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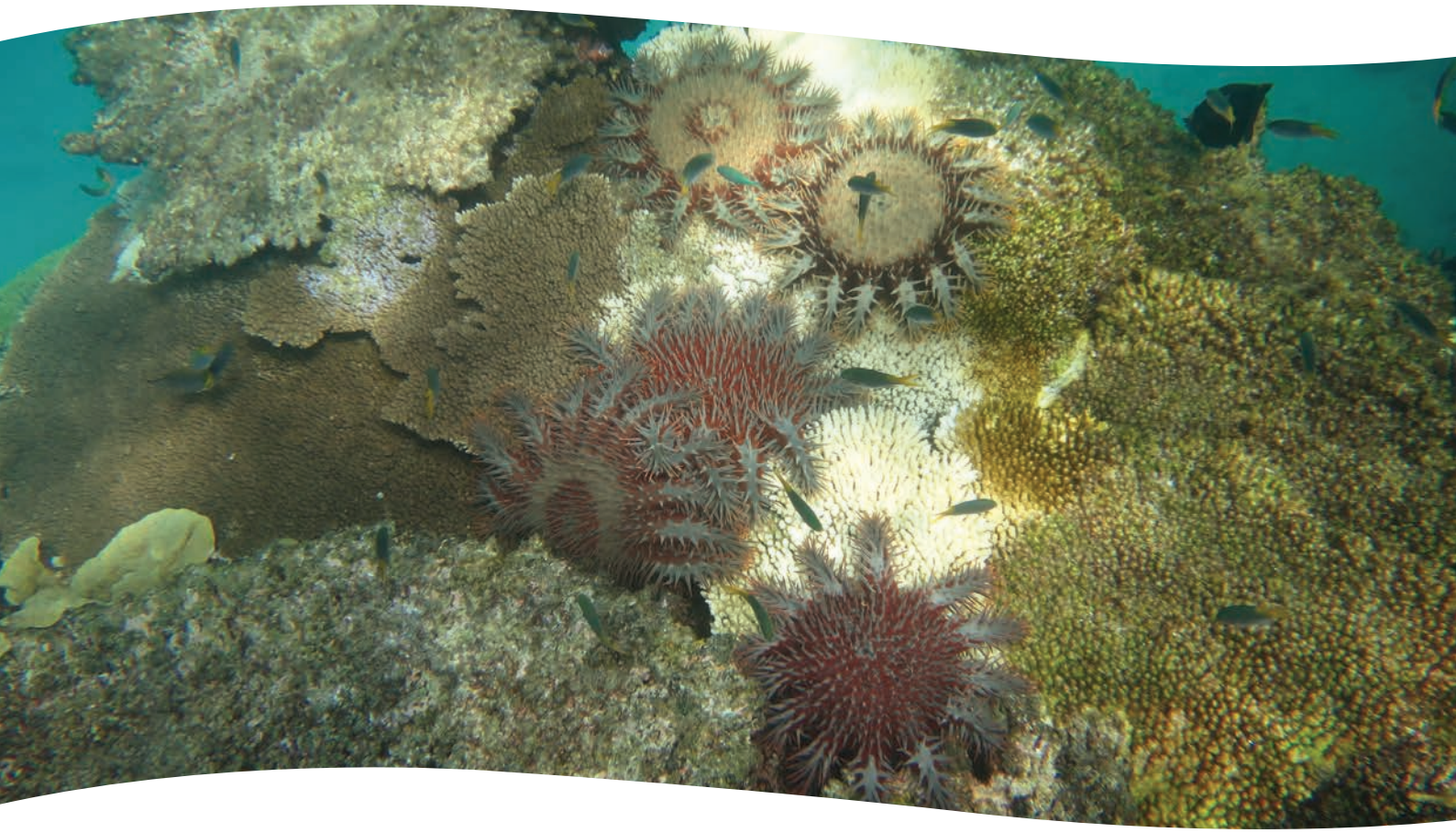
Technical Report

A Review of Biologically Based Control Technologies for Crown-of-Thorns Starfish

Options for Enhancing the Integrated Pest Management Approach

Lone Høj, Maria Byrne, Frederieke Kroon and David Westcott



Australian Government

Australian Institute of Marine Science

A Review of Biologically Based Control Technologies for Crown-of-Thorns Starfish

Options for Enhancing the Integrated Pest Management Approach

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Cover photographs: David Westcott

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ACRONYMS

AAS ..... Australian Academy of Sciences
CCA ..... Crustose Coralline Algae
CoTS..... Crown-of-Thorns Starfish
COTSAC..... Crown of Thorns Starfish Advisory Committee
COTSREC..... Crown of Thorns Starfish Research Committee
CRISPR ..... Clustered Regularly Interspaced Short Palindromic Repeats
CSIRO..... Commonwealth Scientific and Industrial Research Organisation
GBR..... Great Barrier Reef
GBRMPA ..... Great Barrier Reef Marine Park Authority
GMO ..... Gene Modified Organism
IPM..... Integrated Pest Management
Medea..... Maternal Effect Dominant Embryonic Arrest
MPA..... Marine Protected Area
NESP ..... National Environmental Science Program
PCR ..... Polymerase Chain Reaction
RRAP..... Reef Restoration and Adaptation Program
RRRC..... Reef and Rainforest Research Centre Limited
TWQ..... Tropical Water Quality
US EPA..... United States Environmental Protection Agency

ABBREVIATIONS

1-MA……1-methyladenine
3kPZS..... 3-keto petromyzinol sulfate
Bt..... *Bacillus thuringiensis*
Cas9 ..... CRISPR associated protein 9
CpGV..... codling moth (*Cydia pomonella*) granulovirus
crRNA..... CRISPR RNA
ddPCR..... digital droplet PCR
DNA ..... deoxyribonucleic acid
dsDNA..... double stranded DNA
dsRNA..... double stranded RNA
eDNA ..... environmental DNA
HEGs ..... homing endonuclease genes
MCT ..... mutable connective tissue
microRNA..... small non-coding RNA molecule
PGF2α ..... prostaglandin F2α
RGP ..... gonad stimulating peptide
RHDV..... haemorrhagic disease virus
RNA ..... ribonucleic acid
RNAi ..... interfering RNA
shRNA..... short hairpin RNA
siRNA ..... small interfering RNA
SIT ..... Sterile insect technique
SSaDV ..... sea star associated densovirus
SSWD ..... sea star wasting disease
SSWS ..... sea star wasting syndrome
tracRNA..... trans-activating RNA
UV..... ultraviolet
WSSV ..... White Spot Syndrome virus

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We would like to thank all members of the CoTS working group for their workshop contributions and discussions in the lead-up to and during this project. These have been essential when developing our ideas on possible roles of emerging and developing technologies as part of an integrated pest management strategy for CoTS. Cherie Motti and Peter Doherty are thanked specifically for carefully reading drafts of the report and providing constructive comments. This project was funded by the Australian Government's National Environmental Science Program (NESP) Tropical Water Quality (TWQ) Hub.

EXECUTIVE SUMMARY

Successful management of CoTS is increasingly important in light of cumulative pressures experienced by Great Barrier Reef coral, and the threat posed by CoTS to reef restoration efforts that involve coral deployments. Significant investment has been made in an ongoing CoTS control program, with current strategies based on manual culling using the single-injection method and integrated pest management principles.

This report evaluates biologically based technologies that could be developed for CoTS as part of an integrated pest management strategy. While some of these methods have been considered and rejected after previous outbreaks, it is timely to revisit them now as technological development such as next generation sequencing, –omics approaches (genomics, transcriptomics, metabolomics), and ground breaking biotechnology developments (CRISPR/Cas9 gene editing, gene drives), are rapidly reconfiguring the pest management landscape.

We are mindful that the physical properties of water and the biological properties of animals evolved in water are highly relevant when considering the transfer of technologies to open marine systems. The marine environment has a major influence on ecosystem complexity and the manner in which key ecosystem processes operate, with consequences for the life histories and connectivity of individual populations, and on the transmission of potential control agents.

This report considers the following control strategies: 1) Biological control using predators or coral epi-fauna; 2) Biological control using microbial agents; 3) Push-pull strategies using semiochemicals; and 4) Genetic biocontrol. For each strategy we first consider the current state of the technology, drawing on work in terrestrial, aquatic and marine environments. We then consider the potential application of each strategy for CoTS control based on current knowledge of key biological and ecological traits of CoTS. We highlight knowledge and technology gaps, associated risks, and the possible role of each approach in an integrated pest management strategy including the scale and frequency of their application.

Native predators of CoTS considered in this report include fishes that could be protected by fisheries management (conservation), and the giant triton, which could possibly be produced via aquaculture or sea-ranching, followed by deployment on priority reefs (augmentation). The value of coral macro-symbionts (crabs, polychaetes, shrimp, fish) as grazers of early CoTS life stages and defenders of their coral host is also considered. Although reef predators are rarely species specific, the environmental risk of these approaches is deemed to be low (conservation) to medium (augmentative). On the other hand, significant public perception risks are associated with fisheries management, and efficiency would need to be strongly supported by modelling. The time lag until implementation would likely be shorter for conservation approaches (depending on the legislative/management process), as considerable knowledge gaps exist for aquaculture production of these species, in particular for the giant triton. We estimate that the earliest possible implementation of these approaches will be in the mid-2020s and late 2020s, respectively, assuming immediate start and no financing constrains.

Many marine diseases, including the 'sea star wasting disease' (SSWD) (section 6.2.1.1.1), are now recognized as syndromes, where a range of opportunistic microbial agents cause similar symptoms in individuals that are otherwise stressed. This complicates the search for species specific, disease-causing microbial agents for CoTS that transmit horizontally between otherwise healthy individuals. We consider the use of horizontally transmitted pathogens as having relatively high environmental risk due to the lack of control and the potential for species cross-over. As a consequence, this approach also carries significant public perception and regulatory challenges. Knowledge relating to virulence mechanisms and immune responses in CoTS could however lead to insights relevant for genetic biocontrol strategies (see below). A bacterial symbiont present in CoTS gonads is potentially transmitted between parent and offspring. Symbionts are more likely to be species specific than opportunistic pathogens and hold potential as self-replicating entities that could be useful gene carriers in genetic biocontrol strategies. The large knowledge gaps involved imply a long lead time until genetic control strategies based on such approaches could be application ready (see below).

Semiochemicals are defined as compounds that are involved in CoTS chemical communication and signalling. We provide an overview of the available information on semiochemicals that hold potential for use as attractants or repellents for CoTS, and their genetic context. Significant research is however still needed to develop efficient compound(s) mixtures, their large scale production, and deployment ready devices. The efficiency of these approaches in an open water system also has to be confirmed. Attractants could be employed in conjunction with manual control to pull out cryptic CoTS from hiding places, thereby increasing culling efficiencies and reducing revisitation rates. Repellents could potentially be used to delay spawning aggregations, thereby providing increased time for culling before spawning occurs. There is also scope for using repellents for protection of priority sites. We regard these approaches as having low environmental risk as they are applied at a local scale, any unspecific effects can be relatively easily assessed, and the treatment can be discontinued if unwanted consequences are observed. On the other hand, they do carry a financial risk and implementation could be expected in the late 2020s at the earliest under the same assumptions as above.

Genetic control strategies for CoTS would rely on detailed knowledge of CoTS life history traits and biological processes that are unique to CoTS. We highlight significant knowledge gaps, including sex determination and sex differentiation processes in CoTS, and practical challenges relating to agent delivery (via CoTS, symbionts, corals, or externally delivered RNAi) and deployment, and to the scale of the operation. Moreover, we discuss the concept of gene drives and their relevance to biocontrol, where the 'beneficial' genetic modification generally causes a reduction in pest fitness and is selected against by natural selection. Proof-of-concept studies that fill knowledge gaps relevant for genetic control (with and without coupling to a gene drive) would be valuable to ensure that we will be ready if and when safe technologies become available. Studies in this area may also lead to serendipitous discoveries that could lead to innovative control approaches. We note that no gene drives have yet been released in any ecosystem, and their safe use on the GBR would likely be decades away.

For all of the strategies discussed in this report, modelling will be essential to predict the likely outcomes of possible control scenarios, and to identify critical parameter ranges required to reach target levels of CoTS abundance and coral cover as well as enhanced cost effectiveness relative to current operations. Before this can be done, however, the broad range of possible

technologies must be narrowed down to a suite of ‘candidate’ technologies. This is a task for an expert panel. We encourage early engagement with a broad range of stakeholder groups as well as the establishment of multi-disciplinary expert panels that can evaluate the most promising approaches in greater detail including more specific risk assessments, evaluation of relative cost-effectiveness, social acceptability and regulatory hurdles. The current report represents a solid foundation upon which each of these future activities can build.

1.0 BACKGROUND

Crown-of-thorns starfish (CoTS) have long been considered a major threat to coral reef ecosystems in the Indo-Pacific, with four major outbreaks recorded on the central GBR since the 1960s (commencing approximately 1962, 1979, 1993, 2010) (Pratchett *et al.*, 2014). For sites with long-term monitoring data, CoTS have been identified as one of three main contributing factors to loss of coral cover together with storms and bleaching (De'ath *et al.*, 2012; MacNeil *et al.*, 2019). The damage caused by CoTS to sites used for tourism saw early implementation of manual CoTS control to protect sites of high economic value and the longer-term health of these reefs (Gladstone, 1993; Pratchett *et al.*, 2019).

The scientific debate on whether or not CoTS outbreaks are a natural phenomenon or exacerbated by human activities started in the 1960s and is still ongoing (Pratchett *et al.*, 2017; Westcott *et al.*, 2016). This debate was fundamental to GBRMPA's long standing CoTS policy, which was to limit control to small-scale tactical measures in areas important to tourism and science, unless it could be proven that human activities either cause or exacerbate crown-of-thorns starfish (Gladstone, 1993). In light of increased cumulative pressures on coral reefs, some of which are strongly linked to climate change, there is now an increased interest in active intervention methods to protect future coral cover (GBRMPA, 2017b). Hence, the justification for CoTS control on a regional or reef scale is no longer linked to whether or not outbreaks are directly exacerbated by human activities but is dictated by the predicted continued impact of unmitigated outbreaks.

Since initial control efforts on tourism sites, the scope of the CoTS control program was extended in 2013 to include reefs of particular ecological relevance (Westcott *et al.*, 2020). Simultaneously the investment in manual control was elevated to multiple vessels. While the current CoTS control programs is based on divers culling individual CoTS by single-injection of bile salts or vinegar (Boström-Einarsson *et al.*, 2018; GBRMPA, 2017a; Moutardier *et al.*, 2015; Rivera-Posada *et al.*, 2014), decision support systems that optimise control effort by a multi-vessel fleet are being developed (Fletcher *et al.*, 2020; Westcott, Fletcher, 2018; Westcott *et al.*, 2016) and implemented. Nevertheless, given the scale and urgency of the threats to coral reefs, a manual approach to CoTS control is considered by some to be insufficient at the scale of the GBR (Pratchett, Cumming, 2019; Pratchett *et al.*, 2019). On that basis, we review the history of alternative control methods and scan the horizon for emerging technologies that might be considered in future control efforts. We do not focus on the automation of manual culling of CoTS as work in that area is well advanced. Rather, we focus on what are loosely termed biologically based technologies that might be used to suppress CoTS populations or reduce their impacts.

Biological control (or biocontrol) of CoTS using predators or pathogens was raised early as a possible intervention strategy. For instance, the culturing and release of large numbers of giant tritons (*Charonia tritonis*), gastropods that prey on CoTS, was an early recommendation (Endean, 1969). Triton research was funded by COTSREC (Lassig *et al.*, 1995) and is ongoing with more recent projects funded by the National Environmental Science Program (NESP) and Reef2050 (Hall *et al.*, 2017b; Motti *et al.*, 2019; Motti *et al.*, submitted). Investigations into the microbiology and diseases of CoTS were supported by the Crown-of-Thorns Starfish Advisory Committee (COTSAC) research program in the period 1985-1989 as reported by Lassig

(1991). A review of GBRMPAs CoTS management strategy in 1989 by Professor D. T. Anderson recommended that further research into biological control should be pursued (see Lassig *et al.* (1995)). However, the then newly established Crown-of-Thorns Starfish Research Committee (COTSREC) advised against further funding of biological control research due to the uncertainties, costs and risks involved (Gladstone, 1993) and this research was not included under the COTSREC research program (1989-1995) (Lassig *et al.*, 1995).

More recently, the idea of investigating options for biologically based CoTS control methods has been revisited. The Fitzroy Island 2012 Consensus statement on Crown-of-Thorns starfish produced a list of nine priority actions, including research into effective, safe and socially acceptable methods of population-level control based on enhancing natural pathogens, predators, or other novel approaches; chemical discovery of natural attractants, repellents, and spawning inducers; and identification and filling of critical knowledge gaps to locate starfish vulnerability (Doherty, 2012).

Recent rapid developments in molecular methods represent exciting opportunities for the management of marine pests. Molecular methods can generate detailed knowledge of the biology and ecology of pest organisms and potential control agents, including genetic potential (genomics), and the influence of factors such as environmental conditions, species, life stage, physiological condition and tissue type on gene expression (transcriptomics) and metabolism (metabolomics). The information obtained from these studies can lead to new control strategies based on unique aspects of the pest organism's biology that minimise adverse effects on non-target species (Hall *et al.*, 2017a; McCauley *et al.*, 2015). Molecular advances can also improve existing and emerging control strategies by informing site selection and treatment times to attack populations at their most vulnerable stage. For example, cost-effective and sensitive molecular monitoring methods based on environmental DNA (eDNA) are already under development for CoTS (Uthicke *et al.*, 2015a; Uthicke *et al.*, 2018).

This report outlines the state of the art of biologically based technologies for pest control including traditional biological control using predators or pathogens, the use of semiochemicals, and the emerging area of genetic biocontrol. Examples of how these techniques have been applied in terrestrial, freshwater, and marine environments are provided, and lessons that could be relevant for CoTS control, and ultimately prevention of CoTS outbreaks, are emphasized. In light of our current knowledge of CoTS biology and ecology and recent technological advances, we present an overview of strategies that have potential for biologically based CoTS control and prevention. In each case we provide an overview of critical knowledge gaps, as well as a preliminary discussion of the possible risks that are involved and the possible contribution that each could make in an integrated pest management (IPM) strategy for CoTS.

2.0 DEFINITIONS AND PRINCIPLES

Biological control is most often defined as ‘the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be’ (Eilenberg *et al.*, 2001). This typically involves the use of predators, herbivores, parasites or diseases. There has been controversy around this definition, however. A widened definition that accounts for biotechnological advances was proposed by a U.S. National Research Council working group in 1987: ’the use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests), and to favour desirable organisms such as crops, trees, animals, and beneficial insects and microorganisms’(Committee on Science Engineering and Public Policy (U.S.), 1987). This modified definition was met with significant criticism from many researchers in the field who argued that it was incompatible with the historical roots and ecological and philosophical basis of the term, and there were possibly concerns regarding the use of research funding (Garcia *et al.*, 1988; U.S. Congress, 1995). More recently, the term ‘biologically based technologies for pest control’ has been used to cover the intent of the widened definition (U.S. Congress, 1995).

2.1 Integrated pest management

Biological control needs to be part of an integrated pest management (IPM) strategy in order to succeed (Saunders *et al.*, 2010). IPM was developed for use against insect pests in agriculture in response to a growing concern about pesticide use, seeking to optimise the balance between chemical and biological control methods (Thresher *et al.*, 2014b). The term has later been extended to include other control approaches (e.g, mechanical, physical, cultural), and is used in a range of environmental settings. IPM strategies strive to control pest organisms, rather than eradicate them, by applying a combination of control measures at optimal times and locations as determined by monitoring and a thorough understanding of the pest organism’s biology and ecology. As such, IPM is an information intensive approach to pest management. Another important aspect of IPM is that interventions that cause minimum environmental harm and have low cost should be applied first. Generally, the main components of an IPM strategy include 1) Identification of the pest organism; 2) Monitoring numbers and extent of the damage; 3) Establish guidelines for when management action is needed; 4) Prevention; 5) Establishment of a combination of management tools; and 6) Assessing the effect of actions taken.

2.2 Traditional biological control

Predators and herbivores can be used to remove pest organisms either through direct consumption or, in the case of some predators, by altering pest species biology and behaviour by creating a ‘landscape of fear’ (Bleicher, 2017; Hall *et al.*, 2017b). Predator and herbivore-mediated biological control can be achieved by protecting predator stocks or enhancing them from current levels using conservation strategies such as limiting catch quotas or creating protected zones. More common is the use of augmentative strategies, where the abundance of a predator or herbivore is increased through local relocation or enhanced recruitment. The latter strategy often involves the release of stock produced and/or grown-out in specialised facilities.

Microbial agents (viruses, bacteria, yeasts, protists) and multicellular parasites (multicellular fungi, animals) are commonly used in biological control strategies. These agents either counteract other pathogens (e.g. probiotics or bacteriophage), or they target a multicellular pest organism by causing disease or by altering the behaviour or physiology of the pest organism.

Traditionally, biological control approaches have been divided into categories depending on whether the pest species and control agents are native or invasive. Classical and neoclassical biological control use an introduced control agent rather than a naturally occurring species to control an introduced or native species, respectively. Classical biological control aims to establish continued impact, meaning that it is most often irreversible. Practices where exotic control agents are added in high numbers without necessarily achieving establishment, are often referred to as inoculation or inundation biological control, depending on the expected capacity of the control agent to multiply and be efficient in subsequent generations. In contrast, augmentative and conservation biological control aims to increase the abundance of a naturally occurring disease agent or predator. This is achieved through inoculation or inundation, or by limiting harvest, respectively. Augmentative approaches are associated with lower risks and have already been used with some success in marine environments (Atalah *et al.*, 2013b; Hughes *et al.*, 2007) (section 4.3.1.2).

2.3 Push-pull strategies and semiochemicals

Push-pull strategies utilise stimuli to repel organisms away from a protected resource by making it unattractive or unsuitable (push), or attract the target organisms towards a source from which they can subsequently be removed (pull) (Cook *et al.*, 2007). Stimuli used to repel or attract organisms can include visual, auditory and chemical signals. Approaches based on visual and auditory signals often lack specificity and operate on near-scale fields, and large-scale use relies on deployment within large arrays. In contrast, olfaction is one of the most sensitive senses in many species, especially in aquatic species, and operates on near- and far-field scales. Hence, the most commonly applied push-pull strategies are based on semiochemicals.

Semiochemicals are broadly defined as chemical signals that convey information between organisms. In general, semiochemicals can regulate processes such as foraging, attraction, repulsion, reproductive synchrony and regulation, and chemical defence (Cook *et al.*, 2007; Motti *et al.*, 2018; Sorensen, Johnson, 2016). In addition to their use in push-pull strategies, semiochemicals can also be exploited to disrupt mating behaviour by deployment of synthetic sex pheromones. Many semiochemicals provide high specificity and enable selective control of a specific species, life stage and/or sex, thereby providing an opportunity to design highly targeted pest control strategies. On the other hand, high specificity means that the technology is less transferable and targeted research is required for each system.

Efficient use of semiochemicals for pest control requires that the relevant compound(s) can be delivered at a concentration that is detectable by the pest organism or that can camouflage a naturally occurring signal. At the same time, it is crucial that the added signal is not itself camouflaged by naturally occurring signals and this has consequences for the design of both the delivery system and the application strategy (timing and location).

Commonly used strategies that utilise semiochemicals for pest control can be broadly divided into monitoring, mass trapping, lure and kill, push-pull strategies and mating disruption with pheromones (semiochemicals for intraspecific communication) (Reddy, Guerrero, 2010; Smart *et al.*, 2014; Witzgall *et al.*, 2010).

Monitoring of the abundance and distribution of pest populations is often used in integrated pest management of insect to predict the timing of specific treatment options (Rodriguez-Saona, Stelinski, 2009). Many monitoring strategies use traps with an attractant (pheromone, food- or host-attractant, or a combination) to estimate population sizes and sometimes also to assess their reproductive stage. Mass trapping and lure-and-kill strategies utilise an attractant and a trap- and deployment design that is sufficiently effective to control a large proportion of the pest organism in an area. Mass trapping most often incorporate a killing agent such as a pesticide, while lure-and kill strategies incorporate either a pesticide or a pathogen (lure and infect). If the delivery device is not a trap, then the dose delivered to the pest before leaving the lure needs to be sufficient to cause death or reduce fecundity. Lure-and-kill strategies often affect more insects as the attractant and killing agents are spread over a larger area (Reddy, Guerrero, 2010). Traps or lures should affect a large proportion of the population before the mating season and preferably retain efficiency during the entire reproductive period.

It is essential to consider whether the attractants used in traps or lures will attract both sexes or one sex only, and the population biology and life history traits of the pest organism in question. For example, if traps or lures attract males only and they can mate several times, then a very large proportion of the males need to be removed to reduce female fecundity (Smart *et al.*, 2014). If the use of attractants are combined with push-components, the strategy can be classified as push-pull (see above). Host (plant) volatiles can be added to the semiochemical mix for enhanced attractant or repellent effects on the pest organism itself, or to attract their natural enemies (Reddy, Guerrero, 2010; Smart *et al.*, 2014).

In mating disruption, mating behaviour is disrupted by deployment of synthetic sex pheromones. Several mechanisms have been proposed including competitive attraction (males are distracted by false signal), camouflage (boundaries of natural plumes are obscured), desensitization (via adaptation or habituation), and sensory imbalance (ratio of pheromones is distorted) (Rodriguez-Saona, Stelinski, 2009). These mechanisms are not mutually exclusive and in most current applications it is not well understood which mechanisms are in play. A more detailed discussion and examples of applications can be found in the reviews by Rodriguez-Saona, Stelinski (2009), Reddy, Guerrero (2010), and Witzgall *et al.* (2010).

The success of mating disruption is strongly related to population density, with dense populations difficult to control (Reddy, Guerrero, 2010). Moreover, the approach is more likely to be effective if edge effects are relatively small, as is the case for large or enclosed treatment areas (e.g. a mountain valley) (Smart *et al.*, 2014). Mating disruption requires larger amounts of semiochemicals than lures and traps. For example, for codling moth, *Cydia pomonella*, pheromone trap lures typically release 10 -100 times more than calling female, while mating disruption dispensers release up to 10,000 times more than a calling female (release rates of 10-100 mg/ha/h) (Witzgall *et al.*, 2010). Nevertheless, mating disruption has been a very successful approach to control agricultural insects with several examples of successful use (Section 4.1.4).

Pheromone antagonists, or parapheromones, are human-made chemicals that are structurally related to pheromones and that interfere with chemical communication channels of the pest organism (Reddy, Guerrero, 2010). Semiochemicals have also been used to activate host (plant) defences by inducing changes in their metabolism (Smart *et al.*, 2014).

2.4 Genetic biocontrol

The potential use of genetic mechanisms and tools for the control of pest species and disease vectors has a history that stretches back at least to the 1940s (Champer *et al.*, 2016). Over decades the focus has primarily been on genetic strategies to restore or generate novel biocide sensitivity in agricultural pest insects, to control invasive species, and to control insect vectors of pathogenic diseases.

In agriculture the sterile insect technique, which uses radiation to produce sterility, has been used for decades to effect large-scale reductions in the reproductive success of invasive and pest species (Dycke *et al.*, 2005). More recently a range of novel genetic-based strategies that seek to achieve similar outcomes to the sterile insect technique have been explored (Bull, 2015; Thresher *et al.*, 2014a). In these technologies, rather than producing sterility through radiation, at the risk of damaging other aspects of the organism, it is achieved through the introduction of heritable DNA sequences into the species' genome to achieve population suppression or replacement. This might be achieved using viral vectors or alternatively, new gene-editing tools such as CRISPR.

Self-limiting population suppression strategies are based on the introduction of DNA sequences into a population which will disappear from that population without subsequent introductions. Generally, these involve transgenes which either cause lethality to pre-reproductive stages or which skew the population sex ratio in favour of one sex, usually males. In each case the reproductive potential of the population is reduced because the number of females is reduced. Persistence of the DNA sequence in the population is determined by the fitness costs associated with the genetic modification and the effect of laboratory rearing. The benefit of these approaches is that they are inherently self-limiting – if something goes wrong, simply don't add more and the system will return to its original state.

In repressible lethal systems a transgene is inserted with a dominant phenotype that is lethal during larval development. Lethality is suppressed prior to release by providing an antidote, e.g. tetracycline in the case of *Aedes aegypti* (Curtis *et al.*, 2015) in the larval diet. In the field and in the absence of sufficient tetracycline in the diet, progeny inheriting the transgene from released individuals die before reproducing. These systems can also be designed to be lethal to one sex only, usually females, in order to maximise transmission by focusing mate-seeking and reproductive behaviour on wild-type females (Harvey-Samuel *et al.*, 2017).

Alternative approaches to these lethal systems include sex ratio distortion systems. In female-lethal systems the number of offspring carrying the gene sequence is halved each generation as female carriers are killed. This has the effect of diluting the presence of the transgene in the population, and, therefore, its overall effectiveness. In sex ratio distortion systems rather than killing the target sex, sex-ratios are instead biased towards (usually) males, effectively maximizing the number of progeny produced and the frequency of the transgene in the

population. In these systems all individuals develop as a single sex (usually male). These mechanisms can operate through a variety of pathways.

Gene knockouts or inserts (as discussed above), where genes have been permanently inactivated or inserted via genetic engineering, fundamentally differ from gene knockdowns or stimulation, where the expression level of a gene is altered. Gene knockdowns, also referred to as gene silencing, is most often based on the release of interfering RNA (RNAi) that produce a targeted gene knockdown (Bradford *et al.*, 2017; La Fauce, Owens, 2012).

Gene silencing via RNAi is based on a natural cell process where expression of microRNA plays a key role in regulating protein synthesis. By supplying targeted RNA molecules it is possible to interfere with gene expression or translation by neutralizing the targeted mRNA molecules. The RNAi molecules can be supplied externally (small interfering RNA (siRNA) or double stranded RNA (dsRNA)), but an organism can also be genetically engineered to express them (dsRNA or short hairpin RNA (shRNA)). Interfering RNA are used frequently in laboratory studies investigating the effect of knocking down specific genes, and it is also emerging as a strategy for modifying food crops (section 4.1.5). The use of RNAi in wild populations face additional challenges, such as harsh environmental conditions (UV, ribonucleases, dilution), and the development of field suitable delivery methods.

Until recently, very little progress has been made in developing genetic biocontrol methods with few examples moving from technology development to successful implementation in the field. The rapid rise of next generation genomic methods, however, suggests that this situation is likely to change in the near future. The availability of well annotated whole genomes, including for CoTS (Hall *et al.*, 2017a), offers the opportunity to scan the entire genome of the target species for genes that code for particular products and regulators that may be key to its biocontrol. Furthermore, with the development of new and efficient gene editing methods to modify targeted genes and the possible prospect of inserting gene drive mechanisms to rapidly propagate those edits within populations (section 5.0), the early promise of genetic methods in biocontrol seems to be within reach (Dearden *et al.*, 2018; James *et al.*, 2018). While the current situation is both exciting and promising, it is not without complication as discussed in general in section 5.4 and with special reference to potential CoTS control in sections 6.4 and 7.3.

2.5 Biosafety considerations

The risk of any proposed biocontrol strategy needs to be considered carefully in each case. Here we present some general principles, while specific considerations for strategies relevant for CoTS control are presented in section 6.0.

Generally, issues that need to be considered include exact identification and characterisation of the proposed biocontrol agent and pest organism, an evaluation of possible human health risks, an evaluation of environmental risks, and an evaluation of the efficacy of the proposed strategy (van Lenteren *et al.*, 2003). The environmental risk of a biocontrol program is also the most difficult aspect to assess. The host and home range of the proposed biological control agent is critical, but so is the likelihood that they will establish and spread after introduction or augmentation, and the possibility of direct and indirect flow-on effects on other organisms (Solter, Maddox, 1998; van Lenteren *et al.*, 2003).

It can be challenging to predict host specificity based on laboratory results as the physiological host range (predicted by laboratory tests) may be much broader than the true ecological host range (Solter, Maddox, 1998; van Lenteren *et al.*, 2003). van Lenteren *et al.* (2003) outlined factors to consider when designing experiments to test host specificity and highlighted that available knowledge on host spectrum and habitat of the control agent may help determine the non-target organisms to be tested. In general, cross-species transmission and emergence depend on genetics (the genetic basis of a pathogen's ability to infect cells of a new species and spread to new individuals) and ecology (the likelihood that the potential new host species is exposed to the control agent and present at a population density that enables spread on an epidemiological scale) (Holmes, 2013).

Ecological flow-on effects resulting from a proposed biocontrol strategy may include both direct and indirect effects that may be difficult to predict. It is expected that the use of native biocontrol agents will have less severe flow-on effects than the introduction of exotic species, but a detailed environmental risk analysis is required in each individual case.

3.0 DIFFERENCES BETWEEN AQUATIC AND TERRESTRIAL SYSTEMS

The use of biocontrol in aquatic systems is far less common than in terrestrial systems. Several fundamental differences exist between aquatic and terrestrial systems that are relevant for biological control. Some of these are directly related to the physical properties of water and water currents, which provides relative protection against desiccation, rapid temperature fluctuations and UV exposure as compared to air and influences how an entity can move in the surrounding medium. Other differences relate to biological traits of the biota, which in some instances have evolved as a consequence of their aquatic environment.

3.1 Transmission through water

The epidemiology of aquatic diseases reflect additional modes of transmission compared to terrestrial environments, prompting the development of habitat-specific epidemiological models (McCallum *et al.*, 2004; Murray, 2009; Sokolow *et al.*, 2009). While some marine diseases follow traditional density-dependent models (randomly mixing populations) or density-independent models (schooling populations or vectored transmission), others reflect local accumulation of pathogens in the water, or movement of pathogens over long distances with concomitant lack of feedback between pathogen production and disease impact (Murray, 2009). These additional modes of transmission largely result from a lack of dispersal barriers in some oceanic areas and the ability of some marine pathogens to survive for long periods outside of the host (McCallum *et al.*, 2003; McCallum *et al.*, 2004). In addition, the relative vagility of the host animal as well as the colonial nature of some marine invertebrates need to be considered (Sokolow *et al.*, 2009).

The different disease transmission strategies have direct implications for the success of different aquatic disease management actions (Murray, 2009). Similarly, the transmission mode of any candidate biocontrol agent intended for use in marine systems needs to be considered when evaluating their safety, efficacy, and suitable application strategies. For biocontrol purposes, the facilitated spread and dilution of microbial control agents can represent both an opportunity and a challenge, depending on the type of agent and its safety profile.

In aquatic environments, far-field signals are largely restricted to the senses of hearing and olfaction. Semiochemicals are generally released into water and water currents enable gradient formation on larger scales but also affect dilution times (Motti *et al.*, 2018; Zimmer, Butman, 2000). Nevertheless, chemical cues in the marine environment have profound direct and indirect influences on population structure, community organisation and ecosystem function (Hay, 2009). As such any artificially released compound would also have to compete with natural cues. One strategy that has been tested in the field is using implantations that contain a precursor that in turn greatly stimulates natural production of the pheromone of interest (Lim, Sorensen, 2010). Alternatively, low cost production of a simple analogue can in some cases fulfil the role of the natural compound and can be released in larger quantities. Recently, some progress has been made in designing cost-effective emitters for controlled released of pheromones in aquatic environments (Wagner *et al.*, 2018).

3.2 Ecological complexity

Aquatic systems have high diversity with 25 out of the 34 animal phyla found exclusively in aquatic environments (McCallum *et al.*, 2004). With a high diversity of organisms involved in predator-prey and symbiotic or parasitic relationships, the resulting ecological complexity is also high. There are significant knowledge-gaps regarding these ecological interactions including feeding preferences and mechanisms underlying host species range and host range expansions for symbionts and parasites, and the home range of predators and pest species. As a consequence, it has been argued that there is an increased risk of unexpected ecological flow-on effects of biological control in marine systems (Secord, 2003).

3.3 Life histories and population connectivity

Some animal life history traits are found exclusively in aquatic environments, such as clonal life forms and planktonic larvae. The spread of long-lived planktonic larvae is facilitated by the relative openness of many marine systems. Some populations are however dominated by self-seeding due to short larval development times (Jackson, 1986), water current conditions that result in long water retention times (McCallum *et al.*, 2004), or attributes of propagules that facilitate their return to natal grounds (anadromus fishes). In general, self-seeded populations have higher genetic homogeneity, particularly in species that can reproduce asexually. This has consequences for disease and parasite susceptibility as a homogeneous host population may allow for build-up of more virulent strains of pathogens and facilitate epidemic spread of disease (McCallum *et al.*, 2004).

Local connectivity which is partly controlled by marine currents (Riginos *et al.*, 2019), also influences the possible spread of a pest species and its potential biocontrol agents. It is therefore essential to consider together the life history and connectivity of pest organisms when evaluating the risk associated with any marine biocontrol project, in particular where spread to other jurisdictions is possible (section 7.4). As in terrestrial environments, connectivity considerations need to also account for possible human-mediated dispersal. In aquatic environments, this includes domestic and international transfer by commercial (merchant) and Defence vessels, commercial fisheries and tourism operators, recreational fishers and boaters, snorkelers and divers, and scientific operations including collections.

4.0 A HISTORY OF BIOLOGICALLY BASED TECHNOLOGIES FOR PEST CONTROL

4.1 Terrestrial systems

Biological control in terrestrial systems has a long history with many examples of successful and unsuccessful control programs and commercially available control agents. Many of the lessons learnt from terrestrial systems are directly transferable to other systems. Here we present an overview of strategies that are currently in common use, together with those factors that have rendered them suitable for commercialisation.

4.1.1 Predators and herbivores

Predators and herbivores have been used extensively in agriculture, primarily to protect greenhouse crops. Herbivores have also been used in the fight against invasive plants and there are examples of re-introduction of apex predators to areas where they have been hunted to local extinction leading to re-establishment of trophic cascades with flow-on effects on vegetation.

In 2010, a total of 70 predatory agents were produced for use in agriculture, in particular for use on greenhouse crops (van Lenteren, 2012). These included mites (Acari: 30 species), beetles and weevils (Coleoptera: 28 species), true bugs (Heteroptera: 19 species) and a snail (Mollusca: 1 species). For example, predatory mites are used against other (phytophagous) mites, thrips and whiteflies in vegetable and ornamental cultivation systems (Knapp *et al.*, 2018). Generally, predatory biological control agents are less specific than parasitoids and other parasites (sections 4.1.3, 4.3.3) although some are argued to have a limited range of prey (monophagous or oligophagous). In the last two decades there has been a tendency towards using more indigenous rather than exotic species and some of the popular exotic agents are being replaced by indigenous species (van Lenteren, 2012).

Herbivorous insects can be used to control invasive plants if eradication over the entire introduced range is viewed as unlikely, and control by mechanical removal and herbicides, alone, is recognized as impractical and unsustainable (Seastedt, 2015). Australian examples includes the use of stem-boring cactoblastis larvae (*Cactoblastis cactorum*) to control prickly pear (common term for over 10 cactus species, mostly in genus *Opuntia*) (Biosecurity Queensland, 2016), tip and stem borers (e.g. the moth *Carmenta mimosa*) to control *Mimosa pigra* (Natural Heritage Trust, 2004), and the use of Parkinsonia seed beetles (*Penthobruchus germaini*, *Mimosestes ulkei*), Parkinsonia leaf bug (*Rhinacloa callicrates*) and leaf-feeding looper (*Eueupithecia cisplatensis*) to control Parkinsonia (*Parkinsonia aculeate*) (Biosecurity Queensland, 2020).

The reintroduction of wolves (*Canis lupus*) into Yellowstone National Park in 1995/96 led to a decrease in the numbers of elk (*Cervus elaphus*), which had experienced a large population increase in the years since wolfs were extirpated from the park in the mid-1920s (Ripple, Beschta, 2012). Flow-on effects included increases in woody plants such as aspen (*Populus tremuloides*), cottonwoods (*Populus* spp.) and willows (*Salix* spp.) due to reduced herbivory,

and these changes were possibly linked to observed increases in the populations of bison (*Bison bison*) and beaver (*Caster canadensis*) (Ripple, Beschta, 2012). The re-introduction of wolves to Yellowstone is used as the prime example of the concept of 'rewilding', which has received increasing attention as an approach to nature conservation (Lorimer *et al.*, 2015).

4.1.2 Microbial agents

Viral biocontrol of invertebrate pests has a long and relatively successful history in terrestrial agriculture. In particular, the use of baculoviruses (family *Baculoviridae*) to control caterpillars is common worldwide and several preparations are available commercially (Lacey *et al.*, 2015). One reason behind the commercial success of baculoviruses is that they are encased within occlusion bodies (crystallized proteins) that make them relatively robust. In addition, the occlusion bodies make it possible to track and quantify the virus load in the host by light microscopy (Lacey *et al.*, 2015). Baculoviruses are generally regarded as safe, readily mass produced, highly pathogenic and easily formulated and applied. On the other hand, their persistence after application is limited and their kill rate is relatively slow (Lacey *et al.*, 2015). Other viral families that have been used for insect control include the *Reoviridae*, *Parvoviridae,* and *Nudiviridae* as described in detail elsewhere (Lacey *et al.*, 2015).

Biological control programs using exotic viruses to control terrestrial vertebrate pests have a long history in Australia. This includes the rabbit control programs using myxoma virus (MYXV, dsDNA; first released in 1950) and haemorrhagic disease virus (RHDV, ssRNA; first released in 1996) (Strive, Cox, 2019; Wishart, Cox, 2016). While each virus type initially killed large proportions (over 90%) of exposed rabbit populations, some rabbits developed resistance. As a countermeasure, another strain of RHDV (RHDV1 K5) was released in 2017 (Strive, Cox, 2019; Wishart, Cox, 2016). In the meantime an exotic RHDV strain emerged in Australia (RHDV2) that has caused substantial numerical decline in exposed rabbit populations (Mutze *et al.*, 2018; Strive, Cox, 2019; Wishart, Cox, 2016). The current strategy is to ensure that new rabbit biocontrol agents can be released on an ongoing basis (https://invasives.com.au/research/national-rabbit-biocontrol-optimisation/), and to use viral release as part of an integrated pest management program (Wishart, Cox, 2016). Another successful viral biocontrol program in Australia, was the use of feline panleukopenia virus to totally eliminate cats from Marion Island (McColl *et al.*, 2014). In both these cases, none of the native animals present are/were closely related to the target pest organism.

Bacteria that have been commercially developed for control of insect pests and plant pathogenic nematodes include the spore-forming *Bacillus thuringiensis, Paenibacillus* spp., and *Pasteuria usgae*, and the non-spore forming *Serratia entomophila, Burkholderia cepacia* and *Pseudomonas fluorescens* (Lacey *et al.*, 2015; Li *et al.*, 2015). The commercial potential of a bacterial control agent is related to their ease of production and their stability after application. Spores are much more stable than vegetative bacterial cells, which generally increases shelf life and simplifies application protocols.

Subspecies of the spore-forming *Bacillus thuringiensis* (Bt) have a market share of 98% for sprayable bacterial pesticides (Lacey *et al.*, 2015). These bacteria produce crystallized toxins within their spore, which have insecticidal activity. While each strain has a limited host range, the species as a whole has a relatively wide host range among insects and some other pest invertebrates like mites and nematodes. Advantages of using Bt against insect pests include

fast action, low production costs, easy formulation, long shelf life, application via conventional equipment or transgenic plants producing Bt toxins, high selectivity of individual toxins and limited environmental impacts. One drawback is that frequent application is required as it readily degrades in sunlight. Another spore-forming bacterium *Paenibacillus* spp. (milky disease in scarab beetle larvae) requires *in vivo* production and has a relatively narrow host range, limiting its commercial uptake (Lacey *et al.*, 2015). The non-spore forming *S. entomophila,* which is toxic to New Zealand grass grub, is applied using a seed drill rather than by spraying. New technology was developed for *S. entomophila* to produce a stabilised granular product (BioshieldTM) that is stable under ambient conditions for several months, and which has potential for use with other non-spore forming bacteria.

In recent years, biocontrol approaches using the intracellular bacterium *Wolbachia* to control disease-carrying mosquitoes have been developed. The World Mosquito Program (previously called 'Eliminate Dengue') releases male and female *Aedes aegypti* mosquitoes infected by a *Wolbachia* strain that shortens the mosquito's life span. This hinders virus transmission as the virus requires a period of development in the mosquito host and transfer occurs primarily in older mosquitoes (Popovici *et al.*, 2010). An advantage of *Wolbachia*-based biocontrol is that the bacterium naturally induces cytoplasmic incompatibility (section 5.2.5), hence maternally transmitted *Wolbachia* can effectively invade and sustain themselves in the host population providing a method for disease control that is likely to be cost effective (Jeffries, Walker, 2016). While *Wolbachia* has been estimated to be present in 65% of insect species, they are not naturally present in the *A. aegypti* mosquitoes. The *Wolbachia* strains used for virus control in the World Mosquito Program were originally isolated from fruit flies, and were adapted in *Aedes* cell culture lines for three years before stable infection could be established by embryonic microinjection.

Aedes aegypti mosquitoes infected by *Wolbachia* strains have reduced or eliminated transmission efficiency of several medically important viruses including the Dengue (Iturbe-Ormaetxe *et al.*, 2011; Jeffries, Walker, 2016) and Zika viruses (Aliota *et al.*, 2016). The use of *Wolbachia* has also been suggested as a possible control strategy for controlling chikungunya virus and yellow fever virus transmitted by *Aedes aegypti* (Jeffries, Walker, 2016) and Japanese Encephalitis virus transmitted by *Culex* mosquitoes (Jeffries, Walker, 2015). Recent studies in North Queensland have shown that in the field, heat stress may affect the success of *Wolbachia*-based disease control programs and this needs to be taken into account when selecting the location and nature of field breeding sites (Ross *et al.*, 2019).

In a recent development, the 'Debug Innisfail' project (CSIRO, James Cook University, Verily Life Sciences LLC) is currently testing release of male mosquitoes that have been sterilised by infection with another *Wolbachia* strain (https://research.csiro.au/mozzieproject/). This approach is defined as a sterile insect technique (SIT). The released males are non-biting and if they successfully mate with a wild female, the resulting eggs will not hatch. The target is to remove the population of *A. aegypti* rather than produce a population that do not transmit disease.

Concerns regarding *Wolbachia* biocontrol relate to the safety for humans, animals and the environment. More specifically, questions focussed on whether *Wolbachia* can be transferred to 1) humans or induce an immunological response in humans, 2) other animals including mosquito predators, and 3) soil, water or other environments. These concerns were all

addressed, and humans that were experimentally exposed to bites by infected mosquitoes did not develop *Wolbachia* antibodies, there was no evidence of the transfer of *Wolbachia* to non-target hosts, , and there was no detectable transfer to environmental samples (Popovici *et al.*, 2010). However, questions are still being raised about the safety of the approach and the necessity of further experimental work (Silva Loreto, Luz Wallau, 2016). In Australia, there are more than 300 species of mosquitoes and it is not considered likely that removal of the introduced species *A. aegypti* via SIT will have unintended effects on predators or ecosystems (https://research.csiro.au/mozzieproject).

Microsporidia were previously classified as protists but are now recognized as a sister group to fungi or a clade within the fungi (Hibbett *et al.*, 2007). They are unicellular, spore-forming organisms that must be ingested by their host to cause infection. Most are chronic pathogens that kill their host slowly, and many have complex life cycles involving more than one species (Solter, Maddox, 1998). Microsporidia have been used for biocontrol of insects. For example, *Kneallhazia solenopsae* (=*Thelohania solenopsaeis*) is used as a biocontrol agent against fire ants, infecting all life stages and resulting in shortened life-span and barren queens (Williams, deShazo, 2004) . *Nosema locustae* has been used for grasshopper control in specific rangeland situations, (Solter, Maddox, 1998). Two species of microsporidia, *Vairimorpha disparis* (=*V. lymantriae*) and *Nosema lymantrie*, were used against the gypsy moth *Lymantria dispar* (L.) in a classical biocontrol program in the US (Hajek, Delalibera, 2010). However this approach appears to have been unsuccessful, as current pest management strategies for gypsy moths is mainly based on monitoring moth abundance with pheromone-baited traps followed by targeted spraying with *Bacillus thuringiensis* var *kurstaki* (BtK) and mating disruption using synthetic pheromones (Slow the Spread Foundation, 2018) (see also section 4.1.4).

Protists is the common term for eukaryotic organisms that are neither animal, plant nor fungus. The phylum Apicomplexa includes several parasites with biological control potential. For instance, *Sarcocystis singaporensis* is used as part of an IPM strategy for rat control in Thailand (Jäkel *et al.*, 2006) and Laos (Jäkel *et al.*, 2017; Jäkel *et al.*, 2016), where rat bait containing *S. singaporensis* sporocysts is commercially available (Jäkel *et al.*, 2016). The gregarine *Apicomplexa* were also recently proposed to have biological control potential against several insects (Dias *et al.*, 2017; Rueckert, Devetak, 2017). The oomycete *Phytium oligandrum* is registered by the United States Environmental Protection Agency (US EPA) for biocontrol of other pathogenic fungi and other oomycetes and is added as a soil treatment (Ombudsman Biopesticides and Pollution Prevention Division, 2007).

4.1.3 Multicellular parasites

Fungi are the predominant natural pathogens of many terrestrial arthropods (insects, ticks and mites), infecting their host through their cuticle rather than by ingestion. As a consequence, several fungal biocontrol agents have been developed for use against arthropods in terrestrial agriculture and also against mosquitoes transmitting human pathogens such as malaria (Lacey *et al.*, 2015). A meta-analysis of terrestrial biocontrol studies found that fungi increased pest mortality rates to a larger extent than other biocontrol agents (Stiling, Cornelissen, 2005). The most commonly used entomopathogenic fungi for biocontrol purposes include the genera *Metarhizium*, *Beauveria*, *Isaria* (formerly *Paecilomyces*) and *Lecanicillium* (Kwenti, 2017; Lacey *et al.*, 2015). One advantage of using entomopathogenic fungi is that they pose minimal

risk to large groups of beneficial non-target organisms (Lacey *et al.*, 2015). Fungi that infect and kill nematodes have also been tested for managing parasitic nematode infections in ruminants (Kwenti, 2017).

Some anamorphic fungi (asexual stages of fungi) have been developed for mass-production systems with the intention of repeated applications via sprays, granules etc. Other fungal agents have been developed for use in ecology-based biocontrol of insects by manipulating environmental conditions to stimulate natural epizootics and spread, as well as by inoculative (rather than repeated) release to establish long term insect suppression, or by conservation of natural epizootics (Lacey *et al.*, 2015).

Parasitic animals used in agriculture are mainly insect parasitoids in the order Hymenoptera (sawflies, wasps, parasitic wasps, bees and ants) and entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae). In particular, there are 120 species of Hymenoptera (mostly wasps and flies) in commercial production (van Lenteren, 2012), and several are available in Australia (Llewellyn, 2003). In general, parasitoids lay their eggs into or onto a specific life stage of their host and hatched larvae then feed on the host, ultimately killing it. Additional pest control is sometimes provided by adult parasitoids also serving as predators.

Nematodes that infect insects (Steinernematidae, Heterorhabditidae) generally have a broad host range, but are arguably generally safe to non-target organisms and to the environment (Lacey *et al.*, 2015). After the nematode enters the insect host via natural openings or through the cuticle, their symbiotic bacteria are released and proceed to kill the insect host within 24-48 hours. The bacteria also defend against secondary invaders and provide the nematodes with nutrition. Up to three generations of nematodes can exploit the one host before exiting and finding a new host. Nematodes can be mass produced *in vivo* (infected insects) or *in vitro* (solid or liquid fermentation). At least 13 species of insect-infecting nematodes have reached commercial development (Lacey *et al.*, 2015). Nematodes are available commercially in Australia for use against a range of agricultural insect pests and details are presented elsewhere (Lacey *et al.*, 2015; Llewellyn, 2003). Parasitic nematodes were also considered for control of cane toads, rabbits and mice in Australia and possums in New Zealand (Saunders *et al.*, 2010; Singleton, Chambers, 1996) but these approaches do not appear to have been progressed further.

4.1.4 Push-pull strategies and semiochemicals

Push-pull strategies have been developed for many terrestrial systems including for subsistence farming, intensive arable agriculture, horticulture, forestry, and for control of veterinary and medical pests (Cook *et al.*, 2007). Push-componenets are mainly based on semiochemicals including non-host volatiles (e.g. essential plant oils that can mask other odours), pheromones (signals between members of the same species; e.g. anti-aggregation, alarm), oviposition deterrents, and anti-feedants. On the other hand, pull-components can include visual stimulants, host volatiles, pheromones (aggregation, sex), oviposition stimulants, and gustatory stimulants (Cook *et al.*, 2007). In some cases, different types of compounds can act synergistically. For instance, a synthetic apple volatile acts synergistically with a pheromone from the weevil *Conotrachelus nenuphar*, increasing the efficiency of monitoring traps (Pinero, Prokopy, 2003).

An example of successful large-scale use of pheromones for pest control is the ongoing Slow-the-Spread Program, which is a co-ordinated effort by the United States Department of Agriculture (Forest Service, Animal Plant Health Inspection Service) and 10 state governments to limit the spread of the gypsy moth *Lymantria dispar* in the USA. The program was established in 2000 and is managed by the Slow the Spread foundation (www.gmsts.org) (Sharov *et al.*, 2002). Grids of pheromone baited traps (65,000 – 80,000 traps per year) are used for monitoring just ahead of the population front. Areas for treatment are subsequently selected based on discussions between stakeholders, as guided by a computer algorithm. Treatment involves mating disruption induced by controlled-release dispensers with an insect pheromone, or by application of *Bacillus thuringiensis*; with the mating disruption strategy used on a 12 times larger area than the Bt larvicides (ca. 335,000 acres) (Sharov *et al.*, 2002; Slow the Spread Foundation, 2018). The program has reduced the spread of gypsy moths by at least 60%, preventing their establishment on more than 150 million acres since its establishment in 2000 (Slow the Spread Foundation, 2018). Other examples of successful use of mating disruption in terrestrial systems include control of the codling moth *Cydia pomonella* in pome fruit, leafrollers *Platynota stultana* in vineyards and the pink bollworm *Pectinophora gossypiella* in cotton (Reddy, Guerrero, 2010; Rodriguez-Saona, Stelinski, 2009).

Mass trapping strategies have been successfully used against beetles and weevils (Coleoptera) by deployment of traps with aggregation pheromone blends that attract both sexes, resulting in a reduction in the number of damaging larvae that are produced (Smart *et al.*, 2014). Examples of successful use of lure-and-kill strategies include the control of the moths *Ephestia kuehniella* and *Ephestia cautella* in Italian flour mills by using dispensers containing a major pheromone component ((Z,E)-9,12-tetradecadienyl acetate) and an insecticide (cypermethrin), the control of tse-tse flies (genus Glossina) by using blends of attractive cattle volatiles (e.g. acetone, 1-octen-3ol, 3-propylphenol, 4-methulphenol) and visual cues (black and blue colour) to attract flies to screens covered with insecticides, and the control of the Mediterranean fruit fly *Ceratitis capitata* by protein degradation products (e.g. putrecine, trimethylamine) as attractants in lures containing ammonium acetate (Reddy, Guerrero, 2010).

4.1.5 Genetic biocontrol

In agriculture, several transgenic plants have been created, and approved for human consumption, that are themselves toxic to insects (http://www.isaaa.org/gmapprovaldatabase/). These include plants (corn, cotton, cowpea, eggplant, potatoes, rice, soybean, sugarcane and tomato) that have been modified to produce a *Bacillus thuringiencis* (Bt) toxin that targets specific insect groups. Other approved transgenic plants are resistant to specific viruses or oomycetes (papaya, bean, plum, potato, zucchini, capsicum and tomato), or to insecticides (a wide range of plants including corn, cotton, canola, soy beans, sugar beet and alfalfa). Recently, a transgenic apple (Arctic Apples) was approved that uses gene silencing to reduce the expression of polyphenol oxidase, which causes browning after the apple is cut.

In contrast, only two genetically engineered animal products have so far been approved for production of food or pharmaceuticals (https://www.fda.gov/animal-veterinary/biotechnology-products-cvm-animals-and-animal-food/animals-intentional-genomic-alterations) including the triploid salmon AquAdvantage®, which grows to market size in 16 to 18 months rather than

three years (section 4.2.5), and Atryn®, a goat milk product that prevents blood clots. It is expected that more animal products will be approved with the development of more versatile and precise gene editing techniques that enables gene modifications without the introduction of genetic material from other organisms (for example CRISPR/Cas9, see section 5.1 for details).

Significant progress has been made with gene modification of insects that are either disease vectors or agricultural pests. Repressible lethal systems in mosquitoes and agricultural insect pests have been tested in field cages and, in the case of mosquitoes, in field releases in Brazil, the Cayman Island, and Malaysia (Carvalho *et al.*, 2015; Harris *et al.*, 2012; Lacroix *et al.*, 2012). These systems are self-limiting (not linked to a gene drive) and since offspring produced in the wild are not viable, the modification is expected to disappear within a few generations if new releases are not made. Many of the engineered insect systems produce and release only transgenic males, which are not directly implicated in disease transmission (mosquitoes) or production of larvae that damage crops. So far, field cage and field release studies have resulted in 80-95% suppression of the wild type target population (Carvalho *et al.*, 2015; Curtis *et al.*, 2015; Harris *et al.*, 2012; Lacroix *et al.*, 2012). In Australia, a repressible lethal construct in the Mediterranean fruit fly has been tested in cages by the Western Australia Department of Primary Industries and Regional Development and field trials are being discussed with regulatory agencies (https://www.agric.wa.gov.au/news/media-releases/imported-fly-performs-mating-trials).

Another strategy for mosquito control was recently tested in quasi-field trials in Burkina Faso, where malaria is endemic and insecticide resistance is spreading in the mosquito population (Lovett *et al.*, 2019). In this case, spores of a mosquito-specific fungi, *Metarhizium pingshaense*, served as the delivery and expression system for two US EPA approved toxins [calcium activated potassium (KCa) and voltage-gated calcium (CaV) blocker w/k-hexatoxin-Hv1a (Hybrid)], originally derived from spiders, under the control of a haemolymph-specific promoter (Bilgo *et al.*, 2017; Lovett *et al.*, 2019). The modified fungal strain was shown to kill mosquitoes faster and at lower spore doses than the wild type strain in both laboratory studies and quasi-field trials (Bilgo *et al.*, 2017; Lovett *et al.*, 2019).

A recent discussion paper from the Australian Academy of Sciences (AAS) considered the potential use of synthetic gene drive applications in Australia to control human, veterinary and wildlife diseases, to control invasive species, and for agricultural applications (Australian Academy of Science, 2017). They highlighted the possibility of controlling mosquitoes that transmit Dengue, malaria and other mosquito-transmitted diseases. Animals that are regarded as invasive pests in Australia and that could potentially be targeted by gene drives include the mouse *Mus musculus* on islands such as Lord Howe Island. Cane toads and rabbits would be other candidates, with the advantage that there are no native toad species or species closely related to rabbits in Australia. In addition, the AAS discussion paper highlighted that gene drives have potential for controlling agricultural insect pests with short generation times.

4.2 Freshwater systems

Biological control has been more extensively studied in freshwater systems than in marine systems. Compared to marine systems, freshwater systems are relatively contained either as running water within a channel or as a body of water, such as ponds and lakes. Here we

present biological control methods that have been developed, and in some cases implemented in the field, for use as part of IPM strategies for invasive and native disease-transmitting freshwater species.

4.2.1 Predators and herbivores

Control programs of aquatic weeds commonly include the use of herbivorous fish (Chilton, 2018; Hofstra *et al.*, 2014; Varshney *et al.*, 2007) and many species of herbivorous insects (Cock *et al.*, 2009; Varshney *et al.*, 2007). Of these, the insects generally have higher specificity and therefore lower impact on non-target species. The snail vectors of human schistosomiasis can also be partly controlled by predators including crayfish (Mkoji *et al.*, 1999) and the river prawn *Macrobrachium vollenhovenii* (Savaya Alkalay *et al.*, 2014; Sokolow *et al.*, 2015). The use of fish, amphipods and cyclopoid copepods has been tested to control virus-transmitting mosquitoes. For example, in northern Queensland the non-native fish species *Gambusia holbrooki*, and possibly also *Poecilia reticulata*, *Xipophorus maculatus*, *Xipophorus hellerie*, were introduced for biological control of mosquito larvae (Webb, 2007) but any potential effects, either beneficial or detrimental, were not examined. However, recently there have been several warnings against reliance on this approach, in particular for mosquitoes such as *Aedes aegyptii* that can reproduce in small stands of water where it is difficult to introduce insectivores (Azevedo-Santos *et al.*, 2017; Coelho, Henry, 2017).

4.2.2 Microbial agents

The common carp *Cyprinus carpio* is a declared pest in Australia. This species now comprises the majority of fish biomass in south-eastern Australia and causes a wide range of environmental impacts (Smith *et al.*, 2009). A range of management methods have been explored, including the possible release of the cyprinid herpesvirus 3 (CY HV-3, also known as Koi herpes virus) as part of an IPM program. This virus, which is exotic to Australia, is a well-known pathogen that causes large financial losses in the carp aquaculture industry worldwide (Sunarto *et al.*, 2014). The virus has been extensively characterized, including its host range, epidemiology, complete genome sequence, and its effect on immune gene expression in the host (McColl, 2016; McColl, 2013; McColl *et al.*, 2014; Sunarto *et al.*, 2014). A freeze-dried virus preparation has been developed and this will likely be the form used for delivery if the carp control project develops into a full-scale biocontrol program (McColl, 2016). An assessment of the feasibility of using CY HV-3 as a biological control agent for carp was delivered to the Australian Government (Department of Agriculture, Water and the Environment) in January 2020 as part of the National Carp Control Plan (https://www.carp.gov.au), but the plan is not publicly available. The plan will be updated with outputs from still ongoing research and represents only one of several inputs to inform the final decision by the Australian, state and territory governments (https://www.carp.gov.au/en/what-we-are-doing/plan-update).

Invasive freshwater crayfish species are a major threat to native ecosystems in the US and in the UK, and viruses, bacteria, protists, fungi and nematodes have been tested or discussed as possible biocontrol agent (Davidson *et al.*, 2010; Longshaw *et al.*, 2012; Stebbing *et al.*, 2014). In Arizona, Davidson and coworkers (2010) experimentally exposed the introduced crayfish *Orconectes virilis* to a range of potential biocontrol agents. They found that white spot syndrome virus (WSSV, dsDNA) was highly pathogenic to *O. virilis* and easily transmitted by cannibalistic behaviour. Their studies further indicated that WSSV would not be transmitted to

other crayfish individuals via freshwater (Davidson *et al.*, 2010). However, it seems unlikely that this approach will ever proceed given that WSSV is a reportable disease in many jurisdictions (Animal Health Committee, 2016). Thus, intentional release is unlikely to be approved especially in areas currently free of WSSV (Stebbing *et al.*, 2014). The occurrence of related, and in some cases endangered native crayfish in neighbouring states presents additional concerns (Davidson *et al.*, 2010).

Stebbing *et al.* (2014) suggested that some crayfish viruses such as the signal crayfish virus could be host specific, and that these could be of interest if specific crustacean cell lines could be developed and the viruses were fully characterised, including their host range. Most known crayfish pathogens were rejected as good candidates for biocontrol primarily based on their lack of host specificity (Stebbing *et al.*, 2014). A few were recommended for further evaluation, including a *Spiroplasma* bacterium that infects testes in the crayfish *Pacifastacus leniusculus* and appears to reduce sperm production (Longshaw *et al.*, 2012) as well as microsporidia morphologically similar to the genus *Thelohania*, and the mesomycetozoean genus *Psorospermium*. While there are no reports on the use of microsporidia for biocontrol in aquatic systems, a detailed overview of the epizootiology of microsporidia in aquatic invertebrates can be found in Solter (2014). The possibility of using the oomycetes *Saprolegnia* spp. and *Aphanomyces* spp. for biocontrol of crayfish has also been discussed due to their association with diseased crayfish (Stebbing *et al.*, 2014). It was concluded that the former is not a good candidate due to a requirement for epicuticle damage and that very limited knowledge is currently available for the latter.

Mosquitoes that are known vectors of human disease agents have been the focus of several biological control approaches, with varying degrees of success. *Bacillus thuringiensis* subspecies *israeliensis* has been used in aquatic environments such as salt marshes, wetlands, rivers, streams and creeks for control of mosquitoes and black flies. Since the application efficiency and duration of larval exposure is reduced in aquatic relative to terrestrial habitats, frequent re-application is required. It is expected that improvements in formulations and delivery systems will increase efficiencies in aquatic habitats (Lacey *et al.*, 2015). *Lysinibacillus* (*Bacillus*) *sphaericus* has a narrower host range than *B. thuringiensis* subsp. *israeliensis*, being specifically toxic to *Culex* mosquitos. This bacterium is in commercial production, however problems with resistance development in some *Culex* populations have been reported (Lacey *et al.*, 2015). Strategies that involve release of adult mosquitoes are discussed in sections. 4.1.2 and 4.1.5.

The oomycete *Lagenidium giganteum* was approved by the US EPA in 1995 (tradename Laginex) as a biocontrol agent of mosquitoes but was voluntarily deregistered in 2011 by the commercial producer (Vilela *et al.*, 2015). It is unknown if this was linked to the description of several closely related strains that were pathogenic to mammals including humans (Vilela *et al.*, 2015). The use of entomopathogenic fungi against mosquitoes has also been explored (Lacey *et al.*, 2015), and in a recent development a mosquito-specific fungus was genetically engineered to produce scorpion and spider toxins (Bilgo *et al.*, 2017) (see also 4.1.5.).

Invasive zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*) have caused significant ecological and economic damage in North America, including ecosystem disturbances, negative impacts on recreational water use, and severe impacts on industries relying on surface water to produce drinking or irrigation water (Donrovich, 2018; Waller,

Luoma, 2017; Whitledge *et al.*, 2015). A naturally derived molluscicide, Zequanox®, consisting of spray-dried dead cells of the bacterium *Pseudomonas fluorescence* strain CL145A, has been approved by the US Environmental Protection Agency for use on point source discharge and open water systems (Waller, Luoma, 2017). The commercial spray-dried powder formulation is mixed with onsite water and injected into the water system. The mussels consume these particles over several days to weeks, resulting in their death (Donrovich, 2018; Whitledge *et al.*, 2015). Since the bacterial cells in this product are dead, they will not proliferate and establish in the environment, so repeat application may be necessary depending on mussel recruitment. On the other hand, the product represents an example of an alternative way of utilising bacteria and/or their virulence factors to control pests in aquatic environments without the risk of uncontrolled proliferation and spread.

4.2.3 Multicellular parasites

We did not find examples of the use of multicellular parasites in any active biocontrol programs in aquatic systems. It has however been suggested that the oligochaete *Chaetogaster limnaei* has potential use in preventing parasitic diseases vectored by freshwater snails (e.g. fascioliasis, schistosomiasis) as it eats the human-pathogenic trematode larvae in the snail host (Kwenti, 2017; Sokolow *et al.*, 2018). It has also been suggested that other trematodes could out-compete the schistosomiasis-causing trematode or that they could be used to castrate the snail vector, however there is a risk that this would instead facilitate trematode infection via reduced host immune system defences (Sokolow *et al.*, 2018). Another approach that is extensively used in the Caribbean is to outcompete the trematode-carrying snail vectors by adding other snail species that are non-carriers (Sokolow *et al.*, 2018). The latter approach can be long-lasting if the introduced snails are self-sustaining.

4.2.4 Push-pull strategies and semiochemicals

The use of semiochemicals to control invasive species has been explored in aquatic habitats but few strategies have been tested extensively in the field or on an operational scale. One exception is the management of sea lampreys in the Great Lakes of North America, which has included management scale tests of traps baited with a major component of the sea lamprey male mating pheromone, the bile alcohol 3-keto petromyzinol sulfate (3kPZS) (Buchinger *et al.*, 2015; Johnson *et al.*, 2013). This pheromone attracts sexually mature females and can be used to drive them upstream and into traps (Johnson *et al.*, 2009). In years when traps were baited with 3kPZS the trapping efficiency increased by 10% relative to other years (Buchinger *et al.*, 2015; Johnson *et al.*, 2013). A combined push-pull strategy using an alarm cue on one side of the stream and a 3kPZS-baited trap on the other side of the stream decreased the time taken to locate a trap, but did not further increase the trapping efficiency (Hume *et al.*, 2015). While only a modest increase in trapping efficiency has been obtained in the field so far, there is scope for improvement (Buchinger *et al.*, 2015; Johnson *et al.*, 2015). For instance, since the natural whole odour of males catches a larger proportion of sea lampreys relative to 3kPZS alone, identification of additional odour components combined with refined trapping methods may lead to further improvements (Buchinger *et al.*, 2015; Johnson *et al.*, 2015). A cost-effective device where 3kPZS is slowly emitted from polyethylene glycol was recently developed and used as trap bait in the Great Lakes tributaries (Wagner *et al.*, 2018).

In addition to using pheromones to bait traps, it has been suggested that pheromones involved in synchronizing reproductive endocrinology can be used to monitor the presence of fish, or

that they can be added in order to disrupt mating synchrony (Sorensen, Johnson, 2016). Work on sea lamprey pheromone antagonists is ongoing (Sorensen, Johnson, 2016) but field results have not yet been published. It has also been proposed that adding priming pheromones in skewed ratios and quantities could possibly disrupt sexual maturation in sea lampreys and carp (Sorensen, Johnson, 2016).

The use of pheromones has also been tested and explored in the field for control of carp. Researchers at the University of Minnesota were funded via the Invasive Animals Cooperative Research Centre (IA CRC) in Australia to characterise pheromones produced by carp and test delivery strategies (Lim, Sorensen, 2010), followed by field studies in New South Wales and Tasmania (PestSmart, 2014a). The field studies utilised females with a surgical implant that slowly released a synthetic prostaglandin PGF2α to attract male carp into traps. Such females release PGF2α concentrations that are at least 1000 times higher than in naturally ovulating females and can stimulate a volume of water corresponding to an Olympic swimming pool for two weeks (PestSmart, 2014b). Field studies demonstrated that the timing of the deployment is crucial, and so far high capture numbers have not been achieved in the field (PestSmart, 2014a). So while the approach has potential as a species and life-stage specific approach, the technology is still in the development phase (PestSmart, 2014b). The use of semiochemicals is not particularly effective without water flow as diffusion alone gives little in the way of directional information. In contrast, an odour stream generated by current can be received in a far field environment. To improve attractiveness in relatively stagnant water bodies, such as a pond or lake, the use of auditory signals (attractant and repellent sounds) have been tested, albeit so far with limited success (Thwaites, Fredberg, 2013; Zielinski, Sorensen, 2017).

Suggested types of pheromone control for invasive crayfish include the use of attraction pheromones for traps, brood pheromones to attract juveniles or disrupt parental care, and alarm chemicals or repellent stimuli to deter crayfish from certain areas and prevent further spread (Stebbing *et al.*, 2014). Of these strategies, the use of putative attraction pheromones (excreted by sexually receptive females) to specifically lure adult males into traps has been tested in the field (Aquiloni, Gherardi, 2010; Stebbing *et al.*, 2004). Pheromone-baited traps for Signal crayfish (*Pacifastacus leniusculus*) used Phytagel embedded extracts of conditioned water and did attract males only, however the number of males was not significantly higher than the number of males capture in food baited traps (24 hours exposure) (Stebbing *et al.*, 2004). Similar results were found by Aquiloni, Gherardi (2010) who used live sexually receptive red swamp crayfish (*Procambarus clarkia*) or food as attractants in traps (48 h exposure). They found that the live females mainly attracted males, however traps baited with food captured a higher number of individuals. It is possible that trap efficiency could be improved with purification and/or increased concentrations of the putative pheromone(s) (Stebbing *et al.*, 2004) and improved delivery technologies. The putative sex pheromones of signal crayfish were shown to be repellent to native white-clawed crayfish, suggesting that the sex pheromone represents a method to selectively bait the invasive species of interest (Freeman *et al.*, 2010). This is in contrast to food baited traps, which are generally non-selective.

4.2.5 Genetic biocontrol

The potential to use genetic modifications to control invasive fish species as part of an IPM strategy has a relatively long research history, including in Australia (Thresher *et al.*, 2014b).

The production of sterile male or female fish has been achieved by creating triploids, where the odd number of chromosomes leads to meiotic dysfunction (Thresher *et al.*, 2014b). Triploid animals are generally not regarded as genetically modified since there is no modification of individual chromosomes or genes. Triploid individuals can be produced either via physical treatment (thermal or hydrostatic pressure) or via chemical treatment (e.g. cytochalasin B). Triploids are used in aquaculture operations, in recreational fisheries and in biocontrol programs to maximise growth (by minimising investment in gonad development) and minimising the risk of escaped animals being able to establish in the environment. For example, triploid carp are used for aquatic weed management (Chilton, 2018) and the triploid salmon AquAdvantage was recently approved for production, sale and consumption in the US and Canada (U.S. Food & Drug Administration, 2020).

Another strategy that manipulates whole chromosomes in fish is the so-called Trojan Y chromosome approach where Trojan females and super-males, both of which carry two Y chromosomes and therefore produce only male offspring, can be created via selective breeding and use of hormones to sex-reverse juveniles (Thresher *et al.*, 2014b). The possibility that release of super-males may drive the sex ratio to 100% males has not yet been tested in the field, but the survival and reproductive success of brook trout super-males in the wild was recently tested by releasing 2000 super-males in creeks in Idaho (Kennedy *et al.*, 2018). The released fish survived and spawned successfully with wild fish producing only male progeny, confirming the potential of this strategy to eradicate the non-native brook trout.

Importantly, strategies that manipulate whole chromosomes such as triploidy and Trojan Y are suitable only for organisms that have obligate chromosomal sex determination. If autosomal genes or environmental cues are involved in sex determination then alternative strategies have to be found (Thresher *et al.*, 2019).

Repressible lethal systems have been tested in laboratory fish such as zebrafish, channel catfish, medaka and common carp (Thresher *et al.*, 2009; Thresher *et al.*, 2014a; Thresher *et al.*, 2014b). In principle, they use a stage and/or sex-specific promoter, a blocker for a critical developmental gene or a cell-death sequence, and a repressible element. For example, channel catfish (*Ictalurus punctatus*) with integrated transgenic lines over-expressing a dorsalising gene (*BMP2)* showed 95.6% embryo mortality which could be repressed to wild-type control with doxycycline (Thresher *et al.*, 2014b). Further, a tetracycline repressible female-lethal system targeting the vitellogenin gene (*vit1*) was successfully created in zebrafish and carp, and modelling showed the potential for using this approach to control invasive fish species (Thresher *et al.*, 2014a).

The effect of using a female lethal genetic construct (daughterless carp) and the Koi herpes virus (section 4.2.2) was modelled separately and in combination (Brown, Gilligan, 2014; Thresher *et al.*, 2014a). The model suggested that each approach separately will produce limited long-term effect but used in combination they could eradicate carp in fewer than ten generations. It has been argued that carp control in Australia should combine use of the Koi herpes virus with a genetic control mechanism, possibly including a gene drive mechanism (Australian Academy of Science, 2017; McColl *et al.*, 2018). A review of options for synergistic genetic biocontrol of carp (Wedekin, 2019) was recently prepared as part of the National Carp Control Plan process. The Trojan Y technology was considered to be the most promising. Questions relating to social acceptability and legal support were highlighted as major hurdles

that need to be addressed. This includes the acceptability of releasing sex-reversed YY individuals, and of using CRISPR/Cas9 to create a marker phenotype (mirror-type) in locally adapted carp strains. The mirror phenotype occurs naturally elsewhere and can be visually distinguished from wild-type carp enabling monitoring of success and protection from fishing.

Sex ratio distortion systems that uses RNAi knockdown or transgenes of the hormone aromatase, which is required for female sexual development, has been developed for invasive fish and other vertebrates (Harvey-Samuel *et al.*, 2017; Thresher *et al.*, 2014b). In this system genotypic females develop as phenotypically fertile males and are able to mate successfully.

The use of Trojan genes is another genetic biocontrol strategy that has been tested in laboratory fish (medaka) and modelled. The principle is to use a genetic construct that provides a fitness advantage (i.e mating advantage) while at the same time decreasing offspring viability (Muir, Howard, 2001; Muir, Howard, 2002; Muir, Howard, 2004). The theoretical possibility of enhancing mating attractiveness by increasing pheromone production in a sterile male was recently modelled for Sea Lamprey control (Thresher *et al.*, 2019). Their model found that to achieve the same control target (98% reduction within 50 years), the required number of male sterile larvae released annually dropped from 1 million to 200,000 if their competitiveness was enhanced threefold.

Gene silencing strategies have been explored for some freshwater aquaculture species. For example, the production of all-male populations of the freshwater prawn *Macrobrachium rosenbergii* can be achieved by using RNAi to silence a gene encoding an insulin-like androgenic gland hormone, and this technology has been patented (Sagi, 2013). Virus control in freshwater crustaceans via RNAi has also been successful in trials, including for redclaw crayfish (*Cherax quadricarinatus*), which had a 50% reduction in mortality when RNAi silencing a gene (B2) in the virus MrNV (Hayakijkosol, Owens, 2012) and another RNAi could drop the number of Chequa iflavirus five-fold (Sakuna *et al.*, 2018). A major hurdle for the establishment of these approaches in commercial operations is related to the development of efficient delivery methods (La Fauce, Owens, 2012).

4.3 Marine systems

The prospect of adapting biological control methods used in terrestrial environments to marine systems was first discussed in detail by Lafferty, Kuris (1996). Eight years later, Thresher, Kuris (2004) evaluated the efforts that had been made so far, and concluded that while they were low risk, they also had low probability of success. In the meantime, Secord (2003) had argued that classical biological control strategies where an exotic species is introduced to control a pest species, has higher risk in marine environments and should be avoided. This conclusion was based mainly on the fundamental differences between terrestrial and marine systems (section 3.0). The following strategies were highlighted as interesting alternatives for inclusion in IPM programs in marine systems; 1) production of sterile organisms (triploid or otherwise), 2) artificial selection or mass rearing of native enemies (augmentative biocontrol), and 3) population inviability analysis to attack a pest at its most vulnerable life stage. More recently, Atalah *et al.* (2015) concurred with Secord (2003) in discouraging the use of classical biological control for marine systems and arguing that augmentative biocontrol can be successful. Here we provide an updated overview of experimental studies and applied biocontrol programs that have been undertaken in marine systems.

4.3.1 Predators and herbivores

4.3.1.1 Conservation approaches

Conservation biocontrol can involve protection of predators of invasive species by introducing fishing restrictions and other measures.

One of the best studied examples of trophic flow-on effects and interaction webs in marine systems involves sea otters (*Enhydra lutris*), which have relatively small home ranges and have been heavily hunted in several regions. These attributes have enabled detailed, replicated studies to be performed in rocky reefs, soft-sediment sites, and estuaries (Estes *et al.*, 2016). The trophic cascade between sea otters, sea urchins and kelp is linked to strong top-down as well as bottom-up effects. The sea otter population status is linked to the population sizes of orcas, other whales, seals, and oceanic fishes (Estes *et al.*, 2016; Estes *et al.*, 1998; Markel, Shurin, 2015; Reisewitz *et al.*, 2006), to shifts between a kelp-dominated state and urchin barrens (Steneck *et al.*, 2002), and to the prey selection of seabirds such as gulls and eagles (Anthony *et al.*, 2008; Irons *et al.*, 1986). Additionally, sea otters prey on predatory sea stars, with flow on effects on mussel and barnacle populations (Vicknair, Estes, 2012). Other well-studied marine trophic cascades include those linked to the Atlantic cod (*Gadus morhua*), which is heavily fished on both sides of the North Atlantic (Casini *et al.*, 2009; Estes *et al.*, 2016).

The effect of marine protected areas (MPAs) on invasive species has been studied by comparing data from within and outside MPAs. A recent global review found some evidence of negative effects of MPAs on invasive/alien species (Giakoumi, Pey, 2017). However, the evidence is scarce and restricted to a few taxonomic groups and geographical regions. Existing data do not allow disentangling of contributing factors, and reports on the effects of MPAs on invasive/alien species are contradictory (Giakoumi, Pey, 2017). For example, lionfish biomass was negatively correlated with the biomass of grouper in a study that included sites outside and within a MPA (Mumby *et al.*, 2011). A later study (Hackerott et al 2013) in the same area similarly found fewer lionfish within reserves, however this could not be directly attributed to grouper biomass and was instead attributed to targeted removal by managers, dive operations and tourists (Hackerott *et al.*, 2013).

Conservation biocontrol of predators can also provide some protection to coral reefs from phase shifts towards macroalgal dominance resulting from combined effects of over-fishing, reduced water quality and indirect impacts of climate change (Hughes *et al.*, 2007). Large, roofless cages were used to exclude medium and large-sized fishes from selected sites in a protected area of the GBR Marine Park (Pioneer Bay, Orpheus Island) that experienced mass bleaching in 1988. The 30 month study suggested that protection of herbivorous fish not only protects their stocks directly but can also influence on the resilience of coral reefs to climate change by protecting recovering corals against macroalgal overgrowth (Hughes *et al.*, 2007).

4.3.1.2 Augmentation approaches

Predators have so far found little use as biocontrol agents in marine systems, with the exception of biofouling control and the use of a variety of wrasse (Labridae) species to control parasitic sea lice (isopods) in salmon pens (Faust *et al.*, 2018; Skiftesvik *et al.*, 2014; Treasurer, 2002).

Predators and herbivores are both used as a non-toxic approach to control biofouling in small-scale aquaculture operations (Fitridge *et al.*, 2012; Watson *et al.*, 2009). Additionally, competitive exclusion (competition for space) has been suggested as an alternative strategy to prevent biofouling of artificial structures by particularly problematic organisms (Atalah *et al.*, 2013a). There are, however, practical limitations that restrict large-scale use including the availability of sufficient numbers of the biocontrol agent (Watson *et al.*, 2009) and challenges with restraining mobile biocontrol agents to the infrastructure they are intended to clean (Fitridge *et al.*, 2012). It has been argued that while biocontrol can reduce the frequency of net changes, it cannot eliminate them altogether since inedible species will be selected for (Fitridge *et al.*, 2012). On the other hand, if the biocontrol agents have a commercial value in their own right there may be an opportunity for polyculture (Cook, Kelly, 2009; Ross *et al.*, 2004; Sterling *et al.*, 2016), a potential that has been noted in particular for sea urchins and sea cucumbers (Cook, Kelly, 2009; Fitridge *et al.*, 2012; Sterling *et al.*, 2016). In each case it is important to test local species for their suitability for the structure in question. For example, while sea urchins are often found to be well suited for controlling biofouling, Atalah *et al.* (2014) found that *Evechinus chloroticus* was outperformed by the turban snail *Cookia sulcata* and the abalone *Haliotis iris* for reducing and preventing biofouling, in particular on wharf piles.

The potential of using the Australian nudibranch predator (*Phyllodesmium poindimiei*) to control an invasive octocoral (*Carijoa riisei*) in Hawaii was assessed (Wagner *et al.*, 2009). The nudibranch was found to have little impact on the coral biomass and sponge overgrowth protected the coral from predation, hence this approach was not further explored (Wagner *et al.*, 2009). Another case of interest is the observed predatory control of the invasive comb jelly *Mnemiopsis leidyi* by another introduced comb jelly, *Beroe ovata*, in the Black Sea. In combination with eutrophication problems, the introduction of *M. leidyi* via ballast water caused a rapid decline of ecosystem health in the Black Sea in the 1980s (Kideys, 2002; Shiganova *et al.*, 2001). Together with measures to control eutrophication, the (most likely) accidental introduction of the specialized predator *B. ovata* led to a dramatic reduction in the *M. leidyi* population and improved ecosystem health. The disappearance of the two comb-jellies coincided, most likely due to the strong dependency of *B. ovata* on *M. leyidi* for its food supply (Kideys, 2002; Stone, 2005). Nevertheless, *B. ovata* has not been used for biocontrol of invasive *M. leidyi* in other areas such as the Caspian Sea (Stone, 2005). While biological control was listed by Richardson *et al.* (2009) as one possible management option for jellyfish, there have been no studies or trials most likely due to safety concerns.

In contrast, herbivores have been used extensively in the marine environment to control invasive algae as well as to control overgrowth of native algae. In the early 2000's a classical biocontrol approach was considered to control the invasive alga *Caulerpa taxifo*la in the Mediterranean Sea by importing the herbivorous sea slug *Elysia subornata*. Based on mesocosm experiments (Thibaut *et al.*, 2001) and modelling (Coquillard *et al.*, 2000), four major risk areas were identified: dietary switching by the imported herbivore, competition with other important groups of herbivores in the event of eradication of the target food source, introduction of pathogens, and the spread of the introduced herbivore itself. Irrespective of these possible safety risks, it was found that a major practical hurdle was the sensitivity of this slug species to the low winter temperatures of the Mediterranean Sea (Thibaut *et al.*, 2001).
Subsequently, the use of native herbivores to control invasive algae have been tested in small-scale experiments (Chiappone *et al.*, 2003; Conklin, Smith, 2005; Davis *et al.*, 2005; Macia *et*

al., 2007; Stimson *et al.*, 2007; Tomas *et al.*, 2011; Westbrook *et al.*, 2015)), and large-scale field applications (Atalah *et al.*, 2013b; Fiordland Marine Guardians, 2011; Neilson *et al.*, 2018).

In Fiordland, New Zealand, the locally occurring sea urchin *Evechinus chloroticus* was used in combination with manual removal to control the invasive kelp *Undaria pinnatifida* (Atalah *et al.*, 2013b; Fiordland Marine Guardians, 2011). This operation involved a single relocation and release of 30,000 -35,000 urchins (test size ca 11 cm) at a very high densities (>50 individuals m^{-2}), with surveys performed 3 times in the following 16 months. The urchins contributed to eradication both directly by consumption of *U. pinnatifida*, and indirectly by aiding visual searches through denuding of the indigenous kelp canopy (Atalah *et al.*, 2015). However, translocation of such high numbers of *E. chloroticus* caused significant non-target effects, inducing a shift from kelp forest to urchin barren with accompanying reduction in taxa richness (Atalah *et al.*, 2013b). Given that the non-target effects were considered to be localised and reversible, it was argued that the cost was outweighed by the benefit of reducing a more profound and irreversible ecological impact of *U. pinnatifida* establishment in an area with high conservation value (Atalah *et al.*, 2013b). Currently, *U. pinnatifida* is regarded as 'not yet established' in Fiordland. Education and regular diving inspections to remove new individuals before maturation are used to eliminate the 'seed bank' that is present in the Sunday Cove area of Fiordland (Ministry for Primary Industries NZ, 2016).

In Hawaii, the potential use of the native sea urchin *Tripneustes gratilla* to control invasive macroalgae has been extensively studied (Conklin, Smith, 2005; Pan, 2012; Stimson *et al.*, 2007; Westbrook *et al.*, 2015), and was recently tested at operational scale in combination with manual removal aided by an underwater vacuum system (Neilson *et al.*, 2018). A total of 99,000 aquaculture-produced urchin juveniles (test size 2.5 cm) were released in repeated stocking events (stocking density ~4 urchins m^{-2}) and changes in benthic cover was monitored over 5 sampling periods in the following 2 years. The study concluded that the combined approach was successful for use on patch reefs, however it is not known if release of *T. gratilla* would be as effective in continuous reef systems (Neilson *et al.*, 2018).

Several lessons have been learnt from the use of herbivores to control invasive macroalgae. Firstly, herbivore control is more effective for emerging rather than established populations of invasive macroalgae (Neilson *et al.*, 2018). Additional potential challenges and issues with herbivore control include the palatability or possible toxicity of invasive species to local herbivores (Davis *et al.*, 2005; Tomas *et al.*, 2011) and size specific feeding preferences (Neilson *et al.*, 2018; Westbrook *et al.*, 2015). Moreover, herbivore densities that are likely to be 'effective' need to be determined as well as densities that can lead to unintended flow-on effects such as the formation of urchin barrens and population outbreaks of the deployed herbivore. As part of this, it is important to assess the likelihood that released herbivores will reproduce in the wild, and that they can spread by migration (Neilson *et al.*, 2018). For coral reefs, it has been suggested that general grazing pressure on the substratum may reduce the cover of crustose coralline algae (CCA) and the survival success of coral juveniles and recruits, but so far there has been no evidence of this during natural urchin outbreaks or large-scale deployment of urchins (Neilson *et al.*, 2018).

In the Florida Keys and Caribbean coral reefs, the long-spined urchin *Diadema antillarum,* a keystone herbivore, experienced severe population crashes after a disease outbreak (1983-84), and the urchin population is still on average 8.5 times less dense than before the die-offs

(Lessios, 2016). Since then, several studies and technical reports (Chiappone *et al.*, 2003; Macia *et al.*, 2007; Williams, 2016) have investigated the effects of re-introducing *D. antillarum* to coral reefs. Overall, *D. antillarum* reduce the coverage of fleshy algae, while stimulating the coverage of corals and CCA. In particular, the growth rate and densities of juvenile coral increase at sites where *D. antillarum* are reintroduced (Chiappone *et al.*, 2003; Idjadi *et al.*, 2010; Macia *et al.*, 2007; Macintyre *et al.*, 2005). This is likely a result of an increased surface area available for settlement (possibly corresponding to CCA coverage), and reduced mortalities of settled corals from algal over-growth (Chiappone *et al.*, 2003; Idjadi *et al.*, 2010; Macia *et al.*, 2007; Macintyre *et al.*, 2005).

Several approaches and technologies have been developed for deployment of *D. antillarum* including relocation of urchins from other sites (Chiappone *et al.*, 2003; Macia *et al.*, 2007; Miller, Szmant, 2006; Nedimyer, Moe, 2006), deployment of juveniles sourced from laboratory culture (Miller, Szmant, 2006), or deployment of young adults originally settled on settlement plates in the field followed by laboratory rearing to increase their size (Williams, 2016). Challenges to *D. antillarum* augmentation include difficulties with rearing larvae to settlement in the laboratory, and the loss of urchins due to migration or predation within days to weeks. The use of settlement plates and deployment of larger individuals may overcome at least some of these challenges, while at the same time producing additional stock (Williams, 2016).

The available literature on removal of macroalgae to protect coral reefs was recently reviewed (Ceccarelli *et al.*, 2018). Manual removal (possibly assisted by scrapers or suckers) is labour intensive and is recommended that this be combined with enhanced herbivory to suppress regrowth (Ceccarelli *et al.*, 2018). The distinction between grazers that feed primarily on the epithelial algal matrix (fishes and some invertebrates) and browsers that feed on large fleshy macroalgae was found to be important. While grazers can reduce macroalgal development by consuming propagules contained in the epithelial algal matrix, they are not able to remove established stands of macroalgae. In particular, for some macroalgae, the removal of holdfasts is required (Loffler, Hoey, 2018). The ability of urchins to remove holdfasts may be size dependent (Neilson *et al.*, 2018). Other factors that influence on the success of algal removal efforts include the timing relative to the algal growth and the impact of environmental factors such as sediment on herbivore grazing success (Ceccarelli *et al.*, 2018).

Possible negative effects of macroalgal removal include damage to the substratum, injury to benthic organisms including corals, and the clearance of space that can be exploited by even more damaging algae types such as ephemeral algae (Ceccarelli *et al.*, 2018). Additionally, there is a risk of negative effects on other taxa including fishes that recruit into the macroalgae. Algal removal can also affect microbial, physical and chemical parameters (Ceccarelli *et al.*, 2018). These risks all need to be considered and assessed in each case before the decision to remove algae on a large scale is made.

4.3.2 Microbial agents

Viruses are prevalent in marine organisms however, the diversity of marine viruses is poorly known as this area of research has only recently received attention (Laffy *et al.*, 2018; Middelboe, Brussaard, 2017). While it is clear that viral diseases cause significant economic losses in marine aquaculture operations, the implication of viral agents in observed mass mortality events of wild marine species is uncertain (Bateman, Stentiford, 2017; Fey *et al.*,

2015; Hewson *et al.*, 2018). We were not able to find examples of viral biocontrol of multicellular pests in marine systems. In contrast, viruses infecting bacteria (bacteriophage) are regarded as a promising alternative to classical antibiotics to prevent and treat bacterial diseases in aquaculture (Richards, 2014). The use of bacteriophage has also been tested as a treatment option for coral disease caused by the bacterium *Vibrio corallilyticus* (Cohen *et al.*, 2013; Jacquemot *et al.*, 2018).

Bacterial biocontrol is used routinely to control microbial diseases in aquaculture production systems via the application of probiotic bacterial strains (Newaj-Fyzul *et al.*, 2014). This topic has recently been reviewed extensively elsewhere (Hoseinifar *et al.*, 2018; Newaj-Fyzul *et al.*, 2014; Sharifuzzaman, Austin, 2017) and will not be discussed in detail here.

The amoeba *Paramoeba invadens* causes a disease in the green sea urchin *Strongylocentrotus droebachiensis* in Nova Scotia (Feehan *et al.*, 2013) that is characterised by muscle degeneration in tube feet, spines and mouthparts leading to loss of attachment, cessation of feeding, immobility and death (Jones, Scheibling, 1985). The amoeba was suggested as a possible agent for biological control (Jellett, Scheibling, 1988) at a time when high urchin densities produced extensive barrens. Subsequently, recurring natural outbreaks of *P. invadens* have caused urchin mass mortalities that may permanently shift the ecosystem to a kelp bed state (Scheibling, Lauzon-Guay, 2010). This pathogen occurs naturally in the water column and in sediment, and natural outbreak cycles have been linked to seawater temperatures and re-introduction via strong storms and hurricanes (Buchwald *et al.*, 2018).

4.3.3 Multicellular parasites

Early considerations of biological control in the marine environment noted that parasitic castrators could play a similar role in population regulation as parasitoids in terrestrial environments (section 4.1.3). The two groups share a range of traits including that infection by a single castrator or parasitoid always causes reproductive death or kills, respectively, a single host (Kuris, 1974). Due to the success of parasitoids as biocontrol agents in terrestrial systems, the use of introduced castrating parasites were suggested as biocontrol agents against the invasive European green shore crab (*Carcinus maenas*) (Goddard *et al.*, 2005; Kuris *et al.*, 2005; Viney, 1996) and the invasive Northern Pacific Seastar (*Asterias amurensis*) (Viney, 1996). However, these parasites, a barnacle and a ciliate, respectively, are not species specific (Byrne *et al.*, 1997; Goddard *et al.*, 2005; Kristensen *et al.*, 2012) and consequently they are no longer recommended to be used for biocontrol purposes (Bateman *et al.*, 2017; Byrne *et al.*, 1997).

4.3.4 Push-pull strategies and semiochemicals

We did not find any examples of active control programs using push-pull strategies in open marine systems. However, several fisheries utilise traps baited with food, and in effect rely on the scent or signals that emanate from the bait to attract their target species (e.g. hook-and line, pot trap fisheries and baiting for tourism dives). The possibility of utilising specific baits or chemical compounds to repel sharks or reduce fisheries bycatch has also been explored (Jordan *et al.*, 2013; O'Neill *et al.*, 2019). Baited remote underwater video stations (BRUVS) are also routinely used for monitoring of fish in both shallow and deep waters (Whitmarsh *et al.*, 2017).

4.3.5 Genetic biocontrol

Genetic biocontrol for use in marine systems has, as far as we know, only been tested for potential use in aquaculture.

In the Pacific oyster (*Crassostrea gigas*), a proof-of-principle experiment showed that conditional knockdown of a homeobox gene (*HOXCG1)* resulted in 67% larval mortality, while larval survival could be maintained at wild type level in the presence of doxycycline (Thresher *et al.*, 2009).

The use of RNAi to control viruses has been tested for marine prawns including Pacific white shrimp (*Litopenaeus vannamei*), kuruma prawn (*Penaeus japonicas*), and black tiger shrimp (*Penaeus monodon*).For each virus, specific dsRNAs are designed that target one or more essential viral genes. It has been demonstrated that dsRNAs can reduce viral replication and mortalities of prawns exposed to viruses such as White Spot Syndrome virus (Rajeshkumar *et al.*, 2009; Robalino *et al.*, 2005; Xu *et al.*, 2007), Yellow head virus (Posiri *et al.*, 2011; Saksmerprome *et al.*, 2009), and Gill-associated virus (Sellars *et al.*, 2011). As in other environments, a major challenge for siRNA strategies is the development of efficient delivery systems (La Fauce, Owens, 2012) (section 6.4.3.3).

5.0 NEW TECHNOLOGIES: CRISPR/CAS9 AND GENE DRIVES

From a biocontrol perspective, two key advances in recent years have been the development of CRISPR gene editing methods and its combination with artificial gene-drives (Champer *et al.*, 2016; Dearden *et al.*, 2018; Esvelt *et al.*, 2014). CRISPR is a technology that allows for controlled and highly specific editing of genes (Jinek *et al.*, 2012). Unlike previous genetic technologies it does not necessarily introduce new genes to an organism's genome but rather edits the genes that are already present to modify their functioning (Hsu *et al.*, 2014). Not only is CRISPR accurate in terms of identifying specific locations in the genome for editing, but it can be designed to be highly species specific and potentially specific to individual populations (Esvelt *et al.*, 2014). This is significant as it points to an ability to limit spread to non-target species thereby conveying a higher degree of safety in field deployment than previous methods. An additional step-change is provided by the general applicability of the method, enabling genome modification of non-model organisms (Hsu *et al.*, 2014).

To be an effective technology in a biocontrol context, it is desirable that genetic modifications can spread rapidly within the population and become sufficiently represented to achieve the desired outcomes at a population level (Champer *et al.*, 2016). For most biocontrol applications, where the modification would lower fitness and therefore would be pushing against natural selection, this is likely to be too slow a process in its own right. Indeed, even a modification that is neutral in terms of selection, if initially flooded into the population, would under Mendelian inheritance be rapidly reduced to the frequency of its representation within the overall gene pool (Futuyma, 1986).

Gene drives are genetic mechanisms that cause a gene to spread rapidly through a population at rates far greater than would otherwise occur (Burt, 2003). Such biased inheritance mechanisms occur naturally in a range of taxa, however, gene-editing technologies such as CRISPR have made synthetic gene-drives relatively simple to engineer and, as a consequence, accessible for a broader range of systems and researchers. So far a number of concept studies have been published (yeast, mosquitoes and fruit-flies) which demonstrate how CRISPR gene-drives might be applied to the management of a range of issues spanning vector-borne disease, suppressing pest populations, and introducing desired traits in populations. Successful cage experiments with a CRISPR-Cas9 gene drive targeting sex determination pathways in mosquitoes have shown the technology can result in complete population suppression (Kyrou *et al.*, 2018). These trials represent a significant step towards field implementation, however release of a synthetic gene drive outside of highly controlled settings has not yet occurred (Harvey-Samuel *et al.*, 2019). While gene-drive technologies have many potential benefits, they also have unparalleled risks, both ethical, legal and biological, with implications for human health, natural systems and species, and our economies (Esvelt, Gemmell, 2017; Marshall, Akbari, 2018; Webber *et al.*, 2015) (section 5.4).

5.1 How CRISPR-Cas9 gene editing works

The antiviral defences of prokaryotes are based around a family of DNA sequences called 'clustered regularly interspaced short palindromic repeats' (CRISPR) and CRISPR-associated (Cas) genes (Jinek *et al.*, 2012). In the prokaryote immune system, the CRISPR sequences,

which are derived from DNA fragments from previous viral infections, code for nucleases which enable the detection of infection by identifying sequences of DNA that are complementary to their own DNA sequence. The Cas genes associated with these CRISPR elements then cut the DNA strands at that point to eliminate the threat (Hsu *et al.*, 2014). This natural function of the combined CRISPR and Cas proteins provides the mechanisms required for editing genes with high precision and specificity. While there are a number of CRISPR mechanisms that enable gene editing, one Cas protein, Cas9, has proven to be particularly important because it simplifies the molecular components required for gene editing to three components: Cas9, the CRISPR RNA (crRNA) and trans-activating RNA (tracRNA). This has subsequently been engineered into two components, Cas9 and a guide RNA (gRNA) which is the combined crRNA and tracRNA (Hsu *et al.*, 2014; Jinek *et al.*, 2012).

Once CRISPR-Cas9 has identified the appropriate gene and cut the DNA, the cell's repair mechanisms immediately activate to repair the break using available DNA (Ceasar *et al.*, 2016). Different Cas9 variants cut the DNA in different ways and invoke different DNA repair mechanisms, either re-joining the severed strands directly or using available DNA to make the repair. In a synthetic CRISPR-Cas9 gene drive system this latter mode of repair would utilise DNA that modified the target locus' function and which was inserted with the guide DNA to do this. If a desired genomic change is encoded along with the components of the CRISPR system, it will cut and replace the original sequence with the new version in each generation (Ceasar *et al.*, 2016; Hsu *et al.*, 2014).

A more recent development of the CRISPR-Cas9 technology, base editing (Gaudelli *et al.*, 2017), uses aspects of CRISPR-Cas9-based systems to modify individual bases without resorting to the DNA repair mechanisms or donor templates relied upon in classic double-strand breaking CRISPR-Cas9 methods. Base editing is considered an easier means of editing, does not require manipulation of entire DNA sequences, and appears to result in fewer unwanted insertions and deletions than classic CRISPR−Cas9. While still very much a developing technology, base editing may offer the potential for modifying the performance of genes through base-pair substitution rather than through gene or DNA sequence replacement.

5.2 Gene Drives – What are they?

In general, inheritance of genetic traits operates under traditional Mendelian rules of inheritance where each parent contributes one chromosome to each offspring. If each parent has a different allele at a particular locus there is a 50% chance that any one parent's gene will be inherited by any of its offspring (Futuyma, 1986). This has the effect of rapidly diluting that gene's representation in subsequent generations without additional matings with individuals carrying that gene. For example, we would expect 50% of offspring to carry the gene in the first generation, 25% in the second generation, and so on until the gene reaches its equilibrium representation.

Gene drive mechanisms bias inheritance to result in a greater than 50% chance of inheritance of a particular gene (Esvelt *et al.*, 2014). As a consequence, and all other things being equal, rather than reaching the representation in the population that would be expected given its frequency in the gene pool, the allele will spread, even without conferring a fitness advantage (Champer *et al.*, 2016). In natural gene drives the biasing of inheritance might be >50% but is almost always significantly < 100%. The magnitude of the bias determines the rate of spread.

In synthetic gene drive the goal would usually be to achieve as close to 100% as possible to ensure the rate of spread is high and to minimise the opportunity for resistance alleles to arise.

The allure of gene drives in species management contexts is that they may allow us to overcome the evolutionary disadvantage of traits that are desirable for management but not for fitness (Champer *et al.*, 2016; Esvelt *et al.*, 2014). This allows them to spread through wild populations, either to suppress those populations through transmission of disadvantageous traits or to enhance them through transmission of advantageous (Esvelt *et al.*, 2014) traits in cases where population recovery is the goal. In a CRISPR-Cas9 gene drive system the goal would be to use gene editing to target genes that influence fertility or survival and to simultaneously produce a synthetic gene drive system, most likely by mimicking one of the naturally occurring gene drive systems. When it comes to the design of the gene drive system there are a number of potential options (described below) and the choice of gene drive system will be determined by the nature of the problem and the magnitude of the intervention required.

5.2.1 Homing

Homing based drives harness the capabilities of homing endonuclease genes (HEGs). These are naturally occurring gene drives composed of an endonuclease with the ability to copy itself into the opposite chromosome during meiosis, thereby replacing the opposite 'wild-type' allele that was present. HEGs achieve this using a sequence-specific endonuclease to cut their competing allele thereby stimulating a rapid repair by the cell, which in HEG is achieved using the HEG as a template. This results in the HEG copying itself into its competing allele and renders the cell homozygotic. If this occurs in the germline or the early embryo, more than 50% of offspring will receive the HEG along with any payload genes that are associated with it. In a proof-of principle study, a sex ratio distortion system was developed for the malaria mosquito, *Anopheles gambiae* that using the homing endonuclease I-PpoI that selectively cleave sites that primarily occur on the X chromosome and are mostly destroyed during spermatogenesis resulting in a 97% transmission of Y chromosomes during mating (Galizi *et al.*, 2014).

5.2.2 Sex-linked Meiotic drive

Meiotic drives work by interfering with meiotic processes to produce modification of allelic segregation away from Mendelian inheritance patterns (Lyttle, 1993; McDermott, Noor, 2010). Meiotic drive systems are common in nature, having been reported from plants, insects, and mammals (Burt, 2003; Helleu *et al.*, 2015). These systems typically work by limiting the maturation of gametes that lack the meiotic driver resulting in the majority of functional gametes produced by heterozygotes possessing the drive (Helleu *et al.*, 2015).

The mechanisms by which meiotic drives operate are poorly understood, as are the processes producing and sustaining them in natural populations (Helleu *et al.*, 2015). The observation that in some systems the X-chromosome is broken (Newton *et al.*, 1976) has led to the suggestion that an X-chromosome cutting endonuclease could be developed to engineer synthetic sex-linked meiotic drive systems to suppress populations. While this is a possibility, sex-linked meiotic drives are likely to be susceptible to natural selection due to the selective advantage accruing to the limiting sex when sex ratios are unequal. This suggests that any resistance gene that evolves will spread rapidly through the population.

5.2.3 Maternal effect dominant embryonic arrest

Maternal effect dominant embryonic arrest (Medea) systems occur naturally in some insects and behave as modification drives by utilizing an RNA interference (RNAi)-based toxin–antidote combination. In reverse engineered versions of natural Medea systems (Akbari *et al.*, 2014) a microRNA toxin is expressed during oogenesis in Medea-bearing females, disrupting a gene that is essential for development in all embryos, regardless of whether those embryos have inherited a Medea or wild-type allele. An antidote consisting of a recoded version of the target gene that is immune to the effect of the micro RNA toxin is subsequently expressed early in embryogenesis (during the zygotic phase) in those embryos that inherit the Medea element. This results in the survival of only the 50% of the embryos that inherit the Medea element. If the female mates with a Medea-bearing heterozygous male, the antidote from the male will also take effect in the embryo, resulting in 75% of the embryos surviving, all with the Medea element. Consequently, if a payload gene is linked to the Medea element it will possess a frequency-dependent fitness advantage compared to chromosomes lacking Medea and will be rapidly driven through the population (Ward *et al.*, 2011).

5.2.4 Underdominance gene drives

Underdominance refers to situations where heterozygotes have lower fitness than homozygotes and has been documented from nature in a range of species. For example, it plays a role in susceptibility to risk of developing diabetes in humans (Lawson *et al.*, 2011). In a gene drive approach based on underdominance a payload gene that reduced fitness would be linked to the underdominant gene. At low frequencies within a population, individuals carrying the underdominant gene would be unlikely to mate with other underdominant individuals and the trait would be lost. However, at higher frequencies such matings would occur frequently and the trait would move to fixation. The threshold at which this would occur will be determined by the fitness cost of the associated payload gene (Curtis, 1968).

Underdominant gene drives based on combinations of RNA-based toxins and antidotes have been proposed and implemented in the fruitfly (*Drosophila melanogaster*), both as a proof-of-principle system, and as fully functional systems capable of invading wild populations (Akbari *et al.*, 2013; Reeves *et al.*, 2014). However, engineered underdominant systems carrying a payload gene have not yet been constructed. A range of approaches using CRISPR-Cas9 for engineering such a system are possible (Champer *et al.*, 2016) and would likely prove portable across taxa with the potential to generate species-specific underdominant gene drives that are profoundly evolutionarily stable. While the spread and stability of underdominance systems require a high proportion of the population to possess the trait, the release threshold needed for the spread of an underdominance gene drive system with a moderate fitness cost would not be extreme, just under 60% for a translocation homozygote with a 20% fitness cost (Champer *et al.*, 2016). The fact that restriction to local populations is likely due to the requirement for high release thresholds and their ability to be removed completely by the release of large numbers of wild-type organisms, underdominant gene drives are an attractive option.

5.2.5 Heritable Cytoplasmic Incompatibility Agents

This approach doesn't focus on directly manipulating the target species genome but rather uses bacteria or maternally transmitted cytoplasmic factors, such as mitochondria or bacteria,

to spread genes that trigger incompatibility between eggs and sperm, kill male zygotes, or cause male sterility. Such agents are maternally inherited and persist due to a rescue function which allows normal development when infected males and females mate. Heritable cytoplasmic incompatibility agents have already been harnessed in the laboratory and in the field in mosquito (*Aedes aegypti*) control programs using the intracellular bacteria *Wolbachia* (O'Neill *et al.*, 2018). *Wolbachia* are Gram negative bacteria which modify host reproduction in a variety of ways to reduce fitness. While *Wolbachia* have been used successfully to reduce mosquito populations in the wild, genetic engineering of *Wolbachia*, or of any other cytoplasmic factors, to produce particular effects has not been achieved. Recently however, the genetic mechanism of *Wolbachia*-induced cytoplasmic incompatibility was attributed to two prophage WO genes (*cifA* and *cifB*), opening the way for further studies on reproductive parasitism with possible implications for pest control (LePage *et al.*, 2017).

5.3 Inherent limitations: Evolution of resistance

The inheritance and spread of any trait is influenced by selection for or against that trait itself or traits that are associated with it. Indeed, any factor that exerts negative selection on a population will select for the evolution of traits that avoid that negative selection. In the context of IPM this is referred to as resistance. Ecological resistance occurs when the genetic modifications result in changes that influence the success of carriers in securing matings, e.g. by reducing their attractiveness to mates. When the strategy underpinning the manipulation is to develop inter-breeding but genetically incompatible populations, as is the case for underdominance systems, one is attempting to establish precisely the circumstances that lead to the evolution of reproductive isolation (Noor *et al.*, 2001). Molecular resistance refers to the situation where genetic changes impair the performance of the inserted sequence in exerting the desired phenotype.

Different approaches are differently susceptible to resistance and to different types of resistance and as a consequence the approach taken for avoiding the evolution of resistance will vary (Bull, 2015). Perhaps the simplest means of avoiding resistance is to include multiple edits for each outcome thereby increasing resistance by multiplying the requirement for evolved resistance to be effective (Marshall *et al.*, 2017; Noble *et al.*, 2017). The design requires that each targeted mutation must be located within an essential gene and linked to specific sequences that permit cutting. Whether such conditions can be met in the population would have to be determined on a case by case basis and ultimately cannot be easily predicted, however, where population suppression is the goal and a long term solution is required, resistance must be factored into the design (Leftwich *et al.*, 2016).

5.4 Potential Hazards

Gene drives are attractive as tools in conservation and ecosystem management (Gould, 2008; James *et al.*, 2018; Kinnear, 2018; Thresher *et al.*, 2014b) but, the use of these tools in open ecological systems will require careful consideration because the very traits that make them so alluring also make them so potentially risky (Esvelt, Gemmell, 2017; Prowse *et al.*, 2017; Webber *et al.*, 2015).

First, the ability to engineer desirable genetic outcomes carries with it the likelihood that there will be unintended ecological, biological or genetic consequences (Australian Academy of

Science, 2017; Esvelt, Gemmell, 2017; Marshall, Akbari, 2018; National Academies of Sciences, 2016; Prowse *et al.*, 2017; Webber *et al.*, 2015). Such a situation might arise because, for example, the ecological context being managed changes over time. In the case of CoTS outbreaks, control of CoTS may not be warranted outside the outbreak period when CoTS densities are low. Furthermore, while the specific trait change may be desirable, the flow-on effects of this change, e.g. a change in community structure due to the decrease in the abundance of the target species, may ultimately also be undesirable. Similarly, at a genome level little is known about the non-target genetic effects of changing particular genes. An edit of a locus may change its function, e.g. knock it out, but in the process may also modify other genetic or physiological processes, with unknown consequences.

The second concern is the capacity of gene drives to spread a desired trait among populations connected by gene flow or into distant populations due to human-mediated dispersal. This trait is desired in a pest control setting because it can reduce the cost and difficulty of spreading the trait in the system being managed. However, the potential for uncontrolled spread is high, especially in the case of gene drives such as CRISPR-Cas9 based homing drives, which may be capable of unlimited spread within the range of the target species (Alphey, Bonsall, 2014; Burt, 2003; Dhole *et al.*, 2018). This capacity to spread makes the modified organism functionally an invasive organism (Esvelt, Gemmell, 2017). Uncontrolled spread of a gene drive may have significant environmental effects in locations where those effects are not appropriate or, where spread crosses jurisdictional boundaries, to places where the change is not wanted (Esvelt, Gemmell, 2017). Indeed, spread beyond national borders without consent may represent a violation of the Cartagena Protocol (Convention on Biological Diversity, 2016).

5.5 Limiting Spread: Precision Drives, Daisy Chains

A number of options for developing gene drives with limited spread or persistence have been considered and modelling suggests that they may prove to be effective in the field.

The first approach to limiting the spread potential of any gene drive system is to develop precision gene drives, i.e., drives that target loci specific to a particular local population. This of course requires that such loci exist, and, that they are indeed exclusive to that population – both unlikely in highly connected populations such as CoTS. Precision gene drives are likely to be particularly susceptible to evolved resistance because any individuals in the population without the target loci will be at a selective advantage. In theory, increased specificity to a local population and decreased susceptibility to evolved resistance could be achieved by using multiple loci (Esvelt, Gemmell, 2017). Recent developments in the design of guide RNAs create the possibility of including self-limitation through self-targeting single guide RNA (sgRNAs) that limit the duration of Cas9 expression and greater specificity through modulating Cas9 levels (Moon *et al.*, 2019; Petris *et al.*, 2017; Ryan *et al.*, 2017). If transferred to field applications in the future these tools may provide both temporal and geographic limitations.

A second approach to limiting spread is to carefully match the selected CRISPR-Cas9 gene drive systems with the particular pest species and implementation strategy. There are three basic approaches to CRISPR-Cas9 gene drives; 1) threshold dependent drives, e.g. translocations and under-dominance systems, 2) threshold independent drives, e.g. Medea and homing-based systems, and, 3) temporally self-limiting drives, e.g. killer-rescue and daisy chain systems. Both threshold dependent and independent systems could theoretically be

limited under particular population structures and dynamics and careful matching of these drive systems to the particular context may provide a means of achieving limited or temporary spread (Marshall, Akbari, 2018). For example, threshold dependent drives require that an introduction exceeds a critical threshold in order to spread throughout the population, if the threshold is not exceeded, the construct is lost from the population through dilution. Population suppression could be achieved with introductions that don't exceed the threshold but which are sufficient to limit reproductive output. Alternatively, where populations have only limited connectivity, an introduction above the threshold in one population may result in its elimination but connected populations receiving only limited immigration would not exceed the threshold and the drive would not spread. The question would be whether appropriate conditions could be found and maintained in open ecological systems (Marshall, Akbari, 2018).

It is also theoretically possible to engineer CRISPR-Cas9 gene drive systems to be self-limiting. The simplest example of an engineered self-limiting system would be a two-locus system known as "killer-rescue" (Bull, 2015; Thresher *et al.*, 2019). This would comprise two alleles at unlinked loci, one coding for a toxin and the other coding for immunity to that toxin. A release of individuals homozygous for both alleles would result in temporary gene drive as the alleles segregate since the presence of the killer allele in the population confers a benefit to those individuals which also carry the rescue allele. However, as the killer allele declines in frequency due to Mendelian inheritance, the selective benefit of the rescue allele is lost and any fitness cost associated with it will result in selection against it and it should be eliminated from the population (Champer *et al.*, 2016).

An alternative approach is the daisy-chain drive (Noble *et al.*, 2019). These systems are envisaged to comprise of a sequence of linked CRISPR components, e.g. A, B, C, that are introduced to form a single CRISPR-Cas9 gene drive system. Rather than being introduced at a single site they are scattered throughout the genome but, despite this physical separation, they are functionally linked with each element driving the next one in the chain (Noble *et al.*, 2019). In other words, A requires B to be present to function while B requires C to be present. The last element of the chain (e.g. element C) isn't a gene drive and so its abundance in the population is limited by the number of daisy drive organisms released. Furthermore, because edits to the genome will be resisted by natural selection, it can be expected that element C will be lost from the population at a rate determined by the strength of selection against it. As a consequence, the preceding element (e.g. element B, itself a gene drive) will initially increase in abundance while C remains in the population before declining and ultimately being lost as C is lost. The next element (e.g. element A; another gene drive) will follow a similar but more amplified trajectory, initially increasing rapidly before running out of B and disappearing from the population. The more links included in the daisy chain the greater the spread potential of the system. For example, a five-element chain would be hundreds of times more effective in terms of persistence and spread within the population than one with only a single daisy element (Noble *et al.*, 2019).

An alternative form of daisy chain type limiting mechanism would be the daisyfield drive (Min *et al.*, 2017). In this proposed system there would be just two elements. The first would be a non-drive element (guide RNA only) which would have to be present in order for the drive element, the second element, to be activated. By including numerous copies of the non-CRISPR element throughout the genome the time taken to lose these from the genome (with

roughly half being lost with each mating) would be lengthened. Ultimately the non-drive element would become so rare in the population that the drive element would also be lost.

5.6 Consequences of Self-Limitation

While self-limitation is highly desirable, it does not, however, come cost-free; there is a trade-off between the potential for spread of a construct through a population and the need to manage that spread for ecological, legal or ethical reasons. In theory, the greater the spread potential of a construct, the faster and more comprehensive its spread will be and the longer lasting its effect. With no limitation the costs and infrastructure required to achieve an objective should be relatively low, reflecting lower introduction threshold requirements, fewer introductions and longer effect times.

As some degree of self-limitation is introduced the associated costs begin to increase. The greater the degree of self-limitation imposed the fewer generations the effect lasts for and the more-geographically limited its impact. These limitations mean that to treat an area greater than the spread potential of the construct during the period it retains its effectiveness, more genetically modified organisms must be deployed across more sites to ensure coverage. This has significant implications for the scale of the operation required to underpin the exercise, with larger breeding facilities and greater investment in related infrastructure.

6.0 POTENTIAL BIOLOGICALLY BASED TECHNOLOGIES FOR COTS CONTROL

Efficient protection of coral reefs against predation from CoTS has been identified as an integral part of efficient reef conservation and restoration efforts. Here we outline possible biologically based technologies that could be used as part of an IPM strategy for CoTS. In each case, we present the state of knowledge and identify knowledge or technology gaps. We also consider the risk involved with each approach, their likelihood of success in reducing loss of coral cover from CoTS predation, and their likelihood of obtaining social licence and regulatory approval.

6.1. Predators and coral-symbiotic fauna

6.1.1. Background knowledge

The predator-removal hypothesis (Endean, 1969) is one of several hypotheses put forward to explain circumstances that can lead to CoTS outbreaks (Pratchett *et al.*, 2014). Predation may impact recruitment rates of CoTS larvae into settlement (Cowan *et al.*, 2017), and may change growth, reproduction or mortality of CoTS post-settlement (Antonelli, Kazarinoff, 1984; Endean, 1969; Keesing, Halford, 1992a; Keesing, Halford, 1992b; Keesing *et al.*, 2018; Ormond *et al.*, 1990). Despite the lack of field observations of CoTS predation, several independent modelling studies have provided support for the predator-removal hypothesis (Antonelli *et al.*, 1990; Condie *et al.*, 2018; McCallum, 1987).

A recent review identified 80 species of coral reef organisms, including 56 species of coral reef fishes and 24 motile and sessile invertebrates, that prey on different life history stages of CoTS (Cowan *et al.*, 2017). New research has increased this to a total of 80 species of coral reef fish from 17 families reporting to have fed on *Acanthaster* spp. based on field and laboratory observations (Kroon *et al.*, 2020). The majority of these records comprise predation on injured and moribund adult CoTS or dead individuals (Cowan *et al.*, 2017; Kroon *et al.*, 2020). In contrast, only a few field observations document consumption of early life history stages and of healthy adults (Cowan *et al.*, 2017; Kroon *et al.*, 2020).

We do not have a good understanding of predation on early life history stages of CoTS due to the difficulties of observing predation on CoTS early life history stages, such as gametes, larvae, and settled juveniles (Yokochi, Ogura, 1987; Zann *et al.*, 1987). Under controlled laboratory conditions, a wide range of damselfish species readily feed on CoTS eggs and larvae, including in the presence and in preference of Blue Seastar (*Linckia laevigata*) (Cowan *et al.*, 2016; Cowan *et al.*, 2017). Moreover, feeding on pelagic CoTS larvae in the field is probable based on the presence of CoTS DNA in faecal samples collected from six different damselfish species (Kroon et al 2020). For adults, predation can be sublethal as indicated by the large number of CoTS with lost appendages (Budden *et al.*, 2019; Messmer *et al.*, 2017). Sublethal injury has a significant effect on gonad production in CoTS (Budden *et al.*, 2019).

Healthy adult CoTS are unlikely to have many predators, due to their surface cover of toxic spines and their diverse saponin chemical defence profile (Claereboudt *et al.*, 2019; Kitagawa, Kobayashi, 1978; Li *et al.*, 2018). Despite this, the remains of CoTS that had most likely been attacked by a predator have been observed on the reef (Figure 1). Some species are well-

known predators of adult CoTS, including the giant triton snail (Cowan *et al.*, 2017; Endean, 1969; Endean, Stablum, 1973; Moran, 1992), pufferfishes and triggerfishes (Ormond, Campbell, 1974), harlequin shrimp and the lined fireworm (Glynn, 1984). Nevertheless, no single predator that appears to play a disproportionate role in regulating CoTS populations (Cowan *et al.*, 2017). Indeed, the predator-removal hypothesis proposes that the severity of CoTS outbreaks may be mediated by the combined consumption of CoTS by a range of predators at one or more life history stages (Cowan *et al.*, 2017; Endean, 1969).

The relative success of CoTS and their different predators under future climate conditions is uncertain and should be addressed in future studies.

Figure 1: CoTS with injuries likely due to predation (Photo: F. Kroon)

6.1.1.1 Fish

The potential role of fish predators in mitigating the size and frequency of CoTS population outbreaks is currently being evaluated as part of current NESP TWQ CoTS IPM research. Specifically, this project re-examines CoTS consumption by coral reef fish species by using new DNA technologies to detect Pacific Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*) in fish faecal and gut content samples. Using a digital droplet PCR (ddPCR)-based method, the faeces and gut contents of 18 out of 101 coral reef fish species (678 individual fish), captured on reefs with CoTS outbreaks, tested positive to CoTS eDNA (Kroon *et al.*, 2020). These included six damselfish species likely feeding on planktivorous CoTS larvae, and 12 fish species that would have preyed on settled CoTS; nine of these have not been previously reported as potential CoTS predators. Of the fish species known to prey on settled CoTS, samples from five Emperor (Lethrinidae) species, two of which are popular fisheries species, namely *Lethrinus nebulosus* (Spangled emperor) and *Lethrinus miniatus* (Redthroat emperor), were confirmed to contain CoTS eDNA (Kroon *et al.*, 2020).

6.1.1.2 Giant triton

The potential role of the giant triton snail (*Charonia tritonis*) in mitigating CoTS populations has been reviewed and investigated, including in alternate CoTS push-pull control strategies (Hall *et al.*, 2016; Hall *et al.*, 2017a; Hall *et al.*, 2017b).

Little direct information is available on the current population level of giant tritons on the GBR but they are generally reported to be rare, a situation that is in contrast to the early years of the last century (Hall *et al.*, 2017b). It is not clear whether this is due to natural causes or whether harvesting, which ceased in the GBR nearly 50 years ago, caused the population to decline beyond the point of no recovery (Hall *et al.*, 2017b). Either way, focussed restocking and conservation would be required if the giant triton snail were to be used as a biological control agent for CoTS on the GBR (Hall *et al.*, 2017b).

The giant triton primarily feeds on echinoderms with a preference for asteroids, and its presence induces strong evasive behaviour in CoTS. The latter response can be mediated by exposing CoTS to water where tritons have been present, and hence both the giant triton snails themselves and specific chemicals that they produce have potential use in CoTS control by creating a so-called “landscape of fear” (Hall *et al.*, 2016; Hall *et al.*, 2017a; Hall *et al.*, 2017b) (section 6.3.1.4). A study of the movement behaviour of giant tritons on the GBR using acoustic telemetry suggested that they can traverse a reef and that an individual’s range is likely larger than a single reef (Schlaff *et al.*, 2019). The ability of giant tritons to alter CoTS behaviour and possibly prevent or delay aggregations in the field has not yet been established.

The hunting behaviour of the giant triton towards CoTS in captivity has been described in detail, including movement patterns, proboscis extension, penetration of the CoTS central disk, and likely injection of toxins (Bose *et al.*, 2017b). A combined transcriptome and proteome analysis of the giant triton snail salivary gland identified several proteins expected to be involved in saponin neutralization or degradation (arylsulfatases) and prey attack (venom-like proteins, sulfuric acid biosynthesis enzymes) (Bose *et al.*, 2017b). While consumption rates might be relatively low, adult CoTS that have suffered a snail attack but were not eaten are unlikely to survive due to the injection of the snail’s lethal toxin (Motti *et al.*, 2019).

The reproductive biology of the giant triton snail has been described (Motti *et al.*, 2019), and neuropeptides with possible function in reproduction identified from the transcriptome of various tissues (Bose *et al.*, 2017a; Motti *et al.*, 2019).

6.1.1.3 Other benthic predators and coral-symbiotic fauna

Corals prey on CoTS larvae (Chesher, 1969; Yamaguchi, 1974), and epifaunal invertebrates (e.g. polychaetes and shrimp) prey on settling larvae and smaller CoTS within the reef matrix (Cowan *et al.*, 2017; Glynn, 1984). The CoTS-predatory polyp *Pseudocorynactis* sp. has been suggested as helping to control CoTS in locations where it occurs in high abundance (Bos *et al.*, 2008). While it has been shown that CoTS larvae preferentially settle on CCA surfaces where benthic predators have been removed (Cowan *et al.*, 2016), it is not known whether enhanced abundance of invertebrates such as polychaetes, shrimps or crabs could decrease larval settlement success or increase predation on CoTS early life stages.

Interestingly, coral symbionts (fish, crabs, shrimp) can also make coral less attractive to feeding CoTS. For example, (Pratchett, 2001) showed that CoTS displayed coral species

selectivity, however this was no longer significant after removal of coral symbionts. The coral guard crab genus *Trapezia* has been studied in more detail, and larger *Trapezia* species found to protect coral against CoTS attack during an outbreak in Mo'orea, French Polynesia (McKeon, Moore, 2014). It has also been demonstrated that reef patches surrounded by *Pocillopora* that were defended by symbionts could serve as refugia for other corals (Glynn, 1987).

6.1.2 Possible contribution to CoTS IPM strategies

Protecting or augmenting the abundance of predators could be of particular relevance in areas that play a key role in the establishment and spread of outbreaks, e.g. the reefs in the area known as the initiation box and highly connected reefs further south.

Predators are unlikely to be able to protect a reef where CoTS have already reached outbreak densities (Westcott, Fletcher, 2018; Westcott *et al.*, 2016; Westcott *et al.*, 2020). However, enhanced predator populations can potentially play a role in preventing or delaying primary outbreaks by reducing the number of successfully settled larvae and the number of juveniles evading predation, including herbivorous juvenile CoTS which accumulate in the reef infrastructure and may serve as the proximate source of outbreaks (Deaker *et al.*, 2020). Modelling has also suggested that predation can regulate adult CoTS numbers when CoTS are present at naturally low densities between outbreaks (Antonelli *et al.*, 1990; Condie *et al.*, 2018; McCallum, 1987). Predator presence may play a role in reducing or preventing adult CoTS aggregations (for feeding or spawning), but few studies have directly addressed this so far.

Enhancing predator populations could be achieved through indirect and direct means. The indirect method for enhancing fish, triton and other predator populations is the use of fisheries restrictions (temporal closures, reduced take of specific species or groups of species) and MPAs. Marine protected areas have been used in the management of the GBR since 1981 (McCook *et al.*, 2010) and in 2004 no-take zones increased from 4.5% to 33% of the Marine Park. Benefits of this zoning have included increases in the stocks of key fisheries species (Emslie *et al.*, 2015), including potential predators of CoTS, through to enhanced general ecosystem resilience (Castro-Sanguino *et al.*, 2017; McCook *et al.*, 2010; Mellin *et al.*, 2016b; Yates *et al.*, 2016). Recent work suggests that fewer CoTS are observed in no-take zones (Sweatman, 2008; Vanhatalo *et al.*, 2016; Westcott *et al.*, 2020) and that outbreak impacts are reduced in no-take zones (Mellin *et al.*, 2016b). Indirect measures can serve to protect the habitat features that promote populations of predators of smaller size classes and earlier life-history stages as well as the larger predators of adults (Costello, 2014; Mellin *et al.*, 2016b).

The difficulties experienced in the 2004 expansion of MPAs on the GBR suggest that any such move will likely be met with strong opposition (Fernandes *et al.*, 2005). This may be offset to some degree by trade-offs across reefs, e.g. identifying the reefs that contribute the most to CoTS dynamics and spread and swapping zoning on those reefs for reversals of zoning on reefs that contribute less. Identification of interactions between an individual species' predation of CoTS, the extent to which that species is fished, and outbreak risk would inform strategies for limiting catch of those species, e.g., not allowing catch in key areas or at particular times.

The abundance of predators can also be increased through augmentative strategies such as local relocation, restocking and sea-ranching approaches, or aquaculture production. In the context of CoTS control on the GBR, the species suggested as potential candidates include the Giant Triton (*Charonia tritonis*) and species of emperor (Lethrinidae). Restocking might involve deployment of egg capsules or hatched larvae together with settlement, thereby circumventing the challenges related to settlement, while sea-ranching involves captive production and deployment of juveniles. Augmentative strategies have been employed previously in marine environments (section 4.3.1.2) but establishment would require significant research investments and lead-time, infrastructure to allow rearing of sufficient stock and for stock deployment, as well as crucial monitoring programs to assess their success. The survival and retainment of deployed organisms generally depend primarily on their size at deployment, the presence of natural predators, their motility, and the quality/structure of the deployment habitat (Roodt-Wilding, 2007).

The potential for breeding and rearing the giant triton snail in captivity was recently investigated (Motti *et al.*, 2019 and references therein). Captive production of veliger larvae and improvements in larval survival periods were achieved, however settlement in captivity is still elusive and considerable knowledge and technology gaps exist for closed lifecycle production (section 6.1.4, Motti *et al.* (submitted)). Recent identification of neuropeptides with possible function in larval metamorphosis could support advancements in this area (Bose *et al.*, 2017a; Motti *et al.*, 2019).

Aquaculture of emperor species has been attempted, with spangled emperor (*L. nebulosus*) in the United Arab Emirates (Yousif *et al.*, 2009) and pink ear emperor (*L. lentjan*) in India (Anil *et al.*, 2019), however it is uncertain whether these efforts resulted in commercial production. In contrast, Giant Grouper (*Epinephelus lanceolatus*), which prey on CoTS (Cowan *et al.*, 2017) and a variety of other animals, is commercially produced (GFB Fisheries Ltd, 2020).

The peppermint shrimp (*Lysmata* spp), which is a successful predator on CoTS up to approximately 4 months after settlement (Uthicke and Balu, personal communication), is already cultured as a predator for the aquarium trade and commercially available and could be considered as a potential control option for juvenile predation. Very little is known about the protective potential of producing and deploying coral symbiotic shrimp and crabs on priority corals, for example to be deployed with coral produced for restoration purposes.

6.1.3 Associated risks

Protecting predator stocks or enhancing them from current levels using conservation biocontrol strategies such as limiting catch quotas or creating protected zones are approaches currently in place to manage fisheries on the GBR. Overall, we consider conservation biocontrol approaches as carrying relatively low risk (section 8.2, Appendix 1), and it has been argued that predator protection represents a ‘no regrets’ approach (Cowan *et al.*, 2017). There will undoubtedly be opposition to increases in commercial and recreational fishing restrictions, however we consider that social and regulatory licence for these approaches is a realistic prospect if their efficiency in reducing CoTS outbreaks, restoring hard coral cover and flow-on effects on fisheries can be demonstrated.

Augmentative strategies will need to be carefully evaluated including their likelihood of success, the effect of increased predator numbers on community structure and other prey species populations, possible genetic risks if broodstock numbers are limited or differ from the wild-type (Roodt-Wilding, 2007), and the risk of disease transfer to wild populations (Bartley *et al.*, 2006). The human-mediated connectivity of possible release sites and possible regulatory challenges associated with these strategies must be addressed in more detail.

6.1.4 Critical knowledge gaps

The list below includes some critical science and technological knowledge gaps relating to CoTS control via predation, but the list is not exhaustive. Some of the identified knowledge gaps are crucial for developing the required technologies, for instance for aquaculture production of tritons, while others are relevant for a reliable assessment of risk associated with this CoTS control strategy (Appendix 1).

6.1.4.1 Conservation approaches

- The role of predation in preventing or delaying primary outbreaks. Combined analysis of historical relationships between GBR fisheries and CoTS outbreaks including data from paired reefs (green and blue zones), and new surveys and experiments directly addressing the level of predation on CoTS in green and blue zones and a possible link to the incidence and severity of CoTS outbreaks.
- Will a general no-take zone increase the abundance of CoTS predators, or is targeted protection (e.g. of CoTS source and sink reefs) preferable?
- How certain are we about the identity of predators and which (combination of) predators have the greatest impact on CoTS populations?
- The absolute and relative success of CoTS and different predators under future climate conditions. How will this impact the selection of predators to protect or augment?
- Influence of predation on early life history stages on the settlement success of CoTS
- Can CoTS develop resistance to increased predation pressure by increasing their production of predator deterrents (e.g. saponins), or by behavioural changes?
- The public response to increased fishing regulations as a CoTS management approach

6.1.4.2 Augmentative approaches (triton and predatory fish species)

- Identify candidate predators for augmentation that prey on CoTS in the wild
- The movement and prey preference of deployed predators in the wild
- Determinants of predator feeding preferences
- Determine if presence of the candidate predator(s) can reduce or prevent CoTS aggregations in the field
- The natural life cycle of tritons: timing of development stages, nutritional and other cues for development and reproduction, reproduction success in the wild, natural life span etc.
- Aquaculture technologies for production of triton juveniles, including water treatment, nutritional aspects, microbial management, development cues
- The natural life cycle and aquaculture technologies for production of other predator species (fish and invertebrates)
- The optimal strategy for deployment: life stage and size to be deployed, transportation and deployment technology
- Scalability of production and deployment

- Biosafety of deploying aquaculture-produced predators (pathogens, effects on gene pool)
- Human-mediated connectivity of potential release locations
- Can CoTS develop resistance to increased predation pressure by increasing their production of predator deterrents (e.g. saponins), or by behavioural changes?
- Societal drivers or constraints on stocking

6.2 CoTS control using microbial agents (or parasites)

6.2.1 Background knowledge

6.2.1.1 Pathogens and parasites

6.2.1.1.1 Diseases in other asteroids

Asteroids succumb to a range of diseases caused by microorganisms and parasites (Jangoux, 1987a, 1987b, 1987c). These diseases are usually sub lethal, although the health of the infected sea star can be significantly compromised.

Infectious disease can cause mass mortalities of asteroids and other echinoderms and can lead to extirpation of species from vast regions as seen in the *Diadema antillarum* die off Caribbean and the disappearance of the sea star *Heliaster kubiniji* from the Gulf of California (Dungan *et al.*, 1982; Lessios, 2016). The ongoing mass mortality of 20 species of asteroids along the Pacific coast of North America is due to an epidemic of what has been called sea star wasting disease (SSWD), which has caused reduction or disappearance of many species (Bates *et al.*, 2009; Eckert *et al.*, 1999; Harvell *et al.*, 2019; Menge *et al.*, 2016). While the multispecies wasting epidemic has been a major phenomenon along the Pacific coast of North America, sea stars elsewhere around the world also succumb wasting disease including the Atlantic coast of North America, Antarctica, Australia and New Zealand (Bucci *et al.*, 2017; Hewson *et al.*, 2019; Nunez-Pons *et al.*, 2018). Sea stars with wasting symptoms are often seen as isolated cases in populations (Hewson *et al.*, 2019); Byrne, pers obs), but the epidemic along the Pacific coast of North America is unprecedented. It is currently thought that SSWD is a syndrome that may have many causes and involve several different etiological agents. While wasting syndrome (SSWS) is more appropriate term (Hewson *et al.*, 2018) we use SSWD as this continues to be used in recent publications.

Sea star wasting disease starts with the appearance of lesions or small white patches on the body. These patches quickly enlarge and merge, followed by body fragmentation, disintegration and death. The entire process to death takes a few days to a week (Bates *et al.*, 2009; Hewson *et al.*, 2019). The rapid body breakdown in SSWD in the appearance of white degenerating lesions is similar to the extreme ‘self-destruct’ phenomenon of mutable connective tissue (MCT) breakdown that has been documented in sea cucumbers and sea stars (Byrne, 2001; Byrne, O'Hara, 2017; Motokawa, 2011; Wilkie, 2005). The mechanical properties of the echinoderm body wall are unusual because they are controlled by MCT that can be changed from hard to soft over seconds to minutes under neural control. The characteristics of the rapid body degradation during SSWD in sea stars is very similar to and

may involve irreversible MCT softening that contributes to the complete body breakdown (Wilkie, 2005).

In *Heliaster kubiniji*, SSWD appeared in intertidal populations and rapidly spread causing local extinction of the high-density populations of this sea star (Dungan *et al.*, 1982). In the subsequent decades the populations of *H. kubiniji* have not returned. For *P. ochraceus*, recruitment post local extirpation due to sea star wasting disease is associated with a change in population genetics and indicating post-mortality event selection and resilience of this species to disease (Schiebelhut *et al.*, 2018).

The disease agent(s) appears to only affect asteroids and other sympatric echinoderms are not affected. The agent causing SSWD is not clear. In the current epidemic in North America, *Vibrio* was initially implicated as this bacterium is associated with diseased sea stars (Eckert *et al.*, 1999; Staehli *et al.*, 2009). Hewson et al., (2014) linked SSWD to a densoviral cause; the sea star associated densovirus (SSaDV). This finding however has been corrected by the same authors (Hewson *et al.*, 2018). This latter study showed that the densovirus is only associated with the wasting response in one species (*Pycnopodia helianthoides*). Thus, with regard to the mass mortality across 20 sea star species, the presence of the virus is likely to be secondary to the initial cause. An enigmatic group of fungus-like saprobiotic protists was also isolated from sea stars with wasting disease (FioRito *et al.*, 2016).

It is suggested that increased temperature causes stress and compromises the immune system leading the sea stars to succumb to the disease (Eckert *et al.*, 1999) but in some cases the disease increases with cool temperature (Menge *et al.*, 2016). *For Pycnopodia helianthoides*, a detailed analysis of available survey data identified marine heat waves as a major driver (Harvell *et al.*, 2019). The disease is contagious as shown by infection of healthy individuals when they are housed in the same aquarium as a diseased individual. Overall it seems most likely that an environmental trigger caused a change in the health status of the sea star to make it vulnerable to wasting disease. There may also be a change an agent from a being benign to a pathogenic state and once in the ecosystem causes mass mortality through contagious spread.

Mass mortality of asteroids is also reported in Australia, although is not well documented. This includes the massive beaching and die off of *Protoreaster nodosus* on Moreton Island near Tangalooma. This phenomenon of dead starfish has been reported to occur in summer (https://www.facebook.com/rhett.ericsenmiller/videos/850577631722971/?pnref=story).
Mass mortality of sea stars in Port Philip Bay is also reported (Hewson *et al.*, 2019). There are no data on the causes of mass mortality of sea stars in Australia.

Another prominent microorganism that causes disease of asteroids is the ciliate protistan, *Orchitophyra stellarum*. This ciliate enters the testes and causes partial or complete castration through direct consumption of sperm and also stimulates an autoimmune response where the host phagocytes enter the testes and destroy sperm (Byrne *et al.*, 1997). In cases of a high parasite load the testes become infertile. Interestingly, the parasites do not infest ovaries. This ciliate typically infects asteroids in the family Asteriidae (Goggin, Bouland, 1997; Jangoux, 1987a). It was first described from *Asterias rubens* and *A. forbesi* in the North Atlantic (Cepede, 1907). *Orchitophyra stellarum* was later reported in the testes of the Japanese species *Asterias amurensis* (North Pacific Seastar) in Tokyo Bay (Byrne *et al.*, 1997; Byrne *et al.*, 1998) and in

several species along the Pacific coast of North America (Bates *et al.*, 2010; Leighton *et al.*, 1991). Although for nearly 100 years this parasite was only known from *Asterias* species it is now known to parasitise at least 10 other genera, including species outside the Asteriidae (O'Hara, Byrne, 2017). It is suggested that the presence of the parasite in the North Pacific was due to introduction and that the virulence of the pathogen was facilitated by the presence of naïve hosts with a poor defence against infection (Byrne *et al.*, 1998). Population surveys where the parasite occurs reveal the prevalence of females indicating that *O. stellarum* may remove some males from the population (Bates *et al.*, 2010; Leighton *et al.*, 1991).

Ascothoracid 'naked' barnacles such as *Dendrogaster elegans* live as endoparasites in asteroids including *Dermasterias imbricata* (Grygier, 1986). This endoparasite can take over the body, feeding on host cells and fluid. They grow to be enormous females, accompanied by a dwarf male, and their presence causes atrophy of the gonad often castrating their host (Hamel, Mercier, 1994). They are related to the barnacles that castrate the European green shore crab (Goddard *et al.*, 2005) (section 4.3.3).

In conclusion, little is known about microbial agents that have caused mass mortalities in asteroids and it is expected that similar symptoms may be caused by a range of different agents and environmental triggers. Ciliates and barnacles that have been implicated in reproduction control of asteroids are unfortunately not likely to be species specific as concluded from direct evidence in the case of *O. stellarum* and based on data for related organisms in the case of *D. elegans* (Goddard *et al.*, 2005).

6.2.1.1.2 Observed CoTS diseases

Mass-mortality of juvenile CoTS in Fiji was caused by an unknown intracellular parasite, suggested to be a sporozoan pathogen (Zann *et al.*, 1990). This epidemic caused 99% mortality of 10-16 month old juveniles (Zann *et al.*, 1987). For the population in Fiji this mass mortality of juveniles prompted the suggestion that disease plays a role in population control of CoTS and influences the erratic recruitment into the adult population (Zann *et al.*, 1987).

Diseased individuals of *Acanthaster* are occasionally encountered in the field on the GBR (Pratchett, 1999) as illustrated from an individual encountered at One Tree Reef in April 2017 (Figure 2). These CoTS have lesions, a flaccid body and the body wall disintegrates leading to death. Transfer of one of these individual into aquaria with healthy CoTS results in the transfer of the disease, indicating the presence of a water born agent (Pratchett, 1999). The very few reports of diseased CoTS in the field indicate that disease is rare. However, if the animals die quickly evidence of disease may go undetected and would only be seen in serendipitous observations.

Figure 2: Diseased CoTS found in the lagoon at One Tree Reef (Photo: Kennedy Wolfe).

Vibrio species are associated with many marine diseases, and have been isolated from both healthy and diseased CoTS (Reed *et al.*, 1999; Rivera-Posada *et al.*, 2011a; Rivera-Posada *et al.*, 2011b; Sutton, Trott, 1987; Sutton *et al.*, 1988). Rivera-Posada *et al.* (2011b) injected CoTS with thiosulfate-citrate-bile-sucrose-agar (TCBS), a culture medium used specifically for *Vibrio* culture, resulting in the onset of disease symptoms (discoloured and necrotic skin, ulcerations, loss of body turgor, mucus production, loss of spines) and subsequent isolation of several *Vibrio* species. These heavily infected CoTS transmitted their symptoms to healthy CoTS held in the same aquaria (Rivera-Posada *et al.*, 2011b) but also to another sea star species (*Linckia*) (Caballes *et al.*, 2012). It is our opinion that a live *Vibrio* strain is unlikely to be a suitable biological control agent for CoTS as many *Vibrio* species have a broad host range and horizontal gene transfer, including of virulence genes, is relatively common (Espinoza-Valles *et al.*, 2015; Hoffmann *et al.*, 2013).

The stability of CoTS microbiomes during a disease event in the aquarium system at the Australian Institute of Marine Science was assessed by molecular methods. Disease symptoms of sampled CoTS included drooping spines, loss of turgor, and tissue degeneration, but not dermal lesions (Høj *et al.*, 2018). A bacterial imbalance was evident in diseased individuals, with reduced relative abundance of marine spirochetes (likely subcuticular symbionts) in body wall and tube feet, and of *Streptococcus*-, *Rhizobiales*-, and *Enterobacteriaceae*-related bacteria in pyloric caeca. A *Kistimonas*-related bacterium was present in all diseased individuals, in particular their tube feet. *Kistimonas*-related bacteria have previously been implicated in aquaculture diseases (Mendoza *et al.*, 2013; Senapin *et al.*, 2016), but the role of this bacterium as a potential pathogen of CoTS is still unknown. Bacteria belonging to the genera *Arcobacter* and *Vibrio* were found to be opportunistic rather than primary pathogens; but related strains may be primary pathogens of CoTS under other circumstances (Høj *et al.*, 2018).

It is likely that disease outbreaks in aquaria-held CoTS involve stressed and immunocompromised animals, which may suffer microbial imbalance (dysbiosis) followed by proliferation of opportunistic, rather than primary, pathogens (Egan, Gardiner, 2016; Høj *et al.*, 2018; Sutton *et al.*, 1988). This is consistent with the observation that so far, long-lasting disease symptoms have not been seen after injecting healthy, non-stressed CoTS with bacteria isolated from diseased individuals (Høj *et al.*, unpublished data; Sutton *et al.*, 1988). While pathogenic bacteria often occur on the surface or internally in CoTS (Rivera-Posada *et al.*, 2011a) as in other echinoderms, they may not cause disease unless the host becomes immune-compromised (Brothers *et al.*, 2016). This corresponds to opportunistic human pathogens that can be highly problematic in hospital environments but that will not produce disease in healthy individuals.

Nevertheless, it is possible that some CoTS disease events are caused by primary pathogen(s) that can induce disease also in non-stressed individuals. Being present at the correct time and using the correct culture media and culture strategy to obtain isolates of such pathogens represents a major challenge but isolation strategies informed by molecular data hold promise.

6.2.1.2 Viruses in healthy CoTS

A wide range of viruses have been detected in tissues of healthy CoTS (Høj *et al.*, unpublished data) This includes a high diversity of viruses that infect bacteria, so-called bacteriophage, with both double stranded and single stranded DNA viruses detected. In addition, viruses known to infect microalgal cells and amoeba were present in nearly all tested individuals. Several viral groups, including double stranded DNA viruses, retro-transcribing viruses and single stranded DNA viruses, that are known to infect invertebrates and/or vertebrates were also present and these could potentially infect CoTS cells.

Parvoviridae, which includes the densoviruses, have been detected in low relative abundance in apparently healthy CoTS collected from the GBR (Høj *et al.*, unpublished data) and Hawaii (Gudenkauf, Hewson, 2016). Densoviruses were previously implicated in SSWD (Hewson *et al.*, 2014), however recent work suggests that SSWD is most likely a syndrome, that is multiple diseases with similar symptoms but differing etiologies (Hewson *et al.*, 2018) (section 6.2.1.3.1).

Nudiviridae were also detected in healthy CoTS (Høj *et al.*, unpublished data). This is of interest from a biocontrol perspective given that they form a monophyletic sister group with the occluded baculoviruses, which are commercially available for biocontrol of insects (section 4.1.2). In contrast to described terrestrial nudiviruses, the marine nudivirus *Penaeus monodon* nudivirus (PmNV) produces occlusion bodies (Williams *et al.*, 2017), which enable tracking and enumeration of viruses in the host by normal light microscopy. This property has facilitated the development of baculoviruses as commercial biocontrol agents of insects (section 4.1.2).

The high diversity of detected viruses demonstrates that there is scope for isolation of viruses that target specific microbial groups implicated in health and reproductive success of CoTS. If a virus targeting CoTS cells were to be used for biological control *sensu stricto* (i.e. as a replicating entity), then an obvious challenge is the development of technologies for virus production given that no echinoderm cell lines currently exist. The ongoing program for viral control of rabbits and the process towards the possible release of Cyprinid herpesvirus 3 (Koi herpes virus, dsDNA) to control carp in Australian waterways represent a wealth of experience

that need to be consulted if this pathway is to be further explored for CoTS. This includes the complex initial process of multidisciplinary discussions and risk assessments that have to be performed prior to substantial financial investment in the final program (McColl *et al.*, 2014).

6.2.1.3 Symbiotic bacteria in healthy CoTS

Microbiomes of CoTS testes are highly dominated by a novel bacterium related to the genus *Spiroplasma* (class Mollicutes, order Entomoplasmatales, family *Spiroplasmatacea*), and closely related strains are present testes from CoTS collected on both the GBR and Okinawa, Japan (Høj *et al.*, 2018). Female gonad samples have more variable microbiomes, but some samples have a high relative abundance of *Spiroplasma*. It is currently not known if this variability is related to gonad maturation or reflects differences between individuals. The same bacterium as well as other Mollicutes are also present in CoTS pyloric caeca.

Mollicutes are free-living, self-replicating organisms that lack a cell wall. Their genome size is generally small with some being obligate symbionts, many of them able to enter the cells of their host. Preliminary analysis of genome sequencing data suggest that *Spiroplasma* could be an obligate symbiont in CoTS male gonads of Okinawan CoTS (Høj *et al.*, unpublished data), however confirmation is needed for CoTS collected on the GBR. The possibility that the bacterium is vertically transmitted from parent also needs confirmation. Mollicutes source lipids from their host. The dependency of *Spiroplasma* on host lipids can provide a direct link between CoTS nutritional state and/or reproductive investment and symbiont proliferation, as was demonstrated for *Spiroplasma poulsonii* whose proliferation is limited if its fruit fly host is nutrient limited (Herren *et al.*, 2014).

If true, this would open an avenue for CoTS control that specifically targets CoTS in good nutritional condition and/or with high investment in gonad development and gamete production. It also represents a possible mechanism for targeted delivery of transgenes (e.g. dsRNA for gene silencing) to CoTS gonads. At least some *Spiroplasma* strains are suitable as carriers for gene constructs due to their relative host specificity and possible transmission between individuals (Hackett *et al.*, 1992; Klein *et al.*, 2002). Recent culture success of an obligate *Spiroplasma* symbiont of fruit flies (Masson *et al.*, 2018) suggest that it is realistic to grow the organism in culture. However, culturing and genetic modification of the CoTS *Spiroplasma* strains need to be established if this pathway is to be pursued. So far, growth of the CoTS *Spiroplasma* has been confirmed in primary cultures but not in subsequent passages (Høj *et al.*, unpublished data). Another clear knowledge gap relevant for biocontrol purposes relates to the host specificity of the organism.

Other likely symbionts of healthy CoTS include marine spirochetes that are present in body wall and tube feet (Høj *et al.*, 2018; Wada *et al.*, 2020), and bacteria associated with CoTS larvae shift in response to differential feeding (Carrier *et al.*, 2018), however, their functional roles are yet to be confirmed.

6.2.2 Possible contribution to CoTS IPM strategies

Based on current knowledge, it is difficult to envision the use of a self-replicating, horizontally transmitted pathogenic agent in CoTS control. Several challenges have led to this conclusion, including the difficulty of isolating a species-specific primary pathogen that induces disease in healthy individuals, the risk of horizontal transfer of virulence genes, the risk of resistance

development, and the difficulty of containing or reversing the treatment if needed. These risks are higher for a native marine pest than it would be for an invasive pest that may not have close relatives living in the same environment. On the other hand, a vertically transmitted bacterium could be more specific and could, at least in theory, be used as a vector for tissue-specific delivery of RNAi that can silence CoTS specific genes (sections 6.4.2.4, 6.4.3.1).

6.2.3 Associated risks

Biological control using a microbial agent would require extensive research addressing safety concerns in order to obtain social licence and regulatory approval. Factors relating to safety for humans, animals and the environment would have to be considered, as well as expected efficiency, the likelihood for resistance development, and the possibility of reversing or containing transmission.

Evolutionary processes that can lead to species jumping, development of host resistance, and possible reduction in virulence have been best studied in viruses. While it is difficult to make predictions about viral evolution and the likelihood of cross-species transmission and emergence, some general evolutionary principles are worth noting (Holmes, 2013). Firstly, it appears that virus mutation rates but not recombination rates influence their propensity for crossing species-boundaries (Holmes, 2013). Secondly, as for other organisms, viruses are more likely to cross over to a closely related species than to a more distant relative and transmission risk depends on the probability of exposure between the two species. Thirdly, changes in virulence towards the host is linked to the size of the virus genome with large viruses exhibiting several possible routes towards reduced virulence (Di Giallonardo, Holmes, 2015) while the smaller genomes of RNA viruses have limited genomic flexibility and the same mutations can influence on both virulence and transmission.

Resistance against viral infection can also develop in the host. For example, high levels of resistance against the Codling moth (*Cydia pomonella*) granulovirus (CpGV) has developed in certain locations were this virus has been used for biocontrol for more than 20 years (Lacey *et al.*, 2015). This has partly been overcome by introducing new isolates of CpGV, and there is evidence that new products should include a mixture of strains or use alternate strains as part of a resistance management strategy (Lacey *et al.*, 2015).

Factors that determine bacterial host range is less understood due to their more complex molecular composition and cellular structures, but a few principles have emerged from well-studied human pathogens (Pan *et al.*, 2014). Bacterial pathogens have widely different levels of host specificity, which is influenced by at least three factors including specific recognition of host receptors for colonisation and/or dissemination, a specialised ability to evade or overcome the host's immune mechanisms, and the availability of an essential nutrient required for bacterial growth in the host (Pan *et al.*, 2014).

An ideal microbial biocontrol candidate for CoTS would likely depend on specific nutrients present in CoTS and/or be able to recognise CoTS specific receptors. Based on what we currently know about CoTS microbiology, the best candidate to fulfil these criteria is the detected *Spiroplasma* or other intracellular bacteria. It would be necessary to carefully assess the species specificity of any self-replicating candidates, including if they can be transferred between CoTS and corals. The latter would correspond to the situation seen for some plant-

pathogenic spiroplasmas that are transmitted via deltocephaline leafhoppers (Fletcher *et al.*, 2006). It should be noted that the risk profile of a cross-species transfer would differ if the bacterium is naturally virulent and expressing toxins, or if the bacterium on its own is benign but engineered to express an RNAi that can silence a CoTS specific gene (sections 6.4.2.4, 6.4.3.1).

6.2.4 Critical knowledge gaps

No viral, bacterial or parasitic isolates that appear suitable as a microbial biocontrol agent for CoTS in the traditional sense are available. Bacteria of the genus *Vibrio* have been isolated from diseased CoTS and have been shown to induce an immune reaction in healthy CoTS and disease symptoms in stressed CoTS. However, vibrios are generally not species specific and so far the tested strains have not induced disease symptoms in otherwise healthy non-stressed CoTS. On the other hand, a *Spiroplasma*-related symbiont is present in CoTS gonads and represents a possible vehicle for directed delivery of genes (sections 6.2.1.3, 6.4.3.1).

The list below includes some critical science and technology-related knowledge gaps relating to CoTS control via microbial and parasitic agents, but the list is not exhaustive.

- Identification and selection of suitable candidate strain(s)
- Isolation, culture, mass production of candidate strain(s)
- Natural prevalence of candidate(s) in CoTS, other echinoderms, and other reef organisms
- Transfer route between individuals (vertical; horizontal: via water, direct contact, or via vector organism)
- Specificity of candidate(s)
- Can CoTS develop resistance to infection by the candidate?
- Do candidate(s) carry genes of interest for genetic biocontrol?
- Can candidate(s) themselves be genetically manipulated?
- Deployment technology and scalability of deployment
- Human-mediated connectivity of potential release locations

6.3 CoTS control using semiochemicals

6.3.1 Background knowledge

CoTS produce a wide range of complex secondary metabolites (Dong *et al.*, 2011; Xia *et al.*, 2020), and rely on chemosensation and semiochemicals throughout their life history, as do other asteroids (Motti *et al.*, 2018). Chemosensory-mediated behaviours in asteroids include foraging, spawning, larval settlement, growth and metamorphosis, chemical defence, alarm dispersal and predator avoidance. Some of these processes have been studied in detail, while the chemical compounds involved in others are still unknown (Motti *et al.*, 2018). An overview of published CoTS chemosensation studies is presented in Table 1.

6.3.1.1. Foraging

Studies targeting feeding behaviour in CoTS have addressed aggregation, attraction and deterrent responses, as well as stomach relaxation and eversion. In the field, CoTS aggregate in both spawning and non-spawning months (De Dios *et al.*, 2014), and aggregating CoTS themselves secrete molecules that lead to positive chemotaxis (Hall *et al.*, 2016; Hall *et al.*, 2017a). Moreover, actively feeding CoTS attract other individuals, more so than live coral does,

and Y-maze experiments showed that these responses are mediated chemically (Ormond *et al.*, 1973). CoTS prefer feeding on certain coral species and avoid others as shown in field surveys (De'ath, Moran, 1998; Tokeshi, Daud, 2011) and tank experiments (Pratchett, 2001; Wellington, Trench, 1985). Coral macrosymbionts (fish, crabs, shrimp) can influence selectivity, and one study found that CoTS no longer showed coral species selectivity when these symbionts were removed (Pratchett, 2001). Intact corals, coral extracts and several chemical compounds potentially linked to CoTS foraging responses have been tested in animal behavioural assays. Individual compounds that have been tested include amino acids (arginine, glutamic acid, proline, cysteine, isoleucine), betaine, lactic acid, acetylcholine, arachidonic acid, and α-linolenic acid.

6.3.1.2. Reproduction

Spawning CoTS and CoTS gametes attract other individuals (Beach *et al.*, 1975; Hall *et al.*, 2016). The compound 1-methyladenine (1-MA) is routinely used to induce spawning in CoTS and other sea stars (Beach *et al.*, 1975; Caballes, Pratchett, 2017; Hall *et al.*, 2016; Kanatani, 1973; Uthicke *et al.*, 2015b). This compound resumes meiosis of oocytes that are arrested in the prophase, and it is the end product after binding of the relaxin-like gonad stimulating peptide, which is a key reproduction regulator in all sea stars (Kalachev, 2013). Recently, a recombinant version of the CoTS relaxin-like gonad stimulating peptide (RGP) was expressed in yeast and demonstrated to induce maturation and ovulation in CoTS ovary fragments (*ex-vivo*) (Smith *et al.*, 2019). In contrast, glutamic acid inhibits ovulation *ex-vivo* even in the presence of 1-MA (Smith *et al.*, 2018). Interestingly, the abundance of glutamic acid and glutamine was found to increase significantly in food-deprived CoTS (Smith *et al.*, 2018) so starved animals may delay the onset of spawning.

6.3.1.3. Larval settlement, growth and metamorphosis

Crustose coralline algae (CCA), macroalgal detritus and some bacterial biofilms induce settlement of CoTS larvae (Cowan *et al.*, 2016; Henderson, Lucas, 1971; Johnson, Sutton, 1994; Johnson *et al.*, 1991; Keesing, 1992). Interestingly, extracts and filtrates of CCA did not induce settlement (Johnson, Sutton, 1994). Larval development could be accelerated by exposure to the hormone thyroxine (Johnson, Cartwright, 1996), and salinity also influences on larval survival and development (Lucas, 1973).

6.3.1.4. Defence

CoTS show an avoidance response to molecules produced by the predatory giant triton snail, with CoTS moving away from conditioned water in Y-maze trials (Hall *et al.*, 2017a). Other anti-predatory behaviours include the production of toxins that are inflammatory (phospholipase A2), haemolytic (saponin, plancitoxins), membranolytic (saponin), induce apoptosis (plancitoxins), and act as an anticoagulant (plancinin) (Ibrahim *et al.*, 2013; Karasudani *et al.*, 1996; Ota *et al.*, 2006; Savitri *et al.*, 2011; Shiomi *et al.*, 2004) (Table 1). Saponins deter predation by some fish species (Lucas *et al.*, 1979), however other fish species do prey on CoTS despite their production of these compounds (section 6.1.1.1). The giant triton snail is seemingly immune to these toxins, being able to crawl onto and immobilise adult CoTS before injecting and consuming.

Table 1: Chemosensory-mediated behaviours and processes that have been investigated in CoTS. The table is based on the review by Motti *et al.* (2018).

Behaviour	Life stage	Source/chemical stimuli	Bioassay/Observation	Reference
Foraging	Adult	Feeding CoTS; food	Aggregations in non-spawning months	De Dios et al. 2014
Foraging	Adult	Feeding CoTS; damaged prey	Feeding individuals chemically attract hungry starfish. Preferential order: feeding *A. planci*> live coral > dead coral	Ormond et al. 1973
Foraging	Adult	Coral; intact	*Acropora* preferred over *Porites* 14:1	De'ath and Moran 1998
Foraging	Adult	Coral; intact	Non-outbreak populations mostly avoided Poritid species and *Acropora palifera*. Attracted to all other corals, in particular *Favites*, *Goniastrea* and *Montastraea*	Tokeshi and Daud 2011
Foraging	Adult	Coral (*Acropora eurystoma*, *Fungia fungites*, *F. scutaria*, *F. echinata*, *F. horrida*, *Cycloseris cyclolites*); intact or extracts	Corals or extracts produced withdrawal response: *F. scutaria ~ F. echinata ~ F. horrida > A. eurystoma ~ F. fungites > C. cyclolites*	Collins 1975
Foraging	Adult	Coral (*Acropora multicaulis*, *Porites solida*, *Millepora dichotoma*, *Fungia fungites*, *Platygyra lamellina*); intact tissue, nematocyst homogenates	Nematocysts produced weak tube-foot withdrawal indicating other chemistry in whole coral extracts is responsible for arm rearing	Moore and Huxley 1976
Foraging	Adult	Coral; tissue/nematocysts	Activated nematocysts caused tube feet withdrawal	Barnes et al. 1970
Foraging	Adult	Coral (*Acropora gemmifera*, *A. nasuta*, *A. loripes*, *Seriatopora hystrix*, *Pocillopora damicornis*, *Stylophora pistillata*)	Feeding preference of *A. planci*: *A. gemmifera > A. nasuta = A. loripes > S. hystrix > P. damicornis > S.pistillata*. With symbionts (fish, crabs, shrimp) removed, no selectivity was evident	Pratchett 2001
Foraging	Adult	Coral (*Tubastraea micrantha* (non-	Preferential feeding on acroporids and pocilloporids, no feeding on *T. micrantha*	Wellington and Trench 1985

		symbiotic stony coral); *Acropora echinata*, *Pocillopora damicornis*, *Porites lutea*, *P. iwayamensis*, *P. andrewsi*)		
Foraging	Adult	Coral (*Acropora formosa*, *Pocillopora eydouxi*, *Porites iwayamaensis*, *Porites lutea*) tissue slurries, extracts,	Coral tissue slurries (extracts; with or without heat-treatment) injected into mouth cause stomach eversion within 10–15 min	Brauer et al. 1970
Foraging	Adult	*Acropora* sp. large molecular weight molecules	Produced feeding behaviour; reduced response after repeated exposure	Huxley 1976
Foraging	Adult	Small peptides; arginine, glutamic acid, proline, cysteine, isoleucine, betaine, lactic acid, acetylcholine	Betaine and mixture of amino acids produced arm rearing response	Moore and Huxley 1976
Foraging	Adult	Arachidonic acid (AA), α-linolenic acid (LA), Betaine	AA, LA, and betaine attracted asteroids, confirmed by Y-maze. *In situ* 1.5 m^2 traps with LA: 7 asteroids in 9 days (Motobu) and 4 asteroids in 4 days (Onna)	Teruya et al. 2001
Reproduction	Adult	CoTS gonad; intact, extract, extract dialysate	Spawning of single CoTS can induce spawning in other individuals. Attraction to spawning individual. Limited spawning response (10-13%) after exposure to whole gonad, heat treated gonad extract, and gonad extract dialysate	Beach et al. 1975
Reproduction	Adult	CoTS sperm	Release of sperm from reproductive male attracted female cohorts. Females spent twice as much time as males in Y-maze arm delivering sperm	Hall et al. 2016
Reproduction	Adult	Maturation-inducing substance, 1-methyladenine (1-MA)	1-MA detected at 10^{-8}–10^{-9} M when applied externally	Beach et al. 1975

Reproduction	Adult	Maturation-inducing substance, 1-methyladenine (1-MA)	Spawning induced by 1-MA at 10^{-7} M. 1-MA, 1-ethyladenine, 1-methyladenosine, and 1-methyladenosinemonophosphate were also effective in inducing spawning and oocyte maturation	Kanatani 1973
Reproduction	Adult	Maturation-inducing substance, 1-methyladenine (1-MA)	Dissected testes of *A. planci* shed sperm immediately after exposure to 1-MA, while ovaries took ~60 min	Caballes and Pratchett 2017
Reproduction	Adult	Maturation-inducing substance, 1-methyladenine (1-MA)	Males spawned approximately 20 min after injection and females after 40 min	Hall et al. 2016
Reproduction	Adult	Glutamic acid	Inhibition of oocyte maturation and ovulation *ex-vivo*, even in the presence of 1-MA	Smith et al 2018
Settlement and metamorphosis	Larvae	Crustose coralline algae (CCA)	Larvae avoided predators (*P. damicornis* with trapeziid crabs, rubble with polychaete) and were attracted to cleaned CCA rubble and adult *A. planci*	Cowan et al 2016; Keesing 1993
Settlement and metamorphosis	Larvae	Crustose coralline algae (CCA)	No settlement was induced by gamma-amino butyric acid (GABA), KC1 or CCA (*L. pseudosorum* and *P. onkodes*) extracts. Settlement was induced by CCA fragments and by some *Vibrio* sp. biofilms	Johnson et al. 1991
Settlement and metamorphosis	Larvae	Crustose coralline algae (CCA)	Settlement rates were highest on untreated CCA and on reinfected (after antibiotics treatment) CCA. Extracts and filtrates of CCA did not induce settlement	Johnson and Sutton 1994
Settlement and metamorphosis	Larvae	Crustose coralline algae (CCA)	Larvae settled on encrusting algae and macroalgal detritus but not on clean glass dishes, fine siliceous sand, serpulid tubes, *Pocillopora damicornis*, *A. planci* spines or tube feet	Henderson and Lucas 1971; Reviewed in Yamaguchi 1973
Settlement and metamorphosis	Larvae	Thyroxine	Larvae exposed to thyroxine (10^{-7} M) showed accelerated development	Johnson and Cartwright 1996
Settlement and metamorphosis	Larvae	Salinity	Bipinnaria survival increased (from 15 to 51%) as salinity decreased from 35% to 30%. Development completed at 26% but not at 22% salinity	Lucas 1973
Defence	Adult	Plancitoxins	Plancitoxin I and II isolated from spines; venom – haemolytic, induces apoptosis	Ota et al. 2006;Shiomi et al. 2004
Defence	Adult	Phospholipase A2	Phospholipase A2 isolated from spines; inflammatory	Ibrahim et al. 2013; Savitri et al. 2011

Defence	Adult	Plancinin	Plancinin isolated from spines; anticoagulant	Karasudani et al. 1996
Defence	Gametes to adults	Crude saponin extracts from eggs, and adults	All four fish species rejected food particles containing saponins	Lucas et al. 1979
Defence	Adult	*Charionis tritonis*	Avoidance response to *Charonia tritonis*- conditioned seawater in Y-maze trials	Hall et al 2017

6.3.1.5 Recent progress based on –omics analyses

All up, about 400 proteins are secreted by CoTS during aggregation, predator evasion, or both, including enzymes, components of CoTS venom, signalling proteins and structural proteins (Hall *et al.*, 2016; Hall *et al.*, 2017a). For the secreted peptides, corresponding genes are expressed in external tissues such as spines, body wall and the mouth region. Some of these genes appear to be CoTS specific based on comparative genome analysis and hold potential for use in species-specific push-pull control strategies. One example is the ependymin-related proteins (EPDRs), which are suggested to be recently evolved, CoTS specific communication molecules (Hall *et al.*, 2017a). Another example is the G-protein-coupled receptor (GPCR) proteins, which are known chemoreceptors that were highly enriched in external CoTS tissues (Roberts *et al.*, 2017), with some expressed in a sex-specific manner (Roberts *et al.*, 2018).

Recent identification of a wide range of CoTS neuropeptide precursors and neurotransmitters and their corresponding genes may be relevant for the development of specific CoTS control strategies (semiochemicals and/or genetic biocontrol), however a detailed discussion of these datasets are beyond the scope of the current review. Here, we will only point out that combined transcriptome and proteome studies have identified 48 neuropeptide precursors in CoTS, some of which are homologous with neuropeptides found in other echinoderms while others are novel (Smith *et al.*, 2017). Moreover, several small molecule neurotransmitters expressed in the CoTS radial nerve cord show significant differences between well-fed and starved CoTS, and between male and female CoTS (Smith *et al.*, 2018).

6.3.2 Possible contribution to CoTS IPM strategies

If efficient CoTS attractants could be developed, this would have several possible uses in an IPM strategy. A major challenge for manual CoTS control programs is cryptic CoTS that are not spotted by divers during the culling operation. If attractants can mobilise CoTS to leave their hiding spaces such as crevices or under rocks, then the culling efficiency per diving hour may increase, and more importantly, the required revisitation frequency may be reduced. On the other hand, there is a risk that this approach can release herbivorous juveniles from competition from the coral eating stage and promote diet transition (Deaker *et al.*, 2020). Another potential role for attractants is that they can be used in baited traps, and they can therefore potentially assist in delivering other control agents such as microbes or RNAi (sections 6.2, 6.4.3.3) that can cause death or reduce fecundity.

Repellents can play a role in IPM if they can be used to protect priority sites or to prevent or delay the formation of spawning aggregates, potentially providing additional time for manual control before spawning occurs. This could be of value for protection of priority reefs or reef sections, including protection of corals deployed as part of a reef restoration program. The efficiency of such a strategy would likely largely depend on the time period and volume of water where the deployed dispersant would be active.

The use of pheromones for mating disruption by release into the water column would be a direct analogue to the successful use of this approach in terrestrial systems (section 4.1.4). At this stage, this seems less likely to be successful due to the open nature of the area to be treated (large edge effects), the requirement for homogenous plumes of compound, and the high concentrations needed. For aquatic environments, it has also been argued that this approach carries higher risk than the use of attractants and repellents (section 6.3.3).

6.3.3 Associated risks

The use of semiochemicals for specific attraction or dispersion is expected to carry low environmental risk as the compounds will dilute and degrade, hence the effect is reversible. It is also relatively easy to test the efficiency and specificity of each compound/mixture using traps and assessing any by-catch. In contrast, the use of semiochemicals to disturb mating synchrony and maturation carry a higher risk and this approach may be better suited for control of invasive species with no close relatives in the nearby environment (Sorensen, Johnson, 2016).
The approach may carry a financial risk as significant investment in research and development is required and the lead time before the technology is application ready is uncertain, but likely to be 5-10 years depending on funding. Modelling is imperative to assess if the approach is likely to be efficient for use at difficult sub-reefs or reefs, and to define stop-go criteria for the research to continue.

6.3.4 Critical knowledge gaps

Rapid knowledge gains have been made regarding CoTS semiochemicals, however there are still considerable challenges ahead if field applications for efficient CoTS control are to be developed. While some active compounds have been identified, other activities have so far only been demonstrated with crude extracts and the exact nature of the chemical components have not yet been elucidated.

A main hurdle is the demonstration of efficiency at scale in the field. Other hurdles include establishing effective dose, i.e. determination of the volume of water that is activated per unit compound/extract and for how long, application of single compounds versus mixtures that may act synergistically, and the development of cost-effective technologies for compound extraction (single compounds or crude extracts) or production of pure compounds at scale. It is clear that suitable delivery devices also need to be developed, though it is expected that this can build on efforts already undertaken for semiochemical control of sea lampreys (Wagner *et al.*, 2018) (section 4.2.4). Efficient delivery strategies need to be designed as part of an IPM plan, and these need to be guided by modelling to ensure efficiency (section 7.1).

The list below includes some critical science and technology-related knowledge gaps relating to CoTS control using semiochemicals, but the list is not exhaustive.

- Identification and selection of suitable compound(s) and mixtures
- Isolation, mass production of candidate compound(s) or mixtures
- Determination of effective dose, volume of water activated, duration
- Determination of specificity
- Can CoTS develop habituation?
- Are relevant genetic pathways (production, sensing of compound) of interest for genetic biocontrol? (section 6.4.2.3)
- Deployment technology and scalability of deployment

6.4 Genetic biocontrol of CoTS

An overview of how biological and ecological traits of CoTS map onto criteria relevant for genetic biocontrol is presented in Table 2.

6.4.1 Background knowledge

6.4.1.1 CoTS genetics

The CoTS genome was sequenced and analysed from a biocontrol perspective (Hall *et al.*, 2017a; Medina, 2017). Separate draft genome assemblies were produced for two individuals, one collected from the GBR and one from Okinawa, Japan. The 384 Mb genome had low heterozygosity (0.88-0.92%) and the two genomes had 98.8% nucleotide identity (Hall *et al.*, 2017a). Comparison of the CoTS genome sequences with those of other deuterostomes revealed that only a few gene families have increased members in CoTS, providing leads for possible CoTS specific genes and pathways (Hall *et al.*, 2017a), including the species-specific suite of fertilisation genes (Guerra *et al.*, 2020). Combined with transcriptomics and exoprotein analysis, it has been shown that most expanded families encode secreted proteins and receptors that are likely involved in communication between CoTS individuals or in their sensing of the environment, respectively (section 6.3.1.5).

6.4.1.2 CoTS reproduction and developmental biology

CoTS are broadcast spawners and reproduce via seasonal synchronised spawning events, when gametes and larvae are dispersed (Pratchett *et al.*, 2014). On the GBR, spawning has been reported from December to February and is synchronised within but not among populations (Pratchett *et al.*, 2014). It is still unclear if individual starfish can spawn more than once each year (Pratchett *et al.*, 2014). CoTS are dioecous with separate sexes, but in most locations including the GBR their gonads are completely resorbed for parts of the year making morphological distinction between males and females impossible and limiting access to gametes.

The age of sexual maturity of CoTS is estimated to be 2-3 years with senescence, based on laboratory rearing (Lucas, 1984). Gonad mass of individual CoTS increase exponentially with their diameter so larger individuals contribute the majority of gametes during spawning (Babcock *et al.*, 2016b; Budden *et al.*, 2019). The number of oocytes produced by the largest CoTS females has been estimated to be > 100-200 million per season (Babcock *et al.*, 2016b). CoTS develop through a bipinnaria larval stage and then transition to a brachiolaria larval stage that is competent to settle. Brachiolaria larvae readily settle in response to a biofilm on the surface of culture containers or other surfaces such as shell fragments and on aquaculture settlement plates. They appear to settle particularly well in response to the presence of CCA.

Like all asteroid juveniles (Martinez *et al.*, 2017), CoTS start life as herbivores feeding on biofilms and algae with a preference for CCA with some becoming competent predators of corals by approximately 6 months post settlement (Kamya *et al.*, 2018; Kamya *et al.*, 2017). In the absence of coral prey, they can be sustained on an algal diet for at least 6 years as indicated in a laboratory study (Deaker *et al.*, 2020). Recently, it was shown that CoTS bipinnaria larvae can undergo cloning under both low and high food supplies (Allen *et al.*, 2019). The extent of this phenomenon and its impacts on population levels is currently unknown.

As for echinoderms in general, our understanding of sex determination and sex differentiation in CoTS is limited. Recent evidence from gene expression studies and histology of CoTS gonads suggest that some individuals may be facultative hermaphrodites, with small sections of their gonad developing gametes of the other sex, although the role of self-fertilization during

build-up of pre-outbreak populations is still unknown (Guerra *et al.*, 2020; Stewart *et al.*, 2015). Over 5,530 peptides and proteins have been found to be associated with spawning, of which 375 were mapped onto the CoTS genome (Hall *et al.*, 2016). Small molecule neurotransmitter profiles were found to differ between male and female CoTS, with differences seen for instance for gamma-aminobutyric acid (GABA), histamine and serotonin (Smith *et al.*, 2018).

Two critical endogenous endocrine factors implicated in CoTS reproduction and spawning are the gonadotrophin releasing hormone, which stimulates gonad development, and the gonad stimulating peptide (RGP) (previously named gonadal stimulating substance (GSS)), which activates the final gamete maturation before spawning (Hall *et al.*, 2016; Smith *et al.*, 2017). RGP binds to a receptor in the ovary wall, thereby stimulating the release of 1-MA that regulates final maturation of oocytes prior to spawning (Kalachev, 2013). Both the peptide sequence of the RGP and the associated protein coding gene sequence have been characterized for CoTS (Hall *et al.*, 2016; Hall *et al.*, 2017a). The CoTS RGP gene has been cloned and expressed in a yeast expression system, and biological activity was demonstrated for the recombinantly produced protein (Smith *et al.*, 2019).

Insights into the basis of gamete binding in CoTS has been advanced through transcriptomics analysis of gene expression in the testes and ovaries (Guerra *et al.*, 2020). Eight genes were identified that are involved in species specific gamete binding or that encode egg jelly components that attract sperm to eggs. Species specific sequences are now available for nearly the full complement of gamete binding genes known for sea stars and other echinoderms.

Table 2: Overview of traits relevant for development of a genetic biocontrol systems.

Trait	Rationale	Status for CoTS
Sexually reproductive	Required for gene-flow and spread	Yes
Can be reared in the laboratory	Required to produce genetically modified individuals and for controlled trials	Yes
Can be genetically modified in germ-line	Access to eggs and/or sperm for microinjection	Yes
Genetically well characterized	Knowledge of genetic mechanisms and processes to be manipulated is essential	Partly. Genome sequence and some transcriptomes available.
Obligate sexually reproductive	Ensures efficient gene-flow and spread	Clonality in larvae observed, extent is unknown.
Incapable of self-fertilisation	Ensures efficient gene-flow, reduces resistance development	Hermaphrodite individuals observed. Role and relative contribution unknown.
Thorough understanding of molecular-genetic basis of sex determination	To design efficient system.	Not well understood.
Short generation time	Development and production, time required for spread of gene drive	Intermediate – 2-3 years
Simple mating system	Complex mating systems and dormant life stages may extend efficient generation time. Synchronised mating events can provide opportunities for strategic releases.	Synchronised mating event, seasonal.
Good knowledge of ecology (e.g. mating systems, population dynamics, community interactions)	Essential for developing efficient strategy, predicting impact.	Partly. Better than most marine organisms but poor relative to agricultural pests etc.
Dispersal capacity	Relevant for spread. Dispersal of gametes increases risk of interspecific hybrids (spread to non-target species).	CoTS are broadcast spawners, gametes and larvae are widely dispersed.
Dispersal behaviour	Outcome can be influenced if mating behaviour change at low density (in-breeding) or skewed sex ratios	CoTS have widely fluctuating densities. The degree of clonality, inbreeding and sex ratios at different outbreak stages not well documented.

Spatial structuring of population	Spatially explicit sub-populations could be source of wild-type reinvasion.	Northern population is highly connected. Degree of connectedness between populations north and south of Hydrographers Passage not known. Not known if CoTS in deeper waters represents a source of wild-type reinvasion.
Chance of encountering closely related organisms	Trial gene drives suggested used in locations with no native close relatives, e.g. rabbits or cane toads in Australia. Trial gene drives suggested used on islands to reduce chance of 'escape'.	The closest relative to CoTS, *Acanthaster brevispinus*, differs in preferred habitat (deep, sandy areas). While rare, it is present on the GBR. Sea stars from other genera co-occur with CoTS.
Target organism is dominant or sole cause of impact.	Required to achieve and assess efficiency	Partly. CoTS is dominant corallivore but coral loss also due to other factors.
Transferability of technology, cost-sharing	Development costs reduced if pest organism is closely related to other pests and of interest to a variety of stakeholders (human health, veterinary health, agricultural pest).	Echinoderm pests are not common. Stakeholders include tourism, fisheries and related industries, environmental interest groups.

6.4.2 Potential strategies

This section provides an overview and high-level discussion of strategies that have been explored in other systems as well as some alternative CoTS-specific options. For completeness, we have included strategies that are not expected to work or be suitable for CoTS on the GBR. The exception is strategies where whole chromosomes are manipulated, such as triploidy and Trojan Y, which are suitable only for organisms with obligate chromosomal sex determination (Thresher *et al.*, 2019). If autosomal genes or environmental cues are involved in sex determination, as is likely for CoTS, then alternative strategies are needed. Hence, strategies that manipulate whole chromosomes are not discussed further in this report.

For all of the discussed strategies, several major knowledge gaps exist (section 6.4.6) and modelling is required to evaluate their likelihood of success (section 6.5) assuming that they are technically possible.

6.4.2.1 Release of sterile CoTS

Release of sterile individuals is a strategy that has been used successfully in terrestrial (sterile insect technique, sections 2.4, 4.1.2) and freshwater systems (triploid fish, section 4.2.5). Experience from insects suggest that sterile-release programs require that sterile males outnumber fertile males at least 10:1 to be successful (Thresher *et al.*, 2014b), hence any release of sterile CoTS would have to occur just after a culling event or when CoTS populations are naturally at a low level. Our poor understanding of how sex is determined in CoTS makes it challenging to produce and release sterile males only (or sterile females only). If both males and females are released, the efficiency will be reduced further since a proportion of the released individuals will mate with each other rather than with wild type CoTS. An additional challenge is posed by the external fertilisation strategy of CoTS, which means that sterile males would need to produce mobile sperm that can bind to and enter the egg (blocking polyspermy) without producing viable offspring, while sterile females would need to produce eggs that bind sperm without producing viable offspring.

In many insect systems the release of sterile males does not add to the detrimental impact of the pest population, as males do not bite (in the case of mosquitoes) and do not produce caterpillars that can damage crop plants. In contrast, any released sterile CoTS will prey on coral and this need to be factored in when evaluating any beneficial contributions via lowering the reproductive output in the following generation(s).

In conclusion, the release of sterile CoTS is unlikely to be successful unless it is combined with a strategy aiming to bias the sex ratio of the population (section 6.4.2.2).

6.4.2.2 Biasing the CoTS population sex ratio.

Many genetic biocontrol strategies have attempted to bias sex ratios in the pest population, to produce local elimination or depress successful reproduction events (sections 2.4, 4.2.5). A biased sex ratio in favour of males is reported in many natural CoTS outbreak populations on the GBR (Budden *et al.*, 2019; Pratchett *et al.*, 2014) and elsewhere (Caballes, 2017; Nakamura, 1986). This is likely due to the larger reproductive investment made by females, and the resulting sex bias could further lower the reproductive success of low-density populations (Pratchett *et al.*, 2014). This effect has however not yet been quantified. It would

also need to be considered if a strong sex bias could lead to a larger role for hermaphrodite individuals (Guerra *et al.*, 2020), and whether genetic modifications leading to sex bias can be maintained in the population.

An initial hurdle to developing such a strategy in CoTS is that the genes and processes involved in sex determination and sex differentiation are poorly understood (Guerra *et al.*, 2020) (sections 6.4.1.1, 6.4.1.2).

Gamete recognition proteins are species specific and may be worth exploring (Patiño et al., 2016), and these were recently determined for CoTS (Guerra et al. 2020). In contrast, the well-characterized gonad-stimulating peptide RGP is involved in the last step of oocyte maturation (Hall *et al.*, 2016; Smith *et al.*, 2019) may not be species-specific as it mediates the production of 1-MA, which is the spawning hormone in all sea stars. Mining of the CoTS genome (Hall *et al.*, 2016) may identify other candidate targets or genes linked to chemosensory receptors or neurotransmitters that are differentially expressed in male and female CoTS (Roberts *et al.*, 2018; Smith *et al.*, 2018) and this should be investigated further.

6.4.2.3 Trojan genes

Trojan genes are additional modified genes that increase the attractiveness of a gene modified organism that would otherwise have reduced reproductive success. It could be investigated whether the expression level of genes involved in production or reception of pheromones could be targeted in an attempt to increase the mating success of gene modified CoTS.

6.4.2.4 Killing CoTS by gene silencing

Interfering RNA that can induce lethality by gene silencing (section 2.4) has been considered as a direct alternative for chemical pesticides to control mosquitoes (Airs, Bartholomay, 2017), however the approach is not yet ready for field application. The physiology of the RNAi target, the delivery strategy and timing would determine the immediacy and potency of such a strategy. In mosquitoes, RNAi targeting genes involved in programmed cell death (apoptosis) induced rapid mortalities. Other essential genes that have been targeted include tubulins, vacuolar ATPase proton pumps, and genes involved in prophenoloxidase processing, with varying implications for longevity, fecundity and fertility (Airs, Bartholomay, 2017).

Given that CoTS has a much longer life-span than mosquitoes, it is likely that a single delivery of RNAi in an abiotic matrix (e.g. chitosan, nanoparticles) would have to induce rapid lethality, since a slower-acting process may require prolonged exposure to the RNAi. The latter can likely only be achieved if the silencing RNA was supplied via genetic engineering (sections 6.4.3.1, 6.4.3.2).

A strategy aiming to kill CoTS via externally delivered RNAi would not require deployment of additional CoTS. On the other hand, it would have to be demonstrated that this strategy could produce a cost-effectiveness gain that warrants the additional risk represented by genetic manipulation and the potential for non-target impacts.

6.4.2.5 Susceptibility to natural enemies

Control of CoTS is a different challenge than control of an invasive species. Given that CoTS occurs naturally on the GBR, the primary goal is not eradication but rather to keep the population below outbreak levels.

CoTS produce chemical compounds such as saponins, plancitoxins and plancinin that are implicated in protection against predators (section 6.3.1.4) and knockout or knockdown of genes required for their synthesis could render CoTS more vulnerable to predation. Saponins are implicated in predation protection of all life stages, and it could be investigated if this is valid also for other compounds. If CoTS-specific genes that are central in their synthesis pathways could be identified, then this would lower the risk profile of such a modification. An advantage of the approach would be that is not expected to alter mating behaviour or mating success, so individuals that do survive predation are expected to reproduce normally. There is a risk however that individuals with repressed production of one predator deterrent would experience selection pressure towards producing increased levels of other deterrents, with unknown ecological consequences for their current predators. The CoTS microbiome would also likely respond if the chemical environment is altered, and it is so far unknown if some symbionts (e.g. *Spiroplasma*) can influence CoTS fertility.

Predator exposure of juvenile and small CoTS could, at least in theory, be enhanced by drawing them out to feed during the day. The level of melatonin, the main regulator of circadian rhythms in sea stars (Peres *et al.*, 2014) are higher in male than in female CoTS and also drops in food-deprived CoTS (Smith *et al.*, 2018). However, the level of melatonin responds to the trypthophan – serotonin metabolomic pathways, which are also implicated in other physiological processes (Smith *et al.*, 2018). Hence it may not be possible to interfere in the circadian rhythm of CoTS without producing significant unintended and unwanted consequences.

Studies of natural predators (e.g. giant triton) and microbial agents that show virulence towards CoTS or other echinoderms could lead to the discovery of toxins or other virulence mechanisms that could be exploited, for example as part of a ‘killer-rescue’ self-limiting gene drive (section 5.5). The use of toxins from other organisms, such as transgenic spider toxins tested for use in mosquito control (Bilgo *et al.*, 2017; Lovett *et al.*, 2019), could meet significant public opinion and regulatory hurdles.

Interference with the immune system of CoTS is another possible strategy. A genetic modification to make CoTS more vulnerable to naturally occurring opportunistic pathogens would carry a relatively high risk as it would not be possible to control which pathogens would proliferate and potentially spread to other organisms. Alternatively, immune response pathways could potentially be over-stimulated leading to an allergic reaction involving apoptosis/necrosis, as observed after oxbile injections (Grand *et al.*, 2014) currently used to control CoTS on the GBR.

6.4.2.6 Shortening of average lifespan

The fecundity of individual CoTS increases exponentially with size, and hence with age and nutritional condition (Babcock *et al.*, 2016b; Budden *et al.*, 2019). It is therefore possible that shortening the average life span of individuals would reduce their life-span reproductive output significantly. This has parallels with the strategy used for *Wolbachia*-based control of

mosquitoes, where a shortened life span of the mosquito effectively prevents virus transmission (Popovici *et al.*, 2010). As briefly outlined above, gene silencing of certain essential genes has also been shown to reduce the life span of mosquitoes (Airs, Bartholomay, 2017). At this point in time, we are not aware of an obvious gene target that could achieve this in CoTS. Modelling would be required to evaluate to what extent a shortened life span would affect overall CoTS population dynamics and their expected impact on coral cover.

6.4.3 Delivery and deployment

6.4.3.1 Genetically modified CoTS

Direct genetic modifications of CoTS would most likely be made using CRISPR/Cas9 editing by injection into fertilised CoTS eggs or early embryos or by transfection, with subsequent deployment of modified organisms to the reef. The discovery of tissue or life-stage specific transcription promoters and transcription terminators in CoTS would be desirable but not a requirement for the success of such an approach. Deployment would most likely have to occur when wild type CoTS population densities were low to increase the relative abundance of modified CoTS or be conducted along with manual control to reduce the native population to the lowest levels possible. The number of deployments and the number of CoTS required per deployment would have to be estimated by modelling for each genetic system and strategy, including the possible coupling with a gene drive (section 5.0 and below). This strategy would involve an initial addition of CoTS, which would increase the predation pressure on the targeted reef(s).

It is theoretically possible to create transgenic CoTS indirectly by engineering a naturally occurring symbiont to express an RNAi (dsRNA or shRNA) that targets a CoTS specific gene. The *Spiroplasma* bacterial symbiont that is naturally present in CoTS gonads and digestive system (section 6.2.1.2) represents such a candidate but further studies would be required to determine its ecological role and its susceptibility to genetic manipulation. An advantage of this approach is that *Spiroplasma* could possibly provide tissue-specific delivery of RNAi that is responsive to host nutritional state (Herren *et al.*, 2014) or gonad development phase, however this would have to be experimentally confirmed. The transmission pathway of the symbiont would also have to be elucidated to determine if it can be transmitted vertically from parent to offspring. Furthermore, it would be crucial to examine the species specificity of both the symbiont and the siRNA that it would express.

Symbiont delivery could be done in captivity (injection, submersion of eggs or larvae, or feeding of larvae or juveniles), with subsequent release, and the considerations outlined for transgenic CoTS above would then apply. It could also be investigated if the symbiont can be delivered to wild CoTS in the field, either via injection, baited traps, or by release of encapsulated particles. The fact that the symbiont naturally occurs in the CoTS digestive system increases the likelihood that uptake by ingestion in juvenile or adult CoTS could be successful.

Ultimately the scale of infrastructure required and the period over which it has to be maintained will be a function of the characteristics of the construct developed. Coupling of the genetic modification with a completely unconstrained gene drive would require a relatively small (though still significant) investment given that it would have low threshold requirements and should spread rapidly through the entire accessible population. In an extreme case where a construct had unconstrained spread and were lethal, deployment could well require just a few

releases and for these to occur just once. This low cost however would most likely be balanced by higher risks associated with reduced control over the outcomes.

If higher levels of constraint were present naturally or engineered into the gene drive construct, greater investment would be required. A highly self-limited construct would likely have a higher introduction threshold, would remain within the population only temporarily and would have a reduced spread potential. These characteristics would require larger deployments, conducted at more locations and over longer timeframes to achieve their goals. Such a construct would require more regular and higher resolution monitoring of CoTS dynamics on the GBR to detect outbreaks and to allow a timely control response. It may also require greater degrees of support through traditional manual control in order to achieve the required introduction thresholds (Thresher *et al.*, 2014b). Such constructs may require the maintenance of additional facilities and program components indefinitely in order to achieve high levels of control.

6.4.3.2 Genetically modified coral

If production of transgenic corals is progressed under The Reef Restoration and Adaptation Program (RRAP), it could be considered to engineer these corals to also carry genes that can counteract CoTS. This would have parallels with transgenic plants that carry genes for insect toxins, and a recent suggestion that target fish in the Great Lakes could be genetically modified to express molecules that are lethal or sterilise Sea Lamprey (Thresher *et al.*, 2019).

At first consideration, this approach has many challenges and seems unlikely to be successful. Without rapid spread of the coral constructs across the GBR, this strategy would most likely protect the coral constructs themselves rather than control CoTS populations, and non-target effects on other species that feed on or shelter in the modified corals can be expected. If coral were engineered to produce a deterrent or toxin with immediate protective effect, then this could lead to prey-shifting and CoTS could target other coral in the vicinity. On the other hand, if the modification (e.g dsRNA for gene silencing) was created to sterilise CoTS or bias the CoTS population towards one sex, this could lower the reproductive output of CoTS on that reef with possible flow-on effects for coral recovery in the next generation. The efficiency of such an approach would depend heavily on the proportion of modified corals relative to wild-type corals. Another major challenge would be the delivery efficiency to juvenile or adult CoTS feeding on coral, including the delivery of dsRNA to the tissue where its activity is required (e.g. gonads). This approach would not involve the deployment of additional CoTS so it would not directly increase the predation pressure on coral. Modelling would be needed to test the likely efficiency of this approach under different deployment scenarios.

6.4.3.3 External delivery of RNAi

The use of exogenously delivered RNAi represents a reversible strategy that may face fewer regulatory hurdles and less resistance in public opinion than transgenic delivery of RNAis. While this strategy has been used extensively for gene silencing studies in the laboratory, there are considerable challenges to its use in wild populations. These challenges relate to the development of inexpensive methods for production of RNAi at scale and methods of delivery, including the delivery of intact RNAis into the cells where their activity is required.

Bacteria, yeast or microalgae cells can be engineered to produce the RNAi of interest in culture, with subsequent purification or direct use of the intact (live or dead) microbial cells (Abrieux, Chiu, 2016; Itsathitphaisarn *et al.*, 2017). For field delivery, it can be expected that RNAi in microbial cells or protected by a type of nanoparticle will have superior performance as compared to naked RNA, which is rapidly degraded and diluted in the environment. Nanoparticle materials that have been proposed for RNAi delivery in the field include chitosan, carbon quantum dots and cell penetrating peptides (Airs, Bartholomay, 2017; Das *et al.*, 2015; Meade, Dowdy, 2007, 2008). Viral expression systems have also been suggested as a means to deliver and express RNAi in mosquitoes based on the range of entomopathogenic viruses already used for biocontrol and the existence of compatible viral expression systems (Airs, Bartholomay, 2017). While CoTS carry viruses that replicate in eukaryote cells, including densoviruses which have previously been used in RNA silencing (Gu *et al.*, 2011), a major practical hurdle to using a virus for CoTS control is the lack of a CoTS (or echinoderm) cell line required for viral production (section 6.2.1.1).

Externally delivered RNAi is generally delivered to the host via soaking, feeding or injection into the circulatory system. A big challenge with this approach is the subsequent uptake of the RNAi by the cells where they need to be active. The CoTS genome includes a homologue of a dsRNA transporter gene (SID-1) (LOC110988328), which is involved in systemic RNA interference in *C. elegans* (Winston *et al.*, 2002). It is therefore possible that systemic effects would be easier to achieve after oral delivery in CoTS than in mosquitoes, which lack SID-1 (Yu *et al.*, 2013). Possible delivery strategies for RNAi to CoTS include injection, baited traps, or release of encapsulated particles into the water column. Delivery by injection would however not provide an efficiency gain relative to current practices unless the effect is transmissible horizontally or vertically to the next generation. As for all possible strategies, modelling would be required to evaluate the expected effectiveness of the treatment in addition to evaluation of specificity and the cost-effectiveness of the approach.

6.4.4 Possible contribution to CoTS IPM strategies

Most of the genetic biocontrol strategies described above represent long-term strategies that would take several CoTS generations to become effective since they need time to establish in the population. The initial deployment campaigns would have to be combined with manual culling campaigns to increase the proportion of modified CoTS at the deployment sites although the long-term aim would be to drastically reduce revisitation frequencies. Depending on the selected strategy, the genetic biocontrol program could be combined with strategic manual culling, zoning and other fisheries restrictions.

6.4.5 Associated risks

The ability to engineer desirable genetic outcomes carries with it the very real likelihood that those outcomes will not always be desirable. Such a situation could arise in CoTS if, for example, the ecological context being managed changes over time. Similarly, at the genome level, non-target genetic effects could result from changes induced in particular genes – an edit of a locus may change its function, e.g. knock it out, but it may also modify other, potentially unrelated processes, with unknown consequences for the modified animal or its predators.

External delivery of RNAi is a reversible strategy that provides a transient change in gene expression levels but does not introduce a genetic modification. This approach could be

suitable for CoTS in a situation where a transient gene knockdown is the effect that is sought, i.e the effect is only needed at a specific time of the year or at a specific point in the outbreak cycle. However, the approach is not risk free. In particular, the specificity of the RNAi must be carefully evaluated as well as the efficiency of the delivery strategy including uptake into target cells. Moreover, the safety and possible immune-stimulation of any delivery agents would have to be evaluated. There is a significant financial risk associated with rolling this approach out on a large scale as the effect is transient and re-application would be needed. Hence, the efficiency and cost-effectiveness of the strategy would have to be demonstrated by experiments and modelling.

Transgenic expression of RNAi-inducing molecules involves a gene modification and therefore represents a longer-term intervention with a risk profile more similar to that of other genetic modifications.. The regulatory hurdles and public opinion challenges related to this approach will likely be higher than for externally delivered RNAi.

Unless a genetic modification is coupled with a gene drive, it will eventually be lost from the population via natural selection and Mendelian inheritance. While the capacity of a genetic modification to spread a desirable trait among populations connected by gene flow is desirable in a pest control setting, the potential for uncontrolled spread of a gene drive is high (section 5.4). It needs to be recognised that a CRISPR-Cas9 gene drive system in CoTS could spread well beyond the GBR and across national boundaries over a period of generations via natural connectivity processes, or even faster via human-mediated connectivity. Uncertainty about the length of the pelagic phase, northward flowing eddies, and stepping-stone reefs across the Coral Sea and northward along the GBR are factors pointing to a strong potential for natural spread. Hence, any attempt to develop CRISPR-Cas9 gene drives for CoTS control should also consider means of limiting the spread or duration of the control system.

A number of options limiting the spread of gene drives geographically or in time have been considered (sections 5.5, 5.6) and modelling suggests that they are likely to be effective in the field (Min *et al.*, 2017; Noble *et al.*, 2019). Ongoing maintenance of production facilities and surveillance would be an associated cost of increasing levels of self-limitation. The scale of this cost increase might be ameliorated by more strategic deployments, either by targeting i) the locations that maximize spread through the population, e.g. by targeting reefs known to have very high levels of connectivity (Hock *et al.*, 2016), ii) the times and locations that result in the greatest impact on population dynamics, or iii) the initiation box (area where outbreaks start).

It might be suggested that the risk of trial deployments could be reduced by utilising a natural barrier to spread, for example, as provided by strong upwelling currents around Hydrographer's Passage that limit movement northwards. In theory, this means that deployments in the Swains regions could have a reduced risk of uncontrolled northward spread to other jurisdictions. We recommend strongly against this however, as it is likely to be a high-risk approach. Larvae might cross this 'barrier' riding eddies, driven by strong winds pushing surface waters, or through dispersal on gyres out into the coral sea and back again onto the GBR even under current conditions. Furthermore, it is also likely that climate change may destabilise ocean currents and up-wellings (Thornalley *et al.*, 2018; Xiu *et al.*, 2018) and apparent current-based barriers may less reliable than expected as documented by the

penetration of the Antarctic Polar Front by drifting kelp (Fraser *et al.*, 2017). In addition, human mediated spread across a 'natural barrier' is of course possible.

6.4.6 Critical knowledge gaps

The knowledge gaps relating to possible genetic biocontrol of CoTS are extensive and wide ranging. Here we outline some key science and technological areas requiring research and investment over and above the investment in the development of the construct itself.

6.4.6.1 Selection of control strategy

- What kind of outcome or effect (eradication, suppression) is desired?
- Geographical scale
- Temporal scale, duration
- Risk acceptability
- Which modifications would be able to be maintained or spread in the population?
- Possible outcomes of gene-drive strategies in CoTS populations?
- Identification of the types of modifications that fulfil the criteria above
- Human-mediated connectivity of potential release locations

6.4.6.2 Gene technological challenges

- Demonstration of CRISPR/Cas9 modifications in CoTS
- Demonstration of using iRNAs in CoTS
- Tissue-specific transcription promoters or terminators in CoTS?
- Life-stage specific transcription promoters and terminators in CoTS?
- Symbiont-mediated delivery of iRNAs in CoTS?
- Development of suitable self-limiting gene drives (threshold, reversal etc.)

6.4.6.3 Fundamental knowledge gaps, CoTS biology and genetics

- How is sex determined in CoTS?
- Role of larval cloning in CoTS populations
- The age profile of CoTS in nature
- Genetic pathways involved in specific processes, and their regulation
- Development of resistance or selection pressure for altered phenotypes as a result of genetic control strategies
- Species specificity of genetic control strategies
- Possible flow-on effects on natural predators, prey or other reef organisms

6.4.6.4 Development and testing of construct

- Facilities and research to develop and test constructs in contained populations.
- Modelling to determine performance in the field and to underpin the development of appropriate deployment strategies

6.4.6.5 CoTS Aquaculture and deployment

- Husbandry techniques and technologies for breeding and maintaining CoTS.
- Technologies for quick sex determination of CoTS juveniles (if needed)
- Establishment of suitable aquaculture facilities for production of large numbers of larvae or juvenile CoTS
- Development of deployment technologies including methods for larval/juvenile transportation to deployment sites

7.0 OTHER CONSIDERATIONS

7.1 Incorporation into CoTS IPM program

For all of the technologies discussed above, the development of strategies for optimised use as part of an integrated CoTS control program will be crucial. This includes the optimisation of spatial and temporal applications, and modelling of efficiency and relative cost-effectiveness (section 7.2). Monitoring programs will need to be developed and established to assess performance and evaluate the selected program strategy after implementation.

7.2 The role of modelling

Modelling is a means of assessing the outcomes of complex and interacting processes and consequently will play a crucial role in the evaluation of the feasibility and cost-effectiveness of different CoTS control options. A variety of models of reef dynamics that incorporate CoTS population outbreaks have been developed and these differ in their temporal and spatial scale, the factors they consider, as well as their statistical approach (Babcock *et al.*, 2016a; Bozec, Mumby, 2019; Chen *et al.*, 2017; Condie *et al.*, 2018; Fabricius *et al.*, 2010; Hock *et al.*, 2016; Hock *et al.*, 2014; Mellin *et al.*, 2016a; Morello *et al.*, 2014; Pratchett *et al.*, 2017; Rogers *et al.*, 2017; Scandol, 1999; Walshe *et al.*, 2017).

At this point in time, two modelling frameworks, CoCoNet (Condie *et al.*, 2018) and ReefMod (Mumby *et al.*, 2007) are being used to explicitly simulate the dynamics of coral communities and CoTS on the GBR and their responses to different management scenarios (e.g. Condie et al. 2018; Condie et al., submitted). Both CoCoNet and ReefMod are agent-based simulation models but differ in that they model processes differently and make different assumptions. Consequently, deployed in tandem in a consensus approach, they, or similar models, would provide a fundamentally important technology for assessing the likely performance of the different control options. These contributions would include assessing likely performance, identifying critical parameters and parameter value ranges required to reach target levels of CoTS abundance and/or coral cover, and the relative cost-effectiveness of each method. Models should preferably account for climate-mediated changes in the interactions between CoTS and their predators (e.g. tritons, fish) and prey (corals) to enable identification of the most appropriate control options into the future (Mellin *et al.*, 2016a). Such approaches may be used to guide research priorities and set stop-go criteria for continued research into specific technologies.

7.3 Environmental risk analysis

A framework for ecological risk assessment that is suitable for adaptive management was proposed by (Van den Brink *et al.*, 2016), and includes cultural and ecological protection goals, development of ecological scenarios, establishment of the relevant interactions among species, potential sources of stressors, their interactions and the development of cause–effect models. In this report, we have conducted a simplified risk analysis as part of our overall formal assessment of each technology (section 8.0). However, for the more promising control options, a formal and comprehensive environmental risk analysis will be needed involving an expert panel in each case. As an example of a more formal process, we applied a previously

developed framework (Moro *et al.*, 2018) to assess ecological risk and knowledge gaps associated with the potential use of gene drives for CoTS control (Appendix 2).

7.4 Social and institutional support

The level of support for research into the more promising control options needs to be determined for Traditional Owners and stakeholder groups including managers, fisheries and tourism industry groups, environmental groups, and the general public. While there are several challenges to engagement relating to new technologies, early engagement often leads to more useful solutions that are better accepted by both stakeholders and decision-makers (Dana *et al.*, 2014; National Research Council, 2008). In Australia, the stakeholder engagement processes linked to ongoing biological control programs for disease-transmitting mosquitoes (The World Mosquito Program) and rabbits, as well as the possible roll-out of biological control of carp, may serve as templates for a corresponding process for CoTS control. RRAP has initiated engagement with Traditional Owners, and place-based or interest-based stakeholders such as reef communities and reef-based industries (Taylor *et al.*, 2019). It is recommended to engage with these other programs and build on their experiences when planning this process for CoTS control.

Institutional acceptance will be critical from the earliest phases of new technology developments. Of particular importance is early engagement with traditional owners, who have recognised customary connections to identified areas of the Reef (Taylor *et al.*, 2019). Some traditional owner groups in the GBR area have native title rights and related Indigenous Land Use Agreements. In addition, there are several co-governance and management arrangements between Traditional owner groups and National and State government agencies (e.g. GBRMPA, Wet Tropics Management Authority, Queensland Park and Wildlife Services), including several Traditional Use of Marine Resources Agreements that are specific for each individual GBR Traditional Owner group (GBRMPA, 2020b). It is essential that engagement with traditional owners is meaningful and aligned with the multitude of existing plans, strategies and activities, as outlined in more detail by Taylor *et al.* (2019). Traditional Owners have a representative on the Marine Park Authority board and in addition the Indigenous Reef Advisory Committee provides non-binding expert advice to the board (GBRMPA, 2020a). Recently, the Great Barrier Reef Foundation established a Traditional Owner Advisory Group (Great Barrier Reef Foundation, 2020) to guide co-design and co-delivery of activities under the Reef Trust Partnership, which will include the CoTS Control Innovation Program.

The recent Position Statement from GBRMPA on Climate Change (GBRMPA, 2019) indicates a shift to perceiving the GBR as under an existential threat. At the same time resistance to more interventionist approaches to management on the GBR appear to be decreasing with Commonwealth and State agencies actively involved in engineering and translocation programs to support management objectives, e.g. the Raine Island Project (https://parks.des.qld.gov.au/raineisland/) and RRAP (https://www.gbrrestoration.org/home). This engagement signals the potential for active involvement of agencies in considering the potential of novel technologies in the management of CoTS on the GBR.

7.5 Legislative Context

The international, federal and state regulatory frameworks that would apply to each of the prospective control technologies would need to be considered early and thoroughly. RRAP recently assessed the regulatory framework that would apply to the interventions proposed under that program (Fidelman *et al.*, 2019) and their assessment should be used as a starting point.

All technologies discussed in this report would be covered by the Great Barrier Reef Marine Park Act 1975 (Commonwealth), the Environmental Protection and Biodiversity Conservation Act 1999 (Commonwealth) and the related State Acts. Depending on the nature of the technology and of its deployment area, a range of additional Commonwealth and State legislative provisions may also be triggered, including The National Parks and Wildlife Act 1972 (Commonwealth), the Fisheries Management Act 1991 (Commonwealth), the Fisheries Act 1994 (Queensland), the Biological Control Act 1984 (Commonwealth), the Biodiscovery Act 2004 (Queensland), and the Agricultural and Veterinary Chemicals Code Act 1994 (Commonwealth).

Under current Australian legislation any organism created through gene technologies is classified as a Genetically Modified Organism and all related research and field trials would have to be conducted under the conditions of the Australian Government's *Gene Technology Act (2000)*. A recent amendment to the Gene Technology Regulations 2001 (Gene Technology Amendment Regulations 2019) have clarified the definition of a gene modified organism (GMO) under the regulation, excluding organisms that were modified by unguided repair of site-directed nuclease activity as long as no other modifications were introduced by the technology. This amendment further specifies that dealing with a gene drive GMOs requires a licence (Dealings Not Involving Intentional Release: DNIR).

It should also be noted that in spread of any deployed organism beyond national borders without consent may represent a violation of the Cartagena Protocol (Convention on Biological Diversity, 2016).

8.0 SUMMARY ASSESSMENT OF COTS CONTROL APPROACHES

None of the technologies discussed in this report are likely to represent a 'silver bullet', and the challenge is to optimise the combination of tools to develop and strategically apply them to maximise the level of control that can be achieved. Some of the methods already have a strong evidence base and a clear pathway to implementation, while for others one, or both, require clarification.

In this section the technologies previously discussed are formally assessed against a set of criteria relating to effectiveness (short-term, long-term), risk (reversibility, specificity, spatial control, acceptability), and logistical constraints (scalability, revisitation rates, operational cost) based on the approach outlined by (Moro *et al.*, 2018). For each criterion, we considered the current level of knowledge and the extent to which the technology's characteristics fulfill this criterion. For comparison purposes 'Manual control' was included as a reference. Following (Moro *et al.*, 2018) we have used a scale from 0 to 2, where 0 indicates 'knowledge lacking' or 'criterion not fulfilled', and 2 indicates 'knowledge relatively good' or 'criterion fulfilled', respectively. A score of 1 indicates either 'minimal knowledge' or 'criterion partially fulfilled'. We emphasize that the criterion fulfillment scores are based on current knowledge and some will likely change as our knowledge increases.

The underlying reasoning behind each score is presented in Table A1 (Appendix 1) and summarised below. The scores are presented visually in Figure 3, where the level of knowledge used for scoring is represented by the blue line, and the actual score against each criterion is represented by the orange line. Colour coding is used to emphasize the category of each criterion (red - logistics, green - effectiveness, and blue - risk).

The knowledge level and strengths and weaknesses of each technology for the different categories (effectiveness, risk, logistics) is reflected by the respective outline's shape. For example, a category where the blue or orange shape is expanded to the outer ring indicates high levels of knowledge or feasibility, respectively, for that particular category, while one that is collapsed in to the centre indicates a lack of knowledge or uncertainty or risk associated with implementation. This aspect of the graphs can be used to guide the selection of suitable methods (individually or in combination) for specific purposes. For example, if the main purpose is protection of specific sites, then logistics (i.e. scalability) may not be as relevant as other aspects.

In general, the relative area within the resultant blue outline indicates the maturity of our knowledge base for that technology and the relative area within the resultant orange outline the potential for implementing the approach at a large scale.

8.1 Manual control

Manual control is a relatively mature technology, where the level of knowledge and documentation on the outcome of control efforts is much higher than for the alternative technologies. Currently, manual control of CoTS on the GBR is based on ecologically and operationally based IPM approaches that scale from the site to the region, using feedback from

control operations to guide decision making at each of these scales (Fletcher *et al.*, 2020). The approach has been shown to be effective in reducing CoTS numbers and to facilitate coral growth at the site scale, and to reduce CoTS numbers to below ecological thresholds at the reef and regional scale (Fletcher *et al.*, 2020; Westcott *et al.*, 2020).

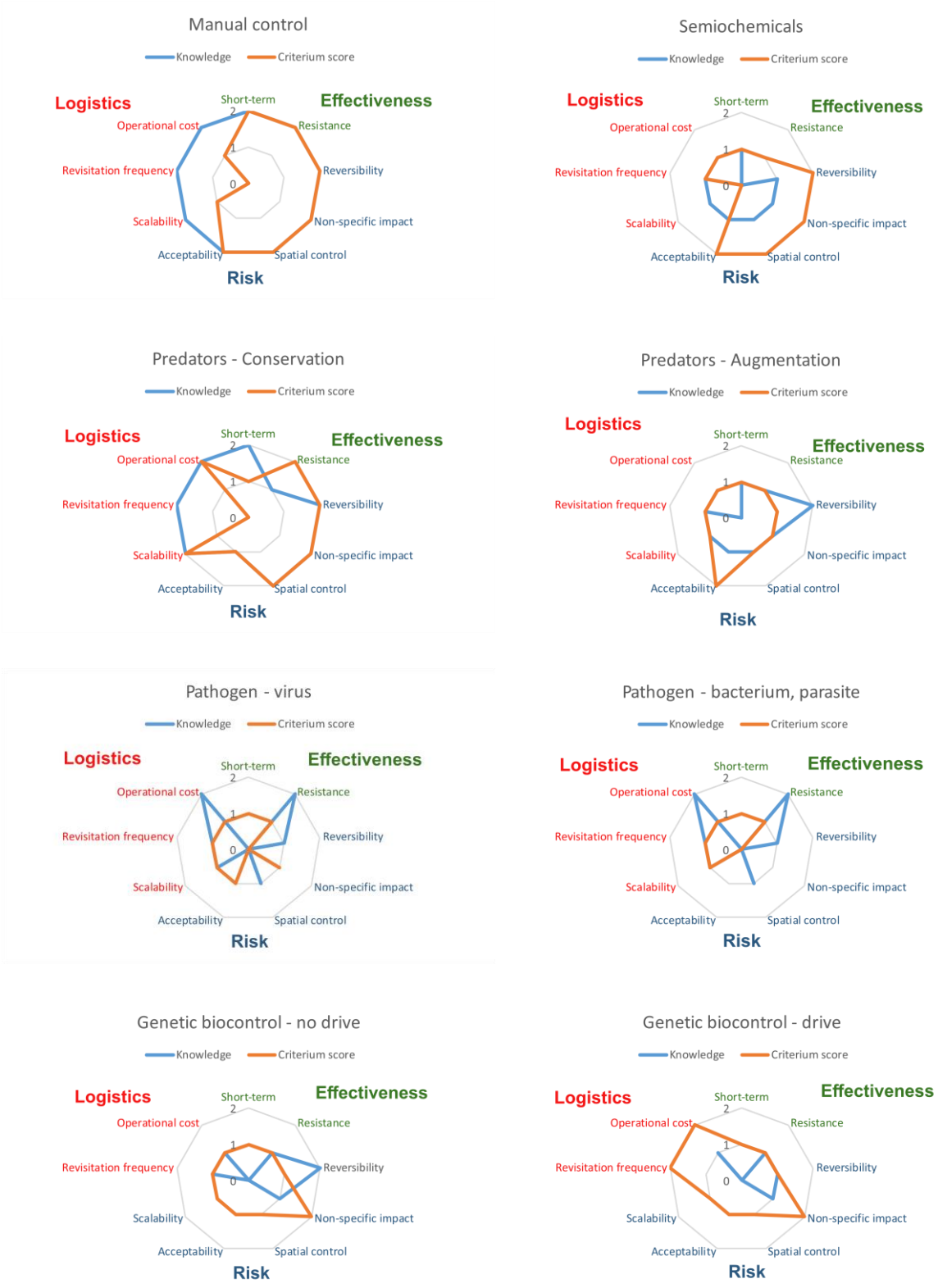

Figure 3: Radar graphs illustrating the knowledge level and score for criteria relating to effectiveness, risk and logistics. Criterion definitions and reasoning behind each score are presented in Table A1.

When used as an adaptive management tool, manual control has demonstrated effectiveness on a regional scale (Fletcher *et al.*, 2020; Westcott *et al.*, 2020). There is also little risk that CoTS will develop resistance to this control method so its effectiveness will be retained over the longer term. Another advantage is the low risk profile; the control is species specific, its geographical footprint can be controlled, and it is expected that effects can be reversed by stopping the control efforts. The method is already approved and in use on the GBR, has relatively good support among stakeholders and has positive social benefits through employment and training. On the other hand, manual control of CoTS has logistical constraints including the need for frequent revisits, the operation is labour and resource intensive, and scalability is limited by resource availability. Strategic deployment can mitigate some of these limitations.

Overall, manual control represents an immediately available approach that has been shown to be effective at the site and reef scale and which is currently being scaled up to the regional scales. It can and should be further refined, including through the integration of some of the other technologies considered here.

8.2 Predator enhancement - Conservation

A predator conservation strategy for CoTS control would operate to maintain and protect populations that prey on one or all CoTS life stages providing top-down control of the CoTS population (sections 2.2, 6.1.2). Predator enhancement through protection based on zoning is conceptually simple and no-take zones (green zones) are already implemented across c. 33% of the GBR. Additional areas are also subject to restricted harvest (http://www.gbrmpa.gov.au/access-and-use/zoning/interpreting-zones) through restrictions such as protected species, size and possession (bag) limits, and seasonal and spawning closures. These restrictions are managed by the Queensland Government via Fisheries Queensland and Department of National Parks, Recreation, Sport and Racing fishing regulations.

Zoning is demonstrated to protect predatory fish and reef community structure (and by implication invertebrate and vertebrate predators of larvae and juveniles). Protected areas have greater resistance to, and resilience in the face of outbreaks and tend to have fewer CoTS (section 6.1.2). However, zoning by itself does not protect a site from outbreaks nor from CoTS damage. Consequently, protection is likely to be used in a complementary fashion to support other approaches and to maximise reef resilience.

The risk-profile of zoning protection is mixed with the possibility to introduce and remove restrictions, and also to target specific fisheries. Many predators are generalists, so the effects are not expected to be CoTS specific, although this does not necessarily mean that the effect is not desirable. Increased predator protection (i.e. increases in areas zoned as no-take) would likely meet strong push-back from groups likely to be impacted, such as commercial and recreational fishers.

The management of zoning and fisheries restrictions require enforcement as well as ongoing monitoring to document the effect of implemented regulations. In theory, zoning and other fisheries regulations could be rapidly implemented but public consultations mean that it is likely a medium-term prospect.

8.3 Predator Enhancement – Augmentation

This approach involves aquaculture of known predators, such as Giant Triton or predatory fish, with subsequent release to supplement existing populations (sections 2.2, 6.1.2). Augmentative biocontrol strategies have been used in other marine locations, but primarily with grazers to control biofouling or invasive algae (section 4.3.1.2). So far, there is no experience with augmentative biocontrol from the GBR.

A variety of invertebrates and vertebrates prey on CoTS, and the likely effectiveness of augmentation in controlling CoTS numbers would depend on prey choice and a range of other factors, which are still undocumented. The risk profile of this approach is difficult to assess in detail due to the limited information available. The most apparent risk is predation on other species than CoTS, the extent of which would depend on the level of augmentation and the fate of released individuals and possible descendants. In addition, unanticipated community level consequences are possible and deployment of aquaculture-reared individuals may represent a biosecurity risk.

The logistical constraints are related to whether development of large-scale aquaculture facilities is possible. The technology faces significant developmental hurdles and will require major research and infrastructure investments.

Overall, this approach would most likely be implemented only at local scales. It is possible that it would be cost-effective only if other benefits, e.g. recovery of populations of an over-harvested species (i.e. Giant Triton) or production of fish for human consumption or recreation (e.g. emperors, Giant Grouper), are regarded as of significant added value or present co-investment opportunities. The significant research and development needs mean that this approach would only be available in the mid- (fish) to long- (Giant Triton) term.

8.4 Pathogen control – viruses, bacteria or parasites

The approach involves culture and broadscale release of viral or bacterial pathogens, or parasites, to control CoTS populations (section 2.2). There is evidence of a wide diversity of viruses in CoTS, however no viral pathogen of CoTS is known, and no echinoderm cell line is available for large-scale virus production. Likewise, no primary bacterial pathogen (i.e causing disease in non-stressed individuals) or suitable species-specific parasite is known (section 6.2.2).

The short-term effectiveness of pathogen biocontrol would be determined by the agent's virulence or ability to interfere with reproduction and its potential for spread. These factors would be agent specific and potentially variable in time and space. Resistance can develop, in particular against viral biocontrol agents, and this risk would have to be managed by developing cocktails of several viruses.

The potential use of viral or microbial pathogens for biocontrol of CoTS was considered by COTSREC in 1989. At that time their assessment was that the methods were too risky due to uncertainties around specificity and control. This assessment remains valid today. A detailed risk analysis is difficult given that no good candidates are available.

The scalability and operational cost of pathogen control would depend strongly on the development of an efficient production system. It is expected that the revisitation frequency would be lower than for manual control, however re-application would likely be required.

Overall, in our assessment the highest value in studying CoTS pathogens lies in the determination of effective virulence mechanisms and CoTS immune responses. This could help in the identification of genes encoding toxins or other relevant virulence factors, or elucidation of CoTS genetic pathways that might be targeted by new genetic technologies. Such work could be done in the short to medium term, 5-10 years.

8.5 Semiochemicals

This approach involves addition of semiochemicals, produced via synthesis or recombination, to the environment either to attract CoTS to a specific location or trap to facilitate culling or to disperse CoTS to protect specific sites or prevent spawning aggregations (sections 2.3, 6.3.2).

Field deployment of CoTS attractants to date are limited to preliminary trials (Teruya *et al.*, 2001) and their effectiveness on the reef is currently speculative. The potential for repelling CoTS by this approach has been shown in lab trials with live predators (section 6.3.1.4), while the potential for attraction is primarily based on Y maze assays with attractants varying from live CoTS and coral, to single compounds (sections 6.3.1.1, 6.3.1.2), and by evidence from proteomics and transcriptomic studies of aggregating CoTS (section 6.3.1.5). Uncertainty exists around how spread on currents will influence the propagation of the signal and how these signals might interact with other signals in the field. Factors such as these will ultimately influence effectiveness.

We expect that the environmental risk profile of using semiochemicals is low if compounds are selected so that they are specific to CoTS, however studies are required to specifically address this for candidate compounds.

With regards to logistics, critical knowledge gaps still exist relating to suitable compounds or mixtures, their production at scale and strategies for field deployment. If combined with gene technologies, there is the potential for manipulating the *in vivo* production levels of chemicals implicated in these processes.

Overall, semiochemicals may provide a means of manipulating the behaviour of CoTS on reefs to support manual control, in particular for attracting CoTS at ‘difficult’ reefs, e.g. John Brewer where a few sites remain at the ecological threshold after 18 visits. There is also a possibility that repellents can be developed to protect priority sites or interfere with spawning aggregations. A focus on identifying and blocking the genetic processes involved in aggregation may also prove fruitful. The development of a strategy for implementation and operationalization would allow quantitative assessment of the potential in the medium term (c. 10 years).

8.6 Genetic Biocontrol

This approach involves creating genetic constructs, most likely using CRISPR-Cas9 gene-editing tools, to reduce CoTS fecundity or survival (sections 2.4, 5, 6.4.2, 6.4.3, 6.4.4). Gene-

drive mechanisms could potentially be included in these constructs to ensure controllable and adequate spread. In general, uncertainties relating to sex determination in CoTS suggest that traditional direct genetic targeting of one or the other sex is likely to be confounded. Instead, developmental pathways such as control of gonad or gamete development, genetic regulation of spawning, or gamete binding genes and proteins (Patiño *et al.*, 2016) may represent more promising targets. Alternative approaches that could be explored include modifying gene expression pathways to alter key behaviours, e.g. blocking responses to semiochemicals, or reducing survival or life span through modifications of key physiological pathways or by mimicking disease effects (section 6.4.2).

The potential effectiveness of these approaches is currently speculative but solidly based in theory and laboratory experience with other species. Genetic biocontrol approaches have the potential to provide high degrees of CoTS specificity and control of spread, depending on the construct. Formalised environmental risk assessments as well as legal, ethical and social considerations in the Australian context need careful evaluation at an early stage (sections 6.4.5, 7.2, 7.3, 7.4).

While the CoTS genome has been described, only limited work has been done to identify candidate genetic mechanisms and processes that could be targeted for editing. These efforts would need to be intensified together with the development of appropriate genetic tools and gene-drive mechanisms, methods for aquaculture of CoTS to provide gametes or larvae for deployment, deployment methods in the wild, and means of minimising predation during the period of spread.

Overall, genetic constructs paired with some form of gene-drive hold future potential for CoTS control that could reduce ongoing logistical requirements for control. However, major investment and a long lead in time required for research and development mean that these methods are unlikely to be deployed in the near future, probably not in the next decade or more. In particular, we note that constructs comprising genetic modifications coupled with a gene drive have as yet not been released anywhere in the world. Despite this, these methods deserve serious consideration and expert input.

8.7 Timelines

Consideration of the risks, knowledge gaps and implementation constraints identified for each of the technologies suggests that there is no “silver bullet” technology that will be available before the next outbreak in the Lizard Island-Cooktown region, expected sometime in the next three to five years based on the frequency of the last four events (Pratchett *et al.*, 2014). While it is impossible to predict when a technology might be ready, we can make some general predictions. Below and in we consider the likely development timeframes for each of the technologies assuming an immediate start to the work and no financing constraints.

Predator conservation - involves known and already implemented management approaches. It could be implemented immediately were it not for the strong likelihood of pushback from stakeholders. Consequently, it is unlikely to be implemented with a consultation and negotiation period of several years. Possible implementation in the mid-2020s.

Predator augmentation – to the extent that the general technologies and processes exist in the aquaculture industry, many of the technological hurdles for this approach have been crossed. However, there remain significant challenges in developing species-specific technologies required for the task, for building infrastructure and operationalizing the process. Testing to assess the biosafety risk and risk of unwanted flow-on effects would also have to be performed. It is unlikely that this approach could be implemented before the late 2020s.

Semiochemicals – significant research is required to identify appropriate candidate semiochemicals, to understand their performance under field conditions, and to develop deployment technologies and strategies. Implementation would be unlikely before the mid to late 2020s.

Viral/Bacterial Mechanism studies – these studies would require extensive field and lab work to identify additional candidate pathogens (in addition to *Vibrio*), and research to describe pathogen impact pathways and how these may be influenced by environmental stress. This information can be fed into the development of genetic biocontrol tools. Unlikely to provide useful information before the mid to late 2020s.

Genetic biocontrol – This technology is, in all of its applications, still very much in its infancy. There remain major research hurdles in identifying and designing appropriate constructs and their mode of operation, in particular in designing tools where a high degree of control and certainty in their operation in the field can be engineered. There is also a major task involved in establishing a social licence for the technologies, in risk assessment, and establishing the legal and ethical frameworks for their use. These latter issues are likely to mean that genetic biocontrol without gene-drive could be implemented somewhat earlier than genetic biocontrol with gene-drive. In either case, comparison with other programs suggest that it is unlikely that implementation would occur before the 2030s.

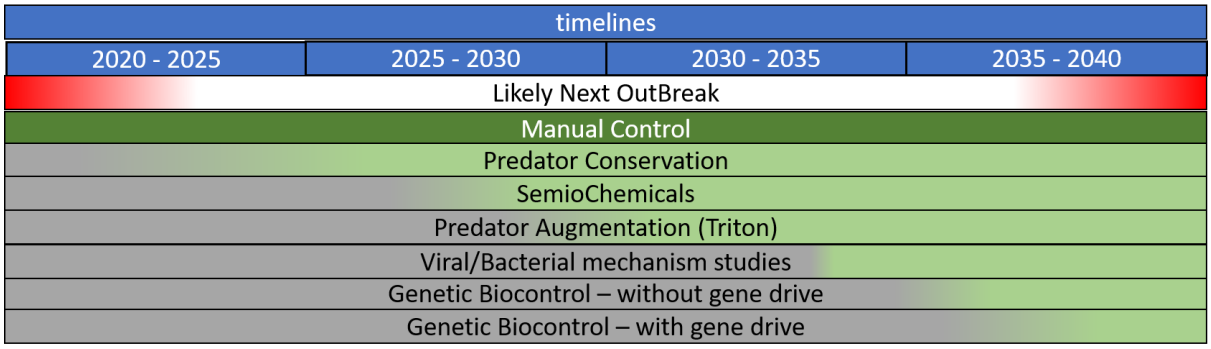

Figure 4: Likely timelines for development and implementation of alternative control techniques. Grey areas represent the period for research, development and consultation prior to implementation of technology. Coloured periods represent implementation period. The likely period of the next outbreak is shown in white as an indicator of the timeliness of each potential technology.

These considerations suggest that by the expected onset of the next outbreak we are likely to only have Manual Control and Predator Conservation as part of our tool set, with other technologies potentially becoming available towards the middle or end of the next outbreak.

9.0 RECOMMENDATIONS

Based on our summary assessments in section 8.0, we make the following recommendations:

1) Manual control will remain central and is the benchmark for control performance

Manual control has proved effective in controlling CoTS at scales from the site through to the GBR (Westcott *et al.*, 2020) and high-resolution temporal and spatial data on control performance and the distribution of CoTS is available. This makes manual control performance an excellent benchmark and method for assessing the performance of other technologies. Currently identified alternatives will not be field deployable until well into the next expected outbreak, and they will either be deployed in a complimentary fashion to manual control or will be enhanced by concurrent manual control. This means that we will continue to rely on manual control for the foreseeable future and should continue to seek to improve how it is implemented. We recommend that monitoring and reactive culling is sustained in the 'initiation box' between Cairns and Lizard Island between outbreaks in the hope of extinguishing primary outbreaks before they generate new waves of secondary outbreaks.

2) CoTS attractants and repellents to complement manual control on difficult and priority reefs.

Further investigation into CoTS attractants is recommended due to their potential for increasing the proportion of CoTS available for manual culling during a visit to a site and for facilitating manual culling by aggregating CoTS at particular sites on a reef. Depending on the development of delivery systems, it is also possible that baited traps could be developed. Further investigation into CoTS repellents is recommended due to their potential use to protect priority sites and in delaying spawning aggregations. The risk profile of these approaches is expected to be low pending tests of candidate compounds (sections 6.3.3, 8.5, Appendix 1).

Recommended actions:

- Identify candidate compounds for aquarium trials and risk assessment
- Synthesize or produce recombinantly compounds with low risk potential
- Field tests to assess effectiveness in an open system
- Modelling to determine critical performance parameter values required for cost-effectiveness relative to additional manual control efforts
- Initiate traditional owner and stakeholder consultation

3) Predator conservation strategies to enhance reef resilience

Consideration of further investigation into fishing restrictions and zoning is recommended as these approaches are already established strategies that increase reef resilience in general. With targeted restrictions (specific fisheries, timing, locations) the CoTS population might be targeted more specifically. Early engagement with traditional owners, stakeholders and regulators would be essential to develop effective strategies with adequate policing and monitoring of outcomes.

Recommended actions:

- Build knowledge base and documentation to determine the likely effectiveness of protection strategies optimised to limit CoTS numbers in terms of specific fisheries, species, seasons, and areas to be targeted
- Initiate traditional owner and stakeholder consultation and implementation processes

4) Planning of possible genetic biocontrol strategies

The possibility of developing genetic biocontrol strategies for CoTS should be further discussed and evaluated by an expert panel. While this approach would have a long time frame for implementation, the scope to develop a species specific control option with lower logistical requirements (in the long term) than manual control is attractive. This is a rapidly evolving field and specific recommendations will require specialist inputs.

Recommended actions:

- Convene an expert group to discuss possible control strategies, technology developments and traditional owner and stakeholder engagement strategies
- Begin examination of legal and regulatory constraints

5) Predator augmentation to control CoTS populations

Predator augmentation combines significant costs and risks with uncertainty over the magnitude and persistence of its impact. We recommend investing in predator augmentation research only if i) significant non-CoTS control benefits will accrue or co-investment is possible, ii) technologies exist or they can be adapted or developed relatively quickly, and iii) a high likelihood of impact on CoTS populations can be demonstrated. It is debatable as to whether there are predatory species which fit these criteria.

Recommended actions:

- Continue work to settle triton veliger larvae, with defined stop-go criteria
- Analyse the environmental risk of augmenting triton populations
    - Non-specific impacts of tritons: feeding preferences, quantification of dietary intake
    - Natural population level of triton: eDNA monitoring?
- Model the costs of development, establishment of infrastructure and operational costs
- Create an overview of predatory reef fish species for which closed life cycle production has already been achieved or can be expected
- Decide if expected risks and costs warrant further research
- Initiate traditional owner and stakeholder consultation

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APPENDIX 1: SUMMARY ASSESSMENT SCORES

Table A1.1: Summary assessment scores. The scores are defined as follows: State of knowledge: 0: Lacking; 1: Minimal; 2: Relatively good.

Criterion score: 0: Criterion not fulfilled; 1: Criterion partially fulfilled; 2: Criterion fulfilled.

MANUAL CONTROL

Category	Criterion	Definition	State of knowledge	Knowledge score	Criterion fulfilment	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable impact at a full suite of relevant temporal and spatial scales.	Good knowledge exists about the performance of single and repeated culls.	2	Demonstrated effectiveness at site, reef and regional scale at priority reefs. Medium and long-term impacts at GBR scale modelled. A small proportion of reefs more difficult (e.g. John Brewer Reef). Limits of detection influences Revisitation frequency (below).	2
	Resistance will not develop	COTS are not likely to become resistant to treatment	Good knowledge exists about the performance of single and repeated culls.	2	There is no suggestion in dataset that resistance will develop.	2
Risk	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Control efforts can be terminated. Good knowledge exists about the performance of single and repeated culls.	2	Control efforts can be terminated and community structure expected to revert to pre-control dynamics. No hypothesised negative effect at the community scale of reduction in COTS densities and no long-term impact of manual control on the potential for future COTS populations.	2
	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Good knowledge exists about the performance of single and repeated culls.	2	Treatment is highly specific.	2

	Spatial control	Treatment can be geographically contained	Good knowledge exists about the performance of single and repeated culls.	2	Local containment demonstrated.	2
	Acceptability	Public and stakeholders will be supportive	Good knowledge exists about public and stakeholder attitudes towards the current control program.	2	Long-term experience demonstrates high acceptability.	2
Logistics	Scalability	Scale of use – treatment suitable beyond reef scale	Good knowledge exists about the performance at different scales.	2	Effective at site and reef scales. Scalability dependent on resourcing.	1
	Revisitation frequency	Treatment to be re-applied when needed. (0 - manual control, 1 – slightly fewer, 2 – many fewer).	Good knowledge exists about the performance of single and repeated culls. Good knowledge exists about the limits of detection of CoTS.	2	Cryptic and small individuals are missed as are CoTS in deep water, exposed reef faces, and complex landscapes; even single sites require multiple visits to maintain low densities. Treatment has to be repeated until threshold is reached and again during new outbreaks. Monitoring required during and between outbreaks.	0
	Operational cost	On-going net cost. (0- more than manual control, 1 – same as manual control, 2 – less than manual control).	Good knowledge exists about the investments required for ongoing culling program.	2	Requires and on-going annual investment of $1-2M/vessel, on-going social benefits	1

PREDATOR ENHANCEMENT – CONSERVATION

Category	Criterion	Definition	State of knowledge	Knowledge score	Criterion fulfillment	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable	Zoning has some effect on COTS densities but does not	2	Efficacy in the field appears to be in ensuring best possible overall conditions	1

		impact at a full suite of relevant temporal and spatial scales	protect from outbreaks. Coral recovery quicker in PAs.			
	Resistance/habituation will not develop	COTS are not likely to become resistant or habituate to treatment	Not known if COTS will increase production of predator deterrents or extent of behavioural changes. It seems unlikely that this will occur in response to conservation approaches given past studies.	1	It seems unlikely that resistance will develop in response to conservation approaches.	2
Risk	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Fisheries restrictions can be lifted, although there is little precedence for this. Fish and benthic community differences between green and blue zones are well documented.	2	Fisheries restrictions can be lifted. Fish and benthic community differences between green and blue zones are well documented.	2
	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Predators are not species specific but general conservation benefits expected. Conservation can target species feeding on pelagic or benthic organisms.	2	Predators are not species specific but general conservation benefits expected. Conservation can target species feeding on pelagic or benthic organisms.	2
	Spatial control	Treatment can be geographically contained	Fisheries regulations can be locally applied. Monitoring data comparing blue and green reefs is available.	2	Fisheries regulations can be locally applied. No negative effects of MPAs outside of the protected zone have been observed.	2
	Acceptability	Public and stakeholders will be supportive	Previous experience has shown the approach is acceptable but likely to be highly contested.	1	The method is acceptable but likely to be highly contested. Efficiency must be documented. Regulatory mechanisms established.	1

			Efficiency must be documented. Regulatory mechanisms established.			
Logistics	Scalability	Scale of use – treatment suitable beyond reef scale	Already in use on many reefs.	2	Already in use on many reefs. Public acceptance will limit the proportion of reefs that can be protected.	2
	Revisitation frequency	Treatment to be re-applied when needed. (0 - manual control, 1 – slightly fewer, 2 – many fewer).	Fisheries regulations can be ongoing or seasonal/when build up towards new outbreak is expected. Enforcement requires frequent revisits.	2	Fisheries regulations can be ongoing or seasonal/when build up towards new outbreak is expected. Enforcement requires frequent revisits.	0
	Operational cost	On-going net cost. (0- more than manual control, 1 – same manual control, 2 – less than manual control).	Development costs, in terms of planning and consultation, and enforcement costs are significant.	2	Development costs, in terms of planning and consultation, and enforcement costs are significant but likely lower than manual control	2

PREDATOR ENHANCEMENT – AUGMENTATION (TRITON, FISH)

Category	Criterion	Definition	State of knowledge	Knowledge score	Criterion fulfillment	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable impact at a full suite of relevant temporal and spatial scales	Efficiency in the field needs to be proven. Lack of specificity extends uncertainty.	1	Efficiency in the field needs to be proven. Lack of specificity extends uncertainty.	1
	Resistance will not develop	COTS are not likely to become resistant to treatment	Not known if COTS will increase production of predator deterrents or extent of	1	Not known if COTS will increase production of predator deterrents or extent of behavioural changes in the field.	1

			behavioural changes in the field. Likelihood depends on level of augmentation.			
Risk	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Augmentation can be stopped. Unlikely to actively remove all deployed predators though fishing/harvesting is possible. The exact effect of remaining predators on community structure is not well documented.	2	Augmentation can be stopped. Unlikely to actively remove all deployed predators though fishing/harvesting is possible. Increased predation on non-target species will remain as long as there are elevated levels of the predator.	1
	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Predators are not species specific. Prey preference is not well documented. The exact effect on community structure is not well documented. Aquaculture-reared individuals could represent a biosecurity risk and this would need to be assessed.	1	Predators are not species specific but this is not necessarily undesirable. The degree of non-target impacts likely depends on level of augmentation and the fate of released individuals and possible descendants.	1
	Spatial control	Treatment can be geographically contained	Initial effects will be local only. The likely migration and reproductive spread of triton is unknown but current evidence suggests likelihood of natural spread is low. Human-mediated spread is possible but little evidence available.	1	Initial effects will be local only. The likely migration and reproductive spread of triton is unknown but current evidence suggests likelihood of natural spread is low. Human-mediated spread is possible.	1

	Acceptability	Public and stakeholders will be supportive	It is expected that acceptability will be high if efficiency and biosafety can be documented. Documentation needed.	1	It is expected that acceptability will be high if efficiency and biosafety can be documented.	2
Logistics requirements	Scalability	Scale of use – treatment suitable beyond reef scale	Aquaculture production likely limited to small scale.	1	Aquaculture production likely limited to small scale.	1
	Revisitation frequency	Treatment to be re-applied when needed. 0 =manual control, 1 – slightly fewer, 2 – many fewer.	It is unknown if triton will be able to rebuild population levels that can be naturally sustained. Fishing impact would require regular stocking.	1	It is unknown if triton will be able to rebuild population levels that can be naturally sustained. Fishing impact would require regular stocking	1
	Operational cost	On-going net cost: 0-more than manual control, 1 – same manual control, 2 – less than manual control	Documentation needed.	0	Establishing protocols, maintaining breeding facility and distributing Triton will be expensive. Cost could be lower for predatory fish.	1

PATHOGEN CONTROL, VIRUS (BASED ON GENERAL PRINCIPLES)

Category	Criterion	Definition	State of knowledge	Knowledge score	Criterion fulfillment	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable impact at a full suite of relevant temporal and spatial scales	No candidate virus is known.	0	Only candidates that effectively kill or reduce fecundity of COTS would be considered.	1
	Resistance will not develop	COTS are not likely to become resistant to treatment	Resistance to virus can develop. This is often compensated for by using	2	Resistance to virus can develop and cocktails or alternate treatment with several viruses may be needed.	1

			cocktails or alternate treatment with several viruses.			
Risk	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Most viruses are difficult to contain once released. No candidate virus is known. Limited documentation from marine systems.	1	Most viruses are difficult to contain once released.	0
	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Viruses can be species specific. No candidate virus is known.	0	Only candidates that are confirmed specific to COTS would be considered.	1
	Spatial control	Treatment can be geographically contained	Practical means of directly controlling geographical spread are generally limited to vaccination and physical separation. Natural current patterns could theoretically limit spread from the southern to the northern GBR. Human-mediated spread is possible but little evidence available.	1	No means of controlling spread. Trial releases could be done in the Southern GBR.	0
	Acceptability	Public and stakeholders will be supportive	It is expected that acceptability may be low. Documentation needed.	0	It is expected that acceptability may be low.	1
Logistics	Scalability	Scale of use – treatment suitable beyond reef scale	Virus production generally require a host cell line or primary cells from the host. A cell line is not yet available for CoTS.	1	Scale of deployment currently limited by lack of cell line for virus production. In theory upscaling assisted by self-propagation.	1

	Revisitation frequency	Treatment to be re-applied when needed: 0 =manual control, 1 – slightly fewer, 2 – many fewer.	Viral biocontrol programs generally require repeat applications.	1	It is likely that repeated application will be necessary	1
	Operational cost	On-going net cost: 0-more than manual control, 1 – same manual control, 2 – less than manual control	The development and implementation of viral biocontrol programs are generally costly. Documentation needed.	2	Significant development and implementation costs expected.	1

PATHOGEN CONTROL, HORISONTALLY TRANSMITTED BACTERIUM, PARASITE (BASED ON GENERAL PRINCIPLES)

Category	Criterion	Definition	State of knowledge	Knowledge score	Criterion fulfillment	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable impact at a full suite of relevant temporal and spatial scales	No candidate pathogen is known.	0	Only candidates that effectively kill or reduce fecundity in COTS would be considered.	1
	Resistance will not develop	COTS are not likely to become resistant to treatment	Resistance to bacterial toxins and parasites can develop.	2	Resistance to bacterial toxins and parasites can develop.	1
Risk	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Most bacteria and parasites are difficult to contain once released. No candidate identified. Limited documentation from marine systems.	1	Most bacteria and parasites are difficult to contain once released.	0

	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Parasites and opportunistic bacterial pathogens rarely species specific. No candidates identified.	0	Parasites and opportunistic bacterial pathogens rarely species specific. Only candidates that are confirmed specific to COTS would be considered.	0
	Spatial control	Treatment can be geographically contained	Practical means of directly controlling geographical spread are generally limited to vaccination and physical separation. Natural current patterns could theoretically limit spread from the southern to the northern GBR. Human-mediated spread is possible but little evidence available.	1	No means of controlling spread. Trial releases could be done in the Southern GBR.	0
	Acceptability	Public and stakeholders will be supportive	It is expected that acceptability may be low. Documentation needed.	0	It is expected that acceptability may be low.	0
Logistics	Scalability	Scale of use – treatment suitable beyond reef scale	Scale of deployment would be limited by production technologies. Ease of production depends on candidate. No candidate identified.	1	Scale of deployment would be limited by production technologies. In theory upscaling assisted by self-propagation.	1
	Revisitation frequency	Treatment to be re-applied when needed. 0 =manual control, 1 – slightly fewer, 2 – many fewer.	Pathogen biocontrol programs generally require repeated applications.	1	It is likely that repeated application will be necessary	1
	Operational cost	On-going net cost: 0- more than manual control, 1 – same	The development and implementation of pathogen	2	Significant development and implementation costs expected.	1

		manual control, 2 – less than manual control	biocontrol programs are generally costly. Documentation needed.			

SEMIOCHEMICALS ATTRACTANTS/REPELLENTS

Category	Criterion	Definition	State of knowledge	Knowledge score	Criterion fullfilment	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable impact at a full suite of relevant temporal and spatial scales	Efficiency in the field and at scale still needs to be proven.	1	Effect on animals in the wild is unknown, effect of currents on concentration and distribution unknown.	1
	Resistance will not develop	COTS are not likely to become resistant to treatment	Little is known.	0	Not expected for attractants. Possible for repellents. Short treatment time and discontinuous use reduce the risk.	1
Risk	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Depending on deployment device, it may be removable. Depending on deployment device, it may have a short treatment time. Released compounds will degrade naturally.	1	It is expected that delivery devices will be designed to fit requirements.	2
	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Depending on the compound, it may be specific for species or life stage. Enhanced concentration relative to normal levels and at other times may alter impact. Documentation needed.	1	It is assumed that species-specific compounds would be used.	2

	Spatial control	Treatment can be geographically contained	A limited body of water will be activated, and dilution will limit geographic distribution of spread. Documentation for specific compounds needed.	1	A limited body of water will be activated. Dilution will limit geographic distribution of spread.	2
	Acceptability	Public and stakeholders will be supportive	It is expected that acceptability will be high but documentation needed	1	High acceptability expected.	2
Logistics	Scalability	Scale of use – treatment suitable beyond reef scale	Scalability likely limited by compound production and deployment resources.	1	Will most likely be applied at site level (sub-reef, individual reef) depending on compound production and deployment resources.	0
	Revisitation frequency	Treatment to be re-applied when needed. (0 - manual control, 1 – slightly fewer, 2 – many fewer).	Depending on deployment device and strategy. Dilution and degradation will limit temporal effect.	1	Repeat/ongoing application needed. Depending on deployment device and strategy (days – months).	1
	Operational cost	On-going net cost. (0- more than manual control, 1 – same manual control, 2 – less than manual control).	Moderate development cost and relatively low on-going deployment costs expected, but documentation needed.	0	Deployed as a complement to manual control or by tour boats.	1

GENETIC BIOCONTROL – NO GENE DRIVE (BASED ON GENERAL PRINCIPLES)

Category	Criterion	Definition	State of knowledge	Knowledge score	Criterion fulfillment	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable impact at a full suite of	Spread in population required and temporal and geographical lags in effectiveness expected.	0	Target would be designed to ensure effectiveness. Manual culling followed by deployment. The deployed COTS would eat coral.	1

		relevant temporal and spatial scales			Modelling needed.	
	Resistance will not develop	COTS are not likely to become resistant to treatment	Resistance will likely evolve through selection unless linked to Trojan gene. Modification may trigger other pathways, processes, behaviours to compensate.	1	Resistance will likely evolve through selection unless linked to Trojan gene. Modification may trigger other pathways, processes, behaviours to compensate.	1
Risk	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Cannot be reversed but modification will be lost via Mendelian inheritance. Timeframes dependent on the degree of selection against the construct. Target not known.	2	Cannot be reversed but modification will be lost via Mendelian inheritance. Timeframes dependent on the degree of selection against the construct.	1
	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Target would be designed to ensure specificity. Target not known. Experimental confirmation needed.	1	Target would be designed to ensure specificity.	2
	Spatial control	Treatment can be geographically contained	Spatial scale of spread determined by dispersal (natural and human-mediated) and degree of negative selection. Target not known.	0	Spatial scale of spread determined by dispersal (natural and human-mediated) and degree of negative selection. Difficult to fine tune.	1
	Acceptability	Public and stakeholders will be supportive	It is expected that acceptability may be low but depends on designed system. Documentation needed.	0	It is expected that acceptability may be low but depends on designed system.	1

Logistics	Scalability	Scale of use – treatment suitable beyond reef scale	Spatial scale of spread determined by dispersal and degree of negative selection. Also depends on delivery system and production of modified entities (gametes, larvae, juveniles, adults). Not yet determined.	0	Spatial scale of spread determined by dispersal and degree of negative selection. Also depends on delivery system and production of modified entities (gametes, larvae, juveniles, adults).	1
	Revisitation frequency	Treatment to be re-applied when needed. 0 =manual control, 1 – slightly fewer, 2 – many fewer.	Likely, since modification will be lost via Mendelian inheritance.	1	Likely, frequency depends on system	1
	Operational cost	On-going net cost: 0-more than manual control, 1 – same manual control, 2 – less than manual control	The development and implementation of genetic biocontrol programs are generally costly. Documentation needed.	1	Significant development and implementation costs, implementation requires field monitoring	1

GENETIC BIOCONTROL –GENE DRIVE (BASED ON GENERAL PRINCIPLES. TECHNOLOGY NOT YET AVAILABLE FOR ENVIRONMENTAL RELEASE).

Category	Criterion	Definition	State of knowledge	Knowledge score	Method characteristic	Criterion score
Effectiveness	Likely effectiveness (short-term)	COTS will be removed/killed and coral protected, demonstrable impact at a full suite of relevant temporal and spatial scales	Spread in population required and temporal and geographical lags in effectiveness expected.	0	Target would be designed to ensure effectiveness and spread in population. Temporal and geographical lags in effectiveness expected. Manual culling followed by deployment. Deployed COTS would eat coral. Modelling needed.	1

	Resistance will not develop	COTS are not likely to become resistant to treatment	Development of resistance is possible. Modification may trigger other pathways, processes, behaviours to compensate. Documentation needed for specific modification.	1	Development of resistance possible. Unknown if modification will trigger other pathways, processes, behaviours to compensate.	1
	Reversibility	Treatment can be stopped and effects reversed with no lasting impacts on future COTS populations or reef community structure and functioning.	Theoretically reversal can be designed either into the system or via a new construct. Target not known.	1	Theoretically reversal can be designed either into the system or via a new construct.	1
	Non-specific impacts	Treatment will not cause detrimental non-target impacts (0 – considerable impacts, 1 – limited impacts, 2 – no impacts).	Target would be designed to ensure specificity. Target not known. Experimental confirmation needed.	1	Target would be designed to ensure specificity.	2
	Spatial control	Treatment can be geographically contained	In theory, gene drives can be designed to spread widely or be geographically contained. Not field tested yet. Spatial scale of spread determined by construct design, dispersal (natural and human-mediated), and degree of negative selection.	0	In theory, gene drives can be designed to spread widely or be geographically contained. Spatial scale of spread determined by construct design, dispersal (natural and human-mediated), and degree of negative selection.	1
	Acceptability	Public and stakeholders will be supportive	It is expected that acceptability may be low but depends on designed system. Documentation needed.	0	It is expected that acceptability may be low but depends on designed system.	1

Logistics	Scalability	Scale of use – treatment suitable beyond reef scale	Spatial scale of spread determined by construct design, dispersal and degree of negative selection. Also depends on delivery system and production of modified entities (gametes, larvae, juveniles, adults). Not yet determined.	0	Spatial scale of spread determined by construct design, dispersal and degree of negative selection. Scalability also depends on delivery system and production of modified entities (gametes, larvae, juveniles).	1
	Revisitation frequency	Treatment to be re-applied when needed. 0 =manual control, 1 – slightly fewer, 2 – many fewer.	In theory, it can be designed to need re-application or to spread indefinitely. Frequency of re-application depends on system. Not yet determined.	0	In theory, it can be designed to need re-application or to spread indefinitely. Frequency of re-application depends on system. Comment: Score of 2 as this could be a characteristic feature of this option	2
	Operational cost	On-going net cost: 0-more than manual control, 1 – same manual control, 2 – less than manual control	The development and implementation of genetic biocontrol programs are generally costly. Documentation needed.	1	Significant development and implementation costs, implementation requires field monitoring	2

APPENDIX 2 : FORMAL ASSESSMENT OF RISK AND KNOWLEDGE GAPS FOR CRISPR-CAS9 GENE DRIVE CONTROL OF COTS

The conceptual model proposed by (Moro *et al.*, 2018) describes key relationships between a targeted species and biological, environmental and logistical parameters (or in the parlance of ecological risk assessment, stressors) that are likely to influence future research on the species, or the survival and release of any modified organism in the environment. The intent is to clarify the potential risks related to the development, persistence and spread of a gene drive organism by identifying knowledge gaps in order to reduce uncertainty in relation to these risks. Following the National Academies (National Academies of Sciences, 2016) their approach focuses on four sets of stressors: 1) the source and genomic information of the target species, 2) the effect of releasing a transgenic species on conspecifics, 3) the effect of releasing a transgenic species on the environment, and 4) the desired outcome (project goal). In Table A2 we provide an initial assessment of these risks for the application of CRISPR-cas9 in the context of CoTS control. This assessment suggests a fairly mixed state of affairs with our knowledge varying from minimal to good and the desirability of the known CoTS traits varying from low to reasonable.

In terms of the inherent characteristics of CoTS (Source stressors) we have a good understanding of the species distribution, a published genome and some understanding of functional sites within the genome. We know that genetic material can be inserted into the nucleus of CoTS but we have limited understanding that could guide decisions about appropriate drive mechanisms. Initial work has focused on the secretome (Hall *et al.*, 2016; Hall *et al.*, 2017a; Motti *et al.*, 2018) but other options might also be appropriate, e.g. manipulating susceptibility to pathogens (Høj *et al.*, 2018; Walshe *et al.*, 2017). We also have only very limited experience in CoTS culture and husbandry that could guide the very large-scale breeding that would be required for any control release of the most likely form of construct, a self-limiting construct. Overall, our knowledge scores at roughly 50%, while the desirability of the CoTS traits in this section also score at 50%. Knowledge gaps can, however, be filled and the desirability of the species' traits will likely change as this occurs. This analysis suggests that further exploration is required.

Our knowledge of the likely effect of a gene drive system on CoTS is sufficient, though many of the traits exhibited by CoTS suggest that it will not be easy to design and implement a CRISPR-cas9 gene drive control technology for this species. While our knowledge of sex determination and differentiation in CoTS is limited, our knowledge of many other aspects of CoTS reproduction and population processes is reasonable, so the overall score for this section is high. Unfortunately, many of the species' traits are of limited desirability. For example, while there are advantageous traits, such as low levels of mate choice, other traits such as mass spawning, pelagic larvae and larval competency periods of c. a month suggest that exceeding thresholds will require very large introductions and that high levels of geneflow, and therefore a very high potential for escape, can be expected. This latter point is supported by the observation that the outbreak moves at a rate of c. 60-80km along the GBR each year (Fletcher *et al.*, 2020; Vanhatalo *et al.*, 2016) and that larval plumes can extend hundreds of kilometres (Uthicke *et al.*, 2015a). However, the observation that Pacific archipelagos show genetically distinct populations (Timmers *et al.*, 2012) may suggest that dispersal south and

east beyond the GBR would likely take longer than northwards through New Guinea. These traits point to a high risk of loss of control of any released construct and to the need to release very high numbers of constructs in order to reach threshold levels at an adequate number of reefs.

Our current knowledge of the potential for adverse effects of CoTS CRISPR-cas9 construct is also mixed. There is limited knowledge about the potential for hybridization and therefore the spread of the construct beyond *Acanthaster* though careful design should be able to limit the construct to the genus if not the species or population. The slow resolution in the taxonomy of the genus (Benzie, 1999; Haszprunar *et al.*, 2017; Vogler *et al.*, 2012) might suggest that spread to congeners may be possible if care was not taken, and, as noted above, the levels of dispersal and geneflow documented to date (Uthicke *et al.*, 2015a; Vanhatalo *et al.*, 2016; Yasuda *et al.*, 2009; Yasuda *et al.*, 2015) suggest that spread beyond Australia's northern border, at least, would be possible. The potential for adverse effects from a successful release are likely to be related to changes in community structure of scleractinian corals and could be modelled.

Finally, what is the endpoint and are there indications that success is likely? Unfortunately, there are no previous examples of echinoderm biocontrol or of successful modification of the echinoderm genome that might provide an indication of the potential for success. There is an alternative control technique to CRISPR-cas9 in the form of manual control, which has been shown to be effective at the scale of individual reefs (Fletcher *et al.*, 2020). This is a real benefit to the prospect of developing genetic control methods as a realistic approach to achieving the required introduction thresholds is to reduce the breeding population, i.e. to lower the threshold (Thresher *et al.*, 2014b). On the other hand, the maximum spatial scale at which manual control is effective will be a function of program objectives (Westcott *et al.*, 2020).

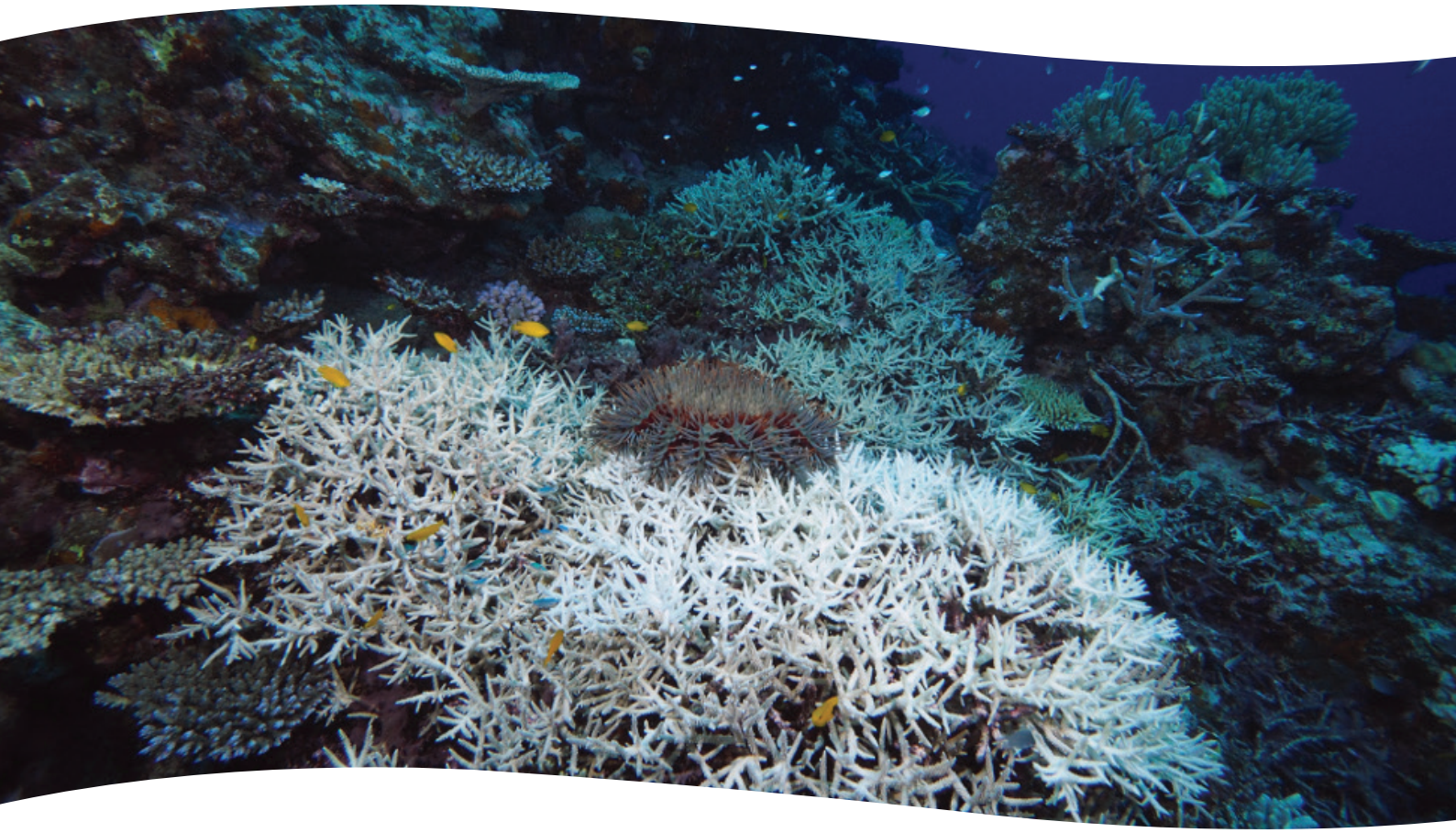
Overall, the risk assessment score for our knowledge of CoTS is relatively good at 23/32 or 72% with the most crucial gaps being in identifying the nature of the genetic approach and the target genome sequence. CoTS traits however are less positive with a total score of 16/32 or 50%. While this score is likely to improve as ideas around the genetic technology to be deployed develop this analysis suggests that caution will be required going forward.

Table A2.1: Assessment of the state of knowledge and the suitability of CoTS traits as currently understood for the application of CRISPR cas9 gene drive technologies using a scoring system based on (Moro *et al.*, 2018). State of Knowledge is scored as 0: lacking, 1: minimal, or 2: relatively good. Desirability of CoTS' traits is scored as 0: highly undesirable (lacking), 1: moderate desirability (partial), or 2: highly desirable (fulfilled).

Category	Trait	Explanation	CoTS Characteristic	State of knowledge	CoTS' trait desirability	Possible Score
Source				**7**	**6**	**12**
	Distribution	Determines potential for control versus eradication with limited distribution being desirable	Broadly distributed throughout the GBR and Indo-Pacific where they are native on coral reef ecosystems (Pratchett et al. 2014)	2	0	
	Genome Sequence	Provides basic framework for designing tool, highly desirable	Genome published (Hall et al. 2017)	2	2	
	Functional sites in genome identified	Determines how a control construct might work	Current research is focused on manipulating the secretome to influence aggregation behaviour and predator responses (Motti et al. 2018). Research into COTS gene expression response to pathogen challenge suggests another potential direction (Høj *et al.*, unpublished data). Other options not yet identified.	1	1	
	Transgenic systems	Can the genome be manipulated?	Genetic material can be inserted into cells suggesting basic physical manipulation is possible (L. Bay, pers. comm. 2016)	1	1	
	Transgenic systems	Candidate drive mechanisms identified, modelled, developed and trialled	Candidate gene drive mechanisms not identified	0	0	
	Culture and Husbandry	Culture and husbandry are fundamental to ensuring that sufficient constructs can be deployed	Aspects of culture and husbandry are understood, e.g. can be reared in captivity, however it is uncertain if sufficient numbers can be bred and raised in captivity	1	2	
Effect on species				**9**	**5**	**10**
	Gene flow	Extent of likely gene flow influences the potential rate of influence spread of gene drive system within and between populations. High rates of gene flow provide rapid spread but low control of spread.	High levels of gene flow expected. Highly fecund, mass spawning, pelagic gametes and larvae. High levels of relatedness within outbreak populations. Genomes of individuals from GBR and Okinawa found to have 98.8% sequence identity.	2	0	

	Reproductive biology / mating system	Influences resistance to spread of gene drive with e.g. low levels of mate choice reducing resistance.	Sexual reproduction, highly polygamous, little mate selection, high gamete #, mass spawning. Larval cloning and hermaphrodites possible. Sex determination and sex differentiation poorly understood.	1	1	
	Spatial Ecology	Influences speed and pattern of spread	Pelagic dispersal, patterns of dispersal determined by currents and wind. Current estimates suggest that the outbreak spreads at c. 50-80km/yr along the reef (Vanhatalao et al. 2016; Fletcher et al. 2020) with significant connectivity between reefs (Hock et al. 2017).	2	1	
	Population regulation	Population structure and drivers of population cycles	Boom bust dynamics (Uthicke *et al.*, 2009), 15 year cycles (Pratchett et al. 2014), multiple generations present at a site, all contribute to rapid population growth under appropriate conditions. Low juvenile survival (Keesing et al. 2018) due to high predations levels. Synchronised mating events reduce efficient generation time.	2	1	
	Translocation stress	Influences potential for release of constructs into wild populations.	No territoriality, sociality, scramble competition for resources, so probably resilient to translocation	2	2	
Effect on environment				**4**	**1**	**6**
	Intraspecific breeding	Potential for spread to non-target species resulting in non-target impacts	Unclear	0	0	
	Border security	Potential to move beyond Australia's borders influences legal and ethical issues	Larval competency in the field unknown, in the lab settlement of 40-day old larvae has been recorded. Dispersal beyond borders highly likely on north-flowing currents and otherwise possible though less likely using Coral Sea reefs as stepping stones. Human-mediated spread is also possible.	2	0	

	Adverse community change	Ideally, the loss of CoTS has few if any flow on negative consequences for reefs.	Interaction networks and their dynamics within coral reef communities are poorly understood but the most likely effect would be a change in the relative abundance of rapidly growing coral species, which are favoured by CoTS, over slower growing species.	2	1	
Endpoint				**2**	**4**	**4**
	Control	Alternative control methods, e.g. manual culling, represent complementary technologies that, by reducing the initial population, amplify the impact of constructs within a population and reduces the number of individuals required to achieve threshold densities.	Manual control is effective in reducing numbers at both the site (Westcott and Fletcher 2018) and the reef scale(Westcott *et al.*, 2020).	2	2	
		Previous examples of fertility or gene drive shown to work in similar species to provide confidence in approach	No previous examples. Mass die offs of echinoderms elsewhere and the sudden decline of outbreaks at some sites in the presence of coral suggest an influence of disease.	0	2	
			Overall CoTS Score	**22**	**18**	
			Maximum Possible CoTS Score	**32**	**32**	



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