Standard Operating Procedures ATLASea for zooplankton

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Zooplankton, the animals drifting in aquatic environments, play a crucial role in marine and freshwater ecosystems. They play an essential role in the food web linking primary producers to higher trophic levels. They also participate in the biological carbon pump through the flow of organic matter to the seabed (faecal pellets, migrations, moults, death, etc.). In addition, zooplankton are a good indicator of water quality, as they react quickly to changes in their environment, which in the long term allows us to assess the effects of climate change.

Definition of objectives by ATLASea

- isolate species whose genome does not exist in the database: GoaT.
- isolate species that are present in large numbers in the nets.

NB: if you isolate larger organisms in your nets, in good shape, that you know how to identify to species and that are not taken into account in GoaT, you can include them to be sequenced (e.g. salps or others...).

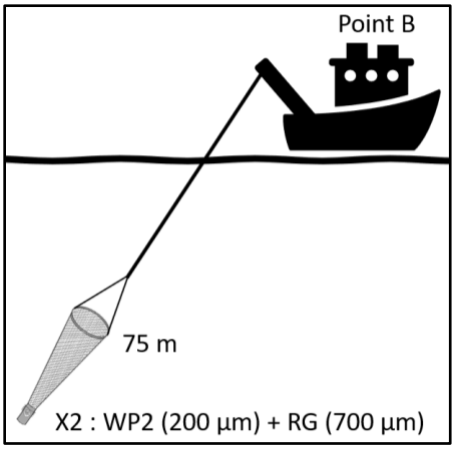
Experimentation on board

Materials

- 1 trolley for transporting equipment between the lab and the boat
- 3 large transparents buckets noted 200-500-1000 with lids
- 2 sieves : 500 µm + 1000 µm
- 2 large funnels with wide neck
- 4 squeeze bottles with seawater filtered 0.2 µm
- 3 5L can of seawater filtered 0.2 µm
- Gloves

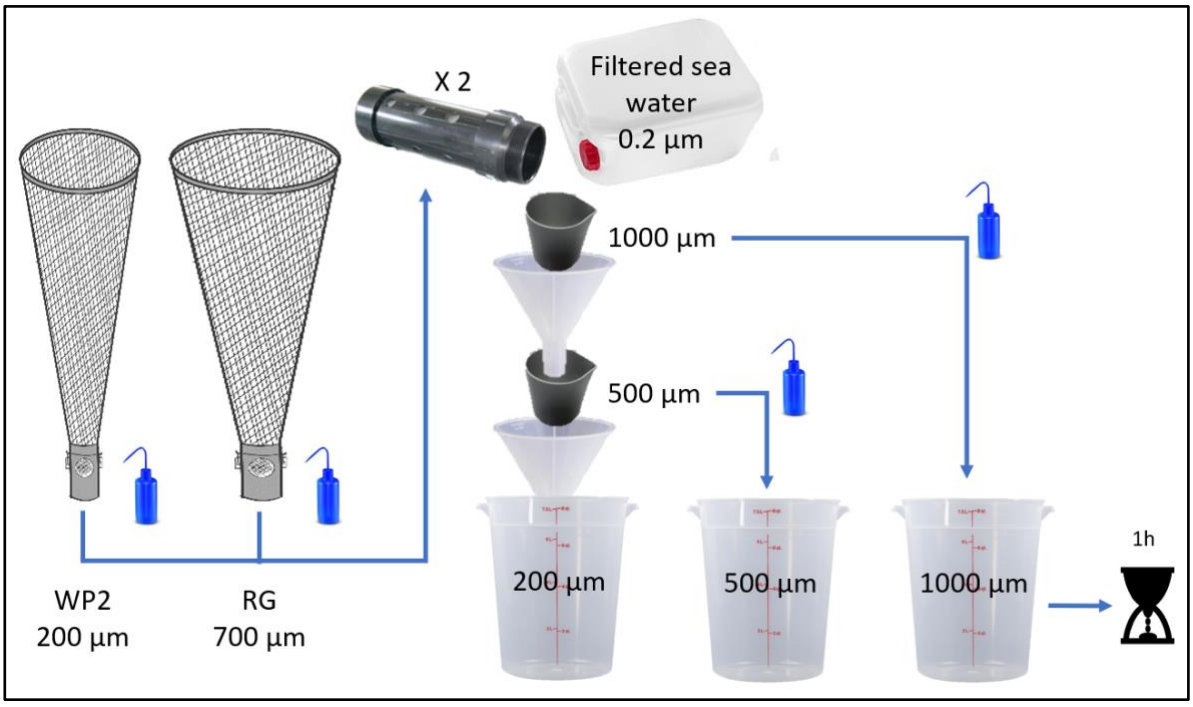
Protocol

1. With Jean-Yves Carval, sailor, carry out two oblique nets to catch as many organisms as possible: 1 WP2 + 1 Régent (RG).



2. Empty the two collectors from the WP2 and RG nets into the pyramid of 500-1000 µm sieves and rinse each sieve thoroughly with 0.2 µm filtered sea water to ensure that the organisms pass through the mesh and that they are cleaned of their detritus.
3. Empty each sieve into their large annotated buckets and let it settle for 1 hour, the time needed for the organisms to digest in order to reduce the diversity of DNA content in the stomachs.

→ These two last operations must be carried out wearing gloves.



Manipulation in the laboratory

Before each manipulation in the laboratory

Clean all equipment with bleach, then rinse thoroughly with sterile Milli-Q water for small objects and fresh water for buckets, funnels and sieves.

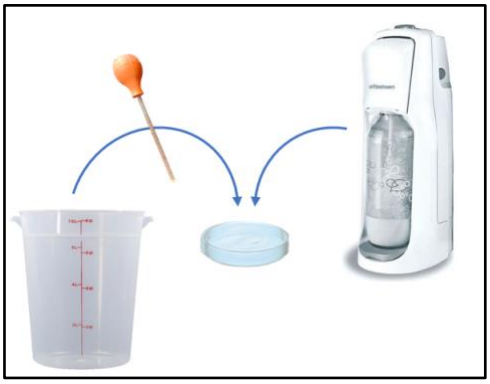
Materials

- 3 5L can of zooplanktonic samples
- Binocular with camera
- Taxonomic kit
- Sodastream kit
- Petri dish
- Glass pipette with bulb
- Autoclaved Eppendorfs tubes 1.5 mL
- Tube rack for Eppendorf tubes
- Nylon bags to store the tube in the dewar
- 1 dewar filled with liquid nitrogen
- Larges ziploc bags to store the Eppendorf tubes in -80°C
- 1 mini spin
- Labels QR codes ATLASea from MNHN (ambassadors@atlasea.fr)
- Metadata excel spreadsheet ATLASea, ask the last version (ambassadors@atlasea.fr)
- Cleaning baths for small equipments (6 mini jars)
- Bleach
- Autoclaved MilliQ water
- Filtered seawater 0.2 µm
- Hair protection
- Gloves

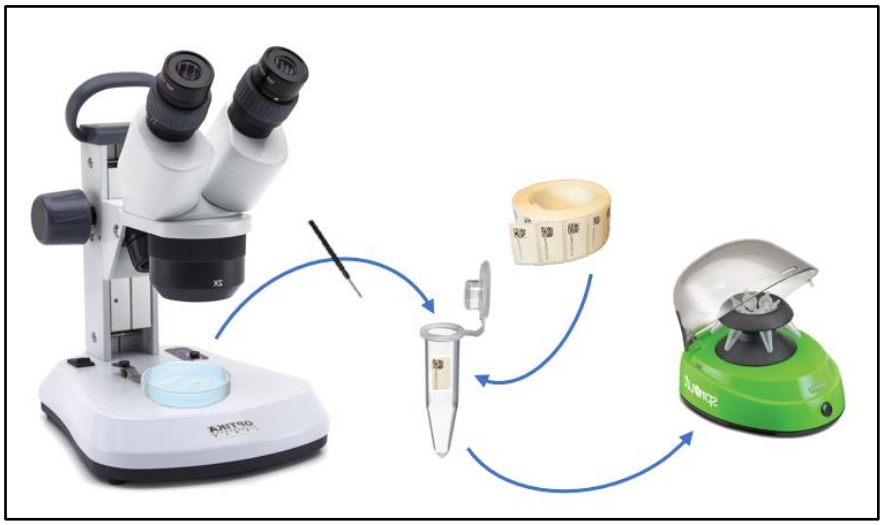
Protocol

The operations described below must be carried out wearing gloves and hair protection.

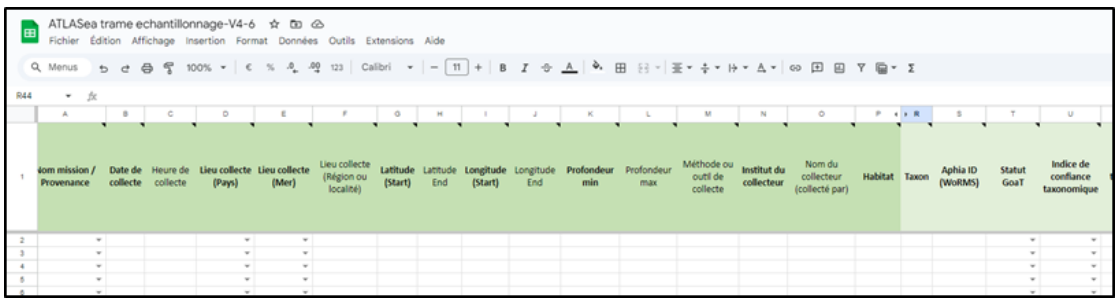
1. Collect the organisms that are still alive using a glass pipette fitted with a bulb and with a wide tip to avoid damaging the organisms. Start with the organisms from the larger fractions, as these contain more adult individuals.
   Place them in a petri dish and add sparkling water prepared by adding gas into the filtered seawater to put the organisms to sleep.



2. Using a taxonomic kit, isolate live individuals 1 by 1 in Eppendorf tubes under binoculars, trying to put as little water as possible in the tube. **There should be one individual per Eppendorf tube.**
   Close the Eppendorf tube tightly.
   Stick a ATLASea QR code label vertically on the tube.
   Place the tube in the mini spin to sediment the organism at the bottom of the tube.

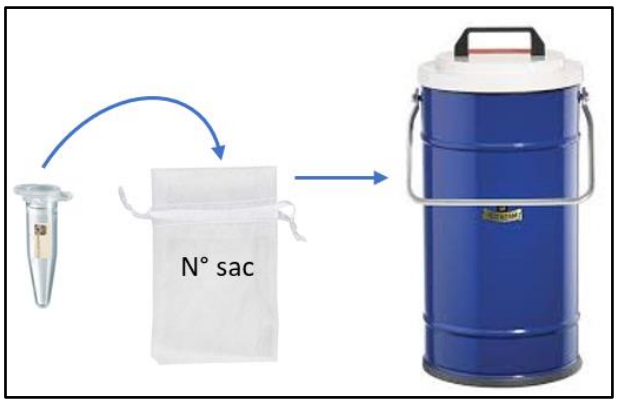


3. Complete the metadata excel spreadsheet ATLASea, remembering to specify if the organism is in the tube. To fill ATLASea QR code label, use the scanner to avoid typos



4. Place the tube in a <u>numbered</u> nylon bag.

Record this number in the metadata excel spreadsheet ATLASea.
Place the nylon bag in a deware filled with liquid nitrogen.
Place between 10 and 15 Eppendorf tubes in a nylon bag before filling a new one.



5. Between each identification, clean the taxonomic kit in bleach and autoclaved MilliQ water baths to avoid cross-species contamination.

6. Once the day is over and the dewar is full, put all the nylon bags containing the tubes into a large ziploc labeled with the ATLASea release date and the first and last name of the person in charge on site.
Store the large ziploc at -80°C and send it to Genoscope.

Sending samples to Genoscope

Always discuss shipping arrangements with a member of Genoscope SeqLab staff:

- Janaina RIGONATO : jrigonat@genoscope.cns.fr
- Karine LABADIE : klabadie@genoscope.cns.fr

Contact Star Service ssh.src@stars-services.com or Éric Bouix directly eric.bouix@stars-services.com to arrange collection of the ziploc bag. When sending frozen samples, they must be placed in dry ice.

Each shipment should include:

- project identification (ATLASea)
- the name of the sender
- a partial description of the samples
- export/import permits for species requiring CITES permits

The samples must be sent to the following address:

SITE CEA EVRY
Réception Marchandises
Janaina RIGONATO
31, Boulevard des Coquibus
91 000 EVRY
FRANCE