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Research Article

Observations of two nuisance stalk-forming diatoms (Bacillariophyta) from a river in Connecticut, Northeastern U.S.A.: *Didymosphenia* sp. and *Cymbella janischii* (A. Schmidt) De Toni

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Abstract

The nuisance taxon *Didymosphenia geminata* was reported in the West Branch of the Farmington River in March, 2011 after a fisherman detected cotton-like tufts attached to rocks. In response, the Connecticut Department of Energy and Environmental Protection (CT DEEP) conducted a comprehensive survey of the river system. After major late summer storms, *Didymosphenia geminata* was not observed again. Surveys in 2012–2013 tracked the spatial and temporal distribution of stalk-forming diatoms at the confluence of the West Branch of the Farmington and Still Rivers, thereby allowing comparison of data from adjacent rivers with distinct water chemistries. Water chemistry and temperature data were collected to characterize nutrient concentrations associated with these diatoms. Surveys showed no evidence of *Didymosphenia geminata* but four native stalk-forming diatom species and a taxon previously unreported in Connecticut, *Cymbella janischii*, were observed throughout the year. Also from November, 2012 through June, 2013, a morphologically distinct diatom in the genus *Didymosphenia* was observed growing prolifically bank to bank with thick mats of long filamentous stalks. Subsequent examination revealed that the taxon previously reported as *Didymosphenia geminata* was instead a different taxon, *Didymosphenia* sp. Furthermore, *Didymosphenia* sp. continued to flourish in the West Branch of the Farmington River, absent from the neighbouring Still River, suggesting that the physiochemical features and in particular higher nutrients may limit the distribution of this diatom. In contrast, *C. janischii* was found growing abundantly further downstream in warmer water and higher nutrient levels.

Key words: *Cymbella janischii, Didymosphenia*, extracellular polymeric stalks, invasive diatoms, mucilaginous tufts, nuisance, rock snot

Introduction

Didymosphenia geminata (Lyngbye) M. Schmidt has become a taxon of interest to ecologists, biologists, anglers, and water quality managers throughout the world since its invasion of New Zealand in 2004 (Spaulding and Elwell 2007; Blanco and Ector 2009). *Didymosphenia geminata*, often referred to as "rock snot", is a putatively invasive, and nuisance species (Kilroy 2004; Spaulding and Elwell 2007; Kuhajek et al. 2014). This species produces extracellular polymeric stalks (EPS) that persist even after the cells are no longer viable, forming mats that have the potential to negatively impact aquatic organisms within rivers and streams (Spaulding and Elwell 2007; CT DEEP 2011).

Didymosphenia geminata natively occurs in cold, oligotrophic waters, in mountainous regions and temperate climates with cold winters and warmer summers, although there are conflicting reports of its habitat preference. Its distribution

now spans diverse conditions from unpolluted to polluted waters (Krammer and Lange-Bertalot 1988; Kilroy 2004; Kilroy et al. 2007; Spaulding and Elwell 2007). The geographical range of *D. geminata* has expanded since it was originally described nearly 200 years ago (Lyngbye 1819; Blanco and Ector 2009; Whitton et al. 2009). This expansion may be because its growth has become more abundant in recent years making it more readily observed where it once may not have been detected (Spaulding and Elwell 2007; Blanco and Ector 2009; Kumar et al. 2009; Bothwell et al. 2014). The current rapid growth and geographical expansion of *D. geminata* may in part be due to seasonal changes, climate change, variation of nutrients such as orthophosphate (SRP), light intensity, rainfall patterns and other environmental factors (Ellwood and Whitton 2007; Kilroy et al. 2007; Spaulding and Elwell 2007; Bothwell and Spaulding 2008; Kilroy et al. 2008; Bothwell and Kilroy 2011; Kuhajek et al. 2014).

In the United States, *D. geminata* was potentially transported by anglers boots, fishing gear and other recreational equipment (Kirkwood et al. 2007; Bothwell et al. 2009) from Western into several Southeastern states, including Virginia, West Virginia, Tennessee, North Carolina (Spaulding and Elwell 2007), and more recently the Northeastern states. Significant growth of *D. geminata* was documented in North America only within the last 20 years (Bothwell and Spaulding 2008; Spaulding et al. 2008; Blanco and Ector 2009). Recently, in the Northeastern U.S.A., *D. geminata* was found in the main stem of the Connecticut River and several of its tributaries in Vermont, New Hampshire and purportedly Connecticut (CT DEEP 2011). In May, 2013 the Massachusetts Executive Office of Energy and Environmental Affairs (MASS EOEEA 2013) announced that *D. geminata* was confirmed in the Green River. Some researchers suggest that *D. geminata* may be indigenous, but rare in the Northeastern U.S.A. (Bothwell and Spaulding 2008; Spaulding et al. 2008; Blanco and Ector 2009) but many state agencies consider *D. geminata* as non-indigenous in the Northeastern U.S.A.

The first observation of *Didymosphenia* in Connecticut was reported to the CT DEEP in 2011 with no earlier record to substantiate this taxon as indigenous to Connecticut (CT DEEP 2011). Uncertainties persist whether *D. geminata* is introduced to or native in Connecticut. Terry (1907) provided a partial list of diatoms found in Connecticut, with *Gomphonema geminatum* (Lyngbye) Ehrenberg (Terry mistakenly used Ehrenberg rather than Agardh as authority) listed as a common species, although no illustrations were provided.

The objectives of this study were to document the presence of two previously unrecorded stalk-forming diatoms in the West Branch of the Farmington River in Connecticut, *Cymbella janischii* (A. Schmidt) De Toni, and an unidentified taxon in the genus *Didymosphenia*. We also contribute information about the environmental conditions that are associated with these and other dominant and native stalk-forming diatom species, including *Cymbella affinis* Kützing, *Gomphonema truncatum* Ehrenberg, *Gomphoneis minuta* (J.L. Stone) Kociolek and Stoermer, and *Encyonema* cf. *minutum* (Hilse) D.G.Mann in the West Branch of the Farmington River over the years 2012–2013. The study site spans the confluence of two rivers with distinct management regimes and characteristics, allowing a comparison of the environmental conditions that are associated with abundant stalk growth of these diatom taxa.

Materials and methods

Study location

The confluence of the West Branch of the Farmington River and the Still River in north central Connecticut provides a unique setting to examine the relationship of water chemistry, temperature, and the presence of stalk-forming diatoms. The upstream catchments for both rivers have markedly different land-use, water quality, and flow regulation.

The West Branch Farmington River (616 km^2) is one of several sub-basins within the greater Farmington River regional basin (1,572 km^2), a significant tributary to the lower Connecticut River (29,184 km^2). The land-use is greater than 85% forested, with no significant population density and has less than 3% impervious cover. The West Branch Farmington River begins in Otis, Massachusetts and is impounded twice after entering Connecticut, first at the Colebrook River Reservoir and then the West Branch Reservoir. It has been suggested that *D. geminata* is more likely to occur in rivers that are regulated by dams because of stable stream flows and constant cooler temperatures (Spaulding and Elwell 2007). The MDC operates West Branch Reservoir and has an agreement to maintain a minimum discharge from the nutrient-poor hypolimnion. This very cold water is essential to support a highly managed destination trout fishery for North American and international anglers, with over 116,000 angling

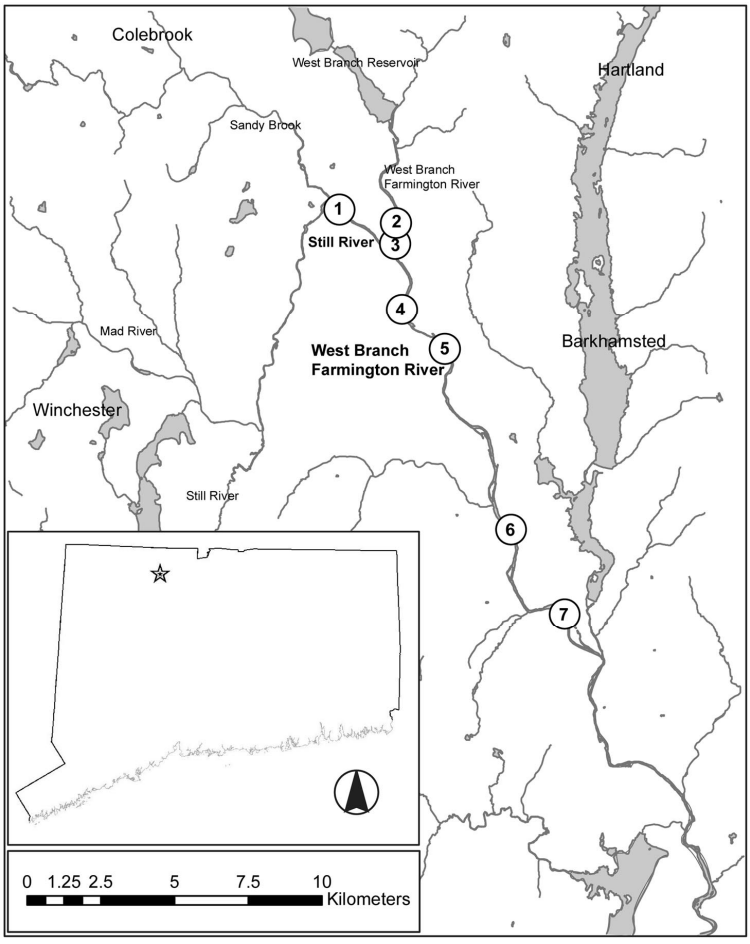

Figure 1. Map of the sampling locations at the Still River and West Branch of the Farmington River in Connecticut (corresponding to sites listed in Tables 1 and 2). Site 1 represents the Still River. Sites 2–7 represent the West Branch of the Farmington River. Site 2 is the location of the mixed stalk-forming diatoms. Site 3 represents the location of the abundant growth of *Didymosphenia* sp. Site 6 represents the location of the abundant growth of *Cymbella janischii.* Site 7 represents the location where *Gomphoneis minuta* was growing.

hours estimated annually (C. Bellucci, CT DEEP, pers. comm.).

Approximately 3 km downstream of the Goodwin dam is the confluence with the Still River (270 km^2). Thirty percent is comprised of the Sandy Brook sub-regional basin, which is over 86% forested and has minimal human disturbance with less than 3.7% impervious surface, minimizing nonpoint source pollution runoff considered a contributing factor for the growth of algae. The remaining 70% comprises the Still River sub-regional basin. At the lower end of this catchment is the city of Winsted, with highly urbanized land use and a waste water treatment facility with a permitted maximum allowable final effluent discharge of 13,250 m^3/d with a 10 year average of 5,678 m^3/d (C. Bellucci CT DEEP, pers. comm., MDC 2013). In addition the permitted maximum daily discharge for total N is 50 mg/l/d.

Water Sampling

Historically, the CT DEEP has monitored the water chemistry of these rivers. At the sites illustrated in Figure 1 and listed in Tables 1 and 2, water samples were taken by grab sampling at depths from 38 – 76.2 cm on 22 May, 2013 and analyzed by the United States Geological Survey (USGS) in East Hartford, Connecticut (Table 2). Also, water samples from the Still and West Branch of the Farmington Rivers were taken and transported to the USGS in East Hartford for analysis of total N and SRP on 12 June, 2013 (Table 2). For the remainder of the sampling dates the authors of this study monitored river water *in situ* for temperature, pH, and conductivity using an YSI® 30 portable hand-held metering probe. Samples were placed in 125 ml sterile wide mouthed poly containers, placed in a cooler and

Table 1. Location and characteristics of sites 1–7 of the Still River and the West Branch of the Farmington River in Connecticut, USA.

Site number	Locality	River and site characteristics
1	Still River, Colebrook 41.967° N, 73.033° W	Larger rocks, open river deeper channel, shaded east and west banks, no impoundments, non-regulated free flowing with an average flow rate of 6.145 m^3/s, mid-sized wastewater treatment plant located 4.7 km upstream from the confluence West Branch of the Farmington and Still River
2	West Branch Farmington River Riverton USGS, Barkhamsted 41.962° N, 73.0176° W	Larger rocks, deeper channel, shaded east and west, regulated flow, impoundments, above the confluence of the West Branch of the Farmington and Still River
3	West Branch Farmington River Riverton Cemetery, Barkhamsted 41.960° N, 73.017° W	Open river channel, shaded - western bank, full morning and afternoon sun, regulated flow, impoundments, above the confluence of the West Branch of the Farmington and Still River, cobbles and boulder substrate, riffles and an average flow rate of 8.9 m^3/s
4	West Branch Farmington River 1 km below confluence, Barkhamsted 41.957° N, 73.015° W	Open river channel, shaded - western banks, morning and afternoon sun, below the confluence of the West Branch of the Farmington and Still River
5	West Branch Farmington River Whittemore Recreation Area, Barkhamsted 41.945° N, 73.016° W	Small vegetated islands with rushes, grasses sedges, shaded - western bank, morning and afternoon sun, shallower channel, cobbles and boulder substrate, riffles
6	West Branch Farmington River Pleasant Valley, Barkhamsted 41.897° N, 72.984° W	Small vegetated islands with rushes, grasses, sedges with full sun, shallower wider channel, cobbles and boulder substrate, riffles
7	West Branch Farmington River Black Bridge, New Hartford 41.877° N, 72.965° W	Open river channel, shaded - western bank, morning and afternoon sun, shallower wider channel, cobbles and boulder substrate, riffles

transported to the Center for Environmental Sciences and Engineering Analytical Services (CESE) at the University of Connecticut in Storrs, a Department of Health certified lab. CESE tested river samples for SRP, total N, and pH (Table 2). Statistical analyses were performed using IBM SPSS Statistics version 21. Log transformation was employed after testing the assumption of normality. Analysis of variance (ANOVA) was used to determine if significant differences existed among samples which contained *Didymosphenia* sp. and those that did not for each of the water quality parameters (SRP, total N, and temperature). Box plots were prepared to illustrate the levels of SRP, total N, and the water temperature across the seven sites, with the presence of different diatoms indicated. We also plotted the frequency of *Didymosphenia* sp. against the levels of SRP, for the 19 distinct water samples (as shown in Bothwell et al. 2014, for *D. geminata*).

Diatom sampling

Benthic samples were collected from several locations in the West Branch of the Farmington and Still Rivers (see Table 1 and Figure 1). Sampling took place in the locations where the putative *D. geminata* had been reported from collections made in 2011. Grab samples of mucilaginous tufts were pulled from rocks and taken from vegetation and placed in Whirl-Pac® bags. The latter were placed on ice and transported to the lab for processing. Sampling took place weekly or two times a month during snow and ice cover. All samples were stored at 4°C until further processing.

Diatom preparation

Diatom samples were simmered on a hot plate in a 1:1 ratio of water and 68% nitric acid to oxidize organic matter, after which the samples were removed from the hotplate to cool. Deionized water was used to rinse the samples of the acid, and then the samples were centrifuged to concentrate the diatom frustules at 600 g to avoid frustule damage. The process of rinsing included the addition of deionized water, centrifuging and the removal of supernatant 4–5 times or until the pH was neutral.

Light microscopy and scanning electron microscopy

Prior to acid washing, samples were placed on a microscope slide with a coverslip overlain and then viewed at ×200 and ×400 magnifications using a BX 60 Olympus microscope. The diatom sample slurry was air dried onto microscope

coverslips, then used to make permanent slides with the mounting medium NAPHRAX®. The diatom frustules were examined at ×1000 magnifications with a BX 60 Olympus microscope. Images were captured using an Olympus DP 25 color digital camera (2560 × 1920 pixels) with Olympus cellSens software. The diatoms on these slides were identified based on their morphological characteristics according to Krammer and Lange-Bertalot (1988), Round et al. (1990), and three online databases, the ANSP Algae Image Database (http://diatom.ansp.org/algae_image/), Diatoms of the United States (http://westerndiatoms.colorado.edu/), and the Great Lakes Image Database: (http://www.umich.edu/~phytolab/GreatLakesDiatomHomePage/top.html).

For SEM, single diatom cells were isolated using a microscope at x100 magnification with a micropipette and transferred onto 25 mm, 3 µm pore polycarbonate Millipore filters (Lang and Kaczmarska 2011). The filters were adhered to SEM stubs with double-sided tape. *Cymbella janischii* and other diatom samples were prepared following the methodology of Morales et al. (2001) the stubs were coated for 1 min at 1.8 kV with gold/palladium using a Polaron sputter coater. The stubs were viewed with the field emission Leo/Zeiss DSM 982 and a field emission FEI Nova Nano 450 scanning electron microscope located at the University of Connecticut Electron Microscopy lab. Image plates were created using Adobe® Creative Suite® 6 Photoshop.

Results

The Still River and the West Branch of the Farmington River are distinct river systems (Figure 1). The water quality data and physical attributes presented in Tables 1 and 2 illustrate that the two rivers have diverse water chemistry, temperature, flow regimes, geomorphology, and sunlight availability due to canopy coverage. These rivers also differ in the benthic diatom taxa present (Table 2). For the Still River (site 1, Tables 1, 2), the mean (antilog ± standard deviation) total N concentration was 413.0 ± 242.5 µg/l, the mean SRP concentration was 13.3 ± 7.4 µg/l, and the mean water temperature was 9.12 ± 4.36°C. *Didymosphenia* sp. and *C. janischii* were not observed at this site over the entire sampling period. At site 2 (Tables 1, 2) the mean total N concentration was 258.0 ± 57.5 µg/l, the mean SRP concentration was 4.12 ± 2.9 µg/l, and the mean water temperature was 8.37 ± 0.65°C, with minimal sunlight exposure from bridge and canopy shading. There was a mixture of the stalk-forming species, *Cymbella affinis, Gomphonema truncatum,* and *Gomphoneis minuta* at this site. Stalk material from a mixture of the three common native diatom taxa covered the substrate from November, 2012, through December, 2012 without *Didymosphenia* sp. Visible growth occurred again in May and June, 2013 with *C. janischii* (although limited and patchy) and *Didymosphenia* sp. present (Figure 2), with the mixture of species restricted to an area of approximately 15 m bank to bank. *Didymosphenia geminata*, described and illustrated by Spaulding and Elwell (2007) and Spaulding (2010), was not detected at this site or during this study. Notably, thick mats with 90% coverage of the morphologically different *Didymosphenia* sp. dominated one segment of the West Branch of the Farmington River above the river confluence (site 3). At site 3 the mean total N concentration was 225.0±31.7 µg/l; the mean SRP concentration was 2.88 ± 1.0 µg/l; and the mean water temperature was 6.09 ± 2.9°C. Site 3 had a wider channel,

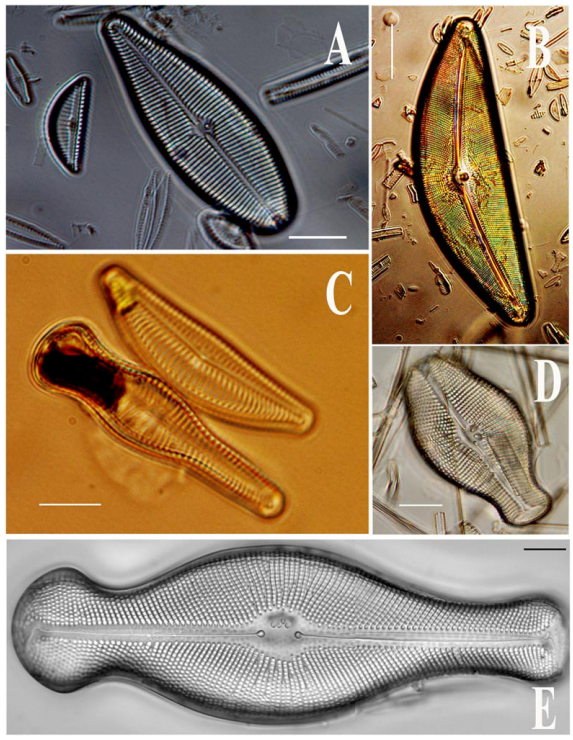

Figure 2. LM images **A**. *Gomphoneis minuta*. **B.** *Cymbella janischii*. **C.** *Cymbella affinis* and *Gomphonema truncatum*. **D.** *Didymosphenia* sp. E. *Didymosphenia geminata* for comparison with **D** (**E** image courtesy of Sarah Spaulding). Scale bars represent 10 µm in **A, C, D**, and **E.** Scale bar represents 20 µm in **B.** Photomicrographs by D. Khan-Bureau.

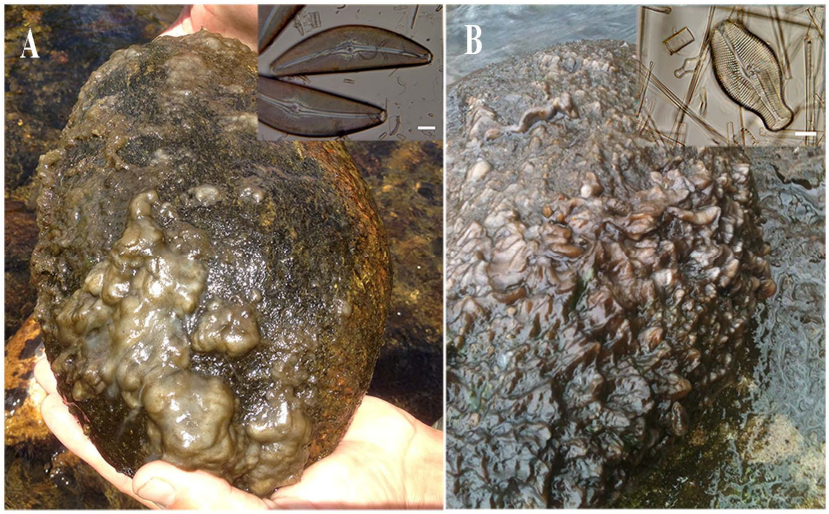

Figure 3. Rocks covered with mucilaginous stalk growth from the West Branch of the Farmington River in Connecticut. **A.** *Cymbella janischii* stalk growth. Inset: cleaned *C. janischii* cells. **B.** *Didymosphenia* sp. stalk growth. Inset: cleaned *Didymosphenia* sp. cell. Scale bar represents 20 µm in image A and 10 µm in image B. Photographs by D. Khan-Bureau.

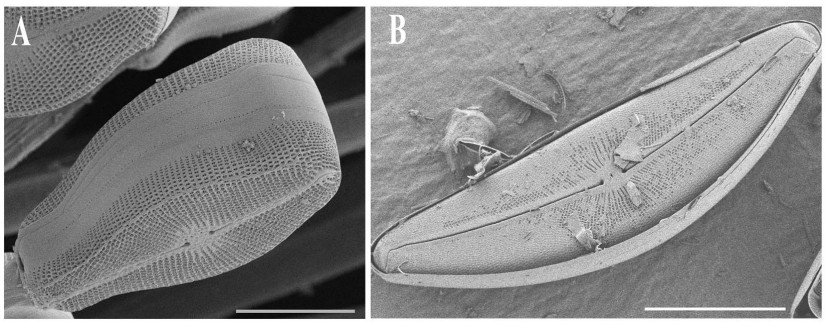

Figure 4. SEM images of **A.** *Didymosphenia* sp. and **B.** *C. janischii*. Scale bar represents 20 µm in A and 50 µm in B. SEM images by D. Khan-Bureau.

is shallower (76 cm) and had abundant sun (Tables 1, 2) unlike sites 1 and 2. The bloom of *Didymosphenia* sp. covered 1 km, 50–60 m bank to bank, with stalked material forming 2.0–5.0 cm thick on the rocky substrate. LM confirmed a combination of other diatoms, benthic macro-invertebrates and river debris within *Didymosphenia* sp. stalked mats. As the river flows further downstream, and particularly after the confluence of the Still and the West Branch of the Farmington Rivers, the nutrient levels and temperatures increase (Table 2). Further downstream, past the confluence (sites 4, 5), *Didymosphenia* sp. was observed in late May, 2013, although the growth was limited to just 1–3 tufts observed. LM and SEM observations confirmed *Cymbella janischii* at site 6 in July, 2012 and stalk growth absent by early October, 2012, as green algae colonized the site. The *C. janischii* mats were thick and covered the substrate bank to bank approximately 40 m wide and 0.5 km each side of the islands (Figure 3). *Encyonema* cf. *minutum* tufts were seen at site 6 further downstream of *C. janischii*, although patchy. Mixing continued below the confluence for approximately 11 km as demonstrated by physicochemical properties (Table 2, site 7). LM confirmed *Gomphoneis minuta* tufts growing at site 7 in early November, 2012, but growth was not observed in December, 2012.

SEM was used to examine the walls of *Didymosphenia* sp. and *C. janischii* for identification purposes because these species have similar mucilaginous growth and both are new records in Connecticut. *Cymbella janischii* cells are asymmetrical whereas *Didymosphenia* sp. cells are not (Figure 4). *Cymbella janischii* cells are large, normally 130–360 µm (Kociolek and Stoermer 1988; Round et al. 1990; Metzeltin and Lange-Bertalot 1995; Bahls 2007; Kociolek 2011). The *C. janischii* cells collected for this study ranged in size from 130–150 µm. The cells of *Didymosphenia* sp. ranged consistently in size from 50–60 µm with few at 38 µm and 68 µm and formed macroscopic mucilaginous strands as long as 18

cm. The size range for *D. geminata* in the U.S.A. is 87–137 µm (Spaulding 2010, Figure 2E). The *Didymosphenia* sp. cells were also unusual because of their compressed morphology in contrast to *D. geminata* (Figure 2D, E).

The water chemistry data were found to be log-normally distributed thus statistical analyses were performed on log-transformed values. ANOVA showed no significant difference among samples that had *Didymosphenia* sp. and those that did not, for total N, SRP, and temperature. A lack of significant difference could indicate no difference or be due to the small sample size, therefore additional water quality sampling would be required to test the relationship between water chemistry and absence/presence of *Didymosphenia* sp. Box plots of total N, SRP, and the T across the seven sites illustrate the distribution of different diatom taxa across the sites and suggest that chemical characteristics (Figure 5) may be linked to environmental preferences but further regular analysis of site water parameters is needed to determine potential correlations. Lastly, we show the occurrence of *Didymosphenia* sp. at low SRP levels (Figure 6) similar to Bothwell et al. (2014) for *Didymosphenia geminata.*

Discussion

Confusion about Didymosphenia geminata *in the Northeastern U.S.A.*

In March, 2011 tufts collected by the CT DEEP from the West Branch of the Farmington River in Connecticut were sent to the Vermont Department of Conservation (VT. DEC) for identification. Using LM, the sample was identified as *D. geminata*, although the cells were on the low end of the size range for this species, having been roughly estimated at 80–90 µm long. Identification cannot be verified since the lab in which the voucher specimen was stored was destroyed during Tropical Storm Irene. No other samples exist (L. Matthews, VT DEC, pers. comm.). The Connecticut DEEP speculates that the purported *Didymosphenia geminata* was not found again in 2011 and in early 2012 because the combination of Tropical Storms Irene and Lee created significant mechanical scouring that may have contributed to the reduction of the population of this diatom (M. Becker CT DEEP, pers. comm.). During these storms the associated rainfall created historic flows and channel alterations even though the river flow is highly regulated. It is hypothesized that stable river flows

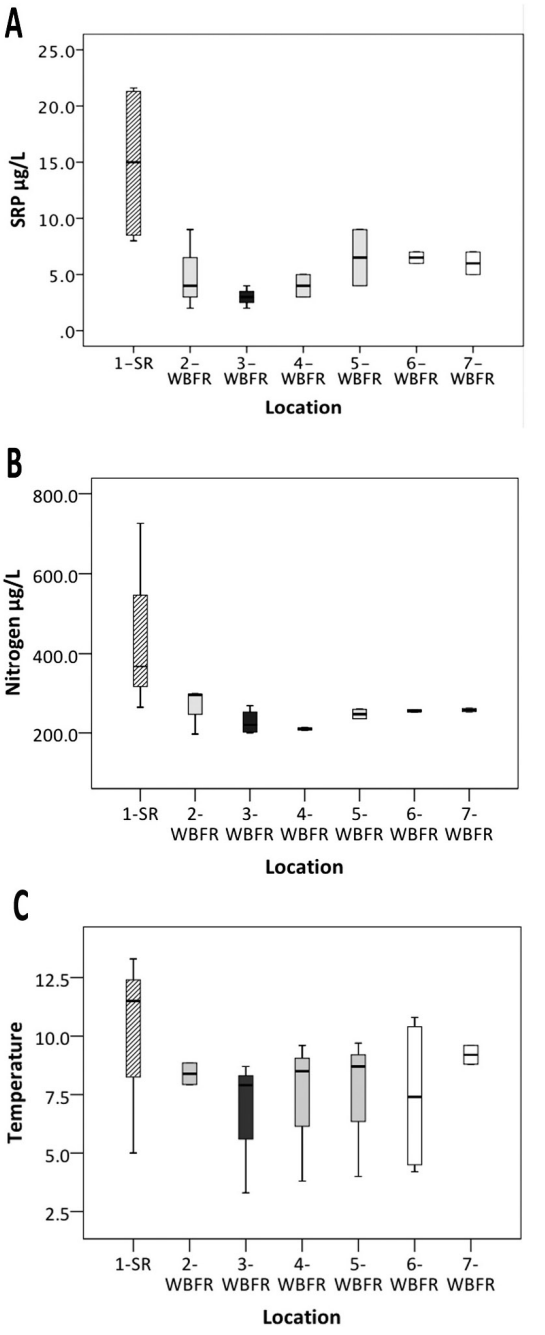

Figure 5. **A**. The distribution relationship of *Didymosphenia* sp. and SRP levels across the 7 sites surveyed. **B.** The distribution relationship of *Didymosphenia* sp. and total nitrogen levels across the 7 sites surveyed. **C.** The distribution relationship of *Didymosphenia* sp. and temperature across the 7 sites surveyed. The hatched lines represent absence of *Didymosphenia* sp. at the Still River site 1. At site 2 light grey boxes represent *Didymosphenia* sp. observed but not abundant and occurred with a mix of stalk-forming diatoms. At site 3 dark grey filled box represent *Didymosphenia* sp. was abundant and blooming 1 km by 50 m. At site 4 and 5 light grey boxes represent only 1–3 tufts observed. At sites 6, 7 unfilled boxes represent native species present, absent of *Didymosphenia* sp. and *C. janischii*.

and secure substrates allow for the establishment of *D. geminata* colonies (Spaulding and Elwell 2007). From the observations made in this study, a stable regulated flow appears to be suitable for this morphologically distinct genus of *Didymosphenia* as well. Many reports of *D. geminata* blooms are from deep, cold, lake-fed, flow restricted and regulated streams (Kilroy 2004; Kumar et al. 2009) and the population of *Didymosphenia* sp. found in this study may share similar environmental tolerances and physio-chemical traits (Figures 5, 6). Further work is underway to understand the morphological and genetic variation of *Didymosphenia* sp. to verify whether this is a new taxon.

Another nuisance stalked diatom, Cymbella janischii

During the summer survey of July 2012, prolific mucilaginous clumps were found (site 6). LM revealed the identification as *Cymbella janischii*. This species, endemic in the Pacific Northwest, was recently reported in Japan and is reported here as present in Connecticut (Bahls 2007; Suzawa et al. 2011). *Cymbella janischii* may have been transported from the Pacific Northwest to the Northeast U.S.A. to as far away as Japan by angler's boots, angler equipment, and by other means (Suzawa et al. 2011). *Cymbella janischii* has similar mucilaginous stalk growth as *D. geminata* (and likewise as *Didymosphenia* sp.) (Pite et al. 2009, Whitton et al. 2009; Suzawa et al. 2011). These taxa are thought to be potentially nuisance, aggressively forming thick gray mats on substrates, even in their native habitats (Spaulding and Elwell 2007). *Didymosphenia geminata* and *C. janischii* have become more problematic in recent years due to expansions of their geographical ranges (Bahls 2007; Kumar et al. 2009; Pite et al. 2009).

Study site habitat preference

The absence of *Didymosphenia* sp. from the Still River, site 1, suggests that environmental factors such as higher levels of SRP and total N with increased water temperatures, and reduced light availability, may limit the growth of this taxon. Discharge from the city of Winsted's waste water treatment plant contributes nutrients to the free-flowing Still River according to the CT DEEP approved permit, CT0101222, for the Town of Winchester 2005 NPDES 2005. Whereas the upper extent (sites 2, 3) of the West Branch of the Farmington is flow-regulated by the MDC Goodwin dam, which discharges very cold oligotrophic waters. Both rivers are heavily visited by anglers throughout the year, and given the close proximity of the two rivers, anglers typically fish in both rivers in one day. Blooms of *Didymosphenia* sp. were recorded only at the upper extent of the West Branch of the Farmington (site 3). Despite the close proximity of the rivers, the spread of *Didymosphenia* sp. by anglers and recreationalists has not occurred in the Still River. Bothwell et al. (2012) reported that the growth of *D. geminata* ceases in river reaches downstream of point source nutrient outfalls. It is possible that *Didymosphenia* sp. does not aggressively grow in higher SRP, total N and warmer waters, as was proposed for *D. geminata* (Bothwell and Spaulding 2011; Kilroy and Bothwell 2011; Bothwell et al. 2012). This hypothesis needs to be tested further.

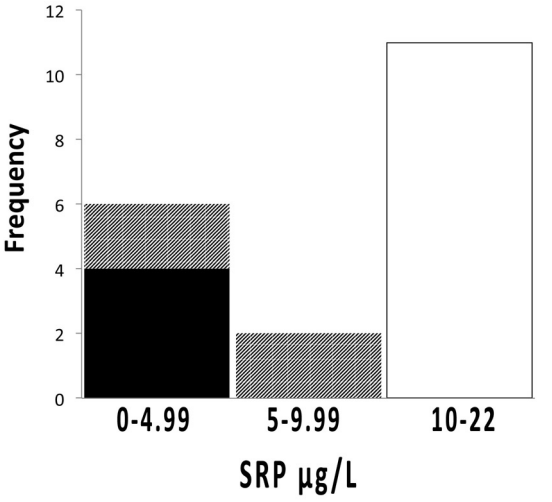

Figure 6. The frequency of occurrence of *Didymosphenia* sp. in 19 river samples as a function of SRP levels. Filled boxes=abundant and blooming, hatched boxes=present but not blooming, unfilled box= not observed.

Analysis of limited grab samples for SRP and total N, and water temperature indicate that the West Branch of the Farmington River may possibly have narrower water chemistry and temperature ranges. Our observations of *Didymosphenia* sp. echoes the recent work by Bothwell et al. (2014), on *D. geminata,* shown to grow prolifically because of SRP limitation. Our location affords a unique opportunity to quantify various physical and chemical variables with blooms of *Didymosphenia* sp. in a natural environmental setting. It may be that the SRP rich waters of the Still River and the mixing at the confluence of the Still River and the West Branch Farmington River

Table 2. Water chemistry and diatom survey data from seven sites the West Branch of the Farmington and Still Rivers in Connecticut, U.S.A. Major stalk-forming diatom taxa were surveyed on all dates shown, and their occurrences are indicated as follows: — = taxon not observed; + = taxon present but at low abundance or of limited distribution; ++ = taxon present and abundant.

Site	Sample Date	SRP µg/L	N µg/L	pH	T °C	Cond. µS/cm	Stalk- forming diatom taxon					
							Didymo-sphenia sp.	*Cymbella janischii*	*Cymbella affinis*	*Gompho-nema truncatum*	*Gomphoneis minuta*	*Encyonema* cf. *minutum*
1	22 Mar. 2013	8	264	6.8	5	n.d.	—	—	—	—	—	—
	14 May 2013	21	367	6.7	11.5	n.d.	—	—	—	—	—	—
	22 May 2013*	21.6	726	n.d.	n.d.	n.d.	—	—	—	—	—	—
	28 May 2013	9	n.d.	6.9	13.3	111	—	—	—	—	—	—
2	29 Nov. 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	—	++	++	++	—
	22 Mar. 2013	n.d.	n.d.	n.d.	3.3	n.d.	—	—	—	—	—	—
	14 May 2013	2	197	6.8	7.93	n.d.	—	—	+	+	+	—
	22 May 2013*	<4	295	n.d.	n.d.	n.d.	—	—	+	+	+	—
	28 May 2013	9	n.d.	6.8	8.85	86.5	+	+	++	++	++	—
	12 June 2013*	<4	298	n.d.	n.d.	n.d.	+	+	++	++	++	—
3	29 Nov. 2012	n.d.	n.d.	n.d.	n.d.	n.d.	++	—	—	—	—	—
	22 Mar. 2013	3	236	6.6	3.3	n.d.	++	—	—	—	—	—
	14 May 2013	2	204	6.9	7.9	n.d.	++	—	—	—	—	—
	28 May 2013	n.d.	200	6.8	8.7	87.1	++	—	—	—	—	—
	12 June 2013*	<4	268	n.d.	n.d.	n.d.	++	—	—	—	—	—
4	22 Mar. 2013	n.d.	n.d.	n.d.	3.8	n.d.	—	—	—	—	—	—
	14 May 2013	3	213	6.8	8.5	n.d.	—	—	—	—	—	—
	28 May 2013	5	207	6.8	9.6	88.6	+	—	—	—	—	—
5	10 July 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	—	—	—	—	—
	4 Dec. 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	—	—	—	—	—
	22 Mar. 2013	n.d.	n.d.	n.d.	4	n.d.	—	—	—	—	—	—
	14 May 2013	9	259	6.8	8.7	n.d.	+	—	—	—	—	—
	28 May 2013	4	235	6.9	9.7	96.4	+	—	—	—	—	—
6	10 July 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	++	—	—	—	—
	4 Dec. 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	—	—	—	—	+
	22 Mar. 2013	n.d.	n.d.	n.d.	4.8	n.d.	—	—	—	—	—	—
	14 May 2013	7	258	n.d.	10	n.d.	—	—	—	—	—	—
	28 May 2013	6	252	n.d.	10.8	100.7	—	—	—	—	—	—
7	10 July 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	—	—	+	+	—
	29 Nov. 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	—	—	—	+	—
	4 Dec. 2012	n.d.	n.d.	n.d.	n.d.	n.d.	—	—	—	—	+	—
	22 Mar. 2013	n.d.	n.d.	n.d.	4.2	n.d.	—	—	—	—	—	—
	14 May 2013	7	261	6.8	8.8	n.d.	—	—	—	—	—	—
	28 May 2013	5	253	7	9.6	88.6	—	—	—	—	—	—

† *Data analyzed by the USGS for this project; SRP listed as <4 µg/L when not detectable; µg/L=1ppb, n.d = no data
Connecticut DEEP water quality data is available upon request

have limited the downstream expansion of *Didymosphenia* sp. Our preliminary results suggest that *Didymosphenia* sp. blooms are related to SRP limitation but other environmental factors may contribute such as the depth and width of the channel, sunlight availability, flow regulation, and other physical and chemical parameters. Collection of additional chemical, stream flow, and algal community structure, cell density and biomass data will help to test our hypothesis.

Whereas many state regulatory agencies consider *D. geminata* to be invasive in the Northeastern U.S.A., the literature supports a native distribution that is circumpolar in the Northern hemisphere (Krammer and Lange-Bertalot 1988; Blanco and Ector 2009; Kumar et al. 2009). The debate continues as to whether *Didymosphenia geminata* should be classified as invasive to the Northeastern states. We documented two previously unreported taxa, *Didymosphenia* sp. and *C. janischii*. Were these taxa transported via anglers boots and equipment and by other vectors (Kirkwood et al. 2007; Bothwell et al. 2009) or are *D. geminata* and *Didymosphenia* sp. native but rare, now becoming nuisance due to changing environmental conditions throughout their ranges (Valéry et. al 2009; Bothwell 2014)? During our study documentation of *C. janischii*, a diatom not previously found east of the Rocky Mountains (Bahls 2007; Kumar et al. 2009; Suzawa et al. 2011) was

confirmed and its presence was adjacent to a well-travelled footpath to the river, suggestive of an anthropogenic source.

Given that diatoms are important biological indicators and are often used for water quality assessments, it is crucial to identify diatoms accurately (Morales et al. 2001; Mann et al. 2010; Pniewski et al. 2010). Further work is needed to identify this unfamiliar *Didymosphenia* sp. including collection of information on the morphological variation present as well as comparison of this taxon to other species of *Didymosphenia* using genetic data. The presence and excessive stalk growth of *Didymosphenia* sp. in the West Branch of the Farmington River suggests a need for further monitoring of these species to determine which environmental conditions are associated with nuisance growth.

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