



MassDEP

Massachusetts Department of Environmental Protection Division of Watershed Management

STANDARD OPERATING PROCEDURE

Field Equipment Decontamination to Prevent the Spread of Invasive Aquatic Organisms

CN 59.6
August, 2015

Prepared by:

Date:

Richard Chase, Assessment and Data
Management Coordinator
David Wong, Environmental Analyst

Approved by:

Date:

Arthur Johnson, Monitoring Coordinator

Approved by:

Date:

Kimberly Groff, Program Supervisor

* see pdf version for valid signatures

NOTE: *References to trade names, commercial products and manufacturers in this SOP does not constitute endorsement.*

List of Revisions

Revision Date	Revision	Pages #s
February, 2008	---	---
December, 2009	Added table for cleaning solutions, DCR brochures, new information regarding zebra mussels, etc.	Throughout
August, 2015	Added table for Mass AIS Waterbody List, New Invasive Species Sightings Reporting Guidance, Updated decontamination information and new references	Throughout

1.0 SCOPE AND APPLICATION

The following SOP has been developed under the assumption that Massachusetts' waterways are threatened by the spread of non-indigenous species. This SOP provides guidance to DWM field staff on the prevention and minimization of the spread of invasive aquatic organisms from one waterbody to another. It covers field and lab decontamination procedures for any equipment and gear used in streams, rivers, lakes, ponds and impoundments throughout Massachusetts **whenever any invasives have been observed or are reasonably suspected to be present and spread to non-contaminated areas by monitoring activity. A complete list of confirmed and suspected aquatic invasive species sightings by WPP and other agencies in Massachusetts can be found from the WPP Toolbox named Mass AIS Waterbody List. If a survey is going to be conducted in any of the listed waterbodies, the decontamination procedures will be implemented.**

This SOP applies to controlling the spread of invasive organisms only. For additional guidance regarding equipment decontamination procedures to prevent and minimize cross contamination of samples, see CN 59.0.

2.0 SUMMARY

By following simple **checking and cleaning steps**, the inadvertent spread of invasive organisms (e.g., Eurasian milfoil, *Didymosphenia geminata* or Didymo, zebra mussel, asian clam, others) from infected to non-infected waterbodies by the actions of DWM field staff can be avoided or minimized. Special procedures are required because infestation can occur from very small amounts of invasive material (in some cases, microscopic), which can remain viable long after being removed from the water.

3.0 SAFETY & LOGISTICAL CONSIDERATIONS

Care should be taken at all times during decontamination activities. Although these procedures do not involve the use of hazardous chemicals, gloves and safety glasses are recommended when using washing solutions. Clean tap water should be available at all times in case of splashes on skin or into eyes. For cleaning at DWM-Worcester, a shower/eyewash station is available, if necessary.

Employ a mitigation-hierarchy approach in implementing this SOP by practicing step-wise decision-making. First, seek to **avoid** contact with invasives such as Didymo and thus the need to decontaminate equipment. If avoidance is not possible, **minimize** contact with invasives and recognize where/when decontamination is required.

Avoidance of the need to decontaminate equipment in the field is preferred. This can be achieved, for example, by using equipment only ONCE at known or potentially-contaminated sites, then bringing all equipment back to the lab for cleaning prior to reuse. It is recognized, however, that this is not always possible for DWM field activities.

Because application of these procedures requires additional time for preparation, use in the field and post-survey cleanup, adequate planning is required to ensure implementation. Specifically, plan to sample known/suspected invasive sites toward the end of the survey, if possible.

4.0 DECONTAMINATION EQUIPMENT AND MATERIALS

The equipment and materials needed to ensure adequate decontamination (DECON) for invasives varies depending on the type of contaminate and the location of decontamination activity. Soaking baths are generally more effective to render organisms non-viable. Spray washing is more useful for physical rinsing off organisms.

In general, the materials that may be needed for decontamination are as follows:

Field DECON “Kit”: EACH CREW/VEHICLE

- cleaning solution bath (cooler): Solution varies (see Appendix B for options; e.g., 5% hand-dishwashing soap solution); in tub with secure lid; minimum non-displaced solution depth of 6 inches); include energy dissipation to avoid excessive splashing, if possible.
- cleaning solution spray: (in pressure sprayer#1)
- decon tubes: containing cleaning solution for unattended deployment tubes and attended multiprobe sondes
- spare cleaning solution (preferably non-P-containing, non-anti-microbial)
- rinse water spray (tap water): in pressure sprayer#2 (Use tap water spray if P-detergent or bleach is the cleaning solution)
- clean rags & paper towels
- trash bags
- PPE (gloves, safety glasses)
- wristwatch
- dedicated plastic sample container (for specimens)
- bleach (1 gallon in reserve if needed)
- portable eye wash (w/ tap water)
- quarters (\$) for manual, pressure-spray car wash

Lab Decontamination:

- hot water (>60°C or >140°F)
- cleaning solution: Varies (e.g., 5% hand-dishwashing soap solution)
- tap water rinse
- brushes, clean rags & paper towels
- trash container
- bleach
- portable pressure hose (boat/trailer washing), if available
- PPE (gloves, safety glasses)

5.0 GENERAL PROCEDURES

Checking and cleaning equipment (e.g., removing plant fragments) prior to moving to another waterbody is a recognized **best management practice** that should normally be employed whenever and wherever appropriate and practicable, regardless of whether invasives are

suspected or confirmed to be present or not. This is especially true for macroscopic plant fragments and visible organisms. The DECON procedures outlined in this SOP represent additional measures to be used when necessary.

STEP 1: Determine presence/absence potential for invasives

In the office and the field, perform background review and pre-survey field reconnaissance adequate to determine the likelihood of invasive organisms being present at each area to be visited. Agencies, watershed groups, etc. can assist in this determination. Based on the results, plan accordingly.

In the field upon arrival at each site, CHECK for the visual presence of invasive organisms in the area to be sampled. **If invasives are found or reasonably suspected to be present and spread to non-contaminated areas by sampling activity (See the Mass AIS Waterbody List in the WPP Toolbox), all activities involving contact with water must employ “DECON” procedures as contained in this SOP.** If invasives are encountered but were not expected, do not perform the activity without the proper decontamination equipment or restrict the sampling equipment to a single waterway, and then clean/dry later at the office/lab.

Example DECON approach involving “Didymo”: As of January 2008, the only watershed in MA. where Didymo has been found (albeit only out of state in upstream NH and VT tributaries) is the Connecticut River watershed. Therefore, the proposed Year-2 monitoring approach by DWM in 2008 will be to:

- Employ DECON procedures at all DWM stations visited in the Connecticut River watershed AND watch for actual Didymo outbreaks in this watershed as much as possible.
- Watch for actual Didymo outbreaks as much as possible in other Year-2 watersheds. If observed in a watershed (in MA. or upstream in other states), employ DECON procedures at all DWM stations visited in that watershed.

Any potential new invasive species observed in any site, whether it is a new site or a site has been infested by other invasive species, needs to be confirmed before it is reported to other agencies. A standard reporting protocol on invasive species sightings are described below (Please also refer to Appendix K about field sampling protocol and verification for suspected invasives).

New Invasive Species Sightings Verification and Reporting Guidance: WPP field survey crew will take an extra sample of the suspicious invasive species. If possible, a photo should also be taken to record the habitat of the suspicious invasive species. If the suspicious species is an animal the sample needs to be preserved with 50% ETOH in a container. For a plant, the sample should be collected as a whole or a segment; if plants have fruits and/or flowers, the plants need to be collected as a whole. Keep the plant sample in a bag or bucket of water at all times: don't leave them out of the water in the sun because they will wither quickly and become useless as specimens. The extra sample will be brought back to the lab and examined by at least one additional WPP biologist for verification. If the sample is not confirmed within WPP, external research experts will be

consulted until the sample is finally confirmed. Upon the final confirmation, the sighting will be added to the list of Mass AIS Waterbody List in the WPP Toolbox. This new invasive sighting will be reported to other agencies and shared with the general public. If the sample is not identified as an invasive species, the waterbody will not be added to the Mass AIS Waterbody list. Contact David Wong for AIS confirmation and Richard Chase and Art Johnson for AIS reporting. Preserve specimens for further analysis if it is needed. Destroy specimens prior to disposal as trash.

STEP 2: Assemble field DECON “Kit”

At the office, prepare the field DECON kit and include it with survey equipment inside each DWM vehicle. The field DECON kit should be available at all times in case it is needed. The cleaning solution can also be made in the field as needed.

STEP 3: Employ Check-Clean-Dry procedures (and DECON as necessary) If you are visiting waterways known or reasonably suspected of containing invasives, you must use **Check, Clean, Dry** procedures. If cleaning or drying in the field is not practical, restrict equipment to a single waterway, and then clean/dry later at the office/lab.

In general, do not wear felt soled boots/kickers or boots with porous surfaces for monitoring activity, especially where Didymo is known or suspected to be present. If it must be done for some reason, restrict felt soles (and other “porous” equipment) to a single waterway, and then thoroughly clean/dry later at the office/lab. For traction (in lieu of felt-soles), use attachable, washable cleats. If cleats are used, do not drive vehicles with them on, and make sure to decontaminate velcro straps.

Ensure that all washwater drains to isolated ground or to the sanitary sewer system. Keep washwater away from storm drains to prevent the spread of invasives (in case decontamination is not 100% effective).

CHECK: Before you arrive at and leave a river or lake site, thoroughly inspect all equipment for plant/algal fragments and debris, AND

CLEAN: Remove all plant fragments and organic debris and dispose as trash. Clean equipment using the most practical treatment for your situation (which will not adversely affect your gear). **See Appendices E-J for specific DECON cleaning procedures**, AND/OR

DRY: Drying will kill many invasive organisms, but must be complete. Slightly moist organisms may survive for months. To ensure effective drying, the item must be *completely dry* to the touch, inside and out, then left dry for at least another 48 hours before use.

STEP 4: Post-survey cleanup

Perform post-survey cleanup to ensure proper disposal of all spent cleaning solutions and to ensure clean equipment for the next users.

6.0 TRAINING

All field staff using field equipment shall be trained in this SOP. Trained staff may also train designated interns in the procedures. Training shall consist of instruction on office and field procedures, including field identification of invasives and decontamination steps. Each trainee will conduct a cleaning procedure under supervision of the trainer. Training in lab microscopy for microscopic invasives (e.g., Didymo and zebra mussel veligers) is also planned.

7.0 QUALITY CONTROL

Random interviews and audits can be performed by DWM QA staff and others to ensure compliance with this SOP.

For surveys involving sample collection, application of these decontamination procedures must not impact sample integrity.

8.0 MAINTENANCE

Survey planning, preparation, and execution with regard to decontamination procedures is the responsibility of the monitoring coordinators and survey crew leads. If problems arise with decontamination equipment or procedures, see DWM's QA Analyst.

9.0 CORRECTIVE ACTIONS

Known failures to follow this SOP will be documented and corrective actions (e.g., communication with applicable staff) taken. Adjustments to this SOP will be made as needed based on hands-on experience, improvements in procedures or the nature and extent of invasives.

10.0 POLLUTION PREVENTION & WASTE MINIMIZATION

- When possible, use prescribed tap water spray and drying procedures as an alternative to chemical use.
- Use the prescribed amounts of cleaning reagents (not more than necessary). Use non-P-containing detergent only.
- Ensure proper disposal of waste materials throughout the decontamination procedure.
- Return spent washwater solutions used in the field to the office/lab for disposal down the sanitary sewer drain.

11.0 REFERENCES

Kilroy, C. February 2005. Tests to determine the effectiveness of methods for decontaminating materials that have been in contact with *Didymosphenia geminata*; Prepared for Biosecurity New Zealand; NIWA Client Report: CHC2005-005, NIWA Project: MAF05501 National Institute of Water and Atmospheric Research Ltd; www.niwa.co.nz. 36 p.

Mass DCR and Mass DFG August 2009. Massachusetts Interim Zebra Mussel Action Plan. <http://www.mass.gov/eea/docs/dcr/watersupply/lakepond/downloads/zebra-mussel-interim-action-plan.pdf>. Accessed on August 5, 2015.

Scholl, C, 2006. Aquatic Invasive Species: A Guide for Proactive and Reactive Management. Report to Wisconsin Department of Natural Resources.
<http://dnr.wi.gov/aid/documents/ais/aisguide06.pdf>. Accessed on August 4, 2015.

Spaulding, S. Elwell, L. 2007. Increase in nuisance blooms and geographic expansion of the freshwater diatom *Didymosphenia geminata*: recommendations for response. US EPA Region 8, Denver, CO & Federation of Fly Fishers, Livingston MT. 33 p.

Special Session on *Didymosphenia geminata*, Western Division American Fisheries Society Meeting, May 15-16, 2006, Bozeman, Montana. post-meeting update.

Warrington P. 1994. Collecting and preserving aquatic plants. Environmental Protection Department, Ministry of Environment, Lands and Parks. Government of British Columbia.
<http://www.env.gov.bc.ca/wat/wq/plants/plantcollect.pdf>. Accessed on August 4, 2015.

Zook, B., Phillips, S. 2015. Recommended Uniform Minimum Protocols and Standards for Watercraft Interception Programs for Dreissenid Mussels in the Western United States. In: Biology and Management of Invasive Quagga and Zebra Mussels in the Western United States (Eds Wong, W.H., Gerstenberger, S. L.). Pages 175-204.

APPENDIX A

MASS. DCR INVASIVES BROCHURES (2010)

(excerpts)

Prevent the Spread of Zebra Mussels

A. Clean, Drain, Dry. All visible plants, mud, or other debris should be removed. All drain plugs should be pulled with any standing water allowed to fully drain. The watercraft and equipment should then be allowed to fully dry for 1 week during July and August, 2 weeks in June and September, and 4 weeks before and after these dates. Dry times should be longer if weather has been unseasonably cool or wet.

Note: Vessels that have been exposed to freezing temperatures for the winter are considered to be decontaminated.

OR

B. Clean, Drain, Decontaminate. After cleaning and draining, use an approved decontamination method or combination of methods on all vessel parts in contact with water and carpeted trailer bunks:

- **Steam or Scalding Hot Wash (>140 degrees):** To achieve this temperature at the surface being cleaned, water temperature must be as close to 155 degrees as possible at the nozzle (Note: Caution should be taken as this temperature may cause scalding to exposed skin). Keep contact for 10 seconds or more. High pressure spray is best to clean the outside surfaces of vessels and low pressure should be used to flush live wells, bilges, ballasts, and engines. Run the water through the craft's cooling system for at least 10 seconds at 140 degrees. Use "ears" for outboards, or garden hose for personal watercraft and inboards as you would normally for winterization or running while out of water.

- **Chlorine/Bleach Solution (1 oz. per gal. water):** Surfaces should be kept "wet" with Chlorine/Bleach solution for at least 10 minutes before rinsing with clean water. Clean all exterior surfaces and flush live wells, bilges, ballasts, and engines with solution. Run outboard engines in a tub, bucket or barrel of solution or use ears to decontaminate engine cooling systems. This is also a good option for soaking ropes, dive gear, or anything else that may be placed into the solution bucket or barrel. Solution may only be used for up to 24 hours after mixing. After that, a fresh chlorine mixture must be mixed.

- **Lysol (As sold, or if using the concentrate, dilute to achieve 1% active ingredient):** Surfaces should be kept "wet" with Lysol solution for at least 10 minutes before rinsing with clean water. Clean all exterior surfaces and flush live wells, bilges, ballasts, and engines with solution. Run outboard engines in a tub, bucket or barrel of solution or use ears to decontaminate engine cooling systems. This is also a

good option for soaking ropes, dive gear, or anything else that may be placed into the solution bucket or barrel. Because of the possible dilution with rinse water, the solution should not be reused. A fresh batch should be used each time if it has been diluted by the cleaning procedure.

- **Vinegar (as sold - 100%):** Surfaces should be kept "wet" with Vinegar solution for at least 20 minutes before rinsing with clean water. This option, however, may not be the most practical or feasible for decontaminating engine cooling systems because of the quantity of vinegar that one would need to purchase in comparison to preparing decontamination solutions from Lysol or bleach concentrate. Vinegar may be a more practical option for cleaning the exterior of the boat and for soaking ropes, dive gear, or anything else that may be placed into the solution bucket or barrel. Because of the possible dilution with rinse water, the solution should not be reused. Fresh solution should be used each time.

Cleaning Solutions

Disinfectant	Concentration	Contact Time
Steam/Scalding hot wash	>140°F	10 Seconds
Chlorine/Bleach Solution	1 oz. per gallon water	10 Minutes
Lysol	1% Solution	10 Minutes
Vinegar	as sold - 100%	20 Minutes
Freezing	<32°F	24 Hours



To report a zebra mussel sighting or for more information, contact Massachusetts DCR Lakes and Ponds Program at 617-626-1250 or www.mass.gov/lakesandponds

The DCR oversees 450,000 acres of parks, forests, beaches, bike trails, watersheds and dams. Its mission is to protect, promote, and enhance the wide variety of natural, cultural, and recreational resources within the Massachusetts state park system. To learn more about DCR and to discover other parks and recreational opportunities within the Massachusetts state park system visit www.mass.gov/dcr or call 617-626-1250 or write DCR, 251 Causeway Street, Suite 900, Boston, MA, 02114.

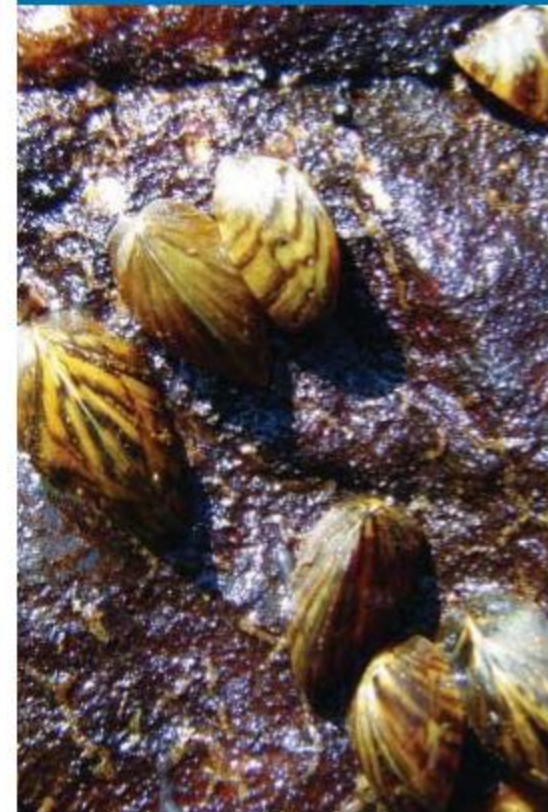
Deval L. Patrick, Governor
Timothy P. Murray, Lt. Governor
Ian A. Bowles, Secretary, EDEEA
Richard K. Sullivan, Jr., Commissioner, DCR

Cover Photo:
Tom Flannery

Printed 2010



Lakes and Ponds Program



Stop the spread of
Zebra Mussels

Dreissena polymorpha
An Invasive Mussel

ATTENTION BOATERS!

What are Zebra Mussels?

Massachusetts lakes, ponds, and streams are a valuable resource for boating, swimming, fishing, and numerous other types of recreation. These water bodies also provide a valuable habitat for a variety of wildlife, including a number of popular sportfish species. Unfortunately, the looming invasion of our waters by exotic mussels and other invasive species threatens to damage these resources.



Zebra Mussel Photo Credit: USGS

The zebra mussel (*Dreissena polymorpha*) has extended its range in the Northeast in recent years and is now found in Laurel Lake (Lee/Lenox), Lure L Brk, Housatonic River, Massachusetts; Twin Lakes in Salisbury, Connecticut; Mary Lakes & Rivers in New York; and several lakes in Vermont, including Lake Champlain, Lake Hortonia & Lake Bomoseen.



Photo Credit: USGS

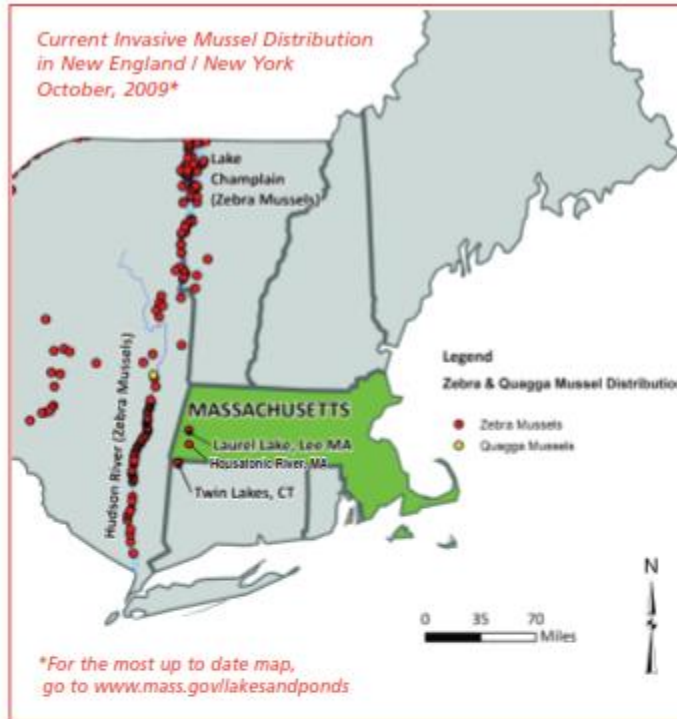
The similar but more aggressive quagga mussel (*D. bugensis*) is rapidly expanding its range and has been found as close as the Mohawk-Hudson River confluence in New York.

Boaters should use caution before launching their boat in Massachusetts water bodies. If you have been boating in western Massachusetts, New York, Vermont or Connecticut, it is especially important that you thoroughly inspect and wash your boat and other equipment following the guidelines in this brochure. Microscopic Larve (Veligers) hitchhike undetected and are a primary way mussels spread from one lake to another.



Photo Credit: Minnesota DNR

Current Invasive Mussel Distribution in New England / New York October, 2009*



*For the most up to date map, go to www.mass.gov/lakesandponds



Photo Credit: Minnesota DNR



Photo Credit: Michigan Sea Grant Archives



Photo Credit: Ontario Ministry of Natural Resources

Why should you be concerned about Zebra Mussels?

Zebra and quagga mussels are some of the most ecologically and economically damaging aquatic organisms to invade the United States. Their destructive power lies in their sheer numbers and ability to attach to solid objects – water intake pipes, propellers, boat hulls, dock pilings, submerged rocks and even other aquatic animals.

- Native mussels, fish and wildlife are threatened. Zebra and quagga mussels consume available food and alter the ecology of infested waters. They compete directly with Juvenile fish for food. They are a direct threat to native mussels, accumulating on their shells in sufficient numbers to smother their hosts. In infested waters, they can wash up on beaches, covering them with thousands of broken sharp shells.
- Boat engines can be ruined by zebra and quagga mussels growing in the cooling system intakes and blocking water flow. They can also jam steering equipment.



Photo Credit: Michigan Sea Grant Archives

How is Didymo Spread?

Anything that comes in contact with water can spread Didymo, including fishing gear, boats, live wells, bait buckets, and most frequently, felt-soled waders. Once Didymo invades a waterway, it is impossible to eliminate. **Please clean all gear that comes in contact with the water. If you cannot clean your gear, dry thoroughly before re-use or restrict gear to a single water body.**



This brochure was prepared by the Department of Conservation and Recreation Lakes and Ponds Program and the Massachusetts Aquatic Invasive Species Working Group (AIS WG). The AIS WG is a collaboration of state environmental agencies, federal agencies and non-profit organizations. This brochure is also made possible by the U.S. Fish and Wildlife Service Aquatic Nuisance Species Task Force.

For more information please visit:
<http://www.mass.gov/czm/invasives/partners.htm>

Prevent the Spread of Didymo

CHECK

Always check your boat and equipment before leaving a water body. Remove any visible plants, clumps of algae or aquatic animals from all gear and empty all bait bucket water, live well water, and cooling water on dry land away from shore.

CLEAN

If you have been in a river or stream, clean:

Non-absorbent Items

Soak item in hot 113°F (uncomfortable to the touch) water for at least 20 minutes. Water must remain at or above 113°F for the entire soaking to be effective.

OR

For one full minute soak or spray a film of either

- 5% solution of dish soap and water (1 cup detergent per gallon of water) or
- 2% solution of bleach (1/2 cup bleach per gallon of water)

Absorbent Items (especially felt-soled waders)

Soak items for 30 minutes in a 5% solution dish soap and hot 113°F (uncomfortable to the touch) water. The water must remain hot during the entire soaking to be effective.

DRY

Whenever possible, especially if gear was not cleaned, allow items to completely dry and wait at least 48 additional hours before re-using.

FREEZE

Freezing any item solid will kill Didymo, but may not kill other invasive organisms.

AVOID FELT-SOLED WADERS

Consider non-absorbent alternatives, including carbide stud replacement soles, detachable cleats and sticky rubber.

♻️ Printed on recycled paper 2008



Lakes and Ponds Program



Stop the Spread of DIDYMO

Didymosphenia geminata
An Invasive Algae
(a.k.a. Rock Snot)

What is Didymo?

Didymo (*Didymosphenia geminata*) is a type of highly invasive algae that attaches to plants, rocks and other hard substrates in rivers and streams. Also called Rock Snot, due to its slimy appearance, Didymo can produce thick mats that cover the stream bed, making fishing, swimming or paddling undesirable, or even impossible.



Infestations of Didymo can cause serious environmental impacts such as loss of habitat for fish and other organisms. Not all species of *Didymosphenia* are invasive.

Where is Didymo?



Didymo is native to the northern hemisphere, and historically only occurred in low-nutrient waters at high latitudes. However, since the mid-1980s Didymo has been taking on the characteristics of an invasive species.

Didymo generally prefers cool, clear, fast-flowing sunlit streams (typical trout streams), but has been expanding its ecological range into lakes and nutrient-rich water bodies in lower latitudes.

Please keep Didymo out of Massachusetts

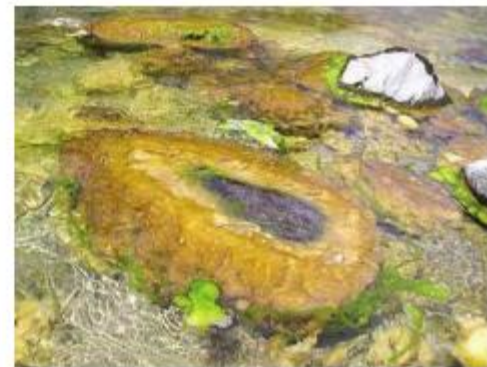
As of 2008, Didymo has not been reported in Massachusetts. However, it has been identified nearby in Vermont's White River and Battenkill River, and in portions of the Connecticut River in both Vermont and New Hampshire.

If you think you have found Didymo, please report it to:

www.mass.gov/czm/invasives/index.htm
617-626-1218

How to Identify Didymo

Didymo begins as small circular brown blotches on rocks and other substrates. These may develop into beige mats that can be up to 10-12" thick. As the stalks lengthen, the tips begin to fray and appear gray or white, and often resemble wet tissue paper. Although Didymo appears slimy, it has a rough damp wool texture and strongly attaches to the substrate. Unlike other algae, Didymo does not come apart when rubbed between your fingers.



APPENDIX B

SELECTED WEB AND AGENCY RESOURCES TO ASSIST IN IDENTIFYING INVASIVE ORGANISMS

General:

<http://www.issg.org/database/welcome/>
<http://nbii-nin.ciesin.columbia.edu/ipane/ipanespecies/noxious.htm>
http://maccweb.org/resources_inv_eco.html
<http://www.invasivespeciesinfo.gov/index.shtml>

***Didymosphenia geminata*:**

http://en.wikipedia.org/wiki/Didymosphenia_geminata
<http://www.biosecurity.govt.nz/pests/didymo>
<http://www.epa.gov/region8/water/didymosphenia/>
http://www.mass.gov/dcr/waterSupply/lakepond/hottopic/ht_didymo.pdf

***Dreissena polymorpha*:**

<http://www.invasivespeciesinfo.gov/aquatics/zebramussel.shtml>
<http://nas.er.usgs.gov/queries/FactSheet.asp?speciesID=5>
http://en.wikipedia.org/wiki/Zebra_mussel
http://wdfw.wa.gov/fish/ans/identify/html/index.php?species=dreissena_polymorpha
<http://dnr.wi.gov/invasives/pubs/protectyourboat.pdf>

Aquatic Plants:

http://www.mass.gov/dcr/waterSupply/lakepond/invasive_1.htm
<http://www.ecy.wa.gov/programs/wq/plants/plantalgaeid.html>
<http://www.maine.gov/dep/blwq/topic/invasives/invpicture.htm>
<http://www.dnr.state.mn.us/invasives/aquaticplants/index.html>
<http://www.deerfieldriver.org/invasives.html>
<http://plants.ifas.ufl.edu/node/21>

Freshwater and Marine Organisms:

<http://mass.gov/czm/invasives/index.htm>
<http://www.mass.gov/czm/invasives/monitor/id.htm>
http://www.oar.noaa.gov/oceans/t_invasivespecies.html
<http://massbay.mit.edu/>
<http://www.northeastans.org/pet/>

Training in Invasives Identification:

APPENDIX C

**CLEANING SOLUTIONS AND/OR ACTIONS TO PREVENT THE SPREAD OF
INVASIVE AQUATIC ORGANISMS**

Invasives of Concern	Disinfectant	Concentration	Contact Time	Reference
Dreissenid mussels (Zebra and Quagga mussels)	Vinegar	100%	20 minutes ^A	1,2,3,4, 5
	Chlorine/Bleach	1 oz. per gallon water	1 hour	
	Power wash with hot wash	>45°C	20 seconds	
	Steam/Scalding hot wash	60°C	10 seconds	
	Freeze	<0 deg. C	4 – 24 hours	
	Salt Bath	Saturated	30 minutes ^A	
	Ethanol	50%	10 minutes	
	Lysol	As sold	10 minutes	
	Drying	Dry to the touch	1 week if kept dry; Up to 4 weeks if subject to cool, wet weather	
Didymo (<i>Didymosphenia geminata</i>)	Dishwashing detergent ^B (non-absorbant)	5% (two large cups or 500ml with water added to make 10 litres)	>1 minute (soak or spray)	6
	Salt	5% v/v (e.g., 1 liter dry salt in 19 liters hot tap water)	>1 minute (soak or spray)	
	Bleach (non-absorbent)	2% (one small cup or 200ml with water added to make 10 litres)	>1 minute (soak or spray)	
	Hot water (non-absorbent)	very hot water <i>kept above</i> 60 °C (140°F; hotter than most tap water)	>1 minute (soak)	
		hot water <i>kept above</i> 45 °C (uncomfortable to touch)	>20 minutes (soak)	
	Hot water (absorbent material)	hot water kept above 45 °C	>60 minutes (soak)	
	Hot water plus detergent (absorbent material)	hot water kept above 45 °C and containing 5% dishwashing detergent	>30 minutes (soak)	
	Freezing	<0 deg. C	12 hours	
	Drying	Dry to the touch	> 2 days after dry	
<i>Other (TBD)</i>	<i>TBD</i>	<i>TBD</i>	<i>TBD</i>	
Other micro-scale invasives	BPJ similar to that for Didymo or mussels			
Aquatic macrophytes (following check-clean procedures)	Drying	Dry to the touch	> 2 days after dry	7
	Power wash with hot wash	≥60°C	2 minutes	

^A It is intended for zebra mussel veligers, not adults.

^B If necessary, use unscented detergent to avoid nausea from prolonged breathing of perfumed air.

- (1) DiVittorio, J., Grodowitz, M., Snow, J., Manross, T. (2012) Inspection and Cleaning Manual for Equipment and Vehicles to Prevent the Spread of Invasive Species. U.S. Department of the Interior, Bureau of Reclamation, Technical Memorandum No. 86-68220-07-05.
- (2) Massachusetts Department of Conservation and Recreation. 2010. Prevent the Spread of Zebra Mussels. Massachusetts DCR Lakes and Ponds Program.
<http://www.mass.gov/eea/docs/dcr/watersupply/lakepond/downloads/zebmussbro10.pdf>. Accessed on August 4, 2015.
- (3) Comeau, S. Ianniello, R.S., Wong, W.H., Gerstenberger, S.L. 2015. Boat Decontamination with Hot Water Spray: Field Validation. In: Biology and Management of Invasive Quagga and Zebra Mussels in the Western United States (Eds Wong, W.H., Gerstenberger, S. L.). Pages 161- 173.
- (4) Wong, W.H., personal observation.
- (5) Waller, D. L., Fisher, S.W., Dabrowska H. 1996. Prevention of Zebra Mussel Infestation and Dispersal during Aquaculture Operations. *The Progressive Fish-Culturist*. 58:77-84.
- (6) New Zealand Ministry for Primary Industries. Didymo: *Didymosphenia geminata*.
<http://www.biosecurity.govt.nz/cleaning>. Accessed on August 4, 2015.
- (7) Blumer, D.L., Newman, R.M., Gleason, F.K. 2009. Can Hot Water Be Used to Kill Eurasian Watermilfoil? *Journal of Aquatic Plant Management*. 47: 122-127.

APPENDIX D
(EXAMPLE)

FIELD DECONTAMINATION EQUIPMENT CHECKLIST
FOR MULTIPROBE DEPLOYMENT SURVEYS

- ❑ **QuickGuide** protocol for decon
- ❑ **non-porous (NP) waders ONLY**
- ❑ **6"-diameter decon soaking tube** (1) filled $\frac{3}{4}$ with decon solution **3"-diameter decon soaking tube with cable notch** (1) filled $\frac{3}{4}$ with 5% decon solution
- ❑ **decon soaking can/cooler** (1) filled $\frac{1}{3}$ - $\frac{1}{2}$ with 5% v/v decon solution (*for misc. items*)
- ❑ **decon sprayer** (1 minimum; 2 each crew if possible) filled with 5% decon solution
- ❑ **extra decon ingredients**
- ❑ **large bags** for used/contaminated **equipment**
- ❑ **watch/timer**
- ❑ **Bungee cords** *sufficient to tie down* decon tubes
- ❑ 2nd set of "new" bungee cords (if needed)
- ❑ 2nd set of "new" concrete blocks (if needed)
- ❑ 2nd set of "new" deploy tubes (if available/needed)

APPENDIX E

GENERAL INSPECTION & CLEANING PROTOCOL FOR DWM BOATING EQUIPMENT

NOTE: Photos of cleaning equipment used below are for illustrative purposes only.
Actual equipment used will vary.

Prior to departure from the DWM office/lab, thoroughly inspect and **check** all watercraft, motors and trailers for the presence of plant/animal remnants and organic debris. Remove all plant fragments, algal clumps and other organisms and dispose of as trash. If necessary, **clean**, rinse & drain (as appropriate) boat motor, trailer, anchors, chains, bilge water, fish tanks, fishing gear and other equipment using portable spray unit or tap water hose or, if a designated wash area is available ¹, use it to rinse all surfaces of boats and equipment. If available, use motor flusher or “muff” device with water hookup to rinse out engine cooling system for 2-3 minutes. If not available, run cold motor out of water for 3-5 seconds MAX to remove most of the water.

When removing boats/trailer from the water, repeat the procedure of checking/cleaning (at the lake just sampled or at a separate washing station prior to entering another waterbody): Check, clean, rinse with salt water, and drain surface areas of a boat, motor, trailer, anchors, chains, bilge water, fish tanks, fishing gear and other equipment using portable spray unit, or tap water hose. If a designated wash area is available, make sure that all water enters the sanitary sewer system. In general, avoid going from one lake (X) to another waterbody (Y) where the same boating equipment must be used AND where invasives are known or reasonably suspected to be present and spread by sampling activity to non-contaminated areas. If this cannot be avoided and after extensive checking and cleaning, DECON by cleaning and rinsing all equipment and surfaces that came in contact with the water using a portable spray unit (containing either tap water or an appropriate non-phosphorus, non-antibacterial solution ²), OR, preferably if available, a designated hot water pressure wash area or manual hot water pressure-spray car wash facility. Drain bilge water on land away from storm drains. To remove cooling water remaining in the motor when exiting the lake, you can pull motor out of water while running and then turn off gas feed or turn motor off after 3-5 seconds. When reaching Lake Y, check for plant fragments again prior to launch.

Example of regional wash facility availability in MA.:

http://maps.google.com/maps?hl=en&source=hp&um=1&ie=UTF-8&q=car+washes+in+Berkshire+County+Massachusetts&fb=1&gl=us&hq=car+washes&hnear=Berkshire+County+Massachusetts&ei=O10pS_zDJ9ve8Aa12_iwDQ&sa=X&oi=local_group&ct=image&resnum=1&ved=0CB8QtgMwAA

¹As of August 2015, DWM does NOT have a designated pressure washing area at the DWM-Worcester location. Use a nearby pressure-spray wash facility and request reimbursement for \$ spent.

²Ensure that cleaning solution used is appropriate for the invasive(s) of concern. Readily available cleaners include bleach, salt and hand-dishwashing detergents. 5% solution (v/v) = 1 part cleaner to 20 parts water. For a 3 gallon spray unit (w/ max fill line of 2 ¼ gallons), add about 1 pint of liquid dishwashing detergent or bleach and fill to max line).

NOTE: **Avoid contaminating waterways with residual cleaning solution by using P-free, non-antimicrobial detergent.** Although most liquid detergents for hand-washing dishes are now phosphorus-free, detergents for automatic dishwashers typically can have a high phosphorus content, as tripolyphosphate is a preferred water-softening agent. Use of soap without anti-bacterial chemicals (e.g., triclosan, triclocarban) also avoids potential pollution with these persistent biocides.

APPENDIX F

DECON PROTOCOL FOR FIELD CLEANING DWM SAMPLING EQUIPMENT & GEAR

NOTE: Photos of cleaning equipment used below are for illustrative purposes only.
Actual equipment used will vary.

Examples of DWM sampling gear that come into contact with ambient water and require checking and cleaning include, but are not limited to: boots and waders, felt-soled kickers, nets, gloves, ropes/tapes, bottle baskets, sediment samplers, Van Dorn samplers, integrated tube samplers, attended multi-probe sondes, deployed multi-probe sonde tubes, anchor/cable/lock assemblies for deployed multi-probe sonde tubes, Secchi disk, velocimeters, boats, motors and trailers

The following procedures apply to checking & cleaning equipment used for all types of DWM surveys in which more than one location is visited on the same day using the same equipment **AND** where invasives are known to be present or suspected to be spread by sampling activity to non-contaminated areas ¹ :

- 1) **Check** for and manually remove plant/animal remnants and organic debris from all equipment prior to and following sampling at each site. Dispose as trash at the site.
- 2) **Clean** gloves, boots and equipment by immersing in a tub(s) containing an appropriate non-phosphorus, non-antibacterial solution ². Keep immersed for the recommended time to kill the invasive of concern. When done, remove the items and rinse with tap water (optional). Cover the tub with the lid for reuse at the next site.



Soak solution



Tap water rinse (optional)

- 3) For equipment that cannot be soaked in the tub, clean by spraying with an appropriate non-phosphorus, non-antibacterial solution for the recommended time using a portable spray device (away from waterbody). Make sure to cover all surfaces thoroughly. Rinse with second sprayer containing tap water (optional).



Spray solution



Tap water rinse (optional)

- 4) **Dry** items to suppress invasives. Where cleaning at the lab is appropriate, these same standards apply, but also include drying (to ensure effective drying, the item must be

completely **dry** to the touch, inside and out, then left dry for at least another 2-30 days, depending on the invasive of concern (see Appendix C).

- 5) Although strongly discouraged, if you wear **felt soled boots/kickers where Didymo and/or other microscopic invasives are known or suspected to be present**, restrict equipment to a single waterway, and then clean/dry later at the office/lab.
- 6) Dispose of all used tub washwater back at the lab down the sink.
- 7) The cleaning solution can be made up at the lab prior to departure or in the field as needed.
- 8) For **attended and deployed multi-probe sondes (and tubes)**, employ the following special procedures:
 - a) In general, **pre-design the deployment surveys to avoid the need to redeploy multi-probe sondes from one location to another**. This will avoid the need for field decontamination, but they will still require decontamination washing at the office/lab. It is recognized that this is not always possible.
 - b) For lab or field washing, scrub & rinse or soak entire apparatuses (sondes, deployment tubes, cables, L-brackets, locks, bungee cords and anchor blocks) with appropriate P-free non-antibacterial solution for the recommended contact time. **DO NOT USE BLEACH ON SONDE UNITS**. For bungee cords (and other porous surfaces), soak for 30 minutes in hot water kept above 45 °C containing 5% dishwashing detergent or other applicable solution (at the lab).
 - c) If unattended deployment tubes must be moved from one location to another, use field decontamination kit at the retrieval site to clean/rinse the entire deployment apparatus using P-free, non-antibacterial solution, followed by tap water rinse), AND use lab-cleaned bungee cords only.
 - d) For attended multi-probe sondes used at multiple sites on the same survey run, clean/rinse sondes and coiled cable after each use with P-free non-antibacterial cleaning solution for the recommended contact time. **DO NOT USE BLEACH ON SONDE UNITS**. Do not clean the datalogger or its comm. ports.

¹ *DECON procedures are also required whenever work is performed outside MA. where Didymo is known or suspected to be present*

² *Ensure that cleaning solution used is appropriate for the invasive(s) of concern. Readily available cleaners include bleach, vinegar, hand-dishwashing detergents and salt.*

APPENDIX G

USE of DECON SPRAYERS

SET-UP: to be performed by lead monitoring coordinator

1. Sprayers have been pre-set to produce a flat spray (no adjustment needed)
2. Fill sprayer unit(s) to max fill line with decon solution. DO NOT EXCEED MAX. FILL LINE.
3. Pressurize unit(s) and test pressure relief valve and spray (to make it ready for use). Approx. 10-20 pump cycles should be sufficient. DO NOT EXCEED MAX. PRESSURE LIMITS.
4. Lock pump top and load sprayer(s) on cart or in vehicle. Protect sprayer wand(s) from damage by securing it inside the vehicle.
5. In case additional decon solution is needed in the field and to avoid having to make it up in the field, a second decon sprayer per crew should be set-up and provided.
6. For maintenance issues, see operator's manual, .
7. For inflation precautions, see below.

USING THE SPRAYER: by survey staff

1. To save time, one person can be spraying boots, gloves and equipment while the other performs other decontamination tasks.
2. If needed, unlock pump and add more pressure to get a better spray.
3. Rinse all "contaminated" items sufficiently for the solution to have the required contact time (not one minute of constant spraying), but be aware of how much spray solution is being used at each site (especially if you don't have a spare sprayer).
4. If solution runs out, use spare sprayer or make up new solution.
5. If you need to open pressurized tank, pull on relief valve first to release pressure before opening.
6. For other precautions, see below.

WARNING AIR COMPRESSOR INFLATION PROCEDURE

An air compressor can be used to pressurize Solo sprayers equipped with a built-in inflation valve. Compressors pressurize sprayers much more rapidly than hand pumping; for this reason extra caution must be exercised when using compressed air. **Sprayers must be functioning properly, unmodified and have all component parts.** The following procedures must be followed. Failure to do so could result in serious injury to the operator or others.



- 1) Before **each** use of a compressor with the sprayer be certain that:
 - a) The umbrella valve (or valve cone on older models) is present, installed and functioning properly. The valve is located on the bottom of the cylinder (see owner's manual, page 2, "Pump Maintenance" item #7).
 - b) The pump assembly is screwed snug to the sprayer tank.
 - c) The pressure relief valve is functioning properly. Check by pulling up on the valve until the red stem shows. The valve stem should move freely and spring back to its original position when released. The O-ring and the valve stem must be greased (see page 2, "Maintenance" in the owner's manual).



If any of the above items or other components is malfunctioning, STOP!, do not use an air compressor to pressurize. Repairs must be made prior to use.



- 2) Additional Precautions:
 - a) Fill the sprayer with liquid formula.
 - b) Do not stand over the sprayer handle while pressurizing with an air compressor.
 - c) Do not stand over the sprayer while releasing the handle from the locked position. Compressed air can cause the pump handle to pop upward if the valve cone or umbrella valve is worn, damaged or if the pressure relief valve is not functioning (see item 1c above).



- 3) When pressurizing, place pump handle in the unlocked position, resting on top of the pump assembly. Begin pressurizing slowly. If the pump handle rises...**STOP!, DISCONTINUE PRESSURIZING.** Pull up on the pressure release valve and hold until all the pressure is released; then remove the pump, check and replace the umbrella valve (or valve cone). Compressed air can cause the pump handle to pop upward if the valve cone or umbrella valve is worn, damaged or not installed completely. Do not pressurize until the worn or damaged part has been replaced, installed properly and the handle does not rise during pressurization. Should you have questions, please call Solo (800) 296-7656.

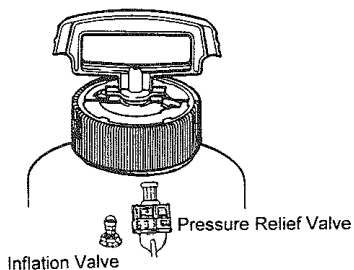


Figure 1 – Handle in the unlocked, resting position.

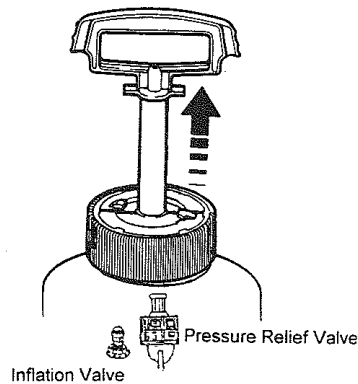


Figure 2 – Handle rising during pressurization; indicating a potentially unsafe condition.

APPENDIX H

EXAMPLE

**QUICKGUIDE FOR DWM FIELD DECONTAMINATION PROCEDURES ¹
FOR MICROSCOPIC INVASIVES (e.g., Didymo and mussel veligers)**

Field Decon Equipment List (qty.):

- this QuickGuide
- non-porous (NP) waders ONLY
- 6” decon soaking tube (1) filled ¾ with 5% detergent solution (800 mls detergent: 16 liters warm water)
- 3” decon soaking tube with cable notch (1) filled ¾ with 5% detergent solution (approx. 150 mls detergent: 2.5 liters warm water)
- decon soaking can/cooler (1) filled ½ with 5% v/v detergent solution
- decon sprayer (2) filled with 5% detergent solution (450 mls detergent: 8.5 liters warm water)
- extra detergent bottle (concentrated)
- 2nd set of “new” bungee cords
- large bags for used/contaminated equipment
- watch/timer
- 2nd set of “new” concrete blocks (if needed)
- 2nd set of “new” deploy tubes (if needed)

Survey Type	Equipment Item	Field Procedure ²	Office Procedure
Multi-probe deployment (DO/T at multiple sites)	concrete blocks (P)	use office-decon’d blocks ONLY, or use site-dedicated block (leave at site).	Return to the office with used blocks and soak for <u>1 hour</u> in hot water (>45C) containing 5% detergent.
	bungee cords (P/NP; any type)	use office-decon’d bungee cords ONLY (2 nd set).	same as above
	cables and locks (NP)	Soak in 5% detergent for at least 1 min., then reuse.	Soak in 5% detergent for at least 1 min., then dry overnight.
	PVC tubes w/ sonde inside (NP/P)	<p>Option 1:</p> <p>A) remove sonde from deploy tube and soak entire sonde with probe guard in decon tube (5% detergent for at least 1 min.)</p> <p>B) redeploy decontaminated sonde in new/office-decon’d tubes (2nd set of tubes needed).</p> <p>C) Keep used tubes separate and isolated</p> <p>Option 2:</p> <p>A) soak deploy tube w/ sonde inside in decon tube containing 5% detergent for <u>at least 5 min.</u> (e.g. to the next site);</p> <p>B) remove sonde from deploy tube and replace storage cup, reuse tube and sonde when needed.</p>	<p>Scrub tubes (especially drilled holes) and sondes (w/ storage cup on) with hot water and detergent, let dry for at least 24 hours.</p> <p>Also, soak probes (in storage cup) in 5% detergent for at least 1 min; rinse with tap water.</p>
Attended multi-probe use (multiple sites)	sonde (NP)	Rinse with 5% detergent for at least 1 min., then reuse sonde.	Rinse with tap water with sonde guard on, replace storage cup.
Temp logger deployments w/ logger (<u>only if re-deployed</u>)	PVC tubes w/ logger inside (NP/P)	<p>Option 1: use office-decon’d temp tubes ONLY (2nd set).</p> <p>Option 2: soak in 5% detergent for <u>at least 5 min.</u>, reuse tube and logger as needed.</p>	<p>Scrub tubes with hot water and detergent (especially drilled holes), let dry for 24 hours before reuse.</p> <p>Also, soak loggers in 5% detergent for at least 1 min; rinse with tap</p>

Survey Type	Equipment Item	Field Procedure ²	Office Procedure
			<i>water. Let dry.</i>
water sample collection	waders (NP; non-felt soled)	<u>Rinse with 5% detergent for at least 1 min.</u> , then reuse after one additional minute (min. 1 minute contact time)	<i>Rinse with 5% detergent for at least 1 min. Let dry.</i>
	gloves, re-usable (NP)	same as above; rinse well in ambient water of the next sampling site prior to next sample collection	<i>same as above</i>
	sampling pole (NP)	same as above, including inside pole. rinse well in ambient water of the next sampling site prior to next sample collection	<i>same as above</i>
	single probes (NP)	same as above; rinse well in ambient water of the next sampling site prior to next sample collection	<i>same as above</i>
	sampling basket, rope and contents (NP/P)	Soak in 5% detergent for <u>at least 5 min.</u> , then reuse. rinse well in ambient water of the next sampling site prior to next sample collection	<i>Scrub entire assembly with hot water and detergent. Let dry overnight.</i>
	misc. NP/P equipment in contact with sediment/water (e.g., anchored guide rope for multi-probe)	Soak in 5% detergent for <u>at least 5 min.</u> , then reuse.	<i>Scrub with hot water and detergent. Let dry overnight.</i>
	Small boats (rivers and streams; NP)	One-time use only, or restrict single boat use only to areas containing invasives. For multiple sites involving potentially unaffected areas, remove visible organic matter, and <u>rinse all contact areas with 5% detergent for at least 1 min.</u> , (min. 1 minute contact time)	<i>Following survey, take boat/trailer to commercial, manual car wash facility. Completely wash (with detergent) all contact areas on boat and trailer. Pour 1 cup of detergent in bilge. Rinse thoroughly.</i>
benthic flora/fauna collection	D-nets; artificial substrates (NP/P)	use office-decon'd artificial substrates ONLY. Soak equipment in 5% detergent for <u>at least 5 min.</u> , then reuse.	<i>Scrub with hot water and detergent. Let dry overnight.</i>
sediment sampling	tube/dredge samplers, spoons, buckets, etc. (NP)	Use site-dedicated equipment if possible. If not, rinse with 5% detergent or salt solution for at least 1 min., then reuse	<i>Rinse with 5% detergent or salt solution for at least 1 min.. Let dry.</i>
Flow survey	velocimeter, wading rod, shovels, etc. (NP/P)	Rinse with 5% detergent for at least 1 min., then reuse	<i>Rinse with 5% detergent for at least 1 min. Let dry.</i>
Backpack electroshocking equipment	Dip nets, buckets, wand (NP/P)	Soak equipment in 5% detergent for <u>at least 5 min.</u> , then reuse.	

¹ for use only where invasives are present or suspected. Some of these field procedures may not be necessary if not re-deploying.

² apply the field procedures in-between sites

P=porous; NP=non-porous

NOTES:

- 1) "Soak" can replace "rinse" if soaking equipment available and appropriate, but rinsing cannot replace the need to soak porous or semi-porous items.
- 2) Where possible, the use of "porous" materials shall be avoided where invasives are present or suspected.
- 3) **Rule of Thumb:** Highly porous(P)=1 hour soak; intermediate materials(P/NP)= 5 min. soak; Non-porous(NP)=1 min. soak or spray
- 4) If and when possible, avoid contact between water sampling devices and bottom substrate.
- 5) Durations listed for rinsing and soaking represent minimum contact times.
- 6) Keep used (contaminated) equipment separate and isolated from new/uncontaminated equipment

APPENDIX I

EXAMPLE

QUICKGUIDE

DECON PROCEDURES (one time use) ¹ AT DWM OFFICES

Office Decon Equipment List (qty.):

- this QuickGuide
- “dirty” bag/container for contaminated equipment brought back (keep separate)
- decon soaking can (1; 32 gallon) filled ½ with 5% detergent solution (*3 liters detergent*) for sonde/tube assemblies
- decon soaking can (1; 32 gallon) filled ½ with 5% detergent solution for concrete blocks
- decon soaking cooler (1) filled ½ with 5% v/v detergent solution for smaller equipment
- decon sprayer (1) filled with 5% detergent solution (*450 mls detergent: 8.5 liters warm water*) for misc. equipment
- scrub brush, detergent and large sink for washing

Equipment Item	Office Procedure
concrete blocks (P)	Option 1: Leave blocks at each site as needed for entire season Option 2: Return to the office with used blocks and soak for <u>1 hour</u> in hot water (>45C) containing 5% detergent (in 32 gallon can).
bungee cords (P/NP; any type)	Soak for <u>1 hour</u> in hot water (>45C) containing 5% detergent (in cooler or can).
cables and locks (NP)	Soak in 5% detergent for <u>at least 1 min.</u> , then dry overnight.
PVC tubes w/ multiprobe sonde inside (NP/P)	Remove storage cup, attach probe guard and soak tube with sonde inside for <u>5 min.</u> in hot water (>45C) containing 5% detergent (in 32 gallon can). Also soak storage cup. After 5 minutes, replace storage cup. Scrub tubes (especially drilled holes) and sondes with hot water and detergent. Rinse with tap water and let dry for at least 24 hours.
PVC tubes w/ temp logger inside (NP/P)	Scrub tubes with hot water and detergent (especially drilled holes), let dry for 24 hours before reuse. Also, soak (DO NOT SCRUB) loggers in 5% detergent for at least <u>1 min.</u> ; rinse with tap water. Let dry.
Other equipment (one time use)	see Appendix H for specific procedures and “rules of thumb”

P=porous; NP=non-porous

¹*for use only where invasives are present or suspected*

APPENDIX J

FIELD SAMPLING PROTOCOL¹ **FOR *Didymosphenia geminata***

¹ *This protocol is for agency use ONLY*

Visual observations

Sites should first be examined for periphyton material before the establishment of transects. Didymo populations may appear as small, circular clumps that are light tan to brown (although suspicious material of any color should be collected) and feels like cotton. As the colonies age and expand the presence of large amounts of stalk material may create the “rock snot” appearance. Material from these clumps should be collected for identification. If possible, transects should include the areas with periphyton clumps.

Scrapes

A marker will be placed at the sampling site (or a GPS reading taken) so that the same location can be relocated at later sampling events. Five transects will be made perpendicular to the flow and through the riffle. A tape measure will be stretched across a stream and the width (from bank to bank) will be divided into five evenly spaced locations where samples will be collected. At each of the five locations, the sampler will reach down and without looking pick up the cobble or rock they first touch. Five samples will be collected from each transect to give a total of 25 per site. The thickness of the microalgal mat on the substrata surface will be estimated or measured according to a scheme described in Barbour et al. (1999). A scrape of the rock surface using a wooden scraper (ice cream stick) will be made and the material will be placed in a sample vial containing a few milliliters of ambient water. All five scrapes from a transect will be composited in this vial. The labeled vial will be added to a larger jar containing ambient water to keep the samples cool. An estimate of the percent cover of the micro and macroalgae on the rock surface will be recorded on the field sheets as well as an estimate of the percent canopy cover. The process will be repeated for the remaining transects so that 5 discrete samples are gathered. Plants and wood should also be checked for didymo and sampled if visible clumps are present.

Plankton net

The use of plankton nets can aid in detecting the presence of didymo at low cell numbers. Plankton nets are deployed for 10 minutes from a rod that had previously been pounded into the substrata. The rods will be put in place midstream, if possible, at the upstream end of a 20 meter reach. When the plankton net is retrieved it will be washed in ambient water by “dunking” the net down to the mouth of the net, but without letting the net fill with water. The samples will be brought back to the lab for identifications and counts.

Algal identifications and counts

The samples will be logged in and put in the refrigerator until they are removed for identification. Any moss or long strands of green filamentous algae will be removed first. The vial will then be shaken and a milliliter removed and added to a Sedgewick Rafter counting chamber. While that sample is settling (15 minutes) a slide will be made to allow observation at 400 x. At least two strips will be examined at 200 x from the Sedgewick Rafter counting chamber and the results recorded as cells per ml.

Sample disposal

All sample material will be decontaminated following the MassDEP-DWM decontamination SOP prior to disposal.



APPENDIX K

**GENERIC FIELD SAMPLING PROTOCOL and VERIFICATION
FOR SUSPECTED INVASIVES**

NOTE: This protocol is for MA agency use only.

- 1) Collect representative sample of suspected invasive into a clean plastic bottle or bag with water.
- 2) If possible, a photo should also be taken to record the habitat of the suspicious invasive species.
- 3) If the suspicious species is an animal the sample needs to be preserved with 50% ETOH in a container. For a plant, the sample should be collected as a whole or a segment; if plants have fruits and/or flowers, the plants need to be collected as a whole. Keep the plant sample in a bag or bucket of water at all times (Don't leave them out of the water in the sun because they will wither quickly and become useless as specimens).
- 4) Label bottle or bag with location, date, time, and collector.
- 5) The sample will be brought back to the lab and examined by at least one additional WPP biologist for identification and verification. Suggested biologists/taxonomists for species verification are listed below.
- 6) If the sample is not confirmed within WPP, external research experts will be consulted until the sample is finally confirmed.
- 7) Upon the final confirmation, the sighting will be added to the list of Mass AIS Waterbody List in the WPP Toolbox. If the sample is not identified as an invasive species, the waterbody will not be added to the Mass AIS Waterbody list. Document results in lab notebook.
- 8) This new invasive sighting or a new invasive species in a waterbody infested by other invasives will be reported to other agencies and the information will be shared with the general public.
- 9) Preserve specimens for further analysis if it is needed. Otherwise, when finished with sample, soak sample for 30 minutes in hot water kept above 45 °C containing 5% dishwashing detergent, then discard as trash or down drain as applicable.
- 10) Recycle sample container or clean with hand-dishwashing soap and bleach solution for reuse.

Contact David Wong for AIS identification/verification and Richard Chase and Art Johnson for AIS reporting.

Potential Biologists/Taxonomists for Invasives Confirmation (macro- and microscopic) *			
Name	Agency	Contact Information	Suspected Invasives
Joan Beskenis	MassDEP-DWM, Worcester, MA.	508-767-2794	Didymo, other microalgae species

Robert Nuzzo	MassDEP-DWM, Worcester, MA.	508-767-2809	Benthic macroinvertebrates
David Wong	MassDEP-DWM, Worcester, MA.	508-767-2892	Aquatic macrophytes, Dreissenid mussels, Asian clam, Crustaceans
Robert Maietta	MassDEP-DWM, Worcester, MA.	508-767-2793	Fish
Dave Worden	Mass. DCR-Quabbin	508-792-7806	Didymo
Sarah Spaulding	U.S. Geological Survey, Denver, Colorado	sarah.spaulding@usgs.gov	Didymo
Adrienne Pappal	Mass. CZM, Boston, MA.	617-626-1218	Didymo, Dreissenid mussels, marine invasives, others
Jim Straub	Mass. DCR	617-626-1411	Dreissenid mussels, Aquatic macrophytes