HIFMB Helmholtz Institute for Functional Marine Biodiversity at the University OLDENBURG

A view on zooplankton diversity in temperate waters inferred from organismal and environmental DNA metabarcoding

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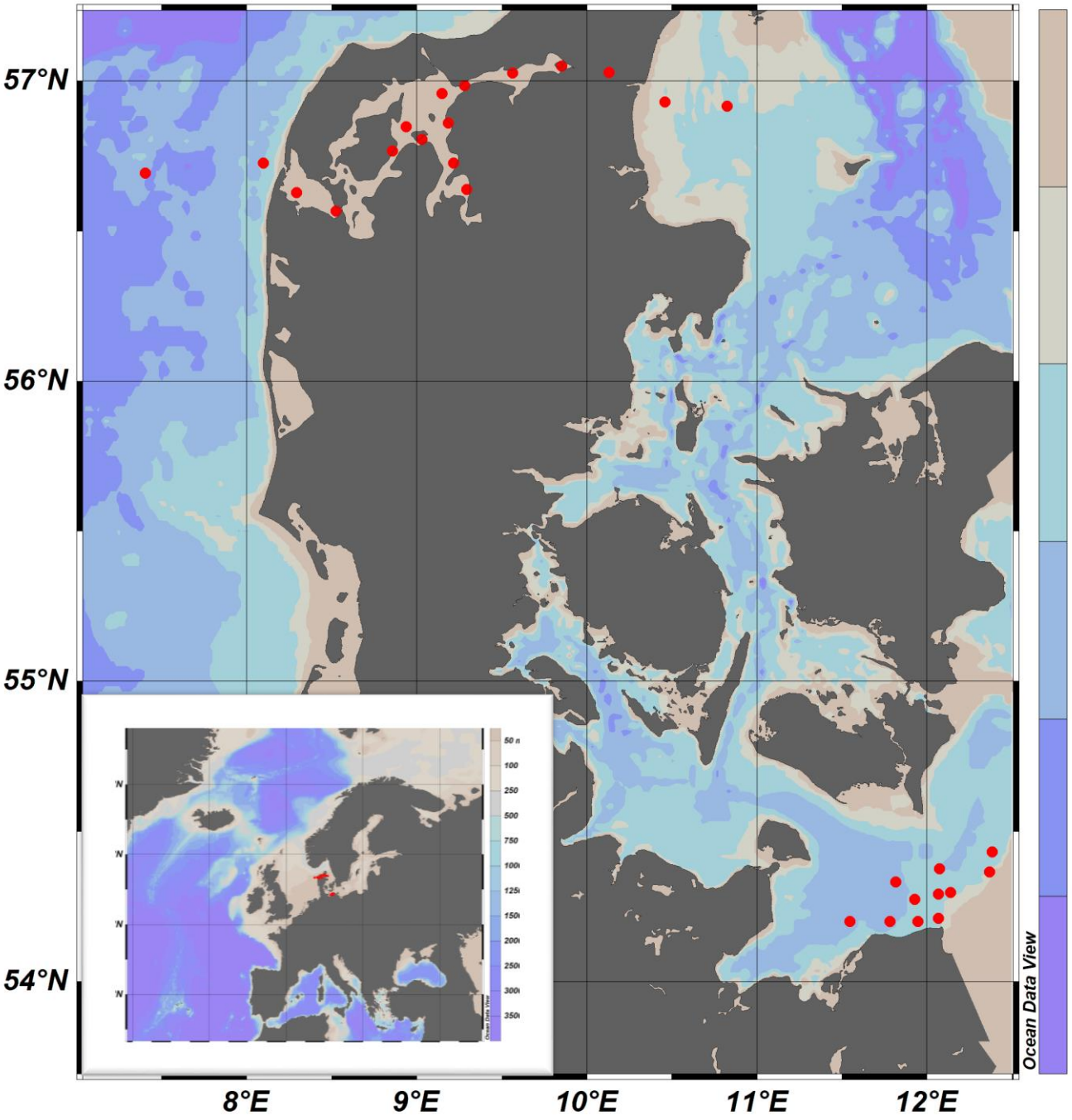
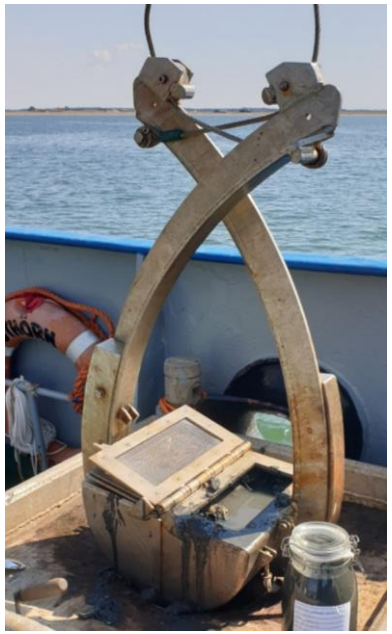
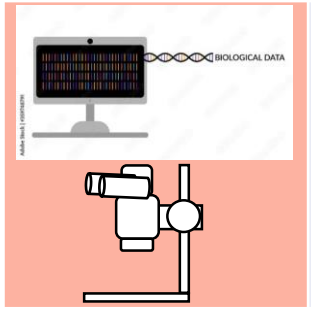
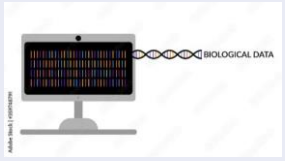
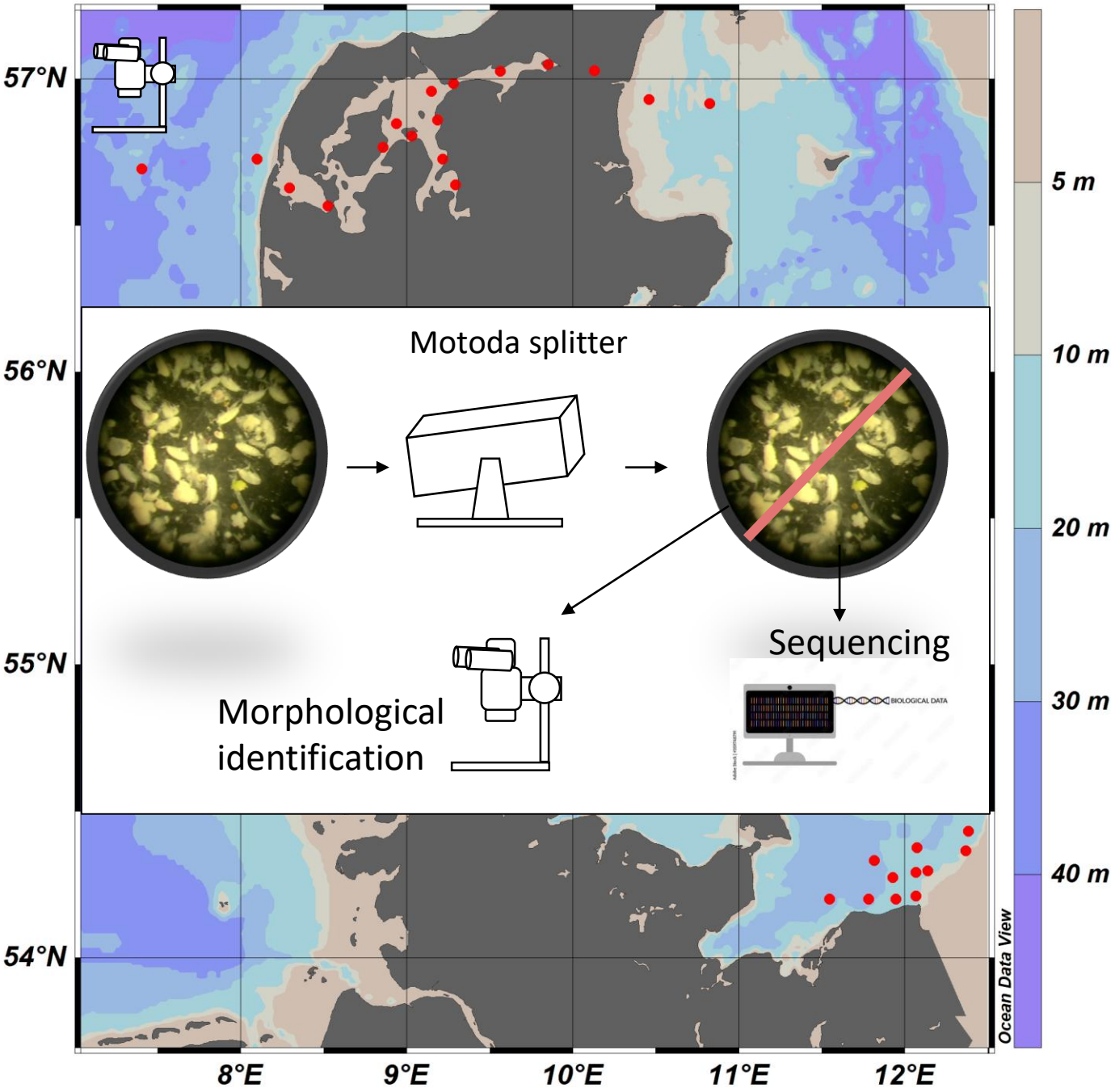
Aims

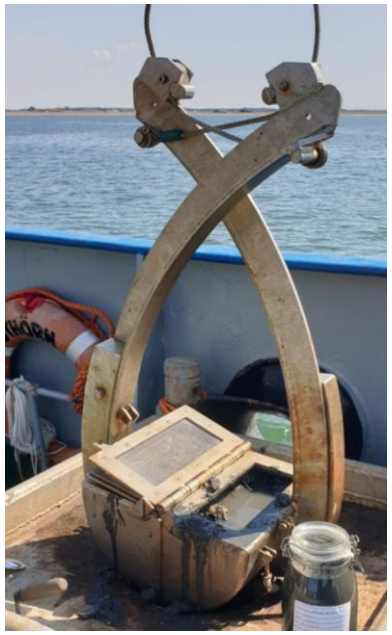
- Marine biodiversity assessment from sequence data
- Identification of factors influencing detection success of species
- Evaluation of MetaZooGene database (MZGdb) to identify diversity in ICES areas the North and the Baltic Sea
- Reliable identification of pelagic communities across regions from eDNA and zooplankton metabarcoding





ZP-Net	eDNA water	eDNA sediment
150 µm	0.2 µm	
	1 L + 1 L (surface + deep)	2 g
17 stations	22 + 19 stations	28 stations
		





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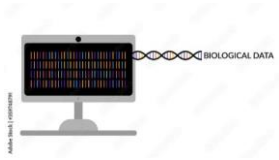
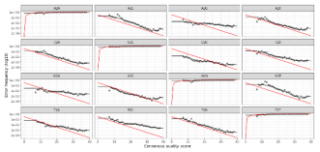
Methods

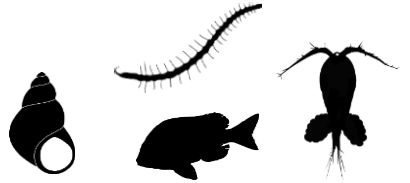
Sample Processing

Assay		Extraction kit
eDNA water		Qiagen DNeasy Blood & Tissue
ZP-Net	split	Qiagen DNeasy Blood & Tissue
eDNA sediment		Qiagen DNeasy PowerSoil

Metabarcoding



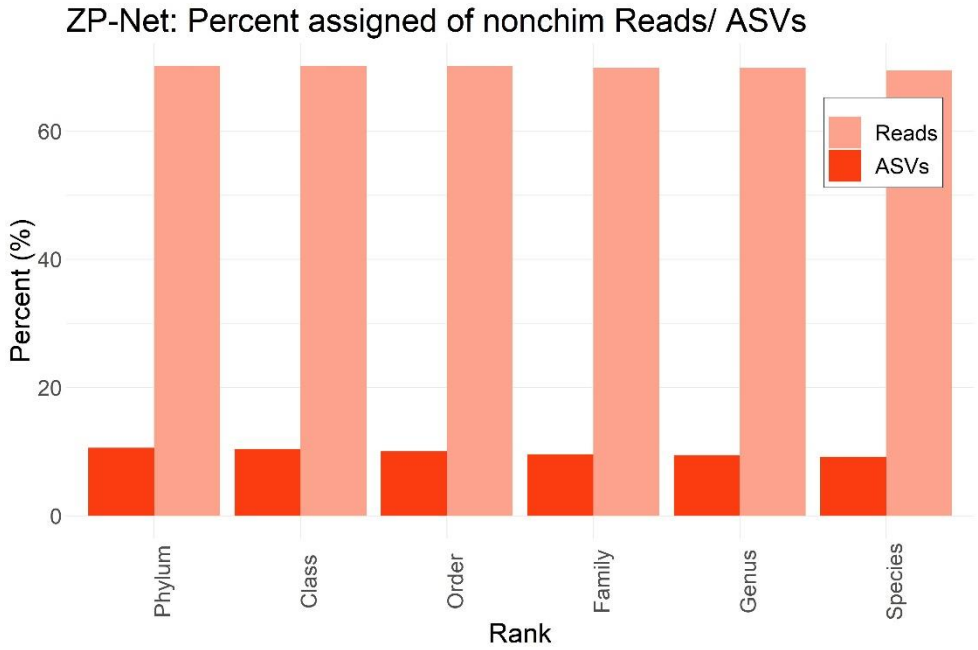


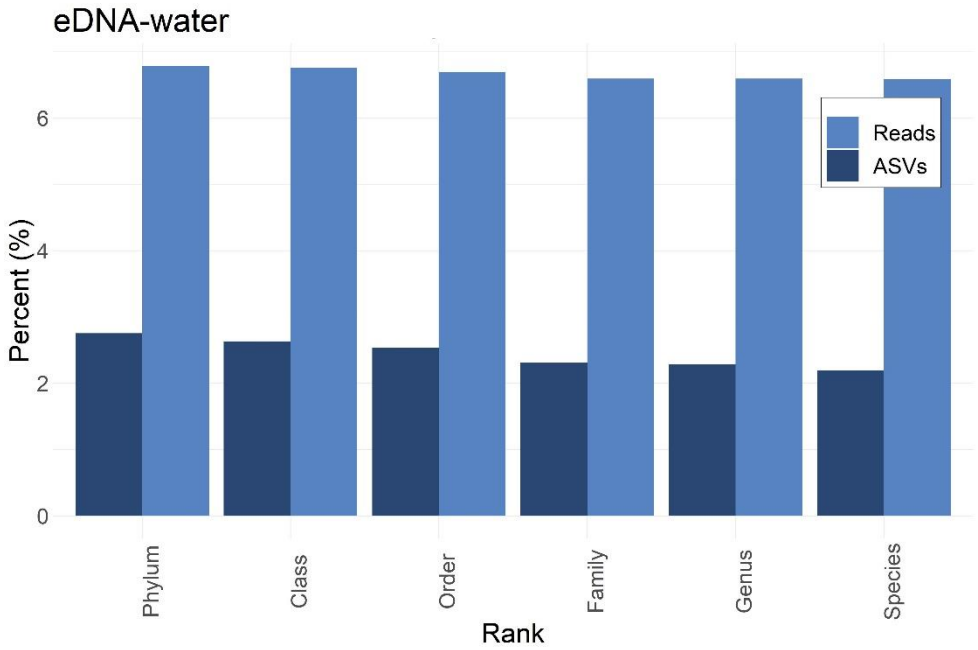


PCR with ‘universal‘ Leray-XT primers (COI) → Sequencing on MiSeq (2* 300 bp) → dada2 & cutadapt pipeline → annotation with MZGdb (World) v2.2 RDP minboot= 95

Sequence Annotation Success

→ Good resolution possible down to species level

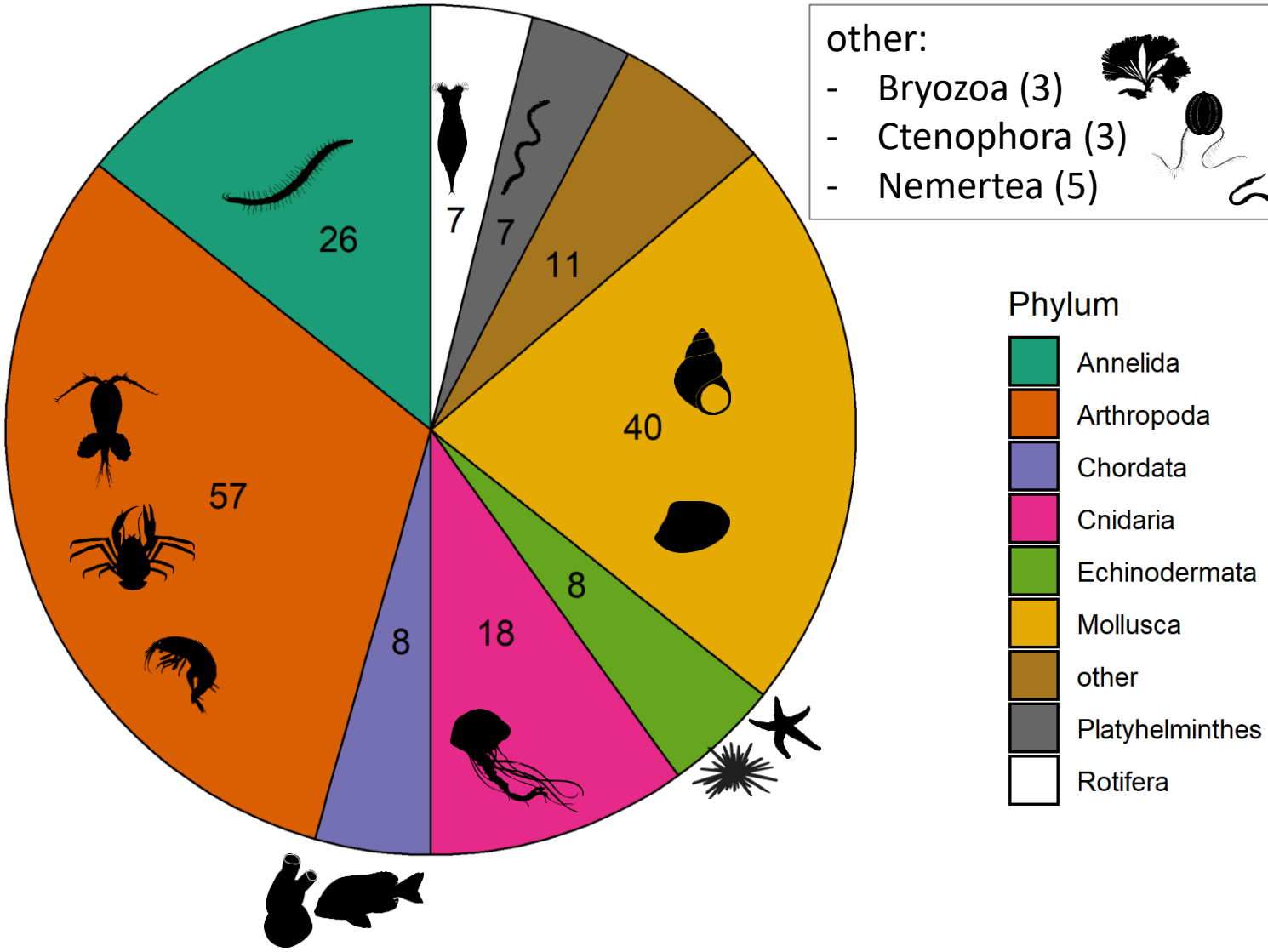




- > 69 % of net sample reads could be assigned to metazoan species

- Unspecific amplification (factor 10!) of non-target taxa

Total Biodiversity



Entire dataset:

- **182 species** in 11 phyla
- Primers & MZGdb adequate for a broad range of taxa

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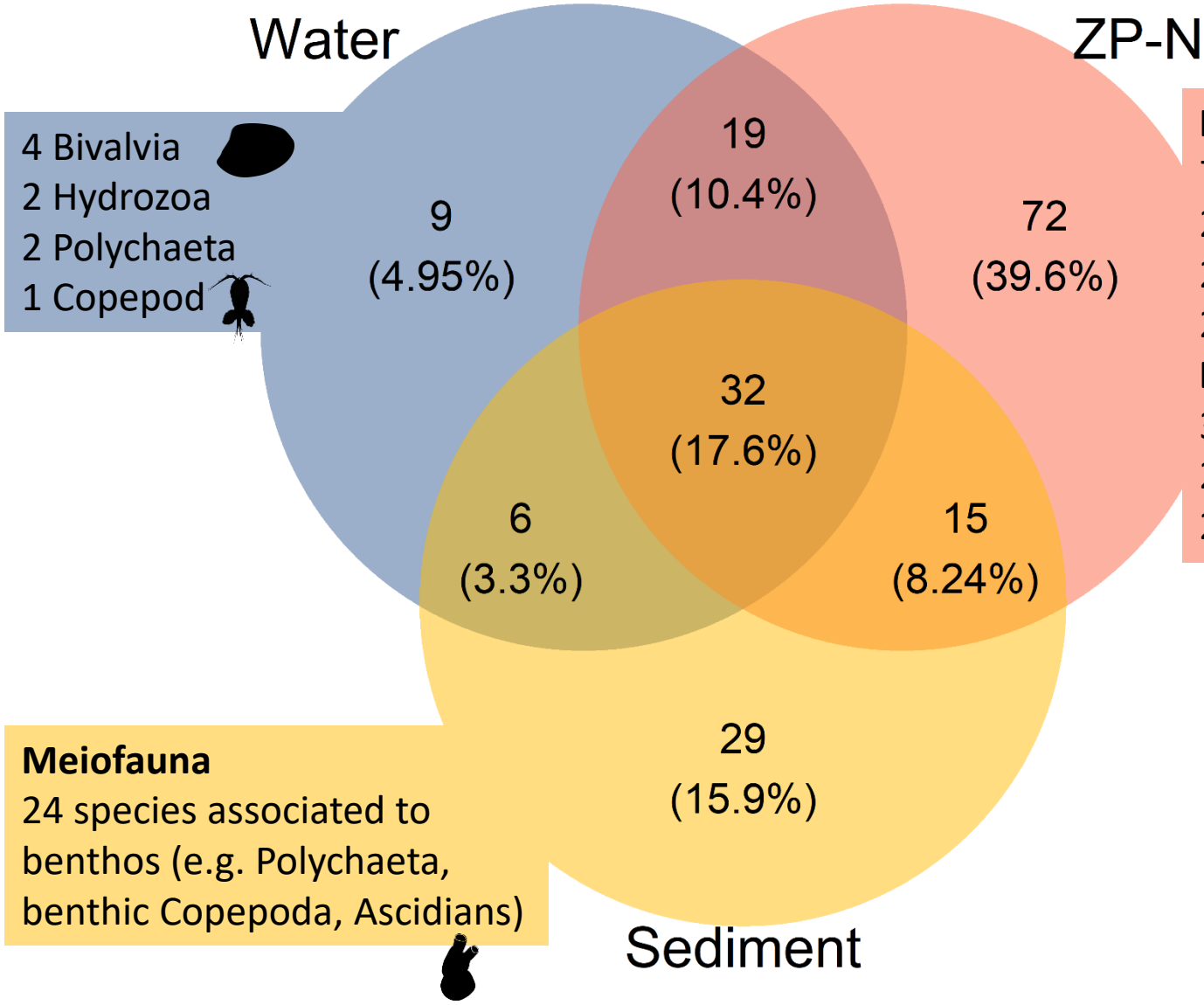
Biodiversity from different Approaches



Holoplankton
7 Hydrozoa
2 Ctenophora
2 Branchiopoda
2 Copepoda

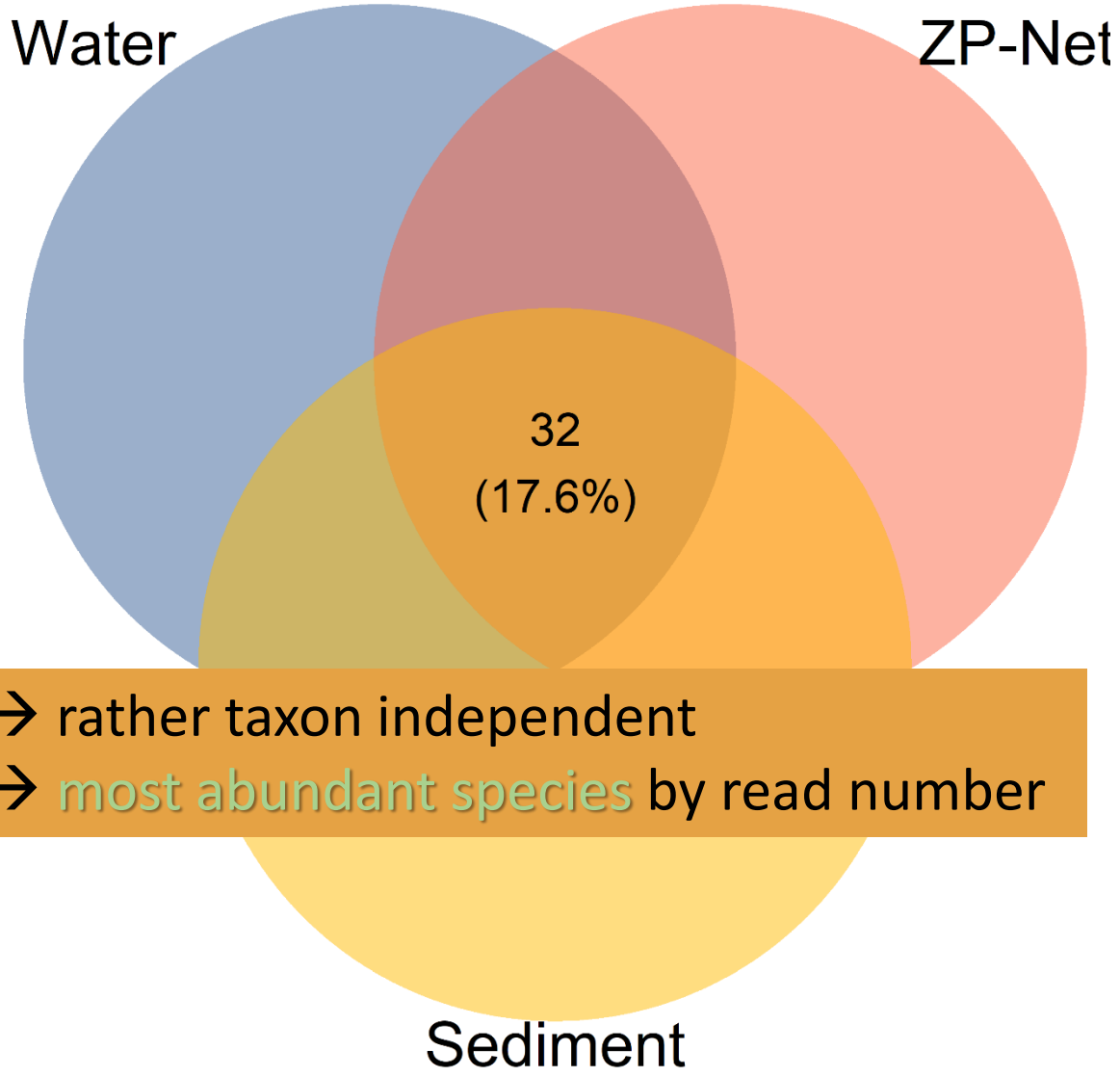
Benthos
3 Nemertea
2 Platyhelminthes
2 harpact. Copepoda

Mero- /Ichthyoplankton
22 Gastropoda
14 Malacostraca
7 Polychaeta
4 Echinodermata
2 Thecostraca
1 Bryozoa
2 Actinopteri

Meiofauna
24 species associated to benthos (e.g. Polychaeta, benthic Copepoda, Ascidians)

- Detected diversity partially depending on sampling strategy
- Methods complement each other well

Biodiversity from different Approaches



→ rather taxon independent
→ most abundant species by read number

Shared Species

Annelida:
Alitta succinea
Hypereteone heteropoda
Lagis koreni
Polydora cornuta
Scoloplos armiger
Streblospio benedicti

Arthropoda:
Penilia avirostris
Pleopis polyphemoides
Acartia (Acanthacartia) tonsa
Centropages hamatus
Oithona similis
Paracalanus parvus parvus
Pseudocalanus elongatus
Temora longicornis
Alpheus bellulus
Hyperia galba
Amphibalanus improvisus

Chordata:
Ascidiella aspersa

Cnidaria:
Aurelia aurita

Ctenophora:
Mnemiopsis leidyi

Echinodermata:
Echinocyamus pusillus
Psammechinus miliaris

Mollusca:
Arctica islandica
Magallana gigas
Mya arenaria
Ruditapes decussatus
Haminella solitaria
Peringia ulvae

Platyhelminthes:
Microstomum rubromaculatum

Rotifera
Synchaeta cecilia
Synchaeta grimpei
Synchaeta triophthalma

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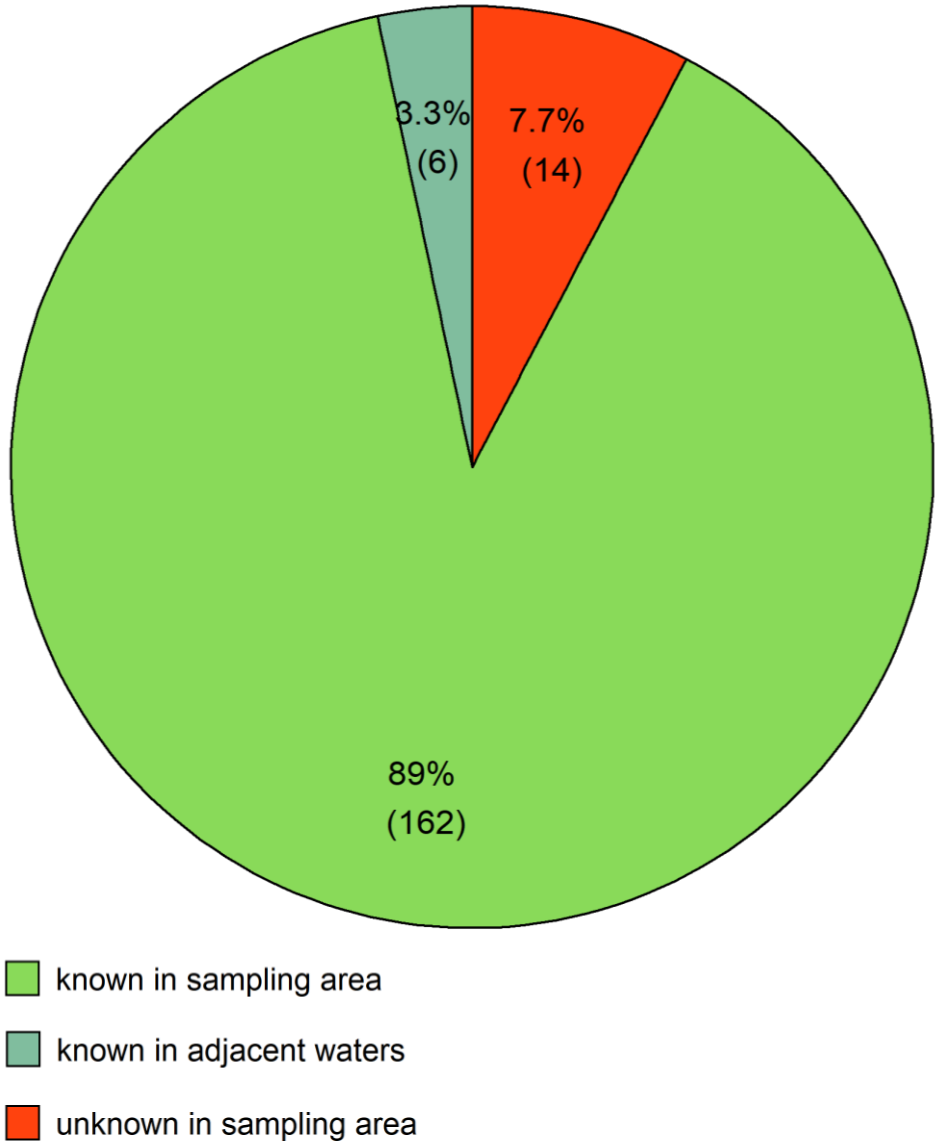
Evaluation of Regional Biodiversity



→ 89 % of species known from sampling area

→ large majority of assignments seems plausible

'Unknown species':

- 5 previously detected non-indigenous species
  - *Amathia tertia, Hypereteone heteropoda* previously sequenced by eDNA in the North Sea [1]
  - *Haminella solitaria* prev. found in the Baltic Sea [2]
  - *Corambe obscura*[3,4] & *Euplana gracilis*[4] previously found in the North Sea
- 7 probably not correctly assigned (BLAST): e.g.: *Calanus euxinus* → *C. helgolandicus*
- 2 new introductions? *Pisione puzae* & *Ercolania viridis*

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Validation against morphology-based species identification

→ Sequence data recover almost all species within Arthropoda

Phylum	Class	Morphology	eDNA (sediment & water) metabarcoding		Zooplankton-Net metabarcoding		Comments
Arthropoda	**Copepoda**	*Acartia sp.*	✓	*A. (A.) tonsa*			
					✓	*A. (A.) clausi*	
		Calanus sp.	X		✓	*C. euxinus*	likely *C. helgolandicus*
		Centropages typicus	✓	*C. typicus*	✓	*C. typicus*	
		Ditrichocorycaeus anglicus	X		✓	*D. anglicus*	
		Oithona sp.	✓	*O. similis*	✓	*O. similis*	
		Paracalanus sp.	✓	*P. p. parvus*	✓	*P. p. parvus*	
		Pseudocalanus sp.	X		✓	*P. elongatus*	
		Temora sp.	X		X		In other samples
		Euterpina acutifrons *	X		✓	*E. acutifrons*	
		Nauplii	✓		✓		
	Malacostraca	Zoea larvae	✓	*Alpheus bellulus*	✓	*Callianassa subterranea*	
					✓	*Upogebia deltaura*	
		Crangonidae (larvae)	X		✓	*Philocheras bispinosus*	
	Branchiopoda	*Evadne nordmanni*	X		✓	*E. nordmanni*	
		Evadne spinifera	X		✓	*E. spinifera*	
		Podon leuckarti	X		O	*P. intermedius*	Misidentification ?

* Confirmed by Sanger-Sequencing

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→ Sequencing and species annotations also worked well without obvious problems within phyla Annelida, Bryozoa and Chordata

Phylum	Class	Morphology	eDNA (sediment & water) metabarcoding	Zooplankton-Net metabarcoding	Comments
Chaetognatha		*Parasagitta setosa* *	X	X	
Cnidaria		*Sarsia sp.*	X	X	
		Ectopleura dumortierii *	✓ *Ectopleura dumortierii*	✓ *Ectopleura dumortierii*	
Annelida	**Polychaeta**	Juveniles	✓ *Polydora cornuta* ✓ *Streblospio benedicti*	✓ *Magelona johnstoni* ✓ *Paramphinome jeffreysii* ✓ *Pisione puzae* ✓ *Polygordius appendiculatus* ✓ *Polygordius lacteus*	
Bryozoa		Cyphonautes larvae	X	✓ *Membranipora membranacea*	
Chordata		Juvenile fish & eggs	X	✓ *Arnoglossus laterna* ✓ *Callionymus reticulatus*	
		Branchiostomata	X	✓ *Branchiostoma lanceolatum*	
		Oikopleura dioica	X	X	
Echinodermata	**Asteroidea**	Bipinnaria larvae	X	X	
	Echinoidea		✓ *Echinocyamus pusillus* ✓ *Echinocardium cordatum*	✓ *Echinocyamus pusillus* ✓ *Brissopsis lyrifera* ✓ *Echinocardium cordatum* ✓ *Echinocardium flavescens*	
	Ophiuroidea		X	✓ *Amphiura filiformis* ✓ *Ophiothrix fragilis*	
Hemichordata		Tornaria larvae	X	X	
Mollusca	**Bivalvia**	Juvenile	X	X	
	Gastropoda	Juvenile	X	✓	9 different species
Phoronida		*Phoronis muelleri*	X	X	

* Confirmed by Sanger-Sequencing

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Trouble Shooting

Taxon	Sequenced in other samples?	Problem caused by ...
Chaetognatha: (*Parasagitta setosa*)	X no	missing in MZGdb v 2.2
Chordata: Tunicata (*Oikopleura dioica*)	X no	missing in MZGdb v 2.2
Phoronida (*Phoronis muelleri*)	X no	missing in MZGdb v 2.2
Echinodermata: Asteriodea	X no	Many common sp. missing in MZGdb v 2.2
Hemichordata	X no	missing in MZGdb v 2.2
Mollusca: Bivalvia	✓ yes	?
Cnidaria (*Sarsia sp.)*	✓ yes	?

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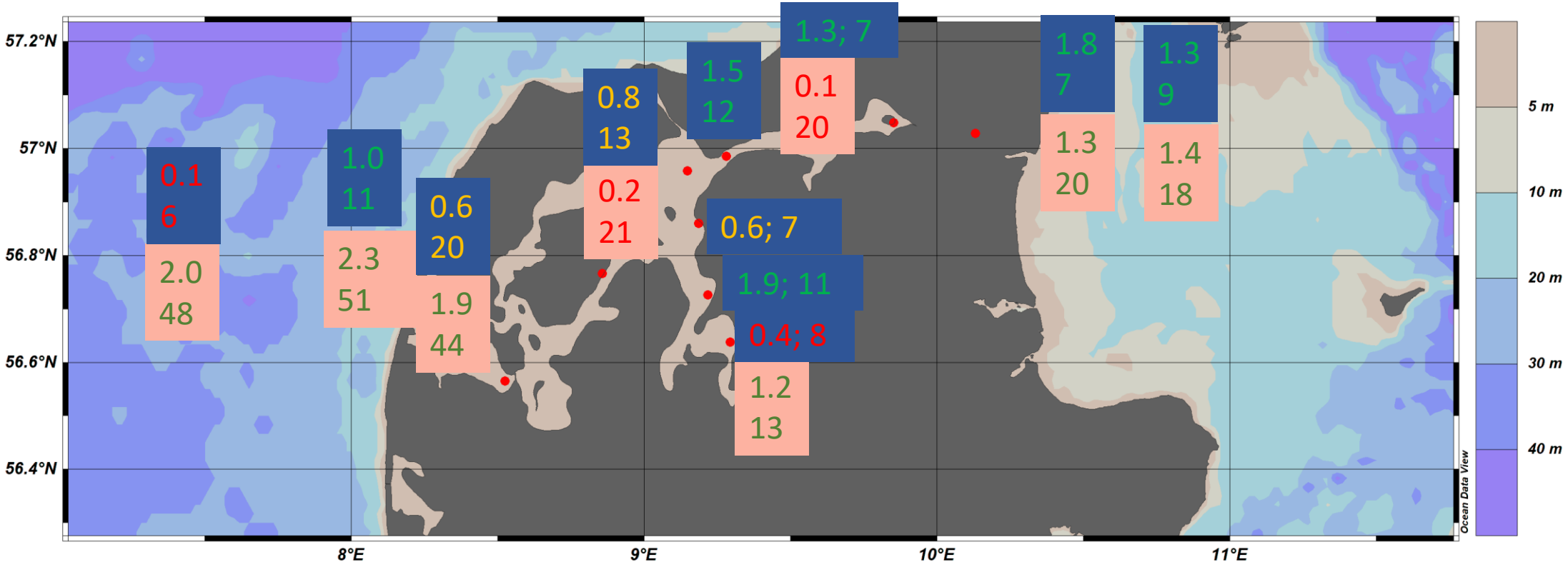
Species diversity shaped by spatial pattern



eDNA water surface

ZP-Net

Shannon Index
Species Count

High Index
Medium Index
Low Index

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Summary and Conclusion

→ Reliable identification of metazoan diversity in ICES area based on sequence data (> 89 %)

→ MZGdb is a valuable tool to identify ICES area fauna

→ Large influence of applied sampling approach on recovered species diversity

→ < 1/5 of species found in all metabarcoding approaches

Combined eDNA and ZP-Net metabarcoding allows a holistic view on marine fauna

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References

- [1] Slijkerman et al. (2017): Monitoring Groningen Sea Ports: non-indigenous species and risks from ballast water in Eemshaven and Delfzijl
- [2] Wranik, Malaquias (2018): Zum Auftreten der Kopfschildschnecke *Haminoea solitaria* (SAY 1822) im Bereich der deutschen Ostseeküste
- [3] Wolff (1988): Exotic invaders of the meso-oligohaline zone of estuaries in the Netherlands: why are there so many?
- [4] Gittenberger et al. (2017): Non-indigenous marine species in the Netherlands