DNA-based monitoring for marine Non-Indigenous Species (NIS)

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Human activities fundamentally change marine biodiversity

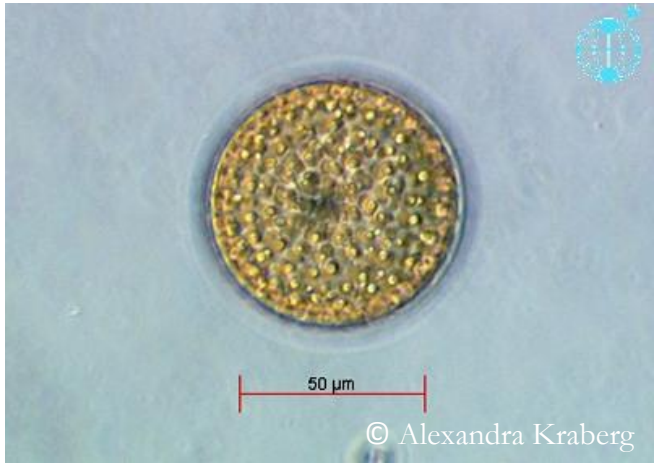
habitat loss and global connectivity

new habitat

pollution and climate change

Marine NIS

- Long-distance travel (on ships) is common
- Monitoring is logistically difficult
- Huge phylogenetic diversity (difficult for standardizing lab routines)



NIS in the harbour of Ostend (Belgium)

- Integration into established methodology: Following OSPAR/HELCOM sampling protocol
- Settlement plates
- Plankton samples (phytoplankton and zooplankton fractions)

★ sampling site

NIS in the harbour of Rostock (Germany)

- van Veen grab & sheet pile wall scratch samples
- sampling according to HELCOM/OSPAR protocols
- three replicates (A-C) at each sampling site
- Identification was focused on benthic organisms
  o taxonomically (IfAÖ)
  o molecular by metabarcoding (SNG-DZMB)

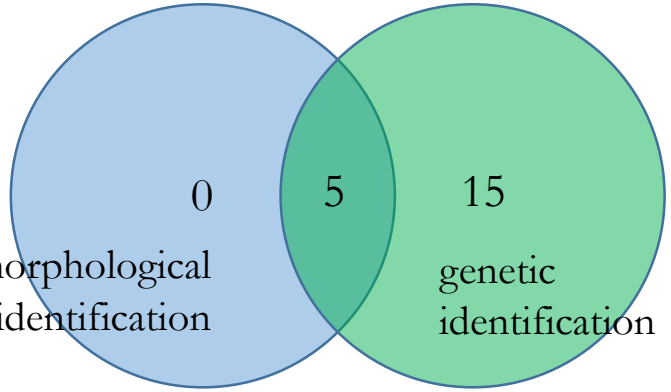
Cost- and time-efficiency

DNA based methods

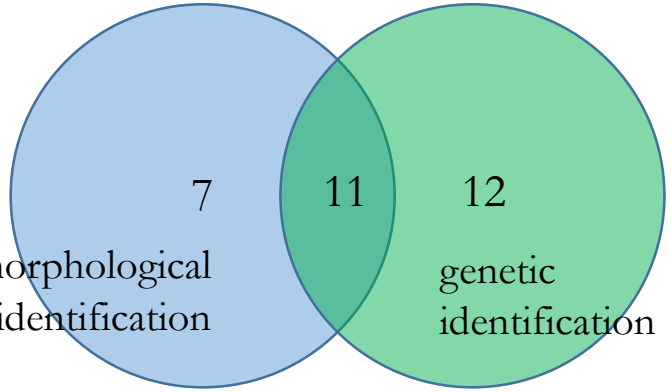
- were 42 % cheaper
- took 75 % less time
- detected 280 % more NIS

Results: NIS detected

Harbor of Ostend (BE)



Harbor of Rostock (D)

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Advantages of DNA-based approach

difficult to observe

insert picture

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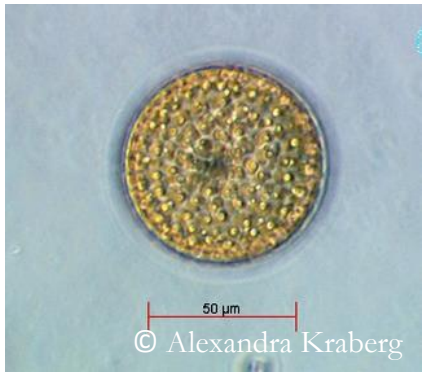
tiny species



rare species

insert picture

difficult to identify

Disadvantages of DNA-based approach

indistinguishable from closely related species

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incomplete reference databases

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little DNA shedding

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primer binding

contamination

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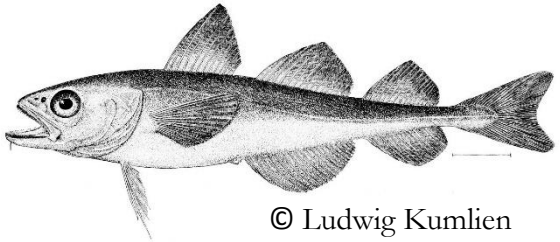


Fundamental differences between morphological and DNA-based monitoring

Morphological monitoring:

- Less cost- and time effective
- Higher sensitivity: high confidence in positive detections
- Lower specificity: false negatives are an issue

DNA-based monitoring:

- More cost- and time effective
- Lower sensitivity: weak points in methodology: false positives are an issue
- Higher specificity: higher detection power: less false negatives

A probabilistic approach

The **probability of the occurrence** of a species given our observation is proportional to the **likelihood that the evidence supports the observation** multiplied with our **prior belief that the detection occurs at the study site**.

Probability of the occurrence

What do we conclude from the study?

Our trust in the methods

Are there contamination sources in the field or in the lab? Can the DNA barcode distinguish among related species? Are there errors in the reference database?

Our prior belief that the species occurs in the study area

Collect all other evidence: distribution maps, dispersal routes, alternative techniques (e.g. morphology)

Example 1: Detection of ivory barnacle (*Amphibalanus eburneus*) in Belgium using DNA-based methods

Our trust in the methods

Negative control revealed no contamination and species can be distinguished with DNA barcodes most of the time

Our prior belief that the species occurs in the study area

Ivory barnacle has been reported repeatedly in neighboring countries

Probability of the occurrence

95 % * 90 % = 86 %

Example 2: Detection of American eel (*Anguilla rostrata*) in Europe using DNA-based methods

Our trust in the methods

Negative control revealed no contamination, but we know that we cannot distinguish between European and American eel with short DNA barcodes

Our prior belief that the species occurs in the study area

American eel is not reported from morphological studies to occur in Europe

Probability of the occurrence

50 % * 10 % = 5 %

Example 3: Detection of scud (*Jassa falcata*) in Belgium using DNA-based methods

Our trust in the methods

Negative control revealed no contamination, but we know that the DNA barcode does not discriminate from the close relative *Jassa marmorata*

Our prior belief that the species occurs in the study area

Species is native to Belgium

Probability of the occurrence

50 % * 100 % = 50 %

Example 4: Detection of polar cod (*Boreogadus saida*) in Belgium using DNA-based methods

Our trust in the methods

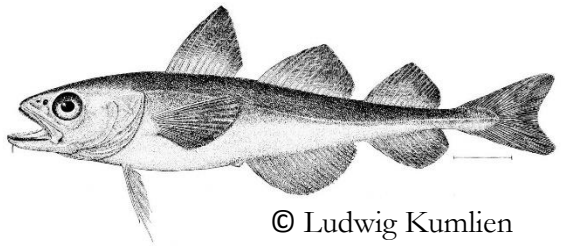
A genetic study on polar cod has been conducted in the laboratory where the samples were processed, imposing a risk of contamination

Our prior belief that the species occurs in the study area

Species occurs in the Arctic

Probability of the occurrence

20 % * 10 % = 2 %



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Example 5: **Not** detecting of Japanese skeleton shrimp (*Caprella muticum*) in Belgium using DNA-based methods

Our trust in the methods

Caprella muticum is often missed in DNA-based studies, probably because PCR amplification does not work well

Our prior belief that the species occurs in the study area

We observed the species in earlier studies in the same geographic area

Probability of the occurrence

(100 % - 30 %) * 90 % = 63 %

Take home messages

- DNA-based methods are often more cost- and time-efficient than traditional approaches
- DNA-based methods typically detect more species
- But some species remain difficult to detect with DNA-based methods
- **Many opportunities, but technical constraints require us to often think in probabilistic terms**

Acknowledgements

Anton Bilsen
Filip Volckaert
Bart Hellemans
Sander Delacauw
Klaas Deneudt
Carolin Uhlir
Sahar Khodami
Magdalini Christodoulou
Pedro Arbizu Martínez
Harbour authorities of Ostend and Rostock
GEANS consortium

Interreg
North Sea Region
GEANS
European Regional Development Fund

EUROPEAN UNION