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# SCALING-UP MARINE eDNA BIODIVERSITY MONITORING: AUTOMATION OF WORKFLOWS FROM SAMPLING TO SEQUENCING

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Image credit: Giacomo d'Orlando / Ronin\_Lab



# OceanOmics Centre



## Aim

- Accelerate and scale the production of marine eDNA biodiversity datasets

## Purpose

- Demonstrate how eDNA data can inform biodiversity discovery and monitoring over large spatial scales
- Develop novel eDNA analytical methods and visualisations

## Focus

- Australian marine estate and neighbouring regions
- Marine vertebrates

# Our Workflow



## Sampling

## Filtration

## DNA Extraction

## Amplification

## Library Preparation

## Next Generation Sequencing



2L seawater  
5 replicates  
per site  
Niskin

0.45  $\mu$ M mixed-  
cellulose membrane  
filters  
Masterflex peristaltic  
pumps

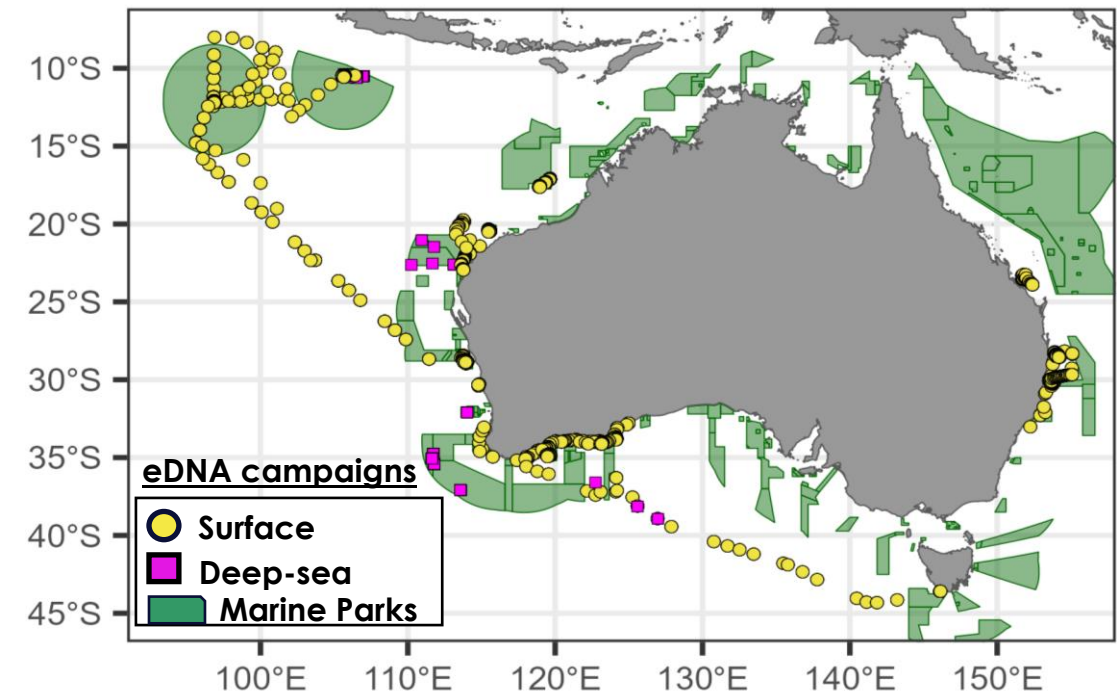
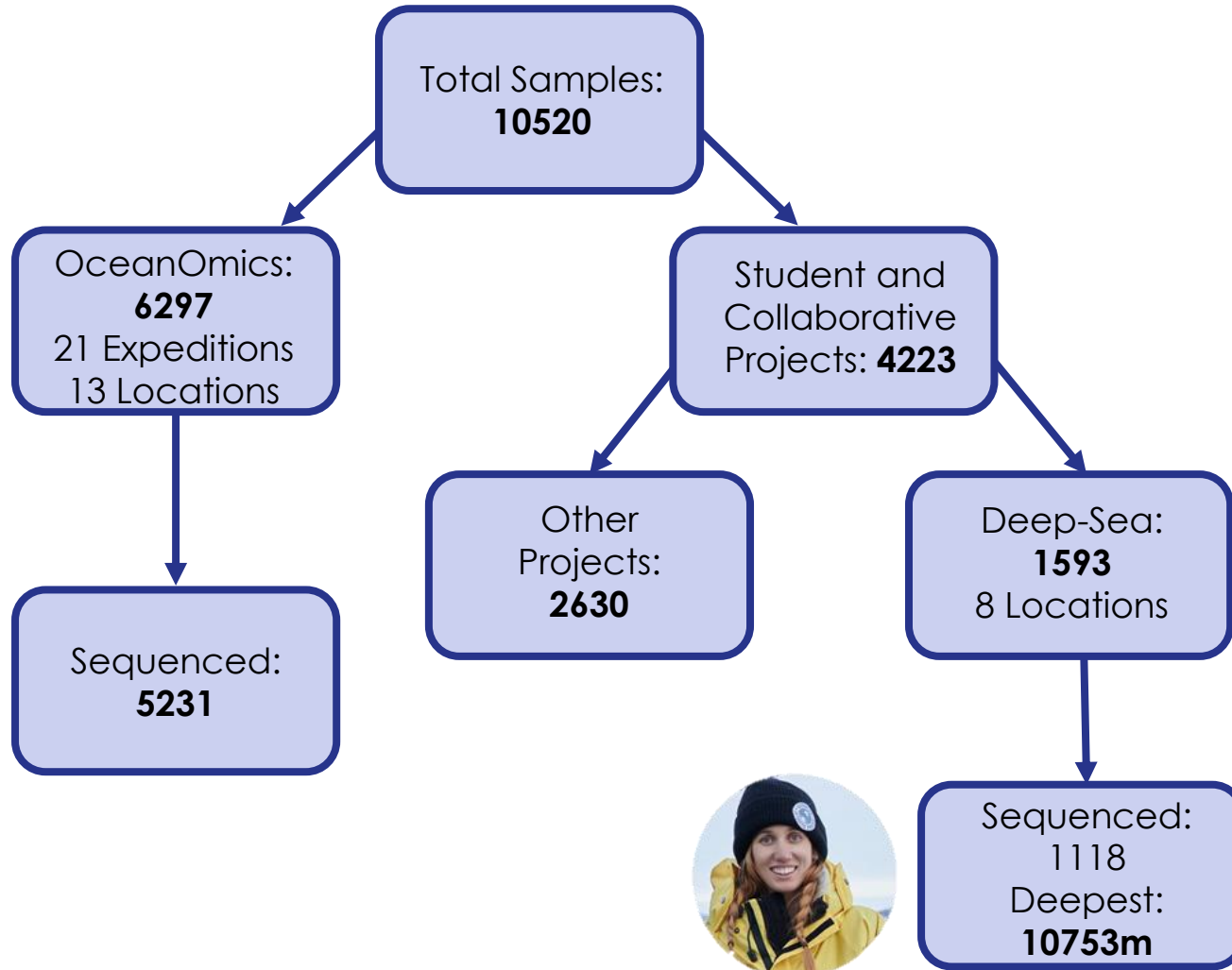
Qiagen  
DNeasy Blood  
and Tissue Kit  
3-hour  
digestion

Assays  
**16S Fish/D**  
**12S MiFish-U**  
12S MarVer01  
12S MiFish-E  
COI-Leray

Illumina TruSeq  
DNA PCR Free

Illumina  
100'000 reads  
per sample

# Sample Processing



# Why Automation?



Scalability



Efficiency



Consistency and Reproducibility

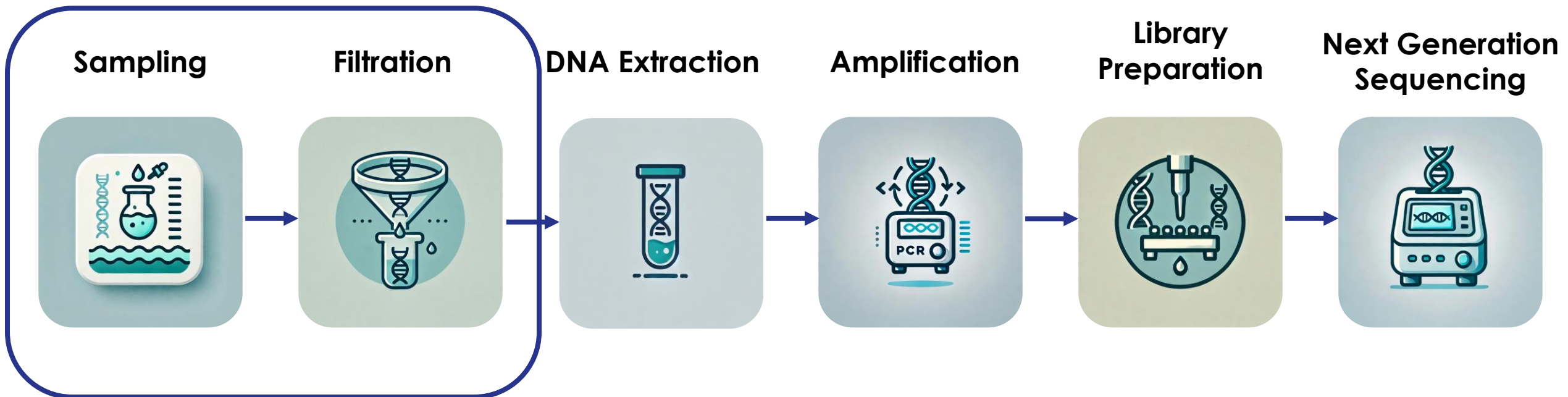


Contamination Control



Cost-Effectiveness

# Sampling and Filtration



# Automated Sampling & Filtration



## Ascension (Ocean Diagnostics)

- In-situ filtration to depths of up to 400m
- Eliminates the need for sample water collection, cold storage and transport, and bench-top filtration
- Minimises contamination risk
- Offers solution to simplify and standardise eDNA sampling, making it more accessible and reproducible



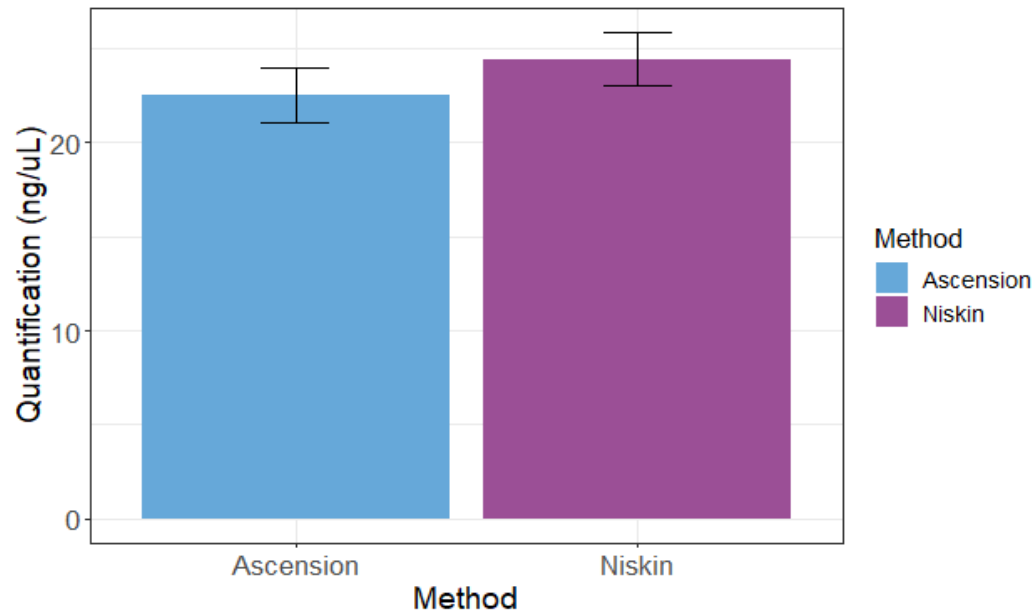
## Method

- Niskin (manual) vs Ascension
- 12 sites along a 1000 km transect
- South-West Western Australia
- 5 x 2L replicates per site (~ 40 minutes)
- Compared DNA yield and fish assemblage

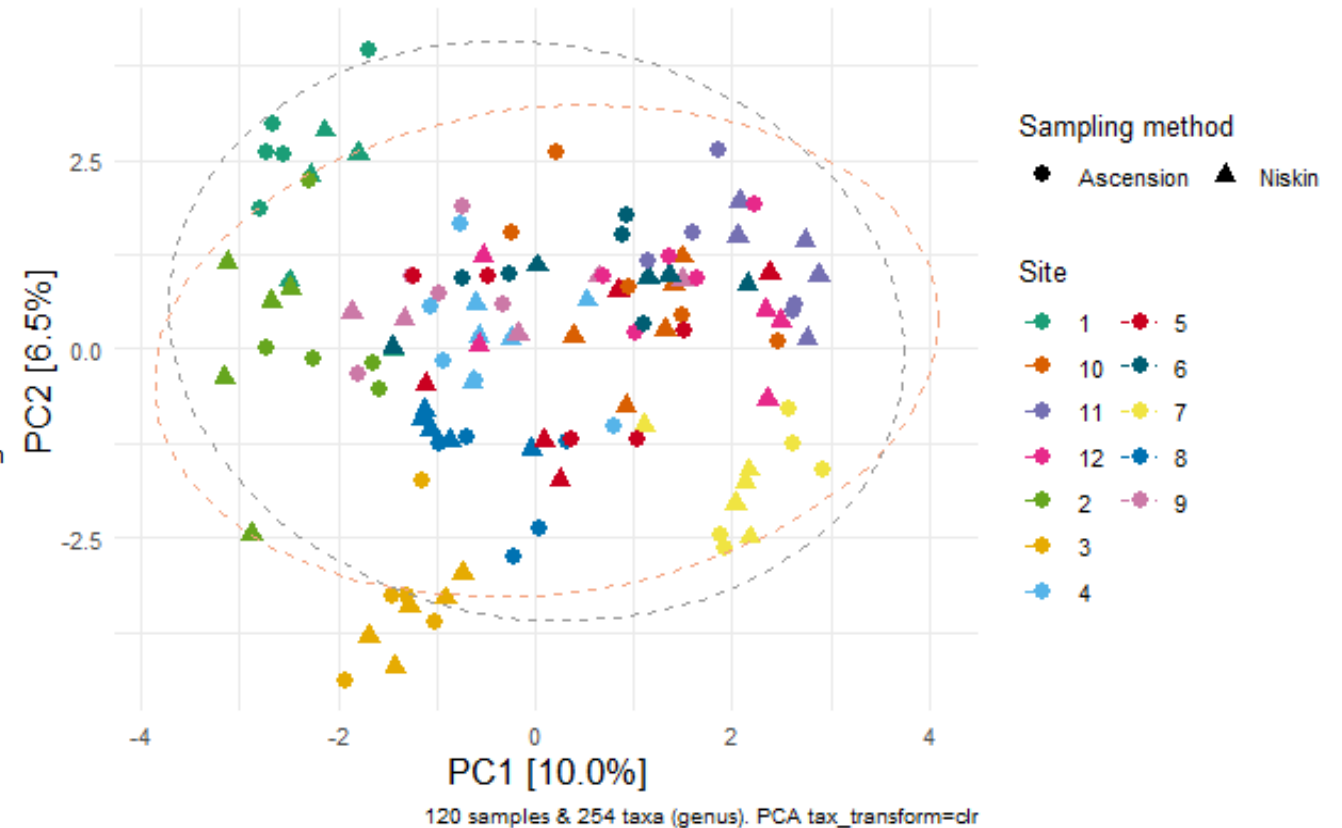
# Manual vs Automated Sampling: Results



## DNA Yield



## Genus Composition



# DNA Extraction



Sampling

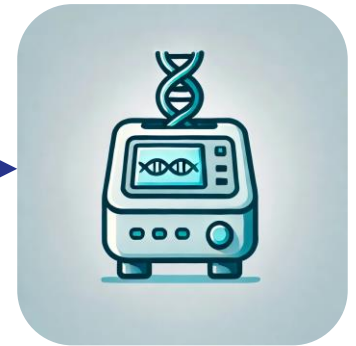
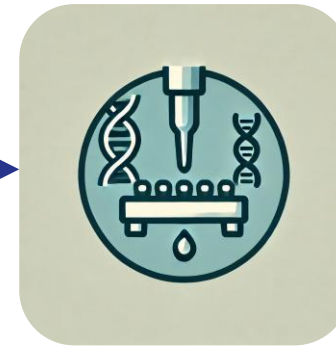
Filtration

DNA Extraction

Amplification

Library Preparation

Next Generation Sequencing



# Automated eDNA Extraction Validation



## **Phase 1:** Preliminary Assessment

Assessing 3 instruments based on key performance factors

## **Phase 2:** Validation at Sea

Testing instruments at sea onboard a research vessel for suitability in the field

## **Phase 3:** Next Generation Sequencing

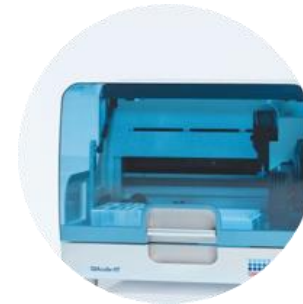
Comparing extraction yield and community composition between manual and automated methods

# Phase 1: Comparing Performance Factors



3 instruments assessed on key performance factors:

- Processing time
- Contamination risk
- Instrument cost
- Cost per sample
- Footprint
- Waste generation



A) QIAcube HT

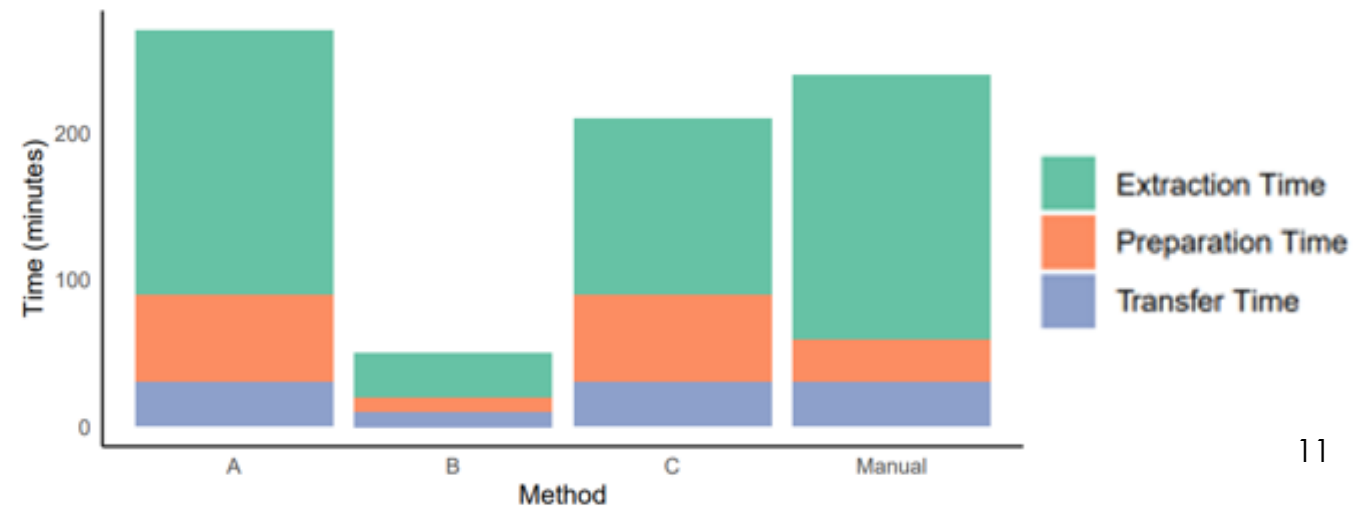


B) Promega Maxwell® RSC 48



C) KingFisher Flex

**QIAcube HT eliminated (A)** due to low efficiency and potential contamination issues



# Phase 2: Feasibility at Sea



## Method

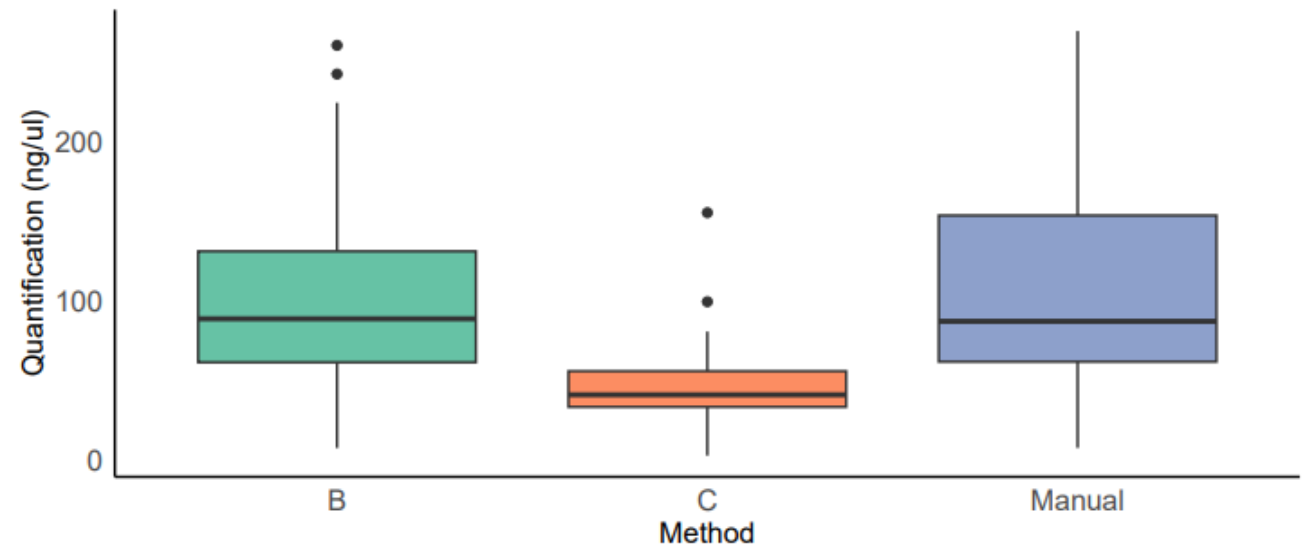
Seawater samples ( $n = 45$ ) were collected and homogenised at 5 sites, then subsampled for extraction manually and on each instrument

## Results

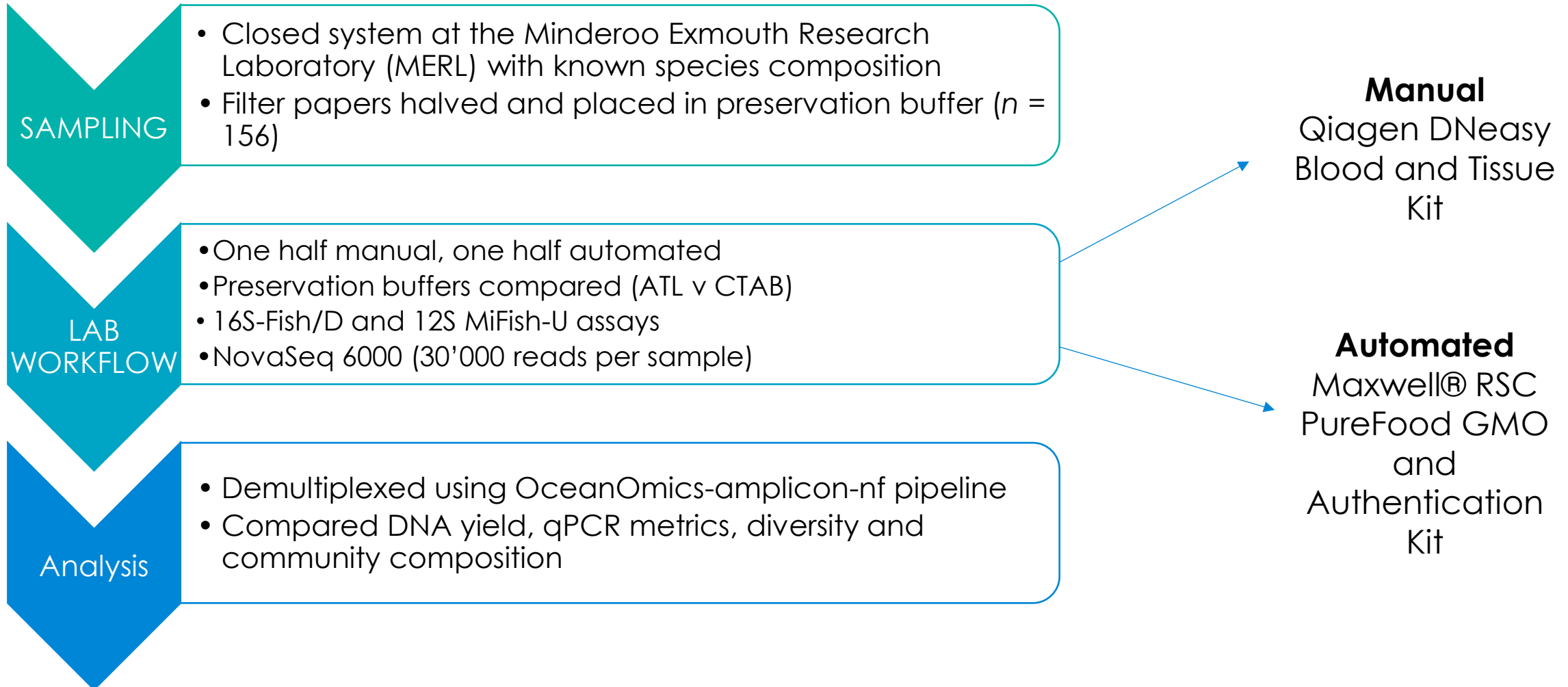
Both performed well at sea- durable and low contamination risk

**KingFisher Flex (C) eliminated** due to low yield

**Promega Maxwell® RSC 48 (B) proceeded** to Phase 3



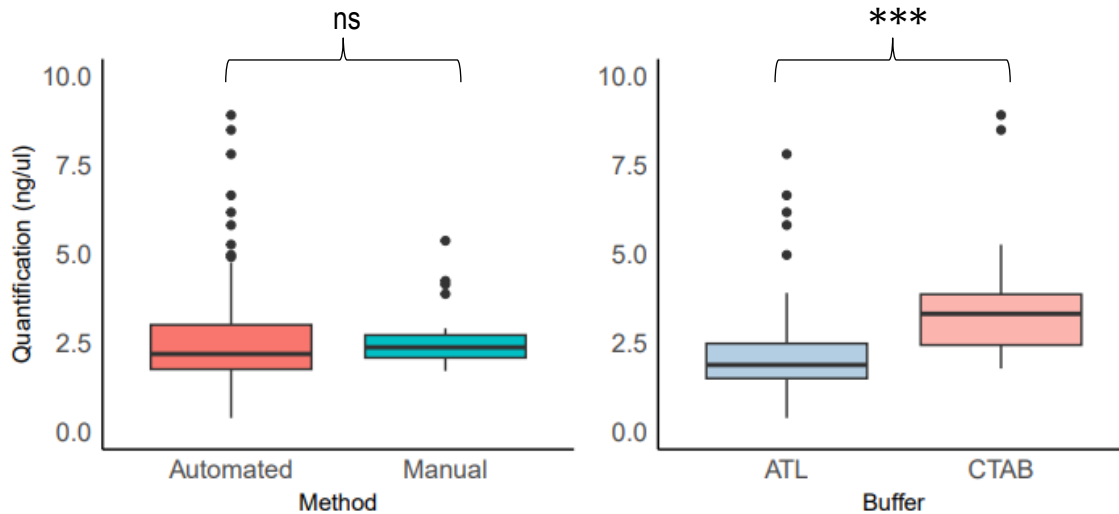
# Phase 3: Positive Detection Control



# No Impact to Assay Performance



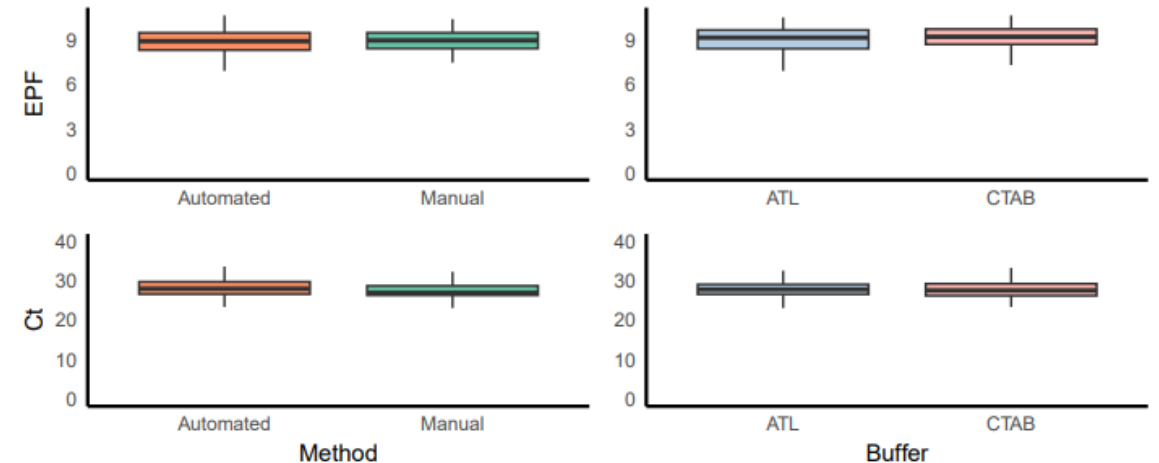
## DNA Yield



No significant difference in DNA yield between manual and automated methods

CTAB buffer outperforms ATL, making it a better choice for maximising DNA yield

## qPCR Metrics



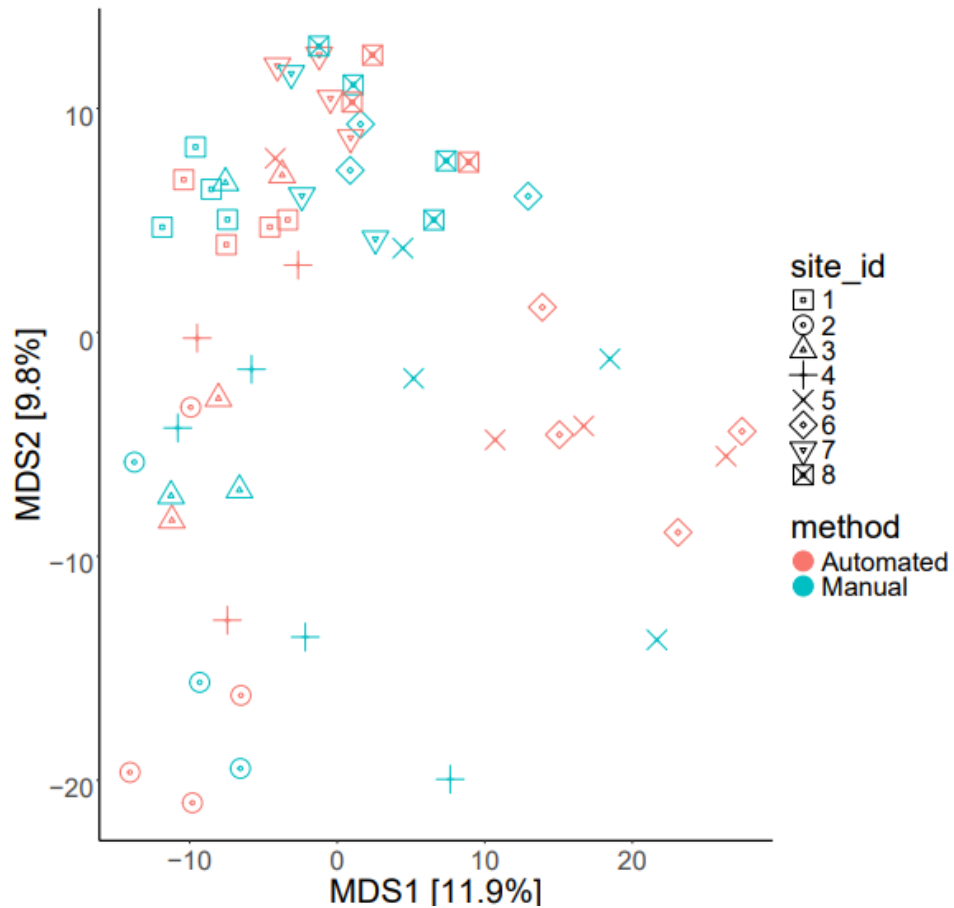
Manual and automated methods are equally effective in qPCR performance.

Despite variation in DNA yield between buffers, there is consistent downstream assay performance

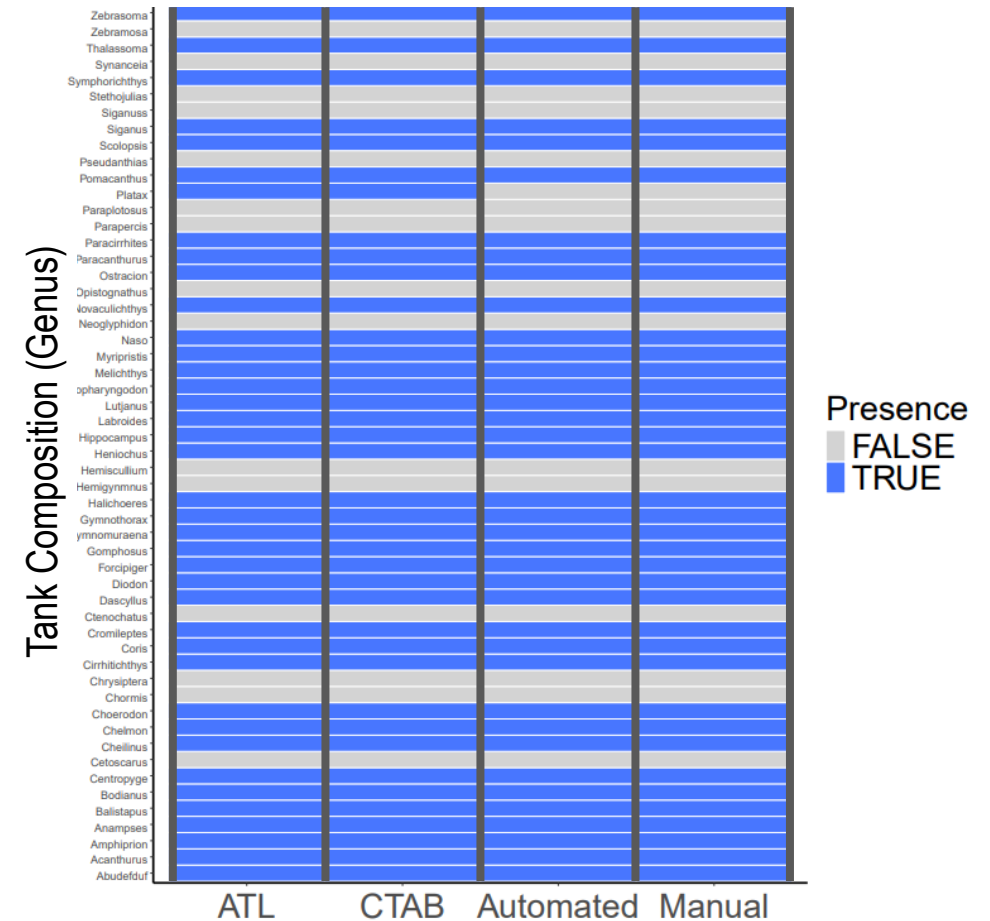
# No Impact to Fish Assemblage



## Diversity



## Genus Composition



# Amplification



Sampling

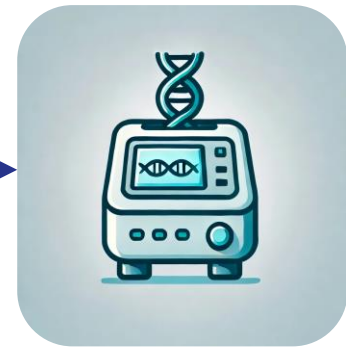
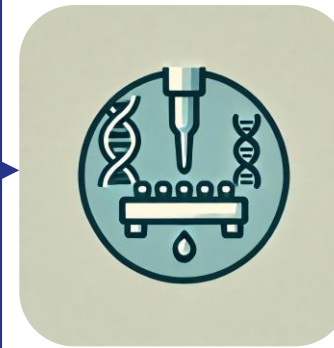
Filtration

DNA Extraction

Amplification

Library  
Preparation

Next Generation  
Sequencing



# Automated Liquid Handling



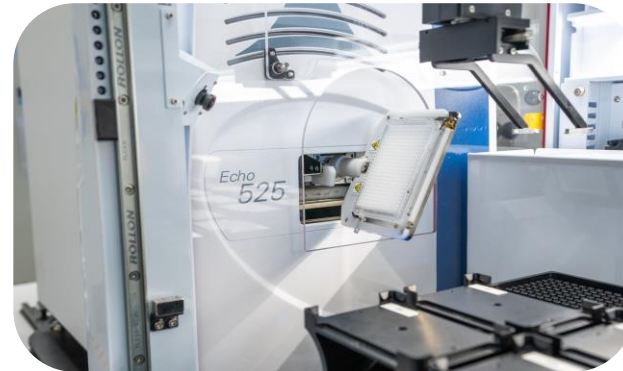
## Biomek i7

Robotic liquid handler for sample preparation and PCR setup



## Echo 525

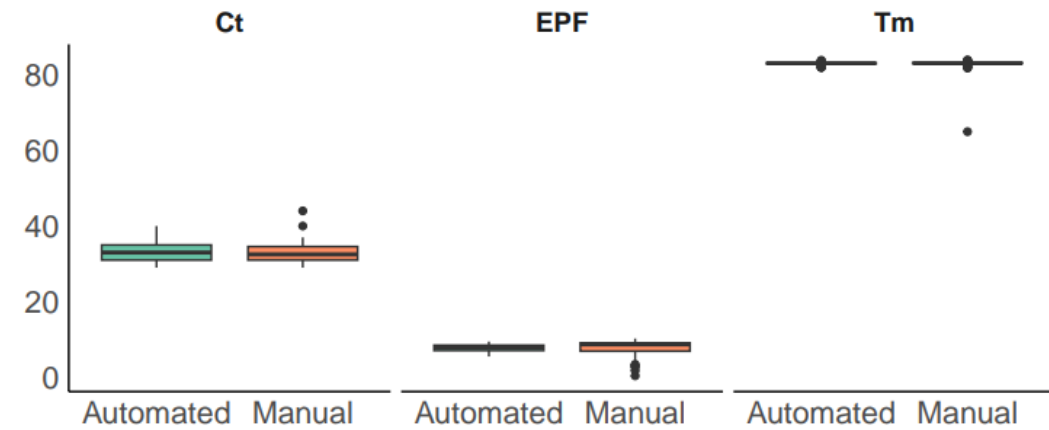
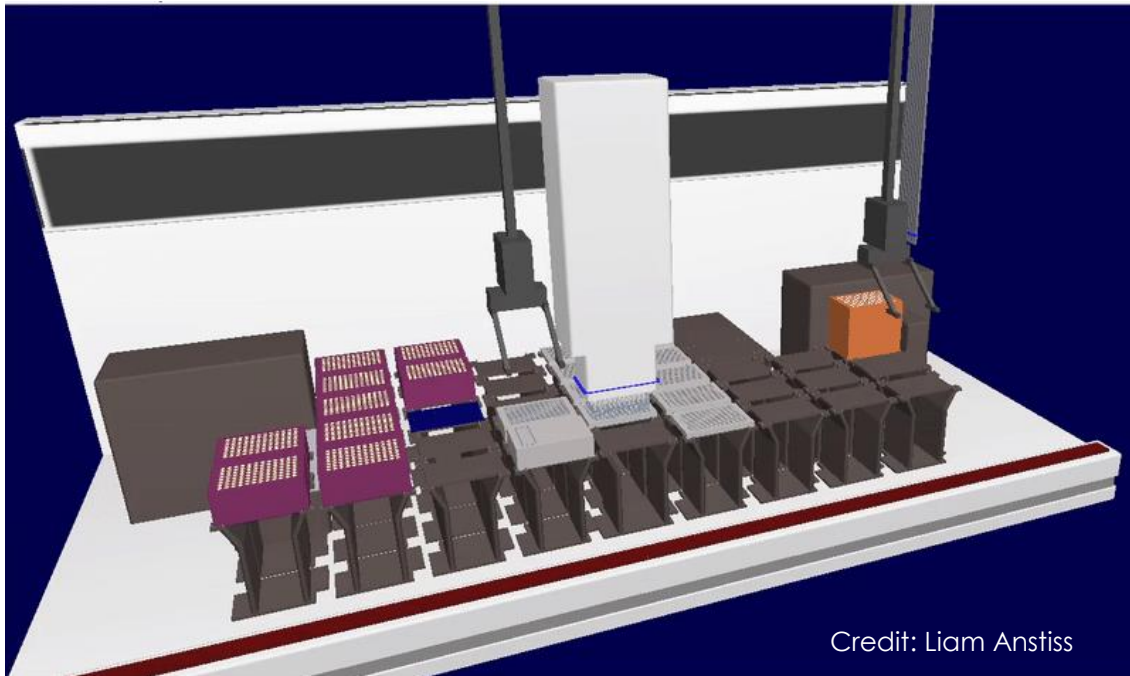
Acoustic liquid handler for precise, contactless sample transfer



1. qPCR Set-up
2. Normalisation
3. Bead Clean-Up

**7 Novel Scripts**

# qPCR and Pooling

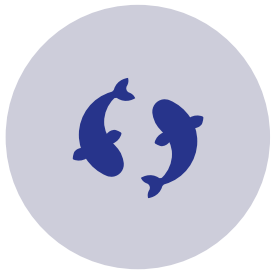


Volumes validated

No contamination identified

Reduced processing times by 46%  
without any impact to qPCR  
performance

# Summary



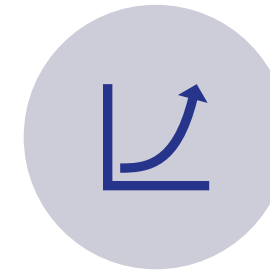
VALIDATED THE ASCENSION FOR LARGE SCALE, HIGH-THROUGHPUT MARINE VERTEBRATE eDNA SAMPLING



QUADRUPLED EXTRACTION EFFICIENCY WITH NO IMPACT TO DNA YIELD OR COMMUNITY COMPOSITION



DEVELOPED SEVEN NOVEL SCRIPTS



REDUCED PROCESSING TIMES BY 46% ENABLING FASTER DATA GENERATION



STRENGTHEN GLOBAL CONSERVATION EFFORTS

# Next Steps



Sampling

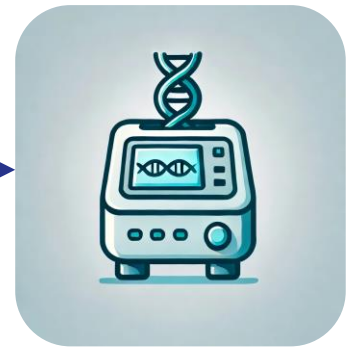
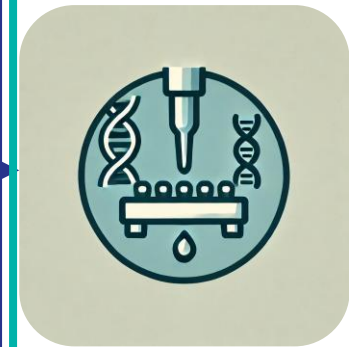
Filtration

DNA Extraction

Amplification

Library Preparation

Next Generation Sequencing



# Outreach



Knowledge Sharing



Publish validated methods,  
scripts & SOPs



Collaboration



protocols.io



GitHub

# Acknowledgements



**UWA:** Professor Amit Chakma, Professor Tim Colmer, Professor Christophe Gaudin, Professor Anna Nowak, Minderoo OceanOmics Centre at UWA team and alumni, UWA Ocean Institute members.

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Sang Huynh   Anna Depiazzi   Marcelle Ayad   Adam Bennett   Shannon Corrigan   Priscila Goncalves   Georgia Nester   Ebony Thorpe   Eric Raes

# OceanOmics Team

## Attendees



Mike  
Bunce



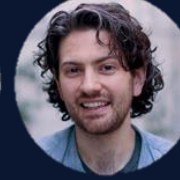
Eric  
Raes



Priscila  
Goncalves



Marcelle  
Ayad



Matt  
Fraser



Adrienne  
Doran



Jessica  
Pearce

## Find out more



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## Presenters



Shannon Corrigan

Ocean Genomes- Reference  
Quality Genome Resources for  
Marine Vertebrates

Thursday- 16:15 Session 17



Georgia Nester

Innovative eDNA strategies for  
advancing deep-sea  
biodiversity research and  
conservation

Friday- 10:35 Session 20



Philip Bayer

The OceanOmics dashboard: A  
visualisation tool for the  
democratisation of eDNA data

Thursday- 15:00 Session 14



Anya Kardailsky

Exploring enrichment and depletion  
techniques using CRISPR and Nanopore  
technologies for enhanced eDNA  
Sequencing

Wednesday- 12:30 Session 3