

Challenging issues for the study of the mesophotic ecosystems by environmental DNA metabarcoding

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On behalf of:

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Plan

1. Challenges for the study of the mesophotic ecosystems

2. Environmental DNA: principles and main steps

3. Main factors (based on experiences)

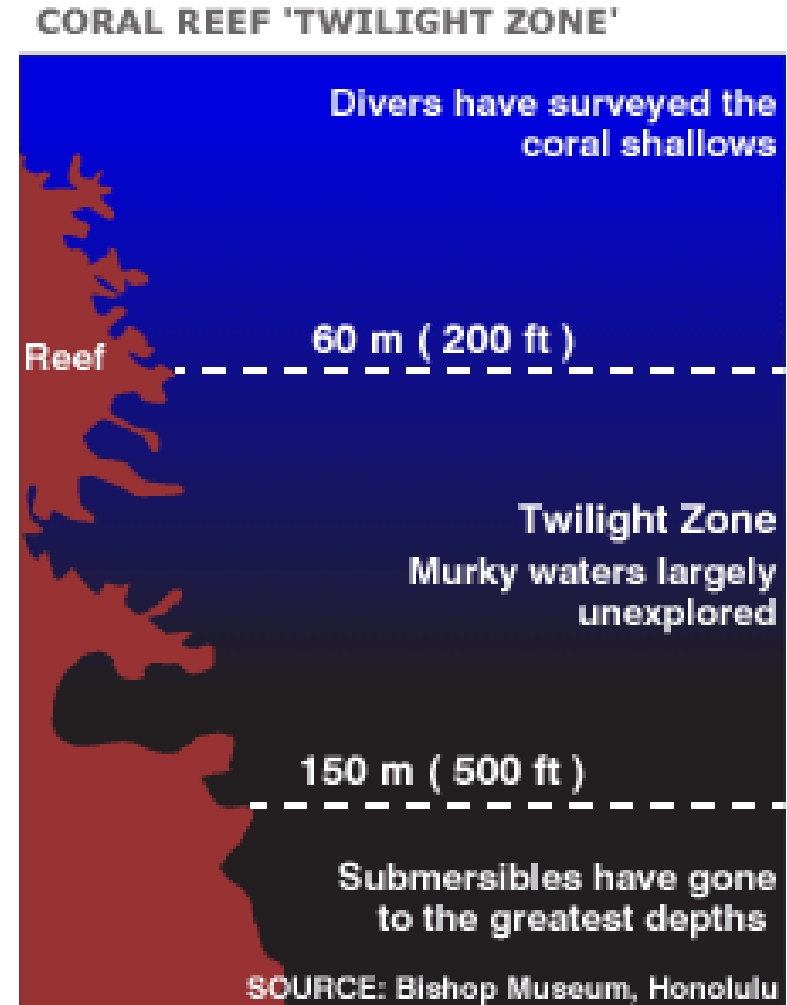
4. Current method

5. Perspectives

1 Challenges for the study of the mesophotic ecosystems

The mesophotic zone (defined as ~30 to 150m depth)

- covers various ecosystems in coral, temperate or cold areas
- specific ecosystems: low light, relatively constant sea water temperature, and less energetic conditions than the subsurface
- therefore constitute unique environmental conditions leading to a specific living community diversity.

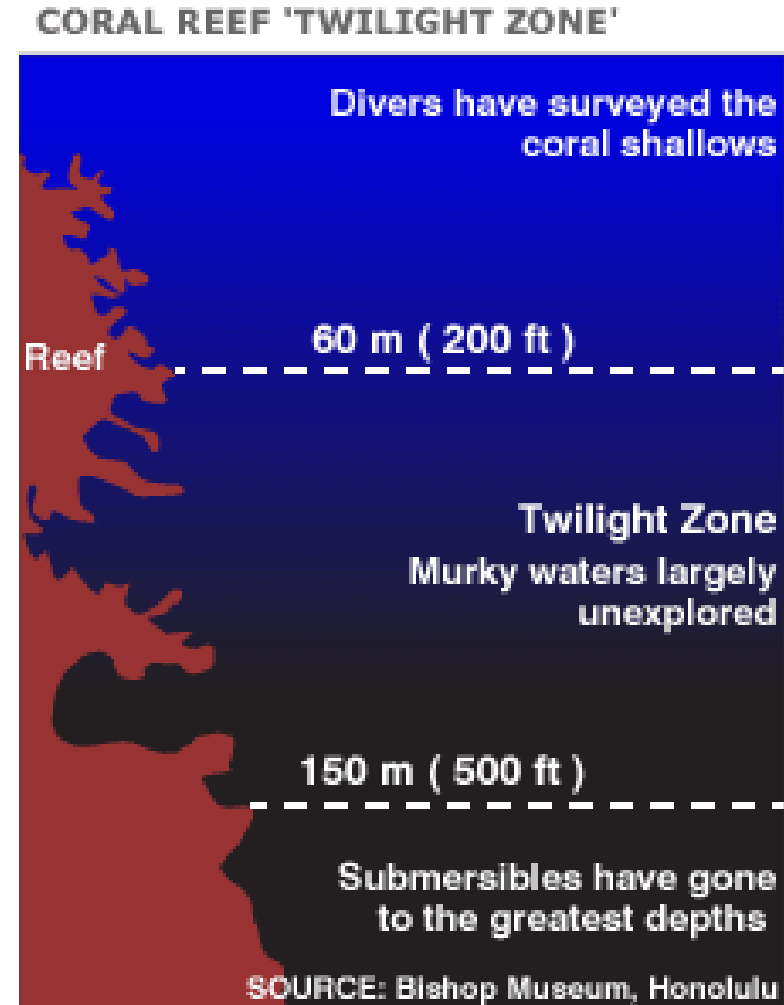


1 Challenges for the study of the mesophotic ecosystems

Moreover, the mesophotic zone faces less anthropogenic pressures and climate change, and constitutes a potential refuge zone on the scale of global changes.

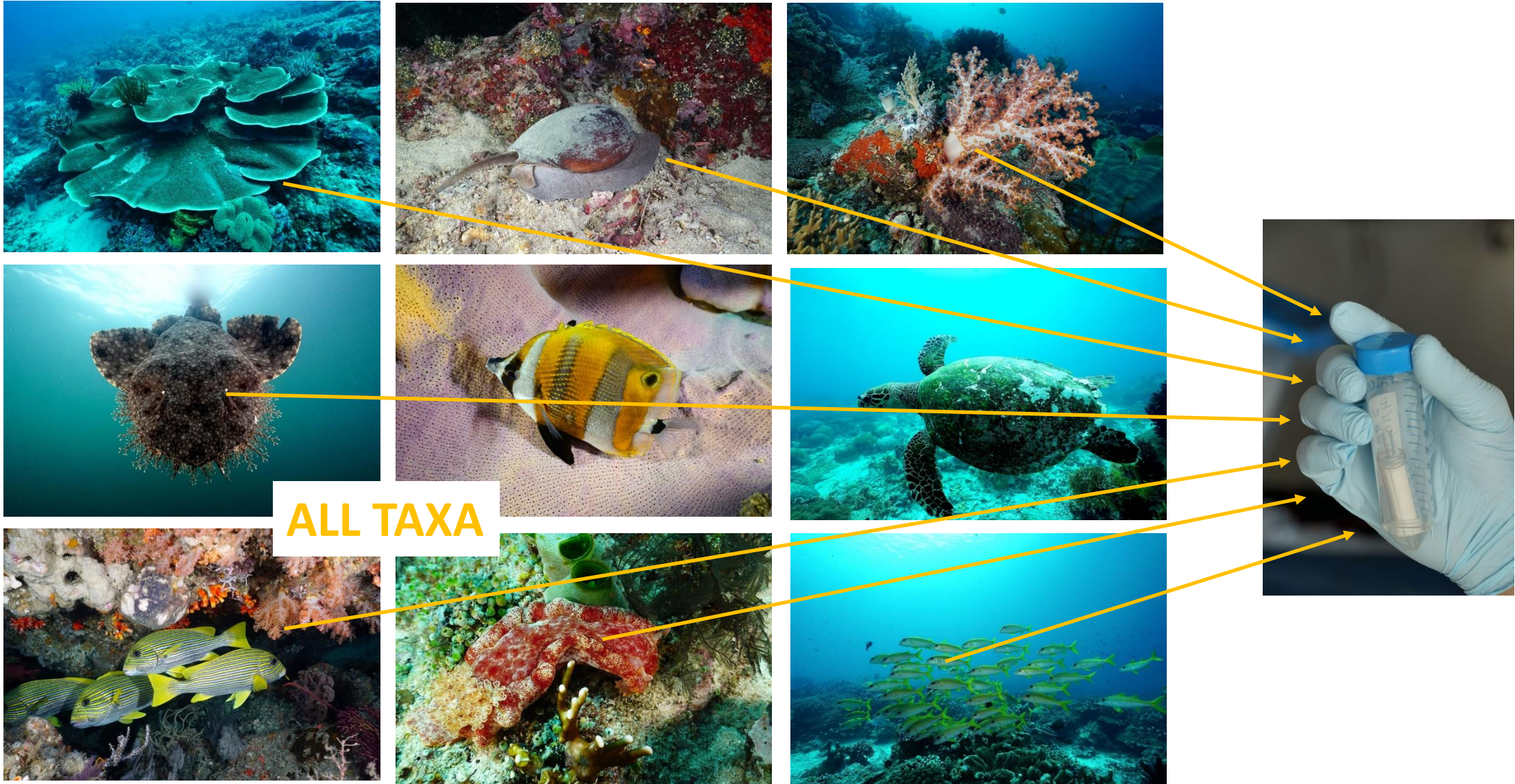
However these coastal areas **remain largely understudied** (Pyle and Copus 2019).

*Some classical methods are not adapted to this area
(by open circuit scuba diving, trawling and dredging...)*



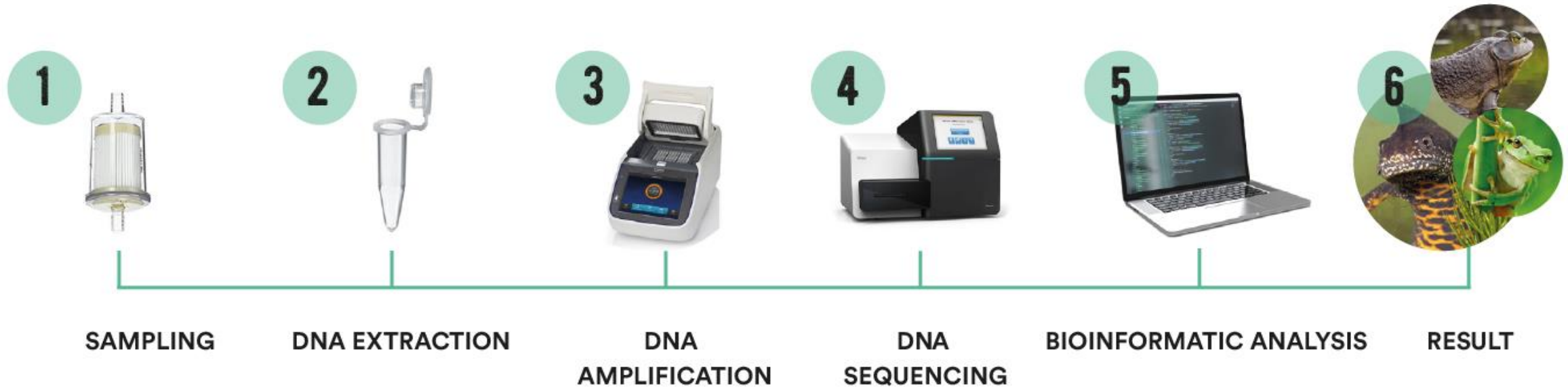
Environmental DNA: principles and main steps

© Photos: Gilles Diraimondo – Lengguru IRD

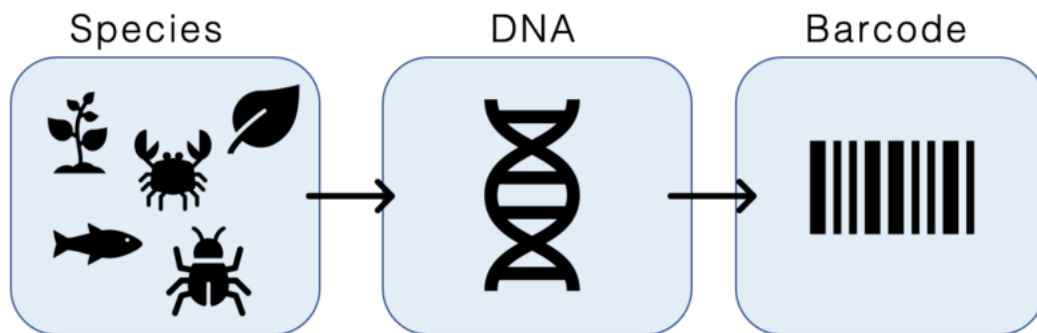


eDNA: detection of traces of organisms' DNA in environmental samples

MAIN STEPS OF THE ANALYSIS



DNA Reference database



Assignment of each DNA sequences

Usefulness of the eDNA (High potential of the method)

Environmental DNA metabarcoding has demonstrated its performance to identify and monitor different taxa in marine environments (Deiner et al. 2017).

Presence/absence: species inventory, biogeography

=> Allow ecological insight / to answer to questions of adaptation, evolution, phenology

&

Looking for a target species

Early detection of invasive species

Identification of spawning sites

Intra-population study

But Limitations and biases of eDNA

Fieldwork

Sampling strategy

Environmental conditions (stratification, currents, energy context...)

Contaminations

Molecular lab.

Choice of microbarcode

Choice of the pair(s) of primers

Number of PCR, number of cycles

Bioinformatics processing

Reference database completeness / adapted to the studied region

thresholds, filters chosen to distinguish chimeras from rare dna

Quality control

Blanks contaminations

- **Use of the tool, taking into account its limitations**
- **Adapt sampling strategy and choices according to the objectives**
- **Methods and protocols in the marine domain not yet stabilized**

Main factors (experiences)

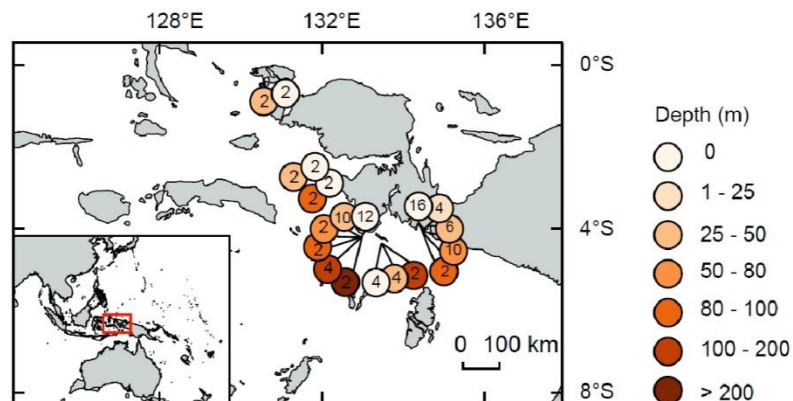
Lengguru 2017 project, in West Papua, Indonesia

3 methods :

- plastic bag in subsurface (*the traditional one*)
- plastic bag by CCR until 100 m depth
- Niskin bottle until 330 m depth

Filtered volume: 2 x 2 L (1 sample + 1 replicate)

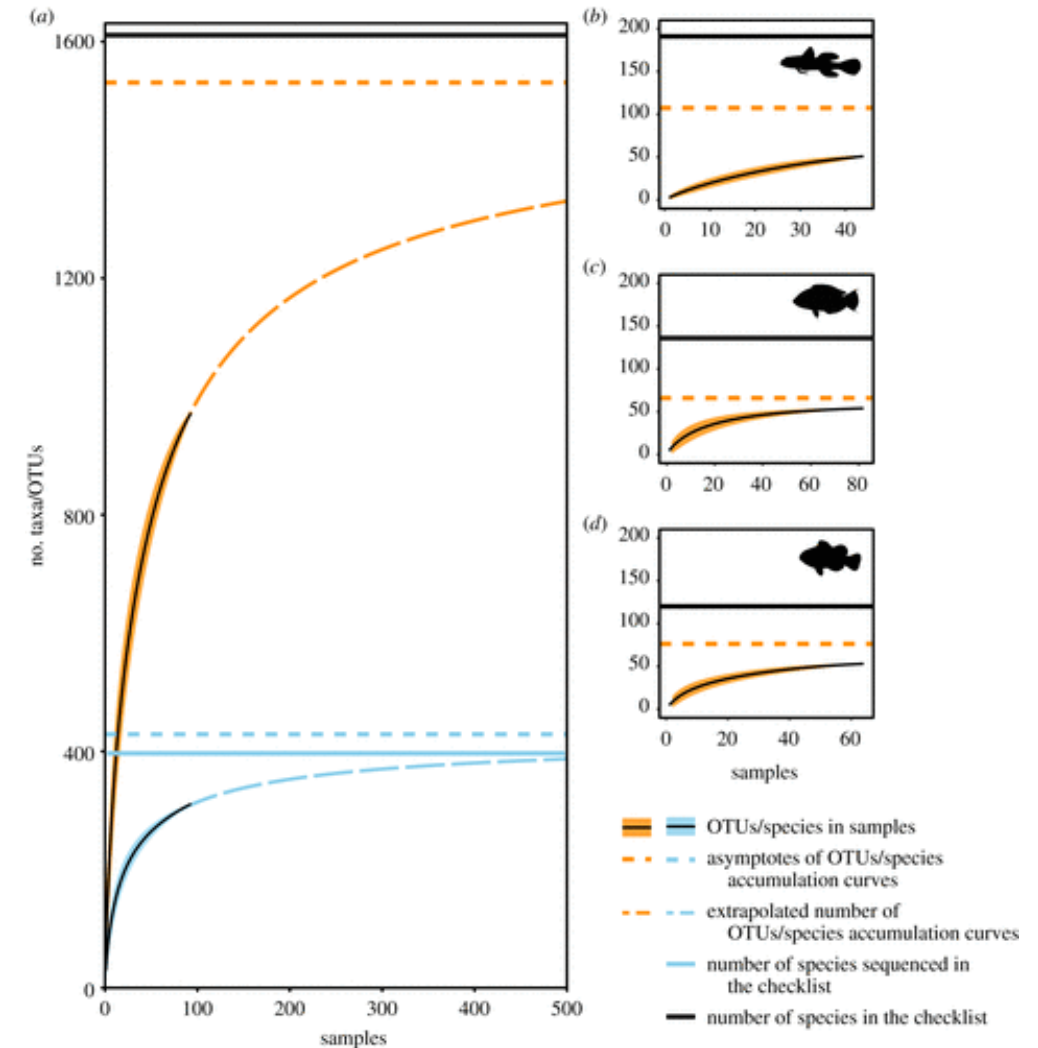
92 locations



Accumulation curves of environmental DNA sequences predict coastal fish diversity in the coral triangle / in a highly diverse ecosystem

Even if... a poorly reference database
(24,5 % sequenced reef fishes of West Papua checklist)

Based on "operational taxonomic units" (OTU)

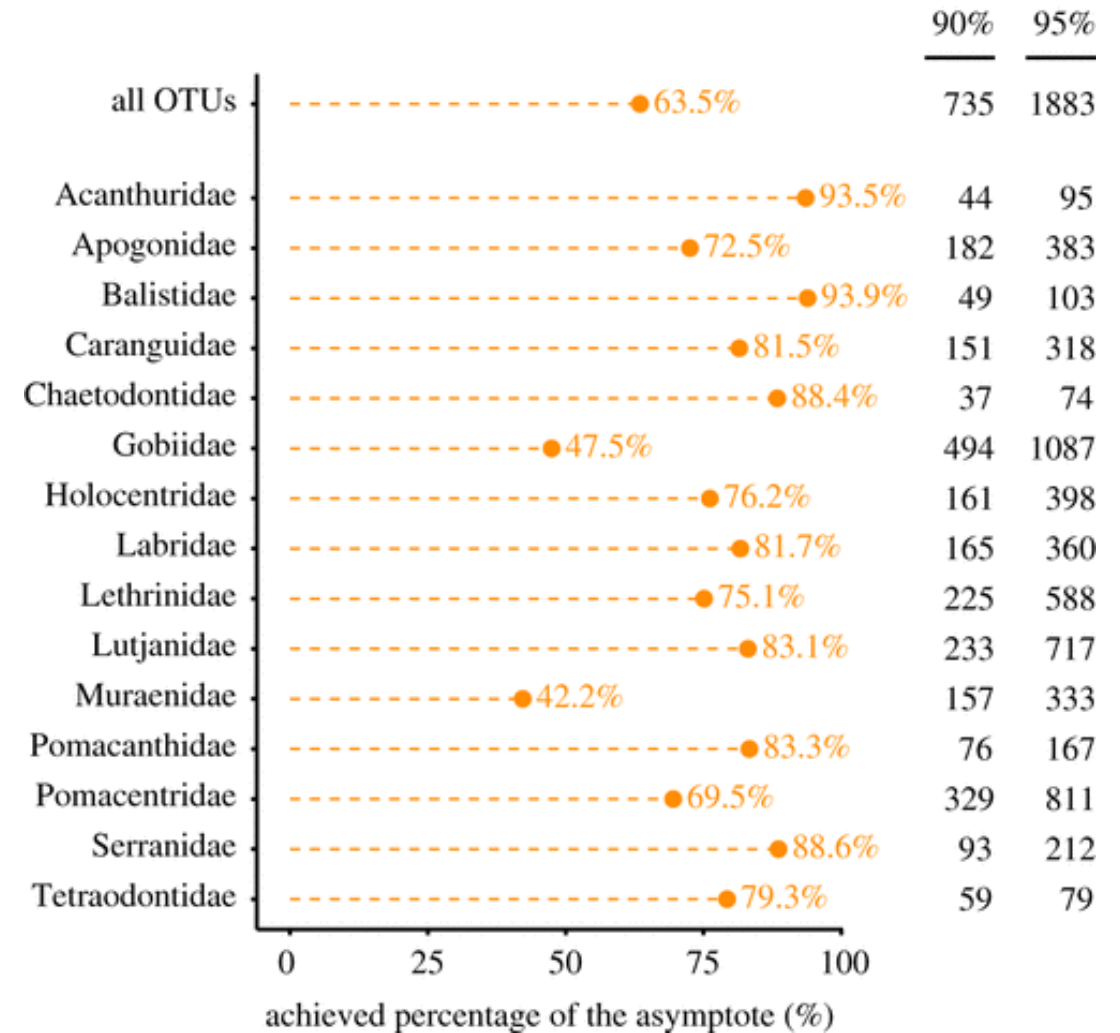


Accumulation curves of species assigned (blue) and the OTUs (orange) obtained in the whole sampling (a) and within the three most diverse families: Gobiidae (b), Labridae (c) and Pomacentridae (d).

High detection capacity with 92 sampling locations (volume: 2 L)

Very close to the habitat/substrate, allowed by CCR diving

High number of sampling locations, geographically distributed, pooled to obtain a regional value



Percentage of the OTUs diversity covered by the current sampling effort (n = 92) in the families of fish (orange) and the estimated sampling effort required to achieve both 90% and 95% of the diversity.

3

Need for completeness of the reference database

12S mitochondrial rDNA => teleo / elasmobranch region defined in Valentini et al. 2016

Both eDNA approach & specimens sampling for barcoding

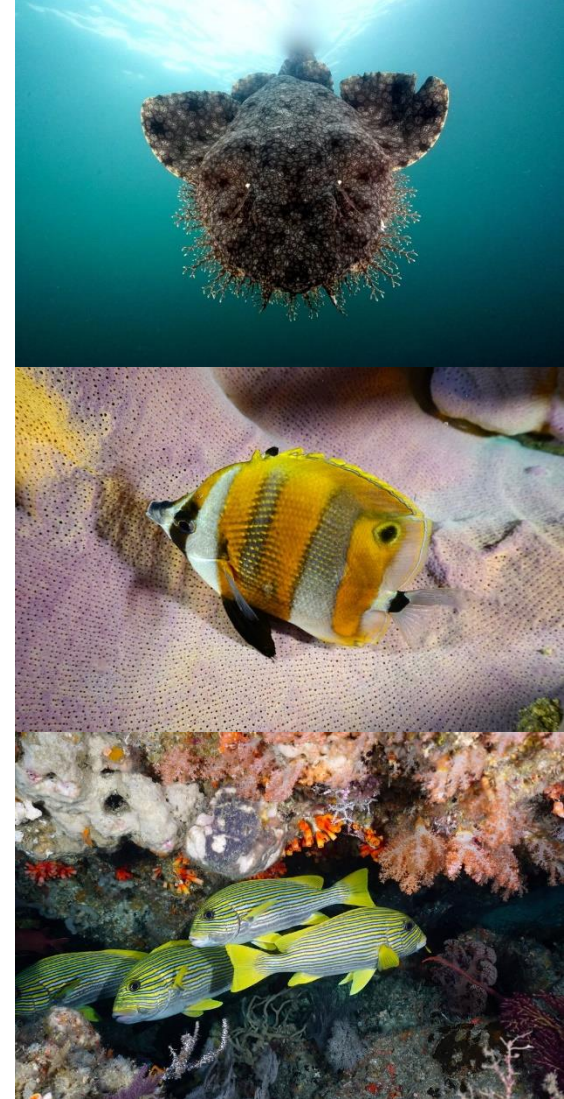
We used to identify the 1466 collected specimens :

1. morphological features
2. genetic sequencing (652 bp CO1 - cytochrome oxidase 1)
(from 413 species, 180 genera and 69 families of fishes/elasmobranch)

Then 3. we amplified and sequenced the 12S region (480 bp) with two distinct pairs of primers respectively designed for teleosts and elasmobranchs

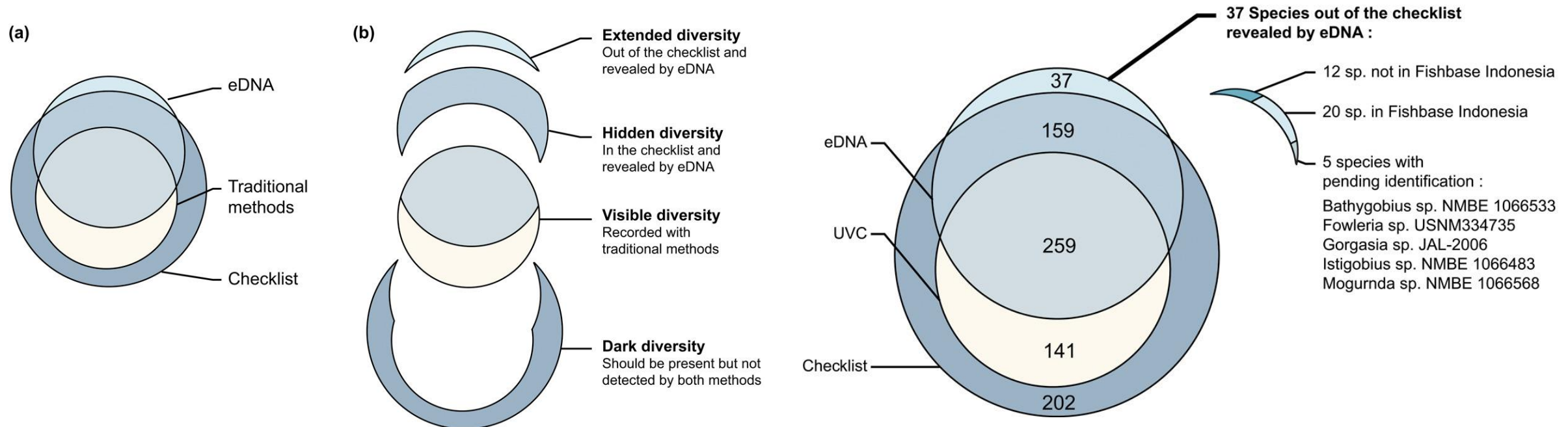
to provide :

- an update of the regional marine species checklist (of the 'Bird's Head Peninsula' (BHP) of West Papua Province' region with 2534 marine fish species)
- the addition of sequences retrieved from our fish sampling **increased this list to 762 sequenced species (43.4%)**



With an improved reference database
and the same eDNA sampling

Better resolving capacity (sequences assignment)
Detection of a hidden diversity (not detected by UVC)
Extension of the known diversity



Other projects: Megafauna (EDM), ...

Surface pumps development

- to filter large volumes (30 L or more)
- to favour dynamic filtering
(to aggregate a “signal” over an area)

Methodological tests

- depth, distance to the habitat : open sea versus coastal environment, combining Niskin bottles and surface pump

Geographical comparaisons

- Highly / poorly diverse ecosystems



© R. Hocdé – IJL SELAMAT - IRD



© O. Borde / Monaco Explorations

3

Refinement of the number of replicates and the water volume ?

The variability of eDNA biodiversity estimates, for some locations, even extensive sampling with 6–20 × 30 L replicates

Recommandations:

30 L / eDNA filter - with 1 replicate (60 L / location)

eDNA studies (**large volume 180 L min., multiple replicates**) could provide **robust broad-scale biodiversity estimates**

Integrative/dynamic sampling, rather than point sampling

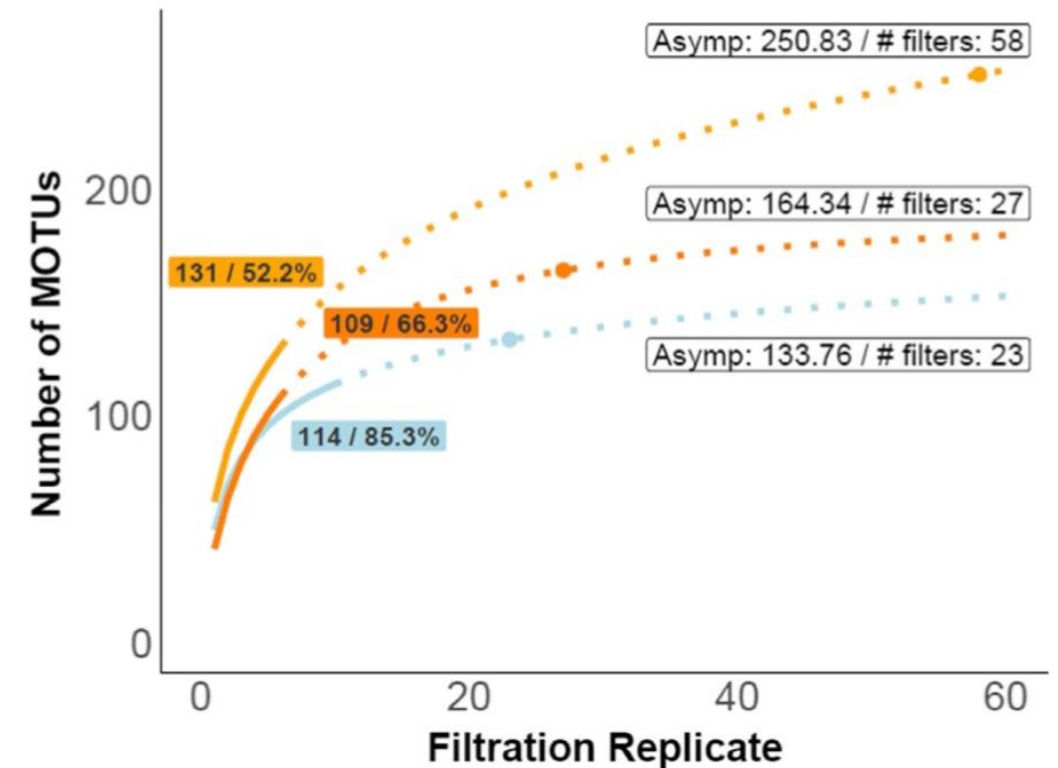


FIGURE 4 Local-scale MOTU richness accumulation analysis of eDNA filtration replicates from Santa Marta and Malpelo. The

LABCOM DIAGeDNA

"Environmental DNA assessment of marine environments"
(UM, IRD... & SPYGEN, Andromède océanologie companies)

Development of several versions of
submersible pumps
specifically developed for deep sea waters
(150 or 1500 m depth)

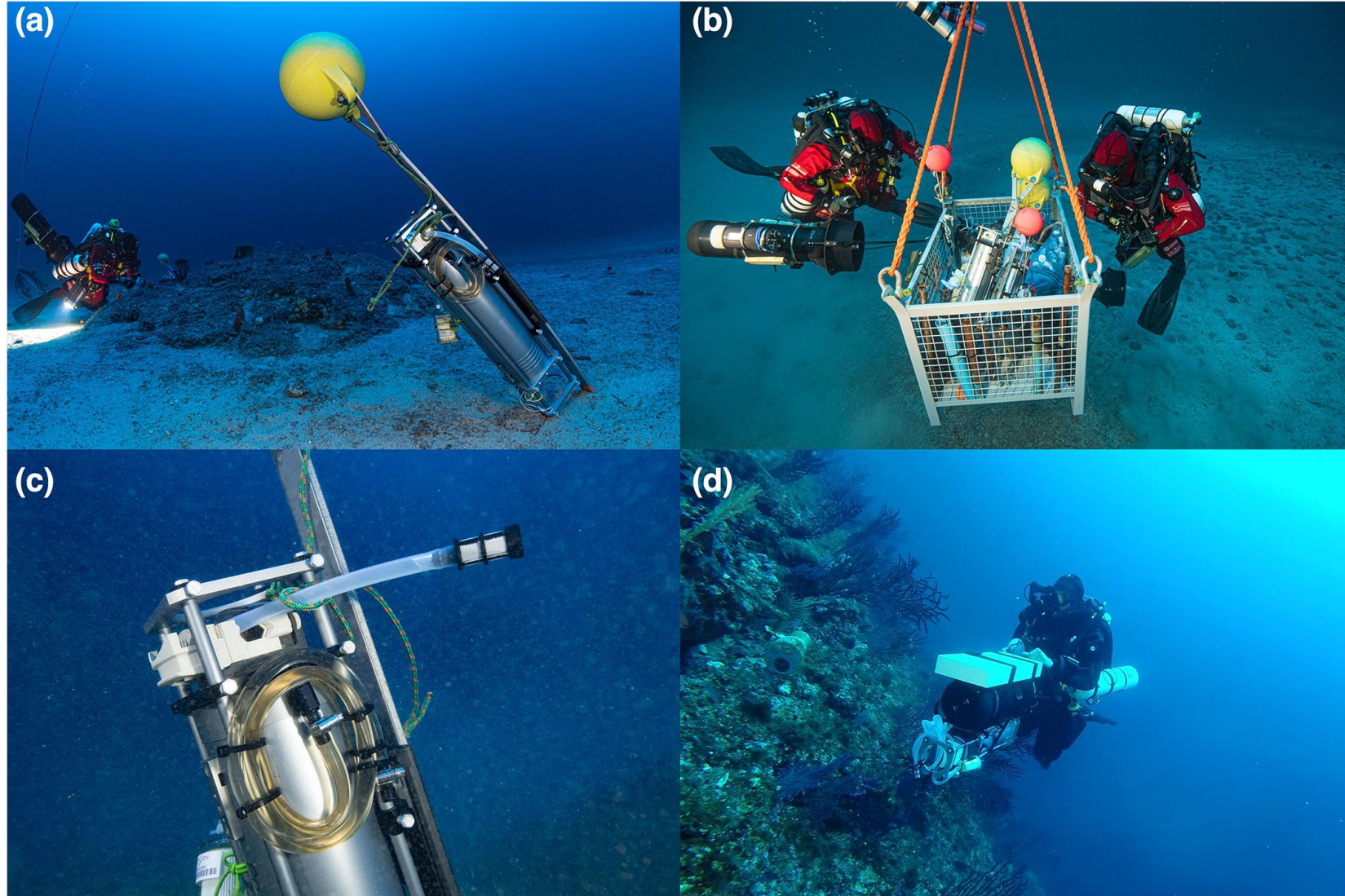
For *in situ* filtering

Deployed by divers or from a ship with a crane and a winch

Aims:

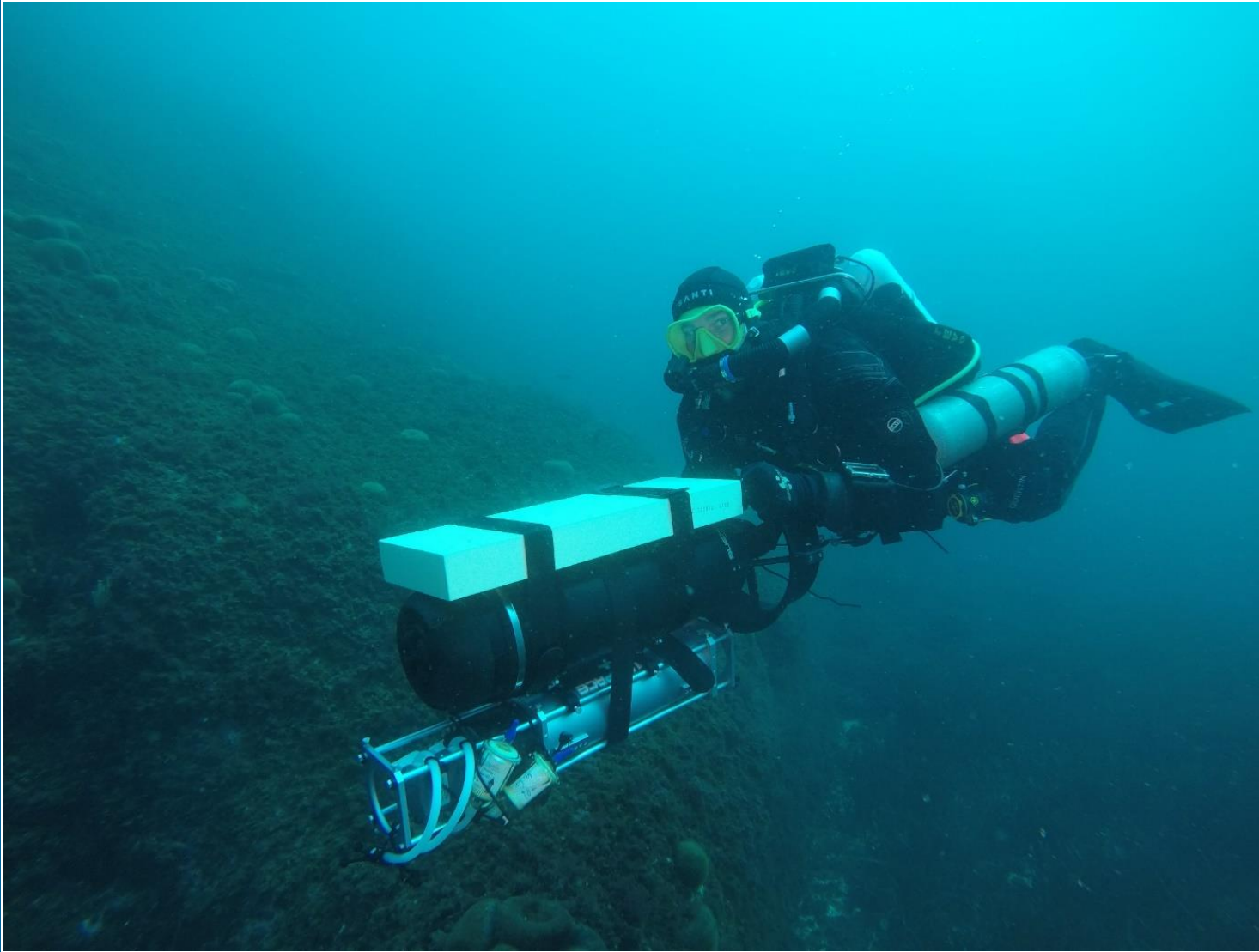
- to catch the best "signal" close to the substrate ⇒ CCR scientific divers
 - Replicate ⇒ 2 CCR scientific divers (+ surface security)
 - Mesophotic area ⇒ Deep CCR divers
 - High volume of filtration ⇒ Submersible pumps (30 L / filter)
 - Integrative / dynamic sampling ⇒ Sea scooters
- + double filtration head to reduce the filtration time and therefore the saturation of the divers (extracts are pooled before amplifications) (15 minutes instead of 30 mm)

Gombessa expeditions, DEEP Heart, DEEP Evo projects



Montage of pictures showing the submersible pump used for sampling along the Provence coast (North-Western Mediterranean Sea) and at the seamount La Pérouse (Western Indian Ocean). Photo credits: (a) Laurent Ballesta, Gombessa expéditions, Andromède Océanologie (2019), (b & c) Laurent Ballesta, Gombessa 6: Mission cap Corse, Gombessa expéditions, Andromède Océanologie (2021) and (d) Régis Hocdé, location: Calanques of Marseille, Riou island

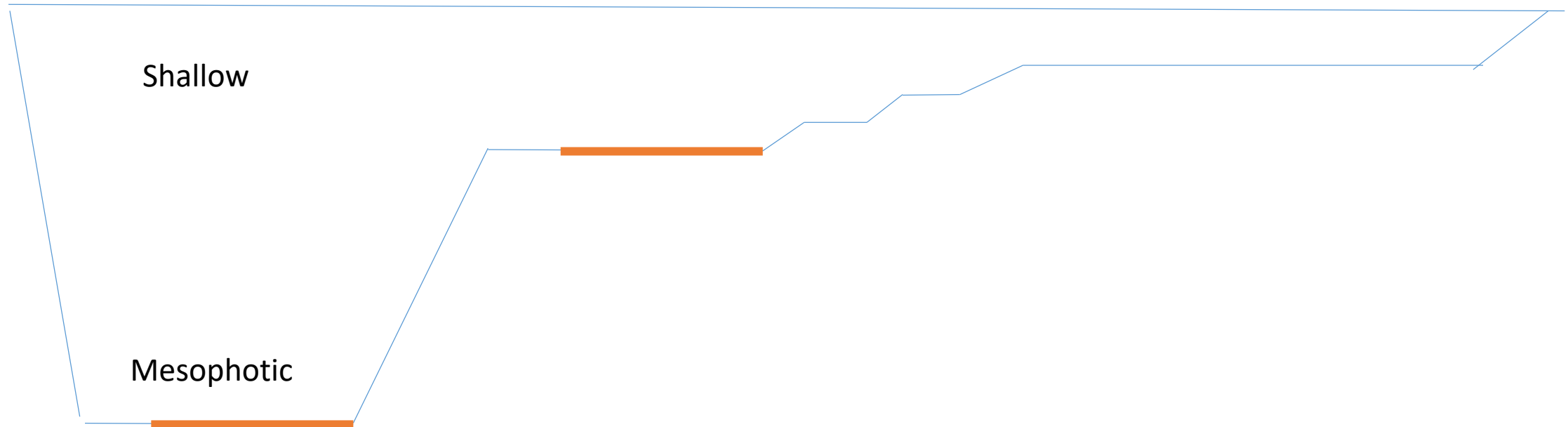
DEEP Heart, DEEP Evo in the 'Calanques', Marseille, France



© Régis Hocdé



DEEP Heart, DEEP Evo in the 'Calanques', Marseille, France



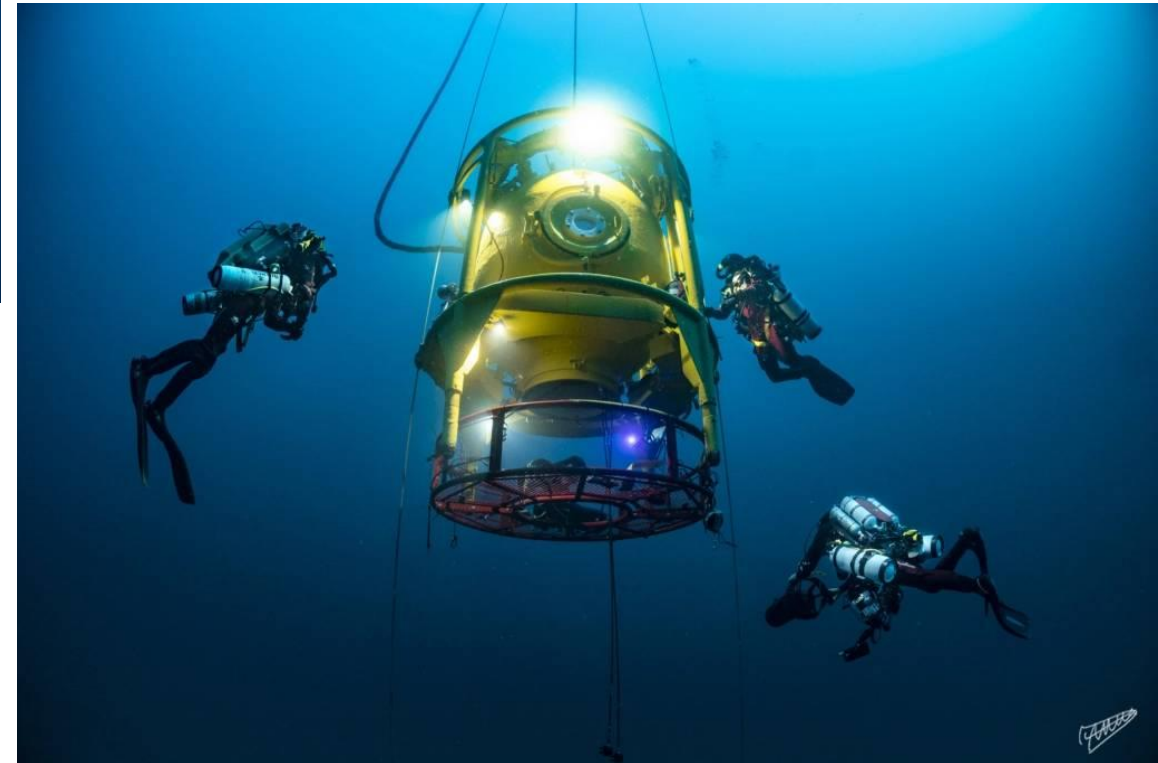
2 pumps – with double head (2 eDNA filters each)
15 mn filtering
= 2 x 30 L

OR
2 pumps – with simple head
30 mn filtering
= 2 x 30 L

Expéditions Gombessa

Combining CCR and saturation

No need of double head for pump



Engineering

Already tested an autonomous underwater vehicle (ou AUV) with pump

Ongoing glider test deployment with pump

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« Calanques »: eDNA-based observatory (already 2 years acquisition,
4 then 2 times/year)

Long-term dimension

**towards a (some) long-term observatory(ies)
of the shallow and mesophotics areas
for the study of vertebrate and invertebrate communities
within No take area / outside MPA**

**To understand the yearly trend evolutions as well as phenology, detection of
invasive species and variations of the seasonal cycles of invertebrate and fish
diversity in the context of global change, .**

THANK YOU FOR YOUR ATTENTION

Hocdé R, Albouy C, Ballesta L, Bianchimani O, Dejean T, Deter J, Duhamet A, Guillemain D, Holon F, Mérigot B, Pélissier L, Rozanski R, Schull Q, Velez L, Zuberer F, Mouillot D

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Thanks to the photographer Laurent BALLESTA / Expéditions Gombessa / Andromède Océanologie



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