Hydrobiologia (2020) 847:1057–1069
https://doi.org/10.1007/s10750-019-04168-y

PRIMARY RESEARCH PAPER

Didymosphenia geminata blooms are not exclusively driven by low phosphorus under experimental conditions

Derek C. West · Jared A. Balik · Mitchell Owens · Brad W. Taylor

Received: 26 April 2019 / Revised: 13 December 2019 / Accepted: 19 December 2019 / Published online: 2 January 2020
© Springer Nature Switzerland AG 2020

Abstract Despite two decades of research, the cause of blooms of the diatom *Didymosphenia geminata* remains uncertain. Blooms have been associated with low nutrient, oligotrophic streams. In this study, we used available data from across the globe and conducted experiments to determine how *D. geminata* responds to soluble reactive phosphorus (SRP) concentrations. Globally, *D. geminata* blooms have been found in streams with SRP below 11 µg P l^{-1}. In North America, blooms only occurred at high equivalent latitudes when SRP was very low, whereas at lower latitudes blooms were observed under higher SRP concentrations. Using an in situ experiment, we found that following physical removal of *D. geminata* from stones, regrowth did not occur despite low SRP concentrations. In a second experiment, we found that there were no differences in *D. geminata* growth between a treatment which depleted SRP and a treatment which maintained elevated springtime SRP levels. These findings indicate that *D. geminata* blooms do not always form when SRP is low, even when cells are present. Bloom formation that is not exclusively related to low SRP suggests additional chemical or biotic factors, specific physical conditions, a seasonal timing requirement, or some combination of these with low P that are necessary to produce blooms.

Keywords Phosphate · Algae · Diatoms · Regrowth · Stalks

Handling editor: David Philip Hamilton

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10750-019-04168-y) contains supplementary material, which is available to authorized users.

D. C. West · J. A. Balik · B. W. Taylor (✉)
Department of Applied Ecology, North Carolina State University, Campus Box 7617, Raleigh, NC 27695-7617, USA
e-mail: brad.taylor@ncsu.edu

D. C. West · J. A. Balik · M. Owens · B. W. Taylor
Rocky Mountain Biological Laboratory, PO Box 519, Crested Butte, CO 81224, USA

M. Owens
Department of Geography, Indiana University, Student Building 120, 701 E. Kirkwood Avenue, Bloomington, IN 47405-7100, USA

Introduction

Changing environmental conditions frequently cause organisms to alter their growth patterns, changing their physiology in ways that impact growth and interactions with the surrounding environment (Davies & Zhang, 1991; Kleiven et al., 1992; Landry-Cuerrier et al., 2008). Limitation of essential nutrients such as nitrogen and phosphorus can drive changes in aquatic primary producers (Smith, 2003), and among colonial

organisms can change colony dynamics (Veldhuis & Admiraal, 1987; Ma et al., 2014). In some diatoms, nutrient-limited cells produce extracellular polymeric substances (EPS) when photosynthetic rates exceed cellular growth rates (Myklestad, 1995; Staats et al., 2000). Through the production of EPS, diatoms can structure their environment in a variety of ways, including by stabilizing sediments, creating marine snow, or modifying benthic habitat (Alldredge et al., 1993; Sutherland et al., 1998; Larned et al., 2011).

The colonial benthic diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt is capable of producing EPS stalk material that can result in dense macroscopic growths on the river bottom (Bothwell et al., 2014). Unlike most algal blooms which result from a proliferation of cells (Smayda, 1997), stalk material dominates *D. geminata* blooms, making up greater than 99% of the biomass of some blooms (Bray, 2014). Blooms often occur in rivers with very low soluble reactive phosphorus (SRP) concentrations (Bothwell et al., 2014), at or below the concentration detectable by many analytical techniques. In New Zealand, a threshold of 2 μg P l^{-1} has been proposed, above which *D. geminata* blooms do not frequently occur (Kilroy & Bothwell, 2012). In Europe and parts of North America, *D. geminata* blooms can also occur in rivers with moderate (4–10 μg P l^{-1}) SRP (Kawecka & Sanecki, 2003; Whitton et al., 2009; Khan-Bureau et al., 2014; Ladrera et al., 2016). In addition to these data from natural streams, experimental channels (Kilroy & Bothwell, 2011; Bray et al., 2017a) and in situ nutrient enrichments (James et al., 2015; Kilroy & Larned, 2016) suggest that increasing nutrient concentrations, particularly phosphorus, can reduce *D. geminata* blooms.

In addition to low SRP concentrations, *D. geminata* blooms generally require cool water temperatures, a stable hydrological regime, and high light conditions (Kilroy et al., 2008). As a result, blooms frequently occur in tailwaters below dams (Kirkwood et al., 2009), but are also conspicuous in what are often otherwise pristine systems. In recent years, blooms of *D. geminata* have appeared, presumably for the first time, in many locations ranging from Vancouver Island in the 1980s (British Columbia, Canada; Bothwell et al., 2009) to New Zealand in 2004 (Kilroy & Unwin, 2011), Chile in 2010 (Reid et al., 2012), and parts of Europe (Beltrami et al., 2008; Whitton et al., 2009). The blooms have altered ecological interactions in the rivers where they occur (Kilroy et al., 2009; Ladrera et al., 2015). Additionally, blooms are undesirable for human use because they are unsightly (as evidenced by their common name of "rock-snot") and pose difficulties to fishermen due to entanglement of gear in the stalks (Beville et al., 2012).

Current theories on the ecological and adaptive value of stalk production by diatoms suggest that stalk production is a stress response to low nutrients (Hoagland et al., 1993; Myklestad, 1995; Staats et al., 2000; Lewis et al., 2002). In the case of *D. geminata,* the dense mats created by stalks can trap particulate organic matter and impede water flow (Larned et al., 2011), resulting in decomposing particulate organic matter and an accumulation of phosphorus within the matrix of the stalks (Sundareshwar et al., 2011; Bray et al., 2017a). One idea is that the accumulation of organic phosphorus within mats may be accessible via extracellular phosphatase enzymes (Ellwood & Whitton, 2007; Bray et al., 2017b). Additionally, by elevating cells above the streambed, the stalks elevate *D. geminata* above the boundary layer allowing the cells better access to inorganic P in the water column (Hudon & Bourget, 1981; Aboal et al., 2012; Bothwell & Taylor, 2017). Despite the presumed function of stalk production, no studies have shown clearly that stalk-producing *D. geminata* have a higher cellular P content (Bothwell et al., 2012) or some other performance advantage, such as a higher frequency of dividing cells (Kilroy and Bothwell, 2012).

In this study, we used global records and localized experiments to characterize the growth of *D. geminata* in relation to phosphorus conditions. We assembled all available published records of *D. geminata* blooms in which SRP concentrations were measured in order to study how the relationship varies geographically. We used field manipulations of *D. geminata* blooms to determine whether abiotic conditions alone could explain bloom growths. Finally, we used flow-through mesocosms to test the effects of source water and phosphorus availability on *D. geminata* growth.

Methods

Didymosphenia geminata: dissolved phosphate survey

In order to characterize the relationship between worldwide blooms of *D. geminata* and SRP, we

surveyed the literature for all published records of *D. geminata* blooms that included a measurement of soluble reactive phosphorus (SRP). We searched the Web of Science database and Google Scholar for "*Didymosphenia geminata*" and either "phosphate," "soluble reactive phosphorus," "dissolved reactive phosphorus," or "filterable reactive phosphorus," as well as searching for references within each of these records. All records which included an observation of *D. geminata* with stalk material, a location, and SRP concentration were included in the analysis. For each record, location, elevation, and phosphate concentration were recorded. Latitude and elevation were given special consideration since they were available for every record and correlate with other abiotic conditions such as light availability, growing season length, and temperature, which have been shown to impact *D. geminata* growth. Latitude and elevation were combined to give a single metric, termed an equivalent latitude by using the relationship of 1 degree latitude for each 200 m of elevation (Montgomery, 2006). If there were multiple records for a single stream, then the values were averaged and used as a single record. If a dam separated the reaches, then we calculated averages for locations above and below dams. For each region, data were analyzed by regressing SRP concentration against equivalent latitude for each stream site using R (R Core Team, 2019) and JMP (SAS Institute Inc., 2018).

In situ *Didymosphenia geminata* regrowth experiment

In order to examine the potential of *D. geminata* cells to produce stalks and form blooms during conditions in which stalk growth was occurring, we removed *D. geminata* cells and stalks from areas of the streambed and followed regrowth of cells and stalks through the growing season. In the summer of 2012 (7 July), we identified reaches of three different streams [East River (ER), Copper Creek (CC), and Washington Gulch (WG)] in the vicinity of Crested Butte, Colorado that were supporting *D. geminata* blooms. In each stream, 8 replicate blocks were randomly assigned; within each block, three similarly sized cobble (110–220 mm diameter) rocks were selected, two with initially high densities of stalked *D. geminata* (minimum 80% *D. geminata* stalk cover, hereafter referred to as bloom rocks) and one with a low density (maximum 20% *D. geminata* stalk cover, hereafter non-bloom rock). Before initiating the experiment, one bloom rock from each block was gently scrubbed with a nylon brush to remove all *D. geminata* cells and stalks. This resulted in three treatments: bloom, non-bloom, and scrubbed rocks; each replicated 8 times in each of 3 streams. The three rocks constituting a block were placed close together in the stream at similar depths and water velocities and were monitored twice weekly for percent cover and thickness of *D. geminata* for a period of 5 weeks. The standing crop index (SCI) was calculated by multiplying the percent cover by the thickness (cm) of the mat (Entwisle, 1989). Prior to the start of the experiment, SRP was measured in each stream with a standard colorimetric method (Ostrofsky & Rigler, 1987) using a Thermo Scientific GENESYS 10S UV–VIS Spectrophotometer (Waltham MA, USA). At the beginning of the experiment, the depth and water velocity were measured at the location of each rock using a SonTek FlowTracker (Xylem Inc, San Diego, CA, USA). Additionally, on day 6 in Copper Creek and the East River and on day 8 in Washington Gulch, we scraped a small sample using a 7.1-cm^2 flexible plastic template from each bloom, non-bloom, and scrubbed rock in three randomly selected blocks and preserved the samples in Lugol's iodine. For each sample, we determined the frequency of dividing *D. geminata* cells following Bothwell & Kilroy (2011) as a metric of cell division rate.

To analyze the growth of *D. geminata* through the course of the experiment, we ran a linear regression on the SCI for each rock individually. For each stream, we used a one-tailed *t* test to test whether the slope of the bloom treatment growth rate was greater than zero. If the slope was greater than 0, indicating growth during the experiment, we then used an ANOVA, followed by a post hoc Tukey–Kramer test to examine the differences between the treatments. Differences in water velocity among the treatments were tested by an ANOVA and a post hoc Tukey–Kramer test. To examine the differences in the frequency of dividing cells among bloom, non-bloom, and scrubbed rocks, we used ANOVA with treatment as a fixed effect and stream as random effect for the analysis across the streams and block as a random effect for the analysis within each stream.

Didymosphenia geminata channel mesocosm experiment

In the summers of 2016 and 2017, an array of 12 experimental channels was erected along the East River at the Rocky Mountain Biological Lab in Gothic, CO. Channels consisted of white plastic rain gutters (3.05 × 0.13 × 0.08 m) placed side-by-side. Water was diverted from either the East River (2016) or an unnamed tributary (2017) to a 200-L settling tank, from which water was piped into each of the channels via vinyl tubing. Water exiting each channel emptied into separate 5-L holding tanks and then a known fraction of this water was recirculated via a magnetic pump to the head of the channel.

Experimental treatments

In 2016, three different experimental treatments were used. In each, water flow was maintained at a rate of 9.3 l min^{-1}. In the first treatment, termed flow-through (FT), 90% of the flow was from the river and the remaining 10% was recirculated water that had already passed through the channel. As this treatment was dominated by stream flow water, it was intended to track the natural conditions in the stream where phosphorus concentrations were expected to fall through the growing season. In the second treatment, termed recirculated (RC), 10% of the flow was from river and 90% was from recirculated water that had already passed through the channel. This treatment was intended to maximize biological uptake, thus reducing phosphorus concentrations. The final treatment, termed phosphorus maintenance (PM), was structured identically to the RC treatment, except that the phosphorus concentration in the channels was maintained near the initial concentration by pumping in a 92 μg P l^{-1} sodium phosphate (Na_2HPO_4) solution at a rate of 5 ml min^{-1}. This rate of phosphorus addition was based on previous measurements of whole-stream phosphorus uptake (Taylor, unpublished) and intended to maintain phosphorus at the concentration when the experiment began.

We used unglazed ceramic tiles (5 × 5 cm) as substrata for diatom colonization and growth. In 2016, tiles were placed in the East River for 12 days to allow colonization by *D. geminata* and other microorganisms. Prior to initiating the experiment, the channels were conditioned by flowing stream water through them, as well as recirculating water with the pumps for a period of 6 days. To further promote microbial colonization of the channels, rocks from the adjacent streambed were placed at the head of each channel for the 6-day period and a slurry of diatoms and other microorganisms was scrubbed from biofilm-covered stream rocks and poured into the recirculating holding tanks at the ends of each channel.

In 2017, the experiment was repeated with several modifications. Water was diverted from an unnamed tributary which does not experience *D. geminata* blooms instead of the East River so that no stalk material could be added from the ambient source water. The phosphorus maintenance treatment was removed, leaving 6 replicates each of the FT and RC treatments. Three channels from each treatment were initially given a single dose of 100 μg P l^{-1} for 1 h before the experiment began, resulting in 4 treatments: FT, FT enriched (FT+), RC, and RC enriched (RC+). Rather than pre-colonizing the tiles by in situ stream incubation, a slurry of *D. geminata* cells was added to each channel and allowed to incubate overnight resulting in an initial density of 300 cells/cm^2. The phosphorus dose was then added to the enriched channels and following the 1-h incubation, all channels were run as recirculating for 24 h to ensure even colonization across treatments.

Sampling

To sample the abundance of *D. geminata* in the channels, three times weekly one tile from the upstream portion and one tile from the downstream portion of each channel were removed, scraped with a razor blade, and then scrubbed with a nylon brush to remove all cells and stalk material. The resulting slurry was preserved in Lugol's iodine and counted at a later date. *D. geminata* density was enumerated using a compound microscope and a Palmer cell, depending on the density of cells either the whole cell was counted, or 20 fields of view were counted at × 100 magnification. *D. geminata* stalks were enumerated under a dissecting microscope at × 40, and the total length and the number of tips of the stalks were counted. Stalk width was measured on three stalks from each channel on each date. The biovolume of stalks was calculated by assuming equal branching within the stalks, such that the average length of a segment is given by the formula:

$$L_S = L_T(\log_2(N) + 1)^{-1}$$

where L_S is the length of the segment, L_T is the total length of the stalk being measured, and N is the number of tips on the stalk. The $\log_2$ term is always rounded up to the nearest integer. The biovolume was then calculated assuming that the stalks were cylindrically shaped (Hillebrand et al., 1999):

$$v = \pi r^2 L_S$$

where v is the biovolume and r is the radius, estimated as the average stalk width for each treatment on each date divided by two.

Nutrients were sampled by taking water samples from the outflow of the channels. All nutrient samples were filtered through Pall Gelman AE filters and placed on ice until being brought back to the lab. SRP contamination from filters was < 0.04 µg l^{-1} following three 30–40 ml rinses of the filter and filter assembly with sample water. P contamination was also minimized by dedicating specific labware for P sampling and analyses and by using new 50-ml centrifuge tubes for each sample. SRP samples were concentrated 70-fold using a Sargasso Seawater magnesium hydroxide coprecipitation (MAGIC) method (Anagnostou & Sherrell, 2008) and then analyzed with the standard colorimetric method (Ostrofsky & Rigler, 1987). Using the MAGIC method, our SRP method detection limit was 0.04 µg l^{-1}. Ammonium samples were taken in 20-ml amber bottles and measured using the fluorometric method established by Holmes et al. (1999) and modified by Taylor et al. (2007), using a Turner Trilogy Fluorometer (Sunnyvale CA, USA). Our ammonium method detection limit was 0.4 µg l^{-1}. Nitrate samples were measured using the second derivative method on the Thermo Scientific GENESYS 10S UV–VIS Spectrophotometer (Crumpton et al., 1992). SRP was measured three times weekly in 2016 and weekly in 2017, while ammonium and nitrate were measured weekly. The nitrogen to phosphorus atomic ratio (N:P) was calculated on the dates on which both N and P were measured using the total N ($NH_4^+ + NO_3^-$) and SRP atomic ratios. Additionally, the trace nutrients such as silica (Si), sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), chlorine (Cl), sulfur (S), and dissolved organic carbon (DOC) were analyzed on days 0, 22, and 44 for the 2016 experiment by the New Hampshire Analytical Lab (J. Potter, UNH, Durham, NH USA). To test for differences between the treatments in N and P and *D. geminata* growth, we used general linear models with treatment and day as fixed effects and channel as a random effect. Differences between treatments for the trace nutrients were tested using an ANOVA for each nutrient on each date. These statistical analyses were performed using R (R Core Team, 2019).

Results

Didymosphenia geminata: phosphate survey

We located records of *D. geminata* blooms and SRP concentration at 50 locations from 15 different studies, including this one, with records from North America, Eurasia, New Zealand, and South America (Table S1). Didymo blooms were observed in streams with SRP concentrations ranging from 0.1 to 10.6 µg P l^{-1} (Fig. 1). Across all locations, there is not a significant relationship between SRP and equivalent latitude ($F_{1,45} = 0.24$, $P = 0.63$), although the pattern differed by region. Within North America, which had the majority (28) of the records in the dataset, there was a negative relationship between SRP and equivalent latitude (slope = − 0.17, $F_{1,26} = 11.15$, $P < 0.003$). Although not significant, Eurasia also had a negative relationship between SRP and equivalent latitude

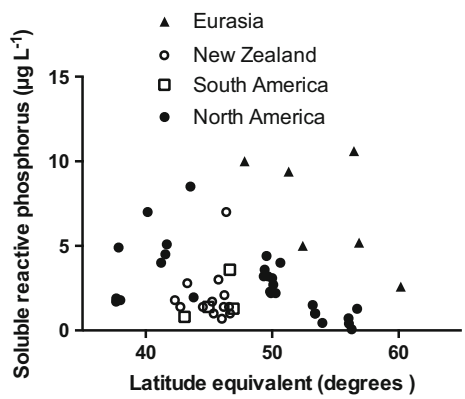

Fig. 1 Worldwide, blooms of *Didymosphenia geminata* have only been observed in rivers with soluble reactive phosphorus (SRP) concentrations less than 11 µg P l^{-1}, within temperate latitudes. Latitude equivalent, an absolute value of latitude, applies a correction to latitude for elevational differences between sites

(slope = − 0.45, $F_{1,4}$ = 2.48, P = 0.19), and in New Zealand, the relationship was influenced by an outlier (with Oreti R. 7 µg P l^{-1}: slope = 0.12, $F_{1,12}$ = 0.14, P = 0.7; without Oreti: slope = − 0.12, $F_{1,11}$ = 0.75, P = 0.4) and in South America, data from only four streams limited assessment of the relationship (slope = 0.42, $F_{1,2}$ = 1.12, P = 0.4).

In situ *Didymosphenia geminata* regrowth experiment

Didymosphenia geminata bloom dynamics were different between the three streams studied in 2012, with only Copper Creek showing evidence of stalk growth during the course of the experiment. SRP was low in all three streams, measuring 1.5 µg P l^{-1} in Washington Gulch and 1 µg P l^{-1} in the East River and Copper Creek at the beginning of the experiment. Copper Creek showed a significant increase in SCI on the bloom rocks between July 6 and August 9, 2012 (t_7 = 4.4, P = 0.003). Meanwhile, in Washington Gulch, the SCI of the bloom rocks decreased significantly between July 12 and August 10, 2012 (t_7 = − 4.19, P = 0.004) and in the East River, there was no significant change (t_7 = − 1.22, P = 0.26). Although the ambient bloom in Copper Creek experienced growth, neither the scrubbed nor non-bloom rocks showed signs of developing blooms (Fig. 2). An ANOVA showed that there were significant differences in the growth rates for the treatments in Copper Creek ($F_{2,21}$ = 13.77, $P < 0.001$). A post hoc Tukey–Kramer test showed that the growth rate was significantly greater on the bloom rocks than the scrubbed or non-bloom rocks ($P < 0.01$), and there were no differences between the growth rates on the scrubbed and non-bloom rocks ($P > 0.05$).

There were no significant differences in water velocities among the treatments in Copper Creek ($F_{2,21}$ = 2.64, P = 0.10). In the East River, water velocity was significantly different among treatments ($F_{2,21}$ = 4.41, P = 0.03) because of 34% higher velocity at the non-bloom rocks compared to the bloom rocks ($P < 0.03$). There were no significant differences in water velocity between the different treatments at Washington Gulch ($F_{2,21}$ = 0.30, P = 0.74).

The frequency of dividing cells was significantly higher on non-bloom rocks (30%) and scrubbed rocks (24%) than bloom rocks (14%) across the three streams ($F_{2,4}$ = 10.22, P = 0.03), and these differences were consistent within Washington Gulch ($P < 0.002$) and Copper Creek ($P < 0.05$), but not East River (P = 0.2).

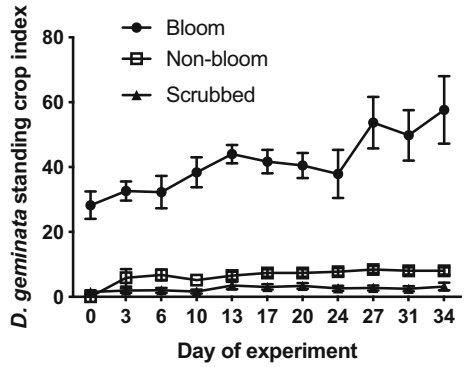

Fig. 2 The average (± SE) standing crop index for *D. geminata* in Copper Creek for bloom rocks increased from July 6, 2012 (day of experiment = 0) throughout the summer, but there was no change in the index on non-bloom and scrubbed rocks during this time. N = 8 rocks per treatment

Didymosphenia geminata channel mesocosm experiment

In 2016, we established differences in phosphorus (P) among the three treatments in the experimental channels (Fig. 3a). The phosphorus maintenance (PM) treatment averaged 2.1 µg P l^{-1}, whereas the flow-through (FT) treatment averaged 1.1 µg P l^{-1} and the recirculated (RC) treatment was depleted to an average of 0.8 µg P l^{-1}. A post hoc Tukey test showed that all of these differences were significant ($P < 0.01$, Table S2). The differences in P did not translate directly to differences in *D. geminata* cell growth, however. The highest density of *D. geminata* cells and stalks was in the FT treatment (Fig. 4), which was higher than either of the other two treatments (Table S2). Despite the differences in P between the RC and PM treatments, there were no significant differences in *D. geminata* cell density between these treatments. There were no significant differences in the frequency of dividing cells between any of the treatments (Fig. 4b, Table S2).

The change in flow regimes and P concentration drove modest changes in nitrogen concentrations in the experimental channels. Ammonium concentration was the highest in the FT treatment, averaging 1.2 µg N l^{-1} followed by the RC treatment

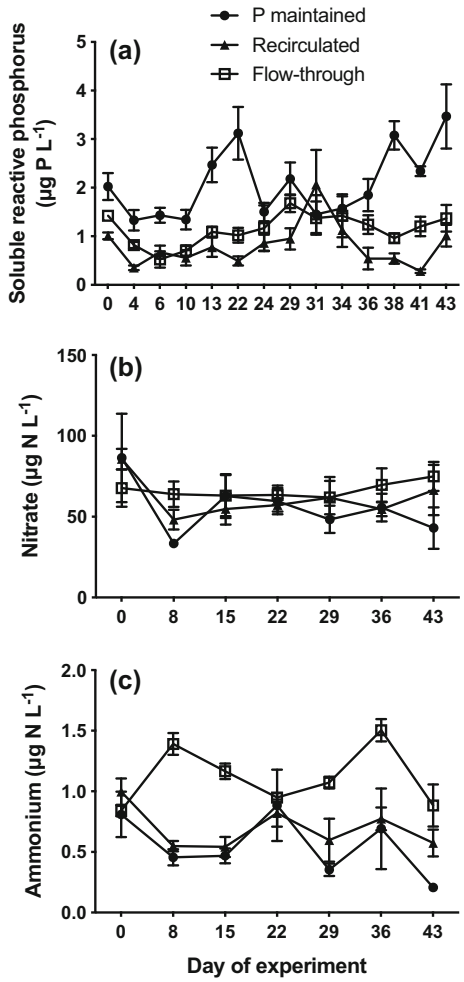

Fig. 3 Time series of concentrations of soluble reactive phosphorus (**a**), nitrate (**b**), and ammonium (**c**) for the recirculated, flow-through, and phosphorus maintained treatments in the channel experiment started on July 12, 2016

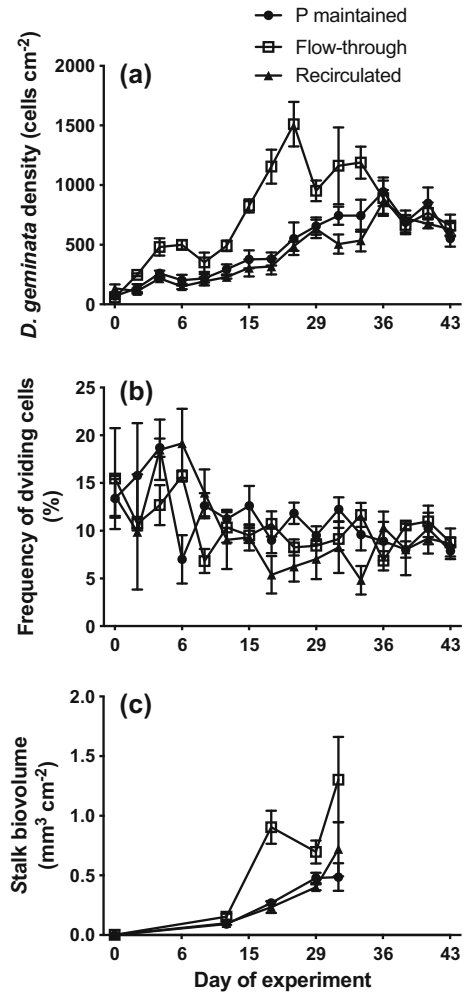

Fig. 4 *Didymosphenia geminata* cell density (**a**), frequency of dividing cells (**b**), and stalk biovolume (**c**) for the recirculated, flow-through, and phosphorus maintained treatments in channel experiment started on July 12, 2016

(0.6 µg N l^{-1}) and the PM treatment (0.5 µg N l^{-1}), and all of these differences were significant (Tukey contrasts $P < 0.03$, Fig. 3b, Table S2). Nitrate followed the same pattern as ammonium, with the FT treatment averaging 66 µg N l^{-1}, followed by RC with 57 µg N l^{-1} and PM with 50 µg N l^{-1}, although these differences were not significant (Fig. 3c, Table S2). The total N to SRP molar ratios were generally high, as expected for a strongly P limited system. N:P was highest in the RC treatment, averaging 276, and lowest in the PM treatment which averaged 53 with the FT treatment intermediate with an average of 122 and all contrasts were significant ($P < 0.04$, Table S2). There were no significant differences in the concentrations of any of the trace nutrients across treatments on any of the three dates ($F_{2,9} = 0.21$, $P > 0.15$ for all tests, Table S3). The difference in the average temperature between the channels was less than 1 °C; nonetheless, the average temperature in the FT channels (11.7 °C) was significantly less than the RC (12.3 °C) or PM (12.4 °C) channels ($F_{2,8} = 21.1$, $P < 0.01$). The temperature differences between the channels were driven primarily by differences in peak daytime temperature, which on average were significantly lower in FT channels

(16.8 °C) than in than the RC (20.9 °C) or PM (21.3 °C) channels ($F_{2,8}$ = 66.2, $P < 0.001$).

The 2017 channel experiment, using an unnamed tributary as a water source had a significantly lower SRP concentration than the 2016 experiment, with SRP concentrations averaging less than 0.3 μg P l^{-1}. The recirculated channels had a slightly lower average SRP concentration (0.25) than the flow-through channels (0.26), but the differences were not statistically significant (Table S4). *D. geminata* was again able to grow in the channels, and concentrations and stalk density increased through time. However, there were no significant differences in the cell density, stalk biovolume, or percentage of dividing cells between the flow regimes or the P enrichments. The recirculated channels (8.3 °C) again were significantly warmer on average than the flow-through (6.7 °C) channels ($F_{1,9}$ = 85.4, $P < 0.001$). The difference in the average maximum temperature was again the reason for the temperature differences, with the recirculated channels (17.4 °C) significantly warmer than the flow-through (11.6 °C) channels ($F_{1,9}$ = 52.4, $P < 0.001$).

Discussion

Low phosphorus concentrations are an important prerequisite for *D. geminata* bloom development, but our results reinforce that low phosphorus is not the sole determinant for bloom development. Low SRP (< 11 μg P l^{-1}) seems to be necessary for blooms to form (Fig. 1), but other factors must be at work in determining the presence of blooms as well. The global distribution of blooms shows that different geographic areas vary in how phosphorus influences bloom occurrence. In South America, blooms have not been documented when SRP exceeds 4 μg P l^{-1}, and in New Zealand, blooms rarely appear when SRP is above 3 μg P l^{-1}. In North America, sites at high equivalent latitudes in the Rocky Mountains only experience blooms at very low SRP concentrations (< 2 μg P l^{-1}), but at sites in the Northeast US, blooms occur at up to 8.5 μg P l^{-1}. The strong correlation in North America between the SRP concentration and the equivalent latitude that blooms form suggests that temperature, or factors that correlate with temperature, such as the onset of spring or light availability, may be important cofactor in bloom development.

The importance of temperature in *D. geminata* bloom development has been shown in observational and experimental studies. Kilroy et al. (2008) used a survey of *D. geminata* growing conditions across New Zealand to show that the blooms are associated with cold water temperatures. Likewise, in another survey, Hix & Murdock (2018) found that blooms and cell densities were associated with colder water temperatures in the southeastern United States and concluded that temperature, light, and SRP were important regulators of bloom biomass but weak predictors of bloom presence or absence. In a survey of over 3000 sites in North America, Kumar et al. (2009) found that *D. geminata* prevalence was negatively related to summer air temperature. Across a series of experiments, Kilroy & Bothwell (2014) showed a strong positive association between temperature and percentage of *D. geminata* cells with stalks, but noted that stalk length was shortest at the warmest water temperature. Jackson et al. (2015) found that a higher biomass, as measured by ash-free dry mass, associated with blooms in warmer temperatures up to 13 °C, although no blooms were observed in four warmest streams surveyed. Climatic differences may help to explain the regional differences in the relationship between SRP concentration and equivalent latitude in the northern hemisphere. Warm ocean currents moderate the temperatures in northern Europe, such that the site in the United Kingdom which has an equivalent latitude of 57° has a summer average high temperature (18.6 °C), higher than the summer average high temperature (17.6 °C) at the North American site in New York at an equivalent latitude of 44°. Although it is possible that temperature alone may explain the relationship between SRP concentration and equivalent latitude of *D. geminata* blooms, other factors correlated with temperature may play an important part as well. Latitude also impacts growing season length, with higher latitudes having a shorter growing season, which could strongly influence a diatom that is considerably larger than other periphytic algal species, meaning that it likely has a longer generation time than its competitors, including algae as well as fungi and bacteria (Peters, 1986). Thus, some other factor(s) combined with or relative to low SRP concentration may explain variation in blooms.

The regrowth experiment revealed that having the appropriate conditions for stalk production was not sufficient to result in bloom formation in our

experimental setting. In the East River and Washington Gulch, the conditions for stalk production may not have been present, or the blooms had already peaked by the time of the manipulation, resulting in no production of *D. geminata* stalk in any of the treatments. In Copper Creek, the *D. geminata* bloom was able to continue growth for a month after the experiment started, indicating that the conditions for stalk growth were present but nearly no regrowth occurred in the scrubbed rock treatment (Fig. 2). The lack of stalk formation on the scrubbed rocks was not from the lack of *D. geminata* cells, as cells were present and actively dividing on the scrubbed rocks. There are a number of factors that could explain the lack of stalk formation on the scrubbed rocks. For instance, as concluded by Flöder & Kilroy (2009), *D. geminata* cell establishment is influenced by periphyton cover and successional stage; therefore, the removal treatment may have altered establishment and bloom development in our study. However, we did find cells and high cellular division of *D. geminata* on scrubbed rocks and cell division was high, suggesting attachment was not limiting for cellular division. If there is an environmental signal which induces stalk formation early in the season, these conditions may have passed, allowing continued growth of the natural stalks, but not causing cells to produce new stalk. Alternatively, by effectively shortening the growing season on scrubbed rocks, the manipulation may not have allowed the cells sufficient time to enter a shift to the stationary growth phase that is associated with increased stalk material production (Underwood & Paterson, 2003). Indeed, Flöder & Kilroy (2009) concluded that *D. geminata* may be a late successional species. However, in Rocky Mountain streams, we have observed that as high springtime flows rapidly recede, stalk material becomes visible on substrates that were briefly inundated early in the season, suggesting that the formation of stalk material is not dependent on the length of the growing season or late successional processes. In fact, these areas inundated briefly by springtime snowmelt are sometimes the only areas to develop visible stalk material, further suggesting that an environmental signal that induces stalk formation early in the season was absent later in the growing season. Thus, identifying whether this environmental signal is water velocity (Larned et al., 2011; Bray et al., 2016, 2017a, b), light (James et al., 2014), nutrients (Bothwell et al., 2014; Kilroy & Larned, 2016), competitors (Bray et al., 2017a), other potential factors (Cullis et al., 2012), or some combination of factors warrants further study.

It has frequently been suggested that *D. geminata* blooms are impaired by high flows or frequent flooding (Kirkwood et al., 2007; Kilroy et al., 2008; Whitton et al., 2009; Jackson et al., 2015). Observational studies have found that high flows early in the season result in lower *D. geminata* densities than in comparable years without the high flows (Richardson et al., 2014; Cullis et al., 2015). Similarly, Jellyman & Harding (2016) present hydrologic evidence for disturbance controlling *D. geminata* biomass, and does Bray et al. (2016) using the Pfankuch substrate stability index. Additionally, at finer-scales, a flow-organism feedback has been proposed by Larned et al. 2011 as a control of *D. geminata* biomass and nutrient exchange within the stalk material and between cells and the water column. However, our removal experiment is the first to show that the effects of a disturbance go beyond physical changes to the system and that physical disturbance has carryover effects that are independent of the growing conditions required for stalk formation.

The channel mesocosm experiment reinforced the conclusion that differences in P concentration are not sufficient to explain patterns of *D. geminata* growth and bloom formation. The flow-through treatment, which had an intermediate P concentration, grew the highest density of *D. geminata* cells and stalk, whereas the addition of phosphate in the P maintenance (PM) treatment resulted in no changes from the P-depleted recirculated (RC) treatment. These differences cannot be explained by differences in nitrogen, as the RC treatment had higher concentrations of N than PM, but no difference in *D. geminata*. N:P ratios do not seem to explain the differences either, as the FT treatment which has the highest *D. geminata* growth has an intermediate N:P ratio. It is also unlikely that another micronutrient caused the growth differences that we saw. The ten percent infusion of stream water into the RC and PM treatments should have been sufficient to prevent depletion of micronutrients which are in low demand and none of the micronutrients we tested for showed any differences between the treatments.

Colder temperatures or immigration of cells from the stream in the FT channels are possible explanations for the better performance of *D. geminata* in this treatment. Though the difference in average water

temperature was less than one degree between FT and the other treatments, the cells could have been stressed by high daytime maximum water temperatures in the RC and PM channels which at 21 °C were more than 4 °C higher than the FT channels on average and peaked at over 27 °C. The survey conducted by Kumar et al. (2009) found that *D. geminata* blooms rarely occurred in locations with mean summer air temperatures greater than 22 °C, and never occurred when it was greater than 26 °C. In the 2017 mesocosm experiment, where differences in *D geminata* between the RC and FT treatments did not emerge, the daytime high water temperatures in the RC treatment averaged less than 18 °C. Immigration of cells coming from the East River into the FT channels in 2016 is another possibility that could explain the disparity in *D. geminata* cell numbers between the treatments. The experiment was designed to minimize the input of cells by building the settling tank with the outflows raised off of the tank bottom to decrease inputs of cells from the stream. Cells migrating from the stream would be expected to preferentially settle in the upstream portion of the channels as they move across the rough surface of the tiles, but the difference in the percentage of cells upstream and downstream was not significantly different from zero in the FT treatment ($\mu = 4\%$, $t_{73} = 0.64$, $P > 0.5$) and there were no significant differences between treatments (Table S2).

There are very few long-term records of SRP concentrations in river ecosystems worldwide, but one such record exists in New Zealand and, importantly, monthly measurements began before the appearance of *D. geminata* blooms. Kilroy & Novis (2018) show that over the period when *D. geminata* blooms have emerged, no trend in SRP concentrations is evident, suggesting that the appearance of blooms cannot be attributed to changing P conditions. However, 50–80% coverage of the streambed by *D. geminata* blooms can develop within < 1 month (see Fig. 3 in Kilroy & Novis, 2018), and other studies show responses of *D. geminata* to SRP and other factors within 7 days (see Fig. 4 in Kilroy & Bothwell, 2012). Therefore, finer temporal scale measurements (e.g., weekly) of SRP concentrations before, during, and after blooms are needed to rigorously test the effects of SRP and others factors on bloom formation. Indeed, research by Hix & Murdoch (2018) supports seasonal variation in SRP and temperature, which are important determinants of *D. geminata* cell division, stalk production, and therefore bloom development. Similarly, a long-term analysis of total P concentrations in two rivers in North America also suggests factors or conditions in addition to low P may be required for mats to form (Kunza et al., 2018).

Although the ultimate drivers of *D. geminata* blooms remain elusive, low SRP remains a consistent factor in the occurrence of blooms (Bothwell et al., 2014). SRP concentrations likely continue to limit *D. geminata* blooms across much of its native range. Extensive observations of SRP are not available across North America, but across England and Wales, only 17% of sites have SRP concentrations below 10 μg P l^{-1} (Mainstone & Parr, 2002). Nonetheless, the evidence we have presented here and the existence of suitable sites in close proximity to existing blooms make it extremely unlikely that phosphorus concentration alone during the summer is controlling the stalk production that results in *D. geminata* blooms. More likely, as proposed previously (e.g., Whitton et al., 2009; Taylor & Bothwell, 2014; Hix & Murdock, 2018), a combination of factors that include low phosphorus, climatic factors, hydrologic regime, other elements, and environmental heterogeneity may be required to create conditions suitable for both stalk production. Cofactors could interact positively with phosphorus to trigger stalk production, such as high light (Kilroy & Bothwell, 2011; James et al., 2014), or cofactors could interact negatively to inhibit phosphorus uptake by diatoms, as has been found with aluminum (Exley et al., 1993), arsenate (Rodriguez Castro et al., 2015), selenium (Moede et al., 1980), and high dissolved CO_2 (Reinfelder, 2012). Future research should explore biotic and abiotic factors that interact positively and negatively with phosphorus, as considering both may further resolve the remaining uncertainty around bloom development.

Acknowledgements We thank E. Binck and J. Fitzgerald for field and laboratory assistance. We are grateful to M. Bothwell for productive discussions and advice. Funding was provided by the University of Texas at Dallas McDermott Scholars program to M. Owens and North Carolina State University and NSF IOS 1641041 to B. Taylor.

References

Aboal, M., S. Marco, E. Chaves, I. Mulero & A. Garcia-Ayala, 2012. Ultrastructure and function of stalks of the diatom *Didymosphenia geminata*. Hydrobiologia 695(1): 17–24.

Alldredge, A. L., U. Passow & B. E. Logan, 1993. The abundance and significance of a class of large, transparent organic particles in the ocean. Deep-Sea Research Part I-Oceanographic Research Papers 40(6): 1131–1140.

Anagnostou, E. & R. M. Sherrell, 2008. MAGIC method for subnanomolar orthophosphate determination in freshwater. Limnology and Oceanography: Methods 6(1): 64–74.

Beltrami, M. E., C. Cappelletti & F. Ciutti, 2008. *Didymosphenia geminata* (Lyngbye) M. Schmidt (Bacillariophyta) in the Danube basin: new data from the Drava river (northern Italy). Plant Biosystems 142(1): 126–129.

Beville, S. T., G. N. Kerr & K. F. D. Hughey, 2012. Valuing impacts of the invasive alga *Didymosphenia geminata* on recreational angling. Ecological Economics 82(Supplement C): 1–10.

Bothwell, M. L. & C. Kilroy, 2011. Phosphorus limitation of the freshwater benthic diatom *Didymosphenia geminata* determined by the frequency of dividing cells. Freshwater Biology 56(3): 565–578.

Bothwell, M. L. & B. W. Taylor, 2017. Blooms of benthic diatoms in phosphorus-poor streams. Frontiers in Ecology and the Environment 15(2): 110–111.

Bothwell, M. L., D. R. Lynch, H. Wright & J. Deniseger, 2009. On the boots of fishermen: the history of didymo blooms on Vancouver Island. British Columbia. Fisheries 34(8): 382–388.

Bothwell, M. L., C. Kilroy, B. W. Taylor, E. T. Ellison, D. A. James, C.-A. Gillis, K. D. Bladon & U. Silins, 2012. Iron is not responsible for *Didymosphenia geminata* bloom formation in phosphorus-poor rivers. Canadian Journal of Fisheries and Aquatic Sciences 69(11): 1723–1727.

Bothwell, M. L., B. W. Taylor & C. Kilroy, 2014. The Didymo story: the role of low dissolved phosphorus in the formation of *Didymosphenia geminata* blooms. Diatom Research 29(3): 229–236.

Bray, J., 2014. The invasion ecology of *Didymosphenia geminata*. University of Canterbury, Christchurch.

Bray, J., J. S. Harding, C. Kilroy, P. Broady & P. Gerbeaux, 2016. Physicochemical predictors of the invasive diatom *Didymosphenia geminata* at multiple spatial scales in New Zealand rivers. Aquatic Ecology 50(1): 1–14.

Bray, J., C. Kilroy, P. Gerbeaux & J. S. Harding, 2017a. Ecological eustress? Nutrient supply, bloom stimulation and competition determine dominance of the diatom *Didymosphenia geminata*. Freshwater Biology 62(8): 1433–1442.

Bray, J., J. O'Brien & J. S. Harding, 2017b. Production of phosphatase and extracellular stalks as adaptations to phosphorus limitation in *Didymosphenia geminata* (Bacillariophyceae). Hydrobiologia 784(1): 51–63.

Crumpton, W. G., T. M. Isenhart & P. D. Mitchell, 1992. Nitrate and organic N analyses with second-derivative spectroscopy. Limnology and Oceanography 37(4): 907–913.

Cullis, J. D. S., C. Gillis, M. L. Bothwell & C. Kilroy, 2012. A conceptual model for the blooming behavior and persistence of the benthic mat-morming diatom *Didymosphenia geminat*a in oligotrophic streams. Journal of Geophysical Research 117: 1–11.

Cullis, J. D. S., D. M. McKnight & S. A. Spaulding, 2015. Hydrodynamic control of benthic mats of *Didymosphenia geminata* at the reach scale. Canadian Journal of Fisheries and Aquatic Sciences 72(6): 902–914.

Davies, W. J. & J. Zhang, 1991. Root signals and the regulation of growth and development of plants in drying soil. Annual Review of Plant Biology 42(1): 55–76.

Ellwood, N. T. W. & B. A. Whitton, 2007. Importance of organic phosphate hydrolyzed in stalks of the lotic diatom *Didymosphenia geminata* and the possible impact of atmospheric and climatic changes. Hydrobiologia 592: 121–133.

Entwisle, T., 1989. Phenology of the *Cladophora-Stigeoclonium* community in two urban creeks of Melbourne. Marine and Freshwater Research 40(5): 471–489.

Exley, C., A. Tollervey, G. Gray, S. Roberts & J. D. Birchall, 1993. Silicon, aluminium and the biological availability of phosphorus in algae. Proceedings of the Royal Society of London Series B: Biological Sciences 253(1336): 93–99.

Flöder, S. & C. Kilroy, 2009. *Didymosphenia geminata* (Protista, Bacillariophyceae) invasion, resistance of native periphyton communities, and implications for dispersal and management. Biodiversity and Conservation 18(14): 3809–3824.

Hillebrand, H., C. D. Dürselen, D. Kirschtel, U. Pollingher & T. Zohary, 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35(2): 403–424.

Hix, L. A. & J. N. Murdock, 2018. *Didymosphenia geminata* habitat requirements are unique and variable for cell establishment and mat accumulation. Hydrobiologia. https://doi.org/10.1007/s10750-018-3809-3.

Hoagland, K. D., J. R. Rosowski, M. R. Gretz & S. C. Roemer, 1993. Diatom extracellular polymeric substances—function, fine structure, chemistry, and physiology. Journal of Phycology 29(5): 537–566.

Holmes, R. M., A. Aminot, R. Kérouel, B. A. Hooker & B. J. Peterson, 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 56(10): 1801–1808.

Hudon, C. & E. Bourget, 1981. Initial colonization of artificial substrate: community development and structure studies by scanning electron microscopy. Canadian Journal of Fisheries and Aquatic Sciences 38: 1371–1384.

Jackson, L. J., L. Corbett & G. Scrimgeour, 2015. Environmental constraints on *Didymosphenia geminata* occurrence and bloom formation in Canadian Rocky Mountain lotic systems. Canadian Journal of Fisheries and Aquatic Sciences 73(6): 964–972.

James, D., K. Mosel & S. Chipps, 2014. The influence of light, stream gradient, and iron on *Didymosphenia geminata* bloom development in the Black Hills, South Dakota. Hydrobiologia 721: 117–127.

James, D. A., M. L. Bothwell, S. R. Chipps & J. Carreiro, 2015. Use of phosphorus to reduce blooms of the benthic diatom *Didymosphenia geminata* in an oligotrophic stream. Freshwater Science 34(4): 1272–1281.

Jellyman, P. G. & J. S. Harding, 2016. Disentangling the stream community impacts of *Didymosphenia geminata*: How are higher trophic levels affected? Biological Invasions 18(12): 3419–3435.

Kawecka, B. & J. Sanecki, 2003. *Didymosphenia geminata* in running waters of southern Poland—symptoms of change in water quality? Hydrobiologia 495(1–3): 193–201.

Khan-Bureau, D. A., M. S. Beauchene, L. Ector, E. A. Morales & L. A. Lewis, 2014. 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. BioInvasions Records 3: 139–149.

Kilroy, C. A. & M. L. Bothwell, 2011. Environmental control of stalk length in the bloom-forming, freshwater benthic diatom *Didymosphenia geminata* (Bacillariophyceae). Journal of Phycology 47(5): 981–989.

Kilroy, C. & M. L. Bothwell, 2012. *Didymosphenia geminata* growth rates and bloom formation in relation to ambient dissolved phosphorus concentration. Freshwater Biology 57(4): 641–653.

Kilroy, C. & M. L. Bothwell, 2014. Attachment and short-term stalk development of *Didymosphenia geminata*: effects of light, temperature and nutrients. Diatom Research 29(3): 237–248.

Kilroy, C. & S. T. Larned, 2016. Contrasting effects of low-level phosphorus and nitrogen enrichment on growth of the mat-forming alga *Didymosphenia geminata* in an oligotrophic river. Freshwater Biology 61(9): 1550–1567.

Kilroy, C. & P. Novis, 2018. Is *Didymosphenia geminata* an introduced species in New Zealand? Evidence from trends in water chemistry, and chloroplast DNA. Ecology and Evolution 8(2): 904–919.

Kilroy, C. & M. Unwin, 2011. The arrival and spread of the bloom-forming, freshwater diatom, *Didymosphenia geminata, in* New Zealand. Aquatic Invasions 6(3): 249–262.

Kilroy, C., T. H. Snelder, O. Floerl, C. C. Vieglais & K. L. Dey, 2008. A rapid technique for assessing the suitability of areas for invasive species applied to New Zealand's Rivers. Diversity and Distributions 14(2): 262–272.

Kilroy, C., S. T. Larned & B. J. F. Biggs, 2009. The non-indigenous diatom *Didymosphenia geminata* alters benthic communities in New Zealand rivers. Freshwater Biology 54(9): 1990–2002.

Kirkwood, A. E., T. Shea, L. J. Jackson & E. McCauley, 2007. *Didymosphenia geminata* in two Alberta headwater rivers: an emerging invasive species that challenges conventional views on algal bloom development. Canadian Journal of Fisheries and Aquatic Sciences 64(12): 1703–1709.

Kirkwood, A. E., L. J. Jackson & E. McCauley, 2009. Are dams hotspots for *Didymosphenia geminata* blooms? Freshwater Biology 54(9): 1856–1863.

Kleiven, O. T., P. Larsson & A. Hobæk, 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. Oikos 65(2): 197–206.

Kumar, S., S. A. Spaulding, T. J. Stohlgren, K. A. Hermann, T. S. Schmidt & L. L. Bahls, 2009. Potential habitat distribution for the freshwater diatom *Didymosphenia geminata* in the continental US. Frontiers in Ecology and the Environment 7(8): 415–420.

Kunza, L. A., C.-A. Gillis, J. Z. Haueter, J. N. Murdock & J. M. O'Brien, 2018. Declining phosphorus as a potential driver for the onset of *Didymosphenia geminata* mats in North American rivers. River Research and Applications 34(8): 1105–1110.

Ladrera, R., M. Rieradevall & N. Prat, 2015. Massive growth of the invasive algae *Didymosphenia geminata* associated with discharges from a mountain reservoir alters the taxonomic and functional structure of macroinvertebrate community. River Research and Applications 31(2): 216–227.

Ladrera, R., J. Goma & N. Prat, 2016. Regional distribution and temporal changes in density and biomass of *Didymosphenia geminata* in two Mediterranean river basins. Aquatic Invasions 11(4): 355–367.

Landry-Cuerrier, M., D. Munro, D. Thomas & M. Humphries, 2008. Climate and resource determinants of fundamental and realized metabolic niches of hibernating chipmunks. Ecology 89(12): 3306–3316.

Larned, S. T., A. I. Packman, D. R. Plew & K. Vopel, 2011. Interactions between the mat-forming alga *Didymosphenia geminata* and its hydrodynamic environment. Limnology & Oceanography: Fluids & Environments 1: 4–22.

Lewis, R. J., L. M. Johnson & K. D. Hoagland, 2002. Effects of cell density, temperature, and light intensity on growth and stalk production in the biofouling diatom *Achnanthes longipes* (bacillariophyceae). Journal of Phycology 38(6): 1125–1131.

Ma, J., J. D. Brookes, B. Qin, H. W. Paerl, G. Gao, P. Wu, W. Zhang, J. Deng, G. Zhu & Y. Zhang, 2014. Environmental factors controlling colony formation in blooms of the cyanobacteria *Microcystis* spp. in Lake Taihu, China. Harmful Algae 31: 136–142.

Mainstone, C. P. & W. Parr, 2002. Phosphorus in rivers—ecology and management. Science of The Total Environment 282–283: 25–47.

Moede, A., R. W. Greene & D. F. Spencer, 1980. Effects of selenium on the growth and phosphorus uptake of *Scenedesmus dimorphus* and *Anabaena cylindrica*. Environmental and Experimental Botany 20(2): 207–212.

Montgomery, K., 2006. Variation in temperature with altitude and latitude. Journal of Geography 105(3): 133–135.

Myklestad, S. M., 1995. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. Science of The Total Environment 165(1): 155–164.

Ostrofsky, M. L. & F. H. Rigler, 1987. Chlorophyll-phosphorus relationships for subarctic lakes in western Canada. Canadian Journal of Fisheries and Aquatic Sciences 44: 775–781.

Peters, R. H., 1986. The Ecological Implications of Body Size, Vol. 2. Cambridge University Press, New York.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Reid, B. L., K. L. Hernandez, M. Frangopulos, G. Bauer, M. Lorca, C. Kilroy & S. Spaulding, 2012. The invasion of the freshwater diatom *Didymosphenia geminata* in Patagonia: prospects, strategies, and implications for biosecurity of invasive microorganisms in continental waters. Conservation Letters 5(6): 432–440.

Reinfelder, J. R., 2012. Carbon dioxide regulation of nitrogen and phosphorus in four species of marine phytoplankton. Marine Ecology Progress Series 466: 57–67.

Richardson, D. C., I. A. Oleksy, T. J. Hoellein, D. B. Arscott, C. A. Gibson & S. M. Root, 2014. Habitat characteristics, temporal variability, and macroinvertebrate communities associated with a mat-forming nuisance diatom (*Didymosphenia geminata*) in Catskill mountain streams, New York. Aquatic Sciences 76(4): 553–564.

Rodriguez Castro, M. C., G. Urrea & H. Guasch, 2015. Influence of the interaction between phosphate and arsenate on periphyton's growth and its nutrient uptake capacity. Science of The Total Environment 503–504: 122–132.

SAS Institute Inc., 2018. JMP Pro. 14.3 edn. SAS Institute, Inc., Cary, North Carolina.

Smayda, T. J., 1997. What is a bloom? A commentary. Limnology and Oceanography 42(5): 1132–1136.

Smith, V. H., 2003. Eutrophication of freshwater and coastal marine ecosystems—a global problem. Environmental Science and Pollution Research 10(2): 126–139.

Staats, N., L. J. Stal & L. R. Mur, 2000. Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions. Journal of Experimental Marine Biology and Ecology 249(1): 13–27.

Sundareshwar, P. V., S. Upadhayay, M. Abessa, S. Honomichl, B. Berdanier, S. A. Spaulding, C. Sandvik & A. Trennepohl, 2011. *Didymosphenia geminata*: algal blooms in oligotrophic streams and rivers. Geophysical Research Letters. https://doi.org/10.1029/2010gl046599.

Sutherland, T. F., J. Grant & C. L. Amos, 1998. The effect of carbohydrate production by the diatom *Nitzschia curvilineata* on the erodibility of sediment. Limnology and Oceanography 43(1): 65.

Taylor, B. W. & M. L. Bothwell, 2014. The origin of invasive microorganisms matters for science, policy, and management: The case of *Didymosphenia geminata*. Bioscience 64(6): 531–537.

Taylor, B. W., C. F. Keep, R. O. Hall Jr., B. J. Koch, L. M. Tronstad, A. S. Flecker & A. J. Ulseth, 2007. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. Journal of the North American Benthological Society 26(2): 167–177.

Underwood, G. J. C. & D. M. Paterson, 2003. The importance of extracellular carbohydrate productionby marine epipelic diatoms. Advances in Botanical Research 40: 183–240.

Veldhuis, M. & W. Admiraal, 1987. Influence of phosphate depletion on the growth and colony formation of *Phaeocystis pouchetii*. Marine Biology 95(1): 47–54.

Whitton, B. A., N. T. W. Ellwood & B. Kawecka, 2009. Biology of the freshwater diatom *Didymosphenia*: a review. Hydrobiologia 630(1): 1–37.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.