

Standard Operating Procedure

Great Salt Lake Water Quality Studies

Egg Breakout

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Introduction

Store eggs in a refrigerator if they cannot be processed immediately after collection. Do not freeze whole eggs because this will 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 standardize procedures for processing avian eggs in order to collect a standardized set of data on whole eggs, embryos, and shells while minimizing the possibility of laboratory contamination of samples. The types of containers and instruments as well as cleaning procedures will vary with the contaminant that is being investigated. Field collection protocols are considered separately and vary with species and study objectives.

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 0.01 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 should 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.
- 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, 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 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 submerged (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 (3) times for each egg and report the average value.
 - b) If using a graduated cylinder, fill it with distilled-deionized water 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 (3) 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).

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.** Tare 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. 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. 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, as follows:
 - i) head between thighs,
 - ii) head in small end of egg,
 - iii) head under left wing,
 - iv) embryo rotated so that the beak is not directed toward air cell,
 - v) feet over head,
 - vi) beak over right wing

- 4) Malpositioned embryos usually do not hatch, and positions I, III, and V 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 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 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), 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. 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) 4 EGG CONTENTS MASS (g): 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.
- 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 shells 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:
$$\text{THICKNESS INDEX} = \frac{\text{EGGSHELL MASS (mg)}}{\text{EGG LENGTH (mm)} \times \text{EGG WIDTH (mm)}}$$

References

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