UN environment programme

ASCOBANS

AGREEMENT ON THE CONSERVATION OF SMALL CETACEANS OF THE BALTIC, NORTH EAST ATLANTIC, IRISH AND NORTH SEAS

ASCOBANS/JG22/Doc.3.7

12 March 2026

22nd MEETING OF THE JASTARNIA GROUP
Online, 14-16 April 2026
Agenda Item 3.7

GUIDELINES FOR GENETIC SAMPLING OF STRANDED HARBOUR PORPOISES

(Prepared by the Coordinator of the Baltic Harbour Porpoise Action Plans)

1. The Jastarnia Group has discussed the need for guidelines on how to take genetic samples from stranded animals, depending on the stage of decomposition. Historically, genetic samples have not been taken from individuals that are severely decomposed, but in the Baltic Proper, given how rare it is to find a stranded animal, all samples are extremely valuable regardless of the stage of decomposition. Also, genetic methods have evolved, and analysis can now be carried out on a wider variety of decomposed tissues. To ensure that there is clear, easily accessible information on how to proceed when an animal is found stranded, these guidelines have been compiled.

2. At the 21st meeting of the Jastarnia Group in March 2025, a first draft of the guidelines was discussed. The 29th Meeting of the ASCOBANS Advisory Committee in September 2025 reviewed the guidelines and made some comments. A revised version has now been prepared and is ready to be endorsed by the 22nd Meeting of the Jastarnia Group in April 2026.

Action requested:

3. The Jastarnia Group is requested to endorse the guidelines in Annex 1.

Annex 1

Instructions for Genetic Sampling of Harbour Porpoise Carcasses

Purpose

This guide provides step-by-step instructions for collecting tissue samples from harbour porpoise carcasses for DNA molecular analysis. The best sampling method depends on the decomposition stage of the carcass.

Ideally, for DNA molecular analysis the carcass should be as fresh as possible. However, for the Critically Endangered Baltic Proper population of harbour porpoises (*Phocoena phocoena*), all carcasses are very valuable and samples should be taken regardless of the stage of decomposition. Also, new methods allow genetic analysis on for example skin samples despite severe decomposition.

The Baltic Proper harbour porpoise can be separated from the neighbouring Belt Sea population through genetic analysis. Therefore, genetic samples are important to gain new knowledge about the occurrence and distribution of the genetically distinct Baltic Proper harbour porpoise population, which will allow to trace the critically endangered population trough time and space.

1. Preparation and Equipment

Before collecting samples, ensure you have:

- Sterile scalpels or knives
- Sterile tweezers/forceps
- Sterile tubes or vials with screw cap, approximately 5 cl volume is enough.
- 95-100% ethanol (non-denatured, preferred preservation medium)
- If possible, cryogenic storage options (dry ice, liquid nitrogen, or -20°C/-80°C freezer). Note though that DNA is also stable in ethanol an ambient temperature for some time.
- Protective gloves
- Labels and permanent markers for documentation
- Data sheet for recording sample details (date, location, carcass condition, etc.)

2. Determining Carcass Decomposition Stage and Sampling Method

- **Avoid contamination by using new tools or sterilizing them between samples**

Fresh Carcass (Code 1-2: Minimal Decomposition)

Best Tissues: Skin, but also muscle, internal organs (liver, heart, kidney)

Possible analyses: Almost any analyses feasible (e.g. full population genomics, microsatellites, SNP panels, mtDNA, sex determination, kinship)

Sampling Method:

- Use sterile tools to extract three samples of 1-2 cm^3, or one sample of approximately 5 cm^3, from the selected tissue (only one tissue necessary). To ensure proper fixation if using ethanol, the piece of tissue can be cut into smaller pieces of approximately 5x5 mm.

Storage:

- Preferably in **95-100% ethanol** (ensures DNA stability at room temperature or refrigerated storage). Preservation in 95% ethanol at a 5:1 volumetric ratio is recommended for barcoding studies to ensure preservation and prevention of DNA degradation. Ensure enough volume of ethanol is used to properly cover the entire sample to fixate it. Short term storage and transport at ambient temperatures is possible but put to **-20°C** for longer storage.

- Alternatively, freeze at **-20°C to -80°C** if ethanol is unavailable. The lower the temperature the better. For optimal preservation, pack each sample separately in vacuum sealed plastic bags.

- If transport of frozen samples is needed, store samples on **dry ice** until freezing, or use ice packs/refrigeration followed by prompt freezing.

- Consider soaking samples in RNAlater before freezing them at –80 °C, as this helps preserve RNA for applications such as transcriptomics.

Moderately Decomposed Carcass (Code 3: Early Bloating, Some Tissue Degradation)

Best Tissues: Skin, for genetic analysis, despite decomposition. Deep muscle (less exposed to degradation), bone (for example ribs) or teeth can also be collected.

Possible analyses: Limited to shorter DNA fragments (e.g. individual genotyping like microsatellites or SNP panels, population genetic analyses, mtDNA, sex determination).

Sampling Method:

- Using sterile tools, excise a skin sample and/or a deep muscle sample from beneath the blubber layer. As above, ensure pieces are small enough to be completely fixated if using ethanol.

- If muscle is severely degraded, also collect a rib or a few teeth.

Storage:

- Skin and muscle: Preserve in **ethanol** or **freeze** as described above.
- Bone/Teeth: Store **dry** or in **ethanol** for later DNA extraction.

Advanced Decomposition (Code 4-5: Bloated, Skin Sloughing, Organs Liquefied)

Best Tissues: Skin for genetic analysis, despite decomposition. Bone (ribs or lower jaw) or teeth can also be collected due to extensive tissue breakdown.

Possible analyses: Limited to shorter DNA fragments (e.g. sex determination, short mtDNA fragments, species ID, limited individual genotyping using short microsatellite loci)

Sampling Method:

- Using sterile tools, excise a skin sample
- Collect a rib or a few intact teeth using sterile tools.

Storage:

- Skin and muscle: Preserve in **ethanol** or **freeze** as described above.
- Teeth: Store **dry** or in **ethanol**.
- Bone: Store **dry** or freeze if possible.

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3. Sample Documentation

See the *Best Practice on Cetacean Post-mortem Investigation and Tissue Sampling* protocol in the Annex of ASCOBANS Resolution 8.10 (Rev.MOP9) *Small Cetacean Stranding Response.*

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4. Storage

The longevity and quality of DNA in tissue samples depend on the preservation method used. Below are general recommendations for storage durations under different conditions:

1. Ethanol Preservation

- **95–100% Ethanol:**
  - Ideal for long-term DNA preservation.
  - Samples can typically be stored for many years (5–10+) at room temperature or refrigerated (4°C) without significant DNA degradation, provided the ethanol is not diluted by water from the tissue, but long-term storage at **-20°C** is preferable**.**
  - Ethanol should fully cover the tissue, and containers should be tightly sealed to prevent evaporation.

- **70% Ethanol:**
    - Acceptable for short- to medium-term storage (weeks to months).
    - DNA quality may begin to degrade after 6–12 months, especially if stored at room temperature.
    - Recommended only if higher concentration ethanol is unavailable. Best stored cold (4°C) to slow degradation.
    - Put to **95–100% Ethanol** for long-term storage (see above).

2. Freezing

- **-20°C (Standard Freezer):**
    - Suitable for medium-term storage (months to a few years).
    - Some DNA degradation may occur over time, especially in tissues with high water content.
    - Avoid repeated freeze-thaw cycles, which accelerate degradation.
- **-80°C (Ultra-Low Freezer):**
    - Best for long-term preservation of high-quality DNA.
    - Samples can be stored for decades with minimal degradation.
    - Ideal for archival tissue collections or where highest DNA integrity is needed for advanced molecular work.

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5. Transport and Submission

- Ensure samples are securely sealed and labeled.
- If shipping frozen tissues, use insulated packaging with dry ice to maintain sample integrity.
- Polystyrene boxes provide optimal protection for transport.

Proper permits may be required for sample collection and transport.