Bird Egg Monitoring for Selenium and Mercury on Great Salt Lake

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Introduction

The study described herein is recommended to address a critical gap in understanding that affects the management of water quality in Great Salt Lake. The intent is for the Utah Department of Environmental Quality, Division of Water Quality (DWQ) to use this as a starting point for further planning and implementation of the study.

Study Objective

The Great Salt Lake (GSL) provides a critical resource for breeding shorebirds and consequently has been identified as a site of Hemispheric Importance. The first numeric water quality standard for selenium for GSL was established in State rule (UAC R317-2-14) as a geometric mean selenium concentration of 12.5 mg/kg in a minimum sample of at least five whole eggs of aquatic-dependent birds that use the waters of Gilbert Bay (Class 5A), GSL. Shorebirds (such as American avocets, black-necked stilts) can be negatively affected by increased selenium contamination. These species are among the more common nesting birds of the GSL, feed largely on macro-invertebrates, and will likely assimilate selenium rapidly.

Information to develop an optimal monitoring plan for implementation of the selenium standard at GSL is not available. For example, data collected in previous GSL selenium studies are not adequate to address the variability of selenium concentrations in shorebird eggs by species, location, and diet. This information is needed to optimize long term monitoring of selenium in shorebird eggs so as to minimize the number of eggs collected and target the appropriate locations and bird species. In addition to selenium, mercury has been identified as a constituent of concern for birds at GSL, so this study will include analyses for both selenium and mercury, primarily in shorebird eggs. This study will focus on developing recommendations for preferred sampling locations, species of shorebird, and associated sampling to document relations to the food chain by answering the following questions:

- Where are the nesting locations avocets and stilts nest at regularly in which selenium and mercury concentrations in their eggs are representative of exposure in GSL?

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- How variable are selenium and mercury concentrations in avocet and stilt eggs and, based on that variability, how many eggs of each species should be the target for monitoring?
- What do the shorebirds eat at GSL, and what are the transfer factors for selenium and mercury from the diet to bird eggs?
- What are the ambient concentrations of selenium and mercury in the water and sediment near shorebird nesting areas and in macro-invertebrates they consume?

Approach

It is expected that this study will have a duration of three to five years for sampling and analysis and six months for development of recommendations following the field study. The following general approach will be used initially, though it may be adjusted annually on the basis of findings during the previous year:

- Survey potential nesting areas for avocets and stilts early in spring to find aggregations of birds where they are likely to nest. Select three areas for sampling where egg selenium and mercury will reflect the birds' foraging in open waters or immediately adjacent to open waters of GSL. The waters in which the birds forage must be directly linked to the open waters of GSL so food items and eggs are representative of selenium and mercury exposure from the open waters of GSL.
- At each of the three locations, collect one egg from each of eight avocet nests and eight stilt nests having full clutches of eggs for selenium and mercury analysis.
- Collect up to five foraging adult avocets and five stilts at each nesting location and qualitatively determine diet of the birds; insofar as possible collect three samples of each food type for selenium and mercury analysis, and collect one sample of water and one composite sample of surface sediment (top 5 cm; each composite composed of five smaller samples) from the foraging area for selenium and mercury analysis. Take a blood sample from each of the avocets and stilts immediately after collection and also save the liver for selenium and mercury analysis.

Variables and characteristics to be measured

- Aggregate percentage and aggregate volume (cc) of food items recovered from the upper digestive tract (pharynx, esophagus, proventriculus, and ventriculus) of avocets and stilts
- Selenium and mercury concentrations in the following:
- Avocet and stilt eggs, blood, and livers
- Macro-invertebrates samples at foraging sites
- Water and sediment near foraging sites

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Other variables

- Incidence of embryo mortality and abnormalities in sampled avocet and stilt eggs

Spatial boundaries

The project area is defined as the open waters of the GSL (also referred to as Gilbert Bay) located north and west of Farmington Bay, west of the Weber River input, and south of Promontory Point, Bear River Bay, and the North Arm (bounded by the railroad causeway).

The following five study areas will be surveyed for nesting aggregations:

- West Carrington Bay (if accessible) – This area has almost no freshwater input and may be influenced by the deep brine layer that forms and depletes at different times than the main body of the lake.
- The South Shore and Eardley Spit – These areas have very little fresh water inflow.
- The Southeast Corner of Gilbert Bay – This area experiences inflows from the Goggin Drain and Lee Creek, both of which are primarily freshwater. Two industrial outfalls are also discharged into the main body of the lake near this area.
- Northern and western sides of Antelope Island (particularly Bridger Bay) – This area was one of the study sites in 2006, and was the location where avocets fed most extensively on brine shrimp, a key organism for food-chain monitoring in GSL.
- Pintail Flats at Ogden Bay – This area is influenced by freshwater from all of the main inflows to the lake.

Temporal boundaries

The period of data collection will be from April to July each year for a minimum of 3 years (2011-2013) to facilitate evaluation of annual variation. Results should be evaluated after 3 years to determine if an additional 2 years of sample collection and analysis is required (total of 5 years). Field and analytical results will be obtained by November each year and an annual report will be submitted by the following January 15 for the previous year. An overall comprehensive evaluation of the data set will be completed by July 2013 (assuming a study period of 3 years) to include recommendations for long-term monitoring of selenium and mercury in GSL shorebird eggs.

Sampling Plan

The sampling plan is summarized as follows:

1. Nesting areas will be surveyed by aerial transect beginning in late April and coordinates of nesting aggregations will be recorded to facilitate the remaining tasks.
2. Once nesting aggregations have been identified, they will be visited to determine the stage of breeding and to identify foraging locations.
3. Five adults of each species will be collected at each site at the beginning of the nesting season, after observing them feed for >15 minutes. Dietary information will

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be obtained by direct examination of gut contents (pharynx, esophagus, proventriculus and ventriculus). Blood and liver samples will be taken from each bird and frozen for potential selenium and mercury analysis.

4. From each of the foraging areas, invertebrates (brine fly adults and/or larvae or pupae, brine shrimp, or other aquatic organisms, depending on what the birds are eating), sediment, and water will be collected for selenium and mercury analysis. If available, three samples of each species and life stage (i.e., larvae, pupae or adult of brine flies) will be collected at each area, with sufficient biomass for analysis (target 5 grams) and additional biomass when that is feasible.
5. A single egg will be collected from each of 8 nests for each species at each location after the clutches are completed (total of 48 eggs per year). Each embryo will be checked for stage of development and late-stage embryos will be examined for developmental abnormalities, including a determination of the embryo's position in the egg. Egg contents will then be placed in a marked chemically-cleaned container and preserved frozen for later analysis.
6. All analytical results for tissue should be reported on a dry-weight basis, along with moisture content for each sample.
7. Sample collection and handling will be consistent with the Quality Assurance Project Plan (QAPP) for Sampling in the Open Waters of Great Salt Lake and following Standard Operating Procedures (SOPs):
    - Avian Diet Sampling
    - Avian Egg Sampling
    - Egg Breakout
    - Harvest of Adult Birds
8. Evaluate data and make recommendations for long-term monitoring of selenium and mercury in GSL shorebird eggs

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Avian Diet Sampling

Introduction

Sampling of macro-invertebrates and other bird food items for analysis can help with the estimation of exposure and risk to birds foraging in the open waters of Great Salt Lake (GSL) from bioaccumulative contaminants. The results may be used to relate water, sediment, and invertebrate concentrations to those observed in birds and bird eggs.

This Standard Operating Procedure (SOP) was adapted and updated from the original SOP (Conover and Cavitt, 2006) prepared as part of the Utah Division of Water Quality's (DWQ's) project, Development of a Selenium Standard for the Open Waters of Great Salt Lake (CH2M HILL, 2008). This SOP assumes a primary objective is to relate contaminant concentrations in food items to concentrations in incubating birds and their eggs. It also assumes that food items are directly linked to the open waters of GSL. Sampling of food items should be adapted to specific study objectives and the diet of the bird species that are targeted.

Pre-sampling Checklist

The pre-sampling checklist of materials needed during macro-invertebrate sampling will include the following:

- A Global Positioning System (GPS) unit
- A map showing sampling sites with coordinates
- Bound field log book
- Field distance measuring instruments
- Digital camera
- Waders and boots
- Aquarium nets or larger kicknets
- Seine
- Whirlpak® or Ziploc® bags with labels
- Nalgene® Bottle
- Sterile and clean 1-mm filtering device
- Gloves
- Labels
- Marker pens and pencils
- Cell phone in case of emergency
- Cooler filled with ice
- First-aid kit
- Distilled water
- White plastic or enamel sorting tray
- Forceps

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Procedures

Samples will be collected as close in time as practical to bird egg collection, wherever applicable. Sampling of water, sediment, and macro-invertebrates should be completed in conjunction with each other if a sampling plan requires the collection of more than one of these matrices. Water and sediment should be collected along with macro-invertebrates to include in the evaluation of contaminants in bird food.

Survey potential nesting areas for birds in late April to find aggregations of birds and identify colonies and/or areas where they are likely to nest. This may be done by aerial transect or ground visits to potential nesting areas. Select areas for sampling where food items will reflect the birds' foraging in open waters or immediately adjacent to open waters of GSL (henceforth called the food-item sampling area, or FISA). The waters in which the birds forage must be directly linked to the open waters of GSL so food items are representative of exposure from the open waters of GSL.

All sampling locations for macro-invertebrates will be represented as delineated areas rather than specific sampling points, to allow samplers to identify regions within each area where the birds are foraging, and to be able to collect sufficient macro-invertebrate biomass for analysis. FISAs will be named after their respective nesting site, and each nesting site will have one FISA associated with it.

A 15-minute feeding observation before sampling within each FISA and collection of foraging birds should provide guidance on where macro-invertebrates will be collected. Preference should be given to collecting macro-invertebrates at the same location as water and sediment samples are collected, wherever applicable. Macro-invertebrates will be collected from the mudflat, benthos, and water column within each foraging area as required by the specific sampling plan.

Invertebrate food items (brine fly adults and/or larvae or pupae and brine shrimp, depending on what the birds are eating) will be collected opportunistically in the general area of each foraging area. If available, three samples of each species and life stage (i.e., larvae, pupae, or adult of brine flies) will be collected at each area, with sufficient biomass for analysis (target 5 grams) and additional biomass when that is feasible. The numbers and types of invertebrates sampled should reflect what the birds are eating. Food items can also be collected by seining behind a boat, using a seine with a proper mesh size to obtain a pure sample of adult brine shrimp if that is the target organism. At those sites where the water is less saline, some corixids may be mixed in with the shrimp. If so, corixids are large enough that they will be removed individually and analyzed as a separate sample f the birds are found to forage on them. Each sample will be stored in a Whirl-pak or Ziploc bag and labeled with its location or sample number and collection date. All food items from a single FISA will be stored together in a 9X 12 envelope upon which is ascribed the date and FISA where the enclosed samples were obtained. If it is not possible to obtain adequate biomass for each representative taxon, samplers will make a visual estimation of relative abundance (by mass) of families of invertebrates within each sample and record this information in their field notes. Samples will be stored in a cooler until transported to laboratory. In the laboratory, they will be frozen until analyzed.

Lack of sufficient organisms for testing requirements at any location will be noted in the field log book. Field notes of all field activity will be recorded in a bound field logbook.

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Field notes will include date and time, names of personnel conducting the survey, the work performed, any problems identified as well as corrective actions taken, and other appropriate general comments or observations.

Water Samples

Wherever applicable per specific sampling plan, a water sample will be collected from each FISA using required methods depending upon the targeted contaminant. Each water sample will be a composite water sample with 20% of the composite water sample coming from 5 different sites systematically distributed across the FISA. Water will be filtered through a 1-mm mesh to remove large items from the sample. All samples will be collected in new Nalgene bottles and stored at room temperature. After 48 hours, the water in each bottle will be decanted and placed in a new Nalgene bottle to separate the water sample from any sediment in the sample.

Sediment Samples

Wherever applicable per specific sampling plan, a sediment sample will be collected for each FISA. The sediment sample will be a composite sample with 20% of the composite sample coming from each of 5 sediment core samples collected from 5 sites systematically distributed across each FISA. Each sediment sample will be taken to a maximum depth of 5 cm being careful to collect any fine organic matter at the surface. The sediment sample will be stored under refrigeration or frozen until they are shipped to the laboratory for selenium, mercury, and total organic carbon analyses.

Field Logbook

Field activities will be documented through journal entries in a bound field logbook, which is dedicated to this project. The field logbook will be water-resistant, the pages will be sequentially numbered, and all entries will be made in indelible ink. Each page of the field logbook will be dated and signed by the person making the entry. The field logbook will contain all pertinent information about sampling activities, site conditions, field methods used, general observations, and other pertinent technical information. Examples of typical field logbook entries include the following:

- Date and time of sample collection
- Name of personnel present
- Referenced sampling location description (in relation to a stationary landmark), GPS coordinates, and maps
- Daily temperature and other climatic conditions
- Field measurements, activities, and observations (e.g., depth of water, condition of water, other relevant conditions)
- Media sampled
- Sample collection methods and equipment
- Types of sample containers used
- Sample identification and cross-referencing
- Types of analyses to be performed
- Site sketches
- Visitors to the site

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- Color photographs taken during sampling activities will be numbered to correspond to photo log entries. The name of the photographer, date, time, site location, and photograph description will be entered sequentially in the photo log as photographs are taken.

Additional information will be recorded in the field notebook as required by DWQ.

References

Cavitt, J. and Conover, M. 2006. Standard Operating Procedure for Avian Diet Sampling; Program Manual for Development of Selenium Standard for Open Waters of Great Salt Lake. Utah Department of Environmental Quality, Division of Water Quality, Salt Lake City, Utah. May 11, 2006.

CH2M HILL. 2008. Development of Selenium Standard for Open Waters of Great Salt Lake. Utah Department of Environmental Quality, Division of Water Quality, Salt Lake City, Utah.

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Avian Egg Collection

Introduction

Sampling of bird eggs for selenium and mercury analysis will provide a direct measure of the bioaccumulation of these constituents in resident nesting birds that are likely to be foraging in the open waters of Great Salt Lake (GSL) during the nesting season. The results may be used to relate water, sediment, and invertebrate concentrations to those observed in the bird eggs. This will aid in the estimation of exposure and risk to the birds from selenium and mercury. It should be noted that **appropriate regulatory agencies should be contacted to ensure that permits and/or documentation are obtained prior to sampling bird eggs**.

This Standard Operating Procedure (SOP) was adapted and updated from the original SOP (Cavitt and Conover, 2006) prepared as part of the Utah Division of Water Quality's (DWQ's) project, Development of a Selenium Standard for the Open Waters of Great Salt Lake (CH2M HILL, 2008).

Pre-sampling Checklist

The materials needed during egg sampling include the following:

- Permits (eggs should be collected under Utah and Federal scientific collecting permits)
- A Global Positioning System (GPS) unit
- A map showing sampling sites with coordinates
- Pre-formatted field data sheets
- Bound field log book
- Binoculars
- Field distance measuring instruments
- Digital camera
- Padded egg collection boxes (hard-sided container [e.g., egg cartons, Tupperware or tackle box, with foam padding])
- Labels
- Marker pens and pencils
- Cell phones in case of emergency
- Cooler filled with ice
- First-aid kit

Procedures

Survey potential nesting areas for birds in late April to find aggregations of birds and identify areas where they are likely to nest. This may be done by aerial transect or ground visits to potential nesting areas. Select areas for sampling where egg selenium and mercury will reflect the birds' foraging in open waters or immediately adjacent to open waters of

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GSL. The waters in which the birds forage must be directly linked to the open waters of GSL so eggs are representative of selenium and mercury exposure from the open waters of GSL. The study area is defined as the open waters of the GSL (also referred to as Gilbert Bay) located north and west of Farmington Bay, west of the Weber River input, and south of Promontory Point, Bear River Bay, and the North Arm (bounded by the railroad causeway).

Once nesting aggregations have been identified, they will be visited to determine the stage of breeding, identify foraging locations, and confirm whether the nesting area meets the food source requirements defined above. Some nests will be located during surveys by watching for the adults leaving the nest, displaying, or "sneaking" away from the nest. Other bird nests will be located by looking for birds displaying behaviors associated with nesting activities, searching for adults on nests with binoculars, and opportunistically finding nests while conducting surveys and other activities. The nesting period varies from year to year and among species. Therefore, it is anticipated that multiple sampling events will be required between April and July to collect the required number of eggs.

Eggs should be collected by randomly selecting one egg from each complete clutch to be sampled. Eggs will usually be collected as soon as a nest is discovered to avoid losing samples to predation and to maximize the number of nests sampled for selenium and mercury. At collection, each egg will be marked with a unique identification code, date, and number of eggs in the clutch using a marker pen. Depending upon the sampling scheme, fresh eggs or eggs with well-developed embryos may be collected. Eggs with late-stage embryos can be recognized at time of collection using egg floatation methods (Custer et al. 1992). Collecting from unfinished clutches (i.e., less than four eggs) or addled eggs (eggs that failed to hatch) should be avoided. The best eggs for contaminants analysis are not cracked, since cracking increases variation in percent moisture, and may lead to leakage or contamination of the contents. Eggs with well-developed embryos can be examined for gross abnormalities and malpositions of the embryo.

The number of eggs collected depends upon the sampling scheme but should not exceed 20 percent of available eggs in the bird egg sampling area. Every attempt will be made to collect eight eggs from each species (avocet and stilt), but if there is a shortage for one species the total number will be collected by taking more eggs of the other species. If the required number of eggs from each species cannot be obtained during a nesting season, and one species is represented more than the other is, this will be noted while reporting and interpreting the data.

A preformatted field data sheet will be filled out that will include a unique nest/egg identification code, bird species, location (using GPS coordinates for the nest), date, number of eggs in the clutch, and initial estimation of incubation stage. The egg will be placed in a container to avoid damage and the container will be placed in a cooler with wet ice. Eggs removed from nests will be transported to laboratory in a hard container with sufficient padding to avoid breakage. Eggs will be refrigerated at 4 degrees Celsius (°C) within 1 to 2 hours of collection and until opened, ideally no longer than seven days.

Field notes of all field activity will be recorded in a bound field logbook. Field notes will include date and time, names of personnel conducting the survey, the work performed, any problems identified as well as corrective actions taken, and other appropriate general comments or observations.

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Field Logbook

Field activities will be documented through journal entries in a bound field logbook, which is dedicated to this project. The field logbook will be water-resistant, the pages will be sequentially numbered, and all entries will be made in indelible ink. Each page of the field logbook will be dated and signed by the person making the entry. The field logbook will contain all pertinent information about sampling activities, site conditions, field methods used, general observations, and other pertinent technical information. Examples of typical field logbook entries include the following:

- Date and time of sample collection
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- Media sampled
- Sample collection methods and equipment
- Types of sample containers usedSample identification and cross-referencing
- Types of analyses to be performed
- Site sketches
- Visitors to the site
- Color photographs taken during sampling activities will be numbered to correspond to photo log entries. The name of the photographer, date, time, site location, and photograph description will be entered sequentially in the photo log as photographs are taken.

Additional information will be recorded in the field notebook as required by DWQ.

References

Cavitt, J. and Conover, M. 2006. Standard Operating Procedure for Avian Egg Collection; Program Manual for Development of Selenium Standard for Open Waters of Great Salt Lake. Utah Department of Environmental Quality, Division of Water Quality. Salt Lake City, Utah. April 22, 2006.

CH2M HILL. 2008. Development of Selenium Standard for Open Waters of Great Salt Lake. Utah Department of Environmental Quality, Division of Water Quality, Salt Lake City, Utah.

Custer, T.W., G.W. Pendleton, and R.W. Roach. 1992. Determination of hatching date for eggs of black-crowned night-heron, snowy egrets, and great egrets. J. Field. Ornithol. 63:145-154.

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Egg Breakout

This Standard Operating Procedure (SOP) was adapted and updated from the original SOP (U.C Davis et al., 2006) prepared as part of the Utah Division of Water Quality's (DWQ's) project, Development of a Selenium Standard for the Open Waters of Great Salt Lake (CH2M HILL, 2008).

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Store eggs in a refrigerator if they cannot be processed immediately after collection. Do not freeze whole eggs because this may crack the shell. Ideally, eggs should be processed as soon as possible after collection, and within seven days of collection. However, because refrigeration arrests development, the vascularization and bright red color of the blood in an egg collected with a living embryo is preserved for a longer period of time.

The following describes the process of harvesting avian eggs in the laboratory. The goal of this description is to collect a standardized set of data on whole eggs, embryos, and shells while minimizing the possibility of laboratory contamination of samples.

All methods and results will be recorded in a bound laboratory logbook. Laboratory notes will include date and time, names of personnel processing the eggs, the work performed, any problems identified as well as corrective actions taken, and other appropriate results and general comments and observations.

Required Supplies

The supplies needed for the procedures include:

1. **Whole Egg Measurements:** distilled-deionized water, volumeter (immersion chamber or graduated cylinder), egg candler, Kimwipes, laboratory balance (to 0.05 g increments), vernier caliper (graduated to 0.0l mm).
2. **Egg Harvest:** glass jars of appropriate size (chemically-cleaned and with TFE cap-liners) or Nalgene jars (depending on contaminant), weigh boats, stainless steel surgical scissors, forceps, blunt probe, lead pencil or waterproof marker.
3. **Shell Thickness:** Micrometer modified for measuring eggshell thickness such as Federal 35 comparator with rounded contacts (graduated measurement to 0.01 mm –estimatable to nearest 0.001 mm) or modified Starrett micrometer (graduated measurement to 0.01 mm – estimatable to nearest 0.001 mm).

Egg Measurement Procedure

1. If possible, eggs will be candled to determine if cracks are present in the shell. Any cracked egg should not be rinsed or immersed in water as this may contaminate the sample.

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2. If an egg is not cracked and is dirty (soil, feces) it should be cleaned with a Kimwipe and distilled-deionized water that is at or near the temperature of the egg.

3. Write the sample ID number on both ends of the eggshell with a dull pencil or waterproof marker (both IDs must be legible).

4. Record any remarkable characteristics of the egg (e.g., cracked, dented, discolorations, small in size, rough surface, etc.).

5. Record the mass (g) of the whole egg; then measure the length (mm) and breadth (mm) of the egg at their greatest dimensions with calipers. (To obtain an accurate measurement of length, one must ensure that the caliper jaws are parallel to the longitudinal axis of the egg. For the breadth measurement, the jaws must be held perpendicular to the longitudinal axis of the egg.)

6. Determine and record the egg volume (cm^3): the method of choice will depend on whether the shell is intact or cracked and what contaminants are being investigated. If determining the fresh weight of the egg is important (e.g., investigating organochlorine contaminants) the "Intact Shell" method is recommended, for investigating contaminants such as selenium and mercury the volume can be calculated from measurements (i.e., "Cracked Shell" method).

7. **Intact Shell:** For eggs with intact shells, determine the egg volume using the water displacement technique outlined below.

    a. If using an immersion chamber, place it next to and above the pan of a laboratory balance. Set a collection vessel on the balance's pan under the side arm of the volumeter. Next, place a wire loop in the volumeter. Fill the volumeter with distilled-deionized water that is slightly warmer than the egg temperature until it flows freely from the volumeter side arm (remember, the temperature of the water should be as close to the temperature of the egg as possible as this will minimize water movement across the eggshell pores). When the water stops flowing, empty the receptacle and return it to the balance pan. Tare the water receptacle. Gently raise the wire loop and place the egg on it. Gently lower the egg until it is completely submersed (lower the egg as quickly as possible without overflowing the volumeter, or breaking the egg). The weight of the displaced water equals the volume (cm^3) of the egg. Repeat this procedure three times for each egg and report the average value.

    b. If using a graduated cylinder, fill it with distilled-deionized water (water temperature should be as described above) and note the starting volume. Using a wire loop, immerse the egg into the graduated cylinder until top of the egg is just below the water surface. Note the final volume and subtract starting volume to determine egg volume. Repeat this procedure three times for each egg and report the average value.

8. **Cracked Shell** (or if an immersion chamber or graduated cylinder is not available): For eggs that are cracked or dented, egg volume is estimated using the length and breadth measurements and an equation from the published literature (e.g., Westerskov, 1950; Stickel et al., 1973; Hoyt, 1979).

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Egg Harvest

(Note: All tools used in egg harvest and embryo exam must be cleaned between egg exams. Investigators should wear surgical gloves and change gloves between eggs.)

1. **Vent egg if necessary.** For eggs with a strong odor (indicating advanced decomposition of the contents), it is advisable to vent the egg before attempting to open it (explosions are possible). With safety glasses in place, gently insert a chemically-clean needle into the blunt end of the egg. Use gentle but steady pressure to pierce the shell.

2. **Open window at blunt end of the egg**. Take a chemically- clean jar and loosen the lid and tare the jar. Work over a clean glass Petri dish or weigh boat. Method 1- using surgical scissors, apply gentle pressure while rotating the scissors so a small hole is made in the shell at the blunt end of the egg just above the air cell. Continue cutting from the hole and cut around the entire egg above the air cell; remove the blunt end of the egg with forceps and place it in a safe place. Method 2—Rest the egg lengthwise on an appropriate surface (compatible with the analyses requested). Using a clean sharp scalpel, gently score the egg about the blunt end of the egg. Apply gentle, steady pressure and make several rotations. If candling of the egg revealed an advanced state of incubation with air cell development, try to remove shell from just above the air cell. Membrane may need to be peeled back to allow further inspection of the embryo.

3. **Inspect embryo position in the egg.** Visually inspect the egg contents through the window and note the size of the air cell. On advanced embryos, this window is used to assess whether the position of the embryo in the egg is normal. Note embryo position and whether the embryo has pipped into the air cell. **Determination of embryo position is not accurate until the embryo is ready to pip the air cell (i.e., the last 4–5 days of incubation).** Shedding the nare caps is a good landmark for avocets and stilts. Normal position of the embryo during the final stages before pipping is with the head in the blunt end of the egg, with the head under the right wing and with the beak pointed toward the air cell. If incubation stage is very late (i.e., just prior to pipping from the shell), the embryo beak is in the air cell to allow pulmonary respiration to begin. There are six malpositions of the avian embryo (Romanoff and Romanoff, 1972), as follows:

    a. head between thighs
    b. head in small end of egg
    c. head under left wing
    d. embryo rotated so that the beak is not directed toward air cell
    e. feet over head
    f. beak over right wing

4. Malpositioned embryos usually do not hatch, and positions a, c, and e are usually lethal.

5. Usually the egg contents can be poured out into the container from the window opened for embryo inspection. If necessary, use the blunt probe to ease the egg contents out through the window or use surgical scissors and make transverse cuts from the blunt end to the narrow end of the egg to facilitate egg opening.

6. **Open egg.** Inspect embryo position and note age of the embryo. To estimate age of the embryo use stages of incubation from literature. The model reference for aging embryos

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is Lillie's development of the chick (Hamilton, 1952) Chapter 3. Good day-by-day embryo stage data with pictures exist for chickens, mallards (Caldwell and Snart, 1974), kestrels (Pisenti et al., 2001), pheasant (Hermes and Woodard, 1987), and cockatiels (Abbott et al., 1991). If no embryo can be found, examine the yolk for the presence of a blastodisc. If fertile and the yolk is intact, this will appear as a white donut shape floating on top of the yolk. If infertile, no distinct donut will be apparent. Note presence (and whether they are of normal size for the stage of development) or absence of eyes and of limbs or limb buds; note presence and number of digits on the feet; measure length of tarsus and upper mandible. Look for evidence of internal hemorrhage, edema, brain swelling, or failure of the body wall to completely close. Photograph any embryos with suspected abnormalities. Minimize handling of the embryo to the degree possible and conduct as much as possible of the above exam in the half shell. Use clean forceps, and beware of cross contamination. Pour the contents into the opened jar. If necessary, use a clean spatula to scrape any remaining contents into the jar (be careful not to tear the shell membrane when using spatula). Record presence or absence of an embryo, estimated age of embryo, other measurements taken, and abnormalities (if any).

7. Measure and record the weight in grams of the tared jar.
8. Label jar with SAMPLE ID and SAMPLE MASS (place one label on the lid and the other on the jar itself), and immediately store the sample in the freezer. Sample shall be kept frozen during transportation to laboratory for analysis.
9. Rinse the interior of the shell halves with tap water being careful not to tear the membrane, or erase the sample IDs. After the shells dry, use a waterproof marker to remark the shells with their sample ID. Store the shell halves together in a cool dry place for at least 30 days, or until they have attained a constant mass. Recycled egg cartons serve as excellent storage containers for egg shells. One tip to ensure that shells do not migrate from their respective compartments is to place a folded sheet of paper over the shells before closing the carton.

Shell Thickness Measurement

1. Determine the eggshell mass (to nearest 0.001 g) of dried shells.
2. Allow eggshells to air dry for at least 30 days and measure eggshell thickness using an appropriate micrometer. Take thickness measurements of each shell along the equator at five places. Minimize influence of shell shape and curvature on the measurement taken. Report the average of all five measurements as the final thickness measurement. If the membrane has separated from the shell, take measurements without the membrane but be sure to make note of this on the data sheet. If possible then obtain measurement of membrane fragments.
3. Calculate the Ratcliffe Index (Ratcliffe, 1967) with the following formula:
4. THICKNESS INDEX = EGGSHELL MASS (mg)/ EGG LENGTH (mm) x EGG WIDTH (mm)

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Harvest and Sampling of Adult Birds

Introduction

Adult birds residing on Great Salt Lake may be harvested and sampled to directly measure bioaccumulation of selenium and/or mercury in resident birds or to determine the diet of the birds. The bird species that are targeted, sampling location, and numbers of birds collected will depend upon the objectives of the sampling effort. The procedures below address collection of American avocets and black-necked stilts with the objective of determining dietary information and collecting blood and liver samples. Methods may be adapted to address other specific study objectives and targeted species. It should be noted that **appropriate regulatory agencies should be contacted to ensure that permits and/or documentation are obtained prior to harvesting adult birds**.

This Standard Operating Procedure (SOP) was adapted and updated from the original SOP (Cavitt and Conover, 2006) prepared as part of the Utah Division of Water Quality's (DWQ's) project, Development of a Selenium Standard for the Open Waters of Great Salt Lake (CH2M HILL, 2008).

Pre-sampling Checklist

The pre-sampling checklist of materials needed during harvesting adult birds will include the followings:

- Permits (**adult birds should be collected only under Utah and Federal scientific collecting permits**)
- A Global Positioning System (GPS) unit
- A map showing sampling sites with coordinates
- Shotgun
- Gloves
- Sterile, non-heparinized syringe
- Sterile Nalgene cryogenic vials
- Whirl-pak or Nalgene bottles and bags
- Sterile Plastic Tray
- 80% Ethanol
- Dissection tools
- Bound field log book
- Binoculars
- Digital camera
- Labels
- Marker pens and pencils
- Cell phone in case of emergency
- Cooler filled with ice
- First-aid kit

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Procedures

Select areas for sampling where the birds are directly linked to the open waters or immediately adjacent to open waters of GSL so they are representative of exposure to the open waters of GSL. The study area is defined as the open waters of the GSL (also referred to as Gilbert Bay) located north and west of Farmington Bay, west of the Weber River input, and south of Promontory Point, Bear River Bay, and the North Arm (bounded by the railroad causeway).

Adult shorebirds will be collected at each sampling site at the beginning of the nesting season by shotgun. Collections will be made as early in the nesting season as possible to minimize disturbance of incubating birds. Because obtaining dietary information is a study objective, American avocets and black-necked stilts will be collected after individuals have been observed feeding for >15min. This will ensure that food items are present within the esophagus. Material collected from the ventriculus is less reliable as an indicator of diet since easily digested items are quickly passed to the intestines whereas hard material can be retained for weeks.

Collection of Samples

Blood

As soon as the adult bird is collected, blood will be collected from the heart or thoracic cavity using a sterile, non-heparinized syringe in field. Attempt will be made to collect at least 2 cc of blood. Once the blood is collected, the syringe will be closed with its cap. To reduce the possibility of contamination, the blood will be stored in the syringe and the syringe will be placed in a freezer or will be transferred to sterile, Nalgene, cryogenic vials and then frozen for analysis.

Liver

The entire liver will be removed from the adult bird, weighed, and frozen in a Whirl-pak or new Nalgene bottle. Color and gross condition (e.g., friable, hemorrhaged, etc). will be recorded on datasheets.

Dietary Information

Immediately following collection, esophageal, proventricular, and ventricular contents will be removed, separated, and placed in individual containers with 80% ethanol. In addition the mouth (pharynx) will be rinsed with 80% ethanol and wash collected. These samples will be examined later using a dissecting microscope to more accurately assess the composition of the samples. Special attention will be given to assess the proportion of the samples made up of brine shrimp, corixids, brine fly adults, and brine fly larvae (or other food items). The volume composition of samples (as percent) will also be determined.

Sample Labels

All samples (food, blood, and liver) will be labeled on the outside with the bird ID number. No internal labels will be used to guard against contamination. For each bird, its blood, liver, and food samples will be placed together in an envelope that also contains the bird's ID number, date, and place collected. Hence, each sample will be doubly labeled. Samples from all birds collected at a single colony will be stored together.

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Bird Carcass

Each bird carcass will be placed in a plastic freezer storage bag and frozen. Before doing so, it will be weighed, sexed, and aged (but all shorebirds are expected to be adults). Females will be checked to see if they are actively producing eggs. Physical measurements will also be taken (body length, wing length, head length and width, and bill length and width).

Field Logbook

Field activities will be documented through journal entries in a bound field logbook, which is dedicated to this project. The field logbook will be water-resistant, the pages will be sequentially numbered, and all entries will be made in indelible ink. Each page of the field logbook will be dated and signed by the person making the entry. The field logbook will contain all pertinent information about sampling activities, site conditions, field methods used, general observations, and other pertinent technical information. Examples of typical field logbook entries include the following:

- Date and time of sample collection
- Name of personnel present
- Referenced sampling location description (in relation to a stationary landmark), GPS coordinates, and maps
- Daily temperature and other climatic conditions
- Field measurements, activities, and observations (e.g., depth of water, condition of water, other relevant conditions)
- Media sampled
- Sample collection methods and equipment
- Types of sample containers used
- Sample identification and cross-referencing
- Types of analyses to be performed
- Site sketches
- Visitors to the site
- Color photographs taken during sampling activities will be numbered to correspond to photo log entries. The name of the photographer, date, time, site location, and photograph description will be entered sequentially in the photo log as photographs are taken.

As required by DWQ, additional information will be recorded in the field notebook.

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