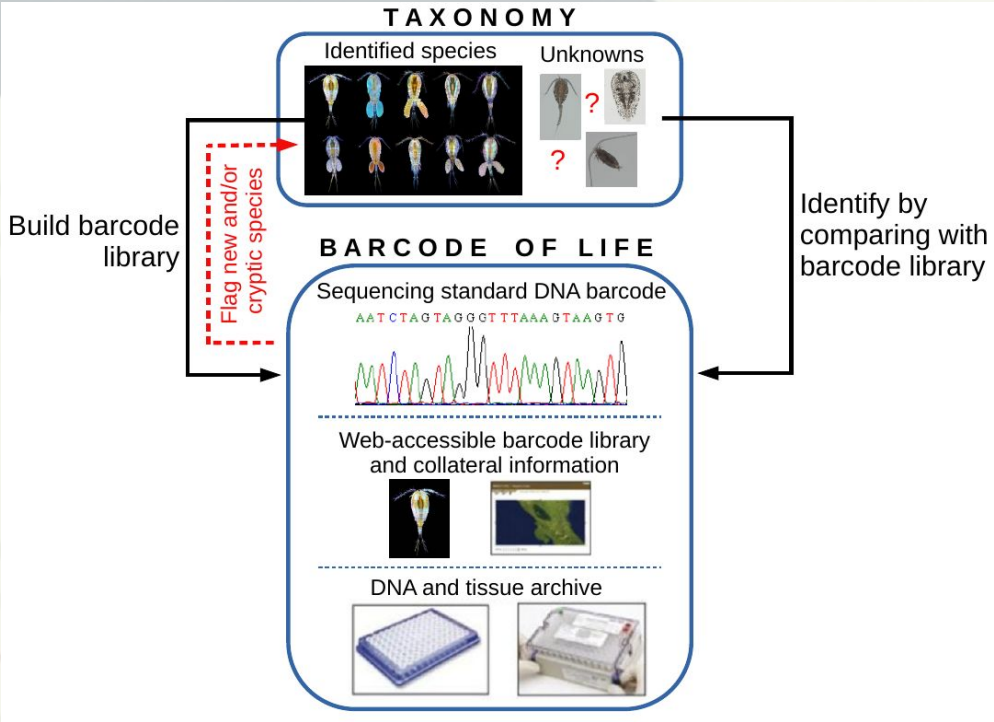
DNA barcoding, metabarcoding & eDNA: a short intro

DNA barcoding

DNA barcoding is a method of identifying species using short sections of DNA or 'barcodes'.

Creating a Reference Barcode

Initially a DNA barcode from a correctly identified specimen is needed as a reference barcode. Creating this DNA barcode involves collecting a specimen and identifying it morphologically to species level. The DNA barcode is then produced by extracting DNA, amplifying a specific segment of the organism's genome by PCR, and sequencing this segment. Once the DNA barcode is produced it is then registered on an international database (such as GenBank). Once the DNA barcode is on a database, other researchers can compare their DNA barcodes to those on the database.



Benefits

- Provides identification of a species where the ID is in question.
- Particularly useful in hard to identify groups.
- Can be used to identify incomplete specimens where key taxonomic features are absent.
- Can be used to identify immature life stages.

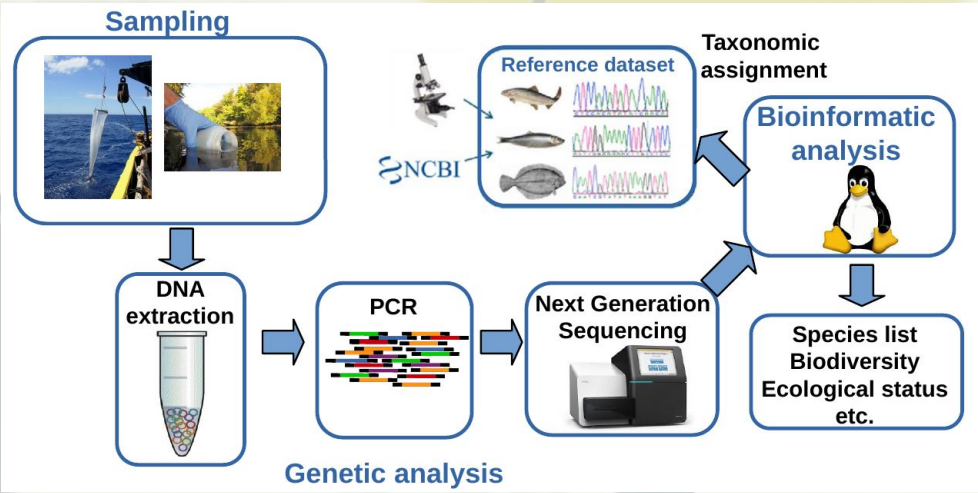
Disadvantages

- A single specimen is analyzed
- You need accurately identified DNA barcodes, which may not always be available, or they may not have been identified properly.
- The specimen must be preserved in such a way as to keep DNA intact (e.g. frozen, dried, preserved in ethanol).

MARBEFES has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement no 101060937

DNA metabarcoding

DNA metabarcoding is the large-scale taxonomic identification of complex environmental samples via analysis of short DNA sequences (called DNA barcodes) of one or a few genes.



- The taxonomic resolution depends on the target gene (barcode) used, and it can reach at the species' level.
- The selection of the PCR primers defines which barcode will be amplified and for which taxonomic group (e.g. bacteria, eukaryotes, plants, arthropods, vertebrates), but this is not absolute (some target species may be missed and some non-target ones amplified).
- Standardization of DNA metabarcoding methods is crucial before routinely applying them to ecological studies.

Using DNA metabarcoding

- Determine species assemblages from various environmental samples
- Detect rare, cryptic or elusive species
- Evaluate management action – whether it is successful or not
- Monitor species abundance changes over time

Benefits

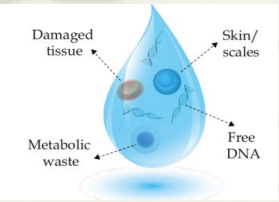
- High throughput
- High accuracy in species' identification (even for difficult taxa, immature stages, cryptic species, degraded samples)
- Standardized procedure
- Cheaper and faster compared to traditional approaches
- No need of a taxonomy expert but requires a well curated and complete reference database for correct taxonomic assignment

Disadvantages

- Results are qualitative or semiquantitative at best due to the PCR
- There can be biases in species' detection and in estimation of the relative abundance mostly due to PCR amplification biases
- Still, incomplete reference databases, especially for non-standard barcodes and for invertebrate taxa

MARBEFES has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement no 101060937

eDNA



Environmental DNA (eDNA) is the DNA released from organisms into their environment (e.g. from skin cells, metabolic waste, mucous, hair, eggs and sperm).
eDNA samples can be collected from seawater, rivers, lakes, snow, soil/sediment and even air.

! eDNA is preserved in the water for a few days, thus reflecting the current communities. In the soil and the sediment it can be preserved for years, even centuries.

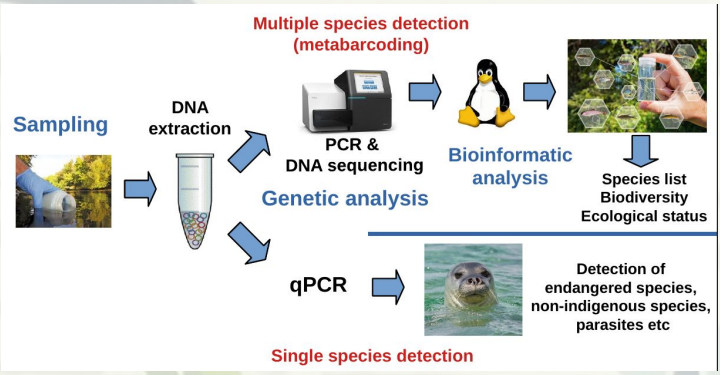
How can it be used in the marine environment?

Seawater samples are collected and filtered through a fine filter. Everything that is on this filter is then extracted using a specialised DNA extraction kit.
eDNA can also be extracted from the sediment.

Main approaches to eDNA analysis

Metabarcoding: multiple species detection.

Quantitative PCR (qPCR): a specific species is targeted and its presence in the sample is tested by using a qPCR assay.



Using eDNA

- Determine species assemblages
- Detect rare, cryptic or elusive species
- Detect migration or spawning behaviour
- Evaluate management action – whether it is successful or not
- Monitor species abundance changes over time
- Monitoring invasion fronts for early detection to allow for rapid response
- Provide data for Blue Carbon analysis

Benefits

- Those of DNA metabarcoding plus
- Non-invasive technique
- Rapid detections for qPCR assays

Disadvantages

- Those of DNA metabarcoding plus
- Different environmental conditions can affect the breakdown of DNA e.g. high UV light

MARBEFES has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement no 101060937

DNA metabarcoding in MARBEFES

Aims:

- Standardize protocols for marine metabarcoding as a biomonitoring tool (in line with other European and International metabarcoding networks)

  Question: which groups to target? (microbes, phytoplankton, zooplankton, benthic communities, fish, marine mammals?)

- Apply these standardized protocols to different BBTs

  Question: to which BBTs?

- Collect and barcode voucher specimens from different BBTs to improve genetic databases

  Question: focus on which taxa? (abundant species that lack barcodes?)

What can DNA metabarcoding offer to other tasks of MARBEFES

- Species lists (and their relevant abundance) for different taxonomic groups from different habitats of the BBTs
- Ecosystem quality indicators (in relation to MSFD)
- Indicators for ecosystem function (based mostly on microbial taxa, and the detection of key ecosystem species)
- Data for Blue Carbon analysis (detection of key species that sequestrate carbon, by performing eDNA analysis of the sediment)

Credits: This leaflet was created by P. Kasapidis (HCMR, Greece), Janette Carlsson and Jens Carlsson (UCD, Ireland)
Pictures and figures were used and modified from the following sources:
Picture of copepods by Andrei Savitsky Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=78800127
DNA barcoding figure modified from Mishra *et al.* 2017
DNA metabarcoding figure modified by Slijkerman *et al.* 2017
eDNA pictures were retrieved from here and here
Monk seal picture by Vasilis Drosakis /Wikimedia Commons/CC BY-SA 4.0 from here