Operationalising eDNA monitoring for early COTS detection

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COTS Control Innovation Program | A research and development partnership to better predict, detect and respond to crown-of-thorns starfish outbreaks

Research Translation Project CCIP-RT-02

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Traditional Owner Acknowledgement

The COTS Control Innovation Program extends its deepest respect and recognition to all Traditional Owners of the Great Barrier Reef and its Catchments, as First Nations Peoples holding the hopes, dreams, traditions and cultures of the Reef.

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Acronyms and Abbreviations

AIMS	Australian Institute of Marine Science
BPM	Blue Planet Marine
COTS	Crown-of-thorns seastar
CPUE	Catch per unit effort
CCIP	Crown-of-thorns starfish Control Innovation Program
CSIRO	Commonwealth Scientific and Industrial Research Organisation
ddPCR	Digital droplet Polymerase Chain Reaction
eDNA	Environmental DNA
GBR	Great Barrier Reef
GBRF	Great Barrier Reef Foundation
PMG	Pacific Marine Group
PP	Proportion Positive
QC	Quality Control
QPWS	Queensland Parks and Wildlife Service
RJFMP	Reef Joint Field management Program
Reef Authority	Great Barrier Reef Marine Park Authority
SALAD	Scooter-assisted large area diver-based visual survey
SOP	Standard Operating Procedure

PROJECT SUMMARY

Project CCIP-D-03 established procedures and proof on concept for an eDNA based monitoring of COTS outbreaks and demonstrated that COTS can be reliably detected at pre-outbreak levels. The current project continues to operationalise the monitoring on platforms other that research vessels.

The main aims of this project were:

1) To operationalise sampling on QPWS vessels

2) To conduct further trials on COTS control vessels

3) To discuss options for streamlining sample analysis with various commercial laboratories and explore options to outsource lab analysis

Collection of eDNA samples from QPWS vessels worked seamlessly and staff were positive about collecting eDNA as a survey method. A total of 18 reefs were successfully monitored over a two-month period using COTS eDNA collection and analysis. Despite contamination issues in the previous CCIP-D-03 project (*Operationalising eDNA monitoring*), improvements in 'eDNA-clean' workflows and changes in the timing of sampling that were trialled during this translation project allowed successful eDNA collections from two different COTS control vessels, resulting in the first eDNA survey result based on control vessel collections.

An eDNA data delivery table was developed that reports eDNA results summarised on the reef level and proposes actions based on inferred COTS density. This data delivery format was applied to eDNA collections from both QPWS and COTS control vessels with intended incorporation into the reef prioritisation processes. Our workflow analysis indicated that the most significant option for streamlining the process is exploring a new DNA extraction method, which would save significant amount of processing time.

Based on quotes from three external companies, outsourcing of the laboratory analysis was not more cost-effective than the current in-house analysis conducted by the Australian Institute of Marine Science (AIMS). In summary, all three project objectives were achieved, and we consider eDNA as a 'ready to implement' operation tool for monitoring COTS across the Great Barrier Reef.

CONTEXT & OBJECTIVES

Acanthaster spp. (Crown of Thorns Seastar, COTS) are several coral eating seastar species in the tropical Indo-Pacific (Uthicke et al. 2024b). Some species undergo occasional or regular population outbreaks that contributes to coral loss and the reef crisis. *Acanthaster* cf. *solaris*, the species occurring on the Great Barrier Reef (GBR), appears particularly prone to outbreaks, with recorded events in French Polynesia (Kayal et al. 2012), Indonesia (Baird et al. 2013), the GBR (Moran 1986; Pratchett 2005), and Okinawa, Japan (Nakamura et al. 2014). The GBR is currently at the end of its 4th outbreak wave since the 1960s, and a 5th

outbreak wave has commenced in the northern section of the GBR (Chandler et al. 2023; Uthicke et al. 2024a).

Early detection of outbreaks and accurate, large-scale COTS monitoring is key for identifying reefs under threat and enabling rapid response. However, this is difficult to achieve with current observational technologies. Manta tow surveys are well suited for large area surveys during active outbreaks to monitor spread and identify locations under impact from increased COTS populations. At natural densities and during early outbreak phases, however, COTS occur in low densities and are difficult to detect with current manta tow observation methods. A monitoring program that has a wider selection of tools to detect COTS is needed to enable detection at low density and early outbreak phases (Babcock et al. 2020). At the Australian Institute of Marine Science (AIMS), we have developed and improved environmental DNA (eDNA) monitoring technology for COTS since 2013. These techniques were applied to detect and quantify COTS larvae (Uthicke et al. 2015; Doyle et al. 2017; Uthicke et al. 2019), settled juveniles (Doll et al. 2021) and post-settlement COTS on the reef at extremely low densities (Uthicke et al. 2018; Doyle and Uthicke 2020; Kwong et al. 2021; Uthicke et al. 2022). For post-settlement COTS, this method is now mature and fully developed. We also demonstrated that this method is quantitative, showing a clear, significant relationship between COTS eDNA analysis results and actual COTS densities on the reef estimated using Scooter-Assisted Large Area Diver-based Surveys (SALAD) (Uthicke et al. 2025). The sensitivity of the method is very high (Uthicke et al. 2022), with detection at the suggested 'Allee threshold' (Rogers et al. 2017) of 3 COTS ha^{-1} feasible (Uthicke et al. 2022; Uthicke et al. 2025). This is distinctly below the 'outbreak levels' currently used for COTS management. Detecting COTS at this or lower levels will be essential to allow intervention at early stages of the outbreaks. In addition, detection of outbreaks at early stages will assist in identifying the locations and extent of outbreak initiation areas, which will allow culling efforts to focus on these areas to prevent future outbreaks and aid our understanding of the causes of (primary) outbreaks.

Supported by the previous Crown-of-thorns starfish Control Innovation Program (CCIP) project CCIP-D-03 (Uthicke et al. 2025), we started operationalising eDNA detection of COTS across various platforms. All initial tests and method developments were conducted on research vessels, and several years of pilot monitoring on a series of reefs were conducted from 2021-2023. Similarly, we established a cost-efficient monitoring effort based on Lizard Island with data from 2019 to 2025 currently available. The value of the location as a 'sentinel station' for initiation of an outbreak wave was demonstrated by detecting a significant increase in eDNA signal after two years of sampling, thus providing an early warning of a developing outbreak at least three years prior to when manta tows were likely to detect an established outbreak (Uthicke et al. 2024a; Uthicke et al. 2025). While AIMS research vessels have provided the necessary platform for method development, with the method now tested and proven it is timely and cost-efficient to transition most of eDNA sample collection to the management vessels involved in COTS monitoring. Some ongoing monitoring of baseline reefs and Lizard Island as a sentinel station by professional scientists is still recommended for quality control. Other platforms include the Queensland Parks and Wildlife Service (QPWS) vessels that conduct annual COTS monitoring across the Great Barrier Reef as part of the Reef Joint Field Management Program (RJFMP), and the COTS control vessels that conduct monitoring and control activities as part of the COTS Control Program. Sampling from QPWS vessels has previously been trialled and is far advanced on

the concept to operationalisation continuum. During project CCIP-D-03, QPWS vessel crews were trained in eDNA collection, and successfully and independently collected samples from ten reefs (Uthicke et al. 2025). In the present research translation project, we aim to finalise operationalisation by providing training and establishing a collection regime on two further RJFMP trips, and by delivering an operational end-to-end workflow from training, collection and fixing of samples to data delivery. The work will be undertaken alongside (and will be integrated with), a further research translation project designed to pilot and operationalise the COTS monitoring design recommendations from project CCIP-D-01 (Lawrence et al. 2025) which provides guidance on which reefs to sample across the GBR.

Methods for eDNA sample collections from COTS control vessels are not as operationally mature as collections from QPWS vessels or research vessels. We conducted several previous tests on COTS control vessels, however, due to the nature of their operation (culling of COTS) we encountered some level of sample contamination when collecting COTS eDNA from control vessels (Uthicke et al. 2025). Although the contamination levels were low, due to the high sensitivity of the method this contamination confounded the interpretation of eDNA data at low COTS levels. Statistical modelling during project CCIP-D-03 suggested that it may be possible to overcome this issue, possibly by collecting a larger number of negative control samples (Uthicke et al. 2025). In the present translation project, we aimed to address the contamination issue by piloting the collection of eDNA from two different COTS control vessels while also adapting the sampling protocol based on the insights gained from the modelling. This included the collection of more negative control samples than in previous trials, to test if this allows quantification. We also collected controls at several locations and times to identify best locations for processing and timing of samples. In addition, we worked with the operators to improve the workflow, with the aim to minimise contamination issues and limit the impact of eDNA collection on the day-to-day management of the operations.

Improving the time and cost efficiency of eDNA monitoring has been another important focus of our research to date. We previously (CCIP-D-03) delivered clear recommendations for the field and laboratory workflow and provided sampling options that could be used to estimate COTS densities using eDNA to achieve different management objectives (Uthicke et al. 2025). An important finding of the previous project was that field sample effort can be reduced compared to our initial trials without sacrificing detection power, thus saving time and funds. While there are now opportunities for non-scientists (e.g. tourism operators, QPWS or COTS control vessels) to collect field samples, currently all laboratory-based sample analysis is conducted at AIMS. Part of the present project is to investigate whether more time and cost-effective methods of analysis exist, for instance outsourcing analysis to commercial laboratories.

CCIP project CCIP-D-03 delivered a method and options for COTS eDNA monitoring. To assess Ships of Opportunity and operationalise eDNA post-settlement was integral to the original CCIP program logic, and CCIP-D-03 had a clear impact pathway by extending the toolbox for COTS detection and improving detection and monitoring to the ultimate impact of coral protection in this project. The **expected benefits** of the present project and **impact pathway** are the same for the proposed follow-up project, with early detection of COTS in low density allowing early intervention. Thus, these methods can be directly taken up and integrated into decision support for COTS control. The **specific objectives** of the current project are:

1) To operationalise eDNA sampling on QPWS vessels, while applying recommendations on sampling design from previous CCIP projects

2) To conduct further trials of eDNA sampling on COTS control vessels to address issues of cross-contamination

3) To investigate options for streamlining of sample analysis with various commercial laboratories and explore options to outsource lab analysis

APPROACH

1) Operationalise sampling on QPWS vessels

Consultation with Great Barrier Reef Marine Park Authority (Reef Authority) COTS Control Program managers and QPWS identified two trips on which COTS eDNA sampling could be tested from QPWS vessels as part of the Reef Joint Field Management Program (RJFMP) COTS Response effort. The same trips were chosen for related translation project CCIP-RT-01 to allow side-by-side comparisons of manta tows (visual observer method), Reefscan (image-based monitoring method), and eDNA data (Lawrence et al. 2026). Decisions on how the eDNA sampling best fitted into the workflow of the daily activities was left to the QPWS cruise leaders.

Training and equipment were delivered to QPWS staff on the 6th January 2025 to enable eDNA collections to be conducted during two field trips, one in January 2025 (FMPCRS 2025-01) targeting reefs in the Swains sector, and one in February 2025 (FMPCRS 2025-02) targeting reefs in the Pompey sector. Sampling design followed the optimised sampling design recommend in CCIP-D-03 (Uthicke et al. 2025), that is, four sites per reef and six samples per site sampling effort. Three types of controls are routinely included to monitor field collections (field controls), laboratory extractions (extraction controls) and digital droplet PCR analysis (PCR controls). Specifically, field controls involve filtering supplied 1 L of purified water through eDNA collection equipment that have been through the same cleaning and preparation process as collection equipment used for samples. Field controls are conducted at a rate of two per reef on the main ship for the duration of the voyage. These controls pass through the entire process of filtration, laboratory extraction and ddPCR. Laboratory extraction controls involve placing a new membrane filter into tubes containing preservation buffer and then extracting these alongside samples at the point of extraction. Extractions are conducted in groups of 12 (due to automation parameters) and thus, each set of 12 comprises 11 samples (including field controls) and one laboratory extraction control. Extraction controls pass through the process of laboratory extraction and ddPCR. PCR controls involve adding water in the place of DNA extract during the digital droplet PCR analysis. This is done at a rate of approximately one per 40 samples undergoing ddPCR analysis. PCR controls pass through the ddPCR process only. This series of controls allows a hierarchical understanding of contamination, if present. For these trips, a total of 36 field controls (two field controls were conducted per reef), 43 extraction controls and 16 PCR controls were analysed.

Sample analysis

As per our standard procedure (Uthicke et al. 2025), subsequent to filtration, filter units were dissembled on board the main vessel and filters placed in ATL buffer by QPWS staff. Upon return to the AIMS lab, eDNA was extracted from samples using a Qiagen Blood and Tissue DNeasy kit on a Qiacube automated nucleic extraction instrument following the method described in Doyle and Uthicke (2020). Digital droplet Polymerase Chain Reaction (ddPCR) was conducted as per Uthicke et al. (2018), with improvements as described in Uthicke et al. (2018; 2024a). Total accepted droplets for each ddPCR reaction were typically above 20,000. Quality control applied to each ddPCR reaction included rejection of ddPCR reaction where the accepted droplet count was below 10,000 (https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf) and consistent thresholding to determine positive and negative droplets.

2) Pilot trials on COTS control vessels

Figure 1. Demonstration and training for COTS eDNA monitoring delivered to PMG (Odyssey, left image) and BPM (Infamis, right image) on the 31st March 2025 in Townsville and 4th April 2025 in Gladstone respectively. Photo credit: Maria Gomez Cabrera

Following detailed consultation and planning involving GBRMPA and Control vessel operators, it was decided to run trial eDNA collections from two COTS Control Vessels, MV Odyssey (Operator: Pacific Marine Group, PMG) and MV Infamis (Operator: Blue Planet Marine, BPM). These vessels were identified as suitable for initial eDNA collection trials mainly due to the available space onboard to conduct eDNA sample processing activities. In addition, operational considerations were discussed in detail with operators and crew of the vessels. For example, eDNA sampling would be conducted separately from culling activities to minimise potential cross contamination, which is most likely to be a direct result of culling activities. For example, staff involved in culling may have COTS DNA attached to their wet suits and skin and bring this on board the tender after operation. Two trial rounds were proposed to allow for potential learnings with respect to controls and contamination. Prior to Round 1 trials it was decided that the cleanest option would likely be to collect eDNA samples in the morning, prior to initialising any culling activities. Thus, vessel crew collected eDNA samples on each reef as their first activity of the day for both trials. The sampling

design was the same as that tested on QPWS vessels (four sites per reef with six samples per site). However, for possible statistical post-hoc correction for contamination and to identify putative contamination sources, several types of negative control samples (described below) were also included with higher replication of controls compared to QPWS eDNA collections or samples collected on previous research voyages.

Round 1 trials, April 2025

Voyages identified for eDNA collections of Round 1 were Odyssey voyage 136 (31st March – 13th Apr 2025) to Townsville reefs and Infamis voyage 41 (4th April – 16th April 2025) to the Swains (Tab. 1). Strong wind conditions during the voyages limited collections to two reefs per voyage.

For each reef in Round 1 trials, field controls were expanded from those conducted with QPWS to assist with tracking contamination and included:

- Tender field controls – pre-culling. Filtering purified water (6 x 1L) supplied by AIMS in the sampling kits while on the tender. This was done directly after eDNA collection, before any culling activity had started. This type of control sample tested whether collecting eDNA samples are less prone for contamination prior to the daily culling activities. In addition, these tender field controls collected from the tender provide the 'standard' procedural negative controls.

- Main ship field controls These are identical to the field controls conducted by QPWS. Filtering purified water (6 x 1L) supplied by AIMS in the sampling kits while on the main ship, at the same location on the ship as sample processing took place. This type of control sample tested for contamination that would be introduced during the sampling fixation process.

- Supplementary main ship field controls using ship's tap water - were also included. These were used to test if the ship's tap water could be utilised as field negative controls. These controls (6 x 1L) were included as it would be logistically easier to use ship water as control samples if they were free of COTS DNA contamination. Thus, these controls are compared to the Main ship field controls described above.

Round 2 trials, August 2025

Odyssey voyage 142 (31st July – 11th Aug 2025, Tab. 1) to Townsville reefs and Infamis voyage 2 (45) (13th Aug – 25th Aug 2025) to the Swains/Mackay region were chosen for Round 2 eDNA collection trials.

Based on results from Round 1 trials, we omitted the control: "Supplementary main ship field controls with water supplied by the vessels". For Round 2, we asked the control vessel operators to take the same number of controls as above per reef. However, to explicitly test for contamination risk post culling, we added:

- Tender field controls – post-culling - Filtering purified water (6 x 1L) while on the tender. These control samples were collected in the afternoon, after other eDNA samples and culling activities.

With the exception of additional control samples described above, sample analysis in the laboratory was as described for samples collected on QPWS vessels (Section Approach 1).

Table 1. Date and location of eDNA sampling from COTS control vessels.

Voyage	Date	Reef
Round 1 trials:		
PMG Odyssey Trip 136	02/04/2025	Banfield Reef (North)
	06/04/2025	Big Broadhurst Reef
BPM Infamis Trip 41	10/04/2025	21-460
	12/04/2025	21-466
Round 2 trials:		
PMG Odyssey Trip 142	04/08/2025	Banfield Reef (North)
	05/08/2025	Judith-Wright
BPM Infamis Trip 2 (45)	19/08/2025	20-386
	22/08/2025	21-057
	23/08/2025	Bushy-Redbill

3) Discuss options for streamlining of sample analysis with various commercial laboratories and explore options to outsource lab analysis

Work under this section consisted of three parts, details of methods are integrated in the Outcome section. First, we conducted a **workflow assessment** of the workflow currently used and searched for efficiencies in each individual step. Second, we conducted an analysis of the current in-house cost at AIMS and obtained external quotes to explore **options to outsource lab analysis**. Third, we collaborated with colleagues at Commonwealth Scientific and Industrial Research Organisation (CSIRO) and GBRMPA to improve **Data delivery and interpretation.**

4) Stakeholder engagement

The project required considerable stakeholder engagement including many discussions with trip leaders and crew of QPWS voyages to streamline sampling, coordinate training, and delivery and retrieval of gear and samples. Similarly, testing eDNA sampling on COTS control vessels required consultation with several control boat operators and crew. Results and approaches were discussed with managers from the COTS Control Program at the Reef Authority.

OUTCOMES & MANAGEMENT IMPACT

1) Operationalise sampling on QPWS vessels and data delivery

The timing of eDNA collection on each reef was decided by QPWS cruise leads, however, guidance was given to avoid any culling activities or COTS collections whilst undertaking eDNA collection.

Time required to complete all activities pertaining to eDNA monitoring in the field (collection, preservation, etc) was approximately two hours per reef.

In total, eDNA was collected on 18 reefs in two Great Barrier Reef (GBR) regions (Tab. 2). All controls returned negative results for CoTS eDNA, thus, it can be concluded that samples collected from QWPS vessels were to the same high standard as we achieved on research vessels or based on research stations, and that positive samples are not due to contamination, but represent real COTS detections.

We use two metrics for estimating COTS density using eDNA samples. These are the proportion of positive samples per reef (PP - the proportion of samples out of the ones collected from a particular reef found to be positive for COTS) and the average copy number (the number of eDNA copies per L of sample) per reef across all samples. We demonstrated in previous work (Uthicke et al. 2022; Uthicke et al. 2024a; Uthicke et al. 2025), that COTS eDNA provides a quantitative measure for COTS densities, with Proportion Positive (PP) being a useful metric for low densities (~ < 10-15 COTS ha^{-1}) and average copy numbers for higher densities (> 10 COTS ha^{-1}). This is because the proportion positive 'saturates' (ie is close to 1, nearly all samples are positive) at densities around 10 Ind. ha^{-1}, and the average copy number is extremely variable at low densities.

Overall, the proportion of positive samples (PP) per reef varied between 0.04 and 0.78 (Tab. 2. The second metric we apply to eDNA samples (the average copy number) assumed relatively low values (< 42 copies L^{-1}).

Our prior research calibrated eDNA results with visual surveys on reefs using the SALAD method, showing that the proportion of samples that tested positive for COTS eDNA correlated well with the estimated densities of COTS from counts (SALAD observed) and counts of COTS plus their feeding scars (SALAD inferred) that were estimated during visual surveys (Uthicke et al. 2024, 2025). In the present study, we have applied and extended that knowledge to develop a conservative interpretation of both PP and average copy number, by grouping eDNA results in four density categories based on conversion to Scooter-assisted large area diver-based (SALAD) (Tab. 3). For example, we consider eDNA results of ~ <0.4 PP and ~ <10 copies per L as indicative of observed COTS densities on a reef being below the COTS Allee threshold of 3 ha^{-1}. Densities above Allee threshold would correspond to eDNA metrics above a PP of ~0.4 and ~10 copies per L. If observed COTS densities on a reef are above the ecological threshold (15 ha^{-1}), we suggest this corresponds to eDNA results where 100% of samples are positive (PP = 1) and the average copy numbers is >100 copies L^{-1}.

COTS density category values based on SALAD inferred data are somewhat lower since inferred SALAD data count scars as well as COTS and assume higher densities. Hence, in case more conservative management decisions are required, interpretation of eDNA results based on SALAD inferred calibrations, should be used.

Table 2. Summary of eDNA results on a per reef basis and suggesting possible follow up action as a simplified example of the data delivery table, the data interpretation given is based on calibrations to SALAD data using observed COTS densities [SO]. Note: actions in this example are suggested based on SO (SALAD observed) and SALAD Inferred. CI: Confidence Interval, SE: Standard Error.

Reef Sampled	Sector	Date	Proportion positive (CI)	Copy Number (± SE)	Calibrated Density (Ind. ha^{-1}) [SO]	Calibrated Density (Ind. ha^{-1}) [SI]	Possible Action
21-444	Swains	8/01/2025	0.46 (0.28 - 0.66)	6.4 (± 1.6)	3 -15	3 - 15	Cull
21-443	Swains	9/01/2025	0.37 (0.21 - 0.58)	6.2 (± 1.79)	3 -15	3 - 15	Cull
21-453	Swains	9/01/2025	0.08 (0.03 - 0.30)	1.3 (± 0.9)	<3	<3	No Action
21-488	Swains	10/01/2025	0.06 (0.01 - 0.26)	0.3 (± 0.3)	<3	<3	No Action
21-489	Swains	10/01/2025	0.06 (0.01 - 0.26)	0.3 (± 0.3)	<3	<3	No Action
21-476	Swains	10/01/2025	0.06 (0.01 - 0.26)	0.7 (± 0.5)	<3	<3	No Action
21-263	Swains	12/01/2025	0.18 (0.07 - 0.38)	1.7 (± 0.8)	<3	<3	No Action
21-270	Swains	13/01/2025	0.14 (0.05 - 0.34)	1.0 (± 0.5)	<3	<3	No Action
21-477	Swains	14/01/2025	0.10 (0.03 - 0.30)	0.7 (± 0.5)	<3	<3	No Action
21-067	Pompey	7/02/2025	0.5 (0.31 - 0.69)	18.4 (± 7.2)	3 -15	3 – 15	Cull
21-071	Pompey	8/02/2025	0.14 (0.05 - 0.34)	1 (± 0.6)	<3	<3	No Action
21-069	Pompey	8/02/2025	0.06 (0.01 - 0.26)	0.3 (± 0.4)	<3	<3	No Action
20-383	Pompey	9/02/2025	0.26 (0.12 - 0.47)	2.3 (± 0.9)	<3	3 – 15	Follow up individual sites
20-387	Pompey	9/02/2025	0.26 (0.12 - 0.47)	3.3 (± 1.4)	<3	3 – 15	Follow up individual sites
20-386	Pompey	10/02/2025	0.42 (0.24 - 0.62)	7.8(± 2.6)	3 -15	3 – 15	Cull
21-092	Pompey	11/02/2025	0.78 (0.57 - 0.90)	41.9 (± 8.7)	3 -15	15 – 25	Cull
21-094	Pompey	11/02/2025	0.66 (0.45 - 0.82)	11.7 (± 2.8)	3 -15	15 – 25	Cull
21-093	Pompey	12/02/2025	0.46 (0.28 - 0.65)	14.8 (± 4.8)	3 -15	3 - 15	Cull

Based on these eDNA calibrated COTS density categories, and in consultation with stakeholders and colleagues (Great Barrier Reef Foundation (GBRF), CSIRO, Reef Authority), we developed a data delivery table (see details in section 3.c; see simplified version in Table 2) summarising eDNA results on a per reef basis and suggesting possible follow up action. Possible follow up action depends on the management objective. In the example (Tab. 2), we assume COTS populations below Allee threshold (3 COTS ha^{-1}) need no action, whereas higher densities either need follow up surveys or culling. Thus, we culling is a possible action on reefs which had estimates over 3 COTS ha^{-1} based on both calibrations to SALAD observed and inferred densities. In some instances, a follow up was recommended because individual sites had elevated eDNA values (data now shown).

Table 3. Proposed interpretation of COTS eDNA data based on two metrics derived from analysis. SALAD observed densities are based on actual COTS numbers seen during underwater scooter surveys, whereas SALAD inferred data include COTS and presumed feeding scars of COTS (Lawrence et al. 2024).

	Proportion Positive		Average copy number	
Density categories (COTS ha^{-1})	SALAD observed	SALAD inferred	SALAD observed	SALAD inferred
<3	~<0.4	~0.2	~<10	~<10
3 -15	0.4-1	0.2-0.6	~10 - <100	~10 - 50
15-25	1	0.6-1	~100 - <1000	~50 - 250
>25	1	1	~>1000	~> 250

Applying these density categories, nine of the 18 reefs sampled by RJFMP are estimated to have low densities (<3 COTS ha^{-1}) (Table 3). On these reefs, the eDNA monitoring results suggest that the risk is low that these reefs have an outbreak or that COTS densities are sufficiently high to disproportionately contribute to larva production. Two additional reefs (i.e. 20-383 and 20-387, located in the Mackay region) were estimated to have low densities overall (<3 COTS ha^{-1}), but some sites within these reefs had an elevated eDNA copy number and proportion positive levels compared to other sites at the same reef, suggesting some site-level reconnaissance could be beneficial. Seven reefs across the two regions sampled are estimated to have elevated densities (3-15 COTS ha^{-1}) based on the eDNA results, which may indicate incipient outbreak and are possible targets for early intervention. In-water follow up surveys are recommended at these seven reefs.

Comparisons of these eDNA monitoring results with other COTS monitoring methods (i.e. manta tow, ReefScan) was undertaken in a related research translation project CCIP-RT-01 (Lawrence et al. 2026). Briefly, manta tow surveys detected COTS on only four of these 18 reefs and ReefScan surveys detected COTS on six of these reefs, demonstrating that eDNA is effective at identifying reefs with low densities of COTS when other methods may be less sensitive. Summary table and individual eDNA data were delivered to GBRMPA for uploading onto the COTS dashboard and made available via AIMS data repository.

2) Pilot trials on COTS control vessels

We conducted two rounds of field trials from COTS control vessels, primarily to improve the workflow and minimise sample contaminations when collecting COTS eDNA samples. Although this part of the project was mainly designed to test and improve sampling strategies from COTS control vessels, the lack of control contamination allowed for reef estimates of COTS densities from eDNA data (PP and average copy number). Data are presented as per QPWS eDNA data (data delivery table, Tab. 6.) with possible actions ranging from no action (COTS likely < 3 ha^{-1}) through to culling.

Round 1 trials, April 2025

For both COTS control operators and on all reefs, Tender field controls – pre-culling did not contain any COTS eDNA contamination (Tab. 4). Similarly, all Main ship field controls returned negative results. Supplementary main ship field control – ship's water contained minor amounts of COTS eDNA on one occasion.

Results from eDNA monitoring detected COTS on all four reefs sampled by COTS Control crews during the April 2025 trials. The eDNA monitoring results revealed intermediate to high values for PP and copy number on Banfield Reef North and Big Broadhurst Reef, indicative of densities exceeding the outbreak threshold. In contrast, eDNA results at reefs 21-460 and 21-466 had lower proportion positive and average copy numbers, indicating that COTS densities where below the reproductive threshold (<3 ha^{-1}) such that investment in culling may not be required at this time (Tab. 6)

Table 4. Results of field controls from Round 1 trials (April 2025) from COTS control vessels. Sample size = 6 for all samples. Standard deviation is given in brackets for eDNA concentrations of positive samples (PP)

	Tender field control -pre culling		Main ship field control		Supplementary main ship field control (ship's water)	
Reef	PP	concentration	PP	concentration	PP	concentration
Banfield Reef (North)	0	0	0	0	0	0
Big Broadhurst Reef	0	0	0	0	0.17	3.13 (7.65)
21-460	0	0	0	0	0	0
21-466	0	0	0	0	0	0

Round 2 trials, August 2025

Round 2 eDNA trials from COTS control vessels included Tender field controls – post culling to test our hypothesis that the best way to avoid contamination is to collect eDNA prior to conducting any culling activity on days of sampling. Similar to Round 1 trials, Tender field controls – pre-culling showed no contamination at any of the five reefs (Tab. 5). By contrast, four out of five Tender field controls - post-culling contained measurable concentrations of COTS eDNA.

Except for the collections on Judith-Wright Reef, none of the Main ship field controls (collected post-culling) were contaminated, indicating a clean sample processing environment and no contamination of personal or material with CoTS DNA.

Table 5. Results of field controls from Round 2 trials (August 2025) from COTS control vessels. Sample size = 6 for all samples. Standard deviation is given in brackets for eDNA concentrations of positive samples

	Tender field control - pre culling		Tender field control – post culling		Main ship field control	
Reef	PP	concentration	PP	concentration	PP	concentration
Banfield Reef (North)	0	0	0.50	10.0 (21.9)	0	0
Judith-Wright	0	0	0	0	0.33	6.7 (10.3)
20-386	0	0	0.33	12.0 (20.5)	0	0
21-057	0	0	0.17	4.0 (9.9)	0	0
Bushy-Redbill Reef	0	0	0.17	3.8 (9.4)	0	0

During the August 2025 trials, with the exception of reef 21-057 which contained low levels of COTS eDNA indicating COTS densities <3 ha^{-1}, eDNA data suggested intermediate to high COTS densities on the reefs investigated (Tab. 6).

In general, these results are comparable to catch per unit effort (CPUE) data (calculated based on culls on the same day as the eDNA collections) (Tab. 7). The lowest two eDNA results (Reef 21-460 and 21-466) correspond to a CPUE of zero, while high results in eDNA PP and copy numbers correspond to high catch rates (e.g. Banfield Reef (North), Judith-

Wright reef and Bushy-Redbill). Despite the low number of comparisons (N=9), both PP (R^2 = 0.59, p = 0.009) and (log transformed) eDNA copy number (R^2 = 0.49, p = 0.0219), significantly predicted CPUE.

Table 6. Summary of eDNA results on a per reef basis and suggesting possible follow up action as a simplified example of the data delivery table for samples collected on COTS control vessels in 2025. The data interpretation given is based on calibrations to SALAD data using observed COTS densities. Note: actions in this example are suggested based on SO (SALAD observed) and SALAD Inferred. CI: Confidence Interval, SE: Standard Error.

Reef Sampled	Date	Proportion positive (CI)	Copy Number (± SE)	Calibrated Density (COTS ha^{-1}) [SO]	Calibrated Density (COTS ha^{-1}) [SI]	Possible Action
Banfield Reef (North)	2/04/2025	0.82 (0.62-0.93)	42.1 (7.7)	3 -15	15-25	Cull
Big Broadhurst Reef	6/04/2025	0.50 (0.31-0.69)	7.6 (1.9)	3 -15	3-15	Cull
21-460	10/04/2025	0.10 (0.03-0.30)	1 (0.7)	<3	<3	No Action
21-466	12/04/2025	0.10 (0.03-0.30)	0.9 (0.7	<3	<3	No Action
Banfield Reef (North)	4/08/2025	0.98 (0.74-1.00)	90.9 (34.9)	3 -15	15-25	Cull
Judith-Wright	5/08/2025	0.78 (0.57-0.90)	131.5 (44.9)	15-25	15-25	Cull
20-386	19/08/2025	0.62 (0.42-0.79)	21.8 (5.3)	3 -15	3-15	Cull
21-057	22/08/2025	0.18 (0.07-0.38)	1.6 (0.7)	<3	<3	No Action
Bushy-Redbill Reef	23/08/2025	0.86 (0.66-0.95)	54.7 (10.1)	3 -15	15-25	Cull

Table 7. Summary of COTS control data on the day of eDNA collection* (data supplied by GBRMPA).

Reef Sampled	Date	Bottom time	Number culled	CPUE
Banfield Reef (North)	2/04/2025	916	140	0.1528
Big Broadhurst Reef	9/04/2025*	904	4	0.0044
21-460	10/04/2025	2136	0	0
21-466	12/04/2025	833	0	0
Banfield Reef (North)	4/08/2025	1324	173	0.1307
Judith-Wright	5/08/2025	1268	112	0.0883
20-386	19/08/2025	420	5	0.0119
21-057	22/08/2025	1710	31	0.0181
Bushy-Redbill Reef	23/08/2025	1199	257	0.214345

* Culling on Big Broadhurst reef was conducted three days after eDNA sampling due to adverse weather conditions.

In summary, trials conducted on two COTS control vessels suggested that issues with field control contamination can be resolved by a combination of thorough training and consideration of how eDNA sampling fits within daily operations such as collecting eDNA samples prior to culling activities. Tender field controls collected post-culling clearly demonstrated that culling introduces an increased risk of contamination. Contamination may be transferred from divers (e.g. from wetsuits or hands) to the tender and would hence impact eDNA measurements. However, given one Main ship field control was still contaminated, in addition to collecting eDNA samples pre-culling we also recommend using the increased number of Field controls (n = 6 per reef) utilised in the COTS control vessel trials compared to field controls from non-culling vessels (n = 2 per reef) to allow for adequate statistical analysis and correction if required. Given this success on both COTS

control vessels with two separate vessel operators we are optimistic that clean eDNA collections could also be done from other culling vessels after appropriate training and consideration of daily operations to enable optimal eDNA collection timing.

3) Discuss options for streamlining of sample analysis with various commercial laboratories and explore options to outsource lab analysis

a) Workflow assessment

Based on experience with COTS eDNA over the last five years we conducted a detailed analysis of the overall workflow (from planning to data delivery), and more detailed review for the laboratory component. All individual work steps from pre-voyage preparation of equipment and data delivery are listed in Table 8. Based on a hypothetical collection and analysis of samples from 10 reefs (~300 samples, including controls), the largest amount of time is needed for laboratory analysis, followed by sample preparation and post-trip sample collection and clean-up. To search for options to increase efficiency we discussed with external parties (e.g. QPWS, control vessel operators, GBRMPA, Bioplatform Australia representatives, commercial eDNA laboratories) and in-house experts. At the pre-trip preparation (e.g. cleaning and preparing filter housings, equipping vessels and training) and collection end, we concluded that, except for further reducing sample size, efficiency is already maximised. All steps outside the laboratory analysis also show some efficiencies with larger batch sizes, i.e. surveying a larger number of reefs makes preparation, training, Standard Operating Procedure (SOP) development, and permit preparation more cost-efficient on a per-reef basis.

Given that the most time is required for laboratory analysis, we conducted a more detailed cost analysis of the different laboratory steps (Fig. 2). The entire process currently takes approximately ten days. The main steps for this are preparation and setup (1d), DNA extraction (4d), ddPCR (3d), and Quality Control (QC) and data analysis (2d). Above a certain number of samples analysed (~ 300 samples equivalent to ten reefs surveyed), the cost savings per sample gained in the laboratory work are relatively small. We did not identify opportunities to reduce time and costs for the preparation or ddPCR steps. The QC and data processing may leave room for improvement through possible automation of the workflow. Automation of data processing and delivery will be the focus of a follow up CCIP bridging project. New technologies provide an opportunity to save time and costs at the DNA extraction process. Magnetic beads are increasingly used for efficient and high-quality DNA extraction and provide efficiencies by enabling a higher rate of batch processing of samples. However, this new method needs to be tested and compared to our standard method. If this extraction technique can be implemented, the time for laboratory analysis can be reduced by an estimated 25%, with a consumable cost reduction by an estimated 15%. Testing this new technique is also part of a follow-up bridging project.

Table 8. Workflow analysis of COTS eDNA monitoring.

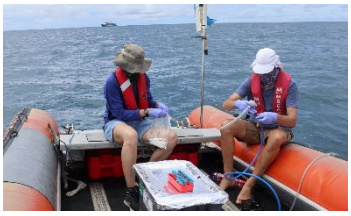
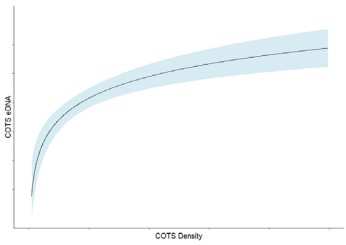
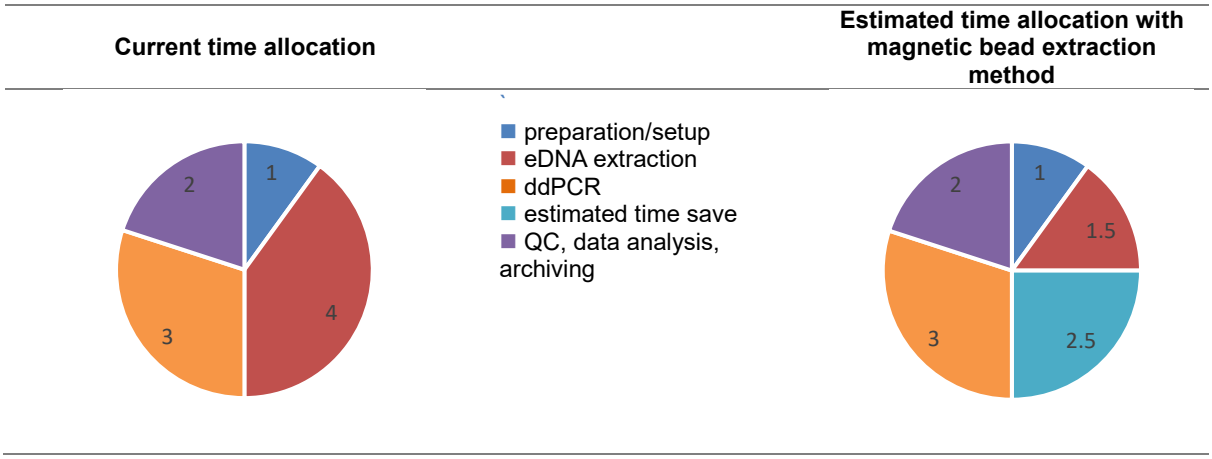
Step	Step in workflow		Who	Time
1	Pre-voyage: Preparation of eDNA collection equipment, training, SOP, permit, risk assessment, freight/travel logistics		Project Coordinator, Collection provider (e.g. QPWS)	5 days
2	Field collection		Collection provider	2 hours per reef
3	Post-voyage: Collection of eDNA gear and samples, debrief and clean-up of eDNA collection equipment		Project Coordinator, Collection provider (eg QPWS)	3 days
4	Laboratory processing (eDNA extraction, ddPCR, QC, data analysis and interpretation)		Laboratory technician	10 days *Preparation/set up: 1 day *eDNA Extraction: 4 days *ddPCR: 3 days *QC, data consolidation, reporting, sample archiving: 2 days
5	Project management, data delivery		Project Coordinator /Reef Authority	2 days

Figure 2. Processing time (days) of individual steps to complete eDNA samples from 10 reefs (300 samples including controls).



b) Options for outsourcing laboratory work

An additional way for cost saving in the eDNA workflow is to outsource the laboratory component from the current in-house analysis at AIMS. Many commercial laboratories exist for DNA analysis, but most of those specialise in next generation sequencing (e.g., for eDNA metabarcoding studies), not digital PCR. We approached several national and international DNA analysis laboratories for quotes for the laboratory analysis of the workflow (DNA extraction and ddPCR). Similar to the analysis of laboratory time (section 3a, above), we costed for analysis of 300 individual filter samples (10 reef surveyed) and compared these to an estimate of the AIMS in-house cost (including consumables and FTE costs, based on full cost recovery). Given digital PCR is required, the number of laboratories providing a suitable service is relatively small. We received quotes from three Australian companies. The price for laboratory analysis of 300 samples (excluding preparatory steps like preparation of equipment for field sampling, training and collection) by external providers was in the range of AUD $25,500 to $42, 000 ($85 - $140 per sample, Tab. 9). These quotes do not consider the need for AIMS to provide standardised CoTS DNA for calibration and tested water or filters as needed as negative controls for field and lab procedures, and handle quality control and data analysis. The estimated AIMS cost using our current method is similar to that of the lower two quotes. Applying the predicted efficiencies gained with the magnetic bead extractions, the cost of analysis at AIMS is expected to be below that of the external quotes

Table 9. Comparison of laboratory analysis for COTS eDNA analysis from 10 reefs (300 samples, given per total batch and per sample) based on quotes from three commercial companies in Australia. Costs do not include sample collection, sending, provision of positive and negative controls and QC.

	Supplier 1 (Adelaide)	Supplier 2 (Melbourne)	Supplier 3 (Perth)
Cost 300 samples	$25,500	$28,200	$42,000
Per sample cost	$85	$94	$140

c) Data delivery and interpretation

Following collection and analysis, data are currently quality controlled, and the available data collated into a delivery spreadsheet for integration into the GBRMPA COTS dashboard and made available at the AIMS web data repository. These datasheets contain readings for each individual sample and not summarised or averaged. In collaboration with Emma Lawrence (CSIRO) and GBRMPA we developed a higher-level data delivery table providing a summary assessment and recommendations for each reef (see Tab. 2). Based on previous calibration (in CCIP-D-02, CCIP-D-03) we suggested a grouping of results into four COTS density categories and provided recommendations (e.g. advised culling, further surveys or no action). These actions depend on density thresholds which are determined by management. An example of the data delivery can be found in Table 3. As stated above, further streamlining of QC, data delivery and interpretation is the subject of a follow-up bridging project.

Impact Pathway

To assess Ships of Opportunity and operationalise eDNA post-settlement was integral to the original CCIP program logic, and CCIP-D-03 had a clear impact pathway by extending the toolbox for COTS detection and improving detection and monitoring to the ultimate impact of coral protection in this project. The **expected benefits** of the present project and **impact pathway** are the same for the proposed follow-up project, with early detection of COTS in low density allowing early intervention. Thus, these methods can be directly taken up and integrated into decision support for COTS control. The **specific objectives** of the current project are:

Early planning, engagement and consultation with GBRMPA, QPWS and COTS Control Vessel Operators was key to the successful implementation of these COTS eDNA monitoring trials. Though a planning workshop involving all parties was attempted but not possible, email and online meetings proved an effective alternative mode to plan the delivery of training and identify voyages for these eDNA monitoring trials.

Reef Joint Field Management Program

Engagement started immediately after project implementation in September 2024, involving GBRMPA, AIMS, and QPWS. Planning needed to incorporate existing operational priorities and specific consultation regarding timing and logistical constraints of conducting eDNA collections alongside other work. This engagement and planning laid the foundation for delivery of training and equipment to QPWS, and successful independent collection by QPWS staff during two voyages. Results reported above identified a range of COTS detections from 18 reefs, with several reefs highlighted as having eDNA derived COTS densities at levels warranting further investigation or culling. Delivery of data from these voyages to GBRMPA, GBRF, CSIRO and QPWS was completed in early April 2025, within 2-3 weeks of receiving the last samples. Subsequently, future support has been indicated by both GBRMPA and QPWS for all RJFMP voyages and a bridging project to begin routine eDNA sampling by QPWS has commenced.

COTS Control Vessels

Similar to QPWS, early engagement was key to achieving research impact for COTS Control Vessel Operators. Initial contact regarding the eDNA trials was made early February 2025 to gauge interest/capacity to undertake trials. Soon after, contact was made with GBRMPA to seek support for these trials to be undertaken on board COTS Control Vessels. Collection of eDNA samples from COTS Control Vessels required further effort with negative controls due to the higher chance of cross contamination of samples. A strategy for collecting samples and controls was developed throughout February/March 2025. During March 2025, information sessions were held with BPM, PMG and INLOC. From these sessions, Odyssey (PMG) and Infamis (BPM) were identified as suitable vessels for conducting the first round of trials. Group email communication (17th March 2025) with GBRMPA, GBRF and COTS Control Vessel Operators with detailed planning, timing, expectations and reporting was provided to ensure all parties were aware of planning progression. eDNA collections were successfully completed for all voyages were eDNA sampling was implemented, with interpretable results obtained.

CONCLUSIONS & NEXT STEPS

The **specific objectives** of the current project were to:

1) 1) To operationalise eDNA sampling on QPWS vessels, while applying recommendations on sampling design from previous CCIP projects

2) To conduct further trials of eDNA sampling on COTS control vessels to address issues of cross-contamination

3) To investigate options for streamlining of sample analysis with various commercial laboratories and explore options to outsource lab analysis

Collections from QPWS vessels worked seamlessly and staff were positive about collecting eDNA as a survey method. Overall, eDNA was collected from 18 reefs and results subsequently reported. The eDNA samples were taken in parallel with Reef Scan Deep tows and Manta tows, with the full comparison of these data provided in the report for CCIP-RT-01 (Lawrence et al. 2026). As detailed in that report, eDNA levels suggesting densities above Allee or ecological thresholds were detected in several cases where the alternative methods could not detect COTS, highlighting the usefulness of eDNA as an early detection method. In collaboration with other agencies and scientists we developed a method to use the proportion of positive samples (PP) and the average eDNA concentration per reef to place COTS densities into four density categories and provide recommendations for possible follow up actions. These are included in a newly developed data delivery table. Data from the RJFMP voyages and COTS control vessels were presented in this format.

Despite some issues with contamination in the previous CCIP-D0-03 project (Uthicke et al. 2025), improved ‘eDNA clean’ workflow and changes in the timing of eDNA sampling with respect to culling activities allowed successful eDNA collections from two COTS control vessels, resulting in the first eDNA survey results from control vessels. It appears a simple change in timing of the sampling (i.e., collecting eDNA prior to culling) was the main factor

now allowing clean collections from these vessels in previous trials. Although this may provide somewhat less flexibility in the daily schedule, as it does not allow using 'degassing' time between dives for eDNA collection, result herein provide a clear way forward should sampling from control vessels be considered for COTS eDNA monitoring.

Our analysis of the workflow indicated that the most significant option for streamlining the process is exploring a new DNA extraction method based on magnetic beads which would save a significant amount of laboratory processing time. As such, we plan to test magnetic bead DNA extraction methods in the follow up CCIP bridging project. Based on quotes from three external Australian companies, outsourcing of the laboratory analysis was not shown to be a more cost-effective method to replace the current in-house analysis. In addition, outsourcing would still require our laboratory to forward samples to external operators, provide positive and negative controls and conduct overall quality control.

We consider operationalising this method of eDNA monitoring on QPWS vessels to be finalised. The next steps, which are occurring in a follow up CCIP bridging project, include 'rolling out' and testing routine monitoring from QPWS vessels by targeting 40-50 reefs within a six-month period. Collections from COTS control vessels has also been successfully trialled on two vessels with specific SOP produced for COTS control vessels should these vessels progress further as operational eDNA monitoring platforms.

Thus, all three objectives were achieved and a variety of options and use cases now exist for using COTS eDNA detection for improved COTS management. Use cases include using eDNA for a) early detection allowing early intervention, b) routine or baseline COTS outbreak monitoring to improve decision making and understanding of outbreaks and c) control vessel-based monitoring to avoid time wastage through diving at low density reefs or to monitoring reefs for re-emerging outbreak after culling activities have finished. Collaboration with GBRMPA on these use cases and further simplifying the data delivery pipeline is part of the follow up bridging project.

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DATA ACCESSIBILITY

Data are deposited at the AIMS data repository and accessible at https://doi.org/10.25845/rxza-nq17

REFERENCES

Babcock RC, Plaganyi EE, Condie SA, Westcott DA, Fletcher CS, Bonin MC, Cameron D (2020) Suppressing the next crown-of-thorns outbreak on the Great Barrier Reef. Coral Reefs 39:1233-1244

Chandler JF, Burn D, Caballes CF, Doll PC, Kwong SL, Lang BJ, Pacey KI, Pratchett MS (2023) Increasing densities of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) at Lizard Island, northern Great Barrier Reef, resolved using a novel survey method. Scientific Reports 13:19306

Doll PC, Messmer V, Uthicke S, Doyle JR, Caballes CF, Pratchett MS (2021) DNA-based detection and patterns of larval settlement of the corallivorous crown-of-thorns sea star (*Acanthaster sp.*). The Biological Bulletin 241:271-285

Doyle J, Uthicke S (2020) Sensitive environmental DNA detection via lateral flow assay (dipstick)—A case study on corallivorous crown-of-thorns sea star (*Acanthaster* cf. *solaris*) detection. Environmental DNA 3:323-342

Doyle JR, McKinnon AD, Uthicke S (2017) Quantifying larvae of the coralivorous seastar *Acanthaster* cf. *solaris* on the Great Barrier Reef using qPCR. Marine Biology 164:176

Kayal M, Vercelloni J, Lison de Loma T, Bosserelle P, Chancerelle Y, Geoffroy S, Stievenart C, Michonneau F, Penin L, Planes S, Adjeroud M (2012) Predator crown-of-thorns starfish (*Acanthaster planci*) outbreak, mass mortality of corals, and cascading effects on reef fish and benthic communities. PLoS ONE 7:e47363

Kwong SLT, Villacorta-Rath C, Doyle J, Uthicke S (2021) Quantifying shedding and degradation rates of environmental DNA (eDNA) from Pacific crown-of-thorns seastar (*Acanthaster* cf. *solaris*). Marine Biology 168:85 (10 pp.)

Lawrence E, Foster S, Williamson D, Matthews S, Schultz D, Pratchett M, Uthicke S, Bainbridge S, Doyle J, Doll P, Armin A, Ewels C (2024) A comparison and calibration of COTS and coral monitoring tools. A report to the Australian Government by the COTS Control Innovation Program.

Lawrence E, Foster S, Gladish D, Matthews S, Williamson D, Uthicke S, Doyle J, Pratchett M, Bainbridge S, Armin A, Crosswell J (2025) Crown-of-thorns starfish (COTS)monitoring and surveillance: Sample design for science and management decisions. A report to the Australian Government by the COTS Control Innovation Program (190 pp).

Lawrence E, Gladish D, Foster S, Kusy B, Chennu A (2026) Operationalizing the Crown-of-thorns starfish and coral monitoring design plan. A report to the Australian Government by the COTS Control Innovation Program:27pp

Moran P (1986) The *Acanthaster* phenomenon. Oceanogr Mar Biol Ann Rev 24:379-480

Nakamura M, Okaji K, Higa Y, Yamakawa E, Mitarai S (2014) Spatial and temporal population dynamics of the crown-of-thorns starfish, *Acanthaster planci*, over a 24-year period along the central west coast of Okinawa Island, Japan. Marine biology 161:2521-2530

Pratchett MS (2005) Dynamics of an outbreak population of *Acanthaster planci* at Lizard Island, northern Great Barrier Reef (1995-1999). Coral Reefs 24:453-462

Rogers JGD, Pláganyi É E, Babcock RC (2017) Aggregation, Allee effects and critical thresholds for the management of the crown-of-thorns starfish *Acanthaster planci*. Marine Ecology Progress Series 578:99-114

Uthicke S, Doyle J, Duggan S, Yasuda N, McKinnon AD (2015) Outbreak of coral-eating Crown-of-Thorns creates continuous cloud of larvae over 320 km of the Great Barrier Reef. Scientific Reports 5:16885

Uthicke S, Lamare M, Doyle JR (2018) eDNA detection of corallivorous seastar (*Acanthaster* cf. *solaris*) outbreaks on the Great Barrier Reef using digital droplet PCR. Coral Reefs 37:1229–1239

Uthicke S, Fisher EE, Patel F, Diaz-Guijarro B, Doyle JR, Messmer V, Pratchett MS (2019) Spawning time of *Acanthaster* cf. *solaris* on the Great Barrier Reef inferred using qPCR quantification of embryos and larvae: do they know it's Christmas? Marine Biology 166:133

Uthicke S, Robson B, Doyle JR, Logan M, Pratchett MS, Lamare M (2022) Developing an effective marine eDNA monitoring: eDNA detection at pre-outbreak densities of corallivorous seastar (*Acanthaster* cf. *solaris*). Science of The Total Environment:158143

Uthicke S, Doyle JR, Gomez Cabrera M, Patel F, McLatchie MJ, Doll PC, Chandler JF, Pratchett MS (2024a) eDNA monitoring detects new outbreak wave of corallivorous seastar (*Acanthaster* cf. *solaris*) at Lizard Island, Great Barrier Reef. Coral Reefs 43:857-866

Uthicke S, Pratchett MS, Bronstein O, Alvarado JJ, Wörheide G (2024b) The crown-of-thorns seastar species complex: knowledge on the biology and ecology of five corallivorous *Acanthaster* species. Marine Biology 171, 32

Uthicke S, Gomez Cabrera M, Patel F, Lawerence E, Tabor B, Foster S, Doyle J (2025) Operationalising and implementing crown-of-thorns starfish (COTS) environmental (eDNA) monitoring on the Great Barrier Reef. A report to the Australian Government by the COTS Control Innovation Program (97 pp).

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