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Predation on the early life stages of the crown-of-thorns starfish (*Acanthaster* cf. *solaris*)

Thesis submitted by

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Abstract

Population outbreaks of crown-of-thorns starfish (CoTS, *Acanthaster* spp.) represent one of the most significant biological disturbances on tropical reefs, contributing to widespread and significant coral depletion throughout the Indo-Pacific. On the Great Barrier Reef, for example, CoTS outbreaks account for 42% of recorded coral loss from 1985-2012. Despite their importance, the demography and biology of CoTS is poorly understood, especially in terms of understanding the potential causes(s) of population outbreaks. For effective management of outbreaks, it is critical to understand the factors that both promote sudden population explosions, and that normally regulate CoTS populations at very low densities.

Predatory release has long been considered a potential contributor to population outbreaks of CoTS, initiating extensive searches for potential predators that may consume large numbers of these starfish at high rates, but are also vulnerable to over-fishing. As such, most research into predation on CoTS has focused on large reef fishes and invertebrates capable of capturing and consuming adult starfish. By contrast, consideration of smaller-bodied and potentially cryptic predators that could consume gametes, larvae or newly settled juveniles has received little attention. Thus, the overarching objective of this research was to explore predation on the early life stages of CoTS by two suites of predators: planktivorous damselfishes, and benthic invertebrates. Specifically, this research compares the predatory responses of a range of damselfishes, testing for interspecific variation in their potential importance as predators of CoTS eggs and larvae, and tests the influence of benthic predators on microhabitat preferences and settlement success of CoTS.

Although CoTS are equipped with anti-predator chemicals at every stage of their life cycle, the effectiveness of these chemicals in deterring predators is increasingly being questioned. In particular, planktivorous damselfishes, which are highly efficient zooplankton predators, may be capable of consuming the early life stages of CoTS in vast quantities, potentially reducing the reproductive and settlement success of this starfish. Nine planktivorous damselfishes are shown to readily consume food pellets that contain concentrations of up to 80% CoTS eggs, however all fishes exhibit increasing rejection of food pellets that contain higher proportions of eggs. This suggests that chemicals within the eggs are unpalatable, however palatability thresholds varied greatly among the damselfishes, indicating species that are likely to be more important as predators of CoTS eggs (or larvae). Notably, *Amblyglyphidodon curacao* consumed food pellets comprising 100% starfish eggs 1.5 times more than any other fish species, and appeared largely insensitive to increases in the concentration of starfish eggs. However, after standardising for size, smaller species, such as *Pomacentrus moluccensis* and *Chrysiptera rollandi*, consume a disproportionate amount of pellets comprising high proportions of starfish eggs and could be particularly important in regulating larval abundance and settlement success of CoTS.

To further explore the role of damselfish predation in potentially structuring CoTS populations, the feeding behaviour and functional responses of eleven damselfishes were examined by offering individual damselfish with increasing concentrations of larvae of either CoTS or *Linckia laevigata* (a co-occurring species with morphologically similar larvae). Consumption rates of CoTS larvae by damselfishes were independent of predator size, however when pooling across all predator species there was a significant negative relationship between predator size and consumption rate of *L. laevigata.* Most predatory species (all except *A. curacao* and *Pomacentrus amboinensis*) exhibited a Type II functional response, whereby the increasing feeding rate decelerated with increasing prey density. In addition, *Acanthochromis polyacanthus* and *A. curacao*, consumed larval CoTS at a greater rate than for *L. laevigata*, and consumption capacity of CoTS by *Dascyllus aruanus* was extremely high (158 larvae h^{-1}). These data reveal that a wide range of planktivorous fishes will prey upon CoTS larvae (at least when offered as the only available prey), and suggest that planktivorous damselfishes may have the capacity to buffer against population fluctuations of CoTS.

Predation rates on specific prey species can vary depending on availability of alternative prey. In the extreme, predators may exhibit prey switching, whereby they preferentially feed on the most abundant, or most readily accessible, prey species. Defining predatory responses to changes in prey availability is critical to evaluating the ability of predators to regulate prey populations. To extend the single-prey experiments, exploring prey preference and testing for prey switching, nine damselfishes were simultaneously offered varying relative densities of CoTS and *L. laevigata* larvae. Again, feeding responses varied among the damselfishes. While no evidence of prey switching was detected, five damselfishes (*A. polyacanthus*, *A. curacao*, *Dascyllus reticulatus*, *P. amboinensis* and *P. moluccensis*) exhibited increased consumption of CoTS larvae with increasing prey density, despite the availability of alternative prey. Moreover, *Abudefduf sexfasciatus* and *P. amboinensis* exhibited preference for CoTS larvae over *L. laevigata* larvae. These findings suggest that planktivorous damselfishes will consume CoTS larvae even in the presence of alternative, and presumably more palatable, prey. Further, most of the damselfishes responded to increasing larval CoTS densities by increasing their prey intake, suggesting that they could be important in regulating successful settlement and recruitment, especially at low (non-outbreak) densities.

CoTS are also expected to suffer high levels of predator-induced mortality during settlement, as they transition to living in the benthic habitat and are exposed to an entirely new suite of predators. Accordingly, these larvae might be expected to exhibit behavioural adaptations, which serve to reduce predation at this critical stage in their life history. Pairwise choice experiments revealed that late stage brachiolaria larvae are able to detect predators in the substrate and where possible, will preferentially settle in microhabitats without predators. Settlement assays (without choices) revealed that larvae do not necessarily delay settlement in the presence of predators, but high levels of predation on settling larvae by benthic predators significantly reduce the number of larvae that settle successfully. Taken together, these results show that CoTS are highly vulnerable to benthic predators during settlement, and that variation in the abundance of benthic predators may significantly influence patterns of settlement and recruitment.

This research shows that eggs, larvae and newly settled CoTS are readily consumed by a wide range of coral reef organisms, such that predation during and soon after settlement may represent a significant bottleneck in their life history. Unfortunately, many of the predators that consume these early life stages are vulnerable to reef degradation and coral loss. Thus it is possible that anthropogenic degradation of reef ecosystems is contributing to the incidence and/or severity of CoTS outbreaks by mediating the abundance of these potentially key predators. Although it seems unlikely that predatory release in and of itself could account for initial onset of CoTS outbreaks, reducing anthropogenic stressors that reduce the abundance and/or diversity of potential predatory species represents a “no regrets” management strategy, but will need to be used in conjunction with other management strategies to prevent, or reduce the occurrence, of CoTS outbreaks.

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Chapter 1: General introduction

1.1 Population regulation

Establishing which factor(s) determine the local abundance and population dynamics of given species remains a central goal of ecology (e.g., Dublin and Ogutu 2015). Natural populations are regulated (moderating rates of change in population size, if not constraining maximum abundance) by a combination of biotic and abiotic factors (Andrewartha and Birch 1954; Hunter and Price 1992). Biotic factors (inter- and intra-specific interactions), such as predation (e.g., Li and Moyle 1981; Jackson et al. 1992) and competition (e.g., Guillou 1996), may be density-dependent (Smith 1935) and either directly, or indirectly affect individual fitness (Morris 1988). In contrast, abiotic factors, such as temperature (e.g., Shuter et al. 1980; Grossman and Freeman 1987) and hydrodynamics (e.g., Mead and Denny 1995), are typically density-independent, and affect the ability of organisms to survive in a specific area or habitat (Roughgarden and Diamond 1986). Although the exact mechanisms acting to regulate particular species and communities may be widely debated (e.g., Jackson et al. 2001) and are highly variable in time and space (e.g., Shears et al. 2008; Ling et al. 2009), identifying and understanding these processes is integral to understanding the distribution and abundance of organisms (Morris 1988).

Much discussion has been centred on whether populations, and consequently communities, are regulated predominantly by bottom-up “resource driven” control or top-down “trophic” control (reviewed by Conversi et al. 2015). Bottom-up control occurs when populations and community structure are regulated by factors that produce variation in the nutrient supply or at lower trophic levels (Menge 1992). Increasing primary production can result in greater productivity at all trophic levels; whilst removal of primary producers (or primary consumers), or reduction in the uptake of energy into the system, can produce resource-limitation at each trophic level (Hunter and Price 1992). Conversely, top-down control occurs when predators at higher trophic levels suppress the abundance, or alter behaviour, of prey items (e.g., Paine 1966; Heithaus et al. 2008; Ling and Johnson 2012). Reducing the number or function of predators in one of the higher trophic levels leads to predatory-release and increased abundance of prey species with subsequent cascading of suppression and release down through the food chain (Hairston et al. 1960; Paine 1980).

Disruption of, or natural variability in, any of the mechanisms that typically regulate population size has the potential to lead to pronounced and acute changes in abundance (e.g., Andrewartha and Birch 1984), especially among species with inherently high intrinsic rates of population growth. Key demographic traits that contribute to high intrinsic rates of population growth are relatively small body size, early maturation, high fecundity and production of offspring that are less energetically costly to produce (MacArthur and Wilson 1967), often referred to as "r-strategists". These particular traits enable species to respond very rapidly to any changes in biotic or abiotic conditions (MacArthur 1960), thus predisposing organisms to major fluctuations in population size. Population fluctuations can be defined as a change in abundance through time, or deviations from a growth pattern (e.g., Keith 1990). These fluctuations can be erratic (e.g., *Strongylocentrotus* spp.; reviewed by Uthicke et al. 2009), and sometimes the number of individuals can increase rapidly, which is often referred to as an outbreak (e.g., *Acanthaster* spp.; Potts 1981).

Rapid increase in population size of a particular species often has detrimental ecological and economic impacts. Whilst such species may play a vital role in ecosystem functioning under normal densities, they are referred to as 'pests' when they negatively impact a human resource, be it agriculture or human health (Flint and van den Bosch 1981). Examples of such species may be found in both native ranges and invaded ranges and may occur as a result of breakdown of any of the biotic or abiotic factors that would typically limit population size, whilst population growth is often enhanced by life history characteristics described above. For example, locusts have the ability to change their physiology and behaviour in response to favourable climatic conditions (drought followed by rapid vegetation growth), becoming gregarious and nomadic, and multiplying rapidly (Cressman 2016). Other examples, such as lionfish in the Caribbean, exhibit rapid population growth as the invaded range is largely free from the regulatory factors, such as top-down, predatory control, that exist in the native range (Hackerott et al. 2013).

Extreme fluctuations in adult abundance are well known among certain marine organisms (e.g., Reese 1966; Warner 1971; Ling et al. 2012), particularly for species with planktonic larvae (e.g., Roughgarden et al. 1988), which rely on successful settlement and recruitment for replenishment of populations. The planktonic larval stage is fundamental in enabling long-distance dispersal for otherwise benthic and sessile organisms (Roughgarden et al. 1988) and larval survival is dependent on the favourability of numerous factors, both in the water column and at the settlement site. Factors that affect larval abundance and distribution in the water column include adult reproductive cycles (Roughgarden et al. 1991), currents, wind patterns and topography (Underwood 1972; Hawkins and Hartnoll 1982), and spatial variation in predation rates (Mileikovsky 1974; Kingsford and MacDiarmid 1988). Additionally, the number of larvae that successfully settle into the adult habitat is affected by factors including predation (Almany and Webster 2006), which may significantly shape the abundance and distribution of adult populations.

Predation at the settlement stage is expected to represent a major bottleneck in the life history of many marine organisms (e.g., corals, Chong-Seng et al. 2014; fish, Hixon 2011) as relatively naïve planktonic larvae are exposed to an entirely new suite of potential predators as they transition to living in benthic reef habitats (Almany and Webster 2006). Predator-induced mortality at this stage could have a significant impact on recruitment levels, such that spatial variation in predators can have a major bearing on the distribution and abundance of benthic marine organisms (Connell 1985; Rowley 1989). Moreover, removal of predators is expected to signifcantly increase the proportion of individuals that successfully settle and recuit back into the population.

In addition to predator abundance, refuge availability may be an important factor that affects vulnerability to predation (e.g., Anderson 2001; Alexander et al. 2015). Increased predator density is expected to increase use of shelter by prey, in order to decrease prey mortality per predator (e.g., Forrester and Steele 2004); whereas increased prey density leads to competition for refuges, with less competitive individuals at greater risk of predation (e.g., Holbrook and Schmitt 2002). Prey may also find refuge from predation in larger body size, such that larger individuals experience reduced predation rates compared to smaller conspecifics (e.g., Keesing and Halford 1992a; Rice et al. 1993; Goatley and Bellwood 2016). Higher growth rates, or larger initial size, may therfore, reduce the risk of predation, especially among early life-history stages (Murdoch and Oaten 1975).

1.2 Population fluctuations among echinoderms

Major fluctuations in abundance have been reported for several species within the echinoderm phylum, including species of Echinoidea (0.89% of species), Holothuroidea (0.70% of species), Asteroidea (0.34% of species), and Ophiuroidea (0.18%) (Uthicke et al. 2009). Many of these species have important functions in marine ecosystems (e.g., Elner and Vadas 1990), and thus, variations in their abundance can have significant ecological and economic consequences (e.g., Moran 1986; Lessios 1988; Elner and Vadas 1990). These variations are reported to follow one of three patterns: catastrophic decrease followed by no, or very slow recovery; rapid increase, before stabilising at a new higher population density; and a fluctuating pattern whereby population density alternates between a high-density state and a low-density state (Uthicke et al. 2009).

Rapid decreases in echinoderm populations are often caused by disease (e.g., Lessios et al. 1984), overfishing (Shepherd et al. 2004), or changes in oceanographic conditions (Verling et al. 2005). The mass die-off of the tropical sea urchin *Diadema antillarum*, attributed to an unidentified pathogen (Lessios et al. 1984), is arguably the most well known example of a population decrease in echinoderms. *Diadema antillarum* is an efficient macroalgal-grazer (Carpenter 1986) and where it occurs in high densities, it is known to overgraze macroalgae, leading to urchin barrens (Tuya et al. 2005). *Diadema antillarum* competes with herbivorous fishes for algal resources (Williams 1981) and prior to the mass mortality event, abnormally high abundance of *D. antillarum* on overfished Caribbean reefs was seemingly mitigating against the loss of these herbivores (Hughes et al. 1987). However, the catastrophic decrease in this species across the Caribbean removed a significant grazing pressure, resulting in a phase shift from coral-dominated to macroalgal-dominated systems (Hughes 1994).

Catastrophic shifts in ecosystem structure as a result of variation in abundance of sea urchins have also been observed in Tasmania, with the range extension of the long-spined sea urchin *Centrostephanus rodgersii*, which is driven by ocean warming (Johnson et al. 2005; Ling 2008). Variations in the abundance of *C. rodgersii* on rocky reefs have mediated the transition from macro-algal dominated systems to urchin barrens (Ling et al. 2009). Similarly to the Caribbean system, overfishing played a role in exacerbating the effects of this disturbance, as extensive removal of sea urchin predators reduced the resilience of kelp beds to invasions by this highly destructive herbivore (Ling et al. 2009). There are numerous other examples of increases in echinoderm population densities associated with introductions (e.g., Byrne et al. 1997; Ling et al. 2012) and release from predation pressure, synonymous with top-down trophic cascade (e.g., McClanahan and Muthiga 1988), however increases in primary productivity (bottom-up trophic cascade) and/or changes in the environment may also play a role in increasing the abundance of echinoderms. For example, population densities of ophiuroids were observed to increase following eutrophication in the

North Sea (e.g., Duineveld et al. 1987) and increased population densities of holothuroids and ophiuroids were correlated with changes in benthic organic carbon supply in the Northeast Pacific deep sea (Ruhl and Smith 2004).

Each of the aforementioned disturbances implicated in increases or decreases in echinoderm abundance may also play a role in the third pattern of population density variation - fluctuation between high-density and low-density states. Fluctuations may be cyclical (e.g., the crown-of-thorns starfish, *Acanthaster* spp. - discussed below) or more irregular, where populations may remain at very high or very low densities for extended periods (e.g., the sea urchin, *Strongylocentrotus* spp., (Steneck et al. 2002)). The exact processes that control *S. droebachiensis* populations are still widely debated, however overfishing of predators of adults is considered the most important factor in initiating massive population increases (Tegner and Dayton 2000; Steneck et al. 2002, 2004). Factors implicated in population decreases include disease outbreaks (Scheibling and Stephenson 1984; Scheibling and Hennigar 1997); fishery pressure (Harris and Tyrrell 2001; Levin et al. 2002; Steneck et al. 2002, 2004); predation on juveniles (Harris and Tyrrell 2001; Levin et al. 2002; Steneck et al. 2004); and ecosystem alteration as a result of introduced species (Harris and Tyrrell 2001; Levin et al. 2002; Steneck et al. 2004). The relationship between presence versus absence of sea otters and the fluctuating population densities of *Strongylocentrotus* spp. has also been widely studied (e.g., Estes and Duggins 1995; Steneck et al. 2002), and is a classic example of top-down trophic cascade. Both hunting (Simenstad et al. 1978; Tegner and Dayton 2000; Tegner 2001; Steneck et al. 2002), and predation by killer whales (Estes et al. 1998), has been observed to reduce the abundance of sea otters, which typically suppress the abundance of *Strongylocentrotus* spp. to near zero (Estes and Duggins 1995), releasing kelp from grazing pressure (Steneck et al. 2002). However in the absence of predators, *Strongylocentrotus* spp. is released from predation pressure, leading to destructive grazing in the kelp forests and the formation of urchin barrens (Tegner and Dayton 2000; Steneck et al. 2002, 2004).

It is clear that a combination of top-down and bottom-up processes are at play in determining the likelihood of boom or bust of key echinoderm populations and resultant phase-shift in benthic habitats (Ling et al 2009; reviewed by Ling et al 2015). Although the exact causes of variations in population density may differ, many of these variations are associated with anthropogenic disturbance (Uthicke et al. 2009).

1.3 Outbreaks of *Acanthaster* spp.

The crown-of-thorns starfish, *Acanthaster* spp. occurs on corals reefs throughout the Indo-Pacific region (Birkeland and Lucas 1990). There are at least four recognised species of crown-of-thorns species (Haszprunar and Spies 2014; Haszprunar et al. 2017), formerly regarded as a single Indo-Pacific species, *A.*

planci. All species of crown-of-thorns starfish feed on scleractinian corals (e.g., Glynn 1988) and at low densities (<10 starfish.ha^{-1}) have negligible impact on the abundance of corals, and associated reef processes (e.g., Glynn 1973; Zann et al. 1990). However, outbreaks of *Acanthaster* spp. (defined as "any large aggregation of many hundreds or thousands of individuals which persists at high densities for months or years and causes extensive mortality among corals over large areas of reef" (Potts 1981)) pose a major threat to coral reefs, as scleractinian corals are fundamental to the productivity and biodiversity of these ecosystems (Wilson et al. 2008). Outbreaks of *Acanthaster* spp. may lead to > 90% reductions in living coral cover (e.g., Chesher 1969; Pearson and Endean 1969; Endean 1973; Endean and Stablum 1973), with the resulting loss of coral cover linked to declines in abundance of coral-associated fishes (e.g., Sano et al. 1984; Williams 1986; Munday et al. 1997) and motile invertebrates (Garlovsky and Bergquist 1970).

One of the most important biological traits that predisposes *Acanthaster* spp. to outbreaks is their enormous reproductive potential (Endean 1982; Conand 1985). Fecundity increases disproportionately with increasing body size, with smaller females (< 30 cm diameter) producing 0.5-2.5 million eggs per year, and females > 40 cm in diameter producing > 100 million eggs per year (Babcock et al. 2016). However, individuals measuring > 60 cm in diameter have also been reported (Pratchett 2005) and it is predicted that these may be capable of producing > 200 million eggs per reproductive season (Babcock et al. 2016).

Acanthaster spp. also exhibit aggregative behaviour (e.g., Babcock et al. 1994), synchronous spawning (Babcock and Mundy 1992), and significantly greater fertilization success when spawning individuals are separated by more than a few metres, relative to other echinoderm species (Yund 1990; Grosberg 1991; Levitan et al. 1991), all of which contribute to high rates of fertilisation success. When spawning individuals are adjacent to each other, fertilisation success may be up to 100% (Benzie et al. 1994). This declines with increasing distance, however fertilisation rates of 50% and 25% may still be achieved over separation distances of 30 m and 60 m, respectively (Babcock and Mundy 1992).

The first well-documented *Acanthaster* spp. outbreaks occurred in southern Japan in the late-1950s (Yamaguchi 1986) and on the Great Barrier Reef in the early-1960s (Pearson and Endean 1969). Outbreaks of *Acanthaster* spp. continue to occur throughout the Indo-Pacific (e.g., Pratchett et al. 2011; De'ath et al. 2012; Baird et al. 2013), and at many locations, their effects far exceed the combined effects of all other major disturbances (e.g., Trapon et al. 2011; De'ath et al. 2012; Pisapia et al. 2016). Of all the disturbances that threaten coral reefs (e.g. mass coral bleaching, coral diseases, increasing seawater temperatures, tropical storms (e.g., De'ath et al. 2012)), outbreaks of *Acanthaster* spp. may be the most amenable to localised intervention and management (De'ath et al. 2012). However, integral to the effective management of outbreaks is understanding the factors that promote sudden population explosions, and this requires greater understanding of processes that otherwise regulate populations of *Acanthaster* spp.

Some authors propose that outbreaks of *Acanthaster* spp. are a natural phenomenon, varying in response to natural environmental perturbations (e.g., Vine 1973; Birkeland 1982). The *natural causes theory* (Vine 1973) suggest that population fluctuations are a natural feature of *Acanthaster* spp. fluctuations, and that high-density aggregations may have simply gone unnoticed prior to the mid-twentieth century due to a lack of awareness of this species among divers. Although there are anecdotal accounts of high *Acanthaster* spp. densities prior to those first recorded by scientists (reviewed in Vine 1973), the absence of systematic, broad-scale monitoring and difficulty in reconstructing a history of numbers that might constitute outbreaks from the sediment record (Fabricius and Fabricius 1992; Keesing and Halford 1992b; Pandolfi 1992) means that the previous occurrence of outbreaks, as seen across the Indo-Pacific since the 1950s, cannot be confirmed. Moreover, the leading hypotheses (*predator removal* (Endean 1969) - the focus of this thesis - and *nutrient enrichment* (e.g., Pearson & Endean 1969; Brodie et al. 2005; Fabricius et al. 2010)) attribute the occurrence of these seemingly recent events to anthropogenic disturbances.

The *nutrient enrichment hypothesis* follows the notion of bottom-up control, suggesting that *Acanthaster* spp. larvae are typically food limited, but experience enhanced survivorship following nutrient enrichment of near-reef waters (e.g., Pearson and Endean 1969; Lucas 1973; Birkeland 1982; Brodie et al. 2005; Fabricius et al. 2010) through run-off from heavily modified catchments. Support for this hypothesis comes from the appearance of adult starfish in the years following major flooding events (Fabricius et al. 2010). However, discrepancies between the occurrence of major flood events and the initiation of outbreaks (reviewed by Pratchett et al. 2014) has placed some doubt on this hypothesis. Moreover, outbreaks of *Acanthaster* spp. certainly occur in areas far removed from any modified catchments and unaffected by coastal eutrophication (Pratchett et al. 2014). There is also no of evidence of increased incidence of outbreaks in areas experiencing nutrient enrichment within the Coral Triangle (Lane 2012). In addition, successful larval development across a broad range of nutrient levels (0.5-5 μg chl-a L^{-1}) (Wolfe et al. 2017), notably including below the levels posited by the *nutrient enrichment hypothesis* (2 μg chl-a L^{-1}) as constraining larval development and survivorship (Fabricius et al. 2010), simultaneously casts doubt on this hypothesis, points to the inherently resilient nature of larvae of this species, and highlights the importance of considering alternative mechanisms (e.g. predatory control) by which *Acanthaster* spp. populations may be regulated (Wolfe et al. 2017). Although nutrient enrichment alone may be unlikely to trigger an outbreak, the combination of nutrient-enriched waters and strong larval retention on a reef, as caused by local hydrodynamics, may promote sufficient larval survival for the initiation of outbreaks (Wooldridge and Brodie 2015).

The *predator removal hypothesis* (Endean 1969) proposes that outbreaks are an unnatural phenomenon, caused by anthropogenic modification and degradation of reef environments (Potts 1981). The first documented outbreak on the GBR followed a period of intense overfishing of the giant triton (*Charonia tritonis*), a reported predator of *Acanthaster* spp. (Endean 1969). While there is no empirical data on their abundance, *C. tritonis* are exceedingly rare on the GBR, and have been since the 1960s, perhaps reflecting the legacy of excessive removals in the 1950s (Endean 1973). Endean (1969) argued that the effective loss of giant triton from reefs in the northern GBR relaxed normally strong regulatory pressure on abundance of juvenile and sub-adult CoTS, leading to increased abundance of large adult starfish that were capable of initiating outbreaks by virtue of their massive combined reproductive output. Support for this hypothesis came from reports of outbreaks from other locations (e.g., Fiji and Western Samoa) where *C. tritonis* had also been extensively harvested, whilst outbreaks had not been reported in areas (e.g. Malaysia, Philippines and Taiwan) where *C. tritonis* were abundant (Endean 1969). The ability of *C. tritonis* to provide the sufficient top-down control necessary to regulate CoTS populations has since been questioned (e.g., Ormond et al. 1990), largely based on their generally low rates of feeding and the apparent reluctance to eat CoTS when provided with alternative prey, however, the *predator removal hypothesis* has evolved through time to place increasing emphasis on fish predators.

More recent investigations of the *predator removal hypothesis* have focused on large predatory fishes capable of consuming adult starfish (e.g., Ormond et al. 1990; Sweatman 1997; Mendonça et al. 2010), which are targeted by fisheries and/or have declined in abundance due to localized fishing activities. There is little explicit or direct evidence that any of the major fisheries target species (e.g., coral trout, *Plectropomus* sp.) are significant predators of *Acanthaster* spp. (Sweatman 1997). However, some studies (Ormond et al. 1990; Dulvy et al. 2004; Sweatman 2008) have reported increased incidence and/or severity of outbreaks of CoTS along gradients of increasing fishing effort. On the GBR, Sweatman (Sweatman 2008) showed that reefs open to fishing were seven times more likely to experience an outbreak of *Acanthaster* spp. (57% of reefs affected) compared to reefs effectively closed to fishing within no-take marine reserves (8% of reefs affected). While the mechanistic basis of these patterns has not been critically tested, increasing evidence of links between fishing and starfish outbreaks (Dulvy et al. 2004; Sweatman 2008) has fuelled significant interest in predation, both to understand the cause(s) and ultimately manage CoTS outbreaks.

Despite decades of research into predation upon *Acanthaster* spp., the extent to which predators may influence population dynamics of this starfish remains equivocal. Scientific interest in predators of *Acanthaster* spp. has traditionally focused on predators of adults (or at least post-settlement) stages (e.g., Endean 1969; Campbell and Ormond 1970; Owens 1971; Dulvy et al. 2004), however predatory regulation might equally occur during spawning and at pre-settlement and settlement stages (e.g., Babcock et al. 1986; Westneat and Resing 1988; Bachiller et al. 2015). Numerous studies (e.g., Rice et al. 1993; Goatley and Bellwood 2016) suggest that smaller individuals will be more vulnerable to predation, as larger individuals reach a refuge in body size. Indeed, rates of predator-induced mortality are observed to decrease with increasing size of juvenile CoTS (Keesing and Halford 1992a), which might suggest that these earlier (pre-settlement and settlement) stages are at even greater risk of predation.

Whilst modelling efforts predict that small changes in the rates of predation and survivorship at the pre-settlement life-stages of *Acanthaster* spp. could have significant effects on adult abundance (McCallum 1988, 1990), there has been little research to establish key predators, or predation rates, at these stages. Here this thesis explores predation on the early life stages of *Acanthaster* spp. as a potentially highly important bottleneck in the life history of this organism.

1.4 Thesis outline

The overarching goal of the research presented in this thesis is to explore predation on the early life-history stages of *Acanthaster* spp., thereby refocusing questions about the potential importance of predation in regulating populations of this starfish. Research is on-going to establish rates of predation on the adult life stage, and the potential effects of predation in constraining individual fitness and reproductive output of adult starfish (e.g., Messmer et al. 2017); however, very few studies have attempted to identify potential predators that will feed on gametes, larvae and/or newly-settled juveniles. Further, the importance of such predators in potentially regulating rates of recruitment and thereby, local abundance of crown-of-thorns starfish is largely unknown. High levels of predation on the pre-settlement and settlement stages will significantly reduce the proportion of survivors through to adulthood, thus spatial variation in rates of predation is likely to significantly augment the distribution and abundance of adult populations. Moreover, extremely high rates of predation can be expected to provide sufficient selection pressure for the evolution of defences (chemical, physical, and behavioural), such that *Acanthaster* spp. can maximise its reproductive fitness, however it is likely that predators will vary in their response to these defences. The objectives of this thesis are therefore to establish predators of the early life stages of *Acanthaster* spp.; identify which of these may have a greater capacity to reduce *Acanthaster* spp. densities; determine patterns of predation by these predators; and identify how benthic predators influence microhabitat preferences and settlement success of this starfish. Consequently, this research extends and adds to the work that has already been published.

Chapter Two of this thesis is a literature review focusing on predation on *Acanthaster* spp. This review synthesizes existing knowledge of potentially important *Acanthaster* spp. predators, considering their individual and collective capacity to influence population dynamics of this starfish. The intention in this review is to differentiate, from the ever-increasing list of putative predators (e.g., Glynn 1984; Moran 1992; Rivera-Posada et al. 2014), between organisms that opportunistically feed on dead or injured starfish (scavengers), versus those predators that feed on live and healthy starfish and either kill them outright or reduce their individual fitness and/or reduce population level fitness. Moreover, this review explicitly considers potential predators at different stages in the life cycle of *Acanthaster* spp., especially pre-settlement (e.g., gametes, larvae) and early post-settlement life stages, which is quite possibly the most significant bottleneck in their life-history (McCallum 1988; McCallum 1990; Morello et al. 2014). Where possible, we report or derive estimates of the rates of mortality due to predation across different life-history stages of *Acanthaster*. Having established the range of putative predators, this chapter considers empirical and theoretical evidence that supports (or refutes) the potential role of predators in moderating (if not preventing) *Acanthaster* spp. outbreaks. Persistent controversy around the role of predation in regulating abundance of *Acanthaster* spp. (e.g., Dulvy et al. 2004; Sweatman 2008; Rivera-Posada et al. 2014) highlights many deficiencies in previous research approaches and points to the definitive need for experimental studies that explicitly test the mechanistic underpinnings of the *predator removal hypothesis*.

The empirical research presented in chapters three to six of this thesis is novel because it focuses explicitly on predators of the early life stages of *Acanthaster* spp., revealing a suite of previously unidentified predators, investigating the patterns of predation by these predators, and establishing how these predators may influence larval settlement patterns. Research for these chapters was conducted under controlled conditions (in aquaria) at the Lizard Island Research Station in the northern Great Barrier Reef (14°40'S; 145°27'E), Australia. Chapter Three examines inter-specific variation in nine species of planktivorous damselfish as predators on *Acanthaster* sp. eggs (i.e. *Amblyglyphidodon curacao*, *Chromis atripectoralis*, *Chromis viridis*, *Chrysiptera* *cyanea*, *Chrysiptera rollandi*, *Dascyllus aruanus*, *Dascyllus reticulatus*, *Pomacentrus amboinensis* and *Pomacentrus moluccensis*). This research is important because it highlights a group of previously overlooked, yet highly abundant predators, which may play an important role in reducing larval *Acanthaster* spp. densities, thus providing some degree of buffering for coral reefs against the devastating impact of *Acanthaster* spp. outbreaks. Furthermore it indicates which damselfish are likely to be more important predators of the early life stages of *Acanthaster*.

Chapter Four examines the feeding behaviour of eleven species of planktivorous damselfish provided with increasing densities of *Acanthaster* sp. larvae. This chapter aims to establish whether planktivorous reef fishes could be important in regulating the abundance of *Acanthaster* sp., or potentially contributing to extreme fluctuations in abundance of this starfish. Specifically, this chapter tests (1) the relationship between predator size and predation rate, (2) consumption rate of *Acanthaster* sp. larvae vs. larvae of a common and co-occurring asteroid *Linckia laevigata*, (3) maximal feeding rates upon both *Acanthaster* sp. and *L. laevigata*, and (4) functional responses of damselfishes feeding upon *Acanthaster* sp. This research provides further support for damselfish predators in providing a buffering capacity against population fluctuations of *Acanthaster*. The patterns of predation primarily exhibited by these predators may contribute to stability of *Acanthaster* spp. populations, however these fishes may be swamped by an abnormally high larval influx, potentially contributing to the characteristic population fluctuations of this starfish.

Chapter Five aims to establish the response of nine planktivorous damselfishes to increasing densities of *Acanthaster* sp. larvae in the presence of alternative prey items. In addition, this chapter specifically tests for prey preference and prey switching behaviour. Predators that feed preferentially upon *Acanthaster* sp. or exhibit prey switching behaviour are expected to play a greater role in reducing high densities of larval starfish. Further, presence of prey switching behaviours amongst *Acanthaster* sp. predators would enhance the buffering potential of coral reefs, as consumption of *Acanthaster* sp. larvae by planktivores would accelerate with increasing larval densities.

Chapter Six examines the influence of benthic predators upon microhabitat preferences and settlement success of *Acanthaster*. Like most marine organisms, *Acanthaster* spp. are expected to be highly vulnerable to predation as they transition from the planktonic larval habitat to the benthic adult habitat, and are exposed to an entirely new suite of potential predators (Almany & Webster 2006). Previously reported high rates of mortality during settlement and metamorphosis (e.g., Yamaguchi 1973) support the suggestion that there will be strong selection for settling larvae to choose microhabitats that minimise predation risk (Lucas 1975), either by avoiding habitats with high abundance of potential predators, or preferentially settling in complex microhabitats that provide greater refuge from predators. The research presented within this chapter aims to test whether *Acanthaster* spp. larvae can detect the presence of benthic predators within settlement substrates (largely based on chemoreception, *sensu* (Johnson et al. 1991)), and thereafter, explore the extent to which larval *Acanthaster* spp. preferentially settle in microhabitats with and without predators present.

Chapter Seven is a general discussion, which provides an overview of all major findings presented in this thesis. This chapter evaluates the significance and implications of these research findings, and highlights key areas of future research that are essential to further understand the effect of predators and the role of predation on population dynamics of *Acanthaster* sp.

Finally, one publication is attached as an appendix at the end of this thesis. This represents additional research related to the topic of *Acanthaster* sp. control in which I was involved during the course of my PhD. My role in this research was in conception and design of the experiment, conducting the fieldwork, and writing and reviewing drafts of the paper. The inclusion of this publication aims to provide a complete picture of all scientific research conducted during my candidature.

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Chapter 2: Known predators of crown-of-thorns starfish (*Acanthaster* spp.) and their role in moderating (if not preventing) population outbreaks[1]

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[1] Cowan Z-L, Pratchett M, Messmer V, Ling S (2017) Known predators of the crown-of-thorns starfish (*Acanthaster* spp.) and their role in mitigating, in not preventing, population outbreaks. Diversity 9:7

2.1. Abstract

Predatory release has long been considered a potential contributor to population outbreaks of crown-of-thorns starfish (CoTS; *Acanthaster* spp.). This has initiated extensive searches for potentially important predators that can consume large numbers of CoTS at high rates, which are also vulnerable to over-fishing or reef degradation. Herein, we review reported predators of CoTS and assess the potential for these organisms to exert significant mortality, and thereby prevent and/or moderate CoTS outbreaks. In all, 80 species of coral reef organisms (including fishes, and motile and sessile invertebrates) are reported to predate on CoTS gametes (three species), larvae (17 species), juveniles (15 species), adults (18 species) and/or opportunistically feed on injured (10 species) or moribund (42 species) individuals within reef habitats. It is clear however, that predation on early life-history stages has been understudied, and there are likely to be many more species of reef fishes and/or sessile invertebrates that readily consume CoTS gametes and/or larvae. Given the number and diversity of coral reef species that consume *Acanthaster* spp., most of which (e.g., *Arothron* pufferfishes) are not explicitly targeted by reef-based fisheries, links between overfishing and CoTS outbreaks remain equivocal. There is also no single species that appears to have a disproportionate role in regulating CoTS populations. Rather, the collective consumption of CoTS by multiple different species and at different life-history stages is likely to suppress the local abundance of CoTS, and thereby mediate the severity of outbreaks. It is possible therefore, that general degradation of reef ecosystems and corresponding declines in biodiversity and productivity, may contribute to increasing incidence or severity of outbreaks of *Acanthaster* spp. However, it seems unlikely that predatory release in and of itself could account for

initial onset of CoTS outbreaks. In conclusion, reducing anthropogenic stressors that reduce the abundance and/or diversity of potential predatory species represents a “no regrets” management strategy, but will need to be used in conjunction with other management strategies to prevent, or reduce the occurrence, of CoTS outbreaks.

2.2. Introduction

Adult crown-of-thorns starfish (CoTS; *Acanthaster* sp.) have numerous long, very sharp and toxic spines (Fig. 2.1). In addition, the dermal tissues of CoTS (and all of their organs) contain high concentrations of chemicals, including saponins (Howden et al. 1975; Barnett et al. 1988) and plancitoxins (Shiomi et al. 1988), which are both unpalatable (Lucas et al. 1979) and highly toxic (Mackie et al. 1975; Shiomi et al. 1990, 2004). Intuitively therefore, one might expect that these starfish are effectively protected and largely immune from predation (e.g., Yamaguchi 1973). In reality, there are few organisms that are completely immune to predation at any or all stages of their life cycle. Rather, well-developed anti-predatory defences reduce the range of predators to which prey species are vulnerable (Bertness et al. 1981), but may or may not affect overall rates of predation and the extent to which prey populations are controlled by predators. Accordingly, there is an increasing number of coral reef organisms (fishes and invertebrates) reported to predate on CoTS (Pratchett et al. 2014; Rivera-Posada et al. 2014a), including some predators (e.g., *Arothron* pufferfishes) that feed almost exclusively on adult CoTS when they are in abundant supply (e.g., during outbreaks). Such predators may be important in supressing the abundance of prey species (Chesson 2000) as well as influencing the behaviour, habitat-associations, and population dynamics of even well-armoured and/or chemically defended prey species (e.g., Pekar et al. 2015).

Despite their physical and chemical defences, post-settlement stages (juvenile and adults) of CoTS often exhibit injuries, largely manifested as missing arms (McCallum et al. 1989; Rivera-Posada et al. 2014a; Messmer et al. 2017). These injuries are believed to occur when predators are only able to remove one or a few arms before the starfish escapes or avoids further damage by hiding within the reef matrix (McCallum et al. 1989). If however, there are high rates of partial predation at specific reef locations (Rivera-Posada et al. 2014a) then it is expected at least some CoTS will also be killed outright and/or consumed in entirety. The cryptic nature and nocturnal behaviour of CoTS, especially when small (<12 cm diameter) or at low densities (Birkeland and Lucas 1990; Rivera-Posada et al. 2014a) further suggests that they must be highly vulnerable to predators. In controlled experiments, survivorship of laboratory reared *Acanthaster* spp. settled to natural substrates is effectively zero, owing to very high rates of predation by naturally occurring predators (Zann et al. 1987; Keesing and Halford 1992a; Cowan et al. 2016a - Chapter 6). Recent research also demonstrates that CoTS larvae are highly vulnerable to predation (Cowan et al. 2016b - Chapter 4), despite having the highest concentrations of anti-predator chemicals (discussed later). Cowan et al. (2016b - Chapter 4) showed that CoTS larvae are readily consumed by many common planktivorous damselfishes, and often in preference to other asteroid larvae.

While there is now general acceptance that CoTS are vulnerable to predation (e.g., Moran 1986; Rivera-Posada et al. 2014a), on-going controversies relate to whether known predators would ever be capable of regulating CoTS populations, and mitigating, if not preventing outbreaks. More specifically, attention is focussed on whether anthropogenic impacts (via fishing or habitat degradation) have supressed the abundance of key predators, thereby accounting for the seemingly recent and/or increasing occurrence of CoTS outbreaks (Pratchett et al. 2014).

Figure 2.1 Adult crown-of-thorns starfish are defended against predators by numerous long, very sharp and toxic spines. Photographic credit: Scott Ling, Dick's Reef, Swains Region, southern Great Barrier Reef (22°18' S, 152°39' E).

2.2.1. The Predator Removal Hypothesis

The *predator removal hypothesis* was one of the first hypotheses proposed to account for CoTS outbreaks (Endean 1969). Following trophic-cascade concepts as a result of ecological extinction of functional echinoderm predators such as sea otters (e.g., Estes and Palmisano 1974; Estes et al. 1978; Estes and Duggins 1995), lobsters (Ling et al. 2009) and large benthic predatory fishes (Steneck et al. 2002), this hypothesis (like many other hypotheses put forward in the 1960s and 1970s, such as the *nutrient enrichment hypothesis* (e.g., Pearson and Endean 1969; Brodie et al. 2005; Fabricius et al. 2010) is predicated on the idea that CoTS outbreaks are an unnatural phenomenon, caused by anthropogenic modification and degradation of coral reef environments (Potts 1981). The initial formulation of the *predator removal hypothesis* related to apparent overfishing of the giant triton (*Charonia tritonis*) on the GBR in the decades immediately preceding the first documented outbreak of CoTS in 1962 (Endean 1969). Notably, ~10,000 giant tritons were removed from the GBR each year from 1947 to 1960 by trochus fishermen and commercial shell collectors (Endean 1969). Densities of triton must have been significant to sustain this level of removal, or at the very least, much higher than they are now. While there is no empirical data on their abundance, *C. tritonis* are exceedingly rare on the GBR, and have been since the 1960s, perhaps reflecting the legacy of excessive removals in the 1950s (Endean 1973).

Endean (1969) argued that the effective loss of giant triton from reefs in the northern GBR relaxed normally strong regulatory pressure on abundance of juvenile and sub-adult CoTS, leading to increased abundance of large adult starfish that were capable of initiating outbreaks by virtue of their massive combined reproductive output. Adding weight to this hypothesis, outbreaks of CoTS were reported from other locations (e.g., Fiji and Western Samoa) where *C. tritonis* had also been extensively harvested, whilst outbreaks had not been reported in areas (e.g., Malaysia, Philippines and Taiwan) where *C. tritonis* were abundant (Endean 1969). The ability of *C. tritonis* to provide the sufficient top-down control necessary to regulate CoTS populations has since been questioned (e.g., Ormond et al. 1990) largely based on their generally low rates of feeding and the apparent reluctance to eat CoTS when provided with alternative prey.

Though the role of giant triton in regulating abundances of CoTS (past, present, or future) is still not resolved, the *predator removal hypothesis* has evolved through time to place increasing emphasis on fish predators. Attention has focused on large predatory fishes capable of consuming adult starfish (e.g., Ormond et al. 1990; Sweatman 1997; Mendonça et al. 2010), which are targeted by fisheries and/or have declined in abundance due to localized fishing activities. Explicit and direct evidence that any of the major fisheries target species (e.g., coral trout, *Plectropomus* sp.) are significant predators of crown-of-thorns starfish is meagre (Sweatman 1997). However, some studies (Ormond et al. 1990; Dulvy et al. 2004; Sweatman 2008) have reported increased incidence and/or severity of outbreaks of CoTS along gradients of increasing fishing effort. On the GBR, Sweatman (2008) showed that reefs open to fishing were seven times more likely to experience an outbreak of crown-of-thorns starfish (57% of reefs affected) compared to reefs effectively closed to fishing within no-take marine reserves (8% of reefs affected). While the mechanistic basis of these patterns has not been critically tested, increasing evidence of links between fishing and starfish outbreaks (Dulvy et al. 2004; Sweatman 2008) has fuelled significant interest in predation, both to understand the cause(s) and ultimately manage CoTS outbreaks.

2.2.2. Objectives of This Review

The purpose of this review is to synthesise existing knowledge of potentially important CoTS predators, considering their individual and collective capacity to influence population dynamics of CoTS. There is an ever-increasing list of putative predators (e.g., Glynn 1984; Moran 1992; Rivera-Posada et al. 2014a; Table 2.1), largely based on anecdotal observations of different coral reef organisms (mainly fishes) feeding on dead or dying CoTS within reef environments. Our intention in this review is to differentiate between organisms that opportunistically feed on dead or injured CoTS (scavengers), versus those predators that feed on live and healthy starfish and either kill them outright or reduce their individual fitness and/or reduce population level fitness by altering patterns in abundance and distribution. It is possible, for example, that the mere presence of benthic predators could disperse adult CoTS that might otherwise aggregate to spawn, and thereby reduce fertilization success. Moreover, this review will explicitly consider potential predators at different stages in the life cycle of CoTS, especially pre-settlement (e.g., gametes, larvae) and early post-settlement life stages, which is quite possibly the most significant bottleneck in their life-history (McCallum 1988, 1990; Morello et al. 2014). Where possible, we report or derive estimates of the rates of mortality due to predation across different life-history stages of CoTS.

Having established the range of putative CoTS predators, this review will consider empirical and theoretical evidence that supports (or refutes) the potential role of predators in moderating (if not preventing) CoTS outbreaks. If predation underlies observed differences in the incidence or severity of outbreaks across gradients of fishing pressure (Dulvy et al. 2004; Sweatman 2008), we would expect to find that the specific predators would be significantly more abundant in areas with little or no fishing, with corresponding increases in effective rates of predation on juvenile and/or adult CoTS within these areas. Persistent controversy around the role of predation in regulating abundance of CoTS (e.g., Dulvy et al. 2004; Sweatman 2008; Rivera-Posada et al. 2014a) highlights many deficiencies in previous research approaches and points to the definitive need for experimental studies that explicitly test the mechanistic underpinnings of the *predator removal hypothesis*.

2.3. Known Predators of Crown-of-Thorns Starfish

A total of 80 species of coral reef organisms are reported to feed on CoTS, including 24 motile and sessile invertebrates versus 56 species of coral reef fishes (Table 2.1). However, most species have been observed feeding on moribund and dead individuals in the field, while observations of predation on healthy, uninjured starfish are comparatively rare. Similarly, field observations of species feeding on the gametes of CoTS are also extremely limited, and field observations of predation upon larvae are simply not feasible. Gut content analysis has also been largely unsuccessful in identifying putative predatory species (e.g., Ormond and Campbell 1974). However, there have been significant advances in the field of environmental DNA (eDNA) analysis in recent years (Thomsen and Willerslev 2015) and it is likely that this technique could be utilised to both identify previously unknown predators, and establish the frequency with which known predators actually consume CoTS. One suggested method to do this would be to collect faeces of presumed CoTS predators and test this for presence of CoTS DNA. However, there are limitations to this technique. Most notably, it is not possible to distinguish between particular life stages of prey species, nor whether specific prey species were alive or dead when consumed (Thomsen and Willerslev 2015), which is important in understanding the role of predators in structuring populations of CoTS. Such experiments should be supplemented with benthic surveys to confirm presence of juvenile or adult starfish, and plankton tows to confirm presence/absence of CoTS larvae (see Uthicke et al. 2015). This could provide an indication of the CoTS life stage from which DNA found in predator faeces has originated, and would be particularly beneficial for predators such as damselfish which may prey upon both pre- and post-settlement life stages (e.g., Pearson and Endean 1969; Cowan et al. 2016b - Chapter 4).

Table 2.1 Species that feed on different life stages and states of health of *Acanthaster* spp. "F" denotes that the particular predator has been directly observed feeding on a particular life stage in the field, which also includes where starfish were made unnaturally available; "L" denotes where feeding is inferred based on studies in a laboratory/aquarium; "G" denotes that *Acanthaster* remains have been recovered from the stomach of the predator; I = not directly witnessed.

Predator	Sperm	Eggs	Larvae	Juvenile	Adult-Healthy	Adult-Injured	Adult-Moribund/Dead	Reference
Fishes								
Angelfish								
Holacanthus passer						L		(Glynn 1984)
Pomacanthus semicirculatus							F	(Moran 1992)
Pomacanthus sexstriatus							F	(Moran 1992)
Bream								
Scolopsis bilineatus							F	(Rivera-Posada et al. 2014b)
Butterflyfish								
Chaetodon aureofasciatus							F	(Rivera-Posada et al. 2014b)
Chaetodon auriga						L	FL	(Moran 1992; Rivera-Posada et al. 2014a, 2014b)
Chaetodon auripes	F							(Keesing and Halford 1992b)
Chaetodon citrinellus						L		(Glynn 1984)
Chaetodon plebeius							F	(Rivera-Posada et al. 2014b)
Chaetodon rafflesi							F	(Rivera-Posada et al. 2014b)
Chaetodon rainfordi							F	(Rivera-Posada et al. 2014b)
Chaetodon vagabundus							FL	(Rivera-Posada et al. 2014b; Boström-Einarsson and Rivera-Posada 2016)
Damselfish								
Abudefduf sexfasciatus		F	L					(Endean 1969; Cowan et al. 2016b)
Acanthochromis polyacanthus			L				L	(Boström-Einarsson and Rivera-Posada 2016; Cowan et al. 2016b)
Amblyglyphidodon curacao		F	L					(Pearson and Endean 1969; Cowan et al. 2016b)
Chromis atripectoralis			L					(Cowan et al. 2016b)
Chromis caerulea						L	F	(Moran 1992; Rivera-Posada et al. 2014a)
Chromis dimidiata			L					(Lucas 1975)

Chromis viridis	L					(Cowan et al. 2016b)
Chrysiptera rollandi	L					(Cowan et al. 2016b)
Dascyllus aruanus	L					(Cowan et al. 2016b)
Dascyllus reticulatus	L					(Rivera-Posada et al. 2014b)
Neoglyphidodon melas					F	(Rivera-Posada et al. 2014b)
Neoglyphidodon oxyodon					F	(Cowan et al. 2016b)
Neopomacentrus azysron	L					(Cowan et al. 2016b)
Pomacentrus amboinensis	L					(Rivera-Posada et al. 2014b)
Pomacentrus chrysurus					F	(Moran 1992; Rivera-Posada et al. 2014a, 2014b; Cowan et al. 2016b)
Pomacentrus moluccensis	L			L	FL	(Rivera-Posada et al. 2014b)
Pomacentrus wardi					F	(Glynn 1984)
Stegastes acapulcoensis				F		(Rivera-Posada et al. 2014b)
Stegastes nigricans					F	(Rivera-Posada et al. 2014b)
Emperors						
Lethrinus atkinsoni		F			FL	(Sweatman 1995; Rivera-Posada et al. 2014b)
Lethrinus miniatus		F				(Birdsey 1988; Sweatman 1995)
Lethrinus nebulosus			G		F	(Birdsey 1988; Rivera-Posada et al. 2014b)
Goatfish						
Parupeneus multifasciatus					F	(Rivera-Posada et al. 2014b)
Gobies						
Cryptocentrus sp.					F	(Moran 1992)
Groupers						
Epinephelus lanceolatus		FG				(Endean 1976)
Pufferfish						
Arothron hispidus		F	FL	L	FL	(Ormond et al. 1973; Ormond and Campbell 1974; Glynn 1984; Rivera-Posada et al. 2014a, 2014b; Boström-Einarsson and Rivera-Posada 2016)
Arothron manilensis					L	(Rivera-Posada et al. 2014b)
Arothron meleagris					F	(Glynn 1984)
Arothron nigropunctatus					F	(Moran 1992)
Arothron stellatus			F			(Keesing and Halford 1992b)

Triggerfish					
Balistapus undulatus				F	(Rivera-Posada et al. 2014b)
Balistoides viridescens	L	FL		L	(Ormond et al. 1973; Ormond and Campbell 1974; Rivera-Posada et al. 2014a, 2014b; Boström-Einarsson and Rivera-Posada 2016)
Pseudobalistes flavimarginatus	L	FGL			(Owens 1971; Ormond and Campbell 1974; Rivera-Posada et al. 2014a)
Rhinecanthus aculeatus				L	(Boström-Einarsson and Rivera-Posada 2016)
Sufflamen verres				F	(Glynn 1984)
Wrasses					
Cheilinus diagrammus				F	(Moran 1992)
Cheilinus fasciatus				F	(Moran 1992)
Cheilinus undulatus		FG			(Chesher 1969a; Ormond and Campbell 1974; Randall et al. 1978; Birdsey 1988)
Coris caudomacula				F	(Rivera-Posada et al. 2014b)
Halichoeres melanurus				L	(Boström-Einarsson and Rivera-Posada 2016)
Thalassoma hardwicke				F	(Moran 1992)
Thalassoma lucasanum			FL		(Glynn 1984; Rivera-Posada et al. 2014a)
Thalassoma lunare				FL	(Rivera-Posada et al. 2014b; Boström-Einarsson and Rivera-Posada 2016)
Thalassoma nigrofasciatum				F	(Rivera-Posada et al. 2014b)
Motile invertebrates					
Acanthaster planci				F	(Moran 1992)
Alpheus sp.				F	(Moran 1992)
Bursa rubeta	I	I			(Alcala 1974) in (Moran 1986)
Cassis cornuta		L			(Chesher 1969a)
Charonia tritonis	F	FL		F	(Endean 1969, 1973; Pearson and Endean 1969; Moran 1992)
Cymatorium lotorium		I			(Ormond and Campbell 1974)
Dardanus sp.		I			(Ormond and Campbell 1974)
Diadema mexicanum				F	(Glynn 1984)

Dromidiopsis dormia			I			(Alcala 1974) in (Moran 1986)
Hymenocera elegans/picta		L	F	F		(Wickler and Seibt 1970; Glynn 1982a, 1984)
Murex sp.			L			(Chesher 1969a)
Neaxius glyptocercus		I	I			(Brown 1970) in (Moran 1986)
Panulirus penicillatus		L				(Zann et al. 1987)
Pherecardia striata		F		F	F	(Glynn 1982a, 1984)
Trapezia flavopunctata	L					(Cowan et al. 2016a)
Trapezia bidentata	L					(Cowan et al. 2016a)
Trapezia cymodoce	L					(Cowan et al. 2016a)
Trizopagurus magnificus					F	(Glynn 1984)
Xanthidae		L				(Lucas 1975)
Sessile invertebrates						
Paracorynactis hoplites		F	F			(Bos et al. 2011)
Platygyra sp.	L					Cowan Pers. obs.
Pocillopora damicornis	L					(Yamaguchi 1973)
Pseudocorynactis sp.			F			(Bos et al. 2008)
Stoichactis sp.			L			(Chesher 1969a)

2.3.1. Pre-Settlement Predation

Most of the putative CoTS predators feed or scavenge on post-settlement life stages (juveniles and adults) compared to pre-settlement stages (Fig. 2.2, 2.3). However, this probably reflects limited research directed at identifying potential predators on CoTS eggs and larvae and/or difficulties in documenting predation on these early life-history stages. Coral reefs typically support very high abundance and diversity of planktivorous species, including many different reef fishes (e.g., Pearson and Endean 1969; Keesing and Halford 1992b; Ciarapica and Passeri 1993; Cowan et al. 2016b - Chapter 4) as well as sessile invertebrates, such as corals (Yamaguchi 1973), which may consume CoTS propagules during spawning, as well as feeding on CoTS larvae when they settle. CoTS are one of the most fecund invertebrates (Babcock et al. 2016b), with very high fertilisation rates (Babcock 1990; Babcock and Mundy 1992), but intuitively, most eggs and larvae must fail to survive. As for other marine species with planktonic larvae, significant rates of pre-settlement mortality are also likely to arise due to predation (Bailey and Houde 1989; Fabricius and Metzner 2004). Yet, given their exceptional reproductive potential (Babcock et al. 2016b), even small changes in the proportion of larvae that survive and settle will lead to vast differences in the absolute number of juvenile and adult starfish.

Early studies suggested that CoTS eggs and larvae were largely immune from predation due to unpalatable chemical defences (saponins) contained within (Lucas et al. 1979). However, more recent examination of predation upon both eggs (Cowan et al. 2017 - Chapter 3) and larvae (Cowan et al. 2016b - Chapter 4) reveals that these are indeed readily consumed by a range of highly abundant, planktivorous damselfishes. Given that this group of predators can be extremely abundant on coral reefs, it is likely that they play an important role in reducing the proportion of CoTS that survive through to settlement, and high densities of damselfishes should be considered important for the buffering capacity of coral reefs. Given that all the planktivorous damselfishes tested in the recent studies (Cowan et al. 2016b - Chapter 4, 2017 - Chapter 3) consumed CoTS material, albeit to varying extents, it is highly likely that there are more predators of the pre-settlement stages that are yet to be identified. Furthermore, the actual suite of predators that prey upon the early life stages of CoTS is likely to span a far greater taxonomic range, from benthic species such as those already identified (e.g., Yamaguchi 1973; Cowan et al. 2016a - Chapter 6; Table 2.1) to large pelagic fishes, such as manta rays and whale sharks.

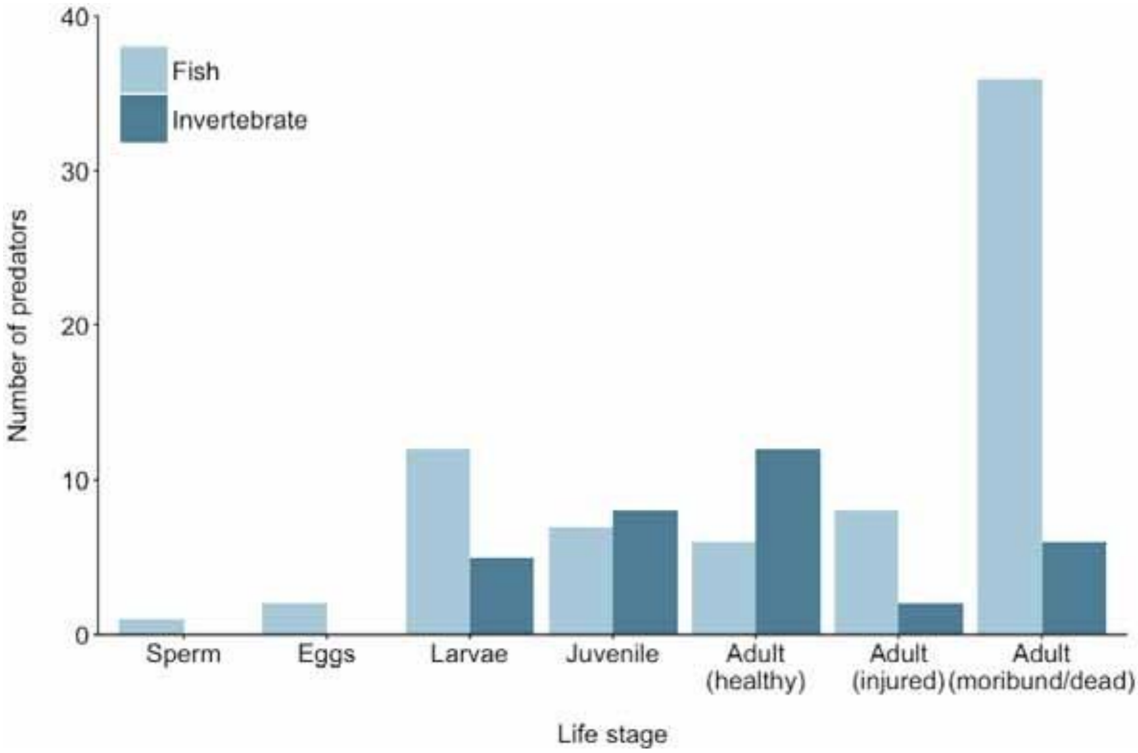

Figure 2.2 Putative predators of crown-of-thorns starfish across each major life stage. Predators at each life stage are not mutually exclusive. This figure is based on references from Table 2.1.

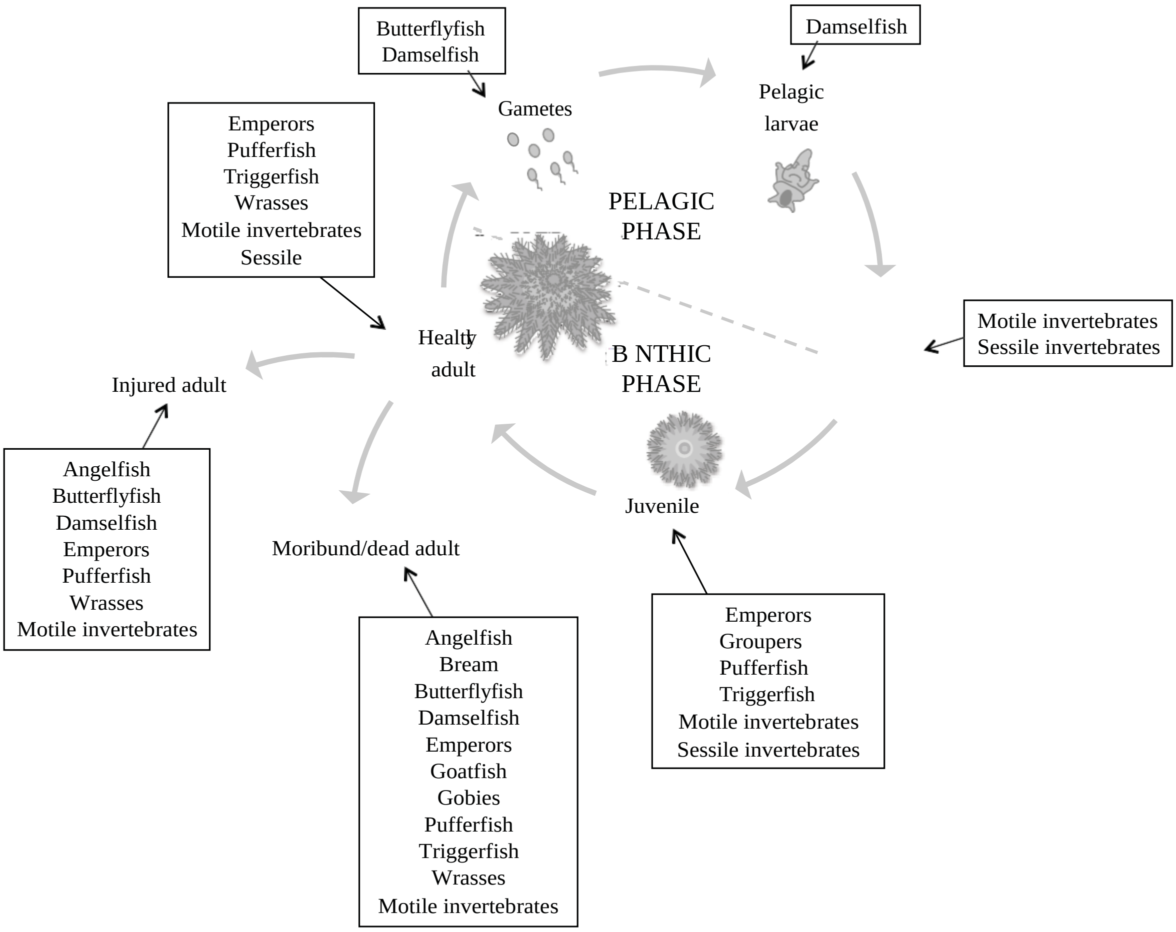

Figure 2.3 Main predatory groups acting at different life stages of crown-of-thorns starfish. This figure is based on references from Table 2.1.

2.3.2. Post-Settlement Predation

A key component of the vulnerability of larvae is their susceptibility to predation during settlement. Larvae preferentially settle in habitats with fine-scale topographic complexity (Lucas 1975), which is likely to be an adaptation to minimise early post-settlement mortality. However, a wide range of potential predators are abundant within the reef matrix (e.g., Glynn 1984; Keesing and Halford 1992a; Keesing et al. 1996; Rivera-Posada et al. 2014). Benthic predators and filter feeders, including corals such as *Pocillopora damicornis*, may exact a heavy toll on the settling larvae of coral reef asteroids, including *Acanthaster* spp. (Yamaguchi 1973). Keesing and Halford (1992b) estimate mortality at settlement and during metamorphosis to be in excess of 85%. Furthermore, Cowan et al. (2016a - Chapter 6) observed that 55% of brachiolaria larvae that settled to rubble with naturally attached crustose coralline had undergone metamorphosis after 48-hours when substrates had been cleaned of potential predators, compared to 0% when polychaete predators were present. If predation represents a significant threat to settling larvae, this should have provided sufficient selective pressure for the evolution of behavioural mechanisms to evade predators, as seen in juveniles and adults in low-density populations. Cowan et al. (2016a - Chapter 6) used static choice chambers to determine whether such mechanisms are present to assist larvae in avoiding settlement on or near predators, revealing that larvae are able to both detect, and respond to, the presence of predators within settlement substrates; larvae were attracted to rubble with naturally attached crustose coralline algae that had been cleaned of predators, but were deterred from these substrates when polychaete predators were present. Whether similar mechanisms are present to enable juveniles to detect and actively avoid predators in the reef matrix remains to be tested.

Following settlement, many marine organisms experience very high early post-settlement mortality (e.g., asteroids (Yamaguchi 1973); bivalves (Roegner and Mann 1995); fish (Almany and Webster 2006); corals (Chong-Seng et al. 2014)), to the extent that this is a recognised demographic bottleneck for some taxa (Chong-Seng et al. 2014). Although Sweatman's (1995) measurements of predation by fishes on laboratory-reared juvenile CoTS (25-79 mm diameter) revealed very low predation rates (0.13% per day), predation on juvenile CoTS is due mainly to epifaunal invertebrate predators, which are highly abundant on coral reefs (e.g., Keesing and Halford 1992a; Keesing et al. 1996). For example, survivorship of laboratory reared CoTS that are settled to freshly collected rubble is effectively zero, owing to very high rates of predation on newly settled larvae by naturally occurring predators (e.g., polychaetes) within the rubble (Keesing and Halford 1992a).

In the eastern Pacific, Glynn (1984) demonstrated that the harlequin shrimp *Hymenocera picta* and a polychaete worm *Pherecardia striata* were significant predators of CoTS (Glynn 1977, 1982a, 1984). Moreover, there have not been any outbreaks, or persistently high numbers of CoTS on reefs in Panama, where these two predators are found in high abundance, and where populations of alternative prey (ophidiasterids) are scarce. Although both field (Glynn 1977) and laboratory studies (Ormond et al. 1973; Wickler 1973; Glynn 1977) demonstrate that the shrimp has difficulties attacking larger and more active CoTS, it is expected that they are highly effective predators of smaller, more cryptic CoTS within the reef matrix (Glynn 1984). The observed preference of *H. picta* for other asteroid prey species, particularly smaller ophidiasterids (Ormond and Campbell 1974), and the strong preference of *P. striata* for crustacean tissue over tissue from CoTS (Glynn 1984) emphasises how the relative scarcity of alternative prey may be an important factor influencing the capacity of a potential predator to manipulate the population dynamics of CoTS (Glynn 1977). If they are important in regulating populations of CoTS, this may occur within a relatively restricted geographical area.

The polyp *Pseudocorynactis* sp. may also play an important role in the population control of CoTS (Bos et al. 2008) in areas where it occurs in high abundances, such as Sogod Bay, Philippines. *Pseudocorynactis* sp. prey on a range of echinoderms and has been observed ingesting adult CoTS up to 250 mm in diameter (Bos et al. 2008). Furthermore, *Pseudocorynactis* sp. preferentially settles under coral ledges and in reef crevices, where it likely predates on cryptic juvenile and sub-adult CoTS (Bos et al. 2008)..

2.4. Rates of Predation on Crown-of-Thorns Starfish

Understanding of the importance of predation in the population dynamics of CoTS is significantly constrained by a lack of empirical data on background mortality rates and natural predation rates. Quantifying mortality rates of CoTS in the field is tractable, but requires significant investment to follow the fate of a large number of uniquely tagged or recognizable individuals (*sensu* Ling and Johnson 2012a) across a broad size range of individuals and in different habitats. The biggest limitation to such experiments is the limited capacity to tag CoTS (e.g., Glynn 1982b). Previous methods used to distinguish individual starfish (staining, branding, tagging and dyeing) were only effective for days to weeks, limiting any capacity to get meaningful long-term data on rates of mortality (Glynn 1982b). Identifying more permanent tagging solutions is essential, but even short-term tagging and tethering experiments could yield important information about the vulnerability of CoTS to predation.

Short-term tagging and tethering experiments have been used to effectively estimate or compare predation vulnerability for a range of echinoderms, especially echinoids or urchins (e.g., McClanahan and Muthiga 1988; Bonaviri et al. 2009; Ling and Johnson 2012a). These studies typically use very short observations (Š3 days) to estimate predation rates, though some recent studies have been conducted over several months; Ling and Johnson (2012a) successfully tagged and tethered the urchin *Centrostephanous rodgersii*, and measured subsequent survivorship >100 days. At present, there are few direct, quantitative estimates of predation or mortality rates for CoTS in the field, which are critical for establishing the importance of predation in limiting the densities of starfish at individual reefs (Moran 1986).

Previous research on CoTS predators has focused on estimating maximum rates of starfish consumption by individual predators and extrapolating this to account for natural densities of these predators (e.g., Chesher 1969b; Pearson and Endean 1969; Potts 1982). This is based on the belief that effective control of outbreak populations is fundamentally reliant on high rates of predation to compensate for rapid population growth of CoTS when outbreaks begin. However, it is not the rate of feeding per se that is important in determining whether a predator can effectively regulate prey densities (Chesson 2000), but changes in the rates of predation in response to spatial and temporal gradients in prey abundance. Predators that are capable of consuming large numbers of CoTS, by virtue of their high abundance and/or individual capacity to consume large numbers of CoTS (Cowan et al. 2016b - Chapter 4), may be important in suppressing local CoTS densities. Cowan et al. (2016b - Chapter 4) showed that a wide range of planktivorous damselfishes will feed on CoTS larvae, some of which (*D. aruanus* and *P. amboinensis*) have very high satiation limits. The capacity of these fishes to consume large numbers of CoTS larvae, combined with high densities of these fishes (as well as many other planktivorous fishes that may also readily feed on CoTS larvae) could be critical in limiting settlement rates (McCallum 1987; Morello et al. 2014) and thereby moderating the local severity of outbreaks. It is unlikely however, that these fishes could actually prevent an outbreak from ever occurring, unless either (i) they selectively target CoTS larvae (over other potentially more abundant prey) even at very low prey densities, and thereby prevent initiation of outbreaks; or (ii) their combined feeding capacity exceeds the very large number of CoTS larvae that can cause the rapid and pronounced onset of some outbreaks.

Early research on putative CoTS predators largely dismissed the importance of generalist reef predators, suggesting instead that important predators would have to be highly specialized (preferentially feeding on CoTS to the exclusion of almost all other potential prey) and feed on adult CoTS (Potts 1982). Importantly, if there are specialist predators that are highly effective in finding and killing CoTS even when they occur at very low densities, then it may be these predators that are key in preventing outbreaks from ever occurring (McCallum 1987). However, effective CoTS predators must be sufficiently generalist to consume alternative prey (McCallum 1987) and thereby sustain themselves during non-outbreak periods when CoTS are scarce. If predation (or lack thereof) is a potential cause of CoTS outbreaks, it seems that we should also be focusing on predators that target pre-settlement, settlement, and post-settlement pre-reproductive stages (McCallum 1990; Morello et al. 2014). Most notably, predation by benthic invertebrates on newly settled starfish appears, at present, to be the most significant bottleneck in their life-history (Morello et al. 2014), but this may be largely attributable to underestimates of predation rates on CoTS during other life-stages.

Quantitative data on predation rates is rare and in most cases comes from experimental studies that aim to determine maximum predation rates by specific organisms (e.g., Pearson and Endean 1969; Keesing and Halford 1992a, 1992b; Bos et al. 2011; Cowan et al. 2016b - Chapter 4), or modelling efforts that predict the rate of predation needed to prevent outbreaks (e.g., McCallum 1987, 1990, 1992; Ormond et al. 1990). Based on caging experiments, the triton shell *C. tritonis* is estimated to consume 0.7 adult starfish per week (Pearson and Endean 1969), however attacks are not always fatal (Chesher 1969a) and this predator prefers to feed on other starfish when given a choice (Pearson and Endean 1969). Furthermore, pre-fishing numbers of *C. tritonis* remain largely unknown and it is unlikely that this invertebrate was ever present in sufficiently high numbers to prevent outbreaks (Ormond and Campbell 1971; McClanahan 1989). Starfish numbers are persistently low in areas where the corallimorph *P. hoplites* (Bos et al. 2011) or harlequin shrimp *H. picta* together with the lined fireworm *P. striata* (Glynn 1982a) are abundant. Consumption rates of adult starfish (up to 340 mm diameter) by *P. hoplites* are estimated to be 29.5 g day^{-1} (Bos et al. 2011). In the eastern Pacific, Glynn (1982a) reported that 5%-6% of the CoTS were being attacked by *H. picta* at any time, and 0.6% of the starfish population were preyed upon by both *H. picta* and *P. striata*. Approximately 50% of CoTS being attacked by

H. picta ultimately died, compared to close to 100% for CoTS being attacked by both *H. picta* and *P. striata*, and these two predators are particularly effective in regulating numbers of juvenile starfish (Glynn 1982a). Keesing and Halford (1992a) measured significant mortality rates on post-metamorphic starfish (5.05% day^{-1} on 1-month old starfish and 0.85% day^{-1} on 4-month old starfish), due to predation by epibenthic fauna contained within dead coral rubble. This is much higher than the attack rate on juvenile starfish (1%-1.5%) that McCallum (1988) suggested would be sufficient to limit the occurrence of CoTS outbreaks, based on demographic modelling. Cowan et al. (2016b - Chapter 4) conducted a series of laboratory experiments, measuring maximal predation rates by a wide range of planktivorous damselfishes upon CoTS larvae, reporting consumption rates ranging from 14 larvae h^{-1} by *C. viridis*, up to 158 larvae h^{-1} by *D. aruanus*.

Ormond and Campbell (1974) suggest that the predatory behaviour of large fishes (the pufferfish *A. hispidus*, and triggerfishes *P. flavimarginatus* and *B. viridescens*) may have the capacity to control densities of adult CoTS in the Red Sea, and may be capable of disbanding aggregations in their early stages. It is however, unlikely that these species would be effective in controlling CoTS populations across the entire Indo-Pacific region as they are not universally common (Endean 1977). However, triggerfishes (particularly, *Balistapus undulatus*) may nonetheless be important maintaining the structure of coral reef ecosystems, by predating on rock-boring urchins (McClanahan and Muthiga 2016). There may be other large predatory fishes capable of regulating densities of adult CoTS; Keesing and Halford (1992b) observed the pufferfish *A. stellatus* to be capable of consuming adult starfish (20 cm diameter) in less than 10 minutes. Further, Ormond et al. (1990) reported greater mean densities of lethrinids and large fish predators from the Red

Sea, where no major outbreaks of CoTS were known to have occurred, compared to the GBR, where two cycles of large scale outbreaks had occurred (Ormond et al. 1990). Further, within the GBR, mean predatory fish densities were found to be reduced on reefs that were experiencing major outbreaks (Ormond et al. 1990). Fish species examined were commercially targeted or frequently caught as bycatch, and variations in population densities of predatory fish between sites was compatible with fisheries data on fishing intensity, therefore the pattern of difference in CoTS populations could be explained by differing fishing intensities between locations (Ormond et al. 1990).

2.4.1. Sub-Lethal Injuries

Discussions to this point have focused on instances of whole animal or lethal predation, but sublethal (or partial) predation is often very apparent and well documented among echinoderms (Lawrence and Vasquez 1996; Ling and Johnson 2012b). For CoTS, very high proportions of adults (up to 60%) have evidence of recent injuries (most apparent as missing arms), which is attributed to partial predation (McCallum et al. 1989). Even if the predation is not immediately fatal, sublethal attacks may still have an important influence on population dynamics. In the short term, open injuries and exposed internal organs may increase the likelihood of pathogenic infections and disease transmission among individuals (Rivera-Posada et al. 2011, 2012; Caballes et al. 2012) and can also increase susceptibility to further attacks (Glynn 1984). Even if starfish effectively repair injuries caused by partial predation, effective declines in the size of individuals caused by sub-lethal predation will reduce food intake (Lawrence 1991, 2010; Lawrence and Vasquez 1996). Crown-of-thorns starfish also regrow damaged or missing arms (Pearson and Endean 1969; Messmer et al. 2013), which will require re-allocation of nutrients to regeneration, which could otherwise have been used for immune defence (Rivera-Posada et al. 2014), reproduction or somatic growth (Lawrence 1991; Lawrence and Vasquez 1996). The removal of arm tips, and consequent removal of the eyespot, may additionally result in reduced foraging efficiency due to the loss of vision, which is important for navigating between reef structures and locating prey (Sigl et al. 2016).

There are strongly opposing views about the inferences of high incidence of partial predation in populations of crown-of-thorns starfish. In general, high incidence of sub-lethal predation has been considered to be generally reflective of high intensity of predation (e.g., McCallum et al. 1989; Rivera-Posada et al. 2014), such that high rates of partial mortality serve as a proxy for high levels of overall mortality. In the Philippines, Rivera-Posada et al. (2014) showed that the incidence of injuries on CoTS was highest within a no-take marine reserve, and supporting information on the high diversity and abundance of reef fishes corroborate the idea that overall intensity of predation would likely be much higher inside versus outside of this reserve. Conversely, high incidence of partial mortality may reflect low intensity of predation pressure (Birkeland and Lucas 1990) because when predation is intense it would be expected that most predation events would result in complete mortality. As such, high incidences of starfish with partial injuries point to the strong regenerative capacity of starfish (Messmer et al. 2017) and may suggest that predation is predominantly sub-lethal for crown-of-thorns starfish. Schoener (1979) suggested that there is no reason to expect any relationship between rates of injuries versus rates of predation-driven mortality, because the efficiency of predation (the proportion of attacks that cause death) is independent of the attack rate or incidence of predation. For the asteroid *Asterias amurensis*, high density populations have been observed to be completely annihilated by an incursion of thousands of spider crabs (*Leptomithrax gaimardii*) that moved to shallow reefs in large numbers (Ling and Johnson 2012b). Additionally, where crab incursions involved fewer individuals, high rates of sub-lethal injury (~70% of starfish population injured) occurred independently of mass predator-driven mortality (Ling and Johnson 2012b). In this example, the high mobility of spider crabs can lead to overwhelming local impacts on starfish populations but this impact is highly variable across space and time (Ling and Johnson 2012b). Ultimately, a dedicated research project is needed to test the relationship between rates of partial versus complete predation mortality. However, in the absence of any empirical data on overall rates of predation mortality, the incidence of injuries serves as the best proxy to test for variation in vulnerability to predation among locations and with size of starfish (McCallum et al. 1989).

A common trend among echinoderm species is for the incidence of sub-lethal injuries to decline with increasing body size (McClanahan and Muthiga 1988; Lawrence 1991; Lawrence and Vasquez 1996; Marrs et al. 2000; Ling and Johnson 2012b; Messmer et al. 2017) reflecting general declines in the vulnerability to predation. Accordingly, Messmer et al. (2017) observed a clear linear decline in both rate and severity of predation with increasing starfish size. Rivera-Posada et al. (2014) reported highest incidence of arm damage in the intermediate (11-20 cm diameter) size class however. This pattern may be explained by changes in behavioural and physical characteristics with increasing size, whereby intermediate sized individuals may have greatest exposure to predators, while smaller starfish (<10 cm) tend to remain hidden but are also more likely to be completely consumed (Birkeland and Lucas 1990). Reduced incidence of sublethal predation in largest individuals (Š21 cm) may be explained by increased length of spines (Rivera-Posada et al. 2014) and satiation of predators following removal of a smaller proportion of the total body mass. Disparity between the trends observed in these studies may be a result of differing sample size or differing suites of predators between the locations on the GBR (Messmer et al. 2017) versus in the Philippines (Rivera-Posada et al. 2014).

Sub-lethal and/or 'trait-mediated' effects of predators (Ling and Johnson 2012b) can include alteration of behaviour and spatial patterns in echinoderms, in addition to changes in adult abundances and local spatial distributions, which are important for the reproductive ecology of free-spawning marine invertebrates as they influence rates of fertilization success (Ling et al. 2012). For CoTS, fertilization success may be close to 100% when spawning individuals are adjacent to each other (Benzie et al. 1994), thus any predator that is capable of dissipating an aggregation, or sufficiently reducing adult density, is likely to have a significant impact on zygote production. Humans have been shown to indirectly alter spatial distributions of asteroids, leading to much higher rates of fertilization in human-driven hotspots of zygote production (Schoener 1979). In this way, sub-lethal predatory effects, leading to reduced individual reproductive performance, in combination with alteration to spatial configurations at the time of spawning may impact on zygote production for asteroids (Ling et al. 2012), potentially influencing the occurrence of secondary outbreaks for CoTS (Endean 1974).

2.4.2. Population Modelling

Population simulation models provide a means of exploring the possible role of predation (or other natural causes of mortality) in regulating populations of CoTS (e.g., McCallum 1987), especially given little or no empirical data on satiation levels and feeding efficiency of potential predators or predation rates on larval and juvenile CoTS. Simulation models indicate that changes to predation rates during the pre-reproductive, post-settlement stage may be particularly relevant in understanding the dynamics of CoTS populations (McCallum 1987, 1988, 1990). Notably, given their exceptional reproductive capacity, small changes in proportional survivorship or settlement success of CoTS larvae will result in large changes in adult abundance. Accordingly, McCallum (1990) argues that relatively subtle changes in the abundance of predators (e.g., caused by exploitation) and/or predation rates, will reduce the level of local recruitment required to overcome (or satiate) predators.

The potential ecological importance of predation as a regulatory factor upon CoTS populations depends largely upon the ability of predators to find and consume prey (Hassell 1978). Quantification of the functional response, described by the intake rate of prey as a function of prey density (Holling 1965), is a common method that provides insight into the dynamics of predator-prey systems (Abrams 1990; Buckel and Stoner 2000; Nilsson and Ruxton 2004). Functional responses may be classified into three types. A type I response assumes a linear increase in the intake rate with increasing food density, generally up to a maximum value, beyond which the intake rate is constant (Holling 1959). Type II is characterized by a decelerating intake rate (Holling 1959) and assumes that the predator is limited by its ability to process food (Kaspari 1990; Baker et al. 2010). Type III is associated with an accelerating intake rate, associated with prey switching behaviour (preferential consumption of the most common type of prey (Murdoch 1969)), up to a saturation point (Holling 1959). Predators that exhibit prey-switching behaviour, feeding more frequently on CoTS in response to a marked increase in their local abundance, may be capable of dissipating an aggregation in its early stages (Ormond et al. 1973). In order to persist when population densities of CoTS are low, predators should be able to take a range of prey, exhibiting an increased feeding response in reaction to a rapid increase in the CoTS population (Ormond et al. 1973). Predators exhibiting these type II and type III functional responses (Holling 1965) are typical of vertebrate predators, reinforcing the focus on fish, and are supported in McCallum's (McCallum 1987; McCallum et al. 1989) population models. In laboratory feeding experiments on *Acanthaster* spp. larvae, planktivorous damselfishes exhibit primarily a type II functional response, indicating their capacity to consume sufficient larvae to suppress settlement rates when larvae are already scarce, thereby contributing to very low natural densities of CoTS recorded outside of outbreak periods (Cowan et al. 2016b - Chapter 4). However, very high densities of larvae, which are a necessary condition for the rapid and pronounced onset of outbreaks, are likely to swamp even the combined consumption capacity of all planktivorous reef fishes (McCallum 1988).

2.5. Conclusions

Crown-of-thorns starfish are vulnerable to predation from a wide range of coral reef organisms, and at all stages of their life cycle. Despite identification of potentially key predators, and groups of predators, natural predation rates in both outbreaking and non-outbreaking populations remain largely unknown. This considerably limits our understanding of the role of predation in structuring the population dynamics of CoTS and of approaches to managing their often-devastating impacts on coral reef ecosystems. Whilst predation is likely to be important in suppressing settlement rates and contributing to naturally low densities of CoTS, the initiation and spread of outbreaks cannot, at present, be definitively attributed to changes (presumably declines) in the abundance of predators and/or changes in predation rates (e.g., Babcock et al. 2016). Babcock et al. (2016) showed that there are likely to be multiple factors that contribute to outbreaks of CoTS, such that a diverse range of management strategies will be required to prevent, or reduce the occurrence, of outbreaks. Maximising the number and diversity of putative CoTS predators is nonetheless, a "no regrets" strategy to minimise the risk of CoTS outbreaks and increase the resilience of coral reef ecosystems, generally. In the meantime, further research into potential predators, as well as estimates of predation and mortality across all life-stages of CoTS, is still warranted. More specifically, rates of pre- and post-settlement predation should be explicitly compared along known gradients in abundance of putative CoTS predators (e.g., inside and outside of marine reserves with marked differences in the abundance and diversity of fishes that feed on CoTS). New technologies provide improved opportunities to explore spatial and temporal variation in the demographics of CoTS populations, for example, DNA screening of diets for large numbers of potential CoTS predators (Redd et al. 2014; Thomsen and Willerslev 2015), plus increased potential to tag and track benthic species within reef environments (MacArthur et al. 2008). Such novel approaches along with remote sensing techniques will provide new insights into changes in the population dynamics and/or environmental conditions during the onset of population outbreaks.

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Chapter 3: Inter-specific variation in potential importance of planktivorous damselfishes as predators of *Acanthaster* sp. eggs[2]

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[2] Cowan Z-L, Ling SD, Dworjanyn SA, Caballes CF, Pratchett MS (2017) Inter-specific variation in potential importance of planktivorous damselfishes as predators of *Acanthaster* sp. eggs. *Coral Reefs* doi 10.1007/s00338-017-1556-y

3.1. Abstract

Coral-eating crown-of-thorns starfish (*Acanthaster* sp.) often exhibit dramatic population outbreaks, suggesting that their local abundance may be relatively unchecked by predators. This may be due to high concentrations of anti-predator chemicals (saponins and plancitoxins), but the effectiveness of chemical deterrents in protecting *Acanthaster* sp., especially spawned eggs, from predation remains controversial. We show that planktivorous damselfishes will readily consume food pellets with low proportions (Š 80%) of eggs of crown-of-thorns starfish. However, all fishes exhibited increasing rejection of food pellets with higher proportions of starfish eggs, suggesting that chemicals in eggs of crown-of-thorns starfish do deter potential predators. Interestingly, palatability thresholds varied greatly among the nine species of planktivorous fish tested. Most notably, *Amblyglyphidodon curacao* consumed food pellets comprising 100% starfish eggs 1.5 times more than any other fish species, and appeared largely insensitive to increases in the concentration of starfish eggs. After standardising for size, smaller fish species consumed a disproportionate amount of pellets comprising high proportions of starfish eggs, indicating that abundant small-bodied fishes could be particularly important in regulating larval abundance and settlement success of crown-of-thorns starfish. Collectively, this study shows that reef fishes vary in their tolerance to anti-predator chemicals in crown-of-thorns starfish and may represent important predators on early life-history stages.

3.2. Introduction

Predation, especially predation on eggs and larvae, is considered among the most important factors that regulate the abundance of marine organisms (Bailey and Houde 1989; Hixon et al. 2002). This is because predation is often the major cause of pre-settlement mortality (e.g. Babcock et al. 1986; Bachiller et al. 2015) and may also be density dependent (Hixon et al. 2012). However, there are few studies that have attempted to quantify predation rates, let alone mortality rates, during pre-settlement life stages of marine organisms (Leis 1991). Moreover, high rates of predation on eggs and pre-settlement larvae are routinely inferred based on the sheer abundance of planktivorous organisms (e.g. Hamner et al. 1988) and the readiness with which such planktivores consume eggs and larvae during experimental feeding studies (e.g. Baird et al. 2001).

The role of predation in regulating populations of crown-of-thorns starfish (*Acanthaster* sp.) is highly controversial. On one hand, over-exploitation of key predators (e.g. giant triton, *Charonia tritonis*) is one of the primary mechanisms put forward to account for the seemingly recent occurrence of outbreaks of crown-of-thorns starfish (Endean 1969). The "predator removal hypothesis" infers that populations of crown-of-thorns starfish are normally regulated by high rates of predation and that outbreaks are due (or partly due) to overharvesting of predators (Endean 1977) and/or resulting trophic cascades (Dulvy et al. 2004). Conversely, crown-of-thorns starfish have well-developed physical and chemical predatory defences and are considered to be largely immune from predation (Lucas et al. 1979; Gladstone 1992). Eggs and larvae of crown-of-thorns starfish have particularly high concentrations of saponins (Barnett et al. 1988), which have been directly implicated in defence against predators (Yamaguchi 1974, 1975; Voogt and Van Rheenen 1979).

The high incidence of injuries among adult *Acanthaster* sp. (e.g. Branham 1973; McCallum et al. 1989; Rivera-Posada et al. 2014) is attributed to sub-lethal predation and suggests that despite their spines and potentially toxic chemicals, adult crown-of-thorns starfish are vulnerable to predation. Accordingly, there are an increasing number of reef fishes and macro-invertebrates that are known to feed on crown-of-thorns starfish (Rivera-Posada et al. 2014). However, it is unclear to what extent these predators kill adult starfish and effectively reduce local densities of crown-of-thorns starfish (Pratchett et al. 2014). Intuitively, predation on smaller starfishes is more likely to be lethal, and population modelling suggests that predation during early life-history stages (e.g. eggs and larvae) is likely to be much more important in regulating abundance of crown-of-thorns starfish than adult predation (McCallum 1990, 1992; Morello et al. 2014). There are several (mostly anecdotal) reports of small-bodied reef fishes, predominantly damselfishes, feeding on the eggs and/or larvae of crown-of-thorns starfish (e.g. Pearson and Endean 1969; Keesing and Halford 1992; Ciarapica and Passeri 1993). However, it does seem that there are marked interspecific differences among fishes in their readiness to eat early life-history stages of crown-of-thorns starfish, and apparent differences in the palatability of eggs versus larvae (Lucas et al. 1979; Cowan et al. 2016).

Reef-based planktivores may exert a significant toll on the reproductive output and settlement success of coral-reef organisms, both during spawning, when planktivores can take advantage of high concentrations of gametes and zygotes (e.g. Pratchett et al. 2001) and during settlement, when competent larvae must run the gauntlet of potential predators in moving from offshore waters to reef environments (Hamner et al. 1988). While a range of different fishes appear to readily consume larvae of crown-of-thorns starfish (Cowan et al. 2016), relatively few fishes have been seen to consume gametes released when starfish are actively spawning in the field (Gladstone 1992; Pearson and Endean 1969). Pearson and Endean (1969) reported that only one species of damselfish (*Amblyglyphidodon curacao*) consumed eggs released by spawning crown-of-thorns starfish, though there were many different damselfishes and other planktivorous fishes in the vicinity when these observations were made.

The purpose of this study was to test for interspecific differences among planktivorous damselfishes in their feeding response to increasing proportions of eggs from crown-of-thorns starfish contained within standardised food pellets. If chemicals (e.g. saponins) contained in the eggs of *Acanthaster* sp. are an effective deterrent of planktivores, we would expect to see fish reduce consumption of pellets as the proportion of starfish eggs they contain increases (sensu Lucas et al. 1979). However, response to chemical defences are likely to vary among predators; those that exhibit only weak responses to increases in the proportion of starfish eggs (and therefore concentrations of anti-predation chemicals) in standardised food pellets, indicate fishes that might be more important as predators of *Acanthaster* sp. eggs. We tested nine species of planktivorous damselfish (*Amblyglyphidodon curacao*, *Chromis atripectoralis*, *C. viridis*, *Chrysiptera cyanea*, *C. rollandi*, *Dascyllus aruanus*, *D. reticulatus*, *Pomacentrus amboinensis* and *P. moluccensis*), selected based on their numerical abundance on mid-shelf reefs in the northern Great Barrier Reef, Australia (Sweatman et al. 2000; Pratchett et al. 2012; Komyakova et al. 2013).

3.3. Materials and methods

3.3.1. Egg collection

Adult *Acanthaster* sp. were collected from Arlington Reef (16°43'S; 146°03'E) in the northern Great Barrier Reef (GBR) during September 2014. Gonads were dissected from two female *Acanthaster* sp. to ensure that a mix of genotypes was used. Gametes were collected following methods described by Kamya et al. (2014): ovaries were rinsed in 1-µm filtered seawater (FSW) to remove immature eggs, then placed in 10^{-5} M 1-methyl-adenine solution to induce ovulation. Spawned eggs were collected after approximately 1 h, rinsed in fresh seawater, checked microscopically for quality (i.e. shape, integrity, germinal vesicle breakdown), then mixed in approximately equal proportions. Eggs were centrifuged on the lowest setting (200 rpm) to remove excess seawater, frozen at -80 ºC and freeze-dried for transport.

3.3.2. Fish collection

The nine species of damselfish (*A. curacao*, *Chromis atripectoralis*, *C. viridis*, *Chrysiptera cyanea*, *C. rollandi*, *D. aruanus*, *D. reticulatus*, *P. amboinensis* and *P. moluccensis*) were collected using a combination of fence nets and clove oil on reefs at Lizard Island (14°40'S; 145°27'E) in the northern GBR in June 2015. Fish were maintained with conspecifics in 32-L flow-through aquaria at the Lizard Island Research Station (LIRS) for a minimum of 48 h to acclimatise to laboratory conditions before being used in feeding trials. Fish were offered commercial dried fish food (New Life Spectrum) and fed to satiation each morning; they were considered acclimatised when they readily fed on the food pellets.

3.3.3. Palatability trials

Experimental feeding trials were conducted using standardised food pellets made by pureeing squid-mantle flesh, an equal volume of distilled water, and sodium alginate at 2% of total wet mass (after Lindquist 1996; Lindquist and Hay 1996; Baird et al. 2001). The mixture was spread into a thin layer and a 0.25 M solution of calcium chloride was poured over it to set the gel. The gel was then rinsed in distilled water and cut into pellets of 0.5 mm diameter, each weighing 600 µg. Five food treatments were produced by substituting unfertilised eggs of *Acanthaster* sp. for 20, 40, 60, 80 and 100% of the squid-mantle flesh. The actual proportional content of *Acanthaster* sp. eggs in food pellets in each treatment was 11%, 24%, 41%, 62% and 89% respectively, after accounting for the volume of distilled water and binding agent.

Feeding trials were conducted in the flow-through (open) aquarium system at LIRS. Fish were placed individually in 32-L flow-through aquaria and starved for 24 h prior to feeding trials. Fish (n = 6-9 per species and treatment level) were randomly assigned to one of six feeding treatments using varying proportions (0%, 20%, 40%, 60%, 80%, and 100%) of eggs of *Acanthaster* sp. versus squid-mantle flesh. Each fish was used only once and trials were run over 16 consecutive days. Aquaria were emptied, cleaned and re-filled between consecutive feeding trials. A control pellet was introduced at the start of every trial to ensure that the fish was ready to eat. If the control pellet was consumed, five pellets from one of the six treatments were introduced in turn into the aquarium. Successive pellets were introduced once the previous pellet had been consumed, or after 2 min if the pellet was rejected. The fate of each pellet of each treatment was recorded as: (1) accepted — the fish immediately retained the pellet; (2) mouthed and accepted —

the fish retained the pellet after spitting it out one or more times; (3) mouthed and rejected — the fish rejected the pellet after capturing and spitting it out several times; (4) rejected — the fish rejected the pellet after capturing and spitting it out only once; or (5) untouched — the fish visually inspected the pellet and rejected it without mouthing. At the end of each trial, a control pellet was added to ensure that any rejection responses were not due to satiation.

To analyse variation in palatability of food pellets across the six treatments with increasing concentrations of eggs from *Acanthaster* sp., we initially compared the proportion of pellets that were consumed (regardless of whether they were mouthed). Transformations did not improve the normality of data, therefore these data were analysed using a permutational analysis of variance (PERMANOVA), using the PERMANOVA+ add-on for PRIMER v.6 with treatment and species as fixed factors, using the Euclidian distance measure, and 9999 permutations of the residuals under a reduced model to calculate the significance of the pseudo-F statistic. In cases where there were not enough unique permutations (<100) to determine permutational *p*-values (p_{perm}), Monte-Carlo asymptotic *p*-values (p_{MC}) were used (Anderson et al. 2008). Data on the proportion of pellets consumed were square-root transformed prior to analysis. Pairwise comparisons were done using Benjamini-Hochberg adjusted p-values to control false discovery rate. We also tested for changes in responses of fishes to successive food pellets (conditioning), using a Kruskal-Wallis test to assess whether there were significant differences in ingestion of pellets across all replicate fish used in each treatment (n = 6), based on the order of pellet.

3.3.4. Size-based dosage responses

To standardise for variation in the size of fish (both within and among species), length of all individuals was recorded (Table 3.1). Weight was estimated using length-weight ratio estimates for each species, obtained from fishbase (www.fishbase.org). The absolute quantity (mg) of eggs in each food pellet was then divided by the weight (g) of each fish to provide a dosage. Standardised response (accounting for the weight of individual fish) was analysed with logistic regression using the binomial GLM routine in R (R Core Team 2015) on RStudio, version 0.99.903 (RStudio Team 2015) and checked for overdispersion. To compare palatability responses among fishes, a median rejection dose (RD_{50}) (cf. median lethal dose) was calculated for each species. This analysis assumes that variation in the sensitivity of putative predatory fishes to anti-predation chemicals in eggs of crown-of-thorns starfish is size-based. However, larger fishes are likely to consume more eggs than smaller individuals or species, and so accounting for size is also necessary in establishing the importance of different planktivorous damselfishes as predators on eggs of crown-of-thorns starfish.

Table 3.1 Size range (total length (TL) in mm) and relative abundance of planktivorous pomacentrid fish predators used in the current study. Data on relative abundance comes from Pratchett et al. 2012 and Komyakova et al. 2013, in which abundances of damselfishes at multiple locations and habitat around Lizard Island were measured. Relative abundance is expressed relative to the abundance of *Pomacentrus amboinensis*, which was surveyed in both studies. Neither study presents data on abundance of *Chrysiptera* spp.

Predator species	Size Range TL (mm)	Relative abundance
Amblyglyphidodon curacao	47-90	0.9
Chromis atripectoralis	38-90	1.0
Chromis viridis	41-80	2.5
Chrysiptera cyanea	37-60	-
Chrysiptera rollandi	25-50	-
Dascyllus aruanus	33-63	0.4
Dascyllus reticulatus	36-75	0.4
Pomacentrus amboinensis	31-70	1.0
Pomacentrus moluccensis	25-55	7.3

3.4. Results

3.4.1. Palatability of Acanthaster *sp. eggs*

There was a significant interaction between predator species and treatment (Pseudo-$F_{40,283}$ = 2.45, $p_{perm} < 0.01$) on the proportion of pellets consumed by fish. All fishes in this study readily consumed food pellets containing up to 80% of eggs from crown-of-thorns. However, the nine species of planktivorous damselfish varied in the extent to which they would consume food pellets containing 100% eggs from crown-of-thorns starfish (Fig. 3.1). Pairwise comparisons indicated that five of the predator species (*Chrysiptera rollandi*, *D. reticulatus*, *P. amboinensis*, *Chromis viridis*, and *P. moluccensis*) consumed significantly fewer of the pellets containing 100% eggs, compared to all other treatments, and two species (*Chrysiptera cyanea* and *Chromis atripectoralis*) consumed significantly fewer pellets containing the highest concentration of eggs, however the proportion of pellets consumed that contained 100% eggs was not significantly different from the proportion consumed that contained 80% eggs (Fig. 3.1c,g). *Dascyllus aruanus* exhibited significant declines in the consumption of food pellets with increasing concentrations of starfish eggs (Pseudo-$F_{5,43}$=6.32, $p_{perm} < 0.01$; Fig. 3.1b); however, pairwise comparisons did not meet Benjamini-Hochberg criteria. While *A. curacao* also consumed fewer food pellets with increasing concentrations of starfish eggs, rates of ingestion did not differ across the six treatments (Pseudo-$F_{5,30}$=1.43, $p_{MC} >$ 0.05; Fig. 3.1a). *Amblyglyphidodon curacao* consumed the highest proportion of food pellets of the nine species of fish tested and consumed 1.5 times more of the pellets containing 100% starfish eggs than any other fish species.

Chrysiptera rollandi was the only species that exhibited significant changes in responses to food pellets (conditioning) depending on order (Kruskal-Wallis, 3^2

(4, $N = 30$) = 11.71, $p = 0.02$); fish that were offered pellets containing 100% eggs of crown-of-thorns starfish tended to reject (after mouthing) pellets one and two, but pellet five tended to be rejected (without mouthing) or left untouched. When considering only the fate of individual pellets (retained vs. rejected), there was no significant difference in response to pellets through time. Results were therefore pooled, disregarding the order in which pellets were offered, to compare the overall proportion of pellets that were consumed.

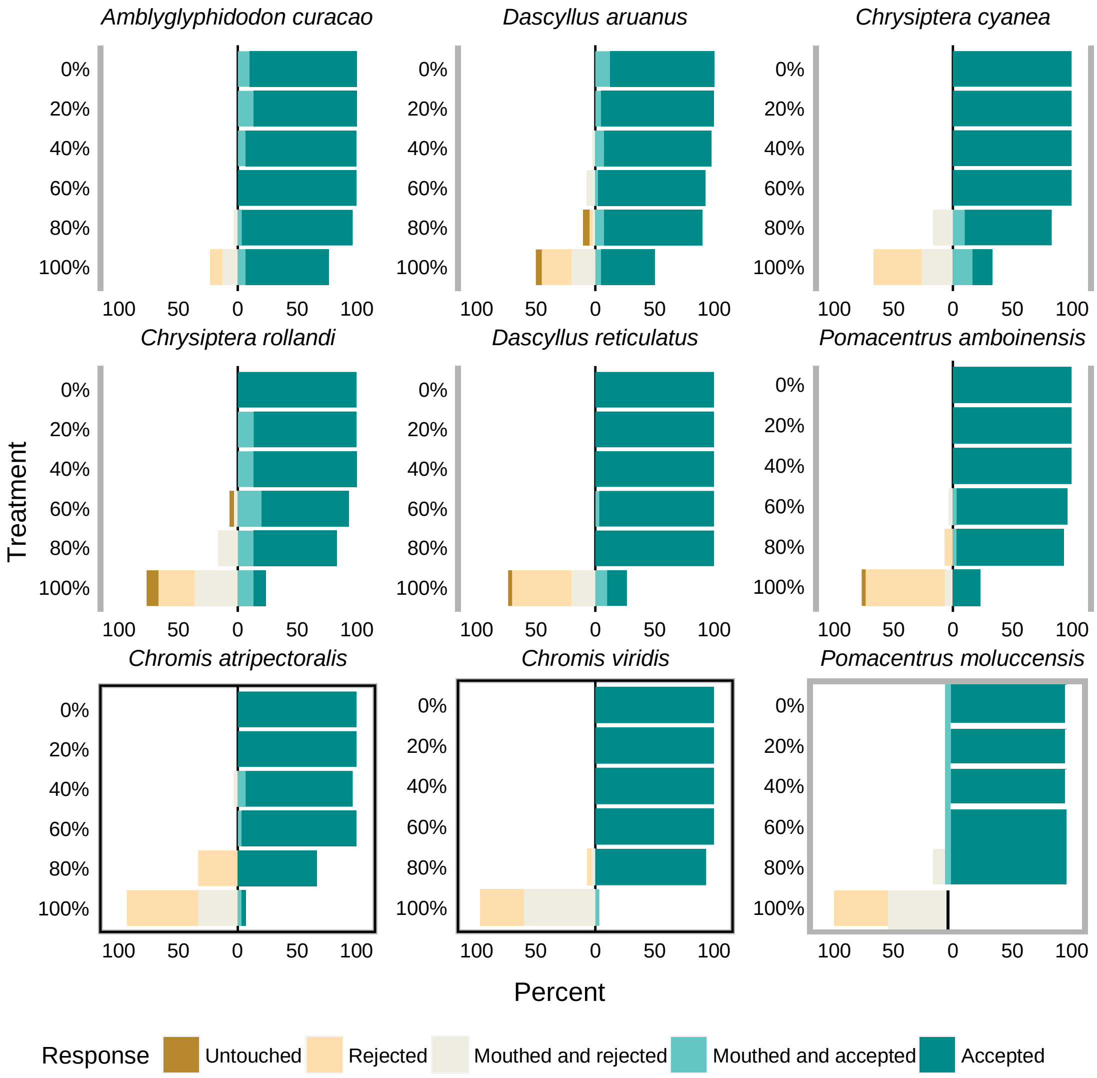

Figure 3.1 Graded response for the palatability of *Acanthaster* sp. eggs by nine planktivorous damselfishes based on five pellets offered to 337 feeding individuals across an increasing treatment gradient of 0, 20, 40, 60, 80 and 100% of feed pellets as *Acanthaster* sp. eggs. Letters represent significant groupings identified by post hoc pairwise comparisons. Accepted = the fish immediately retained the treatment pellet; Mouthed and accepted = the fish spat out the treatment pellet one or more time, before retaining it; Mouthed and rejected = the fish spat out the treatment pellet one or more time, ultimately rejecting it; Rejected = the fish mouthed the treatment pellet once and rejected it; Untouched = the fish approached the treatment pellet and rejected it, without mouthing.

After standardising for size (weight) among fishes, the median rejection doses (RD$_{50}$) to pellets containing starfish eggs ranged from 0.10 ± 0.02 (SE) for *Chromis atripectoralis* to 0.53 ± 0.13 for *Chrysiptera rollandi* (Fig. 3.3a). Increasing the absolute amount of *Acanthaster* sp. eggs relative to the size of fishes significantly increased the probability of rejecting food pellets in seven fish species: *Chromis atripectoralis* (GLM, $3^2 = 2.75$, $p < 0.01$), *C. viridis* (GLM, $3^2 = 2.61$, $p < 0.01$), *P. amboinensis* (GLM, $3^2 = 2.31$, $p = 0.02$), *D. aruanus* (GLM, $3^2 = 2.60$, $p < 0.01$), *Chrysiptera cyanea* (GLM, $3^2 = 2.12$, $p = 0.03$), *P. moluccensis* (GLM, $3^2 = 2.59$, $p < 0.01$), and *C. rollandi* (GLM, $3^2 = 2.37$, $p = 0.02$) (Fig. 3.2). An increase in dosage of 0.011 mg *Acanthaster* sp. eggs g^{-1} fish (equivalent to a single additional egg g^{-1} fish) increased the odds of rejection by these seven species by a factor of 1.39 (*Chromis atripectoralis*, 95% CI = 1.14, 1.86), 1.57 (*C. viridis*, 95% CI = 1.20, 2.43), 1.39 (*P. amboinensis*, 95% CI = 1.10, 2.00), 1.19 (*D. aruanus*, 95% CI = 1.06, 1.39), 1.20 (*Chrysiptera cyanea*, 95% CI = 1.06, 1.51), 1.08 (*P. moluccensis*, 95% CI = 1.03, 1.17), and 1.07 (*C. rollandi*, 95% CI = 1.02, 1.14). Increasing the absolute amount of *Acanthaster* sp. eggs relative to the size of fishes did not increase the probability of rejecting food pellets for *A. curacao* (GLM, $3^2 = 1.44$, $p = 0.15$) or *D. reticulatus* (GLM, $3^2 = 1.92$, $p = 0.05$).

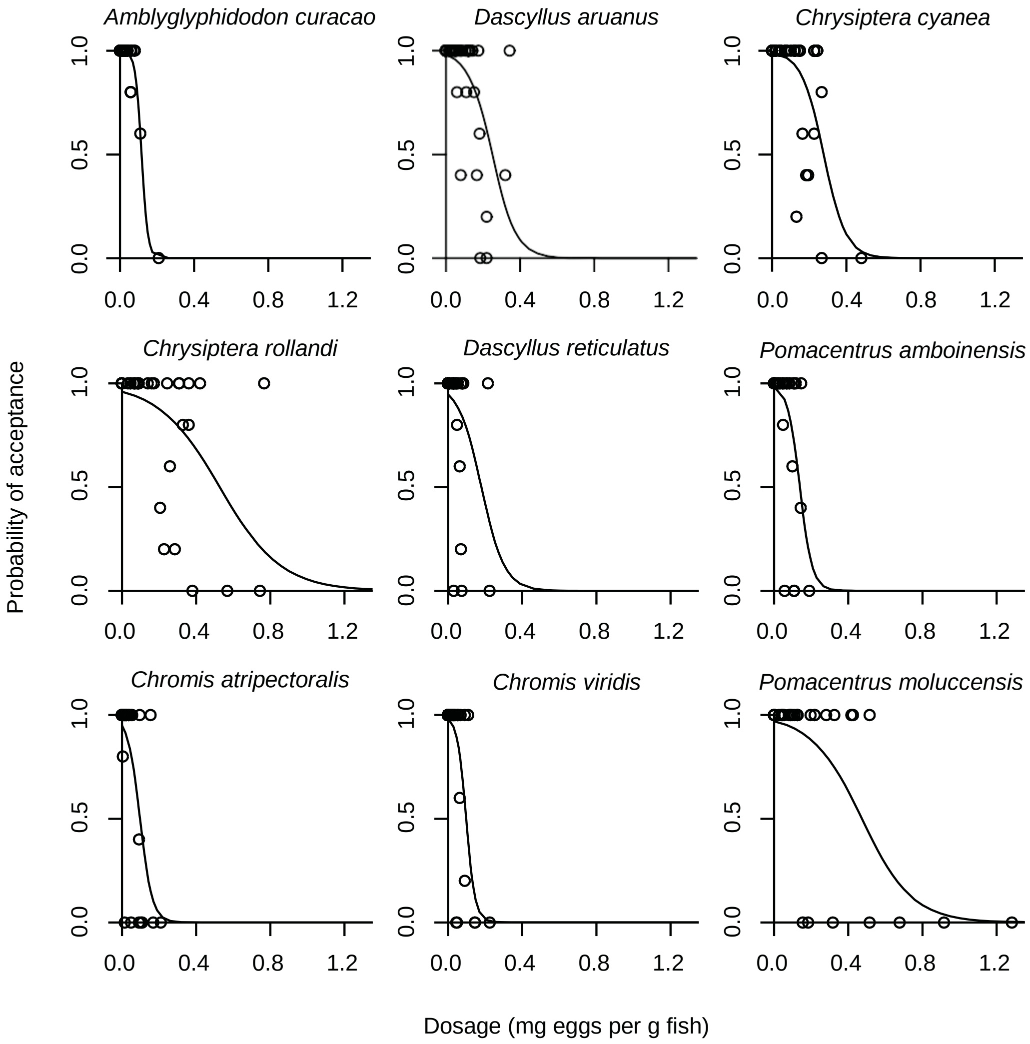

Figure 3.2 Probability of acceptance of feed pellets across increasing dose of *Acanthaster* sp. eggs per gram of fish across nine planktivorous damselfishes.

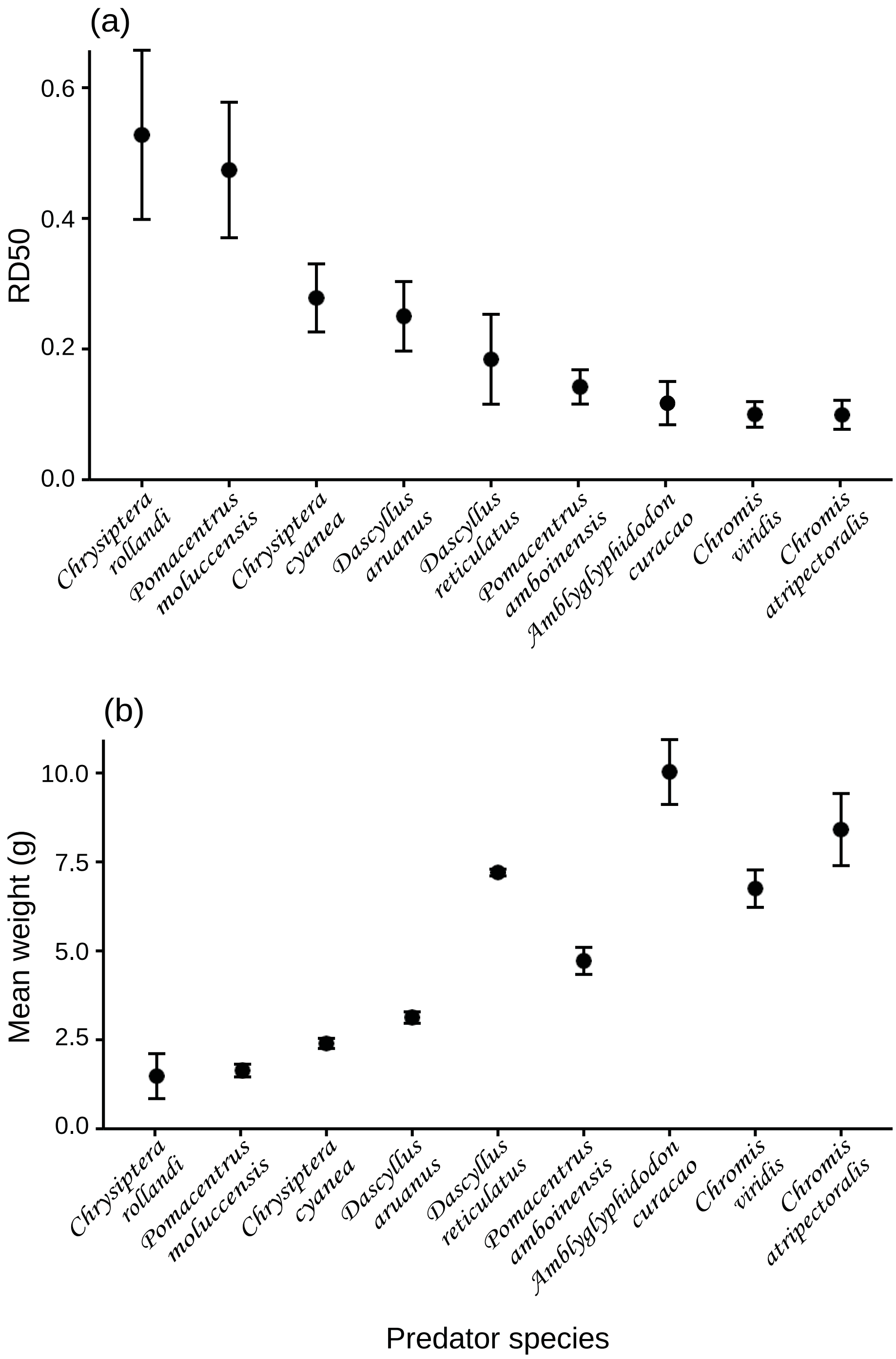

Figure 3.3 Consumption capacity of *Acanthaster* sp. eggs by nine putative planktivorous fish predators. (a) Median rejection dose (RD_{50}) (cf. median lethal dose) (± SE) of *Acanthaster* sp. eggs. (b) Mean observed weight of planktivorous damselfishes.

3.5. Discussion

As for most marine invertebrates, the eggs and larvae of *Acanthaster* sp. are potentially susceptible to a wide range of planktivorous predators (Lucas 1975; Cowan et al. 2016). Despite this, research on the role of predation in regulating populations of crown-of-thorns starfish has focused almost exclusively on rates of predation on post-settlement life stages, predominantly adult starfish (e.g. Endean 1969; Campbell and Ormond 1970; Dulvy et al. 2004). This is mainly attributable to the widely-held assumption that few (if any) potential predators will eat eggs or larvae of crown-of-thorns starfish based on early research that demonstrated the toxicity of saponins (Mackie et al. 1977) and complete avoidance of larvae of crown-of-thorns starfish by planktivorous fishes (Yamaguchi 1974, 1975). However, more recent experimental studies (e.g. Cowan et al. 2016) indicate that pre-settlement life stages of crown-of-thorns starfish are indeed vulnerable to predation. Importantly, experiments have shown that multiple species of common and widespread damselfish readily consumed larvae of crown-of-thorns starfish, and could eat hundreds of larvae before becoming satiated (Cowan et al. 2016). While the effectiveness of saponins was not explicitly tested, the data do not suggest that saponins render larvae sufficiently unpalatable to deter these potentially important predators.

Saponin concentrations in crown-of-thorns starfish are reported to vary with ontogeny, being 2.5-5 times greater in larvae than eggs (Barnett et al. 1988). Given that larvae of crown-of-thorns starfish are readily consumed by a range of common damselfishes (Cowan et al. 2016), it seems unlikely that the lower saponin concentrations in eggs of crown-of-thorns starfish would effectively deter potential predators. However, results from the current study suggest that there are effective anti-predation chemicals contained in the eggs of crown-of-thorns starfish. Notably, there was a decline in the consumption of food pellets with increases in the proportion of starfish eggs, especially when the proportion of starfish eggs in food pellets was Š80% (Fig. 3.1). Moreover, a very high proportion (96%) of the food pellets that were ultimately rejected were first mouthed by fishes, suggesting that food pellets with high concentrations of starfish eggs were rejected based largely on taste. In all, only 28% (78/280) of food pellets made using 100% starfish eggs (with water and binding agents) were consumed by fishes, compared to 98% of pellets with Š60% starfish eggs. It is also noteworthy that no obvious toxic effects were observed in fish that consumed pellets containing eggs of crown-of-thorns starfish.

Despite general declines in acceptance of food pellets with increasing proportions of crown-of-thorns starfish eggs, some fishes appeared much more accepting of anti-predation chemicals, including saponins, contained in the pellets. Importantly, nearly all the food pellets with >80% starfish eggs were consumed by *A. curacao,* and to a lesser extent by *D. aruanus*. Notably, *A. curacao* is the only species of coral-reef fish that has been observed feeding directly on eggs newly released by naturally spawning crown-of-thorns starfish (Pearson and Endean 1969). *Amblyglyphidodon curacao* and *D. aruanus* also had the highest attack rates on larvae of crown-of-thorns starfish (Cowan et al. 2016). This suggests that these species may be particularly insensitive to saponins and could have a comparatively greater role in predation on early life-history stages of crown-of-thorns starfish. It is also likely that consumption capacity of fish will be affected by individual size of the predator, with larger fish expected to be capable of consuming more eggs or larvae before becoming satiated. Thus, in addition to being more accepting of the anti-predation chemicals contained within all life stages of *Acanthaster* sp., the comparatively large size of *A. curacao* (Fig. 3.3b) further suggests that this species would be more important in consuming eggs and larvae of *Acanthaster* sp., and may therefore help to regulate abundance of crown-of-thorns starfish in some circumstances. However, after standardising for size, the smaller damselfish species tend to consume greater quantities of pellets with higher concentrations of starfish eggs (Fig. 3.3). Given that smaller fishes can be extremely abundant on coral reefs (the smallest damselfish considered in this study, *P. moluccensis*, is much more abundant than the larger-bodied species such as *A. curacao* and *Chromis atripectoralis*; Table 3.1), they may have significant capacity to regulate larval abundance and settlement success of crown-of-thorns starfish. However, we should prioritise field-based estimates of survivorship across all life stages of crown-of-thorns starfish, which might be achieved by quantifying declines in the densities of starfish larvae within bodies of water exposed to specific assemblages of planktivorous reef fishes.

A significant issue in testing palatability of food particles, and thereby the effectiveness of potential anti-predation chemicals, is that rates of ingestion will vary with the hunger of the predator tested (Lucas et al. 1979), as well as the size, abundance and nutritional content of different food items. The experimental procedures in this study specifically tested whether fishes detected and responded to changes in the concentration of potential anti-predation chemicals in standardised food particles, but did not necessarily inform whether fishes would or would not eat eggs released by naturally spawning crown-of-thorns starfish. It is clear however, that some species are much more likely to represent important predators on eggs of crown-of-thorns starfish (Pearson and Endean 1969), and explicit consideration needs to be given to distribution and abundance of these fishes relative to the occurrence of outbreaks of *Acanthaster* sp..

Predation by planktivorous fishes is suggested to be an important source of egg mortality for many marine invertebrates such as corals (Babcock et al. 1986; Baird et al. 2001). However, very high levels of fecundity and simultaneous spawning among aggregations of marine invertebrates is likely to overwhelm the consumption capacity of local assemblages of predators (Westneat and Resing 1988), and thereby ensure survival of at least some gametes and zygotes. Crown-of-thorns starfish are extremely fecund (Conand 1984) and it is possible that the quantity of eggs released, especially when starfish occur in high densities and spawn en masse, will far exceed the consumption capacity of local predators. However, planktivorous fish predators may be important in moderating the reproductive success of crown-of-thorns starfish in non-outbreak populations, thereby preventing concentrations of propagules sufficient to cause outbreaks.

Whilst our experiment was conducted using unfertilised eggs, it is unlikely that the results would change if eggs were fertilised, as the damselfish species tested have been shown to readily consume *Acanthaster* sp. larvae (Cowan et al. 2016). Similar methodology, as used by Cowan et al. (2016) could potentially be used to test consumption of *Acanthaster* sp. eggs against eggs of another species, or an alternate food source. However, this would be more difficult due to the very small size of the eggs and the rapid degradation of unfertilised eggs, which would limit the number of replicates and predator species that could be tested. Furthermore, it is more difficult to differentiate *Acanthaster* sp. eggs from eggs of other coral reef species, than it is to differentiate between larvae of different species. The diameter of *Acanthaster* sp. eggs is reported to be in the range of 0.22-

0.25 mm (Caballes et al. 2016), which is approximately half the size of our pellets. This difference in size would presumably mean that actual eggs are more difficult for fishes to discern, especially in the wild. However, the largest species tested in this study (*A. curacao*) has been observed consuming *Acanthaster* sp. eggs in the field (Pearson and Endean 1969), making it likely that other smaller planktivorous fishes would also be capable of consuming eggs. While it is possible that there might be interspecific differences in the readiness with which different fishes consume particles of a specific size, we believe that the biggest constraint on consumption of *Acanthaster* sp. eggs would be the presence of saponins and other anti-predator chemicals, as examined in our study.

Based on consistent declines in acceptance of food pellets containing increasing proportions of crown-of-thorns starfish eggs, our study reaffirms that deterrents (i.e. saponins and/or other chemicals) in eggs of *Acanthaster* sp. limit consumption by at least some potential predators. However, interspecific differences in the responses of planktivorous fishes to *Acanthaster* sp. eggs suggest that comparisons of egg palatability should be expanded to consider a wider range of taxa. *Acanthaster* sp. eggs are slightly negatively buoyant upon release (Birkeland and Lucas 1990), thus benthic organisms that have been considered as predators on settling larvae (Yamaguchi 1973) might also be important as predators on the eggs. Further, spatial distribution of predatory species should be considered as a factor in both buffering against population fluctuations of crown-of-thorns starfish and in the propagation of outbreaks. More specifically, reefs with higher abundance of tolerant species, such as *A. curacao* and *D. aruanus*, may have greater capacity to buffer against outbreaks, but this needs to be explicitly examined.

3.6. References

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Chapter 4: Predation on crown-of-thorns starfish larvae by damselfishes[3]

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[3] Cowan Z-L, Dworjanyn SA, Caballes CF, Pratchett MS (2016) Predation on crown-of-thorns starfish larvae by damselfishes. *Coral Reefs* **35**(4): 1253-1262.

4.1. Abstract

Examining the functional response of predators can provide insight into the role of predation in structuring prey populations and ecological communities. This study explored feeding behaviour and functional responses of planktivorous damselfishes when offered captive reared larvae of crown-of-thorns starfish, *Acanthaster* sp. with the aim of determining whether these predators could ever play a role in moderating outbreaks of *Acanthaster* sp. We examined predatory behaviour of 11 species of planktivorous damselfishes, testing (i) the relationship between predator size and predation rate, both within and among fish species, (ii) consumption rates on larvae of *Acanthaster* sp. vs. larvae of a common, co-occurring coral reef asteroid *Linckia laevigata*, (iii) maximal feeding rates upon both *Acanthaster* sp. and *L. laevigata*, and (iv) functional responses of planktivorous fishes to increasing densities of *Acanthaster* sp. Consumption rates of crown-of-thorns larvae by damselfishes was independent of predator size, however there was a significant negative relationship between predator size and consumption rate of *L. laevigata*, when pooling across all predatory species. Some damselfishes, including *Acanthochromis polyacanthus* and *Amblyglyphidodon curacao*, consumed larval *Acanthaster* sp. at a greater rate than for *L. laevigata.* Most predatory species (all except *A. curacao* and *Pomacentrus amboinensis*) exhibited a Type II functional response, whereby the increasing feeding rate decelerated with increasing prey density. In addition to revealing that a wide range of planktivorous fishes can prey upon larvae of *Acanthaster* sp., these data suggest that planktivorous damselfishes may have the capacity to buffer against population fluctuations of *Acanthaster* sp. Importantly, predators with Type II functional responses often contribute to stability of prey populations, though

planktivorous fishes may be swamped by an abnormally high influx of larvae, potentially contributing to the characteristic population fluctuations of *Acanthaster* sp.

4.2. Introduction

Predatory release has long been considered a potential cause of outbreaks of the crown-of-thorns starfish, *Acanthaster* sp. (e.g., Endean 1969). Essentially, key predators of adult starfishes (such as the giant triton and/or predatory reef fishes) have been subjected to extensive or sustained harvesting pressure, and it is thought that this may have drastically reduced rates of predation and adult mortality, leading to outbreaks of *Acanthaster* sp. (e.g., Endean 1969; Campbell & Ormond 1970; Owens 1971; Dulvy et al. 2004). While scientific interest in predators of *Acanthaster* sp. has traditionally focussed on predators of adult (or at least post-settlement) stages (e.g., Endean 1969; Campbell & Ormond 1970; Owens 1971; Dulvy et al. 2004), predatory regulation might equally occur during spawning and at pre-settlement and settlement stages (e.g., Babcock et al. 1986; Westneat & Resing 1988; Bachiller et al. 2015).

Acanthaster sp. must run the gauntlet of the plankton feeders throughout their planktonic development (Lucas 1975). However, few studies have attempted to identify potential predators on the eggs or larvae of *Acanthaster* sp.; in part, logistic challenges to sampling the early life stages of *Acanthaster* sp. in the field make it difficult to quantify natural rates of predation. Further, high levels of toxicity (saponins) were once thought to effectively protect early life-history stages from predation (e.g., Lucas et al. 1979). However, these may be consumed in large numbers by reef fishes, including some Pomacentridae and Chaetodontidae (e.g., Pearson & Endean 1969; Keesing & Halford 1992; Ciarapica & Passeri 1993). On coral reefs, planktivorous pomacentrid fishes are highly efficient predators, capable of removing a large proportion of the zooplankton in near reef waters (Hamner et al. 1988). If planktivorous fishes readily consume eggs and larvae of

Acanthaster sp., their high densities and high feeding rates may significantly influence reproductive and settlement success (Kingsford & MacDiarmid 1988). Moreover, small changes in rates of predation and survivorship at the pre-settlement life stages of *Acanthaster* sp. could have significant effects on adult abundance (McCallum 1988; McCallum 1990).

The potential ecological importance of planktivorous fish predation as a regulatory factor upon populations of *Acanthaster* sp. depends largely upon the ability of the predators to find and consume prey (Hassell 1978). A common method that provides insight into the dynamics of predator-prey systems is the quantification of the functional response (Abrams 1990; Buckel & Stoner 2000; Nilsson & Ruxton 2004), described by the intake rate of prey as a function of prey density (Holling 1959a). Functional responses are categorised as Type I, II, or III (e.g., Galarowicz & Wahl 2005; Gustafsson et al. 2010). Type I functional response describes a linear increase in feeding rate with increasing prey density, until reaching a saturation point (Holling 1959b). This is most commonly observed in filter-feeding species (e.g., Jeschke et al. 2004), and is assumed to result when handling time is negligible, so that consumption increases in direct proportion to the densities of prey (Jeschke et al. 2002). The Type II functional response is defined by a feeding rate that increases at a decelerating rate towards a satiation point (Holling 1959b) and assumes that handling time and searching time are mutually exclusive (Kaspari 1990; Baker et al. 2010). It typically describes the foraging behaviour of species capable of handling only one prey item at a time, and where there are no increases in capture success with increases in the rate of encounter for given prey items (Real 1977; Abrams 1990). The Type III functional response describes a feeding rate that initially increases with increasing prey densities and then decelerates towards a maximum value, producing a characteristic sigmoidal curve (Holling 1959b; Nachman 2006). This pattern is produced by factors that affect the probability of detection or attack of prey items, including learned behaviour, prey switching, capture success, or aggregation of prey (Murdoch 1973; Morgan & Brown 1996).

The specific functional responses of predators are important because they determine the extent to which predators potentially regulate prey abundance or respond to changes in prey abundance (Eggleston 1990; Eggleston et al. 1992; Taylor & Collie 2003; Ward et al. 2008). Importantly, when predators exhibit a Type II response, prey species occurring at low densities within a closed system suffer an increased risk of mortality and may be driven to extinction (Murdoch & Oaten 1975; Hassell 1978; McCallum et al. 1989). When predators exhibit a Type III response however, prey in low-density populations experience a reduced risk of mortality (Hassell 1978). Thus on a single patch, a Type III response can be stabilising, whilst a Type II response can be destabilising. If however, there is sufficient larval mixing between patches, predators exhibiting a Type II functional response may be capable of stably maintaining prey populations at low levels across most of a metapopulation (McCallum 1988). In such situations, two stable equilibria may exist across patches, where most patches have very low prey densities, though a small number of patches have very high prey densities. Whilst the low density equilibrium is locally stable, sufficiently high larval influx from other patches may lead to a switch in states (McCallum 1988). Larval mixing between starfish populations on the Great Barrier Reef (GBR) is known to occur at very large scales, exceeding that of individual reefs (e.g., Hock et al. 2014). Therefore, sufficiently large numbers of predators exhibiting Type II functional responses could contribute to generally low densities of *Acanthaster* sp. at most reefs and most of the time.

We examined feeding behaviour of planktivorous damselfishes in a series of laboratory assays where fish were provided with varying densities of captive reared larvae of *Acanthaster* sp. This study aims to establish whether planktivorous reef fishes could be important in regulating abundance and/or contribute to extreme fluctuations in abundance of *Acanthaster* sp. Specifically, we tested (i) the relationship between predator size and predation rate, both within predator species and across the planktivorous fish community; (ii) consumption rate of larvae of *Acanthaster* sp. vs. larvae of a common, co-occurring coral reef asteroid *Linckia laevigata*; (iii) maximal feeding rates upon both *Acanthaster* sp. and *L. laevigata*; and (iv) functional response of fishes feeding upon *Acanthaster* sp. The blue starfish, *L. laevigata*, was selected as a comparative species as it co-occurs with *Acanthaster* sp. and has a similar larval development pattern (Yamaguchi 1973). While *L. laevigata* is generally very common (e.g., Williams 2000), it does not exhibit extreme fluctuations in abundance as seen in populations of *Acanthaster* sp. If predation activity upon the early life stages of *Acanthaster* sp. is much reduced compared to predation upon other starfish, this might explain why *Acanthaster* sp. exhibit population outbreaks while most other starfishes have generally much more stable population sizes.

4.3. Materials and methods

4.3.1. Collection and maintenance of study species

This study was conducted at Lizard Island Research Station. Adult *Acanthaster* sp. and *L. laevigata* were collected from reefs at Lizard Island (14°40'S; 145°27'E) in the northern GBR during October-November 2015. Spawning was induced by injecting 1 mL of 10^{-4} M 1-methyladenine into the gonads in each arm, through the aboral side for *Acanthaster* sp. and through the tube feet for *L. laevigata*. Gametes were collected from three to four individuals of each sex to ensure that a mix of genotypes was used. Spawning commenced immediately for males, and within 30 min for females. Sperm was rinsed in 0.2 μm filtered seawater (FSW) and refrigerated at 4ºC prior to use. Eggs were collected from around the arms of females following their release from gonopores and rinsed with 0.2 μm FSW. Fertilization was achieved by adding sperm to reach a final sperm-egg ratio of approximately 100:1. Fertilised eggs were transferred to 16-L larval rearing chambers at a density of approximately 1-2 larvae mL^{-1}. Chambers were maintained at 28.4 ± 1.1ºC (mean ± SD). Larvae were fed twice daily on a mixture of cultured algal species (*Dunaliella tertiolecta* and *Chaetoceros muelleri* at a concentration of 5000 cells ml^{-1} of each species). Water in the chambers was changed daily. Bipinnaria stage larvae were used in experiments. At this stage, the two larval species exhibited minor differences in colour, opacity, shape, and size (Fig. 4.1).

Eleven species of damselfish (*A. sexfasciatus*, *A. polyacanthus*, *A. curacao*, *C. atripectoralis*, *C. viridis*, *C. rollandi*, *D. aruanus*, D. *reticulatus*, *N. azysron*, *P. amboinensis* and *P. moluccensis*), which are among the most common planktivorous fishes on the northern GBR, and commonly occur within habitats occupied by adult *Acanthaster* sp. (e.g., Pearson & Endean 1969), were collected using fence nets or clove oil from reefs at Lizard Island. All fishes were maintained with conspecifics in 32-L flow-through aquaria with PVC pipes for shelter until they were acclimatised to tank conditions. Fish were fed a commercial dried fish food and were considered acclimatised when they readily fed on food provided. This took 1-9 d depending on the species.

4.3.2. Experimental design

To test predatory behaviour on larval starfish, 2 h prior to the experiment individual fish were transferred to 10-L plastic aquaria containing 0.2 µm FSW and a 5-cm open-ended length of PVC tubing for shelter. Throughout the course of the experiment, water temperature ranged between 26.4 and 27.8°C. Fish were starved for 24 h prior to the start of feeding experiments, in an attempt to standardise for hunger within and among species.

During the experimental trials, individual fish were provided with specified densities of *Acanthaster* sp. or *L. laevigata*. Fish were allowed to feed for 1 h before being removed, and aquaria water was then sieved through a 70-µm mesh to capture and count the number of uneaten larvae. Individual fish were used only once. During control trials in which the predator was absent, 100% of larvae were recovered from aquaria.

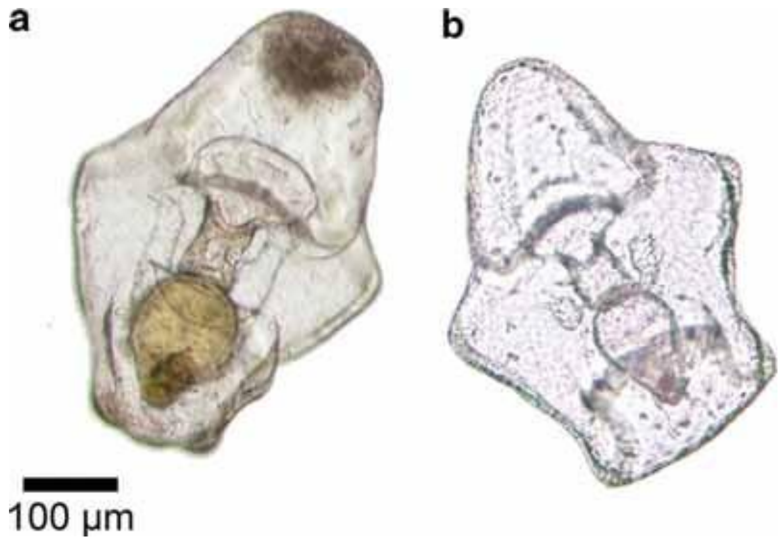

Figure 4.1 Bipinnaria larvae of **a** *Acanthaster* sp. and **b** *Linckia laevigata*

Table 4.1 Weight range in g of planktivorous pomacentrid fish predators, provided with 100 larvae of either *Acanthaster* sp. or *Linckia laevigata*.

Predator species	Range (*Acanthaster*)	Range (*Linckia*)
Abudefduf sexfasciatus	11.56-59.67	23.85-60.18
Acanthochromis polyacanthus	7.00-31.28	15.06-46.75
Amblyglyphidodon curacao	5.75-31.83	5.81-41.55
Chromis atripectoralis	2.65-8.60	2.35-11.35
Chromis viridis	2.10-6.97	1.67-4.35
Chrysiptera rollandi	0.50-1.86	0.58-2.34
Dascyllus aruanus	1.22-5.88	1.59-4.20
Dascyllus reticulatus	2.06-7.51	1.35-4.42
Neopomacentrus azysron	1.85-5.77	1.33-4.69
Pomacentrus amboinensis	0.51-16.05	0.38-7.73
Pomacentrus moluccensis	0.50-6.80	0.65-3.10

4.3.3. Consumption rate

To examine the effect of predator size on predation rate, individual fish of various size (Table 4.1), were provided with 100 larvae of either *Acanthaster* sp. or *L. laevigata* for 1 h. Fish were weighed in a 400-mL beaker filled with seawater immediately after the experiment. Linear regression was then used to test whether the number of larvae consumed over the test period varied with the size (specifically weight) of fish, both within and among predator species. Consumption rates on larvae of *Acanthaster* sp. versus larvae of *L. laevigata* were then compared using t-tests for each of the eleven damselfish species separately.

4.3.4. Functional response and satiation limits

Functional responses were explored by providing individual fish with larvae of *Acanthaster* sp. at one of seven different densities: 2, 5, 10, 25, 50, 75 and 100 larvae 10 L^{-1} seawater. *D. aruanus* and *P. amboinensis* were not satiated when provided with 100 larvae, so additional trials were conducted with up to 300 larvae 10 L^{-1}. Functional responses for each fish species were modelled in the statistical program R (R Core Team 2015). When the response is suspected to be independent of handling time, the Type I response can be modelled (Equation 1), where N is the number of prey eaten, α is the attack rate, N_0 is the number of prey available, and T is the total time available.

$$\mathrm{N} = \alpha \mathrm{N_0 T} \qquad (1)$$

The Type II functional response is most typically represented by the Holling's (1959a) ‘disc equation’ (Equation 2), where h is handling time.

$$\mathrm{N} = \frac{\alpha \mathrm{N_0 T}}{1 + \alpha \mathrm{h N_0}} \qquad (2)$$

Prey replacement could not be achieved in our experiments without disruption to the study species, therefore it was more appropriate to use the 'random-predator equation' (Rogers 1972), which describes a Type II functional response but additionally accounts for prey depletion (Equation 3). As *N* appears on both sides of the equation, this was modified using the *Lambert W* function (*W* in Equation 3; Bolker 2008)) from the emdbook package in R (Bolker 2010), where *T* is the total time available.

$$N = N_0 - \frac{W\left(\alpha h N_0 e^{-\alpha(T - hN_0)}\right)}{\alpha h} \tag{3}$$

When the attack rate is considered as a function of prey density, rather than remaining constant across prey densities, as in a Type II response, the Type III response can be modelled. In the most general form, α is a hyperbolic function of N_0 (Juliano 2001):

$$\alpha = \frac{(d + bN_0)}{(1 + cN_0)} \tag{4}$$

where *b*, *c* and *d* are constants. The Type III functional response, incorporating prey depletion, can be written as per Hassell et al. (1977):

$$N = N_0\left(1 - e^{\left(\frac{d + bN_0}{(1 + cN_0)}(hN - T)\right)}\right) \tag{5}$$

Logistic regression analyses of the proportional mortality of prey as a function of prey density, within the frair package in R (Pritchard 2014), enabled discrimination between Type II or Type III responses. Type II response was indicated by a significantly negative first order term, and Type III response was indicated by a significantly positive first order term, followed by a significantly negative second-order term (Juliano 2001). The suggested Type II or Type III model, along with a Type I model, was then fit to data and AIC values were compared to determine the model of best fit, indicated by lowest AIC value.

Functional response curves were fit to the data using maximum likelihood estimation from the bblme package in R. Parameters of interest relating to a predator's foraging behaviour can be extracted from the fitted functional response models (Jeschke et al. 2002). As the scaling coefficient, the attack rate is one such parameter that describes the initial slope of the curve (Hassell & May 1973; Jeschke et al. 2002). Differences in the extracted attack rate parameters were compared using ANOVA and post hoc Tukey test, providing an indication of the most important predators at low larval densities.

4.4. Results

4.4.1. Consumption rate

There was no significant effect of fish size (specifically, weight) on consumption rates of larvae of *Acanthaster* sp. either among ($F_{1,90} = 3.79$, $p > 0.05$), or within fish species ($F_{10,90} = 1.52$, $p > 0.05$). For *L. laevigata*, increasing weight of the fish predator had a significantly negative effect on consumption rate of larvae when pooling for all fish species ($F_{1,97} = 4.02$, $p = 0.05$), however this was attributable to the low levels of consumption by some larger species (e.g., *A. sexfasciatus*) and there was no significant effect of weight of the predator on consumption rate within fish species ($F_{10,97} = 1.34$, $p > 0.05$).

Consumption rate of *Acanthaster* sp. differed significantly from consumption rate of *L. laevigata* for three of the eleven predatory fish species (Fig. 4.2). *N. azysron*, consumed significantly more larvae of *L. laevigata* than

Acanthaster sp. (t(16.75) = -3.58, $p < 0.01$), whilst consumption rate of larvae of *Acanthaster* sp. was significantly greater than for larvae of *L. laevigata* for *A. curacao* (t(18.68) = 2.25, $p = 0.04$) and *A. polyacanthus* (t(11.79) = 2.58, $p = 0.02$). Consumption rate of the two larval species was not significantly different for seven of the ten predatory species ($p > 0.05$).

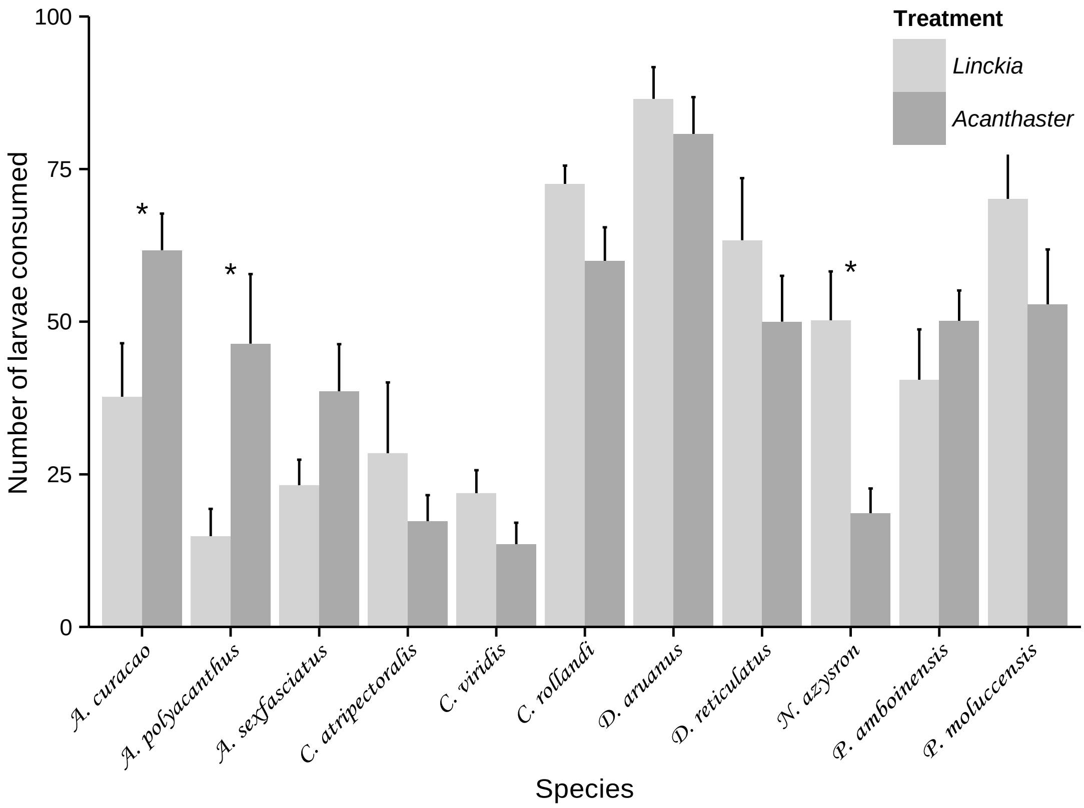

Figure 4.2 Mean number of prey consumed (±SE) by planktivorous fish predators in 1 h when larvae of *Acanthaster* sp. or *Linckia laevigata* were provided at a density of 10 L^{-1}. Asterisks denote a significant difference in consumption rate of *Acanthaster* sp. and *L. laevigata*.

4.4.2. Functional response

Logistic regression suggested that changes in the feeding rates of most predatory species (*A. polyacanthus, A. sexfasciatus*, *C. atripectoralis*, *C. rollandi*, *C. viridis*, *D. aruanus*, *D. reticulatus*, *N. azysron* and *P. moluccensis*) with increasing densities of *Acanthaster* sp. larvae was best represented by a Type II functional response, whereby the increasing feeding rate decelerated with increasing food density (Fig. 4.3). A Type III functional response, described by a significantly positive first-order term followed by a significantly negative second-order term, was exhibited for *A. curacao* and *P. amboinensis* (Fig. 4.4). However, visual comparisons of fitted models suggested the range of prey densities offered to *P. amboinensis* was not sufficiently high to effectively capture satiation; additional replicates, including at higher initial prey density would reveal a satiation point and better capture the full shape of the curve (Fig. 4.4c).

Predicted satiation points, based on the asymptote of the fitted model (Fig. 4.3, 4.4) were highly variable between species, ranging from consumption of approximately 14 *Acanthaster* sp. larvae h^{-1} (*C. viridis*) to a consumption rate of approximately 158 *Acanthaster* sp. larvae h^{-1} for *D. aruanus* (Fig. 4.3).

Parameter estimates of each functional response model with the appropriate data set revealed variable attack rates between predatory species ($F_{10,118} = 13.45$, $p < 0.01$). Attack rates of *D. aruanus* and *A. curacao* were found to be the highest and were significantly greater than the attack rates of *C. viridis* ($p = 0.03$), *A. sexfasciatus* ($p < 0.01$), *N. azysron* ($p < 0.01$), *A. polyacanthus* ($p < 0.01$), *C. atripectoralis* ($p < 0.01$), and *P. amboinensis* ($p < 0.01$, $p < 0.01$) (Fig. 4.5).

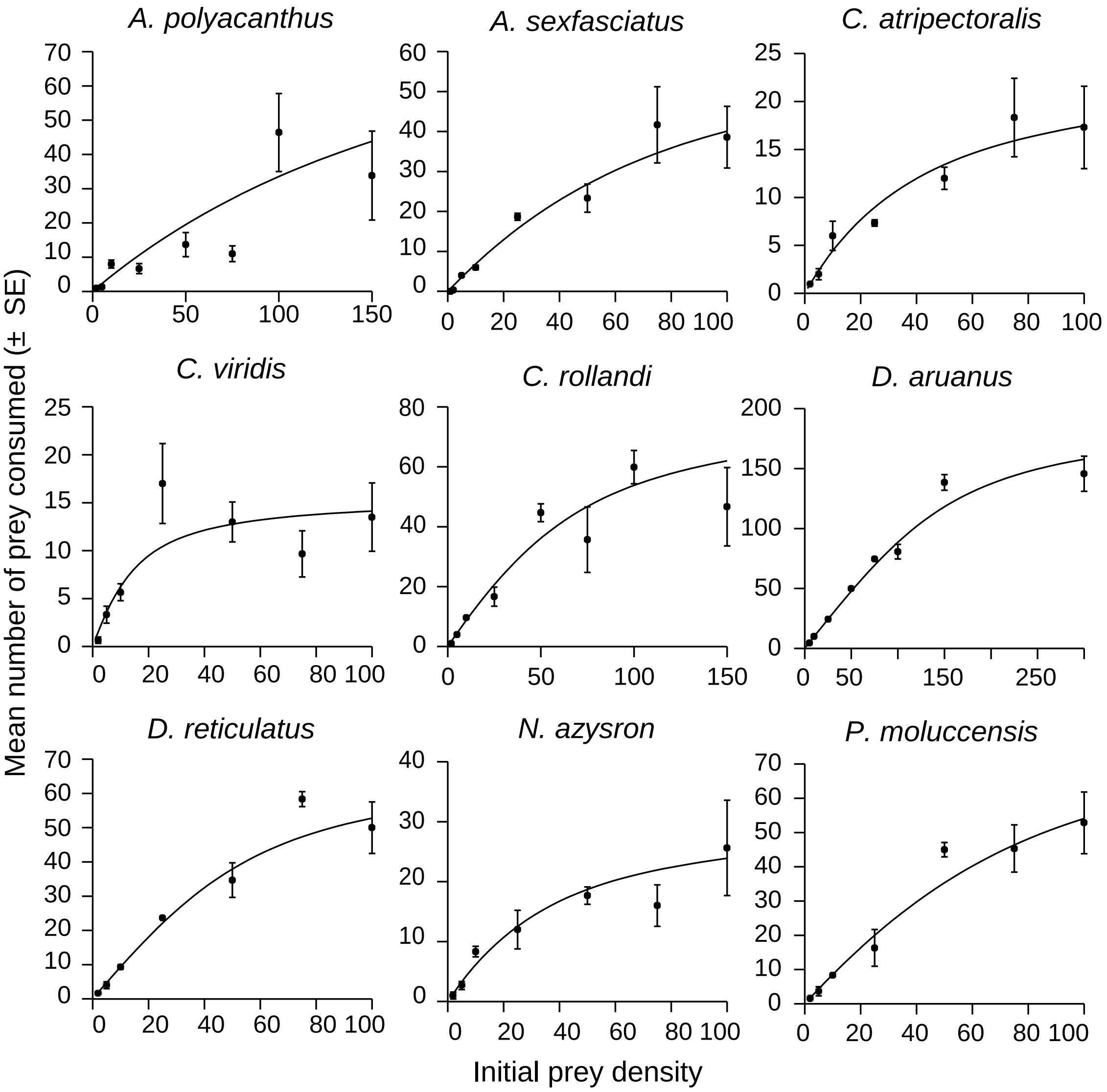

Figure 4.3 Type II functional response curves for planktivorous pomacentrid fishes **a** *Acanthochromis polyacanthus*, **b** *Abudefduf sexfasciatus*, **c** *Chromis atripectoralis*, **d** *Chromis viridis*, **e** *Chrysiptera rollandi*, **f** *Dascyllus aruanus*, **g** *Dascyllus reticulatus*, **h** *Neopomacentrus azysron*, **i** *Pomacentrus moluccensis* preying on larvae of *Acanthaster* sp., modelled by Rogers' random predator equation (Rogers 1972).

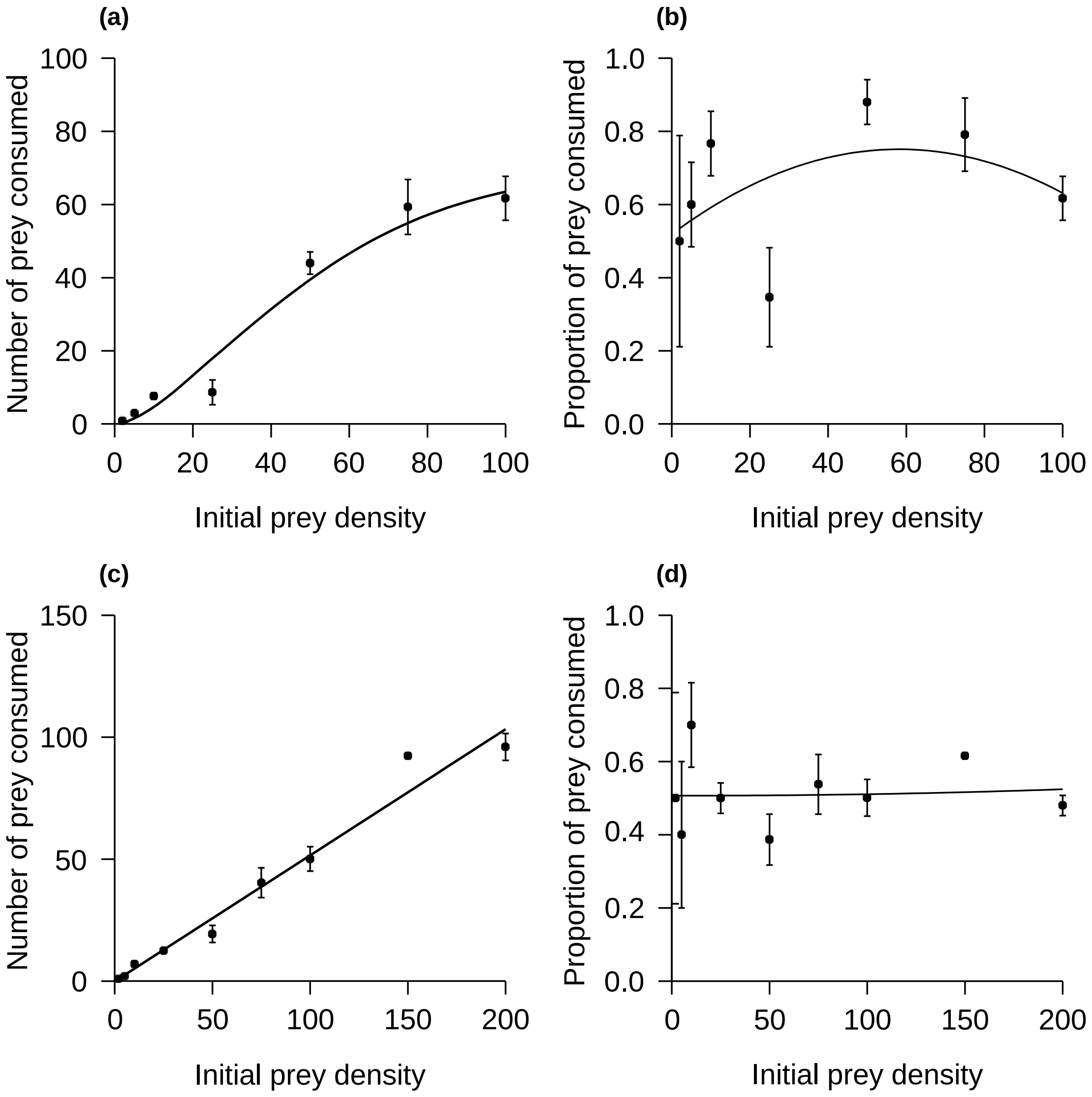

Figure 4.4 Predation of *Acanthaster* sp. larvae *Amblyglyphidodon curacao* (**a**, **b**) and *Pomacentrus amboinensis* (**c**, **d**). **a**, **c** Mean ± SE number of prey consumed at each density; the Type III functional response (Hassell et al. 1977) where attack rate increases with prey density. **b**, **d** Proportional mortality of *Acanthaster* sp. at each density, and curve generated from second-order logistic regression analysis.

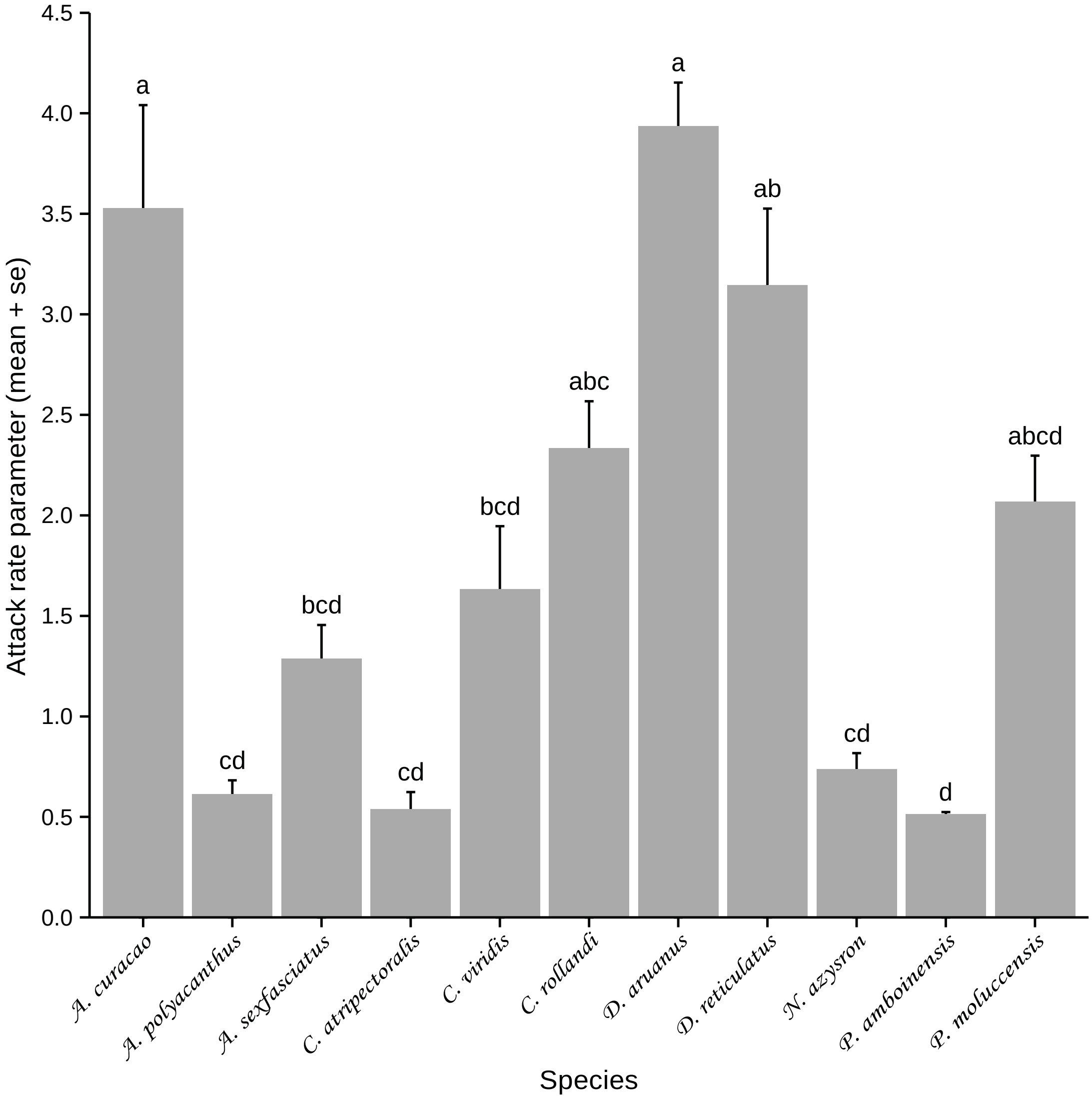

Figure 4.5 Functional response parameter, attack rate a, presented as mean + SE. *Letters* indicate significantly different groupings for attack rate parameters by predatory species, determined by post hoc Tukey test.

4.5. Discussion

This study shows that at least 11 different species of planktivorous damselfish will readily prey upon larvae of *Acanthaster* sp., clearly refuting the idea that these larvae are effectively (chemically) protected from predators (e.g., Lucas et al. 1979), and supporting previous studies (e.g., Pearson & Endean 1969; Keesing & Halford 1992; Ciarapica & Passeri 1993) that suggest larval crown-of-thorns starfish are highly vulnerable to predation. Importantly, most of the fish species tested (all but *N. azysron*) fed upon larvae of *Acanthaster* sp. at equal or greater rates than larvae of a comparable starfish, *L. laevigata*. These results suggest that toxins present in larval crown-of-thorns starfish (saponins) are largely ineffective in reducing predation, at least by common and widespread damselfishes. This directly contradicts the work by Lucas et al. (1979), who showed that all four of the damselfishes tested (*Chromis caerulea*, *A. polyacanthus*, *D. aruanus,* and *N. azysron*) significantly avoided artificial food pellets containing saponins extracted from eggs of *Acanthaster* sp., relative to equivalent food pellets without saponins added. Taken together, these studies suggest that fishes are able to detect saponins, and find them generally distasteful or unpalatable compared to a palatable control, but this is not sufficient to deter fishes from actually feeding on whole larvae of crown-of-thorns starfish.

Rates of predation upon larvae of *Acanthaster* sp. varied among planktivorous damselfishes, but were independent of predator size. This pattern may be due to prey size relative to predator size, which can influence prey preference in generalist predators (Lafferty & Kuris 2002). For most fishes, both the size and range of sizes of prey consumed generally increase with increasing predator size (Keast & Webb 1966; Popova 1967; Popova 1978; Nielsen 1980;

Persson 1990; Juanes 1994): larger predators eat larger prey, whilst the minimum prey size may change only slightly over a wide range of predator sizes. Further, as fish typically prefer larger food particles (e.g., Werner & Hall 1974), the comparatively larger larvae of *Acanthaster* sp. may be consumed in preference to species with smaller larvae, including *L. laevigata*, if they co-occur in the plankton.

Predators consuming more prey at lower densities should have a higher attack rate parameter, owing to the greater initial slope of the line describing their functional response. Predatory species such as *D. aruanus*, *A. curacao*, *D. reticulatus*, and *C. rollandi* which have a higher initial attack rate are likely to be disproportionately important in reducing effective settlement rates when *Acanthaster* sp. populations are at normal, low densities. Of these, *A. curacao* may be particularly important in reducing recruitment of *Acanthaster* sp., as this species was found to consume larvae of *Acanthaster* sp. at a significantly higher rate than it consumed larvae of the comparative species, *L. laevigata*. As densities of larval *Acanthaster* sp. increase on a reef, for example during a mass influx of larvae, those predators with high satiation limits (e.g., *D. aruanus* and *P. amboinensis*) would be most important in potentially reducing the number of larvae and therefore effectively reducing settlement rates. When coupled with field observations that have detected high larval densities of up to 53.3 individuals m^{-3} (Suzuki et al. 2016), our results suggest that planktivores could substantially reduce incoming *Acanthaster* sp. larvae. Regarding both initial attack rates and satiation limits, *D. aruanus* emerges as the most important of the eleven damselfish species tested in this study, representing the most efficient predator of *Acanthaster* sp. larvae across both low and high larval densities. In contrast, *C. atripectoralis* had a low attack rate and a low satiation limit, though we also need to take account of variation in actual abundances of the different predators in different reef environments.

The planktivorous damselfishes considered in this study primarily exhibited Type II functional responses, suggesting that they may be capable of consuming sufficient larvae of crown-of-thorns starfish to effectively supress settlement rates when larvae are already scarce, and thereby contribute to very low natural densities of *Acanthaster* sp. This does not mean however that the initiation and spread of outbreaks can be directly attributed to spatial and temporal patterns in the abundance of these damselfishes (and/or other planktivorous organisms), because very high densities of larvae, which are a necessary condition for the rapid and pronounced onset of outbreaks (e.g., secondary outbreaks), are likely to swamp even the combined consumption capacity of all planktivorous reef fishes (McCallum 1988). At best, high densities of planktivorous fishes, especially those species that selectively target larvae of *Acanthaster* sp. and are capable of eating very large numbers of such larvae, may contribute to low densities of *Acanthaster* sp. recorded outside of outbreak conditions, and thereby reduce (in part) the incidence of new outbreaks.

If damselfishes, and/or other planktivorous reef fishes, are important in suppressing local densities of *Acanthaster* sp., any extrinsic threats to the abundance or composition of planktivorous fish assemblages may in turn, give rise to population outbreaks of crown-of-thorns starfish. Importantly, several of the damselfishes considered in this study are extremely vulnerable to coral depletion (e.g., Pratchett et al. 2012). This means that there is a potentially important feedback loop where high densities of crown-of-thorns starfish, which effectively remove essential habitat for coral-dwelling damselfishes (e.g., *D. aruanus*), may actually contribute to increased survival of larval starfish, and therefore promote even higher densities of crown-of-thorns starfish and ever greater coral loss. This may be important in the formation of primary outbreaks, in which the population builds up gradually over successive years (e.g., Pratchett 2005).

In conclusion, this study shows that coral reef damselfishes, probably like many other planktivorous reef fishes, will readily consume larvae of crown-of-thorns starfish, and each individual fish can eat hundreds of larvae before becoming satiated. Planktivorous fishes may therefore be important in regulating the abundance of crown-of-thorns starfish by moderating settlement success, and especially when starfish are in low abundance, potentially preventing the onset of outbreaks. It remains to be tested whether large-scale variation in the abundance and diversity of planktivorous fishes may account (in part) for spatial and temporal patterns of outbreaks of crown-of-thorn starfish, but high densities of damselfishes are not necessarily, in themselves, insurance against outbreaks of *Acanthaster* sp. What is needed is much more focussed research on the demographics of both low density (non-outbreak) and high density (outbreak) populations of crown-of-thorns, estimating not only the rates of reproduction, settlement, and mortality, but also considering key biological interactions that moderate these rates.

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Chapter 5: Vulnerability of crown-of-thorns starfish larvae to predation by planktivorous damselfishes despite presence of alternative prey

5.1. Abstract

Predation rates on specific prey species will likely vary depending on availability of alternative prey items. Predators may also exhibit distinct prey switching, whereby predators feed disproportionately on the most abundant, most nutritious, and/or most readily accessible, prey species. Defining predatory responses to changes in prey availability is critical for gauging the ability of predators to regulate prey populations. This study explored prey preferences, and tested for prey switching, across nine species of planktivorous damselfish offered varying densities of larvae of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) versus larvae of a common and co-occurring starfish, *Linckia laevigata*. Feeding responses varied among the nine planktivorous predators. While there was no evidence indicating prey switching, five of the damselfish species (*Acanthochromis polyacanthus*, *Amblyglyphidodon curacao*, *Dascyllus reticulatus*, *Pomacentrus amboinensis* and *Pomacentrus moluccensis*) exhibited increased consumption of *A.* cf. *solaris* larvae with increasing density of this ecologically important prey, despite the availability of alternative prey. Moreover, *Abudefduf sexfasciatus* and *P. amboinensis* exhibited preference for larvae of *A.* cf. *solaris* over larvae of *L. laevigata*. These findings show that planktivorous damselfishes will consume larvae of crown-of-thorns starfish even in the presence of alternative, and ostensibly more palatable, prey. Furthermore, most of the damselfishes tested increased their prey intake when densities of *A.* cf. *solaris* larvae increased, suggesting that planktivorous damselfishes could be important in regulating settlement and recruitment of *A.* cf. *solaris*, especially at low (non-outbreak) densities.

5.2. Introduction

It is widely recognised that crown-of-thorns starfish (CoTS, *Acanthaster* spp.) are vulnerable to predation (*reviewed by* Cowan et al. 2017b - Chapter 2). Moreover, predator-induced population regulation underlies one of the earliest and foremost hypotheses put forward to explain the occurrence of outbreaks of CoTS (the *predator removal hypothesis;* Endean 1969). Endean (1969) suggested that CoTS populations are normally maintained at very low densities by high levels of predation by the giant triton (*Charonia tritonis*), which is known to feed on juvenile and adult CoTS. The seemingly sudden and unprecedented occurrence of outbreaks of CoTS on the Great Barrier Reef (GBR) in the early 1960s was thus attributed to excessive harvesting of giant triton during the preceding decades (Endean 1969). Endean (1969) argued that fisheries-induced population collapses of giant triton enabled more CoTS to grow to very large sizes, which led to disproportionate increases in their reproductive output, whereby individual fecundity increases exponentially with body size for *Acanthaster* spp. (e.g., Babcock et al. 2016). The role of giant triton in effectively regulating populations of CoTS has since been challenged (Ormond et al. 1990), but the predator-removal hypothesis has morphed over time to incorporate other potentially important predators (e.g., Glynn 1977, 1982, 1984), especially larger predatory reef fishes (Chesher 1969; Ormond et al. 1973; Randall et al. 1978) that are explicitly targeted by reef fisheries (Dulvy et al. 2004; Sweatman 2008).

Inter-reef differences in the incidence and/or severity of outbreaks of CoTS have been linked to spatial variation in fishing effort in Fiji (Dulvy et al. 2004) and on the GBR (Sweatman 2008). Sweatman (2008) showed that reefs closed to fishing (within no-take marine reserves) were much less likely to be subject to outbreaks of CoTS, compared to reefs open to fishing. These findings suggest that fishes targeted by reef fisheries (e.g., *Plectropomus* spp., Frisch et al. 2016) reduce the incidence of CoTS outbreaks, either by feeding on adult starfish and limiting local reproductive potential, or by effectively reducing settlement and/or recruitment by CoTS, thereby preventing outbreak densities of starfish from becoming established. In many locations, adult CoTS exhibit very high incidence of injuries, that are attributed to fish predation (e.g., Rivera-Posada et al. 2014; Messmer et al. 2017), and will likely constrain reproductive potential of individual CoTS, if not reduce the local densities of adult starfish. Alternatively, exploitation of larger predatory fishes may indirectly facilitate outbreaks of crown-of-thorns starfish through cascading changes in the structure of fish assemblages at lower trophic levels (Dulvy et al. 2004). Exploitation of large predatory species may, for example, influence local abundance of planktivorous fish species (Graham et al. 2003), which are known to feed on larvae of *Acanthaster* spp. (Cowan et al. 2016a - Chapter 4).

Crown-of-thorns starfish possess an array of chemical, physical and behavioural defences (Birkeland and Lucas 1990). Notably, all tissues, organs and life-stages of CoTS contain saponins (Barnett et al. 1988), while the long sharp spines of adult starfish also contain plancitoxins (Shiomi et al. 2004), both of which are presumed to deter potential predators. Given these apparent defences, it has been predicted that predators of CoTS will be highly specialized (e.g., Potts 1981). However, given fluctuations in the abundance of *Acanthaster* spp. at individual reefs (e.g., Pratchett 2005), predators of CoTS must be sufficiently generalist to persist on alternative prey during non-outbreak periods, when densities of CoTS are extremely low. There is increasing evidence that many of the reef fishes and invertebrates reported to feed on CoTS are generalist and opportunistic predators (e.g., Ormond and Campbell 1974; Cowan et al. 2017b - Chapter 2). This points to the potential importance of availability of alternative prey as a determining factor in a predator's ability to exert specific control over CoTS populations. Scarcity of alternative prey has been implicated in the maintenance of CoTS populations at low, or non-outbreak levels, by two invertebrate predators, the harlequin shrimp, *Hymenocera picta* and the lined fireworm, *Pherecardia striata* in the Eastern Pacific (Glynn 1977, 1982, 1984). Both *H. picta* (Ormond and Campbell 1974) and *P. striata* (Glynn 1984) prefer to feed on asteroid species other than CoTS (mainly ophidiasterids), but the paucity of asteroid prey in the eastern Pacific means that even *Acanthaster* spp. are readily and rapidly consumed. As a result, both these species are highly efficient predators of CoTS (Glynn 1984).

Here we explore the feeding response of planktivorous damselfishes to increasing densities of CoTS larvae in the presence of alternative prey. Previous research has defined the functional response of planktivorous damselfishes feeding on CoTS larvae without alternative prey (Cowan et al. 2016a - Chapter 4), showing that these fishes readily consume larval CoTS. However, larval CoTS are thought to be relatively unpalatable (Lucas et al. 1979), and thus planktivorous fishes may preferentially consume larvae of other co-occurring species if available. CoTS have been observed to spawn simultaneously with a wide range of coral reef invertebrates including other asteroids such as *Culcita novaeguineae* and *Linckia laevigata* (Yamaguchi 1973), thus it is likely that an assemblage of alternative echinoderm prey would be simultaneously available in nature. Therefore, the aim of this study is to examine the feeding activity of planktivorous damselfishes in a series of laboratory assays where fish are provided with captive reared larvae of the Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) and/or the blue starfish (*Linckia laevigata*) in varying ratios, to test for prey switching. Prey switching is defined as preferential feeding on the most abundant prey item (Murdoch 1969). If planktivorous damselfishes selectively prey on CoTS larvae in the presence of alternative prey, and/or exhibit prey switching, they may have enhanced capacity to buffer against fluctuating densities of CoTS larvae (*sensu* Murdoch and Oaten 1975; Oaten and Murdoch 1975; Williams and Martinez 2004; Rall et al. 2008) and thereby reduce the incidence or severity of localised outbreaks (Ormond et al. 1973).

5.3. Materials and methods

5.3.1. Collection and maintenance of study species

This study was conducted between October and November 2015. Gravid adults of *A.* cf. *solaris* and *L. laevigata* were collected from fringing reefs around Lizard Island (14°40'S; 145°27'E) in the northern Great Barrier Reef, and transported to the Lizard Island Research Station. Following Cowan et al. (2016a - Chapter 4), spawning was induced by injecting 1 mL 10^{-4} M 1-methyladenine into the gonads in each arm, through the aboral side for *A.* cf. *solaris* and through the tube feet for *L. laevigata*. Gametes were collected from 3-4 individuals of each sex for each species to ensure that a mix of genotypes was used. Spawning commenced immediately for males, and within 30 minutes for females. Sperm was rinsed in 0.2-µm filtered seawater (FSW) and refrigerated at 4ºC prior to use. Eggs were collected from around the arms of females following their release from gonopores and rinsed with 0.2-µm FSW. Fertilization was achieved by adding sperm to reach a final sperm-egg ratio of approximately 100:1. Fertilised eggs were transferred to

16-L larval rearing chambers at a density of approximately 1-2 larvae mL^{-1}. Chambers were maintained at 28.4 ± 1.1°C (mean ± SD). Larvae were fed twice daily on a mixture of cultured algal species (*Dunaliella tertiolecta* and *Chaetoceros muelleri* at a concentration of 5000 cells ml^{-1} of each species). Water in the chambers was changed daily. Bipinnaria stage larvae were used in experiments. At this stage, the larvae of each asteroid species were very similar in size and shape, but were nonetheless distinguishable based on consistent differences in colour (Fig. 5.1).

Nine different species of planktivorous damselfishes (*Abudefduf sexfasciatus*, *Acanthochromis polyacanthus*, *Amblyglyphidodon curacao*, *Chromis atripectoralis*, *Chromis viridis*, *Dascyllus reticulatus*, *Neopomacentrus azysron*, *Pomacentrus amboinensis* and *Pomacentrus moluccensis*) were collected using fence nets or clove oil from reefs at Lizard Island. These species were selected because they are among the most abundant planktivorous fishes on the northern Great Barrier Reef (Table 5.1), and commonly co-occur with *A.* cf. *solaris* in shallow reef environments (e.g. Pearson and Endean 1969). Following Cowan et al. (2016a - Chapter 4), replicate fish of each species were maintained together in 32-L flow-through aquaria until they were acclimatised to tank conditions. Fish were fed a commercial dried fish food and were considered acclimatised when they readily accepted food provided, which took 1-9 days. Fish were then starved for 24 hours prior to feeding experiments, to standardise for hunger within and among species.

5.3.2. Experimental design

Experimental tests of predation rates and feeding preferences were conducted independently for each replicate fish, following Cowan et al. (2016a - Chapter 4):

individual damselfish were placed in a 10-L plastic aquarium containing 0.2-μm FSW with a 5 cm open-ended length of PVC tubing for shelter, two hours prior to the start of the experiment. Throughout the course of the experiment, water temperature ranged between 26.4 and 27.8°C. During the experimental trials, fishes were provided with larvae of *A.* cf. *solaris* and/or *L. laevigata*. The overall prey density in the experiments was kept constant at 100 individuals per replicate, while systematically varying the number of *A.* cf. *solaris* versus *L. laevigata* larvae (0:100, 20:80, 40:60, 60:40, 80:20, 100:0), following Kalinkat et al. (2011) and Jaworski et al. (2013). Fish were allowed to feed for one hour before being removed, and aquaria water was then sieved through a 70-μm mesh to capture, count and identify the number of uneaten larvae. The larvae of *A.* cf. *solaris* were distinguishable from *L. laevigata* based mainly on colour (Fig. 5.1). Differences in the initial and final prey densities were assumed to reflect the number of prey consumed. Individual fish were used only once in each experiment. Each treatment level was replicated between five and 12 times, resulting in a total of 382 trials. In the case of *A. curacao* (n=1), *A. polyacanthus* (n=7), *A. sexfasciatus* (n=3), *C. atripectoralis* (n=2), and *C. viridis* (n=3) replicates were discarded prior to statistical analyses as the total consumption by these individual fish was zero, thus it was not possible to calculate relative consumption. During control trials in which the predator was absent, 100% of larvae were recovered from aquaria.

5.3.3. Data analyses

Variation in the proportion of *A.* cf. *solaris* and/or *L. laevigata* larvae consumed across treatments (ratio of *A.* cf. *solaris* and *L. laevigata* larvae) was analysed using ANOVA and post hoc Tukey tests, providing an indication of species that exhibit increased predation activity with increasing prey density. Data were checked for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test in the "car" package (Fox and Weisberg 2011) within the statistical program R (R Core Team 2016) on RStudio, version 0.99.903 (RStudio Team 2016). Where the assumptions were violated, a logit transformation was used due to the proportional nature of the data (Warton and Hui 2011).

To test for preferential feeding, we compared the number of each prey type that were consumed relative to the frequency in which these prey were offered in each treatment, based on the Chi-square statistic. If larval consumption by fish predators is strictly density dependent and there is no evidence of preferential feeding, the number of larvae of each type consumed would be directly proportional to the relative number of the different larvae in the respective treatment. If predators exhibit prey switching, we expect to see disproportionate consumption of the most abundant prey type in each of the most extreme treatments (20:80 and 80:20), however under the intermediate treatments (40:60 and 60:40) consumption rate may not be significantly different from the pattern predicted by density dependent consumption. The use of a large number of statistical tests has the potential to inflate the chance of a Type I error; therefore a Dunn-Šidák correction was applied to control the family-wise error rate and counteract the problem of multiple comparisons (Šidàk 1967). Accordingly, the critical values (a=0.05 and a=0.01) were adjusted to 0.0014 and 0.003, respectively.

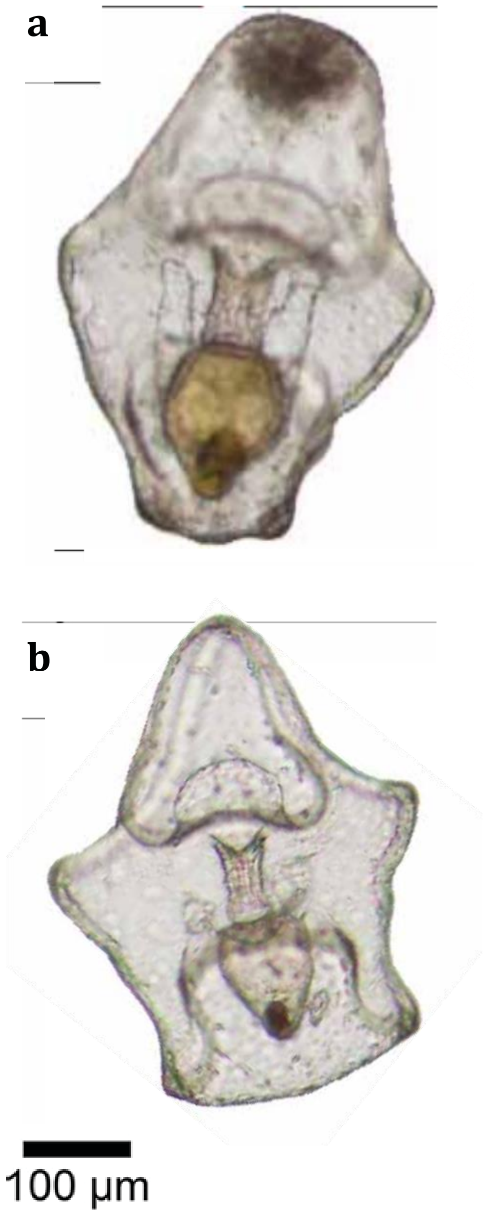

Figure 5.1 Bipinnaria larvae of **a** *Acanthaster* cf. *solaris* and **b** *Linckia laevigata*

Table 5.1 Size (mean weight (± SE) in g and mean length (± SE) in mm) of planktivorous pomacentrid fish predators used in the current study. Data on relative abundance is taken from (Pratchett et al. 2012) and (Komyakova et al. 2013), in which abundances of damselfishes were measured at multiple locations and habitat around Lizard Island. Relative abundance is expressed relative to the abundance of *Pomacentrus amboinensis*, which was surveyed in both studies. Neither study presents data on abundance of *Abudefduf sexfasciatus* or *Neopomacentrus azysron*.

Predator species	n	Weight (g)	Length (mm)	Relative abundance
Abudefduf sexfasciatus	37	29.78 ± 2.43	111.43 ± 2.96	-
Acanthochromis polyacanthus	33	21.18 ± 1.51	105.18 ± 2.53	4.6
Amblyglyphidodon curacao	41	16.30 ± 1.72	85.83 ± 3.06	0.9
Chromis atripectoralis	38	4.91 ± 0.37	67.74 ± 1.64	1.0
Chromis viridis	37	3.34 ± 0.21	58.92 ± 1.31	2.5
Dascyllus reticulatus	39	3.57 ± 0.21	48.95 ± 1.19	0.4
Neopomacentrus azysron	42	3.34 ± 0.17	61.52 ± 1.24	-
Pomacentrus amboinensis	42	4.87 ± 0.43	60.69 ± 1.82	1.0
Pomacentrus moluccensis	48	2.45 ± 0.26	43.96 ± 1.53	7.3

5.4. Results

5.4.1. Predation rates

All nine of the damselfishes tested in this study (*A. sexfasciatus*, *A. polyacanthus*, *A. curacao*, *C. atripectoralis*, *C. viridis*, *D. reticulatus*, *N. azysron*, *P. amboinensis* and *P. moluccensis*) consumed larvae of *A.* cf. *solaris*, even when alternative prey (larvae of *L. laevigata*) were available (Fig. 5.2). Treatment (the proportion of larval type available) had no significant effect on the proportion of larvae consumed for six of the nine predatory species (*A. sexfasciatus*, *C. atripectoralis*, *C. viridis*, *N. azysron*, *P amboinensis* and *P moluccensis*) ($p > 0.05$), indicating that the same proportion of larvae was consumed at each density provided (Fig. 5.2). For *A. polyacanthus*, there was a significant effect of treatment on proportion of both CoTS ($F(4,22) = 2.86$, $p < 0.05$) and *L. laevigata* ($F(4,18) = 3.07$, $p < 0.05$) consumed; however, post hoc tests did not reveal any significant differences between treatment levels ($p > 0.05$), which may have been due to small sample sizes at intermediate treatment levels (n=3-5) as a result of fish that did not feeding during the course of the trial. For the remaining two species (*A. curacao* and *D. reticulatus*), there was also a significant effect of treatment on the proportion of both CoTS (*A. curacao*, $F(4,25) = 5.64$, $p < 0.01$; *D. reticulatus*, $F(4,25) = 5.81$, $p < 0.01$) and *L. laevigata* (*A. curacao*, $F(4,26) = 3.35$, $p < 0.05$; *D. reticulatus*, $F(4,24) = 3.59$, $p < 0.05$) larvae consumed. Proportional consumption of both CoTS and *L. laevigata* larvae was greatest at treatment level 60:40 for both predatory species. Post hoc Tukey tests indicated that the proportion of CoTS consumed was significantly greater at treatment level 60:40 than at treatment levels 20:80 ($p < 0.05$) and 40:60 ($p < 0.01$) for *A. curacao* and at treatment level 100:0 for *D. reticulatus* ($p < 0.01$), and that the proportion of *L. laevigata* consumed was significantly greater at treatment level 60:40 than at treatment levels 100:0 ($p < 0.05$) and 40:60 ($p < 0.05$) for *A. curacao* and at treatment level 80-20 for *D. reticulatus* ($p < 0.05$) (Fig. 5.2).

Dascyllus reticulatus, *P. amboinensis* and *P. moluccensis* were observed to consume more than 50% of the CoTS available at all treatment levels (Fig. 5.2). Further, total prey consumption by *D. reticulatus* and *P. moluccensis* was also high, as these fishes consumed more than 50% of total prey offered across all treatment levels (Fig. 5.2). In contrast, *C. atripectoralis* and *C. viridis* consumed the lowest proportions of available prey, consuming a maximum of 36% and 32% of larvae across treatments, respectively (Fig. 5.2). For *C. atripectoralis*, consumption of CoTS did not exceed 30% of available prey across all treatment levels.

5.4.2. Predation patterns

Four of the damselfish species (*A. sexfasciatus*, *P. amboinensis*, *C. atripectoralis* and *N. azysron*) consistently consumed one larval type disproportionately to its availability, regardless of the relative numbers of different larvae. For *A. sexfasciatus* and *P. amboinensis*, the number of CoTS larvae consumed was greater than expected across the four experimental treatments. This difference was significant for *A. sexfasciatus* across all three treatment levels - 40:60 ($z^2 = 31.71$, df = 1, $p < 0.0003$), 60:40 ($z^2 = 34.16$, df = 1, $p < 0.0003$), and 80:20 ($z^2 = 15.35$, df = 1, $p < 0.0003$), and at one treatment level for *P. amboinensis* -40:60 ($z^2 = 13.91$, df = 1, $p < 0.0003$) (Fig. 5.3). For the other two fishes, *C. atripectoralis* and *N. azysron*, the number of *L. laevigata* larvae consumed was greater than expected across the four experimental treatments; however, this difference was significant at just one treatment level - *C. atripectoralis*, 80:20 ($z^2 = 73.22$, df = 1, $p < 0.0003$) (Fig. 5.3).

Accordingly, these species (*C. atripectoralis* and *N. azysron*) consumed significantly fewer CoTS larvae than expected in most treatments.

Prey switching would be indicated by consumption that is significantly less than predicted by density dependent consumption when the focal larval species is present in low density and significantly greater than predicted when present at high density (e.g., for CoTS, lower at 20:80 and greater at 80:20). *Pomacentrus moluccensis* consumed more CoTS larvae than expected at high density treatments and this was significant at treatment level 60:40 ($z^2 = 12.96$, df = 1, $p < 0.0014$); however, at the highest density treatment (80:20) and lower density treatments (20:80, 40:60), consumption was not significantly different from the pattern predicted by density dependence ($p > 0.0014$) (Fig. 5.3); further the proportion of CoTS consumed was not significantly different across treatment levels (Fig. 5.2). *Dascyllus reticulatus* exhibited an increased feeding response to CoTS larvae at the highest treatment level in which both species were present, consuming significantly more CoTS and significantly fewer *L. laevigata* than expected at treatment level 80:20 ($z^2 = 33.71$, df = 1, $p < 0.0003$); however, consumption was not significantly different from that predicted by density dependent consumption at any other treatment level, for either prey type ($p > 0.0014$). Thus, our results do not indicate that prey switching occurred in any of the predator species (Fig. 5.3).

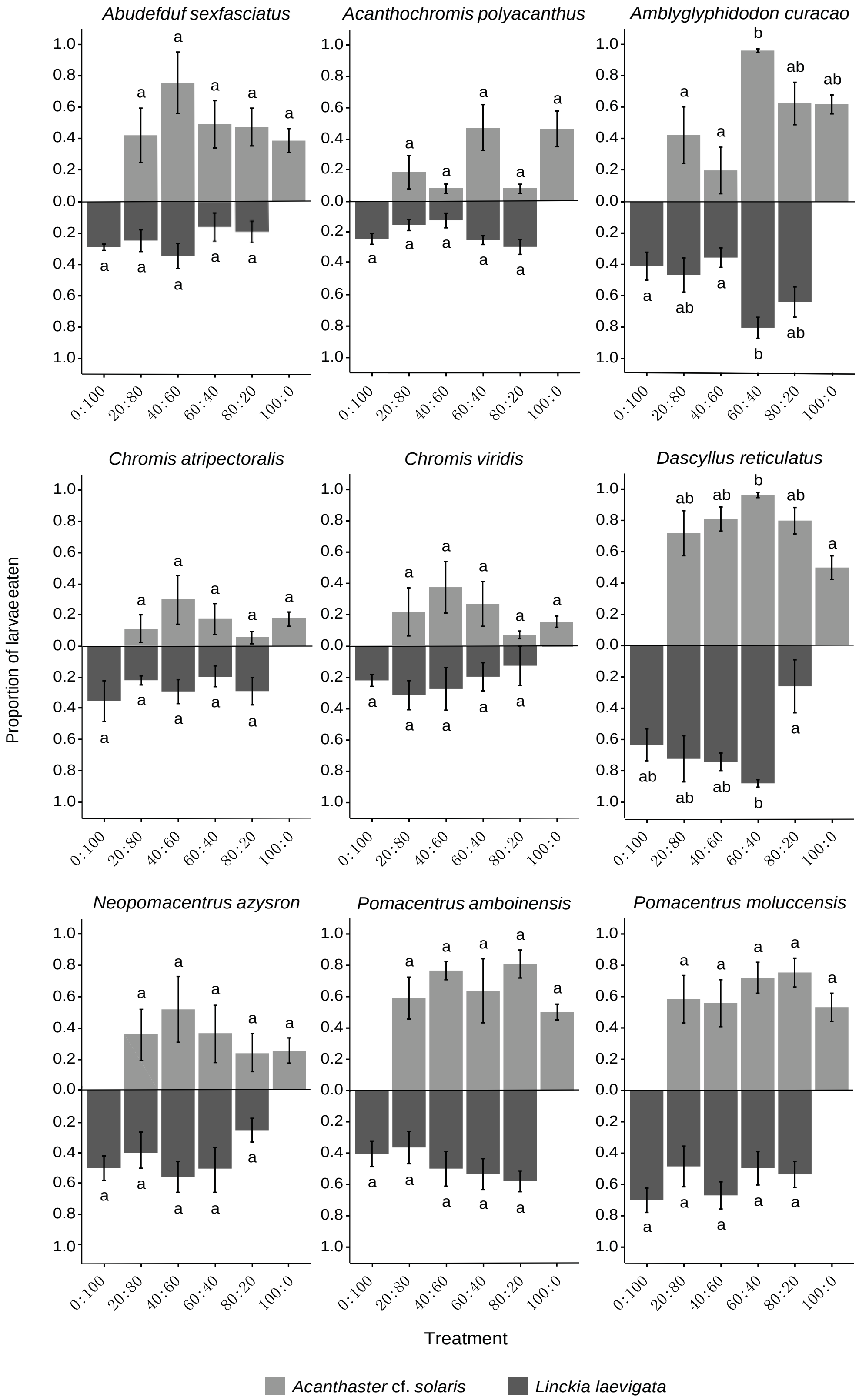

Figure 5.2. Proportion of *Acanthaster* cf. *solaris* and *Linckia laevigata* larvae consumed (mean ± se) by nine species of planktivorous pomacentrid fish across six prey ratios (*A. cf. solaris*: *L. laevigata*): 0:100, 20:80, 40:60, 60:40, 80:20, 100:0. Letters above bars represent significant groupings as indicated by post hoc Tukey tests.

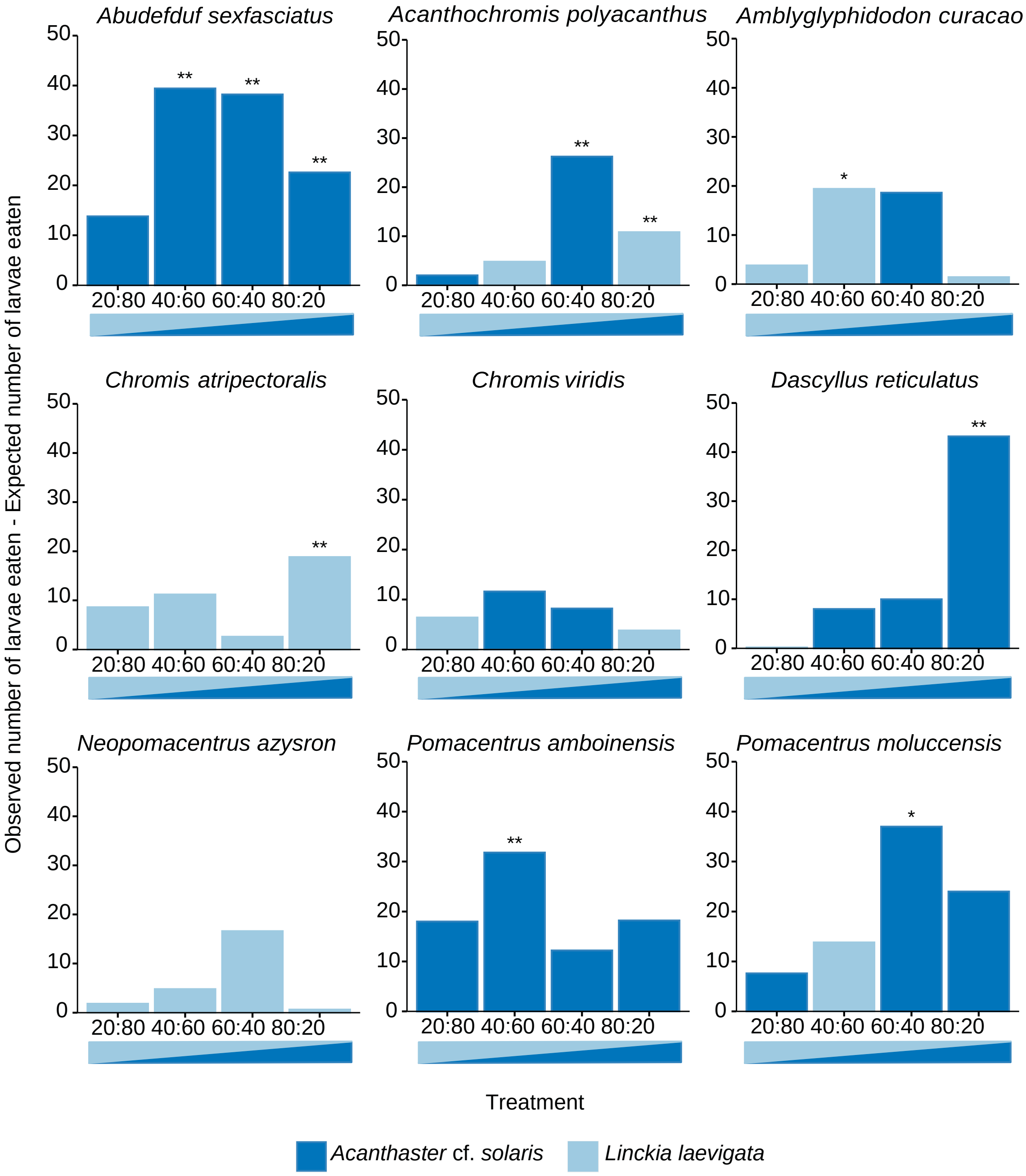

Figure 5.3. Predation activity of nine planktivorous pomacentrid fishes feeding on larvae of *Acanthaster* cf. *solaris* and *Linckia laevigata* across four prey ratios (*A.* cf. *solaris: L. laevigata*): 20:80, 40:60, 60:40, 80:20 (as indicated by relative thickness of shaded horizontal wedges below each x-axis). An asterisk (*) denotes significant values where $p < 0.0014$ and (**) where $p < 0.0003$ (using Dunn-Šidák correction). Significant values indicate deviation from a density dependent pattern of consumption, thus preferential consumption of a particular prey type: dark blue bars indicate preferential consumption of *A.* cf. *solaris*; and light blue bars indicate preferential consumption of *L. laevigata*.

5.5. Discussion

Prey selection is driven by a number of factors including variation in rate of encounter due to differing prey densities (Mathias and Li 1982; Donovan et al. 1997), presence of alternative prey sources (Glynn 1977), anti-predator defences of prey (e.g., Li and Li 1979), and nutritional value of prey (e.g., Meunier et al. 2016). Previous work that examined patterns of predation on larvae of the crown-of-thorns starfish (specifically *A.* cf. *solaris*) in a single prey system across a range of prey densities revealed that the early life stages of this starfish are likely to be vulnerable to predation by a variety of planktivorous damselfishes (Cowan et al. 2016a - Chapter 4). Further, Cowan et al. (2016a - Chapter 4) identified a number of potentially key damselfish species (e.g., *A. curacao* and *A. polyacanthus*, *C. rollandi*, *Dascyllus aruanus*, *D. reticulatus*, *P. amboinensis*, *P. moluccensis*) that may be of greater importance in reducing effective settlement rates and thereby contributing to normal, low adult densities of this starfish, based on high predation rates when prey are at low densities, significantly higher consumption rates of CoTS larvae than *L. laevigata* larvae, high satiation limits, and/or sheer abundance. Our results both support and add to this research, confirming that larvae of crown-of-thorns starfish are vulnerable to predation by a variety of planktivorous damselfishes, even in the presence of morphologically similar alternative prey.

Importantly, all damselfishes tested (*A. sexfasciatus*, *A. polyacanthus*, *A, curacao*, *C. atripectoralis*, *C. viridis*, *D. reticulatus*, *N. azysron*, *P. amboinensis* and *P. moluccensis*) consumed CoTS larvae when provided with alternative *Linckia laevigata* prey, which are presumed to be more palatable (e.g., Glynn 1977). However, the differential consumption of these morphologically similar asteroid larvae suggests some variation in palatability that may be driven by chemical defences. This is highlighted by Cowan et al. (2017a - Chapter 3), who showed that nine damselfish species consumed artificial food pellets containing low concentrations of CoTS eggs, but increasingly rejected pellets as the concentration of CoTS eggs was increased. Although previous authors (e.g., Lucas et al. 1979) allude to the chemical defences contained within the eggs and larvae (and indeed all life stages) of CoTS as being effective in deterring potential predators, planktivorous fishes (Cowan et al. 2016a - Chapter 4) and benthic invertebrates (Cowan et al. 2016b - Chapter 6) have been shown to readily consume these early life stages. Thus, our findings add to the increasing weight of evidence that the actual concentrations of saponins (and other anti-predatory chemicals) in the tissues of CoTS are not wholly effective in deterring many potential reef-based predators that could consume larvae, juvenile or adult individuals (e.g., Pearson and Endean 1969; Keesing and Halford 1992; Ciarapica and Passeri 1993; Cowan et al. 2017b - Chapter 2). This stands to reason as the production of chemical defences is costly (e.g., Dworjanyn et al. 2006), and variation in the level of investment in chemical defences is sensible in the face of variation in predation pressure.

While several of the planktivorous damselfishes considered in this study responded to increasing proportional availability of *A.* cf. *solaris* versus *L. laevigata* larvae by increasing their absolute and proportional consumption of CoTS larvae, we did not detect evidence for prey switching (typically associated with a Type III functional response (Murdoch 1973; Morgan and Brown 1996)). This is in accordance with the findings from single-prey experiments (Cowan et al. 2016a - Chapter 4), which suggest that most planktivorous damselfishes exhibit a Type II functional response when offered increasing densities of CoTS larvae. Under the

Type II functional response, feeding rate on prey increases at a decelerating rate towards a satiation point (Holling 1959) and there are no increases in capture success with increases in rate of encounter (Real 1977; Abrams 1990). If predators primarily exhibit this type of functional response, prey can be maintained at low densities across most of a metapopulation (McCallum 1988), however sufficiently high larval influx may quickly swamp these predators, as they fail to exhibit accelerated feeding rates that might help to buffer against marked increases in larval density (Holling 1959; Nachman 2006).

During outbreaks, recorded densities of CoTS larvae range from 37- 53.3 individuals m^{-3} (Suzuki et al. 2016; Doyle et al. 2017), thus our results suggest that planktivorous fishes would be capable of substantially reducing the number of larvae passing over the reef. In particular, fishes that exhibit an increased feeding response to increasing densities of CoTS larvae (*A. polyacanthus*, *A. curacao*, *D. reticulatus*, *P. amboinensis* and *P. moluccensis*), those that exhibit consistently high feeding rates across a range of densities (*D. reticulatus*, *P. amboinensis* and *P. moluccensis*) and those that feed preferentially on CoTS larvae (*A. sexfasciatus* and *P. amboinensis*) are likely to play the greatest role in reducing effective settlement rates and buffering against a mass larval influx of CoTS (Fig. 5.2). Further, species that are highly abundant (*A. polyacanthus* and *P. moluccensis*; Table 5.1), or consume larger quantities of larvae (*A. curacao*, *D. reticulatus*, *P. amboinensis*, *P. moluccensis*) may also be of particular importance.

Species that did not exhibit an increased feeding response to increasing relative densities of CoTS larvae (*C. atripectoralis*, *C. viridis* and *N. azysron*), especially those that preferentially fed on the alternative prey (*C. atripectoralis* and *N. azysron*; Fig. 5.1), are implicated as being less important in buffering against population fluctuations of CoTS. This is supported by the predicted low satiation limits of these species (18 larvae h^{-1}, *C. atripectoralis*; 14 larvae h^{-1}, *C. viridis*; and 24 larvae h^{-1}, *N. azysron*) when feeding on CoTS larvae in the absence of alternative prey (Cowan et al. 2016a - Chapter 4). Furthermore, *C. atripectoralis* and *C. viridis* did not exhibit an increased feeding response to increasing relative densities of *L. laevigata* (Fig. 5.2), possibly indicating that they are poor predators of asteroid larvae in general. However, the relative abundance of *C. viridis* is 2.5 times greater than *C. atripectoralis* (Table 5.1) and as such this species may have a greater impact on the density of larval CoTS, simply due to its abundance.

The damselfishes considered in this study are not generally vulnerable to overexploitation (Cheung et al. 2007); however, the removal of large fishes, including piscivorous fishes, may indirectly affect population densities of CoTS via trophic cascade mechanisms, in which predators may suppress both the abundance and/or behaviour of prey items (Heithaus et al. 2008; Ling and Johnson 2012). The presence of piscivorous predators suppresses behaviour of planktivorous damselfishes, such that they feed primarily within 1.5 m of the substrate (Motro et al. 2005), where they are able to seek shelter within benthic structures, such as branching corals (Clarke 1992; Beukers and Jones 1997; Bullard and Hay 2002). Intense predation within this layer can lead to near-depletion of zooplankton (Holzman et al. 2005; Yahel et al. 2005), which could be expected to significantly reduce the number of CoTS larvae that are able to reach the substratum. In addition, CoTS exhibit slow larval swimming speeds (Chia et al. 1984) and may be confined to within a few millimetres of the substratum by hydrodynamic forces (Jonsson et al. 1991; André et al. 1993), thus the removal of piscivorous fishes and subsequent release of planktivorous fishes could be expected to allow many more CoTS larvae to reach the benthos.

Coral reefs are at increasing risk of habitat degradation as a result of anthropogenic disturbances and global climate change, which may exacerbate the effects of natural disturbance events (Pratchett et al. 2011; Hughes et al. 2017). Both climate-induced coral bleaching (Pratchett et al. 2016) and tropical cyclones (Halford et al. 2004) have been associated with reduced abundance of damselfishes. Notably, two thirds of the species considered herein (*D. reticulatus, N. azysron, P. moluccensis*, *C. viridis*, *A. sexfasciatus* and *P. amboinensis*) are identified as highly vulnerable to loss of live coral (Pratchett et al. 2016). Whilst obligate coral dwelling damselfishes, such as *D. reticulatus*, may be most affected by coral loss, many facultative- and non-coral dwelling damselfishes also experience strong declines in abundance following 25-60% loss of live coral cover (Pratchett et al. 2016). Thus acute disturbances, which result in loss of live coral cover, including CoTS outbreaks themselves, and concomitant declines in abundances of planktivorous damselfishes, may serve to further increase susceptibility of coral reefs to CoTS outbreaks, however this remains to be explicitly tested.

It would be extremely difficult to quantify rates of predation on the early life stages of CoTS in the field; however, to further investigate the role of damselfishes as potentially key predators on these early life history stages, the experiments described herein, and those by Cowan et al. (2016a - Chapter 4) could be extended to include a broader range of planktivorous predators as well as alternative prey items that are likely to be present alongside CoTS in the plankton. Although not rendering CoTS larvae inedible to planktivorous damselfishes, the concentrations of saponins present in these life stages may be unpalatable (Lucas et al. 1979; Cowan et al. 2017b - Chapter 2), thus it would be worthwhile testing the consumption of these larvae against other plankton, including a broader spectrum of other echinoderms such as ophiuroids, echinoids, and crinoids, which contain only trace amounts of saponins and may thus be regarded as even more palatable than species tested here (Mackie et al. 1977).

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Chapter 6: Benthic predators influence microhabitat preferences and settlement success of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) [4]

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[4] Cowan Z-L, Dworjanyn SA, Caballes CF, Pratchett M (2016) Benthic predators influence microhabitat preferences and settlement success of crown-of-thorns starfish (*Acanthaster* cf. *solaris*). Diversity 8:27

6.1. Abstract

Like most coral reef organisms, crown-of-thorns starfish (*Acanthaster* spp.) are expected to be highly vulnerable to predation as they transition from a planktonic larval phase to settling among reef habitats. Accordingly, crown-of-thorns starfish might be expected to exhibit behavioural adaptations which moderate exposure to predation at this critical stage in their life history. Using pairwise choice experiments and settlement assays, we explored the ability of competent larvae of *Acanthaster* cf. *solaris* to first detect and then actively avoid benthic predators during settlement. Pairwise choice experiments revealed that late stage brachiolaria larvae are able to detect predators in the substrate and where possible, will preferentially settle in microhabitats without predators. Settlement assays (without choices) revealed that larvae do not necessarily delay settlement in the presence of predators, but high levels of predation on settling larvae by benthic predators significantly reduce the number of larvae that settle successfully. Taken together, these results show that crown-of-thorns starfish are highly vulnerable to benthic predators during settlement, and that variation in the abundance of benthic predators may exert a significant influence on patterns of settlement for crown-of-thorns starfish.

6.2. Introduction

As for many benthic reef organisms, settlement is expected to represent one of the major bottlenecks in the life history of crown-of-thorns starfish (*Acanthaster* spp.), whereby relatively naïve planktonic larvae will be exposed to an entirely new suite of potential predators as they transition to living in benthic reef habitats (Almany and Webster 2006). Reef-based predators include both planktivorous fishes and sessile invertebrates (e.g., corals) that intercept larvae as they swim towards benthic habitats (Yamaguchi 1973; Cowan et al. 2016 - Chapter 4), as well as infaunal invertebrate predators that will feed on starfish that settle to specific microhabitats (Keesing and Halford 1992). Both pre- and post-settlement mortality play important roles in structuring populations of marine organisms (e.g., Morgan 2001), but predation rates are generally highest (Š30% day^{-1}) immediately after settlement (reviewed by Gosselin and Qian 1997). Importantly, high rates of early post-settlement mortality can significantly augment patterns of larval supply, having a major bearing on the distribution and abundance of benthic marine organisms (e.g., Connell 1985; Rowley 1989). Moreover, there will be strong selection for settling larvae to choose microhabitats that minimise predation risk (Lucas 1975), either by avoiding habitats with high abundance of potential predators or preferentially settling in complex microhabitats that provide greater refuge from predators.

Predation on crown-of-thorns starfish may be moderated by high concentrations of saponins and other toxins that are presumed to deter potential predators (e.g., Lucas et al. 1979). Notably, three-day-old larval crown-of-thorns starfish have more than two times higher concentrations of saponins than adult starfish (Barnett et al. 1988), which may reflect their increased vulnerability to predation due to limited physical predator defences (e.g., spines). Even so, larval crown-of-thorns starfish are readily consumed by a range of planktivorous reef fishes (e.g., Cowan et al. 2016 - Chapter 4), as well as corals, such as *Pocillopora damicornis* (Yamaguchi 1973). Accordingly, in laboratory-based experiments, >50% of larvae are lost during settlement, and this may be a result of predation by benthic animals which could not be removed from the settlement substrates (Yamaguchi 1973). Even after settlement, juvenile crown-of-thorns starfish experience significant rates of mortality (up to 6.49% day^{-1} for one-month-old starfish), which decreases with size and age, and is largely attributed to predation (e.g., Keesing and Halford 1992).

The purpose of this study was to test whether crown-of-thorns starfish can detect the presence of benthic predators within potential settlement substrates (largely based on chemoreception, (*sensu* Johnson et al. 1991b)), and thereafter, explore the extent to which larval crown-of-thorns starfish preferentially settle in microhabitats with and without predators present. There has been much work on the role of chemoreception in the selection of settlement substrates by marine larvae (reviewed by Pawlik 1992). For example, larval fishes and corals use chemical cues to discriminate between settlement substrates (Dixson et al. 2011) and degraded and healthy reefs (Dixson et al. 2014), as well as respond to the presence of conspecifics (Sweatman 1988). Similarly, brachiolaria larvae of crown-of-thorns starfish respond to chemical cues associated with specific bacterial films, causing them to discriminate between different substrates during settlement (e.g., Johnson et al. 1991a, 1991b; Johnson and Sutton 1994). Moreover, adult crown-of-thorns starfish have been shown to use chemoreception both to locate and orientate towards potential prey (Brauer and Jordan 1970) and feeding conspecifics (Ormond et al. 1973). Given the inherent ability of crown-of-thorns starfish to respond to chemical stimuli, combined with potentially intense predation pressure at settlement, we expect that larval crown-of-thorns starfish are able to both detect and actively avoid predators during settlement.

6.3. Materials and Methods

6.3.1. Collection and Maintenance of Study Species

Adult *Acanthaster* cf. *solaris* (Haszprunar and Spies 2014) were collected from reefs around Lizard Island (14°40’ S; 145°27’ E) in the northern Great Barrier Reef between October and November 2015. All experiments were conducted at the Lizard Island Research Station. Spawning was induced following Cowan et al. (2016 - Chapter 4). One mL 10^{-4} M 1-methyladenine (1-MA) was injected into the gonads in each arm, through the aboral side of each starfish, which immediately induced spawning in males. Females, meanwhile, spawned within 20-30 min of administering 1-MA. To ensure that a mix of genotypes was used, gametes were collected using glass pipettes from at least 3 individuals of each sex. Sperm was rinsed in 0.2-µm filtered seawater (FSW) and refrigerated at 4 °C prior to use. Eggs were collected from around the arms of females following their release from gonopores and rinsed with 0.2-µm FSW. Fertilization was achieved by adding sperm to reach a final sperm-egg ratio of approximately 100:1. Fertilised eggs were transferred to 16-L larval rearing chambers at a density of approximately 1-2 larvae mL^{-1}. Chambers were maintained at 28.4 ± 1.1 °C (mean ± SD). Larvae were fed twice daily on a mixture of cultured algal species (*Dunaliella tertiolecta* and *Chaetoceros muelleri* at a concentration of 5000 cells mL^{-1} of each species). Water in rearing chambers was changed daily. Larval development was monitored daily, and late brachiolaria stage larvae were placed in separate rearing containers prior to use in experiments.

6.3.2. Preparation of Cues

Settlement experiments were conducted using coral rubble encrusted with crustose coralline algae (CCA), which was collected from shallow reef environments (<3 m depth) on the sheltered (north-west) side of Lizard Island. Rubble was broken into 1-2 cm pieces. Prior to experiments, all motile fauna were removed from coral rubble by manually removing fauna and repeatedly rinsing small fragments in saltwater. To ensure that rubble fragments were free of any potential infaunal predators, we also immersed fragments in freshwater for 30 s prior to using them in experiments. Although potential epibenthic predators were physically removed from the rubble, CCA and other encrustations were left intact. The predominant motile invertebrates removed from freshly collected rubble were polychaetes (mainly Nereididae and Amphinomidae). Amphinomidae polychaetes, *Pherecardia striata*, are known to prey upon newly settled *Acanthaster* cf. *planci* (Glynn 1982, 1984), and thus extracted polychaetes were retained for predation experiments. Trapeziid crabs (*Trapezia flavopunctata*, *Trapezia bidentata*, and *Trapezia cymodoce*) were collected by manually removing crabs with plastic forceps from *Pocillopora* corals collected from lagoonal reefs at Lizard Island. Corals were then maintained in a separate aquarium with flow-through seawater, for a minimum period of one week, prior to use in experiments. We did not distinguish between specific crab species or polychaete species in any of the experiments.

6.3.3. Predation Rates by Benthic Predators

To quantify predation rates by polychaetes and trapeziid crabs on brachiolaria larvae of crown-of-thorns starfish, individual predatory organisms (*n* = 16 for polychaetes; *n* = 8 for crabs) were placed in 70 mL specimen containers with 0.2 µm FSW and 10 brachiolaria larvae. No habitat was added in order to minimise the possibility of larval mortality occurring due to factors other than predation by the study organism. Predators were allowed to feed for 12 h through the night (18:30-06:30), and the number of starfish larvae that remained after this period was recorded. Controls were also conducted in which 10 brachiolaria larvae were added to 70 mL specimen containers with no predators.

6.3.4. Static Choice Chambers

To test the ability of settling *Acanthaster* sp. larvae to detect and respond to olfactory cues associated with potential settlement substrates and/or potential predators, we used static choice chambers consisting of two 10-L aquaria (chambers) connected by 150-mm diameter clear acrylic pipe. Substrates, with and without potential predators, were added to 0.2 µm FSW 24 h prior to the onset of experiments. Cues offered were: (i) the coral, *Pocillopora damicornis* without any infaunal organisms; (ii) *P. damicornis* with commensal trapeziid crabs; (iii) cleaned rubble; (iv) rubble with predators (polychaetes); and (v) adult *A.* cf. *solaris.* These cues were added to one or both chambers, in the combinations: (a) Cleaned rubble vs. FSW; (b) Rubble with polychaete predators vs. FSW; (c) Cleaned rubble vs. rubble with polychaete predators; (d) Cleaned rubble vs. coral; (e) Coral vs. FSW; (f) Coral with commensal crabs vs. FSW; (g) Coral vs. coral with commensal crabs; (h) Adult *A.* cf. *solaris* vs. FSW, offering larvae a pairwise choice of water sources. Larvae (n = 10) were individually placed in the centre of the pipe connecting both chambers, allowing horizontal movement towards one or the other aquaria. During trials, both aquaria were covered to minimise wind-driven water movement. Trials were conducted on a single larva, and larvae were not re-used. A choice was scored when the larvae moved well outside of the connecting tube and into one or the other of the two aquaria; there were no instances of larvae swimming back into the tube after entering an aquarium. If no choice was made after one hour, the larva was removed and “no choice” was recorded. After five replicates the chamber was cleaned and the water sources were switched to the opposite side to ensure that preferences were not biased for one side of the chamber. The response of larvae to each pairwise choice of cues was analysed using a Chi-square goodness of fit test against equal expected proportions, using Yates’s correction, as expected counts were Š 5.

6.3.5. Settlement Assays

Settlement assays were conducted to determine whether the presence of benthic predators causes differences in rates of larval settlement. Ten larvae were introduced into 250 mL beakers filled with 0.2 μm FSW and containing one of two treatments: cleaned rubble (n = 7); or rubble with predators (polychaetes) (n = 7). Polychaetes were used as the predators in these experiments (cf. trapeziid crabs), because they naturally associate with rubble (cf. trapeziid crabs that generally associate with live coral). Controls were conducted in which larvae were introduced to 250 mL beakers (n = 7) containing only 0.2 μm FSW. Beakers were visually examined for indication of settlement/predation at 1, 2 and 6 h: when larvae were no longer swimming in the water column, they were assumed to have settled, or been consumed by predators. At 12, 24, 36 and 48 h, the number of larvae still swimming and the number that had settled out of the water column were counted. Larvae detected on the bottom of the beaker were counted as settled and checked under a microscope at the end of the experiment for evidence of metamorphosis. At 48 h, all substrates were also examined under a microscope for evidence of settled and metamorphosed individuals.

A repeated measures permutational analysis of variance (PERMANOVA) with "Treatment" (3 levels, fixed) and "Time" (4 levels, random) was run to test whether the biological habitat and presence or absence of predators had an effect on settlement success of larvae. PERMANOVA is a non-parametric technique that can be used in analysing univariate data (Anderson et al. 2008). Analyses were conducted using the PERMANOVA+ add-on for PRIMER v.6 (Primer-E Ltd., Plymouth, UK), using the Euclidian distance measure and 9999 permutations of the residuals under a reduced model, to calculate the significance of the pseudo-F statistic. Post hoc pair-wise comparisons used Monte-Carlo asymptotic *p*-values (p_{MC}), as the number of unique permutations was low.

6.4. Results

6.4.1. Potential Predators

All three categories of potential benthic predators tested in this study (polychaetes, trapeziid crabs and scleractinian corals) caused elevated rates of mortality among late stage (competent) brachiolaria larvae of crown-of-thorns starfish (Fig. 6.1). Mortality rates for starfish larvae exposed to individual polychaete worms averaged 1.9 larvae (out of 10) ± 0.6 (SE). For starfish larvae exposed to individual trapeziid crabs (ca. 10 mm carapace diameter), mortality rates were 4.5 larvae (out of 10) ± 0.9 (SE). By comparison, 100% of larvae survived across all controls. Mortality rates for starfish larvae exposed to the scleractinian corals, *Pocillopora damicornis*, were not explicitly measured, but all larvae that came into contact with the polyps were immediately consumed..

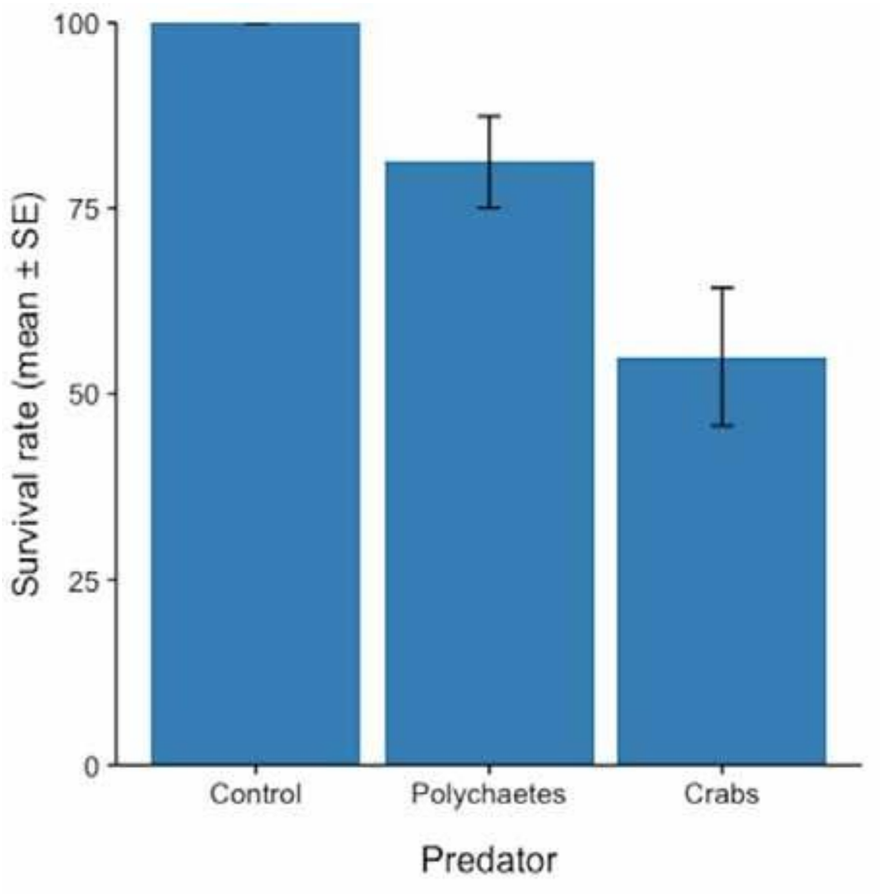
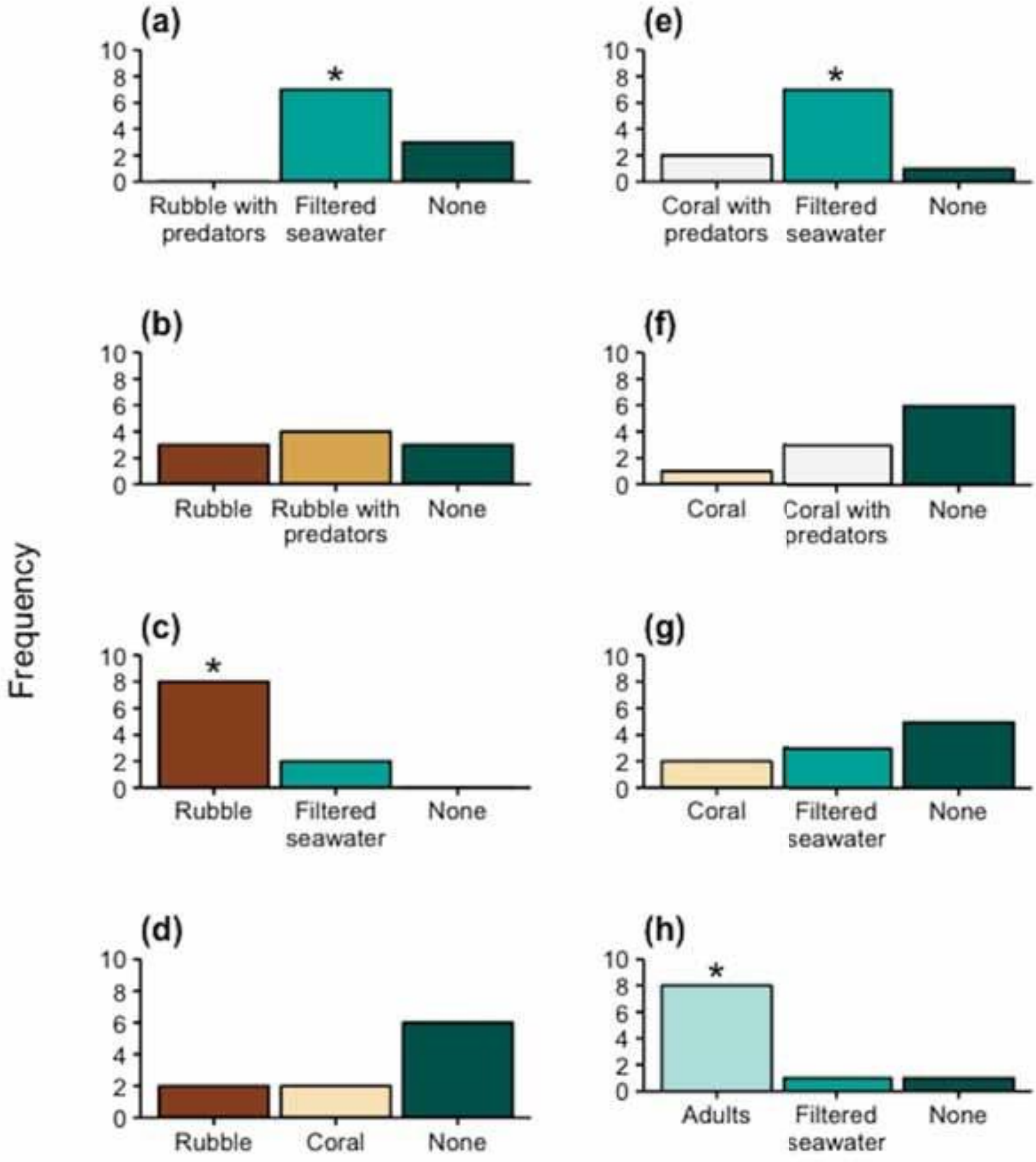


Figure 6.1 Mean survival (+ SE) of brachiolaria larvae during a nocturnal, 12-hour period, in the absence of predators versus when exposed to polychaetes and Trapeziid crabs.

6.4.2. Static Choice Chambers

A total of 80 brachiolaria larvae were used in static choice experiments, across 8 different treatments (Fig. 6.3). Of these 80 larvae, a total of 55 larvae moved outside of the connecting tube, actively swimming towards one or other of the adjoining aquaria containing alternative settlement substrates or cues. Starfish larvae did exhibit significant avoidance of predators (both trapeziid crabs associated with *Pocillopora* coral (3^2 = 6.2, df = 2, p = 0.05), and polychaete predators naturally associated with freshly collected pieces of rubble (3^2 = 7.4, df = 2, p = 0.02) relative to FSW (Fig. 6.2). However, starfish larvae did not discriminate when comparing settlement substrates (rubble with conspicuous CCA and the scleractinian coral, *P. damicornis*) with and without predators (polychaetes: 3^2 = 0.2, df = 2, p = 0.90, crabs: 3^2 = 3.8, df = 2, p = 0.15; Fig. 6.2). When comparing corals with and without trapeziid crabs, 6 (out of 10) of the starfish larvae remained within the connecting tube (and did not venture into either of the adjoining aquaria) for the entire period of observation (60-min).

In the absence of predators (i.e., using rubble and live coral from which all predators had been removed), brachiolaria larvae of crown-of-thorns starfish exhibited strong and significant preference for cleaned rubble with conspicuous CCA over FSW (3^2 = 10.4, df = 2, $p < 0.01$). However, there was no significant difference in response when larvae were offered coral versus FSW (3^2 = 1.4, df = 2, p = 0.50; Fig. 6.2e). Starfish larvae also did not significantly discriminate when offered a choice of cleaned rubble (with CCA) versus coral (3^2 = 3.2, df = 2, p = 0.20; Fig. 6.2c). Larvae exhibited the strongest preference when comparing adult *Acanthaster* cf. *solaris* with FSW (3^2 = 9.8, df = 2, $p < 0.01$), with 8 (out of 10) larvae swimming towards adult conspecifics (Fig. 6.2h).



choice of cues: (a) Cleaned rubble vs. rubble with polychaete predators; (b) Rubble with polychaete predators vs. filtered seawater; (c) Cleaned rubble vs. filtered seawater; (d) Cleaned rubble vs. coral; (e) Coral vs. coral with commensal crabs; (f) Coral with commensal crabs vs. filtered seawater; (g) Coral vs. filtered seawater; (h) Adult crown-of-thorns starfish vs. filtered seawater. * Indicates a significant difference in larval choice frequency indicated by Chi-square goodness of fit test.

6.4.3. Settlement Assays

A total of 210 larvae were used in the settlement assay, across three treatments (Fig. 6.3). Of these 210 larvae, a total of 126 larvae were recovered after 48 h, either still swimming, or in contact with the substrate. There was a significant effect of treatment on the number of larvae still swimming in the settlement assay (Pseudo-$F_{(2,54)} = 13.27$, $p_{perm} < 0.01$). However, there was no significant effect of time (Pseudo-$F_{(3,54)} = 2.17$, $p_{\mathrm{perm}} > 0.05$) and no significant interaction between treatment and time (Pseudo-$F_{(6,54)} = 1.26$, $p_{perm} > 0.05$). In the presence of a settlement substrate, significantly fewer larvae were recorded as swimming, compared to the control ($t = 4.88$, $p_{\mathrm{MC}} < 0.01$), and this was regardless of the presence of polychaete predators ($t = 5.28$, $p_{\mathrm{MC}} < 0.01$). However, there was no significant difference in the number of larvae still swimming in the presence of a substrate with, versus without, polychaete predators ($t = 0.69$, $p_{\mathrm{MC}} > 0.05$; Fig. 6.3a).

A total of 12 larvae were recorded as settled after 48 h, across the three treatments. In the absence of a substrate, 0% of larvae were induced to settle. There was a significant effect of treatment (Pseudo-$F_{(2,54)} = 13.60$, $p_{perm} < 0.01$) and time (Pseudo-$F_{(3,54)} = 3.80$, $p_{perm} = 0.01$), and a significant interaction between treatment and time (Pseudo-$F_{(6,54)} = 2.29$, $p_{perm} = 0.05$) on the number of larvae that were recorded as settled in the settlement assay (Fig. 6.3b). The number of settled larvae that were recovered from the cleaned rubble treatment was significantly greater than for the control at 12 ($t = 3.67$, $p_{\mathrm{MC}} < 0.01$), 24 ($t = 3.43$, $p_{\mathrm{MC}} < 0.01$), 36 ($t = 3.58$, $p_{\mathrm{MC}} < 0.01$), and 48 ($t = 2.46$, $p_{\mathrm{MC}} = 0.03$) hours. There was no significant difference in the number of settled larvae in the cleaned rubble treatment, compared to the rubble with predators treatment after 12 h ($t = 2.01$,

$p_{MC} > 0.05$); however, a significant decrease in the number of settled larvae recorded in the predator treatment from 12 to 24 h ($t = 2.52$, $p < 0.05$) meant that the number of settled larvae in the cleaned rubble treatment was significantly greater than for the rubble with predators treatment at 24 h ($t = 2.71$, $p_{MC} = 0.02$) and 36 h ($t = 2.57$, $p_{MC} = 0.03$) (Fig. 6.3b). Although not significant, the number of settled larvae in the cleaned rubble treatment decreased from 12 to 48 h ($t = 2.26$, $p_{MC} > 0.05$), so the number of settled larvae in the cleaned rubble treatment, versus rubble with predators, was not significantly different after 48 h ($t = 1.53$, $p_{MC} > 0.05$). Notably however, 55% of larvae that settled in the absence of predators had undergone metamorphosis at 48 h, compared to 0% when predators were present.

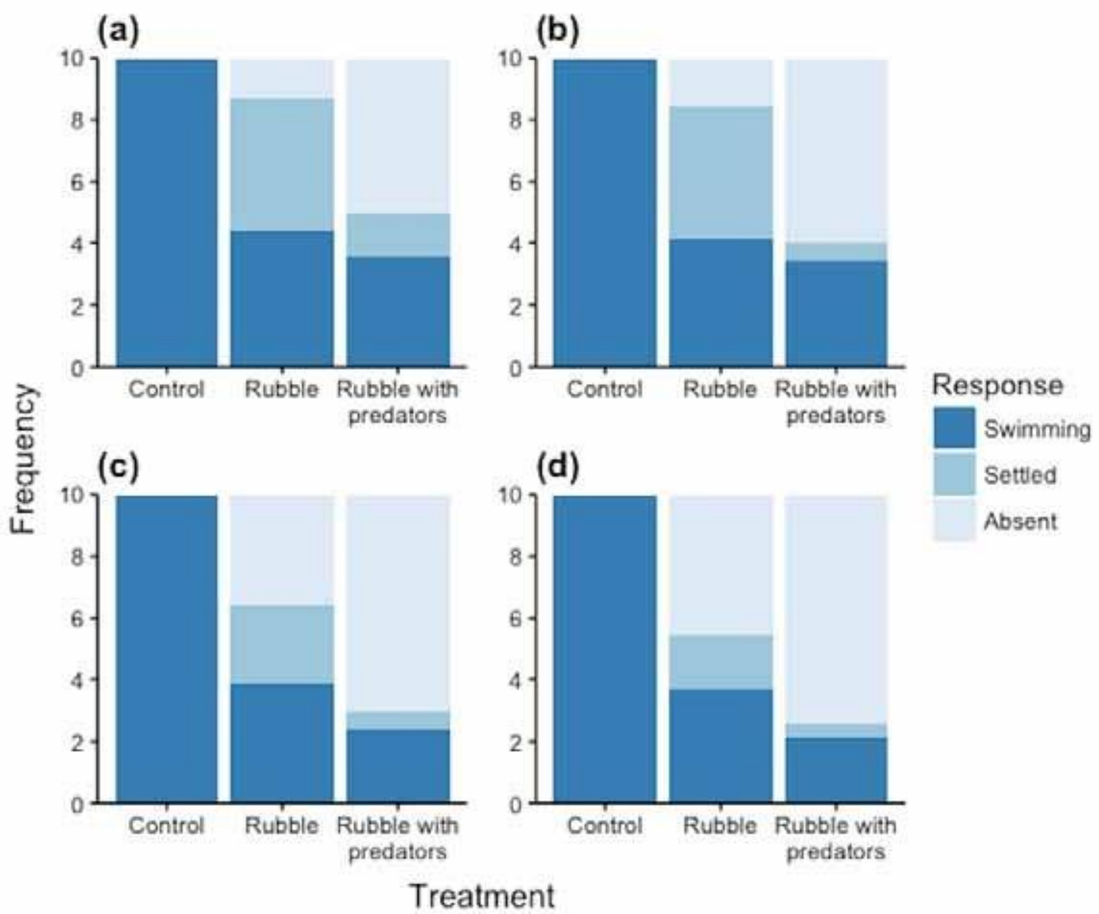


Figure 6.3 Mean number of larvae still swimming, settled and absent in each treatment after (**a**) 12, (**b**) 24, (**c**) 36, and (**d**) 48-h.

6.5. Discussion

Given limited capacity for inter-reef movement of adult crown-of-thorns starfish (Glynn 1982), their abundance on any given reef is fundamentally dependent on rates of successful settlement and recruitment. To maximise settlement success, it would be expected that crown-of-thorns starfish preferentially settle to locations and microhabitats that minimise exposure to potential predators (e.g., Mileikovsky 1974), either settling in locations that are naturally depauperate of potential predators or selecting microhabitats that provide refugia from predators. This study shows that settling larvae of *Acanthaster* cf. *solaris* are highly vulnerable to a range of different benthic predators, including scleractinian corals and their commensals (e.g., trapeziid crabs), as well as polychaete worms that were commonly found on freshly collected pieces of coral rubble, adding to the wide range of predators known to feed on early life-stages of *Acanthaster* spp. (e.g., Yamaguchi 1973; Zann et al. 1987; Keesing and Halford 1992; Cowan et al. 2016 - Chapter 4). Given the vulnerability of crown-of-thorns starfish to predators (reviewed by Cowan et al. 2017 - Chapter 2), it is likely that there are many more predators within the coral reef benthos capable of feeding on settling or post-metamorphic starfish. Once a more complete range of potential predators is established, or those that have the most significant impact on settlement and recruitment success of crown-of-thorns starfish, it will be important to test for spatial variation in abundance and diversity of these predators. For highly fecund species, such as *Acanthaster* spp., small changes to the rates of mortality at these early life stages are likely to have significant knock-on effects, and may account for spatial and temporal variation in the incidence of outbreaks (McCallum 1988, 1990).

Larvae of coral reef organisms, including fishes and corals, use chemical cues to distinguish between healthy and degraded reefs (Dixson et al. 2014). The attraction of brachiolaria larvae of *Acanthaster* cf. *solaris* to cues from cleaned rubble and deterrence from these substrates when polychaete predators were present suggests that larvae are able to detect these predators and may have the capacity to avoid settling to environments with high densities of benthic predators. This indicates a mechanism by which crown-of-thorns larvae can similarly distinguish between healthy and degraded reefs, as degraded reefs can have reduced densities of benthic predators. Polychaetes are amongst the most numerous and abundant component of the macrofauna found within the reef matrix (e.g., Grassle 1973) and are indicator species for marine degradation (Dean 2008). Both abundance and species richness of polychaetes are reduced in fished sites, compared to marine protected areas, as a result of trophic cascades (Pinnegar et al. 2000). Reefs that have been damaged by cyclones also have reduced polychaete abundance (Sukumaran et al. 2016). This may be an important factor in shaping recruitment patterns of *Acanthaster* spp. to reefs, with degraded reefs being more attractive to settling larvae.

Settling *Acanthaster* spp. larvae were not attracted to live coral substrates, and given that corals will prey upon settling larvae, this may explain the rejection of these substrates regardless of the presence, or absence, of commensal predators. Rather, settling crown-of-thorns starfish may actually avoid areas with high coral cover (Chesher 1969). It has been suggested that larvae of crown-of-thorns starfish preferentially settle in areas with high abundance of adult conspecifics because the feeding activities of these adult starfish provide areas of recently dead coral, representing suitable settlement habitats (Pratchett et al. 2014) as well as minimizing the risk of predation by live corals (Chesher 1969). Supporting this, our analyses of settlement preferences based on static choice chambers confirmed that larvae are significantly attracted to adult conspecifics. While other invertebrates, including echinoderms, settle on, or near to, conspecifics (Pawlik 1992; Dworjanyn et al. 2007), this is the first time that it has been demonstrated for *Acanthaster* spp. and work is required to establish the mechanistic basis of this behaviour.

Whilst *Acanthaster* spp. larvae may preferentially settle in habitats with fine-scale topographic complexity to minimise mortality in the early life stages (Lucas 1975), numerous presumed predators are abundant in the reef matrix (e.g., Glynn 1984; Keesing et al. 1996; Rivera-Posada et al. 2014) and results of the settlement assay suggest that settlement is induced in the presence of a cue from substrates (cleaned rubble with naturally attached crustose coralline algae), with the presence of benthic predators unlikely to cause larvae to delay settlement (Fig. 6.3a). Lack of difference in the number of settled larvae recovered from the rubble with predators treatment compared to the control, despite significant difference in the numbers of larvae still swimming in the settlement assays, indicates high levels of predation upon settling larvae, and is supported by our measurements of benthic predation rates. Additionally, no fully metamorphosed juveniles were recovered from treatments containing predators, compared to cleaned rubble treatments, in which 55% of settled starfish had metamorphosed. These data suggest that on a local scale, healthy benthic fauna is highly important in reducing successful recruitment of *Acanthaster* spp., through predation on settling larvae. Settlement substrate and predation by benthic predators may explain high variability in recruitment rates of *Acanthaster* sp. observed by Nakamura et al.

(2015). Acroporids and *Acanthaster* sp. likely prefer to settle on similar substrate types, but variation in local abundance of benthic predators may have a more significant effect on recruitment of *Acanthaster* sp. compared to corals, for which recruitment was observed approximately 1 month after spawning (Nakamura et al. 2015).

In addition to the methods outlined in this study, we tested the ability of *Acanthaster* cf. *solaris* larvae to respond to olfactory cues using two-channel Atema flumes (Atema et al. 2002). The flow rate in the flume chamber was set to the lowest possible speed (0.1 $L \cdot min^{-1}$), which allowed larvae to maintain their position and move across the current, whilst also ensuring laminar flow. Food dye was used to test for laminar flow and confirm separation of water sources. Individual larvae were placed in the centre of the test chamber, allowing choice between the two streams and movement towards a preferred water source. Whilst larvae were able to maintain position and move across the flow of water in the Atema flume chamber, we could not determine with confidence whether larvae were actively choosing one cue over the other: larvae were observed to move from the central starting position; however, they settled against either the outer edges or inner partition of the flume chamber and appeared to become trapped. Adaptation of the flume chamber to exclude edges in which larvae could become trapped would likely overcome this; thus, this may be an efficient method to test the response of *Acanthaster* spp. larvae to a range of environmental cues. Further, this demonstrates an ability of, at least the late-brachiolaria stage, larvae to move against a light current. This could be important in enabling larvae to manoeuvre across the reef matrix when searching for a suitable settlement habitat.

6.6. Conclusions

Variation in the abundance of benthic predators is likely to have a significant influence on settlement patterns of the crown-of-thorns starfish, as indicated by the avoidance of late-stage brachiolaria larvae of substrates that contain predators, and low rates of settlement and metamorphosis on these substrates. Healthy benthic fauna is therefore likely to be important in regulating abundance and moderating settlement success of *Acanthaster* spp. Any extrinsic threat to benthic communities, including disruptive effects that may lead to trophic cascades, are likely to reduce the buffering capacity of coral reefs, increasing susceptibility to devastating outbreaks of crown-of-thorns starfish. Demographic studies of marine invertebrates often reveal particularly intense mortality in the period immediately following settlement (e.g., Gosselin and Qian 1997; Pineda et al. 2002). Studies described herein may also be extended to newly settled, post-metamorphic juveniles, with the aim of determining predation rates and further examining their behavioural responses to environmental cues.

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Chapter 7: General discussion/ conclusions

7.1. Predation on the early life stages of the crown-of-thorns starfish

Predation has long been recognised as a key process in structuring coral reef communities (e.g., fish; reviewed by Hixon 1991). Intuitively, small or young (and potentially naïve) individuals will be more susceptible to predation than larger, older, and comparatively more experienced adults (e.g., Murdoch and Oaten 1975; Goatley and Bellwood 2016). Despite this, and many decades of research into the ability of predators to exert control over populations of *Acanthaster* spp., studies have primarily focused on predators of the adult life stages (reviewed by Cowan et al. 2017b - Chapter 2). Field observations of spawning starfish have shed little light on possible predators, revealing only two species that will readily consume CoTS eggs (*Abudefduf sexfasciatus*, Endean 1969; *Amblyglyphidodon curacao*, Pearson and Endean 1969). Further, early research into predation on eggs and larvae of CoTS reported that predators largely avoid these early life stages (Yamaguchi 1974, 1975), due to anti-predator chemicals contained within tissues of all life-stages (Lucas et al. 1979). In reality, there are few organisms that are completely immune to predation at any or all stages of their life cycle.

The large number of previously unrecognized predators identified in this research (11 damselfishes (Cowan et al. 2016a - Chapter 4), 3 motile invertebrates, and 1 sessile invertebrate predator of larvae (Cowan et al. 2016b - Chapter 6), and up to 8 potential predators of eggs (Cowan et al. 2017a - Chapter 3)), clearly indicate that the early life stages of CoTS are vulnerable to predation. Further, every species that was investigated as a potential predator of *Acanthaster* sp. eggs and/or larvae throughout the course of this research was found to consume these early life stages, albeit to varying extents. This would suggest that there are many more predators of *Acanthaster* spp., yet to be identified, that may play a significant role in reducing reproductive and recruitment success, and ultimately maintaining normal, low-density populations of adult starfish.

Planktivorous damselfishes are highly efficient predators that are capable of removing a significant portion of the zooplankton from near-reef waters (Hamner et al. 1988). High levels of predation on planktonic larvae by these fishes is known to affect local recruitment of benthic organisms (e.g., Gaines and Roughgarden 1987). Thus, the sheer abundance of planktivores on coral reefs (Pratchett et al. 2012; Komyakova et al. 2013) suggests a potentially key role of planktivory in limiting larval survival and settlement success of *Acanthaster* spp., and ultimately constraining local densities of adult starfish. Planktivorous damselfishes are a particularly important group within the planktivores, making up more than 55% of the coral reef fish (36% of the total number of species) reported from Lizard Island (Komyakova et al. 2013). However, predatory responses of damselfishes feeding on the early life stages of *Acanthaster* spp. were found to be highly variable (e.g., Cowan et al. 2016a - Chapter 4), highlighting the importance of diverse communities of predators, and not simply high abundance of individual predator species.

Damselfishes such as *A. curacao* and *Dascyllus aruanus* emerge as potentially key predators of *Acanthaster* spp. as they appear to be most tolerant of anti-predator chemicals contained within eggs and larvae (Cowan et al. 2017a - Chapter 3). However, after standardising for size, smaller bodied species such as *Chrysiptera rollandi* and *Pomacentrus moluccensis* also emerge as potentially important predators, as they consume the greatest proportion of eggs per gram of body mass (Cowan et al. 2017a - Chapter 3). Given that smaller fishes can be extremely abundant on coral reefs (Pratchett et al. 2012; Komyakova et al. 2013), these species may have significant capacity to regulate reproductive success of *Acanthaster* spp. Field observations have detected high densities of *Acanthaster* spp. larvae, up to 53.3 individuals m^{-2} (Suzuki et al. 2016), thus species that consume very large numbers of larvae would presumably play a key role in moderating settlement success. Again, *D. aruanus*, which exhibited a satiation limit of 158 larvae h^{-1}, is highlighted as a potentially key predator of these early life stages, and to a lesser extent *A. curacao* (62 larvae h^{-1}), *C. rollandi* (62 larvae h^{-1}), *P. moluccensis* (54 larvae h^{-1}), and *Dascyllus reticulatus* (53 larvae h^{-1}) may be particularly important in reducing high larval densities (Cowan et al. 2016a - Chapter 4). Further, preferential feeding on *Acanthaster* spp., when alternative prey sources are available suggests that *A. sexfasciatus* and *Pomacentrus amboinensis* might be of particular importance in maintaining low densities of *Acanthaster* spp. (Chapter 5). Considering values for relative abundance of damselfishes reported in Table 3.1 (Cowan et al. 2016a - Chapter 4) and Table 5.1 (Chapter 5), and a mean abundance of 1.06 *P. amboinensis* per 2 m x 2 m quadrat (0.27 individuals m^{-2}) reported from Lizard Island (Komyakova et al. 2013), the planktivorous fish community may be capable of consuming in excess of 230 CoTS larvae.m^{-2}.h^{-1}. This estimate would appear to far exceed the larval density reported by Suzuki et al. (2016) of 53.3 individuals m^{-3}. However, abundance and diversity of damselfishes will vary both spatially and temporally, with this estimate representing just eight damselfish species. Furthermore, the reported maximal consumption rate was measured when damselfish were provided with CoTS larvae as the sole prey item (Cowan et al. 2016a - Chapter 4); whilst many damselfishes will still feed on CoTS larvae in the presence of alternative prey (Chapter 5), a far greater variety of alternative prey items will be available in the natural plankton community that is found on coral reefs and this may impact the prey selection choices made by putative predators.

In addition to suffering substantial levels of predator-induced mortality in near reef waters, *Acanthaster* spp. larvae, like many other marine larvae (e.g., Hines 1986; Stoner 1990) are expected to suffer considerable mortality due to predation at, or soon after, settlement. Indeed, ~50% of competent larvae may be consumed by predators during settlement (Yamaguchi 1973); further, post-settlement mortality rates due to predation appear to be highest for smallest individuals, and have been measured at ~6.5% day^{-1} for small juveniles (~1-mm diameter), decreasing to 1.24% day^{-1} and 0.45% day^{-1} for juveniles measuring ~3 and ~5-mm, respectively (Keesing and Halford 1992). The long period of time (days to weeks) spent developing in the plankton (Pechenik 1979), in addition to the widespread ability of marine larvae, including *Acanthaster* spp. (Pratchett et al. 2016), to delay settlement in the absence of a suitable substrate (Pechenik 1986, 1990), might suggest that risk of predation in the benthos is even higher than would be experienced in the plankton (Pechenik 1979, 1999). *Acanthaster* spp. may be especially vulnerable to predators at the settlement stage, as they exhibit slow larval swimming speeds (Chia et al. 1984), thus may be confined to within a few millimetres of the substrate by hydrodynamic forces (Jonsson et al. 1991; André et al. 1993). Such high risk of predation during settlement should provide sufficient selective pressure for the evolution of mechanisms that enable organisms to reduce mortality at this stage, choosing microhabitats that minimise predation risk (Lucas 1975), either by settling to complex microhabitats that provide a greater refuge from predators, or by avoiding habitats with high abundance of potential predators. Settling *Acanthaster* spp. larvae face risk of predation from a range of benthic organisms, including corals, coral commensals, and polychaetes (Cowan et al. 2016b - Chapter 6). The capacity of larvae to avoid substrates containing high densities of benthic predators (Cowan et al. 2016b - Chapter 6) may serve to reduce predation risk during settlement. However, it seems that *Acanthaster* spp. do not actually delay settlement in the presence of potential benthic predators, reinforcing the importance of these predators in potentially regulating settlement success of *Acanthaster* spp. (Cowan et al. 2016b - Chapter 6).

Significant rates of mortality during and soon after settlement, are fundamental in structuring the settlement preferences and life history dynamics of marine invertebrates (Pechenik 1999; Gallagher and Doropoulos 2017). Clearly *Acanthaster* spp. are vulnerable to high levels of predation during these early life stages, such that the combination of predation by planktivorous damselfishes and cryptic benthic predators almost certainly moderate settlement and population replenishment. This high level of predator-induced mortality likely represents a major bottleneck in the life history of *Acanthaster* spp., as has been shown for many other coral reef invertebrates (e.g., corals; Chong-Seng et al. 2014). Further, any changes to the rates of predation and mortality at these pre-settlement and settlement stages could be expected to have a significant effect on patterns of adult abundance.

The role of predation in regulating CoTS populations remains highly controversial. Over-exploitation of key predators of the post-settlement stages remains a viable means for which CoTS have been able to escape regulatory control, as key predators may have either failed to recover from historic over-exploitation (e.g., the giant triton (Endean 1969)), or continue to be over-exploited (e.g., large predatory fishes (Dulvy et al. 2004)). However, it is increasingly being recognised that CoTS are vulnerable to predation at the pre-settlement and settlement stages, with relatively subtle changes in the abundance of predators and/or predation rates predicted to reduce the level of local recruitment required to overcome (or satiate) predators (McCallum 1990). A reduction in predation pressure on either the pre- or post-settlement stages has the potential to lead to a scenario in which CoTS can saturate top-down control. Whilst this thesis does not explicitly test whether predation on adult vs. pre-settlement stages is more important, it is perhaps more likely to be predators of the planktonic and settlement stages that will be overwhelmed first because the presence of a very large adult population would suggest that predators of these early life stages have already been overwhelmed.

7.2. Overfishing of predators and outbreaks of *Acanthaster* spp.

Reef-wide densities of potentially important predators on *Acanthaster* spp. may be supressed due to excess harvesting (Endean 1969) and overfishing (Dulvy et al. 2004). Endean (1969), for example, suggested that excess harvesting of the giant triton (*Charonia tritonis*) was a primary contributor to outbreaks of *Acanthaster* sp. on Australia's Great Barrier Reef in the 1960s. The removal of predators that can feed on adult starfish both reduces levels of adult mortality, having a direct impact on population density, but also allows adult starfish to achieve larger body size (Endean 1969). Given that individual fecundity increases exponentially with increasing body size (Babcock et al. 2016b), and that *Acanthaster* spp. achieve exceptionally high rates of fertilization success when spawning adults are in close proximity (Yund 1990; Grosberg 1991; Levitan et al. 1991; Benzie et al. 1994), the removal of predators of the adult stage would be expected to significantly increase the reproductive output and success of these starfishes. However, the removal of large fishes, including piscivorous fishes, may also indirectly affect population densities of *Acanthaster* spp., via trophic cascade mechanisms.

On the GBR, Sweatman (2008) described increased incidence of outbreaks on reefs that were open to fishing, compared to reefs within 'no-take' marine reserves. Although many exploited fishes have only rarely been reported to prey directly on adult *Acanthaster* spp., they may mediate starfish populations through top down control and trophic cascades, which favour predators of juvenile starfish (Sweatman 2008). Trophic cascades following the extensive removal of top predators are observed in many marine systems (e.g., Paine 1980). On the GBR, commonly targeted reef fishes, e.g., *Plectropomus* spp. (Frisch et al. 2016) have been found in higher abundance inside no-take areas, compared to fished reefs, whilst prey species including benthic-feeding wrasses have been found in lower abundance in these no-take areas (Graham et al. 2003). Thus, a suggested mechanism by which these exploited fishes could normally mediate *Acanthaster* spp. populations is via suppression of benthic, carnivorous fishes, and subsequent ecological release of invertebrates that prey on juvenile *Acanthaster* spp. (Sweatman 2008).

Although the planktivorous damselfish predators identified herein are not generally subject to fisheries exploitation (Cheung et al. 2007), they may still be affected by the removal of piscivorous fishes. In addition to suppressing the abundance of prey items, trophic cascades may act by suppressing the behaviour of prey items (Heithaus et al. 2008; Ling and Johnson 2012). In the presence of piscivorous predators, planktivorous damselfishes, feed primarily within 1.5m of the substrate (Motro et al. 2005), where they are able to seek shelter within habitat such as branching corals (e.g., Clarke 1992; Beukers and Jones 1997; Bullard and Hay 2002). Within this layer, intense predation can lead to near-depletion of zooplankton (Holzman et al. 2005; Yahel et al. 2005), which could be expected to significantly reduce the number of *Acanthaster* spp. larvae that are able to reach the substrate. The removal of piscivorous fishes and subsequent release of damselfishes may therefore lead to enhanced settlement rates of *Acanthaster* spp., if these planktivores switch to feeding on alternative prey sources that may be present higher up in the water column.

7.3. Susceptibility of predators to habitat loss

Coral reefs are dynamic environments, which naturally experience a range of disturbances (Hoegh-Guldberg and Bruno 2010). However, in recent decades they have suffered more pronounced habitat degradation and ecosystem loss as a result of global climate change and anthropogenic disturbances, which are exacerbating the effects of these natural disturbance events (Hoegh-Guldberg and Bruno 2010; Pratchett et al. 2011; Hughes et al. 2017). Tropical cyclones and coral bleaching, together with outbreaks of CoTS, are responsible for an estimated 50.7 % decline in live coral cover on the GBR between 1985-2012 (De'ath et al. 2012). Each of these events, and indeed any disturbance that results in the partial loss of live coral cover, may produce declines in abundance of predatory damselfishes, potentially serving to increase the susceptibility of coral reefs to *Acanthaster* spp. outbreaks.

Many of the damselfish predators identified in this thesis are extremely vulnerable to coral loss (Pratchett et al. 2016). Following loss of 25-60% live coral cover, 60% of coral reef damselfishes exhibit declines in abundance (Pratchett et al. 2016). Of the damselfish predators identified herein, two thirds are reported to experience declines in abundance with the loss of live coral cover (Pratchett et al. 2016). Obligate coral-dwelling damselfishes are expected to be the worst affected. In particular, many of these fishes inhabit only a narrow range of corals, many of which (e.g. *Pocillopora damicornis*), are highly susceptible to climate-induced bleaching and mortality (Pratchett et al. 2008). Of 64 damselfish species considered, *D. reticulatus* (an obligate coral-dweller) experiences the most significant declines in abundance in response to declining coral cover (Pratchett et al. 2016). However, non-coral-dwelling species such as *C. rollandi* also exhibit significant declines as live coral cover decreases (Pratchett et al. 2016).

Aside from planktivorous damselfishes, sustained and widespread degradation of coral reef ecosystems can impact on local densities of other potential CoTS predators. Cyclones are observed to disturb sediments, leading to habitat alteration and favouring opportunistic species (Gamito and Furtado 2009; Peng et al. 2013). On coral reefs, cyclone damage has been shown to negatively impact on the abundance and diversity of polychaetes (Sukumaran et al. 2016). Although macrofaunal recovery may be observed following these physical disturbance events, speed of recovery is hampered by human interference in the system, such that marine protected status is observed to aid in rapid revival of damaged ecosystems (Sukumaran et al. 2016). Likewise, coral bleaching and coral loss decreases density and fecundity of coral crabs *Trapezia cymodoce* (Stella et al. 2011), which feed on CoTS larvae (Cowan et al. 2016b - Chapter 6), but also increases susceptibility of host corals to predation by adult CoTS (Stella et al. 2011) as the crabs provide a defence against adult starfish (Pratchett 2001).

Predation of live corals by adult CoTS may additionally provide a feedback mechanism by which CoTS populations can increase in density (Fig. 7.1). Firstly, reduction of live coral cover directly removes predators of settling larvae (e.g., coral polyps; Yamaguchi 1973), in addition to removing essential habitat for coral-associated predators, with potential for loss of both coral symbionts (Stella et al. 2011) and coral dwelling damselfishes (Pratchett et al. 2016). This would be expected to reduce predation pressure on these critical early life stages of CoTS, with the potential to significantly increase the proportion of survivors through to settlement, and ultimately adulthood. Furthermore, settling larvae have been shown to be significantly attracted to adult conspecifics (Chesher 1969; Cowan et al. 2016b - Chapter 6), further increasing population densities of adult CoTS, in turn promoting even greater coral loss and ever higher densities of adult starfish. This may be important in the formation of primary outbreaks, in which the population builds up gradually over successive years (e.g., Pratchett 2005).

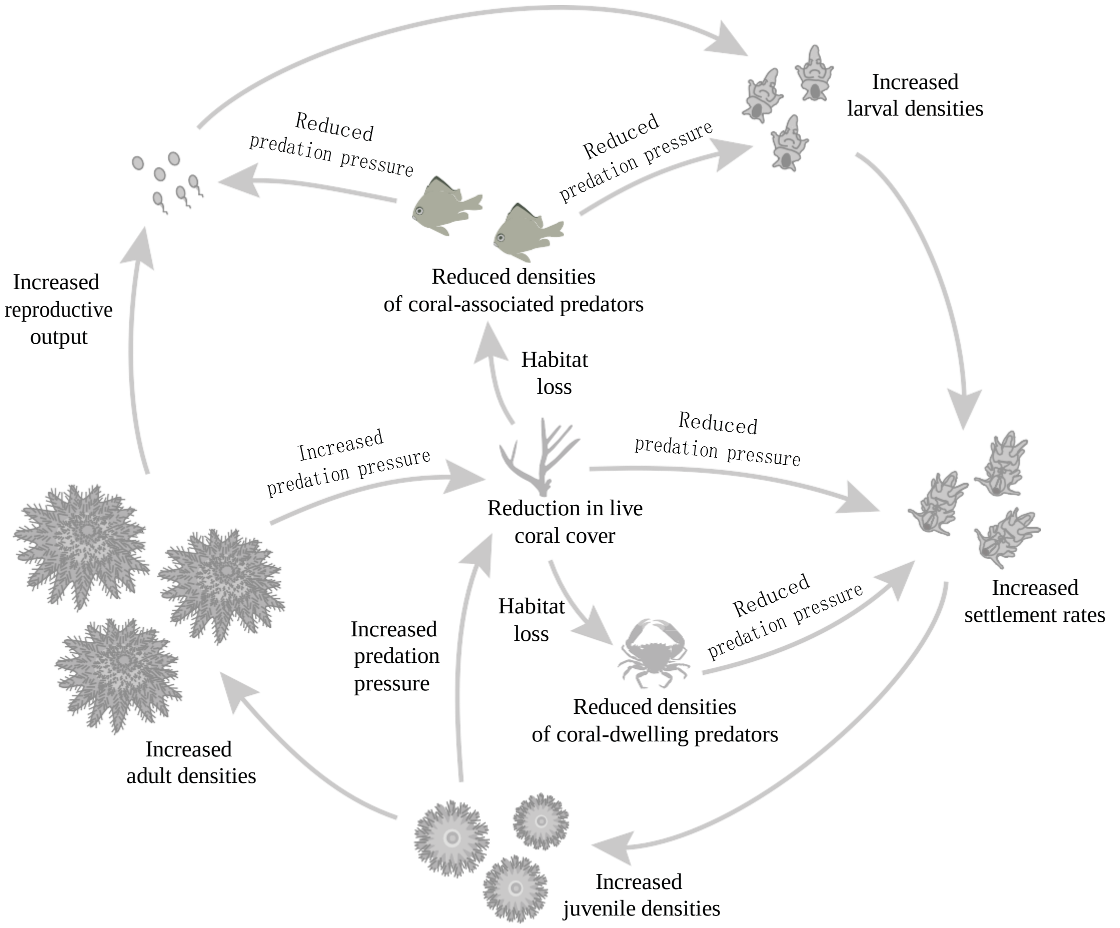

Figure 7.1 Possible feedback mechanisms by which predation by *Acanthaster* spp. on live corals, or reduction in predation pressure on *Acanthaster* spp., may serve to further increase population densities of this starfish.

7.4. Future directions

The exact mechanism(s) by which predators, and predator removal, may impact on CoTS populations remains unclear, however the inherent life-history characteristics of CoTS likely enables them to take advantage of numerous ecosystem disturbances, in order to rapidly increase in population density. Determining the extent to which predation may, or may not, regulate population densities of CoTS is fundamentally reliant on field-based estimates of predation and mortality across all life-stages. DNA screening of diets of large numbers of potential CoTS predators (Redd et al. 2014; Thomsen and Willerslev 2015) and increased potential to tag and track benthic species within reef environments (MacArthur et al. 2008) will provide new insights into predation on CoTS in the field. However, it remains extremely difficult to quantify rates of predation on *Acanthaster* spp. eggs or larvae in the field. Nonetheless, the results presented herein draw attention to the vulnerability of these early life stages to a wide range of predators, notably planktivorous damselfishes, which have been previously overlooked as predators (Yamaguchi 1974, 1975; Lucas et al. 1979). Further, the experiments described herein could be extended, to test a broader range of planktivorous and benthic predators as well as a variety of other alternative prey items that are likely to be present alongside *Acanthaster* spp. in the plankton.

Phenotypic expression of anti-predator morphology during ontogeny may be maximised during larval stages, where vulnerability to predation is greatest (Vuorinen et al. 1989; Harvell 1990; Tollrian 1993). Mean saponin content in *Acanthaster* spp. increases approximately two fold from the egg stage (1.9 % of dry tissue weight x 10) to a one day old larva (5.1 % of dry tissue weight x 10), and again to a three day old larva (9.7 % of dry tissue weight x 10) (Barnett et al.

1988), suggesting independent production of these chemicals and high vulnerability to predation. By comparison, the mean saponin content of a whole adult CoTS is 4.3 % of dry tissue weight x 10 (Barnett et al. 1988). Yet these chemicals, which are presumably costly to produce (Harvell 1990), don't appear to deter reef-based predators from feeding on the early life stages of CoTS (Cowan et al. 2016a, 2016b, 2017a - Chapters Four, Six, Three; Chapter Five). These presumed anti-predator chemicals might serve as a more effective defence against predators found within the plankton, including chaetognaths and larval fishes, however this remains to be tested.

Once a more complete range of potential predators is established, it will be important to evaluate, (a) whether there is spatial variation in abundances of these predators, (b) whether variation in their abundance is indeed correlated with patterns of increased frequency or intensity of outbreaks, and (c) whether patterns of variation correlate with gradients of fishing intensity, and/or in accordance with gradients of habitat degradation. If there is a correlation, then this research may have applications in the management of future outbreaks, to predict reefs that might be at greater risk from invasions of crown-of-thorns starfish.

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Appendix 1 - MacNeil et al. (2016) PeerJ 4:e2310[5]

Joint estimation of crown of thorns (*Acanthaster planci*) densities on the Great Barrier Reef

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ABSTRACT

Crown-of-thorns starfish (CoTS; *Acanthaster spp.*) are an outbreaking pest among many Indo-Pacific coral reefs that cause substantial ecological and economic damage. Despite ongoing CoTS research, there remain critical gaps in observing CoTS populations and accurately estimating their numbers, greatly limiting understanding of the causes and sources of CoTS outbreaks. Here we address two of these gaps by (1) estimating the detectability of adult CoTS on typical underwater visual count (UVC) surveys using covariates and (2) inter-calibrating multiple data sources to estimate CoTS densities within the Cairns sector of the Great Barrier Reef (GBR). We find that, on average, CoTS detectability is high at 0.82 [0.77, 0.87] (median highest posterior density (HPD) and [95% uncertainty intervals]), with CoTS disc width having the greatest influence on detection. Integrating this information with coincident surveys from alternative sampling programs, we estimate CoTS densities in the Cairns sector of the GBR averaged 44 [41, 48] adults per hectare in 2014.

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INTRODUCTION

Outbreaking pests generate extensive environmental degradation and billions of dollars in ecosystem damage annually for resources such as crops (*Oerke, 2006*), forests (*Aukema et al., 2011*), and fisheries (*Knowler & Barbier, 2000*). Among the most ecologically and economically costly are outbreaks of native crown-of-thorns starfish (CoTS; *Acanthaster spp*; *Carter, Vanclay & Hundloe, 1988*), a common coral-eating starfish that outbreaks on many Indo-Pacific coral reefs (*Pratchett et al., 2014*). CoTS are considered a pest due to their destructive impacts on coral populations when outbreaks occur. The causes of CoTS

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outbreaks remain largely unknown, although prominent theories include decreased predation due to fishing of key species (*Sweatman et al., 2008*) and increased larval survivorship due to nutrient pollution (*Fabricius, Okaji & De'ath, 2010*). Of all the major disturbances to coral reefs (e.g., storms, coral bleaching, fishing, pollution, nutrients, and disease) only CoTS outbreaks have the potential to be actively locally managed without negatively impacting other resource users. Yet there is insufficient information available about many aspects of CoTS population dynamics and life history (*Pratchett et al., 2014*) to accurately predict the success of management intervention.

Of the many factors needed to fully understand CoTS population dynamics—including fecundity, larval survival, and settlement—basic information on adult densities is lacking due to inconsistencies in sampling methods that can underestimate local abundance. A key gap in the accuracy of survey data is detectability—the probability of observing individual starfish underwater, given their presence in a surveyed area. Without some estimate of detectability, survey data cannot be indexed to true abundance, limiting the inferences that can be made concerning CoTS population dynamics. Detectability estimation has been widely used among terrestrial and freshwater wildlife studies, including for birds (*MacKenzie et al., 2002*), mammals (*Gu & Swihart, 2004*), and fish (*Peterson & Dunham, 2003*), but these methods have not been widely applied to coral reefs (but see *Kulbicki & Sarramégna, 1999*; *MacNeil et al., 2008*). Quantification of adult CoTS detectability is important for population modeling, spread dynamics, and assessing the feasibility or efficacy of control actions.

K-sample mark-recapture methods for estimating detection probabilities are well suited to repeat visual sampling of most site-attached reef species. These include the application of one or more identifiable marks to individuals within a specific area over a series of K capture occasions, with each observed presence or absence recorded in a K dimensional array of ones and zeros that make up an individual's capture history (i.e., 0, 1, 1, 0, 1, 1 for $K = 6$). In aggregate, an observed population of capture histories allows a distribution of detection probabilities to be constructed and used to estimate how many individuals remained unobserved during sampling.

The Great Barrier Reef (GBR) consists of around 3,000 interconnected reefs stretching more than 2,300 km along the coast of northeastern Australia. There is strong national and international pressure to protect the GBR from increasing degradation due to a series of compounding human and natural disturbances that have eroded average reef conditions over the past 25 years (*De'ath et al., 2012*). While some disturbances are linked to climate change (e.g., storms, bleaching; *Osborne et al., 2011*) and water quality (*Fabricius, Okaji & De'ath, 2010*), CoTS remain a major source of coral mortality (*De'ath et al., 2012*). As such, there is considerable interest in accurately estimating reef-wide CoTS abundance to (a) assess the feasibility of reef-wide controls and (b) project CoTS impacts in terms of ongoing reef degradation.

A key problem in understanding broad-scale CoTS population dynamics on the GBR is that agencies use different survey methods to record the presence, absence, or density of CoTS using different methods that may not be directly comparable. For example, the Queensland Parks and Wildlife Service (QPWS) and Great Barrier Reef Marine Park

Authority's (GBRMPA) joint Field Management Program (FMP) and the Association of Marine Park Tourism Operators (AMPTO) CoTS Control Team conducts reef health surveys to record CoTS abundance, their feeding scars, and the health of their corals prey. This program acts as an early warning system and surveillance tool to help inform cull efforts. AMPTO, in partnership with managing agencies monitors coral cover, culls CoTS and records the number killed within specific areas, with approximately $2.5 million (Australian dollars) committed to the protection of coral in high value tourism areas in 2014–15 (*ReefPlan, 2014*). The Australian Institute of Marine Science (AIMS) long term monitoring program (LTMP) uses a third method of manta-tow surveys to detect whether a reef is suffering CoTS outbreaks above natural densities (*Sweatman et al., 2008*). Currently these different methods of sampling CoTS, while similar in some respects (e.g., AMPTO, AIMS, and the FMP program all use manta tow), cannot be reconciled. Without knowing the true density of CoTS within a survey area the potential bias in each method remains unknown.

To improve abundance estimation for CoTS on the GBR we utilize a K-sample mark-recapture model to estimate detection and infer the density of CoTS in a northern section of the GBR, using covariates thought to impact CoTS detection underwater and a joint model for integration of FMP and AMPTO datasets.

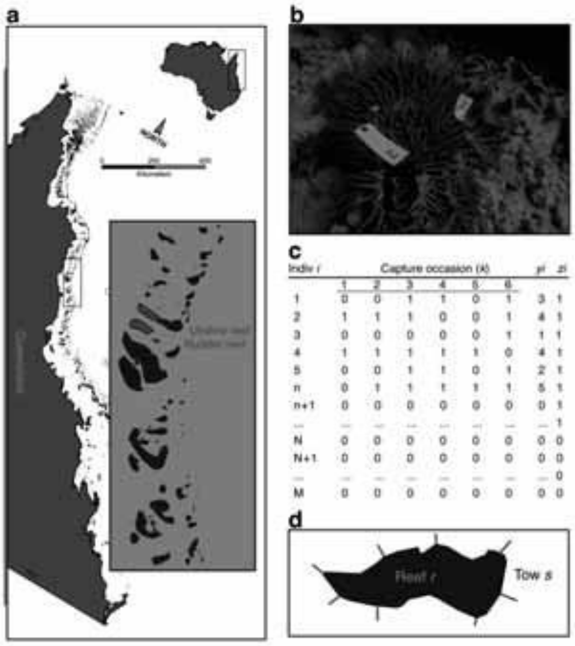
METHODS

Data and analysis consisted of two components. The first involved a dedicated mark-recapture study, conducted by three key data-providing organizations (AIMS, FMP, and AMPTO) that enabled us to estimate the detectability of adult CoTS and the underlying true adult (>14 cm) density within a set of study sites (hereafter 'model-calibrated density'). We then used these values to calibrate broad-scale count (FMP) and kill (AMPTO) datasets and estimate the density of CoTS among reefs in the Cairns Sector of the GBR in 2014. All observations were made under Great Barrier Reef Marine Park Authority Permit G06/19994.1.

Mark-recapture surveys

A dedicated mark-recapture survey was conducted by AIMS, FMP, and AMPTO divers in May 2014 at eight 50 × 5 m transects (sites) spread between two reefs–Undine (Reef 16-023, $n = 3$) and Rudder (Reef 16-020a, $n = 5$)–in the Cairns Sector of the GBR (Fig. 1A); note that reef identification codes are unique identifiers for reefs within the GBR. Sites were selected opportunistically, based on known CoTS outbreaks and were accessible by survey boats. All fieldwork was conducted under the Australian Institute of Marine Science research permit issued by the Great Barrier Reef Marine Park Authority. Surveys were conducted on days with similar sea-state conditions, at depths where sea state was assumed not to have affected detectability.

Within each site, FMP divers conducted initial surveys of the reef surface at between seven to nine meters depth, using standard FMP 5 m-radius point count methods ("Reef Health and Impact Surveys (RHIS)"; *Beeden et al., 2014*) to record the local abundance of



Indiv i	Capture occasion (k) 1	2	3	4	5	6	yi	zi
1	0	0	1	1	0	1	3	1
2	1	1	1	0	0	1	4	1
3	0	0	0	0	0	1	1	1
4	1	1	1	1	1	0	4	1
5	0	0	1	1	0	1	2	1
n	0	1	1	1	1	1	5	1
n+1	0	0	0	0	0	0	0	1
—	—	—	—	—	—	—	—	1
N	0	0	0	0	0	0	0	0
N+1	0	0	0	0	0	0	0	0
—	—	—	—	—	—	—	—	0
M	0	0	0	0	0	0	0	0

Figure 1 **Sampling scheme for crown-of-thorns starfish (CoTS) mark-recapture study.** (A) Study reefs on the Great Barrier Reef; (B) multiple tagged CoTS #62 from the study; (C) parameter-expanded data augmentation (PXDA) matrix of observed (1 to n), unobserved ($n+1$ to N), and not present ($N+1$ to M) individuals within the study superpopulation (M); (D) reef schematic showing partitioning of reef (r) perimeter into manta-tow sections (s), delineated by radiating straight lines. y_i denotes the number of capture occasions over which an individual was observed; z_i indicates an individual was observed in any capture occasion.

CoTS within a total area of 400 m^2 ($n = 5$). These abundances were converted to densities (CoTS/m^2) for comparison with estimates of true densities estimated by mark-recapture.

Next, AIMS divers, using effort and swim speeds typical of AIMS Long-Term Monitoring Program methodology (LTMP), scanned each site looking for CoTS. When a new starfish was spotted it was tagged with two or three clothing tags (plastic t-tags) that included a unique identification number (Fig. 1B). Each transect was re-surveyed $K = 6$ times during both day (60% of observations) and night, by one of three AIMS observer teams. As tag shedding has been reported to be a problem by other researchers we confined sampling of each transect to a single 24 h period, over which CoTS were assumed not to lose tags. In addition, the total percentage of hard coral cover within the transect was recorded as a transect-scale covariate thought to affect detectability.

Finally, after FMP and AIMS surveys were completed, AMPTO divers visited each site and proceeded to kill all observed CoTS within the survey area, using standard cull procedures and recording the number of CoTS killed per unit time of effort (CPUE).

Mark-recapture submodel

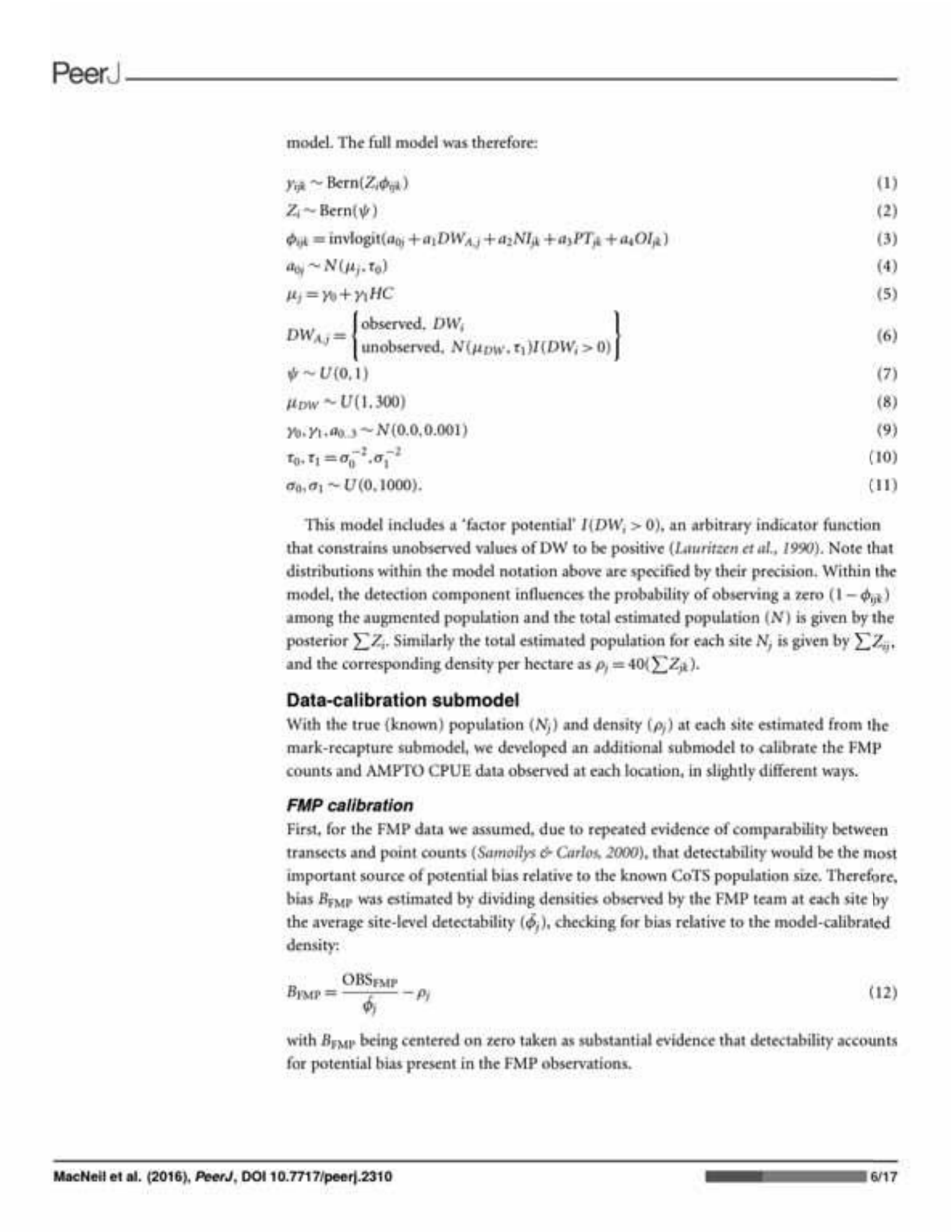
To quantify CoTS detectability and estimate their model-calibrated density within the survey areas we employed a parameter-expanded data-augmentation (PXDA) approach for closed-population mark-recapture (*Royle & Dorazio, 2012*). This flexible class of models allows for individual, spatial, and time-varying heterogeneity in the detection probability of CoTS that is directly integrated into estimates of population size at a given site (N_j). The basic strategy is to use information from the individuals observed at a given site (n_j) to estimate the number of unobserved individuals ($u_j = N_j - n_j$). By conducting repeated sampling of a fixed area and tagging individual CoTS, a set of sampling histories can be built up that records if individual i was observed (recorded as 1) or not (recorded as 0), across a set of k capture occasions (i.e., $y_{ij} = 1, 0, 0, 1, 1, 0$; Fig. 1C). The resulting matrix of observed individuals allows an average probability of detection to be estimated (ϕ), given covariate information within a linear model.

PXDA works by augmenting the observed set of individual sampling histories with an arbitrary number of unobserved sampling histories to create a superpopulation of "pseudo-individuals" (M; Fig. 1C). This superpopulation comprises three components: the observed individuals; a group of unobserved, but present, individuals; and a group of individuals not present in the sample area. The PDXA approach uses probability to assign each of the augmented individuals into unobserved or not present groups using the characteristics from the observed part of the population. Note that this approach will underestimate population size if the detectability of the true unobserved population is different from the observed population due to, for example, physical or behavioural differences.

Unbiased estimation of CoTS densities on a given reef likely depends on habitat characteristics in the survey area and the ability of divers to detect, and therefore count, CoTS underwater. Potentially important habitat factors include substrate type, structural complexity, water clarity, depth, and time of day. Body size and level of activity also likely influence the detection of individual CoTS, especially given that large adult starfish are generally less cryptic than juvenile individuals.

To account for the influence of habitat factors on detection we developed a hierarchical model using available covariates for detection including site-scale hard coral cover (HC), observer team (OT), the disc-width (DW) of individual CoTS, presence of a tag (PT), and a dummy variable for surveys conducted at night (NI), with corresponding individual, sampling-occasion, and transect-scale parameter estimates ($\gamma_1, a_{1,2,3,4}$).

The disc-width individual covariate adds a slightly complicating step to the PXDA approach in that the disc-widths of some observed CoTS were missing and for unobserved individuals are unknown. However, our Bayesian approach allowed us to integrate over this uncertainty by estimating the missing lengths from the observed lengths within the



model. The full model was therefore:

$$y_{ijk} \sim \mathrm{Bern}(Z_i \phi_{ijk}) \tag{1}$$

$$Z_i \sim \mathrm{Bern}(\psi) \tag{2}$$

$$\phi_{ijk} = \mathrm{invlogit}(a_{0j} + a_1 DW_{A,j} + a_2 NI_{jk} + a_3 PT_{jk} + a_4 OI_{jk}) \tag{3}$$

$$a_{0j} \sim N(\mu_j, \tau_0) \tag{4}$$

$$\mu_j = \gamma_0 + \gamma_1 HC \tag{5}$$

$$DW_{A,j} = \left\{ \begin{matrix} \text{observed}, \ DW_i \\ \text{unobserved}, \ N(\mu_{DW}, \tau_1) I(DW_i > 0) \end{matrix} \right\} \tag{6}$$

$$\psi \sim U(0,1) \tag{7}$$

$$\mu_{DW} \sim U(1,300) \tag{8}$$

$$\gamma_0, \gamma_1, a_{0..3} \sim N(0.0, 0.001) \tag{9}$$

$$\tau_0, \tau_1 = \sigma_0^{-2}, \sigma_1^{-2} \tag{10}$$

$$\sigma_0, \sigma_1 \sim U(0,1000). \tag{11}$$

This model includes a 'factor potential' $I(DW_i > 0)$, an arbitrary indicator function that constrains unobserved values of DW to be positive (*Lauritzen et al., 1990*). Note that distributions within the model notation above are specified by their precision. Within the model, the detection component influences the probability of observing a zero $(1 - \phi_{ijk})$ among the augmented population and the total estimated population (N) is given by the posterior $\sum Z_i$. Similarly the total estimated population for each site N_j is given by $\sum Z_{ij}$, and the corresponding density per hectare as $\rho_j = 40(\sum Z_{jk})$.

Data-calibration submodel

With the true (known) population (N_j) and density (ρ_j) at each site estimated from the mark-recapture submodel, we developed an additional submodel to calibrate the FMP counts and AMPTO CPUE data observed at each location, in slightly different ways.

FMP calibration

First, for the FMP data we assumed, due to repeated evidence of comparability between transects and point counts (*Samoilys & Carlos, 2000*), that detectability would be the most important source of potential bias relative to the known CoTS population size. Therefore, bias B_{FMP} was estimated by dividing densities observed by the FMP team at each site by the average site-level detectability $(\bar{\phi}_j)$, checking for bias relative to the model-calibrated density:

$$B_{\mathrm{FMP}} = \frac{\mathrm{OBS}_{\mathrm{FMP}}}{\bar{\phi}_j} - \rho_j \tag{12}$$

with B_{FMP} being centered on zero taken as substantial evidence that detectability accounts for potential bias present in the FMP observations.

AMPTO calibration

While FMP data is broadly comparable to the mark-recapture data in form, the AMPTO CPUE data is a different measure of abundance, where the total number of CoTS observed is standardized over some unit of effort; in this case, the duration of the dive over which the counts were made. CPUE is known from fisheries science to be a notoriously inconsistent index of abundance (*Hartley & Myers, 2001*) that can either decline quickly (hyperdepletion) or remain high (hyperstability) as true abundance declines. Hyperstability is expected to occur where populations are highly clustered and handling time to catch or kill individuals dominates the time spent searching for them. A common model for CPUE data is:

$$\mathrm{CPUE}_t = qN_t^B \tag{13}$$

where CPUE is proportional to true abundance (N_t) given a catchability coefficient (q) and scaling parameter (B). In general, both q and N_t are difficult to estimate; however, given the mark-recapture estimates from our finite survey area, catchability is given by detectability and true abundance is assumed known. Therefore, we estimated the relationship between log(CPUE) and true abundance as:

$$\log(\mathrm{CPUE}_{jk}) \sim N(\theta_j, \tau_\theta) \tag{14}$$

$$\theta_{jk} = \log(\phi_j) + B * \log(N_j) \tag{15}$$

$$B \sim U(0.0, 10) \tag{16}$$

$$\tau_\theta = \sigma_\theta^{-2} \tag{17}$$

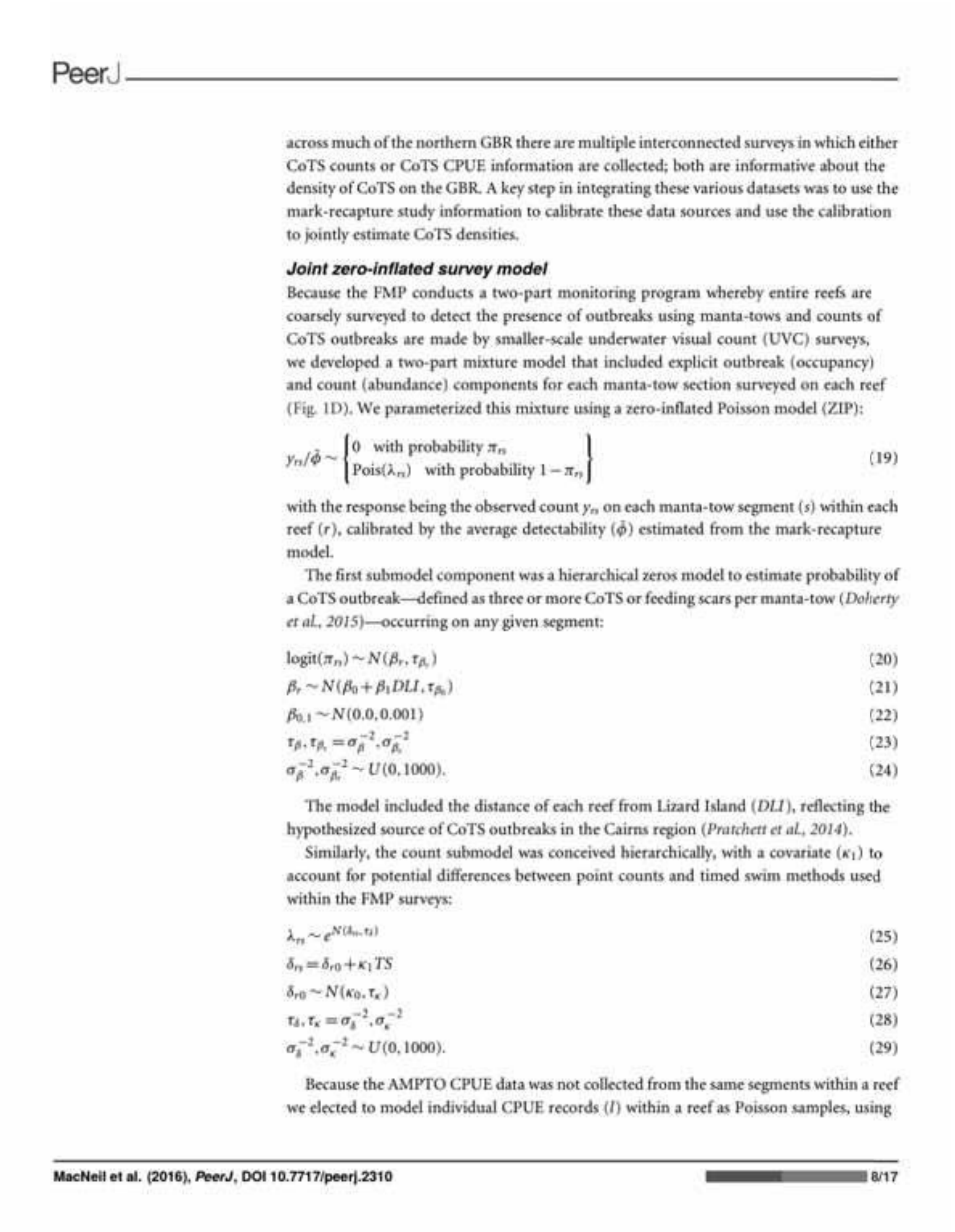
$$\sigma_\theta \sim U(0, 1000). \tag{18}$$

This parameterization essentially defines a log-Normal relationship between true densities and CPUE, estimating parameters on a log–log scale.

Regional surveys

As part of an Australian government-funded effort to control CoTS on high-value tourism and ecological reefs and support the Queensland tourism industry, AMPTO devotes considerable effort to protecting key tourism reefs by applying a lethal injection to the starfish (*Rivera-Posada et al., 2011*). In 2014, this program of targeted control was expanded beyond the high-priority tourism reefs in the Cairns sector to include a subset of 'super-spreader reefs' identified as being highly connected by water currents that receive and then spread CoTS larvae more widely than other reefs (*Hock et al., 2015*).

Operational decisions about where AMPTO can direct their control efforts beyond the primary tourism reefs comes from GBRMPA and the Reef and Rainforest Research Centre, in consultation with the AMPTO Project Manager. GBRMPA monitor average coral cover over time in combination with CPUE to determine if thresholds are breached (e.g., coral cover declining and/or CPUE increasing) and when reefs require re-visitation. In addition, surveys of COTS and coral health from the FMP provide more source information on the distribution of CoTS within the Cairns sector than among other sectors. Therefore,



across much of the northern GBR there are multiple interconnected surveys in which either CoTS counts or CoTS CPUE information are collected; both are informative about the density of CoTS on the GBR. A key step in integrating these various datasets was to use the mark-recapture study information to calibrate these data sources and use the calibration to jointly estimate CoTS densities.

Joint zero-inflated survey model

Because the FMP conducts a two-part monitoring program whereby entire reefs are coarsely surveyed to detect the presence of outbreaks using manta-tows and counts of CoTS outbreaks are made by smaller-scale underwater visual count (UVC) surveys, we developed a two-part mixture model that included explicit outbreak (occupancy) and count (abundance) components for each manta-tow section surveyed on each reef (Fig. 1D). We parameterized this mixture using a zero-inflated Poisson model (ZIP):

$$y_{rs}/\bar{\phi} \sim \begin{Bmatrix} 0 \quad \text{with probability } \pi_{rs} \\ \text{Pois}(\lambda_{rs}) \quad \text{with probability } 1-\pi_{rs} \end{Bmatrix} \tag{19}$$

with the response being the observed count y_{rs} on each manta-tow segment (s) within each reef (r), calibrated by the average detectability ($\bar{\phi}$) estimated from the mark-recapture model.

The first submodel component was a hierarchical zeros model to estimate probability of a CoTS outbreak—defined as three or more CoTS or feeding scars per manta-tow (*Doherty et al., 2015*)—occurring on any given segment:

$$\text{logit}(\pi_{rs}) \sim N(\beta_r, \tau_{\beta_r}) \tag{20}$$

$$\beta_r \sim N(\beta_0 + \beta_1 DLI, \tau_{\beta_r}) \tag{21}$$

$$\beta_{0,1} \sim N(0.0, 0.001) \tag{22}$$

$$\tau_\beta, \tau_{\beta_r} = \sigma_\beta^{-2}, \sigma_{\beta_r}^{-2} \tag{23}$$

$$\sigma_\beta^{-2}, \sigma_{\beta_r}^{-2} \sim U(0, 1000). \tag{24}$$

The model included the distance of each reef from Lizard Island (DLI), reflecting the hypothesized source of CoTS outbreaks in the Cairns region (*Pratchett et al., 2014*).

Similarly, the count submodel was conceived hierarchically, with a covariate (κ_1) to account for potential differences between point counts and timed swim methods used within the FMP surveys:

$$\lambda_{rs} \sim e^{N(\delta_{rs}, \tau_\delta)} \tag{25}$$

$$\delta_{rs} = \delta_{r0} + \kappa_1 TS \tag{26}$$

$$\delta_{r0} \sim N(\kappa_0, \tau_\kappa) \tag{27}$$

$$\tau_\delta, \tau_\kappa = \sigma_\delta^{-2}, \sigma_\kappa^{-2} \tag{28}$$

$$\sigma_\delta^{-2}, \sigma_\kappa^{-2} \sim U(0, 1000). \tag{29}$$

Because the AMPTO CPUE data was not collected from the same segments within a reef we elected to model individual CPUE records (l) within a reef as Poisson samples, using their corresponding reef-scale averages (β_r), after first calibrating using the detectability and CPUE scaling parameter (B) estimates from the mark-recapture model:

$$e^{\frac{\log(\mathrm{CPUE}_{rt})-\log(\hat{\phi})}{B}} \sim \mathrm{Pois}(\lambda_r) \tag{30}$$

$$\lambda_r \sim e^{\beta_{r0}}. \tag{31}$$

In this way, the AMPTO CPUE observations were considered informative of reef-scale average CoTS densities within the Cairns sector, adding information to that present in the FMP surveys.

All models were run using the Metroplis-Hastings algorithm for 10^6 iterations, with a 900,000 burn in period, using the PyMC2 package (*Patil, Huard & Fonnesbeck, 2010*) for the Python programming language. Model convergence was assessed using Gelman–Rubin statistics from multiple model runs (*Gelman & Rubin, 1992*) and model fit was evaluated using Bayesian *p*-values (*Brooks, Catchpole & Morgan, 2000*), with scores lower than 0.025 and 0.975 providing substantial evidence for lack of model fit.

RESULTS

Mark-recapture results

In total, 114 individual CoTS with disc width varying from 15 to 35 cm were observed and tagged over the eight study sites. As intuition would support for a large, slow-moving benthic invertebrate, average detectability of adult CoTS on a reef was high, at 0.82 [0.77, 0.87] (median highest posterior density (HPD) and [95% uncertainty intervals]). Given this high average detectability, the posterior 'true' abundance across all sites was estimated to be 116 [114, 120] individuals.

Starfish size had the greatest overall effect on detectability, with CoTS larger than 30 cm being highly detectable (P(detection) >0.8) and detection declining substantially to near zero for the smallest starfish, none of which were observed in the study (Fig. 2B). While we found little evidence for an effect of hard coral cover (Fig. 2A), some inter-site variability in detection was present, with one site (Undine 3) having markedly lower average detection than the majority of reefs (Fig. 2D). Time of day also had an effect on detectability, with CoTS being more detectable during the day than at night (Fig. 2C).

Other measured factors were found to have limited effects on detection, with little evidence that alternative observation teams were more or less likely to detect individual CoTS (Effect size (ES) −0.13 [−0.63, 0.36] and 0.18 [−0.36, 0.72]). Unsurprisingly, given the bright white tags used, there was a modest, positive effect of tagging (ES 0.30 [−0.22, 0.80]) on CoTS detectability (Fig. 2A).

Data calibration results

Inter-calibration of the AMPTO and FMP datasets showed there were consistent biases present in both. For the AMPTO data, the relationship between CPUE and known density was shown to be hyper-stable, with a scaling exponent B of 0.33 [0.21, 0.46]. Transformed to the original scale, substantial uncertainty in this relationship remained, as only eight data points were available for estimation (Fig. 3A).

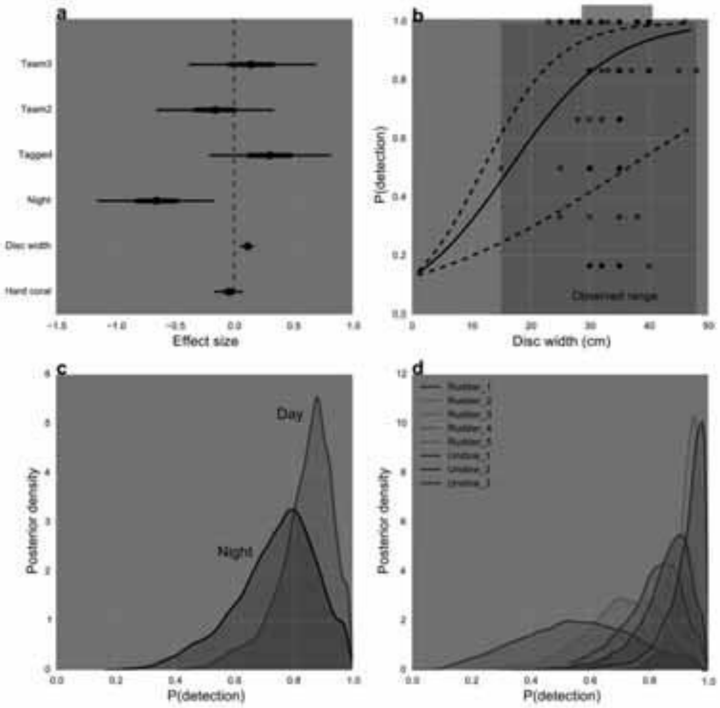


Figure 2 **Factors affecting the detectability of crown-of-thorns starfish on the Great Barrier Reef.** (A) Highest posterior density (HPD) effect sizes for alternative observation team (Team), tagging effects (Tagged), nighttime surveys (Night), animal size (disc with), and the percentage of hard coral present within the survey area. (B) Estimated median relationship between animal size and detectability (solid blue line), with 95% uncertainty intervals (dashed lines) and observed detection rates for $k = 6$ capture-occasions (dots). (C) Posterior probabilities of detection for CoTS, given presence for surveys conducted during the day and night. (D) Posterior probabilities of detection for CoTS among survey sites.

Unlike the AMPTO CPUE data, the FMP data was shown to be primarily biased by detectability, with detection-corrected estimates falling close a 1:1 line (Fig. 3B). Deviations from 1:1 were observed where substantial site-level heterogeneity had been estimated among the site-level random effects (Undine 3 and Rudder 4; Fig. 2D), suggesting some level of site-level variability remained unaccounted for in our model. However, none of our model-fit measures displayed any evidence for lack of fit given our statistical model (Bayesian *p*-value 0.71).

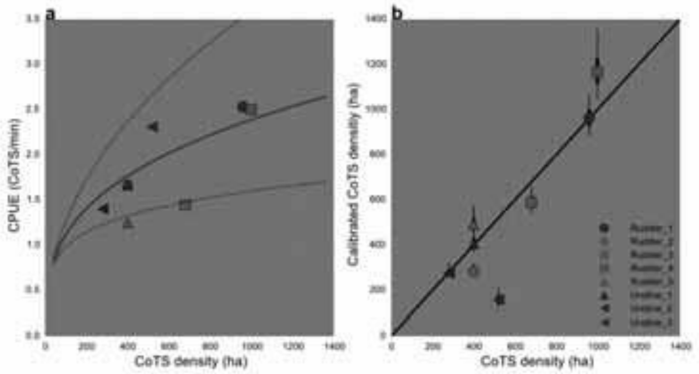


Figure 3 **Data calibration of alternative CoTS survey methods.** (A) Estimated median catch-per-unit-effort (CPUE) calibration curve (solid blue line) and 95% uncertainty intervals (dotted lines) for AMPTO surveys; estimated relationship with CoTS density per hectare was $D_{CoTS} = [\log(0.83) + 0.33 * \log(CPUE)] * 40$; (B) posterior median (dots), 50% (thick lines) and 95% (thin lines) density estimated CoTS densities for detection-corrected FMP surveys. Black diagonal is a 1:1 line in (B).

Regional density estimation

Our joint zero-inflated, Bayesian hierarchical model found that 75 (63%) of the 120 reefs surveyed likely (i.e., P(outbreak) >0.5) experienced outbreaks in 2014 (Fig. 4A), with substantially higher probabilities in the North, near Lizard Island, than among reefs further south (Fig. 4C). Average outbreak probabilities ranged from between 0.95 (i.e., more than three adult CoTS in each manta-tow segment within a reef) to 0.06 (only a 6% average chance of outbreak across manta-tow segments).

Given the estimated outbreak probabilities, the expected density of CoTS among manta-tow segments varied substantially across reefs, with northerly (outbreaking) reefs having 62 or more CoTS per hectare (top 5% of densities), and southerly (non-outbreaking) reefs experiencing densities below 12 CoTS per hectare (bottom 5% of densities; Fig. 4B). Overall, by accounting for detection, we estimate CoTS densities among reefs within the reefs surveyed in the Cairns sector averaged 44 [41, 48] CoTS per hectare in 2014.

DISCUSSION

The problem of imperfect species detectability has been a focus in terrestrial monitoring for decades (*Kéry & Schmidt, 2008*) but has received less attention in marine monitoring programs (*Coggins Jr, Bacheler & Gwinn, 2014*). Without recognizing the true underlying density of CoTS, management actions are much more likely to fail, due to insufficient effort allocated to find remnant populations. Furthermore, understanding and predicting CoTS outbreaks on the GBR and elsewhere has been limited by the quantity and variety of information about basic density estimates and population-level dynamics and coordination among different survey methods and effort; current surveillance for CoTS is not systematic,

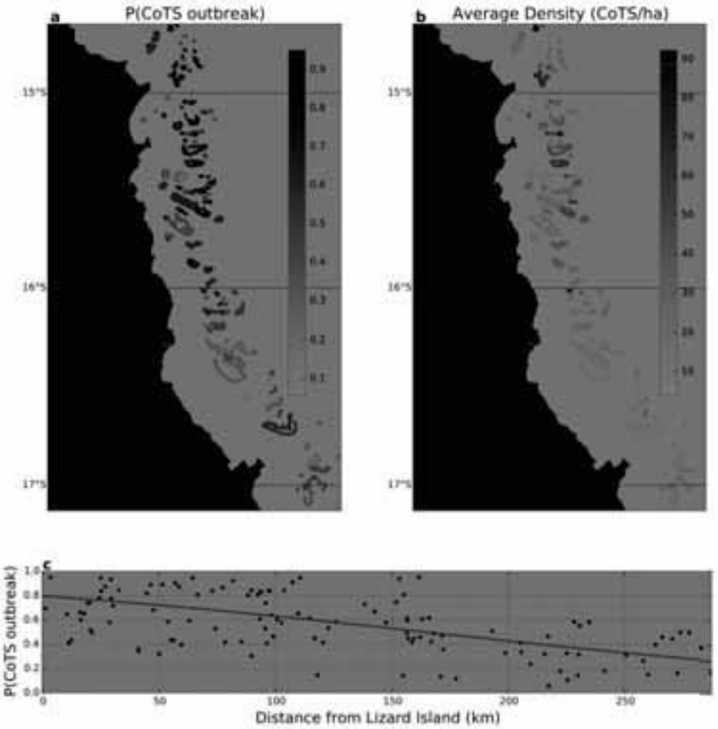


Figure 4 Data-integrated estimated CoTS outbreak densities in the Cairns sector of the Great Barrier Reef, 2014. (A) Reef-wide average probability of a CoTS outbreak; (B) reef-wide average expected CoTS density; (C) estimated relationship between linear distance from Lizard Island and the probability of CoTS outbreak among Cairns-sector reefs.

instead relying primarily on reports made by existing operations. Here we successfully combine the first rigorous estimates of detectability for CoTS with multiple sampling surveys undertaken within the known area of active outbreaks, to estimate the density of adult CoTS within the region. As such, this joint approach should help improve decisions concerning how CoTS outbreak and spread, and the management interventions that may be taken.

Given the tendency for CoTS to hide in crevices and under corals, we were surprised to find that the percentage of hard coral cover had little effect on the detectability of adult CoTS. However, this may be due both to the relatively narrow range of hard coral cover observed (14 to 42%) and the generally large size of CoTS (median = 35 cm); habitat complexity (which we did not measure) may have also affected detectability, however

complexity levels were subjectively considered to be similar among transects. Large adult CoTS (>40 cm) are thought to be most active predominantly in daytime with smaller COTS (<20 cm) tending to be more active at night (*De'ath & Moran, 1998*). Thus, we were not surprised to see that they were somewhat more detectable during the day (0.85) than at night (0.75). Alternatively, increased nighttime movements could have decreased detectability if it led to some CoTS departing the survey area. Although survey divers did not report such activity, it would violate the closure assumption of our models and not allow us to partition nighttime detectability from nocturnal movement.

Detectability among adult CoTS varied most strongly with body size, from highly detectable (up to 0.98) animals with discs 40 to 50 cm wide, declining appreciably (to less than 0.5) among the smallest CoTS observed (<15 cm). This substantial decline in detectability at small body sizes that has the greatest potential to restrict understanding of CoTS population dynamics for two reasons. First, with very low expected detectability (<0.1) at the smallest body sizes it becomes exceptionally difficult to quantify annual recruitment—i.e., CoTS that survive the larval stage and settle on the reef. Secondly, the susceptibility of juvenile CoTS to fish predation is unclear (*Sweatman et al., 2008*), as are natural rates of post-recruitment mortality. Improved understanding of recruitment and post-recruitment mortality will be critical for the assessment of whether cull campaigns can effectively control CoTS outbreaks once they have begun.

The data-integration conducted in our study was sufficient to reveal a strong pattern of diminishing outbreaks from North to South within the Cairns sector, supporting the long held belief that northern GBR CoTS outbreaks originate near Lizard Island and progress southward (*Pratchett et al., 2014*), a pattern strongly dependent on subsequent waves of recruitment. It is the magnitude of annual recruitment that determines the potential spread of a given outbreak, while juvenile survival affects outbreak rate and severity, and is an as-yet untested target for tactical CoTS control.

While there are multiple benefits of increased survey accuracy at the reef level, efficiency gains in directing potential control actions can also be made by formally integrating all available monitoring information. Currently, CoTS control efforts are dedicated toward maintaining coral cover above ecologically important thresholds on a subset of 21 commercially and ecologically important reefs. Despite these efforts, there remain substantial accuracy gains to be made by adopting a formal framework for estimating CoTS densities more generally. This is clear from our illustrative example, where the integration of FMP and AMPTO surveys generated a 15% decrease in the coefficient of variation (CV) of γ_0, the average overall density parameter. Although we integrated only two data sources, others—such as from the AIMS long-term monitoring program and the GBRMPA's Eye on the Reef Program—could be easily added. Such precision increases can only help to direct limited control resources more effectively.

In this study, we have addressed two important gaps in the understanding and quantification of dynamics of CoTS on the GBR, namely in calibrating multiple data sources for bias, primarily due to method and detectability. This increased accuracy can provide immediate benefits in improving the state of knowledge and management of CoTS through the current outbreak. However, a major knowledge gap still remains in that only adult CoTS are counted and killed within current surveys. The juvenile life-history stage, from settlement (0.05 cm) to maturity (~11 cm) (*Pratchett et al., 2014*), remains a crucial black box that should become a focus for future monitoring research. Without the ability to detect and kill juvenile CoTS, adult control operations will only remove the threat to corals for a single year, after which juvenile CoTS will mature and emerge from the reef substrate to feed. It is likely only through early detection of larvae (*Uthicke et al., 2015*), juvenile, and pre-spawn adult COTS that candidate control methods could hope to arrest initial outbreaks.

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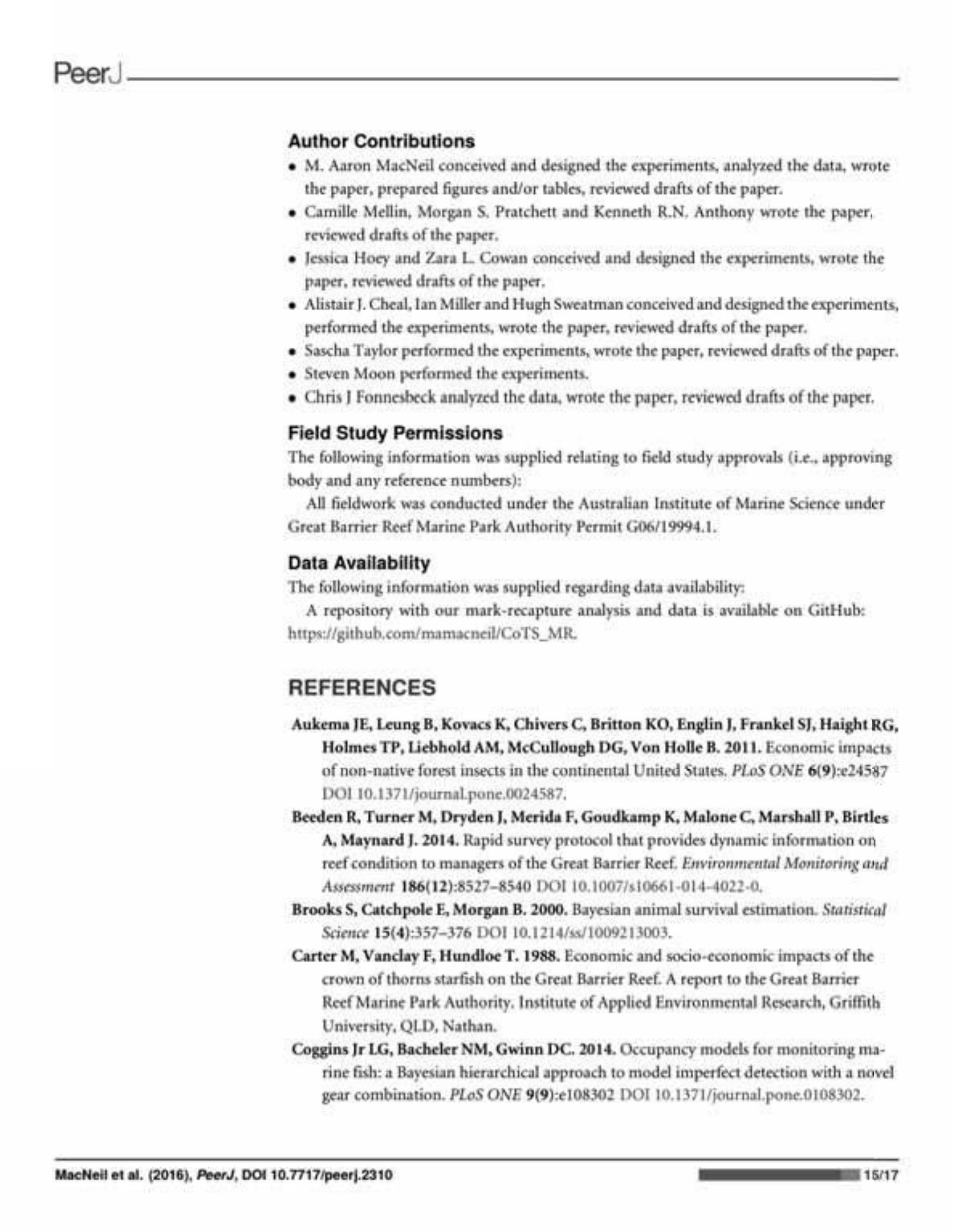
Competing Interests

Steven Moon is an employee of Association of Marine Park Tourism Operators, Cairns, Australia.



Author Contributions

- M. Aaron MacNeil conceived and designed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Camille Mellin, Morgan S. Pratchett and Kenneth R.N. Anthony wrote the paper, reviewed drafts of the paper.
- Jessica Hoey and Zara L. Cowan conceived and designed the experiments, wrote the paper, reviewed drafts of the paper.
- Alistair J. Cheal, Ian Miller and Hugh Sweatman conceived and designed the experiments, performed the experiments, wrote the paper, reviewed drafts of the paper.
- Sascha Taylor performed the experiments, wrote the paper, reviewed drafts of the paper.
- Steven Moon performed the experiments.
- Chris J Fonnesbeck analyzed the data, wrote the paper, reviewed drafts of the paper.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

All fieldwork was conducted under the Australian Institute of Marine Science under Great Barrier Reef Marine Park Authority Permit G06/19994.1.

Data Availability

The following information was supplied regarding data availability:

A repository with our mark-recapture analysis and data is available on GitHub: https://github.com/mamacneil/CoTS_MR.

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