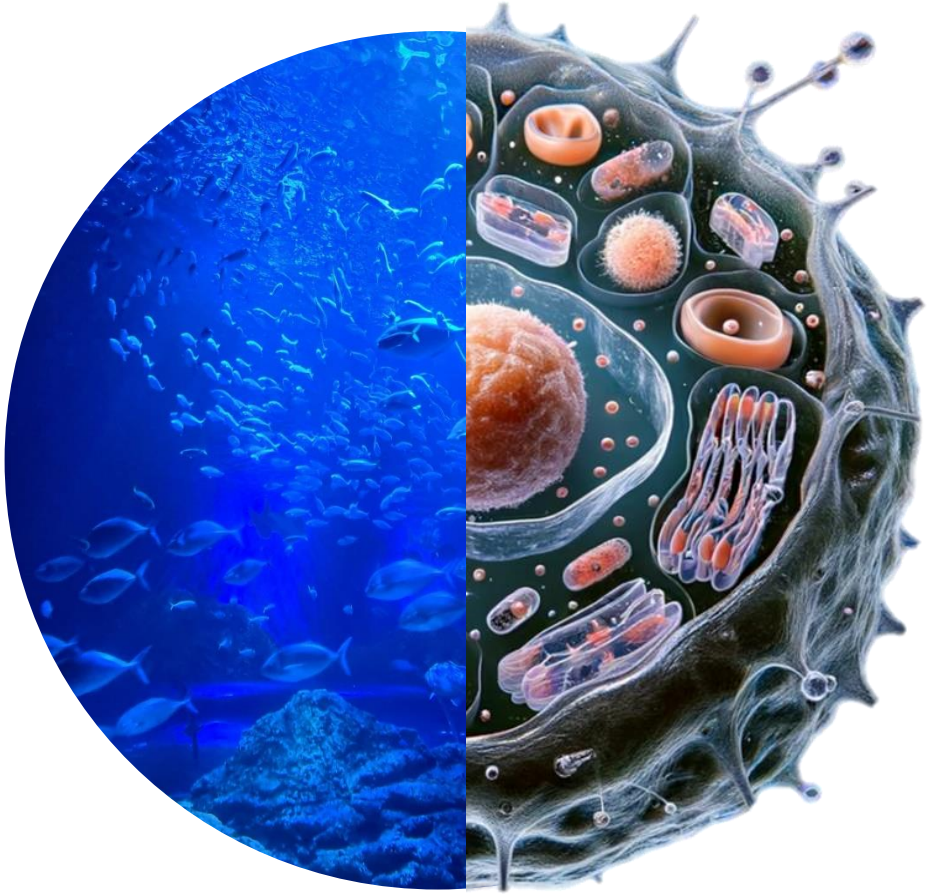
Single cell sequencing is the future of population genetics in marine environments.

Haylea Power
Environomics Future Science Platform
CSIRO
21st February 2025



Genomic analyses from environmental samples

We all do Environmental DNA (eDNA) studies

✓ We already do community diversity surveys or species-specific assays with eDNA samples

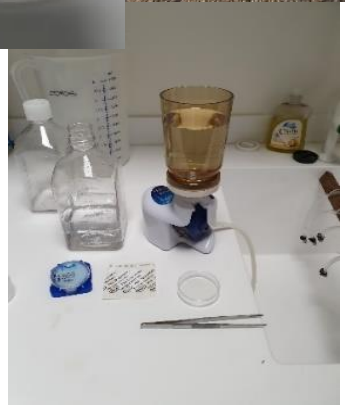
… but can we do more with eDNA samples?

❑ The challenge is obtaining more comprehensive genetic information from eDNA samples which previously required tissue samples.

- ❑ Multi-locus genotypes to identify individuals
- ❑ Measuring animal abundance
- ❑ Population dynamics

Why haven't we done this yet?

eDNA limitations



Unsorted genetic material:

Genomes and fragments from multiple species and individuals.

Highly heterogeneous and degraded:

Material includes degraded tissue to <100 bp fragments.

Limited focus on the physical characteristics of collected material:

Perhaps because current methods seem effective?

Can we improve on this?

eDNA +

Beyond presence-absence: Improving eDNA performance

Exploring the physical components of eDNA samples. Can we:

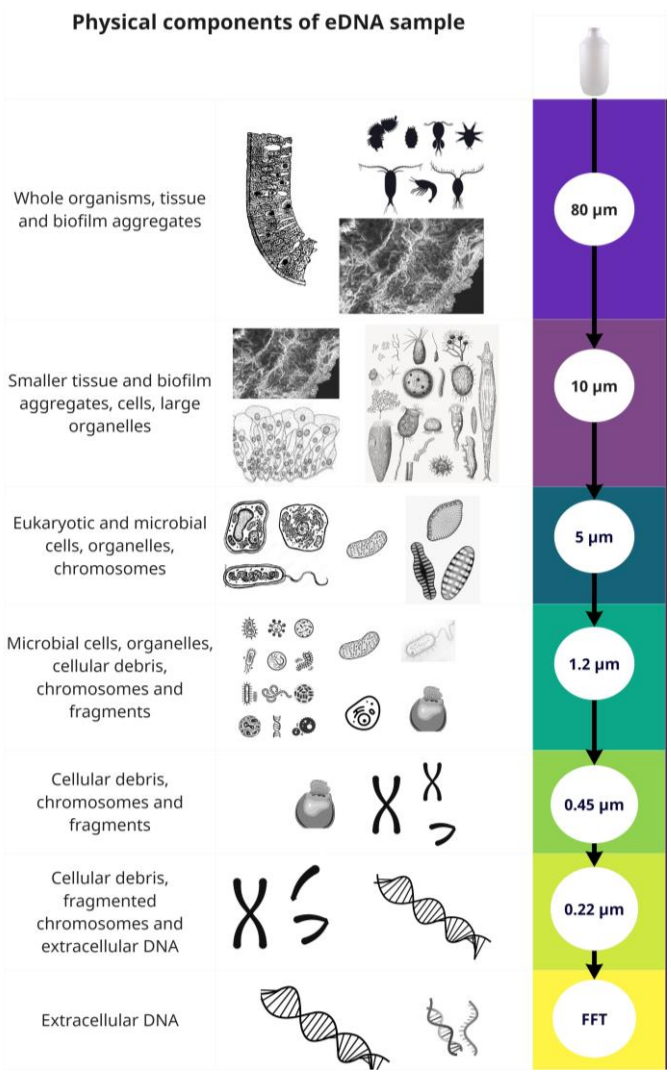
- Physically separate components of eDNA to target taxa?
- Target components of eDNA with fluorescence activated cell sorting (FACS)?
- Enrich eDNA samples without inhibitors and non-target taxa (i.e. bacteria)?
- Enable shotgun or long read sequencing of “cleaner” eDNA samples to retrieve richer genomic information?

The aim:

Develop a non-invasive method to obtain richer information from environmental samples

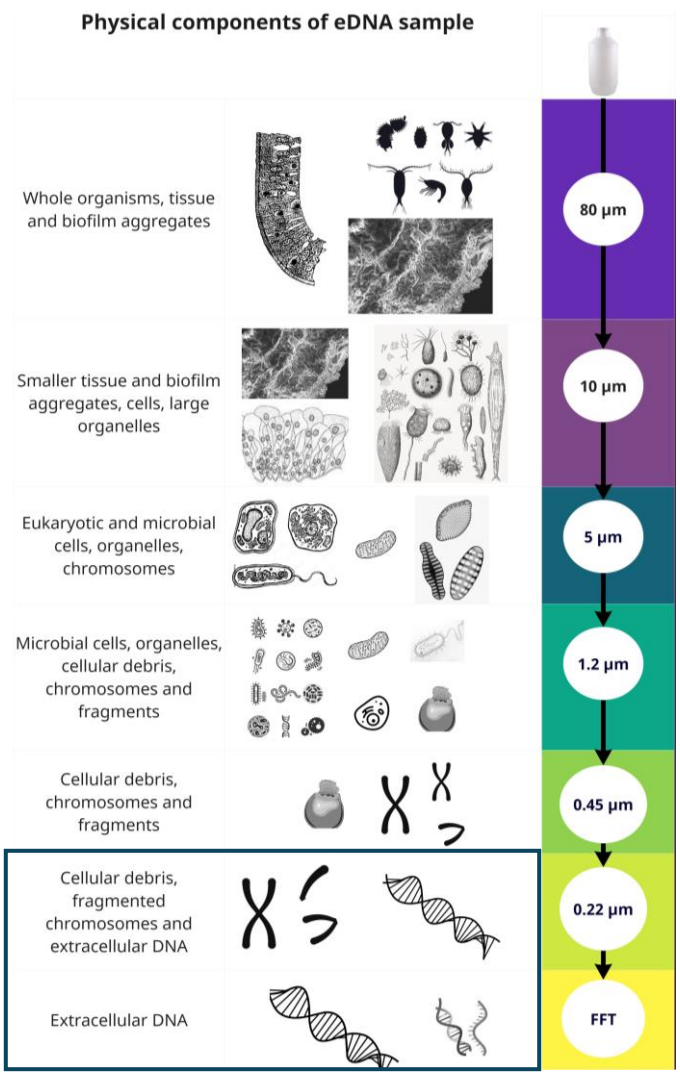
So, What is eDNA?

eDNA components hypothesis



Power *et al.* 2023 What is environmental DNA?

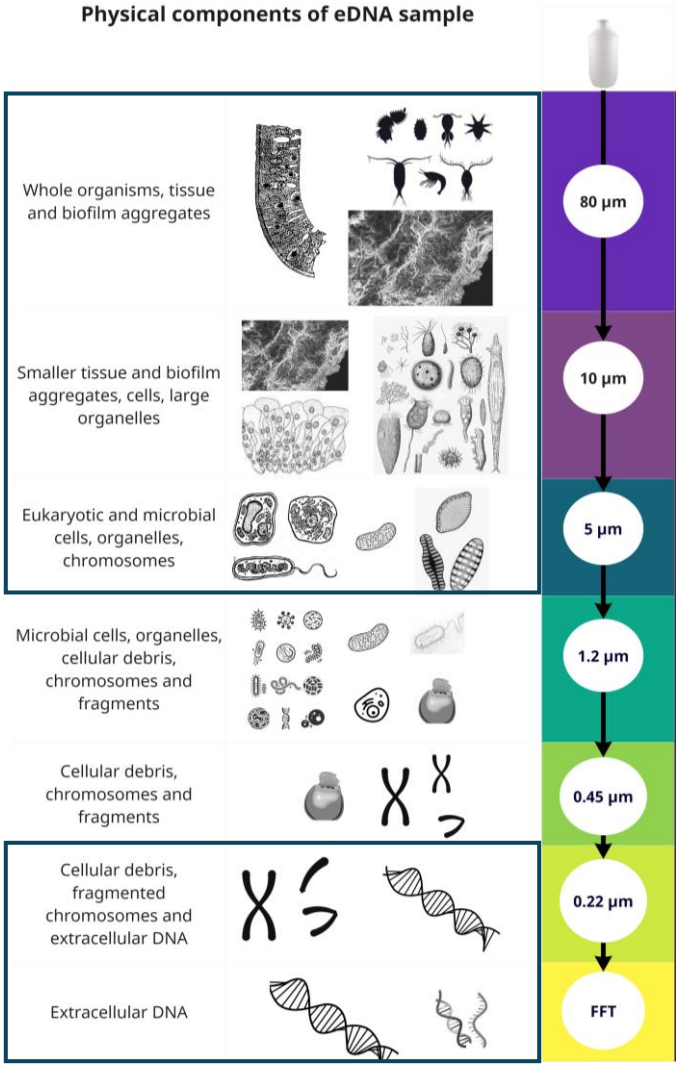
eDNA components hypothesis



What people think they're collecting

Power *et al.* 2023 What is environmental DNA?

eDNA components hypothesis

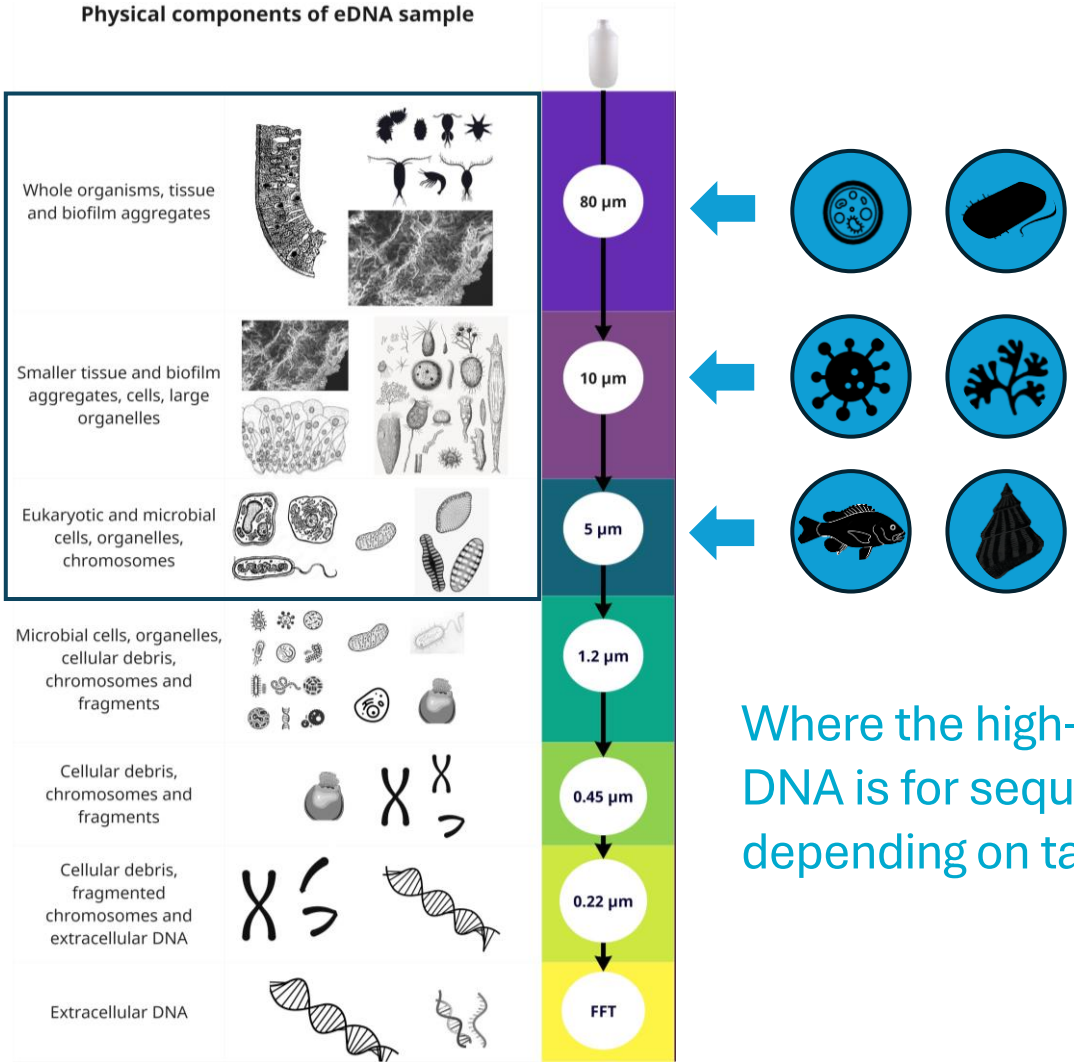


Where the high-quality DNA is for sequencing depending on target

What people think they're collecting

Power *et al.* 2023 What is environmental DNA?

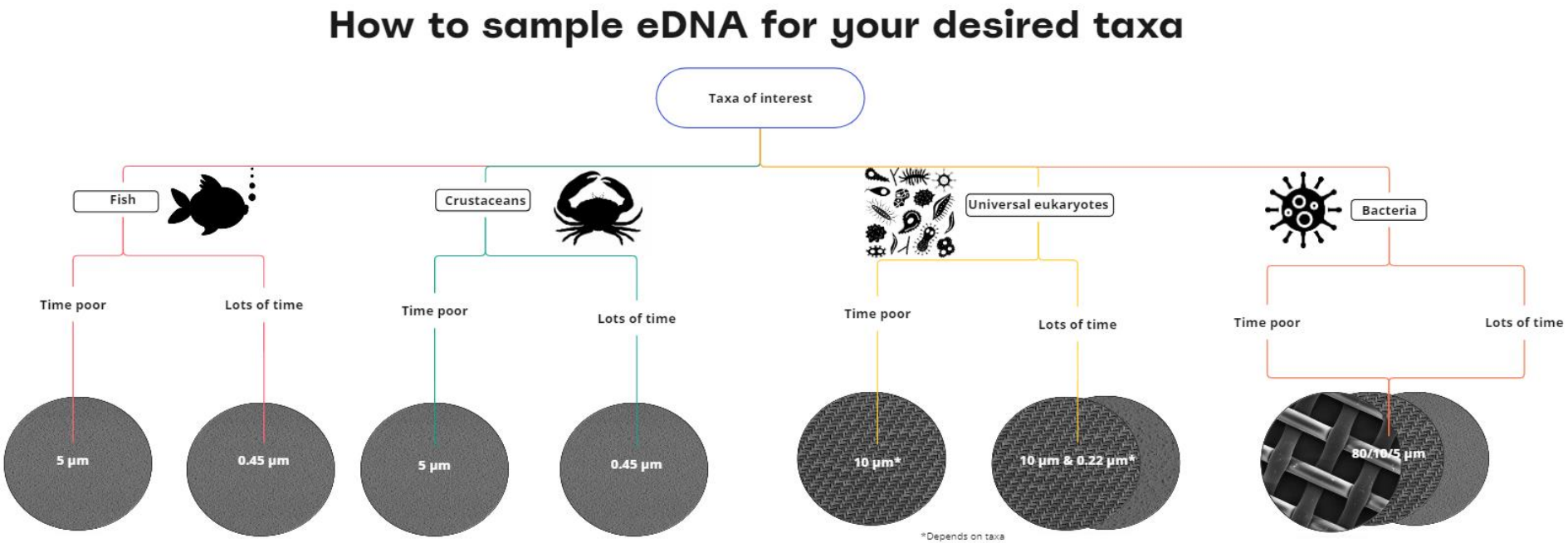
eDNA components hypothesis



Where the high-quality DNA is for sequencing depending on target

Power *et al.* 2023 What is environmental DNA?

How to enrich your eDNA samples

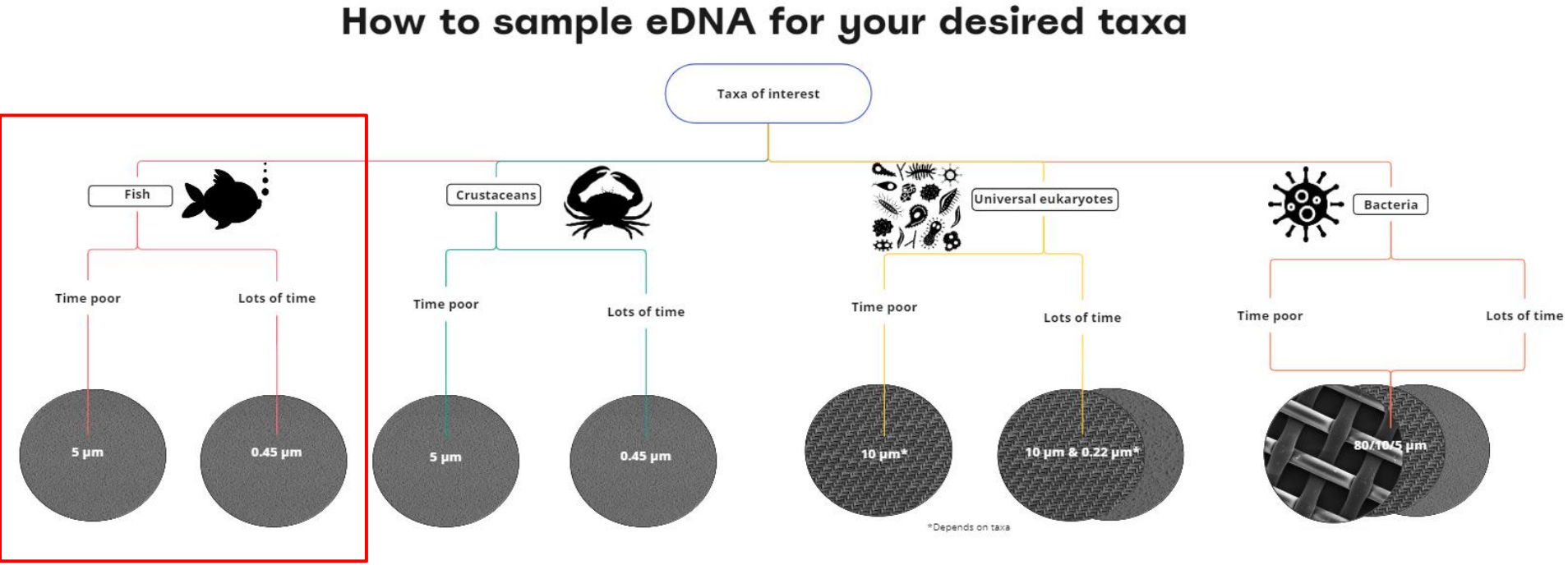


Power *et al.* 2023 What is environmental DNA?

Why does this matter?

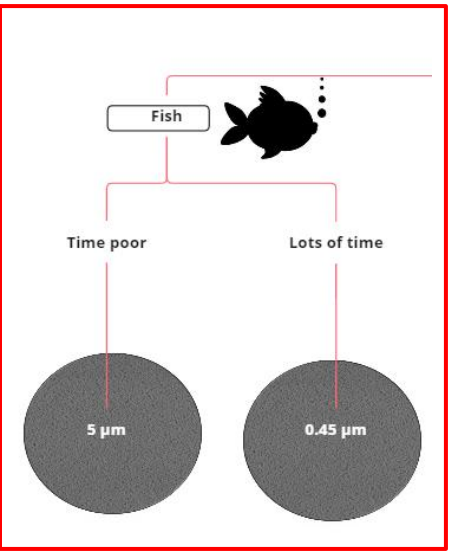
- Can we physically separate components of eDNA to target taxa? (aka enriched eDNA sample)
  - ✓ YES!
- Now, can we target components of eDNA with fluorescence activated cell sorting (FACS)?

Why does this matter?



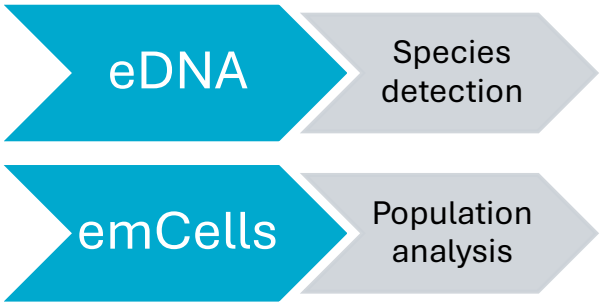
Power *et al.* 2023 What is environmental DNA?

Beyond presence-absence



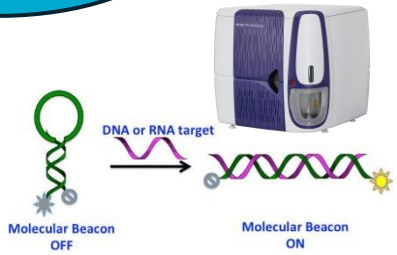
- Can we use just the cellular components?

emCells - *Environmental metazoan cells*



How do we get them?

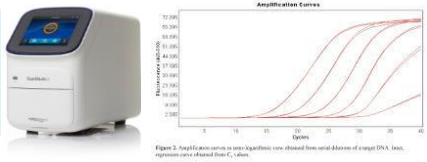
FACS can sort millions of cells per minute



1. Water collection and cell processing

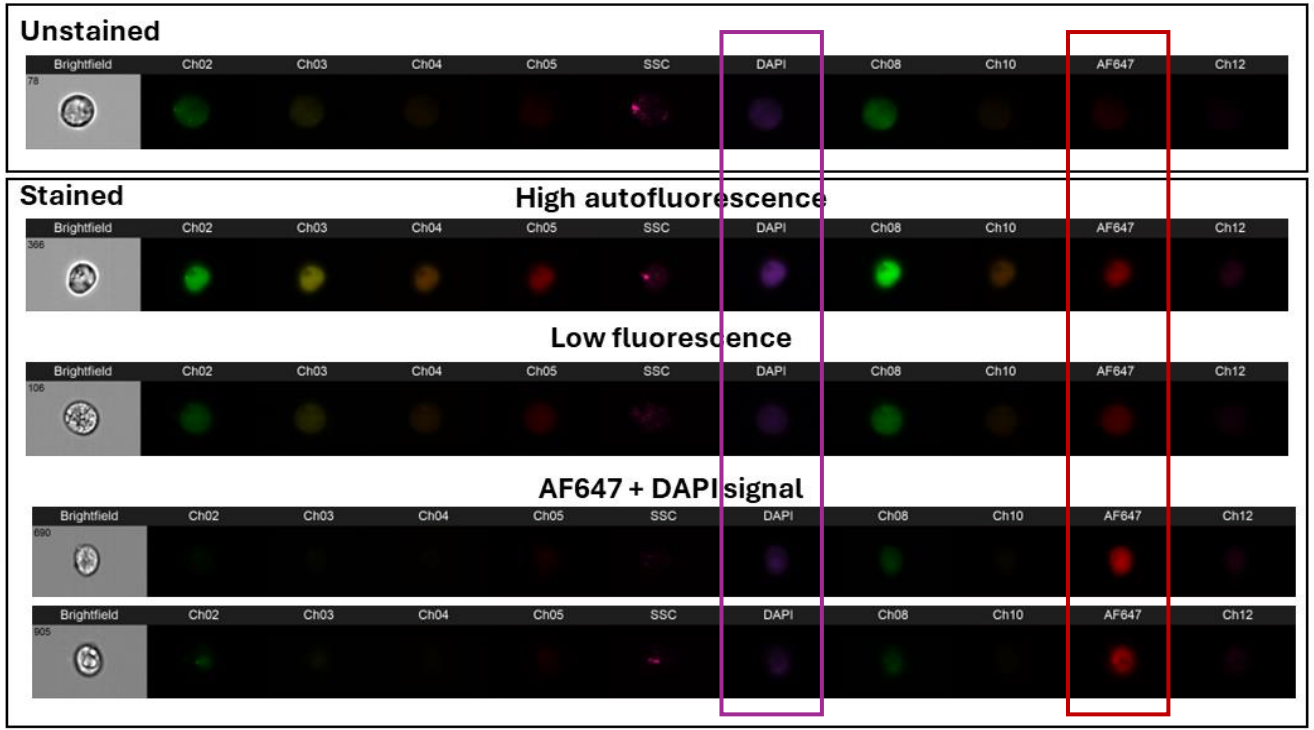
2. Fluorescence-activated cell sorting (FACS)

3. DNA amplify and sequence single cells



4. Obtain more informative markers for population genetics

Imaging flow cytometry

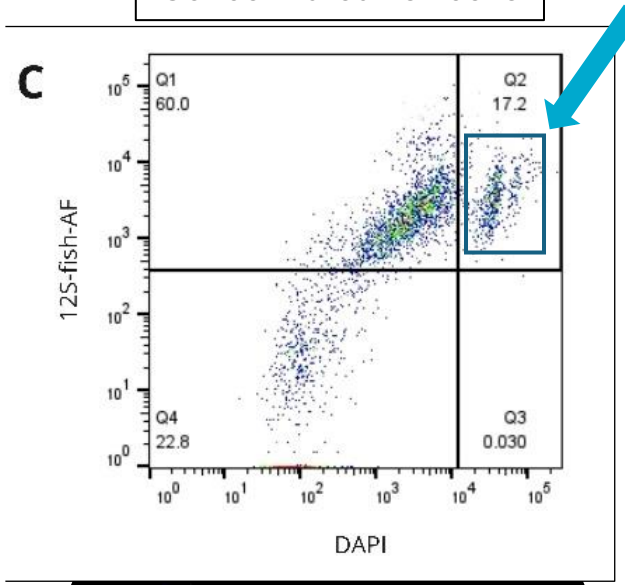


DAPI and probe positive without strong autofluorescence signals

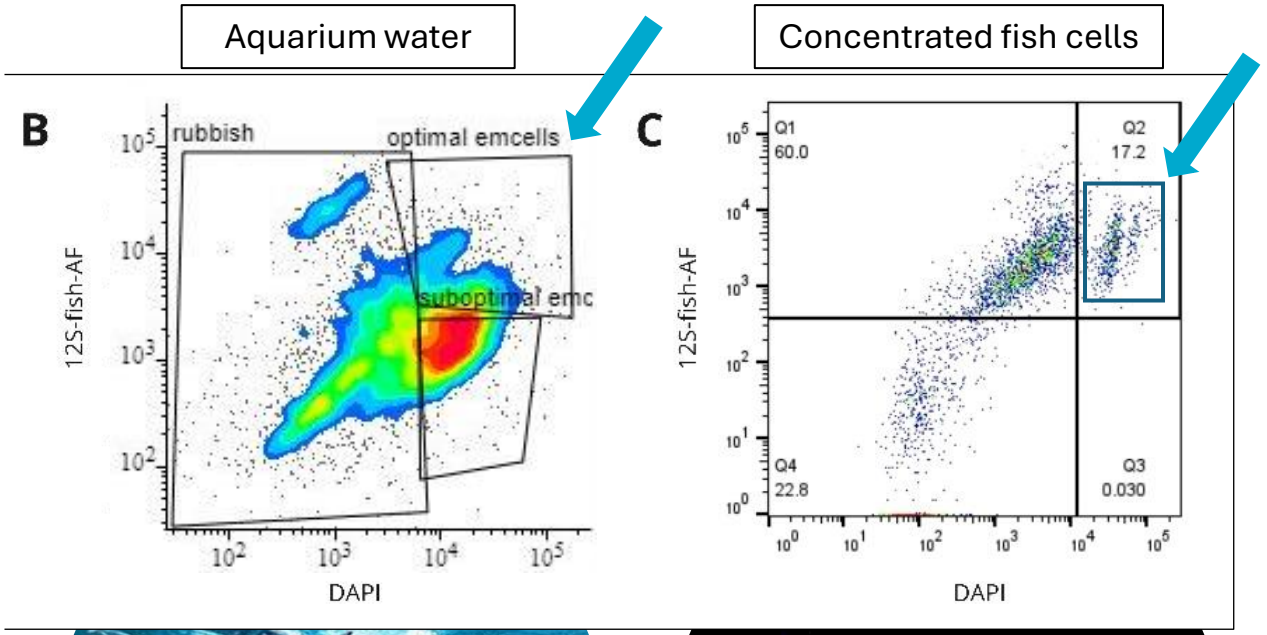
Power *et al.* 2025 Isolation and genetic identification of metazoan cells from seawater. *Under review*.

emCell FACS

Concentrated fish cells



emCell FACS

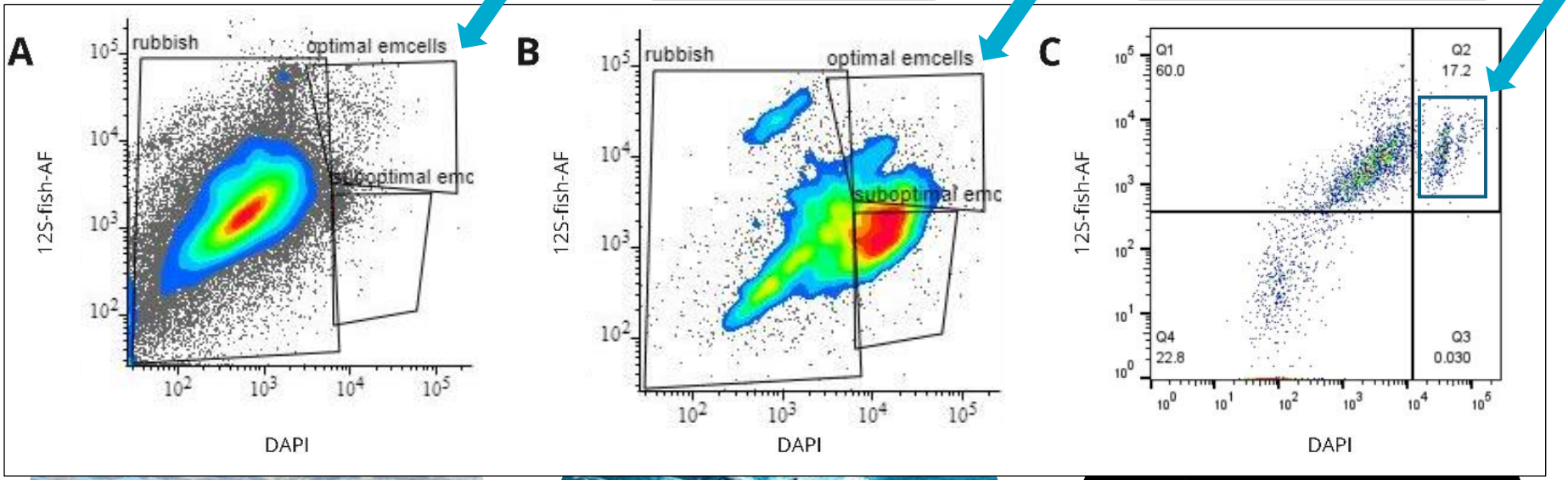

Aquarium water
Concentrated fish cells
B
rubbish
optimal emcells
suboptimal emc
12S-fish-AF
DAPI
C
Q1
60.0
Q2
17.2
Q4
22.8
Q3
0.030
12S-fish-AF
DAPI

emCell FACS

Marine water

Aquarium water

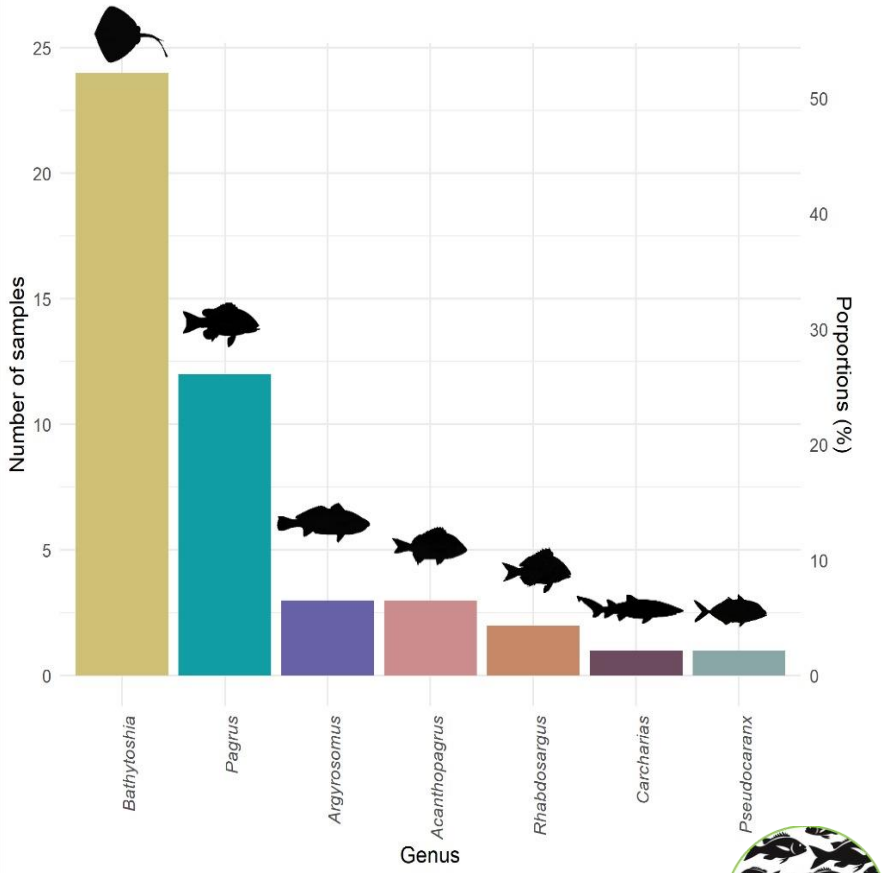
Concentrated fish cells



Isolating emCells

Aquarium of Western Australia (AQWA)

- 3 million L mesocosm
- 38 species of bony fish, sharks and rays
- Two targets: 16S and 18S rRNA
- 25% (n = 49) success rate



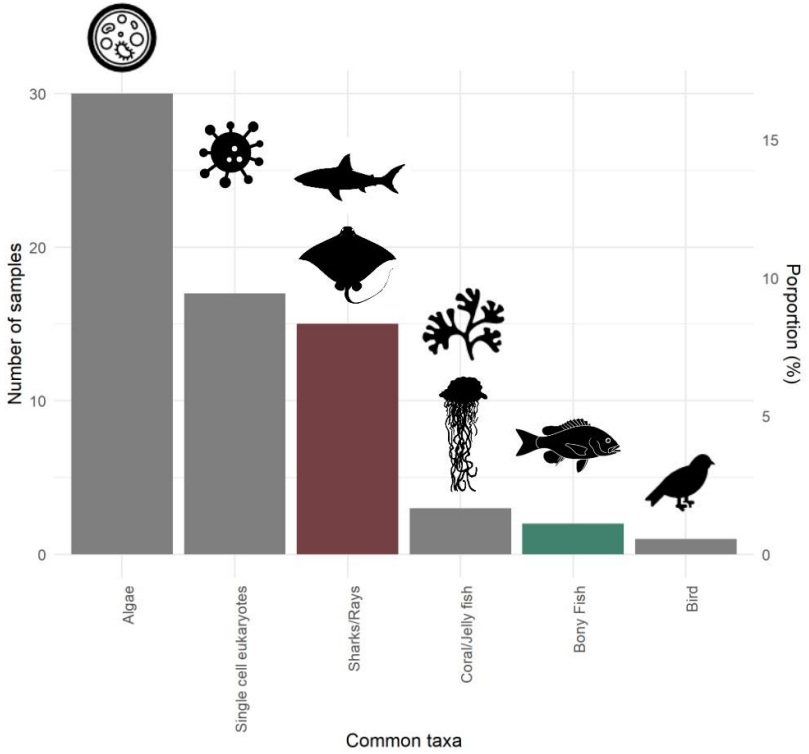
Power *et al.* 2025 Isolation and genetic identification of metazoan cells from seawater. *Under review*.

Off-targets

Autofluorescent cells in the same size range

- **Algae!**
- Single cell eukaryotes
- Coral and jellyfish
- Birds

Working on increasing specificity



Power *et al.* 2025 Isolation and genetic identification of metazoan cells from seawater. *Under review.*

Single cell analyses

Once you have enriched eDNA samples or emCells you can do:

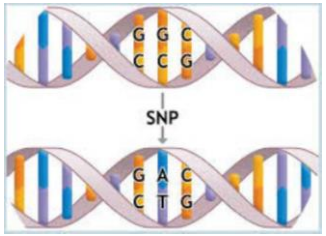
- Multilocus genotyping
- Individual identification
- Mark-recapture population abundance
- Gene flow
- Population structure
- Migrant detection
- Sex ratios
- And more.....

Single cell analyses

Once you have enriched eDNA samples or emCells you can do:

- Multilocus genotyping
- Individual identification
- Mark-recapture population abundance
- Gene flow
- Population structure
- Migrant detection
- Sex ratios
- And more.....

Population genetics 101



- SNP’s (Single Nucleotide Polymorphisms)
- Can use to identify individuals
- Normally need tissue samples
- Difficult from eDNA since they are not linked
- Single cells maintain these linkages

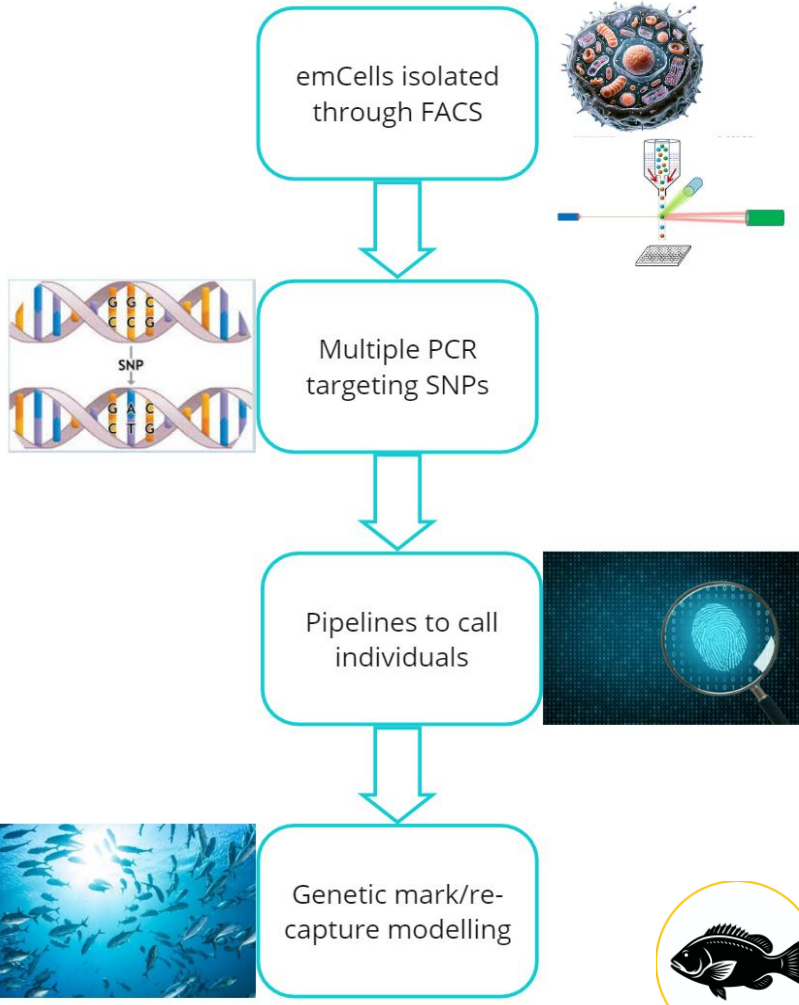
Single cell SNP analysis

Experimental set up

- Tissue samples and single cells
- SNP assay
- Bioinformatic pipeline

Results

- >91% successful
- Minimum 60% allele coverage needed



Power *et al.* 2025 Identification of individual fish by SNP genotyping single environmental cells – emCells. *Under preparation*.

eDNA + Beyond presence-absence

- We can get more from eDNA that just presence-absence!
- Since we can enrich eDNA samples now, we can
  - Reduce background noise
  - Enrich target acquisition
  - Single-cell sequencing is feasible
- Enable non-invasive methods to obtain richer information from environmental samples

Exciting future potential!

Thank you

"Using genomics, bioinformatics and nano-technologies, Environomics is finding new resources in nature and reinventing how we measure and monitor ecosystem health, change and threats" – Olly Berry

Funding

BIOPLATFORMS AUSTRALIA

CSIRO

ENVIRONOMICS
future science platform

Collaborations

THE UNIVERSITY OF WESTERN AUSTRALIA

Translational Cancer Pathology Laboratory & Western Australian Zebrafish Experimental Research Centre

aqwa
THE AQUARIUM OF WESTERN AUSTRALIA

Diversity Arrays Technology Pty Ltd

agrf

National Imaging Facility

The CMCA - Australian Microscopy, Characterisation & Analysis

The University Of Queensland AUSTRALIA
AIBN
Australian Institute for Bioengineering and Nanotechnology

Trau Lab

Curtin University

TrEnD Lab