Appendix IV: USGS SOPs

USGS Standard Operating Procedures			
Page	Procedure/Equipment	SOP number	Revision Date
A	Western Grebe Egg Collection		March 2012
B	Collection of Avian Blood		March 2012
C	Sample Processing		December 2011

Appendix IV A: Western Grebe Egg Collection

Standard Operating Procedure
Western Grebe Egg Collections in CA Lakes 2012

Objectives

(1) Collect up to 30 eggs per colony.
(2) Collect only 1 egg randomly from each nest.
(3) Target eggs from active nests but also collect salvage eggs when necessary.

Equipment

- ☐ Blue sharpies (please use blue to write on eggs)
- ☐ GPS unit (Decimal degrees in NAD83)
- ☐ Float cup & fresh water
- ☐ Soft-sided cooler for egg storage
- ☐ Whirlpaks & plastic Ziplocs for eggs
- ☐ Egg cartons

Data sheets

- ☐ Egg collection data sheet
- ☐ Floatation chart

Egg Collection Data Sheet – write in thin blue Sharpie provided

ID Code: Pre-printed ID codes will be provided prior to leaving for the field.
Date: Record collection date.
Site: Record the lake name
Coordinates Latitude/Longitude: Record nest location in UTMs with NAD83 projection
Number of Eggs in Nest: record the current number of eggs in the clutch before the egg was collected
Float Incubation Stage: record the age the collected egg is floating at, or the incubation age via candling in the field
Nest Status: only collect eggs from viable, currently active nests where the parent(s) are still actively incubating the clutch. If you must (see "Objectives" for only times to do this) collect an egg from an abandoned nest, or a nest with dead or infertile eggs, please note this.
Notes: record any notes about disturbance, failed-to-hatch eggs, abnormal nests, etc.

Egg Storage - Once You Have the Egg Collected

(1) Please carefully write in **thick blue sharpie** on the egg shell:

a. Egg ID Code
b. Date
c. Site
d. Species

(2) Please write in thick blue sharpie <u>on the whirl-pak or Ziplocs</u>:
   a. Egg ID Code
   b. Date
   c. Site
   d. Species

(3) Place the labeled egg into the labeled Ziploc/whirl-pak. **Do NOT** seal the bag; the eggs will mold.

(4) Place the egg wrapped in the Ziploc/whirl-pak into a regular chicken egg carton.

(5) Place the egg carton in a soft-sided cooler in the field (preferably on a small blue ice pack).

(6) At the end of the day, place the egg carton in a large cooler with ice. Do not try to jam the egg carton closed! You may break eggs. It is better to leave it open then try to close it and break eggs.

(7) Write in blue sharpie on the egg carton the general date, species and site where those eggs were collected.

Appendix IV B: Collection of Avian Blood

Standard Operating Protocol for the Collection of Avian Blood

Supplies:

- Syringes (various volumes: 1ml, 3 ml, 5ml)
  - 3ml syringes are the most universal and generally recommended unless working with very small or large veins.
- Needles (various sizes: 27ga., 26ga., 25ga., 23ga., 22ga.)
  - 25 and 23 gauge needles are generally most effective. Use smaller if there is difficulty with 25 ga.
- Cryovials (Pre-labeled; 2.0 ml recommended, but 1.2 OK)
- Cryovial storage boxes
- Alcohol wipes
- Cotton absorbent pads
- Nitrile gloves
- Wet or dry ice
- Sharps/bio-waste container
- Data sheets
- Heat pads
- Folding table
- Sodium heparin

Blood Collection Site

- Several locations can be used for blood collection. Selection should depend upon species, bleeder experience and available supplies. In general, the brachial and jugular veins are preferred. However, the tarsal vein may also be used in some circumstances.

Bleeding Preparation

- Prior to bleeding, be sure to have pre-labeled cryovials and heparinized needles and syringes ready.
  - To heparinize needles/syringes, draw a very small amount of heparin through needle, into syringes and inject back into heparin bottle. Remove needle from heparin bottle and pump the plunger several times vigorously to eject most of the remaining heparin in the syringe.
    - This is important to avoid diluting the sample and skewing the overall weight, thus biasing the concentrations.

Bird Preparation

- After capture hold birds in appropriate sized cage until processing.
- Remove an individual bird from cage, bring to table, and keep head covered with cotton bird bag or pillow case.
- One person should hold the bird on its back and display the ventral surface of the whole wing (for wing bleeding).
- Locate the brachial vein and wipe area with alcohol wipes.

Blood Collection

- Carefully insert needle (with bevel facing up) into selected vein and slowly pull back on plunger making sure there is continuous flow into the syringe.
- When target blood volume has been reached (MAX = 1% of body weight), slowly remove and immediately cap needle and place a cotton pad over the collection site. Place pressure over the collection site until bleeding has stopped.
- Remove needle from syringe, inject blood into cryovial, cap cryovial, and place in storage box on wet or dry ice.
- Discard needle and syringe into sharps/biohazard container.

Blood Storage (in the field)

- Keep blood on dry or wet ice (dry preferred),
    - Freeze upright so blood pools in bottom of cryovial.
    - Freeze as quickly as possible.

Blood Storage (in the lab)

- Upon return to laboratory, immediately place blood in freezer (-20 C).
- Freeze upright.
- Do not allow blood to freeze and thaw, keep frozen.

Appendix IV C: Sample Processing

Sample Processing Standard Operating Procedures

Forest and Rangeland Ecosystem Science Center

CONTAMINANT ECOLOGY RESEARCH PROGRAM

Collin Eagles-Smith

Revised 12/27/2011

USGS
science for a changing world

Quick View Processing SOP

1. Clean work space, tools, analytical equipment.
2. Thaw samples.
3. Prepare data sheets.
4. Generate unique tissue CERP ID Codes (if dissecting tissues from sample).
5. Generate tough tags for tissue samples.
6. Update google docs.
7. Weigh whole body sample (wet weight).
8. Dissect (this process differs by project and sample type and often includes additional steps not identified in this SOP. (For birds, refer to *S:\Projects\Eagles-SmithLab\SOPs\Birds\Egg dissection*).
9. Dry samples.
10. Weigh dry sample.
11. Grind samples.
12. Enter data.
    a. Use ***DataEntryTemplates_EasyAccesssTemplate*** found in *S:\Projects\Eagles-SmithLab\Data*).
    b. Save data in *S:\Projects\Eagles-SmithLab\Active Projects*.
13. Verify (proof) data.
14. Scan data sheets.
    a. Save scans in *S:\Projects\Eagles-SmithLab\Scanned datasheets*
15. Store original data sheets in data repository filing cabinet.
16. Update google docs.
17. Once all data are verified, move folder into the "***Data***" folder (*S:\Projects\Eagles-SmithLab\Data*) indicating data are ready to be uploaded to database.

USGS FRESC Contaminant Ecology Research Program
Standard Operating Procedure (SOP)
Fish Sample Processing
12/20/2011

The sample processing component is the step in the workflow immediately after cataloging, in which samples are dissected (when applicable), dried, and homogenized prior to chemical analyses. This step often occurs over several days as samples are dried, then homogenized. Thus, special care must be taken to ensure that datasheets are carefully tracked.

In some cases, this step is merged with the cataloging step and both are completed simultaneously. Be sure to check with project leader(s) to verify the most appropriate approach for each sample set.

1. **Laboratory conditions and equipment cleanliness**
   - 1.1. Tape clean lab bench paper or aluminum foil to the lab bench.
   - 1.2. Prior to sample processing, the surfaces of all processing locations and tools shall be cleaned and thoroughly rinsed with DI water. All processing tools shall also be rinsed with DI water and wiped with a clean KimWipe between each sample.
   - 1.3. Gently wipe the analytical balance (using care not to apply pressure to the weigh pan) and ensure that it is calibrated daily, prior to use.

2. **Initial sample tracking and cataloging**
   - 2.1. From the freezer, remove only the number of samples that can be initially processed (cleaned, weighed, and inserted into drying oven) in the allotted time for the given day. These samples should not be thawed for ~1 hour before processing.
   - 2.2. Prepare data sheets. Use the processing datasheet template Excel file in the "***Data sheet templates***" folder and add or remove columns as necessary. (See the ***CERP_DATA_database*** or previous project templates for reference).
   - 2.3. Save this file as: "***ProjectID_Proc_MATRIX_##_mmddyy.xls***" (where: ***ProjectID*** is the unique ID for that project (refer to ***tbl CERP_Project List*** in the database: *S:\Projects\Eagles-SmithLab\Databases*), (where ***MATRIX*** is the type of sample being analyzed fish/bird eggs/invertebrates/etc…) and ## is a unique number for that datasheet template). Put a copy of these datasheets in the ***lab datasheet templates*** subfolder within the ***datasheet templates*** folder on the share drive.
   - 2.4. Open the Google Docs file entitled "***Lab_project tracking***" and enter the page numbers you will be using under the Drying and grinding data sheet page numbers column.
   - 2.5. Print datasheets and make sure each datasheet has the current date and page numbers in the header.

2.6. Prepare tough tags with appropriate ***CERP ID Code*** (refer to the ***Master Sample ID codes_ToughTAGs*** (*S:\Projects\Eagles-SmithLab\Sample ID Codes_ToughTags*) Excel sheet and the Access database to identify a list of unique codes. Note: all tissue samples have different codes (refer to the '***tbl TissueCode***' in the database (*S:\Projects\Eagles-SmithLab\Databases*). See ***ID Code SOP*** for more detail.

2.7. Store all data sheets in the respective binder until all samples have been processed.

3. **Sample cleaning and wet weight** – ***This step may seem redundant with the weighing involved in the cataloging step. However, recording the exact wet weight of a sample just prior to drying is critical for data integrity. We often assess these values in comparison to the catalog weights to evaluate desiccation in the freezer due to sublimation.***

3.1. Wearing powderless nitrile gloves remove sample from container and rinse the surface of each thawed sample with DI water and pat dry with clean KimWipe.

3.2. Obtain one drying vessel (e.g. aluminum or plastic weigh boat, sample vial, etc.) for each sample and write the appropriate***CERP ID Code*** on each weigh boat.

3.3. With the balance empty, press the tare button to zero the balance. Place each weigh boat on the balance and record the weight in the "**Drying vessel weight**" cell on the hard copy data sheet.

3.4. With a clean, dry KimWipe, pat the surface of the sample dry and place in weigh boat. Obtain a sample wet weight (be sure that the weight of the drying vessel is included in this – **i.e. DON'T TARE THE WEIGHT BOAT!**) and hand record in the "**Lab wet weight + drying vessel**" cell on the hard copy data sheet.

4. **Dissection** – ***The dissection process varies by project. The following are general procedures outlining the most basic dissection steps. In most cases either 1) the whole organism will be processed and thus no dissection process is warranted, or 2) tissue samples will be extracted and processed for later analyses.***

4.1. With the balance empty, press the tare button to zero the balance.

4.2. Label each drying weigh boat with ***CERP ID Code***.

4.3. Place each weigh boat on the balance and record the weight in the "Drying vessel weight" cell on the data sheet.

4.4. Dissect out appropriate tissue(s). Record ***CERP ID*** with respective tissue code in appropriate column(s).

4.5. For samples in which a muscle tissue is extracted for processing instead of the whole organism, dissect the dorsal portion of muscle between the head and dorsal fin, along the side of the spine (see Figure 1). Remove at least **1 gram** of muscle (both sides of the fish can be pooled if necessary; when applicable make note on data sheet).

4.6. Obtain a sample wet weight for each tissue (be sure that the weight of the drying vessel is included in this – ***i.e. DON'T TARE THE WEIGHT BOAT!***) in the "**tissue wet weight + drying vessel**" cell on the data sheet. Be sure to indicate the tissue type on the data sheet or that the appropriate column exists for the tissue (e.g. IDMuscleAxial, IDKidney, etc.).

4.7. Place tissue in drying oven.

5. Sample drying

5.1. Turn on drying oven and set to 50 degrees C.
5.2. Load samples onto a plastic or aluminum tray, and place the tray in oven.
5.3. Log the sample on the drying oven log sheet (on the front of the oven). .
5.4. Record the dry start date and drying temperature on the processing data sheet.
5.5. Allow sample to dry for 48 hours or until a constant mass is achieved (change in mass in 8 hours is <1%).
5.6. Open the Google Doc file "***Lab_project tracking***" and fill out the appropriate fields with your initials and % completed: (e.g. NB 50%)
5.6.1. Samples dried (N) [N=number]

6. Sample dry weight

6.1. After samples have dried, remove them from the oven (while still warm) and place into large dessicator to cool (~ 20 minutes).
6.2. Log sample removal on drying oven log sheet.
6.3. Record total dry time in the appropriate cell on processing data sheet.
6.4. Remove 3-4 samples from dessicator (NOT the whole tray), place a dried sample **AND** its associated weigh boat onto a calibrated balance and record mass (in grams) in the "**Dry weight + drying vessel**" column on the data sheet.
6.5. Place sample in clean glass vial and label with a ToughTag that has ***Project ID*** and ***CERP ID*** code on it. Tissue may need to be broken into pieces to fit into vials.

7. Sample grinding

7.1. Clean grinding apparatus (Wiley Mill, Cryogrinder, IKA mill, or mortar and pestle). The specific apparatus will vary with sample type and many samples can be ground using a variety of methods (discuss with the project leader(s)).If the sample mass is very small, use mortar and pestle with wax paper, as it reduces sample loss during the grinding process.
7.2. Grind sample to a uniform consistency (fine powder) and record grind method and date on data sheet.
7.3. Carefully pour ground sample back into glass vial, using care not to lose material in the transfer, or contaminate other samples.
7.4. Clean grinding apparatus between samples. Mortar and pestle: clean with DI water and dry with KimWipe; Wiley Mill: clean with compressed air and brush;.Cryogrinder and IKA mill: clean with either DI water and KimWipe or compressed air.

8. Post processing

8.1. Enter all data from hard copy datasheet into the appropriate electronic file and save as "***ProjectID_Proc_MATRIX_data_ddmmyy.xls***" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc…). Use the ***DataEntryTemplate_Easy AccessUpload*** excel file as a template for data entry. This file contains the correct column headings and additional information used in the database but not recorded in the laboratory during dissecting/processing samples. Note: column headings used on laboratory data sheets are identified in row 2 (under the headings used in access). Appropriate column headings may be found on different worksheets for database purposes (i.e., data collected during the "cataloging" process in the lab may actually be found in the "morphology/dissection" worksheet in the DateEntryTemplate excel file). Add and delete columns as necessary for each particular project. All data can be entered on one excel worksheet. For further description of column headings see the access database design view for the respective table

8.2. Proof (verify) all entered data.

8.3. Initial the data sheets following data entry and proofing data.

8.4. Scan hard copy datasheets and save the file as "***ProjectID_Proc_MATRIX_scandata_ddmmyy.xls***" (Where **MATRIX** is the type of sample being analyzed fish/eggs/inverts/etc…) Place PDF of datasheets into the Scanned datasheets folder on the share drive.

8.5. File the hard copy of the original datasheets in the current year's data folder in the data repository filing cabinet.

8.6. Data are ready for uploading to the database, so move excel files to data folder on the share drive (*S:\Projects\Eagles-SmithLab\Data*).

8.7. Open the Google Docs file entitled "***Lab_project tracking***" and update the processing fields with your initials and necessary information. The following fields **must** be completed on the Google Docs project tracking datasheet before moving on to processing:

- 8.7.1. Samples dried (N) N= number of samples
- 8.7.2. Samples ground (N) N=number of samples
- 8.7.3. Drying and grinding data entered
- 8.7.4. Drying and grinding data sheet file names and locations
- 8.7.5. Drying and grinding data sheet page numbers
- 8.7.6. Drying and grinding data sheet file names and locations
- 8.7.7. Processing data proofed
- 8.7.8. Drying data sheets scanned and filed

Sample Data sheet

Project ID#: CERP Project ID
Datasheet ID: ProjectID_Proc_12/21/2011_1

Year "Project Name"
Processing Data

Date (mm/dd/yyyy):_______________

Name(s):_______________

Tissue ID (YY____####_)	CERP ID Code (YY____####) (Note if different)	Sample or Tissue Type (e.g. whole fish)	Drying Vessel (DV) Weight (g)	Tissue Wet Weight +DV (g)	Dry start date	Dry temp (C)	Dry time (hrs)	Tissue Dry Weight + DV (g)	Grind Method	Grind date	Notes

Data Entered by:________ Date:______

Data Verified by:______________ Date:_______