

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

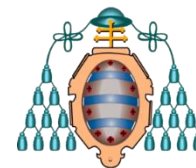
Anastasija Zaiko,

Aurelija Samuiloviene, Alba Ardura, Eva Garcia-Vazquez

Email: anastasija@corpi.ku.lt

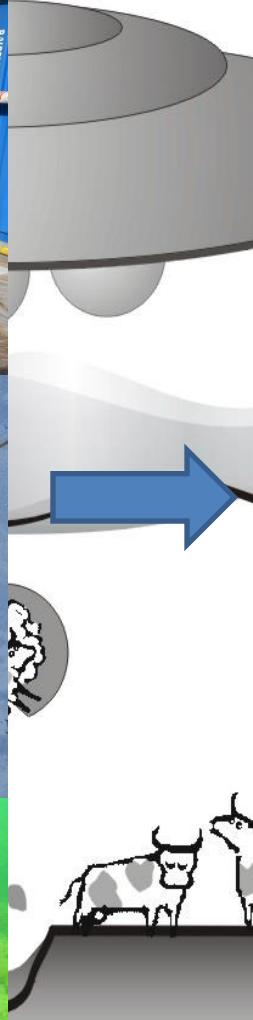
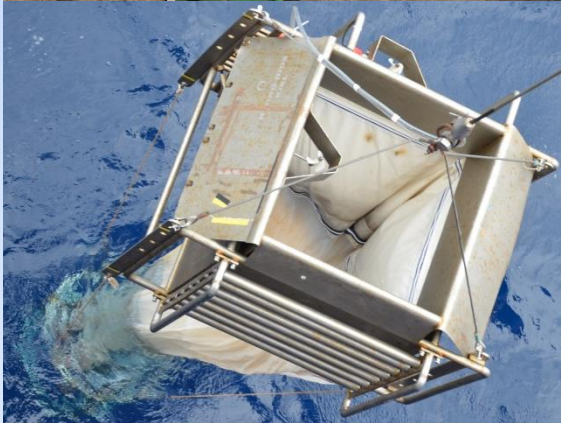


Klaipeda University



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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**

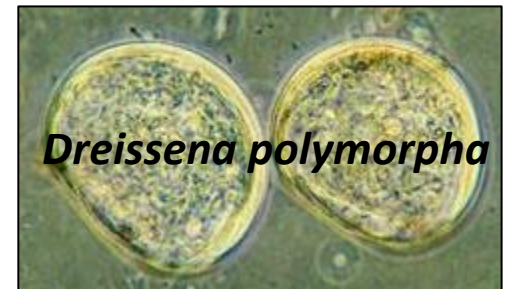


Photo credits:

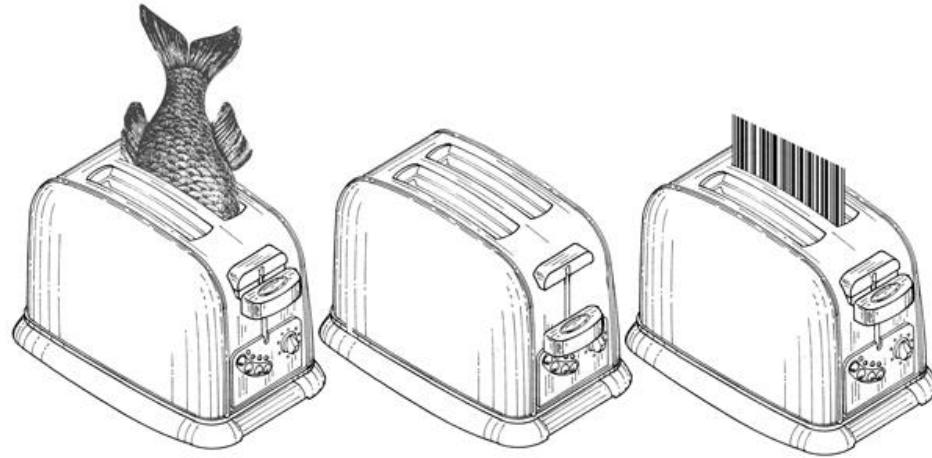
www.mussel.free.org

www.cefas.defra.gov.uk

www.fao.org



Principle of the metabarcoding



Sampling
in the field
(soil, water, etc.)



DNA
extraction



DNA amplification
with universal
primers



High throughput
parallel
pyrosequencing



Reference
database



Species identification
via DNA barcoding

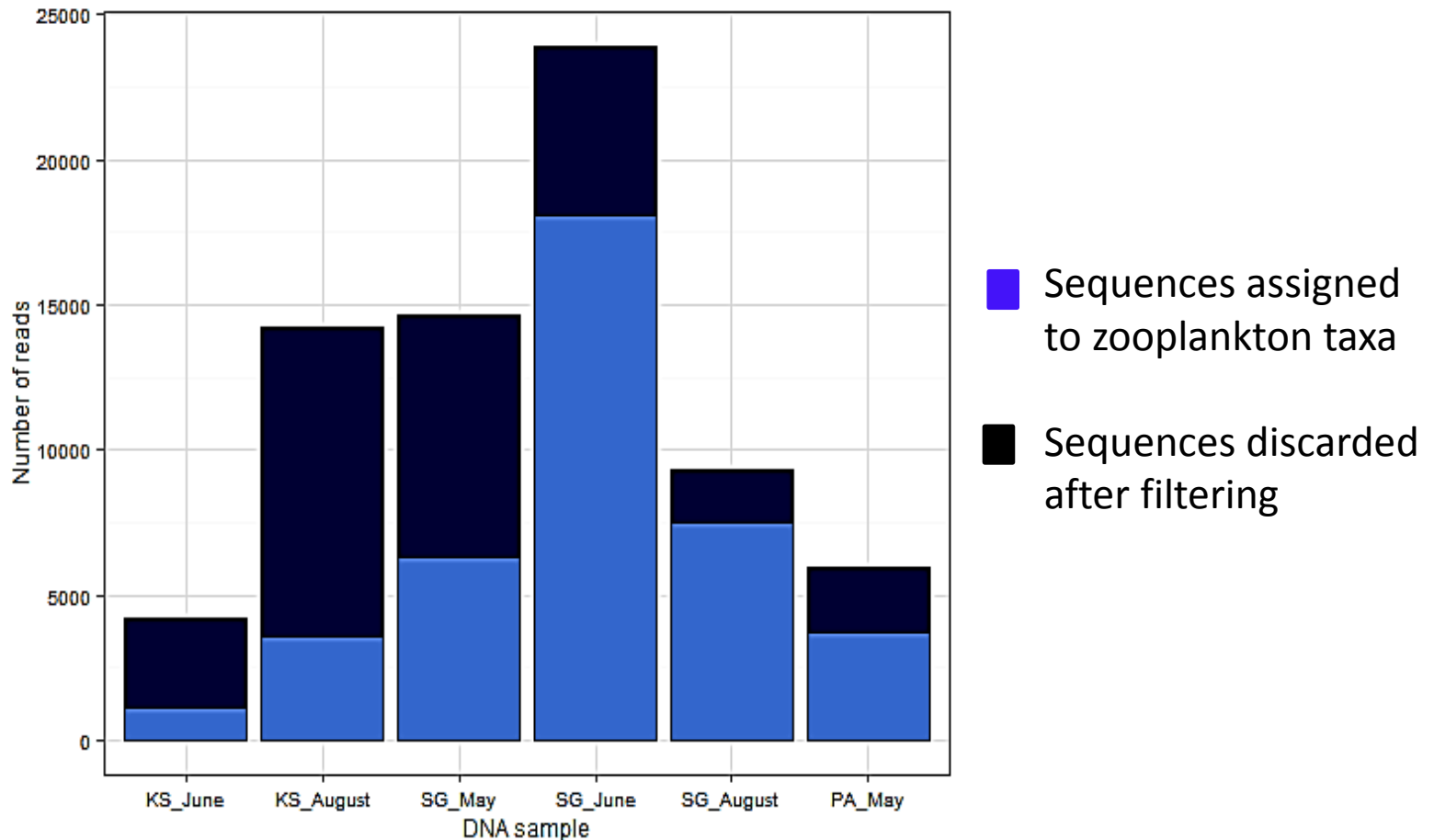
Zooplankton study in the Baltic Sea

- 6 samples collected from ca. 2 m³ 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



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

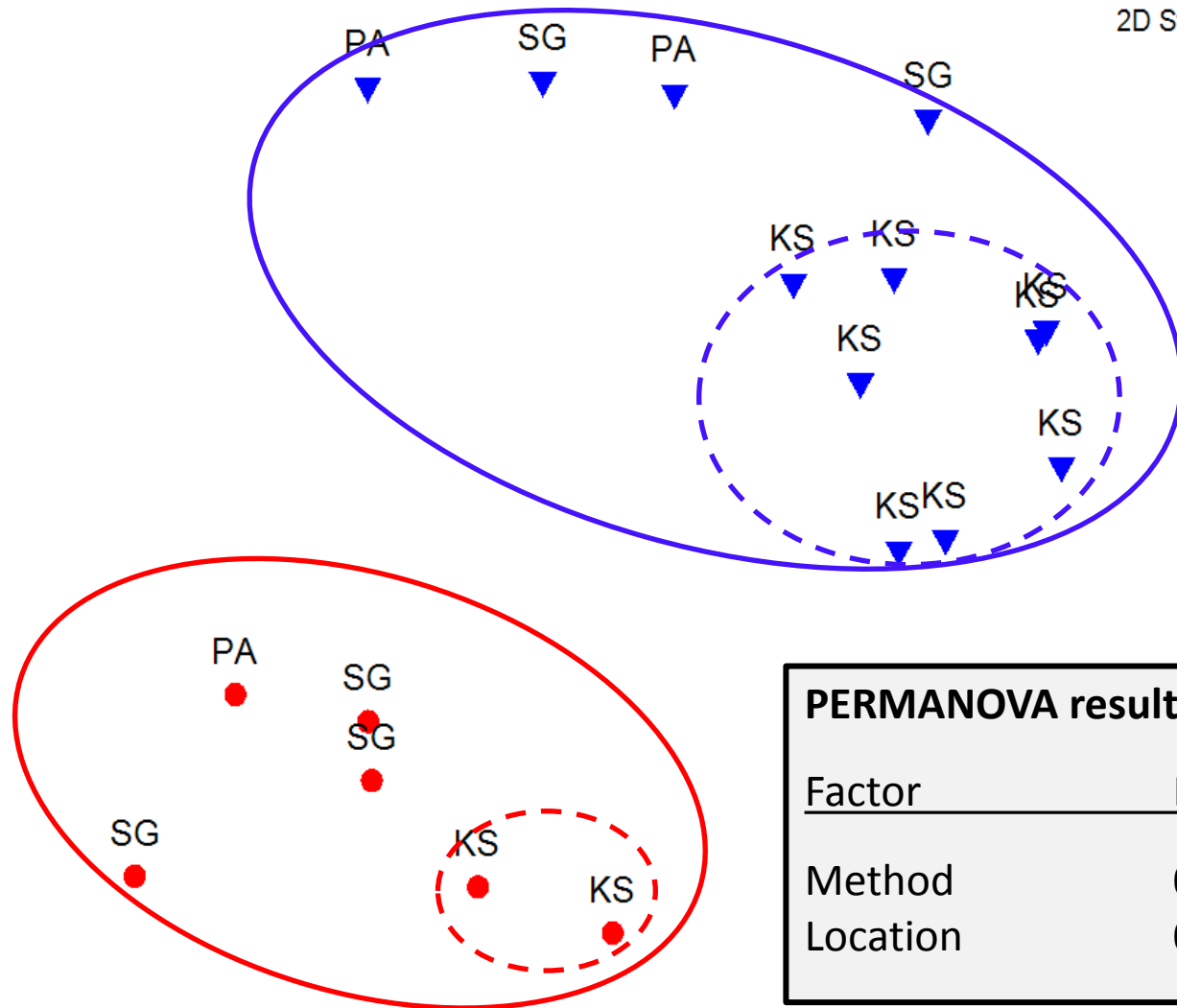
Annelida	Polychaeta	<i>Marenzelleria viridis</i> <i>Marenzelleria neglecta</i>
Crustacea	Cladocera	<i>Bosmina coregoni</i> <i>Bosmina spp.</i> <i>Cercopagis pengoi</i> <i>Daphnia galeata</i> <i>Daphnia pulex</i> <i>Daphnia cucullata</i>
<p>>800 sequences found in all samples, aligned with high confidence (>97% identity, >95% coverage)</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

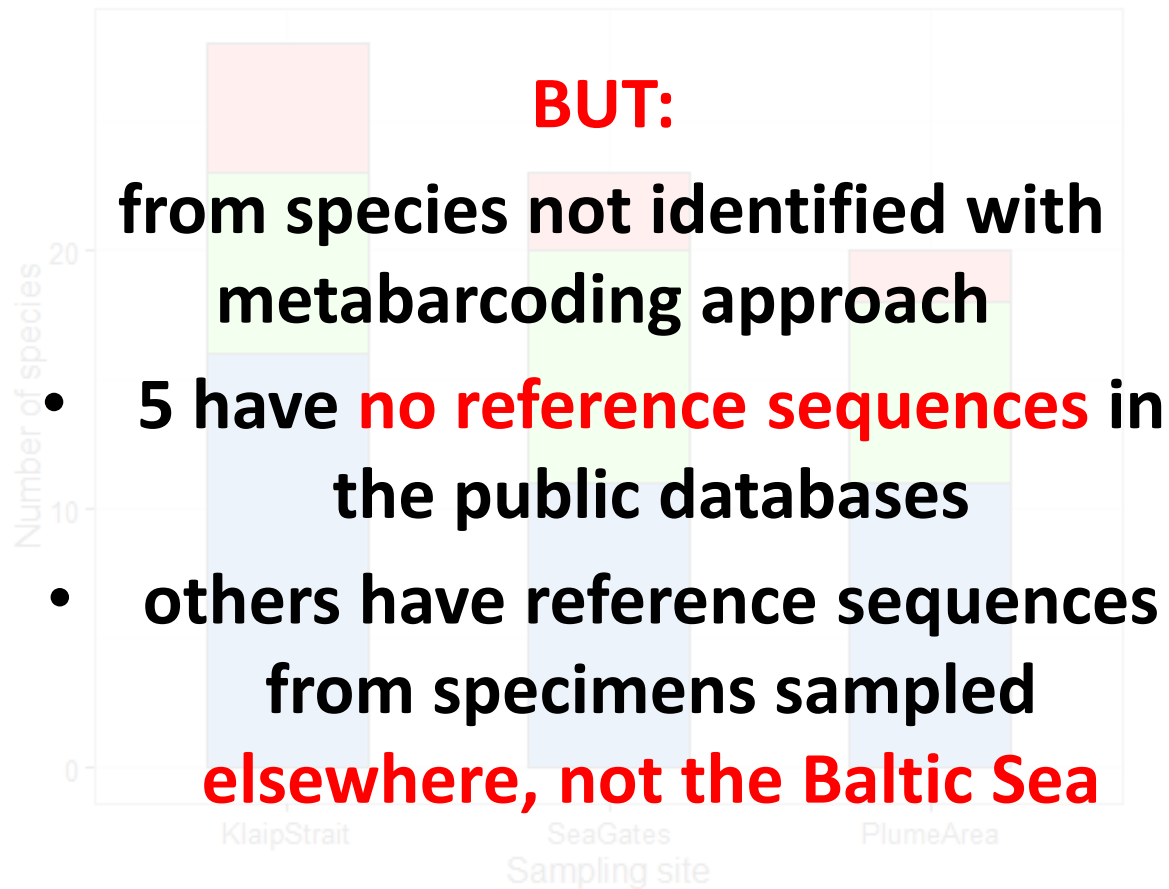
2D Stress: 0,11



PERMANOVA results:

Factor	P
Method	0,001
Location	0,017

Species detected by both techniques



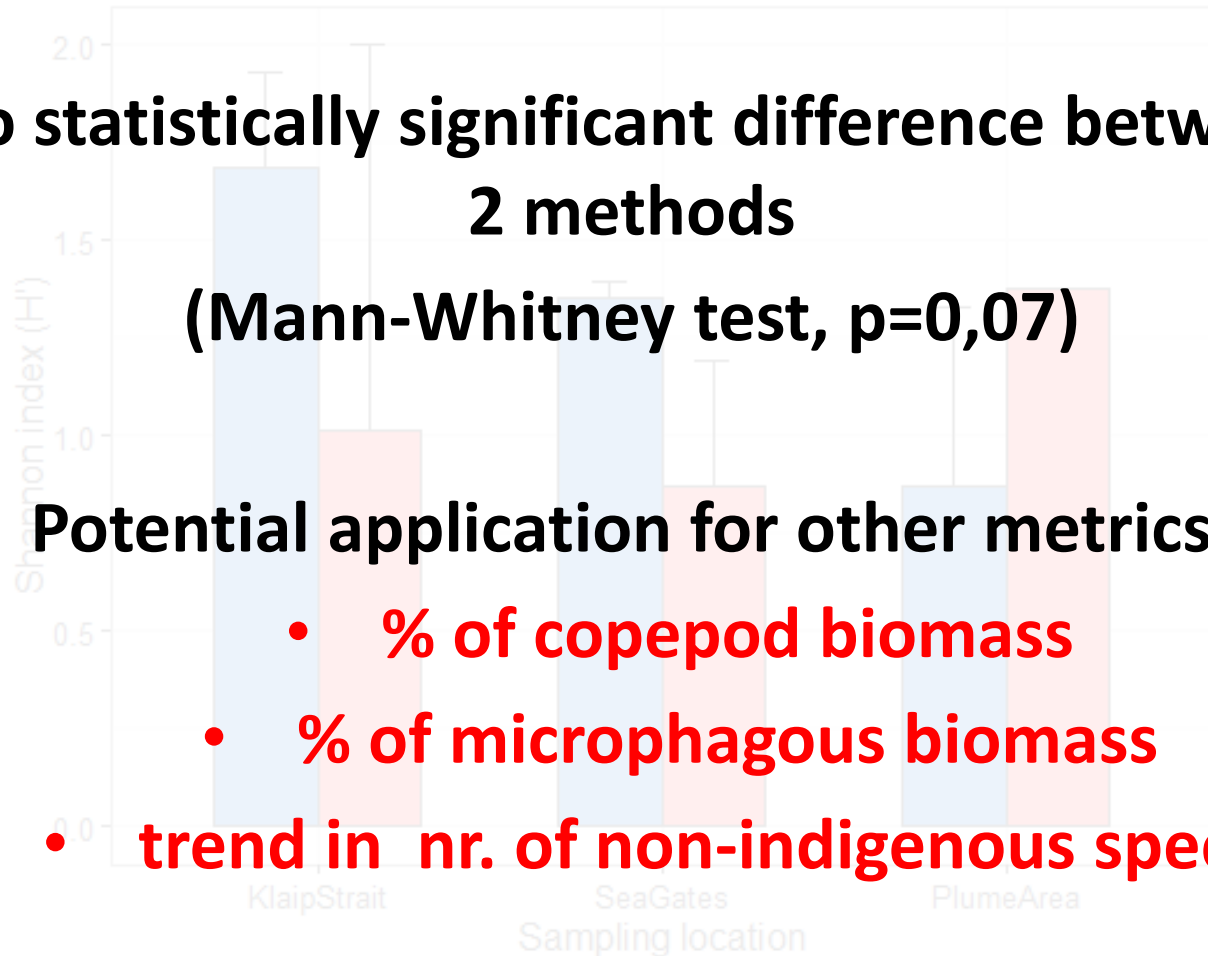
- Species detected only from morphological analysis
- Species detected only from sequencing
- Species shared between two approaches



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!



We thank Yaisel Borell for help with samples,
Xavier Pochon and Susie Wood for critical comments on the abstract.