Deployment of semiochemical control agents to manage crown-of-thorns starfish populations

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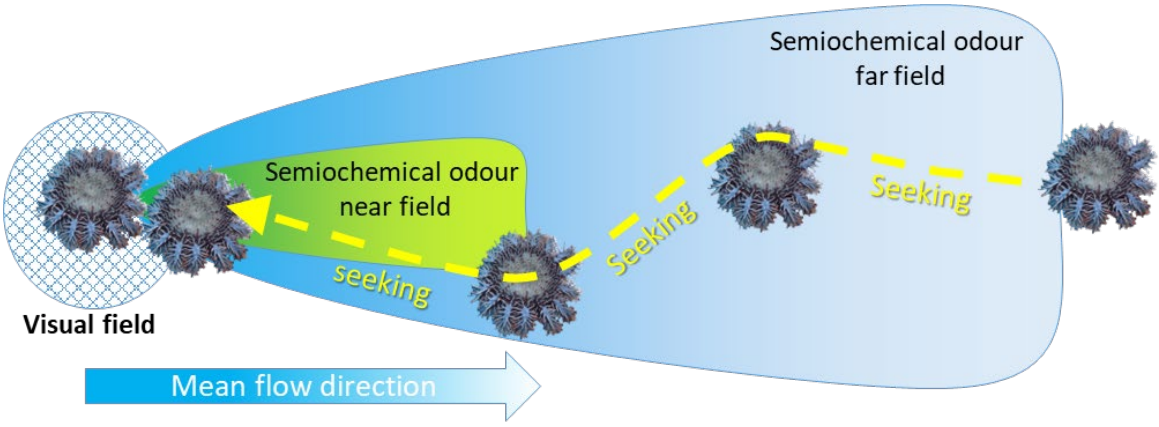

COTS Control Innovation Program | A research and development partnership to better predict, detect and respond to crown-of-thorns starfish outbreaks

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This report should be cited as

Motti CA, Vasile R, Robson B, Høj L, Wang CK, Craik DJ, Degnan BM, Degnan SM, Cummins SF, 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 (80 pp).

Funding Acknowledgement

The COTS Control Innovation Program aims to accelerate the development of innovative surveillance and control methods to manage outbreaks of coral-eating starfish on the Great Barrier Reef. The Program is a collaboration between the Great Barrier Reef Foundation, Australian Institute of Marine Science, Commonwealth Scientific and Industrial Research Organisation, James Cook University and The University of Queensland. The Program is funded by the partnership between the Australian Government's Reef Trust and the Great Barrier Reef Foundation.

Traditional Owner Acknowledgement

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

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Acronyms and Definitions

Acronyms	
COTS	Crown-of-thorns starfish
GBR	Great Barrier Reef
GBRMPA	Great Barrier Reef Marine Park Authority
IPM COTS CP	Integrated Pest Management COTS Control Program
SARs	Structure-activity relationships
Definitions	
Processes	
Control strategy	(referred to as behaviour manipulation strategy) a long-range plan to moderate COTS populations, i.e., attractant; repellent; attractant + repellent; predator enhancement
Push control strategy	The use of repellents to deter or repel a pest from a point-source
Pull control strategy	The use of attractants to lure a pest to a point-source
Push-pull strategy	Combined use of a repellent and an attractant to shepherd a pest to a specific location
Predator enhancement strategy	The use of attractants to lure a predator species to COTS
Alternative control technologies	Technologies expected to displace currently used ones
Supplemental technologies	Technologies expected to be integrated with traditional ones
Delivery method	Procedure (logistics, engineered devices, etc) for transferring the semiochemical to the destination
Collateral damage	The adverse ecological effects of introducing a COTS-targeted (either specific or non-specific) semiochemical into the reef ecosystem and the adverse events associated with continued use.
Point-source	Self-contained, discrete chemoemitting devices applied at specific spatial intervals
Nonpoint-source	Blanket spray or spread across a target area, with limited spatial resolution
(Bio)chemical	
Chemical	A substance formed from two or more elements united in fixed proportions
Semiochemical	A chemical or mixture of chemicals that relays a message to animals and modifies their behaviour

Biological function	The functional or anatomical change resulting from exposure of animals to the semiochemical
Molecular mode of action	Detailed understanding of how the chemical is altered, the specific receptor it interacts with and the physiological changes this causes in the recipient animal
Behaviour	
Passive aggregation	animals gathered together in response to foraging cues or environmental physicochemical parameters
Active aggregation	animals gathered together in response to chemicals released by conspecifics, predators or decaying (often conspecific) tissues
Delivery	
Delivery platform	a suite of engineered or vehicular technologies that are used as a base to deliver equipment or resources
Delivery device	Mechanical or electronic components that transfer the chemical payload from the delivery platform into the environment, i.e., pumps, piston syringes, power supply
Model	
Reef	an individual reef or a collection of reefs around an island in the GBR
Release site	the specific location, identified by GPS coordinates, where the semiochemical is released
Model	(hydrodynamic model for the purpose of this study) a numerical computational model
Model run	(also referred to as simulation) the execution of a model with a unique set of parameter values such as a given spatial domain, time period, chemical release sites, etc.
Model Scenario	the parameters that vary between different simulations of a model for the purpose of comparing those model outputs and inspecting the effect of different parameter values, e.g., different chemical release regimes

EXECUTIVE SUMMARY

Repeat outbreaks of Crown-of-Thorns starfish (COTS) are threatening the survivability of coral reefs. In 2018, the Integrated Pest Management COTS Control Program (IPM COTS CP) was implemented to inform and develop management strategies to reduce the immediate impact of COTS outbreaks and the chance of recurrence. A key component of the IPM COTS CP is the need for supplemental non-manual control technologies to improve the efficiency and advance the effectiveness of current control measures.

COTS life stages and traits are modulated by various tactile, visual and chemical signals. Signalling chemicals, called semiochemicals, have been widely used in terrestrial ecosystems to control pest species, and are now being developed for aquatic ecosystems for invasive species like sea lamprey and common carp. As for these pests, many of the traits of COTS have the potential to be manipulated by semiochemical technologies.

This report provides an overview of semiochemical technologies and learnings from aquatic applications. It includes a knowledge gap analysis and considers delivery strategies, platforms and devices to semiochemically control COTS. Hydrodynamic models explore the spatial and temporal footprint of semiochemical delivery around reefs and reveal the potential for point-source deployment. Finally, case study examples provide context for the use of semiochemical agents under various conceptual COTS control strategies and scenarios, and are intended as tools to initiate engagement with reef managers to consider the implementation and integration of such technology into combined pest management strategies.

The report finds there is great potential for the inclusion of semiochemical technologies in the IPM COTS CP, being inherently eco-safe and tailored specifically for COTS. First, it recommends the implementation of a semiochemical pull control strategy and proposes pheromone attractants produced by conspecifics targeting the adult life history stage be the primary focus of research and development efforts. Raw concentrates or semi-purified fractions of chemicals secreted by captive COTS offer a sustainable, easily prepared and ready supply of COTS-specific semiochemical. Secondly, it found COTS-specific pheromone attractants have great scope for in-water application, in particular, for use in combination with other control techniques, such as traps and cages, to enhance current manual control methods on a localised reef scale. Thirdly, the report recommends application of semiochemical technologies to suppress low- to mid-density populations in the initiation box or during spawning to reduce reproductive success, and to augment control efforts on recalcitrant reefs, such as John Brewer Reef where multiple revisitations have been needed to shift the status to non-outbreak. Finally, the report provides the foundation for developing a complementary control strategy to improve current in-water COTS control, with prospects to test the applicability of raw concentrates immediately.

1. INTRODUCTION

Crown-of-Thorns Starfish (COTS) are highly efficient coral predators, and their population outbreaks have caused extensive loss of coral cover on the Great Barrier Reef (GBR) (Moran 1986, Pratchett et al. 2014) and throughout their Indo-Pacific range (Bruno & Selig 2007, Trapon et al. 2011, Mellin et al. 2016). Repeat outbreaks are threatening the survivability of coral reefs and are impacting on commercial and recreational activities that generate $6.4 billion AUD of economic activity for the GBR each year (O'Mahoney et al. 2017). Significant effort and resources have been expended to understand the causes of these outbreaks and develop management strategies to mitigate further damage (Westcott et al. 2020). The Integrated Pest Management COTS Control Program (IPM COTS CP) was implemented in 2018 and to mid-2020 had reduced COTS densities to below the threshold density of 3 COTS ha^{-1} on 92 of 103 reefs considered requiring intervention (Westcott et al. 2021). The principle aim of this control program is focussed on the protection of coral primarily through suppression of COTS outbreaks, to reduce the immediate impact. Despite this, their persistent recurrence remains of grave concern to reef managers (GBRMPA 2019, AIMS LTMP 2022) and, in the face of mounting pressures from a changing climate, there is now renewed urgency to develop more effective control technologies to maintain reef health (CCIP 2021, 2022).

Semiochemical compounds are ubiquitous, naturally occurring substances that modulate the normal behaviours of animals in the wild (Kost 2008). They are classified according to whether they trigger behavioural and/or physiological responses in the same species (pheromone) or in other species (allelochemical), referred to as conspecific or interspecific communication, respectively. The latter group is further classified based on whether they benefit the receiver (kairomones, including necromones from decaying tissues and apneumones from non-biological origins), the emitter (allomones), or both the emitter and receiver (synomones). In all cases, the semiochemical induces a behavioural response, often through induction of chemotaxis (i.e., the receiver follows a gradient path of the chemical cue over relatively long distances compared with other communication modalities such as mechanosensation or vision) (Atema et al. 2012). The receiver typically detects the semiochemical by chemoreceptors associated with sensory organs or tissues. When stimulated these chemoreceptors activate a signalling cascade that translates this chemical interaction into a range of physiological and behavioural changes.

Semiochemical compounds are by nature relatively non-toxic, often species-specific (especially if a pheromone), have a relatively short half-life and are active at very low (picomolar) concentrations (Civciristov & Halls 2019). They predominantly elicit an innate or hardwired response in the target species, hence, the likelihood of the target population becoming desensitised to continued exposure is low (Wyatt 2010, Stroud et al. 2014). As such, semiochemicals represent innovative tools that can circumvent complications that can arise from the continued use of synthetic toxicants such as pesticides, especially resistance and unintended impacts on non-target species. Within regulatory frameworks for insect and arthropod control (Weatherston & Stewart 2002), semiochemicals are considered toxicologically inactive substances (OECD 2017) posing minimal or no risk to human safety and the environment; in a control scenario the concentrations needed to elicit a response are close to those naturally present in nature. For many terrestrial pests, they are regarded as sustainable alternatives to conventional chemical (i.e., pesticides, herbicides) or engineered control methods (i.e., fencing). These key properties (Vos et al. 2006) and their successful application in terrestrial ecosystems have piqued recent interest in adopting semiochemicals within integrated pest management

strategies to control aquatic pest species (Smart et al. 2014, Hubert et al. 2019, Barber & Steeves 2020).

In the aquatic environment, semiochemical compounds with well-defined biological properties and molecular mode of action have been investigated to enhance more conventional control efforts such as physical barriers and pesticides (Saha et al. 2019, Borowiec et al. 2021). The most advanced aquatic technology to date has been developed to control sea lamprey (*Petromyzon marinus*) in the Great Lakes of North America (Barber & Steeves 2020), with the male-derived sex pheromone attractant (3-keto-petromyzonol sulfate or 3kPZS) now registered by the EPA (Fredricks et al. 2021). Semiochemical control has also been tested for common carp management in Australia (Lim & Sorensen 2012, Invasive Animals CRC 2014). Recently, it has been proposed that semiochemical compounds hold great potential for maintaining COTS populations at levels that negate any impact to coral reef ecosystems (Hall et al. 2017, Motti et al. 2018, Høj et al. 2020) and is based on mounting evidence that water-borne semiochemicals modulate a variety of COTS behaviours, including foraging, spawning, larval settlement, development and metamorphosis, chemical defence, alarm dispersal and predator avoidance (Hall et al. 2017).

This review presents findings from an Early Investment Project funded by the COTS Control Innovation Program (CCIP) to assess the feasibility of deploying semiochemical control agents to manage COTS populations. For context, a brief overview of COTS life stages and traits that may be amenable to control are provided along with the strategies that have been proposed thus far. A more detailed examination of the different types of semiochemical compounds is presented, with emphasis on the factors that are of relevance to their scalable application, including ease of production, monitoring, stability, diffusion, potency and toxicity. This is supported by an overview of formulation alternatives and engineering solutions for delivery. Hydrodynamic modelling of virtual point release scenarios is described, and the effect of different temporal and spatial application strategies considered. Based on these analyses, the possible role of semiochemicals as a supplementary method for COTS control on the Great Barrier Reef is discussed.

2. OVERVIEW OF POSSIBLE SEMICOHEMICAL CONTROL STRATEGIES FOR COTS

The propensity for COTS population explosions has been linked to specific life history traits (Birkeland 1989, Llodra 2002, Deaker & Byrne 2022), and like sea lamprey and common carp, many of these traits have the potential to be manipulated (Motti et al. 2018). Provided in Table 1 is an overview of COTS life history traits and an evaluation of the potential of realised (or field tested) semiochemical control strategies that have been successfully applied to insect (terrestrial) (STS 2022), snail (terrestrial and aquatic) (Tripathi et al. 2013) and fish (aquatic) pest species (Barber & Steeves 2020, Burkett et al. 2021).

Table 1: Inventory of COTS life history traits and the potential to exploit semiochemical-moderated behaviours to control their populations.

Life history stage	Life history trait	Reference to COTS. Relevant examples from other aquatic systems (in ***bold italics***)	Potential for semiochemical exploitation
Larvae	Cloning	(Allen et al. 2019)	No
	Phenotypically plastic growth dynamics	(Deaker & Byrne 2022)	No
	Dietary flexibility	(Deaker & Byrne 2022)	No
	Resilience to variable food conditions	(Deaker & Byrne 2022)	No
	Settlement	(Johnson & Sutton 1994)	Yes – inducers Limited– blockers; fine scale application
Juveniles	Metamorphosis - 2 days post settlement	(Yamaguchi 1973)	No – inducers Limited – blockers; fine scale application
	Chemical defence – toxins, saponins	(Lucas et al. 1979, Cowan et al. 2017)	No
	Peter Pan effect – capable of extending juvenile phase during low food availability	(Deaker et al. 2020)	Limited– possible dietary transition blocker
	Phenotypically plastic growth dynamics	***Carp hunchback morph forms when exposed to constant predator source, they can evade predation but cannot swim as efficiently as the normal phenotype (Kost 2008)***	Limited - predator-induced polyphenism imposes considerable costs under predator-free conditions resulting in deterioration of life history
	Cryptic/camouflage	(De'ath & Moran 1998)	Yes – foraging attraction; fine scale application
	Chemosensation:	(Motti et al. 2018)	Yes – see specific exploitation potential below
	1) anti-predation; chemical defence in the form of toxins and saponins		unknown – specific predator attractant
	2) predator avoidance alarm cue as indicator of the level of predation risk	***Juvenile rainbow trout exposed to predator cue show significantly less fear-related behaviour and higher activity levels but slower acquisition of a learning task (Poisson et al. 2017)***	unknown
	3) predator odour as indicator of the level of predation risk	(Messmer et al. 2013, Hall et al. 2016b, Hall et al. 2017)	unknown – predator kairomone to repel
	4) chemical alarm – saponins	(De'ath & Moran 1998, Campbell et al. 2001)	unknown – conspecific avoidance to warn of predator presence
Adults	Regeneration/cloning	(Messmer et al. 2013)	No
	Vision	(Petie et al. 2016)	Yes – potential combination of control measures
	Mechanosensation	***Sea urchin can be induced to spawn via mechanical shock (Gago & Luís 2010)***	Limited – potential combination of control measures
	Chemosensation:	(Motti et al. 2018)	Yes – see specific exploitation potential below
	1) anti-predation chemical defence – toxins, saponins)	COTS secretome attracts giant triton (Bose et al. 2017b)	Yes – specific predator attractant, COTS defence chemistry to deter fish predators could act as a

		Defence secretion of *Nezara viridula* acts as long-range attractant kairomone for egg parasitoid *Trissolcus basalis* (Mattiacci et al. 1993)	kairomone attractant for another predator such as giant triton
	2) predator avoidance alarm cue as indicator of the level of predation risk	***Descendants of oligochaetes exposed to conspecific alarm cues released after sublethal predation had increased body length (Kaliszewicz 2014)*** ***Diluted extract of crushed individual fish repels conspecifics (Kłosiński et al. 2021)***	Yes - conspecific alarms to drive an alternative reproductive strategy or alter foraging or aggregating behaviours
	3) predator odour as indicator of the level of predation risk	(Hall et al. 2016b, Hall et al. 2017) ***Amphipod Gammarus pulex aggregate in the presence of three-spined sticklebacks (Gasterosteus aculeatus) odour (Kullmann et al. 2008)***	Yes – predator kairomone to drive aggregation
	4) cryptic	(De'ath & Moran 1998)	Yes – to draw out of the reef matrix
	5) passive aggregation – foraging	(de Dios 2015)	Yes – to draw to food cue regardless of dietary specialisation; reproductive state may alter dietary requirement
	6) active aggregation – defence against predation	(Campbell et al. 2001)	Yes – conspecific alarms to drive formation of aggregations
	7) active aggregation – reproduction	(Rogers et al. 2017)	Yes – to draw to mate
	8) high fecundity	(Llodra 2002)	Yes – gonad maturation/spawning inducers/blockers
	9) synchronistic spawning	(Caballes et al. 2021)	Yes – sex specific spawning inducers/blockers
	10) chemical alarm – saponins	(Campbell et al. 2001) ***Echinoderms (Kamyab et al. 2020)***	Yes – conspecific avoidance to disperse aggregations

The application of a semiochemical compound to control a pest species is dependent on target behavioural traits, the compound's biological function and species specificity, hence, in the context of an integrated pest management program, four core control strategies are conceivable to support management decisions.

- **Pull strategy**: Attractants (foraging kairomones, abiotic apneumones, sex pheromones, conspecific disturbance and alarm cues that trigger aggregation) deployed to lure or pull individuals to a point-source for trapping or manual removal, or to entice cryptic individuals from the habitat matrix or depths inaccessible to divers, thereby improving the quality of surveillance data and/or efficiency of manual control.
- **Push strategy**: Repellents deployed to repel or push individuals from a point-source or deter them from entering a location, thereby disrupting the formation or provoking the dispersal of aggregations.
- **Push-pull strategy**: Combined use of repellents and attractants, deployed simultaneously, to push individuals from one area and simultaneously or subsequently pulling them to another for trapping and/or manual removal. A push-pull strategy is currently being investigated for the sea lamprey (Hume et al. 2020) and carp (Sorensen & Stacey 2004).
- **Predator enhancement strategy**: Attractants deployed to entice natural COTS enemies into the area requiring intervention.

Extensive laboratory and controlled in-field bioactivity-guided assays are required to identify, elucidate and establish effectiveness of a semiochemical against the targeted behavioural trait (Klaschka 2009). The challenges of isolating naturally low concentrations of a semiochemical, which requires sophisticated analytical techniques, often represent a bottleneck in the workflow. Similarly, compared with the wide behavioural repertoire exhibited by COTS on the reef, the test regimes and bioassays currently available are limited to the number of behavioural responses that can be accurately measured and translating these laboratory-measured changes in behaviour to the field is often exacerbated by unforeseen environmental factors. To determine whether a particular semiochemical warrants the effort needed to establish it as a COTS control agent, the technical feasibility of its deployment on complex and often remote reef habitats to mediate the interactions of COTS requires critical assessment.

Scenario modelling has the potential to predict semiochemical burden and the extent of impact of semiochemical release within a mathematically defined environment. For coral reefs, understanding the potential impacts of oceanographic and physicochemical factors is paramount to determining the most appropriate COTS behaviour to target and hence the most effective method of semiochemical delivery to adopt. Such an approach can also identify local reef factors that may pose an unsurmountable challenge to the proposed control strategy and suggest alternative delivery methods. The exact nature of a semiochemical (i.e., (bio)chemical properties), its molecular mode of action and specificity for the behavioural trait being targeted, and whether it acts as short to medium-term signal that elicits an immediate but reversible response, or as a signal that triggers long-term irreversible physiological or developmental changes (Ekerholm & Hallberg 2005, Borowiec et al. 2021), are equally critical factors to model. When considered together, different scenarios of the proposed control strategies can be modelled to predict the semiochemical dispersal rate and expected range of influence at a specified reef site.

To facilitate the discovery of genetic, molecular and chemical technologies to mitigate COTS outbreaks and maintain their populations below damaging levels, the COTS genome was sequenced (Hall et al. 2017) and the COTS Consortium subsequently established (Hall et al.

2016b). Mining of the genome revealed specific genetic pathways and molecules implicated in COTS-specific communication and confirmed they possess a sophisticated olfactory system. Seven-transmembrane G protein-coupled receptor (7TM GPCRs) genes were identified, including an array of putative olfactory chemoreceptors (*Ap*ORs) (Hall et al. 2017). The sex-specific expression of approximately 360 *Ap*ORs and their expression in a diversity of COTS tissues highlights the extent of the family of genes. Subsequent studies have demonstrated the localisation of a small subset of these *Ap*ORs in the tube feet and sensory tentacles of COTS (Roberts et al. 2017), considered the chemosensory organs of COTS, as well as their differential expression in males and females (Roberts et al. 2018). These olfactory receptors provide an entry into the molecular and cellular basis of COTS ability to detect and respond to pheromones, allomones and other semiochemicals. Transcriptomic, proteomic and metabolomic studies have subsequently revealed key endogenous neurotransmitters (also referred to as signalling pheromones) associated with COTS feeding and reproduction (Smith et al. 2017, Smith et al. 2018, Smith et al. 2019). Identifying COTS-specific and life stage-specific semiochemicals and corresponding olfactory chemoreceptors remains an on-going interest (Pratchett et al. 2021).

In light of the recent commentary provided by Hall et al. (2017), Motti et al. (2018), Høj et al. (2020) and Pratchett et al. (2021), the inclusion of semiochemical technologies in the COTS control program is gaining traction. However, uptake requires the functional characterisation of candidate semiochemicals, intelligent design and engineering of delivery formulations, methods and chemical release (or chemoemitting) devices, and customisation of the control strategy. In this context, a preliminary examination of the semiochemical literature, focussing on aquatic applications, revealed the knowledge gaps in these areas (Appendix A). From this process, six overarching themes were identified: (i) *properties, biological function and molecular mode of action*, (ii) *spatial and temporal application*, (iii) *formulation and molecular engineering*, (iv) *engineering solutions for delivery in the marine environment*, (v) *spatial and temporal footprint* and (vi) *augmentation of current control methods*. This review will examine recent developments and trends in semiochemical research that are either currently under development or represent possible areas for future investment. Reference will be made to these six themes and a critical evaluation of the feasibility of deploying semiochemical technologies in the marine environment for effective management of COTS provided, disregarding economic viability and possible regulatory and social licence constraints. The review will conclude by offering recommendations concerning the suitability of deploying a semiochemical within the COTS integrated pest management program on Australia's GBR.

3. PROPERTIES, BIOLOGICAL FUNCTION AND MOLECULAR MODE OF ACTION

For COTS, the reef habitat comprises a plethora of highly diverse semiochemical compounds originating from both biotic and abiotic sources (Motti et al. 2018), each with a role in moderating COTS behaviour. To develop effective semiochemical control agents, the focus must be on identifying those semiochemicals that elicit the required behavioural change and assessing their feasibility in the field. Considered here are the opportunities and challenges of identifying and using semiochemicals for COTS control with respect to their properties, biological functions and molecular modes of action.

3.1 Empirical knowledge and assumptions

Screening of the behavioural traits of COTS has revealed three primary targets amenable to semiochemical control: aggregation, spawning and predator avoidance (Table 2). Aggregation plays a major role in survival and reproduction, which can be categorised as being passive (responding to foraging kairomones or environmental apneumones) or active (responding to pheromones, predator kairomones or necromones). In this context, aggregants are often long-range, long-lived, highly potent attractants that encourage grouping of individuals and formation of aggregations (i.e., foraging cues, sex pheromones), or short-range, short-lived less potent signals that retain individuals in the aggregation (i.e., conspecific non-spawning pheromones and disturbance/alarm cues to reduce predation risk). Of the aggregants, sex pheromones are produced at a specific time and emitted by one sex to attract the other for mating. They are active over extraordinary long distances and are species-specific. Hence, aggregants, and especially sex pheromones, represent the most ecologically selective method to control pests, including COTS. Repellents emanating from predators, conspecifics or decaying (usually conspecific) tissues have a similarly wide range of influence and are able to trigger an immediate aversion or avoidance response. They find use in situations where there is a need to disrupt mating or feeding (Bals & Wagner 2012, Stroud et al. 2014). Table 2 summarises the biological function and desired behaviour modification needed for a successful semiochemical control strategy and the potential for application within the COTS Integrated Pest Management Program.

In the aquatic context, semiochemical compounds are typically non-volatile and water-soluble. They range significantly in molecular weight and have varied structural functionality and biological function. For example, attractin is a 58-amino acid residue N-glycosylated protein (6203 g mol^{-1}) secreted by several hermaphroditic aplysiid species from the albumen gland during egg-laying (Painter et al. 1998). It acts, in combination with enticin (69-residues), temptin (103-residues), or seductin (192-residues) proteins, by attracting non-egg-laying individuals into breeding aggregations and stimulates reproductive behaviour (Cummins et al. 2005, Cummins et al. 2006). Similarly, a transcriptomic investigation of male axolotls (*Ambystoma mexicanum*) revealed they produce a suite of high molecular weight proteinaceous pheromones to attract females (Hall et al. 2016a). In the red-bellied newt, *Cynops pyrrhogaster*, females are attracted to the decapeptide sodefrin (1071 g mol^{-1}) secreted from the male abdominal gland into the surrounding water (Kikuyama et al. 1998). Stream-dwelling sea lamprey ammocoetes (larvae) produce the fatty acid derivative (+)-petromyric acid A (488 g mol^{-1}; (+)-PMA) to guide adult migration, while sexually mature spermiating males release the low molecular weight steroid analogue 3kPZS (472 g mol^{-1}) into the water to attract ovulated females (Li et al. 2018a). For the southern pouched lamprey (*Geotria australis*), a quantitative analytical investigation revealed a mixture of three different but closely related steroids petromyzonol sulfate (PS), petromyzonamine disulfate (PADS), and petromyzosterol disulfate (PSDS) released by ammocoetes likely acts synergistically as a migratory pheromone for adults (Stewart & Baker 2012). After extensive field testing, the use of small molecule semiochemicals in the aquatic realm is now being realised for sea lamprey (Burkett et al. 2021) but remains underdeveloped for many other pest species and for larger molecular weight compounds.

Table 2: Semiochemical types for supplementary COTS control and candidate control strategies

Semiochemical type and biological function	Behaviour or physiological effect	Behavioural outcome	Potential COTS control strategy	Delivery method time/duration/scale/strategy	Relevant examples from other systems (aquatic in *bold italics)*
Pull control strategy					
Sex pheromone	Attracts one sex	Active pre-spawning aggregation	Mating disruption via mass trapping or manual culling	(Pre-)spawning season/ Short term/ Localised/ Lure and manual removal or culling	***Largemouth bass (Fujimoto et al. 2020)***
Sex pheromone coupled with toxin/toxicant	Attracts and kills/reduces fitness of one sex	Active pre-spawning aggregation	Mating disruption via mass trapping or lure and kill	(Pre-)spawning season/ Short term/ Localised/ Lure and manual removal or bait and switch	*Coleopteran pests (Mafra-Neto et al. 2014)* ***Common Carp (Hundt et al. 2020)***
Initiator (conspecific)	Triggers signalling and development in one sex	Premature development or gamete release	Mating disruption via asynchronistic reproduction; physiological perturbation strategy	(Pre-)spawning season/ Short term/ Localised/ Implant or injection	***Holothurians (Hamel & Mercier 2004)*** ***Echinoderms (Mercier & Hamel 2009)***
Inhibitor (conspecific)	Blocks signalling and development in one sex	Disruption or stalling of development or gamete release	Mating disruption via asynchronistic reproduction; physiological perturbation strategy	Spawning season/ Short term/ Localised/ Implant or injection or left in situ	
Non-sex attractant (conspecific pheromone, biotic kairomone, apneumones)	Attracts pest species (all) and possibly non-pest species (kairomones and apneumones)	Passive or active aggregation	Lure, and mass trapping and removal; other species can be separated and returned	As needed/ Short term/ localised/ Lure and manual removal	
	Attracts pest species (all) and possibly non-pest species or (kairomones and apneumones)	Passive or active aggregation	Lure, manual culling; other species can be separated and returned	As needed/ Short term/ Localised/ Lure and manual culling	
Non-sex attractant (conspecific pheromone, biotic kairomone, abiotic apneumones) coupled with toxin/toxicant	Attracts and kills/reduces fitness of pest species (all) or non-pest species (kairomones and apneumones)	Passive or active aggregation	Lure and kill; potential collateral damage	As needed/ Long term/ Multiple sites/ Left in situ	**Biomphalaria alexandrina *snail* *(Kenawy et al. 2020)***
Push control strategy					
Conspecific disturbance/alarm cue (conspecific response to predator kairomone, conspecific pheromone repellent or conspecific necromone)	Repels or disperses conspecific	Active dispersal/disruption of aggregation	Dispersal to reduce impact of feeding	As needed/ Short or long term/ Multiple sites/ Left in situ	***Sea Urchin (Chi et al. 2021)*** ***Shark (Stroud et al. 2014)*** ***Sea lamprey (Bals & Wagner 2012)***
	Repels or disperses conspecific	Active disruption of pre-spawning aggregations	Mating disruption to reduce reproductive success	(Pre-)spawning season/ Short term/	***Sea lamprey*** (***Bals & Wagner 2012)***

				Localised/ Left in situ	***Sea lamprey (Dissanayake et al. 2019, Barber & Steeves 2020)*** **Aquatic organisms *(Crane et al. 2022)***
	Sexual rejection	Active disruption of pre-spawning aggregations	Mating disruption to reduce reproductive success	(Pre-)spawning season/ Short term/ Localised/ Left in situ	Mice (Osakada et al. 2019)
Repellent (predator kairomone)	Repels or disperses pest species; may impact non-pest prey species	Active dispersal/disruption of aggregation	Dispersal to reduce impact of feeding; potential collateral damage	As needed/ Short or long term/ Multiple sites/ Left in situ	
	Repels or disperses pest species; may impact non-pest prey species	Active disruption of pre-spawning aggregations	Mating disruption to reduce reproductive success; potential collateral damage	(Pre-)spawning season/ Short term/ Localised/ Left in situ	
Repellent (predator kairomone + conspecific necromone)	Repels or disperses pest species; may impact non-pest prey species	Active dispersal/disruption of aggregation	Dispersal to reduce impact of feeding; potential collateral damage	As needed/ Short term/ Localised/ Guided positioning and manual culling	
Push-pull control strategy					
Repellent + attractant – coordinated (simultaneous) release	Redirects pest species; may modify behaviour of non-pest species	Active attraction and repulsion	Lure and manual culling; other species can be separated and returned	As needed/ Short term/ Localised/ Guided positioning and manual culling	**Sea lamprey *(Hume et al. 2020)*** **Carp *(Sorensen & Stacey 2004)***
Predator enhancement control strategy					
Predator attractant	Attracts predator species	Enhancement of natural predator populations	Predators naturally depress COTS populations; potential collateral damage	As needed/ Long term/ Localised/ Left in situ	Stink bugs, *Podisus maculiventris* (Kelly et al. 2014)

Terrestrial integrated pest management programs are well-advanced, especially for insect species (Brezolin et al. 2018, Barbosa-Cornelio et al. 2019), some to great effect (Witzgall et al. 2010). However, the development of insect-specific semiochemicals (primarily volatile and semi-volatile) and synthetic mimics (Synergy Semiochemicals Corporation 2004) has been complicated by the extraordinary diversity of semiochemical structures and associated biological activities (El-Sayed 2022). To categorise this structural diversity, structure-activity relationships are often utilised. They provide valuable information in the development of pharmaceuticals regarding the chemical properties required to influence a drug's pharmacokinetics and target a specific mode of action, and are also applied in the ecotoxicological assessment of chemical pollutants to predict environmental exposure thresholds of structurally related industrial chemicals (Bradbury et al. 2003). For semiochemicals, their strong similarity in structure but markedly different biological activities makes it difficult to deduce distinct credible structure-activity relationships to accurately predict the biological function and molecular mode of action of closely related semiochemical structures (Francke & Schulz 1999, Bohman et al. 2018, Li et al. 2020) and this has hindered their development into pest control agents.

In the aquatic realm, attempts to establish a structure-activity relationship to describe the small molecule semiochemical pheromones of the sea lamprey has proven challenging. Structurally closely-related sea lamprey semiochemicals elicit different behavioural responses. Semiochemical pheromones are released into the stream environment and act either synergistically or antagonistically (Fissette et al. 2021). For example, ammocoetes, having stayed dormant for up to 15 years in stream sediment, produce the fatty acid (+)-PMA as the primary attractant for migrating adults (active at 10^{-10} to 10^{-13} M) (Li et al. 2018a) within a multicomponent secretion that also includes structurally related (+)-petromyroxol (Li et al. 2015), and the sulfated steroids PS, PADS and PSDS (Sorensen et al. 2005). (-)-PMA and (+)-petromyroxol are also present but are inactive isomers. Once aggregated, the spermiating males secrete the closely related oxidised analogue of PS, 3kPZS, to attract ovulated females (Venkatachalam 2005). The conversion of the alcohol group at position C3 to a ketone significantly alters the biological activity and half-life, with the former being highly potent and long-lived to reach adults downstream, the other shorter-lived. Petromylidenes A–C, with additional substitutions at position C2 of 3kPZS, and which differ from each other in the nature of the alkylidene substituent, have also been isolated from water conditioned with sexually mature males and shown to elicit similarly strong behavioural attraction of ovulated females (ranging from 10^{-9} M, 10^{-11} M, and 10^{-13} M) (Li et al. 2018b). The sulfate at position C24 of these PS analogues directly contributes to its biological function and specificity. Decoupling the biological functions of sea lamprey semiochemicals also faces other challenges, with the structurally unrelated polyamine spermine, initially identified from human semen, also able to attract ovulatory females at 10^{-14} M (Scott et al. 2019).

Semiochemicals can be distinguished by their highly variable modes of action (Table 3) (Eigenbrode et al. 2015). Pheromones form complexes with membrane-bound chemoreceptors (e.g., 7TM GPCRs) through non-covalent close-range interactions (e.g., hydrogen bonding, electrostatic potential or ionic bonding and hydrophilic/hydrophobic regions or van der Walls interactions) which effect a conformational change in the GPCR and activates downstream signalling cascades. Dependent on the dissociation constant (Kd), some interactions are transient or short-term (i.e., high Kd) enabling rapid signalling and dissociation as well as detection at very low concentrations. This ensures repeated semiochemical detection is possible and enables the receiver to quickly and precisely navigate gradient plumes towards the emitter. Other semiochemicals, such as foraging cues and predator kairomones, typically form longer-lasting non-covalent interactions (i.e., low Kd) or stronger covalent bonds with the receiver's

chemoreceptors leading to longer-lasting changes in behaviour and, in some instances, physiology. Yet, there are exceptions and, in reality, little is still understood about the interactions between semiochemicals and their corresponding chemoreceptors much beyond laboratory model species (Amin & Hazelbauer 2010, Bi & Lai 2015).

Interrogation of the COTS genome has revealed 950 new GPCR genes, with 750 belonging to the COTS rhodopsin-class GPCRs (Hall et al. 2017), all potentially encoding for putative chemoreceptors for which the corresponding semiochemicals and biological roles remain undiscovered. To identify and deorphanize the GPCRs that explicitly function as chemoreceptors, an approach that is being applied to sea lamprey control (Zhang et al. 2020), the semiochemical (or combinations of) must first be discovered. In the first instance, identification of COTS-produced semiochemical compounds is critical and a better understanding of COTS chemoreceptors and the genes that encode them will greatly facilitate the analytical challenge of isolating them. In addition, to examine species specificity, such approaches should be extended to include investigation of the closest related species, the short-spined COTS, *Acanthaster brevispinus*, and other corallivore starfish including species of the cushion star, *Culcita* sp. (Montalbetti et al. 2018).

Aside from structural and functional descriptors that determine the molecular mode of action of a semiochemical compound, its intrinsic physicochemical properties are fundamental to understanding and predicting its bioavailability, distribution, behaviour and fate in the environment once released (Lucia & Guzman 2021). Terrestrial semiochemicals are distinguished by high volatility that allows for their diffusion over long distances, application in low concentrations and rapid dissipation. The descriptors commonly considered when profiling terrestrial semiochemicals are vapor pressure (V), Henry's coefficient (H), water solubility constant (W), octanol-water partition coefficient (O) and organic carbon partition coefficient (C); VHWOC (Sanchez Perez et al. 2019).

For the aquatic environment, the desirable properties of low molecular weight semiochemicals are low volatility, intrinsic water solubility and moderate lipophilicity and high polarity (Table 4). Lipophilicity, which is often a consequence of resonance stabilization, affords a stable structure allowing for longer-term bioavailability and broader distribution. For proteins (typically >50 amino acids and >6000 Da in molecular weight) and peptides (typically 5-50 amino acids and 500-6000 Da), their physicochemical properties are determined by the analogous properties of their constituent amino acids. Although proteins are of high molecular weight, their zwitterionic nature and very high ionizability imparts high water solubility through hydrogen bonding (Table 4). Their zwitterionic nature also affords the three-dimensional structure a large degree of flexibility, with stabilising interactions between amino acid constituents just sufficient to maintain the protein structure. This also suggests short-term bioavailability as they are prone to rapid degradation and hence limited distribution, indicating protein semiochemicals are more likely to act as short-range signals. Protein three-dimensional structure can also form internal hydrophobic cavities capable of binding and controlling the release of secondary metabolites and extending their longevity and effectiveness (Wyatt 2014).

Table 3: Desirable and acceptable properties of semiochemicals for COTS control.

Functionality	Desirable properties	Acceptable properties for COTS control	Anticipated outcome	Relevant examples from other systems (aquatic in ***bold***)
Taxa-specificity	Species-specific	Asteroid-specific or Echinoderm-specific		
	Targets a single well-defined species-specific chemoreceptor	Targets uncharacterised chemoreceptors Moderately specific	Signal not conveyed to other species. Limited unintended impacts on other species	
	Selective; induces desired response in target species only	Moderately selective	Signal elicits desired behavioural response	
	No collateral damage	Need to understand direct, indirect, and cumulative species-specific and environmental impacts	Acceptable (but minimal) collateral damage to other species	
	Life history stage-specific	Adult; juveniles and larvae less so	Adult behaviour is modified	
Chemical constituency Half-life	Pure compound	Crude extract/chemical mixtures	Non-target effects may be easier to predict for pure compounds or mixtures of pure compounds. Manufacture is highly intensive	**Cane toad exposure to a single chemical results in avoidance; continuous exposure provides a less reliable signal of predation risk *(Crossland et al. 2019)*** **Uridine diphosphate and uridine triphosphate (4:1) increases mating response in male shore crab *C. maenas* and is specific *(Bublitz et al. 2008)***
	Combination of pure compounds or semi-purified fractions that elicit an additive response	Minor additive increase in modified behaviour	Manufacture is moderately intensive	**Sea lamprey respond to mix of predator (2-phenylethylamine hydrochloride) and conspecific alarm cue *(Di Rocco et al. 2015)***
	Compound/extract highly potent – small amount required	Compound/extract moderately potent – large amount required	Manufacture is simple	**Approximately 500 g of sea lamprey migratory pheromone PADS needed to activate the entire flow of Niagara Falls (~58 km) *(Sorensen et al. 2005)***
	Well-defined chemical properties, i.e., enantiomer specific vs non-specific activity		Non-target effects may be easier to predict for pure enantiomers. Manufacture is highly intensive	Chromatographic resolution of enantiomers *(Keeling et al. 2001)* **Diatom *Seminavis robusta* *(Lembke et al. 2018)*** **Sea Lamprey *(Li et al. 2018a)***
	Specific biochemical interaction on a specific molecular target	Unknown target; specific and/or selective	Known molecular target; no unintended impact on other marine life	
	Short half-life (several days)	< 12 hours		**Sea Lamprey; petromyzonol sulfate active at pg L^{-1} concentrations (or 10^{-12} M) *(Fine & Sorensen 2005)*** **Southern pouched lamprey; half-life 1.5 days *(Stewart & Baker 2012)***

Potency	Highly potent; active at biologically-relevant concentrations - picomolar concentrations; effective over long (reef scale) distances	Moderately potent; readily available; may impart additive effect; likely effective over short (site scale) distances	Low to moderate quantities required for application	**Carp; 704 Da fraction attracts migratory adults at < 0.1 pM** ***(Sorensen et al. 2003)*** **Sea lamprey; exhibited increase in avoidance behaviour in response to increasing concentrations ($5x10^{-10}$ to $5x10^{-8}$ M) of 2-phenylethylamine hydrochloride** ***(Di Rocco et al. 2015)*** Insects (Eigenbrode et al. 2015)
Toxicity	Non-toxic in the marine environment at the effective dose	Mildly toxic with limited impact on non-target species	No unintended impact on other marine life	

Table 4: ***Favourable*** physicochemical properties of secondary metabolite and protein semiochemicals for application in the marine environment

Physicochemical property		**Secondary metabolite**	**Protein**
Chemical	Molecular weight	Low	Medium to High
Solvation	Vapor pressure	Low, non-volatile due to strong intermolecular forces (hydrogen bonding)	Low, non-volatile due to strong intermolecular forces (hydrogen bonding)
	Henry's coefficient	Low to moderate coefficient, hydrophilic (< $5x10^{-5}$ atm-m^3 mol^{-1})	Low coefficient, hydrophilic (< $5x10^{-5}$ atm-m^3 mol^{-1})
	Water solubility product constant (K_{sp})	Intrinsic moderate water solubility at 25°C (logS); high pK_a; solubility > 0.1 M	Intrinsic high water solubility at 25°C (logS); pK_a; solubility > 0.1 M
	Functional groups	Possession of functional groups that favour their solubility	Possession of functional groups that favour their solubility
	Structural integrity	Low degree of flexibility; enantiomeric	Large degree of flexibility stabilising interactions between amino acid constituents just sufficient to maintain structure
	Molecular hydrophobicity (or lipophilicity); measured as octanol/water partition coefficient $LogP_{ow}$	Moderate or low lipophilicity or $LogP_{ow}$. Chemical modification into salt or ester forms to increase solubility	Low lipophilicity; internal hydrophobic cavities suitable for the binding and transport of low molecular weight hydrophobic molecules
	Polarity	Low to moderate; range from no to high level of hydrogen bond donation and/or acceptance, and polar surface area	High; high level of hydrogen bond donation and/or acceptance, and polar surface area
	Ionizability	High pKa	High pKa; zwitterionic
Physical	Freezing point, boiling point, melting point	Unknown; low (150-250°C for insects)	Low
	Viscosity and density	Unknown	Proteins can influence viscosity

3.2 Proteins – emerging semiochemicals

In recent years, proteins and peptides have emerged as semiochemical attractants driving chemical communication in aquatic systems (Kikuyama et al. 1998, Sorensen et al. 2003, Cummins et al. 2006, Touhara 2008, Hall et al. 2016a), including for *Aplysia* spp. (Cummins et al. 2005, Cummins et al. 2006) and COTS (Hall et al. 2017). As candidate COTS semiochemical control agents, proteins and peptides have the potential for highly specific activity, particularly when compared to small molecules (Leader et al. 2008, Craik et al. 2013). With a much larger size compared to small molecules, proteins can use larger binding interfaces tailored to interact with a specific target. The potential for high specificity has been a driving force for their development into therapeutic drugs, where the absence of off-target interactions (i.e., harmful adverse effects) is a primary goal. The specificity at a molecular target level has been seen to translate to the species/organism level, as demonstrated by recently marketed bioinsecticides (Grisham 2000, King 2019, Romeis & Meissle 2020, Sparks et al. 2020). For example, plant fractions containing a suite of natural insecticidal peptides of the cyclotide family have been developed as a world-first bee-friendly agent for control of insects in crop protection (SERO-X®) by Australian company Innovate Ag. Evolutionary analyses provided by Hall et al. (2017), has indeed confirmed that proteins within COTS-conditioned seawater could have highly specific functions, with for example, a group of secreted ependymin-related proteins (approximately 200 amino acids with four conserved cysteines) shown to have diversified uniquely within COTS compared to homologues in other organisms.

4. SPATIAL AND TEMPORAL PATTERNS OF DEPLOYMENT

Integrated pest management relies on a high level of knowledge of the biology and ecology of the pest organism to optimise timing and scale of direct interventions (Westcott et al. 2021). Semiochemical application is a point in case as it relies on mechanistic understanding of semiochemical communication combined with a comprehensive understanding of pest organism ecology. The combined knowledge of the biological function (Table 2), specificity and molecular mode of action (Table 3) and physicochemical properties (Table 4), is relied on to design semiochemical deployment scenarios. The likelihood of success of these scenarios can then be assessed by means of comparisons with successful semiochemical control programs.

4.1 Spatial range

Many of the chemical signals which animals rely on to guide critical behaviours such as migration and reproductive aggregation have extraordinary potency, longevity, and spatial range (Sorensen et al. 2005). It is estimated that only 500 g of the sea lamprey migratory pheromone PADS would be needed to activate the entire flow of Niagara Falls (~58 km), and, with dilution, maintain a > 10^{-13} M active concentration for up to one month (covering the full migration season; May to June in the northern hemisphere). For COTS, being a benthic mobile species that moves on average 2.8 m d^{-1} in areas of high coral cover and up to 10.3 m day^{-1} as coral cover declines (Keesing & Lucas 1992, Pratchett et al. 2020), the spatial range of the chemical signals that guide their aggregations are likely to be effective predominately at the local reef scale (i.e., on average 6.9

km^2 (Hopley et al. 2007)). Furthermore, COTS are able to traverse sandy interstitial reef habitats (Pratchett et al. 2017b), in some instances by rolling at an estimated 0.1 ms^{-1} with 8 revolutions min^{-1} for a duration of 23.5 s to reach the next reef (Cranenburgh & Cranenburgh 2020); a behaviour which may potentially be mediated by long-range semiochemicals emanating from neighbouring healthy reefs. Elucidating the nature of COTS pheromone attractants is critical to establishing the drivers and spatial range of adult COTS migrations leading to aggregation and holds the key for future semiochemical COTS control technologies.

4.2 Timing and duration

The biology of the pest species, its life history stage and the molecular mode of action of the semiochemical has significant influence over the temporal deployment of a semiochemical control agent (Table 2 and Table 3) (Fissette et al. 2021). A prime example is the finfish parasite *Cryptocaryon irritans* which is a major issue in the production of food fish and ornamental aquaculture. Infective theronts are positively chemotactic (attracted) to urea, and examination of their hatching biology revealed the best time to deploy urea-laden traps for capture is prior to sunset (Skilton et al. 2020).

Based on the biology of COTS, numerous temporal deployment scenarios are envisaged for semiochemical control (Table 5). Conspecific attractants, particularly sex pheromones, are likely to deliver greatest success based *a priori* knowledge of semiochemical applications to control the aquatic pests silver carp (*Hypophthalmichthys molitrix*) and sea lamprey (*Petromyzon marinus*) (Sorensen et al. 2019, Siefkes et al. 2021). Field observations over several decades have provided evidence of comprehensive and synchronous spawning by COTS, which on the GBR occurs late December to early January (Babcock & Mundy 1992, Caballes et al. 2021). Therefore, the timing of delivery of a semiochemical technology specifically designed to disrupt spawning should coincide with changes in physicochemical parameters such as increasing water temperature (indicating seasonal change) and biological factors including increased aggregation and gonad maturity (both indicating reproductive preparedness). In this scenario, duration of delivery is inherently limited to the (pre)spawning period and the choice of release mode highly dependent on the desired outcome (Table 5).

Under optimum conditions, COTS larvae settle at approximately 22 days post-fertilization, although they have been maintained in aquaria up to 43 days (Pratchett et al. 2017a). This settlement process is predestined, and favours crustose coralline algae substrate which is also a food source for early juveniles (Johnson et al. 1991). A scenario that specifically targets COTS larvae represents a rigid and finite timeframe in which semiochemical technology could be deployed and would rely on initial in-field confirmation of spawning. For example, the slow, constant release of a highly attractive settlement cue from an impregnated calcium carbonate brick or tile (engineered as a trap) could be deployed at sites adjacent to reefs to attract swimming brachiolaria larvae (17-22 days after fertilisation being the peak settlement window (Pratchett et al. 2017a)). Newly settled COTS could be manually removed but this would require divers to be on-location during the deployment period. Alternatively, deployed substrates could also be impregnated with a toxin/toxicant in a ‘trap and poison’ scenario.

Table 5: Semiochemical deployment scenarios for the control of COTS

Mode of Action	Semiochemical type	Desired behavioural outcome	Specificity	Life stage	Release timing and duration	Scale of release
Spawning disruption	Sex pheromone attractant	Asynchronistic spawning	Species and sex specific	Adult male	1-month leading to spawning; pulsed	Local aggregation
				Adult female		Local aggregation
		Early synchronistic spawning - release of immature gonads, or in conditions not suited to fertilisation or larval survival	Species and/or sex specific	Adults	2-3 months leading to spawning; pulsed	Local aggregation
		Delayed synchronistic spawning - delayed gonad maturation, or release in conditions not suited to fertilisation or larval survival	Species specific	Adults	1-month leading to and during spawning; pulsed	Local aggregation
		Sex pheromone attractant	Species and sex specific	Adult male	1-month up to and during spawning; pulsed or continuous	Reef-wide or local
				Adult female	1-month up to and during spawning; pulsed or continuous	Reef-wide or local
	Mask or repellent	Inability to locate mate (i.e., masking of COTS odour)	Not species specific	Adults	1-month up to and during spawning; pulsed	Reef-wide
Passive aggregation	Kairomone, allomone, synomone or apneumone attractant	Formation of aggregations at a specific location (or in traps)	Not species specific	Juveniles Adults	Anytime; pulsed or continuous	Local aggregation
	Kairomone, allomone, synomone or apneumone repellent	Dispersal of non-spawning aggregations at a specific location		Juveniles Adults	Anytime; low- to mid-density aggregations; pulsed + continuous	Reef-wide
Foraging cue	Non-sex pheromone	Feeding conspecific odour attracts conspecific	Species specific	Juveniles Adults	Anytime; continuous	Reef-wide
	Kairomone attractant	Attractant originating from prey	Not species specific	Juveniles Adults	Anytime; pre-spawning; continuous	Reef-wide
Settlement cue	Kairomone or apneumones attractant	Larval attractant	Species specific	Larvae	~2 months post spawning; on substrate, continuous slow release	Reef-wide
Predator avoidance	Kairomone repellent	Animal repelled from source	Not species specific	Juveniles Adults	Anytime, pulsed or continuous	Reef-wide or local
Combinatorial formulations	Multiple attractants	Conspecific attractant and settlement or foraging cue; additive effects	Species specific; combination	Larvae Juveniles Adults	Anytime; pre-spawning; or post-fertilization, pulsed or continuous	Reef-wide or local
	Repellent + attractant	Animal repelled from source and attracted (guided) to another		Juveniles Adults	Anytime; pre-spawning, controlled pulse	Local

The regulation of feeding behaviour is an essential factor for survival; hence, foraging cues (kairomone attractants and synthetic analogues designed with greater efficacy) also have potential for use as COTS control agents. For adult COTS, these could include long-range persistent attractants (i.e., COTS attracted to specific coral species and coral gardens) or short-range contact cues (i.e., induce stomach eversion and feeding) (Brauer et al. 1970, Teruya et al. 2001). Such technology could potentially be incorporated into traps that could be deployed at the periphery or adjacent to the main reef structure, and be manually removed, or be used in combination with a toxin/toxicant. Given the critical nature of feeding, the timing of delivery is not limited to a particular season, although the strategy may be more effective at attracting COTS

leading up to reproductive periods when energy reallocation drives tissue growth, enrichment of the pyloric cecum and gonadogenesis. As such, monitoring environmental factors and resource availability is critical to determining optimal timing of delivery. However, the justification for such an approach (for either juveniles or adults) is based on the identification of a COTS-specific semiochemical settlement cue and toxin/toxicant (Johnson et al. 1991).

Predator avoidance semiochemicals also have the potential to be used in a multitude of scenarios, from discouraging formation of aggregations and dispersing feeding aggregations to disruption of spawning. Each of these require different deployment timings, the former two at any time, the latter immediately prior to or during spawning. A pulsed-release scenario is likely to have more impact as COTS may become desensitised to the predator kairomone in the absence of any attack (pers. comm Motti) or be less risk adverse as the need to feed increases. To overcome this, additive effects of semiochemicals have been exploited in the control of sea lamprey (Di Rocco et al. 2015), where a minor additive increase in modified behaviour has been achieved with a mix of predator cue (2-phenylethylamine hydrochloride) and conspecific alarm cue.

5. FORMULATION AND MOLECULAR ENGINEERING FOR THE AQUATIC ENVIRONMENT

5.1 Formulation

Susceptibility to degradation is a major factor that can impact the delivery and effectiveness of a semiochemical agent. Degradation can be exacerbated by the interactive effects of multiple environmental biotic and abiotic (Table 6) signals, leading to a dampening or masking of the semiochemical signal, potentially rendering the semiochemical ineffective in the field (Mumm & Hilker 2005, Xu et al. 2017). For example, temperature can increase the diffusion rate of volatile airborne semiochemicals, which affects compound stability leading to decreased molecule life-time in the environment. Another complicating factor is the chemical instability of many volatile semiochemicals against UV light and oxidation. Hence, formulations are often used as a protective vector to stabilise and deliver the semiochemical technology at the required concentration and rate (Lucia & Guzman 2021), yet a major challenge lies in developing formulants that effectively facilitate safe delivery to the receiver. To address this challenge, significant effort has been spent tailoring formulations to protect semiochemicals from these environmental factors and improve pre-release storage, release performance and environmental longevity, as well as reduce the frequency of applications needed to trigger the behavioural response in the receiving animal (Muskat & Patel 2022).

Semiochemical application in the aquatic environment faces a different set of obstacles due to their inherently divergent physicochemical properties, being non-volatile and highly water soluble. The large volumes of water that aquatic species typically inhabit present a different delivery and dilution challenge, with a semiochemical's spatial and temporal distribution patterns also distorted by currents and daily tides (Webster & Weissburg 2009). In laboratory experiments, the sea lamprey male sex pheromone 3kPZS elicits significant activity in ovulated females at concentrations above 10^{-12} M (Siefkes & Li 2004), whereas behavioural responses in the field may occur at concentrations two or more orders of magnitude lower. This example highlights the difficulty in translating laboratory results to large-scale in-water deployment. As for terrestrial

semiochemicals (El-Shafie & Faleiro 2017, Lucia & Guzman 2021), some of the difficultly here lies with the formulation.

Table 6: Environmental conditions may influence semiochemical properties.

Abiotic Influences	**Physicochemical property**	**Impact**	**Relevant examples from other systems (aquatic in *bold*)**
Temperature	Elevated temperatures	Change diffusion rate	**Kairomone production by fish predator increases with rising temp** ***(Lass & Spaak 2003)*** **High temperature stops aggregation of spiny lobster** ***(Ross & Behringer 2019)***
Salinity	River run-off – lower salinity	Change diffusion rate; alters chemical interactions	**Salinity is protective against loss of salmon olfactory function from dissolved copper** ***(Sommers et al. 2016)*** **Senegalese sole olfaction affected by reduced salinity** ***(Velez et al. 2009)***
Ocean acidification	pH		**Low pH reduces shelter seeking in spiny lobster** ***(Ross & Behringer 2019)*** **Marine animals** ***(Porteus et al. 2021)***
Water quality	Pollutants	Chemical masking; adherence /absorption to formulation changing diffusion rate or causing deterioration /degradation	**Metal contamination blocks yellow perch olfaction** ***(Mirza et al. 2009)*** **Metal contamination alters juvenile salmon behaviour** ***(Sommers et al. 2016)***
	River run-off – increased sedimentation	Physical masking; adherence /absorption to formulation changing diffusion rate or causing deterioration /degradation	**Aquatic animals** ***(Webster & Weissburg 2009)***
Turbulence	High fluctuation across diurnal and tidal cycles	Change diffusion rate; alters dispersal rate	**Aquatic animals** ***(Webster & Weissburg 2009)***

Prospects to overcome semiochemical susceptibility are described in Table 7. The formulation ingredients must be able to solubilise, suspend, thicken, dilute or emulsify the semiochemical and stabilise and preserve its purity into efficacious forms (Table 8). Formulants should also facilitate a rapid interaction of the semiochemical with the target chemoreceptor. Of paramount importance, the semiochemical and formulation ingredients must be compatible with one another and it is crucial the toxicity and chemistry of the formulant in combination with the semiochemical meet regulatory guidelines (Weatherston & Stewart 2002, EFSA et al. 2021).

Semiochemical and formulation characteristics that would limit their use in the marine environment include:

1. instability, volatility, and sensitivity of the formulation ingredients resulting from long-term exposure to environmental factors like temperature, salinity, light and UV radiation;

2. inconsistency of semiochemical release rate, especially for those formulations that act by passive dissolution;

3. impact of the physical and/or chemical properties of the formulant on non-target species; and

4. acute toxicity of the formulant(s), acceptable only if it rapidly disappears (degrades or disperses) and has low latency.

Specialized Pheromone & Lure Application Technology (SPLAT®) is a perfect example of the importance of formulation (Mafra-Neto et al. 2014). SPLAT® is designed to lure gravid female mosquitoes to water for oviposition. The formulated semiochemical, comprising of the attractant acetoxy hexadecanolide and an amorphous, flowable, and controlled-release wax emulsion, is deployed at the water surface and has proven to be strongly preferred as an oviposition substrate for more than two weeks post application, imparting an unintended benefit. The addition of bacterial larvicides (which is not being considered for COTS (Høj et al. 2020)), referred to as SPLATbac, caused 100% mortality in newly hatched larvae for at least five weeks after application (Schorkopf et al. 2016).

Formulations are critical to delivery of required concentrations at the required rate. Attractants, such as the plant volatile, anisaldehyde, used to attract host-seeking *Aedes albopictus* mosquitoes, can potentially become unattractive or even repellent if release rates are too high or when they are applied at improper ratios (Hao et al. 2013). This phenomenon is gaining more attention in insect control (Schorkopf et al. 2016) and will require careful assessment with respect to COTS.

Table 7: Summary of semiochemical formulations and modes of delivery with potential for application to COTS control.

Formulation Type	**Nature of formulation**	**Essential property**	**Delivery mode (manual, dynamic or static): device type and relevant examples from aquatic systems in *bold italics***	**Release time-frame**	**Site of release**
Drip emulsion	Dissolved (pre-mixed) semiochemical; high-pressure homogenization, microfluidization or sonication	Stable in pre-mixed solution	Dynamic: Ultra-low volume application; mechanical pump (AD); nanoemulsions	Continuous, pulsed mins/hours	Bottom release; discrete sites on reef bed
Spray emulsion	Dissolved (pre-mixed) semiochemical	Stable in pre-mixed solution	Dynamic: Ultra-low volume application; Hydraulic sprayer (AD)	Continuous, pulsed mins/hours	At depth; discrete sites on reef bed Surface (assumes negative buoyancy)
Oil/grease emulsion	Very viscous preparation; Oil miscible flowable	Stable in pre-mixed solution; stable for days to months	Dynamic: Insects (Lucia & Guzman 2021)	Continuous, days	
Dispersible concentrate	Solid released into aqueous environment	Stable in solid form; Readily soluble upon release	Static:	Single continuous application	Above water (at surface)
Suspension concentrate	Liquid released into aqueous environment	Stable in liquid form	Dynamic:	Continuous, pulsed mins/hours	Directly in-water
Pressurised aerosol; puffer	Gas or liquid		Dynamic: discontinuous dose; ***mimicking shark necromone (Stroud et al. 2014)***	Pulsed, mins/hours	Directly in-water
Augmented delivery via injection	Initiators (inducers) and inhibitors (blockers) delivered directly to the individual (i.e., injection) to induce physiological changes and trigger the release of a semiochemical.		Manual: syringe Dynamic: syringe delivered by (semi)automated robot (not realised); physiological perturbation strategy	Single application	Individual
Augmented delivery via implant	Female implant	Safe for internal release	Manual: Surgical osmotic pump; ***female common carp periodically releases male sex attractant prostaglandin F2α (PGF2α) (0.4 g kg^{-1} of fish) successfully luring males to within 20 m (Lim & Sorensen 2012, Sorensen et al. 2019)***	Continuous inter-peritoneal delivery - weeks	Individual
Encapsulated/granulated beads	Slow dissolution and release		Static: (Weissling & Meinke 1991, Kong et al. 2009, Heuskin et al. 2012)	Continuous slow release - days	Directly in-water
Gel or paste	Slow dissolution and release		Static: Insects (Heuskin et al. 2012, Mafra-Neto et al. 2014) ***COTS (Teruya et al. 2001)***	Continuous slow release - days	Directly in-water

Biodegradable polymer; i.e., high molecular-weight polyethylene glycol	Slow dissolution and release		Static: ***Pheromone-PEG emitters as trap baits for sea lamprey (Wagner et al. 2018)***	Continuous slow release - days	Directly in-water
Non-biodegradable (rechargeable) thermoplastic polymer			Static or dynamic: (Guerret & Dufour 2017)	Continuous slow release - days	
Absorption onto solid matrix, i.e., brick solid block	(Bio)engineered impregnated matrices that facilitates slow dissolution and release	Calcium carbonate composite	Static: zeolite molecular sieves (Muñoz-Pallares et al. 2001) ***BufoTab, aquarium air-stones (CTC 2017)*** Nested wick and two-reservoir design that achieves a constant release of mosquito volatile attractants and repellents over several hundred hours (Kwan et al. 2019)	Continuous slow release – days	Directly in-water
Suitable formulation releases in combination with other sensory stimuli	Mechanosensory; visual		Dynamic: ***snail* Lymnaea acuminata *attracted to photo- and chemo--stimulants to control fasciolosis (Tripathi et al. 2013)***	Continuous or pulsed or single application	Bottom release; discrete sites on reef bed

Table 8: Considerations for the development of marine deployable semiochemicals. TBD = to be determined.

Semiochemical origin	**Manufacture/production**	**Monitoring of release**	**Biodegradability /life-time**	**Diffusion coefficient**	**Toxicity**	**Strengths**	**Limitations**
Natural product – crude extract	Chemical isolation (solvent extraction/ partition)	Behavioural bioassays	Biodegradable TBD: sensitivity to UV, water, oxygen, microbial degradation	TBD Analytical	TBD Bioassays	Fewer regulatory hurdles	Reproducibility and supply Resource intensive to monitor Stability?
Purified natural product – single component	Chemical isolation (solvent extraction/ partition) and purification	Analytical monitoring	Biodegradable TBD: sensitivity to UV, water, oxygen, microbial degradation	TBD Analytical	TBD Bioassays	Fewer regulatory hurdles	Resource intensive to develop Stability?
Synthetic or recombinant natural product	Chemical synthesis Production by recombinant microorganisms Large-scale production may be possible	Analytical monitoring	TBD: sensitivity to UV, water, oxygen, microbial degradation	TBD Analytical	TBD Bioassays	Large-scale production possible	Resource intensive to develop and monitor Stability? Regulatory hurdles?
Synthetic mimic of natural product	Chemical synthesis Large-scale production may be possible	Analytical monitoring	TBD: sensitivity to UV, water, oxygen, microbial degradation	TBD Analytical	TBD Bioassays	Large-scale production possible	Resource intensive to develop and monitor Stability? Regulatory hurdles?

5.2 Molecular engineering

Structure-based chemical design involves pharmacophore mapping (active functional features of a molecule) and generation of analogue mimics based on the three-dimensional structure of the bioactive compound. This approach relies, to some extent, on knowledge of structure-activity relationships, which for semiochemicals is convoluted (as discussed previously). Regardless, efforts are now focussed on the potential of analogue mimics, referred to as parapheromones (Renou & Guerrero 2000, Reddy & Guerrero 2010), to overcome limitations associated with semiochemical bioavailability and efficacy. The manufacture of semiochemical parapheromones also has the potential to substantially increase potency as well as reduce production costs and lessen the reliance on formulations. However, as the design and synthesis of semiochemical parapheromones relies on structural knowledge, identifying, isolating and establishing the structural nature of the active chemical must be the first and primary focus in developing semiochemical COTS control technologies.

5.2.1 Small metabolites

Starfish, including COTS, produce a plethora of low molecular weight secondary metabolites, including alkaloids, terpenoids, steroids, saponins, glycosides, phenazines, natural phenols, polyketides and fatty acid synthase products (McClintock et al. 2013). This structural diversity and complexity in their chemistry presents a significant challenge to their isolation and chemical synthesis, and tailored parapheromones may provide a solution to improve their performance and address supply concerns, respectively. Such an approach has been applied to the control of navel orangeworm (*Amyelois transitella*), tobacco budworm (*Heliothis virescens*) (Xu et al. 2012) and thynnine wasps (*Zaspilothynnus trilobatus*) (Bohman et al. 2016). In all three cases, the desired behavioural response could be manipulated by synthetic parapheromones. For *H. virescens*, (9Z)-tetradecen-1-yl formate, a formate analogue of the natural sex pheromone (11Z)-hexadecenal, induced a stronger response by the pheromone receptor. For *Z. trilobatus*, a sexual response was elicited by 2-hydroxymethyl-3,5-dimethyl-6-ethylpyrazine, the methylated analogue of the active semiochemical, 2-hydroxymethyl-3,5-diethyl-6-ethylpyrazine, yet other closely related analogues were inactive. No examples of parapheromones prepared for the aquatic environment were found.

5.2.2 Proteins and peptides

Protein and peptide semiochemical molecules could, in principle, be formulated in baits to attract COTS for entrapment. However, maintaining them in their active forms is considerably more challenging than for small molecule compounds, although some peptides and proteins have evolved to be ultra-stable. The major challenge relating to the use of proteins is their inherent sensitivity to different types of stresses, specifically enzymatic degradation in proteolytic environments, physical and chemical degradation during long-term storage (e.g. deamidation, oxidation, surface absorption, etc.), and aggregation at high protein

concentrations (Frokjaer & Otzen 2005, Wang 2015, Geraldes et al. 2020). Over the last few decades, approaches for stabilising proteins and peptides have been developed, primarily within the pharmaceutical drug development context, including chemical modification, or reengineering of the protein or formulating the protein within a protective matrix. Some methods used to stabilise therapeutic peptides and proteins could also be applied to develop stable COTS baits because they involve improving the structural or physiochemical properties, which would have a universal effect on preventing degradation. Importantly, most of the stresses noted above that can cause protein degradation are far less prevalent in the marine environment than for terrestrial applications.

Peptides and proteins are well stabilised by the incorporation of multiple disulfide bonds at strategic locations. The reason for this stabilisation is that a disulfide-constrained molecule, or any chemically constrained molecule for that matter, is more conformationally rigid, and therefore more stable against proteolytic degradation (because they are less likely to fit into a non-target protease's active site) and aggregation (because they are less likely to unfold). The ependymin-related proteins discovered within the COTS secretome are rich in disulfide bonds (Hall et al. 2017), suggesting they have privileged stability compared to other proteins, and might have evolved this property so they retain their function whilst diffusing through seawater to reach another COTS some distance away.

One of the most stable classes of peptides known are the cyclotides (Craik et al. 1999), disulfide rich molecules of around 30 amino acids that have three disulfide bonds connected in a knotted framework, a so-called cystine knot, that provides exceptional stability. Cyclotides have the additional feature of a head-to-tail cyclic backbone that reduces their susceptibility to breakdown by exo-proteases. They are thermally stable and stable in a range of chemical conditions, including extremes of pH and in the presence of chemical chaotropes (Colgrave & Craik 2004). For these reasons one potentially valuable approach to the stabilisation of candidate bioactive peptides would be to incorporate, or graft, them into a cyclotide framework as has been described in multiple recent applications in the pharmaceutical arena (Craik & Du 2017, Wang & Craik 2018). For COTS control, biodiscovery research is currently progressing the isolation and identification of large attractant proteins (CCIP 2021) and, once efficacy is verified, will focus on elucidating the bioactive pharmacophore (or chemical functionality). Protein pharmacophores are typically a sequence of 6 to 12 amino acids and are often readily synthesised making them highly amenable to incorporation into a cyclotide framework.

Once the bioactive peptide and protein has been identified and stabilised it would need to be incorporated into a matrix for controlled release into seawater. Once again, most of the technology associated with formulation of peptides and proteins has been in the pharmaceutical area (Frokjaer & Otzen 2005, Wang 2015, Geraldes et al. 2020). One approach has been the use of nanoparticles for sequestration of peptides and proteins. This has already indeed been applied to cyclotide-like molecules (Gerlach et al. 2010), as well as to a host of other peptides and proteins of pharmaceutical interest (Vaishya et al. 2015, Ramos et al. 2022).

While this work is in early stage development, the fact that live COTS secrete peptides and proteins into seawater in a controlled way as attractants suggests that the concentration required to evoke a recognition response by nearby COTS is practically achievable in a

natural physiological setting. Hence, such signalling is likely to be amenable to modulation by exogenous agents in a way that can be exploited for COTS control.

5.2.3 Protein/peptide functionality

One disadvantage of proteins in terms of molecular mode of action is related to their larger size, which prevents them from crossing cell membranes. This is an issue for proteins designed to target a specific intracellular protein, but not for those targeting extracellular receptors. For COTS, we know that behavioural responses can be elicited by conditioned seawater applied externally. This suggests the functional proteins either act on extracellular transmembrane receptors, such as the several GPCRs shown to be highly expressed in COTS tissues (Hall et al. 2017, Roberts et al. 2017), or engage other mechanisms if they need to directly modulate intracellular processes. It would seem the need to cross the cell membrane is not an issue if the objective is to develop COTS secreted proteins into control agents. If COTS proteins do target external receptors such as GPCRs, there is significant opportunities to apply structural, chemical, and biological knowledge from the scientific literature as GPCRs have been heavily studied as targets for therapeutic development (Davenport et al. 2020) and for control of invertebrate pests (Ozoe 2021). Proteins and peptides are excellent starting points for development of specific GPCR modulators because they are often the natural ligands for those receptors, and therefore have been widely used for lead discovery (Swedberg et al. 2016, Davenport et al. 2020, Muratspahic et al. 2020).

5.3 Environmental safety

Odorant-degrading enzymes (ODE) have been found in proximity to chemoreceptors and function to rapidly catabolise semiochemicals into inactive degradation products and inactivate the signal (Chertemps & Maïbèche 2021). Their primary role is to remove redundant molecules and restore the sensitivity of the chemoreceptor for the next signal. The degradation products of semiochemical proteins and peptides are amino acids and are reassimilated into the body as all organisms have physiologies based on these building blocks and so contain intrinsic mechanisms to produce, use and recycle them. Degradation products may also be excreted into the aquatic ecosystem adding to the dissolved organic carbon pool. As such, semiochemicals are naturally degraded and considered eco-safe. This biosafety aspect would be beneficial in COTS control because a control agent might need to be continuously applied, and the accumulated products or their degraded by-products would need to be harmless to the environment and surrounding organisms.

Regardless of their natural origins and low persistence in the ocean, deliberate application of semiochemical technologies in the reef to modify COTS behaviour may be a concern for environmental protection authorities and a thorough risk assessment would be essential to ensure safe deployment and use.

6. ENGINEERING SOLUTIONS FOR DELIVERY IN THE MARINE ENVIRONMENT

6.1 Chemoemission

New innovative chemoemission devices, designed based on foundational methods used in traditional control programs, are now being realised to mimic the emission of semiochemicals in the terrestrial environment (Heuskin et al. 2011, Munoz et al. 2012, Olsson et al. 2015) (Table 7), some with capability to also detect released concentrations (Wei et al. 2017). A limited number of these have been trialled and applied in the aquatic environment (Lim & Sorensen 2012, Wagner et al. 2018, Johnson et al. 2020).

The intent of these chemoemission devices is to:

1. deliver a semiochemical payload;

2. release optimal concentrations of a semiochemical technology to elicit the desired response in a target species over an intended period of time; and

3. create (and maintain – if continuous) artificial odour plumes (i.e., blanket coverage or gradient depending on the behaviour being targeted).

The delivery method can be manual (human intervention), static or dynamic, or a combination of these (Table 7). Manual delivery relies on direct injection or surgical implant of the semiochemical into an individual, which significantly limits the number of individuals able to be targeted. The trade-off is that these methods, while costly and initially resource intensive to implement, aim to alter the physiology of the individual longer-term to indirectly disrupt the local population.

Static chemoemission devices are generally non-powered and tend to be relatively inexpensive, modular, portable, self-contained, and field-deployable (Kwan et al. 2019). They are generally placed in position (point-source) to release the semiochemical in the vicinity of the target species and have the potential to be deployed en masse. They are designed to deliver a dose of formulated semiochemical, either via uncontrolled slow-release (i.e., passive dissolution from eroding/dissolving formulant resulting in emission of decreasing concentrations at an inconsistent rate, often dictated by environmental and physicochemical factors) or semi-regulated slow-release (i.e., controlled by encapsulation or granulation resulting in semi-consistent concentration until depletion). The design and fabrication is aimed at producing a biodegradable device that does not require retrieval. Benefits include the longer-term presence of the semiochemical in the environment, which increases the likelihood of contact with the target species, however, device in-field longevity is limited to a single deployment.

Dynamic chemoemission devices generally rely on a power source and may be programmable, which increases device in-field longevity and enables targeted timing of release (Munoz et al. 2012). They are designed to deliver a consistent dose of formulated semiochemical, whether it be via sustained-release (i.e., at a continuous rate and pattern for a specified duration), pulsed-release (i.e., at a specific frequency for a defined time) or single

shot-release, and can be applied as a point-source or nonpoint-source. Given their design, which is likely to include electronic or battery components, they will require retrieval, although depending on chemical release method could be delivered and retrieved by unmanned autonomous vehicles, reloaded and redeployed.

Some features are considered essential for chemoemitting devices regardless of the specific control scenario or semiochemical properties. In general, the device must:

1. be cost effective within the context of the scale of the pest problem;

2. have minimal environmental footprint and be eco-safe;

3. have longevity in the marine environment relative to the desired duration of the emission; and

4. be reliable or consistent i.e., it will work the same way every time.

Invention of chemoemission devices intended for deployment in the marine environment for COTS control will require inherent design features. Some design features of chemical sampling and biosensor devices deployed to facilitate the assessment and monitoring of chemical concentrations in the environment may be transferrable. In essence, the device must be capable of (programmable) delivery of semiochemicals in required (precise) volumes and concentrations at the desired location.

Optimal device features are determined by the properties of the semiochemical, the desired control scenario (based on knowledge of the behavioural trait and semiochemical biological function), and the infrastructure and resources available to support the deployment and, if necessary, the retrieval. Considered here are the most likely scenarios for COTS control supported by current evidence and standard control methodologies. To understand how such semiochemical control strategies could be integrated into a COTS control program, three case studies are presented to illustrate how different semiochemical agents could be delivered on a reef.

6.1.1 Case study 1: Essential fatty acids as COTS foraging kairomones

Developing coral secondary metabolites as kairomone attractants (Teruya et al. 2001).

Aim: To assess the feasibility of prey-derived kairomone attractant technology for COTS control based on *a priori* knowledge.

A proof-of concept experiment by Teruya et al. (2001) revealed purified arachidonic acid and α-linolenic acid, both produced by coral prey, have an attractive effect for COTS both in aquarium and field experiments. Initially, a simple behavioural bioassay led to the isolation of arachidonic acid and α-linolenic acid as COTS foraging kairomone attractants. These essential fatty acids are present in viscera of the sea urchin *Toxopneustes pileolus* and also in acroporid corals (Teruya et al. 2001). They are bound as glycerophospholipids in the cell membrane and released by enzymes during periods of stress (such as injury to corals under attack) to mediate an inflammatory response. α-Linolenic acid is a confirmed feeding attractant for the corallivore muricid gastropod *Drupella cornus* (Kita et al. 2005). Other closely related unsaturated fatty acids, including eicosapentaenoic acid, docosahexaenoic acid and linoleic acid did not elicit any change in COTS behaviour.

The use of these kairomone attractants to control COTS has not been realised but still represent a sustainable opportunity for the IPM COTS CP if combined with manual injection or physical removal.

Overall control strategy: Pull, foraging prey kairomone attractant, uncontrolled release

Key properties:

- Target life history stage: adult
- Life history trait: chemosensation for foraging
- Mode of action: prey kairomone attractant
- Behavioural outcome: foraging, passive aggregation
- COTS control strategy: lure COTS away from at risk reef sites
    - luring COTS to point-source (trap) for manual injection or physical removal
    - assessing population size and distribution through improved monitoring
- Delivery method:
    - Timing: As needed
    - Duration: uncontrolled continuous long-term release or short-term, or controlled release to lure COTS to traps
    - Scale: localised (?) and reef-wide
    - Mode: left in situ for later retrieval of release device (deploy and retrieve)
- Specificity: not species-specific; will attract other corallivores
- Desirable chemical properties: essential secondary metabolite, water soluble
- Formulation: single molecule (or refined mixture of combination small molecules), active in behavioural bioassays; readily available, simple industrial preparation
- Device: simple single-use device to provide continuous uncontrolled plume via slow dissolution from substrate, be operational over long periods (weeks rather than days) and have no longevity in the environment beyond the deployment timeframe.

6.1.2 Case study 2: Proteins as COTS pheromone attractants

A case study on developing COTS-derived proteins as pheromone attractants (Yap et al. 2022).

Aim: To assess the feasibility of conspecific protein-derived attractant pheromone technology for COTS control based on *a priori* knowledge.

An innovative but not unprecedented approach is to expropriate COTS-specific communication signals into bio-baits. A scalable and environmentally sustainable bioprocessing framework has been developed and has successfully concentrated previous working volumes by 800-fold and refined COTS conspecific signals by 5-fold, enriching evolutionarily specialized attractants. This development has revealed the potential of proteomic and transcriptomic approaches to advance the identification and isolation of candidate protein semiochemicals and support the development of as yet unanticipated technologies for assessment as COTS control agents.

The bioprocessing involved the extraction and concentration of high molecular weight bioactive proteins from seawater readily sourced from captive COTS. The method is highly efficient and requires minimal industrial preparation. The extract matrix, by its very nature, is likely to impart some protection from adverse environmental influences, i.e., degradation by UV light, oxygen, etc. Although the exact nature of the active constituent(s) is unknown, this proteinaceous concentrate represents a new sustainable opportunity for the IPM COTS CP.

Control Strategy 1: Pull, conspecific aggregant, controlled release

Aim: To assess the feasibility of conspecific protein-derived attractant pheromone technology for COTS control

Key properties:

- Target life history stage: adult
- Life history trait: chemosensation for aggregation
- Mode of action: conspecific aggregant (i.e., non-sex pheromone attractant)
- Behavioural outcome: active aggregation
- COTS control strategy: lure COTS for mass trapping; other species (including other echinoderms or predators that may be attracted and inadvertently trapped) can be separated and returned to minimise collateral damage
- Delivery method:
    - Timing: As needed
    - Duration: controlled continuous long-term release for entrapment or short-term in support of COTS monitoring and manual culling, respectively
    - Scale: localised at visitation site
    - Mode: For long-term release to control low- to mid-density populations, left in situ for later retrieval of chemoemission device and trap (deploy and retrieve). For short-term release to support culling, biodegradable device left in situ

- Specificity: species-specific; potent conspecific aggregants would have high control efficacy and low risk perspective
- Desirable chemical properties: water soluble, proteinaceous, potential to stabilise through cyclotides
- Formulation: concentrated extract, active in behavioural bioassays; simple production
- Device: needs to provide continuous plume via continuous controlled dosing (i.e., chemoemitter within a trap). For long-term release device needs to have longevity in the environment, however, deployment will be limited by trap capacity. For short-term release device needs to remain viable for hours, 24 hours at most.

Control Strategy 2: Pull, conspecific attractant, uncontrolled release

Key properties:

- Target life history stage: adult
- Life history trait: chemosensation for aggregation
- Mode of action: conspecific aggregant (i.e., non-sex pheromone attractant)
- Behavioural outcome: active aggregation
- COTS control strategy: pull COTS to a point-source
    - lure cryptic COTS out of reef matrix or towards shallower depths enabling manual culling or monitoring
    - lure COTS to point-source (or trap) off-reef for manual injection or physical removal
    - assess population sizes and distribution through improved monitoring methods
- Delivery method:
    - Timing: As needed or on-going
    - Duration: uncontrolled continuous short-term dissolution or long-term, respectively
    - Scale: localised at visitation site
- Mode: left in situ until biodegraded (deploy and forget)
- Specificity: species-specific; potent conspecific attractants would have high control efficacy and low risk perspective
- Desirable chemical properties: proteinaceous, water soluble, potential to stabilise through cyclotides
- Formulation: concentrated extract, active in behavioural bioassays; simple production
- Device: simple single-use device to provide continuous uncontrolled plume via slow dissolution from substrate, be operational over extended periods and have no longevity in the environment beyond the deployment timeframe.

6.1.3 Case study 3: Predator secretome as COTS kairomone repellents

A case study on developing *Charonia tritonis*-derived secondary metabolites as kairomone repellents (Hall et al. 2016b, Hall et al. 2017).

Aim: To assess the feasibility of predator-derived kairomone deterrent technology for COTS control based on *a priori* knowledge.

COTS is known to respond to the presence of its sympatric predator, the benthic giant triton snail (*Charonia tritonis*). Using behavioural assays, *C. tritonis*-conditioned seawater and trail mucous was confirmed to act as a deterrent signal inducing rapid and aversive responses in adult COTS. Untargeted proteomics has revealed 417 proteins in these exo-secretomes. Untargeted metabolomics has similarly revealed at least 191 metabolites. The chemically rich *C. tritonis* exo-secretome provides an important resource towards the characterisation of a predator pheromone for possible application in the control of COTS populations.

Overall control strategy: Push, predator kairomone repellent, controlled release

Key properties:

- Target life history stage: adult
- Life history trait: chemosensation for predator avoidance
- Mode of action: predator kairomone repellent
- Behavioural outcome: dispersal or disruption of aggregation
- COTS control strategy: deter or repel COTS from a point-source
    - push COTS away from at risk reef sites, flush cryptic individuals out of hiding prior to manual culling
    - flush recalcitrant individuals from the reef matrix for manual injection or physical removal
    - disrupt spawning aggregations and reduce reproductive rates
- Delivery method:
    - Timing: As needed; 24-hours prior to manual culling
    - Duration: controlled short-term release
    - Scale: localised
    - Mode: left in situ for later retrieval of chemoemission device (deploy and retrieve)
- Specificity: not species-specific; potent non-specific repellents could have high control efficacy but also high risk perspective
- Desirable chemical properties: unknown proteins/secondary metabolite(s), water soluble
- Formulation: crude mixture, fractionated mixture retains activity in behavioural bioassays; collected from captive *C. tritonis*, simple industrial preparation
- Device: pulsed-release device (i.e., controlled by a peristaltic pump) to provide specific dose at defined intervals, be operational over short periods (days rather than weeks), retrievable and re-deployable as needed.

6.2 Deployment: lessons from existing technologies deployed in marine environments

6.2.1 Delivery platforms and devices

Technology readiness levels (TRL; Appendix B) are used to assess the maturity of a new technology towards full economic operation, TRL1 being descriptive and TRL9 being 'mission proven'. The operational deployment of semiochemical control agents is undergoing a revolution in terrestrial pest control programs supported by advances in materials science and engineering. New innovative technologies based on foundational control methods are now being applied in the terrestrial environment and include design features to reduce the spatial scale and improve temporal precision of air-borne semiochemicals (Olsson et al. 2015). Hence, chemoemitting devices and deployment technologies already exist at a higher level of maturity. Such sophisticated technology has not yet been realised for application in control scenarios in aquatic environments, however, existing devices and platforms may be amenable to re-purposing for the delivery of control semiochemicals.

Deployment of semiochemicals can be performed using three principal approaches: direct one-off application of the formulated semiochemical, in-field installation, and in-field installation and retrieval. Deployment technologies employed will be dependent on the:

1. Nature of the semiochemical;
2. nature of the chemoemission device;
3. field location for optimal response;
4. site accessibility;
5. spatial coverage;
6. temporal coverage;
7. volume/quantity to elicit the response;
8. duration;
9. installation infrastructure and/or power requirements; and
10. retrieval.

The suitability of different delivery devices to a particular delivery platform will be heavily dependent on the liquid volume/solid mass to be transported, power requirements and endurance, and accessibility of the release site. Delivery devices suited to deployment in marine environments are not 'off-the-shelf' technology ready to be utilised directly; a high level of sizing, customisation, configuration and integration to the platform is required, specific to the individual requirements (as shown in the above Case Studies and discussed by Shah and Singh (2018)). Whilst most individual components will be at high TRL levels, their integration brings more risks that place it more around TRL 6.

Testing technology readiness is critical in deployment design. Existing delivery platforms are assessed for TRL (Table 9) based on the potential for integration with delivery devices. This is not an exhaustive list, but provides a representation of the likely scale (including volume, size of platform and level of complexity) and logistics required to develop an end solution for delivery in the marine environment. A full feasibility analysis for each would be required to confirm and cost each of these potential solutions. Initiatives such as ReefWorks (2022) can provide a suite of secure marine technology testing and evaluation facilities to undertake controlled, safe testing of new uncrewed/autonomous systems and sensor technologies (including proof-of-concept) to confirm their fitness for purpose, safety and environmental compliance before being introduced into control operations. This approach, in conjunction with models to explore release and monitoring scenarios based on real world information, will be required to enable assessment and improvement of platform delivery systems, and provide the perfect framework and test environment against which semiochemical deployment delivery devices can be trialled for technology readiness.

Table 9: Technical readiness level (TRL) assessment of potential delivery platforms for application in COTS control. The assessment covers the breadth of delivery platforms and devices. ¥ most suited to deployment of point-source technology to complement existing manual control methods.

Volume Scale	Delivery Platform	Delivery Device Mode	Logistical/operational considerations	TRL
Near unlimited	Cabled observatory	Dynamic – Pumped	Range limited; localised	6
	Large vessels	Dynamic – Pumped Static – Drainage	Expensive; restricted access to shallow reef release site; broadscale or localised	9
Up to 3600 L	Fixed wing aircraft	Dynamic – Aerial blanket dispersion	Requires expert operator; ship-based docking; broadscale	9
1000 L (intermediate bulk container)	Medium size vessel	Dynamic – Pumped Static – Drainage	Short (week) trip duration; broadscale or localised	7
200 L (drum)	Small boat fleet with or without ROV	Dynamic – Pumped Static – Drainage	Short (day) trip duration; localised	6
Up to ~20 L	Landers / BRUVs	Dynamic – Pumped	Requires expert operator; localised or point-source	6
< 10 L	AUVs, ASVs	Dynamic – Pumped	Requires expert operator; localised	5
	Drone	Solid state – Dropped	Requires expert operator; CASA licence; localised	8
	SCUBA¥	Solid state – manual installation	Requires expert diver; point-source	9

[ROV = remotely operated vehicle, AUV = autonomous underwater vehicle, ASV = autonomous surface vehicle, Lander AUV = autonomous underwater vehicle for seabed landing and persistence, BRUVS = baited remote underwater video system]

6.2.2 Prospects for semiochemical deployment

Controlled static release of semiochemicals represents the least complicated and simplest method to implement in the marine environment and will likely take the form of crystallized pellets or bricks by drop deployment (e.g., drone or ship release). Limitations associated with inconsistent dissolution rates from these solid formulations can be overcome through

improved engineering. For example, Wagner et al. (2018) demonstrated a simple capped PVC pipe housing regulated the water flow/velocity around the emitter consisting of PEG6000 polymer formulant impregnated with the sex pheromone attractant 3kPZS, stabilising the dissolution rate and extending the longevity of the chemoemitting device. Solid-propellent rocket technology similarly relies on achieving a consistent release of energy based on the physical geometry of the exposed propellent. Whilst the ability to formulate a solid-state semiochemical compound and the resulting dissolution rates is still to be achieved, the engineering aspects of controlling the dissolution would start at a high TRL of 8 or 9 (Appendix B), utilising proven technology.

Controlled dynamic release of semiochemicals (pulsed or timed release) at specific sites on the reef will require the integration of a semiochemical delivery device (e.g., pump, large piston syringe, etc. with associated control electronics and power supply) and the delivery platform (e.g., remotely operated underwater vehicle (ROV), autonomous underwater vehicles (AUV), autonomous surface vehicles (ASV), Lander AUV - autonomous underwater vehicle for seabed landing and persistence, baited remote underwater video systems (BRUVS), etc.), and strong knowledge of the release site (discussed in further detail in Section 7). Such an approach is feasible but will require significant planning, testing and in-water resources.

Lure and entrapment technologies are common to most control strategies. An efficient lure and trap design must allow for release and uninterrupted dispersal of the semiochemical lure over the deployment time, and as, COTS are notoriously difficult to entrap and if trapped are masterful escape artists, needs to ensure retention. In addition, optimization of trap design, position (height, orientation) and deployment pattern (density and location) is essential to achieve high trapping efficiency (Hume et al. 2020). Development of lure and trap technologies for the selective capture and retention of COTS should be a primary goal of research efforts, noting that deployment of traps will require regular maintenance to remove captured animals and reset. Trap design must also ensure there is no significant impact on natural enemies and other beneficial reef species that may also be attracted (at least visually) to traps.

The possibility that chemical, physicochemical and visual cues may act synergistically to elicit the behavioural response also needs to be considered (Stephenson 2016, Johnson et al. 2019, Johnson et al. 2020). Since COTS do rely to some extent on visual and tactile stimuli (Petie et al. 2016), initial testing of payload formulations delivered from within, or in the proximity of, lure traps will need to be performed to ensure there is no interference with the animal's ability to detect and respond naturally. COTS use visual cues for close range orientation towards objects. Therefore, repurposing of a tried-and-tested pheromone trapping strategy (Andrews et al. 1996), including the redesign of traps to utilise COTS conspecific aggregants for long-range attraction and enhance visual appeal for short-range attraction, should be explored. Lure traps could be adapted to lure, trap and kill COTS, assuming the formulation is able to effectively and selectively target COTS and not non-target species. As for lure traps, regular maintenance would be required.

6.3 Resource and logistical considerations

As for the current COTS control program, the deployment of semiochemical-based control technologies on the GBR faces logistical and operational constraints relating to the remoteness and offshore locations of many of the afflicted reefs (Fletcher et al. 2020) (Table 9). For purported semiochemical control strategies (Table 2), each has foreseeable constraints that could limit application. For example, a strategy that involves the blanket release of a semiochemical (i.e., to induce out-of-season spawning, or asynchronous spawning of one sex, or to deter from a specific site) may be limited by the weight and volume of chemical to be transported, and dependent on the size of the vessel used (Table 9). Similarly, transport of larger delivery platforms, or platforms that require scheduled docking for maintenance and reloading are also dependent on vessel type and size. The nature of the semiochemical technology and the mechanism of release will together determine the size of the deployment device and the type of delivery platform needed. When operating in the GBR considerations should also be given to the environmental footprint; there should be minimal waste discharged into the environment. Therefore, deployment technologies that are naturally degradable and leave no trace (i.e., formulated bricks, gels or pellets dropped into place) or readily retrievable and reusable (i.e., lure traps positioned and retrieved when full) are preferred.

7. ASSESSMENT OF THE SPATIAL AND TEMPORAL FOOTPRINT OF A SEMIOCHEMICAL ON CORAL REEFS

Hydrodynamic models are useful to describe and explore the theoretical spatial and temporal footprint of a chemical within a defined aquatic system. Presented here are pre-emptive hydrodynamic models describing the dispersal of a virtual chemical after release at select sites within three coral reefs. The ultimate goal of this modelling approach is to provide accurate advice to the IPM COTS CP regarding the suitability of deployment of a semiochemical-based COTS control technology on the GBR.

7.1 Assumptions and empirical knowledge

A directional (or gradient) odour plume is generated by diffusion of a chemical from a point-source in a water current and can be received in a far-field environment dependent on various physicochemical properties. Numerical modelling is a great tool for predicting the extent or range of such plumes. Computer simulations of numerical models describing the release of a chemical into the environment rely on the availability of well-resolved hydrodynamic models in the study area, as well as descriptors of the chemical properties. A series of scenarios can be implemented by varying the model's inputs in addition to the time and place of the simulation, e.g., different chemical release regimes. Comparison of model outputs enables an assessment of the chemical dispersion and the suitability of each release site for deployment.

7.2 Reef selection

The GBR covers 344,400 km^2 from its northern most boundary in Torres Strait (9°08'S 143°52'E) to Lady Elliot Island (24°06'S 152°42'E) in the South, extending up to 300 km off the east coast of Australia. The geographic focus of this modelling study was restricted to three representative GBR reefs (Figure 1) (Table 10), chosen based on the past and current (COTS IPM Dashboard 2022), as well as predicted COTS irruptions (AIMS LTMP 2022):

- Lizard Island (status upgraded from 'no outbreak' to 'potential'),
- John Brewer (status downgraded from 'severe' to 'potential' after extensive culling), and
- Bowden (status currently at 'no outbreak' and is predicted to 'outbreak' as the outbreak wave moves southward).

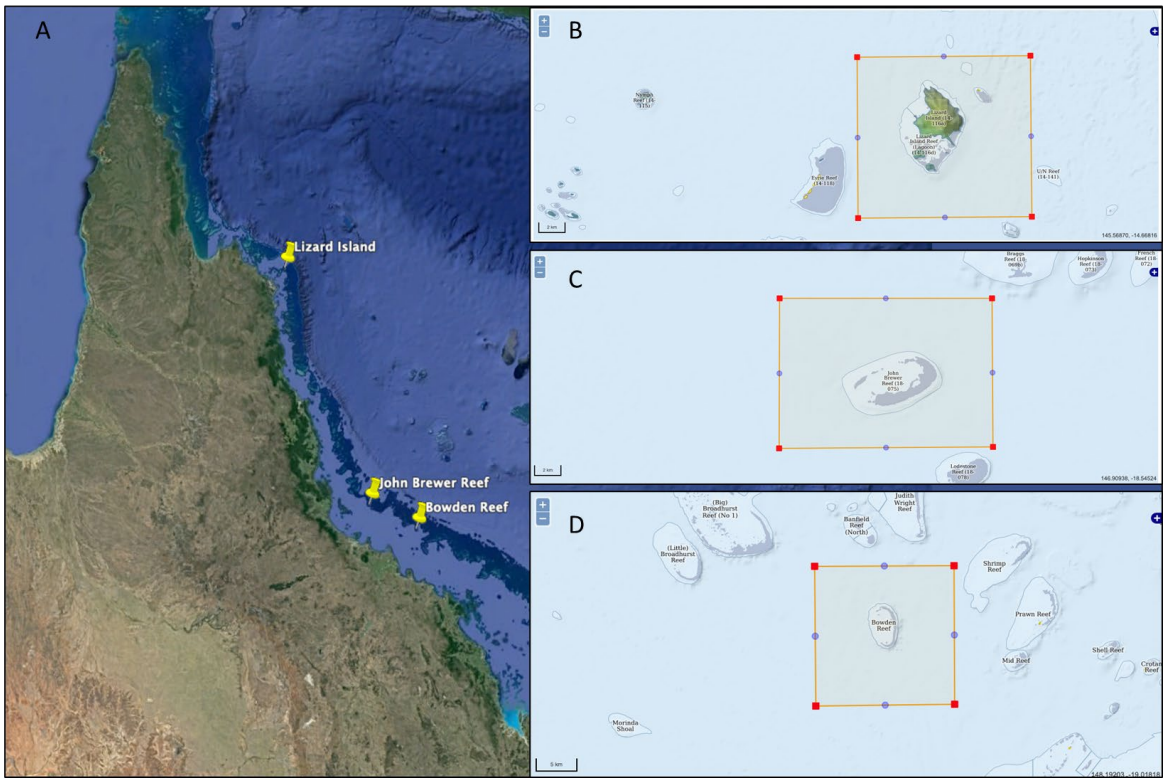

Figure 1 Map showing A. the location on the Great Barrier Reef, and the domain ranges for B. Lizard Island, C. John Brewer and D. Bowden reefs. Source: A. Google Earth, Data SIO, NOAA, U.S. Navy, NGA, GEBCO, Image Landsat / Copernicus; B, C, D. RECOM, CSIRO

Knowledge of reef bathymetry, known reef residence age times (established from visual assessment of prior models (Uthicke et al. 2022) and available high-resolution hydrodynamic models (Chen et al. 2011), supported the selection of these reefs. Lizard Island is a

continental island fringing reef (Hamylton et al. 2015), with high flow water currents and hence lower residency. The southeast-northwest currents in this area are very variable during the COTS spawning season. John Brewer Reef is a closed, circular reef with low current flow and high residency times, with predominant northwest-southeast currents. John Brewer Reef has previously been classified as a self-seeding reef (Black et al. 1991). Bowden Reef is a semi-enclosed lagoon (Wolanski et al. 1989, Spagnol et al. 2002), open on the eastern flank.

Table 10: RECOM model run parameters and usage in chemical release scenarios

Reef name	Run ID	Chemical release start date	Chemical release scenarios
Lizard Island	519	2019-09-01	**1, 2, 3**
	526	2018-10-01	**1**
	521	2017-12-01	**1**
	522	2016-12-01	**1**
	523	2015-11-01	**1**
John Brewer	544	2019-12-01	**1, 2, 3**
	543	2018-11-20	**1**
	547	2017-09-01	**1**
	546	2016-11-01	**1**
	545	2015-10-01	**1**
Bowden	527	2020-09-01	**1, 2, 3**
	539	2019-10-10	**1**
	540	2018-12-01	**1**
	541	2017-10-01	**1**
	542	2015-09-15	**1**

7.3 Model description

The hydrodynamic models were set up using RECOM, the “Relocatable Coastal Ocean Model” that is provided as part of the eReefs marine modelling suite (Steven et al. 2019). RECOM provides a user interface that establishes a nested curvilinear model grid at higher resolution for a small area within the larger domain of the nominal 1 km or 4 km resolution eReefs marine models. The underlying hydrodynamic model used in both the GBR-scale eReefs models and RECOM is SHOC (Sparse Hydrodynamic Ocean Code; (Herzfeld 2006, Herzfeld et al. 2010)), which is part of the CSIRO EMS hydrodynamic-biogeochemical modelling suite, available open source from https://github.com/csiro-coasts/EMS. RECOM draws oceanographic and meteorological boundary conditions from the regional-scale eReefs models and from the Australian Bureau of Meteorology’s ACCESS data products (Puri et al. 2013), respectively, and wave data derived from a local implementation of the SWAN wave model (Booij et al. 1999). SHOC is a three-dimensional (plus time) free-surface baroclinic finite difference hydrodynamic model. RECOM applies a z-coordinate model grid with up to 25 vertical layers that have varying resolution, gradually increasing with depth from 0.5 m near the surface.

For this application, RECOM was run at the three selected reefs (i.e., Lizard Island, John Brewer Reef and Bowden Reef) with a grid resolution of approximately 300 m (for Lizard Island) and 400 m (John Brewer and Bowden Reefs) (Figure 2), over five different time periods during the COTS pre-spawning/spawning season (i.e., from September to January) between 2015 and 2020 (Table 10). These time periods were selected to represent a range of local environmental variations including temperature, water currents and wind, and hence these time periods differed among reefs. Each model run extended for 32 days. The curvilinear grid and boundary condition data provided by RECOM were used to set up a hydrodynamic model using SHOC to simulate the release and dispersal of a virtual semiochemical simulated as a slowly degrading dissolved substance during days 2-31 of each model run. For this purpose, SHOC was run in full hydrodynamic mode using semiochemical tracers released from six sites on each reef (Figure 2).

Three location-specific delivery strategies were considered: 1) attractant (point-source) located on sandy patch adjacent to the coral, 2) repellent (point-source) located near the coral to be protected, and 3) attractant and repellent (push-pull; juxtaposed point-sources). To investigate these, at each reef, three release sites were chosen on sandy patches adjacent to the reef (i.e., not the usual habitat of COTS), and a further three within each reef matrix (i.e., where cryptic COTS are likely to be). These sites were considered suitable for the release of attractants and repellents, respectively (Figure 2; Table 11). Pairs of attractant – repellent sites (1-2, 3-4 and 5-6) were selected in close proximity of each other, to allow future applications of the push-pull strategy.

The models were designed based on existing knowledge of chemical properties that may impact delivery (including release and dispersal) in an aquatic environment (Table 12). Given the current focus of the COTS Control Program is the manual culling of adults in existing outbreaks, the release of the virtual chemical was simulated at 0.25 m above the sea bottom. Three scenarios were considered:

- Scenario 1: The virtual chemical was released continuously at a constant rate of 1 unit s^{-1} during the entire release period.
- Scenario 2: The virtual chemical was released for one hour leading up to each low tide during the release period but with the same total load as in scenario 1 over the simulation period. This scenario represents release during periods when dispersal is likely to be reduced due to low tidal velocities.
- Scenario 3: The virtual chemical was released for one hour leading up to each mid tide during the release period, again with the same total load as in scenario 1 over the simulation period. This scenario represents release during periods when dispersal is likely to be enhanced due to high tidal velocities.

Scenarios 2 and 3 were run only once at each reef, for the most recent time period. These pulsed scenarios were used to assess the importance of timing the release of the virtual chemical at different stages in the tidal cycle. In these two scenarios, the release rate varied slightly between simulations because the number of low tide and mid tide pulses differed among simulations (Table 12).

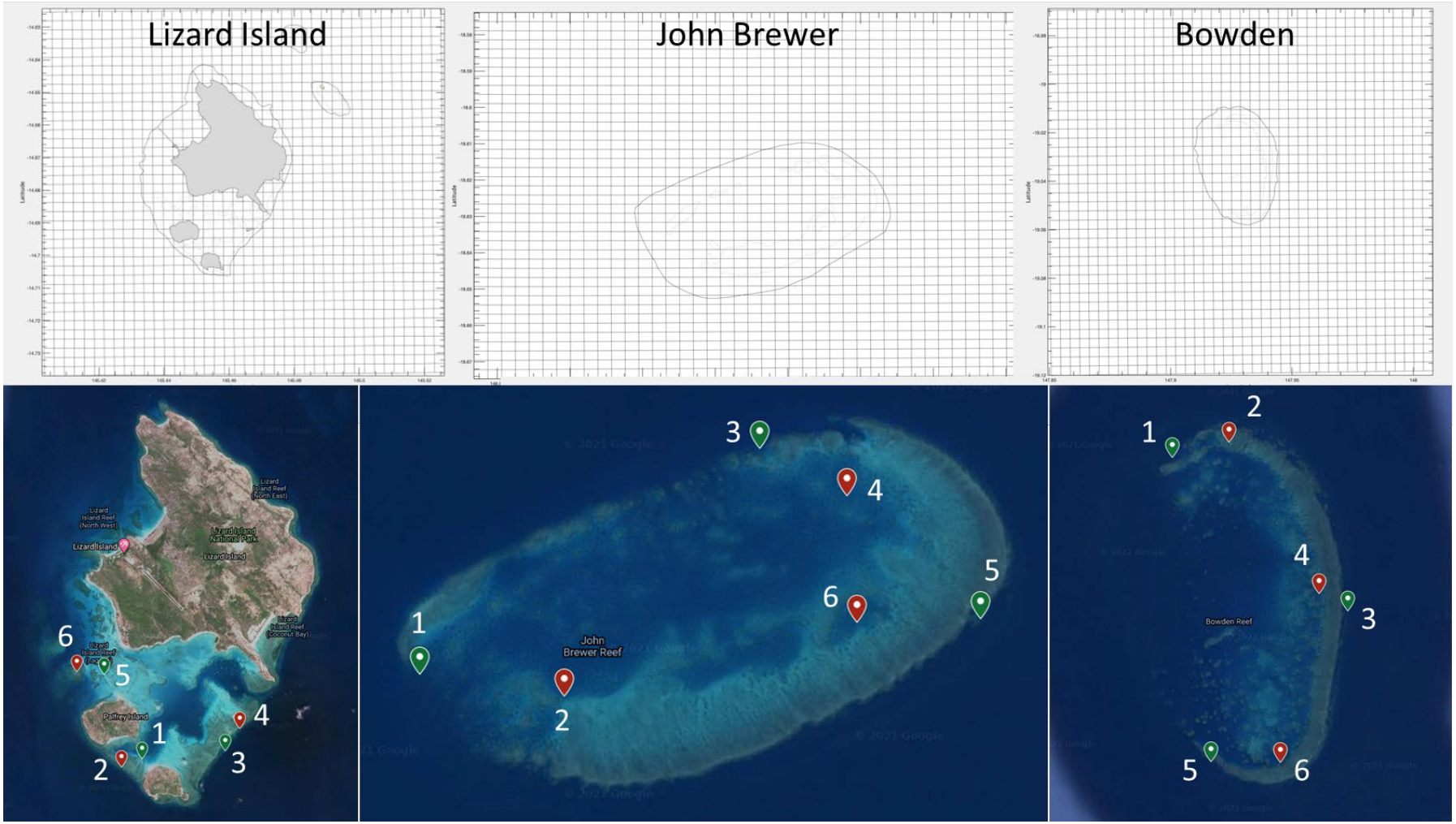

Figure 2 Maps showing the model grid (top panel) and release locations (bottom panel) for Lizard Island, John Brewer and Bowden reefs. In green are the release sites considered suitable for deploying an attractant semiochemical and in red are release sites considered suitable for deploying a repellent semiochemical. Source: Google Earth, Data SIO, NOAA, U.S. Navy, NGA, GEBCO, Image Landsat / Copernicus. Refer to Table 11 for release site coordinates.

Table 11: Chemical release sites

Reef name	Release site	Longitude	Latitude
Lizard Island	Lizard_1	145.45104	-14.69810
	Lizard_2	145.44784	-14.69938
	Lizard_3	145.46375	-14.69697
	Lizard_4	145.46601	-14.69361
	Lizard_5	145.44517	-14.68554
	Lizard_6	145.44094	-14.68515
John Brewer	JohnBrewer_1	147.02859	-18.63611
	JohnBrewer_2	147.04222	-18.63800
	JohnBrewer_3	147.06070	-18.61588
	JohnBrewer_4	147.06893	-18.62017
	JohnBrewer_5	147.08156	-18.63109
	JohnBrewer_6	147.06989	-18.63147
Bowden	Bowden_1	147.92027	-19.01612
	Bowden_2	147.92754	-19.01428
	Bowden_3	147.94282	-19.03482
	Bowden_4	147.93912	-19.03268
	Bowden_5	147.92520	-19.05307
	Bowden_6	147.93420	-19.05316

The properties of the virtual chemical specified in the model parameters were identical across all simulations and release locations. The virtual chemical was assumed to be water soluble and remain in the dissolved state, to be neutrally buoyant, to have a 24-hour half-life, and to be 100% stable. Fluctuations in salinity, temperature, suspended sediment concentrations and light exposure were assumed to have no impact on the properties, stability or longevity of the semiochemical (Table 12). Apart from degradation with a 24-hour half-life, the model did not simulate any chemical reactions or biological interactions that the semiochemical in the real ocean may be subject to.

Table 12: Assumed semiochemical properties and release scenarios

Parameter	Scenario 1	Scenario 2	Scenario 3
RECOM runs	all	519, 544, 527	519, 544, 527
physical state	dissolved		
buoyancy	neutral		
half-life	24-hours		
release depth	at 0.25 m above the modelled sea bottom		
release period	between day 2 and 31 of the run		
release regime	continuous release	one hour pulse leading up to each low tide	one hour pulse leading up to each mid tide
release rate	1 unit s^{-1}	12.86, 12 and 12.63 units s^{-1}	6.21, 6.21 and 6.32 units s^{-1}
total load	2,592,000 units	2,592,000 units	2,592,000 units

7.4 Description of model output files

The raw data together with all outputs are available at: CCIP_SHOC_outputs. Files are organised in subfolders for each scenario and the outputs related to the model comparison are stored in a separate subfolder.

The model outputs the chemical concentration in units m^{-3} in the deepest layer of the water column (the modelled sea bottom) at hourly intervals. The data is presented as:

- animations of the chemical dispersal and its concentration values in each grid cell
- animations of the dose received at each grid cell calculated as the sum of the chemical concentration from the start of the run to each timestep
- timeseries of chemical concentration at the release sites (in the grid cells containing the release sites).
- animations of temporal gradient calculated as the change in the chemical concentration at each time step
- animations of spatial gradient calculated as the maximum gradient in the chemical concentration between each grid cell and its eight neighbouring grid cells
- the maximum concentration of the chemical in each grid cell reached during the entire run plotted against the distance between the release site and the centre of each grid cell
- the total dose received in each grid cell plotted against the distance between the release site and the centre of each grid cell.

7.5 Model results

7.5.1 Timeseries of semiochemical concentration

As an example, presented here are the timeseries of chemical concentrations from all three scenarios at one release site on each reef (Lizard_1, JohnBrewer_1 and Bowden_1; Figure 3). The fluctuations in the virtual chemical concentration in the continuous release scenario 1 (Figure 3) indicate that water currents and daily tides modulate the upper chemical concentration even though the release is consistent and continuous.

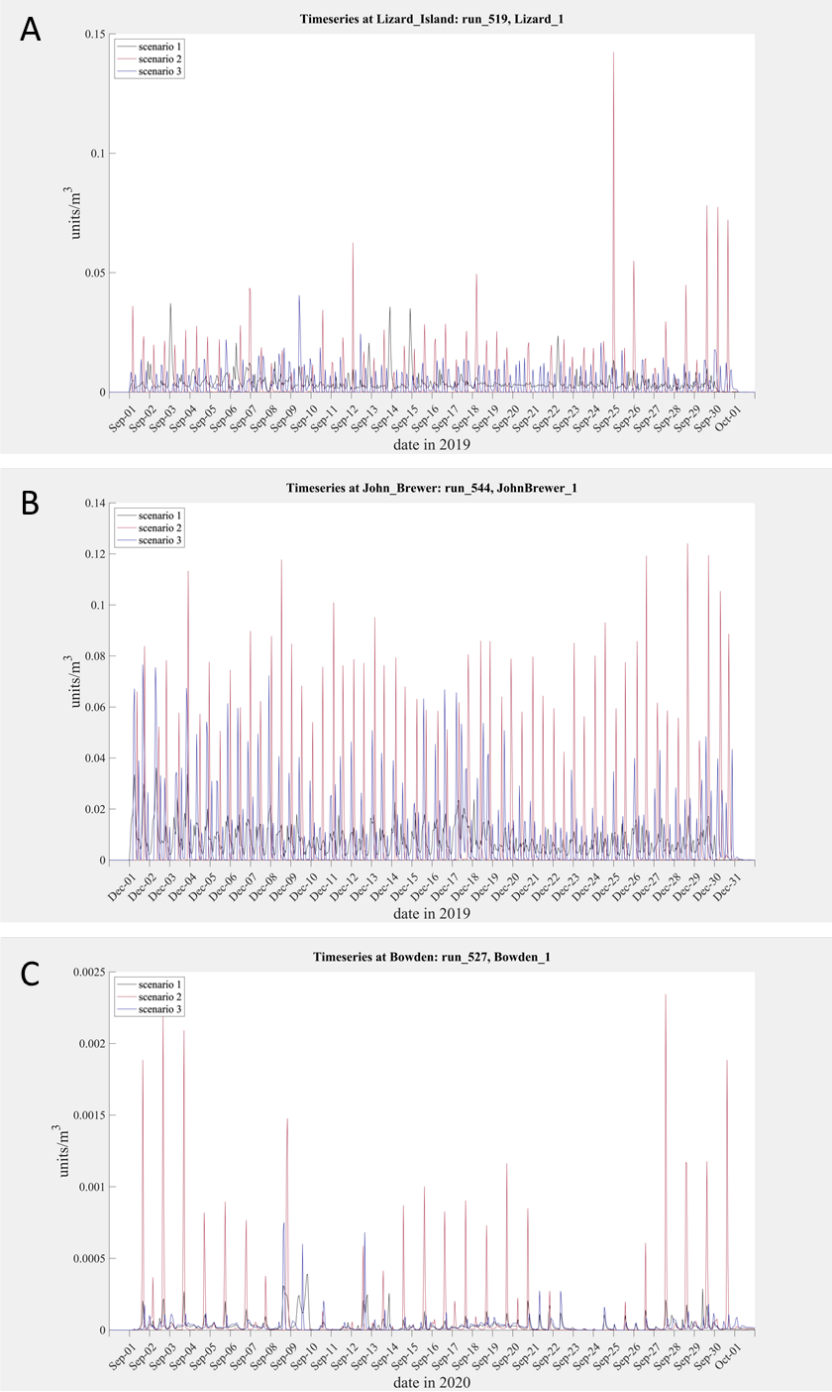

Figure 3 Timeseries of virtual chemical concentration at release site 1 from all three scenarios at A. Lizard Island; B. John Brewer; and C. Bowden reefs. Note the date range and year for each timeseries is different therefore comparisons can only be made per reef and not between reefs.

In the pulsed release scenarios 2 and 3 (released at low and mid tide, respectively), the virtual chemical concentration varied more consistently with the tide at Lizard Island and John Brewer reefs compared to Bowden reef (Figure 3). Timeseries show that tidal advection dominates the signal at the Lizard Island and John Brewer release sites while at Bowden Reef both tides and other undetermined factors affect variations in concentrations, producing a less regular signal (Figure 3).

In all instances, the continuous release scenario 1 (Figure 3) resulted in a higher background concentration of the virtual chemical for the duration of the release period, while in pulsed release scenario 2, and to a lesser extent in scenario 3, the concentration dropped to very low values in between release pulses but reached higher concentrations during pulses (particularly in scenario 2).

7.5.2 Maximum concentration, total dose and spatial gradient

As an example, the maximum concentration and total dose of the virtual chemical from all three scenarios for one release site on each reef (Lizard_1, JohnBrewer_1 and Bowden_1) are presented (Figure 4).

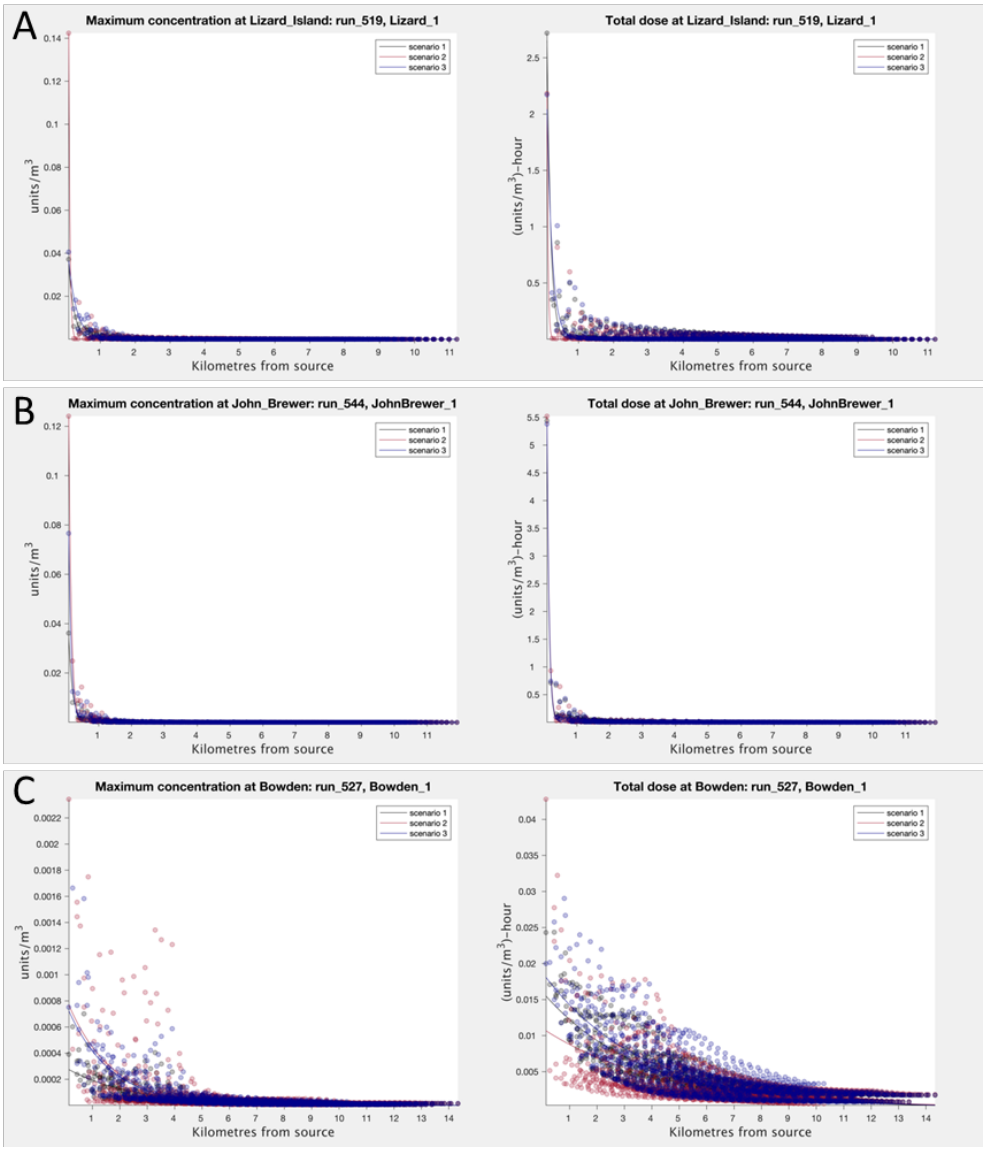

Figure 4 Example of maximum concentration and total dose (with fitted curve) from all three scenarios (1 = grey, 2 = red, 3 = blue) at A. Lizard Island; B. John Brewer Reef; and C. Bowden Reef. Note the scales for each graph are different therefore comparisons can only be made per reef and not between reefs.

As expected, in all cases the highest virtual chemical concentration and total dose were recorded nearest to the release sites. The values decreased exponentially with increasing distance from source as the virtual chemical dissipated with the currents (Figure 4 and Figure 5). This trend was more similar among scenarios at Lizard Island and John Brewer reefs compared to Bowden Reef. The exponential model also had a better fit for Lizard Island and John Brewer reefs (Figure 4). The decrease in concentration and total dose with distance from source was slower at Bowden Reef (i.e., shallower line slope), with the poor line fit indicating a less regular and more variable signal over the release period (as also observed in the timeseries; Figure 3). Overall, the maximum concentration values (Table 13) and total dose (Table 14 and Figure 5) were much lower at this reef compared to Lizard Island and John Brewer reefs indicating a more rapid initial dispersal from the release source.

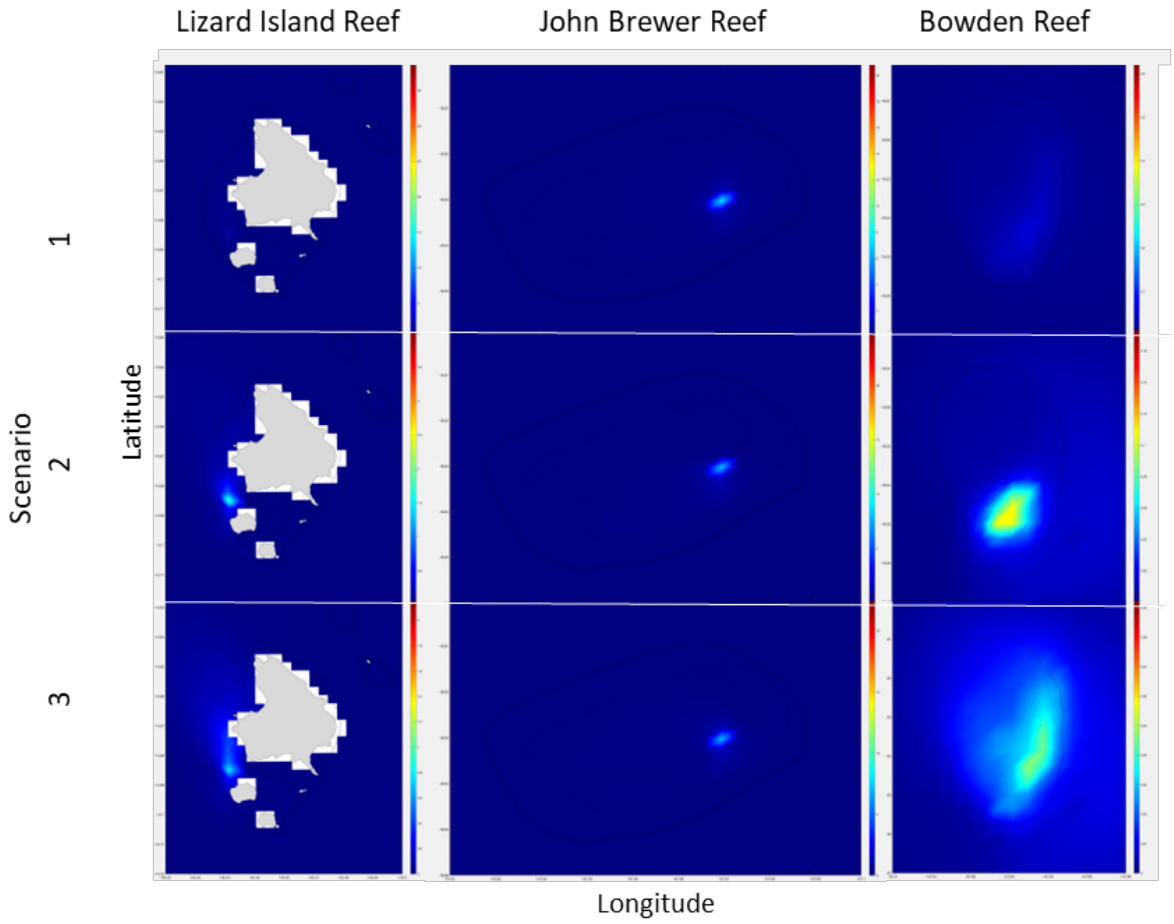

Figure 5 Total dose maps from all three scenarios at release site 6 of Bowden (Run 527 from 2020-09-01 to 2020-10-02), John Brewer (run 544 from 2019-12-01 to 2020-01-01) and Lizard Island (run 519 from 2019-01-01 to 2019-02-01) Reefs. Refer to Table 11 for site 6 coordinates at each reef. Note the heat scales for each domain are different therefore comparisons can only be made per reef and not between reefs.

Across all three reefs, modelled maximum concentrations ranged from 0.001 to 0.15 units m^{-3} in scenario 1, from 0.002 to 0.67 units m^{-3} in scenario 2 and from 0.002 to 0.34 units m^{-3} in scenario 3 (Table 13). Except for one release site at Lizard Island (Lizard_4), the highest maximum concentrations were all achieved in scenario 2 (Table 13). The maximum concentrations recorded in scenario 2 were up to ~6 times that in scenarios 1 and 3: 6.3 times higher at John Brewer and Lizard Island reefs and 6.0 times higher at Bowden Reef. The overwhelming majority of the lowest maximum concentrations were observed in scenario 1; all at John Brewer and Bowden reefs, with only those released from sites 2, 4, 5 and 6 at Lizard Island in scenario 3 being lower.

With respect to the dispersal range, the highest maximum concentrations were recorded at John Brewer Reef, up to 0.051 km from the release site in all scenarios. In scenario 2, the distances at which the maximum concentration was recorded ranged between 0.04 (Lizard_6) and 1.2 (Bowden_3) km from release site. While concentrations above half the maximum concentration were recorded up to 3.9 km (Bowden_1) in scenario 2 compared to shorter distances of 3.3 km (Bowden_1) and 2.1 km (Bowden_5) in scenarios 1 and 3, respectively, the time these concentration values were recorded were the lowest in scenario 2 – only 45 hours compared to 124 and 61 hours in scenarios 1 and 3, respectively.

Table 13: Maximum concentration (conc) vs distance (dist) from release site (6 sites per reef). Scenario 1 = continuous release; scenario 2 = release 1 hour leading to low tide; scenario 3 = release 1 hour leading to mid tide. Blue cells = scenario giving maximum distance to and time at 50% of maximum concentration. Grey cells = scenario giving maximum concentration.

Reef	Run	Release Site	Scenario 1				Scenario 2				Scenario 3			
			Max conc (units m^{-3})	Dist to max conc (km)	Dist to 50% max conc (km)	Time conc was > 50% max conc (hrs)	Max conc (units m^{-3})	Dist to max conc (km)	Dist to 50% max conc (km)	Time conc was > 50% max conc (hrs)	Max conc (units m^{-3})	Dist to max conc (km)	Dist to 50% max conc (km)	Time conc was > 50% max conc (hrs)
Lizard_Island	519	1	0.037	0.10	0.10	11	0.14	0.10	0.10	4	0.041	0.10	0.10	5
		2	0.020	0.069	0.069	10	0.078	0.069	0.069	3	0.018	0.069	0.37	21
		3	0.007	0.15	1.8	124	0.025	0.44	0.77	36	0.014	0.15	0.77	61
		4	0.043	0.055	0.055	4	0.025	0.35	0.68	35	0.015	0.055	0.36	28
		5	0.056	0.11	0.11	4	0.28	0.11	0.11	3	0.044	0.11	0.11	5
		6	0.019	0.041	0.041	3	0.054	0.041	0.37	12	0.014	0.041	0.041	22
John_Brewer	544	1	0.036	0.088	0.088	30	0.12	0.088	0.088	39	0.077	0.088	0.088	38
		2	0.040	0.11	0.11	92	0.23	0.11	0.11	33	0.077	0.11	0.11	40
		3	0.097	0.15	0.15	44	0.59	0.15	0.15	14	0.20	0.15	0.15	20
		4	0.15	0.051	0.051	84	0.67	0.051	0.051	30	0.34	0.051	0.051	35
		5	0.033	0.14	0.14	41	0.21	0.14	0.14	10	0.094	0.14	0.14	14
		6	0.091	0.15	0.15	7	0.42	0.15	0.15	4	0.22	0.15	0.15	3
Bowden	527	1	0.001	0.69	3.3	10	0.002	0.14	3.9	17	0.002	0.28	0.85	8
		2	0.002	0.21	0.39	6	0.008	0.21	0.53	12	0.003	0.21	1.0	12
		3	0.001	0.84	2.1	56	0.003	1.2	2.1	45	0.002	0.84	1.3	23
		4	0.001	0.49	1.4	56	0.008	0.19	1.2	24	0.003	0.49	1.2	17
		5	0.001	0.71	1.7	33	0.005	0.41	0.80	18	0.002	0.71	2.1	37
		6	0.001	1.2	2.1	13	0.004	0.50	1.3	31	0.002	1.2	1.7	17

Table 14: Total dose vs distance (dist) from release site. Scenario 1 = continuous release; scenario 2 = release 1 hour leading to low tide; scenario 3 = release 1 hour leading to mid tide. Blue cells = scenario giving maximum distance to 50% of total dose. Grey cells = scenario giving maximum dose.

Reef	Run	Release Site	Scenario 1				Scenario 2				Scenario 3			
			Max total dose (units m^{-3})-hr	Dist to max dose (km)	Max dist to 50% total dose (km)	Number of grid cells > 50% of total dose	Max total dose (units m^{-3})-hr	Dist to max dose (km)	Max dist to 50% total dose (km)	Number of grid cells > 50% total dose	Max total dose (units m^{-3})-hr	Dist to max dose (km)	Max dist to 50% total dose (km)	Number of grid cells > 50% total dose
Lizard_Island	519	1	2.7	0.095	0.095	1	2.2	0.095	0.095	1	2.2	0.095	0.095	1
		2	1.5	0.069	0.069	1	1.2	0.069	0.52	2	1.2	0.069	0.069	1
		3	1.1	0.44	1.8	11	1.1	0.44	1.75	7	1.1	0.44	1.8	13
		4	1.2	0.055	2.1	10	0.96	0.35	2.2	19	0.86	0.68	2.8	31
		5	2.9	0.11	0.11	1	4.1	0.11	0.11	1	2.1	0.11	0.39	2
		6	1.2	0.041	0.33	2	1.8	0.041	0.041	1	0.80	0.041	1.3	6
John_Brewer	544	1	5.4	0.088	0.088	1	5.5	0.088	0.088	1	5.4	0.088	0.088	1
		2	7.5	0.11	0.11	1	8.6	0.11	0.11	1	5.7	0.11	0.11	1
		3	12	0.15	0.15	1	12	0.15	0.15	1	9.6	0.15	0.15	1
		4	21	0.051	0.051	1	25	0.051	0.051	1	23	0.051	0.051	1
		5	5.0	0.14	0.14	1	5.7	0.14	0.14	1	4.8	0.14	0.14	1
		6	7.4	0.15	0.15	1	7.9	0.15	0.15	1	6.9	0.15	0.15	1
Bowden	527	1	0.024	0.44	3.9	57	0.043	0.14	0.69	5	0.029	0.81	3.9	52
		2	0.11	0.21	0.79	4	0.17	0.21	0.53	2	0.075	0.21	2.3	18
		3	0.078	0.84	2.1	15	0.098	0.84	2.4	26	0.080	1.2	2.1	11
		4	0.11	0.49	1.3	11	0.17	0.49	0.89	4	0.093	0.49	2.3	23
		5	0.064	0.71	2.8	15	0.12	0.41	0.80	2	0.065	1.6	2.8	18
		6	0.056	1.21	2.9	15	0.11	0.86	1.3	8	0.048	1.2	3.4	24

For all reefs and release sites, the total dose recorded ranged from 0.024 to 21 (units m^{-3})-hour in scenario 1, from 0.043 to 25 (units m^{-3})-hour in scenario 2 and from 0.029 to 23 (units m^{-3})-hour in scenario 3 (Table 14). Scenario 2 yielded the highest values of total dose: all release sites at John Brewer and Bowden reefs achieved the highest comparative values, while at Lizard Island Reef the highest values were only achieved at sites 5 and 6. Under scenario 1, release sites 1-4 at Lizard Island Reef were predicted to have the highest total dose. The total dose recorded in scenario 2 was up to 1.5 times higher at John Brewer Reef, 2.3 times higher at Lizard Island Reef, and 2.3 times higher at Bowden Reef, compared to the total doses achieved in scenarios 1 or 3. The maximum total dose in scenario 2 was recorded between distances of 0.041 and 0.86 km from the release site. As for the maximum concentrations, the highest total doses predicted were also at John Brewer Reef, up to 0.051 km from the release point (John_Brewer_4) in all three scenarios (Table 14). At Bowden Reef, values above half the total dose were recorded up to 2.4 km from release site in

scenario 2 in 26 grid cells (Bowden_3), and up to 3.9 km (Bowden_1) in 57 and 52 grid cells in scenarios 1 and 3, respectively.

Table 15: Maximum spatial gradient. Scenario 1 = continuous release; scenario 2 = release 1 hour leading to low tide; scenario 3 = release 1 hour leading to mid tide. Grey cells = scenario giving maximum gradient. Highest maximum gradients at each reef for each scenario are in ***bold italics***.

Reef	Run	Release Site	Scenario 1	Scenario 2	Scenario 3
			Max gradient (units m^{-3} m^{-1})	Max gradient (units m^{-3} m^{-1})	Max gradient (units m^{-3} m^{-1})
Lizard Island	519	1	0.037	0.14	0.041
		2	0.020	0.078	0.018
		3	0.007	0.025	0.014
		4	0.043	0.025	0.015
		5	***0.056***	***0.28***	***0.044***
		6	0.019	0.054	0.014
John Brewer	544	1	0.036	0.12	0.077
		2	0.040	0.23	0.077
		3	0.097	0.59	0.20
		4	***0.15***	***0.67***	***0.34***
		5	0.033	0.21	0.094
		6	0.091	0.42	0.22
Bowden	527	1	0.001	0.002	0.002
		2	***0.002***	***0.008***	***0.003***
		3	0.001	0.003	0.002
		4	0.001	***0.008***	0.003
		5	0.001	0.005	0.002
		6	0.001	0.004	0.002

The highest maximum spatial gradient of virtual chemical concentration between neighbouring grid cells ranged from 0.002 to 0.153 units m^{-3} m^{-1} in scenario 1, from 0.008 to 0.666 units m^{-3} m^{-1} in scenario 2 and from 0.003 to 0.335 units m^{-3} m^{-1} in scenario 3 (Table 15). Except for one release site at Lizard Island Reef (Lizard_4), the strongest spatial gradient in virtual chemical concentration was achieved in scenario 2 for all three reefs (Table 15 and Figure 6). Exploration of the spatial gradient maps revealed the release scenario has significant impact on the dispersal at each reef. The maximum footprint depends on the half-life of the specific semiochemical and the overall duration of the deployment. With a long half-life and long deployment (or high dose and long follow-up time), a large footprint for chemical detectability can be achieved, but perhaps more important is the footprint over which strong spatial gradients are achieved. Figure 6 provides an indication of this given some reasonable assumptions about chemical half-life.

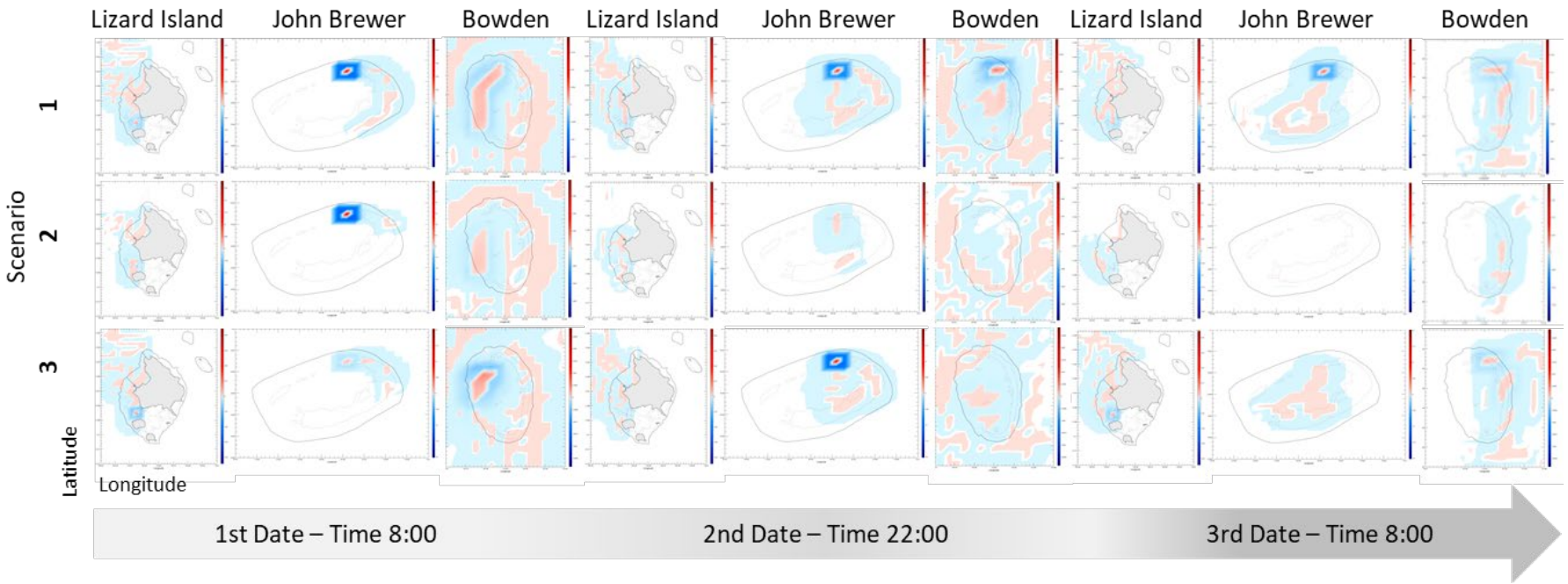

Figure 6 Example maximum spatial gradient maps for all three scenarios modelled at John Brewer (site 3; 2019-12-03 at 08:00, 2019-12-16 22:00 and 2019-12-24 at 08:00), Lizard Island (site 5; 2019-09-03 at 08:00, 2019-09-16 22:00 and 2019_09-24 08:00) and Bowden (site 2; 2020-09-03 08:00, 2020-09-16 22:00 and 2020-09-24 08:00) reefs at three time points each. Release sites chosen randomly, timepoints chosen to represent the full time period. Note the scales for each domain are different therefore comparisons can only be made per reef and not between reefs.

It should be noted that all models are run using an arbitrary unit of concentration. Chemical parameters specific to the semiochemical (i.e., potency, half-life, solubility) will need to be integrated into the models to determine the actual spatial and temporal dispersal footprint.

7.5.3 Influence of currents – temporal gradient

Local currents on the reef are moderated by the major currents and both will have direct impact on the dispersion rate and range of a chemical. For example, at John Brewer Reef, the major persisting current at the time of the model run is easterly, i.e., moving east to west across the reef. Under these hydrodynamic conditions, release site 5 is protected; the current flow is buffered by the reef perimeter resulting in the retention of the chemical plume (mean concentration before pulse $<1x10^{-4}$) close to the point-source (Figure 7). Release site 6 is subjected to current eddies and swirling within the lagoon resulting in a faster rate of dispersal, with mean concentration before pulse just over half that at site 5 ($5x10^{-5}$). Under these conditions it is evident that knowledge of the predominant current is critical to placement of the release device.

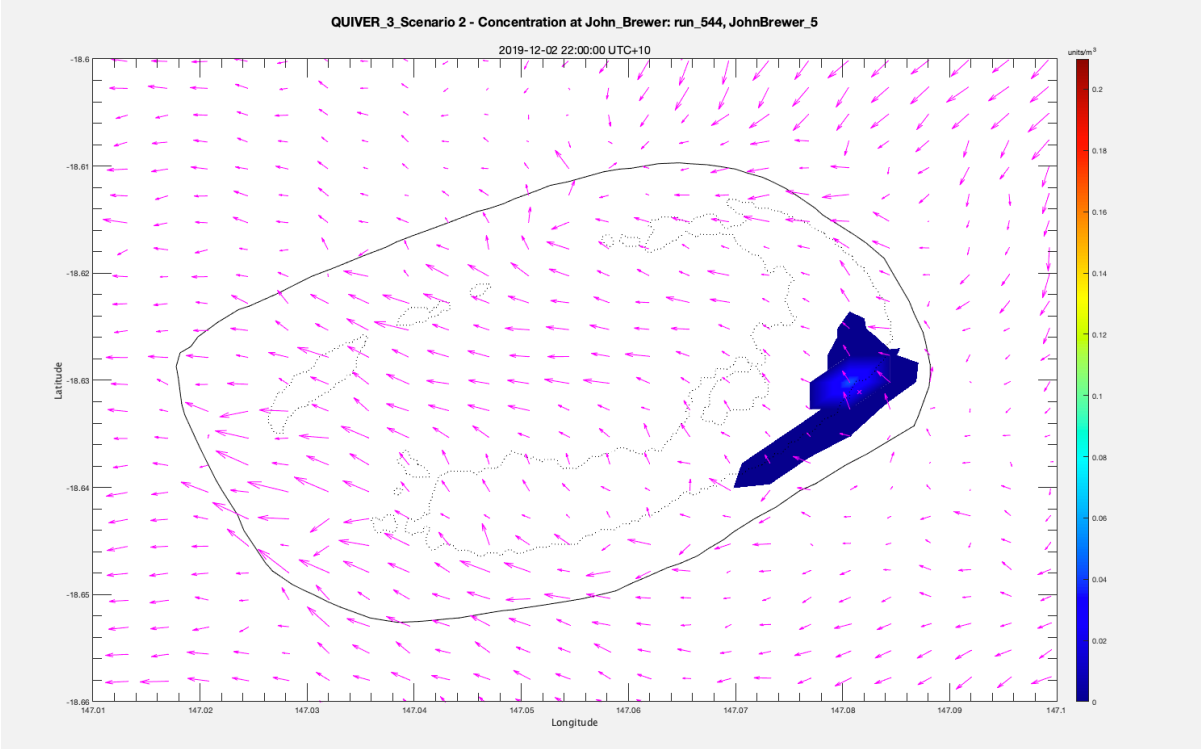

Figure 7 Example of directional currents at John Brewer Reef showing the localised impacts these have on limiting the dispersion of a virtual chemical from site 5 located on the outer perimeter of the reef.

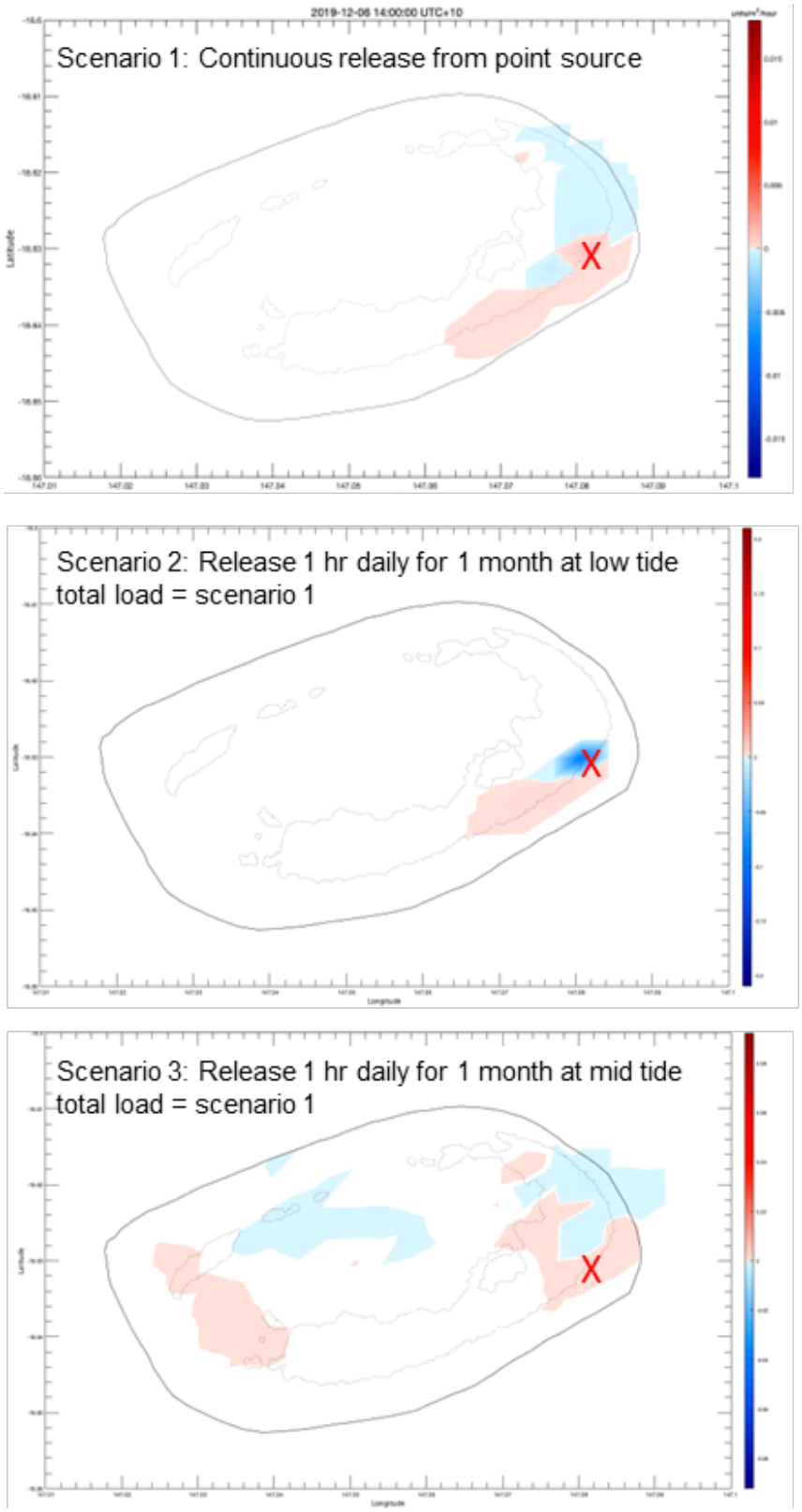

Figure 8 Snapshot at single time point (2019-12-06 at14:00) of the maximum temporal gradient (scales range from low units m^{-3} m^{-1} – blue – to high - red) showing the dispersion of a virtual chemical deployed from site 5 (red X) located on the outer perimeter of John Brewer reef under the three deployment scenarios 1, 2 and 3.

Local currents, influenced by daily tides, will determine the temporal gradient of the released virtual chemical, i.e., the change in concentration at a single location with time. At John Brewer Reef, the model output predicts there is a significant difference in the dispersal pattern and distribution range between a chemical released at the mid tide (scenario 3) compared to either the low tide (scenario 2) or continuous release (scenario 2) scenarios (Figure 8). Further, the site of deployment will also influence the temporal gradient (Figure 9). Comparison of the model outputs for a chemical released at site 5 versus site 6 indicate there is potential for all three deployment strategies. Site 5 represents a suitable location for a pull strategy, attracting COTS from the reef matrix to the outer periphery. Site 6 represents a suitable location for a push strategy, repelling COTS towards the outer periphery. If applied simultaneously, there is potential to induce a push-pull effect.

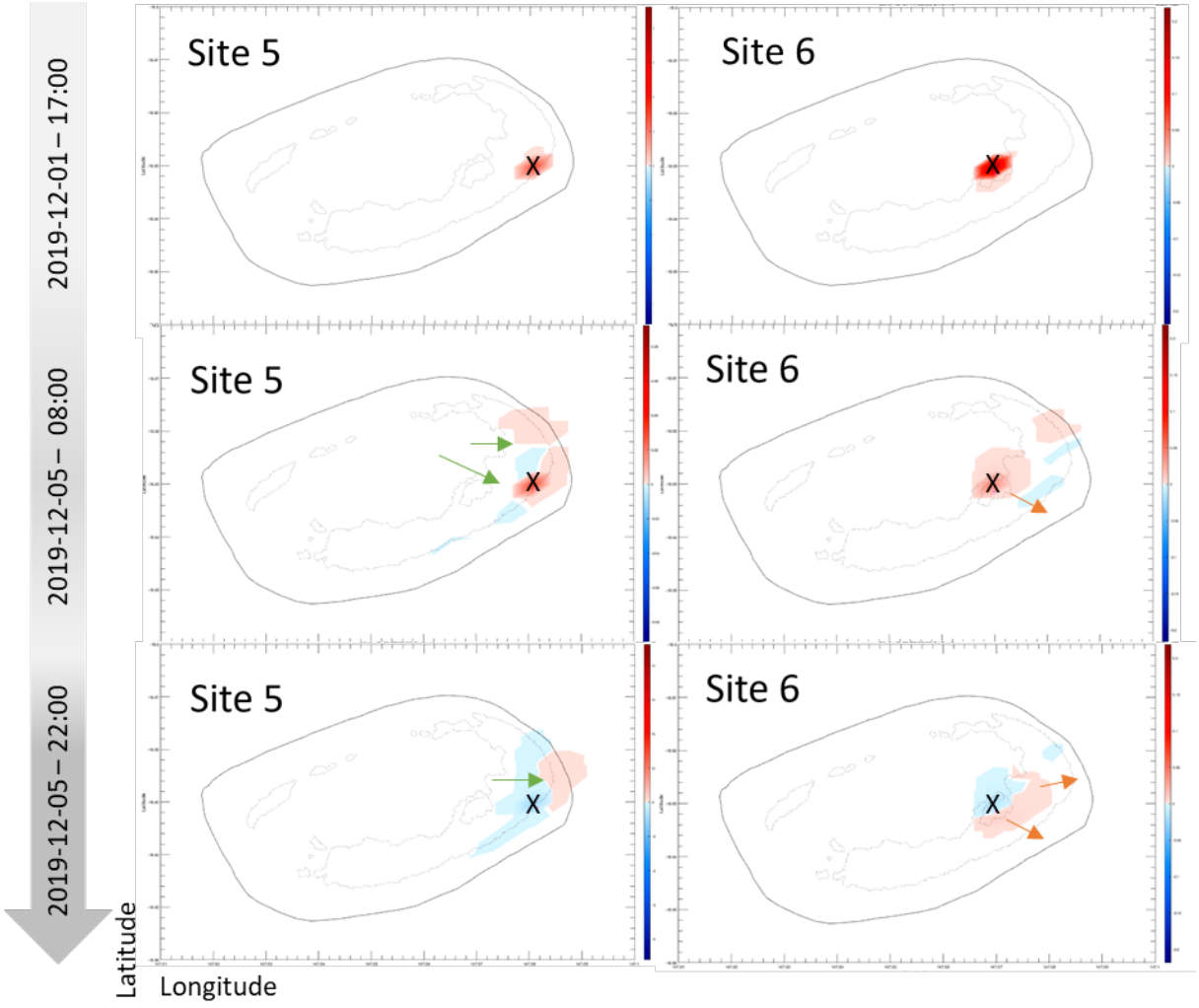

Figure 9 Comparison of the temporal gradient of a chemical deployed from sites 5 and site 6 (black X) on John Brewer Reef over three time points (2019-12-01 at 17:00, 2019-12-05 at 08:00 and 2019-12-05 at 22:00). Dispersal is modelled under scenario 3: release for 1 hr daily for 1 month at mid tide. Site 5 represents deployment of an attractant; site 6 represents deployment of a repellent. The arrows indicate the direction of the behaviour response; green indicates seeking, red fleeing.

7.6 Model interpretation

Release of the virtual chemical over a one-hour period, timed so that the release occurred during the hour leading to each low tide (i.e., scenario 2), resulted in maximum concentrations that were up to 4.4 times those observed with a continuous release of the same total load (scenario 1) and up to 2.0 times those observed with a release timed during the hour leading to mid tide. The maximum gradient achieved in this scenario was up to 5.0 times the maximum gradient achieved in scenario 1 and up to 6.3 times the maximum gradient achieved in scenario 3. Continuous release and release timed at mid tide led to the semiochemical being dispersed faster and further from the release site than in the release timed at low tide.

This scenario comparison demonstrates that careful timing of the virtual chemical release makes a substantial difference to the delivery outcome. Modelling results indicate the following guidelines for semiochemical deployment, depending on the nature of the chemical involved and the mechanism by which it modulates COTS behaviour:

- If high concentrations and strong spatial gradients are more important than a rapid spread of the semiochemical from the release site, scenario 2 would be the first choice. That is, better results would be expected from release timed near low tide. If rapid, wide dispersal of the semiochemical is ideal, then scenarios 1 and 3 (i.e., continuous release and release timed near mid tide) are likely to produce better results;
- If a higher total dose (exposure to the chemical over time) is more important than the speed and distances the semiochemical spreads from the release site, scenario 2 would be the first choice for John Brewer and Bowden. Again, higher doses of the attractant or repellent chemical can be achieved if release is timed near low tides;
- If it is important to maintain a certain background concentration at the release site, scenario 1 would be the first choice;
- If the aim is to create a stronger gradient in space and/or time, this is best achieved in scenario 2.

Ultimately, the choice of scenario will be determined by the chemical parameters and efficacy of the active semiochemical agent. See the sea lamprey literature for examples (Sorensen et al. 2005, Hume et al. 2020, Burkett et al. 2021, Fissette et al. 2021).

7.7 Model synopsis

Here, model simulations are used to examine the potential dispersal patterns of a hypothetical semiochemical deployed on the GBR, giving an indication of how a semiochemical plume is likely to disperse under different conditions from a point-source (i.e., reef system, release sites, release method etc.). Multiple time periods were simulated as ocean currents strongly influence the chemical dispersal rate and subsequent concentration gradients.

For each scenario, the release is simulated for 30 days with the initial virtual chemical concentration set to 0 units and evolving thereafter depending on the release rate and timing of release. The 30-day duration of simulations allows for residual circulation and dispersal to be determined over the course of a spring/summer-neap cycle. While indicative of a semiochemical release and dispersal in the real ocean, they describe a hypothetical semiochemical assumed to have limited chemical or biological interactions. Once a specific candidate semiochemical has been identified, modelling should be repeated using parameters that better reflect the specific characteristics (e.g., decay rates and buoyancy) of the chemical in question. The models presented here consider idealised release scenarios and demonstrate that in principle, modelling can be used to improve design of a deployment program. Model outputs present information regarding the variability between different reefs, time periods or release scenarios, and hence how local concentrations are likely to vary. Ultimately, the effective dispersal distance of the semiochemical is dependent on its properties (stability, potency).

Investigation of COTS movement rates has found the rate of movement in areas of high coral cover is low, i.e., approximately 2.8 m d^{-1}, meaning individual COTS may remain in a restricted area for a period of days (Keesing & Lucas 1992, Pratchett et al. 2017b). However, this rate increases significantly to 10.3 m d^{-1} as coral cover declines. The RECOM model developed here has a resolution of 300-400 m. Future scenario modelling should aim to reduce the resolution to < 50 m using either multiple nested models or a flexible mesh model (e.g., COMPAS (Herzfeld 2021) and DELFT3D (Roelvink & Van Banning 1995)).

The models presented here have improved understanding of the logistical complexities associated with developing a deployable semiochemical technology for controlling COTS on coral reefs. To our knowledge, this is the first time that local-scale hydrodynamic modelling has been employed to guide the design of a semiochemical point-source deployment strategy. As such, it provides the foundation for refinement through the incorporation of chemical descriptors (i.e., of a confirmed semiochemical) and the exploration of other modes of deployment such as broadscale dispersal (e.g., to induce early or asynchronous gamete release of a larger aggregation).

Validation with empirical data is highly recommended for any numerical model. A validation was not possible here due the project being in the theoretical stage. In situ test deployments as well as adequate reef monitoring data describing natural semiochemical dispersal (with emphasis on accurate detection and tracing of their intrinsically low concentrations), are highly recommended.

8. AUGMENTATION OF CURRENT CONTROL METHODS

8.1 Supplemental augmentation

The potential to develop different types of semiochemical control agents that can be implemented in COTS control strategies have been presented and discussed in the sections above. This section will specifically address the question of if and how these strategies can

contribute to the existing COTS control program and consider what would be required to evaluate the possible contribution and success of such strategies in the field.

To evaluate the possible implementation of semiochemical-based control strategies for COTS, much can be learned from the large-scale control program developed for sea lamprey in the Great Lakes of North America (Lewandoski et al. 2021, Siefkes et al. 2021). The scientific success of research into semiochemical-based sea lamprey control has produced an extensive number of scientific publications and numerous semiochemical candidates (refer to citations in Fissette et al. (2021)), of which 3kPSZ has been approved for use in control programs (Fredricks et al. 2021). Despite this progress, in-water implementation into the overall control program is still lacking (Barber & Steeves 2020). In a recent strategic review addressing this disconnect, the Great Lakes Fishery Commission (GLFC) highlighted the difference between alternative and supplemental control methods and their respective evaluation criteria (Siefkes et al. 2021). Since alternative control methods are expected to displace currently used methods (barriers and lamprey-specific pesticides in the case of sea lamprey), their efficacies would have to be equal to or better than current methods at a similar cost to be deemed successful. In contrast, supplemental methods are expected to be integrated with traditional methods, to enhance their efficacy, especially in locations where the traditional methods are less effective due to environmental or societal conditions. It is now recognised that the explicit focus of strategic vision on alternative sea lamprey control methods have hampered the implementation and extent of field testing of technologies that hold promise for integration into combined pest management strategies. Since 2019, the focus has shifted to the development of supplemental methods, such as those targeting migration and reproduction (Siefkes et al. 2021). Additionally, significant effort has been made to better understand and exploit social behaviours of adult carp in lakes through use of semiochemicals to augment control through physical removal (Bajer et al. 2019).

The existing on-water IPM COTS CP has demonstrated success, with COTS irruptions controlled consistently across visited priority reefs and over time to densities below the 3 COTS ha^{-1} reproductive threshold (Westcott et al. 2021). The primary objective of the program remains the implementation of control methods to prevent future outbreaks by permanently suppressing adult COTS densities at well below the ecological and reproductive thresholds. As for sea lamprey (Siefkes et al. 2021), semiochemical-based technologies present a new avenue of control for COTS and has the potential to be easily integrated into the program as a supplemental method. Studies in other systems have shown that for many semiochemical-based strategies, efficacy is higher when applied to low density populations (Smart et al. 2014, Ezzat et al. 2020). This suggests that any enhancement in control efficacy mediated by semiochemical-based strategies is more likely to be realised when applied to monitoring of low-density COTS population, or to control low- to mid-density COTS populations. Despite the overall success of the existing control program, some reefs have proven recalcitrant for manual control and required repeated visits to draw COTS populations down to the ecological threshold. Presented here is a case study that illustrates the challenges associated with control of COTS on John Brewer Reef and the potential for application of supplemental semiochemical technologies.

8.1.1 Case study 4: Enhancing on-water control efforts on John Brewer reef through application of a semiochemical control technology

A case study developing a year-round pull control strategy

Aim: To design a reef-specific pull control strategy to lure COTS to a point-source to reduce dive times required to search for and manually inject individuals, based on *a priori* knowledge.

John Brewer reef has proven recalcitrant for manual control. Repeated visits to draw COTS populations below the 3 COTS ha^{-1} ecological threshold have been futile with COTS numbers frustratingly re-establishing between visits (Fletcher et al. 2020).

For example, in 2018 the outbreak status at John Brewer was classified as 'severe' even after culling, with in-water surveys exceeding 1 COTS tow^{-1} (Figure 10). Culling and survey sites were focussed on the outer perimeter of the reef (Fletcher et al. 2020). Since then, extensive culling focussing on the bommie fields within the reef lagoon has removed 64,243 COTS and the status of the reef downgraded to 'no outbreak', with ≤ 0.1 COTS tow^{-1}. Models of semiochemical release, both inside and outside of the lagoon, suggests John Brewer Reef is amenable to short-term semiochemical control, particularly under scenario 2 – release timed near the low tide, and such technology could be easily integrated into the existing in-water visits/revisits. Therefore, it can be envisaged that semiochemicals could be used on revisitation of difficult reefs such as John Brewer reef once the population has been lowered below a set threshold, possibly prolonging the period required before the next revisitation.

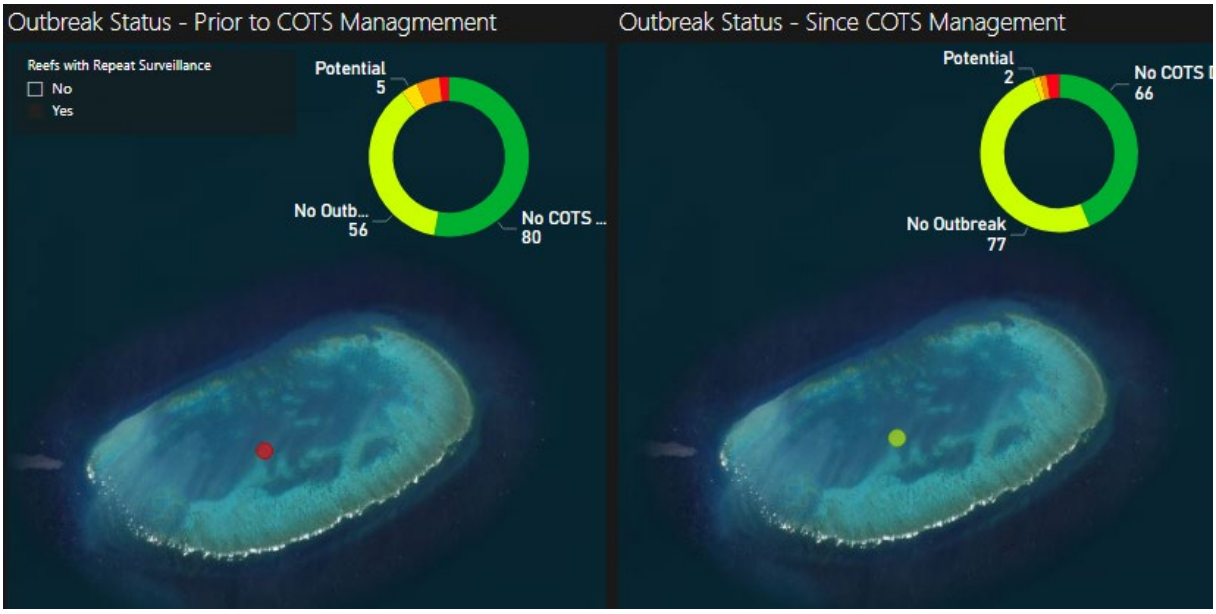

Figure 10 The status of COTS management on John Brewer (COTS IPM Dashboard 2022)

Overall control strategy: Pull, conspecific non-sex attractant, short-term uncontrolled release

Key properties:

- Target life history stage: adult
- Life history trait: chemosensation for feeding aggregation
- Mode of action: conspecific attractant
- Behavioural outcome: passive aggregation
- COTS control strategy: lure COTS to a point-source (either trap or off reef)
    - long-term suppression of a population has been lowered below a set threshold through culling
    - assessing population sizes and distribution through improved monitoring methods
- Delivery method:
    - Timing: 1-day prior to dive and cull
    - Duration: uncontrolled continuous short-term release
    - Scale: localised at dive site
    - Mode: left in situ for later retrieval of chemoemission device (deploy and retrieve)
- Specificity: species-specific
- Formulation: eroding slow-release gel
- Device: simple single-use device to provide continuous uncontrolled plume via slow dissolution from substrate and have no longevity in the environment beyond the 1-day deployment timeframe.

8.2 Challenges of delivery in the coral reef environment

Fundamental challenges associated with delivery of a semiochemical technology on a coral reef include determination of the compound concentration range required to elicit a response in a reef environment (as opposed to in the laboratory), and the volume of activated water and treatment time required to produce an ecologically relevant behavioural response.

Depending on the strategy chosen, the openness of the treated reef area and connectivity between reefs may influence the overall efficacy enhancement that can be expected for the control program. The openness of the reef system is a challenge shared with the existing control program, however, it is particularly relevant for strategies that target fecundity, as the degree of larval seeding from nearby untreated reefs will have to be considered. Other challenges that are shared with the existing control program, and which are still poorly understood, include the possible disconnect between adult numbers and the total reproductive output, and the possibility that removal of corallivorous individuals could release herbivorous juveniles from competition and induce diet transition and rapid growth of remaining individuals (Deaker et al. 2020).

The amount of semiochemical required will depend on its natural degradation rate (half-life) and the degree of dilution by currents. The degradation rate will be impacted by endogenous enzymatic activity (i.e., odour-degrading enzymes associated with signal termination), exogeneous heterotrophic bacteria and/or other abiotic environmental influences, as discussed in section 5, while the dilution rate will vary between deployment sites, and be

susceptible to seasonal and tidal changes in currents, as discussed in section 7. Formulation and delivery platforms and devices are critical to overcoming these issues (Shah & Singh 2018), and experience from other aquatic systems indicates formulation to stabilise the semiochemical is feasible for the marine environment.

8.3 Monitoring performance

As for any control program, the assessment of its success can be challenging and resource intensive. Scientifically, the ideal way to evaluate efficacy is if the technology is used on its own, however, it may be more realistic to compare a combined with the traditional approach only (Siefkes et al. 2021). For COTS, the existing manual control program provides an extensive dataset on COTS numbers over time for a wide range of reefs, including controlled and non-controlled reefs (AIMS LTMP 2022, COTS IPM Dashboard 2022). Hence monitoring data from reefs where semiochemical-based control is used as a supplement to manual control will not exist in isolation, although challenges to robust analysis and data interpretation are presented by natural temporal and spatial variability in COTS abundances. Careful design of an experimental treatment regime has to be combined with careful selection of treatment (release) sites, with possible use of paired sites or paired reefs to allow for comparison between control strategies that include supplementary control technologies and those that exclusively rely on manual control.

Semiochemical technologies that employ attractants to draw COTS from the reef matrix and/or into traps also hold potential for use in monitoring of COTS populations on reefs with low COTS densities. One example would be the use of semiochemical-based technologies to monitor COTS within the initiation box (between Green Island to just north of Lizard Island (Wooldridge & Brodie 2015)) between outbreak cycles in order to inform strategic management decisions and resource prioritisation. This is similar to the way semiochemicals are being used to monitor gypsy moth (*Lymantria dispar*) populations in the USA (STS 2022). Semiochemical-based control strategies can enhance monitoring sensitivity, potentially with a high level of accuracy, but whether this is beyond what is expected to be realised through further development of emerging eDNA- and eRNA-based techniques remains to be shown (Babcock et al. 2020). Another potential use is during early suppression control in the initiation box as the efficacy of the manual control program could be enhanced by pulling COTS from depths that cannot be accessed by divers and by pulling cryptic COTS from within the complex reef matrix.

Alternatively, semiochemical compounds produced by COTS could themselves serve as biomarkers to monitor populations, as has been considered for the New Zealand Southern pouched lamprey (Stewart & Baker 2012). In isolation, or as a suite of compounds released into the environment in specific ratios, semiochemicals could be used to predict the inception (sex pheromone), occurrence (aggregant) and collapse (necromone) of COTS irruptions.

8.4 Semiochemicals as vehicles for adoption of vector augmentation control strategies

The potential to develop combination control agents whereby the formulation delivers both a semiochemical and toxin/toxicant have found application in terrestrial (Mafra-Neto et al. 2014) and aquatic (Schorkopf et al. 2016, Kenawy et al. 2020) control programs. Such an approach relies on species-specificity, preferably for both semiochemical and toxin/toxicant, to minimise impacts on non-target species.

The inclusion of microbial, viral or genetic vectors in integrated pest management programs present serious regulatory, environmental and societal concerns, and for the marine environment currently present an unacceptable risk, as clearly articulated by Høj et al. (2020). Should these concerns be adequately and safely addressed, as they have been for some terrestrial species, semiochemical attractant technologies could act as the vehicle for their transfer to the target COTS life history stage.

Currently there is no appetite to explore these vector augmentation approaches for the control of COTS, yet their potential application should not be completely disregarded. Significant advances are being made in aligned research areas and future prospects may profit from knowledge gained by these endeavours.

9. SUMMARY AND FUTURE ENDEAVOURS

COTS are endemic to the Indo-Pacific region and play an important role in maintaining healthy reefs. To date, management of the too-abundant and widespread distribution of COTS in the GBR has relied on manual intervention to reduce their numbers and abate impacts of predation on hard coral prey. Semiochemical control technologies offer an eco-safe in-water approach to supplement and potentially enhance efficiency of manual control methods within the IPM COTS CP.

The first principle of any integrated pest management program is prevention and suppression of the pest species, prevention being the adoption of measures to reduce the chance of occurrence of the pest, and suppression being the reduction of the impact of the pest species on the natural environment. The review of the semiochemical literature, focussing on delivery in the aquatic environment, and drawing on the expertise of the review panel and the development of hydrodynamic models describing semiochemical dispersal in-water, has established there is great potential for inclusion of a conspecific COTS pheromone attractant to introduce a pull control strategy in the IPM COTS CP.

Key aspects of semiochemical control agents and existing delivery platforms and devices have been considered in the context of their application in a reef habitat for the control of COTS. First and foremost, the review found there are a multitude of COTS life history stages that are amenable to semiochemical control (Section 2), with the adult life stage the preferential target. Conspecific adult aggregation cues were deemed most suited to the needs of the IPM COTS CP and application as a supplemental control strategy to augment

current methods (Section 8). Seasonal deployment is considered the most appealing to curb reproductive success, especially for spawning populations in the initiation box.

The COTS secretome (i.e., waterborne molecules secreted by live COTS) has been confirmed to contain conspecific aggregants (Hall et al. 2017, Yap et al. 2022). The fact that live COTS naturally secrete attractant metabolites, peptides and proteins into seawater in a controlled way suggests that the concentration required to evoke a recognition response by nearby COTS is practically achievable in a natural physiological setting. Hence, application of exogenous semiochemical agents holds great potential to be exploited for COTS control. In addition, concentrates of the COTS secretome represents a sustainable and readily available supply of attractant molecules for unadulterated application as an extract or semi-refined fraction (Case study 2: Proteins as COTS pheromone attractants), or for isolation and identification of behaviour-modifying metabolite, peptide and protein constituents (Section 3). The characterisation and assessment of this chemistry is the focus of CCIP-R-11. Specifically, COTS-secreted peptides and proteins, such as the ependymins which have diversified uniquely within COTS, hold the greatest potential for a COTS-specific attractant (Section 5.2.2). Formulation and molecular engineering of these types of biomolecules will be informed by drug design research (Section 5). It should be noted, however, that alarm or repellent cues, indicating imminent danger, have the potential to elicit a greater behavioural response over much shorter timeframes, as has been observed in sea lamprey (Hume et al. 2020). Given the escape response displayed by COTS exposed to the odour of the predatory giant triton (Bose et al. 2017a), additional efforts should be directed to isolate the active constituents and assess their complementary use, i.e., as part of a push-pull strategy whereby timed release of predator-derived repellent guides COTS into traps containing attractants.

Implementation of push, pull and push–pull semiochemical control strategies were contemplated within the context of the COTS control program (Section 3). The pull-only strategy is preferred given it relies on ‘active’ motivation to attract COTS to a point-source for removal or disposal and is therefore likely to be simpler to implement using the existing infrastructure and resources available within the current program. However, push-pull strategies were also considered, similar to that investigated for the control of sea lamprey in streams (Hume et al. 2020). Traps positioned laterally across the width of the stream, with traps on one side containing a conspecific repellent and the other a conspecific attractant, were successful in redistributing sea lamprey as they locate favourable areas. The issue is intrinsically more complicated in a tidal reef environment with no hard land boundaries. Hydrodynamic models were developed to describe the theoretical spatial and temporal footprint of semiochemical delivery around three coral reefs in the GBR and have revealed variable plume distribution and range, both of which are highly dependent on time and site of release (Section 7). This variability limits the scale at which a semiochemical technology could be effectively deployed. Exploration of three release scenarios at six sites on three reefs found dissipation occurred rapidly with an exponential drop in concentration and total within the first km from the point-source. Models revealed that a pulsed release of a semiochemical over a one-hour period, leading to each low tide (i.e., scenario 2) was the preferred option if high concentrations and strong spatial gradients at the point of release are required, as would be the case if implemented as a supplemental strategy to the existing in-water culling program.

Models have enabled a comprehensive comparison of semiochemical release under different scenarios (Section 7). Incorporating this information into a wider literature review on semiochemical control strategies and delivery systems targeting aquatic systems has shown there is great potential for the application of supplemental semiochemical control agents to augment the existing in-water manual control strategy to manage COTS populations (Section 8). Regardless, before any deployment of a semiochemical on a reef habitat the model will need to undergo refinement, incorporating additional semiochemical-specific physicochemical information as it becomes available and future iterations should aim to simulate possible chemical reactions or biological interactions that the semiochemical may be subject to, as has been done to track the fate of pollutants in aquatic systems. Further, models need to investigate how the amount released influences the range of a semiochemical above a set threshold concentration, i.e., the effective concentration, to guide the choice of delivery platform and device. This first-generation model provides the foundation on which to extend simulation capability to assess the impact of semiochemical release on COTS individuals and populations.

Another factor for consideration is the rate of detection of the semiochemical by COTS. Female sea lamprey sample the odour plume at a relatively slow time scale of seconds, which effectively limits sampling reliability (Johnson et al. 2009). This necessitates the need for the cue to remain available in the immediate vicinity for a greater length of time to intentionally elicit a behavioural response. For example, a longer residency time in stagnant conditions may suit a pulsed release scenario versus a lower residency time in turbulent conditions which may require continuous emission to elicit the same behavioural change. Establishing the rate of detection of conspecific cues, in the first instance, by COTS requires further investigation to establish effective semiochemical concentration thresholds and the requisite life-time. This will also require further hydrodynamic modelling to guide placement of the semiochemical release device for optimal performance.

Existing technologies and platforms, many of which are based on foundational control methods and some of which already have application in the marine environment for other purposes, have immediate potential to be modified-for-purpose to tackle the issue of semiochemical delivery at the spatial scale and temporal precision required (Section 6). Yet what happens in-water under operational conditions is often a compromise between the theoretical and the practically achievable. With this caveat, many existing platforms and release devices have the potential to be developed into innovative in-water COTS control delivery technologies, yet SCUBA (i.e., manual installation of a delivery device) is the most technology ready.

In theory, the advantages of using semiochemicals to control COTS populations are many, and based on findings from this review and the subsequent modelling study, it is recommended that a semiochemical COTS control agent fulfil the below conditions to be considered for further investigation and inclusion in the IPM COTS CP:

- be a conspecific COTS pheromone attractant;
- be COTS specific and target the adult life history stage;
- have minimal impact on non-target species;

- be effective in all types of flow/current conditions, noting changes in flow will influence the pheromone gradient plume and hence its distribution and effectiveness;
- be released to coincide with the low tide to ensure high enough concentrations and adequate retention of the pheromone;
- be amenable to industrial preparation (minimal preparation of an extract or fractions thereof may suit distribution of large quantities, whereas complex synthesis, while expensive, may suit distribution of smaller highly potent quantities);
- readily available supply as needed, i.e., sourced from captive COTS;
- able to be used in combination with other control techniques, such as traps and cages with manual removal or culling, to enhance control efficacy;
- be deployed in the initiation box when COTS are present at low- to mid-density or during spawning to reduce reproductive success; and
- be eco-safe – an inherent property.

Immediate recommendation: development of a COTS-derived pheromone attractant as a formulated composite (i.e., raw concentrate or as a partially purified proteinaceous fraction, formulated with calcium carbonate or an algal-derived gel) for testing in aquaria (i.e., SeaSIM) and in-water (i.e., ReefWorks test range) to assess composite longevity and platforms suited to reef deployment. For a semiochemical technology that performs well and meets the essential criteria mapped out in this review, a deployment architecture design should be developed, tailored for deployment on reefs (via SCUBA) within the initiation box (assumed to seed down-current reefs) by pulsed release from a point-source (i.e., scenario 2) and on recalcitrant reefs like John Brewer Reef which have required numerous repeated (up to 39) visits to control COTS.

Potential for future refinement: Exploration of semiochemical technology for COTS control is very much in the early research phase. This review has identified critical aspects that require further dedicated research and development to realise the potential of this technology within the IPM COTS CP. Identification and isolation of the chemical constituents that act as conspecific aggregants is essential and will enable (i) dose-response concentration thresholds to be determined and (ii) the design of formulations and delivery devices tailored specifically for the reef environment. Existing on-water infrastructure and delivery platforms offer an immediate opportunity to test the deployment of inert proxy formulations under the various deployment scenarios discussed above. Extension of the first-generation hydrodynamic model is critical to (i) establish gradient profiles across priority reefs, including John Brewer Reef, (ii) better understand the impacts of reef and site-specific hydrodynamics and ocean tidal currents on semiochemical dispersal rates and (iii) predict in-water parameters such as optimal dosing concentrations.

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APPENDIX A – KNOWLEDGE GAP ANALYSIS

A virtual workshop held on the 7th July 2021 brought together the project proponents. Using the project title 'Deployment of semiochemical control agents to manage Crown-of-Thorns starfish populations' as inspiration, each person was asked to propose a series of research questions within their area of expertise, following a similar process used by Pratchett et al. (2021) (Table A1). The questions were multifaceted reflecting the broad range of the topic and the multidisciplinary nature of the group. To provide some context, the research questions were organized into eight major themes and then further sorted into distinct categories. Questions were firstly presented in generic terms, relevant to semiochemical use in aquatic ecosystems, and then specifically to address the potential for use in the marine environment to control COTS. For this workshop, the level of maturity of the current status and knowledge of semiochemical use in the marine environment was considered to be low, and as such questions were not ranked, rather they were used to guide the direction of the literature review and establish the outline of the report.

The literature review was conducted using an integrative method by first collating relevant published works on the application of semiochemicals in aquatic ecosystems (based on key search words from the list of questions generated in the workshop) to provide an overview of the knowledge base. A critical review was then conducted, and perspectives and insights combined and used as a foundation to generate concepts for the reef environment and COTS specifically.

Table A1: Research Questions

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.	**COTS specific questions** - what is the knowledge gap? Why is it important?
	Theme 1: Properties and mode of action	
Properties of semiochemical attractants	What properties make a good semiochemical attractant?	What properties would make a good semiochemical COTS attractant?
	What semiochemical properties are amenable to deployment in the aquatic environment? What properties that may impact delivery (i.e., stability, half-life, degradation pathways, dispersion rate, efficacy).	What semiochemical properties are amenable to deployment in the reef environment?
	What type of attractant (i.e., conspecific sex pheromone, foraging kairomone, etc) is best suited for deployment in the aquatic environment?	What type of attractant (i.e., conspecific sex pheromone, foraging kairomone, etc) is best suited for deployment in the reef environment?
	What aggregation pheromone attractants have been deployed in aquatic systems? Are they seasonal, or sex specific (i.e., during spawning time) or are they kairomones?	What properties should a COTS conspecific aggregation pheromone attractant have? What type of attractant should be deployed, i.e., sex specific (i.e., during spawning time and therefore seasonal) or foraging kairomones?

	What life stage is best targeted by attractants, adults, juveniles or larvae?	What COTS life stage should be targeted, adults, juveniles or larvae?
Specificity and selectivity of semiochemical attractants	Which mode(s) of action is amenable to deployment in the aquatic environment? Should the attractant (pheromone or kairomone) have broad spectrum or selective bioactivity?	Which mode(s) of action is amenable to deployment in the reef environment? Should the COTS attractant (pheromone or kairomone) have broad spectrum or selective bioactivity?
	What level of specificity for the target species is required?	What level of specificity for COTS is required?
Efficacy of semiochemical attractants	What level of efficacy of pheromone attractants is required in the marine environment?	What level of efficacy of pheromone attractants is required in the marine environment?
	What biological responses do effective attractants elicit? e.g., movement to point source, increase in activity?	What biological response should the attractant elicit in COTS so as to be suited to IPM? e.g., movement to a point source away from the reef, physiological increase in activity inducing out of season or sync spawning
	Does efficacy change between seasons i.e. as food availability changes, during spawning?	Does efficacy change between seasons i.e. As coral cover changes, during COTS/coral spawning?
Range of semiochemical attractants	What range(s) do semiochemical attractants cover and still be effective in the aquatic environment?	What is the range required for a COTS attractant (pheromone or kairomone), i.e., effective over the transect, reef, or full geographical range of COTS?
Properties of semiochemical repellents	What properties make a good semiochemical repellent/deterrent? What properties that may impact delivery (i.e., stability, half-life, degradation pathways, dispersion rate, efficacy).	What properties make a good semiochemical COTS repellent/deterrent?
	What properties are suited to deployment in the aquatic environment?	What properties are suited to deployment in the reef environment?
	What deterrents (i.e., conspecific alarm pheromone, conspecific injury pheromone, or predator kairomone, or other) have been deployed in aquatic systems? Are they seasonal?	What type of COTS deterrent (i.e., conspecific alarm pheromone, conspecific injury pheromone, or predator kairomone, or other) is best suited for deployment in the reef environment?
	Is it more effective to target adults, juveniles or larvae?	What COTS life stage should be targeted, adults, juveniles or larvae?
Specificity and selectivity of semiochemical repellents	Which mode(s) of action is amenable to deployment in the aquatic environment? Should the deterrent have broad spectrum or selective bioactivity?	Which mode(s) of action is amenable to deployment in the reef environment? Should the COTS deterrent have broad spectrum or selective bioactivity?
	What biological responses do effective repellents/deterrents elicit? e.g., aversive movement, physiological suppression including growth or reproductive maturation, spawning	What biological response should the repellent/deterrent elicit in COTS so as to be suited to IPM? e.g., aversive movement, physiological suppression including growth or reproductive maturation, spawning
	What level of specificity for the target species is required?	What level of specificity for COTS is required?
Efficacy of semiochemical repellents	What level of efficacy of a deterrent (pheromone or kairomone) is required in the marine environment?	What level of efficacy of COTS deterrents (pheromone or kairomone) is required in the marine environment?
	Does efficacy change between seasons i.e. As predator behaviours change, during spawning?	Does efficacy change between seasons i.e. As predator behaviours change, during COTS spawning?
Range of semiochemical repellents	What range(s) do semiochemical deterrents cover and still be effective in the aquatic environment?	What is the range required for a COTS deterrent, i.e., effective over the transect, reef, or full geographical range of COTS?
	Theme 2: Semiochemical formulation and bioengineering	
Analogues - chemical mimetics	Have semiochemicals for deployment in aquatic scenarios been modified (i.e., analogues (bio)synthesised having more potent or prolonged bioactivity? Or with greater species-specificity (if not already)?) To increase efficacy?	What semiochemical properties should be targeted for modification to improve likelihood of efficacy against COTS in the reef environment?
Mode of action	Has the deployment of semiochemicals (either solo or in combination) having different modes of action been effective in aquatic environments to target multiple life-stages and behaviours?	Is there a need to deploy COTS specific semiochemicals (either solo or in combination) having different release modes to target multiple life-stages and behaviours at the same time?

	Have pest species been triggered by semiochemicals to produce altered (i.e., higher or lower) levels of conspecific cues?	What is the potential for COTS to be chemically triggered to produce altered (i.e., higher or lower) levels of conspecific cues?
Formulation strategy	What formulation strategies have been used to deliver semi chemicals in the aquatic environment?	Are there any limitations on the type of formulation suited for deployment of a COTS semiochemical in a reef environment?
	Has a species-specific pheromone (i.e., Will not impact on other closely related species) been modified to have both attractant and toxic properties, and applied in aquatic systems?	Is there potential for a COTS-specific pheromone (i.e., no impact on other asteroids or echinoderms) to be modified to have both attractant and toxic properties, and applied *in situ*? i.e., replace need for divers and single injections. Note: must be highly COTS-specific - or have a level of acceptable collateral damage to other species. Amenable to modification or synthesis.
	Has a structural analogue (mimic) of a confirmed pheromone been used to block the semiochemical receptor and alter pest behaviour? Need to ensure specificity of the mimic.	Is there potential, based on current knowledge of COTS chemoreceptors, a structural analogue (mimic) of a COTS pheromone be used to block the semiochemical receptor and alter COTS behaviour? Need to ensure specificity of the mimic.
	Post translation modification of protein semiochemicals is likely to be important and could complicate production – i.e., amidated. In this case it is crucial to identify the receptors.	
Combination	Have semiochemicals been applied in combination to enhance effectiveness of activity in aquatic systems?	What semiochemicals should be considered in combination to enhance effectiveness of the COTS IPM i.e. A foraging kairomone + conspecific aggregation pheromone?
Genetic modification	Have engineered microbes/animals been used to produce and release semiochemicals at the desired rate over the desired time period? In the aquatic environment?	Could engineered microbes/COTS be used to produce and release semiochemicals at the desired rate over the desired time period? In the reef environment?
	Theme 3: Mechanism of release (engineering)	
Semiochemical application - release mode	What semiochemicals have been applied as a slow-release biocontrol 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)? In the aquatic environment	What type of semiochemical delivery would be most suited to COTS in a reef environment, a slow-release biocontrol agent (i.e., year round), a fast-release single dose (i.e., during spawning), or pulsed application (intermittent to reduce impacts on other species)?
	Has a semiochemical been deployed using multiple release modes to target multiple life-stages and behaviours of pest species? In the aquatic environment?	Would the deployment of a semiochemical using different release modes be suited to COTS given the difference in their three primary life-stages, larvae, CCA eating juveniles and coral eating adults?
	What semiochemical application methods have been used to improve efficacy against the pest species? In the aquatic environment?	Are there any limitations on the mode of application given the nature of the reef environment?
What is available? What is suited to the marine	What are the key engineering considerations for the deployment of semiochemicals in the aquatic environment (i.e., duration, suitable deployment locations, ease of deployment, on-field longevity, delivery via genetically-modified organisms, remote control?	Are there any engineering considerations that would be specific to COTS (i.e., release at night when most active)
	What (if any) semiochemical release devices have been associated with baits/traps in the aquatic environment?	Is there potential to apply semiochemical release devices to baits/traps specific for COTS?
Duration of deployment	What short-term release systems have been deployed in the aquatic environment?	
Delivery device type	What long-term release systems have been deployed in the aquatic environment?	
	What modes of semiochemical delivery have been successful in the aquatic environment (i.e., pulse, continuous, single-shot)	What mode of delivery would be best suited to COTS and under what conditions?
Target life-stage	Which life-stage is most amenable to engineering solutions?	Which COTS life-stage is most amenable to engineering solutions?

	Theme 4: Scale and timing of application	
Hydrodynamic and dispersal modelling	Has the dispersal of (semio)chemicals been modelled in aquatic systems?	What models could be used to understand the dispersal of semiochemicals in the reef environment?
	What scale has the release of (semio)chemicals been applied and how effective has this been (if at all) i.e. broad-scale, localised, or individual?	What scale would be feasible for deployment of a (formulated) semiochemical in the reef environment i.e. A broad scale, or local or individual?
Timing of release	Have semiochemicals been applied long-term and sustained population suppression? In the reef environment?	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/season to deploy semiochemical control agents to ensure optimal results? i.e., attractants could be deployed as baits/lures to complement other control efforts	What would be the optimal time/season to deploy a COTS semiochemical? i.e., attractants could be deployed as baits/lures during an outbreak to complement current culling efforts; deterrents could be deployed to i) discourage aggregation formation especially at key times such as COTS spawning, ii) to disrupt/disperse aggregations at the outbreak initiation phase or ii) during conditions considered stressful to corals i.e. Coral bleaching.
	Will the release times impact or change behaviour?	
	Theme 5: Augmentation of current control methods	
Integration into **IPM**	Have semiochemicals been used to enhance culling success of pest species?	On those reefs where culling is deemed necessary, could semiochemicals be used to enhance culling success? i.e., 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 **or** 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?
	Has a pheromone been used to deliver a lethal agent (either chemical toxin/toxicant or biological agent)? i.e. A lure and kill technology, in the aquatic environment?	Is there potential to use a COTS pheromone be to deliver a lethal agent (either chemical toxin/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.
		What elements are important for implementation in the IPM COTS Control Program and how can semiochemicals complement these?
	Theme 6: Release site prioritisation	
Presence/absence	Has monitoring of (semio)chemicals been done to establish areas that need biocontrol intervention? I.e. A pest-specific chemical biomarker?	Is there an application for COTS (semio)chemicals to identify which reefs should be prioritised for culling? i.e., COTS-specific chemical biomarker used to induce aggregations; possible candidates include: saponins, given the specificity of some; secreted proteins - these compounds are continually/regularly secreted (i.e. not under stress) - presence vs absence.
Monitoring	Has the presence or increase in concentrations of specific semiochemical been used to monitor pest numbers over the longer term? i.e., automated, unmanned, remote sensing of key semiochemicals as an early warning system for pest outbreaks?	Is there an application for COTS (semio)chemicals to monitor COTS numbers in the longer term? i.e., automated, unmanned, remote sensing of key COTS semiochemicals as an early warning system for future outbreaks?

	Theme 7: Interferences	
Climate change	Will environmental change (related to climate change) impact semiochemical efficacy in the aquatic environment rendering them less effective as biocontrol agents? Especially important to consider if activity of semiochemical is seasonal	What is the potential for environmental change (related to climate change) to impact semiochemical efficacy rendering them less effective as COTS control agents? Especially important to consider if activity of semiochemical is seasonal
Declining water quality	Does pollution/sediment/nutrient loading affect efficacy of semiochemicals in the aquatic environment? Particularly relevant to larval phase	What is the potential for pollution/sediment/nutrient loading to affect efficacy of semiochemicals on COTS?
	Theme 8: Target life-stage	
Gametes		Could semiochemicals be effective in disrupting/inhibiting/inducing COTS egg/sperm maturation? There is evidence of chemical activation (1-methyladenine).
		Could semiochemicals be effective in disrupting/inhibiting fertilization? There is evidence of sperm/egg attraction to chemical cues but these have not be 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 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 adults?
Juveniles		Could semiochemicals be effective in changing behaviours of CCA-feeding COTS juveniles? 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?
Spawning		What is the chemical nature of COTS exogenous spawning trigger? Synchronous spawning maximises fertilization rates.
		Could semiochemicals be effective in disrupting synchronous COTS spawning or inducing out-of-season spawning?
		Is the COTS spawning semiochemical species-specific and over what distance?
		Is the COTS spawning semiochemical sex-specific?

APPENDIX B – DEFINITION OF TECHNOLOGY READINESS LEVELS

https://esto.nasa.gov/files/trl_definitions.pdf

TRL 1 Basic principles observed and reported: Transition from scientific research to applied research. Essential characteristics and behaviours of systems and architectures. Descriptive tools are mathematical formulations or algorithms.

TRL 2 Technology concept and/or application formulated: Applied research. Theory and scientific principles are focused on specific application area to define the concept. Characteristics of the application are described. Analytical tools are developed for simulation or analysis of the application.

TRL 3 Analytical and experimental critical function and/or characteristic proof-of-concept: Proof of concept validation. Active Research and Development (R&D) is initiated with analytical and laboratory studies. Demonstration of technical feasibility using breadboard or brassboard implementations that are exercised with representative data.

TRL 4 Component/subsystem validation in laboratory environment: Standalone prototyping implementation and test. Integration of technology elements. Experiments with full-scale problems or data sets.

TRL 5 System/subsystem/component validation in relevant environment: Thorough testing of prototyping in representative environment. Basic technology elements integrated with reasonably realistic supporting elements. Prototyping implementations conform to target environment and interfaces.

TRL 6 System/subsystem model or prototyping demonstration in a relevant end-to-end environment (ground or space): Prototyping implementations on full-scale realistic problems. Partially integrated with existing systems. Limited documentation available. Engineering feasibility fully demonstrated in actual system application.

TRL 7 System prototyping demonstration in an operational environment (ground or space): System prototyping demonstration in operational environment. System is at or near scale of the operational system, with most functions available for demonstration and test. Well integrated with collateral and ancillary systems. Limited documentation available.

TRL 8 Actual system completed and "mission qualified" through test and demonstration in an operational environment (ground or space): End of system development. Fully integrated with operational hardware and software systems. Most user documentation, training documentation, and maintenance documentation completed. All functionality tested in simulated and operational scenarios. Verification and Validation (V&V) completed.

TRL 9 Actual system "mission proven" through successful mission operations (ground or space): Fully integrated with operational hardware/software systems. Actual system has been thoroughly demonstrated and tested in its operational environment. All documentation completed. Successful operational experience. Sustaining engineering support in place.

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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