to most dominant in the cold treatment and less so in ambient and found either not at all or in very low numbers in the warm treatment. Week three showed the opposite trend, and the Eunotiod diatoms preferred the warm temperature over either the ambient or cold treatments. The remaining four groups of diatoms, the Monoraphid, Cymbelloid, Centric, and Araphid, constituted between 50 and 65% of the groups present.

The dominant groups (dominant because they were found to be in high proportion in all three treatments) in week one were the Naviculoids and Gomphonemoids; in week two were the Monoraphids, Nitzschiods, and Naviculoids; and in week three were the Nitzschiods, Naviculoids, and Gomphonemoids.

## **Discussion**

According to other experiments (Patrick 1971) the results in this experiment were opposite to what was expected. The warm treatment had higher species diversity than the cold treatment which suggests that the cold temperature inhibited growth of community structure while the warm enhanced such growth. Because all three treatments displayed a similar community structure, this suggests that the warm treatment simply speeded up the growth process of the diatom communities. Patrick (1971) found that by increasing temperatures, there was a shift in what species were most common which in turn changed

the structure of the diatom communities. This is opposite of what occurred in this experiment because the three treatments experienced a similar community structure – those species that dominated the system were present in all three weeks. What changed were those rare species that grew.

It has been suggested that common species of diatoms are better indicators of the environmental impacts of pollution and water quality compared to that of rare species. Rare diatoms, however, have been found to be some good indicators of water quality (Potapova and Charles 2004). Observations of the presence of these rare species (rare meaning it constitutes of less than 2% of the total of all species counted) can indicate the possible absence of pollution and has the potential for discovering a water quality monitoring method (Potapova and Charles 2004). Therefore it seems plausible to take into consideration the rare species that occurred in this lab because they could have more significant things to tell about water quality compared to the more commonly found species. Potapova and Charles (2004) found the numbers of native as well as rare species to be higher in rivers that were less affected by human activities. Having the presence of rare species can suggest the idea that there is a small niche in which the species can live which could mean that there are other factors influencing the growth of the rare species. This can be extrapolated to the data collected in this experiment because as time progressed, the number of individual rare species increased suggesting that the communities were becoming more diverse and complex throughout the weeks.

Species diversity is characterized by species richness, the number of species in a given collection, and species evenness, a value depending upon the number of both rare and common species. A study by Hairston (1959) showed that rare species tended to

clump and this could be seen in this particular experiment because when one rare species was noted it was more likely to be seen in a given sample. When analyzing the data for species diversity and for species richness the trend shown on graphs displayed significant data. From this observation it can be suggested that species evenness was also a significant difference between all three treatments.

The temperatures in this study are relative depending on what the minimum and maximum numbers were. In some research, the warm temperature in this experiment would be the cold temperature, relative to this experiment, in other research which would explain the results that were obtained from this experiment. In one experiment, it was found that species diversity as well as species richness increased with temperature through a range of 14°C - 25°C. Higher than this temperature the diversity began to decrease and this follows with what this experiment found (Vinson and Rushforth 1989). This experiment had an average temperature between about 18°C and 22°C displaying a median between Vinson and Rushforth's (1989) experiment, resulting in different statistics.

Another possibility for the results obtained could be due to the growth of specific diatom species on the ceramic tile substrates. Because tiles are not part of the natural environment, there was the possibility that these substrates would not yield the same representation as of that in nature. Despite this, studies have shown that communities of representative proportions will indeed grow into a community on artificial substrates as long as there are replicates to count (Fisher and Dunbar 2007).

In fact, ranges that are established through laboratory experiments are likely not to occur in nature due to the effect of other confoundable factors such as light, water

chemistry, or substrate use (Patrick 1971). A continuing current flow was needed to allow for diatom community growth as one experiment found and so this was provided for in this experiment as well (Keithan and Lowe 1985). Current brings nutrients and materials needed to bring about positive productivity of a diatom community but it can also bring about the consequence of removing nutrients from the system as well (Keithan and Lowe 1985).

This experiment can be used to extrapolate what will happen if global warming does indeed increase the temperature of water even only by a couple of degrees. The results of this experiment were substantial considering that there was only about a four degree difference between the lowest and highest temperatures. According to Weckstrom and Korhola (2001), there is an extrapolation of a five to ten degree Celsius increase projected for the year 2100 AD and this could have severe consequences for the fresh water ecosystems and the diversity of the aquatic organisms including their physical and chemical characteristics. Houghton et al. (1990) found that the IPCC (Intergovernmental Panel on Climate Change) estimates that every decade the temperature on earth will increase  $0.3^{\circ}\text{C}$  due to the greenhouse effect. If this is indeed the case, then there will be a dramatic effect on microscopic organisms especially since this experiment shows that even an increase of two degrees makes a significant difference between diatom communities.

Investigating a temperature change of no more than four degrees showed that there was a significant change in species diversity and richness in the diatom communities grown on the tile substrates. Beginning at the microhabitat level and testing a slight change in one environmental factor, such as temperature, has the ability to



restructure and entire system of a diatom community. By taking into account these small-scale ecosystem changes it may be possible to help understand the dynamics of the human to environment action at the local level.

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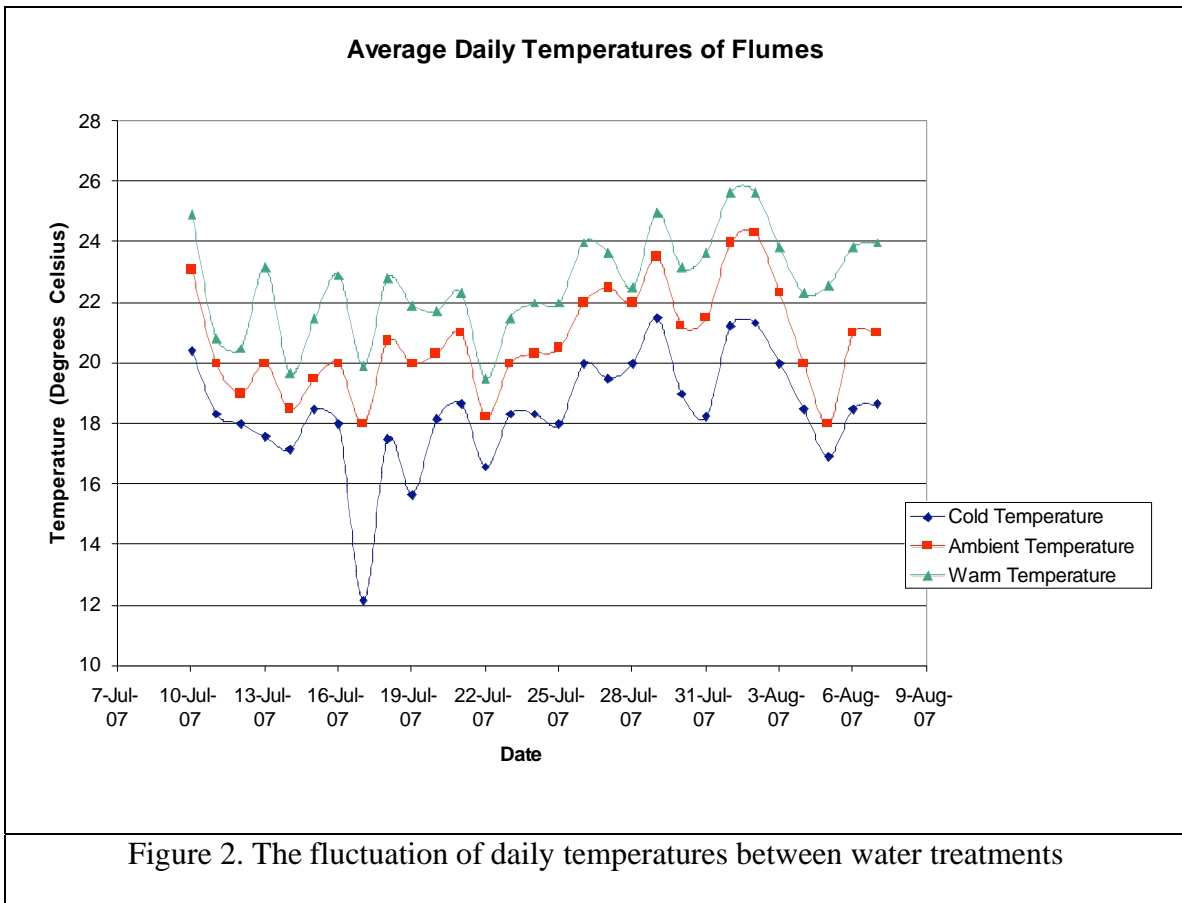


Figure 2. The fluctuation of daily temperatures between water treatments

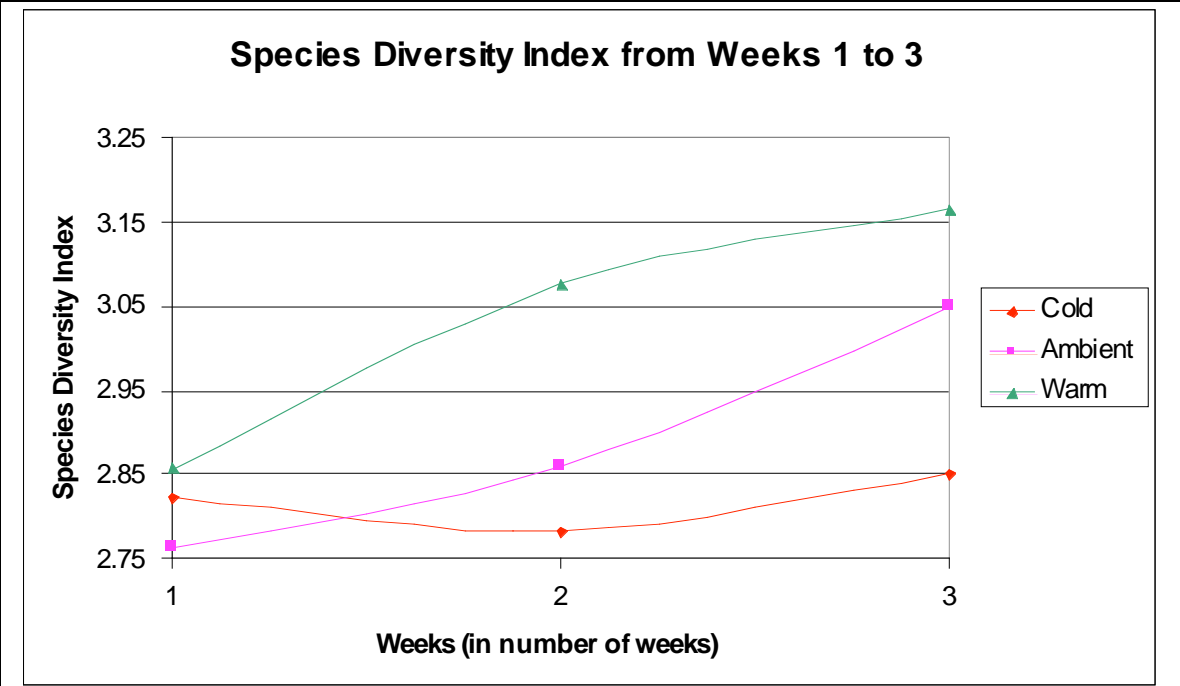


Figure 3. The trend of species diversity from weeks 1 to 3

Species Diversity			
		Week 1	
	C	A	W
Cold		0.3803	0.4177
Ambient			0.2393
Warm			

		Week 2		* = significant below 0.05
	C	A	W	
Cold		0.02296 *	0.04996 *	
Ambient			0.1021	
Warm				

		Week 3	
	C	A	W
Cold		0.03211 *	0.03447 *
Ambient			0.1549
Warm			

	Weeks 1-2	Weeks 2-3	Weeks 1-3
Cold to Cold	0.2969	0.2738	0.3849
Ambient to Ambient	0.3203	0.1281	0.0208 *
Warm to Warm	0.2056	0.1653	0.104

Figure 4. p-values for species diversity



Figure 5. the trend of species richness from weeks 1 to 3

<b>Species Richness</b>			
Week 1			
	C	A	W
Cold		0.437	0.3579
Ambient			0.381
Warm			
Week 2			
	C	A	W
Cold		0.007634 **	0.04451 *
Ambient			0.08659
Warm			
* = significant below 0.05			
** = significant below 0.01			
Week 3			
	C	A	W
Cold		0.06382	0.0009425 **
Ambient			0.03709 *
Warm			
	Weeks 1-2	Weeks 2-3	Weeks 1-3
Cold to Cold	0.3392	0.1499	0.3174
Ambient to Ambient	0.2987	0.1734	0.0537
Warm to Warm	0.1349	0.1777	0.02831 *
Figure 6. p-values for species richness			



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