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

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

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Acronyms and Abbreviations

AGRF	Australian Genome Research Facility
AIC	Akaike's Information Criterion
AIMS	Australian Institute of Marine Science
BLAST	Basic Local Alignment Search Tool
CCA	Crustose coralline algae
CCIP	Crown-of-thorns starfish Control Innovation Program
CI	Confidence Interval
COTS	Crown-of-thorns starfish
DNA	Deoxyribonucleic Acid
eDNA	Environmental DNA
GBR	Great Barrier Reef
GBRMPA	Great Barrier Reef Marine Park Authority
GLM	Generalised Linear Model
HIRS	Heron Island Research Station
LIRS	Lizard Island Research Station
NCBI	National Center for Biotechnology Information
NMSC	National Marine Science Centre
NTC	No Template Controls
OUT	Operational Taxonomic Unit
PCCC	Port Curtis Coral Coast Group
PCR	Polymerase Chain Reaction
RIMReP	Reef 2050 Integrated Monitoring and Reporting Program
RRAP	Reef Restoration and Adaptation Program
RUBS	RUbble Biodiversity Samplers
SCUBA	Self-Contained Underwater Breathing Apparatus
SD	Standard Deviation
SE	Standard Error
SEM	Structural Equation Model
UQ	The University of Queensland

EXECUTIVE SUMMARY

Crown-of-thorns starfish (COTS) are notorious coral predators with a population biology that boasts opportunism. During episodic population booms, COTS can have considerable impacts on entire reefs through their mass consumption of living coral. Several hypotheses have been used to explain COTS outbreaks, though the current consensus is that multiple drivers work in concert to generate outbreaks. It is therefore imperative that these causes of COTS population outbreaks are resolved to enhance the long-term efficacy of reef management and conservation.

One hypothesis that has gained considerable traction among scientists and managers posits that COTS population booms are facilitated by a reduction in their top predators through overfishing. This hypothesis has been interrogated over the years with mounting evidence to suggest that reefs protected from fishing are less prone to COTS outbreaks. However, direct links to predators remain equivocal as observations of predation are sparse and some of the main predators of COTS are not fishery targets. Consequently, the mechanistic understanding of how fisheries-exploited species directly moderate COTS outbreaks is yet to be substantiated clearly.

Prior to this study, there were ~90 species known to consume COTS at various stages of their life cycle. Our understanding of these predators is mainly derived from reef fishes and triton snails that inconsistently consume large COTS. As for many marine species, early life history stages are most vulnerable to predation and can create bottlenecks in population success. Even small variations in predator-induced mortality during early life stages can accumulate to have disproportionate effects on population size. The potential for predator-prey interactions to regulate COTS naturally before their destructive corallivorous stage may stand as one of the most alluring and affordable management approaches. Variability in predation of juvenile COTS in their rubble nursey habitat has great potential to be a proximal cause of population outbreaks but remains a critical knowledge gap.

This project aimed to identify key predators of juvenile COTS in their rubble nursery before they emerge on the reef as corallivorous adults. Of 110 distinct rubble-dwelling taxa tested in experimental feeding trials, 31 new COTS predators were identified. Of these, the red decorator crab, *Schizophrys aspera*, was consistently a voracious feeder on COTS juveniles, while swimming crabs (Portunidae) regularly inflicted injury (e.g. arm and body damage). In mesocosm experiments of natural rubble inclusive of alternate prey, *S. aspera* and portunids detected and consumed newly settled juveniles (2 months old, ~2 mm) at a rate of 5.8 and 0.4 COTS day^{-1}, respectively. For *S. aspera*, this is the highest known rate of predation on benthic COTS, meaning this species alone has great potential to impact COTS population size; though diverse cryptic communities would have the greatest combined impact. Total consumption of juveniles increased with predator size but decreased with COTS size to a size escape threshold ~5 mm and ~10 mm for the Portunidae and *S. aspera*, respectively, close to the size that juveniles transition to eat coral. For *S. aspera*, this was coupled with an increased prevalence of partial predation, which is still likely to curb COTS population success in lieu of immediate mortality.

At a local scale (Heron Island, southern Great Barrier Reef; GBR), there was an increased likelihood of finding *S. aspera* by overturning large rubble pieces overlying thick rubble beds.

S. aspera was positively associated with the relative cover of rubble, hard substrate and soft coral, and its density inverse to that of COTS; sites with the most *S. aspera* had the lowest number of COTS and sites with the fewest *S. aspera* had the most COTS. Whether this correlation is causal requires further exploration but provides the first indication that rubble-dwelling taxa may moderate COTS numbers in nature.

At a regional scale, Heron Island, a protected no-take reef (green zone) in the southern GBR, had the highest densities of *S. aspera* and portunid crabs, while *S. aspera* was rarely found on protected reefs in the northern (Lizard Island Group) and central (Moore Reef) GBR. Moreover, *S. aspera* was smaller in the north and central GBR, which may translate to lower COTS consumption. Fewer rubble-dwelling predators were found at Moore Reef, while the density of portunid crabs was comparable between Lizard Island and Heron Island. There are no data available to address the drivers of these varied predator densities, which are needed to inform COTS management. However, low numbers of cryptic predators, especially *S. aspera*, in the central and northern GBR aligns with regional patterns in COTS outbreak histories. It seems paramount to address whether the few reefs examined here are truly representative of each region, especially compared to reefs in the south that have a history of COTS outbreaks (e.g. the Swains, Fairfax Reef) and reefs open to fishing (blue zones).

Using eDNA methods generated in this project, eight species of wild-caught decapods were documented with detectable concentrations of COTS in their digestive system; 17% (2/12) of *S. aspera* collected at Heron Island and 12% (8/65) of cryptic predators collected at Lizard Island. This is particularly impressive given the short (12–24 h) window that COTS DNA is detectable in the crab gut post-digestion. Whether COTS were consumed in full, injured, or otherwise, is unknown, but this outcome provides evidence that a diversity of cryptic predators regularly interact with COTS in their natural rubble environment. This eDNA method could be added to the COTS management toolbox as an applied method of predator identification and COTS detection.

Lastly, gut content DNA metabarcoding was used to address multi-trophic links involving COTS for the first time. Preliminary results revealed *S. aspera* as a generalist feeder, not a COTS or echinoderm specialist, despite its consumption of juveniles in aquaria and nature. Five species of reef fish known to consume COTS (*Cheilinus chlorourus*, *Oxycheilinus digramma*, *Lethrinus nebulosus*, *Lutjanus russellii*, and *Epinephelus cyanopodus*) returned positive data for *S. aspera*, providing the first information on potential predators of *S. aspera* on the GBR, and eight species of reef fish contained DNA of portunid crabs. Whether fish density corresponds to elevated predation risk for *S. aspera* and other rubble predators, and whether that secondarily modulates populations of juvenile COTS, requires attention.

Through a combination of aquarium experiments, field surveys, and eDNA metabarcoding, we now have a clearer picture of the species responsible for early mortality of COTS that may be important bioindicators of outbreak potential. Understanding the early juvenile predation window could be instrumental to managing COTS outbreaks before episodic pulses of coral-eating adults occur. Maintaining diverse cryptic communities, particularly those involving *S. aspera*, may have disproportionate impacts on COTS population success through high rates of juvenile mortality in their rubble nursery. Strategic management that considers these lower-order taxa as early precursors of COTS population dynamics may be one of the most feasible approaches to preventing or suppressing their impacts.

1. INTRODUCTION

Crown-of-thorns starfish (COTS, *Acanthaster* spp.) are common asteroids in tropical coral reefs of the Indo-Pacific. The large corallivorous adult life stage of COTS, coupled with its propensity to exhibit high density population outbreaks, has been responsible for considerable declines in coral cover across their range (Osborne et al. 2011; De'ath et al. 2012; Mellin et al. 2019). Exactly what causes COTS population irruptions has been a focus of coral reef research and management for decades (Pratchett et al. 2014). It is now agreed that multiple causes spanned across the complex life cycle of COTS are likely required to generate and sustain outbreaks (Babcock et al. 2016; Wolfe and Byrne 2024), making it crucial to quantify and understand these outbreak mechanisms to inform coral reef conservation and management.

The long-standing predator removal hypothesis postulates that overfishing of predators has alleviated top-down control of COTS (Endean 1969). This hypothesis was proposed after the first documented outbreak of COTS on the Great Barrier Reef (GBR), Australia, which was suggested to be associated with depletion of the giant triton, *Charonia tritonis*, through overfishing (Endean and Stablum 1975). The giant triton has long been considered one of the major predators of adult COTS (Hall et al. 2017), however the effectiveness of this species in reducing COTS numbers remains unresolved (Ormond et al. 1990; Motti et al. 2022), as its consumption rates seem too low (<1 COTS week^{-1}) to have an appreciable impact on COTS boom-bust population dynamics (Endean 1969; Birkeland 1989), unless at low density (McCallum 1987).

The predator removal hypothesis gained additional traction in the context of fishery impacts on large reef fishes. Several studies have shown that reefs exploited by commercial and recreational fisheries experience more severe and/or more frequent COTS outbreaks compared to protected areas (Dulvy et al. 2004; Sweatman 2008; Kroon et al. 2020; Westcott et al. 2020; Kroon et al. 2021). These findings are supported by an increase in the prevalence of injured COTS–which provides evidence of predation (McCallum et al. 1989; Budden et al. 2019)–inside protected reef zones (Caballes et al. 2022). But despite the growing number of fish species considered potential predators of COTS (Kroon et al. 2020), direct observations of predation are sparse and often involve non-commercial fisheries target species, such as pufferfishes (Ormond et al. 1973; Cowan et al. 2017). This restricts the mechanistic understanding of how fisheries-exploited species directly moderate COTS outbreaks (Babcock et al. 2016; Pratchett et al. 2021).

Investigations into the predator removal hypothesis have largely been focused on the direct impacts of predators of adult COTS. However, variation in COTS outbreak likelihood and/or intensity with fishing pressure is likely to involve multiple and potentially indirect interactions with early life history stages (Sweatman 2008; Kroon et al. 2020), as characteristic of marine broadcast spawners (Gosselin and Qian 1997), the early life history stages of COTS are most susceptible to predation. Indeed, the gametes and larvae of COTS experience considerable predation pressure from planktivorous fishes and corals (Sano et al. 1987), predators that impede COTS larval settlement and survival (Cowan et al. 2016a; Cowan et al. 2016b; Cowan et al. 2017). Additionally, mortality rates of newly settled juveniles are extremely high at ~5% day^{-1} (Keesing and Halford 1992a; Keesing et al. 2018), with the potential to deplete COTS populations by >90% within weeks of settlement (Wilmes et al.

2018). Juvenile COTS are exposed to predators for much longer (months to years) than gametes and larvae (days to weeks) (Deaker et al. 2020a; Wilmes et al. 2020a), so even small variations in predator-induced mortality during their early benthic life stage would accumulate to have disproportionate effects on COTS population size and outbreak potential (Keesing and Halford 1992b; Morello et al. 2014; Wilmes et al. 2018).

Juvenile COTS primarily settle to coral rubble where they develop as herbivores that consume crustose coralline algae (CCA) before they make the ontogenetic switch to become coral-eaters (Zann et al. 1987; Wilmes et al. 2020b). When coral prey is available, juveniles make the switch to a coral diet 4–12 months post-settlement (Yamaguchi 1974; Zann et al. 1987; Deaker et al. 2020b), depending on the coral species available (Neil et al. 2022). However, juvenile COTS are extremely resilient to food scarcity and can persist for years on low energy foods, such as biofilm (Deaker et al. 2020a). This diet plasticity allows for growth stasis and delays in the transition to corallivory, which may prolong their exposure to predation risk in their rubble nursery but also facilitate accumulation of cohorts as hidden armies that emerge in waves of coral-eaters (i.e. outbreaks) when conditions are favourable (Deaker et al. 2020a; Byrne et al. 2023; Webb et al. 2024). Reef degradation to rubble may therefore promote juvenile COTS populations (Wolfe and Byrne 2024), meaning variability in predation in rubble has great potential to be a proximal cause of population outbreaks, but this remains a critical knowledge gap (Pratchett et al. 2021).

Predator communities in rubble contribute to juvenile COTS mortality (Keesing and Halford 1992a; Keesing et al. 1996; Keesing et al. 2018), but there is poor understanding of the identity of specific predator species operating at this level of the ecosystem, and how COTS mortality varies due to rubble community structure (Cowan et al. 2017; Desbiens et al. 2023). Cryptic fauna that occupy rubble are diverse and span all trophic guilds (Glynn and Enochs 2011), including a suite of predatory species of crustaceans, molluscs and worms (Wolfe et al. 2021). The fireworm, *Pherecardia striata*, and harlequin shrimp, *Hymenocera picta*, have been observed feeding on juvenile COTS on reefs of the Eastern Pacific (Glynn 1984), while the peppermint shrimp, *Lysmata vitatta*, has demonstrated the capacity to consume juvenile COTS in the laboratory (Balu et al. 2021). In addition, polychaete worms and trapeziid crabs can suppress COTS settlement and metamorphosis (Cowan et al. 2016a) and alter juvenile behaviour (Deaker et al. 2021a). Based on these few observations, it is clear that predatory cryptic invertebrates may help to regulate COTS populations through predator-prey interactions with juveniles in their rubble nursery, but a comprehensive evaluation of these rubble-dwelling species has not been conducted.

This project was intended to improve the ecological underpinning of COTS management through identification of key predators in rubble (**Figure 1**). As postulated for the giant triton and some reef fishes (Endean 1969; Kroon et al. 2021), if important cryptic predators are lacking on certain reefs, COTS juveniles may thrive to seed outbreaks. Conversely, few COTS may survive to the coral-feeding adult stage where benthic predators are in high abundance. Understanding what species these cryptic predators are, as well as their biology, ecology and distribution on the reef at local and regional scales, is required to make more accurate predictions of COTS population ecology. This work is an important step in trying to determine the food webs that, if disturbed, may or may not promote COTS outbreaks. This knowledge may then be used to inform improved detection and monitoring with more efficient and effective operational responses (**Figure 1**).

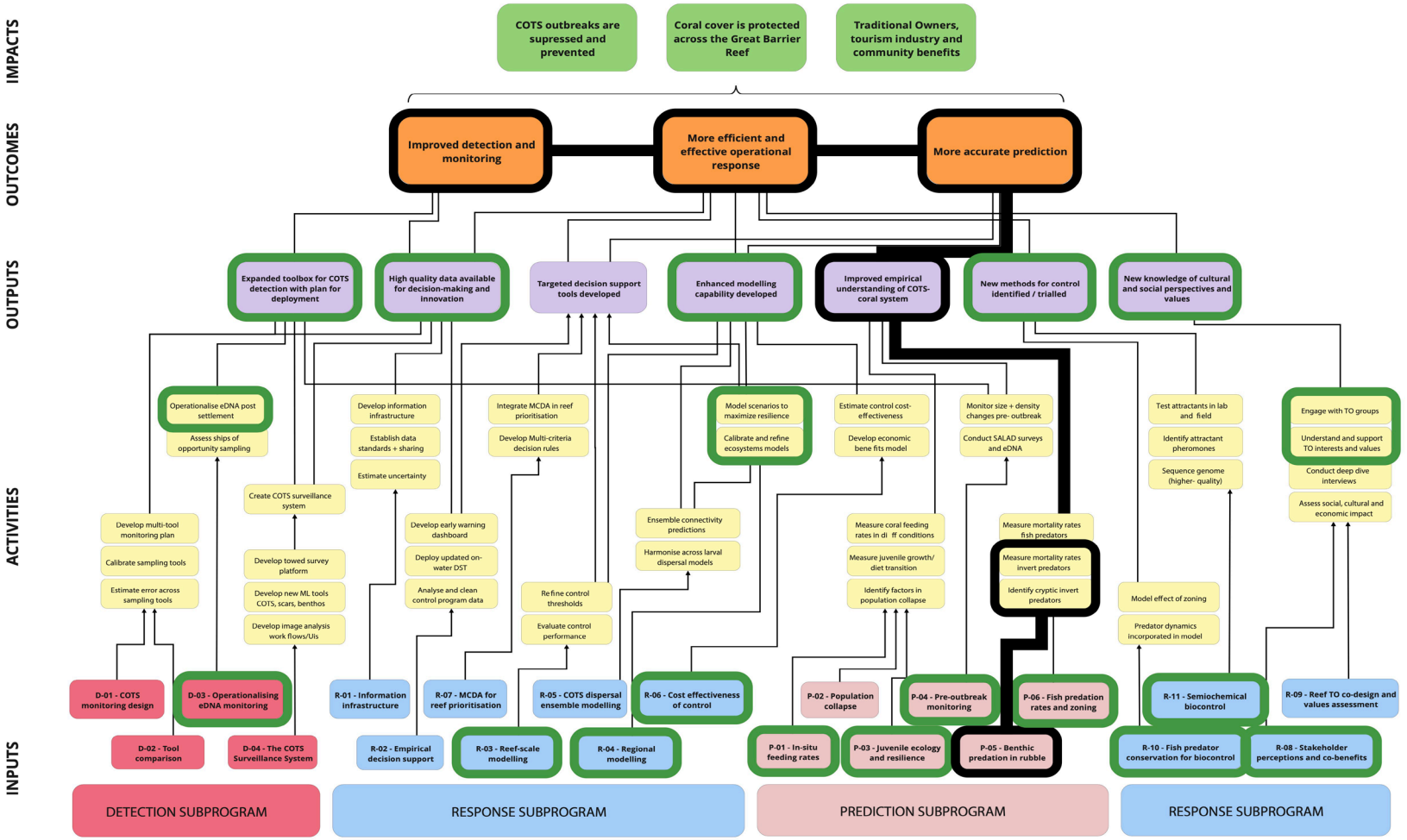

Figure 1. The CCIP program logic model highlighting the intended impact pathway of this project (black) and key synergies generated with other projects across the program (green).

Adequately characterising predation on early juvenile life stages could be instrumental to suppressing and preventing COTS outbreaks. Influential predators were anticipated to emerge as viable bioindicators to predict COTS success and outbreak likelihood at reef and regional scales. Thus, this project formed synergies with a number of COTS Control Innovation Program (CCIP) projects to enhance impact pathways across the program (**Figure 1**), including (1) operationalising eDNA monitoring (CCIP-D-03 Uthicke et al. 2025) to expand the toolbox for COTS detection through consideration of novel cryptic predators, and (2) COTS modelling teams (CCIP-R-03 Rogers et al. 2025; CCIP-R-04 Skinner et al. 2025; CCIP-R-05 Choukroun et al. 2025) so that data generated in this project could be adopted to interrogate whether drivers of early COTS mortality can help to explain and predict current and future outbreaks (i.e. early detection). This project also collaborated with Traditional Owners to understand and support values, ensure respectful use of Sea Country, and so knowledge generated could be beneficial to ranger monitoring and stewardship. Project synergies were recognised, but not realised, with projects on juvenile biology and ecology (CCIP-P-03 Byrne et al. 2025) and semiochemistry (CCIP-R-11 Motti et al. 2025), with interest in addressing whether toxicity increases as juveniles grow to explain predator-prey interactions in this study. Additionally, site selection for surveys in the northern GBR were aligned with other in-water CCIP projects (CCIP-P-01 Pratchett et al. 2025a; CCIP-P-04 Pratchett et al. 2025b; CCIP-P-06 Doll et al. 2025), as well as the external Reef Restoration and Adaptation Program (RRAP), to gain insight on the best rubble sites with contrasting COTS densities to survey cryptic predators.

Overall, this project aimed to provide new information to help resolve the impact of benthic predation on juvenile COTS in their rubble recruitment and nursery habitat. Specifically, this project aimed to:

- **AIM 1:** Identify cryptic rubble-dwelling COTS predators and quantify their contribution to juvenile COTS mortality in a series of experimental feeding trials.
- **AIM 2:** Determine the distribution of key cryptic predators at local and regional scales, and investigate relatedness to COTS densities, outbreak histories, and potential fisheries consequences.
- **AIM 3:** Develop DNA and eDNA detection protocols for key cryptic predators as viable bioindicators and to resolve multi-trophic food webs involving COTS.
- **AIM 4:** Integrate knowledge with Traditional Owners and stakeholders.

2. METHODS

2.1 Fieldwork locations

Three regions representing reefs of the northern, central and southern GBR (**Figure 2**) were visited in this project. All initial surveys and laboratory experiments were conducted at Heron Island Research Station (HIRS) on the southern GBR (**Figure 2C**) over multiple expeditions between September 2021 and April 2023. Additional surveys were conducted at Lizard Island Research Station (LIRS) in the northern GBR (**Figure 2A**) in March 2024, and Moore Reef (and surrounding reefs) in the central GBR (**Figure 2B**) in June 2023. At each location, site selection for predator collections and surveys was influenced by COTS outbreak history, the availability of rubble determined through site reconnaissance, and expert knowledge. Site selection in the central GBR aligned with tourism operations on each day. All in-water surveys were conducted on reefs in green no-take or yellow conservation zones. All collections, surveys and experiments operated under the Great Barrier Reef Marine Park Authority (GBRMPA) permits (G20/44613.1, G22/47448.1, and G23/49210.1) and a University of Queensland (UQ) Animal Ethics permit (2019/AE000388). All statistical analyses described below were conducted in R 4.1.2.

Figure 2. Indication of sites visited in this project in the northern (Lizard Island), central (Moore Reef) and southern (Heron Island) GBR, Australia.

2.2 Novel rubble-dwelling COTS predators and juvenile mortality

2.2.1 Predator candidate collection

Potential COTS predators were collected on SCUBA from coral rubble patches at Heron Island (**Figure 2C**). Initial collections were conducted in February 2022, at 2–12 m water depth, capturing the expected COTS settlement period and juvenile depth range (Wilmes et al. 2020a; Doll et al. 2021), with six additional predator species collected opportunistically in October 2022. Rubble fauna were collected using RUbble Biodiversity Samplers (RUBS) (Wolfe and Mumby 2020) and rubble-filled plastic mesh baskets (4 L) buried in the rubble benthos. RUBS and rubble baskets were collected after ~4 days and were redeployed periodically to sustain rubble community collections. The devices were lifted and sealed immediately in plastic bags underwater, then returned to the laboratory and extensively searched for predator candidates. In addition, active searches for larger mobile taxa were conducted and involved manual searches in rubble patches, overturning rubble pieces by hand to collect conspicuous individuals using small hand nets or by hand.

Potential predators for use in feeding trials were selected from the communities collected based on extensive literature searches and reported diets. Herbivores and those with small body sizes (< 1 mm) were not used. Selected species were housed in 6 L flow-through aquaria with natural rubble supplied with seawater to provide food and shelter until use in predation trials (within days). Larger individuals were separated to ensure predation among predator candidates did not occur. All were sustained with chopped bait prawns fed *ad libitum* every few days but were starved for ~24 hrs before use in experiments.

2.2.2 Juvenile COTS rearing

Adult COTS were collected in the Cairns region and shipped to the National Marine Science Centre (NMSC) in Coffs Harbour, New South Wales, or the Australian Institute of Marine Science (AIMS), Townsville, Queensland, where they were maintained in flow-through aquaria at the approximate temperature of the collection habitat (25–27˚C). COTS were spawned by dissecting gonads from multiple males and females. Ovaries were fertilised and larvae reared in large (≥ 300 L) culture containers of filtered seawater, changed daily. Larvae were provided algal food (e.g. *Proteomonas sulcata*) at a density of 1–5 x 10^4 cells, as needed. After 18–22 days, competent larvae were settled onto polycarbonate plates containing a culture of CCA and mixed algal biofilm. Juveniles were reared on the CCA plates in flow-through aquaria before being transported to HIRS, where they were housed in large (6 L) flow-through aquaria at ~27˚C for use in feeding trials. To obtain a range of juvenile COTS sizes for use in feeding trials, two distinct cohorts were used across three trial periods (September 2021, February 2022, October 2022).

2.2.3 Key predators of COTS

Initial predation trials

To determine the capacity of any given predator to consume COTS, single specimens were placed with an individual juvenile COTS in controlled feeding trials. Predator candidates were photographed (Olympus TG6) for identification to the highest taxonomic resolution and a

phylogenetic tree of all predator candidates constructed using the open-access interactive Tree Of Life tool (Letunic and Bork 2021). Predators were measured and placed in individual 800 mL tanks with flow-through seawater (~0.3 L min^{-1}) with no shelter. One juvenile COTS was then randomly selected from the housing tank, photographed under a dissecting microscope (Olympus SZ61, Dino-Eye AM7025X), and placed into each tank. Juvenile size (maximum diameter, mm) was measured using ImageJ and ranged from 0.8–9.2 mm (mean: 1.99 ± 0.04 mm).

Feeding trials lasted a maximum of three days, with tanks checked periodically each day. When juvenile COTS were not readily visible, the tank was thoroughly searched before all water contents were filtered through a 200 µm mesh and further rinsed with freshwater. If the juvenile was still absent, the predator candidate was examined and rinsed with freshwater to ensure the COTS juvenile was not on the predator itself, which occurred in several instances. COTS were deemed consumed when not found after this extensive search process was repeated several times. At the end of the trial period, COTS were scored as either not consumed, injured (partial predation) or consumed. Predators were aggregated into groups based on the relative frequencies of each outcome (I = incidental, C = consumers, or P = partial consumers). Fisher's exact test was used to compare the incidence of feeding trial outcomes (relative proportion consumed, injured or remaining) among predator species with at least one observed case of COTS consumption or injury using the *fisher.test* function.

Predation likelihood and juvenile size

Five decapod species identified as the most consistent predators of juvenile COTS were used in additional experiments aiming to determine the effects of predator and prey size on the likelihood of juvenile mortality. The decorator crab, *Schizophrys aspera* (n = 89), and four species of swimming crab (Portunidae: *Thalamitoides tridens* (n = 61), *Thalamita pelsarti* (n = 19), *Thalamita admete* (n = 51) and *Thalamita quadrilobata* (n = 20)) were selected. All predators were collected from rubble at Heron Island, as described above. Predators were starved for ~24 hours to standardise hunger, measured (carapace width, mm), and then offered one juvenile COTS for 3 days, as above. Experiments were repeated across three trial periods (September 2021, February 2022, October 2022). COTS were photographed before and after trials (DinoEye AM7025X, Olympus SZ61 microscope) and condition scored as either consumed, injured or intact. Initial size of each COTS was measured post-hoc using ImageJ, ranging 1–14 mm in diameter (mean ± SE: 5.7 ± 0.2 mm) and 2–10 months old. To explore the drivers of variation in consumption relative to predator and prey size, a generalised linear model (GLM) was fit with binomial distributions using the *stats* package. This was repeated to predict the probability of COTS injury (i.e. partial predation). Species were grouped as *S. aspera* (n = 89) or the Portunidae (n = 151) for analyses.

Natural feeding capacity

To address the potential of key predator species to detect and consume juvenile COTS among rubble in a more natural setting, a series of feeding trials were conducted in mesocosms of coral rubble that included natural rubble-dwelling communities. Two decapods, *Schizophrys aspera* (Majidae) and *Thalamita admete* (Portunidae), were used given their prior consistency in initial feeding trials and their high abundance in our rubble sites. To establish the mesocosms, rubble-filled baskets were deployed, collected and

returned to the laboratory in February 2022, as described above. The contents of each rubble-filled basket were then placed in 6 L flow-through tanks (1.15 L ± 0.06 seawater min^{-1}) and left to stabilise for at least 6 hours before trials commenced. Cursory searches of each tank prior to trial commencement were conducted to ensure no confirmed COTS predators were among the sampled rubble community. Each tank was then supplied with 30 juvenile COTS (2 months old, ~2 mm diameter) and one predator, either *S. aspera* or *T. admete* (n = 6 per predator). Control tanks (n = 6) without predator candidates were conducted to capture background COTS mortality caused by the wider rubble community and/or potential searching and handling errors during recovery of remaining cryptic juveniles post-trial. In all treatments, rubble pieces were covered by sessile biota including CCA, turf algae, macroalgae, sponges and ascidians, typical of shallow reef rubble (Wolfe et al. 2021). The natural microhabitat complexity served to amplify the cryptic nature of COTS, which fed on CCA throughout the experiment, as indicated by feeding scars on rubble pieces. The same experiment was repeated in October 2022 with 10-month-old juveniles (~7–10 mm diameter) for *S. aspera* (n = 6) and control tanks (n = 4).

After four days, tanks were searched extensively for COTS. Each rubble piece was removed and rinsed with freshwater at least three times to remove all visible COTS and other rubble fauna. Given the complex morphology of rubble, certain pieces were broken to investigate crevices and holes. All recaptured COTS were retained, photographed and measured, as above. The proportions of COTS that experienced predation (i.e. injured or consumed) in rubble mesocosm trials were compared using GLMs, as above, with time (i.e. juvenile age) as a factor. Partial predation was only observed in *T. admete* treatments. Therefore, the number of consumed and injured COTS were grouped because both outcomes are ultimately representative of predatory interactions.

2.3 Distribution of key cryptic predators

A series of field surveys were undertaken in the northern, central and southern GBR (**Figure 2**) to characterise the density, distribution, and habitat associations of key predators of juvenile COTS in rubble. First, predator surveys were conducted at the local reef scale through targeted surveys of *S. aspera* at Heron Island to explore local habitat drivers of the most formidable cryptic COTS predator (Desbiens et al. 2023). Heron Island was selected to conduct fine-scale assessments as this reef has no reported history of severe COTS outbreaks, so high relative densities of COTS predators were expected. Surveys were then conducted across all three regions in this study (**Figure 2**) to determine broader regional scale patterns in predator distributions and habitat predictors.

2.3.1 Local habitat and reef scale drivers

In February 2023, six sites were established around Heron Island (**Figure 2C**); three along the south-western reef slope where *S. aspera* is common (Desbiens et al. 2023), and three informed by recent (2019–2023) data on COTS densities (unpublished data, COTS Control Program). Two sites were included in the northeast where COTS culling operators have detected and removed the highest numbers of individuals, and one site on the south-eastern reef edge where COTS are rarely, if ever, detected. Together, these sites provided the opportunity to address whether the density of *S. aspera* can help to predict COTS numbers at a local reef scale and thus emerge as a viable bioindicator.

To characterise the density and habitat of *S. aspera*, the abundance of *S. aspera* relative to benthic cover and important microhabitat metrics in rubble biomes (e.g. rubble piece size, rubble bed thickness; Wolfe et al. 2023c) were measured in replicate 4 m x 30 m transects at each site. Transects (120 m^2) were placed parallel to the reef slope to maintain a relatively constant depth. Four transects were completed in shallow (< 8 m) and deep (≥ 8 m) zones at every site except for Coral Gardens, which was represented by six transects per depth (total; n = 52). The 8 m depth boundary was selected as the occurrence of juvenile COTS in rubble is greatest at 8–14 m (Wilmes et al. 2020b). Relative benthic cover per transect was quantified using the point-intercept method (Zvuloni and Belmaker 2016) with categories for live *Acropora*, live coral (other), dead standing coral, dead plating coral, rubble, macroalgae, *Halimeda*, soft coral, sand, and other (including clams and conspicuous sponges). Proportional benthic cover of each category was calculated and mean data determined.

All large rubble pieces (≥ 10 cm diameter) within the 120 m^2 transect area were lifted or overturned to check for the presence of *S. aspera*. Live coral was not overturned, and large rubble pieces consolidated to the substrate were not disturbed. This method was biased towards *S. aspera* living underneath large and accessible rubble pieces on top of the benthos. More rigorous population surveys would be highly invasive and require extensive destruction of habitat. The overturning of large rubble pieces was employed as the least invasive approach to survey *S. aspera* and other cryptic predator populations (Desbiens et al. 2023; Wolfe et al. 2023b).

The density and habitat associations of *S. aspera* were evaluated at several levels of the ecosystem. First, site and mean water depth (m) of transects were used as factors to evaluate the influence of seascape parameters on the local abundance and distribution of *S. aspera*. The size of rubble pieces overturned and rubble bed thickness were log-transformed for use as additional factors. A hurdle regression model was conducted with the *pscl* package to analyse count data via maximum likelihood (Jackman 2020) owing to the rarity (i.e. high proportion of zero counts, 52%) of *S. aspera* among transects. Significant results were explored by Tukey method using *emmeans* (Lenth 2019).

Data on the size of *S. aspera* (n = 99) were used to determine the influence of microhabitat on its biology and ontogeny. A linear model was developed using the base *stats* package of R (Chambers and Hastie 1992), with the size of *S. aspera* as the response variable and rubble piece area, bed thickness, and underlying benthos type as factors. Continuous data were log-transformed for analysis and assumptions checked and confirmed using diagnostic plots with the *DHARMa* package (Hartig 2022). Multiple comparison tests (Tukey's HSD) were conducted post-hoc to explore significant differences using the *agricolae* package of R (de Mendiburu 2021). Then, the influence of benthic cover types on the density of *S. aspera* (100 m^{-2}) was explored using a zero-inflated *glmmTMB* to account for the high proportion of zeros with a gaussian distribution for density data (Brooks et al. 2017; Magnusson et al. 2017). Owing to the number of correlated benthic cover categories (i.e. proportional data), the best-fit model was selected using Akaike's Information Criterion (AIC) values (Sakamoto et al. 1986) within the *step* function of the *lmerTest* package (Kunzetsova et al. 2017). Hard substrate, rubble, and soft coral were retained in the model. All density and cover data were log-transformed for analysis and diagnostics checked and confirmed, as above.

Lastly, COTS culling data from Heron Island (unpublished data, COTS Control Program) were used to explore the hypothesis that *S. aspera* mediate COTS densities through predation of the juvenile stage (Desbiens et al. 2023). The mean number of COTS culled per year in the region was determined and compared to the number of *S. aspera* at each site. We further extracted data on the available mixed coral and rubble habitat around Heron Island using polygon features of the Allen Coral Atlas (Allen Coral Atlas 2022) to convert densities of *S. aspera* to an estimate of COTS consumption at a reef wide scale. Predation capacity was extrapolated based on rates of COTS consumption by *S. aspera* (5.79 ind. d^{-1}) determined in ecologically relevant rubble mesocosm experiments (Desbiens et al. 2023) across the earliest juvenile life stage (≤ 7 mm; Wilmes et al. 2016; Neil et al. 2022). Spatial data were extracted specifically for Eco 1 and Fifth Point to estimate the potential impact of *S. aspera* on COTS for sites with the highest and lowest predator populations, respectively.

2.3.2 Regional patterns

Differences in the density of key rubble-dwelling predators were explored across sites in the northern, central and southern GBR (**Figure 2**) to address whether patterns observed at Heron Island were found in regions further north where COTS outbreaks are more prevalent. Predator species were surveyed in a series of transects placed over the reef and rubble. Transects were 120 m^2 in the south (Heron Island; February 2023) and central (Moore Reef; June 2023) GBR, as above, and 40 m^2 (4 m x 10 m) in the north (Lizard Island; March 2024), with all data standardised to 100 m^2. Presence of all individuals known to consume COTS was recorded, and mean data generated per site and per region. Benthic cover and rubble metrics were recorded, as described for local scale surveys above. Transects were conducted at eight sites at Lizard Island and surrounding reefs (n = 4–11 per site), two sites at Moore Reef (n = 3 per site), and six sites at Heron Island (n = 8–12 per site). Mean densities of *S. aspera* and portunid crabs (species grouped) were compared with linear models in the base *stats* package. Additional searches were conducted at four reefs in the central GBR region (Fitzroy Island, Green Island, Flynn Reef, Milln Reef; **Figure 2B**) in 2–3 hours of SCUBA and/or snorkel time per site. Transects were not conducted as these sites were visited just once governed by short tourist operations each day, but the presence of predator species was noted. More rigorous surveys are needed to generate comparable quantifiable data at these sites.

The extent to which physical and ecological characteristics determine the density of key cryptic COTS predators was then explored in detail using a Bayesian Structural Equation Model (SEM) framework. Structural equation modelling allows evaluation of a network of relationships among ecosystem variables across a range of scales (Grace 2006). To construct the SEM, spatial drivers (reef, site, water depth) were used to predict responses across all levels of organisation, while additional variables were successively added as predictors of variables at lower levels of the ecosystem hierarchy to address structured drivers of habitat (e.g. benthic cover) and rubble microhabitat characteristics (e.g. bed thickness, piece size) on the density of *S. aspera* and portunids (**Table 1**). The Bayesian SEM was developed using the *blavaan* package, which relies on JAGS and Stan to estimate models via Markov Chain Monte Carlo simulation (Merkle and Rosseel 2015). Weakly-informative priors were specified for all fixed effects (Normal distribution with mean = 0 and SD = 1, Table 1). The model was fit with three chains and 11,000 iterations, the first 1,000 of which were discarded as burn-in. Component models within the Bayesian SEM framework

were considered purely additive (**Table 1**) with correlated error structures specified *a priori* to account for confounding variables, such as percent cover of benthic categories. Model convergence was assessed using trace, density, and autocorrelation plots, and was monitored using the $\hat{R}$ convergence criterion (Merkle and Rosseel 2015). Estimate and error values were standardised to scale outcomes for variables of different metrics and used to explore relationships among variables.

Table 1. Component model specification for scaled variables in the Bayesian structural equation model including $\hat{R}$ scores.

Response	Fixed effects	$\hat{R}$
Rubble cover	Reef + Site + Depth	1.00
Sand cover	Reef + Site + Depth	1.00
Live coral cover	Reef + Site + Depth	1.00
Hard substrate cover	Reef + Site + Depth	1.00
Rubble bed thickness	Reef + Site + Depth + Rubble + Sand + Live coral + Hard substrate	1.00
Rubble piece size	Reef + Site + Depth + Rubble + Sand + Live coral + Hard substrate	1.00
Number of rubble pieces	Reef + Site + Depth + Rubble + Sand + Live coral + Hard substrate	1.00
Density of *S. aspera*	Reef + Site + Depth + Rubble + Sand + Live coral + Hard substrate + Bed thickness + Piece size + Number of pieces	1.00
Density of Portunidae	Reef + Site + Depth + Rubble + Sand + Live coral + Hard substrate + Bed thickness + Piece size + Number of pieces	1.00

2.4 Molecular DNA/eDNA detection and cryptic food webs

The use of molecular DNA techniques to detect COTS before they reach outbreak levels is a developing tool in the prediction of COTS success and outbreak likelihood at reef and regional scales. Through multiple molecular approaches, this component aimed to add to the COTS management toolbox through the use of (1) eDNA to recognise key cryptic predators of COTS as bioindicators, and (2) DNA metabarcoding to resolve multi-trophic food web links involving COTS, cryptic predators and higher-order fishes.

2.4.1 eDNA and cryptic COTS predators as viable bioindicators

This eDNA component focused on detection of COTS DNA in the gut contents of cryptic predators, as done for fish gut samples (Kroon et al. 2020), plankton samples for identification of COTS larvae (Uthicke et al. 2015; Doyle et al. 2017; Byrne et al. 2024a), and COTS recruits in settlement traps (Doll et al. 2021). This project first focused on developing suitable methods to detect DNA of consumed COTS within novel predators using a single PCR barcoding approach with COTS-specific markers. To ensure that positive detection of COTS was viable in cryptic predators, a series of pilot studies were conducted at AIMS using decapods known to have consumed juvenile COTS in feeding trials. The methods and outcomes of these pilot studies can be found in Appendix A.

Once best-practice eDNA extraction and detection methods were established, wild-type cryptic predators were collected to address whether COTS DNA could be detected in nature through this predator-prey interaction. Collections of wild-type predators were conducted in March when early-stage juvenile COTS were expected to be in rubble (Wilmes et al. 2020b). At Heron Island (March 2023), *S. aspera* (n = 17) were collected and fixed immediately in 100% ethanol. Five animals were used in methods testing, with the remaining 12 analysed with ddPCR assay (Table S1 in Appendix A). At Lizard Island (March 2024), 65 specimens (Table S2 in Appendix A), including 22 species of decapod and one polychaete (*Pherecardia striata*) previously observed to consume juvenile COTS (Glynn 1984; Desbiens et al. 2023), were collected and analysed with ddPCR assay in the same way. Additional analyses were conducted to determine the Limit of Detection in DNA results (Table S3 in Appendix A). All field and laboratory controls returned no presence of COTS DNA, so positive detection was defined as a sample with one or more positive droplets in ddPCR assay.

2.4.2 DNA metabarcoding to resolve trophodynamics involving COTS

COTS DNA has been detected in the gut and faecal contents of wild-type reef fishes, implying predation of COTS (Kroon et al. 2020), but the role that cryptic predators play in this trophic network has not been determined. Food webs involving COTS are likely to be more complex than currently understood, including a suite of indirect pathways (Sweatman 2008; Kroon et al. 2020). To build a more holistic understanding of food webs involving COTS, we attained the aforementioned fish gut samples (Kroon et al. 2020), supplemented with samples of wild-type cryptic predators to conduct community metabarcoding to map multi-trophic links involving COTS for the first time.

Metabarcoding involves the extraction and amplification of DNA within biological samples, followed by amplicon sequencing and the taxonomic identification of sequences (i.e. barcodes) attributed to each sample. This method enables the simultaneous identification of multiple taxa, including cryptic species that are difficult to observe (Byrne et al. 2024a) and even in ingested/digested matter (Kroon et al. 2020). However, there are extreme taxonomic constraints among cryptic fauna (Wolfe et al. 2023c), which can hinder accurate identification using available DNA repositories (van der Loos and Nijland 2021). Thus, to include cryptic predators in DNA metabarcoding, it was first necessary to confirm species identification and generate unique DNA sequences. Sequences were generated for seven species identified as top predators of COTS in this study: *Schizophrys aspera*, *Thalamita admete*, *T. pelsarti*, *T. prymna*, *T. quadrilobata*, *Thalamitoides tridens* and *Th. quadridens* (n = 2–3 per species). Methods of extraction, amplification and sequencing analysis are outlined in Appendix B.

Once predator sequences were established, DNA metabarcoding was conducted on gut or faecal content of fishes (n = 80) and cryptic predators (n = 12; **Table 2**). Fish faeces were collected as described in Kroon et al. (2020). Only fishes collected during one field trip in Kroon et al. (2020) (#6958, July 2018) were used as this collection yielded the greatest positive detection of COTS DNA in fishes most likely to interact with cryptic predators (**Table 2**). Individual fishes were selected from the inventory based on positive detection of COTS in their faeces and/or known capacity to consume COTS, ensuring replication within species and of individuals from fished (blue zones) and unfished (green zones) reefs, where possible (**Table 2**). As the top cryptic predator of COTS, *S. aspera* was selected for metabarcoding analyses with gut contents extracted as above for eDNA. Unique DNA sequences generated

for the remaining cryptic predators were used to detect their presence in this trophic network as prey. A total of 96 unique samples (including two negative and two positive controls; **Table 2**) were replicated across four plates (two CO1 and two 18S). Due to time constraints, only a portion of metabarcoding data were analysed for this report. These results are therefore preliminary and more rigorous interrogation of the data is required. For ease of presentation, resultant communities identified by DNA were filtered to common marine phyla, including the Porifera, Cnidaria, Annelida, Nematoda, Nemertea, Platyhelminthes, Mollusca, Arthropoda, Echinodermata, and Chordata. Positive detection of n = 54 individuals from other, often microscopic eukaryote phyla were excluded, along with n = 23 cases where taxa were incorrectly assigned to species beyond the study area (e.g. terrestrial insects). Host DNA was also excluded. Full details on methods of extraction, amplification and metabarcoding analysis can be found in Appendix B.

Table 2. List of specimens used in DNA metabarcoding to begin to establish a more comprehensive food web analysis involving known cryptic (*Schizophrys aspera*) and fish predators of COTS. Numbers indicate sample size, coloured cells represent reefs open (green) and closed (blue) to fishing.

Family	Species	Heron Reef	18-025	Kelso Reef	Bramble Reef	Lodestone Reef	Rib Reef	Total
Control	Positive							2
	Negative							2
Majidae	*Schizophrys aspera*	12						12
Balistidae	*Balistapus undulatus*						2	2
Haemulidae	*Diagramma pictum*						2	2
Labridae	*Cheilinus chlorourus*				1	5		6
	Cheilinus fasciatus					5		5
	Oxycheilinus diagramma			1		2		3
	Thalassoma jensenii						1	1
	Thalassoma lunare						1	1
Lethrinidae	*Gymnocranius grandoculis*			1				1
	Lethrinus lentjan						6	6
	Lethrinus miniatus		1		1	2	2	6
	Lethrinus nebulosus			3		2	4	9
	Lethrinus ornatus					3	5	8
	Monotaxis grandoculis						1	1
Lutjanidae	*Lutjanus fulviflamma*				3		4	7
	Lutjanus russellii				1	2	3	6
Nemipteridae	*Scolopsis bilineata*				1		2	3
Serranidae	*Epinephelus cyanopodus*			1				1
	Plectropomus leopardus		3		1		3	7
Tetraodontidae	*Arothon nigropunctatus*		3					4
	Arothon stellatus			1				1
Total		12	7	7	8	21	36	96

2.5 Integration of knowledge with Traditional Owners and stakeholders

As this project was primarily conducted at Heron Island, Gidarjil Development Corporation, a representative organisation of the Taribelang Bunda, Gooreng Gooreng, Gurang, and Bailai Traditional Owner groups of the Port Curtis Coral Coast Group (PCCC) and Capricorn Bunker Region, have been involved in this project throughout its lifetime to ensure respectful use of Sea Country and so data generated could potentially benefit ranger monitoring and stewardship. To bolster this relationship, a workshop on the biology and ecology of coral rubble, COTS and novel cryptic predators was held at HIRS from 4-8th September 2023. Researchers from UQ partnered with four Elders and five Sea Country rangers from Gidarjil to discuss COTS research, monitoring, and the potential integration of scientific survey methods into existing ranger practices.

Beyond primary intentions of relationship- and capacity-building, the focus of the workshop was to discuss coral rubble morphology and stability, cryptic predators of COTS in rubble, and reef fishes as predators of COTS. As impacts to coral reefs continue to amplify, it is now more important than ever to understand and monitor the structural, biological, and ecological components of rubble and how they may contribute to reefs in future (Wolfe et al. 2021; Kenyon et al. 2023). A series of relevant seminars, discussions, and lab and field sessions were conducted with Elders and rangers. Participants contributed to discussions, including sessions delivered by (1) Sea Country ranger, Kelvin Rowe, on the current coral reef monitoring program implemented by Gidarjil in the PCCC region, (2) Dr Tania Kenyon on rubble morphology and stability as part of a project synergy with RRAP's rubble stabilisation project, and (3) Dr Tina Skinner on reef fishes known to consume COTS as a project synergy within CCIP. Practical survey methods that address important research and monitoring questions, which could be aligned with existing ranger protocols, were developed and refined throughout the workshop. This included a series of in-water field exercises on snorkel and SCUBA to gain skills in monitoring rubble bed condition and performing surveys of cryptic COTS predators and reef fishes.

In addition, fieldwork in this project extended to reefs around Cairns (**Figure 2B**). This provided researchers the opportunity to collaborate and engage with end-users (e.g. Reef Magic tourism operators and Mars monitoring teams), and Traditional Owner rangers in-training who joined on several research dives aiming to quantify cryptic COTS predators in suitable habitat at Moore Reef.

3. RESULTS

3.1 Novel rubble-dwelling COTS predators and juvenile mortality

3.1.1 Key predators of COTS

Initial predation trials

A total of 110 distinct taxa from 42 families and 78 genera (**Figure 3**) were collected and used across feeding trials (n = 428). Where possible, taxa were identified to species level, but 59 individuals were only identified to genus. Feeding trials were replicated 1–19 times per species, with low replication owing to rarity. Of the taxa tested, 31 species interacted with COTS on at least one occasion (**Figure 3**), with a total of 24% of juveniles consumed or injured during trials. Confirmed predators were primarily decapod crustaceans (87%), with the greatest representation of species from the Portunidae (**Figure 3E–G**) and Xanthidae (**Figure 3H–J**) families. Two species of annelid worm (*Eurythoe complanata*; **Figure 3A**, and *Lepidonotus cristatus*) and two gastropods (*Latirus polygonus* and *Peristernia reincarnata*; **Figure 3B**) also consumed juvenile COTS. The majority (~72%) of predator candidates did not display capacity to consume juveniles in the feeding trials.

Differences were found between relative proportions of feeding trial outcomes for the 31 confirmed predators ($p < 0.001$). Species that interacted with COTS juveniles on just 1 or 2 occasions were classed as incidental predators (**Figure 4**). Predators in this class were comprised of worms, gastropods, and several decapods, including species of snapping shrimp (Alpheidae), hermit crabs (Calcinidae), and shrimp (Hippolytidae; e.g. *Saron marmoratus*, **Figure 3C**), as well as xanthid crabs (e.g. *Chlorodiella nigra,* **Figure 3H**). Low trial replication for some predators (e.g. hermit crabs: *Dardanus sp.* and Portunidae: *Gonioinfradens paucidentatus*, *Thalamita coeruleipes* and *Zygita murinae*) may have artificially placed them in this category. Partial predators most often inflicted arm and body damage of varying severities (**Figure 3K–M**) over total consumption (**Figure 4**). The partial predator classification was comprised of species of swimming crab (Portunidae; e.g. *Thalamita admete*, *Thalamitoides tridens* and *Thalamita pelsarti*; **Figure 3E–G**), and xanthid crabs (*Cyclodius ungulatus* and *Etisus anaglyptus* (**Figure 3H–J**, **Figure 4**). All predators contrast outcomes for *Schizophrys aspera* (**Figure 3D**), a decorator crab that consumed COTS in 89% of feeding trials (**Figure 4**).

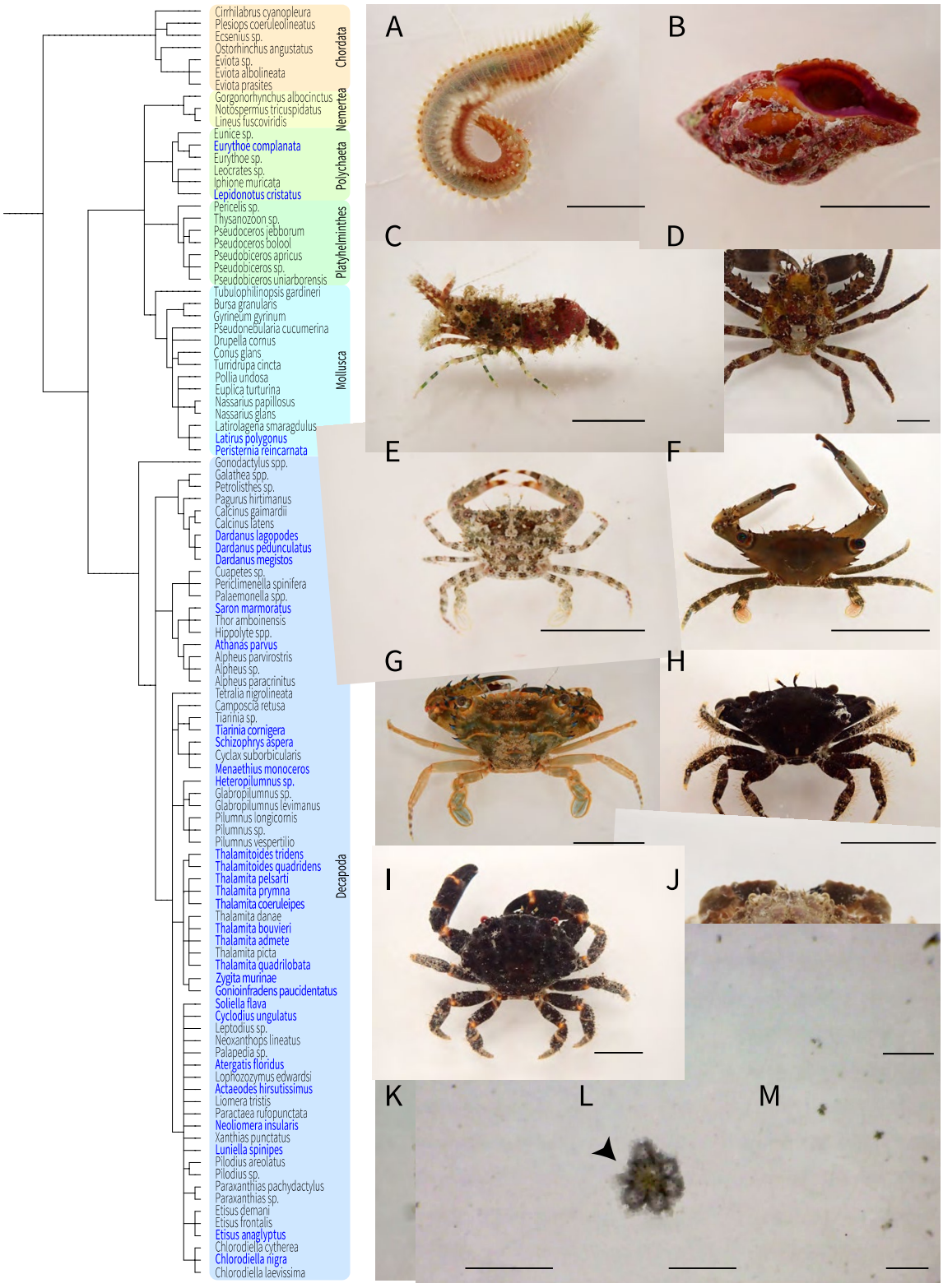

Figure 3. Phylogenetic tree of all potential predators assessed in feeding trials. Blue text denotes confirmed COTS predators. Featured predator candidates (A) *Eurythoe complanata,* (B) *Peristernia reincarnata,* (C) *Saron marmoratus,* (D) *Schizophrys aspera,* (E) *Thalamita admete,* (F) *Thalamitoides tridens,* (G) *Thalamita pelsarti*, (H) *Chlorodiella nigra,* (I) *Cyclodius ungulatus*, and (J) *Etisus anaglyptus.* (K) shows intact juvenile COTS, while (L) and (M) show juvenile COTS damaged by predators. Scale bars equate to 10 mm (A-J), and 2 mm (K-M). Adapted from (Desbiens et al. 2023).

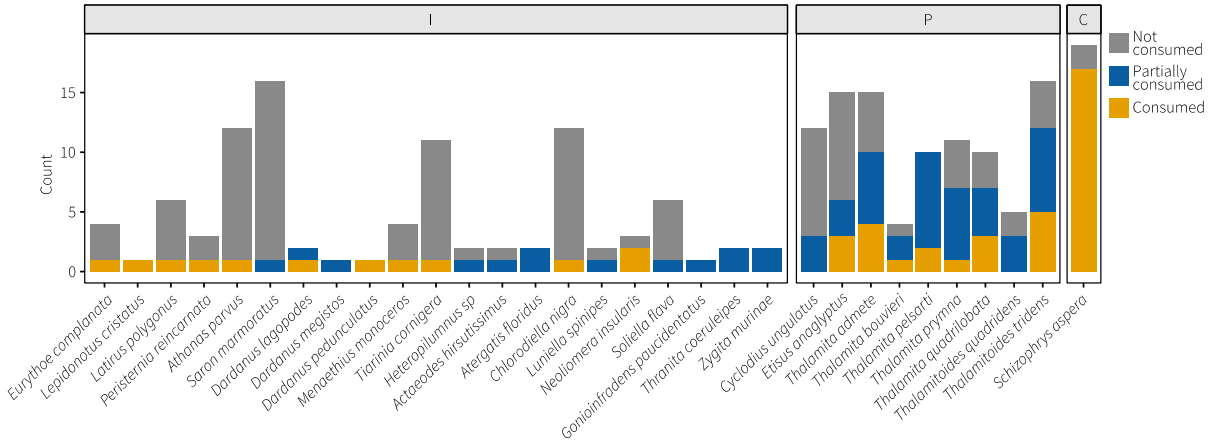

Figure 4. Incidence of outcomes for feeding assays of 31 confirmed juvenile COTS predators. Groups denote species classified as (I) incidental or (P) partial predators, and (C) consumer. Adapted from (Desbiens et al. 2023).

Predation likelihood and juvenile size

The size of juvenile COTS was a negative predictor of consumption probability (β = -0.65, p < 0.001, **Figure 5A**). The probability of total consumption decreased to near-zero at ~10 mm and 5 mm juvenile size for *S. aspera* and the Portunidae, respectively (**Figure 5A**). Predator size was generally a positive predictor of COTS consumption (β = 0.05, **Figure 5B**), though this effect was marginally insignificant (p = 0.065). *Schizophrys aspera* exhibited higher probability of COTS consumption than portunid crabs (β = -3.2, p < 0.001, **Figure 5A,B**). Injury probability was also dependent on predator species, and juvenile and predator size (**Figure 5C,D**). A positive correlation was found between injury and COTS size for *S. aspera*, but for portunids, injury probability decreased with juvenile size (β = -0.9, p < 0.001, **Figure 5C**). Larger predators were more likely to injure juveniles (β = 0.07, p < 0.001, **Figure 5D**). Within the size range of juveniles used (1–14 mm), *S. aspera* appeared to switch from total consumption to partial predation when juvenile size increased to ~10 mm, while portunid crabs reduced both total and partial consumption with COTS size (**Figure 5C,D**).

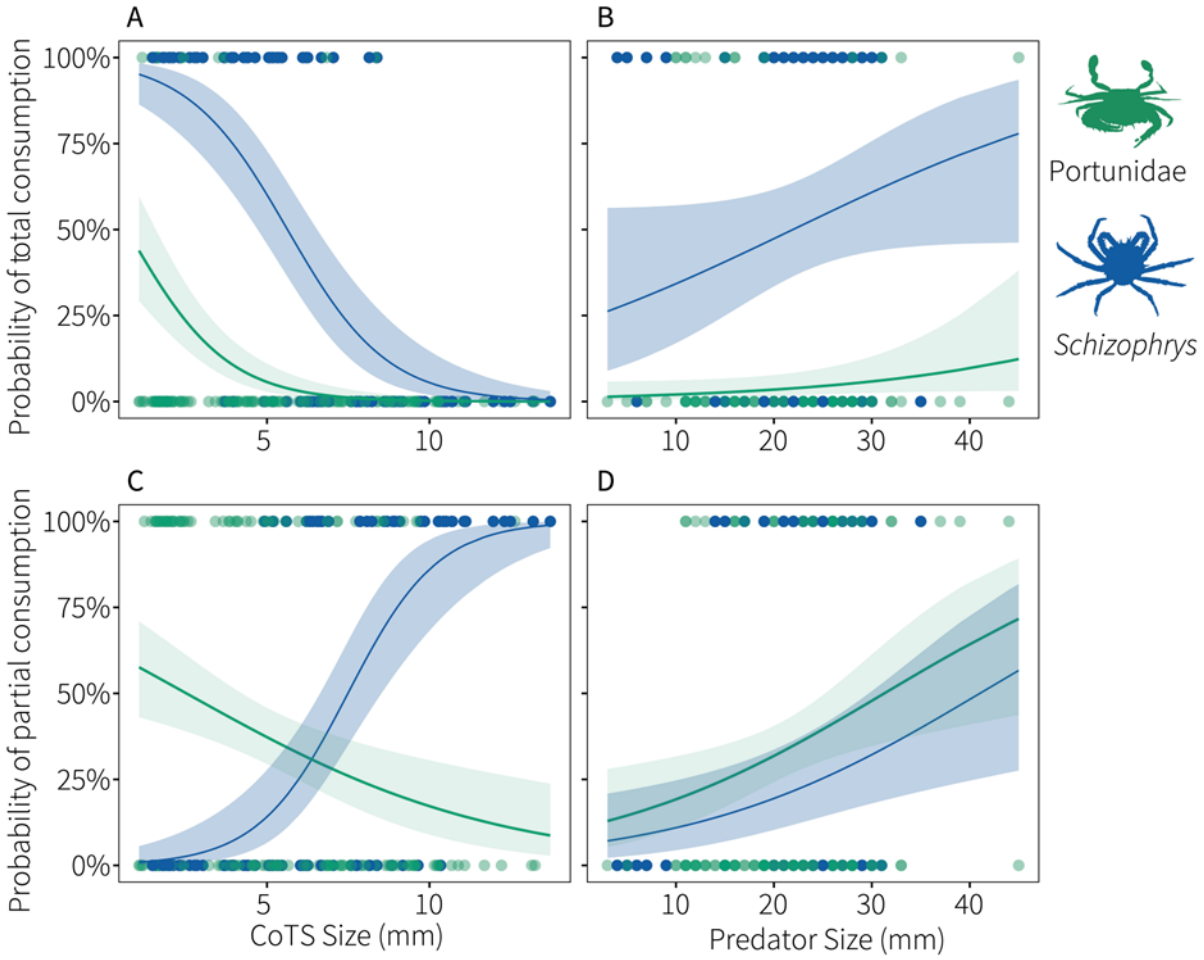

Figure 5. Probability of (A,B) total and (C,D) partial COTS consumption across (A,C) juvenile and (B,D) predator sizes for *Schizophrys aspera* and the Portunidae (species grouped). Bands denote ±95% CI.

Natural feeding environment

Of the 30 juvenile COTS added to control tanks (no added predator), >98% (29–30 ind.) of 2-month-old, and all 10-month-old, juveniles were recaptured (**Figure 6**). Both *S. aspera* ($p < 0.001$) and *Thalamita admete* ($p < 0.05$) consumed more COTS than in the control, and these two predator treatments differed ($p < 0.001$). For 2-month-old juveniles, ~93% of COTS were found intact when *T. admete* was present, with ~2 COTS consumed or injured per replicate (**Figure 6A**). In mesocosms with *S. aspera*, ~21% of COTS were recovered with 24 ± 2 ind. (~79%) consumed from the natural rubble setup (**Figure 6A**). Taking background loss from control tanks into consideration (0.13 ± 0.06 COTS day^{-1}), *T. admete* and *S. aspera* contributed to consumption rates of ~2 mm juveniles at 0.4 ± 0.1 and 5.8 ± 0.4 COTS day^{-1}, respectively. Total consumption of 10-month-old juveniles by *S. aspera* was lower than at 2 months ($p < 0.001$), with an increase in the number of injured juveniles (**Figure 6B**). At ~7–10 mm juvenile size, consumption rate of juveniles by *S. aspera* reduced to 0.8 ± 0.3 COTS day^{-1}, but with an added injury rate of 0.5 ± 0.3 COTS day^{-1}.

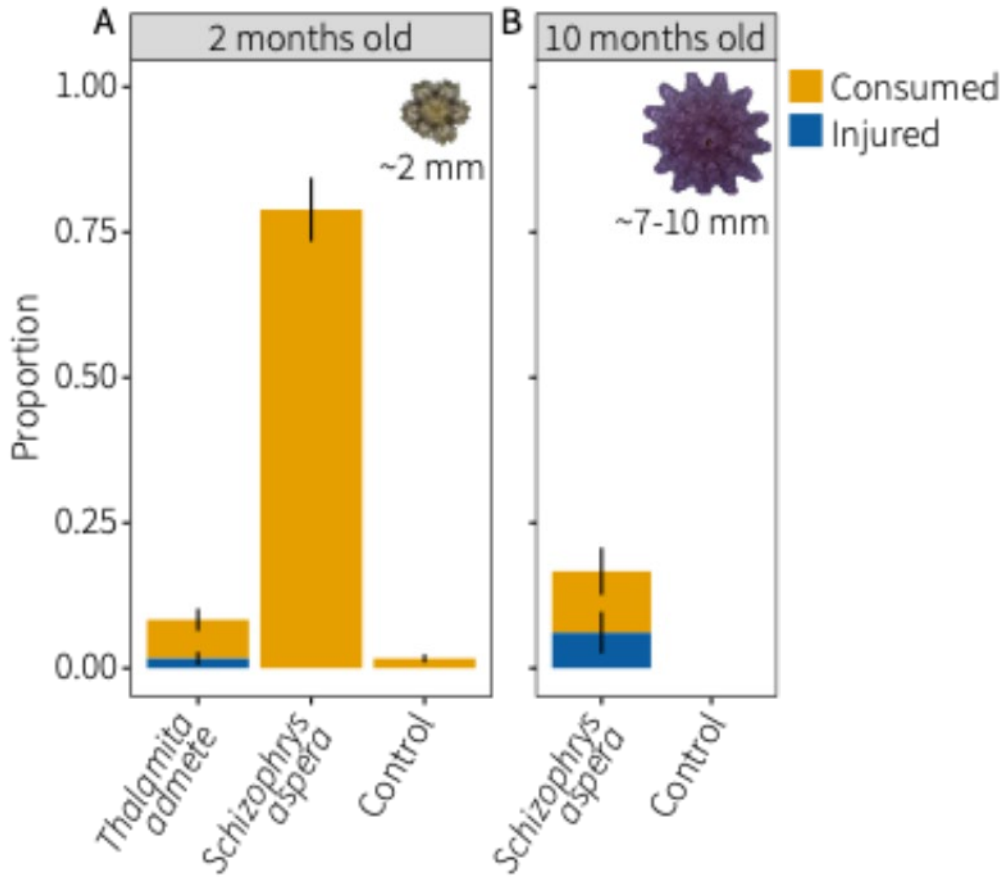

Figure 6. Proportion (± SE) of 2- and 10-month-old juvenile COTS consumed or injured in experimental rubble mesocosms after 96 hours exposure to *Thalamita admete*, *Schizophrys aspera* or control conditions (no predator).

3.2 Distribution of key cryptic predators

3.2.1 Local habitat and reef scale drivers

Schizophrys aspera were found in 48% (25 of 52) of transects. A total of 48 individuals were found through inspection of rubble pieces overlying rubble (n = 607), sand (n = 279), and hard substrate (n = 136). This represents a ~5% success rate in finding *S. aspera* per rubble piece flipped (i.e. effort) at a reef wide scale. The total mean density of *S. aspera* was 0.77 ind. 100 m^{-2} (SE ± 0.16), which was greatest at Eco 1 (2.1 ± 0.5 ind. 100 m^{-2}; **Figure 7**). The highest density in a single transect was 5 ind. 100 m^{-2} in the deep at Halfway while no *S. aspera* were found > 8 m depth at Fifth Point (**Figure 7**). Presence of *S. aspera* was positively associated with the size of rubble pieces ($p < 0.001$) and bed thickness ($p < 0.001$). There was an increased likelihood of encountering individuals by overturning large rubble pieces overlying more chasmic rubble patches but with a concurrent increase in uncertainty (**Figure 8A,B**), which suggests *S. aspera* are rare relative to available preferred habitat. Water depth did not influence the density of *S. aspera* ($p = 0.3$), despite a clear peak in its population in deep areas of Halfway (**Figure 8**).

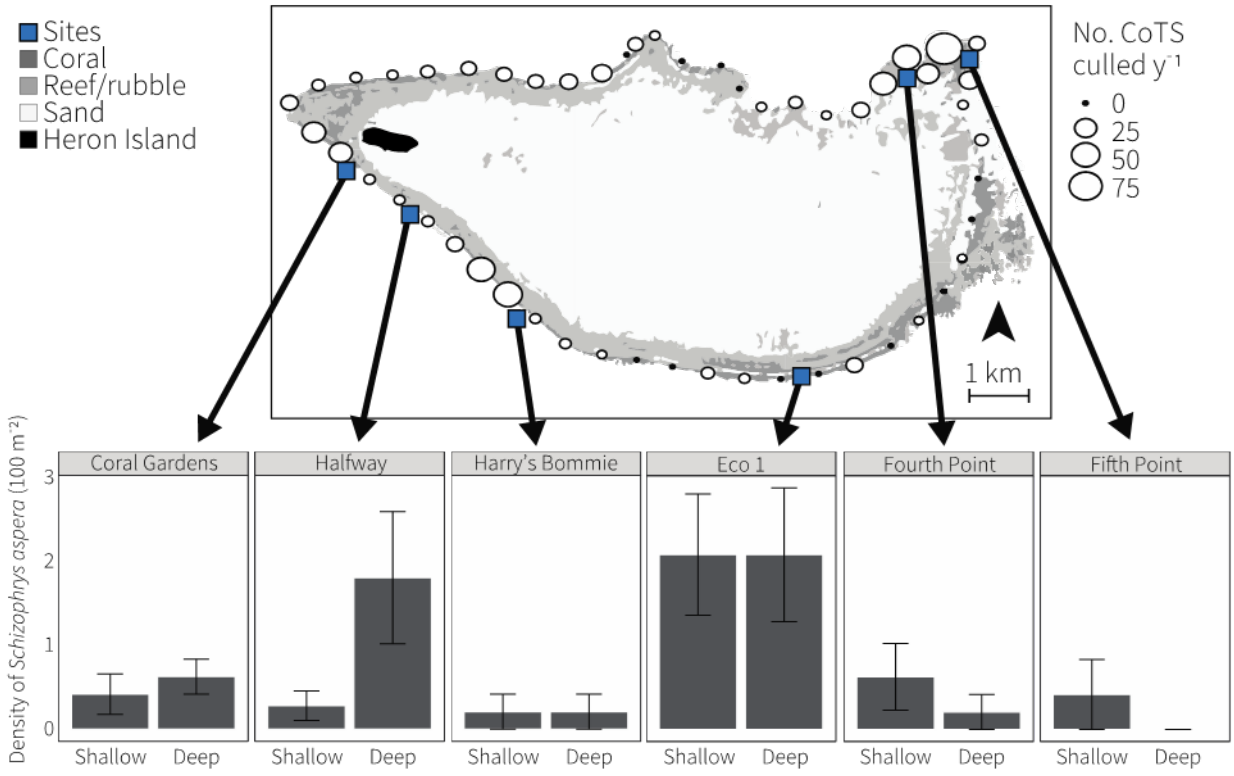

Figure 7. Survey sites around Heron Island, with circles showing mean number of COTS culled from the reef per year, and bars showing the mean density of *Schizophrys aspera* (100 m^{-2}) per site.

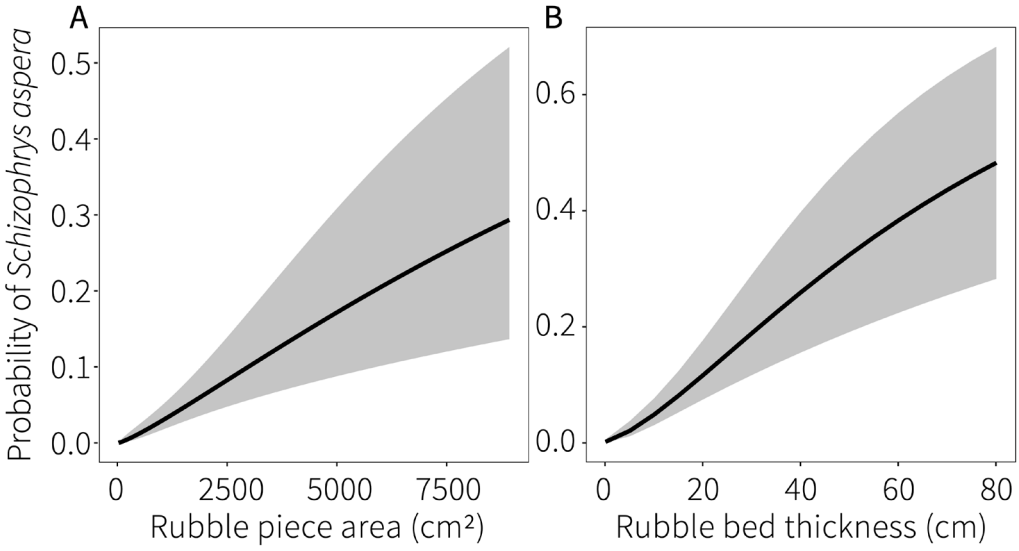

Figure 8. Probability of encountering *Schizophrys aspera* under rubble pieces within transects (n = 1022) based on the (A) size of rubble pieces (cm^2) and (B) rubble bed thickness (cm). Grey area = 95% confidence limits.

All *S. aspera* were 5 to 33 mm (mean: 23.5 ± 0.5 mm; n = 99) with a relatively even spread of males (30%), females (38%), and juveniles (32%). The size of *S. aspera* differed by rubble bed thickness ($p < 0.001$) and underlying benthos type ($p < 0.001$), but not by rubble piece size ($p = 0.63$). Just three individuals were found under rubble overlying sand, which were the smallest individuals (5, 8 and 11 mm) found. Larger individuals were routinely associated

with rubble atop thick patches of rubble. Differences in the density of *S. aspera* were positively associated with the proportional benthic cover of hard substrate ($p = 0.02$), rubble ($p = 0.01$), and soft coral ($p = 0.04$). Halfway and Eco 1 had the highest cover of rubble and hard substrate, respectively, and the highest densities of *S. aspera* (**Figure 7**), and cover of soft coral was highest at Eco 1. Weak negative relationships were detected for the cover of sand and live *Acropora*, though these were not retained in the model.

There was a relationship between the density of *S. aspera* and mean annual number of COTS culled around Heron Reef (**Figure 7**). The lowest number of COTS detected by culling operators was evident at Eco 1 (5.3 ind. y^{-1}) and Halfway (2.1 ind. y^{-1}), the two sites with the highest mean densities of *S. aspera* (**Figure 7**). This contrasts the high numbers of COTS culled at Fourth Point (45.4 ind. y^{-1}), Fifth Point (61.5 ind. y^{-1}), and Harry's Bommie (50.1 ind. y^{-1}), resulting in a negative relationship between the density of *S. aspera* and COTS. Given the amount of available mixed coral and rubble habitat of Heron Reef, we predict from the mean density of *S. aspera* that there could be >25,000 individuals in the region (**Table 3**). If so, it could be possible for this population of *S. aspera* to consume >22 million juvenile COTS across its early benthic life stage to ~10 mm (150 days; **Table 3**). However, the heterogeneous distribution of *S. aspera* may result in localised impacts on COTS. For example, at Eco 1, where *S. aspera* were most abundant, we estimate their potential to consume >7 million juvenile COTS or 18.1 ind. m^{-2} across its early ontogeny (**Table 3**). In contrast, we calculated an order of magnitude lower consumption (1.8 ind. m^{-2}) at Fifth Point where *S. aspera* were rare and COTS numbers high (**Table 3**).

Table 3. Extrapolation of the potential impact of *Schizophrys aspera* on early benthic COTS populations at Eco 1 (high predator, low prey), Fifth Point (low predator, high prey), and at the whole-of-reef scale for Heron Reef (taken from (Wolfe et al. 2023b).

Input data	Eco 1	Fifth Point	Heron Reef
Available habitat (km^2) [a]	0.41	0.14	3.33
Mean density of *S. aspera* ($100m^{-2}$)	2.08	0.21	0.77
Total abundance of *S. aspera*	8,528	294	25,641
Measured COTS consumption (d^{-1}) [b]	5.79	5.79	5.79
Reef-scale COTS consumption (d^{-1})	49,377	1,702	148,461
COTS juvenile duration (d) [c]	150	150	150
Total COTS juvenile mortality	7,406,568	255,339	22,269,208
Total COTS juveniles consumed (m^{-2})	18.1	1.8	6.7

[a] extracted from (Allen Coral Atlas 2022)
[b] mean daily consumption in rubble mesocosms from (Desbiens et al. 2023)
[c] approximate age at ≤7 mm from (Wilmes et al. 2016)

3.2.2 Regional patterns

The density of key juvenile COTS predators in rubble varied among select reefs in the northern, central and southern GBR. The mean density of *S. aspera* was highest in the south, with the fewest found on reefs in the north (**Figure 9**). *Schizophrys aspera* were found at every site in the south but were only found at two (of eight) and one (of two) sites in the northern and central GBR, respectively (**Figure 10**). Of the few *Schizophrys* found at Moore Reef (central GBR), at least one is likely to be a different species to *S. aspera*, evident by its

large size (35 mm) and additional rostral horns, and no *Schizophrys* were found at the four additional reefs opportunistically searched in the central region. Additionally, *S. aspera* were larger in size (p = 0.002) in the south (mean: 22.6 ± 0.8 mm) compared to the central (16.4 ± 4.7 mm) and northern (16.8 ± 1.2 mm) GBR (**Figure 11**). Densities of portunid crabs were comparable in the north and south, while very few decapods were found at Moore Reef overall (**Figure 9**, **Figure 10**). Total crab densities were markedly low at the additional reefs in the central GBR, though several portunids (e.g. *Thalamitoides tridens*, *Thalamita admete*) were found under rubble at Fitzroy Island and Green Island (**Figure 2B**). Whether these patterns for select reefs are truly representative of each region must be clarified with broader surveys.

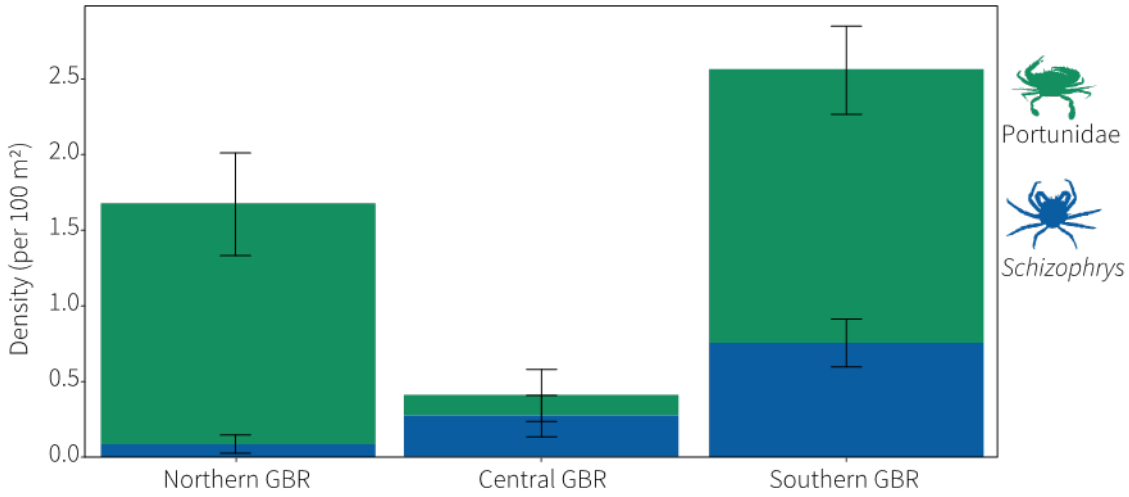

Figure 9. Mean (±SE) density of key predators of juvenile COTS in rubble among survey locations in the north (Lizard Island), central (Moore Reef) and south (Heron Island) GBR.

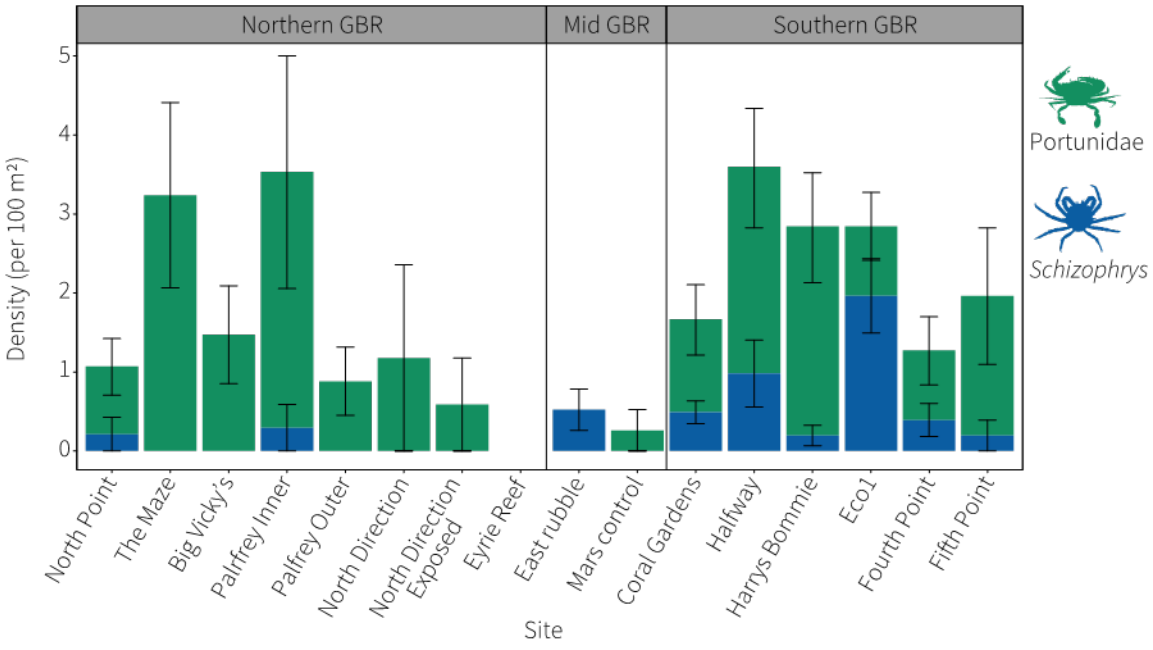

Figure 10. Mean (±SE) density of key predators of juvenile COTS in rubble among survey sites in the north (Lizard Island), central (Moore Reef) and south (Heron Island) GBR.

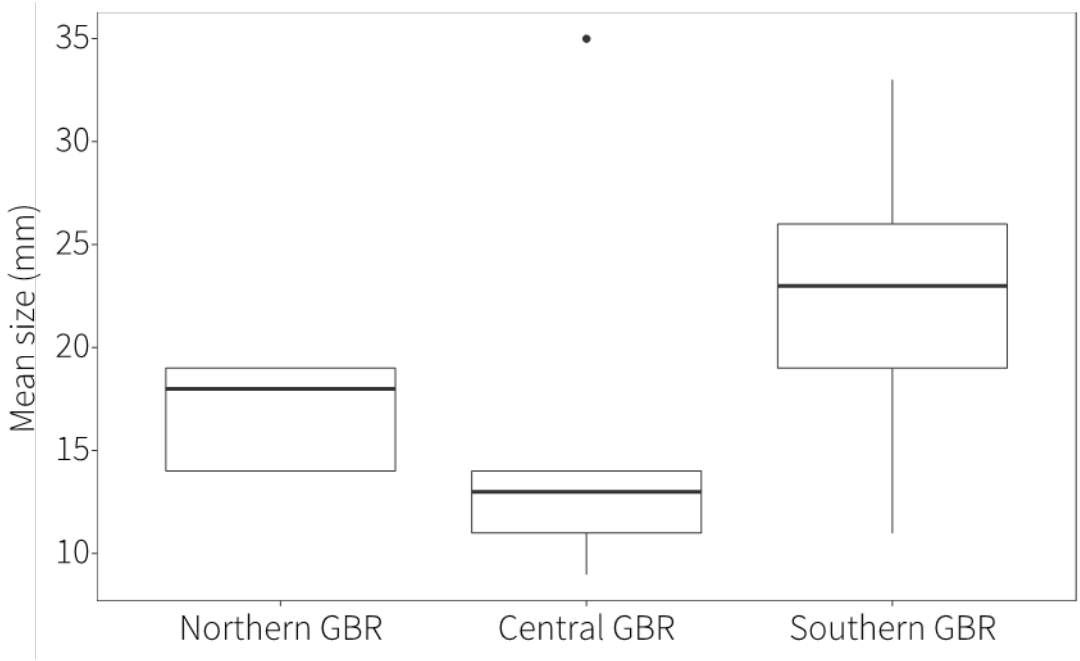

Figure 11. Mean carapace width (mm) of *Schizophrys aspera* across transects and collections in the northern (n = 5), central (n = 6) and southern (n = 41) GBR. Black point represents one large male *Schizophrys* sp. found at Moore Reef.

The SEM model procedure was checked and confirmed, with a posterior predictive *p*-value of 0.5, indicating a good fit, and $\hat{R}$ values consistently ≤1.05 (**Table 1**) indicating chain convergence for all parameters (Vehtari et al. 2021). There was a clear structure to the influence of regional drivers (reef, site and water depth) on the benthic cover of rubble, live coral and sand, including a decrease in rubble cover and increase of sand with water depth (**Figure 12**). The cover of sand had a negative influence on the size and number of rubble pieces, which had flow-on effects on the density of portunid crabs (**Figure 12**). Portunids had a positive relationship with rubble in sandy areas. Rubble cover had a negative effect on rubble piece size, suggesting greater rubble cover was comprised of smaller pieces. The cover of rubble and live coral had positive effects on rubble bed thickness, which was a positive driver of *S. aspera* (Figure 12). *S. aspera* also showed a positive association with hard substrate. At a broader scale, *S. aspera* and portunids were directly influenced by reef (**Figure 12**), indicating strong regional-scale differences in their abundance, particularly for *S. aspera* density (**Figure 9**) and size (**Figure 11**). Although we generalise our findings on the abundance of these predators in the northern, central and southern GBR, we acknowledge that the few reefs explored here may not be fully representative of each region and broader survey data are needed to resolve these spatial-scale outcomes.

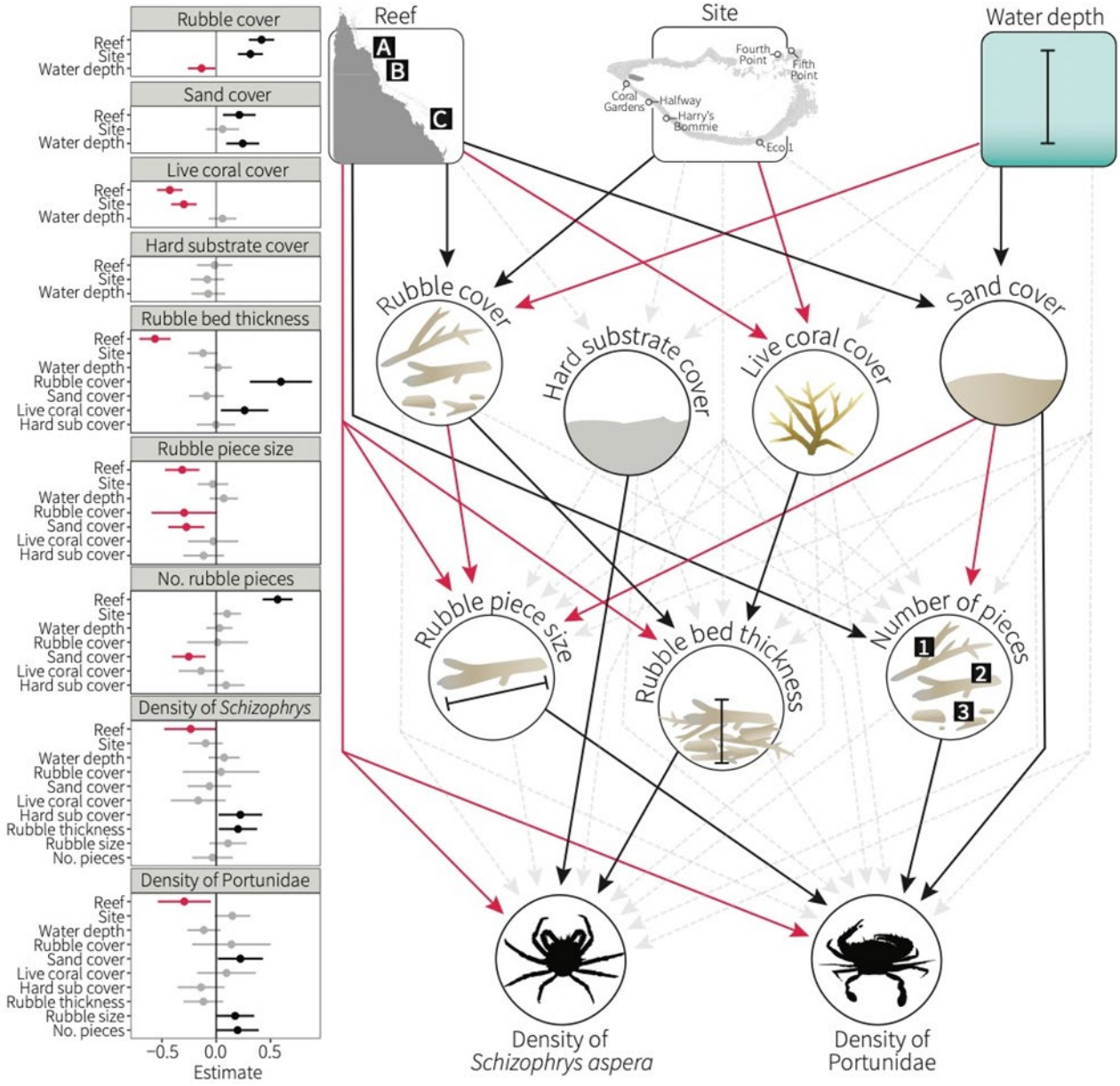

Figure 12. Path diagram of Bayesian SEM describing the physical and ecological drivers of the density of cryptic COTS predators, *Schizophrys aspera* and the Portunidae. Solid black arrows indicate significant positive pathways, solid red arrows indicate significant negative pathways, and grey dotted arrows show nonsignificant pathways. Plots show significant positive (black) and negative (red) scaled estimate and error results of each interaction.

3.3 Molecular DNA/eDNA detection and cryptic food webs

3.3.1 eDNA and cryptic COTS predators as viable bioindicators

The digestive tissue of 17% (2/12) of wild-caught *S. aspera* from Heron Island (Mar 2023) and 12% (8/65) of cryptic predators from Lizard Island (Mar 2024) tested positive for COTS DNA (**Table 4**, and Table S2 in Appendix A). At Heron Island, the two individuals of *S. aspera* were collected from Eco 1 (**Figure 2C**), the site with the highest density of *S. aspera* and lowest number of COTS reported (**Figure 7**). At Lizard Island, the eight individuals with COTS DNA detected represented seven species of decapod, including one shrimp (Hippolytidae), and seven crabs (Epialtidae, Portunidae and Xanthidae; **Table 4**). These decapods were collected from three sites: North Point, Big Vicky's and Palfrey Inner (**Figure 2A**). Zero (of five) *S. aspera* collected at Lizard Island had detectable COTS DNA in their systems (Table S2 in Appendix A).

Table 4. Wild-type invertebrate species collected from Heron Island (Mar 2023) and Lizard Island (Mar 2024) with COTS DNA above Limit of Detection across one or more of the digestive tissues analysed. See Table S2 in Appendix A for full details including species with negative results.

Reef	Year	Family	Species	Site
Heron Island	2023	Majidae	*Schizophrys aspera*	Eco 1
			Schizophrys aspera	Eco 1
Lizard Island	2024	Epialtidae	*Tiarinia cornigera*	North Point
		Hippolytidae	*Saron marmoratus*	North Point
		Portunidae	*Thalamita coeruleipes*	North Point
			Thalamita coeruleipes	North Point
			Thalamita bouvieri	Big Vicky's
			Thalamita admete	Palfrey Inner
		Xanthidae	*Cyclodius ungulatus*	Big Vicky's
			Soliella flava	North Point

3.3.2 DNA metabarcoding to resolve trophodynamics involving COTS

66% of cryptic predator specimens returned unclear taxonomic results against the BLAST sequencing library (Table S4 in Appendix B). For some species, such as *Thalamita admete*, taxonomy was accurate using this cross-reference method, but the top matches for most species were not correct and/or placed at higher taxonomic levels. For example, all specimens of *S. aspera* (Majidae) returned as Decapoda or Xanthidae (Table S4 in Appendix B). Similarly, no results specific to *Thalamitoides quadriens* were available in the library, while for *Thalamita prymna*, it needs to be confirmed whether its BLAST classification as *T.* cf. *rubridens* is indeed correct, though we retain *T. prymna* here for consistency.

Preliminary results from DNA metabarcoding of gut contents indicated that *S. aspera* has a diverse diet. Sponges (Porifera) were the predominant component in the gut content of *S. aspera* (**Figure 13A**), along with worms (Platyhelminthes), decapods (Arthropoda) and reef fishes (Chordata). No COTS DNA was detected in the gut contents of *S. aspera* using this metabarcoding method, indicating a more rigorous assessment of these DNA results is still required. Interestingly, five species of reef fish returned positive results for *S. aspera* in their faecal contents, with the strongest signal detected in *Lutjanus russellii* (**Figure 13B**). Only

one other majid crab, *Tylocarcinus styx*, was detected thus far, in an individual of *Thalassoma jensenii* (Labridae). Portunid crabs were evident in the gut contents of *S. aspera* (**Figure 13A**) and eight species of reef fish (**Figure 13B**), including *Thalamita prymna* and *Thranita pelsarti*, both identified as predators of juvenile COTS in this study. Additional data mining is needed to reveal food web links not yet detected.

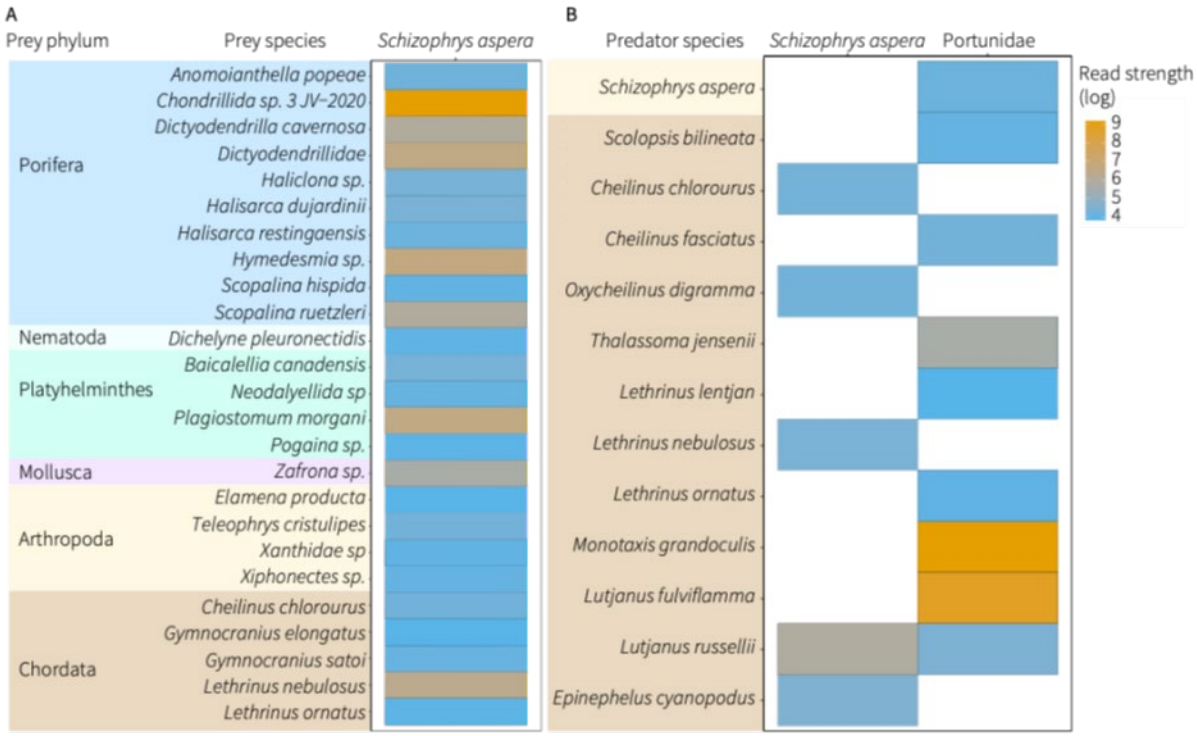

Figure 13. Preliminary results from DNA metabarcoding involving *Schizophrys aspera*, showing (A) prey species in the gut contents of *S. aspera*, and (B) reef fish gut contents with positive detection of *S. aspera* and the Portunidae. Colour code represents the mean strength of DNA reads on the log scale.

Based on these preliminary results, of the 20 species of reef fishes examined, only one individual had detectable traces of an asteroid in its faeces–the sea star, *Linckia laevigata*, found in the starry pufferfish (Tetraodontidae), *Arothron stellatus* (**Figure 14**). COTS DNA has not yet been detected in the faecal contents of reef fishes here. 70% of the fishes examined (14 of 20 species) returned positive results for Decapoda, one of the most commonly detected prey items in faecal matter, along with other reef fishes (**Figure 14**). There were no clear differences in prey items in fishes collected from protected no-take green zones and fished blue zones (Figure S8 in Appendix B), though these data must be more closely examined.

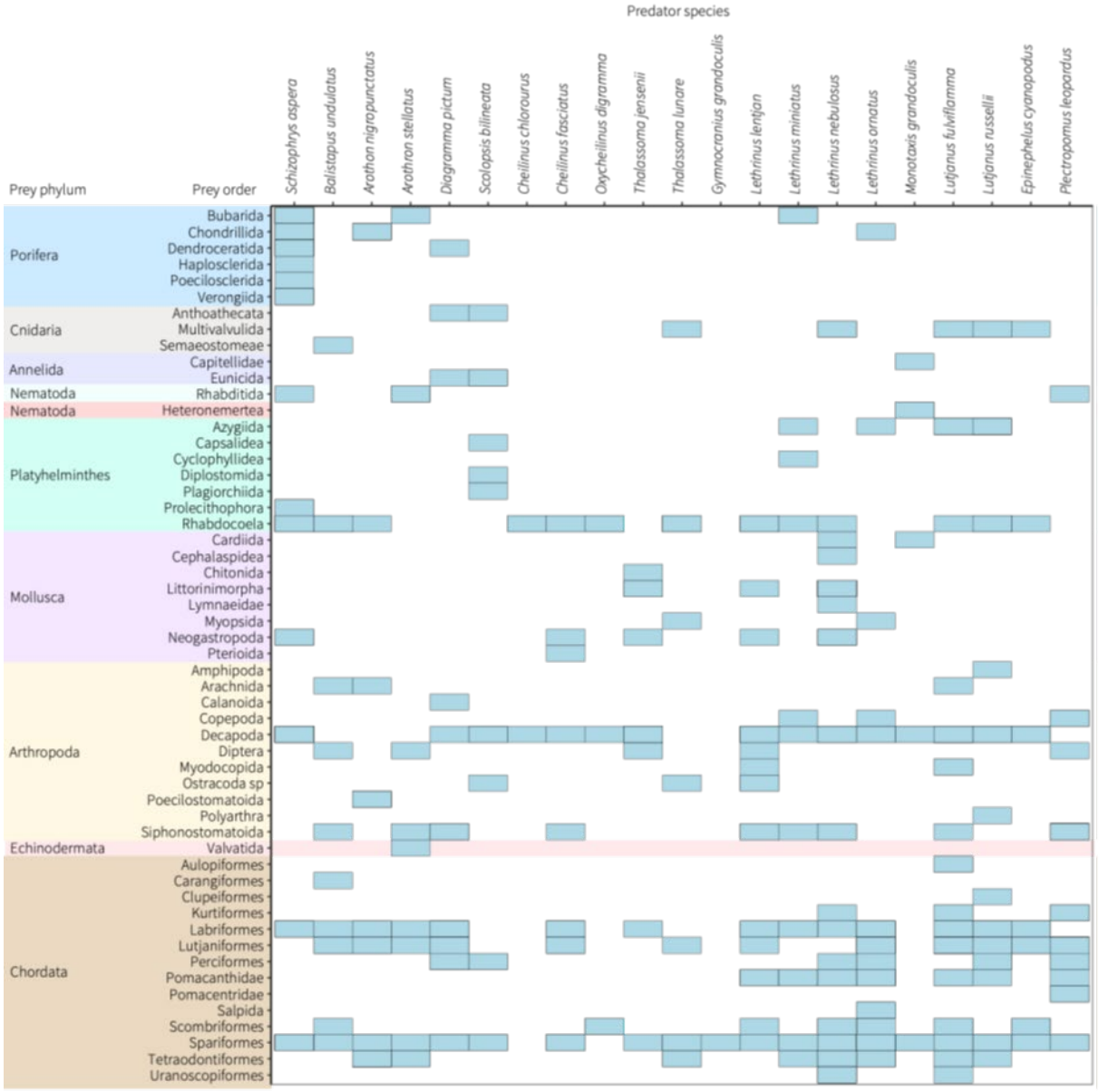

Figure 14. Preliminary results from DNA metabarcoding of the gut contents of *Schizophrys aspera* and faeces of 20 species of reef fish. Coloured cells denote positive detection of prey item at the level of order. Red line at Echinodermata highlights lack of detection of asteroids, including COTS.

4. DISCUSSION AND OUTPUTS

4.1 Novel rubble-dwelling COTS predators and juvenile mortality

The first step to resolving the extent to which predator-prey interactions moderate COTS population dynamics is to identify key predators. Prior to this study, there were ~90 species of predator identified to consume COTS at various life stages, most being reef fishes and just 24 coral reef invertebrates (Cowan et al. 2017; Kroon et al. 2020; Balu et al. 2021). This study more than doubles this knowledge base for invertebrates, with 31 of 110 (28%) novel species of cryptic fauna demonstrating varied capacity to consume juvenile COTS in aquaria (Desbiens et al. 2023).

The most consistent and formidable predator of COTS was the red decorator crab, *Schizophrys aspera*, which consumed juveniles in nearly all feeding trials, including in mesocosms that mimicked the natural rubble environment. This demonstrates the capacity of *S. aspera* to detect and consume COTS from natural rubble complexities and over a range of alternate food options. In this natural rubble setting, the rate of predation by *S. aspera* was 5.8 COTS day^{-1} for 2-month-old juveniles (~2 mm). This is the highest rate of predation on benthic-stage COTS reported, compared to that of the triton snail (~0.2 adults day^{-1}; Endean 1969), reef fishes (~0.02 juveniles day^{-1}; Sweatman 1995) and for mixed rubble communities (~5 juveniles day^{-1}; Keesing and Halford 1992a), but is lower than for planktotrophic predators of larvae (14–158 larvae hour^{-1}; Cowan et al. 2016b). The rate of predation by *S. aspera* decreased to 0.8 COTS day^{-1} for 10-month-old juveniles (~7 mm), which coincided with an increased occurrence of partial predation of 0.5 COTS day^{-1}. Overall, *S. aspera* reduced its tendency to consume whole COTS with juvenile size, transitioning from total consumption to partial predation by ~10 mm juvenile diameter.

Ten species of swimming crabs (Portunidae) consumed juvenile COTS in feeding trials. Their predator style was distinct in their tendency to cause injury over total consumption. The likelihood of total consumption of COTS by portunids neared zero at ~5 mm juvenile size, though partial predation occurred up to the largest juvenile sizes tested (~14 mm). In rubble mesocosms, *Thalamita admete* was able to find and consume (or partially consume) 2-month-old juveniles, but at a lower rate (0.4 COTS day^{-1}) than *S. aspera*. This suggests that juvenile COTS are more likely to be an opportunistic food option for portunids and/or are somewhat unpalatable. Characterising the extent to which COTS toxicology (e.g. Hillberg et al. 2023) develops across juvenile ontogeny may help to explain the consumption size thresholds exhibited by cryptic predators.

Partial predation of juvenile COTS was commonly observed in feeding trials, as previously reported for the harlequin shrimp (Glynn 1982) and peppermint shrimp (Balu et al. 2021). A large proportion of juvenile and adult COTS are found with injury in nature (Glynn 1984; McCallum et al. 1989; Budden et al. 2019; Wilmes et al. 2019; Caballes et al. 2022). The ability of COTS to recover from injuries of varying severity requires attention as partial predation may result in survival and possibly regeneration of multiple individuals (Lawrence and Vasquez 1996). However, evidence for COTS and other echinoderms more broadly, suggests that injury can result in growth stasis, delayed developmental transitions, and reduced reproductive output (Budden et al. 2019; Deaker et al. 2021b). Therefore, while

partial consumers may not immediately contribute to COTS mortality, they are likely to delay growth and development with knock-on impacts to COTS populations.

The remaining predators of juvenile COTS were identified as incidental, injuring or consuming juveniles in just 1–2 trials. For many of these, including portunids (*Gonioinfradens paucidentatus*, *Thalamita coeruleipes* and *Zygita murinae*), xanthids (*Actoeodes hirsutissimus*, *Atergatis floridus*, *Luniella spinipes* and *Neolimera insularis*), and hermit crabs (*Dardanus lagopodes*, *D. medistos* and *D. pedunculatus*), this classification is likely an artefact of low sample sizes owing to their rarity in rubble collections at Heron Island. Greater replication may have resulted in these predators being classed more strongly as COTS predators, as found for the remaining portunids and two xanthids (*Cyclodius ungulatus* and *Etisus anaglyptus*). Other incidental predators included reef shrimp (*Athanas parvus* and *Saron marmoratus*), which showed lower interest in juveniles than other reef shrimp (Glynn 1982; Balu et al. 2021). Similarly, the single instance of predation by the fireworm, *Eurythoe complanata*, contrasts the voracity of another amphinomid worm, *Pherecardia striata*, as a COTS predator (Glynn 1984), while other epialtid (e.g. *Tiarinia cornigera* and *Menaethius monoceros*) and majid (e.g. *Cyclax suborbicularis*) crabs did not show the same interest in consuming juvenile COTS as *S. aspera*.

There were clear differences among and within taxa in the preferences and likelihood of rubble-dwelling taxa to consume juvenile COTS, though we anticipate that rich and diverse rubble communities would have a cumulative and appreciable impact on COTS population success and outbreak potential (Keesing and Halford 1992b; Morello et al. 2014; Wilmes et al. 2018; Desbiens et al. 2023), especially given the prolonged period (~150 days) of early juvenile development in rubble (Wilmes et al. 2016; Deaker et al. 2020a). It may also be true that some species, including the majority (72%) that did not consume COTS in feeding trials, may show greater capacity to do so in nature, which warrants further exploration.

4.2 Distribution of key cryptic predators

At a local scale (Heron Island), *Schizophrys aspera* was predominantly cryptic and required active searches through rubble to detect. This differs to *S. aspera* in the Red Sea that were conspicuous on artificial structures and pylons (Ibrahim 2012, 2014; El-Serehy et al. 2015); though there is taxonomic uncertainty in the *Schizophrys* genus and use of *S. aspera* in some locations needs to be revised (Lee et al. 2018). We demonstrated a 5% (1 in 20) success rate in finding *S. aspera* by overturning large rubble pieces (≥10 cm) in rubble (Wolfe et al. 2023b). The mean density of *S. aspera* was 0.77 ind. 100 m^{-2}, which varied among sites with the highest density at Eco 1 (2.1 ind. 100 m^{-2}) and at Halfway (maximum: 5 ind. 100 m^{-2}). Water depth (2–12 m) did not influence the density of *S. aspera* but there was an increased likelihood of detection by overturning large pieces overlying thick rubble beds.

Schizophrys aspera showed positive associations with the benthic cover of rubble, hard substrate and soft coral, where it camouflages with epibionts (Goodhill et al. 2024). Only three individuals–the smallest (5–11 mm) found–were under rubble pieces atop sand. This indicates that *S. aspera* can settle to sandy environments before ontogenetic migration to the rubble infrastructure, but whether this is a settlement preference before moving towards rubble is unknown. It is probable that *S. aspera* settle also to rubble, as small individuals (≥ 11 mm) were found under rubble atop rubble. It is of interest to explore settlement

preferences and post-settlement survival of *S. aspera* and whether they align with what is known about COTS settlement and early juvenile development (Wilmes et al. 2020b).

The lowest numbers of *S. aspera* were found at Harry's Bommie and Fifth Point, which had low rubble cover and high proportions of live coral and macroalgae, respectively. Whether *S. aspera* were more abundant in these habitats was not resolved here but is expected to be unlikely given its affinity with rubble. It remains unclear how many individuals exist within the reef and rubble infrastructure without extensive destruction of habitat. Standard methods to survey biodiversity in rubble do not seem sufficient to quantify *S. aspera*, as they have not been reported in similar surveys at Heron Island (Wolfe et al. 2023a; Wolfe et al. 2023c) or elsewhere (Takada et al. 2008; Stella et al. 2011; Britayev et al. 2017; Wolfe and Mumby 2020; Stella et al. 2022). This makes our active search and inspection method of large rubble pieces the most effective at present, which could be developed into a monitoring tool to characterise further the distribution and ecology of *S. aspera*. Alternative or complementary survey methods could be explored to estimate *S. aspera* populations from larval supply and recruitment (Doherty 1987; Jones et al. 1999), molecular and environmental DNA approaches, as done to detect and predict COTS (Doyle et al. 2017; Wang et al. 2023), or through attraction to baits (Zimmer-Faust and Case 1982; Anraku et al. 2001; Pezzuti et al. 2002; Spiridonov and Neumann 2008) or pheromones (Ryan 1966; Bamber and Naylor 1997; Ziegler and Forward 2007). Our study provides the first estimate of *S. aspera* populations on the GBR to compare efficacy of other methods in future.

Interestingly, sites with the lowest densities of *S. aspera* (Harry's Bommie, Fourth Point, Fifth Point) had the highest numbers of COTS as reported by culling operators. At this local reef scale, the density of *S. aspera* at Heron Island was inversely correlated to that of COTS, but whether this is causal requires further investigation. There is mounting evidence to suggest that reef zones protected from fishing may experience fewer COTS outbreaks (Dulvy et al. 2004; Sweatman 2008; Vanhatalo et al. 2017; Kroon et al. 2021), but this has not yet been explored in the context of cryptic COTS predators (Desbiens et al. 2023). As a protected no-take reef with high numbers of cryptic predators and no reported COTS outbreak history, these results at Heron Island provide fundamental insight into how this predator-prey interaction may scale to moderate COTS populations naturally. Whether the density of key rubble-dwelling predators could be an informative inverse proxy of juvenile presence in rubble is of interest to determine, perhaps specifically in relation to *S. aspera*.

We extrapolated that *S. aspera* could be responsible for consumption of >22 million COTS during its early post-settlement life stage at Heron Island. This would, of course, be limited by a suite of factors within the rubble, including foraging success, prey availability and detectability, predator and prey size, inter- and intra-specific competition, habitat availability, and so on. Given it has been estimated that 5 COTS recruits m^{-2} are required to seed an outbreak (Keesing et al. 2018), the low density of *S. aspera* at Fifth Point (0.2 ind. 100 m^{-1}) may be insufficient to reduce COTS numbers with an estimated consumption rate of 1.8 ind. m^{-2} over the early juvenile life stage (see **Table 3**). In contrast, the order of magnitude higher density of *S. aspera* at Eco 1 (2.1 ind. 100 m^{-1}) resulted in an estimated consumption rate of 18.1 ind. m^{-2}, which is highly relevant to COTS outbreak suppression and prevention, especially given the homing behaviour of COTS (Ling et al. 2020). Direct field observations of predation and dietary analyses are needed to characterise unequivocally the impact of predator-prey interactions on outbreak potential (Pratchett et al. 2021). This includes

understanding alternate prey of *S. aspera* (e.g. other sea stars, echinoderms, and marine invertebrates) as juvenile COTS are not expected to be its primary food source, along with better estimates of COTS larval influx and settlement rates to help clarify the predator population size necessary to adequately control a given cohort.

Local patterns in the density and distribution of *S. aspera* seemed to be upheld at the few reefs explored at the regional scale. The highest density of *S. aspera* was found at Heron Island in the southern GBR where outbreaks have not been reported, while the fewest were found in outbreak-prone reefs in the northern (Lizard Island) and central (Moore Reef) GBR (Miller 2002; Pratchett 2005; Uthicke et al. 2019; Chandler et al. 2023). As this pattern held true in surveys conducted at local and regional scales, it seems paramount to address this inverse correlation more intensively to resolve whether the presence of cryptic predators directly translates to COTS outbreak susceptibility (Wolfe et al. 2025). Conducting surveys on reefs open to fishing (blue zones) and those in the southern GBR that have history of COTS outbreaks (e.g. the Swains, Fairfax Reef) would provide data necessary to compare with this study in protected no-take reefs (green zones). Broader surveys across multiple reefs within each region are required to resolve whether variation in cryptic predator density can act as an early indicator of COTS outbreak likelihood on the GBR.

At both local and regional scales, *S. aspera* and portunids were positively associated with a series of rubble metrics, including rubble bed thickness and piece size. *S. aspera* was more prevalent underneath rubble overlying thick rubble profiles, which confirms this as a key habitat metric to consider in future monitoring and surveillance. Thick rubble profiles were not as prevalent in the northern and central GBR reefs visited, which may limit habitat availability of *S. aspera* and its capacity as a formidable COTS predator. Data on rubble typology across the GBR are required to better assess these regional scale outcomes. How rubble typology influences juvenile COTS density, growth and survival also seems important to ask, especially in the context of reef degradation (Wolfe and Byrne 2024), as certain habitat metrics should lead to increased predation risk.

Portunid crabs were positively associated with rubble when sand cover was high, suggesting they may occupy a different environmental niche to *S. aspera* and would therefore contribute additively to predation of juvenile COTS if/when encountered. This supports the prediction that a more rich and diverse rubble community would have the greatest cumulative impact on COTS populations (Keesing and Halford 1992b; Morello et al. 2014; Wilmes et al. 2018; Desbiens et al. 2023). Ensuring that rubble-dwelling communities are protected so that a diversity of cryptic predators can contribute to consumption and mortality of COTS seems crucial. Management that protects coral reefs more broadly (e.g. zoning) should be effective in protecting rubble-dwellers, though how predator diversity and density are impacted by anthropogenic and environmental stressors is largely unknown, as is whether extant predator distributions reflect disturbance history. For example, the lower density of predators in the north and central GBR could be related to recent heatwave impacts, which southern regions had largely escaped (Byrne et al. 2024b) until early-2024. Characterisation of cryptic communities at Heron Island now, post-heatwave, may provide crucial information on the response of cryptic COTS predators to thermal events and help to explain regional scale differences in their extant distributions. A more detailed understanding of the biology and ecology of these newfound COTS predators, along with predators unidentified here, is required to adequately monitor, predict and manage them in the context of outbreaks.

4.3 Molecular DNA/eDNA detection and multi-trophic food webs

eDNA methods to detect COTS in the gut contents of key cryptic predators were developed and confirmed in a series of pilot studies. It was determined that if cryptic COTS predators had consumed COTS within ~12 h, there was a high chance (~90%) of detecting this across the three tissue types of the digestive tract (stomach, midgut, abdomen). The stomach portion was effective at detecting COTS that had been consumed within ~1 h, and the midgut and abdomen samples were effective at detecting consumption within 24 h (Appendix A).

Using these methods, 17% (2 of 12) of *S. aspera* collected from Heron Island had detectable concentrations of COTS DNA in their digestive systems. These two individuals were collected from Eco 1, where *S. aspera* were most abundant (Wolfe et al. 2023b) and where COTS numbers were lowest (unpublished data, COTS Control Program). That COTS DNA was detected in *S. aspera* at this site confirms this trophic interaction and adds confidence to the capacity of this majid to consume juveniles and moderate COTS populations in nature.

At Lizard Island, 12% (8 of 65) of wild-type predators showed positive eDNA results. No *S. aspera* collected from rubble around Lizard Island had detectable traces of COTS DNA, however positive detection occurred in one shrimp and seven crabs. Of these, the shrimp (*Saron marmoratus*) along with *Tiarinia cornigera* (Epialtidae), *Soliella flava* (Xanthidae), and *Thalamita coeruleipes* (Portundiae) were classed as incidental predators in our laboratory feeding experiments, suggesting the contribution of these species to COTS mortality may have been underestimated. The remaining wild-type predators, *Thalamita bouvieri* and *Thalamita admete* (Portunidae), along with *Cyclodius ungulatus* (Xanthidae), were classed as partial predators in feeding trials, but whether they partially or wholly consumed COTS in the wild remains unknown.

That any cryptic predators had detectable traces of COTS DNA was an impressive and somewhat unexpected finding given the short (12–24 h) window of detection post-ingestion (Appendix A). Timing predator collections to align with the early settlement period of COTS (March; Wilmes et al. 2020a) proved effective in capturing this ecological interaction. It was even more surprising that eight species of cryptic predator showed positive results for COTS DNA. Based on experimental feeding trials, we had dubbed *S. aspera* the top predator of juvenile COTS, but evidently a range of species operate at this lower trophic level to interact with COTS in nature with a cumulative impact on COTS populations.

It could not be determined whether wild-type predators consumed entire juveniles, caused injury through partial predation, or interacted with juvenile, larval or adult COTS in another manner. But that this trophic interaction was confirmed using eDNA provides strong evidence that *S. aspera* and other cryptic decapods are likely to be natural predators of COTS on the GBR. No new species of COTS predator were detected using eDNA, because our collections targeted species we had previously identified as predators. Positive results across eight species of wild-type predator add confidence in the efficacy of this eDNA method, which could be applied as a detection tool for further novel predator identification and as an inverse proxy for juvenile presence in rubble. Whether the majority (~72%) of predator candidates that did not display capacity to consume juveniles in the feeding trials do so in nature would be interesting to determine, as we anticipate that many rubble-dwelling predators of COTS are yet to be described. It seems plausible to operationalise this eDNA monitoring method to

continue to expand our knowledge of novel COTS predators and trophic interactions that may contribute to natural mechanisms of outbreak suppression.

DNA sequences of cryptic COTS predators were generated for use in multi-trophic metabarcoding analyses. That 66% of cryptic predator specimens returned non-specific results highlights taxonomic uncertainty in lower trophic-level invertebrates and the value of completing this fundamental step to generate our own predator-specific sequences. Without these unique sequences, our newfound predator species would likely have been inaccurately identified in metabarcoding outputs. For example, all specimens of *S. aspera* (Majidae) returned as Decapoda or Xanthidae, which would have misplaced this top cryptic COTS predator at the order or family level, respectively, in community analyses. Moreover, *S. aspera* was the only species of this genus in the BLAST reference library, meaning the single *Schizophrys* sp. collected at Moore Reef yielded the same result and must be assessed closely with a revision of this genus (Lee et al. 2018). Similarly, no results specific to *Thalamitoides quadriens* were available in the library, while the identification of others (e.g. *Thalamita prymna*) must be clarified.

DNA metabarcoding data were partially analysed but the full suite of results was not ready in time to inform this report. For *S. aspera*, preliminary results from gut content metabarcoding suggest this majid consumes a range of prey species beyond COTS, including sponges, worms, molluscs, and arthropods. DNA of portunid crabs was evident in the gut contents of *S. aspera*, but what this predator-predator interaction means for juvenile COTS has not been determined. At the time of this report, no DNA from COTS or any echinoderm species had been detected in the gut contents of *S. aspera* using this metabarcoding approach, which targeted a broader (less COTS-specific) gene to identify a range of species in this trophic network. Sponges (Porifera) were the predominant component of the gut contents of *S. aspera*, as would be expected (Goodhill et al. 2024). Less expected was the presence of five species of reef fish, indicating a generalist, perhaps scavenger component to the diet of *S. aspera*. Potential contamination between samples must be resolved. These data are preliminary but provide an indication of expected outcomes.

Five species of reef fish known to consume COTS (*Cheilinus chlorourus*, *Oxycheilinus digramma*, *Lethrinus nebulosus*, *Lutjanus russellii*, and *Epinephelus cyanopodus*) returned positive data for *S. aspera* DNA in their faecal contents. This is the first documentation of species that may prey on *S. aspera* on the GBR, with the potential to generate interesting top-down effects on benthic predator-prey interactions in rubble. Additionally, portunid crabs, including two COTS predators (*Thalamita prymna* and *Thranita pelsarti*), were evident in the gut contents of eight species of reef fish. Conversely, COTS were not detected in the faecal contents of the 20 species of reef fishes examined, despite having been found in these samples previously with amplification using COTS-specific primers (Kroon et al. 2020), reflecting the broad scope of this metabarcoding approach. It is possible that these higher order predators influence the behaviour or distribution of cryptic predators with flow on effects to COTS juveniles in rubble. How these ecological interactions play out in nature is of considerable interest, including whether predation risk is elevated for *S. aspera* and other cryptic predators in protected reefs where fish biomass is expected to be greater.

Whether the density of *S. aspera* and other COTS predators vary in relation to higher-order predators in response to fishing pressure requires attention so that the possibility of trophic

cascades involving COTS, cryptic predators, and fishes can be addressed. For example, on the GBR, principal fishery targets (i.e. coral trout, *Plectropomus* sp.) demonstrate the greatest benefits from no-take marine reserves, while the density of non-target fishes and benthic assemblages can show no clear differences (Emslie et al. 2015). Here, the fish with the strongest reads of *S. aspera* DNA was the lutjanid, *Lutjanus russellii*. Whether the abundance or behaviours of predators of *S. aspera* are differentially influenced by top predators, such as coral trout, between fished and no-take zones is important to resolve, as this may allow *S. aspera* to persist in protected zones, despite more higher order predators being present. It could be that COTS outbreaks are more prominent on fished reefs (Vanhatalo et al. 2017; Westcott et al. 2020; Kroon et al. 2021) where coral trout are depleted, mid-level fishes are abundant, and thus cryptic predators and their role in the consumption of juvenile COTS are reduced. These nuances will be interrogated further once the full suite of metabarcoding data becomes available, though it is already evident that direct and indirect trophic links between cryptic and higher order COTS predators occur.

This work is an important step in trying to tease out the food webs that, if disturbed, may or may not promote COTS outbreaks. As highlighted by qualitative modelling (Babcock et al. 2016), there is a large amount of uncertainty around the potential role of cryptic invertebrates in COTS outbreaks largely due to their complex (largely unknown) trophic relationships with small predatory fishes and higher order fishes targeted (or not) by fisheries. Part of the problem with pinning these relationships down is that their trophic links are likely to be more specific than currently understood, as evidenced here for *S. aspera* compared to other cryptic COTS predators.

4.4 Integration of knowledge with Traditional Owners and stakeholders

Over five seminars, three practical lab sessions, and four field excursions, workshop participants (**Figure 15A**) contributed to sharing valuable information on their research, monitoring practices, and lived experience. In the lab, Sea Country rangers informed and practiced a series of methods and skills in quantifying rubble condition to help understand reef biodiversity, resilience, and recovery potential (**Figure 15B**), detecting cryptic predators of COTS in rubble as a novel monitoring objective, and identifying and counting reef fishes with a focus on species known to consume COTS. Following seminars and lab sessions, researchers and rangers conducted a series of monitoring and survey exercises on snorkel and SCUBA to apply methods workshopped in the lab (**Figure 15C**). The combination of learning and implementing scientific field methods over several days on the reef provided an invaluable opportunity to streamline data collection protocols to generate effective and tractable monitoring tools.

The workshop highlighted the importance of respectful communication and engagement with Traditional Owner groups, and the need for the development of lasting researcher and institutional relationships to facilitate practical data collection, collaborative grant applications, and importantly, sovereignty of research, monitoring, and decision-making on Sea Country. During the workshop, rangers commented on the possible inclusion of Heron Island, and nearby One Tree Island, in the annual coral reef monitoring exercises in the PCCC region, and incorporation of rubble and COTS predators in existing and new projects on the reef.

Participants from Gidarjil found great value in augmenting protocols and projects to include rubble and COTS predators, and endeavour to partner in future projects that support ranger monitoring and collaborative research.

Figure 15. Images from the workshop held at HIRS, including (A) workshop participants (from left; Uncle Joe, Demond Purcell, Jasmine Pippen, Rehmond Baira, Kenny Wolfe, Aunty Lois, Tania Kenyon, Tina Skinner, Kelvin Rowe, Aunty Lola, Finn Bryant, Uncle Mick, Amelia Desbiens, and Chris Roelfsema), (B) Dr Tania Kenyon (RRAP) explains and practices methods to survey rubble condition and habitat with Sea Country rangers, and (C) Sea Country ranger, Kelvin Rowe, conducting detailed rubble surveys on SCUBA with Dr Kenyon.

4.5 Outputs

Overall, this project provides specific outputs on:

- New knowledge on the identification of cryptic predators of juvenile COTS, including 31 new species of rubble-dwelling taxa, one of which (*Schizophrys aspera*) seemed quite formidable.
- New knowledge on the habitat associations and distribution of key cryptic predators, including inverse correlations with COTS populations at local reef and possibly regional scales, which demonstrates predator-prey outcomes may be appreciable in the wild.
- Methods developed to survey cryptic predators in the field, including standardised transects, manual search methods in rubble, and key habitat metrics that could be adopted in monitoring and surveillance to better predict newfound COTS predators.
- eDNA methods developed to validate cryptic predation in nature, including detection of this trophic interaction in eight species in the wild and viability of key predators as useful bioindicators and precursors of COTS population success.
- Extensive data generated to inform models on COTS populations at high resolution for juveniles, including early mortality rates relative to predator type, and predator-prey sizes, along with predator habitat associations and distributions that may reflect outbreak history.
- Enhanced capacity-building though collaboration and engagement with Traditional Owners and Sea Country rangers, including a workshop held to align research methods and outputs with ranger monitoring practice.

5. RESEARCH SYNERGIES AND NEXT STEPS

Synergies were formed between this and several other CCIP projects to enhance impact pathways across the program. The primary research synergy existed between this project and CCIP-D-03 (Uthicke et al. 2025) on operationalising eDNA monitoring to expand the toolbox for COTS detection through consideration of novel cryptic predators. Lead researchers collaborated across the lifetime of both projects, including a combined field trip to Lizard Island to collect predators for eDNA analyses to detect species with the potential to be viable bioindicators of COTS mortality risk. This research synergy enabled the development of protocols to use cryptic predators in eDNA monitoring, enhancing the toolbox for COTS detection. Through this collaboration, we found ~15% of wild-type predators collected at Heron Island and Lizard Island had detectable traces of COTS in their digestive system. That eight species were found with COTS DNA bolsters outcomes of CCIP-P-05 and CCIP-D-03, and the viability of both detecting COTS predators using this eDNA method and considering cryptic predators as bioindicators of juvenile COTS before they are visibly detectable coral-eaters on the reef that require manual control (i.e. culling).

The primary objectives of this project were to identify new predators of COTS and generate data on their predation rates, size thresholds, and food web interactions to inform existing COTS population models. Over this project, regular meetings and discussions with CCIP modelling teams (CCIP-R-03 Rogers et al. 2025, CCIP-R-04 Skinner et al. 2025, CCIP-R-05 Choukroun et al. 2025) occurred, so that data generated could be useful and applied to population and ecosystem models. With these data, it will now be possible to interrogate whether drivers of early COTS mortality in rubble, as caused by cryptic predators, can be manipulated to reduce COTS numbers before they reach outbreak densities (i.e. early detection). Field data on the density of *S. aspera* indicates that this may be the case at both local (Heron Island) and regional (GBR) scales, and our SEM highlights clear drivers of this outcome from microhabitat to GBR scales. These data can now be applied to spatial models that re-examine the predator removal hypothesis in relation to COTS outbreak likelihood and intensity. It seems important to collect additional data on cryptic predators and rubble condition on reefs open to fishing (blue zones) to address mechanistically whether fisheries directly or indirectly influence COTS outbreaks, and whether extant predator distributions reflect disturbance history (e.g. marine heatwaves) on the GBR.

This project collaborated with Traditional Owners, namely with Gidarjil from the PCCC region, to understand and support values and ensure respectful use of Sea Country. Personal relationships were formed with Gidarjil, so project synergies with internal CCIP projects on Traditional Owner engagement (e.g. CCIP-R-08 Paxton et al. 2025 and CCIP-R-09 Backhaus et al. 2025) were not realised. Discussions with these internal groups were later initiated at CCIP workshops (e.g. Cairns, Nov 2023), but future work is needed to ensure that the activities of both projects generate shared outputs and outcomes. Specifically, experiences and outputs generated from the workshop held at HIRS may be useful to inform outputs on Traditional Owner engagement, and cultural and social perspectives and values.

Synergies were recognised, but not realised, with projects on juvenile biology and ecology (CCIP-P-03 Byrne et al. 2025) and semiochemistry (CCIP-R-11 Motti et al. 2025). It was deemed important for future research to characterise juvenile toxicity across size-age classes to address toxicity thresholds that may help to explain observations of reduced total

consumption of COTS by *S. aspera* and other cryptic predators at ≤ 10 mm COTS size. We recognise the importance of future research and development into semiochemical experiments that address (a) potential aquaculture and rearing of *S. aspera*, and (b) responses of COTS juveniles to predator cues (and predators to COTS) to better understand the mechanisms driving early COTS mortality, including feeding preferences and escape behaviours that may shape predator-prey interactions. For example, chemical cues from adult conspecifics may deter juvenile COTS from transitioning to a coral diet, which suggests that removal of adults through manual control may alleviate adult interference competition and allow cohorts of juveniles residing in rubble to transition into coral-eaters, thereby promoting outbreak generation (Webb et al. 2024). This may help to explain why some reefs require multiple voyages by culling operators to clear COTS, especially of smaller size classes (Westcott et al. 2020). The efficacy of continued manual control has long overlooked the resilient population biology of COTS (Deaker and Byrne 2022), which is typical of echinoderms (Nauen 1978; Ebert 1996; Byrne et al. 2023). Whether cryptic predator density is an early precursor of COTS outbreaks must be considered.

Synergies were formed with external research groups from RRAP through close institutional relationships. RRAP's rubble stabilisation project lead, Dr Tania Kenyon, contributed to the Traditional Owner workshop at HIRS, helping to address the importance of considering rubble in benthic habitat surveys to better understand reef condition and recoverability. This included lab and field sessions on how to design and conduct rubble surveys. Additionally, outcomes from this RRAP project informed site selection in our project, through shared knowledge on sites with good rubble types to survey cryptic predators, as done through an internal synergy with in-water predation projects (CCIP-P-01 Pratchett et al. 2025a, CCIP-P-04 Pratchett et al. 2025b, CCIP-P-06 Doll et al. 2025). Applying this knowledge, this research generated some of the first data available on the density and distribution of key cryptic predators on the GBR to provide a more holistic understanding of predation impacts across COTS life stages. This project also demonstrated the effectiveness of a non-destructive method to survey cryptic decapods and other species more generally that could be widely applied. Monitoring programs may benefit from focusing surveys in areas of reef with large rubble pieces and thick rubble interstices to have the greatest likelihood of detecting *S. aspera* and other rubble-dwellers. These predator-rubble metrics are combined biological and geomorphological features that could be developed as a criterion to inform reef management and rubble stabilisation.

Rubble is a variable habitat with a broad typology that reflects reef condition, disturbance history, and recoverability (Wolfe et al. 2021; Kenyon et al. 2023). This project highlighted specific rubble conditions (i.e. thick rubble beds) that may be used to predict key COTS predators and outbreak likelihood. The thickest rubble beds were found at Heron Island, which has distinct physical and oceanographic regimes compared to Lizard Island and Moore Reef. Juvenile COTS may be more likely to survive in thinner rubble beds where they are exposed to fewer predators. However, the relationship between juvenile COTS and varied rubble typologies is yet to be characterised.

Ultimately, this research indicated that COTS populations may be, at least in part, controlled by predator community type and/or the environmental and disturbance history that affects reef and rubble characteristics, and thus predator density. Data generated here on juvenile predator-prey interactions and their rubble habitat preferences may now be applied to

enhance characterisation of early mortality of COTS in relevant ecological models that include rubble (e.g. ReefMod, in RRAP) to improve predictions of outbreaks and more efficient and effective COTS control. Through this, we have identified top priority areas for further research and development:

- Prior to this study, predator-driven mortality of early post-metamorphic COTS has been generalised at the level of 'cryptic invertebrate fauna'. Through combination of aquarium experiments, field surveys, and eDNA metabarcoding, we now have a clearer picture of the species responsible for juvenile COTS mortality in rubble. With positive results from wild-type predators, it seems viable to operationalise the eDNA method used here to expand the toolbox for COTS predator identification, as we suspect many predators are yet to be described. Additionally, the potential for *S. aspera* to suppress or even prevent COTS outbreaks through predation of juveniles is promising and requires further investigation. Impacts facing *S. aspera* and other cryptic predators are unknown, but if their densities are depleted in certain regions, *S. aspera* may be considered a candidate for biocontrol. We strongly advise that much research is required before considering seeding populations as a management tool, which can cause negative impacts without rigorous interrogation. Overall, determining whether reefs high in cryptic predator (or specifically, *S. aspera*) density can act as a natural safeguard or early indicator of outbreak potential is important to resolve, as this may help to refine manual control effort through selection of priority reefs.

- There remains a large amount of uncertainty around the potential role of cryptic invertebrates in moderating COTS outbreaks due to the complex trophic relationships with higher order fishes. Using data generated in this project (e.g. juvenile mortality rates, predator-prey size, predator density and distribution, rubble habitat metrics, and wild-type predator detection), population and ecosystem-based models can now interrogate whether the magnitudes of variation observed in early juvenile mortality through cryptic predation has an appreciable impact on outbreak dynamics. As natural mechanisms of control, high density cryptic predator communities may be viable bioindicators before COTS are visibly detected. How preferences towards certain rubble conditions influence predator distributions and their interactions with COTS in nature is important to resolve. Ensuring that high post-settlement mortality (>5 ind. m^{-2}) occurs during this vulnerable benthic life stage should be effective in limiting juvenile densities and thus outbreaks, with the greatest path to impact in the initiation box or larval source reefs to suppress primary and secondary outbreaks, which spatial models may now choose to interrogate.

- It is crucial that future projects include Traditional Owners from the onset, and that projects and methods are designed collaboratively, not post hoc. It is a priority to co-design future projects with Traditional Owner groups with the capacity and desire to include cryptic predators and rubble in reef monitoring practices. Working with rangers in their respective Sea Country is necessary to understanding and supporting Traditional Owner interests and values, and to augment research outputs across the reef in an inclusive and respectful manner. Providing rangers the opportunity to expand their knowledge, skillsets, and stewardship of Sea Country through inclusive monitoring programs would generate more data at broader scales, increasing resources for the eDNA COTS detection toolbox and model outputs.

6. MANAGEMENT IMPLICATIONS AND IMPACT

Strategic management that abates early precursors of COTS population outbreaks may be one of the most feasible approaches to preventing or suppressing their impacts. However, long-term solutions first require a more holistic understanding of the intrinsic biology of COTS as resilient echinoderms (Deaker and Byrne 2022; Byrne et al. 2023; Webb et al. 2024; Wolfe and Byrne 2024) and the many extrinsic factors that contribute to COTS outbreaks across a hierarchy of scales (Pratchett and Cumming 2019). This project generated empirical data on overlooked predator-prey interactions operating at lower levels of the ecosystem, which may have considerable impact on COTS populations before they develop into destructive coral-eaters (Wolfe et al. 2025). Outcomes are intended to inform the COTS Strategic Management Framework, including COTS control and its long-term efficacy, to protect the GBR and its environmental, cultural and economic values.

That 31 species of cryptic taxa are newfound predators of juvenile COTS in their vulnerable rubble-dwelling life stage is an impressive outcome that enriches the scientific underpinning and ecological understanding of outbreaks. It is particularly interesting that one species, *Schizophrys aspera*, was a consistent and reliable predator of COTS with the ability to detect and consume juveniles in feeding trials and in nature. That juvenile predator density was inverse to adult COTS at reef and possibly regional scales, and that this trophic interaction was detected in a diversity of wild specimens using eDNA methods generated here, provides certainty to these outcomes. We are now closer to constructing a more realistic and accurate understanding of trophic interactions that may help to control COTS, revealing natural mechanisms of outbreak prevention or suppression through predation of the juvenile stage. It seems reasonable that management ensures these newfound predator species, specifically *S. aspera*, are a research priority and possibly even protected to bolster their role in COTS population regulation on the GBR. However, what stressors impact cryptic predators must be resolved to first assess whether their populations require protection.

This research supports the longstanding hypothesis that predation can moderate COTS outbreaks. Until now, understanding of COTS predators has come from larger species that consume adult COTS (Endean and Stablum 1975; Hall et al. 2017; Kroon et al. 2020). We demonstrated this mechanism of population control from a novel perspective, highlighting overlooked species and processes that occur at cryptic, lower levels of the ecosystem. Outcomes showcase cryptic predators of juvenile COTS (< 10 mm) before they are visually detectable on the reef. Impressively, *S. aspera* demonstrated the highest rate of predation of benthic-stage COTS reported (~6 ind. day^{-1}) despite gut content metabarcoding indicating a generalist diet. This degree of predator-induced mortality is anticipated to accumulate to have disproportionate effects on COTS populations especially where predator abundance is high, as shown here. This makes *S. aspera* an intriguing biocontrol candidate, but we advise that the biology and ecology of this decapod are better understood before considering seeding populations as a management tool. Data on the natural distribution and taxonomy of *Schizophrys* are needed, including their ecological interactions with other species and how a potential boost in their density in the context of COTS biocontrol may have knock-on impacts on other reef species and reef functioning.

The cryptic predator-prey interactions characterised here advance our understanding of trophic pathways involving COTS and are crucial to consider in future research, monitoring,

and management innovation. *Schizophrys aspera* and other cryptic decapods have the potential to be evaluated as tractable management tools and early bioindicators of COTS outbreaks. Data on the local and regional distribution and habitat preferences of cryptic decapods may now be used to refine how and where surveillance is conducted in on-water operations and data collection. For example, culling operators may choose to focus effort at sites, reefs and/or regions with lower density and diversity of cryptic taxa, as low predator abundance may be a precursor to outbreak likelihood. This may be of particular importance if removal of adult COTS does indeed promote juvenile cohorts to transition to coral-eaters, thereby inspiring outbreak generation (Webb et al. 2024). While this study provides the first data on the inverse correlation between cryptic predators in rubble and COTS, we recommend that research continues to advance this understanding to resolve whether this interaction is causal and consistent within each region explored here. We advise that future surveys of cryptic predators focus on reefs in the southern GBR that have a history of COTS outbreaks (e.g. the Swains, Fairfax Reef) to compare with detailed findings for Heron Island here, and in blue zones (fished reefs) at regional scales to contrast with the surveys conducted in green zones (unfished reefs) here.

While cryptic predator density was inversely correlated with local and possibly regional-scale patterns of COTS populations, it is yet to be evaluated whether cryptic predators vary on reefs open and closed to fishing, and how this may scale to be a proximal cause of outbreaks. On the GBR, reefs exposed to fishing pressure appear more prone to outbreaks (Vanhatalo et al. 2017; Westcott et al. 2020; Kroon et al. 2021), but whether this is directly associated with commercial harvest or indirectly through a trophic cascade remains unresolved (Ormond et al. 1990; Pratchett and Cumming 2019; Motti et al. 2022). Preliminary results from gut content metabarcoding here revealed that five species of reef fish known to consume COTS (*Cheilinus chlorourus*, *Oxycheilinus digramma*, *Lethrinus nebulosus*, *Lutjanus russellii*, and *Epinephelus cyanopodus*) returned positive data for *S. aspera*, and eight for portunid crabs. The degree of impact of commercial and recreational fisheries on these fishes warrants investigation, along with how these species interact with top fishery targets, such as coral trout, which are known to vary between fished and unfished reefs (Emslie et al. 2015). It could be that fished reefs are more prone to outbreaks because numbers of coral trout are low, not because coral trout consume high numbers of COTS, but through indirect changes in the abundance and/or behaviours of other predatory fishes less targeted by fishing that would increase predation risk of cryptic predators and diminish their role in early COTS mortality. Understanding and modelling multi-trophic pathways involving COTS predators accurately is key to assessing the mechanisms through which protection from fishing may reduce COTS outbreaks, as it is likely to be more nuanced than currently understood.

This project contributed to relationship- and capacity-building with Traditional Owners, including through a workshop held at HIRS with Elders and Sea Country rangers of Gidarjil. An overarching narrative of the meeting was that many beneficial opportunities are likely to be realised through early engagement with Traditional Owners. For example, Traditional Owner groups that employ rangers to monitor Sea Country may find interest in and benefit from adding COTS and their key predators to existing surveillance protocols. Forming collaborations that support this would empower sovereignty of monitoring and decision-making on Sea Country. Co-development of protocols that augment existing ranger practices with parameters deemed important and informative to COTS control, such as cryptic predator

distributions, is essential, and as demonstrated in the workshop, could be coupled with assessments of reef and rubble condition through partnership with external programs (e.g. RRAP, RIMReP). This would result in an inclusive and effective monitoring strategy to advance local and regional scale data that inform COTS and greater reef management while respecting and supporting the interest and values of Traditional Owners of the GBR.

In terms of surveillance, this project has provided some of the first available data that we know of on the density and distribution of key cryptic predators on the GBR through the use of an effective, non-destructive survey method in rubble. Monitoring programs interested in cryptic predators would benefit from targeting areas of reef with large rubble pieces and thick rubble interstices to have the greatest likelihood of detecting *S. aspera* and other cryptic predators. These predator-rubble metrics are combined biological and geomorphological features that could be upscaled as criteria to inform reef management and rubble stabilisation through external program inputs (i.e. RRAP, RIMReP). For example, rubble bed thickness emerged as an informative benthic habitat metric that could be added to monitoring and surveillance protocols. However, deciding where rubble stabilisation, which is typically focused on facilitating coral recovery, is required may now need to consider cryptic COTS predators, as areas already high in rubble biodiversity may be less desirable for stabilisation intervention. Generation of spatial data on important rubble metrics is key to advancing mapping and modelling projects that are sensitive to benthic habitat type and reef condition in the prediction of COTS outbreaks, especially in the context of reef degradation (Wolfe and Byrne 2024).

This research contributes to achieving the overarching outcomes and impacts identified in CCIP's Impact Plan (**Figure 1**) through an advanced empirical understanding of predator-prey interactions involving COTS. Outcomes of this project help to resolve trophic interactions during the vulnerable early juvenile life stage of COTS, which has great potential to be a proximal cause of outbreaks. The capacity to avail of natural predator-prey interactions in rubble before COTS reaches its destructive corallivorous adult stage is a novel and impactful consideration for future COTS management, especially as rubble may become more prevalent on reefs in the Anthropocene. Outcomes are intended to inform improved detection and monitoring of COTS, including of key rubble-dwelling predators as bioindicator species, of rubble metrics as predictors of predator communities, and of juveniles and their predators in rubble using eDNA methods developed here. We anticipate targeted surveillance and monitoring approaches that consider rubble habitat metrics and bioindicator species to predict susceptibility to and likelihood of COTS outbreaks should provide useful data that increase the efficiency and effectiveness of operational responses, and accuracy of outbreak predictions. This is likely to generate impacts that help to suppress and/or prevent COTS outbreaks through natural mechanisms of population control with knock-on benefits to the protection of coral cover, and safeguarding of cultural, economic and social values, on the GBR.

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8. DATA ACCESSIBILITY

Data are available in several publications (Desbiens et al. 2023; Wolfe et al. 2023b; Wolfe et al. 2025). Data components have also been archived in CCIP research outputs, PowerBI Dashboards and workflows.

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APPENDIX A – PILOT STUDY: eDNA AND CRYPTIC PREDATORS AS USEFUL BIOINDICATORS

A1.1 Determination of COTS DNA detectability in invertebrate predator tissue

In February 2022, three species of decapod, *Thalamitoides tridens* (n = 7), *Schizophrys aspera* (n = 19) and *Thalamita admete* (n = 7), known predators of juvenile COTS (Desbiens et al. 2023), were collected around Heron Island and acclimated to aquarium conditions. Individuals were starved for ~24 h before feeding trials commenced, in which they were fed 3–20 juvenile COTS. After 24 h, predators were fixed in 100% ethanol and tanks thoroughly searched. The number of juvenile COTS purportedly eaten was recorded. Field control animals (n = 3 per species) were also collected, which were fixed immediately with no exposure to other individuals or COTS.

The whole stomach and abdomen were dissected from each decapod. Stomach fullness was recorded as either 0 (empty), 1 (partial contents), or 2 (contents easily visible). Dissection tools were thoroughly cleaned between each dissection. Stomach and abdomen samples were placed in 1 mL extraction buffer (1/10 ATL buffer, Proteinase K 10 mg/mL) and lysed overnight at 56˚C. DNA was extracted on a Qiacube automated extraction instrument or manually from a 600 µL subsample using DNeasy blood and Tissue kit following manufacturers protocol except elution was in 3 X 50 µL TE buffer.

A digital droplet assay (Uthicke et al. 2018) was conducted on samples undiluted and 1/10 dilution. A no template control was included for each batch of dissections (n = 6 batches). This enabled contamination control to be assessed for the entire laboratory workflow. A positive control was also analysed for each batch of dissections. Limit of Detection was defined as the number of positive droplets greater than no template controls (NTC) and field controls. This enabled results to be defined as presence or absence testing from the ddPCR assay results.

COTS DNA was detected in 45% of stomach tissue and 85% of abdomen tissue samples (**Figure S1**), Higher copy numbers were detected in *S. aspera* with several samples having quantifiable levels COTS DNA. No positive droplets were detected in either the field controls or NTC.

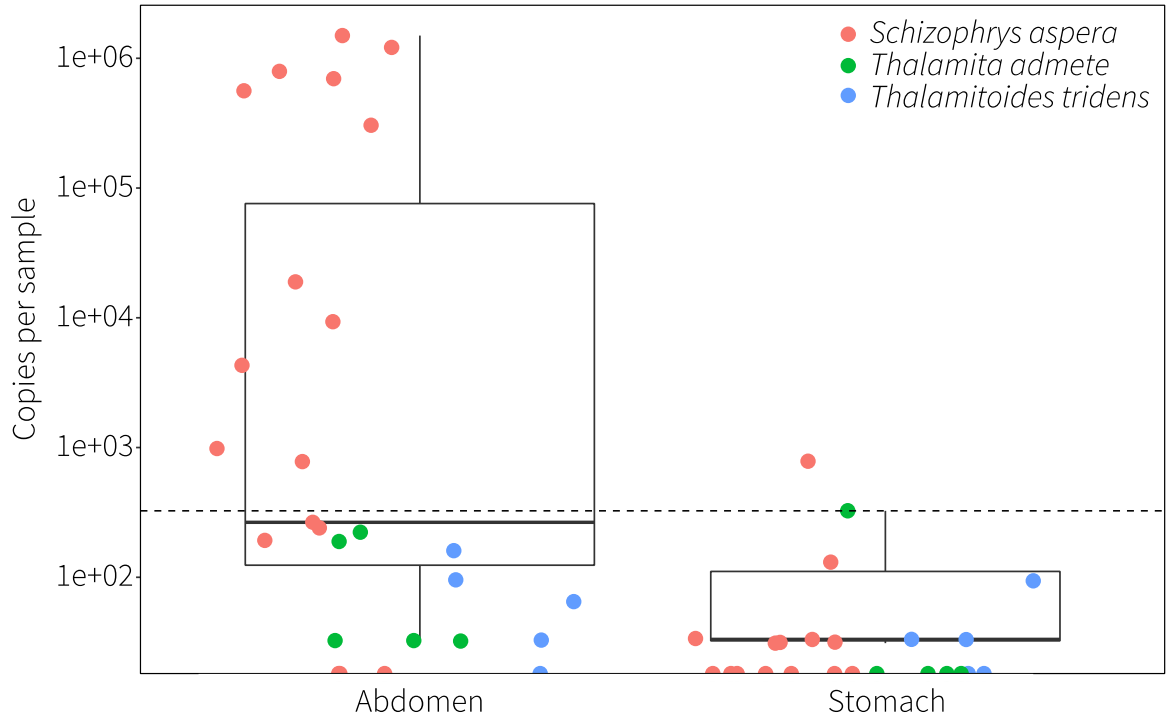

Figure S1. Copies per sample including for all Limit of Detection samples. Black dots represent individual tissue result average from two ddPCR replicates. Y-axis on log scale. Samples with zero COTS DNA detected are shown.

A1.2 Timed feeding aquarium experiments

To determine the length of time COTS DNA can be detected in the digestive system of cryptic COTS predators, a series of timed feeding experiments were conducted in October 2022 and March 2023. *Schizophrys aspera* were collected from reefs of Heron Island, acclimated to aquarium conditions (as above), and fed a single juvenile COTS. Individuals were monitored closely to detect the exact time of consumption and were then fixed in 100% ethanol at intervals of 1 h (n = 3), 3 h (n = 5), 6 h (n = 2), 9 h (n = 2) 12 h (n = 3) and 48 h (n = 3) post-ingestion. The whole stomach, midgut and abdomen were dissected from each predator. The midgut also included part of the surrounding tissue. The size, sex and eggs (if present in females) was also recorded.

Following dissection, samples were placed in 1 mL (1/10 ATL, Protenise K solution 10 mg/mL) or 3 mL (1/5 ATL, Proteinase K solution 20 mg/mL, midgut only) of extraction buffer and DNA extracted following methods described above. No false postives were detected in the no template controls. Thus, postive detection was defined as replicate > 0 positive droplets.

Pooling the three gut tissue types, 100% of *S. apera* tested positive for COTS DNA at 1, 3, 9 and 12 h (**Figure S2A**). The lowest proportion of positive results occurred at 48 h, the longest digestion time tested. For unknown reasons, one specimen fixed at 6 h did not give a signal in any tissue. Excluding this individual, we conclude that measuring all tissues gives a 100% probablility of whether *S. aspera* had consumed juvenile COTS in the last 12 h, with

~50% probability after 24 h, and ~33% after 48h (**Figure S2A**). Stomach samples produced good eDNA signals at 1 h, but with just 35% of the stomach samples positive over the entire time series (**Figure S3**). In contrast, most positive samples were detected in the midgut (50% of samples detected; **Figure S3**). Abdomen and hindgut samples combined were only positive after ~1 h but had a high number of positive samples (45%) overall (**Figure S2B, S3**).

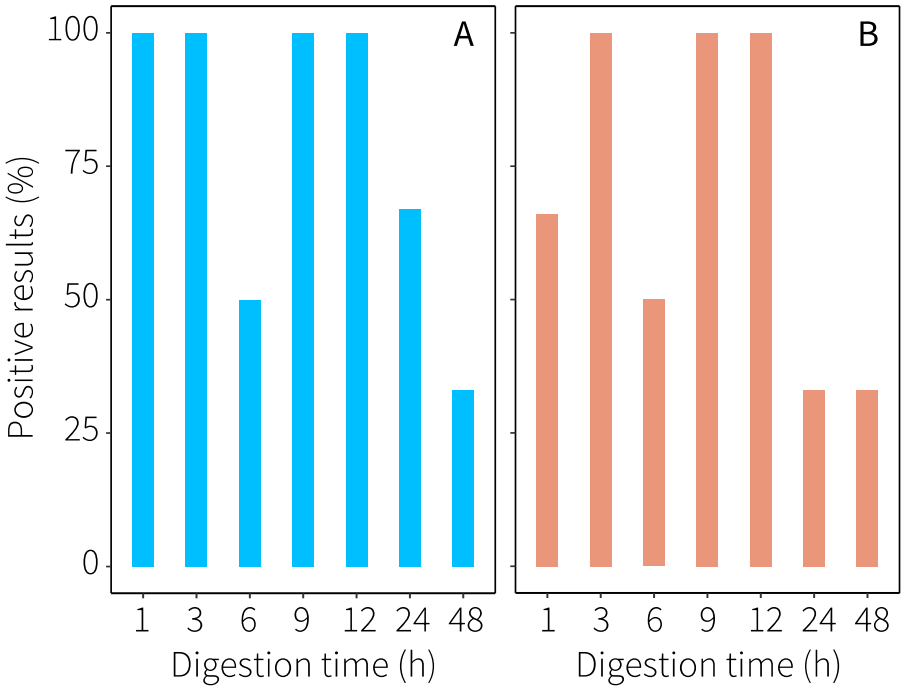

Figure S2. Proportion of positive COTS DNA detection in *S. aspera* digestive tracts from (A) all three tissue types pooled and (B) the midgut and abdomen combined.

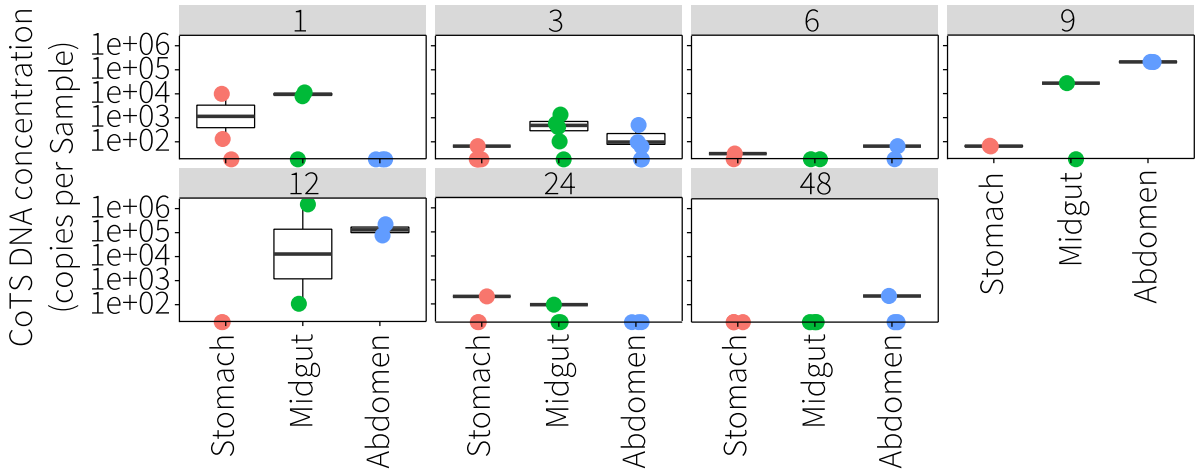

Figure S3. Results for timed feeding trials with *Schizophrys aspera*. Dots represent individual tissue samples, plots shown are hours post-ingestion, y-axis on log scale.

A1.3 Investigation of PCR inhibition and determination of COTS DNA detectability

Although the pilot study indicated that COTS are well detectable in the digestive tissues of cryptic COTS predators, the signal (copy number) was somewhat lower than what would have been expected if entire COTS were consumed. Hence, we investigated occurrence of PCR inhibition.

Seventeen abdomen and stomach samples from the timed feeding trials (above) were PCR clean-up treated (Zymol clean up kit) and spiked with 55 copies µL COTS target DNA (test PCR inhibition and level of COTS DNA detectability in tissue). The same 17 samples were also spiked but untreated (i.e. did not undergo PCR clean-up). All samples returned a postive result (**Figure S4**). One stomach sample showed relatively strong PCR inhibition in both the PCR clean-up and untreated sample while one abdomen sample showed inhibition in the untreated sample (**Figure S4**). In the remaining samples, there was no difference in copy numbers between clean-up and untreated samples. However, measured copy numbers were slightly below those expected (NTC spike result).

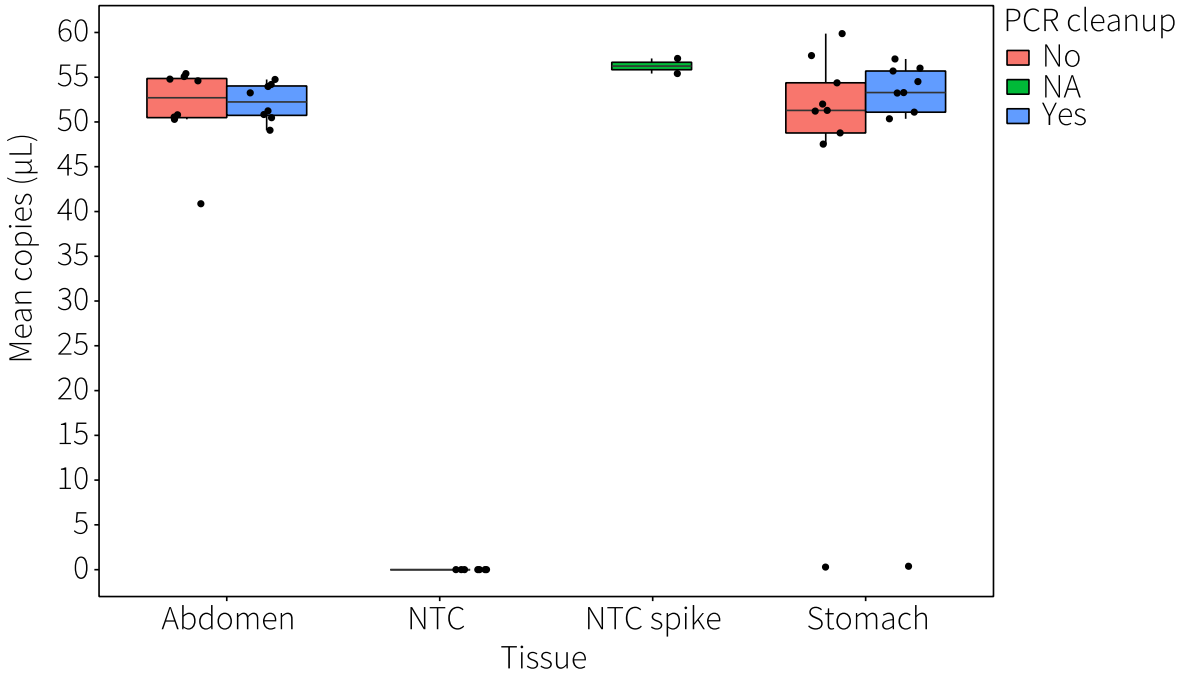

Figure S4. Abdomen and stomach samples tested for PCR inhibition with clean-up and untreated samples.

To test the remaining tissue, eight midgut samples were spiked with 410 copies µL COTS target DNA. All samples returned a postive result. However, similar to stomach and adbomen samples, measured copies were slightly below those expected (**Figure S5**). Overall, some inhibition exists but should not hamper analysis for presence/absence testing. Specifically, if cryptic COTS predators have consumed COTS within 12 h, there is a good chance (~90%) of detecting this across the three tissue types of the digestive tract.

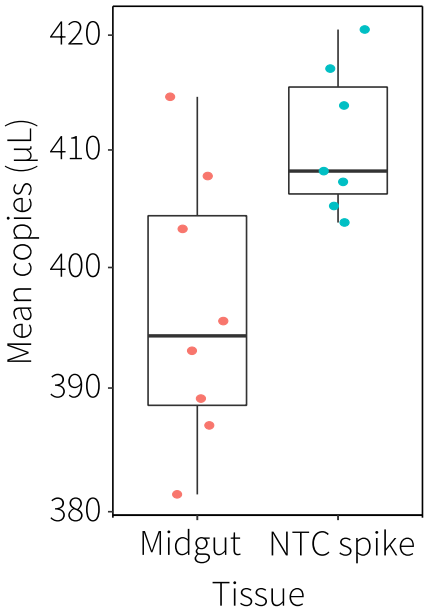

Figure S5. Eight midgut samples tested for PCR inhibition. Expected result = NTC spike.

Table S1. eDNA results from 13 wild caught animals collected around Heron Island in March 2023. Green cells denote positive detection of COTS DNA; sites outlined in **Figure 2C**.

Specimen ID	Tissue	Mean copies (µL)	Copies sample	Site
6	abdomen	0	0	Eco 1
6	stomach	0	0	Eco 1
8	abdomen	0.028	34.530	Eco 1
8	stomach	0	0	Eco 1
13	abdomen	0	0	Eco 1
13	stomach	0	0	Eco 1
17	abdomen	0	0	Eco 1
17	stomach	0	0	Eco 1
18	abdomen	0.027	33.995	Eco 1
18	stomach	0	0	Eco 1
20	abdomen	0	0	Eco 1
20	stomach	0	0	Eco 1
19	abdomen	0	0	Eco 1
19	stomach	0	0	Eco 1
14	abdomen	0	0	Fourth Point
14	stomach	0	0	Fourth Point
15	abdomen	0	0	Fourth Point
15	stomach	0	0	Fourth Point
2	abdomen	0	0	Halfway
2	stomach	0	0	Halfway
1	abdomen	0	0	Halfway
1	stomach	0	0	Halfway
5	abdomen	0	0	Halfway
5	stomach	0	0	Halfway

Table S2. Invertebrate species collected during collection trips at Heron Island (March 2023) and Lizard Island (March 2024) for eDNA analysis to detect presence of COTS DNA. Green cells denote positive detection of COTS DNA; sites outlined in **Figure 2**.

Site	Order	Family	Species	COTS DNA detection
Heron Island				
Eco 1	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Eco 1	*Decapoda*	*Majidae*	*Schizophrys aspera*	+
Eco 1	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Eco 1	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Eco 1	*Decapoda*	*Majidae*	*Schizophrys aspera*	+
Eco 1	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Eco 1	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Fourth Point	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Fourth Point	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Halfway	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Halfway	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Halfway	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Lizard Island				
North Point	*Polychaeta*	*Amphinomidae*	*Pherecardia striata*	-
Palfrey Inner	*Decapoda*	*Xanthidae*	*Actaeodes hirsutissimus*	-
Big Vicky's	*Decapoda*	*Xanthidae*	*Atergatis floridus*	-
North Point	*Decapoda*	*Xanthidae*	*Atergatis floridus*	-
Palfrey Inner	*Decapoda*	*Xanthidae*	*Atergatis floridus*	-
Palfrey Inner	*Decapoda*	*Xanthidae*	*Atergatis floridus*	-
Palfrey Outer	*Decapoda*	*Inachiae*	*Camposcia retusa*	-
Eyrie Reef	*Decapoda*	*Xanthidae*	*Chlorodiella nigra*	-
Big Vicky's	*Decapoda*	*Xanthidae*	*Cyclodius ungulatus*	-
North Point	*Decapoda*	*Xanthidae*	*Cyclodius ungulatus*	-
Big Vicky's	*Decapoda*	*Xanthidae*	*Cyclodius ungulatus*	+
Big Vicky's	*Decapoda*	*Xanthidae*	*Cyclodius ungulatus*	-
Palfrey Inner	*Decapoda*	*Xanthidae*	*Cyclodius ungulatus*	-
Palfrey Inner	*Decapoda*	*Xanthidae*	*Cyclodius ungulatus*	-
Palfrey Inner	*Decapoda*	*Xanthidae*	*Cyclodius ungulatus*	-
Big Vicky's	*Decapoda*	*Xanthidae*	*Etisus sp.*	-
Big Vicky's	*Decapoda*	*Xanthidae*	*Etisus sp.*	-
North Point	*Decapoda*	*Xanthidae*	*Etisus anaglyptys*	-
Big Vicky's	*Decapoda*	*Pilumnidae*	*Heteropilumnus*	-
North Point	*Decapoda*	*Xanthidae*	*Paractaea rufopunctata*	-
North Point	*Decapoda*	*Hippolytidae*	*Saron marmoratus*	+
Palfrey Inner	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
North Point	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
North Point	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
North Point	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
Palfrey Inner	*Decapoda*	*Majidae*	*Schizophrys aspera*	-
North Point	*Decapoda*	*Xanthidae*	*Soliella flava*	-
North Point	*Decapoda*	*Xanthidae*	*Soliella flava*	+

Site	Order	Family	Species	COTS DNA detection
North Direction	*Decapoda*	*Portunidae*	*Thalamita admete*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita admete*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita admete*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita admete*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita admete*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita admete*	+
Big Vicky's	*Decapoda*	*Portunidae*	*Thalamita bouvieri*	+
North Point	*Decapoda*	*Portunidae*	*Thalamita coeruleipes*	+
North Point	*Decapoda*	*Portunidae*	*Thalamita coeruleipes*	+
Big Vicky's	*Decapoda*	*Portunidae*	*Thalamita coeruleipes*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita coeruleipes*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita coeruleipes*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita coeruleipes*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita coeruleipes*	-
Big Vicky's	*Decapoda*	*Portunidae*	*Thalamita pelsarti*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita pelsarti*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita pelsarti*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita pelsarti*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita pelsarti*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamita pelsarti*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita prymna*	-
North Direction Exposed	*Decapoda*	*Portunidae*	*Thalamita prymna*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita prymna*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita sp.*	-
North Point	*Decapoda*	*Portunidae*	*Thalamita sp.*	-
North Direction Exposed	*Decapoda*	*Portunidae*	*Thalamitoides quadridens*	-
Palfrey Inner	*Decapoda*	*Portunidae*	*Thalamitoides tridens*	-
Eyrie Reef	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
North Point	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
North Point	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
North Point	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	+
Palfrey Inner	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
Palfrey Inner	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
Palfrey Inner	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
Palfrey Inner	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
Palfrey Inner	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-
Palfrey Inner	*Decapoda*	*Epialtidae*	*Tiarinia cornigera*	-

Table S3. Data used to determine Limit of Detection. All field controls and laboratory controls returned no presence of COTS DNA. Thus, positive detection was defined as a sample with one or more positive droplets in ddPCR assay (NTC = no template control).

Control type	Year	Sample	Tissue	Positive droplets
Heron Island	2023	*Schizophrys aspera*	abdomen	0
Heron Island	2023	*Schizophrys aspera*	stomach	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Laboratory	2023	NTC	NA	0
Lizard Island	2024	*Atergatis floridus*	chelae muscle	0
Lizard Island	2024	*Etisus anaglyptys*	chelae muscle	0
Lizard Island	2024	*Heteropilumnus sp.*	chelae muscle	0
Lizard Island	2024	*Paractaea rufopunctata*	chelae muscle	0
Lizard Island	2024	*Schizophrys aspera*	chelae muscle	0
Lizard Island	2024	*Thalamita admete*	chelae muscle	0
Lizard Island	2024	*Thalamita prymna*	chelae muscle	0
Lizard Island	2024	*Thalamita pelsarti*	chelae muscle	0
Lizard Island	2024	*Thalamita pelsarti*	chelae muscle	0
Lizard Island	2024	*Thalamita pelsarti*	chelae muscle	0
Lizard Island	2024	*Thalamitoides quadridens*	chelae muscle	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0
Laboratory	2024	NTC	NA	0

APPENDIX B – DNA METABARCODING COTS MULTITROPHIC FOOD WEBS

B1.1 Crab identification

B1.1.1 DNA extraction

Small (match head sized) pieces of tissue were carefully removed from each specimen, placed in 1.5 mL Eppendorf tubes, and dried at room temperature until all residual ethanol had evaporated. The QIAGEN DNeasy® Blood & Tissue Kit was used for DNA extraction following the manufacturer's protocol. Extracted DNA was viewed on a 2% agarose gel to assess DNA quality and estimate concentration.

B1.1.2 DNA amplification

The forward primer LCO1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3') and reverse primer HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA -3') were used to amplify DNA products (Folmer et al. 1994). Amplification was performed in a total volume of 20 µL, containing the following reagents:

(i) 7 µL nuclease-free water,
(ii) 10 µL 2X AmpliTaq Gold™ 360 Master Mix,
(iii) 1 µL forward primer (10 µM),
(iv) 1 µL reverse primer (10 µM), and
(v) 1 µL DNA (10 ng/µL).

Thermocycling parameters involved 10 min at 95°C to activate the polymerase; 35 cycles of denaturation at 95°C for 1 min, annealing at 48°C for 30 sec, elongation at 72°C for 30 sec; and a final cycle at 72°C for 7 min followed by storage at 4°C. Amplified products were viewed on a 2% agarose gel to check that amplification was successful (**Figure S6**).

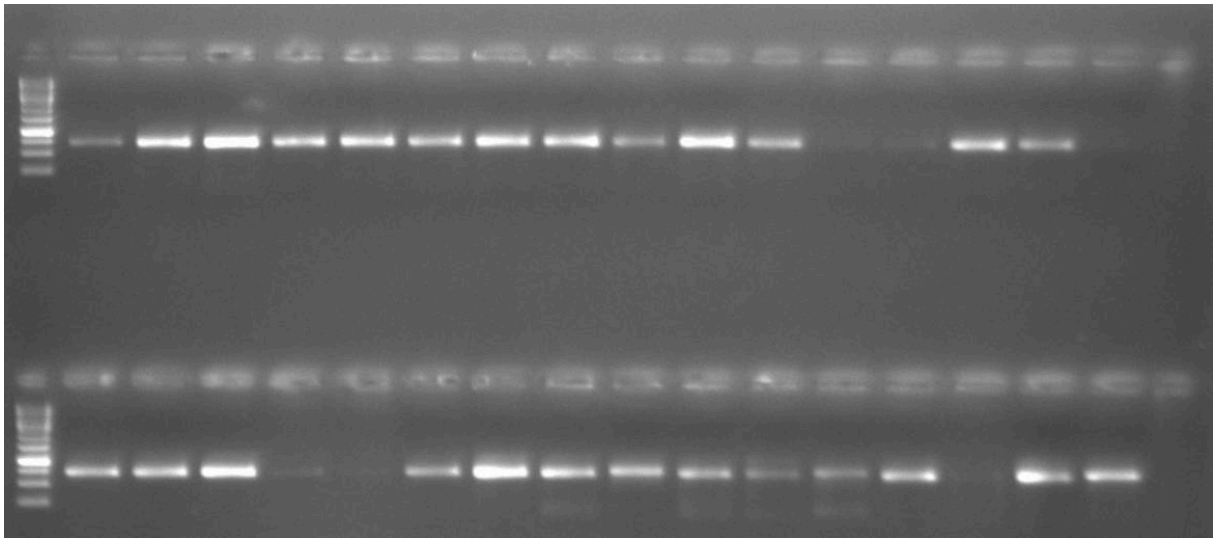

Figure S6. A gel image of a subset of amplified crab DNA. The presence of a white band indicated successful amplification using the CO1 Folmer primers.

B1.1.3 Sequencing and analysis

In preparation for sequencing, the PCR product was cleaned using the ExoSAP-IT® PCR product clean-up reagent. The clean-up followed the manufacturer's protocol, which included incubation at 37°C for 15 min to degrade unwanted products, followed by incubation at 80°C for 15 min to inactivate ExoSAP-IT® (Thermo Fisher Scientific Inc. 2017). The cleaned PCR product was sent to MACROGEN, Korea for standard Sanger sequencing.

The resulting sequence was identified using the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST) with related level of certainty (Table S4). The blastn suite was used to megablast against the nucleotide collection (nr/nt) database, excluding models (XM/XP) and uncultured/environmental sample sequences. Primer sequences were trimmed from sequences in Geneious Prime v 2023.2.1 prior to using BLAST.

Table S4. Sequence outputs for cryptic predators of COTS, including top BLAST matches with NCBI accession, and NCBI and identification certainty (%). Multiple results shown for taxa with top NCBI grades within 1% and then known species selected. Note: the genus *Thranita* has now been synonymised to *Thalamita*.

Species ID	taxa BLAST	NCBI accession	Grade NCBI (%)	Identity (%)
Heron Island				
Schizophrys aspera	Xanthidae sp.	KU285683	97.9	96.4
	Schizophrys aspera	KF452891	95	90.6
Schizophrys aspera	Xanthidae sp.	KU285683	98.4	98.5
	Schizophrys aspera	KF452891	98	92.3
Thalamita admete	*Thalamita admete*	KT365749	99.1	100
Thalamita admete	*Thalamita admete*	JQ180243	99.8	99.5
Thalamita admete	*Thalamita admete*	JQ180243	99.6	99.7
Thalamita pelsarti	*Thranita pelsarti*	MZ393938	99.9	89.7
	Thalamita prymna	MZ559707	99.9	89.7
	Brachyura sp.	HM464280	99.9	89.7
Thalamita pelsarti	Brachyura sp.	HM464280	99.8	100
	Thalamita prymna	MZ559707	99.6	99.8
	Thranita pelsarti	MZ393938	99.4	99.5
Thalamita pelsarti	*Thalamita prymna*	HM464280	99.8	100
	Brachyura sp.	MN184700	99.6	99.8
	Thranita pelsarti	MZ393938	99.4	99.5
Thalamita prymna	*Thalamita cf. rubridens*	KT365756	99.4	99
	Thalamita spinicarpa	KT365787	99	91.3
	Thalamita prymna	MN184700	99	90.9
Thalamita prymna	*Thalamita cf. rubridens*	KT365756	99.9	99.8
	Thalamita rubridens	KT365783	99	91.5
	Thalamita prymna	MN184700	99	91.5
Thalamita prymna	*Thalamita cf. rubridens*	KT365756	99.8	100
	Thalamita spinicarpa	KT365787	99	91.5
	Thalamita prymna	MN184700	99	91.3
Thalamita quadrilobata	*Thalamita quadrilobata*	KT365782	57.5	89.4
Thalamita quadrilobata	*Thalamita quadrilobata*	KT365782	98	91.9
	Thalamita oculea	KT365777	98	82.2
Thalamitoides quadridens	Portunidae sp.	MZ559378	99	88.2
	Thalamitoides sp	MZ539817	99	88
Thalamitoides quadridens	*Thalamitoides sp.*	MT457744	99	84.4
	Portunidae sp.	MZ559828	99	83.9
Thalamitoides quadridens	*Thalamitoides sp.*	MT457744	98	87.6
	Portunidae sp.	MZ559709	98	87.2
Thalamitoides tridens	Brachyura sp.	HM464342	98	89.2
	Thalamitoides tridens	MZ559572	98	88.9

Species ID	taxa BLAST	NCBI accession	Grade NCBI (%)	Identity (%)
Thalamitoides tridens	Brachyura sp. *Thalamitoides tridens*	HM464342 MZ559572	92 92	88 87.8
Thalamitoides tridens	*Thalamitoides tridens*	MZ393932	99.9	88.3
Moore Reef				
Schizophrys sp.	Decapoda sp. Brachyura sp. *Schizophrys aspera*	MH338926 HM465945 KF452891	84 83 74	88.4 87.1 86.5
Schizophrys aspera	Xanthidae sp. *Schizophrys aspera*	KU285683 KF452891	99.9 98	95 89.6

B2.1 Fish and crab gut metabarcoding

B2.1.1 DNA extraction

Crab guts and faeces were extracted by Kroon et al. (2020). Fish guts were preserved in ethanol in 50 mL Falcon tubes and refrigerated until use. Ethanol was drained from each vial prior to performing DNA extractions, by homogenising vials of fish gut and/or faecal contents (drained of ethanol) and transferring ~2 µL of homogenate into a 1.5 mL Eppendorf tube. The QIAGEN DNeasy® Blood & Tissue Kit was used for DNA extraction following the manufacturer's protocol. All DNA extracts were viewed on a 2% agarose gel to check DNA quality (**Figure S7**).

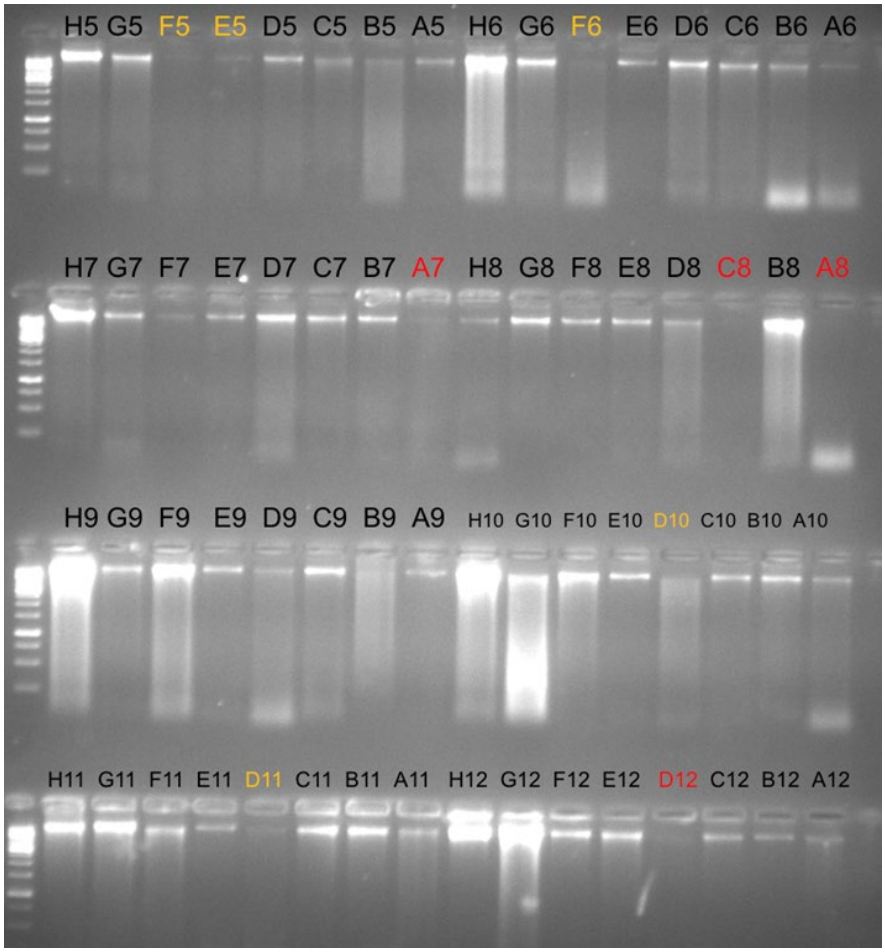

Figure S7. A gel image of a subset of the fish gut DNA that were extracted at the University of Queensland. For the most part DNA quality was good, with only a few samples displayed medium (yellow) to low (red) DNA concentrations. Poor quality DNA samples were not used for metabarcoding.

B2.1.2 DNA amplification

Amplifications were performed using the CO1 primers mlCOIintF/jgHOC2198 and the 18S primers EukF/EukR. A total of 96 unique samples (including two negative controls and two positive controls) were replicated across four plates (two CO1 and two 18S). Each plate was amplified using dual-unique indexed primers so that each sample received a unique forward and reverse primer tags. Each primer set was replicated so that there would be two PCR replicates of each sample per primer assay. Amplification of each sample was performed in a total volume of 25 µL, containing the following reagents:

(i) 8.5 µL nuclease-free water,
(ii) 12.5 µL 2X AmpliTaq Gold™ 360 Master Mix,
(iii) 1.5 µL forward primer (10 µM),
(iv) 1.5 µL reverse primer (10 µM), and
(v) 1 µL DNA (~10 ng/µL).

Thermocycling parameters involved 10 min at 95°C to activate the polymerase; 35 cycles of denaturation at 95°C for 1 min, annealing at 48°C (CO1)/52°C (18S) for 30 sec, elongation at 72°C for 30 sec; and a final cycle at 72°C for 7 min followed by storage at 4°C.

B2.1.3 Library preparation and sequencing

Amplified products were viewed on a 2% agarose gel to confirm the presence of a band ~300–400 bp, indicating successful amplification. If no band was present, amplification was repeated once. The final four plates were sent to the Australian Genome Research Facility (AGRF) for Illumina NovaSeq sequencing (300 cycle). Final library preparation and sequencing was conducted by AGRF. Briefly, library preparation involved amplicon quality checks, amplicon normalisation, pooling of each plate, and Illumina adaptor ligation to result in four libraries for sequencing.

Sequencing reads were demultiplexed, and adapters were removed using QIIME2. Reads with a quality score below Q20 and those outside the expected amplicon length were filtered out using cutadapt. Following quality control, reads were collapsed to unique sequences known as Operational Taxonomic Units (OTU) at 97% similarity using the BBMap tool. For taxonomic assignment, each OTU was compared to the GenBank database (www.ncbi.nlm.nih.gov) using megablast. Species-level assignments required a minimum of 90% identity and >90% query coverage, with all taxonomic assignments manually vetted. In cases where an OTU could not be resolved to a single species (e.g. due to shared haplotypes), a list of multiple species was included, or the OTU was assigned to the best possible taxonomic rank without further classification. Assignments were cross-checked on the Atlas of Living Australia. Species not documented in the study region were assigned a lower order of taxonomic resolution, where possible, or omitted from the dataset (n = 23 individuals). Host DNA (i.e. of the species that the gut originated from) was also excluded.

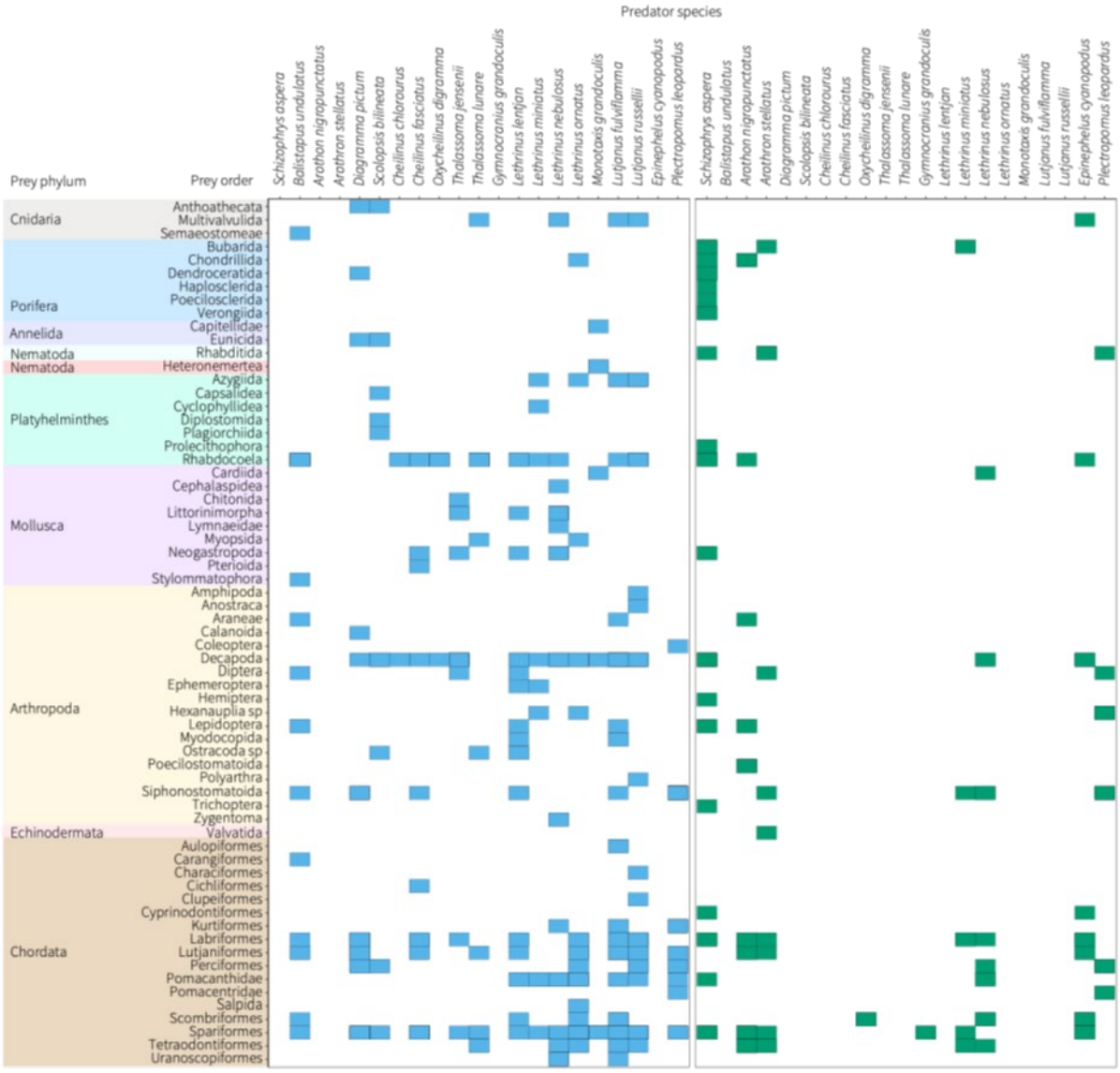

Figure S8. Preliminary results from DNA metabarcoding of the gut contents of *Schizophrys aspera* and faeces of 20 species of reef fish. Individuals collected from fished zones (blue, left) and no-take zones (green, right). Not all species were represented in both zones.

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