

# **Standard Operating Procedure for Mysid Laboratory Analysis**

**LG408**

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## Standard Operating Procedure for Mysid Laboratory Analysis

### 1.0 SCOPE AND APPLICATION

1.1 This standard operating procedure is used to identify, sex, enumerate, and measure mysids from the Great Lakes.

### 2.0 SUMMARY OF METHOD

2.1 The method involves macroscopic and microscopic examination of mysid samples. The entire sample is examined for mysids in a sorting tray. Marsupia of up to 25 gravid female mysids are examined under a stereoscopic microscope for number and stage of brood. Gravid females may have been separated from the bulk sample in the field (see SOP LG409 for sampling). Up to 125 mysids (including up to 25 gravid females) are photographed for digital length measurement.

### 3.0 SAMPLE COLLECTION AND PRESERVATION

3.1 See U. S. EPA GLNPO Standard Operating Procedure (SOP) for Mysid Sample Collection and Preservation (LG409). See the WQS SOP Master List for the current survey to locate the correct version.

### 4.0 APPARATUS

4.1 Most supplies can be acquired from biological supply companies (such as Wildlife Supply). The supplies needed are as follows:

Dissecting microscope with 6x to 50x magnification

600-mL graduated cylinder

Sieve with 200- $\mu$ m mesh

Sieve with 100- $\mu$ m or finer mesh

Funnel (mouth larger than sieve)

Glass sorting tray

Forceps

Microprobe 50-mL

Beaker

250 to 500-mL glass jars

Scintillation or similar vials

Retort stand

Clamp holder

Round burette clamp

Digital camera with ability to take at least 16 megapixel w/ macro, or 10 megapixel with super-macro

ImageJ or similar software (<http://imagej.nih.gov/ij/>)

### 5.0 REAGENTS

5.1 Reagents can be ordered through chemical supply companies. Sugar (food grade sucrose) and borax (laundry additive) can be bought locally.

**5.2** Concentrated Formalin Solution (37% Formaldehyde)

**5.3** For each gallon of formalin, add 227.4 g of sucrose and 34.1 g of borax as described in LG402A zooplankton collection methods. This concentrate has been diluted to 10% (100 ml concentrate added to the 1 L jar and topped off with lab water) in preserving mysid samples (LG409). This dilution can be made in the laboratory if original preservative needs replacement.

**6.0 ANALYTICAL PROCEDURE – MYSID SAMPLE ANALYSIS**

**6.1** Bench Data Sheet

6.1.1 Data sheet (see Appendix 1) records include: Lake, Collection Date, Sample ID, Station, Replicate, Lab Analyst, Processing Date, Location, Camera Name, Analysis Type, Prior Analyst and date [for QA or training counts]. This information should be on the field sheet and sample bottle. Also record processing information associated with this specific lab analysis including: lab analyst, processing date, location of processing, name of camera used, type of analysis (Regular, Quality Assurance, Training Solo, or Training Verification), and (if applicable) lab analyst and processing date for previous lab analysis of this sample. The bench data sheet also includes (1) data on gravid female mysids, with detailed data on up to 25 (separated in field/lab, embryo development stage, embryo count, and picture ID); (2) data on all other mysids in the sample, with detailed data on up to 100 (count and photo IDs of mysids in each of three categories: juvenile, male, and non-gravid female); and (3) count of “extra” mysids (mysids in addition to the 100) which are intact, just heads, just tails, and the total of these [calculated as # intact + max(# just heads, # just tails)]. The total number of mysids in the sample is the sum of the number of gravid females, the measured animals, and the extra animals. Check boxes on presence of other photographs and mysid species, with notes on these. Other notes: the place to record initials and date of entry into electronic database.

**6.2** Initial Sample Processing Prep

6.2.1 The steps of sample preparation listed in this section involve working with 10% formalin and must be performed under an operating fume hood while wearing proper personal protective equipment (eyes and hands). A few specific steps do not involve formalin and may be performed outside a fume hood.

6.2.2 Process samples one at a time. Open sample jar and pour contents through a 200- $\mu$ m mesh or finer sieve over funnel into beaker or formalin waste receptacle. This material may include cryovials with individual gravid females separated in the field. Using a squirt bottle, rinse the emptied sample jar with water to collect any remaining contents and pour rinse water with contents through sieve. Rinse the sample jar thoroughly with water into the sieve to remove any residual organisms adhering to walls of the sample jar. Recap the sample jar, and set aside until section 6.8. The re-sealed sample jar may be set aside outside the fume hood.

6.2.3 Pick up each individual cryovials with forceps, and thoroughly rinse off formalin residue while holding over the sieve, before setting aside in a beaker or other holder (until section 6.3). If necessary, sealed and rinsed-off cryovials may be set aside outside the fume hood.

6.2.4 Thoroughly rinse remaining sample contents with water while in sieve (over waste beaker/receptacle) in fume hood. Be sure to rinse off all formalin residue from sample and sieve.

- 6.2.5 Transfer rinsed-off sieve contents to the sorting tray. Be sure to thoroughly rinse sieve contents into the sorting tray using a squirt bottle. Set the sorting tray aside temporarily (until section 6.4). This step may be done outside the fume hood.

### 6.3 Cryovials Processing Prep

- 6.3.1 The steps of sample preparation listed in this section involve working with 10% formalin and must be performed under an operating fume hood while wearing proper personal protective equipment (eyes and hands). A few specific steps in 6.3 do not involve formalin and may be performed outside a fume hood (These include: 6.3.5, 6.3.6).
- 6.3.2 Cryovials contain individual mysids which were suspected in the field to be gravid females. Occasionally, some of these mysids are not gravid females (mistaken in the field). Place any such mysids back into the sorting tray with the rest of the sample. Make a note on the bench sheet if some cryovials contained animals that were not gravid females. The cryovials are used because embryos can fall out of a gravid female's marsupium during sample transportation, and then we cannot know which gravid female produced the embryos which we find loose in the main sample jar. Storing gravid females in individual cryovials allows any embryos that come loose during transportation to be tracked to a particular gravid female.
- 6.3.3 Process cryovials one at a time. Open a cryovial, empty its contents into a 50-mL beaker, and rinse the cryovial out into the beaker. Make sure to rinse out both the adult mysid and all loose embryos. Visually inspect the beaker (still under the fume hood) to estimate how many embryos have fallen out of the marsupium. Ensure through the rest of the process that these embryos do not get left behind in beakers, sieves, etc.
- 6.3.4 Pour and rinse the mysid, her embryos, and the formalin-water from the 50-mL beaker through a 100- $\mu$ m or finer mesh sieve into the formalin waste beaker/receptacle. Rinse gently but thoroughly with water.
- 6.3.5 Transfer rinsed-off sieve contents to an individual covered petri dish with water squirt bottle. Be sure to rinse out all embryos loose in sieve. Cap petri dish and set aside temporarily (until section 6.5). This step may be done outside the fume hood.
- 6.3.6 Recap cryovial, and set aside until section 6.8. The cryovial may be set aside outside the fume hood.
- 6.3.7 Repeat steps 6.3.2 – 6.3.6 for each cryovial from the sample.

### 6.4 Sample Processing Steps

- 6.4.1 Return to the sorting tray with rinsed biological material to sort mysids in sample. Currently, two species are present in the Great Lakes (native *Mysis diluviana* and shallow dwelling *Hemimysis anomala* introduced in 2006) but almost all mysids in offshore GLNPO samples are expected to be *Mysis diluviana* (Fig. 1). Pothoven et al. (2007) will be helpful for describing species-specific characters that differentiate *Mysis diluviana* and *Hemimysis anomala*. Also relevant is Audzijonyte and Väinölä (2005), who describe the *Mysis relicta* complex (*M. diluviana* was formerly grouped with *M. relicta*).
- 6.4.2 Remove any unusual mysids that might be neither *Mysis diluviana* nor *Hemimysis anomala* (see Fig. 1) and bring these animals to the attention of the project manager or team leader.

They will confirm the taxonomic identification and decide if the animal should be sent out for further taxonomic evaluation. Be sure to note on datasheet if other mysid species than *Mysis diluviana* are confirmed as present in sample. Three Ponto-Caspian mysid species have been introduced to Europe over time and may therefore arrive to the Great Lakes at some time; further information on these species is included below, for reference. For additional details on species-specific characters of *Katamysis warpachowski*, *Limnomysis benedeni*, and *Paramysis lacustris* (synonym: *Mesomysis czerniavskyi*), Sars (1893) is a good reference (see also: Fig. 1). The general reference for mysid biology is Mauchline (1980).

- 6.4.3 Scan the sample for any additional gravid females not in cryovials and remove them carefully, making sure no embryos are lost in the process. It is best to place a glass sorting tray over a black or white surface. A black surface is better for spotting the whole animal, whereas a white surface is better for spotting mysid eyes. Identify gravid females as animals with an embryo-filled marsupium. An embryo-filled marsupium on a preserved gravid female is typically an obvious opaque whitish color. Place these gravid females into individual covered petri dishes (up to 25 total individual gravid females for the sample).
- 6.4.4 If the total number of gravid females (from cryovials and the sorting tray) exceeds 25 for the sample, place gravid females in excess of 25 together in a separate petri dish. Animals in excess of 25 should be photographed and measured but do not need to be further processed.

## 6.5 Gravid mysid processing

- 6.5.1 Examine the individual gravid female mysids in each petri dish from steps 6.3.5 and 6.4.3 under a dissecting scope.
- 6.5.2 The total number of gravid females in cryovials [separated in the field] should have been marked in the field sheet and should also be noted in the bench sheet where specified.
- 6.5.3 Processing of gravid females from the cryovials.
  - 6.5.3.1 Number the gravid female petri dishes and fill in gravid data table on the datasheet according to this numbering.
  - 6.5.3.2 For each of the females from a cryovial (6.3.5), the “Field/Lab” column of the gravid data table on the bench datasheet should be labeled as “Field.”
  - 6.5.3.3 Note whether the pouch looks full or not. Some mysid embryos may have escaped, and mysids are suspected to lose embryos through the embryo development stages in the marsupium (Mauchline 1980).
  - 6.5.3.4 Remove embryos from the marsupium with gentle pressure using a microprobe. Record the number of embryos present (both the ones found loose in the cryovials and the ones removed with the microprobe).
  - 6.5.3.5 Record the stage of the embryos present (E = early, M = mid, L = late, Fig. 2, 3, Mauchline 1980). Early stage embryos are spherical or slightly elongated and contained within the egg membrane which is shed at the end of this stage. These are stage-I embryos (and are often referred to as “eggs”). Mid stage embryos resemble commas. Some may have slight eye development. The thoracic legs and antennae develop during this stage and the eyes become pigmented but are

covered by a thin membrane. These are stage-II embryos (and are often referred to as “larvae”). Late stage embryos resemble adults and have developed eyes that are not constrained by a membrane. These are stage-III embryos (and are often referred to as “eyed larvae”).

6.5.3.6 Set mysid with embryos in covered petri dish aside (with number maintained) until photographed (section 6.7).

6.5.4 Process lab-separated gravid females.

6.5.4.1 Number the gravid female petri dishes continuing the counts for field-separated gravid females (see step 6.5.3.1) and fill in gravid data table on the datasheet according to this numbering.

6.5.4.2 For each of the females from the sorting tray, the “Field/Lab” column of the gravid data table on the bench datasheet should be labeled as “Lab.”

6.5.4.3 Continue processing as in 6.5.3.3 – 6.5.3.6.

6.5.4.4 Set aside numbered petri dishes of individual gravid females (until section 6.7).

**6.6** Enumeration of juveniles, males and non-gravid females.

6.6.1 Stir/swirl sorting tray contents to randomly distribute mysids for sorting. Scan from one end of the tray, carefully removing all mysids (whole or partial) while progressing towards the other end of the tray. Make sure that all mysids, even small ones, are removed as you progress through the tray. Do NOT first remove the most visible mysids. This will ensure that the measured individuals are not a biased subsample of the mysids in the sample. The first 100 whole mysids of each species removed from the tray in this way should be sorted into petri dishes by species and maturity/sex.

6.6.2 Maturity/Sex of mysids

6.6.2.1 Animals smaller than 10 mm (*M. diluviana*) or 4 mm (*H. anomala*) (standard length: rostrum tip to base of telson) can be considered “juvenile” without further investigation of sexual characteristics. It is difficult to distinguish young *M. diluviana* males from females when they are <10 mm long (Pothoven et al. 2004).

6.6.2.2 Assign sex when possible for *M. diluviana* > 10 mm. Determine sex using characteristics of an adult (bifurcated and developed 4<sup>th</sup> pleopod in males; obvious marsupium in females; Fig. 4).

6.6.2.3 If sex characters are not obvious and the animal is larger than 10 mm, closely examine the animal under a microscope for a thickened 4<sup>th</sup> pleopod in early stages of development (sort with males if present), or for expanded flaps of the lamellae on the 6<sup>th</sup> and 7<sup>th</sup> thoracic legs in early stages of marsupium development (sort with females if present). Sometimes the pleopods may be tight against the abdomen; run a fine probe or forceps along the abdomen starting at the uropoda and the pleopods will usually become evident.

6.6.2.4 If neither development of marsupium nor slight development of 4<sup>th</sup> pleopod is evident, sort the mysid with juvenile mysids.

6.6.2.5 Note any unusual features of the animals.

6.6.3 Any mysids remaining after the first 100 are removed should be identified to species and counted, and the number recorded in the data sheet under “Extras: intact”. Any partial mysids (abdomens or cephalothoraxes) should also be counted and recorded as “Extras: heads” or “Extras: tails”. The number in each group should be noted on the appropriate part of the bench data sheet.

6.6.4 Note any unusual features of the sample or the animals in the sample (for example condition of the preservation).

**6.7** Photographing mysids for length measurements (first 100 animals removed from the sample).

6.7.1 Make legible labels for photographs (see Appendix 1) which include sample ID number, photograph date, analyst surname, and whether the photograph is of juveniles (“Juv”), males (“Male”), or females (“Fem”). For gravid female mysids note gravid ID number.

6.7.2 Over a dark countertop or surface, place a flat, transparent, metric ruler and the label. Place the petri dish (transparent) on top of the ruler and label. The ruler should have a sliver of white backing behind at least 10 mm of the edge in the center of the ruler to make tick-marks visible. Arrange the mysids so they are all visible in the field of view from a camera mounted on a retort stand or other device at close range (< 14 cm high; Fig. 5). It is best if no mysids are over white or light-colored surfaces, do not overlap each other, and are lying flat against the surface of the petri dish. (Fig. 6). There may be some animals that are twisted in such way that it is impossible to lay them flat, those animals should still be photographed, but will not be measured.

6.7.3 Record the filename of the photograph as displayed on the camera (e.g., “3574.jpg”) in the appropriate field of the datasheet for gravid females and for random 100 subsample.

6.7.4 Take separate pictures for each species in sample.

6.7.5 Transfer photographs to a computer hard drive and an external or cloud backup drive. Picture names should be maintained as recorded in data notebook, but should have a prefix consisting of a sample ID. Pictures should be placed in folders titled by the sample ID number.

**6.8** Re-Preserving and Archiving Mysid Samples

Each sample will be archived in two 125 ml jars, one for all mysids, and one for all remaining biological material. Standard labels will be used that match the original sample with an additional mention of “mysid” or “non-mysid plankton” and seal labels with packing tape. All mysids including gravid females and embryos will be rinsed into one glass jar with as little rinse water as possible. The original 10% formalin solution can be used to preserve the sample. The rest of the plankton will be rinsed into one or more jars and also preserved with 10% formalin. If more than one jar is required because of high biomass (biomass should represent no more than half the volume of the jar to ensure preservation) use additional jars and be sure to identify the total number of jars used (e.g. 1/2). The “mysid” and “non-mysid plankton” samples will be archived in separate well marked boxes so that future users can easily find the portion they need. Seal each sample jar lid with parafilm.

## 6.9 Mysid Digital Measurement

- 6.9.1 Open the image file in ImageJ. View photo at a magnification appropriate for accuracy.
- 6.9.2 Use the straight line tool to measure the pixel distance of 1 cm on the ruler. Be sure the length of this line is exactly perpendicular to the tick marks of the ruler; this can be done by measuring the distance at the edge of the ruler or along the baseline of the tick marks. Be sure the measuring line begins and ends on the same side of the tick marks. Go to Analysis>Set Scale. Set known distance to “10” and units to “mm” and click OK. Measurements will now be recorded directly in scaled mm. Record the measurement for the scale line (press “M” on keyboard) and draw it on photograph with measurement number for future reference (press “Alt-D” on keyboard). Note that a line will draw simply by pressing “D”, but “Alt-D” is required to print a number on the photograph when using ImageJ.
- 6.9.3 To measure a mysid, use the segmented line tool to draw a line from the tip of the rostrum to the end of the abdomen (same as the base of the telson; Fig. 7). Use as few segments as possible to capture the curvature of the dorsal surface of the mysid. Capturing this dorsal curve is a more consistent method than using a set number of segments which require subjective determination of where to place points and thus introduce substantial variability. Vertices of the line should be along the dorsal surface of the mysid. Recognize that the dorsal surface will not always be on the edge of the mysid as photographed, as it is nearly impossible to get all the mysids in a photograph to lay perfectly on their lateral sides. Record the distance of the line in ImageJ (press “M” on the keyboard). After recording the distance, save the line used for measurement taken on the photograph (press “Alt-D” on the keyboard). This allows mistakes in recording measurements to be noticed both during and after the measurement process. Note that a line will draw simply by pressing “D”, but “Alt-D” is required to print a number on the photograph (ImageJ).
- 6.9.4 Go through the mysids systematically to ensure measurement of all mysids and no duplication of measurements. The total number of measurements should be 1 more than the number recorded on the bench sheet (1 scale measurement + # mysids). If this is not the case, look through each mysid measurement to ensure no mysids were measured twice (two numbers by one mysid on the photo) or that there is a mysid in the photo that was not measured (no number and no line). In the event of a disagreement of number of mysids in photograph and number recorded on bench sheet, accept the photographed number for the data submission unless a specific note on the bench sheet addresses this discrepancy (i.e., “one 3 mm mysid not in photograph”).
- 6.9.5 If an animal cannot be measured, note that on the data file. This occur when the animal is damaged or is not laid flat against the bottom of the petri dish.
- 6.9.6 Save the photograph in ImageJ as a tif file with the same name as the original jpg file. This will preserve the measurement lines used. Saving the file as a tif is better than a jpg file because the tif file preserves the measurement lines. A tif file is also the default image type for ImageJ.
- 6.9.7 Save the measurement results in ImageJ as a \*.txt file with the same name as the saved photograph (this is the photo ID, e.g., “G1404\_003\_245”).

## 7.0 CALCULATION OF MYSID BIOMASS

7.1 Biomass of individual mysids will be calculated using this L-W regression:

$$\ln(W, \text{ dry wt in g}) = -12.27 + 2.72 \ln(L, \text{ mm}); n = 241, r^2 = 0.86 \text{ (Johannsson 1995).}$$

This equation is derived for the length measure used here (tip of rostrum to end of abdomen/base of telson), even though the original reference states otherwise (Johannsson pers. comm.). The equation for tip of rostrum to cleft of telson is:  $\ln(W, \text{ dry wt, g}) = -12.55 + 2.72 \ln(L, \text{ mm})$  (Rudstam et al. 2008).

## 8.0 CALCULATIONS AND REPORTING

8.1 Mysid data are reported as number in the sample, areal density (#/m<sup>2</sup>) and area biomass (g DW/m<sup>2</sup>) which are calculated as follows:

Number in sample = total number of intact animals (gravid+random sample+extra) + the maximum of the number of heads and the number of tails. This is a minimum number of mysids in the sample. Number in sample of separate groups is calculated by assigning the extra animals to groups based on proportions in the random sample. Gravid females are counted in the whole samples and not included in the extra or random samples.

8.1.1 Areal density:

$$D_a = \frac{N}{A}$$

where:

N = Number of organisms in sample

D<sub>a</sub> = Density of organisms in numbers per square meter

A = Area of the mouth of the net (m<sup>2</sup>) = 0.7854 m<sup>2</sup> for 1-m diameter net

8.1.2 Areal biomass: B<sub>a</sub> = D<sub>a</sub> b<sub>av</sub>

where:

b<sub>av</sub> = Average mass of an individual mysid in the sample

B<sub>a</sub> = Biomass of organisms in grams per square meter

Note the average mass is calculated using the calculated individual mass measurements, not from the average length in the sample.

8.2 Two measures of length are common in mysids, (1) from the tip of the rostrum to the end of the abdomen as used here, or (2) from the tip of the rostrum to the cleft of the telson. Because it is easier to measure to the end of the abdomen in images, and because Canada's DFO has a long history of measuring to the end of the abdomen in the Great Lakes, we use this measurement for GLNPO mysid samples. Note that mysids in GLNPO zooplankton samples are measured from the tip of the rostrum to the cleft of the telson (SOP LG403). This second measure is slightly larger than the first one, but there is a strong relationship between them (Fig. 8).

8.3 Data Entry

8.3.1 Data are transferred to an Excel spreadsheet, proof-read, and entered into an Access database. Information on individual mysid, on sample collection, and on analysis procedure are entered

in separate data tables. Test queries in the database includes checks that all samples included in the individual database have entries in the sample and analysis tables and that all lengths entered are within expected ranges. Animals smaller than 10 mm are classified as juveniles. Animals > 11 mm are classified as males or females. Animals >11 mm that were classified as juveniles because of lack of obvious sex characteristics are classified as females in the database. Animals between 10 and 11 mm may be classified as juveniles, males or females in the database.

- 8.3.2 All density and biomass calculations are made in the Access database.
- 8.3.3 All field sheet and bench sheets should be scanned and stored on a secure cloud storage. Original field and bench sheet will be kept with the grantee until both samples and these original sheets are submitted to EPA for long-term archiving.
- 8.3.4 Data should also be backed up on a secure cloud-based storage device.

## 9.0 QUALITY CONTROL AND METHODS PRECISION

### 9.1 Duplicate analyses

- 9.1.1 Ten percent of all samples taken a given year are analyzed in duplicate by a second analyst. The second analyst will scan the remaining sample, count, sex and stage the animals separated by the first analyst, and re-measure lengths on one photograph. Thus, all mysids, and separately all gravid females, are recounted by the second analyst but only a subset are re-measured.
- 9.1.2 These data are not combined with the primary data as only one picture is used for QA length measurements. Instead the counts of total number of mysids, of gravid females, and the average length from one photographs from both analysts are reported in a separate QA table in the data submissions.
- 9.1.3 It is expected that the counts and measurements by two analysts should be no more than 10% different from each other. The QA parameters used for mysids are total counts, counts of gravid females, and average lengths.
- 9.1.4 If there are discrepancies, the project leader will discuss with the analysts and determine if samples need to be recounted, resorted, or pictures re-measured. If the animals are small, even small differences in length measurements may result in lower agreement. Explanations for failed QA data should be given in the submission letter.

### 9.2 Training

- 9.2.1 New analysts will be trained under direct supervision of an experienced analyst on at least 3 samples, at least one of which is from spring, and one of which is from summer. All three embryo stages should be viewed. Other species that may be present (*H. anomala*, other possible invaders) should be viewed using voucher specimens.
- 9.2.2 This phase should be continued until the new analyst recalls the processing steps, accurately determines species, sex and embryo stages, captures good photographs, and takes accurate digital measurements.

- 9.2.3 Then, the next two samples analyzed by the new analyst will also be assessed by the experienced analyst and their results compared as per section 9.1.1 and 9.1.3. If both samples are within the 10% limit in all criteria, then the new analyst can analyze samples on their own.
- 9.2.4 The most difficult aspects of training are the determination of sex for immature animals > 10 mm in length and the digital measurement technique (placing anchor points of segmented line). Trainee should be confident and accurate with these procedures before training is complete.
- 9.2.5 When available, samples from other surveys can be used to train new analysts.

## 10.0 SAFETY AND WASTE DISPOSAL

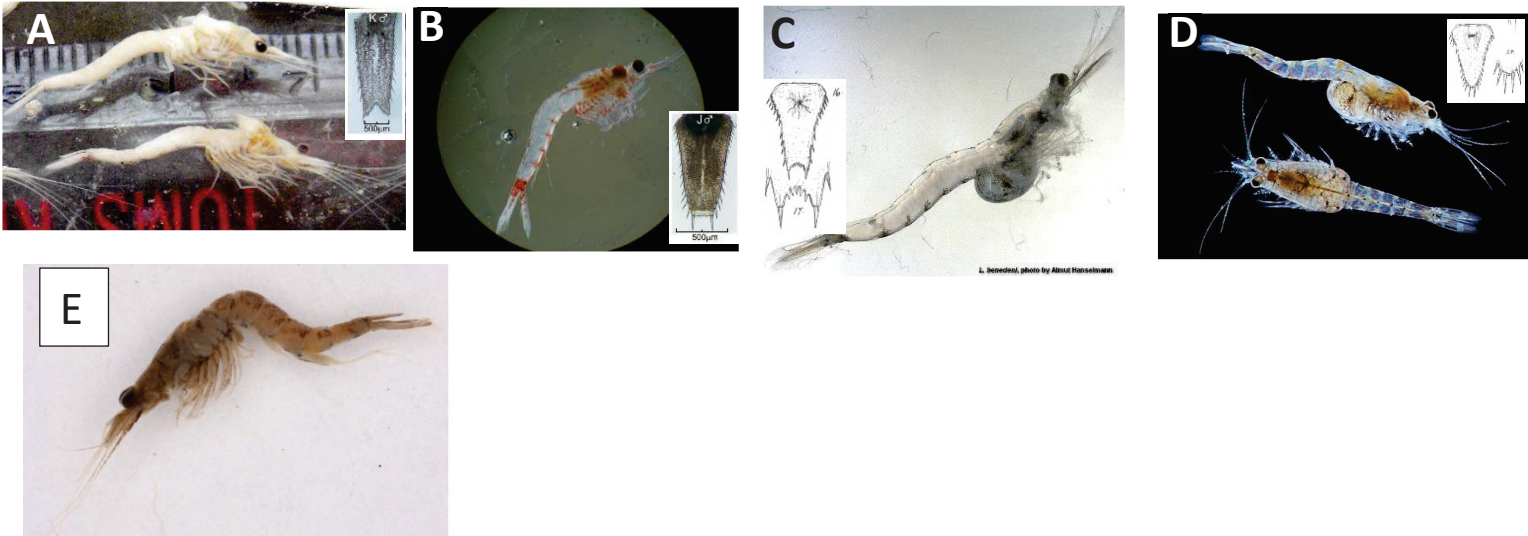
- 10.1 Proper PPE should be worn in the laboratory while handling and preparing samples for analyses (this should include appropriate gloves and safety glasses when handling formalin (10% solution). Follow all laboratory waste disposal guidelines regarding the disposal of the 10% formalin.

## 11.0 REFERENCES

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## FIGURES

FIGURE 1: GREAT LAKES MYSIDS AND TWO POTENTIAL INVADERS



Pictures of mysids that are or may be in the Great Lakes, with respective insets of species telson. A) *Mysis diluviana*, B) *Hemimysis anomala*, C) *Limnomysis benedeni*, D) *Katamysis warpachowski*, and E) *Paramysis lacustris*. Form is more important than color or transparency; most will lack color (as *M. diluviana* in A) when preserved. *Mysis diluviana* (formerly *M. relicta*) is native in the Great Lakes. *Hemimysis anomala* is native to the Ponto-Caspian region, introduced to Europe in the 1950s, and was detected in Lake Michigan in 2006 and has expanded throughout the basin (Pothoven et al. 2007). It frequents shallow rocky habitats and dock pilings and thus does not overlap with *Mysis diluviana*. *Limnomysis*, *Katamysis*, and *Paramysis* are also native of the Ponto-Caspian region and have been introduced to Europe, but have not yet been found in North America.

The following observations are based on voucher specimens held at Cornell and taxonomic resources:

- *Mysis diluviana* have a V-shaped cleft in their telson, with straight-to-convex lateral margins. Antennal scales fully setose margins. Uropoda are also setose all round. (A)
- *Hemimysis anomala* have straight, posterior, setose margin of telson. *Hemimysis* also have the proximal half of the lateral edge of their antennal scales non-setose. (B)
- *Limnomysis benedeni* telson has a rounded, U-shaped cleft, with spinulose concave lateral margins. Brood pouch laterally wider and with markings on lateral surfaces of brood pouch, rather than ventral surface as in *M. diluviana*. Antennal scales tapering in lanceolate form, with narrow base; dorsal texture of scale corrugated like a sea-shell. (C)
- *Katamysis warpachowski* have a convexly-rounded posterior margin of telson. *K. warpachowski* thoracic legs stubby, and held tightly to ventral surface of thorax; thoraxes dorso-ventrally compact. *K. warpachowski* uropoda margins are with short setae. Texture of uropod akin to *L. benedeni* antennal

scale, but less pronounced. (D)

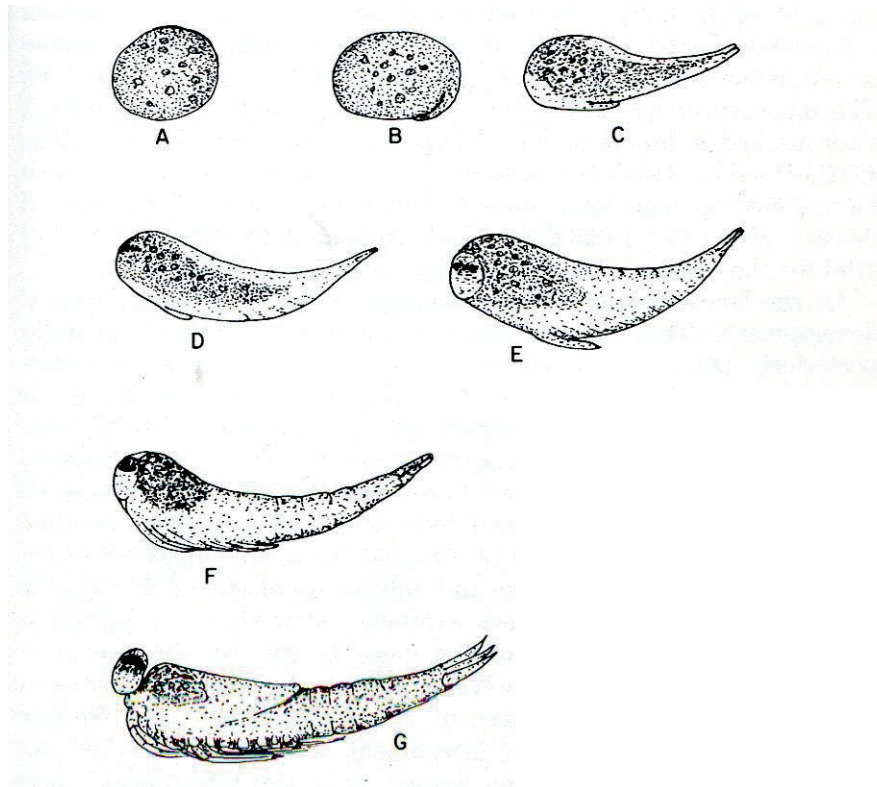
- *Paramysis lacustris* (Syn: *Mesomysis czerniavskyi*) are the most similar to *Mysis diluviana* in appearance. The major difference is in the antennal scale (Sars, 1893, Plate 5). The outer margins are smooth and non-setose, forming into a spiniform projection at anterior end, which is somewhat anteriorly truncated. Anterior and interior margins of antennal scales are setose. (E)

See Pothoven et al (2007) for more information about *M. diluviana* and *H. anomala* characters, and Sars (1893) for more information about *K. warpachowski* and *L. benedeni* characters. *M. diluviana* photograph by Toby Holda; *H. anomala* photograph by NOAA GLERL *L. benedeni* and *K. warpachowski* photographs by Almut Hanselmann; *Paramysis lacustris* image from Alex Borisenko, telson drawings from Sars (1893), and telson photographs from Pothoven et al (2007).

**FIGURE 2: PHOTOGRAPHS MYSIS EMBRYO STAGES**

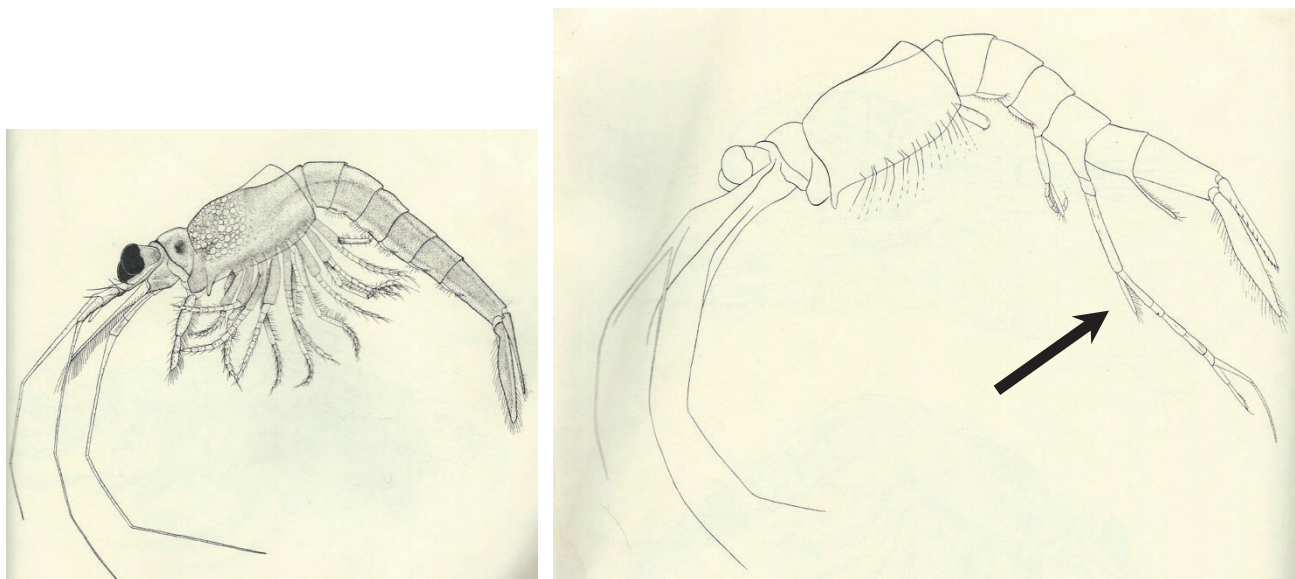


Pictures from Kelly Bowen and Toby Holda

**FIGURE 3: DRAWINGS MYSID EMBRO STAGES**

From Mauchline (1980) Fig 2.17. Larvae of a mysid. A. egg-shaped embryo (stage I or Early stage), B embryo with developing abdomen (stage I, Early stage), C-F stage II (Mid-stage) larvae at successive stages of development, G stage III (Late stage) larva after the first larval molt and with the eyes stalked. Stages I, II, and III correspond respectively to early, mid, and late stages in this protocol.

**FIGURE 4: FEMALE AND MALE MYSIS DILUVIANA**



Drawings by Nancy Korda. Note the enlarged fourth pleopod for the mature male in right photo indicated by the arrow. Gravid females have a distinctive marsupial pouch with embryos.

**FIGURE 5: CAMERA STAND**

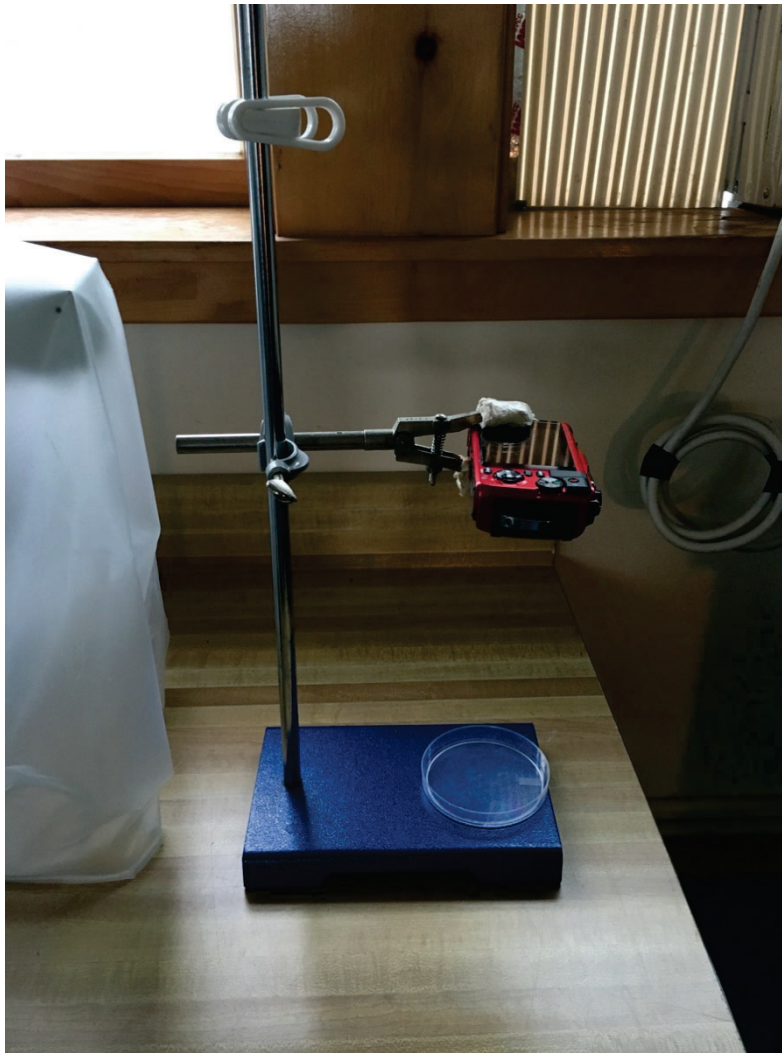
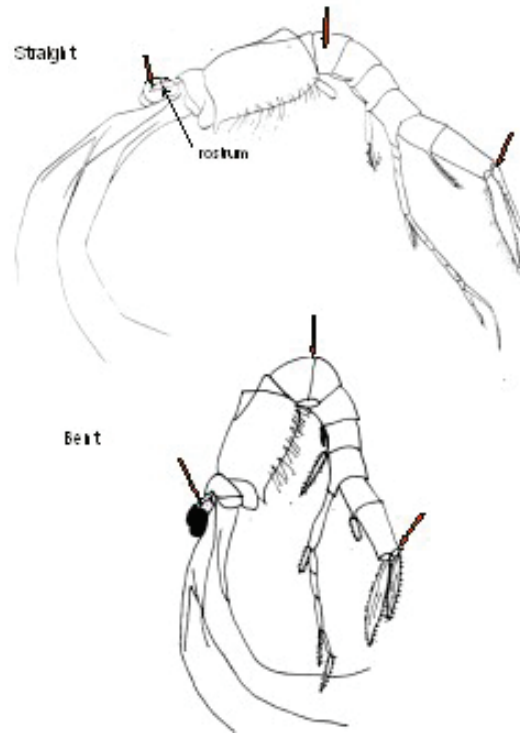


FIGURE 6: MYSID PHOTOGRAPH SETUP



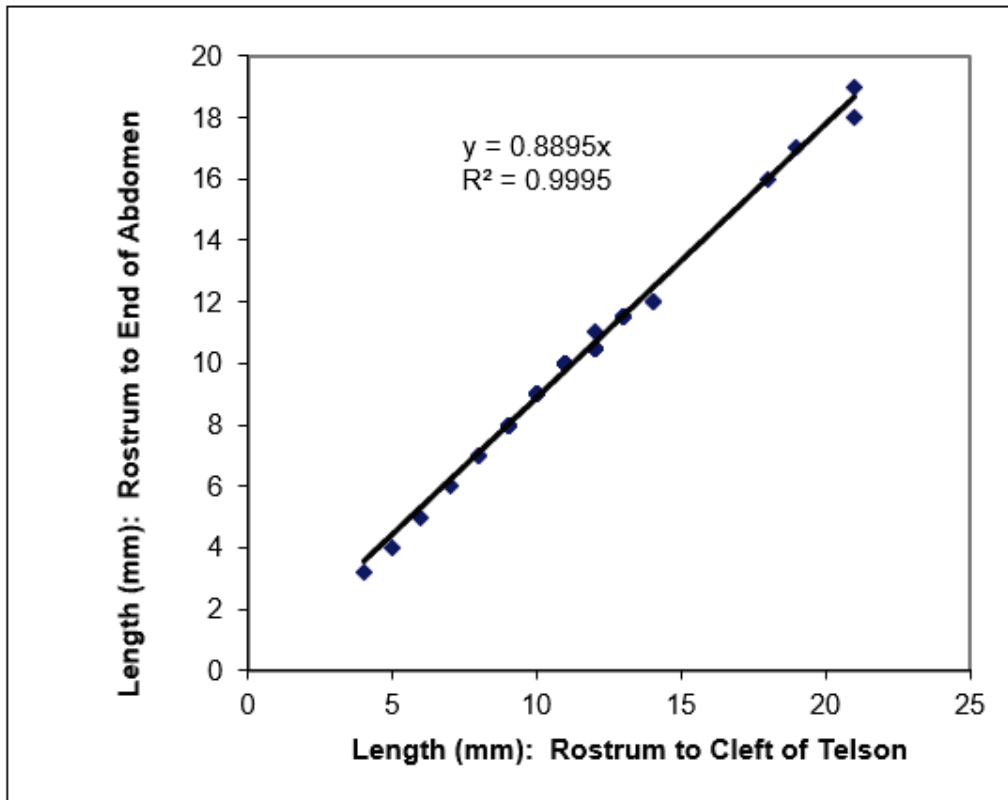
*Mysis diluviana* arranged in petri dish. Note the dark background, the shading of the dish limiting glare in area where there are mysids, and the spacing of mysids so they do not overlap. Also, note the label format needed within image.

**FIGURE 7: MYSID LENGTH MEASUREMENTS**



Measure mysid from the tip of the rostrum to the end of the last abdominal segment.

**FIGURE 8: RELATIONSHIP BETWEEN COMMON MYSID LENGTH METRICS**



Regression of mysid length as measured from tip of rostrum to end of abdomen as a function of mysid length as measured from tip of rostrum to cleft of telson (see Fig. 7). From Ted Schaner and Michael Connerton.

**APPENDIX 1: FORMS**

**Bench Data Sheet Format**

Lake: \_\_\_\_\_ Coll Date: \_\_\_\_\_ Sample ID: \_\_\_\_\_ Station: \_\_\_\_\_ Rep: \_\_\_\_\_  
 Lab Analyst: \_\_\_\_\_ Process Date: \_\_\_\_\_ Location: \_\_\_\_\_ Camera Name: \_\_\_\_\_  
 Analysis Type: R QA TS TV Prior analyst and date (if applicable): \_\_\_\_\_

**Data on Gravid female *Mysis diluviana* in sample:**

Data on photographed Gravid females (up to 25)

ID	Field/Lab	EmbStg	BrdCnt	PicID	PchFull?
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					

ID	Field/Lab	EmbStg	BrdCnt	PicID	PchFull?
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					

# Loose Embryos E\_\_M\_\_L\_\_ Pic ID \_\_\_\_\_

# Vial Yes \_\_\_\_\_ # Vial No \_\_\_\_\_ # >25 \_\_\_\_\_  
 Total Number Gravid (Sum 3 above) \_\_\_\_\_

**Data on all *Mysis diluviana* in sample (except gravid females):**

Random Subsample Counts (up to 100 total) and Photo IDs

Sex & Maturity	<i>Mysis</i> <10 mm	Male	Non-Gravid Female	Total
Count				
Pic ID				

Count "Extra" mysids: # Intact: \_\_\_\_\_ # just heads \_\_\_\_\_ # just tails: \_\_\_\_\_ Total \_\_\_\_\_ (sum of # intact and max of (# heads, # tails))

Total Mysids in Sample (Sum of totals for Gravid Females, Random Subsample, and "Extra") \_\_\_\_\_

Other photographs? Yes/No

If yes, list Pic ID with description and count of items in these photographs:

Other species present? Yes/No (Species name if yes: \_\_\_\_\_)

Details (Pic ID, "Extra count, etc.):

Other Notes:

Entered into Analysis Data Table by (initials) \_\_\_\_\_ on (date) \_\_\_\_\_

### Label Formats

#### Photograph Labels:

[Sample ID]	[Analyst Surname]
[Processing Date] Pic	[Picture # for sample] [Sex/maturity of mysids]

Example:

13GM12S15 Holda 11-24-14 Pic1 Juv
--------------------------------------

#### Vial Labels:

[Sample ID] [Analyst Surname]
[Processing Date] [Vial Type]

Example:

13GM12S15 Holda 11-24-14Sized
----------------------------------

#### Cryovial/Female Gravid Photo Labels:

[Sample ID] FGr [Gravid ID]
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Example:

13GM12S15 FGr 11
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#### Processed Sample:

GLNPO- BMP MysidNet
[Sample ID] [Station ID] Survey
[Collection Date]
[Sample type, mysid or non-mysid]

Examples:

GLNPO – BMP - MysidNet
12GM12S15 MI 41
Spring 2013 3-29-13
Mysids

GLNPO – BMP - MysidNet
12GM12S15 MI 41
Spring 2013 3-29-13
Non-Mysid Plankton