



**EAZA
BIOBANK**



EAZA Biobank Aquatic Animal Sampling Protocol

- NOTE:**
- ALL samples should be collected and shipped in accordance with national legislation
 - Gloves should always be worn when collecting samples to prevent contamination
 - Samples may be taken from live animals opportunistically during routine veterinary or other procedures, or from deceased animals prior to necropsy.
 - Any questions on any of the below can be directed to the [Biobank Coordinator](#).

General sampling procedure

Whole blood: up to 5mL in plastic EDTA (or PAXgene) blood collection tubes.
Invert 5 times to mix.
NOTE: for smaller species, filter paper sample is acceptable.



OR

Tissue: up to 1 cubic cm in plastic tube (such as a 2mL screw-cap tube) or bag.
Any DNA-containing tissue sample is of interest, for example skin, muscle or fin biopsies from live animals, or internal organ samples from dead animals. Umbilical cord can also be collected.
Freeze at -20 or -80 °C immediately or preserve in ethanol (70-90%).



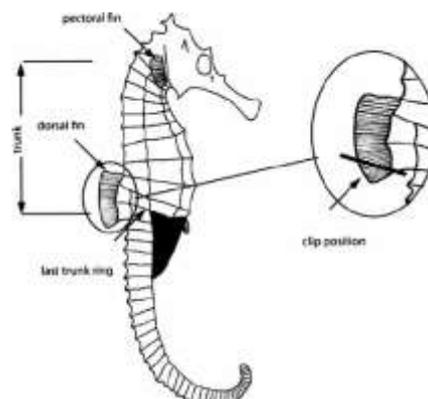
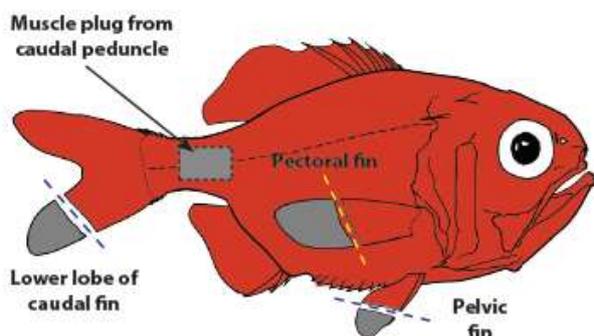
Species-specific sampling considerations

In addition to the samples above

Teleost fish

Fin clip: a small clip, proportional to the size of the fish, can be collection from pectoral, pelvic, dorsal, or caudal fins (can be collected from live animal, anesthesia is usually recommended).

Muscle From a deceased animal, a tissue sample may be removed from the caudal peduncle
Preserve and store both fin clip and muscle plug as a tissue sample (above).



Sharks and Rays

Mucus: epidermal mucus may be scrapped from sharks and rays, non-invasively and opportunistically.
Freeze at -20 or -80 °C immediately or preserve in ethanol (70-90%).



Punch Biopsy: From a live shark, it is possible to collect a skin/muscle sample from the trunk area, just below the dorsal fin.
Freeze at -20 or -80 °C immediately or preserve in ethanol (70-90%).

NOTE: a fin clip is not recommended from elasmobranchs – it is hard material which is difficult to obtain and extract DNA from



Ray barb: The barb, or sting, from rays may be collected, as it contains epidermal tissue.
Freeze at -20 or -80 °C immediately or preserve in ethanol (70-90%).



Sponge/Coral

Tissue: For sponges, cut a piece from the inner part of the sponge, avoiding the surface.
For coral, remove a small fragment with clippers.
Freeze at -20 or -80 °C immediately or preserve in ethanol (70-90%).



Jellyfish

Tissue: For a medusa-stage jellyfish, sample the muscle ring around the margin of the bell, or sample the gonads by holding the jellyfish upside-down with the bell lying flat in hand. Using a plastic transfer pipette, extract a small amount of the gonad and transfer to a plastic tube.
Freeze at -20 or -80 °C immediately (avoid preserving in ethanol if possible).
NOTE: sampling the muscle ring may affect the swimming ability of the jellyfish for a period after the sampling event.

Marine Mammal

See General Sampling procedure above for tissue or blood samples.

Storage and Shipping

Labeling

Label the sample tube or bag with **animal identifier** (transponder, GAN, local ID), **species name, tissue type, and date of sampling**.

Storage

Store samples in a freezer (-20 to -80°C) until shipment is possible. Samples preserved in ethanol may be stored at room temperature. If preferred, collect samples over a period of time for batch shipment. Please ship samples as soon as possible and avoid arrival at the Hub on a weekend.

Packaging

1. **Primary package:** sample tube/ bag
2. **Secondary packaging:** plastic container or bag with absorbent material (enough to absorb sample content)
3. **Tertiary packaging:** reinforced envelope or cardboard box – include a 'UN3373' diamond logo (see left) and the text "**Exempt animal specimen**" and "**refrigerate upon arrival**" on the outside of the packaging.



NOTE: Please enclose the following in your shipment:

- ZIMS specimen report, or list of samples, EAZA Biobank material transfer agreement (MTA; if not sent electronically), and contact details of the sender.
- An icepack or dry ice to keep the samples frozen. Icepacks are not needed for samples in ethanol.



CITES

Some samples from certain species may require CITES Permits. General rules according to CITES regulations:

Within the EU: there is no need for CITES export and import permits (exemptions may apply).

Outside the EU: CITES export permits must be applied for at the national CITES office. **Remember to apply for permits ahead of shipping samples.**



CITES Exemption: exemption is possible for scientific institutions (article VII, §6). All four EAZA Biobank Hubs have the CITES exemption. Your institution may be eligible to apply.

Biobank Hub Addresses

Please send samples to the Biobank Hub relevant for your country

Edinburgh Hub

Shipping country: UK, Ireland, Qatar, UAE, Kuwait



Att: EAZA Biobank Samples
c/o: RZSS WildGenes Biobank
Address: Royal Zoological Society of Scotland (RZSS)
134 Corstorphine Road
Edinburgh EH126TS, UK
Email: biobank@rzss.org.uk

Berlin Hub

Shipping country: Germany, Austria, Croatia, Czech Republic, Hungary, Poland, Russia, Slovakia, Slovenia, Switzerland, Ukraine



Leibniz Institute for Zoo
and Wildlife Research
IN THE FORSCHUNGSVERBUND BERLIN E.V.

Att: Dr. Jörns Fickel
Address: Department of Evolutionary Genetics Leibniz
Institute for Zoo and Wildlife Research (IZW)
Alfred-Kowalke Strasse 17
10315 Berlin, Germany
Email: fickel@izw-berlin.de

Antwerp Hub

Shipping country: Belgium, Luxembourg, The Netherlands, France, Greece, Israel, Italy, Turkey



Att: Dr. Philippe Helsen
Address: Centre for Research and Conservation
Royal Zoological Society of Antwerp (KMDA)
Koningin Astridplein
20-262018 Antwerp, Belgium
Email: Philippe.Helsen@kmda.org

Copenhagen Hub

Shipping country: Denmark, Estonia, Finland, Latvia, Lithuania, Norway, Sweden, Portugal, Spain



Att: Dr. Christina Hvilsom
Address: Copenhagen Zoo
Roskildevej 38
2000 Frederiksberg, Denmark
Email: ch@zoo.dk

Samples may be donated or loaned to the Biobank, please see our Material Transfer Agreement. It is the responsibility of the lending institution to keep the Biobank up to date with contact details.

References

Charnigo, A., Thiele, G., Kong, E.L., et al. Stingray Sting. [Updated 2020 Sep 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. [Figure, Sting ray with barb. Image courtesy S Bhimji MD] Available from: <https://www.ncbi.nlm.nih.gov/books/NBK539785/figure/article-29504.image.f1/>

Domingues, R.R., Garrone-Neto, D., Hilsdorf, A.W.S., Gadig, O.B.F. (2019). Use of mucus as a non-invasive sampling method for DNA barcoding of stingrays and skates (batoid elasmobranchs). *Journal of Fish Biology*. 94(3),512-516. doi: 10.1111/jfb.13919.

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Woodall, L., Jones, R., Zimmerman, B., Guillaume, S., Stubbington, T., Shaw, P., & Koldewey, H. (2012). Partial fin-clipping as an effective tool for tissue sampling seahorses, *Hippocampus* spp. *Journal of the Marine Biological Association of the United Kingdom*. 92(6), 1427-1432. doi:10.1017/S0025315411001810

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