

Standard Operating Procedure for Mysid Analysis

LG408

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1.0 SCOPE AND APPLICATION

- 1.1 This standard operating procedure is used to identify, sex, enumerate, and measure the mysid populations 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 by eye in a sorting tray. Up to 100 mysids are photographed for digital measurement. Marsupia of 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 for sampling).

3.0 SAMPLE COLLECTION AND PRESERVATION

- 3.1 See U. S. EPA GLNPO Standard Operating Procedure (SOP) for Mysid Sample Collection and Preservation.

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- μ m mesh

Sieve with 100- μ m or finer mesh

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.

- 5.2 The reagents needed are as follows:

Ethanol (95% Ethyl Alcohol solution)

6.0 ANALYTICAL PROCEDURE – MYSID SAMPLE ANALYSIS

6.1 Bench Data Sheet Format

6.1.1 Data sheet should record (See Appendix 1): Sample ID; Station ID; Collection Date; Collection Time; Tow Depth Start; Tow Depth End; Replicate; Processing Date; and Analyst Surname; Camera Used; the number of each species of mysid which were not photographed for digital measurement. Also recorded are the numbers of immature, male, female non-gravid, female gravid, and female spent mysids in the first 100 separated individuals of both *M. diluviana* and *H. anomala*. Recorded next to the count are the camera's PicIDs of the photographs associated with each of these groups. Finally, a table of 25 rows for the previously-separated gravid females will record length, embryo stage, brood size, and the camera's PicID.

6.2 Mysid Enumeration and Sorting

6.2.1 Samples should be processed one at a time. Under the hood, rinse the sample from the sample jar through a 200- μ m mesh sieve with water to remove the formalin.

6.2.2 Rinse the sample bottle thoroughly with water into the 200- μ m mesh sieve to remove any residual organisms adhering to walls of the bottle. All containers from which mysids are transferred are to be rinsed thoroughly with water, including sieve, glass sorting tray, and petri dishes. Once mysids have been thoroughly rinsed of the formalin, wash the sample into a glass sorting tray.

6.2.3 Enumeration

6.2.3.1 Start scanning from one end of the sorting tray and remove the first 100 individual mysids encountered with a forceps. This will ensure that the measured individuals are not a biased subsample of the mysids in the sample. If more than one species is present, remove up to 100 individuals of each species in this manner. Place these into separate petri dishes by species. Currently two species are present in the Great Lakes (*Mysis diluviana* and *Hemimysis anomala*). *M. diluviana* is present in open water samples; *Hemimysis* is a shallow water species (Walsh et al. 2012). Other possible species include *Limnomysis benedeni*, *Katamysis warpachowski*. (see Fig. 1). These species are invasive in Europe (Borza 2014) but have not yet been observed in North America. *Paramysis lacustris* is another species to watch for (Borza, pers. comm.). *L. benedeni* is more similar in appearance to *M. diluviana*. More information on *L. benedeni* can be found in Kelleher et al. (1999), who detected this species in shallow waters near the Dutch Rhine. Voucher specimens of all five species are available at the Cornell Biological Field Station (thanks to Peter Borza for providing specimens of *L. benedeni*, *K. warpachowski*, and *P. lacustris*).

6.2.3.2 Remove any unusual mysids, including all mysids that may not be either *M diluviana* or *H anomala* 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.

6.2.3.3 Any mysids after the first 100 should be identified to species, counted and the number recorded in the data sheet. Place them directly into a vial with ethanol ensuring a final

concentration of 70-80% ethanol in the vial (this will require 95% Ethanol as some water will be retained by the mysids). There will be two vials; one for the first 100 animals used for length measurements and another for additional animals. Both vials will be labeled (see Appendix 1) with sample ID, processing date, analyst surname, and either, “Sized,” or, “Extra,” depending on whether the contained mysids were photographed for measurement (sized) or not (extra). Do not fill a vial to more than 30% with mysids to ensure adequate preservation (in 70% ethanol). If one of the vials is too full to add more mysids of its group, use another vial with the same label. When using more than one vial, add vial number to the label (e.g., vial 1 of 2 and vial 2 of 2 when using two vials for a group). Preserve additional mysid species in separate vials. Store all vials within a larger jar with enough 70% ethanol to cover all vials.

6.2.3.4 If there are any mysid heads or bodies (animals damaged during sampling), count the number of heads and bodies separately. The greater of the two counts will be added to the number of mysids in the sample. If there are less than 50 intact mysids, then detached heads may be used for length measurements using the antennal scale length on each head (Grossnickle and Beeton 1979). Mysid heads should go into “Extra” or “Sized” vials as they are unmeasured or measured, respectively (see Appendix 1). Mysid bodies should always go in the “Extra” vial. However, if there are no other “Extra” mysids, bodies and/or heads may go in the “Sized” vial.

6.2.3.5 As desired, other large crustaceans, aquatic insects (e.g., *Chaoborus*), or larval fish should be enumerated and the number entered on the data sheet as a note.

6.2.3.6 Note any unusual features of the sample or the animals in the sample (for example condition of the preservation). Return the rest of the sample to the sample jar and preserve in 70% ethanol.

6.2.4 Individually Preserved Gravid Females.

6.2.4.1 If gravid females were found and removed from the sample in the field, they should be in individual bullet tubes in the sample jar. Note that each individual may have loose young in the individual bullet tube, and these must be included in the brood count specific to each individual. Thus, females must be processed one at a time.

6.2.4.2 Individual bullet tubes of gravid females are stored in formalin, and should be opened under an operating fume hood while wearing proper personal protective equipment (eyes and hands).

6.2.4.3 Begin by pouring and rinsing out a bullet tube into a 50-mL beaker. Ensure enough water is used in rinsing to remove all young that may have fallen out of the female’s marsupium during sample transportation, and ensure that the bullet tube is cleansed of formalin.

- 6.2.4.4 Visually inspect the beaker (still under the fume hood) to estimate how many brood have fallen out of the marsupium; ensure through the rest of the process that these young do not get left behind in beakers, sieves, etc.
- 6.2.4.5 Pour and rinse the mysid, her young, and the formalin-water in the 50-mL beaker through a 100- μ m or finer mesh, ensuring that the filtrate is captured for proper formalin waste disposal. Rinse the filter contents into a large petri dish.
- 6.2.4.6 Under the dissecting microscope, measure the female mysid on a ruler, from the tip of the rostrum to the base of the telson. Record the length (nearest 1 mm, rounding down).
- 6.2.4.7 Gently pry open the marsupium and classify the embryos as E (early-), M (mid-), or L (late-) stage (Fig. 2, 3). Early-stage is spherical and contained within the egg membrane which is shed at the end of this stage. Mid-stage are comma-shaped, and do not have much eye development. The thoracic legs and antennae develop during this stage and the eyes become pigmented but are covered by a thin membrane. Late stage resemble adults and have developed eyes that are not constrained by a membrane. Enumerate the brood and record the count. Photograph female and brood through scope if desired.
- 6.2.4.8 Place the female and all her brood in the appropriate vial. All bullet-tube females and their broods should be returned to the bullet tube they came from. These bullet tubes will be placed in the sample jar with the vials when archived. This will allow QA counts and measurements to be made between analysts concerning lengths, brood stage, and brood counts of these gravid females.
- 6.2.4.9 Repeat steps 6.2.4.2 – 6.2.4.8 for each individually preserved gravid female mysid.
- 6.2.5 Sort first 100 mysids of each species by sexual characters.
- 6.2.5.1 Take individual mysids from the petri dish containing the first 100, and sex them under the scope (see 7.2.6). Place immature, male, female non-gravid, and female gravid/spent mysids in four different petri dishes for digitization.
- 6.2.6 Sexual characters
- 6.2.6.1 Animals smaller than 9 mm (*M. diluviana*) or 4 mm (*H. anomala*) can be considered immature without further investigation of sexual characteristics. It is difficult to distinguish young *M. diluviana* males from females when they are <11 mm long (Pothoven et al. 2004). However, assign sex when possible for *M. diluviana* > 9 mm
- 6.2.6.2 If an individual mysid has obvious characteristics of an adult (highly-developed 4th pleopod in males, and marsupium in females; Fig. 4), sort the animal accordingly. For females, sort into two separate dishes by status as non-gravid or gravid/spent.

- 6.2.6.3 If sex characters are not obvious, closely examine the animal under a stereoscope for a thickened 4th pleopod in early stages of development (sort with males if present), or for expanded flaps of the lamellae on the 6th and 7th thoracic legs in early stages of marsupium development (sort with females if present). Sometimes the pleopods may be tight against the abdomen; if you run a fine probe or forceps along the abdomen starting at the uropoda, they will usually become evident.
- 6.2.6.4 If neither development of marsupium nor slight development of 4th pleopod is evident, sort the mysid with immature mysids for mysids 9 to 11 mm, or with female mysids for mysids > 11 mm. Note any unusual features of the animals.

6.3 Photograph first 100 mysids of each species

- 6.3.1 Make legible labels for photographs (see Appendix 1) which include sample ID number, photograph date, analyst surname, photograph number, and whether the photograph is of immature mysids (“Imm”), males (“Male”), non-gravid females (“FmNG”), or gravid females (“FmGr”), or spent females (“FmSp”).
- 6.3.2 Over a dark countertop or surface, place a flat, transparent, metric ruler, the label, and the appropriate petri dish (see 6.2.6) on top. 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 between 7.5 cm and 14 cm high (Fig. 5). It is preferable to take more pictures at a distance of 7.5 cm than fewer pictures at a distance of 14 cm. Make sure no mysids are over white or light-colored surfaces. Be sure no mysids are overlapping one another, especially near the tip of the rostrum, the end of the abdomen/base of the telson, or the dorsal surface (Fig. 6).
- 6.3.3 Check that the camera’s macro or super-macro setting is enabled. Shade the petri dish as necessary to remove any glare that would obscure mysids. Capture the petri dish of mysids, being careful not to bump or disturb the camera. Ensure the capture was crisp, that there was no obscuring glare, that mysids did not overlap with each other or with a light-colored background and that all mysids were entirely within the picture.
- 6.3.4 Record the camera’s picture ID (e.g., 100-0193) in the appropriate field of the datasheet (PicID for: “Immature”, “Male”, “Female Non-Gravid”, or “Female Gravid & Spent”). Take separate pictures for *M. diluviana* and *H. anomala*.
- 6.3.5 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, and these folders should be placed in a folder for GLNPO_MysidSample>Photographs>SurveyYear.

6.4 Mysid Digital Measurement

- 6.4.1 Open the image file in ImageJ. If necessary, rotate the image until the label appears upright. Set zoom to 100%.
 - 6.4.2 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). If the mysid is straight, use one segment. If the mysid is bent or curved, use up to three segments to capture the length. Some extreme contortions may warrant four segments (Fig. 7). Vertices of the line should be along the dorsal surface of the mysid. Record the distance of the line in ImageJ
 - 6.4.2 Go through the mysids systematically to ensure measurement of all mysids and no duplication of measurements.
 - 6.4.2 Use the straight line tool or one segment of the segmented 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 by measuring the distance at the edge of the ruler or along the baseline of the tick marks. Also, be sure the measuring line begins and ends on the same side of the tick marks. Record the measurement in ImageJ.
 - 6.4.3 Copy the distances recorded in ImageJ to a spreadsheet. Use the final distance entry as a scale to convert all distances to length in mm (at least nearest 0.1 mm). Each photograph should have its own scaling distance.
- 6.5 Taxonomic References
- 6.5.1 The general reference for mysid biology is Mauchline (1980). 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*). For more details on species-specific characters of *Katamysis warpachowski*, *Limnomysis benedeni*, and *Paramysis lacustris* (synonym: *Mesomysis czerniavskiy*), Sars (1893) is a good reference.
- 6.6 Archiving Mysid Samples
- 6.6.1 Sample labels
 - 6.6.1.1 Jars of processed samples should be labeled (see Appendix 1) with the sample ID number, survey, lake, station, date of collection, target organisms (“Mysids”), date of processing, and analyst surname.
 - 6.6.1.2 Vials should be labeled (see Appendix 1) with sample ID number, date of processing, analyst initials, and either, “Sized,” or, “Extra.”
 - 6.6.2 All contents of vials and jars should be preserved in final concentration of ethanol of at least 70%. Vials should be placed in jars with contents of jar, and then jars should be filled with 95% ethanol

until adding water will produce a 70% ethanol solution. Then, they should be sealed with a watertight screw-cap lid. Lids should be sealed with parafilm.

6.6.3 Recording-Keeping

6.6.3.1 When a sample has been processed, an entry should be made in an electronic sample-status file, with the sample ID and date of completion. The lab analyst surname should be entered into a sample database table (See 8.4).

6.6.3.2 Handwritten data sheets (see Appendix 1) should be scanned and stored with the electronic data files in a folder for each survey. A backed-up file should be kept up-to-date as to each sample's location and status as not processed, processed, and measured. A Meta ID denoting stage of processing should be associated with each Sample ID.

7.0 CALCULATION OF MYSID BIOMASS

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

(Equation 2): $\ln(W, \text{ dry wt in g}) = -12.27 + 2.72 \ln(L, \text{ mm})$ $n = 241, r^2 = 0.86$ (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.). See Rudstam et al. (2008) for the equation for tip of rostrum to cleft of telson, $\ln(W, \text{ dry wt, g}) = -12.55 + 2.72 \ln(L, \text{ mm})$.

8.0 CALCULATIONS AND REPORTING

8.1 Mysid data are reported as number and biomass of mysids per square meter, which are calculated as follows:

8.1.1 Areal density:

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

where:

N = Number of organisms
D_a = Density of organisms in numbers per square meter
A = Area of the mouth of the net (m²) = 0.7854 m² for 1-m diameter net

8.1.2 Areal biomass:

$$B_a = \frac{B}{A}$$

where:

B = Biomass of organisms

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- B_a = Biomass of organisms in grams per square meter
- A = Area of the mouth of the net (m²) = 0.7854 m² for 1-m diameter net

8.1.3 *M. diluviana* are highly concentrated in a compressed layer just below the thermocline. So, an evenly distributed population would appear to decline in deeper water if densities or biomass were measured volumetrically; but this would be a mere artifact of a larger denominator, not of more greatly dispersed animals. Therefore, we do not report volumetric density or biomass.

8.2 Two measures of lengths are common in mysids, (1) from the tip of the rostrum to either the end of the abdomen as used here, or (2) from the tip of the rostrum to the end of cleft of the telson. Because it is easier to measure to the end of the abdomen on pictures, 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. There is a strong relationship between the two measures (Fig. 8):

$$[\text{Length to cleft of telson}] = 0.8895 * [\text{Length to end of abdomen}]$$

(Source Ted Schaner and Michael Connerton, R² = 0.9938, N=16).

8.3 Mysids are placed in 1 mm size bins to determine size frequency distributions. Use length categories as [x.0 ≤ Length < y.0] for category x-y mm. An extrapolation example follows:

Length in mm	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	
No. indiv.	10	26	35	11	4	6	7	1	= 100 animals

If there were 211 animals in the sample, the extrapolated size frequency would be:

Length in mm	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	
No. indiv.	21.1	73.9	54.9	23.2	4.2	12.7	14.8	6.3	= 211 animals

If animals were removed in the field, then the removed animals should have been measured as well. However, these removed animals were likely chosen non-randomly (e.g., we were looking for females). For these, calculate the extrapolated size frequency for the preserved sample (random), then add on the removed animals. The same is true for sex ratios. Calculate the extrapolated number of M, F, and I in the sample ignoring the removed animals, and then add the number of removed animals in the categories M, F, and I to the extrapolated values for the sample. An example:

In the example with 211 animals, if we had removed 10 with a size distribution as follows:

Length in mm	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	
No. indiv.	0	0	0	0	0	2	3	5	= 10 animals

Then the sample size distribution would be as follows:

Length in mm	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	
<i>No. indiv.</i>	21.1	73.9	54.9	23.2	4.2	14.7	17.8	11.3	= 221 animals

8.4 Data Entry

8.4.1 Data should be transferred to an electronic database.

8.4.2 All mysid calculations are made using a spreadsheet program such as Excel, a database program, or in R. The following items are to be submitted for data review:

8.4.2.1 A scan copy of all the bench sheets.

8.4.2.2 A digital copy of all the data entered and calculated results.

8.4.3 Backup/duplication must be made of all data submitted to EPA. Data should also be backed up on a secure cloud-based storage device (e.g. CornellBox for Cornell University).

9.0 QUALITY CONTROL AND METHODS PRECISION

9.1 In general, ten percent of all samples are analyzed in duplicate by a second analyst (identified, counted, and measured on the photographs). The second analyst will scan the remaining sample, count, sex and stage the animals separated by the first analyst, and re-measure lengths of one photograph.

9.2 Results from the second analyst are reported under the same sample number as the original sample, with the exception that the last letter is a "Q."

9.3 It is expected that the two analysts should be within 10% of each other for total count in the sample, average length of the mysids (arithmetic mean), immature to adult ratio (counting all *M. diluviana* smaller than 9 mm as immature), and male to female ratio (for *M. diluviana* larger than 9 mm), average length and brood count of analyzed gravid females, and proportion of analyzed gravid females with Early, Mid and Late stage embryos. If not, the reasons for the discrepancies between the analysts should be discussed with the project manager.

9.4 Training

9.4.1 New analysts will be trained by experienced analysts for at least five samples which the experienced analyst will perform, at least two of which are from spring, and two of which are from summer. All three embryo stages should be viewed, and *H. anomala*, *M. diluviana*, and other voucher specimens should be viewed.

9.4.2 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.

9.4.2 Then, the next three samples analyzed by the new analyst will also be assessed by the experienced analyst and their results compared as per section 9.3. If all three are within the 10%, then the new analyst can analyze samples on their own.

9.4.3 When necessary, samples from other surveys available at Cornell University can be used to train new analysts.

10.0 SAFETY AND WASTE DISPOSAL

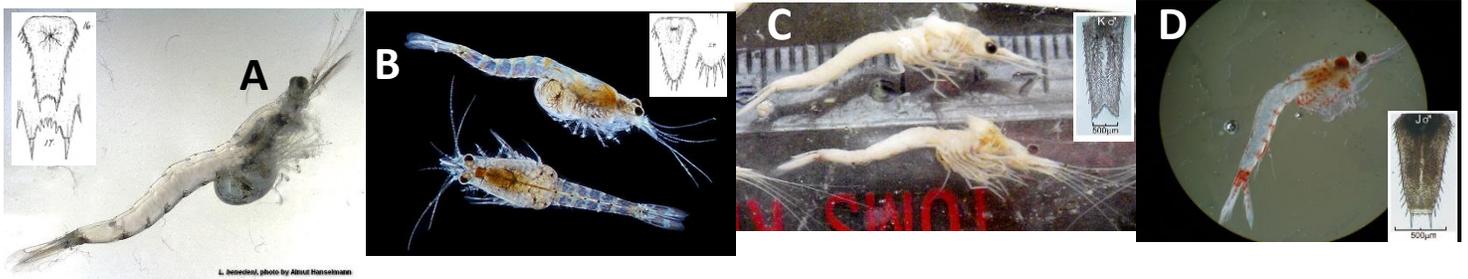
10.1 Proper PPE should be worn in the laboratory while handling and preparing samples for analyses (this should include appropriate gloves and safety glasses when handling formalin (37% formaldehyde). Follow all laboratory waste disposal guidelines regarding the disposal of formalin (37% formaldehyde) and ethanol (95% ethyl alcohol) solutions.

11.0 REFERENCES

- 11.1 Audzijonyte, A., Väinölä, R., 2005. Diversity and distributions of circumpolar fresh- and brackish-water *Mysis* (Crustacea : Mysida): descriptions of *M. relicta* Lovén, 1862, *M. salemaai n. sp.*, *M. segerstralei n. sp.* and *M. diluviana n. sp.*, based on molecular and morphological characters. *Hydrobiologia* 544, 89-141.
- 11.2 Borza, P., 2014. Life history of invasive Ponto-Caspian mysids (Crustacea:Mysida): A comparative study. *Limnologica* 44, 9-17.
- 11.3 Grossnickle, N.E. and A.M. Beeton. 1979. Antennal scale length as a measure of relative size in the opossum, shrimp, *Mysis relicta* Loven. *Crustaceana* 36: 141-146.
- 11.4 Johannsson, O.E., 1995. Response of *Mysis relicta* population dynamics and productivity to spatial and seasonal gradients in Lake Ontario. *Can. J. Fish. Aquat. Sci.* 52, 1509-1522.
- 11.5 Kelleher, B., G. Van der Velde, K. J. Whittmann, M. A. Faasse, and A. Bij de Vaate. 1999. Current status of the freshwater Mysidae in the Netherlands, with records of *Limnomysis benedeni* Czerniavsky, 1882, a Pontocaspian species in the Dutch Rhine branches. *Bulletin Zoologisch Museum University van Amsterdam* 16: 89-94.
- 11.6 Mauchline, J., 1980. The biology of mysids and euphausiids. In: Blaxter, J.H.S., Russell, F.S., Younge, M.s (Eds.), *Advances in Marine Biology*, pp. 1-369.
- 11.7 Pothoven, S.A., G. L. Fahnenstiel, and H.A. Vanderploeg. 2004. Spatial distribution, biomass and population dynamics of *Mysis relicta* in Lake Michigan. *Hydrobiologia* 522: 291-299.
- 11.8 Pothoven, S. A., I. A. Grigorovich, G. L. Fahnenstiel, and M. D. Balcer. 2007. Introduction of the Ponto-Caspian bloody-red mysid *Hemimysis anomala* into the Lake Michigan Basin. *Journal of Great Lakes Research* 33: 285-292.

- 11.9 Rudstam, L.G., Schaner, T., Gal, G., Boscarino, B.T., O'Gorman, R., Warner, D.M., Johannsson, O.E., Bowen, K., 2008. Hydroacoustic measures of *Mysis relicta* abundance and distribution in Lake Ontario. *Aquatic Ecosystem Health and Management* 11: 355-367.
- 11.10 Sars, G. O. 1893. *Crustacea Caspia*, contributions to the knowledge of the carcinological fauna of the Caspian Sea. Part I, Mysidae. Imprimerie de L'academie Imperiale des Sciences, St. Petersburg.
- 11.11 Walsh, M.G., Boscarino, B.T., Marty, J., Johannsson, O.E., 2012. *Mysis diluviana* and *Hemimysis anomala*: Reviewing the roles of a native and invasive mysid in the Laurentian Great Lakes region. *J. Great Lakes Res.* 38 (Supplement 2): 1-6.

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) *Limnomysis benedeni*, B) *Katamysis warpachowski*, C) *Mysis diluviana*, D) *Hemimysis anomala*. Form is more important than color or transparency here; most will lack color as *M. diluviana* in (C) once fixed in formalin and/or preserved in ethanol.

The following observations are based on voucher specimens in the lab. They are confirmed by taxonomic resources unless otherwise noted.

M. 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.

H. anomala have straight, posterior, setose margin of telson. *Hemimysis* also have the proximal half of the lateral edge of their antennal scales non-setose.

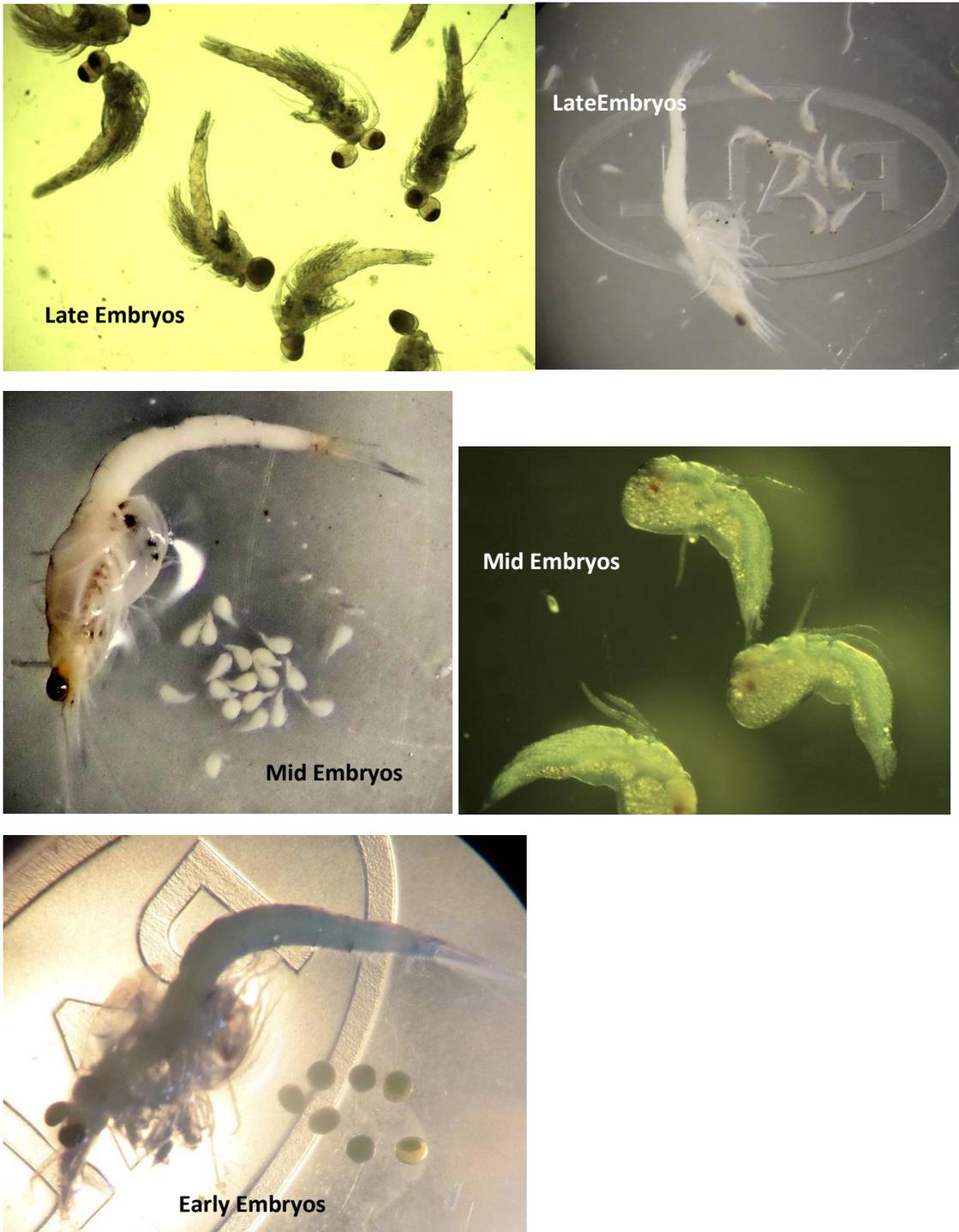
L. benedeni telson has a rounded, U-shaped cleft, with spinulose concave lateral margins. Brood pouch laterally wider and with markings on lateral surfaces of brood pouch, rather than ventral surface as in *M. diluviana*. Antennal scales tapering in lanceolate form, with narrow base; dorsal texture of scale corrugated like a sea-shell.

K. warpachowski have a convexly-rounded posterior margin of telson. *K. warpachowski* thoracic legs stubby, and held tightly to ventral surface of thorax; thoraxes dorsoventrally compact. *K. warpachowski* uropoda margins are with short setae. Texture of uropod akin to *L. benedeni* antennal scale, but less pronounced.

P. lacustris (Syn: *Mesomysis czerniavskiyi*) 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.

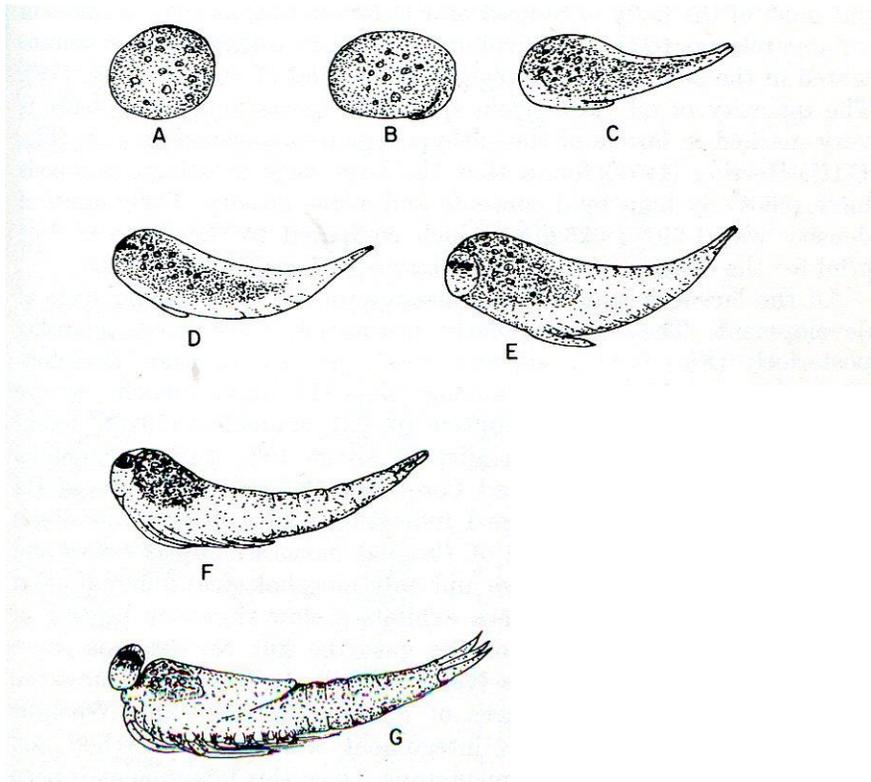
See Pothoven et al (2007) for more information about *M. diluviana* and *H. anomala* characters, and Sars (1893) for more information about *K. warpachowski* and *L. benedeni* characters. *L. benedeni* and *K. warpachowski* photographs by Almut Hanselmann; *M. diluviana* photograph by Toby Holda; *H.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

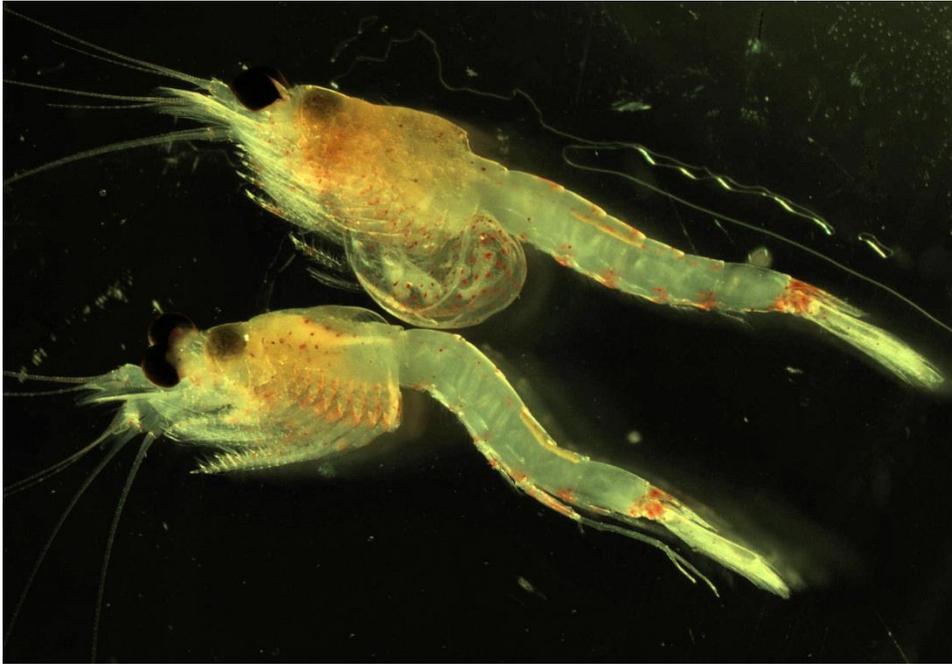


FIGURE 5: CAMERA STAND

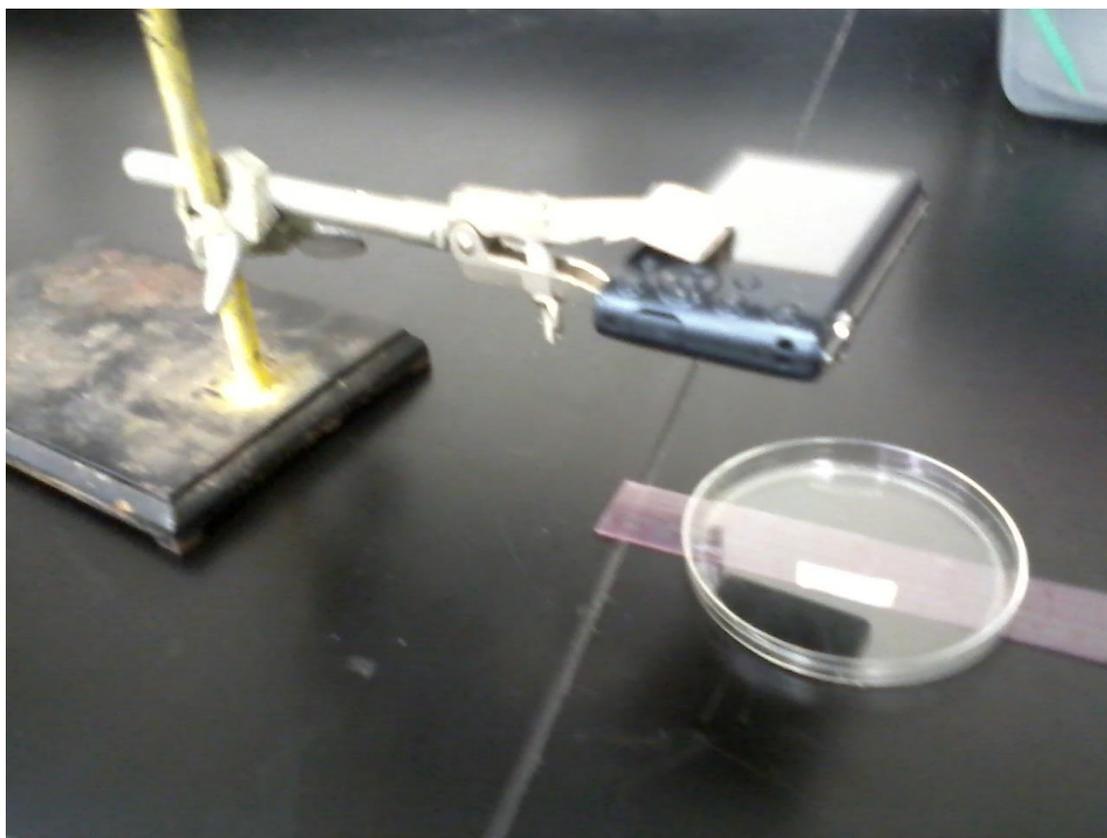
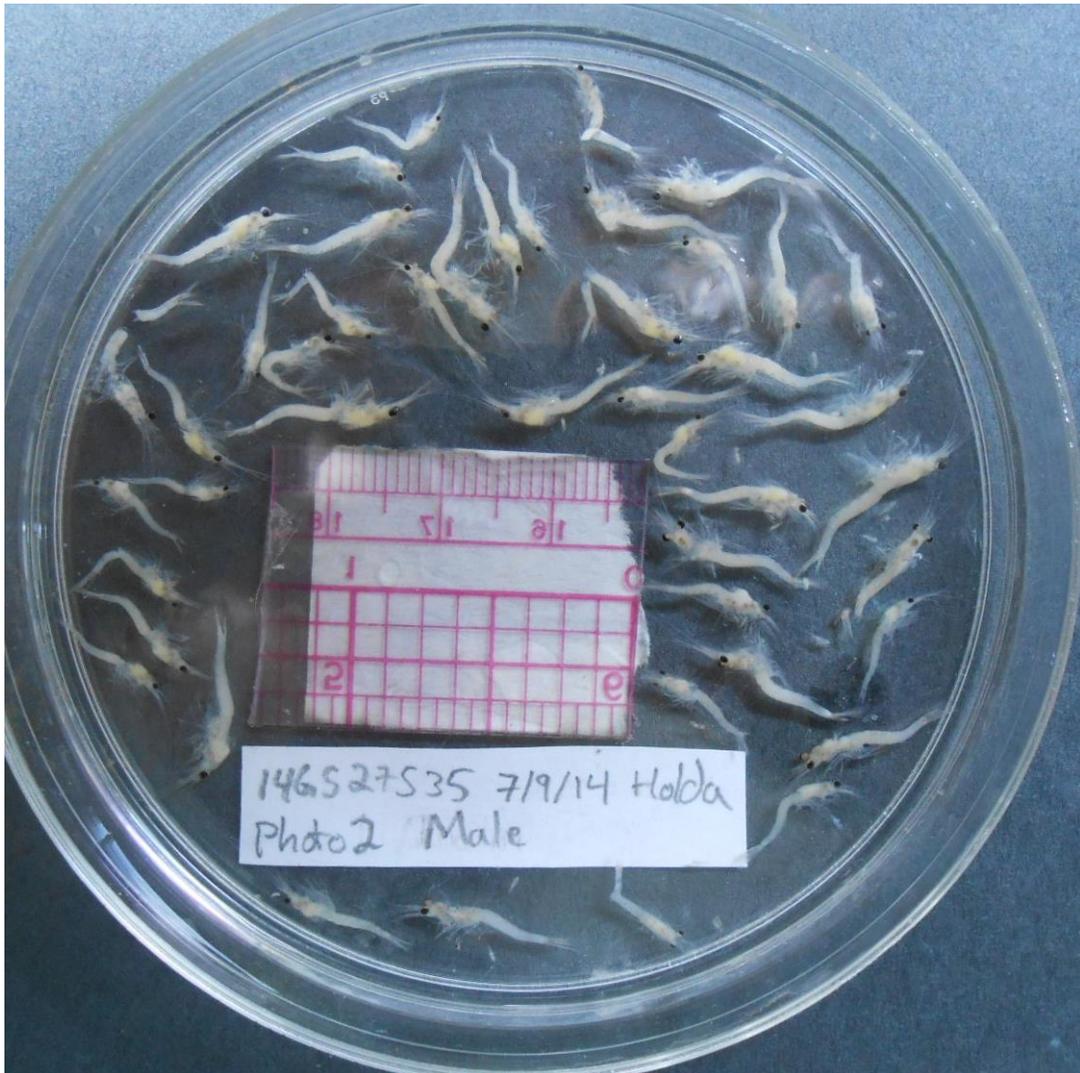


FIGURE 6: MYSID PHOTOGRAPH SETUP

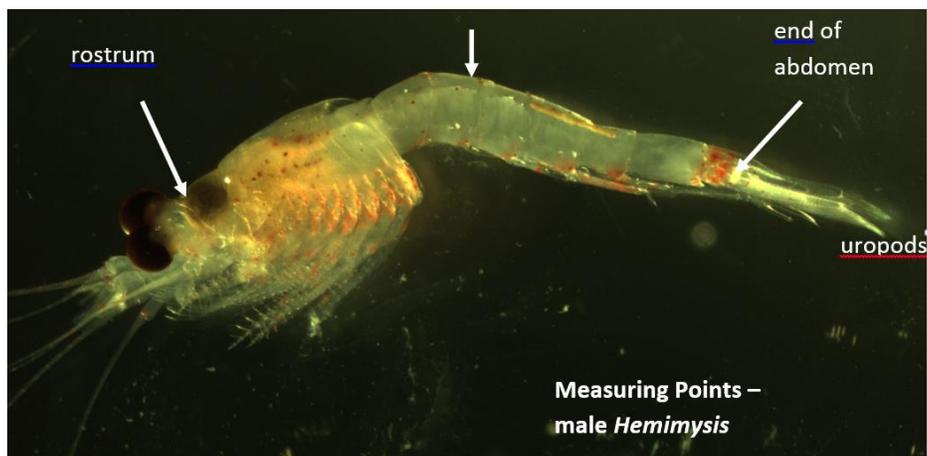
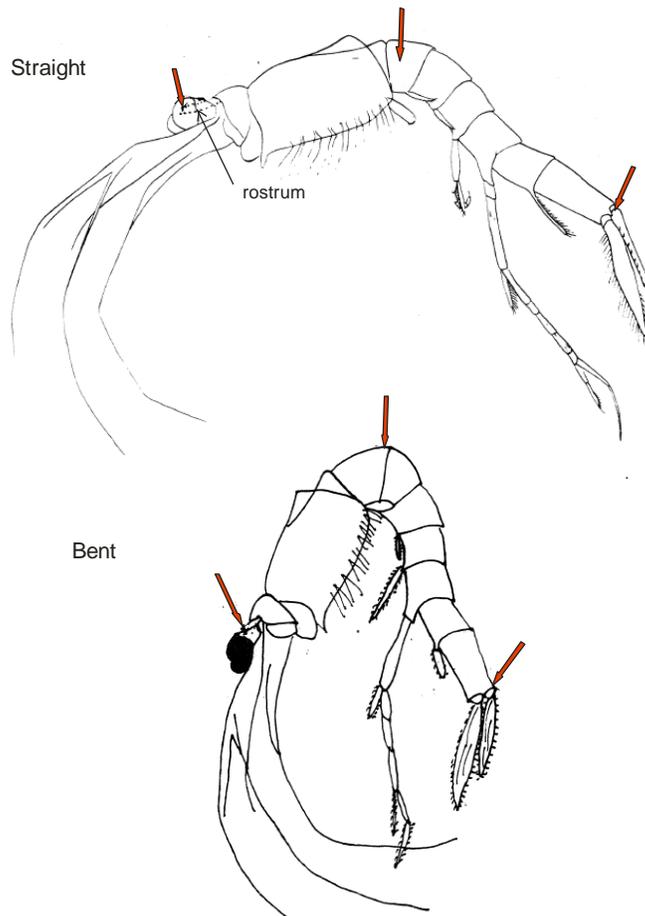


Mysis diluviana arranged in petri dish. Note the dark background, the shading of the dish from glare where there are mysids, and the spacing of mysids so they do not overlap. Also, note the label format.

FIGURE 7: MYSID LENGTH MEASUREMENTS

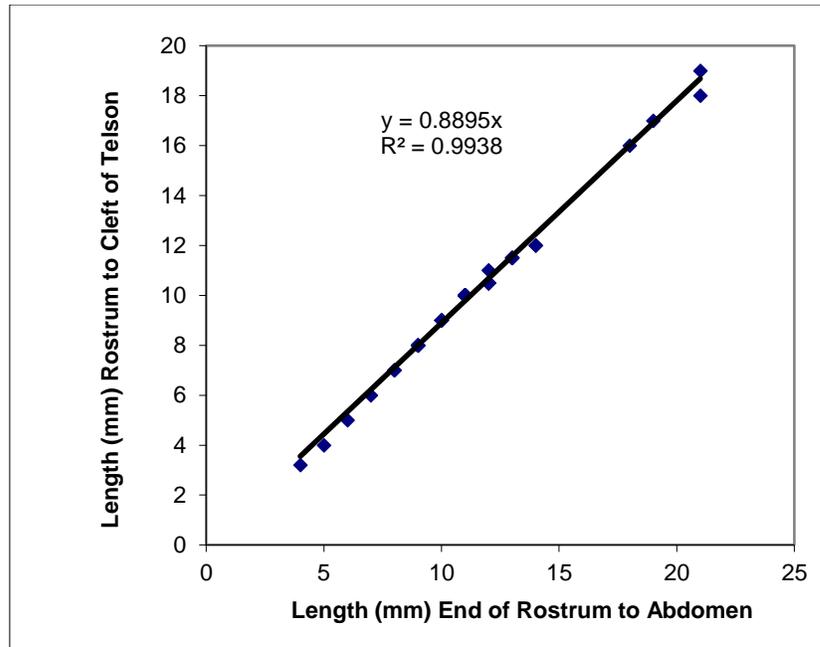
Measuring mysids

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



Pictures from Kelly Bowen

FIGURE 8: RELATIONSHIP BETWEEN COMMON MYSID LENGTH METRICS



Regression of mysid length as measured from tip of rostrum to cleft of telson as a function of mysid length as measured from tip of rostrum to end of abdomen/base of telson (see Fig. 1). From Ted Schaner and Michael Connerton

APPENDIX 1: FORMS

BENCH DATA SHEET FORMAT

Sample ID: _____ Station: _____ Collection Date/Time: _____

Tow Depth Start: _____ Tow Depth End: _____ Rep: _____ Process Date: _____

Lab Analyst Surname: _____ Camera Used: _____

Count "Extra" *M. diluviana*: _____ Count "Extra" *H. anomala*: _____ Count Bytho: _____

Sized *M. diluviana* Counts & Pictures

Sex	Immature	Male	Female: Non-Gravid	Female: Gravid	Female: Spent
Count					
PicID					

Sized *H. anomala* Counts & Pictures

Sex	Immature	Male	Female: Non-Gravid	Female: Gravid	Female: Spent
Count					
PicID					

Field-Removed Gravid *M. diluviana*

ID	Length	EmbStg	BrdCnt	PicID
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				

Field-Removed Gravid *H. anomala*

ID	Length	EmbStg	BrdCnt	PicID
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				

Notes:

LABEL FORMATS

Photograph Labels:

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

Example:

12GA11S75	Holda
11-24-14 Pic1	Imm

Vial Labels:

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

Example:

12GA11S75	Holda
11-24-14	Sized

Processed Sample Jar Labels:

Mysids	[Sample ID]
[Station ID]	[Collection Date]
[Processing Date]	[Analyst Surname]

Example:

Mysids	12GA11S75
MI 40	4-2-12
11-24-14	Holda