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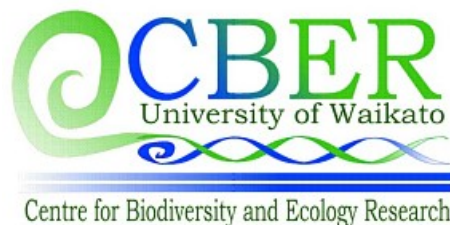
Field guide for didymo DNA sample collection

CBER Contract Report 65

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Didymo drift net sampling preparations

This protocol is designed for work in two-person teams for both safety and to maximise sample integrity.

Sampling equipment

Specialised sampling equipment will be supplied. Further complete kits and components are available from the University of Waikato, Hamilton. Phone 07 838 4022 or email biology@waikato.ac.nz. Common or heavy items should be sourced locally. These items are listed separately below.

A. Didymo DNA drift net sampling kit contents (supplied)

	Item	Item Description	Quantity
1	Sample preservative	70% ethanol in screw cap bottle	500 mL
2	Wash bottle		1
3	Screw cap lid		1
4	Funnel		1
5	Laminated label with address	A5 label taped to inside of lid	1
6	Plastic clip-lid box	Sistema 7 L Klipit	1
7	Sharpened pencil	Use for labelling tubes	1
8	Sharpie permanent felt tip marker	Use for labelling mailing bags	1
9	Stand for Falcon tube		1
	Consumable items - can be replaced		
10	Falcon tubes	14 ml screw top plastic tubes	20
11	Parafilm wax strips 15 x 100 mm	Wrap tightly round cap junction	1 per tube
12	Rubber bands		1 per tube
13	Zip closure plastic bags	small Ziploc bags	20
14	Zip closure plastic bags	large Ziploc bags	3
15	Paper towels	block of paper towels	25
16	Zip loc bag with spares	2 wing nuts, scrap of 40 µm mesh	1
17	Rubbish bags	For disposal of gloves, etc	3
18	Disposable vinyl gloves - pair, large	For handling bleach	5
19	Prepaid mailing bags	A5 bubble bags	3
	Contents of travel box		
20	Large travel box; contains everything	Decontamination	1
21	Handle net	Three-section screw handle, net	1
22	Clip-lid box for net denaturation	Put net in 5% bleach in this box	1
23	Measuring cup	To measure bleach and detergent	1
24	Trigger spray bottle	MAFBNZ Check, clean, dry bottle	1
25	Clip-lid box with kit described above	Contains items 1-19 above	1

B. Drift net sampling equipment (to be supplied locally)

- Sample data sheets and GPS unit.
- Disposable gloves (one pair per site; 5 pair supplied in original kit).
- Rubbish bags for used disposable equipment (3 supplied in original kit).
- Containers with clean tap water for rinsing debris off net after sampling and before denaturation.
- Waders. Preferably rubber rather than neoprene and with rubber soles rather than felt soles to reduce the risk of transferring didymo between sites.

C. Decontamination equipment (to be supplied locally)

- “Janola” household wipes with active ingredient benzalkonium chloride (for disinfecting hands and arms instead of using bleach)
- 2.5 L bottle of Janola bleach (check the use-by date – about 12 months should remain, as bleach loses its potency with storage).
- Block or roll of paper towels
- 10 L bucket for washing detached sampling net
- At least 10 L of town supply tap water
- Sprayer (optional) for spraying 5% detergent on waders and equipment. Make up detergent solution with town supply water before going out into the field.

Sample labelling and pre-packaging before going out in field

To minimise handling and the risk of cross-contamination, please label and pre-package in a large zip-loc bag (one for each site). Put in Falcon tubes, paper towels, rubber band, Parafilm strips, and small zip-loc bag.

Labels must include:

- sampling team names (**NOT JUST INITIALS**)
- sample number (this is the unique site number that appears on the supplied sample form and map for each site)
- sampling method
- date.

Pre-label for each site:

- 1 screw-top 14 mL Falcon tube for DNA sample collection (use pencil, *not* permanent marker. The tubes will have ethanol in them, which, if it leaks, will dissolve permanent marker ink).

The difference between decontamination and denaturation

Decontamination by exposure to 2% bleach or 5% detergent for 1 minute will kill didymo so that live cells cannot be transferred between sites. However, tests at the University of Waikato (Cary et al. 2007) have shown that the DNA from didymo cells killed in this way **CAN STILL BE EXTRACTED**. Thus it is important to **denature** any potential didymo DNA on the sample net, cod end, and the prefilter by exposure to **5% Janola bleach for a minimum of 15 mins**. It is also important to prevent any dead cells from other items (that have not been denatured) reinfesting these items. The appropriate times to do this are explained below.

Drift net sampling

Assign one person with waders to wet duties, e.g., net deployment, and one person to dry duties, e.g., form filling, setting out dry items and new Falcon tubes. Keeping these activities separate will reduce the possibility of cross-contamination. The duties of each person are indicated in the following instructions as WET COLLECTOR and DRY COLLECTOR.

Sampling sites

A “site” is any river reach. Assume that the highest chance of infestation by didymo is at public access points. Sampling should be done downstream of the access point. If the site location suggested by the GPS map reference or site description is unsuitable you may use nearby access points, as long as there are no significant tributaries between the suggested site and the new site.

In each river, sample from upstream to downstream (to eliminate any chance of transferring didymo, if it is present, upstream in a system).

Stay safe at all times. When in the water, velocity (m/s) × depth (m) should be less than 1. A site with water velocity between 0.3 and 0.6 m/s and about 0.5 m deep is ideal.

Site description and inspection

DRY COLLECTOR

Once the sampling site has been decided upon, complete site information on data sheet, as necessary. Ensure that date and time are entered. Site location and GPS coordinates are pre-designated. Check the GPS coordinates and if they differ from those on the form, record the new coordinates. If site is unsuitable for sampling, still fill in the form and return it with the other samples stating clearly why the site was not sampled, e.g., the site was in flood and it was not safe, the site was a slow flowing, muddy bottom stream, etc.

Put on gloves before handling equipment inside the sampling bin.

Set out the bag of pre-labelled containers and disposable equipment for this site and a further zip-loc bag in which you will place the sample collected from this site. A truck tail gate makes an ideal equipment surface.

Set out the equipment that has been decontaminated from the previous site:

- 14 mL Falcon tube in its stand, with plastic funnel in place. If the funnel has been previously used that day and put in the denaturation solution (5% bleach), shake it free of bleach and rinse any residual bleach off with a small squirt of ethanol. Do this away from the Falcon tube.
- 70% ethanol – remove travelling screw cap and replace with squirt nozzle
- decontamination bin
- make up a 5% detergent solution in the spray bottle if not already done.
- fill the net denaturation box with 300 mL Janola bleach followed by clean water to within 1-2 cm of the 6-L fill line

Hicks handle net design

The Hicks handle net (Fig. 1) features removable components such as the main sampling net and the net collar for easy cleaning.

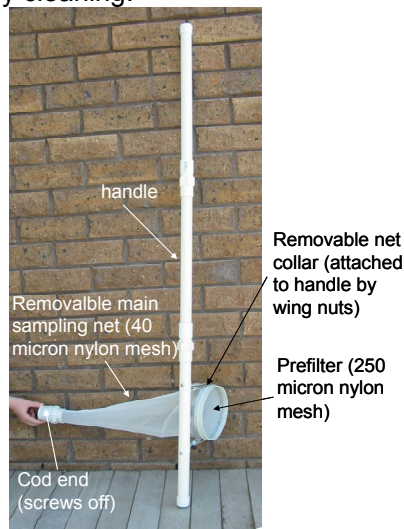


Figure 1. Design of the Hicks handle net.

Hicks handle net drift sampling protocol

WET COLLECTOR

1. Put on disposal gloves before handling any equipment.
2. Set out bucket for washing sampling net.
3. Remove net, collar, prefilter, sampling net and cod end from net denaturation box (denatured by immersion for at least 15 mins in 5% Janola bleach solution if used at a previous site in the same day). Insert pre-filter in to mouth of net.
4. **DO NOT TOUCH PRE-FILTER AT ANY STAGE AFTER ASSEMBLY**
5. Assemble handle (well rinsed inside and out with 5% detergent solution if previously used that day).
6. Attach net collar to handle with bolts and wings nuts supplied.
7. Deploy Hicks handle net with cod end attached in the stream or river in water 0.3-0.6 m/s and about 0.5 m deep, with the 250-micron prefilter facing upstream and the leg of the handle resting on the stream bed. **At no stage should any item or person be upstream of the net.** The handle should be at the side of the net so that no didymo cells from the handle can enter the net (Fig. 2). Stand to one side of the net, not upstream, so that dead didymo cannot wash off waders into net.



Figure 2. Deployment of the Hicks handle net for sampling algal drift.

8. Adjust the angle of the handle of the net to position the net just below the water surface. If the water is more than about 0.4 m deep, hold the handle vertical. Ensure that there are no bubbles in the net and if the water is flowing fast that the net is low enough to stop any air getting into the net. Leave in the water for 10 minutes. **Minimise suspended sediment contamination by ensuring that the net is at least 10 cm above the river bed. If possible, do not sample when the water has an obvious suspended sediment load as shown by discoloration. Sediment interferes with DNA extraction.**
9. After the 10-minute deployment, lift the net from the water. Hold it up so that the water drains out. Wash any material that has accumulated on the sides of the net by rinsing through the water.
10. Most of the sample should have accumulated in the cod end. If water is backing up into the main part of the net because of material blocking the end filter, drain off through the side of the net. Gently tap the cod end mesh to aid draining by breaking the surface tension. Allow the water to drain below the level of the cod end before unscrewing the unit.
11. When the water has fully drained, take the net and cod end back to the Falcon tube and plastic funnel in their stand. Wash out any material left in the cod end with a small amount of 70% ethanol in the wash bottle provided (Fig. 3). Use enough to remove all the material from the cod end, but do not fill the Falcon tube further than

- you need to. **DO NOT TOUCH THE PREFILTER, INSIDE OF THE COD END OR FUNNEL with hands, ethanol bottle, or any other equipment.** You do not need to fill the tube to the 14 mL mark. **Place the funnel in the denaturation box.**
12. Screw the tube cap on as tightly as possible and stretch the Parafilm strip around the cap junction to ensure an air-tight seal. Wrap a paper towel around the tube and secure with a rubber band. Place in the corresponding pre-labelled small zip-loc bag. Put all samples from a single site into one large zip-loc bag (i.e., double bag samples). Note: for the Nov 2007 survey, assume one sample per site).
 13. Place all disposable equipment used in rubbish bag.



Figure 3. Wash the material from the cod end into the Falcon tube with 70% ethanol.

Decontamination and denaturation

It is very important that *all* these hygiene precautions are followed carefully. Decontamination procedures will prevent spreading didymo from one catchment to another, and denaturation procedures will minimise the possibility of contaminating samples with didymo cells or DNA, which could lead to false positives.

Denaturation of main sampling net, prefilter, and cod end

It is most important to strictly follow this procedure to reduce the possibility of false positives.

WET COLLECTOR

1. Remove net from its handle by unscrewing the wing nuts.
2. Remove main sampling net from the net collar by pressure on the plastic ring that retains the net (do not pull on the net itself).
3. Wash the sampling net, net collar, and cod end in bucket of river water with cod end and sample net removed to remove debris.
4. Place the net with the cod end removed, its retaining collar with the prefilter, and the cod end into the clip-top box pre-filled with tap water and 300 mL of Janola bleach. Immerse the net carefully, taking care to allow the air to escape from the open cod end (hold it up and gently push the length of the sampling net under the bleach solution). Once the net and funnel are covered with bleach solution, top up to the fill line with more tap water. **This is a 5% bleach solution; soak the sampling net for at least 15 minutes to denature any didymo DNA. This strong bleach chops the DNA so that it cannot be extracted. Didymo exposed to 5% detergent or 2% bleach will be dead, but its DNA can still be extracted.** Make sure that all parts of the net and prefilter are fully immersed in the solution for the entire soaking time. You can put the lid on and transport the net in the bleach to the next sampling site if you wish.
5. Dispose of the used bleach and detergent solutions carefully (e.g., into gravel or earth well away from the river) where it will not cause environmental damage.
6. To prevent recontamination, keep net, prefilter, and cod end in denaturation box when not in use

Decontamination of general equipment

WET COLLECTOR

1. **CLEAN ALL sampling items as detailed in the following sections, including all buckets, containers, waders, and anything that has come in contact with the water.** Remove all dirt and mud.
2. Unscrew Hicks handle into its three parts and spray all parts inside and out with 5% detergent. Place the handle components and net soaking in its box into the large bin for transport to the next site.
3. Spray waders and any other items that have been in the river thoroughly with 5% detergent solution. Absorbent items must be sprayed until fully saturated or soaked in solution.
4. Use paper towels soaked in the decon solution to scrub any surfaces, as necessary.
5. Place all items that have been cleaned back into large bin ready for transport to next site, including the denaturation box containing the funnel, sample net, prefilter, and cod end.
6. Dispose of gloves in rubbish bags. Wipe arms and hands with "Janola" wet wipes or similar with benzalkonium chloride as the active ingredient. Put lid back on large bin.
7. At the end of each sampling day, visually check all potentially contaminated boots/waders, clothing and equipment to ensure they are cleaned, decontaminated, and ready for the next sampling day.
8. After collecting all the samples required for this survey, rinse the sampling net and prefilter in tap water to remove all traces of bleach and hang out to dry in the shade. This is to prolong the life of the net as it will degrade and disintegrate if exposed to strong bleach and sunlight for too long. This equipment may require a denaturation treatment before the next deployment.

Sample return

Enter sampling site and sampling details into the MAFBNZ didymo database.

If samples have to be stored before despatch (e.g., over weekends), keep in a cool, dark place (fridge). DO NOT FREEZE.

All samples, together with sample sheets, should be sent in the prepaid postage bags provided or couriered to the following address:

Prof. Craig Cary or Assoc. Prof. Brendan Hicks Science Store School of Science and Engineering The University of Waikato Gate 8, Hillcrest Rd Hamilton 3240
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Acknowledgements

We acknowledge valuable editorial revisions provided by Frances Velvin (MAF Biosecurity NZ), and the framework provided by the sampling protocol developed in the following report:

Duncan, M., C. Kilroy, and C. Vieglais. 2007. Protocol for the collection of samples for delimiting surveys for *Didymosphenia geminata* for microscopic and DNA analysis. Prepared for MAF Biosecurity New Zealand. NIWA Client Report: CHC2007-102. National Institute of Water and Atmospheric Research Ltd, Riccarton, Christchurch.

Reference

Cary, S.C., B.J. Hicks, K.J. Coyne, A. Rueckert, C.E.C. Gemmill, C.M.E. Barnett. 2007. A sensitive genetic-based detection capability for *Didymosphenia geminata* (Lyngbye) M. Schmidt: Final report. Client report prepared for MAF Biosecurity New Zealand. *CBER Contract Report No. 62*. Centre for Biodiversity and Ecology Research, Department of Biological Sciences, School of Science and Engineering, The University of Waikato, Hamilton, New Zealand.