

Paleolimnology and molecular investigation to understand blooms of *Didymosphenia hullii*, *Didymosphenia geminata*, and *Cymbella janischii*, nuisance stalk-forming diatoms in the West Branch of the Farmington River, Connecticut, USA.

Introduction

Diatoms are ubiquitous microscopic algae found wherever there is water, including in lotic or lentic ecosystems, the ocean and in moist sands. Diatoms form significant components of most aquatic ecosystems, especially as primary producers in rivers. In recent years and for reasons not fully understood, stalk-forming diatoms are believed to have expanded their global range. This expansion and other unknown processes have triggered prolific blooms with thick mats of long filamentous stalk material causing adverse conditions to river ecosystems worldwide. These conditions include the biological deterioration of habitats, loss of biodiversity and significant negative impacts on the sport fishing industry. Until recently, these nuisance diatom species have not posed problems for rivers in Connecticut. However, three especially problematic and nuisance stalk-forming species are now known to be actively growing in the West Branch of the Farmington River, in Connecticut. The first species, *Cymbella janischii*, is documented as regionally endemic in the Pacific Northwest. The second species, *Didymosphenia hullii*, was recently described from the West Branch of the Farmington River. The third recently confirmed species is *Didymosphenia geminata*. This taxon is globally distributed and has caused undesirable impacts to countless river ecosystems. The distribution of these problematic species, and the extent to which they could spread, poses a potential risk not only for the West Branch of the Farmington River, but other rivers in the state, which boasts an economic value of millions of dollars annually from fishing and fishing related activities. *Didymosphenia* taxa are known to grow abundantly

in stable flowing, regulated, cold, oligotrophic waters, which are similar conditions to portions of the West Branch of the Farmington River where they grow today. During the drought summers of 2015–2017, these species expanded their range within the river. Preliminary data indicated changes in size and morphology of *D. hullii*, but this later was determined to be due to the presence of an additional species, *D. geminata*.

Cymbella janischii grows more prolifically further downstream of the *Didymosphenia* taxa, in slightly warmer waters with higher levels of nutrients. However, all three taxa were found sporadically together. Will these diatoms continue to migrate to other rivers in Connecticut and the Northeast?

Research Objective

This study will examine the three stalk-forming diatom phylogenetic relationships using DNA analyses. We will primarily use core sampling, and microscopy to verify whether the three taxa historically have been present, but are rare or absent in the West Branch of the Farmington River, assess the expansion of their geographical range in the river and evaluate what environmental and anthropogenic factors that triggers these diatoms to bloom.

The specific objectives are the following:

1. Implement paleolimnological methods in different locations to determine if any of the three species, *Didymosphenia hullii*, *D. geminata*, or *C. janischii*, were historically present in the river.
2. Provide detailed morphological descriptions of each organism using light and scanning electron microscopy.
3. Determine molecular descriptions of each taxon, including DNA sequences for specific genes. Sequences will be compared to other populations in North America and elsewhere, and contributed to the national sequence repository (GenBank).
4. Monitor physico-chemical conditions, at several sites where bloom formations occur.
5. Further investigate the impacts of dams and river flow relative to blooms.

6. Provide guidance for best management planning to help reduce and control the extracellular mucilaginous stalk growth in the river.
7. Provide community college students with research opportunities.

Methods/Procedures

Sampling methods in the West Branch of the Farmington River Several locations have been chosen where the substrate is no more than 76 cm deep. The sampling locations have continual flow throughout the year, and ample rock and cobble substrate conducive for diatom growth. Randomized collections of mucilaginous stalk tufts will be placed in Whirl-Pak bags, stored on ice, and taken back to the lab for analysis. Each sample will be divided into aliquots for morphological and molecular components of the study. Samples will be brought to the lab, morphologically examined, and stored at 4°C until processing. Live samples will be processed for DNA extraction or will be stored at -20°C until processing. Preserved samples will be stored in RNA Later for 5–21 days then refrigerated at 4°C until single cell isolation (Hamilton et al., 2015) is performed. The live and preserved samples will be used for sequence collection and DNA analysis. An additional sample will be used for morphological analyzes. Part of the sample will be preserved in *RNA Later*® for 5–21 days and then refrigerated at 4°C after which single cells will be cleaned with deionized water and placed for the final time in a PCR tube containing 100 µL of 10% (w/v) Chelex R 100 solution (Richlen & Barber, 2005, Hamilton et al., 2015). The single cells will be ruptured using a rigorous glass bead homogenization procedure and DNA extracted in the Chelex using a thermocycler for 20 minutes at 95°C. From the fresh samples several cells will be isolated using a micropipette and placed in a 0.2 ml PCR tube. From these tubes, 1–10 individual cells of *Didymosphenia geminata*, *D. hullii* or *Cymbella janischii* will be placed in PCR tubes

and washed 3–5 times (Lang & Kaczmariska, 2011). After the final wash and centrifugation, the supernatant will be removed and replaced by 1 µl of sterile water. The samples will then be heated at 95°C on a thermocycler for 10 min prior to PCR to open the frustules for DNA extraction (Lange & Kaczmariska, 2011). The PCR mix for both fresh and preserved samples will consist of 10 µl GoTaq® Green Master Mix, 0.5 µl of each primer (Hamilton et al. 2015, Khan-Bureau et al., 2016, Richlen & Barber, 2005, Zimmermann et al., 2011), and sterile deionized water for a final volume of 20 µl in the PCR tubes, each containing cells of *Didymosphenia hullii*, *D. geminata*, and *Cymbella janischii* cells in separate PCR tubes. PCR products will be sequenced, and resulting sequences will be compared to GenBank, the public sequence database, using BLAST searches. Sequence alignments of new and related published data will be used for phylogenetic analysis, which will assist in determining identities (Khan-Bureau et al., 2016). The methods of Hamilton et al. (2015), Zimmermann et al. (2011), Richlen and Barber (2005), and Jahn et al. (2007) will be used to examine the efficacy of a collective approach for molecular analysis and contrasting LM, SEM, and DNA examination of diatoms. All DNA work and analyses will be performed at UCONN in the Lewis lab.

Morphological Analysis Using Light and Scanning Electron Microscopy For LM, diatom samples will be simmered on a hot plate in a 1:1 ratio of water and 68% nitric acid to oxidize organic matter until the sample's liquid is reduced to half, after which the samples will be removed from the hot plate to cool. Deionized water will be used to rinse the samples of the acid, subsequently the samples will be centrifuged to concentrate the diatom frustules at 600 g to avoid frustule damage. The process of rinsing includes the addition of deionized water, centrifuging and the removal of supernatant 4–5 times or

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until the pH is neutral. After air-drying the diatom samples overnight on coverslips, frustules will be mounted on glass microscope slides in the mounting medium naphrax, heated on a hot plate and then cooled to produce permanent vouchers. The diatom frustules will be examined at 600 and 1000× magnifications and observed with an Olympus CX 41 Phase Contrast microscope. Images will be captured using an Olympus DP 25 color camera 2560x 1920 pixels or a MicroFire CCD Color Camera at 1600x 1200 pixels. LM preparation can be accomplished at both Three Rivers Community College and at UCONN. FY2019 SEM work will take place at UCONN Electron Microscopy Lab and at the Canadian Museum of Nature. Aliquots of each slurry sample will be dried onto several pieces of aluminum foil, which will be trimmed to fit and mounted on stub with double sided tape (Morales et al. 2001). The stubs will be prepared by being coated for 30 seconds at 1.8 kV with gold/palladium using a Polaron sputter coater (Morales et al. 2001). The stubs can then be viewed with a FEI Nova Nano 450 scanning electron microscope. Image plates will be created using Adobe® Creative Suite® 6 Photoshop. The physical characteristics of the river will be described such as water depth, geomorphology, riparian zone description, canopy and other physical attributes that could influence the diatom species and population. The USGS has sampling stations at one location on the Still River before the convergence of the Still and West Branch of the Farmington Rivers and that information will be utilized as needed.

Core Sampling

We will use core samples to examine presence or absence of these taxa. Core sampling has been used successfully to examine paleo-records of frustules from lakes, though hasn't been frequently used in rivers because of limitations and challenges of flow and

sedimentation. However, preliminary core samples may provide enough information to determine the historical presence or absence of these nuisance stalk-forming diatoms. The core samples can be archived for future lead-210 dating. Depending on the conditions of the sediment, we will use either a gravity or piston corer to retrieve sediment cores from multiple locations in the river at and below the sites known to harbor the nuisance species. Since we anticipate the sites not being deep, we will be able to facilitate penetration of the core tubes into sediments using the gravity corer (Glew, 1989). Core samples will be cut on site using an extruder (Glew, 1988). The cores will be used to examine if the taxa were present historically, and if so, when they became part of the river ecosystem. Core samples will be prepped at Connecticut College in New London and SEM work performed at UCONN Electron Microscopy Lab with some samples being examined at the Canadian Museum of Nature. Core samples will be archived at Connecticut College for future lead-210 dating, if the taxa are found in historical sediments.

Significance of Research

Invasive stalk-forming diatoms, also known as rock snot, produce mucilaginous stalks, which grow on rocks and other substrates in rivers (Bahls 2007, Blanco & Ector, 2009, Khan-Bureau et al., 2016). When rock snot diatoms bloom, their copious extracellular stalk growth (extracellular polysaccharide substances, ESP) negatively impacts benthic macroinvertebrates and native algae, which in turn alter fish populations and food webs (Blanco & Ector 2009, 2013, Bothwell et al. 2014, Spaulding & Elwell2007). In addition to altering the ecosystem, heavy concentrations of rock snot are unpleasant for recreationists, tourism, and anglers. The West Branch of the Farmington River is the most

fished river in Connecticut, with over 116,000 man-hours of fishing and adds approximately 152 million dollars annually (freshwater fishing and fishing activities) to the economy of Connecticut (M. Beauchene CT DEEP, pers. comm.). Thus, blooms of these diatoms could result in a potential economic dilemma (Khan-Bureau et al., 2014). There are no known or recorded historical episodes of invasive blooms of these diatoms in the West Branch of the Farmington River or anywhere else in the state. As a result, these problematic taxa are believed to be recent invaders, now finding conditions in the West Branch of the Farmington River conducive to forming blooms. Alternatively, it is possible that one or more of the species were historically present, but rare and previously undetected in the river (Khan-Bureau et al., 2014). Recent growth has ecologists, anglers, and other concerned groups questioning where these organisms originated, what triggers the blooms, and why they continue to bloom prolifically in the West Branch of the Farmington River. Presently, there are no known safe control methods that can eliminate the prolific stalk growth (Kilroy & Bothwell, 2011, Spaulding & Elwell 2007).

Project Impact

Unfortunately, COVID-19 stopped much of the work. One of the Co-PIs had major ankle surgery and the project slowed. However, things picked back up after Dr. William Ouimet agreed to assist in doing the core sampling in July 2020. Dr. Ouimet, brought with him a wealth of knowledge in river geomorphology, GIS, and expertise drill exploration in rivers. He also operates the UConn Sediment Coring Facility at UConn. His coring methodology, commonly referred to as vibracoring, incorporates using a vibratory machine, that is operated with a generator, which vibrates the 3"x 8' pipe as deep as allowable into the river. This technology is more conducive to river coring than

the gravity or piston corer. We didn't realize the importance of this method and the equipment necessary until we tried using the gravity corer. We are very grateful for Dr. Ouimet's and his graduate students help. We will continue to work on this project with Dr. Ouimet.

The TRCC students who have worked on this project took preliminary core sediment samples. We found *Didymosphenia* taxa as well as *Cymbella janischii* however the frustules were not found in the deep sediment of the core but in the upper core sediments, about 5 cm. This was not unexpected since rocks and sediments in rivers continuously are eroded and pushed downstream at a much faster rate than lake sediments. Further analyses indicate that of the three sampling sites the deeper core sediment samples revealed no *Didymosphenia* taxa or *C. janischii* frustules thus far. Further microscopy work on our core samples and coring in the WB of the Farmington River will need to be explored when the weather permits. The cores taken to the UConn Sediment Coring Facility will be studied further and dated. The sediment from the cores alone can inform us if these diatom taxa have been in this river for a significant time or if they are newly brought here by fisherman's boots, tackle and other means. DNA analysis will continue once the snow and ice have melted and warmer weather permits us to take samples. Samples will be split between Dr. Khan-Bureau, TRCC/UConn, and Dr. Paul Hamilton of the Canadian Museum of Nature in Canada. SEM and LM will commence once the core sediments have been dated and split for further microscopy work. Dr. Peter Siver and Dr. Khan-Bureau will perform the SEM work at UConn.

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