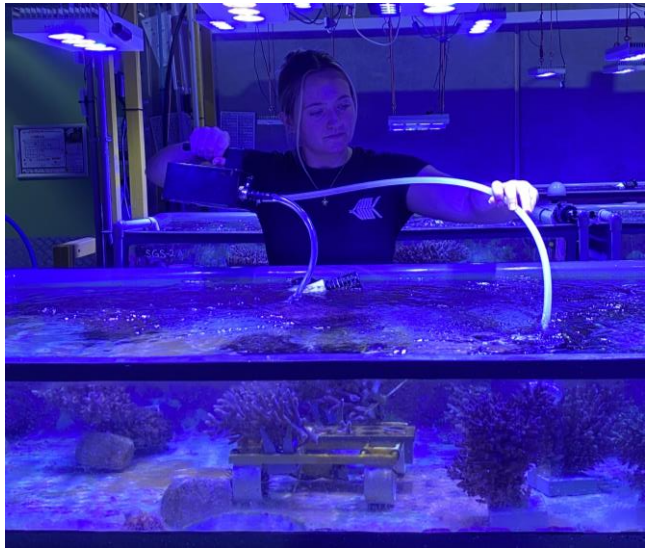

Investigating eDNA to expose silent infiltrations of Acropora-Eating Flatworms (*Prosthiomum acroporae*) in coral aquaculture

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Clare Grimm[1], Yui Sato[1,2], Jonathan Barton[1,2], David Bourne[1,2], Jason Doyle[1]

[1]Australian Institute of Marine Science

[2]James Cook University

[3]The National Sea Simulator, AIMS

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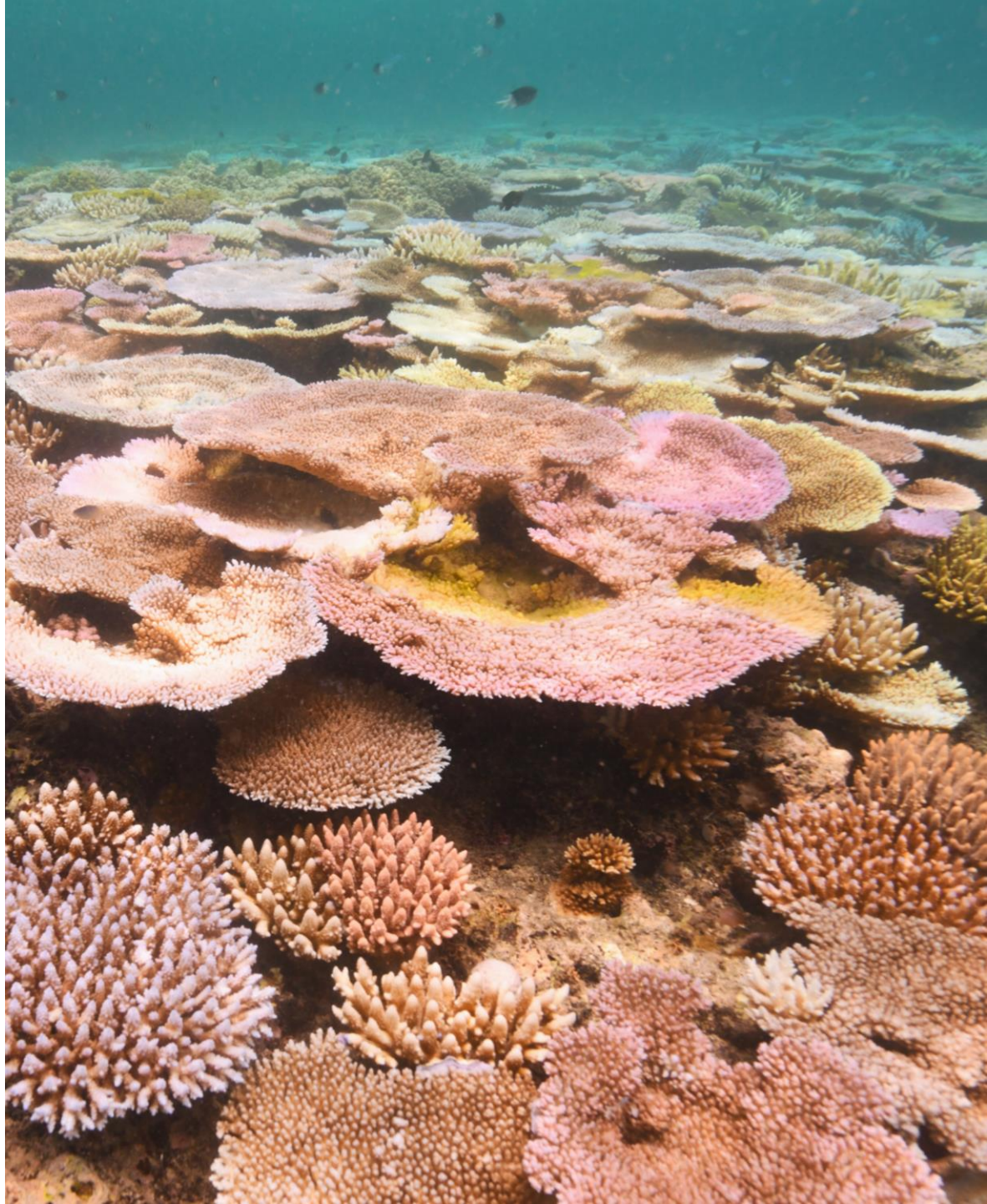
I acknowledge the Bindal and Wulgurukaba Peoples as the traditional owners and custodians of the land and sea countries that our studies take place. I pay my respects to Elders, past, present and emerging generations.

The State of Coral Reefs

The Great Barrier Reef is globally recognized for its environmental, social, economic, and cultural significance

Coral Reefs around the world are increasingly undergoing anthropogenic threats.

Future projections of coral reef health is predicted to decline – intervention is needed.



G. Frank, Australian Institute of Marine Science

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RRAP Coral Aquaculture and Deployment

This intervention aims to seed aquaculture-grown corals onto the Great Barrier Reef (GBR) using human assisted delivery methods.

RRAP goal: 10 million – 100 million corals deployed on the GBR per year.

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RRAP Coral Aquaculture and Deployment – Coral Seeding

1) Gravid Coral Broodstock Collection
Annually ~Oct.

3) Coral Larvae Culture
5-7 days post spawning

5) Coral Device Assembly
1-3 days

2) Coral Spawning
2-7 days post full moon

4) Coral Recruit Settlement
7-14 days growth

6) Coral Deployment
~1 month after spawning event

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Large-scaled coral aquaculture challenges

Diseases in young corals

Pests on coral broodstock

Genetic diversity of offspring corals

Quality Assurance and Quality Control (QA/QC) team

David Bourne

Jason Doyle

Yui Sato

Ramona Brunner

Elena Pfeffer

Jonathan Barton

Clare Grimm

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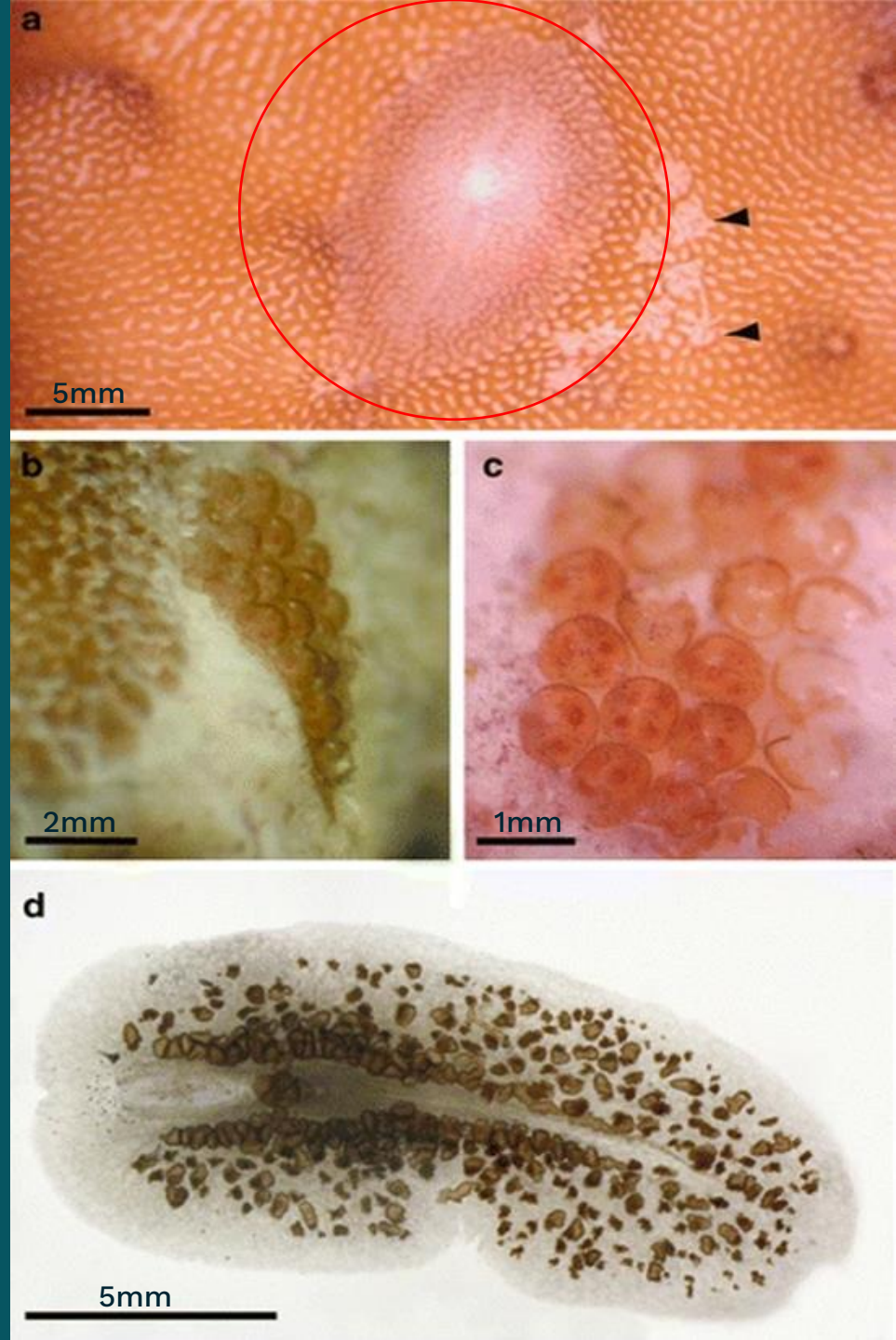
Acropora coral-eating flatworm (AEFW)
(*Prostiostomum acroporae*)

Consequences of Predation on *Acropora* hosts:

- Irreversible tissue damage
- Rapid tissue necrosis (RTN)
- Ultimately colony mortality if left unchecked

Aquaculture Control Challenges:

- High fecundity
- Cryptic camouflage (a)
- Small bodied (d)
- Complex life history traits (intracapsular larva) (b/c)



Rawlinson, 2011

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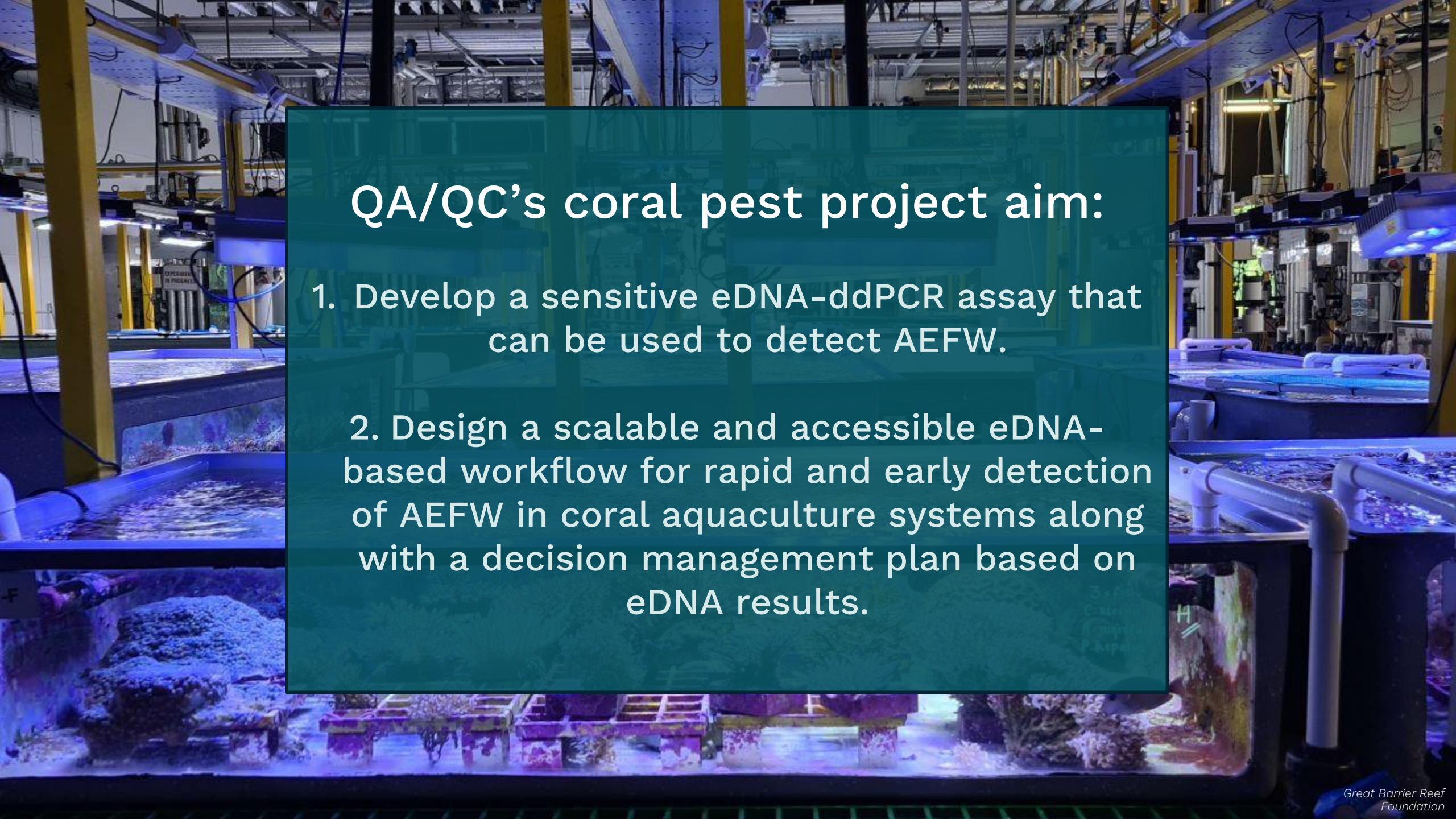

QA/QC's coral pest project aim:

1. Develop a sensitive eDNA-ddPCR assay that can be used to detect AEFW.

2. Design a scalable and accessible eDNA-based workflow for rapid and early detection of AEFW in coral aquaculture systems along with a decision management plan based on eDNA results.

Great Barrier Reef Foundation

eDNA Assay Development

Collected *Acropora*-associated flatworms (*Prosthiostomum* sp.) for DNA extraction and sequencing.

Using Geneious Prime, we designed specific, PCR F&R primers, with a hydrolysis probe, targeting a consensus section of the mtCOI gene (192bp.)

```
1   cataaagata ttggtacktt atatttarty ttaggkgtat gggcaggmtt kataggyaca
61  ggtttmagwt ttataattcg tttagaactm ggtcaacccg gwagsctttt mcaraaatct
121 caaatatata atagarttat tacggcacat ggtttagtaa tgatattttt tttkgtaatg
181 cctgttatga taggwggktt tggwaattga ttaattccta twtatatggg agttgcygat
241 atgaayttyc cacgrttaaa taatttwagt ttatgattat taatgccttc rgtaacwtta
301 ttaataggtt cyttctttgt wagaaaagga gtaggaggrg gwtgaacmat atatcctcca
361 ctatcwagaa aaatagctca cgckggaagw agtgttgatt tagcaatatt tgcyttacac
421 ttagcwggyg tgagrtctat tttaggwtcw attaatttta ttagtacagt wggwnaaagt
481 aataaggggg gaatggcatg ataycgttty ccwttattta tatgagcwat gackataaca
541 gcatatatgt tagtattatc wttaccagtw ctagctgcag gaattacwat gttattaacs
601 gatcgaaaat ttaaaacaac atttttgat ccttcaggkg gaggtgatcc wattttattt
661 caacatattt tctgattttt g
```

Consensus sequence for Prosthiostomum acroporae (AEFW) used for designing specific primers and probe for ddPCR.

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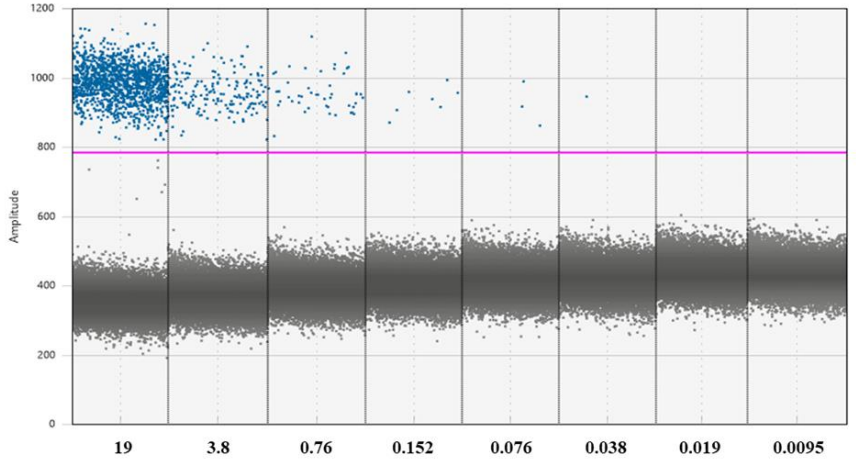
eDNA Assay Development

ddPCR validation and testing done on the Bio-Rad QX200 ddPCR system.

ddPCR optimization included:

- **Annealing/extension temperature**
- **Primer and probe concentration**

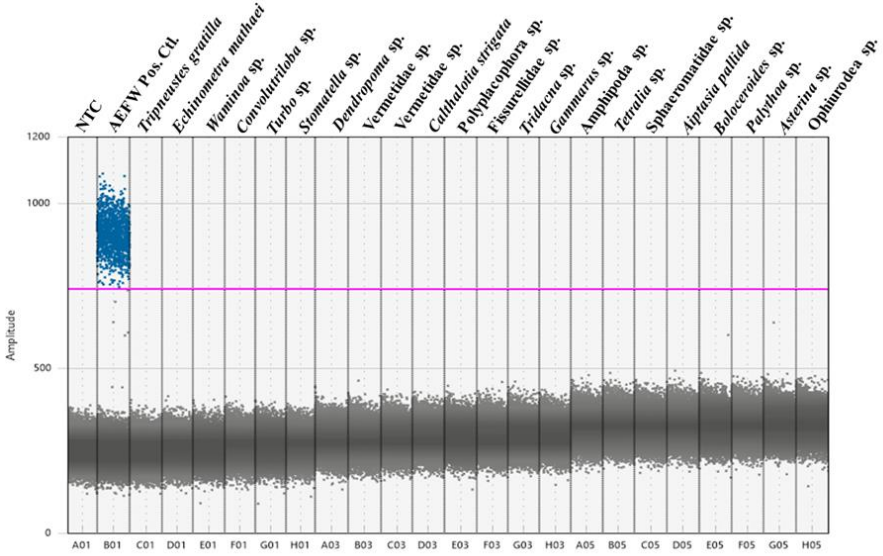


ddPCR assay sensitivity

Dilution series of AEFW gDNA, with concentrations ranging from 19 pg to 0.0095 pg per reaction

Concluded LOD:

0.0152 pg/µL (2.26 ± 1.18 copies per ddPCR).

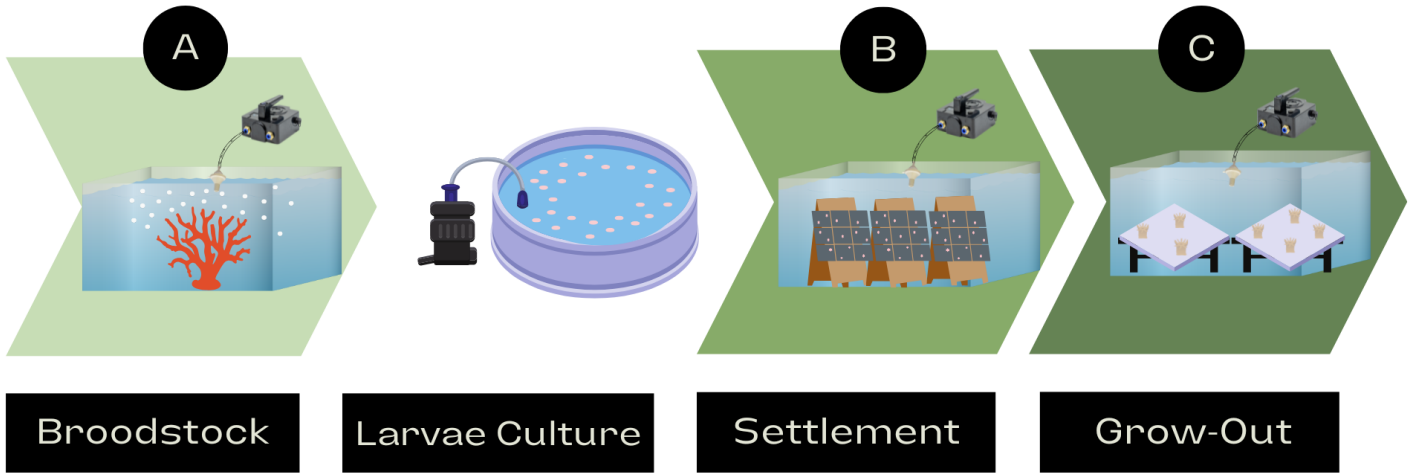


ddPCR assay specificity

DNA extracted from non-target marine invertebrate species found within the AIMS Sea Simulator aquarium facility.

No amplification of any non-target species above the positive control threshold.

eDNA Application to Coral Aquaculture



- 10L seawater samples filtered through eDNA pump (Grover) onto 1.2um MCE membrane filter.
- eDNA samples extracted using the Qiagen Blood and Tissue kit using the QiaCube robot.
- Extracted eDNA samples were analysed with optimized ddPCR assay.

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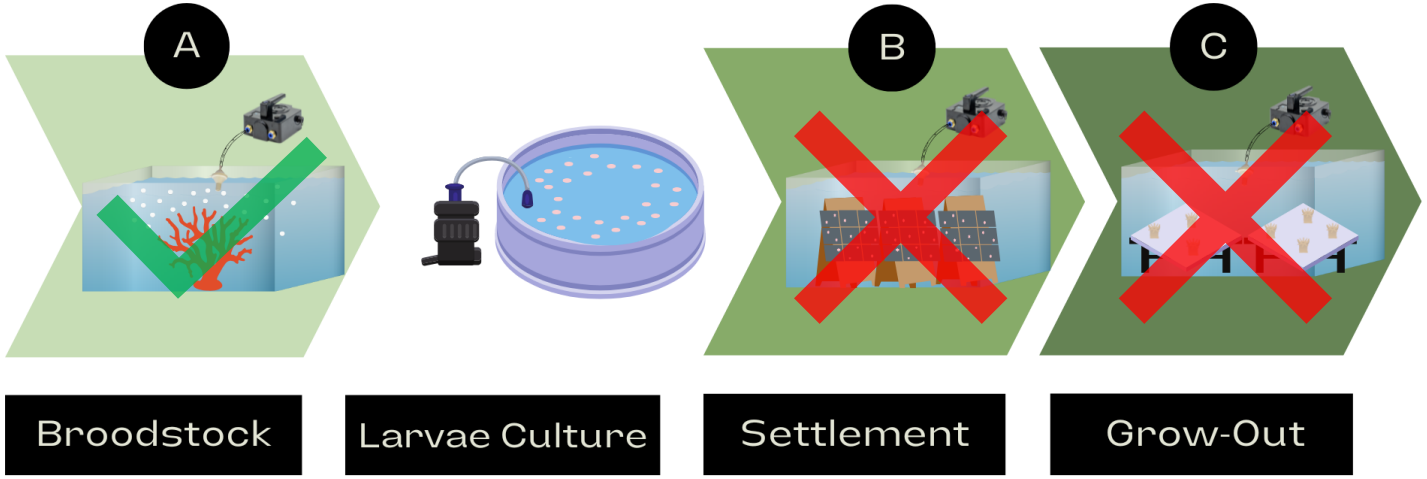
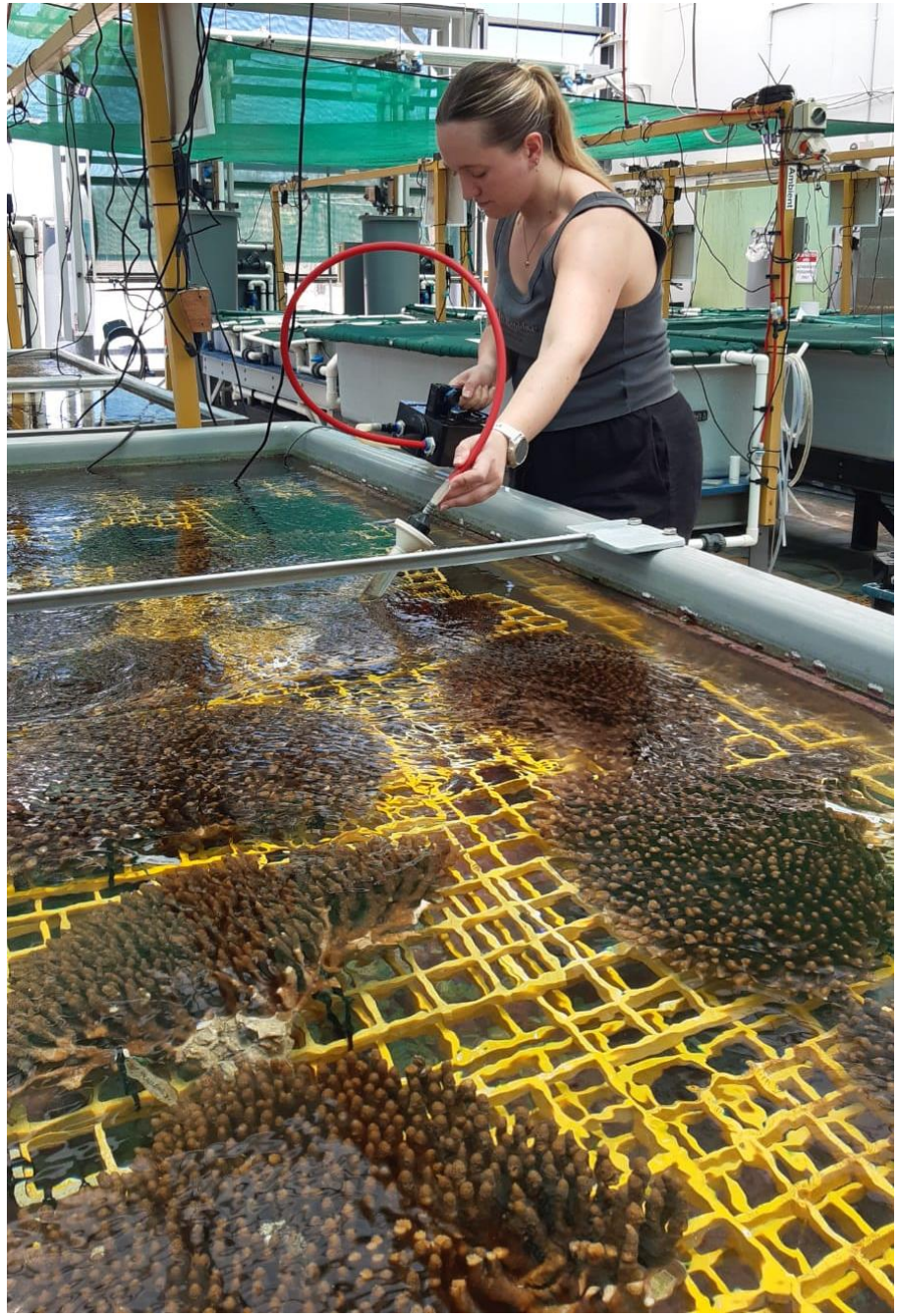
eDNA Application to Coral Aquaculture

- NO AEFW eDNA detected in the coral settlement tanks or coral grow-out tanks – expected.
- Positive AEFW eDNA detection in 2/3 brookstock tanks
  - *Acopora millepora*
  - *Acropora loripes*
- Observations of Rapid Tissue Nerosis (RTN) alongside AEFW predation and eggs in *A. loripes* brookstock.





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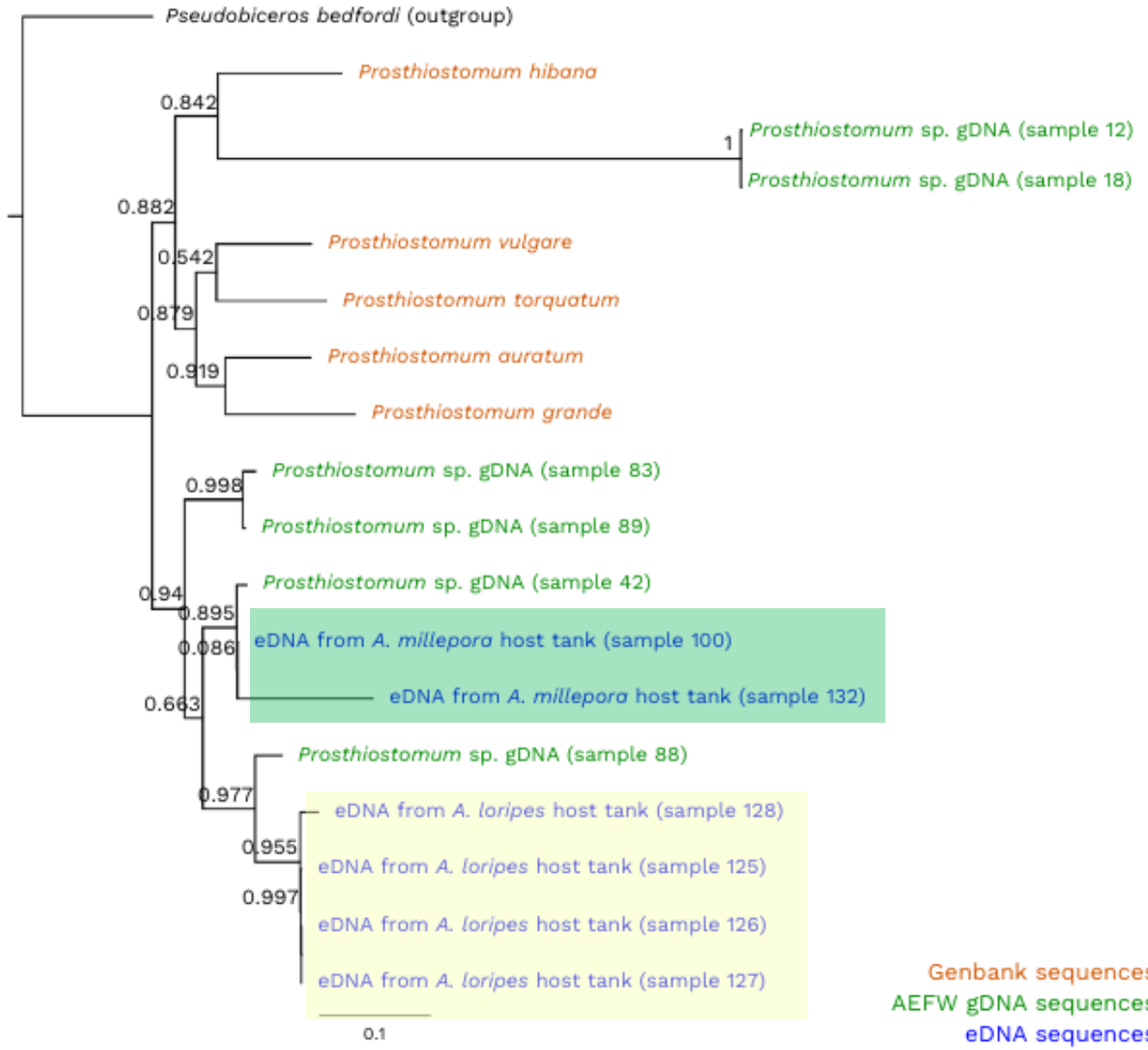
eDNA Application to Coral Aquaculture

Coral Host Species	Sample Date	Mean copies per ddPCR	SD	AEFW detection*
A. millepora	5-Dec-23	5.1	5.2	Yes
	14-Dec-23	3.0	0.6	Yes
A. spathulata	5-Dec-23	0.7	0.7	No
	14-Dec-23	0	0	No
A. loripes	8-Dec-23	0	0	No
	14-Dec-23	3.2	4.9	Yes

*above LOD (2.26 ± 1.18 copies per ddPCR)



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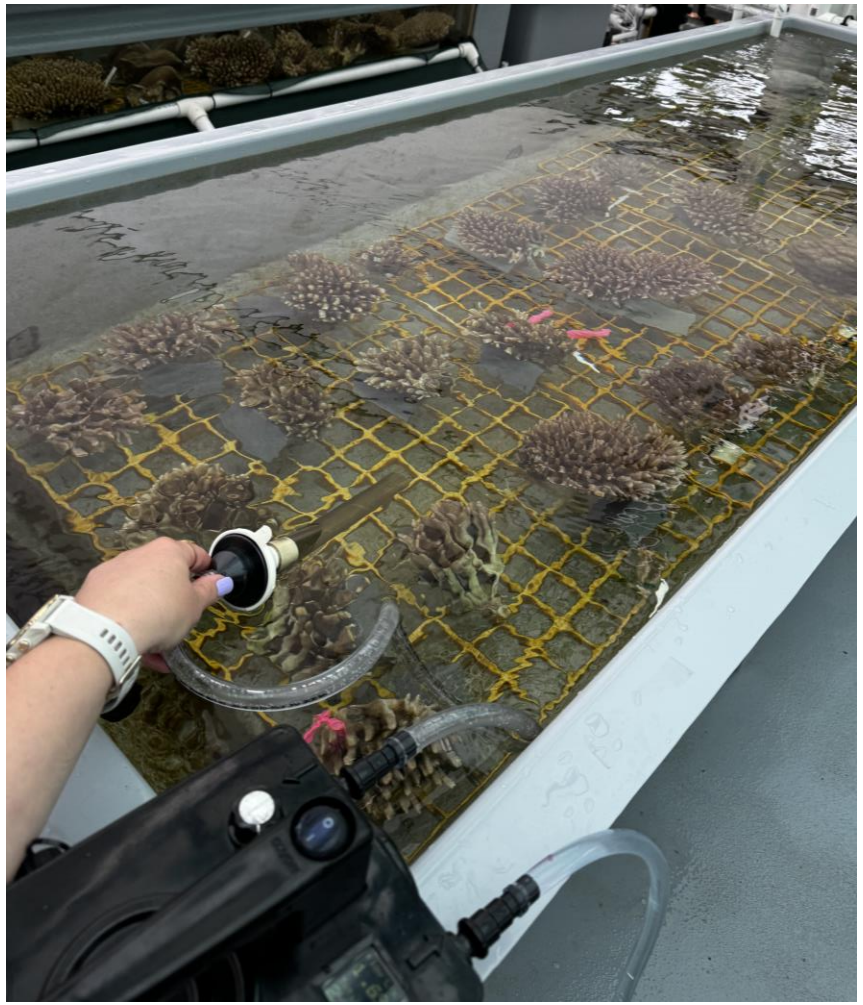
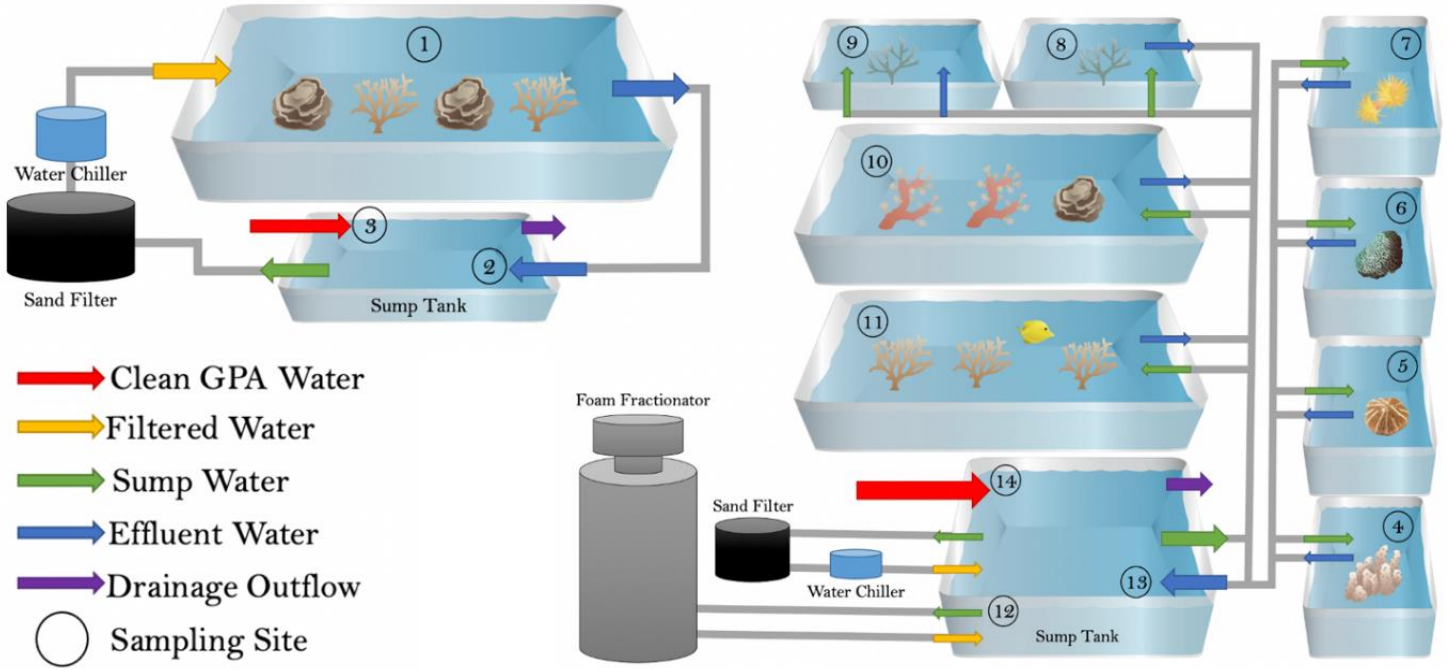
eDNA Application to Coral Aquaculture – Future Research



- AEFW eDNA shedding and decay studies
- Understanding the ecology of AEFW eDNA in coral aquaculture systems
  - Effect of systems designs
  - Location of sample points



	Scalability	Accessibility
eDNA sample collection *(Grover eDNA sampler)*	?	✓
eDNA extraction method *(QiaCube)*	?	?
eDNA analysis *(Bio-Rad ddPCR)*	✗	✗

Future QA/QC Workflow Projections...

eDNA sample collection *(Subsurface Automated Sampler[1])*	✓	✓
eDNA extraction method: *(bead-based automated extraction[2])*	✓	?
eDNA analysis: *(LAMP-assay and dipstick detection[3])*	✓	?

Formel et al (2021)[1]
QA/QC SOP (unpublished)[2]
Doyle & Uthicke (2021)[3]

Australian Government | Australian Institute of Marine Science

RRAP

OUR MISSION: SAVE EARTH'S REEFS

Acknowledgement – thank you.

Thank you to AIMS and the SeaSim staff.

Special thanks to the QA/QC team: David Bourne, Yui Sato, Jason Doyle, Ramona Brunner, Jonathan Barton, Elena Pfeffer, and wonderful students and volunteers.

We extend our deepest respect and recognition to all Traditional Owners of the Great Barrier Reef and First Nations Peoples globally holding the hopes, dreams, traditions and cultures of this world.

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