

NISTIR 7678

**SEABIRD TISSUE ARCHIVAL AND MONITORING
PROJECT (STAMP): Project Overview, and Updated
Protocols for Collecting, Processing and Banking
Seabird Eggs**



**Lauren B. Rust¹
Rebecca S. Pugh¹
Amanda J. Moors¹
Stacy S. Vander Pol¹
Paul R. Becker¹
And David G. Roseneau²**



¹U.S. Department of Commerce
National Institute of Standards and Technology
Chemical Science and Technology Laboratory
Analytical Chemistry Division
Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, SC 29412



²U.S. Department of the Interior
²U.S. Fish and Wildlife Service
Alaska Maritime National Wildlife Refuge
95 Sterling Hwy. #1
Homer, AK 99603

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ACKNOWLEDGEMENTS

The following villages, organizations, and agencies are acknowledged for their egg collection, processing and funding contributions to STAMP: Bureau of Indian Affairs, Alaska Regional Subsistence Branch, Anchorage; U.S. Fish and Wildlife Service, Alaska Maritime National Wildlife Refuge, Homer; U.S. Fish and Wildlife Service, Office of Migratory Bird Management, Anchorage; U.S. Fish and Wildlife Service, Marine Mammals Management, Anchorage; U.S. Geological Survey Biological Resources Division, Alaska Science Center, Anchorage; U.S. Geological Survey Biological Resources Division, Western Regional Office, Seattle; Minerals Management Service, Anchorage; Alaska Department of Fish and Game, Dillingham; University of Alaska; University of Washington, Seattle; Oregon State University; World Wildlife Fund and Perdue University; Alaska Sea Otter & Sea Lion Commission, Anchorage; Alaska Native Science Commission, Anchorage; Transboundary Ecologic LLC, Anchorage; State Nature Biosphere Preserve ‘Komandorskiy’, Commander Islands, Russian Far East; Village of Point Hope, Point Hope; Maniilaq Association, Kotzebue; Native Village of Deering, Deering; Natural Resources Division, Kawerak, Inc., Nome; Bering Straits Native Corporation, Nome; Native Village of Shaktoolik, Shaktoolik; Native Village of Savoonga, St. Lawrence Island; Paimiut Tribal Council, Village of Paimiut, Hooper Bay; Native Village of Mekoryuk, Mekoryuk; St. George Traditional Council, St. George Island; St. George Island; Togiak Traditional Council Environmental Program, Togiak; Native Village of Nanwalek, Nanwalek); Seldovia Village Tribe, Seldovia; Tatitlek Village IRA Council, Tatitlek; Sitka Tribe of Alaska, Sitka; and the residents of the Native Village of Diomedes, Little Diomedes Island.

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.

ABSTRACT

The Seabird Tissue and Archival Monitoring Project (STAMP) is a collaborative effort by the U.S. Fish and Wildlife Service (USFWS), the Alaska Maritime National Wildlife Refuge (AMNWR), and the National Institute of Standards and Technology (NIST) to monitor long-term (decadal) trends in persistent, potentially harmful bioaccumulative contaminants in the Bearing and Chukchi seas and Gulf of Alaska using seabird tissues, i.e., primarily eggs (York *et al.* 2001). The cryogenic banking of eggs for retrospective analysis is a major component of STAMP. Before STAMP, data and information on contaminants in Alaskan seabirds were very limited. Analysis of banked eggs is helping to develop baseline data on contaminants in major Alaskan Marine Regions. To date, approximately 14,000 egg sample aliquots representing over 1,453 eggs from 44 sampling sites have been banked at NIST's Marine Environmental Specimen Bank in Charleston, SC. These data are also significant in providing information on contaminants in a human subsistence resource that is prominent in many rural Alaskan diets. This report is to provide an up-to-date description of the STAMP protocols for collecting, processing and banking seabird eggs.

SEABIRD TISSUE ARCHIVAL AND MONITORING PROJECT:

Project Overview, and Updated Protocols for Collecting, Processing and Banking Seabird Eggs

Introduction

The Seabird Tissue and Archival Monitoring Project (STAMP) is a collaborative effort by the U.S. Fish and Wildlife Service Alaska Maritime National Wildlife Refuge (USFWS-AMNWR), the National Institute of Standards and Technology (NIST) and the Bureau of Indian Affairs Alaska Region Subsistence Branch (BIA-ARSB) to monitor long-term (decadal) trends in persistent, potentially harmful bioaccumulative contaminants in the Bearing and Chukchi seas and Gulf of Alaska using seabird tissues, i.e., primarily eggs (York *et al.* 2001). This approach is similar to that of the Alaska Marine Mammal Tissue Archival Project (AMMTAP), started in 1987 by NIST and the National Oceanic and Atmospheric Administration (NOAA) as part of the Outer Continental Shelf Environmental Assessment Program sponsored by the Minerals Management Service. It was developed to cryogenically bank tissues collected from marine mammals harvested by Alaskan subsistence hunters (Becker *et al.* 1993). Specimens from both AMMTAP and STAMP are banked in the Marine Environmental Specimen Bank (Marine ESB) at the Hollings Marine Laboratory, Charleston, SC.

Northern seabirds feed near the top of the food chain and have the potential for accumulating anthropogenic contaminants. Before STAMP, data and information on contaminants in Alaskan seabirds were very limited. Analysis of banked eggs is helping to develop baseline data on contaminants in major Alaskan Marine Regions, monitor long-term trends in these potentially harmful substances, and conduct retrospective studies. These data are also significant in providing information on contaminants in a human subsistence resource that is prominent in many rural Alaskan diets.

This report provides up-to-date description of the STAMP protocols for collecting, processing and banking seabird eggs (York *et al.* 2001). For recent findings on contaminant levels and the geographic differences that have been found in them see Roseneau *et al.* (2008) and Vander Pol *et al.* (2009).

STAMP Project History

STAMP was initiated in 1998 when protocols for collecting, processing, and banking seabird eggs were first developed by USFWS, the U.S. Geological Survey Biological Resources Division (USGS-BRD), and NIST with funding support from the U.S. Department of Interior's Minerals Management Service (MMS). The project was designed to track trends in environmental quality by 1) collecting Alaskan seabird eggs using standardized protocols so to minimize contamination, 2) processing and banking the contents under conditions that ensure chemical stability during long-term (decadal) storage, and 3) analyzing aliquots of the stored

material to determine current baseline levels of persistent bioaccumulative contaminants (e.g., chlorinated pesticides, polychlorinated biphenyls [PCBs], brominated flame retardants [PBDEs], butyltin compounds [organotins], and mercury concentrations and isotopes (Vander Pol *et al.* 2009). Methods and protocols were developed for STAMP in 1998 (York *et al.* 2001), and specimen collections began in July 1999. The USGS-BRD stopped participating in the project in 2007 and AMNWR took over all of the processing responsibilities in addition to their original duties of collecting eggs. *Note: eggs were not obtained in 2007 because AMNWR needed to obtain additional equipment, supplies, and lab space, and lacked the time to process them.*

Since its beginning, STAMP has involved many Federal and State of Alaska agencies, regional and local organizations, and individuals from local Alaskan villages that have an interest in the project. Funding support has come from MMS, USGS-BRD, USFWS AMNWR, Bureau of Indian Affairs Alaska Region Subsistence Branch (BIA-ARSB), and the North Pacific Research Board (NPRB), with in-kind contributions from USFWS, USGS-BRD, and NIST. A major expansion began in 2005 when the NPRB provided three years of funding to STAMP through NPRB Project 0543, “Expanding the Seabird Tissue Archival Project (STAMP) in the North Pacific: Geographic Patterns in Contaminant Residues in Seabird Eggs used in Rural Subsistence Diets.” The results of this work were published in Roseneau *et al.* 2008 and Vander Pol *et al.* 2009. In 2008, additional support was provided by the NPRB to STAMP for a joint study by USFWS AMNWR, NIST, and Kawerak Inc. in Nome, Alaska, to investigate “Mercury levels in murre and gull eggs harvested for food in the Norton Sound region and potential sources of contamination” (NPRB Project 0822).

STAMP has received recognition from the 2007-2008 International Polar Year (IPY) Program and as a contributor to both the Arctic Monitoring and Assessment Programme (AMAP) and the Conservation of Arctic Fauna and Flora (CAFF), both working groups of the International Arctic Council. In 2008, STAMP was designated as an AMAP/CAFF Coordinated Monitoring Effort (CME), the goals of which are to: (1) form a more complete picture of the overall state of the Arctic ecosystems, and their extent of structural integrity, resiliency, and sustainability; (2) identify and/or quantify stressors affecting sustainability of Arctic ecosystems, and therefore the Arctic’s living resources; and (3) seek efficiencies of operation as directed by Senior Arctic Officials (AMAP/CAFF 2007).

Numbers of seabird colonies, colony locations, and species targeted by STAMP has evolved since 1999. In 1999, STAMP began collecting common and thick-billed murre eggs, and in 2001, the program was expanded to include black-legged kittiwake eggs. In 2004, glaucous gull (*Larus hyperboreus*) and glaucous-winged gull (*L. glaucescens*) eggs were added to the project due to the request by and funding contributions from the BIA-ARSB. Gulls were included because their eggs are an important subsistence food in many coastal regions of Alaska. Also, these two species of gulls feed on a wide variety of fish and invertebrates and also scavenge on marine mammal carcasses and refuse in community dumps and land-fills where they have opportunities to be exposed to relatively high levels of anthropogenic contaminants (Vander Pol *et al.* 2009). Currently, STAMP criteria for selecting study sites and species include the overall geographic distribution and regional importance of the nesting colonies; their locations relative to onshore, nearshore, and offshore environments; the logistical feasibility of collecting eggs at them; the trophic position and foraging strategies of the birds; the foraging habitats used by the

birds; and the use of the birds' eggs in rural subsistence diets. (*Note: STAMP temporarily stopped collecting kittiwake eggs in 2005 to concentrate efforts on completing the murre and gull work*).

Use of Seabird Eggs for Monitoring

Little was known about contaminants in Alaskan seabirds before the advent of STAMP. More than 95% of the seabirds breeding in the continental United States nest at colonies in the Bering and Chukchi seas and Gulf of Alaska (USFWS 1992). The Bering and Chukchi seas are not only under the influence of airborne contaminants transported across the pole from Eastern Europe, but also from atmospheric and oceanic transport from Asia across the Pacific Ocean. Overall contaminant patterns and levels in Alaskan seabirds are probably influenced by atmospheric transport of contaminants from Asia eastward and northward into the Gulf of Alaska, oceanic transport from Asia via the eastward flowing North Pacific Current, and the transport of substances into the Bering and Chukchi seas from the Northern Gulf of Alaska via the westward moving Alaskan Stream and Alaskan Coastal Current (Stabeno *et al.* 1999; Li *et al.* 2002). Existing and former military installations may also contribute to local point source "hot spot" pollution patterns in Alaska (Vander Pol *et al.* 2003).

During its early planning stages, STAMP participants identified the seabird egg as the first tissue of choice for study by the project. There is a relatively long history of using bird eggs for environmental monitoring and for investigating the health status of bird populations (Vander Pol *et al.* 2003). 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 (Hatch *et al.* 1993).

Eggs are particularly useful for the temporal and spatial monitoring of persistent organic pollutants (e.g., polychlorinated biphenyls (PCBs), chlorinated pesticides, dioxins) and mercury (York *et al.* 2001). POPs are long-lived organic compounds that bioaccumulate and have potential harmful effects. Examples include polychlorinated biphenyls (PCBs), chlorinated pesticides, (i.e., DDT, chlordane, toxaphene, hexachlorobenzene [HCB], hexachlorocyclohexane [HCH], and dieldrin), dioxins, and furans. Although most of these substances are restricted or banned in most developed countries, they are still manufactured for export and remain in use in many developing countries. POPs can be found in the environment many thousands of kilometers from their points of release because they can be transported through the atmosphere as gases and aerosols (Vander Pol *et al.* 2003). Mercury is another contaminant that is atmospherically transported to remote regions of the globe. Bioavailability of mercury depends on microbial conversion of inorganic mercury to methylmercury, which is fat-soluble, bioaccumulates much like POPs, and is toxic. Although most of the mercury emission to the atmosphere is natural (e.g., volcanic emissions), the greatest source of anthropogenic emission is fossil fuel combustion (particularly coal) and waste incineration (AMAP 2002).

Currently, STAMP is focused on collecting, banking, and analyzing murre and gull eggs because they are harvested in many rural Alaskan coastal communities where they play important roles in local diets (Iknokinok and Georgette 1997). Common and thick-billed murre eggs are of particular value for monitoring long-term trends in environmental quality. Both of these

species feed at upper trophic levels and have the potential to accumulate and store contaminants in relatively large amounts (Springer *et al.* 1984, 1986, 1987; Roseneau *et al.* 2000). However, some resource partitioning occurs between the two species that is probably reflected by species differences in contaminant loads. Both common and thick-billed murres feed on Pacific sand lance (*Ammodytes hexapterus*), capelin (*Mallotus villosus*), and small cod (e.g., walleye pollock, (*Theragra chalcogramma*); Pacific cod, (*Gadus macrocephalus*); saffron cod, (*Eleginus gracilis*); and Arctic cod, (*Boreogadus saida*). However, thick-billed murres tend to forage farther from shore and at greater depths than common murres and they also feed on a variety of benthic species, including invertebrates that they catch on or near the bottom. In contrast, common murres usually forage closer to shore at shallower depths on small mid-water fishes (Swartz 1966, 1967; Springer *et al.* 1984, 1986, 1987; Roseneau *et al.* 2000; Vander Pol *et al.* 2009).

Initial data from murre eggs obtained at colonies associated with deep oceanic habitats in the Bering and Chukchi seas and Gulf of Alaska suggested that there were north-south and east-west geographic gradients in contaminant levels (Christopher *et al.* 2002; Vander Pol *et al.* 2003; Vander Pol *et al.* 2004; Day *et al.* 2006). In 2005, the North Pacific Research Board (NPRB) provided funding support to STAMP to verify the presence of these patterns, by adding more coastal and mainland colonies to the project and analyzing subsets of these egg collections (see Figure 4 and Appendix 3). This work confirmed that there were north-south and east-west gradients in contaminant levels in the Bering and Chukchi seas and Gulf of Alaska, but it also found relatively high mercury levels in murre eggs from Norton Sound that were similar to those initially reported by STAMP for murre colonies in southeastern Alaska (Roseneau *et al.* 2008, Vander Pol *et al.* 2009).

In 2003, STAMP expanded the murre component of the project and created a new gull module because of the importance of their eggs in rural subsistence diets. The gull component was initiated in May 2004, when the BIA-ARSB began funding local village residents to collect eggs at several coastal gull colonies. The BIA had become interested in STAMP when a number of rural organizations indicated that they wanted more information on contaminants in the eggs of these birds (e.g., Point Hope IRA Council Parks and Wildlife, Point Hope; Maniilaq Association Subsistence Division, Kotzebue; Natural Resources Division, Kawerak Inc., Nome; Native Village of Mekoryuk; St. George Traditional Council Island Sentinel Program; Togiak Traditional Council Environmental Program; Seldovia Village Tribe Environmental Program; Tatitlek IRA Council; and Sitka Tribe of Alaska) (Roseneau *et al.* 2008, Vander Pol *et al.* 2009)

Specimen Banking for STAMP

The contents of seabird eggs collected by STAMP are stored cryogenically (-150 °C) at the Marine Environmental Specimen Bank (Marine ESB) at the Hollings Marine Laboratory, in Charleston, South Carolina. The Marine ESB is a cryogenic facility that stores well-documented environmental specimens collected as part of ongoing research and monitoring programs conducted in marine and coastal environments. Aliquots of the STAMP egg specimens provide the samples needed to determine current baseline levels of contaminants. Remaining portions of these specimens plus unanalyzed egg specimens are maintained in the Marine ESB for future retrospective analyses.

Cryogenically banking egg contents for retrospective analysis is an important component of STAMP. The long-term storage of carefully selected, representative samples in a specimen bank is an important complement to real-time contaminant analysis in an environmental monitoring program. For example, the Canadian Wildlife Service successfully documented temporal changes in PCBs and pesticides in the Great Lakes region by analyzing herring gull (*L. argentatus*) eggs that were collected and banked as part of its Wildlife Toxicology Program (Mineau *et al.* 1984; Elliott 1985; Wakeford and Kasserra 1997; Norstrom and Hebert 2006). Also, the decrease in PCBs and chlorinated pesticides and the increase in mercury shown by Braune *et al.* (2001) for the Prince Leopold Island area are based on a reanalysis of archived samples of seabird eggs collected between 1975 and 1998. Eggs collected, processed, and banked by STAMP using standardized protocols will allow researchers to identify and study new analytes of interest in future years and they will also allow analytical techniques to be compared over time. These comparisons will provide valuable information on the accuracy and effectiveness of current techniques versus new, more sophisticated methods that may be developed in upcoming years.

To date, approximately 1,453 eggs representing over 14,000 egg sample aliquots have been banked in the Marine ESB as a part of the STAMP.

Management System

Eggs are collected for STAMP by participating agencies (e.g., USFWS, USGS-BRD, BIA-ARSB), rural organizations (e.g., tribal and village councils) and rural residents (e.g., subsistence hunters), and air-freighted to Homer, Alaska where they are processed by AMNWR personnel at the AMNWR laboratory. Collection and processing procedures are described in detail in the Methods and Materials section of this report.

Processing procedures result in (1) frozen egg contents (egg specimens) that are expressed shipped frozen along with the corresponding seabird data forms to the Marine ESB for baseline analyses and banking, and (2) dried eggshells that are shipped to the University of Alaska Museum of the North in Fairbanks, Alaska, for long-term storage.

After the Marine ESB receives the frozen specimens and seabird data forms, they are checked for potential problems (lost labels, thawed specimens, lost or incomplete data forms, etc.) and then logged into the Marine ESB specimen tracking database. The samples are kept frozen until all cataloging procedures are complete and then they are put in Liquid Nitrogen (LN₂) vapor freezers for long-term (decadal) storage at -150 °C. After the samples have been formally entered into long-term storage and the data base tracking system, aliquots are available for analyses. Cataloging and banking procedures for STAMP specimens are the same as those used by the Marine ESB for other programs, including the Marine Mammal Health and Stranding Response Program (MMHSRP), the Alaska Marine Mammal Tissue Archival Project (AMMTAP), the Mussel Watch Program, and the Peregrine Falcon Monitoring Program. Details on these procedures have been described elsewhere (Pugh *et al.* 2007).

Organizations and individuals responsible for collecting, processing, and banking eggs are listed in Table 1.

Table 1: Organizations and individuals responsible for collecting, processing and banking eggs for STAMP.

Activity	Location	Responsible Party
Egg Collecting	<u>US Fish and Wildlife Service</u>	<u>Alaska Maritime NWR</u> D. Roseneau, G. Thomson J. Williams, A. Kettle L. Slater
	<u>US Fish and Wildlife Service</u>	<u>Selawik NWR</u> Refuge Biologist
	<u>US Fish and Wildlife Service</u>	<u>Togiak NWR</u> Refuge Biologist
	<u>US Fish and Wildlife Service</u>	<u>Office of Migratory Birds</u> D. Irons
	<u>Alaska Biological Science Center</u>	<u>USGS Biological Resources Division</u> S. Hatch
	<u>University of Alaska</u>	<u>Institute of Arctic Biology</u> E. Murphy
	<u>University of Washington</u>	<u>Aquatic & Fishery Sciences</u> J. Parrish
	<u>Kawerak Inc.</u>	<u>Subsistence Resources Program</u> S. Tahbone, E. Trigg
Egg Processing (1999-2006)	<u>Alaska Biological Science Center</u> Anchorage, Alaska	<u>USGS Biological Resources Division</u> K. Simac, G. York
Egg Processing (2006-2009)	<u>Alaska Maritime NWR</u> Homer, Alaska	<u>USFWS</u> D. Roseneau
Eggshell Archiving	<u>University of Alaska Museum</u> Fairbanks, Alaska	<u>University of Alaska - Fairbanks</u> K. Winker
Egg Content Banking	<u>Marine Environmental Specimen Bank</u> Charleston, South Carolina	<u>NIST</u> R. Pugh, A. Moors, L. Rust

Access Policy

Researchers not associated with STAMP can request aliquots of banked specimens; however, release of these materials to outside investigators requires approval from the project managers of the collaborating agencies and is contingent upon the determination that enough material will be left in the Marine ESB to allow STAMP to conduct retrospective analyses and monitor long-term trends. Requests for samples must include clear, concise descriptions of the proposed work and coincide with STAMP's long-term goals. The following information must be supplied with the sample requests:

1. The name(s) of the principal investigator(s) and affiliated research or academic institutions.
2. The specific sample material requested and quantity desired.
3. A brief description of the proposed research and its funding source.
4. A brief justification for use of the banked tissues.
5. The name of the research facility where the analytical work will be conducted.
6. A brief description of the analytical quality control procedures that will be used and a signed agreement stating that the research facility will participate in NIST directed interlaboratory comparison exercises for quality assurance (QA) purposes.
7. The estimated date for completion of research, and preparing/submitting subsequent reports and publications.
8. A signed agreement stating that copies of all results and findings, including analytical data, will be provided to the collaborators of STAMP, along with a data submission schedule.
9. A signed agreement stating that credit and acknowledgment will be given to USGS, USFWS, NIST's Marine ESB, BIA-ARSB, AMNWR and STAMP for collecting and processing seabird eggs.

The requesting parties will be responsible for all shipping charges. Additional charges may also be required to cover the costs of retrieving, documenting, and packaging the samples for shipment.

PROJECT GOALS AND OBJECTIVES

STAMP's current goals are to collect and bank tissues (primarily eggs) from Alaskan colonial seabird species to determine current baseline contaminant levels, track long-term trends in environmental quality, and provide material for retrospective analysis. At present, specific objectives are to:

1. Develop and use standardized protocols for collecting, processing, analyzing, and banking seabird eggs.
2. Collect eggs from a broad range of Alaskan thick-billed murre, common murre, glaucous gull, and glaucous-winged gull colonies scattered throughout the Bering and Chukchi seas and Gulf of Alaska for several nesting seasons to determine current baseline contaminant levels and verify the presence/absence of geographic patterns.
3. Process the eggs under controlled laboratory conditions to ensure the sample material does not become contaminated by outside sources.
4. Cryogenically bank the egg contents in the Marine ESB under controlled laboratory conditions to ensure chemical stability during long-term (decadal) storage.
5. Send the eggshells to the University of Alaska Museum of the North for long-term (decadal) storage.
6. Analyze subsamples (aliquots) of the egg contents under controlled laboratory conditions to determine current baseline contaminant levels.
7. Describe current geographic and temporal patterns in the Alaskan and northeastern Pacific marine environments.
8. Use the contaminant patterns observed in eggs to improve our understanding of the cycling and transport of these chemicals in the environment.

MATERIALS

STAMP developed the first protocol for collecting seabird eggs in 1999 (York *et al.* 2001). In 2004, these protocols were revised and updated (Roseneau *et al.* 2008, Vander Pol *et al.* 2009). The following details represent the most current protocols that are being utilized to date and reflect new techniques for collecting, processing, packaging, and transporting samples.

Egg collecting kits are provided by AMNWR and mailed to participating partners free-of-charge. AMNWR also pays the return shipping costs.

Materials in Egg Collecting Kits

- 50-quart coolers or Rubbermaid Action Packers with collection protocols
- Foam padding to protect the eggs (Figure 1)
- All necessary state and federal permits
- Laminated photographs to help differentiate between common and thick-billed murre and Glaucous-Winged and Glaucous Gulls (Figures 2 and 3 respectively)
- Data recording forms
- Pre-printed shipping labels
- Egg labels
- Several “keep upright-handle with care” and “keep cool-do not freeze” stickers
- Plastic bags
- Disposable talc-free gloves
- Permanent markers and pencils
- Duct tape

Permits: State and federal permits needed to collect eggs from Alaskan seabird colonies are obtained by STAMP’s AMNWR project manager. The permits are state-wide and cover almost all of the nesting locations in the Beaufort, Chukchi and Bering seas, Gulf of Alaska, and Aleutian Islands. They allow up to 15 common murre, 15 thick-billed murre, 36 glaucous gull, and 36 glaucous-winged gull eggs to be obtained from the listed colonies every year. The permits were reviewed by the USFWS permit committee and Alaska Department of Fish and Game biologists during January-March 2009. They were renewed on 1 April 2009 and are currently in effect until 31 March 2012. They can be renewed every three years until 2020, and new collecting locations can be added at any time. Annual reports, due by 15 January every year, are compiled and submitted by STAMP’s AMNWR project manager. *Note: eggs obtained from locations outside Alaska (e.g., Tatoosh Island) are covered by permits held by the participating collectors.*



Figure 1: Packing eggs for transport. Top left: eggs placed on the foam padding; Top right: layering the foam pads in the provided coolers; Middle right: Supplies provided in the kit, and Bottom: Cooler ready to be shipped. (Note: The eggs in the top left picture are not shown in their individual bags.)

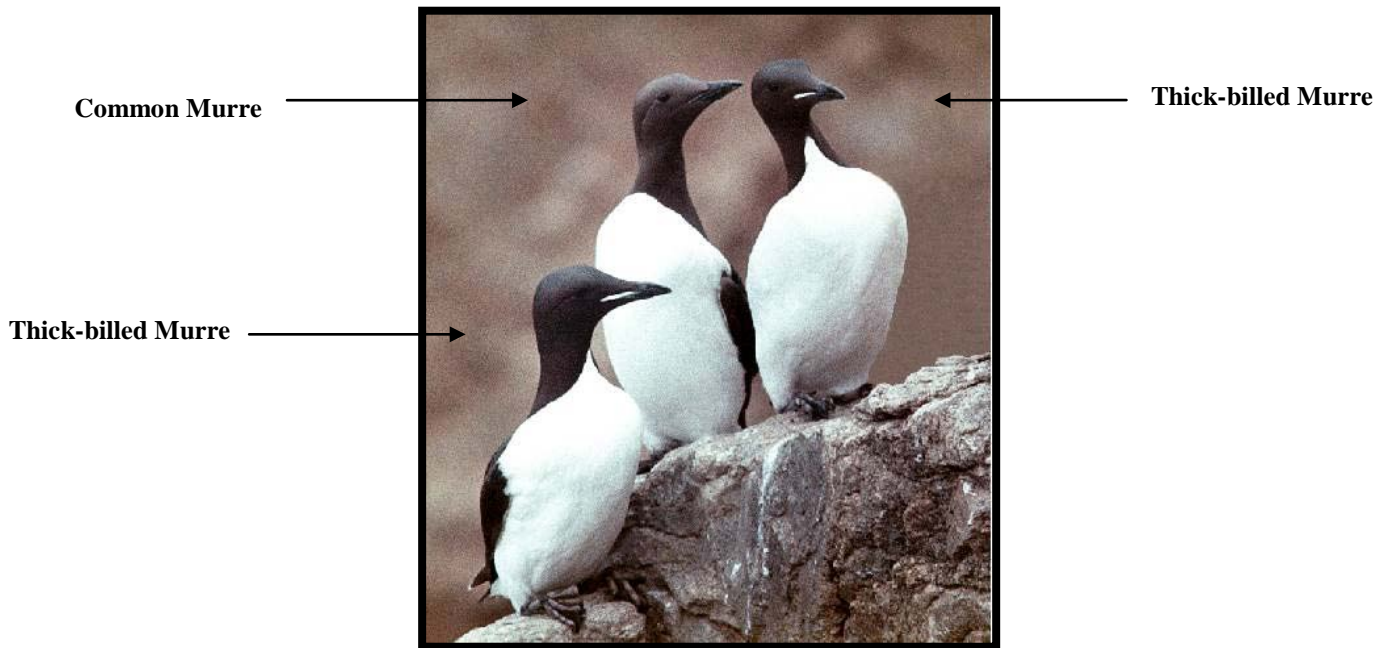


Figure 2: Murre identification photo guide.

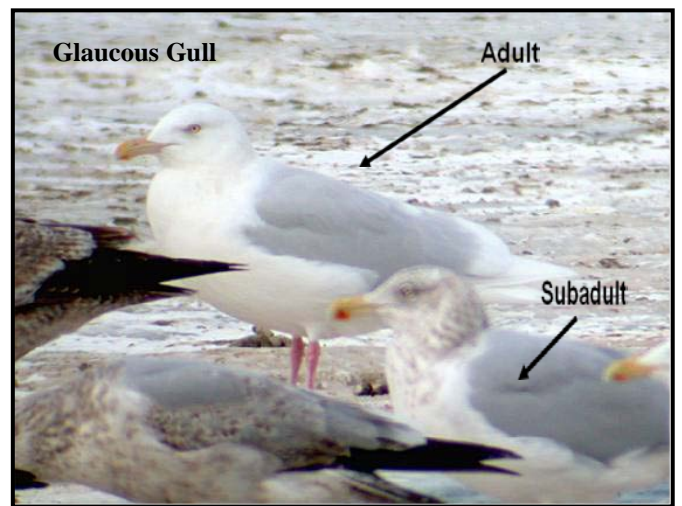
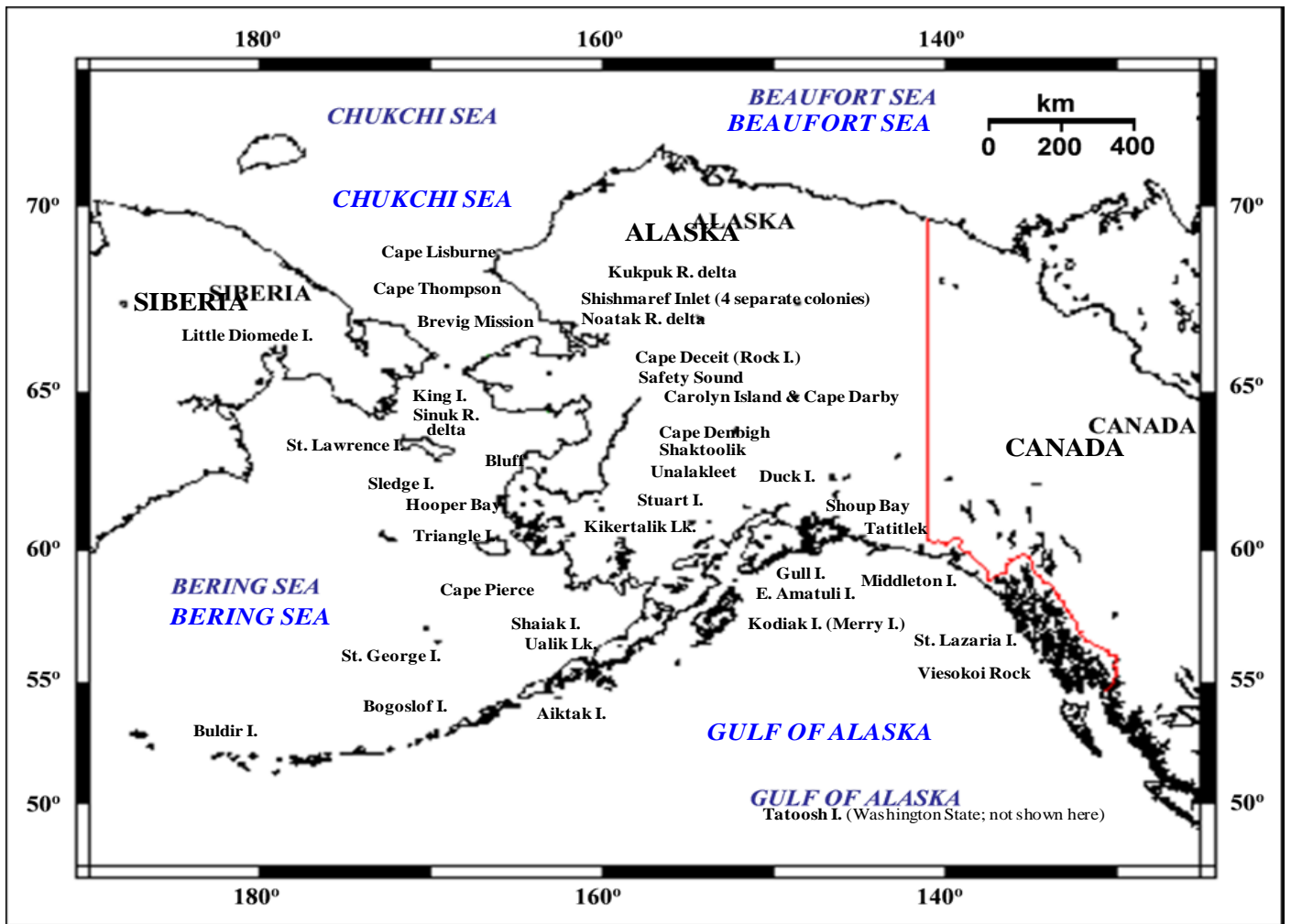


Figure 3: Gull identification photo guide.

METHODS

Study Area and Sampling Sites

The STAMP study area encompasses sites in the Bering and Chukchi seas, Aleutian Islands, Gulf of Alaska, and northeastern Pacific Ocean, and by the close of the 2009 breeding season, the project successfully sampled 44 colonies in these regions (Figure 4).



Map courtesy of Dave Roseneau.

Figure 4: The 1999-2009 Seabird Tissue Archival and Monitoring Project (STAMP) seabird egg collecting sites.

The seabird egg collecting and banking protocols developed by STAMP for the Marine ESB is based on methods 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. The egg banking protocol is based directly on procedures used by Marine ESB staff to catalog and bank AMMTAP and MMHSRP tissue samples (Becker *et al.* 1988, 1991, and 1999).

The murre and gull egg collecting protocols were designed to obtain uncontaminated fresh eggs during the early incubation period to avoid obtaining replacement eggs. They have continued to evolve over the years to become less complex and minimize egg breakage during transport. The intent of these updated protocols is to make them more user-friendly for rural residents to use at remote locations under typical Alaskan field conditions and 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 Marine ESB, including storage under LN₂ vapor at -150°C.

Egg Collection Field Procedures

The procedures below have been reproduced from NISTIR 7562 with modifications and updates.

Collecting murre eggs

Each murre egg collection kit consists of 1 plastic Coleman cooler capable of holding 15 murre eggs.

- 1) The permit allows 15 murre eggs to be collected. Use the egg labels to identify which species of eggs are being collected.
- 2) Collect the eggs as soon as possible after the birds have started laying eggs. If not possible, indicate in the 'Comments' section on the datasheet when they were collected (for example, "about 2 weeks after egg laying began").
- 3) Using talc-free vinyl gloves, remove the eggs from the nest. Always wear gloves and try not to touch the eggs with bare hands, particularly if there's a chance that there might be any residual outboard fuel, oil, or insect repellent on them as this will contaminate the shell and contents. Note on the datasheet if no gloves were available and the eggs were picked up with bare hands.

Labeling the eggs

The collection kit contains pre-printed labels for the eggs (Figure 5). Use the enclosed pencil to fill out one label for each murre egg that is collected (DO NOT use ball point pens). Assign a different number for each nest you collect eggs from (1-15). If more than 1 egg is present in a

nest, use the same nest number for each egg collected from that particular nest, so it's known that the eggs labeled with the same nest numbers came from the same clutch of eggs.

- 1) Identify and label eggs individually. The species and location will be pre-printed on the labels. Fill in the missing information (name, egg collection date, assigned nest number, and any comments (e.g. "collected late in the season", "did not use gloves", "eggs almost ready to hatch"). The labels should be printed using soft to medium pencils (No. 2 or 3) and placed between the two plastic bags.
- 2) One Seabird Data Form should be filled out **per clutch** (see Appendix 1). In the comments section on page 2, make a note if more than one egg was found in that clutch. The assigned 12-digit sample ID number should be hand-printed on the Seabird Data Form 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). Refer to Figure 7 below for instructions on how to assign the identification codes.

Collected by: <u>Tom Jones</u>
Date: <u>July 1, 2009</u>
Species: <u>Common Murre</u>
Location: <u>Duck Island</u>
Egg No.: <u>6</u>
Comments: <u>Collected late in season</u>

Figure 5: Pre-printed murre egg labels.

Bagging the eggs

Place each egg in individual plastic bags and seal it with clean non-contaminating tape.

- 1) Gently squeeze the air out of the plastic bag before closing it and gently wrap the closed bag around the egg.
- 2) Place the bagged egg into a second plastic bag along with a completed pre-printed label.

- 3) Gently squeeze the air out of the second plastic bag before closing it and gently wrap it around the egg. The double-bagged, labeled egg is now ready to be packed for shipping. *Note: squeezing the air out of the bags before sealing them is important—it prevents the eggs from rolling around loose in bubbles of trapped air.*

Packing the eggs for shipping

Use the Coleman coolers as shipping containers for the eggs. Each cooler is designed to safely hold up to 15 murre eggs (Figure 1). If collecting both common and thick-billed murre eggs, use the cooler marked “COMU Egg Collection Kit” for the common murre eggs and the cooler marked “TBMU Egg Collection Kit” for the thick-billed murre eggs (check the egg labels in the kits—they also indicate which species the kit is for).

Each cooler contains 3 large loose pieces of foam padding (about 10 cm thick) labeled as: Layer #1, Layer #2, and Layer #3, and a foam block glued to the underside of the lid to fill the recessed cavity. Pack the bagged eggs in the coolers as described below.

- 1) Remove foam Layers #1 and #2 from the cooler and leave Layer #3 in the bottom of the container.
- 2) Lay 8 of the doubled-bagged eggs on their sides in the two grooves in layer #3 in the 8 marked spots. Don’t let the eggs touch each other or the walls of the cooler—try to space them about 2.5 cm apart. Put small pieces of foam between the eggs and use some tape to secure them in place.
- 3) Put Layer #2 back in the cooler, grooved side up, and gently push it down onto the first layer of eggs to hold them firmly in place.
- 4) Lay 7 of the doubled-bagged eggs on their sides in the two grooves in Layer #2 in the 7 marked spots. Again, don’t let the eggs touch each other or the walls of the cooler—try to space them at least 2.5 cm apart.
- 5) Put Layer #1 back in the cooler following the directions marked on it and gently push it down onto the second layer of eggs to hold them firmly in place. Layer #1 should stick up a little above the lip of the cooler (about 2.5 cm). If it sticks up more than that, remove it and try to re-adjust Layer #2 (in other words, gently push it down a little more firmly onto the eggs in Layer #3).
- 6) When the cooler is ready to ship, fill out the enclosed collection form and lay it on top of Layer #1 in the container.
- 7) Close the lid and tape the cooler shut by wrapping 4-5 layers of tape completely around it about 15 cm in from each end, just like it was originally taped shut.
- 8) Tape the pre-printed shipping label securely to the top of the lid, and stick the “Keep Cool” and “Handle with Care” labels on the top and sides of the containers. The cooler is now ready to ship.

Storing and shipping the eggs

Ship the eggs to Homer, see address below, as soon as possible after collecting them. If that is not possible, keep the eggs as cool as possible but do not freeze them. Keeping them stored in a refrigerator is best, otherwise, keep them in the cooler and place it outside in a cool shady place.

If an egg cracks during packaging and freezer facilities are available (e.g., Cape Lisburne), the egg can be salvaged by freezing it as soon as possible and shipping it frozen to the processing laboratory. 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.

Contact Dave Roseneau at the Alaska Maritime National Wildlife Refuge in Homer, AK, before sending the eggs and specify the date the samples are to be shipped as well as the name of the air carrier. If the cooler cannot be sent directly to Homer, Dave or Vern will request it to be sent to a hub community like Kotzebue, Nome, Bethel, Fairbanks, Anchorage, or Kodiak where they can arrange to have it transferred to another air carrier that will bring it to Homer. After the samples are shipped, contact Dave again and provide him with the actual date of shipment as well as the air carrier's airway bill number so the shipment can be tracked.

Dave Roseneau,
USFWS Alaska Maritime National Wildlife Refuge
95 Sterling Highway #1
Homer, Alaska 99603-7472
Phone (907-226-4613)
Fax (907-235-7783)

E-mail: dave_roseneau@fws.gov

Vern Byrd can be contacted if Dave is not available (phone 907-235-6546, fax 907-235-7783, or e-mail <vernon_byrd@fws.gov>). *Note: the government phone system will not accept collect calls. Please call direct, give them your number, and tell them to call you back.*

Use the air carrier servicing your community that is willing to ship the eggs freight collect if possible. Call or e-mail Dave at the number above if there are shipping problems.

Collecting gull eggs

The collection kit for gull eggs consists of 2 plastic Coleman coolers large enough to hold up to 18 gull eggs each for a total of 36 eggs.

Some kits also contain disposable cameras for taking pictures of nesting areas and birds. If there is a disposable camera in the collection kit, please use it to photograph the nesting area where the eggs are collected, as well as the collecting activities. Close up pictures of the birds will help confirm the species identity for the long-term database. Send the camera back with the egg shipment.

The permit allows the collection of up to 36 gull eggs from a total of 12 nests. Gulls typically lay 2-4 eggs per clutch. Do NOT take eggs from more than 12 nests and do NOT collect more than a total of 36 eggs. Collect the eggs during the first 2 weeks of the laying season starting a few days after the birds have finished laying eggs and have completed their clutches. If that's not possible,

collect the eggs when possible and note how early or late it was compared to when the birds first started laying eggs (for example, “right when they first started laying eggs”, “about three weeks after they started laying eggs”, “just before the eggs hatched”, etc.).

Here are some tips when deciding which nests to collect from:

First Choice: In the gull nesting area, look for nests that have 3 eggs in them. Try to find 12 nests that have 3 eggs in each one of them. In this particular case, the total number of eggs collected will be 36 (36 eggs from 12 nests containing 3 eggs each) and the collection will be complete.

Second Choice: If 12 gull nests with 3 eggs in each are not observed, take all of the eggs from as many 3-egg nests as possible, and then look for nests that have 2 eggs in them. Take all of the eggs from enough of the 2-egg nests to meet the sampling quota of 12 nests. For example, if only 3 nests that contain 3 eggs are found, 9 more nests that contain 2 eggs each will need to be located. In this particular case, if 9 nests containing 2 eggs each are found, the total number of eggs collected will be 27 (9 eggs from 3 nests containing 3 eggs each and 18 eggs from 9 nests containing 2 eggs each).

Third Choice: If it is not possible to find any nests with 3 eggs, try to find 12 nests that have 2 eggs in each one of them, and collect all of these eggs. In this particular case, the total number of eggs collected will be 24 (24 eggs from 12 nests containing 2 eggs each).

Fourth Choice: If only nests with 2 eggs per nest are found, take all of the eggs from as many nests as possible and then look for nests that have 1 egg in them. Collect eggs from enough of the 1-egg nests to meet the sampling quota of 12 nests. For example, if 5 nests containing 2 eggs each are found (10 eggs), 7 more nests that contain 1 egg each to equal 12 nests will need to be collected. In this particular case, if 7 nests containing 1 egg each are collected, the total number of eggs collected will be 17 (10 eggs from 5 nests containing 2 eggs each and 7 eggs from 7 nests containing 1 egg each).

Fifth Choice: If it happens to be a year when nesting conditions are poor and the gulls only manage to lay 1 egg per pair, try to collect 12 eggs from 12 nests that contain 1 egg each. In this particular case, if 12 nests that have 1 egg each are found, the total number of eggs collected will be 12 (12 eggs from 12 nests containing 1 egg each). *Note: if at least 6 nests that contain 1 egg each cannot be found, don't collect any eggs and wait until the next year. Also, contact Dave Roseneau and let him know if the birds failed to lay a normal number of eggs.*

Always wear a set of gloves provided in the collection kit when handling the eggs. Wearing gloves ensures that contamination from gasoline, oil, or insect repellent, possibly on your hands, does not come in contact with the eggs. All of these substances can penetrate the egg shells and contaminate the egg contents. As a general rule, always try to avoid touching the eggs with bare hands.

Labeling the collected gull eggs

The collection kit contains pre-printed labels for the eggs. Use the enclosed pencil to fill out one label for each egg collected. Do not use ball point pens. When filling out the labels, use a different number for each nest where an egg was collected. Number the nests from 1 to 12 in collection order and use these numbers to label the eggs. If more than 1 egg is present in a nest, use the same nest number for each egg collected from that particular nest. Eggs labeled with the same nest numbers were collected from the same clutch of eggs. For example, if nest no. 5 at the Kukpuk River contained 3 eggs and all 3 were collected, each one of the three separately bagged eggs from that nest should have a label with the number “5” written on it like shown below in Figure 6.

- 1) Identify and label the collected eggs individually. The species and location will be pre-printed on the labels in the collection kit. Fill in the required information: name, egg collection date, assigned nest number, and any comments. For example, “collected late in the season”, “did not use gloves”, “eggs almost ready to hatch”, etc. The labels should be printed using soft to medium pencils (No. 2 or 3) and placed between the two plastic bags.
- 2) Enter the identification codes in the “Sample ID Number” space on the Marine ESB Seabird Data Form. 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.

Collected by: <u>Tom Jones</u>
Date: <u>July 1, 2009</u>
Species: <u>Glaucous Gull</u>
Location: <u>Kukpuk River</u>
Nest No.: <u>5</u>
Comments: <u>did not use gloves</u>

Figure 6: Pre-printed gull egg labels.

Bagging the eggs

Put each egg in individual plastic bags found in the collection kit (never put more than 1 egg in a single bag). After the egg is placed in a bag, please do the following:

- 1) Gently squeeze the air out of the plastic bag and seal the bag closed. Gently wrap the closed bag around the egg.
- 2) Put the bagged egg into a second plastic bag with a completed label.
- 3) Gently squeeze the air out of the second plastic bag and seal it closed. Gently wrap the bag around the egg. The double-bagged, labeled egg is now ready to be packed for shipping.

Packing the eggs for shipping

Use the Coleman coolers for shipping containers and carefully pack the bagged eggs in them following the directions listed below.

The coolers are designed to safely hold up to 18 gull eggs each. Each cooler contains 3 large pieces of foam padding about 10 cm thick labeled Layer #1, #2, and #3, and 1-2 smaller pieces of foam glued into the recessed cavity in the underside of the lid (Figure 1). Pack the eggs in the coolers as follows:

- 1) Remove foam Layers #1 and #2 from the cooler and leave Layer #3 in the bottom of the container.
- 2) Lay 10 of the doubled-bagged eggs on their sides in the two grooves in layer #3 in the 10 designated spots. Do not let the eggs touch each other or the walls of the cooler. Space them about 2.5 cm apart. Use pieces of tape to secure the eggs to the foam to keep them in place.
- 3) Put Layer #2 back into the cooler, grooved side up, and gently press it down onto the first layer of eggs to hold them firmly in place.
- 4) Lay 8 of the doubled-bagged eggs on their sides in the two grooves in Layer #2 in the 8 designated spots. Again, do not let the eggs touch each other or the walls of the cooler. Space them at least 2.5 cm apart and use a piece of tape to secure them to the foam.
- 5) Put Layer #1 back into the cooler following the directions marked on it and gently press it down onto the second layer of eggs to hold them firmly in place.
- 6) Put the shipping form on top of Layer #1.
- 7) If a disposable camera is sent in the collection kit, fit it into one of the gaps next to the foam glued to the underside of the lid and tape it in place.
- 8) Close the lid and tape it shut by wrapping 2-3 layers of duct tape completely around the cooler about 15 cm from each end.
- 9) Tape the pre-printed shipping label securely to the top of the lid, and stick the “Keep Cool” and “Handle with Care” labels on the top and sides of the container. The cooler is now ready for shipping (Figure 1).

Keep the packaged eggs as cool as possible (ideally at 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 the egg if it cannot 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.

Ship to Dave Roseneau. Please refer to the murre ‘egg storing’ and ‘shipping’ instructions above. PLEASE CONTACT DAVE ROSENEAU OR VERN BYRD BEFORE SHIPPING TO HOMER, AK.

EGG PROCESSING PROCEDURES

The updated processing protocol currently being used by AMNWR personnel is summarized below. Procedures used to process murre and gull eggs are the same—the only difference is that the contents of multi-egg gull clutches are pooled before they’re homogenized (murre lay 1-egg clutches).

All processing is done under controlled laboratory conditions by personnel wearing vinyl talc-free gloves using chemically cleaned equipment (e.g., titanium knives; stainless steel blender shafts, blades, forceps, and spatulas; glass measuring cups, glass beakers and pipettes) and an AirClean® Systems positive-pressure laminar flow hood lined with UHV foil and equipped with a HEPA 0.3 micron filter to minimize chances of contamination. Egg contents are removed and weighed under the positive-pressure hood. Contents put in chemically cleaned measuring cups and beakers are covered with clean UHV foil before they’re transferred to a VirTis high-speed blender station to be homogenized. The foil covering is kept in place during blending operations (blender shafts and blades are inserted into the contents through small slits cut in the foil with titanium knives), and the covered containers are returned to the positive-pressure hood before the foil is removed and the homogenized contents are pipetted into clean Teflon® jars and cryovials. Chemical cleaning of glassware, knives, blender shafts and blades, forceps, spatulas, and other processing tools is done under a standard negative-pressure laboratory fume hood lined with UHV foil. All of the cleaned items are covered by foil or wrapped in protective layers of it before they are transferred back to the positive-pressure hood and used for processing samples.

The AMNWR laboratory in Homer, Alaska, became fully functional in 2008. It contains both NIST and AMNWR supplied equipment. Items provided by NIST include an AirClean® Systems positive-pressure laminar flow hood, a stainless-steel Interscience BagMixer 400, a high-speed VirTis blender with stainless steel shaft and blade capable of turning 30,000 RPM, a heat sealer for sealing Teflon bags®, a cordless pipette gun, two serrated titanium knives, a large 10-L Nalgene container for storing a supply of Type 1 DI water. AMNWR-supplied items include a standard negative-pressure laboratory fume hood for working with volatile chemicals; a secure chemical storage locker; a Type 1 DI water making system; a drying oven; two battery-powered digital calipers capable of measuring eggs to thousandths of centimeters (e.g., three decimal places—0.000 cm); a digital Ohaus scale capable of weighing eggs and their contents to hundredths of grams (e.g., two decimal places—0.00 g); several glass 1-L beakers; glass funnels; stainless steel tongs, forceps, spatulas, and wire; a 18 cubic foot refrigerator/freezer for temporarily storing whole eggs and homogenized samples; and a computer and printer to enter and compile data and print data forms, protocols, and address labels.

Material 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, plastic bags, and cardboard labels for storing egg shells
- Cardboard shipping tubes
- Labels for exterior of shipping tubes
- Tape for securing exterior labels
- High purity distilled (HP) water or best available water for rinsing samples
- High grade methanol (99 %) (MeOH) for rinsing instruments
- High grade acetone (99 %) for rinsing instruments
- High grade hexane (95 %) for rinsing instruments
- Micrometer, (0 to 25 mm) accurate to 0.01 mm
- Calipers, accurate to 0.01 mm
- Titanium blade knife, serrated
- BagMixer
- Stainless steel hand blender
- Hexane-rinsed aluminum foil
- Lab coats (disposable)
- Balance for weighing samples
- Surgical scissors and forceps
- Insulated gloves, safety glasses, and tongs for handling the liquid nitrogen (LN₂) and frozen samples
- LN₂ in container for freezing samples
- Dewar and lid

Seabird Data Form

During processing, a 2-page Seabird Data Form must be filled out for each clutch of eggs (Appendix 1). Standard biological/environmental information at the time the egg is collected is recorded on the first page. The boxes at the top of the first page provide space for the NIST/Marine ESB identification numbers. These are assigned to the samples when they arrive at the Marine ESB. The additional information to be recorded on the first page is listed below:

1. Sample ID Number: Assign a 12-digit alphanumeric code to the egg and enter this code here using the following protocol: The first four characters (letters) identify the colony, the next two characters (numbers) identify the clutch (e.g., 01, 02, 03). The next four characters (letters) consist of the standard common name acronyms for the species the egg was collected from (Appendix 3), and the last two characters (numbers) specify the year the egg was collected (e.g., '99) (Figure 7).

NOTE: From 1999-2006, the sample ID consisted of 14-digits. The year was written out (e.g.1999). In 2008, due to space constraints, the code was shortened to 12-digits and only the last two numbers of the sampling year is now written out.

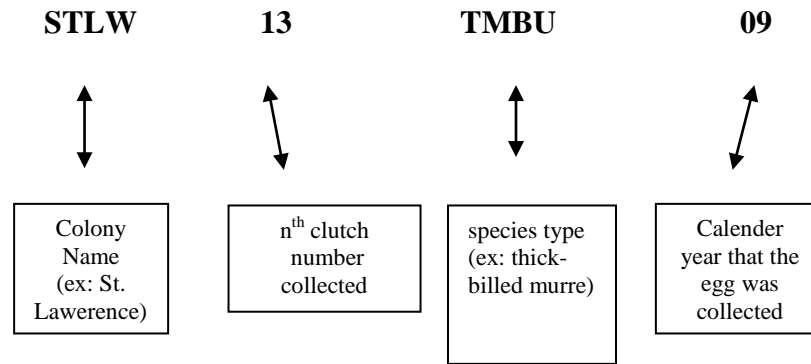


Figure 7: 12-digit Sample ID number.

2. Species: Enter the genus/species name.
3. Geographic Area: Enter the general location where the egg is collected (e.g., Cook Inlet, northeastern Chukchi Sea, southeastern Bering Sea, Bering Strait).
4. Lat.&Long.: The latitude and longitude of the nesting colony where the eggs were obtained. In most cases, the person receiving the eggs at the processing facility determines these coordinates by looking the location up in the USGS Dictionary of Alaska Place Names by Donald Orth, or by using USGS place names and Google Earth to calculate them. In some cases, they are calculated by using Google Earth in conjunction with maps or written descriptions provided by the egg collectors.
5. Colony Name: Enter the common name of the seabird nesting colony where collection occurs (e.g., Cape Lisburne, St. George Island, East Amatuli Island, St. Lazaria Island).
6. Sample Source: General information on who was responsible for obtaining the clutch of eggs (e.g., USFWS seabird monitoring program, local subsistence harvest).
7. Site ID Name/Number: Enter the study name or plot number 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. Sample Type: The type of tissue collected (e.g., liver, kidney, muscle, feather, blood, egg, other).

9. 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).
10. Method of Collection: Describe the method used to collect the egg (e.g., “collected by hand using vinyl glove”, “collected by local subsistence harvesters without gloves”; refer to “Collecting murre/gull eggs” section).
11. Date and Time of Death: Record the time of death as day, month, year, and hour, if known, using the same format described in No. 9 above.
12. 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).
13. 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.
14. 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 (e.g., “shipped to Kotzebue at 1000 hrs on 07/07/2009 on Bering Air, shipped to Fairbanks at 1400 hrs on 08/11/2009 on Alaska Airlines, shipped to Homer at 1700 hrs on 08/12/2009 on ERA Airlines”).
15. Pre-Shipment 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 Marine ESB.
16. Protocol: Indicate the protocol that was used to collect or process the eggs. If the “Standard” approved protocol was used, state this. If the protocols were “Modified” described them in detail.
17. Comments: Enter any additional remarks pertinent to the collection, processing, or archiving of the egg. If body tissues (e.g., flesh, bone, feathers) are collected from dead seabirds for later analyses, note the date and time they were collected.
18. Additional Samples: A brief comment about other samples that may have been obtained from the same sampling site in the same year (e.g., “10 other useable samples (clutches) were collected from the same location on the same date”). Also, any information that might indicate eggs were collected from the same nest site in previous years.

19. Collected by: The full name of the person that collected the eggs or a general statement about how they were obtained (e.g., “collected by unnamed subsistence hunters from Point Hope”).)

The second page of the Seabird Data Form pertains to biological data and information that are specific to the egg and the processing of the egg:

20. Sample ID Number: The same 12-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.
21. Date and Time of Sample Preparation: Enter the date and start time that the egg sample was processed and prepared for LN₂ freezing.
22. Processor: Enter the full name of the individual(s) who processed the sample.
23. Date and Time of Freezing: Enter the date and time at which the egg sample is frozen at – 80 °C or below in LN₂ vapor (-150 °C) in preparation for shipping to the Marine ESB.
24. Date and Time of Shipping to Marine ESB: Enter the date and time that the sample was shipped to the Marine ESB. This information should be reported here as day, month, year, and hour using the format described in No. 9 above, and should reflect the time that the sample left the processing site.
25. Shipper: Enter the full name of the person responsible for shipping the sample from the processing site to the Marine ESB.
26. Date and Time Received at Marine ESB: This information is recorded by the receiver at the Marine ESB. Enter the date and time that the sample was received at the Marine ESB.
27. Receiver: The full name of the individual that received the sample at the Marine ESB.
28. Egg Measurements: Whole egg measurements (weight, expressed as 000.00 g; length, expressed as 0.00 cm); breadth (expressed as 0.00 cm), egg content weights (expressed as 000.00 g), and eggshell weights (expressed as 000.00 g).
29. Sample Aliquots: Record the number of polypropylene vials, Teflon jars, and Teflon bag (s) that the sample has been aliquoted into (e.g., 6 polypropylene vials—B01P, B02P, B03P, B04P, B05P, B06P; 5 Teflon jars—B01T, B02T, B03T, B04T, B05T; Teflon bags—1).
30. Form Prepared by: Enter the full name and signature of the person that filled out the 2-page form.

31. Shipping Information: The shipping address for the form and processed samples are listed at the bottom of the second page of the form, including the name and phone number of the person responsible for receiving the samples.

Receiving Whole Eggs

When a cooler containing eggs arrives, open it and carefully put the bagged eggs and the shipping form in a USPS Priority Mail 12" x 12" cardboard box (2 boxes may be required for gull eggs), tape the box shut and write the collecting location, collector's name, arrival date, and number of eggs on its top; place in the STAMP refrigerator.

Measuring Whole Eggs

- 1) When the eggs are ready to be processed, pick one of the sampling sites (e.g., the Sinuk River delta) and take the box(s) that contain the specific eggs out of the STAMP refrigerator and open it. Sort through all of the bagged eggs looking at the nest numbers written on the egg labels. Assemble a full clutch of eggs (some clutches only contain 1 egg and some may contain 2 or 3 eggs), take it out of the box(s). Close the box(s), put it back in the refrigerator, and take the clutch of eggs into the lab.
- 2) Turn the AirClean® Systems positive-pressure laminar flow hood on and leave it on during processing. Keep the folding doors closed until you are ready to use it and close them between clutches of eggs.
- 3) Check to make sure that the STAMP Ohaus digital scale is sitting level on a piece of UHV foil under the right front corner of the AirClean® Systems positive-pressure hood. If it's not, wipe it off with an alcohol-moistened TexWipe, put it on a piece of foil under the right front corner of the hood, and level it by adjusting the legs until the level bubble is centered. *Note: always check to make sure the bubble is centered and the scale is level before using it.*
- 4) After the scale is in place and the hood is on and running properly, follow the directions listed below. *Note: these directions assume you are working with a clutch of 3 eggs; however, in most cases, you'll be working with clutches that only contain 1 or 2 eggs, so be prepared to modify the steps accordingly.*
 - A) Using a pencil, fill out the blank seabird data form with the species (glaucous gull, thick-billed murre, etc.), colony location (Safety Sound, Norton Sound, etc.), date collected, collector's name, egg processor's name, the date you froze the samples (when the samples were placed in the STAMP freezer), 12-digit alphanumeric ID code, shipper's name, receiver's name, and any unusual features about the egg (e.g. cracked, dented, pitted, discolored).
 - B) Place one TexWipe per egg on a piece of foil near a sink to help keep the eggs in place.

- C) Put on a pair of gloves and remove the egg(s) from the plastic bag(s) and place them on the TexWipes.
- D) Remove the paper labels from the plastic bags and place them with the datasheet. Once you remove your gloves, staple them to the data form.
- E) Rinse the eggs with DI water then wipe them dry with TexWipes (1 TexWipe per egg). Place them back on the TexWipes on the foil in a line, and designate them Eggs A, B, and C. Use the convention that the left-most egg is always Egg A, the right-most egg is always Egg C, and the egg in the middle is always Egg B.
- F) Replace your gloves.
- G) Put a piece of TexWipe on the scale and zero it by pushing “TARE”. The TexWipe will help keep eggs from rolling off.
- H) Place Egg A on the scale (Figure 8), weigh it to the nearest tenth of a gram (i.e., 00.0 g), and record the weight on the data form in column A on the whole egg (g) line (Figure 9, Appendix 2).
- I) Measure Egg A using calipers at its greatest (longest and widest) part (Figure 10). Turn the calipers on, turn it to “millimeter” mode. The millimeter mode allows you to make measurements to 2 decimal places (e.g., 00.00 mm). After measuring each egg, convert the millimeter (mm) readings to centimeters (cm) by moving the decimal place 1 place to the left (record length and breadth to nearest thousandth of a centimeter—i.e., 0.000 cm) and record the information in column A on the length and breadth lines (Figure 9).
- J) Repeat steps F through I for Eggs B and C and record the data on the appropriate lines in columns B and C, respectively (Figure 9).

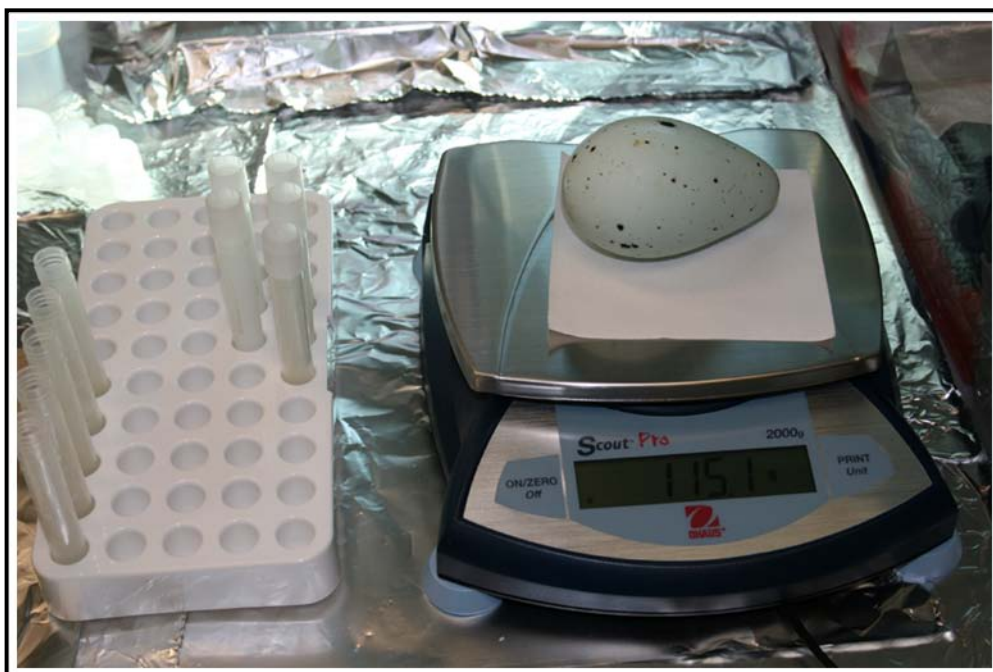


Photo courtesy of Dave Roseneau

Figure 8: Weighing an egg on a TexWipe on a digital scale.

EXAMPLE

IV Egg Measurements:	A	B	C
Whole egg (g)	94.6 g	95.2 g	broken – thrown away
Length (cm)	7.391 cm	7.302 cm	NA
Breadth (cm)	5.053 cm	5.124 cm	NA
Egg contents (g)	80.0 g	85.0 g	NA

Figure 9: Filling out Part IV on the Seabird Data Form.



Photo courtesy of Dave Roseneau

Figure 10: Measuring a rinsed and dry egg with digital calipers.

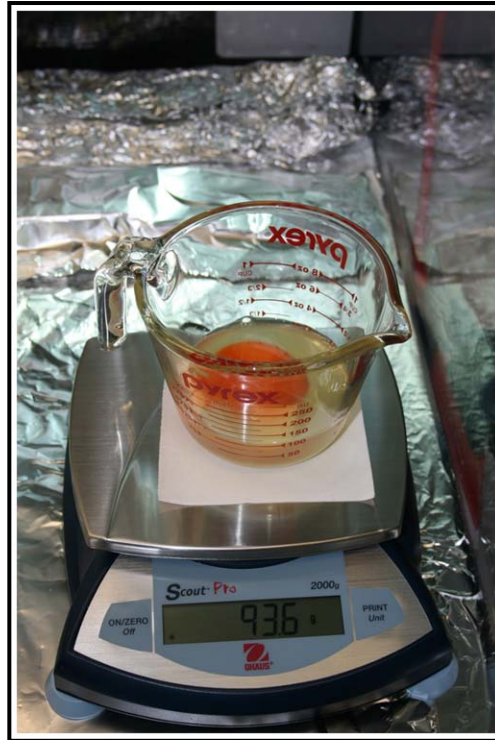
Removing Egg Contents from Shells

- 1) All of the tasks must be performed under the AirClean® Systems positive-pressure hood, clean room or equivalent. Wear talc-free vinyl gloves while handling the eggs and change gloves between clutches.
- 2) Remove the contents of each egg using the following procedure:
 - A) Turn on the master switch on the back of the AirClean® Systems unit, open the hood's folding door to turn the light and filter fan on and let the unit run for about 30 seconds. Be sure all the chemically clean glassware needed is available under the hood. If not, put on a pair of vinyl talc-free gloves, clean the items under the negative-pressure fume hood according to the 'cleaning protocols' below, wrap them in clean pieces of UHV foil, and put them against the back wall of the hood behind the working area. Be sure there are several pieces of UHV foil in the hood.
 - B) Go to the heat sealing station, take the plastic clamp off the heat sealer's arm and turn it on by setting the temperature dial to 4 so it will be ready when needed.
 - C) Put on a pair of vinyl talc-free gloves, pick up a clean glass measuring cup from the back of the hood, set it down on the left side of the hood about 10 inches back from the folding door, and remove the UHV foil covering.

- D) Fold a small piece of clean UHV foil firmly over the rim of the cup. It should cover about half of the rim and extend 4 cm down the side.
- E) Set the cup on the scale, press the “TARE” button to zero the readout, and return the cup to its spot on the foil several inches back from the door.
- F) Pick up Egg A, large end up, in one hand; take it to the open hood; hold the cup steady with your free hand; and crack it open on the cup’s foil covered rim, just like you would crack a chicken egg open on the rim of a mixing bowl (2-3 firm taps should do it; rotate the egg slightly between taps to extend the crack about 1/3 of the way around its widest point).
- G) After the shell is cracked, quickly move the egg over the center of the cup, hold the large half in your free hand, and break the egg open by applying upward pressure and pulling them gently apart. *Note—practice cracking open some chicken eggs on the foil-covered rim of a measuring cup. Gull egg shells are fairly thin and they’re not that much harder to break open than chicken eggs. However, murre egg shells are thicker and stronger and they’ll require firmer, more forceful taps on the rim of the measuring cup to crack them open cleanly. To practice breaking a murre egg open, use an egg from a colony where 15 eggs were collected—if contents are lost or pieces of shell fall in it, there will still be 14 eggs left from the sampling site.*
- H) Hold the shell halves at steep angles over the cup until the contents have drained into it. If some of the albumen persists and clings to the insides of the shells, set them down on a clean piece of UHV foil, unwrap one of the clean stainless steel spatulas and gently scrape the remaining material into the cup. Try not to tear the shell membrane and get pieces of it in the contents.
- I) When all of the contents from Egg A are in the cup, take the shell halves to the lab sink, rinse them out with Type 1 DI water, put them down on a clean piece of foil or TexWipe, and designate them “Egg A”.
- J) Go back to the hood and put the measuring cup containing Egg A’s contents on the scale (Figure 11) and pencil in the weight on the appropriate line of the data form (Figure 9).
- K) Push the “TARE” button on the scale to zero it. Place the cup in the open space on the left side of the hood.
- L) Write the unique 12 character alphanumeric code assigned to the clutch and “Egg A” on a paper lunch bag, put the shell halves in it, and set it aside.
- M) Repeat steps E through L with Eggs B and C, using the same measuring cup. Remember to “TARE” the scale after Egg B is weighed. *Note: if the clutch only contains one egg, you can skip this step.*

- N) After all of the egg contents are in the measuring cup, place the cup back in the hood and cover it tightly with UHV foil. Make sure the foil extends about 3 cm below the rim and put a large rubber band around the cup just above the handle to hold the foil in place.

NOTE: If the egg contains an embryo >2cm, put the egg contents into a Teflon bag, heat seal the bag, place in a labeled cardboard tube and send to Charleston where it will be cryohomogenized.



Photos courtesy of Dave Roseneau

Figure 11: Egg contents in a glass measuring cup ready to be weighed.

Homogenizing Egg Contents

- 1) Take the covered cup to the VirTis blender station and clamp it in with the handle facing out (make sure the clamps are tight and secure).
- 2) Remove a foil-wrapped blender shaft with attached blade and a foil wrapped titanium knife from the back of the AirClean® Systems hood and take them to the blender station.
- 3) Unwrap the blender shaft/blade and insert it into the blender and tighten the knob holding it in as much as you can. Make sure it is tight.

- 4) Lower the blender blade until it's just above the foil covering the cup. Unwrap one of the titanium knives and cut a small slit in the foil slightly longer than the blade and widen it slightly with the tip of the knife.
- 5) Lay the knife back down on its foil wrapping and guide the blender blade through the slit in the foil. Lower the blades until it touches the bottom of the cup, and then raise it up about 1.5 cm and secure it in place by tightening the knob that controls blade height. Re-tighten the knob.
- 6) Fold a small piece of clean UHV foil loosely around the blender shaft to cover the slit in the foil (i.e., "tent" the foil around the shaft).
- 7) Put a pair of safety glasses on, set the blender speed dial to 1, and turn it on (Figure 12). After it runs for a few seconds, increase the speed to 3, let it run for a few more seconds, and then increase the speed to 5 and dial it back to 1. Blending is complete when the contents at the bottom are a uniform color and no albumen can be seen swirling around. Turn the blender off and reset the dial to 1. If pieces of albumen are still present, turn the blender back on and repeat the speed settings one more time, turn it off, and recheck the contents—any remaining pieces of albumen should be gone. *Note: do not over-blend the contents. The longer you blend it, the frothier it will become and will make pipetting difficult.*
- 8) Loosen the knob controlling blade height, gently pull the blade up through the slit in the foil cover, trying not to rip the foil, and place a small clean piece of foil over the hole.
- 9) Remove the shaft/blade from the blender and take it and any other tools you used (e.g., titanium knives, forceps, spatulas, wire loops) to the sink and put them in a beaker of tap water to soak until you're ready to clean them.
- 10) Unclamp the foil-covered measuring cup and move it to the work area under the positive-pressure hood. Let the homogenized contents stand for a few minutes to let any froth that formed dissipate.

Pipetting Egg Contents into Sample Jars and Vials

- 1) Take out 5 Teflon® 15 mL jars and labels, neatly print the 12 character alphanumeric code assigned to the clutch and "B01T" on the first label, and then repeat this process with the 4 remaining labels, numbering them B02T, B03T, B04T, and B05T, respectively. Attach the labels to the jars by bending them slightly in the middle and sliding the ends under the rims on top of the lids. *Note: homogenized samples from eggs obtained in 1999-2006 were labeled in a different order. Teflon® jars were labeled B01T-B05T followed sequentially by the polypropylene cryovials labeled B06P-B10P. This was originally thought to make banking easier, but it didn't and the new numbering system described here was adopted in 2008.*

- 2) Take out 5-6 15 mL polypropylene cryovials, depending on how much homogenized material there is, and neatly print the 12 character alphanumeric code assigned to the clutch and “B01P” in the frosted area of the first vial, and then repeat this process with the 4-5 remaining vials, numbering them B02P, B03P, B04P, B05P, and B06P, respectively. Put the cryovials in one of the vial holders.
- 3) Put a 16 cm-wide piece of clean UHV foil in of the hood beside the scale.
- 4) Put the cryovial holder containing the labeled vials on the foil. Remove the caps from the vials and set them open side down on the foil just in front of the holder.
- 5) Put the labeled Teflon® jars on the foil in a line next to the cryovial holder. Remove the lids and set them open side down on the foil by the jars.
- 6) Place the covered measuring cup containing the homogenized sample to the front of the hood near the vials and jars, remove the foil covering, and discard it (don’t reuse it).
- 7) Remove the pipette gun from its protective plastic bag and set it on or near the scale under the hood (make sure the scale is turned off).
- 8) Take a clean glass pipette from the storage area at the back of the hood and slide it into the pipette gun. *Note: Always keep a supply of clean pipettes wrapped in a layer of clean UHV foil at the back of the hood and remember to keep the pipette gun charged by plugging it in after each processing session.*
- 9) Set the pipette gun on the negative pressure setting, put the pipette tip about 1 inch below the surface of the homogenized sample in the measuring cup, press the trigger gently and suck up about 9-10 mL of the liquid, reset the gun to the positive pressure setting, put the pipette in the first Teflon® jar, press the trigger gently, and carefully fill it about **half** full. *Note: when filling the pipette—press the trigger gently and let the liquid flow into it slowly and steadily. Never overfill the pipette because if liquid gets into the mechanism that holds the pipette in place, the gun will have to be disassembled and cleaned before it can be used again. Avoid sucking up any froth that might be floating on top of the sample for the same reason.*
- 10) Repeat the above process with jars B0T2-B0T5, but fill them to the 15 mL line just below their tops.
- 11) After the jars are full, begin filling the cryovials to the 15 mL line just below their tops. Always fill them in sequence (i.e., B01P→B06P), in case you run out of sample material before you can fill the last vial.
- 12) After the jars and vials are filled, pull the used pipette from the gun and put it on a TexWipe outside the hood. Lay the pipette gun down on a clean piece of UHV foil near the hood.

- 13) Screw the lids back on the jars and carefully set them upright in a small tray beside the hood. Screw the caps back on the vials and carefully set the holder containing them in the tray with the Teflon® jars (Figure 13). *Note: keep the jars and vials upright until the samples have been frozen.*
- 14) Take the tray containing the aliquots to the freezer. Set the Teflon® jars upright along one edge of the freezer and transfer the cryovials to the cryovial holder that's already in place.
- 15) Bring the tray and empty cryovial holder back to the lab and pencil in the date and time the aliquots were frozen on the seabird data form. Pencil in the number of aliquots of Teflon® jars and cryovials that were filled on the appropriate lines of the data form and list them by their numbers. *See Appendix 1—e.g., 5 (B01T, B02T, B03T, B04T, B05T); 6 (B01P, B02P, B03P, B04P, B05P, B06P).*
- 16) Take the used pipette to the sink, rinse it out with tap water, and put it in the used glass disposal receptacle.
- 17) Return to the positive-pressure hood. If 10 mL or more of the homogenized sample remains after filling the jars, do the following: place a Teflon® bag in a 1000 mL glass beaker and prop the mouth open with one of the stainless steel wire loops. Carefully pour the contents of the cup into the center of the bag without letting it touch the loop or run down the sides of the bag. *Note: contents on the sides of the bag will prevent the heat sealer from sealing them properly.* More than 1 bag may be needed to contain the remaining material.
- 18) After the excess contents are in the Teflon® bag, remove the loop, lift the bag out of the beaker, squeeze the air out of it, hold it closed, and take it to the heat sealing station. Gently pull the top of the closed bag across the unit's Teflon®-coated element until the homogenized liquid in the bottom is about half an inch below it, pull the unit's arm down and hold it closed on the bag for about 3 seconds, release the arm, wait a few seconds, and then take the bag off of the element by gently pulling up one side. *Note: at a temperature setting of 4, hold the unit's arm down on the bag for about 2-3 seconds—any longer could melt the bag. Always separate sealed bags from the Teflon®-coated element slowly to avoid tearing the seams open. Practice on an empty Teflon® bag if needed.*
- 19) After the bag is sealed, roll it up, put it in a white cardboard cryotube, and neatly print the 12 character alphanumeric code assigned to the clutch on the side with a cyropen.
- 20) Place the tube(s) in the freezer. Record the number of Teflon® bags used on the appropriate line of the seabird data form.
- 21) Check over the seabird data form for the clutch of eggs you just processed for any obvious errors or omissions and file in the data form drawer. When all of the clutches

- from the same collecting site are processed, use the penciled in versions to make complete electronic copies in MS Word.
- 22) Take the measuring cup and any beakers used to the sink and fill them up with tap water until you're ready to begin cleaning them.
 - 23) Replace the UHV foil under the AirClean® Systems positive-pressure hood and the negative-pressure laboratory fume hood with fresh ones.
 - 24) Be sure all of the chemicals, Type 1 DI water, tools, glassware, and pieces of UHV foil are replenished to process another clutch of eggs.

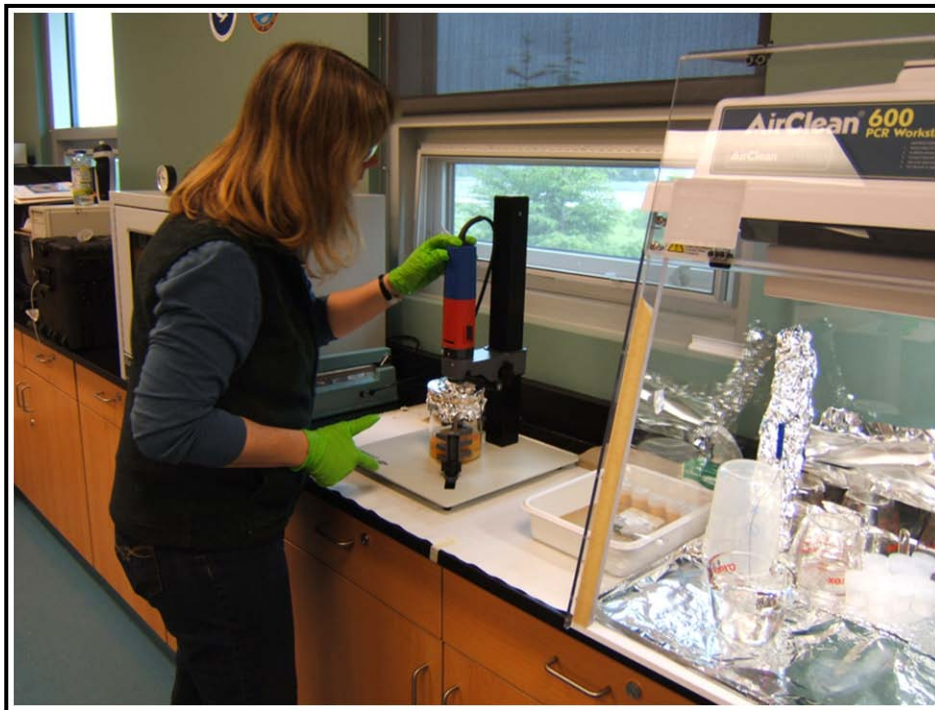


Photo courtesy of Dave Roseneau

Figure 12: Homogenizing egg contents in a pre-cleaned, UHV foil-covered 1-L glass beaker with the high-speed VirTis blender.

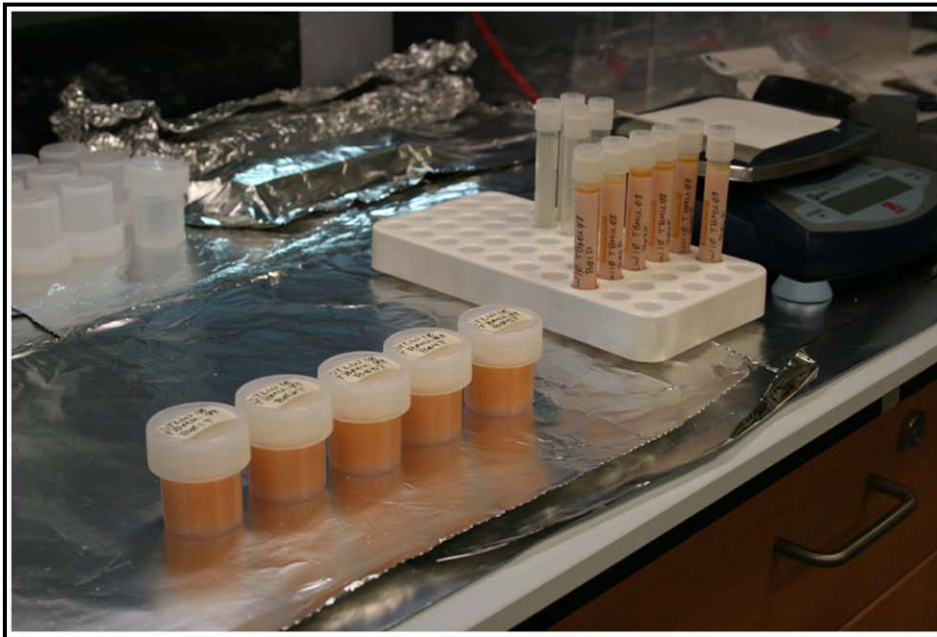


Photo courtesy of Dave Roseneau

Figure 13: Homogenized egg content aliquots in 5-15 mL Teflon® jars and 6 polypropylene cryovials ready to be frozen.

Cleaning Glassware

All glassware and stainless steel tools used to process the eggs must be chemically cleaned under the negative-pressure fume hood using the procedures listed below between each new clutch of eggs. Always wear safety goggles and talc free vinyl gloves when working with chemicals under the hood.

- 1) Before cleaning, prepare the negative-pressure fume hood. Turn it on, wipe the work surface down with methanol-dampened TexWipes and cover the work surface with a layer of clean UHA foil. Wipe down one of the medium-sized Rubbermaid® containers, line it with UHV foil, and set it on the foil at the back of the hood. Retrieve the 4-L glass waste bottle, methyl alcohol, acetone, and hexane squeeze bottles from the chemical storage locker. Fill the labeled squeeze bottles about half full, and return the large bottles to the locker. Open the waste bottle and set it aside in the back of the hood. Take a short-stemmed glass funnel and put it in the open mouth of the waste bottle. Tear off about 20 inches of UHV foil and lay it down near the front of the hood to provide a work area (replace this piece of foil with a fresh piece after it has been used a few times). Tear off several pieces of foil about 8 inches wide, tear them in thirds, and set them at back of the hood. Tear off several more pieces of foil about 12 inches wide and put them in the back of the hood. Close the hood and leave it running until cleaning is ready to begin. *Note: the larger pieces of foil can be torn into smaller pieces, as needed, to wrap clean tools in, and the smaller pieces will be used to cover the mouths of the measuring cups after you clean them.*

- 2) Using a TexWipe, wash the measuring cups, blades, spatulas, wire loops, and titanium knives with tap water and a drop or two of non-phosphate soap, and then give them a good rinse under the tap.
- 3) Rinse the tools and the inside of the measuring cups and beakers with Type I DI water using one of the squirt bottles.
- 4) Open the fume hood and place the rinsed items on a piece of clean aluminum foil under it.
- 5) When cleaning stainless steel tools (blender, knife blades, wire loops, shafts, spatulas, titanium knives) hold them over a spare glass measuring cup, one at a time, and using the squeeze-bottles rinse them with three squirts of methanol, three squirts of acetone, and then three squirts of hexane and let them dry on a piece of foil for a few minutes. Pour excess liquid into the waste container. Once dry, wrap each in a clean piece of UHV foil and place them at the back of the positive-pressure AirClean® Systems hood until they're needed again.
- 6) To clean the glass measuring cups and glass or polypropylene beakers, hold them upright in one hand and use the squeeze-bottles to rinse them out three separate times with methanol, acetone, and hexane by squeezing each of the chemicals around them just below their rims and letting the chemicals run down inside them. After each three-chemical rinse, empty the excess liquid into the waste bottle. Once rinsed, set them upright on the foil in the work area, close the hood, and let them dry for a few minutes. Once dry, open the hood, cover their mouths with pieces of UHV foil, and take them back to the AirClean® Systems hood and store them against the back wall.
- 7) Back under laboratory hood, pour the waste chemicals into it using the glass funnel and close the bottle. Place the STAMP squeeze-bottles and funnel in one of the STAMP trays and close the hood window down leaving it open a few inches. Let the fan run for about 10-15 minutes and then turn it off and close the hood all the way.
- 8) To clean pipettes, hold several of them at a time over a spare glass measuring cup and squirt them inside and out with three squirts of the three chemical mentioned above and place them in the foil-lined Rubbermaid® container (lean them against the side of the container so they're standing at an angle). Close the hood and let them dry for several minutes, then wrap them in UHV foil and take them to the AirClean® Systems hood and store them against the back wall.
- 9) Back under to the laboratory hood, pour the waste chemicals into it using the glass funnel and close the bottle. Remove the glass funnel from the waste bottle, hold it over a spare measuring cup, rinse it several times with hexane, and set it down on a clean piece of UHV foil. Retrieve the 4-L bottle of hexane from the chemical storage locker, put it under the hood, remove the cap, insert the funnel, pour the contents of the hexane squeeze bottle into it, remove the funnel, replace the cap, and take it back to the storage locker. *Note: never leave hexane in the squeeze bottle over night because it will*

eventually ruin the cap—always leave the cap off of the squeeze bottle after you empty it so it can dry out.

Drying and Shipping Eggshells

Once the egg contents are removed, rinse the shell halves out with Type 1 DI water; set them open side down on a clean TexWipe and let them drain dry for several minutes. Place the eggshells in individual paper lunch bags that has the alphanumeric identification number of the clutch and “Egg A”, “Egg B”, or “Egg C” (as appropriate) written on it; close the bag by folding the top over and creasing it; and set it aside on the counter top near the drying oven (see “Removing contents from shells” section above). Then, as time allows over the next few days, do the following:

- 1) Open the drying oven; Take 12 small TexWipes out of their packet and put 6 of them on the top rack and 6 of them on the bottom rack, spacing them about 1 inch apart. Assign them the numbers 1-12, using a left-to-right, back-to-front, and top-to-bottom scheme as shown below.

Top Rack

1	2	3
4	5	6

Bottom Rack

7	8	9
10	11	12

- 2) Pick up one of the paper bags containing the shells from one of the eggs, write #1 on it, remove the shell-halves, and put them on TexWipe square #1 in the back left-hand corner of the top rack (see above).
- 3) Choose 11 more bags and repeat the above process until there are shells on all of the TexWipe squares on both oven racks.
- 4) Close the drying oven’s door, turn it on, and set the temperature dial to 3.
- 5) Leave the shells in the oven for 48 hrs. Once dry, refer to the empty paper bags and write the eggs’ 12 character alphanumeric identification numbers on 12 clean plastic bags.
- 6) Remove the shells from the oven, put each in a plastic bag, and put the sealed bags in a 12” x 12” x 12” USPS Priority Mail box padded with bubble wrap and tape it shut.
- 7) Repeat steps 1 through 6 with the rest of the clutches.

- 8) Temporarily store the boxes containing the shells in one of the lab cabinets under the counter until you are ready to ship them to Dr. Kevin Winker at the University of Alaska Museum in Fairbanks, Alaska.

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

- 9) When all the shells are ready to be shipped to the museum in Fairbanks, pack the boxes in the larger cardboard cartons stored in the lab (four boxes per carton), address them to Kevin, put several “Fragile—Handle with Care” and “Priority Mail” stickers on them, weigh them, print out the postage on the postage meter, and take the metered cartons to the Post Office.

- 10) Call or e-mail Dr. Kevin Winker to let him know a shipment of eggshells is on the way via Priority Mail.

Shipping Frozen Egg Samples

The Marine ESB provides LN₂ vapor dry shippers in protective fiberglass containers to transport frozen samples to Charleston. When properly charged, these “dry-shippers” will keep samples frozen for several days.

To prepare the samples for shipping, put all of the Teflon® jars and polypropylene vials from each clutch into individual Teflon® bags (e.g. one bag should contain 5 Teflon® jars and 5-6 cryovials) and seal them shut two or three times with the heat sealer. Bag the clutches one at a time and run them through the heat sealer as soon as you can to avoid moisture condensing on the sides of the bags. If moisture is present, the seams will not seal properly. If this happens, seal the bag again 1-2 more times just above the original seam to help keep it from popping open when it’s shipped.

When you’re ready to send samples to the specimen bank, contact Rebecca Pugh in Charleston at (843)762-8952 (rebecca.pugh@noaa.gov) and ask her to send you a fully charged dry-shipper via FedEx. If Rebecca is unavailable, contact Amanda Moors at (843)762-8953 (amanda.moors@noaa.gov) or Lauren Rust at (843)762-8951 (lauren.rust@noaa.gov). Always try to contact Rebecca on Wednesday, so she’ll have plenty of time to charge the shipper over the weekend and send it out via FedEx on Monday morning. When it arrives, put the Teflon® bags containing the samples in it and then arrange to have FedEx pick it up the following morning (Wednesday; it must be ready by 9 AM). If it leaves Homer Wednesday morning, Rebecca will receive it Thursday afternoon. Do not send shippers to the Marine ESB late in the week. If the samples cannot be sent out by Wednesday, call a Marine ESB personnel to discuss further options.

The dry-shippers consist of heavy cylindrical fiberglass containers that have LN₂ vapor-charged flasks secured inside them. The flasks are charged with nitrogen vapor by filling them three-quarters of the way up with LN₂, letting them stand for 24 hours, refilling them to the half way mark, letting them stand for another 3-4 hours, and then pouring off the excess LN₂. The fiberglass containers containing the charged flasks have hinged lids that latch closed with stainless steel clamps. The LN₂ flasks are sealed with large foam stoppers (plugs). When you're ready to put samples in a flask, be sure to put on a heavy long-sleeved shirt, sweater, or light jacket, have a pair of insulated gloves handy, and follow the directions below (the vapor in the flasks is about -70 °C and it will freeze bare skin fast!).

- 1) Bring the dry shipper into the laboratory and open it, removing the foam stopper.
- 2) Wearing cryogloves, gently place the frozen bags containing aliquoted eggs and the cardboard tubes containing remaining sample into the dry shipper(s). Leave enough room to return the foam stopper.
- 3) Double check the seabird data forms for completeness and accuracy. Any deviations or modifications of the protocol must be noted on the form. *Note: please also send the datasheets by e-mail/disk.*
- 4) Place a copy of the completed and signed forms in a plastic bag or manila envelope. Place this bag or envelope between the protective shipping cover and the dry shipper; close and latch and secure with cable ties.
- 5) Fill out a FedEx label and attach it to the top of the shipper. Ship the dry shipper(s) with the samples to the NIST Marine ESB in Charleston, South Carolina, within 48 hours or as soon as possible using 24-hour express package service to:

NIST Charleston Lab
Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, South Carolina 29412
Attn: Rebecca Pugh
(843) 762-8952

- 6) Notify the NIST Marine ESB personnel by telephone as soon as possible after the frozen samples are shipped:

Rebecca Pugh (843) 762-8952 or
Amanda Moors (843) 762-8953 or
Lauren Rust (843) 762-8951 or
Paul Becker (843) 725-4815

Maximum holding time for the shippers is 10 to 12 days. Shipping costs will be paid by NIST. Never ship samples late in the week, or before holidays, unless special arrangements have been made with the shipping service and the NIST Marine ESB laboratory personnel.

Egg Sample Archival Procedures

When the dry-shippers arrive at the Marine ESB, the egg samples are unpacked, checked for temperature and packaging problems, and put in temporary cold storage. 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 ready to be stored in a long-term location in the LN₂ vapor-phase freezers (-150°C).

When a sample is moved to the long-term freezer storage location, a Marine ESB Storage Form (Appendix 2) is completed documenting the archiving process and the specific storage location of the sample. Each egg sample is given a unique Marine ESB Storage ID Number (Figure 14), according to the format described in Pugh *et al.* (2007). This number is entered onto the seabird data form as well as the storage datasheet to act as a cross reference. In general, the first two characters (letters) represent the project (e.g. “ST” for STAMP), the next two characters (numbers) represents the year of the project the eggs were collected (e.g. “01” is the first year of the project and the eggs were collected in 1999, “02” is the second year of the project and the eggs were collected in 2000, etc.). The next character (letter) indicates the tissue type (e.g. “E” for egg, “M” for embryo), the following character(s) (number(s)) identifies the next sequential number of egg or embryo that has been collected since the project started (e.g. “001” is the first egg banked, “250” is the 250th egg banked), and the last character (letter) is always a “C”, indicating the eggs are banked in Charleston.

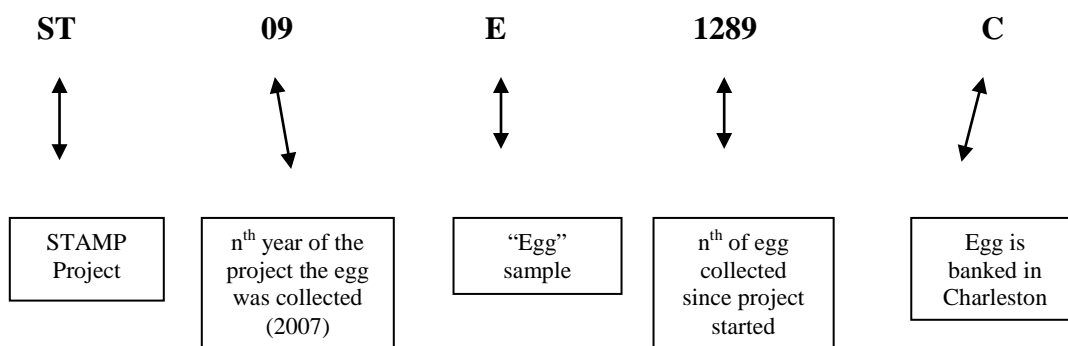


Figure 14: Storage ID Number.

The storage datasheet also includes the number of eggs in a clutch (e.g. A, B, and C), their corresponding weights, the date they were banked, the date they were homogenized, the storage locations of the aliquots and the personnel's initials. When a subsample is requested, the date the subsample is removed is also recorded here. The information above is entered into the computerized Marine ESB's Paradox and Freezerworks databases, and hard copies are filed away. After a request for samples is received and approved, Marine ESB personnel will remove one 5g egg subsample (Teflon jar or polypropylene cryovial) for each requested sample from its LN₂ storage location and give them to the requesting parties. When this occurs, the corresponding storage forms and computerized databases are updated to include the dates the samples were pulled from the freezers, the name of the person receiving them, and a description of the analyses that will be run on them.

The seabird field datasheet (Appendix 1) containing detailed information from the time the egg was collected to the time it is received at the Marine ESB is also entered into Paradox, Freezerworks and Excel databases. The field datasheet is scanned, given the file name corresponding with the Storage ID number and saved in a secure STAMP folder. The storage ID numbers are added to the field datasheet to act as a cross reference; the hardcopy is filed away at the Marine ESB, and upon request, will be made available to interested parties. The samples will remain in the LN₂ vapor-phase storage freezers at approximately -150° C until they are requested and released for future analysis.

SUMMARY

This report provides detailed information on the most current updated versions of the protocols used to collect, process, and bank seabird egg samples for STAMP. Current plans are to store all samples collected to date and in upcoming years in the Marine ESB indefinitely so that they can be used by NIST personnel and other researchers to track long-term trends in contaminants in Alaska's marine environments and serve as a source of material for retrospective analyses.

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Appendix 1: Seabird Data Form.

SEABIRD TISSUE ARCHIVAL MONITORING PROGRAM

SEABIRD DATA FORM

I Sample ID Number: KUKP01GLGU08

Species: Glaucous Gull

Geographic Area: Eastern Chukchi Sea, Alaska **Lat.:** 68° 22' 52.25" N **Long.:** 166° 19' 32.20" W

Colony Name: Kukpuk River Delta **Sample Source:** _____

Site ID Number/Name: _____

II Sample Type: Liver Kidney Muscle Feather Egg Blood Other: Egg

Date and Time of Death (If Applicable): _____

Date and Time of Collection: 07/05/2008 (time unknown) **Method of Collection:** Gloved hand

Weather Conditions: Unknown

Field Storage Conditions: Stored outside in cool place until 07/06/2008 and then shipped to Anchorage via Kotzebue at about 1100 hrs on 07/07/2008 (Frontier Air). Arrived in Anchorage on 07/08/2008 and kept in a cool place in the Frontier hanger until they were picked up by ERA Airlines and shipped to Homer about 0700 hrs on 07/09/2008. They arrived in Homer at about 0750 hrs and were picked up and delivered to FWS headquarters and put in the STAMP refrigerator at about 0900 hrs that same morning.

Pre-shipment Storage Conditions: (see above).

Date and Time Shipped from Study Site: Taken to Point Hope via ATV on 07/05/2008, shipped to Anchorage on 07/07/2008, and then shipped to Homer on 07/09/2008 (see above).

Protocol: Standard.

Comments: Latitude and longitude calculated using Google Earth (approximate center of the river delta—see Google image)

Additional Samples: 14 other useable samples (clutches) were collected from the same location on the same date.

Collected by: Elijah Lane

III Sample ID Number: KUKP01GLGU08 **Storage ID Number:** _____

Date and Time of Sample Preparation: 07/09/2008 at 1335 hrs. **Processor:** Karen Corbell

Date and Time of Freezing (-80° C or below): 07/09/2008 at 1520 hrs (about -30° C)

Date and Time of Shipping to MESB: 16 July 2008 at 1000 hrs **Shipper:** Karen Corbell

Date and Time Received at MESB: _____ **Receiver:** Rebecca Pugh

IV Egg Measurements:

	A	B	C
Whole egg (g)	89.40 g		
Length (cm)	6.951 cm		
Breadth (cm)	4.967 cm		
Egg contents (g)	79.40 g		
Egg shell weight (g)	9.70 g		

Comments: 1-egg clutch. The difference between the whole egg weight and the sum of the egg contents and shell weight reflects the amount of contents that were lost during processing.

V Sample Aliquots:

Number of Polypropylene Vials: 5 (B01P, B02P, B03P, B04P, B05P)

Number of Teflon Jars: 5 (B01T, B02T, B03T, B04T, B05T)

Number of Teflon Bags: 0

VI

Form Prepared by: _____
David G. Roseneau
(Print Name)

(Signature)

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

Rebecca Pugh
Research Biologist
NIST, 331 Fort
Johnson Road
Charleston, SC 2941

Appendix 2: Marine ESB Storage Data Form

NIST SPECIMEN BANK													
Sample Storage													
	Storage ID				Wt (g)	Date In			Date Out			Initials	
	Fz	Rack	Box			Day	Mo	Yr	Day	Mo	Yr		

Comments _____													
Subsamples		Storage ID				Wt (g)	Date In			Date Out			Initials
LN2	Other	Fz	Rack	Box	XY		Day	Mo	Yr	Day	Mo	Yr	
B001													
B002													
B003													
B004													
B005													
B006													
B007													
B008													
B009													
B010													
B011													
B012													
B013													
B014													
B015													
B016													
B017													
B018													
B019													
B020													

Appendix 3: Abbreviations for colony sites and bird species.

STAMP Egg Collecting Sites	Abbreviation
Cape Lisburne, eastern Chukchi Sea	CLIS
Kukpuk River delta, eastern Chukchi Sea	KUKP
Cape Thompson, eastern Chukchi Sea	CTOM
Noatak River delta, Kotzebue Sound	NOAT
St. Lawrence I. northern Bering Sea	STLW
Sledge Island, Norton Sound	SLED
Safety Sound, Norton Sound	SAFE
Cape Denbigh, Norton Sound	CDEN
Hooper Bay, eastern Bering Sea	HOOP
Triangle Island, eastern Bering Sea	NUNI
Shaiak Island, Bristol Bay	TOGI
St. George I., southeastern Bering Sea	STGE
St. Paul I., southeastern Bering Sea	STPA
Bogoslof Island, southeastern Bering Sea	BOGO
Aiktak I., eastern Aleutian Is.	AIKT
Buldir Island, western Aleutian Is.	BULD
East Amatuli I., northern Gulf of Alaska	EAAM
Gull I., Kachemak Bay	GULL
Duck I., western Cook Inlet	DUCK
Kodiak I., northwestern Gulf of Alaska	CHBY
Tatitlek vicinity, northeastern Prince William Sound	TATI
St. Lazaria I., southeastern Gulf of Alaska	STLA
Viesokoi Rock, southeastern Gulf of Alaska	SITK
Cape Deceit, Kotzebue Sound	DEER
Cape Pierce, Bristol Bay	CPEI
Ualik Lake, Bristol Bay	UALK
Kikertalik Lake, Bristol Bay	KILK
Little Diomed I., Bering Strait	DIOM
Shoup Bay, northern Prince William Sound	SHBY
Middleton I., northern Gulf of Alaska	MIDD
Bluff, Norton Sound	BLUF
Tatoosh I., northeastern Pacific Ocean	TATO
Shishmaref Inlet (2 nd East Channel), eastern Chukchi Sea	SHFA
Shishmaref Inlet (Egg I. and Arctic Lagoon), eastern Chukchi Sea	SHFB
Shishmaref Inlet (Tin Cr.), eastern Chukchi Sea	TINC
Brevig Mission, northern Bering Sea	BREV
King Island, northern Bering Sea	KING
Sinuk River delta, Norton Sound	SINU
Carolyn I., Golovin Bay, Norton Sound	GOBA
Cape Darby, Norton Sound	CDAR
Shaktoolik, Norton Sound	SHAK
Unalakleet River delta, Norton Sound	UNAL
Stuart I., Norton Sound	STUA
Seabird Species	Acronyms
Black-legged Kittiwake (<i>Rissa tridactyla</i>)	BLKI
Glaucous Gull (<i>Larus hyperboreus</i>)	GLGU
Glaucous-winged Gull (<i>L. glaucescens</i>)	GLGW
Common Murre (<i>Uria aalge</i>)	COMU

Thick-billed Murre (*U. lomvia*)

TBMU

Unidentified Murre species (*U. spp.*)

UNMU
