



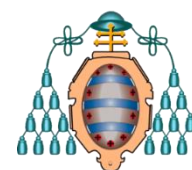
# Metabarcoding approach in biodiversity and biosecurity surveys: a pilot study from the Baltic Sea

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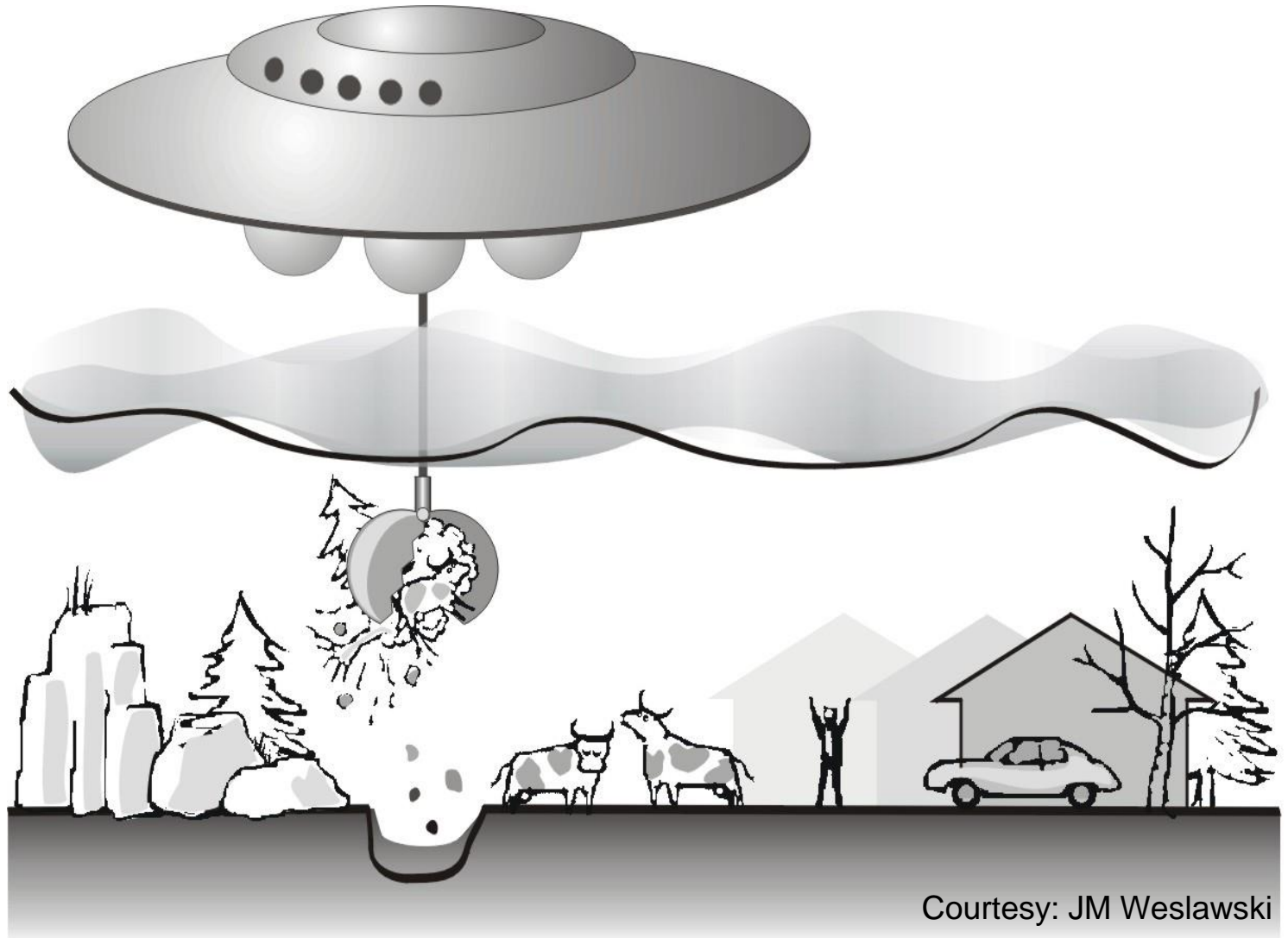


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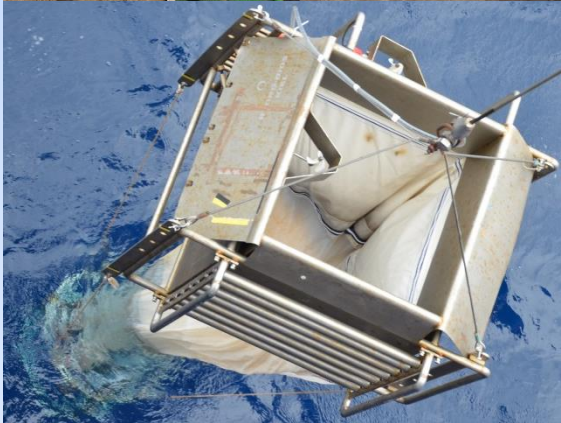
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# Challenges of marine surveys



Courtesy: JM Weslawski

# Challenges of marine surveys





# Increasing human pressures, declining taxonomic expertise

- Morphological identification methods are laborious
- Require considerable taxonomic expertise
- Often fail to identify cryptic species
- Or species at the larval stage



**Risk to overlook or misidentify  
non-indigenous, pathogen or indicator  
species**

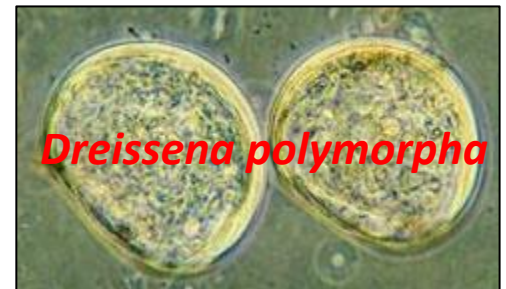
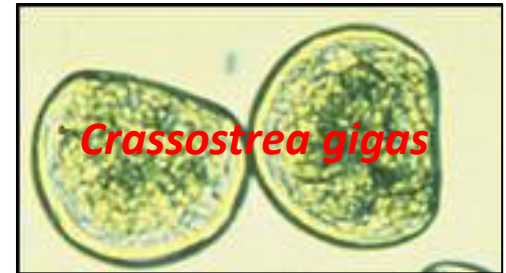
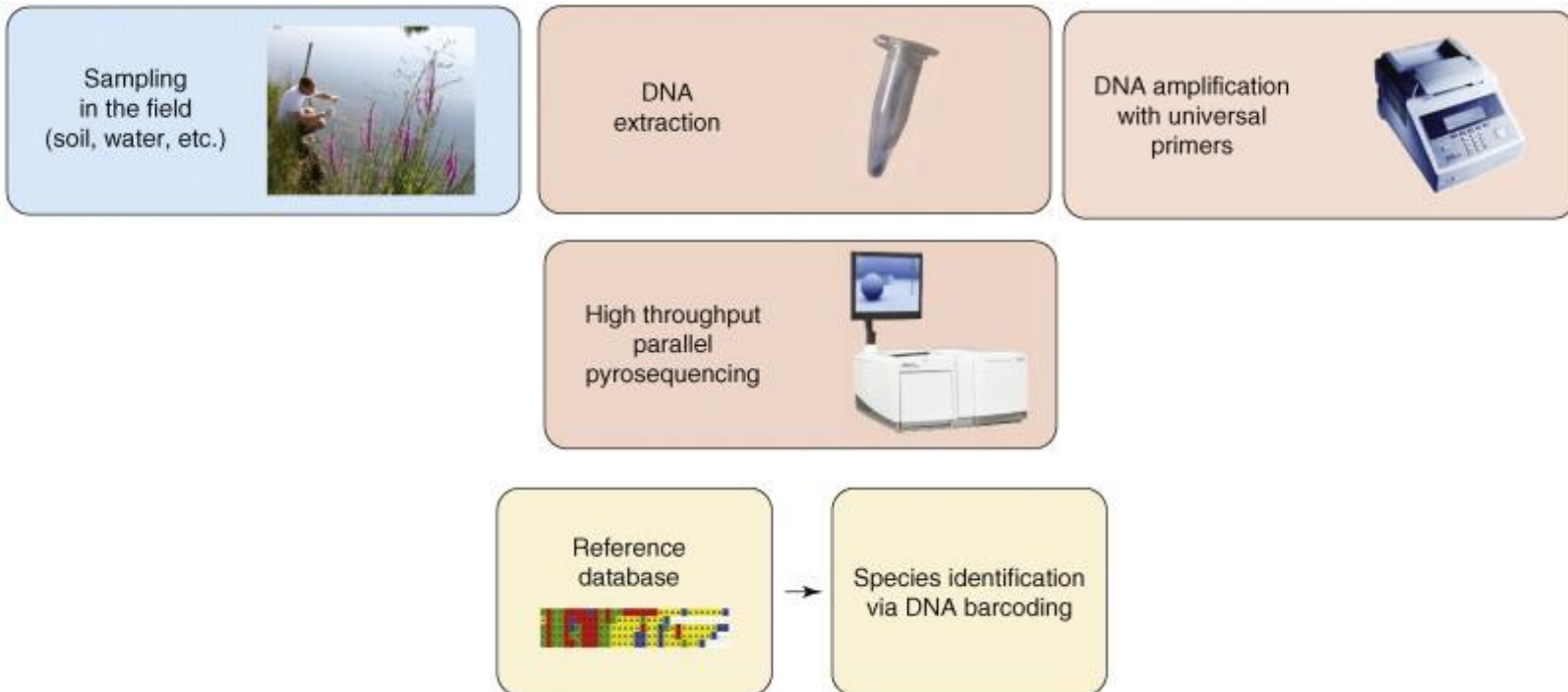
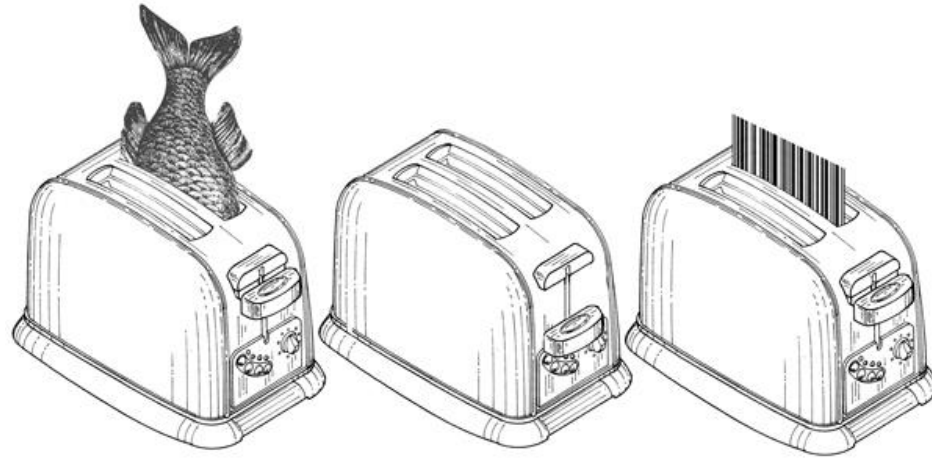


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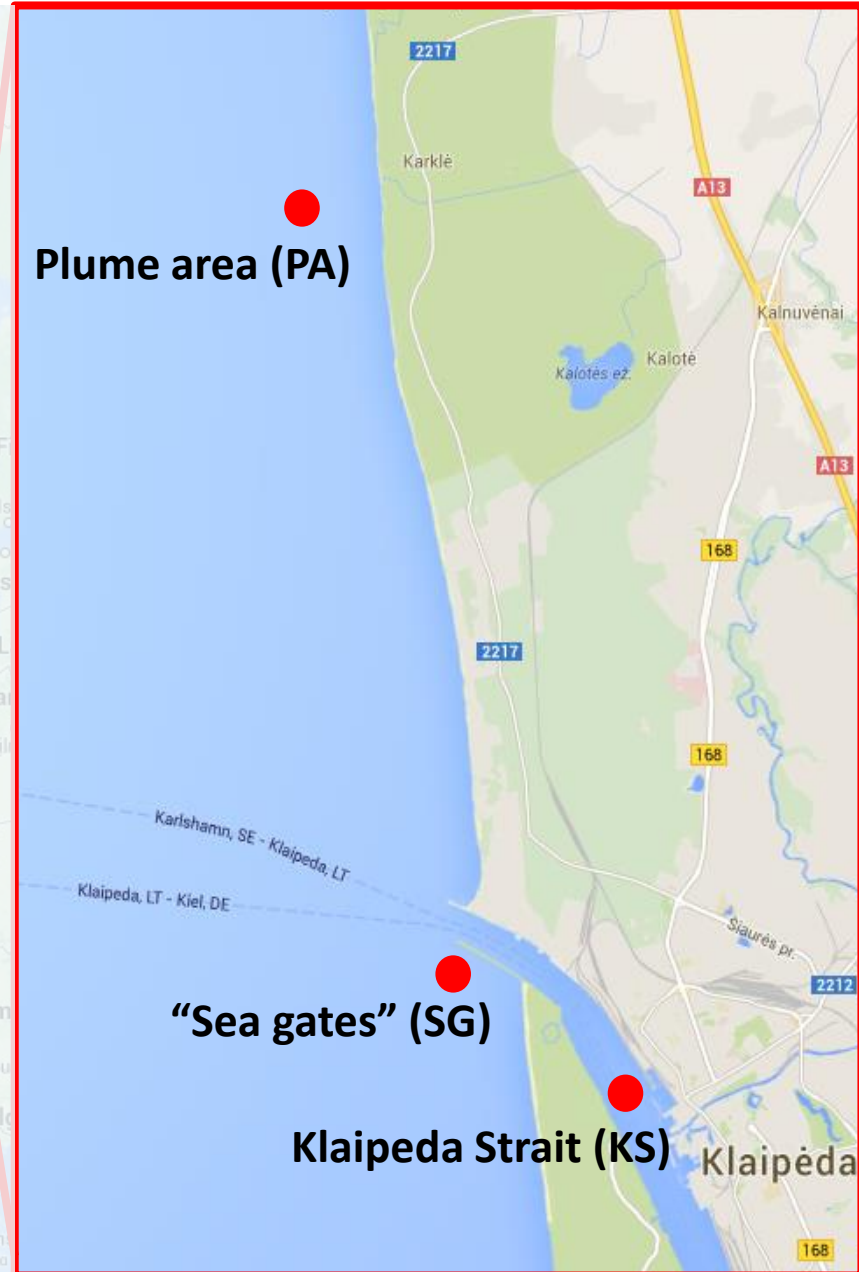


# Principle of the metabarcoding



# Zooplankton study in the Baltic Sea

- 6 samples collected from ca. 2 m<sup>3</sup> water each
- bulk DNA amplified with universal COI primers
- sequenced with a Genome Sequencer FLX (Roche)
- aligned against NCBI database
- compared to the morphologically analyzed samples

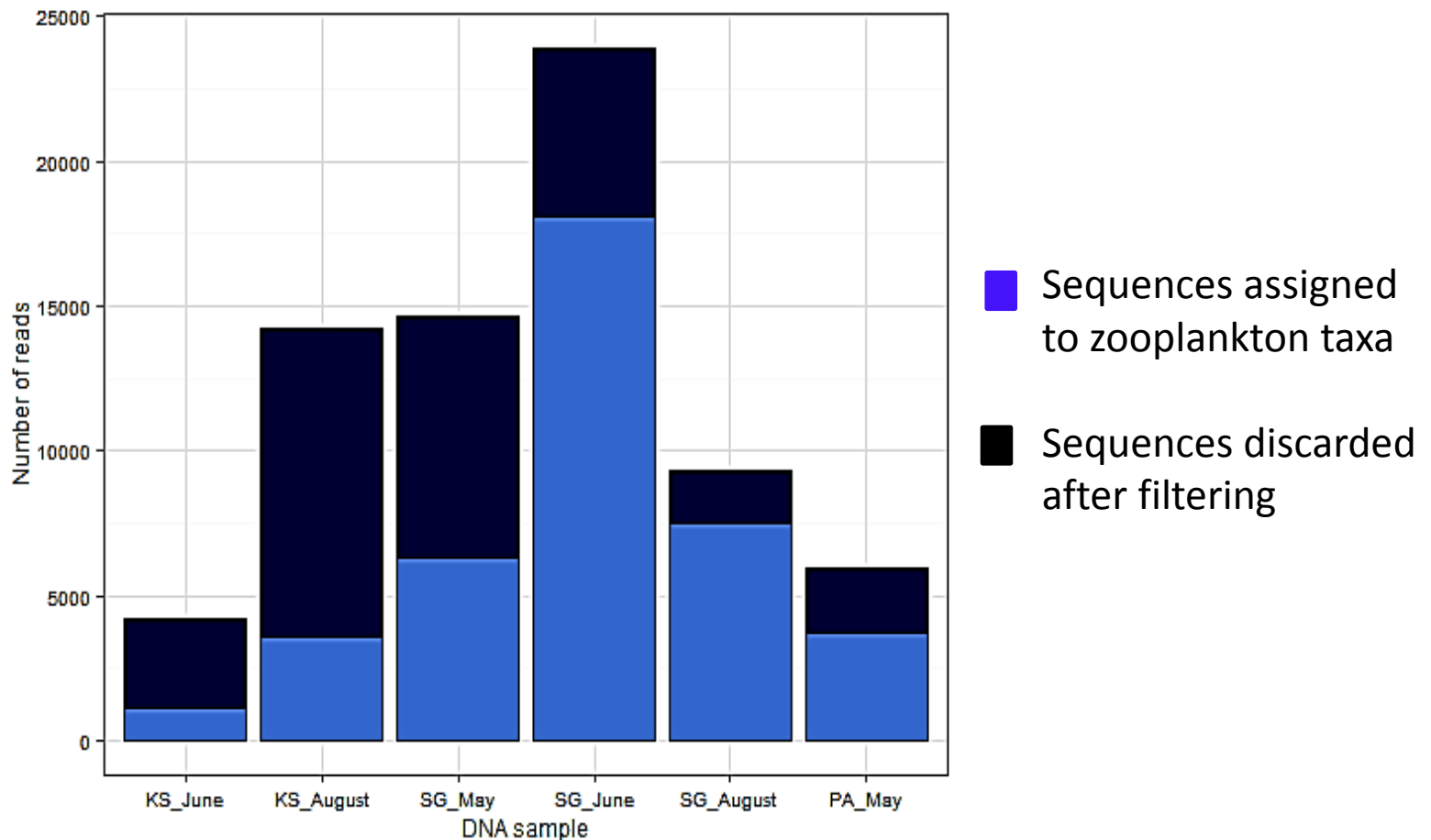


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# Sequencing results

- Approx. 100 000 good-quality sequences retrieved
- About 75% of those resulted in positive alignment hits
- 40 291 assigned ( $\geq 97\%$  homology and  $>90\%$  coverage) to 18 zooplankton taxa (species or genus level)



# Species detected by metabarcoding

(in red – those, detected exclusively by meabarcoding)

<b>Annelida</b>	<b>Polychaeta</b>	<i>Marenzelleria viridis</i> <i>Marenzelleria neglecta</i>
<b>Crustacea</b>	<b>Cladocera</b>	<i>Bosmina coregoni</i> <i>Bosmina spp.</i> <i>Cercopagis pengoi</i> <i>Daphnia galeata</i> <i>Daphnia cucullata</i> <i>Evadne nordmanni</i> <i>Leptodora kindtii</i> <i>Pleopis polyphemoides</i> <i>Podon leukartii</i>
	<b>Copepoda</b>	<i>Acartia tonsa</i> <i>Mesocyclops leukarti</i>
<b>Mollusca</b>	<b>Bivalva</b>	<i>Dreissena polymorpha</i> <i>Macoma balthica</i> <i>Mytilus sp.</i>
	<b>Gastropoda</b>	<i>Hydrobia ulvae</i>
<b>Rotifera</b>	<b>Eurotatoria</b>	<i>Keratella quadrata</i>





# Invasive species in the Baltic

<b>Annelida</b>	<b>Polychaeta</b>	<i>Marenzelleria viridis</i> <i>Marenzelleria neglecta</i>
<b>Crustacea</b>	<b>Cladocera</b>	<i>Bosmina coregoni</i> <i>Bosmina spp.</i> <i>Cercopagis pengoi</i> <i>Daphnia galeata</i> <i>Daphnia cucullata</i> <i>Evadne nordmanni</i> <i>Leptodora kindtii</i> <i>Pleopis polyphemoides</i> <i>Podon leukartii</i>
	<b>Copepoda</b>	<i>Acartia tonsa</i> <i>Mesocyclops leukarti</i>
<b>Mollusca</b>	<b>Bivalva</b>	<i>Dreissena polymorpha</i> <i>Macoma balthica</i> <i>Mytilus sp.</i>
	<b>Gastropoda</b>	<i>Hydrobia ulvae</i>
<b>Rotifera</b>	<b>Eurotatoria</b>	<i>Keratella quadrata</i>



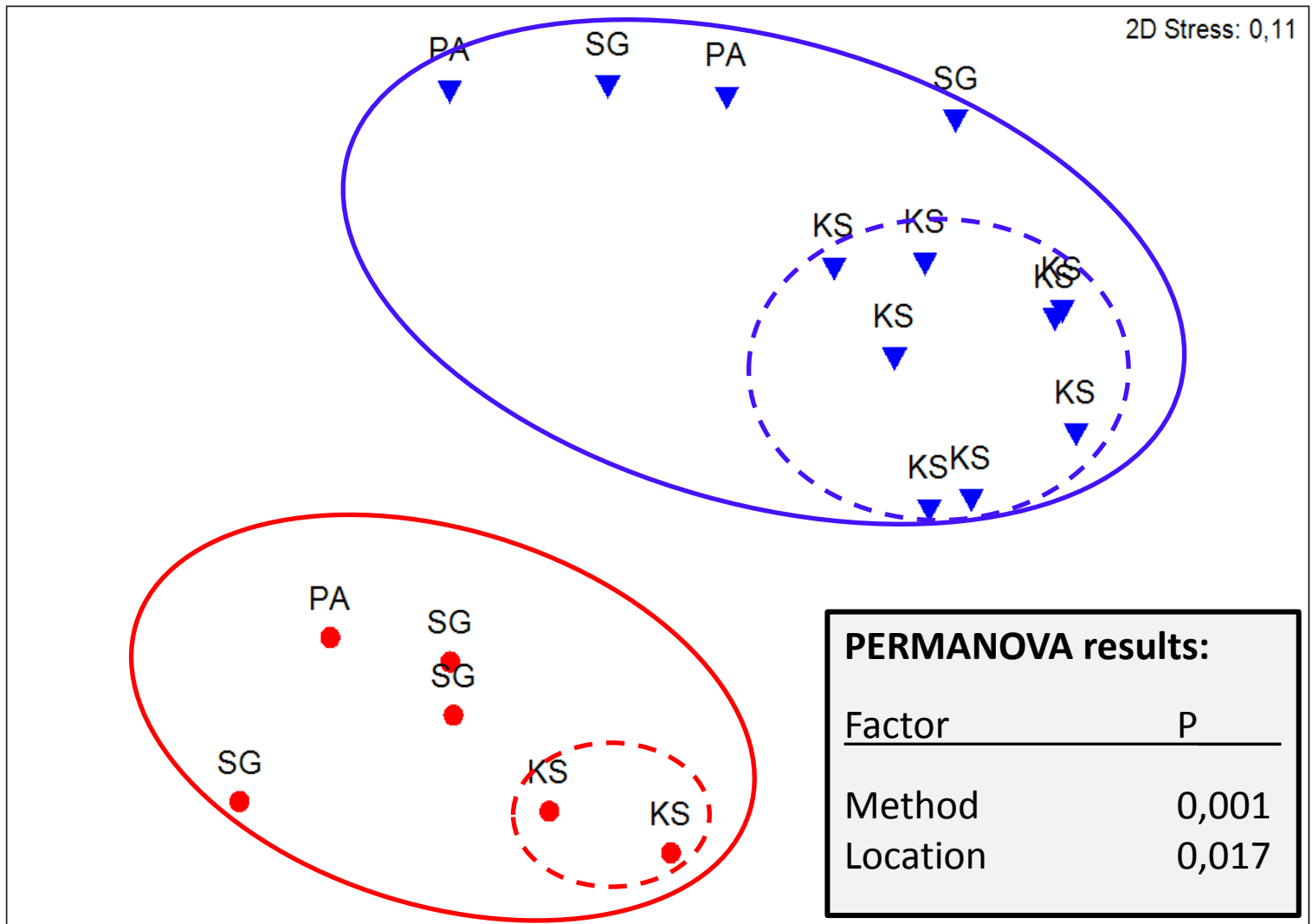
# Considered to be absent from the study area

Annelida	Polychaeta	<b><i>Marenzelleria viridis</i></b> <i>Marenzelleria neglecta</i>
Crustacea	Cladocera	<i>Bosmina coregoni</i> <i>Bosmina spp.</i> <i>Cercopagis pengoi</i> <i>Daphnia galeata</i> <i>Daphnia pulex</i> <i>Daphnia caudata</i>
<p><b>&gt;800 sequences found in all samples, aligned with high confidence (&gt;97% identity, &gt;95% coverage)</b></p>		
	Copepoda	<i>Acartia tonsa</i> <i>Mesocyclops leukarti</i>
Mollusca	Bivalva	<i>Dreissena polymorpha</i> <i>Macoma balthica</i> <i>Mytilus sp.</i>
	Gastropoda	<i>Hydrobia ulvae</i>
Rotifera	Eurotatoria	<i>Keratella quadrata</i>



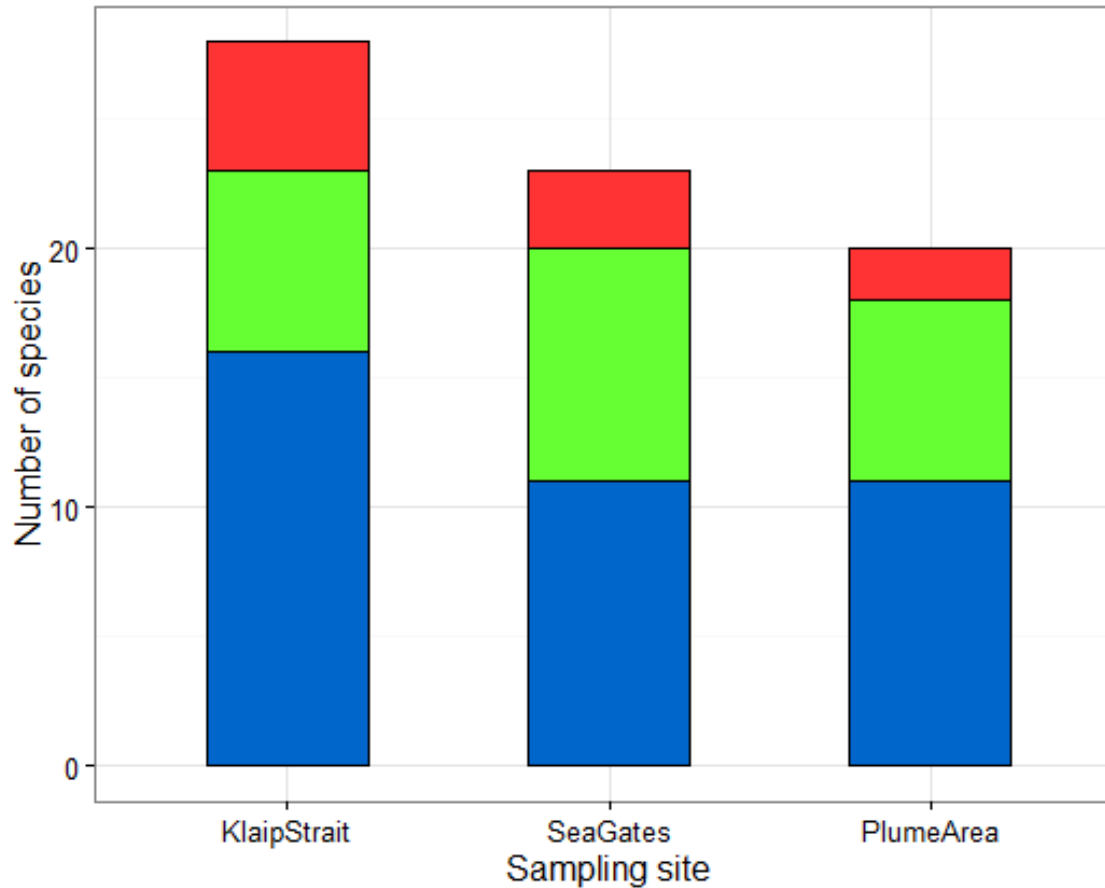
# Traditional monitoring vs metabarcoding

Transform: Presence/absence  
Resemblance: S7 Jaccard



# Species detected by both techniques

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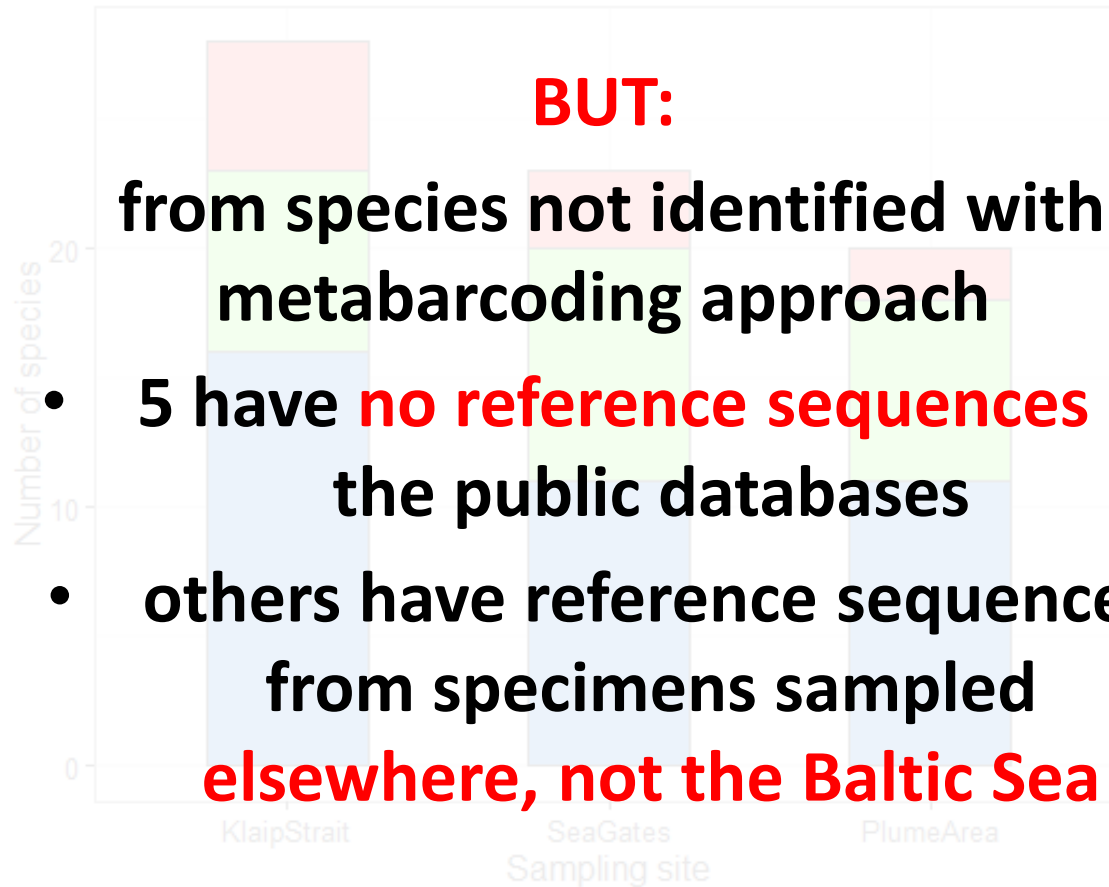
- Species detected only from morphological analysis
- Species detected only from sequencing
- Species shared between two approaches





# Species detected by both techniques

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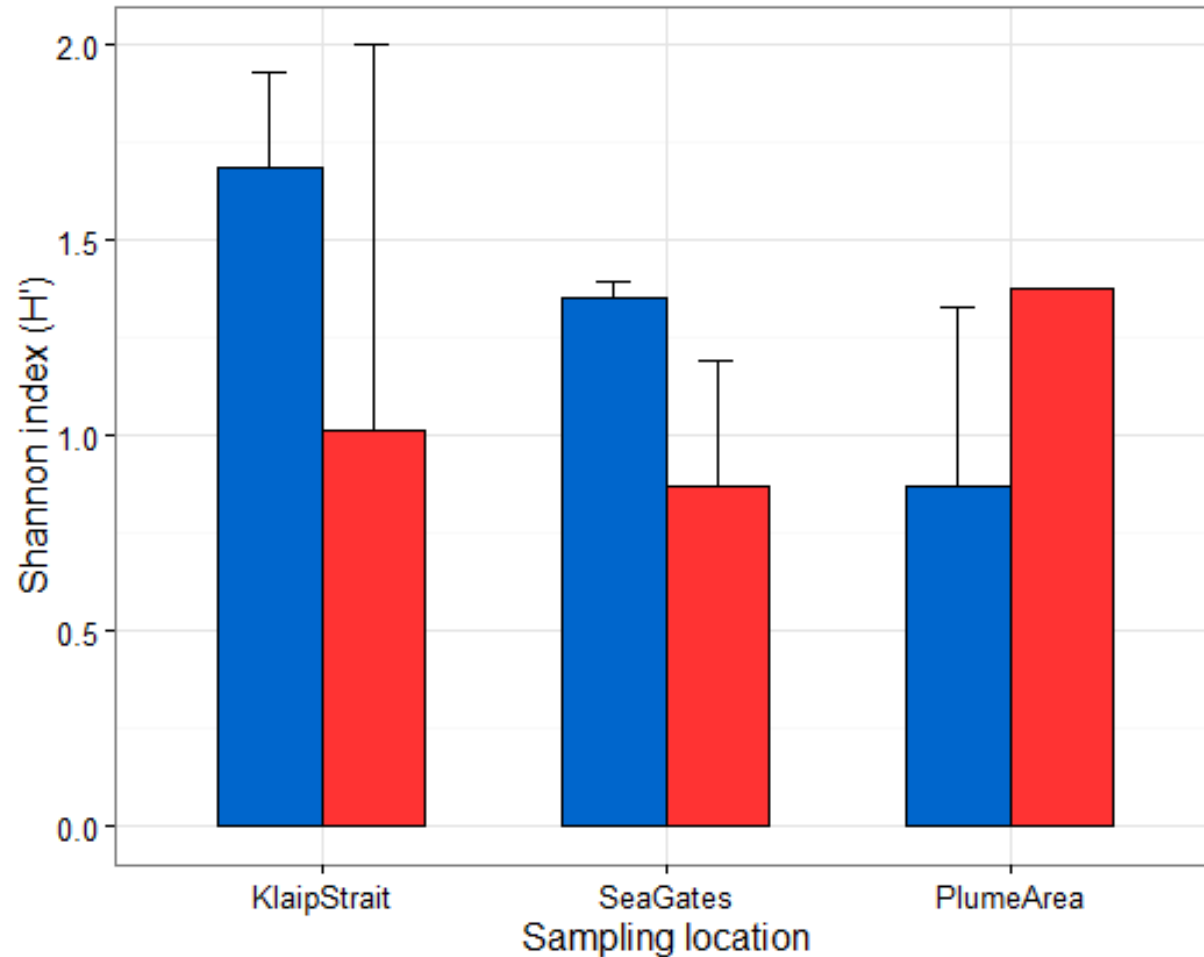
- BUT:**
- from species not identified with metabarcoding approach
- 5 have **no reference sequences** in the public databases
  - others have reference sequences from specimens sampled **elsewhere, not the Baltic Sea**

- Species detected only from morphological analysis
- Species detected only from sequencing
- Species shared between two approaches



# Biotic metrics and ecosystem health

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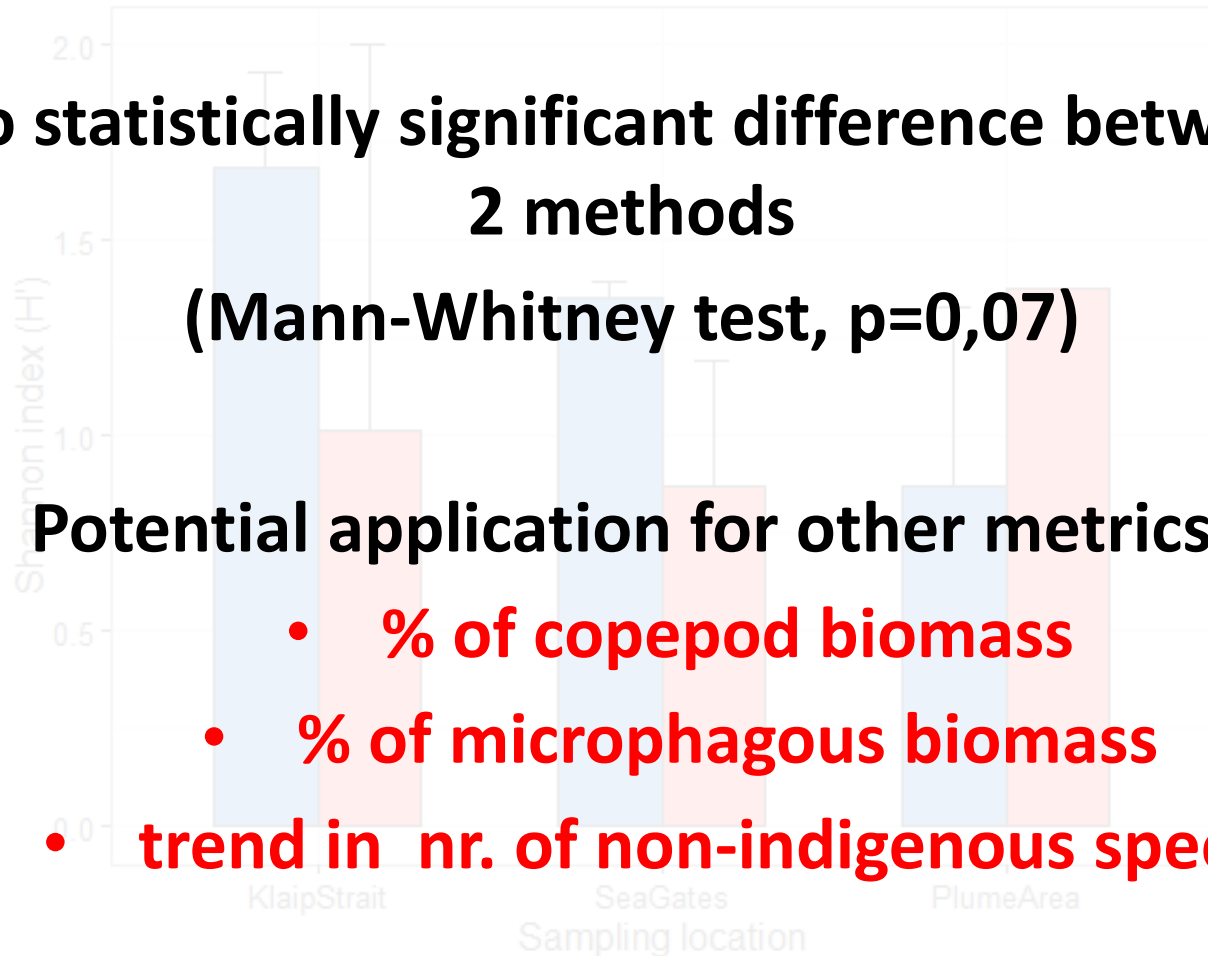
- Index calculated based on biomasses from morphological analysis
- Index calculated based on reads nr. from metabarcoding



# Biotic metrics and ecosystem health

No statistically significant difference between  
2 methods

(Mann-Whitney test,  $p=0,07$ )



- Index calculated based on biomasses from morphological analysis
- Index calculated based on reads nr. from metabarcoding



## Summing up...

- Metabarcoding is a prospective tool for marine surveillance
- It does not require particular taxonomic expertise
- It allows identification of cryptic life stages (eggs or larvae), detection of rare and sparsely distributed organisms
- It is applicable for early detection of environmental pests or indicator species
- Suitable for general biodiversity assessment and development of environmental quality metrics

### **BUT**

- **Comprehensive reference databases needed**
  - **Quantification should be elaborated**
- **Marker validation / application of multiple markers**





# Thank you!

DEVOTES 

 BIO-C3



**BONUS**  
SCIENCE FOR A BETTER FUTURE OF THE BALTIC SEA REGION



Lietuvos  
mokslo  
taryba

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Xavier Pochon and Susie Wood for critical comments on the abstract.