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# Estimating the Frequency of Sexual Reproduction in the Diatom Stephanodiscus niagarae

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# Estimating the frequency of sexual reproduction in the diatom *Stephanodiscus niagarae*

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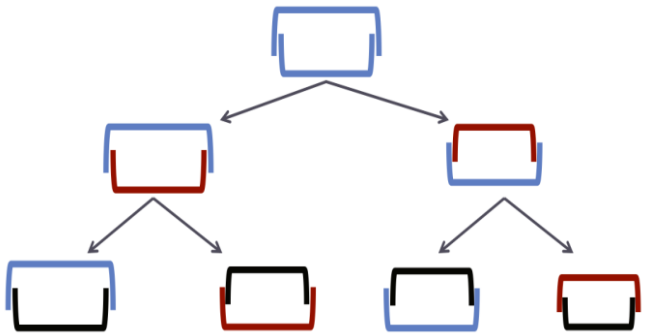
**Abstract**

Diatoms are single-celled micro-algae with cell walls composed of silica, that reproduce in a way that results in a decrease in cell size after each round of mitotic (asexual) division. A cell cannot continue to shrink indefinitely, so when the average size of the population reaches a critical threshold, diatoms reproduce sexually and restore their maximal size. It is unclear, however, how frequently diatoms undergo sexual reproduction in nature. As a proxy for sexual reproduction, I monitored changes in cell size of a population of the diatom *Stephanodiscus niagarae* from the plankton community of Lake Fayetteville (Arkansas, USA). Weekly sampling during the Fall 2015-Spring 2016 period revealed that *S. niagarae* is a winter species, occurring after a shift in the planktonic community of Lake Fayetteville, in which populations of warm-water green and blue-green algae decline and are replaced by a diatom-dominated community. *S. niagarae* first occurred in December, in clear colder water at 10 $^oC$ or below. Within weeks, this population grew steadily from a few hundred to several thousand cells per milliliter, reaching a maximum density in late January. The population declined rapidly thereafter. Although this decline coincided with increased lake temperature and the proportion of cells infected by a chytrid parasite, it is not clear if these two factors are the proximal cause for *S. niagarae* declines. Asexual reproduction resulted in substantial variation in average cell size, and an increase in variance towards the end of the growth period. However, the modest variation in median cell diameter of about 5 $\mu m$ indicated that a synchronous population-wide sexual reproduction event did not occur during the course of the study. These results suggest that the frequency of sexual reproduction in *S. niagarae* in Lake Fayetteville might be on the order of several to dozens of years. However, the possibility of asynchronous sexual reproduction and a population with overlapping generations cannot be ruled out.

# Introduction

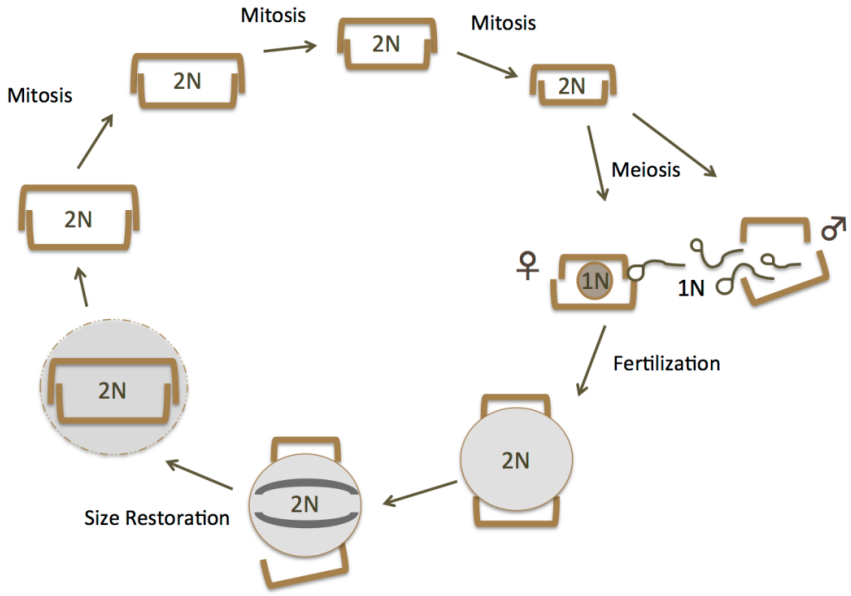
All species reproduce, whether it is sexually or asexually. Bacteria reproduce strictly asexually, and with very few exceptions, eukaryotes reproduce sexually. Sexual reproduction requires considerable energy and allows an organism to contribute only half of its genetic material to its offspring. The benefits of sexual reproduction, however, are thought to far outweigh the costs. For example, recombination during meiosis creates novel combinations of alleles, some of which may be beneficial to the organism. Recombination during meiosis also allows organisms to rid themselves of deleterious mutations while preserving beneficial ones (Song et al., 2012). This is not possible in strictly asexual species, resulting in a phenomenon known as Muller's Ratchet, in which a population is predicted to eventually suffer a 'mutational meltdown' after the irreversible accumulation of numerous deleterious mutations over time (Roach & Heitman, 2014). Thus, each time sexual reproduction takes place, a genetically unique set of offspring is created. Some of these offspring may have allele combinations that better suit them to new and changing environmental conditions, essentially allowing sexually reproducing organisms to evolve more rapidly than their asexual counterparts. Parasites constitute one part of an organism's environment, which is broadly defined. In the continual 'arms race' between a parasite and its host, sex allows a host species to adapt more quickly to constant pressure from parasites. This hypothesis, known as the Red Queen Hypothesis, is considered one of the best-supported explanations for the origin and maintenance of sexual reproduction (Van Valen, 1973). Mathematical models of the Red Queen effect suggest that the adaptive edge conferred by sex will cause asexual individuals to become rare in a parasitized population, while sexual individuals become more common – a prediction that has been supported by empirical data (Lively, 2010).

Figure 1: The characteristic change in cell size as diatoms undergo a series of mitotic cell divisions. A cross-section of bipartite diatom cell wall – similar to a petri dish – is shown. Each daughter cell receives one-half of the parent cell wall and must fabricate the smaller half *de novo*, resulting in one daughter cell that is equal in size the parent and one that is slightly smaller.



Diatoms are single-celled, typically planktonic algae, with cell walls composed of silica. This silica cell wall causes diatoms to reproduce in a distinctive way (Fig. 1). Namely, constraints imposed on the architecture of their inorganic cell wall causes the population to decrease in size after each round of asexual reproduction. Each mitotic division results in one cell that is the same size as the parent cell and one cell that is slightly smaller than the parent. Each diatom will continue to go through mitotic divisions, creating one cell line that gets smaller and smaller

Figure 2: Diagrammatic representation of the diatom cell cycle. A series of mitotic divisions results produces smaller cells (see Fig. 1). Small cells eventually undergo meiosis, resulting in the production of haploid (1N) gametes, which combine to produce diploid (2N) zygotes, called auxospores (bottom). Sexual reproduction is necessary to restore the maximum cell size.




after each round of cell division. As a result, the average cell size of the population decreases, while the variance in size increases, over time (Edlund & Stoermer, 1991).

A diatom cannot, of course, shrink indefinitely and continue to survive. When the diatom reaches a critical size threshold – typically when a cell is roughly one-third of its maximum cell size – sexual reproduction is stimulated (Fig. 2). Diatoms reproduce sexually, a process that joins the familiar male and female gametes (Fig. 2). The resulting zygotes, also called auxospores, expand to restore the maximal cell size (Fig. 2). These cells will then reproduce asexually until they again reach their minimum size threshold, and the cycle repeats (Fig. 2; Edlund & Stoermer (1991)). This predictable change in cell size is called the MacDonald-Pfitzer rule (Round et al., 1990).

Although this process is well understood, it remains unclear how frequently diatoms undergo sexual reproduction in nature. This is important because, for the reasons outlined above, it may limit their ability to adapt to changing environmental conditions to which they must continually respond, including infections by viruses and, in Lake Fayetteville, chytrid fungi (unpublished data). On a larger scale, diatoms are faced with an ever-changing ocean environment, in which pH and salinity are both expected to decrease in response to global climate change (Trimborn et al., 2008). Understanding the frequency of sexual reproduction of diatoms, one of the earth's foremost primary producers, is therefore of paramount importance.

The purpose of this study was to understand how frequently diatoms undergo sexual reproduction in nature. I focused on one particular diatom species, *Stephanodiscus niagarae*, which is a dominant phytoplankton species in Lake Fayetteville. This species is a good candidate for this study due to its large cell size, characteristic and easily recognizable morphology, and because its reproductive biology is well understood (Edlund & Stoermer, 1991). By knowing

how frequently diatoms undergo sexual reproduction will allow a better understanding of how quickly diatoms can respond and adapt to environmental change.

# Material and Methods

Each week, from September 2015 through March 2016, I collected phytoplankton from Lake Fayetteville using a 10 $\mu m$ mesh plankton net and returned to the lab to process the samples that day. Using a 100 $\mu m$ filter, I filtered and then analyzed a 1 mL sample of water from Lake Fayetteville using a FlowCam VS-I-B B3 Particle Counter, which photographed, counted, and measured each cell that passed through a 200 $\mu m$ flow cell in a thin fluid stream. The instrument is able to process tens of thousands of cells in minutes, making a once labor-intensive task simple and easy, while providing more accurate measurements.

In addition to my dataset, Dr. Thad Scott from the Department of Crop, Soil, and Environmental Science at the University of Arkansas provided 15 months of Lake Fayetteville phytoplankton samples over a period spanning two years. I analyzed these as well, allowing us to determine when *S. niagarae* is most abundant and if there is any evidence of sexual reproduction in recent years.

Although the FlowCam provides accurate results at a very high rate, it cannot take images of the quality necessary to distinguish between species with similar morphologies, shape, and size. In addition to *S. niagarae*, two other diatoms with very similar overall morphology are common in Lake Fayetteville. Using FlowCam images, it is difficult to reliably identify specimens of these three species, especially when they are of similar size. Therefore, in order to better characterize the population of *S. niagarae* in Lake Fayetteville, I prepared permanent microscope slides from each sample and took high-resolution light microscope images. First, I added warm nitric acid to 3 mL of filtered lake sample to digest any organic matter, and then rinsed the sample to bring the pH to near neutral. Next, I dropped 1 mL of the treated sample onto a cover slip. Once the cover slip was dry, I mounted it to a glass slide using a resin with high light refractive index (Naphrax; Brunel Microscopes Ltd.) that enables high-resolution microscope imaging. I then viewed the slide on a Zeiss Axioscope microscope at magnification of 100x, ten times higher than that of the FlowCam.

I measured the diameter of 207 *S. niagarae* present in the sample with highest *S. niagarae* abundance using the program ImageJ, which provided a range of size measurements. This range was used to eliminate any questionable specimens taken with the FlowCam. Taking images with the light-microscope also allowed me to document and record subtle changes in cell wall morphology as the diatoms proceeded through their life cycle. This allowed me to track weekly changes in the size of this diatom population in Lake Fayetteville. All statistical comparisons were performed in the freely-available R Statistical Software (R Development Core Team, 2016).

# Results

The first sample that I collected and ran through the FlowCam was from the end of October 2015, which is still a fairly warm part of the year; the temperature of the lake was $18^oC$, and the sample was very dense, which is what lead us to filter the samples. This sample did not contain any *S. niagarae*, but was very abundant in other phytoplankton groups, including green and bluegreen algae, both of which are known to be warm water algae (Trentacoste et al., 2015). The lake temperature remained at $18^oC$ for the first two weeks of sampling, and then dropped down to $16^oC$ at the beginning of November, and then to $14^oC$ by the fourth sampling week

(Table 1). The lake became clearer as winter approached, but no *S. niagarae* specimens were identified, and our samples were still dominated by other species of phytoplankton. Although our diatom of interest had not yet been spotted, having these samples allowed us to track the community through time, seeing how the plankton community was affected by the seasonality of the year. The temperature of the lake was $12.5^oC$ at the end of November, but it was not until the very beginning of December, when the temperature of the lake was $10^oC$, that the first *S. niagarae* were identified in our samples (Table 1). At this time, the lake was substantially colder than it had been in earlier sampling, the cyanobacteria were less abundant, and the phytoplankton samples were much sparser (Table 1).

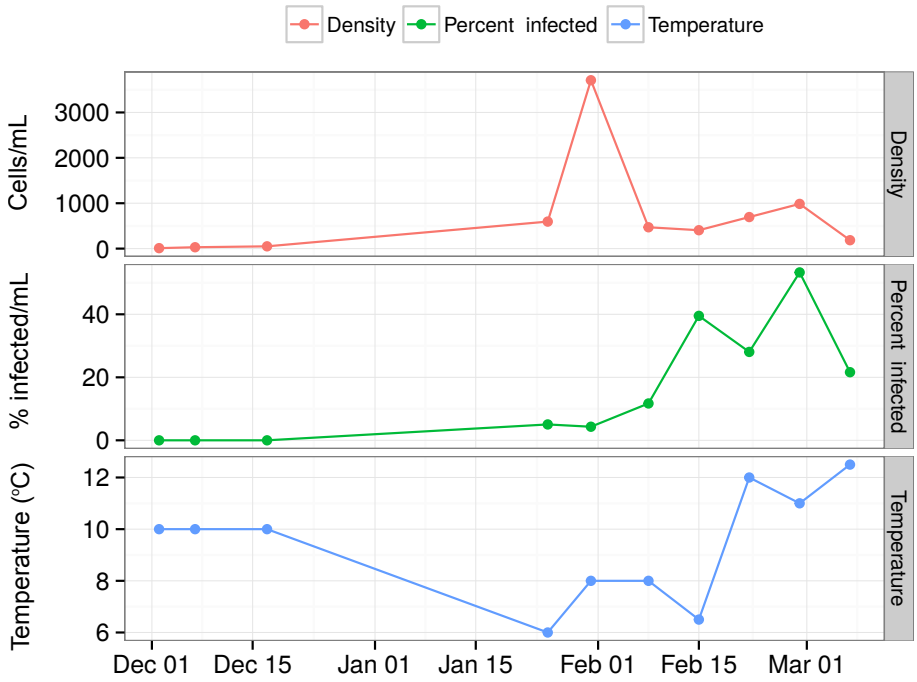
| Date | Temp ($^oC$) | Median diameter | Mean diameter | SD diameter | Cell/mL | % infected by chytrid |
|---|---|---|---|---|---|---|
| 2015-12-02 | 10 | 34.7 | 34.7 | 4.4 | 10 | 0 |
| 2015-12-07 | 10 | 36.4 | 35.5 | 5.8 | 30 | 0 |
| 2015-12-17 | 10 | 38.2 | 39.7 | 5.9 | 50 | 0 |
| 2016-01-25 | 6 | 33.6 | 34.0 | 5.3 | 595 | 5.04 |
| 2016-01-31 | 8 | 33.8 | 34.5 | 5.2 | 3710 | 4.31 |
| 2016-02-08 | 8 | 33.2 | 33.4 | 5.1 | 470 | 11.70 |
| 2016-02-15 | 6.5 | 37.9 | 38.4 | 5.7 | 405 | 39.51 |
| 2016-02-22 | 12 | 37.3 | 38.0 | 5.9 | 695 | 28.06 |
| 2016-02-29 | 11 | 37.8 | 39.3 | 7.3 | 985 | 53.30 |
| 2016-03-07 | 12.5 | 38.8 | 40.0 | 6.2 | 185 | 21.62 |

Table 1: Abundance and size of *Stephanodiscus niagarae* throughout the growth season in Lake Fayetteville. Lake temperature and the percent of cells infected by chytrid are also shown for comparison.

The abundance of *S. niagarae* increased over the month of December, but their overall density was very low, starting with 10 cells/mL in the first samples, and rising to 50 cells/mL by the third sample. A large increase in the abundance of *S. niagarae* was recorded by the third week of January, when the density was more than ten times that of the previous sample, 595 cells/mL (Table 1; Fig. 3). The next sample, taken at the end of January when the lake temperature had increased to $8^oC$, contained over six times as many *S. niagarae* as the previous sample, reaching the maximum observed density of 3710 cells/mL (Table 1; Fig. 3). Since lake access was restricted for the first two weeks of January, we were unable to follow in detail the increase in population size up to the maximum cell density observed in the second half of January. *S. niagarae* cells infected with a chytrid parasite were for the first time recorded during this period of peak *S. niagarae* density. Between 4% and 5% of *S. niagarae* individuals in both samples were found to be infected by chytrids (Table 1). The following weeks were marked with an increase in number of cells infected by chytrids and a substantial drop in *S. niagarae* population size (Table 1). In early February, the lake temperature remained at $8^oC$, but the number of *S. niagarae* plummeted to 470 cells/mL of which 11.7% were infected by chytrids (Table 1). Over the following weeks the density of *S. niagarae* varied between 400 and 985 cells/mL with anywhere between 39% and 54% of cells infected by chytrids (Table 1). The last sample, taken in March, had the highest lake temperature of $12.5^oC$, and had a large drop in *S. niagarae* abundance, having 185 cells present, with about 12.62% of these affected by chytrids (Table 1).

During the periods of growth and decline, the *S. niagarae* population in Lake Fayetteville changed both in its average cell size and the amount of variation in size (Fig. 4; Table 1). The mean diameter ranged between 34.7 $\mu m$ and 40 $\mu m$ and displayed significant differences across

Figure 3: Abundance of *Stephanodiscus niagarae*, percent of infected cells and surface lake temperature during the study. Lake access was closed between December 15 and January 15, so no data are available for this period.

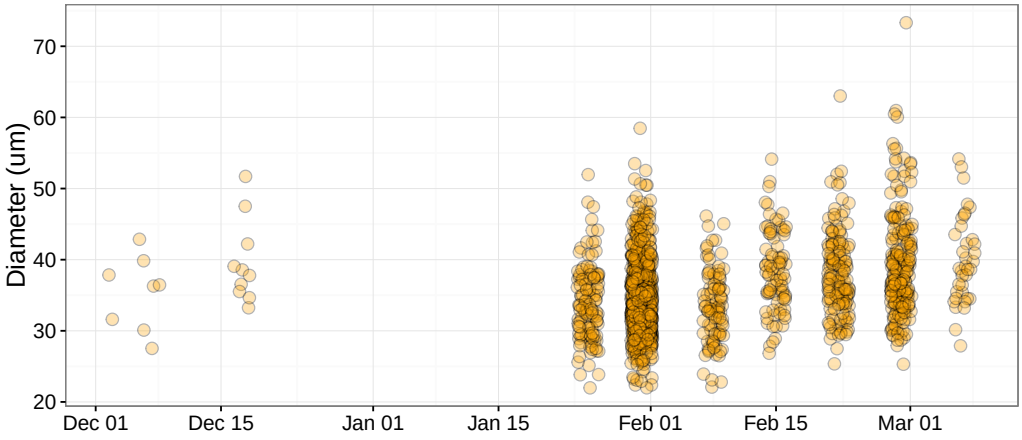



samples (ANOVA: F-value=22.98, df=9, p-value $< 0.001$). The change in variances was more pronounced, starting at 19.36 $\mu m^2$ and reaching 53.29 $\mu m^2$ towards the end of the growth season and was also significantly different through time (Levene's test for homogeneity of variances with median center: F-value=2.78, df=9, p-value $< 0.01$). Although the mean diameter (cell size) doesn't appear to decline substantially as the growth season progresses, the increase in variance is consistent with diatom population growth Edlund & Stoermer (1991); Fig. 4; Table 1). The diameters were also compared to samples taken from Lake Fayetteville one year ago and two years ago; the sizes were about the same, indicating that the population size has not increased much over the last three years.

# Discussion

The peculiar way in which the diatom cell wall is put together causes an inevitable reduction in cell size though the process of vegetative (mitotic, asexual) reproduction (Edlund & Stoermer, 1991; Kaczmarska et al., 2013; Windler et al., 2014). Since diatoms cannot reduce in size indefinitely, their vegetative life histories have to be interrupted by an event in which maximal cell size is restored. The most common mechanism in which diatoms achieve size restitution is sexual reproduction involving the fusion of two haploid gametes to form a zygote without a rigid cell wall that has the ability to expand and increase in size relative to the parent cells (Davidovich et al., 2015; Kaczmarska et al., 2013). Aside from size restitution, sexual reproduction is the primary way of introducing genetic variability in a diatom population through a combination of genetic crossing-over, independent assortment of chromosomes, and combining alleles from unrelated gametes. The cues and frequency of sexual reproduction are important

Figure 4: Diameter of *Stephanodiscus niagarae* cells throughout the course of the study. The vertical position of each point represents the cell diameter in $\mu m$. Within a particular sample (date) the measurements are plotted with a horizontal jitter to help with overplotting of points with similar y-axis values.

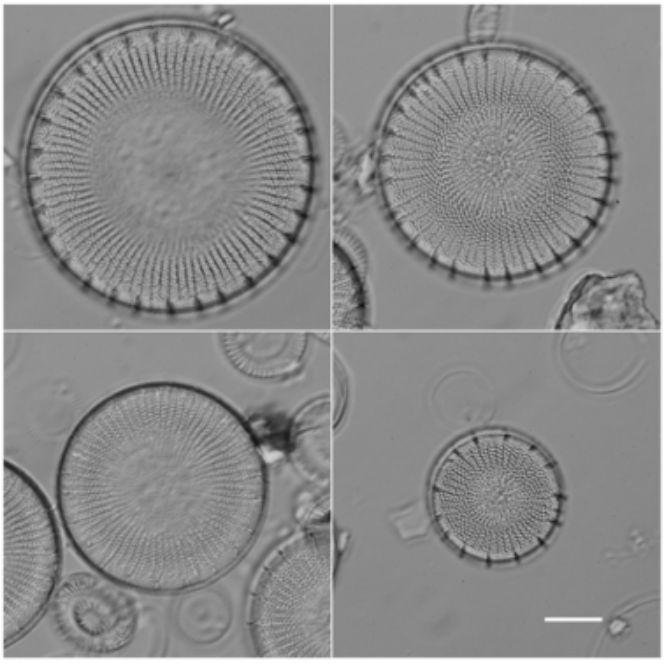



but understudied aspects of diatom ecology and evolutionary biology.

The diatom *S. niagarae* is widespread throughout the United States and occurs commonly in lakes and reservoirs with moderate nutrient content (Kilham & Fritz, 1996). I studied the population dynamics of *S. niagarae* in Lake Fayetteville, a small urban reservoir in Northwest Arkansas with above average productivity relative to other lakes in the state and a summer water transparency of about two feet (ADEQ, 1999). The main goal of my study was to document the population growth and changes in cell size due to vegetative divisions throughout the growth season of *S. niagarae*. The proximity and ease of access to the lake, as well as the availability of high-throughput imaging equipment, allowed me to monitor the species weekly and provided a fine resolution of changes in population density and size. I used these data to approach the question of frequency of sexual reproduction in *S. niagarae*. Since the cues for sexual reproduction in this species are unknown and the frequency of sex is unpredictable, approximations based on the rate of size diminution of the population are, perhaps, the best way to estimate how often *S. niagarae* undergoes sexual reproduction.

In Lake Fayetteville, *S. niagarae* grew in the winter months when the water temperature dropped bellow $10^oC$ and green and blue-green algae were not as abundant as in the preceding warmer period (Fig. 3; Table 1). The occurrence of *S. niagarae* overlapped with the winter turnover in Lake Fayetteville, which is a warm monomictic lake characterized with summer stratification in temperature and dissolved oxygen and winter mixing (Hoffman et al., 1955). *S. niagarae* cells first appeared in December at a relatively low density of a few dozen cells per mL, and reached maximum density about two months later in the second half of January (Table 1). Based on this period of increase, and assuming simple density-independent growth, the population doubling time was about once per week (Fig. 3; Table 1), i.e. the density of *S. niagarae* in Lake Fayetteville nearly doubled every week. However, this estimate is somewhat uncertain because samples are unavailable for late December to early January due to closed lake access (Fig. 3). After the maximum cell density of 3700 cells/mL in Late January, the population declined rapidly and fluctuated in the range of few hundred cells/mL (Table 1; Fig. 5-6). However, the main factors controlling the density of *S. niagarae* in the lake are uncertain.

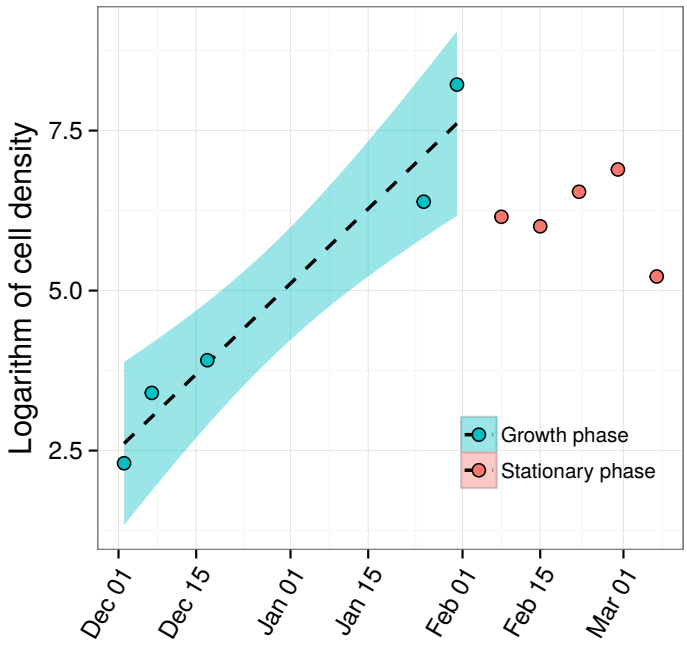
Figure 5: Size diminution in *Stephanodiscus niagarae* as a result of vegetative division. The diatom in the top left having the largest diameter recorded in my study, and the diatom in the bottom right having the smallest. Scale bar = 10 $\mu m$.



In addition to cell density, I was able to measure surface lake temperature and the percent of *S. niagarae* cells infected with a chytrid parasite. The drop in density of *S. niagarae* coincided with both increase in lake temperature and increase in the number of cells affected by chytrids (Fig. 3, 6; Table 1). It is possible that chytrid abundance is dependent on lake temperature and might increase as temperature rises, and it is also possible that high densities of *S. niagarae* (and other diatoms) might promote an increase in abundance of chytrid parasites (Gsell et al., 2013a,b). Therefore, it is difficult to disentangle whether the abiotic (temperature) or biotic factor (chytrid abundance) is the proximal cause of *S. niagarae* decline. A number of additional factors might also play a role in *S. niagarae* population dynamics. Resource availability in lakes is dependent on the season, as well as abiotic factors including sunlight, water depth, rainfall, and water transparency (Gsell et al., 2013a,b). None of these were taken into account in this study, so it remains unclear how much of an effect they have on the *S. niagarae* population and its reproductive patterns.

The growth dynamics of the population of *S. niagarae* in Lake Fayetteville were accompanied by changes in the average cell size (Table 1; Fig. 5-6). As the population grew and declined, there was a tendency for the dispersion of cell sizes around the mean to increase (Fig. 6) and both the mean and variance were significantly different across samples. Although the increase in variance is consistent with expectations of diatom population growth, the finding that the average cell size did not decrease as the growth season progressed was unexpected. Indeed, the mean and median size of the population were higher towards the end of the growth season compared to the beginning, but this pattern is difficult to interpret, due to the fact that data for vertical distribution of *S. niagarae* within the lake are unavailable. The larger average size of *S. niagarae* in the surface plankton of the lake could, for example, be a byproduct of

Figure 6: Regression of the natural logarithm of S. niagarae cell density by time (weeks) during the study. Black line is the regression line and blue shaded area is the 95% confidence interval for the regression. The coefficients of the regression were: $ln(Density) = 1.7 + 0.65 \times Time$




vertical migration of cell of *S. niagarae* towards deeper layers as water temperature increased. It is also possible that by the end of the growth season, the vertical mixing of the lake was complete and the lake began to stratify. In such conditions phytoplankton are commonly found in deeper water near the thermocline, where deep chlorophyll maxima are formed (Cantin et al., 2011). As a result, the data obtained at the tail end of the study period might not be a very accurate representation of the size distribution of *S. niagarae* throughout the depth profile of Lake Fayetteville.

These considerations make it difficult to estimate the rate of size decrease of the population of *S. niagarae* in Lake Fayetteville and by extension, the frequency of sexual reproduction. However, comparisons to samples taken two seasons before (March 2013-2014) reveal that the population from 2013-2014 to 2016 is overlapping in size and have comparable mean diameters. Therefore, despite the potential limitations of not having data for the vertical distribution of *S. niagarae*, it appears that the average size of this species varies within a relatively narrow range over a period of three growth seasons. This amount of variation implies that the overall cell size of the population does not reduce substantially over the course of a growth season and might take several years, or potentially up to a few decades, for the population to decrease in size enough so that a population-wide event of sexual reproduction occurs. Finally, it is also possible that sexual reproduction occurs asynchronously and continuously in the lake. The fraction of cells that have reached the lower size threshold might undergo size restitution when necessary, while larger cells might continue to grow vegetatively. This would produce a population with overlapping generations of parental and offspring genotypes. The size distribution of such a population might not display a pattern of extended periods of size reduction interrupted by dramatic spikes in cell size, but instead be fairly homogeneous through time. Such growth

dynamics would complicate the quantification of the frequency of sexual reproduction in this species, requiring exhaustive documentation of zygotes in wild samples over several growth seasons.

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