General recommendations for the Classes Osteichthyes and Chondrichthyes

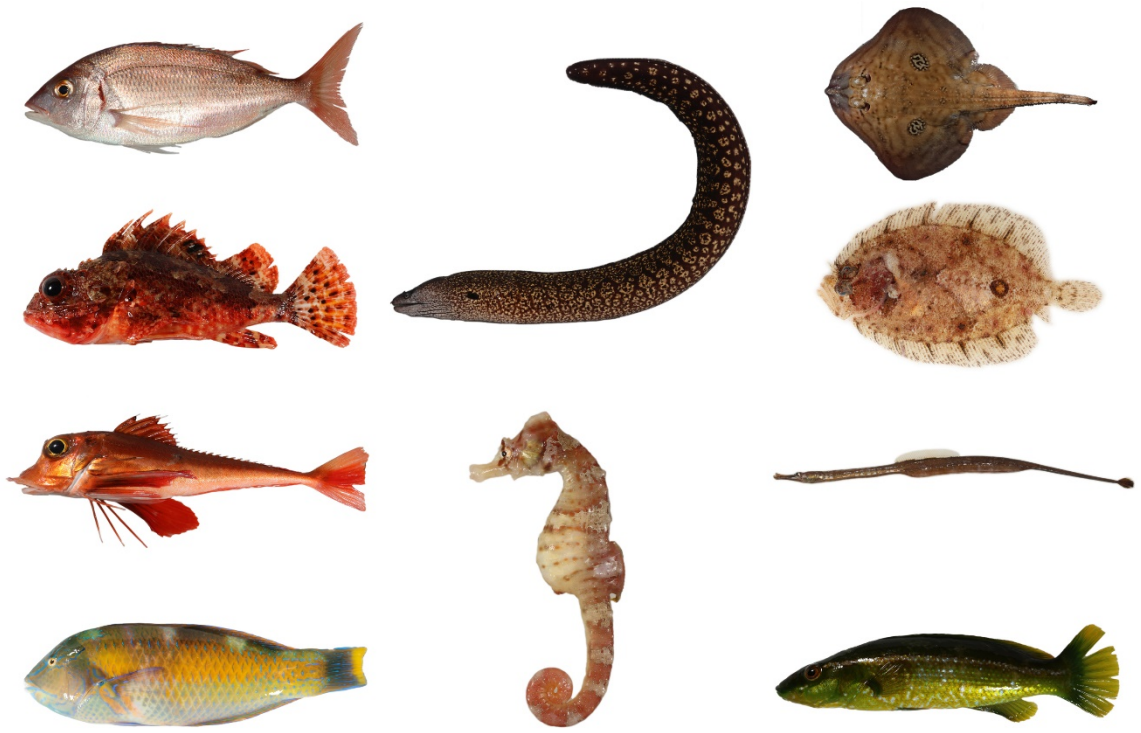
For general instructions see 0 – SOP Sampling General Recommendations

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Chondrichthyes are fishes that have skeletons made of cartilage, while *Osteichthyes* have skeletons made of bone. Chondrichthyes do not possess swim bladder and have exposed gill slits, whereas Osteichthyes typically have a swim bladder and gills covered by an operculum.

It is reminded that using vertebrates animals for scientific purposes has specific and mandatory regulatory compliance.



Legends (left to right, to to bottom): *Pagellus erythrinus*, *Muraena elera*, *Leucoraja naevus*, *Scorpaena notata*, *Zeugopterus regius*, *Trigla lyra*, *Hippocampus hippocampus*, *Syngnathus rostellatus*, *Halichoeres radiatus*, *Labrus viridis*.

Prior to the preparation of tissues, the operations carried out on the **Vertebrata** and Cephalopoda (collection, anesthesia, euthanasia) must be carried out in accordance with European directive

n°2010/63/UE, transposed in France under decree n°2013-118 of February 1, 2013 relating to the protection of animals used for scientific purposes.

Guidelines

Sampling

After collection (by hand, dredge/trawl, RAv, others…), specimens are placed in seawater containers equipped with an aeration system (ex. aquarium air pump) and maintained to a temperature adequate for their conservation.

They must be kept individually or in group of individuals from the same species and station. To avoid or limit stress, if possible, elements allowing specimens to hide from the operator's view will be installed (ex. rocs or shells for small specimens, partial cover/lid, etc…). Fishes must be maintained in an environnement as calm as possible, and manipulated with care and caution during transfers in between containers, with appropriate material (fish nets).

At this step, specimens are identified with:

- Sampling date
- Station number allowing to know sampling date, GPS coordinates, sampling method, harvesters, habitat (via a field sheet completed during sampling).
- Tracking sheet with the name of the taxa (specie, genus, … rank), the taxonomist name, the sex …
- A warning in case of venomous or otherwise dangerous species, in order to avoid accidents during manipulations.

If a specimen dies during sampling, it must placed immediately on ice or refrigerated blocs to keep it fresh. Genetical sampling must then be performed as soon as possible, to avoid any degradation. The specimen shouldn't be sampled after 2 hours post-mortem.

Anesthesia

Les dosages de benzocaïne pour l'anesthésie sont les suivants :

- Téléostéens : 50-75mg de benzocaïne par litre d'eau de mer, soit 3 à 5mL de solution de benzocaïne pour 10L d'eau de mer ;
- Elasmobranches : 25mg de benzocaïne par litre d'eau de mer, soit 1.7mL de solution de benzocaïne pour 10L d'eau de mer.

Photography

Ideally, images should be taken in the highest quality resolution (macro lens recommended). ¶

In cases where no part of the specimen can be conserved as a voucher, the photographs will serve as a voucher. These should therefore include relevant identifying features, when possible. This must be discussed with the taxonomist.

Furthermore, specimens should be photographed alive if possible, since colors can be altered after death. However, while dead specimens can (and should) be photographed immediately, alive ones must be anesthetized before photography.

The first picture is the reference photo (see SOP – 0) taken with a **ruler**, the **code identifiers** (e.g. 1 ATLASea QR code, 1 **CE-00XX** barcode for each specimen), the tracking sheet and the station label with the specimen.

Taking photographs with the specimen in a glass container with water helps to avoid reflections, allowing clearer pictures. Yet, it presents a risk of awakening for anesthetized specimens, it is hard to setup for large specimens, and does not allow to photograph some relevant anatomical details (for example, the fins are hard to spread while in water.)

Accordingly, if possible, one general photograph should be taken in water (Water must be clean, and changed between specimens), and other shots should be taken following the instructions below :

- Once taken out of the water, gently wipe the specimen to remove mucus and left-over water.
- Depending on the color of the specimen, place it on a uniform white or matte black background (be careful not to kill the specimen during this operation for anesthetized specimens).
- In most cases the specimen should be laid on its right side, its head facing left, <u>because the left side is the one that must be photographed</u>.
- Depending on the morphology of the species, other view should be must be taken instead :
    - Round, or laterally compressed fishes : left side ;
    - Dorso-ventrally flattened fishes : side of the eyes ;
    - Elasmobranches with flattened body (ex. ray) : Dorsal and ventral view ;
    - Fishes flattened by gravity (ex. anglerfish) : left side and dorsal view ;
    - Gobies and other similar species : left side, dorsal and ventral view ;
- If the species presents characteristics necessary for taxonomic determination, close-up of these characteristics should be made.

4- Euthanasia

Le poisson doit être euthanasié avant tout prélèvement biologique en l'absence de DAP.

Protocole :

- le placer dans un bain de benzocaïne à une concentration de 250-300 mg/L d'eau de mer, soit 17-20 mL de la solution de benzocaïne pour 10L d'eau de mer.
- Le poisson est considéré comme euthanasié en l'absence totale de mouvements et de réactions au toucher.
- Une fois sorti un bac, La mort des animaux doit est **confirmée** (réglementation) par l'observation par l'utilisation d'une des méthodes suivantes (dans l'ordre de priorité)
- Destruction du cerveau (enfoncer une tige métallique adaptée -aiguille ou pointe- dans la partie supérieure du crâne la plus dure, généralement entre les deux yeux)
- Exsanguination (couper les arcs branchiaux)

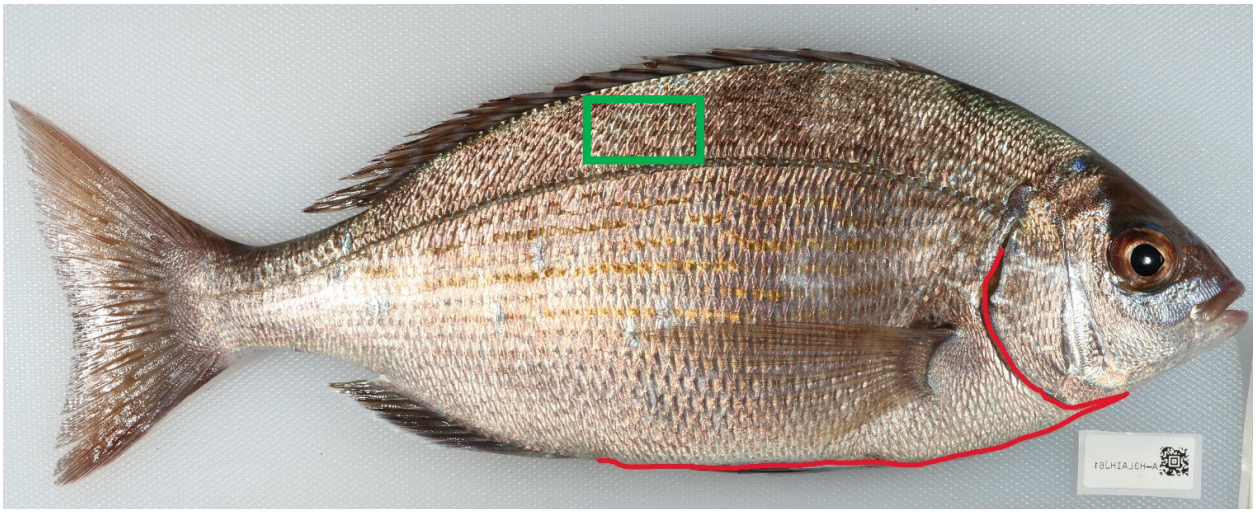
Dissection for Genome Sequencing

Depending on the size of the fish, different types of samples can be collected:

- If dissection yields more than 2 g of tissue, prioritize organs for sampling in the following order:
    - **Heart** (>200 mg) – **spleen** (>200 mg) – **muscle** (at least 8 tubes 300 mg each).
    - Gonad – brain (>200 mg).
    - Fin – eye – liver (potentially contaminated by other organisms).
    - Do not collect gills or digestive tract.
- If dissection does not field at least 2 g of tissue, see details in Section Small-sized individuals.

Dissection for tissue sampling

1. Rinse the fish using filtered seawater, then gently blot it dry with a paper towel before placing it on the dissection table.
2. Position the fish with its right side facing upward and the head oriented to the right. All tissue sampling must be performed on the **right side** to preserve the left side as the taxonomic voucher.



3. Example of incision for right-side dissection (red line indicates the incision) and area to be sampled for muscle tissue (green rectangle). For **muscle** sampling, remove the scales from the dorsal flank above the lateral line, where the flesh is thickest. Using a scalpel, cut out a rectangular section approximately 1 × 3 cm, remove the skin, and extract the muscle tissue. Rinse, blot dry, and place the sample in a sterile Petri dish.
4. Cut off the pectoral **fin**, and if insufficient material is obtained, also collect the pelvic fin (only sample additional fins if the total material is not enough to fill several collection tubes). Rinse, blot dry, and place in a sterile Petri dish.
5. Carefully cut the membrane covering the **eye**, sever the optic nerve, and remove the eye. Rinse, blot dry, and place it in a sterile Petri dish.
6. Open the visceral cavity by cutting from the anus toward the mouth, then make an incision along the gills to facilitate access to the **internal organs**.
7. Lift the flank to access the organs and collect the **heart** and a piece of the **liver**. If visible parasites are present on the liver, remove them using forceps. Rinse the samples with filtered seawater, blot dry on clean absorbent paper, and place each in a sterile Petri dish.
8. Cut each organ into small pieces and fill 3–5 cryotubes with approximately **300 -500 mg** of tissue per tube.
9. Do not mix different types of tissues even if the minimun mass is not reach.
10. Thoroughly disinfect the dissection tools between each organ.
11. **Flash-freeze** the tubes in liquid nitrogen or a dry shipper. Once frozen, group them in the same nylon bag containing the blood collection tubes, so that all tubes from a single individual are kept together. Store either in liquid nitrogen, a dry shipper, or at −80 °C, depending on available conditions.

Small-sized individuals

For small individuals, the entire specimen should be collected.

1. Gently brush the skin with seawater to remove as many attached organisms and impurities as possible.

2. Carefully open the visceral cavity by making an incision and remove the viscera, which may contain parasites, taking care not to rupture them.
3. Rinse the abdominal cavity with filtered seawater and dry the specimen with clean absorbent paper.
4. Cut the individual into 2 or 3 sections (head/body or head/abdomen/tail) and place them in separate cryotubes.
5. Flash-freeze the cryotubes in liquid nitrogen, then store them in liquid nitrogen, in a dry shipper, or at −80 °C, depending on available conditions.

Voucher samples conservation:

For Generals instructions see SOP – 0.

Voucher will be stored at MNHN.

Every part of the specimen that are not taken during the dissection for genetical analysis must be kept as voucher.

Place the specimen in a ziplock bag of matching size, add with it the following labels : ATLASea, MNHN, and Station label, (try to keep them as visible as possible from the outside). Then, seal the bag hermetically, and place it in a freezer (-20°C or -80°C preferably), taking care to lay it as flat as possible, with the specimen in a straight line, to ensure as many features as possible are visible once frozen. Indicate on the bag if the specimen is venomous, as to avoid any accident during subsequent manipulation and strengthen the ziplock bag

Send to the MNHN.