

**Protocol for Acquiring Zooplankton for Mercury Analysis  
for the  
REMAP Assessment of Mercury in Vermont and New Hampshire Lakes**

Collections of zooplankton within the  $\geq 201 \mu$  size fraction are to be collected with specially designed project nets which are fabricated of non-metal materials. The dimensions of these nets are 30 cm by 125 cm,  $\geq 201 \mu$ , equipped with a detachable 200ml 'Dolphin' reduction bucket (®Wildlife Supply Company, Saginaw MI). Collections are to be performed during a constrained time period to control for seasonal variation in the zooplankton assemblage. In the present study, sampling will be performed during August.

**Summary:**

For the HgT sample, a minimum of 5 tows will be collected from the immediate vicinity of the lakes' REMAP project sampling station, and the contents composited, reduced, then decanted to a pre-weighed, graduated 50ml sample vial, after which the total volume of the sample will be constituted to 50ml. The length of each individual tow composited will be recorded on the field sampling sheet. This sample will be used to measure HgT in the plankton, as well as total planktonic biomass. A modified clean-hands dirty hands protocol will be used for this collection, which is described in the detailed steps below.

Two additional samples will be collected for the purpose of taxonomic analyses. The first sample, the  $\geq 201 \mu$  size fraction, will be composited from two individual tows, which is then decanted to a 50ml sample vessel, narcotized with CO<sub>2</sub>, and preserved with formalin solution. The second sample, the 45 - 200  $\mu$  size fraction, will be composited from two individual tows, which is then decanted to another 50ml sample vessel, narcotized with CO<sub>2</sub>, and preserved with formalin solution. The length of each composite contributing tow will be recorded on the field sampling sheet.

Zooplankton-HgT samples will be handled in the same method as sediment samples, and in accordance with the REMAP Quality Assurance Project Plan.

**Equipment:**

- 201  $\mu$  zooplankton net described above
- 45  $\mu$  zooplankton net
- 200 ml lot-certified PETE 'compositing vessel'
- 500ml acidcleaned squeeze bottle (this should be re-cleaned after every tenth sampling event).
- 500ml squeeze bottle for CO<sub>2</sub> water (seltzer)
- CO<sub>2</sub> (seltzer) water
- 1 pre-weighed, pre-coded, lot-certified 50ml polycarbonate sample vessel
- 2 non-weighed 50ml polycarbonate vessels
- powder-free vinyl gloves
- protective plastic sheet 4'x 4' or larger
- field sampling sheet

### **Preparatory Steps:**

Prior to going out into the field, a pre-coded 50ml sample vessel is weighed to the nearest 0.001 g, and the weight and code recorded.

In the field, after the vessel has arrived at station and has been securely anchored, 'clean hands' and 'dirty hands' are designated. 'Clean hands' and 'dirty hands' don regular-length powder-free vinyl gloves.

A plastic sheet is draped over the gunwale of the sampling boat, such that the net will not have the opportunity to contact the boat.

'Dirty hands' removes and assembles the non-metallic net, and 'clean hands' and 'dirty hands' jointly backflush the net 3X in lake surface water. The dolphin bucket is similarly rinsed.

### **Tows for HgT and Biomass Determination:**

'Dirty hands' lowers the net to within 1 meter of the lake bottom, and rests the net 30 seconds to allow the water column to recolonize.

'Dirty hands' records the depth of this tow on the field sampling sheet.

'Dirty hands' retrieves the net at a rate of < 1 m per second.

When the net-hoop breaches the surface, 'dirty hands' lifts the net, and rinses the contents down along the net-sides using lake water and an acidcleaned squeeze bottle.

Once the sample is condensed into the dolphin bucket, 'clean hands' removes the bucket, further reduces the sample, and decants it into the 100 ml 'compositing vessel.'

This tow collection procedure is repeated until a minimum of 5 tows are collected. The field coordinator will determine if additional tows are necessary to obtain sufficient material for biomass and HgT analyses.

The contents of the compositing vessel is decanted to the 201 $\mu$  dolphin bucket, and the contents reduced to < 50ml volume.

'Clean hands' opens the 50ml sample vessel, rinses it 3X with lake water, and decants the reduced composite plankton material into the vessel. The vessel is then filled to 50ml with lake water, and capped tightly<sup>1</sup>

'Dirty hands' opens a zip-bag, and 'clean hands' drops the filled 50ml vessel into the bag.

'Dirty hands' closes the bag and places it into the designated cooler for submission to the VTDEC LaRosa laboratory for analysis.

### **Tows for Taxonomic Analyses - $\geq 201 \mu$ :**

<sup>1</sup>Available REMAP project data indicates that epilimnetic water HgT concentrations are > 2 orders of magnitude smaller than plankton concentrations which were determined during the planktonHgT method demonstration. Such concentrations are unlikely to contaminate the zooplankton samples. Thus, it is recommended that the plankton sample be kept in the lake water from was obtained until the sample is dried for biomass determination and digestion in the laboratory.

Two additional tows are composited, using the 201  $\mu$  net, into the compositing vessel using the procedure outlined above.

The contents of the compositing vessel is then covered with seltzer water, capped, and allowed to sit 60 seconds. At this time, the contents are returned to the dolphin bucket, reduced to the maximum extent possible, rinsed using the seltzer-squeeze bottle into a labeled 50 ml sample vessel, to approximately 25ml volume.

The sample is capped and allowed to sit 5 minutes. The sample is then opened, and filled to 50 ml with formalin-solution.

**Tows for Taxonomic Analyses - 45-200  $\mu$ :**

Two tows are composited using the 45  $\mu$  net, following the procedure outlined directly above.

While the 201  $\mu$  dolphin bucket is held above the assembled 45  $\mu$  net, the contents of the 45  $\mu$  composite is passed through the 201  $\mu$  dolphin bucket, and allowed to run out into the 45  $\mu$  net. This step removes plankton in the  $\geq 201 \mu$  fraction from the 45 -200  $\mu$  fraction.

The 45 $\mu$  sample is then recondensed, and transferred back to the compositing vessel.

The contents of the compositing vessel is then covered with seltzer water, capped, and allowed to sit 60 seconds. At this time, the contents are returned to the dolphin bucket, reduced to the maximum extent possible, rinsed using the seltzer-squeeze bottle into a labeled 50 ml sample vessel, to approximately 25ml volume.

The sample is capped and allowed to sit 5 minutes. The sample is then opened, and filled to 50 ml with formalin-solution.

The taxonomy samples are submitted to Dartmouth University.

Vermont - New Hampshire REMAP Mercury Project

Zooplankton Field Form

Lake \_\_\_\_\_ Date \_\_\_\_\_ Station Depth \_\_\_\_\_

**Sampling Team Initials:**

***HgT Tows:***

50ml Vessel Code: \_\_\_\_\_ 50 ml Vessel Weight (g) \_\_\_\_.

50ml Vessel Code: \_\_\_\_\_ 50 ml Vessel Weight (g) \_\_\_\_\_. (Use for duplicate)

Use for normal sample		use for duplicate sample	
Tow #	Tow length (m)	Tow #	Tow length (m)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

***Taxonomy Tows:***

201 $\mu$ Fraction		45 $\mu$ to 200 $\mu$ fraction	
Tow #	Tow length (m)	Tow #	Tow length (m)
1		1	
2		2	
Use this space for duplicate tows			
1		1	
2		2	