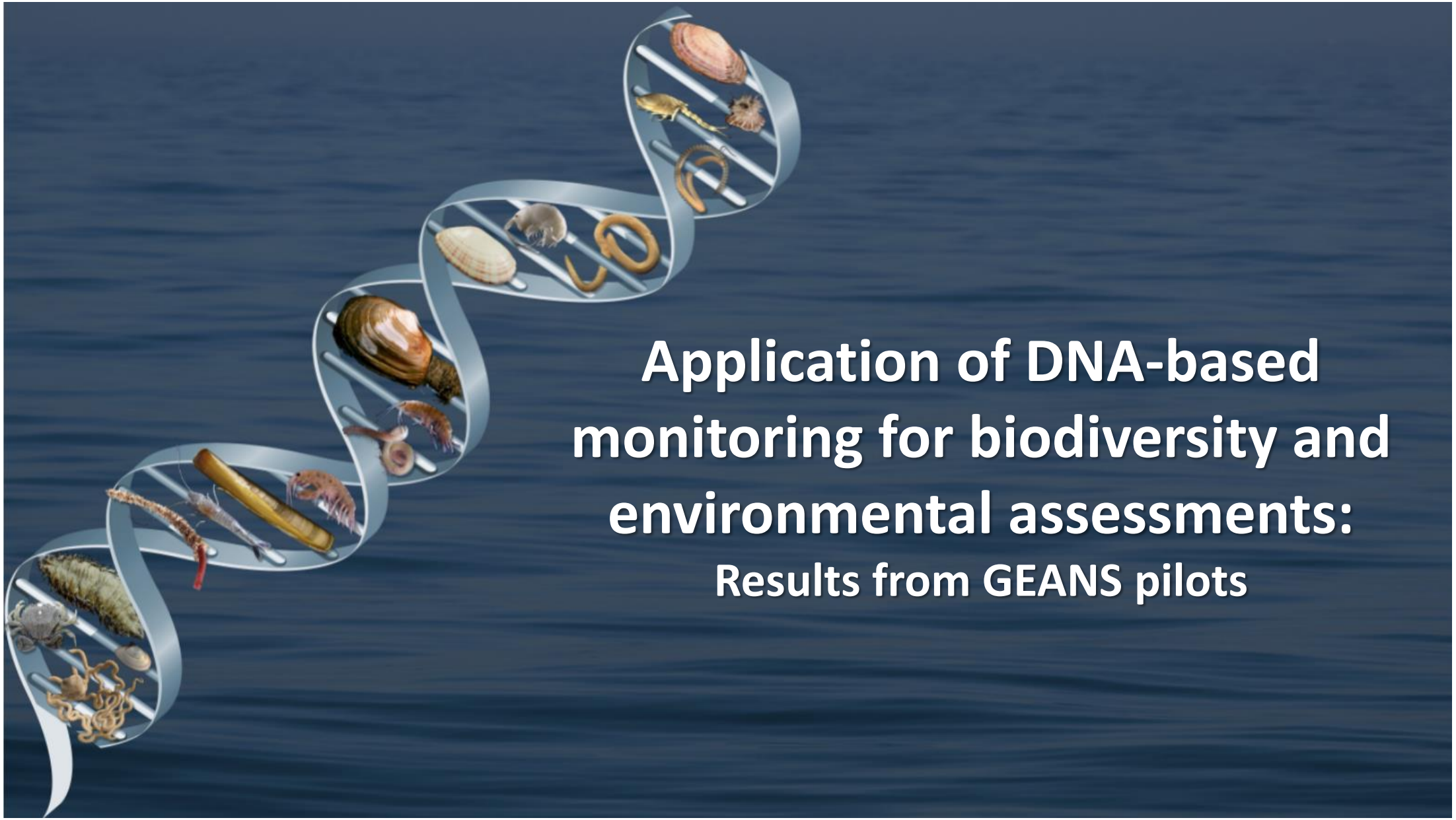
TOR C4: To review the development of new molecular genetic techniques and the application of the methods for biodiversity and environmental assessments

BEWG 2022, 10 May

Annelies De Backer

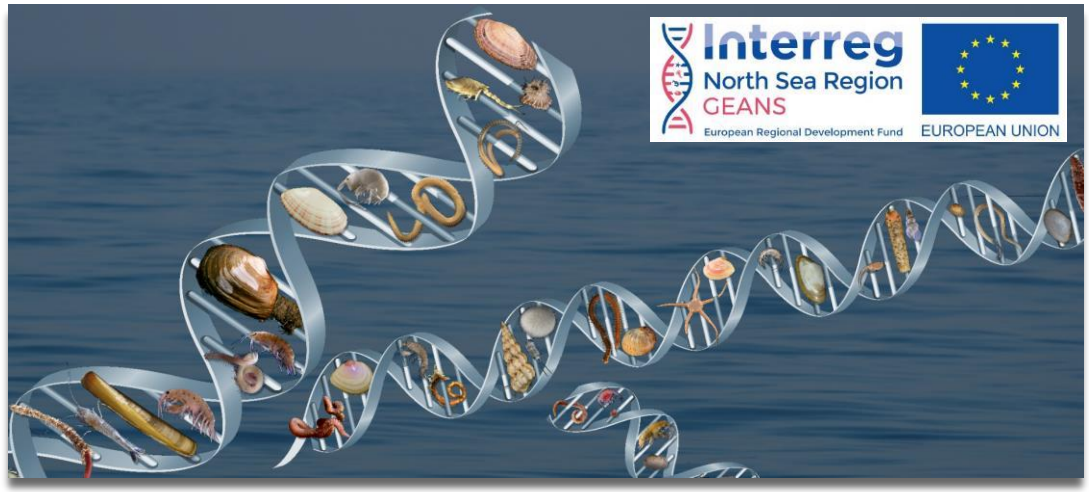
ILVO



Application of DNA-based monitoring for biodiversity and environmental assessments:

Results from GEANS pilots

Genetic tools for Ecosystem health Assessment in the North Sea region (GEANS)



Website: https://northsearegion.eu/geans

or: https://geans.eu

@GEANS_Interreg

Objective: GEANS strives to implement DNA-based tools in routine monitoring programs in support of policy and decision making concerning ecosystem health.

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Interreg North Sea Region GEANS
European Regional Development Fund
EUROPEAN UNION

ILVO · Cefas · Aarhus University · SEAnalytics AB · NORD University · SENCKENBERG world of biodiversity · VLIZ · Wageningen University & Research · Naturalis Biodiversity Center

WP1 - Project management

WP2 – Communication

WP3 – DNA reference library

WP4 – Protocols and tools

WP5 – Pilots

- Non-indigenous species
- Hard substrates
- Soft substrates

WP6 - Policy Decision Framework

Add-on Reference library
- Filling gaps through historical collections
- Taxonomist meeting
- Full mitogenomes

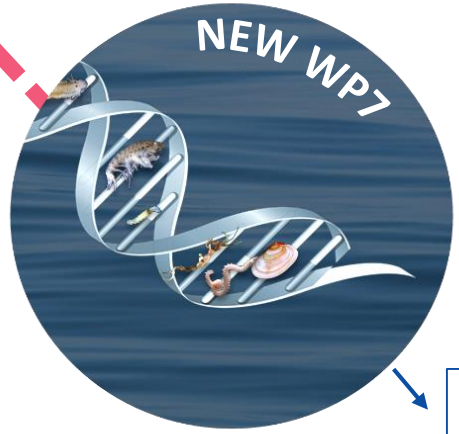
Towards a *reliable* and *future-proof* DNA reference sequence library

Add-on Protocols and tools
- Transnational ring test
- Standardised analytical tool

Towards consolidation of harmonized protocols, for *scientists and stakeholders*

WP7 – Towards zero impact monitoring

- Pilots to validate eDNA in
  - offshore wind farms
  - ecosystem restoration
  - non-indigenous species



Applying developed protocols to '*new*' methods

GEANS pilots

- Goals:
  - Establish harmonized protocols for molecular monitoring
  - Proof their effectiveness in pilot studies
  - Remove barriers for implementation
- Three '*sensu lato*' pilots – mostly comparison conventional with molecular methods
- Often steered through stakeholders and/or in parallel with existing monitoring programs

Pilot led by ILVO
Involved partners:
- Senckenberg
- Naturalis
- Aarhus University

Pilot led by SeAnalytics
Involved partners:
- Aarhus University
- Nord University
- VLIZ

Pilot led by VLIZ
Involved partners:
- Senckenberg
- Nord University
- Naturalis

Pilot to study the effects of marine aggregate extraction on benthic communities comparing traditional and DNA-based methods

Derycke S. , Van den Bulcke L., Maes S., Wittoeck J., Hillewaert H., De Backer Annelies

Interreg North Sea Region GEANS
European Regional Development Fund

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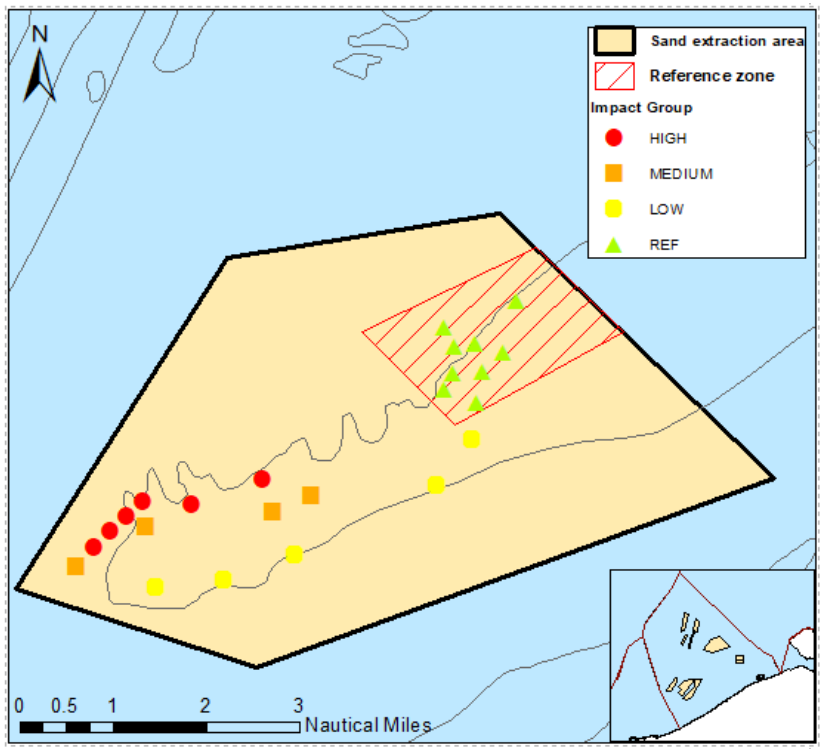
Biological monitoring…

can we increase throughput and reduce costs?

Thorntonbank: epicenter of aggregate extraction since 2015 (150 000 m^3/month) (Van Veen grabs)

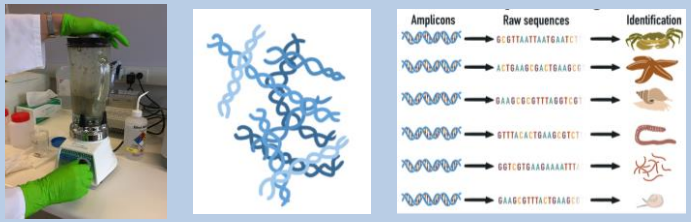


Morphology based analysis

67 hours

6 514,37 euro

DNA-based analysis (bulk metabarcoding)



38 hours

4 848,39 euro

Lab protocol available here: https://www.geans.eu/protocols/sbs

DNA-based identification is 45% faster and 27% cheaper

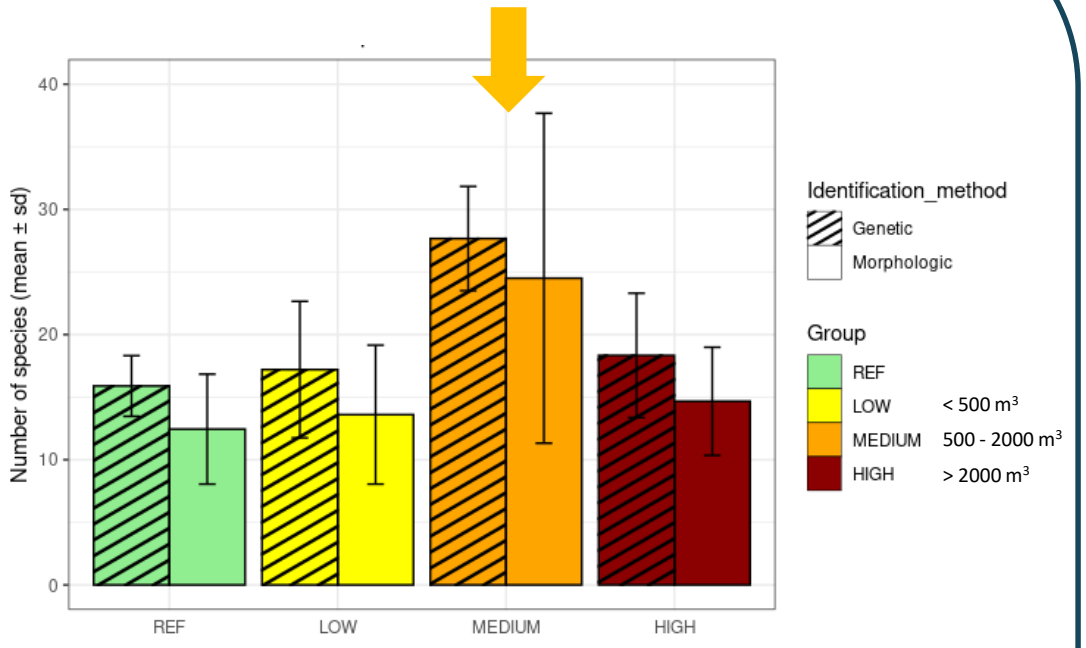
(50% faster and 44% cheaper for 96 samples)

Biological monitoring…

can we increase throughput and reduce costs…

without losing ecological information?

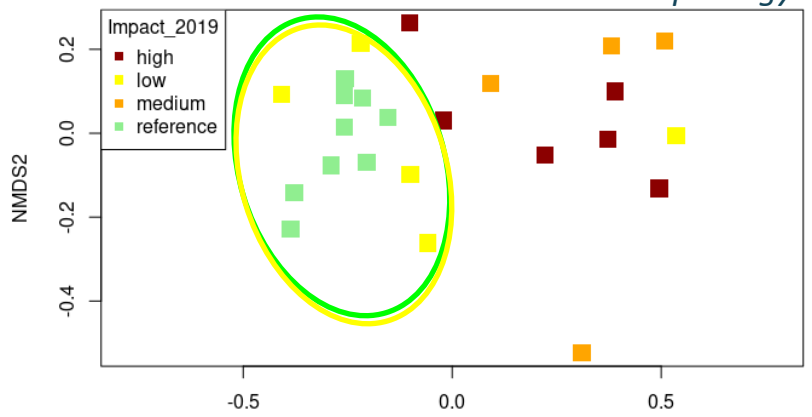
Number of species



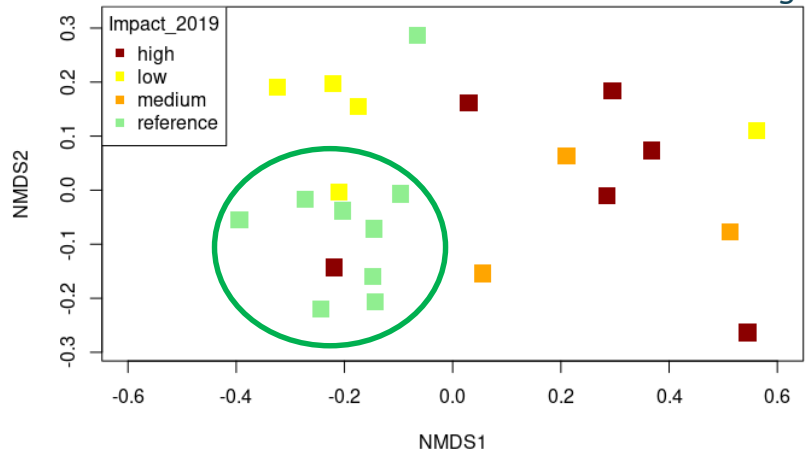

```
Analysis of Variance Table

Response: nr_species
             Df  Sum Sq Mean Sq F value   Pr(>F)
Group         3  632.57 210.857  6.6086 0.001054 **
Method        1  146.17 146.174  4.5813 0.038802 *
Group:Method  3    0.11   0.038  0.0012 0.999941
Residuals    38 1212.44  31.906
---
```

Differences between communities

Morphology



DNA metabarcoding



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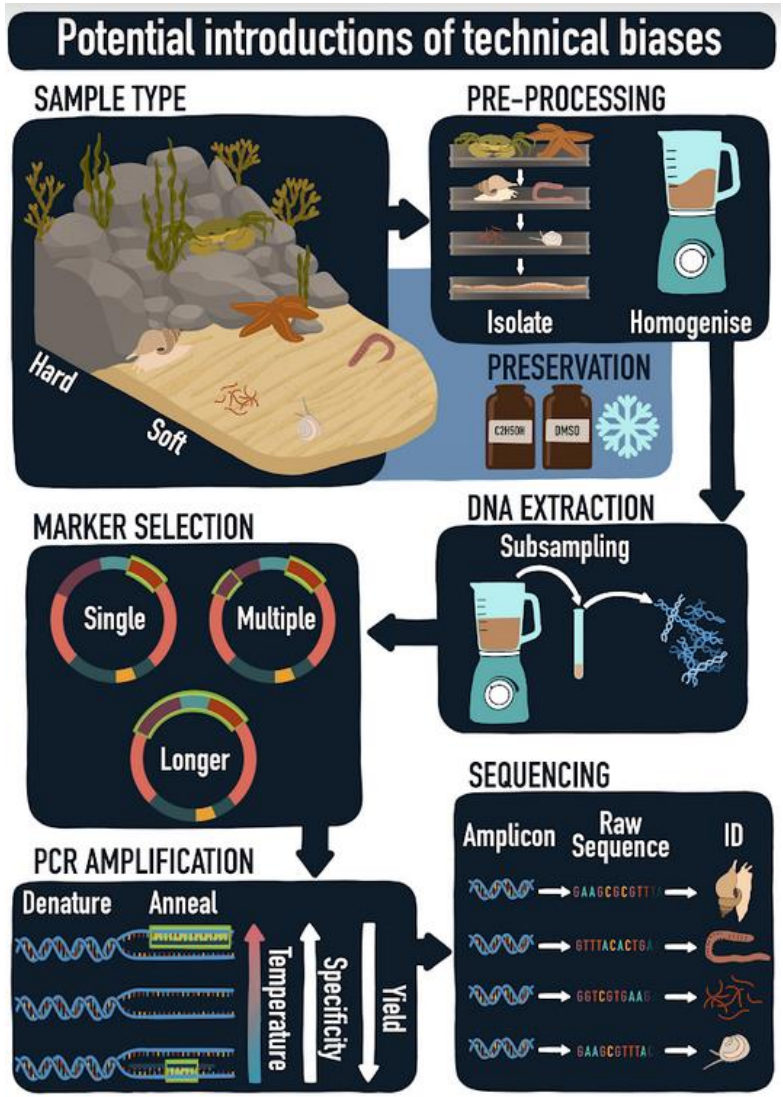
Is DNA metabarcoding data robust and repeatable?



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Loos & Nijland (2020). Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. Molecular Ecology

ORIGINAL RESEARCH article

Front. Mar. Sci., 15 June 2021 | https://doi.org/10.3389/fmars.2021.637858

Detection of Macrobenthos Species With Metabarcoding Is Consistent in Bulk DNA but Dependent on Body Size and Sclerotization in eDNA From the Ethanol Preservative

Sofie Derycke[1,2*], Sara Maes[1], Laure Van den Bulcke[1], Joran Vanhollebeke[1], Jan Wittoeck[1], Hans Hillewaert[1], Bart Ampe[3], Annelies Haegeman[4], Kris Hostens[1] and Annelies De Backer[1]

MBMG
Metabarcoding & Metagenomics

Metabarcoding and Metagenomics 5: 233–247
DOI 10.3897/mbmg.5.71107

Research Article

Towards harmonization of DNA metabarcoding for monitoring marine macrobenthos: the effect of technical replicates and pooled DNA extractions on species detection

Laure Van den Bulcke[1,2], Annelies De Backer[1], Bart Ampe[1], Sara Maes[1], Jan Wittoeck[1], Willem Waegeman[2], Kris Hostens[1], Sofie Derycke[1,2]

Bulk DNA = most robust & Body size and abundance do not explain species detection using DNA metabarcoding, but diversity of the sample does & rare species harder to detect

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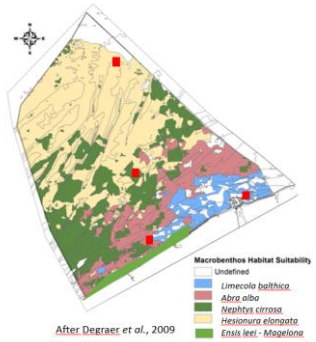
Is DNA metabarcoding data robust and repeatable?

RINGTEST



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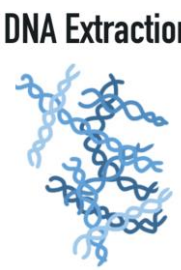

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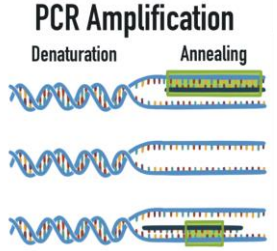

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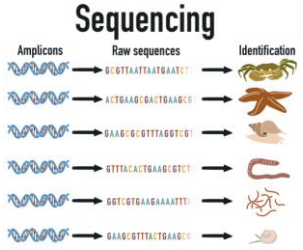

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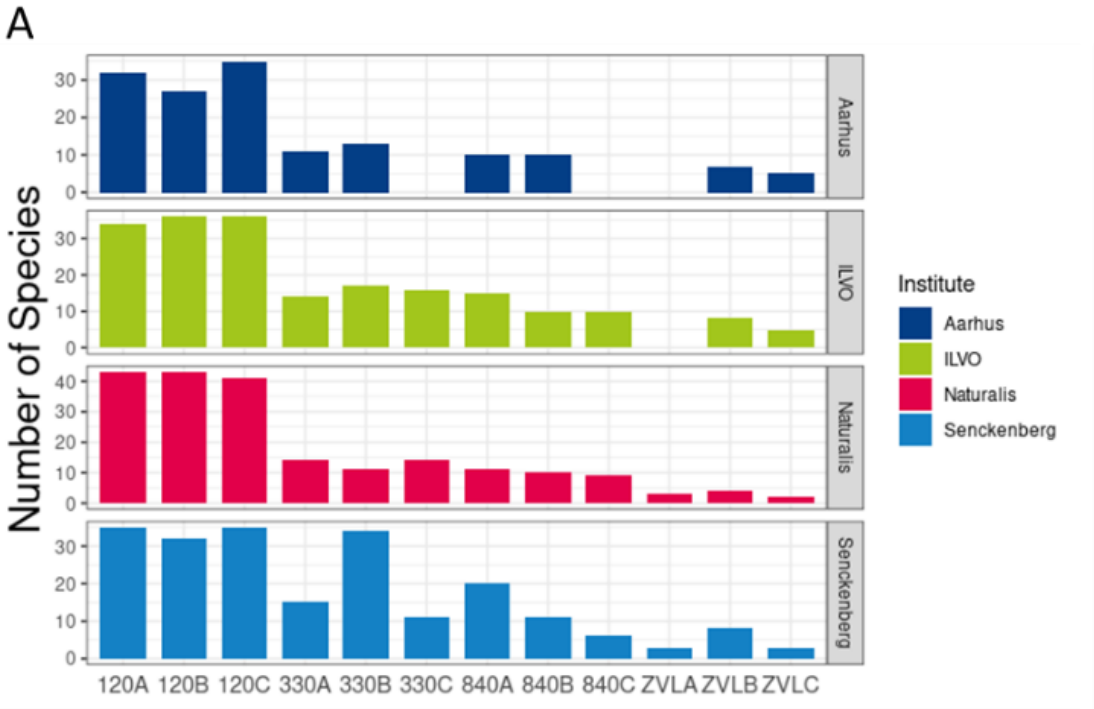

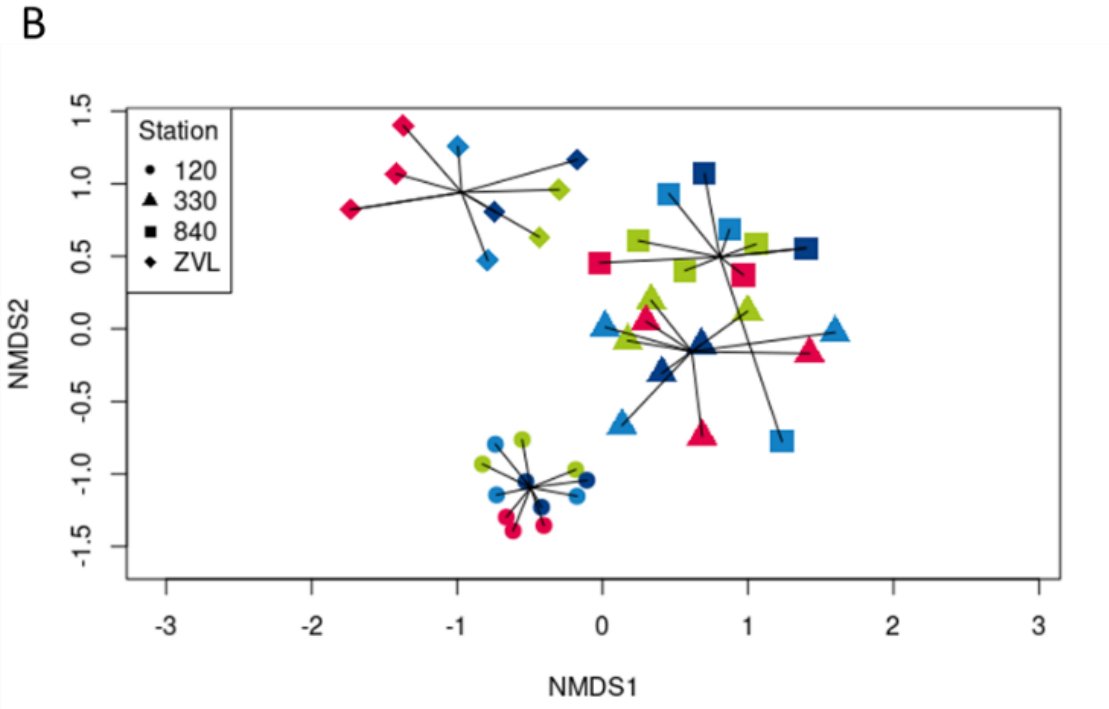

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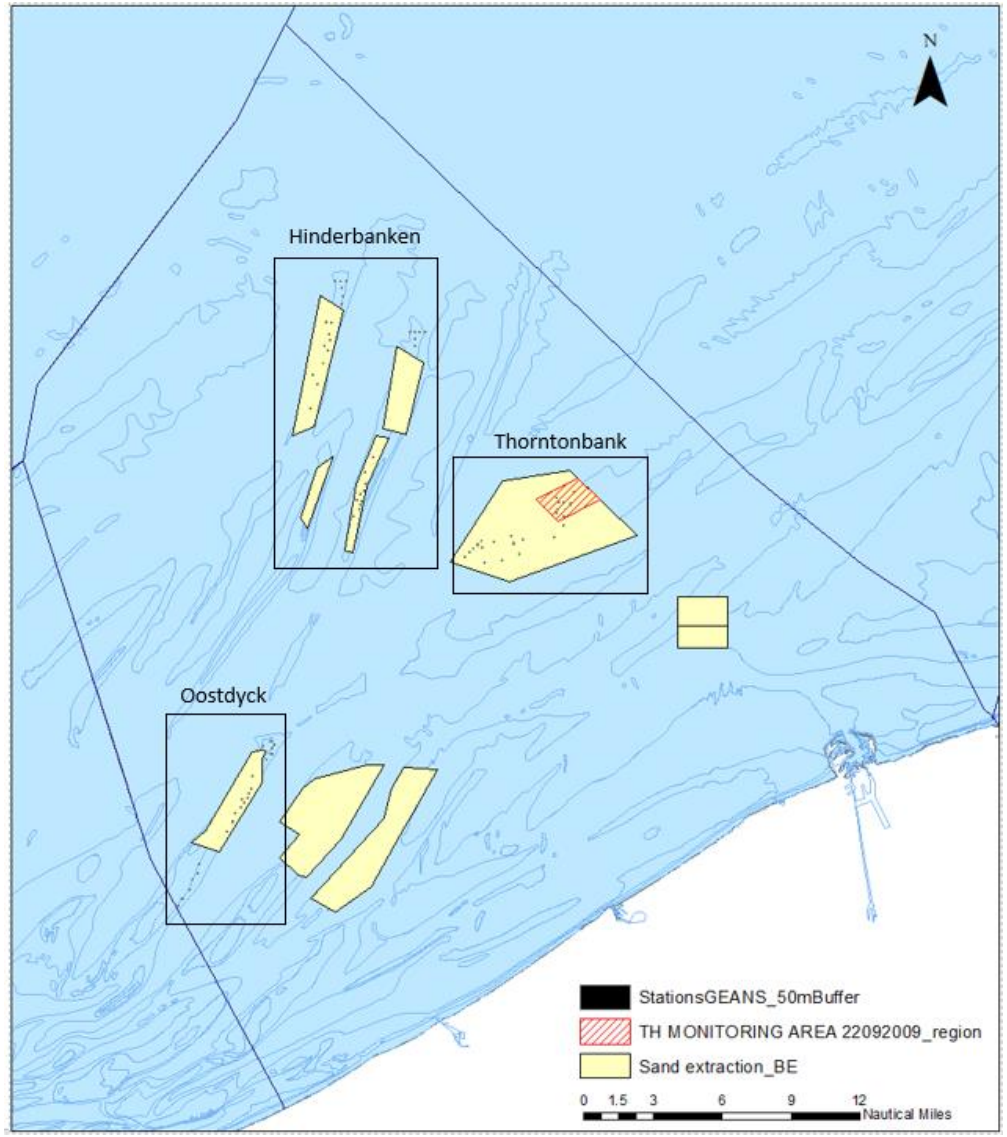
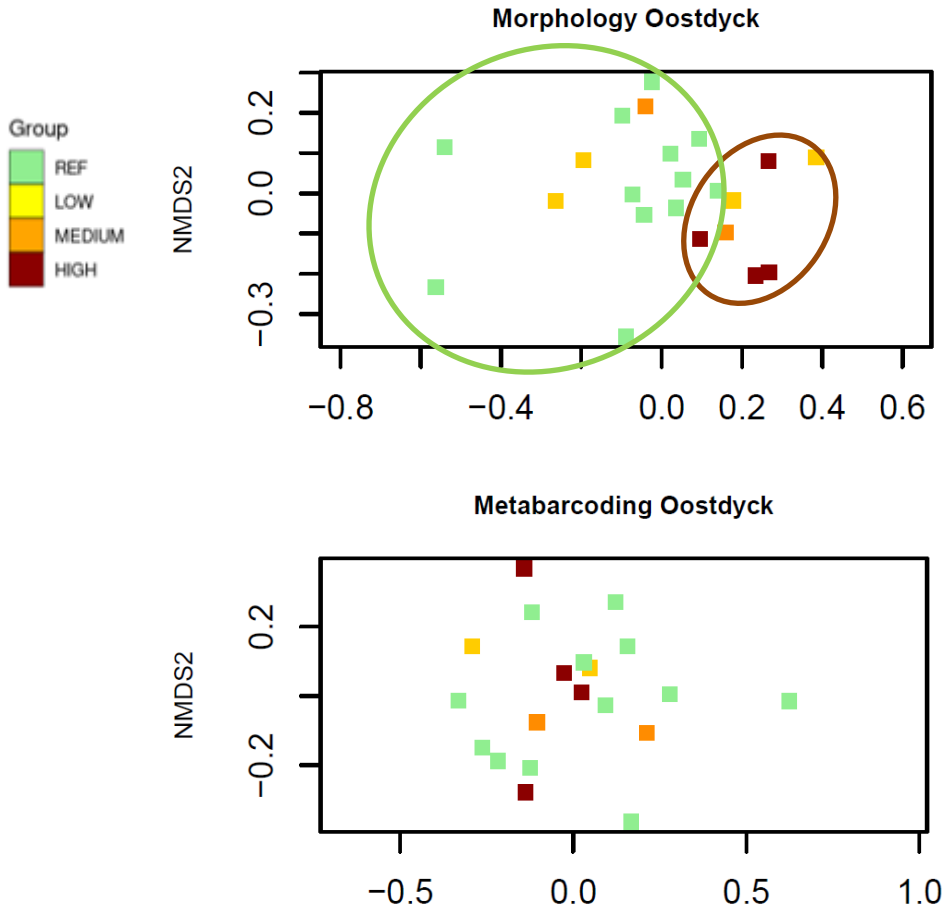
DNA-based monitoring for aggregate extraction



Oostdyck: continuous but low extraction intensity (30 000 m^3/month)



High ≠ Ref

High number of juveniles in high impact sites, which are considered an extra taxon!

No significant ≠

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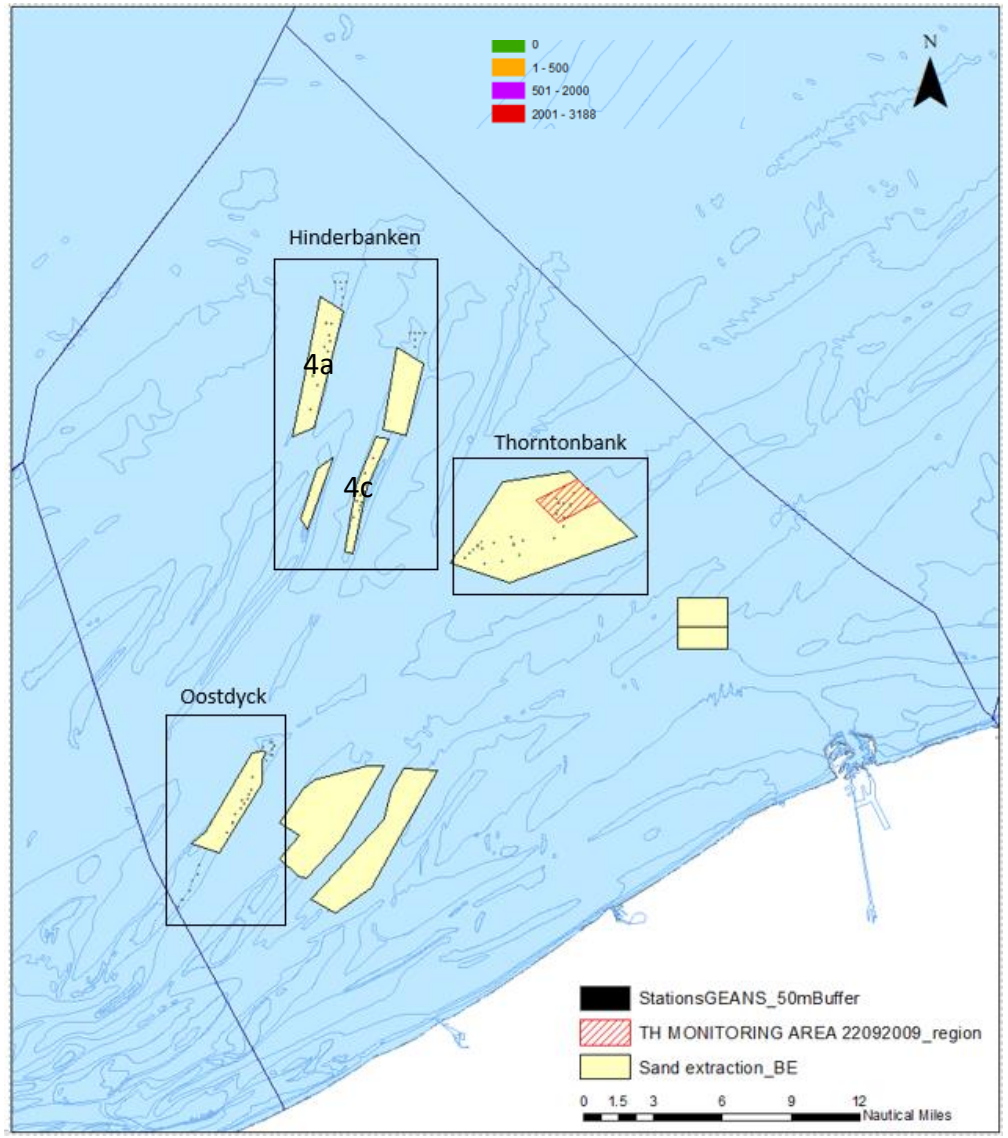
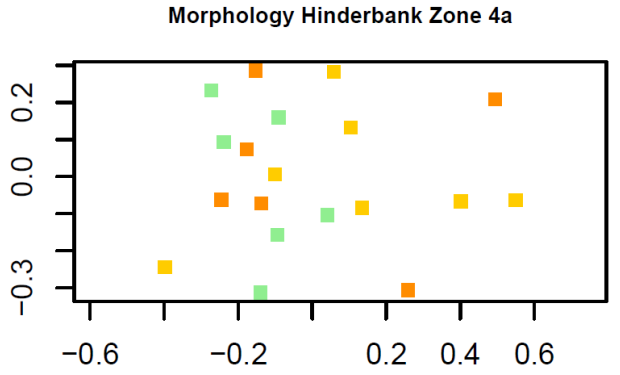
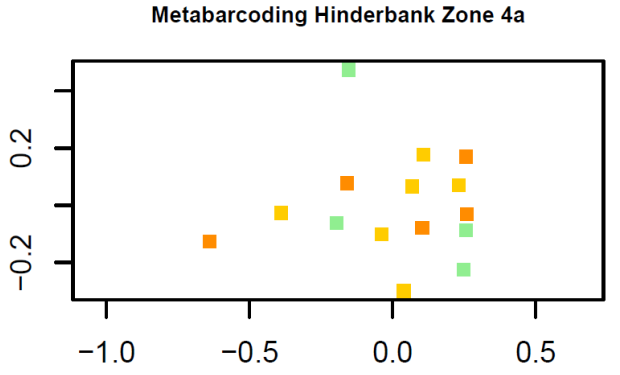
DNA-based monitoring for aggregate extraction

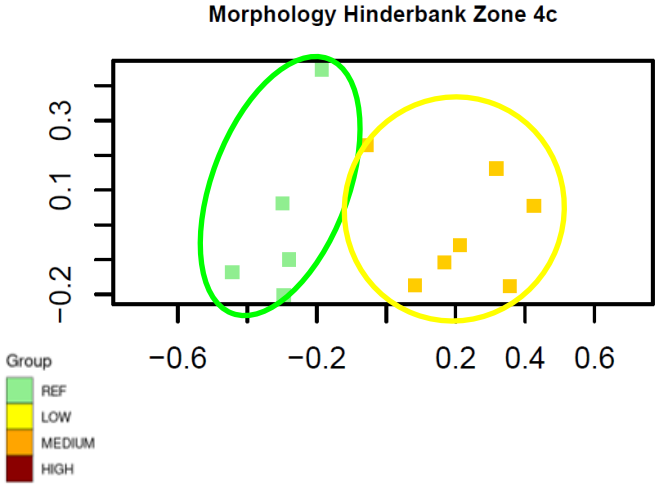


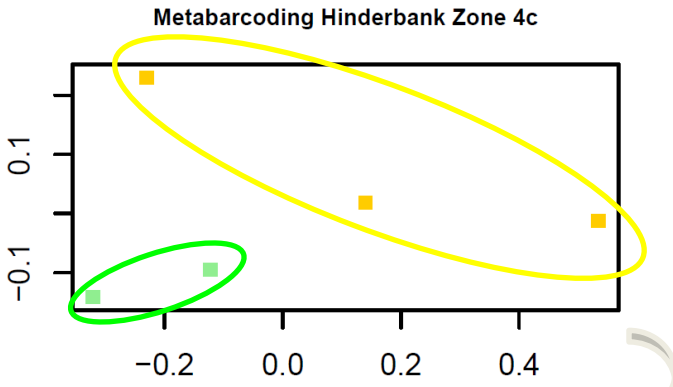
Hinderbanken 4a: high extraction intensity (600 000 m^3/month) Feb-Apr 2019





Hinderbanken 4c: very low extraction in 2019, but intense in previous years





Low number of samples in DNA metabarcoding

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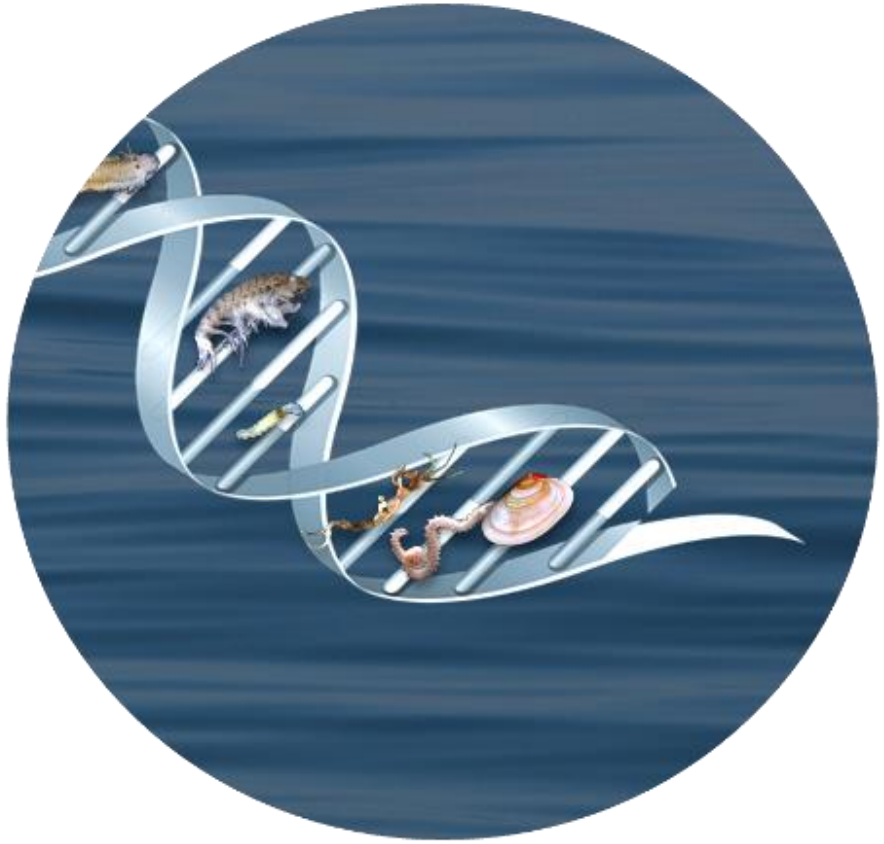
Conclusion

DNA-based monitoring vs morphological monitoring

- Cheaper +
- Faster +
- High throughput +
- Robust +
- Repeatable +
- Ecological patterns +



- No life stage information -
- Failure of library preparation -
- No biomass or density info -

A QUICK SCREENING AND WARNING TOOL

COMPLEMENTARITY

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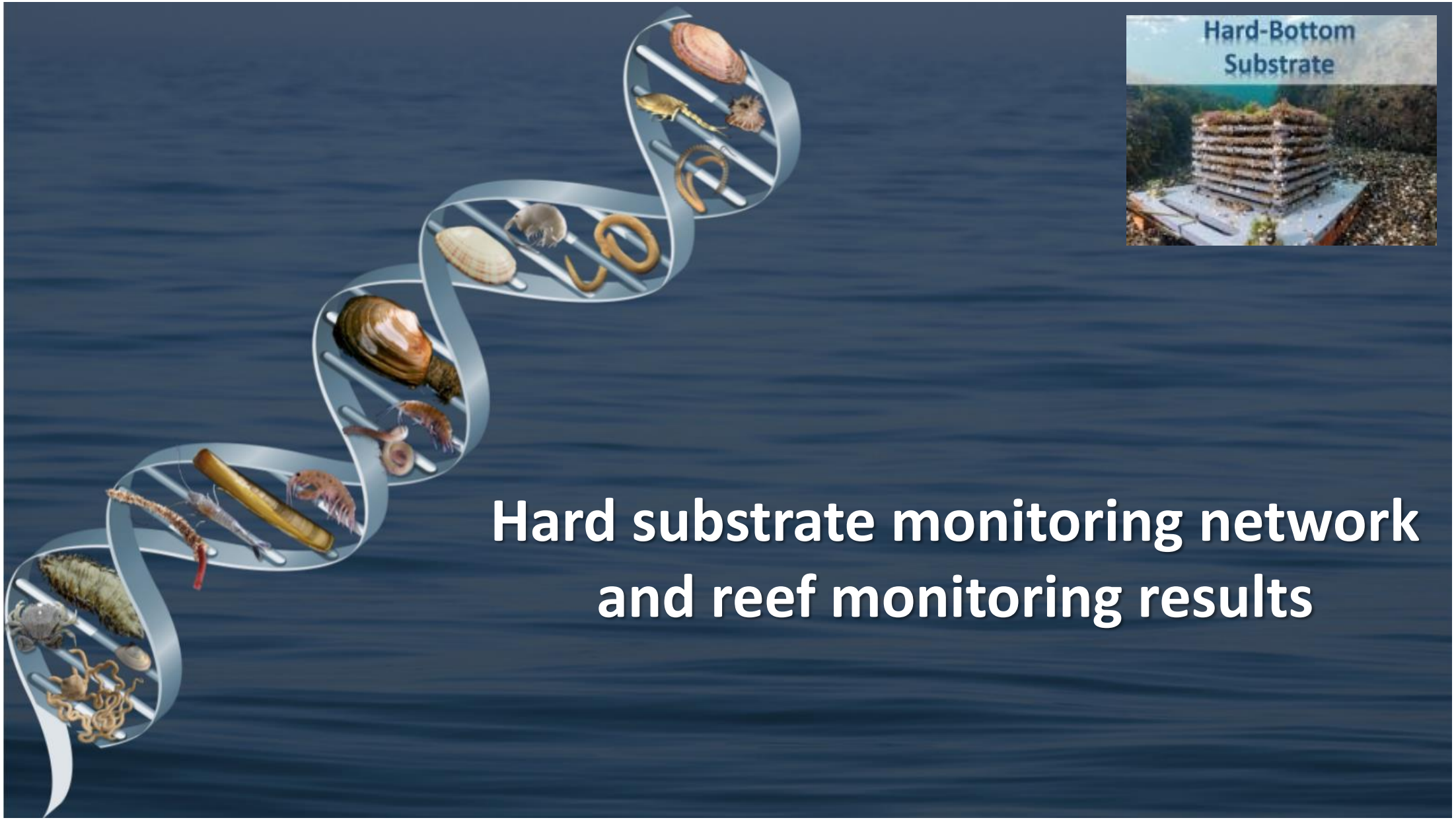

Hard substrate monitoring network and reef monitoring results

Interreg North Sea Region GEANS European Regional Development Fund
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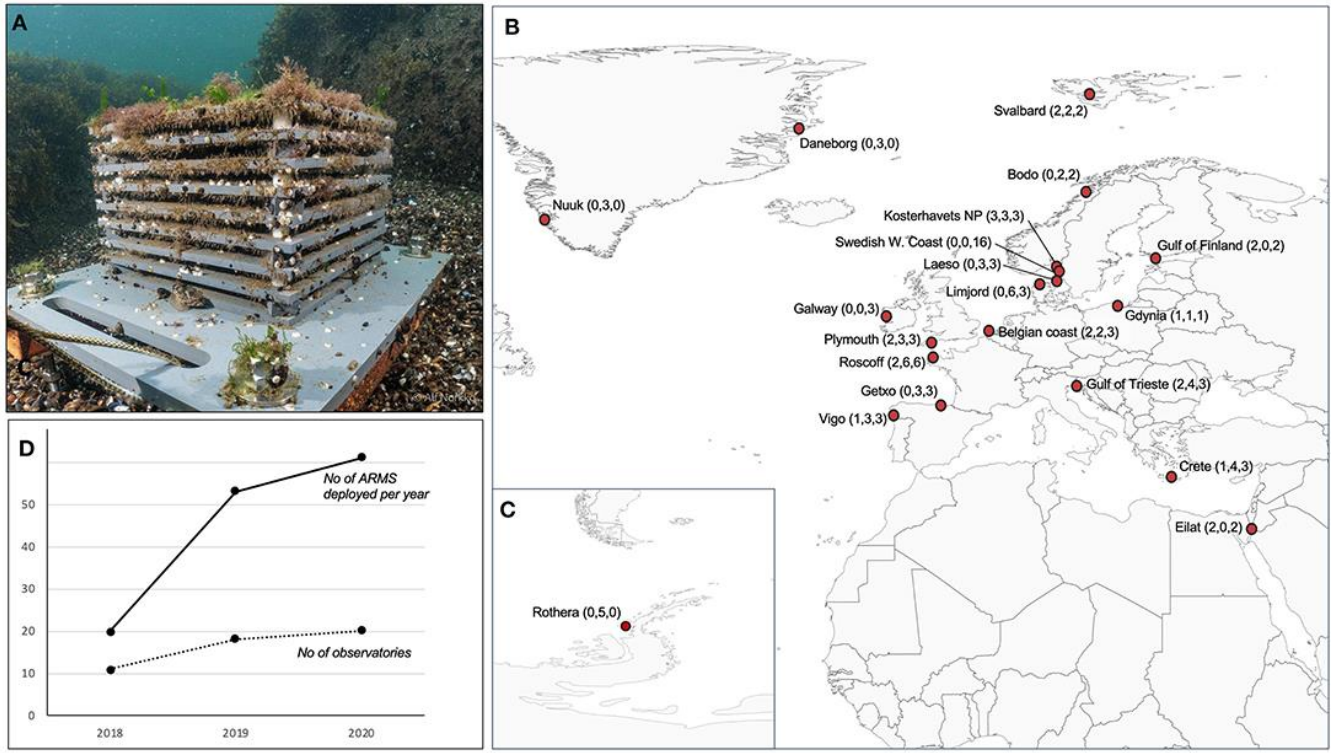
Marine Biodiversity Observation Network for genetic monitoring of hard-bottom communities

Aligned with Assemble Plus – Autonomous Reef Monitoring Structures (ARMS) (http://www.arms-mbon.eu/)



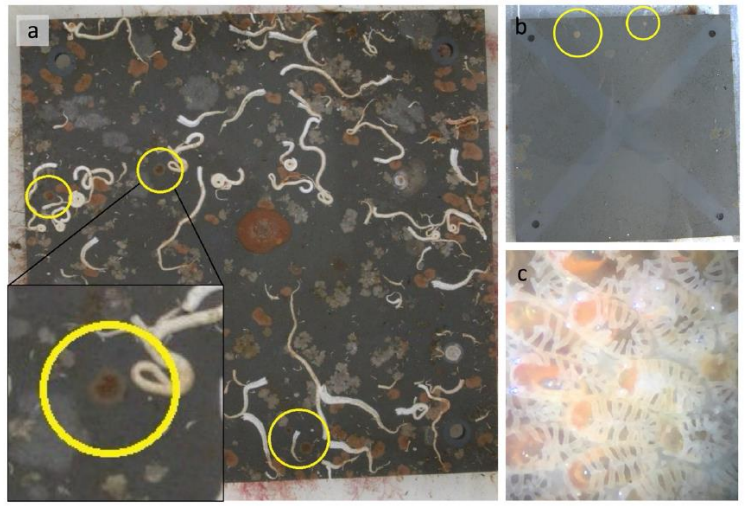
European Marine Omics Biodiversity Observation Network (EMO BON) Handbook (Version 1.0) including sampling protocols, Data Management plan (DMP), Access and Benefit Sharing guidelines (ABS) and Molecular Standard Operating Procedures are available here: https://www.geans.eu/protocols/hbs

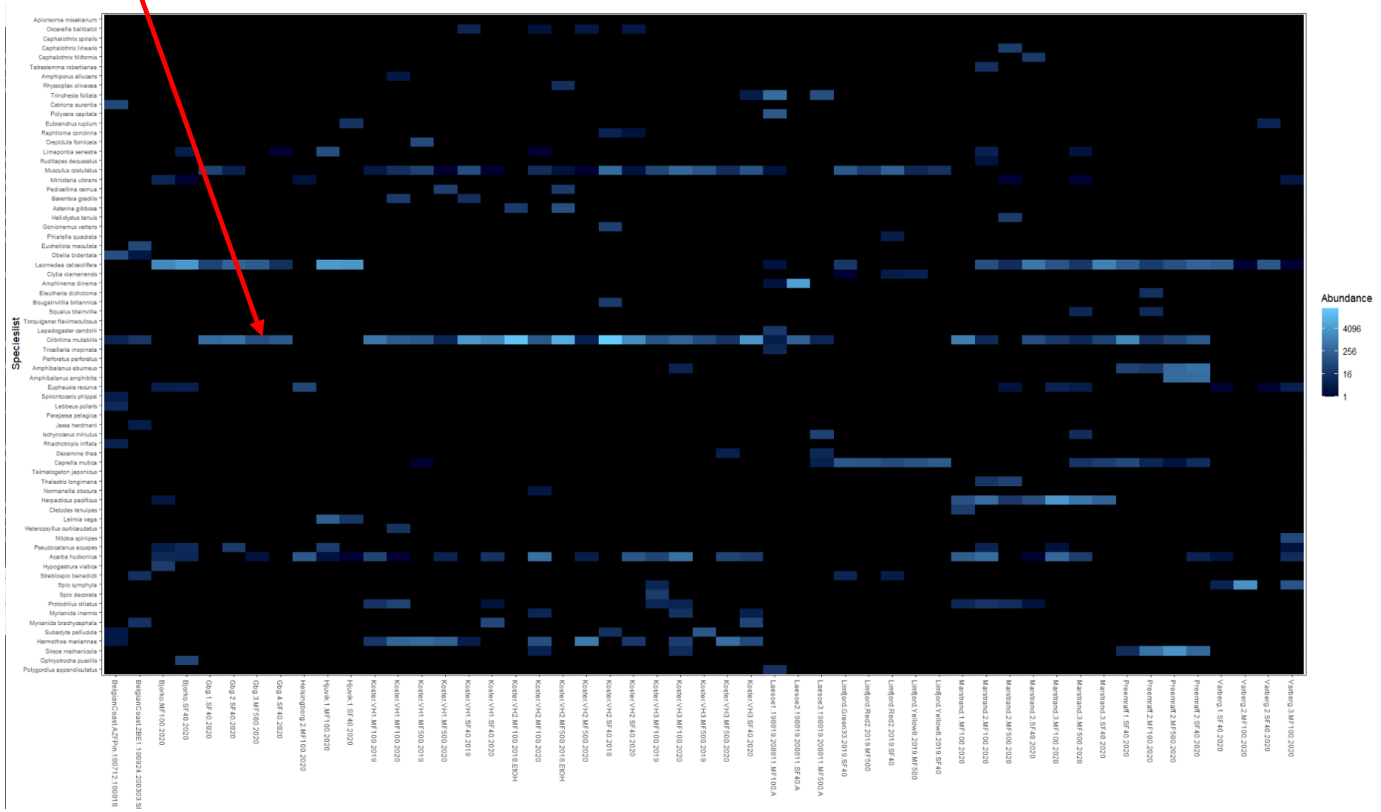
Obst *et al.* (2020) https://doi.org/10.3389/fmars.2020.572680

Example of possible use of ARMS data

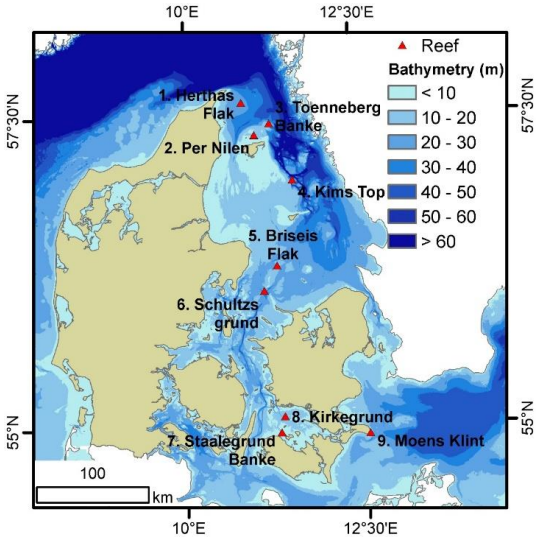
Heatmap scan showing the presence of alien species (y-axis) over ARMS sampling locations in NSR (x-axis). Abundance is represented by number of reads in an ARMS sample.

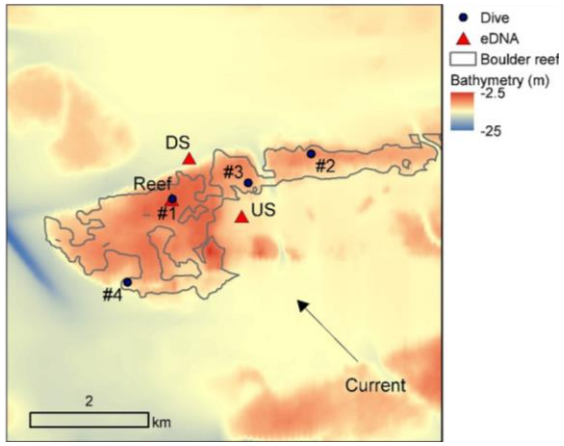
Juxtacribrilina mutabilis (a newly arrived bryozoan) – data now published on GBIF DOI [10.15468/y3upe9](https://doi.org/10.15468/y3upe9)



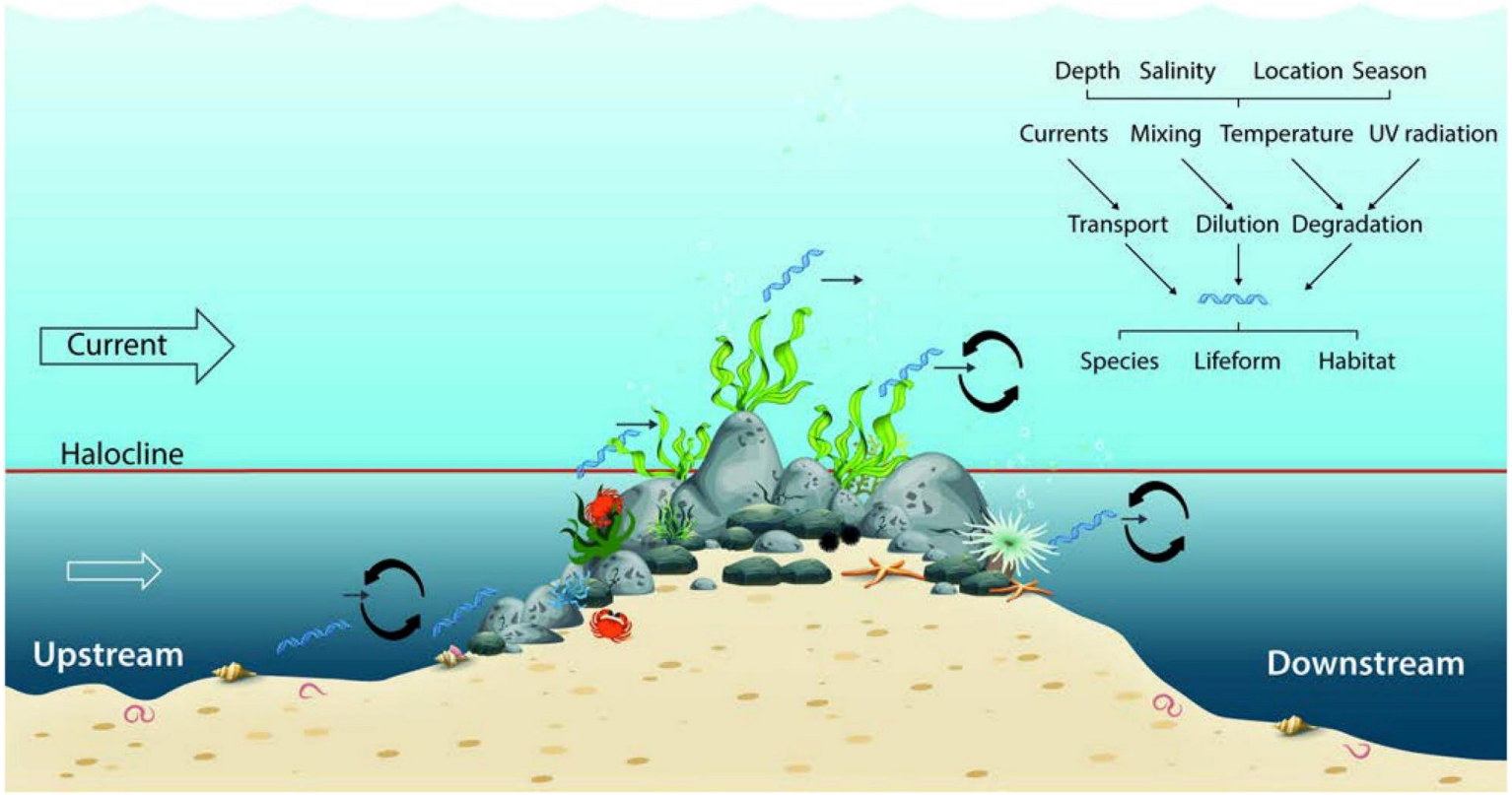


Environmental DNA Monitoring of Biodiversity Hotspots in Danish Marine Waters





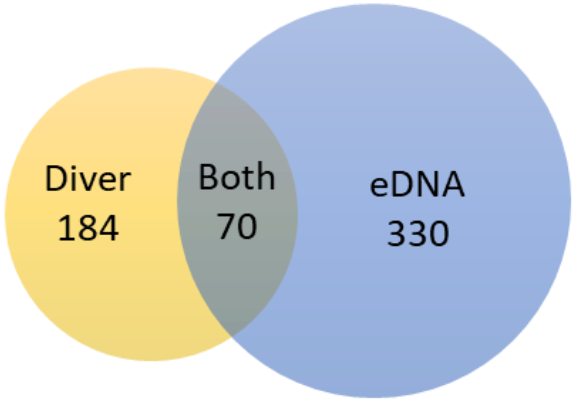
Danish reef monitoring program – conventional diver-based monitoring combined with eDNA monitoring



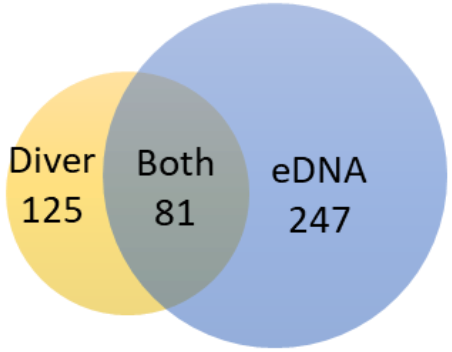
Staehr *et al.* 2022 https://doi.org/10.3389/fmars.2021.800474

Sensitivity in detection of species

Species

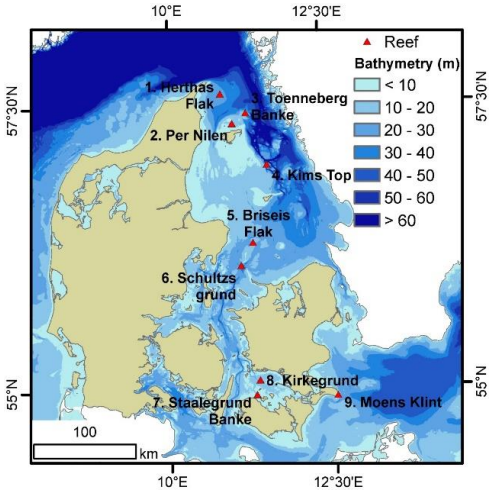


Genus

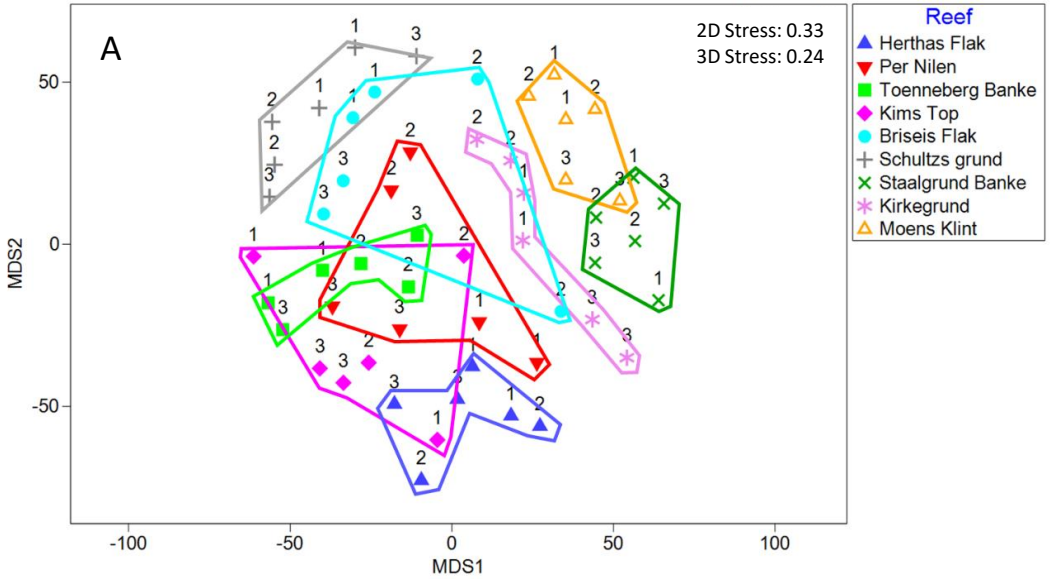


Taxonomic level	Functional groups	Only diver	Only eDNA	Both (% of total)	Total
Species	Macroalgae	78	33	16 (13)	127
	Epifauna	94	143	48 (17)	285
	Fish	8	36	4 (8)	48
	Infauna	4	118	2 (2)	124
	Total	184	330	70 (12)	584
Genus	Macroalgae	49	18	22 (25)	89
	Epifauna	69	105	50 (22)	224
	Fish	5	26	6 (16)	37
	Infauna	2	98	3 (3)	103
	Total	125	247	81 (18)	453

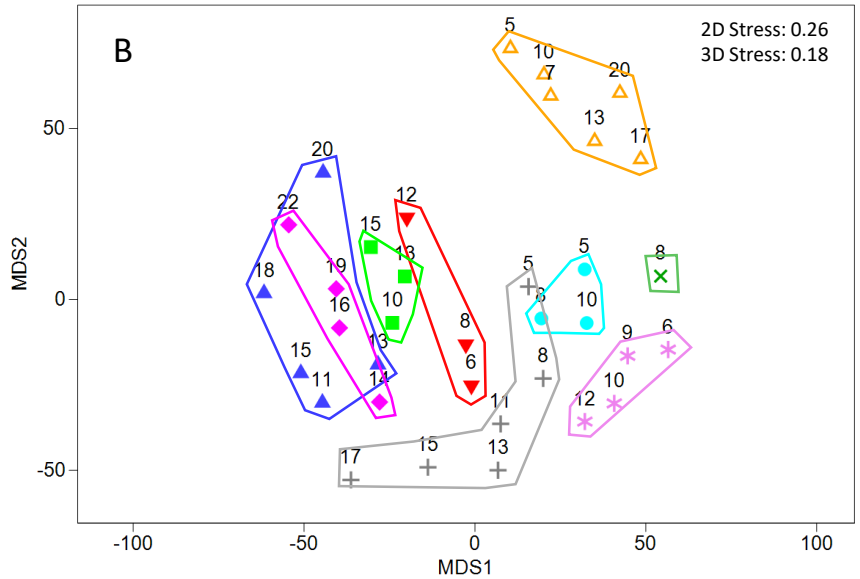
Environmental DNA Monitoring of Biodiversity Hotspots in Danish Marine Waters



eDNA

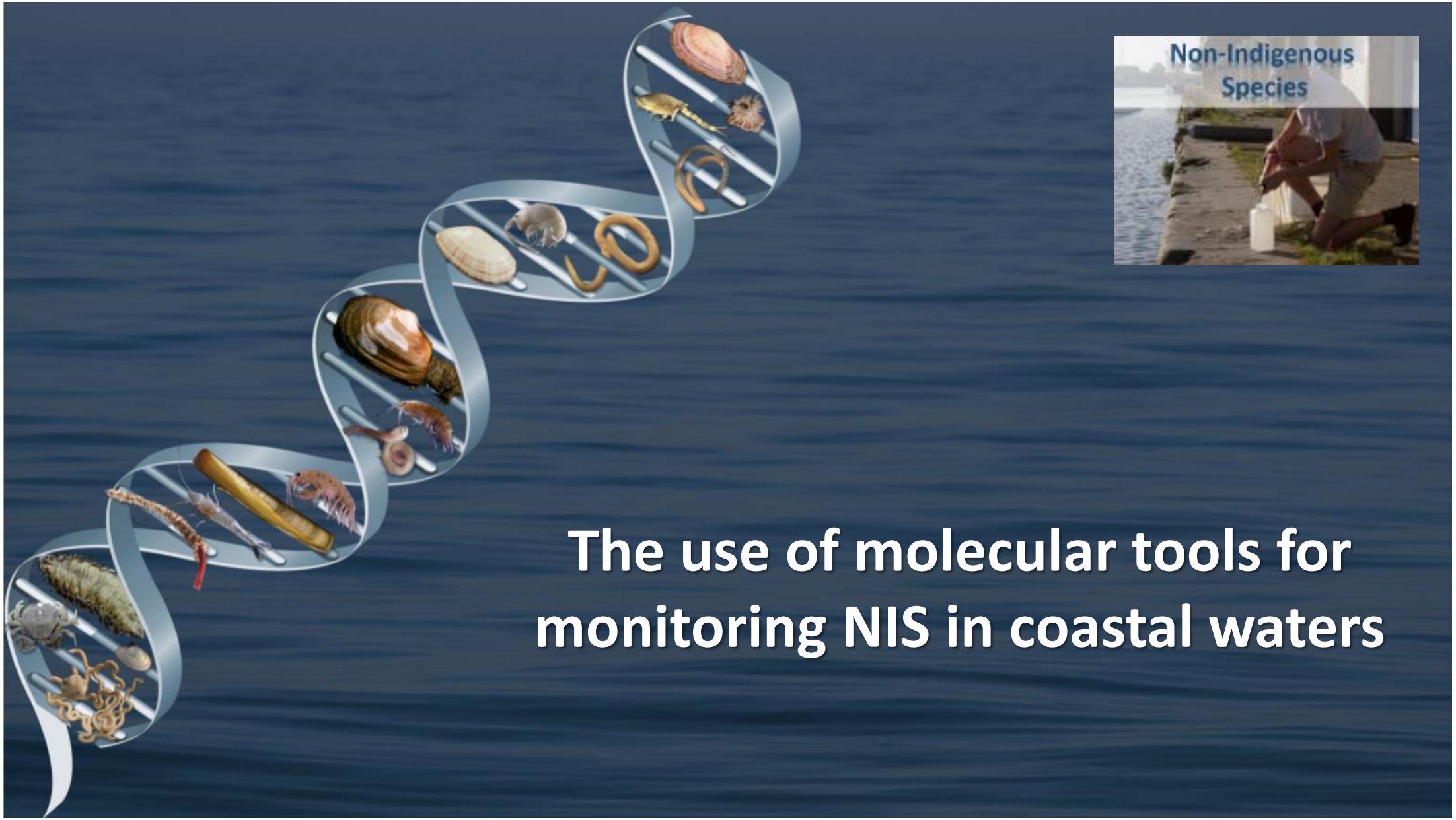


Diver based



Summary

- eDNA ≠ diver. Different sensitivity towards macroalgae and infauna
  - **eDNA = good supplement to diver observations, provides more complete picture**
- Only the diver based method is quantitative, but both can provide relative abundance
- Both eDNA and diver method documents significant differences among reef locations
- eDNA method is capable of separating upstream – over reef and downstream sites
  - Would be good to supplement with CTD profiles
- Both methods provide interesting data on species distribution that can be related to environmental conditions



The use of molecular tools for monitoring NIS in coastal waters

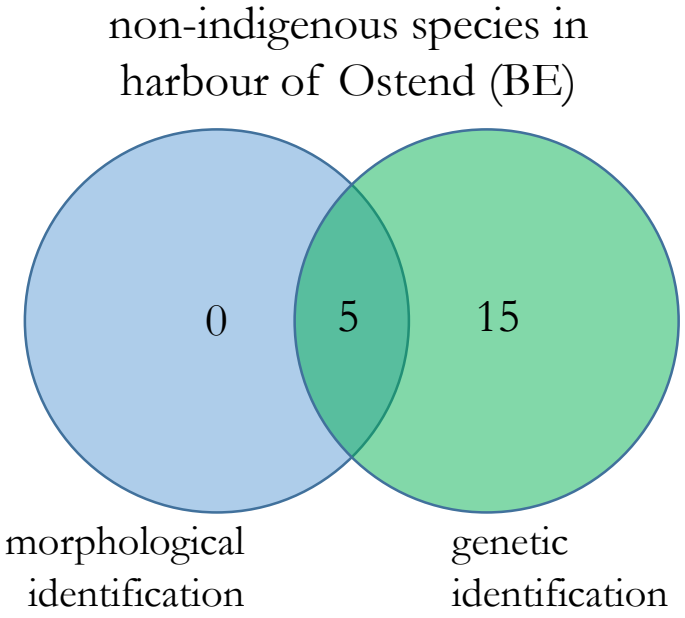
dPCR assay: Round goby *(Neogobius melanostomus)*

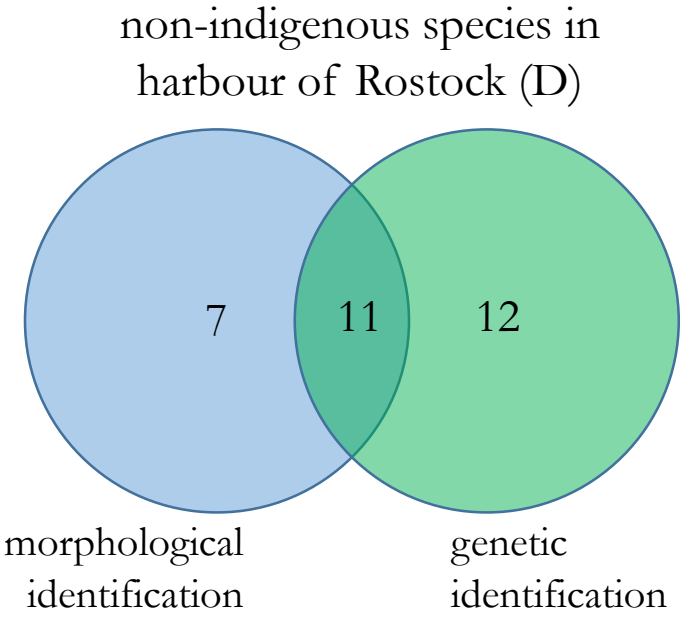
- eDNA (water sample) comparison with test fishing
- dPCR identified gobies in all sites where test fishing found the species
- dPCR identified gobies in three additional locations where test fishing was negative, but where the species was reported earlier

From: https://www.seanalytics.se/publications/reports

Results:While cost- and time-effective, genetic methods also detect many species that were missed in morphological assessments

OSPAR/HELCOM protocol for NIS in harbours





Biofouling on recreational rafts

Sampling site & Sample size:

- 2 marinas in the North Sea (Accumersiel, Büsum) and 1 in the Baltic Sea (Kappeln)
- 12 Biofouling-scrape samples from private recreational crafts
- Focus on benthic organisms

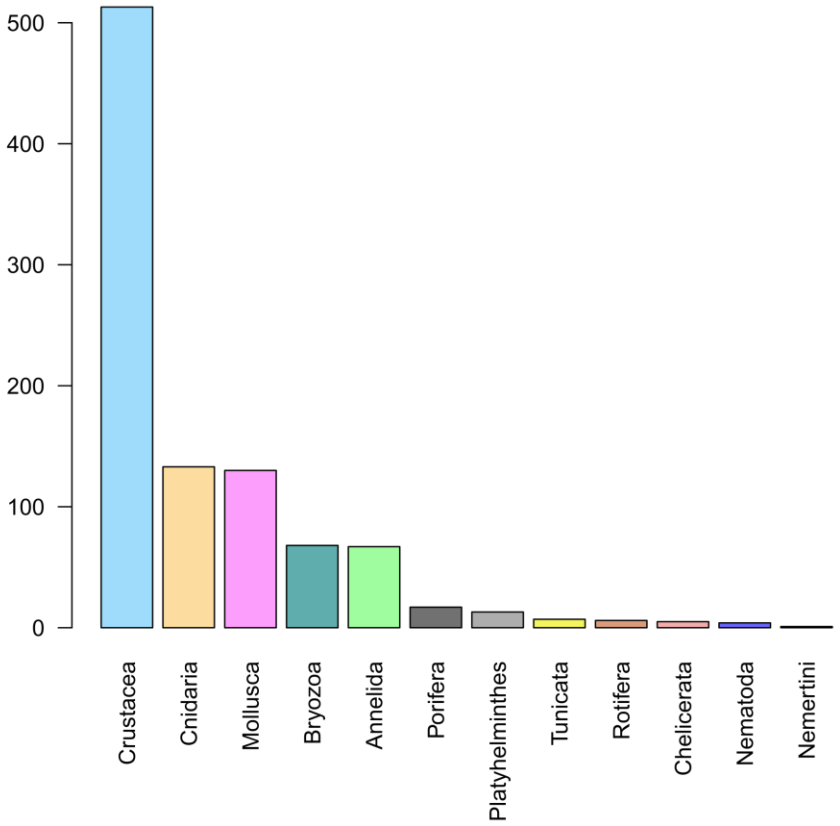
Identification was conducted

- taxonomically (IfAÖ)
- molecular by metabarcoding (SNG-DZMB)

Biofouling on recreational rafts

Results

1. Which organisms were detected using the metabarcoding method?



Amount of amplicon sequence variants (ASVs) per target groups.

Most abundant species with largest amount of assigned haplotypes: *Amphibalanus improvisus, Austrominius modestus, Polydora cornuta, Magallana gigas, Blackfordia virginica, Alitta succinea, Alcyonidium verrilli, Caprella mutica*

NIS!

In total **~25 % of haplotypes (ASVs)** were assigned to species level

→ **75% of ASVs do not have a genetic reference sequence on publicly accessible libraries** and could, thus, not be determined to species level!

Biofouling on recreational rafts

Results

2. Which method shows higher specificity particularly with regard to the detection of non-indigenous species?

Combining both methods resulted in the detection of **32 non-indigenous species:**

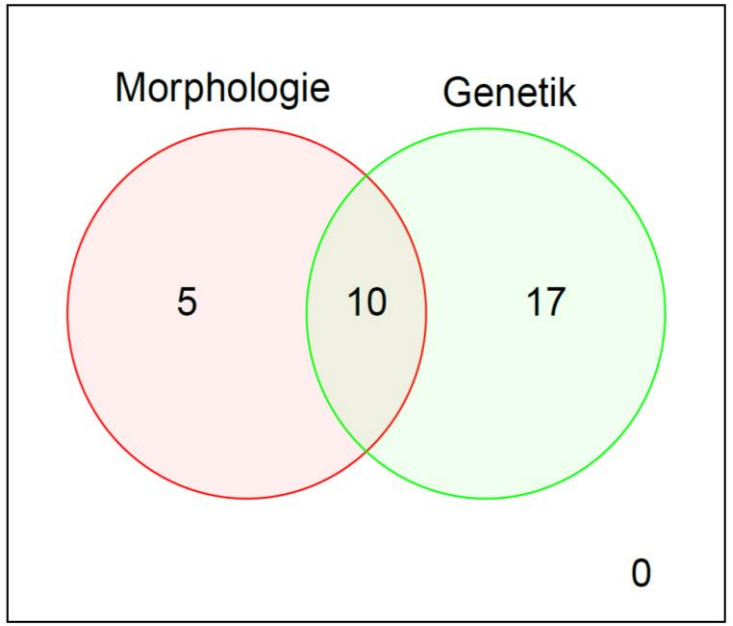
Traditional methods: 15 NIS
Genetic methods: 27 NIS
In total, **10 NIS were shared**:

Alitta succinea, *Amphibalanus improvisus*, *Austrominius modestus*, *Botryllus schlosseri*, *Bugulina stolonifera*, *Caprella mutica*, *Jassa marmorata*, *Magellana gigas*, *Molgula manhattensis* & *Polydora cornuta*.

Species detected only by genetic tools are the ones usually difficult to identify based on morphology (e.g. Bryozoa, Cnidaria, Annelida & Amphipoda).

! Caution: Genetic results also contain a few meiofaunal or planktonic groups (morphologically only benthic macrofauna considered) **!**

Shared non-indigenous species



Veen-diagramm showing the number of NIS detected by both methods.

Conclusions NIS

Strengths

- Efficient, accurate and scalable
- More information by lower impact
- Less taxonomic training → automatic taxonomic assignment
- Look at different ecosystem components in one sample
- Genetic tools very useful as an addition to morphological assessment (2x amount of species detected)
- High potential of detecting „difficult“ taxa

Weaknesses

- Reference libraries need to be completed (only 25% match)
- Quantitative information unreliable at present
- False positives/negatives
- Every new, genetically detected NIS must be validated morphologically
- DNA extraction or –amplification fails for some species
- Taxon-specific similarity-based identification thresholds

Recommendations:

→ Metabarcoding + unprocessed subsample as backup for morphological „ground truthing“

→ Use molecular tool for rapid screening (check specific cases based on genetic findings)

→ Remove barriers to assure faster exchange of data among institutions or countries

Overall conclusions

- High potential for fast and cost-efficient monitoring that is scalable
- Could be used as **rapid screening tool**
- **Complementarity** with conventional methods
  - E.g. identification of difficult species, more NIS detected,…
- Applicable for community analyses (metabarcoding) and single species interest (dPCR)
  - Ecological patterns mostly highly similar
- Relative abundances can be determined but not absolute abundance
- How/whether to **use** them **depends on** the **monitoring question**
  - E.g. interested in ecological patterns or in species list, bulk samples versus eDNA samples,…

Challenges

- With current techniques **reliable curated reference databases** important!
  - WP3 – but still difficult to find less common species
- Actual **implementation in monitoring programmes** for e.g. legislative drivers
  - Any ideas on how to accelerate this?
- **Sociological** – acceptance of methods = still a barrier
- Step away from one on one comparisons and acknowledge strengths of either method
- Some species groups/samples harder to amplify than others – primer free methods/multiple primers?
- Develop more sophisticated DNA-based detection methods that overcome current weaknesses

Thank you!

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