

Protocol for Sampling Sea Duck Tissues for DNA analysis

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Characterization of genetic structure within and among breeding and wintering areas of sea ducks

Molecular genetic markers are providing another tool to help characterize breeding and wintering areas of sea ducks along with assessing relationships among individuals residing within the same population. Breeding and wintering areas can be characterized by examining differences in allelic frequencies among sampled sites. In addition, individuals can be assigned back to putative populations of origin to assess admixture of breeding sites on the wintering grounds. Molecular markers can assess inter-individual relationships among putative family groups (i.e. paternity) or the familial relationships among nesting individual (i.e. kin-based groupings/microgeographic structure). They can also be used to determine the sex of individuals when sex is unknown (that is, if feathers or eggshell membranes only are available). Molecular markers can also be used to understand adaptive evolution if differences in functional genes, such as those associated with immune response, are assayed.

Sample Collection

DNA samples can be collected in a variety of ways. The following table lists various sample collection methods and appropriate storage.

Collection Method	DNA preservation technique	Short-term Storage
Blood	Blood Buffer* (Longmire) in vials	Room Temperature
Blood	Filter Paper in envelopes (silica gel)	Dry, Room Temperature
Blood quills	Tissue or Longmire Buffer*	Room Temperature
Bone	Envelopes (silica gel) or Dry Microtubes	Dry, Room Temperature
Buccal swab	Longmire Buffer	Room Temperature
Egg shell membranes	Envelopes (silica gel) or Dry Microtubes	Dry, Room Temperature
Feathers	Envelopes	Dry, Room Temperature
Muscle	Tissue Buffer	Room Temperature

*Longmire Buffer (Longmire et al. 1988) is the recommended blood preservation buffer for storage of samples in the field. Researchers have determined experimentally that “Queen’s Buffer” (Seutin et al. 1991) and “SET” lysis buffer (0.15M NaCl, 0.05M Tris, 0.001M EDTA, pH 8.0) can cause difficulties in preservation and extraction of DNA from some species and sample types, if samples are not extracted within a few days (Conrad et al. 2000), and particularly when working with microsatellite loci. We have not observed this difficulty with blood samples stored in Longmire Buffer at ambient temperatures for up to several years (Talbot et al. unpublished data).

Sample Archival

Place sample in the above recommended technique. Be sure to label vials or envelopes with species ID, a unique number (i.e. band number) or collection name (i.e. nest ID), locality, collection date, sex and age if known, and collector.

1. Check to ensure that your sample collection kit contains 1.7 mL microcentrifuge tubes containing the appropriate preservation buffer, a Sample Collection Form, and a Sharpie marker.
2. On the Sample Collection Form, record data associated with the respective sample (i.e. species, sample ID or band number, collection location, collection date, sex and age if known, and collector). Additional data may be required, depending upon study.
3. Label the outside of the box (i.e. species, collection location, collection dates, collector name, and contact telephone number).
4. Samples collected in the United States can be mailed via regular post to the Alaska Science Center, USGS, Molecular Ecology Laboratory for storage and archival. Samples collected outside of the United States require a USDA import permit included in the shipment that is on file at the Molecular Ecology Laboratory. Please contact us before shipping samples collected outside of the US so we can provide you with the proper permits and additional instructions. Please notify Kevin Sage (ksage@usgs.gov; phone 907-786-7193) when you have mailed the samples.

Please address samples to:
Kevin Sage
Alaska Science Center, USGS
4210 University Dr.
Anchorage AK 99508

Collection Methods for DNA Analysis

■ **Blood** (*Blood/1 Vial/Blood Buffer/Room Temperature*)

Only a few drops of blood are needed from each bird, enough to turn the buffer red (usually about 5 - 10 drops, about 30 to 60 μ L).

Blood samples can be taken from the brachial, jugular, or tarsus veins. Brachial and jugular veins require drawing blood using a new sterile syringe for each individual. Samples should only be collected in this manner by an experienced person. Do not use heparinized needles, syringes, or vials. Heparin inhibits DNA amplification making molecular data collection difficult or impossible. Therefore if blood clotting is an issue, consider using EDTA instead of heparin.

Blood can be obtained by pricking the tarsus vein and transferring the drops to the tubes by way of capillary tubes. Use a capillary tube bulb to expel blood from capillary tubes (do not use your mouth) into the buffer after collection. If the blood clots inside the capillary tube break off the tube inside the buffer vial and just leave it there.

We have provided 1.7 mL Eppendorf tubes with a blood buffer storage solution (Longmire Buffer, Longmire et al. 1988). The chemicals are not toxic, but this solution should not be ingested.

The buffer and any blood/buffer combination can be stored at room temperature until you return from the field, at which point they should be frozen.

■ Blood on filter paper (*Blood/Whatman Filter Paper/Dry (Silica gel)/Room Temperature*)

Blood samples can be taken from the brachial, jugular, or tarsus veins. Brachial and jugular veins require drawing blood using a new sterile syringe for each individual. Samples should only be collected in this manner by an experienced person. Do not use heparinized needles, syringes, or vials. Heparin inhibits DNA amplification making molecular data collection difficult or impossible. Therefore if blood clotting is an issue, consider using EDTA instead of heparin.

Blood can be obtained by pricking the tarsus vein and transferring the drops to the tubes by way of capillary tubes. Use a capillary tube bulb to expel blood (5–6 drops) from capillary tubes (do not use your mouth) onto filter paper (Whatman).

The paper should be kept separate from other samples to avoid contamination. Allow the damp filter paper to dry and store separately in either: (1) a ziploc bag with silica gel, or (2) a separate envelope. In a pinch (field situations), paper towel can be used, following the same protocol.

If samples can not be kept dry, a drop or two of 95-100% ETOH on the blood stain on filter paper will preserve the DNA.

■ Blood Quills (*Blood Quills/1 Vial/Tissue or Blood Preservation Buffer/Room Temperature*)

Blood quills are put into tubes containing either tissue or blood preservation buffer, provided by our lab. Pull two or more blood quills from the bird. We recommend sampling wing coverts rather than emerging primaries and secondaries, which are more critical to flight. If the quills will not fit into the tube, trim off the feather tips, leaving the bloody ends (calamus) in the tube.

We strongly suggest that latex gloves be used when sampling, and that instruments be cleaned with 10% bleach between sampling. This prevents between-sample contamination and protects the collector from infectious diseases and any preservatives that may have been used in the skin's preparation.

Tubes can be stored at ambient temperature for shipping.

■ Bone (*Bone/1 Envelope/Dry (Silica gel)/Room Temperature*)

Hard tissue samples, such as bone, should be kept as dry as possible. These can be stored in containers or envelopes.

■ Buccal Swab (*epithelial cells/1 swab/original packaging, Longmire Buffer, or silica gel/Room Temperature*). See Handel et al. (2006) for details.

To collect buccal cells from sea ducks, roll the collection swab firmly on the inside of the “cheek”, approximately 20 times on each side. Make certain to move the brush over the entire “cheek” side.

The samples can be stored dry if you are returning to the lab right away. If storing dry, air dry the swab at for 10-15 minutes at room (field) temperature. Store the dry swab in the original packaging at ambient temperature for up to one week. If you are going to be in the field longer than a week, place the swab in a 1.7 mL vial containing 350 mL of blood preservation buffer (Longmire Buffer). You will need to cut the swab to size, cap and label the vial.

An alternative method of storing for longer periods is to place the swab (after collection of cells and drying) into a small coin envelope, cutting the end of the stick so it will fit, close the envelope, and place the envelope into a Ziploc bag containing silica gel (provided). We prefer the Longmire Buffer storage procedure.

Samples stored in silica gel or blood preservation buffer can be stored at ambient temperature.

■ *Egg Shell Membranes (Egg shell membranes/1 Envelope/Dry (Silica gel)/Room Temperature)*

Collect each membrane (preferably vascularized membranes) and place it in a separate envelope, then into a plastic ziploc bag containing silica gel. Placing all membranes in the same envelope causes cross contamination of samples.

We do not use the hard shell at all, so that portion can be left in the field.

Do not store feathers and eggshells from the same nest in the same envelope.

Give the nest a number, and then label each feather or egg sample with that number (e.g., nest number 100 has feather sample number 100 and membrane sample numbers 100(1), 100(2), etc.).

■ *Feathers from nests or live birds (Feathers/1 Envelope/Dry(Silica gel)/Room temperature)*

We get the best results with contour or tail/wing feathers (those with a substantial sheath or rachis) deposited in nests or shed by birds during molt (or plucked from live birds). However, feathers from any portion of the bird will suffice. The DNA is actually in the calamus, so feathers without the calamus cannot be used to extract DNA.

Although in the past, we were unable to obtain DNA from down feathers, we have recently been able to extract DNA from down feathers of raptors and are doing experiments to see if our techniques are more broadly applicable (Latta et al., in prep).

Please collect as many contour feathers from each nest as possible (we use 5 feathers per DNA extraction from geese and at least that many for passerines, but like to have extra in case it does not work the first time around). Feathers can be removed after the nest has failed or hatched, or when first discovered if you don't plan to revisit the nest.

Keep feathers dry after collection, since moisture can cause decay of feathers and subsequently the DNA. Place feathers in paper coin envelopes or, if bone dry, in plastic bags. Store feathers from different nests or birds in separate bags/envelopes.

Envelopes can be placed in a plastic bag with 2 Tbsp. silica gel to aid in maintaining a dry environment.

■ *Muscle (Tissue/1 Vial/Tissue Preservation Buffer/Room Temperature)*

Among muscle tissue samples, heart is the most preferred for birds, since the mtDNA yield is very high relative to nuclear yield. DNA can also be extracted from tongue, skin, hair, teeth and bone. Soft tissue samples can be stored at room temperature in the field in Tissue Preservation Buffer.

Any muscle or skin tissue will work and can be stored in this buffer solution. Please make sure that the storage buffer completely covers the tissue sample. Also, make sure to clean instruments between sampling different birds to prevent cross-contamination, using a 10% bleach solution followed by a water rinse. A sample about the size of a pencil eraser is all that is needed, but make sure the sample is entirely submersed in the buffer.

Storage Chemical Descriptions and Hazards

(For more specific details on each chemical ingredient, see the attached Material Safety Data Sheets.)

■ *Blood Preservation Buffer (Longmire et al. 1988)*

Recommended for field storage of blood, blood quills, and buccal swabs.

100mM Tris HCl pH 8.0
100mM EDTA
10 mM NaCl
0.5% SDS (Sodium Dodecyl Sulfate)

Recipe: 100 mL 1M Tris- HCl, pH 8.0.
200 mL 0.5M EDTA
2.5 mL 4M NaCl
5.0 grams SDS
Bring to 1 Liter with dH₂O

Storage: Room temperature (sometimes Longmire buffer precipitates at cooler temperatures. We typically store ours in a 37°C incubator.

Disposal: Sink

Hazards:

Tris/HCL. May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. Ingestion of large doses may cause interior irritation, nausea, weakness and collapse. If ingested, drink copious amounts of water and call a physician.

EDTA. May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, drink copious amounts of water and call a physician

NaCl (Sodium chloride). May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, drink copious amounts of water and call a physician

SDS (Sodium dodecyl sulfate). May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, do not induce vomiting. Drink copious amounts of water and call a physician.

■ Tissue Preservation Buffer

Recommended for Field storage of tissue.

4.0 M Urea
0.2 M NaCl
10mM EDTA
0.5% N-Lauroyl-Sarcosine
100mM Tris HCl pH 8.0

Recipe: 240.24 grams Urea
 11.69 grams NaCl
 5.0 grams N-Lauroyl-Sarcosine
 3.72 grams EDTA
 100 mL 1M Tris- HCl, pH 8.0.
 Bring to 1 Liter with dH₂O

Storage: Room temperature

Disposal: Sink

Hazards:

Tris/HCL. See above.

EDTA. See above.

NaCl (Sodium chloride). See above.

N Lauroyl sarcosine. May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, do not induce vomiting. Drink copious amounts of water and call a physician.

Urea. May cause irritation to skin or eyes. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, do not induce vomiting. Drink copious amounts of water and call a physician.

■ *Materials used in preserving Feather and Blood on filter paper Hazards*

Silica gel or beads. Silica can be an inhalation hazard, especially for those with asthma. Do not breathe silica dust or leave in open areas.

Ethanol. Ethanol is highly flammable; do not place near an open flame. High vapor concentrations or consumption can cause narcotic effects. Do not breathe vapors or consume this ethanol.

References Cited

- Conrad, K. F., R. J. Robertson and P. T. Boag. 2000. Difficulties storing and preserving tyrant flycatcher blood samples used for genetic analyses. *Condor* 102:191-193.
- Handel, C. M., L. M. Pajot, S. L. Talbot and G. K. Sage. 2006. Use of buccal swabs for sampling DNA from nestling and adult birds. *Wildlife Society Bulletin* 34: 1094-1100.
- Longmire, J. L., A. K. Lewis, N. C. Brown, J. M. Buckingham, L. M. Clark, M. D. Jones, L. J. Meincke, J. Meyne, R. L. Ratliff, F. A. Ray, R. P. Wagner and R. K. Moyzis. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics* 2:14-24.
- Seutin G., B. N. White, P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analysis. *Canadian Journal of Zoology* 69:82-90.