Didymosphenia geminata in Pennsylvania: an investigation of current and historic distribution, habitat suitability, and nutritional content

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TABLE OF CONTENTS

EXECUTIVE SUMMARY .......................................................................................................................... 1
INTRODUCTION ........................................................................................................................................ 3
METHODOLOGY ....................................................................................................................................... 5
RESULTS ................................................................................................................................................... 14
CONCLUSIONS......................................................................................................................................... 41
ADDITIONAL RESEARCH INDICATED ............................................................................................... 42
ACKNOWLEDGMENTS .......................................................................................................................... 42
CITATIONS ............................................................................................................................................... 43

TABLES

Table 1. Physicochemical Variables Influencing Didymo Habitat Suitability, Criteria Promoting Didymo Presence, Relative Weights, and Associated Literature Sources........................................... 8
Table 2. Didymo Cell Density at Longitudinal Study Sites on Pine Creek in Spring and Fall 2015........................................ 17
Table 3. Total Number of HUC10 Watersheds within Major Drainages in Pennsylvania, and Number and Percent (in parentheses) of HUC10 Watersheds with at Least One and Zero SRP Records...................................................... 23
Table 4. Two-Way ANOVA Results Examining Didymo Presence and Season of Fatty Acid Relative Abundance........................................................................................................................................... 36

FIGURES

Figure 1. Microscopic View of a Didymosphenia geminata Cell Collected from West Branch Pine Creek, Potter County, Pennsylvania in November 2015........................................................................... 4
Figure 2. Map of Remote Water Quality Monitoring Stations in Pennsylvania......................................................... 9
Figure 3. Photosynthetically Active Radiation (PAR) Sensor (Foreground) and YSI 6600 sonde in Protective Casing in Background at the West Branch Pine Creek RWQMN Station........................................ 11
Figure 4. Didymo Presence / Absence in Pennsylvania from June 1993 – November 2015........................................ 16
Figure 5. Presence of Didymo in Algal Samples in the Pine Creek Watershed Collected (a) Before and (b) After 18 June 2013............................................................................................................. 18
Figure 6. Didymo Cell Density and Soluble Reactive Phosphorus Concentrations at Monitoring Locations in the Pine Creek Watershed........................................................................................................ 19
Figure 7. Boxplot of Soluble Reactive Phosphorus (SRP) and Total Dissolved Phosphorus (TDP) Concentrations from 249 Samples Collected in Pennsylvania in 2014 and 2015, by Season. 20
Figure 8. Boxplot of Soluble Reactive Phosphorus (SRP) Concentrations from 249 Samples Collected in Pennsylvania in 2014 and 2015, by Site........................................................................................................ 22
Figure 9. Mean Soluble Reactive Phosphorus in HUC10 Watersheds and Didymo Presence in Pennsylvania............................................................................................................................ 24
Figure 10. Results of Habitat Suitability Index Presented in Order of Most Suitable to Least Suitable............... 26
Figure 11. Photographs of Didymo Growth on Substrate and Instream Conditions During 2014 and 2015 at the West Branch Pine Creek Monitoring Station ............................................................. 28
Figure 12. Continuous Data Collected at West Branch Pine Creek 21 October 2014 – 31 December 2015......................................................................... 31
Figure 13. Hydrograph of Modeled Streamflow Data at West Branch Pine Creek from 1 January 2013 to 31 December 2015 ........................................................................................................................................ 32
APPENDICES

Appendix A. Staff, Students Supported, and Outreach................................................................. 50
Appendix B. Impact Statement ...................................................................................................... 52
Appendix C. Habitat Suitability Scoring Matrix ............................................................................. 53
EXECUTIVE SUMMARY

Didymosphenia geminata (Lyngbye) M.Schmidt (herein referred to as didymo) is a benthic freshwater diatom capable of producing nuisance blooms that impact the recreational, ecological, and aesthetic value of freshwater ecosystems. Recently, didymo has expanded its range and ecological tolerances leading to massive blooms in areas where it was previously undocumented or had existed in low abundance (Spaulding and Elwell, 2007; Blanco and Ector, 2009; Whitton et al., 2009). Since 2007, these blooms have been increasingly observed in Pennsylvania (PA), yet currently no inventory of its distribution or assessment of watershed vulnerability exists for the state (PFBC, 2011). The intent of this study was to conduct a comprehensive assessment of didymo throughout PA and provide relevant technical information that could help inform management efforts. This study addresses three important research questions regarding didymo in PA streams; each of which are summarized below, along with their associated results and implications.

Research Question 1) What is the current and historic distribution of didymo in Pennsylvania?

We combined literature and historical collections with comprehensive field surveys to assess the current distribution of didymo. In total, 1752 historical records were compiled and supplemented with 40 samples collected in streams throughout PA. Of these, 96 records detected didymo from six watersheds within or intersecting the political boundary of PA, including the Delaware River and East Branch Dyberry Creek in northeastern; Pine Creek and Trout Run in northcentral; Gunpowder Falls in southern; and Youghiogheny River in southwestern PA.

We also conducted a more intensive survey within the Pine Creek Watershed, a region where didymo blooms have been recently discovered (June 2013). Currently, didymo can be found on the substrate along a 24-mile reach from ranging from West Branch Pine Creek at Crippen Run, downstream to the Tiadaghton access in the Pine Creek gorge. Didymo coverage was greatest near the mouth of West Branch Pine Creek, with cell densities decreasing rapidly progressing downstream. All tributaries to Pine Creek other than the West Branch, including Ninemile Run, Babb Creek, Cedar Run, Slate Run, and Little Pine Creek, were not colonized by didymo.

Research Question 2) What is the potential habitat suitability for nuisance didymo blooms in Pennsylvania?

We assessed water chemistry, stream morphology, and other data to examine the potential habitat suitability of nuisance didymo blooms throughout PA. We incorporated targeted water quality sampling in concert with compilation of existing data sources to construct a database of Soluble Reactive Phosphorus (SRP; >18,000 samples), which is a nutrient known to limit didymo abundance. SRP concentrations were negatively correlated to the distribution of didymo across PA, with didymo restricted to watersheds with mean SRP concentrations <10 µg/L. This pattern was complex, however, and potentially linked to water management practices.
(regulated versus unregulated streams). The link between SRP and didymo was apparent in the Pine Creek Watershed. Didymo abundance was greatest in the upstream reaches of Pine Creek where SRP was lowest, then appeared to decline downstream as SRP increased.

We created an in-depth didymo habitat suitability index using 12 physicochemical parameters known to influence didymo blooms. Results suggest that, at least in PA, suitable didymo habitat is marginal at best, largely due to lack of flow regulation. Episodic nuisance didymo blooms may arise in unregulated streams when environmental conditions permit, but enduring blooms are most likely restricted to habitats downstream of bottom-release dams in this region. We then coupled high frequency sampling with continuous data collection at West Branch Pine Creek to elucidate relationships between physicochemical parameters and didymo coverage. Streamflow, temperature, and pH appeared to exert control over didymo coverage throughout the study period, with streamflow appearing to exert the most control at this location with consistently low SRP concentrations. Antecedent periods of stable flow were required for accumulation of didymo stalk material at West Branch Pine Creek.

Research Question 3) How does didymo presence affect the nutritional content of biofilms?

We collected periphyton from 44 sites across PA in the summer and fall of 2015. Fatty acid analysis was conducted on the periphyton to assess the nutritional relevance of biofilms colonized by didymo. To date, the fatty acid results are provisional, due to an unforeseen bias in the analytical chemistry methodology. The presence of didymo in the biofilm and season of collections (summer or fall) best explained variation in physiologically important fatty acid profiles (sensu essential fatty acids). Individual fatty acids also varied in with respect to season and didymo presence. When grouped according to fatty acid function group (e.g., saturated versus unsaturated), the effect of season was strongest, which is unsurprising given seasonal differences in photoperiod, temperature, nutrients, and carbon loading. Significant differences in saturated and mono-unsaturated fatty acids also suggest differences in nutritional quality between didymo and didymo-free biofilms.

This study served to supplement knowledge related to didymo in PA streams. Here, we have characterized the current distribution of didymo and the physicochemical parameters that govern its occurrence and propensity to bloom at different scales. We also assessed how the nutritional content of biofilms at the base of the food web may be influenced by the presence of didymo, which may have implications at higher trophic levels. We conclude that, based on current information, unregulated streams in the Susquehanna Basin generally have marginal didymo habitat and are unlikely to support year-round nuisance blooms. These streams, however, could support periodic blooms with potentially negative impacts on recreation and ecology. Based on our analysis, tailwater stream habitats appear to be most susceptible to extended and intense didymo blooms. Didymo was only observed in discrete patches in specific regions of PA, which somewhat refutes the hypothesis that didymo is present across a large geographic area in low abundances. Nevertheless, data are still limited and not adequate to unequivocally characterize didymo as native or introduced to PA. Further studies are required to definitively address origin of didymo and its associated relevance to stream ecosystem health.
INTRODUCTION

Didymosphenia geminata (Lyngbye) M.Schmidt (herein referred to as didymo) is a relatively large, single-celled diatom known for its characteristic ‘coke bottle’ shape when viewed microscopically (Figure 1). When conditions are suitable, didymo can form blooms up to 8 inches (20 centimeters) thick, which in addition to degrading the experience of recreationists, has the potential to alter physical and biological conditions in aquatic ecosystems (Spaulding and Elwell, 2007; Whitton et al., 2009). Globally, didymo is considered to be one of the most problematic invasive species currently threatening lotic systems (Blanco and Ector, 2009).

Historically, didymo’s range was restricted to relatively pristine oligotrophic lakes, streams, and rivers in circumboreal regions. Recently, however, didymo has been observed expanding both its range and ecological tolerances, resulting in massive blooms where it was previously undocumented or existed in low abundance (Spaulding and Elwell, 2007; Blanco and Ector, 2009). Didymo has received significant attention in contemporary scientific literature due to its dramatic range expansion, more frequent nuisance blooms, and its unusual ability to produce large blooms in low nutrient environments. As a result, multiple hypotheses that address its origin and dominance have been formulated including: anthropogenic spread of didymo (e.g., Bothwell et al., 2009), prevalence of a bloom-forming genetic strain of didymo (e.g., Whitton et al., 2009), and anthropogenic impacts from climate change and oligotrophication (Bothwell et al., 2014; Taylor and Bothwell, 2014). Regardless of the reason for the recent globalized spread of didymo, impacts experienced by streams are real. Impacts to biota have been documented in fish (James and Chipps, 2016), macroinvertebrate (Gillis and Chalifour, 2010), and algal (Gillis and Lavoie, 2014) assemblages. In spite of its contentiously argued impacts, much uncertainty remains with respect to how nuisance blooms arise and the mechanism for which it impacts freshwater ecosystems. Moreover, the fact that once established, there is no current viable option for treatment or eradication is a real concern among water resource managers (Clearwater et al., 2011, James et al., 2015).

There are historical records of didymo in the Delaware River and in the vicinity of Philadelphia, Pennsylvania (PA) dating back to the early 20th century (Boyer, 1916; Boyer, 1927). Throughout the mid-Atlantic, didymo remained largely undetected until the early 2000s, when didymo began to garner international attention. In 2007, didymo blooms were observed on the East and West Branches of the upper Delaware River near the New York (NY) and PA border (PASG, 2015). Large didymo blooms were observed throughout a 100-mile stretch of the Delaware River in spring 2012 (DRBC, 2014). Additional didymo observations were made in 2012 in Dyberry Creek in the Delaware River Watershed and on the Youghiogheny River in southwestern PA (PFBC, 2012). In June 2013, didymo was detected on the middle portion of the mainstem of Pine Creek in Lycoming County near Hamilton Bottom, PA (PFBC, 2013). Interestingly, this initial observation comprised didymo cells collected using a plankton tow net in the water column (Jeff Butt, personal communication) rather than attached substrate. A few months later in October 2013, didymo was found attached to the substrate 50 miles further upstream on West Branch Pine Creek in Potter County near Galeton, PA (SRBC, 2013).

To date, there has been no comprehensive study of didymo in PA, leaving many important management questions unanswered. Moreover, the statewide distribution of didymo has yet to be formally inventoried leaving many potentially vulnerable watersheds unidentified (PFBC, 2011). Additionally, little research has addressed the ecological impacts of didymo
colonization, and what it may mean for PA streams. This study involved three main research questions that are intended to address these lingering uncertainties, which will be addressed independently throughout this document.

![Microscopic View of a Didymosphenia geminata Cell Collected from West Branch Pine Creek, Potter County, Pennsylvania in November 2015](image)

**Research Question 1) What is the current and historic distribution of didymo in Pennsylvania?**

This study coupled the compilation of existing, contemporary algal assemblage records with targeted field collection of additional algal data to construct a database of the current didymo distribution in PA. Targeted data collection was also employed to examine the spatial and temporal abundance of didymo in the Pine Creek Watershed, where didymo has most recently been observed.

**Research Question 2) What is the potential habitat suitability for nuisance didymo blooms in Pennsylvania?**

Recent studies suggest that dissolved phosphorus may be one of many factors that constrain nuisance didymo blooms. As such, we compiled existing data and targeted the collection of new data to build a robust database that reflects the variability of stream phosphorus concentrations throughout PA. Moreover, we also conducted an in-depth examination of habitat suitability in 17 watersheds using continuous monitoring equipment that have been deployed for numerous years. These 17 watersheds were all located within the PA portion of the Susquehanna River Basin (SRB) and were selected based on proximity to recent didymo observations and
priori knowledge of phosphorus concentrations. Additionally, it is currently unknown if didymo will respond to environmental conditions in the mid-Atlantic region in a similar manner to where the majority of didymo research has been conducted. For this reason, high frequency data were collected within the West Branch of Pine Creek, which was intended to determine which physicochemical variables were associated with didymo stalk production associated with nuisance blooms.

Research Question 3) How does didymo presence affect the nutritional content of biofilms?

From a taxonomic perspective, didymo is a member of the class Bacillariophyceae, a group of diatoms often revered as an indicator of good water quality (Berger et al., 2009; Bothwell et al., 2014). Yet, despite this classification, there is concern that the presence of didymo in freshwater systems may negatively influence aquatic food webs. Researchers contend that the complex mat structures created by didymo blooms may also impact consumers directly by altering habitat suitability, or indirectly by provisioning nutritionally poor resources (Kilroy et al., 2009; Cullis et al., 2015). While studies that examine the effects of didymo on physical habitat are increasingly prevalent, research that examines the nutritional significance of didymo in aquatic ecosystems remains surprisingly understudied (Whitton et al., 2009).

Fatty acids (FAs) are a fundamental building block of life and a potentially useful research tool from a variety of ecological perspectives (Brett and Müller-Navarra, 1997; Honeyfield and Maloney, 2015). As a source of carbon, they reflect an important nutritional resource that is not easily available to all organisms (Napolitano, 1999). Unlike saturated FAs, which comprise a suite of double bonds, unsaturated FAs are considered more nutritious. Many consumers cannot directly generate polyunsaturated FAs via de novo synthesis, and thus the compound must be integrated into their diet via consumption (Torres-Ruiz et al., 2007). Those that can make use of biosynthesis pathways via elongation and desaturation of the carbon chain, do so at high energetic costs (Arts et al., 2001; Dalsgaard et al., 2003). As such, unsaturated FAs, especially highly unsaturated FAs, are an important dietary commodity in aquatic ecosystems (Twining et al., 2015). Here, using FA analysis as an indicator of nutritional quality, we evaluated the nutritional relevance of benthic biofilms that differentially comprise didymo across two sampling periods and a variety of sampling sites.

METHODOLOGY

The study area was defined as 332 ten-digit hydrologic unit code (HUC10) watersheds that were within, or intersected with, the political boundary of PA. Data were compiled and collected within the study area and are described below as they relate to each research question.

Research Question 1) What is the current and historic distribution of didymo in Pennsylvania?

There were two components to this research question. First, the current spatial distribution of didymo across the study area was determined using contemporary data. Second, the spatial distribution and temporal abundance of didymo in the Pine Creek watershed was
examined in depth. To complete these objectives, a systematic search for contemporary periphyton/algal assemblage records was completed from numerous sources. Records from 1993 to present were obtained from state and federal agencies, academic institutions, non-profit organizations, and citizen scientists. Data sources included Academy of Natural Sciences PA (ANSP), Susquehanna River Basin Commission (SRBC), PA Department of Environmental Protection (PADEP), Delaware River Basin Commission (DRBC), U.S. Environmental Protection Agency (USEPA) National Rivers and Streams Assessment (USEPA, 2016a), PA and NY iMap Invasives databases (imapinvasives.org), PA Trout Unlimited, Potapova (2010), Keller and Hilderbrand (2015), and Robert Volkmar (University of Duquesne, retired). These data were collected using various techniques including natural substrate scrapes, tow net deployment in the water column, and artificial tiles. Data processing also varied in type, including microscopic examination of material or polymerase chain reaction (PCR) tailored to didymo DNA (Cary et al., 2014). All records included were determined to be valid, as institutional quality control procedures provided ample confidence in the accuracy of results.

In addition to literature surveys, 40 periphyton samples were collected from 29 sites using modified Rapid Bioassessment Protocols (Barbour et al., 1999). At each site, eleven pieces of natural substrate (cobbles) were removed randomly from the entire width of the stream site. Periphyton within a 12 cm² delimiter was disturbed from each particle using a soft brush and rinsed into a composite sample bottle and the corresponding sample volume was recorded. A 50 mL subsample was then extracted from the composite sample and preserved with formaldehyde. Ten samples were collected from a 24-mile longitudinal section of Pine Creek in spring and fall 2015 to examine periphyton assemblage spatial and temporal dynamics, including density of didymo cells. Eight additional samples, which were collected from the Pine Creek Watershed prior to the first observation of didymo in 2013, were examined for didymo presence or absence. The remaining 22 samples were collected from throughout the SRB, including the habitat suitability stations (discussed below), were also examined for didymo presence or absence. All periphyton sample processing procedures followed The Academy of Natural Sciences (2002) protocol.

Research Question 2) What is the potential habitat suitability for nuisance didymo blooms in Pennsylvania?

Statewide nutrient concentrations

Soluble reactive phosphorus (SRP; also known as dissolved orthophosphate) concentrations have been shown to be a causative factor in determining the habitat suitability for didymo. Specific thresholds of SRP (below ~2 µg/L) have been documented that limit cell division, but stimulate stalk production, often resulting in nuisance blooms (Kilroy and Bothwell, 2011; Bothwell et al., 2014). Due to the significance of phosphorus to didymo ecology, and its importance to watershed management, we characterized the association of SRP and didymo throughout the study area. Water samples were collected from 51 Remote Water Quality Monitoring Network (RWQMN) stations within the PA portion of the SRB (Figure 2) and from additional locations in the Pine Creek Watershed during 2014–2015. Samples were collected and processed for SRP and total dissolved phosphorus (TDP) by the University of Maryland Center for Environmental Science Appalachian Laboratory. Reporting detection limit was 1.1
micrograms/Liter (µg/L) for SRP and 7.8 µg/L for TDP. In addition, SRP data were compiled from the PADEP, DRBC, Maryland Department of Natural Resources (MD DNR), and the STORET data warehouse (USEPA, 2016b). Data were cleaned using the following guidelines: all non-detect SRP records with detection limit of ≥ 10 µg/L or not specified were deleted, all records flagged as trip blanks or lab matrix spikes were deleted, and data older than 1986 were deleted. Non-detect results, where detection limits were specified and < 10 µg/L, were assigned a value equal to the detection limit. The remaining records were summarized by HUC10 watersheds in the study area in ArcGIS version 10.1 (ESRI, Inc.; http://www.esri.com). Mean SRP by HUC10 watershed was used to determine potential didymo habitat suitability on a broad scale.

**Habitat suitability index**

Additional physicochemical variables have been shown to influence didymo blooms (Cullis et al., 2012; Bothwell et al., 2014; Bray et al., 2016). Rigorous statistical modeling (i.e., logistic regression) of didymo presence or absence in response to environmental variables is not currently possible in the Susquehanna Basin due to the small sample size of didymo presence. Instead, an extensive literature search was conducted to determine physicochemical variables that influence didymo habitat suitability established for other regions. Distinct thresholds or specific criteria were rarely defined in the literature, instead, most emphasized a relationship between variables and the likelihood of didymo presence or increased abundance (e.g. Rost et al., 2011; Bray et al., 2016). These published observations also suggest that, while many factors can exert control over didymo, some are more important than others. For this reason, we weighted criteria according to their relative importance in terms of didymo habitat suitability. Table 1 summarizes the variables, their criteria or threshold that supports didymo presence/bloom formation, their relative weight, and their respective literature sources. Existing SRBC datasets at the 17 habitat suitability stations are large, due to collocation with SRBC RWQMN stations where sondes have been deployed for a number of years. Data compiled at these stations included: water chemistry, land use, geomorphology, and physical habitat data. Specific parameters included in the habitat suitability index included long-term median values of turbidity and pH for the entire period of record at all sites (> 3.3 years). Mean SRP was calculated from samples collected during 2014-2015, with a range of 4-19 samples collected at each site. The remaining water chemistry variables (sulfate, calcium, nitrate, and total organic carbon) represented mean values of samples collected four times a year at SRBC RWQMN stations, for the period of record of each station. Flow regulation was determined by examining the NHD waterbody GIS shapefile for lakes and/or impoundments controlling a significant portion of flow upstream of each station. Channel width, substrate size, and stream gradient (m elevation difference/100 m stream) were measured at each site during 2014 or 2015 using modified U.S. Geological Survey (USGS) National Water-Quality Assessment Program methods (Fitzpatrick et al., 1998). Our measured variables were compared against published didymo habitat preferences (Table 1), and were ranked using a simple scoring system where a value of 1 given to variables within the documented preferred range and a value of 0 to variables outside of the range. These scores were then multiplied by the weight of each variable. The sum of all the individual scores then represented the total score out of a possible 23 points. A larger score represents a higher habitat suitability for didymo.
Table 1. Physicochemical Variables Influencing Didymo Habitat Suitability, Criteria Promoting Didymo Presence, Relative Weights, and Associated Literature Sources

<table>
<thead>
<tr>
<th>Variable</th>
<th>Criteria</th>
<th>Weight</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow regulation</td>
<td>Lakes/impoundments upstream</td>
<td>5</td>
<td>Whitton et al., 2009; Kirkwood et al., 2009; Bray et al., 2016</td>
</tr>
<tr>
<td>Temperature</td>
<td>&lt;10% days &gt;18ºC</td>
<td>2</td>
<td>Lindstrøm and Skulberg, 2008; Whitton et al., 2009</td>
</tr>
<tr>
<td>Light</td>
<td>&gt; 10 m wide channel^</td>
<td>2</td>
<td>Bothwell and Kilroy, 2011; James et al., 2014; Bray et al., 2016</td>
</tr>
<tr>
<td>Substrate</td>
<td>rocky/hard; large gravel to cobble</td>
<td>1</td>
<td>Whitton et al., 2009; Bergey et al., 2010</td>
</tr>
<tr>
<td>Turbidity</td>
<td>&lt;5 NTU*</td>
<td>1</td>
<td>Kirkwood et al., 2007; Bothwell et al., 2014</td>
</tr>
<tr>
<td>Gradient</td>
<td>low gradients (&lt;0.5%)^</td>
<td>1</td>
<td>James et al., 2014</td>
</tr>
<tr>
<td>Soluble Reactive Phosphorus</td>
<td>&lt;2 µg/L</td>
<td>5</td>
<td>Bothwell and Kilroy 2011; Kilroy and Bothwell 2012</td>
</tr>
<tr>
<td>Soluble Reactive Phosphorus</td>
<td>&lt;5 µg/L</td>
<td>2</td>
<td>Silldorff and Swann, 2013; Bothwell et al., 2014; Klauda et al., 2015</td>
</tr>
<tr>
<td>Soluble Reactive Phosphorus</td>
<td>&lt;10 µg/L</td>
<td>1</td>
<td>Stoddard et al., 2005; Lindstrøm and Skulberg, 2008</td>
</tr>
<tr>
<td>pH</td>
<td>&gt;6.7</td>
<td>2</td>
<td>Stoddard et al., 2005; Lindstrøm and Skulberg, 2008</td>
</tr>
<tr>
<td>Sulfate</td>
<td>&gt;2.5 mg/L</td>
<td>1</td>
<td>Lindstrøm and Skulberg, 2008</td>
</tr>
<tr>
<td>Calcium</td>
<td>&gt;1.8 mg/L</td>
<td>1</td>
<td>Lindstrøm and Skulberg, 2008</td>
</tr>
<tr>
<td>Nitrate</td>
<td>&lt;1 mg/L</td>
<td>1</td>
<td>Stoddard et al., 2005; Spaulding and Elwell, 2007</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>&lt;6.5 mg/L</td>
<td>1</td>
<td>Lindstrøm and Skulberg, 2008</td>
</tr>
</tbody>
</table>

^ Streams with mean width of >10m were preferred by didymo in a study in the Black Hills of SD (James et al., 2014).
* This threshold was based on Lloyd et al. (1987) that found a 3-13% decrease in primary productivity in clear Alaska streams in response to 5 NTU turbidity values.
+ Streams with gradients >0.5% were devoid of didymo in a study in the Black Hills of SD (James et al., 2014).
Figure 2. Map of Remote Water Quality Monitoring Stations in Pennsylvania (Watersheds in purple (numbers 1-17) were chosen as habitat suitability stations for this study. Soluble reactive phosphorus data were collected at all stations.)
West Branch Pine Creek case study

A detailed analysis was conducted at the West Branch Pine Creek RWQMN station (station no. 7 in Figure 2) from 21 October 2014 through 31 December 2015 to assess the association of didymo abundance with physicochemical and hydrological variables. Didymo bloom intensity was measured 11 times during the study period at the West Branch Pine Creek. Didymo bloom intensity was measured as standing crop index (SCI; Kilroy and Bothwell, 2012), which is the product of percent didymo coverage and mat thickness (mm). Mean SCI was calculated by averaging percent coverage x mat thickness from 11 pieces of substrate that were removed from the entire width of the stream at the sampling site during each site visit. Continuous data were also collected, including pH, specific conductance, temperature, turbidity and dissolved oxygen, using a YSI 6600 data sonde as part of the SRBC RWQMN (http://mdw.srbc.net/remotewaterquality/). In addition, underwater photosynthetically active radiation (PAR) was characterized with a sensor (single LI-COR 2-pi, wiped) fastened to the substrate using a rebar stake and integrated into the existing YSI 6600 continuous data sonde (Figure 3).

Streamflow was measured at the West Branch Pine Creek RWQMN station during 35 discrete sampling events since the site was established in 2010. Measurements spanned a large range of hydrologic conditions. Average daily streamflow (ADF) was modeled in order to make this variable continuous. First, the Baseline Streamflow Estimator (BaSE) software (Stuckey et al., 2012) was used to select the most spatially correlated, un-regulated USGS stream gage for comparison of conditions at West Branch Pine Creek, which was determined to be Kettle Creek at Cross Fork, PA (01544500). The relationship of log10 transformed observed discharge measurements at West Branch Pine Creek and ADF at Kettle Creek was determined to have the best fit of all candidate models examined (R² = 0.82). Therefore, the linear regression equation (y = 0.9703x - 0.1535) was used to predict ADF at West Branch Pine Creek, using ADF time series data available for Kettle Creek from the USGS PA Waterwatch website (http://waterdata.usgs.gov/pa/nwis/inventory/?site_no=01544500). To avoid the potential biases associated with extrapolating high flow outside of the range where measurements were used to derive the regression equation, all ADF values greater than the maximum observed discharge were determined using a simple drainage-area ratio method. Here, ADF at West Branch Pine Creek was determined by adjusting the observed ADF at the Kettle Creek gage by the drainage area ratio of the sites (Equation 1). The regression based streamflow record extension and drainage-area ratio methods are described more fully in Hirsch (1982) and Hirsch (1979), respectively.

**Equation 1:**

\[ Q_{ungaged} = \frac{DA_{ungaged}}{DA_{gaged}} x Q_{gaged} \]

- \( Q_{ungaged} \): Average daily streamflow at the ungaged location (West Branch Pine Creek)
- \( Q_{gaged} \): Average daily streamflow at surrogate USGS gage station (Kettle Creek)
- \( DA_{ungaged} \): Drainage area of the ungaged location (70.3 mi²)
- \( DA_{gaged} \): Drainage area at surrogate USGS gage station (136.0 mi²)
Sixteen discrete water chemistry samples (SRP and TDP) were collected during the study period at the West Branch Pine Creek RWQMN station. In order to determine how didymo SCI was associated with SRP concentrations, these discrete measurements were used to predict continuous concentrations. Although total phosphorus concentrations in streams are known to correlate with streamflow, it is less clear whether SRP concentrations are likewise correlated. So, Pearson correlation was used to quantify the relationship between SRP concentration and measured continuous variables at West Branch Pine Creek. The only variables significantly correlated with SRP were turbidity and streamflow ($\alpha=0.05$). Streamflow was positively correlated with SRP and had the best relationship (Pearson $r = 0.648$, $P= 0.007$). The composite method (function loadComp) in the loadflex package (Appling et. al, 2015a) in the R software environment (R Development Core Team, 2006) was then used to predict daily SRP concentrations based on empirical observations and streamflow. This composite method “combines the predictions from a regression model with an empirical ‘residuals correction’ to bring predictions closer to observations during the period of interest. This two-step process can reduce short term biases and thereby lead to more accurate estimates of total fluxes or mean concentrations.” (Appling et al., 2015b).

Figure 3. Photosynthetically Active Radiation (PAR) Sensor (Foreground) and YSI 6600 sonde in Protective Casing in Background at the West Branch Pine Creek RWQMN Station
Research Question 3) How does didymo presence affect the nutritional content of biofilms?

We sampled 12 different sites in the summer and a subset of 5 sites in the fall throughout the study area to collect a total of 44 samples for inclusion in this study. At each site, a suite of subsites (2-4) within 100m reach were selected. From each subsite, rocks (~10) were randomly collected and scrubbed clean of all biofilm material. The resulting slurry was then placed into 2L Whirlpacks®, and frozen at -20 or -80°C. Frozen Whirlpack® samples were then transported to the USGS Leetown Science Center Northern Appalachian Research Laboratory facility and subsequently underwent lyophilisation using a Virtis Genesis 25ES Freeze dryer (SP Industries, Gardiner, NY).

Data generation caveat

Our initial analysis indicated that the methodology employed for the fatty acid component of the study may have had an analytical bias that underestimates the saturated component of the fatty acid profile (SAFA). As a consequence, because the data are normalized to lipid content and expressed as a percentage of total fatty acids, the unsaturated component of the fatty acid profile (mono-unsaturated [MUFA], polyunsaturated [PUFA], and highly unsaturated [HUFA]) may have been artificially inflated. As of right now, we cannot state with confidence that the entire dataset is accurate. Due to time constraints associated with grant reporting deadlines, we are not able to correct the issue and are presenting the current data on a provisional basis. To ameliorate this issue, we are going to re-analyze all samples and offer all subsequent analyses at our own costs to ensure completion of the project in a scientifically rigorous manner.

Brief synopsis of fatty acid characterization

Periphyton FA determination was performed using gas chromatography methodology. Briefly, lipids were extracted from the periphyton and subsequently methylated with a propane solution. The process of methylation created a fatty acid methyl ester (FAME), which facilitated combustion and detection by a gas chromatograph (GC). Based on carbon chain size and extent of desaturation, FAMES differentially passed through the column and their peaks were detected at different times. These characterized peaks were then compared to known standards.

Detailed methods for fatty acid determination

The process of lipid hydrolysis, FA methylation, and extraction was a modification of the one-step procedure described by Garcés and Mancha (1993). Briefly, freeze-dried periphyton samples (35-200 mg) were heated to 60-65°C in Teflon-lined capped, conical test tubes mixed with 3.7 mL hexane, 1.0 mL toluene, and 2.3 mL aqueous reagent. Aqueous stock reagent consisted of methanol (21 mL), 2,2-dimethoxypropane (3 mL), and concentrated sulfuric acid (1 mL). Samples were heated and intermittently vortexed for two hours at 15 min intervals. Samples were then shaken and cooled to room temperature, after which 2 ml of saturated sodium chloride solution was added, the tube vortexed and then centrifuged for 10 min at 600 x g relative centrifugal force (rcf). The top layer was transferred to a clean test tube with a Pasteur pipette. Two mL of hexanes was then added to the lower layer and again vortexed, centrifuged,
and the top layer added to the clean test tube. Tubes containing the combined hexane extracts were reduced to dryness under stream of nitrogen gas. Two ml of hexane was added to resolubilize the lipid material and duplicate 150 μL subsamples were gravimetrically assessed for total lipid concentration (lipid mass/dry weight material extracted). The remaining sample was dissolved in hexane (1.7 mL) and diluted to 1 mg lipid/mL for GC analysis.

GC separation and quantification of FAs employed a Hewlett-Packard 6890N Series GC fitted with Supelco column; 100 m x 0.25 mm ID x 0.20 μm thick film (SP-2560; Bellefonte, PA). Eluted peaks were determined by comparing their retention times to those of the known FA methyl ester standards using a five-point standard curve. FA methyl ester standards consisted of i14:0, i15:0, a15:0, i16:0, a16:0, and i17:0 (Sigma-Aldrich, St. Louis, MO); 18:1ω11, 18:4ω3 (Cayman Chemical, Ann Arbor, MI); Docosapentaenoic acid, 22:5ω3 (DPA: Supelco #47563-U, Bellefonte, PA) and a 37-component FA methyl ester standard (Supelco #47885-U, Bellefonte, PA). Cholestane, a synthetic lipid was added to all samples as an internal standard to assess extraction efficiency (Iverson, 2009). The remaining known FA concentrations were calculated based on the standard curve of its nearest neighbor.

Data analysis

Individual FAs were expressed as a percentage of the total FAs summed across all samples. FAs that comprised greater than 0.1% by mass of the total known FA community were retained and subsequently normalized to a 0-100% scale (Maranto et al., 2011). Overall, these 29 FAs accounted for 76-87% of the total biomass of all known FAs characterized across samples. Individual FAs were also compiled and classified on a percentage basis according to their structural groups (SAFA, MUFA, PUFA, HUFA, ω3, and ω6). We used nonmetric multidimensional scaling (NMDS) to constrain the complement of FAs to two axes and analysis of similarity (ANOSIM) to compare the variation of FA community composition across season (summer and fall) and didymo treatments (presence or absence). Moreover, we used the envfit function in the vegan package (Oksanen et al., 2013) in R as a data exploration tool to examine which environmental factors best correlated to the community similarities of the ordination. We examined the following variables: (1) how the samples were taken (sample description); (2) visual presence of didymo via microscopy (didymo present or absent); (3) visual observation of didymo stalk (didymo stalk); (4) Trophic state of the site (Trophic); (5) Season collected (Season); and (6) a Season by didymo present interaction (Season x didymo). We used Similarity Percentage Analysis (SIMPER) to evaluate which particular FAs contributed the greatest similarities across treatments (i.e., Didymo presence versus absence in the summer). We also conducted two-way ANOVAs evaluating the influence of: (1) Didymo present via microscopy (present versus absent); (2) Season (summer versus fall); and subsequent interaction of the two main effects on each FA and FA guild (i.e., SAFA, MUFA, PUFA, HUFA, ω3, ω6, ω3:ω6).
RESULTS

Research Question 1) What is the current and historic distribution of didymo in Pennsylvania?

Statewide didymo spatial distribution

A total of 1792 total algal records were compiled throughout the study area. The earliest record was from June 1993, while the most recent record was November 2015. The majority of these records (n=1696) did not detect the presence of didymo. These negative records were spread across a large portion of the state, but there are noticeable gaps in coverage in western PA (Ohio River drainage). There were, however, 96 records that positively detected didymo. The positive observations were from six major watersheds within or intersecting with the political boundary of PA. The presence and distribution of didymo has been well documented in four of these watersheds: Delaware River in northeastern, Pine Creek in northcentral, Gunpowder Falls in southern, and Youghiogheny River in southwestern PA (Figure 4).

Didymo has been observed in the East Branch, West Branch, and in the upper mainstem Delaware River since 2007. Didymo blooms with large amounts of stalk material were observed downstream to near Callicoon, NY, in the reach of the river that is thermally and hydrologically governed by bottom-release dams located upstream. In April 2012, a significant bloom was observed throughout an approximately 100-mile stretch of the Delaware River, from below Dingmans Ferry at river mile 239 upstream to above the confluence of the East and West Branches at river mile 330. Didymo coverage was spatially variable, but this was a notable event that demonstrated the potential for large scale nuisance blooms when environmental conditions were suitable (Figure 4; DRBC, 2014). The influence of the Lehigh River, which adds significant loadings of SRP to the nutrient poor upper section of the Delaware River, is notable in the context of didymo bloom formation. Didymo has only been observed in short stalk form below the mixing zone of the Lehigh River, where SRP concentrations are increased (Silldorff, 2012; Silldorff and Swann, 2013).

Didymo was observed in the Youghiogheny River in 2012 (PFBC, 2012) and was systematically surveyed in June and July of that year. Didymo was found attached to the substrate with some locations exhibiting blooming characteristics along a 49-mile reach from just below Youghiogheny River Lake in southwestern PA along the Maryland border, to just downstream of Smithton, PA (Figure 4; Rick Spear, personal communication). Our study also confirmed the presence of didymo just downstream of Youghiogheny River Lake in 2015, attached to the substrate and in the water column (Keller and Hilderbrand, 2015). Like the Delaware River, the Youghiogheny is also regulated by a reservoir with a hypolimnietic release.

Currently, there is no evidence of didymo in Maryland (MD) prior to 2008. In January 2008, didymo was first observed in Gunpowder Falls downstream of the Prettyboy Reservoir, which is another large reservoir with a hypolimnietic release (Figure 4). Subsequent monitoring revealed that blooms most often occurred annually between December and May along a 16 mile stretch of Gunpowder Falls below Prettyboy Reservoir. Interestingly, didymo has been constrained to the MD portion of the Gunpowder Falls watershed, with no observed occurrences upstream of Prettyboy Reservoir. Didymo has also been documented in three additional MD
watersheds outside of our study area, Savage River, North Branch Potomac River, and Big Hunting Creek (Klauda et al., 2015).

East Branch Dyberry Creek and Trout Run in northeastern and northcentral PA, respectively, have both confirmed the presence of didymo, yet only in one sample (Figure 4). In East Branch Dyberry Creek, didymo was observed on 3 May 2012 in one small patch in a sunlit riffle. It was present in the early-stage growth form of small tufts. A search upstream and downstream from this location did not result in any additional observations of didymo on stream substrate (Tim Daley, personal communication).

The positive didymo record in Trout Run comes from a single environmental DNA (eDNA) sample taken on 4 April 2015 (Keller and Hilderbrand, 2015). This positive record indicates that didymo cells were present in the water column at the time of sampling. Only one of two samples run in duplicate indicated didymo presence, suggesting that didymo abundance may be very low. The possibility that this was a false positive is unlikely due to stringent lab procedures and quality control assurances (Stephen Keller, personal communication).

Rock scrape samples collected in Trout Run in April and October were both examined microscopically without detection of didymo, despite apparent presence in the water column. While seemingly in conflict, the disparity between didymo cell abundance on the substrate and in the water column has been documented in previous studies. For instance, in a recent study in New Zealand 27% of sites confirmed didymo suspended in the water column but not in benthic sampling (Bray et al., 2016). Additionally, Kilroy and Dale (2006) demonstrated an ability to detect didymo cells using drift sampling techniques up to 14 miles downstream of sparse didymo colonies. Therefore, aside from methodological error or contamination, the most likely explanation is that didymo was present in the water column in the lower section of Trout Run in April 2015, yet not present on the substrate. Tributaries impacted by abandoned mine drainage enter a short distance upstream of the sampling site on Trout Run, which could make site-specific physicochemical conditions inhospitable for didymo (i.e., elevated acidity, metals, low pH). Viable populations upstream of the impacted tributaries may provide a source of cells that remain in the water column and pass through areas of poor water quality. Repeated sampling at this site and additional sampling upstream in the Trout Run Watershed would elucidate this uncertainty.

Pine Creek didymo temporal and spatial abundance

The distribution of didymo throughout Pine Creek was explored in detail. A total of 112 historical algal records and archived samples were compiled to determine if the presence of didymo could be detected prior to the initial finding. Notably, three archived SRBC samples from June and July 2012 were examined for the presence of didymo in the upper section of Pine Creek, in the reach where didymo was first found attached to the substrate in 2013. None of the compiled records or archived samples examined showed any evidence of the presence of didymo in the Pine Creek watershed prior to June 2013 (Figure 5a). Didymo cells were first detected by PADEP in the water column on 18 June 2013 on lower Pine Creek near Hamilton Bottom, PA. Didymo was then found attached to the substrate later in October 2013, more than 50 miles upstream in West Branch Pine Creek and upper reaches of Pine Creek (Figure 5b). Since the initial discovery of didymo in Pine Creek, 84 additional algal samples have been, 25 of which
Figure 4. Didymo Presence / Absence in Pennsylvania from June 1993 – November 2015
confirmed the presence of didymo on the substrate or in the water column. Cell density and didymo coverage have been highest in upstream reaches, including West Branch Pine Creek and upper Pine Creek. However, didymo cells have been observed attached to the substrate as far downstream as the Tiadaghton access in the Pine Creek gorge, which is 24 miles from the upstream extent of colonization. Additional samples from further downstream have not revealed didymo presence on the substrate, but samples filtered from the water column have detected the presence of didymo cells. Repeated sampling conducted in 2014 and 2015 indicates that valuable Pine Creek tributaries, including Ninemile Run, Genesee Forks, Phoenix Run, Long Run, Elk Run, Marsh Creek, Babb Creek, Cedar Run, Slate Run, Blockhouse Creek, and Little Pine Creek are not currently colonized by didymo (Figure 5b).

Five algal samples were collected from substrate at the monitoring station on West Branch Pine Creek at Crippen Run, which is about 2 miles upstream of the known colonization (Figure 5), between November 2013 and May 2015. Interestingly, these samples and two eDNA samples collected in April of 2014 and 2015, did not detect the presence of didymo on the substrate or in the water column (Keller and Hilderbrand, 2015). Didymo was later observed from substrate samples collected in November 2015 at this location. This serves as evidence that didymo is expanding its range in the Pine Creek Watershed.

We recognize that conventional sampling protocols for microorganisms, such as didymo, are considered inadequate to definitively determine which taxa are truly absent at any given point in time. Consequently, it becomes difficult to determine if didymo was definitively absent based on historical samples and/or data (Spaulding and Elwell, 2007; Lavery et al., 2014). It is perplexing, however, that when similar methods were employed to collect samples after the first observation of didymo in 2013, detection of cells was quite easily accomplished.

To further examine the spatial and temporal abundance of didymo in the Pine Creek Watershed, quantitative data were collected from a 24-mile reach of Pine Creek on 28 May and 10 November 2015. Algal samples were collected from the substrate at five sites from West Branch Pine Creek at Crippen Run downstream through the mainstem of Pine Creek to the Tiadaghton access in the Pine Creek gorge (sites A-E in Figure 6). Didymo was present at three sites in the spring and two sites in the fall. Cell densities were highest at the West Branch Pine Creek RWQMN station during both sampling events, and decreased at downstream sites (Table 2).

Table 2. **Didymo Cell Density at Longitudinal Study Sites on Pine Creek in Spring and Fall 2015**

<table>
<thead>
<tr>
<th>Site*</th>
<th>Spring didymo cell density (cells/cm²)</th>
<th>Fall didymo cell density (cells/cm²)</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A – West Branch Pine Creek at Crippen Run</td>
<td>0.0</td>
<td>104.5</td>
<td>52.3</td>
<td>73.9</td>
</tr>
<tr>
<td>B – West Branch Pine Creek at RWQMN station</td>
<td>5768.2</td>
<td>492.4</td>
<td>3130.3</td>
<td>3730.5</td>
</tr>
<tr>
<td>C – Pine Creek downstream of Galeton along Rt. 6</td>
<td>50.0</td>
<td>0.0</td>
<td>25.0</td>
<td>35.4</td>
</tr>
<tr>
<td>D – Pine Creek at Rexford</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>E – Pine Creek 1.6 mi upstream of Tiadaghton access in</td>
<td>13.3</td>
<td>0.0</td>
<td>6.6</td>
<td>9.4</td>
</tr>
</tbody>
</table>

*Letter corresponds to site labels in Figure 6
Figure 5. Presence of Didymo in Algal Samples in the Pine Creek Watershed Collected (a) Before and (b) After 18 June 2013
Figure 6. Didymo Cell Density and Soluble Reactive Phosphorus Concentrations at Monitoring Locations in the Pine Creek Watershed
Research Question 2) What is the potential habitat suitability for nuisance didymo blooms in Pennsylvania?

Statewide nutrient concentrations

A total of 249 SRP and TDP samples were collected from the Susquehanna River drainage, at 51 RWQMN and two additional stations (Figure 2). Between 3-19 samples were collected at each site between 22 April 2014 and 23 November 2015. At a minimum, three SRP samples were collected from all RWQMN stations in PA during the spring, summer, and fall of 2015. Concentrations in 24 and 154 of these samples were below the detection limit for SRP and TDP, respectively. Median concentrations of SRP were highest in summer and lowest in fall and winter. Median TDP concentrations did not appear to vary across seasons, which may be explained by the greater number of samples below detection limits. Upper quartiles TDP concentrations, however, were noticeably higher in the spring and summer compared to fall and winter (Figure 7).

![Figure 7. Boxplot of Soluble Reactive Phosphorus (SRP) and Total Dissolved Phosphorus (TDP) Concentrations from 249 Samples Collected in Pennsylvania in 2014 and 2015, by Season (Dashed line indicates detection limit. Bold line indicates median value, lower and upper extent of boxes indicate first and third quartiles, respectively.)](image)

Interestingly, mean SRP concentrations were lowest in West Branch Pine Creek (1.95 µg/L) and Trout Run (1.96 µg/L), which are the only two watersheds where didymo is present in the Susquehanna Basin portion of PA. The highest mean concentration of SRP occurred at Sugar
Creek (54.37 µg/L). The remaining streams displayed a large range of SRP concentrations (Figure 8).

The pattern of SRP concentrations and didymo occurrence throughout Pine Creek is somewhat consistent with findings from other published studies. SRP concentrations are very low in West Branch Pine Creek, where didymo colonization begins and is most dense. A short distance downstream West Branch Pine Creek meets Upper Pine Creek to form the mainstem of Pine Creek in Galeton borough. Incrementally higher mean SRP concentrations have been observed (4.4 µg/L) at the mouth of Upper Pine Creek. A short distance downstream in Galeton, a sewage treatment plant discharges treated effluent into Pine Creek, which is another source of phosphorus. Moving downstream, two tributaries, Long Run (4.2 µg/L) and Elk Run (10.1 µg/L), enter Pine Creek and raise phosphorus concentrations even further (Figure 6). In Ansonia, PA, where Pine Creek turns south and flows through the PA grand canyon, Marsh Creek enters and contributes additional phosphorus. Marsh Creek has among the highest mean SRP concentrations of all RWQMN watersheds (19.4 µg/L; Figure 8). Along this journey through Pine Creek, didymo cell density is highest in the oligotrophic portion of West Branch Pine Creek and then decreases markedly as Pine Creek flows downstream and becomes more mesotrophic. Mean SRP nearly triples along this 24-mile section of Pine Creek, from the site where didymo colonization originates (2.0 µg/L) to the Tiadaghton access in the Pine Creek gorge (5.7 µg/L) (Figure 6). SRP concentrations appear to exert control over didymo distribution and abundance in Pine Creek, effectively creating a narrow window of habitat suitability. As such, nuisance blooms may occur with some regularity depending on other physicochemical variables (discussed below) in the downstream portion of West Branch Pine Creek. Moreover, due to phosphorus contributions from downstream tributaries and point sources, nuisance blooms are less likely to affect much of the remaining portion of Pine Creek. To verify this conclusion, better estimates of SRP concentration and didymo occurrence are needed in the mainstem portion of Pine Creek from Marsh Creek to its confluence in the West Branch Susquehanna.

In total, 18,583 of the SRP records that were compiled throughout the study area met data screening criteria and were subsequently included in this analysis, including the samples discussed above and data from other sources. At least one SRP record was present in 173 out of 332 HUC10 watersheds (52%) located within or intersecting with the political boundary of PA. The majority of HUC10 watersheds (304/332) were located in the three major drainages in PA: Susquehanna, Ohio, and Delaware Rivers, respectively. The Susquehanna had the best data coverage, with nearly 68% of HUC10s having at least one SRP record. Data collection resulting from this study was the leading contributor of the enhanced spatial resolution of SRP data in the Susquehanna River drainage. The Delaware River, Potomac River, and Chesapeake Bay drainages had good SRP data coverage, while the Ohio River, Lake Erie, and Genesee River drainages had poor coverage (Table 3). The maximum number of records in one HUC10 watershed was 1534, which was the Elk River HUC10 in southeast PA. Five of the 332 total HUC10 watersheds had only one record of SRP, which was used to calculate a mean SRP value for the entire watershed. This small sample size has a greater likelihood of misrepresenting actual SRP conditions, but we chose to include all HUC10s with at least one SRP record for the purpose of visually examining the relationship of didymo distribution and SRP concentration. Caution should be exercised before conducting more advanced analytical techniques due to this potential source of bias.
Figure 8. Boxplot of Soluble Reactive Phosphorus (SRP) Concentrations from 249 Samples Collected in Pennsylvania in 2014 and 2015, by Site (The number preceding the stream name on the x axis corresponds with the numbered watersheds in Figure 2. Streams in blue on the x axis are also habitat suitability stations in this study. Box plots are shown in ascending order of mean values. Dashed line indicates detection limit (1.1 µg/L). In each respective box, bold line indicates median value, lower and upper extents indicate first and third quartiles, respectively. The y axis was cropped to display the variation in low concentrations.)
Table 3. Total Number of HUC10 Watersheds within Major Drainages in Pennsylvania, and Number and Percent (in parentheses) of HUC10 Watersheds with at Least One and Zero SRP Records

<table>
<thead>
<tr>
<th>Major Drainage</th>
<th>No. of HUC10 watersheds</th>
<th>No. and (percent) HUC 10s with ≥1 SRP records</th>
<th>No. and (percent) HUC 10s with 0 SRP records</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susquehanna River</td>
<td>140</td>
<td>95 (67.9)</td>
<td>45 (32.1)</td>
</tr>
<tr>
<td>Ohio River</td>
<td>109</td>
<td>20 (18.3)</td>
<td>89 (81.7)</td>
</tr>
<tr>
<td>Delaware River</td>
<td>55</td>
<td>37 (67.3)</td>
<td>18 (32.7)</td>
</tr>
<tr>
<td>Potomac</td>
<td>17</td>
<td>17 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Lake Erie</td>
<td>5</td>
<td>0 (0.0)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Chesapeake Bay</td>
<td>4</td>
<td>4 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Genesee River</td>
<td>2</td>
<td>0 (0.0)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>332</td>
<td>173 (52.1)</td>
<td>159 (47.9)</td>
</tr>
</tbody>
</table>

The PA nutrient dataset allowed us to evaluate the distribution of didymo from the perspective of SRP-specific habitat constraints. SRP concentrations were generally lower in the forested West Branch Susquehanna subbasin in north-central PA compared to watersheds in central and southeastern PA. Data were lacking throughout much of western PA in the Ohio River and Lake Erie drainages (Figure 9). Data were available to calculate mean SRP concentrations throughout five of the six watersheds where didymo is present (all except East Branch Dyberry Creek). In all watersheds where didymo colonization originated, mean SRP concentrations were <10 µg/L. The West Branch Pine Creek and Lower West Branch Susquehanna River (Trout Run) HUC10s had the third and ninth lowest mean SRP concentration of all 173 HUC10 watersheds where data were available. Moreover, mean SRP in the tailwaters of three streamflow regulated, hypolimnetic release streams (West Branch Delaware River, Gunpowder Falls, and Youghiogheny River) was higher (5.1-10 µg/L) than other free-flowing systems (Pine Creek and Trout Run, < 5 µg/L) that supported didymo (Figure 9). This pattern could inform the role of water regulation and management of didymo.
Figure 9. Mean Soluble Reactive Phosphorus in HUC10 Watersheds and Didymo Presence in Pennsylvania
Using data from a suite of 17 sites (see Figure 2) we conducted a modeling effort to incorporate physicochemical variables that, in addition to SRP, might be relevant to characterizing didymo habitat suitability. We applied data characterized from each site to a compiled list of thresholds and criteria used in other didymo habitat suitability indices (Table 1). In terms of physical criteria, the majority of sites had suitable rocky substrate (16) and non-turbid water (15); yet only a few sites met the flow regulation (1), low temperature (3), and shallow gradient (4) deemed preferable for didymo. Aside from SRP, chemical suitability yielded little discriminatory power among sites as sulfate, calcium, nitrate, and total organic carbon (TOC) were deemed suitable across all sites. Soluble reactive phosphorus was only low enough at a handful of sites, with 2 sites meeting the <2 µg/L and 8 sites under <5 µg/L threshold. Site specific score can be viewed in Appendix C.

According to our analysis, Little Pine Creek was most suitable for didymo colonization, scoring 18 out of 23 possible points (Figure 10). Interestingly, didymo has not been detected at this site during numerous sampling events in 2014 and 2015. Little Pine Creek is downstream of a riverine impoundment with flow attenuation capability (Little Pine Lake) and has low SRP concentrations. Little Pine Lake has an epilimnetic release, which in combination with its large size, causes temperature at this site to be warmer than preferred didymo habitat. Also, Little Pine Creek has the highest sulfate concentrations (mean = 28.4 mg/L) of all habitat suitability sites. The sulfate criteria included in the habitat suitability index is a minimum criterion derived from the literature, but no mention of a maximum preferred limit could be found. It is possible that a legacy of coal mining in the Little Pine Creek Watershed, which results in elevated sulfate levels today, is preventing didymo colonization. Second, our index is based on habitat suitability assuming that didymo can colonize the site. Didymo simply may not have had the opportunity to colonize this site.

The two sites where didymo has been observed in the Susquehanna basin had the second and third highest scores, which were West Branch Pine Creek and Trout Run, respectively (Figure 10). West Branch Pine Creek met every chemical criteria, but did not have adequate flow regulation upstream and warmed above 18ºC for a substantial portion of the year. These factors appear to manifest themselves in temporal observations at this station, where didymo coverage responded negatively to high flow events and warm summer temperatures. If flows become stable in the fall as water temperature decreases, didymo stalk growth occurs (see further discussion of habitat suitability at this site below). West Branch Pine Creek appears to be an example of didymo colonization in marginal habitat, as it is subjected to greater natural variation in flow and temperature than a flow regulated regime provides.

Trout Run also lacks flow regulation and a consistently cold thermal regime. However, pH is depressed at this site with a long-term mean of 5.9, which is below the suggested minimum didymo tolerance of 6.7-7.0 (Stoddard et al., 2005; Lindstrøm and Skulberg, 2008). While didymo has not been detected on the substrate at this location, the most plausible explanation for the presence of didymo in the water column is that the eDNA sampling event in 2015 detected DNA that had drifted from an upstream population. Other possibilities that must be considered, however unlikely, include cross-contamination and false positive PCR results.

Kettle Creek is equally suitable habitat for didymo compared to Trout Run, but didymo has not been detected during repeated sampling in 2014-2015. This site on Kettle Creek, which is located about 17 miles upstream of Alvin R. Bush dam (Kettle Creek Reservoir), lacks flow
regulation upstream and has slightly higher SRP concentrations than is preferred (mean = 3.78 µg/L), but otherwise represents suitable habitat. Other sites that scored relatively high include: Bowman Creek, Ninemile Run, Snake Creek, Upper Pine Creek, and Pine Creek (Figure 10). Of these sites, didymo has only been observed at Pine Creek, which is directly downstream of the didymo colonization at West Branch Pine Creek. This site has had only a few cells observed attached to the substrate, with no bloom formation noticed to date.

Figure 10. Results of Habitat Suitability Index Presented in Order of Most Suitable to Least Suitable (The number preceding the stream name on the x axis corresponds with the numbered watersheds in Figure 2. Red font on x axis indicates documented didymo presence in the watershed.)
Sites included in the habitat suitability index are representative of streams throughout PA, were distributed throughout a large geographic area, and were preferentially chosen based on a priori knowledge of low nutrient concentrations (Figure 2). As such, we can draw a general conclusion that unregulated PA streams generally do not have adequate flow stability to support long-term nuisance didymo blooms. Yet, at sites where didymo is or becomes established, blooms may occur during natural periods of stable hydrologic conditions.

Streams that may have adequate thermal regimes to support didymo are often small, too narrow to allow adequate light infiltration through the canopy, steep in gradient, and poorly buffered. This combination of conditions, while broadly categorized, may forecast unsuccessful didymo establishment in streams below 10 m wide. Most larger streams with adequate gradient and width may also be too warm and eutrophic to sustain didymo blooms. Interestingly, these larger streams which include West Branch Pine Creek, represent marginal habitats that may have periodic nuisance didymo blooms, but likely will not experience blooms year-round. Truly preferred habitat, which is oligotrophic reaches downstream of bottom-release dams, is not common in the Susquehanna Basin. This habitat is present in adjacent PA drainages, including the Delaware River, Youghiogheny River, and Gunpowder Falls.

**West Branch Pine Creek case study**

We conducted high frequency discrete sampling and characterized didymo standing crop index (SCI) at the West Branch Pine Creek RWQMN station from 21 October 2014 through 31 December 2015. Separate studies have established that SCI strongly reflects measured periphyton ash free dry mass (AFDM), where an AFDM threshold of 35 g/m² for nuisance periphyton corresponds to an SCI of 220 (Kilroy and Bothwell, 2012). We only observed one instance (19 November 2014) where observed SCI at West Branch Pine Creek exceeded the nuisance periphyton threshold (Figure 11). Didymo SCI remained high throughout the winter of 2014-2015, but persistent ice cover prevented access to the substrate for an accurate SCI estimate. A high flow event that coincided ice-out in the spring of 2015 removed much of the didymo stalk material that had been attached to the substrate during winter, which left substrate nearly bare in April 2015 (Figure 11). Evidence of growth of didymo stalk material was present in May 2015, but summer low flows coincided with the disappearance of didymo stalk material from the substrate in August 2015. Didymo stalk material began to form on substrate once again in November 2015 and had grown to near nuisance levels by December 2015 when this study concluded (Figure 11).
Figure 11. Photographs of Didymo Growth on Substrate and Instream Conditions During 2014 and 2015 at the West Branch Pine Creek Monitoring Station (Standing Crop Index (SCI) values and date of photographs are displayed for each series of pictures.)
We examined the relationship among a suite of continuous variables and didymo standing crop index (SCI) to identify potential physicochemical parameters that may be responsible for controlling didymo bloom intensity. A number of variables appeared to influence didymo SCI, with streamflow, temperature, and pH appearing to be most correlated (Figure 12), which concurred with literature sources used to construct the habitat suitability index. The thick didymo mats that persisted at West Branch Pine Creek throughout the fall and winter of 2014-2015 were virtually eliminated after a high flow event that coincided with ice-out in April 2015. An uncharacteristic period of stable flow in the spring of 2015 (May – June) allowed didymo SCI to rise slightly, but rising water temperature and high flow events during the summer of 2015 reduced SCI to zero through early October 2015. Although didymo SCI increased during fall 2015, a series of high flow events restricted SCI to values much lower than fall 2014, which had much more stable streamflow (Figure 12). The high streamflow events in fall 2015 also depressed pH to below-neutral values, which stands in contrast to the alkaline conditions experienced in fall 2014. SRP values also increased with increasing streamflow, in effect causing concentrations to increase in fall 2015 in contrast to the low concentrations of SRP that persisted throughout fall 2014 (Figure 12).

Although didymo stalk production has been associated with high light environments (Bothwell and Kilroy, 2011), mean daily photosynthetically active radiation (PAR) and daylight length did not appear to control didymo SCI at the West Branch Pine Creek monitoring site. The underwater PAR sensor was installed on 8 May 2015 and operated continuously through the end of the study period. Day length appeared to decrease steadily after the summer solstice on 21 June 2015. Day length also noticeably decreased during a high flow event in October 2015, when instream turbidity increased dramatically (>70 NTU). Mean daily PAR was highly variable throughout the study period, but in general values were highest in spring and decreased as the seasons progressed (Figure 12). Mean daily PAR was significantly, negatively correlated with streamflow (Pearson $r = -0.37$, $P < 0.001$) The relationship between PAR and cloud cover are of interest but were not examined as part of this study. Mean daily PAR decreased to its lowest observed levels during the period of highest didymo growth during the study period, which was October through December of 2015. It appears that even the lowest observed levels of PAR are sufficient to support the photosynthetic requirements of didymo stalk production.

Specific conductance did not vary greatly during the study period, with all observed values falling between 30 and 60 microsiemens/cm, which did not appear to affect didymo SCI. Dissolved oxygen was elevated throughout the study period at West Branch Pine Creek, with the lowest recorded values (~8 mg/L) occurring in the summer of 2015, and also did not likely exert control over didymo SCI.

Streamflow appears to exert the most control over didymo SCI. Periods of stable flow preceded observations of abundant didymo stalk material in fall 2013 and 2014. However, there were numerous high flow events in 2015, especially during the late fall (Figure 13), which serves as the growing season for didymo in West Branch Pine Creek. High flow events result in increased water velocities, sheer stresses, suspended sediment loads, and in some cases, bed mobility, which are the primary controlling factors controlling benthic algal biomass at local scales (Biggs and Close, 1989; Cullis et al., 2013).
Figure 12. Continuous Data Collected at West Branch Pine Creek 21 October 2014 – 31 December 2015
Figure 13. Hydrograph of Modeled Streamflow Data at West Branch Pine Creek from 1 January 2013 to 31 December 2015 (Red dots correspond with sampling events and associated didymo abundance observations.)
Research Question 3) How does didymo presence affect the nutritional content of biofilms?

A total of 12 unique sites were sampled in summer 2015 (28 May to 9 July) across the study area. A subset of 5 sites were sampled in the fall (2 – 10 November) in the Pine Creek Watershed (Figure 14).

![Fatty Acid Sampling Locations in Summer and Fall 2015](image)

**Figure 14. Fatty Acid Sampling Locations in Summer and Fall 2015**

Multivariate analysis of fatty acid profiles

NMDS ordination was constrained to two axes and displayed significant clusters related to both the seasonality and presence of didymo (stress = 0.16) (Figure 15). For example, sample description ($R^2 = 0.32$, $p < 0.001$), visual microscopy ($R^2 = 0.07$, $p = 0.051$) and visual stalk ($R^2 = 0.09$, $p = 0.025$) were all correlated to the ordination; which highlights a potential didymo signature. Season was also highly important ($R^2 = 0.33$, $p < 0.001$), yet the interaction of season and didymo presence were most relevant ($R^2 = 0.485$, $p < 0.001$). The SIMPER analysis showed that C18:3n3, C20:5n, and C16:1n7 cumulatively explained over half of the variation in the summer (50%); while C20:5n3, C16:1n7, and C16.0 explained 38% in the fall.
**Individual fatty acids**

Published studies that have examined periphyton FA profiles report a strong presence of C14:0 FA (6-15% of total FAs) (Sushchik et al., 2007; Hill et al., 2011; Guo et al., 2015). To our surprise, our method did not detect C14:0. Nevertheless, of the saturated FAs that were detected, some differed with respect to season and didymo presence (Table 4). The relative amount of C15:0 was higher, and both C17:0 and C18:0 lower in periphyton samples that comprised didymo (Figure 2). C24:0 was also greater in samples that comprised didymo, yet this pattern was more pervasive in the fall as evidenced by the season x didymo interaction (Table 4, Figure 16).

Mono-unsaturated FAs were highly influenced by season. C16:1n7 was the only MUFA that differed with respect to didymo without a confounding seasonal interaction. C18:1n9c and C22:1n9 were both greater in biofilms that did not contain didymo, but only in the summer (Table 4, Figure 17). Individual PUFAs were strongly influenced by didymo composition, and many of these were dependent upon season. For example, C18:2n6c and C18:3n6 were both more abundant in the summer, yet overall, C18:2n6c was highest and C18:3n6 lowest in biofilms that comprised didymo respectively. C18:3n3, C18:4w3, C20:5n3, and C24:5n3c were all subject to season x didymo interactions, but in unique ways. For instance, both C18:3n3 and C22:6n3 were higher in biofilms where didymo was absent, but only in the summer and fall respectively. Similarly, C18:4w3 and C20:5n3 were higher in biofilms where didymo was present, but only in the fall and summer respectively (Table 4, Figure 18).

**Fatty acid metrics**

Despite a strong effect of didymo on the relative abundance of individual FAs, pooling into “fatty acid functional groups” was less informative. The relative abundance of both SAFA and MUFA fatty acid groups were higher in the summer, yet biofilms that comprised didymo were lower in SAFA and greater in MUFA. Similar seasonal effects were observed across seasons for poly- and highly-unsaturated fatty acids, w3, w6 fatty acids; and the commonly used w3:w6 estimate of nutritional condition (Table 4, Figure 19).
Figure 15. Results of Non-metric Multidimensional Scaling of Fatty Acid Composition Across Season and Didymo Treatments (Arrows reflect relevant environmental factors correlated to the analysis.)
Table 4. **Two-Way ANOVA Results Examining Didymo Presence and Season of Fatty Acid Relative Abundance** (‡ Denotes physiologically important fatty acids. Asterisks reflect level of statistical significance (* >0.05, **>0.01, *** >0.001).)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Didymo F(1,40)</th>
<th>Season F(1,40)</th>
<th>Interaction F(1,40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C15:0</td>
<td>4.31 *</td>
<td>0.54</td>
<td>2.41</td>
</tr>
<tr>
<td>aC16:0</td>
<td>1.02</td>
<td>3.26</td>
<td>0.97</td>
</tr>
<tr>
<td>C16:0</td>
<td>5.97*</td>
<td>35.35</td>
<td>10.65**</td>
</tr>
<tr>
<td>iC17:0</td>
<td>0.37</td>
<td>3.68</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>SAFA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17:0</td>
<td>12.30***</td>
<td>30.96***</td>
<td>1.16</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.92*</td>
<td>0.47</td>
<td>1.60</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.07</td>
<td>77.23***</td>
<td>23.20***</td>
</tr>
<tr>
<td>C23:0</td>
<td>1.99</td>
<td>0.07</td>
<td>6.40*</td>
</tr>
<tr>
<td>C24:0</td>
<td>5.29*</td>
<td>1.36</td>
<td>12.92***</td>
</tr>
<tr>
<td>C14.1n5</td>
<td>3.49</td>
<td>0.12</td>
<td>1.84</td>
</tr>
<tr>
<td>C15:1</td>
<td>1.62</td>
<td>0.33</td>
<td>1.12</td>
</tr>
<tr>
<td>C16:1n7</td>
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<td>0.54</td>
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<tr>
<td>C18.1n97</td>
<td>1.18</td>
<td>10.06**</td>
<td>0.40</td>
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<tr>
<td><strong>MUFA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1n9c</td>
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</tr>
<tr>
<td>C20:1n9</td>
<td>0.41</td>
<td>0.17</td>
<td>8.89**</td>
</tr>
<tr>
<td>C22:1n9</td>
<td>18.74***</td>
<td>5.75*</td>
<td>8.19**</td>
</tr>
<tr>
<td>C24:1n9</td>
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<td>8.05**</td>
<td>0.86</td>
</tr>
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</tr>
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<td>9.70 **</td>
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</tr>
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<td>14.69***</td>
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<td>C20:2</td>
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</tr>
<tr>
<td><strong>PUFA</strong></td>
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<td></td>
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</tr>
<tr>
<td>C18:4w3</td>
<td>10.09**</td>
<td>32.14***</td>
<td>4.74*</td>
</tr>
<tr>
<td>C20:3n6</td>
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<td>5.65*</td>
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</tr>
<tr>
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<td>0.44</td>
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</tr>
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<td>4.52*</td>
<td>6.22*</td>
<td>0.80</td>
</tr>
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<td>C20:5n3‡</td>
<td>6.94*</td>
<td>51.30***</td>
<td>5.23*</td>
</tr>
<tr>
<td>C22:4w6‡</td>
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<td>0.18</td>
</tr>
<tr>
<td>C22:5n3c</td>
<td>0.32</td>
<td>15.63***</td>
<td>4.30*</td>
</tr>
<tr>
<td>C22:6n3</td>
<td>8.12***</td>
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<td>HUFA</td>
<td>8.40*</td>
<td>19.84*</td>
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<tr>
<td>w3</td>
<td>6.12*</td>
<td>7.22*</td>
<td>0.51</td>
</tr>
<tr>
<td>w3</td>
<td>0.53</td>
<td>34.40***</td>
<td>0.04</td>
</tr>
<tr>
<td>w3:w6</td>
<td>1.04</td>
<td>116.90**</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Figure 16. Boxplots Representing the Relative Abundance of Saturated Fatty Acids (SAFA) in Periphyton Samples Collected in the Summer and Fall (Presence of didymo cells in periphyton was confirmed by microscopic analysis.)
Figure 17. Boxplots Representing the Relative Abundance of Mono-unsaturated Fatty Acids (MUFA) in Periphyton Samples Collected in the Summer and Fall (Presence of didymo cells in periphyton was confirmed by microscopic analysis.)

Figure 18. Boxplots Representing the Relative Abundance of Poly-unsaturated Fatty Acids (PUFA) in Periphyton Samples Collected in the Summer and Fall (Presence of didymo cells in periphyton was confirmed by microscopic analysis.)
We characterized fatty acid profiles across a suite of seasons and sites in periphyton differentially impacted by didymo. We then used the fatty acid profiles to evaluate the nutritional context of biofilms from a variety of perspectives. First, using multivariate ordination techniques, we were able to identify key periphyton fatty acid profiles that differentiate season and didymo presence or absence. For example, in the summer, α-linolenic acid (ALA, C18:3n3), eicosapentaenoic acid (EPA, C20:5n3), and palmitoleic acid (PAO, C16:1n7) mutually explained over 50% of the variation in the fatty acid groups separating biofilms with didymo from those without. Interestingly, the relative abundance of ALA and EPA, both considered physiologically relevant fatty acids, also differed with respect to didymo presence; albeit in different ways. Periphyton ALA was highest in the summer in samples where didymo cells were absent. Conversely, although EPA was highest in the fall, its relative abundance differed most among didymo and non-didymo periphyton in the summer. PAO was also a relevant indicator among didymo and non-didymo groups, which is not surprising since it is often used as an ecological indicator for diatom abundance (Napolitano 1999). In samples collected in the fall, PAL, EPA, and palmitic acid (PAL, C16:0) cumulatively explained variation in community similarities among didymo present versus absent periphyton, but to a much lower extent (~38%).

In addition to suites of fatty acid profiles, we also evaluated individual fatty acid relative abundances across season and didymo presence. Overall, specific SAFA fatty acids (C15:0, C16:0, C17:0, C18:0, and C24:0) differed as a function of didymo presence in the periphyton. Patterns of periphyton C16:0 and C24:0 SAFAs were more complex and reflected differences among didymo samples that were season-specific. C24:0 only differed among didymo samples (present versus absent) in the fall. While overall, the relative abundance of C16:0 was highest in

Figure 19. Boxplots Representing the Relative Abundance of Pooled Fatty Acid Metrics in Periphyton Samples Collected in the Summer and Fall (Presence of didymo cells in periphyton was confirmed by microscopic analysis.)
the summer, it was more abundant in didymo-present samples in the summer and lower in the fall. Caution should be taken not to over-interpret these results however, as our current dataset may reflect biases against SAFA fatty acid groups. In addition to methodological biases, the disparate sample sizes of in the summer and fall may also be relevant.

To specifically evaluate the hypothesis that periphyton that comprise didymo differ nutritionally from those that are didymo absent, we evaluated a suite of nutritionally important fatty acids and pooled fatty acid guilds. Here we define nutritionally important fatty acids as an analogue to essential fatty acids, realizing that in nature, there are a variety of pathways to get the desired nutritional product. Interestingly, the relative abundance of many fatty acids deemed nutritionally important was greatest in biofilms that comprised didymo. For example, both α-linolenic acid (ALA, C18:3n3) and Stearidonic acid (SDA, C18:4w3) were higher in biofilms that comprised didymo, regardless of season. The two fatty acids, however, are biochemically linked to each other. Stearidonic acid (SDA) may be biosynthesized from ALA via elongation and desaturation mechanisms (enzyme delta-6-desaturase)(Wacker and von Elert, 2001). Arachidonic acid (ARA, C20:4n6) and EPA were also greater in the didymo-present periphyton, but only in the summer. Finally, Docosapentaenoic acid (DPA, C22:5n3c) and Docosahexaenoic acid (DHA, C22:6n3), both nutritionally important ω-3 fatty acids were greatest in didymo-present periphyton samples in the fall and marginally lower in the summer. These results indicate that there is no pervasive pattern in terms of nutritionally important fatty acids and didymo context. Some fatty acids suggest didymo is more nutritious, while others suggest otherwise. Biosynthesis of fatty acids is a complex process however, with many direct and indirect pathways that may not be readily apparent in our study. More work on biosynthesis networks is needed to tease out these relationships.

Combining specific fatty acids into respective functional guilds yielded surprisingly little information. All guilds responded strongly to season, which is unsurprising given differences in photoperiod, temperature, nutrients and carbon loading (dissolved and leaf material) (Mulholland and Hill, 1997; Bossio et al., 1998; Honeyfield and Maloney, 2015). Interestingly, only the relative abundance of SAFAs and MUFAs differed with respect to didymo presence and this was in the summer. SAFA was higher and MUFA lower in the didymo-absent periphyton samples, indicating potentially greater nutritional quality of periphyton that contains didymo. While not statistically significant, periphyton comprised of didymo also exhibited greater HUFA, ω3, and ω3:ω6, further supporting the notion that the nutritional quality of didymo in biofilms may be a resource that is sufficient or ever beneficial to consumers. This is not surprising, since as a group, diatoms are generally considered more nutritious than cyanophytes or green algae.

While seemingly biologically relevant, caution should be taken when comparing this dataset for two important reasons. First, we are currently unaware of the extent which our methodological of bias extends to the interpretation of our results. Unfortunately, we were unable to remedy this issue before the grant funding deadline, however the issue will be remedied and the new dataset re-assessed. Second, nutritional quality can be characterized from many different perspectives not included in this study. For instance, the stock nature of the didymo may reflect unpalatable components including lignins and cellulose or toxins designed to inhibit consumption by grazers. Here, indicators of total carbohydrate relative to lipid may prove to be insightful. Other stoichiometric indices including carbon:phosphorus (C:P) and (carbon:nitrogen) may resolve the issue of palatability. Nevertheless, our results demonstrate the potential for didymo to influence the nutritional context of benthic biofilms and more studies that directly link consumer grazing to periphyton nutritional indices are warranted.
CONCLUSIONS

The ecological paradox that didymo represents, where nuisance blooms are restricted to nutrient poor, often pristine settings, presents a unique paradigm for the management of freshwater ecosystems. Moreover, the current debate surrounding its native or non-native status further confounds our understanding of what an appropriate response should be from a policy makers.

The intent of this study was to (1) compile current knowledge and augment the amount of information available to managers regarding the distribution of didymo in PA; and (2) identify gaps where relevant information and data exist. The physicochemical parameters examined in concert the spatial didymo distribution database serve to facilitate a better understanding of factors that govern the distribution of didymo across Pennsylvanian watersheds. These analyses highlight the potentially strong influence of both SRP concentration and flow regulation on didymo habitat suitability in this region. Additionally, the examination of didymo abundance temporally using continuously monitored physicochemical variables also serves as a novel approach that effectively elucidated dynamics of didymo colonization in an unregulated stream.

One major gap in our analysis relates to the temporal and spatial patchiness of didymo blooms and the ability to detect them when they arise. As is the case for all environmental issues, limitations in funding and manpower prohibit sampling all sites at all times. While preliminary, the methodological approach linking synoptic measurements with continuous data outlined in our report is a promising tool that can be used to potentially identify sites and time periods prone to didymo blooms. This tool may facilitate monitoring and management of streams that are either unmonitored or monitored infrequently.

We conclude that in general, unregulated streams in the Susquehanna basin serve as marginal didymo habitat and are unlikely to support year-round nuisance blooms. They could however, support episodic blooms that may periodically impact recreation and ecological health in a negative manner. We also highlight habitat deemed susceptible to didymo colonization and proliferation that may benefit from targeted management efforts.

It is unclear how a changing climate or increased anthropogenic watershed stressors will impact the varied physicochemical parameters relevant to habitat suitability of didymo. These factors, which may increase or decrease didymo habitat suitability, should be considered in future management plans aimed at controlling the spread of didymo.

Further, in light of growing concern over potential didymo effects on streams, there is a pressing need to assess its functional relevance to stream ecosystem health (Spaulding and Elwell 2007, Taylor and Bothwell 2014). This study is the first of its kind, to our knowledge, as we examined impacts of didymo through the lens of nutritional content of didymo colonized biofilms. Some insightful and unexpected relationships between fatty acid content and didymo presence and seasonality were revealed that could lead to a greater understanding of aquatic ecosystems.
ADDITIONAL RESEARCH INDICATED

Numerous monitoring projects and management actions, such as bans on felt sole waders, decontamination strategies, and public outreach campaigns, have been implemented in the mid-Atlantic region in order to document and/or slow the spread of didymo, which was until recently considered to be a classic example of an invasive species. As a result of recent research, didymo’s status as an introduced or native species is currently uncertain, and hotly debated. The highest priority should be placed on future research that definitively determines the origin of didymo in the mid-Atlantic region. Studies involving paleolimnological analysis of sedimentary diatom assemblages would provide insight into the origin of didymo with a high degree of certainty for the region (e.g., Lavery et al., 2014).

The single record of didymo presence in Trout Run in north-central PA should be investigated further. The water chemistry conditions at Trout Run appear to be outside of didymo’s preferred ecological tolerances, which suggests upstream portions of the watershed may be more likely to support didymo. Repeated sampling at the location of the 2015 observation and additional sampling upstream should be completed to elucidate the current presence and/or distribution of didymo in the Trout Run Watershed.

The didymo spatial database constructed for this study and shown visually in Figure 4 has noticeable gaps in data coverage, especially in the Ohio River and Lake Erie drainages. Additional effort should be targeted at collecting and/or compiling algal assemblage data where it is currently lacking. Data collection should be prioritized in watersheds where SRP concentrations are low (Figure 9); however, SRP data are generally lacking in the same areas and should also be collected when possible.

The fatty acid data collected and examined as part of this study represents a novel approach for evaluating the roles of seasonality and didymo colonization on the nutritional content of biofilms. The small sample size included herein shows promise but should be expanded to include samples collected over a larger spatial and seasonal gradient to promote increased understanding of how didymo affects ecosystem function.

ACKNOWLEDGMENTS

We thank Pennsylvania Sea Grant for providing funding for this study. The Susquehanna River Basin Commission funded earlier data collection activities that improved the quality and scope of this effort.

The contributions of many dedicated individuals were instrumental in adding value to this study. Steven Keller (University of Vermont), Bob Hilderbrand, Regina Trott (University of Maryland), and Jason Cessna (MD DNR) enabled eDNA sampling in Pennsylvania that provided clarity on the current distribution of didymo. Robert Volkmar (University of Duquesne, retired) also provided his expertise to aid the investigation of didymo distribution. Jeff Zimmerman (SRBC) provided invaluable GIS expertise that allow clear presentation of results. Dawn Hintz (SRBC) leveraged RWQMN resources and Graham Markowitz (SRBC) aided streamflow modeling efforts for the benefit of this study. Katie Kline (University of Maryland Center for Environmental Science) facilitated laboratory analysis of much of the water chemistry data included herein. Andrew Leakey, Matthew Elsasser, Aaron Henning, Blake Maurer, Luanne Steffy, David Haklar, and John Balay (SRBC) assisted with data collection. The following
individuals and their respective institutions provided data that added value to this study: Erik Sildorff and Bob Limbeck (DRBC); Tim Daley, Jeff Butt, Josh Lookenbill, and Rick Spear (PADEP); Michael Kashiwagi and Katherine Hannah (MD DNR); Heidi Krahling (NY Natural Heritage Program); Amy Jewitt (PA Natural Heritage Program); Katie Dunlap and Jake Lemon (Trout Unlimited). Additional support for this study was provided by Robert Morgan (PA Fish and Boat Commission); Lori Maloney and Erica Tomlinson (Tioga County Conservation District); Ron Comstock (Pine Creek Headwaters Protection Group); and John Dillon (Pine Creek Outfitters).

CITATIONS


Klauda, R.J., K.V. Hanna, M.T. Kashiwagi, and J.L. Saville. 2015. *Didymosphenia geminata* in Maryland trout streams: is extreme phosphorus limitation a necessary condition for


APPENDIX A

a. Staff
   i. Number of individuals
      • 21
   ii. Number of full-time employees (as part of the grant):
      • 10
   iii. Number of full-time employees (as part of match):
      • 16

b. Students Supported
   i. Number of Undergraduate Students
      • 0
   ii. Number of Graduate Students
      • 0
   iii. Number of Ph.D. Students
      • 0
   iv. Degrees Awarded (please indicate level)
      • 0

c. Outreach/Extension
   i. Number of meetings, workshops, or conferences, and number of attendees

   ii. Number of public or professional presentations, and number of attendees
      • Trout Unlimited Aquatic Invasive Species Workshop. February 28, 2015. Rec Park Center, Lewistown, PA. Oral Presentation:


APPENDIX B

Impact Statement

*Didymosphenia geminata* (didymo) is a freshwater diatom that is considered one of the most problematic invasive species currently threatening streams and rivers globally. Didymo is capable of producing nuisance blooms in pristine streams that could impact recreational, ecological, and aesthetic value of aquatic ecosystems. Although didymo blooms have been documented in Pennsylvania since 2007, there has been no comprehensive inventory of didymo distribution or identification of vulnerable watersheds within the state. Researchers from the Susquehanna River Basin Commission, U.S. Geological Survey, and Academy of Natural Sciences Pennsylvania collaborated to address lingering uncertainties to benefit future management of aquatic resources.

We compiled databases of didymo distribution and nutrient concentrations for Pennsylvania that characterized the physicochemical parameters that govern didymo presence and abundance. Results suggest that water chemistry (dissolved phosphorus) and streamflow regulation exert influence on didymo distribution at broad and local scales. We identified currently didymo-free watersheds where suitable habitat for didymo colonization exists and used continuous monitoring to elucidate relationships between didymo coverage, water chemistry, and hydrology in a free-flowing stream. Further, provisional data suggest the presence of didymo and season best explained variation in physiologically important fatty acid profiles, which is an indicator of nutritional quality in streams.

Our novel and interdisciplinary methodological approaches serve to increase technical knowledge related to didymo that is available to inform aquatic ecosystem management in Pennsylvania. We encourage future research that definitively determines the status of didymo as native or introduced to Pennsylvania.
### APPENDIX C

Habitat suitability scoring matrix. The number preceding the stream name on the x axis corresponds with the numbered watersheds in Figure 2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Criteria</th>
<th>Weight</th>
<th>1 Snake Creek</th>
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+ FMG = fine/medium gravel (2-16 mm)  
+ CG = coarse gravel (16-32 mm)  
+ VCG = very coarse gravel (32-64 mm)  
+ SC = small cobble (64-128 mm)  
+ LC = large cobble (64-128 mm)
### Appendix C (cont.)

<table>
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+ FMG = fine/medium gravel (2-16 mm)
+ CG = coarse gravel (16-32 mm)
+ VCG = very coarse gravel (32-64 mm)
+ SC = small cobble (64-128 mm)
+ LC = large cobble (64-128 mm)
## Appendix C (cont.)

<table>
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| Physical score (/12) | 2 10 4 4 |
| Chemical score (/11) | 6 8 8 6 |
| Total (/23)          | 8 18 12 10 |

**Percent**: 34.8 78.3 52.2 43.5 56.5

+ FMG = fine/medium gravel (2-16 mm)
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