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Innovative Training Network

24th of October, 2017

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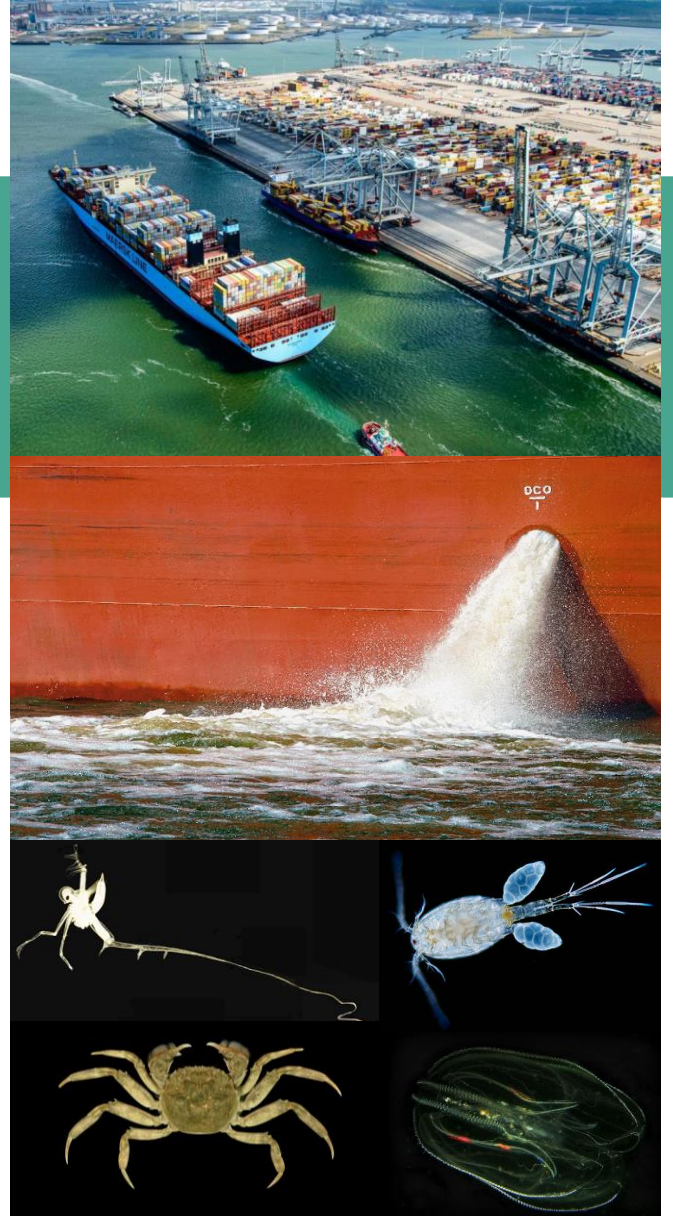
Implementing DNA metabarcoding as cost-effective tool to provide biological data for port baseline survey



Presented by : Anaïs Rey (Ph.D student)
Supervised by : Dr Naiara Rodríguez-Ezpeleta and Dr Oihane C. Basurko

20th International Conference on Aquatic Invasive Species

Introduction: Non-indigenous Species introduced with ballast water into ports

- Shipping carries about **90% of world trade**

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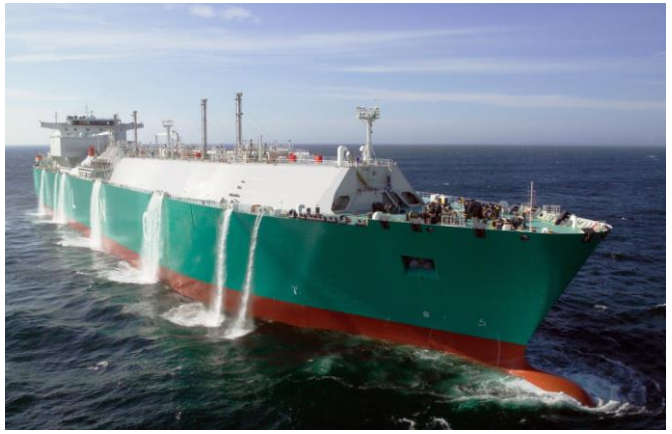
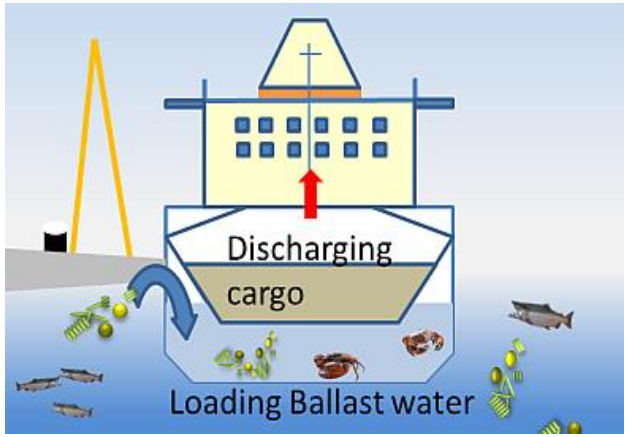
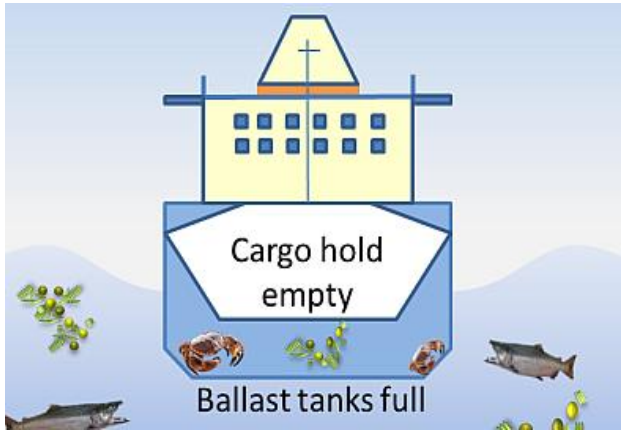


- Shipping carries about **90% of world trade**
- **10 billon tones of ballast water** are moved globally each year
- **7,000 species** are daily transferred around the world with ballast water

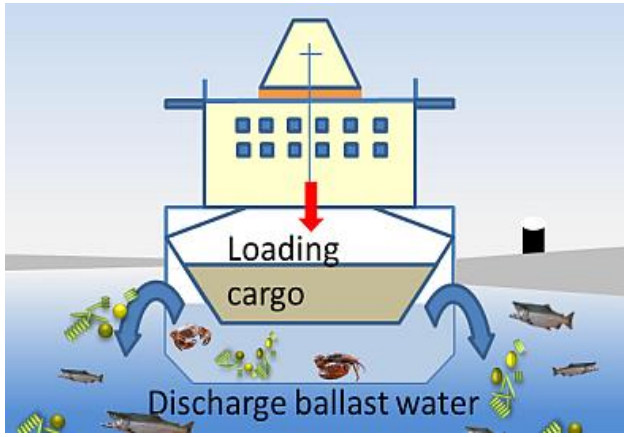
At source port



During voyage

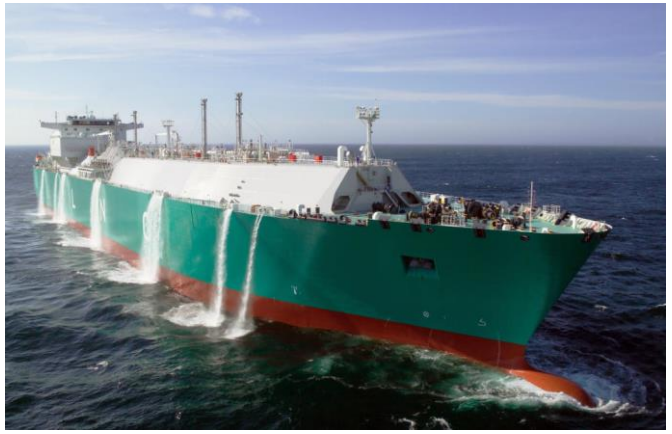


At recipient port



https://www.nioz.nl/ballastwater-en

Introduction: Non-indigenous Species introduced with ballast water into ports



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- **10 billon tones of ballast water** are moved globally each year
- **7,000 species** are daily transferred around the world with ballast water
- Major vector of **introduction of Non-Indigenous Species, pathogens and harmful algae**

Economical impact

Zebra mussel (*Dreissena polymorpha*)

- Costs for management $500 millions/year in Great Lakes

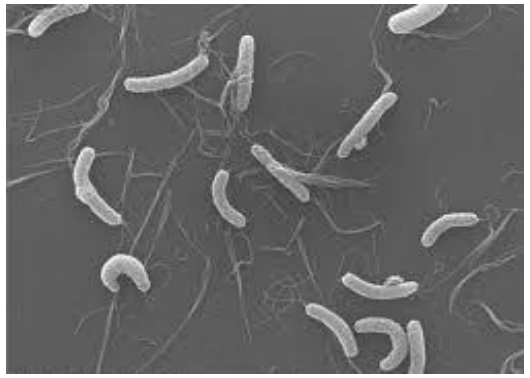
Environmental impact

Chinese crab (*Eriocheir sinensis*)

- Food web altered
- Native biodiversity reduced
- Physical disturbances

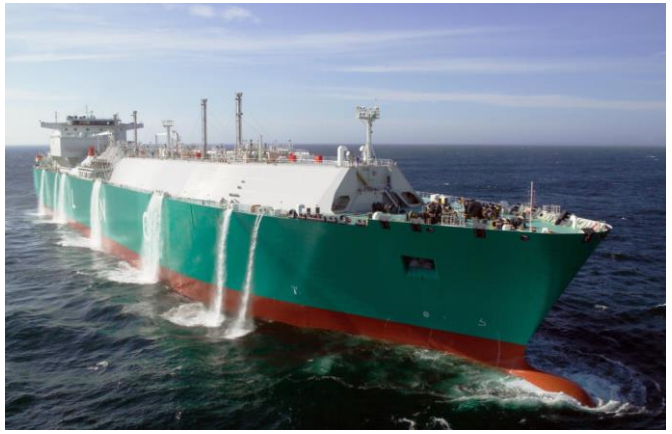
Social impact

Cholera (*Vibrio cholera*)



- Human disease

Introduction: Non-indigenous Species introduced with ballast water into ports



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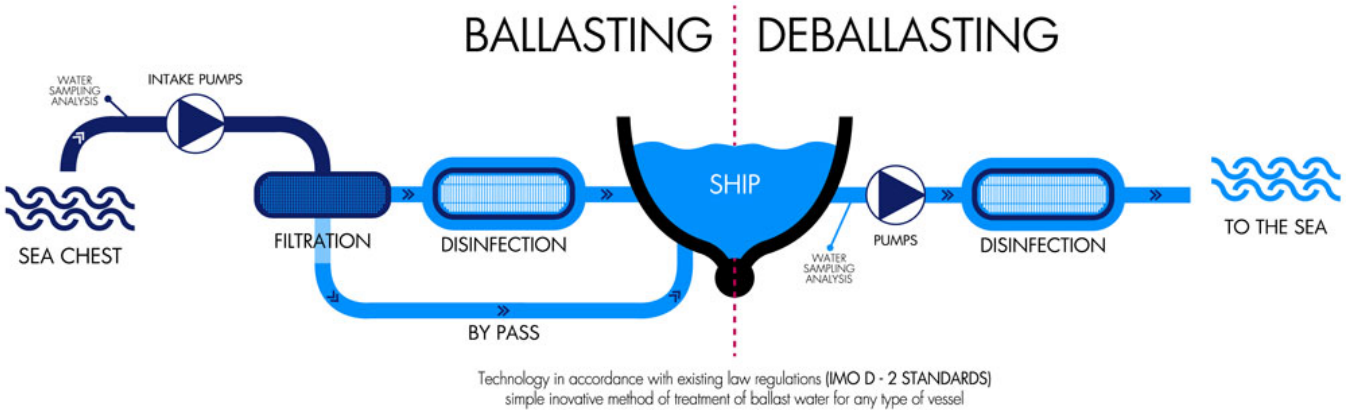
- To prevent further biological invasions, **the Ballast Water Management Convention has been established and is currently into force**

IMO
Ballast Water Management Convention

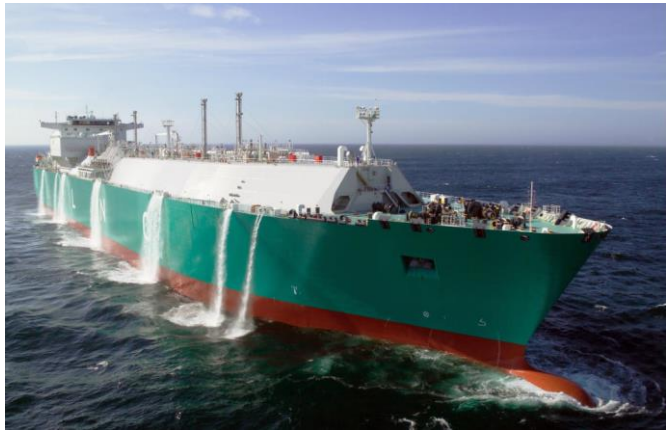
All ships in international travel must manage their ballast waters and sediments:

- Ballast Water Exchange in open sea
- Ballast Water Treatment Systems



Technology in accordance with existing law regulations (IMO D - 2 STANDARDS)
simple inovative method of treatment of ballast water for any type of vessel

Introduction: Non-indigenous Species introduced with ballast water into ports



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Ballast water monitoring needed:
Developing tools that will provide biological data to assess the compliance to the Ballast Water Management Convention

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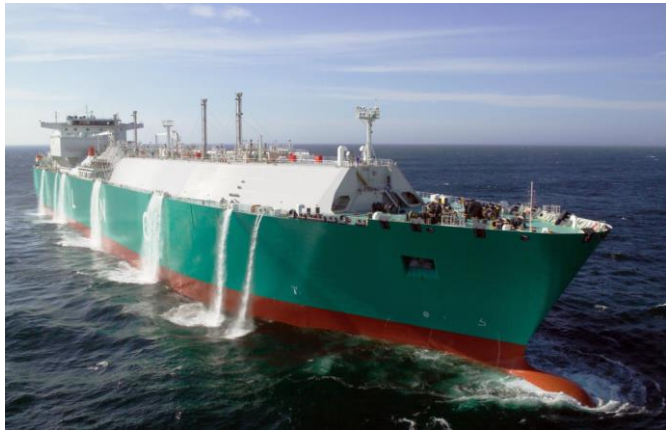

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Focus on DNA-based detection of species as a solution:

Assessing the usefulness of genetic tools for ballast water and ports monitoring

Contents lists available at ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

The challenges and promises of genetic approaches for ballast water management

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AZTI, Marine Research Division, Sukarrieta, Txatxarramendi ugartea z/g, 48395 Sukarrieta, Bizkaia, Spain

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Introduction: Port baseline surveys

Monitoring applications extracted from Guidelines

✓ **Risk assessment for granting exemptions: port baseline survey & target species detection**

Example:

Shipowner looking an exemption for the specific voyage Rotterdam (Netherland) and Le Havre (France)

↳ Ask for an exemption

↳ **Scientific Risk Assessment**

→ **Is there a risk of harmful organisms introduction ?**

↳ Risk

Low | Medium | High

Biological data from ports are needed:

✓ **Native species**

✓ **Non-Indigenous species**

Exemption granted ← **Further study** → **Exemption declined**

Objectives

To ease complex and expensive port baseline surveys

By developing of a protocol based on the use of DNA metabarcoding

- The taxonomic assignment of individuals from an environmental sample based on their DNA sequences

Where specific objectives will be assessed:

❖ **Reliability of the DNA metabarcoding port baseline survey to**:

- Detect Non-Indigeneous Species
- Retrieve the biological communities of the port

❖ **The potential of using environmental DNA to retrieve similar communities**

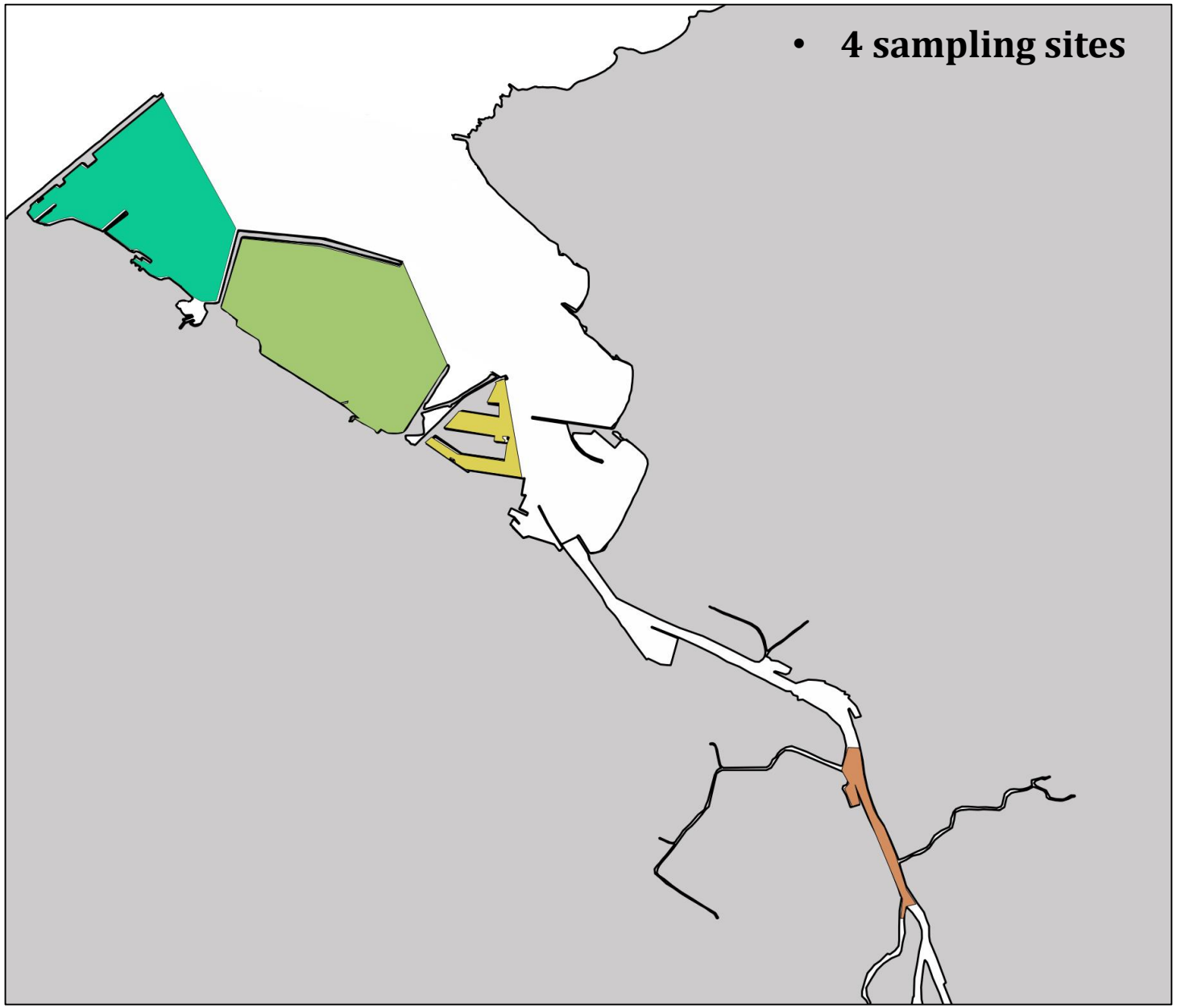
❖ **The importance of seasonal sampling**

Materials and methods: The port studied

→ Port of Bilbao, open on the Atlantic ocean (Bay of Biscay), Northern Spain, Europe

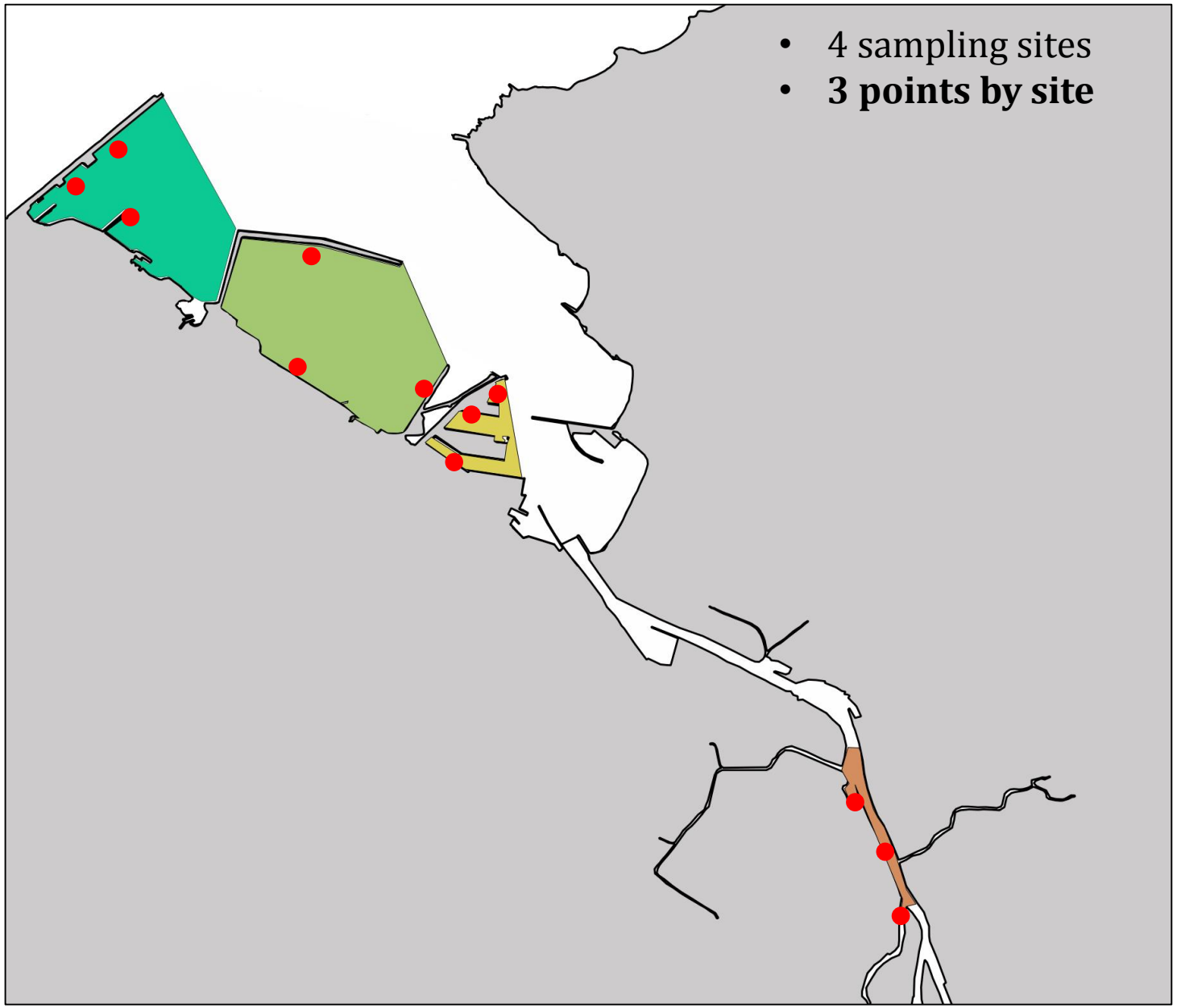
http://www.breakbulk.com/bilbao-port-traffic-rises

Materials and methods: The sampling design



4 Sampling periods:
Autumn 2016
Winter 2017,
Spring 2017
Summer 2017

Materials and methods: The sampling design



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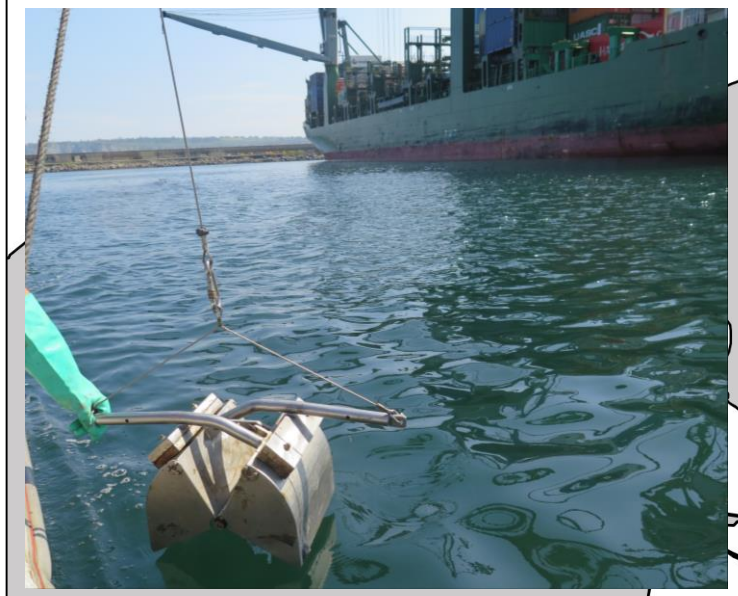

- **Sediment**

Target: benthic macrofaunal

4 Sampling periods:
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Summer 2017

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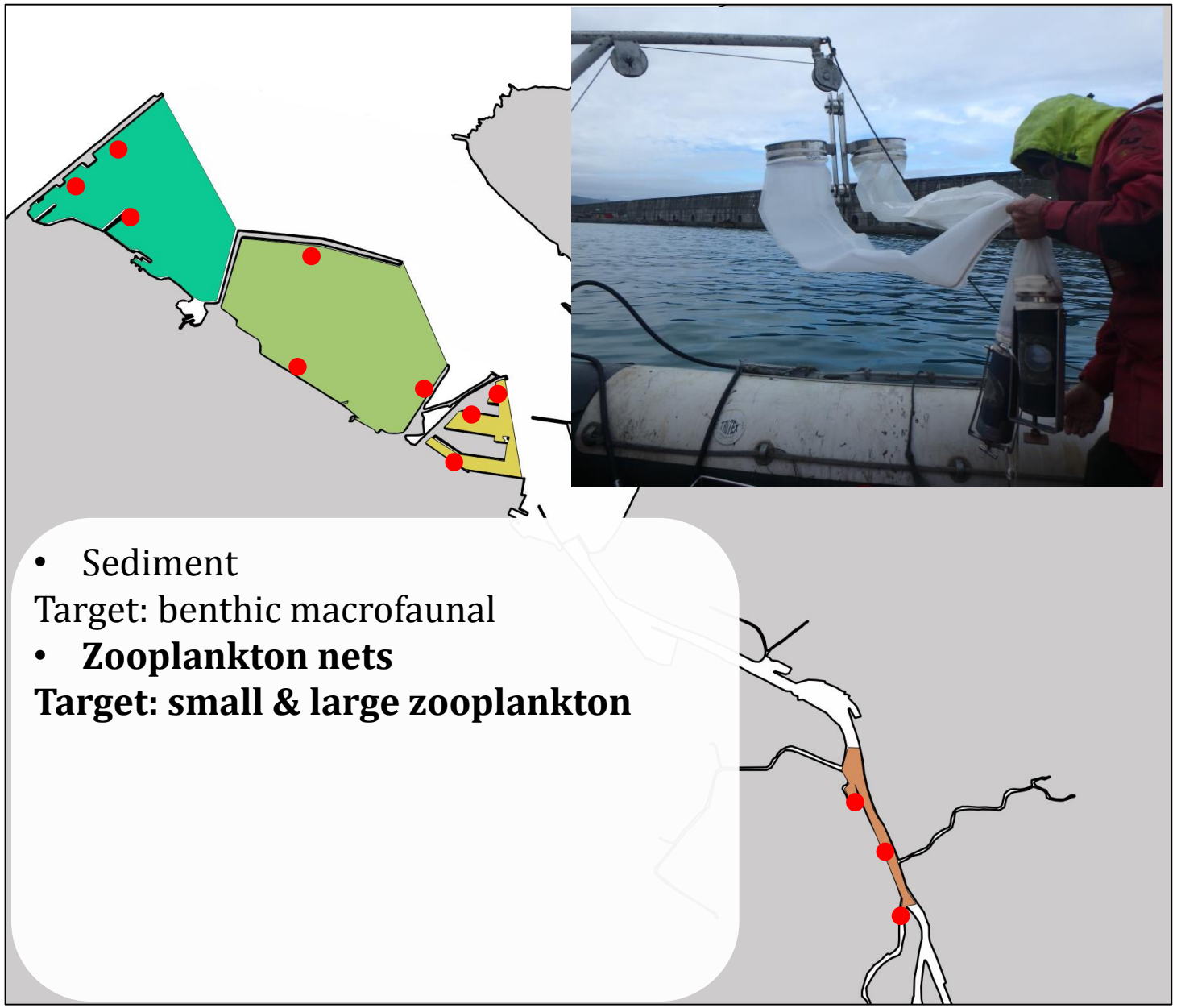
Materials and methods: The sampling design



- Sediment

Target: benthic macrofaunal

- **Zooplankton nets**

Target: small & large zooplankton

4 Sampling periods:
Autumn 2016
Winter 2017,
Spring 2017
Summer 2017

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Materials and methods: The sampling design

- Sediment

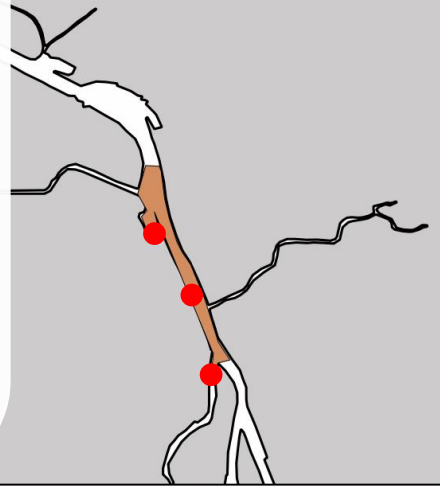
Target: benthic macrofaunal

- Zooplankton nets

Target: small & large zooplankton

- **Scrapping surfaces & PVC plates:**

Target: fouling organisms



2 Sampling periods:
Winter to Spring 2017,
Spring to Summer 2017

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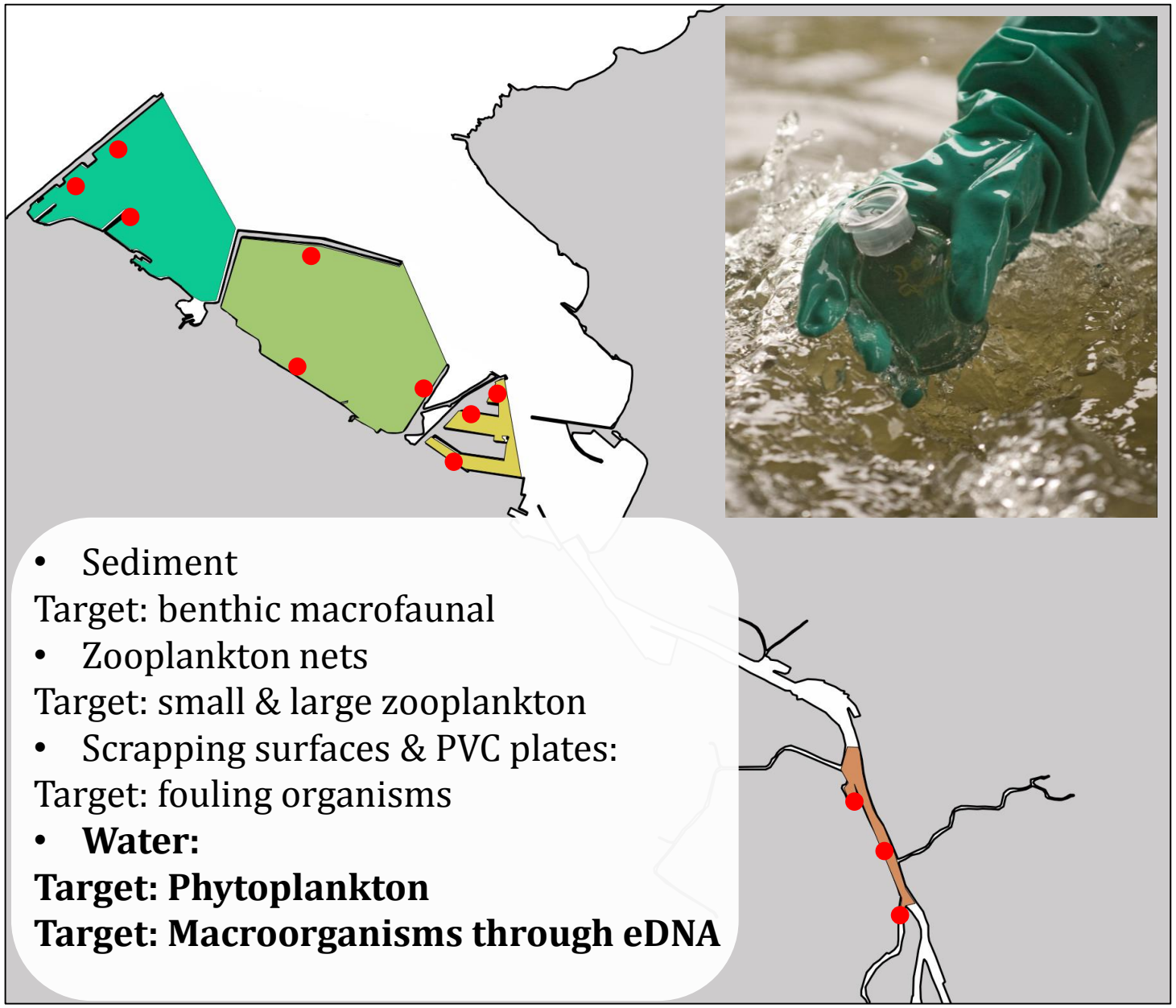
Materials and methods: The sampling design



- Sediment

Target: benthic macrofaunal

- Zooplankton nets

Target: small & large zooplankton

- Scrapping surfaces & PVC plates:

Target: fouling organisms

- **Water:**

Target: Phytoplankton

Target: Macroorganisms through eDNA

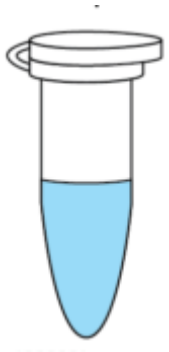
4 Sampling periods:
Autumn 2016
Winter 2017,
Spring 2017
Summer 2017

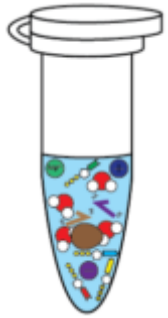
Materials and methods: The metabarcoding workflow

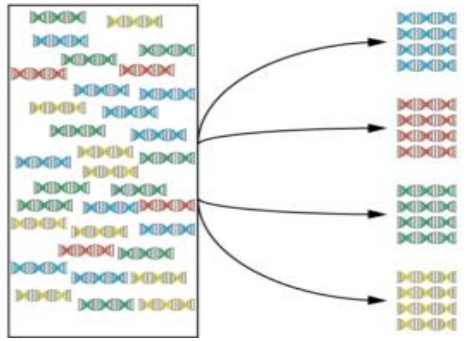
- **In total, 160 samples were processed**
- **From filtered water:**

To retrieve macroorganisms

Environmental sample → Filtration → DNA extraction → Amplification of DNA barcode: **COI** → Next-Generation Sequencing: Illumina MiSeq → Bioinformatic processing → Species identification







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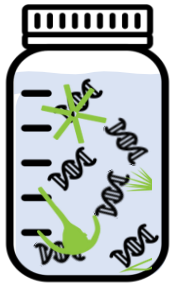
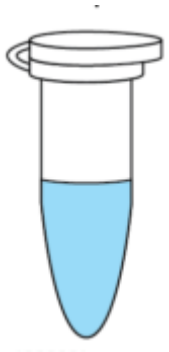


Filtration

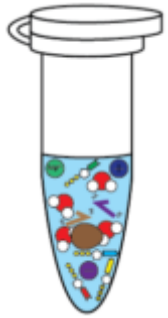
DNA extraction



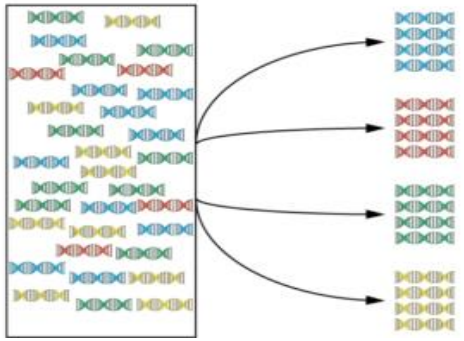
To retrieve macroorganisms

Amplification of DNA barcode:

COI



Next-Generation Sequencing: Illumina MiSeq

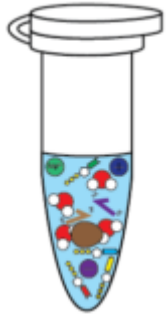


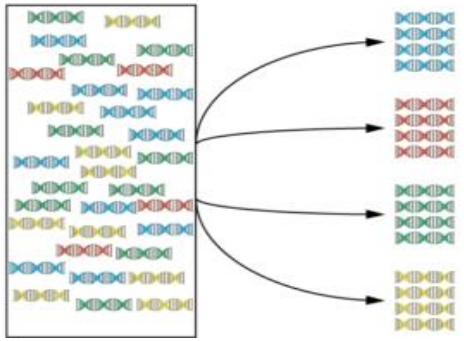
Bioinformatic processing

Species identification

To retrieve phytoplankton

18S





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Materials and methods: The metabarcoding workflow

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To retrieve benthic macrofaunal

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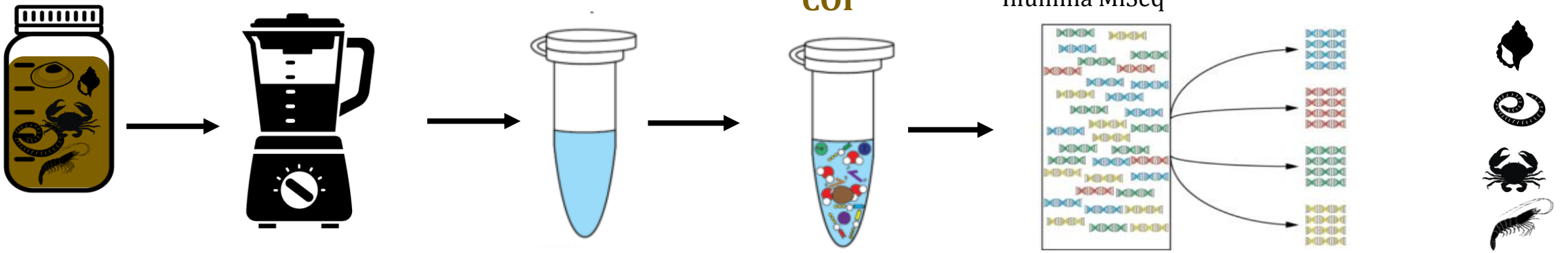

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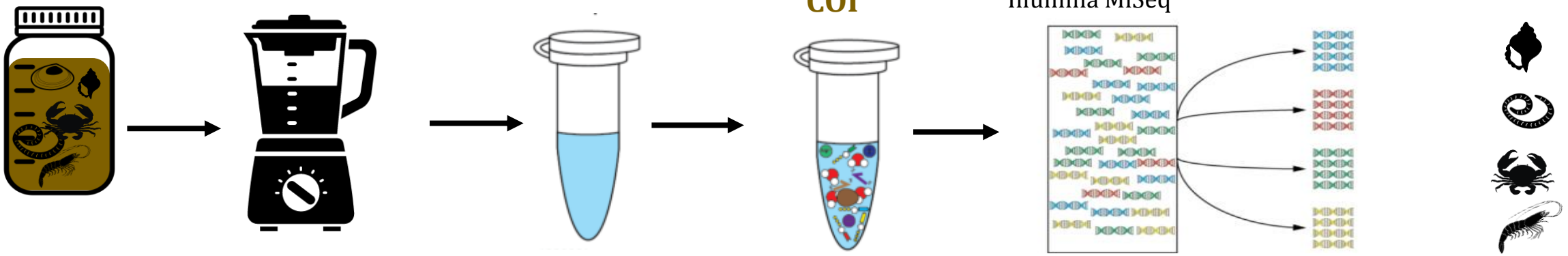
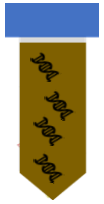
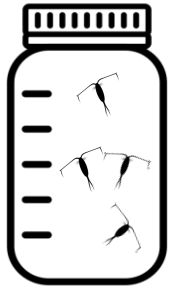




To retrieve benthic macrofaunal: COI
To retrieve harmful algae species: 18S

To retrieve fouling organisms: COI & 18S

To retrieve zooplankton: COI and 18S



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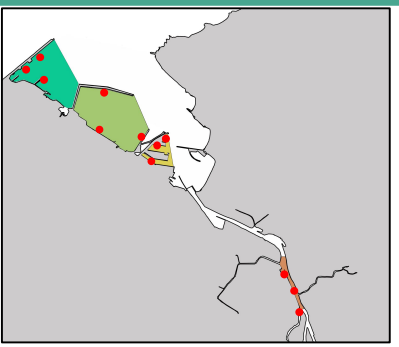
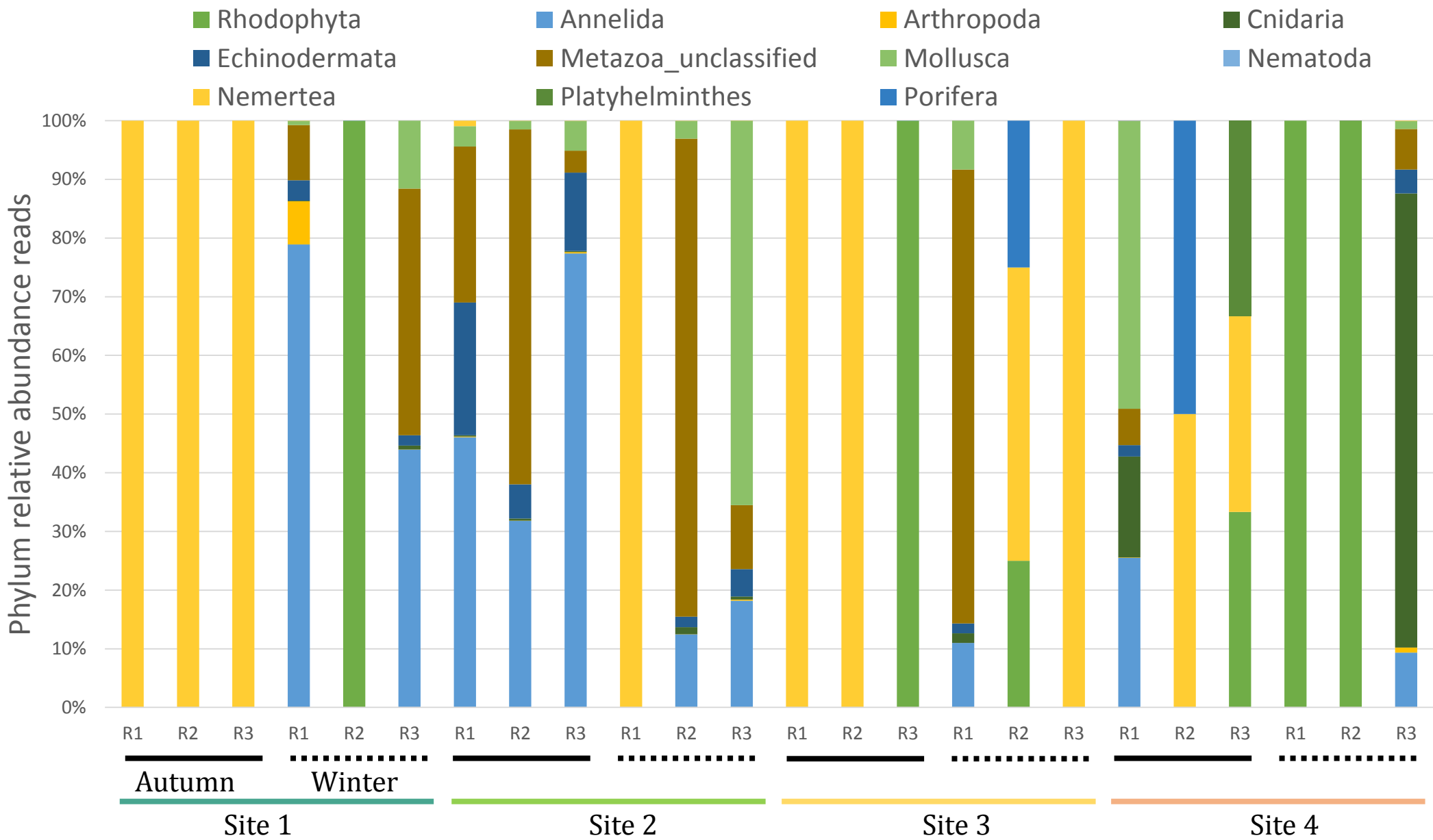
Preliminary results for the benthic macrofauna sampling:



Autumn and Winter periods :



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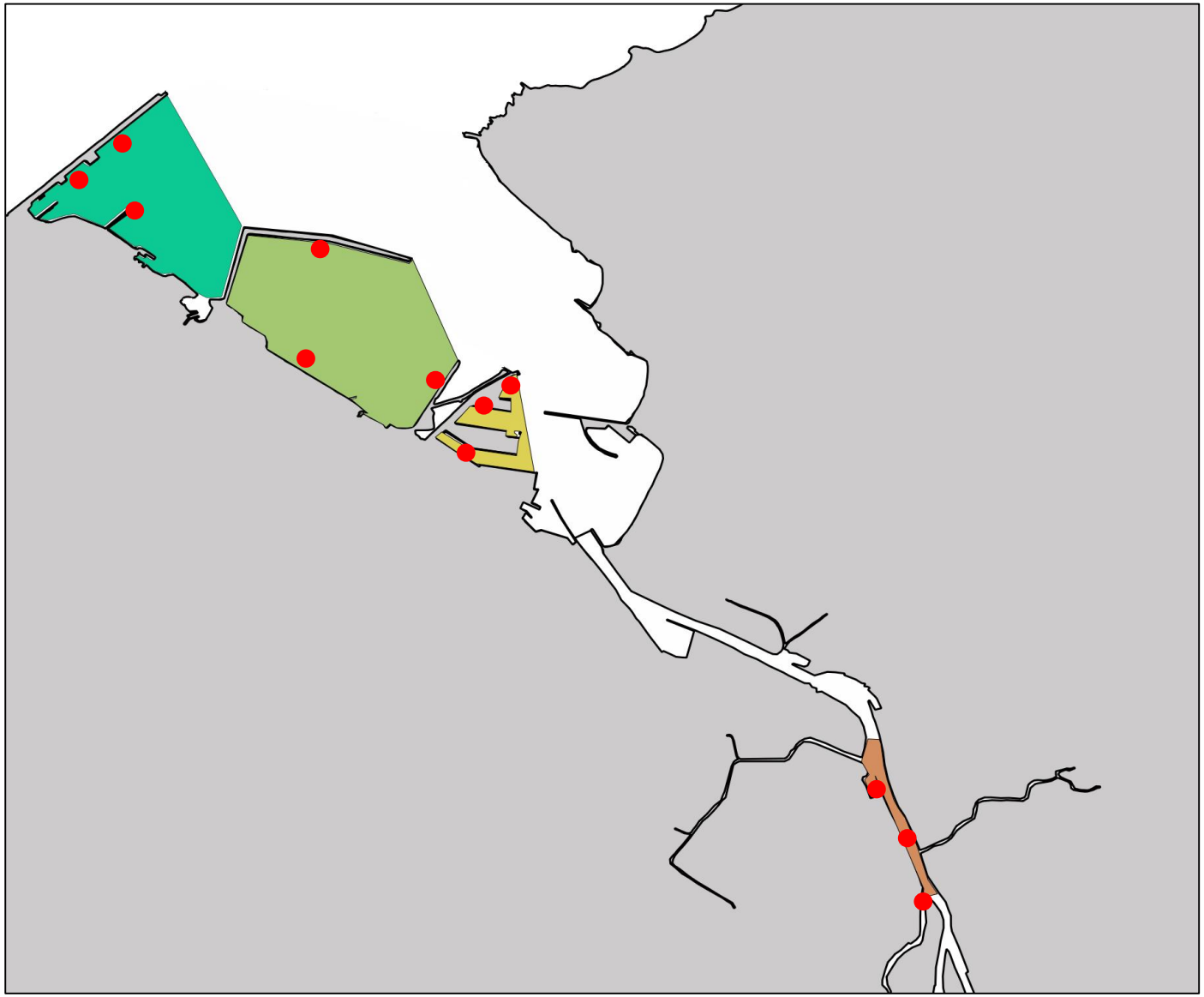
Preliminary results for the benthic macrofauna sampling:

Total OTU α-Diversity : 104

- **Autumn period :** 88 (84%)
- **Winter period :** 78 (75%)
- **Shared OTU between 2 periods :** 62 (60%)

OTU identified to :

- **Species level→** 73 (70 %)

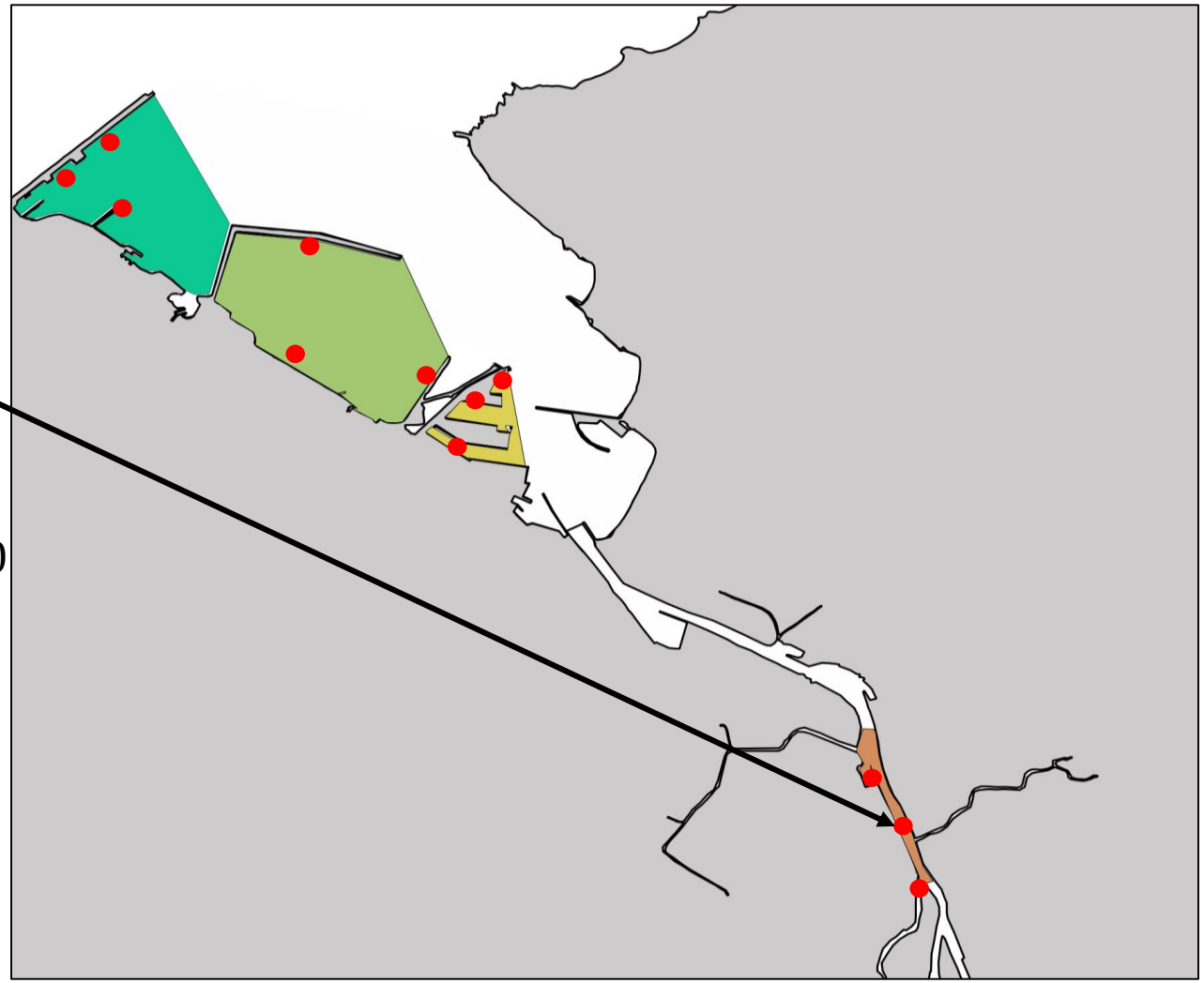


Preliminary results for the benthic macrofauna sampling:

Non-Indigenous Species detected in the benthic macrofauna communities:

Black-pygmy mussel (*Xenostrobus securis)*

- First report in 2010
- Native from Japan
- Likely maritime transport



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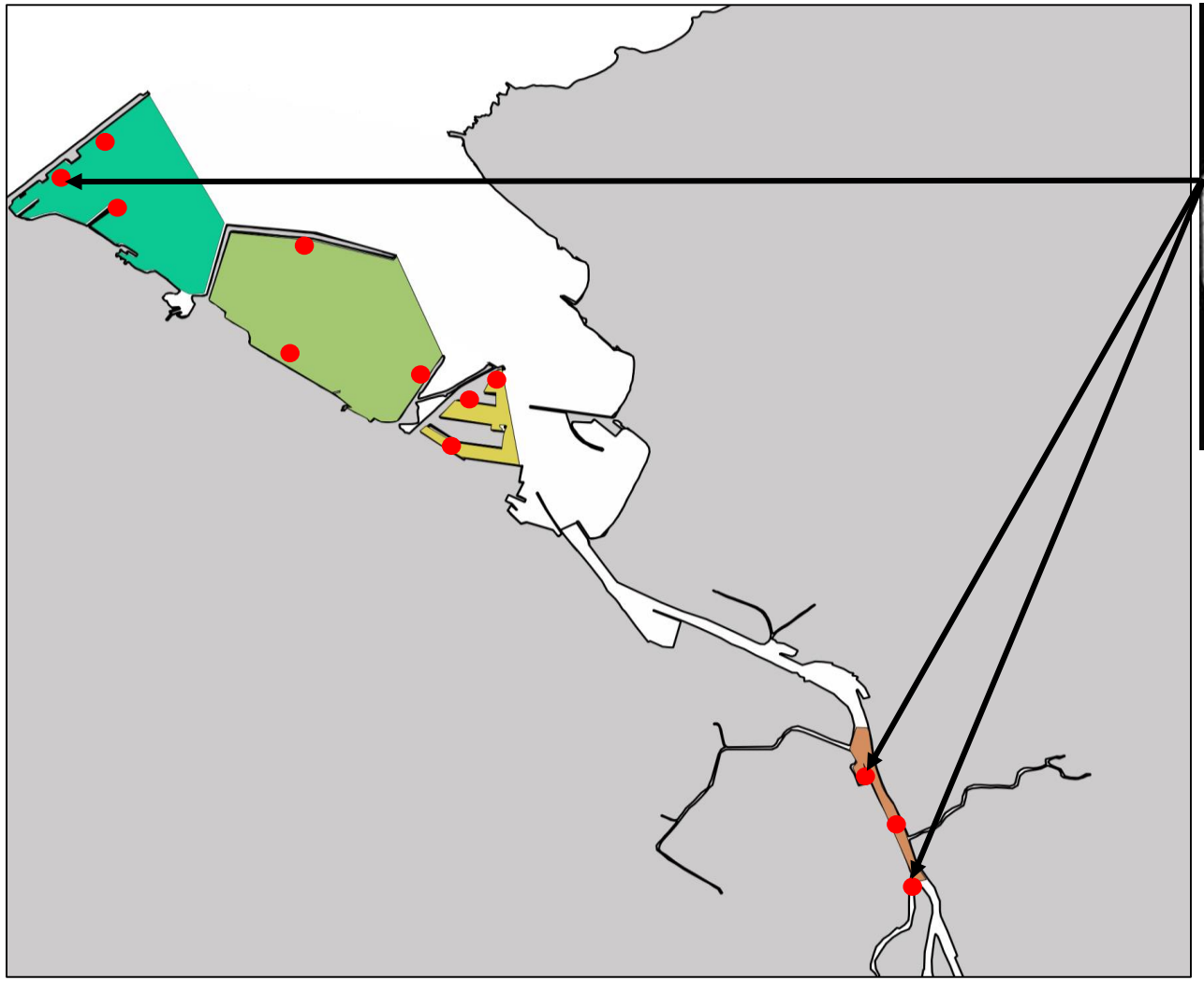

Manila clam *(Ruditapes philippinarum)*

- First report 2005
- Native from Pacific coast of Asia
- Introduced with aquaculture in Europe

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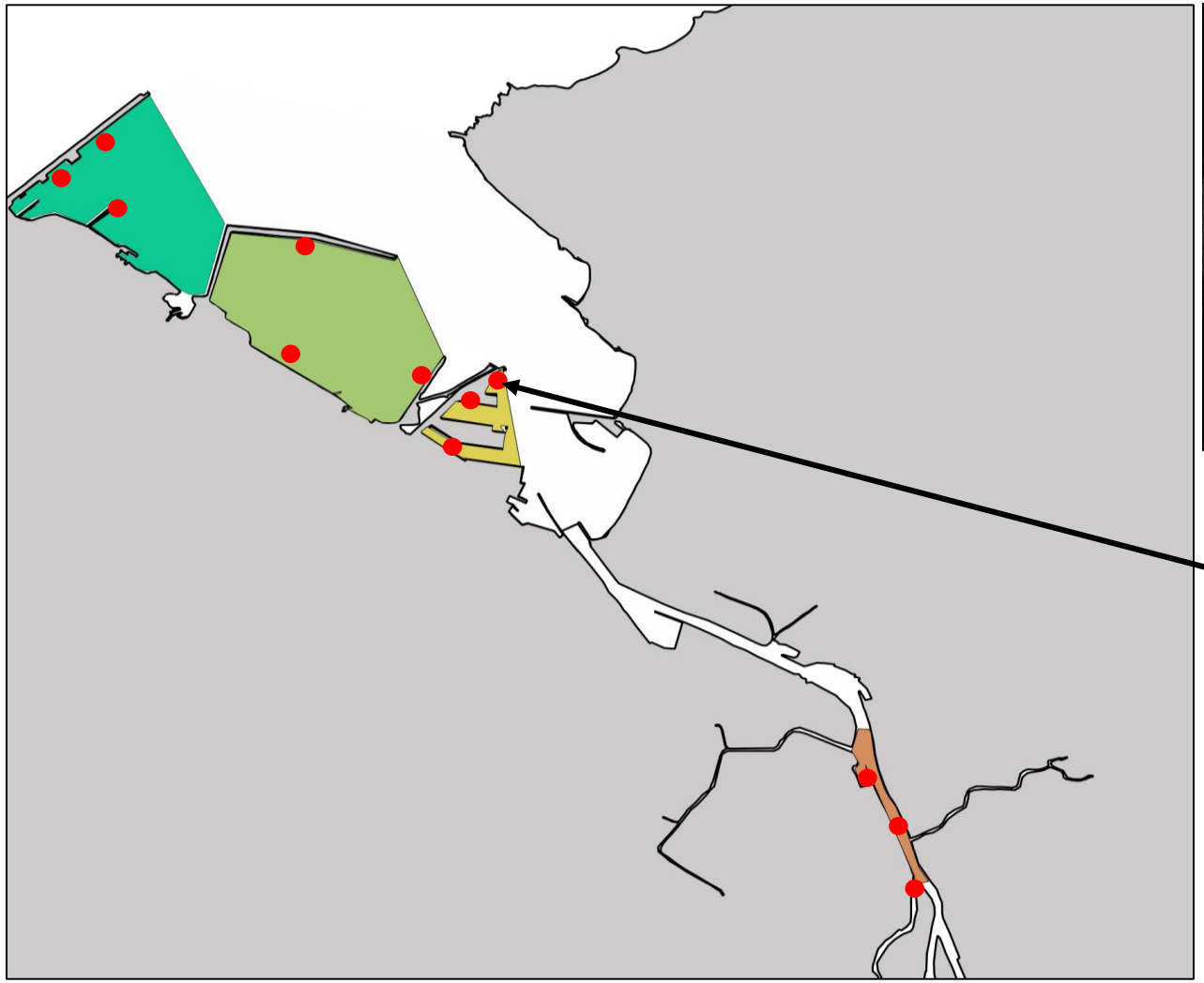


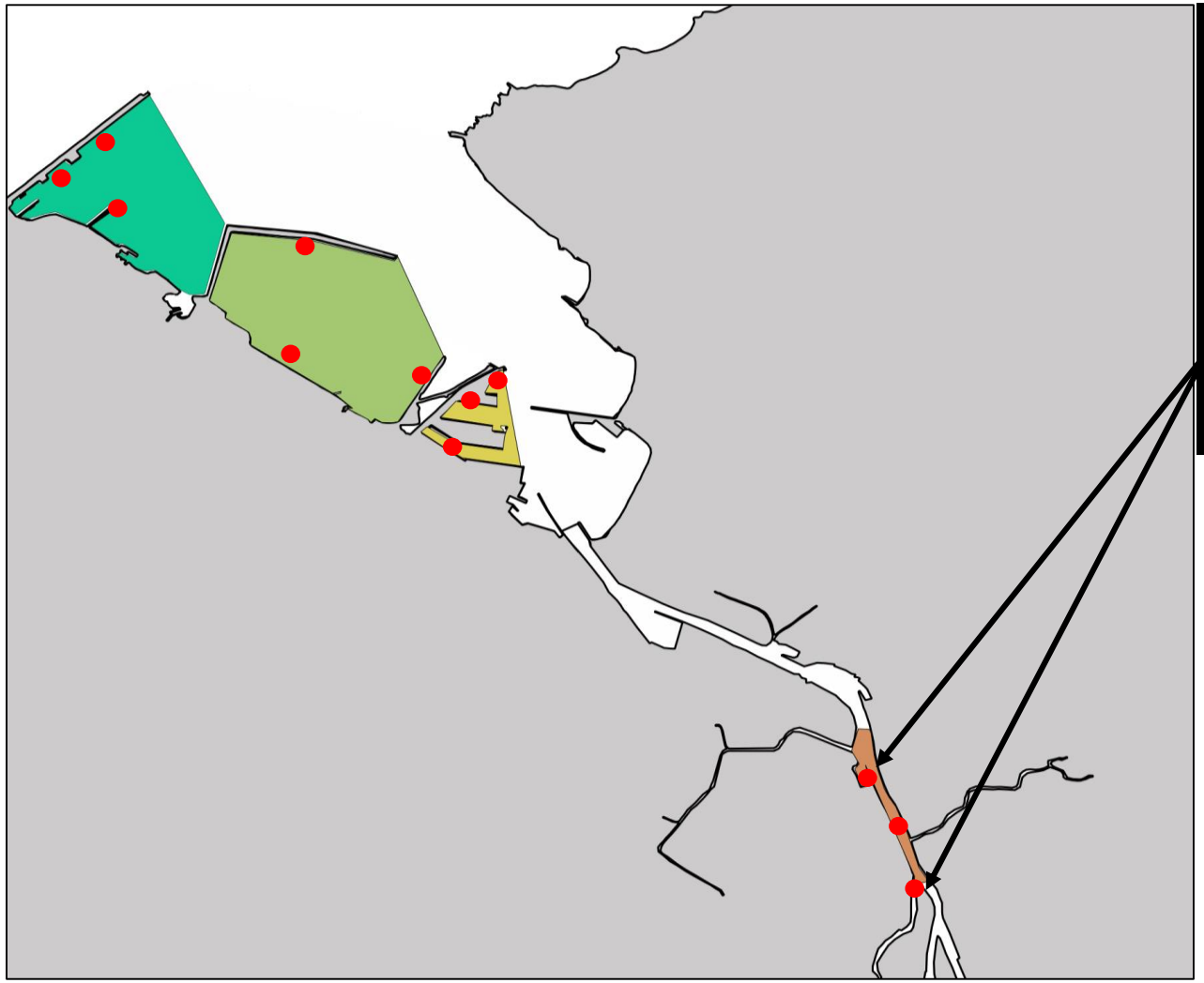
Manila clam *(Ruditapes philippinarum)*

Amphipoda *(Grandidierella japónica)*

- FIRST RECORD in Northern Spain
- Native from Japan
- Likely maritime transport

Preliminary results for the benthic macrofauna sampling:

Non-benthic macrofauna Non-Indigenous species recovered with the sampling:



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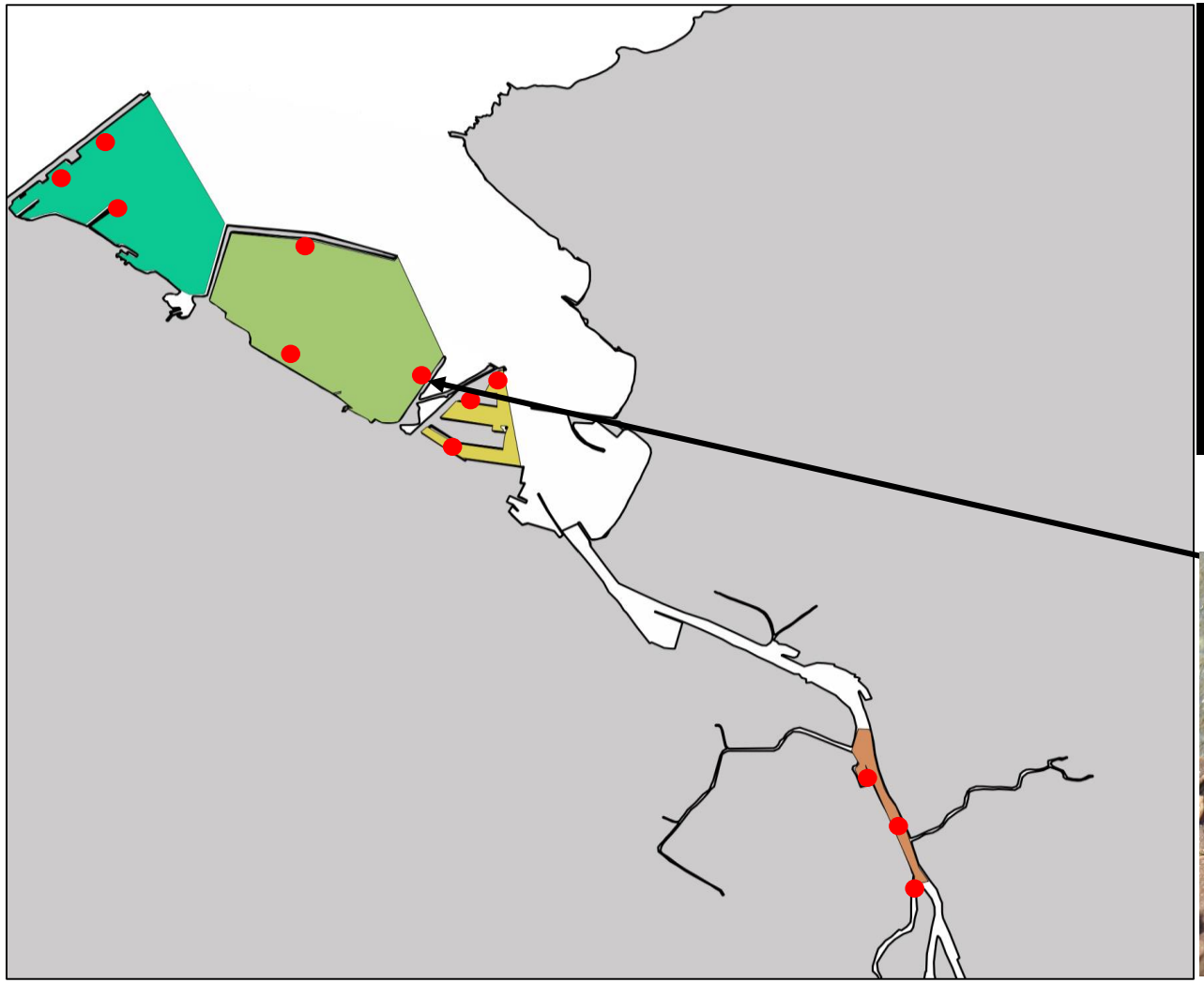

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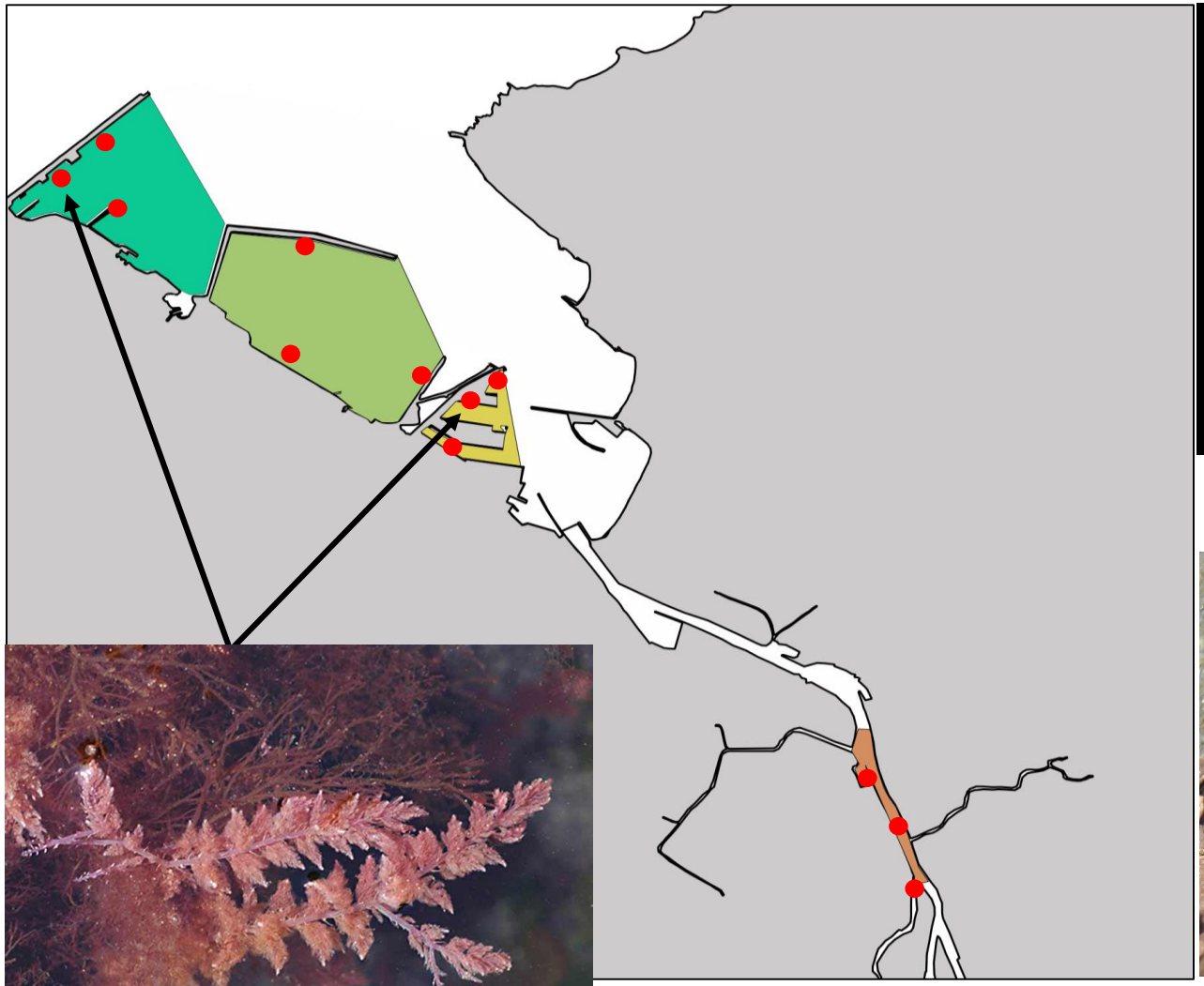

DISCUSSION & CONCLUSION:

What have we learned so far?

- First results suggest DNA metabarcoding as cost-effective tool to provide biological data for port baseline survey
- Good species level recovery in general
- Seasonal sampling is needed
- Some samples are dominated by one phylum → Representativity?

DISCUSSION & CONCLUSION:

What have we learned so far?

- First results suggest DNA metabarcoding as cost-effective tool to provide biological data for port baseline survey
- Good species level recovery in general
- Seasonal sampling is needed
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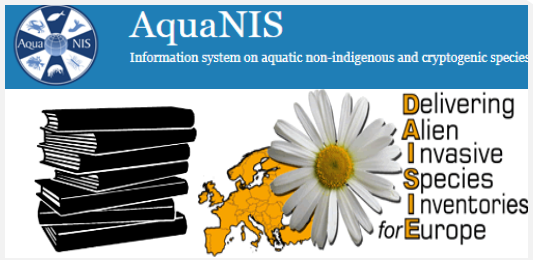
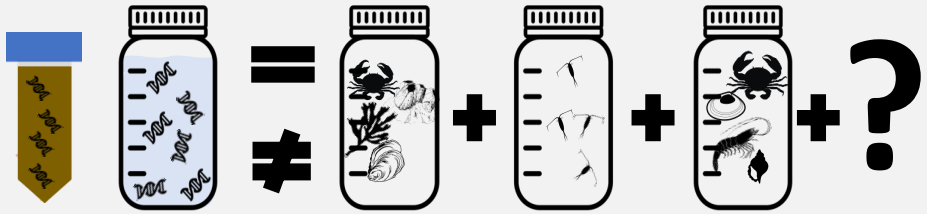
What is next?

- Work in progress 

- Comparison of the DNA metabarcoding port baseline survey with previous monitoring surveys and Non Indigenous Species databases



- Assessment of environmental DNA (from water and sediment) to retrieve the community of the biodiversity components



- Cost and time analysis 

- Propose the next steps to optimize:
- Barcoding of Non-Indigenous Species?
- Further benchmarking of metabarcoding protocols to minimize overlooking taxa?

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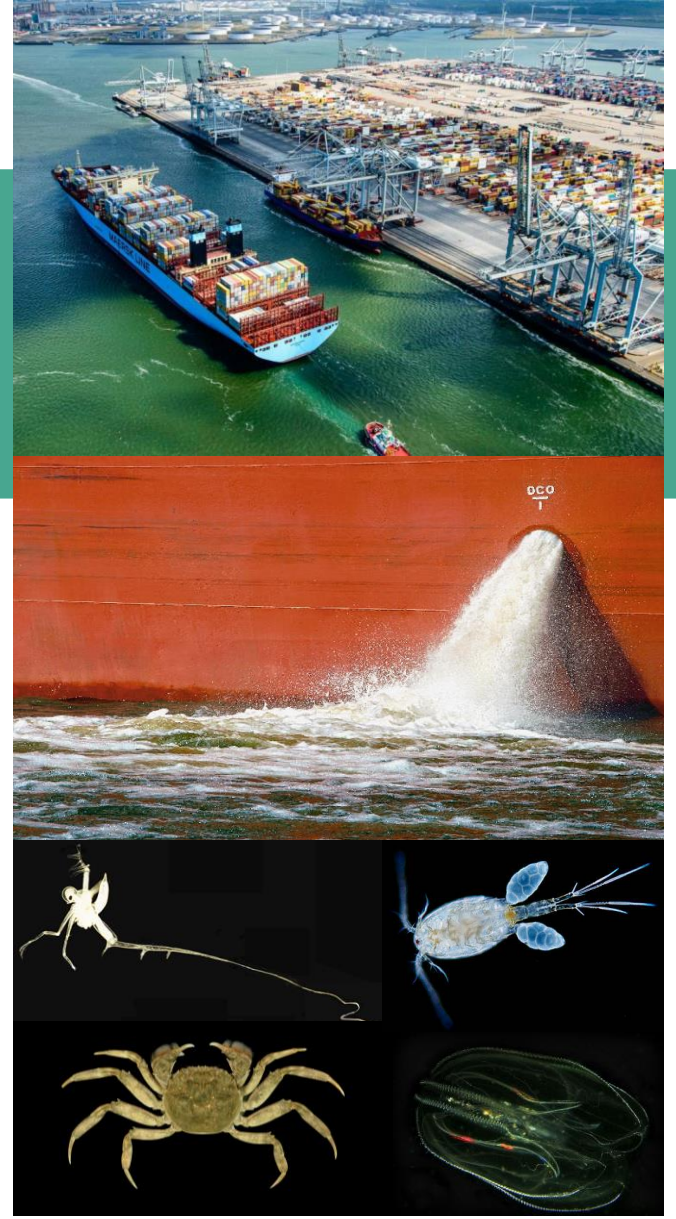
Thank you for your attention!

Questions?



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Introduction: Making the link between ballast water monitoring & genetic tools

Methodology: Review of the literature, guidelines and regulations of the BWM Convention

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The challenges and promises of genetic approaches for ballast water management

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Overview

- **36 studies in ballast water and ports which targeted:**

Pathogens, Bacteria, Protists
Zooplankton, Phytoplankton
Invertebrates in sediment

- **Approaches:**

Targeted species and biodiversity assessment surveillance

Monitoring applications extracted from Guidelines

- Test the efficacy of ballast water management
- Assess the alive biodiversity discharged via ballast water
- Risk assessment for granting exemptions: port baseline survey & target species detection

Future investments before possible implementation

- Standardization
- Increasing portability of genetic tools
- Improvement of reference databases
- Exploring alternative sampling strategies (environmental DNA)