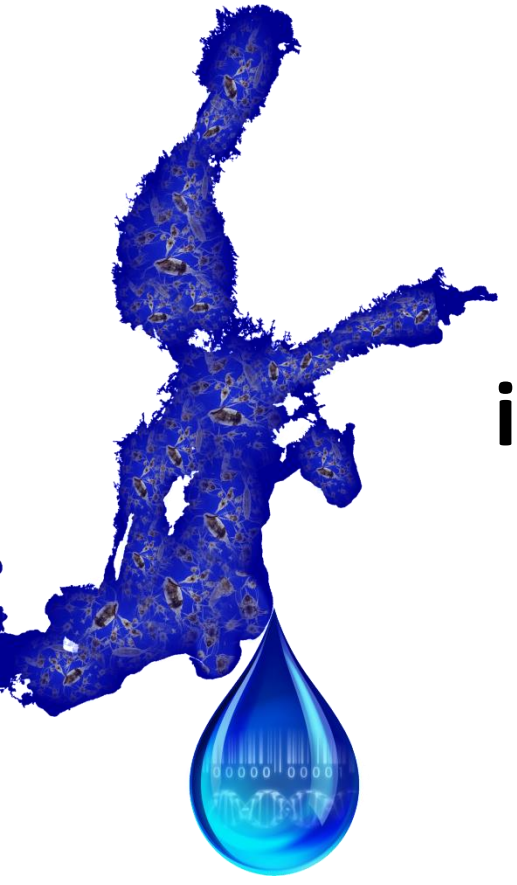
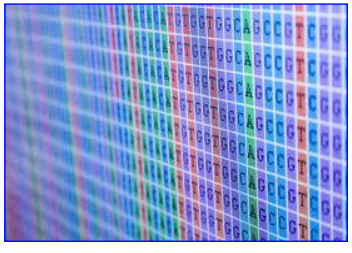


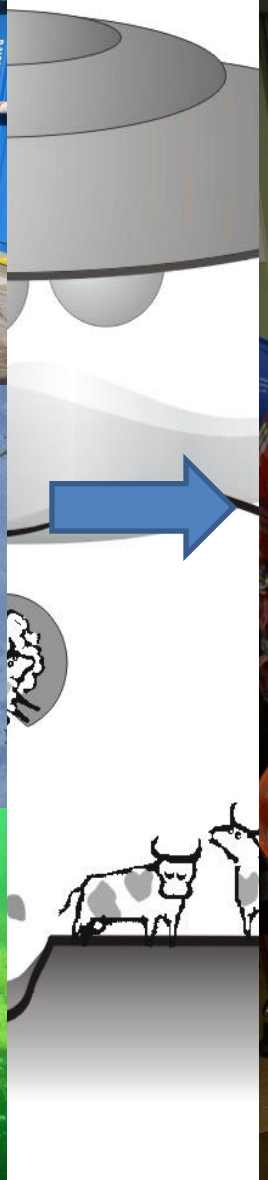
Molecular approaches for non-indigenous species surveillance – from introduction pathways to established populations

Anastasija Zaiko,
Aurelija Samuiloviene, Alba Ardura, Eva Garcia-Vazquez,
Xavier Pochon, Susie Wood

Email: anastasija@corpi.ku.lt

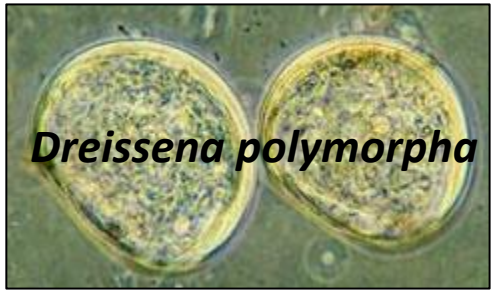


Challenges of surveying marine ecosystem



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

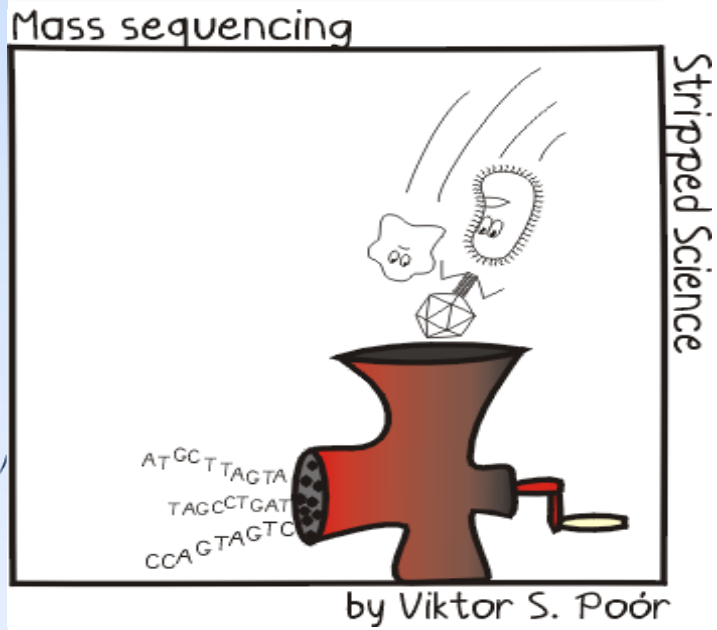


Risk to overlook or misidentify non-indigenous, pathogen or indicator species

Photo credits:
www.musselfree.org
www.cefas.defra.gov.uk
www.fao.org

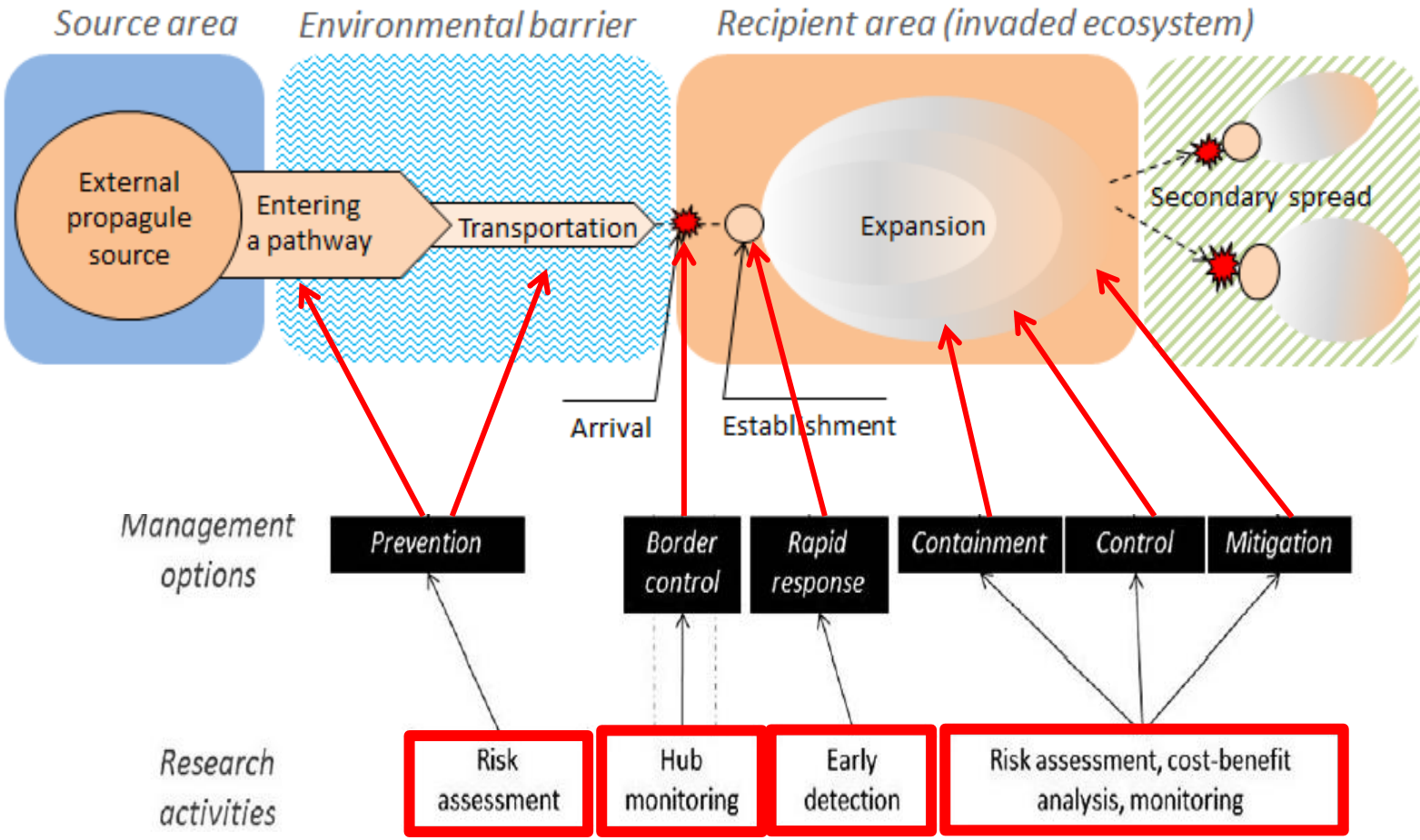
Opportunities of the eDNA analysis with next-generation sequencing

MiSeq Illumina™ Technology



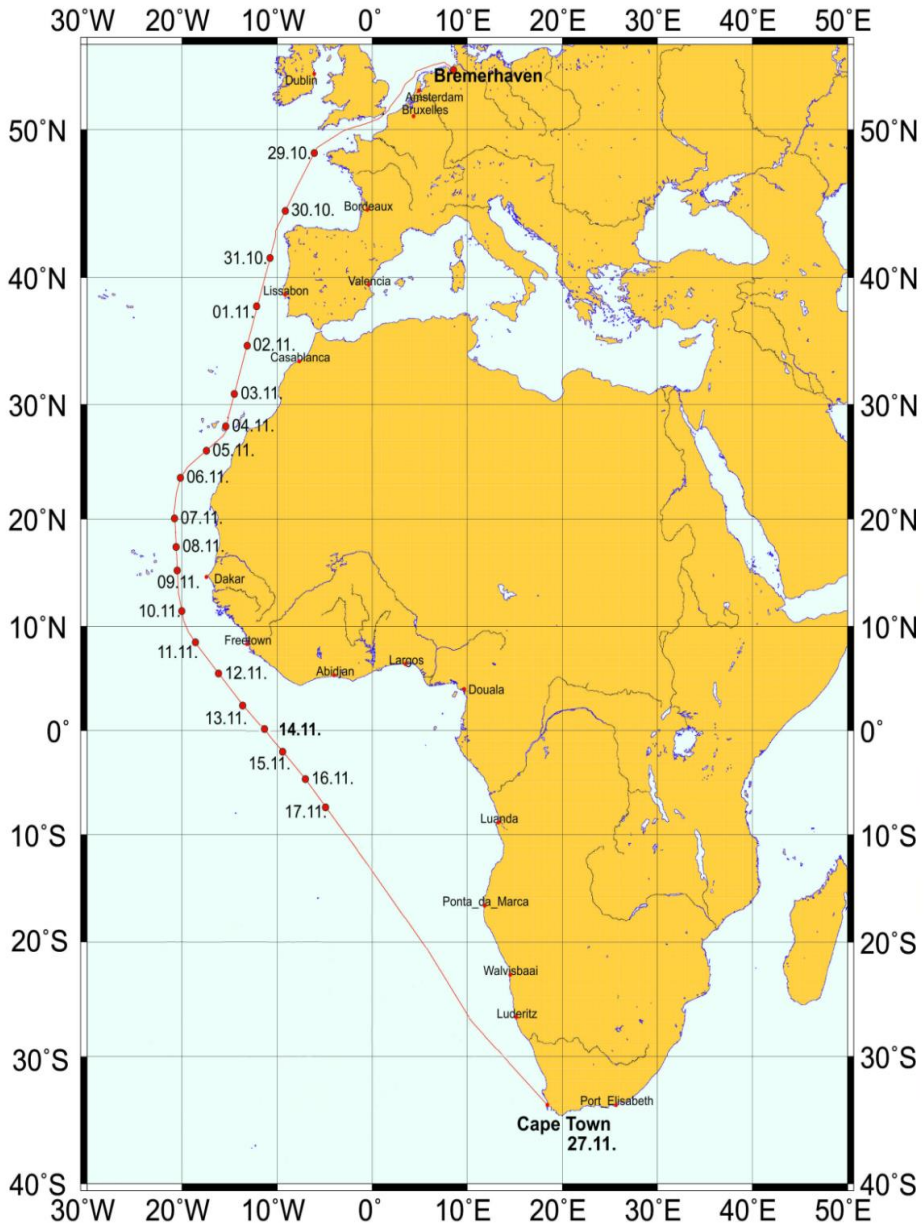
- High-throughput
- Parallel sequencing
- Millions of reads at once
- Low-cost sequencing
- Multiplex capability
- Multiple species detection (DNA Metabarcoding)

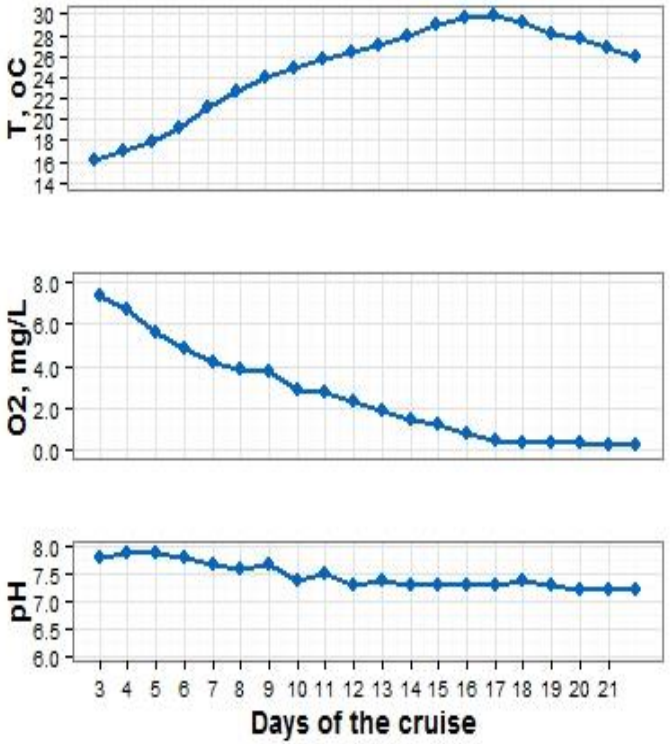
The consecutive stages of NIS introduction

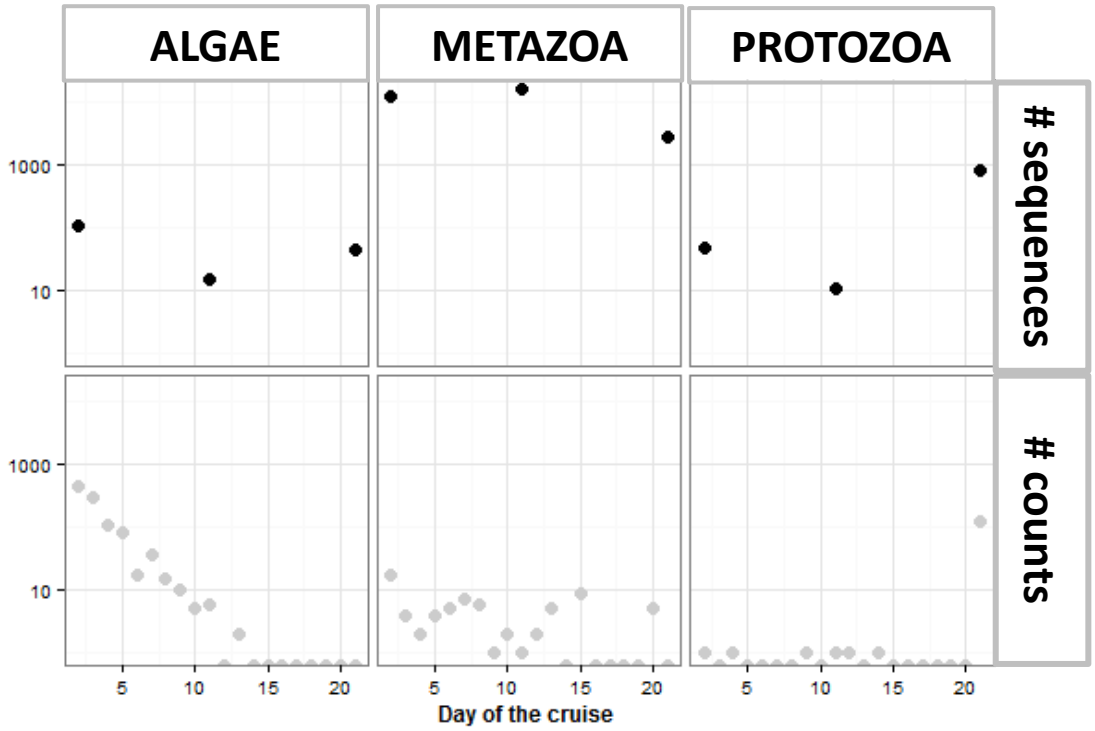


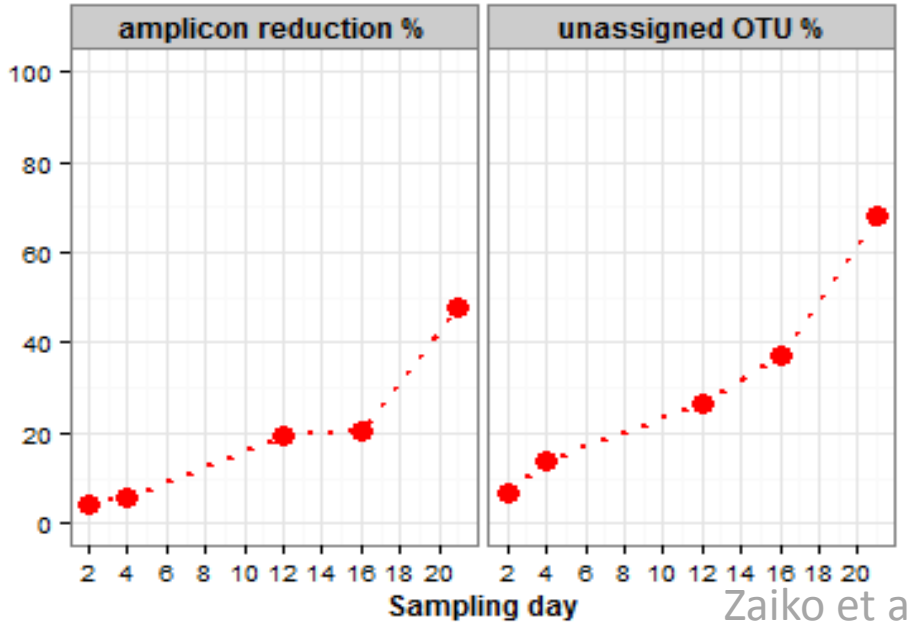
Olenin et al. (2011) *Mar Poll Bull* 62: 2598-2604

1. NIS detection on a pathway: BW survey









Peringia ulvae – an European hydrobiid gastropod seemed to withstand the harsh BW conditions

Zaiko et al. (2015) *Mar Poll Bull* 92: 25-34

2. Early detection of NIS: biofilm study

Diversity

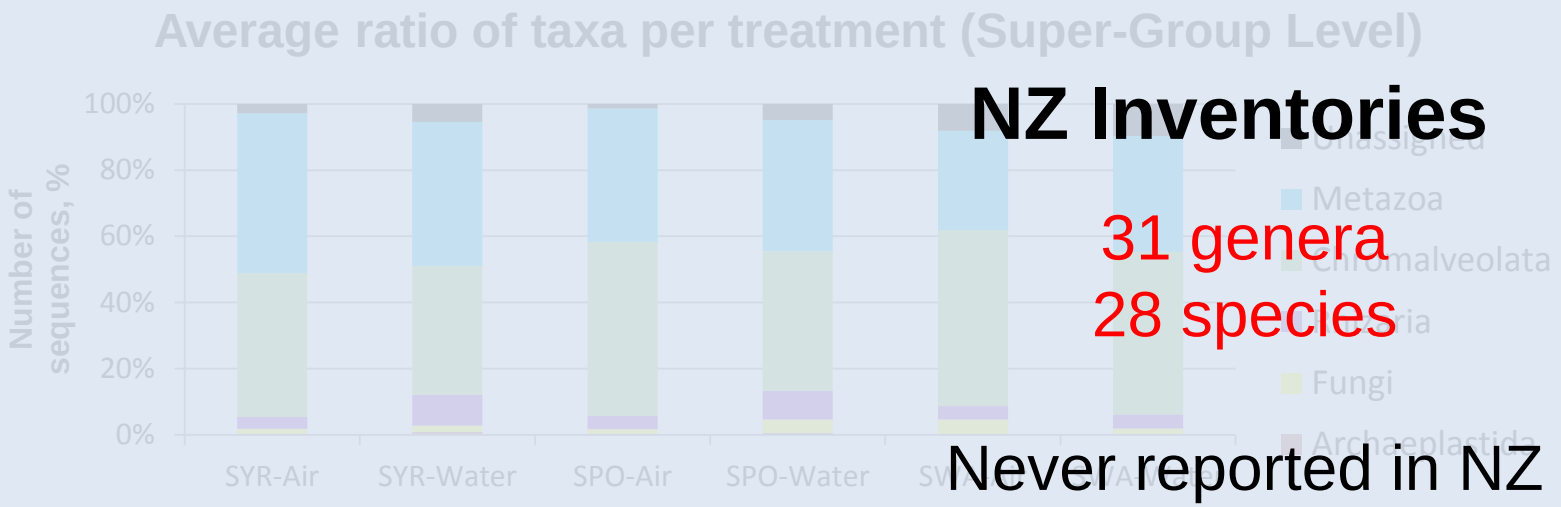
5 Supergroups
22 Phyla
42 Classes
83 Families
96 Genera
46 Species

MiSeq Illumina™ Technology => 450 Operational Taxonomic Units

No statistical differences in biodiversity
between devices nor sampling environments

NIS

Ciona savignyi



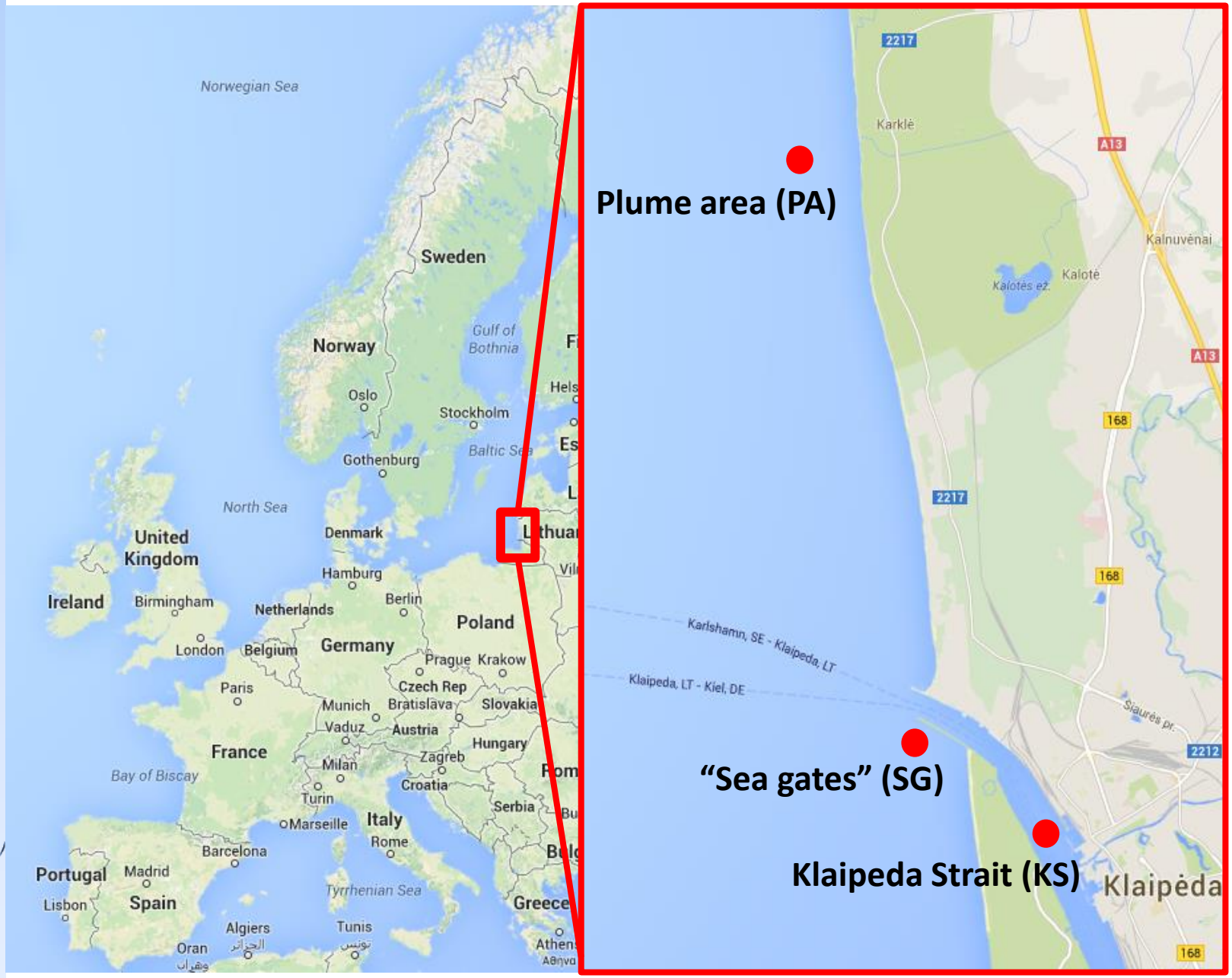
NZ Inventories

31 genera
28 species

Never reported in NZ

Pochon et al. (2015) *Biofouling* 31: 241-251

3. Early detection of NIS: plankton study



Species detected by metabarcoding

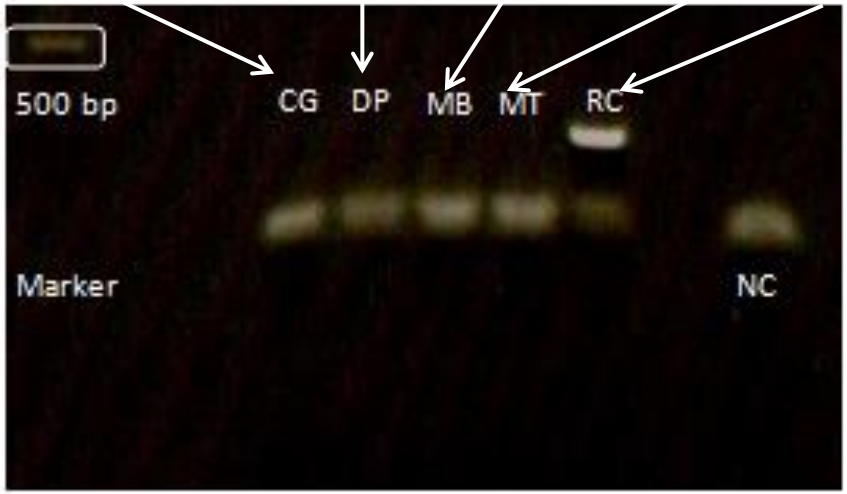
Annelida	Polychaeta	***Marenzelleria viridis***
		Marenzelleria neglecta
Crustacea	Cladocera	*Bosmina coregoni*
		Bosmina spp.
		Cercopagis pengoi
		Daphnia galeata
		Daphnia cucullata
		Evadne nordmanni
		Podon intermedius
		Podon leuckarti
	Copepoda	*Acartia tonsa*
		Eurytemora affinis
		Mesocyclops leukarti
Mollusca	Bivalva	*Dreissena polymorpha*
		Macoma balthica
		Mytilus sp.
	Gastropoda	*Hydrobia ulvae*
Rotifera	Eurotatoria	*Keratella quadrata*

>800 sequences found in all samples, aligned with high confidence (>97% identity, >95% coverage)

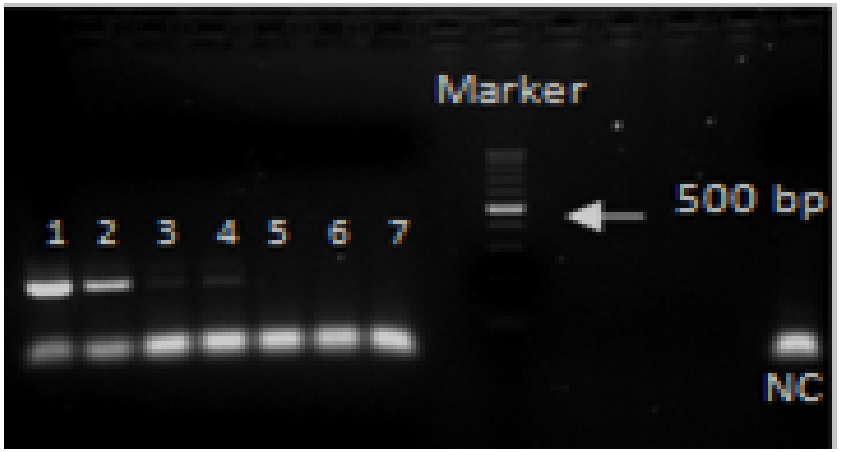
4. Detection of a target NIS from plankton

Rangia cuneata – a recent invasive species, abundant in the Vistula lagoon and with the potential to spread further

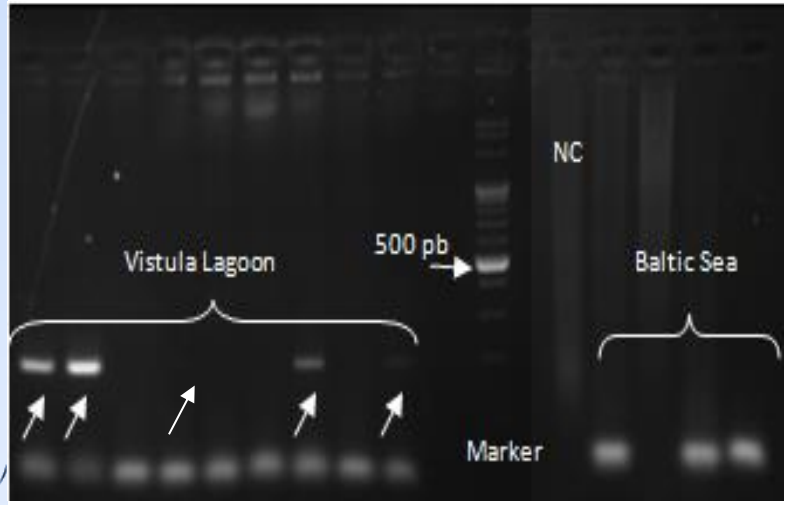
Cerastoderma Dreissena Macoma Mytilus Rangia

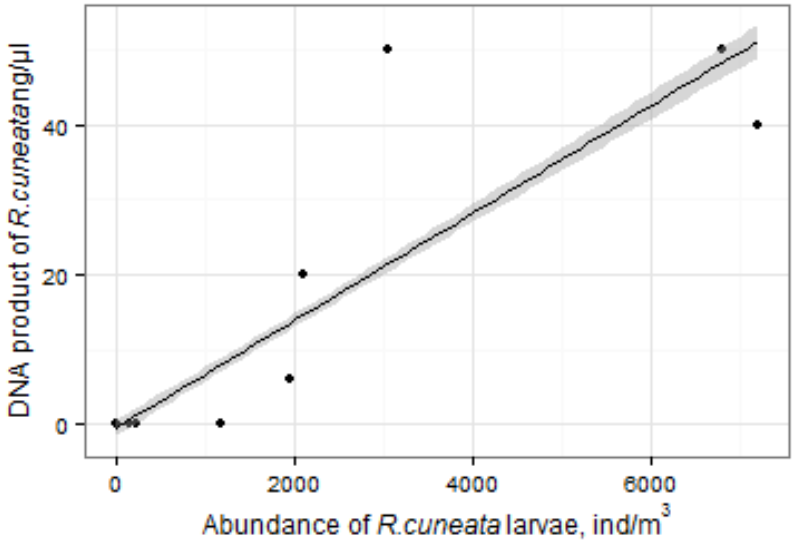


Marker sensitivity ~ 40ng/uL DNA



Location	*R. cuneata* larvae density, ind/m^3
Juodkrante	Not detected
Nida	Not detected
1	6791
3	3058
7	1168
9	1946
4	20262





Ardura et al. (2015) *Mar Environ Res,* in press

Summing up:

- Molecular techniques can assist in NIS detection and surveillance
- They may help to overcome the issues with the lack of taxonomic expertise
- They allows identification of cryptic life stages (eggs or larvae), detection of rare and sparsely distributed organisms
- Suitable for general biodiversity assessment, detection of target organisms
- May help to obtain information necessary for development of environmental quality metrics or pressure indicators

BUT

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

Thank you!

Collaborators: Yaisel J. Borell, Jose L. Martinez, Deni Ribicic, Julia Schmidt-Petersen, Anna Semenova, Jonathan Banks