Time Specific Population Dynamics of Diatoms in Artificial Environments

Sydney M. Brown and Katie M. Johnson

Department of Biological and Environmental Sciences, Georgia College & State University, Milledgeville, GA 31061, USA

INTRODUCTION

Phycological research is important because algae serve as the base trophic level of aquatic systems, energetically supporting entire aquatic communities. Culturing in a laboratory setting requires significant phycological background and ecological knowledge to mimic the complex environmental conditions experienced by diatoms. *Gomphenema parvulum* Kützing is an important monitoring diatom. Diatoms are a group of algae defined as primary producers with cell walls composed of silica. Glass is permeable for light, brittle and impermeable for gasses and liquids. Due to the unique properties of glass, asexual reproduction results in a diminution of cell size within a part of the population. Each generation restores larger size through the formation of an auxospore via sexual reproduction.

STUDY SITE

Figure 1: Pictures (top) of deployed diatometer at collection location and (bottom) of surrounding habitat slightly downstream. Map (right) showing the sampling location (Lat. 33.370750, Long. -81.627738) on Upper Three Runs Creek (UTRC), which is located on SRS, ©2017 Google. (Johnson thesis, 2020)

Our study site, Upper Three Runs Creek (UTRC) is a headwater stream of the Savannah River. The Savannah River is one of Georgia's largest rivers, providing an estimated 1.4 million people with potable water. The following industries are also located along the Savannah River: International Paper, Plant Vogtle (nuclear power), and the Department of Energy's Savannah River Site, which processes nuclear waste. Because UTRC is considered a biodiversity hotspot and a control site for the Savannah River Site, we chose this site to create voucher flora that could be used for future studies assessing anthropogenic impacts downstream. (Johnson thesis, 2020).

Five different monocultures were inoculated from Upper Three Runs Creek (UTRC) at differing times providing for differing growing periods for each diatom population. By establishing monocultures, confounding variables such as interspecific competition on resources were eliminated. An attempt at providing ideal growing conditions as light and liquid nutrients were provided. The goals of this study were: 1) to establish and maintain for several generations 5 monocultures of *G. parvulum* from UTRC with differing growing periods, 2) assess whether time served as a function for influencing morphologies within a population through diminishing frustule sizes through generations, and 3) investigate whether there was evidence that natural sizes were restored via sexual reproduction

METHODS

- 5 Monocultures of *G. parvulum* were inoculated on a modified agar medium using species from UTRC on the following dates: March 22, 2019- May 1, 2019; December 19, 2018- February 20, 2019; May 3-4, 2019; November 28, 2018; February 20, 2018- May 1, 2019
- Samples were assigned to labels SB1-5: SB signifying the first author's initials- corresponding dates of inoculation with sample names shown in Figure 3 & Table 1
- Samples were grown in a sequential fashion, the oldest (SB2) being first inoculated from an individual from UTRC, following samples were inoculated by from the previous sample. New samples inoculated to avoid interspecific competition from cyanobacteria.
- Samples were left to desiccate once new sample inoculated, initiating cell death across sample
- Monocultures were digested according to standard protocol in December 2019, neutralized, and placed into 20mL vials
- Samples were dried onto cover slips and mounted onto permanent slides using Naphrax® (Brunel Microscopes, Ltd., Chippenham, Wiltshire, UK) mounting medium
- Using oil immersion at 1000x on a light microscope, 100 units were measured length by width and recorded
- Unit values compared to lower end of morphological definition of *G. parvulum* using chi-squared analysis
- Samples were also compared to each other using paired t-tests

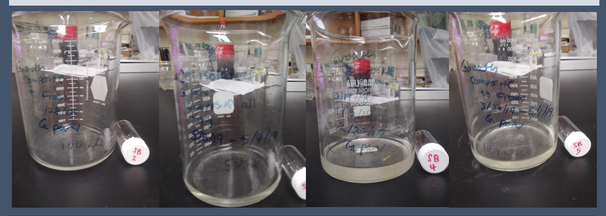

Figure 2. Sample processing and concentration after digestion

RESULTS

- χ^2 (df=3) = 10.8, p<0.05
- Paired t-test for each samples used
- t-values range from 2.6-9.5, with df=198 for all paired t-test; p<0.05

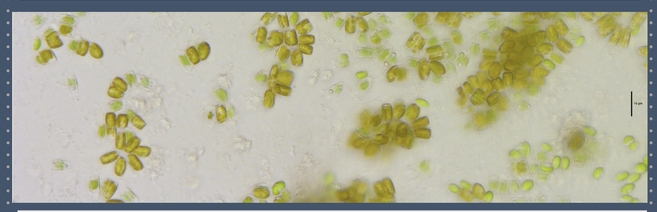

Figure 3. Live *G. parvulum* (Kützing) Kützing monoculture (September 2018 –May 2019) brown colored cells assessed at highest physiological state with visible coloration form chlorophyll c and pheophytin, green colored frustules have chlorophyll a visible and are assessed as less physiologically active. Clumped growth patterns visible. (Johnson thesis 2020)
Scale bar = 10 µm at 400X mag.

RESULTS cont.

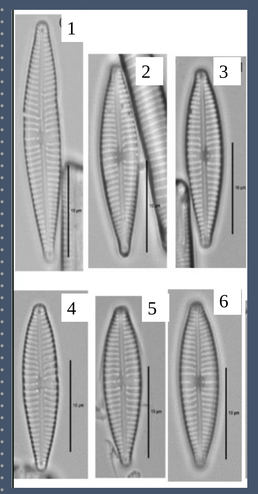

Plate1. Cleaned diatom material, *G. parvulum* natural population collected from Upper Three Runs Creek (Johnson thesis 2020); Scale bars = 10 µm at 1000X magnification.

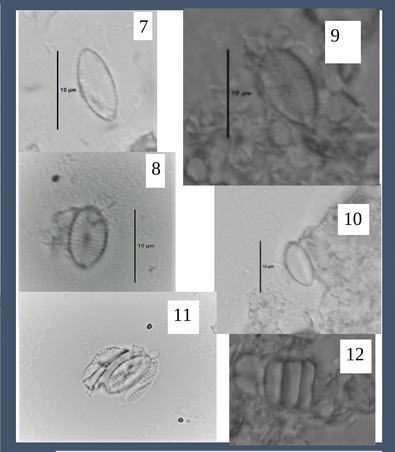

Plate2. Cleaned diatom material, *G. parvulum* inoculated population; ***7-10*** rounded morphology of clonal diatoms; ***11-12.*** shows their tendency of frustules to stick together after divisions in chains; Scale bars = 10 µm at 1000X magnification.

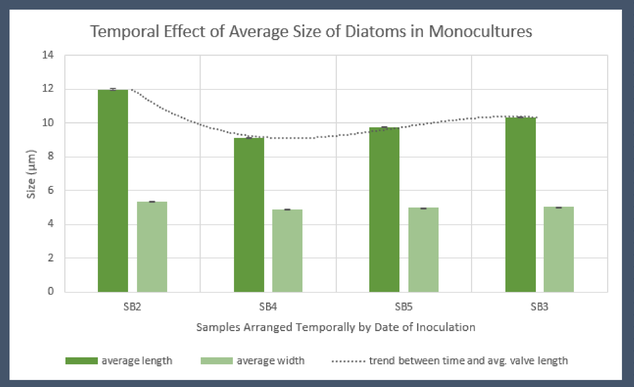

Figure 4. Morphological features of *Gomphonema parvulum* grown in artificial conditions arranged temporally from oldest to youngest
(n=100, mean ± standard error)

t-value SB2-SB3	t-value SB2-SB4	t-value SB2-SB5	t-value SB3-SB4	t-value SB3-SB5	t-value SB4-SB5
5.407	9.452	7.229	5.894	2.644	3.179

Table 1. t-values for each paired t-test between cell length of all samples; all calculated t-values significant at p<0.05

CONCLUSIONS

- Each sample started from an individual which reproduced asexually to create a monoculture population, leading to a diminution of cell size until reaching a point in which auxosporulation is triggered- restoring natural sizes
- Auxospores were not observed
- all the clonal samples were significantly smaller than defined values for *G. parvulum (chi-square significant)*
- The oldest clonal colony (sample 2; SB2) had the largest range of cell lengths, 8-20 µm compared to natural population
- Using paired t-tests, each sample was significantly different in lengths to each other
- oldest colony was the largest, hosting generations the least temporally removed from the natural population
- A pattern of average cell length significantly increasing in newer generations emerges, likely due to diminution of cell sizes. Older samples likely had not had enough time to restore sizes via sexual reproduction
- Morphologically frustules become more rounded with lengths shortening
- Cell widths did not change significantly between all samples (p>0.05)

References

Johnson, K., & Manoylov, K. (2020). Creating Diatom Voucher Floura, Investigating Sampling Methods and Possible Gomphonema Parvulum Morphotypes for Southeastern Tributary Upper Three Runs Creek.

Lange-Bertalot, H. (2016). The Diatom Genus Gomphonema from the Republic of Macedonia. Koeltz Botanical Books. 8, 98-99.

Rose, D., & Cox, E. (2014). What Constitutes Gomphonema parvulum? Long-Term Culture Studies Show That Some Varieties of G. parvulum Belong with Other Gomphonema Species. Plant Ecology and Evolution. 147(3), 366-373. http://dx.doi.org/10.5091/plecevo.2014.1059

Armbrust, E., & Chisholm, S. (1992). Patterns of Cell Size Change in Marine Centric Diatom: Variability Evolving From Clonal Isolates. Journal of Phycology. 28, 146-156. https://doi.org/10.1111/j.0022-3646.1992.00146.x

ACKNOWLEDGEMENTS

Thank you Dr Kalina Manoylov for her mentorship, Georgia College and the Manoylov Phycology Lab, for providing the resources for this research. Special thanks to Dr. Kalina Manoylov for providing mentorship throughout the process of this research.