Addenda to:

Great River Ecosystems Field Operations Manual Angradi, T.R. (editor). 2006. Environmental Monitoring and Assessment Program: Great River Ecosystems, Field Operations Manual. EPA/620/R-06/002. U.S. Environmental Protection Agency, Washington, D.C.

The following tables replace the corresponding tables in the document cited above. These changes only apply for the 2006 field season.

Subsequent addenda will be posted at this site. Contact T. Angradi for more information. Angradi.theodore@epa.gov; 215-529-5243

Table 5-1. Procedure for collecting water chemistry samples.

1. Fill in the site ID and date on the front and back of the water chemistry and plankton form (Figure 5-1 and 5-2).

2. Calibrate the DO meter (if the meter has not already been calibrated at the base location), record calibration details in the log book. Indicate on the water chemistry and plankton form (Figure 5-1) whether or not the DO and pH meter have been calibrated on the day of sampling.

3. Using a fine-point waterproof marker (e.g., Sharpie), fill out a pre-printed sample label (Figure 5-3) for one 4-L cubitainer, one 500-m L bottle, and one 10ml vial (these containers will be shipped to the lab). On each label, circle the sample type (WCC = water chemistry composite in cubitainer; ALK = 500-mL bottle, DIC=10ml vial), affix the labels to the cubitainer, bottle, and vial, and cover them with clear tape. Record the sample ID numbers (from the labels) on the forms (water sample ID for the 4-L cubitainer; alkalinity sample ID for the 500-mL grab). On a second 4-L cubitainer, write the site ID and date directly on the cubitainer with a waterproof marker (this container is not shipped).Containers m ay also be labeled at the base location prior to departing for the sample site. If the site has been identified as a QA/QC (Section 5.4) site for water chemistry, additional containers will be needed for field blanks and duplicate samples.

4. Attach the sounding weight to the winch cable. Attach the end of the hose from the peristaltic pump (Tygon size 24) and the Guzzler pump (garden hose with check valve on the end) and the DO/conductivity and pH probe(s) (or datasonde) to the cable above the sounding weight (Figure 5-4).

5. Using the coordinates provided in the site dossier, navigate to the first sampling location located halfway between the thalweg sample location and one of the shorelines (river-right or river-left).

6. At the sample location, anchor if possible. Otherwise, the driver should hold the boat on the sample location facing upriver. Remove the lids from both of the 4-L amber cubitainers and pull them open. One cubitainer (label covered with tape) will hold composite water sample 1; the other cubitainer (site ID written on side) will hold composite water sample 2. Do not blow into the cubitainers to inflate them; this will contaminate the samples. Sun block, insect repellent, etc., will contaminate the sample so avoid touching the inside of the cubitainer or cap.

7. From the sonar unit, determine and record the depth under the boat on the form. If the depth is > 2 m, a subsample will be collected at 0.5 m off the bottom, at mid-depth and at 0.5 m from the surface (each subsample is about 445 mL (Table 5-2): 4L composite volume/three sample sites/three subsample depths). If the depth is < 2 m and > 1 m, subsamples will be collected only at 0.5 m off the bottom and 0.5 m below the surface (each subsample is about 665 mL). If the depth is < 1 m, the entire 1300-mL subsample for the location is collected at mid-depth. The pumps may lack sufficient lifting power beyond about 6 m. If a sample location on the cross-channel transect cannot be sampled, the subsample volume for each remaining location should be adjusted so that the total sample volume for the site is maintained.

Table 5-1. Procedure for collecting water chemistry samples, continued.

8. Lower the hose/sensor cluster to the deepest subsample depth using the depth dial on the winch, by counting marks on the cable, or by allowing the sounding weight to contact the bottom and then raising it to the proper depth. Adjust the depth to account for the distance between the bottom of the weight and the sensors. Turn on the peristaltic pump and the DO/conductivity/pH meter(s). Make sure the sounding weight is not bouncing along the bottom during sampling. Use plastic cable ties to secure hoses and the sensor cable(s) to the winch line as the weight is lowered. Pump overboard more water than the entire length of the peristaltic pump hose can hold (determined beforehand). Once the hose is refreshed, pump about 100 mL into each cubitainer to rinse it out. Repeat this two more times making sure rinse water comes in contact with all interior surfaces.

9. Pump the first subsample (of 445, 665, or 1300 mL depending on the number of subsample depths) into cubitainer 1 and cap it. Use graduations on the cubitainer to estimate when the subsample volume is attained. Pump a second subsample of equal volume into cubitainer 2 and cap it (total of 890, 1330, or 2600 mL per depth at each sample location). Record sample depth, DO(mg/L) and conductivity (:S/cm), temperature and pH following procedures in the instrument operating manuals. Record the actual sample depth (from the surface). Flag depths (K flag) for which no instrument readings are made.

10. Collect phytoplankton and zooplankton subsamples using the procedures described in Table 5-5.

11. Repeat steps 6-9, as appropriate, for the other subsample depths at the sample location. Record the total depth or sample depth each sample location on the form. Estimate Secchi depth (Table 5-3).

12. Repeat steps 6-10 for the two other water quality sample locations at the site. By sliding the cable ties down the winch line as the line is winched up, it should be possible to move between stations without having to disassemble the sampling apparatus.

Table 5-1. Procedure for collecting water chemistry samples, continued.

13. At the thalweg sample location only, collect samples for dissolved inorganic carbon (DIC) and alkalinity/pH (described below).

13a. Dissolved inorganic carbon (DIC). Samples collected for DIC and pH are essentially dissolved gas samples, and any contact with atmosphere, even small bubbles, will contaminate it. Therefore, sample vials need to be flushed with sample and capped with no air bubbles. DIC is collected in a 10 mL glass screw cap vial, which has a light coating of silicone vacuum grease pre-applied to the vial rim and which contains 2-4 mg of blue copper sulfate crystals as a preservative. DO NOT RINSE THE VIAL. The preferred method is to overfill a vial 2x from a pump that produces a bubble-free stream of water. In detail, connect a T-fitting to the end of the peristaltic pump tube, such that a smaller (1/4" OD) tube flows at about 75-150 mL/min (or 10 mL per 4-8 seconds). Make sure there are no air bubbles in any of the tubes. Pinch the small tube to stop flow and insert it to the bottom of the 10 mL glass vial. Let the tube flow until the vial fills and overfills. Count approximately how long it takes to fill the vial and let the water spill out of the vial for twice that time (i.e. if it takes 5 seconds to fill the vial, let it spill for 10 seconds). Keep the tube upright and don't flush too fast, in order to prevent the copper sulfate crystals from flushing out of the vial. Gently remove the tube, such that the water continues to spill out of the vial while removing the tube. As the end of the tube exits the vial, make sure no bubbles are introduced (this may require pinching the tube at the last instant) and make sure that there is reverse meniscus (water bulging beyond vial rim). Cap the vial. There should be no air bubbles visible when the vial is inverted, but if there is a tiny one (1/100 of volume of vial), it is OK. Copper sulfate crystals should still be visible. An alternative method is to fill and flush the vial submerged in bucket of river water. This method requires that the bucket is filled with minimum of turbulence and bubbles, which may be difficult from a strong current moving past the boat. Once the bucket is filled properly, the sample vial is submerged and flushed with a syringe. In detail, remove the cap from the 10 mL glass vial. In the same hand, hold the vial with the opening facing up and the 60 mL plastic syringe with tubing facing down (plunger up). The syringe should be empty (plunger in) and have 12-14" of silicone tubing (1/4" OD) attached to it with the free end of the tubing cut at a sharp angle. With on hand, submerge vial and syringe/tubing and let the vial fill. As soon as the vial is full of water, tap it to remove any air bubbles and insert the silicone tubing to the bottom of the vial, keeping everything submerged. Use your free hand to pull back on the syringe plunger, drawing about 30 mL of water out of the vial (which exchanged with air bubbles as the bottle filled) and replacing it with new water from the bucket. While keeping the vial submerged, remove the tube and syringe from the vial and out of the water. Place the vial cap underwater and shake out any bubbles. Screw it tightly onto the vial while both are still underwater. As above, there should be no visible bubbles. DIC sample preservation. After the vial is capped, blue copper sulfate crystals should be visible in the bottom of the vial. These will dissolve in a few minutes and inhibit biological activity. If the crystals are not visible, the vial was either flushed to fast, forcing the crystals out of the vial, or it took too long to fill the vial and cap it, allowing the crystals to dissolve. If this occurs, collect a second sample (but keep us both, noting which is which).

13b. Alkalinity and Laboratory pH. pH is also affected by dissolved gases, and this sample

should be collected in the same way as the DIC sample. Alkalinity is collected in a 500 mL bottle, which should be RINSED WITH RIVER WATER THREE TIMES (there is not preservative in the vial). Take the sample by filling and capping the bottle under water at arms-length; cap the bottle underwater with no head space. Alternatively, fill a clean bucket with river water from the surface at the thalweg location and take the sample by filling and capping the bottle under water.

14. After all the water and plankton has been collected, the boat can be beached at the first shoreline sample station for sample processing. Place the water samples in a cooler with ice.

Table 5-4. Base-location procedure for filtering a water sample for chlorophyll a, TSS, geochemical markers, DIC and DOC (based, in part, on personal communication with Anthony Aufdencampe, Stroud Water Research Center, Avondale, PA)

1. Shake Composite water sample 2 and 3 vigorously and completely pour both composites into an 8-L plastic churn splitter. Use a subsample dispensed from the churn to rinse all the sediment out of the cubitainers into the churn. Churn for 10 strokes with the dasher touching the bottom of the churn on each stroke. Dispense subsamples while continuing to churn. Rinse out the cubitainers with DI water; they can be reused for Composite water samples 2 and 3 at other sites.
2. Turbidity. Dispense about 75 mL of composite from the churn into a beaker for turbidity analysis. Follow the operating instructions for the turbidimeter and make 3 replicate readings (3 subsamples from the 75 mL of composite). Record the values in NTUs on the Water Chemistry and Plankton Form. Record the temperature of the sample. Clean sample tubes as required in the turbidimeter operating instructions
3. Set up the filtering apparatus by connecting a vacuum pump to the filter manifold with tubing. Use an in-line wastewater reservoir.
4. Total suspended solids (TSS). Place the filter holder on the reservoir and position a pair of preweighed 47-mm membrane filters on the manifold (they will be prepackaged as a pair). Handle filters with forceps and make sure the top filter from the filter holder is on top in the manifold. Wetting the manifold screen with DI water will allow the filter to adhere better.
5. Record the filter pair ID number on the Water Chemistry and Plankton Form. Secure the top of the apparatus. Wetting the manifold screen with DI water will allow the filter to adhere better. Filter the sample using a vacuum of not more than 15 psi. If the water is turbid or green, dispense (while churning) and filter about 50 to 100 mL at a time using a graduated cylinder; keep track of the amount dispensed. If the water is clear, dispense and filter 500 mL at a time for a maximum of about 1.5 L per filter (4 filters need to be filled from 8 L churn). Pour the entire contents of the graduated cylinder into the filter funnel each time. Continue dispensing until the filters begins to clog and filtration slows to about 1 drip per 2-5 seconds (work on other tasks while waiting). If the filter clogs completely before all the sample in the filter funnel has been filtered, discard the sample and filters and prepare a new sample with a smaller volume of water
6. Record the final volume filtered on the form and on the sample label. After filtering, return the filter pair to its container (dirty side up) using forceps.
7. Repeat step 4-6 for a second filter pair.

8. Geochemical markers. Place the filter holder on the reservoir and position a pre-weighed and pre-combusted (450° C, 4-6 h) 47-mm Millpore AP40 glass-fiber filter on the manifold. Handle filters with forceps The top of the filter is the side opposite the “checked” or “gridded” side. Record the filter ID number from the filter container on the Water Chemistry and Plankton Form. Secure the top of the apparatus. Filter as for TSS samples, using 15 psi vacuum and about 1.5-2.0 times the volume filtered for the TSS samples (these glass fiber filters have a much higher capacity than membrane filters). Pour the entire contents of the graduated cylinder into the filter funnel each time. Continue dispensing until the filter begins to clog and filtration slows. Maximum filtered volume should be 3.0 L, in order to leave 2,0 L for chlorophyll. If the filter clogs completely before all the sample in the filter funnel has been filtered, discard the sample and filter and prepare a new sample with a smaller volume of water. Save this water for the DOC sample (step 9b).

9. Record the final volume filtered on the form and the sample label. After filtering, return the filter to its container (dirty side up) using forceps.

   9a. Fill the DOC vial with the water filtered through the glass fiber filter for geochemical markers. Be careful to not touch the inside of the cap or the vial rim with your hands, as DOC samples are easily contaminated with finger grease. Do not overfill or drain this vial, as a few drops of sodium azide preservative have been placed in the bottom of the vial and should not be discarded. Recap tightly.

10. Chlorophyll a. Filter chlorophyll in shade or subdued light. Place the filter holder on the reservoir and position a 47-mm Whatman GF/F glass-fiber filter on the m anifold (not pre-weighed). Handle filters with forceps. The top of the filter is the side opposite the “checked” or “gridded” side. Wetting the manifold screen with DI water will allow the filter to adhere better. Secure the top of the apparatus. If the water sample appears clear, dispense up to 1000 mL of river water from the churn into a graduated cylinder. Filter the sample using a vacuum of not more than 7 pounds per square inch (psi). If the water is turbid or green, dispense (while churning) and filter about 50 to 100 mL at a time using a graduated cylinder; keep track of the amount dispensed. Pour the entire contents of the graduated cylinder into the filter funnel each time. Continue dispensing until the filter begins to clog and filtration slows. If the filter clogs completely before all the sample in the filter funnel has been filtered, discard the sample and filter and prepare a new sam ple with a smaller volume of water.

11. Record the final volume filtered on the form and sample label. Remove the filter with forceps and place it in a foil-wrapped scintillation vial. The filter may be folded in half.

12. Rinse the filter apparatus, churn and graduated cylinder with DI water and store in a plastic bag to keep clean.

13. Fill out a sample label (Figure 5-1) for each filter or filter pair (TSS), place labels on the chlorophyll vial and cover with tape. Write the sample ID from the label directly on the “Petrislide” containers (TSS and geochemical marker filters) and set labels aside (they will be re-associated with the filters after the filters are dried). Keep chlorophyll filters cold (near 4° C) until they can be frozen.

Table 5-4. Base-location procedure for filtering a water sample for chlorophyll a, TSS, geochemical markers, DIC and DOC, continued.

14. Chlorophyll filters are preserved by freezing. Geochemistry and TSS filters are preserved by drying in an oven. Dry filters overnight in a drying oven at 30 - 50° C with the Petrislide container face up and the lids ajar (the Petrislide containers will melt at >70° C). Drape a sheet of aluminum foil over the filters to keep out dust. If a drying oven is not available, filters will usually dry over 24 to 48 hours in an air conditioned room/dehumidified room just by setting the filters out on a table with lids ajar and foil draped on top to keep out dust. DIC and DOC sample vials are chemically preserved (pre-dispensed in vial), and should be shipped within a week in order to minimize gas permeation through the stopper. Also, DIC samples should be kept at room temperature, as temperature extremes may cause leaking or gasses to come out of solution. DOC samples can be refrigerated (but not frozen) until being shipped.
15. After the filters are dried, place the label on the petrislide container (but do not obscure other information on the container). Store dried filters in a dry location prior to shipment.

Table 8-5. Processing fish for fin-tissue DNA analysis.

1. Refer to Table 8-5.1 for the number of sites to be sampled for fish DNA. In order to obtain a broad geographic spread of sites, crews should either sample their first or second sites and last sites of the season or use sites in numerical order by Site ID. At selected sites, check "DNA sample site?" box at the top of the fish sampling form (Figure 8-2).

Table 8-5.1 Distribution of and crews responsible for fish DNA samples in 2006

River	Number of Fish DNA sites to sample
Ohio River	2 sites each in upper, middle, and lower reaches Total = 6 sites
Missouri River	2 sites in upper (Garrison) reach 2 sites in middle (SD-NE, NE-IA)) 2 sites by CPCB crew (lower reach) 8 sites in lower reach (USGS/MDC) Total = 14 sites
Mississippi River	2 sites by each crew (LCM, OLM, BVM, HSM, ORM, GRM) Total = 12 sites

2. Large fish. Fishing should be completed as usual. All large fish should be identified and sized and data recorded. For fish DNA samples, each of the first 20 large fish of each species (>12 cm; size class 5 or larger; including fish retained for fish tissue contaminants – Section 9) across both electrofishing transects should have a 0.5-1.0 cm^2 punch or clip of the caudal (tail) removed. It may work best to punch the fish while it is held in the net. Keep a running count so that no more than 20 fish of each species are punched. Once 20 fish of each species (across both transects) are sampled, it is not necessary to collect more fish from that site (i.e., it is acceptable for all DNA samples to come from the first transect).

3. Place the fin tissue sample in a #1 paper coin envelope (provided by EPA). Fill out and affix an Fish Tissue DNA sample label (Figure 8-7) to the envelope. Affixing blank labels to the coin envelopes prior to sampling is recommended to save time during sampling. It is important to make every attempt to keep the coin envelopes containing the fin samples dry during the sampling process. DNA preservation is accomplished by wicking away of moisture from the tissue and onto the envelope.

4. Digital photographs are only needed for representative fish of each species and particularly unusual specimens (e.g. suspected hybrid catostomids) at a site. It is not necessary to photograph every large fish. Place the labeled envelope next to the fish and take a digital photograph. Release all fish unless they are to be retained as a fish tissue contaminant samples. Between fish, rinse hands and punch/scissors by vigorous agitation in river water.

5. Transfer the sample ID from each envelope to the field form and the NERL Sample Tracking Form. Specify the sample type as DF [fin]. A separate tracking form will be used for each type of sample. Use as many tracking forms as necessary.

6. Place all the sample envelopes for a site in a zip-lock plastic bag labeled with the site ID. Refrigerate or ice-down (while maintaining in a dry state) immediately. Until shipped, samples should remain refrigerated or frozen. Ship the samples (with completed

tracking forms) weekly to Tammy Goyke at NERL (contact information is on tracking form). Be sure to update the SWIM sample tracking database.

7. Download and back-up digital images. Record the image file name (and fish common name) on the sample tracking form. (It is recommended that prior to recording the image file name, files be renamed to the six digit EMAP Sample ID number (e.g., 172933.jpg – this will provide a simple and direct link from the photo to the sample). At the end of the season, send the fish DNA digital photograph files and all other digital photograph files (fish vouchers, site photographs, etc.) to USEPA/MED in Duluth, MN

8. Place all the sample envelopes for a site in a zip-lock plastic bag labeled with the site ID and refrigerate immediately. Ship the samples weekly to the appropriate person at NERL.

9. Small fish. All small fish (<12 cm; size class 4 or smaller; excluding fish retained for fish tissue contaminants – Section 9) are preserved in the field as a composite sample (Sample type: DC [composite]) in 85% ethanol. All fish must be placed in fresh 75% ethanol within 2-5 days of collection. Allowing the fish to stay in the initial preservation media for more than a few days may result in sample degradation, hampering morphological and genetic analyses. In the lab, the fish should be processed (identified and counted and data recorded) as usual (Table 8-2). Fish are then sorted to species. Each species is placed in a separate jar(s) with new 75% ethanol.

10. Label each jar with a Fish Tissue DNA label. Each species receives a unique sample ID. Additional jars of the same species are labeled with continuation labels with the same sample ID for the species. Con-specific small fish samples are designated as “DW [whole]” sample types. Cover the labels with clear tape and seal the jar(s) with electrician’s tape. Transfer the sample ID of species to the fish sampling form (Figure 8-2) next to the first record for that species.

11. Transfer the sample ID, species common name, and sample type “DW [whole]” to the NERL sample tracking form. (Note, at the top of the NERL tracking form, the composite sample ID from which the separated species samples were derived should be recorded to show the sample lineage. The “parent” sample should show a sample type of ‘DC’). Update SWIM sample tracking database. Retain samples until they can be transferred to NERL. Contact information is on the tracking form.

12. In addition to the samples specified in Table 8-5.1, crews may collect additional fin-tissue samples (i.e. large fish only) of unknown or unusual specimens at any other site and submit them for DNA analysis using the same sample tracking procedures outlined above. This would be of great benefit to the scientists who are researching the use of this technology for phylogeography and hybridization issues that may be related to stressors in Great Rivers.