

MONITORING CONTAMINANTS IN SEABIRDS: THE IMPORTANCE OF SPECIMEN BANKING

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SUMMARY

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Historically, contaminants in seabird tissues were monitored by considering physically visible effects; however, subtle, sub-lethal effects are also important. Several long-term monitoring and specimen banking programs are in place for seabirds, and the present paper reviews recommendations for appropriate samples for banking. Current applications of specimen banking programs are discussed, including temporal comparisons (especially of emerging contaminants), comparison of analytical techniques, and development of reference materials. Recommendations for seabird tissue specimen banks include publication of available samples; standardized methods for collection, processing and storage of samples; and inclusion of information about sample collection conditions.

Key words: Contaminants, biomonitoring, specimen bank, tissue bank, seabirds

INTRODUCTION

During the 1960s and 1970s, monitoring the effects of contaminants on birds was straightforward. The very visible effects of contaminants are seen in the well-known cases of DDT and other chlorinated pesticides causing eggshell thinning and subsequent population collapse of raptors and pelicans; the Great Lakes Embryo Mortality, Edema, and Deformities Syndrome (GLEMEDS), most visibly identified with cross-billed cormorants (see Gilbertson *et al.* 1991); and even bird deaths from oil pollution. For the most part, the production and use of contaminants that cause very visible effects have been banned or restricted, and the occurrence of the effects have declined. However, other current use pesticides and anthropogenic chemicals produce more subtle, sub-lethal effects, the significance of which for avian health may be difficult to determine. Many papers have been published on sub-lethal effects of currently used contaminants in birds, including effects on immune, nervous and endocrine systems; hepatic, renal and thyroid function; and reproductive and behavioral abnormalities (see Briggs *et al.* 1996, Davis *et al.* 1997, Brunström and Halldin 2000, Bustnes *et al.* 2000, Gabrielsen & Henriksen 2001, Monteiro & Furness 2001, de Roode *et al.* 2002, Kuzyk *et al.* 2003). Thus the need for monitoring contaminant concentrations continues.

An important component of any monitoring program is the relevance and accuracy of the measurements. Avoiding the “shifting baseline syndrome” (Sheppard 1995) requires historical knowledge that may be obtained from literature searches. However, for emerging contaminants such as brominated flame retardants (BFRs) and perfluorinated compounds (PFCs), little or no historical knowledge exists. For polychlorinated biphenyls (PCBs), analytical techniques have changed, making comparisons with historical data tenuous. Historically, PCBs were measured using Aroclor standards, but now

individual congeners are measured. Using the sum of congeners as a measure of total PCBs results in values that are less than half of those historically reported for PCBs, solely because of differences in analytic techniques (Turle *et al.* 1991). Recognition of such factors, therefore, emphasizes the importance of having a resource of banked samples to investigate changes in contaminant exposure and accumulation over time.

The present paper reviews the application of biological specimen banking to seabird monitoring and research, and describes the role of banking in an ongoing seabird monitoring program in Alaska. It also presents some recent results from that program. The Seabird Tissue Archival and Monitoring Project (STAMP) began collecting, banking and analyzing Alaskan seabird eggs in 1999. STAMP began as a collaboration among the US Fish and Wildlife Service–Alaska Maritime National Wildlife Refuge (USFWS–AMNWR), the US Geological Survey–Biological Resources Division (USGS–BRD), and the National Institute of Standards and Technology (NIST). The Bureau of Indian Affairs–Alaska Region Subsistence Branch (BIA–ARSB) later joined the effort, and the North Pacific Research Board recently provided additional funding support.

SELECTING SEABIRDS AND TISSUES FOR MONITORING

As was illustrated by Mallory *et al.* (2006) and described in the Arctic Monitoring and Assessment Programme (AMAP 1998), marine birds are useful as indicators of physical change in the environment with implication for climate changes and of changes in contaminant levels in the environment. Hollamby *et al.* (2006) evaluated the process of selecting an appropriate avian species for biomonitoring. Based on Hollamby *et al.* (2006) and the recommendations of Wise and Koster (1995) for the kinds of specimens that should be banked for long-term studies, the following criteria should be

used to determine the seabird species and tissue types that are most appropriate for monitoring and banking:

- The species and tissue should bioaccumulate contaminants in concentrations that can be measured in relatively small amounts, but the tissue should be large enough for multiple analyses for future research.
- The species and tissue should be easy and relatively inexpensive to sample so that multiple-year collections at the same sites and times may be obtained.
- The tissue samples must be properly collected and stored to eliminate extraneous contamination or changes in the contaminants of interest.
- The species and tissue must be representative of the area to be monitored, consistent with the goals of the project.

Several seabird species and tissues have previously been used for long-term monitoring and banking programs (Table 1). In 1998, AMAP identified eggs from the seabird family Alcidae (murre, murrelets, auklets, guillemots, puffins, dovekeys, and razorbills—all diving species) as key tissues for circumpolar monitoring of persistent organic pollutants by all Arctic nations (AMAP Scientific Experts Workshop, Girdwood, Alaska, April 1998). Based on this information, STAMP selected Common Murre *Uria aalge* and Thick-billed Murre *U. lomvia* eggs as the priority matrix for long-term (100-year) monitoring and banking. Eggs from Black-legged Kittiwakes *Rissa tridactyla* were also included to represent a surface-feeding species to compare with the deep-diving murre, thus obtaining representation from different parts of the food web. The banking of Black-legged Kittiwake eggs was later suspended because of difficulties in obtaining entire multi-egg clutches and the potential complications of contaminant level variations attributable to differences in first-laid as compared with second- and third-laid eggs (see Pastor *et al.* 1995). Two species were added to the project at the request of local Alaskan subsistence harvesters and to correlate with other studies (e.g. see Muir *et al.* 1999, Riget & Dietz 2000, Gabrielsen & Henriksen 2001, Verreault *et al.* 2005). Glaucous Gull *Larus hyperboreus* and Glaucous-winged Gull *L. glaucescens* egg collections began in 2004 with the entire clutch banked as one sample.

Common and Thick-billed Murre eggs are ideal for monitoring contaminants in the Alaskan marine environment. These piscivorous species are near the top of the food web and bioaccumulate contaminants to levels that are easily measured. Murres lay a single large egg, the contents providing on average 88 g of sample with only 4 g needed for current analyses; thus, a large banked sample can be retained for future research. The single-egg clutch limits the effect of laying order on variability in contaminant loads (Pastor *et al.* 1995). Eggs are relatively easily collected from birds breeding on cliffs in regions where researchers or subsistence harvesters have access. Minimal training and equipment are required for proper collection and storage before shipment for processing and banking. Each egg represents a complete, distinct unit, and the banked, easily homogenized contents are protected from extraneous contamination by the eggshell and membrane.

Murres stay in the northern latitudes year-round and arrive on their breeding grounds several weeks before egg-laying (e.g. Ehrlich *et al.* 1988, Gaston & Hipfner 2000). Therefore, contaminant concentrations in the eggs reflect contaminant conditions in the local region and are representative of the adult female at the time

of laying (Braune *et al.* 2001). Murres are abundant, and about 80% of the pairs that lose eggs early in the breeding season re-lay eggs within about 15 days (e.g. see Tuck 1960, Cramp 1985), so that collecting small numbers of eggs early in the season does not detrimentally affect nesting populations and as replacement eggs may have different contaminant profiles (see Bignert *et al.* 1995), the timing of egg collection needs to be early in the egg-laying season. Finally, monitoring contaminants in murre eggs is important, because the eggs are used for human subsistence food in Alaska and throughout the Arctic.

SPECIMEN BANKING FOR SEABIRDS

The importance of specimen banking is well established, with several past journal issues being devoted to this topic [*Science of the Total Environment* 1993 Vol. 139–140, *Chemosphere* 1997 Vol. 34(9–10), and *Journal of Environmental Monitoring* 2006 Vol. 8(8)]. Specimen banks are distributed worldwide with samples collected from a wide variety of geographic regions, including Antarctica (see Riva *et al.* 2004). The type of biological samples maintained in the specimen banks are also quite diverse and include materials such as moss and lichen, pine needles, vegetables, rodents, tissues of large game animals, krill, mussels, fish, marine mammal tissues, and human tissues; a few also include birds.

Not all of the research using banked specimens is focused on contaminant monitoring. Some banked materials are stored for genetic research, including possible re-introduction of species and for medical development, including determining biomarkers and development of new drugs. The number of specimen banks with seabird materials is relatively small (see Table 1), but the estimated large number of private researcher collections—although they may not necessarily follow the stringent standardized collection and archival protocols of the formal specimen bank—may also provide valuable samples for future research. Some current specimen banks are actually extensions of museums of the past, and researchers have been able to integrate samples stored in museums and specimen banks (see Bignert *et al.* 1995, Dietz *et al.* 2006).

Museum feather samples have long been used to monitor for mercury, and recently, they were determined to be feasible for analysis of organic contaminants (Jaspers *et al.* 2007). However, some questions regarding contamination from storage techniques have arisen (Hogstad *et al.* 2003), reinforcing the need for standardized procedures that attempt to eliminate extraneous contamination.

Recent applications of banked seabird tissues

As mentioned earlier, specimen banks do far more than just act as short-term storage or holding for samples before analyses. Seabird samples from specimen banks have been used to retrospectively analyze for new contaminants of interest.

Flame-retardant compounds have received much recent attention. Analysis of banked specimens have shown that BFRs increased in Common Murre eggs collected from the Baltic Sea from 1969 until the mid-to-late 1980s and then decreased again through 2001 (Sellström *et al.* 2003). In Double-crested Cormorant *Phalacrocorax auritus* eggs collected from British Columbia coast in Canada between 1979 and 2002, BFR concentrations peaked in 1994 (Elliott *et al.* 2005), but BFRs only increased in Herring Gull *L. argentatus* eggs collected from the Great Lakes between 1981 and 2000 (Norstrom *et al.* 2002).

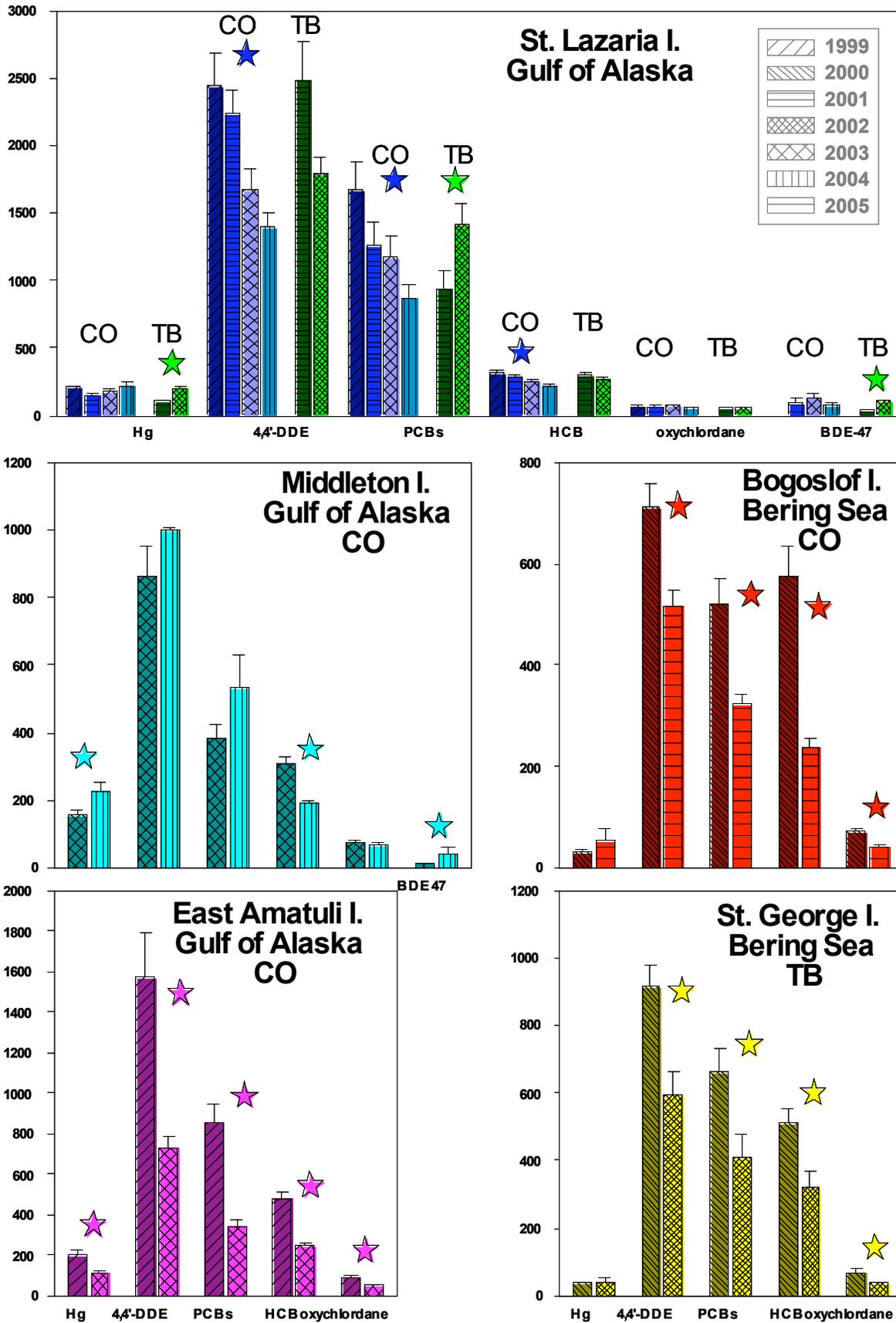


Fig. 1. Temporal comparisons of selected contaminants for Common Murre *Uria aalge* (CO) and Thick-billed Murre *U. lomvia* (TB) eggs collected as part of Seabird Tissue Archival and Monitoring Project (STAMP) between 1999 and 2005. Concentrations are based on lipid mass, with the exception of mercury, which uses wet mass. Stars indicate significant ($P < 0.05$) differences between years based on analyses of variance.

Another new contaminant of interest is PFCs used in Teflon (DuPont, Wilmington, Delaware, USA) and Scotchguard (3M, St. Paul, Minnesota, USA). Butt *et al.* (2007) demonstrated an overall increase in PFCs using archived livers of Thick-billed Murres and Northern Fulmars *Fulmarus glacialis* collected from Prince Leopold Island, Canada, between 1975 and 2004.

Herring Gull eggs banked in Germany were used to retrospectively monitor for synthetic musk compounds (Rüdel *et al.* 2006) and organotins (Rüdel *et al.* 2003).

The use of banked seabird samples has also been used to re-examine the effects of changing analytical techniques (Turle *et al.* 1991) and earlier conclusions regarding concentrations and temporal changes. For example, Norstrom and Hebert (2006) used banked Herring Gull eggs to reanalyze older samples for direct comparison with current samples for PCBs, the insecticide hexachlorobenzene (which had poor recoveries using earlier analytical techniques), and contaminants that were not routinely measured during 1971–1982, such as dioxins, furans, photomirex, and octachlorostyrene. Their retrospective analysis provided data for modeling trends and effects during that highly contaminated period.

Retrospective analyses of banked seabird specimens for contaminants may also provide new insights into issues such as global fractionation and effects of control measures (Bignert *et al.* 1998) and dietary influences (Hebert *et al.* 1997). The influence of climate change on the global patterns of contaminant transport, deposition and bioaccumulation is another factor that will increase interest in banked environmental and biological specimens for retrospective analysis. Mercury research has already revealed some interesting differences in contamination patterns. Koster *et al.* (1996) showed declining mercury concentrations in Herring Gull eggs from the Great Lakes between 1972 and 1992. Levels in eggs of Northern Fulmars and Thick-billed Murres increased between 1975 and 1998 in the eastern Canadian Arctic, but eggs of Black-legged Kittiwakes were unchanged during that period (Braune *et al.* 2001). Common Murre eggs from the Baltic showed a declining mercury trend between 1969 and 1993 (Odsjö *et al.* 1997). Recent research from STAMP indicates

patterns that are changing with time for mercury and several organic contaminants (Fig. 1). These trends appear to be related to study location, species and sampling year.

One benefit of specimen banks is that they can provide all specimens for analysis by a single laboratory, thus eliminating the differences arising from inter-laboratory quality assurance. However, another important aspect of specimen banks has been the development of matrix-matched reference materials for the analysis of banked tissues to ensure inter-laboratory comparability (Reddy *et al.* 1993). Researchers in Germany have developed internal reference material for contaminant analyses of Herring Gull eggs (Emons *et al.* 1998). Likewise, Canadian researchers have developed control materials for Herring Gull egg and Double-crested Cormorant egg analyses (Wakeford & Turle 1997, B. Wakeford pers. comm.). The development of a murre egg control material for use in conjunction with STAMP has been previously described (Vander Pol *et al.* 2007) and is available on request for use by analytical laboratories.

Recommendations

If researchers are to be able to access banked specimens, they need to know what is available and where it is stored. Specimen bank inventories and analytical results from banked specimens have to be readily available to interested researchers. A published policy for accessing specimens should also be established by the banks maintaining the specimens. Banked specimens should be carefully chosen to monitor the ecosystem of interest and should contain all information about collection conditions. Ideally, the collection and processing conditions of banked samples should be consistent over time and should eliminate extraneous contamination. If relevant information and specimen bank inventories are available, carefully chosen, collected, and banked seabird tissues will allow future researchers a method to determine past changes in the global environment.

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TABLE 1
List of seabirds and tissues used in long-term banking and monitoring programs

Species	Tissue	Location	Banking started	Reference	
Common name	Scientific name				
Common Murre	<i>Uria aalge</i>	Eggs	Sweden	1969	Bignert <i>et al.</i> 1995
Herring Gull	<i>Larus argentatus</i>	Eggs	Great Lakes, Canada	1974	Hebert <i>et al.</i> 1999
Northern Fulmar	<i>Fulmarus glacialis</i>	Eggs and liver	Prince Leopold Island, Canada	1975	Braune <i>et al.</i> 2001 and Butt <i>et al.</i> 2007
Black-legged Kittiwake	<i>Rissa tridactyla</i>	Eggs	Prince Leopold Island, Canada	1975	Braune <i>et al.</i> 2001
Thick-billed Murre	<i>Uria lomvia</i>	Eggs and liver	Prince Leopold Island, Canada	1975	Braune <i>et al.</i> 2001 and Butt <i>et al.</i> 2007
Black Guillemot	<i>Cephus grylle</i>	Breast muscle and liver	Iceland	1976	Ólafsdóttir <i>et al.</i> 2005
Double-crested Cormorant	<i>Phalacrocorax auritus</i>	Eggs	British Columbia, Canada	1979	Elliott <i>et al.</i> 2005
Herring Gull	<i>Larus argentatus</i>	Eggs	Germany	1988	Marth <i>et al.</i> 2000

collected eggs, including for the samples reported here: Alexis Paul, Susan Hatch, Dean Kildaw, Arthur Kettle, Laurie Ness, Nora Rojek, Vernon Byrd, Leslie Slater, Julie Snorek, Andrew Ramey, Jessica Fisher, and J. Williams. Leslie Chappel and Kristin Simac (USGS) processed the eggs, while Rebecca Pugh, Michael Ellisor and Amanda Moors (NIST) provided specimen banking support that included cryohomogenizing egg specimens and coordinating specimen shipments. Mercury analysis was conducted by Rusty Day (NIST). STAMP would probably not exist without the tireless support and work, including obtaining permits and co-ordination of collections, conducted by Dave Roseneau (USFWS). This is publication number 158 of the North Pacific Research Board which provided funding under grant number 0534. This is publication number 158 of the North Pacific Research Board which provided funding under grant number 0534.

DISCLAIMER

Any mention of commercial products is for information only; it does not imply recommendation or endorsement by NIST.

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