SWEDISH MUSEUM OF NATURAL HISTORY

Manual for collection, preparation and storage of bird eggs

for the Swedish National Monitoring Program for Contaminants in Marine Biota

Report 3:2024

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Contents

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Translated from Swedish by Anne L. Soerensen

Collection

Common guillemot (Uria aalge), common tern (Sterna hirundo) and Eurasian oystercatcher (Haematopus ostralegus) are the three species of bird eggs collected within the Swedish National Environmental Monitoring Program for Contaminants in Marine Biota (Figure 1) [*Soerensen and Faxneld*, 2020].

Annually, 20 common guillemot eggs are collected, 10 goes for contaminant analyzes (the addition eggs are saved in the Swedish Environmental Specimen Bank (ESB) after preparation [*Odsjö*, 2006]). Annually, 10 egg from common tern and 10 eggs from Eurasian oystercatcher are collected and goes for contaminant analyzes.

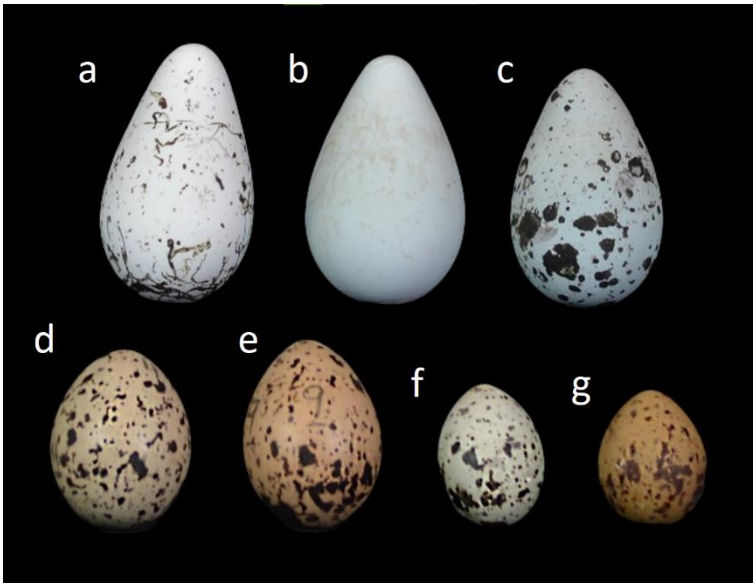

Figure 1. Eggs from the three species collected within the Swedish National Monitoring Program for Contaminants in Marine Biota. *a-c*) common guillemot (Uria aalge), *d-e*) Eurasian oystercatcher (Haematopus ostralegus), *f-g*) common tern (Sterna hirundo).

Area of collection

Sites used for collection within the Swedish National Environmental Monitoring Program for Contaminants in Marine Biota area: Stora Karlsö (Gotland, Sweden; approximate latitude 57.28 and longitude 17.97) for common guillemot and Tjärnö at the Swedish west coast (Skagerrak; approximate latitude 58.89 and longitude 11.03) for common tern and Eurasian Oystercatcher.

Time of collection

Collection should be carried out as early as possible during the breeding period, not later than 14 days after the laying of the first egg in the colony. For each site, the time and place of collection should be mirrored as close as possible between years to reduce the impact of external drivers on the contaminant concentration.

Collection and transportation

At collection, each egg should be labelled with species, location, date and name of the collector. The eggs should be placed in a refrigerator, as soon as possible. The eggs should be packed carefully prior to transportation in order to avoid problems during transportation to the Swedish Museum of Natural History. Every egg should be placed individually in a plastic bag (polythene) after which they are placed separately in a crush absorbing material in stiff boxes of wood, plastic or cardboard.

Accession number and sample ID

All specimen that arrive at the museum are given a unique identifier, an accession number. The accession number follow the individual egg and samples that comes from that egg. When samples are made out of several eggs, the content is most often sampled from a series of eggs with forth going accession numbers. A unique “sample ID” is further assigned to each sample that is prepared for chemical analysis.

Arrival and preparation of jars

Upon arrival, check the eggs at the laboratory to decide if any eggs are in such a shape that they should be discarded before getting an accession number. As a matter of routine, eggs are discarded if a large part of the egg's contents has leaked and there is a risk that the egg content have been contaminated.

Create an accession number for each egg. Write the accessions number on a small manila tag together with information on the species and site of collection, which follow the egg in the egg carton, where the eggs are temporarily stored (see Figure 2). This, and other information associated with each accession number, is also stored in a database where all biological and contaminant information for each specimen and samples are later linked.

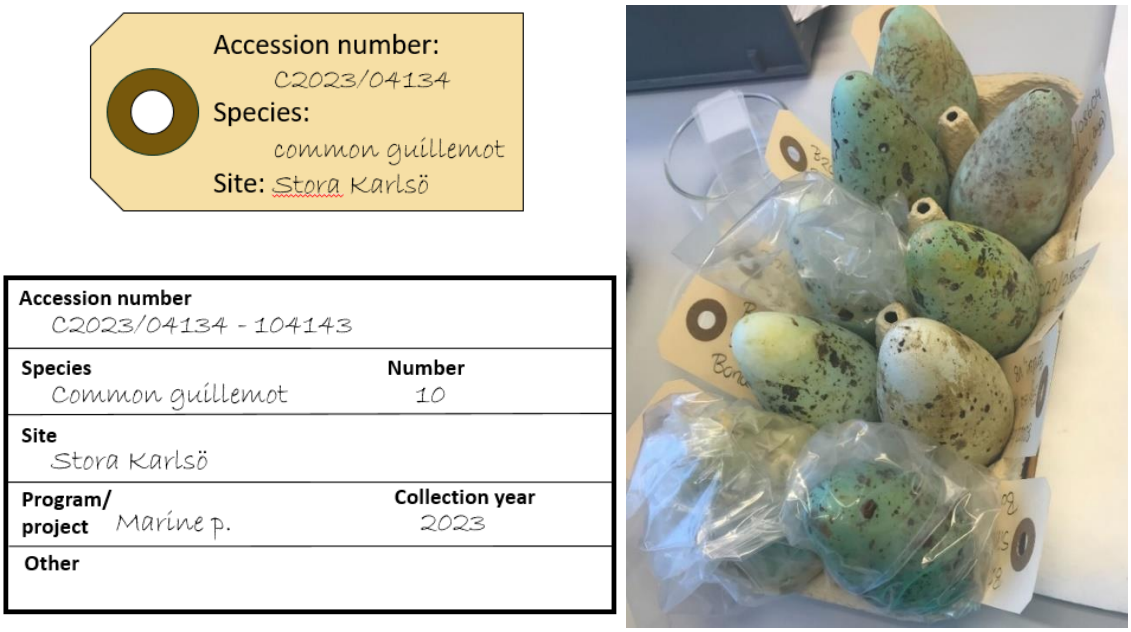

Figure 2. Manila tag for accession number, information sheet and egg carton with common guillemot eggs and their manila tags.

For the common tern and Eurasian oystercatcher, create another two labels with accession numbers for each egg (one for a glass jar and one for a lid for the egg content). For common guillemot, create six labels for each egg, as the egg content will be distributed in three jars (Figure 3). Further, create an "information sheet" containing information on the eggs that will be stored together in the ESB (these will be from the same species/site/year). The sheet should include accession number series, species, number of eggs, site, program material is collected for, and collection date (Figure 2).

Prepare burned (brown) glass jars and a lid with aluminum foil underneath for each egg. For common tern and Eurasian oystercatcher, a 60 ml jar is used (marked with "p.a."). For common guillemot, two 60 ml and one 30 ml jar is used. The labels for the two 60 ml jars should be marked with "MPB" and "LT", and the 30 ml jar should be marked with "p.a." (Figure 3).

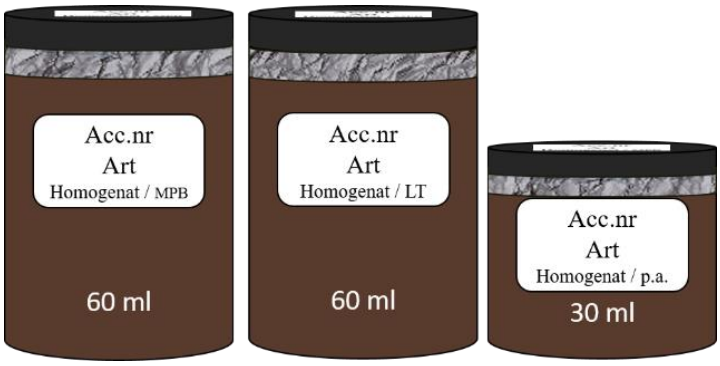

Figure 3. Brown glass sample jars for common guillemot egg content including label information needed (Acc. nr = accession number, art = species, MPB = Swedish Environmental Specimen Bank (-25°C), LT = Low temp freezer (-80°C), p.a.= på analys = for analysis.

Biological measurements – weight and size

Weigh the whole egg (shell and content) while it is still intact, and measure the length and width of the egg in mm with a caliper (Figure 4). Record the measurements with two decimal places. In addition, make a note if the egg is cracked and/or has leaked. The egg is then placed back in the egg carton.

Note, cracked eggs that have leaked can be weighed and measured while in the plastic bag. In such cases, tare the scale with a bag similar to that the egg is in before weighing the egg.

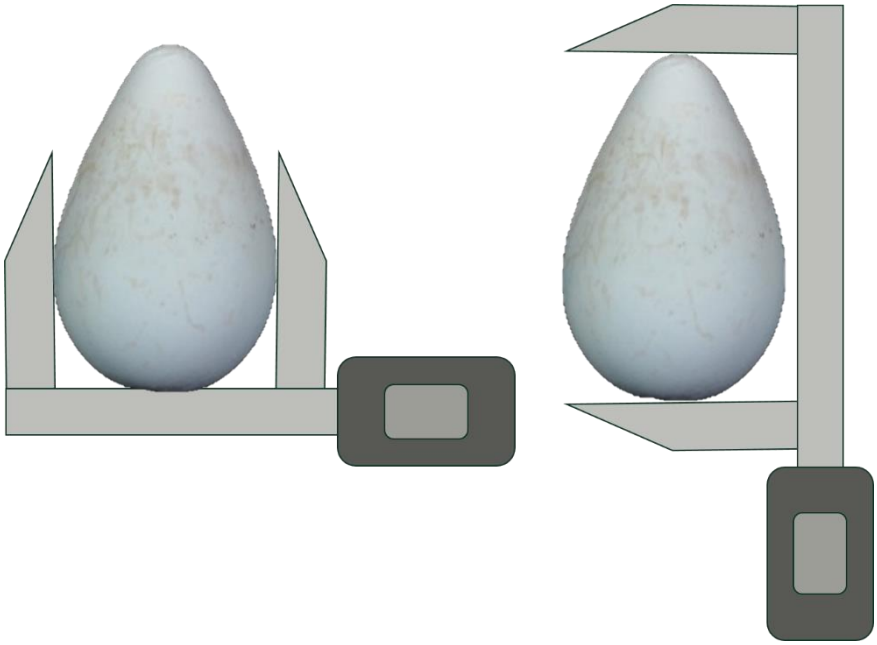

Figure 4. Measurement of length (left) and width (right). The egg used in this example is from a common guillemot (*Uria aalge*).

Extraction of egg content

The egg needs to be opened to extract the content. The incision and sampling of the egg content should be performed in a fume hood to avoid possible splashes and odor. Before opening the egg, prepare the glass jar that will hold the egg content by placing it on a scale before reset the scale. Use a hand drill to create a small hole just above the equator of the eggshell (Figure 5). This is done to reduce the pressure in the egg by releasing any gases that may have formed within (if this is not done, there is a risk that the eggshell will crack during the incision). For the incision, use a multi-tool (Dremel) equipped with a thin saw blade (0.6 mm thick and 22 mm in diameter). Begin the first incision at the small whole and remove a 1×1 cm square with clean stainless steel forceps (Figure 5). Set the square aside until the eggshell has to be cleaned.

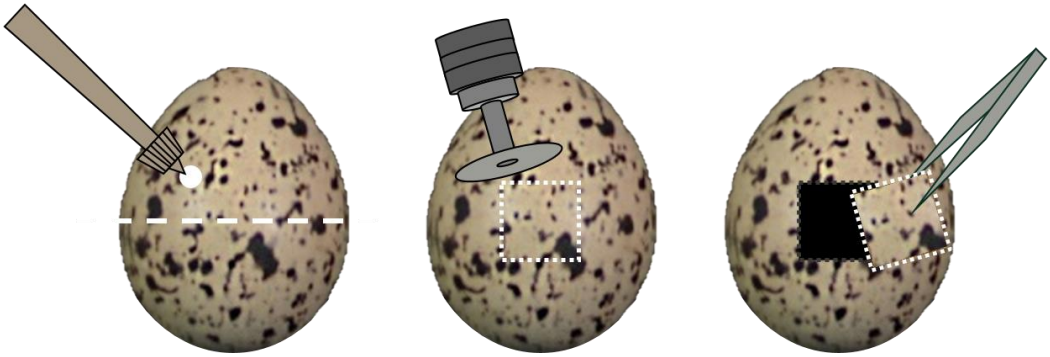

Figure 5. The procedure for the incision (from left to right); placement of a drill hole done with a hand drill just above the equator (the dashed line), placement of incisions in the egg (the small dashed lines) with the multi-tool, removal of shell square with forceps. The eggs is from an Eurasian oystercatcher (*Haematopus ostralegus*).

Sample the egg by emptying the content directly in the prepared glass sample jar for common tern and Eurasian oystercatcher, while for common guillemot the content is emptied in a 250 ml glass beaker (as the content will be distributed to several glass sample jars after homogenization). Scrape gently with a stainless steel spatula to remove all content. Add a note on the color, consistency, and smell as well as presence of any fetal tissue.

Well-developed fetuses are sometimes encountered as part of the egg content. These are saved separately in aluminum foil, and not in the glass jar with the other content. Weigh and measure the length of the fetus (”*crown to tail*”) in grams (two decimals). Wrap the fetus in aluminum foil and label it with accession number, species and type of material (in this case “fetus”).

Homogenization of egg content

Eggs that will go for contaminant analysis should be homogenized. The egg content is homogenized in the brown glass sample jar for common tern and Eurasian oystercatcher while for common guillemot the homogenization is done in the 250 ml glass beaker. The homogenization is conducted with an IKA Turrax homogenizer. Between every egg that is homogenized, take apart and clean the homogenizer to avoid contamination between samples.

Mark sample jars with homogenized egg content that will go to analysis laboratories for contaminant analysis with "p.a." The contaminant samples can either be sampled directly (see "Sample preparation for contaminant analysis") or can be placed in a thin plastic bags in the freezer until contaminant sample preparation. Sample jars that are going straight to the ESB are marked with "MPB" or "LT" (Figure 3).

Pack the glass jar with the egg content and, in the cases where a fetus was encountered, the aluminum package with the fetuses in a thin unsealed plastic bag and place it in the freezer. Let these sit in the freezer for at least some hours to let the content freeze.

If samples should go to the analysis laboratories for contaminant analysis go to "Sample preparation for contaminant analysis". If the samples, should go straight to storage in the ESP see "Storage".

Sample preparation for contaminant analysis

Take the sample jars with homogenized egg content marked "p.a." and let them thaw if they are frozen. Prepare "contaminant sample" jars (Table 1). Stir each "p.a." sample jar before the content is distributed in the "contaminant sample" jars. The contaminant samples can be individual or pooled (10 eggs). Indicate on the contaminant sample jars what type of analysis the samples are for, if they are individual or pooled, what accession numbers are part of the sample and how big the sample is in gram (one decimal place).

Record the weight of the leftover egg content after the samples for contaminant analysis has been taken. Pack the leftover material according to instructions in "Homogenization of egg content" and store it in the ESB. This step is not necessary for common guillemot as there are extra samples from 10 eggs every year and therefore a lot of material that goes straight to the ESB.

Table 1. The general contaminant analysis that the egg sample go for (see *Soerensen and Faxneld* [2022]). One jar for each individual sample and one jar for each pooled sample.

Common guillemot	Common tern and Eurasian oystercatcher
▪ Metals + Hg – individual samples, 3 g ▪ CIC + BFR – individual samples, 10 g ▪ PFAS – pooled sample, 1.5 g (0.15 g/egg) ▪ Dioxin – pooled sample, 30 g (3 g/egg)	▪ Metals + Hg – pooled sample, 3 g (0.3 g/egg) ▪ CIC + BFR – pooled sample, 10 g (0.1 g/egg) ▪ PFAS – pooled sample, 1.5 g (0.15 g/egg) ▪ Dioxin – pooled sample, 30 g (3 g/egg)

Post sampling work on eggshells

Clean the eggshells (including the square that was cut out) carefully on both the inside and outside by rinsing them in lukewarm water. Be careful so that the outer membrane on the shells inside is not removed during this step (the inner membrane can be lost in this step). Then carefully wipe off moist of the eggshells with household paper. Place the shells, including the cut out squares, back in the egg carton together with the associated manila tags with information on accession numbers (Figure *2*). When all the relevant eggshells from the sampling are cleaned, let them dry at room temperature for three months.

Biological measurements – shell thickness

The thickness of the eggshell is measured in mm on the cut-out square after the eggshells have dried for three months. A Mitutoyo micrometer is used for the measurements. For each measurement, the measured thickness is recorded together with information on whether the outer shell membrane is attached with the inside of the eggshell or not. Calculate the thickness (in mm) of the eggshell based on the average value of five measurement points (Figure 6), and with an accuracy of two decimal places. Both the width of the shell and outer membrane has to be measured. If the outer membrane is not attached with the shell, the outer membrane should be measured separately in the same way as the shell.

Be aware that there sometimes is a yellow layer of deposition on the inside of the shell. This should be carefully scraped off before doing the weight measurement. Weigh the eggshell, including the square and any other loose parts, and record the weight in grams (two decimal places).

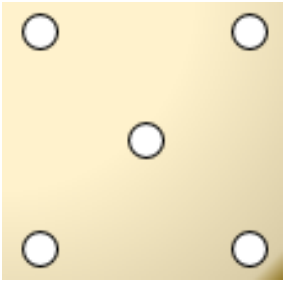

Figure 6. 1x1 cm square cut from the eggshells – the five circles indicate the locations where the shell thickness should be measured.

Storage

Egg contents and eggshells are stored in the ESB.

Egg content

Combine the frozen samples belonging to the same species/site/sampling event in a thicker vacuum bag together with the “information sheet” containing sample information. Vacuum pack the samples and place them in the ESB

When specimens are expected to be used for biochemical studies or for analyses of non-persistent compounds, the ambition is to store the material at as low temperature as possible, from collection to the final storage (-80°C). When specimens are intended to be used for analyses of highly persistent compounds, storage at -25°C is used.

Eggshell

Eggshells can be stored at room temperature, temperature <20°C, if nothing else is prescribed. The shell fragments are dried at room temperature and stored dark at a temperature <20°C in zip lock bags with a manila tag.

Checklist for sampling of egg for contaminant analysis

☐ Inspect incoming eggs and remove bad eggs if necessary.

☐ Create accession numbers for incoming eggs, register in database and create information labels.

☐ Label the eggshells and sample jars.

☐ Line up burned glass sample jars with burned aluminum foil to place underneath the lids for the eggs. Add labels to jars (jar and lid).

☐ Measure biological data; weight (g), length (mm) and width (mm).

☐ Sample the egg content.

- Tare the scale with the sample jar on the scale
- Cut out a 1x1 eggshell square
- Empty the egg content into the labelled sample jars
- Describe the content (color, consistency, smell and presence of fetal tissue

Presence of fetus?

☐YES ☐NO

- Weight in gram
- Length in mm (crown to tail)
- Pack in aluminum foil
- Pack in thin plastic bag and freeze

Need to homogenize egg contents?

☐YES ☐NO

☐ Pack the egg content

- Place the sample jar in a thin plastic bag and place in the freezer

Need to send samples for contaminant analysis?

☐YES ☐NO

- If yes, take jars with sampled egg content (directly or from the freezer) and distribute them into relevant “analysis sample” jars (individual or pooled samples)
- If no, place the jars with sampled egg contents in vacuum bags with other samples from same sites and year (including any fetus) and the information sheet. Vacuum the bag and store

☐ Clean the shells in lukewarm water.

☐ Dry the eggshells for three months

☐ Measure eggshell

- Measure eggshell thickness
  - Measured with shell membrane?

    ☐YES ☐NO
- Weight of the eggshell

☐ Pack the eggshell in a zip lock bag including an ”information sheet”.

References

Odsjö, T. (2006), The environmental specimen bank, Swedish Museum of Natural History—a base for contaminant monitoring and environmental research, *J. Environ. Monitor.*, *8*(8), 791-794.

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