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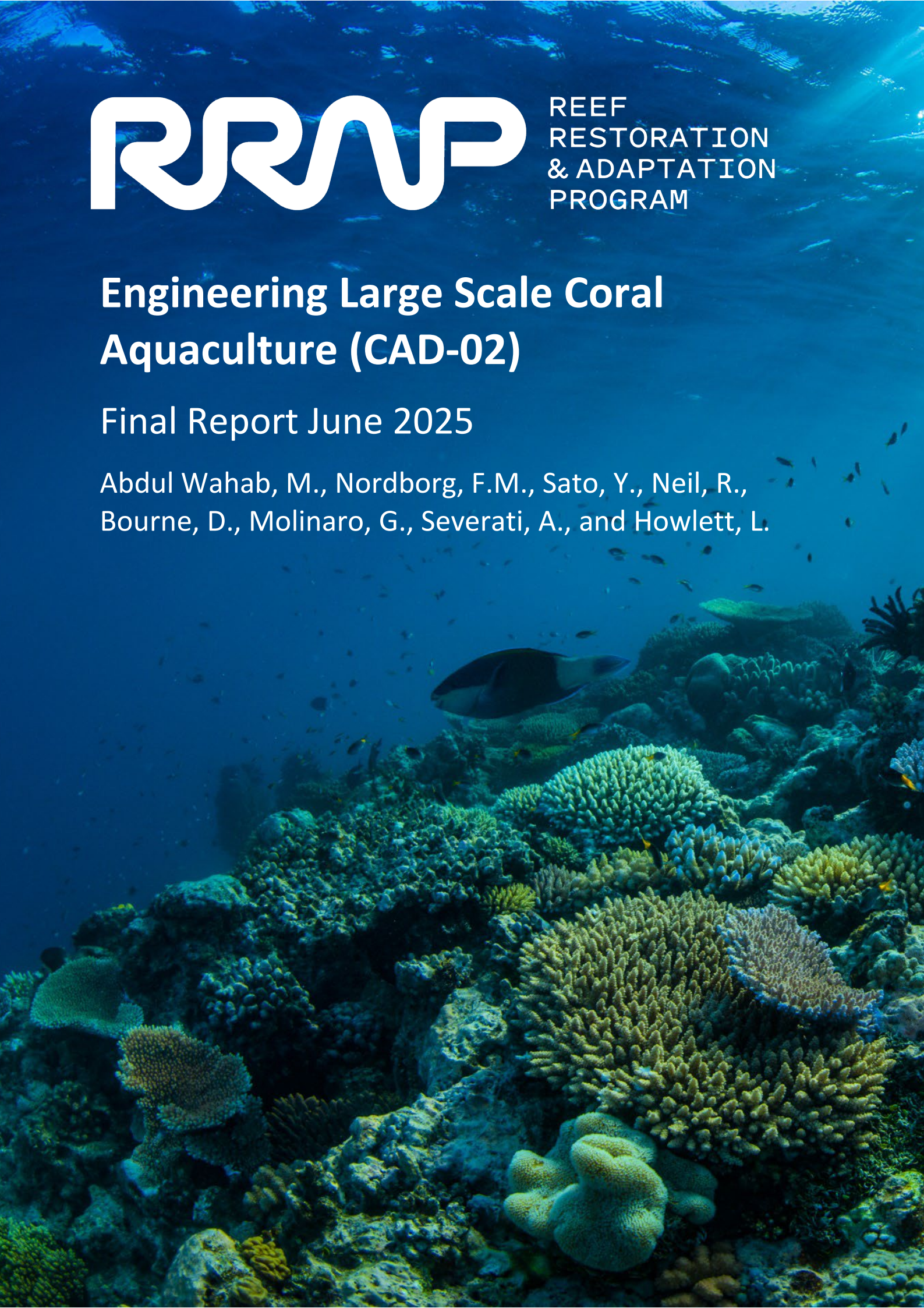
Engineering Large Scale Coral Aquaculture (CAD-02)

Final Report June 2025

Abdul Wahab, M., Nordborg, F.M., Sato, Y., Neil, R., Bourne, D., Molinaro, G., Severati, A., and Howlett, L.



RRAP Engineering Large Scale Coral Aquaculture (CAD-02) Final Report June 2025

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This report summarises work undertaken under *Engineering Large Scale Coral Aquaculture (CAD-02)* in accordance with the Reef Restoration and Adaptation Program's *Coral Aquaculture and Deployment* Project Agreements. It provides a summarised, point-in-time synopsis of activities, methods, findings and outcomes completed in accordance with the approved project scope up to 30 June 2025.

All information reflects project scope and outcomes as of May-June 2025. Subsequent updates, analyses, or scientific developments are not included. This report should be read alongside any associated and publicly available technical reports, datasets, and publications for full detail. This report does not provide scientific inferences, policy guidance or operational instructions beyond the project's defined scope and duration.

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The RRAP partners acknowledge Aboriginal and Torres Strait Islander Peoples as the first marine scientists and carers of Country. We acknowledge the Traditional Owners of the places where RRAP works, both on land and in sea Country. We pay our respects to elders; past, present, and future; and their continuing culture, knowledge, beliefs, and spiritual connections to land and sea Country.

We specifically acknowledge and thank the following Traditional Owners of sea Country that this report relates to:

Location	**Traditional Owner Group**
AIMS Cape Cleveland	Bindal
Davies Reef cluster	Bindal
Townsville (south of Ross River)	Bindal
Palm Islands group	Manbarra
Townsville (north of Ross River)	Wulgurukaba

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1 Executive Summary

The overarching objective of the RRAP Project Engineering Large Scale Coral Aquaculture (CAD-02) within the Reef Restoration and Adaptation Program (RRAP) was to enhance the efficiency, scalability, and cost-effectiveness of culturing both asexually and sexually derived coral propagules for reef restoration applications. Research efforts focused on optimising aquaculture processes by drawing on established practices from commercial aquaculture industries and building on prior coral spawning research conducted at the Australian Institute of Marine Science (AIMS) and National Sea Simulator (SeaSim) in Townsville. To address the various challenges associated with large-scale coral propagation, the research was structured across three integrated sub-projects:

- **CAD-02.1:** Coral aquaculture facility prototype and design
- **CAD-02.2:** Companion species and co-culture methods
- **CAD-02.3:** Health assurance and quality control

Throughout the first phase of RRAP, the CAD-02.1 Sub-project has led to substantial advancements in coral aquaculture system design and production capacity. The newly developed systems can produce up to seven million coral eggs per system, per night and can achieve settlement densities of up to 16,000 settlement units per square metre. Designed for compatibility with automation, these systems support streamlined operations and greater scalability. Both sea and land transport options have been successfully trialled, confirming logistical flexibility, and a prototype facility has been established as a feasible model for future expansion.

The CAD-02.2 Sub-project focused on refining co-culture methods to improve coral production in a cost-effective manner. Results showed that species-specific combinations of microherbivores yield better outcomes; for example, small gastropods were most effective when paired with Acroporid corals, while juvenile urchins showed better compatibility with *Goniastrea* species. Among grazing fish, those with a cropping grazing strategy provided the optimal trade-off between algae control and minimising recruit mortality. It is recommended that fish be introduced into co-culture tanks only after coral recruits have reached the 4/5 polyp stage to reduce the risk of early-stage mortality. Furthermore, fish-derived dissolved wastes were found to benefit more autotrophic coral species, potentially due to the uptake of nitrogen and phosphate by their photosymbionts.

The CAD-02.3 Sub-project contributed key insights into coral genetics, eukaryotic pest and microbial factors towards ensuring the success in producing healthy coral progenies in aquaculture for conservational success. It was found that parent genotypes play a major role in shaping offspring diversity, highlighting the importance of broodstock genotyping. In addition, droplet digital polymerase chain reaction (ddPCR) techniques based on environmental deoxyribonucleic acid (eDNA) contributed to the development of model quality-control assays, enabling the sensitive detection of coral-eating flatworms, total bacterial load and disease-associated *Vibrio* bacteria. Notably, extremely low concentrations of total bacteria in aquaculture systems were predictive of potential coral health compromise along with increased *Vibrio*. Machine-learning models using this data demonstrated the potential use for early risk identification and management of disease threats in coral culture environments.

Future research in coral aquaculture should prioritise the optimisation and scaling of *ex situ* production systems to enhance coral survival during cultivation and post-deployment. Key areas for development include rapid phenotyping tools to improve broodstock selection, expansion of automated monitoring systems for larval and recruit stages, and refinement of high-density grow-out techniques that maximise facility efficiency without compromising coral health. To address the seasonal limitations of coral spawning, research into buffering strategies such as engineered larval storage substrates and out-of-season spawning protocols is recommended. These innovations would enable year-round production and deployment, increasing operational flexibility and reducing reliance on short, natural spawning windows.

Further investigations are needed to refine co-culture strategies, focusing on the functional roles of different companion species in supporting coral growth and survival. Mixed-species combinations should be assessed for both performance and pest control effectiveness, with an emphasis on developing robust biosecurity protocols to mitigate potential risks to reef ecosystems. In parallel, advancing health assurance and quality control will require deeper exploration of microbial indicators, including *Vibrio* spp., as early warning signals of compromised coral health. Studies towards the development of rapid, easy-to-use diagnostic assays and genetic tools for assessing broodstock diversity and tracking offspring will be essential for both production efficiency and ensuring deployed corals are genetically diverse. Together, these research priorities form a pathway toward reliable, scalable, and ecologically responsible coral aquaculture for restoration applications.

2 Background and Justification for the Research

CAD-02.1: Coral aquaculture facility prototype and design

Prior to the commencement of the Reef Restoration and Adaptation Program (RRAP) in 2021, the feasible scale of coral propagation, both sexual and asexual, limited the scope of reef restoration research and implementation of interventions. Up to this point, methods were not able to supply coral at the scale necessary for ecologically relevant restoration outcomes. Therefore, a step change in scalability of coral aquaculture was needed, requiring high density coral aquaculture methods to efficiently and cost-effectively propagate and deploy corals recruits at medium to large scales. Through the CAD-02.1 Sub-project, refinement and optimisation of processes developed underpin improved production cost estimates and tested designs for high throughput, cost-effectiveness and health assurance of corals at scale.

The design for upscaled production was based on the following assumptions, which formed the basis of CAD-02.1 research objectives:

- A focus on up to three target coral species across multiple reproductive modes.
- Operator-free gamete handling, from spawn capture to free-swimming larvae.
- A combined production model, integrating sexual and asexual techniques in a versatile design approach of the grow-out facility.
- The innovation of a division of settlement and deployment substrates.
- A short captive grow-out period (three to twelve weeks) and early deployment, to minimise the loss of diversity due to selective pressures in the captive environment.
- Minimise adverse domestication processes throughout the life cycle of the cultured species.

CAD-02.2: Companion species and Co-Culture methods

Corals follow a Type III survivorship pattern, characterised by high mortality in early life stages and low survival rates to adulthood. Therefore, post-settlement survival, particularly during the first six months, is a critical bottleneck for coral propagation.

Co-culturing, a common and important technique in aquaculture, has been successfully applied to improve survival and growth of coral spat and juveniles. Previous studies have demonstrated that co-culturing of *Acropora* spat with juvenile sea urchins significantly increases survival, with coral survival rates increasing by an order of magnitude at the highest urchin densities. In these systems, corals exhibited significantly faster growth, reaching size-escape thresholds earlier (Craggs et al. 2019). More recently, similar benefits have been observed in co-culturing trials using gastropods, resulting in higher (>26 times) survival rates for coral juveniles reared in in the National Sea Simulator (SeaSim). In synergy with the engineering and logistic developments, co-culture will contribute to cost-effective coral production at medium to large scales.

These promising results, however, had largely been demonstrated at small scales. For co-culture to support medium- to large-scale restoration efforts within RRAP, further research was required to adapt and optimise these systems for integration into high-throughput coral aquaculture facilities. RRAP Sub-project CAD-02.2 aimed to address these knowledge gaps via the following objectives:

- Develop knowledge and methods to underpin co-culture practices that enhance early coral survival and growth during the aquaculture phase of propagation.
- Identify the key drivers of early coral survival in controlled aquaculture environments, with a particular focus on understanding the role of co-cultured organisms and their interactions with coral recruits.

- Evaluate critical co-culture parameters, including stocking density, organism size, behavioural interactions, and system engineering considerations (e.g. hardware configuration, water flow, and substrate design), to inform optimal integration into the prototype-scale aquaculture production system.

CAD-02.3: Health Assurance and Quality Control

Strict health and quality assurance protocols are essential in coral aquaculture, particularly when propagated corals are intended for release into natural reef environments as part of conservation or restoration efforts. Ensuring the health, genetic integrity, and pathogen-free status of cultured corals is critical to avoid introducing risks to already vulnerable reef ecosystems.

A comprehensive quality assurance and quality control (QA/QC) framework is therefore required to ensure that propagated coral stock possess the desired genetic traits and remain below defined thresholds for pests and pathogens. However, the development and implementation of such a program presents several challenges. One of the primary limitations is the current lack of detailed understanding of coral genetic diversity and the specific genes associated with key physiological traits such as stress tolerance and disease resistance. Moreover, our knowledge of the biotic (e.g. microbial communities, symbionts) and abiotic (e.g. water chemistry, temperature fluctuations) factors contributing to coral disease remains limited.

To effectively implement a QA/QC system in coral aquaculture, the development of targeted diagnostic assays is necessary. These assays must provide reliable and informative metrics to assess genetic diversity and detect pathogen or pest presence at biologically relevant thresholds. Given the resource constraints and complexity of this task, the CAD-02.3 Sub-project aimed to adopt and adapt established diagnostic and scientific approaches from other fields to suit the specific requirements of coral aquaculture.

The initial suite of diagnostic assays focussed on coral species identified as priorities within the aquaculture program, as well as previously recognised pathogens and pests. The methods were designed with flexibility in mind, allowing for adaptation and expansion to accommodate newly identified organisms of concern arising from this sub-project or related work under the RRAP Enhanced Corals and Treatments (ECT) and Coral Aquaculture and Deployment (CAD) Sub-programs.

3 Research Objectives and Key Findings

A current list of project outputs are listed on the RRAP website: gbrrestoration.org. Key research objectives and findings are detailed below.

Table 1: Key findings of the Project aligned to the overarching and specific research questions for each sub-project.

Objective		Key Findings and/or Outcomes
1. Sub-project: CAD-02.1: Coral aquaculture facility prototype and design		
1 (a)	Applying knowledge developed in other areas of the sub-programs to base the design of a prototype aquaculture production facility capable of automated large output of high-quality corals.	CAD synthesis paper - Sub-project CAD-02.1 has continuously integrated learnings from other RRAP teams and sub-programs when developing and testing new coral aquaculture systems, and collaborated with representatives from across the RRAP Coral Aquaculture and Deployment (CAD), Enhanced Corals and Treatments (ECT), Cryopreservation (CP) and Translation to Deployment (T2D) Sub-programs to ensure systems are fit for purpose and compatible with methods and processes developed in other sub-projects. - The systems developed by the Sub-project CAD-02.1 have been used in the production of a prototype, containerised aquaculture facility, the ReefSeed. Additionally, systems (AutoSpawners, 500 litre (L) larval culture tanks, mass settlement tanks and recruit rearing tanks) are currently in production and will be used as a prototype, high-throughput aquaculture production facility (with a planned production of 60,000 deployment devices during the 2025 summer coral spawning event) to support the Pilot Deployments Program (PDP). - A review of efficiency and scaling gains from the development and implementation of CAD technologies into a prototype production facility (the National Sea Simulator as a testing bed), showed the potential for a 1.4- to 28.6-fold increase in production per unit area of facility, compared to pre-innovation scenarios (Abdul Wahab et al. in submission).
1 (b)	Systems and methods for the automated handling and processing of coral gametes, including collection, fertilisation, rearing and settlement of spawning and brooding species.	- A modular coral aquaculture system, the AutoSpawner, for automated holding of broadcast spawning hard corals and harvesting, fertilisation and cleaning of their eggs was developed and tested. Testing showed that coral larvae produced using the AutoSpawner were of equal quality to larvae produced using traditional, manual methods while reducing the amount of time and labour required to produce a given quantity of larvae (Severati, Nordborg, et al. 2024). The system has been successfully

Objective	Key Findings and/or Outcomes
	used with nine hermaphroditic (individual coral colonies produce both eggs and sperm) and one gonochoric (individual coral colonies produce only eggs or sperm) species to date. - The fertilisation and egg cleaning-module from the AutoSpawner system (the AutoFertiliser; Severati, Nordborg, et al. 2024) was adapted and tested for use as an independent fertilisation system for bulk fertilisation of coral eggs where automated harvesting is unsuitable. The AutoFertiliser was used for example when specific colony crosses, or smaller batches of bulk fertilisation, were required. The AutoFertilisers have been successfully used independently from the AutoSpawner system for five coral species to date. - The design of the 500 L coral larval culture tanks was updated to improve the movement of corals (from fertilised eggs through to free-swimming larvae) in the tank, reduce the husbandry requirements (including cleaning frequency), increase the holding density, and make the harvesting procedure easier and more efficient. The performance of the new larval culture tanks was tested by the CAD-01.1 Sub-project through two large-scale experiments, confirming that larvae can be held at densities up to 500,000 larvae m^{-2} of facility space (Ramsby et al. 2026a), and that water usage can be reduced (without reducing larval quality) by switching to semi-recirculating operation after initial embryonic development has been completed (approximately 24 to 48 hours after introduction into larval tanks; Abdul Wahab et al. 2024). - Systems for automated, continuous monitoring of coral larval culture density in 500 L larval culture tanks using computer vision and machine learning algorithms were developed and tested in collaboration with the RRAP Translation to Deployment (T2D-01) Project (Coral Spawn and Larvae Imaging Camera System (CSLICS) Tsai et al. 2025). Top- and side-mounted versions of the system hardware were developed and tested in the National Sea Simulator during the Great Barrier Reef summer coral spawning events in 2022, 2023 and 2024. The performance of the machine learning algorithms was validated against replicated manual counts for each deployed system and coral species. The CSLICS can provide tank density estimates every 0.4-4 seconds, a 300-3,000-fold efficiency gain compared to performing manual larval density counts and has been used for 10 species to date. - A desktop version of the CSLICS, with the capability of estimating the developmental stage during early coral embryonic development and fertilisation success in samples collected from AutoFertilisers or recently stocked larval culture tanks, was also developed in collaboration with the RRAP Translation to Deployment (T2D-01) Project.

Objective		Key Findings and/or Outcomes
		The developed algorithms can distinguish between multiple developmental stages, including eggs, embryos undergoing first cleavage, 2-cell stage, 4-8-cell stage and >16-cell stage, resulting in a 225-2,250-fold efficiency gain compared to manual development stage assessment.
1 (c)	Develop systems to reliably mass culture newly recruited corals to the age of deployment at sea, to minimise early life stages mortality.	Coral recruits require the right environmental conditions to survive and thrive, including the right light conditions, feeds and enough water movement to support gas exchange. To be able to develop systems for large-scale rearing of coral recruits that minimise mortality, an understanding of what the optimal conditions for recruit survival are required. While some of these conditions were investigated by other RRAP Sub-programs and projects (e.g. light by CAD-01.2, Ramsby et al. 2024; feeds by ECT-03.2 and co-culture to reduce competition by CAD-02.2, Neil et al. 2024 a,b,c), and information produced there could be incorporated in the CAD-02.1 large-scale experimental work, the effects of water movement (or lack thereof) on coral recruits remained largely unknown. Additionally, testing of early prototypes of mass-rearing systems for *Acroporidae* coral recruits indicated that a lack of water movement within the tanks can result in widespread recruit mortality during grow out. - Survival of *Acropora abrolhosensis* recruits reared for 7 weeks at water velocities between five and 20 cm s^{-1} showed close to no mortality (survival >97%) in small-scale experimental systems (Nordborg et al. 2024). While no difference in survival was observed, the growth of recruits was higher at higher water velocities for the same rearing period, indicating a higher water velocity was more beneficial for recruit health (Nordborg et al. 2024). - To optimise the space usage in land-based coral aquaculture facilities it would be advantageous to be able to rear coral recruits on vertical substrates, instead of the traditionally used horizontal surfaces. Experimental comparisons of *Acropora millepora* recruit survival when reared on settlement tiles held at 0°, 60° and 90° angles in a large, coral rearing system showed that recruits reared on vertical surfaces (90°) had significantly higher survival than recruits reared on horizontal (0°) or 60° surfaces (Nordborg et al. 2024). Tiles held vertically also had less fouling from algae, and other benthic organisms that may compete with coral recruits, than tiles held horizontally or at 60°, indicating that less labour may be required to maintain systems when recruits are reared vertically. - Long-term rearing of coral recruits (up to 12 weeks) in land-based facilities can be cost-prohibitive, for example costs of seawater usage and labour required for maintenance. Experimental comparisons of *Acropora spathulata* recruit survival over 12 weeks of rearing in aquaculture systems with either flow-through or semi-

Objective		Key Findings and/or Outcomes
		recirculating seawater showed that there was no increase in mortality when recruits are reared in semi-recirculating systems (Nordborg et al. 2024). If anything, the recruits appeared to do better in the semi-recirculating systems (Nordborg et al. 2024). Additionally, observations of fouling organisms appeared to be lower overall and occurring later during the rearing period in the semi-recirculating systems, and the measured bacterial loads tended to be lower than in the flow-through systems (refer to Sub-project CAD-02.3 for further details; Brunner et al. in prep). The use of semi-recirculating systems (all other things being equal) reduces water usage by 9.5-fold per 24 hours.
1 (d)	Systems for the transport to sea of settlement units and devices for deployment.	- Removable concrete tile holders from machined polyvinyl chloride (PVC) (for concrete settlement tiles poured using separate moulds) and semi-permanent, PVC moulds (for pouring and handling) concrete settlement tiles were developed and tested to facilitate space-efficient holding and transport of concrete settlement tiles post-settlement. Additionally, PVC racks for vertical holding and transport of concrete settlement tiles in semi-permanent moulds (up to 10 per rack) were developed. When used in combination, the semi-permanent tile moulds and tile racks reduced the risk of physical damage to coral recruits and breakage of tiles, while allowing for sufficient water flow across tile surfaces to ensure recruit survival, during transport. The racks were designed to be modular, to allow for use with both small (e.g. 70 lite) and large (up to pallet sized) transport systems. - The design of the deployment devices was iteratively adjusted and improved over the course of the program (refer also to the CAD-01.2 Sub-project, in RRAP Project Coral Propagation and Deployment (CAD-01)), including in relation to cost effective transport of devices to sea for deployment. The latest device designs allow for the use of a spigot or rod through the centre of the device without interfering with the loading of settlement units or the attachment of units to the deployment device. A modular transport system using the spigots (consisting of modular trays that can be placed inside transport systems and secure the spigots of devices in place vertically) was developed in parallel and were successfully used for deployments of devices in early 2025. - Systems for automated deployment of devices when at sea are being developed by the RRAP Translation to Deployment (T2D) Sub-program (with input from CAD-02.1), including the development of the Deployment Guidance System (DGS). The device and transport systems developed by CAD-02.1 are fully compatible with the DGS.

Objective		Key Findings and/or Outcomes
1 (e)	Develop systems to settle coral larvae effectively and homogenously on large surfaces, suitable for large scale production.	- Shallow, mass settlement systems for settlement of coral larvae on settlement tiles held horizontally were designed and tested to reduce the labour requirements during mass coral settlement operations and ensure tanks are suitable for large-scale production activities. Two tank designs were developed, each capable of holding nine or 30 concrete settlement tiles per tank (3,600 and 12,000 settlement units per tank, respectively). The tanks can be racked vertically, increasing the space efficiency during settlement onto concrete settlement tiles by up to 24-fold compared to traditional methods of settlement on aragonite plugs placed in horizontal trays in 50 L aquaria. The tanks have been successfully used for large-scale settlement of over 10 coral species to date. - The potential of performing mass settlement with the settlement tiles held on a 60° or 90° angle (to improve the efficiency of space use in a coral aquaculture facility), compared to horizontally, was also assessed experimentally. Preliminary results indicate that the angle of the settlement tiles during settlement does not affect how many coral larvae settle onto the tiles, or how homogenously they are spread across the tile surfaces, as long as the biofilm with biochemical cues on the tiles (estimated by measuring the percent of the settlement unit surfaces that were covered by crustose coralline algae (CCA) is similar across the entire tile surface. By settling coral larvae on tiles held at a 60° angle approximately 28 tiles (11,200 settlement units) could be settled per m^2. This is similar to the number of tiles that can be settled horizontally using the shallow mass settlement systems described above when three tanks are racked vertically. - Free-swimming coral larvae require biochemical cues to undergo settlement (Heyward et al. 1999), i.e. the attachment to a surface and metamorphosis into the primary polyp which can then grow into a new coral colony. Different coral species respond to different cues, but most species will undergo settlement when exposed to the live biofilms formed on surfaces during conditioning in seawater, in particular to certain species of CCA. Depending on the system used for conditioning, and the desired species of CCA the conditioning may take between four and 12 weeks (Abdul Wahab et al. 2022). As a result, the development of conditioning systems where concrete settlement tiles can be conditioned at a higher density, without a reduction in the number of larvae settling per settlement unit or changes to the distribution of settlement across the tiles, was identified as a priority. The CAD-02.1 Sub-project experimental work investigating the effect of the angle of the settlement tiles during conditioning indicates that settlement tiles can be conditioned on up to a 60° angle

Objective		Key Findings and/or Outcomes
		without any significant changes in the total number of larvae settled, or their distribution across the tile surface when tiles are held horizontally during settlement. - A Standard Operating Procedure (SOP) for large-scale settlement of coral larvae was produced, incorporating learnings from this sub-project and other sub-projects across the RRAP Coral Aquaculture and Deployment (CAD) Sub-program (Nordborg et al. 2024).
1 (f)	Design systems suitable for high level of automation for the handling of coral settlement substrates and integration into deployment devices.	- After performing the first experiments using large numbers of concrete settlement tiles (>100 tiles, or 40,000 settlement units) several potential issues for large-scale coral settlement, and integration of settlement units into deployment devices, using high levels of automation were identified. These included unintentional breakage of tiles, the need to increase holding settlement and holding densities in the facility while reducing the labour required to perform settlement, the process for loading and securing settlement units into deployment devices and the labour required for monitoring of recruit survival post-settlement. The CAD-02.1 Sub-project have worked to resolve the identified issues by refining and further improving on system designs and workflows in an iterative way in collaboration with other RRAP Projects and Sub-projects, CAD-01.2, T2D-01 and CAD-01.1, as well as additional experts from industry and academia. - To address the unintentional breakage of tiles during handling, and to facilitate increased holding densities, several options for semi-permanent moulds that are suitable for industrial manufacturing processes were developed and tested. Semi-permanent moulds manufactured from PVC can withstand extended submersion in seawater, reduce the unintentional breakage of tiles, enable the use of increased holding densities in facility by supporting tiles during vertical racking in aquaculture systems, are lighter compared to PVC tile holders to allow for easier manual handling, are compatible with tile cutting devices (e.g. the assisted tile cutting jigs developed in collaboration with the RRAP Translation to Deployment (T2D-01) Project; see also below), and integrate with both assisted manual (developed in collaboration with T2D-01) and automated (McLaren-the Great Barrier Reef Foundation (GBRF)-AIMS collaboration; see also below) deployment device assembly machine. - Monitoring of recruit survival in aquaculture facilities, including assessment of which settlement units should be loaded into deployment devices, is very labour intensive. Therefore, an automated monitoring system, the Coral Growout Robotic Assessment System (CGRAS) was conceived by CAD-02.1 and then developed in collaboration with T2D-01. The system includes a robotic image capture system and software for

Objective		Key Findings and/or Outcomes
		counting the number of live corals on each settlement unit across the concrete settlement tiles (using machine learning). A hardware prototype has been successfully tested by the sub-project team, and the machine learning algorithms are able to identify coral recruits and coral juveniles for three species, with results validated using counts performed by coral experts. Validation of the software also showed that the use of CGRAS can reduce the labour required to assess settlement tiles (including cleaning, handling and imaging of the tile, and the manual counting of corals from captured images) from approximately 140 minutes to approximately 12 minutes per tile (~11.7-fold efficiency increase). The software side of CGRAS is now also being adapted for integration with the automated device assembly machinery (see below for details) through the OCTAV system (Observations for Coral Tab Assessment and Verification system). - CAD-02.1 has been working closely with the CAD-01.2 Sub-project, in RRAP Project Coral Propagation and Deployment (CAD-01) and the RRAP Translation to Deployment (T2D) Sub-program to optimise the design of the deployment devices for automated loading of settlement units. This has also included providing input and testing of the assisted tile cutting jigs (which also spread out the settlement units for easier handling) as well as the device assembly jigs (DAJ), both in facility and in the field, alongside CAD-01. - Device assembly is a bottleneck in the coral production workflow. A collaboration was established between the Great Barrier Reef Foundation (GBRF), McLaren Racing and AIMS to develop an automated Device Assembly Machine (DAM) that would increase the assembly throughput of the facility – a concept of the DAM was developed. Additional funds were received from the GBRF to contract a manufacturer for the DAM to complete the design and build the system in Australia, whereby a tender process was completed early 2025. Bosch Australia was selected and contracted to perform the work. As of June 2025, the fully design work is nearing completion, with the build completion of the system scheduled for March 2026.
2. CAD-02.2: Companion species and co-culture methods		
2 (a)	Develop knowledge and methods to underpin co-culture to increase the survival and early growth of propagated corals.	- Companions can be added to coral culture to provide fouling control, pest management or nutrient enrichment. We have identified compatible companion organisms for different coral species and/or growth-forms from immediate post-settlement to the juvenile stage.

Objective		Key Findings and/or Outcomes
		- We have developed methods to sustainably culture appropriate species of micro-herbivores within coral aquaculture facilities, to control fouling control in recruit grow-out environments.
2 (b)	Help identify the drivers of early coral survival in an aquaculture environment, and seek to evaluate co-culture factors such as stocking density, organism size, interaction with hardware and system engineering to optimise co-culture for integration into the prototype scale aquaculture system.	- Competition with algae represents a major driver of early mortality. On live settlement substrates CCA and turf algae posed a threat when left uncontrolled, while on frozen tiles colonisation by filamentous algae could directly compete with corals and cause mortality. - Our experiments showed that factors such as tile condition, the species of corals to be cultured, and size of the grow-out system must be considered when selecting companion organisms. - Suggested stocking densities are provided for the different companion species. System requirements necessary for companion well-being (e.g. hides for fish or snails) also presented.
3. CAD-02.3: Health assurance and quality control		
3 (a)	To identify problem organisms (disease, pests) that need monitoring within a coral aquaculture setting.	- Quality-assurance and quality-control (QA/QC) work targeted bacterial and eukaryotic pests of corals during coral spawning experiments to assess their links to coral mortality in aquaculture breeding facilities. - Given the general uncharacterised nature of coral diseases in captive aquaculture environments, the abundance of total *Vibrio*, a genus commonly associated with disease causation in marine organisms (Vandeputte et al. 2024), was assessed along with quantification of total bacterial abundance. - Coral-eating flatworms were targeted as a pest organism target of primary concern, which has demonstrated health impacts for coral in captive environments. The presence of flatworms were identified associated with broodstock corals showing signs of predation. - Corallivorous nudibranchs were found to not be a major risk to corals held in captive aquaculture environments due to their limited host range. However, poorly described species boundaries for coral-associated nudibranchs was a challenge for assay development.

Objective		Key Findings and/or Outcomes
3 (b)	Experimentally validate appropriate diagnostics tests to detect and quantify selected coral pests and potential pathogens.	- A sensitive molecular detection and quantification method targeting coral-eating flatworms was established using droplet digital polymerase chain reaction (ddPCR) based on environmental DNA (eDNA). - The flatworm ddPCR assay can detect low copies of eDNA in sample collected from aquarium water, enabling identification of cryptic flatworms in parent coral tanks. Detection was validated by visual observations of infestation. - Flatworms were not detected in coral recruit settlement and grow-out tanks. Broodstock tanks holding adult corals for spawning were the only locations where flatworms were detected with the sensitive eDNA assay. This result highlighted that a specific focus of pest flatworm detection on broodstock is sufficient. - A duplex ddPCR assay to quantify total bacterial and total *Vibrio* abundance simultaneously was developed for aquarium water samples. The assay was applied across the operational stages of the coral aquaculture system, in conjunction with coral spawning experiments, and tested across various settings (e.g. water flow rates, larval stocking densities, semi-recirculation system). - Using a machine-learning model based on bacterial occurrence and coral survivorship, links between coral mortality and high *Vibrio* abundance were identified. - Assessment of mortality prediction using the model suggested that a risk of compromising coral health with high *Vibrio* proliferation may be forecasted from detection of low total bacterial abundance.
3 (c)	Experimentally validate and implement robust assessments of coral genetic diversity across the coral breeding program to inform best practice.	- Genetic diversity of offspring corals produced from coral spawning experiments was assessed across developmental stages, comparing different broodstock coral numbers and diversity, gamete handling and rearing environment. - Parental assignment, effective population size, heterozygosity were found to be comparable estimators that can capture genetic diversity dynamics within coral aquaculture settings. - Genetic diversity of progeny was assessed during larval culturing stages, post settlement and after two years in the field, in addition to testing different rearing environments (tanks versus in-water nets). Results highlighted the importance of

Objective		Key Findings and/or Outcomes
		broodstock diversity in progeny genetic diversity with little evidence of major genetic bottlenecks during aquaculture breeding. - The number of broodstock corals required to produce a level of offspring genetic diversity was found subject to the genetic diversity of the broodstock colonies, highlighting the importance of population-genomic assessment of the source corals and optimisation of broodstock sampling strategies accordingly. - Parental assignment to offspring corals indicated uneven parental contributions using sperms and eggs that were separated and normalised. However, a strong positive correlation was obtained between gamete volumes and parental assignments in progenies when gamete bundles were simultaneously added and gently homogenised, but not going through the laborious processes of sperm/egg-separation, cell quantification and normalisation.
3 (d)	Develop the quality assurance and quality control standard operating procedures to meet the project objectives.	- Scalable extraction methods for bacterial and pest DNA from water samples were developed for ddPCR quantification. Application of the ddPCR assays to water samples containing coral-loaded seeding devices returned data in a three- to four-day window and showed low *Vibrio* and pest abundance prior to coral deployment; a timeframe that is required for screening of problematic organisms for biosecurity. It is important to understand that the context of this turn-around time relies on exclusive access to laboratory personnel and equipment to undertake diagnostic activities and needs to scale to the aquaculture operation. - Guidance of breeding operations based on molecular sequencing of broodstock and progeny was compiled. The importance in genetically characterising broodstock corals to target high effective population size and to avoid clonality and contaminating cryptic species was highlighted.

Adjustments to key research objectives

Table 2: Variation in the Project over time.

Initial Research Question	Explain when, how and why the research question changed
No adjustments to report	

4 Future Research Recommendations

4.1 CAD-02.1: Coral aquaculture facility prototype and design

Several key residual knowledge gaps, and challenges, to further optimise and scale the production of corals in an *ex-situ* facility for restoration and to improve the survival of corals both during production and post-deployment were identified and are detailed below.

4.1.1 Optimising methods to improve the performance of the conservation aquaculture processes

Methods to settle, inoculate (Symbiodiniaceae and probiotics), and feed young corals have been successfully developed for several coral species in the families Acroporidae and Merulinidae. To achieve better production efficiency in the future, and thus higher returns on investments (better cost efficiency), we identified key future research and development pathways to improve the settlement, survival, growth and health of young corals in the facility, including across a broader species range with different larval behaviours, symbiotic relationships and feeding strategies. Further improvements could be achieved by engaging in the following activities:

1) *Development of systems for rapid phenotype assessment of broodstock.*
    a. **Potential for future gains:** The selection of suitable broodstock is critical for the downstream success of coral production facilities and deployments of produced material. The development of a CGRAS-like phenotyping system utilising pulse amplitude fluorometry, image collection and automated, machine learning data extraction and analysis would significantly improve the efficiency of, and reduce bottlenecks for, the start of the aquaculture production pipeline.
2) *Upscaling of cost-effective modular production systems.*
    a. **Potential for future gains:** The first phase of RRAP developed effective technologies for the production of corals that are hermaphroditic and release buoyant gametes, but did not focus on corals with other reproductive modes. To ensure that high-throughput methods and systems for cost-effective production are available for a diverse range of coral species, the development and testing of AutoSpawner systems suitable for use with additional gonochoric species, and species with non-buoyant eggs and sperm is strongly recommended.
3) *Further development and optimisation of automated monitoring systems for pelagic early coral life stages.*
    a. **Potential for future gain:** During the initial phase of RRAP the first automated, computer vision and machine learning-based monitoring system for coral larval culture operations was developed (CSLICS) in collaboration with the RRAP Project Translation to Deployment (T2D-01). This system offers a significant efficiency gain for coral aquaculture monitoring and an expansion of the machine learning algorithms to additional species will be critical to the success of large-scale programs such as the Pilot Deployments Program (PDP). Additionally, development of additional monitoring functions, including trend analysis and alarm functions, is highly recommended to support future at-scale production and facilitate rapid responses to changes in high-value larval cultures. The continued development of the desktop-CSLICS concept, and machine learning algorithms, will also be critical for the high-throughput assessment of culture stocking densities and fertilisation success required for at-scale coral production.
4) *Optimise holding conditions, and maximise growing space, while in facility to improve survival and growth across a range of species.*

a. **Potential for future gains:** Facility space is valuable and its optimisation through the development of high density holding and grow-out aquaculture systems, which do not compromise on the survival and health of the resulting spat would be required to upscale production. Through the current RRAP, we have developed methods to increase the density of holding and grow-out of spat, for example through vertical tile growing conditions – however, this has not been tested at the full-scale capacity. The upscaled production of coral seeding units in facility for the Pilot Deployments Program would provide an opportunity for assessing the performance of high density grow-out conditions at a scale that is realistic. Maintaining linkages with the scope of work currently in RRAP Coral Aquaculture and Deployment (CAD) Sub- projects, CAD-02.1, CAD-01.1 and CAD-01.2 will be crucial.

5) *Further development and expansion of automated monitoring of early coral life stages post-settlement.*

a. **Potential for future gain:** During the first phase of RRAP a prototype semi-automated, robotic imaging and computer vision system for assessment of recruit survival was developed and tested (CGRAS). Further refinement and expansion of the use-cases for this system (including the diversity of coral species with trained machine learning algorithms) would significantly improve the efficiency of the aquaculture pipeline and reduce the risk of coral recruit loss by identifying the need for early intervention during rearing. By incorporating deep learning to reduce the required input into the algorithm development process by ecologists, further expanding the use case. Additionally, developing the capability to identify pest and nuisance species would increase husbandry efficiency by alerting operators and allowing the focus of efforts on the systems and issues that are of the highest priority without labour intensive manual monitoring.

4.1.2 Developing buffering methods for step-change in production of sexual propagules and year-round deployment by removing time constraint of larval supply

The production of coral recruits through sexual propagation, at scale, is currently limited to the summer spawning, and thus comes under a batch production model. If we are able to expand /buffer the production of sexual recruits year-round, this will 1) allow for year-round deployment of corals, 2) utilise the facility year-round. This activity is aimed at developing buffering strategies for year-round coral production and deployment by:

1) *Developing novel space and water efficient technologies for maximising facility production throughput.*

a. **Potential for future gains**: Novel methods for the holding of larvae and spat that reduces the use of *ex situ* facility footprint and resources, e.g. water and electricity, would make the production of coral seeding units more cost effective. Novel engineered substrate that could store larvae or spat in closed pods (e.g. <2x2 cm dimensions), with formulated seawater that contains provisions for nutrition and symbionts, and with gas exchange facilitated by bespoke environmentally suitable polymer-film, could eliminate the need of costly facilities for larval culturing, settlement and nursery grow-out, and serve as a buffering mechanism for protracted deployment. If these pods could also be utilised directly for deployment, then this would eliminate the need for seeding devices. Maintaining linkages with the scope of work currently in the Sub-project, CAD-02.1 – upscaling coral propagation, will be crucial.

2) *Upscaled out-of-season spawning for the supply of larvae throughout the year.*

a. **Potential for future gains**: Out-of-season spawning has successfully been performed at AIMS and elsewhere globally, by offsetting the spawning cycle of corals through the manipulation of temperature, photoperiod and lunar profiles. To date, the AIMS SeaSim team has been able to mass spawn and produce larvae of coral species in the Acroporidae and Merulinidae, that typically spawn in the summer months, in autumn and during the daylight hours in

controlled room settings, albeit at small- to medium-scale. There is potential for upscaling this process, which needs to be balanced with the cost of production and also needs to consider the implications of deploying spat outside of their natural, temporal recruitment window. Maintaining linkages with the scope of work currently in the RRAP Coral Aquaculture and Deployment (CAD) Sub- projects, CAD-02.1, CAD-01.1 and CAD-01.2 will be crucial.

4.2 CAD-02.2: Companion species and co-culture methods

Future co-culture research should test the potential functional impacts of different mixes of companions on their ability to deliver their respective services. Past research has indicated that in mixed cultures individual organisms may perform worse than in single-companion set-ups, which leads to lower overall survival/growth of the corals. As part of this, the effectiveness of pest control companions should be examined more thoroughly, as research on this aspect of co-culture remains relatively limited. Importantly, biosecurity aspects of using companion organisms need to be explored, and protocols developed to ensure the use of companion organisms does not pose a risk to the reefs that will be reseeded with cultured corals.

4.3 CAD-02.3: Health assurance and quality control

Links between bacterial abundance and coral health should be further investigated by collecting microbial data across more scenarios where morality of young corals is high. During the current research experiments, there were only sporadic early-life-stage coral mortality events. Total abundance of bacteria and *Vibrio* spp. provide a relatively robust indicator for health and disease dynamics of corals in aquaculture. The capacity of the indicator to predict coral loss beforehand needs to be further studied, allowing time to apply potential interventions. The further development of rapid, point-of-care assays are desirable to support coral production and biosecurity screening. Current assays while robust are not real-time and hence easy-to-use rapid assays (e.g. ‘dipsticks’-types) need to be developed.

If/when coral-rearing technologies and procedures are established to effectively suppress larval mortality below ‘natural’ larval loss, operational importance and benefit of bacterial and pest QA/QC assays in coral aquaculture will mostly stem from pre-deployment screening of potentially problematic organisms as a biosecurity measure. Further studies of QA/QC tools with a greater focus on risk management of restoration interventions, rather than on supporting coral production efficiency, are recommended. Building upon our implementations as models, assays targeting other potential organisms can be developed easily if definitive links to disease in corals and other marine organisms is identified.

Genetic assays to characterise broodstock coral genetic diversity and their successful parental contributions to offspring would introduce significant benefits for responsible coral restoration interventions. Further studies are recommended to characterise how offspring genetic makeups are reflected by breeding practice variations, such as how gamete bundles are handled and cross-fertilised in bulk. To maximise the benefit of genetic knowledge in aquaculture, research towards development of a rapid genetic characterisation workflow (sequencing assay and analysis pipeline) will be desirable to better align to aquaculture operation timeframe and the use of genetic data.

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