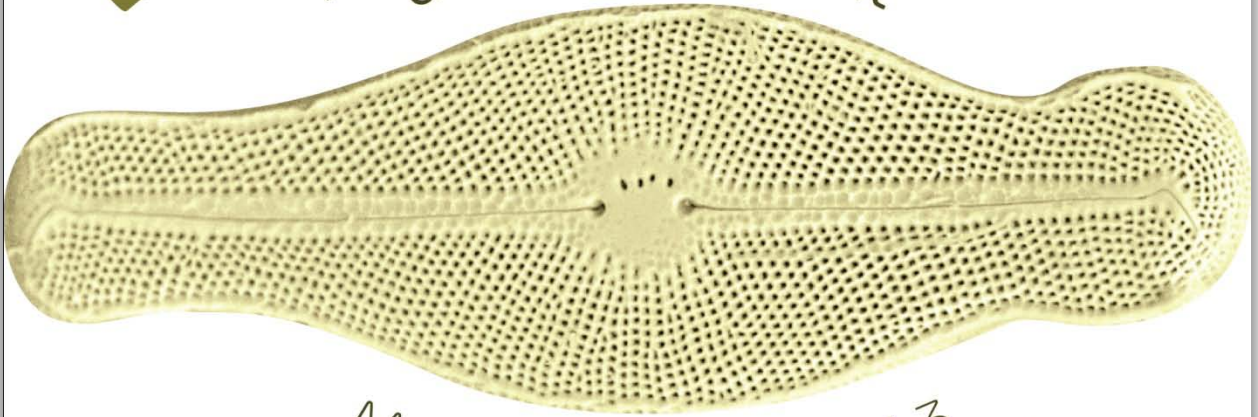


# International Didymo Conference

New horizons in science  
and management



March 12-13, 2013  
Providence, Rhode Island

Hosted by the Invasive Species Action Network and the Northeast Aquatic Nuisance Species Panel  
Fiscal management provided by the Northeast Aquatic Nuisance Species Council



## **International Didymo Conference Committee**

Leah C. Elwell; Invasive Species Action Network - Chair

Sarah Spaulding; University of Colorado and US Geological Society

Michele L. Tremblay; naturesource communications

Meg Modley; Lake Champlain Basin Program

Don Hamilton; National Park Service

Tim King; US Geological Survey

Tim Schaeffer; PA Fish and Boat Commission

Bob Wiltshire; Invasive Species Action Network

Brian Whitton; University of Durham

Sarah Whitney; Pennsylvania Sea Grant



**Plenary Speaker Dr. Cathy Kilroy:** Cathy Kilroy has been a researcher at New Zealand's National Institute of Water and Atmospheric Research since the mid 1990s, with an additional major role in science writing and editing until the early 2000s. Prior to 2004 her focus was mainly on (a) stream periphyton and its use in biomonitoring, and (b) the taxonomy and ecology of endemic and cosmopolitan diatoms in New Zealand freshwaters (especially wetlands), which was the topic of her PhD. Following the discovery of the first blooms of *Didymosphenia geminata* in a South Island river in 2004 Cathy switched focus and became almost completely immersed in projects aimed at understanding

the biology, ecological effects and distribution of *D. geminata* as it gradually spread to more and more South Island rivers. She served on the Biosecurity New Zealand Technical Advisory Group during the NZ Government response to didymo (up to 2008) and lead or participated in research projects ranging from investigating decontamination methods, to designing survey strategies, to modeling/predicting the potential range of *D. geminata* in New Zealand, to studies of ecological effects. She has provided advice relating to didymo to authorities in Australia and Chile, and to individuals from a range of countries. Recent research has included investigating factors that affect *D. geminata* growth in natural systems, in collaboration with colleagues from Canada and from various institutions in New Zealand.



**Plenary Speaker Dr. Max Bothwell:** Max Bothwell has been a research scientist with Environment Canada since 1979. He received academic training at the University of California-Santa Barbara (BA, MA) in phytoplankton ecology, algal cell physiology, nutrient chemistry/dynamics and ultraviolet photobiology and at the University of Wisconsin-Milwaukee (PhD) in limnology. His long interest in nutrient control of attached algal communities in rivers led to landmark publications in the 1980s that established the mechanistic and quantitative relationships between periphyton growth rates and standing crop limitation by phosphorus in rivers. In the 1990s his research on solar ultraviolet radiation effects on algal

communities and trophic level interactions led to numerous accolades including the Provasoli Award.

The phenomenon of *Didymosphenia geminata* blooms on Vancouver Island, Canada, caught his attention in 1993. He led investigations to determine the cause of those blooms and in 2006-2007 served as a Technical Advisor to BioSecurity New Zealand on the incursion of *D. geminata*. The interpretation that *D. geminata* blooms in North America are a result of a new variant with bloom-forming tendency is largely based on circumstantial evidence. Now, collaborative experiments and observations in New Zealand and North America provide evidence that the proximate cause of *D. geminata* blooms is very low phosphorus. This new understanding provides a potential ecological trigger for *D. geminata* blooms suggesting that they are not simply recent introductions of *D. geminata* cells into new areas. Collaborations are now shifting to explore the various mechanisms for low P or oligotrophication of rivers.



**Keynote Speaker Kirk Deeter:** Kirk Deeter is the editor of *TROUT* magazine and an editor-at-large for *Field & Stream*. He is known for his off-beat adventure story angles: Chasing mako sharks from kayaks... teaching Eskimos to be fly fishing guides... fishing for arapaima in the jungle of Guyana, etc. His work has also appeared in the *New York Times*, *Outside*, *Men's Journal*, *Garden & Gun*, *Big Sky Journal*, *The Drake*, *Fly Rod & Reel*, *Fly Fisherman*, and elsewhere. His F&S feature "Carp Crazy" (illustrated by Ralph Steadman) was listed in *America's Best Sports Writing*.

Deeter is co-host of *Field & Stream's* "Fly Talk" weblog at [fieldandstream.com](http://fieldandstream.com), the "Fly Fishing Jazz" columnist for [MidCurrent.com](http://MidCurrent.com), and also the editor-in-chief of *Angling Trade* (the business magazine covering the fly fishing industry). He is the author of five books, including *The Little Red Book of Fly Fishing* (co-authored with the late *Denver Post* outdoors editor Charlie Meyers). His next, *Fly Fishing for Carp*, will be released in April.

## Program Agenda

Day 1 Tuesday March 12, 2013		
7:30 am		<b>Registration &amp; Continental Breakfast-- Foyer</b>
8:30 am	Leah Elwell, Invasive Species Action Network and Meg Modley, Northeast Aquatic Nuisance Species Panel	<b>Welcome</b>
8:40 – 9:00 am	Kirk Deeter, Editor Angling Trade and Trout Magazine	Keynote Speaker
9:00 - 10:00 am	Plenary Speaker: Cathy Kilroy, National Institute of Water and Atmospheric Research of New Zealand	<i>Didymosphenia geminata</i> : an extraordinary organism
<b>Session I: <i>Didymosphenia geminata</i>, the New Zealand Experience</b> <i>Moderator: Meg Modley, Lake Champlain Basin Program</i>		
10:00 am	Phoebe Zarnetske, Yale School of Forestry and Environmental Studies	Integrating invasion history with environment to model <i>Didymosphenia geminata</i> hotspots across New Zealand
10:20 am	John Sanson and Philippe Gerbeaux, Ministry for Primary Industries of New Zealand and Dept of Conservation	<i>Didymosphenia geminata</i> in New Zealand: an update on current management and research approaches
<b>10:40 – 11:00</b>	<b>Break – Sponsored by Trout Unlimited</b>	
11:00 am	Craig Cary, University of Waikato	A sensitive genetic-based detection and enumeration method for <i>Didymosphenia geminata</i>
11:20 am	Jeanne Kuhajek, Cawthron Institute	Laboratory-based experiments to investigate didymo distribution patterns in New Zealand

**Session II: *Spatial Distribution***

*Moderator: Michele L. Tremblay,  
naturesource communications*

11:40 am	Vivian Montecino, Universidad de Chile	On the biogeography of <i>Didymosphenia geminata</i> in Chile: niche requirements and potential habitats
<b>12:00 - 1:15 pm</b>	<b>Lunch</b>	<b>Buffet served in Ballroom</b>
1:20 pm	David Richardson, SUNY New Paltz	Spatial distribution and ecosystem effects of a nuisance, bloom-forming diatom ( <i>Didymosphenia geminata</i> ) in Catskill Mountain streams, New York
1:40 pm	Sarah Spaulding, University of Colorado and US Geological Survey	Paleolimnological records of <i>Didymosphenia geminata</i> in North America
2:00 pm	Robert Pillsbury, University of Wisconsin	Are the recent blooms of <i>Didymosphenia geminata</i> in Lake Superior (USA) caused by an aggressive strain or environmental changes?
2:20 pm	Paula Furey, Saint Catherine University	Consequences of <i>Didymosphenia</i> and other mucilaginous diatoms common downstream of hydropeaking dams as food for tadpoles

**Session III: *Biology and ecological processes***

*Moderator: Leah Elwell, Invasive Species Action Network*

2:40 pm	Carole-Anne Gillis, Institut National de la Recherche Scientifique	Bottom-up effect of <i>D. geminata</i> presence on juvenile Atlantic salmon foraging behavior and habitat selection
3:00 pm	Brad Taylor, Dartmouth College	Tritrophic effects of <i>Didymosphenia geminata</i> blooms on fish

**3:20 pm Break – Sponsored by Simms Fishing Products**

- 3:40 pm Andy Rost, Sierra Nevada College Ecological consequences of extensive *Didymosphenia geminata* growth in headwater streams of the Sierra Nevada, USA
- 4:00 pm PV Sundareshwar, South Dakota School of Mines and Technology Does sulfation of Didymo stalks facilitate iron adsorption and phosphorus concentration in mats?
- 4:20 pm Nicholas Bach, University of Wisconsin The effects of disturbance and nutrient addition on a periphyton community dominated by *Didymosphenia geminata*
- 4:40 pm Michael Gretz, Michigan Technological University Comparative analysis of Didymo stalks and other diatom extracellular matrix components: Structure, synthesis, and degradation
- 5 pm Adjourn

**Day 2 Wednesday March 13, 2013**

8:00 am **Registration & Continental Breakfast-- Foyer**

**8:00 – 9:30 am Breakfast Poster Session**

Beth Flagler – New Hampshire Rivers Council, River Runners: Spreading the word about Didymo

Carole-Anne Gillis, Institut National de la Recherche Scientifique - Volunteer based observations to understand the growth, persistence and removal of rock snot blooms

Katherine Hanna, Maryland Dept of Natural Resources - Statewide Occurrence and Seasonal Abundance Patterns for Didymo in Maryland Waters

Daniel James, US Fish and Wildlife Service – Use of phosphorus enrichment to reduce bloom coverage of the benthic diatom *Didymosphenia geminata* in an oligotrophic stream: Rapid Creek, South Dakota

Diba Khan-Bureau, University of Connecticut -Monitoring the Distribution of *Didymosphenia geminata* and other “Rock Snot” species found in the Connecticut River and its Tributaries in Connecticut

8:00 – 9:30 am

### Breakfast Poster Session

Ron Klauda, Maryland Dept of Natural Resources - An Inexpensive, Easy to Build Stream-side Wash Station for Cleaning Wading Boots

Lisa Kunza, South Dakota School of Mines and Technology - *Didymosphenia geminata* presence in Grand Teton National Park and a nuisance bloom

Samantha Root, Bard College - Controlling Didymo: effective decontamination strategies and recommendations for public outreach

Maurice Rodway, Southland Fish and Game New Zealand - Long term management of *Didymosphenia geminata* in a controlled river

Christine Sandvik, South Dakota School of Mines and Technology -The effects of iron on growth of *Didymosphenia geminata*

### Session IV Genomics and detection

*Moderator: Don Hamilton, National Park Service*

9:30 am

Tim King, US Geological Survey

Determining the ecological and evolutionary processes associated with distribution and behavior of the nuisance freshwater diatom *Didymosphenia geminata*

9:50 am

Deborah Iwanowicz, USGS National Fish Health Research Laboratory

Initial report on whole genome amplification from a single cell of *Didymosphenia geminata*

10:10 am

Harry Nelson, Fluid Imaging Technologies

Identification, enumeration and measuring cell dimensions of *Didymosphenia sp.* using an imaging particle analyzer (FlowCAM)

10:20 am

**Break**

10:30-11:30 am

Max Bothwell, Environment Canada

Plenary: The Didymo Story: A changing view of the causes

11:30-12:45 pm

**Lunch**

**Buffet served in Ballroom**

### Session V Management

*Moderator: Bob Wiltshire, Invasive Species Action Network*

12:50 pm

Christina Vieglais, USDA APHIS

Assessing the risks of *Didymosphenia*: potential vs. observed impacts



1:15 – 2:45 pm	<p><b>Panel Discussion – The Management of <i>D. geminata</i>: Reactions and Responses</b></p>	<p>1:20 – 1:30 <b>Ron Klauda</b> - Didymo Infestation in Maryland USA: A State Agency’s Reactions, Responses, and Results</p>
	<p><i>Moderator:</i> Bob Wiltshire, Invasive Species Action Network</p>	
	<p>Panel Participants  <b>Beth Flagler</b>, New Hampshire Rivers Council  <b>Ron Klauda</b>, Maryland Dept of Natural Resources  <b>Dave Kumlein</b>, Trout Unlimited  <b>Tom McMahon</b>, Arizona Game and Fish Department  <b>Brad Taylor</b>, Dartmouth College  <b>Mark Van Patten</b>, Missouri Dept of Conservation</p>	<p>1:30 – 1:40 <b>Mark Van Patten</b> – Missouri’s Success in Educating before Regulating</p> <p>1:40 – 1:50 <b>Brad Taylor</b>– Investigating <i>Didymosphenia geminata</i> contamination at White River National Fish Hatchery after flooding of the facility by Tropical Storm Irene</p> <p>1:50 - 2:45 Facilitated discussion with all panel members</p>
<b>2:45 pm</b>	<b>Break</b>	
3:00 pm	<p>Sarah Spaulding – University of Colorado and US Geological Survey</p>	<p>Moving Forward: research and management needs</p>
4:30 pm	<p>Leah Elwell – Invasive Species Action Network</p>	<p>Closing/Adjourn</p>

## Plenary

**Cathy Kilroy.** National Institute of Water and Atmospheric Research, New Zealand.

### ***Didymosphenia geminata*: an extraordinary organism**

The stalked diatom *Didymosphenia geminata* has a remarkable capacity for producing massive proliferations of carbohydrate stalk material in water bodies with very low nutrient concentrations. Perhaps even more remarkable is the expanding range of these “blooms” over the last 25 years. *D. geminata* has undergone a transformation from a relatively rare diatom reported mainly in northern and mountainous areas of the Northern Hemisphere to a nuisance organism with a global range. The profile and notoriety of *D. geminata* was raised substantially when blooms were reported in New Zealand for the first time in 2004. Nine years on, *D. geminata* is now firmly established in South Island rivers. Although the impacts of the blooms on New Zealand river ecosystems (especially fish) are still not clearly documented, impacts on recreational and aesthetic values in many rivers are well-recognised. In many rivers, the blooms show no sign of abating.

*D. geminata* blooms in New Zealand may be different from those in some Northern Hemisphere locations because *D. geminata* is almost certainly non-indigenous in New Zealand. It has flourished in some South Island rivers that provided apparently perfect conditions for blooms. The evidence supporting the notion that *D. geminata* is a new organism in New Zealand is explored, drawing on pre- and post-didymo data and recent experimental results and surveys. Experiments in New Zealand have demonstrated that *D. geminata* proliferations occur because of very low phosphorus (P) rather than in spite of low P. Conversely, elevated dissolved P generally corresponds with the absence of blooms, or complete absence of *D. geminata*, from certain areas in New Zealand. Other aspects of *D. geminata* biology may also contribute to its ability to produce such large accumulations of material in oligotrophic systems. There is still much to learn about this extraordinary diatom.

**Phoebe L. Zarnetske<sup>1</sup>, Cathy Kilroy<sup>2</sup>, Philippe Gerbeaux<sup>3</sup> and John Leathwick<sup>3</sup>.** <sup>1</sup>Yale University School of Forestry & Environmental Studies, New Haven, Connecticut; <sup>2</sup>National Institute of Water and Atmospheric Research, New Zealand; <sup>3</sup>New Zealand Department of Conservation.

### **Integrating invasion history with environment to model *Didymosphenia geminata* hotspots across New Zealand**

The benthic diatom “didymo” (*Didymosphenia geminata*) rapidly invaded New Zealand’s South Island, with recent invasions in Chile and the United States. Predictive distribution models have used static environmental drivers such as landuse type, and water quality to help identify areas susceptible to invasion. However, invasions are dynamic processes, often driven by propagule pressure and interactions between the invader, its environment, and other species. New Zealand didymo surveys currently span 2005-2012, providing a means to examine the influence of previous didymo upstream sources (as established benthic or floating cells) on new downstream populations. In addition, recent flume experiments uncovered important interactions between nutrients and didymo growth and survival. We combine these new insights within boosted regression tree models to determine the relative influence of different drivers, and to generate updated predictions of didymo occurrence across New Zealand. We hypothesized that upstream propagule source and in situ environmental variables should have the largest effect on didymo occurrence. Our model results consistently show that a large portion of the variation in didymo occurrence is explained by the previous occurrence of didymo cells upstream of a stream reach. A smaller portion is consistently explained by certain in situ environmental variables including phosphorus, which was recently identified through experiments. By incorporating information on the spread of didymo and new insights on didymo growth, this model captures essential components of the invasion process. The predictive map output shows potential invasion hotspots which will be useful for focusing control and monitoring efforts on the North and South Islands.

**John Sanson<sup>1</sup> and Philippe Gerbeaux<sup>2</sup>.** <sup>1</sup>Ministry for Primary Industries, Wellington City, New Zealand;  
<sup>2</sup>Department of Conservation, Christchurch, New Zealand;

***Didymosphenia geminata* in New Zealand: an update on current management and research approaches**

Since the first confirmed record in New Zealand of the Northern Hemisphere diatom *Didymosphenia geminata* nine years ago, the species has spread to many catchments in the South Island. In many of the locations where it has become established, its invasive epithet has been justified. In other rivers, *D. geminata* has been observed microscopically only, and some rivers and streams are still free of the species, despite proximity to affected sites. Assessments of impact on freshwater biota including benthic invertebrate and fish communities, as well as economic evaluation of potential costs associated with invasion of freshwater habitats will be reported on. These clearly justify the need for on-going management actions. The Ministry for Primary Industries, as the lead management agency, and the Department of Conservation, along with other key stakeholders including Iwi, Fish and Game, Regional Councils and Power companies, have worked closely together to identify means to reduce the spread (still no record of didymo in the North Island) and prioritize research needs. Some of the research outcomes will be reported on in other presentations at this conference. Our presentation will focus on the management lessons learnt, ranging from strategies in place to respond to new incursions, an operations framework for the long-term management of Didymo on public conservation land, a predictive model (recently updated and also presented separately) and tools that were developed to raise public awareness and optimize their efficiency. This includes annual surveys of users that help us identify the best awareness tools and target key user groups.

S. Craig Cary<sup>1</sup>, Brendan J. Hicks<sup>1</sup>, Andreas Rueckert<sup>1</sup>, Susanne Wood<sup>2</sup>, and Kathryn J. Coyne<sup>3</sup>.

<sup>1</sup>University of Waikato, Hamilton, New Zealand; <sup>2</sup>Cawthron Institute, Nelson, New Zealand; <sup>3</sup>University of Delaware.

### **A sensitive genetic-based detection and enumeration method for *Didymosphenia geminata***

The freshwater benthic diatom *Didymosphenia geminata* is emerging globally as an organism with an extraordinary capacity to impact stream ecosystems by forming persistent blooms of dense mucilaginous mats. Ongoing surveillance to monitor the distribution of didymo in New Zealand's North Island is a key component of Biosecurity New Zealand's response efforts. Our objective was to develop a sensitive molecular-based detection method for *Didymosphenia geminata* that can be used as the basis of an early detection protocol before new infestations are visible. Early detection offers the best chance of mitigation of new incursions of the organism. Low numbers, patchy distribution and the existence of morphologically similar diatoms have the potential to confound early detection and monitoring of *Didymosphenia*. Developments in molecular technology now allow rapid and highly specific low-level detection of eukaryotic and prokaryotic microorganisms. These techniques involve gene amplification technologies that provide greater sensitivity and specificity of detection and enumeration of algal species compared to microscopy, and are ideally suited for routine monitoring and surveillance of field samples. We will present a highly sensitive quantitative real-time PCR (Q-PCR) protocol designed specifically for high-throughput detection and enumeration of *Didymosphenia geminata*. Our specific requirements for this new methodology were that it has 1) robust field capabilities from collection to quantification, 2) species or strain level specificity that has been environmentally validated, 3) extreme sensitivity for low-level detection (<1 cell/ml), 4) a broad dynamic range of detection (> 5 orders of magnitude), and 5) the highest degree of reproducibility. This protocol has been developed along with optimized field collection and preservation procedures designed specifically for diatoms inhabiting river ecosystems and is currently the primary surveillance tool used in the North Island. Data recently collected from New Zealand and other global sites will be presented.

**Jeanne M. Kuhajek<sup>1</sup> and Susanna Wood<sup>1</sup>.** <sup>1</sup>Cawthron Institute, Nelson, New Zealand.

### **Laboratory-based experiments to investigate didymo distribution patterns in New Zealand**

Didymo was first detected in New Zealand in 2004 and has since spread to over 150 rivers throughout the South Island. The distribution of didymo in New Zealand is enigmatic: didymo grows prolifically in many rivers throughout the South Island yet is absent in the groundwater-fed tributaries of these rivers, despite known introductions. Furthermore, it remains undetected in the North Island, despite the presence of numerous habitats predicted to be suitable for the diatom and the high potential for didymo introductions there. The reasons for these distribution patterns are unknown. Both water chemistry and biofilm composition are thought to be important in defining didymo distribution and abundance, however, research has been limited by the lack of laboratory techniques for studying the diatom. Results from experiments designed to explore the influence of water chemistry and bacteria isolated from didymo mats on survival of the diatom will be presented. A laboratory-based method was developed for assessing didymo survival and attachment under controlled laboratory conditions. Using this method, didymo was grown in water collected from rivers and groundwater-fed creeks with and without didymo throughout New Zealand, as well as in water systematically spiked with select nutrients at a range of concentrations. Using standard microbiology techniques, over 100 bacterial strains were isolated from didymo mats and unique strains identified using molecular methods. Co-culturing experiments were undertaken and strains that enhanced or decreased didymo survival were identified. Additionally, biofilm communities at geographically-similar sites with and without didymo were characterised using molecular fingerprinting techniques. The results of these studies provide insight into factors that affect the establishment and survival of didymo, furthering our understanding of the invasive nature of this organism. The development of a laboratory-based method for the study of didymo provides potential to investigate thus-far unexplored aspects of this organism.

**Montecino Vivian<sup>1</sup>, Ramiro Bustamante<sup>1, 2</sup>, Sunil Kumar<sup>3</sup>, Loreto Castillo<sup>1</sup>, Paola Muñoz<sup>1</sup>, Nicole Tapia<sup>1</sup> and Ximena Molina<sup>1,4</sup>.** <sup>1</sup> Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile; <sup>2</sup>Instituto de Ecología y Biodiversidad, Universidad de Chile, Santiago, Chile; <sup>3</sup>Colorado State University, Fort Collins, CO, USA; <sup>4</sup>POCH Ambiental S.A., Santiago, Chile.

**On the biogeography of *Didymosphenia geminata* in Chile: niche requirements and potential habitats.**

*Didymosphenia geminata* was reported more than 4 decades ago in the Chilean Patagonia. In 2010, the first register of *D. geminata* as a plague was informed for the Futaleufu River. Currently, its presence has been reported south of 41°41'S; 72°6' W, in the watersheds of Rio Palena, Aysen and Baker. In Chile, as elsewhere, anthropogenic activities are recognized as the principal causal factor of *D. geminata* invasion. Since *plague* containment is very difficult, it is urgent to predict habitat preferences and quantify dispersal vectors as a way to anticipate invasion in non-invaded zones. We used Maxent, to identify key niche environmental variables and to predict the potential distribution for this species in Chile. For environmental data, we considered a total of 27 variables including climatic, topographic, hydrological, and remotely sensed variables. Nineteen bioclimatic data layers were obtained from the WorldClim dataset (~1-km resolution). Topographic and hydrological variables included elevation, flow accumulation, flow direction and compound topographic index. Remotely sensed variables included percent tree cover and percent herbaceous cover. We also included growing degree days and geologic age. Maxent was chosen because it performs better than other species distribution models methods and it is relatively robust to small sample sizes. Our study covered 174 sites while the whole available data bases are from 225 rivers from Northern Patagonia to the Magellan Region, according to this, until early 2012 we have 55 presences and 170 absences.

The model accurately predicted current distribution of *D. geminata* in Chile with an AUC value of 0.85. We found that geologic age of the rocks, flow accumulation, and growing degree days were the top predictors with 37.1%, 29.2% and 14.1% contributions. Other environmental predictors included flow direction, percent herbaceous cover, percent tree cover, mean annual temperature, and compound topographic index. Jackknife tests showed that geologic age had the highest training gain when used in isolation and with higher AUC. Despite no potential distribution is predicted for Mediterranean zones of Chile, future geographic expansion is expected toward the Patagonian regions. Therefore, these results can be used for early detection and management of *D. geminata* spread and for prioritizing monitoring and surveillance sites in Chile.

**Financed by:** CORFO-INNOVA 11BPC-10019

**David C. Richardson<sup>1</sup>, Timothy Hoellein<sup>2</sup>, David B. Arscott<sup>3</sup>, Catherine Gibson<sup>4</sup>, and Samantha Root<sup>5</sup>.**  
<sup>1</sup>SUNY New Paltz; <sup>2</sup>Loyola University, Chicago; <sup>3</sup>Stroud Water Research Center; <sup>4</sup>Skidmore College; <sup>5</sup>Bard College.

**Spatial distribution and ecosystem effects of a nuisance, bloom-forming diatom (*Didymosphenia geminata*) in Catskill Mountain streams, New York.**

*Didymosphenia geminata* (didymo) is a nuisance stream alga that has increased in New York streams and rivers in recent years. In the summers of 2010-2012, we investigated the spatial distribution of didymo in Esopus Creek (Catskill Mountains), the chemical and hydrological causes of blooms through water chemistry analysis and microscopy, and the effects of didymo on biofilm and macroinvertebrate communities. We sampled 7 sites weekly in June and July 2010-2012, ranging from the headwaters to just above the inflow of the Ashokan Reservoir, as well as several tributaries of the Esopus. Additionally, we sampled Catskill watersheds with reservoir systems (Rondout Creek and Neversink River) to identify didymo presence and absence. Finally, we examined the effects of didymo densities on macroinvertebrate communities in 10 sites during 2011 and 2012. Spatially, didymo was distributed throughout the Catskills and has been rapidly spreading, although didymo densities were higher below reservoirs than above. Didymo cell densities increased concurrently with increases in conductivity. In Esopus Creek, didymo was consistently absent at the furthest upstream site and had the highest densities near a tributary that contributed wastewater effluent from a ski resort. Didymo blooms developed throughout the summer 2011, but blooms were scoured away by a flood in early July. In general, didymo coverage was lower in 2011 than in summer of 2010 or 2012, likely as a result of higher and flashier flows. Overall, the magnitude of didymo blooms was linked to hydrologic patterns, water chemistry, and human activity. Didymo densities had a negative effect on total macroinvertebrate family richness, biodiversity, and number of Ephemeroptera, Plecoptera, and Trichoptera families. This project details the rapid proliferation of didymo in New York and suggests the need for communication with researchers and recreational users of these ecosystems to prevent further spread. We also begin to address hydrologic and chemical controls on didymo blooms and the deleterious effects of blooms on aquatic ecosystems.



**Sarah Spaulding.** US Geological Survey / INSTAAR, University of Colorado.

**Paleolimnological records of *Didymosphenia geminata* in North America**

Nuisance blooms of the diatom *Didymosphenia geminata* occur in many streams and rivers of North America. However, questions remain concerning the degree to which blooms are natural events, or whether they are in response to recent environmental change and spread of invasive genotypes. Although *D. geminata* is known from lakes, the species is most often recorded in flowing waters where it reaches its greatest biomass. Determining the history of diatoms in streams and rivers, however, is more problematic than in lakes, as streams are high flow systems that typically do not leave a continuous sedimentary record that can be interpreted. In sites where streams and rivers flow into lakes, records of historical change in river systems may be archived in lake sediments. Paleolimnological reconstructions have now been completed on lake sediments from a number of sites in Alaska, Montana and Wyoming. This presentation will summarize the results of *D. geminata* abundance from these sites. In a number of sites, *D. geminata* has been present for a very long time, up to 10,000 years before present. These results are important because they demonstrate that 1) *D. geminata* formed large populations in a newly deglaciated site within a relatively short period of time, 2) the large populations were present absent human transport, and 3) maximum cell concentrations show a strong relationship to the period of warm, dry climate in the region. The historical record is informative for understanding the expansion of *D. geminata* from its presumed Siberian origin, the relationship to modern nuisance blooms and recommendations for appropriate management response.

**Robert Pillsbury<sup>1</sup>, Jo Thompson<sup>2</sup> and Mark Edlund<sup>3</sup>.** <sup>1</sup>University of Wisconsin Oshkosh; <sup>2</sup>U.S. Environmental Protection Agency; <sup>3</sup>St. Croix Watershed Research Station.

**Are the recent blooms of *Didymosphenia geminata* in Lake Superior (USA) caused by an aggressive strain or environmental changes?**

Within the past two decades *Didymosphenia geminata* has spread broadly and colonized mainly oligotrophic rivers producing nuisance blooms. But why is this expansion occurring now? One hypothesis is that an aggressive strain of *D. geminata* has mutated/developed. The quick expansion of such a strain could produce a high degree of valve shape similarity between populations. Another hypothesis is that *D. geminata* was present although rare in most habitats but recent environmental changes have made these blooms more likely to occur (producing blooms with more distinct morphological characteristics). Ten morphological characteristics were measured from 9 separate populations of *D. geminata*. At least 30 individuals were measured from each population. Correspondence Analysis was used to explore the differences in morphological variation between populations. Multiple samples from recent large-scale blooms from Lake Superior were also analyzed over time and distance. Historical accounts of possible *Didymosphenia* blooms were also considered. These patterns found here suggest that while environmental factors play a role in determining cell shape, samples from different populations often seem to represent a fairly distinct morphology. This pattern is consistent with the environmental change hypothesis.

**Paula C. Furey<sup>1</sup>, Sarah J. Kupferberg<sup>2</sup>, and Amy J. Lind<sup>3</sup>.** <sup>1</sup>Department of Biology, Saint Catherine University; <sup>2</sup>Department of Integrative Biology, University of California, Berkeley; <sup>3</sup>USDA Forest Service - Tahoe and Plumas National Forests.

**Consequences of *Didymosphenia* and other mucilaginous diatoms common downstream of hydropeaking dams as food for tadpoles.**

In Sierra Nevada, California, *Didymosphenia* and other mucilage-producing diatoms can dominate near-shore river environments when hydrologic and thermal regimes are altered by dams. Tadpoles of the declining Foothill yellow-legged frog (*Rana boylei*) endemic to the flowing waters of this region prefer to graze periphyton in near-shore habitats. We assessed periphyton assemblages from rocks from varial-zones and continuously-wetted-zones of rivers where flow fluctuates daily with short-term power generation. We then examined how different algal assemblages impact food consumption and growth by rearing tadpoles on diets of periphyton from a regulated river with *Didymosphenia*, a regulated river with mucilaginous diatoms, and an unregulated stream with high food-quality periphyton as the control. Food sources were crossed with temperature treatments that mimicked cool, hypolimnetic water released from an upstream reservoir, or matched tadpole thermal preference. In the rivers, mucilaginous algal taxa tolerated the varial zones better than non-mucilaginous algal taxa. In the grazing experiment, both food source and temperature had significant effects on tadpole food consumption and growth. Overall, tadpole growth performance on mucilaginous taxa was poor relative to those grazing on high quality periphyton. These effects were magnified by temperature. Under cool conditions, tadpoles gleaned little food and only maintained body weight on high food-quality algae. In contrast, in warmer conditions, tadpoles ingested *Didymosphenia*-dominated periphyton at a rate similar to control tadpoles, but did not grow, with a 72 hr relative weight gain of  $4.3 \pm 5.4\%$ , versus a  $30.7 \pm 3.4\%$  for controls. Tadpoles grazing on other stalked mucilaginous diatoms had a weight loss of  $21.0 \pm 9.2$  (cold) and  $16.6 \pm 5.6\%$  (warm). These results encourage a holistic approach to dam management that integrates consideration of multiple abiotic and biotic factors to conserve amphibians.

**Carole-Anne Gillis<sup>1</sup> and Normand Bergeron<sup>1</sup>.** <sup>1</sup> Institut National de la Recherche Scientifique, Centre ETE, Québec.

### **Bottom-up effects of *D. geminata* presence on juvenile Atlantic salmon foraging behavior and habitat selection**

In 2006, blooms of *Didymosphenia geminata* Schmidt (didymo) were officially identified in the Matapedia River, an Atlantic salmon (*Salmo salar*) river of the Gaspé Peninsula (Québec, Canada). This alga can produce extensive thick benthic layers in stable flow oligotrophic rivers. The local economy relies heavily on the Atlantic salmon fishery and concerns have been raised that didymo could act as an additional stressor to this anadromous population. Existing research on the effect of didymo on native fisheries are mainly post-hoc investigations and mechanisms by which *D. geminata* alters ecosystem function and Atlantic salmon habitat are still unknown. In a previous study, Gillis and Chalifour (2010) showed that the presence of didymo caused significant shifts in macroinvertebrate community structure and prey abundance. Despite an increase in overall prey production, prey location and availability may be altered by dense didymo mats. Therefore, this alga is suspected of altering juvenile Atlantic salmon (JAS) foraging behaviour and habitat selection through food-web interactions. This study aimed to evaluate the effects of didymo on JAS by assessing their foraging behaviour in didymo-affected and didymo-free sites. Data was collected in the Patapedia River by visual observation of young-of-the-year salmon conducted by one person while snorkelling. Results show that juveniles make a higher proportion of benthic forays than drift forays with increasing didymo cover ( $R^2 = 0.54$ ,  $p < 0.001$ ). Benthic forays are known to be more energetically consuming than drift forays (Shearer *et al.*, 2007). Current analysis is underway to examine whether the shift in foraging behaviour is triggered by limited prey availability induced by the didymo mats or if *D. geminata* presence enhances profitability. Further research is now underway to evaluate the effects of didymo on habitat selection to determine whether juveniles prefer or avoid sites with *D. geminata*. Preliminary observations show that juveniles tend to be resilient to the presence of mats possibly due to behavioural plasticity. This research project provides insights to the role of didymo on JAS future in eastern Canada by investigating unexplored areas of didymo impact research: foraging behaviour and habitat selection.

**Brad Taylor.** Department of Biological Sciences, Dartmouth College, New Hampshire and The Rocky Mountain Biological Laboratory, CO.

### **Tritrophic effects of *Didymosphenia geminata* blooms on fish**

Theory and empirical studies predict that increasing primary productivity should positively affect higher trophic levels. The diatom *Didymosphenia geminata* produces massive amounts of stalk material, or nuisance blooms, in rivers around the world. The recent emergence of *Didymosphenia* blooms has generated concern about their possible effects on river food webs, particularly at higher trophic levels occupied by fish. Yet our understanding of the effects of *Didymosphenia* blooms on river food webs and fish, in particular, is limited. Here I used comparative experimental and observational approaches to test how *Didymosphenia* blooms affect stream invertebrates and whether they propagated up the foodweb to affect trout in Rocky Mountain rivers where *Didymosphenia* is considered native. Observations from rivers with and without blooms and *Didymosphenia* removal experiments showed that blooms changed stream insect density, diversity, composition, and body size, but not biomass. Experiments with non-edible material that mimicked *Didymosphenia* revealed that the primary mechanism explaining increases in particular groups, chironomids and oligochaetes, was that *Didymosphenia* stalks provided a refuge from invertebrate predators. In contrast, similar experiments showed that the decrease in large-bodied mayflies was due to *Didymosphenia* stalks increasing their susceptibility to invertebrate predators. Feeding trial experiments in cattle tanks also revealed that trout ate fewer insects when communities resembled those in streams with *Didymosphenia* blooms. Mark-recapture studies of ~600 trout in multiple rivers with and without blooms over three years showed that trout growth rates were 44% lower in rivers with *Didymosphenia* blooms, which could not be explained by other factors, such as temperature or density dependence. Moreover, rivers with blooms had more oligochaetes, and the oligochaete *Tubifex tubifex* is the only known intermediate host for the whirling disease parasite, *Myxobolus cerebralis*; thus, based on polymerase chain reaction analyses, whirling disease prevalence in trout was 50% higher in rivers with blooms (73%) versus without blooms (20%). These results show strong negative tritrophic effects of *Didymosphenia* blooms on salmonids in Rocky Mountain rivers, a result not predicted by theory.

**Andy L. Rost<sup>1</sup>, Christian H. Fritsen<sup>2</sup> and Sudeep Chandra<sup>3</sup>.** <sup>1</sup>Sierra Nevada College, Science and Technology Department; <sup>2</sup>Desert Research Institute, Division of Earth and Ecosystems Sciences; <sup>3</sup>Department of Natural Resources and Environmental Science, University of Nevada

**Ecological consequences of extensive *Didymosphenia geminata* growth in headwater streams of the Sierra Nevada, USA**

Growth of the stalked diatom *Didymosphenia geminata* may pose an ecological threat to stream ecosystems, but the extent and mechanisms of potential impacts remain largely unknown. A comparative ecosystems assessment on two similar and adjacent streams (one with and one without *D. geminata*) and their confluence reach was conducted to help identify potential effects of *D. geminata* growth on (1) benthic community structure, (2) trophic pathways (3) food quality, and (4) spatial patterns of potential impacts. Differences in benthic community structure in both biomass and community composition were observed throughout the study. Impacts to trophic pathways, assessed using  $\delta^{13}\text{C}$  signatures in a two-end mixing model, showed that the relative contribution of autochthonous carbon in the *D. geminata* streams was 50% to 75% less compared to the non *D. geminata* stream. Impacts to food quality, assessed by comparing the CN ratios of food resource and consumers between streams, showed the elemental imbalance between the primary producers and the primary consumers was three times higher in the *D. geminata* stream. Downstream from the confluence of the two streams, benthic community structure and function rapidly became more similar to the non *D. geminata* stream. Results show that extensive growth of *D. geminata* can be ecologically disruptive, altering benthic community structure, trophic pathways, and food quality; however, an unaffected tributary stream can quickly assuage impacts. This study can provide insight into the extent and mechanisms by which growth of *D. geminata* affects stream ecosystems and under what conditions affected streams recover.

**P. V. Sundareshwar<sup>1</sup>, Lakshminarayan M. Iyer<sup>2</sup>, L. Aravind<sup>2</sup>, L. Kunza<sup>1</sup>, S. Gautam<sup>1</sup> and C. Sandvik<sup>1</sup>.**  
<sup>1</sup>South Dakota School of Mines and Technology; <sup>2</sup>NCBI, NLM, NIH.

### **Does sulfation of Didymo stalks facilitate iron adsorption and phosphorus concentration in mats?**

Recently, it was proposed that iron (Fe) likely plays an important role in *Didymosphenia geminata*'s (Didymo's) ability to grow prolifically in unpolluted streams and rivers where phosphorus (P) availability is low. Didymo blooms consist of copious amounts of stalk material and cells. However, stalk production and cell division occur under opposite conditions of P bioavailability, with stalk elongation occurring under P starvation, while cell division occurring under P surplus conditions. The recently proposed Fe mediated P pulsing requires adsorption of Fe onto stalks which upon oxidation adsorbs phosphate from water. Redox dynamics in the mat results in pulsing of P within the mat creating alternating conditions of P surplus and starvation. It is plausible that mechanism of Fe mediated P concentration is an active response to P starvation. We present evidence from wetland plants that exhibit similar morphological response to P starvation – analogous to the stalk elongation in Didymo, and demonstrate active adsorption and oxidation of Fe on the plant roots in response to P starvation.

In Didymo, we postulate that the Fe-mediated P adsorption on to stalk is facilitated by the sulfation of the stalk. In eukaryotes, the primary enzymes involved in sulfation of proteins, polysaccharides and a wide range of small molecules belong to the sulfotransferase superfamily. These enzymes catalyze the transfer of sulfate from a 3'-phosphoadenosine 5'-phosphosulfate to amine and alcohol functional groups. Sulfation is implicated in forming ionic cross-links and contributes to the anionic nature of the stalk, which facilitates interactions with cations such as ferrous and ferric ions. Indeed sulfated polysaccharides in Phaeophytes are known to possess ferrous ion-chelating activity. Using comparative genomics, we present a survey of sulfotransferases across completely sequenced diatom genomes, some of which are bacillariophytes related to *Didymosphenia*. At least five or more families of secreted sulfotransferases were recovered, of which one is specifically related to the heparan-sulfate sulfotransferases, a family of enzymes that modifies the polysaccharide heparan. Phylogenetic analyses of diatom sulfotransferases reveal a mixed heritage, with some families present in diverse eukaryotes, and others only limited to diatoms and bacteria, suggestive of recent lateral acquisition.

Nicholas Bach<sup>1</sup> and Robert Pillsbury<sup>1</sup>. <sup>1</sup>University of Wisconsin Oshkosh.

**The effects of disturbance and nutrient addition on a periphyton community dominated by *Didymosphenia geminata***

The invasive diatom *Didymosphenia geminata* can successfully compete with native periphyton communities in cold-water, oligotrophic streams, often degrading the utility of trout streams. In Rapid Creek, SD, *D. geminata* occurs in discreet, high density patches while visually absent from other sections. Starting in 2007, nutrient additions (N and P) were continuously added to a section of Rapid Creek for another study. This allowed us to place a series of ceramic tiles in 4 distinct habitats; 1) native periphyton with no nutrient addition, 2) *D. geminata*-dominated periphyton with no nutrient addition, 3) native periphyton with nutrient addition, and 4) *D. geminata*-dominated periphyton with nutrient additions. Tiles from each habitat were also subjected to three disturbance patterns (low, medium, and high). All tiles were scraped, measured for chlorophyll a, and algal species composition determined. The experiment ran for 21 days in July, 2008. Low disturbance tiles had significantly ( $p = 0.001$ ) greater periphyton growth rates compared with medium and high disturbance tiles. Algal biomass determined from rocks suggest that the stream is nutrient limited. However results from the tiles indicate that short-term, periphyton, growth rates are greater without nutrient addition ( $p=0.001$ ). This may be due to shifts in the species composition and architecture of the periphyton mats, in which the community is changing from one dominated by araphids (*Diatoma* sp. and *Fragilaria* sp.) to one of primarily *Achnantheidium* sp. A pilot experiment comparing an existing periphyton communities to bare rock suggest that *D. geminata*, in Rapid Creek, is slow to colonize and prefers established periphyton communities ( $p = 0.001$ ) in order to attach. Implications for the management of *D. geminata* will be discussed.



**Michael R. Gretz.** Michigan Technological University.

**Comparative analysis of Didymo stalks and other diatom extracellular matrix components: Structure, synthesis, and degradation**

Investigation of stalk production in *Didymosphenia geminata* reveals several similarities to that of the closely related diatoms *Cymbella cistula*, *C. mexicana* and *Gomphonema olivaceum*. The sulfated xylogalactan which is the primary polysaccharide component in all of these diatom stalks is in some ways structurally similar to agars and carrageenans reported from marine and freshwater red algae including *Bangia*. The sulfated xylogalactan from *C. cistula* has been shown to be intrinsically hydrophilic and ionic cross bridging with  $\text{Ca}^{+2}$  is indicated. Other aspects of extracellular polymer production important in consideration of *D. geminata* control are cell processes closely related to stalk biogenesis. For the marine diatom *Achnanthes longipes*, a definite sequence of colonisation of substrata and stalk production is observed. Adhesion occurs in a predictable sequence: 1) gliding motility; 2) cessation of motility; 3) pad production with permanent attachment; 4) stalk production, elevating the cell from the substratum; 5) cell division; and 6) gliding motility of daughter cells. A variation of this type of sequence occurs in *D. geminata*, with detachment from the stalk important in dispersal. Comparative analysis also allows for estimates of the potential efficacy of mechanical, chemical, and enzymatic methods of stalk degradation.

**Tim L. King<sup>1</sup>, Don Hamilton<sup>2</sup>, Aaron Aunins<sup>1</sup>, Debra Iwanowicz<sup>1</sup>, Michael R. Gretz<sup>3</sup>, Leslie J. Matthews<sup>4</sup>, Marina Potapova<sup>5</sup> and Andy L. Rost<sup>6</sup>.** <sup>1</sup>U.S. Geological Survey-Leetown Science Center, Kearneysville, WV; <sup>2</sup>National Park Service, Upper Delaware Scenic and Recreational River, Beach Lake, PA; <sup>3</sup>Michigan Technological University; <sup>4</sup>Department of Environmental Conservation, Vermont Agency of Natural Resources; <sup>5</sup>Academy of Natural Sciences, Philadelphia, PA; <sup>6</sup>Science and Technology Department, Sierra Nevada College.

**Determining the ecological and evolutionary processes associated with distribution and behavior of the nuisance freshwater diatom *Didymosphenia geminata***

In spite of their tremendous ecological importance, the molecular mechanisms that enable diatoms to succeed in a range of diverse environments remain largely unexplored. Results from the first diatom genome projects (e.g., *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*) showed the presence of various genes needed for efficient management of carbon and nitrogen encoding urea cycle components. The comparative study of the two diatom genomes revealed that only 57% of genes are shared and that horizontal gene transfer from prokaryotes appears pervasive in diatoms. Thus, the necessity for functional genomics and reverse genetics approaches to further explore diatom gene repertoires is clear. Through a multidisciplinary approach, our research project is designed to develop a better understanding of the ecological and evolutionary processes associated with the growth and spread of the nuisance diatom *Didymosphenia geminata*. We are working to assemble DNA sequences generated via next generation genomic shotgun sequencing and robust de novo assembly of the transcriptome of *D. geminata* through RNA-Seq analyses. These methodologies will provide genetic markers for the study of neutral and adaptive genetic variation to identify genetic associations with observable traits (e.g., physiological tolerance, metabolic and immunological variation, mucopolysaccharide stalk production). This research will lead to identification of metabolically (functionally) critical genes, as well as, genes that can assist in elucidating taxonomically-, phylogeographically-, and demographically-informative processes. Lastly, we will team with the NPS's and the Delaware River Basin Commission's extensive water quality assessment in the Delaware River to compare water quality parameters with sites where *D. geminata* blooms occur. We seek to identify ecological components from sampled water quality parameters that correlate with demographic shifts in *D. geminata* populations. It is our hope that this research will determine whether genetic differentiation (e.g., reproductive isolation and differential gene expression) occurs between sites where *D. geminata* populations exist as nuisance blooms and or exist in relative obscurity. This talk will provide the diatom research community with an update on these research efforts.

**Deborah Iwanowicz<sup>1</sup>, Cierra Hardt<sup>1</sup>, Aaron Aunins<sup>1</sup>, and Timothy King<sup>1</sup>.** <sup>1</sup>USGS Leetown Science Center.

**Initial report on whole genome amplification from a single cell of *Didymosphenia geminata***

*Didymosphenia geminata* is a stalked penate diatom for which basic biological, ecological, and evolutionary knowledge is lacking. This is a concern since within the last few decades *D. geminata* blooms have been reported with increased frequency and intensity. *D. geminata* blooms can cover up to 100% of surfaces with mats of thickness greater than 20 cm, greatly altering physical and biological conditions within streams. These mats have resulted in a massive disturbance of freshwater ecosystems and have been documented to exhibit a significant impact on regional economies through alteration of stream hydraulics and overall ecosystem health and function. While a molecular technique for identifying the presence of *D. geminata* based on the highly conserved gene encoding the small subunit ribosomal RNA (18S rRNA) in water samples exists, no genetic markers applicable to phylogeographic or population structuring are available. In fact, no nucleotide sequences of any type (DNA or cDNA) for *D. geminata* have been deposited in the NCBI database. This is not surprising as the difficulty associated with the cultivation, collection and extraction of genetic material from *D. geminata* hinders genome construction. No method for developing pure cultures of didymo, let alone axenic cultures presently exists necessitating new approaches for genetic analysis. Here, we discuss modified DNA extraction and amplification techniques that were used to obtain didymo DNA from 1-10 individual cells while minimizing bacterial contamination in subsequent whole genome sequencing runs. Using these techniques, we hope to obtain high-quality genome assembly of *D. geminata*. Next generation sequencing for this project was completed on the IonTorrent platform, a relatively new approach to sequencing that uses semiconductor chips. With these tools we hope to obtain whole genome amplification and prove it as a useful tool for investigating the genetics of unculturable diatoms.

**Harry Nelson.** Fluid Imaging Technologies, Yarmouth, Maine.

**Identification, enumeration and measuring cell dimensions of *Didymosphenia sp.* using an imaging particle analyzer (FlowCAM)**

The ability to detect, identify and enumerate *Didymosphenia* in natural samples is often a difficult and time consuming process when using a light microscope. Similarly, difficulties can arise when measuring cell dimensions and conducting size analysis for the purpose of understanding *Didymosphenia* population dynamics and phylogenetic relationships using microscopy. A new technique has been developed using an automated imaging particle analyzer (FlowCAM) that can rapidly capture images of microscopic particles in a fluid medium and provide accurate identification information and morphological measurements. Because the valve of *Didymosphenia* is large, unique in appearance, and relatively symmetrical, it is ideally suited for detection and identification with the FlowCAM. With over 400 in use worldwide since 2000, the FlowCAM has been proven as a reliable tool for plankton research. Here we describe how the FlowCAM can detect, identify and enumerate *Didymosphenia geminata*. An overview of the technology, along with images and data from wild samples, will be presented.

**Christina Vieglais.** USDA Animal and Plant Health Inspection Service, Riverdale, Maryland

**Assessing the risks of Didymosphenia: potential vs. observed impacts**

When didymo was first discovered in New Zealand in 2004, information about its potential impacts to the New Zealand people, environment and economy was both sparse and anecdotal. A retrospective review of the risk and impact assessments that were conducted as part of the New Zealand government's incursion response to didymo will be compared with impacts that have been recorded to date in NZ, Chile and the US. With didymo, the exposure side of the risk equation is increasingly evident while the hazard side remains more elusive.

Panel: The Management of *D. geminata*: Reactions and Responses

**Ronald J. Klauda<sup>1</sup>, Katherine V. Hanna<sup>1</sup> and Theaux M. Le Gardeur<sup>2</sup>**; <sup>1</sup>Maryland Department of Natural Resources; <sup>2</sup>Gunpowder RIVERKEEPER.

### **Didymo Infestation in Maryland USA: A State Agency's Reactions, Responses, and Results**

In January 2008, a "strange growth" was observed by anglers in Gunpowder Falls, a moderately-enriched (mean total N=2.59 mg/L), tail-water trout stream below the 610 hectare Prettyboy Reservoir in north-central Maryland USA. Maryland Department of Natural Resources (MDNR) reacted quickly and confirmed the growth to be *Didymosphenia geminata* ("didymo" or "rock snot") in April 2008---the first documented occurrence of this diatom in Maryland. Press releases were issued and "Prevent the Spread" signs were posted during May. In late May, MDNR staff designed, built, and deployed six wader wash stations along the Gunpowder, followed by other stations along other streams in June. To date, 39 wash stations have been deployed along 21 Maryland trout streams. A monthly survey at nine stations in the middle Gunpowder began in July to assess the distribution and abundance of didymo. A tenth station in the Gunpowder upstream from Prettyboy Reservoir was added in July 2009. To determine if didymo occurred outside the Gunpowder, water samples were collected from 20 trout streams across the State in June 2009 (repeated in 2010 and 2012). These samples were analyzed by Dr. Kathryn Coyne (University of Delaware) for the presence of didymo cells using a QPCR assay developed in New Zealand. Beginning in October 2009, benthic macroinvertebrate samples are collected about every other month at one didymo-infested station in the Gunpowder and also in a nearby, non-infested reference stream. In response to the discovery of didymo in Maryland, the presence of whirling disease, and concerns about the chytrid fungus, New Zealand mud snails, and golden algae, MDNR applied the Precautionary Principle and banned felt-soled wading boots statewide in March 2011, the first U.S. state to take such action. Once anglers fully understood the threats posed by didymo, most embraced this ban and have become better stewards of their favorite fishing spots. Didymo is a seasonal nuisance in the first 3-4 km of the Gunpowder tail water below the dam that renders the use of nymphs and other subsurface flies by anglers ineffective to futile. Despite these management actions, didymo has infested three other Maryland trout waters: the lower Savage River (2009), Big Hunting Creek (2012), and the north Branch Potomac River (2012); although abundances there have not yet reached nuisance levels as they have in portions of the Gunpowder.

Panel: The Management of *D. geminata*: Reactions and Responses

**Mark Van Patten.** Missouri Department of Conservation- Fisheries Division.

### **Success in Educating before Regulating**

The alga *Didymosphenia geminata* commonly referred to as “Didymo,” has adapted to diverse water quality conditions, demonstrating increasing tolerance in a variety of habitats and has been expanding its range since the 1980s.

Didymo grows to create extensive underwater carpets negatively impacting stream ecosystems. Waders and watercraft are considered pathways for spread. The most likely pathway is the felt soled wader worn by many trout anglers. Felt soles are porous, hold moisture for days, and can allow Didymo to survive in the sole of the boot, increasing the potential for introduction in the next trout stream the angler visits.

Didymo is already in the White River, just south of Missouri’s border in Arkansas. With the threat of invasion so close to our southern border, the Missouri Department of Conservation (MDC) enacted a comprehensive proactive public outreach effort to reach our angling constituents who are the most likely vectors for introducing Didymo in Missouri’s spring-fed, cold water streams.

A series of Open House Public Forums were held to educate Missouri trout anglers about Didymo and to instill the message: ***Check and Clean or Dry***. Staff also took the opportunity to provide information about the newly installed wader/boot wash stations and to educate the angling public about the need for a ban on porous soled waders in Missouri trout streams.

A specific outreach plan and a twelve (12) month time line were established. The following bullets generalize this plan:

- schedules for Open House Forums sent to media outlets;
- numerous follow up articles were generated for and by reporters from various media outlets including electronic media such as pod casts, blogs and web-based forums;
- development and production of signs and banners were developed for display at various venues and used at a variety of events and public stream access sites;
- production and dissemination of a Didymo fact sheet for the public; and
- *You Tube* video produced for treating porous soled waders rendering them non-porous to comply with a ban on porous soled waders and boots

Through this outreach effort the angling public provided nearly uncontested support for a regulation banning the use of porous soled waders in Missouri trout streams. The ban went into effect March 1, 2012 and, to date; we have documented no invasions of Didymo in Missouri.

Panel: The Management of *D. geminata*: Reactions and Responses

**Michael Goehle<sup>1</sup>, Dave Tilton<sup>1</sup>, and Brad Taylor<sup>2</sup>.** <sup>1</sup>US Fish and Wildlife Service; <sup>2</sup>Dartmouth College.

**Investigating *Didymosphenia geminata* contamination at White River National Fish Hatchery after flooding of the facility by Tropical Storm Irene**

*Didymosphenia geminata* was confirmed in the White River in July, 2007, with nuisance blooms reported in 2007 and 2008. Following Tropical Storm Irene on August 28, 2011, it was unknown if didymo entered the water supply of the White River National Fish Hatchery, either through direct flooding of circular tanks or through well water supply contamination. The U.S. Fish and Wildlife Service was concerned about the potential of spreading didymo if fish at the hatchery were to be stocked. In an effort to confirm the presence of didymo within hatchery water systems, a series of detection protocols was used throughout the system. A molecular genetics lab in Colorado (Pisces Molecular LLC), which has developed a qPCR technique for detecting didymo DNA in water samples, was contracted to analyze the samples. The lab did not detect didymo in any of the samples taken, including six plankton net samples from within the hatchery as well as the ambient river sample and additional scrapings from drum filter effluent solids. Although the hatchery waters tested negative for didymo, fish destined for the Great Lakes were not stocked.



Plenary

**Max L. Bothwell.** Environment Canada, Pacific Biological Station, Nanaimo, BC.

### **The Didymo Story: A changing view of the causes**

Blooms of the river benthic diatom *Didymosphenia geminata* began appearing in rivers on Vancouver Island in the early 1990s and within a decade had spread to many nutrient-poor rivers. During that time investigations into the probable environmental cause of these blooms proved inconclusive. Following the Vancouver Island episode, *D. geminata* blooms began appearing in other places in the Northern Hemisphere and in the 2000s in the Southern Hemisphere (New Zealand and the Patagonia region of South America). British Columbia Steelhead Tag data were analyzed to explore the possibility that the spread of *D. geminata* on Vancouver Island might have resulted from the introduction of a bloom-forming variant during a period of intense river recreational fishing pressure in the late 1980s. The view of *D. geminata* as an invasive bloom-forming variant, often moved by people among watersheds, has been widely accepted.

Now the idea that *D. geminata* in North America is a new variant with bloom-forming tendencies is in question. Studies in New Zealand during 2008-2010 on factors controlling the cellular growth rates and bloom formation by *D. geminata* established the counterintuitive fact that blooms only form under conditions of very low phosphorus. Increased extracellular polysaccharide stalk production under P-starvation provides a possible mechanism for bloom formation. Since *D. geminata* is native to the Northern Hemisphere and the only demonstrated trigger for blooms is extremely low phosphorus, blooms may actually signal the onset of oligotrophication. The potential mechanisms reducing P concentrations in rivers are multi-fold and probably vary between regions. Some studies indicate that atmospheric N-deposition can lead to greater P deficiency in pristine waters while in other cases direct anthropogenic enrichment of landscapes with nitrogen seems a likely culprit behind the blooms. The implications that extremely low phosphorus concentrations are the proximate cause of *D. geminata* blooms will be discussed in the context of increased occurrences of *D. geminata* around the world.

Poster Session

**Beth Flagler.** New Hampshire Rivers Council.

**River Runners: Spreading the word about Didymo**

The New Hampshire Rivers Council is leading the educational movement in the Granite State to stop the spread of Didymo. Paid staff and interns were employed as *River Runners* to look for and document locations of the algae during 2008, 2009, and 2010. In 2012 a new approach to the program was introduced, training volunteers to identify and report Didymo occurrences. The Didymo outreach problem is significant in New Hampshire because funding is limited and many people have never seen Didymo in bloom. Without signs posted the algae is invisible to unknowing recreational paddlers or anglers. Proper planning and behavioral changes are necessary so that river and stream users treat every trip as though it was contaminated event. The new *River Runners* program offers a “train the trainer” model that works with groups already involved in water quality monitoring, state appointed Local Advisory Committees for rivers designated in the State’s Rivers Management and Protection Program, conservation commissions, and other watershed entities. Recruiting and keeping committed individuals engaged is the critical component to the success of the River Runners program. A primary motivator to increase participation is the economics behind healthy rivers for recreation and fishing. In New Hampshire a 2007 study showed a potential decline in the state’s economy of \$51 million in lost sales, \$18 million in lost income, and 800 lost jobs if the perception of the water quality were to decrease. The study, *What’s Our Water Worth*, cosponsored by the New Hampshire Rivers Council, gets the attention that is needed to prove the significant potential of the Didymo problem.

Findings from River Runners 2012 were that volunteers had heard of Didymo, many were more familiar with its common name, Rock Snot, but did not have a full understanding of its life cycle or proper decontamination techniques. Of the nineteen volunteers trained in the field, twelve followed through with their commitment to the program during the 2012 season. No new incidences of Didymo were found by River Runners in 2012, but nearly six hundred Stop Rock Snot brochures were distributed and approximately ten informational signs were posted.

Poster Session

**Carole-Anne Gillis<sup>1</sup> and Normand Bergeron<sup>1</sup>.** <sup>1</sup>Institut National de la Recherche Scientifique, Centre ETE, Québec.

### **Volunteer based Observations to understand the Growth, Persistence and Removal of Rock Snot Blooms**

Since 2006, the Restigouche River watershed has been affected by the presence of *Didymosphenia geminata*, commonly called “didymo” or “Rock snot”. Not only is the alga suspected of altering the environment, it also decreases the aesthetic value of affected rivers. Local economy has a heavy reliance on Atlantic salmon fishery and concerns have been raised that didymo could act as an additional stressor to this anadromous population. Throughout the years, didymo blooms have varied significantly in occurrence and intensity making study site selection quite difficult. Therefore, since 2009, a volunteer network has engaged citizens in participating in weekly monitoring of didymo proliferations on multiple sites along different rivers. We targeted key organizations involved in Atlantic salmon fisheries who were interested in understanding how this nuisance alga affected their local economy and stock recruitment. Data obtained ensured an efficient monitoring of didymo. Currently, this network involves 70 volunteers from 22 different organizations. To this day, more than 1000 observations of didymo bloom extensiveness were reported on more than 20 catchments. This data has helped optimize sampling site locations and has given valuable information with regards to building and testing a conceptual model of the growth, persistence and removal of didymo blooms (Cullis *et al.*, 2012). Public participation is key in conservation outcomes in this case. By involving the public, we achieve a higher success of awareness-building.

Poster Session

**Katherine V. Hanna<sup>1</sup> and Ronald J. Klauda<sup>1</sup>.** <sup>1</sup>Maryland Department of Natural Resources.

### **Statewide Occurrence and Seasonal Abundance Patterns for Didymo in Maryland Waters**

Presence of the freshwater diatom *Didymosphenia geminata* in Gunpowder Falls was reported to the Maryland Department of Natural Resources (MDNR) in January 2008 by anglers. That April, MDNR confirmed the finding and has been charting the extent and spread of didymo ever since. In June 2008, nine stations along the middle region of Gunpowder Falls below Prettyboy Reservoir were established to determine the extent of the didymo infestation and to describe seasonal abundance patterns. These stations are being visited monthly to visually survey for didymo presence, collect substrate samples for microscopic examination, and measure water temperature, current velocity, and turbidity. The data reveal a seasonal pattern in the abundance and spatial distribution of the diatom in Gunpowder Falls. Peak monthly abundance occurs from January through May at the five most upstream stations. An additional station was added in the river above the dam in September 2009, but this location is still didymo-free. In October, benthic macroinvertebrate sampling was included in the survey and a nearby stream without didymo was set up as a control site, to provide insight into the effects of didymo on invertebrates. In June 2009, didymo was found in western Maryland's Savage River, 1.8 km upstream from its confluence with the Potomac River. Through several control measures instituted by MDNR and an effort to educate the public, the spread of didymo was halted until 2012, when new occurrences were confirmed in Hunting Creek and North Branch Potomac River. DNA testing has been done in several streams across the state since June 2009. Results point to the containment of didymo, so far, to these four known areas. Data analyses are under way to determine which chemical and/or physical factors can explain spatial differences in didymo abundance in Gunpowder Falls.

Poster Session

**Daniel A. James<sup>1</sup>, Steven R. Chipps<sup>2</sup>, Max L. Bothwell<sup>3</sup>, and John Carreiro<sup>4</sup>.** <sup>1</sup>U.S. Fish and Wildlife Service; <sup>2</sup>U.S. Geological Survey; <sup>3</sup>Environment Canada; <sup>4</sup>South Dakota Game, Fish and Parks.

**Use of phosphorus enrichment to reduce bloom coverage of the benthic diatom *Didymosphenia geminata* in an oligotrophic stream: Rapid Creek, South Dakota**

*Didymosphenia geminata* was first observed in Rapid Creek of South Dakota's Black Hills during 2002 and since then has been associated with changes to invertebrate and fisheries resources. Because extremely low levels of phosphorus (P) have been associated with *D. geminata* stalk development (i.e., blooms), increasing P availability to *D. geminata* cells was considered as a possible method to reduce bloom severity. We conducted two, whole-stream P enrichment experiments in a *D. geminata* impacted section of Rapid Creek to determine if enrichment would result in reductions of *D. geminata* coverage. After enrichment with a slow-release fertilizer (Osmocote: 14-14-14) during a pilot study conducted in 2007, *D. geminata* coverage increased in sites above enrichment (mean = +60%) while coverage decreased in sites below enrichment (mean = -44%) from May to July. Throughout the summer (May to September), coverage decreased more below enrichment sites (mean=82%) relative to sites above the enrichment (mean=27%). Additionally, as distance from the enrichment source increased, the reduction in coverage was less pronounced, indicating that the negative effect of P augmentation on *D. geminata* coverage was commensurate with proximity to the source. In 2008, a quick-release fertilizer (MAP: 11-52-0) was used for enrichment. We used a conservative, robust before-after-control-impact (BACI) study design to evaluate the impact of P enrichment in 2008 and found that addition of P in amounts ~60% above ambient levels resulted in measurable decreases in *D. geminata* coverage. We were able to attribute decreases in coverage to the addition of P rather than natural variation in coverage using the BACI design. The addition of P to oligotrophic streams with blooming *D. geminata* appears to be a viable option for control of this nuisance species.

Poster Session

**Diba Khan-Bureau<sup>1</sup>, Michael Beauchene<sup>2</sup> and Louise Lewis<sup>1</sup>.** <sup>1</sup>University of Connecticut; <sup>2</sup>Department of Energy and Environmental Protection, Inland Fisheries Division.

**Monitoring the Distribution of *Didymosphenia geminata* and other “Rock Snot” species found in the Connecticut River and its Tributaries in Connecticut.**

*Didymosphenia geminata*, often referred to as “rock snot” or “didymo” is considered nuisance and invasive. This diatom species produces copious extracellular stalks that persist even after the cells have died, forming mats that have the potential to negatively impact the aquatic organisms within rivers and streams.

*Didymosphenia geminata*'s geographical range has expanded throughout the world. Over the past 5 years in the northeastern U.S., didymo has been found in the main stem of the Connecticut River and several tributaries in Vermont, New Hampshire and Connecticut. *Didymosphenia geminata* was confirmed by the Connecticut Department of Energy and Environmental Protection (DEEP) in the West Branch of the Farmington River in 2011 after a fisherman collected and reported cotton-like tufts attached to rocks. In response the DEEP immediately conducted a longitudinal survey within the river system to define the most upstream extent. Additional surveys in January through April 2012, July and September 2012 were conducted at locations observed to have the highest density during the initial survey in May of 2011. Surveys in 2012 indicated no evidence of *D. geminata*. However other diatom species closely related to didymo that produce similar mucilaginous polysaccharide stalks and habit were confirmed, creating confusion for future visual monitoring. On-going surveys are needed.

Monitoring of invasive species is essential but can be challenging for state agencies. Sharing monitoring efforts with state and local agencies, educational institutions and NGOs can help alleviate costs and promote a consistent monitoring protocol. In addition, this shared monitoring effort can contribute to an educated angler and boating community, provide real world job experience and launch an interest in science for college students, and reduce resource demands on resource management. Faculty from Three Rivers Community College, the University of Connecticut, and staff from the DEEP are partnering to design a “**Citizen’s River Monitoring Program**” and develop a website and citizens science program to locate and identify bloom forming nuisance and invasive diatoms in Connecticut.

Poster Session

**Ronald J. Klauda<sup>1</sup>, Jonathan A. McKnight<sup>1</sup> and Katherine V. Hanna<sup>1</sup>.** <sup>1</sup>Maryland Department of Natural Resources.

### **An Inexpensive, Easy to Build Stream-side Wash Station for Cleaning Wading Boots**

Less than two months after the diatom *Didymosphenia geminata* was confirmed for the first time in a Maryland stream, Gunpowder Falls, in April 2008, Maryland Department of Natural Resources (MDNR) staff designed, built, and deployed six wader wash stations along this blue ribbon, tail-race trout stream in central Maryland, USA. Each wash station includes a sign that informs anglers about the presence and threat posed by didymo, and also asks them to take 60 seconds to help protect the river and avoid carrying didymo cells to other streams. Didymo blooms in some portions of the Gunpowder are achieving seasonal nuisance levels for anglers and are reducing the effectiveness of fishing with nymphs and other subsurface artificial flies. The sign asks the anglers to soak and scrub their boots in a 5-10% salt solution provided at the wash station. The wash stations are constructed of pressure-treated lumber, with a plastic masonry tub to hold the salt solution. Each wash station is also equipped with a long-handle scrub brush. The total cost for materials is less than \$40 per wash station. A wash station can be constructed in about 90 minutes, with the aid of a circular saw and a battery-powered drill for driving screws. Since May 2008, MDNR staff and our angler partners have built and installed 39 wash stations along 21 trout streams across central and western Maryland. The wash stations are being maintained by DNR staff and volunteers from angling organizations and the Gunpowder RIVERKEEPER. The deployment of wash stations has been met with positive encouragement from anglers, and is also serving an important outreach function for non-anglers such as hikers and boaters who share the same access points along the river. MDNR staff has shared our wash station design with many angler organizations and management agencies in several states.

Poster Session

**Lisa Kunza<sup>1</sup>, Angela Ostrander<sup>2</sup> and Robert Hall<sup>2</sup>.** <sup>1</sup>South Dakota School of Mines and Technology;  
<sup>2</sup>University of Wyoming.

***Didymosphenia geminata* presence in Grand Teton National Park and a nuisance bloom**

*Didymosphenia geminata* is a nuisance freshwater diatom that is altering physical and biological conditions in streams and rivers. The stalk material *D. geminata* produces causes epilithic mats which can cover up to 100% of stream substrate with thicknesses of up to 20 cm or greater. In 2008, we surveyed 24 streams and rivers for *D. geminata* in Grand Teton National Park, Wyoming. *D. geminata* was present in 7 locations including: Lake Creek both at the inlet ( $2.6 \times 10^6$  cells  $m^{-2}$ ) and outlet ( $1.5 \times 10^8$  cells  $m^{-2}$ ) to Phelps Lake, Taggart Lake Outlet ( $2.4 \times 10^7$  cells  $m^{-2}$ ), Jenny Lake Outlet ( $8.4 \times 10^5$  cells  $m^{-2}$ ), Flat Creek ( $5.9 \times 10^5$  cells  $m^{-2}$ ), Leigh Lake Outlet ( $1.3 \times 10^4$  cells  $m^{-2}$ ), and Fish Creek ( $9.2 \times 10^3$  cells  $m^{-2}$ ). *D. geminata* was present in lake outlets and spring streams all of which were visited by people during our sampling. The mat of *D. geminata* in the Phelps Lake outlet portion of Lake Creek was up to 2.5cm thick and covered >70% of substrate for >1km. The epilithic biomass of the Lake Creek nuisance bloom was greater than the epilithic biomasses of 21 of our other streams, but lower than the epilithic biomass of 2 spring streams without *D. geminata* present. Occurrence of *D. geminata* in lake outlets and spring streams suggests that *D. geminata* prefers stable flow, substrate, and temperature. The presence of *D. geminata* in high traffic areas suggests that people may be the primary vector for dispersal.



Poster Session

**Samantha Root<sup>1</sup>, Catherine O'Reilly<sup>2</sup> and Dave Kumlien<sup>3</sup>.** <sup>1</sup>Bard College; <sup>2</sup>Illinois State University; <sup>3</sup>Trout Unlimited.

**Controlling Didymo: effective decontamination strategies and recommendations for public outreach.**

With the increasing appearance of nuisance blooms caused by *Didymosphenia geminata* (didymo), there has been a need to determine and assess effective strategies for reducing contamination and spread. Our study experimentally tested several common decontamination treatments and used surveys to determine the response of state agencies and fishermen to decontamination procedures. In testing decontamination products, we found that dish liquid detergent was the most effective, followed by bleach, Virkon, and salt. We also found that recommendations for decontamination varied widely from region to region. Recreational fishermen and other stream users found it difficult to know which of these decontamination methods was the most effective for use. Ultimately, fishermen claimed that they learned their decontamination methods primarily from sources other than state agencies. From the fishermen's perspective, didymo was the aquatic invasive species of highest concern, but there was a wide range of approaches to didymo control. We recommend that environmental agencies concentrate on the importance of comprehensive and consistent information sources and standards for didymo decontamination and education. Agencies could increase the effectiveness of their outreach efforts by working more closely with each other and with related national nonprofit organizations (e.g., Trout Unlimited), local economic venues (e.g., fly fishing stores), or through existing regulatory mechanisms (e.g., licensing, signage) to directly provide information.

Poster Session

**M. Rodway<sup>1</sup>, W. Jarvie<sup>1</sup> and S. Sutherland<sup>1</sup>.** <sup>1</sup>Southland Fish & Game, New Zealand

**Long term management of *Didymosphenia geminata* in a controlled river**

The diatom *Didymosphenia geminata* (didymo) is native to the Northern Hemisphere and was accidentally introduced into the South Island of New Zealand. It was discovered there in 2004. Didymo forms extensive mats on river beds, attaining thicknesses of 50 mm or more, and transforming the appearance and ecosystem function of the affected rivers. Rivers with clear water, low nutrients, long periods of low flows, and boulder/cobble substrates provide the most suitable habitat for didymo. Lake outlet rivers and those controlled for hydropower generation are the most affected. A programme to identify, and provide, increased flows to remove the didymo biomass to levels that do not adversely affect angling opportunities and fish biomass has been developed with Meridian Energy, holders of consents to dam and divert river flows to generate electricity in the Waiau River Catchment, South Island, New Zealand. Flows at least 10 times the median at least 4 times per year, with increased frequency in the summer months, are required to reduce didymo biomass to acceptable levels on a continuing basis.

Poster Session

**Christine Sandvik<sup>1</sup>, P.V. Sundareshwar<sup>1</sup>, Robert Pillsbury<sup>2</sup> and Lisa Kunza<sup>1</sup>.** <sup>1</sup>South Dakota School of Mines and Technology; <sup>2</sup>University of Wisconsin Oshkosh.

**The effects of iron on growth of *Didymosphenia geminata***

Iron (Fe) availability may contribute to phosphorus (P) acquisition by *Didymosphenia geminata*. Since Fe binds to P in oxic environments, P can be removed from the water. Due to mat development by *D. geminata*, anoxic zones may develop that allow for release of the Fe bound P. Interrupting P and Fe binding may, therefore inhibit growth of *D. geminata*. Alum may compete with Fe to bind P and interrupt Fe binding of P. Ferrozine binds Fe removing reactive Fe from the water and prevents P binding to Fe. To examine the effects of Fe on *D. geminata*, we incubated *D. geminata* in recirculating chambers with nutrient treatments. Our recirculating chambers were one gallon and lined with marble tiles seeded with *D. geminata*. To seed the tiles, we soaked them in blended *D. geminata* mat from Rapid Creek, SD for 6 hours. We setup four recirculating chambers per treatment of: control (no nutrients added), Fe + P + N, Fe + P + N + Al, and Ferrozine. We incubated the recirculating chambers at 8.6 degrees C with 12 hours of light per day. To examine the change in algal assemblage over time for each treatment, we randomly removed 2 tiles for cell counts every four days for 104 days. Treatments containing Alum and Ferrozine had decreased algal growth.

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