

Sampling Approaches and Designs Using Environmental DNA & RNA for Marine Biosecurity Surveillance

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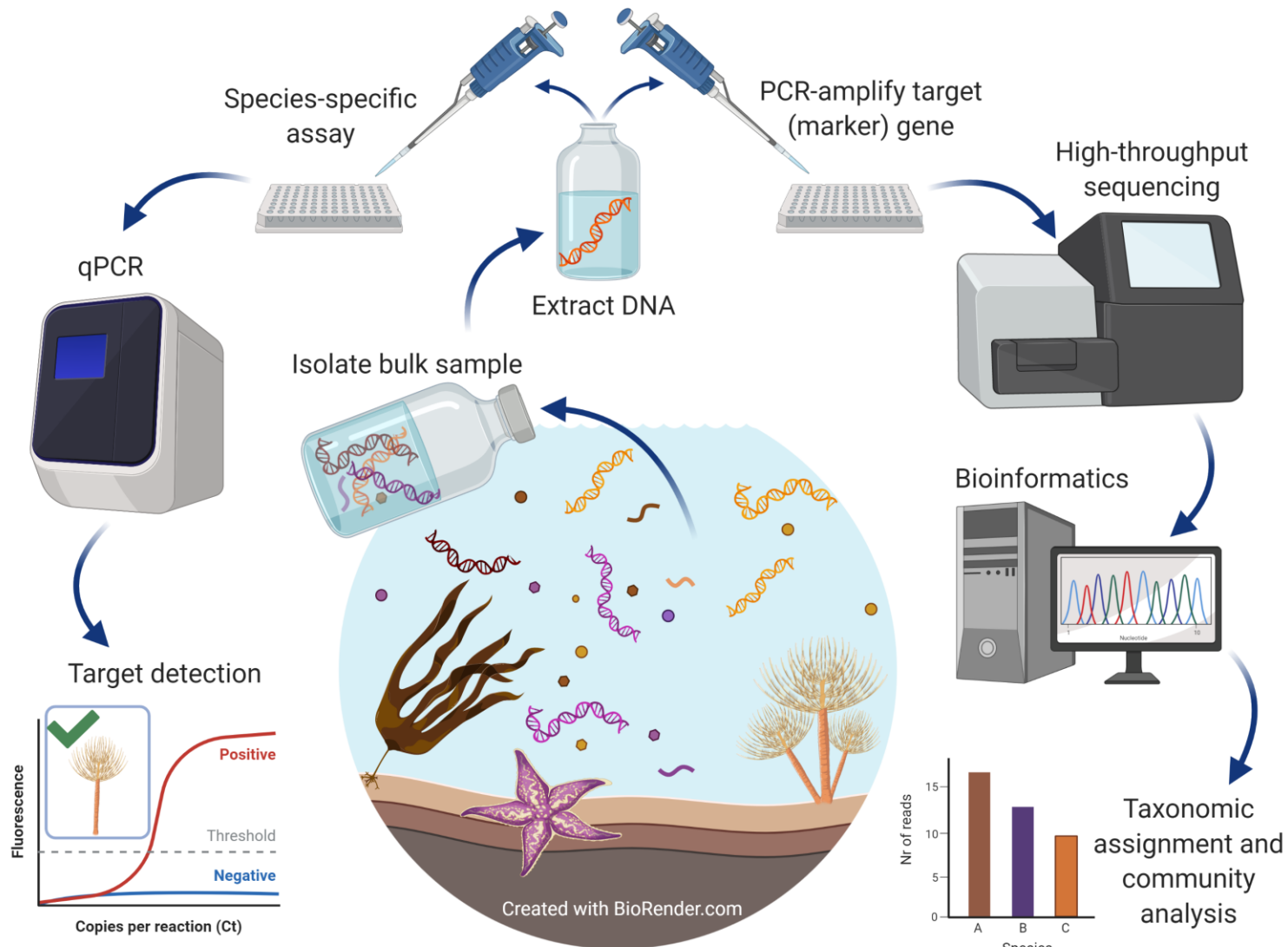
³ *Sequench Ltd, Nelson, New Zealand*

⁴ *Department of Anatomy, University of Otago, Dunedin, New Zealand*



MARINE BIOSECURITY TOOLBOX

Kia tirotiro mangōpare, arā ko ngā tai e whā
Look through the eyes of the mangōpare,
observing in all directions



PROTECT



DETECT



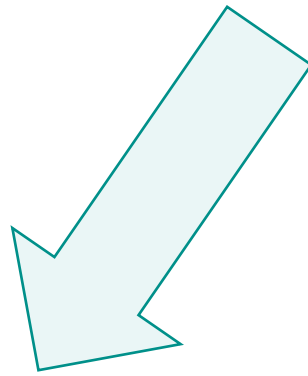
MANAGE &
RESPOND



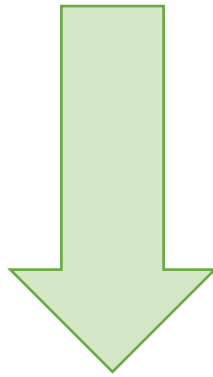
ECONOMICS &
DECISION-SUPPORT



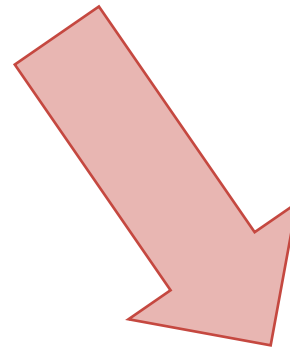
Optimise eDNA/eRNA sampling approaches and designs for marine biosecurity applications



How

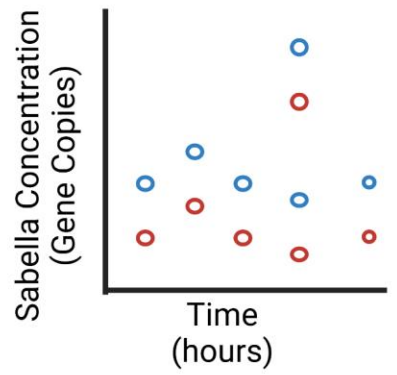


Where

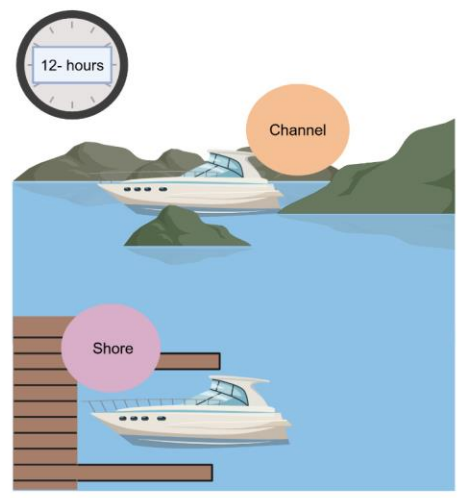


When

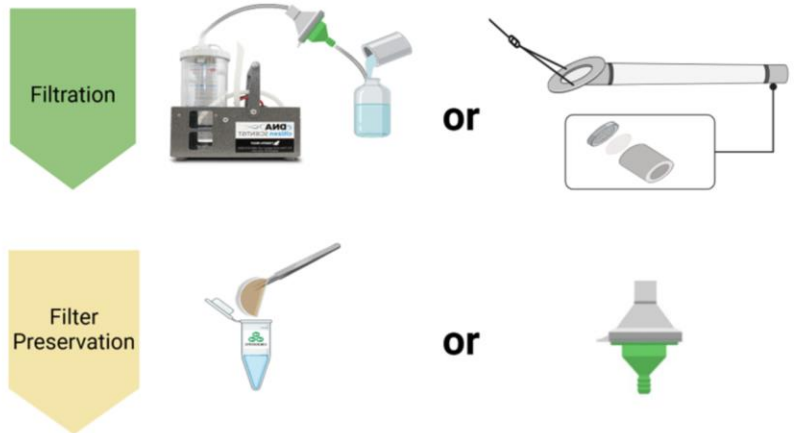
In Situ Dynamics



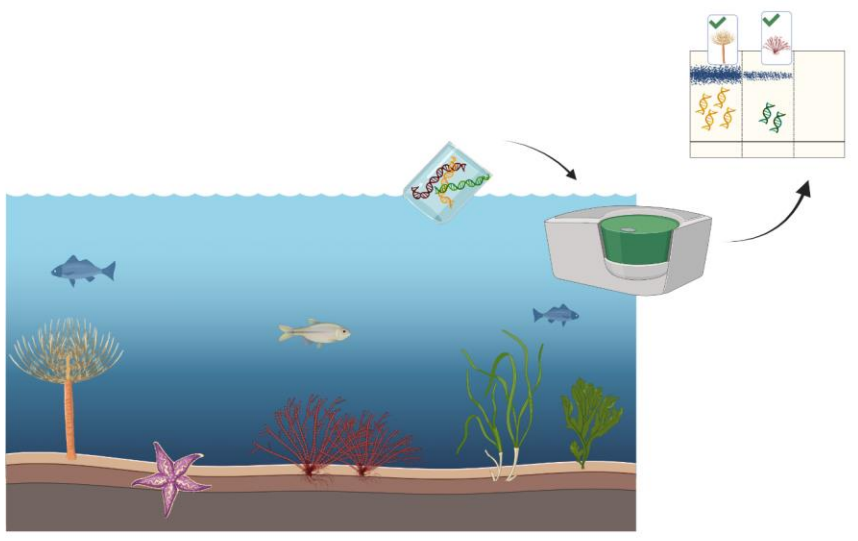
Distribution & Dispersal



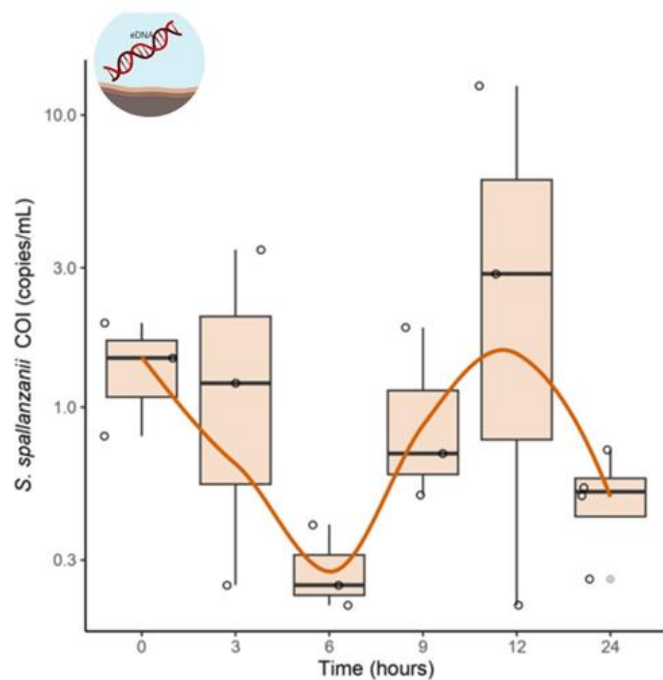
Optimal Sampling Workflows



Innovative Sampling Methods



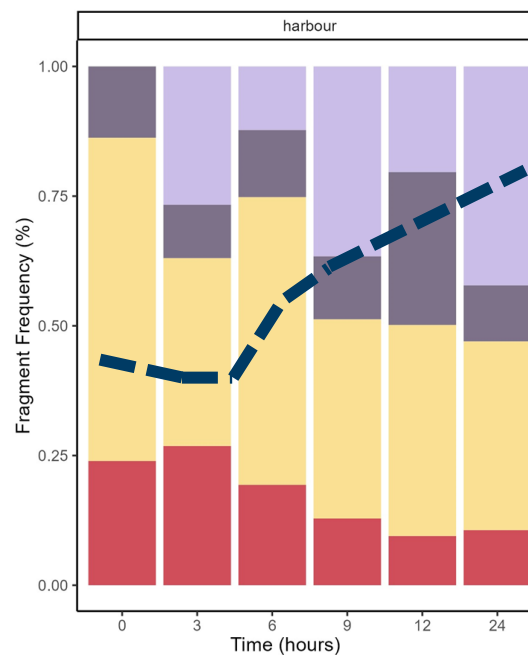
In Situ Dynamics



When: In-Situ Dynamics

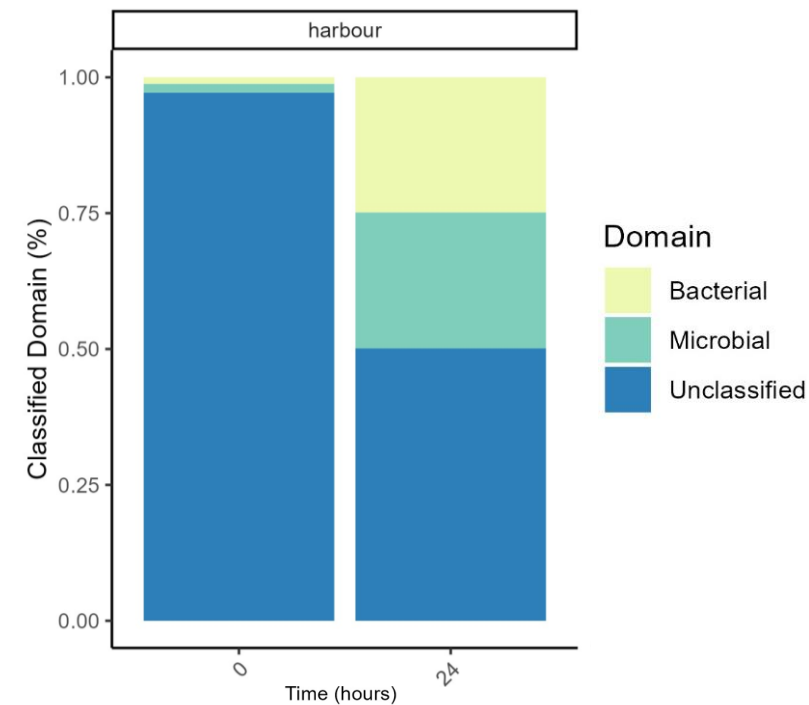
Total eDNA Fragment Length Frequency

Fragment Length (bp) >10,000 5,000-10,000 1,000-5,000 <1,000



What: More insight with new technologies

Mean Percentage of Domains in Control Samples Over Time

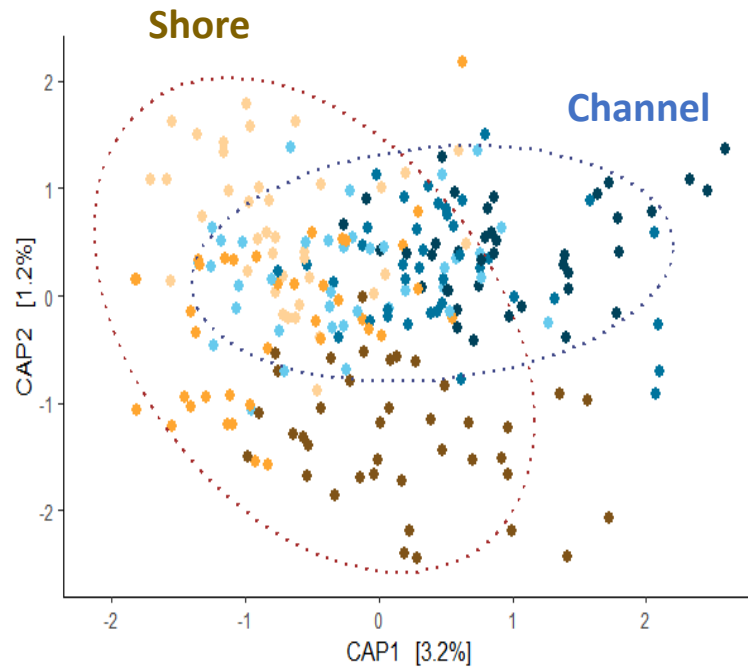


Distribution & Dispersal

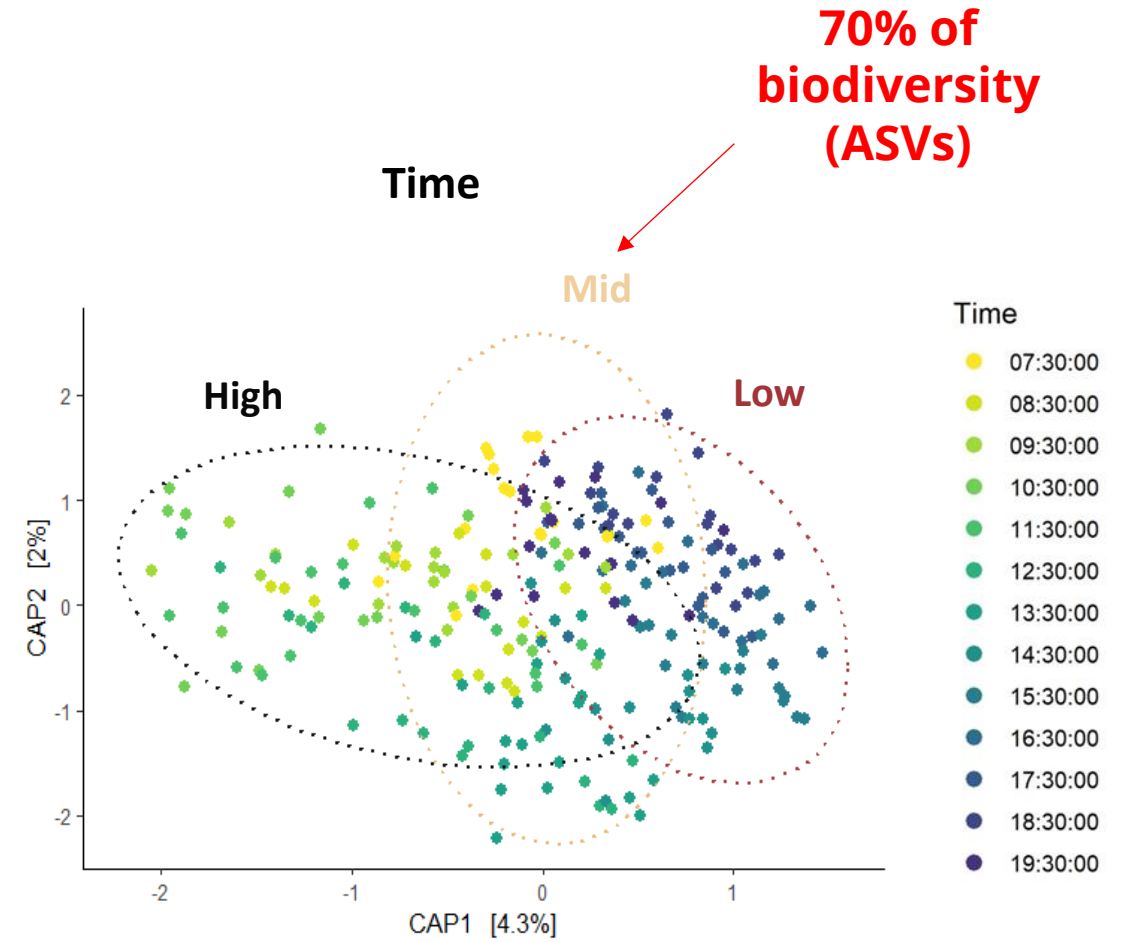
Mitochondrial COI



Location



Time

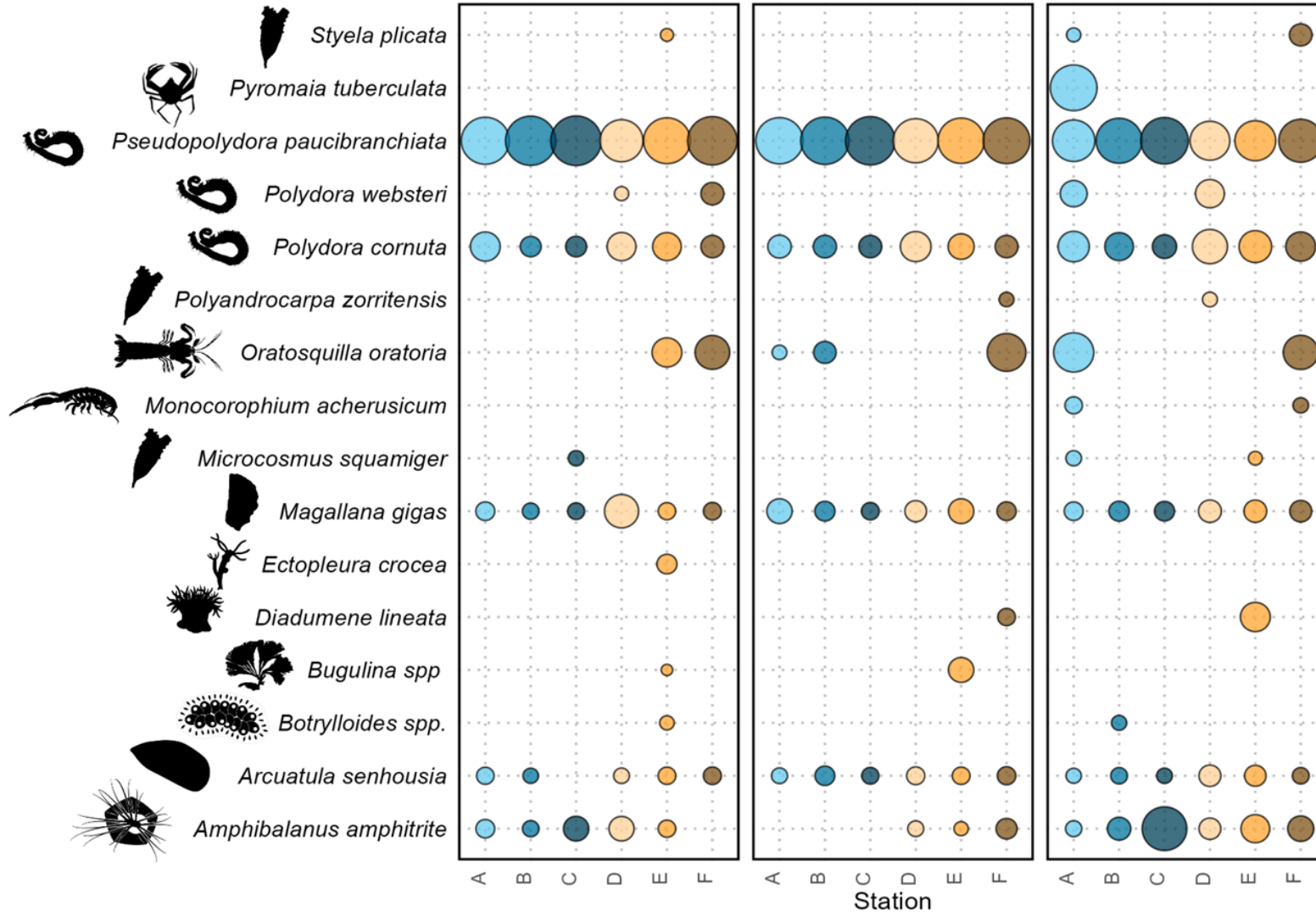


Distribution & Dispersal

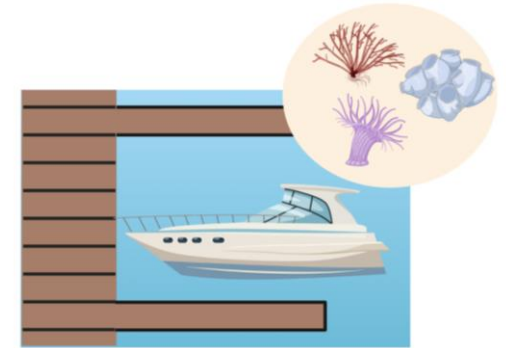


Pest Alert Tool

Mitochondrial COI-Metazoans



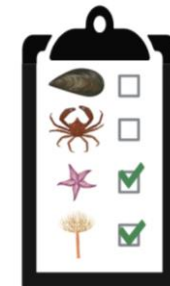
Where: Target species habitat



When:

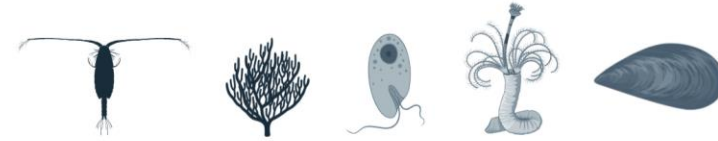
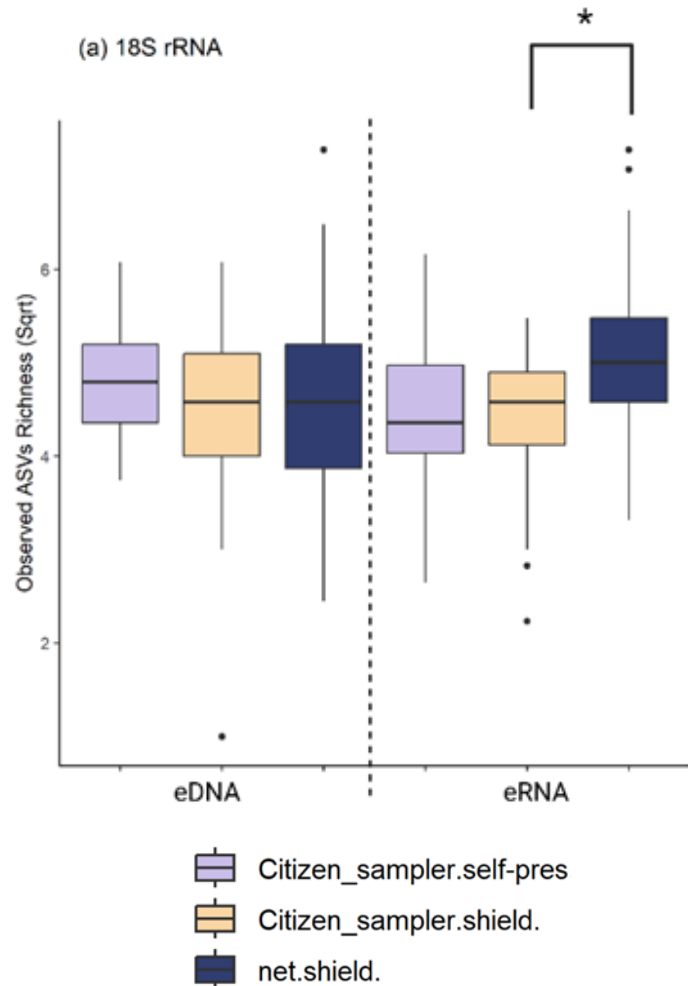
Low Tide

Screening for NIS

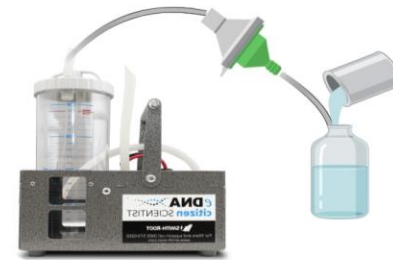


Optimal Sampling Workflows

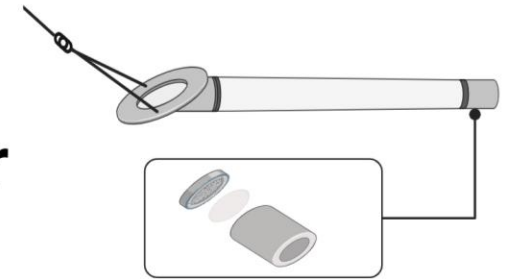
How: Adaptive Sampling for Highly Abundant Taxa (18S rRNA)



Filtration



or



Filter Preservation

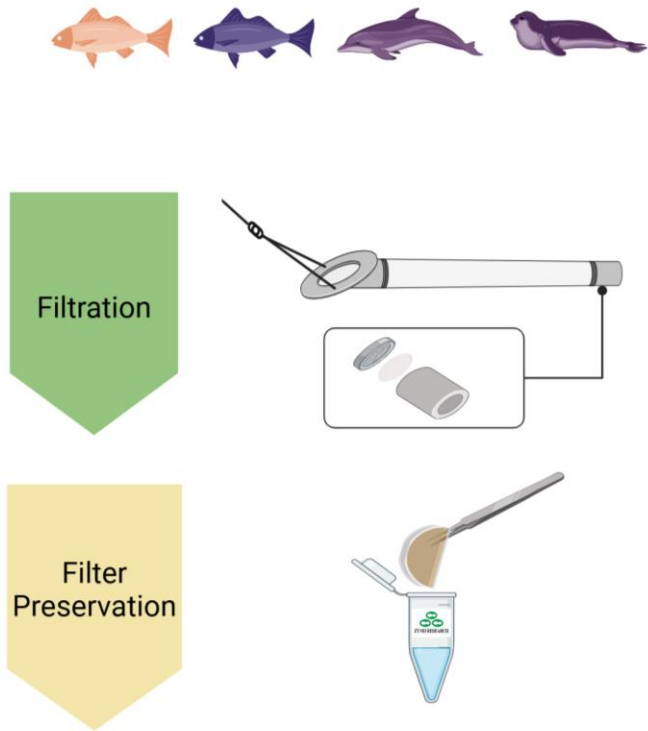


or

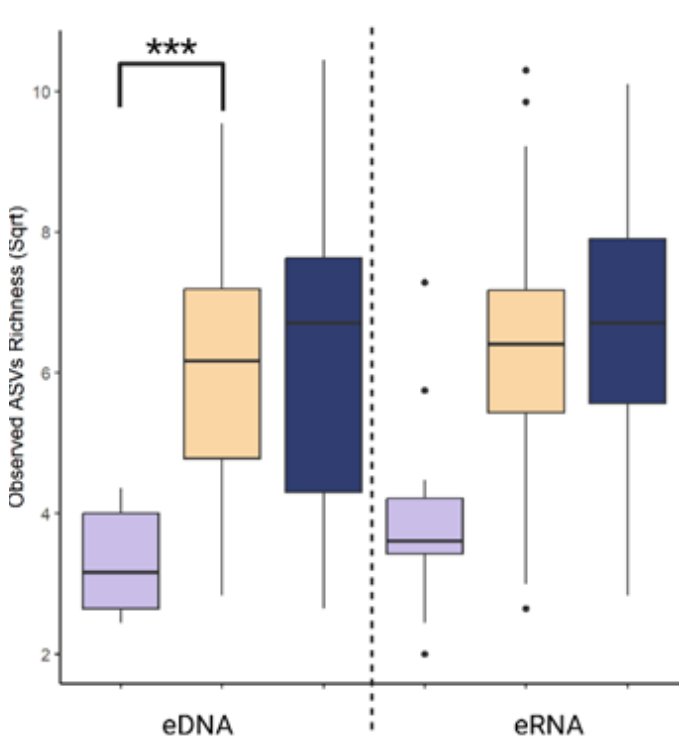


Optimal Sampling Workflows

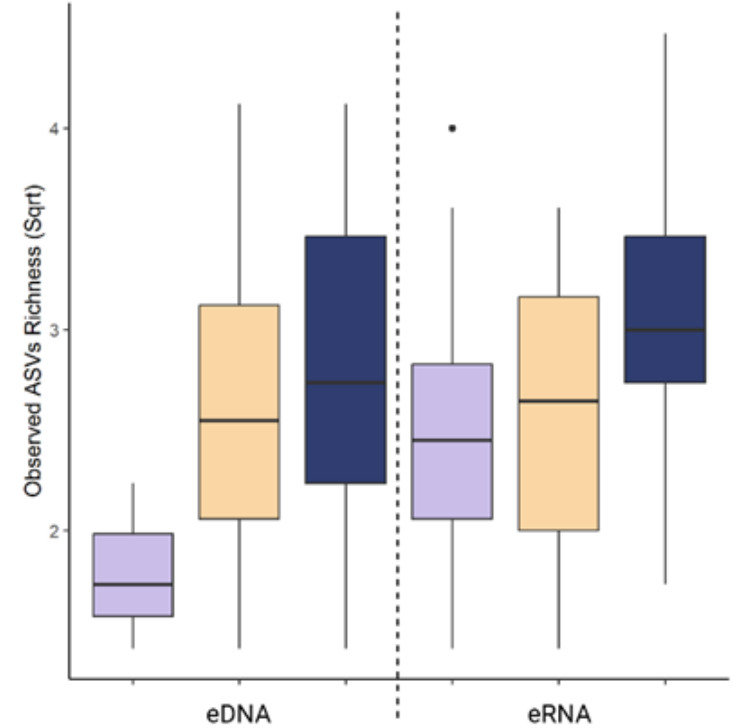
How: Careful Consideration for Low-Abundance and Sparsely Dispersed Taxa (16S rRNA for Fish and Vertebrates)



(b) 16S rRNA-Fish



(c) 16S rRNA-Marine Vertebrate

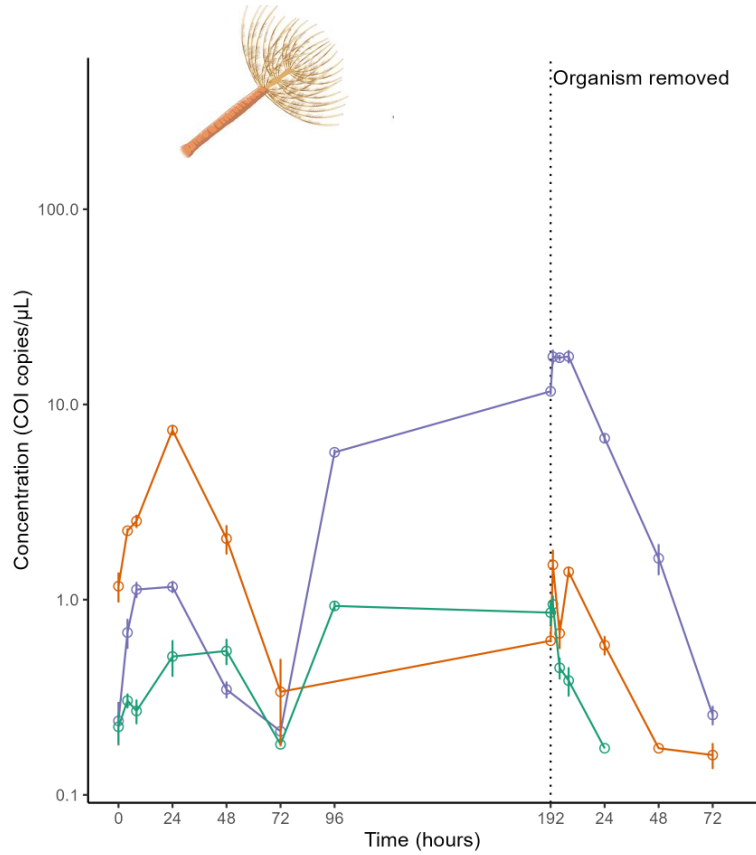


- Citizen_sampler.self-pres
- Citizen_sampler.shield.
- net.shield.

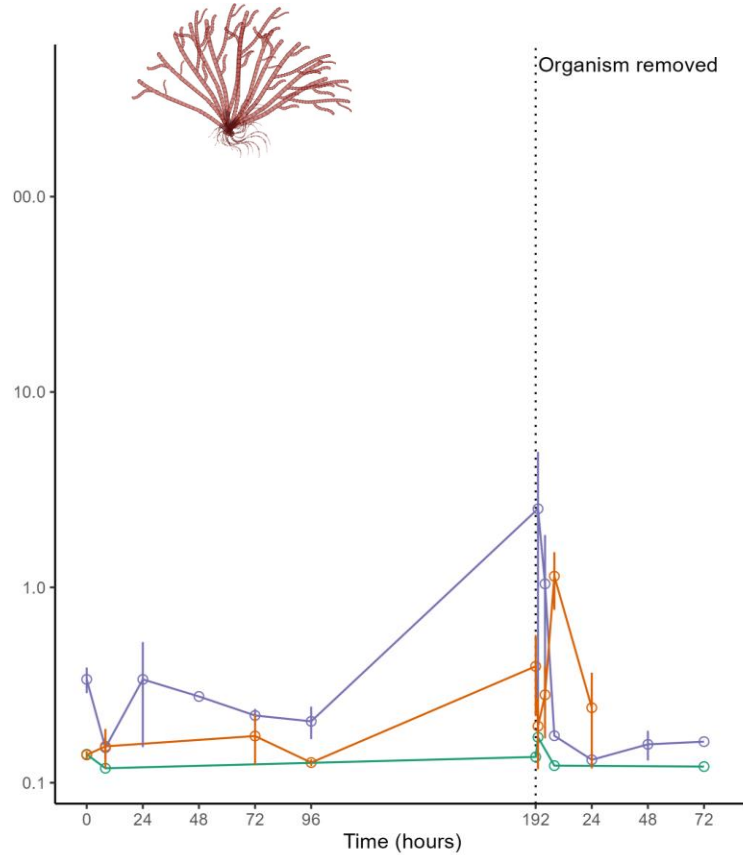
Innovative Sampling Methods

How: direct-ddPCR is possible

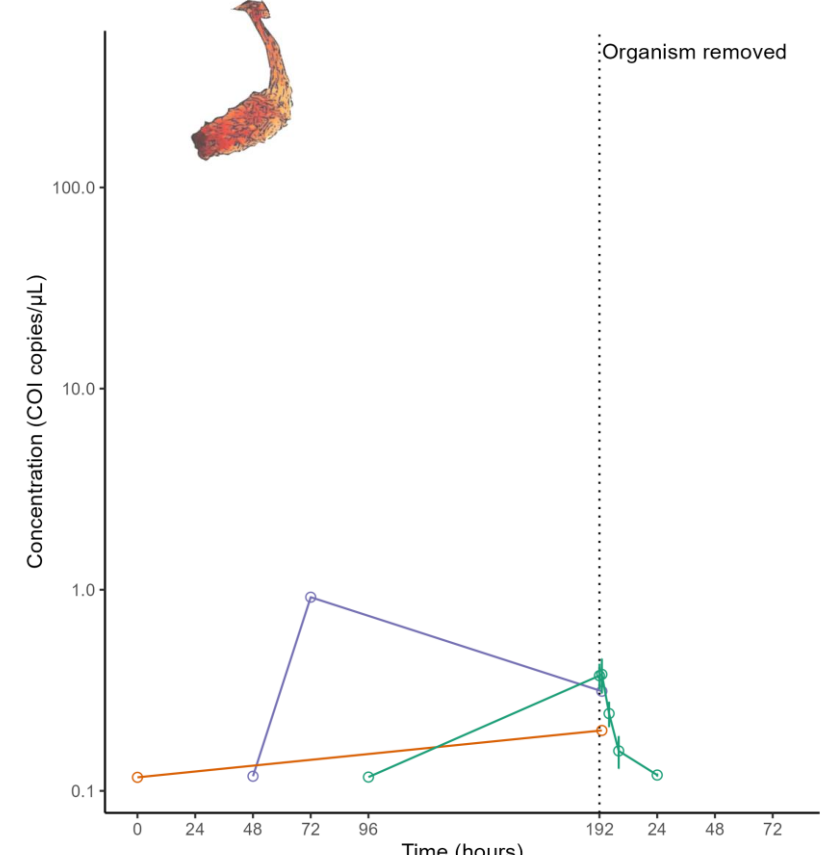
Considerations:



Sabella spallanzanii

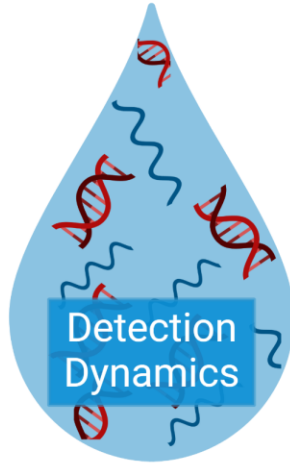
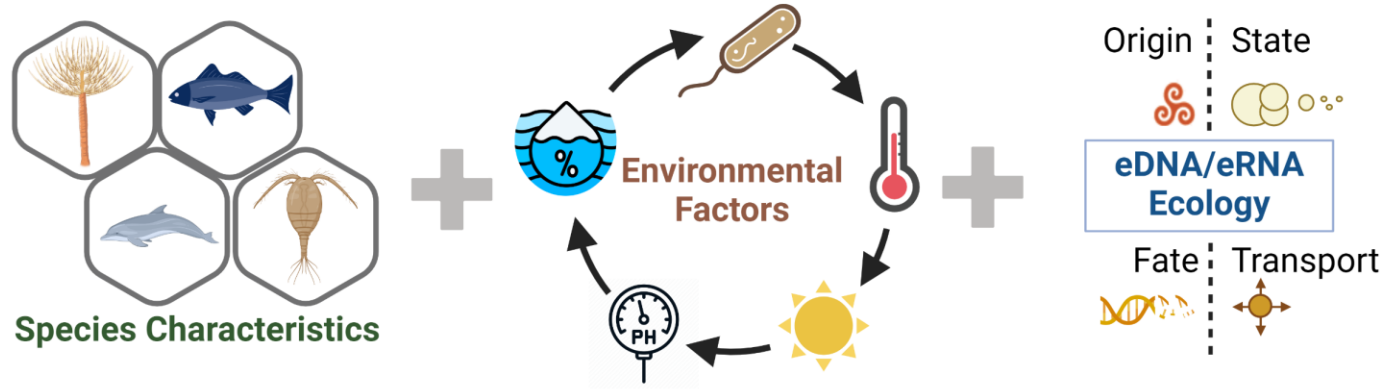


Bugula neritina

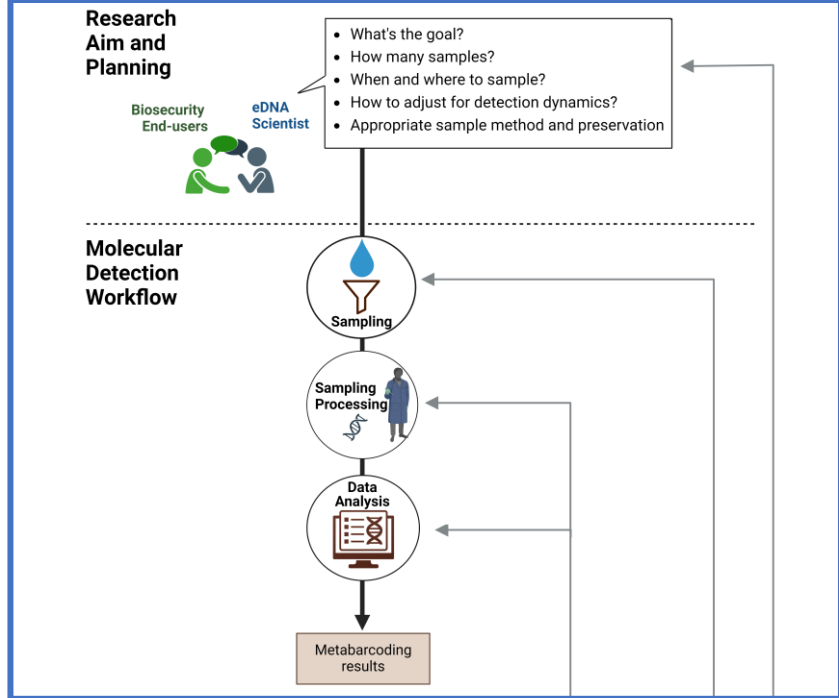


Styela Clava

Biomass — High — Medium — Low



eDNA/eRNA Sampling Approaches and Design

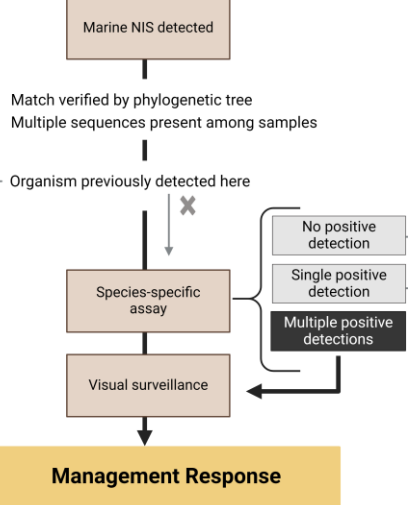


Quality Check



- Appropriate bioinformatics pipelines X
- Up-to-date reference databases X
- Adequate sequencing depth
- Validated lab protocols used
- Best lab practise/guidelines followed
- Valid negative/positive controls X
- Process designed to minimize errors
- Science/publication quality data produced

Risk Assessment

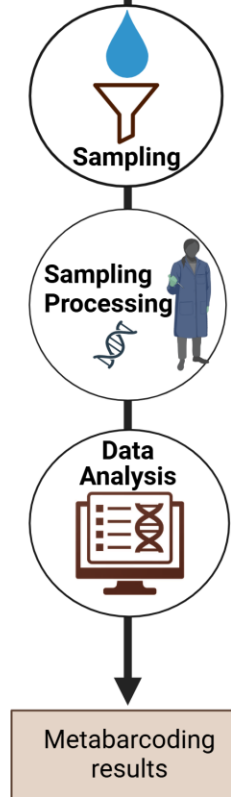


Research Aim and Planning



- What's the goal?
- How many samples?
- When and where to sample?
- How to adjust for detection dynamics?
- Appropriate sample method and preservation

Molecular Detection Workflow

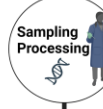


Research Aim and Planning



- What's the goal?
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Molecular Detection Workflow



Metabarcoding results

Quality Check



- Appropriate bioinformatics pipelines X
- Up-to-date reference databases
- Adequate sequencing depth
- Validated lab protocols used
- Best lab practise/guidelines followed
- Valid negative/positive controls X
- Process designed to minimize errors
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Risk Assessment



Marine NIS detected

Match verified by phylogenetic tree
Multiple sequences present among samples

Organism previously detected here X

Species-specific assay

Visual surveillance

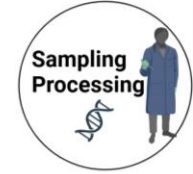
Management Response

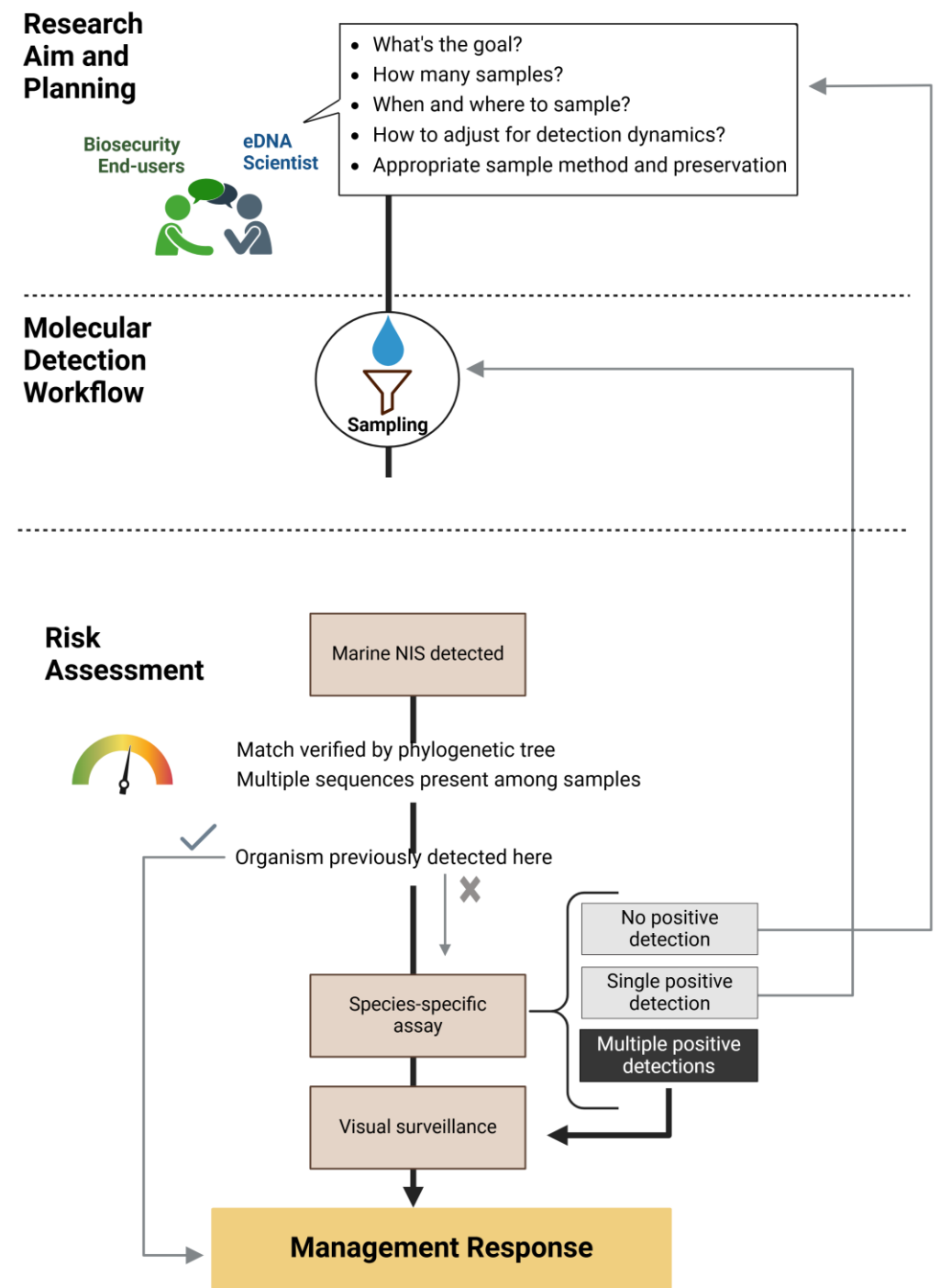
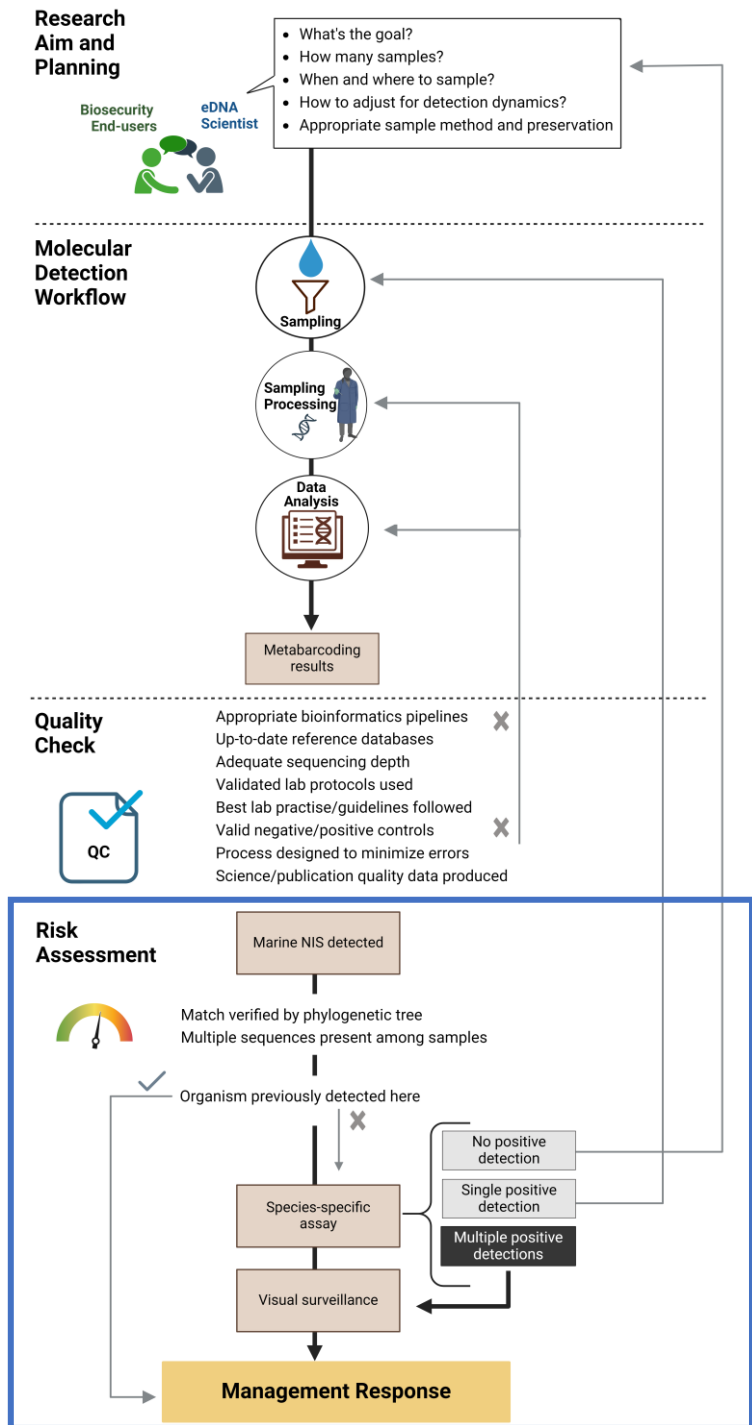
- No positive detection
- Single positive detection
- Multiple positive detections



Inappropriate bioinformatics pipelines

Invalid negative/positive controls





Acknowledgments



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Look through the eyes of the mangōpare,
observing in all directions

Many thanks to the fantastic team at Cawthron and all the partnerships with the Marine Biosecurity Toolbox



Joint Graduate Centre



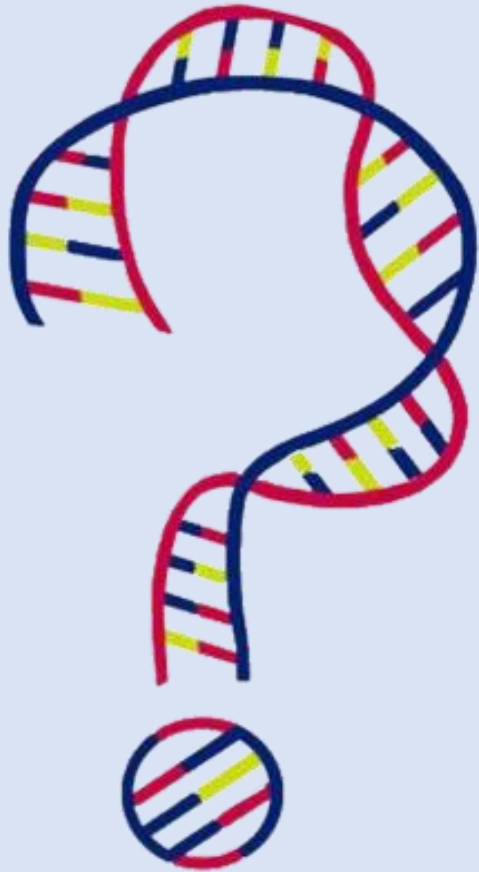
UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND



www.biosecurity-toolbox.org.nz



Thank-you! Any Questions?



Contact Me



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