TEMPERATURE ELEVATION DRIVES BIOMASS AND COMMUNITY SHIFTS IN PERIPHYTON

By

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ABSTRACT

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The influence of temperature on periphyton was studied in experimental streams over a range of temperature from 13.5 to 38 °C. Response of periphytic algae was measured as changes in biomass, diversity, biovolume, and growth rate during a seven-day colonization period. Maximum biomass developed at 27 °C during early stages of colonization and optimum temperature associated with maximum biomass shifted later to 33 °C. Species richness decreased rapidly as temperature increased from 13.5 to 33 °C. The community shifted as temperature increased from evenly co-dominating low-temperature diatom taxa to a few dominant intermediate-temperature diatom taxa, and finally to a few taxa of cyanobacteria. Considerable differences among species growth rates were observed across the temperature gradient. Low-temperature diatoms had high growth rates within a low temperature range and intermediate-temperature diatoms maintained a relatively constant growth rate from low to intermediate temperatures but failed to grow at high temperatures. Cyanobacteria grew poorly or not at all at low temperatures and grew most rapidly at high temperatures.

These results demonstrate the importance of the direct effects of climate change forcing by temperature on periphytic community composition in streams and suggest that warmer climates will increase cyanobacterial abundance and reduce diatoms in stream periphyton, which may lead to changes in food webs and degradation of ecosystems.

This thesis is dedicated to

My parents

For their selfless love, infinite confidence and firm support

ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. R. Jan Stevenson, for his endless inspiration and patient guidance. I feel grateful for his earnest participations in discussions that lead to completion of this thesis. He provided very important insights to my thesis research and gave help to every aspect of the thesis accomplishment. I also would like to thank my committee members, Dr. Stephen K. Hamilton and Dr. Patricia A. Soranno, for their comments on my research design and their suggestions on the early drafts of the thesis. I feel grateful to everyone in Stevenson's algae lab, including Seth Hunt, Jason Zalack, Stephanie Miller, Julianne Heinlein, Linda Novitski, Rui Chen, Ionel Ciugulea, Allison Rober and Kevin Wyatt, since they taught me every detail of experiments and techniques. I could not accomplish this research without their great assistance.

I also want to share this accomplishment with my parents, for their spiritual support and encouragements. It has never been enough to express my deepest gratitude to them.

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INTRODUCTION

There is a great concern about the ecological impacts of climate change on algae. Temperature is accepted as one of the factors driving occurrence of harmful algal blooms in various aquatic habitats, from freshwater to marine. Many studies have documented effects of elevated temperature on algae (Paul, 2008; Lassen et al., 2010). Massive algal growth under high temperature can cause problems with nutrient depletion, resource (i.e., light, oxygen) limitation, toxin production, habitat alterations, and undesirable changes in community structure and function (Butterwick et al., 2005), which lead to instability and degradation of ecosystems. Excessive algal growth can potentially affect water supply for human uses, aesthetic appeal, and recreation (Biggs, 1996). In some cases, temperature effects have been found to be more important than other factors. McQueen and Lean (1987) found that when temperature was below 21 °C, no matter how the nitrogen to phosphorus ratio changed, a blue-green algal bloom never occurred. Moreover, Lewandowska and Sommer (2010) found the timing of the spring algal bloom was advanced by warming. Thus the problem of harmful algal blooms due to increasing temperature has become a major concern and numerous experiments examining phytoplankton temperature response have been conducted over the years.

In contrast to phytoplankton, much less is known about the effects of temperature on periphyton. Most studies of seasonal variation in periphyton pay more attention to effects of other physical factors (i.e., nutrients, light and hydrology) when it comes to periphyton. The only study regarding temperature effects on periphyton (Bothwell, 1988) does not include soft algae (i.e., blue-green algae, green algae). Therefore, much information is lacking regarding the effects of elevated temperatures on periphytic diatoms and soft algae. Understanding how increased temperature affect the biomass, diversity and community composition in periphyton could help assess functional alterations in streams that may be

driven by climate change.

Temperature is an important determinant of algal biomass and algal species composition in aquatic ecosystems (Rutherford et al., 1995). Increasing temperature has a direct positive effect on the rate of photosynthesis within a tolerable range. The typical response is for photosynthesis to increase progressively with increasing temperature (DeNicola, 1996) with a Q_{10} of approximately 2.0, where Q_{10} is the ratio of metabolic rates at temperatures with differences of 10 °C. However, very high temperatures can denature enzymes causing photosynthetic rates to decline rapidly.

Given saturating nutrients (i.e. nitrogen, phosphorus, and micronutrients) and light, temperature enhancement of photosynthesis is hypothesized to be completely transferred to cellular growth rates. Therefore increased rates of photosynthesis at elevated temperatures enable faster algal growth. Generally speaking, growth rates are highest at an optimal temperature, and decline as temperature deviates from the optimum, with a more abrupt decline towards higher than lower tolerance limits (Epply, 1972; Dauta et al. 1990; Rutherford et al. 2000; Lassen et al. 2010). The tolerance limits as well as the optimal temperatures can vary between different species (Biggs, 1996; Smayda, 1997; Butterwick et al. 2005). Even though a periphyton community is an assemblage of multiple species, each with potentially unique thermal responses, I predict that a similar unimodal relationship between biomass and temperature would occur.

Despite the large variability in methods and types of lotic ecosystems studied, maximum areal net primary production increases exponentially with temperature for temperatures below 30 °C (DeNicola, 1996). Measurements of lotic net primary productivity at temperatures greater than 30 °C show a general decline in productivity from 30 to 40 °C. Williamson et al. (2010) also observed that algal production increases significantly with temperature in the lower range and decreases significantly in the higher range. Furthermore, Morin et al. (1999)

found that primary production was more closely related to water temperature in stream periphyton (Q_{10}=2.5) than in either lake phytoplankton (Q_{10}=1.4) or ocean phytoplankton(Q_{10}=1.2). Other studies also found periphyton were more affected by elevated water temperature in contrast with phytoplankton (e.g., Chuang et al., 2009).

Because different species exhibit different growth responses to temperature, temperature has been hypothesized to be an important factor driving diversity variation and community shifts in algae (Patrick, 1969; Wilde and Tilly, 1981; Dauta, 1982; Lamberti and Resh, 1985; Dauta et al., 1990; Stevenson et al., 1996; Rutherford et al. 2000; Bouterfas et al., 2002; Butterwick et al. 2005; Chuang et al. 2009; Elliott, 2010). Species diversity has been found to increase with temperature up to 25-30 °C, then decrease above 30 °C, as the species-rich diatom flora in periphyton are replaced by a few species of green algae or cyanobacteria. Several studies have shown that diatoms tend to dominate at approximately 5-20 °C (Dauta, 1982; Rutherford et al., 2000; Butterwick et al., 2005), green algae and xanthophyte dominate at 15-30 °C (Bouterfas et al., 2002; Dauta, 1982; Dauta et al., 1990; Butterwick et al., 2005), and cyanobacteria dominate at > 30 ºC (Patrick, 1969; Wilde and Tilly, 1981; Lamberti and Resh, 1985; Elliott, 2010).

In addition, temperature can exert an indirect effect on algal growth through other physical factors. Periphytic algal biomass in streams depends upon the interaction of many factors, which can operate simultaneously, such as light, nutrients, and grazing (Rutherford et al., 2000). Dauta et al. (1990) demonstrated a temperature-denpedent shift of light optima for growth in several algal species. McQueen and Lean (1987) noted that the optimal nitrogen to phosphorus ratio was related to temperature. Agawin et al. (2000) found a strong covariation between temperature and nutrient concentrations in natural ecosystems. Also, high temperature can reduce grazing stress (Rutherford et al., 2000). Thus the confounding effects of temperature and other environmental factors often make it difficult to interpret field

observations.

The objectives of my study were to use experimental streams to determine how increasing temperature would affect the production and composition of the periphyton community. I conducted an experiment in a culture room with six temperature treatments, I hypothesized: the relationship between temperature and periphyton biomass would be unimodal with the optimal temperature near 30 ºC; diversity would decrease as temperature increased and community composition would shift from species-rich diatom flora to a few species of cyanobacteria; and all of these changes could be contributed to differences in temperature regulation of algal growth rates that varied interspecifically and among diatoms, green algae, and cyanobacteria as major taxonomic groups.

MATERIAL AND METHODS

Experimental streams

The experiment was set up in a culture room with 18 experimental streams. The experimental streams were 135×45 cm loops of 6 cm diameter plastic pipes with the top piece cut lengthwise. These loops were filled with water from the Red Cedar River. Current was generated by pumping air into the bottoms of the upstream ends of the loops through a hose with HAILEA® diaphragm air pump. Water was lifted by air bubbles and produced current velocities of 10 cm s^{-1} in the open channel. To minimize effects of turbulence on algal colonization caused by air bubbles at the upper end of the open channels, unglazed ceramic tiles (2.3×2.3cm) were placed along the entire length of the open channel, but periphyton was only collected from tiles placed at the downstream ends. Temperature was manipulated in each channel by inserting HYDOR THEO 300-W heater. The heaters can change water temperature from 13 to 40 ºC. Temperature treatments were 13.5, 17, 23.5, 27, 33 and 38 ºC.

Three replicate channels were run for each treatment. The six temperature treatments were assigned randomly across the 18 channels.

Solutions of $NaNO_3$, KH_2PO_4 and $NaSiO_3$ were added to each stream channel every day of colonization to ensure periphyton were not nutrient limited. My goal was to maintain nutrient concentrations above saturating levels. According to a previous study, periphytic diatom growth saturated at approximately 16 µg SRP L^{-1} and 86 µg NO_3-N L^{-1} (Rier and Stevenson, 2005). 30 µg TP L^{-1} and 1000 µg TN L^{-1} were considered the thresholds to prevent nuisance accrual of *Cladophora* (Stevenson et al., 2006). Bothwell (1989) found periphytic diatoms were no longer phosphorus limited at 30-50 µg SRP L^{-1}.

The algal inoculum was produced by scraping rocks from the Red Cedar River in May when water temperature was around 15℃. The Red Cedar runs through the Michigan State University campus in East Lansing, Michigan. According to measurements in lab, the Red Cedar River has total phosphorus (TP) and total nitrogen (TN) concentrations roughly around 10 and 500 µg/L, respectively. Scrapings were combined in a single container and homogenized before aliquots were added to each stream.

Sampling and measurement

Water samples were collected from each channel every day and stored in a cold room before analysis. Total nitrogen and TP were assayed as nitrate-nitrite and soluble reactive phosphorus, respectively, with a Spectronic® GENESYS™ 2 spectrophotometer after persulfate digestion according to standard methods (APHA 1998). Silica concentration was estimated with a Skalar® auto-analyzer following standard methods (APHA 1998). To minimize conductivity effects on algae, distilled water was added to replace evaporated water,

and conductivity was determined daily in each channel using a YSI 556 Multi-Probe System™. Temperature was monitored every day with thermometers. Since the accuracy of temperature control of the aquarium heaters was limited, intervals between temperature treatments were not equal to each other, but the temperatures were stable over time.

After the algal inocula were added, tiles were randomly collected from all channels every day for 7 days. Periphyton was scraped from unglazed tiles into beakers using a toothbrush. Samples were diluted to known volumes with deionized water, homogenized with a Biospec® biohomogenizer, and then sub-sampled with a mechanical pipette. Chlorophyll-*a* (chl *a*) was determined using a Turner Designs TD-700 fluorometer after a 24 hour extraction in 90% ethanol at cold temperature (APHA 1998). Subsamples for counts were preserved in M3 for algal counts (APHA 1998). For algal enumeration, semi-permanent syrup mounts were prepared by drying 1 mL sample on cover glass (22mm×22mm) with one drop of Taft's syrup medium for the first day, with addition and drying of 1 ml Taft's Mounting syrup on the second day, and inversion of algae in concentrated syrup (modification of Stevenson, 1984).

A minimum of 300 natural units of algae were identified and enumerated to the lowest taxonomic level for diatoms and non-diatom algae. For those species with dominance in one treatment but with less than 10 units in another treatment, counting continued until the counts of each species reached 30 units. The number of fields of view was recorded separately for successively longer counts to determine cell densities. Counts were performed at 1000× on a Leica DMLB light microscope. Also light microscopy was used for determining algal biovolumes. Twenty-five cells of each species were measured, which was excepted to reduce the standard error (SE) in species biovolume estimates to less than 10% of the mean (Hillebrand et al., 1999). Species with cell numbers greater than 30 cells in any one sample or with average cell count greater than 1% in all samples were selected for analysis and

designated as “common species”.

Data analysis

Periphyton biomass on the tiles was estimated as area-specificdensities of chlorophyll (µg chl *a* /cm^2) and cell volumes (µm^3 biovolume /cm^2) (Stevenson et al. 1996). Accumulation rates of biomass were calculated using the following equation:

Biomass accumulation = (chl a_2 – chl a_1) / ($t_2 – t_1$)

where t was day of colonization and both chl *a* and t were estimated at two times. Growth rates were estimated as the daily per capita changes in benthic algal cells (Stevenson et al., 1996) in each channel and for each colonization period between successive sampling times using the following equation:

$\mu = (\log_e B_2 - \log_e B_1) / (t_2 - t_1)$

where B is biovolumes of species and t is again the time.

Effects of temperature on biomass were tested by one-way analysis of variance (ANOVA) using a significance level of 0.05 in SPSS v. 17.0. Polynomial regression analysis was conducted to develop predictive models of the influence of temperatureon periphyton biomass. The significance of temperature coefficients and model regression were tested at a 0.05 significance level.

The Shannon-Wiener Diversity Index (H') was calculated to characterize communities (Stevenson et al., 1996) for the six temperature treatments using the following equation:

$$H' = -\sum_{i=1}^{S}(p_i \ln p_i)$$

where S is the number of species; p_i is the relative abundance of each species, calculated as

the proportion of individuals of a given species relative to the total cell number of individuals of all species: n_i/N, where n_i is the cell number of individuals in species i and N is the total cell number of all individuals.

In order to examine general temperature effects on community taxonomic composition, non-metric multidimensional scaling (NMDS) of the square-root transformed biovolume of common taxa was analyzed using R (R-2.9.2). Non-metric multidimensional scaling (NMDS) creates a geometric configuration of distances among samples based on differences in species composition. The Bray-Curtis coefficient was used to produce a similarity matrix among species for different temperature treatments. The similarity matrix was ordinated using NMDS to visualize patterns in the community composition. The degree of reliability of the NMDS plot was indicated by a stress value. Stress values less than 0.05 give an excellent representation with little distortion of the data (Clarke and Warwick, 1994). Algal species were aggregated into four general groups based on NMDS distances and a histogram of group biovolume in different treatments was produced to visualize the taxonomic composition shifts among temperature treatments.

RESULTS

Experimental stream conditions

Temperatures in channels varied little around initial treatment levels throughout the experimental period (Fig. 1). Treatments with maximum temperature variation were found to be 38 ± 1.5 ºC (SE), followed by 16 ± 0.9 ºC (SE). The remaining variations in temperature were low, with an SE less than 0.67 ºC. According to the results of biomass, community composition and species response, 13.5 and 16 ºC were classified as low temperatures, 23.5 and 27 ºC were classified as intermediate temperatures, and 33 and 38 ºC as high

temperatures.

Total nitrogen and TP were both saturating for periphyton growth throughout the experiments with minimum concentrations of 100 μg/L and 60 μg/L, respectively (Fig. 2-A, Fig. 2-B). Total nitrogen concentration was saturated for periphyton growth in all treatments within colonization periods. Total phosphorus continually increased until the day 5, after which the concentration declined rapidly because of the exponential growth of periphyton. Among treatments, low temperatures had the highest concentration of phosphorus. The minimum silica concentration was 4 mg/L (Fig.2-C). Silica concentration in the 38 ºC treatment maintained at a roughly constant level, which was dominated by cyanobacteria dominance. In all the other treatments, silica concentration decreased obviously after day 4, which was associated with diatom dominance in those treatments. For intermediate temperature treatments, although silica decreased most severely and reached the lowest level among treatments, periphyton growth in those treatment was not limited by silica because the silica concentration leveled off after day 6. Moreover, conductivity was within the intermediate range of values (Fig. 2-D) and was assumed to affect periphyton growth in a similar way. The Red Cedar River was also within the intermediate range with value of 518 μS/cm in May.

°C

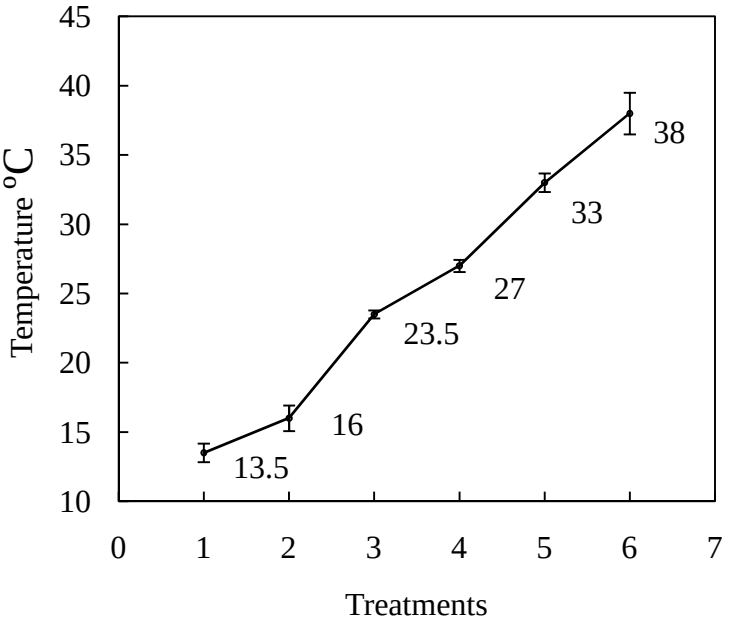
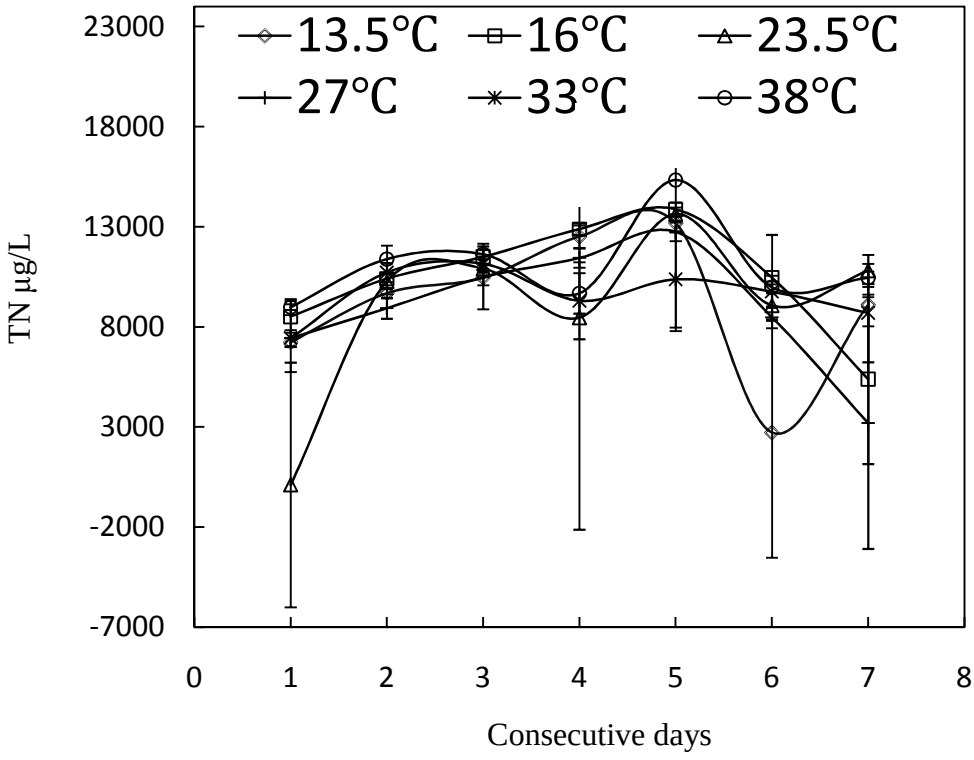


Figure 1: Temperatures over the experiment across the 6 treatments (triplicates of 18 artificial channels). Error bars show standard error of the mean.

Figure 2: Nutrient concentrations and conductivity in the artificial streams during the experiments.



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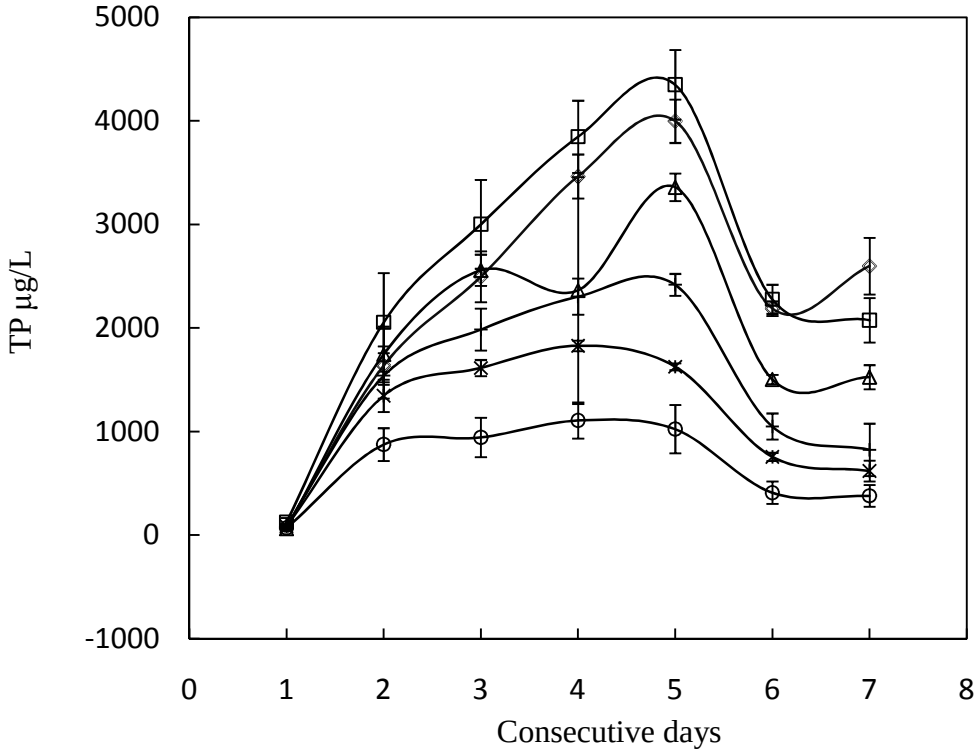

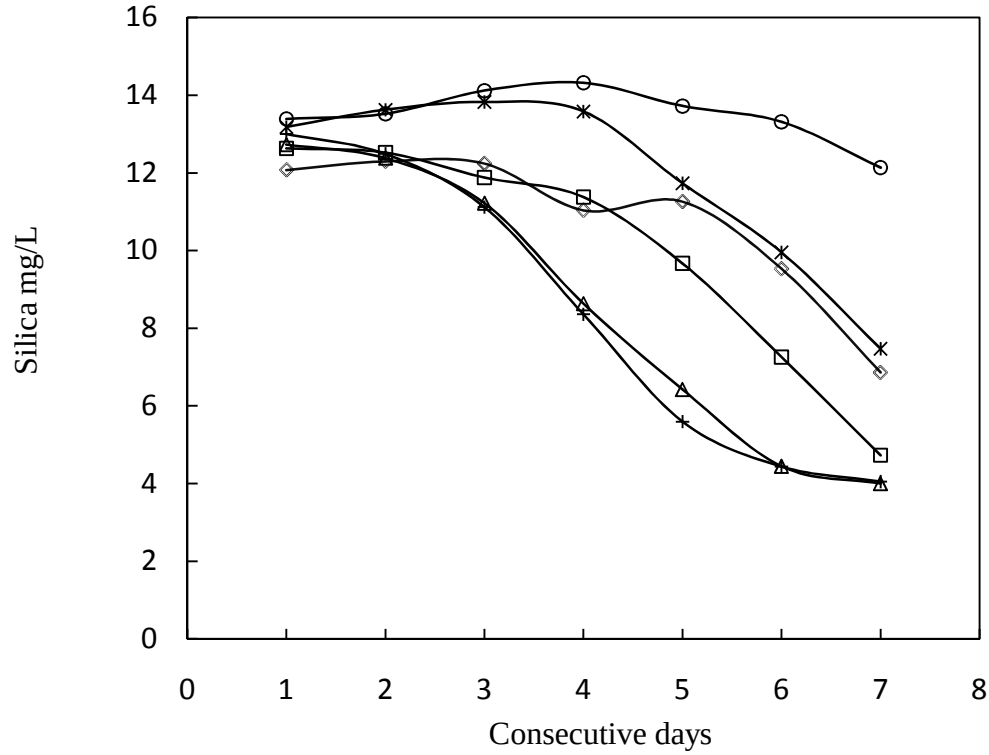

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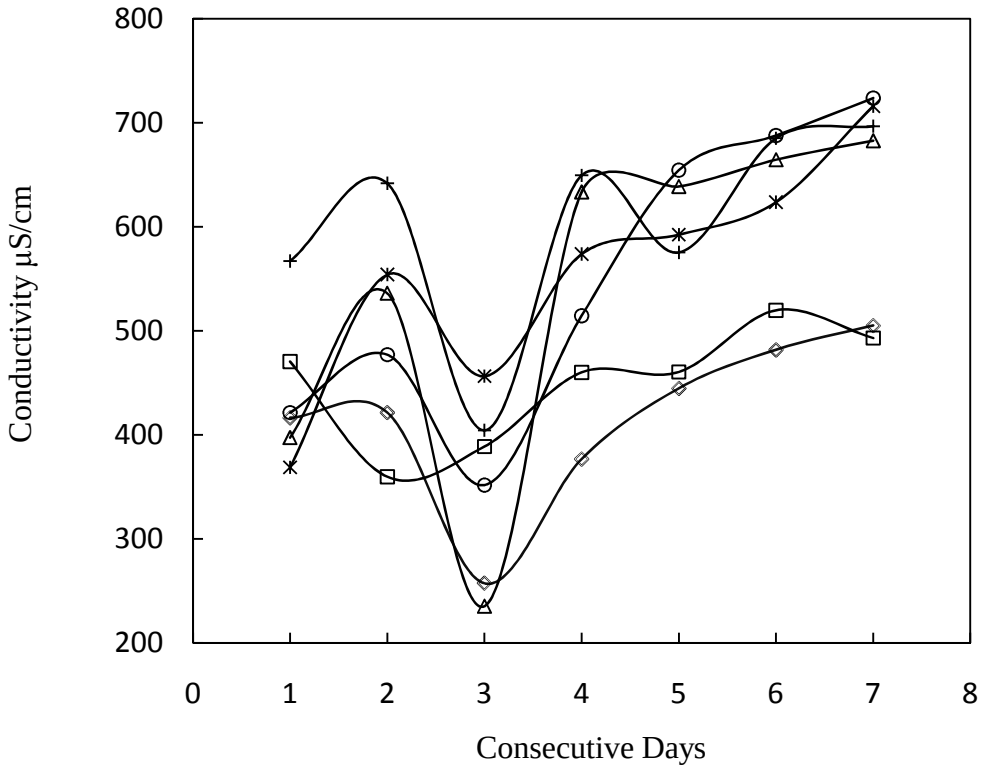


Biomass development during colonization periods

Variability in algal biomass development was great during colonization periods. Accumulation of periphyton biomass went through five different phases during colonization (Fig. 3). In the early period, which was also considered an adaptation period or immigration period, biomass did not change much through time. Then there was an exponential growth period. In some treatments, peak biomass, sloughing and recovery periods were also observed. The timing and occurrence of these phases varied among temperature treatments. The intermediate temperature treatments had an early exponential growth phase occurring between day 3 and day 4 followed by sloughing and recovery. Both low and high temperature treatments had longer adaptation periods than the intermediate temperature, with exponential growth between the day 5 and day 6. During the exponential growth period in the low and intermediate temperature treatments, the corresponding total phosphorus concentration

dropped dramatically from 4500 µg/L to 2000 µg/L and silica dropped from 12 mg/L to 4 mg/L (Fig. 2). The 38 ºC treatment had the longest adaptation period, followed by exponential growth and no apparent peak biomass period.

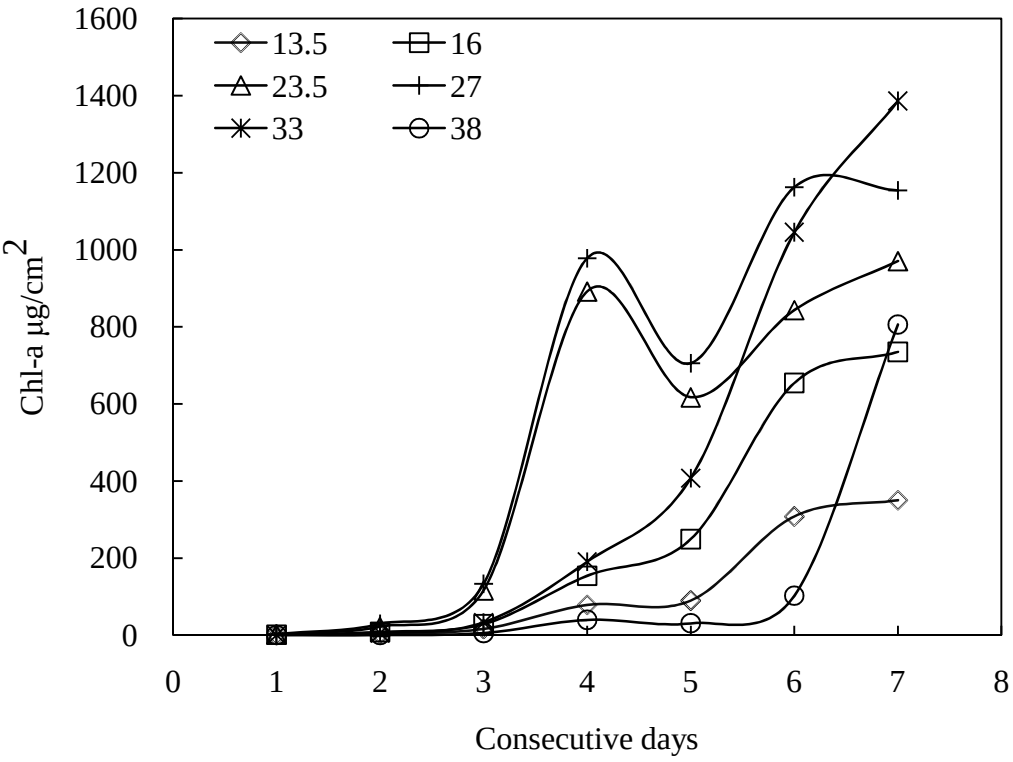


Figure 3: Average chlorophyll -a for six temperature treatments during the experiments.

To better understand the process of periphyton biomass development, accumulation rates over the first 7 days were calculated at daily intervals (Fig. 4). In the intermediate temperature treatments (23.5 and 27 ºC), the accumulation rates reached peaked on day 4, and became negative on day 5, indicating early peak biomass followed by sloughing phases. For low and high temperature treatments, the curve showed a gradual increase in accumulation rate before day 6, delaying the exponential growth period in those treatments. After day 6, the accumulation rates began to decrease but were not negative, and thus the biomass leveled off (Fig. 3). In the 38 ºC treatment, the biomass increased through time but the increases were not considerable until day 6. This treatment did not level off at a peak biomass (Fig. 3).

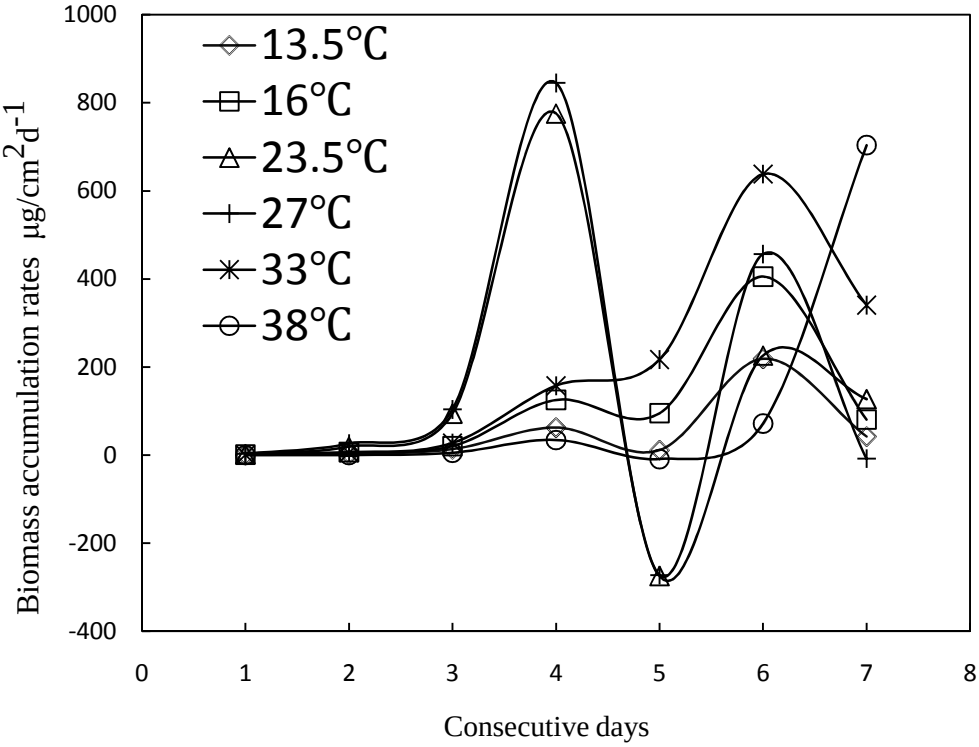


Figure 4: Chlorophyll-a (algal biomass) daily accumulation rates across the experimental temperature gradient.

Biomass -temperature relationship

The algal biomass densities among temperature treatments were significantly different (pairwise ANOVA, $p < 0.039$). Chlorophyll-*a* varied from 0.2 to 1386 µg/cm^2. It took 5 days for the diatom-dominated periphyton community to reach the biomass of 1000 µg/cm^2 at 27 ºC and one week for the cyanobacteria-dominated periphyton community to reach 1386 µg/cm^2 at 33 ºC. The relationship between chl -*a* and temperature was unimodal for all 7 consecutive days with peak production in treatments ranging from 27 to 33 ºC (Fig. 5). Periphytic biomass was usually lower in low and high temperature treatments compared with the intermediate temperature treatments. Polynomial regression model revealed a significant unimodal relationship between chl-*a* and temperature with P-values < 0.1 for the model and < 0.05 for the temperature coefficients (Table 1) for each of the 7 days of the experiment. The

model explained 79-98% of the variation in biomass. The intermediate and high temperatures had stronger temperature enhancement on periphytic biomass development than low temperatures, as indicated by the significantly higher β_1 values in the polynomial model.

Optimal temperatures with maximum production increased over the course of the colonization period. From the day 1 to day 5, the optimum was between 25 to 27 ºC, while after day 5, the maximum biomass developed in successively higher (33 and 38 ºC) temperature treatments. According to the polynomial regression model, the optimal temperature was around 25 ºC from the day 1 to day 5, and shifted to 33 ºC on the day 6 and day 7.

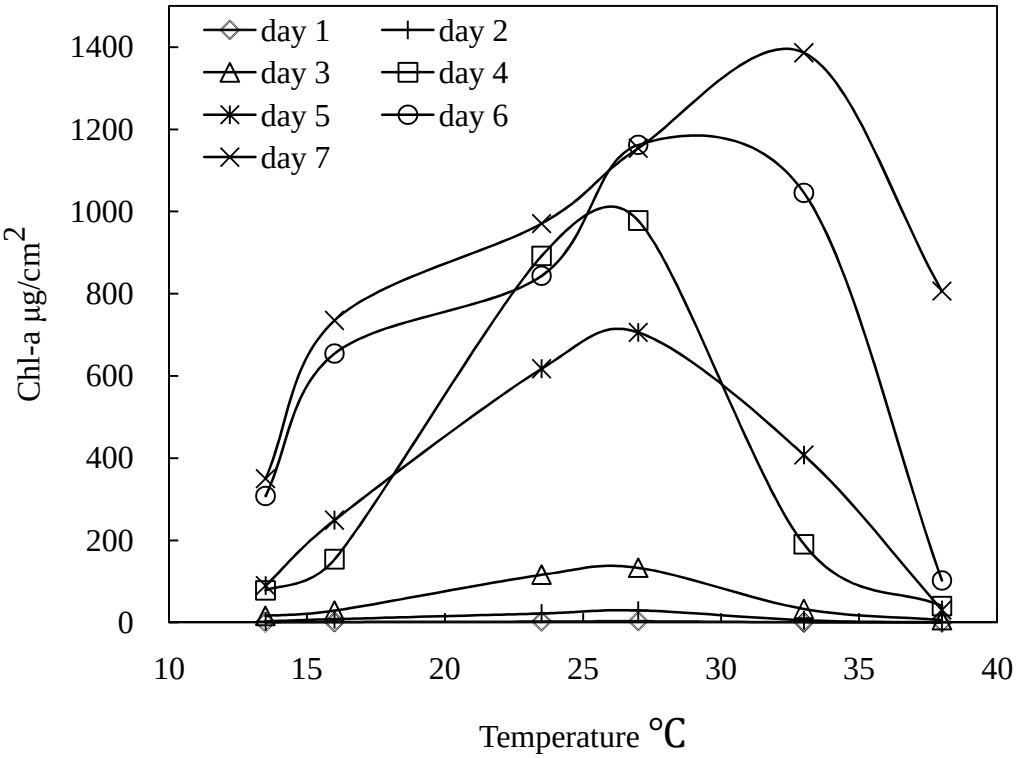


Figure 5: Chlorophyll-a concentration across the different temperature treatments on each of the 7 days.

Table 1: Polynomial regression results for chl-*a* – temperature relationship for each of the 7 days of the experiment.

days	coefficients			R-square	p-value				optimal
	constant	T	T^2		constant	β_1	β_2	model	T
1	-7.35	0.779	-0.016	0.789	0.079	0.047	0.063	0.097	25.1
2	-75.4	7.84	-0.156	0.816	0.061	0.038	0.031	0.079	25.1
3	-369	38	-0.751	0.839	0.048	0.03	0.001	0.064	25.3
4	-2899	293	-5.76	0.809	0.058	0.039	0.038	0.084	25.4
5	-2021	210	-4.1	0.984	0.002	0.001	0.029	0.002	25.6
6	-2593	290	-5.68	0.831	0.062	0.032	0.036	0.07	25.5
7	-1735	205	-3.55	0.843	0.101	0.048	0.045	0.062	28.9

Community response

Thirty-two species were present in the periphytic algal community based on microscopic observation of samples, including twenty-five diatoms, four cyanobacteria and three green algae. According to the Shannon-Wiener Diversity index H' (Table 2), community richness decreased dramatically from H'=2.55 at 13.5 ºC to H'=0.57 at 33 ºC. The diversity index increased slightly to 1.35 at 38 ºC.

ANOVA showed there was no significant difference in diversity within low temperature treatments, intermediate temperature treatments, or high temperature treatments, but diversity was significantly lower in intermediate temperature treatments than low temperature treatments (P=0.073), and significantly lower in high temperature treatments than intermediate temperature treatments (P=0.024). When temperature jumps from intermediate to high temperatures, the community lost 45% of the species; whereas from low to intermediate temperatures, the community lost 29% of the species.

Strong evidence of community shifts with temperature treatments was evident from NMDS ordination and proportional changes in biovolumes of functional groups based on 18 common species (Fig. 6, Fig. 7). The NMDS analysis had a low stress value, 0.0027, which indicated a good classification of species. Cluster 1 included *Navicula lanceolata*, *Navicula*

gregaria, Fragilaria crotonensis, Nitzschia acicularis, Surirella minuta, Coccoids sp.1 and Coccoids sp.2. Cluster 2 included *Nitzschia palea, Nitaschia capitellata, Nitzschia dissipata, Thalassiosira weissflogii, Synedra ulna* and *Cyclotella meneghiniana*. *Pseudanabaena sp., Anabaena sp.* and *Phormidium sp.* were the third cluster. Also comparison of functional groups of periphyton in terms of percent biovolume showed distinctness between six temperature treatments. The proportional contribution of different clusters to total biovolume was highly affected by temperature. Seven of 14 species dominated with great evenness in low temperature treatments. The senven species, which were diatom species from cluster 1, all contributed 10-20% to the community. Thus cluster 1 was called low-temperature diatoms. In contrast, the intermediate temperature treatments were composed of so-called intermediate temperature diatom species from cluster 2, and species were much less evenly distributed. *Nitzschia palea* and *Synedra ulna* were the two most abundant species with contributions of 30-40% to the community. *Navicula lanceolata, Nitzschia acicularis, Navicula gregaria* and *Surirella minuta*, which were all cluster 1 species, were more or less suppressed above 23.5 ºC. Green Coccoids spp. had proportions about 5%-10% across low to intermediate temperature treatments and lost importance at high temperatures. Cyanobacteria gained importance in high temperature treatments. *Leptolyngbya sp.* prevailed and accounted for 83% of the taxa at 33 ºC, followed by *Nitzschia palea* contributing 14%. At 38 ºC, *Leptolyngbya sp.* and *Pseudanabaena sp.* co-dominated the community with proportions of 54% and 26.9%, respectively.

Table 2: Shannon-Wiener Diversity Index (H') for six temperature treatments.

Temperature treatments	H'
13.5	2.55
16	2.44
23.5	1.91
27	1.59
33	0.57
38	1.35

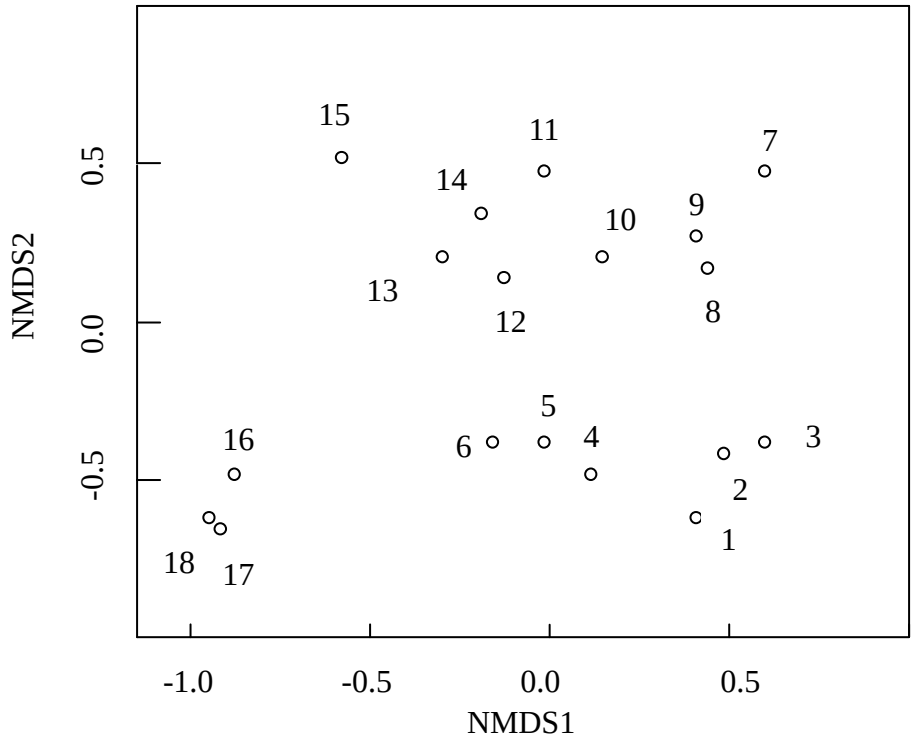


Figure 6: NMDS ordination of Bray-Curtis similarities from square-root transformed cell biovolumes for each species in the peripyton community for six temperature treatments on the day 3. 1: N. lanceolata; 2: S. minuta; 3: N. gregaria; 4: F. crotonensis; 5: Coccoid sp.1; 6: Coccoid sp.2; 7: T. weissflogii; 8: N. aciularis; 9: M. varians; 10: C. meneghiniana; 11: S. ulna; 12: N. capitellata; 13: N. dissipata; 14: N. palea; 15: Leptolyngbya spp.; 16: Pseudanabaena spp.; 17: Anabaena spp.; 18: Phormidium spp.

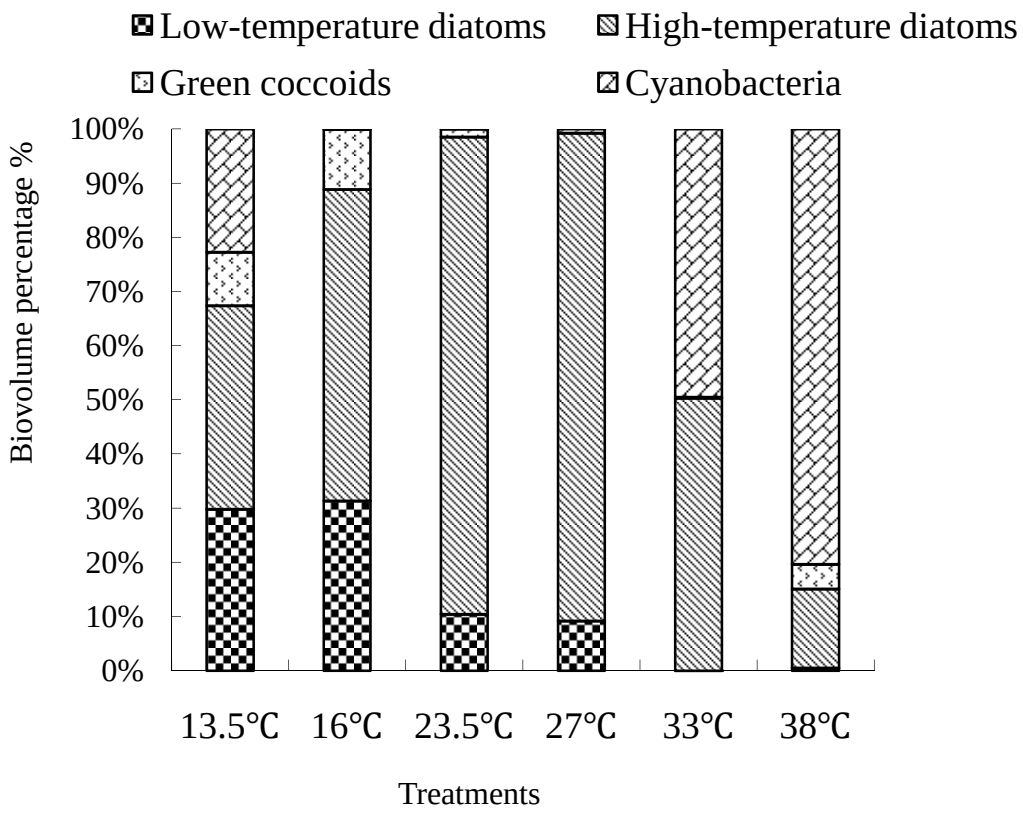


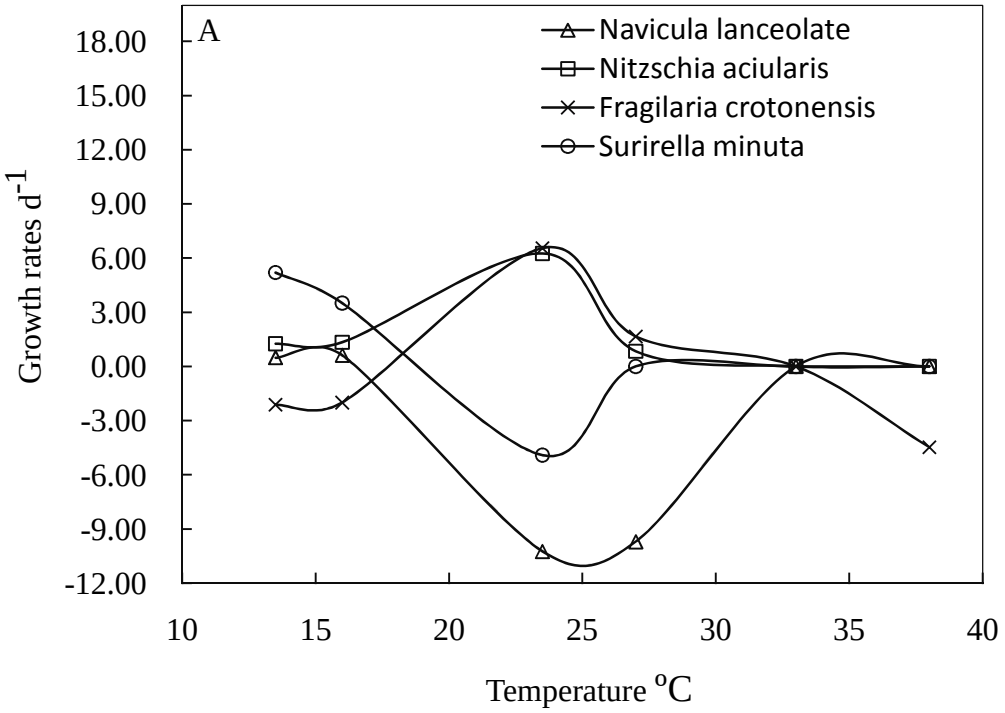
Figure 7: Percentage of biovolume of 18 common species among different temperature treatments on day 3.

Species growth rates response to temperature variation

Analysis of growth rates showed that different species had different growth rates among temperature treatments. The specific growth rates during exponential growth (μ_{max}) from day 3 to day 5 for the 18 common species are listed in Table 3. Low-temperature diatoms showed two patterns of the growth rate-temperature relationship (Fig.8-A). In the case of *N. Lanceolata* and *S. minuta*, growth rates were negatively affected by temperature and negative values occurred above 17.5 ºC. In contrast, growth rates of *N. acicularis* and *F. crotonensis* first increased with temperature and then decreased above 23.5 ºC. Overall the low-temperature diatom species had no or negative growth rates beyond 23.5 ºC. Intermediate temperature diatom species *N. palea, S. ulna, N. capitellata* and *N. dissipata*

maintained growth rate between 1 and 1.5 d^{-1} in low to intermediate temperature treatments (Fig.8-B). Only high temperature exerted negative effects on these intermediate temperature species. Green Coccoids spp. increased slowly in growth rate with temperature up to 27 ºC, and then above that, their growth rate decreased dramatically (Fig.8-C). For blue-green algae, the pattern was complex, especially for *Leptolyngbya* (Fig.8-D). *Leptolyngbya* grew poorly at low temperature. Since low and high temperature diatom and green algae grew actively within range from 13.5-23.5 ºC, *Leptolyngbya* hardly survived until the temperature was up to 27 ºC. When temperature reached 33 , *Pseudanabaena sp., Anabaena sp.* and *Phormidium sp.* had high growth rates of 3 to 6 d^{-1}, and thus suppressed *Leptolyngbya sp.* However, *Leptolyngbya sp.* was the dominant species in the high temperature treatments where other species were absent.

Figure 8: Growth rate for 18 common species along temperature gradien. A: low temperature diatom species; B:intermediate temperature diatom species; C: green coccoids; D: cyanobacteria



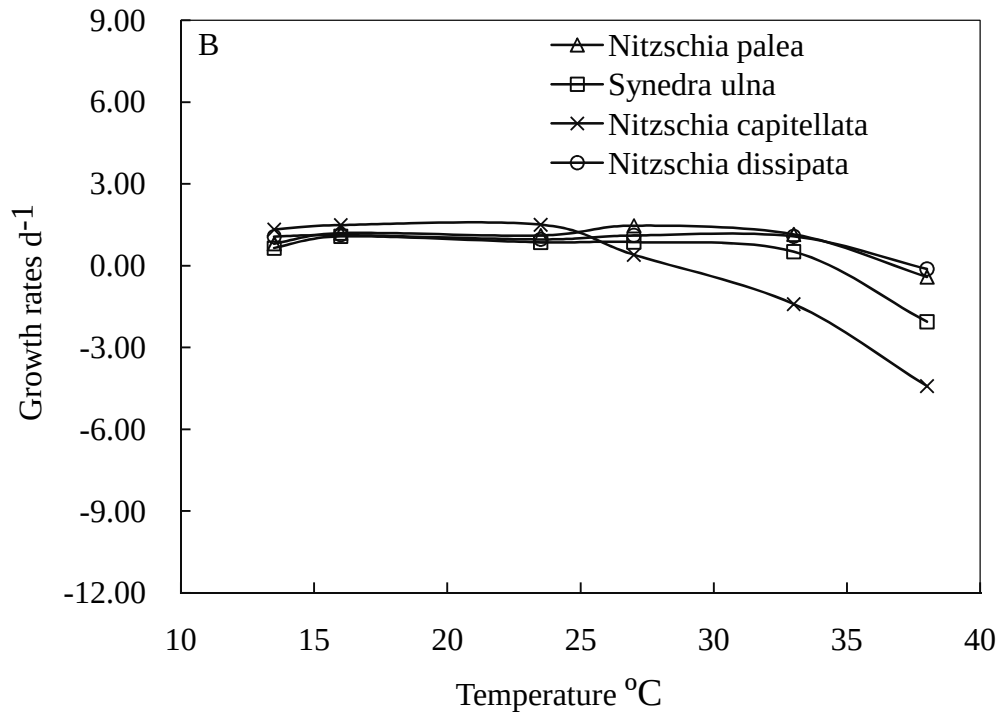
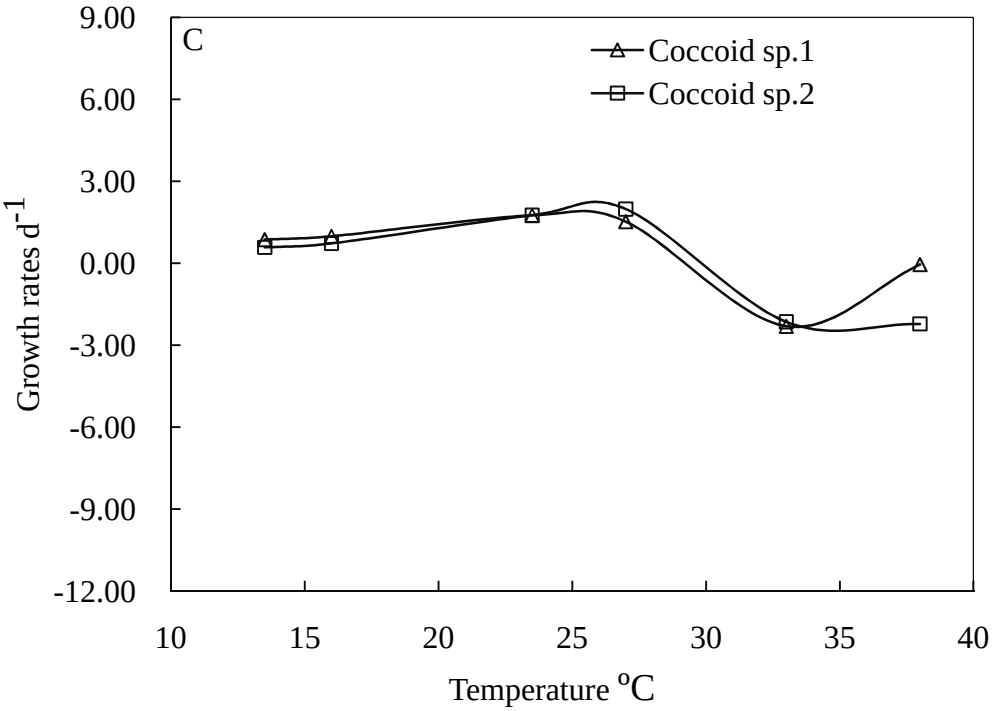


Figure 8 continued


C
Coccoid sp.1
Coccoid sp.2
9.00
6.00
3.00
0.00
-3.00
-6.00
-9.00
-12.00
10
15
20
25
30
35
40
Growth rates d-1
Temperature oC

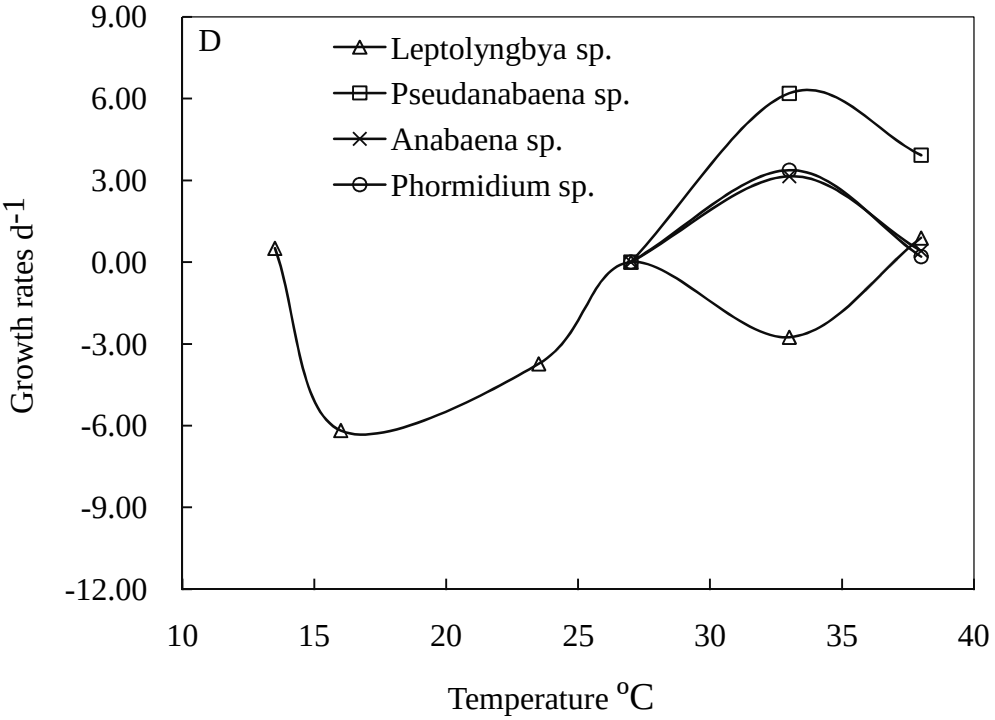

D
Leptolyngbya sp.
Pseudanabaena sp.
Anabaena sp.
Phormidium sp.
9.00
6.00
3.00
0.00
-3.00
-6.00
-9.00
-12.00
10
15
20
25
30
35
40
Growth rates d-1
Temperature oC

Table 3: Values of growth rate (d^{-1}) obtained in artificial streams for 18 common periphytic species from 13.5 to 38 ºC. Growth rates were determined from day 3 and day 5, and were averaged over the experiment.

Species name	Temperature ^{o}C					
	13.5	16	23.5	27	33	38
C.meneghiniana	0.74±0.09	0.87±0.41	1.29±0.05	1.65±0.20	-1.77±2.64	-6.81±0.26
F. crotonensis	-2.12±3.21	-2.00±3.03	6.55±12.08	1.66±10.10	0	-4.48±2.28
M. varians	0.63±0.37	1.55±0.33	0.85±0.59	6.92±5.91	-2.69±2.69	0
N. gregaria	0.16±0.37	0.26±0.26	0.46±0.11	5.82±5.30	0	0
N. lanceolate	0.48±0.07	0.61±0.07	-10.24±5.14	-9.71±5.39	0	0
N. aciularis	1.26±0.24	1.34±0.24	6.26±5.43	0.84±1.26	0	0
N. capitellata	1.32±0.26	1.49±0.12	1.50±0.49	0.40±0.69	-1.41±3.17	-4.41±1.99
N. dissipata	1.06±0.10	1.13±0.13	0.96±0.14	1.11±1.24	1.07±0.24	2.12±0.44
N. palea	0.81±0.25	1.19±0.10	1.11±0.42	1.47±0.50	1.15±0.20	-0.41±0.49
S. minuta	5.2±2.50	3.51±2.44	-4.91±5.14	0	0	0
S. ulna	0.64±0.12	1.07±0.08	0.85±0.44	0.86±0.52	0.51±0.30	-2.06±2.19
T. weissflogii	2.86±2.19	0.67±0.34	-4.72±4.89	-4.80±5.19	0	0
Coccoid sp.1	0.86±0.28	0.98±0.43	1.75±0.58	1.52±0.22	-2.31±2.47	-0.05±0.3
Coccoid sp.2	0.58±0.23	0.72±0.29	1.75±0.23	1.98±0.34	-2.15±2.70	-2.22±2.65
Anabaena sp.	0	0	0	0	3.15±3.15	0.41±0.31
Leptolyngbya sp.	0.51±1.06	-6.18±0.19	-3.73±3.73	0	-2.76±3.81	0.89±0.18
Phormidium sp.	0	0	0	0	3	0
Pseudanabaena sp.	0	0	0	0	6.19±2.82	3.92±2.78

Strong temperature enhancement was observed over different temperature intervals for different species (Table 4). Low temperature diatoms had negative Q_{10}. For example, *N. lanceolata* had Q_{10}= -21.48 when temperature increased from 13.5 to 23 ºC. Intermediate temperature diatoms and green coccoids had positive temperature coefficients within the low to intermediate temperature range, while their Q_{10} was negative at high temperatures. In contrast, *Leptolyngbya sp.* had the opposite result with negative Q_{10} at low temperatures and positive Q_{10} at high temperatures.

Table 4: Temperature coefficient (Q_{10}) for common species .

Species		Q_{10}			
		23.5/13.5 ^{o}C	27/16^{o}C	33/23.5 ^{o}C	38/27 ^{o}C
Low temperature diatoms	*N.lanceolate*	-21.48	-15.82	0.00	0.00
	S. minuta	-0.95	0.00	0.00	N/A
	N. aciularis	4.98	0.63	0.00	0.00
	F. crotonensis	-3.09	-0.83	0.00	-2.70
Intermediate temperature diatoms	*N. palea*	1.37	1.23	1.04	-0.28
	S. ulna	1.33	0.80	0.60	-2.39
	N. capitellata	1.14	0.27	-0.94	-11.08
	C. meneghiniana	1.74	1.89	-1.37	-4.13
	M. varians	1.35	4.47	-3.15	0.00
	N. dissipata	0.91	0.98	1.11	-0.11
Green coccoids	*Coccoid sp.1*	2.02	1.54	-1.32	-0.03
	Coccoid sp.2	3.04	2.75	-1.22	-1.12
Cyanobacteria	*Leptolyngbya sp.*	-7.33	0.00	0.74	N/A

DISCUSSION

Results summary

Temperature elevation had strong but different effects on species growth, which produced

shifts in community composition and changes in biomass. Elevated temperature eventually cause a shift to cyanobacteria from diatoms, which is exemplified by growth rates of several species in each functional group (i.e., low temperature diatoms, intermediate temperature diatoms and cyanobacteria). Increasing temperatures generate low growth rates and negative Q_{10} for many diatom species while promoting fast growth and positive Q_{10} for cyanobacteria (Table 3, Table 4, Fig. 8). The shift to cyanobacteria with warming is believed to reflect the competitive advantages of cyanobacteria associated with higher growth rates at high temperatures in streams.

In addition, periphyton diversity decreased dramatically with temperature. If temperature increased from low to intermediate, or from intermediate to high temperature range, the community diversity decreased severely. Within the tolerable range, periphyton co-exist with equitable species evenness and less changes in diversity.

According to my results, the periphytic biomass peaked earlier at the intermediate temperatures than the low or high temperatures. However, later during colonization periods, an even larger peak biomass occurred at high temperature (Fig. 3). The shift in the optimal temperature and the different timing of maximum biomass were results of initial community compostion and different species-specific growth rates. Since the Red Cedar River stayed around 15 ºC when I sampled periphyton, cyanobacteria were likely relatively rare and the initial community was mostly composed of low to intermediate temperature diatoms and green coccoids. Diatom taxa are capable of rapid colonization (Biggs, 1996). As a result, diatoms quickly colonized in the experimental channels and created the early peak biomass, while cyanobacteria spent longer time in immigration and adaptation period before they grew fast to create the peak biomass late in the experiment. In addition, the cyanobacteria-dominated community generated a larger peak biomass at high temperatures than the diatom-dominated community did at intermediate temperatures because

cyanobacteria had higher growth rates than diatoms at their own optimal temperatures. Therefore the shift to cyanobacteria at high temperatures may enhance the total production of algal biomass in streams.

Based on the previous discussion, the initial community composition may influence the biomass response and community response to temperature in the same habitat, but I will argue that the influence of initial community composition is restricted to time scale. The unimodal relationship between biomass and temperature, and the fact that community shift from diatoms to cyanobacteria will not change because the photosynthesis-temperature relationship and the species-specific tolerance range for growth rate are the main reasons for biomass and community response, which are not changed by the initial community.

Comparison between periphyton and phytoplankton

Comparison between my results and other studies in phytoplankton shows that temperature exerts different effects on periphyton and phytoplankton. A previous study using batch cultures suggested that the most marked enhancement of growth rate for phytoplankton occured in the range from 8 to17 ºC (Butterwick, 2005). In the present study of periphyton, the differences in growth rates were most pronounced at intermediate to high temperature, in the range from 23 to 33 ºC. Another study focusing on phytoplankton using batch cultures (Dauta, 1990) found that optimal temperature ranged from 27 to 35 ºC in cyanobacteria, but were lower than 20 ºC in diatoms. I got higher optima for periphyton than phytoplankton on average. The optimal temperature was around 33 ºC and ranged from17.5 to 23 ºC for periphytic cyanobacteria and low-temperature diatoms, respectively. There was no obvious optimum for intermediate temperature diatoms, which maintained relatively constant growth rates as temperature increased up to 33 ºC. In addition, Morin et al. (1999) reported that stream periphyton growth (Q_{10}=2.5) was more strongly correlated with temperature than

ocean phytoplankton (Q_{10}=1.2) and lake phytoplankton (Q_{10}=1.4) based on field observations. The authors speculated that the differences could result from differences in colinearities between temperature and other factors (i.e., light, nutrients, inorganic carbon) affecting algal growth. In my study, a temperature coefficient greater than 2 was observed for five species and was not restricted to a single functional group, which supported the idea that periphyton growth was more strongly related to temperature, and thus I would argue that this phenomenon might have little to do with confounding effects of environmental factors because temperature was the only factor influencing algal growth in my experiment. Besides, the widespread occurrence of some phytoplankton (e.g. *C. marssonii*, Butterwick et al. 2005) indicated an insensitivity of growth over the temperature range. The width of the temperature-insensitive range can be up to 10 ºC for phytoplankton (e.g. *Asterionella*, *Cryptomonas*) (Butterwick, 2005). However, according to my results, the relationship between temperature and growth rates varied and temperature always exerted a significant effect on growth rates, except that some intermediate-temperature diatoms had relatively constant growth rates over the temperature range up to 13.5 to 27 ºC. Overall, this comparison leads to the conclusion that stream periphyton have higher temperature optima and stronger relationships with temperatures than phytoplankton.

Implications for algal growth in natural streams and other aquatic ecosystems

Considering the fact that most rivers and streams in Michigan have low temperatures, around 10 ºC in spring and fall, and above 20 ºC in summer (USGS data 2007-2009), which are lower than the optimal temperatures (i.e., from 25 to 33 ºC), I will argue that climate changes will provide a chance for both diatom and cyanobacteria to approach optimal temperatures for production. Besides, a field study indicated that algal blooms occurred

earlier in years of higher mean annual air temperature (Marshall and Peters, 1989). Since the periphytic biomass increases with temperature up to the optimal temperature, I will expect that a slight increase in temperature will trigger an earlier periphyton bloom in spring.

Similar to the situation in lakes, in which hydraulic retention time (i.e. flushing rate) was a critical factor determining phytoplanktonic algal biomass (Paerl and Huisman, 2008; Elliott 2010), periphyton biomass is affected by colonization periods. With the dominance of diatoms in the initial community, short colonization periods lead to diatom dominance in the community. In other words, two requirements are essential for cyanobacteria dominance in streams: one is a considerable initial high density of cyanobacteria and the other is a sufficiently long colonization period.

According to my results, as temperature increases, the risk of losing native species increases. However, on the other hand, low initial diversity of cyanobacteria could generate a low diversity at high temperatures. Since new species were prevented from entering the experimental streams, I could not evaluate impacts of high temperatures on natural streams that likely have a greater range of taxa to colonize the benthic surfaces.

Also the low and intermediate temperature treatments had high levels of TP and low levels of silica while the high temperature treatments had an opposite situation with low levels of TP and high levels of silica. The variation in nutrients was due to different community composition associated with different nutrient demands. Thus the community shifts in periphyton will cause changes in biogeochemistry of streams.

The results from the present study may be applied to periphyton in other aquatic ecosystems, like wetlands, but there are some restrictions. Biomass will probably be unimodally related to temperature and community will shift from low-temperature diatoms, to intermediate-temperature diatoms, and to cyanobacteria as temperature increases. However, different kinds of species in each functional groups will be expected, which are determined

by habitat types. In contrast, we should extend the result of diversity-temperature relationship to other aquatic ecosystems with more caution. Decreased in diversity with temperature may to related to starting colonization with taxa adapted to low temperatures.

Implication of temperature effects on ecological function and seasonal variation

Temperature caused important impacts on ecological functions. Since most of stream grazers prefer diatoms over cyanobacteria (Lampert, 1987), food webs will be changed dramatically with functional groups shifting from diatoms to cyanobacteria. Benthic insect larvae and crustaceans which prey on diatom will be negatively impacted as well as the food weds they support. Thus the ecosystem services decrease. Furthermore, high richness of periphyton leads to structurally complex food webs with a high degree of connectedness. In contrast, low richness leads to short and simple food chains (Thompson and Townsend, 1999). Thus stream ecosystems may become unstable and be degraded by warming. Structural and functional attributes indicate that temperature effects are similar to those found in polluted streams (Chuang et al., 2009).

Our experimental work is notable to show some consistency with seasonal patterns of periphyton in streams. Rosemond (1994) found periphyton biomass was lowest in early spring and highest in summer. Biomass-temperature relationships derived from the present study is in agreement with the robust pattern observed in nature that elevated temperatures are required for high biomass. With regards to the community composition, Biggs (1996) found that late winter/early spring was characterized by the development of diatom-dominated communities (e.g., *Synedra, Navicula, Diatoma*) and communities often became dominated by cyanobacteria as summer started (e.g., *Phomidium, Homeothrix*). Patch growths of large filamentous algae, such as *Cladophora*, developed in late summer. Our experimental results also demonstrate similar community composition at the genus level, with

Navicula and *Synedra* at low temperatures, and *Phomidium* at high temperatures.

However, it should be stressed that these temperature effects do not necessarily take place in natural settings. The ability of periphyton to grow and prosper in streams is the outcome of a complex series of interactions between water quality, hydrological and biotic factors (Biggs, 1996). For example, grazers can consume increased biomass resulting in a lack of a relationship between biomass and temperature (Rosemond, 1994; Hillebrand and Kahlert, 2001). Many of these factors were not reproduced in the experimental streams.

CONCLUSION

In summary, over the range of 13.5 to 38 ºC, temperature had a significant direct effect on periphyton in terms of biomass, diversity, community composition and species- specific growth rate. Both the highest and lowest temperature adversely affected algal biomass development. Algae shifted from a low-temperature diatom dominated community to intermediate-temperature diatoms and finally to a cyanobacteria dominated community as temperature surpassed 35 ºC. Meanwhile the community lost species diversity. Temperature had a species-specific effect on growth rates. Currently Michigan streams are still within the optimal temperature range of periphyton growth, but climate change threatens to degrade stream ecosystems.

REFERENCES

REFERENCES

APHA 1998. Standard methods for examination of water and wastewater. Washington DC, American Public Health Association.

Agawin, N. S. R., C. M. Duarte, and S. Agusti, 2000. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnology and Oceanography 45: 591-600.

Bothwell, M.*L. 1989.* Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1293-1301.

Bouterfas, R., M. Belkoura and A. Dauta, 2002. Light and temperature effects on the growth rate of three freshwater algae isolated from an eutrophic lake. *Hydrobiologia* 489: 207-217.

Butterwick, C., S. I . Heaney and J. F. Talling, 2005. Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshwater Biology* 50: 291-300.

Chuang, Y. L., H. H. Yang and H. J. Lin, 2009. Effects of a thermal discharge from a nuclear power plant on phytoplankton and periphyton in subtropical coastal waters. *Journal of sea research* 61: 197-205.

Clarke, K. R. and R. M. Warwick, 1994. Changes in marine communities: An approach to statistical analysis and interpretation. Plymouth marine Laboratory, Plymouth.

Dauta, A., J. Devaux, F. Piquemal and L. Boumnich, 1990. Growth rate of four freshwater algae in relation to light and temperature. *Hydrobiologia* 207: 221-226.

Denicola, D. M., 1996. Periphyton responses to temperature at different ecological levels. Pages 149-181 in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe (editors). Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego.

Eppley, R. W., 1972. Temperature and phytoplankton growth in the sea. *Fishery and bulletin* 70: 1063–1085.

Elliott, J.A., 2010. The seasonal sensitivity of Cyanobacteria and other phytoplankton to changes in flushing rate and water temperature. *Global change biology* 16: 864-876.

Hillebrand, H., C. Durselen, D. Kirschtel, U. Pollingher and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Pycology* 35: 403-424.

Lampert, W., 1987. Laboratory studies on zooplankton-cyanobacteria interactions. *N.Z. J. Mar. Freshwater Res.* 21: 483-490.

Lassen, M.K., K. D. Nielsen, K. Richardson, K. Garde and L. Schlüter, 2010. The effects of temperature increases on a temperate phytoplankton community–A mesocosm climate change scenario. *Journal of Experimental Marine Biology and Ecology* 383: 79-88.

Lewandowska, A. and U. Sommer, 2010. Climate change and the spring bloom: a mesocosm study on the influence of light and temperature on phytoplankton and mesozooplankton. *Marine ecology-progress series* 405: 101-111.

McQueen, D. J. and D. R. S. Lean, 1987. Influence of Water Temperature and Nitrogen to Phosphorus Ratios on the Dominance of Blue-Green Algae in Lake St. George, Ontario. *Canadian Journal of Fisheries and Aquatic Science* 44(3): 598-604.

Paerl, H. W., and J. Huisman, 2008. Blooms like it hot. *Science* 320: 57-58.

Paul, V. J., 2008. Global warming and cyanobacterial harmful algal blooms. *Advances in Experimental Medicine and Biology* 619: 239-257.

Rier, T. S. and R. J. Stevenson. 2005. Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. *Hydrobiologia* 561:131-147.

Rosemond, a.d., 1994. Multiple factors limit seasonal-variation in periphyton in a forest stream. *Journal of the north american benthological society* 13: 333-344.

Rutherford, J. C., M. R. Scarsbrook, and N. Broekhuizen, 2000. Grazer control of stream algae: modeling temperature and flood effects. *Journal of environmental engineering* 126: 331-339.

Smayda, T. J., 1997. Harmful Algal Blooms: Their Ecophysiology and General Relevance to Phytoplankton Blooms. *Limnology and Oceanography* 42: 1137-1153.

Stevenson, R.J., 1984. Procedures for mounting algae in a syrup medium. Trans. Amer. Microsc. Soc. 103:320-321.

Stevenson, R. J., S. T. Rier, C. M. Riseng, R. E. Schultz and M. J. Wiley. 2006. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications fro developing nutrient criteria. *Hydrobiologia* 561: 149-165.

Thompson, R. M. and C. R. Townsend, 1999. The Effect of Seasonal Variation on the Community Structure and Food-Web Attributes of Two Streams: Implications for Food-Web Science. *Oikos* 87: 75-88.

Williamson, C. E., C. Salm, S. L. Cooke and J. E. Saros, 2010. How do UV radiation, temperature, and zooplankton influence the dynamics of alpine phytoplankton communities. *Hydrobiologia* 648:73-81.