Didymosphenia geminata Infestation in Maryland: Reactions and Responses by the Maryland Department of Natural Resources, 2008-2014

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Introduction

Didymosphenia geminata (hereafter, didymo) was not the first non-native aquatic organism that showed up, uninvited, in Maryland's waters. But when it did, the Maryland Department of Natural Resources (DNR) Invasive Species Matrix Team evaluated the discovery, discussed management options, embraced the Precautionary Principle (Foster et al. 2000, Science and Environmental Health Network 2000, Sachs 2011) and quickly acted, to the extent that available staff and limited resources would allow.

The purpose of this report is to document how DNR staff reacted and responded between 2008 and 2014, with the help of many willing participants (see Acknowledgements section), to this potential ecological and economic threat. A question/answer format will be used to describe what occurred, what was learned, and what we still do not understand. This report is not intended to be a comprehensive review of the available scientific literature on didymo. Many other published documents (e.g., Spaulding and Elwell 2007, Blanco and Ector 2009, Whitten et al. 2009) have already done that and more completely than what we could hope to achieve in this report. Rather, what is known and not known about didymo, relevant to Maryland waters, is discussed herein----with citations of the most relevant scientific literature listed in the References section.

Question #1: What's known about didymo and why should we be concerned about its presence in Maryland?

Didymo is a freshwater diatom, a single-celled benthic alga thought to be native to pristine habitats in mountainous areas of circumpolar Asia, Europe, and North America. There, didymo is found in cool/cold, very low nutrient, clear streams and rivers. Unlike most other diatoms, individual didymo cells can grow a yellow-brown or grayish-white mucopolysaccharide stalk (or strand) up to 2 feet long. When didymo abundance is high and a 'bloom' occurs, many stalks are produced, they entangle, and the result is growth of large mats (up to 10 inches thick) that resemble wet toilet paper, but which actually feel gritty and more like wet wool. The appearance of these extensive mats helped earn didymo's other monikers: 'rock snot' and 'boulder boogers'. The mats pose an ecological threat to aquatic plants, macroinvertebrates, and fish. In addition, the mats diminish the aesthetic qualities of pristine trout waters, hamper anglers, and can negatively affect recreational fishing and tourism (Beville et al. 2012).

Didymo cells are microscopic but much larger than other freshwater diatoms. The siliceous didymo cell is shaped like an old-fashioned Coke bottle, a distinguishing characteristic. Didymo cells attach securely to large pebbles, cobble, boulders, other coarse substrates, and sometimes to woody debris, plants, and the odd beer can. Although didymo cells can attach, grow, and proliferate on finer and softer substrates such as mud, sand, and gravel, they are much less likely to do so than on harder surfaces.

For reasons not well understood, didymo is expanding its range and habitat tolerances to include more southern waters in warmer climates. Didymo is now found in British Columbia, Canada, New Zealand, South America, and parts of the United States where it had not been reported before. In the mid-1980s, didymo began to exhibit characteristics of an invasive species, forming nuisance-level blooms in streams and rivers. For this report, we accept this definition of an 'invasive species' approved by the Invasive Species Advisory Committee of The National Invasive Species Council: *"A species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health".*

There are some reports suggesting that large didymo blooms occurred even earlier, during the 19th century. In its "new" locations, didymo is a highly variable diatom and unpredictable in its seasonal growth patterns between years and from river to river. It is possible that more research will lead to a better understanding of didymo ecology and reduce the current level of unpredictability.

The behavior of didymo in areas of the world where it appears to be a newcomer suggests it could alter lotic ecosystems. To date, however, the evidence for negative ecological impacts is limited. Reported nuisance-level didymo blooms have occurred in larger streams and rivers, most often in colder tailwater areas below impoundments. Didymo is also found in some lakes, ponds, and non-regulated streams and rivers; but it does not seem to achieve nuisance blooms in lentic waters.

Like many non-native aquatic species, didymo poses both ecological and economic threats. Wherever one or more of these threats are manifested, didymo can justifiably be labeled as 'invasive', in addition to being non-native. From an ecological perspective, a large biomass of stalk material produced by didymo cells that forms thick mats that can completely cover the substrate and trap sediments has the potential to disrupt aquatic food webs. Extensive bottom coverage by mats of didymo stalks threaten the biodiversity of streams and rivers if they smother macroinvertebrate species, native diatoms, and aquatic plants----thereby possibly reducing food and habitat for fish. Conversely, oxygen-rich didymo mats also create additional habitat that favors some aquatic insect larvae. Hence, the documented ecological impacts of didymo thus far are mixed.

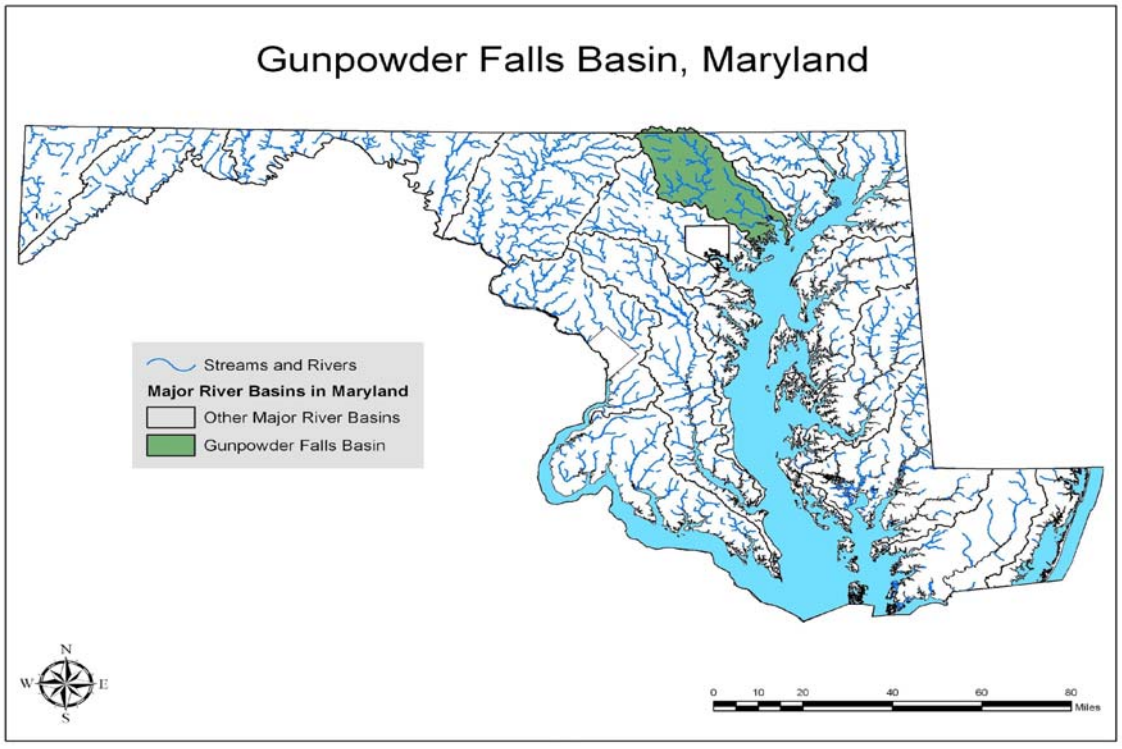
More research focused on better understanding the ecological effects of didymo blooms is clearly needed. Didymo presence has caused shifts in the community composition of the macrobenthos. Midge larvae and worms increase while caddisfly, stonefly, and mayfly larvae decrease. Some studies have also observed higher overall macroinvertebrate densities after didymo becomes established, but average organism size is smaller. There is even less information on the effects of didymo blooms on fish communities. Some studies report no changes in fish growth or production in didymo-infested waters. Others have observed declines in native fish populations following a didymo infestation.

Question #2: When was didymo first confirmed in Maryland waters and where?

Alert anglers fishing for trout in Gunpowder Falls (hereafter, the Gunpowder) below Prettyboy Reservoir, Baltimore County, in January 2008 noticed "something strange looking" clinging to the river bottom. They suspected it was didymo and contacted DNR. Samples were collected and the first ever reported occurrence of didymo in Maryland was confirmed microscopically by Walter Butler, then one of DNR's benthic macroinvertebrate experts. On May 1, DNR biologist Ron Klauda estimated that 20-25% of the river bottom in a 50-m long section of the Gunpowder upstream from the Falls Road bridge was covered by a didymo bloom. In July, staff from DNR and Baltimore County launched a monthly survey in the Gunpowder to document the spatial distribution of didymo, estimate the extent of bottom coverage at several locations along the river, describe seasonal bloom patterns, and track its spread. See the answers to Question #6 for more information on this survey.

The Gunpowder is a 53 mile long tributary of the Chesapeake Bay that drains portions of southeastern Pennsylvania and central Maryland (see Map 1).

Map 1: The Gunpowder Falls watershed



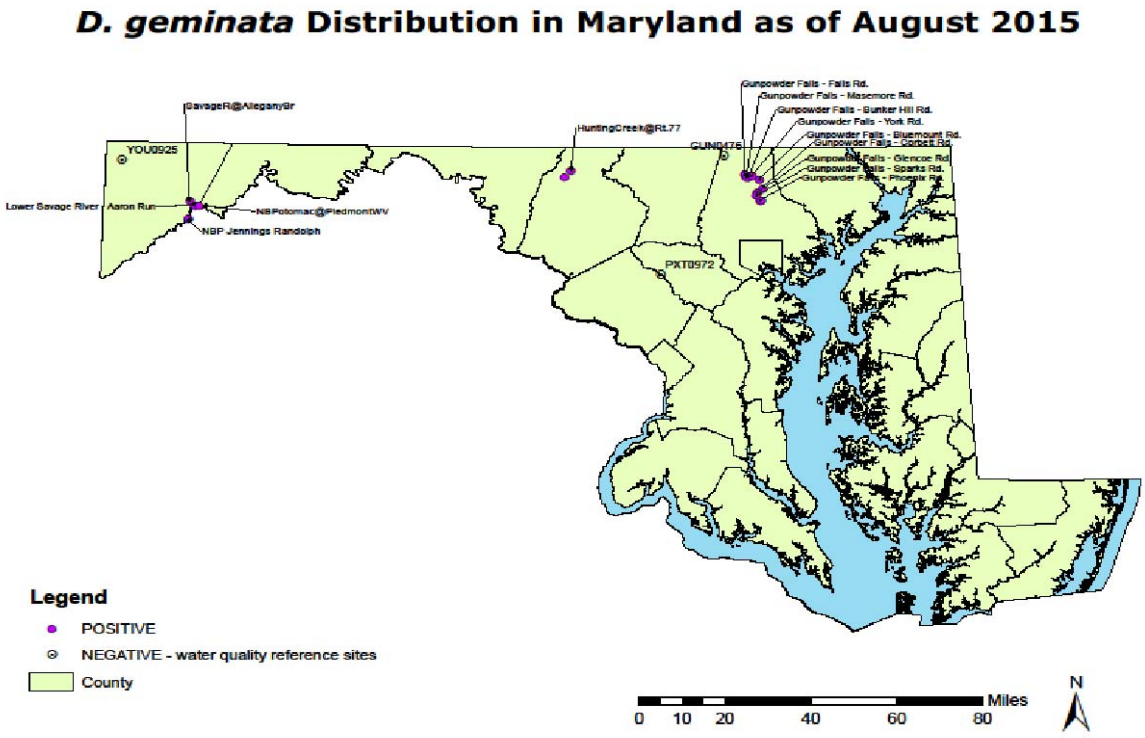
The Gunpowder watershed lies mostly in the Piedmont region, encompasses over 450 square miles, and contains 217 miles of streams. Controlled water releases from Prettyboy Reservoir, a 1,500 acre water supply impoundment, creates almost 20 miles of excellent cold-water habitat for brown trout---all within a half-hour drive from downtown Baltimore.

Anglers from all over the United States., especially the northeastern and mid-Atlantic states, and also from several other countries, come to the Gunpowder to experience this catch-and-release blue-ribbon tailwater fishery. The upper portion of the Gunpowder tailwater, with the best brown trout habitat and where didymo was first discovered, lies within Gunpowder Falls State Park. Phosphorus and nitrogen levels there are relatively low, making it an oligotrophic to almost mesotrophic stream----near ideal conditions for didymo to establish and flourish.

Question #3: What is the current distribution of didymo in Maryland?

Since didymo was first confirmed in the Gunpowder in early 2008, it has also been found in three other Maryland waters: first reported in the Savage River downstream from the reservoir (Garrett County) in June and November 2009, first reported at three locations in the North Branch Potomac River (Allegany and Garrett Counties) in August 2011 and September 2012 (two locations near the Savage River confluence and a third location further upstream---from the Jennings-Randolph Dam downstream to Barnum), and first reported in May 2012 in Big Hunting Creek downstream from Hunting Creek Lake in Cunningham Falls State Park (Frederick County); see Map 2.

Map 2: Distribution of didymo in Maryland as of 4/7/2016



Seasonal didymo blooms are still being observed in the Gunpowder, primarily from

December through March (personal communication with Theaux LeGardeur, Gunpowder Riverkeeper, on 4/4/16) and also in the entire Savage River tailwater area and at the three North Branch Potomac River locations (personal communication with Alan Klotz, DNR, on 2/11/16). No didymo blooms have been seen in Big Hunting Creek since 2012 for reasons that are unclear (communication with Mark Toms, DNR, on 2/23/16).

In late September 2016, a clump of material with a yellow tint was observed floating in an alcohol-preserved benthic macroinvertebrate sample jar that was being processed by Ellen Friedman and Neal Dziepak of DNR's Resource Assessment Service (RAS). Some of the material was examined microscopically and appeared to contain didymo cells. This preliminary identification was confirmed by Jennifer Wolny, the RAS algal expert. The benthic macroinvertebrate sample was collected by Friedman and Dziepak on July 12, 2016, in the Youghiogheny River in the town of Friendsville, Maryland (39° 39' 47.8" N, 79° 24' 27.6" W). This is the first reported collection of didymo in the Maryland portion of the Youghiogheny River. No didymo bloom was observed by Friedman and Dziepak on July 12 when it was unknowingly collected, nor has a bloom been observed and documented at this location or at any other locations in the Maryland Youghiogheny River since then. So, at this time, it is uncertain if didymo is established and thriving in the Maryland Youghiogheny River.

Two methods were used to document the distribution of didymo in Maryland: light microscopy examination of substrate samples or suspected didymo stalks and a quantitative real-time qPCR assay on plankton net samples collected from the water column aimed at detecting didymo DNA (Cary et al. 2014). Microscopy was used whenever visual assessment of a suspected didymo bloom occurred. Used as a screening tool, the qPCR assay for didymo DNA at low cell densities allowed DNR staff to monitor many more stream and river sites. Most of the sites screened with the qPCR assay were negative for didymo (see Map 2); however, the first discovery of didymo in the lower Savage River (in June 2009) was confirmed by a qPCR assay. The qPCR assay used is very sensitive and capable of detecting didymo cell abundance as low as 1 per ml of plankton net sample (Cary et al. 2014).

Question #4: Is there any evidence to show that didymo is native (or indigenous) to Maryland?

At the International Didymo Conference held in Providence, Rhode Island on March 12-13, 2013, two of the many interesting topics discussed by the attendees were the origins of didymo and possible explanations for the apparent increase in nuisance-level blooms. Didymo is almost certainly a new organism in New Zealand and Chile, two southern hemisphere countries, and human transport is the most likely introductory pathway. However, paleolimnological records from several northern hemisphere lakes in Alaska, Montana, and Wyoming show that didymo has been there for up to 10,000 years. Hence, human transport from somewhere else was probably not involved in establishing these didymo populations. One presenter at the 2013 conference stated that he considers didymo to be a native species in many Rocky Mountain rivers of the United States. Klauda and

Hanna, the co-authors of this report, both attended the 2013 didymo conference. In his oral paper delivered there, Klauda reported that DNR considers didymo to be a non-native species in Maryland, until proven otherwise, and a seasonally-nuisance species with the potential to become invasive. Spaulding and Elwell (2007) stated that didymo was historically reported in only one state in the United States, Virginia, by Patrick and Reimer in a 1975 publication.

Soon after returning from this conference, Klauda looked for any evidence that didymo is native to Maryland. Starting locally, he contacted Dr. Susan Gresens at Towson University in Towson, Maryland, on 3/18/13. She suggested that he contact Dr. Marina Potapova, Assistant Professor/Curator of the Diatom Herbarium at the Academy of Natural Sciences, Drexel University, Philadelphia, Pennsylvania. Dr. Potapova has done extensive research on the taxonomy, ecology, and biogeography of freshwater, mostly riverine, diatoms. She is a council member on the International Diatom Society and an Associate Editor of two international journals: Phycologia and Diatom Research. The Academy's Diatom Herbarium holds one of the largest diatom collections in the world.

Klauda contacted Dr. Potapova on 3/20/13 and asked if she thinks didymo is native to Maryland. He also asked if she or her colleagues at the Academy are updating the 1975 monograph by Patrick and Reimer by re-sampling streams in the eastern U.S., including Maryland. Dr. Potapova responded to Klauda's questions in an email dated 3/21/13: *"Yes, this is true that Didymo was reported by Patrick and Reimer from Virginia only. As far as I know it was also found in the beginning of 20th Century in the sediments of the Delaware River in Philadelphia. It does not grow in brackish water, of course, and definitely grew somewhere upstream. The problem is that it's quite difficult to say now where Didymo was occurring historically because stream diatom samples were rarely collected in the past. We have diatom samples from 182 sites in Maryland collected in the past 20 years, and no Didymo was reported in any of them. Most of the samples in our collection were collected by other people or agencies, but diatoms are usually identified here, at the Academy. I did not know that Didymo was ever found in Maryland, but it looks from your message that it was."* Klauda's email to Dr. Potapova dated 3/20/13 told her about the international didymo conference and that he had reported on the early 2008 discovery of didymo in the Gunpowder and subsequent discoveries of didymo blooms in three other Maryland waters at this conference. Dr. Potapova also stated in her 3/21/13 email to Klauda that *"Didymosphenia geminata underwent a name change.....but that was a long time ago. It obtained its current name in 1899, so all of the more or less reliable records are under the current name."*

Klauda met Paul Bugas, an aquatic biologist with the Virginia Department of Game and Inland Fisheries, at the March 2013 didymo conference. On 3/20/13, Klauda sent Mr. Bugas an email asking him if he knew where in Virginia Patrick and Reimer (1975) apparently found didymo sometime during the 1960s. Klauda also asked Mr. Bugas if his agency considers didymo to be non-indigenous or indigenous to Virginia waters. Mr. Bugas responded in an email dated 3/29/13 and said that the 1975 publication by Patrick and Reimer did not mention the stream or county or even the region of Virginia where they apparently collected didymo. Mr. Bugas also concluded, "*Given that information, I would hesitate to call didymo indigenous to Virginia waters."*

Although there is some uncertainty, we can find no clear evidence to support a conclusion that didymo is native (indigenous) to Maryland. Therefore, until convinced otherwise, we think DNR is justified to say that didymo is a non-native species and to conclude that it was introduced into the state some time prior to its discovery in the Gunpowder in early 2008. DNR's management actions in response to didymo are therefore consistent and appropriate with an assumption of its non-native status. How it probably got to Maryland will be addressed in our answers to the next question.

Question #5: What is the most plausible pathway for the introduction of didymo into Maryland waters?

There is no way to prove how didymo found its way into the Gunpowder or the other three Maryland waterways. But it is probably safe to say that the least likely pathway was on the feet or legs of waterfowl and wading birds. The more likely pathway of introduction involves humans: specifically tubers, kayakers, canoeists, and anglers. We have not investigated the potential recreational floater pathways. But it is our impression that most people who tube or kayak or canoe the Gunpowder are not bringing their watercraft into Maryland from other states.

It is possible that one or more recreational floaters on the Gunpowder could have transported their kayaks or canoes to the lower Savage or North Branch Potomac Rivers and inadvertently introduced didymo. Most likely, though, didymo was introduced into the Gunpowder, Savage River, North Branch Potomac River, and Big Hunting Creek, Maryland, via the waders and gear of trout anglers who were probably unaware they were serving as vectors. The Gunpowder is a popular trout stream for anglers from Maryland and other states.

The pattern of didymo spread into waters where it probably did not exist previously is closely associated with patterns of human water-based recreation, especially angling. In British Columbia, nuisance blooms of didymo appeared in the mid-to late 1990s, about the same time felt-soled waders became available to and popular with anglers. Only a few didymo cells are needed to inoculate new waterways where, if the conditions are right, didymo cells will survive, divide, and persist. Felt soles can and do absorb water, debris, and didymo plus other microorganisms. Felt soles provide a moist, temporary habitat where didymo cells can survive until an angler visits another fishing spot. Didymo cells can survive out of water in a cool, damp and dark place for 40-60 days or longer.

With the advantages of hindsight, DNR staff might have foreseen didymo's arrival in Maryland before 2008 and taken a more proactive approach to preventing its introduction. Bur, with limited resources this would have been difficult to accomplish. What was being reported in other nearby states could have served as warning signs. Didymo blooms were reported for the first time in the Mid-Atlantic Region, in Virginia, in 2006. Then in June 2007, didymo was reported in the northern reaches of the Connecticut River and also in the

White River, Vermont. By October 2007, there were reports of didymo occurrences in the East and West Branches of the upper Delaware River in New York and Pennsylvania. So, it was not a complete surprise to DNR staff when didymo was discovered in Gunpowder Falls, Maryland only a few months later in early 2008.

A similar and likely also an angler-associated transfer from another state or states is plausible for the appearances of didymo blooms in the lower Savage River, North Branch Potomac River, and Big Hunting Creek, Maryland---discoveries that were reported not long after didymo was first reported in the Gunpowder. Didymo cells could also have been picked up in the Gunpowder on anglers' boots and/or gear and unintentionally moved to these other Maryland locations.

Question #6: How did DNR react and respond to the confirmation of didymo infestations in Maryland?

The bases for DNR's relatively aggressive reactions and responses to the confirmation of a didymo bloom in the Gunpowder in early 2008 were the assumptions that: 1) Didymo is not native in Maryland, and 2) Didymo was inadvertently introduced to Maryland by anglers. DNR's management responses were developed by the agency's Invasive Species Matrix Team (ISMT). Klauda was a member of this multi-disciplinary group. The ISMT acknowledged that DNR could have initiated a public education/outreach campaign sooner to prevent didymo's introduction into Maryland. But the team moved quickly to take actions that could determine the extent of didymo's distribution, assess the possibility that it might be eradicated, and act to stop or slow the spread of didymo to other waters of the state.

Specifically, the ISMT took these actions in response to the didymo infestation:

1. Beginning in May 2008, a series of press releases were issued to notify and educate the public.

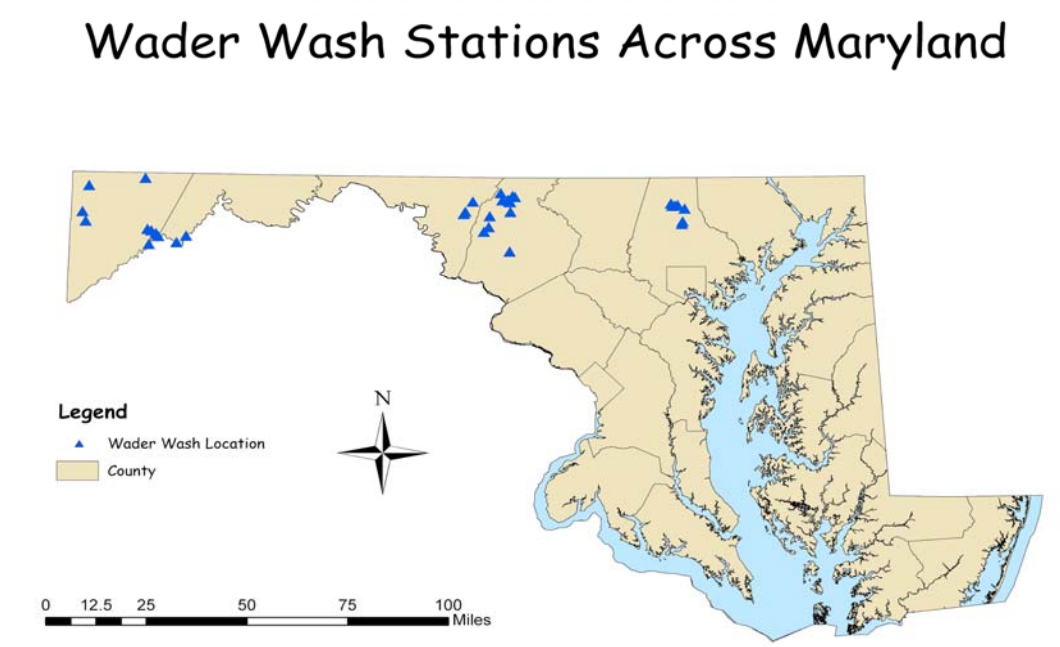
2. Fact sheets were written and circulated about didymo biology, probable introduction pathways, potential ecological/economic threats, and methods that anglers could use to decontaminate their boots and gear.

3. Signs were posted at major angler access points in May 2008 to alert anglers and others about didymo.

4. Six wader wash stations were designed, constructed, and deployed along the Gunpowder in May and June 2008 to educate anglers and other recreational water users about the potential threats from didymo, and also to provide places near river access points where anglers could decontaminate their boots before entering the river to fish and after leaving the river---to perhaps travel to another trout stream (https://drive.google.com/open?id=0B684NRP4uOmfSWVGa3laUEtLUnc). At last count, there are at least 45 wader wash stations, with educational signage,

deployed along Maryland streams and rivers (see Map 3).

Map 3: Wader wash stations in Maryland



5. A monthly didymo survey was started in the Gunpowder in July 2008 to better understand seasonal distribution and abundance patterns. In October 2009, the survey was expanded to include the collection of benthic macroinvertebrates at one didymo-infested station in the Gunpowder and also at a non-didymo infested reference station in nearby Little Falls.

6. After careful evaluation, deliberation, and solicitation of public comments, DNR announced a statewide ban on felt-soled wading boots that took effect on March 22, 2011.

7. In planning and implementing these management actions, the ISMT embraced the Precautionary Principle. Even in the face of some uncertainty about the relative importance of felt soles in transporting didymo and other organism cells, DNR decided to act with caution and protect Maryland's aquatic resources.

Each of these management actions is discussed in more detail below.

Press Releases

The first of at least seven didymo-related press releases was issued by DNR on May 6, 2008--less than a month after DNR staff confirmed didymo for the first time in Maryland. In

addition to announcing this unfortunate discovery, the first press release briefly described didymo as looking slimy, but feeling more "*like wet cotton or wool*". The press release called didymo a "*new, invasive, non-native algae*". In hindsight, calling didymo invasive in Maryland may have been premature. This first release was, however, accurate in stating that didymo "*has the potential to disrupt ecosystems*" and can be transported unknowingly by anglers on felt-bottom boots and their fishing gear. Anglers were urged, in this press release, to clean, disinfect, and thoroughly dry their boots and gear to help prevent the spread of didymo to other state waters. Contact information was provided in the press release, and anyone who saw what they thought could be didymo was encouraged to notify DNR.

A second DNR press release was issued on May 28, 2008. Anglers were urged to use six wader wash/sterilization stations that DNR staff had constructed and deployed throughout the tailwaters of the Gunpowder. In this press release, DNR again urged anglers (and also kayakers and canoeists) to sterilize anything they use that comes into contact with river water. Suggested sterilization procedures were mentioned in this press release. Anglers were also advised against using felt-soled boots and waders, and urged to replace them with non-porous soled boots.

DNR issued a press release on December 14, 2009, announced the confirmation by DNR staff in late November of a didymo bloom in the lower Savage River, below the reservoir and just downstream of the Allegany foot bridge. This press release also asked anglers and other outdoor enthusiasts who enjoy Maryland's waters to help prevent the spread of didymo and other unwanted aquatic invaders by checking, cleaning, and drying all gear that has been in contact with river water. Once again, DNR encouraged anglers to replace their felt-soled boots "*with new sticky rubber soled models which are much easier to clean and disinfect.*"

A DNR press release on March 17, 2011, announced that felt soles on wading boots would be banned statewide on March 22, 2011, "to protect and preserve native wildlife and habitats." In this press release, Jonathan McKnight, head of the ISMT, said, "*Felt is porous and can remain damp for weeks, keeping harmful microscopic organisms alive and making it virtually impossible to disinfect. After reviewing the science and spending a year on outreach, public meetings and citizen response, we concluded that the only responsible action was to ban this material to halt the spread of harmful invasive organisms. The 'do nothing' response just would not cut it when the health and beauty of our rivers is at stake."* The press release also stated that DNR field biologists have been successfully using wading boots with new non-porous, rubber material soles. These same messages were repeated in a DNR press release issued on March 22, 2011---the date when wearing felt-soled waders/wading boots within five feet of any body of water in Maryland became illegal. Maryland was the first state to take this management action. Vermont soon followed with a similar ban on April 1, 2011. Alaska, Maryland, Missouri, Nebraska, Rhode Island, and South Dakota have enacted similar felt bans.

On July 1, 2016, the Vermont Department of Environmental Conservation repealed the statewide ban on using felt-soled footwear. This decision was apparently based on new research that suggests didymo is native to the northeastern United States, and that recent changes in environmental conditions are stimulating the formation of nuisance blooms.

Since no evidence has been found suggesting that didymo is native to Maryland or other mid-Atlantic states' waters, use of felt and other absorbent material soles on wading boots is still illegal everywhere in Maryland.

DNR issued another press release on April 1, 2011, to remind the public that felt-soled waders and wading boots are illegal in any body of water in Maryland. The press release also stated that, *"We understand that some anglers could be unaware of this new law. For this reason, Natural Resources Police will initially focus on education, issuing a warning providing information to anyone wearing felt-soled boots or waders."*

DNR issued a press release on May 3, 2012, to announce the confirmation of a didymo bloom in another Maryland tailwater trout stream, Big Hunting Creek. John Mullican, DNR's Regional Fisheries Manager, stated, *"We observed the heaviest growth of didymo at the Joe Brooks Memorial, with lighter growth areas downstream to just below the canyon."* This press release again reminded anglers about the felt-soled boot ban.

Fact Sheets and DNR Website Links

The first didymo-related press release issued by DNR (May 6, 2008) included a link to DNR's invasive species website (*www.dnr.state.md.us/invasives/*). The public was encouraged to visit this website for more information about didymo. The current website address for invasive species information is: *www.dnr.maryland.gov/invasives*. Several fact sheets on didymo were also prepared by DNR staff for posting on the website and for distribution via other public education/outreach communication channels. The titles of three fact sheets are:

1. *JUST SAY "NO!" TO DIDYMO* (by Ron Klauda, DNR, 11/29/08, *http://dnr.maryland.gov/streams/Publications/Didymo_info.pdf*)

2. *COMPILATION OF INFORMATION ON DIDYMOSPHENIA GEMINATA* (by Ronald J. Klauda, DNR, 3/29/09, *https://drive.google.com/open?id=0B684NRP4uOmfSHJYcFhhUDVnNjA*)

3. *Chronology of 'Didymo'-related Events in Maryland* (by Ron Klauda, DNR, 12/9/09, *https://drive.google.com/open?id=0B684NRP4uOmfQ21ySWpCQlJ0TnM*).

DNR also made an effort to more widely publicize an internal agency policy on boots and equipment that DNR's field crews had been following since 2007, before didymo was first confirmed in the State. The equipment disinfection policy was implemented by DNR because of the potential for our field crews to unknowingly transfer non-native and invasive organisms from one stream to another during their sampling activities. Whirling disease, amphibian chytrid fungus, rana virus, largemouth bass virus, viral hemorrhagic septicemia, avian influenza, and didymo were and still are organisms of concern that can be easily transported on wading boots and sampling gear. The boot and equipment disinfection procedures required of DNR field crews between sampling sites initially consisted of soaking and scrubbing items in a 10% bleach solution for at least one minute, and then rinsing

everything thoroughly with fresh water at a location at least 50 yards from the nearest water body. The 10% bleach solution was soon replaced with the now preferred disinfectant: a 2% solution of Virkon Aquatic. At the end of each sampling day, all disinfected boots and gear should be dried for at least 48 hours.

Posted Signage

In early May 2008, DNR staff posted "*PREVENT THE SPREAD OF DIDYMO"* signs at angler access points along the Gunpowder where didymo was confirmed less than one month earlier. Also in May 2008, DNR staff posted *"DON'T SPREAD WATER INVADERS!"* signs at angler access points along other Maryland trout streams where didymo was not known to occur, but were places that could be fished by anglers who had recently been in the Gunpowder. Among other messages designed for public education/outreach, these signs recommended that anglers not use felt-soled boots.

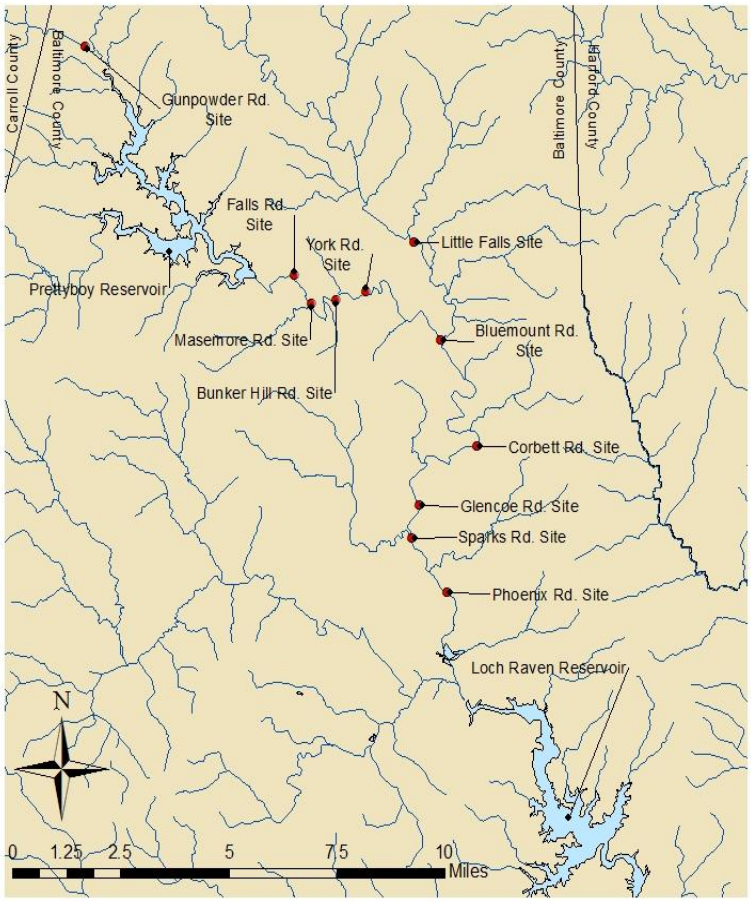
Wader Wash Stations

Another important part of DNR's public education/outreach campaign, initiated soon after didymo was confirmed in the Gunpowder in April 2008, was led by Jonathan McKnight and other ISMT members. They constructed and deployed six wader wash stations along the Gunpowder during May and June 2008. Each wash station had a sign that explained to the angler why they were being asked to wash (disinfect) their wading boots, a pan filled with a saturated salt (NaCl) solution, and a scrub brush (*https://drive.google.com/open?id=0B684NRP4uOmfSWVGa3laUEtLUnc*). The wash stations were relatively inexpensive (~$36 for materials) and could be easily built by two people in a little over an hour. Additional wader wash stations were built and deployed along other trout streams across Maryland, with a peak number of 45 wash stations in 2014.

Didymo Surveys

Beginning in July 2008 and extending through June 2014, monthly surveys were conducted along the Gunpowder to gain a basic understanding of didymo's spatial distribution and seasonal abundance patterns. The survey was led by Klauda and Hanna (DNR), with valuable support from staff with Baltimore County's Department of Environmental Protection and Sustainability (key investigators were Dennis Genito and Kevin Brittingham). During their involvement with the survey, the Baltimore County team surveyed four stations in the Gunpowder downstream from Prettyboy Reservoir: Bunker Hill Rd., York Rd., Corbett Rd., and Phoenix Rd. DNR staff initially surveyed five additional stations: Falls Rd., Masemore Rd., Bluemount Rd., Glencoe Rd., and Sparks Rd.--also downstream from Prettyboy Reservoir. In addition, beginning in July 2009, DNR staff began monthly surveys at Gunpowder Rd., on the Gunpowder upstream from the reservoir--a location that was then and continues to be didymo-free. In April 2010, DNR staff established a reference station in Little Falls, a didymo-free stream about 5 miles north of the didymo-infested middle Gunpowder (see Map 4).

Map 4: Locations of all didymo survey sites in the Gunpowder and the Little Falls reference site



When involvement of the Baltimore County team ended in mid-2011, DNR did not have enough staff to continue monthly surveys at all stations in the Gunpowder, plus conduct occasional surveys in other Maryland streams and rivers that were infested with didymo. Therefore, the York Rd., Corbett Rd., Sparks Rd., and Phoenix Rd. stations in the Gunpowder were dropped from the didymo survey after June 2011. DNR continued to survey monthly at the Bunker Hill Rd. station, previously monitored by the Baltimore County team. See Table 1 for a summary of stations and years that were surveyed by DNR or Baltimore County staffs in the Gunpowder for didymo.

Table 1: Stations included in MD/DNR didymo survey in Gunpowder Falls.

Station	Year Surveyed						
	2008[a]	2009	2010	2011	2012	2013	2014[b]
Gunpowder Rd.		√	√	√	√	√	√
Falls Rd.	√	√	√	√	√	√	√
Masemore Rd.	√	√	√	√	√	√	√
Bunker Hill Rd.	√	√	√	√	√	√	√
York Rd.	√	√	√	√ [d]			
Bluemount Rd.	√	√	√	√	√	√	√
Corbett Rd.	√	√	√	√ [d]			
Glencoe Rd.	√	√	√	√	√	√	√
Sparks Rd.	√	√	√	√ [d]			
Phoenix Rd.	√	√	√	√ [d]			
Little Falls[c]			√	√	√	√	√

a: Started in July 2008 b: Ended in June 2014 c: Reference station (no didymo) d: Through June 2011

Survey stations along the Gunpowder were located at road crossings that also serve as angler access points. Two person survey teams visually examined the river bottom at each station with a bathyscope, usually in a 100-m long section bisected by the road crossing. However, because of high current velocities that made wading difficult during normal flows and hazardous during high flows, 50-m long sections were surveyed monthly at the Falls Rd. station (upstream from the road crossing) and Bluemount Rd. station (downstream from the road crossing). Each survey team walked parallel paths, in an upstream zig-zag direction, so the river bottom across the entire channel width could be visually examined for didymo presence. Percent visible bottom coverage by didymo at each station was estimated by the survey teams and scored as follows: 0 = no visible didymo growth, 1 = very sparse to sparse bottom coverage less than or equal to 20%, 3 = moderate coverage greater than 20% but less than or equal to 60%, and 5 = abundant coverage equal to or greater than 60%. In addition, about 10 substrate samples were collected from each surveyed river section, transported to the laboratory inside labeled zip-top bags on ice in an insulated cooler, and kept in the cooler until they were examined microscopically for the presence of didymo cells within 24 hours after collection--usually within 15 hours.

To ensure that the survey teams did not transport didymo cells from infested to non-infested areas, survey stations not infested with didymo were surveyed at the start of each sampling day, before infested stations were surveyed. In addition, boots and sampling gear used by the didymo survey teams were disinfected and/or dried for at least three weeks between monthly surveys.

In addition to the visual substrate examinations, water temperature, current velocity, and turbidity were measured at each station along the Gunpowder and at the Little Falls

reference station during each monthly survey. There are two USGS stream gages operating on the Gunpowder: at Falls Rd. near Parkton, Maryland (USGS number 01581920) and at Glencoe Rd. (USGS number 01582500). Additionally, DNR has a long-term water quality monitoring station at the Glencoe Rd. bridge where an array of parameters are measured monthly. Continuously-recording water and air temperature data loggers were deployed at all didymo survey stations.

The Baltimore County survey team started collecting benthic macroinvertebrate samples at the Bunker Hill Rd. station in October 2009. Macroinvertebrate sampling continued at this station about every other month through April 2014. During several sampling events in 2009-2011, macroinvertebrate collections at the Bunker Hill Rd. station were taken with a Hess sampler (by the Baltimore County team) and with a D-net (by the DNR team). Beginning in May 2010, DNR started collecting benthic macroinvertebrates at the Little Falls reference station with a D-net, about every other month through April 2014.

Substrate composition was assessed by the DNR team at the Falls Rd., Masemore Rd., Bluemount Rd. and Glencoe Rd. stations in the Gunpowder, and also at the Little Falls reference station, in July 2012. We used a modified Wolman Walk method (Wolman 1954) to assess substrate type (characterized as silt, sand, gravel, pebble, cobble, boulder, bedrock, wood, and other) at 100 locations within each 50-m or 100-m long survey section at each station.

The most intensive survey efforts for didymo occurred in the Gunpowder, as just described. And, as mentioned briefly in the answer to Question #3, a statewide study was launched by DNR staff in 2009 with the goal of screening other trout streams in the State that could be vulnerable to didymo infestation. Six streams were screened, using a qPCR assay, in 2009, with many more streams screened in 2010, 2011, 2012 and 2015 (see Table 2).

Table 2: Sites screened for didymo using qPCR assay

SITE	sampled 2009	sampled 2010	sampled 2011	sampled 2012	sampled 2015
Antietam Creek		y	y	y	y
Bear Creek	y	y	y	y	y
Beaver Creek		y	y	y	y
Bee Tree Run		y	y	y	y
Big Hunting Creek			y	y	y
Casselman River	y	y	y	y	y
Catoctin Creek		y	y	y	y
Deer Creek		y	y	y	y
Fishing Creek		y	y	y	y
Gunpowder Falls	y			y	y
Jones Falls		y	y	y	y
Little Falls		y	y	y	y
Little Gunpowder Falls		y	y	y	y
Little Hunting Creek		y	y	y	y
Little Seneca Creek		y	y	y	y
Morgan Run		y	y	y	y
North Branch Potomac River	y	y	y	y	y
Patapsco River		y	y	y	y
Patuxent River		y	y	y	y
Savage River Reservoir	y	y	y	y	y
South Branch Patapsco River		y		y	
Upper Savage River		y	y	y	y
Youghiogheny River	y	y	y	y	y

A total of 91 sites in 23 streams were sampled and assayed for didymo DNA using a qPCR assay. Streams were sampled by DNR staff and assayed in multiple years.

In a separate but relevant study, Keller and Hilderbrand (2015) sampled 76 Maryland stream sites in March through May 2014. Their study had two main objectives: 1) Determine the spatial extent of the didymo infestation in Maryland streams using environmental DNA (eDNA)-based assays that are specific to the presence of didymo DNA sloughed off into their environment, and 2) Test how the presence/absence of didymo is related to native stream biodiversity, land use, urbanization, and water quality measures. Three of their sampling sites were in streams/rivers known to have didymo blooms: the Gunpowder, Big Hunting Creek, and the lower Savage River. Their study obtained positive results for didymo at only two of the 76 sampled sites: the Gunpowder and lower Savage River. Hence, Keller and Hilderbrand concluded that didymo "*is not currently widespread* [in Maryland], *nor is it naturally occurring throughout the region at background levels that are detectable by our qPCR assay.*" These results lend support to DNR's current position that didymo is not native to Maryland waters.

DNR staff also visually surveyed the following streams and collected substrate samples that were microscopically examined for didymo cells in the laboratory: the lower Gunpowder below Loch Raven in August 2008 (negative for didymo) and again in July 2009 (negative), Cunningham Falls Creek (above the falls and reservoir) and two sites in Big Hunting Creek (below the reservoir) in May 2011 (all negative), Cunningham Falls Creek and Big Hunting Creek again in November 2011 (both negative), Little Hunting Creek (off Catoctin Hollow Rd) in December 2011 (negative), Fishing Creek (off Mountaindale Rd. above the reservoir) in December 2011 (negative), Cunningham Falls Creek and Big Hunting Creek again in December 2011 (both negative), Big Hunting Creek again in May 2012 (positive for didymo) and in November 2012 (negative), two sites in the Patuxent River below Brighton Dam (about 100 m below the dam and downstream from Haviland Mill Rd.) in April 2013 (both negative), and again in Big Hunting Creek (below the reservoir) in July 2013 (negative).

Statewide Ban on Felt-Soled Wading Boots

Because of concerns about introducing and spreading harmful organisms like whirling disease and chytrid fungus, DNR field crews stopped using felt-soled wading boots in 2007---before didymo was first discovered in Maryland. In 2008, soon after didymo was confirmed in the Gunpowder, the ISMT started talking about a possible statewide ban on felt-soled boots. When didymo was confirmed in the lower Savage River in June 2009, DNR began planning for a ban on 'felts', and the agency decided that the most responsible action was to apply The Precautionary Principle. Simply stated, The Precautionary Principle says that when there are threats of serious or irreversible damage to public health or the environment, lack of full scientific certainty should not be used as a reason for postponing cost-effective measures that could prevent the damage. In the summer of 2010, DNR requested public comment on a draft felt-soled boot ban. After clarifying and refining the language, the ban took effect on March 22, 2011, and is worded as follows: *"An individual may not use footgear with external felt soles in State waters or within five feet of State waters. ('Felt sole' means a sole to which felt or any other natural or synthetic material capable of absorbing liquid is attached.)"*

Maryland was the first state to ban felt-soled wading boots, followed closely by Vermont on April 1, 2011. As of February 2015, five additional states have enacted bans on the use of porous-soled footgear: Alaska, Missouri, Nebraska, Rhode Island, and South Dakota. Several other states encourage but don't require anglers to use boots with non-porous soles.

During the first year or so after Maryland enacted the ban, DNR's enforcement officers issued warnings rather than citations, and also passed out educational cards to help the angling public understand the reasons for the ban. Most Maryland anglers seemed to accept what DNR was trying to achieve with the ban on 'felts' and why, even though to comply with the ban meant that they might have to buy a new pair of boots. But, as expected, some anglers were less than supportive of the ban, at least initially. Their reactions were similar to the five stages of grief, as described by Felicity Barringer in her August 16, 2010, article in the New York Times.

1. Denial: *The science that says 'felts' can absorb and harbor live didymo cells, and carry them from stream to stream, is wrong.*

2. Anger: *Why should I fall on my butt for the good of the environment?*

3. Bargaining: *I will use my felt-soled boots only in my favorite stream and nowhere else.*

4. Depression: *I can't afford to discard a perfectly good pair of felt-soled waders and buy a new hard-sole pair.*

5. Acceptance: *OK, I'll go felt-less if I must, but I won't like it.*

We also heard about other more rebellious reactions from a few anglers. Some said they won't give up their 'felts', but will just pay the fine if they get caught. The fine for a felt-sole violation was $125 in April 2011 and still is.

The leaders of several angler groups across Maryland understood and accepted the 'felts' ban from the outset, and helped garner support for the ban from their membership. One example of such stakeholder support occurred on the Gunpowder, where didymo was first discovered in Maryland. Theaux LeGardeur, owner of a fly-fishing shop and guide service in Monkton, and the Gunpowder Riverkeeper, was an important ally of DNR in gaining acceptance of the ban in the trout fishing community. He also recruited a group of volunteers to help set-up, maintain, and repair wader wash stations along the river. LeGardeur is continuing to support DNR's efforts to stop the spread of didymo from the Gunpowder. In an email dated 4/14/16, he told Klauda that he and his volunteers rebuilt four wader wash stations this year and they are still maintaining 12 wash stations. He also said that it "*amazes me that so many newcomers to the shop associate the Gunpowder with didymo--especially out of state anglers. I think it is a testament to how seriously and thoughtfully MDDNR took the threat early on. It has surely impacted our winter nymph fishing but the fish are healthy.....we still have plenty of insects and the* [felt] *sole ban and the wash stations are working to contain the spread into other waterways."* Similar informal partnerships between DNR and trout anglers were formed around the didymo threat across the State.

Question #7: What have we learned about didymo ecology in Maryland waters?

As mentioned above and described in the answers to Question #6, discovering a didymo bloom for the first time in Maryland in early 2008 triggered the implementation of a monthly survey in the Gunpowder that started in July 2008 and ended in June 2014. The major objectives of this survey were to determine the spatial extent of didymo distribution and describe seasonal abundance patterns. A one-time characterization of substrate composition was completed at five survey stations to shed light on preferred habitat conditions for didymo. Ancillary water temperature, current velocity, and chemistry data were also collected during the monthly surveys to better understood the environmental conditions associated with peak bloom periods. What we know at this time about didymo ecology in

Maryland comes primarily from six years of monthly surveys in the Gunpowder.

Although this is the only report that attempts to describe and discuss all of DNR's reactions and responses to didymo infestations in Maryland, six posters were also prepared to communicate survey findings at scientific conferences. Highlights from these posters are provided below. For more details and to see maps, photographs, data tables, and graphs included in these posters, go to the links provided below.

1. *Just Say 'NO' to Didymo: A Collaborative Survey of 'Rock Snot' Occurrence in the Gunpowder Falls, Maryland*
   [https://drive.google.com/open?id=0B684NRP4uOmfcEZYSE83WU0yWXc]

This poster reported on the results of monthly surveys in the Gunpowder conducted between July 2008 and March 2009. Didymo was found at all nine surveyed stations, with the highest abundances (expressed as mean monthly bottom coverage scores, 0-5) observed at the three most upstream stations closest to Prettyboy Dam: Falls Rd. (3.5), Masemore Rd. (3.1), and Bunker Hill Rd. (2.1). Some didymo growth was observed at the three most downstream survey stations (Glencoe Rd., Sparks Rd., Phoenix Rd.), but mean monthly bottom coverage scores were very low (0.1-very sparse), probably due mostly to the dominance of silt, sand, and gravel substrates at these three stations. We found didymo present during each monthly survey between July 2008 and March 2009. But didymo was most abundant and in bloom condition during February and March 2009.

River discharge measured at the USGS gaging station at Glencoe Rd. was relatively low and stable during February and March 2009. Water temperatures during these two months of didymo blooms at the three most upstream stations ranged from 4.0 to 6.2 C. The middle Gunpowder, where the nine didymo survey stations were located, was slightly alkaline (mean pH = 7.7) between July 2008 and March 2009, well oxygenated (mean D.O. = 10.6 mg/L), clear (maximum turbidity = 1.7 NTU), and borderline mesotrophic (mean total nitrogen = 2.59 mg/L, mean total phosphorus = 0.02 mg/L). Summer temperatures at the three most upstream stations with the highest didymo abundances were below 16 C. At the most downstream survey stations with the lowest didymo abundances, peak summer water temperatures were higher, between 18 and 21 C.

2. *Rock Snot Revisited: An Update on the Presence of Didymo in Maryland*
   [https://drive.google.com/open?id=0B684NRP4uOmfZ2Q3ZFVLTEpQeUU]

This poster reported on the results of monthly surveys conducted in the Gunpowder between July 2008 and March 2010. This poster also reported that didymo was confirmed at two sites in the lower Savage River, below the reservoir, in June and November 2009. A map showing the locations of 34 wader wash stations (as of 3/12/10) was presented. Based on 20 months of survey data, didymo abundance in the Gunpowder was highest at the three most upstream stations and lowest at the most downstream station. Didymo abundance was highest in February through May. Average bottom coverage scores for didymo blooms during the winter months at the six most upstream stations were lower during the second

year of the survey, perhaps due to higher current velocities, generally higher monthly peak river discharge, and somewhat warmer water temperatures in the fall months. Benthic macroinvertebrate data collected at the Bunker Hill Rd. station in the Gunpowder in October and December 2009 were summarized and presented in this poster.

3. *Three Years After Didymo Infested the Gunpowder Falls, Maryland: Any Apparent Impacts?* [https://drive.google.com/open?id=0B684NRP4uOmfTzRpb1Z3ZXlJWHc]

This poster reported on the results of monthly surveys conducted in the Gunpowder between July 2008 and February 2011. The statewide ban on felt-soled wading boots that became law in Maryland on March 22, 2011, was mentioned in this poster. The most abundant didymo blooms in the Gunpowder consistently occurred from January through April, when water temperatures were lowest, below 8 C. Monthly winter to spring water temperatures were similar across all nine survey stations downstream from Prettyboy Reservoir. Didymo was most abundant at the three upstream stations (Falls Rd., Masemore Rd., Bunker Hill Rd.), with didymo abundance at the Bluemount Rd. station during the third year of the survey being similar to abundances at the Falls Rd. and Masemore Rd. stations. Didymo abundances appeared to be higher during the first year of the survey (July 2008-June 2009), compared to the second and third years. One plausible partial explanation is that current velocities were higher during fall, winter, and spring at all stations during the second year of the survey. High current velocities could scour less stable substrates and thereby reduce didymo abundance.

This poster also reported on information gleaned from two benthic macroinvertebrate data sets for the Gunpowder: (a) several years of data collected pre-didymo infestation (1986-2006) and two years of post-didymo infestation data (2008-2009), and (b) 13 months of data (October 2009-January 2011) collected at the Bunker Hill Rd. station and four months of data (October 2010-January 2011) collected at the didymo-free reference stream, Little Falls. Several benthic macroinvertebrate metrics calculated from these data sets were highly variable between 1986 and 2009. We found no evidence of any changes in these data sets that could be associated with the didymo infestation. For the shorter time series (2009-2011), EPT taxa appeared to be more dominant at the reference stream, while Chironomidae taxa were more dominant at the Bunker Hill Rd. station. Additional analyses of the benthic macroinvertebrate collected from 2011 through April 2014 may shed more light on the question of didymo impacts on benthos in the Gunpowder.

4. *Didymo Response to Near-Record Flows in Gunpowder Falls, Maryland* [https://drive.google.com/open?id=0B684NRP4uOmfMm1CbkpsSTdRdDA]

This poster presents the results of the didymo survey conducted in the Gunpowder between July 2008 and March 2012. In late August-early September 2011, the mid-Atlantic region was hit with a double whammy of storms. First, Hurricane Irene roared through and dumped 10.3 inches of rain on the Baltimore, Maryland area. Then along came Tropical Storm Lee and the Baltimore area received an additional 13.3 inches of rain. This combination produced the wettest two month period on record. Very high flows were

measured at several USGS gages across Maryland. Freshwater flows into the Chesapeake Bay were the highest for any September on record. Flows during September in the Gunpowder reached record highs, with peak discharges on September 8, 2011, of 9000 cfs at Falls Rd. and 13,000 cfs at Glencoe Rd.--by far, the largest river discharges to occur there since the monthly didymo surveys began in July 2008. Flows were also well above normal during November and December 2011. The periods of record are 2000 to the current year for the USGS gage at Falls Rd. and 1982 to the current year for the gage at Glencoe Rd. These very high flows in the Gunpowder from September through December 2011 gave us the opportunity to see how didymo would respond.

For five months after the high flow event in September 2011, we observed very low didymo abundance at all survey stations. But when we surveyed the Gunpowder in February 2012, a large didymo bloom was underway. Then in March, an even more abundant didymo bloom was observed---one that exceeded any blooms we had observed in previous survey years. The monthly didymo surveys conducted by DNR have shown that didymo abundance in the Gunpowder typically peaks between January or February and May. So, the didymo bloom pattern we observed in early 2012, five months after near-record flows, was what we could expect in a typical year but was surprising after the flood event. Hence, the effects of the record scouring flows during September 2011 on didymo abundance were only temporary and certainly did not eliminate this benthic diatom from the Gunpowder.

5. *Statewide Occurrence and Seasonal Abundance Patterns for Didymo in Maryland Waters* [*https://drive.google.com/open?id=0B684NRP4uOmfcjhBNzAzVUZnMGs*]

Although the monthly didymo surveys in the Gunpowder continued through June 2014, this is the last poster prepared so far that reports on the statewide distribution of didymo and summarizes what we learned about seasonal abundance patterns since 2008. This poster was presented at the International Didymosphenia geminata Conference held on March 12-13, 2013, in Providence, Rhode Island.

This poster includes a map that shows the stream/river sites across Maryland that were sampled to learn if didymo was present. To date, didymo blooms are still being observed in three Maryland rivers: Gunpowder Falls, lower Savage River, and the North Branch Potomac River. As mentioned above, a didymo bloom occurred in Big Hunting Creek in late winter-early spring of 2012. But no blooms have been observed there since.

Based on water temperature measurements collected by an in situ data logger (HOBO Water Temperature Pro v2 Data Logger - U22-001) from mid-May through mid-November 2012, it appears that Big Hunting Creek became too warm for didymo to survive and thrive beginning in late May and extending through the summer into mid-October (Table 3). The upper temperature tolerance of didymo can be variable, but peak biomass (i.e., a bloom) typically occurs only when and where water temperatures do not exceed 18 C. Water temperatures were consistently above 18 C in Big Hunting Creek from May 25 through June 4, with temperatures also exceeding 20 C during that time period. Water temperatures also exceeded 18 C a few times in late June to early July. From mid-July

through September, water temperatures were almost always above 18 C, frequently above 20 C, and sometimes above 22 C--with a peak temperature in late August of almost 25 C. Anyone interested in receiving a plot of the water temperature readings in Big Hunting Creek from mid-May through mid-November 2012 that are summarized in Table 3 should contact co-author Hanna (katherine.hanna@maryland.gov).

In Gunpowder Falls, where we documented didymo blooms every year from 2008 through 2014 at three survey stations in the Prettyboy Reservoir tailwater, water temperatures never exceeded 16 C. The locations of all didymo blooms are downstream from impoundments in areas that support tailwater trout fisheries. Areas downstream from dams also had a higher frequency of didymo blooms in the Red Deer River, Canada (Kirkwood et al. 2009). So far, less than 4% of Maryland's wild trout stream miles are infested with didymo. But the places that are experiencing didymo blooms are important trout waters of the State.

Table 3: Summary of water temperature readings collected by in situ data loggers in Big Hunting Creek from mid-May through mid-November 2012

Month	Number of Water Temperature Readings	Average Water Temperature (C)	Percent of Readings ≥ 18 C	Percent of Readings ≥ 20 C	Percent of Readings ≥ 22 C
May (partial)	1188	17.3	54.5	6.5	0
June	2160	16.7	17.2	0.5	0
July	2232	17.4	33.0	3.8	0
August	2232	19.9	98.6	44.3	2.3
September	2160	19.5	77.8	40.9	5.3
October	2232	14.4	5.4	0	0
November (partial)	1410	7.8	0	0	0

In the Gunpowder, didymo occurs from the dam on Prettyboy Reservoir downstream to at least the Phoenix Rd. bridge (the most downstream station that was regularly surveyed for several years), a distance of 26 river km. However, nuisance-level blooms are confined to the upper 12 km of the river, from the dam downstream to Bluemount Rd. The upper most station at Falls Rd. consistently had the highest didymo abundance of the nine stations that were regularly surveyed. Beginning in September 2009, monthly surveys were also conducted at one station in the Gunpowder just upstream from Prettyboy Reservoir, at the Gunpowder Rd. bridge. We never confirmed didymo at this station.

Substrate size, composition, and stability appear to be important factors in determining where didymo will establish and flourish. The substrate assessment that Klauda and Hanna conducted at four survey stations in the Gunpowder plus the Little Falls reference stream in July 2012 supported our observations that didymo prefers larger, more stable cobble, boulders, and bedrock---all substrate types that are dominant at the Falls Rd., Masemore Rd., and Bluemount Rd. stations. At the station where didymo was consistently most abundant

(Falls Rd.), substrate composition was 32% cobble/boulders/bedrock, 28% pebbles, and 26% gravel. By comparison, at the Glencoe Rd. station that was further downstream and where monthly didymo abundances were consistently low, silt/sand/gravel made up 79% of the substrate, much less stable, with only 18% pebble/cobble/bedrock.

Peak didymo blooms in the Gunpowder occurred between February and May, although didymo cells were found on substrate samples collected during all months at the nine survey stations. Didymo abundance tended to increase following rapid declines in water temperatures in November through February. Abundance then decreased as water temperatures warmed in March through June. The lowest didymo abundances in the Gunpowder were consistently observed from July through October or November. The months of peak didymo abundance also coincide with those months when deciduous trees on the river bank have dropped most or all of their leaves. As a result, the river channel becomes less shaded and light availability in the water column and on the stream bottom should increase----compared to the summer months when channel shading is at its peak and didymo abundance is low. So it's likely that water temperature and light availability, in addition to other possible factors, play important roles in determining when didymo blooms occur.

6. *Didymosphenia geminata in Maryland Trout Streams: Is Extreme Phosphorus Limitation a Necessary Condition for Seasonal Bloom Formation?*
   [https://drive.google.com/open?id=0B684NRP4uOmfaVJlRkZOZVNVeGM]

This poster presented the results of analyses of soluble reactive phosphorus or SRP data (measured as orthophosphate in field-filtered water samples) collected monthly between 2006 and 2013 in the Gunpowder, Savage River, and North Branch Potomac River. These three Maryland rivers were and still are infested with didymo. The objective of this poster was to explore the hypothesis that extreme phosphorus limitation is a trigger for stalk growth and mat development that characterize nuisance didymo blooms. The analyses showed that didymo blooms can form in Maryland waters when SRP concentrations typically exceed 2 ppb. Like several other studies have shown, low phosphorus levels are one key requisite for a didymo bloom in Maryland waters. But, unlike the reported situation in New Zealand rivers (Bothwell et al. 2014), extreme phosphorus limitation into the ultra-oligotrophic (0-4 ppm total phosphorus) does not appear to be a necessary condition for seasonal bloom formation in Maryland rivers.

This conclusion was confirmed by two recent studies in other Mid-Atlantic watersheds. Silldorff and Swann (2013) surveyed the Delaware River between October 2012 and May 2013 to document the phenology of didymo blooms and evaluate the effects of different nutrient regimes on stalk formation and bloom development. Areas of the river with stalk formation were generally associated with SRP concentrations below 10 ug/L. But they also observed didymo stalk formation and blooms in areas where the SRP concentrations were well above the 2 ug/L threshold reported for New Zealand (Bothwell et al. 2014). We agree with the conclusion by Silldorff and Swann (2013) that variations in phosphorus concentrations may not completely, or may not alone, drive the dynamics of didymo blooms.

Shank et al. (2016) reached similar conclusions from their studies in Pine Creek and five other Pennsylvania watersheds where didymo was detected. In the tailwaters of three regulated, hypolimnetic release streams, they found didymo growing where SRP concentrations ranged from 5.1 to 10 ug/L. What we observed in the Gunpowder and what these other investigators observed in the Delaware River and six Pennsylvania watersheds show that streams in the Mid-Atlantic region of the U.S. are different from streams in New Zealand. So it should not be surprising that we see phosphorus thresholds for didymo stalk formation that are higher than those reported for New Zealand streams.

In addition to these six posters, an invited talk titled "*Didymo Infestation in Maryland, USA: A State Agency's Reactions, Responses, and Results*" was presented by Klauda in a panel discussion that was part of the International Didymosphenia geminata Conference in March 2013. [*https://drive.google.com/open?id=0B684NRP4uOmfUFNtNTdPNG1oakk*]

We did not routinely measure the thickness of didymo mats during the monthly surveys in the Gunpowder. But at 1130 on 3/22/15, during a didymo bloom in the Gunpowder at Falls Rd., Klauda measured mat thickness at 12 locations in the river channel, on cobble and boulder substrates, distributed along the left bank, mid-channel, and right bank. The river was running clear at 157 cfs and the water temperature was 5.0 C. The range of didymo mat thicknesses was from 2 to 14 mm, with average and median thicknesses of 6.3 mm and 5 mm.

Question #8: Is there evidence that didymo is causing ecological or economic impacts in Maryland?

Between July 2008 and June 2014, DNR staff and our partners conducted six years of monthly didymo surveys and collected about four years of benthic macroinvertebrate data in the Gunpowder, conducted screening sampling/qPCR assays at 91 sites in 22 streams/rivers, and intermittently checked the status of didymo infestations in Big Hunting Creek and in the Savage and North Branch Potomac Rivers. Separate from this didymo-focused effort, DNR staff also conducted fisheries surveys and collected benthic macroinvertebrate samples in the Gunpowder, Savage, and North Branch Potomac. We looked at all these data for evidence of any ecological or economic impacts of didymo infestations in Maryland waters. So far, no evidence of major impacts have emerged.

The time series of benthic macroinvertebrate data collected in the Gunpowder before didymo was first reported there in early 2008 and since then, using comparable sampling methods and also including a non-didymo infested reference stream, is not yet long enough to yield any meaningful insights into possible didymo effects on aquatic food webs. Hopefully, benthic macroinvertebrate sampling at the Bunker Hill Rd. survey station in the Gunpowder and also at the Little Falls reference stream will be continued at least annually for many years. Future analyses of these data will shed more light on this important ecological impact question.

From a trout perspective, the available data for addressing this ecological impact question are most abundant for the Gunpowder. The upper 12 km of the river below Prettyboy Reservoir is managed as a catch-and-release, wild brown trout, tailwater fishery. DNR staff have been collecting annual data on trout population numbers and recruitment each fall since 2001 at three locations in the Gunpowder that have been infested with didymo since 2008: Dam/Falls Rd., Masemore Rd., and Bluemount Rd. If there are any adverse impacts from didymo blooms on brown trout, the earliest life stages should be the most vulnerable. Young-of-year brown trout densities (numbers per hectare) in the Gunpowder have fluctuated since 2001 (see Table 4).

Table 4: Young-of-year brown trout densities and confidence intervals (number/hectare +/- 95% CI) for the Dam/Falls, Masemore, and Bluemount electrofishing stations in the Gunpowder Falls tailwater, 2001-2015

Year	Dam/Falls	Masemore	Bluemount
2001	31+/-14	410+/-116	253+/-4
2002	365+/-37	1447+/-50	283+/-147
2004	208+/-57	536+/-28	No Sample
2005	289+/-650	1284+/-54	634+/-125
2006	371+/-124	1189+/-49	193+/-13
2007	925+/-111	811+/-83	67+/-16
2008	509+/-77	1296+/-76	131+/-43
2009	44+/-13	505+/-49	229+/-53
2010	31	289+/-53	421+/-34
2011	0	75+/-8	77+/-17
2012	245+/-20	502+/-11	131+/-12
2013	19	235+/-76	156+/-14
2014	88+/-9	808+/-32	243+/-10
2015	358+/-91	1573+/-155	200+/-15

But there is no discernable declining or increasing trend since 2008 at any of these three sampling stations. On 1/15/16, Mark Staley (DNR's Central Region Manager, Fisheries Service) told Klauda that there is "*Still no 'smoking gun' in the* [Gunpowder Falls] *trout data that point to didymo having an impact in a positive or negative way in 2015. Trout reproductive success reached levels not seen since 2008 at the Dam/Falls station and Masemore station."* Given the 'noise' of annual variability in brown trout reproduction in the middle Gunpowder between 2001 and 2015 (49-fold at the Dam/Falls station, 20-fold at the Masemore station, and 9-fold at the Bluemount station), it will require many more years of data to see if any strong 'signal' of didymo effects on brown trout in the Gunpowder emerges.

Personal communications between Klauda and Alan Klotz (DNR's Western Region 1 Manager, Fisheries Service) on 3/9/15 and 2/11/16 revealed no clear evidence that didymo has had any effects (negative or positive) on Maryland's two western rivers where didymo

blooms are occurring: the lower Savage River below the reservoir and in the North Branch Potomac River from just downstream of the Jennings Randolph Dam for about a mile to Barnum. According to Klotz, "*The North Branch Potomac has very little natural* [trout] *reproduction in our sample station close to the tail race downstream of Jennings Randolph dam. In the Savage, poor year classes* [of trout] *have been correlated with the number of flow events > 800 cfs during the critical egg/fry stage (as well as the draining of the reservoir and associated sediment deposits* [downstream] *in 2009."* Klotz also stated that the frequency and magnitude of flows in the Savage River during the critical period for trout (between October and June) appear to be much more important for determining reproductive success than didymo blooms.

So what are the anglers seeing and saying about didymo in Maryland waters? In heavily-infested waters, didymo blooms could cause declines in freshwater angling--particularly fly fishing for trout, a $0.9 billion industry in the United States, avid anglers spend a lot of time fishing in their favorite trout streams. They see what's happening more regularly than do DNR staff. So the observations of anglers who have been fishing Gunpowder Falls for many years, before and after didymo arrived, are important.

Theaux LeGardeur, trout angler, owner of a tackle shop and fishing guide service in Monkton, Maryland (near the Gunpowder) and the Gunpowder Riverkeeper, told Klauda the following in January 2013: *"The good news is that the river* [Gunpowder Falls] *is intact, we have not lost any measurable insects hatches and the wild fish are getting along fine."* More recently (on 4/14/16), he told Klauda that didymo blooms are still occurring and "*Fishing activities* [in the Gunpowder] *are impaired especially in low flows and when the* [didymo] *blooms are most pervasive."*

From the perspectives of Jeff Lewatowski, a fishing guide on the Gunpowder who has been fishing this river for 20+ years, Klauda heard this in January 2013: *"From a non-scientific standpoint, in late winter and early spring, when it* [didymo] *blooms, it's only a nuisance when nymph fishing subsurfaces."* On 4/15/16, Lewatowski agreed with Le Gardeur's statement that didymo blooms are still occurring and also told Klauda that, *"I find that the insect hatches have been as good or even better than when didymo first appeared. I personally believe that the mayfly nymphs use the algae as habitat. I find that looking at rocks in the stream in spring the nymphs are actually living on top of the rocks within the algae* [didymo]. *Short of it being a nuisance, I have not recognized any visible affect to the fishing or stream health."*

Micah Dammeyer, another fishing guide on the Gunpowder, told Klauda on 4/14/16 that, "*I would say that the* [didymo] *blooms I see are about the same in density* [as 2008-2014] *or somewhat less intense.* He also said, "*It's hard to tell if it's the didymo or other influences like major storm-spillover* [from Prettyboy Reservoir] *that have changed the hatches. While the storm that hit in 2011 cleaned a lot of the didymo out, it also shifted the gravel bed and removed much of the beneficial detritus from the river. In any case, the hatches have diminished slightly though some anglers I encounter would say that the decline has been great. I think* [their] *opinion is biased. I have seen fantastic hatches some days and other days couldn't buy a mayfly. I think, as anglers, our memories always drift to a boom time."*

On 2/11/16, Klotz replied to Klauda's questions about didymo-related reports from trout anglers who fish the lower Savage River and stated, "*I can't say that I have received complaints from*

anglers about poor fishing or insect hatches. The biggest complaint I get regarding didymo in the lower Savage River tail-water is the fouling of nymphs or wet flies by fly fishing anglers."

So, it would appear that didymo blooms in Maryland are, so far, a seasonal nuisance to trout anglers but not having any discernible effects on the benthic macroinvertebrate assemblages or the trout populations. We don't know if the didymo nuisance to trout anglers is causing any economic impacts. If the nuisance level increases to the point where anglers avoid their favorite trout streams or are forced to use dry flies instead of nymphs and wet flies during peak didymo blooms (late winter to early spring), then it's possible that some adverse economic impacts could result.

Question #9: What options are available to DNR for managing didymo blooms?

The ideal option for dealing with a non-native, potentially-invasive species like didymo in Maryland would be to prevent its introduction. But with its discovery in the Gunpowder in early 2008, that option went away. DNR had to shift to public education/outreach efforts and promulgating new regulations, as described above, in an effort to stop or slow the dispersal and introduction of didymo into non-infested waters. An eradication option was considered by DNR staff, but quickly rejected as not possible. We could find no reports that didymo has ever been successfully eradicated once it infested a natural water body. Management actions taken by DNR to stop or slow dispersal and new introductions included the design and implementation of monitoring efforts (also described above) to learn more about the statewide distribution of didymo and its potential environmental and economic impacts to assess the effectiveness of public education/outreach efforts and new regulations.

Are there any other management actions that DNR should have taken or could take in the future to deal with didymo? Our survey results and the scientific literature indicate that didymo blooms most often occur below dams/impoundments, in clear regulated rivers where temporal variations in flows and temperatures are dampened. These areas also have low nutrient levels (especially phosphorus), high light levels, and coarse substrates. Such 'ideal' conditions raise one obvious question: Could well-timed flushing flows that mobilize the stream bed be used to reduce didymo blooms to levels that pose lower threats to the aquatic community and anglers?

In late August-early September of 2011, Mother Nature provided some answers to this question; i.e., a field test of the hypothesis that flushing flows in the Gunpowder would have no measurable effects on didymo abundance. Hurricane Irene and Tropical Storm Lee gave Maryland a 1-2 punch and together dumped about 24 inches of rain on the watershed. Record flood flows were recorded in the Gunpowder in early to mid-September. For five consecutive months after this high flow event, we saw very low didymo abundance in the Gunpowder. But when we surveyed the river in February 2012, didymo was blooming again and heavily. This bloom persisted through April 2012.

Could a second flushing flow event, either from another storm event or an intentional release of water from Prettyboy Reservoir in December 2011 or January 2012, have prevented or at least dampened the abundant didymo bloom we observed and documented in February through April 2012? Possibly, but another large storm event did not occur and DNR would not have allowed an intentional flushing flow from Prettyboy Reservoir during fall-winter because that's when brown trout eggs are incubating in the gravel.

So, for now, the use of flushing flows is not likely to become a viable management option to control didymo blooms in the Gunpowder or in any other tailwater river in Maryland where didymo occurs. Even if such a control strategy was desirable, more information is needed on the critical flow requirements and shear stress resistance of didymo blooms. Two studies that investigated hydrodynamic control of didymo mats were recently published (Cullis et al. 2013, 2015). They found that didymo is well adapted to survival in high-shear environments and not easily removed by flood flows.

Could water temperatures be manipulated to depress didymo blooms in areas downstream of dams? The upper temperature tolerance of didymo appears to be variable, but peak biomass typically occurs when water temperatures do not exceed 18 °C. Our observations of one late winter-early spring didymo bloom in Big Hunting Creek in 2012 that has not reoccurred since (see page 23) supports an 18 C water temperature threshold for bloom formation. If reservoir releases were managed to maintain downstream water temperatures above 18°C or preferably 20°C during the summer and thereby exceed the preferred range for didymo, nuisance blooms would probably not occur often, if at all. However, the areas in Maryland where didymo is known to occur and bloom are being maintained as tail-water trout fisheries. So, summer water temperatures cannot be allowed to exceed 20°C. Therefore, water temperature manipulation is not a viable management option to control didymo in Maryland.

What about increasing phosphorus levels in didymo-infested waters to reduce or even eliminate blooms? This question was addressed in a study conducted during 2007 and 2008 in a tail-water section of Rapid Creek, an oligotrophic stream, in South Dakota (James et al. 2015). They added 6 ug/L of phosphorus (P) and observed a significant decrease in didymo biomass up to only 0.6 km downstream of the P-addition point. Blooms of other algal groups did not occur in the P-augmented areas; i.e., no undesirable eutrophication effects were observed. P additions did not eliminate didymo from the manipulated stream and didymo biomass was reduced only as long as P augmentation occurred. James et al. (2015) concluded that this approach is not a long-term solution to eliminating didymo bloom formation. But it may offer some hope for at least a temporary mitigation option in a relatively localized area of didymo infestation.

Could reducing light availability, another key environmental requisite for didymo proliferation, offer a feasible management option to reduce stalk growth and bloom formation? The roles of turbidity, suspended sediments, and shading in limiting the growth, density, and distribution of didymo are not well studied nor understood. In Maryland's Gunpowder Falls, didymo blooms typically appear in February and then essentially disappear in late April or mid-May, when leaf out is at or near 100% and canopy cover is at or near the

seasonal maximum. James et al. (2014) reported that didymo bottom coverage in Rapid Creek, South Dakota, was inversely related to canopy coverage. Lindstrom and Skullberg (2008) also reported that didymo appears to depend on unshaded habitats to thrive in Norwegian rivers. Maintaining healthy riparian corridors with large shoreline trees should increase shading and help prevent or at least minimize didymo establishment and proliferation in narrow to moderately-wide rivers and streams.

Two studies by Kirkwood et al. (2007) and George and Baldigo (2015) found that moderate to high levels of turbidity (4.3 - 119.5 NTUs) and suspended sediments (3.0 - 136.0 mg/L) likely restricted didymo growth in the Red Deer River (Alberta, Canada) and in the upper Esopus Creek (New York, USA). From a management perspective, we would not recommend intentionally increasing either turbidity or suspended sediments in a stream to reduce problematic didymo blooms. But knowing the levels of light availability in streams and rivers from measurements of turbidity and suspended sediments could help resource managers predict where didymo will or will not likely thrive.

Are there any effective, safe, and acceptable chemical or biological controls for didymo available now or in the near future? Laboratory studies conducted in New Zealand (Jellyman et al. 2010) screened ten biocides and identified four that significantly reduced didymo cell viability and showed promise of being effective during a range of contact times up to one hour and over time periods up to 15 days. None of the ten biocides caused 100% mortality of didymo cells. Gemex, a chelated copper formulation and a photosynthetic inhibitor, was the most effective biocide. A 5 mg/L solution killed 94% of the didymo cells during a one-hour exposure. Artificial stream trials (Jellyman et. 2011) revealed unacceptable effects on non-target organisms from three of the four biocides most effective on didymo. These researchers concluded that Gemex was cost-effective, easily manufactured, and algal-specific enough to warrant use in a full river trial.

Clearwater et al. (2011) reported that a one-hour pulse dose of 20 mg of copper/L (as chelated copper--Gemex) in Princhester Creek, New Zealand, significantly affected a well-established didymo infestation. The test stream had an average width of 6m and a median flow of ~0.2 m^3/s. The target Gemex dosage was achieved up to 1.5 km downstream of the injection point. They reported that the elimination of early-stage didymo infestations is possible and suppression of well-established infestations could be achieved, although repeated application of Gemex would be required. Overall, the single Gemex application had minimal long-term effects on non-target algae, invertebrates, and fishes, except for significant localized trout mortalities that occurred on the treatment day. Clearwater et al. (2011) concluded that further trials with Gemex are needed in larger rivers, on earlier-stage didymo infestations, and using repeated applications to determine how to treat didymo infestations effectively.

From a possible biological control perspective, Wood and Kuhajek at Cawthorn Institute in Nelson, New Zealand, were the first researchers to successfully culture didymo in the laboratory in 2011. Earlier, Wood and Kuhajek discovered that didymo cells must attach to a surface before they can divide. If they don't attach, they die. And without cell attachment, there is no stalk production and therefore no bloom. These researchers are hoping that

being able to culture didymo will enable them to determine if some naturally-occurring bacteria or other organisms that live in aquatic biofilms might inhibit didymo cell attachment and growth, and thereby reveal a potential biological control strategy.

In summary, the only viable management actions currently available for dealing with didymo and acceptable for use in Maryland are public education/outreach efforts and the promulgation of focused regulations to prevent its introduction and dispersal. Once didymo infests a natural water body, eradication is impossible. A few control methods aimed at reducing the severity of didymo blooms have been proposed and some of these are being studied. But their potential value as viable management tools are uncertain and remain to be seen. For most infested waters in Maryland, didymo is probably here to stay at some level of abundance for the foreseeable future.

Question #10: What questions remain unanswered about didymo in Maryland waters?

Even though the survey efforts conducted by DNR staff and their partners, plus the research projects completed to date on didymo, have certainly enhanced our understanding of this freshwater diatom's ecology in Maryland, there are still unanswered questions. The following list is not exhaustive, but includes some key questions that remain.

1. Is the didymo infestation in Maryland still in its early stages? If yes, what other management actions should be taken to control its spread?

2. Has didymo become established and is it producing blooms in the Youghiogheny River in Maryland downstream from Deep Creek Lake?

3. What are the most important habitat conditions (preferences and tolerances), other than low phosphorus, that are critical to didymo survival, growth, and bloom development?

4. Are didymo blooms in Maryland having any negative or positive effects on the biota in infested waters?

5. Are the didymo blooms that have been occurring in three Maryland rivers causing economic impacts or are they only seasonal nuisances to anglers?

6. Are the 45 or so wader wash stations deployed along several Maryland trout streams/rivers to educate anglers and encourage them to clean/decontaminate their wading boots and gear to reduce the chances for didymo dispersal being adequately maintained and are they doing what they were designed to do?

7. What additional investigations are needed (e.g., analysis of sediment cores collected from impoundments/ponds in the central and western regions) to determine if didymo is or is not native to Maryland?

8. Should a Pest Risk Assessment be conducted for didymo in Maryland, similar to what was done in Oregon in 2009, that would yield a Relative Risk Rating on a scale of 1-9 and an associated measure of uncertainty to guide future management actions?

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