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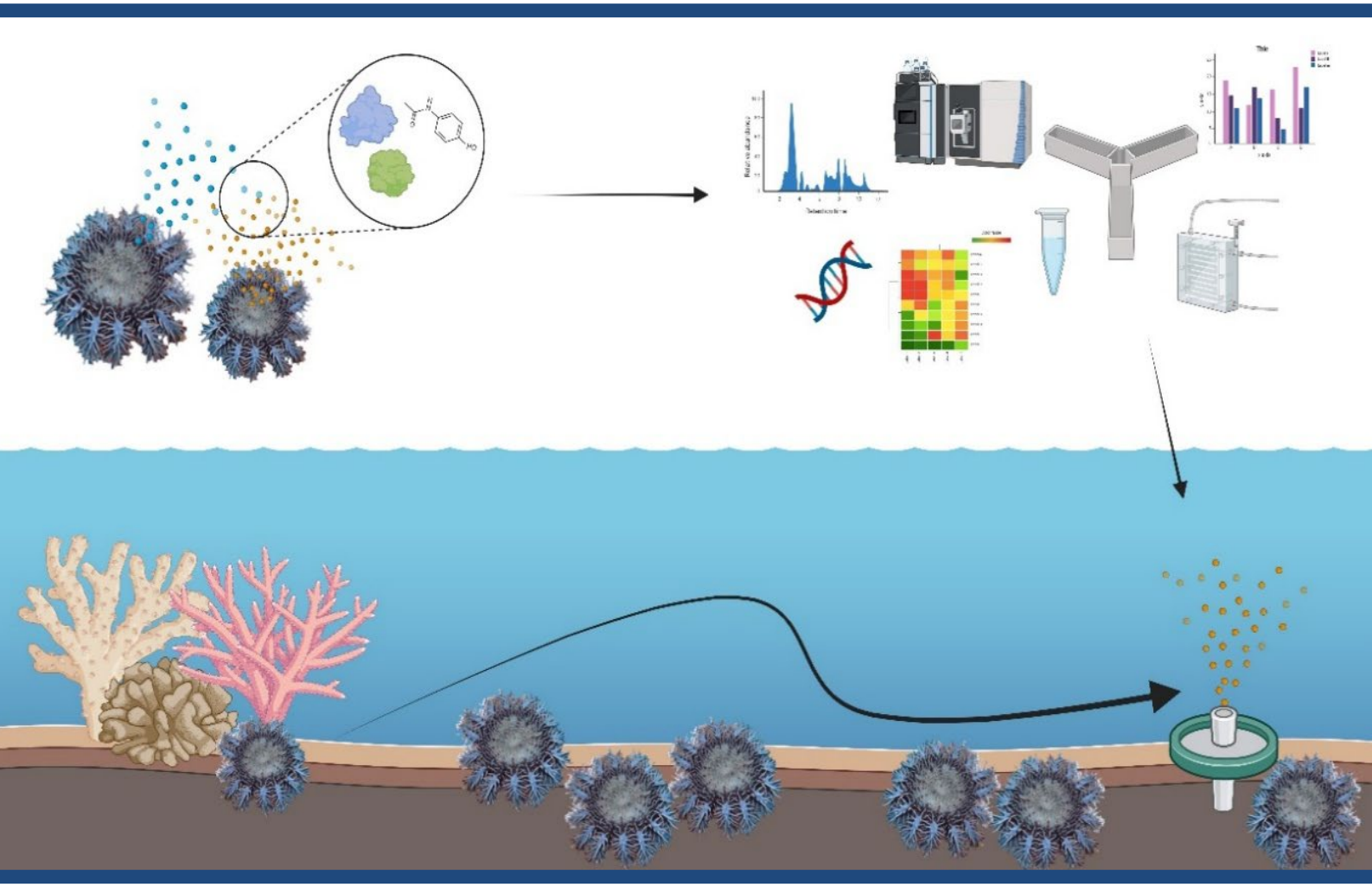

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COTS Control Innovation Program | A research and development partnership to better predict, detect and respond to crown-of-thorns starfish outbreaks

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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, Abbreviations and Key Descriptions

Entities	
AGI	Applied Genomics Initiative
AIMS	Australian Institute of Marine Science
AMPTO	Association of Marine Park Tourism Operators
AMSA	Australian Marine Sciences Association
ANU	Australian National University
ANZMBS	Australia New Zealand Marine Biotechnology Society
APMBC	Asia-Pacific Marine Biotechnology Conference
APVMA	Australian Pesticides and Veterinary Medicines Authority
BPM	Blue Planet Marine
CCIP	Crown-of-thorns starfish Control Innovation Program
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAWE	Department of Agriculture, Water and the Environment (2020-2022)
GBR	Great Barrier Reef
GBRF	Great Barrier Reef Foundation
GBRMPA	Great Barrier Reef Marine Park Authority
NCBI	National Center for Biotechnology Information
OIST	Okinawa Institute of Science and Technology
ONT	Oxford Nanopore Technologies
PMG	Pacific Marine Group
QCIF	Queensland Cyber Infrastructure Foundation
RRAP RPE	Reef Restoration and Adaptation Regulatory and Policy Environment Subprogram
SeaSim	AIMS National Sea Simulator (www.aims.gov.au/seasim)
UniSC	University of the Sunshine Coast
UQ	University of Queensland
Terminology	
CCA	Crustose coralline algae
CCM	Central carbon metabolism
COTS	Crown-of-thorns starfish
DEG	Differentially expressed gene
EPDR	Ependymin-related proteins
GPCR	Guanine-protein-coupled receptors
GVBD	Germinal vesicle breakdown
IPM	Integrated Pest Management
KEGG	Kyoto Encyclopedia of Genes and Genomes
mRNA	Messenger RNA
RGP	Relaxin-like gonad-stimulating peptide
RNA	Ribonucleic acid
RNC	Radial nerve cord
SAR	Structure Activity Relationship
Semiochemical	Derived from the Greek word semeion, meaning "signal", a semiochemical is an essential natural signalling (or informative) molecule produced and emitted by an organism to moderate interactions with other individuals (either intraspecific, i.e., same species, or interspecific i.e., different species) and their environment.

	They do not directly kill pests but interfere with their communication and disrupt or alter normal behaviour. Sometimes referred to as an infochemical.
SSP	Spine-secreted proteins
TPM	Transcripts per kilobase million
TRL	Technology readiness level
Methodologies	
ACN	Acetonitrile
ANOVA	One-way analysis of variance
dpf	Days post-fertilisation
eDNA	Environmental DNA
EtOH	Ethanol
FrA	> 30 kDa fraction
FrB	10–30 kDa fraction
FSW	Filtered Seawater
GO	Gene Ontology
Hi-C	Ligated Chromosome Conformation Capture method
IDA mode	Information Dependant Acquisition mode
IR	Infrared
LC-QQQ-MS	Liquid Chromatography Triple Quadrupole Mass Spectrometer
MS/MS	Tandem mass spectrometry
MW	Molecular Weight
MWCO	Molecular weight cut-off
PacBio HiFi	Pacific Biosciences highly accurate long-read method
PCA	Principal component analysis
PCDL	Personal Compound Database Library
PLS-DA	Partial least squares discriminant analysis
PSU	Practical salinity unit
QC	Quality Control
QToF-MS	Quadrupole Time-of-Flight Mass Spectrometer
SEM	Scanning electron microscopy
SpE	Crude spine extract
SPPS	Solid-phase peptide synthesis
StWE	Crude stress-conditioned water extract
TFA	Trifluoroacetic acid
TFF	Tangential flow filtration method
UHPLC	Ultra high-pressure liquid chromatography

EXECUTIVE SUMMARY

Innovative and transformative approaches are urgently needed to manage coral reefs under global change. The adult life phase of the corallivorous crown-of-thorns starfish (COTS; *Acanthaster* cf. *solaris*) plays a key role in maintaining coral reef health, however, its propensity for population outbreaks, likely due to anthropogenic causes, is a major driver in the loss of coral in the Indo-Pacific. Management intervention in the form of manual culling has achieved a 44% reduction in COTS numbers on target outbreaking reefs within the Great Barrier Reef (GBR), yet this has been at significant cost and effort.

Semiochemicals are being realised as important control and monitoring tools in the management of pest species. They are natural chemical compounds emitted by organisms into the environment as a means of communicating with conspecifics (i.e., pheromones) or to interact with and sense other organisms (i.e., allelochemicals). Their deliberate and controlled application has the potential to activate or disrupt the communication process and lead to a modification of the organism's natural behaviour. The use of semiochemicals to sustainably maintain COTS populations, e.g., attracting individuals to a specific location for easy removal, has genuine potential to build resilience into the system while ensuring that the species, which is indigenous to the Indo-Pacific, is still able to thrive in a balanced ecosystem.

The grand challenge for the COTS Control Innovation Program (CCIP) is to develop an environmentally safe and highly effective semiochemical attractant to control COTS populations while ensuring it is publicly acceptable and economically viable. Despite previous fragmented research efforts, the unifying framework of the CCIP Response subprogram Semiochemical Biocontrol project (herein CCIP-R-11) has enabled a coordinated search for new supplementary and complementary control tools designed to suppress and prevent future outbreaks, with the longer-term objective of delivering a step-change in COTS population control. Here, the convergence of expertise and technologies has created a collaborative forum to drive innovation and revolutionise the COTS Integrated Pest Management (IPM) program, with CCIP-R-11 generating valuable research synergies and findings, and positioning the program to reshape how COTS populations may be managed into the future.

CCIP-R-11 applied a multifaceted strategy to maximise the likelihood of identifying a species-specific COTS-derived lead pheromone attractant. The strategy employed the latest multi-omic technologies (genomics, transcriptomics, proteomics, and metabolomics), compound separation and whole animal behavioural assays to investigate COTS chemistry, biology, function and behavioural ecology, which ultimately led to the identification of leads for further assessment. This systems-biology approach used top-down exploration of the genome and transcriptome to identify genes encoding for chemoreceptors and proteins with features reminiscent of known semiochemicals. In parallel, bottom-up exploration of the proteome, glycome, lipidome, and metabolome of COTS secretions (i.e., the secretome) identified lead protein, peptide and small molecule ligands to support gene predictions.

The discovery of these leads, coupled with aquarium behavioural trials showing consistent orientation and movement toward the pheromone point source, provides clear proof-of-concept for this newly actualised pipeline and demonstrates the potential for these

compounds to be developed into targeted tools for COTS monitoring and control. Yet, raw material supply often presents a significant hurdle in the production of natural extracts and biomolecules. To address this, methodologies were established to produce, at scale, pheromone attractant leads to support functional assessment and future in-field testing. These included recombinant yeast-based protein expression and solid-phase peptide synthesis to produce proteins and peptides, and tangential flow filtration to concentrate and refine the natural secretome. With many millions of COTS currently predicted to be on the GBR alone, source material for the latter is only limited by the logistics in accessing adult COTS for secretome collection.

The directed discovery and development strategy employed here proved successful in identifying and producing, at scale, lead pheromone attractants, and has provided baseline data to guide regulatory and permitting applications and assessments. A rapid review of Australia's regulatory process, governed by the Australian Pesticides and Veterinary Medicines Authority, established that, for a semiochemical formulated for deployment in the marine reef environment, the existing decision tree and guideline criteria are ambiguous, and that 'Pre-Application Assistance' will be required to clarify and establish the submission workflow and guide in-field benchmark testing. The workflows and processes underpinning this strategy were formalised into a semiochemical discovery pipeline with utility for discovery and assessment of allelochemicals (i.e., kairomone attractants and COTS-derived and predator-derived alarm cues) to modify COTS behaviour and may also find application in the search for semiochemicals to control other COTS life stages and other pest marine species.

In summary, this body of work identified COTS-derived protein, peptide and small-molecule leads that reliably elicited movement toward the cue source, demonstrated scalable production routes (recombinant expression, chemical synthesis and refined secretome), and established a clear pathway toward in-field validation and regulatory engagement. Overall, these outcomes reinforce the immense potential of semiochemical control agents as an innovative solution to the long-standing and recalcitrant COTS problem, although realising this potential will require time and sustained support to refine large-scale production, navigate regulatory approval processes and undertake robust in-field trials.

1 INTRODUCTION

1.1 Background

The *Acanthaster* species complex (class: Asteroidea) (Vogler et al. 2013; Yuasa et al. 2021), also known as the crown-of-thorns starfish (COTS), is a cluster of at least four distinct species: *A. benziei* (Red Sea), *A. mauritiensis* (South Indian Ocean), *A. planci* (North Indian Ocean), and *A.* cf. *solaris* (Western Pacific). They are widely distributed across tropical Indo-Pacific coral reefs, with the genus native to the reefs of Australia (including the Great Barrier Reef; GBR), Fiji, French Polynesia (Bora Bora, Moorea, Raiatea, and Tahiti), Japan, Marshall Islands, Micronesia, Papua New Guinea, Philippines, Vanuatu, Vietnam, and the USA (Hawaii and California). Through concerted research efforts over the past few decades, motivated by the scale (> 40%) of coral cover loss (De'ath et al. 2012), a substantial body of information is now available regarding the biology and ecology of COTS (> 940 research articles 1965 to 2016 (Pratchett et al. 2017; Pratchett et al. 2021) and 208 from 2017 to June 2024 (Appendix A **Table A 1**)).

COTS inhabit the benthic zone on coral reefs and, through several remarkable adaptations, have become a formidable corallivore with the propensity for population outbreaks (Birkeland and Lucas 1990; Pratchett et al. 2017). These adaptations include the ability to: i) autotomise and regenerate arms, ii) defend themselves with sharp toxin-laden spines and haemolytic saponins, and iii) navigate their environment through photoreception (Petie et al. 2016a; Petie et al. 2016b) and mechano- and chemosensation (Motti et al. 2018; Harris et al. 2025a). They are also dioecious, highly fecund (releasing in excess of 100 million eggs per female per season (Babcock et al. 2016)), and capable of synchronised spawning (Caballes and Pratchett 2014). Following fertilisation, at 2–3 days post-fertilisation (dpf; bipinnaria), the planktonic larval stage feeds on phytoplankton (Lucas 1982) as they are transported via Indo-Pacific oceanic currents across reefs (Black et al. 1995; Yasuda et al. 2009). Notably, they can survive for several weeks in the planktonic zone even in the absence of food (Wolfe et al. 2015). Under both food limited and plentiful conditions, cloning of the bipinnaria larval stage has been observed (~3–10%) (Allen et al. 2019). Settlement occurs once they reach the late brachiolaria stage (13–17 dpf), thought to be induced by environmental cues (i.e., coralline algae and/or associated microbial communities) signalling the location of a suitable benthic habitat (Doll et al. 2023). Metamorphosed benthic juvenile COTS feed on crustose coralline algae and biofilms before transitioning to a coral diet at 4–12 months and 7.5–8.5 mm in size (Neil et al. 2022). This dietary transition is moderated in part by food availability (Deaker et al. 2020a) and in part by the presence of adults (Deaker et al. 2020b); the juveniles can effectively lie in wait for favourable conditions. As adults, COTS have a voracious appetite and are capable of consuming up to 478 cm^2 of living coral per day in summer, the equivalent to 10 m^2 of coral per year (Foo et al. 2024). They show a preference for scleractinian corals, in particular *Acropora* species, but will also feed on *Pocilloporidae* spp. and *Porites* spp. if *Acropora* are not in abundance (De'ath and Moran 1998; Pratchett 2010). In captivity, adults can survive on detritus or will revert to cannibalism (Harris et al. 2025a). Starved COTS have been kept for up to six months with marked reduction in size and tissue condition (Hall et al. 2016), alongside up-regulation of genes associated with sleep promotion, immunity, lysosome and glucose supply, indicating they enter a state of dormancy (Yang et al. 2022). Larvae, benthic juveniles, and adults have also all been shown

to tolerate elevated temperatures and CO_2 levels (Lang et al. 2022; Byrne et al. 2023; Mos et al. 2023).

The draft genome sequences for COTS from Australia (GBR) and Japan (Okinawa) (Hall et al. 2017), together with subsequent advances in molecular and genetic techniques, have enabled population-level and phylogenomic analyses that have since revealed a greater level of taxonomic complexity within the genus. Although COTS were previously thought to be a single species, *A. planci*, with *Acanthaster brevispinus* the only other congener (Haszprunar et al. 2017), current data now suggests *A. planci* represents a species complex of at least four species, with the GBR and Pacific Ocean species being tentatively named *Acanthaster* cf. *solaris* (Kool et al. 2011; Haszprunar and Spies 2014; Haszprunar et al. 2017; Chen et al. 2021; Yuasa et al. 2021). Beyond its role in resolving population structure and speciation, the *A.* cf. *solaris* genome has been pivotal in advancing functional COTS biology by enabling transcriptomic and proteomic investigations. These genome-anchored studies have provided insights into the molecular mechanisms underlying key biological functions and behaviour, including chemosensory signalling, secretion, and neural processes (Hall et al. 2017; Jönsson et al. 2022; Morin et al. 2023; Smith et al. 2023; Morin et al. 2024; Zhu and Lu 2024), and established a strong foundation for further -omic investigations.

The GBR Marine Park Authority's (GBRMPA) COTS Control Program currently relies on water quality management, zoning, and manual lethal injection (using ox-bile (Rivera-Posada et al. 2014) or citric acid (Buck et al. 2016)) to mitigate COTS outbreaks. Manual lethal injection expends significant resources at significant cost (Westcott et al. 2020) and, while its coordinated application has alleviated the impacts of COTS at specific targeted locations (achieving a six-fold reduction in starfish numbers and a 44% increase in coral cover in the Townsville region (Matthews et al. 2024)), the risk to corals remains significant across the entire GBR (Li et al. 2024). The manual culling effort is also labour-intensive and time-consuming (Birkeland and Lucas 1990; Pratchett and Cumming 2019; Westcott et al. 2020). Although these targeted management methods have proven to be effective, model projections under warming scenarios suggest more frequent and severe outbreaks are possible (Castro-Sanguino et al. 2023), therefore, it is of utmost importance that new economical, efficient, and scalable technologies are developed to enable a step-change in their control at reef-wide scales.

Supported by the rapid emergence of using pheromone attractants in terrestrial pest control strategies (e.g., synthetic female sex pheromones to disrupt mating in major moth pests (Cardé 2007)), there is growing interest in semiochemicals (i.e., non-living nature-based bioprotection controls (Stenberg et al. 2021)) for aquatic ecosystems, where the goal is to alter behaviour rather than directly kill pests. Although this approach is proving promising for controlling freshwater pests (Stacey 2014; Sorensen and Johnson 2016), it remains unrealised in the marine environment (Harris et al. 2025a). Most recently, the application of semiochemicals to modify COTS behaviour has been explored (Hall et al. 2017; Høj et al. 2020; Motti et al. 2022). However, because chemical cues disperse and dilute rapidly in the marine environment (Weissburg 2011), and because attractants must be both COTS-specific and potent enough to remain effective under field conditions (Motti et al. 2022), the discovery process has been inherently slow. As demonstrated by the decades-long development of sea lamprey pheromone controls (Sorensen and Hoye 2007; Sorensen and Johnson 2016),

translating fundamental research into a viable semiochemical management tool will likely require sustained, long-term effort (Harris et al. 2025a).

Early exploration of the COTS draft genome, combined with proteomics and behavioural experiments (Hall et al. 2017), provided the first insights into the molecular chemosensory signalling components that drive adult COTS behaviours in response to conspecifics and predators. Central to this was the discovery that COTS behaviour is mediated through secreted semiochemical biomolecules that may act as COTS-specific aggregation factors (Moore and Huxley 1976; Teruya et al. 2001; Motti et al. 2018). Since then, efforts have focused on the systematic unveiling of the chemical repertoire of COTS, in particular those compounds that facilitate aggregation, and on identifying chemoreceptors and understanding their roles (Hall et al. 2017; Roberts et al. 2017; Smith et al. 2017; Motti et al. 2018; Roberts et al. 2018; Smith et al. 2019).

Technological development of COTS-specific pheromone attractants has been protracted, advancing only incrementally as new analytical and molecular technologies improve. This reflects, in large part, the absence of a structured development pipeline to guide the process. Currently, there are a number of elements that are impeding advancement:

1) Starfish genomic resources are incomplete, thereby limiting our ability to confidently identify species-specific genes with functions directly related to COTS chemosensation and moderation of behaviour; and
2) There are significant analytical and logistical unknowns, as well as seasonal time constraints, that affect both the isolation of secreted semiochemicals and the assessment of their efficacy, in particular:
    a. solating or producing this chemistry in a reliable and sustainable manner over time and seasons; and
    b. in ample quantities for behavioural testing and large-scale field deployment.

To address these challenges, the COTS Control Innovation Program (CCIP; a scientific consortium of core research partners) embarked on an ambitious research and development project, the CCIP-R-11 Semiochemical Control project within the CCIP Response Subprogram. The aim of the CCIP-R-11 project was to establish a verified pipeline for the delivery of step-change innovation in supplementary and complementary semiochemical tools and operational strategies aimed at improving the efficiency and effectiveness of the COTS Control Program through suppression of future outbreaks. In parallel to the discovery and testing activities that build on prior knowledge of COTS, the research also sought to inform other CCIP projects to better understand the socio-economic perspectives of using COTS-derived semiochemical control agents, the cost benefit of producing and deploying these agents on the GBR and the regulatory implications of semiochemical control methods (**Figure 1**; pathways having synergy with the CCIP-R-11 project are emphasised in **bold**). Presented herein is the progress made towards this lofty goal.

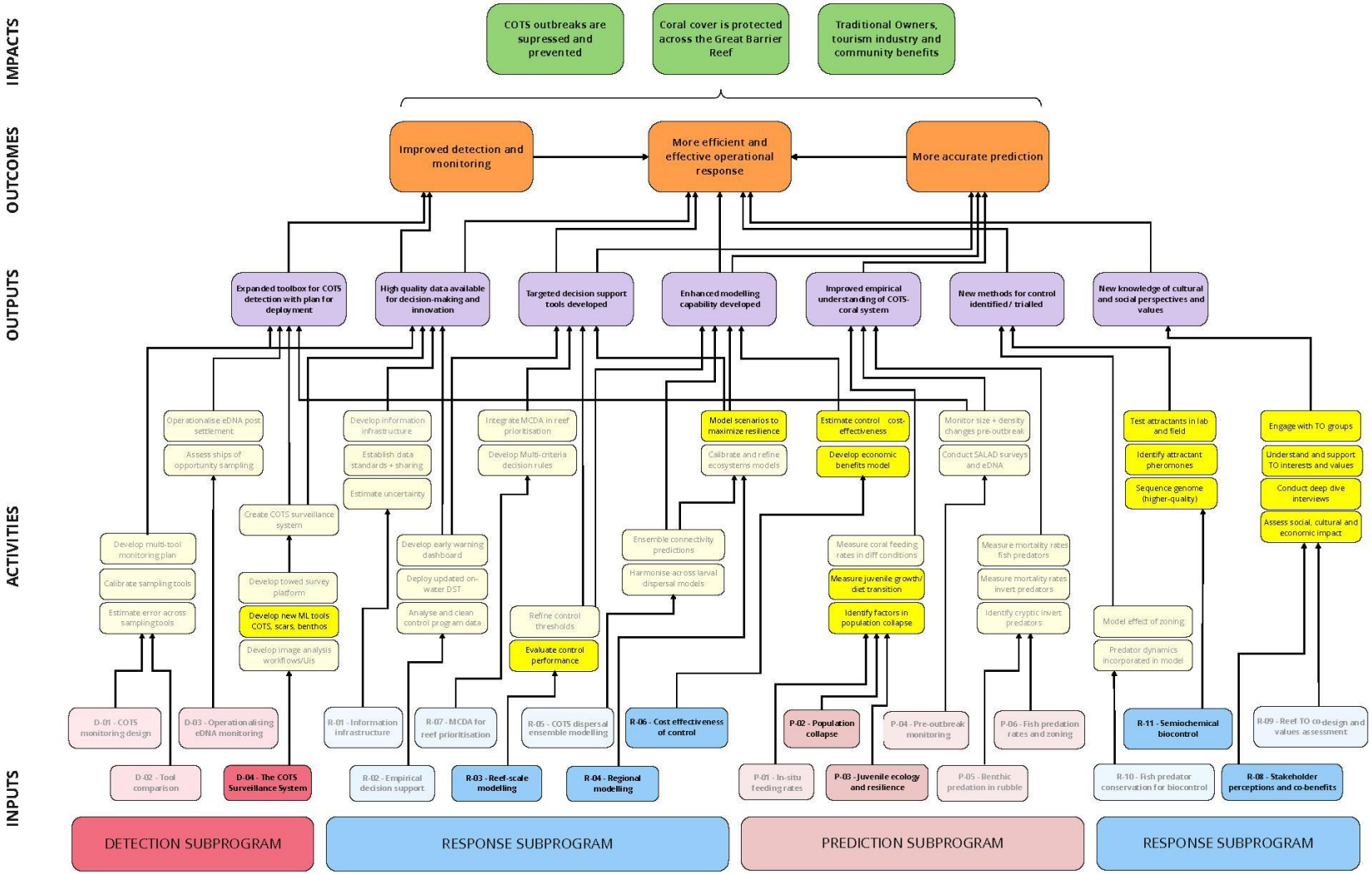

Figure 1. Project impact pathway, including key synergies with other projects (in **bold** colour) across the CCIP program.

1.2 Aims of the CCIP-R-11 project

The grand challenge is to develop a revolutionary COTS control method to supplement and complement individual lethal injection. To address this challenge, the scope of the CCIP-R-11 project included developing a COTS semiochemical pheromone attractant with properties amenable to deployment within the framework of the COTS Integrated Pest Management (IPM) program. An expert working group was convened, the state of knowledge assessed (Motti et al. 2022) and a strategy developed to overcome knowledge gap impediments (Appendix B **Table A 2**). This was achieved through the establishment of a toolkit of methodologies and workflows designed to identify, assess, produce, and deploy at scale a COTS-specific attractant semiochemical control agent. Specifically, the project aimed to:

1. Substantially improve existing COTS genomic resources by:
    a. improving the GBR COTS genome assembly and establishing a pipeline to facilitate interrogation for the identification of genes and proteins associated with chemosensation;
    b. understanding within-species genetic variation; and
    c. generating a draft genome for *Acanthaster brevispinus* to improve understanding of inter-species genetic/gene expression profile variation.
2. Refine robust large- (e.g., behavioural maze) and small-scale (e.g., tissue/receptor) assays to monitor COTS behavioural and physiological responses, respectively.
3. Apply in silico and assay-guided approaches to identify lead COTS pheromone attractants.
4. Produce/purify lead attractants at scale and determine their efficacy. and
5. Assess the potential of candidate attractants for (rapid) deployment within the COTS IPM using criteria established by the CCIP early investment project “Deployment of semiochemical-based control agents to manage COTS populations and outbreaks” (Motti et al. 2022).

1.3 Expected Outcomes and Benefits

In the terrestrial environment, naturally occurring species-specific semiochemicals have proven successful in controlling pest species (Agelopoulos et al. 1999; Mitchell 2012; Progar et al. 2013; Sharma et al. 2019; Abd El-Ghany 2023). The associated risks of using semiochemicals are considered minimal as:

1. No modification of the environment is needed for them to be effective.
2. There are no concerns regarding introduction of a foreign and potentially toxic chemical.
3. The likelihood of impact on other non-target species is limited.
4. They are not intended to kill the pest organism directly and therefore there is no chance of total eradication of target species or cohabiting communities.

Improved genomic resources for *Acanthaster* spp. are expected to facilitate the discovery and development of naturally occurring COTS-specific pheromone attractants; the expectation is that these will have negligible impact on non-target species. In turn, methods to produce these at scale for whole animal testing will enable efficacy testing and feasibility assessment for reef scale deployment. Confirmed efficacy of conspecific COTS pheromone attractants (extended to include juveniles) will advance our understanding of COTS communication and provide a critical parameter to strengthen predictive models (including those being developed within the broader CCIP research portfolio) of reef-scale distribution. Finally, the use of COTS pheromone attractants capable of modifying conspecific behaviour (of both adults and juveniles) has the potential to add to and complement the arsenal currently available to reef managers to control COTS populations (Motti et al. 2022). If proven effective in aquaria tests, COTS pheromone attractant technology has enormous potential to significantly improve culling efficiency by reducing reliance on, enhancing, or replacing aspects of manual control by injection; and enhancing monitoring and surveillance efforts, which ultimately, through suppression, will prevent future population outbreaks. The successful application of semiochemical technologies in terrestrial pest control strategies provides confidence that similar COTS-specific semiochemical technology could be integrated into the COTS IPM strategy, for example via:

1. Targeted seasonal application, e.g., before spawning, to lure and remove adults, reducing the number of gametes released synchronously and suppressing fertilisation rates.
2. Targeted application, luring to a point source, to increase culling efficiency on:
    a. the first visit to a priority site, i.e., ‘initiation’ and ‘super-spreader’ reefs, or
    b. recently visited sites experiencing recurring outbreaks, thereby suppressing outbreak severity.
3. Targeted application at monitoring and surveillance sites by luring adults into the open for easy detection and observation.
4. Non-targeted application (e.g., continuous slow-release deployment) by luring COTS
    a. away from the reef; or
    b. into baited traps for delivery of other control agents.

Undoubtedly, knowledge gained of the chemical repertoire of adult COTS, alongside the improved genome and additional data obtained from juveniles (CCIP-P-03, Byrne et al. 2024), will not only provide insight into their biological and ecological function but also support control efforts more broadly (i.e., across the GBR and other marine habitats) and for other pest echinoderms (i.e., the Northern Pacific seastar) (Dommisse and Hough 2004).

Studying semiochemicals in the marine environment is essential for understanding the interactions and dynamics of marine ecosystems. Yet, the highly complex process of discovering marine semiochemicals (Motti et al. 2022) ideally requires the integration of various techniques to expedite their identification, from genomic and proteomic database generation to functional assay testing. A pipeline to guide this research is currently lacking, with existing research workflows applied in an ad hoc manner and without a coordinated framework to support comprehensive evaluation of semiochemical candidates. Hence, the

principal outcome of this project will be a validated pipeline, spanning from the COTS outbreak problem at its input to a COTS-specific pheromone attractant at its output.

2 METHODS

A multifaceted approach was applied to fast-track identification of COTS-derived attractants. This capitalised on current multi-omic capabilities and pre-existing/published -omics resources generated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), the University of Queensland (UQ) and the University of the Sunshine Coast (UniSC), as well as the behavioural testing capabilities of the AIMS National Sea Simulator (SeaSim; www.aims.gov.au/seasim). Where applicable, protocols used previously have been followed or adapted, and the reader is directed to relevant citations and/or appendices for details.

To identify lead attractants, four primary activities were undertaken:

1. Assessment of the current knowledge of COTS protein and metabolite chemistry and functionality (Appendix B Table A 2).
2. Review, compilation and comprehensive exploration of multi-omics datasets to identify putative signalling proteins, peptides and small metabolites (sections 2.1 and 2.2).
3. Assay(sensory)-guided chemical isolation and chemical characterisation (section 2.3).
4. Predictive mapping of functional COTS chemistry against the genome to identify corresponding chemoreceptors (section 2.4).

2.1 Refinement and establishment of omics databases

This project exploited the bioinformatic resources at CSIRO, UQ and UniSC, as well as collaborating parties at the Australian National University (ANU) Foley Institute, to:

1. Democratise the COTS genome (level = 3-star contig assembly).
2. Generate a 5-star full assembly at the chromosome level using Hi-C technology.
3. Generate a high-quality draft *A. brevispinus* genome based on short- and long-read sequencing.
4. Conduct transcriptome (gene expression) analysis of existing libraries for *A.* cf. *solaris* and *A. brevispinus* to improve gene identification and function prediction (i.e., annotation), and to associate expression of genes of interest with tissue, life stage and condition.
5. Generate the first tissue-specific COTS metabolomic, glycomic and lipidomic database.

2.1.1 Democratisation of the COTS (*Acanthaster* cf. *solaris*) genome

Funded by the UQ Genome Innovation Hub, the UQ Marine Genomics Lab collaborated with the Queensland Cyber Infrastructure Foundation (QCIF) to produce a publicly available WebApollo-JBrowse COTS genome browser. Built on the Apollo platform and hosted by the

Australian Biocommons, the browser provides an interactive repository for large and complex multi-omic datasets designed to significantly enhance the collaborative value of genomics projects for non-model species. Specifically, it integrates genomic and transcriptomic datasets related to the COTS genome into a comprehensive, visual, standardised and publicly accessible system. This democratisation workflow allows for the easy management, searching, visualisation, exploration and sharing of multi-omic information by any interested party.

Following this workflow, the existing GBR COTS genome (Hall et al. 2017) was uploaded to the Apollo online genome repository. The purpose-built configuration was then used to integrate previously published (i.e., podia, spines, and body wall (Hall et al. 2017)) and newly generated transcriptomic (Jönsson 2023; Morin 2023; Morin et al. 2023) datasets. These combined resources were then mapped to the COTS genome, and visualisation and online accessibility assessed.

2.1.2 COTS Hi-C sequencing

Hi-C sequencing is a high-throughput genomic technique that captures chromatin conformation and provides the three-dimensional (3D) architecture of the genome (Belton et al. 2012). The organisation of the genome in 3D space is directly correlated to its functionality, and is crucial for regulating gene expression and cell differentiation, including gametogenesis, embryogenesis, organ development and disease processes (Bonev and Cavalli 2016; Kong and Zhang 2019). In addition to enabling studies of genome function, Hi-C contact information is widely used to scaffold de novo genome assemblies to chromosome scale, particularly in non-model species lacking genetic maps. For COTS, establishing chromosome-scale genome organisation using Hi-C will improve assembly contiguity and gene annotation, thereby facilitating the identification and genomic context of chemoreceptor gene families implicated in behavioural modulation.

The CSIRO Applied Genomics Initiative (AGI), whose mission is to optimise and apply genomics to deliver reference genomes and enable translational research and support real-world decision making (Appendix C **Figure A 1**), committed to sequencing and assembling the male and female *A.* cf. *solaris* genomes using a combination of Oxford Nanopore Technologies (ONT) long-read and Illumina short-read sequencing, with Hi-C for chromosome-scale scaffolding consistent with current best practice for high-quality reference genomes.

Various tissues from male and female COTS were subjected to four different extraction protocols in an effort to extract high-quality DNA. After extensive quality control, preliminary extraction results indicated the Qiagen Genomic Tip protocol – designed for gentle, high-molecular weight DNA extraction - gave the best output, with testes of the male and tube feet of the female yielding the highest-quality DNA. This protocol-tissue combination was subsequently selected for Hi-C library preparation.

Kit choice and protocol parameters can materially impact library quality, long-range signal and scaffolding outcomes (Kadota et al. 2020; Yamaguchi et al. 2021); therefore as COTS is a non-model organism, cross-kit (Arima Genomics Arima-Hi-C kit, Qiagen Epitect Hi-C kit and Phase Genomics Proximo Hi-C Kit) and cross-lab (UQ, CSIRO and ANU) evaluation

was undertaken to ensure robustness. Additional extraction and cross-linking strategies were also investigated in collaboration with ANU and the Foley Institute, including protocols developed for other marine invertebrates (Srivastava et al. 2010; Gaiti et al. 2017). Fresh extractions from COTS gonads (eggs and sperm) were prepared, and sperm DNA (selected due to its high DNA concentration and enriched long-range contacts for improved assembly scaffolding (Battulin et al. 2015)) was additionally processed using extended digestion times (1.5 hr) to optimise fragment quality. All Hi-C library preparations were evaluated for suitability for downstream sequencing.

2.1.3 *Acanthaster brevispinus* draft genome

To support further investigations into the evolutionary traits unique to the *Acanthaster* genus, and to COTS (*A.* cf. *solaris*) specifically, sequencing of the *A. brevispinus* genome was attempted to generate a comparative resource.

Tube feet from a female *A. brevispinus* (**Figure 2**) were used for initial extractions, but, unlike COTS, DNA quality and yield were poor. Despite this limitation, a long-read sequencing library was prepared using a custom low-input protocol for evaluation on the PacBio Revio and the Oxford Nanopore Technologies PromethION platforms. Where high-molecular weight input is limited, PacBio provides validated low- and ultra-low-input options (e.g., Ampli-Fi) that employ amplification and enable sequencing from 1–50 ng DNA, albeit with potential amplification bias.

Figure 2. *Acanthaster brevispinus*, sibling species to *Acanthaster* cf. *solaris*.

A second DNA extraction was performed, prioritising the Qiagen Blood and Tissue kit to generate a short-read sequencing library. The ANU sequencing centre assessed library preparation performance and identified the presence of inhibitors that interfered with transposase-based kits. In marine invertebrates, polysaccharides and other secondary metabolites are common and can compromise enzymatic steps or sequencing yield, therefore, both enzymatic and mechanical shearing approaches were tested to improve library quality.

In parallel, short-read RNA-seq data, derived from radial nerve cord (RNC), tube feet and stomach (deposited in the National Center for Biotechnology Information (NCBI) BioProjects PRJNA548418 and PRJNA16358), were used to generate predicted coding sequences

(CDSs; open reading frames spanning one or more exons) to support annotation. CDSs inferred from RNA-seq are an important analysis resource for discovering interspecies variation and gene expression changes (e.g., changes in gene expression during cell differentiation or in response to a chemical cue) (Eghbalnia et al. 2020).

The approach used for Hi-C sequencing of COTS (refer to section 2.1.2) will be applied to *A. brevispinus* tissues, contingent on obtaining high-quality high-molecular weight DNA and intact nuclei.

2.1.4 Non-targeted and targeted metabolomic and lipidomic profiling of COTS tissues

Small-molecule (< 1.5 kDa) metabolites play important roles in organism function (i.e., as hormones, signalling molecules, and secondary metabolites) and are a direct representation of the response of the organism to external stimuli. Leveraging the CSIRO collaboration and building on prior knowledge (Appendix D **Table A 3**), a comprehensive metabolic and lipid investigation was undertaken to profile the following COTS tissues: eyes, eggs from one female, gonads from one female and four males, pyloric caeca, pyloric stomach, RNC, skin, spine, sensory tentacle, tube feet and the ambulacral skeleton (or vertebrae). Eggs were obtained through biopsy of ovary tissue (Smith et al. 2018) and addition of the ovulatory hormone 1-methyladenine (1-MeAde; a potent inducing factor for oocyte maturation and spawning in seastars (Mita 1999).

COTS tissue and egg extracts were prepared (Beale et al. 2022) and targeted analysis of central carbon metabolism (CCM) metabolites conducted on an Agilent Infinity Flex II ultra-high pressure liquid chromatography (UHPLC) coupled to an Agilent 6470 Triple Quadrupole Mass Spectrometer (LC-QQQ-MS) (Gyawali et al. 2021). Untargeted polar metabolites were measured on an Agilent Infinity Flex II UHPLC coupled to an Agilent 6546 Quadrupole Time-of-Flight Mass Spectrometer (LC-QToF-MS) (Beale et al. 2022). The non-polar lipid fractions were prepared (Beale et al. 2021) and analysed on an Agilent Infinity Flex II UHPLC coupled to an Agilent 6546 Quadrupole Time-of-Flight Mass Spectrometer (QToF-MS) (Shah et al. 2021).

Raw spectral datasets of CCM metabolites were processed using MassHunter Quantitative Analysis Software v0B.10.0. Untargeted polar CCM metabolite data and non-polar lipid data were processed by MassHunter Profinder Analysis Software and data analysis was performed using MetaboAnalyst 5.0 (Pang et al. 2021). Chemometric analyses including unsupervised principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) were used to visualise the natural groupings of all COTS samples and assess the discrimination amongst tissue groups and the individual variation between harvested eggs from different females. Univariate data analysis with one-way analysis of variance (ANOVA) was conducted to identify metabolites and lipids that were significantly ($p < 0.05$) different amongst tissues using a post-hoc Dunn's significance test. Egg analysis was carried out from a qualitative perspective. A heatmap of the top 50 different features was generated to visualise differences in their relative abundance and the significant metabolites mapped on the KEGG metabolic network. Saponin analysis in tissues and eggs was independently carried out using an in-house customised Personal Compound Database Library (PCDL) based on Stonik et al. (2020).

2.2 Identification of lead semiochemicals

Understanding underlying molecular mechanisms associated with chemosensation and monitoring the molecular (i.e., gene, protein, and metabolite) perturbations that occur in response to a stimulus is key to the identification of lead semiochemicals. The integration of multi-omics data provides the foundation for this approach and, in combination with tailored isolation strategies, was applied here to identify putative COTS attractants and generate quantities for behavioural and functional assays. Guided by prior findings (Hall et al. 2017):

1. A method to process the COTS secretome was developed and demonstrated, and the product structurally characterised (section 2.2.2),
2. Interrogation of the refined COTS genome and tissue-specific transcriptomes was performed to identify COTS-specific ependymin-related proteins (EPDRs) and a method developed to express these (section 2.2.3).
3. Transcriptomic and proteomic analyses were undertaken on COTS spine, previously identified as a possible source of semiochemicals, and lead semiochemical peptides synthesised (section 2.2.4).

2.2.1 Animal collection and husbandry

Adult COTS (15–30 cm in diameter) were collected as required (between 2021 and 2024) from the central GBR by COTS Control Program divers operating from the Association of Marine Park Tourism Operators (AMPTO) and Pacific Marine Group (PMG) vessels, under GBRMPA Permits G17/38293.1, G18/41332.1 and G21/38062.1. Animals were transported to the AIMS SeaSim aquarium facility and kept in an aerated outdoor flow-through seawater tank (4 m diameter x 0.75 m height, 9.4 m^3, round, seawater filtered to 0.45 µm used throughout SeaSim) at ambient conditions (26–29°C, salinity averaging 35 practical salinity unit (PSU)). For tissue and spine harvesting, COTS (~25 cm in diameter) were transported by air freight to the UniSC Aquaculture facility, where they were housed in a protein-skimmed isolated 1,500 L capacity seawater tank. At least five days prior to behavioural testing, animals were isolated in individual 40 L tanks to ensure naivety (ambient temperature with flowthrough and aeration). Where needed, needle biopsy of gonad tissue was performed by sampling through a small incision at the base of the arms. Microscopy (Leica M80 stereoscope) was used to confirm reproductive status of individuals (Smith et al. 2018).

Additionally, two adult short-spined COTS (*A. brevispinus*; GBRMPA Permit G21/38062.1), housed together in an aerated 116 L flow-through tank, and confirmed previously by biopsy to be females, were investigated as a comparative study.

2.2.2 Non-targeted processing and characterisation of COTS-conditioned water

Selective tangential flow filtration purification of secreted COTS proteins

Natural semiochemicals from COTS can potentially be harvested and formulated as attractants, but this requires new ecofriendly and scalable approaches for semiochemical isolation. Tangential flow filtration (TFF) chromatography separates biomolecules in solution based on their molecular size and is a gentle chromatographic technique well-suited to

maintaining biomolecular integrity. This method has global application in the food, pharmaceutical, and agriculture industries (Musumeci et al. 2018) and unlike other extractive methods, does not produce hazardous by-products, hence it is an environmentally sustainable, safe, reliable, and rapidly scalable technique. Here, TFF was applied for the first time to rapidly sequester active signalling molecules from COTS-conditioned seawater (first reported in Hall et al. (2017)), circumventing the need for extensive sample processing and expensive isolation of individual active components, and thereby overcoming the processing hurdle. However, the reality of filtering and processing hundreds of litres of seawater enriched with COTS secretions was untested. Therefore, ample effort was spent planning, designing, refining operating parameters, membrane choice selection, and establishing a reproducible process.

For preparation of COTS-conditioned seawater, adult COTS, collected in the summer of 2020, corresponding to the annual spawning season, were placed in a tank (static, aeration) for 12 hr to simulate aggregation conditions (n=5 COTS per tank, n=18 tanks; individuals were randomly selected to ensure population representativeness, sex of each individual was not determined). The COTS-conditioned seawater was tandem-filtered (5.0 µm Aqua Medic sediment filters and 0.1 µm Sawyer filters) to remove particulate matter and then fractionated using TFF over a 10 kDa molecular weight cut-off (MWCO) membrane to yield a < 10 kDa and a > 10 kDa fraction, the latter of which was further fractionated over a 30 kDa MWCO membrane to yield a > 30 kDa fraction (FrA) and a 10–30 kDa fraction (FrB).

Proteomic analysis

Proteomics is the comprehensive study of the structure and function of proteins and peptides, and along with glycomics (described below), provides a detailed characterisation of the active signalling molecules. These approaches are complementary to genomic approaches but are essential for the unequivocal confirmation of the identity of the active molecules so they can be reproducibly isolated from independent experiments. To characterise the proteinaceous chemistry secreted into the water, the TFF-derived FrA (active fraction – refer to results section 3.2.1) was concentrated, and proteins/peptides reduced (using 0.1 M dithiothreitol), alkylated (with iodoacetamide), and trypsin digested. The trypsin-liberated peptides were analysed by nano-high-performance liquid chromatography coupled to an Ekspert nano LC400 and Sciex Triple Time-of-Flight (ToF) 6600 mass spectrometer with PicoView nanoflow ion source and Analyst 1.7 software. The peptides were washed over a SGE C18 trap column and bound peptides eluted by gradient from a ChromXP C18 column. Full-scan ToF-MS product ion data was acquired in Information Dependant Acquisition (IDA) mode over a mass range of 350–2000 *m/z* and for production of MS/MS ions, over 250–1500 *m/z*. Peptide identification was performed using Protein Pilot 5.0.2 and the resulting peptide sequences were compared with available COTS transcriptomes and genome data (Hall et al. 2017) and the NCBI database.

Glycomic analysis

Glycomics is the comprehensive study of sugars and carbohydrates. The glycome can be highly complex with glycans often forming complex polysaccharides, being conjugated with lipids (glycolipids), or bound to proteins (glycoproteins), the latter of which includes the G-protein-coupled receptors (GPCRs) which are found in echinoderms (Arey 2012).

Glycomic analysis was undertaken in collaboration with Adelaide Glycomics (University of Adelaide). The proposition that the presence of complex glycans on glycoproteins might hinder access to trypsin digest sites was established from preliminary glycomic profiling of the summer 2020 FrA (> 30 kDa) (Appendix E Figure A 2), therefore, to increase the number of proteomic hits, the protease digestion process was further optimised by first deglycosylating the surface glycan via glycan-hydrolases. The deglycosylated samples were then treated with trypsin prior to analysis by MS/MS (as described above).

Lyophilised fractions were subject to mild acid hydrolysis (1 M sulphuric acid treatment for 3 hours at 100°C) and the liberated monosaccharides derivatised (1-Phenyl-3-methyl-5-pyrazolone) and products quantified by C18 reversed-phase chromatography (1260 Agilent HPLC coupled with a diode array detector).

2.2.3 Targeted recombinant expression of COTS ependymin-related proteins, identified through multi-omic mining

Previously, 26 ependymin-related proteins (EPDR) were identified from COTS and hypothesised to play a role in COTS conspecific communication (15 potentially associated with aggregation and 11 with alarm) (Hall et al. 2017). Here, a more comprehensive interrogation of the refined COTS genome (version 2) was undertaken and the protein Accession ID GBR.60.100 identified as a promising lead for attraction. Exploration of the proteomic dataset (Hall et al. 2017) revealed GBR.60.100 to have the highest proteomic abundance based on MS peptide matches (BLAST search against the COTS GBR v1.1). Specifically, it has a high level of conservation with the EPDR domain and has six cysteine residues, suggesting it may be a signalling protein.

The ability to produce desired proteins in sufficient amounts is critical for their assessment and application at scale as control agents, and can be achieved by microbial fermentation (Yap et al. 2020). This method involves insertion of the gene coding for the desired protein into the host genome. Here, the yeast, *Pichia pastoris*, was chosen as the host to recombinantly express and secrete GBR.60.100.

Yeast-based recombinant bioproduction was performed following the workflow previously established by Yap et al. (2021). DNA encoding GBR.60.100 was synthesised and cloned into the pPICZ-alpha vector. The vector was transformed into *P. pastoris* before small-scale expression optimisation was performed to identify high expressors. The colony with the highest expression was selected for bioreactor scale-ups in a 10 L bioreactor vessel. The bioreactor spent-media was harvested 72 hours post induction and purified using affinity chromatography.

2.2.4 Targeted proteomic and transcriptomic profiling of COTS spines

The distinctive aboral spines that inspire the common name (crown-of-thorns) are well recognised as both a physical and chemical defence for COTS. They produce secretory bioactive molecules, including plancitoxins (toxic DNase II proteins of 20,000–25,000 Da (Shiomi et al. 1988; Shiomi et al. 2004), amphipathic glycosides or saponins (Thao et al. 2013a; Thao et al. 2013b; Ngoan et al. 2015), phospholipases A_2 (Shiomi et al. 1988), plancinin (Karasudani et al. 1996), and other small biomolecules, e.g., 2,2-azinobis-3-

ethylbenzothiazoline-6-sulphonic acid, 1,1-diphenyl-2-picrylhydrazyl, Fe^{2+} and butanol (Shiomi et al. 1990; Lee et al. 2014).

A previous transcriptomic investigation of COTS tissues also found there were high levels of expression of genes encoding rhodopsin GPCRs and species-specific secretome proteins in spines, as well as reproductive and neural associated tissues (Hall et al. 2016; Hall et al. 2017). As such, their presence in spines suggested a role in COTS communication, possibly influencing aggregation and/or spawning-associated behaviours. Here, COTS aboral spines were analysed by high resolution scanning electron microscopy (SEM), differential gene expression, and proteomics to examine for spine-associated biomolecules (Hillberg 2024) with properties desirable of a pheromone attractant (Motti et al. 2022).

SEM and histology

For SEM, aboral spines from unperturbed adult COTS were fixed (10% formalin then glutaraldehyde and paraformaldehyde buffer) and then processed as described in Hillberg et al. (2023). For histology, unperturbed COTS aboral spines were fixed (4% paraformaldehyde and stored in 70% EtOH), rehydrated (50% EtOH, washed with water and decalcified using Morse's solution - 10% sodium citrate and 50% formic acid) and sectioned as per Smith et al. (2018). Sections were viewed under a Leica DM550 microscope equipped with a Leica camera.

Screening COTS spines and spine secretome for peptide and protein semiochemical leads

To obtain aboral spine secretome proteins, aboral spines dissected from unperturbed non-reproductive COTS were placed in Milli-Q water and agitated to facilitate venom release (crude spine extract; SpE) (Hillberg et al. 2023). The solution was further fractionated through a 10 kDa cut-off Amicon ultra-15 centrifugal filter unit (4000 x g for 10 min). To identify lead semiochemicals (i.e., those secreted into the water), COTS were stressed by intermittent mechanical agitation to produce crude stress-conditioned seawater (StWE) which was also further fractionated through a 10 kDa cut-off filter (as described above). Protein quantification of the retentate (> 10 kDa) and eluate (< 10 kDa) of both sets of extracts was done using a Nanodrop 2000 instrument at 280 nm. All samples (crude, and < 10 and > 10 kDa fractions) were tested in the brine shrimp lethality assay and activity observed at 48 hr post-exposure (refer to section 2.3.2).

To determine the nature of chemistry typically secreted by COTS spines, spines were excised from unperturbed and mechanically stressed COTS and acidified (immersed in 0.1% aqueous trifluoroacetic acid; TFA). Acidified samples were loaded onto a C18 cartridge (5 g Sep-Pak Vac 20cc) and eluted with 0.1% TFA acidified 60% acetonitrile (ACN). Dried eluent was reconstituted in aqueous 0.5% formic acid and trypsin digested as per Ni et al. (2018). For comparison, COTS-conditioned water was collected from non-reproductive unperturbed COTS individuals and chromatographed over a C18 Sep-Pak with 0.1% TFA acidified 60% acetonitrile (ACN). The lyophilised eluate was spilt in half, and one-half trypsin digested.

Tryptic and non-tryptic sample preparations were analysed by µHPLC-MS/MS on an ExionLC liquid chromatography system coupled to a Quadrupole Time-of-Flight AB SCIEX X500R Mass Spectrometer (µHPLC QToF-MS/MS). Samples were injected onto a 100 mm ×

1.7 µm Aeris PEPTIDE XB-C18 100 µHPLC column and gradient eluted with 0.1% aqueous formic acid and 100% ACN. MS data was acquired in the Information Dependant Acquisition mode and full scan QToF-MS data was acquired over the mass range 350–1400 *m/z*; product ion MS/MS data was acquired over 50–1800 *m/z*. Ions observed in the QToF-MS scan exceeding a threshold of 100 cps and a charge state of +2 to +5 were set to trigger the acquisition of product ion. The data was acquired and processed using SCIEX OS software. The µHPLC QToF-MS/MS data was analysed using PEAKS v7.0 against the protein database assembled from the available COTS transcriptomes (including spine data obtained here) and genome data (Hall et al. 2017).

Spine differential RNA-seq analysis, annotation and peptide synthesis

RNA-seq analysis is key to identifying differentially expressed and co-regulated genes, and predicting the proteins that are likely to be produced. To understand the level and distribution of COTS gene expression across tissues, heatmaps of available transcriptome data (http://marinegenomics.oist.jp/cots) were constructed (R version 3.1.1; https://www.r-project.org/). An in-depth RNA-seq comparative quantification was then performed specifically using COTS RNC, tube foot and sensory tentacle data derived from Roberts et al. (2017) and Smith et al. (2017). Here, RNA-seq data for COTS spine was obtained to expand this resource and allow for comparative analysis. Aboral spines from unperturbed COTS were immersed in RNAlater, then extracted with TRIzol™ Reagent and total RNA quantified using a Nanodrop 2000 and integrity assessed with a Bioanalyzer RNA 6000 Nano mRNA kit. RNA-seq was then performed following the Novogene standard workflow (https://en.novogene.com/) on an Illumina 2500 sequencing platform (GenBank accession: PRJNA901199). To assess the possible role of spine proteins in semiochemical communication, further Illumina RNA-seq of spines from non-reproductive and reproductive stage COTS was done (as above). Relative expression of aboral spine genes was determined using the CLC Genome Workbench (Version 20) based on transcripts per kilobase million (TPM), with the GBR COTS genome as reference.

Putative secreted spine proteins were predicted based on the presence of signal peptide and absence of transmembrane domain in the protein precursor sequences, using SignalP (V.5.0) and TMHMM (V.2.0) webtools (Krogh et al. 2001; Almagro Armenteros et al. 2019). Differential gene expression was visualised in a heatmap using the ClustVis webtool (Metsalu and Vilo 2015). General functions of identified putative proteins were predicted by performing Gene Ontology (GO) annotation against a non-redundant database on the NCBI using a BLASTp search on the Omicsbox software (BioBam).

Peptide sequences were selected for synthesis and testing based on evidence for genus specificity, expression level, changes between reproductive stage and their confirmed presence in the excretory-secretory proteome. Peptides were synthesised to 95% purity by ChinaPeptides (China), lyophilised and stored at -20°C until use.

2.3 Behavioural and functional testing of promising lead semiochemicals

Multi-omic knowledge of a species alone does not reliably inform whole animal response. Here, a comprehensive approach was taken, coupling multi-omics knowledge with

behavioural assays, focussed on the whole animal, and functional assessments, to establish semiochemical lead activity and efficacy.

2.3.1 Whole animal behaviour assays: design, validation and testing of cues

Whole animal behavioural assays simulate the behavioural response of a living organism to a stimulus within a controlled environment. Behavioural testing of COTS at the AIMS SeaSim, using a large-scale Y-maze (also referred to as a bifurcation maze), has previously confirmed COTS are capable of chemosensation and can detect waterborne chemical cues emanating from living coral prey, conspecifics, and giant triton predators (Hall et al. 2016; Bose et al. 2017; Hall et al. 2017). As per previous work, prior to each behavioural assay, COTS were segregated into individual tanks (40 L, ambient temperature, aeration and flow-through) and held naïve. Water temperature used for the naïve holding tanks was applied to the assay system. Between each experiment all surfaces of the assay system were brushed and the entire system flushed for 20 min at high flow rate followed by a longer flush (up to 4 h) at 20 L min^{-1} to remove any trace of previous chemical stimuli. Robust studies (Hall et al. 2016; Hall et al. 2017) have established that COTS can display thigmotaxis but do not show left or right handedness, therefore, for ease of statistical assessment, the test cue was delivered via the right arm only and 'no movement' data was considered as a response.

Large-scale Y-maze

Y-maze systems have a long and extensive history of use in the assessment of an organism's ability to discriminate between two choices, usually a stimulus and a control. Here, the large-scale Y-maze system was applied to test COTS-derived waterborne lead pheromone attractants (refer to detailed methods in Hall et al. (2017)). Briefly, water temperature and photoperiod simulated ambient Townsville summer conditions, aligning with the timing of COTS collection and preparation of the summer cue. The Y-maze arena consisted of a main channel (1.75 m length x 0.6 m width), and two arms (2.35 m length) which were separated by a 0.8 m divider to ensure no backflow between arms. The system was illuminated with infrared (IR) lamps and filmed with an infrared camera (Basler AG acA1300-60gmNIR) fitted with an IR lens. The tanks were exposed to regional photoperiod changes in Townsville with full sunlight spectrum plasma units (Luxim Model GRO-41-01, Luma America) and crepuscular twilight ramping. The chemical cue was released into the 'cue' arm at a constant flow rate using a peristaltic pump. Each arm of the Y-maze was supplied by a constant flow of seawater at 5 L min^{-1}. Video footage was automatically captured and analysed with Ethovision® XT11 to reveal COTS behaviours over spatial and temporal scales (Noldus et al. 2001; Spink et al. 2001).

Here, to allow for retrospective comparisons, large-scale Y-maze assays were conducted on COTS-conditioned water, as previously described (Hall et al. 2017). Test samples (n=20) and seawater controls (total volume 500 mL per trial, n=20) were introduced at constant rate of 2 mL min^{-1} via a peristaltic pump over 4 hrs, starting late afternoon (16:00 hrs) coinciding with the greatest period of COTS movement (Ling et al. 2020). Each COTS subject was removed from the Y-maze after every assay and a new animal used for the next experiment.

Statistical analyses of COTS behaviour in the large-scale Y-maze assay were conducted in EthoVision® XT11 as per Hall et al. (2017). Eight dependent variables, using centre-point detection, were defined to interpret COTS behaviour (Noldus et al. 2001):

1. Distance moved (total and mean cm);
2. Velocity (mean distance moved, cm sec^{-1});
3. Distance to zone (total and mean shortest distance to target arm, cm);
4. Heading to zone (movement relative to target zone, deg);
5. In zone (cumulative duration spent in target arm, sec; frequency in zone, latency to first visit and number of times individuals moved from outside the plume into the cue plume);
6. Meander (total and mean change (variance) in direction relative to distance moved, degrees cm^{-1});
7. Movement (average velocity at > 0.06 cm sec^{-1} and at > 0.25 cm sec^{-1});
8. Mobility state (cumulative duration considered mobile if > 20% of movement and highly mobile if > 70% of movement).

All data was analysed (applying the variables listed above) through an independent sample t-test (GraphPad Prism® 9.4.1).

Mid-scale Y-maze

A mid-scale portable whole animal Y-maze system (**Figure 3**) was designed and constructed to assess COTS acute response to semiochemicals (i.e., over shorter distances and timeframes). The Y-maze arena consisted of a main channel (0.5 m length x 0.6 m width), and two arms (0.5 m length). The system was operated in flow-through mode and under the same conditions as for the large-scale Y-maze trials. Dye tests, using fluorescein, were performed to confirm laminar flow; an optimal constant flow rate of 5 L min^{-1} per arm was established. The cue (refer to specific methods below) was delivered via peristaltic pump. COTS behaviour was recorded using the same camera and lighting as described above, with EthoVision® v.15.8 software. To reduce interference from light reflection, the transparent walls of the Y-maze were covered with self-adhesive matt-black film. To assess COTS behaviour in response to the cue, EthoVision® XT monitored six detection zones within the Y-maze over 1 hr (**Figure 3**). All data was analysed (applying the variables listed above) through independent sample t-test (GraphPad Prism® 9.4.1).

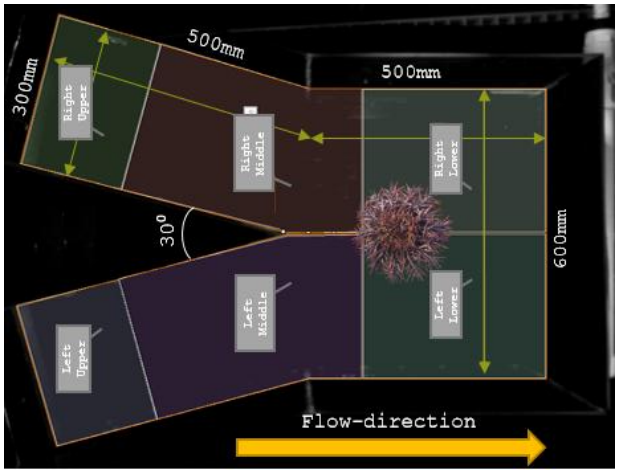

Figure 3. Schematic of mid-scale Y-maze system used to monitor COTS behaviour in response to cues. Notional detection zones are illustrated divided into the lower, middle, and upper left and right sides.

Two-current flume system

Based on the recommendation by Motti and Hall (2018) (Appendix F) and best practices (Jutfelt et al. 2017), an alternate two-current choice linear flume system was designed and constructed to assess COTS behavioural responses under controlled yet realistic hydrodynamic conditions. The following assay parameters were optimised:

1) Reduce
    a. seawater flow;
    b. total seawater volume;
    c. experiment duration;
    d. water turbulence;
    e. movement restrictions, thereby eliminating bias;
    f. impact of thigmotaxis (Hall et al. 2016);
    g. animal stress; and
2) Increase
    a. number of assays that can be performed per week;
    b. control over cue plume, i.e., concentration gradient;
    c. reproducibility of animal responses.

The design features a 6 m large-scale black arena with honeycomb collimators to establish a laminar flow (**Figure 4**, Appendix F **Figure A 3** and **Figure A 4**), optimised by the water flow rate. The collimators ensure separation and even distribution of the introduced chemical cue across an open arena creating two separate flow lanes designated ‘cue’ and ‘non-cue’ arm without the need for a physical barrier (as is required in the Y-maze). This design allows the animal free movement and choice and provides alternative/different but complementary behavioural data than the classic Y-maze. As for the Y-maze system, the chemical cue is delivered at a constant flow rate using a peristaltic pump. Animal behaviour was tracked

under infrared light using the same system as above and COTS movement recorded and analysed (applying the variables listed above) using EthoVision® XT17 software.

Dye tests, using fluorescein, were performed to optimise the water flow rate and to ensure laminar flow consistency post the collimator. Additional testing of the cue delivery system was performed to optimise the cue flow rate and ensure even distribution of the cue within the arm post the collimator. To test the validity of the flume system, a positive control trial was conducted (n=20) using glycine betaine (GlyB), a known coral-derived foraging cue for starfish (McClintock and Lawrence 1984) including COTS, and results compared to previous Y-maze assay results (Hall et al. 2016). COTS-derived attractant cues were subsequently tested in this system.

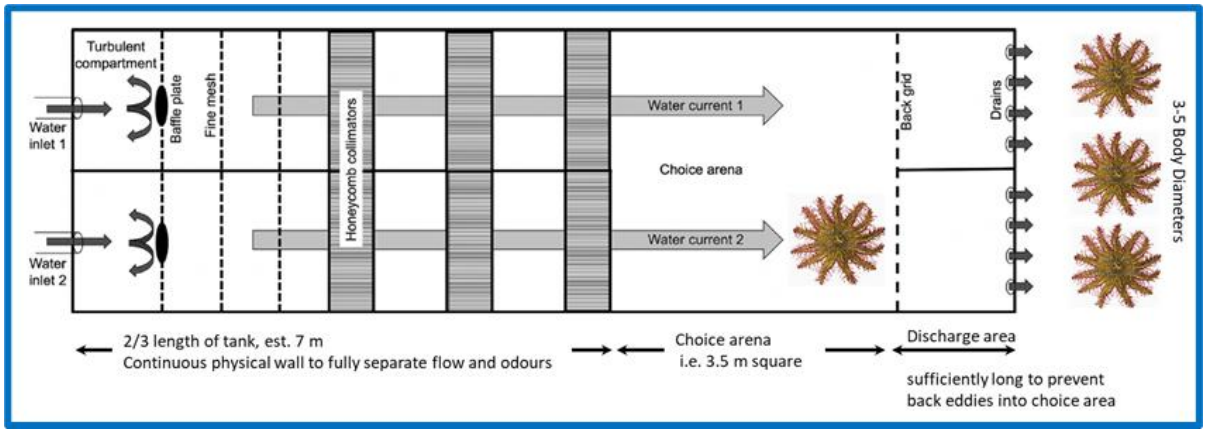

Figure 4. Schematic of flume system used for COTS behaviour assays.

2.3.2 Assessing efficacy and toxicity of lead attractants

The efficacy of the potential lead attractants was determined by quantifying COTS responsiveness and establishing their impact on spawning/reproductive functioning and non-spawning aggregation behaviour. The toxicity of the leads was assessed using the brine shrimp lethality assay. Chemical properties of leads were considered in the context of Motti et al. (2022).

COTS behavioural response to tangential flow filtration concentrates

Test fractions (500 mL of summer 2020 FrA and FrB per trial, n=20 COTS per fraction) and seawater controls (500 mL per trial, n=20 COTS) were introduced into the large-scale Y-maze system and the response of COTS monitored according to Hall et al. (2017). To provide sufficient evidence of reproducibility in COTS response, and as a means of validating the new flume system, COTS behavioural assays were repeated using fresh preparations of the > 30 kDa fraction (FrA) from COTS collected in summer (2023) and winter (2023) (n=20 COTS for both). Analysis of movement data was performed using Ethovision® XT17 software and R (ggplot2 and tidyverse) and GraphPad Prism®.

COTS behavioural response to recombinant COTS ependymin-related protein GBR.60.100

To assess the behavioural response of COTS to recombinant COTS EPDR GBR.60.100, large-scale Y-maze behavioural testing (n=12, trials limited by sample quantity; n=20 controls

– as above) was conducted as above. Each experiment was performed by placing one COTS in the Y-maze starter box and exposing it to recombinant GBR.60.100 (or control) delivered to the right Y-arm by peristaltic pump (2.2 mL min^{-1}). The 'time spent' by the COTS in each zone of the Y-maze was measured, as was the accumulative time spent between the right- and left-hand side of the Y-maze. Analysis of movement data was performed using Ethovision® XT17 software.

COTS behavioural response to synthetic COTS spine-secreted peptides

To assess the acute behavioural response (i.e., within 1 h) of non-reproductive COTS to spine-derived peptides, mid-scale Y-maze behavioural testing was conducted following methods adapted from Hall et al. (2017) and Hillberg (2024). Each experiment was performed by placing one COTS in the Y-maze starter box (**Figure 3**) and exposing it to the peptide cocktail (or control) delivered to the right Y-arm by peristaltic pump (2.2 mL min^{-1}). The peptide cocktail (n=6 synthetic peptides) was prepared to 1 nM per peptide, equivalent to a single peptide concentration in the Y-maze ranging from 100 pM–44 fM. The 'time spent' by the COTS in each zone of the Y-maze was measured, as was the accumulative time spent between the right- and left-hand side of the Y-maze. Analysis of movement data was performed using Ethovision® XT17 software.

Exploration of the endogenous relaxin-like gonad-stimulating peptide (RGP) as a possible control agent by disrupting reproduction

In starfish, endogenous RGP regulates oocyte meiosis (i.e., egg maturation) prior to spawning. Comparative RNA-seq analysis showed RGP gene expression is elevated in female COTS during the reproductive season. The recombinant COTS RGP, generated in a competent yeast (*P. pastoris*) expression system, proved capable of inducing germinal vesicle breakdown (GVBD; the process of nuclear envelope dissolution and chromosome condensation) of oocytes from ovarian fragments, followed by ovulation (Smith et al. 2019). Of note is the high levels of RGP expressed in the sensory tentacles, suggestive of a role in chemoreception.

Here, the potential of RGP as a possible COTS control agent was further assessed. The predicted mature RGP structure (Smith et al. 2019) (**Figure 5**) was synthesised (Florey Institute; verified by MS/MS) in quantities that allowed for subsequent behavioural testing.

Figure 5. Illustration of the predicted relaxin-like gonad-stimulating peptide (RGP) in the crown-of-thorns starfish (COTS), showing the regions of a signal peptide (grey), B- and A-chains, dibasic cleavage sites (red) and disulfide cross-linkages (green lines) between the cysteine residues.

Spawning response of COTS and Acanthaster brevispinus *to synthetic COTS RGP*

The effect of the synthetic RGP on COTS oocyte maturation was investigated using *in vitro* GVBD assays (Hillberg et al. 2025). Ovary tissue (~500 mg) was excised from healthy female COTS (n=3) and confirmed by inverted microscopy (Leica M5600) to be mature (oocytes ~200 µm in diameter) with no signs of prior GVBD. Ovary fragments were incubated (in 90 µL filtered seawater (FSW) 96-well plates, ambient temperature,1 hr) in one of four solutions: FSW (10 µL; negative control), 1 mM and 10 mM 1-MeAde per well (as positive controls) and 1 µM synthetic COTS RGP. Oocytes were monitored by inverted microscopy every 10 min and those undergoing GVBD counted. Statistical comparison was performed using GraphPad software. A pairwise comparison was performed using the t-test (two-tailed) method with significant difference considered at p-value of ≤0.05.

The effect of the synthetic RGP on COTS spawning activity was also investigated by *in vivo* assay over three spawning seasons (summer 2021, summer 2022 and winter 2023) (Hillberg et al. 2025). Adult gravid COTS were sexed by biopsy (Smith et al. 2018) then segregated in flow-through 100 L aerated tanks (FSW; 0.04 µm, 25–26°C and PSU 35.5) for a minimum of 24 hr to recover.

In 2021, two males and two females were intra-coelomically injected (1 ml syringe with a 27 G needle) with 100 µL Milli-Q water (negative control) or 10 mM 1-MeAde at the arm junction close to the location of the gonads. Behaviour changes and spawning were documented by video (GoPro HERO 8 Black) for a period of 3 hrs. All experiments were conducted during the late afternoon until early evening (summer 2021; between 16:00 and 23:00). No spawning behaviour was observed, or release of gametes 3 hr post-injection, therefore, a further 300 µL of MilliQ-water or 30 mM 1-MeAde was administered. Synthetic RGP (1 µM, 100 µL) was administered to both male and female COTS (7 males and 6 females).

At completion of the summer 2021 tests, in vitro fertilisation was performed and viability of COTS gametes assessed (Hillberg et al. 2025). In brief, eggs from three females and sperm from one male COTS, release induced by injection with 1 µM RGP, were collected, gently mixed and fertilisation monitored by microscopy periodically for 3 hrs.

To ensure reproducibility of spawning induction, RGP injections were repeated in summer 2022 on a new cohort. COTS are known to stress spawn upon handling (Fraser et al. 2000), an aspect that was not considered in the 2021 trials. Therefore, to assess for any behaviour changes induced when administering control treatments, all animals (n=20) were first injected with 100 µL Milli-Q (negative control) 7 days prior to RGP injections. These animals were then injected with synthetic RGP (1 µM, 100 µL) and monitored for 3 hrs. The effect of the synthetic RGP (1 µM, 100 µL) on *A. brevispinus* spawning activity was similarly tested in summer 2022 on two female *A. brevispinus* opportunistically held at AIMS. Both were assumed to be gravid based on a time of year consistent with a previous study (Lucas and Jones 1976).

To assess for any seasonality difference, RGP injections were repeated for COTS in winter 2023.

Toxicity testing of lead attractants - brine shrimp lethality assay

The brine shrimp lethality assay was performed based on existing protocols (Meyer et al. 1982). Brine shrimp (*Artemia salina)* larvae were exposed to samples prepared at 50% of the original concentration of a given extract/fraction plus two additional dilutions (1:10 and 1:100). For negative controls, 20 µL FSW was added to the well, for positive control 20 µL TFA. Live *A. salina* were counted at 0, 24, and 48 hrs post-treatment to calculate percent mortality. Data was visualised by applying a log10 function to the peptide concentrations (x-axis) and plotting against the average mortality rate converted into probit (y-axis (Finney 1971)). The lethal dose required to cause 50% mortality in *A. salina* (LC_{50}) was calculated via a 3-point linear regression. A one-way ANOVA (univariate analysis) and subsequent Scheffe post-hoc tests were used to establish significance at a confidence threshold of 95% ($P \leq 0.05$). Where data did not contain an equal variance of error according to Levene's test ($P \leq 0.05$), the Games-Howell post-hoc test was used to evaluate significant differences between different groups at a confidence threshold of 95% ($p \leq 0.05$).

Assay 1: The TFF active summer FrA was tested neat and across a serial dilution series of 1 in 2, 4, 8, 16 and 32 with FSW (800:200 µL FSW:FrA). A positive control of 800:200 µL FSW:TFA, and a negative control of 1 mL FSW were used.

Assay 2: The crude spine extract (SpE) was tested at 110 µg mL^{-1} and dilutions of 1:10 and 1:100. The size fractionated SpE (< 10 kDa and > 10 kDa SpE fractions) were tested at 93.3 µg mL^{-1} and dilutions of 1:10 and 1:100. The crude stress-conditioned water extract (StWE) was tested at 107 µg mL^{-1} and dilutions of 1:10 and 1:100 while size-fractionated StWE (< 10 kDa StWE and > 10 kDa StWE fractions) were tested at 98.2 µg mL^{-1} and dilutions of 1:10 and 1:100.

Assay 3: A 1 nM stock solution of each of six synthetic peptides (1 mg), inspired by COTS spine-secreted proteins (SSP), was prepared in Milli-Q water. A cocktail of the six synthetic peptides was also tested at 100 pM, 10 µM and 100 µM.

2.4 Investigation of COTS betaine receptors towards a receptor-based assay

Reverse chemical ecology represents an alternative strategy to identify semiochemicals (Leal 2005,2017). It relies on knowledge of the semiochemical receptor and evaluates its affinity for biomolecules (or ligands that bind to the receptor) via 3D computational modelling of the ligand:receptor interaction. However, the expression of highly specific semiochemical receptors allows for functional analysis by screening libraries of putative semiochemicals.

In the marine environment and specifically for corals, glycine betaine (*N*,*N*,*N*-trimethylglycine; GlyB) and betaine derivatives function to protect cells against osmotic and temperature stress, and to support coral-dinoflagellate symbiosis (Ngugi et al. 2020). Betaine derivatives and betaine lipids have been shown to be highly genus specific (Reddy et al. 2023), with *Pocillopora* spp. containing GlyB, *Millepora* cf. *platyphylla* containing hydroxyproline betaine (HProB) and *Porites* spp. alanine betaine (AlaB) and taurine betaine (TauB) (Hill et al. 2010). Betaines, including GlyB, are released by corals under predation (Moore and Huxley 1976), and GlyB is a confirmed COTS attractant (Moore and Huxley 1976; Suenaga 2004), hence

its routine use as a positive control in COTS functional assays; it induces robust behavioural responses in test animals ((Hall et al. 2017) and section 3.3.1). As such, it is highly probable that COTS have betaine receptors and that GlyB acts as a chemosensory ligand. To elucidate the mechanism of action of betaines in COTS chemosensation and their utility in behavioural assays, the GBR COTS genome was interrogated for the presence of betaine-like receptors and a workflow to express lead receptors established.

2.5 Stakeholder engagement

Stakeholder knowledge has been, to date, largely an untapped entity with respect to the search for semiochemical control agents designed to complement and supplement existing COTS control methods. Early engagement with multidisciplinary scientists (Pratchett et al. 2017; Pratchett et al. 2021) and reef-based operators (i.e., AMPTO, PMG and Blue Planet Marine (BPM); refer to section 2.5.1) as academic and industry stakeholders, has enabled early investigative studies, including the inception and planning for CCIP-R-11. However, CCIP-R-11 recognised that to take this concept to the next level where the use of semiochemical technologies is widely accepted, engagement with and participation of stakeholders who are familiar with decision-making contexts and existing corporate and community knowledge (and datasets) is crucial (Gerlak et al. 2023) (section 5.2). Provided below are the outputs from CCIP-R-11 stakeholder engagement.

2.5.1 Operations and Logistics

The success of CCIP-R-11 is reliant on an established, long-term relationship with the GBRMPA reef-based operators. CCIP-R-11 sources live adult COTS for tissue sampling and assay testing from PMG and BPM crews, without whom the costs of procuring specimens would have been significant and inhibitive. To foster and grow these relationships, CCIP-R-11 has and continues to maintain regular dialogue (i.e., via email and in person) with PMG and BPM crews and GBRMPA and, over the course of the project, has:

1. Hosted the PMG crew at AIMS (24th August 2021) involving a presentation ‘The search for COTS pheromone attractants to control Crown-of-Thorns starfish’ and site tour, including of the SeaSim and the Behavioural Facility.
2. Met with PMG and BPM representatives at CCIP workshops and conferences and discussed supply logistics, protocols for handling and transporting COTS and in-field observations of COTS behaviours (CCIP forums: 6th-7th October 2022, 15th-16th May 2023, 13th-14th November 2023, 15th April 2024 COTS Workshop; 16th-18th April 2024 Reef Resilience Symposium).
3. Met with BPM representatives and agreed to develop a project to design and test traps (15th April 2024 COTS Workshop).

2.5.2 Regulatory Meetings and Workshops

Regulatory licence to operate is critical to secure and needs to be considered alongside social and cultural licence to operate. CCIP-R-11 has begun to explore the regulatory requirements towards realising a semiochemical-based COTS control method:

1. Met with CCIP-R-08 project team members (Fidelman, Bartelet):
    a. Hosted CCIP-R-08 (Fidelman; 29th May 2023 CCIP-R-11 1-day workshop) and discussed regulatory implications of innovative COTS control methods.
    b. Facilitated by CCIP-R-08, CCIP-R-11 presented at the GBR Regulators Forum 22nd June 2023 'The search for Crown-of-Thorns Starfish pheromones: modifying conspecific behaviour to control outbreaks'.
    c. Met with representatives from the Australian Pesticides and Veterinary Medicines Authority (APVMA 22nd June 2023) and discussed workflows and procedures to assess semiochemical technologies.
2. Met with GBRMPA representatives and discussed permit requirements for applying semiochemicals on the GBR, including a presentation 'The search for COTS pheromone attractants to control Crown-of-Thorns starfish' (21st June 2023).
3. Hosted CCIP (Bonin) at AIMS (6th September 2023) and discussed project status and regulatory requirements for field trials.
4. Met with CCIP-P-08 project team members (Paxton, Lockie) (November 2023) - engagement resulted in translation of CCIP Early Investment Project report (Motti et al. 2022) into a co-authored review publication (Harris et al. 2025a).

2.5.3 Community and Traditional Owner engagement

Social and cultural licence to operate is critical to secure and needs to be considered alongside a permit licence to operate. CCIP-R-11 has begun to explore the societal landscape towards realising a semiochemical-base COTS control method.

1. Cultural engagement:
    a. Participated in 'The Art and Science of Oceans' initiative established by AIMS and the Torres Strait-based Ghost Net Collective (https://www.ghostnetcollective.com.au/education/projects), Erub Arts, and the Torres Strait Regional Council. Involvement was through workshops (3rd and 9th September 2021) with Erub artists and educators, AIMS tours, contribution to collaborative woven marine debris and ghost net art installations (commissioned to commemorate AIMS' 50th year) and input into development of educational material for the Australian Science and Visual Art Years 1-12 school curriculum (March 2022), both featuring COTS, to integrate art, science and environmental conservation.
    b. Hosted Torres Strait Regional Authority (October 2021) to discuss COTS problem.
    c. Participated in the inaugural Indigenous Education Resource Centre (IERC) Winter School (a joint AIMS and JCU activity for Year-12 students) (June 2022) at AIMS, including development of resources and booklets and 2-hr COTS workshop and tour.
    d. Participated in the Aboriginal and Torres Strait Islander in Marine Science (ATSIMS) Program (a joint AIMS and JCU activity for Year 9 and10 students

from local and regional Queensland) (June 2022) at AIMS, including development of resources and booklets and 1-hr COTS workshop and tour.

e. Met with Wulgurukaba Elder Eddie Smallwood (8th September 2022) to discuss COTS control research. This is the first engagement to seek retrospective approval to undertake collections of COTS and to conduct SeaSim experiments.

f. Hosted Townsville Aboriginal artist Jaquanna Elliot (10th May 2023), including a tour of the AIMS Behavioural Facility and discussions regarding local Bindal knowledge, and bringing science (i.e., the COTS problem) into the narrative.

g. Hosted trainee Gudjuda Indigenous Rangers and Elders at AIMS; including a tour of the AIMS Behavioural Facility and hands-on presentation (14th June 2024).

2. Media engagement:

   a. UQ Media report (Genetics provide key to fight crown-of-thorns starfish - UQ News - The University of Queensland, Australia) (Morin et al. 2024).

   b. AIMS Facebook feed – 16th October 2023 – featured Dr. Richard Harris and the newly commissioned behavioural flume system.

   c. Channel TV 10 interview (2nd June 2023) for 'Planet Shapers' documentary, showcasing Australian Government-driven initiatives for the GBR, including CCIP.

   d. AIMS website COTS content updated March 2023 to include CCIP project summary.

   e. Department of Agriculture, Water and Environment (DAWE now DCCEEW) Reef Branch interview (May 2022) showcasing the interconnected relationships between the Reef, the people committed to caring for it and the communities that depend on it.

2.5.4 Conference and CCIP Workshop Presentations

CCIP-R-11 has presented research findings on COTS semiochemicals at three project workshops and various conferences:

1. CCIP-R-11 Project Knowledge Gaps Workshop #1 (29th May 2023; online).
2. Motti et al. (23rd May 2022) Deployment of semiochemical control agents to manage Crown-of-Thorns starfish populations. CCIP Steering Committee, oral.
3. Hillberg et al. (August 2022) An investigation of semiochemicals responsible for attraction, feeding and aggregation behaviours in the Crown-of-Thorns Starfish (*Acanthaster planci*). AMSA, oral.
4. Jönsson et al. (August 2022) Gravid Male and Female Crown-of-Thorns Starfish Differentially Express Pheromones and Neural Signalling Molecules. AMSA, oral.
5. Morin et al. (August 2022) A Novel Secreted Gene Family in Crown-of-Thorns Starfish. AMSA, oral.

6. Motti et al. (August 2022) Deployment of semiochemical control agents to manage Crown-of-Thorns starfish populations. AMSA, oral.
7. Smith et al. (August 2022) Purification and identification of saponins produced by Crown-of-Thorns Starfish. AMSA, oral.
8. Smith et al. (September 2022) Purification and identification of saponins produced by Crown-of-Thorns Starfish. AIMS COTS Workshop, oral.
9. Degnan et al. (6th-7th October 2022) CCIP-R-11 update. CCIP 2-day Innovation for Impact Workshop, oral.
10. Motti et al. (15th-16th May 2023) CCIP-R-11 update. CCIP 2-day Integration Opportunities Workshop, oral.
11. CCIP-R-11 Midpoint project status update Workshop #2 (29th May 2023; in person).
12. Yap et al. (2nd-6th October 2023) Biocontrol of crown-of-thorns starfish through sustainable bioprocessing and bioproduction. Combined 13th APMBC and 5th ANZMBS. Poster, runner-up for the best poster prize award.
13. CCIP-R-11 Project status update Workshop #3 (12th December 2023; in person).
14. Cummins et al. (13th-14th November 2023) CCIP-R-11 update. CCIP Integration and Translation Workshop, oral.
15. Yap et al. (May 2024) Semiochemical control of Crown-of-Thorns starfish through scalable bioprocessing. Reef Resilience Symposium, oral.

2.5.5 Theses

1. (Morin 2023)
2. (Jönsson 2023)
3. (Hillberg 2024)
4. (Yap 2026) submitted

2.5.6 Publications

CCIP-R-11 members have utilised the -omics resource library and published findings that extend our knowledge of COTS biology and chemistry:

1. Yasuda et al. (2022) Two hidden mtDNA-clades of Crown-of-Thorns Starfish in the Pacific Ocean. Frontiers in Marine Science, 9:831240.
2. Jönsson et al. (2022) Sex-specific expression of pheromones and other signals in gravid starfish. BMC Biology, 20(1):288.
3. Smith et al. (2023) Structure and proteomic analysis of the crown-of-thorns starfish (*Acanthaster* sp.) radial nerve cord. Scientific Reports, 13:3349.
4. Mendoza-Porras et al. (2023) Biochemical metabolomic profiling of the Crown-of-Thorns Starfish (*Acanthaster*): New insight into its biology for improved pest management. Science of the Total Environment, 861:160525.

5. Morin et al. (2023) Captivity induces a sweeping and sustained genomic response in a starfish. Molecular Ecology, 32(13):3541-3556.
6. Hillberg et al. (2023) Crown-of-Thorns starfish spines secrete defence proteins. PeerJ, 11:e15689.
7. Morin et al. (2024) Seasonal tissue-specific gene expression in wild Crown-of-Thorns starfish reveals reproductive and stress-related transcriptional systems. PLOS Biology, 22:e3002620.
8. Harris et al. (2025a) The future of utilising semiochemical pest control methods to manage the destructive crown-of-thorns starfish outbreaks on coral reefs. Biological Conservation, 302:110984.
9. Harris et al. (2025b) A family of crown-of-thorns starfish spine-secreted proteins modify adult conspecific behavior. iScience 28:112161.
10. Yap et al. (in submission) A generalizable, scalable multi-omic appraoch to identify biomimetics for controlling marine pests: the crown-of-thorns starfish as a test case. One Earth

3 RESULTS

3.1 Refinement and establishment of omics databases

3.1.1 Democratisation of the COTS genome

This genome browser contains revised annotated coding sequences (initially reported in Hall et al. (2017)) with 160+ mapped transcriptomes generated as part of ARC Linkage Project (LP170101049) with support from the Great Barrier Reef Foundation (GBRF). Rather than publishing via peer review, given this is a refinement exercise, UQ opted for rapid communication. The democratised COTS is now publicly accessible via Degnan marine genomics lab | Apollo Portal (genome.edu.au) and is accompanied by documentation on how to set up and use the tool, including how to manage, search, visualise, explore and share transcriptomic, epi-genomic and proteomic data (both COTS and other organisms). This COTS genomic resource adds to the resources already published and available via the Okinawa Institute of Science and Technology (OIST) Genome Browser Information: Acanthaster planci ver. 1.0 (oist.jp) (funded by OIST, AIMS and Department of the Environment Reef Rescue 'Caring for Country'). The outputs of this are summarised in (Appendix D **Table A 3**).

3.1.2 A draft chromosome-scale genome assembly of COTS

Sequencing data generated for both the male and female COTS individuals were subjected to quality control (QC) and cleaned to remove adapters and low-quality or contaminant sequences using PoreChop v0.2.4 and TrimGalore v0.6.10. The two genomes were assembled using SMARTdenovo v. 1 and error corrected using Racon (v1.4.3) and Masurca (polca) v. 4.0.9. A coverage of 60X was achieved for each individual and produced high-quality contig assemblies. The refined male (GCA_030586445.1) and female (GCA_030586425.1) genomes have been submitted to NCBI (refer to genome statistics in **Error! Reference source not found. Table A 4** to **Table A 7**). Although these assemblies are not chromosome-scale, the COTS genome was of sufficient quality to support targeted exploration of COTS function. Incorporation of these refinements resulted in an exemplar starfish genome that was subsequently used to facilitate the identification of lead protein pheromones.

Initial attempts at Hi-C sequencing using COTS tube feet tissues (stored at -80°C) and standard protocols developed for mammalian systems were unsuccessful. In parallel runs, Hi-C library generation from tick, mosquito and plant species was successful. The primary impediment was the nature of the COTS DNA material, proving recalcitrant to Hi-C library construction by interfering with the cross-linking between the COTS chromatin (DNA + histone proteins) and formaldehyde. COTS produce a plethora of secondary metabolites (Mendoza-Porras et al. 2023) that possibly result in cross-linking, i.e., saponins or mucous-associated polysaccharides or even nucleic acids, thereby blocking sites for protein-protein linking. High salinity levels and the harshness of the fixing protocol were also identified as possible compounding factors. Alternative strategies using modified extraction and cross-linking approaches were further investigated with ANU and the Foley Institute.

Sequencing protocols, based on UQ methodologies designed for other marine invertebrates, were trialled to improve DNA extraction and cross-link DNA for ChIP- and ATAC-Seq. Fresh DNA extractions from COTS gonads (eggs and sperm) were performed. Given its high DNA load, sperm DNA samples were also prepared applying longer digestion times (1.5 hr), with the intention to produce a higher-quality cross-linked material for Hi-C library construction. All commercial kits tested (Arima Genomics Arima-Hi-C kit, Qiagen Epitect Hi-C kit and Phase Genomics Proximo Hi-C Kit) were unsuccessful in producing a viable library for Hi-C sequencing. While the PacBio Revio system gave good results for fish species, COTS DNA remained problematic.

These results demonstrate that while a high-quality draft COTS genome assembly has been achieved, generation of chromosome-scale assemblies using Hi-C will require further optimisation of tissue selection, DNA purification and chromatin cross-linking protocols. Continued efforts to refine these protocols for COTS, building on new knowledge of COTS chemistry (refer to Section 3.1.4), will be critical for enabling 3D genome mapping and for advancing the identification of new candidate semiochemicals and chemoreceptors, and supporting investigations into key aspects of their biology functional investigations, e.g., limb autotomisation and regeneration. Improved protocols will also support the investigation of a broader range of non-model marine organisms of economic and biosecurity value.

3.1.3 A draft *Acanthaster brevispinus* genome

Several methodological issues were encountered when performing DNA extractions of *Acanthaster brevispinus* tissues for Hi-C sequencing. DNA extractions of female tube feet consistently produced low yields and poor DNA integrity, highlighting a notable species-specific difference in DNA extraction outcomes. These limitations impeded successful Hi-C library construction. Enzymatic shearing resulted in low-quality sequence data with insufficient coverage, and although mechanical shearing improved library quality, it remained inadequate for reliable sequencing. As Hi-C library construction also proved equally challenging for COTS species more broadly, Hi-C library preparation for *A. brevispinus* was paused pending future optimisation and access to higher-quality DNA.

Long-read sequencing of *A. brevispinus* DNA was attempted following the low-input PacBio Revio protocol, however, extracted DNA quality was again insufficient to support robust sequencing. As a result, the research focus was shifted to short-read Illumina RNA sequencing in an effort to generate a draft assembly. The *A. brevispinus* RNA-seq data aligned strongly (75 to 98%) with COTS protein annotations, confirming a high degree of homology between the sibling species. Annotation of the RNA-seq data is ongoing and, once finalised, will be uploaded to the WebApollo-JBrowse COTS genome browser. Importantly, integrating DNA sequencing and RNA-Seq data has already enabled the generation of CDSs for *A. brevispinus*, providing a valuable comparative dataset for future evolutionary and chemosensory investigations.

Building on these results, and to enable future species comparisons aimed at accelerating the search for species-specific semiochemicals and receptors, efforts should now focus on: (i) finalising and releasing the annotated short-read draft genome, (ii) leveraging the RNA-seq evidence to refine gene models and guide targeted gap-filling, (iii) extracting higher-quality, high-molecular weight DNA by optimising tissue collection, preservation and

extraction workflows, and (iv) re-attempting long-read sequencing once high-quality DNA is available. These steps will support the generation of chromosome-scale assemblies needed for 3D genome mapping and downstream functional inference.

3.1.4 Metabolomic and lipidomic profiling of COTS tissues

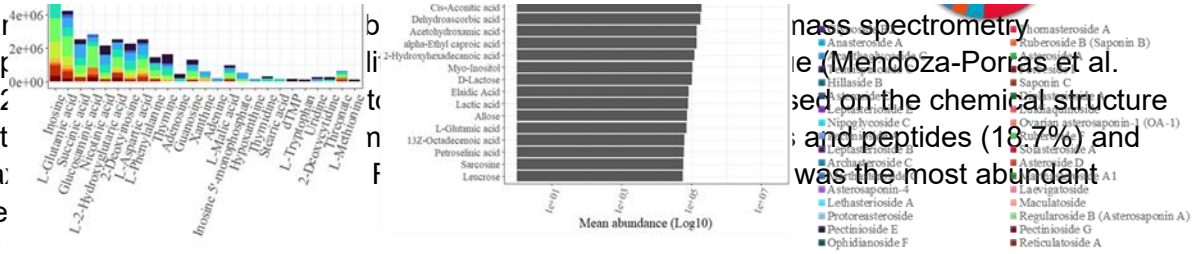

Figure 6. Crown-of-thorns starfish metabolites. **(A)** most abundant central carbon metabolites that enrich purine and pyrimidine metabolism in the tissues: pyloric caeca (PC), eyes (EY), gonad (GN), pyloric stomach (PS), radial nerve (RN), skin (SK), spines (SP), sensory tentacles (ST), tube feet (TF) and vertebrae (VE); **(B)** top twenty most abundant metabolites in eggs; and **(C)** abundance of asterosaponins identified in eggs.

3.2 Identification and production of lead attractants

Application of multi-omics techniques to COTS-conditioned water and tissue samples yielded information on their transcriptome, proteome, metabolome, and other identified leads that were then further assessed for behavioural activity and toxicity.

3.2.1 Putative pheromone attractants from COTS-conditioned water isolated and characterised

Tangential flow filtration (TFF) method development

The COTS-conditioned seawater was successfully harvested, concentrated, and fractionated using TFF and regenerated cellulose nominal molecular weight cut-off membranes. Application of the optimised process yielded two fractions: > 30 kDa (FrA) and 10–30 kDa (FrB), in sufficient volumes to allow for multiple large-scale Y-maze attraction assays (**Figure 7**), lethality testing and chemical characterisation by mass spectrometry. These experiments have now established a TFF workflow for future isolation of semiochemicals from COTS-conditioned water that can routinely process hundreds of litres of seawater within a day, increasing throughput by over an order of magnitude compared to chromatographic methods that are reliant on harsh chemicals.

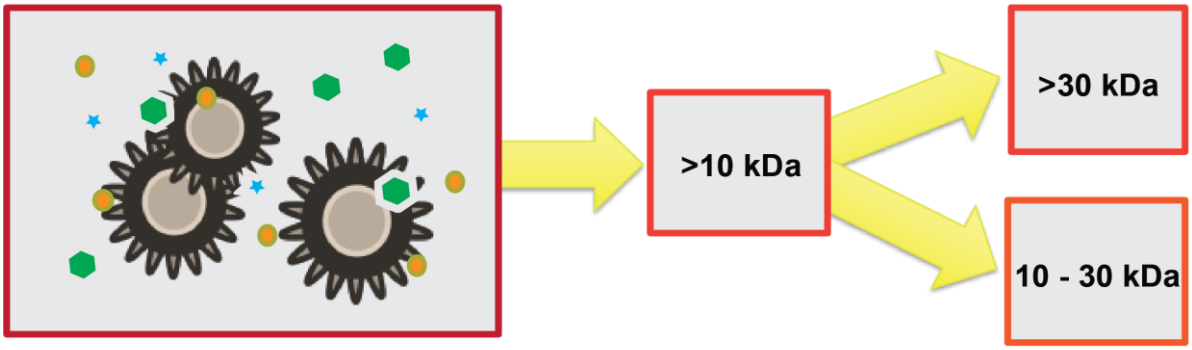

Figure 7. Schematic of the tangential flow filtration fractionation workflow.

Proteomic analysis reveals presence of COTS-specific ependymin-related proteins

Mass spectrometry of the active summer fraction, TFF FrA, revealed at least 19 secreted proteins, 12 being structural or signalling proteins, including two ependymin-related proteins (EPDRs; Appendix G **Table A 8** and **Figure A 6**) potentially involved in communication. Phylogenetic comparison of EPDRs determined these two EPDRs to be COTS-specific, but different to GBR.60.100, and with little sequence similarity to other members of the asteroid clade (Appendix G **Figure A 6**) (Hall et al. 2017).

Glycomic analysis reveals seasonal fluctuation in the COTS glycome

Analysis of the winter and summer TFF FrAs (post acid hydrolysis) revealed a preliminary glycan profile of monosaccharides (Appendix E Figure A 2). The major components found in both samples correlate to galactosamine, glucosamine, galactose and fucose, corroborating previous findings (Bahrom et al. 2013), although full characterisation and quantification remains to be done. An overall increase in the concentration of glycans was observed in the summer FrA (**Figure 8**). Based on the monosaccharide composition, the predominant polymer is most likely a sulfated galactoaminogalactan.

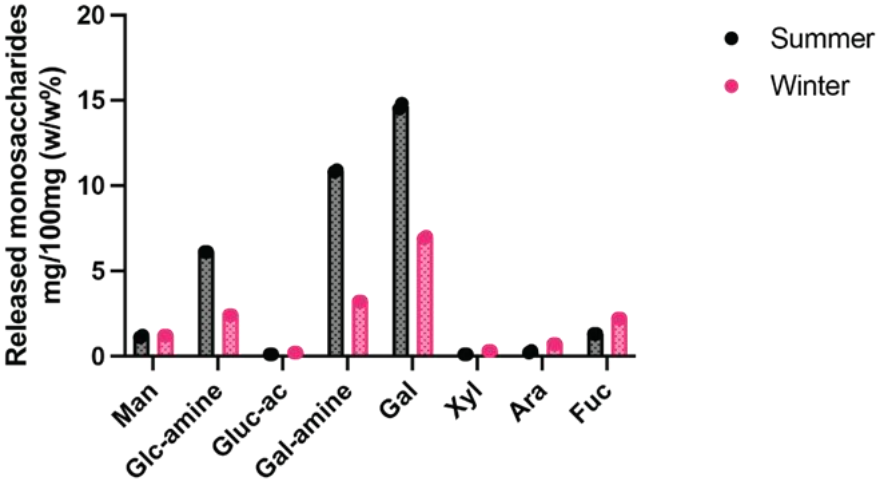

Figure 8. Seasonal difference in the glycan profile of the > 30 kDa COTS secretome.

3.2.2 Recombinant expression of COTS EPDRs

Here ntified through mining of the GBR COTS geno r with parameters as reported by (Yap et al. inant GBR.60.100 in yields of 120 mg L^{-1} cult gerprinting confirmed the sequence of the yeast-derived recombinant GBR.60.100. The SDS-PAGE analysis indicated that the recombinant product is between the size of 30–40 kDa (

Figure 9), which is as predicted. Treatment with PNGase F, an enzyme that specifically removes N-glycosylation, proved that the recombinant EPDR GBR.60.100 was glycosylated based on the band shift when treated with PNGase F. These experiments have established the workflow for recombinant production of individual putative attractants to deconvolute semiochemical mixtures.

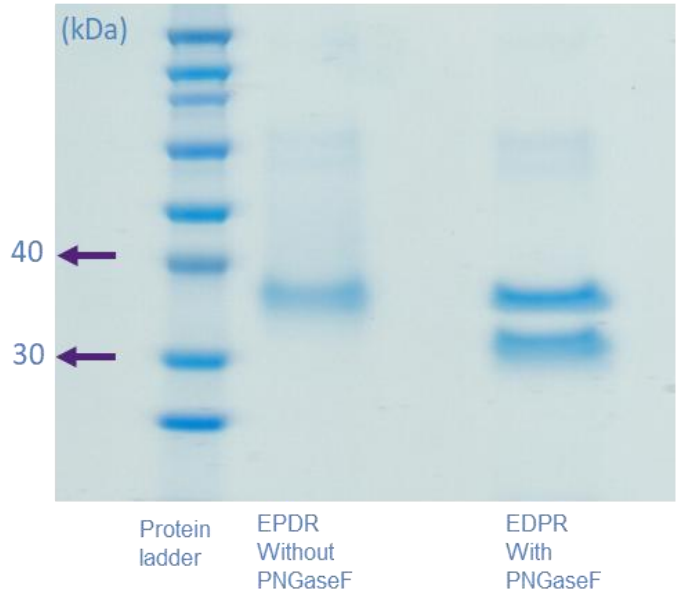

Figure 9. SDS-PAGE purification of recombinant ependymin-related protein (EPDR) GBR.60.100 showing protein ladder based on molecular weight standards, protein band for glycosylated EPDR and deglycosylated EPDR (treated with PNGase F enzyme).

3.2.3 Putative pheromone attractants identified from the COTS spine

COTS spine ultrastructure

SEM images of COTS aboral spines provided a view of the basic ultrastructure of the outer surface of COTS spines with progressive magnifications (**Figure 10A, B**), revealing a topography of craters and pores (2–10 µM in diameter) covering the entirety of the spine. Imaging of a longitudinal sectioned spine revealed the underlying components, including an inner core consisting of a porous 3D meshwork of endoskeleton plates (trabeculae), which aligned longitudinally and parallel to the spine's length, and an outer epithelial layer (**Figure 10C**). This was similarly observed through histological section analysis (**Figure 10D**) and further demonstrated an outer columnar epithelium with a thick basement membrane. A well-defined nucleus was present and granular-like dots could be observed, dispersed throughout the basement membrane.

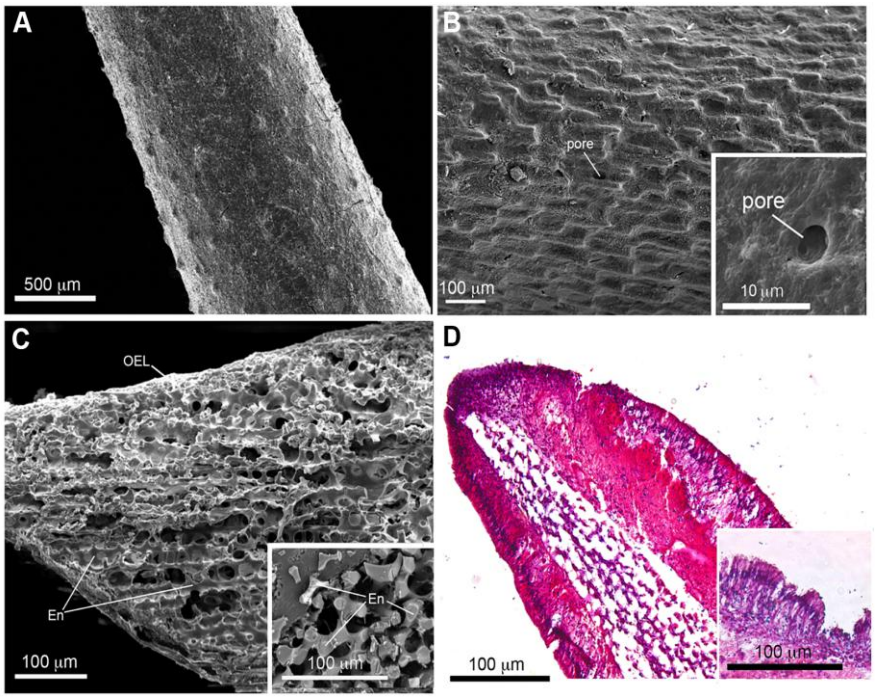

Figure 10. Scanning electron microscopy (SEM) and histological analysis of crown-of-thorns starfish (COTS) aboral spine. **(A)** SEM images showing COTS aboral spine outer surface, and at **(B)** higher magnification. Pores located on the spine surface are indicated. **(C)** SEM image of longitudinally sectioned aboral spine showing the core structure of spine, including endoskeletons (En) and outer epithelial layer (OEL). **(D)** Hematoxylin and eosin (H&E) stain of COTS aboral spine longitudinal section.

COTS secrete high molecular weight spine-derived biomolecules relevant to defence

At 110 µg mL^{-1}, crude COTS spine extract (SpE) induced significantly higher mortality in brine shrimp (52.93±14.63% mean±SD) when compared with the control group (FSW as negative control showed a mortality of 3.48±3.28%) (**Figure 11**), however, dilutions of 1:10 and 1:100 did not. Testing of size fractionated SpEs (< 10 kDa and > 10 kDa), demonstrated that only > 10 kDa at 93.3 µg mL^{-1} induced significant toxicity (51.33±13.13%), comparable to that of crude SpE. The crude stress-conditioned water extract (StWE) induced significant mortality at 107 and 10.7 µg mL^{-1} (88.21±9.64% and 50.08±11.45%, respectively), while size-fractionated > 10 kDa StWE caused significant mortality (81.52±9.81%) at 98.2 µg mL^{-1}. Although < 10 kDa StWE did induce mortality at the highest concentration tested (10.0 µg mL^{-1}), the data showed an unequal variance of error according to Levene's test (Games-Howell post-hoc test was considered insignificantly different to the control group (P= 0.063)). The lethal concentration (LC_{50}) of each extract and the extract toxicity are summarised in **Table 1**. All extracts, except the < 10 kDa SpE, were considered toxic based on Meyer's criterion. Following Clarkson's criterion, the SpE and > 10 kDa SpE exhibited medium toxicity, whereas all StWE treatments were considered highly toxic.

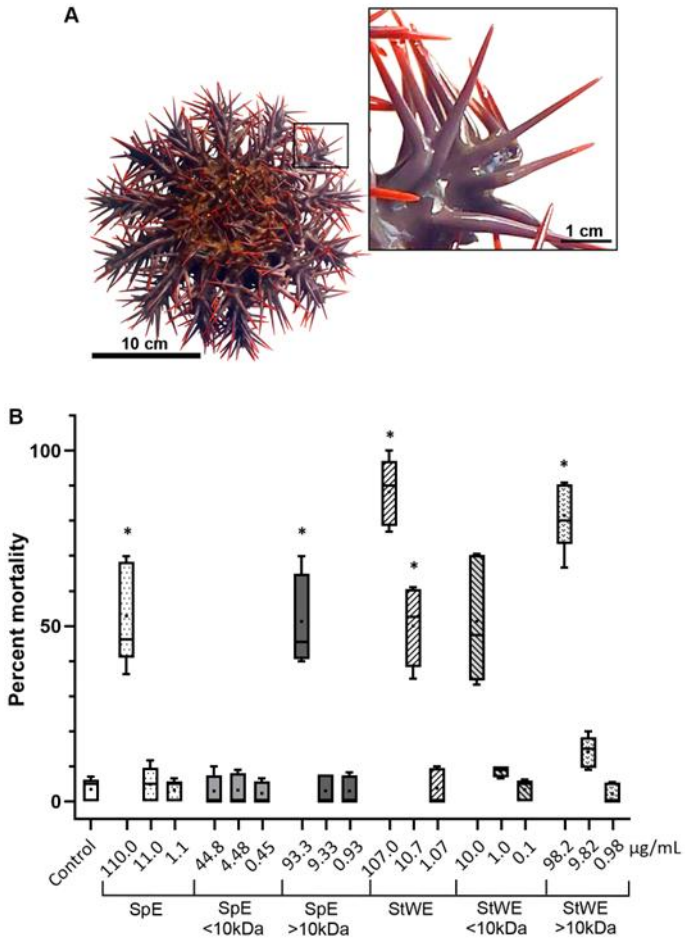

Figure 11. Toxicity of crown-of-thorns starfish (COTS) spine venom extracts based on a brine shrimp (*Artemia salina*) lethality assay. **(A)** Anatomical view of COTS aboral spines. Inset shows the morphology of spines at higher magnification. **(B)** Percent mortality 48 hrs post-exposure was determined for COTS spine extract (SpE), size-fractioned SpE (< 10 kDa and > 10 kDa SpE), stress-conditioned water extract (StWE) and size-fractioned StWE (< 10 kDa and > 10 kDa StWE), as compared to FSW, n = 5 per treatment. Square symbols and horizontal lines within the boxes indicate mean and median values, respectively. Error bars show standard deviation (SD) of data. * indicates significant difference from the control group ($p \leq 0.05$).

Table 1. A summary of the venom extracts prepared from the crown-of-thorns starfish (COTS) and their cytotoxicity based on a brine shrimp lethality assay.

Extract	**LC_{50} (µg mL^{-1})**	**Toxicity class (Meyer's/Clarkson's)**
Spine extract (SpE)	153.65	Toxic/Medium toxic
< 10 kDa SpE	1.43E+23	Non-toxic
> 10 kDa SpE	187	Toxic/Medium toxic
Stress-conditioned seawater extract(StWE)	14.42	Toxic/Highly toxic
< 10 kDa StWE	12.98	Toxic/Highly toxic
> 10 kDa StWE	30.74	Toxic/Highly toxic

Spine proteome analysis reveals proteinaceous toxins

Mass spectral analysis of aboral spine proteins extracted from relaxed (SpRE) and stressed (SpSE) COTS identified a total of 157 protein sequences, i.e., that matched to at least one mass spectral peptide signal in at least one extract. Peptide sequence determination identified 56 different proteins, and GO annotation indicated they were mainly associated with cellular processes, lipid transport, cytoskeleton organisation and modulation of processes in another organism (e.g., induces haemolysis in another organism) (Appendix I Figure A 8). Sixteen of these proteins were consistently identified in spine extracts and were predicted to encode a signal peptide (Appendix I Table A 9). Besides oki.111.24, which was annotated as an uncharacterised protein, other secreted proteins had high confidence matches to known proteins, including EPDRs, plancitoxin-1, phospholipase A2, cysteine-rich secretory protein, pancreatic triacylglycerol lipase-like protein and vitellogenins. A cluster analysis based on gene expression pattern, revealed a distinct group of genes that were highly expressed in the spine (**Figure 12**). Gene description of each gene ID is provided in Appendix I Table A 10.

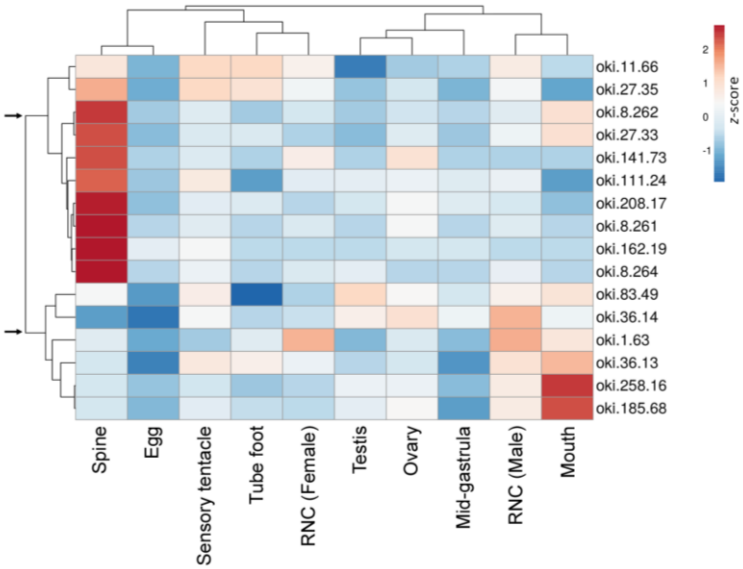

Figure 12. General biological functions of total proteins and gene expression of putative secreted proteins identified in the proteomic analysis of crown-of-thorns starfish (COTS) spines. Heatmap shows relative expression of genes encoding putative secreted proteins in various tissues. Two distinct clusters of genes are observed (arrows).

Three plancitoxin genes were predicted from the OKI COTS genome model, *oki.27.33*, *oki.27.35* (both full length; 356 amino acids) and *oki.27.34* (partial length; 192 amino acids C-terminal region), that encode proteins with deoxyribonuclease (DNase) II domain organisation (**Figure 13**). *Oki.27.33* shared highest similarity to *oki.27.35* and plancitoxin I (84.12%; GenBank accession number: BAD13432). *Oki.27.34* shared ~36–38% similarity to *oki.27.33* and *oki.27.35*. A DNase II domain (domain ID: IPR004947), catalytic domain repeats 1 and 2 (CD09120 and CD09121), and seven conserved cysteine residues were recognised in the full-length COTS plancitoxins (**Figure 13A**). Gene expression of all forms was relatively abundant in the spine, although were also detected in other adult COTS tissues such as tube foot, sensory tentacle, RNC and stomach (**Figure 13B**). Within the sensory tentacle, expression of *oki.27.34* was notably higher than other forms. Only *oki.27.33* and *oki.27.35* were identified from aboral spine protein preparations. Phylogenetic analysis showed that COTS plancitoxins share similarity with plancitoxins reported in other

echinoderms and molluscs yet formed a distinct clade from vertebrate DNase II-alpha proteins and their homologous proteins in nematodes (**Figure 13C**).

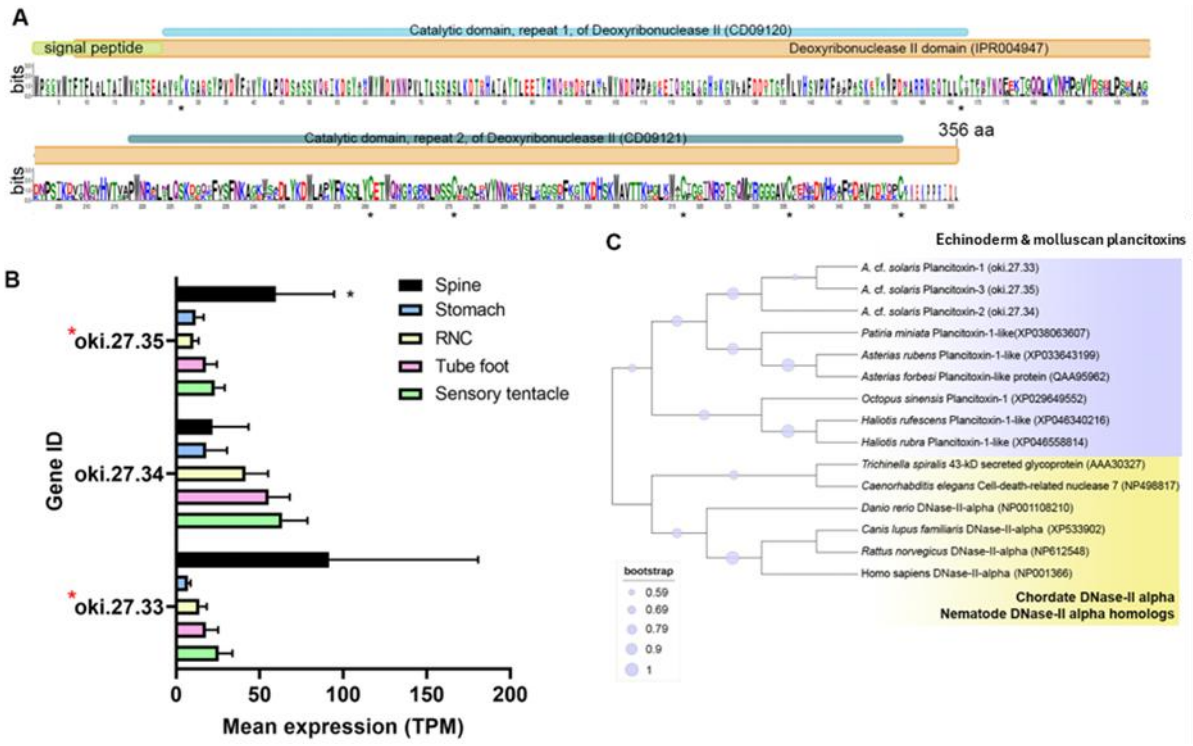

Figure 13. Plancitoxins in the crown-of-thorns starfish (COTS). **(A)** Schematic representation of deduced COTS plancitoxins (oki.27.33, oki.27.34, and oki.27.35). Sequence logo shows the conservation of amino acid sequences among all forms of COTS plancitoxins. Coloured bars at the top of sequence logo display the conserved domains and signal peptide region, and black asterisks show the position of conserved cysteine residues. **(B)** Graph showing relative plancitoxin gene expression (transcripts per million, TPM) in COTS tissues. *, indicates significantly higher ($p < 0.05$) gene expression in spine compared to other tissues. Red asterisks denote those identified in this study by aboral spine protein analysis. **(C)** Phylogenetic tree of COTS plancitoxin proteins with plancitoxin/plancitoxin-like proteins, deoxyribonuclease-2-alpha (DNase-II-alpha) and DNase II-alpha homologs from other species (model, Whelan and Goldman; 1,000 bootstraps). Purple circles on the clades indicate level of bootstrap confidence (≥50%).

The COTS genome contained eight genes encoding secreted-type PLA2 (sPLA2) proteins: *oki.8.260*, *oki.8.261*, *oki.8.262*, *oki.8.264*, *oki.8.268*, *oki.8.269*, *oki.8.270*, and *oki.36.73*. All genes except *oki.36.73* were located on scaffold 8. Deduced proteins from sPLA2 genes on scaffold 8 shared ~50–58% similarity, whereas oki.36.73 showed highest similarity (41.29%) to oki.8.268. All COTS sPLA2 precursors (159–175 amino acids in length) displayed a signal peptide sequence, a PLA2 domain (domain ID: IPR016090), a catalytic PLA2 histidine active site (PROSITE patterns ID: PS00118), and 14 conserved cysteine residues (**Figure 14A)**. The deduced PLA2 from *oki.8.261* and *oki.8.262* shared 98.73% and 100% identity, respectively, to the previously reported COTS PLA2-I and -II (GenBank: BAE46765 and BAE46766) (Ota et al. 2006). Relative gene expression of COTS sPLA2 indicated that the *oki.8.260*, *oki.8.261*, *oki.8.262*, and *oki.8.264* were abundant in the spine, whereas *oki.8.268* was predominant in the sensory tentacle, tube foot, and RNC (both sexes), but also detected in the spine tissue (**Figure 14B**). Gene expression levels of *oki.8.269* and *oki.8.270* were very low or not detected in any tissues investigated, while *oki.36.73* showed moderate expression in stomach tissue. Among all sPLA2 identified, only protein products from

oki.8.261, *oki.8.262*, and *oki.8.264* were detected from COTS aboral spine sample preparations. Phylogenetic tree analysis of COTS sPLA2 proteins with different groups/types of sPLA2 in other species showed that echinoderm sPLA2 (including those of COTS) formed a distinct cluster, which was then rooted with PLA2 groups I (e.g., type IA PLA2 toxin in a cobra and type IB PLA2 in humans) and IX (i.e., PLA2 venom in a marine snail, *Conus magus*) (**Figure 14C**). Of note, oki.36.73 formed a separate root from other proteins and appeared to be distinct from other secreted PLA2 proteins. Another distinct clade (including humans) contained a group of sPLA2 proteins from various species which had been classified as PLA2 groups II, III, V and X.

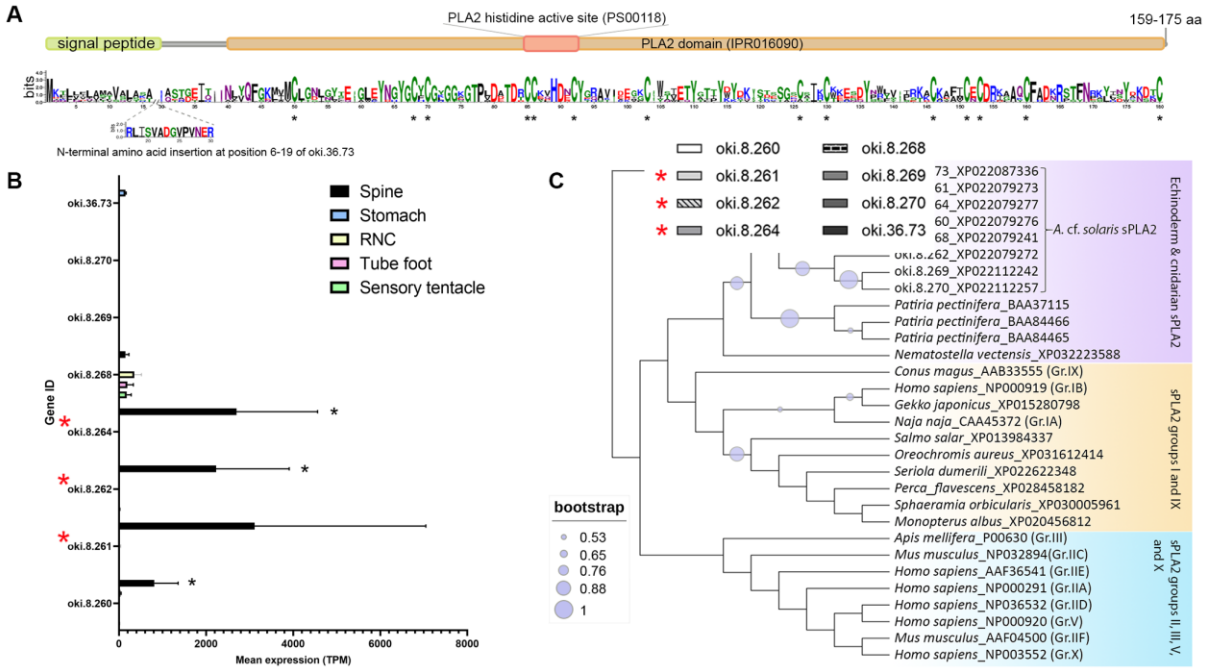

Figure 14. Secreted phospholipase A2 (sPLA2) proteins in the crown-of-thorns starfish (COTS). (**A**) Schematic representation of deduced sPLA2 proteins identified in the COTS genome (gene IDs included). Sequence logo shows an amino acid sequence conservation among all forms. Coloured bars above a sequence logo shows the conserved domains and signal peptide region. An N-terminal insertion for oki.36.73 is shown from positions 6 to 19. Asterisks indicate highly conserved cysteine residues among COTS sPLA2, as well as sPLA2 in other animals. **(B)** Histogram showing relative COTS sPLA2 gene expression in TPM (transcripts per million) in COTS tissues. *, indicates significantly higher ($p < 0.05$) gene expression in spine compared to other tissues. Red asterisks denote those identified in this study by spine protein analysis. **(C)** Phylogenetic tree of COTS and other sPLA2 proteins. sPLA2 from different groups/types are included. The tree was constructed based on maximum likelihood method (model, WAG; frequency=5; 1,000 bootstraps). Purple circles on the clades indicate level of bootstrap confidence (≥ 50%).

Oki.208.17 was annotated as a peptidase inhibitor 16-like protein (PI16), sharing greatest similarity to a PI16-like protein from *Patiria miniata* (accession number: XP038075979; 63.32%identity) and a PI16 found in *Asterias rubens* (accession number: XP033625744; 52.36% identity). The oki.208.17 precursor was 220 amino acids in length and contained a signal peptide, cysteine-rich secretory protein (CRiSP) domain (IPR001283), a venom allergen 5-like domain (IPR002413) and an allergen V5/testis-specific protein (Tpx-1)-related conserved site (IPR018244), corresponding to the 'D_{142}HYTQLVWAKS$_{152}$' motif (**Figure 15A**). Ten cysteine residues were present, eight of which were within the CRiSP domain. Expression of *oki.208.17* was high in the spine tissue (almost 700 TPM), with low expression (< 10 TPM) also detected in other tissues (**Figure 15B**). Phylogenetic tree analysis indicated that COTS oki.208.17 was most closely related to echinoderm PI16s, which formed a sister clade with vertebrate PI16/PI16-like proteins (**Figure 15C**). Snake venom-type CRiSPs and insect venom allergen 5, were more distantly related.

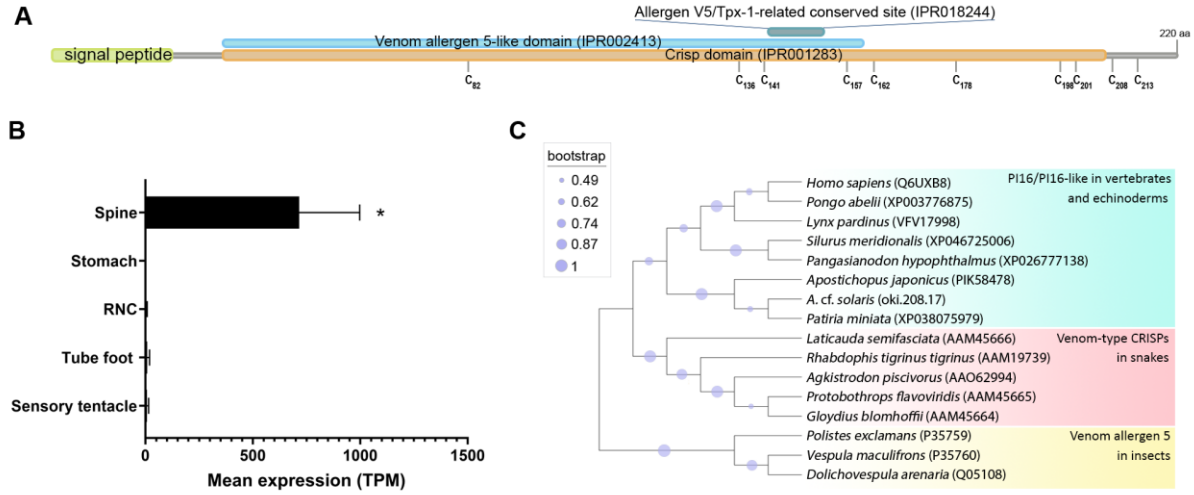

Figure 15. A peptidase inhibitor 16-like protein (PI16) in the crown-of-thorns starfish (COTS). **(A)** Schematic representation of COTS PI16 (oki.208.17). Coloured bars show the conserved domains and signal peptide region. Cysteine residues ('C') and their amino acid positions are indicated. **(B)** Histogram showing relative gene expression (transcripts per million; TPM) of oki.208.17 in COTS tissues. *, indicates significantly higher ($p < 0.05$) gene expression in spine compared to other tissues. **(C)** Phylogenetic tree of PI16s and CRiSPs. Tree was constructed based on maximum likelihood method (model, WAG; frequency=5; 1,000 bootstraps). Purple circles on the clades indicate level of bootstrap confidence (≥45%).

Multiple EPDRs were identified from COTS spine extracts, with gene expression of oki.11.66 and oki.141.73 highest in the spines. Similarly, a pancreatic lipase-related protein (oki.162.19) and an uncharacterised protein (oki.111.24 which is rich in cysteines (n=18) with no match in the NCBI non-redundant protein database or having any recognised domains) were also highly expressed in spines. Although identified from spine extracts, expression of a single DMBT1-like (oki.83.49 which contained a F5/8 Type C domain, a calcium-binding EGF-like domain, and a scavenger receptor Cys-rich domain, which were repeated throughout the entire protein) and kielin/chordin-like proteins (oki.258.16), as well as three Vtg-like proteins (oki.36.13, oki.36.14, and oki.185.68) that contained a 'vitellogenin_N superfamily' and 'VWD superfamily' motifs, was highest in the mouth and sensory tentacles.

Spine differential RNA-seq analysis and annotation reveals non-toxin-like proteins

Comparative differentially expressed gene (DEG) analysis of spines from non-reproductive and reproductive COTS found that 686 genes were up-regulated in the reproductive state, while 228 genes were up-regulated in the non-reproductive state (**Figure 16**).

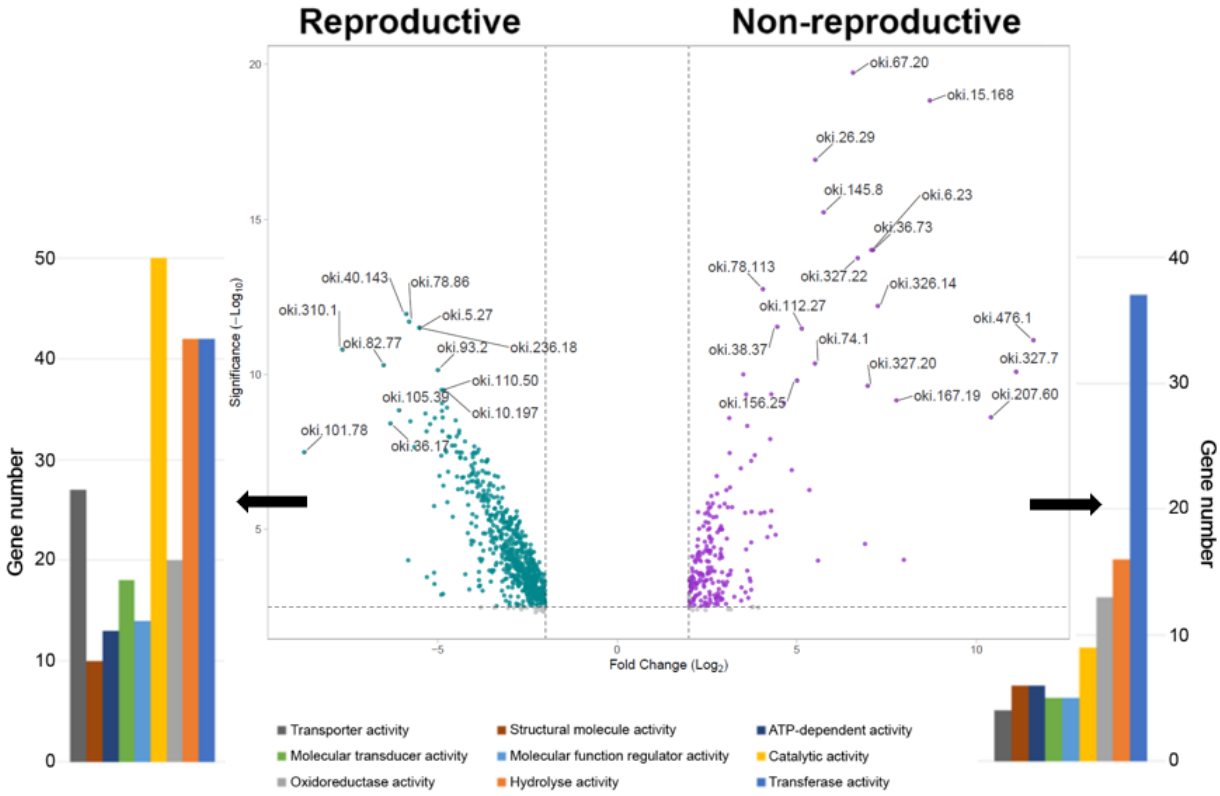

Figure 16. Differential gene expression of crown-of-thorns starfish spine tissue in the reproductive (n=686) and non-reproductive state (n=228). Volcano plot shows significant (sig. $-\text{Log}^{10} > 2.2$; Log^2 FC -1.8–1.8) differentially expressed genes (30 top hits labelled with oki. identifier), during reproductive and non-reproductive states, and graphs showing associated biological function GO annotation.

Of the 686 genes up-regulated in reproductive stage COTS spines, those associated with catalytic, hydrolyse and transferase activity were most abundant. Further investigation found that the most highly up-regulated genes encoded for enzymes (e.g., cathepsin L1 and cathepsin B), defence proteins (e.g., cystatin-A, bactericidal permeability-increasing protein, DMBT1-like) and other proteins involved in signalling processes. Of those involved in signalling processes, 56 were receptors, represented by adhesion GPCRs, angiopoietin-1 receptors, glutamate receptors, low-density lipoprotein receptors, metabotropic glutamate receptors and tyrosine kinase receptors (Appendix J **Table A 11**). Glutamate receptors demonstrated the highest level of expression in spines of reproductive COTS. Also identified were 64 genes exclusively expressed in reproductive state spines (i.e., < 5 TPM in non-reproductive stage spines); those with the highest fold-change (> 100 TPM) included genes encoding protocadherin Fat 4, CD226 antigen, macrophage-expressed gene 1 and HRAS-like suppressor 3.

Of the 228 genes up-regulated in spines of non-reproductive COTS, the majority had predicted functions related to transferase activity; far fewer were associated with hydrolyse and catalytic activity. The most highly up-regulated gene expression was observed for those

predicted to be enzymes (e.g., prostaglandin reductase 1, cholinesterase 1) or structural (e.g., perlucin, SpP19), the remaining being novel. Thirty-three genes were also identified that showed exclusive expression within non-reproductive spines (i.e., < 5 TPM in reproductive stage spines); those with the highest fold-change (> 100 TPM) included genes encoding a coiled-coil domain-containing protein 3, flavin-containing monoamine oxidase A, ATP-dependent RNA helicase DHX40 and two novel proteins (*oki.327.20, oki.327.22*). These two novel proteins were predicted to be secreted based on the presence of a signal peptide, along with 16 other non-reproductive state spine proteins.

The *oki.327.20* and *oki.327.22* genes matched closely to five additional genes annotated in the COTS genome (and located in close genome proximity: oki.327.14, *oki.327.15*, *oki.327.17*, *oki.327.18* and *oki.327.20*). These did not match with any other sequences present in the NCBI database, suggesting genus or even species specificity. Upon sequence alignment, the highest level of similarity was found within the N-terminal predicted signal peptide region, as well as several residues dispersed throughout the length of the precursor proteins (**Figure 17A**); most notably E28, E35, R/K40, R47, D65, R70, V74 and A82 (based on *oki.327.14*). While expression of both *oki.327.20* and *oki.327.22* was significantly up-regulated during the non-reproductive state, other protein family members showed relatively high levels of expression during both non-reproductive and reproductive states (**Figure 17B, C**). Given that gene expression appeared to be primarily confined to the spines, they are termed COTS spine-secreted proteins (SSPs).

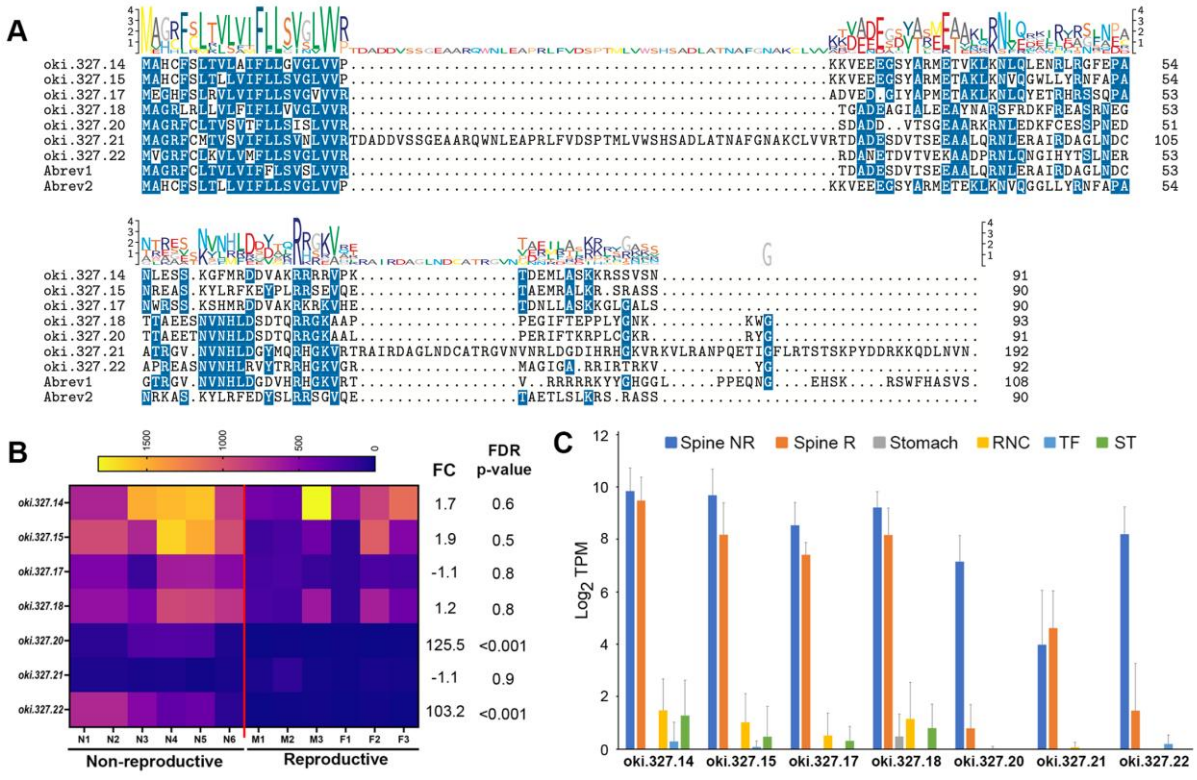

Figure 17. Molecular analysis of a novel family of secreted crown-of-thorns starfish (COTS) proteins associated with spine tissues. **(A)** Multiple sequence alignment of the seven novel COTS spine proteins, and two *Acanthaster brevispinus* homologs. **(B)** Heatmap showing relative gene expression (TPM) in spine tissue in the non-reproductive and reproductive states, including fold-change (FC) and false discovery rate (FDR) *P*-value. **(C)** Graph comparing gene expression across various COTS tissues: spine non-reproductive (*NR*), spine reproductive (*R*), stomach, radial nerve cord (*RNC*), tube foot (*TF*) and sensory tentacle (*ST*).

The *oki.327.14* had previously been identified from COTS-conditioned water following exposure to its predator, the giant triton snail, *Charonia tritonis* (Hall et al. 2017). Here, three different COTS SSPs were identified, with high confidence, in COTS-conditioned water during the non-reproductive season, *oki.327.14, oki.327.15* and *oki.327.18* (**Figure 18**). Each contain dibasic (and *oki.327.14* a pentabasic) residues that could be cleaved by prohormone convertases prior to secretion. Results here further support findings reported in (Jönsson et al. 2022; Morin et al. 2024).

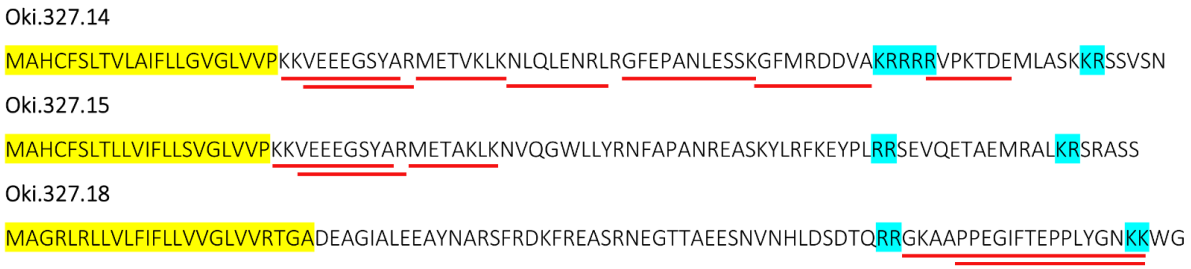

Figure 18. Amino acid sequences of crown-of-thorns starfish (COTS) spine-specific proteins identified from non-reproductive COTS-conditioned water. Sequences highlighted in yellow indicate predicted signal peptide; those in blue indicate predicted cleavage sites; and those underlined in red indicate peptides detected through mass spectral analysis.

3.3 Behavioural and functional testing of promising leads

3.3.1 Validation and performance of the whole animal flume system

A two-current choice laminar flume system was constructed (Appendix E **Figure A 3**). Initial dye tests of the flume system established the operational system parameters needed to produce stable, parallel flow lanes for COTS behaviour assays (Error! Reference source not found.; **Table 2**; Appendix F **Figure A 5**). In the presence of GlyB, the system's performance was validated: individuals displayed nearly a two-fold increase in distance moved (3036.2±757.0 vs. 1515.9±757.0 cm for control; $p < 0.01$), and meandering (2366.1±1173.5 vs. 1165.9±744.2 degrees for control; $p < 0.05$) as compared to controls (**Figure 20**). There was also a trend towards more frequent entries into the GlyB plume (5.7±2.2 vs. 3.1±2.8 frequency for control, $p > 0.05$), although this difference was not statistically significant. Collectively, these metrics indicate a change in behaviour from controls, demonstrating increasing rates of movement and time spent in the highest concentration of the cue, patterns consistent with that expected of a foraging animal (Hall et al. 2017). This flume assay provides the most realistic assessment of COTS responses in a laboratory setting, allowing free unrestricted movement and is an essential tool for the development and assessment of COTS attractants.

Figure 19. Image of the two-current choice laminar flume testing arena showing the trajectory of the laminar flow.

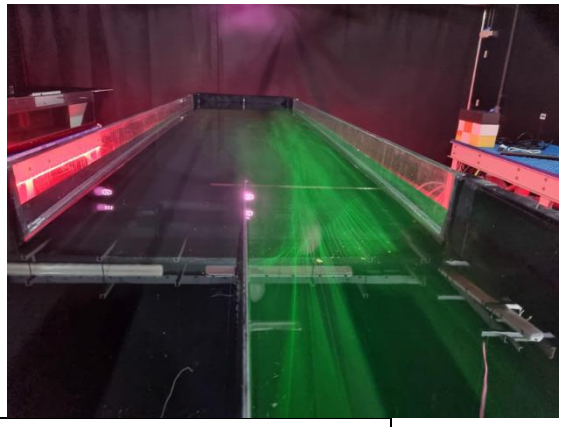

Table 2. AIMS SeaSim Behavioural flume system.

Experimental parameter	Optimised value
Flume dimensions	Arena total length: 6 m Arena total width: 1 m
	Active zone total length: 3 m Active zone total width: 1 m
	Flume 'arm' length: 3 m Flume 'arm' width: 0.5 m
Baffle dimensions	Total height: 0.30 m Total thickness: 5 mm Total width (as per arena width): 1 m Position from proximal wall: 0.6, 0.7, 0.8 m Aperture of individual openings: 5 mm
Collimator dimensions	Total height: 0.30 m Total thickness: 0.1 m Total width (as per arena width): 1 m Total length: 0.5 m Position from proximal wall: 1.3 m Aperture of individual openings: 4 mm (circular)
Water delivery	Flow rate: 25 mL min^{-1} per arm Total volume per 3-hour exposure: 4.5 L
Cue delivery – peristaltic pump	Flow rate: 2.7 mL min^{-1}

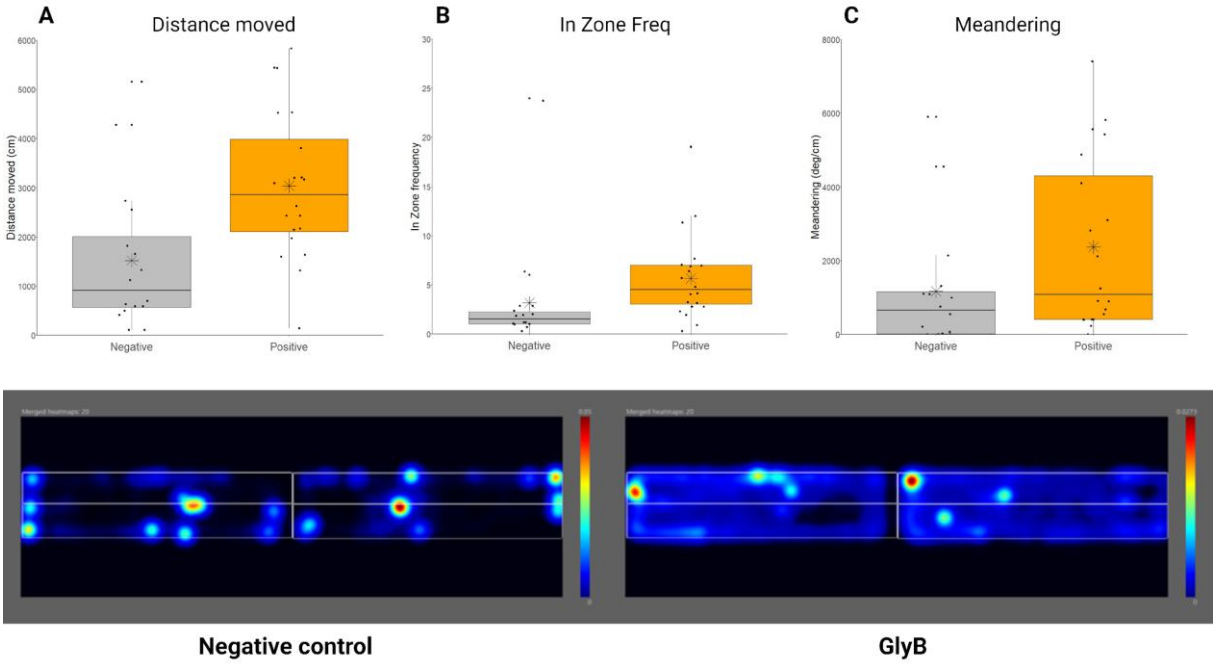

Figure 20. Movement of crown-of-thorns starfish (COTS) in response to glycine betaine (GlyB). Graphs (GlyB positive = orange, n=20; negative control = grey, n=20) show **A)** an increase in distance moved (p = 0.0004), **B)** increased time spent in the cue plume (p = 0.06) and **C)** increased meandering (p = 0.03). Dots indicate individual data points. The centre line is the median; the box shows the interquartile range (IQR); whiskers extend to 1.5×IQR; ✳ indicates the mean; points outside the whiskers are outliers. Heatmaps allow visualisation of increased movement in response to controls (filtered seawater; left panel) and GlyB (right panel); warmer colours indicate higher occupancy (blue→red scale). Vertical white lines denote zone boundaries used for in-zone calculations.

3.3.2 TFF-derived semiochemicals from COTS-conditioned water elicit a response in COTS

TFF-derived high molecular weight COTS fraction modifies COTS behaviour

Four endpoints were measured to interpret the response of COTS to TFF fractions: direction, lane preference, meandering and mobility (

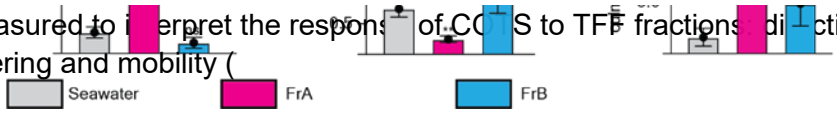

Figure 21). COTS exposed to summer 2020 FrA displayed attraction behaviour by moving with clear intent to locate the source of the cue (17.54±0.12 degrees vs. -22.15±0.07 for Fr B and -10.75±0.07 for control). In addition, COTS entered the treatment arm with higher frequency (3.92±1.55 frequency) compared to those exposed to FrB or seawater only (0.30±0.15 and 0.59±0.16 frequency, respectively). FrA also evoked a 65-73% reduction in meandering (249.10±51.89 degrees cm^{-1}) compared to FrB and seawater only (712.30±170.1 and 932.80±152.0 degrees cm^{-1}, respectively) and a ~6-fold increase in mobility (6.18±2.87 mins vs. 2.85±1.27 for Fr B and 0.94±0.51 for control). Collectively, the data show FrA consists of biomolecules capable of modifying COTS behaviour, with attractant properties.

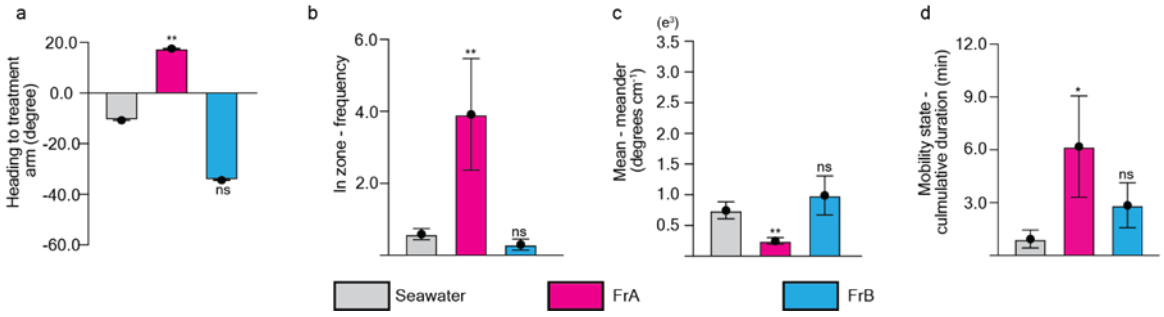

Figure 21. Demonstration of behavioural response of crown-of-thorns starfish (COTS) to COTS-conditioned seawater fractions showing the > 30 kDa fraction (FrA; pink) **(a)** motivates COTS to move towards the cue and **(b)** spend more time (i.e., higher frequency) in the cue plume with **(c)** minimal meandering but **(d)** heightened mobility. Error bars indicate standard error of the mean; ns = not significant, p > 0.05; *p <0.05; **p < 0.01, unpaired t-test.

COTS secretome exhibits seasonal variation

In subsequent behavioural assays using the flume system, the seasonality difference between summer 2023 FrA and winter 2023 FrA was evaluated, with seawater as control. Again, two endpoints were quantified: meandering and lane preference. For meandering, the summer 2023 FrA evoked minimal meandering (475.7±190.20 degrees cm^{-1}; $p < 0.05$), consistent with that observed above for the summer 2020 FrA, indicating a directional bias. In contrast, a higher level of meandering was observed when exposed to the winter 2023 FrA (1132.0±213.9 degrees cm^{-1}; not significant, $p > 0.05$), the response comparable with the seawater only control (1244±452.4 degrees cm^{-1}) (**Figure 22**) and indicating more exploratory, non-directed movement. For lane preference, COTS showed a clear attraction to the 2023 summer FrA, i.e., a significant preference to enter and re-enter the treatment lane (0.17±0.13 frequency; $p < 0.01$). No lane preference was observed for the winter FrA or seawater control (-0.08±0.08 and -0.11±0.09 frequency, respectively; not significant, $p > 0.05$). Taken together, these results confirm that there is a seasonal difference, with the summer FrA acting as a directional cue (low meandering, significant lane choice).

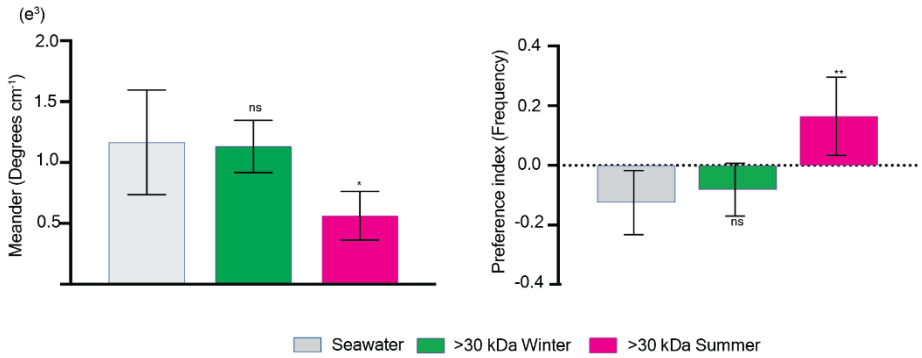

Figure 22. Demonstration of seasonal behavioural response of crown-of-thorns starfish (COTS) to summer and winter seawater fractions > 30 kDa showing the summer fraction reduces the meandering (left) and attracts COTS to the cue plume (right). Error bars indicate standard error of the mean; ns = not significant, p > 0.05; *p <0.05; **p < 0.01, unpaired t-test.

TFF-derived high molecular weight COTS fractions do not display toxicity in vitro

At the highest concentration tested (neat) the active TFF summer fraction FrA did not induce any mortality in brine shrimp. Positive TFA controls induced 100% mortality.

3.3.3 Recombinant GBR.60.100 does not elicit a response in COTS

Exposure of COTS to the recombinant COTS EPDR GBR.60.100 did not evoke any significant movement of COTS towards the stimulus as compared to the control. COTS exposed to recombinant GBR.60.100 spent equal time in each arm of the large-scale Y-maze. No significant difference was observed in the mean distance moved or velocity or mobility between controls and COTS exposed to the recombinant GBR.60.100.

3.3.4 COTS spine-secreted proteins elicit a response in COTS

Based on mass spectrometry and tertiary models, each of *oki.327.14, oki.327.15, oki.327.17* and *oki.327.18* had predicted cleavage sites. This provided an avenue for the synthesis of peptides (**Table 3**) and functional assays.

Testing of the cocktail of synthetic peptides derived from COTS SSPs at 100 pM, 100 mM and 10 mM over 48 hrs did not indicate toxicity against brine shrimp as compared to FSW control (P = 0.727; ≥ 0.05) (**Figure 23**). Exposure of COTS to the cocktail in the mid-scale Y-maze assay evoked significant movement of COTS towards the stimuli (one-tailed P = 0.0364), measured as a function of mean time spent in each arm; COTS exposed to FSW controls spent equal time in each arm (
Figure 24A, B) whereas those exposed to the cocktail spent > 70% in the stimulus arm (
Figure 24C, D). No significant difference was observed in the mean distance moved between controls and the cocktail, however, a significant difference in the velocity with which COTS moved (P = 0.0257; independent sample-*t* two-tailed) was noted.

Table 3. List of peptide sequences synthesised, including gene ID, amino acid sequence and molecular weight (MW) in Daltons (Da).

Gene ID	Amino acid sequence	MW (Da)
***oki.327.14*-A**	KKVEEEGSYARMETVKLKNLQLENRLRGFEPANLESSKGFMRDDVA	5315.02
***oki.327.14*-B**	VPKTDEMLASK	1218.43
***oki.327.15*-A**	KKVEEEGSYARMETAKLKNVQGWLLYRNFAPANREASKYLRFKEYPL	5624.46
***oki.327.15*-B**	SEVQETAEMRAL	1363.51
***oki.327.17*-A**	DVEDGIYAPMETAKLKNLQYETRHRSSQPANWRSSKSHMRDDVA	5119.64
***oki.327.18*-A**	DEAGIALEEAYNARSFRDKFREASRNEGTTAEESNVNHLDSDTQ	4945.13

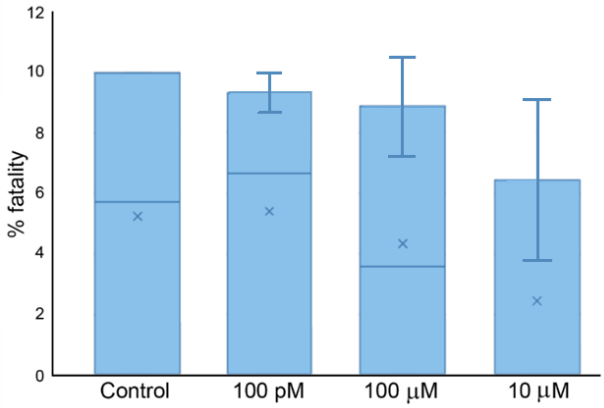

Figure 23. Toxicity of the synthetic crown-of-thorns starfish spine-secreted protein cocktail against brine shrimp at 48 hr post-exposure. Error bars are representative of 95% confidence intervals; × indicates the mean. Control was filtered seawater.

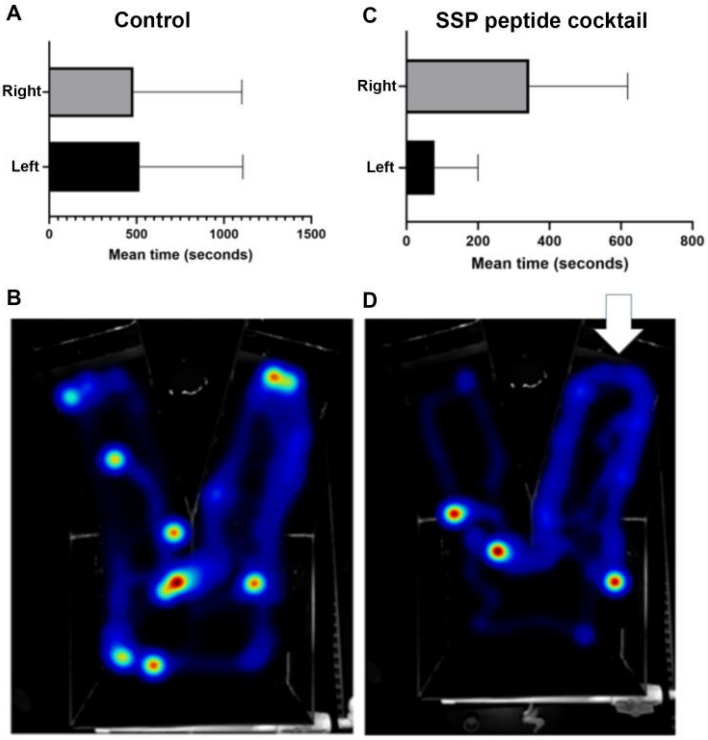

Figure 24. Mid-scale Y-maze behaviour assay assessing the effect of the synthetic crown-of-thorns starfish (COTS) spine-secreted protein (SSP) cocktail on adult COTS. (**A**) Mean time spent in the right versus left arm for control (filtered seawater delivered via both Y-arms), and (**B**) overlay heatmap image (n = 5). (**C**) Mean time spent in the right versus left arm; SSP peptide cocktail (1 nM) delivered to the right arm, FSW to the left arm, and (**D**) overlay heatmap image (n = 5). Significant (*P* = 0.043) preference exists in the sample population exposed to the SSP stimuli. For heatmaps, warmer colours indicate higher occupancy (blue→red scale).

3.3.5 Synthetic COTS RGP induces spawning in COTS and *Acanthaster brevispinus*

Synthetic A- and B-chains of the predicted COTS RGP were synthesised and chemically linked, via disulfide cross-linkages, to produce a heterodimer RGP (MW 4.78 kDa) (Wu et al. 2024), verified by MS/MS.

In vitro *synthetic RGP assay: germinal vesicle breakdown (GVBD)*

No GVBD was observed following treatment of oocytes with Milli-Q water (**Figure 25**). All (100%) freshly excised female gonads incubated with 1 µM synthetic RGP underwent GVBD within 1 hr post-treatment. For 1-MeAde, both 1 and 10 mM were equally effective.

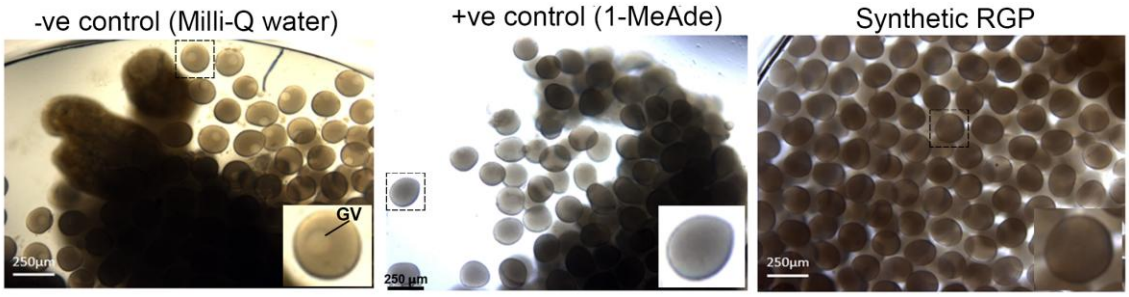

Figure 25. Oocytes from freshly excised crown-of-thorns starfish ovary fragments. Germinal vesicle (GV) breakdown was assessed by presence and absence of GV at 1 hr post-treatment with Milli-Q water (left panel), 1-methyladenine (1-MeAde; middle panel) and 1 µM synthetic COTS relaxin-like gonad-stimulating peptide (RGP; right panel). Inset images show digital magnifications of boxed oocytes. -ve, negative control; +ve, positive control.

In vivo *COTS assay: spawning induction*

In negative Milli-Q water control treatments (n = 4), no behaviour changes or spawning were observed 3 hrs post-injection. In positive 1-MeAde control treatments, 100 µL did not induce spawning (**Figure 26A,** n = 4), while 300 µL induced spawning behaviours (i.e., arching) and gamete release (n = 2) at 55- and 59-mins post-injection. All COTS injected with synthetic RGP (n = 13) spawned within 1 hr post-injection. Spawning induction times differed significantly between the sexes (independent sample t = 2.162; df=13; two-tailed P = 0.0499): male = 19.88 mins, female = 33.00 mins; 95% CI: 0.009109 to 26.24; difference between means: 13.13±6.071 mins) (**Figure 26B**). RGP-spawned eggs derived from three injected females were fertilised with RGP-spawned sperm from one injected male, resulting in a > 95% fertilisation rate, with early phase of embryonic development observed 3 hrs post-fertilisation (**Figure 26C**). Negative controls and non-reproductive COTS (i.e., out of season) did not exhibit spawning-like behaviour or release gametes during these trials.

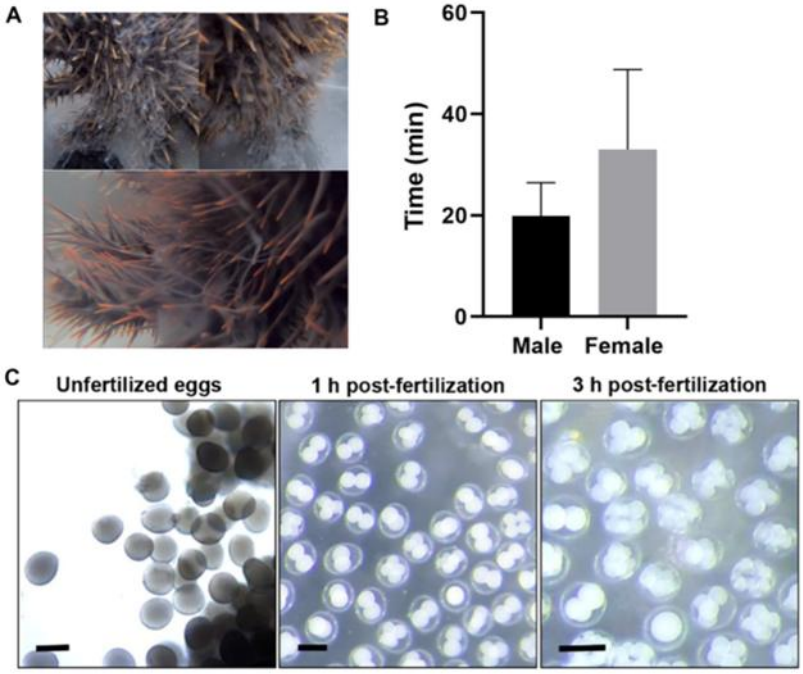

Figure 26. Spawning induction in crown-of-thorns starfish (COTS) using the synthetic COTS relaxin-like gonad-stimulating peptide (RGP). **(A)** Spawning (i.e., gamete release) was evident by the water changing from clear to opaque. **(B)** Histogram shows the spawning induction time for male (n = 7) and female (n = 8) COTS following administration of 1 µM synthetic COTS RGP. **(C)** Representative sequential images taken of unfertilised eggs, then 1 and 3 hrs post-fertilisation. Scale bar represents 200 µm.

In vivo Acanthaster brevispinus *assay: spawning induction*

Intra-coelomic injection with 1 µM COTS-derived synthetic RGP induced spawning in the two female *A. brevispinus* (**Figure 27**), with an average induction time of 40.50±10.61 mins. Note that as only two *A. brevispinus* were available, neither were subjected to injection with Milli-Q water or 1-MeAde.

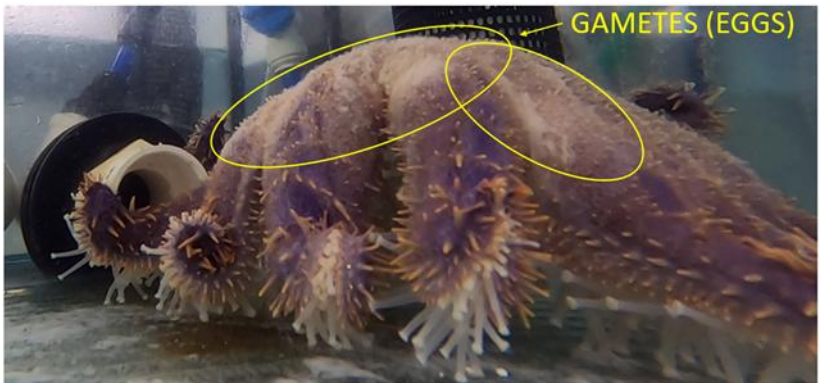

Figure 27. Female *Acanthaster brevispinus* (~35 cm in diameter) was induced to spawn by injection with 1 µM synthetic crown-of-thorns starfish relaxin-like gonad-stimulating peptide. Arching behaviour, as seen here, is typical before spawning.

3.4 Recombinant expression of COTS receptors: preliminary experiments on the betaine receptor

Genomic and transcriptomic analyses of COTS have consistently highlighted elevated expression of chemoreceptor candidates likely responsible for their response to semiochemical stimuli. In development of pest control agents (such as in insecticidal design), expression of these target receptors and development of targeted activity assays is essential. Here, betaine-like receptors were investigated based on relevant literature from homologous organisms (Peden et al. 2013; Hardege et al. 2022) and because betaine elicits a robust attractant response in COTS behavioural assays (section 3.3.1), thus making the betaine receptor an excellent example for method optimisation until bona fide COTS ligand:receptor interactions are validated in the near future. While betaine does not activate a COTS-specific response, molecular functionalisation of betaine could produce a COTS-specific analogue. This goal would also require expression of the receptor to understand molecular and structural differences to related species and other marine organisms.

From a rapid review of the literature, studies on the model organism *Caenorhabditis elegans* revealed betaines act on ligand-gated ion channels (Peden et al. 2013; Hansen et al. 2022; Hardege et al. 2022). Interrogation of the COTS genome identified three genes that were up-regulated in COTS tube feet (GO:0015276/GO:0022834; GO:0005230) and RNC (GO:0048018) and which have homology to betaine receptors in *C. elegans*. Based on this information, a workflow to express these putative betaine receptors in a functional format was devised using standard insect cell lines, specifically, *Spodoptera frugiperda* (SF9) and *Trichoplusia ni* (High FiveTM) (Grisshammer and Tateu 1995). The method for transferring the gene encoding for the betaine receptors into a bacmid (a shuttle vector based on a functional fertility plasmid, or F-plasmid, that can be propagated in insect cells) is currently under development at UQ.

4 DISCUSSION AND OUTPUTS

On the GBR, the GBRMPA COTS Control Program monitors and removes, via single injection culling, adult COTS from economically or ecologically important reefs. On targeted reefs in the Townsville region, the program has achieved a six-fold reduction in starfish numbers and a 44% increase in coral cover (Matthews et al. 2024). Even with this success, the resources required remain significant and new approaches are desperately needed to control future COTS outbreaks and conserve coral cover and impart resilience. The use of species-specific semiochemicals to modify COTS behaviour is showing great promise (Hall et al. 2017; Motti et al. 2022; Harris et al. 2025a; Harris et al. 2025b) with outcomes of this study providing further supporting evidence.

4.1 COTS multi-omics resources

A refined genome, extensive tissue- and stage-specific transcriptomes, proteomes and a lipidome, glycome and metabolome have now been generated for COTS. Sequencing the genome of the closely related short-spined sibling species *A. brevispinus* has proven challenging, with method development and optimisation ongoing.

4.1.1 Association of transcriptomics profiles with COTS tissue type and stage

Associated with this project, an additional 175 high-quality transcriptomes generated from wild COTS tissues and organs, as well as six from COTS spines and three from *A. brevispinus*, were collected. Seasonal (section 3.3.2) and physiological differences between reproductive and non-reproductive COTS and the sexes were identified ((Jönsson et al. 2022; Morin et al. 2024); section 3.2.3), as well as the physiological impacts of being translocated to captivity (Morin et al. 2023). The raw RNA sequences generated in this study have been made publicly available in the NCBI Sequence Read Archive (SRA) under BioProjects PRJNA821257, PRJNA901199, PRJNA548418 and PRJNA16358. These new GBR v1.1 gene models and transcriptomes (RNA-seq tracks) can be visualised at: Degnan Marine - Crown of thorns starfish | Apollo Portal (genome.edu.au). Combined with broader availability of other -omic technologies, in depth exploration of the genome and these additional transcriptomes has aided the identification of lead pheromone attractants.

Information from the assembled transcriptomes, considered along with previously published research from CCIP-R-11 project members (Appendix D **Table A 3**), has provided useful insight for the development of a semiochemical control technology and the parameters that will guide operational deployment. For example, questions such as ‘When is the best time to deploy a semiochemical?’ can draw on the knowledge that COTS exhibit seasonal variation in their chemical profiles that coincides with seasonal behaviours, i.e., they consume significantly greater quantities of coral in summer months immediately before spawning. This knowledge can inform and advise on the strategic deployment of the semiochemical technology in combination with culling in late winter/early spring to limit impact to corals.

4.1.2 Association of baseline metabolomic profiles with COTS tissue type

LC-MS metabolomic analyses established baseline metabolite and lipid profiles in a range of COTS body tissues and eggs (Mendoza-Porras et al. 2023). The metabolite and lipid profiles of the pyloric caeca, RNC and gonads differed markedly from those of other tissues, with many compounds present in substantially higher concentrations.

The nucleoside, inosine was the most abundant metabolite in COTS tissues, and highest in the gonads. Inosine is involved in purine biosynthesis (i.e., de novo synthesis of adenosine and guanosine), gene translation, and modulation of the fate of RNAs (Srinivasan et al. 2021), and in the polychaete *Nereis succinea*, is a component of a pheromone complex involved in spawning (Zeeck et al. 1998). Its high abundance in COTS gonads suggests a similar reproductive role. Further functional assessment of inosine is needed to confirm a role in COTS behaviour.

In COTS eggs, 2-hexyldecanoic acid was the most abundant metabolite. Along with inosine and L-glutamic acid (also in high abundance in both COTS gonads and eggs), it is a co-component of the *N. succinea* pheromone. The presence of these metabolites in COTS gonads and eggs suggests a possible synergistic relationship whereby they act as a pheromone complex to trigger COTS spawning. A comparative study of naturally spawned eggs would assist in identifying key metabolites underpinning spawning in female COTS in the field (Wang et al. 2024).

4.2 Potential of COTS-derived semiochemicals as control agents

Here, the knowledge gained through associated genomic, transcriptomic, proteomic and metabolomic investigations, in parallel with *in vivo* behavioural analyses, aided the identification of lead pheromone attractants that influence COTS movements. Based on this information a database of potential attractant semiochemicals has been compiled (**Table 4**) and efforts are ongoing to fully characterise and test these *in vitro*, *ex vivo*, and *in vivo*.

Table 4. Potential COTS-derived pheromone attractants. EPDR = Ependymin-related proteins; RGP = Relaxin-like gonad-stimulating protein; SSP = Spine-Secreted Proteins; TFF = Tangential flow filtration; *in vivo* = tested in whole animal behavioural assay; *in vitro* = tested on animal tissues; *ex vivo* = tested on live animal tissue; NT = not tested.

Semiochemical	State	Method of identification	Status
TFF summer Fraction A > 30 kDa	Crude fraction	TFF; Proteomic knowledge; *in vivo*	Active; repeatable
EPDR GBR.60.100	Recombinant pure peptide	Prior genomic knowledge (Hall et al. 2017); *in vivo*	Inconclusive
Inosine, 2-hexyldecanoic acid and L-glutamic acid	Detected but not isolated	Metabolomic knowledge	NT
Synthetic COTS SSP	Synthetic pure peptides	Transcriptomic and proteomic knowledge; *in vivo*	Active; repeatable
Synthetic COTS RGP	Synthetic pure peptide	Prior knowledge *in vitro* (Smith et al. 2019; Mita et al. 2022)	Active *ex vivo*; repeatable

4.2.1 Sequestering active semiochemicals from COTS-conditioned seawater to overcome the processing obstacle

Here, the application of an optimised TFF method to harvest, concentrate, and fractionate COTS-secreted attractants from large volumes of COTS-conditioned seawater proved successful. It efficiently reduced the volume of COTS-conditioned seawater by 1000-fold and yielded fractions in sufficient quantities that allowed for multiple Y-maze behavioural assays and with the potential to achieve viable volumes for technology development and deployment at reef scale. Quantitative behavioural analysis established the > 30 kDa summer FrA, collected over two summers, as having COTS attractant properties, proving the TFF method to be both repeatable and reproducible. The summer FrA elicited the lowest meandering value, suggesting naïve sedentary COTS followed the gradient plume with greater focus and intention; the raised mobility state also indicates heightened motivation to locate the source.

This bioprocessing approach reduced the putative lead COTS-attractants from 108 (Hall et al. 2017) to 19 and implicated two COTS-specific EPDRs in COTS communication, consistent with a previous finding that EPDRs have evolved into species-specific communication biomolecules (Hall et al. 2017). Hence, the presence of COTS-specific EPDRs could confer FrA its COTS attracting properties, validating the TFF approach for isolating COTS attractants. In parallel, the testing of the recombinant GBR.60.100 EPDR, identified through genome mining, did not evoke a change in COTS behaviour. Recombinant expression of the two candidate EPDRs should be undertaken to assess their bioactivity and allow for further comparative functional studies against recombinant GBR.60.100.

Traditional approaches to develop semiochemicals routinely apply further chromatographic purification to yield the individual biomolecules, yet the isolation and testing of pure biomolecules may not be necessary. Recently, crude extracts prepared from natural sources have been commercialised as baits for semiochemical control of invasive cane toads (BufoTabs; (CTC 2018)) and as bio-pesticides for crop protection (Sero-X; Research and Supply Permit: PER93075; NEW 2023: Research and Supply Permit: PER93075 (APVMA 2016)). The scalable TFF method, which is the same as that used to produce Sero-X, will allow for more extensive field testing of summer FrA, and, if successful, could pave a way for its use to control COTS on a regional scale. Similarly, the slow release of female cane toad toxin from BufoTabs is an exemplar of how a semiochemical might be deployed on the reef, having an effective lifetime of 24 hrs.

Raw material supply is often a limiting factor in the production of semiochemical extracts and biomolecule purification (Immaraju 1998). With COTS numbers predicted to be in the tens of millions on the GBR despite intensive culling efforts, raw material is not limited, rather, the limiting factor is the logistics required to perform TFF at scale *in situ*, which must be considered in any upscaling of this approach. To secure reliable, raw material for TFF at scale, a structured supply partnership, supported by GBRMPA permits, could see licensed on-water operators and Traditional Owner ranger groups supply COTS on a fee-for-service basis to defined QC specifications (i.e., established collection, handling and delivery Standard Operating Procedures). In parallel, vessel capacity, COTS holding, on-water or near-shore TFF processing, Occupational Health and Safety (including venom-handling), waste management, and sample traceability systems would need to be developed. Such an approach would also need to incorporate safeguards for reef values, including site-selection

rules, cultural approvals, low-impact methods, supply-chain and QC standards, auditable sample and waste handling, and the establishment of a culturally appropriate benefit stream in sea Country.

Producing an active extract is considered the most promising direction for further investigation, not only because of the issues discussed above regarding source material and upscaling, but also because it is likely that there is not one single component that is active. In *Petromyzon marinus*, females are attracted to males when the two compounds, 3-keto petromyzonol sulfate (3kPZS) and petromyzonol sulfate (PZS), are present at certain ratios, however, when petromyzonol tetrasulfate (3sPZS) is present, female attraction is disrupted resulting in a 97% reduction in spawning success (Scott et al. 2023). For *Aplysia*, attractin is secreted to attract a mate, yet when in combination with any of three other molecules also secreted, enticin, seducin and temptin, is more attractive (Cummins et al. 2004; Cummins et al. 2005; Cummins et al. 2007b; Cummins et al. 2007a). More thorough chemical profiling of the COTS secretome is needed to reveal whether synergistic effects are playing a role in semiochemical communication and, if so, whether the ratio of active components can be manipulated to modify and/or disrupt COTS behaviour.

4.2.2 COTS spine-secreted proteins as COTS control agents

Synthetic COTS SSP-inspired peptides were confirmed to modify COTS behaviour, likely acting as priming-pheromones (i.e., inducing aggregation for spawning and with potential roles in the activation of gamete maturation) and positioning them as strong candidates for field testing (Hillberg 2024; Harris et al. 2025b). In the context of COTS control, these cues could be applied to aggregate early-gravid adults for rapid removal before the optimal spawning period, thereby reducing the number of gametes released and hence the number of larvae. Their design was informed by the observed large gene expression changes in the spine that correlated with reproductive maturation, i.e., COTS spine genes were differentially expressed in male versus female and non-reproductive versus reproductive states. Genes encoding chemosensory adhesion regulators, including G-protein (D1, G2, and G7-like) coupled receptors, and two glutamate receptors (isoform X1 and X6), were up-regulated in reproductive COTS, proteins already identified as leads for future chemosensory reception-type studies (Croset et al. 2010; Ferreira et al. 2014). Non-reproductive COTS spines demonstrated an increase in a novel family of genes expressed exclusively in spines and encoding the COTS-specific spine-secreted proteins (SSPs). Further, a comparative analysis of the genome of the northern Pacific seastar (a predatory Japanese species that is invasive in Australia) did not find any homologs of the COTS oki.327 gene cluster, indicating COTS specificity and elevating the COTS SSPs to candidate status (Wang et al. 2023).

Temporal expression of genes in COTS spines and protein profiling of COTS-conditioned seawater indicated the active secretion of defence-associated proteins. These included plancitoxin-1, phospholipase A2, allergen venom-type proteins and PI16. SEM observations suggest they are likely released from secretory-type cells. Together with the identification of COTS SSPs, these results highlight the spine as a chemically active secretory organ that releases both defence and behaviour-modifying biomolecules. Moreover, in other taxa, venom toxins can also serve communication roles, e.g., social wasp venoms are multifunctional secretions that include alarm and sexual pheromone activities, beyond defence, illustrating that toxicity and signalling are not mutually exclusive functions (Turillazzi

2006). In the aquatic context, bufadienolide-type toxins of the female cane toad, released to deter predators from egg masses, also act as a cane toad tadpole attractant (CTC 2018; Crossland et al. 2021). This species-specific attractant has been developed into a commercial lure, illustrating how a discovery approach can deliver precise, behaviour-modifying cues. Given the well-recognised value of toxins as molecular tools, as lead molecules for therapeutics (King 2011; Pennington et al. 2018) and as semiochemical agents (Crossland et al. 2021), further investigation of COTS venom-type proteins for semiochemical activity should be pursued (Harris et al. 2025b).

4.2.3 Revealing the mode of action of COTS RGP in the search for a COTS control agent

Spawning of gravid male and female COTS can be induced by injection of a recombinant COTS RGP, with oocytes undergoing GVBD (Smith et al. 2019). Furthermore, recombinant (Smith et al. 2019) and synthetic (Mita et al. 2022) COTS RGP can trigger ovulation *in vitro* within ovarian fragments. Here, injection with synthetic RGP was confirmed to induce spawning behaviour and gamete spawning in both males and females, triggering GVBD in eggs, with spawned gametes successfully fertilising. It also triggered spawning in gravid female *A. brevispinus* suggesting COTS RGP is not species-specific but can act interspecifically at the RGP GPCR, corroborating findings by Mita et al. (2022) that RGPs from *Patiria pectinifera* and *Asterias amurensis* were able to induce COTS ovary ovulation and providing mechanistic insight into the success of *in vitro* hybridisation between *A. brevispinus* and *A. planci* (now referred to as *A.* cf. *solaris*) (Lucas and Jones 1976).

Although RGP itself, as an endogenous signalling cue, is unlikely to find utility as a COTS control agent, these results provide valuable insights into its mode of action, i.e., how spawning is initiated and regulated. Future efforts should focus on elucidating the exogenous cue(s), the neural cascade that regulates its production, and receptors that could be targeted by future semiochemical tools. By informing the timing of reproductive readiness and fertilisation likelihood, this understanding can provide inputs for population-dynamic models and help guide when and where to intensify on-water control, thereby strengthening the foundation for developing safe and effective semiochemical-based approaches.

4.2.4 Revealing the mode of action of COTS betaine receptors towards development of a receptor-based assay

Characterisation of the molecular mechanism of action of GlyB on COTS is key to developing GlyB-inspired species-specific small molecule attractants. High-resolution structural information is needed to identify how GlyB (and potentially other betaines) binds to the COTS receptors, and, using homologous sequences, pinpoint opportunistic and species-specific binding sites around where GlyB binds. This knowledge will guide chemical functionalisation of betaines to engineer conspecific analogues. Even if structural information cannot be obtained, the ability to express betaine receptors has enormous potential, including opening up opportunities for high-throughput screening of natural product libraries to identify new semiochemical leads. The benefit of small molecules as control agents is that they are cheap to manufacture, a critical consideration in any large-scale application as COTS attractants or baits. The technologies under development here for the expression of COTS-derived betaine receptors therefore set the foundation for future exploration of other COTS receptors

important for conspecific communication, including those that have been identified through the transcriptomic and genomic studies supported by this research project.

Operationalising this plan, once the bacmid is constructed, would involve the insect cell being transfected, incubated, and the culture medium containing the recombinant baculovirus (P1) harvested and used as seed virus stock to infect SF9 and Hi5 cells. To achieve protein yields suitable for detailed biochemical (e.g., size-exclusion chromatography) and behavioural characterisation, culturing of infected SF9 and Hi5 cells would need to be optimised under varied temperature, agitation, and time parameters. The resulting expression profiles would then guide scaled culturing in a bioreactor. Harvesting from this sustainable source could yield ample biomass for protein purification, and subsequent use in ligand-binding assays. Should these experiments be successful, they would establish a receptor-based assay platform for screening large natural-compound libraries to discover selective binders of this and other COTS receptors. This approach to the discovery of COTS attractants is distinctly different to those presented earlier: starting from the molecular level and drawing on the vast knowledge and procedures currently adopted in the pharmaceutical and agrochemical industries.

4.3 Pipeline for the discovery of COTS-derived semiochemical candidates as control agents

The primary goal of this study was to identify a COTS pheromone attractant, verified through behavioural analysis. In advancing this effort, a framework was established focussed on identifying and assessing COTS-derived semiochemicals as agents to mediate COTS behaviour as well as a pipeline - from establishment of a species-specific multi-omics database to the discovery of biomolecules through to bioproduction and validation - aimed at affording reef protection against COTS outbreaks. The discovery and development of a semiochemical pest management agent logically follows a similar pipeline as for drug discovery (Zanders 2011). Despite other successful semiochemical pest control agents having been developed (Harris et al. 2025a), to our knowledge, the pipeline discussed herein is the first formalised into a logical workflow that can be utilised and adapted by other researchers to fully characterise and understand semiochemicals and their application as pest control agents. The pipeline encompasses a series of stages, each relying on complex multifaceted and multidisciplinary processes, that involve a host of scientific, regulatory, and financial challenges. Here, through synergy with and effective strategic collaboration across disciplines and science institutions, scientific methods have expanded our knowledge of COTS biology and chemistry and have led to the discovery of several lead semiochemical COTS attractants. With these leads now progressing from the early stages of characterisation to larger-scale production and testing, the search for more continues. Notwithstanding the need for further concerted research effort to progress lead discovery and optimisation, significant effort is also required to address the concerns and requirements of regulatory bodies and of the tourism industry and the wider community before a semiochemical control agent is realised.

Preliminary literature investigations to better understand the biological, ecological, and environmental factors that moderate COTS behaviours and to identify the gaps in knowledge

and determine the type of behaviour (or mode of action) to target (Appendix B **Table A 2**), were essential in developing a roadmap of the research.

This was then followed with a desktop review (Motti et al. 2022; Harris et al. 2025a) and investigation of the chemistry of COTS with the aim to identify a promising molecular semiochemical candidate. Depending on the behaviour being targeted, the semiochemical can be a specific large MW biomolecule or protein, or a small MW primary or secondary metabolite. Research utilising semiochemicals as pest control agents for aquatic organisms such as sea lamprey and carp has made positive progress (Sorensen et al. 2019; Fredricks et al. 2021; Hume et al. 2021; Siefkes et al. 2021), yet this research has been ongoing for multiple decades, possibly hampered by a focus on chemical isolation. Therefore, here, a more holistic approach was taken. At the project onset, computational mining of the available genomic, transcriptomic, proteomic and metabolomic resources (Appendix D **Table A 3**) provided the first insight into possible leads, i.e., gene expression and the secretome protein profile indicated COTS spines were a major source of signalling proteins and identified the EDPRs as prime leads (Hall et al. 2017). However, many of these resources were either not readily accessible in a form that allowed for their integration with other -omics datasets or were limited in number and therefore restricted the scope of biological and behavioural investigations. To address this**, a pipeline was developed outlining an efficient roadmap from semiochemical identification to pest control management.**

The first stage of the pipeline (**Figure 28**) aimed to improve and expand the available COTS -omic resources. Hence, the COTS genome was refined and democratised to facilitate access and interrogation (Degnan marine genomics lab | Apollo Portal (genome.edu.au)) and methods assessed towards generating a draft genome for *Acanthaster brevispinus* which will ultimately allow for comparative assessment and discovery of similarities and differences between the sibling species. Tissue transcriptomic resources were expanded (Hillberg et al. 2023; Jönsson 2023; Morin et al. 2023,2024) following standardised RNA-seq methodologies, leading to the establishment of comprehensive gene, protein, and metabolite datasets based on matches to public (NCBI and KEGG) or customised PCDL databases. Next, stage two involved the semiochemical purification from COTS-conditioned water, which must take into consideration the potential complexity and dynamicity of biomolecules when preparing samples and selecting the most appropriate LC separation and MS detection methods (Gulcicek et al. 2005). Similarly, metabolomic studies of COTS tissues represent a particular challenge as more than one analytical platform is required to capture the full diversity of chemistry (from polar to non-polar). Here, an LC-MS/MS approach was taken as a first assessment. This approach also requires screening of chemical libraries, however, databases for secondary metabolites tend to be defragmented (Go 2010) and not easily searchable (MarinLit for COTS secondary metabolites); hence to support metabolomic profiling an in-house customised saponin PCDL was created (Mendoza-Porras et al. 2023). With these additional COTS datasets, and with more specific chemical and biological knowledge, **several leads with possible attraction activity were identified** (**Table 4**).

Once a lead is identified, the semiochemical discovery process moves to the third stage. Here, this involved assessing, under controlled lab conditions, the ability of the lead to modify COTS behaviour, specifically to attract COTS to a point source. A series of *in vitro*, *in vivo* and *ex vivo* testing (whole animal – as used here, and cell, receptor or enzyme assays) were conducted to evaluate the mode-of-action, safety, and efficacy of the lead (as a crude,

fraction and/or pure compound – depending on the isolation approach taken) and further establish its potential impacts when released into the environment. It is essential that semiochemical leads are tested for their toxicity upon non-target species; here a simple brine shrimp lethality assay was employed as a measure of toxicity, and a free-movement assay (either Y-maze or flume system) as a measure of behaviour modification. **Two candidates, TFF summer FrA > 30 kDa and COTS SSP > 10 kDa, were confirmed to be non-toxic to brine shrimp and elicit attraction behaviour in adult COTS.**

The third stage also involves the production of the lead (bio)molecules, often a major hurdle in the development of any candidate chemical product. Traditional methods used in drug discovery include synthesis of the lead molecule or optimised structural analogues (designed to enhance the potency, selectivity, and specificity). Inspired by COTS SSPs > 10 kDa, **peptides were commercially synthesised and the peptide cocktail confirmed to be non-toxic and act as a COTS attractant.** With the characterisation of each new lead, a structure-activity relationship (SAR) can be established and used to guide the design of semiochemical-inspired active mimetics with the necessary attributes required of a control agent, including being safe to deploy. SARs can also facilitate pharmacophore-based virtual screening to predict more leads (Liu et al. 2016). Characterising the associated chemoreceptor is also key and allows for the bioengineering of pheromone-ligand conjugates (Lengger and Jensen 2020), whereby the pheromone lures the COTS and the ligand acts on COTS with specific activity. Here, the development of a workflow to support characterisation of key betaine receptors has begun, and recombinant expression of these will be trialled towards developing an *in vitro* chemoreceptor assay for betaine-like ligands. A method was also established to recombinantly express the lead EPDR GBR.60.100, although testing in the Y-maze assay found it to be inactive at the concentration tested, with no clear attraction to the point source. Unlike the application of drugs, the formulation of semiochemicals for the purpose of controlling pest species has less well-defined requirements (section 5.2), for example, crude extracts are now being considered as ecofriendly alternatives (i.e., Sero-X; (Abaho et al. 2021; Aioub et al. 2024)). Applying this approach, **a TFF method was developed to produce, at scale, the non-toxic crude summer FrA > 30 kDa, which was subsequently confirmed to modify COTS movement.**

The fourth and final stage involves consultation and engagement with government regulatory bodies, environmental managers, Traditional Owner groups, marine industries, and the community. Regulatory bodies (i.e., Great Barrier Reef Marine Park Authority; GBRMPA – environmental; and Australian Pesticides and Veterinary Medicines Authority; APVMA - pesticides) require comprehensive data on safety and efficacy of new and novel technologies to initiate in-field trials. Often there are a clear set of guidelines that dictate what and how new technologies, including chemical, can be used. APVMA also considers information pertaining to the mode of action of the chemistry (and any products) and worker health and safety (i.e., exposure during handling and application). Here, early engagement with the APVMA has established that the use of semiochemicals as an ecofriendly alternative is gaining traction, in line with national and international decisions and activities (Fredricks et al. 2021; APVMA 2022).

In-field pilot trials are essential to determine the efficacy of the semiochemical agent under real-world conditions and different biological scenarios (i.e., pre-outbreak vs outbreak; spawning vs non-spawning seasons; refer to Appendix B **Table A 2**, (Lundgren 2013; Motti

et al. 2022)). If successful, findings from such pilot studies will provide the evidence base needed to support submissions to regulatory authorities for implementation at reef scale. They will also inform the development and operationalisation of semiochemical delivery mechanisms within the COTS IPM. In addition, reef-scale surveillance is also required to assess effectiveness, analogous to that done to assess the current culling effectiveness.

It is anticipated that it will take several years for the pipeline to realise a fully validated, safe and effective COTS-specific attractant, particularly as it has yet to be successfully applied end-to-end. **Successfully navigating this pipeline will require an ongoing, concerted, focused, and collaborative effort, as well as significant financial investment** (Grunder et al. 2021). However, the survival of the GBR and other Indo-Pacific coral reefs is at risk, and with extensive efforts now being put into coral reef restoration projects globally (Anthony et al. 2017; Anthony et al. 2020; Suggett et al. 2024), this investment is critical and has real potential to improve reef health long-term.

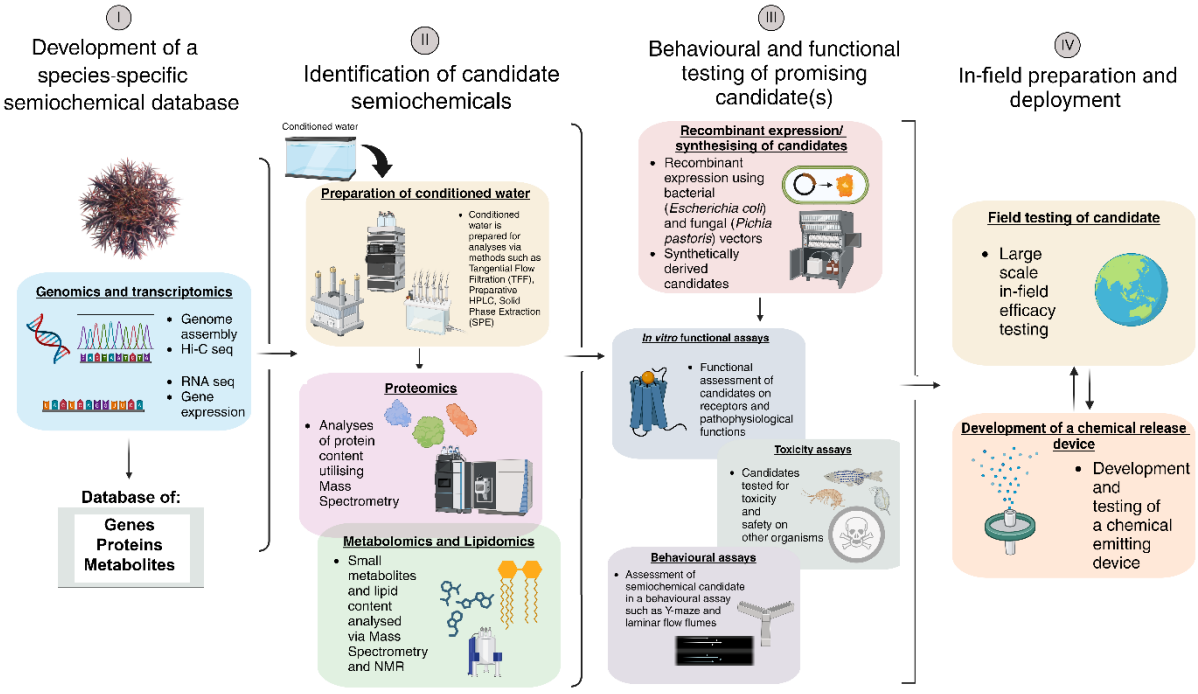

Figure 28. Pipeline to realise a semiochemical control agent for the control of crown-of-thorns starfish.

4.4 Project outputs

A summary of the final outputs of the CCIP-R-11 project are listed below.

- Dataset: Democratised COTS genome (browser publicly available).
- Dataset: New genomic data for *Acanthaster brevispinus* (related short-spined sibling species).
- Dataset: New transcriptomic, proteomic, metabolomic, lipidomic and glycomic data for adult (CCIP-R-11) and juvenile (CCIP-P-03 Byrne et al. 2024, Appendix K **Table A 12**) COTS.

- Method / Technology: Validated two-current choice behavioural flume system, with Standard Operating Procedure.
- Method / Technology: Pipeline developed for the discovery, characterisation and production of candidate semiochemicals for behavioural and functional testing.
- Dataset: Database of lead and candidate pheromone attractants.
- Knowledge / Recommendation: New knowledge on the response of adult COTS to a range of attractant molecules (in lab).
- Knowledge / Recommendation: Feasibility of using attractant baits to enhance COTS control established (translation of Early Investment Project report (Motti et al. 2022) into a review publication (Harris et al. 2025a)), and a roadmap for securing regulatory approvals outlined.

5 RESEARCH SYNERGIES AND NEXT STEPS

It is widely recognised that effective collaboration which is inclusive, adaptive, and interdisciplinary can prevail over the inherent underinvestment in the development of new technologies to prevent and manage pest species (Littlejohn and Delepine 2021). For the GBR, recurring outbreaks of COTS populations pose a direct threat to its survival, and it is now recognised that supplementary and complementary control tactics are needed to mitigate this (Pratchett et al. 2021). Innovative novel semiochemical technologies that act to suppress COTS populations is the focus of CCIP-R-11 (**Figure 1**). CCIP-R-11 has taken inspiration from the Great Lakes Fishery Commission (Hume et al. 2020), a consortium that has applied a multidisciplinary science-based management strategy to control the sea lamprey (i.e., an exemplar IPM that utilises semiochemicals), and established a collaborative working group to expand our knowledge of COTS behavioural ecology and develop COTS semiochemical control agents. Our robust expansion of previous semiochemical pest management research and the development of a purposeful discovery pipeline will allow for more efficient research into semiochemical candidates for IPM.

5.1 Synergies

CCIP-R-11 has reviewed the core aspects of COTS biology (survival, foraging, and reproduction) and past solutions, and identified the knowledge gaps (Appendix B **Table A 2**) and challenges in controlling their populations as well as the development opportunities for semiochemical-inspired control agents (Motti et al. 2022; Harris et al. 2025a). The interconnectedness between collaborating CCIP scientists, and between CCIP-R-11 scientists and reef-based industries, reef managers, tourism operators, and more recently Traditional Owners, is a key aspect for the science innovation and technology development within CCIP-R-11. At the onset of the project, it was anticipated that the analytical abilities, innovative thinking and active transfer of knowledge that occurs during these interactions will deliver impact across the CCIP and more broadly to the COTS IPM. Motivated by mutual concern and the urgent need to improve COTS control on the GBR, CCIP-R-11 has engaged closely with CCIP-P-03 (Byrne et al. 2024) and CCIP-R-08 (Lockie et al. 2025) to create a knowledge-sharing environment (**Figure 1**; pathways emphasised in **bold**). These two collaborations integrated adult- and juvenile-based experimental approaches (extension of CCIP-P-03), and aligned scientific knowledge with societal perceptions (CCIP-R-08 Lockie et al. 2025) (Harris et al. 2025a).

For example, the workflow established to investigate the COTS metabolome revealed a plethora of primary and secondary metabolites in adult tissues and was the motivation to examine, as part of the CCIP-P-03 Value add project (Byrne et al. 2024), the metabolome of COTS juveniles across their ontology (from 12 to 27 months old). Through RNA-seq, a greater insight was gained into the composition of spine protein secretome in adult COTS, and with CCIP-P-03 (Byrne et al. 2024) this technique is now being applied to investigate the spine chemistry of the crustose coralline algae (CCA)-feeding juveniles to eliminate co-occurring molecules and help pinpoint adult-specific pheromone attractants. In support of this, and to extend the MS-based proteomic library, a reference juvenile COTS transcriptome, with gene expression levels and annotation included (Appendix K **Table A 12**), was generated from the RNA-seq data of three juveniles and plancitoxins found to be

present (Byrne et al. 2024), but seemingly not in high levels, and potentially different from the adult. Most recently, CCIP-R-11 has engaged with CCIP-P-03 to apply the TFF workflow to collect juvenile COTS secretome allowing for a comparative study of the secreted proteins. This will aid in the identification of biomolecules derived from adults that suspend the juvenile ontogenetic transition to corallivory. Research here has also increased knowledge of the cascade that mediates COTS reproductive biology through *ex vivo* assays and confirmed the utility of synthetic COTS RGP, which acts on both males and females, and will support future efforts by CCIP-P-03 researchers (and others) to rear and study juvenile progeny from live captive adults. The consequence of these ongoing synergistic activities will be an improved understanding of the semiochemicals that induce spawning aggregations and spawning of COTS and regulate the development of juveniles.

The synergy between fundamental molecular-based scientific approaches applied here and exploring the societal perspective offered by CCIP-R-08 (Lockie et al. 2025) has been profoundly informative. The need to control COTS on the GBR is widely recognised within the research and tourism communities, however, the potential application of pheromone attractants as a control agent is less so. Arising from the synergy between CCIP-R-11 and CCIP-R-08, discussions with GBRMPA, the GBR Regulators Forum, and APVMA (facilitated by the Reef Restoration and Adaptation Regulatory and Policy Environment subprogram; RRAP RPE) revealed there is public acceptance and preference for distribution of attractants for COTS management (Harris et al. 2025a) over traditional chemical pesticide methods and genetic and microbial interventions (Høj et al. 2020), however, informal discussions with reef-based operators and Traditional Owners (CCIP symposia, Reef Resilience Symposium 2024 COTS Workshop) indicated an unfamiliarity of what a semiochemical is; the term semiochemical is not widely recognised and has negative connotations primarily because it contains the word ‘chemical’ (Motti pers. comms.). There was also an underlying level of misapprehension regarding the nature and safety of any application within the GBR, i.e., no one wants an unsafe ‘chemical’ introduced into the environment (Motti pers. comms.). To address these societal misperceptions, it is critical that future communications with stakeholders and the community focus on establishing a clear and concise narrative based on a strong definition. Equally important is ensuring that evidence is presented in a digestible format that reiterates the biological function of the pheromone attractant and demonstrates that it has no unintended impacts on non-target species, particularly if bioengineered delivery strategies are employed.

The regulatory landscape for semiochemical-based COTS control is unknown. With novel semiochemical leads now identified and some potentially transitioning into the in-field testing phase (i.e., as promising candidates), the need for regulatory approval to assess their feasibility and viability as control agents is imminent. Synergy exists with the RRAP RPE subprogram which is currently developing a robust regulatory and policy environment to enable the testing and deployment of novel unconventional interventions on the GBR and produce best-practice guidelines for GBR coral restoration projects (Fidelman et al. 2019). Learnings from this subprogram advise that interventions involving the collection and use of biological material native to Queensland and its waters for biodiscovery research (i.e., collection of the COTS secretome), and the manipulation of reef organisms (i.e., modification of COTS behaviour), will need to be considered under the Queensland Biodiscovery Act 2004 (2023), and may require permit approval from both GBRMPA and the Queensland Department of Environment, Science and Innovation. Although there is no certainty, the

natural source of COTS-derived semiochemicals and their non-toxic mode of action means assessments are likely to indicate low environmental risk and recommend approval. However, deployment conditions (e.g., scale, location, delivery mode and timing) will still need to be optimised and validated.

During an opportunistic observation following unintentional exposure of COTS to elevated temperatures, CCIP-R-11 recorded high mortality and evidence of an immune response. Reporting of this to the CCIP initiated a fast-tracked approval of CCIP-P-02 Population Collapse project (Høj et al. 2024), initially proposed but not funded. It was hypothesised that the Banfield Reef population (site of COTS collection for the CCIP-R-11) may be on the precipice of a natural collapse, a phenomenon that has not been captured before and for which the mechanisms are poorly understood. Therefore, with field support provided by MV Odyssey, male and female COTS were collected, and various tissues dissected and preserved immediately for future -omic analyses (refer to CCIP-P-02 report for further details). These samples will allow changes in expression level of genes implicated in COTS immune and stress responses, potential shifts in COTS-associated microbiomes, and changes in COTS proteomes, metabolomes and lipidomes to be correlated with collapse, should that eventuate at Banfield Reef. These findings may also improve the parametrisation of models used to guide the implementation of the COTS culling program.

Synergies, based on information sharing, exist with (i) CCIP-R-06 (Cost effectiveness of control, Scheufele et al. 2025), which supports assessments of cost benefit (Appendix L **Table A 13** and **Table A 14**), (ii) CCIP-R-04 (Regional modelling, Skinner et al. 2025), and (iii) CCIP-R-03 (Reef-scale modelling, Rogers et al. 2025), which support the evaluation of the benefits of integrating new management interventions into the COTS IPM and assessing their efficacy under different ecological conditions at the reef scale (**Figure 1**). For the latter, demonstrated in-field efficacy will ultimately be required to realise these benefits. Collectively, this information sharing will guide any future deployment of COTS attractant control agents.

Although field trials were not feasible here, CCIP-R-11 engaged with CCIP-D-04 (COTS Surveillance System, Bainbridge et al. 2025) to support in-field testing of robotics and imaging technologies, which may have application in the deployment of semiochemical technologies and monitoring of their effectiveness. This link provides the basis for future collaboration to conduct in-field attraction trials.

Another layer of synergy includes links with the reef-based operators BPM and PMG. This project engaged closely with these reef-based operators to secure a regular supply of live animals, critical to conduct whole animal assays and obtain fresh COTS tissues. This engagement also facilitated on-board scientific activities, with CCIP-R-11 researchers joining field trips for the purpose of field sampling (Jönsson et al. 2022; Morin et al. 2023; Høj et al. 2024; Morin et al. 2024). Through attendance at the CCIP symposia and the 2024 Reef Resilience Symposium, CCIP-R-11 engaged with reef-based operators to discuss logistics for continued supply and, while a product is not yet available, formulate early plans for in-field testing and eventually deployment of a semiochemical control agent.

These synergies have bolstered the efforts of the collaborative working group and identified new avenues to pursue towards establishing a COTS semiochemical control agent. CCIP-R-

11 will continue to collaborate with these and other stakeholder groups (i.e., agricultural sector, regulators, community and Traditional Owners) and, where possible, engage and, through education, raise the profile of the plight of the reef and the need to develop smarter (innovative and effective) COTS control technologies.

5.2 Next steps - Operationalisation of candidate pheromone attractants

5.2.1 Navigation of regulatory approval frameworks for in-field testing and deployment of a COTS pheromone attractant

Globally, the use of semiochemicals as supplementary and complementary pest control agents is growing. Regulation of their use was initially assessed against criteria designed for pesticides, yet as semiochemicals are inherently biological in origin, these criteria were often found to be inapt (Robin and Marchand 2019). The OECD 12 (2017) consensus document on semiochemicals summarises environmental impact and human health risks of semiochemicals and concluded that reduced data requirements, and the use of reasoned cases in lieu of data, are appropriate for semiochemicals.

Any deployment of novel and effective COTS semiochemical control methods in the GBR will be reliant on existing policy and regulatory frameworks, however, it is unclear as to whether these are well equipped. The GBRMPA Policy on Great Barrier Reef Interventions (Interventions-Great-Barrier-Reef-Policy (gbrmpa.gov.au)) states that “Restoration and/or adaptation intervention(s) […] mean an action, or actions, actively undertaken in the Marine Parks to support ecosystem recovery, build resilience and achieve conservation benefits for the Great Barrier Reef” and clearly articulates support for “other measures” to control COTS to protect corals. From a regulatory perspective, however, and without any precedence in the marine environment, manipulation of COTS using a natural semiochemical agent will first require controlled large-scale lab experiments and small-scale field trials. Given the delivery will be directly into the environment and not via intracoelomic injection, field trials will require a heightened level of rigour compared to those conducted for citric acid and bile salts (Lundgren 2013; Buck et al. 2016; Boström-Einarsson et al. 2018). The methodology will consider learnings from field trials on sea lamprey (Wagner et al. 2006; Meckley et al. 2012; Wagner et al. 2018), noting that the marine environment poses fundamentally different challenges than confined river and lake systems. For example, in open marine systems, factors such as tidal cycles, wave action, and general water movement need to be considered as they will undoubtedly lead to increased dilution and dispersion of the semiochemical (Motti et al. 2022). It should also be noted that for such trials, purpose specific GBRMPA permits (GBRMPA 2020) (i.e., ‘A program to take animals or plants that pose a threat’, the objective being a Control Program for COTS; and ‘Conduct of a research program’, the objective being collection of COTS for the purpose of research) will require additional criteria be met depending on the nature of the formulation (i.e., extract or pure or pure with a co-formulant). Consideration must also be given to the impact, if any, on the environment resulting from the introduction of the semiochemical, i.e., reduced water quality (Productivity Commission 2003) (Appendix M **Table A 15**). Given the semiochemical candidate is naturally present in the environment, is not intended to kill COTS directly and will dissipate rapidly (Motti et al. 2022), any environmental impacts and collateral damage to

non-target species are expected to be negligible but this needs to be demonstrated as the degree of impact will inform permit decision-making.

Although semiochemical products are expected to pose lower potential risk to human health and the environment than conventional pesticides, with the benefit of hindsight from other IPM programs, further environmental risk assessment and consultation with stakeholders and Traditional Owner groups will be required. Currently, deployment of semiochemicals in the marine environment has no clear regulatory decision pathway in Australia.

5.2.2 APVMA regulatory approvals for use

Semiochemicals fall within the Australian Government's Agricultural and Veterinary Chemicals Code Act 1994 (agvet code) and must be evaluated and registered by the APVMA with the National Registration Scheme for Agricultural and Veterinary Chemicals (NRS; Commonwealth and the states and territories).

The APVMA classifies purified semiochemical pheromones as Group 1 'Biological Chemicals' (APVMA 2022) that induce 'modifying effects in target species' whose use results 'in less exposure to humans and the environment than conventional pesticides' because of their very low application rates, high dissipation and application in bait, trap, or encapsulated formulation. These criteria were created to assess terrestrial applications; currently, there are no specific criteria for the deployment of a semiochemical in the marine environment. Hence, semiochemicals and their synthetically derived analogues are assessed by the APVMA on a case-by-case basis. Naturally occurring enzymes, i.e., high molecular-weight non-toxic proteins that may also contain non-protein functionality such as carbohydrates, lipids, phosphate groups, and metallic ions, are also categorised as a Group 1 'Biological Chemical'. The safety of these proteins is evaluated based on the potential risk to the environment, exposure to workers and safety on the target organism. In addition, unpurified or partially purified extracts derived from "other" organisms (i.e., Sero-X produced by TFF) are listed within Group 2 'Plant and Other Extracts'. Depending on the nature of the semiochemical pheromone candidate and the pathway for production, a COTS-derived semiochemical could potentially be categorised in one or both groups. The APVMA also specifically distinguishes lures and attractants (APVMA 2024) and has an established decision tree with guidelines to support product assessment and registration (Appendix M **Table A 17**). Furthermore, it recognises the need for flexibility in determining the data requirements (APVMA 2019) for 'Biological chemical products' that do not have direct toxicity to the target (and non-target co-habiting) species, allowing for their evaluation on a case-by-case basis, as well as providing specific guidance (i.e., via a Pre-Application Assistance) for when reduced information requirements apply. However, the more novel the product, i.e., COTS-derived semiochemical control agent, the APVMA, which takes a risk-based approach, is likely to require more extensive data on safety and efficacy (Ehlers 2011; APVMA 2019).

From a regulatory perspective, APVMA deems a semiochemical to be the active ingredient when it is 'primarily responsible for the biological' effect, with consideration given to its chemical composition (i.e., pure compound or racemic mixture or extract from natural source, synthetic compound, or mimic). If they are used in formulated 'attract and kill' products, they are considered to be co-formulants, not active substances. Taking this approach, preliminary

consideration of the guidelines with respect to a COTS-derived attractant control agent (Appendix M **Table A 17**) identified that the existing decision tree and guideline criteria are ambiguous, and that 'Pre-Application Assistance' will be required to clarify and establish the submission workflow.

5.2.3 Technology Readiness Levels towards operationalisation of a COTS semiochemical control agent

There are nine conventional technology readiness levels (TRLs) used by the chemical industry starting with the idea, and progressing through to concept, proof-of-concept, preliminary process development, detailed process development, pilot trials, demonstration and full-scale engineering, commissioning, and finally production (Buchner et al. 2019) (Appendix M **Table A 16**). Each step entails specific criteria with detailed indicators that provide technical solution clarity and guide the chemical innovation from basic research through to applied research, development, and deployment. The evidence-based data encompassed within each TRL facilitates preliminary technical assessment, and eventually evaluation (within an adaptive assessment plan, i.e., tailored specifically to each candidate and considering the chemical properties and type of behaviour that is modified) and communication of the chemical technology's maturity, providing the necessary data for regulatory bodies to assess whether they are fit for purpose and safe to use.

Product technical assessment by the APVMA follows a workflow based on TRL's with a key component being the early identification of any knowledge gaps that need to be addressed to secure an approval, registration, license and permit to deploy a chemical control. This workflow includes assessment of the semiochemical against the following statutory criteria:

1) toxicology of both the active constituent and product;
2) residues and trade assessment;
3) occupational exposure aspects;
4) environmental fate, toxicity, potential exposure, and hazard; and
5) efficacy and target crop or animal safety.

The ability to source adequate quantities of the pheromone attractant is critical for the assessment process and of course ultimately for deployment *in situ*. Here, relatively cheap manufacturing methodologies (i.e., TFF protein separation, direct peptide synthesis, recombinant peptide/protein expression) were developed and applied for the production of promising leads, with all found to be feasible, i.e., all capable of generating ample quantities of the semiochemical for assessment against the five criteria and to support small- and large-scale in-field testing. Cost estimates for both traditional solid-phase peptide synthesis (SPPS) and recombinant expression using *P. pastoris* (Appendix L **Figure A 9**, **Table A 13** and **Table A 14**) have been previously calculated (Yap et al. 2020); findings indicate that recombinant expression offers a cost-effective option, producing high yields of the target peptide. These costs are considered reasonable as compared, for example, against the production of a pharmaceutical or nutraceutical (Farid et al. 2020). With production deemed to be feasible both in quantity and cost, effort should now be focussed on designing and testing engineering mechanisms to deliver these pheromone attractants for the purposes of inducing aggregations for trapping and culling. Design of the deployment technologies should

consider the resources already available and in use for culling by lethal injection as well as the potential for population monitoring.

5.2.4 Research Priorities

Three key priority areas for further research and development have emerged from this project:

- **Research priority 1** is to continue to apply the discovery pipeline (-omic profiling and mining, behavioural assays, candidate production/synthesis) to validate and identify other new lead semiochemical moderators of COTS behaviour. Activities to consider include:
    - Progressing identified lead and candidate pheromone attractants with extensive COTS behaviour tests, including decoupling sex as a factor in their attraction activity.
    - Identifying and characterising new lead semiochemical moderators (i.e., allelochemicals emitted by conspecifics and other organisms, that act as alarm cues, spawning cues, foraging cues, deterrents/repellents) through comparative studies with *Acanthaster brevispinus*.
    - Developing further the methodology to identify and produce COTS chemoreceptors for translation into *in vitro* assays for detecting semiochemical ligands and, in the long-term, biosensors for environmental monitoring of semiochemicals post application (Tilmaciu and Morris 2015; Maran 2022).
    - Applying the pipeline to both adult and juvenile life stages, in the first instance, expanding the -omics resource library to include larval and juvenile life stages.
- A small-scale pilot field study, planned within CCIP-R-11 and which was dependent on rapid identification of a suitable candidate(s) and secured regulatory approvals, proved to be unachievable within the project timeline. Therefore, **Research Priority 2** is to conduct a review of in-water field trials assessing the efficacy of releasing semiochemical agents to modify the behaviour of an organism and design and conduct a small-scale field trial of the most promising candidate to determine optimal deployment parameters and demonstrate efficacy against COTS and environmental safety. Activities to consider include:
    - Establishing a roadmap to navigate the regulatory approval process and achieve effective implementation that also includes a communications strategy to engage with stakeholders.
    - Upscaling production from lab to field scale.
    - Chemically profiling reef waters from a naturally-occurring high-density aggregation as a comparison with lab-based studies (reported here) and to guide APVMA.
    - Designing methodologies to assess variability in effectiveness in the field.
    - Devising methods to detect the semiochemical *in situ*

- Assessing the effects of candidate semiochemicals on congener in lab (AIMS SeaSim) and field (reef-based operators) trials.

- In parallel, **Research priority 3** is to design engineering solutions to attract and trap COTS. Activities to consider include:
  - Reviewing, designing and engineering prototype delivery systems and traps (building on knowledge from Motti et al. (2022) and experience of reef-based operators).
  - Assessing their utility in lab (AIMS SeaSim) and field (reef-based operators) trials using candidate semiochemicals with confirmed activity against COTS.
  - Developing deployment strategies (based on modelling in Motti et al. (2022)) considering location and number of devices to deploy.

6 MANAGEMENT IMPLICATIONS AND IMPACT

Here, at the interface of scientific endeavour, reef management policy and on- water management practice, CCIP-R-11 has realised a strategy to identify and tap into the potential of COTS-specific pheromone attractants for application within the COTS IPM program.

6.1 Integrated pest management framework

Development of a semiochemical control agent suited to aquatic scenarios is technically challenging (Sorensen and Johnson 2016; Motti et al. 2022). Realised here was the potential for combining multi-omic profiling of adult (CCIP-R-11) and juvenile (CCIP-P-03, Byrne et al. 2024) COTS with functional and behavioural analyses. Outputs (Appendix D **Table A 3**) represent a key resource to improve understanding of COTS biology and ecology and reveal key chemistries associated with tissue type, life stage and seasonal variation with a role in COTS communication. These solution-oriented findings contribute to the base knowledge of COTS chemosensory capacity (having identified low and high MW lead biomolecules from different pathways, i.e., top-down and bottom-up interrogation of -omic resources) and have allowed for the development and validation of an all-encompassing semiochemical discovery pipeline founded on the systematic application of methodological workflows. However, at this early stage, there is no immediate or direct entry point to effect change in the COTS IPM management strategy. Notwithstanding, these findings and the pipeline framework are the catalyst for future assessment of semiochemical candidates against COTS and co-habiting species, and reef-based evaluations to assess spatial efficacy and guide future deployment scenarios. In addition, the pipeline provides the framework to explore COTS semiochemical control beyond just conspecific attractants.

6.2 On-water Operations and Data collection

While not yet realised, an effective semiochemical control agent has the potential to improve the efficiency and effectiveness of the on-water operations, e.g., using an attractant that lures adults from within the reef and concentrates them for rapid removal or enhancing detection during surveys. It should be noted, however, that the efficacy of the semiochemical candidates identified here needs to be substantiated, and that their application is likely to be most effective for suppressing non-outbreak populations or those in early outbreak phase, where targeted behavioural disruption can have the greatest impact. Knowledge of the chemistries that drive and regulate COTS population change, i.e., cues capable of inducing spawning aggregations (summer TFF FrA) or regulating oocyte maturation (synthetic RGP), both of which influence reproductive capacity, may also prove useful for models exploring population dynamics of COTS to better inform on-water operations (including detection and monitoring). Furthermore, new knowledge of semiochemical communication in juveniles (CCIP-P-03, Byrne et al. 2024) also opens a new avenue of exploration to control COTS populations, especially relevant during and after culling of adults at priority reefs.

6.3 Overarching Outcomes and Impacts

Findings from CCIP-R-11 contribute directly to **new methods for control identified and trialled** and **improved empirical understanding of COTS-coral system** (**Figure 1**) and has value-added to the -omics resources available for COTS (Appendix D **Table A 3** and references therein). This comprehensive resource positions COTS as an exemplar and may extrapolate well to other marine pest species including the Northern Pacific Seastar as well as marine invertebrate species in need of urgent conservation (i.e., pipeline to discover semiochemicals that enhance their reproductive capacity, or that deter predators).

There is also a direct contribution to **new knowledge of cultural and social perspectives and values** (**Figure 1**) with the inclusion of opinions and insight of policy makers, reef-operators, Traditional Owners and communities through CCIP-lead workshops, conference forums and education outreach proving to be effective in raising awareness about the benefits of including semiochemical control agents in the COTS IPM program as well as highlighting the perceived impediments to community buy in.

As illustrated in **Figure 1**, there is a pathway for indirect contribution of CCIP-R-11 concepts and findings to modelling and decision-support outcomes, although these will only be realised once the efficacy of semiochemical candidates has been verified in field deployment trials.

The outcomes from CCIP-R-11 have advanced the concept of pheromone attractant control agents from theory (i.e., TRL 1) to discovery and proof-of-concept (i.e., TRL 3 to 4) and has advanced efforts to innovate and devise effective supplementary and complementary control methods for both adults and juveniles to ensure future COTS outbreaks are suppressed and prevented. Continued and coordinated research effort is required to assess and, if effective, undertake in-field testing of the candidate semiochemical pheromone attractants identified here, progress lead semiochemicals, and discover new semiochemical pheromones and allelochemicals (i.e., produced by other species), towards developing agents suitable for use as a control or for monitoring purposes within the COTS IPM program. Through engagement with APVMA, GBRMPA, and industry, community and Traditional Owner groups, a roadmap to translate innovation to product needs to be created to ensure readiness for when a product exits the pipeline, and to provide assurance of the safety and sustainability of the product and articulate benefits to the reef.

6.4 Scope for Future Research

Semiochemical agents in COTS IPM offer a revolutionary approach to control COTS populations. Semiochemicals represent a promising control tool due to their unique properties: being naturally occurring in the environment, often species-specific, do not cause adverse effects on non-target organisms, are active at low concentrations, do not persist or accumulate in the environment, and do not pose any environmental risks. While significant progress has been made here to discover semiochemicals that can meet all the biological, chemical and regulatory criteria outlined herein, and which are highly effective and suitable for practical application to control COTS in the reef environment (Motti et al. 2022; Harris et al. 2025a), no one candidate has yet successfully traversed the pipeline. As such, there

remains a need for fundamental continued research to identify COTS chemosensory receptors and semiochemical ligands capable of moderating behaviour. In parallel, engineering of application technologies (i.e., chemical formulations that can ensure semiochemical integrity during deployment, controlled release systems, traps) which are suited to the reef environment, (i.e., are robust, economical, non-toxic, and biodegradable or readily retrieved) as well as a ratified roadmap to secure regulatory approvals are needed to ensure semiochemical agents and delivery device prototypes are ready for testing in field deployment trials. To harness the momentum and excitement created here and guide these future research efforts, a horizon scan is recommended, bringing together new partners (national and international; scientists and governing bodies) to review strategies used, and understand what has worked, what hasn't, and why. This engagement will establish a global network to extend the vision, expertise and capability of the CCIP and facilitate interdisciplinary innovation towards realising the potential of semiochemical agents to control COTS populations.

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8 DATA ACCESSIBILITY

Raw data are available in the supporting information associated with the primary research articles (see sections 2.5.5 and 2.5.6).

Degnan marine genomics lab | Apollo Portal (genome.edu.au)

COTS: NCBI Sequence Read Archive (SRA) under BioProjects PRJNA821257, PRJNA901199 (spine).

Acanthaster brevispinus RNA-seq: NCBI BioProjects PRJNA548418 and PRJNA16358

9 REFERENCES

Abaho I, Masembe C, Akoll P, Jones CLW (2021) The use of plant extracts to control tilapia reproduction: Current status and future perspectives. Journal of the World Aquaculture Society 53:593–619

Abd El-Ghany NM (2023) Semiochemicals for controlling insect pests. Journal of Plant Protection Research 59:1–11

Agelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA, Pow EM, Smart LE, Smiley DWM, Wadhams LJ, Woodcock CM (1999) Exploiting semiochemicals in insect control. Pesticide Science 55:225–235

Aioub AAA, Ghosh S, Al-Farga A, Khan AN, Bibi R, Elwakeel AM, Nawaz A, Sherif NT, Elmasry SA, Ammar EE (2024) Back to the origins: biopesticides as promising alternatives to conventional agrochemicals. European Journal of Plant Pathology 169:697–713

Allen JD, Richardson EL, Deaker D, Agüera A, Byrne M (2019) Larval cloning in the crown-of-thorns sea star, a keystone coral predator. Marine Ecology Progress Series 609:271–276

Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H (2019) SignalP 5.0 improves signal peptide predictions using deep neural networks. Nature Biotechnology 37:420–423

Anthony K, Bay LK, Costanza R, Firn J, Gunn J, Harrison P, Heyward A, Lundgren P, Mead D, Moore T, Mumby PJ, Oppen MJHv, Robertson J, Runge MC, Suggett DJ, Schaelke B, Wachenfeld D, Walshe T (2017) New interventions are needed to save coral reefs. Nature Ecology and Evolution 1:1420–1422

Anthony KRN, Helmstedt KJ, Bay LK, Fidelman P, Hussey KE, Lundgren P, Mead D, McLeod IM, Mumby PJ, Newlands M, Schaffelke B, Wilson KA, Hardisty PE (2020) Interventions to help coral reefs under global change-A complex decision challenge. PLoS One 15:e0236399

APVMA (2016) Public Release Summary on the Evaluation of the new active clitoria ternatea in the product Sero-X Insecticide. Licensed from the Australian Pesticides and Veterinary Medicines Authority (APVMA) under a Creative Commons Attribution 3.0 Australia Licence.

APVMA (2019) APVMA risk assessment manual Chemistry and manufacture. Licensed from the Australian Pesticides and Veterinary Medicines Authority (APVMA) under a Creative Commons Attribution 4.0 Australia Licence

APVMA (2022) Guideline for the regulation of biological agricultural products. Australian Pesticides and Veterinary Medicines Authority (apvma.gov.au)

APVMA (2024) Lures and attractants. Australian Pesticides and Veterinary Medicines Authority (apvma.gov.au)

Arey BJ (2012) The Role of Glycosylation in Receptor Signaling. In: Petrescu S (ed) Glycosylation. InTechOpen, London.

Babcock RC, Dambacher JM, Morello EB, Plaganyi EE, Hayes KR, Sweatman HPA, Pratchett MS (2016) Assessing different causes of Crown-of-Thorns starfish outbreaks and appropriate responses for management on the Great Barrier Reef. PLoS One 11:e0169048

Bahrom NA, Sirajudeen K, Yip GW, Latiff AA, Ghazali FC (2013) Sulfated glycosaminoglycans from crown-of-thorns *Acanthaster planci* - extraction and quantification analysis. Food Science and Nutrition 1:83-89

Bainbridge S, Armin MA, Page G, Tychsen-Smith L, Coleman G, Oorloff J, Harvey D, Do B, Marsh B, Lawrence E, Kusy B (2025) The crown-of-thorns starfish (COTS) Surveillance System (CSS): end-to-end technology for the detection of reef pests. A report to the Australian Government by the COTS Control Innovation Program.

Battulin N, Fishman VS, Mazur AM, Pomaznoy M, Khabarova AA, Afonnikov DA, Prokhortchouk EB, Serov OL (2015) Comparison of the three-dimensional organization of sperm and fibroblast genomes using the Hi-C approach. Genome Biology 16:77

Beale DJ, Hillyer K, Nilsson S, Limpus D, Bose U, Broadbent JA, Vardy S (2022) Bioaccumulation and metabolic response of PFAS mixtures in wild-caught freshwater turtles (*Emydura macquarii macquarii*) using omics-based ecosurveillance techniques. Science of the Total Environment 806:151264

Beale DJ, Shah R, Karpe AV, Hillyer KE, McAuley AJ, Au GG, Marsh GA, Vasan SS (2021) Metabolic Profiling from an Asymptomatic Ferret Model of SARS-CoV-2 Infection. Metabolites 11:327
Belton JM, McCord RP, Gibcus JH, Naumova N, Zhan Y, Dekker J (2012) Hi-C: a comprehensive technique to capture the conformation of genomes. Methods 58:268-276
Birkeland C, Lucas J (1990) *Acanthaster planci*: major management problems of coral reefs. CRC Press, Boca Raton, Florida
Black K, Moran P, Burrage D, De'ath G (1995) Association of low-frequency currents and crown-of-thorns starfish outbreaks Marine Ecology Progress Series 125:185–194
Bonev B, Cavalli G (2016) Organization and function of the 3D genome. Nature Reviews Genetics 17:661–678
Bose U, Wang T, Zhao M, Motti C, Hall M, Cummins S (2017) Multiomics analysis of the giant triton snail salivary gland, a crown-of-thorns starfish predator. Scientific Reports 7:6000
Boström-Einarsson L, Bonin MC, Moon S, Firth S (2018) Environmental impact monitoring of household vinegar-injections to cull crown-of-thorns starfish, *Acanthaster* spp. Ocean & Coastal Management 155:83–89
Buchner GA, Stepputat KJ, Zimmermann AW, Schomäcker R (2019) Specifying Technology Readiness Levels for the Chemical Industry. Industrial & Engineering Chemistry Research 58:6957–6969
Buck A, Gardiner N, Boström-Einarsson L (2016) Citric Acid Injections: An Accessible and Efficient Method for Controlling Outbreaks of the Crown-of-Thorns Starfish *Acanthaster* cf. *solaris.* Diversity 8
Byrne M, Deaker DJ, Gibbs M, Selvakumaraswamy P, Clements M (2023) Juvenile waiting stage crown-of-thorns sea stars are resilient in heatwave conditions that bleach and kill corals. Global Change Biology 29:6493–6502
Byrne M, Beale DJ, Cummins SF, Dworjanyn SA, Mos B, Motti C (2024) Juvenile resilience hypothesis: the potential that reserve populations of herbivorous crown-of-thorns sea stars across a range of ages may seed outbreaks. A report to the Australian Government by the COTS Control Innovation Program
Caballes CF, Pratchett MS (2014) Reproductive biology and early life history of the crown-of-thorns starfish. Nova Science Publishers, New York
Cardé RT (2007) Using pheromones to disrupt mating of moth pests. In: Kogan M, Jepson P (eds) Perspectives in Ecological Theory and Integrated Pest Management. Cambridge University Press, pp122–169
Castro-Sanguino C, Bozec YM, Condie SA, Fletcher CS, Hock K, Roelfsema C, Westcott DA, Mumby PJ (2023) Control efforts of crown-of-thorns starfish outbreaks to limit future coral decline across the Great Barrier Reef. Ecosphere 14:e4580
Chen B, Yu K, Yao Q, Liao Z, Qin Z, Yu X, Wu Q, He B (2021) Insights Into the Environmental Impact on Genetic Structure and Larval Dispersal of Crown-of-Thorns Starfish in the South China Sea. Frontiers in Marine Science 8
Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, Benton R (2010) Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. PLoS Genetics 6:e1001064
Crossland MR, Salim AA, Capon RJ, Shine R (2021) Chemical cues that attract cannibalistic cane toad (*Rhinella marina*) larvae to vulnerable embryos. Scientific Reports 11:12527
CTC (2018) Cane Toad Challenge Fact Sheet. In: IMB UoQ (ed)
Cummins SF, Nichols AE, Warso CJ, Nagle GT (2005) Aplysia seductin is a water-borne protein pheromone that acts in concert with attractin to stimulate mate attraction. Peptides 26:351–359
Cummins SF, Nichols AE, Amare A, Hummon AB, Sweedler JV, Nagle GT (2004) Characterization of *Aplysia* enticin and temptin, twonovel water-borne protein pheromones that act in concert with attractin to stimulate mate attraction. Journal of Biological Chemistry 279:25614–25622
Cummins SF, Xie F, Misra M, Amare A, Jakubowski JA, Vries MRd, Sweedler JV, Nagle GT, Schein CH (2007a) Recombinant production and structural studies of the Aplysia water-borne protein pheromone enticin indicates it has a novel disulfide stabilized fold. Peptides 28:94–102
Cummins SF, Xie F, de Vries MR, Annangudi SP, Misra M, Degnan BM, Sweedler JV, Nagle GT, Schein CH (2007b) Aplysia temptin – the 'glue' in the water-borne attractin pheromone complex. The FEBS Journal 274:5425-5437

De'ath G, Moran P (1998) Factors affecting the behaviour of crown-of-thorns starfish (*Acanthaster planci* L.) on the Great Barrier Reef: 2: Feeding preferences. Journal of Experimental Marine Biology and Ecology 220:107–126
De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27-year decline of coral cover on the Great Barrier Reef and its causes. Proceedings of the National Academy of Sciences of the United States of America 109:17995–17999
Deaker DJ, Mos B, Lin HA, Lawson C, Budden C, Dworjanyn SA, Byrne M (2020a) Diet flexibility and growth of the early herbivorous juvenile crown-of-thorns sea star, implications for its boom-bust population dynamics. PLoS One 15:e0236142
Deaker DJ, Aguera A, Lin HA, Lawson C, Budden C, Dworjanyn SA, Mos B, Byrne M (2020b) The hidden army: corallivorous crown-of-thorns seastars can spend years as herbivorous juveniles. Biology Letters 16:20190849
Doll PC, Uthicke S, Caballes CF, Patel F, Gomez Cabrera MDC, Lang BJ, Pratchett MS (2023) Induction of larval settlement in crown-of-thorns starfish is not mediated by conspecific cues. Scientific Reports 13:17119
Dommisse M, Hough D (2004) Controlling the Northern Pacific seastar (*Asterias amurensis*) in Australia. In: The Australian Government Department of the Environment and Heritage (ed). Department of Sustainability and Environment, Victoria, Australia 1–52
Eghbalnia HR, Wilfinger WW, Mackey K, Chomczynski P (2020) Coordinated analysis of exon and intron data reveals novel differential gene expression changes. Scientific Reports 10:15669
Ehlers R-U (2011) Regulation of Biological Control Agents. Springer Dordrecht, Biomedical and Life Sciences
Farid SS, Baron M, Stamatis C, Nie W, Coffman J (2020) Benchmarking biopharmaceutical process development and manufacturing cost contributions to R&D. MAbs 12:1754999
Ferreira T, Wilson SR, Choi YG, Risso D, Dudoit S, Speed TP, Ngai J (2014) Silencing of odorant receptor genes by G protein βγ signaling ensures the expression of one odorant receptor per olfactory sensory neuron. Neuron 81:847–859
Fidelman P, McGrath C, Newlands M, Dobbs K, Jago B, Hussey K (2019) Regulatory implications of coral reef restoration and adaptation under a changing climate. Environmental Science & Policy 100:221–229
Finney DJ (1971) Probit Analysis, 3rd ed. By D. J. Finney, Cambridge University Press, 333 pp.
Foo SA, Millican HR, Byrne M (2024) Crown-of-thorns seastar (*Acanthaster* spp.) feeding ecology across species and regions. Science of the Total Environment 930:172691
Fraser N, B.Crawford, Kusen J (2000) Best practices guide for Crown-of-Thorns clean-ups. Proyek Pesisir Special Publication Coastal Resources Center Coastal Management Report #2225, Coastal Resources Center, Narragansett, Rhode Island 38:1–30
Fredricks K, Johnson N, Hubert T, Siefkes M (2021) Registration and application of sea lamprey pheromones for sea lamprey control in the United States and Canada. Journal of Great Lakes Research 47:S448–S454
Gaiti F, Jindrich K, Fernandez-Valverde SL, Roper KE, Degnan BM, Tanurdžić M (2017) Landscape of histone modifications in a sponge reveals the origin of animal cis-regulatory complexity. eLife 6
GBRMPA (2020) COTS Strategic Management Framework. GBRMPA, Townsville
Gerlak AK, Guido Z, Owen G, McGoffin MSR, Louder E, Davies J, Smith KJ, Zimmer A, Murveit AM, Meadow A, Shrestha P, Joshi N (2023) Stakeholder engagement in the co-production of knowledge for environmental decision-making. World Development 170
Go EP (2010) Database Resources in Metabolomics: An Overview. Journal of Neuroimmune Pharmacology 5:18–30
Grisshammer R, Tateu CG (1995) Overexpression of integral membrane proteins for structural studies. Quarterly Reviews of Biophysics 28:315–422
Grunder SA, Markham JL, Sullivan WP, Eilers C, Tallon K, McGarry D (2021) A review of sea lamprey control in Lake Erie, 2000–2019. Journal of Great Lakes Research 47:S506-S522
Gulcicek EE, Colangelo CM, McMurray W, Stone K, Williams K, Wu T, Zhao H, Spratt H, Kurosky A, Wu B (2005) Proteomics and the Analysis of Proteomic Data: An Overview of Current Protein-Profiling Technologies. Current Protocols in Bioinformatics 10:13.11.11-13.11.31
Gyawali P, Karpe AV, Hillyer KE, Nguyen TV, Hewitt J, Beale DJ (2021) A multi-platform metabolomics approach to identify possible biomarkers for human faecal contamination in Greenshell™ mussels (*Perna canaliculus*). Science of the Total Environment 771:145363

Hall MR, Bose U, Cummins SF, Motti CA, Wang T, Zhao M, Roberts R, Smith M, Rotgans BA, Wyeth RC, Thomas-Hall P (2016) The Crown-of-Thorns secretome: towards a control technology. In: Australian Federal Government Department of the Environment Reef Rescue (ed). Australian Institute of Marine Science, Canberra

Hall MR, Kocot KM, Baughman KW, Fernandez-Valverde SL, Gauthier MEA, Hatleberg WL, Krishnan A, McDougall C, Motti CA, Shoguchi E, Wang T, Zhao M, Bose U, Shinzato C, Hisata K, Fujie M, Kanda M, Cummins SF, Satoh N, Degnan SM, Degnan BM (2017) The crown-of-thorns starfish genome as a guide for biocontrol of this coral reef pest. Nature 544:231–234

Hansen TVA, Sager H, Toutain CE, Courtot E, Neveu C, Charvet CL (2022) The Caenorhabditis elegans DEG-3/DES-2 Channel Is a Betaine-Gated Receptor Insensitive to Monepantel. Molecules 27:312

Hardege I, Morud J, Yu J, Wilson TS, Schroeder FC, Schafer WR (2022) Neuronally produced betaine acts via a ligand-gated ion channel to control behavioral states. Proceedings of the National Academy of Sciences 119:e2201783119

Harris RJ, Barnard dAL, Paxton G, Lockie S, Craik DJ, Cummins SF, Wang CK, Motti CA (2025a) The future of utilising semiochemical pest control methods to manage the destructive crown-of-thorns starfish outbreaks on coral reefs. Biological Conservation 302:110984

Harris RJ, Hillberg AK, Bastin LD, Lausen BS, Suwansa-Ard S, Wang T, Elizur A, Kikuchi S, Nakashima K, Satoh N, Motti CA, Cummins SF (2025b) A family of crown-of-thorns starfish spine-secreted proteins modify adult conspecific behavior. iScience 28:112161

Haszprunar G, Spies M (2014) An integrative approach to the taxonomy of the crown-of-thorns starfish species group (Asteroidea: Acanthaster): A review of names and comparison to recent molecular data. Zootaxa 3841:271-284

Haszprunar G, Vogler C, Wörheide G (2017) Persistent gaps of knowledge for naming and distinguishing multiple species of Crown-of-Thorns-Seastar in the *Acanthaster planci* species complex. Diversity 9

Hill RW, Li C, Jones AD, Gunn JP, Frade PR (2010) Abundant betaines in reef-building corals and ecological indicators of a photoprotective role. Coral Reefs 29:869–880

Hillberg A (2024) Investigation of Crown-of-Thorns Starfish (*Acanthaster* species complex) semiochemicals. University of the Southern Cross

Hillberg A, Smith M, Lausen B, Suwansa-ard S, Johnston R, Mitu SA, MacDonald L, Zhao M, Motti C, Wang T, Elizur A, Nakashima K, Satoh N, Cummins S (2023) Crown-of-thorns starfish spines secrete defence proteins. PeerJ 11:e15689

Hillberg AK, Harris RJ, Motti CA, Elizur A, Wu H, Hossain MA, Bathgate RAD, Cummins SF (2025) In vivo induction of crown-of-thorns starfish (*Acanthaster* spp.) spawning using a synthetic relaxin-like gonad-stimulating peptide. General and Comparative Endocrinology 373:114804

Høj L, Byrne M, Kroon F, Westcott D (2020) A Review of Biologically Based Control Technologies for Crown-of-Thorns Starfish: Options for Enhancing the Integrated Pest Management Approach. Report to the National Environmental Science Program, Reef and Rainforest Research Centre Limited, 133 pp.

Høj L, Harris R, Yau G, Kuek F, Motti C (2024) COTS population collapse. Sampling from Banfield reef. A report to the Australian Government by the COTS Control Innovation Program. Townsville.

Hume JB, Luhring TM, Wagner CM (2020) Push, pull, or push–pull? An alarm cue better guides sea lamprey towards capture devices than a mating pheromone during the reproductive migration. Biological Invasions 22:2129–2142

Hume JB, Bracken FSA, Mateus CS, Brant CO (2021) Synergizing basic and applied scientific approaches to help understand lamprey biology and support management actions. Journal of Great Lakes Research 47:S24–S37

Immaraju JA (1998) The commercial use of azadirachtin and its integration into viable pest control programmes. Pesticide Science 54:285–289

Jönsson M, Morin M, Wang CK, Craik DJ, Degnan SM, Degnan BM (2022) Sex-specific expression of pheromones and other signals in gravid starfish. BMC Biology 20:288

Jönsson NMM (2023) Chemosensory Communication in the Crown-of-Thorns Starfish: Towards a Green and Species-specific Biocontrol program. University of Queensland.

Jutfelt F, Sundin J, Raby GD, Krang A-S, Clark TD (2017) Two-current choice flumes for testing avoidance and preference in aquatic animals. Methods in Ecology and Evolution 8:379–390

Kadota M, Nishimura O, Miura H, Tanaka K, Hiratani I, Kuraku S (2020) Multifaceted Hi-C benchmarking: what makes a difference in chromosome-scale genome scaffolding? GigaScience 9
Karasudani I, Koyama T, Nakandakari S, Aniya Y (1996) Purification of anticoagulant factor from the spine venom of the crown-of-thorns starfish, *Acanthaster planci*. Toxicon 34:871–879
King GF (2011) Venoms as a platform for human drugs: translating toxins into therapeutics. Expert Opinion on Biological Therapy 11:1469–1484
Kong S, Zhang Y (2019) Deciphering Hi-C: from 3D genome to function. Cell Biology and Toxicology 35:15–32
Kool JT, Paris CB, Barber PH, Cowen RK (2011) Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. Global Ecology and Biogeography 20:695–706
Krogh A, Larsson B, Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. Journal of Molecular Biology 305:567–580
Lang BJ, Donelson JM, Caballes CF, Uthicke S, Doll PC, Pratchett MS (2022) Effects of elevated temperature on the performance and survival of pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*). Marine Biology 169:43
Leal WS (2005) Pheromone Reception. The Chemistry of Pheromones and Other Semiochemicals II. Springer Berlin, Heidelberg
Leal WS (2017) Reverse chemical ecology at the service of conservation biology. Proceedings of the National Academy of Sciences 114:12094–12096
Lee C-C, Hsieh HJ, Hsieh C-H, Hwang D-F (2014) Spine venom of crown-of-thorns starfish (*Acanthaster planci*) induces antiproliferation and apoptosis of human melanoma cells (A375.S2). Toxicon 91:126–134
Lengger B, Jensen MK (2020) Engineering G protein-coupled receptor signalling in yeast for biotechnological and medical purposes. FEMS Yeast Research 20:p.foz087.
Li Y, Hao R, Yu K, Chen X (2024) Short-Term Impact of Decomposing Crown-of-Thorn Starfish Blooms on Reef-Building Corals and Benthic Algae: A Laboratory Study. Water 16:190
Ling SD, Cowan ZL, Boada J, Flukes EB, Pratchett MS (2020) Homing behaviour by destructive crown-of-thorns starfish is triggered by local availability of coral prey. Proceedings of the Royal Society B: Biological Sciences,287:20201341
Littlejohn RJ, Delepine JM (2021) Effective Collaboration to Prevent and Manage Invasive Pests: Lessons Learned from the Pacific Northwest (United States). In Invasive Alien Species: Observations and Issues from around the World, pp339-352
Liu J, Tian Z, Zhang Y (2016) Structure-based discovery of potentially active semiochemicals for *Cydia pomonella* (L.). Scientific Reports 6:34600
Lockie S, Fidelman P, Paxton G, Bartelet H, Brooksbank L, Graham V, Draaisma L, Backhaus V (2025) Perceptions of crown-of-thorns starfish (COTS) management in the Great Barrier Reef, economic and social co-benefits, and regulatory considerations. A report to the Australian Government by the COTS Control Innovation Program.
Lucas J, Jones M (1976) Hybrid crown-of-thorns starfish (*Acanthaster planci* x *A. brevispinus*) reared to maturity in the laboratory. Nature 263:409–412
Lucas JS (1982) Quantitative studies of feeding and nutrition during larval development of the coral reef asteroid *Acanthster planci* (L.). Journal of Experimental Marine Biology and Ecology 65:173–193
Lundgren PB, Ayling T, Ayling A, Page C, Simmonds A, Doherty P (2013) Report from the operational-scale field trial of the new crown-of-thorns control method (injection with Bile salts). Report to the Australian Government Reef Programme. Reef and Rainforest Research Centre Limited, Cairns 17
Maran SPM (2022) Application of Artificial Intelligence (AI) Tools in Integrated Pest Management (IPM) - An Insect - Plant Interaction Perspective. Acta Scientific Agriculture 6:11–16
Matthews SA, Williamson DH, Beeden R, Emslie MJ, Abom RTM, Beard D, Bonin M, Bray P, Campili AR, Ceccarelli DM, Fernandes L, Fletcher CS, Godoy D, Hemingson CR, Jonker MJ, Lang BJ, Morris S, Mosquera E, Phillips GL, Sinclair-Taylor TH, Taylor S, Tracey D, Wilmes JC, Quincey R (2024) Protecting Great Barrier Reef resilience through effective management of crown-of-thorns starfish outbreaks. PLoS One 19:e0298073

McClintock JB, Lawrence JM (1984) Ingestive conditioning in *Luidia clathrata* (Say) (Echinodermata: Asteroidea): Effect of nutritional condition on selectivity, teloreception, and rates of ingestion. Marine and Freshwater Behaviour and Physiology 10:167–181
Meckley TD, Wagner CM, Luehring MA (2012) Field evaluation of larval odor and mixtures of synthetic pheromone components for attracting migrating sea lampreys in rivers. Journal of Chemical Ecology 38:1062–1069
Mendoza-Porras O, Nguyen TV, Shah RM, Thomas-Hall P, Bastin L, Deaker DJ, Motti CA, Byrne M, Beale DJ (2023) Biochemical metabolomic profiling of the Crown-of-Thorns Starfish (*Acanthaster*): New insight into its biology for improved pest management. Science of The Total Environment 861:160525
Metsalu T, Vilo J (2015) ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Research 43:W566-W570
Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 45:31–34
Mita M (1999) 1-Methyladenine: a starfish oocyte maturation-inducing substance. Zygote 8:S9–S11
Mita M, Osugi T, Kawada T, Satake H, Katayama H, Kitamura T, Miura T, Miura C (2022) Characterization and localization of relaxin-like gonad-stimulating peptide in the crown-of-thorns starfish, *Acanthaster* cf. *solaris*. General and Comparative Endocrinology 328:114107
Mitchell ER (2012) Management of Insect Pests with Semiochemicals: Concepts and Practice. Springer New York, NY
Moore R, Huxley C (1976) Aversive behaviour of crown-of-thorns starfish to coral evoked by food-related chemicals. Nature 263:407–409
Morin M, Jönsson M, Wang CK, Craik DJ, Degnan SM, Degnan BM (2023) Captivity induces a sweeping and sustained genomic response in a starfish. Molecular Ecology 32:3541–3556
Morin M, Jönsson M, Wang CK, Craik DJ, Degnan SM, Degnan BM (2024) Seasonal tissue-specific gene expression in wild crown-of-thorns starfish reveals reproductive and stress-related transcriptional systems. PLoS Biology 22:e3002620
Morin MA (2023) Characterising the crown-of-thorns starfish secretome to identify factors for future biocontrol systems. University of Queensland.
Mos B, Mesic N, Dworjanyn SA (2023) Variable food alters responses of larval crown-of-thorns starfish to ocean warming but not acidification. Communications Biology 6:639
Motti C, Hall M (2018) Review of whole animal behavioural testing facilities. Report prepared for AIMS Appropriation Task 002714. Australian Institute of Marine Science, Townsville. 47pp
Motti C, Vasile R, Robson B, Høj L, Wang C, Craik D, Degnan B, Degnan S, Cummins S, Martini A, Edwards O (2022) Deployment of semiochemical control agents to manage Crown-of-Thorns starfish populations. A report to the Australian Government by the COTS Control Innovation Program. 80pp
Motti CA, Bose U, Roberts RE, McDougall C, Smith MK, Hall MR, Cummins SF (2018) Chemical ecology of chemosensation in Asteroidea: Insights towards management strategies of pest species. Journal of Chemical Ecology 44:147–177
Musumeci T, Leonardi A, Bonaccorso A, Pignatello R, Puglisi G (2018) Tangential Flow Filtration Technique: An Overview on Nanomedicine Applications. Pharmaceutical Nanotechnology 6:48–60
Neil RC, Gomez Cabrera M, Uthicke S (2022) Juvenile age and available coral species modulate transition probability from herbivory to corallivory in *Acanthaster* cf. *solaris* (Crown-of-Thorns Seastar). Coral Reefs 41:843–848
Ngoan BT, Hanh TT, Vien le T, Diep CN, Thao NP, Thao do T, Thanh NV, Cuong NX, Nam NH, Thung do C, Kiem PV, Kim YH, Minh CV (2015) Asterosaponins and glycosylated polyhydroxysteroids from the starfish Culcita novaeguineae and their cytotoxic activities. Journal of Asian Natural Product Research 17:1010–1017
Ngugi DK, Ziegler M, Duarte CM, Voolstra CR (2020) Genomic Blueprint of Glycine Betaine Metabolism in Coral Metaorganisms and Their Contribution to Reef Nitrogen Budgets. iScience 23:101120
Ni G, Liang D, Cummins SF, Walton SF, Chen S, Wang Y, Mounsey K, Wei MQ, Yuan J, Pan X, Liu X, Wang T (2018) Comparative Proteomic Study of the Antiproliferative Activity of Frog Host-Defence Peptide Caerin 1.9 and Its Additive Effect with Caerin 1.1 on TC-1 Cells Transformed with HPV16 E6 and E7. Biomed Research International 2018:7382351

Noldus LPJJ, Spink AJ, Tegelenbosch RAJ (2001) EthoVision: A versatile video tracking system for automation of behavioral experiments. Behavior Research Methods, Instruments, & Computers 33:398–414
OECD 12 (2017) Guidance document on semiochemical active substances and plant protection products. Series on pesticides, OECD Publishing, 93pp
Ota E, Nagashima Y, Shiomi K, Sakurai T, Kojima C, Waalkes MP, Himeno S (2006) Caspase-independent apoptosis induced in rat liver cells by plancitoxin I, the major lethal factor from the crown-of-thorns starfish *Acanthaster planci* venom. Toxicon 48:1002–1010
Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques P, Li S, Xia J (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Research 49:W388-w396
Peden AS, Mac P, Fei YJ, Castro C, Jiang G, Murfitt KJ, Miska EA, Griffin JL, Ganapathy V, Jorgensen EM (2013) Betaine acts on a ligand-gated ion channel in the nervous system of the nematode *C. elegans*. Nature Neuroscience 16:1794–1801
Pennington MW, Czerwinski A, Norton RS (2018) Peptide therapeutics from venom: Current status and potential. Bioorganic & Medicinal Chemistry 26:2738–2758
Petie R, Garm A, Hall MR (2016a) Crown-of-thorns starfish have true image forming vision. Frontiers in Zoology 13:41
Petie R, Hall MR, Hyldahl M, Garm A (2016b) Visual orientation by the crown-of-thorns starfish (*Acanthaster planci*). Coral Reefs 35:1139–1150
Pratchett MS (2010) Changes in coral assemblages during an outbreak of *Acanthaster planci* at Lizard Island, northern Great Barrier Reef (1995–1999). Coral Reefs 29:717–725
Pratchett MS, Cumming GS (2019) Managing cross-scale dynamics in marine conservation: Pest irruptions and lessons from culling of crown-of-thorns starfish (*Acanthaster* spp.). Biological Conservation 238:108211
Pratchett MS, Caballes CF, Wilmes JC, Matthews S, Mellin C, Sweatman HPA, Nadler LE, Brodie J, Thompson CA, Hoey J, Bos AR, Byrne M, Messmer V, Fortunato SAV, Chen CCM, Buck ACE, Babcock RC, Uthicke S (2017) Thirty years of research on Crown-of-Thorns Starfish (1986-2016): Scientific advances and emerging opportunities. Diversity 9
Pratchett MS, Caballes CF, Cvitanovic C, Raymundo ML, Babcock RC, Bonin MC, Bozec Y-M, Burn D, Byrne M, Castro-Sanguino C, Chen CCM, Condie SA, Cowan Z-L, Deaker DJ, Desbiens A, Devantier LM, Doherty PJ, Doll PC, Doyle JR, Dworjanyn SA, Fabricius KE, Haywood MDE, Hock K, Hoggett AK, Høj L, Keesing JK, Kenchington RA, Lang BJ, Ling SD, Matthews SA, McCallum HI, Mellin C, Mos B, Motti CA, Mumby PJ, Stump RJW, Uthicke S, Vail L, Wolfe K, Wilson SK (2021) Knowledge Gaps in the Biology, Ecology, and Management of the Pacific Crown-of-Thorns Sea Star, *Acanthaster sp*., on Australia's Great Barrier Reef. The Biological Bulletin 241:330–346
Productivity Commission (2003) Industries, Land Use and Water Quality in the Great Barrier Reef Catchment, Research Report, Canberra
Progar RA, Gillette N, Fettig CJ, Hrinkevich K (2013) Applied Chemical Ecology of the Mountain Pine Beetle. Forest Science 60:414–433
Queensland Biodiscovery Act 2004 (2023) Biodiscovery Act 2004: Current as at 1 March 2023, Queensland.
Reddy MM, Goossens C, Zhou Y, Chaib S, Raviglione D, Nicolè F, Hume BCC, Forcioli D, Agostini S, Boissin E, Boss E, Bowler C, Vargas Cd, Douville E, Flores M, Furla P, Galand PE, Gilson E, Lombard F, Pesant S, Reynaud S, Sullivan MB, Sunagawa S, Troublé R, Thurber RV, Wincker P, Zoccola D, Voolstra CR, Allemand D, Planes S, Thomas OP, Banaigs B (2023) Multi-omics determination of metabolome diversity in natural coral populations in the Pacific Ocean. Communications Earth & Environment 4:281
Rivera-Posada J, Pratchett MS, Aguilar C, Grand A, Caballes CF (2014) Bile salts and the single-shot lethal injection method for killing crown-of-thorns sea stars (*Acanthaster planci*). Ocean & Coastal Management 102:383–390
Roberts RE, Motti CA, Baughman KW, Satoh N, Hall MR, Cummins SF (2017) Identification of putative olfactory G-protein coupled receptors in Crown-of-Thorns starfish, *Acanthaster planci*. BMC Genomics 18:400–415
Roberts RE, Powell D, Wang T, Hall MH, Motti CA, Cummins SF (2018) Putative chemosensory receptors are differentially expressed in the sensory organs of male and female crown-of-thorns starfish, *Acanthaster planci*. BMC Genomics 19:853

Robin DC, Marchand PA (2019) Evolution of the biocontrol active substances in the framework of the European Pesticide Regulation (EC) No. 1107/2009. Pest Management Science 75:950–958
Rogers JGD, Plagányi ÉE, Blamey LK, Desbiens A, Murphy N, Fletcher C (2025) Dynamic models to inform crown-of-thorns starfish (COTS) intervention strategies at the reef-scale. A report to the Australian Government by the COTS Control Innovation Program.
Scheufele G, Skinner C, Fletcher CS (2025) Economic assessment of manual crown-of-thorns-starfish (COTS) control scenarios on the Great Barrier Reef. A report to the Australian Government by the COTS Control Innovation Program.
Scott AM, Johnson NS, Siefkes MJ, Li W (2023) Synergistic behavioral antagonists of a sex pheromone reduce reproduction of invasive sea lamprey. iScience 26:107744
Shah RM, Hillyer KE, Stephenson S, Crosswell J, Karpe AV, Palombo EA, Jones OAH, Gorman D, Bodrossy L, van de Kamp J, Bissett A, Whiteley AS, Steven ADL, Beale DJ (2021) Functional analysis of pristine estuarine marine sediments. Science of the Total Environment 781:146526
Sharma A, Sandhi RK, Reddy GVP (2019) A Review of Interactions between Insect Biological Control Agents and Semiochemicals. Insects 10, 439
Shiomi K, Yamamoto S, Yamanaka H, Kikuchi T (1988) Purification and characterization of a lethal factor in venom from the crown-of-thorns starfish (*Acanthaster planci*). Toxicon 26:1077–1083
Shiomi K, Yamamoto S, Yamanaka H, Kikuchi T, Konno K (1990) Liver damage by the crown-of-thorns starfish (*Acanthaster planci*) lethal factor. Toxicon 28:469–475
Shiomi K, Midorikawa S, Ishida M, Nagashima Y, Nagai H (2004) Plancitoxins, lethal factors from the crown-of-thorns starfish *Acanthaster planci*, are deoxyribonucleases II. Toxicon 44:499–506
Siefkes MJ, Johnson NS, Muir AM (2021) A renewed philosophy about supplemental sea lamprey controls. Journal of Great Lakes Research 47:S742-S752
Skinner C, Condie SA, Fletcher CS, Mumby PJ (2025) Assessing Alternative Control Scenarios for Crown-of-Thorns Starfish on the Great Barrier Reef: An Ensemble Approach. A report to the Australian Government by the COTS Control Innovation Program.
Smith M, Rotgans BA, Wang, Phan, Bose U, Egertova M, Hall MR, Byrne M, Elizur A, Elphick MR, Motti C, Cummins S (2023) Structure and proteomic analysis of the crown-of-thorns starfish (*Acanthaster* sp.) radial nerve cord. Scientific Reports 13:3349
Smith MK, Chieu HD, Aizen Y, Motti C, Elizur A, Cummins SF (2019) A Crown-of-Thorns Seastar recombinant relaxin-like gonad-stimulating peptide triggers oocyte maturation and ovulation. General and Comparative Endocrinology 281:41–48
Smith MK, Bose U, Mita M, Hall MR, Elizur A, Motti CA, Cummins SF (2018) Differences in small molecule neurotransmitter profiles from the Crown-of-Thorns Seastar radial nerve revealed between sexes and following food-deprivation. Frontiers in Endocrinology 9:551
Smith MK, Wang T, Suwansa-Ard S, Motti CA, Elizur A, Zhao M, Rowe ML, Hall MR, Elphick MR, Cummins SF (2017) The neuropeptidome of the Crown-of-Thorns Starfish, *Acanthaster planci*. Journal of Proteomics 165:61–68
Sorensen P, Rue M, Leese J, Ghosal R, Lim H (2019) A Blend of F Prostaglandins Functions as an Attractive Sex Pheromone in Silver Carp. Fishes 4:27
Sorensen PW, Hoye TR (2007) A critical review of the discovery and application of a migratory pheromone in an invasive fish, the sea lamprey *Petromyzon marinus* L. Journal of Fish Biology 71:100–114
Sorensen PW, Johnson NS (2016) Theory and application of semiochemicals in nuisance fish control. Journal of Chemical Ecology 42:698–715
Spink AJ, Tegelenbosch RAJ, Buma MOS, Noldus. LPJJ (2001) The EthoVision video tracking system—A tool for behavioral phenotyping of transgenic mice. Physiology & Behavior 73:731–744
Srinivasan S, Torres AG, Ribas de Pouplana L (2021) Inosine in Biology and Disease. Genes (Basel) 12:600
Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier ME, Mitros T, Richards GS, Conaco C, Dacre M, Hellsten U, Larroux C, Putnam NH, Stanke M, Adamska M, Darling A, Degnan SM, Oakley TH, Plachetzki DC, Zhai Y, Adamski M, Calcino A, Cummins SF, Goodstein DM, Harris C, Jackson DJ, Leys SP, Shu S, Woodcroft BJ, Vervoort M, Kosik KS, Manning G, Degnan BM, Rokhsar DS (2010) The *Amphimedon queenslandica* genome and the evolution of animal complexity. Nature 466:720–726
Stacey N (2014) Hormonally Derived Pheromones in Teleost Fishes. In Fish Pheromones and Related Cues, pp33–88

Stenberg JA, Sundh I, Becher PG, Björkman C, Dubey M, Egan PA, Friberg H, Gil JF, Jensen DF, Jonsson M, Karlsson M, Khalil S, Ninkovic V, Rehermann G, Vetukuri RR, Viketoft M (2021) When is it biological control? A framework of definitions, mechanisms, and classifications. Journal of Pest Science 94:665–676

Stonik VA, Kicha AA, Malyarenko TV, Ivanchina NV (2020) Asterosaponins: Structures, Taxonomic Distribution, Biogenesis and Biological Activities. Marine Drugs 18:584

Suenaga K (2004) Bioorganic Studies on Marine Natural Products with Bioactivity, Such as Antitumor Activity and Feeding Attractance. Bulletin of the Chemical Society of Japan 77:443–451

Suggett DJ, Guest J, Camp EF, Edwards A, Goergen L, Hein M, Humanes A, Levy JS, Montoya-Maya PH, Smith DJ, Vardi T, Winters RS, Moore T (2024) Restoration as a meaningful aid to ecological recovery of coral reefs. npj Ocean Sustainability 3:20

Teruya T, Suenaga K, Koyama T, Nakano Y, Uemura D (2001) Arachidonic acid and α-linolenic acid, feeding attractants for the crown-of-thorns sea star *Acanthaster planci*, from the sea urchin *Toxopneustes pileolus*. Journal of Experimental Marine Biology and Ecology 266:123–134

Thao NP, Cuong NX, Luyen BTT, Thanh NV, Nhiem NX, Koh Y-S, Ly BM, Nam NH, Kiem PV, Minh CV, Kim YH (2013a) Anti-inflammatory asterosaponins from the starfish *Astropecten monacanthus*. Journal of Natural Products 76:1764–1770

Thao NP, Cuong NX, Luyen BTT, Nam NH, Cuong PV, Thanh NV, Nhiem NX, Hanh TTH, Kim EJ, Kang HK, Kiem PV, Minh CV, Kim YH (2013b) Steroidal Constituents from the Starfish *Astropecten polyacanthus* and Their Anticancer Effects. Chemical & Pharmaceutical Bulletin 61:1044–1051

Tilmaciu CM, Morris MC (2015) Carbon nanotube biosensors. Frontiers in Chemistry 3:59

Turillazzi S (2006) *Polistes* venom a multifunctional secretion. Annales Zoologici Fennici 43:488–499

Vogler C, Benzie JAH, Tenggardjaja K, Ambariyanto, Barber PH, Worheide G (2013) Phylogeography of the crown-of-thorns starfish: genetic structure within the Pacific species. Coral Reefs 32:515–525

Wagner CM, Jones ML, Twohey MB, Sorensen PW (2006) A field test verifies that pheromones can be useful for sea lamprey (*Petromyzon marinus*) control in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 63:475–479

Wagner CM, Hanson JE, Meckley TD, Johnson NS, Bals JD (2018) A simple, cost-effective emitter for controlled release of fish pheromones: Development, testing, and application to management of the invasive sea lamprey. PLoS One 13:e0197569

Wang J, Liang W, Gao Z, Zhang L, Deng B, Chen X, Sha Z, Ru X (2024) Potential sexual pheromones of sea cucumber *Apostichopus japonicus* and their functions on aggregative and spawning behavior. Aquaculture Reports 36:102186

Wang Y, Wang Y, Yang Y, Ni G, Li Y, Chen M (2023) Chromosome-level genome assembly of the northern Pacific seastar *Asterias amurensis*. Scientific Data 10:767

Weissburg MJ (2011) Waterborne Chemical Communication: Stimulus Dispersal Dynamics and Orientation Strategies in Crustaceans. In: Breithaupt T, Thiel M (eds) Chemical Communication in Crustaceans. Springer New York, New York, NY, pp63–83

Westcott DA, Fletcher CS, Kroon FJ, Babcock RC, Plaganyi EE, Pratchett MS, Bonin MC (2020) Relative efficacy of three approaches to mitigate Crown-of-Thorns Starfish outbreaks on Australia's Great Barrier Reef. Scientific Reports 10:12594

Wolfe K, Graba-Landry A, Dworjanyn SA, Byrne M (2015) Larval starvation to satiation: influence of nutrient regime on the success of *Acanthaster planci*. PLoS ONE 10:e0122010

Wu H, Praveen P, Handley TNG, Chandrashekar C, Cummins SF, Bathgate RAD, Hossain MA (2024) Total Chemical Synthesis of Aggregation-Prone Disulfide-Rich Starfish Peptides. Chemistry – A European Journal 30:e202400933

Yamaguchi K, Kadota M, Nishimura O, Ohishi Y, Naito Y, Kuraku S (2021) Technical considerations in Hi-C scaffolding and evaluation of chromosome-scale genome assemblies. Molecular Ecology 30:5923-5934

Yang L, Zhang Y, Chen H, Gao Q, Luo P, Hu J, Zheng F, Yuan Y, Fu L, Chen C (2022) Physiological and transcriptomic responses to starvation in the corallivorous crown-of-thorn starfish. Frontiers in Marine Science, 9

Yap K (2026) A molecular platform for design and production of next-generation peptides. University of Queensland.

Yap K, Du J, Rehm FBH, Tang SR, Zhou Y, Xie J, Wang CK, Veer SJd, Lua LHL, Durek T, Craik DJ (2021) Yeast-based bioproduction of disulfide-rich peptides and their cyclization via asparaginyl endopeptidases. Nature Protocols 16:1740–1760

Yap K, Motti C, Chan LY, Thomas-Hall P, Bastin L, Morin M, Jönsson M, Lua LHL, Degnan SM, Degnan BM, Wang CK, Craik DJ (2026) A generalizable, scalable multi-omic approach to identify biomimetics for controlling marine pests: the crown-of-thorns starfish as a test case. One Earth submitted

Yap K, Du J, Looi FY, Tang SR, Veer SJd, Bony AR, Rehm FBH, Xie J, Chan LY, Wang CK, Adams DJ, Lua LHL, Durek T, Craik DJ (2020) An environmentally sustainable biomimetic production of cyclic disulfide-rich peptides. Green Chemistry 22:5002–5016

Yasuda N, Nagai S, Hamaguchi M, Okaji K, Gérard K, Nadaoka K (2009) Gene flow of *Acanthaster planci* (L.) in relation to ocean currents revealed by microsatellite analysis. Molecular Ecology 18:1574–1590

Yasuda N, Inoue J, Hall MR, Nair MR, Adjeroud M, Fortes MD, Nishida M, Tuivavalagi N, Ravago-Gotanco R, Forsman ZH, Soliman T, Koyanagi R, Hisata K, Motti CA, Satoh N (2022) Two Hidden mtDNA-Clades of Crown-of-Thorns Starfish in the Pacific Ocean. Frontiers in Marine Science 9

Yuasa H, Kajitani R, Nakamura Y, Takahashi K, Okuno M, Kobayashi F, Shinoda T, Toyoda A, Suzuki Y, Thongtham N, Forsman Z, Bronstein O, Seveso D, Montalbetti E, Taquet C, Eyal G, Yasuda N, Itoh T (2021) Elucidation of the speciation history of three sister species of crown-of-thorns starfish (*Acanthaster* spp.) based on genomic analysis. DNA Research 28

Zanders ED (2011) Drug Discovery Pipeline Overview The Science and Business of Drug Discovery: Demystifying the Jargon. Springer US, Boston, MA, pp93–95

Zeeck E, Harder T, Beckmann M (1998) Inosine, L-glutamic acid and L-glutamine as components of a sex pheromone complex of the marine polychaete *Nereis succinea* (Annelida: Polychaeta). Chemoecology 8:77–84

Zhu Y, Lu Z (2024) Proteome structuring of crown-of-thorns starfish. Frontiers in Marine Science, 11:1487904

10 APPENDICES

Appendix A

Table A 1 Web of Science Citation report of publications reporting on the crown-of-thorns starfish (COTS) between January 2017 and June 2024. There are 146 publications reporting directly on COTS and a further 62 reporting indirectly.

Authors	Article Title	Journal	Year	Volume	Page	DOI Link
Direct reporting on Crown-of-Thorns Starfish (n=146)						
Smith et al.	The neuropeptidome of the Crown-of-Thorns Starfish, *Acanthaster planci*	J Proteomics	2017	165	61-68	http://dx.doi.org/10.1016/j.jprot.2017.05.026
Kamya et al.	Indirect effects of ocean acidification drive feeding and growth of juvenile crown-of-thorns starfish, *Acanthaster planci*	Proc. Royal Soc. B Biol Sc.	2017	284	-	http://dx.doi.org/10.1098/rspb.2017.0778
Vanhatalo et al.	Spatiotemporal modelling of crown-of-thorns starfish outbreaks on the Great Barrier Reef to inform control strategies	J Appl. Ecol.	2017	54	188-197	http://dx.doi.org/10.1111/1365-2664.12710
Allen et al.	The Effects of Salinity and pH on Fertilization, Early Development, and Hatching in the Crown-of-Thorns Seastar	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010013
Brodie et al.	Potential Enhanced Survivorship of Crown of Thorns Starfish Larvae due to Near-Annual Nutrient Enrichment during Secondary Outbreaks on the Central Mid-Shelf of the Great Barrier Reef, Australia	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010017
Caballes et al.	Environmental and biological cues for spawning in the crown-of-thorns starfish	Plos One	2017	12	-	http://dx.doi.org/10.1371/journal.pone.0173964
Caballes et al.	Interactive Effects of Endogenous and Exogenous Nutrition on Larval Development for Crown-Of-Thorns Starfish	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010015
Wilmes et al.	Modelling Growth of Juvenile Crown-of-Thorns Starfish on the Northern Great Barrier Reef	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010001
Harrison et al.	Microsatellites Reveal Genetic Homogeneity among Outbreak Populations of Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*) on Australia's Great Barrier Reef	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010016
Pratchett et al.	Thirty Years of Research on Crown-of-Thorns Starfish (1986-2016): Scientific Advances and Emerging Opportunities	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9040041

Authors	Article Title	Journal	Year	Volume	Page	DOI Link
Pratchett et al.	Body size and substrate type modulate movement by the western Pacific crown-of-thorns starfish, *Acanthaster solaris*	Plos One	2017	12	-	http://dx.doi.org/10.1371/journal.pone.0180805
Caballes et al.	Environmental Tipping Points for Sperm Motility, Fertilization, and Embryonic Development in the Crown-of-Thorns Starfish	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010010
Roberts et al.	Identification of putative olfactory G-protein coupled receptors in Crown-of-Thorns starfish, *Acanthaster planci*	BMC Genomics	2017	18	-	http://dx.doi.org/10.1186/s12864-017-3793-4
Chen et al.	Bayesian semi-individual based model with approximate Bayesian computation for parameters calibration: Modelling Crown-of-Thorns populations on the Great Barrier Reef	Ecological Modelling	2017	364	113-123	http://dx.doi.org/10.1016/j.ecolmodel.2017.09.006
Hall et al.	The crown-of-thorns starfish genome as a guide for biocontrol of this coral reef pest	Nature	2017	544	231-	http://dx.doi.org/10.1038/nature22033
Bose et al.	Multiomics analysis of the giant triton snail salivary gland, a crown-of-thorns starfish predator	Scientific Reports	2017	7	-	http://dx.doi.org/10.1038/s41598-017-05974-x
Bose et al.	Neuropeptides encoded within a neural transcriptome of the giant triton snail *Charonia tritonis*, a Crown-of-Thorns Starfish predator	Peptides	2017	98	3-14	http://dx.doi.org/10.1016/j.peptides.2017.01.004
Cowan et al.	Known Predators of Crown-of-Thorns Starfish (*Acanthaster* spp.) and Their Role in Mitigating, If Not Preventing, Population Outbreaks	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010007
Vercelloni et al.	Crown-of-thorns starfish undermine the resilience of coral populations on the Great Barrier Reef	Glob. Ecol. BioGeograph.	2017	26	846-853	http://dx.doi.org/10.1111/geb.12590
Rogers et al.	Aggregation, Allee effects and critical thresholds for the management of the crown-of-thorns starfish *Acanthaster planci*	Mar. Ecol. Prog. Ser.	2017	578	99-114	http://dx.doi.org/10.3354/meps12252
Pratchett et al.	Larval Survivorship and Settlement of Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*) at Varying Algal Cell Densities	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010002
Messmer et al.	Variation in Incidence and Severity of Injuries among Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*) on Australia's Great Barrier Reef	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010012
Mellin et al.	Selective Feeding and Microalgal Consumption Rates by Crown-Of-Thorns Seastar (*Acanthaster* cf. *solaris*) Larvae	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010008
Medina et al.	BIOCONTROL Crown-of-thorns no more	Nature	2017	544	168-170	http://dx.doi.org/10.1038/nature21905
Kayal et al.	Colonies of the fire coral *Millepora platyphylla* constitute scleractinian survival oases during *Acanthaster* outbreaks in French Polynesia	Marine Biodiversity	2017	47	255-258	http://dx.doi.org/10.1007/s12526-016-0465-6
Wolfe et al.	Superstars: Assessing nutrient thresholds for enhanced larval success of *Acanthaster planci*, a review of the evidence	Mar. Poll. Bull.	2017	116	307-314	http://dx.doi.org/10.1016/j.marpolbul.2016.12.079

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Kayal et al.	Bias associated with the detectability of the coral-eating pest crown-of-thorns seastar and implications for reef management	Royal Soc. Open Sci.	2017	4	-	http://dx.doi.org/10.1098/rsos.170396
Haszprunar et al.	Persistent Gaps of Knowledge for Naming and Distinguishing Multiple Species of Crown-of-Thorns-Seastar in the *Acanthaster planci* Species Complex	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9020022
Tian et al.	Artificial breeding and culture of the crown-of-thorns starfish *Acanthaster planci* (Linnaeus, 1758) larvae in China	Inverteb. Reprod. Devleop.	2017	61	157-163	http://dx.doi.org/10.1080/07924259.2017.1315342
Cowan et al.	Interspecific variation in potential importance of planktivorous damselfishes as predators of *Acanthaster* sp eggs	Coral Reefs	2017	36	653-661	http://dx.doi.org/10.1007/s00338-017-1556-y
Sparks et al.	Paternal identity influences response of *Acanthaster planci* embryos to ocean acidification and warming	Coral Reefs	2017	36	325-338	http://dx.doi.org/10.1007/s00338-016-1505-1
Scott et al.	Population dynamics of corallivores (*Drupella* and *Acanthaster*) on coral reefs of Koh Tao, a diving destination in the Gulf of Thailand	Raffles Bull. Zoo.	2017	65	68-79	
MacNeil et al.	Age and Growth of An Outbreaking *Acanthaster* cf. *solaris* Population within the Great Barrier Reef	Diversity	2017	9	-	http://dx.doi.org/10.3390/d9010018
Doyle et al.	Quantifying larvae of the coralivorous seastar *Acanthaster* cf. *solaris* on the Great Barrier Reef using qPCR	Marine Biology	2017	164	-	http://dx.doi.org/10.1007/s00227-017-3206-x
Kamya et al.	Enhanced performance of juvenile crown of thorns starfish in a warm-high CO2 ocean exacerbates poor growth and survival of their coral prey	Coral Reefs	2018	37	751-762	http://dx.doi.org/10.1007/s00338-018-1699-5
Smith et al.	Differences in Small Molecule Neurotransmitter Profiles From the Crown-of-Thorns Seastar Radial Nerve Revealed Between Sexes and Following Food-Deprivation	Front. Endocrin.	2018	9	-	http://dx.doi.org/10.3389/fendo.2018.00551
Roberts et al.	Putative chemosensory receptors are differentially expressed in the sensory organs of male and female crown-of-thorns starfish, *Acanthaster planci*	BMC Genomics	2018	19	-	http://dx.doi.org/10.1186/s12864-018-5246-0
Wilmes et al.	Contributions of pre- versus post-settlement processes to fluctuating abundance of crown-of-thorns starfishes (*Acanthaster* spp.)	Mar. Poll. Bull.	2018	135	332-345	http://dx.doi.org/10.1016/j.marpolbul.2018.07.006
Boström-Einarsson et al.	Environmental impact monitoring of household vinegar-injections to cull crown-of-thorns starfish, *Acanthaster* spp.	Ocean Coast. Manag.	2018	155	83-89	http://dx.doi.org/10.1016/j.ocecoaman.2018.01.023
Keesing et al.	Mortality rates of small juvenile crown-of-thorns starfish *Acanthaster planci* on the Great Barrier Reef: implications for population size and larval settlement thresholds for outbreaks	Mar. Ecol. Prog. Ser.	2018	597	179-190	http://dx.doi.org/10.3354/meps12606
Motti et al.	Chemical Ecology of Chemosensation in Asteroidea: Insights Towards Management Strategies of Pest Species	J Chem. Ecol.	2018	44	147-177	http://dx.doi.org/10.1007/s10886-018-0926-4

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Høj et al.	Crown-of-Thorns Sea Star *Acanthaster* cf. *solaris* Has Tissue-Characteristic Microbiomes with Potential Roles in Health and Reproduction	A Env. Microbiol.	2018	84	-	http://dx.doi.org/10.1128/AEM.00181-18
Chak et al.	Effectiveness of the removal of coral-eating predator *Acanthaster planci* in Pulau Tioman Marine Park, Malaysia	J Mar. Biol. Assoc. UK	2018	98	183-189	http://dx.doi.org/10.1017/S002531541600117X
Uthicke et al.	Effects of larvae density and food concentration on Crown-of-Thorns seastar (*Acanthaster* cf *solaris*) development in an automated flow-through system	Scientific Reports	2018	8	-	http://dx.doi.org/10.1038/s41598-017-19132-w
Lowe et al.	The crowns have eyes: multiple opsins found in the eyes of the crown-of-thorns starfish *Acanthaster planci*	BMC Evol. Biol.	2018	18	-	http://dx.doi.org/10.1186/s12862-018-1276-0
Boström-Einarsson et al.	Dead enough? The thorny issue of culling crown-of-thorns starfish using vinegar injections. A reply to Dumas et al. The chaotic history of using vinegar injections to control *Acanthaster* spp. populations, Ocean & Coastal management, 2018, Volume 165, Page number 434-435	Ocean Coast. Manag.	2018	165	436-437	http://dx.doi.org/10.1016/j.ocecoaman.2018.05.016
Dumas et al.	The chaotic history of using vinegar injections to control *Acanthaster* spp. populations. A comment to Bostrom-Einarsson L., Bonin M. C., Moon S. and Firth S. (2018). Environmental impact monitoring of household vinegar injections to cull crown-of-thorns starfish, *Acanthaster* spp. Ocean & Coastal Management 155: 83-89	Ocean Coast. Manag.	2018	165	434-435	http://dx.doi.org/10.1016/j.ocecoaman.2018.05.017
Saponari et al.	Monitoring and assessing a 2-year outbreak of the corallivorous seastar *Acanthaster planci* in Ari Atoll, Republic of Maldives	Environ. Monitor. Assess.	2018	190	-	http://dx.doi.org/10.1007/s10661-018-6661-z
Adjeroud et al.	Ephemeral and Localized Outbreaks of the Coral Predator *Acanthaster* cf. *solaris* in the Southwestern Lagoon of New Caledonia	Zoological Studies	2018	57	-	http://dx.doi.org/10.6620/ZS.2018.57-04
Uthicke et al.	eDNA detection of corallivorous seastar (*Acanthaster* cf. *solaris*) outbreaks on the Great Barrier Reef using digital droplet PCR	Coral Reefs	2018	37	1229-1239	http://dx.doi.org/10.1007/s00338-018-1734-6
Allen et al.	Larval cloning in the crown-of-thorns sea star, a keystone coral predator	Mar. Ecol. Prog. Ser.	2019	609	271-276	http://dx.doi.org/10.3354/meps12843
Smith et al.	A Crown-of-Thorns Seastar recombinant relaxin-like gonad-stimulating peptide triggers oocyte maturation and ovulation	Gen. Comp. Endocrin.	2019	281	41-48	http://dx.doi.org/10.1016/j.ygcen.2019.05.009
Korsvig-Nielsen et al.	Eyes and negative phototaxis in juvenile crown-of-thorns starfish, Acanthaster species complex	Biology Open	2019	8	-	http://dx.doi.org/10.1242/bio.041814
Pratchett et al.	Managing cross-scale dynamics in marine conservation: Pest irruptions and lessons from culling of crown-of-thorns starfish (*Acanthaster* spp.)	Biological Conservation	2019	238	-	http://dx.doi.org/10.1016/j.biocon.2019.108211

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Wilmes et al.	Incidence and severity of injuries among juvenile crown-of-thorns starfish on Australia's Great Barrier Reef	Coral Reefs	2019	38	1187-1195	http://dx.doi.org/10.1007/s00338-019-01845-1
Haywood et al.	Crown-of-thorns starfish impede the recovery potential of coral reefs following bleaching	Marine Biology	2019	166	-	http://dx.doi.org/10.1007/s00227-019-3543-z
Budden et al.	Effect of sublethal predation on reproductive output of the crown-of-thorns starfish *Acanthaster* sp., with an overview of arm damage	Mar. Ecol. Prog. Ser.	2019	629	103-116	http://dx.doi.org/10.3354/meps13111
Keesing et al.	Two time losers: selective feeding by crown-of-thorns starfish on corals most affected by successive coral-bleaching episodes on western Australian coral reefs	Marine Biology	2019	166	-	http://dx.doi.org/10.1007/s00227-019-3515-3
Reimer et al.	Crown-of-thorns starfish outbreak at oceanic Dongsha Atoll in the northern South China Sea	Marine Biodiversity	2019	49	2495-2497	http://dx.doi.org/10.1007/s12526-019-01021-2
Uthicke et al.	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	2019	166	-	http://dx.doi.org/10.1007/s00227-019-3582-5
Deaker et al.	The hidden army: corallivorous crown-of-thorns seastars can spend years as herbivorous juveniles	Biology Letters	2020	16	-	http://dx.doi.org/10.1098/rsbl.2019.0849
Wilmes et al.	Habitat associations of settlement-stage crown-of-thorns starfish on Australia's Great Barrier Reef	Coral Reefs	2020	39	1163-1174	http://dx.doi.org/10.1007/s00338-020-01950-6
Cowan et al.	Crown-of-thorns starfish larvae are vulnerable to predation even in the presence of alternative prey	Coral Reefs	2020	39	293-303	http://dx.doi.org/10.1007/s00338-019-01890-w
Ling et al.	Homing behaviour by destructive crown-of-thorns starfish is triggered by local availability of coral prey	Proc. Royal Soc. B Biol Sc.	2020	287	-	http://dx.doi.org/10.1098/rspb.2020.1341
Wilmes et al.	Contrasting size and fate of juvenile crown-of-thorns starfish linked to ontogenetic diet shifts	Proc. Royal Soc. B Biol Sc.	2020	287	-	http://dx.doi.org/10.1098/rspb.2020.1052
Babcock et al.	Suppressing the next crown-of-thorns outbreak on the Great Barrier Reef	Coral Reefs	2020	39	1233-1244	http://dx.doi.org/10.1007/s00338-020-01978-8
Kroon et al.	DNA-based identification of predators of the corallivorous Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*) from fish faeces and gut contents	Scientific Reports	2020	10	-	http://dx.doi.org/10.1038/s41598-020-65136-4
Westcott et al.	Relative efficacy of three approaches to mitigate Crown-of-Thorns Starfish outbreaks on Australia's Great Barrier Reef	Scientific Reports	2020	10	-	http://dx.doi.org/10.1038/s41598-020-69466-1
Dumas et al.	Citizen Science, a promising tool for detecting and monitoring outbreaks of the crown-of-thorns starfish *Acanthaster* spp.	Scientific Reports	2020	10	-	http://dx.doi.org/10.1038/s41598-019-57251-8
Plagányi et al.	Ecological analyses to inform management targets for the culling of crown-of-thorns starfish to prevent coral decline	Coral Reefs	2020	39	1483-1499	http://dx.doi.org/10.1007/s00338-020-01981-z
Matthews et al.	COTSMod: A spatially explicit metacommunity model of outbreaks of crown-of-thorns starfish and coral recovery	Pop. Dyn. Reef Crisis	2020	87	259-290	http://dx.doi.org/10.1016/bs.amb.2020.09.001

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Matthews et al.	Larval connectivity and water quality explain spatial distribution of crown-of-thorns starfish outbreaks across the Great Barrier Reef	Pop. Dyn. Reef Crisis	2020	87	223-258	http://dx.doi.org/10.1016/bs.amb.2020.08.007
Hue et al.	Temperature affects the reproductive outputs of coral-eating starfish *Acanthaster* spp. after adult exposure to near-future ocean warming and acidification	Mar. Environ. Res.	2020	162	-	http://dx.doi.org/10.1016/j.marenvres.2020.105164
Wada et al.	A ubiquitous subcuticular bacterial symbiont of a coral predator, the crown-of-updates thorns starfish, in the Indo-Pacific	Microbiome	2020	8	-	http://dx.doi.org/10.1186/s40168-020-00880-3
Rodríguez-Villalobos et al.	Temporal and Spatial Abundance of *Acanthaster* cf. *solaris* (Echinodermata: Asteroidea) in a National Park in the Gulf of California, Mexico: 2005-2016	Pacific Science	2020	74	269-282	http://dx.doi.org/10.2984/74.3.5
Kroon et al.	DNA-based identification of predators of the corallivorous Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*) from fish faeces and gut contents (vol 10, 8184, 2020)	Scientific Reports	2020	10	-	http://dx.doi.org/10.1038/s41598-020-75953-2
Inoue et al.	An Investigation into the Genetic History of Japanese Populations of Three Starfish, *Acanthaster planci*, *Linckia laevigata*, and *Asterias amurensis*, Based on Complete Mitochondrial DNA Sequences	G3-Genes	2020	10	2519-2528	http://dx.doi.org/10.1534/g3.120.401155
Burn et al.	Biogeographical variation in diurnal behaviour of *Acanthaster planci* versus *Acanthaster* cf. *solaris*	Plos One	2020	15	-	http://dx.doi.org/10.1371/journal.pone.0228796
Kroon et al.	Fish predators control outbreaks of Crown-of-Thorns Starfish	Nature Communications	2021	12	-	http://dx.doi.org/10.1038/s41467-021-26786-8
Chen et al.	Insights Into the Environmental Impact on Genetic Structure and Larval Dispersal of Crown-of-Thorns Starfish in the South China Sea	Front. Mar. Sci.	2021	8	-	http://dx.doi.org/10.3389/fmars.2021.728349
Pratchett et al.	Reproductive investment and fecundity of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) on the Great Barrier Reef	Marine Biology	2021	168	-	http://dx.doi.org/10.1007/s00227-021-03897-w
Doll et al.	DNA-Based Detection and Patterns of Larval Settlement of the Corallivorous Crown-of-Thorns Sea Star (*Acanthaster* sp.)	Biological Bulletin	2021	241	271-285	http://dx.doi.org/10.1086/717539
Deaker et al.	Echidnas of the Sea: The Defensive Behavior of Juvenile and Adult Crown-of-Thorns Sea Stars	Biological Bulletin	2021	241	259-270	http://dx.doi.org/10.1086/716777
Iguchi et al.	Genetic structure of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) in southern Japan based on genome-wide RADseq analysis	Coral Reefs	2021	40	1379-1385	http://dx.doi.org/10.1007/s00338-021-02145-3
Deaker et al.	Coral defences: the perilous transition of juvenile crown-of-thorns starfish to corallivory	Mar. Ecol. Prog. Ser.	2021	665	115-125	http://dx.doi.org/10.3354/meps13660
Sill et al.	Climate change impacts on the ecological dynamics of two coral reef species, the humphead wrasse (*Cheilinus undulatus*) and crown-of-thorns starfish (*Acanthaster planci*)	Ecological Informatics	2021	65	-	http://dx.doi.org/10.1016/j.ecoinf.2021.101399

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Martínez-Sarabia et al.	Damage caused by crown-of-thorns starfish (*Acanthaster* cf. *solaris*) outbreak to restored corals in the southern Gulf of California, Mexico	Bull. Mar. Sci.	2021	97	329-336	http://dx.doi.org/10.5343/bms.2020.0034
Yuasa et al.	Elucidation of the speciation history of three sister species of crown-of-thorns starfish (*Acanthaster* spp.) based on genomic analysis	DNA Research	2021	28	-	http://dx.doi.org/10.1093/dnares/dsab012
Caballes et al.	Temporal variability in gametogenesis and spawning patterns of crown-of-thorns starfish within the outbreak initiation zone in the northern Great Barrier Reef	Marine Biology	2021	168	-	http://dx.doi.org/10.1007/s00227-020-03818-3
Kwong et al.	Quantifying shedding and degradation rates of environmental DNA (eDNA) from Pacific crown-of-thorns seastar (*Acanthaster* cf. *solaris*)	Marine Biology	2021	168	-	http://dx.doi.org/10.1007/s00227-021-03896-x
Balu et al.	Is predation of juvenile crown-of-thorns seastars (*Acanthaster* cf. *solaris*) by peppermint shrimp (*Lysmata vittata*) dependent on age, size, or diet?	Coral Reefs	2021	40	641-649	http://dx.doi.org/10.1007/s00338-020-02047-w
Hernández-Morales et al.	Variability of size and food type of Ac*anthaster planci* (Echinodermata: Asteroidea) in the southern Gulf of California, Mexico	Revista de Biol. Trop.	2021	69	185-201	http://dx.doi.org/10.15517/rbt.v69iSuppl.1.46352
Caragnano et al.	A snapshot of reef conditions in North Ari Atoll (Maldives) following the 2016 bleaching event and *Acanthaster planci* outbreak	Mar. Fresh. Res.	2021	72	987-996	http://dx.doi.org/10.1071/MF20119
Tkachenko et al.	Extensive coral reef decline in Nha Trang Bay, Vietnam: *Acanthaster planci* outbreak: the final event in a sequence of chronic disturbances	Mar. Fresh. Res.	2021	72	186-199	http://dx.doi.org/10.1071/MF20005
Ha et al.	Asterosaponins from the tropical starfish *Acanthaster planci* and their cytotoxic and anticancer activities in vitro	Nat. Prod. Res.	2021	35	548-555	http://dx.doi.org/10.1080/14786419.2019.1585845
Yang et al.	Physiological and transcriptomic responses to starvation in the corallivorous crown-of-thorn starfish	Front. Mar. Sci.	2022	9	-	http://dx.doi.org/10.3389/fmars.2022.1021377
Yasuda et al.	Two Hidden mtDNA-Clades of Crown-of-Thorns Starfish in the Pacific Ocean	Front. Mar. Sci.	2022	9	-	http://dx.doi.org/10.3389/fmars.2022.831240
Caballes et al.	Prevalence and severity of sublethal injuries in crown-of-thorns starfish relative to marine reserves in the Great Barrier Reef	Aqua. Conserv. Mar. Fresh. Ecosys.	2022	32	993-1004	http://dx.doi.org/10.1002/aqc.3762
Mita et al.	Characterization and localization of relaxin-like gonad-stimulating peptide in the crown-of-thorns starfish, *Acanthaster* cf. *solaris*	Gen. Comp. Endocrin.	2022	328	-	http://dx.doi.org/10.1016/j.ygcen.2022.114107
Qin et al.	Significant Changes in Bacterial Communities Associated with *Pocillopora* Corals Ingestion by Crown-of-Thorns Starfish: An Important Factor Affecting the Coral's Health	Microorganisms	2022	10	-	http://dx.doi.org/10.3390/microorganisms10020207
Clements et al.	Freshening of Great Barrier Reef waters is deleterious for larval crown-of-thorns starfish, counter to the terrestrial runoff hypothesis	Mar. Ecol. Prog. Ser.	2022	696	1-14	http://dx.doi.org/10.3354/meps14150

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Neil et al.	Juvenile age and available coral species modulate transition probability from herbivory to corallivory in *Acanthaster* cf. *solaris* (Crown-of-Thorns Seastar)	Coral Reefs	2022	41	843-848	http://dx.doi.org/10.1007/s00338-022-02255-6
Lang et al.	Effects of elevated temperature on the performance and survival of pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)	Marine Biology	2022	169	-	http://dx.doi.org/10.1007/s00227-022-04027-w
Deaker et al.	The relationship between size and metabolic rate of juvenile crown of thorns starfish	Invert. Biol.	2022	141	-	http://dx.doi.org/10.1111/ivb.12382
Tkachenko et al.	Concurrent effect of crown-of-thorns starfish outbreak and thermal anomaly of 2020 on coral reef communities of the Spratly Islands (South China Sea)	Mar. Ecol. Evol. Perspect.	2022	43	-	http://dx.doi.org/10.1111/maec.12717
Jönsson et al.	Sex-specific expression of pheromones and other signals in gravid starfish	BMC Biology	2022	20	-	http://dx.doi.org/10.1186/s12915-022-01491-0
Hue et al.	Impact of near-future ocean warming and acidification on the larval development of coral-eating starfish *Acanthaster* cf. *solaris* after parental exposure	J Exp. Mar. Biol. Ecol.	2022	548	-	http://dx.doi.org/10.1016/j.jembe.2021.151685
Heng et al.	Crown-of-thorns starfish outbreak at Taiping Island (Itu Aba), Spratlys, South China Sea	Bull. Mar. Sci.	2022	98	101-102	http://dx.doi.org/10.5343/bms.2021.0030
Abbasi et al.	A Cooperative Dynamic Task Assignment Framework for COTSBot AUVs	IEEE Trans. Automat. Sci. Eng.	2022	19	1163-1179	http://dx.doi.org/10.1109/TASE.2020.3044155
Mondal et al.	Decadal status of *Acanthaster planci* (Linnaeus, 1758) along the coral reef habitat of Andaman and Nicobar Islands	Indian J. Geo-Mar. Sci.	2022	51	753-759	http://dx.doi.org/10.56042/ijms.v51i09.2331
Kuo et al.	What is for dessert? Crown-of-thorns starfish feeds on non-scleractinian anthozoans at Taiping Island (Itu Aba), Spratlys, South China Sea	Marine Biodiversity	2022	52	-	http://dx.doi.org/10.1007/s12526-021-01240-6
Horoiwa et al.	Integrated Population Genomic Analysis and Numerical Simulation to Estimate Larval Dispersal of *Acanthaster* cf. *solaris* Between Ogasawara and Other Japanese Regions	Front. Mar. Sci.	2022	8	-	http://dx.doi.org/10.3389/fmars.2021.688139
Rogers et al.	Culling corallivores improves short-term coral recovery under bleaching scenarios	Nat. Comm.	2022	13	-	http://dx.doi.org/10.1038/s41467-022-30213-x
Uthicke et al.	Developing an effective marine eDNA monitoring: eDNA detection at pre-outbreak densities of corallivorous seastar (*Acanthaster* cf. *solaris*)	Sci. Total Environ.	2022	851	-	http://dx.doi.org/10.1016/j.scitotenv.2022.158143
Hillberg et al.	Crown-of-thorns starfish spines secrete defence proteins	Peerj	2023	11	-	http://dx.doi.org/10.7717/peerj.15689
Desbiens et al.	Novel rubble-dwelling predators of herbivorous juvenile crown-of-thorns starfish (*Acanthaster* sp.)	Coral Reefs	2023	42	579-591	http://dx.doi.org/10.1007/s00338-023-02364-w
Peng et al.	Phytoplankton community structure and environmental factors during the outbreak of Crown-of-Thorns Starfish in Xisha Islands, South China Sea	Environmental Research	2023	235	-	http://dx.doi.org/10.1016/j.envres.2023.116568

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Wang et al.	Ultrasensitive and on-site eDNA detection for the monitoring of crown-of-thorns starfish densities at the pre-outbreak stage using an electrochemical biosensor	Biosen. Bioelec.	2023	230	-	http://dx.doi.org/10.1016/j.bios.2023.115265
Keesing et al.	The short spined crown-of-thorns starf*ish Acanthaster brevispinus* is a corallivore too	Coral Reefs	2023	42	399-404	http://dx.doi.org/10.1007/s00338-023-02351-1
Mos et al.	Variable food alters responses of larval crown-of-thorns starfish to ocean warming but not acidification	Communications Biology	2023	6	-	http://dx.doi.org/10.1038/s42003-023-05028-1
Doll et al.	Settlement cue selectivity by larvae of the destructive crown-of-thorns starfish	Biology Letters	2023	19	-	http://dx.doi.org/10.1098/rsbl.2022.0399
Smith et al.	Structure and proteomic analysis of the crown-of-thorns starfish (*Acanthaster* sp.) radial nerve cord	Scientific Reports	2023	13	-	http://dx.doi.org/10.1038/s41598-023-30425-1
Doll et al.	Induction of larval settlement in crown-of-thorns starfish is not mediated by conspecific cues	Scientific Reports	2023	13	-	http://dx.doi.org/10.1038/s41598-023-44422-x
Khan et al.	Identification of Crown of Thorns Starfish (COTS) using Convolutional Neural Network (CNN) and attention model	Plos One	2023	18	-	http://dx.doi.org/10.1371/journal.pone.0283121
Kroon et al.	The effect of catchment load reductions on water quality in the crown-of-thorn starfish outbreak initiation zone	Mar. Poll. Bull.	2023	195	-	http://dx.doi.org/10.1016/j.marpolbul.2023.115255
Milne et al.	Preparing for and managing crown-of-thorns starfish outbreaks on reefs under threat from interacting anthropogenic stressors	Ecological Modelling	2023	484	-	http://dx.doi.org/10.1016/j.ecolmodel.2023.110443
Li et al.	Impacts of selective feeding of crown-of-thorns starfish on the coral community in the South China Sea	Mar. Fresh. Res.	2023	74	982-993	http://dx.doi.org/10.1071/MF22133
Castro-Sanguino et al.	Control efforts of crown-of-thorns starfish outbreaks to limit future coral decline across the Great Barrier Reef	Ecosphere	2023	14	-	http://dx.doi.org/10.1002/ecs2.4580
Chandler et al.	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	2023	13	-	http://dx.doi.org/10.1038/s41598-023-46749-x
Mendoza-Porras et al.	Biochemical metabolomic profiling of the Crown-of-Thorns Starfish (*Acanthaster*): New insight into its biology for improved pest management	Sci. Total Environ.	2023	861	-	http://dx.doi.org/10.1016/j.scitotenv.2022.160525
Lang et al.	Impacts of ocean warming on the settlement success and post-settlement survival of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)	Coral Reefs	2023	42	143-155	http://dx.doi.org/10.1007/s00338-022-02314-y
Sasayama et al.	Neuritogenic steroid glycosides from crown-of-thorns starfish: Possible involvement of p38 mitogen-activated protein kinase and attenuation of cognitive impairment in senescence-accelerated mice (SAMP8) by peripheral administration	Bioorg. Med. Chem.	2023	78	-	http://dx.doi.org/10.1016/j.bmc.2022.117144
Kwong et al.	Telomere dynamics in the Pacific crown-of-thorns seastar (*Acanthaster* cf. *solaris*): effect of age, diet, and tissue type	Coral Reefs	2023	42	977-985	http://dx.doi.org/10.1007/s00338-023-02405-4
Morin et al.	Captivity induces a sweeping and sustained genomic response in a starfish	Mol. Ecol.	2023	32	3541-3556	http://dx.doi.org/10.1111/mec.16947

Authors	Article Title	Journal	Year	Volume	Page	DOI Link
Byrne et al.	Juvenile waiting stage crown-of-thorns sea stars are resilient in heatwave conditions that bleach and kill corals	lob. Change Biol.	2023	29	6493-6502	http://dx.doi.org/10.1111/gcb.16946
Smith et al.	Structure and proteomic analysis of the crown-of-thorns starfish (*Acanthaster* sp.) radial nerve cord (vol 13, 3349, 2023)	Scientific Reports	2023	13	-	http://dx.doi.org/10.1038/s41598-023-34881-7
Li et al.	Short-Term Impact of Decomposing Crown-of-Thorn Starfish Blooms on Reef-Building Corals and Benthic Algae: A Laboratory Study	Water	2024	16	-	http://dx.doi.org/10.3390/w16020190
Wei et al.	A new strategy based on a cascade amplification strategy biosensor for on-site eDNA detection and outbreak warning of crown-of-thorns starfish	Sci. Total Environ.	2024	927	-	http://dx.doi.org/10.1016/j.scitotenv.2024.172258
Zhao et al.	Stochastic dynamics of coral reef system with stage-structure for crown-of-thorns starfish	Chaos Sol. Frac.	2024	181	-	http://dx.doi.org/10.1016/j.chaos.2024.114629
Morin et al.	Seasonal tissue-specific gene expression in wild crown-of-thorns starfish reveals reproductive and stress-related transcriptional systems	Plos Biology	2024	22	-	http://dx.doi.org/10.1371/journal.pbio.3002620
Matthews et al.	Protecting Great Barrier Reef resilience through effective management of crown-of-thorns starfish outbreaks	Plos One	2024	19	-	http://dx.doi.org/10.1371/journal.pone.0298073
Foo et al.	Crown-of-thorns seastar (*Acanthaster* spp.) feeding ecology across species and regions	Sci. Total Environ.	2024	930	-	http://dx.doi.org/10.1016/j.scitotenv.2024.172691
Abe et al.	Simulated connectivity of crown-of-thorns starfish around Ashizuri-Uwakai National Park (western Japan) based on a high-resolution hydrodynamic modeling	Coral Reefs	2024	43	371-390	http://dx.doi.org/10.1007/s00338-024-02471-2
Sánchez-Luna et al.	The crown-of-thorns starfish *Acanthaster planci* as a predator of black coral *Antipathes galapagensis* in the Gulf of California	Bull. Mar. Sci.	2024	100	107-108	http://dx.doi.org/10.5343/bms.2023.0090
Wu et al.	Total Chemical Synthesis of Aggregation-Prone Disulfide-Rich Starfish Peptides	Chem A. Eur J	2024		-	http://dx.doi.org/10.1002/chem.202400933
Uthicke et al.	eDNA monitoring detects new outbreak wave of corallivorous seastar (*Acanthaster* cf. *solaris*) at Lizard Island, Great Barrier Reef	Coral Reefs	2024		-	http://dx.doi.org/10.1007/s00338-024-02506-8

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Indirect reporting on crown-of-thorns starfish (n=62)						
Russ et al.	Partitioning no-take marine reserve (NTMR) and benthic habitat effects on density of small and large-bodied tropical wrasses	Plos One	2017	12	-	http://dx.doi.org/10.1371/journal.pone.0188515
Clements et al.	Size matters: Predator outbreaks threaten foundation species in small Marine Protected Areas	Plos One	2017	12	-	http://dx.doi.org/10.1371/journal.pone.0171569
Fraser et al.	Fertiliser management effects on dissolved inorganic nitrogen in runoff from Australian sugarcane farms	Environ. Monitor. Assess.	2017	189	-	http://dx.doi.org/10.1007/s10661-017-6115-z

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Hairsine et al.	Review: Sediment-Related Controls on the Health of the Great Barrier Reef	Vadose Zone Journal	2017	16	-	http://dx.doi.org/10.2136/vzj2017.05.0115
Chazottes et al.	Impact of an experimental eutrophication on the processes of bioerosion on the reef: One Tree Island, Great Barrier Reef, Australia	Mar. Poll. Bull.	2017	118	125-130	http://dx.doi.org/10.1016/j.marpolbul.2017.02.047
Hock et al.	Connectivity and systemic resilience of the Great Barrier Reef		2017	15	-	http://dx.doi.org/10.1371/journal.pbio.2003355
Hornbeak et al.	Marine Envenomation	Emerg. Med. Clinic. North Amer.	2017	35	321-	http://dx.doi.org/10.1016/j.emc.2016.12.004
Wolff et al.	Vulnerability of the Great Barrier Reef to climate change and local pressures	Glo. B.	2018	24	1978-1991	http://dx.doi.org/10.1111/gcb.14043
McDougall et al.	The evolution of ependymin-related proteins	BMC Evo. Biol.	2018	18	-	http://dx.doi.org/10.1186/s12862-018-1306-y
Lam et al.	Acute drivers influence recent inshore Great Barrier Reef dynamics	Proc. Royal Soc. B Biol. Sci.	2018	285	-	http://dx.doi.org/10.1098/rspb.2018.2063
Meirelles et al.	Metagenomics of Coral Reefs Under Phase Shift and High Hydrodynamics	Front. Microbiol.	2018	9	-	http://dx.doi.org/10.3389/fmicb.2018.02203
Ceccarelli et al.	Rehabilitation of coral reefs through removal of macroalgae: state of knowledge and considerations for management and implementation	Res. Eco.	2018	26	827-838	http://dx.doi.org/10.1111/rec.12852
Condie et al.	Great Barrier Reef recovery through multiple interventions	Conserv. Biol.	2018	32	1356-1367	http://dx.doi.org/10.1111/cobi.13161
Needleman et al.	Environmental and Ecological Effects of Climate Change on Venomous Marine and Amphibious Species in the Wilderness	Wild. Environ. Med.	2018	29	343-356	http://dx.doi.org/10.1016/j.wem.2018.04.003
Pisapia et al.	Changes in the population and community structure of corals during recent disturbances (February 2016-October 2017) on Maldivian coral reefs	Scientific Reports	2019	9	-	http://dx.doi.org/10.1038/s41598-019-44809-9
Cowburn et al.	Evidence of coral bleaching avoidance, resistance and recovery in the Maldives during the 2016 mass-bleaching event	Mar. Ecol. Prog. Ser.	2019	626	53-67	http://dx.doi.org/10.3354/meps13044
Vercelloni et al.	Exposure, vulnerability, and resiliency of French Polynesian coral reefs to environmental disturbances	Scientific Reports	2019	9	-	http://dx.doi.org/10.1038/s41598-018-38228-5
MacNeil et al.	Water quality mediates resilience on the Great Barrier Reef	Nat. Ecol. Evol.	2019	3	620-627	http://dx.doi.org/10.1038/s41559-019-0832-3
Ren et al.	Isolation of a New PAK1 Gene from Sea Cucumber (Apostichopus japonicus) and Its Expression Analysis and Function Characterization	J. Ocean Uni. China	2019	18	1147-1157	http://dx.doi.org/10.1007/s11802-019-4034-z
Lopez et al.	Multiple Facets of Marine Invertebrate Conservation Genomics	Ann. Rev. Animal Biosc.	2019	7	473-	http://dx.doi.org/10.1146/annurev-animal-020518-115034
Mellin et al.	Spatial resilience of the Great Barrier Reef under cumulative disturbance impacts	Global. Change Biol.	2019	25	2431-2445	http://dx.doi.org/10.1111/gcb.14625

Authors	Article Title	Journal	Year	Volume	Page	DOI Link
Callaghan et al.	Near-reef and nearshore tropical cyclone wave climate in the Great Barrier Reef with and without reef structure	Coast. Eng.	2020	157	-	http://dx.doi.org/10.1016/j.coastaleng.2020.103652
Sato et al.	Changes in the potential stocks of coral reef ecosystem services following coral bleaching in Sekisei Lagoon, southern Japan: implications for the future under global warming	Sustainability Sci.	2020	15	863-883	http://dx.doi.org/10.1007/s11625-019-00778-6
Pratchett et al.	Size-specific recolonization success by coral-dwelling damselfishes moderates resilience to habitat loss	Scientific Reports	2020	10	-	http://dx.doi.org/10.1038/s41598-020-73979-0
Thompson et al.	Development of the coral index, a summary of coral reef resilience as a guide for management	J Environ. Manag.	2020	271	-	http://dx.doi.org/10.1016/j.jenvman.2020.111038
Zuo et al.	Using Landsat Data to Detect Change in Live to Recently (<6 Months) Dead Coral Cover in the Western Xisha Islands, South China Sea	Sustainability	2020	12	-	http://dx.doi.org/10.3390/su12135237
Schlaff et al.	Acoustic tracking of a large predatory marine gastropod, *Charonia tritonis*, on the Great Barrier Reef	Mar. Ecol. Prog. Ser.	2020	642	147-161	http://dx.doi.org/10.3354/meps13291
Cannon et al.	Coral reefs in the Gilbert Islands of Kiribati: Resistance, resilience, and recovery after more than a decade of multiple stressors	Plos One	2021	16	-	http://dx.doi.org/10.1371/journal.pone.0255304
Abe et al.	Climate-induced species range shift and local adaptation strategies in a temperate marine protected area, Ashizuri-Uwakai National Park, Shikoku Island, western Japan	Ocean Coast. Manag.	2021	210	-	http://dx.doi.org/10.1016/j.ocecoaman.2021.105744
Castro-Sanguino et al.	Reef state and performance as indicators of cumulative impacts on coral reefs	Ecol. Ind.	2021	123	-	http://dx.doi.org/10.1016/j.ecolind.2020.107335
Hammerman et al.	Variable response of Red Sea coral communities to recent disturbance events along a latitudinal gradient	Marine Biol.	2021	168	-	http://dx.doi.org/10.1007/s00227-021-03984-y
Mumby et al.	Reconnecting reef recovery in a world of coral bleaching	Limnolo.Oceanog. Met.	2021	19	702-713	http://dx.doi.org/10.1002/lom3.10455
Condie et al.	Large-scale interventions may delay decline of the Great Barrier Reef	Royal Soc. Open Sci.	2021	8	-	http://dx.doi.org/10.1098/rsos.201296
Stoeckl et al.	Assessing changes to ecosystem service values at large geographic scale: A case study for Australia's Great Barrier Reef	Ecosystem services	2021	51	-	http://dx.doi.org/10.1016/j.ecoser.2021.101352
Ortiz et al.	Important ecosystem function, low redundancy and high vulnerability: The trifecta argument for protecting the Great Barrier Reef's tabular *Acropora*	Conserv. Lett.	2021	14	-	http://dx.doi.org/10.1111/conl.12817
Sassa et al.	Divalent metal transporter-related protein restricts animals to marine habitats	Commu. Biology.	2021	4	-	http://dx.doi.org/10.1038/s42003-021-01984-8
Turak et al.	Impacts of coastal land use change in the wet tropics on nearshore coral reefs: Case studies from Papua New Guinea	Mar. Poll. Bull.	2021	168	-	http://dx.doi.org/10.1016/j.marpolbul.2021.112445
Pérez-Rosales et al.	Documenting decadal disturbance dynamics reveals archipelago-specific recovery and compositional change on Polynesian reefs	Mar. Poll. Bull.	2021	170	-	http://dx.doi.org/10.1016/j.marpolbul.2021.112659

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Klein et al.	Development and Interrogation of a Transcriptomic Resource for the Giant Triton Snail (Charonia tritonis)	Mar. Biotech.	2021	23	501-515	http://dx.doi.org/10.1007/s10126-021-10042-7
Zhang et al.	Molecular and Functional Characterization of a Novel Kunitz-Type Toxin-like Peptide in the Giant Triton Snail Charonia tritonis	Marine Drugs	2022	20	-	http://dx.doi.org/10.3390/md20110686
Xiao et al.	Recent deterioration of coral reefs in the South China Sea due to multiple disturbances	PeerJ	2022	10	-	http://dx.doi.org/10.7717/peerj.13634
Tkachenko et al.	Coral reefs degradation under complex impact of natural and anthropogenic factors with Nha Trang Bay (Vietnam) as an example	Zhu. Obs. Bi.	2022	83	462-480	http://dx.doi.org/10.31857/S0044459622060082
Cline et al.	Fish community structure and dynamics are insufficient to mediate coral resilience	Nat. Ecol. Evo.	2022	6	1700-1709	http://dx.doi.org/10.1038/s41559-022-01882-0
Guo et al.	Binding Pattern Reconstructions of FGF-FGFR Budding-Inducing Signaling in Reef-Building Corals	Front. Phys.	2022	12	-	http://dx.doi.org/10.3389/fphys.2021.759370
Tkachenko et al.	Phase shift from a stony-coral to a soft-coral community on a coral reef: a case study of an alternative state	Mar. Biol. Res.	2022	18	544-554	http://dx.doi.org/10.1080/17451000.2022.2153869
Bozec et al.	Cumulative impacts across Australia's Great Barrier Reef: a mechanistic evaluation	Ecolo. Monogra.	2022	92	-	http://dx.doi.org/10.1002/ecm.1494
Motti et al.	A Review of the Giant Triton (*Charonia tritonis*), from Exploitation to Coral Reef Protector?	Diversity	2022	14	-	http://dx.doi.org/10.3390/d14110961
Clark et al.	Reconstructing past disturbance in coral communities using U-Th dating of dead coral skeletons	Geology	2023	51	983-987	http://dx.doi.org/10.1130/G51419.1
Patricio-Valerio et al.	Meteorological Satellite Observations Reveal Diurnal Exceedance of Water Quality Guideline Thresholds in the Coastal Great Barrier Reef	Remote Sensing	2023	15	-	http://dx.doi.org/10.3390/rs15092335
Zhao et al.	Ecological Effects of Predator Harvesting and Environmental Noises on Oceanic Coral Reefs	Bull. Math. Biol.	2023	85	-	http://dx.doi.org/10.1007/s11538-023-01166-z
Jia et al.	Identification and Expression of the Conotoxin Homologous Genes in the Giant Triton Snail (*Charonia tritonis*)	J. Ocean Uni. China	2023	22	213-220	http://dx.doi.org/10.1007/s11802-023-5147-y
Anderson-King et al.	Branching coral growth and visual health during bleaching and recovery on the central Great Barrier Reef	Coral Reefs	2023	42	1113-1129	http://dx.doi.org/10.1007/s00338-023-02403-6
MahmoudZadeh et al.	Distributed task allocation and mission planning of AUVs for persistent underwater ecological monitoring and preservation	Ocean Eng.	2023	290	-	http://dx.doi.org/10.1016/j.oceaneng.2023.116216
Tkachenko et al.	Coral reef collapse in South-Central Vietnam: a consequence of multiple negative effects	Aquatic Ecology	2023	57	65-83	http://dx.doi.org/10.1007/s10452-022-09994-2
Rogers et al.	Improving coral cover using an integrated pest management framework	Eco. Appl.	2023	33	-	http://dx.doi.org/10.1002/eap.2913
Smith et al.	Drivers of coral mortality in non-acute disturbance periods	Mar. Ecol. Prog. Ser.	2023	717	37-50	http://dx.doi.org/10.3354/meps14362

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Mentzel et al.	Evaluating the effects of climate change and chemical, physical, and biological stressors on nearshore coral reefs: A case study in the Great Barrier Reef, Australia	Int. Environ. Assess. Manag.	2024	20	401-418	http://dx.doi.org/10.1002/ieam.4871
Dennis et al.	Isolation by disturbance: a pattern of genetic structure of the coral *Pocillopora grandis* in the Gulf of California	Mar. Ecol. Prog. Ser.	2024	733	43-57	http://dx.doi.org/10.3354/meps14553
Emslie et al.	Changing dynamics of Great Barrier Reef hard coral cover in the Anthropocene	Coral Reefs	2024	43	747-762	http://dx.doi.org/10.1007/s00338-024-02498-5
Byrne et al.	DNA metabarcoding as a tool for characterising the spatio-temporal distribution of planktonic larvae in the phylum Echinodermata	Coral Reefs	2024	43	717-731	http://dx.doi.org/10.1007/s00338-024-02496-7
Han et al.	THREE-DECADE changes of reef cover in Pulau Layang-Layang, Malaysia using multitemporal Landsat images	Mar. Environ. Res.	2024	197	-	http://dx.doi.org/10.1016/j.marenvres.2024.106454
Bellwood et al.	Studying functions on coral reefs: past perspectives, current conundrums, and future potential	Coral Reefs	2024	43	281-297	http://dx.doi.org/10.1007/s00338-024-02474-z

Appendix B

Table A 2 Summary of the current knowledge and gaps that should be bridged to enhance the application of semiochemicals as a novel alternative technology for monitoring and controlling crown-of-thorns starfish populations.

Topics	Categories	General Questions – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	Mode of Action – general comments	Specific comments
Attractants – General Considerations	*Source and nature of attractants*	What is the chemical nature of COTS foraging attractants? Are they emitted by coral prey (including microbial biofilms) items (foraging kairomones) or feeding conspecifics (pheromones or released foraging kairomones)? What is the chemical nature of COTS conspecific aggregation pheromone attractants? Are they seasonal, or sex specific (i.e. during spawning time) or are they kairomones? Is it more effective to elucidate and use attractants for adults, juveniles or larvae?	Semiochemicals are highly diverse and range from small primary metabolites to complex secondary metabolites, to peptides and large proteins – hence their isolation and elucidation requires a variety of chemical techniques, assays (*in vitro* and whole animal behaviour), field testing and broad range of expertise. No distinct structure–activity relationships can be deduced from known semiochemicals – from insect literature (Francke 1999). Several coral metabolites have been confirmed as foraging cues, but a more extensive investigation of coral chemistry is warranted. Reliant on biological and ecological knowledge of each life-stage. Primarily lab-based; extensive receptor/animal assay	Several coral metabolites have been confirmed as foraging cues, but a more extensive investigation of coral chemistry is warranted. Primarily lab-based; extensive receptor/animal assay
	Specificity and Selectivity	What is their mode of action? Does the attractant (pheromone or kairomone) have broad spectrum or selective bioactivity? What is the level of specificity for COTS?	There is no quantitative data to establish the effectiveness of specific semiochemicals in influencing COTS outbreaks or suppressing populations. Behaviour assays need to be established or developed.	
	Efficacy	What is the efficacy of adult COTS pheromone attractants in the field? Does efficacy change between seasons? Can the pheromone or kairomone be modified for increased or prolonged bioactivity? Or species-specificity (if not already)		What dosage is required in-field? Need extensive research for field deployment (suitable compounds; specificity; habituation; deployment) Definitely done in insect world

Topics	Categories	General Questions – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	Mode of Action – general comments	Specific comments
	Range	Is the attractant (pheromone or kairomone) effective over the full geographical range of COTS? i.e. is it effective across the entire GBR, Japan, etc?		Need extensive research for field deployment (suitable compounds; specificity; habituation; deployment)
Repellents – General Considerations	*Source and nature of repellents/deterrents*	What is the chemical nature of COTS repellents/deterrents? What type of deterrent are they, conspecific alarm pheromone, conspecific injury pheromone, or predator kairomone, or other? What predators produce COTS kairomone deterrents? Giant triton is confirmed, but what about corals not widely predated on? What about macroalgae? What about other carnivorous Mollusca? Is it more effective to elucidate and use deterrents for adults, juveniles or larvae?		Primarily lab-based
	Specificity and Selectivity	What is their mode of action? Does the repellent/deterrent (conspecific or predator-derived) have broad spectrum or selective bioactivity? What biological response does the repellent/deterrent elicit in COTS? E.g. aversive movement, physiological suppression including growth or reproductive maturation, spawning. What is the level of specificity for COTS?	Requires that the semiochemical have a half-life that is long enough to be effective in the reef environment but short enough to not cause irreparable impacts. There is no quantitative data to establish the effectiveness of specific semiochemicals in influencing COTS outbreaks or suppressing populations. Behaviour assays need to be established or developed.	
	Efficacy	What is the efficacy of COTS deterrents (pheromone or kairomone) in the field? Does efficacy change between seasons i.e. as predator behaviours change, during COTS spawning?		What dosage is required in-field? Need extensive research for field deployment (suitable compounds; specificity; habituation; deployment)
	Range	Is the deterrent effective over the full geographical range of COTS? i.e. is it effective across the entire GBR, Japan, etc?		Need extensive research for field deployment (suitable compounds; specificity; habituation; deployment)
Target life-stage	*Gametes*	Could semiochemicals be effective in disrupting/inhibiting/inducing egg/sperm maturation? There is evidence of chemical activation (e.g., 1-Methyladenine).		What dosage is required in-field?

Topics	Categories	**General Questions** – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	**Mode of Action – general comments**	**Specific comments**
		Could semiochemicals be effective in disrupting/inhibiting fertilisation? There is evidence of sperm/egg attraction to chemical cues, but these have not been identified – would need to be highly specific given spawning occurs at the same time as coral spawning.		
	Larvae	What semiochemicals are effective in changing behaviours of COTS larvae i.e. attractants such as foraging kairomones (prey)? What semiochemicals are effective in changing behaviours of COTS larvae i.e. avoidance allomones (i.e. from adult COTS or recently settled juveniles – competition allomones) that induce avoidance of unsuitable settlement substrate? Or settlement kairomones that induce settlement/metamorphosis on suitable cues (substrate)? Or pheromone attractants emitted by conspecific adults?	Behaviour assays need to be established or developed, design of trap	
	Juveniles	Could semiochemicals be effective in changing behaviours of CCA-feeding COTS juveniles? No definitive evidence – need to consider CCA derived foraging kairomones. Could a semiochemical, or semiochemical mimic interrupt the dietary transition of COTS? i.e., to delay transition into coral feeding adults, would need to be deployed in a very specific time frame ~6–8 months after spawning.		
	Sub-adult/adult	Could semiochemicals be effective in changing behaviours of coral-feeding sub-adult and adult COTS? Evidence of foraging kairomones, conspecific pheromone attractants, spawning pheromone attractants, conspecific alarm pheromones and predator alarm kairomones. Could a COTS semiochemical (kairomone) with specificity to attract parasite species with a very narrow host range, possibly limited to COTS, be applied as a control method?	Behaviour assays need to be established or developed, design of trap	Are there any COTS specific parasites? Limited risk profile due to specificity
	Spawning	What is the chemical nature of COTS exogenous spawning trigger? Synchronous spawning maximises fertilisation rates.	No specific semiochemical available; To date only crude extracts have shown stimulation	Low; Lab-based; extensive receptor/animal assay; Semiochemical spawning triggers could be used to

Topics	Categories	General Questions – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	Mode of Action – general comments	Specific comments
		Could semiochemicals be effective in disrupting synchronous spawning or inducing out-of-season spawning? Is the spawning semiochemical species-specific and over what distance? Is the spawning semiochemical sex-specific?	Reliant on mature animals A semiochemical would need to be highly specific to induce out-of-season spawning for COTS only, or disrupt synchronous spawning	facilitate asynchronous spawning or spawning of immature gametes. There is possibility of triggering mass spawning of COTS in the field, dosages and time of delivery of cue likely to be critical There are differences in the neuropeptides between the sexes
Semiochemical Formulation	*Release mode*	Can and should the semiochemical be applied as a slow-release control agent (i.e. year-round), as a fast-release single dose (i.e. during spawning), or in pulses (intermittent to reduce impacts on other species)? Would the deployment of different release modes (of one or several semiochemicals) enable targeting of multiple life-stages and behaviours? Could COTS be triggered to produce altered (i.e., higher or lower) levels of conspecific cues?	Requires the semiochemical be readily available, or readily modified or synthesised. Mechanism of field release to be established e.g., slow release, fast release, pulse release	
	Application method	How best can the semiochemical be applied to ensure efficacy? On what scale can the semiochemical formulation be applied i.e. a broad scale, or local or individual? What methods of formulation are suitable? Could engineered microbes/animals be used to produce and release peptide semiochemicals at the desired rate over the desired time period?	Development of formulas, traps	

Topics	Categories	**General Questions** – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	**Mode of Action – general comments**	**Specific comments**
	Combination	Could semiochemicals be used in combination to enhance effectiveness i.e., a foraging kairomone + conspecific aggregation pheromone?		
Chemoreceptors	*Source + nature of attractants*	Can we exploit the COTS genome and their behavioural responses to pheromones or kairomones to identify target chemoreceptors? Can COTS chemoreceptors be exploited? i.e., can we identify receptor-specific ligands from both interspecies and intraspecies sources. Could these chemoreceptors be used to identify signalling functions of metabolic intermediates? Using knowledge of COTS chemoreceptors, could target-specific molecular assays be developed for rapid screening of semiochemicals (natural and synthetic mimics)? Given that many chemoreceptors remain functionally un-annotated this would require extensive research to identify and characterise chemoreceptors. Could we produce recombinant chemoreceptors to rapidly identify and characterise semiochemical ligands? Is It more effective to elucidate chemoreceptors for adults, juveniles or larvae?	Receptors may need co-proteins for *in vitro* functionality	
	Genetic modification	Will genetic mutations of target-specific chemoreceptors abolish activity? Would require extensive research and implementation to introduce mutation into the COTS population in a controlled manner.		
Reef Prioritisation	*Presence/ absence*	Can semiochemicals help to identify which reefs should be the focus of culling efforts? i.e., a COTS-specific chemical biomarker such as that used to induce aggregations; possible candidates include: saponins, given the specificity of some; secreted proteins–- these compounds are		

Topics	Categories	**General Questions** – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	**Mode of Action – general comments**	**Specific comments**
		continually/regularly secreted (i.e. not under stress)–- so looking for presence vs absence.		
	Monitoring	Could presence/increases in concentrations of specific semiochemicals be used to monitor COTS numbers in the longer term? i.e., automated, unmanned, remote sensing of key semiochemicals as an early warning system for future outbreaks?		
Scale of application		Could semiochemicals be broadly applied on priority reefs? i.e., would require a long half-life of the semiochemical, would need to be COTS-specific. Will environmental change (related to climate change) impact semiochemical efficacy rendering them less effective as COTS control agents? Especially important to consider if activity of semiochemical is seasonal. Does pollution/sediment/nutrient loading affect efficacy of semiochemicals? Question is particularly relevant to larval phase.		
Manage population thresholds		On those reefs where culling has returned COTS numbers below reproductive (3 COTS ha^{-1})/ecological (4–5 COTS ha^{-1}) thresholds, could predator kairomones or alarm pheromones be used to ensure continued and sustained population suppression? i.e., mimic predator odours that alter behavioural/phenotypic/physiological traits leading to sub-optimal performance of the prey, i.e. slow growth and delayed maturity. What is the best time to deploy pheromone attractant controls to ensure optimal results? i.e., attractants could be deployed as baits/lures during an outbreak to complement current culling efforts.	By mediating behaviour through natural products, especially with high specificity, populations can be suppressed but not eliminated–- COTS is a native species and plays an important role on the GBR. E.g. in general, pheromones form complexes with receptors through non-bonding close-range interactions, which facilitates processing and dissociation mechanisms to avoid permanent signals at the receiver's site. In the case of	

Topics	Categories	General Questions – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	Mode of Action – general comments	Specific comments
		What is the best time to deploy semiochemical repellent/deterrent controls to ensure optimal results? i.e., used during non-outbreak periods to i) discourage aggregation formation especially at key times such as COTS spawning, ii) to disrupt/disperse aggregations at the outbreak initiation phase or iii) during conditions considered stressful to corals i.e., coral bleaching.	kairomones, they often form covalent bonds with the receptors resulting in a longer lasting signal.	
Augmentation of current culling methods	*Attractants as lures*	On those reefs where culling is deemed necessary, could semiochemicals be used to enhance culling success? i.e., use of a pheromone or foraging kairomone attractant to lure COTS into an area away from the reef substrate for easy access. Note this may prove useful on reefs where the outbreak is in the later stages and many individuals are seeking prey. Could a pheromone be used to deliver a lethal agent (either chemical toxicant or biological agent)? i.e., a lure and kill technology would replace the need for divers and single injections. Note this would have to be highly COTS-specific–- or a level of acceptable collateral damage to other species be established. Could a COTS-specific pheromone (i.e., will not impact on other asteroids or echinoderms) be modified to have both attractant and toxic properties, and applied *in situ*? i.e., replace the need for divers and single injections. Note this would have to be highly COTS-specific–- or a level of acceptable collateral damage to other species be established. The semiochemical would need to be amenable to modification or synthesis. Could a structural analogue (mimic) of a confirmed pheromone be used to block the semiochemical receptor and alter COTS behaviour? Need to ensure specificity of the mimic.	Development of traps specific to life-stage and season required Primarily lab-based; extensive receptor/animal assay	
	Deterrents to elicit escape response	On those reefs where culling is deemed necessary, could semiochemicals be used to enhance culling success? i.e., predator kairomone to flush COTS from cryptic sites for easy access by SCUBA. Note this may only prove useful on reefs where the outbreak is in the initial stages and many individuals, including sub-adults are cryptic–- or would animals just retreat further into the reef structure?		

Topics	Categories	General Questions – What is the knowledge gap? Why is it important? Ensure that all options, irrespective of their readiness, have been considered and prioritised.	Mode of Action – general comments	Specific comments
		Could a more potent structural analogue (mimic) of a confirmed semiochemical deterrent be developed and alter COTS behaviour?		
Chemical profile of COTS	*Gametes*	Does the chemical profile of COTS change across the life-stages? Could mining of the COTS metabolome assist in the identification of functional semiochemicals?	Elucidation and mining of the COTS metabolome may help to improve understanding of the complexities that exist in conspecific chemoreception that enhance or hinder aggregations.	
	Larvae			
	Juveniles			
	Sub-adult/ adult			

Appendix C

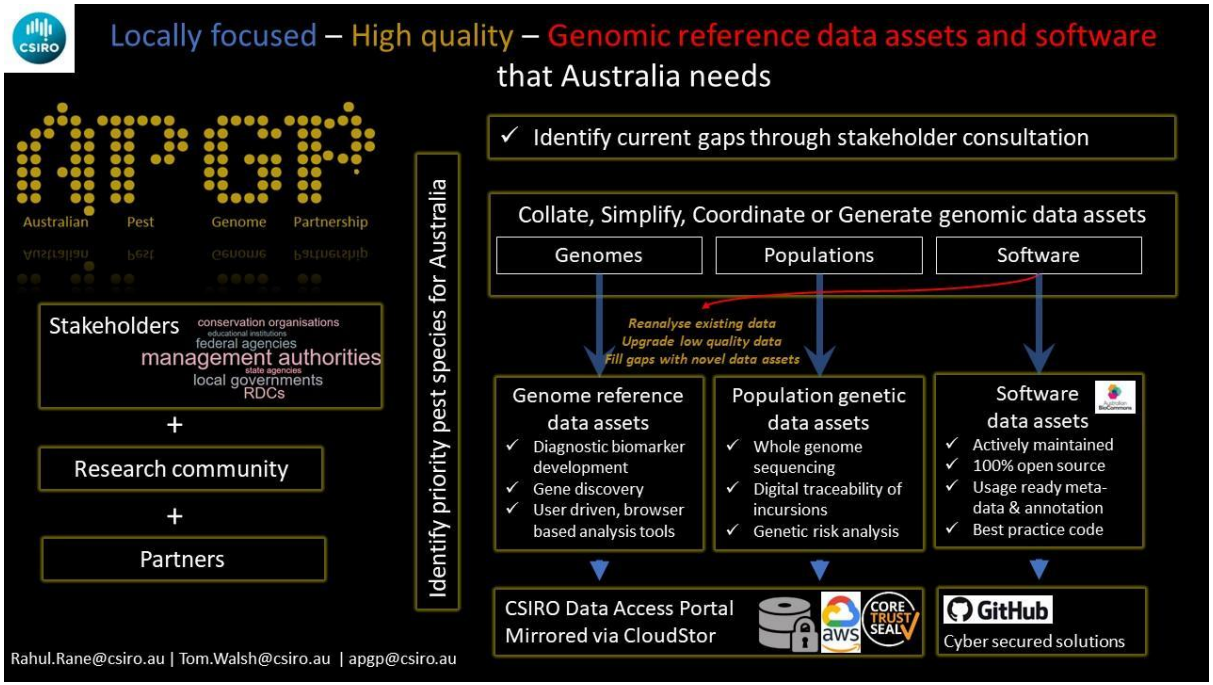


Figure A 1 Australian Genomics Initiative.

Appendix D

Table A 3 Summary of the multi-omics resources available for the crown-of-thorns starfish. Products in **bold** have been completed within the project timeframe, utilising project resources and databases.

Category	Gender	Location	Status	Time	Tissue Type	References
Reference Genome v1.0 EVM2 OIST	female	Captivity	Aggregation	0 mins	Coelomic Fluid; Coelomocytes	Hall et al. (2017)
Reference Genome v1.1 PASA UQ	male	Wild Davies Reef	Solitary	15 mins	Gonads	**Jönsson et al. (2022)**
	unknown	Wild Lynch Reef	fed	30 mins	Papula	**Morin et al. (2023, 2024)**
			unfed	summer	Radial Nerve Cord	
				winter	Sensory Tentacles; Tube Feet	
					Skin	
					Spines	
Draft ***A. brevispinus* genome CSIRO**					Tube feet	**Motti et al. (2024)**
Reference transcriptomes UQ/USC	female	Captivity			Coelomic Fluid;	Roberts et al. (2017)
	male	Wild Davies Reef			Egg; Egg Jelly; Gonads	Smith et al. (2017)
	unknown	Wild Lynch Reef			Mouth; Stomach	Roberts et al. (2018)
improves genome					Papula	Smith et al. (2018)
supports proteomics					Radial Nerve Cord	Smith et al. (2019)
					Sensory Tentacles; Tube Feet	**Jönsson et al. (2022)**
					Skin	**Morin et al. (2023, 2024)**
					Spines	
Draft transcriptome *A. brevispinus* USC	unknown	Captivity	Solitary		Radial nerve Cord	**Smith et al. (2023)**

Category	Gender	Location	Status	Time	Tissue Type	References
Reference Proteome USC	female	Captivity	Aggregation	summer	Egg; Egg Jelly; Gonads	Roberts et al. (2017)
	male		Solitary	winter	Mouth; Stomach	Smith et al. (2017)
	unknown		fed		Podia	Roberts et al. (2018)
supports metabolomics			unfed		Radial Nerve Cord	Smith et al. (2018)
					Sensory Tentacles; Tube Feet	Smith et al. (2019)
					Skin	**Hillberg et al. (2023)**
					Spines	
Reference Adult Metabolome CSIRO	female	Captivity	Solitary	3 days	Ambulacral skeleton	Hall et al. (2016)
	male		unfed	summer	Eggs; Gonads	**Mendoza-Porras et al. (2023)**
					Eyes	
confirms protein expression					Pyloric caeca; Pyloric stomach	
					Radial Nerve Cord	
					Sensory Tentacles; Tube Feet	
					Skin	
					Spines	
Reference Juvenile Metabolome CSIRO	unknown	Captivity	CCA-feeding	12-27 months	Whole animal	**Byrne et al. (2024)**
			Coral-feeding	transitioned	Whole animal	underway
					spines	underway

Appendix E

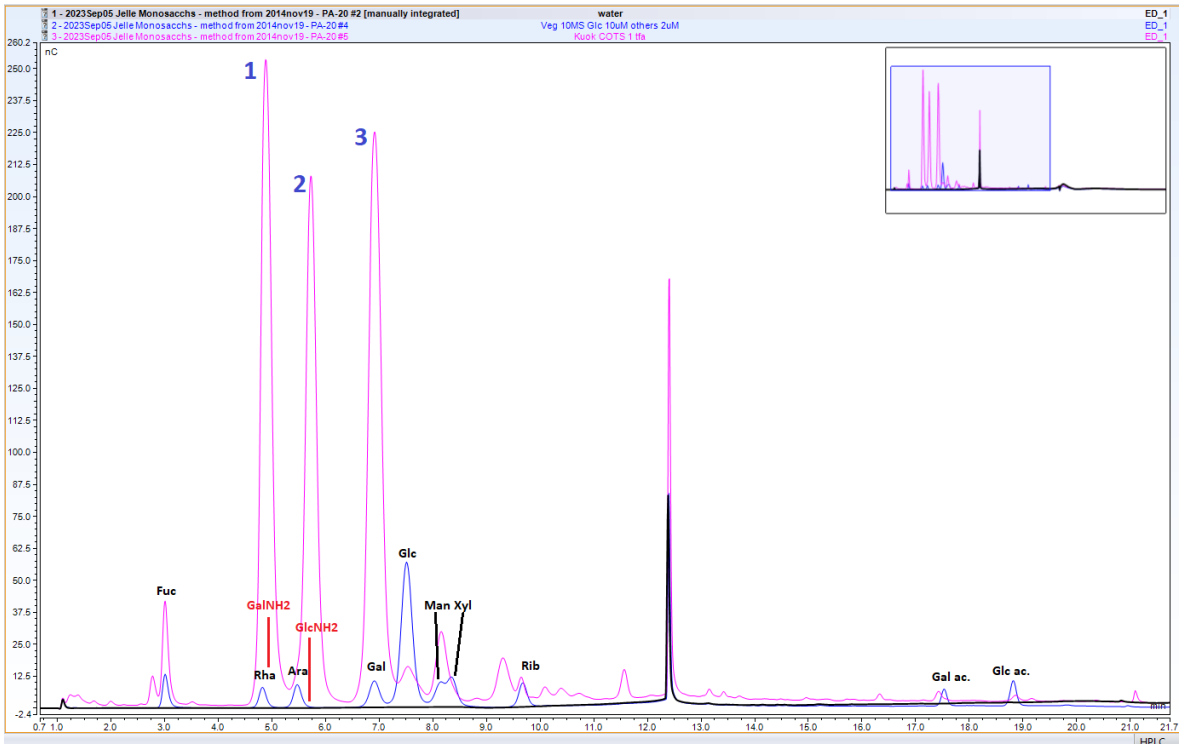


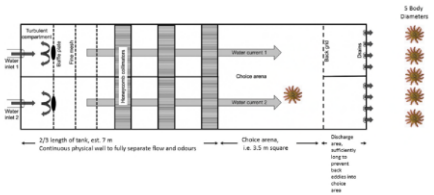
Figure A 2 Monosaccharide profile of > 30 kDa summer 2020 fraction FrA. Fuc = fucose, Rha = rhamnose, Ara = arabinose, GalNH2 = galactosamine (peak 1), GlcNH2 = glucosamine (peak 2), Gal = galactose (peak 3), Glc = glucose, Man = mannose, Xyl = xylose, Rib = ribose, Gal ac. = galacturonic acid, Glc ac. = glucuronic acid.

Appendix F

Excerpt from Motti and Hall (2019) Review of whole animal behavioural testing facilities. Report prepared for AIMS Appropriation Task 002714. Australian Institute of Marine Science, Townsville (47 pp).

Preliminary Design of Two-current Choice Flume

Two-current flume original design for CoTS and Triton choice behavioural research. Two-current flumes also applicable to research on impacts of sensory biology and ecophysiology including dredging, hypoxia (O_2 depletion and CO_2 elevation) climate change research, ecotoxicology, etc. of sessile and motile organisms.



Summary

1. Tank with total width to give 2 choice 'virtual channels', i.e. no physical separation within the choice arena virtual channels created through simultaneous release of two adjacent (but physically separated) laminar flow currents
2. Assume average COTS diameter 0.35 m
3. Tank total width = 3.5 m
   Each 'virtual channel' 4.5 times COTS diameter = 1.575 m (per channel)
4. Entire choice arena, 3.5 m square
5. Tank total length = 9 m (may be shorter depends on model output)
6. Operational water depth = 0.2 to 0.4 m
7. Tank wall height = 1 m
8. Water velocities 1 to 20 cm/s (Storlazzi et al. 2004 – flow on coral reefs) – higher flow rates are potentially better to maintain separation of the two currents as they flow through the entire length of the choice arena.

Specifications

Design based on review by Jutfelt et al. (2017)
https://besjournals.onlinelibrary.wiley.com/doi/10.1111/2041-210X.12668 – however it is emphasized that their design might be scalable there is at least a modelling requirement to test and validate that the virtual flume channels will remain intact as is possible with small scale flumes, i.e. 0.4 m wide x 1.2 m length.

Examples of small scale two-current flume designs.

https://www.youtube.com/watch?v=oQZnm7WWqRM

https://www.youtube.com/watch?v=jrtyc-rLGWc

The requirement for the total tank length to be 2-3 times that of the choice arena is questionable but has to be modelled and tested. It may well be that with sufficient bank of collimators that the laminar flow structuring part of the tank can be shorter than the recommended 2-3 times.

Parameter	Recommended	Operational Conditions /Uncertainties
Dimensions	Total length 3 times choice arena area Width 4-5 times diameter of CoTS to so animal can travel in any direction up to 5 times its diameter	Average COTS diameter 0.35 m (30 to 40 cm) 3.5 m wide, each channel width 1.75 m
Choice Arena	3.5 m^2	Allows free movement of animal for at least 5 body lengths.
Seawater quality	Flow through, cannot recirculate water due to 'odour' pollution.	Discharge seawater can be used to supply other tanks
Water Inlet	Must be stable flow (L/min), either gravity head or constant pressure pumps Immediately disperse inlet jet with baffle plate. Must be automated so short term experiments with high flow (for example 30-60 minutes) can be triggered and then flow reduced.	Inlet baffle to produce fully turbulent water before entry into collinators. Experiments typically will be for a short period sometime during the night. Activities video recorded for later image analysis.
Fine mesh walls	To act as preliminary moulding of water flow towards laminar conditions	
Collimators	Honeycomb – comb diameters and length determined by model? May require multiple units, i.e. 3 to produce final laminar flow.	Slot in/out units Plascore *, Castenaso ** Unknown long these need to be – need to model actual required length.
Water current in choice area	Need to demonstrate that current entering choice arena are fully separated for at least half (preferentially the full) the length of the choice arena.	Objective is to have one choice arena but two different water columns, i.e. one control/blank and one with test odour.
Discharge grid	Far enough away from the discharge manifolds to prevent flow back up which would disrupt flow within the choice areana.	Discharge area with physical divider, i.e. trying to get 2 currents totally isolated to flow out of collimator that is maintained across the choice arena until it reaches the physically divided discharge area.
Outflow	Water to discharge and NOT recicrculating.	
Additional Requirements		
Length of Experiments	30-60 minutes likely sufficient to determine response	
Illumination	Infra-red lighting.	

	Walls/ceiling must be non-reflective as biggest problem is reflection of infrared light off water surface producing 'white outs'
Overhead camera	Infra-red camera sufficiently high over tank for lens to view entire choice arena.
Automation	Ability to inject odour water into flume when required (automated). Injection may be by dosing pump/syringe when testing known concentrations of specific chemicals. Inject may be flow from header tank containing other organisms/scents feeding into one or the other channels.
Headed tank	Tank to hold experimental odours that can be injected into the water inlet pipe feeding the turbulent compartment of the flume.
Drain (to empty tank)	Drain level with bottom of tank to allow complete drainage for cleaning. Large diameter to allow rapid draining.
Doppler flow monitor	Ability to accurately measure current flow through the choice arena, i.e. Doppler current meter. This may need electronic feedback to constant flow pumps as minor changes may be needed at each water inlet to balance the flow that enters the choice arena.
Thigmotaxis	Organisms like structure, i.e. COTS will sit on wall rather than bottom. Not sure if there is any fix to this, i.e. to maximise the likelihood that the animal stays on the flat bottom.
Acclimatization	Ability to have test animal first 'caged' in centre of one current channel which can be automatically removed a short time before the 30-60 experiment begins, i.e. the animal is introduced into the flume at the end of the working day but the actual experiment and injection of odour plume may not happen for some hours later in the night. Animals needs to be acclimatized to tank conditions before conducting any experimentation.

- https://www.plascore.com/honeycomb/honeycomb-cores/thermoplastic/pc2-polycarbonate-honeycomb/
- https://www.cel.eu/

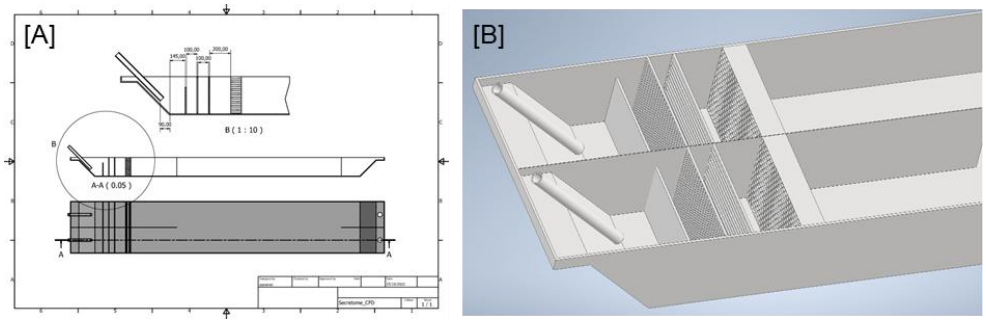


Figure A 3 Schematics showing (A) design draft and (B) computer-generated simulation of two-current choice laminar flume design.

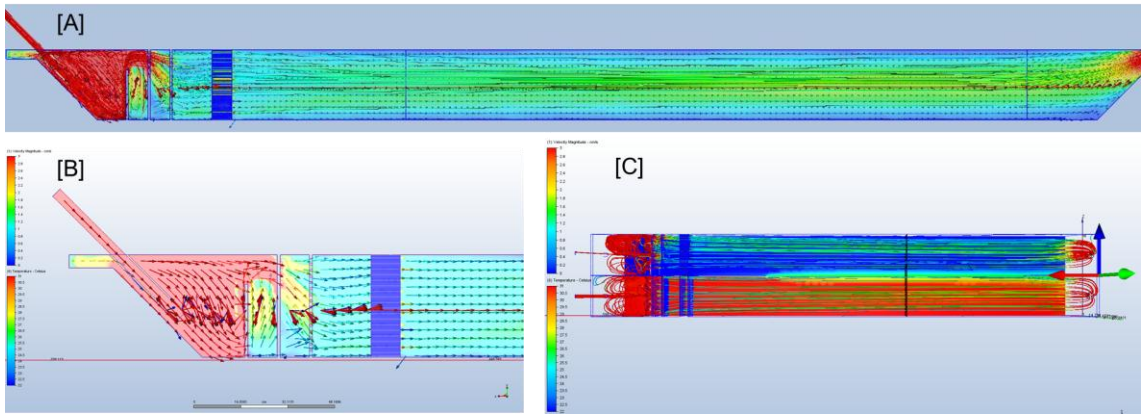


Figure A 4 Computer-generated flow showing (A) side-on simulation of plume as it moves the length of the tank, (B) predicted path of introduced plume as it passes through the series of baffles and the collimator (dark blue) and (C) top view of the two plumes as they move the length of the tank.

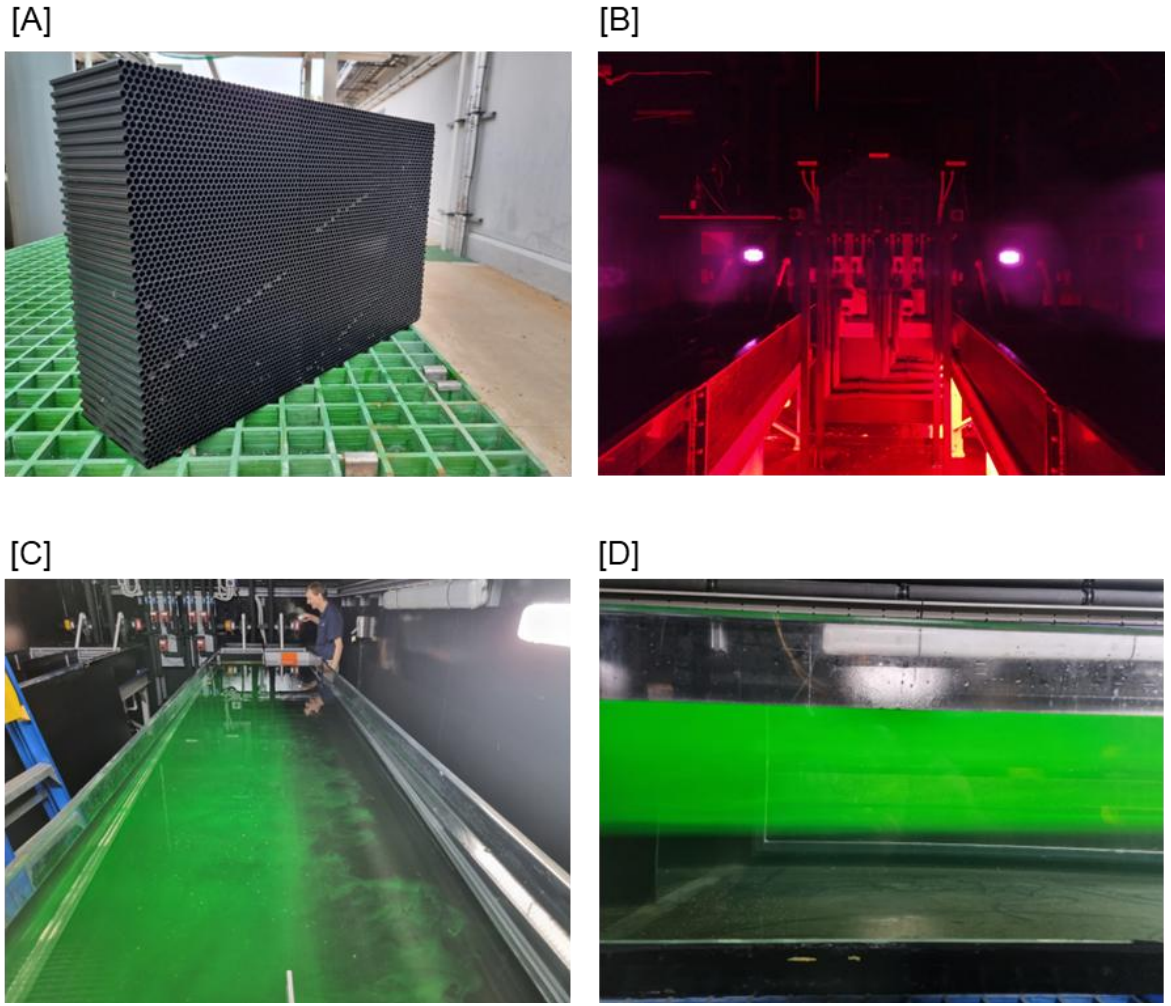


Figure A 5 Testing phase, showing (A) final design of the collimator and (B) infrared lighting to enable safe working under 'night' conditions. Initial trials of the flume using fluorescein (green) to track the movement of the chemical plume (C) showed vertical stratification at 5 mL min^{-1} flow rate with some bleeding and (D) undesired horizontal stratification of the plume.

Statistical summary of the crown-of-thorns starfish genome assembly

Table A 4 COTS genome assembly statistics for Male and Female COTS. Megabase = mb (1 mb = 1,000,000 base pairs or bp), kilobase = kb (1 kb = 1000 bp).

	Male – GCA_030586445.1	Female – GCA_030586425.1
Genome size	403.9 mb	402.6 mb
Number of contig/scaffolds	633	1169
N50	1.7 mb	979 kb
GC content	41	41
Genome coverage	45x	59x

Table A 5 Busco statistics for Male and Female COTS

	Male – GCA_030586445.1	Female – GCA_030586425.1
Complete	99.48%	97.59%
Duplicated	1.47%	1.99%
Fragments	0.1%	0.21%
Missing	0.42%	0.21%
N	954	954

Table A 6 Annotation statistics for Female COTS. Base pair = bp, megabase = mb (1 mb = 1,000,000 base pairs).

	% GC	% of genome	Average size (bp)	Median size (bp)	Number	Total length (mb)
Exon	45	17	375	150	187,327	70
Gene	41	74	16,470	9,456	18,125	299
Intron	40	58	1,470	623	158,180	233

Table A 7 Annotation statistics for Male COTS. Base pair = bp, megabase = mb (1 mb = 1,000,000 base pairs).

	% GC	% of genome	Average size (bp)	Median size (bp)	Number	Total length (mb)
Exon	45	17	374	150	187,528	70
Gene	41	75	16,709	9,551	18,104	303
Intron	40	59	1,493	624	158,371	237

Appendix G

Table A 8 List of the proteins identified in the > 30 kDa summer fraction (FrA). The two ependymin-related proteins (EPDRs) are highlighted in red. * represents the EPDR found in the *Asteroid*-specific clade. The sequence names have been adapted from Hall et al. (2017).

Sequence name	Description	Classification
gbr.332.7.t1	Uncharacterised protein	Conserved Uncharacterised Protein
gbr.504.1.t1	Uncharacterised protein LOC110982186	Conserved Uncharacterised Protein
gbr.19.105.t1	Beta-glucuronidase	Hydrolytic Enzymes
gbr.147.31.t1	Plancitoxin-1-like	Hydrolytic Enzymes
gbr.558.4.t1	Phospholipase A2 AP-PLA2-I-like	Hydrolytic Enzymes
gbr.58.65.t1	Trypsin alpha-3-like	Hydrolytic Enzymes
gbr.13.85.t1	Peroxidasin homolog isoform X2	Other Enzymes
gbr.108.39.t1	Fibrocystin-L-like	Structural/Signalling Proteins
gbr.11.89.t1	Melanotransferrin 2	Structural/Signalling Proteins
gbr.156.37.t1	Putative defence protein 3	Structural/Signalling Proteins
gbr.198.25.t1	Vitellogenin 1	Structural/Signalling Proteins
gbr.2.190.t1	Pentraxin fusion protein-like	Structural/Signalling Proteins
gbr.289.10.t1	Neuronal cell adhesion molecule-like isoform X1	Structural/Signalling Proteins
gbr.35.36.t1	Vitellogenin 2	Structural/Signalling Proteins
gbr.36.17.t1	Contactin-2-like isoform X1	Structural/Signalling Proteins
gbr.56.80.t1	Von Willebrand factor-like	Structural/Signalling Proteins
gbr.72.115.t1	Mitochondrial import inner membrane translocase subunit TIM50-like	Structural/Signalling Proteins
gbr.60.102.t1	Development-specific protein LVN1.2-like	Structural/Signalling Proteins
gbr.218.34.t1*	Development-specific protein LVN1.2	Structural/Signalling Proteins

gbr.60.102.t1
MDIKLAALLLLVGLATSYAQPAEGPCCFPDQFVVGVDSEAFLGQGYPWQ
LEEEQSRESASKIQPEGVVLGSETAVGGRGGTKRLAIVGQSAVDVNKNM
IGNEFTLFQSDEERPSRQRLIYDYEQGFQYIIDTDELKCSKTKLQGDIP
RCVPPGVDFNTTVYLGDRQLFIDSYHYQIQQLFKNGRSSVSVTKEGCIP
NSASFSGTTFRTSILSFAGYFNYEAGIADPDRFFEVPEYCPTVRL

gbr.218.34.t1
MKDHGLLCVSTIVIQIIDLGNLVLRACRSESWSVALGLKTARHTHHNPY
QGPPIAAMYAAVILCILVVAASASPEKFIFGNKLDQPAPEKCCAEPYYT
FQADSVFNTLQEGSLLTELIRARGAYDSIDKKFGLKVDLHISNGTVELY
QLINDFKEGLGYYIYTEEEETKCVEFPITSGFPYNCIPEGSTYVGSVTI
GDRALRAANWYFNDKTDPTKDMHIVFSIKEEECIDLGYLARTFDPETGT
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PRGQRLFQ

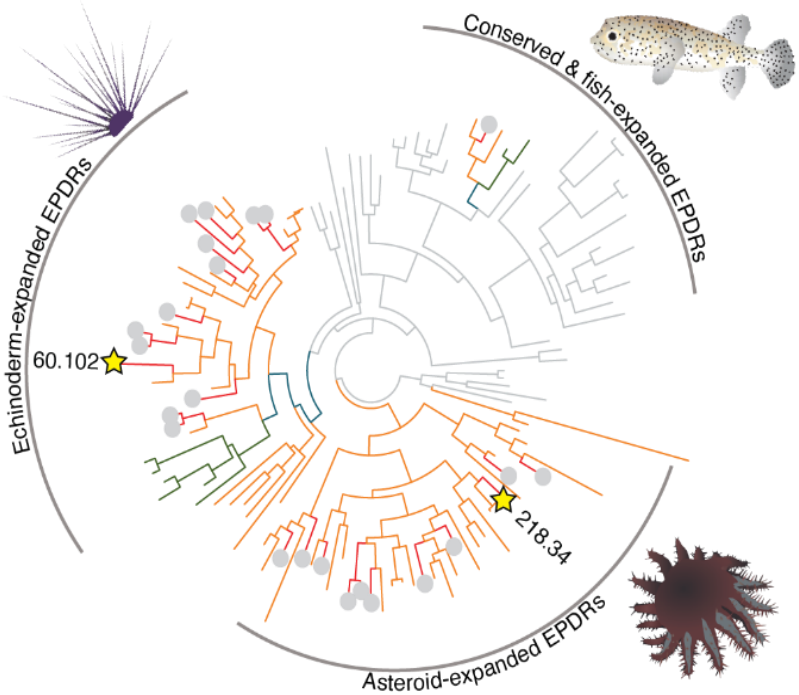

Figure A 6 Amino acid sequences of the two ependymin-related proteins (EPDRs) discovered in the > 30 kDa summer fraction (FrA). Phylogenetic comparison of EPDRs divided into three major clades, echinoderm, asteroid, and conserved and fish-expanded. Grey circles represent all COTS EPDRs. Yellow stars represent COTS EPDRs identified in the > 30 kDa summer fraction FrA. COTS genes are highlighted in red; asteroids, orange; sea urchin, green; and echinoderms, blue-green (adapted from Hall et al. 2017). Images of COTS, sea urchin and puffer fish are from T. Saxby, J. Hawkey, and J. Woerner (Integration and Application Network, Center for Environmental Science, University of Maryland).

Appendix H

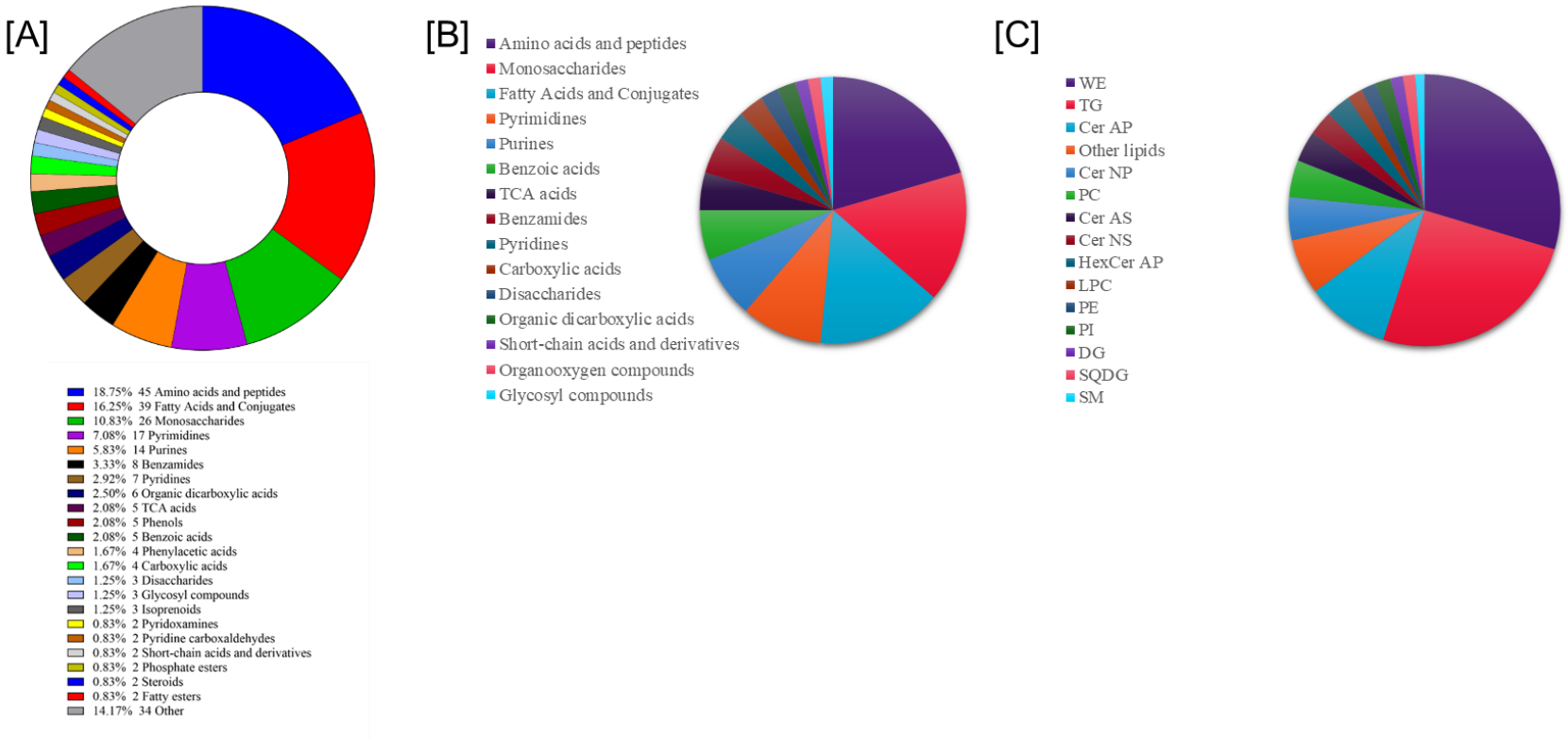

Figure A 7 Metabolites in tissues and eggs of crown-of-thorns starfish (COTS). Classification of A) metabolites in COTS tissues; and B) metabolites and C) lipids in eggs (Mendoza-Porras et al. 2023).

Appendix I

Table A 9 Annotation and expression of significantly up-regulated receptors within spines from reproductive and non-reproductive crown-of-thorns starfish. Gene expression reported in transcript per million (TPM). Level of gene expression: High = red, Medium = yellow and Low = green.

Gene ID	Best BLAST hit	R	NR	Gene ID	Best BLAST hit	R	NR
oki.71.34	adhesion G-protein coupled receptor D1-like	36.8	8.0	oki.70.95	MAM and LDL-receptor class A domain-containing protein 1	49.4	12.0
oki.44.114	adhesion G-protein coupled receptor G2-like	197.5	31.5	oki.163.34	Metabotropic glutamate receptor 3	68.9	10.1
oki.44.112	adhesion G-protein coupled receptor G2-like	129.2	21.0	oki.1.199	metabotropic glutamate receptor 3	45.0	13.3
oki.153.3	adhesion G-protein coupled receptor G2-like	93.8	8.5	oki.106.12	metabotropic glutamate receptor 4-like	40.4	18.0
oki.10.196	adhesion G-protein coupled receptor G7-like	380.3	74.9	oki.321.26	mitochondrial import receptor subunit TOM34	24.5	14.1
oki.90.57	alpha-1D adrenergic receptor-like	59.7	29.3	oki.43.109	muscarinic acetylcholine receptor M2	43.9	35.0
oki.93.71	angiopoietin-1 receptor-like	178.2	32.7	oki.63.14	netrin receptor UNC5C-like	115.7	25.5
oki.93.72	angiopoietin-1 receptor-like	125.6	5.3	oki.31.75	nuclear receptor corepressor 1-like isoform X2	69.8	41.2
oki.93.3	angiopoietin-1 receptor-like	81.3	1.8	oki.104.33	nuclear receptor subfamily 1 group D member 2	27.6	25.3
oki.35.33	atrial natriuretic peptide receptor 1-like	78.5	31.6	oki.139.29	peripheral-type benzodiazepine receptor-associated protein 1-like	33.4	11.6
oki.49.20	cation-independent mannose-6-phosphate receptor	28.2	12.9	oki.8.112	peroxisome proliferator-activated receptor gamma isoform X1	36.3	18.1
oki.12.168	fibroblast growth factor receptor 1 isoform X4	77.5	15.0	oki.46.93	probable G-protein coupled receptor 133	6.9	3.4
oki.18.15	fibroblast growth factor receptor 1-like isoform X3	65.6	8.5	oki.156.18	probable G-protein coupled receptor CG31760	41.0	22.3
oki.37.101	galanin receptor type 1	44.2	15.0	oki.308.2	proto-oncogene tyrosine-protein kinase receptor Ret-like	75.6	28.5
oki.191.38	glutamate receptor 1 isoform X1	1342.2	173.5	oki.71.29	receptor expression-enhancing protein 2 isoform X2	60.3	29.7
oki.191.39	glutamate receptor 1 isoform X6	1618.1	187.7	oki.93.2	receptor-type tyrosine-protein phosphatase alpha-like	157.2	9.6
oki.31.225	glutamate receptor 2 isoform X3	43.1	11.3	oki.547.1	receptor-type tyrosine-protein phosphatase alpha-like isoform X2	253.9	11.4
oki.112.52	hepatocyte growth factor receptor	45.2	15.6	oki.6.213	receptor-type tyrosine-protein phosphatase beta isoform X2	214.7	18.9
oki.13.95	hepatocyte growth factor receptor-like	84.5	30.0	oki.136.27	receptor-type tyrosine-protein phosphatase F-like isoform X1	33.0	2.9
oki.82.77	interleukin-17 receptor A-like	252.0	10.0	oki.68.14	renin receptor-like	275.1	80.4
oki.136.59	interleukin-6 receptor subunit beta-like isoform X1	56.4	19.1	oki.2.283	short transient receptor potential channel 5	22.7	18.9
oki.14.52	Lamin-B receptor	23.5	13.6	oki.229.13	sortilin-related receptor	114.0	31.6
oki.24.72	leucine-rich repeat and	60.9	4.0	oki.117.41	TGF-beta receptor type-2	83.7	20.1
oki.84.40	low density lipoprotein receptor adapter protein 1-A-like isoform X1	43.5	13.9	oki.45.78	thyroid receptor-interacting protein 11	13.9	10.6
oki.20.124	low-density lipoprotein receptor-related protein 12-like	58.0	8.6	oki.126.21	transient receptor potential cation channel subfamily A member 1 homolog	53.1	6.6
oki.170.8	low-density lipoprotein receptor-related protein 2	160.5	23.9	oki.70.85	tyrosine kinase receptor Cad96Ca	140.7	15.0
oki.104.32	low-density lipoprotein receptor-related protein 2 isoform X2	137.7	9.5	oki.93.14	tyrosine-protein kinase receptor Tie-1-like	203.6	8.1
oki.142.35	macrophage scavenger receptor types I and II isoform X1	421.0	43.0	oki.1.232	tyrosine-protein phosphatase non-receptor type 13-like isoform X1	13.1	3.3

Table A 10 BLAST annotation of significantly differentially expressed genes in crown-of-thorns starfish spine tissue between reproductive males and females. * indicates those encoding a protein with signal peptide.

Gender	Name	Log_2 fold change	FDR p-value	BLAST annotation
Male	oki.659.1	11.85	0	uncharacterised protein LOC110987300
	oki.376.14*	11.55	0	ly6/PLAUR domain-containing protein 2-like
	oki.265.20	10.08	0.01	organic solute transporter subunit alpha-like
	oki.145.50	7.1	0	inter-alpha-trypsin inhibitor heavy chain H2
	oki.8.266	6.82	0	phospholipase A2 AP-PLA2-I-like
	oki.263.28*	6.78	0	nephrin-like isoform X4
	oki.7.158	6.64	0.01	ketimine reductase mu-crystallin-like
	oki.67.1	6.43	0	cell death activator CIDE-3 isoform X2
	oki.145.52*	6.29	0	inter-alpha-trypsin inhibitor heavy chain H4
	oki.91.77	6.29	0	FAS-associated death domain protein-like isoform X1
	oki.96.44	6.19	0	peroxisomal sarcosine oxidase-like
	oki.58.134	6.1	0	uncharacterised protein LOC110979978
	oki.6.241	6.04	0	pathogen-related protein-like
	oki.400.2*	5.98	0	plexin-B-like isoform X2
	oki.132.12	5.95	0.05	predicted protein
	oki.58.132	5.62	0	---NA---
	oki.33.18	5.57	0	deoxycytidylate deaminase
	oki.412.8	5.1	0.05	uncharacterised protein LOC110990579
	oki.688.1*	4.62	0	putative ferric-chelate reductase 1
	oki.564.1	4.4	0.02	protein NLRC5-like isoform X4
	oki.228.35*	3.77	0	microfibril-associated glycoprotein 4-like
	oki.352.12	3.63	0.04	very long-chain acyl-CoA synthetase-like
	oki.803.1	3.55	0.01	neurogenic locus notch homolog protein 1-like isoform X1
	oki.1.327	3.52	0	acyl-CoA-binding domain-containing protein 4-like isoform X2
	oki.156.14	3.29	0	cytochrome P450 4F1-like
	oki.52.79*	2.84	0	cell wall protein DAN4-like
	oki.86.73*	2.62	0	cAMP-dependent protein kinase regulatory subunit
	oki.94.11	2.62	0.03	prolyl endopeptidase-like
	oki.40.2	2.55	0.02	uncharacterized protein LOC110981726
	oki.1.167	2.53	0	steroid 17-alpha-hydroxylase/17,20 lyase-like isoform X2
	oki.143.30	2.53	0.03	oxidoreductase NAD-binding domain-containing protein 1
	oki.68.113	2.48	0.01	uncharacterised protein LOC110980999
	oki.90.5	2.45	0	mediator of RNA polymerase II transcription subunit 16
	oki.29.36	2.24	0.02	E3 ubiquitin-protein ligase RNF25 isoform X1
	oki.32.14	2.17	0.04	contactin-associated protein-like 2
Female	oki.300.12	7.34	0.01	CD226 antigen-like
	oki.142.38	6.82	0.01	sacsin-like
	oki.45.92	5.9	0.03	sushi domain-containing protein 2-like

	oki.406.3	5.76	0	proprotein convertase subtilisin/kexin type 9
	oki.7.120*	5.75	0.03	5'-nucleotidase-like
	oki.7.121	5.52	0.05	5'-nucleotidase-like
	oki.537.1	5.35	0	angiopoietin-1 receptor-like
	oki.186.27	4.93	0	uncharacterised protein LOC110987300
	oki.22.3	4.67	0.03	deoxynucleoside triphosphate triphosphohydrolase SAMHD1-like isoform X1
	oki.300.15	4.52	0	CD226 antigen-like
	oki.73.25	3.8	0.04	uncharacterised protein C1orf112-like
	oki.34.1	3.65	0	NLR family CARD domain-containing protein 4-like isoform X1
	oki.37.5*	3.36	0	adhesive plaque matrix protein 2-like
	oki.50.52*	2.92	0.04	flocculation protein FLO11-like isoform X2
	oki.98.13	2.9	0	cytochrome c
	oki.70.124	2.83	0.04	NLR family CARD domain-containing protein 4-like isoform X1
	oki.132.46	2.7	0.04	THAP domain-containing protein 10-like
	oki.96.91	2.01	0.05	CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase-like isoform X1

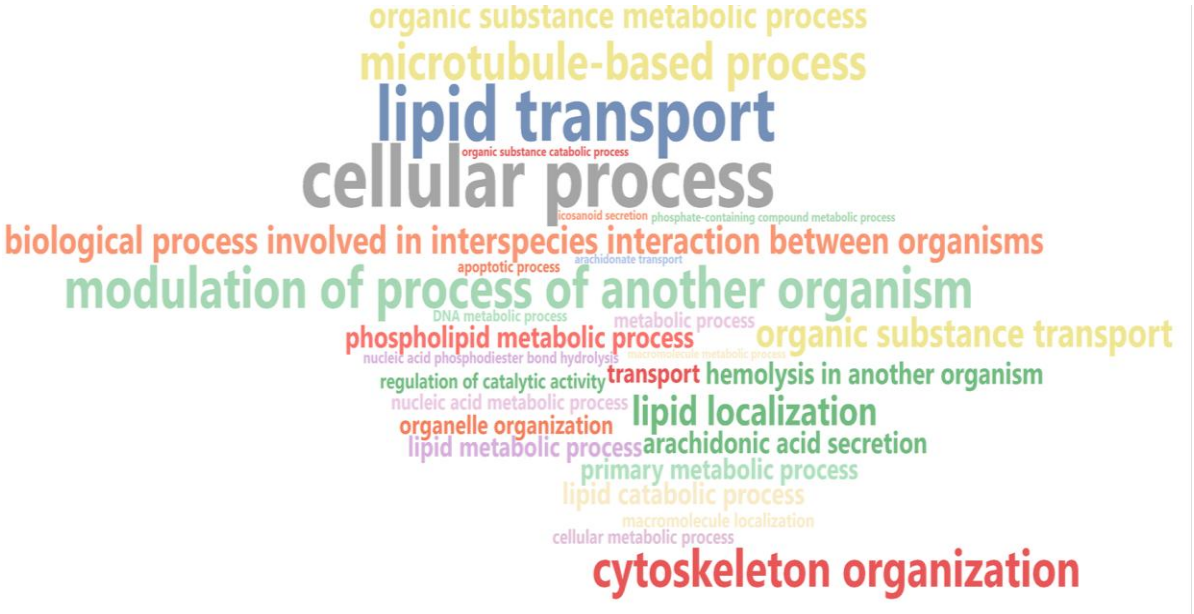

Figure A 8 WordCloud displaying the top 40 most abundant Gene Ontology (GO) terms (limited to the biological process category) for total proteins identified in the crown-of-thorns starfish spine extracts. The font size indicates the node score of each GO term.

Appendix J

Table A 11 Summary of putative secreted proteins identified in the spines of crown-of-thorns starfish. The numbers of total and unique mass spectral (MS) peptide match, BLAST hit proteins with corresponding species, e-value and GenBank accession numbers, and identified protein domains for each protein precursor are included.

Protein ID	MS peptide matches	unique MS peptide matches	BLAST hit protein and species (NCBI accession number)	e-value of BLAST hit	Identified protein domains
oki.208.17	1	1	Cysteine-rich secretory protein LCCL domain-containing 2-like [*Acanthaster planci*] (XP0022106668)	9.94E-133	Signal, SCP domain
oki.83.49	3	2	Deleted in malignant brain tumors 1 protein-like [*Lytechinus variegatus*] (XP041455825)	0	Signal, SR, FTP, EGF domains
oki.141.73	1	1	Ependymin [*Patiria miniata*] (XP038059830)	2E-15	Signal, Epdr domain
oki.11.66	1	1	Ependymin-related protein 1-like [*Patiria miniata*] (XP038069568)	1E-71	Signal, Epdr domain
oki.258.16	26	26	Kielin/chordin-like protein [*Patiria miniata*] (XP038067966)	0	Signal, von Willebrand factor type C domain
oki.162.19	3	3	Pancreatic lipase-related protein 2-like [*Acanthaster planci*]	3.23E-158	Signal, Pancreatic lipase-like domain
oki.8.264	11	3	Phospholipase A2-II-like [*Acanthaster planci*] (XP022079277)	1E-102	Signal, PA2c domain
oki.8.261	5	1	Phospholipase A2-II-like [*Acanthaster planci*] (XP022079273)	7E-103	Signal, PA2c domain
oki.8.262	7	7	Phospholipase A2-I-like [Acanthaster planci] (XP022079272)	8E-102	Signal, PA2c domain
oki.27.33	7	1	Plancitoxin-1-like [*Acanthaster planci*] (XP022085064)	0	Signal, Dnase II domains
oki.27.35	23	17	Plancitoxin-1-like [*Acanthaster planci*] (XP022085063)	0	Signal, Dnase II domains
oki.111.24	2	2	Uncharacterised protein LOC110983903 [*Acanthaster planci*] (XP022099251)	0	Signal
oki.36.13	6	4	Vitellogenin 2 [*Patiriella regularis*] (AHK12748)	0	Signal, d1gw5a, LPD N, DUF1943, VWD domains
oki.36.14	2	2	Vitellogenin 2 [*Patiriella regularis*] (XP022087200)	0	Signal, d1gw5a, LPD N, DUF1943, VWD domains
oki.185.68	56	5	Vitellogenin-like [*Acanthaster planci*] (XP022105422)	0	Signal, d1gw5a, LPD N, DUF1943, VWD domains
oki.1.63	2	2	Pentraxin fusion protein-like [*Acanthaster planci*] (XP_022090826)	0	Signal, F5/8 type C domain

Appendix K

Table A 12 List of gene clusters identified in the reference juvenile crown-of-thorns starfish transcriptome, annotated against the nucleotide (NT), SwissProt, KO not (KEGG Orthology), PFAM, MF and EuKaryotic Orthologous Groups (KOG) protein databases, reveal the presence of plancitoxins.

Gene ID Cluster	Gene Length	NT Description	Swissprot	KO/PFAM/MF/KOG	Biological Process
11131.0	399	PREDICTED: *A. planci* plancitoxin-1 (LOC110976258), transcript variant X3, mRNA	--	--	--
12038.0	1612	PREDICTED: *A. planci* plancitoxin-1-like (LOC110976372), mRNA	Plancitoxin-1 OS= *A. planci* OX=133434 PE=1 SV=1	Deoxyribonuclease II	DNA metabolic process//DNA catabolic process
16982.0	400	PREDICTED: *A. planci* plancitoxin-1 (LOC110976258), transcript variant X3, mRNA	--	--	--
2378.0	665	PREDICTED: *A. planci* plancitoxin-1 (LOC110976258), transcript variant X3, mRNA	--	--	--
4220.10986	8574	PREDICTED: *A. planci* uncharacterized LOC110976371 (LOC110976371), mRNA	Plancitoxin-1 OS= *A. planci* OX=133434 PE=1 SV=1	Deoxyribonuclease II	DNA metabolic process//DNA catabolic process
4220.11142	1348	PREDICTED: *A. planci* plancitoxin-1-like (LOC110976260), mRNA	Plancitoxin-1 OS= *A. planci* OX=133434 PE=1 SV=1	Deoxyribonuclease II	DNA catabolic process//DNA metabolic process
4220.1233	2212	*A. planci* plan-I mRNA for plancitoxin I, complete cds	Plancitoxin-1 OS= *A. planci* OX=133434 PE=1 SV=1	Deoxyribonuclease II	DNA metabolic process//DNA catabolic process
4220.15457	1036	PREDICTED: *A. planci* plancitoxin-1-like (LOC110976261), mRNA	--	--	--
4220.2375	941	PREDICTED: *A. planci* plancitoxin-1-like (LOC110976261), mRNA	Plancitoxin-1 OS= *A. planci* OX=133434 PE=1 SV=1	Deoxyribonuclease II	DNA metabolic process//DNA catabolic process
4220.2994	3266	PREDICTED: *A. planci* plancitoxin-1-like (LOC110976259), mRNA	Plancitoxin-1 OS= *A. planci* OX=133434 PE=1 SV=1	Deoxyribonuclease II	DNA metabolic process//DNA catabolic process

Appendix L

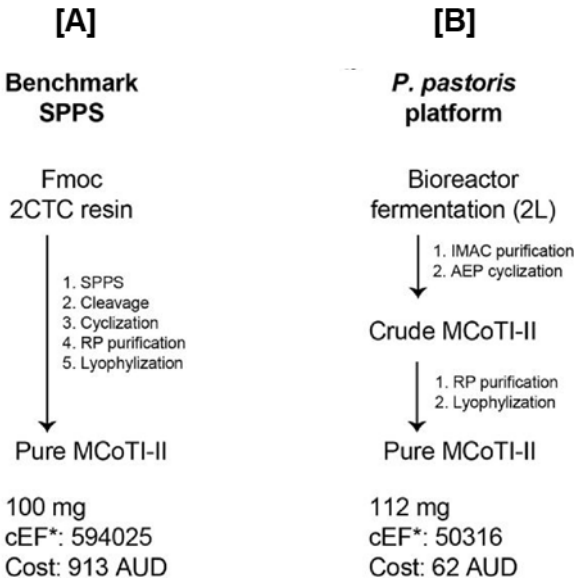

Figure A 9 Workflow for the (A) benchmark solid-phase peptide synthesis (SPPS) and (B) recombinant expression of MCoTI-II cyclic peptide (CP) using the *Pichia pastoris platform* (Yap et al. 2020), applied here to EPDR. Flowchart shows comparison of the two methods with cEF and associated costs. Based on the current exchange rate of 1 AUD = 0.70 USD the benchmark SPPS cost is 639 USD and the *P. pastoris* platform cost is 43 USD. *cEF (complete E factor) = (Σm (solvents) + Σm (reagents) - m (product))/ m (product).

Table A 13 Summary of amount and cost of materials, reagents, solvents and water used in the synthesis of 100 mg of MCoTI-II cyclic peptide using benchmark solid-phase peptide synthesis (Yap et al. 2020).

Materials, reagents, solvents and water	Weight (kg)	Cost (AUD)
∑ weight of amino acids	0.192	118.20
∑ weight of resin	0.002	8.77
∑ weight of DMF	44.000	420.96
∑ weight of DCM	1.203	7.63
∑ weight of piperidine	0.958	89.18
∑ weight of HCTU	0.154	92.62
∑ weight of DIPEA	0.142	55.28
∑ weight of MeOH	0.031	0.19
∑ weight of ACN	1.144	13.93
∑ weight of TIPS	0.006	13.34
∑ weight of TFA	0.533	93.74
∑ weight of H_2O	11.039	0.00
Total	**59.403**	**913.84**

Table A 14 Summary of amount and cost of materials, reagents, solvents and water used in the recombinant production of 112 mg MCoTI-II. The calculations include *Pichia pastoris* production and purification of MCoTI-II CPs as well as production and purification of MCoAEP2 (Yap et al. 2020).

Materials, reagents, solvents and water	Weight (kg)	Cost (AUD)
∑ weight of yeast extract	0.030	8.94
∑ weight of peptone	0.060	7.10
∑ weight of glycerol	0.082	1.29
∑ weight of methanol	0.438	1.83
∑ weight of biotin[%]	0.000	0.10
∑ weight of yeast nitrogen base	0.027	38.01
∑ weight of methanol	0.003	0.06
∑ weight of ACN	0.401	4.88
∑ weight of TFA[%]	0.000	0.00
∑ weight of imidazole[%]	0.001	0.50
∑ weight of H_2O	4.595	0.00
Total	**5.637**	**62.71**

[%]Trace amounts used in downstream purification buffers

Appendix M

Table A 15 Excerpt from the Productivity Commission 2003, Industries, Land Use and Water Quality in the Great Barrier Reef Catchment, Research Report identifying current chemical management practices relevant to GBR water quality (WQ) including herbicides and pesticides.

WQ concerns & possible causes	Main industries/ activities	Potentially harmful practices	Potentially beneficial practices
Overuse or misapplication of herbicides and pesticides	• Sugarcane • Horticulture • Cotton	Over application of chemicals	• **Weed and pest monitoring** • **Integrated Pest Management** • **Use of more benign chemicals** • Coordinating application with irrigation activities

Table A 16 Technology readiness levels (TRLs) attributed to the innovation phases in the chemical industry, from “basic research”, “applied research” and “development” to “deployment”. Modified from Buchner et al. 2019.

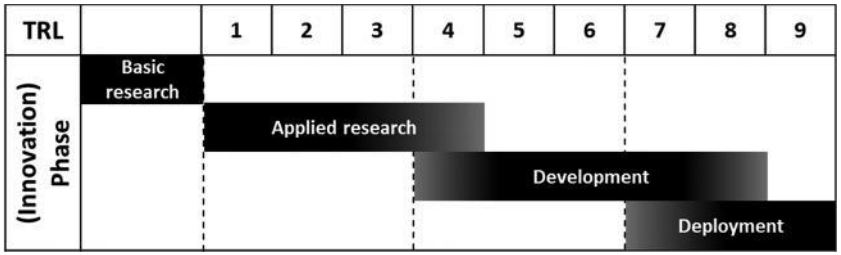

Table A 17 APVMA attractant product registration decision tree (modified from APVMA), applied to determine whether registration of a COTS-derived pheromone is required for use in COTS integrative pest management.

APVMA Categorisation and considerations for lures and attractants			**Categorisation and considerations for COTS semiochemical[2] attractants**	
Desired outcome	**Attractant source**	**Registration required?**	**Attractant source**	**Registration required?**
Invertebrate pest management	Food; not containing any active constituent[1]	No; may require permit of use	Non-food; COTS-derived	Unknown; Possibly
Attract pest for the purpose of destroying it – non-active constituent[1]	Non-food	No	Non-food; COTS-derived; destruction via manual injection of bile salts	Unknown; Possibly
Attract pest for the purpose of destroying it - active constituent[1] or used with another agvet product		Yes	Non-food; COTS-derived; destruction via active constituent	Yes
● Stupefy, ● repel, ● inhibit feeding of, ● prevent infestation/attack by, ● modify pest physiology, ● modify effect of an agricultural chemical		Yes	Non-food; COTS-derived; prevention of infestation and attacks achieved via other methods/agents	
Attractant and agvet product are marketed, packaged and supplied: as a single product or separately, but with the intention or claim to use the attractant and the agvet product together		Yes	Non-food; COTS-derived: ● extract (i.e., TFF), ● recombinantly expressed peptides and proteins ● synthesised (i.e., peptides), ● synthetically modified (i.e., mimics)	

[1] An active constituent is the component(s) in a chemical preparation or formulation that is responsible for bringing about a biological or therapeutic effect. By definition this excludes behavioural effect.

[2] Currently, deployment of semiochemicals in the marine environment has no clear regulatory definition. The European Union definition states semiochemicals can be classified as the active ingredient or as a co-formulant (ECHFS 2024).

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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