Standard Operating Procedures for Aquatic Ecological Sampling and Analysis

Submitted in Support of the Savannah Electric and Gulf Power 316(b) Impingement and Entrainment Characterization Studies in Compliance with Section 316 (b) Phase II Rules

Savannah Electric

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1.0 Field Impingement Sampling

1.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform impingement sampling for 316 studies conducted by the EAT Department MACTEC Gainesville.

1.2 *Scope*

This SOP is applicable to all impingement sampling conducted by MACTEC Gainesville EAT Department personnel.

1.3 *Guidelines*

1.3.1 Impingement Sampling

Impingement sampling at each station will be conducted on a bi-weekly or monthly basis for a one-year period. Impingement sampling will be conducted by utilizing a collection basket fitted with 3/8" mesh placed within the screen-wash trough or under the screen wash discharge located at the cooling water intake structure (CWIS).

1.3.1.1 Prior to Sampling

Prior to the initiation of an impingement sampling, the traveling screens shall be operated and backwashed to remove any trash or debris that may have accumulated on the screens or in the screen wash trough. MACTEC will coordinate with plant personnel concerning the operation and cleaning of the screens.

1.3.1.2 Sampling methods

Impingement samples will be collected using collection baskets fitted with net inserts having a 3/8" mesh. The baskets will be placed under the screen wash discharge at the on-set of each 24-hour sampling event. If required, an additional fish collection basket will be placed in the sampling area as each basket is emptied so that the water is continuously filtered. The important task of the impingement sampling is to account for all fish impinged during the sampling interval and to take steps to ensure that this is accomplished.

1.3.1.3 24 Hour Sampling Events

During the 24-hour sampling event, contents of the baskets will be emptied into holding containers, as necessary, to prevent overflow and the baskets will be cleaned of all organisms at the end of the sampling period.

Sampling date, sampling interval (2400-0600, 0600-1200, 1200-1800, 1800-2400 hrs), start time, and stop time shall be recorded for all samples. *(NOTE: start time/stop time shall be particularly important in recording actual sampling intervals in the event of breaks in continuous sampling.)*

1.3.1.4 Processing of Samples

Competent fisheries biologists will process the impingement samples. Each sample will be processed by counting and identifying all fish to the lowest practical taxonomic level. Individual fish that cannot be identified to species in the field will be preserved for identification by taxonomic specialists. Dead fish will be placed on ice and sorted and identified in the lab. Live fish will be sorted and identified in the field and returned to the source water body.

1.3.1.5 Treatment of Live Fish

Any live fish will be placed in an aerated live well and processed in advance of other specimens which may be dead or moribund.

1.3.1.6 Fish Processing

Fish in the sample will be sorted by species. Following sorting, up to 30 randomly chosen individual specimens within each size category will be measured to the nearest mm total length (TL) and their condition will be recorded as live, dead or stunned. Fish total length (TL) will be measured to the nearest mm using a measuring board. Weight will be measured to the nearest gram using a spring scale or digital top-loading scale. The spring scale will be used for larger fish (>2000g or 4 1bs) and the digital top-loading scale will be used for smaller fish (<2000g or 4 lbs). If partial fish (head only, tail only) are present in the sample, measurements will be accompanied by a description of the fish body part. Fish will be recorded as live if the fish is able to maintain equilibrium in the aerated live well. Fish will be recorded as moribund or stunned if they are unable to maintain equilibrium, cannot swim down, are lying on the bottom of the live well or have extruded the swim bladder. Moribund or stunned fish will still move their fins and operculum (gills flaps) occasionally. Signs that a fish is dead include unmoving gill flaps, no body movement and clouded eyes. Fish will be recorded as previously dead if they show any signs of decomposition or extensive fungus growth. Fish parts that have become detached from the body (head only, tail only) will be described, identified (if possible), measured, and weighed. A total weight measurement will be taken for each species. During periods of high impingement MACTEC will make observations and document causes for impingement other than plant withdrawal.

1.3.1.7 Batch Samples

If the number of specimens in the sample for a particular species and size category is large, then the species/size category count will be estimated by sub-sampling. A sub-sample of 30 individuals will be weighed and length measured and the total sample will be weighed. The number of individuals in the whole sample will be estimated from the ratio of the total sample weight to the sub-sample weight total and the count within the sub-sample.

1.3.1.8 Collection of Non-Fish Organisms

Shellfish found in the impingement sample, such as native freshwater mussels, Asiatic clams, zebra or quagga mussels, and crayfish, will be identified to a

practical taxonomic level and will be counted (in the case of few specimens such as native freshwater mussels or crayfish) or weighed in bulk (in the case of numerous Asiatic clams or zebra and quagga mussels).

1.3.1.9 Treatment of Live Fish

Any live fish will be returned to the source body of water in a suitable area where they are not subject to re-impingement, subsequent to processing. Sampled fish will be examined for presence of tags. Any tags will be reported to the appropriate agency in a timely manner. Tagged fish will be stored frozen and shipped to the appropriate agency (if applicable).

1.3.1.10 Recording of Sampling Data

Relevant ancillary information will be recorded during the sampling event, as is indicated in the SOP for Sampling Documentation.

1.3.1.12 Reporting Problems

Any problems that arise during sampling will be reported to the Project Manager immediately. The Project Manager will provide guidance if the sampling plan needs to be amended or sampling needs to be rescheduled.

1.3.1.13 Health and Safety Procedures

Proper health and safety procedures will be followed during all sampling events. Formalin used to preserve specimens will be stored in a tightly sealed container and kept away from ignition sources. Formalin will be handled in well-ventilated areas to avoid inhalation. Protective gear (i.e. latex gloves and protective eyewear) will be worn when handling formalin. Thicker cloth gloves will be worn for fish handling to reduce the possibility of puncture wounds and cuts from fish spines, scutes and teeth.

2.0 Field Entrainment Sampling

2.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform entrainment sampling for 316b studies conducted by MACTEC.

2.2 *Scope*

This SOP is applicable to all entrainment sampling conducted by MACTEC personnel.

2.3 *Guidelines*

2.3.1 Entrainment Sampling

The purpose of entrainment sampling is to determine the abundance, seasonality, and species composition of selected fish and shellfish eggs, larvae, and post-larvae entrained through the cooling water intake structure (CWIS) at each CWIS at the power plant.

2.3.2 Coordination

a. Prior to the initiation of sampling, continuous chlorination treatment of all units shall be discontinued.
b. At the outset of sampling, notification will be made to shift supervisor.
c. The shift supervisor will provide a plant operator who will assist in valve actuation (see Section 2.3.6, below).

2.3.3 Sampling Methods

Samples shall be obtained using taps from each unit.

2.3.4 Sampling Volumes

Sample volume will be determined with an in-line flow meter and shall not be less than 100 m^3. The flow meter will be of a paddle-wheel type with a resettable accumulating flow feature.

2.3.5 Sampling Locations

Samples shall be obtained by diverting water from a 4" tap previously installed on the water box. A composited sample will be collected from the Units by sampling from the 4" tap.

2.3.6 Sample Collection

a. The sampling apparatus shall consist of a hose and pipe system fitted with a flow control valve and an in-line flow meter to convey water from the tap location to a centralized sampling container equipped with an ichthyoplankton net. Ichthyoplankton nets shall have a 500 µm mesh and be equipped with removable cups or cod ends. Nets shall be suspended in water within a tank to reduce velocities and extrusion of sampled

organisms. The tank shall be equipped with two overflow outlets to convey filtered water to appropriate floor drains or sump locations.

b. Four-inch flex pipe delivering sample water to the collection net shall be connected to each sample location. The valve assembly shall be in the closed condition.

c. Flow meters shall be "zeroed" on each in-coming tap line.

d. MACTEC technicians shall then initiate sampling by opening the flow control valve on each tap.

e. When a sufficient sample volume has been achieved as indicated on the accumulating flow meter, sample collection shall cease by closing the flow control valve on the incoming line.

f. The net shall be removed from the sampling tank and positioned for external wash-down. Washing of the net shall be accomplished using a low-pressure wash consisting of previously filtered raw water. All organisms and detritus shall be washed into the collection bucket at the cod end of the net.

g. After the net has been thoroughly washed down, the collection bucket shall be carefully removed. A large white sorting tray shall be position under the collection bucket when it is removed and as the contents of the bucket are transferred to labeled sample containers. Collection buckets shall be carefully rinsed to ensure that all organisms have been properly transferred to the sample container. Any organisms collected in the sorting tray shall also be transferred to the sample jar.

h. Cleaned and rinsed collection buckets shall be reinstalled on the cod end of each net in preparation of the next sampling interval.

i. After transfer of the sample to the appropriate, labeled sample container, the sample shall be preserved in 10% formalin solution with Rose Bengal stain. Each sample will be properly labeled.

2.3.7 Sample Intervals/Timing

Entrainment samples will be collected from each CWIS at night.

2.3.8 Sample Processing

Entrainment samples will be processed through MACTEC's EAT Laboratory according to EAT Laboratory SOPs for Laboratory Sample Processing.

All fish eggs, larvae and juveniles will be sorted from the sample using a 10X magnifying lamp and submitted for taxonomic analysis. If samples contain a large number of specimens or large amounts of detritus, samples may be split using a Folson plankton splitter or other appropriate device. Sub samples will be processed until a minimum of 200 identifiable specimens are found, but counts for individual sub samples will be maintained.

2.3.9 Recording of Sample Data

At the completion of sample collection, the flow meter readings and any relevant ancillary information will be recorded as indicated in the SOP for Sampling Documentation.

2.3.10 Sample Labeling

Entrainment samples will be labeled with the client name (i.e. Savannah Electric or Gulf Power), plant name, date, gallons filtered, and sample container number (ex. 1 of 2).

3.0 Ecology Laboratory Sample Logging

3.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform logging of field samples into the EAT Laboratory conducted by MACTEC Gaineseville.

3.2 *Scope*

This SOP is applicable to all samples being sent to the EAT Laboratory for sample processing conducted by MACTEC Gainesville.

3.2.1.1 Sample Labels

A label indicating the sample code should be placed inside and outside of each jar or vial. From this point on all samples will be referred to by the sample code.

3.2.1.1.1 Outside Label

On each jar/vial place a strip of blue tape and copy over client name, plant name, sample date, sample volume, and jar number. If more than one jar was used for the sample, place a tag on each jar.

- For ichthyoplankton samples use blue tags.
- Write sample code legibly with a waterproof pen.

3.2.1.1.2 Inside Label

Inside of each jar place a label with the same information.

- Use waterproof paper.
- Write in pencil only.

4.0 Ecology Laboratory Sample Sorting

4.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform sample sorting for the EAT Laboratory conducted by MACTEC Gainesville.

4.2 *Scope*

This SOP is applicable to sample sorting conducted by the MACTEC Gainesville EAT Department Laboratory personnel.

4.3 *Guidelines*

4.3.1 Sample Sorting

4.3.1.1 Laboratory Sample Processors

Find the sample to be sorted. On the lab sheet, place initials in the appropriate sorted/picked columns at the beginning of the sample sorting process and place the completion date in the column when you have completed sorting the sample.

4.3.1.2 Obtain Sample

Find the appropriate sample on the project shelf. Take the next sample in order of date, that needs to be processed (**Do Not Skip Samples**).

4.3.1.3 Sample

The following steps should be performed in the fume hood. Rinse contents of sample into the project appropriate size sieve (try to use the #120 sieve, if possible)

- Rinse jar and lid thoroughly with deionized water.
- If sample volume is substantial, split with Folsom plankton splitter so as to limit picking time to 4 hours or less (unless otherwise indicated by project manager).
- Transfer waste formalin to appropriate bottle (see Formalin Handling SOP)
- Rinse sample off sieve with deionized water into collecting pan/bowl.
- Using forceps and magnification, transfer sample contents to labeled vials containing 40% ethanol.

4.3.1.4 Sample Starting

Place a small amount of sample from the sieve into a white sorting pan with enough water to cover the material. Usually 1/4 inch of water is enough.

- AT LEAST 3/4 of the white pan should be visible in the bottom of the pan.
- The paper label from the formalin jar should stay with the sample until the sorting step is finished.

4.3.1.5 Sample Sorting

Sort through the white pan completely while looking through the magnifier lamp. Move all detritus and sand around with forceps. The white pan has sections on the bottom of it that can be used as a grid to follow to ensure that the entire pan has been sorted. Once the entire pan has been sorted through then swish the pan contents around and sort through it again. Repeat the swishing and sorting of the pan until the pan has been sorted through twice without finding any animals. Add a few granules of Rose Bengal stain. The solution should turn light pink. Sort through the sample again, looking for organisms that have turned pink-red with the stain. Transfer all organisms to labeled vial with 40% ethanol.

4.3.1.5.1 *Animal Placement*

Pick out of the sample all animals or parts of animals found and place animals into appropriately labeled vials

- Put ichthyoplankton specimens in blue-labeled jar.

Place any large animals that do not fit the vials into a larger jar - place the sample information on the jar using the appropriate type label. Use a counter while picking to enumerate all specimens collected from the sample.

4.3.1.5.2 *Ichthyoplankton Samples*

When processing ichthyoplankton samples use a counter to count the number of ichthyoplankton placed in to the sample vial/jar.

4.3.2 Sample Processing Completion

Rinse all debris left in the white sorting tray back into the original sample jar. Put an additional yellow label on the jar indicating the following information – sorters initials, date sorted, and the number of organisms obtained from the sample. Fill the jar with 70% ethanol. If sample takes up more than half the jar, decant water off the top once debris has settled. Place the jar on the project specific shelf marked for QA/QC jars. Place jars back on the shelf in chronological order.

4.3.2.1 Sample Contents Collected

Place the sample vials on the project shelf in the taxonomy lab. (Put empty labeled vial in slot if no animals were found). Place original sample jar with yellow label onto project QA shelf (top shelf in taxonomy lab).

4.3.3 Sorting Documentation

Fill out appropriate log book accordingly indicating:

- Your initials and date.
- Time required for sorting.
- Total number of organisms collected from the sample.

5.0 Title: Ecology Laboratory Sample Splitter

5.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform sample splitting for the EAT Laboratory conducted by MACTEC Gainesville.

5.2 *Scope*

This SOP is applicable to sample splitting conducted by the MACTEC Gainesville Ecology Department Laboratory personnel using the Folsom Plankton Splitter.

5.3 *Guidelines*

Use the Folsom Plankton Splitter to split samples that have already been sorted from the debris but to numerous to count and identify all organisms. The project manager must approve the splitting of any samples before it is split.

5.3.1 Sample Splitting Preparation

- Set up the Folsom Plankton Splitter with the troughs in place
- Rinse vial of organisms into wheel.
- Dilute sample in wheel by adding 300 mL of water. Do not exceed 500 mL.
- Cautiously rotate wheel back and forth, mixing the solution until a good distribution is reached.
- Slowly pour sample into the wheel's troughs. This will separate the sample in two equal parts.
- Add a slight amount of water to wheel to rinse the remaining organisms out of the splitter and pour into the same two troughs.
- Sieve each trough separately, designating one trough as the "taxonomic sample" and the other as a "saved portion."
- Pour saved portion back into original vial.
- "X" vial.
- Check one trough to see if more than 200 organisms remain. If so, repeat splitting with one of the trough's samples.
- If a second split is necessary, pour the "taxonomic sample" in the wheel, split sample again and choose one of the troughs for a new "mount sample" and add the other portion to the previously split "saved portion" in the vial.
- After splitting is complete, return the saved portion to the original vial, add alcohol and mark the splitting code on the vial. The splitting code is as follows:
    - Split once = 1x
    - Split twice = 2x
    - Split thrice = 3x
- Mark the splitting code in the log book in column 18 or 22, respectively.

5.3.2 Mark Sample Vial Processed

- Put an "X" on vial once sample is analyzed.

5.3.3 Problem Situations to Avoid

- Too much water in wheel--contents dump out.
- Forgetting to mark split codes in log book, on vials, and on slides.
- Putting alcohol in the plankton splitter.

5.3.4 Re-Store Sample

Rinse all remaining debris in the sample processed sieve back into the original sample jar. Put an X on the sample label. Place the jar on project shelf marked QA/QC. Place all jars back on the shelf in numerical order.

6.0 Title: Ecology Laboratory Sorting Quality Control

6.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform quality control for sample sorting for the EAT Laboratory conducted by MACTEC Gainesville.

6.2 *Scope*

This SOP is applicable to Quality Assurance and Quality Control (QA/QC) of sample sorted by the MACTEC Gainesville EAT Department Laboratory personnel.

6.3 *Guidelines*

QA/QC is a critical aspect of laboratory processing of samples and is necessary to ensure that such processing is completed in a technically proficient manner and that sample integrity is not compromised. As a general rule, 10 percent of all samples sorted by each sorter will be checked for sorting efficiency.

6.3.1 Sample Sorting

6.3.1.1 QA/QC Assessment

Check the log book for the project and note the number of samples that have been denoted as sorted. Designate a subset of ten samples that have been processed to do a QA/QC check on the sample sorting. Randomly pick one of the ten samples in the set to be the QA/QC sample.

6.3.1.2 QA/QC Sample

Obtain the QA/QC sample from the project shelf marked for QA/QC and process the same for a second time following the SOP for Sample Sorting. The individual performing the resorted is to be an EAT Laboratory person other than the original sorter. Any animals found by QA/QC person during the resorting process will be counted and recorded.

6.3.1.3 QA/QC Check

The number of animals found by original sorter for the sample that is being checked will be counted and recorded. The number of animals found by the QA/QC sorting process will be added to that original number to obtain a total sample number. Efficiency will then be calculated by dividing the number of animals found by the original sorter by the total number of animals found for the sample. The efficiency number/result will be recorded on the QA/QC column and samples will be noted on the project Resort Logsheets in the logbook. The QA/QC entries of log book will be filled in.

6.3.1.4 QA/QC Efficiency Standards

A 90 percent efficiency is required for the designated sample group to pass the QA/QC check.

6.3.1.5 Sample Fate

6.3.1.5.1 *Passing Samples*

If the sample checked passes the QA/QC check then that group of samples will be marked with an "N" in the log sheet column. These samples can now be finished processing following the SOPs for Sample Identification.

6.3.1.5.2 *Failing Samples*

If the checked sample fails the QA/QC check:

1. The designated samples in the sample group will be placed back on the shelf for resorting.
2. A "Y" will appear in the log sheet.
3. A list of the samples to be resorted and the original sorter's initials will be placed on the resort logsheet of the project logbook.

6.3.1.6 Sample Resorting

All samples listed on the resort logsheets will be resorted. Place all animals from the resort in to the original sample vials. If vial has been marked X'ed, then make up a new vial. Put the new vial in to the large/oversized animal box and fill out the large/oversized animal form. Place the resorted samples back on to the projects QA/QC shelf. Mark "resorted" on the sample jar label. Fill in all appropriate columns of project logbook sheet for resorting.

6.3.1.7 Resort QA/QC Check

Ten percent of resorted samples will be QA/QC'ed using the above procedure.

6.3.1.8 QA/QC Corrective Actions

If a sorter continues to fail the QA/QC check, it is the responsibility of the sorter and the QA/QC person to identify the problem and set up a corrective action.

6.3.1.8.1 *Possible Problems and Corrective Actions*

<u>Possible Problems</u>

- Too much detritus in pan.
- Unfamiliarity of sorter with animals.
- Not swishing pan sufficiently to dislodge animals from detritus.

<u>Corrective action</u>

- QA/QC person may ask to check a pan after the sorter believes they have found all animals.
- The sorter may be asked to look through animals sorted from other samples and become more familiar with taxa.

In addition to the procedures outlined above, a QA/QC Audit Form should be completed by the auditor to detail the work subjected to the QA/QC review, the assessment of the technical accuracy of the work, and corrective measures required (if appropriate).

7.0 Ecology Laboratory Sample Identifications

7.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform sample identification for the EAT Laboratory conducted by MACTEC Gainesville.

7.2 *Scope*

This SOP is applicable to sample sorting conducted by the MACTEC Gainesville EAT Department Laboratory personnel.

7.3 *Guidelines*

Sample Identification

No identifications should begin until the sample has passed the QA/QC sorting check

7.4 Obtain Sample

Obtain sample listed in the project book that is next to be identified.

7.4.1 Others Identification

a. All ID's should be done using one of the stereoscopes; polarized light set up should be used for ichthyoplankton taxonomy.
b. All ID's should be to the lowest practical taxonomic level (check with the lab manager or project manager for special cases).
c. Check misplaced animal form for additional specimens.
d. All identifications should be recorded on a bench sheet (Figures 6-1 and 6-2).
e. A reference collection of animals should be kept for each project.
   Use vials with tan labels.
   Indicate on bench sheet which animals were referenced.
f. After finishing a sample, return all animals to original "other" vial and place an "X" on label. (All misplaced animals should be put in original vial and additional vials cleaned out).
g. Fill in columns of log sheet.
h. Return vial of identified animals to its appropriate slot.
i. Fill out bench sheets completely. Include numbers and animal codes.
j. Put all bench sheets for that project in numerical order in a file.

7.4.2 Storage of Samples

Place all project samples upon completion of identification into a project specific storage box, labeled with the project specific information and documentations and place in the warehouse completed project storage area.

8.0 Title: EAT Laboratory Identification Quality Control

8.1 *Purpose*

The purpose of this document is to establish a consistent procedure to perform quality control for sample identifications for the EAT Laboratory conducted by MACTEC Gainesville.

8.2 *Scope*

This SOP is applicable to Quality Assurance and Quality Control (QA/QC) of sample identifications by the MACTEC Gainesville EAT Department Laboratory personnel.

8.3 *Guidelines*

QA/QC is a critical aspect of laboratory processing of samples and is necessary to ensure that such processing is completed in a technically proficient manner and that sample integrity is not compromised.

8.3.1 Sample Identification

8.3.1.1 QA/QC Assessment

Check the log book for the project and note the number of samples that have been denoted as voucher specimens. Obtain the voucher collection for the project to be QA/QC checked from the project laboratory designated location.

8.3.1.2 QA/QC Vouchers

The individual performing the verification of the voucher collection is to be an EAT Laboratory person other than the individual who performed the original identification. Alternatively, an independent third party known to be an expert in the taxonomic field subject to consideration, shall be identified and shall be contacted to verify the taxonomic identification of the voucher specimen.

8.3.1.3 QA/QC Check

The voucher collection specimens will be identified to confirm the original identification process of the organisms for the project. The verification of the voucher specimens will be noted in the sample logbook. The QA/QC entries of log book identification will be filled in.

8.3.1.4 QA/QC Efficiency Standards

A 100 percent efficiency is required for the designated project voucher collection to pass the QA/QC check.

8.3.1.5 Sample Fate

8.3.1.5.1 *Passing Samples*

If the voucher specimens pass the QA/QC verification then all sample identifications have been confirmed and that finishes the laboratory sample processing. The sample data collected can then be used for the project report following the SOPs for data processing and report generation.

8.3.1.5.2 *Failing Samples*

If the voucher specimen fails the QA/QC verification:

1. The designated specimen will be noted on the voucher specimen logsheet.
2. The specimen identification will be checked by a third party taxonomist.
3. The sample identification that is confirmed by the third party will be used.
4. If the identification is not that of the original taxonomist the data sheets will be corrected to the verified identification.

8.3.1.6 Overall QA/QC Check

Datasheets are reviewed to verify that information is filled out completely on the forms before they are sent for data processing.

8.3.1.7 QA/QC Corrective Actions

If a taxonomist continues to fail the voucher specimen QA/QC check, it is the responsibility of the project manager and the QA/QC person to identify the problem and set up a corrective action.

8.3.1.7.1 *Possible Problems and Corrective Actions*

Possible Problems

- Unfamiliarity of taxonomist with particular species of a geographical area.
- Organisms identified to a taxonomic level that is to low for confirmation.

Corrective action

- QA/QC person may ask to assist the taxonomist with particular aspects of the identification process.
- Taxonomist may work with various other/additional taxonomic keys to assist in an accurate identification.