ATLASea Standard Operating Procedures

General recommendations for sampling

These Standard Operating Procedures (SOPs) contain guidance on processing various taxa within the scope of the ATLASea project. The guidance specifically refers to the tissue samples needed for taxonomic identification and specimen vouchering (which takes place at the Muséum national d'Histoire naturelle (MNHN)) and outlines the dissected tissues required for DNA barcoding and whole genome sequencing, which takes place at the Genoscope.

The available SOPs are listed below. Please note other taxonomic groups may be added in the future:

1- Crustacea
2- Bryozoa
3- Tunicata
4- Anthozoa
5- Medusozoa and Ctenophora
6- Echinodermata
7- Mollusca
8- Porifera
9- Annelida
10- Algae – to be written*
11- Fish – to be written*
12- Culture collection – to be written*
13- Zooplankton – to be written*

Future plans for this SOP:

This SOP will be reviewed (on a quarterly/*ad hoc* basis) by ATLASea team members to incorporate feedback from the community.

We are still refining our best practices and fine-tuning elements of this SOP, making it subject to change. We welcome your questions, comments, and suggestions. If you have any advice, comments, techniques, or lessons learned that you would like to contribute, we would greatly appreciate it (please contact: cse@atlasea.fr).

ATLASea sampling selection

ATLASea teams collect marine organisms of ecological, endangered, and economical interest observed in the French EEZ. GoaT database must be consulted before a species entering in the ATLASea program, to avoid conflicts with other genomics projects. Only species not taken in charge for other programs or already sequenced will be treated in ATLASea program, for any question and clarifications send an email to cse@atlasea.fr

****Sampling permits are strictly required.**

A. Sampling

1. For each taxon, provide enough aliquots:
    - 1 for taxonomic identification vouchering (in ethanol, formalin, etc., depending on the specimen)
    - 1 for the specimen biobanking (Flashfrozen)
    - at least 10 for High Molecular Weight (HMW) DNA extraction, RNA extraction and long-range data production (Flash-frozen or in ethanol depending on the organisms)
2. Each vial should be handled with gloves and correctly labeled:
    - Only use labels provided by WHEEL-Sea:

    Contact: Kamil SZAFRANSKI

    E-mail: kamil.szafranski@bio.ens.psl.eu

    to order printed labels.


    - Labels must be stuck along the tube (not around the tube).
    - Be sure that tubes and gloves are dry before handling the labels (the labels can be detached from the tubes during the flash freezing if wet).

3. Fresh material is critical for obtaining HMW-DNA. If possible, specimens should be sampled alive. However, specimens will deteriorate even in these conditions so sampling should occur as soon as possible.
4. Anesthetic methods can be applied to calm animals. Some animals (example molluscs) can be anesthetized with ethanol 1% bath for few minutes. Filtered sparkling seawater can be prepared in the field using a machine such as “SodaStream” and applied to calm organisms such as annelids and crustaceans. See specifics SOPs for more details.
5. Avoid freeze-thaw cycles to prevent unwanted cell disruption or degradation;
6. Samples should be flash-frozen in several aliquots as soon as they are prepared.
7. Always handle input material in a manner that minimizes nuclease exposure and activity, e.g. wear clean gloves (change regularly) when handling input material, storage tubes etc., use nuclease-free buffers and filtered and autoclaved seawater bottles and keep the input material as cold as possible during handling.

8. Before dissection and preservation remove all visible contaminants and epibionts as much as possible (washes, brushing… with stereomicroscope).
9. **Never pool different individuals** (even from the same species) in the same tube.
10. Dissection should be done as quickly as possible, maintaining the cold chain until flash-freezing (for example, by dissecting individuals on a glass board placed on an ice tray).
11. Decontaminate instruments (forceps, scalpels, etc.) between **EACH** organism by immersing them for 1 min in sterile water, transferred for 30 minutes in a bleach bath (2.6% active chlorine solution diluted ¼), then rinsing them for 5 minutes in a sterile water bath, finish with ethanol immersion to dry the instrument quickly.

    Wash surfaces (Aniospray + water…) and change gloves between each organism.
12. Collect "fleshy" parts when possible (avoiding shell, digestive contents, embryos/brooded progeny, ...).
13. Dissect **at least 10 pieces** (approx. **300mg** each for animals, ideally **1g** each for algae and plants). More details for each phylum in dedicated SOPs.

    Cut each piece into smaller fragments before putting them in separate tubes (with unique identification labels).

    For small individuals, put 1 specimen per tube.
14. Avoid compacting the sample in the bottom of the tube, and never fill the tube to the top (to facilitate sample recovery for subsequent handling and to prevent the cap from popping off during thawing).
15. In the case of very small specimens, certified that it is placed in the bottom of the tube.
16. After conditioning the sample in the tubes weigh the tube with the cap (tare with the same model of tube already labelled).
17. Scan the labels on the vials and fill in the log sheet dedicated to the ATLASea sampling. Ask cse@atlasea.fr for the latest version. The barcode must be scanned (before flash freezing) to avoid typos. Ensure that all tissues from the same individual are correctly identified on the log sheet.
18. Once scanned the barcode the sample must be immediately preserved. Most biological samples should be flash-frozen (FF) in liquid nitrogen to minimize nucleic acid degradation by nucleases, then stored at -80°C and shipped on dry ice (see Section D). Depending on the sample, it can also be preserved in ethanol 80%, stored at -20°C and shipped in cold box.

19. During campaigns, vials should be grouped (around 10 tubes) and placed in a nylon bag identified with a unique number. When possible, place tubes corresponding to the same taxon in the same bag.

Bags should be properly sealed using twist-ties to avoid loss of tubes during the transportations.

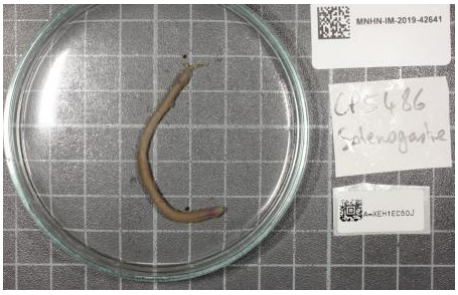
Sealed bags should be then immersed in liquid nitrogen (in Dewar).

20. If immediate storage of the samples in a -80°C freezer is not feasible due to field conditions, an alternative is to place them in a larger liquid nitrogen container specifically designed for storage. This container should have a large opening to facilitate sample retrieval. The samples can then be transported to a facility where they can be stored under ultra-freezer conditions.
21. Place the nylon bags into a Zip-Seal bag, label it with the campaign information, and prepare it for shipping.

B. Photography

1. The specimens must be photographed alive before dissections.
2. The specimen can be placed in a container (glass Petri dish, crystallizer, watch glass) filled with seawater on a black background. If the animal is dark, use a light/white background.
3. It is possible to place the specimen directly on a black/white background without a container, for example Asteroidea, Echinoidea, Ophiuroidea.
4. The time required to take the photos may vary depending on the liveliness of the specimen.
5. Return the specimen to the seawater from time to time (between adjustments, information collection, etc.) to avoid animal stress and deterioration.
6. No anaesthesia is used during the photo shoot, with the exception of Annelids and Crustaceans (see the corresponding sections).
7. The photos are saved in JPEG format and in the best possible resolution (different depending on the types of cameras. Example: phone, professional camera).

8. The photos are named using the ATLASea identifier assigned to the specimen and incremented.
    a. Example: Specimen barcode: A-765YDNQJ7
        i. Photo 01: A-765YDNQJ7_01
        ii. Photo 02: A-765YDNQJ7_02
        iii. Photo 03: A-765YDNQJ7_03
9. Once the photo is taken, the specimen can be processed for dissection and sample preservation.
10. **First photo:** overall view of the animal, dorsal side.
11. **Other photos:** Close-up view of parts, patterns, etc.
    a. head, pygidium, polyps, tentacles, oral disc,
    b. for bivalves: both valves, exterior and interior of the shell, you can recover the shell after the animal was dissect.
12. **Ventral view:** if the animal is too lively, you may use an anaesthetic method to calm down the animal. You can add few mL of sparkling seawater into the container, this is a good option since it does not interfere in the DNA quality for extraction and sequencing. For Holothuroidea: mouth, anus, underside, tentacles if visible. If an anaesthetic is added to the medium, the photos must be taken quickly to prevent the animal deterioration before the dissection and preservation for genomic protocols.
13. **Lateral view** (shrimp, lobster, some Peracarids) → if the specimen is not very lively, but necessary for taxonomic identification.
14. **Last photo:** Animal + a scale (ruler, stative scale, etc.) + labels (MNHN, ATLASea, Station, other labels)



Example Annelida last photo

C. Voucher

1. It is very important to have as many taxonomic vouchers as possible (correction/validation of identification post-mission, collection duty), minimally damaged or with interesting taxonomic parts.

2. Once the specimen dissection is finished, the rest of the animal must be fixed in ethanol 80%, except for molluscs (see SOP Mollusca).
3. In field conditions, the vouchers are stored in one or more labelled drums filled with 80% alcohol. The alcohol volume in the drums should be checked frequently to ensure proper preservation of the specimens (due to evaporation or dilution from water-filled specimens like sponges and holothurians).
4. If the specimen is too small and nothing is left after dissection and sample preservation, another specimen can be fixed as species representative. **ATTENTION**: assure that they have the same morphological traits of the preserved specimen.
5. Vouchers must be labelled properly; labels must be ethanol resistant if put inside the container.
6. If nothing remains of the specimen after dissection and there is no other specimen to be called as voucher, you need to keep the labels: ATLASea, MNHN, STATION, etc... These labels should be placed in a bag labeled "ATLASea specimen labels WITHOUT VOUCHER". Send them to ATLASea team with the others vouchers.
7. In the case of CITES specimens, count and group them in the same drum.
8. All the permits are mandatory; we cannot include a species in the MNHN collection without the permit.
9. **Contact Mélanie Van Weddingen to arrange details for voucher shipment**:
    a. Mélanie Van Weddingen
    b. Email : melanie.van-weddingen@mnhn.fr
    c. Phone : 01 40 79 56 34

D. Shipment of Genomic Samples

1. Always arrange shipping details with a staff member of Genoscope SeqLab and send the ATLASea log sheet via e-mail.
2. Please contact:

- **Janaina RIGONATO** : jrigonat genoscope.cns.fr
- Karine LABADIE : klabadie@genoscope.cns.fr
- Pedro OLIVEIRA : pcoutool@genoscope.cns.fr

3. Shipment of **frozen samples** should be performed either in dry-shippers or on dry ice via a suitable carrier (e.g.: Transportéo, Cryoexpress, etc).
4. The samples preserved in -80°C should be placed in the middle of dry ice, samples preserved in ethanol -20°C should be placed in the top of dry ice.
5. Shipment should be favored on Mondays or Tuesdays between 8.30 a.m. and 5.00 p.m. Outside this time frame, please contact Genoscope SeqLab to arrange for the reception.
6. The package should be sent to the following address:

SITE CEA EVRY

Réception Marchandises

To : **Janaina RIGONATO**,

Emmanuelle Petit or Pedro H OLIVEIRA

31, Boulevard des Coquibus

91 000 EVRY

FRANCE

7. Each shipment should include:
    - project identification (ATLASea)
    - the name of the sender
    - a partial description of the samples
    - permits for species requiring CITES permits
    - ATLASea log sheet completed

Appendix

Material models:

Tubes:

- 1 ml Cryotube à jupe, pas de vis externe avec bouchon naturel (couleur possible), fond conique (ClearLine) [ref Dutscher # 390700]
- 2 ml Cryotube à jupe, pas de vis externe avec bouchon naturel (couleur possible), fond rond (ClearLine) [ref Dutscher # 390701]
- 2 ml Cryotube à jupe, pas de vis externe avec bouchon coiffant, fond rond (Corning) [ref Dutscher # 430659]
- 5 ml screw tube sterile (Eppendorf) [ref Dutscher # 934683]
- 25 ml screw tube sterile (Eppendorf) [ref Dutscher #934685]
- 50 ml Conical Centrifuge Tubes (Greiner) [ref Dutscher #227261]

Bags:

- Nylon protection net, reusable with drawstring (15x10 cm) [Amazon #https://www.amazon.fr/Anti-Insectes-Protection-Plantes-R%C3%A9utilisables-Anti-Oiseaux/dp/B08XXJMY4C]
- Nylon protection net, reusable with drawstring (15x25 cm) [Amazon #https://www.amazon.fr/AMZMUKAUP-Protection-Raisins-R%C3%A9utilisables-Rangement/dp/B09N7PV2LD/ref=sr_1_1_sspa?crid=2VTI1EVYUW654&keywords=sachet+filet+nylon&qid=1690200116&sprefix=sachet+ny%2Caps%2C213&sr=8-1-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1]
- Twist attaches [Amazon #Twist Attaches Or, 800 Pcs Métallique Pince pour Fermeture Sachet Alimentaire, Attache Sac pour Paquet de Bonbon, Sachet Plastique, Transparent Cello]
- Zip-Seal Bags (grands sachets) 350x250 mm [ref VWR #129-0307]
- Zip-Seal Bags (petits sachets) 170x120 mm [ref VWR #129-0297]

Scan:

- Bluetooth QR & Barcode to PC application to scan labels with your phone and import the code to the Excel sheet via Bluetooth: https://play.google.com/store/apps/details?id=dev.fabik.bluetoothhid
- Honeywell Voyager Extreme Performance 1470g-2D [Ref. Fabricant #1470G2D-2USB-1-R]