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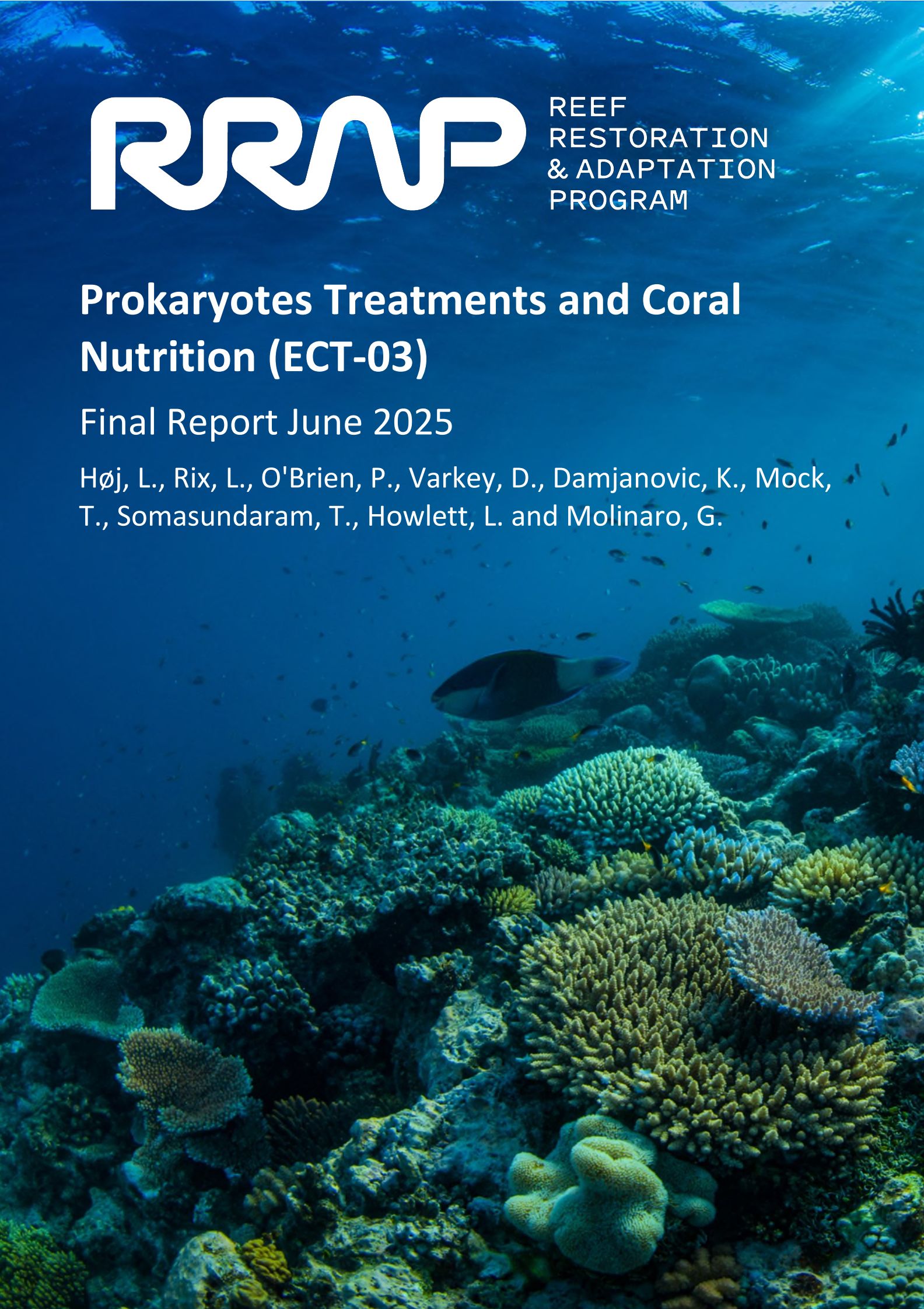
Prokaryotes Treatments and Coral Nutrition (ECT-03)

Final Report June 2025

Høj, L., Rix, L., O'Brien, P., Varkey, D., Damjanovic, K., Mock, T., Somasundaram, T., Howlett, L. and Molinaro, G.



RRAP Prokaryotes Treatments and Coral Nutrition (ECT-03) Final Report June 2025

Enquiries should be addressed to:

Dr Lone Høj, l.hoj@aims.gov.au

Dr Lorna Howlett, lh.howlett@aims.gov.au

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This report summarises work undertaken under *Prokaryotes Treatments and Coral Nutrition (ECT-03)* in accordance with the Reef Restoration and Adaptation Program's *Enhanced Corals and Treatments* 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**
Palm Islands	Manbarra
Davies Reef	Bindal

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1 Executive Summary

Prior to the first phase of the Reef Restoration and Adaptation Program (RRAP), scalability in coral aquaculture was limited by the lack of reliable, broad-spectrum cues to induce larval settlement, particularly in non-Acroporid coral species, and other technologies to support the survival, growth and health of corals to reduce their holding time in aquaculture systems, and hence costs, prior to deployment. Therefore, under the Enhanced Corals and Treatments Sub-program, the Prokaryotes Treatments and Coral Nutrition Project (ECT-03) addressed three key goals to advance coral aquaculture and support upscaled reef restoration efforts: (i) enhance and control the settlement of core non-Acroporid coral species in an aquaculture setting through the use of microbial inducers; (ii) develop the resources and know-how required to apply bacterial probiotics to improve coral settlement, growth, nutritional condition, and resilience to stress and disease; and, (iii) establish diets and supplements, including probiotics, to support healthy, fit, and reproductively active corals throughout their life cycle in captivity, including following asexual propagation.

Sub-project ECT-03.1.1 used a targeted, community-based approach to characterise microbial communities in high and low settlement biofilms and identify microbial inducers of larval settlement. The findings demonstrated that biofilm communities are highly diverse, dynamic, and responsive to different substrate conditioning treatments with variations in microbial community composition associated with significant differences in coral larval settlement. Optimal settlement outcomes were achieved under natural day-night light cycles and with extended conditioning durations (two months compared to one-month). Numerous candidate microbial inducers were identified and targeted for experimental validation and development of bacterial settlement treatments. More than 500 bacterial isolates were cultivated from settlement biofilms and 201 candidate inducers were screened for settlement activity with nine coral species. Approximately 50% of the screened bacterial isolates successfully induced larval settlement, highlighting the success of the approach in identifying novel microbial inducers. Effective inducers were identified for all tested coral species, including difficult to settle non-Acroporids, with some isolates outperforming traditional settlement substrates such as crustose coralline algae (CCA) and coral rubble. Moreover, bacteria were successfully applied to RRAP settlement devices, and protocols were developed to increase the shelf-life of the bacterial treatments to improve feasibility of upscaling for coral aquaculture workflows.

Sub-project ECT-03.2.1 isolated, cultured and tentatively identified over 900 new bacterial isolates from coral colonies and early life stages to produce a collection of potential candidates for probiotic development. Notably, several isolates exhibited antibacterial activity against the coral pathogen *Vibrio coralliilyticus* and produced digestive enzymes, suggesting potential for broader applications in coral health and resilience. Single-candidate inoculations indicated that the microbiomes of *Acropora kenti* and *Platygyra daedalea* were amenable to manipulation to varying degrees, with individual candidates eliciting different microbiome responses and coral health outcomes. For *Acropora kenti*, a probiotic consortium consisting of four strains was used to test probiotic delivery strategies (via water column or diet; number of deliveries) and whether probiotic application could improve health outcomes and resilience to an acute heat stress followed by a recovery period. The experiment demonstrated differing uptake of probiotic candidates, with one candidate forming prevalent and stable associations irrespective of the delivery strategy and stress exposure. While no consistent health or resilience benefit of probiotic application was observed in this month-long experiment, the results are consistent with emerging evidence that coral juveniles are relatively resilient to acute temperature stress.

In sub-project ECT-03.2.2, encapsulated diets with and without essential fatty acid enrichment were developed and compared with live feeds over a three-month period. While encapsulated diets and live feeds supported survival and growth of *Acropora kenti* equally, only live feeds supported growth of *Goniastrea retiformis*. There was also no evidence of enrichment of supplied essential fatty acids in coral tissues of either species. In a separate experiment, feeding protocols using live feeds (Artemia and rotifers) and a commercial liquid supplement were validated to support the development of an RRAP Standard Operating Procedure (SOP) for feeding coral juveniles.

Collectively, results from the three sub-projects contribute critical insights into microbial and nutritional interventions for enhancing coral aquaculture outcomes and inform scalable solutions for reef restoration applications.

Future research should focus on advancing microbial treatments and nutritional interventions to enhance the efficiency, scalability, and resilience of coral aquaculture for reef restoration. Building on the promising outcomes of the first phase of RRAP, priority will be given to the continued development of bacterial settlement treatments, with an emphasis on bacterially produced cues and stabilised treatments over live bacterial cultures to minimise risk and enhance logistical feasibility. Identifying and isolating the specific bioactive compounds responsible for coral settlement therefore remains a critical research need, as does the optimisation of treatment protocols for large-scale application across diverse coral species. Parallel efforts will refine the use of probiotics in aquaculture, targeting single-strain inoculation strategies and testing efficacy across species to streamline operational integration. Long-term experiments will be essential to validate benefits to coral health, survival, and resilience, while fundamental research will elucidate the molecular mechanisms underlying probiotic function. In coral nutrition, continued refinement of live feed protocols including automated dosing systems should be pursued and include multi-species trials and assessment of post-deployment performance. Further development of formulated feeds should target refinement of physical feed characteristics that enable use with automated dosing systems and test the use of increasing feed doses. Across all three Prokaryotes Treatments and Coral Nutrition (ECT-03) sub-projects, future work will be guided by scalability, regulatory considerations, and industry partnership readiness to ensure these innovations translate into tangible outcomes for reef restoration at scale.

2 Background and Justification for the Research

The large-scale restoration of coral reefs required the development of scalable and cost-effective aquaculture methods for producing both sexually and asexually propagated corals. Progress in this area had been limited by two major challenges:

i. the absence of reliable, broad-spectrum cues to induce larval settlement and metamorphosis—particularly in non-Acroporid coral species; and
ii. limited knowledge of how to optimise early growth, nutrition, and disease resistance in juvenile corals to maximise survival and reduce their time in aquaculture prior to out planting.

To address these limitations, the Prokaryotes Treatments and Nutrition Project (ECT-03) was initiated under the Reef Restoration and Adaptation Program (RRAP).

Sub-project ECT-03.1 focused on identifying and characterising microbial communities capable of inducing larval settlement with the aim of developing microbial treatments to enhance coral settlement in aquaculture systems. The lack of reliable settlement cues was a bottleneck in coral aquaculture, particularly for many important and diverse non-Acroporid species (Banaszak et al. 2023, Bostrom-Einarsson et al. 2019, Randall et al. 2020). While crustose coralline algae (CCA) had long been recognised as a settlement cue for Acroporid corals, they were less effective for many other genera (Whitman et al. 2020). Microbial biofilms, by contrast, had demonstrated broader potential across a range of coral genera (Randall et al. 2024). Moreover, since microbes can be produced at scale, they presented a promising opportunity for the development of scalable settlement treatments, either through use of inductive microbes themselves or application of microbially produced settlement cues (Banaszak et al. 2023). However, the lack of knowledge of the specific microbial taxa, chemical signals, and genetic pathways responsible for settlement prevented the development of effective treatments. The sub-project therefore focused on identifying and cultivating microbial consortia from biofilms and validating their ability to induce settlement in a controlled aquaculture setting.

In parallel, sub-projects ECT-03.2.1 (Microbial Probiotics) and ECT-03.2.2 (Coral Nutrition) addressed the second bottleneck: developing treatments and diets to enhance coral growth and survival post-settlement.

While bacterial probiotics are commonly used in commercial aquaculture facilities, coral probiotics was an emerging field of research (Peixoto et al. 2017). Proof-of-concept studies had shown that supplying bacterial probiotics to captive adult corals could alter their microbiome and increase bleaching tolerance in some species (Rosado et al. 2019), but little was known about the possible benefits of applying probiotics during coral early-life development. Sub-project ECT-03.2.1 therefore aimed to develop the resources and know-how needed to use bacterial probiotics to improve coral nutritional condition, growth, health and resilience to stress and disease. The project focused on isolating, cultivating and screening probiotic candidates, followed by inoculation experiments with coral juveniles to test if probiotic application could enhance their performance.

Refined heterotrophic feeding regimes for captive corals have the potential to improve survival, growth, and health in the facility and following deployment (Banaszak et al. 2023, Conlan et al. 2017, Conlan et al. 2019, Osinga et al. 2011). While *Artemia nauplii* and microalgae are established food sources for captive corals (Barton et al. 2017, Osinga et al. 2012), their impact on coral nutritional status were found to be limited and vary between coral species (Conlan et al. 2018, Conlan et al. 2019). Moreover, data from experimental feeding studies with rotifers were sparse and inconclusive (Conlan et al. 2017, Da Ros et al. 2022, Osinga et

al. 2012). At the outset, formulated heterotrophic feeds for coral aquaculture had received little research attention.

Formulated heterotrophic diets hold several advantages for large-scale aquaculture relative to live feeds, including being less prone to batch-variability, no loss of nutritional components due to metabolism by the live feed organism, greater biosecurity, and access to scaled production that can meet demands of multiple facilities (Hardy et al. 2022). In sub-project ECT-03.2.2 the development of microencapsulated diets, which protects bioactive compounds against oxidation within various coating or “shell” materials (Nedovic et al. 2011), were explored as a possible technology to encase essential macro- and micro-nutrients for delivery to captive corals. The project focused on identifying ingredients and supplements with expected benefits, followed by the development of palatable microencapsulated diets and testing of their ability to promote growth and health of coral juveniles.

These efforts were carried out in close collaboration with other RRAP components—including RRAP Sub-program Coral Aquaculture and Deployment (CAD) sub-projects CAD-01.1 (Optimising coral propagation), CAD-01.2 (Deployment devices), Engineering Large Scale Coral Aquaculture (CAD-02) (Facility design), and Assisted Evolution (ECT-02) sub-project ECT-02.2 (Algal symbiont treatments)—to ensure integration with broader aquaculture and restoration frameworks.

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
1.1 Sub-project ECT-03.1.1: Microbial Inducers for Coral Larval Settlement		
1.1 (a)	Enhance and control the settlement of core non-Acroporid coral species in an aquaculture setting using microbial inducers	This was the overarching aim of the sub-project and not intended as a specific objective. It was achieved through the two specific objectives listed below. Through objectives 1.1(b) and 1.1 (c), microbial inducers were identified and developed into bacterial treatments to successfully enhance and control the settlement of nine coral species, including six non-Acroporid species and three Acroporid species (details below): – *Acropora anthocercis* – *Acropora kenti* (midshelf) – *Acropora kenti* (inshore) – *Dipsastraea speciosa* – *Echinophyllia aspera* – *Echinophyllia orphonensis* – *Goniastrea retiformis* – *Lobophyllia corymbose* – *Platygyra daedalea* The project findings were communicated through national and international conferences, RRAP reports, student theses, and peer-reviewed publications.
1.1 (b)	Identify microbial taxa that induce settlement and metamorphosis of three to five non-Acroporid coral species for reef restoration, to enable aquaculture production at scale.	A targeted community-based approach was used to identify microbial taxa associated with coral settlement. Microbial biofilms conditioned under different treatments were analysed to characterise high and low settlement biofilms and identify specific microbial taxa and functions correlated with settlement: • Biofilm communities are highly diverse, dynamic, and vary according to conditioning treatment, with differences in community composition linked to significant changes in larval settlement. – Biofilms were extremely diverse with 69 prokaryotic phyla and 525 families represented across all treatments and 36,128 unique amplicon sequencing variants (ASVs) identified, highlighting the challenge of identifying individual settlement-inducing taxa.

Objective		Key Findings and/or Outcomes
		– Light and biofilm age are critical factors in the development of settlement-inducing biofilms. Biofilms conditioned under natural day: night light cycles consistently have higher settlement than dark conditioned biofilms. Older biofilms (two- versus one-month) were also associated with higher settlement across all coral species tested. – Biofilms from the same treatment but conditioned in separate replicate tanks were also significantly different, and this tank effect significantly influenced settlement. This highlights that even minor environmental differences can lead to changes in community composition with cascading impacts on settlement success. • Numerous bacterial taxa were found to be correlated with high and low settlement across the four non-Acroporid coral species investigated indicating the presence of both settlement inducers and inhibitors and highlighting the complex interactions governing settlement in microbial biofilms. – We identified 197 ASVs correlated with high settlement across the four coral species investigated. These taxa associated with high settlement were taxonomically diverse, encompassing 15 phyla and 74 families. – High settlement taxa were largely species specific with no single taxa correlated with high settlement across all coral species tested. Nevertheless, at a higher taxonomic level bacteria from the families Flavobacteria, Rhodobacteraceae, Rhizobiaceae and Pirellulaceae were consistently correlated with settlement across multiple non-Acroporid coral species. • Metagenomic analysis of biofilms identified putative microbial functions involved in attracting larvae to settlement surfaces and initiating settlement. – Findings suggest coral larvae are initially attracted to settlement sites by specific pigmented and gaseous compounds, while biofilm contact may trigger settlement through cues that likely include neuropeptides, secondary metabolites, and effector proteins. We also identified toxins and other inhibitors that may deter settlement. – Identification of the specific bacterially derived cues and mechanisms involved in coral settlement provides information that could be used to engineer high settlement biofilms as well as providing additional targets for the development of bacterial treatments to control coral settlement. These findings were used to inform the selection of targets for the development of bacterial settlement treatments in objective 1.1(c).
1.1 (c)	Develop a suite of bacterial treatments that can be applied to devices to enhance settlement success.	To develop bacterial treatments to enhance settlement, a culture collection of >500 bacterial isolates was established and a shortlist of ~200 candidate microbial inducers identified from 1.1(b) were screened for settlement activity: • A culture collection of more than 500 new bacterial isolates was established with bacteria isolated from conditioned settlement tabs, coral rubble, and seawater. – In total, 517 isolates were sequenced for taxonomic identification and 371 were unique at 100% nucleotide identity. – The culture collection was taxonomically diverse including representatives from five bacterial phyla and 41 families. – Approximately 200 of the bacterial isolates had greater than 97% sequence similarity to taxa found to be correlated with high settlement in 1.1(b) and were shortlisted as candidate microbial inducers.

Objective		Key Findings and/or Outcomes
		• The ~200 shortlisted candidate microbial inducers were screened for settlement activity in settlement assays with nine coral species (six non-Acroporid and three Acroporid species). – In total, 104 of the 201 screened isolates (~50%) successfully induced a settlement response in at least one coral species. Of these 34 induced a medium (20-40%) or high (>40%) settlement response. – These confirmed inducers were taxonomically diverse, including taxa from three bacterial phyla and 25 families, and represent species and families not previously associated with coral settlement, such as the families Pirellulaceae Erythrobacteraceae, and Sphingomonadaceae. – While no isolate induced settlement across all nine coral species, several were able to induce settlement in as many as five different species, including across both Acroporid and non-Acroporid species. In particular, the families Flavobacteraceae, Roseobacteraceae, Rhodobacteraceae, Erythrobacteraceae, Sphingomonadaceae, and Pseudoalteromonadaceae all contained numerous taxa that induced settlement in a range of coral species. – Effective microbial inducers were identified for all nine coral species tested, including the difficult to settle non-Acroporid species that had low settlement in the positive controls (CCA and coral rubble), with several inducing higher settlement than the positive controls. – Overall, approximately 50% of the candidates tested were confirmed to induce settlement in vivo, confirming our targeted approach of using molecular data to guide the isolation and selection of candidate inducers was effective in identifying novel settlement inducers. • Methods were established to effectively apply bacterial treatments to settlement devices. – The application of mono-species bacterial biofilms to different settlement substrates (plastic or glass coverslips, well-plates, and concrete settlement tabs) was tested. While all were effective, the concrete settlement tabs were the most practical to work with and typically produced the highest settlement, demonstrating our microbial treatments are compatible with RRAP settlement devices. – Optimisation of cell concentrations and biofilm growth conditions can significantly increase settlement rates. However, cell concentrations must be optimised for each isolate to maximise settlement and avoid negative consequences for the larvae as some bacteria can be lethal above threshold concentrations. – Methods were developed to improve the stability and shelf-life of the bacterial treatments to make them more practical for use in large-scale coral aquaculture. Using this novel approach, bacterial treatments can be applied to settlement devices and stored at room temperature for weeks with no decrease in settlement success. This eliminates the challenge of needing to produce bacterial treatments on demand to coincide with larval competency and demonstrates that settlement devices could instead be prepared in advance.

Objective		Key Findings and/or Outcomes
		• The genomes of ~90 settlement inducing isolates and closely related non-inducing strains were selected for whole genome sequencing to uncover the mechanisms by which these bacteria induce coral settlement and for genomic screening to ensure these strains are safe for use in coral aquaculture. – Use of live bacteria poses challenges for upscaling and bacterially produced cues may be more practical for large-scale application. However, this requires further investment into identifying the specific settlement cues produced by bacteria and developing cost-effect approaches to synthesise these cues. – Analysis of the ~90 sequenced bacterial genomes is underway to identify specific bacterially derived settlement cues that could be targeted for further treatment development. – The genomes will also be screened to eliminate potential pathogens and ensure the selected strains do not pose any off target or environmental risks. This genomic screening is the first step to ensure these bacteria are safe for use in coral aquaculture. Overall, we have developed bacterial treatments to enhance and control the settlement of nine coral species. These treatments are now ready to be tested in larger-scale settlement trials.
2.1 Sub-project ECT-03.2.1: Microbial Probiotics to Enhance Growth and Survival		
2.1 (a)	Develop the resources and know-how needed to use bacterial probiotics for enhancing coral settlement, growth, nutritional condition, and resilience to stress and disease	This was an overarching aim of the sub-project and not intended as a specific objective. It was achieved through the two specific objectives listed below. – A bacterial culture collection was established including >500 candidates isolated from seawater, biofilm and coral rubble (ECT-03.1), and > 900 candidates isolated from coral (ECT-03.2). – Experience and expertise in the isolation and characterisation of coral bacteria, and in the application of probiotics to coral early life stages, were built through the bacterial isolation and in vitro screening programs, followed by four inoculation experiments using newly settled spat. – Requirements for upscaled applications of microbial probiotics were evaluated and reported in internal and external RRAP reports. Potential national and international partners for upscaling the production of bacterial probiotics were considered and relevant contacts established. The established resources and results were communicated at national and international conferences, RRAP reports, student theses and peer-reviewed publications.
2.1 (b)	Produce a library of >100 identified probiotic candidates that have been screened *in vitro* for phenotypic traits with	**This objective was fully achieved:** • A large, taxonomically diverse culture collection of more than 900 new bacterial isolates was established.

Objective		Key Findings and/or Outcomes
	expected nutritional and health benefits for coral.	– Isolates were recovered from the coral species *Acropora millepora*, *Acropora kenti* (formerly *A. tenuis*), *Acropora hyacinthus*, *Platygyra daedalea*, *Goniastrea retiformis* and *Porites lobata*, including about 250 isolates from gamete bundles and spawning water. – Isolates include representatives from at least four phyla, 15 orders, and 27 families, as identified by partial 16S ribosomal ribonucleic acid (rRNA) gene sequencing. • Antibacterial activity and production of enzymes that may enhance digestion were confirmed in several isolates included in an *in vitro* screening program – Antibacterial activity against the coral pathogen *Vibrio coralliilyticus* was found in 72 out of 277 screened isolates. – Production of one or more digestive enzymes including amylase, caseinase, gelatinase, chitinase, lipase, and phospholipase was found in 36 out of 51 screened isolates. • Select candidates were further characterised by whole genome sequencing (33 isolates) and *in vivo* screening (see 2.1 c below) Shortlisting of candidates for testing in coral spat was based on their taxonomic identity, *in vitro* screening results, and current knowledge of coral microbiology and bacterial probiotics.
2.1 (c)	Assemble probiotic consortia and test their ability to enhance the performance of at least two coral species (one Acroporid, one non-Acroporid) that are part of the agreed RRAP target coral species.	**This objective was achieved in full for *Acropora kenti* and in part *for Platygyra daedalea*:** • *A. kenti* spat were amenable to microbiome manipulation, identifying early life stages as a window of opportunity for such approaches. – Single-strain inoculations in well-plates of newly settled *A. kenti* showed that individual candidates resulted in differing microbiome responses, stability of formed bacteria-host associations, and host health outcome (assessed by survival, growth, immune response activation, photosymbiont counts). The experiment supported the selection of candidates for inclusion in a probiotic consortium for *A. kenti*. • For *A. kenti*, a consortium of four probiotic candidates was assembled. – The resulting consortium included the bacterial strains *Roseivivax lentus* 3-ATT-3, *Endozoicomonas acroporae* 2-ATT-W5, *Halomonas smyrnensis* 2-ATT-W4, *Pseudoalteromonas rubra* ATM06, all of which were originally isolated from adult *A. kenti* colonies. • The *A. kenti* consortium was tested in larger-scale flow-through systems, investigating different delivery routes (via water column or diet) and number of applications (two or six deliveries). with all treatments exposed to an acute temperature stress challenge after three weeks followed by one week recovery. – *Roseivivax lentus* 3-ATT-3 formed stable associations with *A. kenti* spat across all experiments and the association was retained after exposure to temperature stress. This association was stable after only two applications (the lowest number tested), providing a logistical advantage over candidates that need repeated applications.

Objective		Key Findings and/or Outcomes
		– *Endozoicomonas acroporae* 2-ATT-W5 produced coral associated microbial aggregates (CAMAs) in *A. kenti* spat in the controlled well-plate system, demonstrating potential for establishing long-term symbiosis. However, CAMAs did not form after inoculation with the consortium and further studies would be required to establish suitable inoculation procedures for *Endozoicomonas*-based probiotics. – *Halomonas smyrnensis* 2-ATT-W4 formed a transient association in flow-through systems where the strain was detected at low levels once re-applications ceased. The candidate holds potential as a transient probiotic in aquaculture, with reduced environmental risk after deployment. – *Pseudoalteromonas rubra* ATM06 was detected at very low levels or not at all in the coral microbiome but induced community shifts. This candidate holds potential as a transient probiotic in aquaculture, with reduced environmental risk after deployment. – While no clear health benefit of probiotic application was observed, this is consistent with emerging evidence that coral juveniles are relatively resilient to temperature stress. Future experiments should investigate if microbiome manipulation of newly settled corals can mediate delayed benefits and stress resilience in older juveniles or colonies. • The microbiome of *P. daedalea* was amenable to manipulation but only one of the eight candidates tested individually induced a significant microbiome shift. – This *Ruegeria* candidate (*Ruegeria* P1 27, isolated from an adult *P. daedalea* colony) was not tested in a flow-through system due to low settlement success of *P. daedalea* for the summer spawning in 2022 (Table 2).
2.2 Sub-project ECT-03.2.2: Coral Nutrition		
2.2 (a)	Develop diets and supplements (probiotics) to support healthy, fit, and reproductively active corals throughout their life cycle in captive aquarium conditions, including after asexual propagation	This was the overarching aim of the sub-project and not intended as a specific objective. It was achieved through the two specific objectives listed below. • A standard operating procedure for feeding both Acroporid and non-Acroporid coral juveniles with a zooplankton mixture was experimentally validated. Ongoing monitoring of deployed corals will provide evidence to determine if combination feeding with a liquid supplement containing minerals, vitamins, pigments and marine lipids can further enhance survival and growth after deployment. Due to altered priorities, long-term feeding experiments were not performed with asexually propagated corals (Table 2). The project findings were communicated through national conferences, RRAP reports and literature reviews, student theses, and peer-reviewed publications in preparation.
	Identify live feed enrichments, nutritional formulations, and supplements with	• The project developed encapsulated diets that were enriched with specific fatty acids for subsequent experimental validation in three-month long feeding experiments with coral spat. – Novel microencapsulated diets were produced by complex coacervation to stabilise and prevent oxidation of valuable nutrients (e.g. oils) and control sensory issues.

Objective		Key Findings and/or Outcomes
	expected nutritional and health benefits for experimental validation	– A baseline encapsulated diet was made with fish oil (2022, 2023), and enriched encapsulated diets were made using fish oil enriched in docosahexaenoic acid (DHA: 22:6n-3) (2022) or arachidonic acid (ARA: 10:4n-6) (2023). – Enrichment of specific fatty acids were confirmed analytically in the diets themselves. • Supplementation of the encapsulated diet with a probiotic consortium was tested as a probiotic delivery strategy as part of ECT-03.2.1 (see 2.1 (c) above). • A commercial liquid coral feed (Continuum Micro Blast; including minerals, vitamins, pigments and marine lipids) and live zooplankton (Artemia, rotifers) were provided separately or in combination to juveniles of *Acropora millepora* and *Platygyra daedalea*, which differ greatly in their relative reliance on autotrophy and heterotrophy, for three months. – Combined feeding with zooplankton and the commercial liquid feed did not enhance survival or growth relative to the zooplankton mixture on its own for either coral species. Supplementation with the liquid feed did however produce *P. daedalea* spat with stronger pigmentation. – Corals from this experiment have been deployed and will be monitored through 2025/26 to assess whether the feed treatments produced differences in spat survival and growth after deployment.
2.2 (c)	Develop a palatable microencapsulated diet and test its ability to promote growth and maintain health of at least two coral species (one Acroporid, one non-Acroporid) that are part of the agreed RRAP target coral species.	• Microencapsulated feeds were ingested by multiple species of captive corals when combined with an attractant (fish protein hydrolysate) to enhance palatability and adjust buoyancy. – Ingestion confirmed via video analysis for coral juveniles (*Acropora kenti, Platygyra sinesis, Platygyra daedalea, Goniastrea retiformis, Lobophyllia corymosa*), adult colonies (*Galaxea fascicularis*, *Pocillopora acuta*), and asexually produced corals (*P. daedalea*, *Porites lutea*). • In two 3-month feeding experiments, encapsulated diets supported survival and growth of juvenile *A. kenti*, but no statistically significant benefit was recorded relative to live feeds (Artemia, rotifers). • Encapsulated diets enriched in specific fatty acids did not produce corresponding enrichment in fatty acid profiles of fed coral (*A. kenti*) juveniles. • In one 3-month feeding experiment, encapsulated diets did not support growth of *G. retiformis* juveniles. In contrast, the Artemia treatment in the same experiment supported significant growth.

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
Assemble probiotic consortia and test their ability to enhance the performance of at least two coral species (one Acroporid, one non-Acroporid) that are part of the agreed RRAP target coral species.	**A probiotic consortium was tested with one coral species only, *Acropora kenti*.** Single probiotic candidates were tested in a well-plate system with newly settled spat of two coral species, *A. kenti* and *P. daedalea*, following the summer spawning of 2021. These experiments identified four promising candidates for use with *A. kenti*, which were tested as a consortium in a flow-through system following the summer spawning of 2022, and individually in a flow-through system following the 2023 summer spawning. In contrast, out of the eight candidates tested individually with *P. daedalea* spat in 2021, only one formed a stable association with the host. Nevertheless, we selected candidates that were compatible (no cross-inhibition) that we intended to test as a consortium with *P. daedalea* in a flow-through system. For the summer spawning in 2022, we had very low settlement success with *P. daedalea* however, and too few spat were available to run an experiment with this coral species. From this point we focused our efforts on developing the *A. kenti* probiotic treatment given the four promising candidates that were identified for this coral species.
Develop diets and supplements (probiotics) to support healthy, fit, and reproductively active corals throughout their life cycle in captive aquarium conditions, including after asexual propagation (overarching goal ECT-03.2.2).	**Long-term feeding experiments were performed with coral juveniles only.** Coral fragments of *Galaxea fascicularis*, *Pocillopora damicornis* and *Acropora hyacinthus* were used in experiments performed in 2021 to document feeding behavioural responses and diet uptake using three live feeds (Artemia, copepods, rotifers), four attractants (Artemia homogenate, copepod homogenate, commercial amino acid mixture, microalgae mixture), and two alginate bead sizes. Using a prototype encapsulated diet soaked in attractant (fish protein hydrolysate, krill protein hydrolysate) and Artemia, video analysis of diet ingestion was performed with coral juveniles settled after the summer spawning in 2021 (a range of coral species) and asexually produced sheets of *Platygyra daedalea* and *Porites lutea* in 2022. From this point onwards, our focus shifted to longer-term feeding experiments with coral juveniles, reflecting a similar shift in research focus towards asexual coral production in the RRAP Sub-program Coral Aquaculture and Deployment (CAD) projects.

4 Future Research Recommendations

4.1 Microbial inducers for coral larval settlement

Microbial settlement treatments have great potential to improve the efficiency and success of large-scale sexual propagation of corals for reef restoration. During the first phase of RRAP, we have made significant progress in identifying bacterial inducers for a range of coral species. We recommend continuing the development of these inducers into treatments suitable for use in reef restoration. Here we provide next steps and recommendations for research priorities:

- **Prioritise bacteria-produced cues over live bacteria.** Live bacteria pose greater risks and logistical challenges for implementation at scale. Efforts should therefore focus on bacteria-produced cues or methods to preserve bacteria to ensure treatments are practical for use in large-scale coral aquaculture.
- **Identify and isolate specific bacteria-produced cues.** Further research will be required to identify and isolate the specific bacteria-produced cues responsible for inducing coral settlement. The potential benefits of this research are expected to outweigh the additional efforts and cost.
- **Focus on feasibility of upscaled production and implementation.** Selection of settlement cues for treatment development should consider the factors outlined in this report to ensure development of a product with potential for upscaling. Feasibility for upscaled treatment production and implementation will depend on securing a suitable industry partner and require a full cost-benefit analysis.
- **Test on a wider range of coral species.** Treatments should be tested and optimised to target as wide a range of species as possible to support restoration of biodiverse reefs. However, specific treatments that target endangered or difficult to settle species may also prove valuable for restoration efforts.
- **Conduct larger scale settlement experiments.** The best methods for applying treatments to desired settlement devices will need to be established and settlement experiments should be scaled up to test efficacy of treatments during mass settlement in aquaculture
- **Conduct longer-term experiments for risk assessments.** Treatments will need to be evaluated to ensure they are safe to use in an aquaculture settling. This should include rigorous genetic and experimental screening as well as longer-term studies to assess health and survival of coral spat and juveniles.
- **Fundamental research to understand mechanisms of bacteria-induced coral settlement.** Studies should investigate bacterial and host gene expression and metabolites to understand the molecular mechanisms underpinning how bacteria influence coral settlement. This fundamental knowledge would inform treatment development and risk assessment.

4.2 Probiotics for aquaculture production of coral

Probiotics delivered to coral spat and juveniles in aquaculture facilities were demonstrated to modulate, and in some cases were incorporated into, the coral microbiome. Probiotics application in *ex situ* aquaculture can therefore introduce beneficial bacterial traits into the coral holobiont and we recommend continuing the

development of these treatments as initiated in the first phase of RRAP. Specifically, we recommend that future work include the following activities.

- **Develop single inoculation protocols.** A single inoculation would reduce the resources required for longer-term experiments and reduce logistical challenges for probiotic use in upscaled coral aquaculture. Efforts should focus on identifying the time window during coral ontogeny that is suitable for a single inoculation.
- **Validate host benefits in longer term experiments.** Benefits to coral survival, growth, health and resilience should be validated in longer term experiments, preferably including deployments with assessment of survival after a natural heat wave or after a longer timeframe (e.g. one year).
- **Test probiotic strains with a broader range of coral species.** If the same strains can be used for multiple coral species, this would reduce logistical challenges for probiotics use in upscaled coral aquaculture to support restoration of biodiverse reefs.
- **Investigate further the potential to upscale production of probiotic candidates.** The activity of probiotic candidates after upscaled production would need to be confirmed. Assessments of the costs and logistics of production would be contingent on production volume and securing a suitable industry partner for manufacture and distribution.
- **Conduct fundamental research to understand probiotic mechanisms after inoculation.** Studies should include analyses of bacterial and host gene expression and metabolites, and other advanced methods to assess the localisation of specific probiotics and their metabolic activities. This fundamental knowledge would inform treatment development and risk assessment.
- **Prioritise experiments required to inform risk assessments and the permitting process.** This includes tracking the possible transfer of probiotic strains to non-target organisms, longer-term studies of host performance, and fundamental understanding of probiotic mechanisms (as above).

4.3 Coral nutrition

The development of formulated feeds optimised for captive corals is expected to support reef restoration efforts. Given the results summarised herein on the use of novel microencapsulated feeds, we recommend that future work include the following activities:

- **Conduct feeding trials with a broader range of coral species.** This would aid in developing feeds, supplements and feeding protocols tailored to meet species-specific and age-specific feeding behaviours and nutritional requirements to support upscaled coral aquaculture for restoration of biodiverse reefs.
- **Validate the benefits of feeding coral juveniles by monitoring survival and growth after deployment.** The benefits of developed feeds, supplements and feeding protocols on the survival and growth of cultured corals after deployment needs validation.
- **Refine the physical and nutritional properties of microencapsulated feeds.** The ability to adjust the composition, feed particle size, buoyancy and feed palatability or attractiveness of microencapsulated feeds would enhance the delivery of exogenous nutrients to captive corals via formulated feeds.

- **Further investigate the potential to upscale the production of formulated feeds.** Feasibility for upscaled treatment production and implementation will depend on securing a suitable industry partner and require a full cost-benefit analysis once further details are known.

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