OCS Study, MMS 2001-031

NISTIR 6735

SEABIRD TISSUE ARCHIVAL AND MONITORING PROJECT: Protocol for Collecting and Banking Seabird Eggs

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NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

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U.S. Department of Commerce
Donald L. Evans, Secretary

National Institute of Standards and Technology
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DISCLAIMER

Certain commercial equipment or instruments are identified in this paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the National Institute of Standards and Technology nor does it imply that the equipment or instruments are the best available for the purpose.

SEABIRD TISSUE ARCHIVAL AND MONITORING PROJECT: Protocol for Collecting and Banking Seabird Eggs

INTRODUCTION

Environmental Specimen Banking

Archiving biological and environmental samples for retrospective analysis is a major component of systematic environmental monitoring. The long-term storage of carefully selected, representative samples in an environmental specimen bank is an important complement to the real-time monitoring of the environment. These archived samples permit:

1. The use of subsequently developed innovative analytical technology that was not available at the time the samples were archived, for clear state-of-art identification and quantification of analytes of interest,

2. The identification and quantification of analytes that are of subsequent interest but that were not of interest at the time the samples were archived, and

3. The comparison of present and past analytical techniques and values, providing continued credibility of past analytical values, and allowing flexibility in environmental monitoring programs.

Seabirds, including albatrosses, pelicans, cormorants, terns, kittiwakes, murres, guillemots, and puffins spend most of their lives at sea and have special adaptations for feeding in the marine environment, including the ability to excrete the excess salt obtained from ingesting seawater. Many species nest in dense groups (colonies) on steep, precipitous sea-cliffs and headlands.

Seabirds are long-lived and slow to mature. They occupy high positions in the marine food web and are considered sensitive indicators for the marine environment (prey includes krill, small fish, and squid). Breeding success, timing of nesting, diets, and survival rates may provide early indications of changing environmental conditions (e.g., see Hatch et al., 1993). Chemical analysis of seabird tissues, including egg contents, can be particularly useful in determining whether contaminants (and potential biological effects) associated with human industrial activities, such as offshore petroleum and mineral exploration and development, are accumulating in marine environments. The collection and archival of seabird tissues over a period of several years will be a resource for future analyses, providing samples that can be used to determine historical baseline contaminant levels.

The specimen bank for the Seabird Tissue Archival and Monitoring Project (STAMP) is maintained by the National Institute of Standards and Technology (NIST) in the satellite facility of the National Biomonitoring Specimen Bank (NBSB) at the NIST Charleston Laboratory, Charleston, South Carolina. The Charleston banking facility is designated for marine specimens as part of the National Marine Analytical Quality Assurance Program. The NBSB design for long-term cryogenic storage,

which has been described by Wise and Koster (1995), is a result of 20 years of experience involving cooperative efforts between NIST and the Environmental Protection Agency (EPA), and several years of comparative studies with specimen banking programs in Germany, Japan, Sweden, and Canada. Agencies using the NBSB include EPA, U.S. Department of Agriculture (USDA), Food and Drug Administration (FDA), National Cancer Institute (NCI), National Oceanic and Atmospheric Administration (NOAA), and U.S. Geological Survey, Biological Resources Division (USGS/BRD). Both NOAA and USGS/BRD are primary participants in the use of the NBSB for marine environmental research and monitoring. The two NOAA programs using the NBSB are the National Status and Trends Program and the Marine Mammal Health and Stranding Response Program. The USGS/BRD is collaborating with both NIST and NOAA in banking tissues from Alaska marine mammals for the Alaska Marine Mammal Tissue Archival Project (AMMTAP).

Project Goal and Objectives

The goal of STAMP is to archive a representative collection of tissues from Alaskan colonial seabird species for future contaminant analyses and documentation of long-term trends in environmental quality. The Project has two objectives:

1. Collect specimens from representative Alaskan colonial seabird species suitable for determining levels of organic and inorganic contaminants.

Tissues collected for archival are limited to freshly killed birds taken by researchers or subsistence hunters. Eggs are collected as part of routine colony monitoring by USFWS personnel and processed by USGS/BRD personnel according to NIST standard operating procedures (SOP). The reader is referred to the Materials and Protocols Sections.

2. Transport, catalog, and archive samples in conditions that are suitable for long-term storage and eventual contaminant analyses.

After collection, samples are packaged, transported, cataloged, and archived according to protocols established by the NBSB. Storage is in liquid nitrogen vapor at -150 °C, which provides the best conditions for minimizing sample degradation. Samples will be available to project partners for future contaminant analyses. Requests for archived samples by other researchers and agencies will be considered by project partners on a case-by-case basis.

Banking Seabird Eggs

Seabird tissues, particularly eggs, have played important roles in environmental monitoring in Europe and Canada. The Canadian Wildlife Service (CWS) collects, banks, and analyzes eggs and tissues from seabirds of the Atlantic and Pacific coasts as part of its Wildlife Toxicology Program. (See Mineau et al., 1984; Elliott, 1985; and Wakeford and Kasserra, 1997). Eggs are particularly

useful for the temporal and spatial monitoring of persistent organic pollutants (e.g., polychlorinated biphenyls (PCBs), chlorinated pesticides, dioxins) and mercury. For example, the CWS successfully documented temporal changes in PCBs and pesticides in the Great Lakes by analyzing banked herring gull (*Larus argentatus*) eggs (Mineau et al., 1984). Also, eggs from alcids (seabirds belonging to the family Alcidae that includes murres, murrelets, auklets, guillemots, and puffins) were identified as key indicators for circumpolar monitoring of persistent organic pollutants (POPs) by all Arctic nations participating in the International Arctic Monitoring and Assessment Programme (AMAP) Phase II - Years 1998 - 2003 (AMAP Scientific Experts Workshop, Girdwood, Alaska, April 1998).

The AMAP report on the state of the Arctic environment summarizes knowledge on POPs in seabirds living in arctic environments. However, the report is limited to the Canadian and Scandinavian Arctic (AMAP, 1998). It presents data indicating that piscivorous seabirds feeding at the top of the marine food web (e.g., cormorants, puffins, kittiwakes) have higher levels of PCBs in their eggs than those feeding at lower levels (e.g., eiders). Levels in seabird eggs were higher in the Scandinavian Arctic than in the Canadian Arctic and, within Canada, levels were higher in the high eastern Arctic than in the lower western Arctic. Also, levels of PCBs approaching concentrations known to affect hatching success were found in thick-billed and common murre (*Uria lomvia* and *U. aalge*), puffin, black guillemot (*Cepphus grylle*), and black-legged kittiwake (*Rissa tridactyla*) eggs from the Canadian and Norwegian regions of the Arctic (AMAP, 1998).

Presently, there are few data on the concentrations of POPs in colonial seabirds nesting in Alaska. Kawano et al. (1988) reported chlordane concentrations in thick-billed murres from the North Pacific and Gulf of Alaska. However, the most comprehensive information on organochlorine residues in Alaskan seabird eggs was obtained 25 years ago (Ohlendorf et al., 1982). Extrapolating levels of POPs from the western Canadian Arctic database is not appropriate, because atmospheric and oceanic transport of contaminants from Southeast Asia eastward and northward into the Gulf of Alaska and Bering Sea, and the oceanic transport of other substances eastward along the northern and eastern coasts of Siberia and into the Chukchi and Bering seas probably affect overall contaminant patterns and levels in Alaskan seabirds. Local sources from existing and former military installations also have to be considered.

More than 95 % of the seabirds breeding in the continental United States breed at Alaskan colonies in the Gulf of Alaska and Bering and Chukchi seas (USFWS, 1992), and about 70 % of the Alaskan birds nest on Alaska Maritime National Wildlife Refuge (AMNWR) lands (G. V. Byrd, personal communication). In 1998 and 1999, the USGS/BRD, AMNWR, and NIST initiated a joint program to develop and test protocols for collecting, processing, transporting, and banking eggs collected from selected colonies of common murres and thick-billed murres (*Uria aalge* and *U. lomvia*) on AMNWR lands.

Murres spend about 80 % of their time at sea (USFWS, 1992). They are capable of diving to depths of up to 200 m to capture prey, feeding on a wide variety of small fish, including capelin (*Mallotus villosus*) and Pacific sand lance (*Ammodytes hexapturus*), other osmerids and gadids, sculpins,

pricklebacks, shannies, ronquils, and some larger invertebrates (e.g., shrimp, squid). Murres only come ashore to breed in large colonies on precipitous sea cliffs and headlands, where they lay single eggs on bare rock ledges in dense aggregations. The eggs are relatively large, yielding contents ranging from 75 g to 95 g, which is sufficient for multiple analyses. Murres are also capable of laying replacement eggs about 13 to 14 days after losing first eggs.

A protocol for collecting and banking seabird eggs was tested at murre colonies in the AMNWR in 1998 - 1999 (York et al., 1999). Six colonies, ranging from the Arctic Ocean (Chukchi Sea) to the North Pacific (Gulf of Alaska), were selected for routine sampling. These colonies are located at Cape Lisburne in the Chukchi Sea, Little Diomede Island in Bering Strait, Bluff in Norton Sound (northern Bering Sea), St. George Island in the southern Bering Sea, Amatuli Island in the northern Gulf of Alaska, and St. Lazaria Island in the southeastern Gulf of Alaska near Sitka (Fig. 1). During this pilot phase, the draft collection, sample processing, transportation, and banking procedures were evaluated for practicality and suitability for obtaining uncontaminated samples and were modified where necessary. The revised protocol is found in the Methods section of this report and serves as the SOP for obtaining future samples.

Using the protocol developed in 1998, murre eggs were collected in 1999 from colonies on Little Diomede, St. George, Amatuli, and St. Lazaria islands. The eggs were processed and transported to the NBSB at NIST in Charleston, South Carolina, where they were archived at temperatures of -150 °C. The egg contents provide a good source of material for conducting preliminary chemical analyses to establish baseline concentrations of POPs and other contaminants. The results will be compared among colonies, and with published Alaskan and Canadian seabird data and data from other species in Alaska. This work represents the first step toward developing a database that will quantify and document the transfer of persistent organic compounds through seabird food webs in Alaska.

Protocols will continue to be developed and refined throughout the life of the project as additional species (e.g., kittiwakes, auklets, guillemots, and petrels) and tissues (e.g., liver, kidney, muscle, feathers, and blood) are incorporated into it. The eggs of black-legged kittiwakes, *Rissa tidactyla*, will probably be incorporated into the project in the near future. This species is a surface feeder that preys on small fish, particularly sand lance, capelin, and at higher northern Arctic latitudes, Arctic cod *(Boreogadus saida)*. They also occasionally take invertebrates (e.g., surface schooling euphausiids). As in the case of murres, kittiwakes nest in large colonies on coastal and insular sea-cliffs and headlands. They build grass nests and lay up to 3 eggs (in Alaska, the typical clutch size is 2 eggs). The eggs are smaller than murre eggs and provide less material for analyses. Work will be conducted in the future if kittiwake clutches can be combined for banking and analyses and still meet project objectives. Selected tissue samples may also be analyzed to determine the suitability of the tissue types for determining levels of inorganic and organic contaminants.

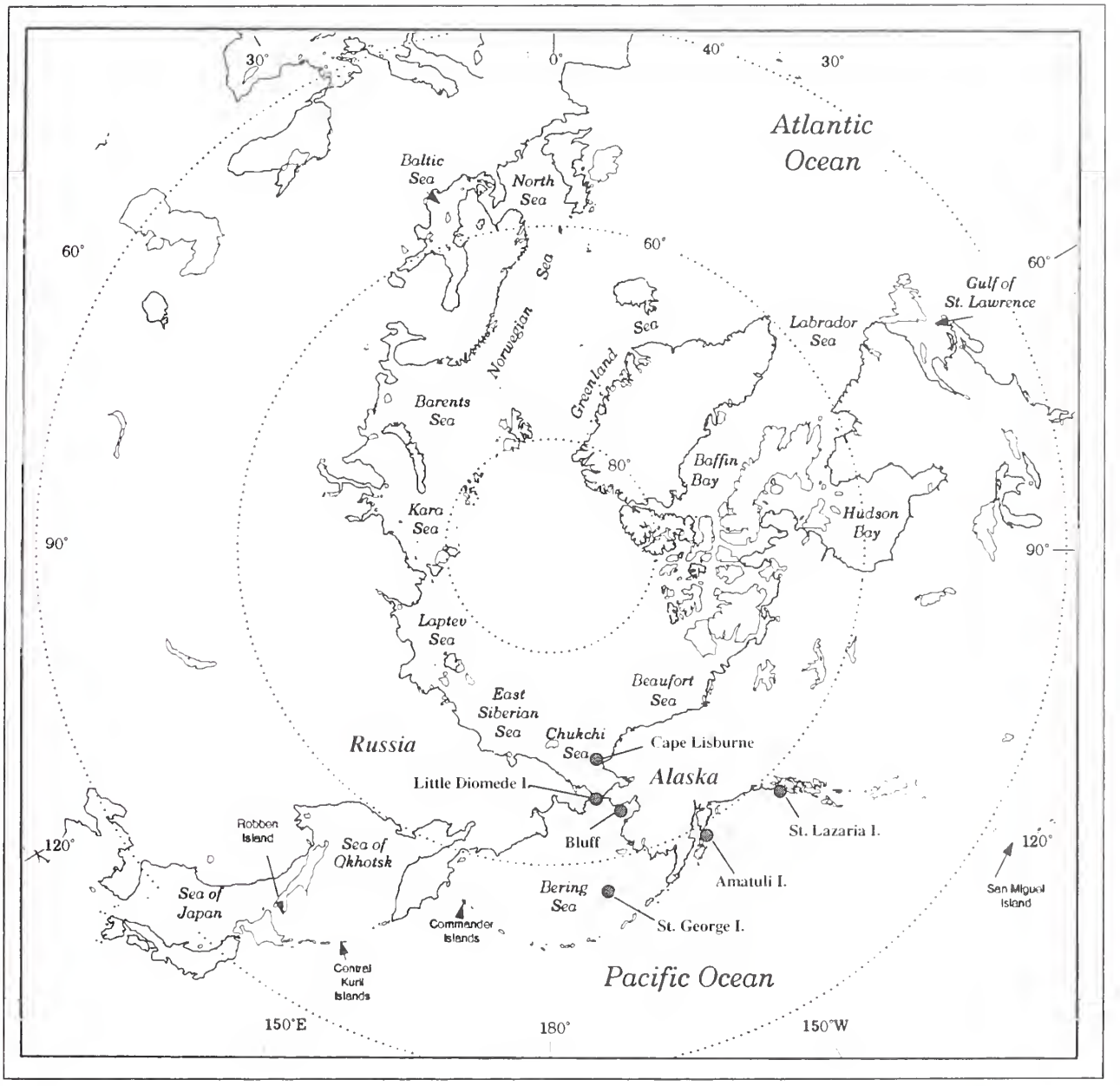

Figure 1. Locations of murre (*Uria* spp.) colonies selected for egg collections for STAMP (indicated by •): Cape Lisburne (Chukchi Sea), Little Diomede Island (Bering Strait), Bluff (northern Bering Sea), St. George Island (southern Bering Sea), Amatuli Island (central Gulf of Alaska, and St. Lazaria Island (Eastern Gulf of Alaska).

MANAGEMENT SYSTEM

The STAMP specimens are collected, transported, processed, and banked by collaborating researchers from the USGS/BRD, USFWS, and NIST. Eggs are collected by USFWS, Alaska Maritime NWR personnel during routine monitoring studies at the colonies. The egg processing laboratory is located at the USGS/BRD Alaska Biological Science Center, Anchorage, Alaska, where USGS/BRD personnel are responsible for removing the contents from the shells and shipping the frozen processed samples to the NBSB at the NIST Charleston Laboratory in Charleston, South Carolina. The remaining eggshells are shipped to the University of Alaska in Fairbanks, where they are archived at the University of Alaska Museum.

After arrival at the NIST Laboratory, the samples are checked, cataloged, and banked by NBSB personnel. The NBSB cataloging and archiving procedures are consistent with those employed by NIST for the banking of marine mammal tissues for the Marine Mammal Health and Stranding Response Program (MMHSRP) and Alaska Marine Mammal Tissue Archival Project (AMMTAP) (Becker et al., 1997)

A list of seabird colonies, individuals collecting the eggs, and individuals responsible for specimen processing and banking is shown in Table 1.

Table 1. Responsibilities for collecting, processing, and banking eggs from Alaskan seabird colonies for STAMP.

Activity	Location	Responsibility
Egg Collecting	Alaska Maritime NWR	USFWS
	Cape Lisburne, Chukchi Sea	D. Roseneau, G.V. Byrd
	Little Diomede I., Bering Strait	D. Irons
	Bluff, Northern Bering Sea	E. Murphy, G.V. Byrd
	St. George I., Southern Bering Sea	A. Sowls, G.V. Byrd
	Amatuli I., Central Gulf of Alaska	A. Kettle, G.V. Byrd
	St. Lazaria I., Eastern Gulf of Alaska	L. Slater, G.V. Byrd
Egg Processing	Alaska Biological Science Center	USGS Biological Resources Division
	Anchorage, Alaska	G. Weston York
		K. Simac
Eggshell Archiving	University of Alaska Museum	University of Alaska - Fairbanks
	Fairbanks, Alaska	K. Winker
Egg Content Banking	NBSB, NIST	NIST
	Charleston, South Carolina	R. Pugh

Requests for banked samples by researchers that are not associated with STAMP will be considered; however, release of the tissues to outside investigators will be contingent upon the approval of STAMP program managers from each of the collaborating agencies. Sample release will depend on a determination that a surplus of requested sample material exists beyond anticipated sampling or analytical needs of STAMP. Requests for samples must include a clear and concise statement of the proposed work and be consistent with the goal and purposes of STAMP. The following specific information should be included in the request for samples:

1. Name of principal investigator and affiliated research or academic organization
2. Specific sample and quantity desired
3. Explanation of proposed research to be conducted, including funding source
4. Justification for use of banked tissue
5. Research facility where analyses will be conducted
6. Analytical quality control procedures to be used and agreement to participate in NIST-directed interlaboratory comparison exercises for quality assurance (QA) purposes
7. Estimated date for completion of research, and schedule/date of subsequent reports
8. Agreement that all results and findings, including analytical data, be provided to the NBSB and STAMP (this includes a data submission schedule)
9. Agreement that credit and acknowledgment will be given to USGS, USFWS, NIST, and STAMP for use of banked tissues. The following acknowledgment will be inserted in all publications, abstracts, or presentations:

   The samples used in this study were provided by the National Biomonitoring Specimen Bank at the National Institute of Standards and Technology and the Seabird Tissue Archival and Monitoring Project (STAMP). STAMP is operated through the collaboration of the United States Geological Survey, the United States Fish and Wildlife Service, and the National Institute of Standards and Technology.

Shipping charges will be borne by the individual or institution requesting the samples. Additional charges to cover costs associated with sample preparation and distribution may also be required.

STAMP will publish annual tissue inventory reports. These reports will provide information on the status of seabird egg samples stored at the NBSB and the results of any chemical analyses that may have been conducted on them.

MATERIALS

The following materials are provided by the NBSB, unless noted otherwise.

Materials Required for Collecting Eggs and Shipping Them to the Processing Laboratory

- Data recording forms
- Talc-free vinyl gloves
- Teflon FEP (fluorinated ethylene propylene) bags for transporting individual eggs from field
- Tape for sealing Teflon bags
- Ziplock bags
- [1]Water resistant cardboard egg labels
- [1]Plastic containers (e.g., 5-gallon [18.9 L] paint buckets); one container per 5 to 10 eggs
- [1]O-ring sealing lids with two 12 mm holes per lid (for plastic container)
- [1]Packing material (e.g., styrofoam peanuts) for plastic container
- [1]Shipping labels

Materials Required for Processing Eggs

- Talc-free vinyl gloves
- 356 mm x 406 mm Teflon FEP bags or sheets to provide clean working surfaces
- Lint-free Tex-wipes
- 250 ml beaker to hold Teflon bags used to hold egg contents
- 76 mm x 203 mm Teflon FEP bags, ziplock bags, and cardboard labels for storing egg shells
- Cardboard shipping tubes
- Labels for exterior of shipping tubes
- Tape for securing exterior labels
- [1]1 L bottle containing high purity distilled (HP) water or best available water for rinsing samples
- [1]1 L bottle high grade ethanol (95 %) (EtOH) for rinsing instruments
- Micrometer, (0 to 25 mm) accurate to 0.01 mm
- Calipers, accurate to 0.01 mm
- Titanium blade knife, serrated
- Teflon spatula for emptying egg contents
- Lab coats (disposable)
- [1]Balance for weighing samples
- Surgical scissors and forceps
- Insulated gloves, safety glasses, and tongs for handling the liquid nitrogen (LN_2) and frozen samples
- LN_2 in container for freezing samples
- Dewar and lid

[1]Items provided by the processing laboratory or cooperating individuals.

Materials Required for Packaging Samples and Shipping Them to the Specimen Bank

- Dry shippers (LN_2-cooled) with protective shipping covers
- Shipping labels

Seabird Data Form

An example of the data recording form is provided on pages 13 to 14. This 2-page form must be filled out for each egg. Standard biological/environmental information is recorded on the first page (page 13). The boxes at the top of this page provide space for the NIST identification codes. These are assigned to the samples when they arrive at the NBSB. Other information to be recorded on this page is listed below:

1. Sample ID Number: Assign a 14-digit alphanumeric code to the egg and enter this code here using the following protocol: The first four letters (e.g., see below) should be used to identify the colony and the first two numbers should be used to identify the egg (e.g., 01, 02, 03). The next four letters should consist of the standard common name acronyms for the species the egg was collected from (e.g, COMU = common murre, TBMU = thick-billed murre, BLKI = black-legged kittiwake). The last four numbers should be used to identify the year that the egg was collected (e.g., 1999). Examples of the alphanumeric codes that should be used for the six study sites listed in Table 1 are shown here: Cape Lisburne (CLIS), e.g., "CLIS01TBMU1999"; Little Diomede I. (LIDI), e.g., "LIDI02TBMU2000"; Bluff (BLUF), e.g., "BLUF03COMU2001"; St. George I. (STGE), e.g, "STGE01BLKI1999"; St. Lazaria I. (STLA), e.g, "STLA02COMU2000"; East Amatuli I. (EAAM), e.g, "EAAM01BLKI2002". All questions regarding proper labeling, including assigning alphanumeric codes, should be directed to the NBSB contact listed on page 14.

2. Species: Enter the genus/species name.

3. Geographic Area: Enter the most common name of the general location where the egg is collected. Egg collectors should be as specific as possible (e.g., Cook Inlet, northeastern Chukchi Sea, southeastern Bering Sea, Bering Strait).

4. Lat. Long.: Record the latitude and longitude of the location where the egg is collected. Latitudes and longitudes should be reported to the nearest tenth of a minute, if possible.

5. Colony Name: Enter the common name of the seabird nesting colony where collection occurs (i.e, Cape Lisburne, St. George Island, East Amatuli Island, St. Lazaria Island).

6. Sample Source: Record any information pertinent to the agencies, persons, and research or management programs responsible for egg collections (e.g., USFWS seabird monitoring program, local subsistence harvest).

7. Site ID Name/Number: Enter a name or number code to identify the sections or subsections

of the colonies where the egg was collected. If numbers or names do not exist, they should be created and recorded in the field notes (e.g., West Arch, Spire Rock). This name/number code will help ensure that eggs will be obtained from the same parts of the colonies each sampling year.

8. Date and Time of Collection: Record this information as the day, month, year, and hour. The month should not be numbered, but should be written in abbreviated form (Jan, Feb, Mar, Apr, May, Jun, Jul, Aug, Sep, Oct, Nov, Dec) and the hour should be on a 24-hour basis (i.e., 6:00 PM is reported as 1800).

9. Method of Collection: Describe the method used to collect the egg (e.g., collected by hand using vinyl gloves, collected by local subsistence harvesters without gloves; see Egg Selection and Handling section).

10. Date and Time of Death: If body tissues (e.g., flesh, bone, feathers) are collected from dead seabirds for later analyses, record the time of death as day, month, year, and hour, if known, using the same format described in No. 8, above.

11. Weather Conditions: Note the general weather conditions (e.g., wind speeds and directions, temperature, precipitation) that were present when the egg was collected, along with anything that might be pertinent to potential contamination sources (e.g., radar sites that may have used or are using large transformers, hydraulic fluids, and other chemicals).

12. Field Storage Conditions: Report field storage conditions and the length of time that the egg was stored in the field before being shipped to the processing facility.

13. Date and Time Shipped from Study Site: Record the date and time that the egg was shipped from the field study site to the processing facility. This information should be recorded here as day, month, year, and hour using the same format described in No. 8, above.

14. Post-Processing Storage Conditions: Report the storage conditions at the processing facility and the length of time that the egg was stored at the site before being shipped to the NBSB.

15. Protocol: Indicate the protocol that was used to collect or process the eggs. If the standard approved protocol was used, state this. If modifications were made, described them in detail.

16. Comments: Enter any additional remarks pertinent to the collection, processing, or archiving of the egg.

17. Additional Samples: Note if other types of tissue samples were obtained that were related to the collected egg (e.g., body tissues from a dead member of the nesting pair). Information should include types of samples, their purpose, and names and locations of individuals receiving the samples. This space should be used to report if the egg was part of a multi-egg clutch that was collected that year (e.g., in the case of kittiwakes). If it was, the identification codes for the other eggs collected from the clutch should be noted here. This space should also be used to report if the egg was collected from a nest site that was known to have been

used as a sampling site in previous years (i.e., a fact suggesting that the egg may have come from the same breeding pair).

On the second page of the data recording form (page 14) enter data and information that are specific to the egg:

18. Sample ID Number: The same 14-digit alphanumeric code found on the first page of the form (Item No.1) is entered here in case the two pages become accidentally separated.

19. Date and Time of Sample Preparation: Enter the date and time (start time) that the egg was processed and placed in a container in preparation for LN_2 freezing. This information should be reported here as day, month, year, and hour using the format described in No. 8, above.

20. Processor: Enter the name of the individual(s) who processed the sample for LN_2 freezing and packed and shipped it to the NBSB.

21. Date and Time of Freezing: Enter the date and time at which the egg sample is frozen at - 80 °C or below in liquid nitrogen vapor. This information should be reported here as day, month, year, and hour using the format described in No. 8, above.

22. Date and Time of Shipping to NBSB: Enter the date and time that the sample was shipped to the NBSB. This information should be reported here as day, month, year, and hour using the format described in No. 8, above, and should reflect the time that the sample left the processing site.

23. Shipper: Enter the name of the person responsible for shipping the sample from the processing site to the NBSB.

24. Date and Time Received at NBSB: Enter the date and time that the sample was received at the NBSB. This information should be entered as day, month, year, and hour using the format described in No. 8, above.

25. Receiver: Enter the name of the individual receiving the sample at the NBSB.

26. Histological Samples: Record any information on any related samples that were collected for histological slides. This information should include: the Individual/Organization making the slides, the Final Destination of the slides, the kind of Tissues Sampled, and the method of sample preservation (if appropriate).

27. Sample Weight: Record the weight of the sample (whole egg and eggshell contents).

28. Egg Measurements: Record the length and breadth of the egg, and the eggshell thickness as per the laboratory protocol (pages 17 to 18).

29. Form Prepared by: Enter the name of the person that filled out the 2-page form.

All of the appropriate information for express shipping of the samples is provided in the lower right hand corner of the second page. This includes the full address of the NBSB, as well as the name and telephone number of the individual responsible for receiving the samples.

SEABIRD TISSUE ARCHIVAL MONITORING PROGRAM
NATIONAL BIOMONITORING SPECIMEN BANK
SEABIRD DATA FORM

I

Sample ID Number:_____ Species:_____

Geographic Area:_____ Lat.:_____ Long.:_____

Colony Name:_____ Sample Source:_____

Site ID Number/Name:_____

II

Sample Type: Liver Kidney Muscle Feather Egg Blood Other_____

Date and Time of Death (If Applicable):_____

Date and Time of Collection:_____ Method of Collection:_____

Weather Conditions:_____

Field Storage Conditions:_____

Pre-shipment Storage Conditions:_____

Date and Time Shipped from Study Site:_____

Protocol: Standard Modified (Please note modification below)

Comments:

Additional Samples:

Collected by:_____

III Sample ID Number:_____

Date and Time of Sample Preparation:_____ Processor:_____

Date and Time of Freezing (-80° C or below):_____

Date and Time of Shipping to NBSB:_____ Shipper:_____

Date and Time Received at NBSB:_____ Receiver:_____

IV Histological Samples:

Individual/Organization:_____

Final Destination:_____

Tissues Sampled:_____

V

Sample Weight:	A	B		
Liver			Egg (whole)	
Kidney			Egg (contents)	

Egg Measurements: Length:_____cm Breadth:_____cm

Eggshell Thickness:_____mm (mean of ten measurements)

VI

Form Prepared by:_____
(Print Name)

_____
(Signature)

A copy of this form should be shipped with samples to:

Rebecca Pugh
Research Biologist
NIST
219 Fort Johnson Road
Charleston, SC 29412
(843) 762-8647

METHODS

The seabird egg collection and banking protocol developed by STAMP for the NBSB is based on protocols used by the Canadian Wildlife Service Specimen Bank (Elliott, 1985) and Nordic Countries (Nordic Council of Ministers, 1995). However, some modifications have been made to reflect input from the USFWS Alaska Maritime NWR. This protocol is also consistent with those used by the NBSB for the AMMTAP and MMHSRP (Becker et al., 1988; 1991; 1999).

Taking into consideration field conditions during egg collections, the intent of the protocol is to obtain fresh, well-defined specimens uncontaminated by extraneous sources of trace elements and organic compounds, and to package and transport these samples as quickly as possible under conditions that eliminate or minimize specimen degradation prior to storage. Sample storage and inventory procedures follow those routinely performed at the NBSB, including storage under liquid nitrogen vapor at -150 °C. A generalized scheme for the protocol used by STAMP is presented in Figure 2.

EGG COLLECTION FIELD PROCEDURES

Selecting and Handling Eggs

Ten eggs are collected from each target species at the designated nesting colonies. To avoid obtaining eggs with large embryos, collections should be made as early as possible during the laying period and preferably no more than 14 days after the first eggs are laid at the colonies. Collections should be made under conditions that will minimize sample degradation prior to shipping and storage. For species that lay single eggs (e.g., common and thick-billed murres), eggs suspected of being replacement eggs should not be collected. For species that normally lay multiple eggs (e.g., kittiwakes), complete clutches will be collected whenever they contain more than one egg. The following procedures are used to handle the eggs:

1. Remove eggs from the nest using talc-free vinyl gloves. Immediately, place each egg in individual Teflon bags. Seal each Teflon bag with clean non-contaminating tape and place the sealed bag containing the egg in two individual ziplock bags (i.e., one inside the other).

2. Identify and label eggs individually. Fourteen-digit alphanumeric identification codes (refer to page 9) should be hand-printed on water resistant paper labels cut from "Rite in the Rain" field notebook paper and placed inside the outer-most ziplock bag (i.e., the label should reside between the two ziplock bags containing the Teflon-bagged egg). The labels should be printed using soft to medium pencils (No. 2 or 3) or indelible ink (pencil is preferred because it is not as vulnerable to alcohol and other solvents as is "permanent" ink).

3. Enter the identification codes in the "Sample ID Number" space of the NBSB Seabird Data Form (refer to pages 9 and 10). This form should be filled out completely as soon as possible after the eggs have been collected (storage and shipping times should be added to the forms just before the samples are shipped to the processing laboratory).

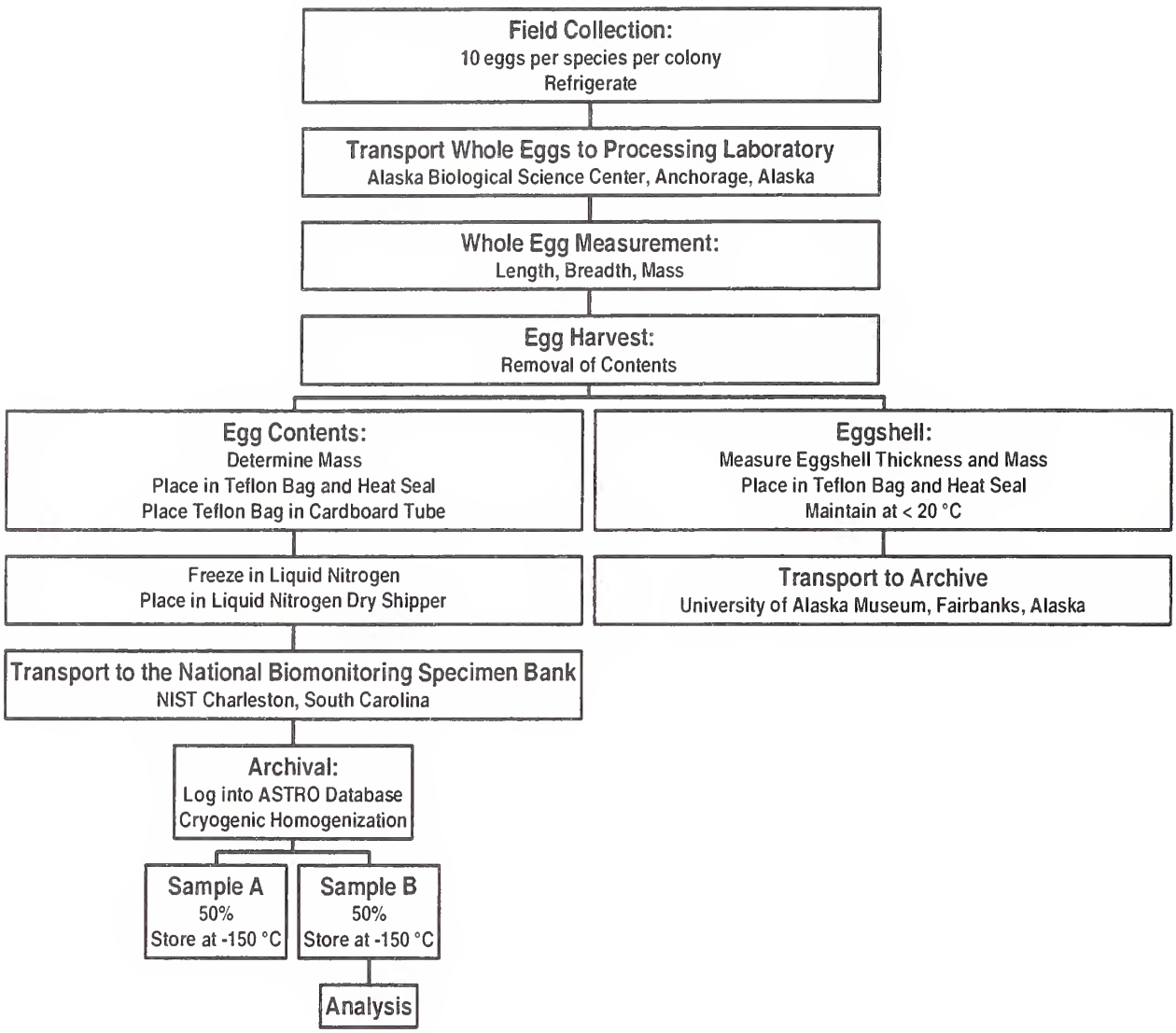

Figure 2. Generalized scheme for the egg collection and banking protocol used by STAMP.

4. Refrigerate the eggs or keep them cool until they are shipped to the processing laboratory.

Transporting Eggs to the Processing Laboratory

1. Carefully pack individually bagged eggs from the same colony in solid plastic containers with enough shock- resistant packing material (e.g., styrofoam peanuts, crumpled paper, or dry grass) to protect them during shipping. The plastic containers that are provided by the processing laboratory or cooperating individuals (Table 1), consist of new, clean 5-gallon (18.9 L) paint buckets with o-ring sealing lids or double-walled plastic cooler-type totes. If buckets are used, they will have two approximately two 12 mm holes drilled in the lids to allow for air pressure changes during shipment by aircraft. Two buckets (5 eggs per container) or one tote (10 eggs per container) will be required to safely transport 10 eggs.

2. Keep the packaged eggs as cool as possible (ideally at about 4 °C) until they are shipped. If an egg cracks during packaging and freezer facilities are available (e.g., Cape Lisburne), it can be salvaged by freezing it as soon as possible and shipping it frozen to the processing laboratory. Do not discard it if it can not be frozen, but keep it in the shipment. The decision to retain such an egg in the collection will be made by the processing laboratory personnel based on the condition of the egg after shipment.

3. Ship packaged eggs and Seabird Data Forms to:

USGS/BRD
Alaska Biological Science Center
1011 East Tudor Road
Anchorage, Alaska 99503
Attn: Geoff York
(907) 786-3928

Egg Processing Laboratory Procedures

The seabird egg processing procedures are based on USFWS, Portland Field Office, SOP:F-003 protocol. Egg processing should take place within a laboratory under the cleanest conditions available. At a minimum, processing should be done in an enclosed covered area free of obvious sources of potential contamination such as cigarette smoke, fuel oil fumes and smoke, and laboratory chemicals such as formaldehyde. The work area should be thoroughly cleaned to remove dust and other residues, and work surfaces covered with Teflon sheeting before processing begins. Processing the eggs consists of six steps: measuring whole eggs, collecting egg contents ("egg harvest"), measuring eggshell thickness, archiving eggshells, packaging and freezing egg contents, and shipping frozen egg contents (egg sample) to the NBSB.

Measuring Whole Eggs

1. Wear talc-free vinyl gloves while handling the eggs and change gloves between each egg.

2. If possible, "candle" the eggs by placing each in front of a bright light source to determine the approximate stage of embryo development and to determine if cracks are present in the shell.

3. Store the eggs in a refrigerator if they cannot be processed immediately after they arrive at the laboratory. DO NOT FREEZE the eggs unless they are already cracked because freezing whole unbroken eggs will crack the shells.

4. Clean each intact egg with a lint-free cloth and HPLC-grade distilled-deionized water that is at or near the temperature of the egg. Change cloths between eggs.

5. Record any unusual features (e.g., cracked, dented, pitted, or discolored shells; unusual sizes or shapes).

6. Weigh (g) each whole egg and record this value on the Seabird Data Form.

7. Measure the length (cm) and breadth (cm) of each egg with calipers at their greatest, longest, and widest points and enter these measurements on the Seabird Data Form. To obtain accurate measurements of length, make sure that the caliper jaws are parallel to the longitudinal axis of the egg. For breadth, hold the jaws perpendicular to the longitudinal axis of the egg.

8. Carefully rinse each egg again with HPLC-grade water following the measurements and allow them to air dry.

Collecting Egg Contents (Egg Harvest)

1. Wear talc-free vinyl gloves while handling the eggs and change gloves between eggs.

2. Remove the contents of each egg using the following procedure:
   a. Line a clean 250 mL beaker with a clean 76 mm x 203 mm Teflon bag. Place it on a scale and tare (g).
   b. Place the egg lengthwise on a clean Teflon sheet. Using a knife with a titanium blade, gently score around the egg (about its equator) using the serrated titanium blade knife. Apply gentle, steady pressure and make several rotations around the egg. Once through the shell, insert the tip of the knife blade to cut the membrane and begin separating the two halves. Carefully cut 1/2 to 2/3 the distance around the egg. Invert the egg while pulling apart the shell halves and pour the contents into the opened Teflon bag.
   c. If necessary use a clean Teflon spatula to scrape any remaining contents into the jar (BE CAREFUL not to tear the shell membrane when using the spatula).
   d. Rinse the spatula with HPLC-grade water followed by 95 % EtOH after each use and allow to air dry.
   e. Weigh the beaker containing the Teflon bag with egg contents and record the tared weight under EGG (contents) on the Seabird Data Form.
   f. If the egg contains an embryo, carefully remove the embryo from the separated shell halves. Embryo and egg contents, if any, are stored separately. Length of the embryo

(crown - tail) is recorded.

g. Heat-seal the Teflon bag. Place it in another Teflon bag along with a "Rite in the Rain" field notebook paper label containing the egg contents, mass, and identifying sample number, and heat-seal the second bag.

Measuring Eggshell Thickness

1. Wear talc-free vinyl gloves while handling the eggshells and change gloves between eggshells.

2. Rinse shell halves with HPLC-grade water, taking care not to tear the membrane. Shell halves should be stored in a cool dry place for a minimum of 30 days to attain a constant weight prior to taking additional measurements.

3. Weigh dried eggshells and record this measurement on the Seabird Data Form.

4. Measure eggshell thickness with a micrometer having rounded contacts. The micrometer contacts should be rinsed with HPLC-grade water followed by a EtOH rinse between the eggshells of the individual eggs. The measurements should be made at five separate equidistant points around the circumference of both shell halves and 0.5 cm from the cut edge that separated each half.

5. Record the mean of the 10 measurements on the Seabird Data Form for each egg. If the membrane is absent or has separated from the shell, note this fact in the Comments section on the form.

Archiving Eggshells

1. Place the eggshells of each egg (the two halves) in a small Teflon bag and heat-seal it. Next, place the Teflon bag and a "Rite in the Rain" field notebook paper label containing the sample number, eggshell thickness, and mass data inside a small ziplock bag. Maintain the bagged shells at <20 °C until they are ready to be shipped to the University of Alaska Museum in Fairbanks, Alaska, where they will be permanently archived.

2. Package the eggshell samples and a copy of their respective Seabird Data Forms in a durable container filled with appropriate shock-resistant material and ship to:

University of Alaska Museum
907 Yukon Drive
Fairbanks, AK 99775-6960
Attn: Kevin Winker
(907) 474-7027

Packaging and Freezing Egg Contents Samples

1. Record the egg contents sample number and mass on the label designed for the cardboard shipping tube. Place the double-bagged egg contents sample in an individual cardboard tube and affix the sample label to the outer cardboard tube with the wide clear tape.

2. Freeze the labeled cardboard tubes containing the samples by immersing them in LN_2 for 10 minutes. If LN_2 is not immediately available, freeze the samples in a laboratory freezer capable of maintaining a temperature of -80 °C or lower. (Special Note: LN_2 should not be stored in sealed containers. Personnel handling LN_2 should wear boots, cuffless trousers, non-absorbent aprons, loose insulating gloves, and safety glasses).

Shipping Frozen Egg Samples

Frozen samples are transported in liquid nitrogen vapor dry shippers that are provided by the NBSB. These shipping containers will keep specimens frozen for 2 days to 5 days, depending on proper "charging" and proper handling of the containers. To properly charge a dry shipper, fill the container ¾ of the way with liquid nitrogen and let it stand for 24 hours. Refill the shipper ½ of the way and let it stand for approximately 3 hours to 4 hours then pour off the excess LN_2. This is required to fully saturate the absorbent material that is located in the lining of the container. The dry shipper is now ready to receive samples.

1. Place the frozen cardboard tubes containing the samples in the dry shipper.

2. Double check the Seabird Data Forms for completeness and accuracy. Any deviations or modifications of the protocol must be noted on the form.

3. Place a copy of the completed forms in a ziplock bag or manila envelope. Place this bag or envelope between the protective shipping cover and the dry shipper; close and latch the shipping cover.

4. Ship the dry shipper(s) with the samples to the NBSB at NIST in Charleston, South Carolina, within 48 hours or as soon as possible after sample collection using 24-hour express package service to:

   NIST
   219 Fort Johnson Road
   Charleston, South Carolina 29412
   Attn: Rebecca Pugh
   (843) 762-8647

The dry shipper(s) must not contain excess LN_2 when shipped, i.e., all LN_2 must be absorbed in the container lining. Maximum holding time for the shippers is 10 days to12 days. Shipping costs will be paid by NIST. Never ship samples late in the week, i.e., Thursday, or Friday, or before holidays, unless special arrangements have been made with the shipping service and NBSB/NIST laboratory personnel.

5. The NIST/NBSB personnel should be notified by telephone as soon as possible after the frozen samples are shipped:

Rebecca Pugh (843) 762-8647 or
Paul Becker (843) 762-8503

Egg Sample Archival Procedures

After the samples have been received at NIST, they are transported to the NBSB facility. The dry shippers are unpacked and the egg contents samples are inspected for packaging problems and for proper temperatures. The Seabird Data Forms and samples are compared to ensure that they are complete and correspond with one another. After the samples and forms have been checked the samples are assigned an NBSB number, stored in liquid nitrogen vapor freezers, and logged into the Archival Specimen Tracking Retrieval Operations (ASTRO) database and Paradox database.

Before a sample is moved to its permanent, long-term freezer storage location, it is cryogenically homogenized using a cryogenic procedure designed to reduce the likelihood of changes in sample composition due to thawing and refreezing (Zeisler et al., 1983). The resulting homogenate is then divided into several aliquots depending on the species (e.g., B001, B002, B003; contents of common and thick-billed murre eggs average about 80 g and one egg from these species provides about 16 5-g aliquots). Some of the aliquots from each sample are stored in different freezers to provide additional long-term security. Half of the aliquots from each sample are placed in long-term storage and the other half are available for immediate and near-term analyses.

When a sample is moved to the long-term freezer storage location, a storage form is filled out documenting the archiving process and the specific storage location of the sample. This information is also entered into the computerized NBSB Paradox and ASTRO databases.

The samples will remain in the liquid nitrogen vapor storage freezers at approximately -150 °C until they are requested and released for future analysis. Detailed information describing each sample and its source is recorded and maintained in the NBSB Paradox database. Hard copies of this information will also be kept in the NBSB files, and upon request, will be made available to interested parties.

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