Effective Date ___O8 AUG 2016___

Approved By ___~Mark Gee___

___Julie Munchie___

SOP 1.01: <u>Plankton Sampling Site Selection</u>

I. <u>Summary</u>

Originally, the criteria for routinely sampled sites were based primarily upon likely locations of *Dreissenid* mussel introduction and propagation. The routine site list is comprised of Wahweap Marina, Stateline Marina, Antelope Point Marina, Glen Canyon Dam, Bullfrog Marina, Halls Crossing Marina, and Hite Marina. In the recent past when water levels were too low to access and sample Hite Marina, Good Hope Bay was introduced as a replacement uplake routine site.

As the *Dressenid* population in Lake Powell has spread throughout the body of water, sampling protocols have evolved to meet the new needs of veliger monitoring and the abilities of the laboratory. The below procedures reflect the changes.

II. <u>Procedures</u>

A. Primary Sampling Sites:

   i. The sites that are sampled on a monthly basis are areas where the risk of introducing *Dreissenid* mussels were deemed highest prior to infestation. These areas of high risk included marinas, with the exception of Dangling Rope Marina, because of the frequent launching of vessels that may have been in infested waters previously. Because of the extensive and consistent sampling efforts over time in these areas, they will remain part of the Primary Sampling Sites.

   ii. Outside the breakwater at Glen Canyon Dam is also considered a routine sampling site. The water flow, hard substrates, and relative location on the lake increased its potential exposure to settling veligers and its susceptibility to *Dreissenid* colonization. Because of its extensive and consistent sampling efforts over time, this location will remain a Primary Sampling Site.

B. Secondary Sampling Sites:

   i. As Lake Powell has become infested, there is less need for random sampling and more need for monitoring the spread of the infestation, but sampling every infested Visitor Use Zone on a monthly basis is not feasible. Instead, each infested Zone outside of the
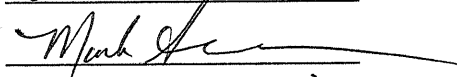
Primary Sampling Sites' Zones will be sampled on a quarterly basis. Each Zone will have a dedicated routine site location, similar to the Primary Sampling Sites.
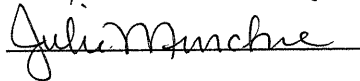
C. Random Sampling:

    i.   Random sampling is also done periodically to reduce sampling bias. Random sampling sites are generated using GIS (refer to SOP 2.01: Random Plankton Sampling Site Selection Using ArcMap).

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Approved By _(signature) Mark ___

(signature) Julie Munchie

Attachments:
Plankton Tow Data Sheet (A)
Plankton Sampling Site ID Key (C)

SOP 1.02: Preparation for Plankton Sampling

I. **Summary**

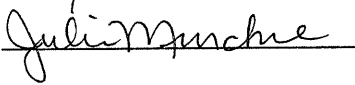The necessary preparations for plankton sampling are described below.

II. **Equipment**

Field sampling binder	DI water
Plankton Tow Data Sheets	DI water wash bottle
Plankton Sampling Site ID Key	GPS unit
Black permanent marker & pen	Plankton net & cod-end
Field thermometer	Line (marked every 1 meter) & reel
Sample bottles (125ml, 250ml)	Hach meter handheld unit
Ethanol, 95% denatured with methyl &	Hach meter dissolved oxygen probe
IPA	Hach meter pH probe
White distilled vinegar	Hach meter conductivity probe
Vinegar spray bottle	12 AA batteries

III. **Procedures**

A. Reserve the boat you wish to use on Google Calendar.

B. Determine the sites to be sampled, and the number of samples per site to be collected – bring enough sample bottles for these samples and a few extras.

C. Bring a spray bottle with vinegar to decontaminate the net after each sample site and about 2 liters extra, depending on the number of sample sites.

D. Bring at least 2 liters of ethanol to preserve the collected samples. More, up to 1 or 2 gallons, may be necessary if it's a multi-day sampling trip.

E. Bring a wash bottle with DI water and about 2 liters of extra DI water.

F. Make sure the field sampling binder has enough Plankton Tow Data Sheets (Attachment A), black permanent markers and pens, and a field thermometer.

G. Bring the handheld Hach meter, appropriate probes to measure pH, dissolved oxygen, and conductivity, the GPS unit, and batteries.

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Attachments:
Plankton Tow Datasheet (A)
Plankton Tow Water Volume Sampled (W)

SOP 1.03: <u>Plankton Sampling Techniques</u>

I. <u>Summary</u>

Sampling shall be performed in such a manner as to minimize the amount of variation in the data due to the technician performing the sampling. Record any environmental conditions that may affect the test results or any departure from the procedures in the comments section of the Plankton Tow Datasheet. The following field sampling procedures shall be followed.

II. <u>Equipment</u>

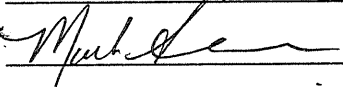|	
Field sampling binder	DI water
Plankton Tow Data Sheets	DI water wash bottle
Plankton Tow Water Volume Sampled	GPS unit & 4 AA batteries
Sheet	Handheld Hach meter
Black permanent marker & pen	Hach pH probe
Field thermometer	Hach dissolved oxygen probe
Sample bottles (125ml, 250ml)	Hach conductivity probe
Ethanol, 95% denatured with methyl & IPA	Plankton net & cod-end
Ethanol wash bottle	Line (marked every 1 meter) & reel
White distilled vinegar	Boat
Vinegar spray bottle	

III. <u>Procedures</u>

A. Locate the site to be sampled and bring the boat to rest. If boat continues to drift, tie off to a structure if it is safe to do so. If there is no structure to tie off to, have one technician operating the boat to keep it in place.

B. Record the Site ID, Date, Time, Location Description, Bottom Depth, and Depth of Tow on the Plankton Tow Datasheet (Attachment A).

C. Hang a field thermometer from the boat so that the bulb of the thermometer is about 4 inches below the surface of the water. Read the water temperature after the field thermometer has adjusted to the water temperature and record on the Plankton Tow Datasheet.

D. Record the GPS position during the field collection using the procedure described in SOP 2.01: Electronic Recording of Sample Sites Using GPS. Record the GPS coordinates on the Plankton Tow Datasheet.

E. Record the dissolved oxygen, pH, and conductivity levels as described in SOP 1.16: Measuring Dissolved Oxygen, pH, and Conductivity with the Hach Meter.

F. Record the turbidity using a Secchi disk, as described in SOP 1.17: Recording Turbidity with the Secchi Disk.

G. Rinse net and cod-end in the lake before connecting cod-end to bottom of net.

H. Plankton samples are collected using the following procedure:

    i. Label sample bottles as described in SOP 1.04: Sample Bottle Labeling.

    ii. Drop the net down to a maximum of 50 meters, or 3 meters above the bottom depth if bottom depth is 50 meters or less. Hold line at desired depth until added tension and weight is felt on the line before pulling the net up.

    iii. Manually retrieve net using a hand-over-hand technique at a constant rate of 0.5m/s. Slow and steady retrieval is the key to collecting a good plankton tow.

    iv. After the top of the net has breached the surface, rinse the organisms into the cod-end piece of the net. This is accomplished by lowering the net back into the water, keeping the opening above the water surface, then quickly raising the net straight up. This action will move the collected plankton into the cod-end. Repeat this procedure several times to ensure all the organisms inside the net are in the cod-end.

    v. Unscrew the cod-end and drain the water out through the mesh, leaving the plankton behind. Using a gentle swirling motion can help speed up the process.

    vi. Using the DI water wash bottle, gently rinse the sides of the cod-end to condense the plankton. After letting excess water drain out, pour plankton into labeled sample bottle. Continue to rinse plankton into sample bottle until all plankton has been transferred from the cod-end and into the sample bottle.

I. If the collected samples are especially dense and cannot be condensed to fit into a 125ml sample bottle, use a labeled 250ml sample bottle instead. If there are no 250ml sample bottles available, use two 125ml bottles, and label appropriately "1 of 2" and "2 of 2".

J. Preserve samples as described in SOP 1.05: Plankton Sample Preservation.

K. Follow steps H-J to collect a replicate sample for PCR analysis.

L. Decontaminate net and cod-end with vinegar as described in SOP 1.06: Plankton Net Decontamination.

M. Record all remaining information on the Plankton Tow Datasheet. Refer to Plankton Tow Water Volume Sampled (Attachment W) for the calculated amount in liters of lake water sampled.

Effective Date ___O8AUG2016___

Approved By _[signature]_

[signature]

Attachments:
Plankton Sampling Site ID Key (C)

SOP 1.04: Plankton Sample Bottle Labeling

I. Summary

  A. Proper protocol must be followed when labeling bottles used for plankton sample collection. These site identifications are used in conjunction with the date to form a unique identification code to each sample received in the laboratory. It serves as an identifier for all subsequent activities associated with each sample.

II. Equipment

  Sample bottles (125ml or 250ml)
  Permanent black marker

III. Procedures

  A. Using a black permanent marker, each sample bottle will be labeled with:

      1. Site Identification
      2. Collection Date (format example: "28JUL14" ddMMMyy)
      3. Depth of Tow
      4. Preservation Technique

  B. Refer to the Plankton Sampling Site ID Key (Attachment C) for routine, secondary, random, and reported site names and identification codes.

  C. Each Site ID will be followed by a number indicating the order in which the samples were collected at a particular site. When replicate samples are taken, they will be designated by capital letters in alphabetical order at the end of the Site ID (ex: WWM1A; WWM1B). Samples that will be analyzed by microscopy will be labeled with an "A" and those for PCR analysis will be labeled with a "B."

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SOP 1.05: <u>Plankton Sample Preservation</u>

I. <u>Summary</u>

To avoid degradation, samples need to be preserved in a 70% final ethanol solution.

II. <u>Equipment</u>

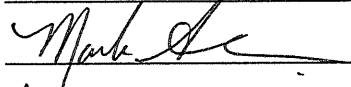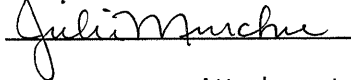Sample bottle
Black permanent marker
Ethanol, 95% denatured with methyl &IPA

III. <u>Procedures</u>

A. Immediately after collection, the sample is rinsed from the cod-end into a sample bottle as described in SOP 1.03: Plankton Sampling Techniques.

B. Mark the level of sample with a permanent marker on the outside of the bottle.

C. Add three times the volume marked with ethanol. This will result in a nearly 70% ethanol solution (66.67%).

D. It is important that the amount of raw sample does not exceed 1/3 of the sample bottle before the ethanol is added. At this amount, you would need to add enough ethanol to fill the sample bottle completely.

E. If the sample is too large to be contained in a single sample bottle, it may be split into two bottles. Make sure to record this on the Plankton Tow Datasheet (Attachment A) in the comments section.

F. Record the preservative and the final solution percent (70% EtOH) on the Plankton Tow Datasheet.

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Approved By _____

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Attachments:
Plankton Tow Datasheet (A)

SOP 1.06: Plankton Net Decontamination

I. Summary

To prevent cross-contamination of plankton samples, the plankton net and cod-end need to be decontaminated with vinegar between sample sites. If collecting a replicate at the same site, decontamination does not need to be performed. Decontamination takes place after the plankton sample has been rinsed from the cod-end into a sample bottle.

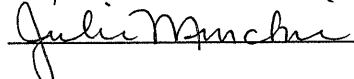II. Equipment

Plankton net and cod-end
Spray bottle filled with distilled white vinegar

III. Procedures

A. With the cod-end disconnected, hold up the top of the plankton net and spray vinegar liberally to the inside and outside black portion of the net.

B. Flip the net over and spray the inside and outside of the plastic at the end of the net where the cod-end connects. Hold the net upside-down and spray the entire outer part of the mesh until it is saturated with vinegar.

C. Finally, spray the inside and outside of the cod-end liberally.

D. Upon arriving at the next site, rinse the plankton net and cod-end separately and thoroughly in the lake before collecting another sample.

E. Record the pre-sample net decontamination procedure on the Plankton Tow Datasheet (Attachment A).

F. After each day of sampling is completed, each net and cod-end must be rinsed thoroughly with water. This can be done either by hanging by the laboratory shower or with a hose outside. Let plankton net hang to air dry.

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Approved By _M̄ʳᵇ ᵈ̲_

Julie Manchin

Attached Documentation:
Veliger Monitoring Laboratory Form (B)
Veliger Ostracod Comparison Chart (D)

SOP 1.07: Sample Processing by Compound Microscope

I. Summary

Procedures for processing a plankton sample with the Axiostar *plus* Zeiss Microscope are described below.

II. Equipment

Plankton sample	KimWipes
63μm sieve	2 Petri dishes
250μm sieve	Disposable graduated pipette
Wash bottle filled with RO water	Glass beaker (at least 250ml)
Spray bottle filled with white distilled vinegar	Sedgewick-Rafter cell
	Mechanical tally counter
Wash bottle filled with ethanol, 95% denatured with methyl & IPA	Axiostar *plus* Zeiss Compound Microscope

III. Procedures

A. Filter sample to remove larger particles.

1. Place a 250μm sieve on top of a 63μm sieve, aligning the rivets and rotating the sieves so that the sample will not catch on the rivets.

2. Keeping the sieves together, pour the sample into the 250μm sieve over the sink.

3. Rinse out the sample bottle at least three times to ensure that the entire sample is in the sieve.

4. Gently run tap water over the sieves to allow any smaller organisms caught on the larger sieve to pass through to the 63μm sieve.

5. Separate the sieves from one another. Using the RO wash bottle, condense all of the particles on the 250μm sieve to one side and rinse the plankton out of the sieve into a beaker.

B. Condense the smaller organisms on the 63μm sieve using the RO water bottle and pour into a Petri dish, using as little water as possible, as a fairly condensed sample is preferred.

C. Complete the Site ID, Collection Date, Analysis Date, and Tech sections on the Veliger Monitoring Laboratory Form (Attachment B). Indicate whether the sample was Filtered, Split, the Split Method, and Number of Splits.

D. Clean the Sedgewick-Rafter cell with a KimWipe and place on microscope stage. The sample is analyzed in successive 1ml aliquots taken by disposable pipette from the Petri dish. Note : It is helpful to put a very small amount of liquid soap into the tip of the pipette to help reduce the amount of sample that is left behind in the pipette.

E. Make sure the adjustable polarizing filter is in place and adjust the light settings so that planktonic organisms are highlighted against a dark background.

F. Set the microscope to the 5x objective and the phase to H.

G. Scan the Sedgewick-Rafter slide by moving through the fields of each row and repeating for all the rows to inspect the whole aliquot. Note : Repeat the same pattern for every slide in every sample.

H. Look for luminescent calcium carbonate shells that could be ostracods, *Corbicula*, or *Dreissenid* veligers. Refer to the Veliger Ostracod Comparison Chart (Attachment D) and the Zebra and Quagga Mussel Identification binder for classification.

I. To get a closer look at organisms, the 10x objective can be used. A magnification higher than the 10x objective cannot be used with the Sedgewick Rafter cell. The organism of interest will first have to be transferred to a microscope slide with a cover slip.

J. Tally each positively identified ostracod, veliger, or *Corbicula* onto the Veliger Monitoring Laboratory Form as the sample is processed, as well as the number of slides analyzed. Alternatively, the mechanical tally counter can be used to keep track of these items and then transfer the final count onto the Veliger Monitoring Laboratory Form.

K. Take one photograph (under non-polarized light at 100X magnifications) of each *Dressenid* veliger or unidentifiable organism with the AxioCam (refer to SOP 1.08: Photomicroscopy Using AxioCam). For already known contaminated sample sites, photographs are not needed.

L. After visually inspecting the whole slide, pour it into a glass beaker and rinse any residual sample with the RO water bottle. Dry the slide with a KimWipe before adding the next sample aliquot.

M. Once processing in complete, fill out the rest of the Veliger Monitoring Laboratory Form. If the sample was split, see SOP 1.14, Section IV. "Cheat Sheet."

N. After bottling analyzed sample into glass vial, thoroughly spray the sieves, glassware, and funnel with vinegar to decontaminate and let stand for at least 30 minutes before

scrubbing clean with water. To clean the Sedgwick-Rafter cell, do not use vinegar as it will quickly dissolve the adhesive under the gold frame. Wash the slide under running water and gently scrub around the inside of the frame with a brush.

Effective Date ___ O 8 AUG 2016 ___

Approved By ___ *[signature]* ___

___ *[signature]* ___

Attached Documentation:
Veliger Monitoring Laboratory Form (B)

SOP 1.08: <u>Photomicroscopy Using AxioCam</u>

I. <u>Summary</u>

Procedures for taking pictures of organisms using *AxioVision* software are described below.

II. <u>Equipment</u>

Axiostar *plus* Zeiss Microscope   Computer with *AxioVision* software
Axiocam ERc5s   Zeiss camera cable

III. <u>Procedures</u>

A. Turn on computer and connect the camera cable from the USB port on the left side of the FlowCAM to the port on the back of the AxioCam (blue box) located on the top of the microscope. *Note 1: On the top of the blue box, there is a small light that will be either green or red. Wait until the light is green to connect the cable or the software will not recognize the camera.

B. On the computer desktop, open the *AxioVision Rel. 4.8* software.

C. Click the "Live" icon on the toolbar. The Live view window will open.

D. Pull out the metal rod located on the right side of the microscope's binocular tube to switch the light path from the eyepiece to the camera and swivel the polarizing disk to the left to remove from the field of view.

E. The Live view window should now show the sample with a slightly smaller field of view.

F. Make sure the magnification listed in the drop-down menu at the bottom left of the screen is the same as the magnification of the objective.

G. To perform an automatic adjustment of image properties such as exposure and white balance click on the associated buttons on the bottom toolbar. To enter in desired settings click on the Properties button and go to the Display or Adjust property pages.

H. Focus on the object you want to photograph and click on "Snap".

I. Close out of the Live view window and the image will be displayed.

J. Select "Measure" from the toolbar, and then "Length" from the menu. Click on one side of the organism and then click on the opposite end to get the length. Again, make sure the software is set to the same magnification as the objective so any measurements will be displayed correctly.

K. Each image will be stored as a tab with a default filename. It is best to rename each photo as it is taken to help keep track of the number of organisms with the mechanical tally counter.

L. Save images in a new folder labeled with the collection date followed by the Site ID. The new folder should be located in the "Microscope Images" folder for the current year. Save the images as JPEGs and rename the image files to reflect what was imaged and at what magnification.

M. Record the folder name containing the photos on the Veliger Monitoring Laboratory Form (Attachment B).

N. Transfer image folders to the network drive. The destination folder is located at U:\Aquatic\WaterLab\Water Lab - ZM Microscopy\Microscope\Veliger Sample Photos.

Effective Date ___O8AUG 2016___

Approved By ___~Mark Cle___

___Julie Munchie___

SOP 1.09: <u>Assembling FlowCAM Hardware</u>

I. <u>Summary</u>

Procedures for setting up the hardware components of the FlowCAM are described below.
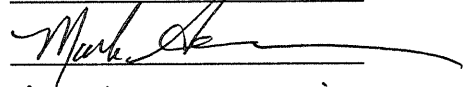
II. <u>Equipment</u>

FlowCAM
4x objective
300FC or 300FOV5 flow cell
QCFC300 or QCFC SP flow cell holder
Collimator
Two polarizing filters

Mini funnel
Silicone tubing with collars
Short segment of silicone tubing
Beaker (at least 250ml)
Disposable graduated pipette
Kimwipes

III. <u>Procedures</u>

A. Install the microscope objective by threading it into the optics cube located on the left side of the FlowCAM. When analyzing a plankton sample the 4x objective should be used.

B. Thread the collimator into place in front of the light source on the right side.

C. Make sure both polarizing filters are in place. One of the filters is located in the slot in front of the camera on the left. The other one is positioned on the slide bar as close to the collimator as possible.

D. Install the mini funnel holder into place above the objective.

E. Wrap silicone tubing with collars around the peristaltic pump wheel while placing collars in holders. Attach the short segment of outflow tubing to the collared tubing where the OUT arrow is and feed it into a beaker.

F. Unscrew the retaining cap of the flow cell holder. Wipe flow cell with a Kimwipe and place inside holder and replace cap. Tighten gently to avoid breaking the flow cell. The 300FC flow cell goes in the QCFC300 flow cell holder. The 300FOV5 flow cell goes in the QCFC SP flow cell holder. Both must be used with the 4x objective.

G. Install flow cell holder onto the mount in front of the 4x objective with the thumb screw facing upwards and tighten.

H. Attach the inlet tube from the top of the flow cell to the funnel or pipette tip in the holder. Next, attach the outlet tube from the bottom of the flow cell to the pump tubing where the IN arrow is.

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SOP 1.10: <u>Configuring VisualSpreadsheet 4X Software Program</u>

I. <u>Summary</u>

Procedures for configuring VisualSpreadsheet 4X to process plankton samples in cross-polarized light are described below.
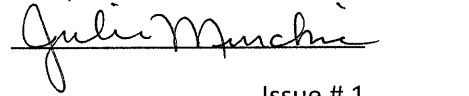
II. <u>Equipment</u>

FlowCAM
VisualSpreadsheet 4X Version 2.4.1

III. <u>Procedures</u>

A. Make sure the 4x objective is installed in the FlowCAM.

B. Double click and open the VisualSpreadsheet 4X icon located on the desktop.

C. Click Setup > Context...

D. Select the "Load" tab and click "Load a Context File..."

E. If using the 300FOV flow cell load the "4x_XPL_Veliger_Settings_FOV_GLCA.ctx" context file. If using the 300FC flow cell load the "4x_XPL_Veliger_Settings_Standard_GLCA.ctx" context file.

F. Select "OK" to close out of the Context window.

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Approved By _____

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SOP 1.11: Focusing the FlowCAM

I.   Summary

Procedures for focusing the FlowCAM are described below.

II. Equipment

FlowCAM   Filtered plankton Sample
VisualSpreadsheet 4X Version 2.4.1   DI water in wash bottle

III. Procedures

A. Add DI water to the pipette tip and disconnect the flow cell silicone tubing from the tubing that wraps around the pump. This will cause the DI water to flow freely from the pipette tip through the flow cell and prevent bubbles from forming in the flow cell chamber. Reconnect the tubing when the DI water has passed through the glass chamber.

B. If there are any remaining bubbles in the glass chamber, turn on the pump then pinch and hold the tubing downstream of the flow cell for a few seconds and then release. This will remove any air bubbles trapped within the flow cell.

C. In VisualSpreadsheet, go to "Setup" and select "Setup and Focus."

D. If using the 300FC flow cell, continue to Step E. If using the 300FOV (cuvette) flow cell, use the horizontal thumbscrew to slightly adjust the position of the 300FOV flow cell so that the two white lines defining the acceptable region are superimposed over the edges of the flow cell.

E. Rotate the polarizing filter to raise the intensity mean to 100 so particles are easier to see.

F. Add a small amount of plankton sample to the pipette tip and turn the pump on FORWARD and FAST.

G. As organisms begin to appear in the camera frame in the Setup and Focus window, switch the pump to SLOW.

H. Focusing the FlowCAM on large stationary organisms yields the best results. By starting and stopping the pump, it is possible to keep an organism in the frame.

I.   Focus the image by turning the fine focus knob. Find the clearest focus on several particles and then run the sample on SLOW and see how well particles are imaged.

J.   Once sufficiently focused, reverse the pump to bring all the particles used to focus back into the pipette. Keep an eye on the cascading particles in the Setup and Focus window to see if they are mostly in focus. If not, stop the pump and re-focus.

K.   Rotate the polarizing filter to back down to lower the intensity mean to 25.

Effective Date _____ 08AUG2016 _____

Approved By _____ (signature) _____

_____ (signature) _____

Attachments:
Veliger Monitoring Laboratory Form (B)

SOP 1.12: <u>Sample Processing by FlowCAM</u>

I. <u>Summary</u>

Procedures for processing a plankton sample with the FlowCAM are described below.

II. <u>Equipment</u>

FlowCAM	DI water
VisualSpreadsheet 4X Version 2.4.1	250µm sieve
Plankton sample	63µm sieve
Mini funnel	Disposable graduated pipette
Ethanol	Vinegar

III. <u>Procedures</u>
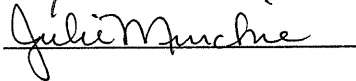
A. On the Veliger Monitoring Laboratory Form (Attachment B), indicate the Analysis Date, Tech, type of Flow Cell, whether the sample was split, Split Method, Number of Splits, and the Photo Folder ID.

B. There should already be DI water and some sample in the mini funnel from focusing the FlowCAM.

C. Go to "Analyze" and select "AutoImage Mode." Confirm that the "Stop only when user terminates" box is checked and click OK.

D. The Save screen will open and prompt a filename. Name the file in the following format: CollectionDate_4x_FlowCell_SiteID. Record the Folder ID on the Veliger Monitoring Laboratory Form (Attachment B). Before pressing save, start the pump on FAST 5 (5 referring to the dial) and then press Save.

E. After the background has been calibrated, pipette sample from beaker into the pipette tip/small funnel. Adding small amounts of sample at a time minimizes the chance of clogging.

   1. If a clog does occur, gently pinch and release the tubing below the flow cell until the clog has broken up and passed through. This, however, may rotate the 300FOV flow cell and cause the acceptable region lines and flow cell edges to misalign. The software will then begin to image the edges of the flow cell repeatedly. To realign the

flow cell, in the AutoImage window, go to "Tools" and then "Recalibrate." This should prevent further imaging of the flow cell edges.

F. Adjust the intensity mean if necessary so that only birefringent particles are imaged (~25).

G. Continue adding sample at a steady rate.

H. When all the sample is in the pipette tip/small funnel, let the FlowCAM run until there is only a little sample left in the tip.

I. Add DI water to rinse residual sample from the funnel and to help flush particles through tubing and flow cell.

J. Watch the AutoImage screen for particles as water runs through the flow cell, adding more water as needed, until nothing is being imaged.

K. As soon as the last of the sample passes through the flow cell and bubbles appear on the screen, click "Pause" and record the FlowCAM run time (bottom left corner of the AutoImage window) on the Veliger Monitoring Laboratory Form (Attachment B), and then close the AutoImage window. This will end the run.

L. After the sample has run completely through the tubing, turn off the pump.

M. Remove the pipette tip/small funnel and unhook silicone tubing from around the pump. Attach a DI wash bottle to the top of the flow cell tubing and run DI water through it to flush any remaining particles into the beaker.

N. Remove the remaining tubing and fill all tubing with vinegar and let stand for at least 1 hour before rinsing with DI water. Replace bigger plastic tube cover over the 300FC flow cell to protect it.

O. After bottling analyzed sample into glass vial, thoroughly spray the sieves, glassware, and funnel(s) with vinegar to decontaminate and let stand for at least 30 minutes before scrubbing and rinsing clean with water.

Effective Date ___08AUG2016___

Approved By _____

_____

Attached Documentation:
Veliger Monitoring Laboratory Form (B)

SOP 1.13: <u>Sample Post-Processing by VisualSpreadsheet</u>

I. <u>Summary</u>

Procedures for plankton sample post-processing with VisualSpreadsheet software are described below.

II. <u>Equipment</u>

FlowCAM
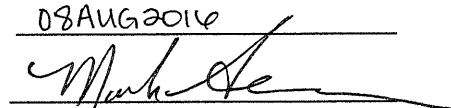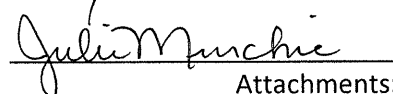VisualSpreadsheet 4X Version 2.4.1
Mechanical tally counter

III. <u>Procedures</u>

A. After completing the run, press "F3" to show all data and then "F2" to open the view window.

B. Scan through all the collage images and click on those those that look like ostracods, *Dressenid* veligers, or *Corbicula*. The frame around the image will turn red, indicating it is selected. After all the images are selected, click the "Show Selected" button. Only the selected images should now be displayed.

C. Go to "File" and select "Export Data." An Excel spreadsheet with the list of selected images will open. Navigate to the column with the Source Image Number.

D. Open the current sample's folder in Windows Explorer. From the Excel spreadsheet Source Image Number Column, locate each image in the current sample's folder and copy, cut, and paste each one into the new temporary file.

E. In the temporary folder, review the raw images to verify that the image is of an ostracod, veliger, or *Corbicula*. Delete the image if it is not. Use the mechanical tally counter as images are identified.

F. After reviewing all the pertinent raw images, delete the rest of the raw images left in the current sample's folder. Make sure to keep all collage images.

G. Replace the selected raw images from the temporary folder back into the sample folder.

H. Empty the recycle bin.

I. Record the number of raw images of ostracods, *Corbicula*, *Dressenid* veligers, and unknown on the Veliger Monitoring Laboratory Form (Attachment B). If the sample was split, please refer to SOP 1.14 Sample Splitting by Folsom and Motodo Sample Splitters, Section IV. "Cheat Sheet." *Note 1: Maximum number of splits is not limited to 5.

J. If there are images of suspicious or unknown organisms (besides images of poor quality where identification is not possible), make copies of the raw image files and label the images with the collection date and site ID.

1. Open the copied image file in *AxioVision* to add a scale bar or measurements to the image.

2. Go to "Measure" and select "Scalings." The Scalings Control window will open. Choose "FlowCAM 4x" then click on "Apply selection to image." Close the Scalings Control window.

3. Select "Measure" and then "Length." Click on one side of the organism and then click on the opposite end to get the length.

4. Go to "File" and "Save As" to save the image as a JPEG.

5. Transfer any JPEG images of suspicious or unknown organisms to the network for review by the Laboratory Director and/or Aquatic Ecologist. destination folder is located at U:\Resources\Aquatic\WaterLab\VeligerSampling\FlowCAM\FlowCAM Images.

Effective Date   D8AUG2016

Approved By

SOP 1.14: <u>Sample Splitting by Folsom and Motodo Sample Splitters</u>

I.   <u>Summary</u>

Procedure for splitting plankton samples, after the sample is filtered, with 2 different plankton splitters: Folsom Plankton Splitter and Motodo Plankton Splitter

II.   <u>Equipment</u>

Folsom:
Base/stand
Drum
Peg
2 boats

Motodo:
Base/stand
Splitter box

Both:
RO water
Tap water
Mild soap
Filtered sample
2 beakers (at least 250ml

III.   <u>Procedures</u>

A. Folsom

1. Assemble the drum, peg, and boats on the base.

2. Level the base by tightening/loosening the pegs in each corner of the base to place the air bubble in the center of the circle in the level mounted on the base.

3. Pour the filtered sample into drum and rinse the beaker a minimum of three times.

4. Rotate the drum 120° back and forth several times to ensure thorough mixing of the sample.

5. Rotate the drum to pour the sample into the boats and rinse the inside of the drum while keeping it tilted so any remaining particles are flushed out. This complete one split.

6. Keep track of the number of splits performed by making tally marks after each split and record the number on the Veliger Monitoring Laboratory Form (Attachment B). See table below for percentage of sample analyzed "Cheat Sheet."

7. To continue splitting, take one of the two boats and repeat steps 3-5 to complete as many splits as needed to reduce sample to an acceptable size.

8. Hand-wash drum and boats with tap water and mild soap. Do not use any type of alcohol for cleaning as the acrylic is not alcohol-resistant.
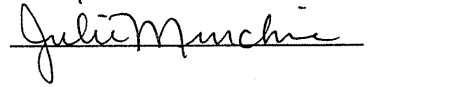
B. Motodo

1. Place the box with the groove fitted to the peg on the stand.

2. Ensure the base is level by tightening/loosening the pegs in each corner of the base to place the air bubble in the center of the circle in the level mounted on the base.

3. Pour the filtered sample into box, washing beaker free of residual sample with RO water. Slowly tilt the box back and forth several times, until sample is mixed. Leave box tilted with the enclosed end resting on the base. Rinse residual sample with RO water down into the resting end.

4. Pick up box and pour the half of the sample from the triangular hole in the corner of the enclosed end into a large beaker. Be aware of the half of the sample on the other side of the separating blade so it doesn't overflow into the emptying compartment when pouring.

5. While holding the box upright with triangular hole over beaker, squirt RO water into box until all the residual sample has been washed down into the compartments. This completes one split.

6. Keep track of the number of splits performed by making tally marks after each split and record the number on the Veliger Monitoring Laboratory Form (Attachment B). See table below for percentage of sample analyzed "Cheat Sheet."

7. Repeat steps 3-5 to complete as many splits as needed to reduce sample to an acceptable size.

8. Once finished with the splitter, hand-wash box with tap water and mild soap. Do not use any type of alcohol for cleaning as the acrylic is not alcohol-resistant.

IV. "Cheat Sheet"

# of Splits	% of Sample Analyzed	Multiply organism count by:
0	100	-
1	50	2^1
2	25	2^2
3	12.5	2^3
4	6.25	2^4
5	3.125	2^5

Effective Date ___O8AUG2016,___

Approved By ___(signature)___

___(signature) Julie Munchie___

SOP 1.15: <u>Post-Processing Sample Preservation and Bottle Labeling</u>

I. <u>Summary</u>

Procedures for preserving a plankton sample post-processing and for labeling the sample bottle.

II. <u>Equipment</u>

Fine point black permanent marker	DI water
30ml clear glass French square bottle	Ethanol, 95% denatured with methyl & IPA
¾" round white label	Tap water
Square white label (about 1"x1")	Short-stemmed funnel
Processed plankton sample	White distilled vinegar
63µm sieve	
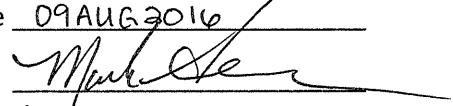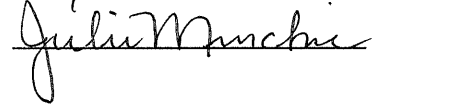
III. <u>Procedures</u>

A. Pour the sample into the 63µm sieve. Using tap water, rinse the beaker several times to make sure no sample is left behind.

B. In the sieve, condense sample down to one corner and rid sample of any excess water.

C. Place the short-stemmed funnel in the glass bottle. Using ethanol, rinse the sample into the bottle, leaving room at the top of the bottle to add enough DI water to make a final 70% ethanol solution.

D. Add DI water and cap bottle.

E. Place a ¾" round white label on the cap and a small square white label on the side of the bottle.

F. On the cap, write the Site ID, collection date, and ">63µm."

G. On the side, write the Site ID; collection date; sample depth' "70% EtOH"; "Analyzed" and technician's initials; the date the sample was analyzed; and ">63µm."

H. Additionally, if more than one glass bottle was used, write on each label the individual bottle's number out of the total number of bottles used to store that sample (ex: 1 of 3).

Effective Date ___09 AUG 2016___

Approved By _____

SOP 1.16: Hach Meter Use

I. Summary

Procedures for using the Hach meter to collect pH, conductivity, and dissolved oxygen in the field, and equipment storage.

II. Equipment

Hach Multi-Parameter Meter, HQ40D
Hach IntelliCAL CDC401 Conductivity Probe
Hach IntelliCAL LDO101 Luminescent/Optical Dissolved Oxygen Probe
Hach IntelliCAL PHC101 Gel Filled pH Electrode
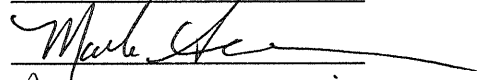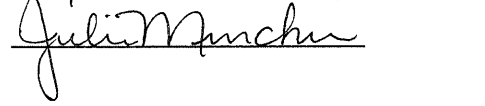Hach pH Electrode Storage Solution

III. Procedures

A. Begin by plugging in up to two probes in the outer two ports on the top of the meter. Align the pins and the notch, insert, and tighten the metal collar around the port.

  i. The pH electrode has a translucent, plastic cap with a small, yellow sponge to hold storage solution that will need to be removed each time it is used, and replaced between uses to protect the small bulbs in the end of the electrode. Storage solution does not need to be used in the short periods of time between sites, but should be used if the electrode will not be in use for several hours (overnight, between a morning sample and an evening sample, etc.).

B. Press the power button in the center, bottom of the face of the meter. The meter will go through a quick startup process and will display the units of measurement of the probe/electrode plugged in.

C. Lower the probe/electrode into the water until the entire metal piece of the probe/electrode is submerged. Adjust the depth as needed for wave action so the probe/electrode stays submerged.

D. To begin taking measurements, press the green button on the face of the meter. Above this button on the screen says, "Read."

E. The meter will begin taking measurements, and will display a progress bar for each probe connected. When the meter stabilizes, the progress bar will be filled in gray, and when the meter locks onto a measurement, the meter will sound a quiet beep, and will display a small padlock symbol next to the measurement on the screen.

F. To preserve battery life, turn off the meter between uses.

G. Before long-term storage, make sure the equipment is dry. This can be accomplished either by hand drying or air drying.

Effective Date ___09AUG2016/___

Approved By ___*Mark Afc*___

___*Julie Munchin*___

SOP 1.17: <u>Secchi Disk Use</u>

I. <u>Summary</u>

Procedure for the use of a Secchi Disk, as a measurement of water turbidity. The quality of Secchi depth data is user-dependent; it varies from person to person depending on vision. The depth of visibility also depends on factors such as sunlight and wave action, which can both produce extra glare on the water. It is encouraged to take several measurements and measurements by multiple people for more precision.

II. <u>Equipment</u>
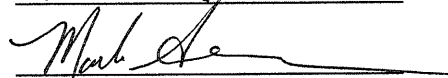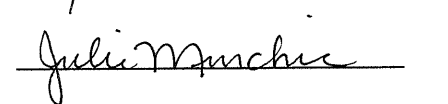
Secchi Disk

III. <u>Procedures</u>

A. Begin by locating the shaded side of the boat or dock.

B. With your back to the sun, lower the disk into the water off the shaded side of the boat or dock until you can no longer see it.

C. Raise the disk until it is visible again, and lower it again until it's not visible. Repeat this process until you are confident in the point at which you lose sight of the disk. Taking the average of the depths at which it is visible and not visible can be an alternative option to raising the disk and lowering it again.

D. Report the depth in meters, rounded to the nearest hundredth (##.## meters).

Effective Date _08 AUG 2016_

Approved By _[signature]_

[signature]

SOP 2.01 <u>Random Plankton Sampling Site Selection Using ArcMap</u>

I.   <u>Summary</u>

Random sampling sites are generated based on current lake elevation using ArcMap software. Map sheets showing random sample locations are created and printed for field documentation.

II. <u>Equipment</u>

Computer with network access
ESRI ArcMap 10
Color printer

III. <u>Procedure</u>

A. Start ArcMap software and open the random map template file: **Plankton_Random_yyyy.mxd** , where **yyyy** is the current year.

   1. The ArcMap – Getting Started window will appear after starting up ArcMap. On the left side of the window under "Existing Maps," choose "Browse for more" to open the map template file in the Map Library for the current year: **U:\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\Map_Library\ Plankton_Random_yyyy.mxd**

B. Save the map as a new document so the template is not modified.

   1. Click File > Save As.

   2. Browse to the Map Library for the current year: **U:\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\Map_Library**

   3. Change the map name to: **RZ_yyyymmdd_Zxx_elev,** where **yyyymmdd** is the current date, **xx** is the random zone number, and **elev** is the current lake elevation rounded to nearest 10 feet (for example: **RZ_20110630_Z01_3640)**. Click Save.

C. Make sure the "Spatial Analyst" extension is enabled. If not, the "Random Plankton Points" tool will not function.

1. Go to the "Customize" menu in the toolbar, click "Extensions…"
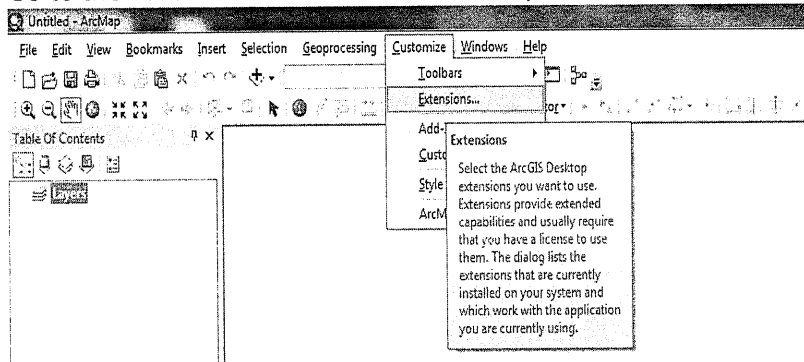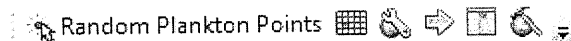
2. The Extensions window will open. Check mark the box next to "Spatial Analyst." Close the window.
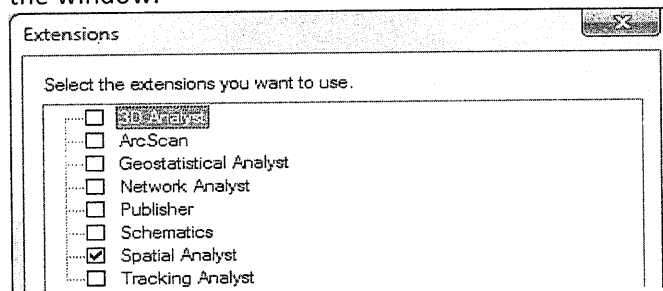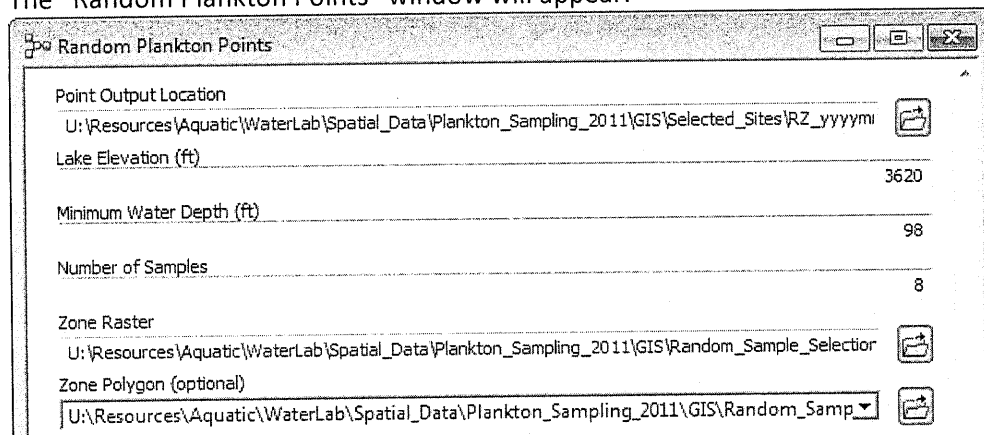
D. Generate random sampling points.

1. Click on "Random Plankton Points" located on the Random Sampling toolbar:

2. The "Random Plankton Points" window will appear.

Point Output Location
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_2011\GIS\Selected_Sites\RZ_yyyymi

Lake Elevation (ft)
3620

Minimum Water Depth (ft)
98

Number of Samples
8

Zone Raster
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_2011\GIS\Random_Sample_Selectior

Zone Polygon (optional)
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_2011\GIS\Random_Samp
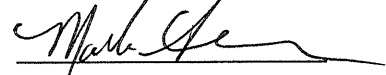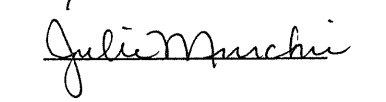
3. Verify the proper "Point Output Location" is listed for saving the shapefile:
   U:\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS\Selected_Sites
   where **yyyy** is the current year. Click the folder icon to browse to the appropriate
   folder. Rename the shapefile in the format: **RZ_yyyymmdd_Zxx_elev.shp** as previously
   saved for the map file.

4. In the "Lake Elevation" box, type in the current lake elevation rounded to the nearest 10
   feet.

5. In the "Minimum Water Depth" box, set the desired minimum tow depth. The
   minimum water depth defaults to 98 feet or 30 meters. Note: For most zones the
   minimum depth of 30 meters is sufficient. For inflow zones (Zones 8, 9, 13) the
   minimum depth will need to be decreased.

6. In the "Number of Samples" box, set the number of points to be generated. Note: Not
   all points generated have to be sampled. Generally 6 samples are collected on a given
   trip so the number of samples to be generated should be at least 6.

7. Select the "Zone Raster" for the appropriate random zone to be sampled. Click the
   folder icon to locate the desired file. The zone raster files are located at:
   U:\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS
   Random_Sample_Selection\Baseline\Random_Plankton_Sampling_Baseline_yyyy.gdb

8. Select the "Zone Polygon" for the appropriate random zone to be sampled. Click the
   folder icon to locate the desired file. The polygon files are located at:
   U:\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS
   Random_Sample_Selection\Baseline\Random_Plankton_Sampling_Baseline_yyyy.gdb

9. Click OK. Random points are generated by the tool and the **RZ_yyyymmdd_Zxx_elev**
   shapefile is added to the Table of Contents under "Index Map" in the Map data frame as
   a new layer. Close the Random Plankton Points box when finished.

10. Right click on the newly created **RZ_yyyymmdd_Zxx_elev** shapefile and select "Zoom To
    Layer" and the random points should be displayed.

E. Display the bathymetry layer for lake contours.

1. In the Table of Contents, check the "**bathy08_xxxx**" layer where **xxxx** is the current lake
   elevation rounded to the nearest 10 feet. Lake contour lines will be displayed.

F. Set symbology for the random sampling points.

1. In the Table of Contents, double-click the newly created symbol, under
   RZ_yyyymmdd_Z12_elev. The symbol selector window will open.

2. Choose a symbol that is visible with the bathymetry layer. The suggested symbol is a red "Circle 16" or red "Circle 17." Click OK.

G. Update the map sheets text block in Layout View.

   1. Click View > Layout View (if not already in layout view).

   2. Double-click the text block in the lower left-hand corner of the map to open the Properties dialog box. Note: The black arrow will need to be selected from the Tools toolbar in order to change text.

   3. Enter the Random Zone number, Lake Elevation (**actual** current lake elevation), and the Shapefile and GPS Jobfile names. The shapefile is named as before: **RZ_yyyymmdd_Zxx_elev.shp**. The GPS jobfiles are named using the **anticipated** sampling date, not the creation date in the following format: **RZyymmdd**. Click OK.

H. Prepare the document for printing.

   1. Go to File > Page and Print Setup. Select the designated printer. Select "landscape" for the orientation. (Note: The boxes for "Use Printer Paper Settings" and "Scale Map Elements proportionally to changes in Page Size" may need to be checked to ensure that the entire map is displayed on the printed page). Click OK.

   2. Print the mapsheet. Go to File > Print, select Print.

   3. Save the ArcMap document.

I. Export the set of map sheets (optional).

   1. Click File > Export Map.

   2. Change "Save as type" to PDF.

   3. Save PDF to the appropriate Map_Library using the current map name: **U:\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\Map_Library**

J. Close ArcMap when finished.

Effective Date ___08AUG 2016___

Approved By _~Mark Ife_____

Julie Manchin

SOP 2.02 <u>Electronic Recording of Sample Sites using GPS</u>

I.   <u>Summary</u>

The location of all plankton samples collected will be recorded using the Thales MobileMapper handheld GPS unit. GPS files and point locations will be labeled in a standardized manner.

II.   <u>Equipment</u>

Thales MobileMapper GPS unit
4 AA batteries

III.   <u>General Procedures for GPS Units</u>

A. Each day of sampling will be given its own GPS job file. All GPS files should contain only periods of time that are less than 24 hours. All points taken that day will be recorded in the GPS and stored under that file, unless some samples are taken for special circumstances and a separate file is justified.

B. GPS job files with selected random sample locations should be created and loaded on the GPS prior to sampling to assist in navigation to random sites (refer to SOPs 2.01 and 2.04). Alternatively, map sheets or a laptop with a GPS can be used as a navigational aid for random sites.

C. Prior to field use, ensure that a secure digital (SD) memory card and batteries are properly installed in the unit, and that extra batteries are put in the GPS case.

D. To turn on the unit, press the red "PWR" button once quickly, and press the "ENTER" button three times in a row to cycle past the initial startup, copyright, and warning screens.

E. To turn off the unit, press red "PWR" button once, and the unit will shut off at the end of a 5-second countdown. Note: if the GPS unit is turned off, the active job file is closed and will need to be reopened to save new points.

F. Pressing and holding the "PWR" button will adjust the backlight settings.

G. The "NAV" button cycles the GPS display between the map, compass, position, and satellite status screens.

map screen ——→ compass screen ——→ position screen ——→ satellite status screen
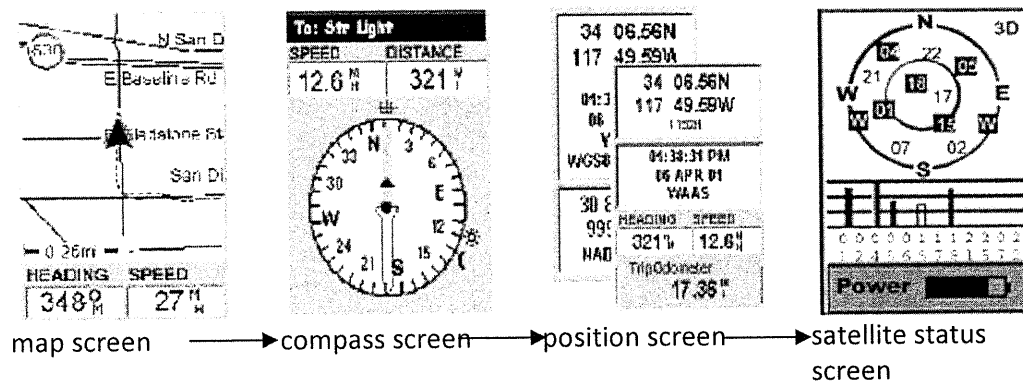
H. The map screen shows current position and heading with a triangle. The center scrolling button can be used to move a cursor around the screen for selecting features. Press "ESC" to hide the cursor and return to the current position marker. Pressing the "IN" and "OUT" buttons will adjust the level of zoom displayed on the map screen. Note: the position cursor will display as an hourglass if the unit has not established adequate satellite connections.

I. The compass screen shows speed, distance, and direction to a waypoint.

J. The position screen can be used to manually record the current GPS position in NAD83 UTM format (xxxxxxxxE xxxxxxxN), and also displays the current time and date.

K. The satellite status screen shows how many satellites the GPS unit has established connections with, and the strength of the satellite signals (shown as black bars). An accurate position fix cannot be determined unless the unit has connected to at least 3 satellites. This screen also shows remaining battery power.

L. The "MENU" button is used to create or open GPS job files that sample point locations are saved into.

M. The "LOG" button is used to create a new point location in the open job file.

N. Pressing "ENTER" will accept the highlighted selection.

O. The "ESC" button will take you back to the previous screen without making any changes.

IV. GPS Job File Naming Conventions

A. Plankton Sampling job files created in the field for routine sample collection are given a 7-character name which has a prefix to designate the sample type (Z for zebra mussel) followed by the current date in yymmdd format (**Zyymmdd**). Example: locations of plankton samples collected on 21AUG10 would be saved in a job file named "Z100821."

B. Selected site job files created prior to random sampling and uploaded to the GPS unit for navigation are given a "RZ" prefix (for random zebra) followed by the **anticipated** sampling date in yymmdd format (**RZyymmdd**). Example: A random sampling map is prepared on 07AUG10 for sampling on 14AUG10. The selected points will be uploaded to the GPS in a job file named "RZ100814."

C. If the job file in use becomes corrupted and new points cannot be recorded in it, a new job file is created with the "Z" prefix, the current date in yymmdd format, and an additional 1-letter suffix starting with "A" (Example: job file "RZ100814" becomes corrupted while sampling. A new file named "Z100814A" is created and used for subsequent points).

V. <u>Creating and Opening GPS Job Files</u>

A. Turn on GPS unit and wait for the GPS to establish a satellite connection.

B. To create a new GPS job file, press the "MENU" button, then highlight and select "New Job." Enter the job name by using the scrolling center button to move around the alphanumeric display, and the "ENTER" button to register each character. When the entire name has been entered, highlight "Ok" and press "ENTER."

C. On the feature library screen, highlight "ZM_SAMPL.MMF" and press "ENTER." The "ZM_SAMPL" template contains the attribute fields necessary for recording plankton sample data associated with each location (SiteID and CollDate).

D. On the job mode screen, highlight "Post-processing" and press "ENTER." Selecting "Post-processing" allows the points collected in the field to be differentially corrected on a computer to improve their accuracy.

E. Press "NAV" to return to the map screen.

F. To open an existing or uploaded GPS job file, press the "Menu" button, then highlight and select "Open Job." Highlight the desired job file on the job list screen and press "ENTER".

VI. <u>Recording Point Features at Sample Sites</u>

A. If the GPS unit is not on, power it up and wait for the GPS to establish a satellite connection.

B. If the correct job file is not active, create or open an existing GPS job file, as appropriate.

C. To record the sample site location, press the "LOG" button to bring up the new feature screen. The feature type will be "ZM_Sample" for jobs created on the GPS, and "RZ_yyyymmdd_Zxx_elev" for uploaded random sampling jobs.

D. Press "ENTER" on the new feature screen to log the sample site location. The GPS display will show the logging screen and the unit will start recording positional data. To stop collecting point data, highlight the "Close" icon on the logging screen and press "ENTER." Log points for 15 seconds.

VII.   Editing Feature Attributes

A. After recording a sample location, press "ESC" or "NAV" as necessary to return to the map screen.

B. Move the cursor on the map screen directly over the new point feature with the scrolling center button. When the feature has been highlighted, the feature name ("ZM_Sample" or "RZ_yyyymmdd_Zxx_elev") will be displayed at the bottom of the screen. Press "Enter" to open the selected feature screen for that point.

C. The selected feature screen will show the feature attributes (SiteID and CollDate) and their values. If you are entering data for new point, these attributes should be blank. If they are not blank, press "ESC" to return to the map screen and select the correct point. The "IN" and "OUT" buttons can be used to adjust the zoom to an appropriate level for selecting the correct feature.

D. To enter or edit feature attribute values for the selected feature, highlight the "Edit" icon and press "ENTER." Highlight the SiteID attribute and press "Enter" to bring up the alphanumeric display. Type in the SiteID then highlight the "OK" icon and press "ENTER" when finished to return to the selected feature screen. Highlight the CollDate attribute and repeat the process to enter the current date in the appropriate format (e.g. 14AUG10).

E. After editing both the SiteID and the CollDate fields, highlight the "Close" icon on the selected feature screen and press "ENTER."

F. If a point feature is accidentally created at a location where no sample is taken, the SiteID should be set to "NOSAMPLE" or "NONE."
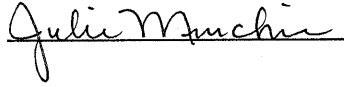
VIII.   Changing the GPS Background Map Display

A. To select a background map, press the "Menu" button, then highlight and select "Setup."

B. On the setup menu screen, highlight "Select Map" and press "ENTER."

C. On the change map screen, highlight the desired detail map or select "Empty" to use only the default background map. Detail map names reflect the lake elevation contours used to create their symbology. (e.g. WQ3640.IMG shows the shoreline at 3640 feet and should be selected for lake elevations close to 3640 feet).

D. After selecting a detail map, highlight "Save" and press "ENTER." The detail map will now display on the map screen.

E. Additional detail maps can be created in MobileMapper Office and uploaded to the GPS as Disc Image files.

Effective Date _08 AUG 2016_

Approved By _(signature)_

Julie Munchin

SOP 2.03 <u>Downloading and Differentially Correcting GPS Points</u>

I.   <u>Summary</u>

Raw sample site data recorded in the field are downloaded from the GPS using MobileMapper Office. GPS points are then differentially corrected to improve accuracy. Copies of the raw and corrected GPS data are stored in folders specific to the GPS unit, and a shapefile is exported for GIS analysis.

II.   <u>Equipment</u>

Thales MobileMapper GPS Unit   MobileMapper Office
Computer with network access   ArcCatalog 10
Secure digital (SD) card reader

III.   <u>Downloading Raw GPS Data to the Network</u>

A. Remove the secure digital (SD) memory card from the Thales MobileMapper handheld unit. The card is located in the battery compartment.

B. Connect card reader to the computer and insert the GPS card.

C. In Windows Explorer, transfer all the files associated with a given date from the GPS card to the appropriate raw GPS data folder location on the network.

Raw data from the Wahweap GPS unit is stored at:
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\WW_Unit\Raw
where yyyy is the current year.

Raw data from the Bullfrog GPS unit is stored at:
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\BF_Unit\Raw

Highlight the MobileMapper job file (.MMJ) and all associated files (.R00, .M00, .G00, etc.) then right-click and choose "Copy." Go to the folder location for the Bullfrog or Wahweap GPS unit, then right-click and choose "Paste."

IV.   <u>Transferring Raw GPS Data to Corrected Data Folder</u>

A. MobileMapper Office **must** be used to transfer the raw data to the corrected data folder. Raw data copied from the SD card using Windows explorer will not be properly formatted for differential correction.

B. Start MobileMapper Office.

C. Select "File" from the menu bar and select "Download from GPS." The MobileMapper Transfer window will appear.

D. Set the left (source) and right (destination) MobileMapper Transfer windows to the appropriate directories.

Source directories for Wahweap and Bullfrog units (respectively) are:
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\WW_Unit\Raw
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\BF_Unit\Raw

Destination directories for Wahweap and Bullfrog units (respectively) are:
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\WW_Unit\Corrected
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\BF_Unit\Corrected

Choose "File" from the menu bar and click on "Connect" then "PC Drive." Navigate to the appropriate directories if they are not displayed in the left and right windows.
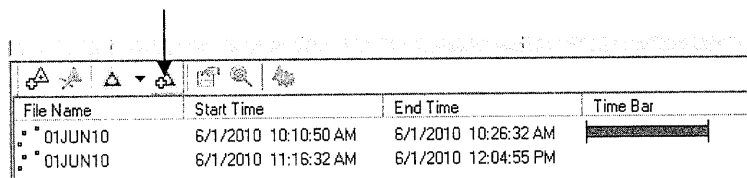
E. Click and drag the selected MobileMapper job file from the left window to the right window. The "Copying File" window will appear. In order to transfer more than one file at a time, hold down the "Ctrl" key on the keyboard while selecting.

source ("Raw" folder) ⟶ destination ("Corrected" folder)
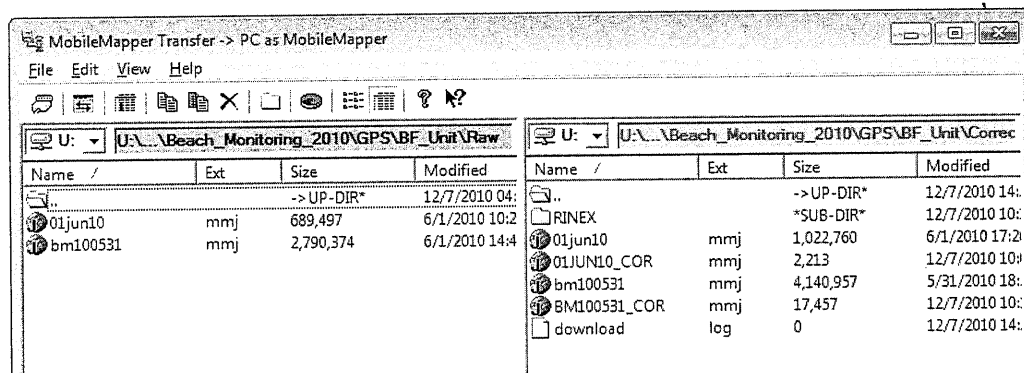
F. When the transfer is finished, close the MobileMapper Transfer window. The file(s) transferred will be displayed in the MobileMapper Office window.
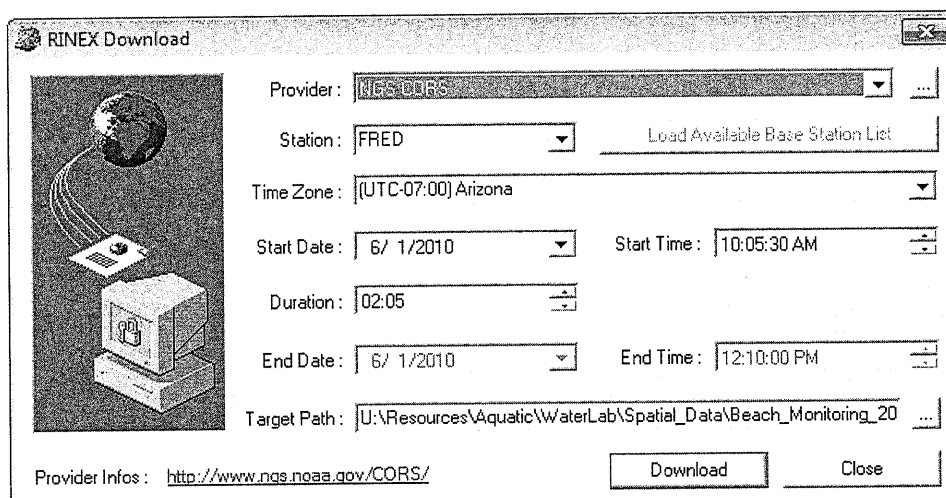
V. Differentially Correcting GPS Data

A. Wait at least 24 hours after completion of sampling before differentially correcting a file.

B. Start MobileMapper Office and open the GPS job file to be differentially corrected. Select "File" from the menu, then "Open" from the drop down list. Navigate to and select the job file from the appropriate "Corrected" data folder. The file will now be displayed in the MobileMapper Office window.

C. Before differentially correcting the job file, resave it as "filename_COR.mmj" in the "Corrected" folder. Select "File" from the menu, then "Save As" from the drop down list.

D. Download reference station data for the open job file. Click on the "Download Reference Station" button found on the lower toolbar.

E. The "RINEX Download" window will appear. Set Provider, Station, and Time Zone as shown below. The start time and end time should be adjusted to completely encompass the time when points were logged (if necessary).

Target Paths for Wahweap and Bullfrog units (respectively) are:
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\WW_Unit\Corrected
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\BF_Unit\Corrected

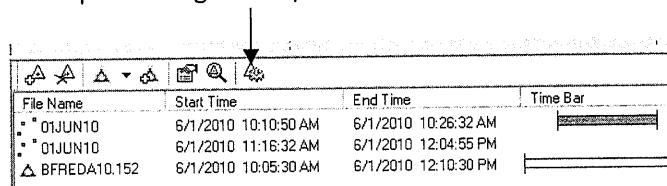Click "Download" and then click "Close" when the download is complete.

F. In the box on the bottom of the MobileMapper Office window, a yellow bar will appear beneath the green bars of the GPS file. Make sure this yellow bar extends beyond both ends of the all the green bars. If not, download additional RINEX files to cover the missing time periods.

File Name	Start Time	End Time	Time Bar
01JUN10	6/1/2010 10:10:50 AM	6/1/2010 10:26:32 AM	
01JUN10	6/1/2010 11:16:32 AM	6/1/2010 12:04:55 PM	
BFREDA10.152	6/1/2010 10:05:30 AM	6/1/2010 12:10:30 PM	

F. Set the coordinate system for the job. Select "Options" from the Menu bar. Click "Select Coordinate System." The "Select Coordinate System" window will appear. Select "NAD1983_UTM_Zone12N" from the drop-down list. The coordinate system will then be displayed in the lower right hand corner of the screen.

> Note: If this coordinate system does not appear in the drop-down list, it can be added by importing any shapefile that uses the NAD1983_UTM_Zone12N projection into MobileMapper Office. Click File>Import to open the shapefile and the projection will become available. Once the projection has been added, reopen the "filename_COR.mmj" to finish differential correction.

G. Differentially correct the data. Click on the "Process data" icon, and then click "Okay" when processing is complete.

File Name	Start Time	End Time	Time Bar
01JUN10	6/1/2010 10:10:50 AM	6/1/2010 10:26:32 AM	
01JUN10	6/1/2010 11:16:32 AM	6/1/2010 12:04:55 PM	
BFREDA10.152	6/1/2010 10:05:30 AM	6/1/2010 12:10:30 PM	

The "Correction" field in the Job Properties box should change from "Real-time" to "Post-processed" to indicate that the correction has been applied.

Job Properties	
Property	Value
Job Name	01JUN10
Receiver	MobileMapper 6.56
s/n	000017295655
Start Time	6/1/2010 10:11:31 AM
End Time	6/1/2010 11:51:35 AM
Duration (hh:mm:ss)	01:40:04
Correction	Real-time
Number of Features	4
Features per Feature Type	
WQ_Sample	4

Job Properties	
Property	Value
Job Name	01JUN10_COR
Receiver	MobileMapper 6.56
s/n	000017295655
Start Time	6/1/2010 10:11:31 AM
End Time	6/1/2010 11:51:35 AM
Duration (hh:mm:ss)	01:40:04
Correction	Post-processed
Number of Features	4
Features per Feature Type	
WQ_Sample	4

H. Save the job file.

VI.   Exporting Corrected GPS Data

    A.   Export the corrected points to a shapefile for GIS analysis. In MobileMapper Office, choose "File" from the menu bar and click "Export...".

        Export directories for Wahweap and Bullfrog units (respectively) are:
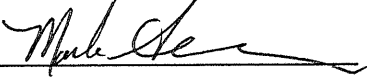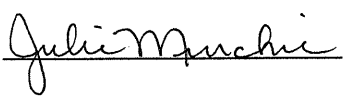**U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\WW_Unit\Export**
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\BF_Unit\Export

        Click "Export."

    B.   Rename the exported shapefile to match the job file it originated from in **RZyymmdd** or **Zyymmdd** format. Start ArcCatalog and locate the exported shapefile. Job files created on the GPS unit will default to "ZM_Sample" and random sample jobs uploaded to the GPS will default to "RZ_yyyymmdd_Zxx_elev." Select the file and press F2 to rename it.

Effective Date ___O8AUG2016___

Approved By ___(signature)___

___(signature)___

SOP 2.04 <u>Uploading Random Sample Points to GPS</u>

I.   <u>Summary</u>

      Random sample site shapefiles created in ArcMap are converted to GPS job files. Job files are then uploaded to a Thales MobileMapper GPS unit to assist with field navigation.

II.   <u>Equipment</u>

      Thales MobileMapper Handheld GPS Unit

      Computer with network access

      MobileMapper Office software

      SD memory card from GPS

      SD card reader

III.   <u>Procedure for Uploading Random Sample Point to GPS</u>
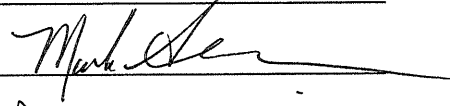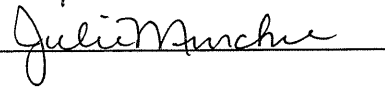
    A. Start MobileMapper Office.

    B. Click File > Import.

    C. Navigate to the selected shapefile to be uploaded and click Open. Random sample shapefiles are stored at:
       U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS\Selected_Sites
       where **yyyy** is the current year.

    D. Click Import in the Import GIS Data window to add the point features.

    E. Save the point features in a GPS job file. Go to File > Save As.

    F. Navigate to the **Uploads** folder for the Wahweap or Bullfrog GPS Unit. The Uploads folder is located at:
       U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GPS\WW_Unit
       for Wahweap or **\BF_Unit** for Bullfrog.

    G. Name the GPS job file in the format: **RZyymmdd** where **yy** is the 2-digit year, mm is the 2-digit month, and dd is the 2-digit day. Then click Save.

    H. Proceed to Section IV if uploading using a thin client computer and a SD memory card reader. Proceed to Section V if uploading using a computer with a serial port and MobileMapper Office.

IV.   Upload Using a Thin Client and SD Card Reader

    A. Remove the SD memory card from the GPS battery compartment.

    B. Connect the SD card reader to the computer USB port and insert the SD memory card from the GPS into the reader.

    C. In Windows Explorer navigate to the **Uploads** folder and right-click on the desired GPS job file and choose Copy.

    D. In Windows Explorer open the drive associated with the GPS memory card. Right-click within the GPS memory card window and choose Paste to transfer the desired GPS job file to the GPS memory card.

V.   Upload Using a Computer Serial Port Cable and MobileMapper Office

    A. Connect the GPS unit to the computer with the serial port communication cable.

    B. Press "PWR" to turn the GPS unit on.

    C. In MobileMapper Office, click File > Upload to GPS > Job.

    D. The "Uploading to GPS" window will open while MobileMapper Office connects to the GPS and uploads the file. The window will close automatically once the upload is complete.

    E. Press "PWR" to turn the GPS unit off.

    F. Disconnect the GPS unit from the computer.

Effective Date 08AUG2016

Approved By

SOP 2.05: <u>GIS Data Management</u>

I. <u>Summary</u>

Plankton sample shapefiles exported from MobileMapper Office are copied into the GIS directory and organized by sampling date. Shapefiles are projected and edited to ensure attribute data is correct and all points are accounted for. Edited shapefiles are appended to a geodatabase, and the locations of missing points are estimated. GIS data is then loaded into the GLCA Plankton Samples geodatabase and is joined with tabular laboratory data stored in an Access database.

II. <u>Equipment</u>

Computer with network access   Microsoft Access
ESRI ArcGIS 10.x   Plankton sampling datasheets

III. <u>Procedure for Editing GIS Points</u>

A. Follow these steps to copy shapefiles from the GPS export directory and organize by sample date.

1. Start ArcCatalog and browse to the WaterLab\Spatial_Data directory.

2. In the **Plankton_Sampling _yyyy\GIS\Sampled_Sites** folder for each laboratory, create a new folder named **\yyyymmdd** for each day that water samples were processed in that lab.

3. Create a new **\edited** subfolder in each of the new sample date folders.

4. Reference the original field data sheets to determine which GPS jobfile(s) were used to collect GPS points for each sample date.

5. Locate the shapefiles exported from those GPS jobfile(s) in the **\GPS\WW_Unit\Export** or **\GPS\BF_Unit\Export** directory.

6. Copy and paste the shapefiles from the GPS export directory into the GIS sample date **(\yyyymmdd)** folder for the correct laboratory.

B. Project all shapefiles in the sample date folders, and copy to the **\edited** subfolder.

1. Click Windows > Search to open the search window in ArcCatalog.

2. Type Project and press Return.

3. Click Project (Data Management) to open the projection tool.
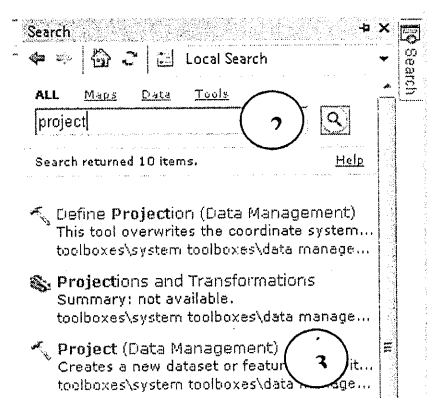
4. Drag a shapefile from the sample date folder to Input Dataset.

5. An Output Dataset name is automatically assigned (filename_Project.shp).

6. Set the Output Coordinate System to NAD_1983_UTM_Zone_12N.

   a. Select... > Projected Coordinate System > UTM > NAD 1983

   b. Click Add > Ok.

7. Click Ok to create a new projected file.
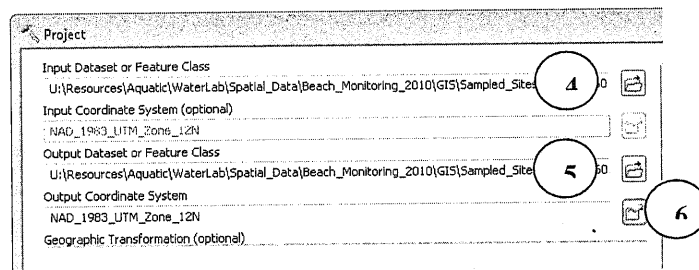
8. Copy and paste the projected file **filename_Project.shp** from the sample date folder **\yyyymmdd** to the edited subfolder **\yyyymmdd\edited**.

9. Repeat if necessary for additional shapefiles in the sample date folder.

C. To project and copy many files concurrently, use the **Project and Copy** model builder tool.

   1. In ArcCatalog, locate the **Project and Copy** tool in the **PlanktonSamplingDBToolbox.tbx** at:
   U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS\ Random_Sample_Selection\Tools

   2. Right-click Project and Copy>Edit to open the Model Builder interface.

   3. Double-click Iterate Feature Classes.

4. Set the Workspace or Feature Dataset to the folder that you want to process (**Plankton_Sampling_yyyy\GIS\Sampled_Sites\WW or BF)**. All shapefiles in the folder you select will be projected and then copied to the same directory they are currently in.

5. Check "Recursive" to process shapefiles in all subfolders of the designated Workspace. This option allows the tool to project and copy files in sample date subfolders of **Plankton_Sampling_yyyy\GIS\Sampled_Sites\WW or BF**. Click "OK."
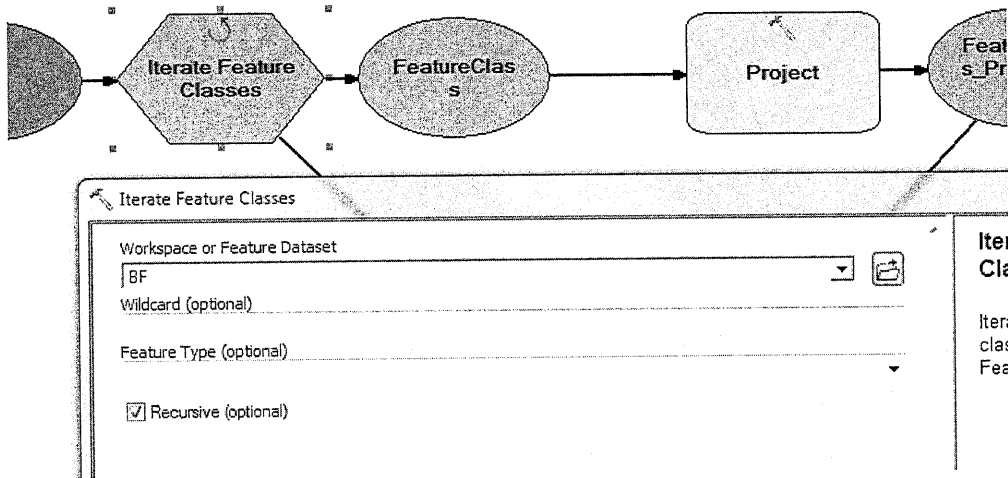
6. On the ModelBuilder toolbar, click Model>Run Entire Model.

7. When the Project and Copy tool has finished running, use ArcCatalog to move the copied files **filename_Project_Copy.shp** from the sample date folder **\yyyymmdd** to the edited subfolder **\yyyymmdd\edited**.

D. Merge shapefiles for sample dates with multiple files in the **\edited** folder.

1. Click Windows > Search to open the search window in ArcCatalog.

2. Type Merge and press Return.

3. Click Merge (Data Management) to open the merge tool.

4. Drag all files in the \edited folder to the Input Datasets textbox. The filenames will appear in the Datasets list.

5. The Output Dataset name will default to **filename_Merge.shp.**

6. Press Ok to run the merge tool.

7. In ArcCatalog, delete all files from the \edited folder except for **filename_Merge.shp.**

E. Edit point features to remove extra points and correct attributes.

  1. Start ArcMap and press "Cancel" to work in an empty map.

  2. Click Customize > Toolbars and verify that the Editing toolbar is checked.

  3. Click ✛ Add Data to add the shapefile to be edited from the sample date edited folder (**filename_Copy.shp, filename_Merge.shp** or similar).

  4. Click Editor > Start Editing on the Editing toolbar.

  5. In the Table of Contents, right-click the shapefile and click "Open Attribute Table."

  6. Compare the features listed in the table to the original field data sheet for the sample date.

  7. Delete any features that don't represent an actual sample location, such as selected sites, or technician GPS errors.

     a. Click the small box left of the feature FID to highlight the feature.

     b. Press "Delete."

  8. Correct technician entry errors in the formatting of the SiteID and CollDate fields. All characters in these fields should be uppercase with no spaces allowed. CollDate follows the ddmmmyy format where mmm is the 3-character alphanumeric abbreviation.

     a. Double-click in the field that is incorrect to bring up a cursor.

     b. Correct the error.

  9. Annotate any missing GIS points on the field data sheet. If data entry has already been completed, the Access database and printouts should also be annotated following the relevant SOPs.

  10. Save edits made to the template shapefile. Click Editor > Stop Editing > Yes on the Editing toolbar.

  11. In ArcCatalog, rename the completed shapefile **ZM_yyyymmdd_BF.shp** or **ZM_yyyymmdd_BF.shp**.

IV.   Procedure for Appending Edited GIS Points to Geodatabase

  A. To append edited GIS shapefiles to the geodatabase, use the **Append to DB** model builder tool.

1. In ArcCatalog, locate the tool in the **PlanktonSamplingDBToolbox.tbx** at:
   **U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS\
   Random_Sample_Selection\Tools**

2. Right-click Append to DB > Edit to open the Model Builder interface.

3. Double-click the Append box.

4. Input Datasets can be remain blank.

5. Set the Target Dataset that files will be appended to by navigating to the desired
   folder. Choose **ZM_All_Sites_yyyy_BF** or **ZM_All_Sites_yyyy_WW** as appropriate
   from: **U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS\
   Sampled_Sites\All_Sites\ZM_All_Sites_yyyy.gdb.** Click OK once the Target Dataset
   has been selected.
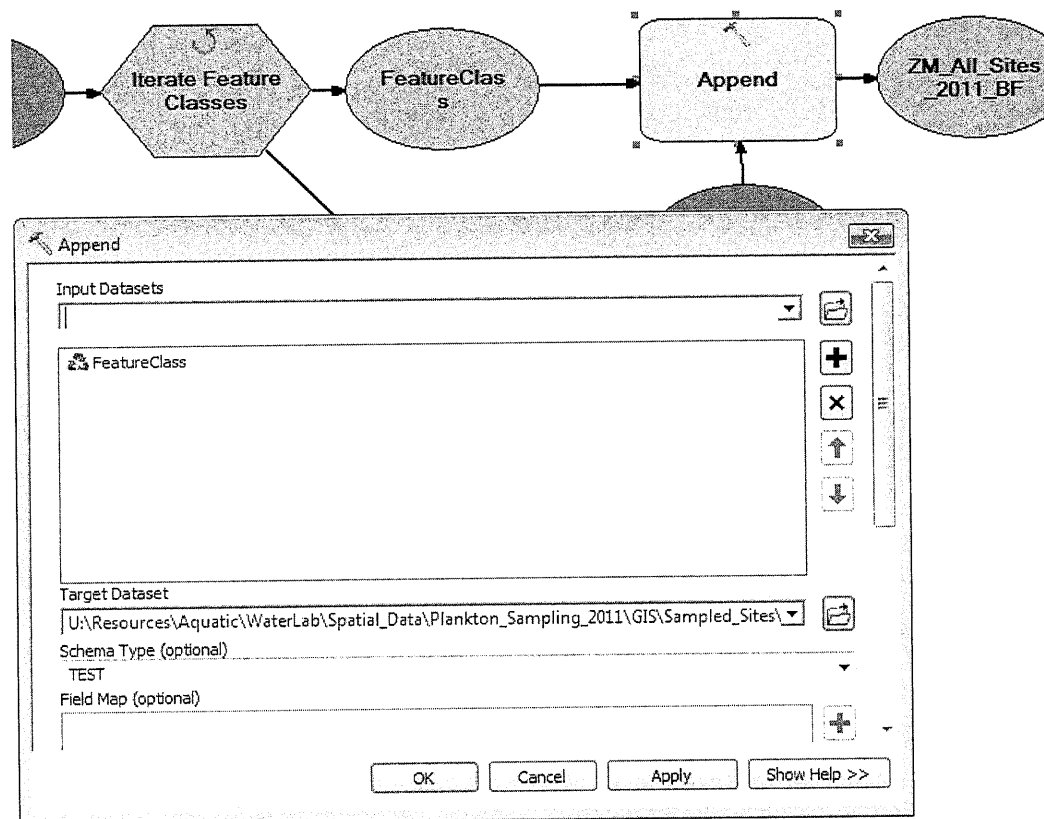
6. Double-click Iterate Feature Classes.

7. Set the Workspace or Feature Dataset to the folder that you want to append files
   from (**\Plankton_Sampling _yyyy\GIS\Sampled_Sites\WW or BF**).

8. Check "Recursive" to process shapefiles in all subfolders of the designated Workspace. This option allows the tool to append files in sample date\edited subfolders of **\Plankton_Sampling _yyyy\GIS\Sampled_Sites\WW or BF**.

9. Set the Wildcard to "ZM*" to ensure that only shapefiles that have been edited and renamed will be appended to the database. Click OK.
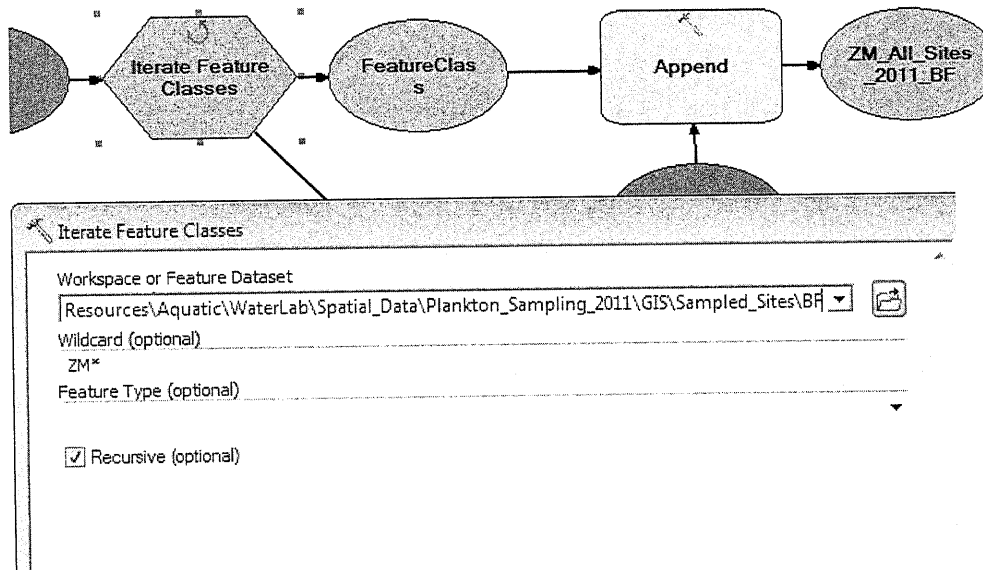
10. On the ModelBuilder toolbar, click File > Run Entire Model.

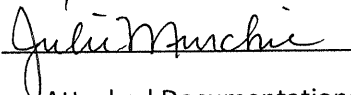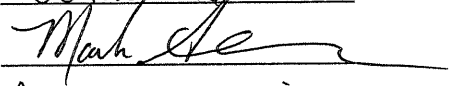V.   Procedure for Estimating Missing GIS Points

A. Point estimates of sampling site locations not recorded with a GPS unit can be saved in the **ZM_All_Sites_yyyy_estimated** feature class located at:
U:\Resources\Aquatic\WaterLab\Spatial_Data\Plankton_Sampling_yyyy\GIS\ Sampled_Sites\All_Sites\ZM_All_Sites_yyyy.gdb

B. Annotate estimated GIS points added to the database on the field data sheet. If data entry has already been completed, the Access database and printouts should also be annotated following the relevant SOPs.

C. Selected site locations can be used as random sampled site estimates.

1. Determine which selected site location was used for the random site from the field data sheets and maps.

2. In ArcMap, open the appropriate selected site shapefile and estimated feature class.

3. Click Editor > Start Editing on the Editing toolbar, and select the **ZM_All_Sites_yyyy_estimated** feature class.

4. Select the Edit tool from the Editing toolbar, and click on the selected site point feature corresponding to the missing random point.

5. In the Table of Contents, right-click the selected site shapefile and click "Open Attribute Table". Verify that the correct point is highlighted.

6. Right-click the selected site shapefile and select Copy.

7. Right-click the selected site shapefile and select Paste. Choose **ZM_All_Sites_yyyy_estimated** as the layer to paste into.

8. Open the attribute table for the estimated layer and modify the SiteID and CollDate fields to the correct values.

9. Click Editor > Stop Editing > Yes on the Editing toolbar to stop editing and save edits.

D. Routine site locations can be estimated based on previous sampling locations or from the description of the site location.

1. In ArcMap, open the estimated feature class and add any necessary reference layers.

2. Click Editor > Start Editing on the Editing toolbar, and select the **ZM_All_Sites_yyyy_estimated** feature class.

3. Select the Create Features tool from the Editing toolbar, and select the Point ⊡ Point construction tool from the Create Features window.

4. Click at the desired location on the map to create a new point.

5. Open the attribute table for the estimated layer and modify the SiteID and ProcDate fields to the correct values.

6. Click Editor > Stop Editing > Yes on the Editing toolbar to stop editing and save edits.

VI. <u>Procedure for Updating the GLCA Plankton Sampling Geodatabase</u>

A. The GLCA_Plankton_Samples geodatabase is loaded with new data after all recorded and estimated points have been edited and appended to the **ZM_All_Sites_yyyy** geodatabase.

1. In ArcCatalog, navigate to the GLCA_Plankton_Samples geodatabase located at: U:\Resources\Aquatic\WaterLab\Spatial_Data.

2. Right-click the GLCA_Plankton_Samples_2008_present feature class and select Load>Load Data.

3. In the Input Data box, navigate to and select the **ZM_All_Sites_yyyy_BF** feature class. Press Open and then Add to add the file to the list of source data to load. Add the **ZM_All_Sites_yyyy_WW** and **ZM_All_Sites_yyyy_estimated** feature classes to the list.

4. Click Next to advance to the summary page, and then click Finish to load the data.

B. Calculating the GPS_ID field for joining tabular data.

1. Open ArcMap and add the **GLCA_Plankton_Samples_2008_present** geodatabase feature class to the layer.

2. Open the attribute table of the geodatabase.

3. Highlight all points added for the current year.

4. Right-click the GPS_ID field header and select "Field Calculator."

5. In the "GPS_ID =" window add the following equation string:
[CollDate] &"-"& [SiteID]. Then click "OK."

Effective Date _O8AUG2016_

Approved By _~M̶a̶r̶k̶ ̶L̶e̶e̶_

Julie Munchie

Attached Documentation:
Veliger Monitoring Laboratory Form (B)

SOP 3.01: Internal Audit for Precision

I. Summary

At the beginning of every summer sampling season and every month thereafter, the Quality Assurance (QA) Officer performs an internal audit to test the precision between technicians in the analysis of plankton samples for *Dreissenid* veliger detection and enumeration. An internal audit must be performed by all technicians for both microscope and FlowCAM techniques.

II. Equipment

Axiostar *plus* Zeiss Compound Microscope
FlowCAM
Two previously analyzed plankton samples with veligers
Plankton sample processing and analysis equipment (Refer to SOPs 1.07–1.13)
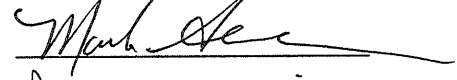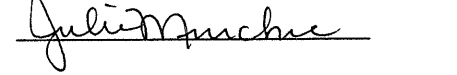
III. Procedures

A. The QA Officer chooses a previously analyzed sample with a known number of veligers.

B. Technicians then analyze the sample and record results on the Veliger Monitoring Laboratory Form (Attachment B).

C. The QA Officer (or designee) then runs a sample through the FlowCAM in accordance with SOP 1.12: Sample Processing by FlowCAM and SOP 1.13: Sample Post-Processing by VisualSpreadsheet and counts the number of veligers imaged.

D. The technicians, in turn, look through the collage images to determine the number of images veligers and record their findings on the Veliger Monitoring Laboratory Form (Attachment B).

E. The QA Officer then compares the results and writes an Internal Audit for Precision Report.

The Internal Audit of Precision Report contains: 1) A copy of each technician's Veliger Monitoring Laboratory Form (Attachment B); 2) A conclusion. The audit passes if all of the technicians' results are reasonably close in veliger counts. If the audit fails a corrective action report must be written.

F. The Internal Audit for Precision Report must be retained by the QA Officer.

Effective Date ___O8AUG 2016___

Approved By _____

_____

Attached Documentation:
Veliger Monitoring Laboratory Form (B)
Demonstration of Capability Certification Statement (Q)
Demonstration of Capability Notes (R)

SOP 3.02: Demonstration of Capability

I. Summary

A key component in the Glen Canyon Microscopy Laboratory's Quality Assurance Plan is the performance of a Demonstration of Capability (DOC) Test by each technician. Annually, each technician must perform a DOC before independently performing laboratory techniques.

II. Equipment

Axiostar *plus* Zeiss Compound Microscope
FlowCAM
Previously analyzed plankton samples
Veligers to spike sample(s)
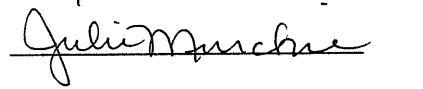Plankton sample processing and analysis equipment (Refer to SOPs 1.07–1.13)

III. Procedures

A. The Quality Assurance (QA) Officer or Laboratory Supervisor will need to set up a Demonstration of Capability Test when a laboratory technician has completed their training (Refer to SOP 4.03: Laboratory Technician Training).

B. Prior to the test, the QA Officer or Laboratory Supervisor selects three previously analyzed plankton samples with *Dreissenid* veligers.

C. Under the supervision of the QA Officer, the technician must prepare the plankton samples as if they were a routine plankton sample.

D. The plankton samples must then be analyzed using the compound microscope and FlowCAM by the technician to determine the presence or absence of *Dreissenid* veligers in each of the three samples (Refer to SOPs 1.07–1.13).

E. Results must be recorded on the Veliger Monitoring Laboratory Form (Attachment B). Also, fill out the Demonstration of Capability Notes (Attachment R).

F. The QA Officer compares the results achieved with the results expected. If the results do not match, the test is failed – the QA Officer must write a Corrective Action Report (Attachment I) which identifies the problem and the technician must take another Demonstration of Capability Test.

G. Upon successful completion of the DOC Test, the Laboratory Director and QA Officer must properly complete a DOC Certification Statement (Attachment Q) for the technician.

H. All documentation of this process, expected results, achieved results, laboratory notes, and the DOC Certification Statement must be retained by the QA Officer in the Demonstration of Capability binder. A copy of the DOC must be given to the technician.

Effective Date ___08AUG2016___

Approved By ___[signature]___

___[signature]___

Attached Documentation:

Signature Sheet and Ethical Policy Attestation (P)

SOP 3.03: Data Integrity System

I. Summary

The Glen Canyon National Recreation Area Laboratories' data integrity system consists of several components. All laboratory personnel must annually complete Data Integrity Training, provide their official signature and initials that will be used on all required laboratory documentation, and sign an ethical policy attestation statement. Laboratory management is responsible for in-depth periodic monitoring of data integrity and maintaining a procedure for employees to report data integrity issues.

II. Procedures

A. Data Integrity Training is provided to new laboratory personnel and annually for all current personnel by laboratory management. An attendance sheet must be signed by those in attendance.

B. Topics covered in Data Integrity Training must include a discussion of how and when to report data integrity issues, record keeping, and the organizational mission and its relationship to the critical need for honesty and full disclosure in analytical reporting.

C. Following Data Integrity Training, new personnel must provide their official signature and initials that will be used on all required laboratory documentation. All personnel must sign an Ethical Policy Attestation (Attachment P) that states:

"I will uphold the commitments of the Glen Canyon National Recreation Area Microscopy Laboratory as listed in the Quality Manual. I hereby acknowledge that the falsification of data is unethical, and I shall not do so while working in Glen Canyon National Recreation Area Laboratories. I understand that not upholding the aforementioned commitments and/or behaving unethically may lead to my termination. I understand that any deviation from a Standard Operating Procedure must be documented with a corrective action report."

D. Copies of official signatures, initials, and Ethical Policy Attestations must be retained by the employee and laboratory management.

E. Laboratory management assures a receptive environment in which all employees may privately discuss ethical issues or report items of ethical concern. Potential issues will be handled in a confidential manner until a follow up evaluation, investigation, or other appropriate actions have been completed and the issues clarified.

F. An employee may report an ethical concern either verbally or in writing to the Laboratory Director or Quality Assurance Officer.

G. It is the responsibility of the laboratory management to investigate and take action in response to any reports of unethical behavior. Findings of inappropriate activity must be documented and include any disciplinary actions involved, corrective actions taken, and notification to the client.

H. Other Quality Assurance activities such as the Quality Assurance Audits (SOP 4.07) also provide opportunities to detect unethical behavior.

I. Unethical behavior will result in a detailed investigation that could lead to disciplinary action including termination.

Effective Date ___O8 AUG 2016___

Approved By ___(signature)___

___(signature)___

Attached Documentation:
Signature Sheet and Ethical Policy Attestation (P)
Example Data Sheet (V)

SOP: 4.01 Recording Data

I. Summary

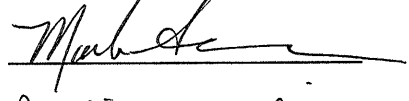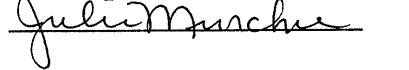   Guidelines for recording data are described below.

II. Procedure

   A. Record data directly, promptly, and legibly in permanent black ink.

   B. All data sheets will have a minimum left margin of 0.8 inches. Additional space needs to be left at the bottom of data sheets for any notes or entry errors that need to be recorded. No entries are to be made in the left-hand margin.

   C. When recording data, one should sign his/her initials as they appear on the Signature Sheet (Attachment P).

   D. Changes in data shall be made by crossing out any errors with a single line so as not to obscure the original entry. Place an asterisk paired with a number beside the crossed-out entry to indicate the footnote with the corrected record. The correction footnote must indicate the reason for correcting the record, the information to correct the record, and be dated and initialed by the technician. See the attached Example Data Sheet (Attachment V) for examples of data entries and changes to entries.

   E. When recording time of events use Mountain Standard Time (Arizona, no daylight savings) on a 24-hour clock. For example, one thirty in the afternoon would be recorded as "1330".

   F. Record dates as day month year with the month written out or abbreviated. Do not indicate the month with numbers. For example, the 4th of July in 2010 would be recorded as "04 Jul 10" or "04 July 10". (Note: the year can also be written out as 4 digits.)

G. There should be no blank spaces on a completed data sheet. Where applicable, enter "x", "0", "N/A", or place a straight line through the blank space if a field is not applicable. Using a ruler, cross out (X) or draw a line through the remainder of any unused portion of the data sheet.

H. All data generated must be retained. The Quality Assurance Officer will review all of the data before it is archived.

Effective Date ___08AUG2016___

Approved By _____

_____

Attached Documentation:

Plankton Tow Datasheet (A)

Veliger Monitoring Laboratory Form (B)

SOP 4.02: Data Entry in Microsoft Access

I. Summary

Plankton Tow Datasheets, Veliger Monitoring Laboratory Forms and any maps associated with sampling are stored in the Veliger Monitoring Data binder. The field and laboratory data is entered into the Veliger Monitoring database computer file on the U: drive. The procedure for entering data is described below.
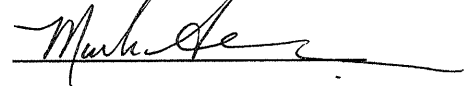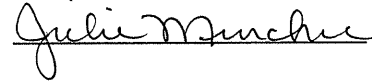
II. Equipment

Computer with network access

III. Procedures

A. A properly trained Laboratory Technician must log onto the Glen Canyon network.

B. Open the current Veliger Monitoring database in Microsoft Access. The database is located at U:\Resources\Aquatic\WaterLab\Veliger Sampling\Veliger Monitoring Database.

C. Obtain the data sheets (Attachments A and B) from the Veliger Monitoring binder.

D. Open the table "Plankton Tow Data" to enter data from the Plankton Tow Datasheet (Attachment A).

E. Open the table "Analysis" to enter data from the Veliger Monitoring Laboratory Form (Attachment B).

F. Enter the requested information into each of the tables using the data recorded on the associated Plankton Tow Datasheets and Veliger Monitoring Laboratory Forms.

G. The Veliger Monitoring Database should be updated monthly to keep track of plankton samples collected and analyzed by microscope and FlowCAM.

Effective Date ___O8AUG 2016___

Approved By ___*Mark Ofe*___

___*Julie Munchu*___

Attached Documentation:
Microscopy Laboratory Training Document (I)

SOP 4.03: <u>Laboratory Technician Training</u>

I. <u>Summary</u>

Laboratory technicians must be adequately trained in the methods and procedures of the laboratory. A Training Document is used to record training in specific methods and procedures. Laboratory personnel must successfully complete a Demonstration of Capability before performing analysis techniques unsupervised. Laboratory management must maintain records of all laboratory personnel that include qualifications, experience, training records, Demonstration of Capabilities, Signature Sheet and Ethical Policy Attestations, and documentation of any courses or workshops attended.

II. <u>Equipment</u>

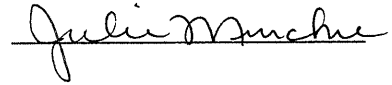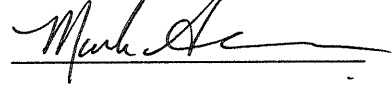Laboratory Quality Manual
Current SOPs

III. <u>Procedures</u>

A. New laboratory personnel must be given a copy of the current Microscopy Laboratory Quality Manual and current Standard Operating Procedures. Additionally, a copy of this manual is available in the laboratory at all times.

B. The Laboratory Technician must read and understand this manual.

C. A Microscopy Laboratory Training Document (Attachment I) is used to record training in specific methods and procedures that are needed by an employee to perform their duties.

D. The Laboratory Director, QA Officer, or a previously trained technician must train new technicians in each procedure. It is the responsibility of the trainer to observe and review the work of the new technician.

E. The date of initial training will be recorded on the technician's Training Document.

F. When the *trainer* feels the trainee can perform the procedure unsupervised, the *trainer* will sign and date the Training Document.

G. When the *trainee* feels that they can perform the procedure unsupervised, the *trainee* will sign and date the Training Document.

H. Training Documents must be completed and maintained for all key personnel and technicians. Each technician is responsible for making sure their training record is accurate and current. Each technician must give a copy of their Training Document to the Laboratory Director and Quality Assurance Officer whenever the document is updated.

I. Each technician must complete a Demonstration of Capability successfully (refer to SOP 3.02) for each method before performing the procedure unsupervised.

Effective Date ___O8AUG 20X0___

Approved By ___*(signature)*___

___*(signature)*___

Attached Documentation:

Plankton Sampling and Microscopy Supply Log (F)

Plankton Sampling Supplies Sent To Bullfrog (G)

SOP 4.04: Maintaining a Lab Supply Log and Chemical Inventory

I. Summary

An accurate record of all plankton sampling, microscopy, and FlowCAM supplies and chemicals must be kept for future reference.

II. Procedures

A. Plankton Sampling and Microscopy Supply Log

1. When supplies specifically for the Veliger Monitoring Program are received by the laboratory, document the date received, technician initials, and description of each item including company, quantity, lot number, and reason for ordering the item on the Plankton Sampling and Microscopy Supply Log (Attachment F). When items are received at the Bullfrog Laboratory, they are to be recorded in the Bullfrog Laboratory Supply Log in the same fashion as in Wahweap.

2. When supplies are transferred from Wahweap to Bullfrog or vice versa, document the date, items, quantity, company, lot number, and the method by which it was sent (plane, boat, vehicle) on the Plankton Sampling Supplies Sent to Bullfrog (or Wahweap) Log (Attachment G).

3. When chemicals and disposable labware are received write the date received and technician initials on each box/container with a permanent marker. When these items are opened write the date opened and technician initials on the box/container.
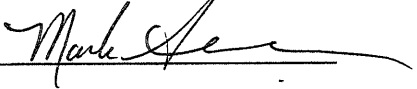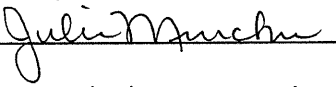
B. Chemical and Supplies Inventory

1. The laboratory must maintain a Chemical and Supplies Inventory documenting all of the chemicals and disposable items used in the lab. Items used for plankton sampling and microscopy are documented in the Beach Monitoring Chemical and Supply Inventory.

2. When chemicals or disposable labware are received, the following will be documented in the Chemical and Supplies Inventory: the name of the product, manufacturer, lot number, maximum container quantity, current quantity, received date, opened date, expiration date, and hazard warnings/special precautions.

3. There should be no blank spaces on a completed Chemical and Supplies Inventory sheet. Enter "x", "0", "N/A", or place a straight line through the blank space if a field is not applicable.

4. The Chemical and Supplies Inventory is an electronic document that will be printed out as a hard copy as it is updated.

5. The Chemical and Supplies Inventory will be updated as needed and a hard copy will be filed in the Supply Log. The Chemical and Supplies Inventory is used to order needed products.

Effective Date ___08AUG2016___

Approved By ___*Mark Sfe*___

___*Julia Munchu*___

Attached Documentation:
Corrective Action Index (J)
Corrective Action Report (K)
Preventive Action Index (L)
Preventive Action Report (M)

SOP 4.05: Corrective and Preventive Actions

I. Summary

All activities conducted by laboratory personnel with concern to the Veliger Monitoring Program are outlined in the Quality Manual. The Quality Manual consists of an annual Quality Assurance Manual and all current Standard Operating Procedures. Corrective Action is taken for nonconforming work, departures from the Quality Manual, and equipment malfunctions. Preventive Action is a pro-active process to identify opportunities for improvement. All staff members are responsible for conducting corrective and preventive actions. When a corrective or preventive action is identified, a Corrective or Preventive Action Report is completed and given to the Quality Assurance Officer. After monitoring the results, the Quality Assurance Officer determines if they are acceptable. If they are not acceptable, a new action report must be completed.

II. Procedures

A. Corrective Action:

1. Identify the nonconforming work, departure from the Quality Manual, or the equipment malfunctions.

2. Create a log entry in the Corrective Action Index (Attachment J) by filling in the discovery date, corrective action number (CA#), and subject. The CA# must be the two digit year followed by the next whole number (for example: 11-001).

3. Create a Corrective Action Report (Attachment K) using the same date, CA#, and subject as in the Corrective Action Index. Also, record the problem, cause, records affected (including previous records), and technician initials.

4. Record the CA# on any affected record.

5. If possible, record any potential solutions to the problem, the solution selected for implementation and date of implementation, outcome, and technician initials. Refer to equipment manuals for possible solutions.

6. Inform the Laboratory Supervisor, Quality Assurance Officer, and the Laboratory Director of the situation.

7. Deliver photocopies or send electronic copies of the Corrective Action Report to the Laboratory Director and the Quality Assurance Officer.

8. The Quality Assurance Officer must monitor the results and determine if they are acceptable, as well as initial, and date all Corrective Action Reports and the Corrective Action Index promptly. If the results are not acceptable, a new Corrective Action must be taken, and the unsatisfactory report must refer to the new CA#.

9. The Quality Assurance Officer must photocopy the reviewed report and deliver it to the Aquatic Ecologist.

10. Procedure for equipment malfunction:

    a. Identify the equipment malfunction.

    b. Place a note on the equipment identifying the malfunction. Until the equipment is repaired, it may not be used.

    c. Follow steps 2 through 9 in Section A.

    d. The effect of the equipment malfunction on previous tests must be examined. New Corrective Action Reports are necessary if previous tests may have been affected.

    e. After the equipment has been repaired, return the equipment to service and remove the malfunction note. Repairs must be noted in the Equipment Maintenance logbook.
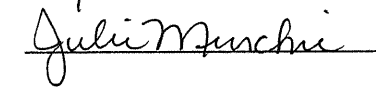
B. Preventive Action:

1. Identify an area for improvement.

2. Create a log entry in the Preventive Action Index (Attachment L) by filling in the discovery date, preventive action number (PA#), and subject. The PA# must be the two digit year followed by the next whole number (For example: 11-001).

3. Create a Preventive Action Report (Attachment M) using the same date, PA#, and subject as in the Preventive Action Index. Also, record the area in need of improvement, reason, records affected, and technician initials.

4. Record the PA# on any affected record.

5. If possible, identify potential improvements, the improvement selected for implementation and date of implementation, outcome, and technician initials. Refer to equipment manuals for possible solutions.

6. Inform the Laboratory Supervisor, Quality Assurance Officer, and the Laboratory Director of the situation.

7. Deliver photocopies or send electronic copies of the Preventive Action Report to the Laboratory Director and the Quality Assurance Officer.

8. The Quality Assurance Officer must monitor the results and determine if they are acceptable, as well as initial, and date all Preventive Action Reports and the Preventive Action Index promptly. If the results are not acceptable, a new Preventive Action must be taken, and the unsatisfactory report must refer to the new PA#.

9. The Quality Assurance Officer must photocopy the reviewed report and deliver it to the Laboratory Director.

Effective Date ___O8AUG2016___

Approved By ___*Mah Ue*___

___*Julie Munchie*___

SOP 4.06: Equipment Documentation

I. Summary

The laboratory is furnished with all the items needed to perform microscopic analyses and to perform quality control activities. All equipment is maintained in good working order. Each piece of equipment is documented and uniquely identified by a serial number and/or National Park Service property number. Documentation is maintained in the Equipment Maintenance Log. If a piece of equipment performs unsatisfactorily, the instrument is removed from service, tagged, and repaired with proper documentation.
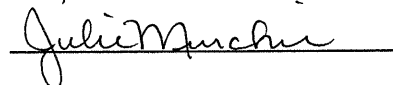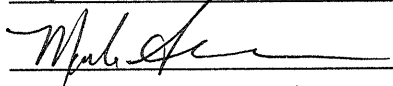
II. Equipment

All microscopy laboratory equipment

III. Procedures

A. Laboratory Equipment

1. An Equipment Documentation Form (Attachment H) must be maintained for each piece of equipment. Information on this form includes: the date, preparer's name, name of item, manufacturer name, model number, serial number, NPS property number, electrical information, location of the manufacturer's instructions (must be within the laboratory building), date received, condition when received, date placed in service, current physical location, date and description of each maintenance activity, and date and description of each repair. The current physical location must be indicated on the Wahweap Outer Laboratory Map (Attachment U).

2. The equipment documentation must be current. Any changes to the status of the equipment must be documented on the Equipment Documentation form.

3. Whenever equipment performs unsatisfactorily, a Corrective Action Report (Attachment K) must be completed. Refer to SOP 4.05: Corrective and Preventative Actions.

4. All equipment must be cleaned regularly. Cleaning dates must be recorded in the Equipment Maintenance and Cleaning Log (Attachment S).

Effective Date ___08AUG2016___

Approved By ___(signature)___

___(signature) Julie Munchu___

Attached Documentation:
Quality Assurance Audit (O)
Corrective Action Report (K)

SOP 4.07: <u>Quality Assurance Audits, Managerial Review, and the Quality Manual</u>

I. <u>Summary</u>

The combination of Monthly Quality Assurance Audits, annual Managerial Reviews, and the Quality Manual are the centerpieces of the Microscopy Laboratory's Quality System. The Monthly Quality Assurance Audits ensure that laboratory management is aware of the work being conducted in the laboratory. The annual managerial review evaluates the continuing suitability and effectiveness of the Veliger Monitoring Program along with any changes or improvements to the program. The Quality Manual describes all policies and procedures to ensure and document the quality of the analytical data.

II. <u>Procedures</u>

A. Monthly Quality Assurance Audits

1. When the Microscopy Laboratory is in operation, the QA Officer must review the work conducted on a monthly basis.

2. All forms must be checked for proper documentation and usage errors. The following must be reviewed: Corrective and Preventive Action Reports, Veliger Monitoring Laboratory Forms, Plankton Tow Datasheets, and Equipment Maintenance and Cleaning Logs.

3. Errors discovered on the forms, with the exception of transcription errors, must be corrected through the use of Corrective Action Reports (Attachment K). Refer to SOP 4.05: Corrective and Preventive Actions.

4. The QA Officer records "Proofed" followed by the date, and initials on every log that has been reviewed. If the log sheet is only partially used, the QA Officer draws a line and records the information above it or notes the area and records the information at the bottom of the sheet.

5. Monthly Quality Assurance Audits should be kept as both electronic and hard copies.
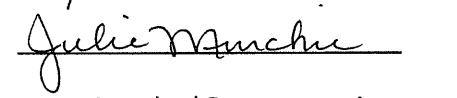
B. Managerial Review

1. Annually the Laboratory Director or delegate must complete a Managerial Review.

2. This document reviews the quality assurance and analytical activities to ensure their continuing suitability and effectiveness. Furthermore, it documents that the Quality Manual was up to date and met any new work requirements necessary for the volume and type of work undertaken.

3. The Managerial Review must include the following topics:

    a. A summary of quality assurance activities and results including: Monthly Quality Assurance Audits, Corrective and Preventive Actions, Demonstration of Capabilities, Internal Audits for Precision, and Equipment Maintenance.

    b. A summary of laboratory activities and assessments, and any other comments from external bodies.

    c. A summary of Standard Operating Procedure alterations that have occurred since the last Managerial Review.

    d. A summary to indicate that the Quality Manual continues to meet necessary standards.

4. The Laboratory Director and QA Officer must sign and date the final version of the Managerial Review.

C. Quality Manual

1. It is the responsibility of the QA Officer to create and submit to the Laboratory Director necessary changes to any areas of the Quality Manual.

2. All areas of the Quality Manual must be uniquely identified by including the date of issue, page numbering, and the total number of pages.

3. The Laboratory Director and QA Officer must sign and date the final version of the Quality Manual.

Effective Date ___O8AUG 2016___

Approved By ___(signature)___

___(signature) Julie Manchu___

Attached Documentation:
Corrective Action Report (K)
Correspondence Index (N)

SOP: 4.08 Complaint and Correspondence Policy

I. Summary

This policy has been created in order to serve the Microscopy Laboratory's client and outside parties better. The Correspondence Index serves as a record of solicited client feedback, unsolicited correspondence, as well as a record of all correspondences occurring between laboratories, clients, and other outside parties. The Correspondence Index and associated documentation is stored in the Correspondence Logbook in the Laboratory Director's office.

II. Procedure

A. In the event staff should receive a comment (including complaints) about the Microscopy Laboratory, staff members should instruct the addresser to write a letter with return address information and send it to:
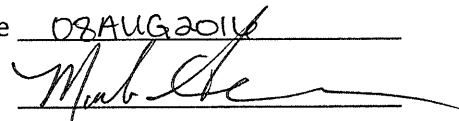
Glen Canyon NRA
Lake Powell Water Laboratories, Laboratory Director
P.O. Box 1507
Page, AZ 86040

B. The Laboratory Director responds to all incoming correspondence.

C. All correspondences, incoming and outgoing (solicited or unsolicited), must be properly documented in the Correspondence Index (Attachment N). The date of incoming or outgoing correspondence, correspondence ID number (CID), "To" or "From" must be indicated under contact information along with the address (if available) of the contact, and subject of the correspondence must be entered into the Correspondence Index.

1. All hard copies of correspondences must be stored in the logbook, chronologically, by CID number with newest correspondences in the back of the logbook.

2. A correspondence ID number is assigned using "CID" and the current two digit year followed by the next whole number (example: CID 11-001). The correspondence ID number must also be indicated on the hard copy of the correspondence. If the correspondence is a response to or continuation of a previous correspondence, the correspondence ID number will be that of the original correspondence followed by the next lowercase letter (example: 11-001a, 11-001b, etc).

3. If the nature of the written correspondence is casual or in memoranda form, such as e-mail notifications or facsimile transmissions, a hard copy of the correspondence must be entered into the Correspondence Index. In the case of verbal correspondences, a synopsis of the correspondence must be synthesized and entered into the Correspondence Index.

4. If the correspondence addresses a previously unknown laboratory problem, a Corrective Action Report (Attachment K) must promptly be completed and included with the correspondence. Refer to SOP 4.05: Corrective and Preventative Actions.

5. If an official-type response is required, the response must be drafted and given to the Resource Management Secretary to finalize so that National Park Service correspondence guidelines are met.

6. The response must be signed and sent to the addresser.

7. Laboratory Director's initials, Glen Canyon Response Number (if an official response), and CA# must be recorded in the Correspondence Index. Draw a single line through the CA# box if corrective action or official response was not necessary.

8. Copies of all correspondence must be retained in the Correspondence Logbook under the CID Number and Glen Canyon Response Number (if applicable).

Effective Date ___O3AUG2014___

Approved By ___(signature)___

___(signature)___

Attached Documentation:
Current SOP Log (Y)

SOP 4.09: <u>SOP Creation and Revision</u>

I. <u>Summary</u>

A Standard Operating Procedure (SOP) is maintained for each common laboratory activity. If ever the Microscopy Laboratory institutes or changes a procedure, a new SOP or revision must be created. Each SOP has an effective date and issue number, and is signed by the Laboratory Director and QA Officer. The laboratory and all laboratory personnel must possess a current edition of the laboratory SOPs. A list of current issue numbers is maintained and displayed to keep all copies of the SOP current. As part of the document control system, old issues are stored at the Glen Canyon National Recreation Area Headquarters building for at least five years. All staff must follow SOPs without deviation, if there is a deviation from a SOP, a Corrective Action Report must be completed. Refer to SOP 4.05: Corrective and Preventative Actions. Technical errors within an SOP should be brought to the Laboratory Director and QA Officer's attention immediately, and a Corrective Action Report must be completed. Grammatical errors should be highlighted and given to the QA Officer. The QA Officer reviews all of the SOPs annually.

II. <u>Procedures</u>

A. The QA Officer must create a new SOP or revision every time a procedure is instituted or changed. Electronic copies of the SOPs are maintained on the Glen Canyon NRA computer network.

B. The Laboratory Director must review the document.

C. When the document is error-free the QA Officer and Laboratory Director sign and record the effective date on the SOP.

D. The Current SOP Log (Attachment Y) must be updated along with the Quality Manual if necessary.

E. The QA Officer must photocopy the SOP and the Current SOP Log and distribute copies to all laboratory personnel.

F. The QA Officer must remove the old version of the SOP from the Master SOP file and placed it into the Old SOP file.

G. The new SOP is placed into the Master SOP file.