Environmental influences on the reproductive biology and early life history of the crown-of-thorns starfish

Thesis submitted by

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Statement on the contribution of others

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	Jairo Rivera-Posada[2]	manuscript writing and editorial assistance
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Chapter 3	Morgan Pratchett[1]	conceptual / editorial assistance
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Abstract

Population outbreaks of the coral-eating crown-of-thorns starfish, *Acanthaster* spp., often result in extensive coral mortality with highly extended recovery times, thereby contributing significantly to sustained and ongoing declines in coral cover across the Indo-Pacific. Long-term or permanent solutions depend on filling crucial gaps in our knowledge of the biology of crown-of-thorns starfish, particularly its reproductive biology and early life history, to understand the initiation and spread of outbreaks. Populations of crown-of-thorns starfish are typically predisposed to major fluctuations due to inherent properties of their life history such as high fecundity, high fertilization rates, and short generation times. However, densities vary enormously in space and time, pointing to major fluctuations in reproductive success. The overarching question therefore is: what limits recruitment success in crown-of-thorns starfish and which stages or processes in its life cycle are most vulnerable to these constraints? Small environmental perturbations that trigger life-stage-specific responses can have pronounced effects on recruitment success and hence, on the dynamics of adult populations. My research explored the role of environmental factors on (1) gametogenesis and reproductive timing; (2) spawning induction and synchronicity; (3) fertilization and embryonic development; and on (4) larval vitality, in relation to maternal provisioning and larval nutrition.

To assess gametogenic activity and reproductive timing in crown-of-thorns starfish, intensive and extensive sampling of crown-of-thorns starfish from Australia's Great Barrier Reef (GBR) was conducted. This study revealed marked inter-annual variation in reproductive timing and output, possibly depending on local environmental conditions. In the first sampling season (September 2013 to March 2014), there was only minor and repeated spawning that occurred over a highly protracted spawning

period, while in the second sampling season (September 2014 to March 2015), there was evidence of comprehensive and synchronous spawning by crown-of-thorns starfish.

I then examined the role of environmental and biological cues for spawning in crown-of-thorns starfish. For gonochoric and broadcast spawning species such as crown-of-thorns starfish, spawning synchrony is fundamental for achieving high rates of fertilization. Highly synchronized gamete release within and among distinct populations is typically the result of the entrainment of neurohormonal endogenous rhythms by cues from the environment. In this study, I conducted multiple spawning assays to test the effects of temperature change, reduced salinity and nutrient enrichment of seawater, phytoplankton, gametes (sperm and eggs), and the combined effect of sperm and phytoplankton on the likelihood of spawning in male and female crown-of-thorns starfish. I also investigated sex-specific responses to each of these potential spawning cues. I found that (1) abrupt temperature change (an increase of 4°C) induced spawning in males, but less so in females; (2) males often spawned in response to the presence of phytoplankton, but none of the females spawned in response to these cues; (3) the presence of sperm in the water column induced males and females to spawn, although additive and synergistic effects of sperm and phytoplankton were not significant; and (4) males were more sensitive to the spawning cues tested and most likely spawn prior to females. These results suggest that environmental cues act as spawning 'inducers' by causing the release of hormones (gonad stimulating substance) in sensitive males, while biological cues (pheromones) from released sperm, in turn, act as spawning 'synchronizers' by triggering a hormonal cascade resulting in gamete shedding by conspecifics. Given the immediate temporal linkage between the timing of spawning and fertilization events, variability in the extent and synchronicity of gamete

release will significantly influence reproductive success and may account for fluctuations in the abundance of crown-of-thorns starfish.

Following spawning, larval stages develop in the water column for at least 14-16 days, where environmental factors could constrain survivorship and effective development. The persistence and success of marine invertebrate populations is fundamentally dependent upon larval survival and settlement; hence the variable sensitivity of planktonic stages and processes (i.e. gametes, fertilization, embryonic development) to environmental stressors (e.g. temperature, salinity, pH) may be a potential population bottleneck. Here, I compared sperm swimming speeds and proportion of motile sperm and rates of fertilization and early development under a range of environmental variables (temperature: 20-36°C, salinity: 20-34 psu, and pH: 7.4-8.2) to identify environmental tipping points and thresholds for reproductive success. I also tested the effects of water-soluble compounds derived from eggs on sperm activity. This study demonstrated that gametes, fertilization, and embryonic development are robust to a wide range of temperature, salinity, and pH levels that are outside the range found at the geographical limits of adult distribution and can tolerate environmental conditions that exceed expected anomalies as a result of climate change. Water-soluble compounds associated with eggs also enhanced sperm activity, particularly in environmental conditions where sperm motility was initially limited. These findings suggest that fertilization and embryonic development of crown-of-thorns starfish are tolerant to a wide range of environmental conditions, though environmental constraints on recruitment success may occur at later ontogenic stages.

Previous studies on crown-of-thorns starfish have primarily focused on the effects of water quality and nutrient availability on larval growth and survival, while the role of maternal nutrition on reproduction and larval development has been overlooked. To

examine the effects of maternal nutrition on oocyte size and early larval development, I pre-conditioned females for 60 days on diets of preferred coral (*Acropora abrotanoides*) versus non-preferred coral prey (*Porites rus*) and compared resulting gametes and progeny to those produced by females that were starved over the same period. Females fed *ad libitum* with *Acropora* increased in weight, produced heavier gonads and produced larger oocytes compared to *Porites*-fed and starved females. Fed starfish (regardless of whether it was *Acropora* or *Porites*) produced bigger larvae with larger stomachs and had a higher frequency of normal larvae that reached the late bipinnaria / early brachiolaria stage compared to starved starfish. Females on *Acropora* diet also produced a higher proportion of larvae that progressed to more advanced stages faster compared to *Porites*-fed starfish, which progressed faster than starved starfish. These results suggest that maternal provisioning can have important consequences for the quality and quantity of progeny.

Based on these findings, I proceeded to test whether maternal provisions from the egg were able to offset limitations imposed by limited access to exogenous sources of nutrients during the formative stages of larval development. This study examined the individual, additive, and interactive effects of endogenous (maternal diet: *Acropora*, *Porites*, mixed, and starved) and exogenous (larval diet: high concentration at 10^4 cells·mL^{-1}, low concentration at 10^3 algal cells·mL^{-1}, and starved) nutrition on the survival, growth, morphology, and development of larvae of the crown-of-thorns starfish. Female starfish on *Acropora* and mixed diet produced bigger oocytes compared to *Porites*-fed and starved treatments. Using oocyte size as a proxy for maternal provisioning, endogenous reserves in the oocyte had a strong influence on initial larval survival and development. This suggests that maternal reserves can delay the onset of obligate exogenous food acquisition and allow larvae to endure prolonged periods of

poor environmental nutritive conditions or starvation. The influence of exogenous nutrition became more prominent in later stages, whereby none of the starved larvae reached the mid-to-late brachiolaria stage 16 days after the onset of the ability to feed. There was no significant difference in the survival, development, and competency of larvae between high and low food treatments. Under low algal food conditions, larvae compensate by increasing the length of ciliated feeding bands in relation to the maximum length and width of the larval body, which improve food capture and feeding efficiency. However, the effects of endogenous nutrition persisted in the later developmental stages, as larvae from starved females were unable to develop larger feeding structures in response to food-limiting conditions. Phenotypic plasticity influenced by endogenous provisions and in response to exogenous food availability may be an important strategy in boosting the reproductive success of crown-of-thorns starfish, leading to population outbreaks.

The tolerance of early life history stages and processes to a suite of environmental stressors and the plasticity in reproductive behavior and larval morphology add to a growing list of traits that predispose crown-of-thorns starfish to pronounced fluctuations in abundance. Taken together, these results demonstrate that variable sensitivity of early life history stages and processes to environmental factors can have flow-on effects that disproportionately impact recruitment success and population replenishment in crown-of-thorns starfish. The cumulative effects of environmental variables on the success of different stages and processes in the life cycle of crown-of-thorns starfish ultimately dictate the available number of larvae that settle and recruit on reefs, and consequently, the patterns of abundance of adult crown-of thorns starfish.

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Appendix A

Chapter 1

General Introduction

The corallivorous crown-of-thorns starfish is perhaps one of the most well known coral reef organisms, notorious for episodic population explosions that have contributed to widespread and accelerating degradation of Indo-Pacific coral reefs (Pratchett et al. 2014 – **Chapter 2**). Although generally regarded as a single species throughout its entire geographical range, recent molecular sampling has revealed that there are at least four strongly diverged mitochondrial clades, largely restricted to: i) the Red Sea (*Acanthaster* sp.); ii) the Pacific and Coral Triangle (*Acanthaster* cf. *solaris*); iii) the Northern Indian Ocean (*Acanthaster planci*); and iv) the Southern Indian Ocean (*Acanthaster mauritiensis*) (Vogler et al. 2008; Haszprunar and Spies 2014). However, there remains a degree of uncertainty in distinguishing between the various 'species' of crown-of-thorns starfish that are yet to be resolved (Haszprunar et al. 2017). Past studies have used *Acanthaster planci* to describe crown-of-thorns starfish belonging to the Pacific clade. For this reason, *Acanthaster planci* was used when referring to the Pacific 'species' in **Chapter 2** and **Chapter 3** of this thesis, while "crown-of-thorns starfish" or *Acanthaster* spp. were used when referring to the entire species complex (excluding *Acanthaster brevispinus*). For all experiments in this thesis (**Chapters 4, 5, 6, 7, 8**), crown-of-thorns starfish from various locations in the Pacific (Great Barrier Reef, Australia and Guam, Micronesia) were used and referred to as *Acanthaster* cf. *solaris*, as suggested by Haszprunar and Spies (2014) (see **Appendix A – Figure A1**).

Outbreaks of crown-of-thorns starfish represent one of the most significant biological disturbances on coral reefs and remain one of the principal causes of

widespread declines in live coral cover on Indo-Pacific reefs (Bruno and Selig 2007; De'ath et al. 2012; Pratchett et al. 2014 – **Chapter 2**). Excluding the recent (2015/16) global bleaching event (Hughes et al. 2017), regional declines in coral cover during severe outbreaks of *Acanthaster* spp. on Indo-Pacific reefs are equivalent to or exceed coral loss caused by any other category of disturbances, such as severe tropical storms, coral disease and mass-bleaching episodes (Osborne et al. 2011; Trapon et al. 2011; De'ath et al. 2012). Morevoer, the time taken for coral assemblages to recover after outbreaks of crown-of-thorns starfish is higher than for any other type of disturbance (Mellin et al. 2016). Increasing frequency and intensity of major disturbances (including outbreaks of crown-of-thorns starfish) have resulted in progressively slower recovery, which have in many instances led to directional changes in the structure of reef habitats (Pandolfi et al. 2003), including phase shifts towards algal-dominated reef communities (Done 1992b; Hughes et al. 2010). This coral loss is further resulting in fundamental changes in ecosystem structure and function (Seymour and Bradbury 1999; Bellwood 2004). Reducing or reversing sustained coral loss is therefore, the foremost global priority for coral reef scientists and managers (Birkeland 2015), and must include effective management of outbreaks of *Acanthaster* spp. (De'ath et al. 2012).

Managing crown-of-thorns starfish populations (specifically, containing or preventing outbreaks) and mitigating their effects on coral reefs are conditional upon identifying the proximal causes of outbreaks (Fabricius et al. 2013; Pratchett et al. 2014 – **Chapter 2**). The three most prominent hypotheses put forward to explain population outbreaks of crown-of-thorns starfish all involve natural variation and constraints on the reproductive biology and early life history of crown-of-thorns starfish (reviewed in Caballes and Pratchett 2014 – **Chapter 3**). The 'natural causes hypothesis' is based on the assumption that population sizes of highly fecund organisms with planktotrophic

larvae, such as *Acanthaster* spp., are inherently unstable (Vine 1973). The 'predator removal hypothesis' suggests that *Acanthaster* spp. populations are normally regulated by high rates of predation on post-settlement juvenile starfish and that outbreaks arise as a consequence of the release from predation pressure due to overharvesting of predators (Sweatman 2008). The 'larval starvation hypothesis' suggests that terrestrial runoff brought with flood plumes during heavy rainfall events causes elevated nutrient levels and leads to phytoplankton blooms, which provide nutrition for otherwise starved larvae of crown-of-thorns starfish (Birkeland 1982; Lucas 1982; Brodie et al. 2005; Fabricius et al. 2010). These hypotheses (discussed further in **Chapter 2** and **Chapter 3**) are not always mutually exclusive and will most likely vary spatially and temporally and to date, none have universal or unequivocal support. Clearly however, the high incidence and severity of outbreaks at many reef locations cannot be sustained, because anthropogenic changes to marine environments have either caused fundamental shifts in the population dynamics of crown-of-thorns starfish or have undermined the capacity of reef ecosystems to withstand these periodic disturbances (Pratchett et al. 2014 – **Chapter 2**).

Reducing the incidence and or severity of outbreaks of *Acanthaster* spp. is critical for reversing widespread declines in coral cover throughout the Indo-Pacific. Improved efficiency of direct controls has provided opportunities to limit the progression and spread of outbreaks if detected early (Bos et al. 2013; Rivera-Posada et al. 2014; Dumas et al. 2016), but long-term and permanent solutions really depend on definitive knowledge and appropriate action to address the ultimate causes of outbreaks. There is widespread recognition that the reproductive biology and early life history of crown-of-thorns starfish are key to understanding when, how, and why outbreaks occur (Caballes and Pratchett 2014 – **Chapter 3**).

Outbreaks are manifestations of inherent instability within certain systems, attributed to either unique life-history features (e.g. high fecundity, short generation times, high mortality during their early life-history, and generalized patterns of prey and habitat use) which predispose crown-of-thorns starfish to major fluctuations in population size, or major changes in the physical and biological environment that release populations from usual regulating factors (Uthicke et al. 2009). The overarching aim of this thesis was to fill crucial gaps in our knowledge of the environmental influences on the reproductive biology and early life history of crown-of-thorns starfish in order to establish key limitations in recruitment and population replenishment. In **Chapter 2**, I compiled and analysed extensive literature on the biology and ecology of crown-of-thorns starfish to identify crucial knowledge gaps. I also examined the evidence for and against the principal hypotheses put forward to explain spatial and temporal patterns of outbreaks, as well as explored whether it was possible or feasible to intervene and limit ongoing degradation caused by crown-of-thorns starfish. In **Chapter 3**, I reviewed the key features of the reproductive biology and early life history of crown-of-thorns starfish that predispose it to population fluctuations and discuss factors that regulate gametogenesis, fecundity, spawning, fertilization, larval development, and post-settlement survival. Information on the reproductive biology and early life history of crown-of-thorns starfish covered in **Chapter 2** may be repeated in **Chapter 3**, albeit with more detail. Although these reviews comprehensively identified key knowledge gaps in our understanding of the biology and ecology of crown-of-thorns starfish, the scope of this thesis is limited to the role of environmental factors in driving variation in processes relevant to the reproductive biology and early life history of crown-of thorns starfish. Field sampling and experimental studies were conducted to assess the role of environmental factors on gametogenesis, spawning, fertilization,

embryonic development, and larval development, which constitute the subsequent chapters of this thesis.

In **Chapter 4**, I examined the reproductive biology and behavior of crown-of-thorns starfish in Australia's Great Barrier Reef (GBR) through intensive and extensive sampling, specifically considering: i) broadscale differences in size-structure and diameter-weight relationships; ii) the size at sexual maturity and sex ratio of discrete populations; and iii) inter-annual variation in the timing and progression of gametogenesis, based on monthly changes in the gonadosomatic index, as well as in the size and stages of oocytes in female gonad tissues. I also discussed which environmental variables (i.e. temperature, day length, salinity, amount of rainfall, and chlorophyll-*a* concentration) were correlated with observed gametogenic patterns.

In **Chapter 5**, I experimentally tested potential environmental and biological cues for spawning in crown-of-thorns starfish. I explicitly tested the effects of temperature change, reduced salinity and nutrient enrichment of seawater, phytoplankton, addition of spawned gametes (sperm and eggs), and the combined effect of sperm and phytoplankton on the likelihood of spawning and examined sex-specific responses to these proximal spawning cues. Given the immediate temporal linkage between the timing of spawning and fertilization events, variability in the extent and synchronicity of gamete release will significantly influence reproductive success and may account for fluctuations in the abundance of crown-of-thorns starfish.

In **Chapter 6**, I compared sperm behavior and rates of fertilization, cleavage, and gastrulation under a range of environmental variables (temperature, salinity, pH) to identify environmental tipping points and thresholds for reproductive success. Reproductive failure in echinoderms has been reported at different levels of these environmental parameters, but few have explicitly tested whether this is due to the

sensitivity of gametes, failure of fertilization, or failure of fertilized eggs to cleave or hatch (Byrne et al. 2009; Allen and Pechenik 2010). I also tested the excitatory effect of water-soluble egg extracts on sperm behavior to add a maternal dimension to the characterization of sperm motility. Sperm swimming speeds and proportion of motile sperm are discussed in relation to fertilization rates. Developmental arrest in response to multiple environmental stressors at the earliest stages can be used to define lower and upper limits for normal development. Quantifying environmental regulation of initial elements of reproductive success is important in understanding the spatial and temporal dynamics of populations of crown-of-thorns starfish, as well as understanding vulnerability to environmental changes.

In **Chapter 7**, I examined the role of experimental variation in maternal nutrition (comparing between individuals that were starved, fed on preferred corals and fed on generally non-preferred coral prey) on the larval growth and early development prior to exogenous feeding by larvae. The effects of maternal nutrition on the following aspects of reproduction and larval development in crown-of-thorns starfish were specifically addressed in this study: (1) adult female morphometrics before and after treatment; (2) gonad and pyloric caeca indexes; (3) oocyte size and shape; (4) fertilization rates; and (5) early larval growth, survival, and development.

Building upon the results of the previous chapter, I evaluated the individual, additive, and interactive effects of endogenous ("Maternal") and exogenous ("Larval") nutrition on larval vitality and morphology in **Chapter 8**. The purpose of this study was to determine whether the effects of maternal provisioning disappear through compensation or persist throughout development under different conditions of food availability for larvae.

Finally, I consolidated the results of these independent experiments and summarized them in the context of the spatiotemporal patchiness of recruitment success and population densities of crown-of-thorns starfish. I also discussed possible limitations in existing connectivity and management models in the absence of maternal effect parameters in relation to coral cover and community structure. Management implications were also discussed with emphasis on the need to ensure the persistence of coral reef ecosystems, especially given other emerging threats associated with global climate change.

Chapter 2

Limits to understanding and managing outbreaks of crown-of-thorns starfish[1]

2.1 Introduction

Coral reefs are increasingly regarded as one of the world's most threatened ecosystems. Not only have reef ecosystems suffered a long history of degradation (e.g., Pandolfi et al. 2005), but climate change is also expected to have a greater effect on coral reefs than almost any other ecosystem (Walther et al. 2002; Hoegh-Guldberg and Bruno 2010). Anthropogenic degradation of coral reef ecosystems began centuries ago with extensive exploitation and harvesting of large vertebrate species (Pandolfi et al. 2005). More recently, there have been sustained declines in the abundance of corals (e.g., Gardner et al. 2003; Bellwood et al. 2004; De'ath et al. 2012), and associated shifts in the biological and physical structure of benthic habitats (Hughes et al. 2010). Across the Caribbean, average coral cover has declined from approximately 50% in 1977 to <10% in 2001, representing an average annual loss of 1.67% (Gardner et al. 2003). In the Indo-Pacific, average annual coral loss was 1.05% between 1982 and 2003, and is accelerating (Bruno and Selig 2007). Approximately 19% of the world's coral reefs have been effectively destroyed, meaning that >90% of coral has been lost and there is little prospect of recovery (Wilkinson 2008). Moreover, 35% of reefs face a

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similar fate within the next 10-40 years unless there is effective management action to halt or reverse ongoing coral loss (Wilkinson 2008).

Scleractinian corals are fundamental to the geomorphology, biodiversity, and productivity of coral reef ecosystems (e.g., Hoegh-Guldberg 2004; Wild et al. 2004; Pratchett et al. 2008; Stella et al. 2011). Most importantly, corals contribute to both biological and physical habitat structure (Pratchett et al. 2008), providing essential resources (food and shelter) for many reef organisms (Jones et al. 2004; Cole et al. 2008; Stella et al. 2011), high diversity of distinct microhabitats (e.g., Messmer et al. 2011), and habitat structure that mediates important biological interactions, such as competition (e.g., Munday 2001; Holbrook and Schmitt 2002) and predation (e.g., Caley and St John 1996; Beukers and Jones 1998; Coker et al. 2013). There is considerable correlative and experimental evidence showing that reef locations with high cover and diversity of scleractinian corals support greater abundance and diversity of coral reef organisms, especially fishes (e.g., Carpenter et al. 1982; Jones 1988; Munday 2000; Jones et al. 2004; Holbrook et al. 2000, 2002, 2008; Messmer et al. 2011). Accordingly, extensive loss of live corals leads to marked declines in the abundance and diversity of coral reef fishes (e.g., Wilson et al. 2006; Pratchett et al. 2008, 2011). Effects of coral loss are even more pronounced and affect a greater diversity of reef organisms when combined with loss of structural complexity, either due to direct physical disturbances (e.g., severe tropical storms) that damage coral skeletons, or the gradual decomposition and erosion of corals killed by biological disturbances (Pratchett et al. 2008).

Major causes of coral loss vary geographically. Most notably, the current status of coral reefs throughout the world is strongly reflective of the timing and extent of human colonisation (e.g., Pandolfi et al. 2005). The most degraded reef environments (east

Africa, southeast Asia, and the Caribbean) are in areas with very large human populations (Wilkinson 2004), which reflect the overarching effects of chronic (press) disturbances, such as overfishing, pollution, sedimentation and eutrophication. There are also a range of acute (pulse) disturbances that strongly influence the structure and dynamics of coral reef assemblages (e.g., Gilmour et al. 2013). At the global scale, the most pronounced acute disturbances are episodes of mass bleaching, linked to increasing ocean temperatures (Hoegh-Guldberg 1999; Hughes et al. 2003), severe tropical storms (Gardner et al. 2003), or rapid and pronounced increases (termed "plagues", Vine 1973; "outbreaks", Weber and Woodhead 1970; or "infestations", Endean 1977) in the abundance of crown-of-thorns starfish and other coral predators.

In the Caribbean, high levels of coral mortality since 1977 are variously attributed to severe tropical storms (e.g., Gardner et al. 2003), increasing incidence of coral disease (e.g., Aronson and Precht 2001), and/ or recent episodes of mass bleaching (Williams and Bunkley-Williams 2000). In reality, it is the combination of different disturbances that is responsible for sustained and ongoing declines in the cover of scleractinian corals across much of the Caribbean, and associated shifts in the biological and physical structure of reef habitats. For example, phase-shifts from coral- to macroalgae-dominated systems in Jamaica can be traced back to overfishing of herbivorous fishes, which had already occurred by the 1960s (Hughes 1994). However, marked changes in the biological and physical structure of reef habitats were not apparent until severe storms (e.g., Hurricane Allen in 1981; Hurricane Gilbert in 1988), and the mass mortality of the sea urchin *Diadema antillarum* in 1983 (Lessios et al. 1984). Each of these disturbances had an important and independent contribution to the resulting degradation of coral reef systems (Hughes 1994).

In the Indo-Pacific, outbreaks of crown-of-thorns starfish (*Acanthaster* spp.) have long been considered one of the major causes of coral loss (e.g., Pearson 1981; Bruno and Selig 2007; De'ath et al. 2012). The first well-documented outbreaks occurred in southern Japan in the late 1950s (Yamazato 1969 in Yamaguchi 1986) and on Australia's Great Barrier Reef (GBR) in the early 1960s (Pearson and Endean 1969). However, there are several earlier reports (as far back as the 1930s) of very high densities of *Acanthaster* spp., which probably represented outbreaks (Dana 1970, Vine 1973). The severity and extent of coral loss caused by outbreaks of *Acanthaster* spp. in the 1960s and 1970s generated considerable concern about the fate of coral reefs (e.g., Cornell and Surowiecki 1972). In reviewing recovery of coral communities from different disturbances, Pearson (1981) reported that "damage caused by *Acanthaster* infestations during the last 10 to 15 years has been more extensive and dramatic than that caused by any other natural or man-made disturbance" (Page 110, Pearson 1981). Since that time, emerging threats associated with climate change, such as coral bleaching (Hoegh-Guldberg 1999) and disease (Bruno et al. 2007), has become the major focus of coral reef science and management (Hughes et al. 2003). However, outbreaks of crown-of-thorns starfish 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 the effects of severe outbreaks have been far greater than combined effects of all other major disturbances, including climate-induced coral bleaching (e.g., Trapon et al. 2011, De'ath et al. 2012). Importantly, it is the combined effect of outbreaks of *Acanthaster planci* and other diverse disturbances (e.g., sedimentation, cyclones and bleaching) that have caused sustained and accelerating degradation of coral reef ecosystems throughout the Indo-Pacific (e.g., Jones et al. 2004, Pratchett et al. 2006).

The purpose of this review is to synthesize established knowledge and insights on the causes and consequences of outbreaks of crown-of-thorns starfish, focussing on the increased understanding and research undertaken in the last two decades (mostly since 1990), given that there were several substantive reviews of the research on the biology and ecology of *Acanthaster* spp. between 1960-1990 (e.g., Potts 1981; Moran 1986, Birkeland and Lucas 1990; Endean and Cameron 1990). Since 1990, there have not been any major comprehensive reviews on the biology and population dynamics of *Acanthaster* spp., but numerous commentaries on specific issues (e.g., Brodie 1992; Brodie et al. 2005) related to causes or consequences of population outbreaks. In the last few years, there has also been renewed interest in outbreaks of *Acanthaster* spp., largely attributable to fresh outbreaks of crown-of-thorns starfish at many locations throughout the Indo-Pacific (e.g., Kayal et al. 2012; Baird et al. 2013). Moreover, there is an increasing realization that urgent action is needed to reverse sustained and ongoing declines in live coral cover that are occurring throughout the world (e.g., Gardner et al. 2003; Bellwood et al. 2004; De'ath et al. 2012), and of all the factors that are contributing to degradation of coral reef ecosystems in the Indo-Pacific, outbreaks of *Acanthaster* spp. are considered to be the most amenable to direct and immediate intervention (cf. climate induced coral bleaching, increasing prevalence of coral disease, increasing severity of tropical storms). Controlling outbreak populations of *Acanthaster* spp. is considered one of the most promising strategies to halt or reverse widespread declines in live coral cover (e.g., De'ath et al. 2012) and thereby improve the capacity of reef systems to cope with inevitable threats due to sustained and ongoing climate change as well as other more direct anthropogenic disturbances. A second purpose of this review is to highlight gaps in our knowledge of the biology of *Acanthaster* spp. that

persist despite five decades of research, and constrain both understanding of the causes and effective management of outbreaks.

2.2 Biology of crown-of-thorns starfish

The crown-of-thorns starfish, specifically *Acanthaster planci* (Linnaeus 1758) was first described based on the original description by Plancus and Gualtieri (Vine 1973). Crown-of-thorns starfish have since been reported on coral reefs throughout the tropical Indo-Pacific from the Red Sea (e.g., Goreau 1964) to Panama (e.g., Glynn 1973), but have never been recorded in the Caribbean or Atlantic Ocean. Crown-of-thorns starfish are also found in a wide range of latitudes, from 34^{o}N on sub-tropical reefs in the Ryukyu Islands, Japan (Yamaguchi 1986), to 32^{o}S at Lord Howe Island (DeVantier and Deacon 1990). Marked geographic differences in appearance and allelic frequencies within the broad geographic range suggest that there may be at least two species of crown-of-thorns starfish (Benzie 1999), distributed within the Indian and Pacific oceans, respectively. Recent molecular sampling (632 bp from the COI region) of crown-of-thorns starfish (nominally, *Acanthaster planci*) from throughout its entire range revealed that there are four strongly differentiated clades from distinct geographical regions: 1) Red Sea, 2) southern Indian Ocean, 3) northern Indian Ocean and 4) Pacific, which probably represent distinct species (Vogler et al. 2008). However, Vogler et al. (2013) found no genetic differentiation between the crown-of-thorns starfish in the far eastern Pacific (sometimes considered to be a distinct species, *Acanthaster ellisii*; e.g., Barham et al. 1973, but see Glynn 1974) from the remainder of the Pacific. For this review, *Acanthaster* spp. will be used when referring to the entire species complex, whereas *A. planci* is reserved for use when referring explicitly to

crown-of-thorns starfish from the Pacific. Another well described species, *A. brevispinus* Fisher 1917, is known from deep-water habitats in the western Pacific, but this species is rarely found in coral reef habitats (Birkeland and Lucas 1990) and is not considered in this review.

Moran (1986) described crown-of-thorns starfish as "one of the most well-known animals in coral reef ecosystems" (Page 1, Moran 1986), and went on to say that the biology of this animal has been particularly well studied. It is true that there was considerable research on the basic biology of *Acanthaster* spp. in the 1970s and 1980s, including studies on reproductive biology (e.g., Lucas 1973), diet (e.g., Brauer et al. 1970; Branham et al. 1971; Ormond et al. 1973; Glynn 1974) and behavior (e.g., Barnes et al. 1970; Moran et al. 1985). However, crown-of-thorns starfish remain something of an enigma, with relatively little known about their demography and population dynamics. Moore (1990) suggested that intermittent outbreaks may be attributable to fundamental switches in the inherent life-history characteristics between endemic and epidemic characters, but this has never been explicitly tested.

Most of what is known about the reproductive biology and life cycle of crown-of-thorns starfish (**Figure 2.1**) comes from detailed studies of *Acanthaster planci* in the western Pacific (e.g., Lucas 1973; Yamaguchi 1973a; Nishihira and Yamazato 1974; Conand 1984; Babcock and Mundy 1992a,b). While the reproductive biology and life history is likely to be broadly similar for other *Acanthaster* spp. from the Indian Ocean, this needs to be verified as geographical (and taxonomic) differences in their biology may account for marked geographical differences in the incidence and severity of outbreaks (as discussed later). Comprehensive and detailed information about well-studied aspects of the biology of *A. planci* was provided by Moran (1986). Rather than

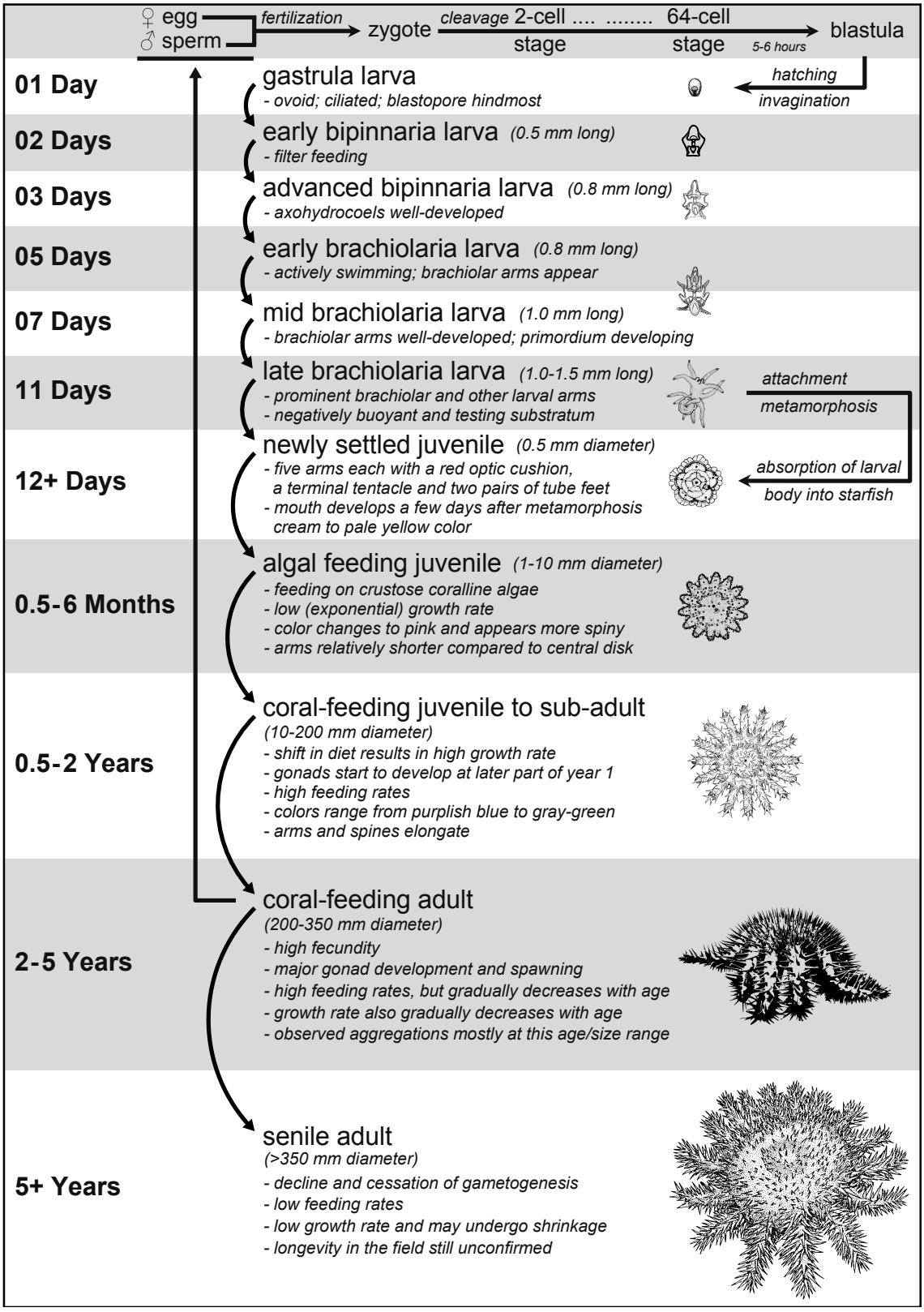

Figure 2.1 Complete life cycle of crown-of-thorns starfish. Adapted from laboratory rearing studies by Yamaguchi (1973a) and Lucas (1984) and compilations by Moran (1986) and Birkeland and Lucas (1990).

repeat this information, this review will limit discussion to advances in the biological knowledge since 1986, as well as considering key aspects of the biology that are fundamental in understanding the proximal causes of outbreaks, including inherent constraints of reproductive success, and the structure (age and size) of normal versus outbreak populations. There is widespread recognition that the biology (especially, the reproduction and early life-history) of crown-of-thorns starfish is key to understanding when and why outbreaks occur (e.g., Birkeland 1982, 1989a).

2.2.1 Fecundity

One of the most important biological traits of *Acanthaster* spp., which is particularly relevant to major population fluctuations, is their enormous reproductive potential (Endean 1982; Conand 1984). Large female starfish can produce up to 65 million eggs per season (Conand 1984; Kettle and Lucas 1987). It has long been recognized that *Acanthaster* spp. release millions of eggs each time they spawn (e.g., Pearson and Endean 1969), but Conand (1983, 1985) provided the first quantitative analysis of size-based fecundity for *Acanthaster planci*, following detailed studies in Noumea. These data correspond closely with similar research undertaken by Kettle and Lucas (1987) who also found disproportionate increases in fecundity with increasing size, ranging from 0.5-2.5 million eggs per year for individuals <30cm up to 46-65 million eggs per year starfish that are 40cm diameter (Birkeland and Lucas 1990).

Initiation of gametogenesis is clearly related to both age and size of *Acanthaster* spp. Gonad development in laboratory-reared starfish (Yamaguchi 1973a, Lucas 1984) and in a field population in Fiji (Zann et al. 1987) started before the animals reached the age of 2 years, and the largest individuals in any given cohort were also the first to exhibit gametogenesis (Lucas 1984, Zann et al. 1987). Gonads appear as aciniform rows

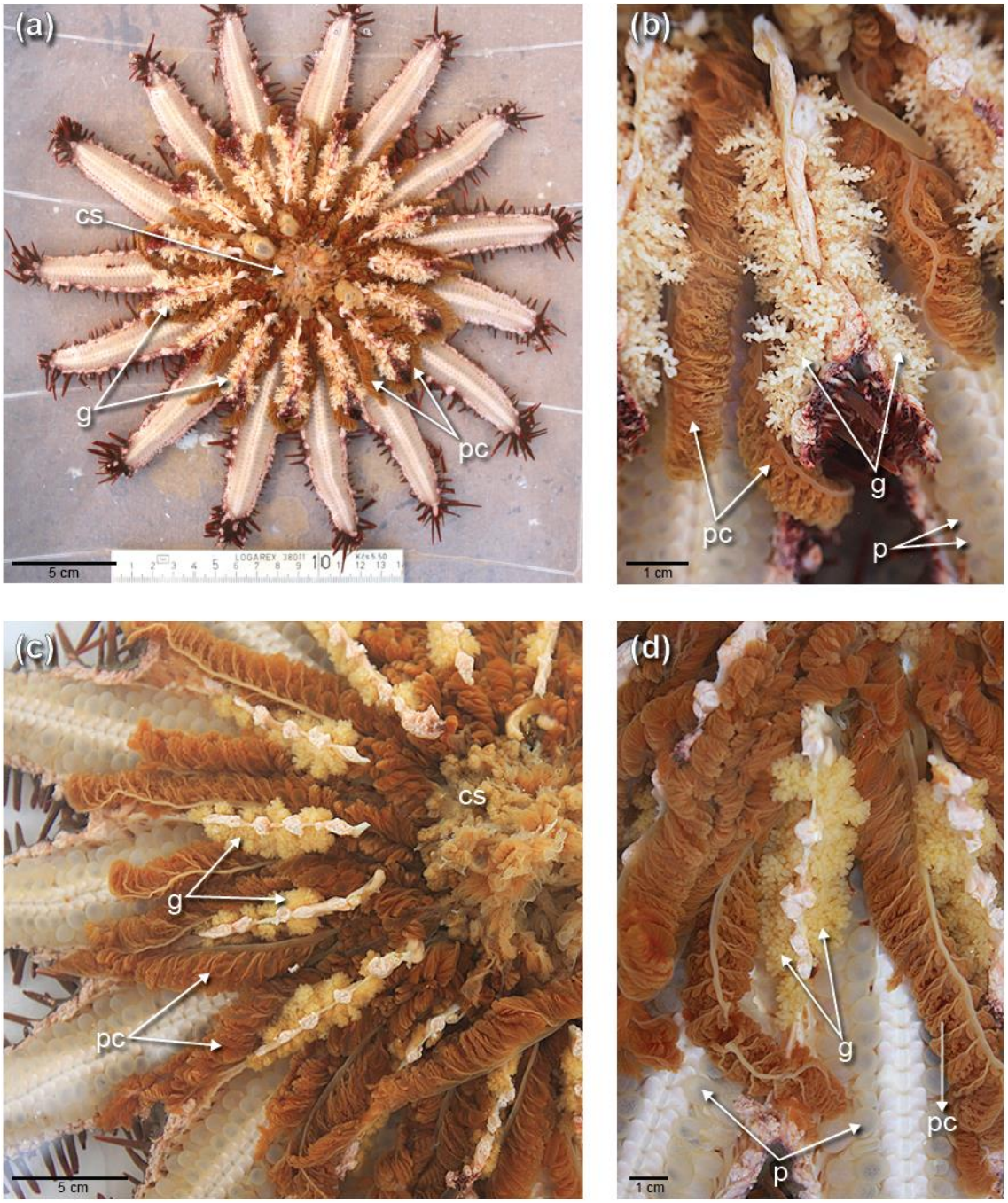

Figure 2.2 Aboral view of crown-of-thorns starfish showing internal arrangement of gonads **(g)**, pyloric caeca **(pc)**, podia **(p)**, and cardiac stomach **(cs)** in male (**a-b**) and female **(c-d)** specimens. (Photographs taken by C.F. Caballes).

along each side of the inner wall of the proximal part of each arm (**Figure 2.2**). When arms are dissected to expose internal digestive and reproductive organs, male and female starfish are readily distinguishable as testes are cream or pale yellow in colour and have smaller, more numerous lobes (**Figure 2.2a**, **2.2b**) compared to ovaries, which appear as larger, spherical, yellow (sometimes almost orange) lobes (**Figure 2.2c**, **2.2d**). It is also apparent that the size (weight or volume) and maturation stage of gonads are very consistent among arms, such that gonadosomatic indices are generally based on sub-sampling of only 1-3 arms (Lucas 1973; Conand 1984; Ogura et al. 1985; but see Yokochi and Ogura 1987). Changes in the size of gonads parallels changes in gonad index and size of oocytes (Yamazato and Kiyan 1973) and swelling in the proximal region of arms. Larger starfish increasingly partition energy towards reproduction (ova production) at the expense of the body wall and pyloric caeca (Kettle and Lucas 1987). Gonad indices in female *Acanthaster planci* are usually higher compared to males and this disparity becomes more pronounced at the peak of the breeding season (Cheney 1974; Conand 1984; Yokochi and Ogura 1987; Babcock and Mundy 1992a). Spermatogenic development in testes is mainly reflected in the thickness of the germinal layer (Yamazato and Kiyan 1973). In females, the stage of gametogenic cycle can be assessed by looking at the size of oocytes, the presence or absence of layers of connective tissue that bind oocytes together, and ovulation (Babcock and Mundy 1992a).

While the gametogenic cycle of asteroids may be regulated by endogenous (intrinsic) factors such as age, size, and nutritional status, or by exogenous (extrinsic) factors such as temperature, photoperiod, and food availability (reviewed in Mercier and Hamel 2009), there are very few studies on the role of nutritional status on gametogenesis and fecundity in *Acanthaster* spp. Cheney (1974) found that starving

Acanthaster planci (by placing them in a cage for one month without food) resulted in reabsorption of gonads, atrophy of the pyloric ceaca, and a decrease in the overall size (diameter and weight) of individual starfish. Other environmental factors (e.g., extreme temperatures, reduced salinity, and limited food availability) may also exert exogenous control on gametogenesis and fecundity, but this has not been investigated. For the large part, scientists are still coming to terms with spatial and temporal variation in occurrence of spawning, let alone understanding variation in the fecundity of individual starfish, and relating this to local environmental conditions.

2.2.2 Spawning

Like most asteroids, *Acanthaster planci* is a gonochoristic species, whereby male and female individuals must be in close proximity and spawn simultaneously to effectively reproduce (Babcock et al. 1994). This is important because reproductive success may be greatly constrained when there is a highly biased sex ratio (e.g., Stump 1994) or if densities of starfish are low and the distance between individuals is large (Vine 1973). Initial studies on the sex ratio of *A. planci*, based on sampling of outbreak populations (e.g., Pearson and Endean 1969, Nishihira and Yamazato 1974), suggested that there are generally equal number of males and females. However, strongly male biased sex ratios have been recorded in several populations (e.g., Stump 1994; Caballes et al. unpublished data). In September 2011, 93 large starfish (>30cm diameter) were sampled in Guam (Hospital Point), of which only 12 were female (Caballes et al. unpublished data). Similarly at Lizard Island (northern GBR) in March 2013, 115 starfish were collected across a range of different locations (ranging in size from 15 to 47cm), of which only two individuals were female (Caballes et al. unpublished data). Of the remaining individuals, 76 were male and 37 were immature or non-reproductive.

Both these populations were sampled in the aftermath of peak outbreak densities, and the strong male bias may reflect generally lower survival of females, which invest much more energy in reproduction (Stump 1994). However, if there is strong male sex bias in low-density populations generally, then this has the potential to greatly limit reproductive success.

Broadcast spawners (e.g., fishes, corals and many marine invertebrates) release copious quantities of gametes during spawning, but typically achieve low fertilization rates unless 1) individuals are highly aggregated, 2) spawning is synchronized, and 3) spawning occurs in low to moderate flow conditions (e.g., Mercier and Hamel 2009). In Okinawa, Okaji (1991) found that dispersed populations spawned later, and over a much longer period compared with aggregated populations. Okaji (1991) suggested spawning is also more synchronised within aggregated populations, which results in much higher reproductive success. Moreover, Cheney (1974) found that aggregated individuals had consistently higher gonadosomatic indices (indicative of fecundity and reproductive potential) compared with those from dispersed populations. More importantly, fertilization rates for *Acanthaster* spp. and other free-spawning marine invertebrates decline precipitously with increasing distance between male and female individuals (Levitan et al. 1992). For *Acanthaster* spp., fertilization success is close to 100% when male starfish spawn adjacent to spawning females (Benzie et al. 1994), but declines with increasing distance between spawning individuals (**Figure 2.3**). However, fertilization rates recorded when males and females are separated by relatively large distances (>20m) are significantly greater than those of many other marine invertebrates (Yund 1990, Grosberg 1991, Levitan et al. 1991; **Figure 2.3**). This disparity could be due to the greater size and fecundity of *Acanthaster* spp., the large quantity of gametes released during spawning (Babcock and Mundy 1992a; Babcock et al., 1994) or greater

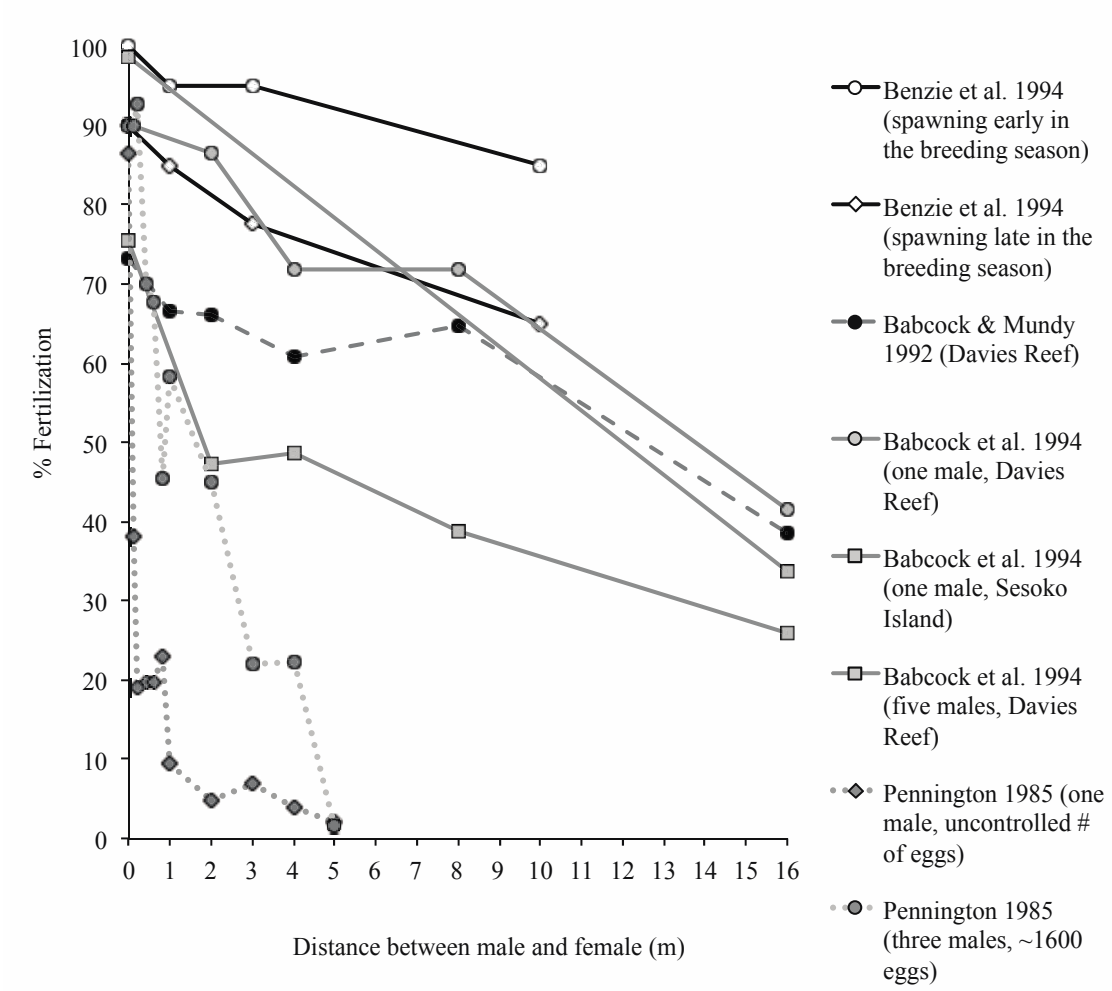

Figure 2.3 Fertilization rates for female *Acanthaster planci* (solid and dashed lines) versus *Strongylocentrotus droebachiensis* (dotted lines) at varying distances downstream from spawning males.

capacity for fertilization at low sperm concentrations (Benzie and Dixon 1994). Even so, minimising the distance between spawning individuals by aggregating will lead to a marked increase in reproductive success, suggesting that chance aggregation of adults on reefs with very low overall densities of *Acanthaster* spp. may be sufficient to precipitate an outbreak (e.g., Vine 1973).

During spawning, gametes are shed from aboral rows of gonopores along the sides of each arm. Exudates from spawning females appear as translucent spherical grains, while males exude milky clouds of sperm. Despite the conspicuousness of spawning starfish, there have been relatively few observations of natural spawning in the field (**Table 2.2**). It is still unclear whether *Acanthaster* spp. spawns just once each year, or whether there are multiple spawning events concentrated within a particular spawning period. At Lodestone Reef in the GBR, Lucas (1973) showed that periods of most active gametogenesis correspond to periods of increasing temperature and marked changes in the photoperiod. As for many other marine invertebrates (e.g., corals; Baird et al. 2009), temperature appears to be the most important cue for seasonality in spawning. In warmer locations, there is a tendency for starfish to reach maximum gonad maturity and begin spawning whenever sea surface temperatures exceed 27°C (**Figure 2.4**). At higher latitudes, where temperatures never reach this threshold (e.g. Hawaii, New Caledonia), gametogenesis and spawning are often concentrated in months when the temperature first starts to rise (**Figure 2.4**). Breeding and spawning seasons of *Acanthaster* spp. at higher latitudes are mostly shorter, well-defined events compared with more protracted gametogenesis and spawning at lower latitudes (e.g. Guam, Palau) (**Table 2.1**). Mature gonads have been reported year round in *A. planci* from Guam (e.g., Cheney 1974), but tend to be found in only a few months of each year at most other locations (**Table 2.1**).

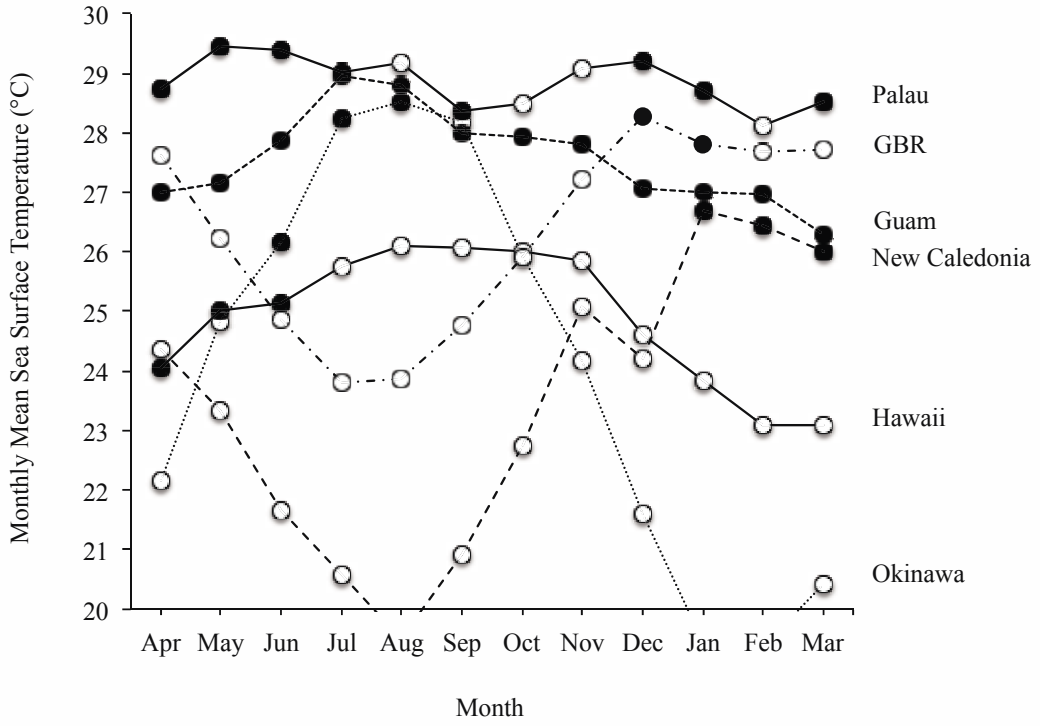

Figure 2.4 Seasonal variation in sea-surface temperatures and spawning times for *Acanthaster planci* at different locations throughout the Pacific. Spawning (filled circles) tends to occur only when temperatures are above 27°C, although at locations where temperatures never reach this level (e.g., Hawaii), spawning is restricted to months when temperatures first start to rise. Data sources: Palau, Idip 2003; Lizard Island, GBR, L. Vail unpublished data; Guam, Cheney 1974; New Caledonia, Conand 1984; Hawaii, Branham et al. 1971; and Okinawa, Japan, Yamazato & Kiyan 1973.

Compilation of the limited observations of natural spawning of *Acanthaster* spp. (**Table 2.2**) shows that spawning occurs mostly in the late afternoon, though it has occasionally been observed in the early morning (Gladstone 1987; Kishimoto 1989) and after dark (Babcock and Mundy 1992b). There is no apparent link between the timing of spawning and lunar phases or tidal cycles (Babcock and Mundy 1992a), suggesting that spawning is not linked to environmental cues. It is known that spawning by one individual will often instigate spawning by other individuals in the local proximity (e.g., Babcock and Mundy 1992a) or within aquaria. However, this does not explain the synchronous behavioral changes observed in many aggregations of *Acanthaster* spp., whereby individuals become particularly active, move to shallow promontories, and adopt the characteristic arched posture prior to the release of gametes (Babcock and Munday 1992a). Laboratory experiments by Beach et al. (1975) revealed that pheromones extracted from *Acanthaster planci* ovaries and testes synchronize spawning among neighbouring animals and also induce movement towards spawning individuals. Beach et al. (1975) argued that gametogenic cycles are influenced by local environmental conditions (mainly, temperature), but chemically mediated communication between gravid individuals is necessary to coordinate gamete release. Consistent with this hypothesis, spawning by *Acanthaster* spp. is often synchronised at very localised scales, within populations, but not among populations (Babcock and Mundy 1992a, Yasuda et al. 2010). Babcock and Mundy (1992a) saw *A. planci* spawning in one area of Davies Reef on the GBR, while *A. planci* in other parts of the same reef were not.

Spawning by *Acanthaster* spp. is concentrated in summer months at most locations, but the evidence is contradictory as to whether individual starfish spawn once annually (e.g., Babcock and Mundy 1992a, 1992b) or spawn multiple times (e.g., batch

spawn) each year (e.g., Conand 1983). In Noumea, Conand (1983) found that all female starfish examined contained oocytes at vastly different stages of maturity, suggesting that individual *Acanthaster planci* spawn sequentially over several months. However, in the central GBR Babcock and Mundy (1992a) suggested that *A. planci* spawn just once, mainly in early December. Babcock and Mundy (1992a, 1992b) recorded changes in the size and density of oocytes in *A. planci* at Davies Reef over much of the year, but concentrated sampling over the austral summer (October to February). They saw a marked drop in the proportion of females that were gravid, as well as marked reductions in mean size of gonads, in early December. Accordingly, spawning was observed in the evening of December 7th 1990, at 2145h, where 88 (out of 129) starfish were seen spawning along a single, shallow (1-4 m) transect. Elsewhere on the GBR, spawning has been reported from December, January and into February (**Table 2.2**), but it is unclear whether this reflects geographic variation in the timing of annual spawning, or whether crown-of-thorns starfish on the GBR can spawn multiple times in one year. It is clear that starfish at some locations (e.g., Guam) are gravid year round (Cheney 1974), and reproductive behavior is likely to vary geographically in response to environmental regimes and food availability. More detailed studies on the gametogenic cycle, maturation and spawning of *Acanthaster* spp. are needed, combined with systematic sampling of individually tagged starfish to establish spawning behavior of individuals as well as populations. This information is critical for potentially linking the precise timing of spawning behavior and associated reproductive success to spatial and temporal anomalies in local conditions, such as nutrient dynamics (e.g., Fabricius et al. 2010).

Table 2.1 Peak seasons in annual reproductive cycle of *Acanthaster* spp. from different locations based on gonad development and spawning. ○ = months surveyed/sampled, ● = spawning/breeding season, ◉ = mature gonads but only partial or no spawning, ◌ = not surveyed/sampled but spawning assumed.

Latitude	Location	Month (April to March)												Reference
		04	05	06	07	08	09	10	11	12	01	02	03	
33 °N	SW Honshu [a]				●	●								Hayashi 1975
28 °N	Amami-Ohshima [d]		○	○	●	○								Yasuda et al. 2010
26.5 °N	Okinawa [b, c, e]	○	○	○	●	●	○	◉		○		○	○	Yamazato and Kiyan 1973
	Okinawa [b, c]	○	○	●	●	○	○	○	○	○	○	○	○	Okaji 1989
	Okinawa [d]		○	○	●	○	○							Yasuda et al. 2010
26 °N	Kerama Islands [d]		○	●	●		○							Yasuda et al. 2010
25 °N	Gulf of California [c]	●												Dana and Wolfson 1970
24.5 °N	Miyako Island [d]		○	●	○			○						Yasuda et al. 2010
24 °N	Iriomote [b]	○	○	●	○	○	○	○	○	○			○	Yokochi and Ogura 1987
	Iriomote [b]	○	●	●	○	○	○	○	○	○			○	Habe et al. 1989
24 °N	Sekisei Lagoon [d]		●	○	○	○								Yasuda et al. 2010
20-21 °N	Red Sea [b]					●	●							Crump 1971
	Red Sea [b]					●	●							Moore, 1985
21 °N	Hawaii [a, d]	●	◉	●		○			○		○			Branham et al. 1971
13 °N	Guam [f]								●	●				Chesher 1969
	Guam [d]	◉	◉	◉	◉	◉	●	●	●	◉	◉	◉	◉	Cheney 1974
9 °N	Panama [f]										●			Glynn 1974
7 °N	Palau [b]	●	●	●	●	○	●	○	○	◉	◉	○	●	Idip 2003
7 °N	Philippines [b]	●	●		○		○		○		○		○	Bos et al. 2013
5-15 °N	Micronesia [c]					●	●							Eldredge 1970
	Micronesia [b]	◉	◉	◉	◉	◉	◉	◉	◉	◉	◉	◉	◉	Cheney 1972
4 °N	Maldives [a]	●											●	Ciarapica and Passeri 1993
9 °S	Solomon Islands [c]												●	Eldredge 1970
14 °S	W Samoa [c]									●	●			Garlovsky and Bergquist 1970
17-19 °S	Central GBR [a, b]					○	○	○	○	●	●	○	○	Pearson and Endean 1969
	Central GBR [b, e]	○		○		○	○	○	○	●	●			Lucas 1973
	Central GBR [a, b, c]							○	○	●	●	●		Babcock and Mundy 1992a
	Central GBR [a, b]					○	○	○	●	●	●	○		Babcock and Mundy 1992b
	Central GBR [a]									●				Gladstone 1992
18 °S	Fiji [a]											●		Owens 1971
19-23 °S	SE Polynesia [c]									●	◉	◉	◉	Devaney and Randall 1973
20 °S	NW Australia [d]		○					○	●	●	●	●		Wilson and Marsh 1974
22 °S	New Caledonia [b, d]	○	○		○			○	●	●	●	●	○	Conand 1984
27 °S	South Africa [c]											●	●	Schleyer 1998
32 °S	Lord Howe Island [c]												●	DeVantier and Andrews 1987

Method used to describe annual reproductive cycle of *Acanthaster* spp. : [a]field observation of spawning; [b]changes in gonad index; [c]gonad condition or histology of ovaries and testes; [d]changes in proportion of mature and spent gonads; [e]changes in oocyte size and frequency; [f]no method given.

Table 2.2 Observations of spontaneous natural spawning in the field.

Region	Location	Date			Time of day	Depth	Extent of spawning	Reference
Japan	Kii Peninsula	17	Jul	1973	evening	–	–	Hayashi 1975
	Kii Peninsula	15	Aug	1973	evening	–	–	Hayashi 1975
	Iriomote Island	9	Jun	1984	afternoon	4-8 m	3 males and 1 female spawned	Yokochi 1985
	Okinawa	9	Jun	1988	daytime	15 m	several individuals spawned	Kishimoto, 1989
	Okinawa	04-05	Jul	1988	1500	–	–	cited in Babcock and Mundy 1992a
	Okinawa	13	Jun	1990	1500	–	3 male individuals spawned	cited in Babcock and Mundy 1992a
	Okinawa	17	Jun	1990	1425-1645	–	6 males and 1 female spawned	cited in Babcock and Mundy 1992a
	Okinawa	19	Jun	1990	afternoon	–	single male	cited in Babcock and Mundy 1992a
Red Sea	N Gulf of Aqaba	24	Jul	2004	1823, 1859	10 m, 21 m	two individuals spawned (water temperature: 24.5°C)	D. Zakai (pers. observation)
Hawaii	Kalohi Channel	23-24	Apr	1970	–	–	Several starfish spawned	Branham et al. 1971
Guam	Tanguisson Reef	21	Apr	2006	1500	5 m	single female (~ 35 cm diameter) in a massive aggregation spawned for 30 min	C.F. Caballes (pers. observation)
Maldives	North Malé Atoll		Apr	1991	–	–	–	Ciarapica and Passeri 1993
PNG	Yule Island	04	Jul	1989	1630		50-100 individuals spawned	cited in Babcock and Mundy 1992a
Central GBR, Australia	Arlington Reef	10	Jan	1968	1330	3 m	Dense aggregation; spawning lasted 30 minutes and involved several males and only one female spawned	Pearson and Endean 1969
	John Brewer Reef	20	Jan	1983	1500	–	single female	cited in Babcock and Mundy 1992a
	Lizard Island		Feb	1983	0900	–	20-30 males; no females	Gladstone 1987
	Rib Reef	21	Jan	1984	1615	–	2-3 males spawned on top of boulder	cited in Babcock and Mundy 1992a
	Rib Reef	13	Dec	1984	1600	4 m	more than 50 clustered individuals	cited in Birkeland and Lucas 1990
	Wheeler Reef	5	Jan	1987	1520-1800	3-8 m	40-100 starfish spread over a 2400 m^2 area	cited in Birkeland and Lucas 1990
	Wheeler Reef	5	Jan	1987	1600	–	single female (adjacent starfish didn't spawn)	cited in Babcock and Mundy 1992a
	Bowden Reef	17	Jan	1988	–	–	3-4 starfish	cited in Babcock and Mundy 1992a

	Bowden Reef	18	Jan 1988	1430	–	1 starfish	cited in Babcock and Mundy 1992a
	Hayman Island	5	Dec 1990	1534-1706	2.5 m	10 males, single female was last to begin spawning and spawned for the shortest time	Gladstone 1992
	Davies Reef	7	Dec 1990	2145	≤ 7 m	68% of 129 starfish (38 females and 50 males) spawned over a 2-hr period	Babcock and Mundy 1992a
	Davies Reef	17	Dec 1990	1700	≤ 7 m	3 male individuals spawned	Babcock and Mundy 1992a
	Davies Reef	11	Dec 1991	1630	1-4 m	single female initially spawned, followed by >50 starfish (mostly males)	Babcock andMundy 1992b
	Davies Reef	12	Dec 1991	1930	1-4 m	8 males and 1 female spawned	Babcock andMundy 1992b
	Davies Reef	13	Dec 1991	2030	1-4 m	single male spawned	Babcock andMundy 1992b
	Davies Reef	23	Jan 1992	2030	1-4 m	2 males released gametes through gonopores from only a few arms	Babcock andMundy 1992b
	South/Palfrey Island	18	Dec 2009	1630	1 m	12 males releasing copious amounts of sperm, most were exposed and individuals were well-spaced from each other (3-10 m apart)	L. Vail and A. Hoggett (pers. observation)
Fiji	Muaivuso	01	Feb 1970	1400-1600	0.5-4 m	Dense clusters, many individuals spawned	Owens 1971

2.2.3 Larval development

The life cycle of *Acanthaster* spp. is typical of most asteroids, with larval development divided into two distinct bipinnaria stages (pelagic feeding larva characterized by bilateral arrangement of the pre- and post-oral ciliated swimming and feeding bands) and three brachiolaria stages (feeding larva characterized by the presence of brachiolar arms and attachment disk on the pre-oral lobe), prior to metamorphosis and settlement (**Figure 2.1**). Fertilized embryos develop to the blastula stage after 8-9 hours, then hatch after approximately one day as free-swimming gastrula larvae (Lucas 1982). After 2-4 days, larvae have a completely formed alimentary canal and start filter feeding on unicellular algae and other suspended particulate matter (Yamaguchi 1973a). Larvae then proceed to the brachiolaria stage, developing brachiolar arms, which will eventually be used to locate favourable substrate prior to settlement (Henderson and Lucas 1971). The typical planktonic larval duration (PLD) for *Acanthaster planci* is 11 days (**Figure 2.1**), though, like many marine invertebrates (Richmond 1987; Graham et al. 2008), these starfish can delay settlement and significantly extend their PLD (Yamaguchi 1973a). The rate of development also varies with temperature (Henderson and Lucas 1971) and food availability (Lucas 1982), such that the PLD can range from 9 to 42 days.

Factors that limit the rate and success of larval development of *Acanthaster* spp. (mainly, *Acanthaster planci*) have received a great deal of attention in the past years, as this was considered key to understanding the initiation of population outbreaks (e.g., Lucas 1982; Olson and Olson 1989). Much of this research has concentrated on larval nutrition, and the extent to which larval survivorship is constrained by abundance of phytoplankton, which in turn depend on the levels of nutrients. Phytoplankton are generally considered to be the main food source for larval *Acanthaster* spp., as both

natural phytoplankton and cultured unicellular algae (single or mixed species) have been successfully used to rear larvae under laboratory conditions (Henderson and Lucas 1971; Lucas 1975, 1982; Uchida and Nomura 1987; Okaji 1996; Keesing et al. 1996; Fabricius et al. 2010). However, Lucas (1982) suggested that the amount of phytoplankton required to maintain cultured larvae was much higher than what generally occurs in near reef waters (e.g., within the GBR lagoon) leading to suggestions that larvae are severely food-limited except during major phytoplankton blooms ("larval starvation hypothesis"; Lucas 1982). Similarly, Fabricius et al. (2010) reported minimal survival of larval *Acanthaster planci* at chlorophyll concentrations below 0.25 $\mu g.l^{-1}$, whereas larval survival increased approximately eightfold with each doubling of chlorophyll concentrations up to 3.0 $\mu g.l^{-1}$. These experiments were designed to answer specific questions about the larval nutrition of *A. planci* and were very limited in their representation of model ecosystems. For instance, any potential predators on larval crown-of-thorns starfish ($\geq$100 μm length) were explicitly excluded from the experimental system by filtering (25 μm filters) incoming seawater. Even so, these data are used to argue that outbreaks of *A. planci* may arise from pulses of recruits produced by temporary increases in productivity linked to high rainfall events and associated floods (Fabricius et al. 2010). It is not clear whether predators and/ or competitors would alter the survivorship of larval crown-of-thorns starfish sufficiently to affect the relationship with nutrient concentrations, but these experiments need to be repeated and extended before any conclusions about limitations to larval survivorship can be made. It is also controversial whether *Acanthaster* spp. are food limited, as several studies have successfully reared larvae at normal (low) chlorophyll concentrations (Olson 1987; Johnson et al. 1991).

Olson and Olson (1989) suggested that larval *Acanthaster planci* are capable of exploiting a diversity of different sources of nutrition. Under normal conditions of low plankton abundance, high levels of survivorship were facilitated by the ability of *A. planci* to utilize dissolved organic matter (DOM) and bacteria (Olson and Olson 1989). Importantly, larvae reared in *in situ* culture chambers showed no sign of food limitation and were able to develop at near-maximal rates despite low levels of phytoplankton (Olson 1987). Moreover, nutrient enrichment did not result in increased survivorship, but did result in a slight increase in the rate of development (Olson 1987). Accordingly, Hoegh-Guldberg (1994) showed that dissolved free amino acids (DFAA), can supply significant amounts of energy for developing larvae. Microscopic analyses by Ayukai (1994) showed that *A. planci* larvae generally consume only large phytoplankton, as there was limited evidence of ultraplankton (<5 μm) or bacteria within stomach contents. However, *A. planci* may rely on alternative sources of prey (including ultraplankton and bacteria) when preferred prey (large phytoplankton) are in limited abundance. Given increased knowledge of patterns of resource use, food limitation experiments with developing larvae should be repeated to test explicitly whether high concentrations of DFAA, free-living bacteria, or ultraplankton, may compensate for the limited abundance of phytoplankton under field conditions. Experimental work needs to be corroborated with field assessment of the nutritional condition of crown-of-thorns larvae before, during and after major flood events, so as to specifically relate this to local phytoplankton concentrations. In the past it was difficult to distinguish the larvae of *Acanthaster* spp. from those of other echinoderms, but Roper (1997) developed a technique to stain crown-of-thorns larvae with fluorescently labelled monoclonal antibodies, which readily distinguish *Acanthaster* spp. within plankton samples.

As for many marine organisms, predation on larval *Acanthaster* spp. is expected to be very high, especially during the late brachiolaria stage, when larvae come within the vicinity reefs and attempt to settle. However, logistical challenges to sampling *Acanthaster* spp. larvae in the field make it difficult to quantify natural rates larval mortality and rates of predation. Unlike coral eggs, which are heavily preyed upon by planktivorous fishes (e.g., Pratchett et al. 2001), *Acanthaster* spp. gametes and larvae are often avoided by planktivorous fishes and invertebrates (Yamaguchi 1974a, 1975). Chemical analyses by Lucas et al. (1979) showed that eggs and larvae of *Acanthaster* spp. contain saponins, which presumably make them less palatable. During a spawning event at Blue Pearl Bay (Hayman Island, GBR), Gladstone (1992) observed that planktivorous fish feeding nearby ignored gametes released by spawning *Acanthaster planci*. Reef fishes within the vicinity of a spawning *A. planci* at Arlington Reef, central GBR, also ignored gametes, except for one species of damselfish, *Abudefduf curacao*, which was observed feeding on eggs shed by the spawning female (Pearson and Endean 1969). Butterflyfishes, *Chaetodon auripes* and *Chaetodon falcula* have also been observed to feed on *A. planci* gametes in Okinawa and the Maldives, respectively (Keesing and Halford 1992, Ciarapica and Passeri 1993). Keesing and Halford (1992) suggest that spawning usually occurs late in the afternoon or at night, when the impact of visual predators would be minimised. However, little is known about when or where developed larvae actually settle, or whether natural rates of predation on larvae that are trying to settle are high or low. Chesher (1969) suggested that predation by filter feeders, such as corals, would inflict significant mortality on settling larvae. Yamaguchi (1973a) documented that *Pocillopora damicornis* will feed on the larvae of *A. planci* and other coral reef asteroids. However, predation by corals is likely to have limited influence on overall survivorship for two reasons. Firstly, Ormond and Campbell (1974)

found that *A. planci* larvae could detect and readily avoid live corals. Secondly, there are many areas of coral reef substrata that have relatively low cover of live coral where starfish larvae could settle without being consumed by coral polyps (Reichelt et al. 1990b). Chesher (1969) suggested that larvae are attracted to aggregations of adult *A. planci* because they have already removed corals that would otherwise prey on the larvae. More likely, however, is that the feeding activities of adult *A. planci*, increase the availability of microhabitats (dead but intact coral colonies) that are conducive to settlement.

Abiotic factors play an important role in development and survivorship of larval *Acanthaster* spp. (e.g., Henderson and Lucas 1971; Lucas 1973). Laboratory rearing experiments suggest that *Acanthaster planci* have a very narrow temperature tolerance. Optimal temperatures for larval development appear to be between 26 and 31°C (e.g., Lucas 1973). Temperatures $\geq 32^{o}$C appear lethal, while larvae simply did not complete development at temperatures <25°C (Henderson and Lucas 1971; Lucas 1973). In Guam, larvae were successfully reared at 27-29°C, while larvae reared at temperatures below 25°C did not advance to brachiolaria stage and showed regression to earlier stages, even though they were observed to feed vigorously (Yamaguchi 1973a). Similar temperature ranges were used to rear larvae through to settlement in the Red Sea (28 and 29°C; Ormond and Campbell 1974) and southern Japan (fluctuating between 25-30.3°C; Uchida and Nomura 1987). Importantly, temperature tolerance of *A. planci* varies with developmental stages (Johnson and Babcock 1994). The late brachiolaria stage appears to be the most temperature-sensitive (Habe et al. 1989), and this may constrain settlement to areas with relatively warm temperatures (26-31°C). However, greater temperature tolerance in early larval stages allows larvae to withstand exposure to cooler waters and slowly continue normal development during oceanic transport.

Hatched gastrula larvae can tolerate temperatures between 13 and 34°C, and bipinnaria larvae can tolerate temperatures of 14.5-32°C (Habe et al. 1989). However, the rate of development is greatly accelerated at higher temperatures: Habe et al. (1989) found that embryonic development is completed in 31 hours at 20°C but only 11 hours at 32°C.

Although echinoderms are generally very sensitive to changes in salinity (Diehl 1986), the early larval stages of *Acanthaster* spp. appear to tolerate very wide ranges in salinity. Gastrula larvae can tolerate a salinity range of 21‰-45‰ and bipinnaria larvae can tolerate 21‰-50‰ salinity (Habe et al. 1989). Henderson (1969) also found that bipinnaria larvae can tolerate abrupt salinity changes from 36‰ down to 21‰ and they developed more rapidly at lower salinities. Despite the robust early larval stages, late brachiolaria and metamorphosing stages are less tolerant to salinity and rupture with 2‰ changes in salinity (Henderson and Lucas 1971). Larval development and metamorphosis was completed at 26‰, but not at 22‰ (Lucas 1973). On the GBR, salinity in near shore water is influenced by river discharge and during times of flooding and heavy rainfall, salinity levels ≤30‰ are often recorded (Brodie et al. 2005). Lucas (1973) showed that larval survival was threefold higher at 30‰ compared to ambient conditions, suggesting that temporary declines in salinity brought about by periods of heavy rainfall and runoff may actually enhance larval survivorship (Birkeland 1982). The tolerance of unfertilized gametes, especially sperm, to lowered salinity is unknown. Sperm of *Acanthaster planci* have been shown to be extremely sensitive to changes in temperature and seawater chemistry (C. Caballes and J. Rivera-Posada, unpublished data), and may therefore be sensitive to changes in salinity (but see Greenwood and Bennett 1981).

2.2.4 Settlement

Towards the end of the brachiolaria stage, the brachiolar arms of *Acanthaster* spp. larvae elongate to improve locomotion while supporting the weight of the starfish primordium (Olson et al. 1988). At this time, larvae start to drift downward and flex the anterior body dorsally to orient the brachiolar arms against the substratum to test its suitability for settlement (Yamaguchi 1973a). Based on laboratory experiments (Henderson and Lucas 1971; Ormond et al. 1973, Yamaguchi 1973a; Ormond and Campbell 1974; Lucas 1975; Johnson et al. 1991; Keesing and Halford 1992; Johnson and Sutton 1994) and field observations (Zann et al. 1987; Yokochi and Ogura 1987), larval *Acanthaster planci* are very particular about where they settle. However, Lucas (1975) suggested that these strong settlement preferences do not necessarily limit settlement success so long as larvae are transported over coral reef habitats while they are still competent to settle. Larvae appear to settle preferentially in habitats with fine-scale topographic complexity, so that the larvae are completely hidden within carbonate matrix, or amongst coral rubble, prior to metamorphosis (Lucas 1975). This may be an adaptation to minimise larval mortality at settlement and during metamorphosis, which is suggested to be in excess of 85% (Keesing and Halford 1992). Natural rates of post-settlement mortality are extremely difficult to measure, but as for many coral reef organisms (e.g., Trapon et al. 2013), there is increasing realization that biological interactions (competition and predation) are probably very important in determining patterns of settlement (Keesing and Halford 1992). Ormond and Campbell (1974) showed that skeletons of dead colonies of *Acropora hyacinthus* were favoured over the other coral skeletons they tested (*Acropora diversa*, *Pocillopora verrucosa*, and *Stylophora pistillata*) probably because the branch spaces more closely match the size of the larvae. However, other work has suggested that settlement cues for *A. planci* are

more strongly influenced by biofilms (e.g. encrusting algae, and associated bacteria and detritus), rather than the microhabitat complexity. For example, *A. planci* will not generally settle on glass or ceramic tiles (Henderson and Lucas 1971; Ormond and Campbell 1974; Johnson et al. 1991), unless these substrates are first conditioned in natural environments to generate fine growth of microalgae (Henderson and Lucas 1971).

Observations of recently settled *Acanthaster planci* in Suva Reef, Fiji (Zann et al. 1987) and Ryukyu Islands, Japan (Yokochi and Ogura 1987) revealed a strong association with coralline algae (e.g. *Porolithon onkodes*), which is expected given that newly settled starfish feed almost exclusively on coralline algae (Yamaguchi 1973a; Lucas 1984; Zann et al. 1987). Yamaguchi (1973a) observed *A. planci* larvae settling directly on dead coral encrusted with coralline algae (*Porolithon* sp.), but found no settlement on bleached coralline algae or on pieces of beach rock covered with filamentous algae (but see Henderson and Lucas 1971). Johnson et al. (1991) also found high rates of settlement on coral rubble with high coverage of the coralline algae, *Lithothamnium pseudosorum*, but reported significantly lower settlement on tiles colonised by non-calcareous crustose red algae (*Peyssonellia* sp.), and other species of coralline algae (*Porolithon onkodes*, *Neogoniolithon foslei*). Techniques developed for large-scale culture of *A. planci* larvae have achieved high rates of settlement on thalli of *L. pseudosorum* (Ayukai et al. 1996, Keesing et al. 1996). Treatment of highly inductive shards of *L. pseudosorum* with antibiotics reduced settlement to low levels, signifying that induction of settlement and metamorphosis of *A. planci* may be mediated by chemical cues produced by epiphytic bacteria (Johnson et al. 1991). Settlement and metamorphosis was inhibited in the absence of bacteria; larvae always settled on sections of thallus with high densities of bacteria, but not in areas where epiphytic

bacteria were sparse (Johnson et al. 1991; Johnson and Sutton 1994). However, surface bacteria did not induce settlement when isolated from soluble algal compounds, suggesting that bacteria require the algal substratum to produce inductive compounds or that compounds from both the bacteria and coralline algae are required to induce settlement and metamorphosis (Johnson and Sutton 1994).

2.2.5 Juvenile ecology

Following settlement, metamorphosis of *Acanthaster* spp. brachiolaria larvae occurs with the absorption of the anterior part of the larval body into the starfish primordium (Yamaguchi 1973a), which emerges two days later as a five-armed juvenile starfish 0.3 to 0.7 mm in diameter with two pairs of tube feet, a terminal tentacle, and a red optic cushion on each arm (Henderson and Lucas 1971, Yamaguchi 1973a, Lucas 1975). After three weeks, these juvenile starfish start adding arms at two-week intervals and the body turns pink, which camouflages the juvenile starfish against the coralline algae on which it is feeding (Lucas 1975; Yamaguchi 1973a; Birkeland and Lucas 1990). During this phase, juvenile starfish do not feed on coral tissue, possibly to avoid damage caused by mesenteric filaments when coming in to contact with coral polyps (Yamaguchi 1973a). Whilst feeding on coralline algae, growth rates of *A. planci* are very slow (1.5-2.6mm/ month), but increase rapidly when starfish switch to feeding on scleractinian corals at around 6 months (Yamaguchi 1974a; Zann et al. 1987). Maximal growth rates (16.7-25.0mm/ month) occur between 5-18 months after *A. planci* switch to feeding on live corals, but slow substantially after this period, presumably because starfish begin diverting energy from somatic growth as they become sexually mature (Birkeland and Lucas 1990).

Although juvenile *Acanthaster* spp. contain saponins, they are still likely to be extremely vulnerable to predation (Keesing and Halford 1992). Small juveniles are cryptic and are mostly active at night (Zann et al. 1987), presumably to avoid visual predators like reef fishes. This highly cryptic and nocturnal behavior continues until starfish reach at least 15cm diameter, at an age of approximately 20 months (Zann et al. 1987), after which starfish are much more active during daylight hours. As a consequence, relatively few juvenile *Acanthaster* spp. have been found, despite extensive field sampling (Doherty and Davidson 1988; Johnson et al. 1992). Estimates of recruitment (e.g., Doherty and Davidson 1988; Zann et al. 1990) are often based on the emergence of relatively old (e.g., 1-2 year old) individuals. The abundance and distribution of these older juveniles are likely to differ greatly from patterns at settlement, due to high rates of post-settlement mortality (e.g. Keesing and Halford 1992) and likely movement of larger juvenile starfish as they switch to eating scleractinian corals (Endean and Cameron 1990).

2.2.6 Adult growth and longevity

The demography of *Acanthaster* spp. is extremely plastic, in that adult growth and longevity seem to be strongly dependent on local environmental conditions, such as food availability, temperature and wave exposure (e.g., Kenchington 1977; Ormond and Campbell 1971; Lucas 1984). For this reason there has been considerable controversy surrounding even the most basic demographic questions, such as whether growth of *Acanthaster* spp. is determinate (or more precisely, asymptotic; e.g., Yamaguchi 1974a; Lucas 1984)) or indeterminate (Kenchington 1977). This issue was discussed at length in Moran (1986), but never resolved. Lucas (1984) argued that *Acanthaster planci* from the GBR reached a maximum size of approximately 340 mm diameter at approximately

3 years of age (**Figure 2.5**), after which they entered a period of senescence and had a maximum longevity of 4-5 years. These assertions are clearly at odds with extensive and increasing records of *A. planci* that are up to 750 mm in diameter and 8 years of age (Endean and Cameron 1990; Stump 1996). Based on their regenerative ability, as well as physical and chemical defences, Endean (1982) suggested that adult crown-of-thorns starfish would have very low mortality and should live for decades (see also Ebert 1973). Very large *A. planci* have been recorded, up to 750mm diameter (Lucas 1984), but mostly on the GBR and often outside of active outbreaks. At high densities, *Acanthaster* spp. may have highly constrained, finite growth and survivorship which is possibly linked to strong intraspecific competition and rapid depletion of prey resources during major outbreaks (Kettle 1990; Mills 2012). However, recent studies (e.g., Pan et al. 2010) clearly show that starfish in outbreak populations can grow well beyond 350 mm and can live for more than 8 years. Substantial variation in growth rates among individuals from a single cohort will obscure any relationship between size and age, and that this alone accounts for the range of sizes within outbreaking populations (Stump and Lucas 1990). To test this, size-independent proxies of individual age have been explored, including spine length, age pigments, and pigment bands (equivalent to growth rings) on spines (Birkeland and Lucas 1990). The most reliable and widely adopted technique involves estimating individual ages based on pigment banding on spines, developed by Stump and Lucas (1990). Mark-recapture and tetracycline staining of *Acanthaster planci* in the field (Stump and Lucas 1990; Stump 1994) confirmed that growth bands on longest spines taken from the aboral surface of upper arms were laid down seasonally, and thus banding couplets are reflective of age in years. However, growth bands only become apparent after sexual maturity, at around 2 years of age (Stump 1996). Moreover, an important question for demographic studies (and

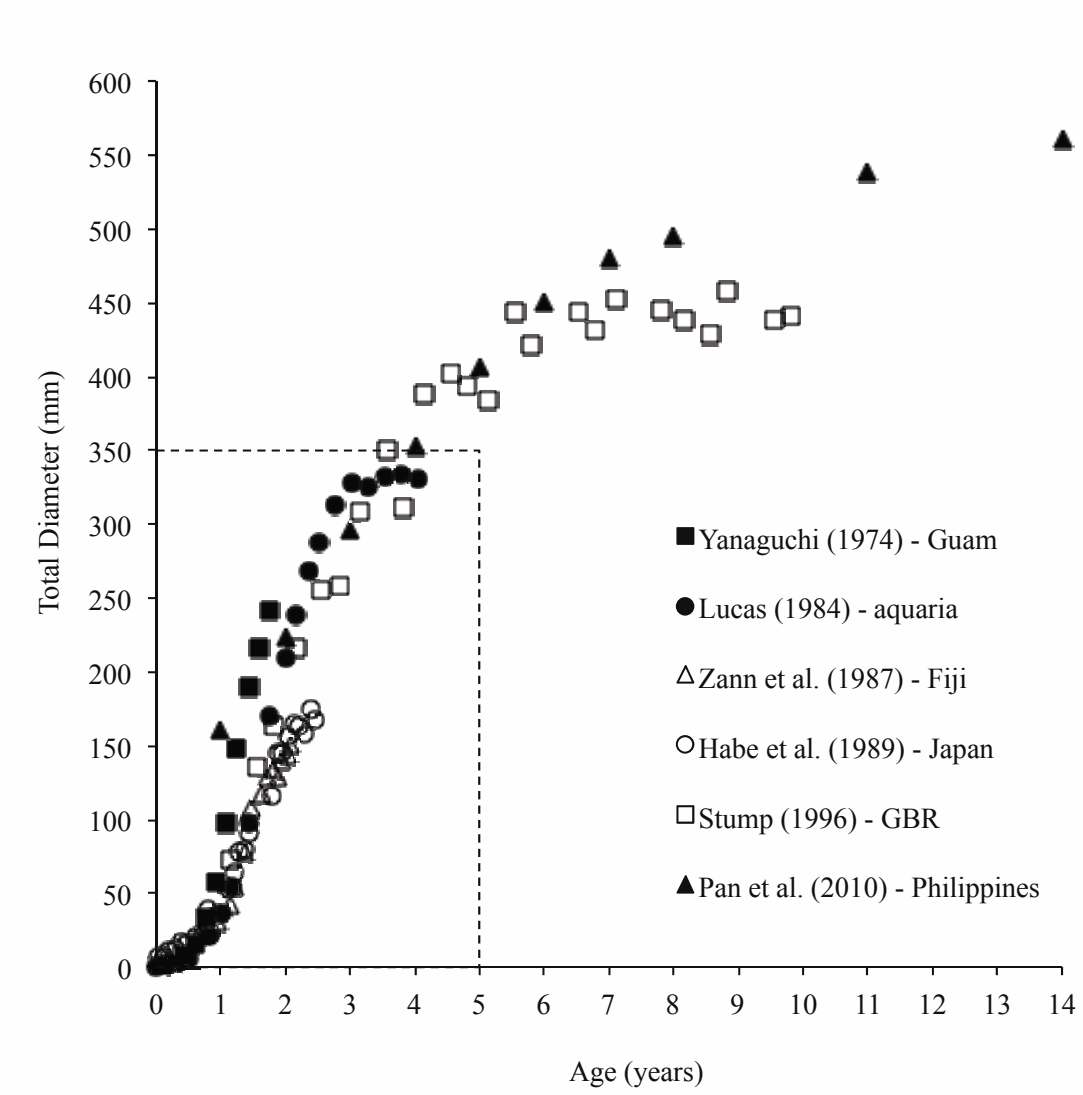

Figure 2.5 Relationship between total diameter (millimeters) and age (years) for *Acanthaster planci* based on data from throughout the western Pacific. There is a strong and consistent size-age relationship, despite initial suggestions that marked plasticity in initial growth, combined with rapid attainment of asymptotic size, would obscure any such relationship. The growth curve in the formative part of the life history (incorporating the initially slow growth in the first 6 months) is best explained by a sigmoidal function. After 1 year, however, the generalized growth curve is largely consistent with von Bertalanffy growth equations. The dashed line indicates the presumed maximum size and age of *A. planci* prior to 1990, based on the apparent asymptote at 340-mm diameter for laboratory-reared starfish (Lucas 1984). Field-based studies, however, suggest that there is a much larger asymptotic size.

improved understanding of the timing and therefore possible causes of outbreaks) of *Acanthaster* spp. is whether variation in the sizes of starfish within a given population reflects distinct cohorts, and therefore the range in ages of individuals (e.g., Pratchett 2005). The alternative is that caution must be taken in selecting the most appropriate spines (Stump and Lucas 1999) as marked differences in putative ages (ranging from 1-17 years) may be obtained from spines taken randomly across the surface of individual starfish (Souter et al.1997).

Initial studies of post-settlement growth of *Acanthaster planci* revealed marked changes in growth rates at different life-stages (e.g., Lucas 1984). The growth pattern is sigmoid (e.g., Lucas 1984; Stump 1996), with slow growth both when starfish first settle and feed on calcareous algae and when starfish attain sexual maturity at approximately 2+ years of age. However, when considering only the 1+ individuals, growth can be described effectively using von Bertalanffy growth functions (**Figure 2.5**), where the L_∞ (asymptotic size) is between 474 mm based on data from Stump (1996), and 580 mm based on data from Pan (2010). By combining size at age data across all previous studies, including laboratory-based measurements of Lucas (1984), it is apparent that there are distinct differences in the reported size (diameter) of starfish in consecutive age classes: 30-100mm for starfish with an estimated age between 1and 2 years, 100-250mm for 2-3 year old starfish, 250-300mm for 3-4years, 300-400 for 4-5 years, and >380 for 5+ years (**Figure 2.5**). Increased research and further validation of growth relationships in different locations (especially outside of the Pacific) may be needed in order to provide increased resolution of ages, necessary to establish the precise timing for the initiation of different outbreaks. However, it is generally accepted that wide ranges in the size of individuals, especially within a single populations, reflect

marked differences in ages, rather than extreme variation in growth of starfish from the same cohort (Stump 1996).

Lack of an effective method of tagging individuals has greatly impeded field studies of the demography of *Acanthaster* spp. (Glynn 1982). Early attempts to tag starfish involved embedded tags, but starfish quickly ejected tags (within weeks) or dropped the arm to which tags were attached. Some tags also lead to high rates of infection, or greatly modified individual behavior, undermining efforts to document 'natural' rates of growth and survivorship (Moran 1986). Later tagging efforts avoided damaging the dermal tissues, instead attaching harnesses around the oral disk, or coloured bands to individual spines (e.g., Keesing and Lucas 1992), but again tags had a limited lifespan. Stump (1996) marked *Acanthaster planci* using tetracycline injections to stain their spines. In combination with counts of arms, and records of the position of madreporites this could be used to confirm the identity of individual starfish. However, this technique did not allow easy recognition of individuals in the field. In 2012, Rivera-Posada (unpublished data) used loops of relatively inert nylon monofilament that were passed through pre-drilled holes in the skeletal elements of the aboral disk and between the ambulacral ridge of the arms. Holes were drilled with Kirschner wires at low speed to avoid thermal osteonecrosis or fracture that could lead to a rapid ejection of tags. Needles and cardio catheters were then used to pass the nylon through the arm and skeletal elements, securing the two ends with small brass connector sleeves. These tags last at least 4 weeks in aquaria, but are yet to be tested in the field. By threading coloured plastic beads onto the nylon loop it will be possible to identify a large number of tagged *Acanthaster* spp., with wide application in studies of demography and individual behavior (e.g., movement).

2.2.7 Feeding behavior

The crown-of-thorns starfish is just one of many different coral-reef organisms that feed on scleractinian corals (Glynn 1988; Cole et al. 2008; Rotjan and Lewis 2008). However, the capacity of *Acanthaster* spp. to deplete local cover of scleractinian corals is far greater than for any other corallivorous species (Glynn 1988; Birkeland 1996a; Carpenter 1996). Most corallivores are limited in their rate of feeding because scleractinian corals have only a very thin veneer of living tissue over the surface of an indigestible calcareous skeleton (Keesing 1990). As a consequence, corallivores must selectively pick live tissues from the surface of corals (which tends to limit the rate of feeding), or else ingest large quantities of calcium carbonate, which is energetically costly (Motta 1988). In contrast, *Acanthaster* spp. (and other corallivorous starfish) are extremely well adapted to feed on scleractinian corals as they can digest tissue from a large area of coral surface at once. These asteroids feed by everting their stomach through their oral opening and spreading it over the surface of live corals or any other benthic prey (Jangoux 1982). Enzymes are then secreted through the gastric tissues that digest coral tissues within 3-5 hours (Goreau 1964; Brauer et al. 1970). *Acanthaster planci* has a much larger stomach for its size than other corallivorous asteroids (e.g., *Culcita novaeguineae*), enabling it to consume scleractinian corals 2-5 times faster than other starfish of equivalent size (Birkeland 1989a). Moreover, Benson (1975) suggested that *Acanthaster* spp. possess the most specialised enzyme system for digestion of wax esters, which are a major component of coral tissues. Even so, crown-of-thorns starfish consume a maximum of 150-250 cm^2 of live coral per day, depending on their body size (Chesher 1969; Glynn 1973).

A key facet of the feeding behavior of *Acanthaster* spp., which has a major bearing on their ecological impact, is their feeding preferences (Moran 1986). Moran

(1986) identified many factors that influence feeding preferences of *Acanthaster* spp., including 1) the nutritional content and growth form of corals, 2) coral defences (e.g., mesenterial filaments, nematocysts and secondary metabolites), 3) coral defence by commensal infauna (mostly, trapezid crabs), 4) the distribution of corals, 5) local environmental conditions, and 6) prior conditioning of individual starfish (see also Birkeland and Lucas 1990). This combination of different factors was expected to lead to complex patterns of feeding preferences, that vary with geographical variation in the composition of coral assemblages (Birkeland and Lucas 1990) and with size and abundance of the starfish (Moran 1986). To test this, I compiled published data on the proportional consumption of different coral genera by *Acanthaster* spp., relative to their availability at different locations throughout the Pacific (**Figure 2.6**). These data do not represent feeding preferences *per se*, because they do not strictly assess the selection of one prey type over another (e.g., Keesing 1990; De'ath and Moran 1998b; Pratchett 2007). Moreover, the relative consumption of different coral genera is generally inferred from changes in the abundance or mortality of different coral genera during outbreaks, though many factors (e.g., routine coral mortality, coral disease, predation by other corallivores, and/or bleaching) may have contributed to coral loss (e.g., Pratchett et al. 2009a). However, studies that directly compared mortality rates between sites with and without outbreak densities of *A. planci* (e.g., Pratchett et al. 2009a; Pratchett 2010) found that coral mortality was negligible in sites where *A. planci* were rare or had only recently been reported.

Variation in forage ratios, averaged over seven studies (**Figure 2.6**) and six different geographical locations across the Pacific (Uva Island, eastern Pacific, Glynn 1974; Hawaii, Chess et al. 1997; French Polynesia, Bouchon 1985; Papua New Guinea, Pratchett et al. 2009a; GBR, Pratchett 2010; Indonesia, Baird et al. 2013), show that

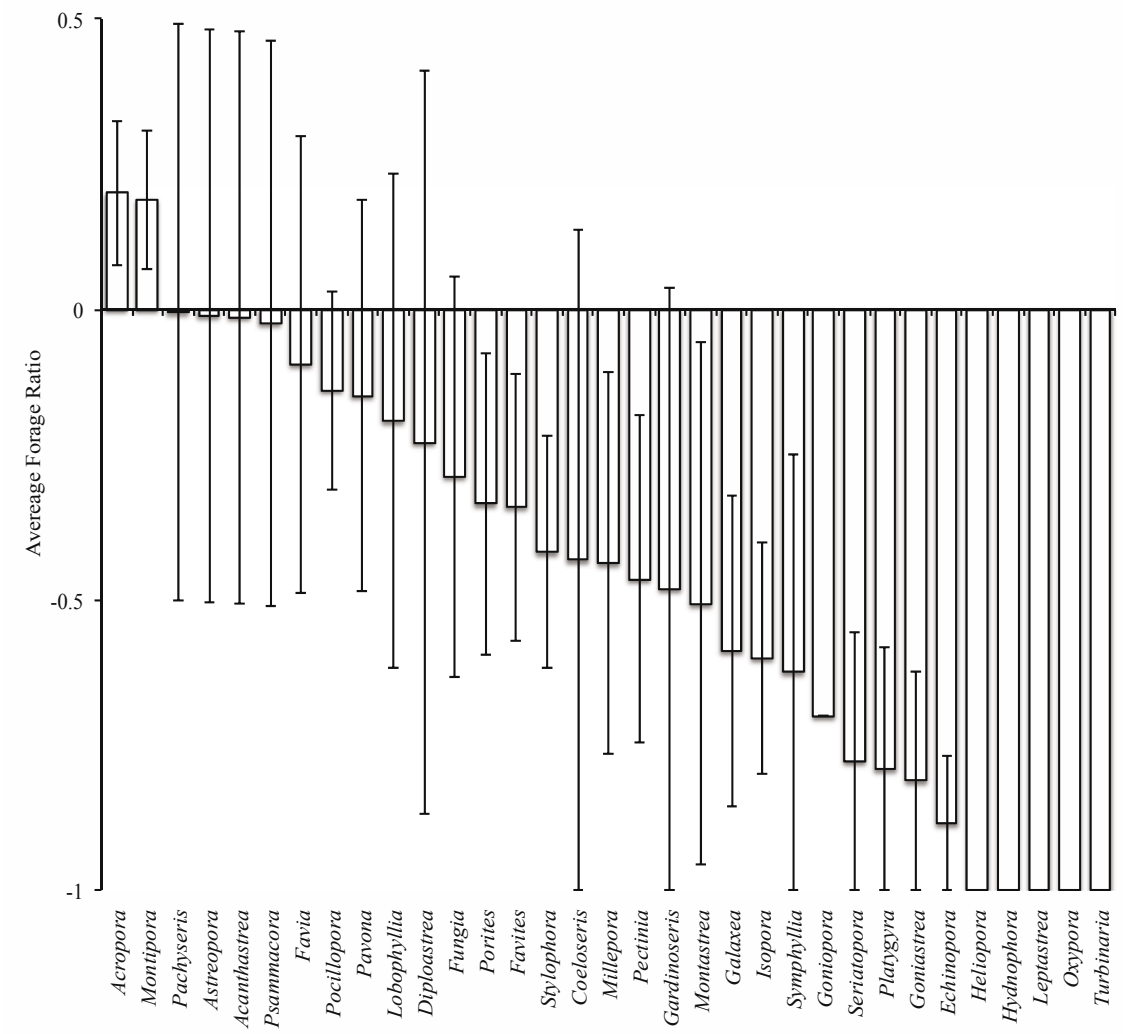

Figure 2.6 Variation in forage ratios (averaged across seven studies in the Pacific) for different coral genera, indicative of general feeding preferences. Forage ratios compare proportional consumption of coral genera to their relative abundance in the local area, following the work of Tokeshi & Daud (2011). Data sources: Glynn 1974, Bouchon 1985, Keesing 1992, Chess et al. 1997, Pratchett et al. 2009a, Pratchett 2010, Baird et al. 2013.

Acropora and *Montipora* are the most preferred coral genera. These two coral genera were consistently consumed in greater proportions than would be expected from their availability. At the other extreme, there were four genera of scleractinian corals (*Hydonophora*, *Leptastrea*, *Oxypora*, and *Turbinaria*) as well as *Heliopora*, which did not decline in abundance during outbreaks of *A. planci*, presumably because they are non-preferred coral prey. Average forage ratios for virtually all other corals (all except *Acropora* and *Montipora*) were negative, though there was variation in apparent preference for individual coral genera among studies and locations (**Figure 2.6**). Reported forage ratios for *Pachyseris, Astreopora, Acanthastrea, Psammocora*, *Lobophyllia*, and *Diploastrea* ranged from -1 to close to 1, indicating that abundance did not change during outbreaks of *Acanthaster planci* at some locations, while there was extensive depletion at other locations. For example, there was a 63% decline in abundance of *Acanthastrea* during an outbreak of *A. planci* at Moorea, French Polynesia, in 1980 (Bouchon 1985), but there was no apparent change in abundance of *Acanthastrea* during a recent outbreak of *A. planci* in Sumatra, Indonesia (Baird et al. 2013).

Despite marked variation in forage ratios for some coral genera, the rank order of coral genera revealed by comparing average forage ratios are in accord with more detailed studies of feeding preferences conducted under laboratory conditions (e.g., Brauer et al. 1970; Collins 1975; Ormond et al. 1976; Pratchett 2007), and field-based studies of specific feeding behavior (De'ath and Moran 1998b). Prior studies on the feeding preferences of *Acanthaster* spp. have mostly compared among *Acropora*, *Pocillopora* and *Porites*, showing that *Acropora* is most preferred and *Porites* is least preferred (reviewed by Moran 1986). Explanations for these overarching feeding preferences (e.g., nutritional content, chemical deterrents, or colony defence) are

lacking, and they are likely to vary in their importance depending on the coral taxa (Pratchett 2007). Comparing *Acropora* spp. with *Pocillopora* spp. (and other Pocilloporidae), Pratchett (2001) showed that differential feeding preferences were largely attributable to differences in the effectiveness with which commensal infauna (and especially trapezid crabs) defend their coral hosts (see also Glynn 1987). Notably, *A. planci* did not distinguish between *Acropora* spp. and pocilloporid corals when commensals were removed from all corals (Pratchett 2001). *Porites* spp. also contain symbiotic organisms (*Pedum spondyloideum* and *Spirobranchus giganteus*); rather than preventing *A. planci* from eating their host colony, these organisms enhance the survivorship of a few adjacent coral polyps which may enable subsequent regeneration of the colony (e.g., DeVantier and Endean 1988). The avoidance of *Porites* spp. by *Acanthaster* spp. is generally ascribed to a combination of low nutritional value, chemical deterrents to feeding (De'ath and Moran 1998a), and also the inability of starfish to attach to large, smooth colonies, leading to high probability of dislodgement except in very calm conditions.

The relative preference of *Acanthaster* spp. for certain corals (e.g., *Acropora* spp.) could result in shifts in the structure of coral assemblages during outbreaks. However, even the least preferred corals are consumed during extremely severe outbreaks, or when coral prey is scarce (e.g., Chesher 1969; Pearson and Endean 1969). This is particularly apparent when comparing effects of outbreaks of *A. planci* at Moorea in 1980-81 (Bouchon 1985) with those of subsequent outbreaks in 2007-2012 (Kayal et al. 2012). In 1980-81, Bouchon (1985) recorded proportional declines in the abundance of the major coral genera, *Acropora*, *Pocillopora, Porites*, of 45.5%, 0% and 0%, respectively. In contrast, Kayal et al. (2012) reported comprehensive extirpation of *Acropora* spp. and *Pocillopora* spp., and greater than 95% decline in local cover of

Porites spp. Kayal et al. (2012) did report that there was sequential depletion of these major genera, with *Acropora* removed first, then *Pocillopora*, and then *Porites*. Clearly, selective effects of *A. planci* are most apparent during outbreaks that cause only moderate loss of corals (Birkeland and Lucas 1990). The relative consumption of different coral genera also appears to be very different for low-density (non-outbreak) populations of *Acanthaster* spp. (e.g., Tokeshi and Daud 2011), where feeding preferences appear to be largely dictated by the proximity of corals to appropriate shelter.

2.3 Outbreaks of crown-of-thorns starfish

Extreme variability in adult abundance is very common among marine organisms, particularly those with planktonic larvae (e.g., Roughgarden et al. 1988). However, few marine organisms show changes in abundance of the magnitude, or rate, shown by crown-of-thorns starfish. The abundance of *Acanthaster* spp. can increase by as much as six orders of magnitude within one to two years (reviewed by Birkeland and Lucas 1990). At Tutuila Island, American Samoa, the overall abundance of *A. planci* increased from 1-2 starfish in 1976 to more than 200,000 starfish in late 1977 (Birkeland and Randall 1979). Similarly, at Tanguisson Reef, Guam, densities of *A. planci* increased from less than 0.1 starfish.ha^{-1}, to more than 1,000 starfish.ha^{-1} during the course of 1967 (Chesher 1969). More recently, Kayal et al. (2012) reported maximum densities of 151,650 starfish per km^2 along the northern barrier reef of Moorea, French Polynesia, with densities increasing more than 10-fold over the course of just one year. The combined feeding activities of high densities of crown-of-thorns starfish also cause extensive coral depletion (e.g., Chesher 1969; Kayal et al. 2012), leading to widespread

concern that outbreaks of *Acanthaster* spp. are becoming more frequent and more prevalent across the Indo-Pacific (e.g., Brodie et al. 2005).

Despite extensive and increasing reports of "outbreaks" of *Acanthaster* spp. across the Indo-Pacific, there are major inconsistencies and deficiencies in published reports that constrain rigorous and comprehensive analyses of the geographical extent and recurrence of outbreaks. These problems are partly due to inherent complexities in defining and comparing the extent and severity of outbreaks among different locations. On the GBR, for example, outbreaks are reported at the scale of the entire reef system (Sweatman et al. 2008), recognising inherent coupling of outbreaks among the well-connected reefs that make up this vast reef system (Reichelt et al. 1990a; Moran et al. 1992). Elsewhere, however, outbreaks are often reported from individual locations, let alone individual reefs (e.g., Koonjul et al. 2003). The incidence of outbreaks of crown-of-thorns starfish on small, isolated, or unpopulated reefs is important for understanding the potential causes of (and anthropogenic contribution to) outbreaks of *Acanthaster* spp., but there are likely to be many different factors that influence the initiation and impacts of outbreaks, which are rarely considered in published reports of localized outbreaks. In the Maldives, for example, outbreaks of *Acanthaster planci* were reported at many different atolls in the early 1990s (Ciarapica and Passeri 1993), which runs counter to Birkeland's (1982) assertions that outbreaks really only occur on high islands (discussed later). However, no attempt was made to quantify temporal and spatial patterns in starfish densities, nor assess the population structure of localized outbreaks, to understand how these outbreaks were initiated and/ or spread among nearby atolls.

2.3.1 Defining outbreaks of crown-of-thorns starfish

While there are conspicuous differences in the densities of *Acanthaster* spp. between outbreaking and non-outbreaking populations, rigorous definitions of outbreaks are elusive. Potts (1981) defined outbreaks of crown-of-thorns starfish as "any large aggregation of many hundreds or thousands of individuals which persist at high densities for months or years and causes extensive mortality among coral over large areas of reef" (Potts 1981, pg 65). This definition encompasses aspects of both the biological (pronounced and unexplained increases in the abundance of a species) and ecological definition (rapid increases in the abundance of a species beyond that which can be sustained by local resources) of population outbreaks, but these qualitative definitions are not particularly useful when attempting to rigorously account for the incidence and occurrence of outbreaks. Normal, background densities of *Acanthaster planci* may be spatially variable (e.g., Glynn 1990), and so it is important to distinguish between periodic outbreaks, versus highly localised and chance aggregations of starfish from populations with persistent moderate densities (Moore 1990).

Moran and De'ath (1992) proposed an operational definition of 1,500 starfish.km^{-2} for outbreaks of *Acanthaster planci* on Australia's GBR by estimating the actual densities of starfish (after accounting for sampling bias) that exceed sustainable limits (**Table 2.3**). Substantial coral mortality was only observed at reefs with >1,500 starfish.km^{-2} (equivalent to 15 starfish.ha^{-1} or 0.22 starfish per 2-minute manta tow), suggesting that this is the maximum density of starfish that can be sustained by reefs with average coral cover (Moran and De'ath 1992). Similar estimates of the maximum sustainable density of *A. planci* (1,000 starfish.km^{-2}) were obtained by relating feeding rates of starfish to the average annual turnover in well-established coral assemblages (Keesing 1990). It was recognized, however, that the density of *Acanthaster* spp. that

Table 2.3 Operational criteria used to distinguish between outbreak and non-outbreaking (normal) densities of *Acanthaster* spp.

Minimum threshold	Reference
30-40 starfish per km^2	Clark and Weitzman 2008
14 starfish per $1000m^2$	Endean and Stablum 1975
40 starfish per 20 min swim	Pearson and Endean 1969
100 starfish per 20 min swim or manta tow	Chesher 1969
10 starfish per 1 min spot check	Pearson and Garrett 1978
30-50 starfish in 20 minutes	Faurea 1989
1 starfish per 24 m^2	Pearson and Endean 1969
100 starfish per hectare	Dana et al.1972
260 starfish per hectare	Glynn 1973
150 feeding scars per 250 m^2	Lison de Loma et al. 2006

can be sustained on any given reef will vary enormously in time and space, depending upon the abundance and composition of scleractinian corals, as well as the size and distribution of starfish. Keesing and Lucas (1992) estimated that densities of 10-15 starfish per hectare could be sustained in areas with >20% coral cover. However, sustained declines in coral cover on reefs across the Pacific (Bruno and Selig 2007) mean that outbreaks of *Acanthaster* spp. that could once be sustained, would now contribute to accelerating coral loss. On Australia's GBR, for example, average coral cover has declined from 28.0% in 1982 to 13.8% in 2012, due in large part to outbreaks of *Acanthaster planci* (De'ath et al. 2012). Sustained declines in mean coral cover could also increase vulnerability to current and future outbreaks.

While density of *Acanthaster* spp. provides the most relevant measure to compare the extent and severity of outbreaks (Moran 1986), it is not easy to obtain precise estimates of starfish abundance. Notably, density estimates for *Acanthaster* spp. depend greatly on the temporal and spatial scales of sampling (Endean and Cameron 1990). Even during outbreaks, the density of starfish varies within and among reefs, among habitats, and may also vary on very short timescales within any given location. Changes in the behavior of starfish after they attain adult size (switching from generally cryptic and nocturnal, to diurnally active) also significantly affects the ease with which starfish can be surveyed, and the resulting estimates of starfish densities (Endean and Cameron 1990). Outbreak populations of *Acanthaster* spp. tend to be highly aggregated, such that maximum recorded densities may be very large, whereas there are very few or no starfish at nearby locations (e.g., Pratchett 2005). It is possible therefore, that densities of starfish on just one or a few transects may exceed threshold densities indicative of an outbreak (e.g., 1,500 starfish per km^2; Moran and De'ath 1992), while densities averaged across the entire reef or reef system remain well below these levels. For this

reason, some monitoring studies distinguish between 'spot' versus 'reef-wide' outbreaks (Engelhardt 1999), or 'incipient' versus 'active' outbreaks (e.g., Sweatman et al. 2008). Moran (1986) called for standardized methods for quantifying the abundance of *Acanthaster* spp., recommending repeated and intensive surveys of starfish at individual reefs, using area-based surveys to quantify densities, combined with measurements of maximum diameter, to clearly establish the fine-scale temporal and spatial dynamics of outbreak populations. The few studies that have conducted intensive sampling at a single reef throughout the course of an outbreak (e.g., Pratchett 2005; Kayal et al. 2012) have provided significant new insights into the causes and consequences of outbreaks of *Acanthaster* spp.

2.3.2 Primary versus secondary outbreaks

Outbreaks of *Acanthaster* spp. are thought to arise in two different ways (e.g., Potts1981; Johnson 1992), which will be reflected in marked differences in population structure (**Figure 2.7**). In many cases, outbreak populations comprise individuals of similar size (<150mm variation between the smallest versus largest individuals) with a unimodal size structure (**Figure 2.7a**) suggestive of a single massive influx of new recruits (Birkeland and Lucas 1990). These outbreak populations generally comprise only a single cohort or year class (e.g., Dana et al. 1972; Glynn 1973; Zann et al. 1987) and appear as dramatic increases in the abundance of starfish within weeks to months (e.g. Chesher 1969; Branham et al. 1971). However, there are many cases where outbreak populations include individuals of a great range of sizes with a multi-modal size structure (**Figure 2.7b**). In some instances, outbreak populations have been shown to include starfish from at least 5-6 different cohorts (e.g., Stump 1996; Engelhardt et al. 1999; Pratchett 2010), and there has been a gradual increase in the density of

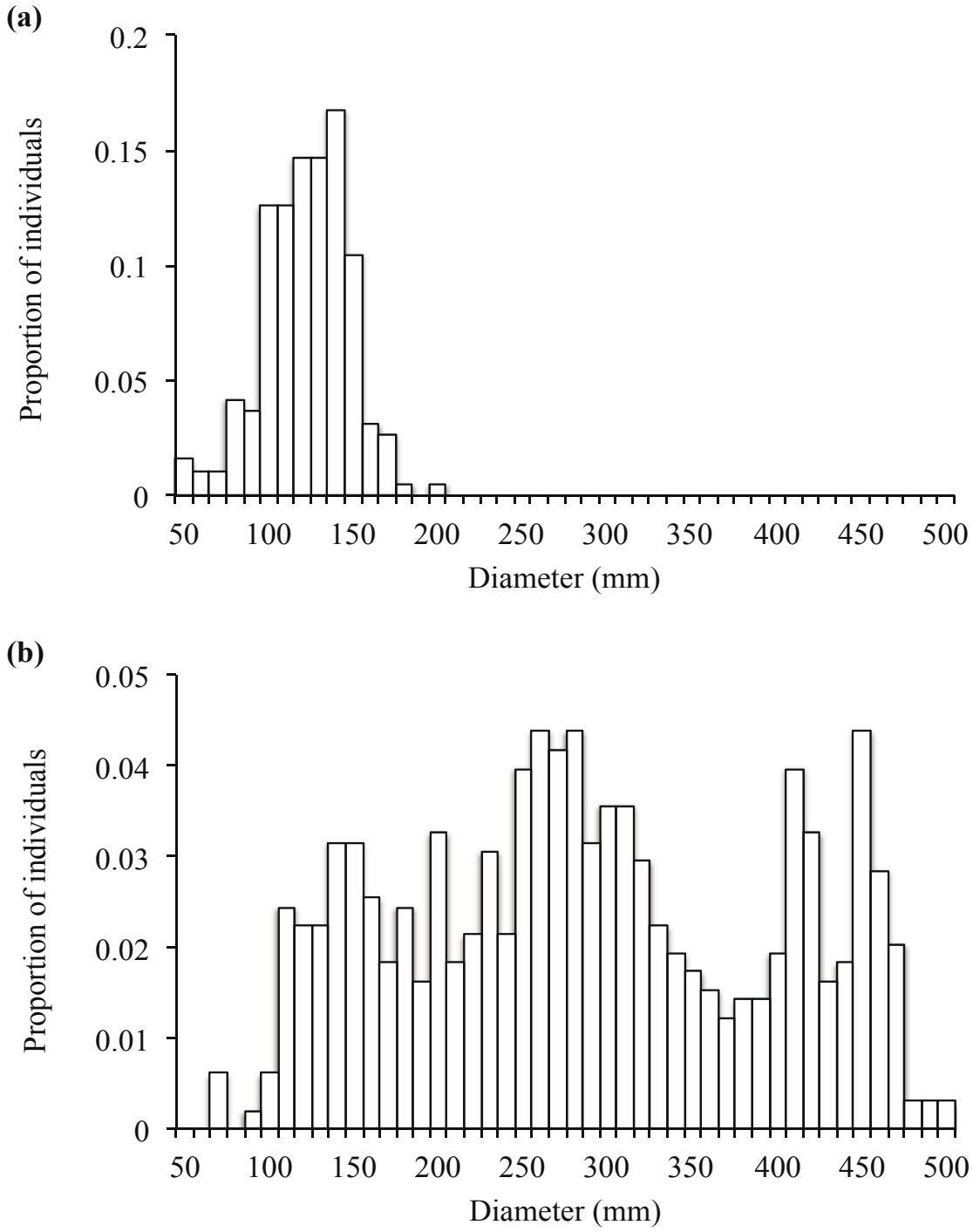

Figure 2.7 Contrasting size structure of outbreak populations of *Acanthaster planci* in (**a**) September 1985, at Suva, Fiji (Zann et al. 1987), and (**b**) July 1995 at Lizard Island, on Australia's Great Barrier Reef (Stump 1996). In Fiji, the entire population was comprised of starfish ranging in size by <150 mm (n = 158), dominated by a single year class, and indicative of a single mass-recrutitment (i.e. 'secondary outbreak'; Endean 1977). At Lizard Island, the population comprised individuals ranging in size by >430 mm (n = 317) and multiple age classes, suggestive of gradual accumulation of starfish of several years (i.e. 'primary outbreak'; Endean 1977).

Acanthaster spp. over many years, indicating the progressive accumulation of individuals over several successive recruitment events (e.g., Zann et al. 1987, 1990; Stump 1996; Pratchett 2005).

Factors which contribute to instantaneous increases in the recruitment of *Acanthaster* spp. (e.g., increased survivorship of starfish larvae; Lucas 1973; Birkeland 1982), leading to rapid and dramatic increases in starfish densities, are likely to be very different from those which cause slow, progressive increases in starfish densities (Johnson 1992). Importantly, the sustained and gradual accumulation of crown-of-thorns starfish from multiple successive recruitment events may represent a mechanism by which outbreaks are initiated ('primary outbreaks', Endean 1974; Johnson 1992; Stump 1996), which then give rise to massive numbers offspring causing subsequent outbreaks on nearby and downstream reefs ('secondary outbreaks', Endean 1974) and cause massive devastation over very large areas (Reichelt et al. 1990a). Birkeland and Lucas (1990) suggested that gradual increases in the densities of *Acanthaster* spp. over several years (regardless of final densities) do not really fit with the definition of an outbreak, arguing that 'outbreaks' must arise suddenly. The distinction between primary versus secondary outbreaks may, however, be fundamental to understanding the initiation of outbreaks of *Acanthaster* spp. (e.g., Pratchett 2005; Fabricius et al. 2010), as distinct from waves of outbreaks that are a predictable consequence of large, established breeding populations.

Endean's (1974) distinction between primary and secondary outbreaks, while logical, is not easy to apply because ecologists rarely have sufficient information to identify the sources of outbreaks with certainty, so the identification of primary outbreaks is largely conjectural. Intensive monitoring of *Acanthaster planci* at Lizard Island, in the northern GBR, during the 1990s provided a good example of a primary

outbreak, where the number of starfish increased very gradually until reaching outbreak densities in 1996 (Sweatman et al. 1998; Pratchett 2005). Moreover, at peak densities of crown-of-thorns starfish, the local population comprised of individuals ranging in size from 110 to 620 mm, and there was evidence of repeated annual recruitment from 1992 to 1997 (Pratchett 2005). The source of recruits settling at Lizard Island is not known, but they may represent the progeny of the adult starfish that were already present on reefs around Lizard Island (albeit in very low densities) prior to the outbreak. With high levels of self-recruitment, these outbreaks may have resulted from incremental increases in the reproductive output of the initial reproductive population and all of their subsequent progeny, leading to exponential growth in population size. Detailed studies of this outbreak (e.g., Stump 1996; Pratchett 2005, 2010) did not provide any real clues as to the cause(s). However, given the gradual accumulation of starfish over more than 5 years, any factor responsible for the initial onset of outbreaks is likely to be very subtle and difficult to detect.

Secondary outbreaks of *Acanthaster* spp. are known mainly from the GBR and southern Japan (Potts 1981), where waves of outbreaks spread among well-connected reef systems after the initiation of one or more primary outbreaks (e.g., Kenchington 1977; Yasuda et al. 2009). However, outbreak populations with highly constrained size structure, characteristic of secondary outbreaks, have also been reported at many small and isolated reef systems throughout the Pacific (e.g., Birkeland 1982) suggesting that the extensive dispersal of larvae from primary outbreaks may initiate successive outbreaks throughout the Pacific. To evaluate this, Timmers et al. (2012) explored genetic structure and connectivity among outbreak populations of *Acanthaster planci* at 23 sites across the Pacific Ocean, including the north-western (e.g., Guam), north central (e.g., Hawaii), and south central Pacific region (French Polynesia). Strong

regional, archipelagic, and inter-reef genetic structuring of *A. planci* populations indicate that larval dispersal is highly constrained, and that high densities of larvae do not spread across open ocean expanses to initiate secondary outbreaks at distant reefs (see also Vogler et al. 2013). These data suggest that primary outbreaks may have gone undetected within many small archipelagos, or that in some circumstances, primary outbreaks may result from a single massive influx of larvae (Birkeland 1982). Recent advances in genetic research, including a now extensive library of microsatellite markers specific for *Acanthaster planci* (Yasuda et al. 2006b, 2007; Wainwright et al. 2012) provide greatly improved opportunities to study the initiation (e.g., source populations) and subsequent spread of outbreaks. As such, future studies of outbreak populations should not only record the density of *Acanthaster* spp., but also measure maximum diameter and obtain genetic samples (6-10 tube feet placed in 95% ethanol) for a representative sample of individual starfish.

2.3.3 Geographical incidence of recent outbreaks (1990-2013)

Since 1990, at least 246 outbreaks of *Acanthaster* spp. have been reported from across the Indo-west Pacific (**Figure 2.8**), which is three times the number of outbreaks reported (82) prior to 1990 (e.g., Moran 1986; Birkeland and Lucas 1990). Though mostly qualitative, these recent reports represent outbreaks or infestations that are distinct in space and time, at either a particular group of reefs (mostly at the scale of small island nations in the Pacific) or discrete episodes of outbreaks within a given geographic location (e.g., outbreaks of *Acanthaster planci* were reported in the Society Islands, in French Polynesia in 1969-1970, 1979-1982, and 2006-2009). This apparent increase in the number of reported outbreaks may or may not indicate a real increase in the spatial and/or temporal incidence of outbreaks; it is surely attributable in part to an

increase in surveys and monitoring of reef environments, combined with increased awareness of *Acanthaster* spp. in this more recent period (see also Baird et al. 2013). One of the key arguments put forward as evidence of the role of humans in causing or exacerbating outbreaks of crown-of-thorns starfish is that the incidence of devastating outbreaks has increased in recent times (e.g., Brodie et al. 2005). However, in locations where there have been recurrent outbreaks since the 1960s (e.g., GBR, Japan, and Society Islands), there is no evidence of increased frequency of outbreaks (or more specifically, a decrease in the period between successive outbreaks). On the GBR, it was 17 years between the start of the first recorded outbreak (1969) and the emergence of the second major outbreak (1979), and it was the same period (17 years) between the start of the third (1993) and start of the most recent, fourth major outbreak (2010). In French Polynesia, it was only 10 years between the first two reported outbreaks (1969-1970 versus 1979-1982), but then 27 years until the devastating outbreak that occurred in 2006-2009 (Kayal et al. 2012). Similarly, in Japan it was almost 20 years between the most recent wave of outbreaks and previous high frequency, almost chronic outbreaks that occurred throughout the 1960s and early 1970s (Yasuda et al. 2009). If anything, the period between successive outbreaks is getting longer, as might be predicted if the initiation of renewed outbreaks requires substantial recovery of coral prey (e.g., Bradbury et al. 1985; Bradbury and Antonelli 1990; Fabricius et al. 2010).

Global degradation of coral reef ecosystems, especially sustained declines in the abundance or cover of scleractinian corals, is widely attributed to the increasing frequency of acute disturbances (e.g., Seymour and Bradbury 1999; Fabricius et al. 2010), such that major disturbances occur too often to allow effective (though not necessarily complete) recovery of coral assemblages between successive disturbances. Moreover, the time required for coral assemblages to recover from periodic

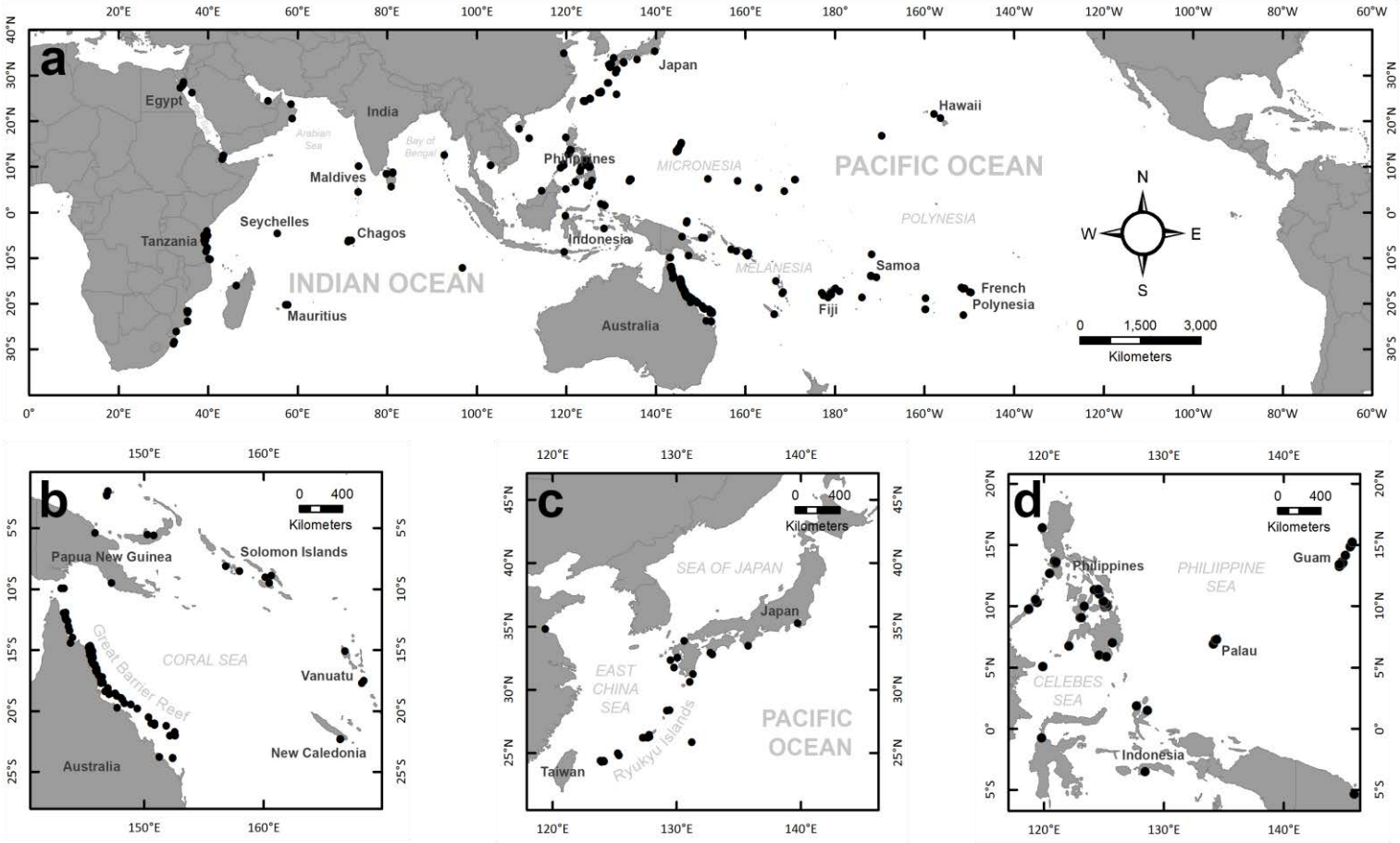

Figure 2.8 Geographical spread of reported outbreaks of *Acanthaster* spp.: (**a**) global distribution; (**b**) Great Barrier Reef; (**c**) southern Japan; (**d**) Coral Triangle and western Pacific Ocean. Each dot indicates a specific reef where outbreaks of crown-of-thorns starfish have been reported since 1990. No account is made of the extent, severity or recurrence of outbreaks at each location.

disturbances may be increasing (Seymour and Bradbury 1999). The minimum interval between outbreaks of *Acanthaster* spp. at individual reefs will be set by local recovery of coral assemblages (Bradbury et al. 1985; Fabricius et al. 2010), since very low levels of coral cover would prevent outbreaks from becoming established. This implies that the overall incidence of outbreaks of *Acanthaster* spp. should decline with sustained and increasing degradation of coral reef ecosystems. In particular, there should be longer periods between successive outbreaks on individual reefs. Alternatively, limited abundance of coral prey may constrain the overall abundance or persistence of *Acanthaster* spp. while outbreaks continue to occur at equivalent or increasing frequency (Fabricius et al. 2010) or may even become chronic (e.g., Zann et al. 1990; Mendonça et al. 2010), thereby causing accelerated degradation of reef ecosystems. The current wave outbreaks on the GBR provides a good opportunity to test whether outbreaks of *Acanthaster planci* can become established on reefs with very low coral cover, as many reefs in the central GBR were impacted by severe tropical storm (category 5 Cyclone Yasi) that tracked across the GBR in early 2011 (e.g., Lukoschek et al. 2013). Coral cover remains at or below 5% on many reef exposed to this disturbance, and preferred coral prey of *Acansthaster* spp. (e.g., *Acropora*) are particularly scarce (e.g., Lukoschek et al. 2013).

Despite increases in the overall number of reported outbreaks, the geographical distribution of outbreaks reported in 1990-2012 is fairly consistent with the record prior to 1990 (e.g., Moran 1986; Birkeland and Lucas 1990). Most notably, the vast majority (167/ 246) of outbreaks of *Acanthaster* spp. are reported from the Pacific Ocean (**Figure 2.9**), and mainly from the west Pacific, including Australia's GBR, southern Japan, Micronesia, and Melanesia. There has been a slight increase in the proportion of outbreaks reported from the Indian Ocean, increasing from 11.0% in 1956-1989 to

15.4% in 1990-2012, with unprecedented outbreaks of *Acanthaster* spp. recorded in some isolated locations, such as Chagos (R. Roche, Unpublished data). In most locations, outbreaks have now been reported at least twice, and up to four times, since the 1960s within the same area, if not the same reef, showing that outbreaks of *Acanthaster* spp. are not rare, singular events. The greatest concentrations of outbreaks have occurred within extensive reef systems of the GBR, southern Japan, and also within the Philippines (**Figure 2.8**). However, there have also been recent outbreaks on many small, isolated and relatively unpopulated reef systems.

Analyses of all reported outbreaks since 1990 show that the majority of outbreaks (139 out of 246) have occurred on arrays of platform reefs along continental shelves, such as the GBR. A further 72 (56%) outbreaks have been reported on high islands, such as Moorea in French Polynesia. The remaining outbreaks (35 out of 246) have been reported from low-lying islands, atolls or completely submerged reef platforms. It is likely that the causes, especially anthropogenic contributions to the initiation of outbreaks, are likely to vary among these different reef systems (Birkeland 1982). Notably, continental and high island reefs will be much more subject to terrestrial runoff (Birkeland 1982), which is one possible cause of outbreaks (see *Nutrient Enrichment Hypothesis*). The flip side to this is that human populations on low-lying islands and atolls have reduced capacity for agriculture and are much more reliant on coral reef fisheries (McClanahan et al. 2002), and excessive exploitation of predatory fishes has also been linked to increased severity of outbreaks of *A. planci* (Dulvy et al. 2004). In order to assess whether outbreaks occur more or less than expected on different types of reefs, the relative frequency of outbreaks need to be compared to the proportional area of continental and high island reef versus low lying atolls and submerged reefs, but such data are not readily available.

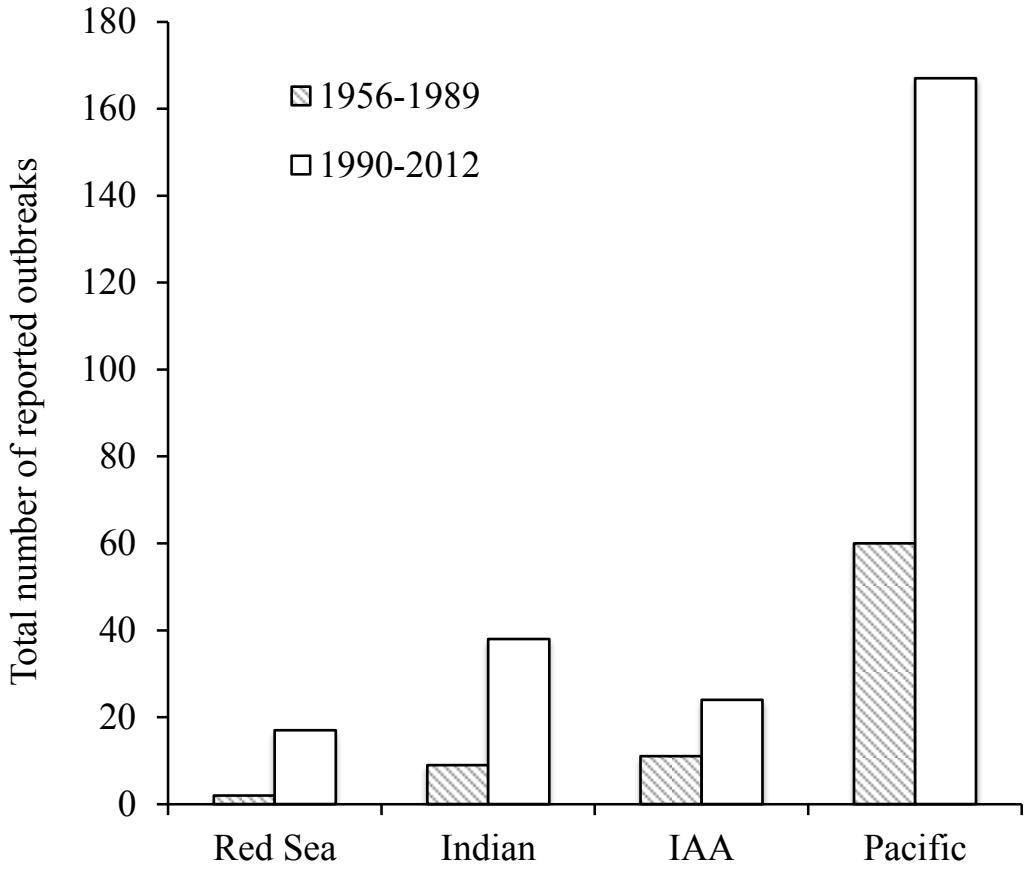

Figure 2.9 Number of reported outbreaks in different regions of Indo-Pacific (Red Sea, Indian Ocean, Indo-Australia Archipeligo, and Pacific Ocean) pre-1990 versus post-1990. No account is made of the extent, severity or recurrence of outbreaks at each location.

2.3.4 Finescale patterns of outbreaks

Australia's Great Barrier Reef - The Great Barrier Reef, extending 2,000 km along Australia's east coast is the world's largest reef system and has the dubious distinction of supporting both the most extensive and greatest frequency of outbreaks of *Acanthaster* spp. The first documented outbreak was detected in 1962 at Green Island (Pearson and Endean 1969), though high densities of starfish were reported on other GBR reefs in the 1950s and earlier (Vine 1973; Ganter 1987). Since 1962, there have been three additional outbreak episodes on the GBR, commencing in 1979, 1993, and 2010. The initiation and progression of the first two outbreaks, based on extensive but largely uncoordinated sampling across the GBR, was discussed at length by Moran (1986). The publication of Moran's review (Moran 1986) roughly coincided with the advent of systematic monitoring on the GBR to document patterns of outbreaks of *Acanthaster planci* in time and space (e.g., Sweatman et al. 2008). When monitoring began in 1985 there were active outbreaks on many reefs near Townsville (~18.5 ^{o}S) and also in the Swain reefs well offshore from Gladstone (~22 ^{o}S) (**Figure 2.10**). Subsequent monitoring documented the progressive spread in the southward extent of active outbreaks (**Figure 2.10**), consistent with patterns reported in previous outbreaks (Reichelt et al. 1990a). However, outbreaks in the Swains region (southern GBR) appear to occur independently of the wave of outbreaks that affect reefs from Cooktown to Mackay (www.aims.gov.au/docs/research/biodiversity-ecology/threats/cots-animation.html).

The initiation and spread of outbreaks of *Acanthaster planci* on the GBR has been fairly consistent in all four recorded outbreaks, including the current outbreak that started in 2010. Each of the outbreaks appears to have been initiated on mid-shelf reefs in northern central region of the GBR (the 'initiation box') between Lizard Island

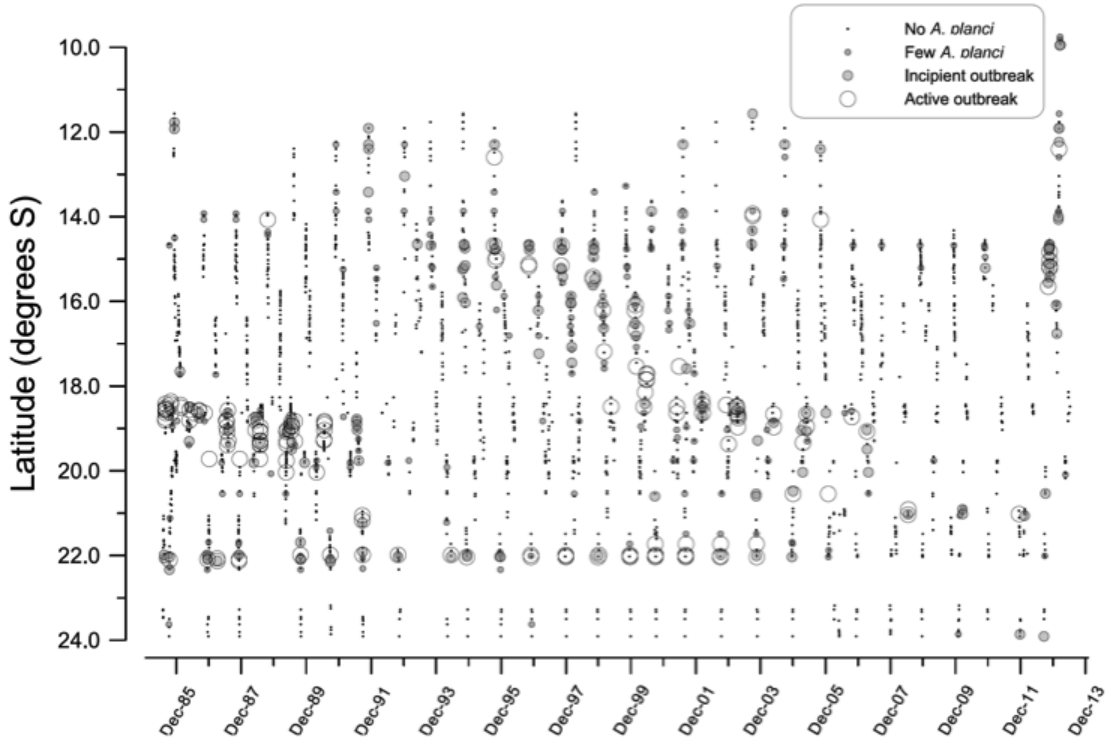

Figure 2.10 Occurrence of *Acanthaster planci* on reefs of the Great Barrier Reef (arranged by latitude) between 1985 and 2013, based on manta tow surveys of reef perimeters. Note that the same reef may be represented in several years. Reefs are divided into four categories: dots indicate reefs with very low densities of *A. planci* (≤3 per reef); smaller grey symbols indicate reefs with >3 starfish but less than 'Incipient' outbreak densities (mean ≥0.22 *A. planci* per 2-minute tow); larger grey symbols indicate reefs with 'Incipient' outbreak densities of *A. planci* (mean ≥0.22 *A. planci* per tow); large unfilled symbols indicate reefs with 'Active' outbreak densities (mean ≥1.0 *A. planci* per tow).

(14.6°S) and Cairns (17°S), and mainly in the northern portion of this area. During the initial waves of outbreaks (in 1962 and 1979), high densities of *A. planci* were first detected (or at least reported) on reefs close to Cairns (e.g., Green Island), though increases in densities of *A. planci* may have occurred even earlier on reefs to the north, as recorded during the start of outbreaks in 1993 and 2010. Limited temporal and spatial resolution of monitoring of GBR reefs (e.g., Sweatman et al. 1998) mean that it is still unclear whether outbreaks start from a single reef within this area, or arise simultaneously on a suite of closely positioned reefs (Pratchett 2005). In 1994, outbreaks of *Acanthaster* spp. were first recorded at Lizard Island, but were reported soon after (within the same year) at several other reefs within the immediate area, including Linnet, North Direction, and Rocky Islet (Sweatman et al. 1998). The exact timing and location of primary outbreaks is critical to understanding and identifying potential causes or triggers of outbreaks on the GBR, which suggests that this area should be monitored intensively whenever initiation of a new wave of outbreaks seems possible. Alternatively, the pattern of initiation and spread of outbreaks may be reconstructed using the size frequency distribution of high density populations on disparate reefs (*sensu* Kenchington 1977), combined with newly developed genetic markers (Yasuda et al. 2006a; Wainwright et al. 2012) to potentially map the directionality of dispersal among reefs within the initiation box.

The recurring pattern on the GBR is the southward progression of outbreaks from 17-20 °S, at a rate of 1 degree of latitude every three years (Kenchington 1977; Reichelt et al. 1990a). This is attributed to progressive colonization of reefs by larvae spawned at the outbreak front, which disperse only 1-2 degrees of latitude (Kenchington 1977). Outbreaks of crown-of-thorns starfish also appear to spread northwards from the initiation area, but the northward progression is slower and less consistent (Moran

1992), though reefs in the northern region have not been surveyed as frequently as those in other regions of the GBR. The progression of outbreaks northward and southward on the GBR has been attributed to movement of adult starfish between reefs (e.g., Talbot and Talbot 1971), though differences in the size-frequency distribution of disparate populations seem more likely to be the result of larval dispersal (Kenchington 1977), with prevailing currents (Black and Moran 1991).

2.4 Disturbances caused by outbreaks of crown-of-thorns starfish

Acanthaster spp. have gained considerable notoriety, not only because of their tendency to undergo rapid and dramatic increases in populations size, but because outbreak populations can cause extensive and widespread depletion of scleractinian corals (e.g., Pearson and Endean 1969; Chesher 1969; Randall 1973; Colgan 1987). At low densities (<10 starfish. ha^{-1}), *Acanthaster* spp. have negligible impact on scleractinian coral cover (e.g., Glynn 1973; Zann et al. 1990). However, the combined feeding activities of very high densities of crown-of-thorns starfish (up to 151,650 starfish per km^2; Kayal et al. 2012) can cause rapid and pronounced coral loss. In Guam, for example, high densities (>1,000 starfish per hectare) of *Acanthaster planci* persisted for 30 months in 1967-1969 and killed virtually all scleractinian corals (>90% coral mortality) from 1-68 m depth along a 38-kilometre stretch of coastline (Chesher 1969). Similarly, in French Polynesia, very large aggregations of *A. planci* systematically moved around the entire circumference of Moorea, and removed >96% of coral (Kayal et al.2012). While climate-related disturbances (e.g., bleaching and disease) are a major concern for reef managers around the world, especially since 1998 (Knowlton 2001), outbreaks of *Acanthaster* spp. continue to occur throughout the Indo-

Pacific region (e.g., Pratchett 2005; Baine 2006) and have so far been responsible for far more coral mortality than has been attributed to climate related disturbances (Bruno and Selig 2007; Osborne et al. 2011; Trapon et al. 2011; De'ath et al. 2012).

Although outbreaks of *Acanthaster* spp. can cause massive and widespread coral depletion (e.g. Pearson and Endean 1969; Chesher 1969; Randall 1973; Colgan, 1987) this is not always the case. Outbreaks of *Acanthaster* spp. vary greatly, not only in the number, size and density of starfish, but also in their effects (Moran 1986). For example, high densities of *Acanthaster planci* persisted for more than 18 months (1969-70) at Molokai Island, Hawaii, but had negligible impacts on the abundance of scleractinian corals (Branham et al. 1971). Similarly, in Panama, high-density populations of *A. planci* caused minimal reductions in the live cover of scleractinian corals (Glynn 1974, 1976). Large aggregations of many hundreds to thousands of crown-of-thorns starfish have been reported on reefs throughout the Indian and Pacific oceans, including Panama (Glynn 1974, 1976), Samoa (Birkeland and Randall 1979), Micronesia (Chesher 1969; Colgan 1987), southern Japan (Nishihira and Yamzato 1974; Keesing 1992), the GBR (e.g., Moran et al. 1988; Reichelt et al. 1990a), Cocos-Keeling Islands (Colin 1977); and the Red Sea (Ormond and Campbell 1974). However, incidences of large-scale destruction of scleractinian corals by outbreaks of *Acanthaster* spp. have occurred primarily within the south and west Pacific (Moran 1986; Birkeland and Lucas 1990). More specifically, devastating outbreaks of *Acanthaster* spp. have been most apparent in southern Japan (Nishihira and Yamazato 1974; Keesing 1992), on Australia's GBR (Pearson and Endean 1969), in Micronesia (Chesher 1969; Colgan 1987), and French Polynesia (Trapon et al. 2011; Kayal et al. 2012).

Variation in the effects of outbreaks of *Acanthaster* spp. in different regions of the Pacific might be explained by the relative dominance of *Acropora* in local coral

assemblages (Birkeland and Lucas 1990); *Acropora* tends to dominate in the south and west Pacific and is consistently among the corals that are first and worst affected during outbreaks (e.g., Pratchett et al. 2009a, Pratchett 2010). In the north and east Pacific (e.g., the main Hawaiian Islands and Panama) *Acropora* is relatively scarce and coral assemblages tend to be dominated by *Pocillopora*, which is much less susceptible to crown-of-thorns attack (e.g., Glynn 1974, 1976) though it can be damaged extensively by extremely severe outbreaks (e.g., Adam et al. 2011; Kayal et al. 2012). Geographic variation in the effects of *Acanthaster* spp. may also result from differences in the population dynamics and behavior among the four nominal sister species distributed in different parts of the Indo-Pacific (Vogler et al. 2008). *Acanthaster* spp. from throughout the Indo-Pacific ostensibly look and behave the same way, but devastating impacts of crown-of-thorns starfish appear to be confined to the Pacific, which is the geographical range of *Acanthaster planci*. This warrants explicit comparisons of demographic rates, feeding rates and feeding preferences among *Acanthaster* spp. from each of the four distinct sub-populations identified by Vogler et al. (2008), extending the studies in the Pacific to the Red Sea and both southern and northern Indian Ocean regions.

Effects of *Acanthaster* spp. can also vary at much smaller scales, within and among adjacent coral reefs (e.g., Pratchett 2010). Highest densities of *Acanthaster* spp., hence the greatest depletion of live coral cover, tends to occur on the leeward side of reefs (Laxton 1974; Pratchett 2005), which is due either to 1) high abundance of preferred coral prey (e.g., monospecific stands of staghorn *Acropora*) in these habitats, or 2) reduced water flow and turbulence in back reef environments, which can potentially dislodge starfish whilst feeding in more exposed locations (Endean and Stablum 1973). However, crown-of-thorns are rarely found within shallow, semi-

enclosed lagoons, which often support many suitable coral prey (e.g., Pratchett 2005; Pratchett et al. 2011), suggesting that other environmental variables also influence their finescale distribution. *Acanthaster* spp. have very rarely been observed on mesophotic (>30 m deep) reefs of the GBR (T. Bridge, pers. comm.), even though these habitats are very extensive (Harris et al. 2013) and can support very high cover of scleractinian corals (Bridge et al. 2013). These habitats could provide refuges for coral species to reseed shallow water reef habitats in the aftermath of devastating outbreaks of *Acanthaster* spp. (Bridge et al. 2013).

2.4.1 Directional shifts in coral composition

Disturbances influence the structure of ecological communities either through selective mortality of particular species, or by random, localized mass mortality across a wide range of different species (often termed "catastrophic mortality") that clears space for recolonization (Petraitis et al. 1989). Small-scale or relatively discrete disturbance events (e.g., predation events) usually have a disproportionate impact on certain individuals or species (Petraitis et al. 1989), and so exert a strong structuring influence on populations and communities. Such events may for example, increase diversity and promote coexistence of species by reducing the abundance of competitively dominant species, and allowing inferior competitors to persist (e.g., Porter 1972, 1974). However, disturbances that differentially affect species may also reduce diversity by disproportionately affecting rare species, thereby increasing the dominance of already abundant species (e.g., Glynn 1974, 1976). Catastrophic disturbances meanwhile, eliminate most (if not all) of the species in an area, and may contribute to increased species diversity by preventing dominant species from monopolizing all available resources (Petraitis et al. 1989). While effects of *Acanthaster* spp. on coral assemblages

are essentially a predatory interaction, resulting loss of scleractinian corals can be catastrophic (e.g., Chesher 1969), and the specific effects will depend upon the frequency and severity of major outbreaks.

Endean and Cameron (1985) suggested that the severity of any given disturbance should be judged not by how much coral is killed, but by the type of coral killed. Notably, the removal of very long-lived and slow growing species (e.g., *Porites* spp.) is likely to have longer-term impacts on community structure, compared with selective removal of short-lived, fast-growing species (Endean and Cameron 1985). Like most disturbances (e.g. freshwater plumes, Jokiel et al. 1993; coral bleaching, Marshall and Baird 2000), outbreaks of *Acanthaster* spp. tend to have a disproportionate impact on fast-growing coral species (e.g., *Acropora*), which also recruit abundantly and often recover rapidly in the aftermath of major disturbances (e.g., Linares et al. 2012). However, *Acanthaster planci* do sometimes feed on very large and very old colonies of massive *Porites* (Done 1985). The specific effects of crown-of-thorns outbreaks (and other recurrent disturbances) on the composition of coral assemblages will depend on the frequency and severity of these disturbances. Severe, but infrequent disturbances are likely to have a disproportionate effect on slow growing species that are incapable of recovering between successive disturbances (Done 1985). However, frequent moderate outbreaks (especially in isolated locations) are likely to cause the localized extirpation of corals that are favored by *Acanthaster* spp. (e.g., Berumen and Pratchett 2006; Pratchett et al. 2011).

A striking example of persistent shifts in the structure of coral assemblages has been documented in Moorea, French Polynesia, where a major outbreak of *Acanthaster planci* in 1980-1981 led to the disproportionate loss of *Acropora* corals (Bouchon 1985). Ongoing disturbances (including cyclones, coral bleaching and a further

outbreak of *A. planci*) have since prevented recovery of *Acropora* spp., such that the dominant coral genera in 2009 were *Pocillopora* and *Porites* (Berumen and Pratchett 2006; Adjeroud et al. 2009; Pratchett et al. 2011, 2013), which were relatively unaffected by outbreaks of *A. planci*. Given sufficient time between disturbances, *Acropora* might be expected to recover eventually and regain its former dominance in Moorea. However, Pratchett et al. (2011) measured recruitment rates for *Acropora* and other dominant coral genera (*Porites* and *Pocillopora*) in Moorea, and showed that the relative abundance of new recruits strongly reflected the current patterns of adult abundance. This is evidence of a positive feedback mechanism, which is likely to reinforce and sustain the altered community structure (Knowlton 1992; Nyström et al. 2008).

Selective depletion of certain coral species, linked to marked feeding preferences of *Acanthaster* spp. (e.g., Brauer et al. 1970; Collins 1975; Ormond et al. 1976; Colgan 1987; Keesing 1990; De'ath and Moran 1998b) has the capacity to greatly alter coral diversity (e.g., Porter 1972, 1974; Glynn 1974; Colgan 1987) and therefore, habitat heterogeneity. In the eastern Pacific, *Acanthaster planci* tend to avoid the most abundant coral, *Pocillopora* (Glynn 1974, 1976, 1980). By feeding mostly on rare coral species, this further increases the dominance of *Pocillopora,* leading to declines in coral diversity (see also Branham et al. 1971). In the Indo-west Pacific, however, *A. planci* feeds predominantly on *Acropora* spp. and *Montipora* spp. (e.g., Ormond et al. 1976; Colgan 1987; Keesing 1990; De'ath and Moran 1998b; Pratchett 2010), which are relatively abundant and often competitively dominant corals. This presumably increases the prevalence of other less abundant coral species, which Porter (1972, 1974) suggested might lead to increases coral diversity. Pratchett (2010) explicitly tested for changes in coral diversity during a moderate outbreak of *Acanthaster planci* at Lizard

Island, northern GBR, and showed that despite disproportionate effects on preferred corals, coral diversity declined (rather than increased) with declines in coral cover. Contrary to Porter's (1972) suggestion it seems unlikely that outbreaks of *A. planci* (even relatively moderate outbreaks) would ever cause increases in coral diversity, because crown-of-thorns starfish are not sufficiently averse to rare corals. In the absence of preferred corals, *Acanthaster* spp. will certainly feed on other less preferred corals (Moran 1986; Keesing 1990; Cameron and Endean 1990), so further outbreaks on highly degraded reefs are likely to lead to even more extensive depletion of corals, with collateral effects on the local diversity of other reef-associated organisms.

2.4.2 Indirect effects of outbreaks of crown-of-thorns starfish

Extensive coral depletion caused by outbreaks of *Acanthaster* spp., as well as directional shifts in the composition of coral assemblages, have broad impacts on a wide variety of coral reef organisms. Outbreaks of crown-of-thorns starfish have been linked to increased abundance of soft corals (e.g., Endean 1971; Chou and Yamazato 1990), algae (Larkum 1988), urchins (Belk and Belk 1975), and herbivorous fish species (Endean and Stablum 1973; Wass 1987), while causing declines in abundance of coral-dependent fishes (e.g., Williams 1986; Sano et al. 1984, 1987; Munday et al. 1997), and motile invertebrates (Garlovsky and Bergquist 1970). Changes in the abundances of these reef organisms are the indirect result of massive reductions in the abundance of scleractinian corals. For example, increases in the abundance of urchins (specifically, *Echinometra mathaei* and *Diadema* spp.) following outbreaks of *Acanthaster planci* have been related to increased food availability, as algae colonise skeletons of dead, but intact corals (e.g., Belk and Belk 1975; Larkum 1988). Given the potential severity of

starfish outbreaks, it is not surprising that many coral reef organisms are indirectly affected.

Most studies that have considered secondary effects of outbreaks of *Acanthaster* spp. have measured changes in the abundance of coral reef fishes associated with localized coral loss (e.g., Williams 1986; Sano et al. 1987; Hart et al. 1996; Munday et al. 1997; Adam et al. 2011; Pratchett et al. 2012). Aside from herbivorous fish species (e.g., Endean and Stablum 1973; Wass 1987; Adam et al. 2011), most fishes tend to decline in abundance in the aftermath of major outbreaks of *Acanthaster* spp. that cause extensive coral loss (e.g., Bouchon-Navaro et al. 1985; Williams 1986; Sano et al. 1987, Munday et al. 1997). These effects are most pronounced for specialist fishes that depend on corals for food (e.g., butterflyfishes; Williams 1986) or habitat (e.g., coral-dwelling gobies; Munday et al. 1997; coral-dwelling damselfishes, Pratchett et al. 2012). At least 133 species (and 11 different families) of coral reef fishes feed on scleractinian corals (Cole et al. 2008), the majority (69 species) of which are butterflyfishes (family Chaetodontidae). Also, 320 species (from 39 different families) have been shown to use live corals as habitat (Coker et al. 2013). These data suggest that 8-10% of coral reef fishes will be directly and adversely affected by extensive coral depletion (see also Munday et al. 2008). Localized depletion of preferred coral may ultimately lead to extirpation of fishes that are directly reliant on corals (e.g., Kokita and Nakazono 2001; Munday 2004). This is particularly so for highly specialized fishes that rely on only a very limited suite of coral species, though it is important to look at other biological traits that may offset extinction risk in these species (Lawton et al. 2011).

The effects of very severe outbreaks of *Acanthaster planci* also extend well beyond the few fishes that are directly dependent on live corals (e.g., Sano et al. 1987),

especially where coral depletion is associated with loss of habitat structure. Extensive coral depletion caused by large or persistent outbreaks of *A. planci* (e.g., Pearson and Endean 1969; Chesher 1969; Colgan 1987; Sano et al. 1987) almost invariably leads to marked declines in habitat and topographical complexity, which are critical for sustaining high diversity of reef fishes and other reef-associated organisms (Wilson et al. 2006; Pratchett et al. 2009b). Once dead, the exposed skeletons of scleractinian corals are susceptible to biological and physical erosion (Glynn 1997; Hutchings 2011). Over time, skeletons of erect branching corals (e.g., *Acropora* and *Pocillopora*) break down into coral rubble (Sheppard et al. 2002; Graham et al. 2006), whereas more robust skeletons of massive corals (e.g., *Porites*) may become dislodged or gradually eroded *in situ* (Sheppard et al. 2002). The structural collapse of dead coral skeletons takes 4-7 years (Pratchett et al. 2008) and if there is no substantial recovery of corals in the meantime then the corresponding loss of habitat structure and topographic complexity can have far reaching effects on the abundance and diversity of fishes (Sano et al. 1984, 1987; Pratchett et al. 2009b). In southern Japan, for example, Sano et al. (1987) reported >65% fewer individuals and species of fishes at a reef that had been devastated by a localized outbreak of *Acanthaster planci*, compared with nearby reefs with extensive growth of staghorn *Acropora*. On the reef that had been devastated by *A. planci* extensive stands of *Acropora* corals were rapidly eroded, converting once highly complex three dimensional habitats into flat, homogenous rubble fields (Sano et al. 1987).

2.5 Causes of crown-of-thorns starfish outbreaks

Unifying theories for population outbreaks were proposed by scientists working in terrestrial environments long before outbreaks of the crown-of-thorns starfish were even known to occur (e.g., MacArthur 1955; Elton 1958). Both MacArthur (1955) and Elton (1958) argued that population outbreaks are manifestations of inherent instability within certain systems, attributed to either: i) particular life-history characteristics (e.g., high fecundity, short generation times, high mortality during their early life-history, and generalized patterns of prey and habitat use), which predispose an organism to major fluctuations in population size; or ii) major changes in the physical and/ or biological environment that release the outbreaking population from usual regulating factors (e.g., Andrewartha and Birch 1984; Berryman 1987). Numerous hypotheses have been put forward to explain the occurrence of population outbreaks of *Acanthaster* spp. (reviewed by Moran 1986; Birkeland and Lucas 1990). These hypotheses generally fall into two groups that place importance either on factors affecting recruitment rates (i.e. 'Natural Causes hypothesis', Vine 1973; 'Larval Recruitment hypothesis', Lucas 1973; 'Terrestrial-Runoff hypothesis', Birkeland 1982), or on changes in the behavior and/ or survivorship of post-settlement individuals (i.e. 'Predator-Removal hypothesis' Endean 1969; 'Adult Aggregation hypothesis', Dana *et al*. 1972; 'Prey-Threshold hypothesis', Antonelli and Kazarinoff 1984). While several of these hypotheses have been considered biologically improbable (e.g., Potts 1981; Birkeland and Lucas 1990), no single hypothesis has universal support. Sudden and dramatic increases in the abundance of starfish must involve successful recruitment (Birkeland and Lucas 1990), but both pre- and post-recruitment processes are likely to contribute to the dynamic nature of *Acanthaster* populations (Bradbury and Antonelli 1990), as has also been shown for many other marine organisms (e.g., Jones 1987, 1991; Hughes 1990). Many

biologists and theoretical ecologists concur that single factor hypotheses that seek to explain the occurrence of crown-of-thorns outbreaks in all locations and at all times are likely to oversimplify the population dynamics for this organism (reviewed by Birkeland and Lucas 1990; Bradbury and Antonelli 1990). It is also important to recognize that *Acanthaster* spp., probably more so than any other coral reef organism, are predisposed to major fluctuations in population abundance (Birkeland 1989b).

Given the life history characteristics of *Acanthaster* spp., it is almost harder to explain the persistence of low-density populations than it is to explain outbreaks (Endean and Cameron 1990). On any given reef, it is likely that outbreaks will occur periodically, through the effects of random environmental variation on reproductive success and/or larval survival. As discussed previously, *Acanthaster* spp. has extremely high fecundity (e.g., Conand 1983, 1985), while fertilization rates, as well as developmental rates and survivorship of larvae are highly subject to the vagaries of local environmental conditions. Moore (1990) examined the characteristics of key locations where *Acanthaster* spp. occur, but never in outbreak densities, and suggested that a combination of 1) low and fragmented coral cover (causing individuals to be dispersed), 2) hydrodynamic conditions that cause larvae to be retained rather than exported, and 3) relatively high populations of predators (reducing starfish numbers and also causing individuals to disperse) that prevents outbreaks from occurring.

Understanding of the causes of crown-of-thorns outbreaks has been greatly hindered by a lack of data on finescale temporal and spatial changes in the population structure and dynamics of *Acanthaster* spp. (Moran 1986). In particular, there are few data on changes in the distribution, density and spawning behavior of *Acanthaster* spp. in the period immediately preceding an outbreak. This is because most studies of outbreak populations (e.g., Pearson and Endean 1969; Chesher 1969; Branham et al.

1971; Sakai 1985) are initiated after starfish densities have already increased to outbreak levels. Also, very few studies have continually monitored changes in the structure and dynamics of *Acanthaster* populations at regular intervals over an extended period, encompassing an entire outbreak cycle (Moran 1986). On the GBR, for example, long-term and very extensive monitoring of the distribution and abundance of *Acanthaster planci* is undertaken by the Australian Institute of Marine Science (Sweatman et al. 2011), but the methods developed to sample over vast reef areas prohibits the collection of detailed information on population structure and reproductive condition. Inherent trade-offs in the collection of finescale biological information versus broadscale surveys to detect changes in the abundance of *Acanthaster* spp. across extensive reef areas represent one of the greatest challenges to understanding the processes that contribute to the initiation of new and distinct outbreaks. On the GBR, it may be possible to focus detailed surveys in the area where primary outbreaks are known to occur. Elsewhere, however, the distribution of primary versus secondary outbreaks is largely unknown.

2.5.1 Natural versus anthropogenic drivers

Reviews on the effects of disturbances on coral reefs (e.g., Pearson 1981) invariably distinguish between natural (e.g., storms and other weather events) versus anthropogenic (e.g., overfishing and pollution) disturbances. The connotations of this are obvious, in that it is the anthropogenic disturbances that are considered responsible for the recent (anthropocene) degradation of coral reef ecosystems (e.g., Hughes et al. 2003), and need to be managed. However, the distinction between natural and anthropogenic disturbances is not always clear (e.g., Potts 1981). Severe tropical storms (cyclones, hurricanes and typhoons), for example, are recurrent disturbances that have

impacted coral reefs throughout their evolution and development. However, Webster et al. (2005) reported that anthropogenic climate change is increasing the severity, if not the frequency of severe tropical storms (but see Klotzbach 2006; Landsea et al. 2006), with obvious ramifications for coral reef ecosystems. The role of anthropogenic activities in causing or exacerbating outbreaks of crown-of-thorns starfish is also highly controversial (e.g., Potts 1981).

When extensive outbreaks of *Acanthaster* spp. were documented in the late 1960s (Chesher 1969; Pearson and Endean 1969) it was immediately assumed that these were new and unprecedented phenomena linked to human activity, such as coastal development (Chesher 1969), pesticides and pollutants (Randall 1972), or excessive harvesting or predatory organisms (Endean 1977). In support of this, Endean (1982) pointed out that the first affected reefs (e.g., Green Island) on the GBR were those that had greatest human visitations. Several scientists (e.g., Weber and Woodhead 1970; Dana 1970; Vine 1970, 1971) put forward the contrary view that outbreaks of *Acanthaster* spp. were a natural occurrence that had simply gone unnoticed prior to the 1960s. Rapid increases in the number of reports of 'infestations' and 'plagues' of *Acanthaster* spp. from throughout the Indo-Pacific following initial awareness of the issue were taken as evidence for this (Vine 1971). Some argued that outbreaks had occurred in both the recent (e.g., Vine 1971, 1973) and distant past (e.g., Walbran et al. 1989a,b).

There are many anecdotal accounts of crown-of-thorns starfish occurring in high densities at locations across the Indo-Pacific well before outbreaks were reported by scientists (Vine 1970, 1973). Fishermen in Santa Ysabel in the Solomon Islands recalled a time in the 1930s when night-fishing was hazardous due to the abundance of *Acanthaster planci* (Vine 1970). Former trochus and pearl shell divers on the GBR

recalled seeing large numbers of the starfish on individual reefs going back to the 1930s (Ganter 1987). In 1913, H.L. Clark collected three *A. planci* from the reef flat of Mer Island in the eastern Torres Strait (far northern GBR) without diving (Clark 1921), suggesting that starfish must have been common. These anecdotal records suggest that localized outbreaks have occurred in the past (Vine 1973). However, the previous occurrence of waves of outbreaks as seen on the GBR in recent decades cannot be confirmed in the absence of systematic, broadscale monitoring.

Further evidence of past outbreaks (over geological time) has been sought in the form of mesodermal skeletal elements of *Acanthaster* spp. in the sediment record. Skeletal elements from *A. planci* have been found in numerous sediment cores from GBR reefs (Frankel 1977, 1978; Walbran et al.1989a, b), but reconstructing a history of *A. planci* numbers and drawing conclusions about the existence of past outbreaks is not simple because of disturbance by burrowing organisms, varying sedimentation rates and differential compaction of the sediments (Pandolfi 1992; Keesing et al. 1992; Fabricius and Fabricius 1992). Only very few cores from a limited number of reefs have been studied intensively and the resulting reconstructions are quite variable. Even if outbreaks did occur prior to the 1950s (which appears likely), the key question is whether outbreaks are occurring more frequently in recent times, and if this reflects increasing anthropogenic changes to marine and coastal environments (Brodie 1992).

While debate continues about the role of anthropogenic activities in causing or exacerbating outbreaks of *Acanthaster* spp., it is clear that the current regime of disturbances to which most coral reefs are subject cannot be sustained (Gardner et al. 2003; Bruno and Selig 2007; De'ath et al. 2012; Fabricius 2013). On the GBR, for example, De'ath et al. (2012) reported a 50.7% decline in mean coral cover across 214 reefs that have been repeatedly and comprehensively surveyed (using reef-wide manta

tows) from 1985 to 2012. The timing and rates of coral loss varied spatially (see also Sweatman et al. 2011), but these data are evidence of significant reef-wide habitat degradation, largely attributable to recurrent outbreaks of *Acanthaster planci* that compound upon other large-scale and persistent disturbances (Osborne et al. 2011; De'ath et al. 2012). Over this 27-year period, outbreaks of *A. planci* affected 49% of reefs and hind casting showed that coral cover would have increased at 0.89% per year (as opposed to annual declines of 0.53%) were it not for impacts of crown-of-thorns starfish (De'ath et al. 2012). Similarly, in the central Pacific, the reefs surrounding Moorea Island in French Polynesia have been subject to a high frequency of different disturbances, including seven distinct episodes of mass coral bleaching, two major cyclones and two outbreaks of *A. planci* since 1979 (Trapon et al. 2011). Despite this frequency and diversity of disturbances in Moorea, significant long-term coral loss and degradation of reef environments are clearly attributable to the devastating effects of *A. planci* outbreaks in 1980-81 and 2007-11 (Adam et al. 2011; Trapon et al. 2011; Kayal et al. 2012). It has been suggested that if disturbances of this frequency and magnitude had occurred throughout the geological period (Holocene) during which contemporary coral assemblages evolved and coral reefs developed, then the biological and physical structure would be very different (Randall 1972). Most notably, large colonies of slow-growing massive corals (mostly, *Porites*) could not withstand extremely severe outbreaks of *Acanthaster* spp. that cause high rates of mortality across all preferred and non-preferred corals (e.g., Endean and Cameron 1985; Done et al. 1988; Done 1992a).

A significant and increasing effect of *Acanthaster* spp. on coral assemblages and reef ecosystems is not in itself evidence that outbreaks are unnatural (Birkeland and Lucas 1990). Rather, other anthropogenic disturbances (e.g., fishing and harvesting, sedimentation, eutrophication and pollutants) may have undermined the capacity of reef

ecosystems to withstand these periodic disturbances, eroding their resilience and leading to changes in the ecosystem responses to persistent and ongoing disturbances. Even more likely is that the pervasive effects of humans on coastal ecosystems have fundamentally altered the structure and function of both crown-of-thorns populations and reef ecosystems, forever altering any semblance of a natural system. Managing ongoing effects of *Acanthaster* spp. is conditional upon identifying the specific factor(s) that cause or exacerbate contemporary outbreaks, and much of the current discussion is centred around one of two alternative hypotheses; 1) nutrient enrichment and 2) predatory release (e.g., Birkeland and Lucas 1990; McClanahan et al. 2002; Brodie et al. 2005; Mendonça et al. 2010; Fabricius 2013).

2.5.2 Nutrient enrichment hypothesis

The notion that outbreaks of *Acanthaster* spp. may arise due to enhancement of larval survivorship through nutrient enrichment has been proposed several times (e.g., Pearson and Endean 1969; Lucas 1973; Nishihira and Yamazato 1974; Birkeland 1982; Brodie 1992; Brodie et al. 2005; Fabricius et al. 2010). Birkeland (1982) suggested that outbreaks of *Acanthaster planci* at several locations in Micronesia and Polynesia tended to occur three years after extremely heavy rainfall events, often preceded by extended droughts. Birkeland (1982) argued that such events provide a pulse of nutrients that stimulate phytoplankton blooms, which supplement otherwise limited food for crown-of-thorns larvae (Lucas 1973). However, enhanced survival of larval crown-of-thorns starfish may also be related to specific environmental conditions (low salinity and high temperatures) at times and in locations affected by river runoff (Henderson 1969, Lucas 1973). Fundamental to this 'terrestrial runoff hypothesis' is the notion that outbreaks occur suddenly, and three years following heavy rainfall periods (Birkeland 1982),

which accounts for the time required for larval starfish to settle on the reef, metamorphose into the adult form, begin feeding on corals, and attain sufficient size (200-300 mm) to emerge from the reef matrix and become readily apparent (**Figure 2.1**). However, some of Birkeland's (1982) findings have since been questioned, based on inconsistencies in either the initiation of outbreaks or the timing of severe tropical storms and peak rainfall events (Endean and Cameron 1990). In Guam, for example, major outbreaks were first reported in 1967 (Chesher 1969), meaning that the larval recruitment event that led to this outbreak would have preceded the drought breaking rains in July 1965 by 18 months (Endean and Cameron 1990). Similarly for the GBR, Fabricius et al. (2010) argued that each of the major episodes of outbreaks was initiated by a major flooding event (in 1958, 1974, 1991 and 2008) that contributed to increased survival of larvae (see also Day 2000). However, the time between flooding events and subsequent outbreaks of *A. planci* ranges from 2-5 years (**Table 2.4**). The purported years of major flooding events in Fabricius et al. (2010) also do not correspond to the documented incidence of major drought-breaking floods, based on Barium/ Calcium ratios in long-lived colonies of *Porites* from Havanah and Pandora reefs (McCulloch et al. 2003). Some of the biggest floods (1968 and 1981) recorded using this method (McCulloch et al. 2003) did not appear to initiate outbreaks (**Table 2.4**), though Fabricius et al. (2010) did stress that floods must occur in November to January to benefit crown-of-thorns larvae. Also, floods may not cause outbreaks of *Acanthaster* spp. if they occur too soon after the preceding outbreak so that the coral cover has not had time to recover sufficiently to sustain a new outbreak (Fabricius 2013).

Table 2.4 Timing of major flood events on Australia's Great Barrier Reef (specifically, peak flow events from the Burdekin River, located 19.6°S), relative to the agreed start of each of the four major waves of outbreaks.

Peak Flood Events		Start of corresponding outbreak	Time between flood and outbreak	Location
McCulloch et al. 2003	**Fabricius et al. 2010**			
1958	1958	1962	4 years	Green Island (16.7°S)
1968	-	-	-	
1970	-	-	-	
-	1974	1979	5 years	Green Island (16.7°S)
1981	-	-	-	
1988	-	1993	5 years	Lizard Island (14.6°S)
1991	1991	1993	2 years	Lizard Island (14.6°S)
1998	-	-	-	
NA	2008	2010	2 years	Lizard Island (14.6°S)

Extended delays (>3 years) between flood events and reported outbreaks on the GBR in 1962 and 1979 (**Table 2.4**) may be attributed to limitations in the detection of outbreaks prior to the implementation of reef-wide monitoring in 1985. For more recent outbreaks however, high densities of *A. planci* would have already recruited on reefs around Lizard Island when flooding events occurred in 1991 and 2008. Moreover, these recent outbreaks almost certainly developed through several consecutive years of high recruitment from 1994 to 1998 (e.g., Pratchett 2005), making links to individual flooding events somewhat tenuous. In addressing these observations, Fabricius et al. (2010) report that "floods have reached or crossed this part of the shelf in 1991, 1994, 1995 and 1996" (page 603), inferring that this was an unusual period with a high frequency of flooding events. It is possible that initial outbreaks that occurred on the GBR in 1958, 1971, and 2008 also comprised multiple cohorts and consecutive years of high recruitment, but there are no data on population structure to explicitly assess this.

Further complexities associated with linking outbreaks of *Acanthaster planci* on the GBR to periodic major flooding events relate to the spatial patterns of outbreaks (Brodie et al. 2005). Notably, outbreaks of *A. planci* tend to occur predominantly on mid-shelf reefs (Moran 1986), rather than inshore reefs where the influence of terrestrial runoff is greatest (Brodie et al. 2005), or on offshore reefs. Also, outbreaks are initiated north of Cairns (close to either Cairns at 17.0^{o}S or Lizard Island at 14.5^{o}S), rather than on reefs in immediate proximity to major river systems (Brodie 1992). Fabricius et al. (2010) argue that the confluence of high nutrients and mid-shelf reefs is limited to northern latitudes, between 14.5^{o}S-17.0^{o}S, and it is only here that nutrient concentrations exceed minimal thresholds (>0.25-0.5 $\mu g.l^{-1}$) necessary for survivorship of crown-of-thorns larvae. They suggest that flood plumes from major rivers (particularly the Burdekin River) travel northwards staying close to the coast, but are

deflected offshore by Cape Grafton, a promontory just south of Cairns. There are however, large areas of the GBR (e.g., in the far northern section, and Swains) that have long-term average chlorophyll concentrations >0.5 $\mu g.l^{-1}$ (**Figure 2.11**). Also, chlorophyll concentrations in summer months (November-May), which is when *A. planci* spawn, are generally >0.5 $\mu g.l^{-1}$ throughout the Wet Tropics (16 to 19^{o}S), independent of any major flooding events (Brodie et al. 2005).

If the productivity of waters on the GBR is consistently below levels (0.25-0.5 $\mu g.l^{-1}$) needed to sustain larval growth and survivorship (e.g., Fabricius et al. 2010), it makes it hard to explain the southward propagation of waves of outbreaks that cause widespread devastation. The conventional wisdom (Brodie 1992; Brodie et al. 2005; Fabricius et al. 2010) is that once primary outbreaks have become established, the immense numbers of larvae produced by high densities of well-fed starfish will generate enough late-stage larvae to successfully settle on downstream reefs (to the south) regardless of very low rates of larval survival. It is certainly true that three waves of outbreaks have propagated effectively through the mid-shelf reefs (>25 km offshore) north and south of Townsville (~19° S), where chlorophyll concentrations rarely exceed 0.25 $\mu g.l^{-1}$. However, it is unclear whether this is due to sheer volume of larvae spawned on reefs to the north (of which only a very small proportion actually survive), or evidence of the capacity of larvae to successfully develop and settle despite relatively oligotrophic conditions (e.g., Olson 1987). It does seem illogical that low nutrient levels would prevent the formation of primary outbreaks on reefs south of 16^{o}S, and yet allow for the extensive formation of devastating secondary outbreaks. Moreover, simulation models do not reproduce the southward propagation of waves of outbreaks when using high levels of larval mortality expected to occur when chlorophyll concentrations are <0.25 $\mu g.l^{-1}$ (Fabricius et al. 2010). A more parsimonious explanation might be that

primary outbreaks are established over several years and independent of any flood events, but the subsequent spread of outbreaks may be conditional upon years of very high larval survivorship, which is facilitated by major flood events that enhance food availability.

A logical extension of the 'terrestrial runoff hypothesis' is that outbreaks would be expected to occur more frequently on high islands compared to atolls (the 'high island hypothesis'; Birkeland 1982), due to localized effects elevated nutrient concentrations. Tsuda (1971) and Pearson (1975) noted that outbreaks occurred predominantly on reefs near high islands or along continental shelves. Birkeland (1982) showed that 'large populations' of *Acanthaster planci* were reported on 19 (out of 23) high islands compared to 2 (out of 22) atolls across Micronesia and Polynesia based on data presented by Marsh and Tsuda (1973). Analyses of all reported outbreaks since 1990 show that outbreaks have occurred on at least 35 low islands or atolls across the Indo-Pacific (e.g., Eniwetok Atoll and Majuro Atoll, in the Marshall Islands), although the majority of outbreaks (29% and 56%, respectively) are reported from high islands (e.g., Moorea, French Polynesia) and continental shelves (e.g., the GBR). Outbreaks on low islands and atolls cannot be readily linked to terrestrial runoff. However, there may be other sources of nutrients that cause plankton blooms and thereby enhance larval survival away from high islands or major rivers, including 1) upwellings (e.g., Sweatman et al. 2008; Mendonça et al. 2010, 2) bioturbation and resuspension of sediments by severe tropical storms, and 3) oceanographic features that create high productivity fronts (Houk et al. 2007). On the GBR, it is evident that here have been outbreaks on reefs in the Swains region that occur almost independently and asynchronously with waves of outbreaks propagating from north of Cairns (Sweatman et al. 1998). Since the Swain Reefs are >100 km offshore they are rarely if ever exposed

to flood plumes, though there is some evidence that upwelling occurs in the region (Kuchler and Jupp 1988).

Houk et al. (2007) described inter-annual fluctuations in ocean productivity in the northern Pacific associated with the Transition Zone Chlorophyll Front (TZCF), which may explain the irregular occurrence of outbreaks across this region. However, these periods of peak productivity coincide with the "emergence" (presumably from deeper water) of high densities of adult *Acanthaster planci* (Houk et al. 2007; Houk and Raubani 2010) rather than settlement of larvae, which must have occurred ~2 yrs earlier; it is unclear how this phenomenon relates to secondary outbreaks. Despite the growing enthusiasm for the nutrient enrichment hypothesis, several authors have cautioned against its broad applicability (e.g., Potts 1981; Olson and Olson 1989; Endean and Cameron 1990; Lane 2012). Lane (2012) points out that outbreaks of *Acanthaster* spp. have been occurring despite overarching declines in global ocean productivity. Also, there is no evidence of increased incidence of crown-of-thorns outbreaks in areas with enhanced nutrient concentrations (either due to periodic high precipitation and high erosion rates, or areas with upwellings) within the Indo-Australia archipelago or 'Coral Triangle' (Lane 2012). It is also important to remember, that much of the potential importance of nutrient enrichment depends on larvae of *Acanthaster* spp. being generally food limited (Lucas 1982; Okaji et al. 1997; Fabricius et al. 2010). Olson (1985, 1987) set out to test Lucas' suggestion that larval *A. planci* rarely complete development at phytoplankton concentrations that are typical of GBR waters in the absence of flood plumes. He developed an apparatus that allowed larvae to be supplied with seawater with controlled concentrations of phytoplankton while being held in chambers underwater in the field. He found that *A. planci* larvae developed at near maximal rates at ambient chlorophyll levels in the absence of

phytoplankton blooms (Olson 1987). He therefore suggested that fluctuations in larval food resources have limited role in explaining inter-annual variation in larval recruitment, which must underlie sudden increases in the abundance of *Acanthaster* spp. (Olson 1987). Okaji (1996) tried to continue this line of research using the same apparatus, as well as a modified form with inline filters, to further manipulate phytoplankton densities. In spite of changing the chambers every two days, he found that chlorophyll concentrations within the chambers increased over the course of his experiment to be well above ambient levels, presumably through contamination and retention of phytoplankton. He concluded that the apparatus was unreliable and abandoned the approach in favour of the laboratory experiments described later in Fabricius et al. (2010). Olson (1987) did not measure chlorophyll concentrations in the chambers in the course of his main experiments, but he did specifically test for such an effect in a pilot experiment (Olson 1985) and found that chlorophyll concentrations in the experimental chambers remained at or below ambient levels after two days in shallow water and bright sunlight. Based on this observation, the larval chambers were changed every two days during the main experiment (Olson 1987) specifically to prevent any accumulation of phytoplankton. These divergent results reinforce the importance of repeating and extending the quite limited studies relating growth and survival of *Acanthaster* larvae to phytoplankton concentration; and they also emphasise the need to confirm the findings in the field.

Despite discrepancies and inconsistencies in the spatial and temporal occurrence of outbreaks (e.g., **Table 2.4**), high rainfall, terrestrial runoff and elevated nutrients are likely to increase the likelihood that outbreaks of *Acanthaster* spp. will actually occur, but they do not necessarily account for all recorded outbreaks. Importantly, the nutrient enrichment hypothesis provides one of the only plausible mechanisms by which

anthropogenic activities may have exacerbated outbreaks of *Acanthaster* spp. (increasing their severity and/ or frequency) over recent decades (Brodie 1992; Brodie et al. 2005). When proposing the terrestrial runoff and high island hypotheses, Birkeland (1982) maintained that outbreaks were essentially a natural phenomenon triggered by irregular rainfall events. He did acknowledge that land clearing might increase nutrient concentrations in coastal environments following terrestrial runoff (Birkeland 1982). If so, there may also be a signal whereby outbreaks of *Acanthaster* spp. are greatest in areas closest to heavily populated coastlines (but see Lane 2012), or coinciding with periods of extensive settlement and clearing of coastal land. However, outbreaks have been reported on isolated and unpopulated reefs (e.g., Chagos), but they maybe less frequent or less severe than on reefs subjected to increased anthropogenic influences. Unfortunately, inconsistencies in monitoring and reporting of nominal outbreaks make it virtually impossible to test these ideas explicitly. This should be a priority for future monitoring studies.

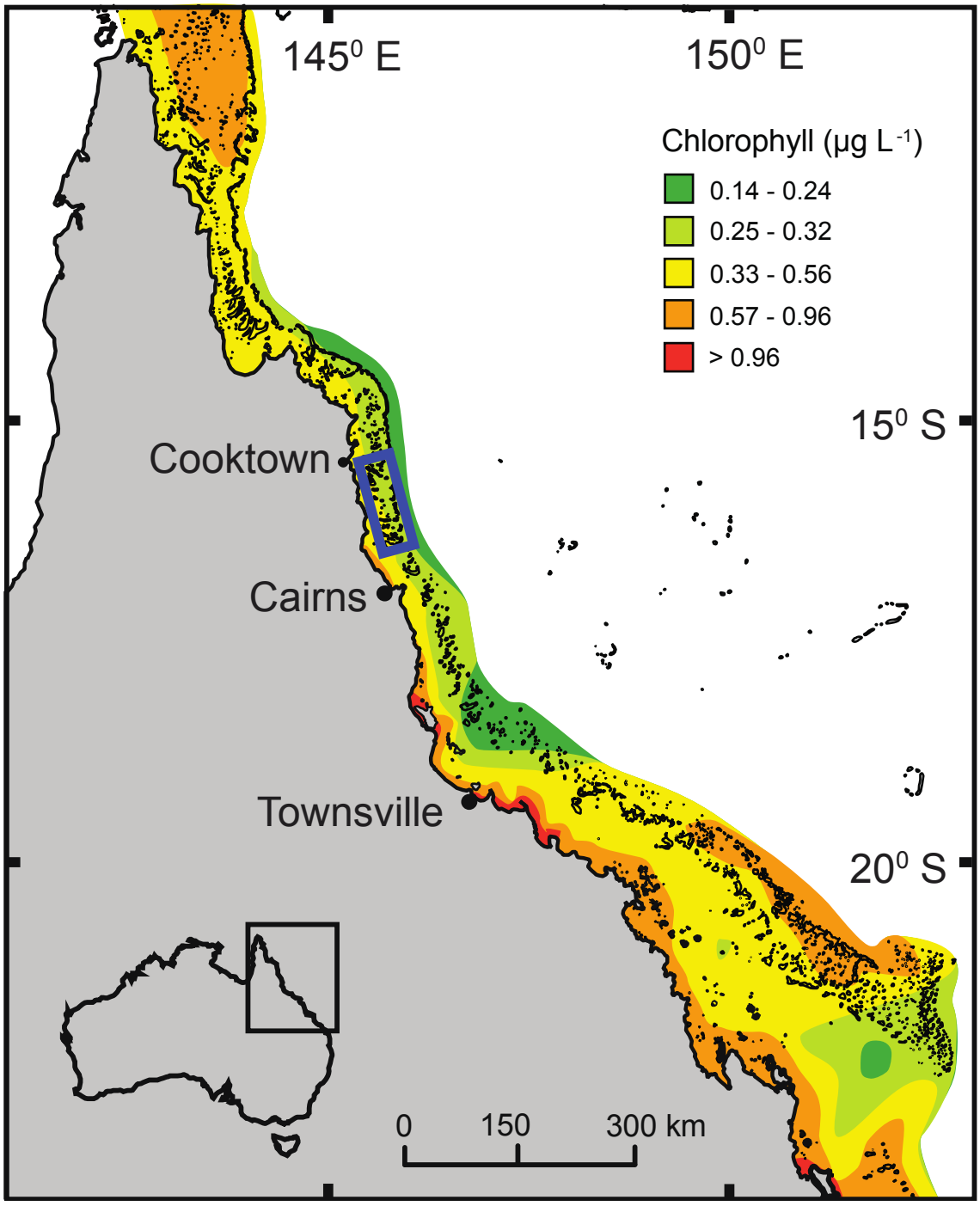

Figure 2.11. Map of Australia's Great Barrier Reef showing the variation in average ('normal') patterns of productivity (chlorophyll-*a* concentrations). Data source: E-atlas (http://maps.e-atlas.org.au/). Also shown, is the small area north of Cairns (the BLUE 'initiation box') in which primary outbreaks of *Acanthaster planci* are thought to be initiated and then spread (mostly, southwards), causing progressive waves of secondary outbreaks (e.g., Fabricius et al. 2010).

2.5.3 Predator removal hypothesis

One of the earliest hypotheses to account for outbreaks of *Acanthaster* spp. was the 'predator removal hypothesis*'* (Endean 1969), which assumed that populations of crown-of-thorns starfish are normally regulated by high levels of post-settlement or adult predation (Endean 1969; McCallum 1987, 1990). This hypothesis was given increased credibility by two recent studies (Dulvy et al. 2004; Sweatman 2008) that reported increased incidence and/ or severity of outbreaks of crown-of-thorns in areas subject to fisheries exploitation (see also Ormond et al. 1990). Sweatman (2008) compared the rates of occurrence of starfish outbreaks on GBR reefs that were open to fishing and on reefs where fishing was prohibited. Because outbreaks come in waves, only those reefs that were close to known outbreaks were considered; 75% of reefs that were open to fishing suffered outbreaks, compared to 20% for reefs that had been closed to fishing for a minimum of 5 years (Sweatman 2008). These studies (Ormond et al. 1990; Dulvy et al. 2004; Sweatman 2008) do not reveal the mechanistic basis for the observed results, but suggest that harvesting of coral reef fishes may increase the likelihood that outbreaks of *Acanthaster* spp. will occur, by removing one of the key regulatory mechanisms that prevent extreme population fluctuations.

When the predator removal hypothesis was initially proposed, the principal known predator of adult *Acanthaster* spp. was the giant triton, *Charonia tritonis* (Pearson and Endean 1969). Harvesting of tritons for sale as curios was suggested to have reduced predator numbers in the decades leading up to the first recorded outbreak in the 1960s (Endean 1969). However, the little that is known of the ecology of *C. tritonis* suggests that even at pre-harvest densities they would not be effective in controlling outbreak populations of crown-of-thorns starfish. For example, when large

C. tritonis were placed in a cage and supplied with abundant adult *A. planci*, average consumption was only 0.7 starfish per triton per week (Pearson and Endean 1969).

More recently, attention has focussed on predation by fishes, particularly emperors (family Lethrinidae) because these are relatively large, generalist benthic carnivores that feed on and around areas of coral (e.g., Sweatman 1997; Mendonça et al. 2010). They are also widely fished, so there is the possibility that their numbers have been reduced through overexploitation, which could be related to the apparent increase in frequency of outbreaks on the GBR. Other known predators of *Acanthaster* spp. are triggerfishes and pufferfishes (Campbell and Ormond 1970, Owens 1971), though numbers of these fishes are unlikely to have changed through exploitation, at least on the GBR. Direct evidence that any fisheries target species are major predators of *Acanthaster spp.* is meaer (Sweatman 1997). Remains of *Acanthaster* spp. have been found in gut contents of some fisheries target species (Birdsey 1988; Randall et al. 1978, **Table 2.5**), but only rarely. Ormond et al. (1990) point out that prey switching behavior, which is a critical component of the population models, means that a lack of observations of predation or the absence of *Acanthaster* spp. in predators' gut contents when starfish densities are low does not necessarily mean that predation by fishes is not important in regulating starfish populations. Few studies have sampled the gut contents of potential predators in locations where *Acanthaster* spp. are known to be present. However, Sweatman (1997) examined the gut contents of 98 lethrinids that were caught close to an area with outbreak densities of adult *Acanthaster planci* on the GBR, and did not find any starfish remains. Similarly, Mendonça et al. (2010) examined gut contents for >20 large (~50 cm total length) individuals of potential starfish predators, including snappers *Lutjanus bohar* and *Lutjanus johni*, emperors *Lethrinus* spp., *Cheilinus lunulatus* at reefs infested with *Acanthaster* spp., but failed to detect starfish remains in

Table 2.5 Fishes that feed on *Acanthaster* spp.

Family	Species	Reference(s)
Serranidae	*Epinephelus lanceolatus*[L,K]	Endean 1976
Lethrinidae	*Lethrinus miniatus*[L]	Keesing and Halford 1992; Sweatman 1995
	Lethrinus nebulosus[L]	Birdsey 1988; Keesing and Halford 1992
	Lethrinus atkinsoni[L]	Sweatman 1995
Pomacanthidae	*Euxiphipops sexstriatus*[r]	Moran 1992
	Pomacanthus semicirculatus[r]	Moran 1992
	Holacanthus passer[r]	Glynn 1984
Chaetodontidae	*Chaetodon auriga*[r]	Moran 1992
Labridae	*Thalassoma lunare*[r]	Rivera-Posada et al. 2013
	Thalassoma lucasanum[r]	Glynn 1984
	Thalassoma hardwicki[r]	Moran 1992
	Chaetodon citrinellus[r]	Glynn 1984
	Cheilinus diagrammus[r]	Moran 1992
	Cheilinus fasciatus[r]	Moran 1992
	Cheilinus undulatus[L,K]	Ormond and Campbell 1974; Keesing and Halford 1992; Randall et al. 1978
Pomacentridae	*Abudefduf curacao*[e]	Pearson and Endean 1969
	Pomacentrus moluccensis[r]	Moran 1992
	Chromis caerulea[r]	Moran 1992
	Chromis dimidiatus[l]	Lucas 1975
Gobiidae	*Cryptocentrus* sp.[r]	Moran 1992
Tetraodontidae	*Arothron hispidus*[L,K]	Ormond et al. 1973
	Arothron manilensis[r]	Rivera-Posada et al. 2013
	Arothron nigropunctatus[r]	Moran 1992
	Arothron stellatus[L,K]	Keesing and Halford 1992
Balistidae	*Balistoides viridescens*[L,K]	Ormond et al. 1973
	Pseudobalistes flavimarginatus[L,K]	Ormond and Campbell 1974; Owens 1971

Note: We clearly distinguish those animals that prey on live starfish (**L**) and have the capacity to kill (**K**) juvenile or adult starfish, as these species may be important in regulating abundance of *Acanthaster* spp. Fishes that have only been seen to feed on remains of dead or dying starfish (**r**), eggs (**e**), or larvae (**l**) may also feed on live juvenile and adult starfish, but this is unconfirmed.

guts of any of the fishes. Possibly, these fishes rarely feed on adult *Acanthaster* spp., but instead target juvenile starfish (Endean 1976). Endean (1976) recorded remains of juvenile *A. planci* in the guts of a Queensland grouper, *Epinephelus lanceolatus*, though predation rates on juvenile starfish are difficult to quantify.

The cryptic behavior of small (<200 mm diameter) *Acanthaster* spp. is often attributed to predator avoidance (Yokochi and Ogura 1987; Zann et al. 1987; Birkeland and Lucas 1990). Moreover, many small and large starfish have missing or regenerating arms (**Table 2.6**), which is considered evidence of recent predator attacks (McCallum et al. 1989). Given the high proportion of starfish with missing arms, reflective of partial or incomplete predation, it may be that mortality rates attributable to predation are also very high (McCallum et al. 1989). In the Philippines, Rivera-Posada et al. (2014) showed that >70% of juvenile (110-200 mm diameter) *Acanthaster planci* had missing or regenerating arms. Rates of injury were much lower in both smaller (probably due to highly cryptic behavior) and larger starfishes, which have increased protection from longer more sturdy spines (Rivera-Posada et al. 2014) compared with starfish of intermediate sizes. Moreover, the rates of injury were much higher within areas closed to fishing, compared to nearby fished areas, providing a potential mechanism to explain why outbreaks are less prevalent in areas closed to fishing (Dulvy et al. 2004; Sweatman et al. 2008). However, observations of lethal predation on *A. planci* are rare and the relationship between partial predation and rates of mortality is completely unknown. Field experiments have failed to find intense predation by fishes on juvenile *Acanthaster* spp. When small (25-79 mm diameter), laboratory-reared *A. planci* were placed in a semi-natural setting in an area of one reef on the GBR where likely fish predators were present, predation rates were only 0.13% of starfish per day (Sweatman 1995). Also, when similarly small juvenile *Acanthaster planci* were presented to

emperors on a nearby reef, only a minority of juveniles were consumed and many were rejected after mouthing and survived the encounter (Sweatman 1995). These results suggest that predation is unlikely to constrain survivorship of juvenile *A. planci*, though they are only based on a single reef and single time.

The circumstances in which predators could regulate *Acanthaster* spp. have been investigated using population models (McCallum 1987, 1990, 1992; Ormond et al. 1990), which focus on the feasibility of fish predators controlling the numbers of crown-of-thorns starfish on an individual reef, rather than considering their role in the initiation and perpetuation of waves of outbreaks. Potential predators need to be able to take a range of prey so they can persist when densities of *Acanthaster* spp. are low, but then switch their attention to crown-of-thorns starfish as numbers rise. These type II and type III functional responses (Holling 1965) are generally characteristic of vertebrate predators, reinforcing the focus on large fishes (e.g., Lethrinidae). Most of the parameters in the models have never been measured in the field, but using plausible estimates for numbers of starfish that recruit and for rates of prey consumption by fishes, the models suggest that realistic densities of fishes may regulate local populations of *Acanthaster* spp. over a range of recruitment rates, and might therefore be important in preventing the gradual development of primary outbreaks. However, mass-recruitment of *Acanthaster* spp. would quickly swamp fish predators (McCallum 1987, 1992; Ormond et al. 1990), limiting the capacity to prevent sudden or secondary outbreaks.

Table 2.6 Geographical variation in the proportion of *Acanthaster* spp. with missing or damaged arms, which is considered to be a proxy for differing levels of predation.

Location	Year	% with arm damage	Reference
Western Australia	1985	64%	Simpson and Grey 1988
Hawaii	1972	60%	Branham 1973
Philippines	2012	59%	Rivera et al. (unpub. data)
Guam	1991	59%	Lawrence 1991
Papua New Guinea	1970	50%	Pyne 1970
GBR	1994	50%	Stump 1996
Western Australia	1985	47%	Simpson and Grey 1988
Ryukyu Islands	1984	46%	Nakamura 1986
Guam	1981	43%	Glynn 1982
GBR	1987	40%	McCallum et al. 1989
Ryukyu Islands	1985	35%	Nakamura 1986
Ryukyu Islands	1986	33%	Nakamura 1986
GBR	1967-1968	33%	Pearson and Endean 1969
Ryukyu Islands	1985	32%	Nakamura 1986
Sudan	1984	30%	Moore 1985
Sudan	1969	29%	Ormond and Campbell 1971
Western Australia	1985	25%	Simpson and Grey 1988
Ryukyu Islands	1984	20%	Nakamura 1986
Sudan	1970	20%	Ormond 1971
Panama	1980-1981	17%	Glynn 1982
Fiji	1984-1985	13%	Zann et al. 1987
Sudan	1970	4%	Ormond 1971
Sudan	1984	2%	Moore 1985

Note: Levels of damage are generally highest among smallest individuals, but few studies explicitly distinguished between size classes, so data are presented as the overall percentage of individuals with any evidence of damage. Data are arranged from highest to lowest, rather than by geographical location.

2.5.4 Pathogenesis and outbreak cycles

Outbreaks of *Acanthaster* spp. often end with precipitous declines in starfish densities (e.g., Chesher 1969; Moran et al. 1985; Moran 1986; Pratchett 2005). In many cases (e.g. Endean 1969; Chesher 1969; Pearson and Endean 1969) these declines follow extensive depletion of scleractinian corals, prompting suggestions that starfish either die from starvation, or move *en masse* to find food (Endean 1969). An alternative explanation, however, is that *Acanthaster* spp. are highly vulnerable to pathogenesis (e.g., Zann et al. 1987; Pratchett 1999), and that rapid transmission of disease(s) among high density populations ultimately leads to population collapse. Echinoderms generally, are highly susceptible to disease (Jangoux 1987a, b), and disease has been implicated in mass-mortalities of numerous species of urchins and starfish (e.g. Dungan et al. 1982, Lessios et al. 1984). Moreover, the operation of disease (independent of any other regulatory mechanisms) can lead to predictable fluctuations in the abundance of host animals (e.g., May and Anderson 1979), possibly accounting for cyclical outbreaks of *Acanthaster* spp., such as have been observed on the GBR.

There is extensive evidence that crown-of-thorns starfish are particularly prone to disease, and are often seen to exhibit symptoms of pathogenesis (Birkeland and Lucas 1990). Burkholder (1973), for example, showed that *Acanthaster planci* had much lower resistance to bacterial infection compared with many other starfish. Importantly, *A. planci* had weak resistance to Gram-positive bacteria, and almost no resistance against Gram-negative bacteria (Burkholder 1973). Accordingly, crown-of-thorns starfish often succumb to bacterial infection when kept in captivity (Lucas 1984; Sutton et al. 1988). Symptoms of disease in *Acanthaster* spp. include lesion formation, tissue degeneration, loss of turgor, and collapsed spines, which are indicative of bacterial infection (Sutton et al. 1988). Sutton et al. (1988) isolated several potential pathogens,

including *Vibrio harveyi*, *V. tubiashi*, *V. campbellii*, *Pseudomonas* and *Moraxella* bacteria from starfish exhibiting aforementioned symptoms. Similar symptoms were also seen in a crown-of-thorns starfish found at Lizard Island (northern GBR) in 1999 (Pratchett 1999). While attempts to identify the pathogenic organism(s) causing these symptoms were unsuccessful, Pratchett (1999) did show that tissues removed from the sick and dying starfish, could be used to infect other seemingly healthy starfish in close proximity, but without direct contact. Perhaps more importantly, the detection of this dying starfish in 1999 coincided with the rapid disappearance of starfish in the aftermath of a significant outbreak (Pratchett 2005), and preceded the local depletion of scleractinian corals (Pratchett 2010).

Rapid declines in abundance of crown-of-thorns starfish following almost total consumption of coral prey (e.g., Chesher 1969; Moran 1986) may also be partly attributable to pathogenesis, whereby severe limits to energetic, nutrient, or vitamin intake increases susceptibility to opportunistic pathogens that are ubiquitous in the marine environment. Mills (2012) demonstrated that *Acanthaster planci* at high densities with *ad libitum* access to food invest far greater energy into immune defence compared to individuals living at low densities, presumably as a response to the increased likelihood of infection at high densities. If *A. planci* continue to invest considerable energy in immune defence even when prey are scarce, energy reserves may be rapidly depleted and actually make starfish more prone to disease (Rivera-Posada 2012). Accordingly, Sutton et al. (1988) showed that bacteria isolated from diseased *A. planci* did not induce disease when injected into healthy (well fed) individuals. Sutton et al. (1988) also showed that animals collected from reefs with limited coral cover were particularly prone to disease, as opposed to starfish collected prior to extensive depletion of coral resources. Similarly, significant incidence of

disease was recorded in Fiji during the late 1980s when *Acanthaster* spp. settled onto reefs that had been almost completely denuded of potential prey (Zann et al. 1990).

Despite anecdotal and opportunistic observations of pathogenesis in *Acanthaster* spp., there has been very little rigorous research on the vulnerability of these starfish to disease. Several potentially pathogenic organisms, including *Vibrio owensii*, *Vibrio rotiferianus*, *Vibrio fortis*, *Vibrio harveyi*, *Vibrio natriegens* and *Photobacterium eurosenbergii* have been isolated from *Acanthaster planci* (Rivera-Posada et al. 2011a, b). Birkeland and Lucas (1990) also reported a marine bacterium described as Type A bacterium, which was found on >50% of individuals examined, and appears to be a specific symbiont of *Acanthaster planci*. Zann et al. (1990), meanwhile, attributed the mass mortality of juvenile *A. planci* in Fiji to a sporozoan pathogen. However, this pathogen was not isolated or identified. Ongoing research on pathogenesis in *Acanthaster* spp. may provide critical insights for understanding outbreak dynamics, but may also be utilized to control outbreak populations (e.g., Rivera-Posada et al. 2012, 2013) and thereby, reduce ongoing degradation of coral reef habitats in the Indo-Pacific.

2.6 Managing outbreaks of crown-of-thorns starfish

Managing coral reefs in the face of significant and increasing disturbances (e.g., climate induced coral bleaching and coral disease) and increasing anthropogenic pressures associated with burgeoning populations in many tropical nations (e.g., Bell et al. 2009) represents a considerable challenge; more so, because most major disturbances cannot be managed directly. Outbreaks of *Acanthaster* spp. are one of the few disturbances on coral reefs that may be amenable to direct intervention, either by increased efficiency of direct control (Rivera-Posada et al. 2012), development of

effective biological controls (Endean 1969), or by ultimately addressing the anthropogenic factors (e.g., eutrophicaton and/ or overfishing) that may have initiated or exacerbated outbreaks near heavily populated or highly modified coastal environments (Kenchington and Kelleher 1992; Brodie and Waterhouse 2012). Irrespective of their cause(s), control of outbreaks of *Acanthaster* spp. may provide the best opportunity to reverse ongoing coral loss and reef degradation throughout the Indo-Pacific (Endean and Cameron 1990), which is important for sustaining not only the ecological integrity of reef systems, but also in sustaining the critical goods and services that are derived from coral reef environments (Pratchett et al. 2008). Further, emerging threats posed by climate change provide a renewed imperative to try and reverse the ongoing degradation of coral reef ecosystems, in order to maximize the adaptive capacity and resilience of coral reef organisms (Hughes et al. 2003). If outbreaks of *Acanthaster* spp. could be prevented, it is suggested that this alone could reverse sustained declines on the GBR (De'ath et al. 2012). The same is probably true for other locations in the western Pacific, where outbreaks of *Acanthaster planci* have recurring and devastating effects on coral assemblages.

2.6.1 Direct control

Since the first documented outbreaks of *Acanthaster* spp. in the 1950s and 1960s, the immediate response has been to try and collect or kill the burgeoning numbers of starfish (e.g., Barnes 1966; Raymond 1986). These responses reflected the pervading view, that outbreaks of *Acanthaster* spp. were a direct consequence of anthropogenic changes in the marine environment and posed a real and immediate threat to the future of coral reefs. Despite subsequent controversy about the extent to which outbreaks may or may not be natural (Sapp 2000), there have continued to be very extensive and

widespread control efforts aimed at eradicating or excluding *Acanthaster* spp. from reef environments and thereby minimizing damage caused to coral assemblages (**Figure 2.12**). The most common methods used to protect reef areas from crown-of-thorns starfish are hand collections of individual starfish for disposal onshore (e.g., Yamaguchi 1986), or injections with toxic chemicals to kill starfish *in situ* (e.g., Johnson et al. 1990). Early eradication programs also cut *Acanthaster* spp. into small pieces and left them on the reef, but this process is not recommended due to their phenomenal capacity to survive and regenerate after significant physical damage (Owens 1971; Sweatman and Butler 1993; Messmer et al. 2013). Several other initiatives, such as the construction of physical barrier or fences to exclude starfish from accessing areas of live coral have also been attempted (e.g., Bell et al. 1986), but are only feasible on very small scales. In the past five decades (mostly since the 1970) at least 17 million starfish have been killed or removed from reefs across the Indo-Pacific, mainly in southern Japan and on Australia's GBR (**Figure 2.12**), at an estimated cost of US$15-44 million. Given the extensive and widespread efforts to eradicate outbreaks of crown-of-thorns starfish it is clear that the collective of coral reef scientists and managers have already come to a decision about whether we *should* intervene and control outbreak populations of *Acanthaster* spp., therefore the question now is whether we *could* intervene and control outbreak populations of *Acanthaster* spp.

The largest and longest running control program for *Acanthaster planci* was undertaken in Japan, where approximately 13 million starfish were collected between 1970 and 1983, at an estimated cost of US$6-7 million (Yamaguchi 1986). Despite this prolonged effort, chronic infestations of *Acanthaster* spp. still killed in excess of 90% of coral across vast areas of fringing reefs. The few examples where control programs have been effective, either in eradicating crown-of-thorns starfish from areas of reefs, or

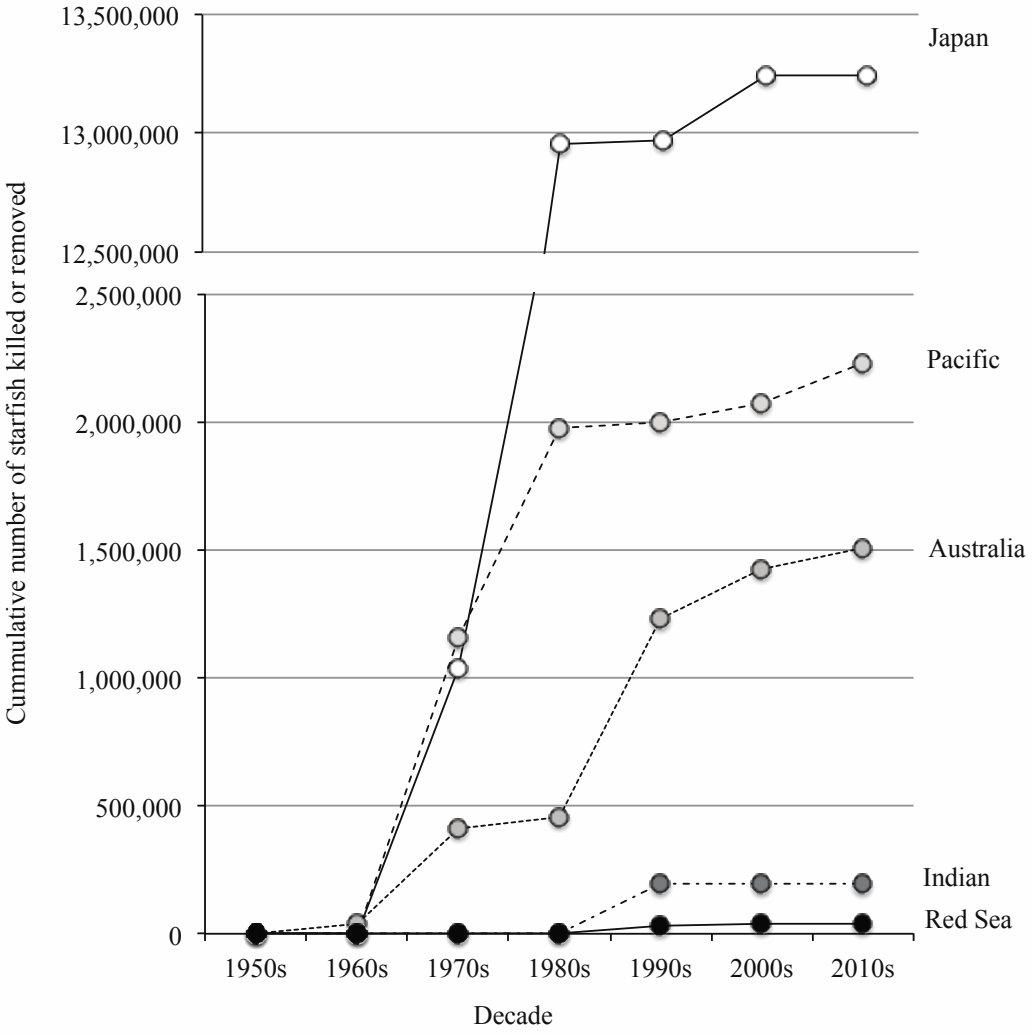

Figure 2.12 Cumulative number of crown-of-thorns of starfish that have been killed *in situ* or removed from coral reefs across the Indo-Pacific, beginning with eradication programs on Australia's Great Barrier Reef and in Southern Japan in the 1960s.

preventing significant declines in local coral cover, have tended to be very small in spatial scale (Moran et al. 1988; Birkeland and Lucas 1990). Perhaps even more important is the early detection and rapid response to apparent increases in the density of *Acanthaster* spp. Fisk and Power (1999) suggested that effective controls are only ever going to be feasible on very small areas of reef, and considered starfish control to be important tactical responses to maintain coral cover at important tourism sites. However, this requires a significant longterm commitment to detect and eradicate starfish that continue to invade from outside of the areas being protected. Fisk and Power (1999) showed that control efforts would need to be undertaken every 1-2 weeks throughout the course of an outbreak, while effectiveness of localised control efforts is greatly increased by explicitly targeting known aggregations of starfish that occur outside the area being protected. The feasibility and effectiveness of large-scale (e.g., reef wide) control programs has been continually questioned (e.g., Kenchington and Pearson 1982), because it remains unclear whether measures required to effectively protect small patches of reefs can be achieved simply by scaling up efforts (e.g., number of diver hours) in proportion to reef area. Even if such scaling were relevant, it would require an inordinate effort to protect entire reef systems, such as the GBR (Birkeland 1989b; Endean and Cameron 1990; Bos et al. 2013). However, it may be feasible to protect individual reefs; and for the GBR, effective control of primary outbreaks on a small number of reefs may be all that is required, if these localized episodes are the basis of subsequent reef-wide outbreaks (Kenchington 1977; Moran et al. 1988; Reichelt et al. 1990a; Fabricius et al. 2010).

A widely used method for eradicating *Acanthaster planci* is to inject starfish with sodium bisulphate (e.g., GBR, Hoey and Chin 2004; Papua New Guinea, Pratchett 2009a), which is considered to have limited side effects for other reef organisms and

also one of the most efficient methods for directly killing starfish *in situ*. Even so, each starfish has to be extracted from the reef matrix and then injected multiple times all over the oral disk in order to ensure complete mortality. Significant increases in efficiency could be achieved by using a toxin that could be administered with a single dose, and anywhere on the starfish. Rivera-Posada et al. (2012) demonstrated that single injections of low concentrations of oxbile and oxgall induced rapid death of *A. planci*, representing a novel and potentially efficient method for controlling crown-of-thorns starfish. The recommended protocol involves injecting each starfish just once, preferably in the upper portion of one of the arms, with 10 ml of 8-10 $g.l^{-1}$ of oxbile mixed in freshwater (Rivera-Posada et al. 2013). In tank-based trials 100% of starfish injected with 10 ml of 8 $g.l^{-1}$ of oxbile died within 24 hours, (Rivera-Posada et al. 2013). Moreover, there was no evidence of unintended side effects for other coral reef organisms (e.g., scleractinian corals, echinoderms and fishes). This method could increase the number of starfish that can be killed on a single dive by a factor of ten, since each starfish need only be injected once and it is not necessary to extract the animals from the coral. Field trials are currently underway; if successful, this will be a significant advancement in improving the efficiency of control programs, which has been deemed necessary before attempting large-scale reef-wide controls of *Acanthaster* spp. (e.g., Bos et al. 2013).

This discovery that oxbile could be used to effectively kill *Acanthaster planci* arose from research on the pathogenesis of crown-of-thorns starfish (Rivera-Posada 2012). Initially, thiosulfate-citrate-bile-sucrose agar (TCBS), which is the primary plating medium used for the isolation and growth of *Vibrio* bacteria (Kobayashi et al. 1963), was injected into starfish in order to explore naturally occurring bacteria on and in *A. planci* (Rivera-Posada 2011a, b). However, *A. planci* that were injected with

TCBS rapidly developed symptoms of bacterial infection (Rivera-Posada et al. 2011a), which under the right conditions was spread among nearby starfish. Initiation of disease following injection of TCBS into *Acanthaster planci* was attributed to increased activity of resident *Vibrio* bacteria. However, the proteins in TCBS (peptone, oxgall and yeast) also caused an allergic reaction in crown-of-thorns starfish, thereby contributing to their rapid mortality (Rivera-Posada et al. 2012). Similarly, gastric mucin from pigs was shown to be toxic to *Acanthaster* spp. in the 1980s (Lassig 1991).

Current research (e.g., Rivera-Posada et al. 2013) is focussed on the use of oxbile to control outbreaks of crown-of-thorns starfish, which does not invoke transmissible disease, and thereby ensures that only injected starfish will actually die. Ultimately, however, it may be possible to exploit the inherent susceptibility of echinoderms to disease (Rivera-Posada 2012) in order to control outbreak populations of *Acanthaster* spp. The use of transmissible diseases would greatly increase the effectiveness of control efforts, but also introduces the risk of interspecific transmission (mainly to other species of echinoderms). Caballes et al. (2012) tested for disease transmission between *Acanthaster* spp. and another common starfish on coral reefs, *Linckia guildingi*. After exposure (and direct contact) to diseased *A. planci*, four out of the five *L. guildingi* developed skin lesions. Moreover, *Vibrio rotiferianus*, which was previously reported as a pathogen isolated from lesions of experimentally infected crown-of-thorns starfish, was also isolated from lesions on the surface of *L. guildingui*. Importantly, all *L. guildingi* fully recovered after 14-46 days, but these findings highlight the need for extensive testing of potentially pathogenic organisms, before they can be used for biological control.

There are several other organisms such as sporozoans, fungi and ciliates that could potentially induce pathogenesis in *Acanthaster planci*. The key is to identify

potential pathogens that have selective effects on *Acanthaster* spp., while minimizing the risk of secondary infections among other coral reef organisms (e.g., Byrne et al. 1997a). Protozoan parasites, for example, often have very high host specificity, which reduces the risk of interspecific transmission of inducible diseases. There are also several other parasitic organisms that are known to infect echinoderms that warrant further investigation. Three genera of gregarine sporozoans that infect the digestive tracts of echinoderms are *Cystobia*, *Lithocystis* and *Urospora*. *Cystobia* species (*Cystobia grassei*, *Cystobia holothuriae*, *Cystobia irregularis*, *Cystobia schneiden*) causes disease in gut-associated haemal system and coelomic cavity of echinoderms (Jangoux 1987a, b) inducing the same clinical signs of disease described by Zann et al. 1990. Another potential parasite is the ciliate *Orchitophyra stellarum* that is found in the testes of the asteroid *Asterias rubens*; which mostly parasitizes male gonads causing a progressive breakdown of germinal tissue (Byrne et al. 1997a), thereby rendering the starfish sterile. There are also protophytan algae (e.g., *Coccomyxa ophiura* and *Coccomyxa astericola*) that cause lethal lesions in the body wall of asteroids and ophiuroids. It is currently unknown whether these pathogens can infect *Acanthaster* spp., or whether infection will necessarily lead to increased mortality or reduced reproductive output. However, the potential for greatly increasing the efficiency and effectiveness of existing control efforts warrants further research, all the while recognising the need for careful and rigorous tests of the risks involved.

2.6.2 Addressing ultimate causes of crown-of-thorns starfish outbreaks

Irrespective of increases in the efficiency of direct controls, these solutions will only ever provide temporary or short-term solutions for minimizing effects of *Acanthaster* spp. on coral reef ecosystems (Birkeland and Lucas 1990). Longterm or

permanent solutions meanwhile, will require improved understanding of the ultimate cause(s) of outbreaks. In situations where outbreaks are initiated or exacerbated by anthropogenic effects, be it elevated nutrient concentrations associated with terrestrial runoff (Fabricius et al. 2010) or overfishing (e.g., Dulvy et al. 2004), these causes need to be explicitly addressed in order to reduce the likelihood that outbreaks will recur, or at least reduce the frequency of future outbreaks (Fabricius et al. 2010). Changes to water quality and fisheries management are already being implemented in some locations, as they represent 'no-regret' strategies: Improvements in water quality (by minimizing run-off of sediments, nutrients and pollutants), and reductions in fisheries exploitation (largely through the increasing establishment of no-take marine protected areas) are likely to increase the resilience of reef ecosystems (e.g., Fabricius 2005; Wooldridge 2009; McCook et al. 2010), even if they do not actually limit outbreaks of *Acanthaster* spp.

Declining water quality in coastal environments (due to land clearing, urbanization and coastal development, and increased fertilizer use in adjacent catchments) is a pervasive threat to coral reefs throughout the world (Fabricius 2005). On the GBR, changed land use in adjacent catchments is suggested to have resulted in a six-fold increase in nitrogen and a nine-fold increase in the amount of phosphorus entering the GBR lagoon (Kroon et al. 2012). Increasing nutrients directly affect the abundance, composition and resilience (especially, reproductive capacity) of reef-building corals, and increase relative abundance of macroalgae or heterotrophic filter feeders (Fabricius 2005; Fabricius et al. 2005), leading to overall degradation of reef ecosystems. For this reason, the Australian Governement has invested $375 million in 'Reef Plan', which aims to reduce inorganic nitrogen loads in runoff from catchments along the Queensland coast down to 50% by 2020, along with equivalent reductions in

pesticide loads, and thereby eliminate detrimental effects on the health and resilience of the GBR (Anon 2013). Spatially explict modelling suggests that improvements in water quality across the GBR will reduce macroalgal cover by 39% and increase diversity of hard corals by 16% on a subset of reefs (28%) that are currently exposed to water quality conditions outside of recommended thresholds (De'ath and Fabricius 2010). Moreover, these benefits are additional to any reductions in the frequncy of outbreaks of *A. planci* that might occur as a consequence of improved water quality (De'ath and Fabricius 2010).

The number of no-take marine protected areas on coral reefs has increased considerably in recent decades, coincident with accelerating reef degradation and increasing expectations of the benefits that no-take areas can provide (Graham et al. 2011). No-take areas are one of the foremost strategies used to reduce exploitation of coral reef fishes, and have definitive benefits for abundance and biomass of fishes, especially large-bodied, predatory fishes (Graham et al. 2011). The ecological consequences of removing large predatory fishes from coral reef ecosystems are poorly understood, but may include cascading effects upon ecosystem structure, function and diversity (Dulvy et al. 2004). Most notably, Dulvy et al. (2004) revealed a direct relationship between densities of predatory fishes (associated with differences in local fishing pressure) and the local severity of outbreaks of *Acanthaster planci* in Fiji. Similarly, Sweatman (2008) showed that there was a lower frequency of outbreaks of the *A. planci* in no-take areas on the GBR, compared to fished areas. Despite these observations, the mechanistic basis for an ecological link between exploited fishes and the local incidence of crown-of-thorns outbreaks remains uncertain. It may be that predatory fishes are important in regulating the abundance of *Acanthaster* spp., but only

at very specific and early stages in their life history (Rivera-Posada et al. 2014), but this requires further research across a range of different locations.

Whatever the underlying mechanism, Sweatman (2008) suggests that increases in the areal extent of fisheries closures may reduce the reef-wide incidence of outbreaks of *Acanthaster* spp. This effect may also be amplified if fewer reefs with starfish outbreaks mean less effective propagation of outbreaks from reef to reef through the central GBR. More importantly, coral cover was also shown to be higher inside no-take areas on the GBR, compared to fished areas (McCook et al. 2010), which is an obvious consequence reduced incidence of outbreaks. More generally, the evidence that no-take areas benefit coral assemblages is limited (Graham et al. 2011), and differences in coral cover between areas that are open versus closed to fishing are most apparent where destructive fishing methods lead to declines in coral cover on fished reefs (e.g., Baird et al. 2005). However, no-take areas are likely to have benefits extending far beyond the protection of exploited fish species, and are considered fundamental to sustaining the resilience of coral reef ecosystems (Hughes et al. 2007).

2.7 Conclusions

Outbreaks of *Acanthaster* spp. are considered to be one of the most significant disturbances affecting coral reefs in the Indo-Pacific (Pearson 1981; Birkeland 1996b; Bruno and Selig 2007). In his review of especially influential species and potentially 'keystone species' on tropical coral reefs, Birkeland (1996b) devoted a considerable portion of his discussion to *Acanthaster* spp. The importance of crown-of-thorns starfish is also reflected in the plethora of studies (at least 1,200 published studies) on this single species or species complex (see reviews by Potts 1981; Moran 1986; Birkeland and

Lucas 1990, and references therein). In spite of this considerable research effort, many questions about the biology of *Acanthaster* spp. remain unanswered, which greatly limits the understanding and hence the potential to manage outbreaks. The most fundamental question is what regulates the normal abundance of *Acanthaster* spp., thereby preventing chronic infestations of starfish throughout the Indo-Pacific.

There is no doubt that the *Acanthaster* spp. are predisposed to major population fluctuations, due to inherent properties of their life history (e.g., immense fecundity, short generation times), and it is easy to conceive how small changes in distribution or behavior could lead to rapid and pronounced increases in the abundance of starfish (Potts 1981; Moran 1986; Birkeland and Lucas 1990; Fabricius 2013). Most notably, there is likely to be very limited reproductive success (specifically, fertilization success) in low density, pre-outbreak populations of *Acanthaster* spp. where individuals tend to be highly dispersed (Endean and Cameron 1990). This may be further exacerbated by strongly male-biased sex ratios observed in some low-density populations (e.g., Caballes et al. unpublished data). If however, there is a chance aggregation of male and female crown-of-thorns starfish, then the number of resulting progeny will go from effectively zero to many millions virtually overnight. However, this alone cannot explain periodic outbreaks, because there is likely to be very strong selection for *Acanthaster* spp. to aggregate every time they spawn, whereas outbreaks tend to occur at most every 7-15 years (Potts 1981). It is more likely that slow and progressive increases in the abundance of *Acanthaster* spp. on reefs that have very small persistent populations, are a necessary precursor to a marked increase in reproductive success, which may then initiate outbreaks (Moore 1990). The reproductive biology of *Acanthaster* spp. is geared towards extensive but infrequent spawning, which would be expected to lead to marked inter-annual fluctuations in reproductive success and

recruitment, yet for much of the time and on most reefs within their range, crown-of-thorns starfish occur at very low (almost negligible) densities (Birkeland and Lucas 1990). This suggests that there are other regulatory factors that limit reproductive success, larval survival, settlement rates, and/or post-settlement growth and survivorship.

The many hypotheses used to explain the initiation of outbreaks (or relax normal regulatory processes) of *Acanthaster* spp. are not mutually exclusive and their importance is very likely to vary both spatially and temporally (Birkeland and Lucas 1990; Bradbury and Antonelli 1990). The critical question is what, if anything, can be done to reduce the frequency and/ or severity of crown-of-thorns outbreaks, and thereby minimize or even reverse sustained declines in abundance of corals in many parts of the Indo-Pacific? The threat of the ensuing reef degradation to coastal fisheries and other reef-based industries (e.g., tourism) suggests that tropical nations should invest heavily in the eradication of *Acanthaster* spp., especially if reef-wide controls are potentially feasible. The effectiveness of control programs will be further enhanced by focussing on detecting and eradicating primary outbreaks before they can initate widespread secondary outbreaks (Fabricius 2013). Longterm or permanent solutions depend on definitive knowledge and appropriate actions to address the ultimate cause(s) of outbreaks. However, no-regret strategies can be implemented even in the absence of complete knowledge: improvements in water quality and reductions in fisheries exploitation should benefit the resilience of reef ecosystems, and may reduce long-term effects *Acanthaster* spp. whether or not they reduce the incidence of outbreaks.

Chapter 3

Reproductive biology and early life history of the crown-of-thorns starfish[2]

3.1 Introduction

Coral reefs are one of the most productive and diverse, yet also one of the most fragile, ecosystems on the planet. Global degradation of coral reef ecosystems has been increasing at an alarming rate over the past several decades (Bruno and Selig 2007; Gardner et al. 2003). Human activities such as overexploitation and destructive fishing practices, coastal pollution (leading to declining water quality, increased sedimentation, and eutrophication), habitat destruction through unsustainable development, and the spread of invasive species have been the major drivers of widespread and accelerating declines in coral cover and diversity (Mumby and Steneck 2008; Wilkinson 2008). Aside from causing increasing mortality of reef-building corals, the degradation of reef ecosystems is also linked to increasing prevalence of disease (Bruno et al. 2007; Harvell et al. 2007), algal blooms resulting from overfishing (Hughes et al. 2007; Mumby et al. 2006) or mass mortalities of herbivores (Lessios 1988), and outbreaks of coral predators (Pratchett et al. 2009a; De'ath et al. 2012; Baird et al. 2013), all of which are undermining the resilience of reef ecosystems (Hughes et al. 2003). These perennial anthropogenic pressures, are also now being compounded by emerging threats associated with climate change, such as ocean acidification (Hoegh-Guldberg et al.

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[2] Published as:

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2007), sea-level rise (Blanchon and Shaw 1995), bleaching and increasing thermal stress (Pratchett et al. 2013; Reynolds et. al. 2014), and increasing frequency and intensity of tropical cyclones (Fabricius et al. 2008).

Throughout the Indo-Pacific, one of the most important disturbances on coral reefs continues to be outbreaks of crown-of-thorns starfish, *Acanthaster* spp.[3] (Bruno and Selig 2007; De'ath et al. 2012; Pratchett et al. 2014; **Figure 3.1**), which can cause extensive coral loss over very large scales with major consequences for the structure of coral assemblages, but also many other reef-associated organisms. Around the island of Guam in Micronesia, for example, it was reported that in a 2 ½ year period, 90% of corals were killed along a 38-km shoreline (Chesher 1969). On the Great Barrier Reef (GBR), outbreaks that commenced in 1962 killed 80% of scleractinian corals down to a depth of 40 meters at Green Island (Pearson and Endean 1969). Similar levels of coral loss were recorded in reefs around the Ryukyu Islands in southern Japan during the same period (Yamaguchi 1986). More recent surveys and reports indicate that crown-of-thorns starfish outbreaks still remain as one of the principal causes of widespread decline in live coral cover in these locations and in many other locations such as Samoa, French Polynesia, Papua New Guinea, Fiji, Vanuatu, Philippines, and Indonesia (Pratchett et al. 2014).

Despite increasing research attention and management concern around emerging threats to coral reefs from global climate change (e.g., Hoegh-Guldberg and Bruno 2010), the impact of crown-of-thorns starfish outbreaks at many locations has been far greater than the combined effects of all other acute disturbances. The GBR has lost over

[3] Molecular sampling from populations covering the entire known range of crown-of-thorns starfish revealed that there are four strongly differentiated clades with restricted locations (Pacific, northern Indian Ocean, southern Indian Ocean, Red Sea), which probably represent distinct species (Vogler et al., 2008).

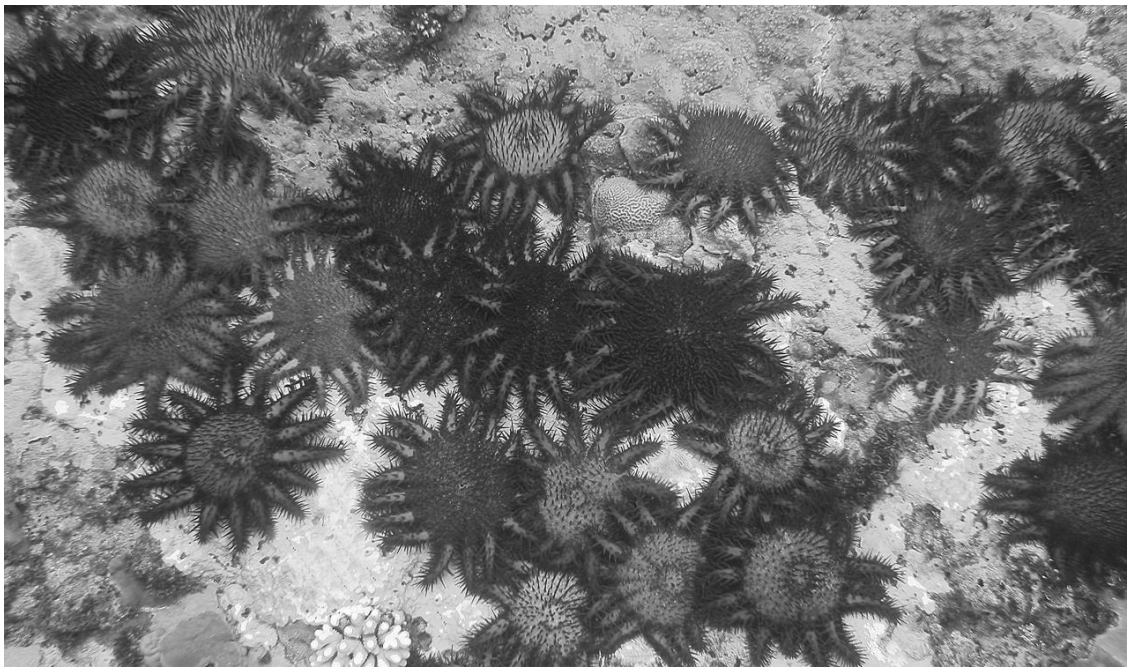

Figure 3.1 Massive aggregation of crown-of-thorns starfish during an outbreak in 2006 at Tanguisson Reef, Guam, Micronesia. (Photograph by C.F. Caballes)

half its coral cover since 1985 and a significant proportion of these estimated losses have been due to crown-of-thorns starfish predation (De'ath et al. 2012; **Figure 3.2A**). Osborne et al. (2011) also show that among the various agents of coral disturbance in the GBR, crown-of-thorns starfish outbreaks were associated with the greatest coral decline and largest distribution of coral loss (**Figure 3.2B**). Similarly, in Moorea, French Polynesia, Trapon et al. (2011) found that the greatest rates of coral loss recorded since 1979 coincided with outbreaks of crown-of-thorns starfish (**Figure 3.2C**). High coral mortality resulting from crown-of-thorns starfish predation can also have effects that cascade throughout the coral reef. Benthic macroalgae tend to immediately colonize newly available space following coral mortality (Belk and Belk 1975), and over longer time frames, habitat-forming hard corals may be replaced by sponges or soft corals (Birkeland and Lucas 1990). Following high levels of coral mortality, inevitable bioerosion of coral skeletons also leads to fundamental changes in the physical structure of coral reef habitats (Seymour and Bradbury 1999) and declines in structural complexity can have major effects on reef fish assemblages (Sano et al. 1987; Graham et al. 2006; Pratchett et al. 2008), typically leading to 60-70% declines in abundance and diversity.

Models suggest that in the absence of crown-of-thorns starfish outbreaks, sustained declines in coral cover could be reversed, despite continuing losses from tropical cyclones and coral bleaching (De'ath et al. 2012). This provides strong incentive for direct and immediate control of crown-of-thorns starfish outbreaks, to minimize ongoing declines in live coral cover and maximize resilience to other major disturbances. Novel methods in controlling outbreaks have improved the efficiency of direct intervention programs (Rivera-Posada et al. 2012, 2013). However, longterm solutions that directly address the proximal cause(s) of outbreaks depend on

understanding key aspects of the life history of crown-of-thorns starfish to establish limitations in recruitment and population replenishment. Despite being one of the most well known reef organisms and despite several decades of research into the biology and ecology of crown-of-thorns starfish, there is still no unified hypothesis on what causes devastating outbreaks. Nevertheless, there is a general consensus among researchers that exploring the reproductive biology and early life history of crown-of-thorns starfish is essential in understanding mechanisms that lead to outbreaks. This is evident in that most hypotheses put forward to explain the initiation and geographical incidence of outbreaks of place considerable importance on factors affecting the reproductive biology and early life history of crown-of-thorns starfish, such as fertilization success, larval ecology, and/or post-settlement survival (Pratchett et al. 2014).

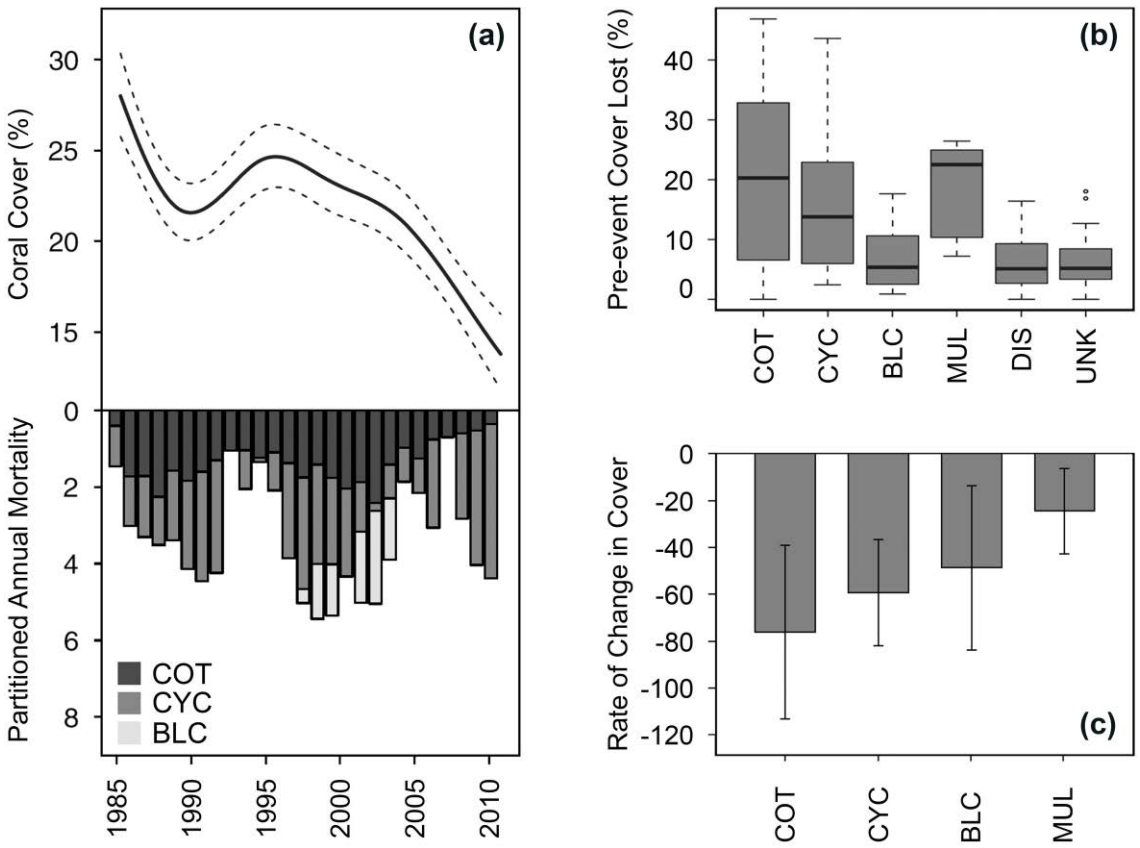

Figure 3.2 Contribution of crown-of-thorns starfish predation to coral loss. **(a)** Coral decline in the Great Barrier Reef, Australia from 1985-2012 and attributed cause of decline (adapted from De'ath et al. 2012 with permission from the National Academy of Sciences of the United States of America); (**b)** Loss of coral cover following major disturbances in the Great Barrier Reef from 1995-2009 (adapted from Osborne et al. 2011); (**c)** Annual geometric rate of change in coral cover following major disturbances in Moorea, French Polynesia (adapted from Trapon et al. 2011). COT = crown-of thorns starfish outbreak, CYC = cyclones/storms, BLC = bleaching, MUL = multiple disturbances, DIS = disease, UNK = unknown causes.

3.2 Hypotheses on the causes of outbreaks

The cause(s) of population outbreaks of crown-of-thorns starfish remains contentious; the three major hypotheses that have been proposed to account for the initiation of outbreaks, including i) the 'natural causes hypothesis' (Vine 1973), ii) the 'predator removal hypothesis' (Endean 1977; Sweatman 2008), and iii) the 'larval starvation hypothesis' (Birkeland 1982; Lucas 1982; Brodie et al. 2005; Fabricius et al. 2010) are based, at least in part, on aspects relating to the reproductive biology and early life history of crown-of-thorns starfish.

Outbreaks can arise from progressive aggregation of adult individuals from different cohorts (Pratchett 2005) or mass settlement of planktonic larvae (Yokochi and Ogura 1987; Zann et al. 1987), but these events are not mutually exclusive. The 'natural causes theory' postulates that outbreaks are inherent population instabilities expected from highly fecund organisms with planktotrophic larvae, such as crown-of-thorns starfish (Uthicke et al. 2009). Fine-scale monitoring throughout the course of an outbreak by Pratchett (2005) demonstrated that outbreaks at Lizard Island, in the northern GBR resulted from a prolonged build-up of starfish numbers through multiple successive recruitment events. Moreover, the accumulation of starfish arose independently from any sudden or substantial increase in rates of recruitment.

With the frequency and intensity of recent outbreaks, doubts have been raised whether the current regime of population outbreaks (e.g., on Australia's GBR) could have been sustained over evolutionary timeframes (Randall 1972; Birkeland and Lucas 1990). It has also been pointed out that almost all of the major outbreaks have occurred near centers of human populations (Chesher 1969; Randall 1972; Nishihira and Yamazato 1974). Therefore, there is a strong belief that anthropogenic activities and the degradation of coastal environments have either caused or exacerbated outbreaks of

crown-of-thorns starfish (e.g., Fabricius et al. 2010). Major hypotheses supporting this view include the 'predator removal hypothesis' and the 'larval starvation and terrestrial runoff hypothesis' (Birkeland 1982; Lucas 1982; Brodie 1992; Brodie et al. 2005)

The 'predator removal hypothesis' infers that crown-of-thorns starfish populations are normally regulated by high rates of predation on post-settlement life stages and that outbreaks arise as a consequence of the release from predation pressure due to overharvesting of actual predators (Endean 1977), or resulting from subsequent trophic cascades (Dulvy et al. 2004). While it has never been explicitly considered, it is also possible that there are important predators on larvae, especially during settlement. Several species (mostly coral reef fishes) have been recorded to prey upon juvenile and/ or adult crown-of-thorns starfish (reviewed by Pratchett et al. 2014). Significant levels of predation by triggerfishes and pufferfishes on adult crown-of-thorns starfish were observed in the Red Sea and feeding rate calculations demonstrated that this could account for reductions in numbers from outbreak densities of approximately 2000 adults down to around 5-20 starfish per kilometer reef face (Ormond et al. 1990). The pufferfish, *Arothron stellatus*, had been observed to consume entire small adults (20 cm) in less than 10 minutes (Keesing and Halford 1992a). In addition, Rivera-Posada et al. (2014) observed very high frequencies of sublethal arm damage on medium-sized crown-of-thorns starfish (11-20 cm), which can be used as an index of predation (McCallum et al. 1989). It is expected however, that predation rates would be highest immediately after settlement. Benthic epifauna have been found to be important predators of small crown-of-thorns starfish that are very cryptic and are often inaccessible to fish predators (Keesing and Halford 1992a, b). Indirectly, fishing pressure on large piscivores can lead to outbreak populations by reducing the densities

of benthic carnivorous fishes and relieve predation pressure on invertebrates that feed on small crown-of-thorns starfish (Sweatman 2008).

The larval starvation and terrestrial runoff hypothesis suggests that enhanced nutrient supply from river runoff, usually after periods of extremely heavy rainfall around high islands and continental land masses, elevates levels of primary production resulting in a phytoplankton bloom, which enhances the survival of crown-of-thorns starfish larvae through decreased mortality from starvation (Lucas 1982) or through more rapid larval development, decreasing exposure to other sources of mortality such as predation (Birkeland and Lucas 1990). Importantly, given the very high fecundity of crown-of-thorns starfish (e.g., Conand 1984; Kettle and Lucas 1987), very slight changes in larval survival could lead to substantial differences in local rates of recruitment. Aside from river runoff, upwellings and sediment resuspension during storms (Furnas and Mitchell 1986), and broad oceanographic features like the transition zone chlorophyll front (Houk et al. 2007) could also be responsible for enhanced phytoplankton levels. On the other hand, *in situ* culturing experiments by Olson (1987) showed that crown-of-thorns starfish larvae develop at near-maximal rates in the absence of phytoplankton blooms, suggesting that fluctuation in larval food resources may be of little importance in explaining interannual variation in larval recruitment.

3.3 Reproductive biology

3.3.1 Gender differentiation and gonad morphology

Crown-of-thorns starfish are dioecious, sexually reproducing starfish (Yamaguchi 1973a). While it is not possible to distinguish sexes based on external morphology, the gender of individuals can be determined by drawing contents from gonads along the

arm junction using a syringe with a large-bore biopsy needle. Individual eggs from female specimens are visible while sperm appear as white streaks that turn the solution cloudy. Alternatively, sexes can be distinguished by making a small incision on the proximal region of the arm and examining gonad clusters, so long as the individuals are gravid and mature (e.g., Pratchett et al. 2014). Rows of gonad clusters are found along each side of the inner wall of each arm. Testes usually appear cream or pale yellow in color and have smaller, more numerous lobes compared to ovaries, which appear as larger, more spherical, yellow or orange lobes. Immature gonads are often cream to pale yellow in color while spent gonads are usually brown. The sex of individuals with immature or spent gonads can only be determined histologically (Birkeland and Lucas 1990).

Gonads increase in size as individuals get bigger and become sexually mature. When examined histologically, the width of spermatozoa zones in testis lobules and the oocyte diameter in ovaries are correlated with variations in gonad size (Lucas 1973). Each arm has 14-18 gonad clusters of similar size on each side, except for those closest to the distal portion of the arm, which are usually smaller than the rest (Conand 1983). There is typically minimal variation in gonad maturity and size (i.e. weight or volume) between arm rays of individual starfish. Kettle and Lucas (1987) recommended using the average weight of gonads from three arms to calculate the gonadosomatic index. However, Yokochi and Ogura (1987) found considerable variation in gonad weight between arms, suggesting that entire starfish must be dissected and processed to estimate total reproductive allocation. At the very least, small regenerating arms must be excluded as the size and maturity of gonads may significantly deviate from normal arms (but see Bos et al. 2013).

3.3.2 Gametogenesis

The asteroid gametogenic cycle usually consists of three major stages: accumulation of nutrients to be utilized during gametogenesis, proliferation of gonial cells and differentiation into gametes, and a spent period where residual gametes are reabsorbed (Mercier and Hamel 2009). Histological observations of testes (**Figure 3.3**) reveal that mature spermatozoa are present throughout the year, even when the gonad indices are lowest (Yamazato and Kiyan 1973). However, there is a cyclic change in the thickness of the spermatogenic layer surrounding the tubule, which contains spermatogonia, spermatocytes, and spermatids. Changes in the thickness of the spermatogenic layer reflect the spermatogenetic activities of the testes, i.e. it is thinnest right before spawning when the large central region of mature testis lobes are packed with spermatozoa. With the release of mature spermatozoa, the spermatogenic layer gradually thickens while the proliferation of spermatogonia, mitotic and meiotic divisions resulting in spermatocytes and spermatids, and differentiation into spermatozoa continue. Examination of histological sections of ovaries (**Figure 3.4**) show that changes in the size and modality of oocytes within ovary lobes reflect the stages of oogenesis (Lucas 1973). Developing ovaries contain a full range of oocyte sizes, with the larger oocytes concentrated in the center of the ovarian tubule and the smaller oocytes attached to the ovarian wall. Mature ovary lobes contain large oocytes with a single, well-defined mode (Yamazato and Kiyan 1973).

The length, timing, and patterns of the reproductive cycle in echinoderms may be regulated by the interplay of hormonal (intrinsic) and environmental (extrinsic) factors (Mercier and Hamel 2009). Gametogenesis is controlled by the nervous and/ or endocrine systems of the starfish. Oocyte maturation in crown-of-thorns starfish is induced by the release of a neurohormonal peptide, also known as a gonad-stimulating

substance (GSS), produced by the radial nerves (Henderson 1969; Henderson and Lucas 1971). When stimulated by GSS, the ovarian tissues produce a 1-methyladenine, which initiates meiosis in oocytes and generates gonad wall tension to expel gametes (Kanatani 1969).

High intraspecific variability in reproductive cycles between populations exposed to different environmental variables suggests that extrinsic factors also play an important role in modulating gametogenic processes. Temperature is considered as one of the principal factors that control gametogenesis in crown-of-thorns starfish, such that peak reproductive activity is often observed during the warmest months of the year or when temperatures are close to 28°C (Pratchett et al. 2014). On the GBR, where there is a clear annual reproductive cycle for crown-of-thorns starfish that peaks during the Austrsl summer (November to December), gametogenesis takes around three months (Lucas 1973). However, the influence of temperature is less clear in areas where seawater temperatures undergo minimal seasonal changes. For example, in low-latitude locations such as Guam and Palau, gametogenesis seems to be staggered among different individuals so that reproduction appears protracted throughout the year (Cheney 1974; Idip 2003). Moreover, food availability and nutrient storage can also influence gamete production in echinoderms (see Mercier and Hamel 2009). In asteroids, nutrients are processed and stored in the pyloric caecum (Lawrence 1987). Prior to gonad growth, active feeding by starfish results in an increase of pyloric caecum size (Mauzey 1966). Several studies have demonstrated an inverse relationship in the growth of pyloric caeca and gonads in starfish, suggesting an energy transfer from pyloric caeca to gonads during gametogenesis (see Chia and Walker 1991). However, very few studies have examined the influence of food availability on crown-of-thorns starfish gametogenesis. Cheney (1974) reported that total deprivation of coral

food for one month by caging resulted in shrinking of gonads, deterioration of pyloric caeca, and decrease in total diameter. Conversely, gravid crown-of-thorns starfish collected from Okinawa, which were starved for 90 days, showed no change in the size and condition of gonads even though pyloric caeca were reduced to thin ribbons (Okaji 1991). Understanding the effect of food intake on reproductive potential of crown-of-thorns starfish, is potentially very important in understanding the initiation of outbreaks and should be a priority for future research.

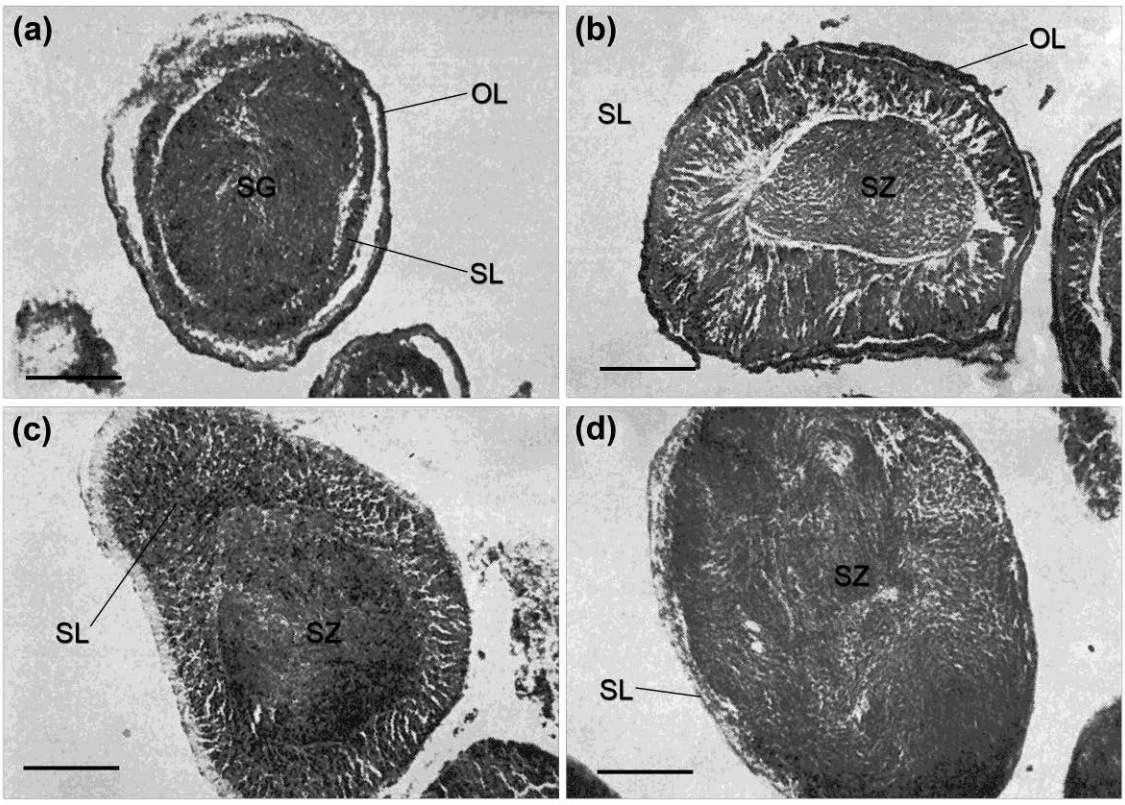

Figure 3.3 Histological sections of crown-of-thorns starfish testes representing different stages during spermatogenesis: **(a)** immature testis with three distinct layers: **OL** = outer layer, **SL** = spermatogenic layer; **(b)** developing testis with thickening spermatogenic layer and **SZ** = spermatozoa accumulating in lumen; **(c)** spermatogenic layer reaching maximum thickness; **(d)** mature testis packed with spermatozoa and with negligible spermatogenic layer (Adapted from Yamazato and Kiyan 1973 with permission from the University of Guam). Scale bars = 0.1 mm.

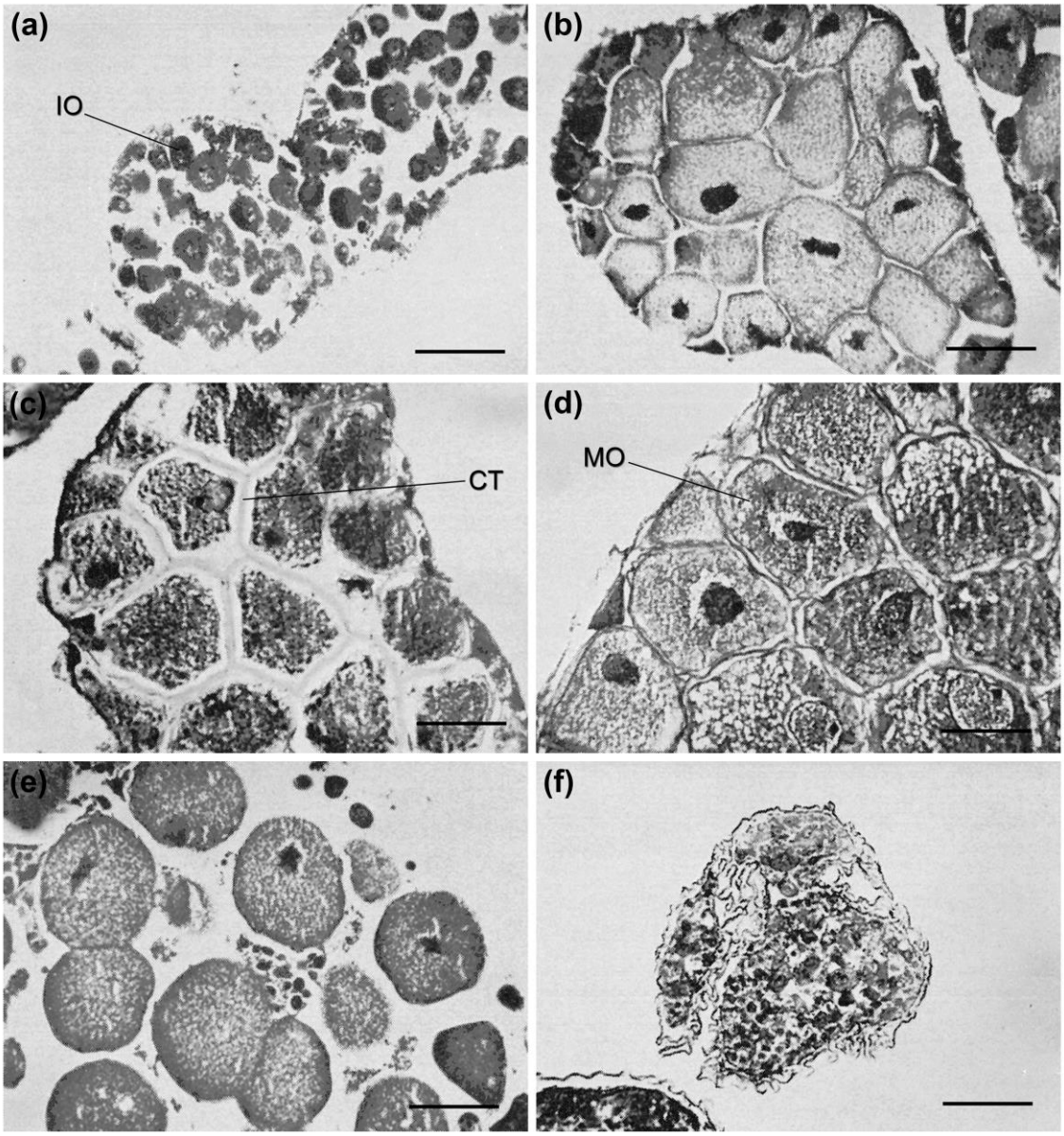

Figure 3.4 Histological sections of crown-of-thorns starfish ovaries representing different stages during oogenesis: **(a)** immature ovary with previtellogenic oocytes; **(b)** developing ovary with a range of oocyte sizes, smaller oocytes attached to the ovarian wall; **(c)** connective tissues surrounding mature oocytes are conspicuous; **(d)** mature ovary densely packed with mature oocytes of generally uniform size; **(e)** mature oocytes liberated from lumen; **(f)** spent ovary. **IO** = immature oocytes; **CT** = connective tissues; **MO** = mature oocytes (Adapted from Yamazato and Kiyan 1973 with permission from the University of Guam). Scale bars = 0.1 mm.

3.3.3 Fecundity

Like most asteroids with planktotrophic larvae and large body size, crown-of-thorns starfish are highly fecund – a single mature female is capable of producing over 60 million eggs in a single season (Conand 1985). Fecundity increases exponentially as the starfish grows; therefore larger individuals have a higher reproductive input per spawning period, especially in populations that are multimodal (**Figure 3.5**). The ratio of gonad weight over total body weight also increases exponentially, indicating that reproduction is progressively prioritized, at the expense of the body wall and pyloric caeca (Kettle and Lucas 1987). These fecundity values, however, come from aggregated populations on reefs with high coral cover and from locations where there are distinct seasonal patterns of seawater temperature. Fecundity may be influenced by the nutritional status of individuals, as it is related to growth. To date, no study has looked into possible tradeoffs between fecundity (i.e. no of eggs) and egg size in a low food availability scenario. Studies on the fecundity of crown-of-thorns starfish from low latitude reefs with relatively stable temperatures are also warranted.

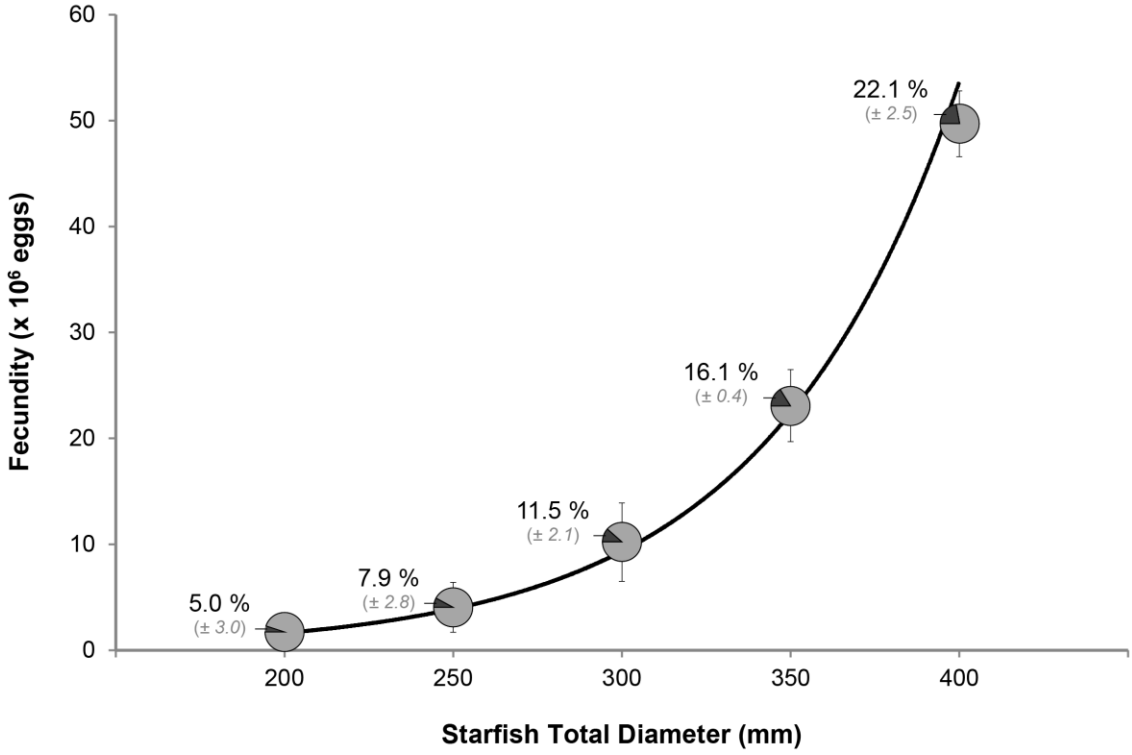

Figure 3.5. Relationship between starfish size and fecundity. Dark grey sections of pie charts represent proportion of gonad weight (values ± SD) in relation to total body weight. Data compiled from studies by Conand (1985) in Noumea, New Caledonia and by Kettle and Lucas (1987) in the Great Barrier Reef, Australia.

3.3.4 Spawning

Spawning behavior of crown-of-thorns starfish is very conspicuous. Individuals show characteristic arching posture on top of elevated coral heads or rocks, waving of arms, and vigorous tube feet activity before and during spawning (Babcock 1990; Babcock and Mundy 1992a, b; Gladstone 1992). Slight increases in gamete release height could potentially enhance downstream dispersal, especially at rapid flow rates (Metaxas et al. 2002). Gametes are shed from aboral rows of gonopores along the sides of each arm. Exudates from spawning females appear as translucent spherical grains (**Figure 3.6A**), while males exude milky clouds of sperm (**Figure 3.6B**). Eggs are slightly negatively buoyant while sperm are neutrally buoyant (Benzie et al. 1994). Spawning has been observed in both aggregated and dispersed populations (Pratchett et al. 2014**)**. Babcock and Mundy (1992a) noted that crown-of-thorns starfish were unusually active 2 hours prior to spawning and counted more exposed individuals during spawning compared to after spawning, where several starfish retreated back under corals. Gladstone (1992) also observed starfish coming out from under the ledges and climbing on top of coral heads to spawn, and then moved back beneath ledges after spawning. In Okinawa, Okaji (1991) found that a dispersed population spawned later, but only partially, and had a more prolonged spawning period compared to aggregated populations, which synchronously spawned at the peak of the spawning season.

Observations of spawning of crown-of-thorns starfish in the field are rare and often fortuitous. Most observations come from relatively higher latitudes (e.g. Japan and Central Great Barrier Reef, Australia), where major spawning periods are shorter and more predictable (Pratchett et al. 2014). Babcock and Mundy's (1992a, b) work at Davies Reef in the GBR during the 1990 to 1992 spawning seasons has been the most extensive monitoring of natural spawning in the field to date. On the evening (2145 hrs)

of 7 December 1990, Babcock and Mundy (1992a) witnessed the spawning of 38 female and 50 male starfish out of the 129 starfish they counted along their transects at a maximum depth of 7 m. Ten days after this major spawning event, a minor spawning involving three male crown-of-thorns starfish was witnessed at 1700 hrs, where there was no arching and sperm were exuded from gonopores between only a few arms (Babcock and Mundy 1992a). Other starfish near the spawning male did not spawn. Around the same time (5 December 1990), Gladstone (1992) also observed natural spawning of crown-of-thorns starfish at Blue Pearl Bay, Hayman Island in the Whitsunday region of the GBR. An individual male starfish, hunched on top of a coral head at 2.5 m depth, started spawning at 1510 hrs and successive spawnings by other starfish began 4-55 minutes later. Spawning lasted for 36-92 minutes and the sole female spawned for the shortest time and was the last to begin spawning. Sampling of gametes during and after the spawning season also revealed that spawning individuals may not necessarily shed all gametes at one time (Babcock and Mundy 1992a; Yasuda et al. 2010).

Inferring from the few *in situ* observations of spontaneous spawning (Pratchett et al. 2014), crown-of-thorns starfish seem to spawn during the warmest months or when sea surface temperatures are around 28°C, and during the falling tide, around late afternoon to evening. However, there are several exceptions and to date, no predictable timing cue to explain spawning synchrony has been discovered. Although the role of temperature in timing reproductive cycles is well recognized, it does not directly explain the synchronous commencement of spawning. Other environmental cues and chemically mediated communication between individuals may act as final triggers for spawning. Previous studies linking peaks in phytoplankton abundance to spawning induction in some species of chiton, mussels, and sea urchins (Himmelman 1975; Starr

et al. 1990) has led to the suggestion that crown-of-thorns starfish may use chemical cues from phytoplankton blooms as the final trigger for spawning. Phytoplankton associated cues may be more reliable signals to synchronize spawning because it integrates other factors that improve larval success. For marine invertebrates with planktotrophic larvae, such as crown-of-thorns starfish, larval survival is often strongly influenced by food availability (Fabricius et al. 2010), thus one critical advantage of phytoplankton as a spawning cue is ensuring that there is abundant food for larvae. However, this mechanism has not been explicitly tested among crown-of-thorns starfish and there is very little evidence that peak abundance of larval food supply induces spawning in asteroids (see Mercier and Hamel 2009). Chemically mediated communication between gravid individuals may be crucial in the final stages right before and during gamete release. The difference in the timing of spawning between males and females can also be inferred from *in situ* observations. Males usually spawn before females (see Pratchett et al. 2014), but there are also examples of females initiating mass spawning events (Babcock and Mundy 1992b). It is possible that chemical cues associated with sperm can induce spawning in females or vice versa. Laboratory experiments by Beach et al. (1975) revealed that pheromones extracted from crown-of-thorns starfish ovaries and testes synchronize spawning in neighboring starfish and induce movement towards the spawning individual. In comparing spawning between dispersed and aggregated populations, Okaji (1991) suggested that aggregated individuals receive spawning stimuli at a higher frequency and magnitude compared to dispersed individuals, thereby accounting for better synchronization and higher reproductive output.

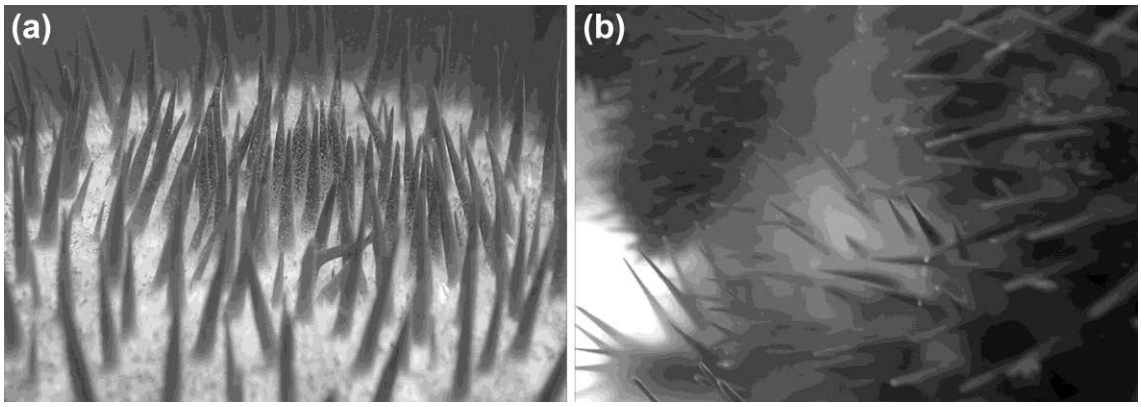

Figure 3.6 Gamete release in crown-of-thorns starfish. (**a**) female starfish shedding egg granules; (**b**) male starfish shedding a cloud of sperm. (Photographs by C.F. Caballes)

3.3.5 Fertilization success

Rapid dilution and diffusion of gametes presents a major challenge for the reproductive success of broadcast spawning benthic invertebrates, such as crown-of-thorns starfish. Broadcast spawners release copious amounts of gametes during spawning, but often suffer from low fertilization rates unless individuals aggregate, synchronize gamete release, and are located in low to moderate flow conditions (Mercier and Hamel 2009). Observations of natural spawning in the field have involved isolated or lone individuals or only a small proportion of the population (e.g., Pearson and Endean 1969; Babcock and Mundy 1992a,b; Gladstone, 1992). Despite these constraints, crown-of-thorns starfish still attain high rates of recruitment, which suggests that these animals can achieve exceptionally high fertilization rates (**Figure 3.7**). Fertilization rates during natural spawning of crown-of-thorns starfish could reach up to 83% at the peak of a major spawning event (Babcock and Mundy 1992a). Moreover, Benzie et al. (1994) suggest that fertilization rates for spawning events that happen early in the breeding season of crown-of-thorns starfish from the GBR are slightly higher compared to spawning events at end of the breeding season. In induced spawning experiments, fertilization rates were close to 100% when male and female starfish are next to each other (Babcock et al. 1994). As expected, fertilization rates drop significantly as the distance between spawning individuals increases. Nevertheless, 70% fertilization success was still achieved at distances of up to 8 m between spawning individuals and more than 20% at a distance of 60 m (**Figure 3.7** and citations therein). Fertilization success in crown-of-thorns starfish is slightly higher compared to other asteroid species (**Figure 3.7**) and significantly greater than those reported for other invertebrates (Yund 1990; Grosberg 1991; Levitan et al. 1991; Babcock and Keesing 1999). Fertilization rates increased at higher sperm concentrations (Uthicke et al. 2013).

Although high fecundity and high production of gametes may increase the overall number of fertilized zygotes produced (Babcock and Mundy 1992a; Babcock et al. 1994), it does not appear to influence fertilization rate, which is defined as zygote production per capita. Benzie and Dixon (1994) suggest that high fertilization rates reflect the inherent capacity of crown-of-thorns starfish sperm for enhanced fertilization success at given sperm concentrations, at greater distances, and at longer durations from the point of gamete release. There is some evidence for variation in fertilization success between eggs with normal (i.e. spherical) and irregular morphology (Ayukai et al. 1996), but the role of parental nutrition on egg quality and the presence of endogenous chemicals in gametes that can enhance fertilization still need to be thoroughly investigated. There are also very few studies on the role of abiotic factors on crown-of-thorns starfish fertilization success. In looking at the potential effects of near-future ocean acidification on crown-of-thorns starfish recruitment, Uthicke et al. (2013) found that low pH (down to 7.6) reduced sperm motility and velocity, which resulted in much lower fertilization rates. However, cross-factorial experiments showed no significant difference between different combinations of temperature and pH treatments (Kamya et al. 2014). Thermal enhancement of fertilization, as an effect of increased swimming speeds and sperm-egg collisions, has been previously demonstrated in other echinoderms (Mita et al. 1984).

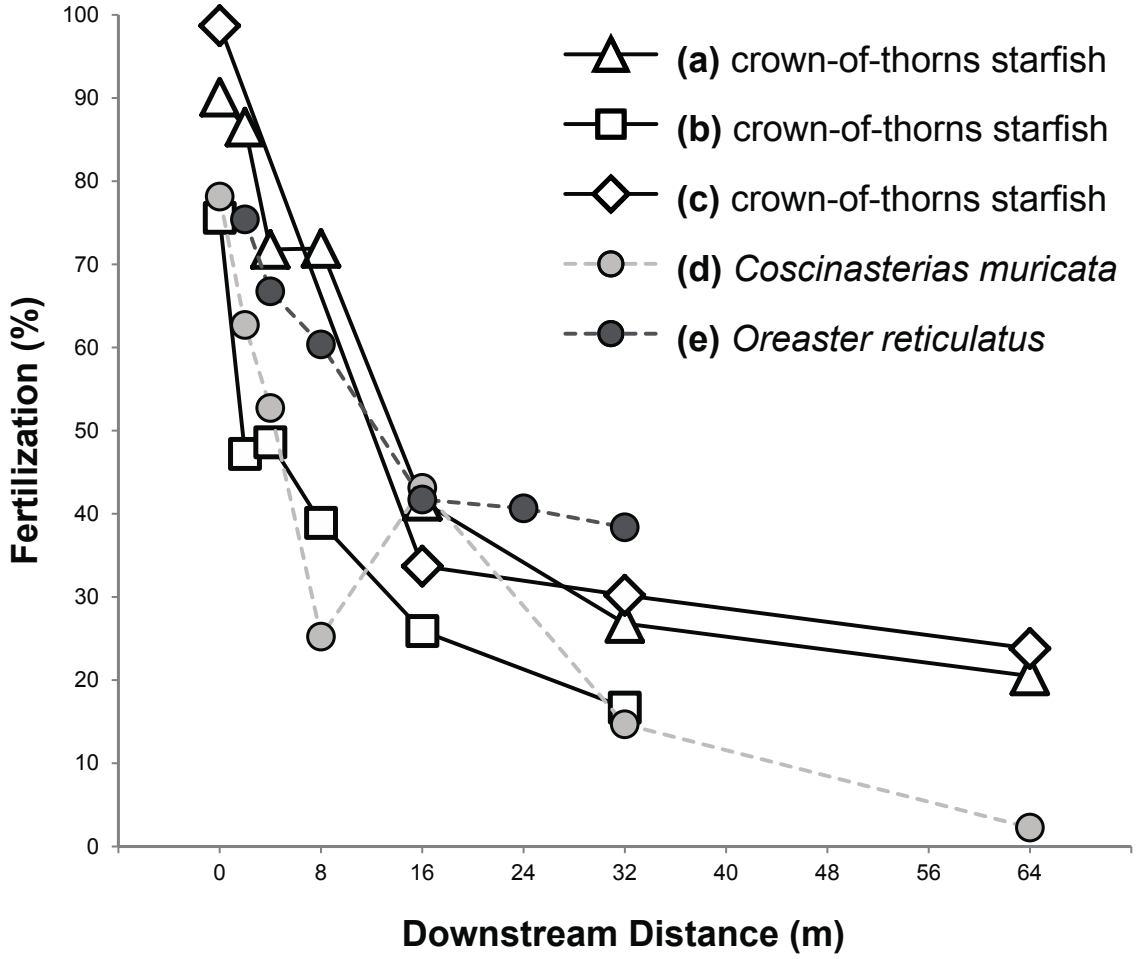

Figure 3.7 Fertilization success in crown-of-thorns starfish compared to other asteroids. (**a**) crown-of-thorns starfish, one male, Davies Reef, GBR, Australia (Babcock et al. 1994(; (**b**) crown-of-thorns starfish, one male, Sesoko Island, southern Japan (Babcock et al. 1994); (**c**) crown-of-thorns starfish, five males, Davies Reef, GBR, Australia (Babcock et al. 1994); (**d**) *Coscinasterias muricata*, one male, Whangateau Harbor, Auckland, New Zealand (Babcock et al. 2000); (**e**) *Oreaster reticulatus*, one male, Norman's Pond Cay, Lee Stocking Island, Bahamas (Metaxas et al. 2002).

3.4 Larval Ecology

3.4.1 Planktonic larval stages

Following fertilization, zygotes undergo rapid changes and go through cleavage divisions, forming morulae, blastulae, and gastrulae (Birkeland and Lucas 1990). Free-swimming larvae hatch during the early stages of the gastrula stage and initially pause on the bottom before swimming to the surface via ciliary movement that causes the body to rotate on its long axis (Yamaguchi 1973a). There are two major planktonic larval phases in the crown-of-thorns starfish life cycle: (1) the bipinnaria, which develops from endogenous resources invested by the parent into the egg, and (2) the brachiolaria, which takes in food from its environment and develops the starfish primordium (McEdward and Miner 2001). Bipinnaria larvae are characterized by bilateral arrangement of the pre- and post-oral ciliated swimming and feeding bands, while brachiolaria are characterized by the presence of brachiolar arms and attachment disk on the pre-oral lobe (**Figure 3.8**). After 2-4 days, early bipinnaria larvae complete the alimentary canal and start filter feeding on unicellular algae and other suspended particulate matter (Yamaguchi 1973a). Larvae then proceed to the brachiolaria stage where it develops brachiolar arms (**Figure 3.8**), which will be eventually used to locate favorable substrate prior to settlement (Henderson and Lucas 1971). The factors and processes that affect these planktonic and planktotrophic stages have received a great deal of attention in the past years mostly to test hypotheses based on the notion that larval survival leads to increases in the number of crown-of-thorns starfish recruits. Among the biological, ecological, physical, and environmental factors and processes that impact the planktonic larval stages of crown-of-thorns starfish, larval nutrition (affects growth and survival) and larval transport (dispersal of larvae to suitable

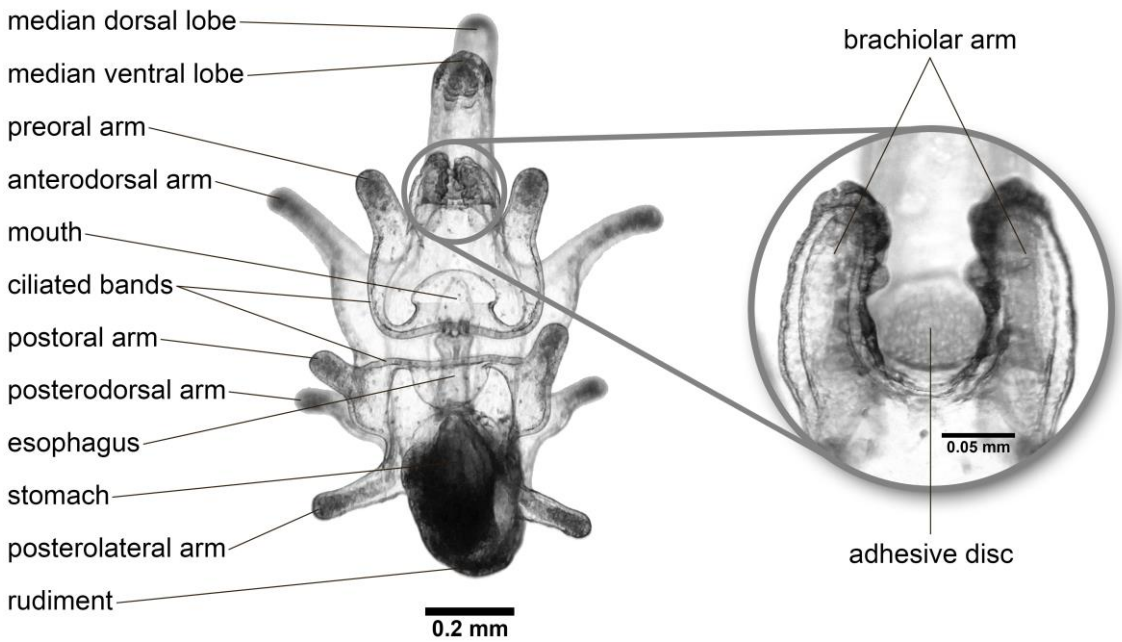

Figure 3.8 General anatomy of the brachiolaria larval stage of crown-of-thorns starfish prior to settlement and metamorphosis. (Photograph by C.F. Caballes)

locations for settlement and recruitment) are perhaps the most studied. Although equally important, only a few studies have investigated the role of predation and abiotic factors (*e.g.* temperature and salinity) on larval development.

3.4.2 Nutritional requirements for larval stages

Phytoplankton have generally been considered as the main food source of crown-of-thorns starfish larvae and have been used in the laboratory to rear larvae through complete development, either as natural phytoplankton or as single or mixed species of cultured unicellular algae (Henderson and Lucas 1971; Lucas 1975 1982; Uchida and Nomura 1987; Okaji 1996; Keesing et al. 1997; Fabricius et al. 2010). Large amounts of phytoplankton food were required to maintain cultured larvae in the laboratory, but phytoplankton concentrations reported for Great Barrier Reef waters were generally low or marginal for the nutritional requirements of crown-of-thorns starfish larvae (Lucas 1982). From this, Lucas (1982) suggested that larvae must be normally food-limited and that it is only during irregular phytoplankton blooms that there would be sufficient food available to complete larval development and actually settle on the reef (Lucas 1982). Furthermore, Birkeland (1982) proposed that enhanced nutrient supply from river runoff, usually after periods of extremely heavy rainfall preceded by droughts around high islands and continental land masses, result in phytoplankton blooms that enhance the survival of crown-of-thorns starfish. However, larvae reared in *in situ* culture chambers showed no sign of food limitation and were able to develop at near-maximal rates under normal conditions (Olson 1987). Artificial enrichment of seawater did not result in increased survivorship and only a slight difference in development rate was recorded. Olson and Olson (1989) concluded that the divergence of their results compared to those from Lucas (1982) might be due to the ability of crown-of-thorns

starfish larvae to utilize other food sources, such as dissolved organic matter (DOM) and bacteria. Hoegh-Guldberg (1994) showed that dissolved free amino acids (DFAA), which are relatively minor components of DOM in seawater, can supply significant amounts of energy for developing larvae. However, natural concentrations of DFAA are probably too low to have a meaningful contribution to the nutritional requirements of larvae (Ayukai et al. 1996). Moreover, microscopic analysis by Ayukai (1994) showed that the diet of crown-of-thorns starfish larvae almost exclusively comprises relatively rare, large phytoplankton, and ultraplankton (<5 μm), but not bacteria.

The extent to which larvae of crown-of-thorns starfish are normally food-limited remains unclear. Brodie et al. (2005) presented the following as evidence on the role of nutrient enrichment in triggering primary outbreaks in the GBR: 1) increased discharge of dissolved inorganic nutrient from rivers associated with agricultural development; 2) discharge of river runoff into coral reef areas in the GBR; 3) elevated nutrient content of river discharge causing phytoplankton blooms and altering phytoplankton community assemblage; 4) aforementioned conditions occasionally coinciding with initiation sites; and 5) increased chlorophyll levels significantly increasing larval survival. Fabricius et al. (2010) also provided further support for the hypothesis that phytoplankton availability predominantly controls primary outbreaks by combining laboratory experiments, historical river discharge and chlorophyll concentration data for the GBR, and crown-of-thorns starfish – coral population model simulations. Experiments showed that the proportion of larvae completing development increases 8-fold with every doubling of chlorophyll concentrations up to 3 μg l^{-1}, while field data and the population model simulations show that increased river discharge during floods and regional differences in phytoplankton availability are strongly related to spatial and temporal patterns in crown-of-thorns starfish outbreaks on the GBR (Fabricius et al. 2010). Aside

from terrestrial runoff, oceanographic features such as the transition zone chlorophyll front can enhance phytoplankton levels and provide ideal conditions for larval survival and has been found to coincide with the spawning season of crown-of-thorns starfish and trigger primary outbreaks in the North Pacific Ocean (Houk et al. 2007). Furthermore, Houk and Raubani (2010) also suggest that high regional productivity associated with anomalous oceanographic conditions coincided with outbreaks in Vanuatu. Despite the growing evidence linking crown-of-thorns starfish outbreaks to enhanced phytoplankton levels, Lane (2012) cautions about the broad applicability of this hypothesis because outbreaks have been occurring despite overarching declines in global oceanic phytoplankton in the past century. Also, there is no evidence of increased incidence of outbreaks in the 'Coral Triangle' area despite frequent phytoplankton blooms associated with periodic high precipitation, upwelling, and high erosion rates (Lane 2012).

3.4.3 Predation on planktonic stages

Larval predation is one of the least studied ecological factors that can potentially regulate larval survival and consequently the population size of adult crown-of-thorns starfish. Prior to settlement, released gametes, swimming larvae, and late brachiolaria searching for suitable settlement substrates must avoid predation. Unlike coral eggs, which are heavily preyed upon by planktivorous fishes, crown-of-thorns starfish gametes and larvae are often avoided upon visual recognition or rejected after tasting by fish and invertebrate predators (Yamaguchi 1973a, 1975). Laboratory experiments by Lucas et al. (1979) showed that eggs and larvae contain saponins, which act as chemical defenses detected by and unpalatable to planktivorous fish. Keesing and Halford (1992b) suggest that because spawning events usually occur late in the afternoon or at

night, the impact of visually orienting planktivores as predators is significantly reduced. Another potential predator of crown-of-thorns starfish gametes and larvae are the corals themselves. Chesher (1969) proposed that predation by filter feeders (e.g. corals) can inflict significant mortality on settling larvae. Ormond and Campbell (1974) found that larvae typically move away from contracted coral polyps and settling brachiolaria larvae are eaten when they come in contact with coral polyps. The polyps of the widely distributed coral, *Pocillopora damicornis*, were observed feeding on the larvae of crown-of-thorns starfish and other coral reef asteroids (Yamaguchi 1973a). The likelihood of this interaction being of major importance is diminished by the fact that there are many areas of coral reef substrata that have relatively low cover of live coral where starfish larvae could settle without being consumed by coral polyps (Reichelt et al. 1990b). The small size of these stages and the rarity of encountering abundant larvae in the field make larval predation logistically difficult to study, thus the lack of empirical data on larval mortality. Further studies are warranted to assess the extent at which predation regulates larval mortality in crown-of-thorns starfish.

3.4.4 Environmental Constraints on Larval Development

Aside from biological and ecological regulation of larval populations, abiotic factors such as temperature and salinity also play an important role in larval development. Crown-of-thorns starfish larvae from the GBR survive over a narrow temperature range; Laboratory-reared larvae completed development at 28-29 °C while those remaining at 24-25 °C did not advance beyond brachiolaria (Henderson and Lucas 1971). Ideal temperature for larval development, at least those from the GBR, seems to be between 28 and 30 °C, where maximum survival and completion were found in laboratory studies (Lucas 1973). Larvae died rapidly at 32 °C, none developed beyond

bipinnaria at 24 °C, and few reached brachiolaria at 26 °C (Lucas 1973). In Guam, larvae were successfully reared at 27-29 °C, while larvae reared at temperatures below 25 °C did not advance to brachiolaria stage and showed regression to earlier stages, even though they were observed to feed vigorously (Yamaguchi 1973a). Similar temperature ranges were used to rear larvae to complete development in the Red Sea (28 and 29 °C; Ormond and Campbell 1974) and southern Japan (fluctuating between 25-30.3 °C; Uchida and Nomura 1987).

The narrow temperature tolerances reported above do not explain the presence of crown-of-thorns starfish populations in areas where temperatures do not reach this range (Yamaguchi 1987). Johnson and Babcock (1994) suggest that the abovementioned narrow developmental temperature tolerances do not apply to all developmental stages. More recent research into the thermal tolerance of crown-of-thorns starfish shows that development rate, normal development and larval size were optimal at 28.7 °C, but development rates remained relatively constant up to 31.6 °C (Lamare et al. 2013). Holoblastic radial cleavage proceeded normally over a 10 °C range, depending on the geographic source or recent history of temperature exposure of parent starfish (Johnson and Babcock 1994). Hatched gastrula larvae can tolerate temperatures between 13 and 34 °C with very minimal mortality (Habe et al. 1989). Gastrula larvae completed normal development to bipinnaria throughout a temperature range of 13 °C (Johnson and Babcock 1994) and bipinnaria larvae can tolerate temperatures at 14.5-32 °C with less than 50% mortality (Habe et al. 1989). The rate of development is also strongly influenced by temperature. Habe et al. (1989) found that embryonic period is 31 hrs at 20 °C but only 11 hrs at 32 °C. The brachiolaria stage appears to be the most temperature-sensitive (Habe et al. 1989), but early larval

hardiness allows larvae to be swept to less cooler waters and slowly continue normal development during larval transport.

Initial studies on the effect of salinity (Lucas 1973) show that larval survival was enhanced up to threefold when salinity was lowered from 35‰ to 30‰; complete larval development and metamorphosis was achieved at 26‰ but not at 22‰ (Lucas 1973). Gastrula larvae tolerated a salinity range of 21‰-45‰ and bipinnaria larvae tolerated 21‰-50‰ salinity (Habe et al. 1989). Henderson (1969) also found that bipinnaria larvae can tolerate abrupt salinity changes from 36‰ down to 21‰ and developed more rapidly at lower salinities. Despite the robust early larval stages, late brachiolaria and metamorphosing stages are less tolerant to salinity and rupture with 2‰ changes in salinity (Henderson and Lucas 1971). In the GBR, salinity is influenced by river discharge (at times of flooding and heavy rainfall) to reef systems closer to the northern Queensland coast and goes down to presumably favorable levels of 30‰ (Brodie et al. 2005).

3.4.5 Larval competency and dispersal distances

Mortality during the pelagic larval stage may not be necessarily due to nutrition, predation, or environmental factors mentioned above, but rather as a result of unfavorable dispersal, which results in the failure of larvae to encounter favorable reef habitat within a limited competency period (Dight et al. 1990). The maximum planktonic larval duration (PLD) for crown-of-thorns starfish is estimated to be 42 days (Lucas 1982), though the optimal period for larval settlement may be much shorter. This aspect of larval survival has many stochastic components that it can account for a large amount of spatial and temporal variation in settlement (Keesing and Halford 1992a). The negatively geotactic larvae of crown-of-thorns starfish are incapable of

settling within the first week after fertilization (Lucas 1973; Olson 1987) and once advected off their natal reefs, may be transported for up to one month before settling (Yamaguchi 1973a), leading to dispersal distances of >1,000 km (Nash et al. 1988; Timmers et al. 2011). The spatial and temporal distribution patterns of crown-of-thorns starfish in the GBR led to the supposition that populations are propagated through larval dispersal resulting from physical and hydrodynamic processes (Kenchington 1977; Pearson and Garrett 1978). Similar assumptions on the role of larval dispersal have been made when correlating the distribution of crown-of-thorns starfish population over time with prevailing hydrodynamic patterns in other regions, such as southern Japan (Yamaguchi 1986), central Red Sea (Moore 1988), and Guam (Caballes 2009).

Based on survey and size-frequency distribution data, Kenchington (1977) postulated that the southward movement of outbreaks in the GBR was the result of a cascade of recruitment down the GBR through larval dispersal from the epicenter in the northern part of Green Island off Cairns, suggesting that there is very limited larval dispersal. Alternatively, Talbot and Talbot (1971) suggested that this pattern is due to the southward migration of adult starfish. Ebert (1983) also argued that Kenchington's (1977) size-frequency analysis could be explained by latitudinal variation in growth rates rather than as a large-scale wave of recruitment. However, graphical modeling of outbreak patterns for the period 1966-1989 supports the idea that transport of starfish larvae by ocean currents is the principal mechanism by which starfish populations disperse over large distances and found that the southward drift of outbreak activity is consistent with speed and direction of average summer currents on the GBR, although the concept of a discrete seed area was not supported. Moran et al. (1992) analyzed the pattern of movement of outbreaks for the periods 1966-1974 and 1979-1991, and confirmed the southward drift of outbreaks in the GBR and also found a 'weak' pattern

of progressive northward movement of outbreaks over time, both originating from latitude 16°S. Moreover, modeling of variation in ocean circulation within the GBR (Dight et al. 1990) has identified asymmetries in reef connectivity resulting from larval dispersal and are able to account for: 1) the southward spread of crown-of-thorns starfish populations from the region of Green Island; 2) the high incidence of outbreaks on mid-shelf reefs in central GBR; and 3) the susceptibility of some reefs to repeated recruitment, notably Green Island and Feather reefs (Dight et al. 1990). Similarly, broadscale patterns of larval dispersal generated from model simulations were in strong qualitative agreement with observed spatial and temporal distribution of adult crown-of-thorns starfish populations in the GBR (James and Scandol 1992). Using the same models, Scandol and James (1992) also found that outbreaks generally occur more frequently in the inner and central matrix reefs in the GBR and the overall impact of starfish populations undergoing an outbreak on the reef system decreases with a southward shift in the location of initial outbreaks. Hydrodynamics may also affect recruitment densities on different parts of individual reefs, particularly in areas characterized by retention cell and eddies (Black and Moran 1991). Periods of slow, low frequency, longshore currents result in abnormally high natal larval recruitment and may be a critical factor associated with primary outbreaks in the GBR (Black et al. 1995).

Phylogeographic studies have shed light on the long-range dispersal of crown-of-thorns starfish larvae and also considered as the most efficient of the very few means of providing information on the origin of recruits to outbreaking populations (Benzie and Stoddart 1988; Benzie 1992). Genetic data have been obtained from the GBR (Nash et al. 1988; Nishida and Lucas 1988; Benzie and Stoddart 1992a, b; Benzie and Wakeford 1997; Benzie 1999; Vogler et al. 2008; Yasuda et al. 2009; Vogler et al. 2013), from the

Red Sea (Vogler et al. 2008), from several sites throughout the Indian Ocean (Benzie 1999; Yasuda et al. 2009; Gérard et al. 2008; Vogler et al. 2008; Vogler et al. 2012), and from various locations throughout the Pacific Ocean (Nishida and Lucas 1988; Benzie 1999; Katoh and Hashimoto 2003; Vogler et al. 2008; Yasuda et al. 2009; Timmers et al. 2011; Timmers et al. 2012; Vogler et al. 2013). High larval dispersal and gene flow was implied in earlier population genetic studies using allozyme genetic markers, which showed low levels of differentiation within the GBR and across the Pacific. Nash et al. (1988) examined the genetic structure of crown-of-thorns starfish populations in the GBR and found evidence of considerable gene flow among all the populations surveyed – from Lizard Island in the north to One Tree Island in the south, which are separated by about 1,300 km. Subsequent studies by Nishida and Lucas (1988) demonstrated low genetic divergence among populations throughout the Pacific, although the crown-of-thorns starfish population from Hawaii was most differentiated from all the other populations. Benzie and Stoddart (1992b) likewise found that the Lord Howe Island population formed a discrete outlier from all other Australian populations. Gene flow to these peripheral populations is most likely restricted and sporadic with only a few migrant recruits reaching these islands, thus producing a strong founder effect (Benzie 1992). Regardless of these outliers, low levels of divergence among Pacific populations have been interpreted as indicators of high larval dispersal and widespread reef connectivity. There was however, a marked genetic discontinuity between Pacific and Indian Ocean populations, which were also congruent with observed distribution of color morphs (Benzie 1999).

Population genetic studies have also sought to test the southern drift hypothesis and whether or not outbreaks are independent events. Benzie and Stoddart (1992b) examined the level of inter-population variation among outbreaking and non-

outbreaking populations in the GBR and found greater differentiation among non-outbreaking populations, suggesting that most outbreaks are directly propagated from initial (primary) outbreaks and are not independent events. Benzie and Wakeford (1997) also found no significant genetic differentiation between populations of a given age class, between age classes within populations, and between GBR outbreak populations 10 years apart (1986 and 1996), all suggesting that outbreak populations were derived from the same genetic source. Similarly, Katoh and Hashimoto (2003) found high genetic similarity between samples from two outbreaks in Okinawa that were 15 years apart (1982-1983 in Chatan; 1997 in Onna). They explained that allele frequencies were kept similar because relatively large populations of crown-of-thorns starfish were maintained around Okinawa even in non-outbreak periods during that span of time.

The conclusions drawn from allozyme data, however, may be misleading, as an absence of genetic structure does not necessarily imply high gene flow and widespread larval dispersal (Williams and Benzie 1997; Gérard et al. 2008; Vogler et al. 2013). Recent studies have developed novel methods to address these shortcomings. For example, the complete mitochondrial genome of *Acanthaster* spp. has been sequenced (Yasuda et al. 2006a) and a total of 16 polymorphic microsatellites have been isolated from *A. planci* (Yasuda et al. 2006b, 2007). Using these microsattelite markers, Yasuda et al. (2009) detected genetically distinct groups in accordance with ocean current systems and restricted gene flow among samples in accordance with geographical distances. Similar to previous studies (Nishida and Lucas 1988; Benzie and Stoddart 1992b; Benzie 1999), there was a large genetic break between Indian and Pacific Ocean populations, high gene flow within northwestern Pacific and GBR groups, and some structure in relation to peripheral groups (Yasuda et al. 2009). Gérard et al. (2008) assessed the variability and accuracy of phylogeographic signal of three mitochondrial

loci (COI 16S rDNA, and tRNA) and found that tRNA genes are three times less divergent than COI and 16S rDNA genes. Using COI sequences from samples covering the entire distribution of crown-of-thorns starfish, Vogler et al. (2008) described four deeply diverged clades that form a pan-Indo-Pacific species complex. Using the highly variable mitochondrial control region, Timmers et al. (2012) found substantial genetic differentiation among central Pacific populations and less genetic exchange among regions and archipelagos, compared to within archipelagos (Timmers et al. 2010). This is in contrast to previous reports of high gene flow throughout the Pacific that were assumed to reflect high dispersal potential (Nishida and Lucas 1988; Yasuda et al. 2009). Moreover, Vogler et al. (2013) reported high levels of genetic structure and significantly limited genetic exchange among Pacific Ocean populations, although the high larval dispersal potential of crown-of-thorns starfish may also be achieved as evidenced by gene flow between populations isolated in the past and high levels of genetic connectivity among distant populations. Vogler et al. (2012) also reported a very strong and modest genetic structure in the northern Indian Ocean and southern Indian Ocean species, respectively. These patterns of divergence are hypothesized to arise from past and present ocean circulation patterns, regional differences in ocean primary productivity and varying demographic histories (Vogler et al. 2012).

3.5 Settlement and metamorphosis

Larvae usually develop to brachiolaria stage competent for settlement after 11 days, although the rate of development may vary from 9 to 42 days depending on temperature (Henderson and Lucas 1971) and food availability (Lucas 1982). As larvae increase in size, brachiolar arms also elongate to improve locomotion while supporting

the weight of the starfish primordium (Olson et al. 1988). Towards the end of the brachiolaria stage, larvae start to drift downward and flex the anterior body dorsally to orient the brachiolar arms against the substratum (**Figure 3.9**) to test its suitability for settlement (Yamaguchi 1973a). Based on laboratory experiments (Henderson and Lucas 1971; Ormond et al. 1973; Yamaguchi 1973a; Ormond and Campbell 1974; Lucas 1975; Johnson et al. 1991; Keesing and Halford 1992a; Johnson and Sutton 1994) and field observations (Zann et al. 1987; Yokochi and Ogura 1987), larvae seem to show strong settlement preferences for certain kinds of substrates. Lucas (1975) suggested that settlement preference is not a major factor affecting the survival of crown-of-thorns starfish larvae as long as larvae are transported over a coral reef while they are still competent to settle. However, laboratory experiments by Ormond and Campbell (1974) show that larvae do not settle on live corals and coral polyps have been observed to prey on settling larvae (Ormond et al. 1973; Yamaguchi 1973a). Chesher (1969) suggested that aggregations of adult crown-of-thorns starfish attract settling larvae by feeding on coral polyps that would otherwise be predatory to larvae. Dead coral skeletons of *Acropora hyacinthus* were favored by brachiolaria larvae over skeletons of *Acropora diversa*, *Pocillopora verrucosa*, and *Stylophora pistillata* (Ormond and Campbell 1974). Larvae seem to thrive in rough surfaces or depressions that enclose the metamorphosing larvae (Lucas 1975). Ormond and Campbell (1974) suggested that *A. hyacinthus* skeletons were favored over the other coral skeletons they tested because the shape, size, and texture of calices suit metamorphosing larvae. There is little or no settlement on clean glass or clean ceramic tiles (Henderson and Lucas 1971; Ormond and Campbell 1974; Johnson et al. 1991), although larvae settle on the bottom of glass culture dishes with biological film (e.g. algal detritus, encrusting algae) on the surface (Henderson and Lucas 1971).

Observations of recently settled crown-of-thorns starfish in Suva Reef, Fiji (Zann et al. 1987) and Ryukyu Islands, Japan (Yokochi and Ogura 1987) indicate that larvae settle on crustose coralline algae (CCA). Yamaguchi (1973a) observed larvae directly settling on dead coral encrusted by CCA (*Porolithon* sp.), but found no settlement on bleached coralline algae or on pieces of beach rock covered with filamentous algae (but see Henderson and Lucas 1971). Johnson et al. (1991) also found high rates of settlement on coral rubble and the CCA, *Lithothamnium pseudosorum*, but reported significantly lower settlement on fouled ceramic tiles, non-calcareous crustose red algae (*Peyssonellia* sp.), and other species of CCA (*Porolithon onkodes*, *Neogoniolithon foslei*). Techniques developed for large-scale culture of crown-of-thorns starfish larvae from the GBR have achieved high rates of settlement on thalli of *L. pseudosorum* (Ayukai et al. 1996, Keesing et al. 1997).

Substratum specificity in crown-of-thorns starfish settlement appears to be chemically mediated. Chesher (1969) earlier implied conspecific chemoattraction of settling larvae towards feeding aggregations of adult crown-of-thorns starfish. However, extracts of adult crown-of-thorns starfish applied to coral skeleton substrate did not increase rates of settlement (Ormond and Campbell 1974). Likewise, recently depredated *Pocillopora damicornis* corals and addition of crown-of-thorns starfish spines and tube feet on the substrate did not increase settlement rates (Henderson and Lucas 1971). When larvae were separated from *L. pseudosorum* by mesh, high rates of settlement was still observed, suggesting that settlement induction can occur without contact to CCA and may be mediated by compounds consisting of large molecules (Johnson et al. 1991). However, bioassays with common marine invertebrate settlement inducers, γ-amino butyric acid (GABA) and potassium chloride (KCl) at different

concentrations, did not induce settlement and metamorphosis in crown-of-thorns starfish larvae (Johnson et al. 1991).

Treatment of highly inductive shards of *L. pseudosorum* with antibiotics reduced settlement to low levels, signifying that induction of settlement and metamorphosis of crown-of-thorns starfish may be mediated by chemical cues produced by epiphytic bacteria (Johnson et al. 1991). Settlement and metamorphosis was inhibited in the absence of bacteria and larvae always settled on sections of thallus having high densities of bacteria, but not in areas where epiphytic bacteria are sparse (Johnson et al. 1991; Johnson and Sutton 1994). However, surface bacteria were not inductive when isolated from soluble algal compounds, suggesting that bacteria require the algal substrate to produce inductive compounds or that compounds from both the bacteria and CCA are required to induce settlement and metamorphosis (Johnson and Sutton 1994).

Larvae experience high mortality during settlement and metamorphosis. Yamaguchi (1973a) suggested that high mortality during settlement of laboratory-cultured larvae was due to predation by benthic epifaunal organisms. Still, Keesing and Halford (1992a) reported 85% mortality on apparently competent larvae on *L. pseudosorum* substrate that were carefully cleaned of epifauna. Further examination of the processes that regulate survival during settlement and metamorphosis will be important in determining how this stage in the early life history of crown-of-thorns starfish can influence recruitment patterns at much larger spatial scales (Johnson 1992).

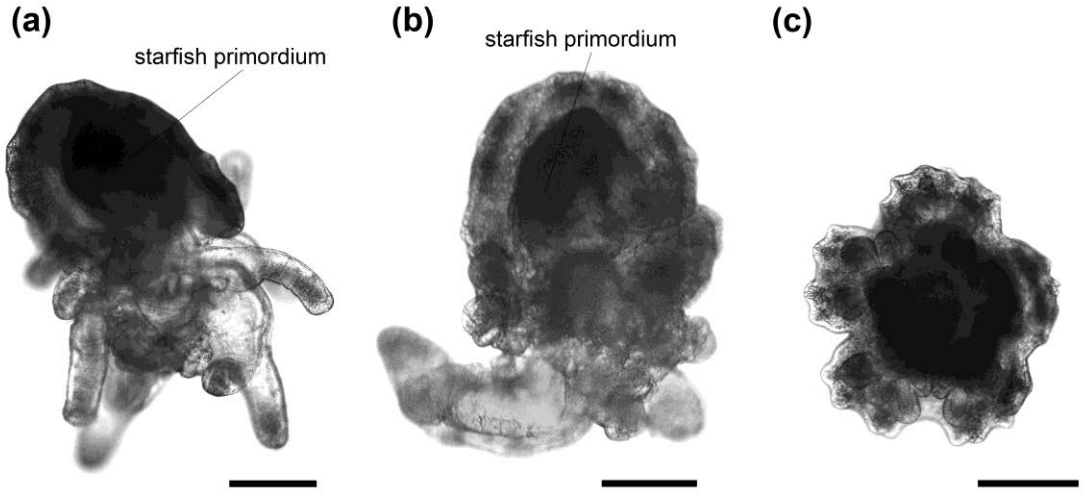

Figure 3.9. Settlement and metamorphosis of crown-of-thorns starfish: (**a**) Negatively buoyant late brachiolaria larvae exploring suitable settlement substrate; (**b**) Newly settled and metamorphosing larvae starting to absorb larval body; (**c**) Juvenile starfish, 24 hrs after metamorphosis. Scale bar = 2 mm (Photographs by C.F. Caballes)

3.6 Post-settlement growth and survival

Following settlement, metamorphosis of crown-of-thorns starfish brachiolaria larvae occurs with the absorption of the anterior part of the larval body into the starfish primordium (Yamaguchi 1973a) and emerges two days later as a five-armed juvenile starfish 0.3 to 0.7 mm in diameter with two pairs of tube feet, a terminal tentacle, and a red optic cushion on each arm (Henderson and Lucas 1971; Yamaguchi 1973a; Lucas 1975; **Figure 3.10A**). Three weeks after metamorphosis, crown-of-thorns starfish start adding arms at two-week intervals and the body color turns pink (**Figure 3.10B**), which camouflages the juvenile starfish against the CCA it is feeding on (Lucas 1975; Yamaguchi 1973b; Birkeland and Lucas 1990; **Figure 3.10C**). During this phase, juvenile starfish do not feed on coral tissue even if it is around the vicinity to avoid damage caused by mesenteric filaments when placed in contact with coral polyps (Yamaguchi 1973a). This CCA-feeding stage is characterized by slow growth but the ubiquitous occurrence of CCA on coral reefs suggests that food availability is unlikely to be limiting in most locations. However, at around four to six months, food availability may be more significant as juveniles switch their diet to corals and begin to grow rapidly (Yamaguchi 1974a; Zann et al. 1987; **Figure 3.10D**). The effect of feeding by juveniles on coral is very minimal but consumption rates increase with size (Kettle and Lucas 1987). Feeding preferences of juveniles may also be influenced by the size of coral polyps as nematocysts and mesenteric filaments can cause severe damage on the aboral surfaces of juveniles (Yamaguchi 1973a). In addition, certain coral species promote faster growth rates in juveniles, with average growth rates ranging from 12 mm per month for those feeding on *Acropora formosa* and only 0.1 mm per month for those feeding on *Porites lichen* (Keesing & Halford 1992a).

Early juvenile crown-of-thorns starfish have eluded researchers in the field despite extensive search efforts (Doherty and Davidson 1988; Johnson et al. 1992). Based on the few observations of natural populations of post-settlement juveniles in the field, rubble beds and boulders encrusted with CCA seem to be preferred habitats during this stage (Yokochi and Ogura 1987; Zann et al. 1987). Significant predation rates by epibenthic carnivores, as high as 5% per day, have been recorded for juvenile starfish in these habitats (Keesing & Halford 1992b). Moreover, the cryptic and nocturnal behavior of small juveniles has led some researchers to suggest that this is a clear adaptation to avoid visually orienting predators, like reef fishes. This cryptic behavior continues during the early coral-feeding stage around 13-18 months with an estimated diameter of less than 10 cm (Zann et al. 1987). After 20 months (diameter >10 cm), crown-of-thorns starfish shift from cryptic to daytime feeding and at 24 months (~ 15 cm), sexual maturation and active migration will commence (Zann et al. 1987). This exposes the starfish to visually searching predators and with developed gonads, crown-of-thorns starfish at this stage could be energetically rewarding as prey (Sweatman and Butler 1993).

Sweatman (1995) placed small, laboratory-reared juvenile crown-of-thorns starfish in a semi-natural setting where suspected fish predators were present and found that losses attributable to predation were low (0.13% of starfish per day) – much lower than levels of predatory mortality (1.5% of starfish per day) predicted to have an impact on population regulation (McCallum 1988). In addition, when small juvenile crown-of-thorns starfish were made unnaturally accessible to putative predators (i.e. lethrinid fishes), some, but not all, juveniles were consumed and some juveniles were rejected after mouthing (Sweatman 1995). These results suggest that predation by large reef fishes did not significantly influence the population dynamics of juvenile crown-of-

thorns starfish and there may be a need to consider the role of relatively small invertebrate feeders in regulating local populations of crown-of-thorns starfish. Conversely, Rivera-Posada et al. (2014) found high frequencies of sublethal arm damage in medium-sized crown-of-thorns starfish (11-20 cm), which coincides with the phase when crown-of-thorns starfish shift from cryptic to exposed daytime feeding followed by the onset of sexual maturity and migration (Keesing 1995). The high incidence of arm damage within this size class suggests that predators may be able exercise some level of regulation on crown-of-thorns starfish populations at a local scale (Rivera-Posada et al. 2014).

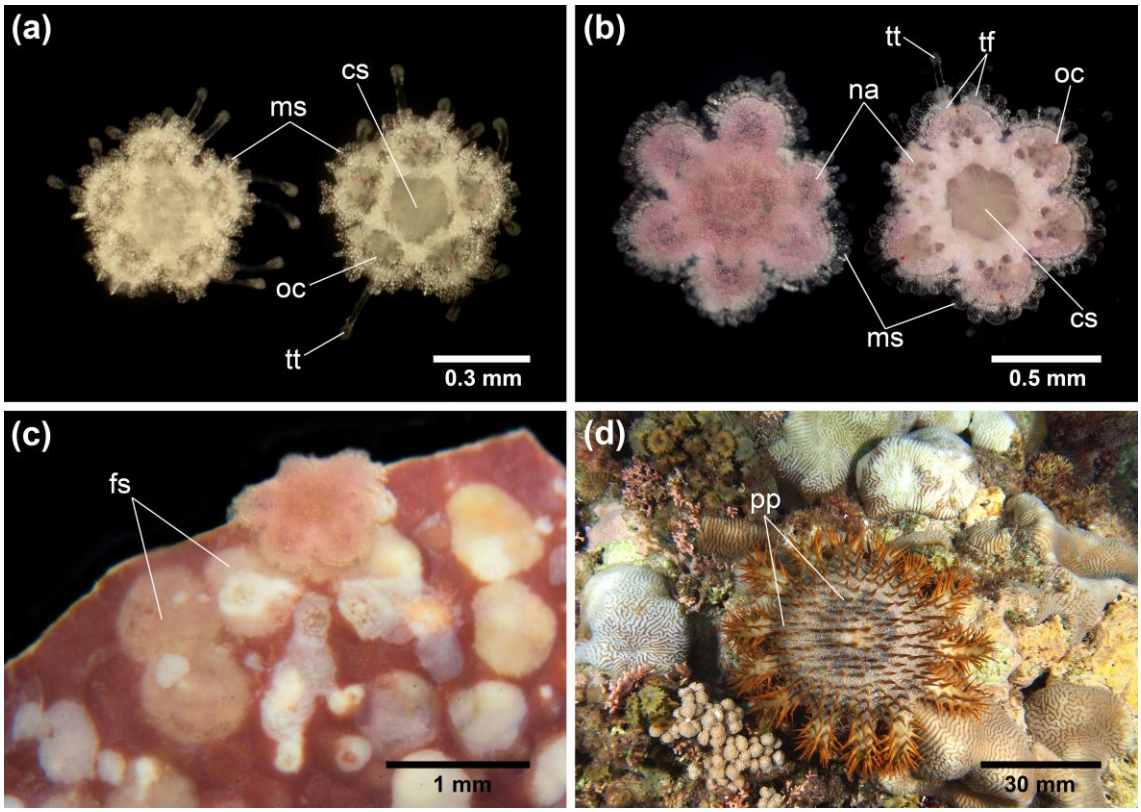

Figure 3.10 Juvenile stages of crown-of-thorns starfish. (**a**) 1 week after metamorphosis; (**b**) 4 weeks after metamorphosis – starting to add new arms; (**c**) 6-week old juvenile feeding on crustose coralline algae; (**d**) coral-feeding juvenile with complete number of arms. ms = marginal spines; cs = cardiac stomach; oc = optical cushion; tt = terminal tentacle; tf = tube feet; na = new arms; fs = feeding scars; pp = papillae (Photographs by C.F. Caballes)

3.7 Perspectives for future research and management

Despite significant research into the biology of crown-of-thorns starfish throughout the last four decades (reviewed by Moran 1986; Birkeland and Lucas 1990; Pratchett et al. 2014), it is clear that there remain many enigmas about this organism. This is largely attributable to the waxing and waning of scientific interest in crown-of-thorns starfish in line with the coming and going of population outbreaks (Pratchett et al. 2014), and what is needed is a comprehensive and detailed comparison of outbreak and non-outbreaking populations, ideally within the same location. In addition, the background information presented in this chapter provides a framework for future studies of the mechanisms underlying reproductive biology and larval ecology of crown-of-thorns starfish and can be used as a guide for conducting manipulative experiments in the laboraotory and in the field to gain insights into the ultimate cause(s) of population outbreaks. This fundamental biological information is not only important in better understanding the dynamics of crown-of-thorns starfish populations and establishing key limitations in recruitment and population replenishment, but has significant utility in designing appropriate management responses. For example, understanding how exogenous and endogenous factors interact to promote gamete competence, fertilization success, larval development, and post-settlement growth is important not only from an ecological standpoint but also for developing control measures that directly address the proximate causes of outbreaks and for assessing how populations may be affected by, and respond to, natural and man-made disturbances. The frequency of outbreaks and magnitude of resulting coral damage cannot be sustained by coral reefs as it continues to face ever increasing threats associated with climate change. Although direct intervention programs to control crown-of-thorns starfish populations are temporary, these activities will certainly help mitigate coral

mortality and allow recovery if done properly and efficiently. Knowledge on the reproductive biology of crown-of-thorns starfish is crucial in timing control efforts to minimize further recruitment (Bos et al. 2013). Furthermore, implementing land use practices that improve water quality (reduce nutrient runoff) and establishing protected areas (reduce exploitation of potential predator species) may possibly limit further outbreaks and at the same time provide definite benefits for the resilience of reef ecosystems (Fabricius 2005; McCook et al. 2010).

Chapter 4

Temporal variability in gametogenesis and reproductive behavior of crown-of-thorns starfish in the Great Barrier Reef, Australia

4.1 Introduction

The reproductive biology and behavior of crown-of-thorns starfish is fundamental in understanding their population dynamics (Caballes and Pratchett 2014). Importantly, all of the major hypotheses put forward to account for the periodic incidence of rapid and pronounced increases in abundance of crown-of-thorns starfish (*outbreaks*; Potts 1981) are predicated on pronounced increases in the reproductive success caused by natural or anthropogenically-induced changes in densities of spawning starfish (e.g., 'predator-removal hypothesis', Endean 1977), fecundity, fertilization success ('natural causes hypothesis', Vine 1973), or larval survivorship (e.g., 'larval starvation hypothesis', Lucas 1982). Understanding, and effective management, of outbreaks of crown-of-thorns starfish is therefore, reliant on improved information about what drives individual and population-level differences in reproductive biology and behavior e.g., size and age at sexual maturity, size-fecundity relationships, annual gametogenic times, and synchrony of spawning), particularly those factors that may contribute to step-changes in reproductive success.

Crown-of-thorns starfish are predisposed to rapid and pronounced increases in local population size (e.g., Chesher 1969) owing to their inherent life-history characteristics, such as early maturation and high fecundity (Uthicke et al. 2009). However, reproductive output and/or population replenishment must be highly constrained in most instances to account for normally very low densities of crown-of-

thorns starfish (Moran 1986). Babcock and Mundy (1992a) suggested that reproductive success of crown-of-thorns starfish is generally constrained by low levels of fertilization success owing to overdispersion of individuals in low-density populations and limited evidence of spawning synchrony. While the gametogenic cycle of crown-of-thorns starfish may be regulated by neurohormonal mechanisms (Giese and Kanatani 1987) and modulated by other intrinsic factors such as age, size, and nutritional status (Yamaguchi 1974a; Lucas 1984; Zann et al. 1987), high intraspecific variability in reproductive cycles within and between populations exposed to different environmental variables suggests that extrinsic factors also influence gametogenic processes. Exogenous (extrinsic) factors such as temperature, photoperiod, rainfall, salinity, and phytoplankton concentration have been shown to be important in regulating reproductive events in asteroids (reviewed in Mercier and Hamel 2013).

Most of what is known about the reproductive biology of crown-of-thorns starfish come from intensive field-based studies conducted at specific locations on Australia's Great Barrier Reef (Green Island - Pearson and Endean 1969, Lodestone Reef - Lucas 1973; Davies Reef- Babcock and Mundy 1992a, b). These studies provided important insights into the spawning behavior of crown-of-thorns starfish and identified a relatively discrete season (November to January) of reproductive activity (Pearson and Endean 1969; Babcock et al. 1992a), at least for the Pacific species, *Acanthaster* cf. *solaris*, in the GBR. While the seasonality of crown-of-thorns starfish gametogenesis in the GBR is well-established, temporal and spatial variation in reproductive timing and output remains poorly understood (Babcock and Mundy 1992a, b). Most notably, it is unknown whether individual crown-of-thorns starfish spawn just once per season (Lucas 1973), or spawn repeatedly (e.g. batch or dribble spawning; Eckelbarger and Watling 1995) throughout the reproductive season (Babcock and Mundy 1992a,b).

Early onset of maturation is widely regarded as one of the foremost attributes of crown-of-thorns starfish that enable rapid population growth (e.g., Babcock and Munday 1992a). Lucas (1973) reported that individuals begin to reproduce after 2-years, based on observations from laboratory-reared individuals (see also Yamaguchi 1973b, Lucas 1984). Based on observations of field specimens, Birkeland and Lucas (1990) suggested that most individuals ≥200 mm in total diameter appear sexually active. Overall, there has been relatively limited sampling of smaller crown-of-thorns starfish during spawning periods, which is necessary to test for spatial variation in the size at sexual maturity.

While crown-of-thorns starfish can become sexually mature very early (Lucas 1973), larger individuals make a disproportionate contribution to overall reproductive potential (Conand 1985; Babcock et al. 2016b), given exponential increases in individual fecundity with increasing size. The size-structure of local populations will therefore have a major bearing on reproductive potential. In many cases, outbreak populations of crown-of-thorns starfish exhibit very limited size-ranges, reflecting the rapid initiation of outbreaks through a single mass-recruitment (Yokochi and Ogura 1987; Zann et al. 1987), such that reproductive capacity of most individuals is very limited, at least in the first few years following the initiation of the outbreak. However, some outbreak populations comprise starfish from multiple distinct cohorts, as evident based on broad size ranges and observations of repeated annual recruitment over several successive years (e.g., Pratchett 2005). In these instances, overall reproductive capacity will be strongly influenced by the proportional abundance of larger individuals, as well as total densities of mature individuals. There is also likely to be additional variation among individuals independent of size (total diameter) due to differences in food availability and feeding history (Caballes et al. 2016), potentially reflected in

differences in weight (standardised for diameter) during reproductive periods. Variation in length-weight relationship has often been used in fisheries science as indicators of fat content, condition, and gonad development (Froese 2006). Kettle and Lucas (1987) demonstrated that crown-of-thorns starfish increasingly partition energy towards gonad production as it grows, which could be reflected in changes in parameters of the diameter-weight relationship in crown-of-thorns starfish.

This study examined the reproductive biology and behavior of *A*. cf. *solaris* on the GBR, specifically considering i) broadscale differences in size-structure and diameter-weight relationships; ii) the size at sexual maturity and sex ratio of discrete populations; and iii) inter-annual variation in the timing and progression of gametogenesis, based on monthly changes in the gonadosomatic index (*GSI*), as well as the size and stages of oocytes in female gonads. Spatial and temporal variation in reproductive timing and output will be related, where possible, to local environmental conditions (i.e. temperature, day length, salinity, amount of rainfall, and chlorophyll-*a* concentration). This information is critical for potentially linking the precise timing of spawning behavior and associated reproductive success to spatial and temporal anomalies in local conditions, which may explain the patchiness of crown-of-thorns starfish populations in the GBR.

4.2 Materials and Methods

4.2.1. Size structure and growth

Specimens of the Pacific species of the crown-of-thorns starfish, *Acanthaster* cf. *solaris*, were collected by snorkeling or SCUBA from 55 reefs along the Great Barrier Reef, Australia (**Figure 4.1**). The diameters of starfish collected from 55 reefs were measured (to the nearest mm) and wet weight (the nearest g) was measured for starfish collected from 22 reefs (**Appendix B – Table B1**). Diameter–weight relationship was estimated by combining data from 22 reefs (**Table B1**) where both diameter and weight were measured. The relationship between diameter and weight was expressed using a two-parameter power function:

$$W = \beta D^{\alpha} \tag{4.1}$$

where W is total body weight (g), D is maximum diameter (mm), β is the intercept and α is the allometric coefficient. Model fitting was done using the 'FSA' package in R (Ogle 2016). Diameter-weight relationships were used to test whether growth was isometric ($\alpha = 3$) or allometric ($\alpha \neq 3$) for mature and immature starfish, as well as for every sampling month. T-test was used to test the null hypothesis that $\alpha = 3$. An analysis of covariance (ANCOVA), implemented in R (R Core Team 2016) was used to test whether there was a variation in the parameters of the diameter-weight relationship between mature (males and females) and immature starfish, and between sampling months having n > 100 starfish sampled (May, September to February), with the log-transformed diameter as covariate.

4.2.2. Size at first maturity and sex ratio

Individuals were classified as 'immature' (sexually undifferentiated) if no gonads were detected upon inspection of arms following dissection. For mature individuals, sex

was determined firstly by examining gonads through a small incision along the proximal section of intact arms –testes were cream or pale yellow in color and have smaller, more numerous lobes while ovaries appeared as larger, spherical, yellow (sometimes almost orange) lobes (Pratchett et al. 2014) and secondarily by drawing contents from gonads along the arm junction using a syringe with a large-bore biopsy needle – eggs appear as translucent spherical grains, while sperm make the water appear cloudy when observed against the light (Caballes and Pratchett 2014). The proportion of sexually mature starfish collected from September to December was modeled using a generalized linear model (GLM) with binomial errors and logit link function in R (R Core Team 2016). Confidence intervals for the parameters of the logistic equation were estimated using 1000 bootstrap samples. Using the fitted logistic curves, size at first sexual maturity was determined by estimating the diameter (D_{50}) and weight (W_{50}) at which 50% of the starfish sampled were sexually mature. Sex ratio was calculated for crown-of-thorns starfish populations that were sampled from September to December (it is difficult to reliably determine sex macroscopically after spawning) during the 2013 and 2014 sampling seasons. For each reef, deviation from the expected ratio of 1:1 was tested with the chi-square (χ^2) test.

4.2.3. Gametogenesis and spawning

For finescale assessment of gametogenic cycle and reproductive timing, repeated sampling of a dispersed but persistent population within the GBR initiation zone was conducted. Monthly samples (approximately around the middle of each month, except for December, which was sampled at the end of the first week for each spawning season) of 10-35 starfish were collected from reefs between Palfrey and South Island in the Lizard Island group (**Figure 1, inset**) during the breeding season – from September

2013 to March 2014, then again from September 2014 to March 2015. Samples were immediately transported to the Lizard Island Research Station for dissection. Incisions were made on both sides of each arm from the proximal to the distal region to expose gonads. Gonads were examined macroscopically (*sensu* Yasuda et al. 2010) and classified into four gametogenic stages: (1) "pre-spawning" – all dissected arms contained gonads that were not yet full-sized; (2) "mature" – arms packed with full-sized gonads; (3) "partial spawning" – reduced gonad volume and presence of full-sized and shrunken gonad lobes; and (4) "post- spawning" – almost all gonads lost and remaining gonads reduced to shrunken lobes. Subsequent histological examination of the same gonads was done to corroborate macroscopic examination of gonads. Temporal (monthly and annual) variation in the frequency distribution of gametogenic stages was analyzed as a contingency table using log-linear models with log link and Poisson error terms (Agresti 1996). Deviance statistics (χ^2) were used to compare models in R (R Core Team 2016). Pairwise comparisons were done using G-test of independence (or with Fisher's exact test when minimum expected values are < 5) with correction for false discovery rate (Benjamini & Hochberg 1995). After obtaining the total body weight of each starfish, all gonads were carefully removed and weighed. Gonadosomatic index (*GSI*) was calculated for each specimen using the formula:

$$GSI = (W_{gonad} / W) \times 100\% \quad (4.2)$$

where $\boldsymbol{W_{gonad}}$ is gonad weight (g) and $\boldsymbol{W}$ is the total body weight (g) of individual starfish. The mean *GSI* ± SD was calculated for each sampling month. A two-way ANOVA was used to analyze the difference in *GSI* (response variable) between months and sampling years (fixed categorical predictors). Inspection of quantiles of normal distribution plots (Q-Q plots) and residual plots were used to assess normality and homogeneity of variance, respectively. Pairwise post hoc tests were subsequently

performed using the Tukey's method in 'lsmeans' function in R (R Core Team 2016). Only female gonads were assessed for gametogenic stages and *GSI*, as spermatogenesis has been shown to proceed in parallel with spawning, such that some testes may retain a mature appearance even though some sperm have been released (Yamazato and Kiyan 1973; Byrne et al. 1997b).

4.2.4. Gonad histology and oocyte size frequency distribution

To examine the histology of gametogenesis and to document the pattern of gonad maturity, a portion of the gonad clusters from starfish sampled monthly were stored in 10% phosphate-buffered seawater formalin and retained prior to histological analysis. Gonads from at least 10 female and 5 male crown-of-thorns starfish from monthly samples were haphazardly selected for histological analysis. For each gonad sample from individual starfish, three gonad clusters were transferred to a 70% ethanol solution and dehydrated through a graded ethanol series, cleared in benzene, embedded in paraffin wax, sectioned at 5 μm. Sections were stained with Mayer's haematoxylin and eosin and examined under a microscope to assess the stage of maturity of gonads (Byrne 1992; Byrne et al. 1997b). Ten digital photographs each at 10x and 40x magnification were taken of each slide using a camera mounted on a microscope.

Information on the size frequency distribution of oocytes was obtained by measuring the maximum and minimum diameter of at least 50 oocytes using image analysis of ovary slide photographs in Image J. Only eggs sectioned through the nucleolus were selected to ensure that oocytes were measured at the center. Histograms of the average oocyte diameter for each sampling month were generated and temporal variation in oocyte diameter analyzed using a two-way ANOVA with sampling month

and year as fixed factors, followed by pairwise post hoc comparisons using 'lsmeans' function in R (R Core Team 2016).

4.2.5. Environmental conditions

Seawater temperature, salinity, and rainfall data for waters around Lizard Island were obtained from the Australian Institute of Marine Science Data Centre (http://data.aims.gov.au). Data used for daylength calculations were sourced from Geosciences Australia (http://www.ga.gov.au). Daily mean chlorophyll-*a* concentration for midshelf waters in the Cape York and Wet Tropics regions, which covers waters around Lizard Island, were acquired from e-Reef Marine Water Quality Dashboard (http://www.bom.gov.au). Mean *GSI* was used as a quantitative proxy of the gametogenic patterns observed throughout the study. Correlations between *GSI* and contemporary environmental variables (i.e. temperature, daylength, rainfall, salinity, and chlorophyll-*a* concentration were calculated using Pearson product-moment correlation coefficient (ρ).

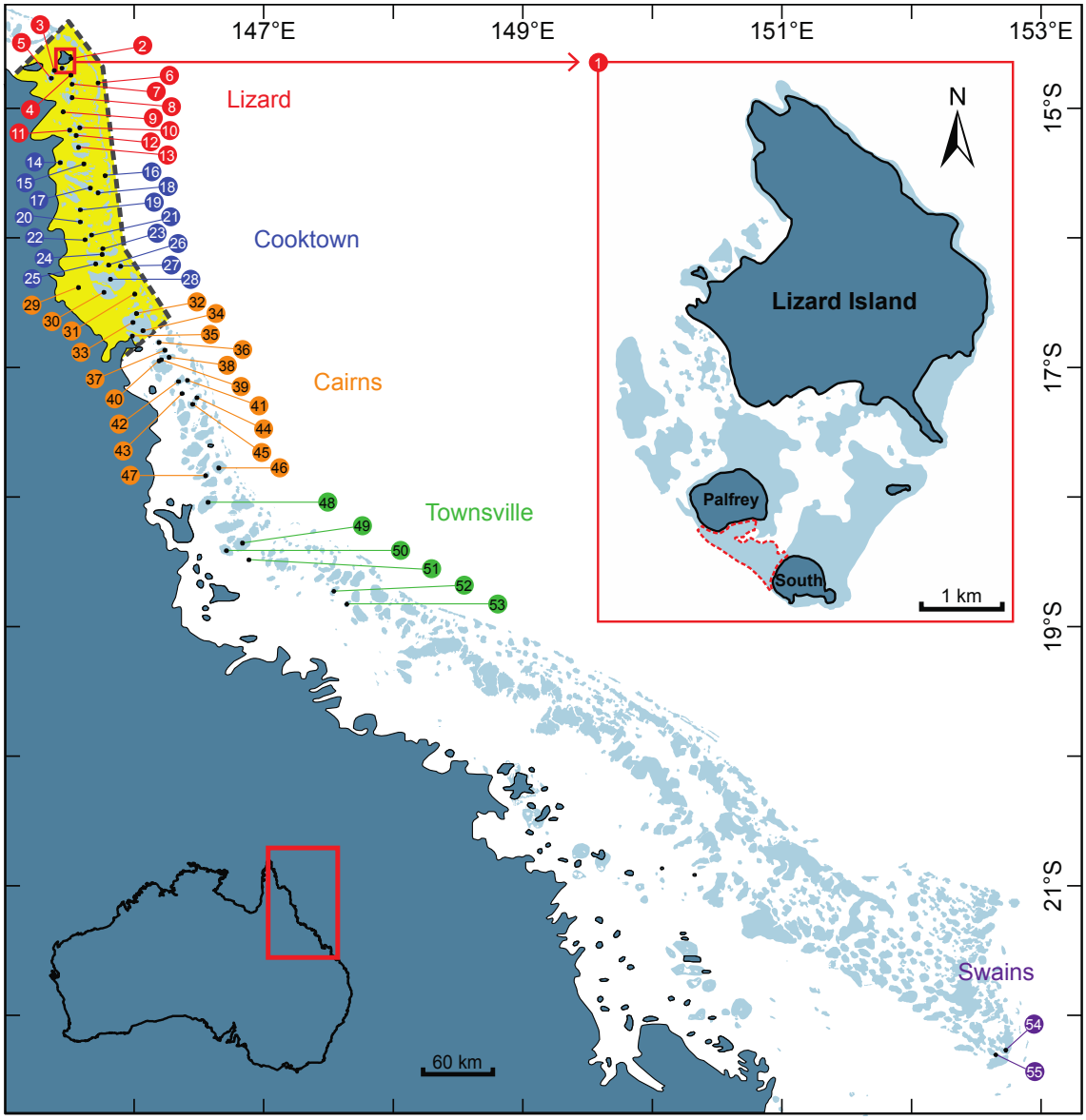

Figure 4.1 Map of sampling locations along the Great Barrier Reef, Australia. Monthly gonad samples for the 2013-2014 and 2014-2015 spawning seasons were dissected from crown-of-thorns starfish collected from the reef between Palfrey and South Island (inset: red outline). Yellow box with dashed lines demarcates reefs within the "outbreak initiation box" defined by the Australian Institute of Marine Science (AIMS, see Pratchett et al. 2014). Reef names are shown in **Appendix B – Table B1**.

4.3 Results

4.3.1. Size structure and diameter-weight relationships

Specimens collected from 55 reefs in the northern GBR ranged from 15 to 550 mm total diameter (mean = 280 ± 75 mm; n = 5100) and 10 to 4705 g in drained wet weight (mean = 844 ± 510 g; n = 3871). Females were bigger and heavier on average (D = 305 ± 59 mm; W = 1035 ± 549 g) compared to males (D = 285 ± 62 mm; W = 847 ± 473 g). Overall, crown-of-thorns starfish exhibit negative allometery in weight (W) relative to total diameter (D) as shown by the slope of the fitted equation: $W = 0.0007 D^{2.652}$ based on data for a total of 3,871 individuals (**Figure 4.2a**). There was no significant difference in the slopes ($F_{2,2994} = 0.32$; p = 0.7287; **Table 4.1a**) of the diameter-weight relationship between mature females (**β**: 95% CI = 2.584–2.684; $p < 0.0001$), males (**β**: 95% CI = 2.580–2.655; $p < 0.0001$), and immature starfish (**β**: 95% CI = 2.439–2.734; $p < 0.0001$), all of which exhibited allometric growth (p-values indicate that the exponent parameter, **β**, was significantly different from 3) . However, there was a significant difference in intercepts ($F_{2,2994} = 37.86$; p < 0.0001), showing mature crown-of-thorns starfish (female or male) were larger than immature starfish (**Figure 4.2b**). Slopes of the diameter-weight relationship were significantly different between sampling months ($F_{6,3799} = 17.06$; p < 0.0001). The rate of change in weight in relation to diameter varied between months, whereby exponent parameters (**β**) were highest in November (95% CI = 2.765–2.923) and December (95% CI = 2.618–2.965), and lowest in February (95% CI = 2.505–2.592) and May (95% CI = 2.422–2.541), which correspond with pre-spawning and post-spawning periods, respectively.

Table 4.1 Results of analysis of covariance (ANCOVA) comparing the parameters of the diameter-weight relationship **(a)** between female, male, and immature starfish, and **(b)** between sampling months

Source of Variation	*df*	*F*	*P*
(a) Sexual Maturity			
log (Diameter)	1	49547.02	**< 0.0001**
Sexual Maturity	2	37.86	**< 0.0001**
log (Diameter) x Sexual Maturity	2	0.32	0.7287
Residuals	2994		
(b) Sampling Month			
log (Diameter)	1	62464.15	**< 0.0001**
Month	6	41.38	**< 0.0001**
log (Diameter) x Month	6	17.06	**< 0.0001**
Residuals	3799		

4.3.2 Size at first maturity and sex ratio

Minimum size at first sexual maturity was estimated at a diameter of 125 mm and weight of 45 g. Sexually undifferentiated starfish ranged from 60-230 mm in diameter (160 ± 29 mm) and 10-365 g in weight (166 ± 76 g). Estimated diameter at first sexual maturity (D_{50}) was 172.72 mm (95% CI: 168.49–176.76 mm; **Figure 4.2b**) and weight at first sexual maturity (W_{50}) was 203.47 g (95% CI: 191.04– 216.19 g; **Figure 4.2c**).

Sex ratio was close to 1:1 for most of the populations, albeit slightly skewed towards males (**Figure 4.3**). Out of 13 populations sampled during the 2013-2014 and 2014-2015 spawning seasons, five were significantly skewed towards males. Populations of crown-of-thorns starfish samples from MacGillivray Reef ($\chi^2 = 33.22$; $df = 1$; $p < 0.0001$), North Direction Island Reef ($\chi^2 = 13.34$; $df = 1$; $p = 0.0003$), and South Direction Island Reef ($\chi^2 = 5.73$; $df = 1$; $p = 0.0167$) during the 2013-2014 spawning season (**Figure 4.3a**), and from Palfrey/South Island Reef ($\chi^2 = 5.43$; $df = 1$; $p < 0.0197$) and McCulloch Reef ($\chi^2 = 5.12$; $df = 1$; $p < 0.0236$) during the 2014-2015 season (**Figure 4.3b**) had significantly more males than females.

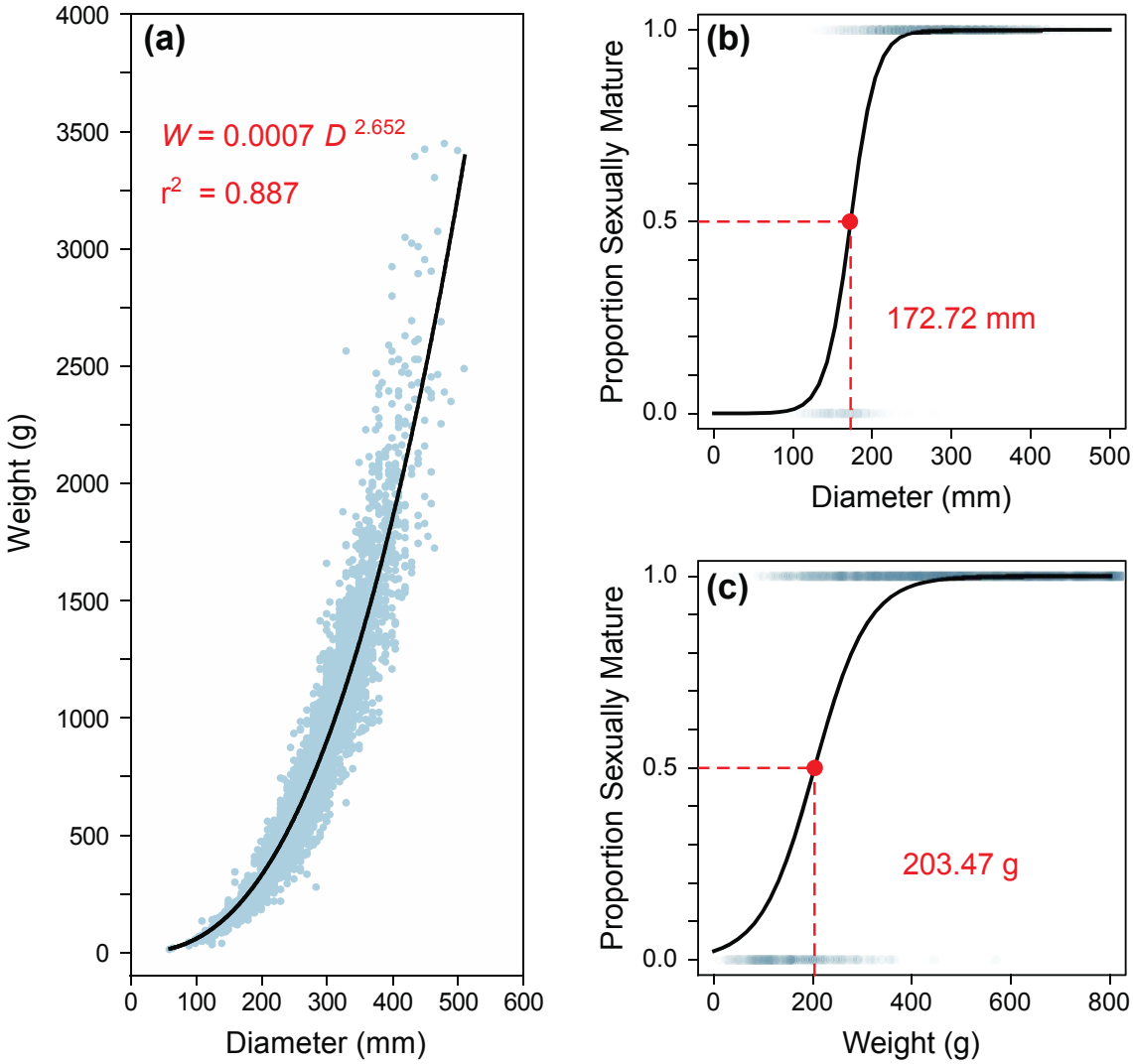

Figure 4.2 Allometric growth and size at first sexual maturity: **(a)** pooled data on diameter-weight relationship of sexually mature and immature starfish fitted with a two-parameter power equation; **(b)** diameter at first sexual maturity; and **(c)** weight at first sexual maturity estimated using logistic curves.

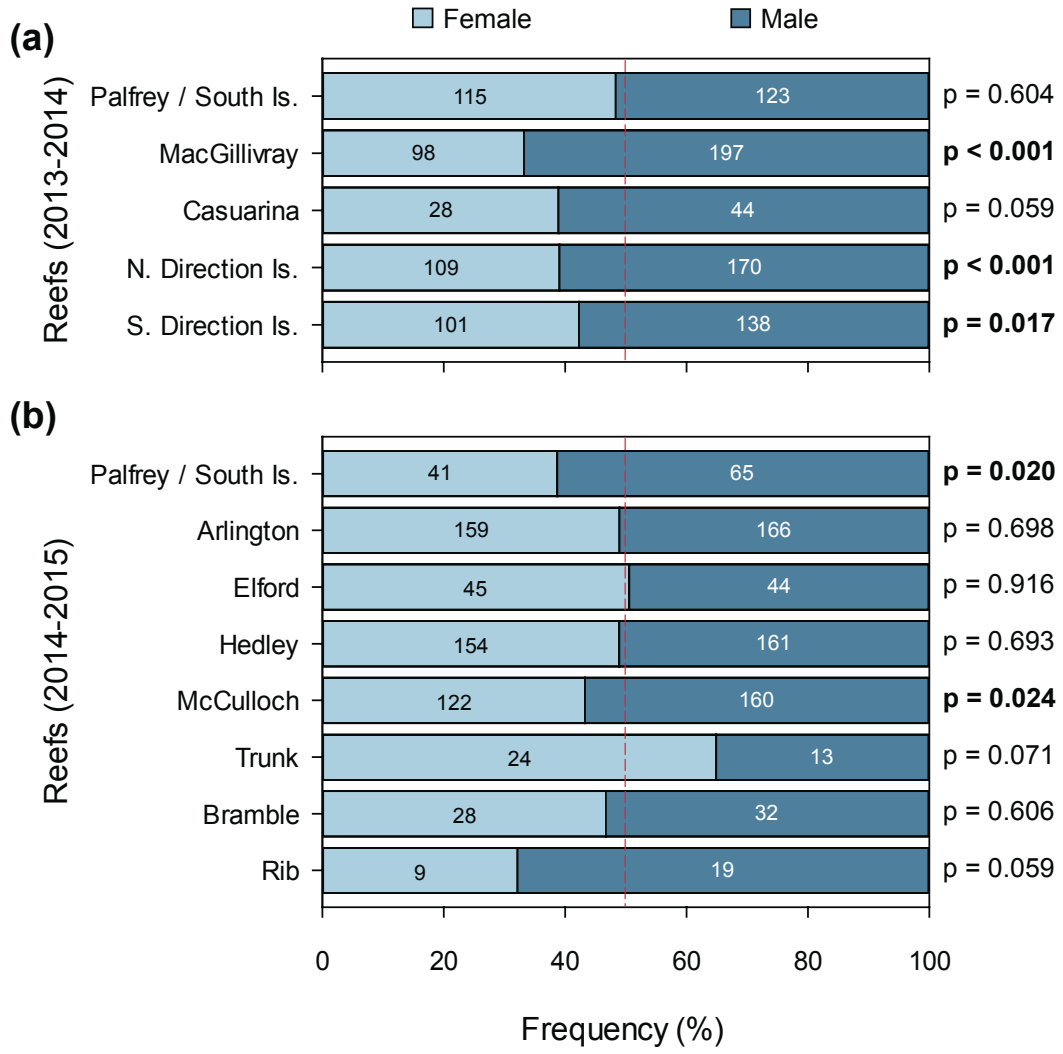

Figure 4.3 Sex ratios within populations sampled during two successive spawning seasons. *P*-values are based on chi-square goodness-of-fit tests. Refer to **Figure 4.1** and **Appendix B – Table B1** for reef locations.

4.3.3 Gametogenesis and spawning

Hierarchical log-linear model comparisons show that the relative frequencies of gametogenic stages of the ovaries were conditionally dependent on the spawning season ($\chi^2 = 43.60$; $df = 21$; $p < 0.0001$) and the sampling month ($\chi^2 = 509.71$; $df = 36$; $p < 0.0001$). This indicates that the frequency distribution of gametogenic stages varied between years, and was also significantly different between months; except for September and October, October and November, and February and March (**Figure 4.4a**). Majority of the ovaries were still in the pre-spawning / growing stage from September (65%) to October (67%) 2013. By November 2013, 67% of ovaries examined have matured, and some starfish have partially (10%) or completely (8%) spawned by December 2013. Over 50% of the sampled starfish have spawned by January 2014, and majority of ovaries were at "partial spawning" or "spent" stage in February and March 2014. Remarkably, there were still mature female gonads that showed no signs of spawning this late in the spawning season. Frequency distribution of gametogenic stages was unimodal in September 2014 (84% at "pre-spawning" stage) and bimodal in October 2014 (44% "pre-spawning, 56% "mature") and November 2014 (42% "pre-spawning", 58% mature"). Reproductive maturity peaked in December 2014 and 21% of sampled starfish have either partially or completely spawned at this time. All sampled starfish have spawned by January, and 81% and 90% of starfish were at spent stage in February and March, respectively.

Patterns of monthly variation in *GSI* were mostly consistent with changes in the frequency distribution of gametogenic stages. Results of two-way ANOVA are summarized in **Table 4.2a**, where a significant interaction term indicated that interannual variation had to be assessed at the level of sampling months, while monthly differences in *GSI* were assessed within each spawning season. Monthly variation in

mean *GSI* (± SD) within each spawning season are shown in **Figure 4.4b**. Mean *GSI* was relatively high (0.089 ± 0.020) in September 2013 and gradually peaked to 0.123 ± 0.024 in November 2013. These *GSI* levels were maintained in December 2013 (0.118 ± 0.016), then progressively decreased after spawning from 0.083 ± 0.018 in January 2014, down to 0.022 ± 0.010 in March 2014. For the 2014-2015 spawning season, *GSI* was relatively low at the early stages of the gametogenic cycle in September 2014 (0.048 ±0.010), but consistently increased in the subsequent months, until *GSI* drastically peaked in December 2014 (0.144 ± 0.023). Mean *GSI* values declined dramatically after spawning, which was most likely indicative of complete spawning. There was a significant interannual difference in mean *GSI* for the months of September, January, and February. Mean *GSI* was significantly higher in September 2013, although the increase in *GSI* approaching the peak gametogenic period was more gradual in 2013 compared to the following year. After spawning in December, several individuals with relatively high *GSI* were still present in January and February 2014, while all of the starfish sampled from January to March 2015 had very minimal amounts of gonad tissue left.

Table 4.2 Results of two-way ANOVA comparing mean (**a**) GSI and (**b**) oocyte diameter between 2013-2014 and 2014-2015 spawning season ('Year') and between sampling months (September to March).

Source of Variation	***df***	***F***	***P***
(a) GSI			
Year	1	21.60	**< 0.0001**
Month	6	30.08	**< 0.0001**
Year x Month	6	3.68	0.0014
Residual	485		
(b) Oocyte diameter			
Year	1	262.39	**<0.0001**
Month	6	427.06	**<0.0001**
Year x Month	6	44.32	**<0.0001**
Residual	3550		

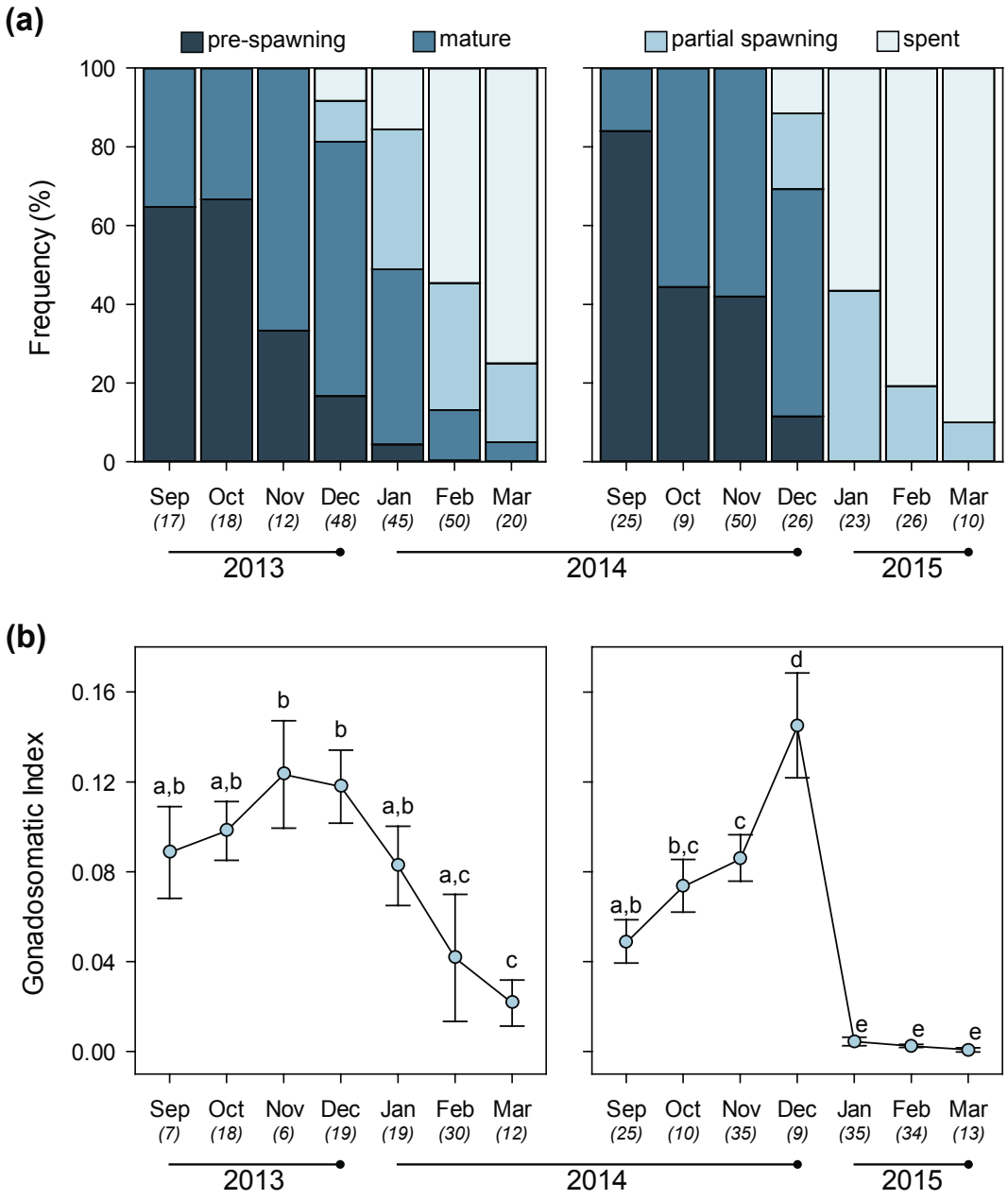

Figure 4.4 Development of female gonads shown as monthly changes in the (**a**) percentage of individuals in each development stage and (**b**) monthly variation of gonadosomatic index. (n = numbers in parentheses below each month).

4.3.4 Oocyte size frequency distribution and gonad histology

Interannual and monthly patterns in *GSI* were mirrored, for the most part, in variation of oocyte size frequency distribution (**Table 4.2b**). Almost all oocyte size classes were represented in gonad samples from September and October 2013, while the proportion of smaller oocytes (20-40 μm) was higher in September 2014, then the mode shifted to the 50-80 μm size class in October 2014 (**Figure 4.5**). Large oocytes (> 80 μm) were abundant during peak period of the crown-of-thorns starfish reproductive cycle in November and December for both spawning seasons, although patterns of size-frequency distribution did not coincide with the drastic increase in *GSI* in December 2014, followed by a steep drop in January 2015. The bimodal oocyte size frequency distribution in February and March 2014 corresponded with presence of unspawned individuals with relatively high *GSI* during the first spawning season. The high proportion of small oocytes in February and March 2015 shows that fully grown oocytes were shed during spawning, but immature oocytes remained.

Histological analysis of ovaries (**Figure 4.6**) also verified the gametogenic cycle, as indicated by the relative frequency of maturity stages of gonads and *GSI* values, and oocyte-size frequency distribution. Ovaries were at the growing stage ("pre-spawning") in September and October 2013, and contained oocytes at various stages of development. Relatively larger oocytes are situated in the central position, while smaller oocytes line the ovarian wall. In the second spawning season (2014-2015), majority of growing oocytes were smaller in September, but advanced oocytes have started to accumulate in the lumen in October. For both seasons, ovaries were filled with fully-grown vitellogenic oocytes by November, while few basophilic pre-vitellogenic oocytes lined the outer epithelial layer. Partial spawning had occurred by December 2013, as demonstrated by the spaces in the lumen left by released oocytes. A large proportion of

fully-grown oocytes remained by January 2014 and was loosely arranged in the lumen. Some fully-grown relict oocytes were not released and were still present in the ovaries by February and March 2014. In December 2014, ovaries were packed with fully-grown mature oocytes and the ovarian wall was thin and distended. Spawning occurred much later, but was more comprehensive compared to the previous year; by January 2015, only a few relict oocytes remained in the lumen. Gonads were almost completely empty in February 2015, except for smaller immature oocytes that were not spawned, which have degenerated and resorbed through phagocytosis by March 2015.

The development of testes (**Figure 4.7**) was characterized by the thickness of the germinal layer and the amount of spermatozoa in the lumen. At the beginning of the gametogenic cycle (between August and September in the GBR), the outer epithelial layer was relatively thick and spermatocytes in the germinal epithelium started to form basophilic columns (**Figure 4.7a**). Between early September and early November, gametogenesis intensified and the spermatogenic layer extended towards the lumen, which was starting to get filled with spermatozoa at this point (**Figure 4.7b**). Around late November to early December, the lumen of mature testes was densely packed with spermatozoa and the spermatogenic layer was almost negligible (**Figure 4.7c**). For partially spawned starfish, the lumen in the testis was less densely packed and empty spaces within the lumen were left by released sperm. Spent testes were shrunken in appearance and the haemal sinus started to expand (**Figure 4.7d**). Relict spermatozoa and phagocytes were also present in the lumen of spent testes.

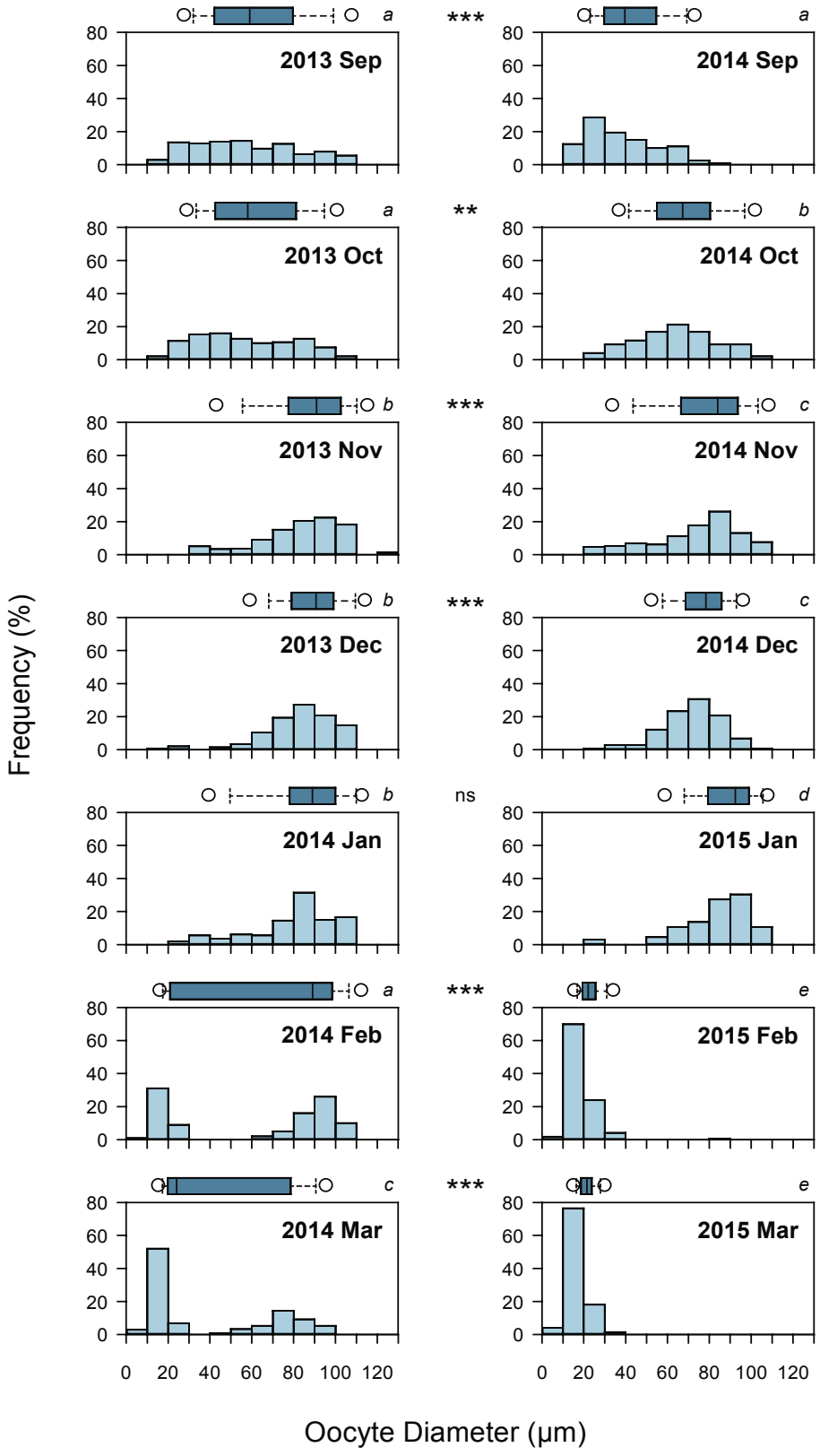

Figure 4.5 Oocyte size-frequency distribution. Histograms show frequencies of different oocyte size-classes each month. Boxplots cover the oocyte diameter range. Letters indicate significant differences between months, within years, as determined by post hoc comparisons. Variation between years (*** $p < 0.001$, ** $p < 0.01$, *ns* $p > 0.05$), within sampling months, are also shown.

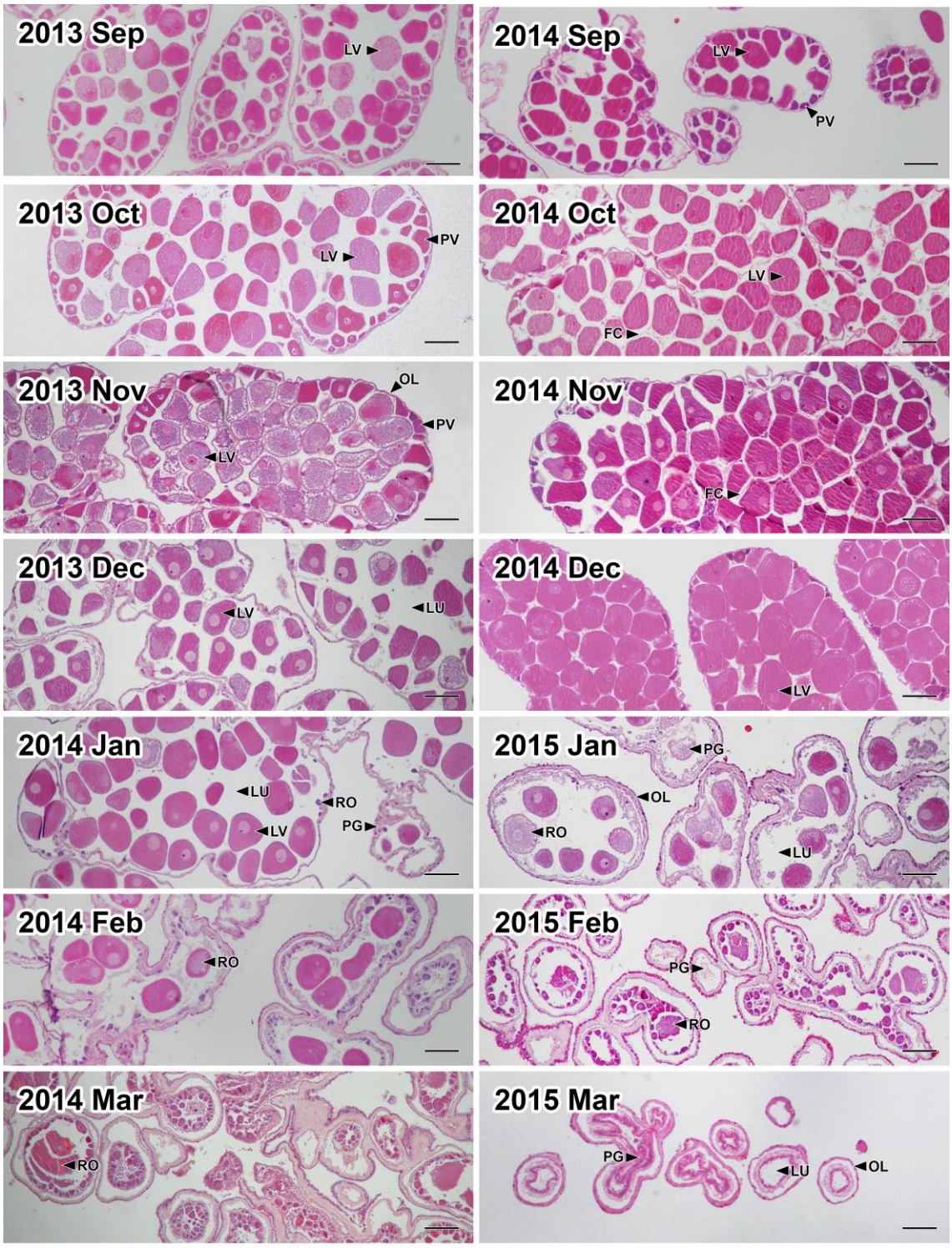

Figure 4.6 Histology of ovaries showing monthly patterns in gametogenic activity and interannual variation in reproductive timing, and spawning extent. **PV** = pre-vitellogenic oocytes ; **LV** = late vitellogenic oocytes; **OL** = outer epithelial layer; **FC** = follicle cells around oocytes; **RO** = relict oocytes; **LU** = lumen; **PG** = phagocytes (scale bar = 200 μm)

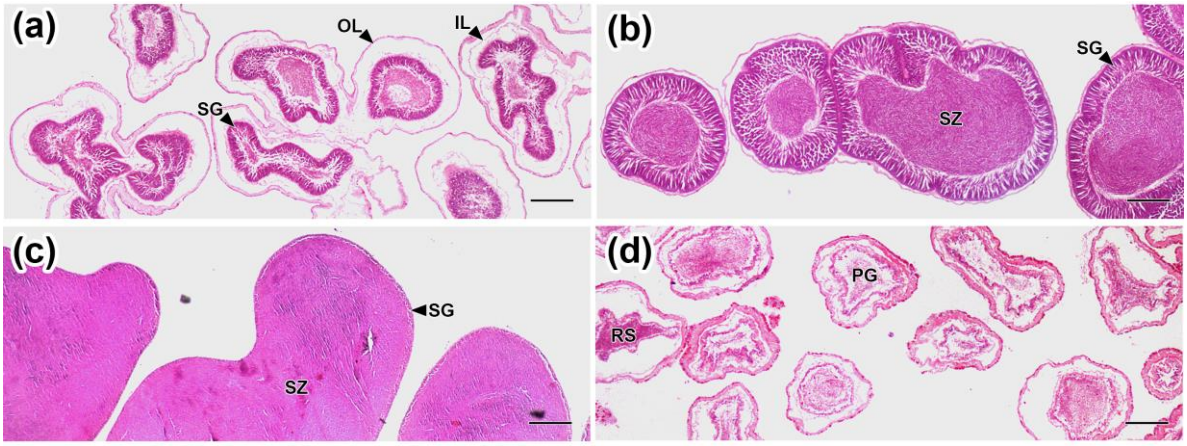

Figure 4.7. General histology of testes showing spermatogenesis: (**a**) early development; (**b**) late development, spermatocyte layer thickens; (**c**) mature, pre-spawning, spermatocyte layer almost negligible; (**d**) spent. **SG** = spermatogonia (spermatocyte columns); **OL** = outer epithelial layer; **IL** = inner epithelial later; **SZ** = spermatozoa (scale bar = 200 μm).

4.3.5. Environmental conditions

Patterns in *GSI* prior to spawning were positively correlated with temperature (Pearson's $\rho = 0.93$; $p = 0.0007$) and daylength (Pearson's $\rho = 0.79$; $p = 0.0198$). There was no significant correlation between *GSI* and rainfall, salinity, or chlorophyll-*a* concentration. Average monthly rainfall (< 3 mm), salinity (~35 psu), and chlorophyll-*a* concentration (< 0.4 μg L^{-1}) were steadily at 'normal' levels from September to December for both years. High levels of rainfall and associated reductions in salinity and increases in primary production (chlorophyll-*a* concentration) were not recorded until after peak spawning – around January and March in 2013, and between February and March in 2014. Variability in photoperiod (daylength) between spawning seasons was almost negligible. Increase in average monthly seawater temperature around Lizard Island from September until putative spawning in December was more gradual in 2013 (September: 24.82 ± 0.15°C; October: 25.99 ± 0.06°C; November: 27.31± 0.18°C; December: 27.97± 0.08°C) compared to the following year (September: 23.99 ± 0.08°C; October: 25.00 ± 0.08°C; November: 26.45± 0.08°C; December: 28.42 ± 0.13°C). For the first spawning season sampled (2013-2014), average monthly temperature peaked in February (28.38 ± 0.04°C), while average temperature peaked in January (28.66 ± 0.09°C) during the second spawning season (2014-2015).

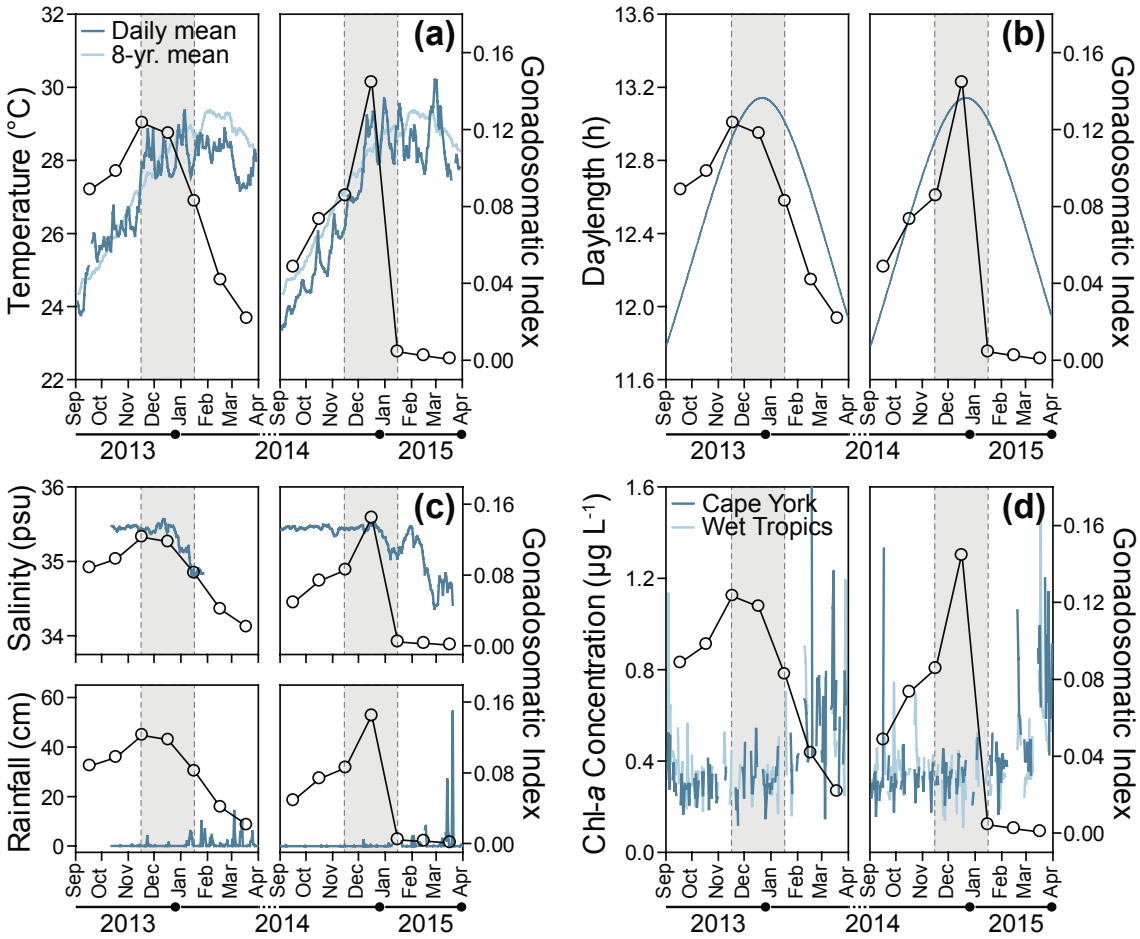

Figure 4.8. Variation in relevant environmental parameters around Lizard Island: (**a**) Daily average and 8-year average of daily seawater temperature; (**b**) Daylength measured as the number of hours between sunrise and sunset per day; (**c**) Salinity and rainfall accumulation; (**d**) daily mean chlorophyll-*a* concentration in Wet Tropics and Cape York regions in the GBR. Gonadosomatic index is overlaid and shown as outlined circles. Grey bars indicate estimated spawning season from late November to mid-January.

4.4 Discussion

This study reveals striking inter-annual variation in the reproductive biology of crown-of-thorns starfish from the northern GBR, suggesting that the timing of final maturation and gamete release can vary, possibly depending on local environmental conditions. In the first sampling period (September 2013 to March 2014), standardized gonad weight (*GSI*) was highest in November and December, but then exhibited protracted and gradual declines over the subsequent few months. This suggests that either the starfish failed to spawn, and ultimately resorbed their gonads, or there was only minor and repeated spawning that occurred over a highly extended spawning period. In the second sampling period however, there was evidence of comprehensive and synchronous spawning by crown-of-thorns starfish, which occurred some time in late December or early January, as per Babcock and Mundy (1992b). This study highlights that there are likely to be considerable complexities in the reproductive biology of crown-of-thorns starfish, partly due to the influence of exogenous factors, which may be fundamental in understanding temporal and spatial variation in the reproduction, recruitment, and ultimate adult abundance of crown-of-thorns starfish.

4.4.1. Allometric changes in weight versus diameter

Body mass of reproductive crown-of-thorns starfish was negatively allometric, whereby the mass of larger individuals was lower than expected based on isometric scaling (scaling component <3). This result is consistent with previous studies that examined diameter-weight relationships for crown-of-thorns starfish (Nishihira and Yamazato 1972; Yamaguchi 1974a; Conand 1985; Kettle and Lucas 1987; Bos et al. 2013), and is largely attributed to reduced investment in skeletal ossicles with increasing size, despite increasing reproductive investment in larger individuals

(Yamaguchi 1974a; Kettle and Lucas 1987). Accordingly, the pattern of negative allometery was the same even for non-reproductive (immature) individuals. There is however, evidence of reproductive contributions, based on monthly differences in slope coefficients. Slope parameters were highest in November and December and lowest in February and March, which corresponded with highest gonad indices prior to spawning and lowest gonad indices after spawning, respectively. This study sampled a wider range of diameters (60 to 510 mm) and ontogenic stages (immature and mature) compared to previous studies (Kettle and Lucas 1987). Morevoer, this is the first study to demonstrate that changes in the parameters of the diameter-weight relationship may be used as a proxy for gonad production in echinoderms. This is important as diameter and weight are usually the easiest parameters to measure with available equipment in the field.

4.4.2. Size at maturity

The size at sexual maturity measured in this study (172.72 mm; 95% CI: 168.49–176.76 mm) was smaller than previous estimates from laboratory-raised juveniles: < 250 mm (Yamaguchi 1974a), ~200 mm (Lucas 1984); but bigger than estimates from animals in the field: > 120 mm (Zann et al. 1987) and 130-160 mm (Bos et al. 2013). However, the smallest starfish that had developing gonads was 125 mm in diameter, while the largest individual with no discernible gonad was 230 mm in diameter. From a large sample of crown-of-thorns starfish in the GBR, Kenchington (1977) reported that the smallest starfish with gonads was 99 mm and the largest individual without gonads was 159 mm. Initiation of gametogenesis in crown-of-thorns starfish is clearly related to both age and size of *Acanthaster* spp. Gonad development in laboratory-reared starfish (Yamaguchi 1973a, Lucas 1984) and in a field population in Fiji (Zann et al. 1987)

started before the animals reached the age of 2 years, and the largest individuals in any given cohort were also the first to exhibit gametogenesis (Lucas 1984; Zann et al. 1987). Using derived growth curves in Pratchett et al. (2014), the estimated age sexual maturity is also < 2-years old based on the calculated D_{50} value. Size at sexual maturity may vary due to genetic predisposition and environmental factors (Birkeland and Lucas 1990), but any variation would have been averaged when the data were pooled for the whole GBR.

4.4.3. Sex ratio

Sex ratio was slightly skewed towards males in all but two populations, although only five out of the 13 reefs samples were significantly different from the expected 1:1 ratio. It can be inferred that male-biased populations may be an adaptive strategy to offset sperm limitation. Levitan (2004) demonstrated that there was sexual dimorphism in response to changes in sex ratio, whereby male standardized variance in fertilization success increased with male-skewed sex ratio, while female standardized variance in fertilization success decreased with male bias in sex ratio. Field fertilization assays conducted by Babcock et al. (1994) also showed that fertilization rates were almost always higher, at any given distance from the female, in experiments where five males were used compared to when only one male was used. Conversely, incidence of polyspermy, which results in reproductive failure, may be high for free-spawning invertebrates, even in low-density populations where sperm may be limited (Franke et al. 2002). Further studies are needed to establish the effective sex ratio for crown-of-thorns starfish by measuring fertilization rates at different sex ratios. The effects of sperm limitation and polyspermy should also be investigated.

Previous studies on the sex ratio of crown-of-thorns starfish, based on sampling of outbreak populations (e.g., Pearson and Endean 1969; Nishihira and Yamazato 1974), have reported relatively equal abundance of males and females. However, strongly male biased sex ratios have been recorded in several populations (e.g., Stump 1994; Caballes et al. unpublished data). For example, only 12 out of the 93 starfish (>30cm diameter), sampled from Hospital Point in Guam, were female (Caballes et al. unpublished data). Strong male bias may reflect generally lower survival of females (Stump 1994), which invest much more energy in reproduction (Cheney 1974; Babcock and Mundy 1992a). Moreover, if there is strong male sex bias in low-density populations generally, then this has the potential to greatly limit reproductive success, unless this is an adaptive strategy to offset sperm limitation in the field (Levitan 2004).

4.4.4. Gametogenic cycle

Although, temporal patterns in the relative frequency of gonad maturation stages, *GSI*, oocyte size frequency, and gonad histology were generally consistent with predicted austral summer breeding season in the GBR (reviewed by Caballes and Pratchett 2014), marked interannual variation in gametogenesis and reproductive timing was apparent. The size-frequency distribution of oocytes accounted for observed variations in gonad indices. In the 2014-2015 spawning season only small oocytes were left in the lumen, while ovary samples from the previous season still had a relatively high proportion of mature oocytes in the lumen that were starting to degenerate by March 2014 as confirmed by histological examination of gonads. This indicates that resorption of undischarged gametes begins regardless of whether spawning takes place or not (Zhadan et al. 2015). Babcock and Mundy (1992b) measured the *GSI* of crown-of-thorns starfish populations at Davies Reef during the spawning season between

August 1990 and February 1992. They recorded high *GSI* values for the population in 1990-1991 just before a major spawning and noted that the drop in *GSI* appeared to be rather more rapid in the second season compared to the first season, reaching levels of less than 5% in early January.

4.4.5. Environmental conditions

Among the contemporary environmental variables tested, temperature and daylength were the only factors that were significantly correlated with variation inthe *GSI* values within and between years. Rainfall, salinity, and chlorophyll-*a* concentration did not influence gametogenic activity. The highest amount of rainfall, associated with slight reductions in salinity and elevated concentrations of chlorophyll-*a*, were not recorded until after spawning (around March and April). Although the reproductive cycle of crown-of-thorns starfish in the GBR generally follows photoperiod (Lucas 1973), interannual variation in daylength during the two spawning seasons sampled was almost negligible, so it is less likely to be the driver of observed interannual variation in reproductive timing and output. Temperature also modulates gametogenic development in crown-of-thorns starfish and spawning usually occurred when seawater temperatures were around 28°C, which is optimal for fertilization and early embryonic development (Caballes et al. 2017a), and larval survival (Lucas 1973). The observed interannual variation in reproductive timing and output may have been influenced by fluctuation and magnitude of temperature change between the two spawning seasons. Mean monthly temperature increments from September to December 2013 were as follows: 1.17°C, 1.32°C, 0.66°C, which represents 3.15°C rise in temperature within a 3-month period. In 2014, mean monthly temperature increments from September to December were as follows: 1.01°C, 1.45°C, and 1.97°C, which

represents a total increase in temperature of 4.43°C within 3 months. The strong spawning response observed in December 2014, which resulted in the release of almost all mature gametes, may be attributed to the steep increase in temperature (~2°C) between November and December 2014. Consistent with this, laboratory experiments by Caballes et al. (2017b) showed a significantly high proportion of crown-of-thorns starfish releasing gametes in response to an abrupt increase in temperature.

While temperature clearly has an effect on reproductive timing, the ultimate cues for gamete release may be entirely different. Explicit tests of potential environmental and biological cues for spawning will be necessary to accurately predict spawning periodicity in the GBR. Based on the information presented here, control efforts should ideally be initiated well before the breeding season (around June to July), as reproductive timing will likely vary between spawning seasons.

Chapter 5

Environmental and biological cues for spawning in the crown-of-thorns starfish[4]

5.1 Introduction

Population outbreaks of the coral-eating crown-of-thorns starfish often result in extensive coral mortality (Pratchett et al. 2014) with highly extended recovery times (Mellin et al. 2016), thereby contributing significantly to sustained and ongoing declines in coral cover across the Indo-Pacific. Given that crown-of-thorns starfish mature quickly (within two years; Caballes and Pratchett 2014) and can have very high fecundity (>100 million oocytes per season for a single female starfish; Babcock et al. 2016b) they are capable of very rapid increases in population size. However, densities of crown-of-thorns starfish vary enormously in space and time (Uthicke et al. 2009), pointing to major fluctuations in reproductive success. Despite being one of the most studied species in coral reef environments, rates of reproductive success (and variation therein) for crown-of-thorns starfish are virtually unknown. Previous studies have shown that variation in the number and arrangement of spawning individuals, as well as the prevailing flow conditions, dictate the local concentration of gametes (Denny and Shibata 1989; Levitan et al. 1992; Babcock et al. 1994). However, the extent to which spawning is synchronized (within and among populations) is the most fundamental constraint on the fertilization success of broadcast spawning, gonochoric species

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(Babcock et al. 1986; Levitan 1995; Olive 1995), such as crown-of-thorns starfish (Babcock and Mundy 1992a; Babcock et al. 1992).

Gametogenesis and spawning in asteroids is, in part, regulated by endogenous neurohormonal mechanisms (Giese and Kanatani 1987). Relaxin-like gonad-stimulating peptides (Mita et al. 2015) produced by supporting cells beneath the outer layer of starfish radial nerves induce the production of a maturation-inducing hormone, 1-methyladenine (Kanatani 1973). Production of 1-methyladenine in ovarian follicle cells around oocytes (Mita 1993) and interstitial cells in testes (Kubota et al. 1977) begins immediately upon detection of gonad-stimulating peptides. This maturation-inducing substance induces the breakdown of the follicular envelope and germinal vesicle of the oocyte, thereby leading to oocyte maturation and spawning of gametes by contraction of the gonad wall (Kanatani 1973). The timing of gamete release is the result of the entrainment of these often tightly programmed endogenous rhythms by cues from the environment.

Environmental factors influencing the course of reproductive events in echinoderms are complex and spawning has been correlated with changes in temperature, photoperiod, lunar cycles, salinity, food abundance, and phytoplankton concentrations (Giese and Kanatani 1987; Mercier and Hamel 2009). Exact triggers of synchronous spawning in marine invertebrates are not well known, partly because of the challenges involved in identifying spawning cues (Mercier and Hamel 2009). Spawning may be synchronous at the scale of meta-populations, where spawning is likely influenced by regional cues (e.g., lunar cycle, day length, temperature), or at scale of local populations ("epidemic" spawning), where gametogenic cycles are likely influenced by generic cues (e.g., water temperature), but actual spawning is largely determined by very localized phenomena (Babcock et al. 1986, 1992; Mercier and Hamel 2009). For crown-of-thorns

starfish, synchronous spawning has been observed among dense aggregations of adults, but the timing appears very unpredictable and it is unknown to what extent spawning is synchronized across discrete populations (Caballes and Pratchett 2014; Pratchett et al. 2014). Notably, there have not been any specific studies that test for spawning synchrony at the scale of meta-populations of crown-of-thorns starfish, which would be possible based on intensive sampling of reproductive condition at multiple locations. Furthermore, there have been reports that spawning by crown-of-thorns starfish coincides with spawning by other sympatric asteroids (Babcock et al. 1992; Babcock 1995; Scheibling and Metaxas 2008), suggesting that there might be general heterospecific cues that initiate spawning.

On Australia's Great Barrier Reef (GBR), the peak spawning period of crown-of-thorns starfish (between November and February) has been deduced from changes in gonad index, gonad condition or histology of ovaries and testes, and changes in oocyte size frequency distribution (see **Table 2** in Pratchett et al. 2014). However, proximate cues that trigger gamete release are difficult to infer from periodic sampling (often done monthly) and analysis of gonads (Mercier and Hamel 2009). Systematic observations of spontaneous spawning in the field has also provided valuable information on the spawning behavior of crown-of-thorns starfish and levels of synchrony in relation to prevailing environmental conditions (Babcock and Mundy 1992a, b; Gladstone 1992). However, observations of spawning of crown-of-thorns starfish in the field are rare. Inferring from the few *in situ* observations of spontaneous spawning by crown-of-thorns starfish (Pratchett et al. 2014), synchronous spawning occurs most often during the falling tide, around late afternoon to evening.

Chemoreception is well documented among asteroids, and despite the absence of a central ganglion in the asteroid nervous system, its radial symmetry and disk-like

body covered with receptor units provide an ideal mechanism for gross chemosensory perception and simultaneous monitoring of stimulus intensity at different positions on its surface (Sloan and Campbell 1982). Unspecialized epithelial cells, innervated by a plexus of the ectoneural system, have been proposed to be receptive to a wide range of stimuli (Pentreath and Cobb 1972). Sloan and Campbell (1982) also described chemically mediated responses in the terminal or sensory tube feet. Pearse et al. (1986) also suggested that asteroid ocelli might be involved in the detection of spawning cues. Previous studies on the foraging behavior of crown-of-thorns starfish have documented its chemosensory ability (Brauer et al. 1970; Ormond et al. 1973), which may allow them to likewise perceive potential spawning cues such as changes in seawater temperature and quality, exudates from phytoplankton, and pheromones from conspecific gametes. Babcock and Mundy (1992a) noticed that starfish that ultimately spawned at Davies Reef in the GBR were unusually active for two hours prior to spawning, which might indicate the time period over which starfish respond to environmental spawning cues.

Effective cues for synchronized spawning within and among distinct populations must be distinguishable from background environmental variation and might also be expected to indicate periods that will maximize fertilization rates and/ or larval survival (Babcock and Mundy 1992b; Himmelman 1999; Baird et al. 2009). The summer spawning season of crown-of-thorns starfish in the GBR have coincided with peak seawater temperatures (Babcock and Mundy 1992a; Pratchett et al. 2014), increased diurnal temperature range (Berkelmans and Willis 1999; Jones et al. 2000; Berkelmans 2001), reduced salinity and high nutrient input from heavy freshwater runoff during flood events (Devlin et al. 2012; Schroeder et al. 2012; Wooldridge and Brodie 2015), and elevated densities and changes in community structure of phytoplankton (Revelante

and Gilmartin 1982; Devlin et al. 2013). Spawning events in multiple echinoderm species have been reported to follow abrupt changes in temperature (Selvakumaraswamy and Byrne 2000; Himmelman et al. 2008). Although temperature appears to influence local gametogenic cycles in crown-of-thorns starfish (reviewed in Pratchett et al. 2014), there is currently no evidence that temperature (either absolute temperatures or rapid changes in temperature) induce spawning. Mass spawning events in some temperate species of chiton, mussels, and sea urchins have also been linked to peaks in phytoplankton abundance (Himmelman 1975; Starr et al. 1990, 1993). Phytoplankton blooms associated with high flow events, usually following cyclones have been documented in the GBR (Devlin et al. 2013). In marine invertebrates with planktotrophic larvae, such as crown-of-thorns starfish, larval survival is often strongly influenced by food availability (Fabricius et al. 2010), thus one critical advantage of phytoplankton as a spawning cue is ensuring that gamete release is timed when environmental conditions are favorable for larval development and survival. Conversely, flood events associated with phytoplankton blooms are often coupled with significant reductions in salinity (Devlin et al. 2012; Schroeder et al. 2012), which may have maladaptive consequences for fertilization success and early development (Caballes et al. 2017a). The role of peak abundance of larval food supply (phytoplankton) on spawning induction in tropical asteroids remains poorly understood (Reuter and Levitan 2010). Inter-individual chemical communication through sex pheromones from conspecific gametes has also been proposed in several marine invertebrates (Mercier and Hamel 2009). Spawning by one individual in an aggregation of the sea urchin, *Sphaerechinus granularis*, induced other conspecifics to spawn (Unger and Lott 1994). The presence of sperm in the water column has been experimentally demonstrated to induce spawning in sea urchins (Starr et al. 1990;

Reuter and Levitan 2010) and starfish (Beach et al. 1975; Miller 1989). Further studies suggested a synergistic relationship between sperm and phytoplankton cues, where spawning response depends on whether sea urchins have been in contact with phytoplankton or phytoplankton extracts (Starr et al. 1992). Conversely, Reuter and Levitan (2010) found that phytoplankton alone did not induce spawning, but when a phytoplankton cue was followed by the addition of sperm, response time to sperm was significantly reduced.

The purpose of this study was to experimentally test potential spawning cues for crown-of-thorns starfish. To the best of my knowledge, explicit tests of spawning cues have never been undertaken for crown-of-thorns starfish, potentially due to logistic challenges associated with experimenting with crown-of-thorns starfish. Alternatively, previous such studies may simply have never been published due to null results or inconclusive findings. In this study, I tested the effects of temperature change, reduced salinity and nutrient enrichment of seawater, phytoplankton, addition of spawned gametes (sperm and eggs), and the combined effect of sperm and phytoplankton on the likelihood of spawning in males versus females. Apart from determining the proximate cues for spawning, these experiments were intended to better understand sexual dimorphism in response to cues and establish whether males or females spawn first. Despite its importance in understanding the mechanisms of synchronous spawning in marine invertebrates (Levitan 1998, 2005), few studies have examined sex-specific responses to spawning cues.

5.2 Methods

5.2.1 Collection and maintenance of specimens

This study was carried out in strict compliance with the guidelines set out by James Cook University and the Lizard Island Research Station. Collection of crown-of-thorns starfish was conducted under Great Barrier Reef Marine Park Authority (GBRMPA) Permit No.G13/36401.1. Adult specimens of the Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*), ranging from 250 to 350 mm diameter, were collected in late November 2014 from Unnamed Reef 14-133 (14° 55.147' S, 145° 30.492' E) located 15 nautical miles (28 km) south of Lizard Island, in the northern Great Barrier Reef, Australia. Average seawater temperature at the collection site during the time of collection was 27.66 °C. Starfish were promptly transported to the Lizard Island Research Station and placed in a 5000-l round fiberglass tank and maintained at ambient conditions (28.30 ± 0.67 °C; 35.46 ± 0.07 psu; pH 8.17 ± 0.01) with continuous flow of fresh seawater. Individuals that were damaged due to handling and/or prematurely spawning due to stress were immediately separated and not used in any experiments. Sexes were also separated, whereby sex identification was done by making a small incision on the proximal region of the arms to collect and examine gonad contents (Caballes and Pratchett 2014). Ovary and testes lobes were placed in 1-methyladenine to check if starfish were ready to spawn. Incisions were allowed to heal and close off for three days prior to undertaking spawning experiments (Ayukai et al. 1996).

5.2.2 Bioassays for spawning induction

Experiments were conducted from late November to early December 2014, which is the likely period of peak spawning of crown-of-thorns starfish on the GBR (Babcock

and Mundy 1992a). Five sets of experiments were conducted to quantify the spawning response of crown-of-thorns starfish to (1) temperature, (2) seawater enrichment, (3) phytoplankton species, (4) addition of spawned gametes, and (5) synergistic effects of gametes and phytoplankton. Starfish were individually placed in plastic aquaria with 50-l seawater in a closed system and provided with constant aeration. Experiments were conducted in shaded wet benches so sunlight from 1500 to 1800 hours was able to penetrate and amount of light was evenly distributed among aquaria. Each bioassay ran for 12 h, from 1500 hours to 0300 hours to coincide with the times of day when spontaneous spawning was previously observed in the GBR (Pratchett et al. 2014). Average photoperiod during the experiments was 13 h. A visual examination of released gametes was done every 15 min and when gametes were released from gonopores along most arms it was scored as "spawned" and the time of spawning was recorded. All replicates were completely independent and each individual sea star was only tested in a single treatment (i.e. one sea star per aquarium for a given treatment condition). Sea stars that have been exposed to a given treatment were not reused for other experiments.

Experiment 1. Spawning response to ambient northern GBR summer temperature (28°C), moderate temperature change (28°C to 30°C), and abrupt temperature change (26°C to 30°C) were assessed for this bioassay. Starfish in plastic aquaria with 0.45-µm filtered seawater were allowed to adjust to initial temperatures (28°C, 28°C, 26°C) for 3 h prior to changing to final temperature settings. Temperature treatments in the closed recirculating system were set using aquarium chillers (Hailea, Guangdong, China) or heaters (Eheim Jäger, Deizisau, Germany) attached to digital temperature controllers (Aqua Logic Inc., CA, USA). Five independent replicates of each sex were used per treatment (N=30).

Experiment 2. This was conducted as procedural control experiments to evaluate spawning response to filtered seawater (control), low-salinity filtered seawater, and nutrient-enriched filtered seawater. Controls were prepared by filtering seawater through a 0.2-µm filter (FSW) to exclude microalgae. For the low-salinity treatment (LS-FSW), filtered freshwater was added until salinity was down to 25 psu. Nutrient-enriched seawater (NE-FSW) was prepared by adding 2 ml of AlgaBoost™ f/2 medium (AusAqua Pty., Ltd., Wallaroo, Australia) to 20 l of 0.2-µm filtered seawater, which was devoid of phytoplankton. Natural phytoplankton blooms are likely to be associated with reduced salinity and high nutrient inputs (Devlin et al. 2013). This experiment isolated the effects of salinity and nutrients from phytoplankton. Eight independent replicates of each sex were used per treatment (N=48).

Experiment 3. This bioassay was used to test spawning response to monocultures of three species of common marine phytoplankton: the dinoflagellate *Dunaliella tertiolecta* (strain CS-175), and the diatoms *Skeletonema pseudocostatum* (strain CS-252) and *Chaetoceros muelleri* (CS-176). Axenic strains of microalgae were supplied by the Australian National Algae Culture Collection (CSIRO, Hobart, Tasmania). Monospecific cultures were maintained in exponential growth with the use of 0.2-µm filtered seawater enriched with AlgaBoost™ f/2 medium. The cultures were grown at 20°C under a 16-hour light: 8-hour dark cycle (daylight fluorescent lighting). Air filtered at 0.2-µm was continuously bubbled through the cultures. Sodium metasilicate pentahydrate (13 mg l^{-1}) was added to seawater medium used to culture diatoms. Cell density was quantified daily using a haemocytometer. Concentrated cultures were placed in sealed glass bottles and allowed to sit in a water bath set at 28°C for 3 h before being added to each aquarium to reach a final concentration of 5 x 10^8 cells l^{-1}, based on previous spawning induction experiments on sea urchins (Starr et al. 1990;

Reuter and Levitan 2010). Filtered seawater (FSW) was used for controls and eight independent replicates of each sex were used per treatment (N=64).

Experiment 4. This bioassay was conducted to examine the spawning response of crown-of-thorns starfish to conspecific gametes. Eggs were collected from two female starfish induced to spawn by injecting 1 x 10^{-4} M 1-methyladenine on each arm junction 90 min before the experiment started. Eggs were transferred to clear containers with FSW and the number of eggs per mL was counted using a gridded slide under a dissecting microscope. Eggs were added to aquaria to achieve a final concentration of ~2 eggs ml^{-1}. Sperm was collected from two male starfish 15 min before the experiment started using the same method employed for females above. Sperm concentration was quantified by haemocytometer counts and added to aquaria to achieve a concentration of 1 x 10^4 sperm ml^{-1} (Benzie and Dixon 1994). Filtered seawater (FSW) used for controls was devoid of gametes and eight independent replicates of each sex were used per treatment (N=48).

Experiment 5. This experiment was performed to determine whether sperm and high phytoplankton concentrations had a synergistic or additive effect on spawning response in crown-of-thorns starfish. It was not possible to test for synergies across all combinations of potential spawning cues (due to limitations in aquarium space and the number of starfish that could be housed), and this synergy was prioritized based on previous studies showing evidence of synergism between sperm and phytoplankton (Starr et al. 1990, 1992; Reuter and Levitan 2010); as well as limited evidence for threshold temperature and salinity (Mercier and Hamel 2009). Seawater (FSW) in control aquaria had no gametes or phytoplankton, while sperm treatments were the same as above. For sperm and phytoplankton (PP) treatments, a mixture of three species of phytoplankton (*D. tertiolecta*, *S. pseudocostatum*, *C. muelleri*), each at a

concentration of 1.67 x 10^8 cells ml^{-1}, was added to the sperm suspension. Eight independent replicates of each sex were used per treatment (N=48).

5.2.3 Statistical analyses

The number of starfish that spawned in response to different treatments was arranged as a Model II contingency table, where marginal totals for each treatment (replicates) were fixed (Quinn and Keough 2002). Contingency tables for each set of experiments were analyzed using log-linear models with log link and Poisson error terms (Agresti 1996) to examine the spawning response of crown-of-thorns starfish in relation to 'Sex' and 'Treatment'. Spawning response was considered a response variable so all models included the interaction between 'Sex' and 'Treatment' (Quinn and Keough 2002). Deviance statistics (G_2) were used to compare models in R (R Core Team 2016.). Odds ratio (OR) calculations for cells with zero observed counts were corrected by adding 0.5 to each cell (Agresti 1996). Asymptotic standard errors were also obtained to calculate 95% confidence intervals for odds ratios. Pairwise comparisons were done using Fisher's Exact Test implemented in R (R Core Team 2016). Distributions of spawning response time after exposure to independent treatments were compared using the Log-rank test, which is a widely used non-parametric test to compare time-to-event (time until spawning from initial treatment) distributions, while adjusting for right-censoring (termination of experiment after 12 h) (Walker and Shostak 2010). This was followed by Holm-Šídák *post hoc* multiple comparisons (α=0.05) implemented in Sigmaplot 12 (Systat Software, Inc., CA, USA).

5.3 Results

5.3.1 Effects of threshold temperature versus temperature change

Across all treatments, 40% of all males spawned compared to only 6.7% of female starfish ($G_2 = 9.954$, df = 3, p = 0.019). Spawning response was found to be dependent on temperature change treatments ($G_2 = 17.530$, df = 4, p = 0.002), where a +4°C temperature shock (26°C to 30°C) resulted in significantly higher spawning frequency in males (100%) compared to control (0%; OR = 121.000, 95% CI 2.017–7259.723) and +2°C temperature change treatment (20%; OR = 33.000, 95% CI 1.064–1023.620) treatments (**Figure 5.1a**). Females did not spawn under 'no change' and 'moderate change' treatments, and only spawned at a single instance when exposed to a +4°C temperature shock. Male spawning response time distribution (**Figure 5.2a**) was also significantly different among treatments (Log-rank $\chi^2 = 8.623$, df = 2, p = 0.013), but there was no significant treatment effect on time-to-spawning in female starfish (Log-rank $\chi^2 = 2.000$, df = 2, p = 0.368; **Figure 5.2a**). Male starfish spawned 240 min (±SE = 122 min) after exposure to temperature change from 26°C to 30°C. All log-linear model comparisons to test for complete dependence and conditional dependence for this experiment and subsequent experiments are summarized in **Table 1**. Odds ratios and 95% confidence intervals for pairwise comparisons of all treatments tested in each experiment are listed in **Appendix C – Table C1**.

5.3.2 Effects of water quality properties

FSW control, low-salinity FSW, and nutrient-enriched FSW were ineffective in inducing high rates of spawning in crown-of-thorns starfish (**Table 1**). Spawning was not dependent on procedural treatments ($G_2 = 0.395$, df = 4, p = 0.983), but an association with 'Sex' exists ($G_2 = 10.976$, df = 3, p = 0.012), as 16.7% of males

spawned and none of the female starfish spawned under all the treatments (**Figure 5.1b**). Only 12.5% of males spawned under FSW control and nutrient-enriched FSW treatments, and only 25% spawned under low-salinity FSW. For the males that spawned, response time distributions were also not significantly different from controls (Log-rank $\chi^2 = 0.567$, df = 2, p = 0.714; **Figure 5.2b**).

5.3.3 Effects of phytoplankton monocultures

The incidence of spawning when crown-of-thorns starfish were exposed to monocultures of phytoplankton are significantly higher among males (37.5%; OR = 26.277, 95% CI 1.456–474.208) compared to females, where no spawning was observed across all treatments ($G_2 = 13.802$, df = 4, p = 0.008). Among the phytoplankton species tested, exposure of males to *S. pseudocostatum* resulted in the highest frequency of spawning (62.5%), but was not significantly different from controls (12.5%) and other phytoplankton species (*Dunaliella*: 12.5%; *Chaetoceros* 25%) ($G_2 = 10.379$, df = 6, p = 0.110; **Figure 5.1c**). Overall, different phytoplankton taxa had a significant effect on spawning response time distributions (Log-rank $\chi^2 = 8.440$, df = 3, p = 0.038), but none of the pairwise comparisons had enough power to meet the Holm-Šídák criterion (**Figure 5.2c**).

5.3.4 Effects of conspecific gametes

Regardless of sex ($G_2 = 4.186$, df = 3, p = 0.242), there was a significant increase in the incidence of spawning following addition of gametes ($G_2 = 17.008$, df = 4, p = 0.002). The presence of sperm in the water column induced 75.0% of males and 37.5% of females to spawn, while only 12.5% of males and females spawned when exposed to eggs (**Figure 5.1d**). None of the starfish spawned under 'Controls'. Among males, the

incidence of spawning when exposed to sperm was 13 (95% CI 1.329–127.168) times higher than when exposed to eggs. Moreover, there was a significant difference in the cumulative probability of spawning and response times of males (Log-rank $\chi^2 = 12.887$, df = 2, p = 0.002); in particular, spawning rates (incidence and response time) were significantly higher in response to sperm compared to eggs and controls (**Figure 5.2d**). There was no significant difference in female spawning response time (Log-rank $\chi^2 = 2.050$, df = 2, p = 0.150) among gamete treatments (**Figure 2d**).

5.3.5 Sperm and phytoplankton

Experiments to test the synergistic effects of phytoplankton and sperm showed that spawning response was dependent on 'Treatment' ($G_2 = 16.412$, df = 4, p = 0.003) for both males and females. Sperm (50%) and phytoplankton-and-sperm (43.8%) treatments did not differ significantly, but spawning frequencies of males and females under both treatments were significantly higher than controls (**Figure 5.1e**). There was an overall difference in male spawning rates among treatments (Log-rank $\chi^2 = 7.984$, df = 2, p = 0.018), as response time under sperm and phytoplankton-and-sperm treatments was significantly faster compared to controls (**Figure 5.2e**). Although spawning response time between sperm and phytoplankton-and-sperm treatments did not differ significantly, starfish exposed to phytoplankton-and-sperm had shorter average response times (81 ± 15 mins) compared to sperm treatments (216 ± 51 mins). There was no difference in female response time among treatments (Log-rank $\chi^2 = 4.277$, df = 2, p = 0.181; **Figure 5.2e**).

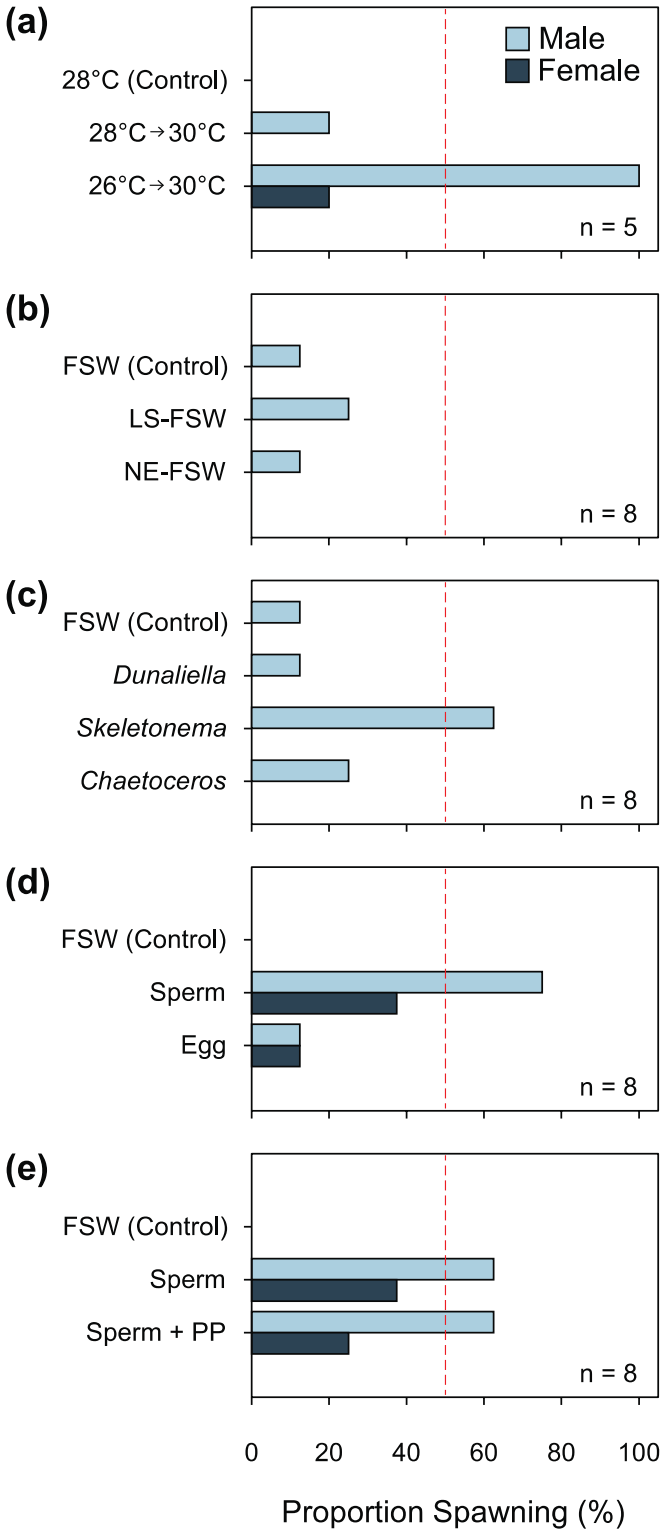

Figure 5.1 Proportion of starfish that spawned in response to cues: (**a**) seawater temperature, (**b**) water quality, (**c**) phytoplankton, (**d**) conspecific gametes, (**e**) sperm and phytoplankton. FSW = 0.2-µm filtered seawater; LS-FSW = low salinity filtered seawater; NE-FSW = nutrient-enriched filtered seawater; PP = combination of three phytoplankton species. Bars traversing the dashed lines represent spawning of more than 50% of individuals exposed to a given treatment.

Table 5.1. Analysis of deviance table for hierarchical comparisons of log-linear models to test for patterns of complete dependence and conditional independence of variables inducing spawning response in crown-of-thorns starfish. 'Spawning' was considered to be a response variable, so all fitted models included the 'Treatment' by 'Sex' interaction term.

Source	*df*	G_2	*P*
(a) *Temperature*			
Treatment	4	17.530	**0.002**
Sex	3	9.954	**0.019**
Treatment × Sex	2	3.400e-10	1.000
(b) *Water Quality*			
Treatment	4	0.573	0.966
Sex	3	5.965	0.113
Treatment × Sex	2	4.887e-10	1.000
(c) *Phytoplankton (PP)*			
Treatment	6	10.379	0.110
Sex	4	13.802	**0.008**
Treatment × Sex	3	3.307e-10	1.000
(d) *Gamete*			
Treatment	4	18.962	**0.001**
Sex	3	2.348	0.503
Treatment × Sex	2	0.736	0.692
(e) *Sperm* + *PP*			
Treatment	4	16.412	**0.003**
Sex	3	3.358	0.340
Treatment × Sex	2	0.153	0.926

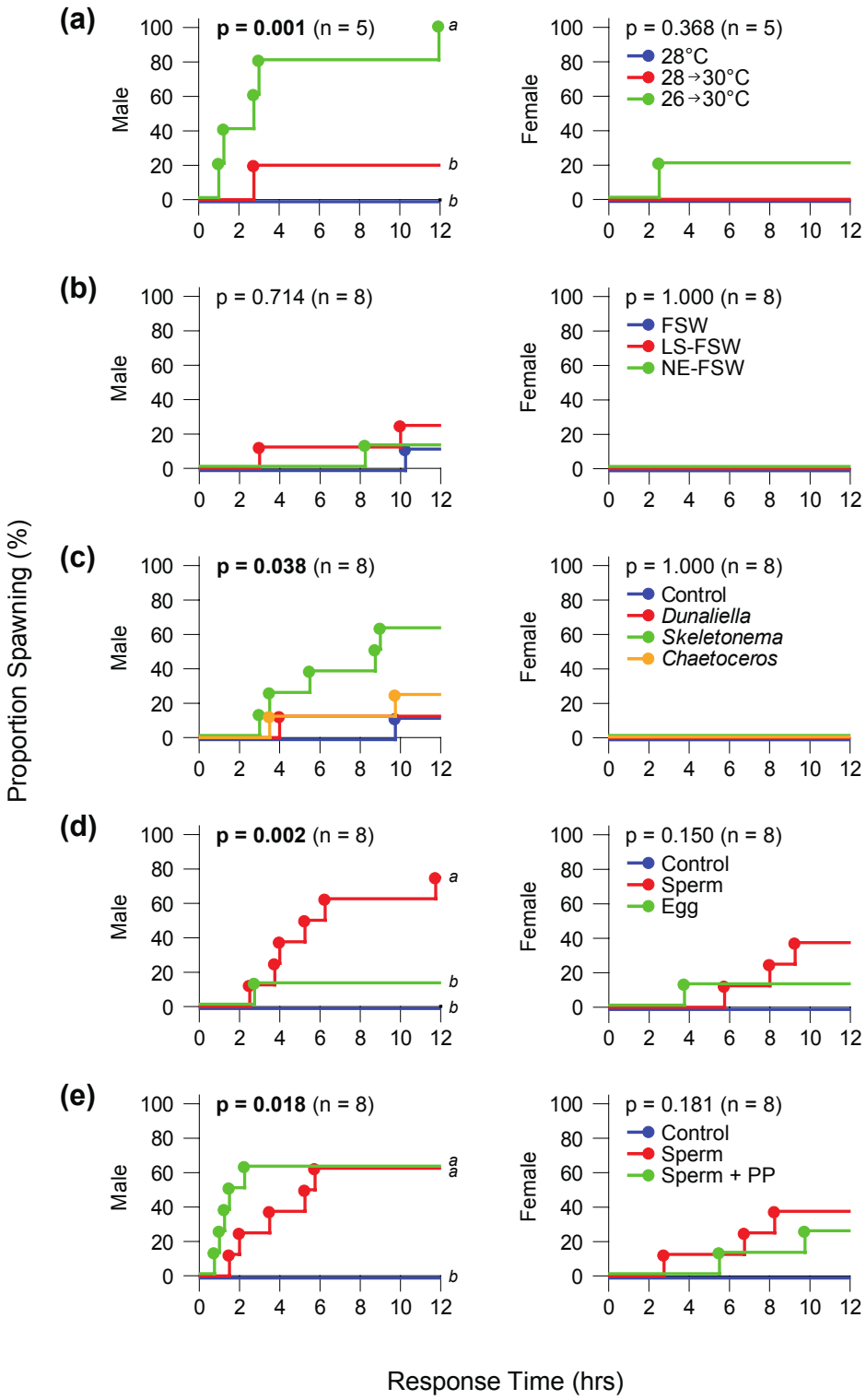

Figure 5.2 Response time and cumulative probability of spawning in male and female crown-of-thorns starfish after exposure to environmental and biological cues: (**a**) seawater temperature, (**b**) water quality, (**c**) phytoplankton, (**d**) conspecific gametes, (**e**) sperm and phytoplankton. Solid circles are individual spawning events and different letters indicate significant differences based on multiple comparisons (Holm-Šídák) after Log-rank analyses. FSW = 0.2-µm filtered seawater; LS-FSW = low salinity filtered seawater; NE-FSW = nutrient-enriched filtered seawater; PP = combination of three phytoplankton species.

5.4 Discussion

While there have been no explicit tests of spawning cues for crown-of-thorns starfish, geographical differences in gametogenic cycles (reviewed by Pratchett et al. 2014) suggest that temperature is an important determinant of seasonal maturation, if not actual spawning. Temperature has been one of the most discussed potential spawning cues in the extensive literature available for marine invertebrates; despite this, very few studies have provided convincing evidence on the proximal role of temperature in gamete discharge (Mercier and Hamel 2009). In several echinoderm species, including crown-of-thorns starfish in the GBR, gametogenesis is clearly linked to local temperature regimes, but few studies have shown that specific changes in temperature or absolute temperatures stimulate gamete release (e.g., Hamel and Mercier 1995; Mercier and Hamel 2008). On the GBR, the long-term average sea surface temperature during the annual summer spawning season (mid November to mid January) is 28.00 ± 0.5 °C. In my spawning experiments none of the gravid starfish spawned when maintained at 28 °C, suggesting that threshold temperatures are not sufficient in their own right to induce spawning. However, gamete release in male crown-of-thorns starfish was triggered by an abrupt increase in seawater temperature, independent of any changes in nutrient concentrations, phytoplankton abundance, photoperiod, or conspecific interactions. Sea surface temperatures can vary > 4 °C throughout the summer spawning season on the GBR, but within the course of a single day, temperatures usually vary within 1 °C in the relatively deeper reef slope and within 1-2 °C in the shallower reef flats (Berkelmans and Willis 1999). Although rare, abrupt temperature changes have been reported in some parts of the GBR (Jones et al. 2000; Berkelmans 2001; Berkelmans et al. 2010). Temperature spikes from normal diurnal temperature variation have been associated with intense summer upwelling events in the

GBR (Berkelmans et al. 2010). On a number of occasions, temperature change at a rate of 1 °C per hour over a 6-hour period have been documented in inshore reefs around Magnetic Island. Diurnal temperature variation was usually more pronounced in reef flats, and varied on average by 4 °C at more offshore reefs around Heron Island (Berkelmans 2001), but can vary by up to 5-7 °C when tidal range is at its maximum (Jones et al. 2000). Spawning observations of crown-of-thorns starfish *in vivo* have mostly been reported in shallow depths, where changes in seawater temperature are likely to be greatest. Babcock & Mundy (1992a) reported that all spawning starfish were found between 1 and 4 m deep during the spawning event of crown-of-thorns starfish observed at Davies Reef on the GBR. Although not very common in tropical reefs, these rapid increases in temperature may be important in triggering spawning.

Minchin (1987) suggested that rapid increases in seawater temperature, caused by local moderate onshore winds on sunny days, induced spawning in the starfish *Marthasterias glacialis* in shallow waters (< 4-m depth). Himmelman et al. (2008) reported that the mass spawning of several echinoderm species off the Mingan Islands in northern Gulf of St. Lawrence (eastern Canada) coincided with sharply increasing seawater temperatures brought by the incursion of warm surface waters. In natural settings, abrupt fluctuations in temperature may also result in alterations of seawater chemistry and may be associated with increased abundance of phytoplankton. Fine scale monitoring of concurrent environmental data during natural spawning events is needed to provide conclusive evidence for the role of temperature in gamete release.

Flood plumes in the GBR are characterized by medium to low salinity, high nutrient levels, increased chlorophyll-*a* concentration, and elevated phytoplankton abundance (Devlin et al. 2013). Reduced salinity and elevated nutrient levels did not induce gamete release in females and spawning frequency and response time in males

was not significantly different from controls. Evidence for the role of salinity in spawning induction in echinoderms is scant and salinity fluctuations are typically minimal and short-lived (reviewed in Mercier and Hamel 2009). It would also seem to be maladaptive to use salinity as a cue for gamete release as low salinity has been shown to have detrimental effects on osmotic balance in the eggs of crown-of-thorns starfish, resulting in reduced cleavage and gastrulation rates (Caballes et al. 2017a). Consistent with my results, less than 5% of green sea urchins (*Strongylocentrotus droebachiensis*) and blue mussels (*Mytilus edulis*) responded to addition of f/2 culture medium in the absence of phytoplankton (Starr et al. 1990). These results suggest that water quality parameters (low salinity, high nutrients) typically associated with high phytoplankton abundance (Devlin et al. 2013) do not directly induce spawning in crown-of-thorns starfish.

Frequency of spawning in male starfish in response to the three phytoplankton species tested was not significantly above control levels and none of the females spawned. This is consistent with results of work on the sea urchin, *Lytechinus variegatus*, where only a very small proportion of males and none of the females spawned in response to phytoplankton (Reuter and Levitan 2010). The duration of my experiment (720 min) may not have been enough to stimulate a significant spawning response, although phytoplankton cues must be detected on the onset of blooms for it to be advantageous to planktotrophic larvae since these events are often short-lived. I also cannot rule out that spawning response of crown-of-thorns starfish may be dependent on the concentration of phytoplankton, as previously shown for *S. droebachiensis* and *M. edulis* (Starr et al. 1990). Nevertheless, phytoplankton concentrations used in this study were higher than concentrations that induced maximum spawning in experiments by Starr et al. (1990) and maximum phytoplankton abundances from flood plume samples

in the GBR (Devlin et al. 2013). In addition, mass spawning by crown-of-thorns starfish has also been observed in the absence of peaks in phytoplankton abundance in the GBR (Babcock et al. 1992). Although putative cues isolated from phytoplankton were found to be present in a variety of algal species, it is worth noting that *Skeletonema* induced 62.5% of males to spawn, compared to only 25% and 12.5% when exposed to *Chaetoceros* and *Dunaliella*, respectively. This variation may indicate a qualitative difference in the exudates of this microalgae species. Monitoring of flood plumes in the GBR has shown that elevated chlorophyll-*a* concentrations during high flow events are associated with the highest phytoplankton abundances, driven predominantly by high counts of nanoplankton species, particularly the diatoms *Skeletonema*, and *Chaetoceros* (Devlin et al. 2013). Further studies are warranted on the possible role on synchronous spawning of these abundant diatoms associated with flood plumes in the GBR. For echinoderms with planktotrophic larvae, such as crown-of-thorns starfish, it would be advantageous to time gamete release when environmental conditions are favorable for larvae (Starr et al. 1990; Brodie et al. 2005; Fabricius et al. 2010). However, apart from phytoplankton blooms induced by nutrient enrichment, flood events are also associated with environmental stressors, such as reduced salinity, which may have maladaptive consequences for gametes, fertilization, and embryonic development in crown-of-thorns starfish (Caballes et al. 2017a).

The presence of sperm or chemical cues associated with sperm and/or spawning induced gamete release in a large proportion of male and female starfish. In an aggregation of the sea urchin, *Sphaerechinus granularis*, one-third of the group immediately spawned after gamete release was induced in an individual and sea urchins downstream also started shedding gametes within 20 minutes (Unger and Lott 1994). My results are also consistent with spawning induction assays where conspecific sperm

triggered gamete release in *L. variegatus* (Reuter and Levitan 2010). Previous laboratory experiments have shown that pheromones extracted from ovaries and testes of crown-of-thorns starfish attract movement towards the spawning individual and triggers synchronous spawning among neighboring starfish (Beach et al. 1975). Miller (1989) also demonstrated that female starfish (*Asterias forbesi* and *Orthasterias koehleri*) produced long-lived sperm chemoattractants and proposed a model where males respond by migrating towards females and as the concentration of attractants increases (through aggregation and increased production by females with ripening ovaries), males are induced to spawn, releasing sperm that stimulates spawning in females. This is further supported by the finding that homogenates of ovaries from the brittle stars, *Ophiocoma dentata* and *Ophiocoma scolopendrina*, induced spawning in conspecific males, while sperm did not elicit any response (Soong et al. 2005). My results, however, show that eggs in the water column did not induce a significant proportion of starfish to release gametes. Combining sperm and phytoplankton did not increase the likelihood of spawning in both males and females, but it did slightly reduce the spawning response time in male starfish when compared to sperm treatments. This warrants further studies as sperm and phytoplankton have been shown to have synergistic effects in sea urchin spawning assays (Starr et al. 1990; Reuter and Levitan 2010).

Across all experiments, males were more likely to spawn in response to potential cues tested compared to females; and even if the females did spawn, males responded much faster. Sexual dimorphism in spawning has been reported in numerous broadcast spawning marine invertebrates, and in most cases, males initiate spawning before females (Levitan 1998). This pattern is consistent with observations of *in situ* spawning by crown-of-thorns starfish, where some males initiate spawning followed by gamete

shedding by females and other males (reviewed in Pratchett et al. 2014), albeit with some exceptions (see Babcock and Mundy 1992b). If sperm is limited, females will most likely spawn first and induce males to spawn so that sperm dilution is minimized (Soong et al. 2005). Alternatively, when sperm competition exerts a strong selective pressure, males typically spawn earlier to reach unfertilized eggs first (Levitan 2005). Some males in a given population may be more sensitive to exogenous cues and gamete shedding by these males subsequently causes the release of pheromones that induce spawning in conspecifics (Babcock and Mundy 1992a; Unger and Lott 1994). Delay in female spawning may reflect constraints on the mechanism of egg release compared to sperm release in males, as it has to go through maturation (Giese and Kanatani 1987; **Figure 5.3**). When placed in seawater with 1-methyladenine, testes tend to shed sperm immediately, while ovaries take 30-60 min (Caballes and Pratchett 2014). *In situ* spawning experiments using the red sea urchin, *Strongylocentrotus franciscanus*, show that early-spawning males gained higher average fertilization, more extensive spatial cover of fertilization, and far fewer cases of reproductive failure compared to males that spawned later (Levitan 2005). The delay in spawning by females may allow males to accumulate sperm to a critical concentration and eggs are not shed until this threshold sperm concentration in the water column is reached (Levitan 1998). The optimal interval between the initiations of male and female spawning is influenced by flow conditions and the degree of sperm competition and aggregation (Levitan 2005). Sperm of crown-of-thorns starfish has been shown to age more rapidly than eggs and must come in contact with eggs within 2 h from release to avoid wastage and fertilization failure (Benzie and Dixon 1994).

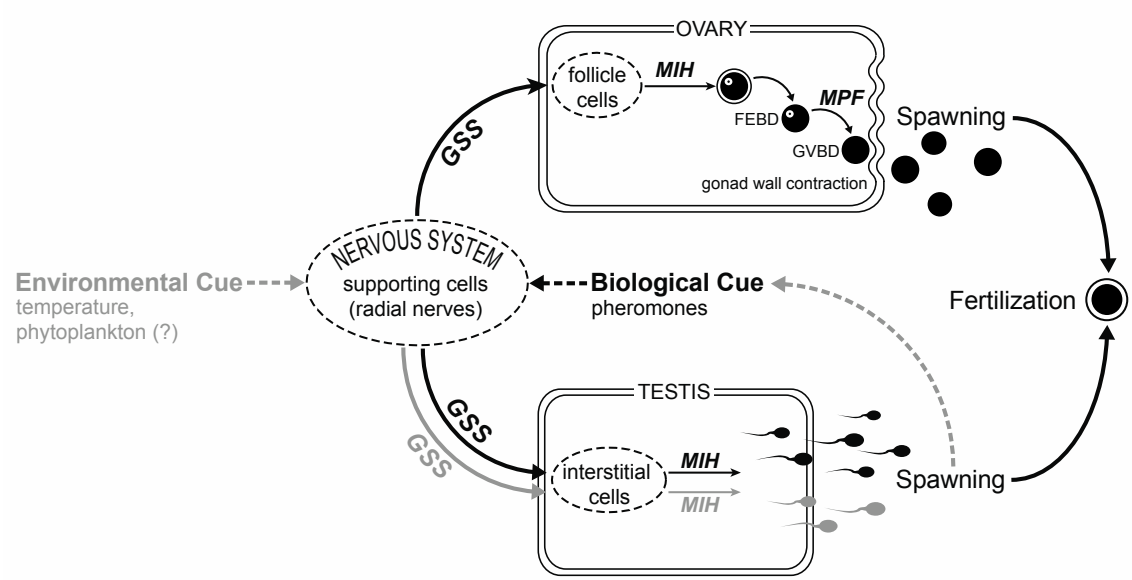

Figure 5.3 Schematic diagram of proposed cascade model for spawning induction and synchrony in response to environmental and biological cues. Grey arrows are responses to environmental cues and black arrows refer to biological cues. Neurohormonal mechanisms are based on Giese and Kanatani (1987) and Mita et al. (2015). **GSS** = gonad-stimulating substance (relaxin-like gonad stimulating peptide); **MIH** = maturation-inducing hormone; **MPF** = maturation-promoting factor; **FEBD** = follicular envelop breakdown; **GVBD** = germinal vesicle breakdown.

The proportion of starfish spawning in response to the cues tested in this study may be comparable to the proportion of spawning observed in the field. For example, the most substantial natural spawning observed at Davies Reef involved only 60% of all individuals, but gamete density was enough to significantly reduce water visibility (R. Babcock, pers. comm.; Babcock and Mundy 1992a). Taken together, my experiments suggest that male crown-of-thorns starfish initiate spawning in response to environmental cues (e.g. temperature change), which subsequently synchronizes spawning by inducing females and other males to spawn via biological cues (pheromones) from sperm in the water column (**Figure 5.3**). I propose that environmental cues act as spawning 'inducers' by causing the release of hormones (gonad stimulating substance) in sensitive males. Biological cues (pheromones: Beach et al. 1975; Miller 1989) from released sperm, in turn, act as spawning 'synchronizers' by triggering a hormonal cascade resulting in gamete shedding by conspecifics. The ultimate environmental cue that induces gamete release remains unclear. Other environmental cues that were not tested here, such as length of photoperiod, light intensity, tides, and currents could also play a role in spawning induction (Mercier and Hamel 2009). Here I showed that an abrupt rise in temperature, rather than a defined threshold temperature, triggered spawning in male starfish. Majority of males also spawned in response to the presence of the diatom, *Skeletonema*, which is known to be abundant during high flow events in the GBR (Devlin et al. 2013). Marine invertebrates may use a hierarchy or combination of environmental cues to trigger synchronous spawning in a population (e.g. Watson et al. 2000; Gaudette et al. 2006). Crown-of-thorns starfish have been observed to participate in synchronous multi-specific spawning events in the GBR (Babcock et al. 1992) and may respond to a common spawning signal released by other species that are shedding gametes. It is difficult to

separate the stimuli for gametogenesis from the actual spawning cue, since the culmination of gamete production may itself stimulate spawning, as the pressure of gravid gonads may stimulate the gonadal musculature, thereby exciting the hormonal mechanisms (Giese and Kanatani 1987). My experiments were conducted with isolated individuals, and the degree of synchrony might increase further if starfish were in close contact, so that cues could accumulate and be magnified among individuals. In comparing spawning between dispersed and aggregated populations, Okaji (1991) suggested that aggregated individuals receive spawning stimuli at a higher frequency and magnitude compared to dispersed individuals, thereby accounting for better synchronization and higher reproductive output. Spawning was also minimal in small populations of *S. droebachiensis* compared to a large and dense population, implying that sperm concentration may not have been high enough to trigger pheromone-mediated spawning in less responsive urchins (Gaudette et al. 2006). Differences in the physiological condition of individuals and temporal or spatial variation in the concentration or magnitude of environmental cues may also explain the unpredictability of crown-of-thorns starfish spawning events. Given the immediate temporal linkage between the timing of spawning and fertilization events, variability in the extent and synchronicity of gamete release may significantly influence reproductive success and explain marked fluctuations in the abundance and distribution of crown-of-thorns starfish populations.

Chapter 6

Environmental tipping points for sperm motility, fertilization, and embryonic development in the crown-of-thorns starfish[5]

6.1 Introduction

Outbreaks of the coral-eating crown-of-thorns starfish are one of the most significant biological threats to coral reefs and account for a substantial proportion of coral mortality in the Indo-Pacific region (De'ath et al. 2012; Baird et al. 2013; Pratchett et al. 2014). Crown-of-thorns starfish are predisposed to major population fluctuations, whereby local densities may vary by several orders of magnitude (Uthicke et al. 2009), due to inherent features of their reproductive biology and behavior (Babcock et al. 2016b; Caballes et al. 2016). Reproductive success is central to explaining periodic increases in local densities (Caballes and Pratchett 2014). Understanding the critical events in the early life history of crown-of-thorns starfish is key to identifying population bottlenecks that could be strategically targeted to improve control programs and mitigate coral mortality (Hoey et al. 2016). Despite this, environmental drivers of variation in reproductive success for *Acanthaster* spp. remain poorly understood.

Achieving high fertilization rates is vital in ensuring reproductive success (Levitan 1995). Fertilization had initially been thought to be non-limiting, given that broadcast spawners, such as crown-of-thorns starfish, release copious amounts of

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gametes during spawning (Conand 1985; Babcock et al. 2016b). Population replenishment in crown-of-thorns starfish is believed to be largely regulated by larval provisioning, larval delivery, post-settlement competition and predation (Cowan et al. 2016; Yamaguchi 1973a; Fabricius et al. 2010; Hock et al. 2014). However, a host of factors, at the gamete, individual, and population levels, as well as prevailing environmental conditions, can influence fertilization success (Levitan 1995). For example, changes in sperm swimming speeds and the proportion of motile sperm affect fertilization success in crown-of-thorns starfish (Uthicke et al. 2013) and other echinoderms (Havenhand et al. 2008; Schlegel et al. 2012). Previous studies have shown that the number and distribution of individuals and the prevailing flow conditions during spawning dictate the local concentration of gametes (Denny and Shibata 1989; Levitan et al. 1992; Babcock et al. 1994). Fertilization rates of crown-of-thorns starfish have been reported to reach up to 83% at the peak of a major spawning event (Babcock and Mundy 1992a). In induced spawning experiments in the field, fertilization rates can be as high as 95% when male and female starfish are in very close proximity (Babcock et al. 1994). As expected, fertilization rates drop significantly as the distance between spawning individuals increases. Nevertheless, 70% fertilization success was still achieved at distances of up to 8 m between spawning individuals and more than 20% at a distance of 60 m (Babcock et al. 1994). Fertilization success per unit distance in crown-of-thorns starfish is higher compared to other asteroid species and significantly greater than those reported for other marine invertebrates (Caballes and Pratchett 2014). Despite achieving high fertilization rates at given sperm concentrations, at greater distances, and at longer durations from the point of gamete release (Benzie and Dixon 1994), very little is known on the tolerance of gametes,

fertilization, and early development of crown-of-thorns starfish to a wide range of environmental conditions.

For broadcast spawning invertebrates such as crown-of-thorns starfish, early life history stages occur in the water column where environmental factors could disrupt the initial phases in the process of population replenishment. The persistence and success of populations require that all developmental stages be completed successfully and the variable sensitivity of planktonic stages (i.e., gametes, fertilization, early development) to environmental stressors (e.g., temperature, salinity, pH) may be a potential population bottleneck (Byrne 2012; Przeslawski et al. 2015). Evaluating the effects of environmental stress on gametes and early life history stages is important as this can result in detrimental flow-on effects where physiological performance and cellular responses of subsequent ontogeny depend on the success of preceding stages (Byrne 2012). In addition, marine organisms are exposed not only to natural environmental stressors, but also the compounding effects of anthropogenic stressors, notably increasing global temperatures, pulses of decreased salinity brought about by higher frequency of cyclones and freshwater runoff, and reduced pH (Przeslawski et al. 2015). Climate change causes changes in baseline environmental conditions, such that inherent fluctuations of temperature, salinity, and pH, particularly in nearshore waters, may increasingly exceed tolerance thresholds, especially for populations currently living at physiological limits (Helmuth et al. 2006).

Recent studies on the response of early life history stages of marine invertebrates to ocean warming and acidification, have improved our knowledge on environmental thresholds of several species (Przeslawski et al. 2015). Generally, temperature affects everything an organism does through its pervasive physiological impact on all biological functions (Hoegh-Guldberg and Pearse 1995). Ocean acidification has

negative impacts on development due to direct pH effects and hypercapnic suppression of metabolism, and is a major threat to marine calcifiers because acidification decreases carbonate saturation with a negative impact on skeleton formation (Przeslawski et al. 2008; Byrne 2012). Pulses of reduced salinity brought by heavy rainfall or freshwater lenses of river plumes have been reported to result in decreased growth and reproduction rates in some invertebrates (Roberts et al. 2006) and affect the cellular osmoregulation in gametes and embryos (Greenwood and Bennett 1981). The responses of echinoderms to these environmental stressors are stage- and species-specific, but gametes and fertilization appear to be robust to a wide range of temperature, salinity, and pH levels (Rupp 1973; Kashenko 2005; Dupont et al. 2010). Environmental tolerances of echinoderm embryos are generally narrower than for gametes and fertilization (Johnson and Babcock 1994; Kashenko 2005; Havenhand et al. 2008).

Spermatozoa of free-spawning marine organisms remain immobile at the time of gamete release but become motile spontaneously upon dilution in seawater. Evaluating the response of spermatozoa to environmental factors is important since activation is influenced by seawater temperature, osmotic pressure, extracellular pH, ultraviolet radiation, and the concentration of specific ions relative to that in the seminal plasma in echinoderms (Shirai et al. 1982; Mita and Nakamura 1998; Lu and Wu 2005a, 2005b). Sperm swimming speeds in the polychaete, *Galeolaria caespitosa*, have been reported to be enhanced under increased water temperatures (Kupriyanova and Havenhand 2005), but comparable research is yet to be undertaken for most echinoderms (Przeslawski et al. 2008; Byrne 2012). Decreased motility and inactivation of sperm at low salinities has also been reported in sea urchins (Greenwood and Bennett 1981; Dinnel et al. 1987). Previous studies on echinoids also show reductions in the percentage of motile sperm at decreased pH and ultimately reproductive success [18–

20]. There is also evidence that oocytes from conspecifics release attractants that induce chemotaxis toward the egg (Miller 1985; Cook et al. 1994; Nishigaki et al. 1996). In some marine invertebrates, chemoattractants may not only change the direction of sperm swimming but also increase sperm swimming speeds and the proportion of motile sperm (Bolton and Havenhand 1996; Litvak and Trippel 1998; Kupriyanova and Havenhand 2002). The interactive or additive effects of environmental stressors and egg-derived chemoattractants warrant further attention, especially given potential impacts of climate change on fertilization success.

The purpose of this study was to compare sperm behavior and rates of fertilization, as well as early development under a range of environmental variables to identify environmental tipping points and thresholds for reproductive success (Caballes and Pratchett 2014). Here, I examine temperature, salinity, and pH thresholds of sperm motility, fertilization, cleavage, and gastrulation. Reproductive failure in echinoderms has been reported at different levels of these environmental parameters, but few have investigated whether this is due to the sensitivity of gametes, failure of fertilization, or failure of fertilized eggs to cleave or hatch (Byrne et al. 2009; Allen and Pechenik 2010; Allen et al. 2017). We also tested the excitatory effect of water-soluble egg extracts on sperm behavior to add a maternal dimension to the characterization of sperm motility. Sperm swimming speeds and proportion of motile sperm are discussed in relation to fertilization rates. Previous studies on the impacts of these environmental variables on marine invertebrates have mostly set experimental conditions with respect to projections by the Intergovernmental Panel on Climate Change (IPCC. 2014) for temperature rise (2°C to 4°C above ambient), pulses of decreased salinity (regionally variable), and ocean acidification (0.2 to 0.4 pH units below ambient) (Byrne 2011). Here, I included extreme environmental stressor treatments to determine how far gametes, fertilization,

and early development can be pushed to identify tipping points and thresholds for deleterious effects. Developmental arrest in response to multiple environmental stressors at the earliest stages can be used to define lower and upper limits for normal development. Quantifying environmental regulation of initial elements of reproductive success is important in understanding the spatial and temporal dynamics of populations of *Acanthaster* spp., as well as understanding vulnerability to environmental changes.

6.2. Methods

6.2.1 Collection and maintenance of animals for experiments

Adult individuals of the Pacific species of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) were collected from aggregations in reefs around Puntan Dos Amantes (13° 32.346' N, 144° 48.200' E) on the northwest coast of the island of Guam, Micronesia in October 2013. Starfish were immediately transported to the University of Guam Marine Laboratory and allowed to acclimatize to ambient conditions for 48 hours (28.79 ± 0.23°C; 34.19 ± 0.04 psu; pH 8.23 ± 0.02) in 1000-L concrete tanks with flow-through seawater. Individuals were sexed by drawing contents from gonads along the arm junction using a syringe with a large-bore biopsy needle (Caballes and Pratchett 2014). Male and female crown-of-thorns starfish were placed in separate tanks prior to experiments. Gametes from gravid individuals were examined under a compound microscope to generally assess reproductive maturity of oocytes and sperm motility.

6.2.2 Preparation of experimental seawater

Water-soluble egg extracts. Water-soluble egg extracts and seawater solutions (EES) were prepared by incubation of unfertilized eggs from five females (standardized to 100 egg ml^{-1}) for 60-90 min (Bolton and Havenhand 1996; Kupriyanova and Havenhand 2002) under different levels of temperature, salinity, or pH as described below. Eggs were filtered through a 0.22-μm syringe filter (Millipore, Darmstadt, Germany) and immediately used in experiments. Filtered seawater (0.2-μm) was used as controls and incubated under different levels of environmental treatments. Pre-treated experimental seawater were kept in sealed Nalgene® glass containers prior to experiments.

Temperature. Preliminary pilot studies have shown that temperature below 20°C resulted in zero fertilization and cleavage. Temperatures ranging from 20°C to 36°C, at 2°C intervals, were tested in this study. This experiment was done inside a temperature-controlled room set at 16°C. Parafilm®-sealed beakers with 0.2-µm filtered seawater were placed in water baths with aquarium heaters (Eheim Jäger, Deizisau, Germany) connected to digital controllers (Aqua Logic Inc., CA, USA) to maintain set temperatures. Pre-calibrated digital thermometers were placed in each water bath to monitor and stabilize set temperatures.

Salinity. Initial rangefinder experiments showed zero fertilization at 18 psu. Eight salinity levels were tested in this study: 20, 22, 24, 26, 28, 30, 32, and 34 psu. Salinity treatments below ambient conditions (<34 psu) were prepared by adding distilled freshwater to 0.2-µm filtered seawater until set levels were reached. This experiment was done in an incubator (VWR International, PA, USA) set at 28°C. Beakers were fitted with plastic lids that had a 12-rpm synchronous motor attached to a plastic stirrer to maintain set conditions and prevent the formation of artificial haloclines within beakers. Salinity of seawater samples from experimental beakers was also measured before and after experiments using HI 96822 Seawater Refractometer (Hanna Instruments, RI, USA) with automatic temperature compensation.

pH. This experiment was conducted to test the tolerance of fertilization and embryonic development in crown-of-thorns starfish to different pH_{NIST} levels: 7.4, 7.6, 7.8, 8.0, and 8.2. Experimental seawater pH levels (below ambient pH 8.2) was achieved by gently bubbling CO_2 into reservoir overhead tanks, using a pH computer (Aqua-Medic of North America, CO, USA) connected to a solenoid valve, until programmed levels were reached. Experimental 0.2-µm filtered seawater was gravity-fed to containers with 45-mm mesh windows enclosed by a plastic jacket placed in

water baths set at 28°C. Seawater pH in experimental containers were measured before and after experiments using Orion 3-Star benchtop pH meter (Thermo Scientific, MA, USA), which was triple calibrated with NIST-certified buffers (pH 4.01, 7.00, 10.01).

6.2.3 Sperm speed and motility

Sperm speed (sperm point-to-point velocity = total distance traveled per second) and sperm motility (percentage of motile sperm) were measured from five male starfish, using techniques described for crown-of-thorns starfish (Uthicke et al. 2013) and sea urchins (Schlegel et al. 2012). Experimental seawater treatments were prepared as described in the previous section. For each dilution, 2 µl of dry sperm were diluted with 4 ml of experimental seawater. One drop (~100 µl) of this sperm suspension was placed on an albumin-coated microscope slide and a coverslip, which were separated by a 0.75 mm thick O-ring and focus set midplane to minimize wall effects on sperm swimming speed (Havenhand et al. 2008). Sperm behavior was captured using a Canon EOS 60D single lens reflex camera coupled with a Zeiss Axio Scope A1 (A-Plan ph1 10×/0.25 objective). The videocamera was remotely controlled using Canon EOS Utility and set to take 25 frames per second over a two second period. All recordings were made within 10s of the sperm suspension being placed on the slide. For each male, three replicate observations (slides) were made for three independent sperm dilutions under each temperature, salinity, or pH level and water-soluble egg extract treatment combination. Video recordings were post-processed with Sony Vegas Movie Studio HD (Sony Creative Software Inc., Middleton, WI), and 1s video clips from each slide (replicate) were analyzed using computer-assisted sperm analysis (CASA) plugin in Image J (Wilson-Leedy and Ingermann 2007). From an average of 200 sperm tracks

analyzed per slide, mean sperm speed and percentage of motile sperm was determined for each replicate (slide) and standard deviation (SD) was calculated.

6.2.4 Bioassays for fertilization and embryonic development

Three sets of experiments were conducted to quantify fertilization, cleavage, and gastrulation rates in response to different levels of (1) temperature, (2) salinity, and (3) pH. Mature ovary lobes were dissected from two female starfish and gently placed in glass dishes with 0.2-µm filtered seawater (FSW) at 28°C, to which, 1-methyladenine (1-MA) was added at a final concentration of 1×10^{-4} M. Eggs were spawned after 60 min and pooled by transferring to a large glass beaker with FSW. For each experiment, eggs were split into triplicate containers (with 150-mL experimental seawater) for each treatment level. Approximately 300 eggs were rinsed with experimental seawater and transferred to beakers so that final density was ~2 eggs ml^{-1}. Testes lobes were dissected from three male starfish and sperm that were shed after ~3 min were pooled together and placed in experimental seawater for ~10 s at a concentration of 1×10^{4} sperm ml^{-1} to ensure appropriate treatment conditions when added to containers with eggs. There was no water movement in the beaker at this point to minimize immotile sperm from artificially coming in contact with eggs. After 30 min, eggs were rinsed three times in experimental FSW to remove excess sperm and resuspended in experimental FSW. Gametes were pooled to reflect a population of spawners, as might occur in nature, and to record the mean response of the system under investigation. Gamete concentrations used in this study resulted in high fertilization rates (> 95%) during procedural control experiments and none of the eggs showed fertilization envelopes without the addition of sperm, demonstrating that there was no contamination during the preparation and handling of gametes. After two hours, ~100 eggs from each replicate were placed in a

scintillation vial and 7% formalin was added to prevent further development. Fertilization (presence of fertilization envelope, **Figure 6.1a**) and/or holoblastic radial cleavage (cell division, **Figure 6.1b**) were assessed in the first 50 eggs seen across a gridded slide viewed under a compound microscope at low power. Beakers containing the remaining embryos were then resealed and maintained in experimental temperature, salinity, or pH conditions. After 24 hours, 50 embryos were scored as either "gastrula" if they had developed archenteron, or "non-gastrula", where invagination had not occurred (**Figure 6.1c**). Five independent runs using different sets of gamete sources were undertaken with full replication for each treatment. Mean values from three containers within runs were used as replicates in each experiment (n = 5) and SD calculated. Temperature, salinity, and temperature-compensated pH measurements of seawater in experimental beakers were monitored using a HI 9828 multiparameter handheld probe (Hanna Instruments, RI, USA), with only minimal fluctuation from set values (<0.1).

6.2.5 Statistical analyses

Statistical comparisons of sperm speed between combinations of environmental treatment (temperature, salinity, or pH) and water-soluble egg extracts was performed using a two-factor analysis of variance (ANOVA) followed by post hoc pairwise comparisons using the 'lsmeans' function in R with Tukey's adjustment (R Core Team 2016). No significant departures from normality and homogeneity of variance were detected for all data. A generalized linear model (GLM) with binomial errors and logit link function was used to analyze the effect of each environmental treatment and water-soluble egg extracts (fixed categorical predictors) on the proportion of motile sperm. Significant overall tests were followed by post hoc pairwise comparisons between

different levels of temperature, salinity, or pH with corrected p-values (Benjamini and Hochberg 1995) using the 'glht' function from the 'multcomp' package in R (Hothorn et al. 2008). Mean sperm speed and motility for each male (n = 5) across 3 replicate dilutions (slides) were used in these analyses.

A generalized linear model (GLM) with binomial errors and logit link function was used to analyze the effect of temperature, salinity, or pH (categorical predictors) on fertilization, cleavage, or gastrulation rates (binomial response variables). Quasibinomial error distributions were used in place of binomial errors to correct for overdispersion when detected (Crawley 2013). This was followed by post hoc multiple comparisons with corrected p-values (Benjamini and Hochberg 1995) using the 'glht' function from the 'multcomp' package in R (Hothorn et al. 2008). Data from within treatments that had zero variance were excluded in the analyses.

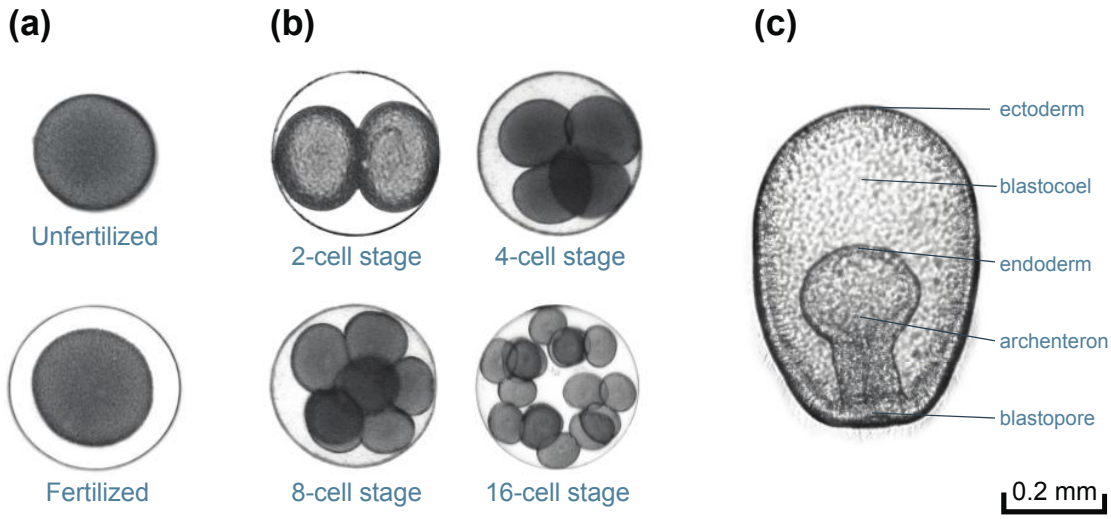

Figure 6.1 Early life history processes or stages assessed in this study: (**a**) fertilization, (**b**) early cleavage, and (**c**) gastrulation.

6.3. Results

6.3.1 Temperature

Seawater temperature ($F_{8, 72}$ = 85.96, p < 0.0001) and exposure to water-soluble egg extracts ($F_{1, 72}$ = 13.16, p = 0.0005) had a significant effect on sperm swimming speeds in crown-of-thorns starfish (**Appendix D – Table D1**). Sperm velocity was lowest at the minimum temperature tested, 20°C (FSW: 100.75 μm s^{-1} ± 9.48 SD, here and in all instances hereafter; EES: 127.05 ± 14.40 μm s^{-1}), and peaked at a temperature range of 28°C to 34°C (FSW: >221 μm s^{-1}; EES: >225 μm s^{-1}) before slightly dropping back to 219 ± 96 μm s^{-1} (FSW) and 228.01 ± 25.59 μm s^{-1} (EES) at 36°C (**Figure 6.2a**). Sperm exposed to water-soluble egg extracts had consistently faster swimming speeds compared to controls, but this difference was most prominent between 20°C and 26°C where sperms swimming speeds were relatively slow in controls (**Figure 6.2a**). We found a significant variation in sperm motility between temperature treatment levels (χ^2 = 1233.07, df = 8, p < 0.0001) and between control and water-soluble egg extract treatments (χ^2 = 31.34, df = 1, p = 0.0008). The proportion of motile sperm was steadily increasing from a minimum of 8.80 ± 3.02% (FSW) and 21.47 ± 7.49% (EES) at 20°C then peaking at >65% (FSW) and >70% (EES) for temperatures between 28°C and 34°C (**Figure 6.2b).**

Water temperature, ranging from 20 to 36°C, had a significant effect on fertilization, cleavage and gastrulation for *A* cf. *solaris*, whereby reproductive performance would be maximized at intermediate temperatures (26-30°C). For fertilization, there was significant variation across the full range of temperatures tested (**Appendix D – Table D1**; χ^2 = 1316.20, df = 8, p < 0.0001), mainly due to low fertilization under low and high temperature extremes. Fertilization rates were >89% between 24°C to 32°C (**Figure 6.2c**). For cleavage, there was significant variation with

temperature (**Appendix D – Table D1**; $\chi^2 = 521.09$, $df = 7$, $p < 0.0001$). Cleavage was > 75% for 26°C to 32°C (**Figure 6.2d**), but greatly reduced at lower and higher temperatures. Temperature also had a significant effect on gastrulation rates (**Appendix D – Table D1**; $\chi^2 = 822.66$, $df = 7$, $p < 0.0001$). The proportion of embryos undergoing gastrulation was maximized between 26°C and 32°C (**Figure 6.2e**).

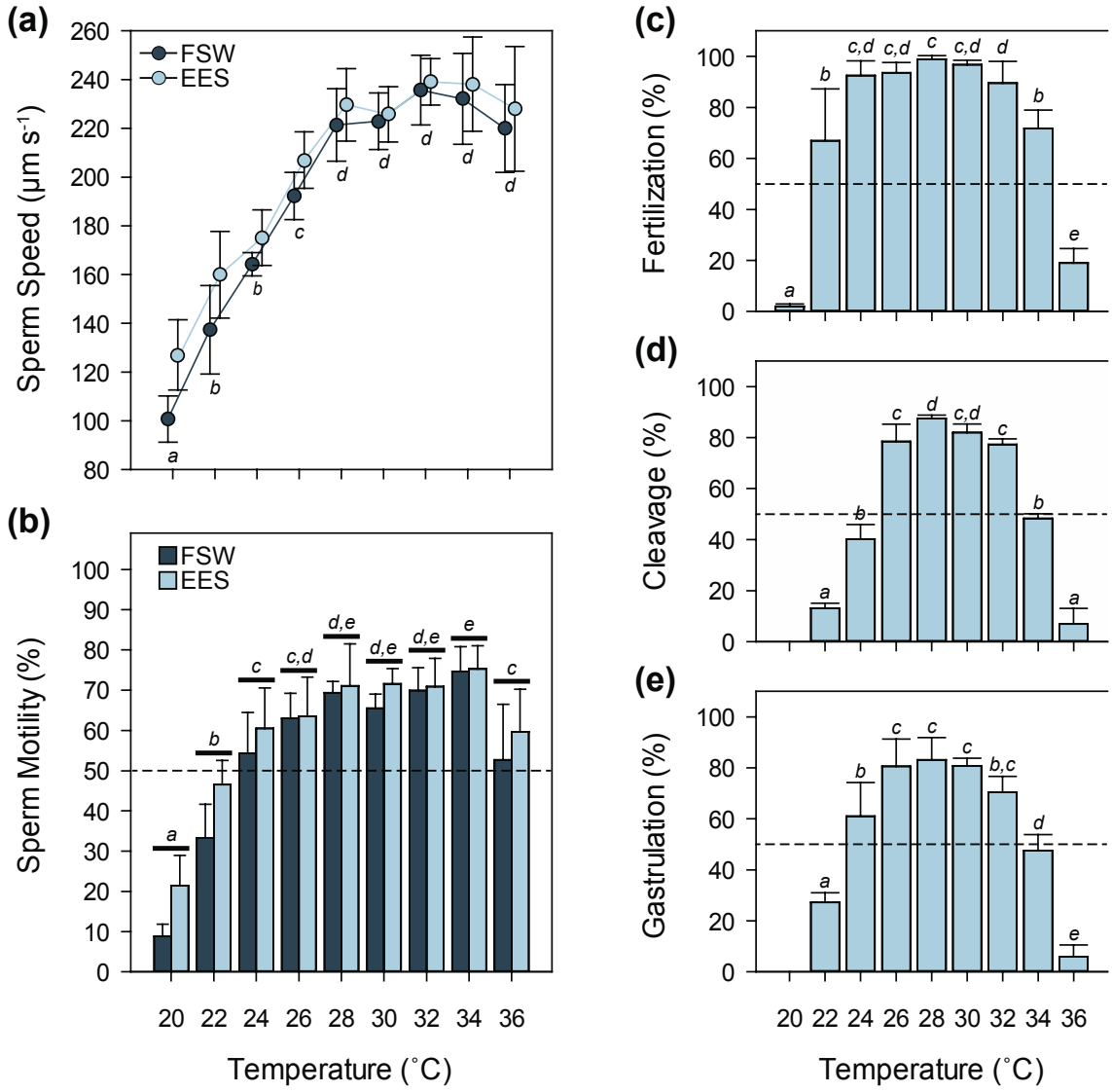

Figure 6.2. Thermal tolerance of sperm, fertilization, and embryonic development: (**a**) sperm speed (points slightly displaced for clarity), (**b**) sperm motility, (**c**) fertilization, (**d**) cleavage, and (**e**) gastrulation (n = 5). Letters next to error bar caps (± SD) indicate significant differences based on *post hoc* pairwise comparisons with corrected p-values. FSW = 0.2-µm filtered seawater (control); EES = solution with water-soluble egg extract.

6.3.2 Salinity

Salinity ($F_{7, 64}$ = 5.83, p < 0.0001) had a significant effect on sperm swimming speeds in crown-of-thorns starfish (**Appendix D – Table D1**). The disparity in sperm velocity between treatments exposed to water-soluble egg extracts and controls was progressively wider from high to low salinity, but differences were not statistically significant ($F_{1, 64}$ = 2.93, p = 0.0918) (**Figure 6.3a**). Variation between salinity treatments was mainly driven by differences between three groups: low sperm swimming speeds for treatments ranging from 20 to 22 psu, intermediate velocity at 24 and 26 psu, and significantly higher sperm velocity from 28 to 34 psu (**Figure 6.3a**). Sperm swimming speeds were relatively high across all treatments, with mean sperm velocity all above 170 μm s^{-1}. Salinity (χ^2 = 523.43, df = 7, p < 0.0001) also had a significant effect on sperm motility, but not water-soluble egg extracts (χ^2 = 16.42, df = 1, p = 0.0682) (**Appendix D – Table D1**). The proportion of motile sperm was above 40% for salinities ranging from 24 to 34 psu.

Salinity had a significant effect on overall fertilization rates (χ^2 = 597.86, df = 7, p < 0.0001). Fertilization envelopes did not form at salinities < 20 psu in preliminary experiments, while 31.43 ± 13.89% and 36.67 ± 12.41% of eggs were fertilized in 20 psu and 22 psu treatments, respectively. Highest fertilization rates were achieved at 30 psu (89.33 ± 8.29%), 32 psu (97.60 ± 2.34%), and 34 psu (96.40 ± 3.35%). The proportion of embryos undergoing cleavage was significantly different between salinity treatments (**Appendix D – Table D1**; χ^2 = 369.59, df = 5, p < 0.0001). Fertilized eggs did not cleave at 20 and 22 psu, while only 15.03 ± 8.76% cleaved under the 24-psu treatment. Percentage of normal cleavage in crown-of-thorns starfish was optimal (> 85%) when exposed to salinities ranging from 30-34 psu. Cleavage rates at 26 psu (57.04 ± 14.64%) and 28 psu (65.80 ± 11.50%) treatments were significantly lower than

those under 30-34 psu (**Figure 6.3d**). There was also a significant variation in the proportion of embryos undergoing gastrulation after 24 hours between salinity treatments (**Appendix D – Table D1**; $\chi^2 = 504.40$, $df = 5$, $p < 0.0001$). As with cleavage rates, no gastrulation occurred at 20 and 22 psu, and the proportion of embryos at gastrula stage was significantly higher at salinities between 30 and 34 psu compared to 26 psu (56.80 ± 8.81%) and 28 psu (65.20 ± 10.07%) treatments, which were also significantly higher than 24 psu treatment (8.80 ± 5.55%) (**Figure 6.3e**).

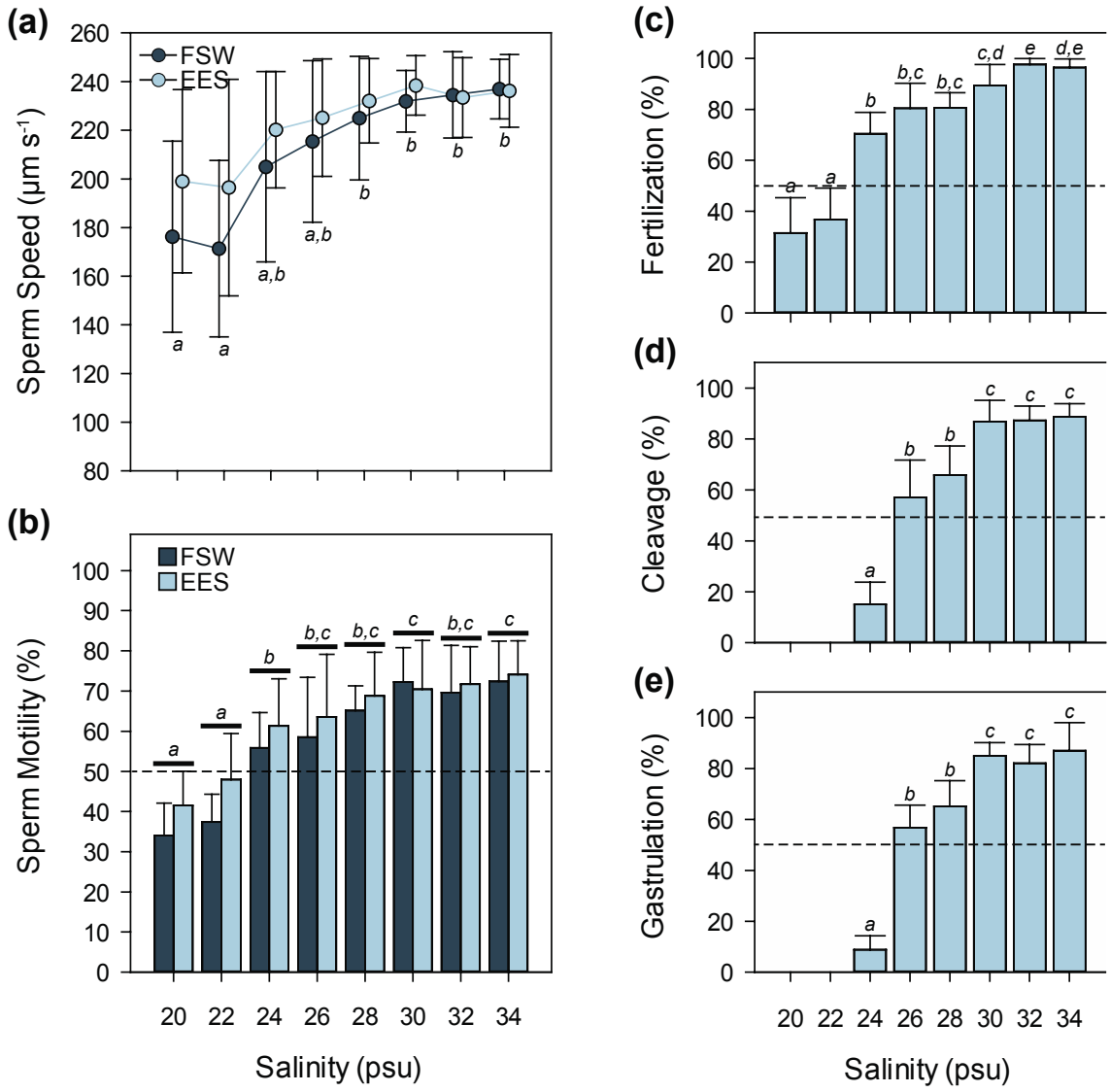

Figure 6.3. Effect of salinity on sperm behavior, fertilization, and early development: (**a**) sperm speed (points slightly displaced for clarity), (**b**) proportion of motile sperm, and proportion of eggs undergoing (**c**) fertilization, (**d**) cleavage, and (**e**) gastrulation (n = 5). Letters above error bars (± SD) indicate significant differences based on *post hoc* pairwise comparisons with corrected p-values. FSW = 0.2-µm filtered seawater (control); EES = solution with water-soluble egg extract.

6.3.3 pH

Mean sperm swimming speeds differed significantly (**Appendix D – Table D1**) between pH treatments ($F_{4,\ 40}$ = 28.57, p < 0.0001), but not between egg-derived extracts and controls ($F_{1,\ 40}$ = 3.85, p = 0.0568). For this experiment, sperm velocity was highest at pH 8.2 (FSW: 228.89 ± 17.89 μm s^{-1}; EES: 235.40 ± 15.44 μm s^{-1}) and pH 8.0 treatments (FSW: 224.23 ± 24.05 μm s^{-1}; EES: 222.23 ± 27.65 μm s^{-1}). Apart from pH 7.4 treatments, where sperm velocity was lowest (FSW: 118.69 ± 31.73 μm s^{-1}; EES: 147.52 ± 30.22 μm s^{-1}), sperm swimming speeds were relatively high (> 180 μm s^{-1}) for pH levels ranging from 7.6 to 8.2 (**Figure 6.4a**). We also found significant variations in the proportion of motile sperm under different pH (χ^2 = 669.24, df = 4, p < 0.0001) and egg extract (χ^2 = 38.11, df = 1, p = 0.0033) treatments (**Appendix D – Table D1**). The proportion of motile sperm was consistently higher for treatments exposed to water-soluble egg extracts (**Figure 6.4b**). For sperm under pH levels ranging from 7.6 to 8.2, motility was over 50%, while the proportion of motile sperm was relatively low at pH 7.4 (FSW: 14.93 ± 6.74%; EES: 29.87 ± 13.50%).

Percentage of fertilization was high across all pH levels tested (**Figure 6.4c**), except for eggs in pH 7.4 (46.09 ± 13.73%), which was significantly lower than fertilization success at pH 7.6 to pH 8.2 (> 88%). The effect of low pH levels was more evident when looking at the frequency of normal cleavage (**Figure 6.4d**) and gastrulation (**Figure 6.4e**). Cleavage (45.48 ± 13.17%) and gastrulation rate (40.13 ± 10.75%) at pH 7.4 was lowest among all the pH levels tested. The range of pH levels for optimum normal cleavage and gastrulation (> 89%) was between pH 8.0 and pH 8.2. Proportion of embryos undergoing cleavage and gastrulation was significantly higher at optimum pH levels (8.0-8.2) compared to pH 7.6 and pH 7.8 (**Appendix D – Table D1**).

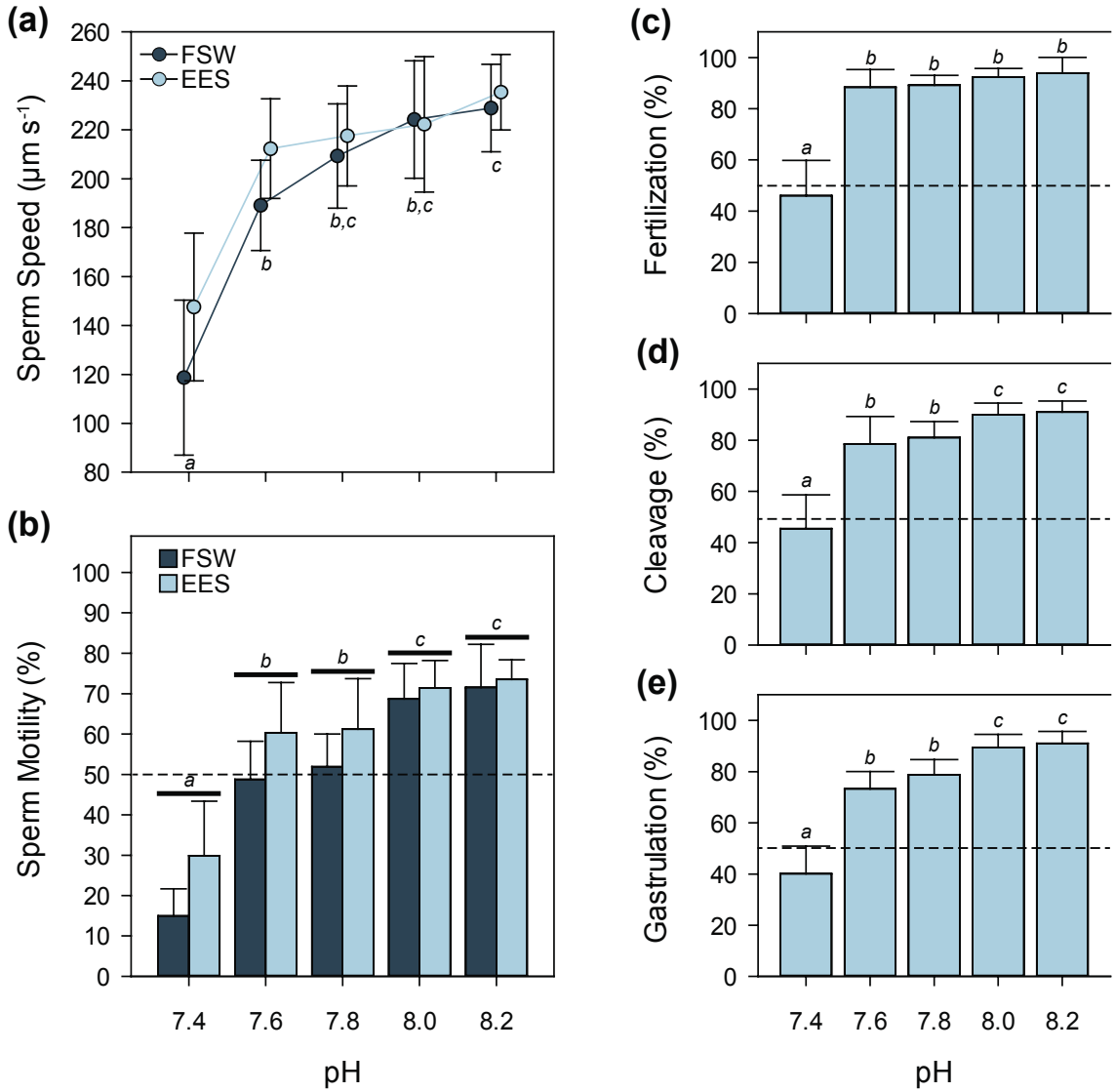

Figure 6.4. Influence of pH on sperm behavior, fertilization, and early development: (**a**) sperm speed (points slightly displaced for clarity), (**b**) proportion of motile sperm, and proportion of eggs undergoing (**c**) fertilization, (**d**) cleavage, and (**e**) gastrulation. Letters above error bars (± SD) indicate significant differences based on *post hoc* pairwise comparisons with corrected p-values. FSW = 0.2-µm filtered seawater (control); EES = solution with water-soluble egg extract.

6.4 Discussion

This study shows that crown-of-thorns starfish gametes, fertilization, and embryonic development are robust to a wide range of environmental conditions. Notably, these early life-stages could tolerate temperature, salinity, and pH conditions well beyond those experienced by *Acanthaster* spp. across their normal geographic range, even accounting for extreme anomalies in contemporary environmental conditions and predicted climate change impacts that are likely to occur at the end of this century (IPCC. 2014). If general to all populations, these findings have important implications for the reproductive success and dispersal of crown-of-thorns starfish. A common pattern observed in this study was that sperm motility, fertilization, cleavage, and gastrulation were maximized at local summer temperature, salinity, and pH conditions, which generally coincides with periods of peak reproduction for crown-of-thorns starfish (Cheney 1974; Pratchett et al. 2014)(**Figure 6.5**). This suggests that spawning in crown-of-thorns starfish occurs at an optimal time when environmental conditions favor enhanced fertilization and early development. Our results also show that chemoattractants (water-soluble egg extracts) play some role in sperm activity across all environmental parameters tested.

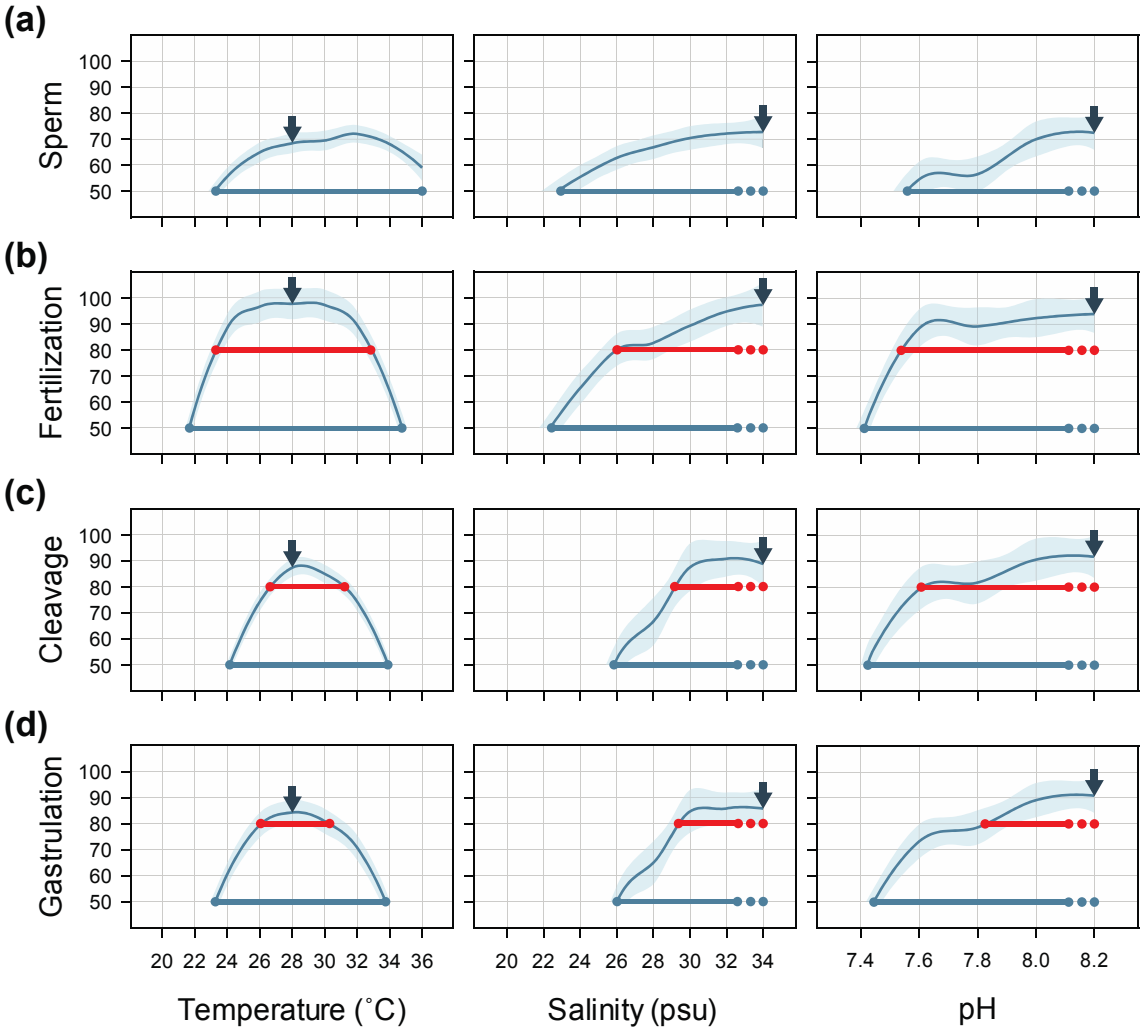

Figure 6.5. Environmental tipping points for (**a**) sperm motility, (**b**) fertilization, (**c**) cleavage, and (**d**) gastrulation. Arrows signify mean ambient levels during spawning. Curves are loess smoothers fitted to dataset with proportions >50%; bold lines cover the range where (a) proportion of motile sperm, (b) fertilization, (c) cleavage, and (d) gastrulation rates were >50% (dark blue) or >80% (red). Ellipses indicate that upper limits (above ambient) were not examined.

6.4.1 Temperature

Fertilization rates for crown-of-thorns starfish were high (>80%) over a wide temperature range (24-32 °C), but does appear to be adversely affected by even higher temperatures (34-36 °C), as shown for many other tropical echinoderms (Rupp 1973). Thermal enhancement of fertilization as a result of increased motility and respiratory rates of spermatozoa, with concomitant decrease in ATP concentration, has been previously demonstrated in other echinoderms (Mita et al. 1984). Thermal robustness of fertilization may be also due to the loading of protective maternal factors (*e.g.* heat shock proteins) during oogenesis (Yamada and Mihashi 1998; Hamdoun and Epel 2007). This protection may be enhanced in species with large eggs – crown-of-thorns starfish for example, have larger eggs compared to other planktotrophic tropical asteroids and maternal provisioning to the egg influences early larval development (Caballes et al. 2016). Increased temperature and associated decrease in viscosity increases fertilization success due to increased sperm swimming speeds (Kupriyanova and Havenhand 2005). This was evident for high fertilization rates achieved at temperatures above ambient levels (28°C) and low fertilization at lower temperature extremes (zero fertilization at 18°C and below). However, reduced sperm activity at 22°C and 24°C still resulted in relatively high fertilization rates, while heightened sperm activity at 34°C and 36°C did not correspond with significant reductions in fertilization rates. The limiting factor appears to be the restriction placed on the viability of sperm subjected to temperature extremes (Mita et al. 1984). At temperature extremes above normal, reduced fertilization was associated with increases in the incidence of polyspermy and granular fertilization membranes that adhere to the egg (Hagström and Hagström 1959). Increased sperm activity due to elevated temperature, as observed in this study, could also result in mechanical damage to the sperm and incur metabolic

costs and exhaustion of energy reserves (Greenwood and Bennett 1981). Physiological and viscosity-based aspects of high temperatures can influence sperm longevity, and hence fertilization success, by directly affecting sperm velocity (Kupriyanova and Havenhand 2005).

Temperatures that do not restrict fertilization may nonetheless be detrimental for embryonic development (Andronikov 1975). Embryos of the temperate sea urchin, *Strongylocentrotus purpuratus*, subjected to seawater 8°C above ambient showed normal fertilization, but subsequently resulted in abnormal cleavage (Farmanfarmaian and Giese 1963). This was also consistent with earlier work by Rupp (1973) where fertilization rates of crown-of-thorns starfish decreased by 20% while cleavage fell by 60% at 34°C. Similarly, more recent work by Sparks et al. (2016) showed that the proportion of cleaved embryos was significantly lower at 31°C compared to 27°C and 29°C treatments. Our study revealed that cleavage and gastrulation for crown-of-thorns starfish, were maximized over a relatively narrow temperature range (26-32^{o}C) and closely reflects the range of temperatures to which crown-of-thorns starfish are likely to be exposed throughout their geographic range (Pratchett et al. 2014). Conversely, Habe et al. (1989) showed that gastrulation was possible at a wider temperature range (13 and 34°C) than cleavage. This suggests that if post-gastrula embryos are swept into cooler waters, normal development can proceed during transport and will have important implications for long-range dispersal. However, the proportion of embryos that successfully cleave limits the proportion of embryos undergoing gastrulation. In this study, embryonic development in crown-of-thorns starfish ceased at 20°C and below, which was slightly higher than the lower thermal limit for embryonic development reported for crown-of-thorns starfish from the GBR, which was between 18 and 19°C (Johnson and Babcock 1994; Lamare et al. 2014). This might reflect the less variable

thermal environment of adult crown-of-thorns starfish from Guam used in this study compared to crown-of-thorns starfish from the GBR (Pratchett et al. 2014). Thermal acclimatization of adults, particularly during gametogenesis, has been found to shift the thermotolerance of echinoderm embryos (Johnson et al. 1990; Johnson and Babcock 1994).

6.4.2 Salinity

Out of the three pervasive environmental stressors investigated in this study, response to salinity is perhaps the least studied for crown-of-thorns starfish. Here, I found that the lower salinity limit for successful fertilization (>50%) in *Acanthaster* spp. was about 24 psu (**Figure 6.5**). No fertilization occurred after 2 hours at salinities below 20 psu. At 20 and 22 psu, less than 10% of eggs produced fertilization envelopes. This range and lower salinity limit appears to be common in asteroids (24 to 32 psu in *Asterias amurensis* (Kashenko 2005); 22 to 34 psu in *Asterina pectinifera* (Kashenko 2006)), echinoids (26 to 36 psu in *Echinocardium cordatum* (Kashenko 2007); 24 to 32 psu in *Echinarachnius parma* (Allen and Pechenik 2010)), and holothuroids (24 to 32 psu in *Eupentacta fraudatrix* (Kashenko 2000)). Fertilization was highest at mean ambient salinity conditions experienced by adults in their natural habitat throughout most of the year. Dinnel et al. (1987) found that fertilization of gametes of the sea urchin, *Strongylocentrotus purpuratus*, was best at the salinity at which the adults were held. Contrary to these observations, Roller and Stickle (1993) found no evidence of acclimation of echinoid gametes when *Lytechinus variegatus* were exposed to different salinities prior to spawning.

Developmental failure at low salinity is often thought to reflect limited fertilization, possibly due to substantial reductions in sperm motility. There is a paucity

of work on the response of echinoderm spermatozoa to salinity fluctuations and most examples come from research on sperm activity in commercially valuable teleost fishes (Griffin et al. 1998; Litvak and Trippel 1998; Elofsson et al. 2003). Sperm swimming speeds and sperm motility were relatively high between 24 and 34 psu and decreased slightly at 20 and 22 psu, which partly mirrored the range observed for fertilization. Minor improvements in sperm activation when exposed to water-soluble egg extracts were observed, but were not significant. The influence of egg extracts on sperm velocity and motility was greater at lower salinities.

Although there was some fertilization at 20-22 psu, eggs failed to cleave at these salinities and less than 20% cleaved at 24 psu. The failure of eggs to develop at low salinity largely reflects an inability of fertilized eggs to complete meiosis and cleave, rather than simply an inability of eggs to become fertilized at these low salinities. Salinity changes appear to have most detrimental effects for ova, which are unable to control water flow in and out of the cell. Osmotic shock experiments on the spermatozoa and ova of the echinoid, *Parechinus angulosus*, prior to fertilization under optimal temperature and salinity conditions indicated that temperature gradients exerted a greater effect on spermatozoa while low salinity was more deleterious to ova – at salinities below 15 psu, water was imbibed by the ova, which swelled and lysed. Salinity tolerance of gastrulation mirrored that of cleavage. Salinity levels as low as 10 psu have been observed to persist in nearshore and mid-shelf waters in the GBR after flood events (Devlin et al. 2013). Since embryos were not as tolerant to low salinities as previously expected (Habe et al. 1989), the timing of reduced salinity events would be critical in predicting the population response.

6.4.3 pH

Our results show that fertilization in *Acanthaster* spp. was robust to reduced pH. Patterns of fertilization success in relation to pH were coincident with relatively high sperm swimming speeds and proportion of motile sperm down to 0.6 pH units below ambient (pH 8.2) and significant reductions at pH 7.4 (**Figure 6.5**). In looking at the potential effects of near-future ocean acidification on crown-of-thorns starfish recruitment, Uthicke et al. (2013) found that low pH reduced sperm motility and velocity, which resulted in reduction of fertilization rates by 0.7% at pH 7.9 and 25% at pH 7.7 across a wide range of sperm concentrations. It was not clear whether impaired sperm motility, resulting in reduced fertilization at low pH, may be due to acidosis or the narcotic effect of hypercapnia on sperm (Johnson et al. 1983). For the sea urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei*, seawater acidified by CO_2 had a more severe effect on fertilization compared to HCl-acidified seawater, suggesting that hypercapnia may be more influential to fertilization. However, cross-factorial experiments showed no significant difference in fertilization rates between different combinations of temperature and pH (7.6 to 8.1) treatments (Kamya et al. 2014). This is consistent with my findings wherein no significant differences in fertilizations rates were found for pH ranging from 7.6 to 8.2. The mechanism of hypercapnic stress on sperm involves the control intracellular pH; although these effects may be overcome through respiratory dilution effects when sperm is released into the water column (Chia and Bickell 1983). Coelomic fluid surrounding crown-of-thorns starfish gonads has a mean pH of 7.49 (Uthicke et al. 2013), which is relatively low, hence may be activated when seawater pH levels are above this. This could explain the robustness of sperm motility and fertilization in crown-of-thorns starfish even at relatively low pH. In addition, my results also demonstrated that water-soluble compounds derived from eggs

also promoted sperm motility at low pH. Activation of nonmotile sperm by egg-derived compounds may provide a mechanism by which the energy reserves of sperm can be conserved in the absence of eggs, thereby maintaining sperm viability for extended periods (Bolton and Havenhand 1996; Kupriyanova and Havenhand 2005). This response has been reported for many species of corals, molluscs, echinoderms and ascidians (Byrne 2011).

The pH tolerance range for cleavage and gastrula embryos coincided with that of fertilization, albeit with slight reductions in frequency. Similarly, Kamya et al. (2014) reported that pH had no significant effect on gastrulation in crown-of-thorns starfish. Marine invertebrates that do not calcify during early developmental stages are generally robust to reduced pH (Dupont et al. 2010; Byrne 2012). Later stages (bipinnaria and brachiolaria) in the life history of crown-of-thorns starfish are more sensitive to reduced pH and have been shown to suffer high rates of larval abnormality and mortality at low pH (Uthicke et al. 2013; Kamya et al. 2014).

6.4.4 Interactive effects and implications for subsequent larval development

Our results show that absolute sperm velocity (221–237 μm s^{-1}), at ambient temperature (28°C), salinity (34 psu), and pH (8.2) levels, was slightly higher compared to previous estimates on crown-of-thorns starfish sperm swimming speeds (210 μm s^{-1} in Uthicke et al. 2013). These values are generally higher compared to estimates of sperm swimming speeds in other marine invertebrates, *e.g.* echinoids (*Heliocidaris erythrogramma*, 26–38 μm s^{-1} (Havenhand et al. 2008; Schlegel et al. 2012); *L. variegatus*, 153–275 μm s^{-1} (Levitan 2000)), bivalves (*Macoma calcarea*, ~60 μm s^{-1} (Vihtakari et al. 2016); *Mytilus galloprovincialis*, ~50 μm s^{-1} (Vihtakari et al. 2016); *Crassostrea gigas*, 94 μm s^{-1} (Havenhand and Schlegel 2009)), and polychaetes (*G.*

caespitosa, 45–114 μm s^{-1} (Kupriyanova and Havenhand 2002; Schlegel et al. 2014)). High sperm velocity over a wide range of temperature, salinity, and pH levels partly explains high fertilization rates of crown-of-thorns starfish in the field (Babcock and Mundy 1992a). However, there is a possible tradeoff between sperm velocity and sperm longevity, which also influences fertilization success (Levitan 2000). Sperm longevity was not quantified in this study, but previous studies have shown that crown-of-thorns starfish sperm can also remain competent for longer periods relative to other echinoderm species, resulting in relatively higher fertilization rates at greater distances (Benzie and Dixon 1994).

The response of gametes and early life history stages to multiple environmental stressors may have significant flow-on effects on the survival and development of subsequent larval stages, and thus, on successful recruitment. In the GBR, spawning of crown-of-thorns starfish have usually coincided with peak summer temperatures, as well as high precipitation. Although fertilization and embryonic development may be robust to high temperatures (up to 34°C), survival may be low when salinities drop (below 25 psu) during heavy rainfall events that result in high freshwater discharge from rivers. Disregarding the influence of other variables (*i.e.* predation, dispersal), the proportion of embryos progressing to subsequent larval stages will be substantially reduced. Tolerance of crown-of-thorns starfish larvae has also been shown to be stage-specific and may constrain successful recruitment further (Caballes and Pratchett 2014). Bipinnaria larvae of crown-of-thorns starfish can tolerate temperatures between 14.5 and 32°C for up to 48 hours, while the brachiolaria stage is more sensitive to temperature variation (Habe et al. 1989). In terms of tolerance to salinity, bipinnaria larvae can tolerate abrupt salinity changes down to 21 psu (Henderson 1969; Habe et al. 1989), while brachiolaria larvae rupture even with a decrease in salinity of 2 psu

(Henderson and Lucas 1971). High flow events have also been associated with elevated nutrient levels and phytoplankton densities, which have been shown to improve larval survival and development (Fabricius et al. 2010; Wolfe et al. 2015a), even more so when modulated by increased temperatures up to 30°C (Uthicke et al. 2015).

Here I showed that CO_2-acidified seawater (down to pH 7.6) did not have a significant effect on fertilization and early embryonic development. Similarly, Allen et al. (2017) found that reduced pH, whether in isolation or in combination with lower salinity, had no detectable effects on fertilization and early development in crown-of-thorns starfish. However, the detrimental effects of ocean acidification have been shown to be more apparent in subsequent larval stages. Uthicke et al. (2013) found that normal development and settlement in crown-of-thorns starfish larvae kept at pH 7.6 was significantly reduced compared to pH 8.1 treatments. Low pH (7.6) couple with elevated temperatures (30°C) also had an additive negative effect on larval size and development (Kamya et al. 2014). However, the positive effects of increased temperature on larval growth (Uthicke et al. 2015) may ameliorate the detrimental effects of low pH.

6.5 Conclusions

Taken together, my results show that crown-of-thorns starfish gametes, fertilization, and embryonic development are robust to a wide range of temperature, salinity, and pH levels, well beyond environmental conditions found within the current geographical distribution of *Acanthaster* spp. Majority of sperm are motile at temperatures between 24 and 36°C, salinities between 24 and 34 psu, and pH between 7.6 and 8.2. Over 50% of eggs are fertilized at wide range of temperature (22-34),

salinity (24-34), and pH (7.6-8.2) levels. The robustness of fertilization to these pervasive environmental stressors may be attributed to the molecular predisposition of crown-of-thorns starfish sperm (Stewart et al. 2015), which possesses an enhanced capacity for high fertilization rates, compared to other echinoderms (Benzie and Dixon 1994). Compared to fertilization, tolerance range for cleavage was mostly narrower for temperature (26-32°C), salinity (26-34 psu), and pH (7.6-8.2). Gastrulation under salinity and pH levels tested coincided with cleavage rates, while thermotolerance range for gastrulation was slightly wider than cleavage (24-32°C). In general, the effects of temperature and pH on fertilization and early development mostly corresponded with the sensitivity of sperm to these stressors, while response to salinity was largely due to detrimental effects on osmotic balance in eggs. Water-soluble compounds associated with eggs also enhanced sperm activity, particularly in environmental conditions where sperm motility was initially limited. Although the response to multiple environmental stressors was tested in this study, these pervasive environmental parameters impact marine organisms simultaneously. Future work should include cross-factorial studies to tease out additive, antagonistic, and synergistic interactions between these factors (Przeslawski et al. 2015). The tolerance of the earliest stages of development to a wide range of environmental stressors suggests that later ontogenic stages (larvae, juveniles, adults) may be more vulnerable to small fluctuations in environmental conditions.

Chapter 7

The role of maternal nutrition on oocyte size and quality, with respect to early larval development in the coral-eating crown-of-thorns starfish[6]

7.1 Introduction

Episodic population outbreaks of the crown-of-thorns starfish have resulted in widespread degradation of Indo-Pacific coral reefs (Pratchett et al. 2014). While the ultimate cause of outbreaks is still the subject of debate, most researchers agree that exploring the reproductive biology and early life history of crown-of-thorns starfish is essential in understanding mechanisms that lead to outbreaks. The two most prominent hypotheses that seek to explain the cause(s) of outbreaks, the 'terrestrial runoff hypothesis' (Birkeland 1982; Lucas 1982; Brodie et al. 2005; Fabricius et al. 2010) and 'predator removal hypothesis' (Endean 1977; Sweatman 2008) are built upon variations in larval survival, growth, and development in response to starvation and predation. Populations of crown-of-thorns starfish are predisposed to major fluctuations due to inherent properties of their life history such as high fecundity (Conand 1985; Kettle and Lucas 1987), high fertilization rates (Babcock et al. 1994; Benzie and Dixon 1994), and short generation times (Yamaguchi 1973b; Caballes and Pratchett 2014). Small environmental and biological changes, therefore, could potentially lead to rapid increases in the abundance of crown-of-thorns starfish (Uthicke et al. 2009).

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Fluctuations in larval survival and development can have pronounced effects on recruitment rates and hence the dynamics of adult populations (Balch and Scheibling 2001; Fabricius et al. 2010). Increased larval nutrition has a positive effect on the condition of the larvae of crown-of-thorns starfish, as it does on other marine organisms with planktotrophic larvae. (Lucas 1982). Past studies on the survival and development of larvae of crown-of-thorns starfish have primarily been centered on the direct effects of nutrient concentrations and exogenous food availability in the water column (Lucas 1982; Olson 1987; Okaji et al. 1997; Wolfe et al. 2015a). However, the role of maternal nutrition on reproduction and larval development of crown-of-thorns starfish has generally been overlooked. The few studies that have explored effects of maternal condition in crown-of-thorns starfish examined the effects of food availability on gametogenesis. Cheney (1974) reported that total deprivation of coral food for one month by caging resulted in shrinking of gonads, deterioration of pyloric caeca, and decrease in total diameter. Conversely, gravid crown-of-thorns starfish collected from Okinawa and starved for 90 days showed no change in the size and condition of gonads even though pyloric caeca were reduced to thin ribbons (Okaji 1991).

The quantity and quality of food available to adult starfish can have flow-on effects on overall reproductive capacity (George 1996). Coral composition and abundance are often variable in nature (Veron 1997; Burdick et al. 2008) and local conditions can influence the nutritional status of corallivores, like crown-of-thorns starfish. It is well established that crown-of-thorns starfish have distinct feeding preferences and *Acropora* (along with *Montipora*) are among the most preferred genera, consistently eaten in preference to other locally abundant corals (De'ath and Moran 1998b; Pratchett et al. 2009a, 2014). Although *Porites* are much less preferred, they are not totally avoided and are often consumed when more preferred species have been

depleted (Kayal et al. 2012). Starved individuals have also been observed towards the end of outbreak events when live coral prey becomes scarce (Zann 1992).

Natural variation in food availability and nutrient assimilation can influence gamete production in echinoderms (Mercier and Hamel 2009). Starvation has been found to result in the failure of gonads to achieve normal size increments (Nimitz 1976). In asteroids, most nutrients used in gametogenesis are processed and stored in the pyloric caecum and delivered directly to the gonads (Shirai and Walker 1988). Echinoderms found in favorable habitats with abundant supply of preferred food items often respond by increasing body weight, gonad size, and pyloric caeca index (George 1996). For example, Scheibling and Lawrence (1982) found that starfish (*Echinaster* sp.) found on seawalls with abundant supply of oysters, sponges, ascidians, and bryozoans were almost three times heavier than individuals from less favorable sites. Moreover, green sea urchins (*Strongylocentrotus droebachiensis*) from shallower depths, where preferred macroalgal food was more abundant, had larger gonads compared to females from food-limited deeper sites (Bertram and Strathmann 1998). The weight of the pyloric caeca for *Pisaster ochraceus* from wave-exposed sites, where food items are more abundant, was significantly higher compared to starfish from wave-protected sites (George 1999). Laboratory studies also confirmed that adults on rich food diets during active gametogenesis have higher body weight, gonad size, and pyloric caeca index (George 1996). This was demonstrated by experimental manipulation of diet in the New Zealand starfish, *Sclarasterias mollis*, where body weight, and gonad and pyloric caeca index increased significantly compared to starved starfish (Xu and Barker 1990a, 1990b). The lipid content of fed starfish was also higher than in starved groups, although protein and carbohydrate contents in gonads did not vary significantly between feeding treatments (Xu and Barker 1990b).

Maternal nutrition can alter resource allocation mechanisms and affect nutrient investment in oocytes (Russell 1998; Lawrence et al. 2003). Oocyte size, fecundity, and oocyte quality can vary with the nutritional history of adults (George 1990; George et al. 1990, 1991; de Jong-Westman et al. 1995). Because oocyte size is a function of maternal investment, selection on oocyte size is a function of maternal fitness (Levitan 1996). Echinoderms found at sites with abundant food supply and in high food laboratory treatments mostly produced higher numbers of large, high quality oocytes (George 1996). Female starfish (*Leptasterias epichlora*) from high food availability, exposed sites produced higher numbers of larger oocytes with higher protein content compared to those from sheltered sites (George 1994b). Starfish collected from less favorable sheltered sites subsequently placed under high food ration treatments in the laboratory had more oocytes with larger diameter compared to starfish in low food treatments (George 1994b).

For free-spawning invertebrates, the entire maternal contribution to subsequent generations is provided in the oocyte (Jaeckle 1995). Following fertilization and gastrulation, the digestive tract differentiates (early bipinnaria stage) and larvae enter the facultative feeding period (FFP); at this stage, larvae are able to feed but do not necessarily require food because maternal provisions from the oocyte are still available (Byrne et al. 2008b). During the initial larval stages, maternal provisioning can have important consequences for starvation resistance, mortality risk from predation, and developmental rates (Reitzel et al. 2005; Prowse et al. 2008), ultimately leading to reduced planktonic duration and increased settlement success In the absence of exogenous food, larvae from large oocytes (high maternal investment) have significant buffering from longer periods of starvation during the FFP (Byrne et al. 2008b).

Predation rates during the vulnerable stage of larval development are also moderated by rapid larval development (Sinervo and McEdward 1988).

In this study, I examined the role of experimental variation in maternal nutrition (comparing between individuals that were starved, fed on preferred corals and fed on generally non-preferred coral prey) on the larval growth and early development prior to exogenous feeding by larvae. The effect of maternal nutrition on the following aspects of reproduction and larval development in crown-of-thorns starfish are specifically addressed in this study: (1) adult female morphometrics before and after treatment; (2) gonad and pyloric caeca indexes; (3) oocyte size and shape; (4) fertilization rates; (5) early larval growth; (6) larval survival; and (7) larval development. Few studies have investigated the effect of the nutritional state of the adult crown-of-thorns starfish on oocyte size, fertilization, larval growth and development. This has important implications on the survival of larvae when exogenous food supply is low or absent. Development and growth rates of the larvae of crown-of-thorns starfish are predicted to be low in the absence of enhanced phytoplankton levels (Lucas 1982; Brodie et al. 2005; Fabricius et al. 2010; Wolfe et al. 2015a). However, it is not known whether increased maternal investment allows larvae to withstand prolonged periods of starvation and proceed with normal development. Because food quality (coral community structure) and quantity (coral abundance) varies widely between adult populations of crown-of-thorns starfish in coral reefs, any effect of maternal nutrition on larval quality and survivorship may influence the overall reproductive success of crown-of-thorns starfish and help explain marked fluctuations in abundance.

7.2 Methods

7.2.1 Specimen collection and ethics statement

All experiments were conducted at the University of Guam Marine Laboratory (UOGML) in accordance with regulations set out by the University of Guam and James Cook University. Crown-of-thorns starfish (*Acanthaster* cf. *solaris*) were collected on SCUBA from reefs at the southern end of Ague Point (13.565360°N, 144.819119°E) on the northwest coast of Guam and immediately transported to UOGML. No permits were required to collect crown-of-thorns starfish. Sex was determined by examining contents drawn from gonads along the arm junction using a syringe with a large-bore biopsy needle (Caballes and Pratchett 2014). Male and female crown-of-thorns starfish were maintained in separate flow-through tanks. Male crown-of-thorns starfish were maintained on a mix of *Acropora* spp., *Porites* spp., *Pocillopora* spp. corals as soon as they were introduced to holding tanks, while female crown-of-thorns starfish were starved for 10 days prior to being assigned to one of three different feeding treatments (described below). Coral collections were done under a special license issued by the Guam Department of Agriculture – Division of Aquatic and Wildlife Resources to UOGML (in accordance with Section 63123 of Title 5, Guam Code Annotated). *Acropora abrotanoides* colonies were collected from Pago Bay and *Porites rus* colonies were collected from Western Shoals, Guam. Aside from being abundant in these collection sites (Burdick et al. 2008), these species were selected because *A. abrotanoides* is among the most highly preferred prey coral species in Guam, whereas *P. rus* is one of the least preferred species (Caballes 2009). Columnar and tabular/plate growth forms of these corals were collected to make surface area measurements easier. Coral infauna (e.g., *Trapezia* crabs) were physically removed from all coral fragments so as not to deter feeding by crown-of-thorns starfish (Pratchett 2001).

7.2.2 Feeding treatment

Nine female crown-of-thorns starfish with intact arms and approximately similar diameter (ca. 320 ± 7 mm) were placed in individual plastic bins with flow through ambient seawater (temperature = 29.07 ± 0.47 °C; salinity = 32.97 ± 0.06 psu; pH = 8.30 ± 0.05). All females were nearing reproductive maturity based on microscopic examination of oocytes drawn from starfish using the biopsy procedure described in the previous section. Oogenesis in crown-of-thorns starfish usually takes between two and three months, although some oocytes can complete oogenesis within a month (Lucas 1973). Starfish were assigned to one of three different feeding treatments (n = 3) for 60 days: i) Starved (no food), ii) *Acropora* (fed with *Acropora abrotanoides*), and iii) *Porites* (fed with *Porites rus*). Supply of coral food for fed treatments was replenished as soon as the piece of coral provided has been completely consumed. Since live coral was used in the diet of *ad libitum* fed starfish, the only way to reduce the amount of coral used was to minimize the sample size. Growth of each individual starfish was quantified based on changes in diameter (Δd) and weight (Δw) from day 0 to day 60. At the end of the feeding experiment (day 60) I also calculated the gonad index (GI) and the pyloric caeca index (PCI) for each individual. The average weight of gonads and pyloric caeca from three arms was multiplied with the total number of arms of each starfish to estimate the total gonad or pyloric caeca weight. GI and PCI were expressed as the ratio of gonad or pyloric caeca weight to the total weight of the starfish (Conand 1985).

To relate differences in physiological and reproductive condition to food intake, I calculated the rate of feeding for each individual starfish by measuring the total surface area of coral consumed throughout the 60 day period. Consumed coral fragments were weighed dry and the surface area of each fragment was estimated following the foil-

wrapping technique. Each piece of coral was tightly molded with heavy-duty aluminum foil to fit depressions and projections, following Marsh (1970). The aluminum foil molds were flattened and digital photographs were taken using a ruler as scale. These pictures were analyzed by calculating the area (cm^2) of the flattened molds using the image analysis software, Image J (Schneider et al. 2012).

7.2.3 Spawning induction and oocyte dimensions

To test the effect of feeding treatments on oocyte metrics, gonads were dissected from the nine females and ovaries were rinsed in 0.2-µm filtered seawater (FSW) to remove loose oocytes. Ovary lobes were treated in 10^{-5} M 1-methyladenine to induce ovulation. Released oocytes were transferred into containers with filtered seawater and wet mounted on glass slides for microscopic examination. Oocytes were photographed with a camera (Canon EOS 60D) mounted on a microscope (Leica DM300) with a calibrated ocular micrometer. Ayukai et al. (1996) developed a criterion to estimate oocyte quality in crown-of-thorns starfish based on morphometric characteristics. Oocytes that were relatively large (> 0.15 mm in diameter), spherical (round), and uniform in size and shape often achieved successful fertilization, embryogenesis, and gastrulation (Ayukai et al. 1996). Diameters (d_{oocyte}) of the long and short axes of 100 randomly selected mature oocytes (have undergone germinal vesicle breakdown) from each treatment were measured using Image J (Schneider et al. 2012). Oocyte volume (v_{oocyte}) was calculated using the formula for an oblate spheroid: $4/3 \times \pi \times (\text{long axis radius})^2 \times \text{short axis radius}$. Oocyte sphericity is the ratio of the long and short axis diameter measurements.

7.2.4 Fertilization

Oocytes from each female were placed in separate 1 L beakers with FSW kept at 28˚ C. Approximately 200 oocytes from each female were transferred into triplicate 250-ml beakers using a glass pipette. Spermatozoa were collected from the testes of 5 males and checked for motility under a microscope. Approximately equal amounts of spermatozoa from each male were combined and counted using a haemocytometer. Oocytes were fertilized with spermatozoa diluted to achieve a spermatozoa-to-oocyte ratio of 100:1. After 10 minutes, an aliquot of eggs was subsampled from each replicate and further development halted with 7% formalin before examination. One hundred eggs were examined and eggs with raised fertilization envelopes were counted and percent fertilization was calculated.

7.2.5 Larval rearing

Fertilized eggs from the nine females were separately reared in triplicates at 28˚ C and after 24 hours, 50 actively swimming gastrulae were siphoned into separate glass culture jars with 200 ml FSW. Each jar was equipped with a plastic stirring paddle attached to a 20-rpm synchronous motor. Water changes with fresh FSW were performed three times daily. Surviving larvae in each jar, regardless of developmental stage, were counted daily for eight days during the second water change. After four days, 10 normally developing larvae from each jar were placed in a relaxing agent (7% $MgCl_2$) for 10 minutes and fixed in 10% formalin in FSW. Larvae were immediately photographed using a camera mounted on a microscope shortly after fixation and total length, width, and stomach area were measured using ImageJ (**Figure 7. 1**).

After 8 days, all surviving larvae were categorized into the following developmental stages: (1) early bipinnaria – preoral and anal lobes present, coelomic

pouches below or close to mouth; (2) advanced bipinnaria – coelomic pouches above the mouth and almost touching, anterodorsal and posterolateral arms start to form; (3) late bipinnaria / early brachiolaria – anterior extension of fused coelomic sacs, anterodorsal and posterolateral arms longer, posterodorsal arms start to elongate, preoral arms start to form; and (–) abnormal (stunted, deformed) development (Yamaguchi 1973a; Byrne and Barker 1991; George 1999; Caballes and Pratchett 2014). The percentage of normally developing larvae after eight days was also calculated. No food was provided to the larvae during the entire duration of the experiment to control for variation in the food intake of individual larvae.

7.2.6 Data analyses

Mean daily consumption by each female was analyzed using one-way analysis of variance (ANOVA) to test for differences in total area of live coral consumed on exclusive diets of *Acropora* versus *Porites*. Change in diameter and weight (= post-treatment – pre-treatment), post-treatment gonad index, and post-treatment pyloric caeca index were compared between feeding treatments (3 levels, fixed) using one-way ANOVA followed by post hoc Tukey's pairwise comparisons in SPSS 22.0 (IBM Corporation, NY, USA). Proportion of fertilized eggs were arcsine square-root transformed prior to nested ANOVA analysis with 'Maternal Nutrition' as fixed effects and 'Female' nested as a random factor nested under 'Maternal Nutrition'. Normality and homogeneity of variance of data on oocyte dimensions, proportion of normal larvae, and proportion of larvae reaching late bipinnaria / early brachiolaria stage after eight days did not improve after transformations. A nested (hierarchical) permutational analysis of variance (PERMANOVA) with 'Female' (3 levels, random) nested within 'Maternal Nutrition' (3 levels, fixed) was run to analyze differences between and within

feeding treatments. PERMANOVA is a non-parametric technique that may also be used in analyzing univariate data (Anderson et al. 2008). Analyses were conducted using the PERMANOVA+ add-on for PRIMER v.6 (Primer-E Ltd., Plymouth, UK), and used the Euclidean distance measure, Type III sums of squares, and 9999 permutations of the residuals under a reduced model to calculate the significance of the pseudo-F statistic. In cases where not enough unique permutations (< 100) were possible to determine permutational p-values (p_{perm}), Monte-Carlo asymptotic p-values (p_{MC}) were used instead (Anderson et al. 2008). Pairwise comparisons between Maternal Nutrition treatments were analyzed using Benjamini-Hochberg corrected p_{MC}-values (Benjamini and Hochberg 1995). Data on the proportion of surviving larvae were arcsine square-root transformed prior to daily comparisons using nested ANOVA using a similar model described for fertilization data. Statistical comparison of larval length, width, and stomach area were made using a three-factor nested ANOVA with 'Maternal Nutrition' as a fixed effect (3 levels), 'Female' (3 levels, random) nested within 'Maternal Nutrition' and 'Jar' (3 levels, random) nested within 'Female' and 'Maternal Nutrition'. A *post hoc* Tukey Test was used for pairwise comparisons of fixed factor means. Variation in the proportion of larvae under 4 larval development categories between feeding treatments was analyzed using G-test of independence followed by *post hoc* pairwise comparisons applying Benjamini-Hochberg correction for multiple comparisons (Benjamini and Hochberg 1995).

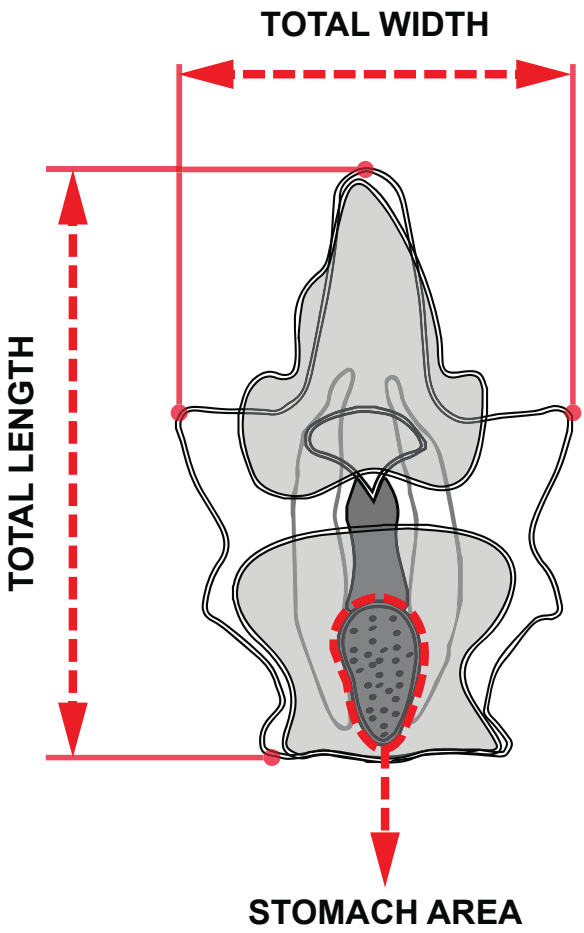

Figure 7.1. Bipinnaria larva morphometrics: Image analysis measurements of length, width, and stomach area of four-day old larvae.

7.3 Results

7.3.1 Coral consumption and morphometrics

Female crown-of-thorns starfish provided with *ad libitum* rations of coral food exhibited a significant difference in consumption rates depending on the coral species ($F_{1,5} = 55.309$, $p = 0.002$). Starfish provided with *A. abrotanoides* consumed an average of 157.64 ± 10.71 cm^2 of coral tissue per day while *P. rus* was consumed at rate of 101.81 ± 7.37 cm^2 per day (**Table 7.1**). There was no significant difference in Δd between treatments ($F_{2,8} = 3.413$, $p = 0.102$); however, the change in weight (Δw) between treatments was significantly different ($F_{2,8} = 5.816$, $p = 0.039$) and *post hoc* pairwise comparisons show that weight gain in *Acropora*-fed females was significantly higher than in starved females (**Table 7.1**). Post-treatment gonad index (GI) was also significantly different between treatments ($F_{2,8} = 7.530$, $p = 0.023$), with values from *Acropora*-fed crown-of-thorns starfish significantly higher than for starved starfish. Mean Δw and GI was not significantly different between *Acropora*- and *Porites*-fed starfish, nor between *Porites*-fed and starved females. Maternal treatments also had a significant effect on pyloric caeca index (PCI) values ($F_{2,8} = 12.846$, $p = 0.007$) and pairwise comparisons between treatments indicate that coral-fed starfish (regardless of whether they were maintained on *Acropora* or *Porites*) had higher pyloric caeca index values compared to starved crown-of-thorns starfish. There was no significant difference in PCI between starfish in coral food treatments.

Table 7.1. Diameter and weight of females pre- and post-treatment, gonad index (GI) and pyloric caeca index (PCI) after feeding treatments.

Maternal Diet	♀	CC	d_0	d_1	Δd	w_0	w_1	Δw	GI	PCI
Starved	1	0	321	316	-5	1205	1154	-51	12.76	3.24
	2	0	330	327	-3	1022	997	-25	14.31	3.63
	3	0	314	313	-1	1069	1056	-13	15.15	5.26
Acropora	4	148	325	324	-1	1224	1219	-5	16.53	6.88
	5	155	311	314	3	1028	1046	18	18.22	8.66
	6	169	319	321	2	1154	1170	16	18.69	7.77
Porites	7	97	320	318	-2	1183	1172	-11	15.22	5.72
	8	98	313	316	3	964	971	7	16.02	6.97
	9	110	328	330	2	1257	1268	11	17.59	7.16

CC = mean daily coral consumption (cm^2)

$\mathbf{d_0}$ = pre-treatment diameter (cm), $\mathbf{d_1}$ = post-treatment diameter (cm); **Δd** = diameter change

$\mathbf{w_0}$ = pre-treatment weight (g); $\mathbf{w_1}$ = post-treatment weight (g); **Δw** = weight change

% GI = post-treatment gonad weight (g) / post-treatment body weight (g) × 100%

% PCI = post-treatment pyloric caeca weight (g) / post-treatment body weight (g) × 100%

7.3.2 Oocyte dimensions and fertilization

Significant variation in oocyte diameter (pseudo-$F_{2,891}$ = 11.463, p_{perm} = 0.009) and calculated oocyte volume (pseudo-$F_{2,891}$ = 15.316, p_{perm} = 0.007) was noted among starfish in each of the three feeding treatments. The fixed effects accounted for 31% and 33.5% of variation in oocyte diameter and oocyte volume, respectively, while around 50% of variation was due to differences at the replicate level. Pairwise comparisons (**Figure 7.2**) showed that the diameter and volume of oocytes from *Acropora*-fed females (d_{oocyte} = 0.25 ± 0.02 mm; V_{oocyte} = 8.27×10^{-3} ± 1.95×10^{-3} mm^3) was significantly higher than oocytes from *Porites*-fed (d_{oocyte} = 0.23 ± 0.02 mm; V_{oocyte} = 6.54×10^{-3} ± 1.77×10^{-3} mm^3) and starved females (d_{oocyte} = 0.22 ± 0.04 mm; V_{oocyte} = 5.52×10^{-3} ± 2.63×10^{-3} mm^3). Oocyte diameter in this study was relatively higher compared to previously reported oocyte sizes of crown-of-thorns starfish and other coral reef asteroids with planktotrophic mode of development (**Table 7.2**). Maternal nutrition also had a significant treatment effect on oocyte sphericity (pseudo-$F_{2,891}$ = 9.675, p_{perm} = 0.012), which accounted for 33.7% of variation while 47.1% of variation was due to differences at the replicate level. Oocytes from fed females (*Acropora* = 0.97 ± 0.02; *Porites* = 0.95 ± 0.03) were predominantly more spherical (ratio of long and short axis ≈ 1) than oocytes from starved females (0.90 ± 0.07), which were mostly ellipsoidal in shape. Oocyte diameter (pseudo-$F_{2,891}$ = 9.759, p_{perm} < 0.001), volume (pseudo-$F_{2,891}$ = 8.625, p_{perm} < 0.001), and sphericity (pseudo-$F_{2,891}$ = 17.709, p_{perm} < 0.001) also differed significantly among females within maternal nutrition treatments, but only accounted for less than 20% of the total variation in each parameter. Overall, the size and shape of oocytes was less uniform in starved starfish compared to oocytes from fed starfish.

Fertilization rates of eggs from individual females were high (88%-100%) across all treatment levels (**Figure 7.3**). Maternal nutrition did not have a significant effect on fertilization rates ($F_{2,18)} = 2.318$, $p = 0.180$), despite significantly different oocytes sizes. There was also no significant variation in fertilization rates of eggs from individual female starfish within treatments ($F_{2,18)} = 2.477$, $p = 0.063$).

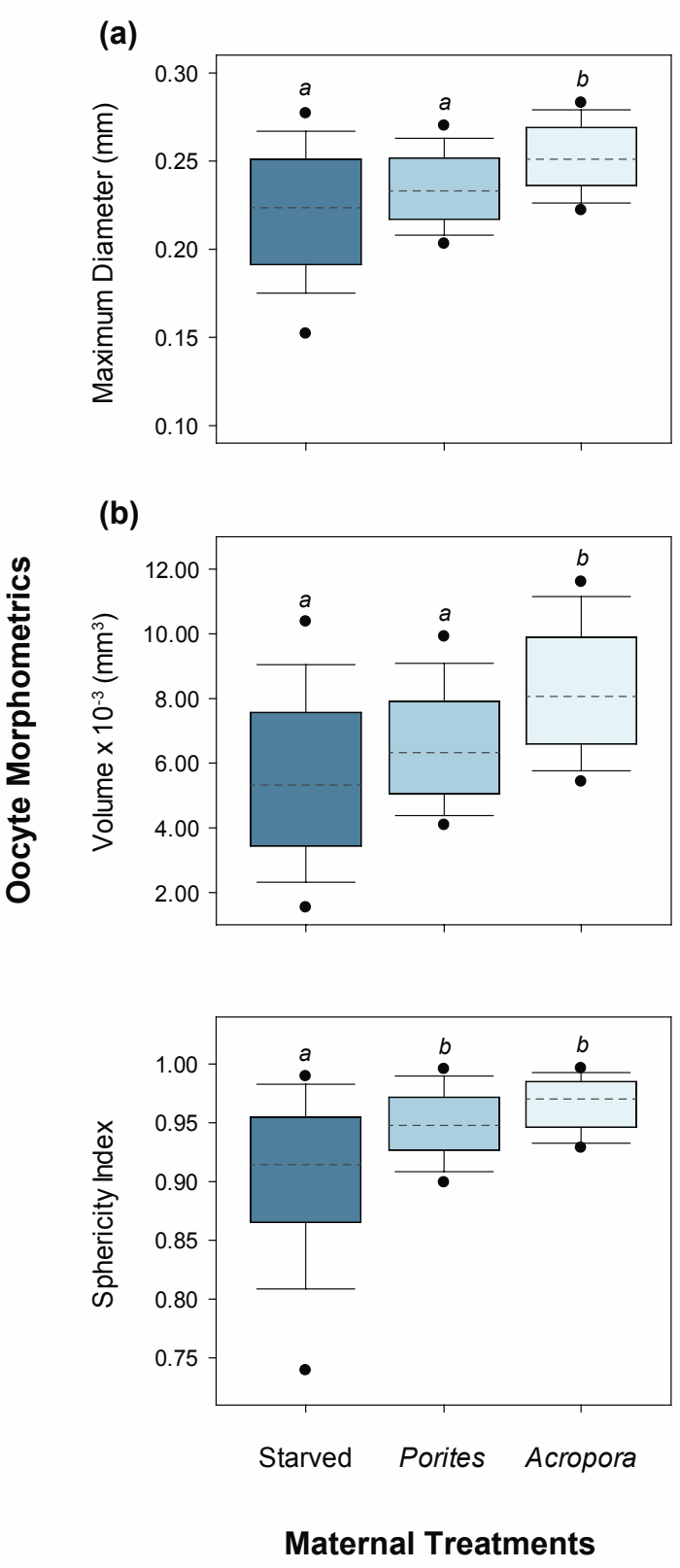

Figure 7.2 Size and shape of oocytes from females under different maternal nutrition treatments. Plots show median (dashed line), 25th and 75th percentile range in the grey box, 5th and 95th percentile range as error bars, and outliers as solid circles for oocyte (**a**) maximum diameter, (**b**) volume, and (**c**) sphericity index (n = 100). Different letters are significantly different based on *post hoc* pairwise comparisons.

Table 7.2. Reported oocyte size of crown-of-thorns starfish and other coral reef asteroids (Order Valvatida) from different locations.

Species	Diameter[a]	Mode[b]	Location	Reference
Acanthasteridae				
Acanthaster planci	0.200 – 0.260	P	New Caledonia	(Conand 1985)
	0.100	P	GBR, Australia	(Henderson 1969)
	0.175	P	GBR, Australia	(Lucas 1982)
	0.180	P	GBR, Australia	(Hoegh-Guldberg and Pearse 1995)
	0.200 – 0.205	P	GBR, Australia	(Wolfe et al. 2015b)
	0.100	P	Java, Indonesia	(Mortensen 1931)
	0.189 – 0.205	P	Palau, Micronesia	(Yamaguchi 1977)
	0.190	P	Guam, Micronesia	(Yamaguchi 1973a)
	0.125 – 0.287	P	Guam, Micronesia	This study[c]
	0.191 – 0.278	P	Guam, Micronesia	This study[d]
	0.214 – 0.288	P	Guam, Micronesia	This study[e]
	0.190	P	Kushimoto, Japan	(Hayashi et al. 1973)
Goniasteridae				
Fromia ghardaqana	1.000	L	Red Sea	(Mortensen 1938)
Ophidiasteridae				
Gomophia egyptiaca	0.650	L	Guam, Micronesia	(Yamaguchi 1974b)
Linckia laevigata	0.150	P	Guam, Micronesia	(Yamaguchi 1973a)
Ophidiaster granifer	0.600 – 0.650	L	Guam, Micronesia	(Yamaguchi and Lucas 1984)
Oreasteridae				
Culcita novaeguineae	0.184 – 0.198	P	Palau, Micronesia	(Yamaguchi 1977)
	0.180		Guam, Micronesia	(Yamaguchi 1973a)
Protoreaster nodosus	0.201	P	Palau, Micronesia	(Yamaguchi 1977)

[a] Oocyte diameter (mm)
[b] Developmental modes**: P** = planktotrophic, **L** = lecithotrophic
[c] Starved (from 3 females, n = 100 oocytes per starfish)
[d] *Porites*-fed (from 3 females, n = 100 oocytes per starfish)
[e] *Acropora*-fed (from 3 females, n = 100 oocytes per starfish)

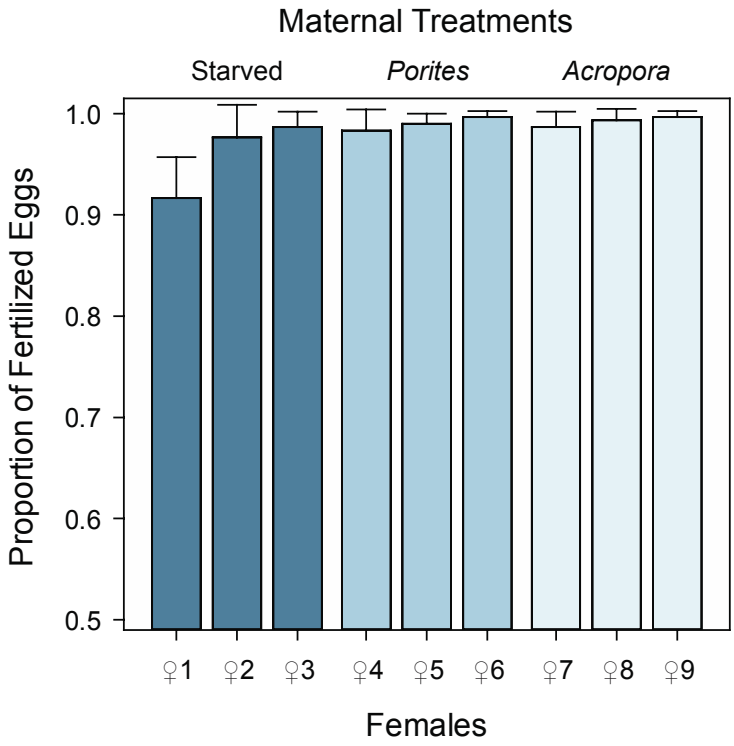

Figure 7.3 Fertilization success across all females under each maternal nutrition treatment. Proportion of fertilized eggs calculated from the number of eggs with raised fertilization envelopes out of 100 randomly selected eggs (n = 3). Error bars represent +1 standard deviation (SD).

7.3.3 Larval growth, survival and development

After four days, surviving larvae were in the bipinnaria stage. Maternal nutrition had a significant effect on larval morphometrics at this stage (**Figure 7.4**). Larvae from females fed with *Acropora* (0.75 ± 0.12 mm) and *Porites* corals (0.72 ± 0.15 mm) were significantly longer than larvae from starved females (0.53 ± 0.14 mm) (**Table 7.3**: $F_{2,243} = 20.351$, $p = 0.002$; **Figure 7.4a**). Maternal nutrition also had a significant effect on the width of larvae at this stage (**Table 7.3**: $F_{2,243} = 23.321$, $p = 0.001$, **Figure 7.4b**). Larvae from fed females (*Acropora*: 0.52 ± 0.09 mm; *Porites*: 0.50 ± 0.11 mm) were also significantly wider than larvae from starved females (0.37 ± 0.10 mm). A similar pattern was also observed in terms of stomach area (**Table 7.3**: $F_{2,243} = 23.321$, $p = 0.001$, **Figure 7.4c**), where larvae from *Acropora*- ($5.28\times10^{-3} \pm 1.49\times10^{-3}$ mm^2) and *Porites*-fed ($4.94\times10^{-3} \pm 1.64\times10^{-3}$ mm^2) females had larger stomachs than larvae from starved females ($4.10\times10^{-3} \pm 1.68\times10^{-3}$ mm^2). It is interesting to consider that 4 days may not have been sufficient time to discern larval growth trajectories between the two fed treatments.

Table 7.3. Results of mixed model hierarchical ANOVA for length, width, and stomach size of larvae from females under three treatments of maternal nutrition

Source	*df*	*MS*	*F*	*P*
Larval Length				
Maternal Nutrition	2	1.267	20.351	0.002
Female (Maternal Nutrition)	6	0.062	2.128	0.100
Jar (Female (Maternal Nutrition))	18	0.029	1.666	0.046
Error	243	0.018		
Larval Width				
Maternal Nutrition	2	0.624	23.321	0.001
Female (Maternal Nutrition)	6	0.027	2.000	0.119
Jar (Female (Maternal Nutrition))	18	0.013	1.491	0.094
Error	243	0.009		
Stomach Area				
Maternal Nutrition	2	33.405	32.564	0.001
Female (Maternal Nutrition)	6	1.026	0.522	0.784
Jar (Female (Maternal Nutrition))	18	1.964	0.736	0.772
Error	243	2.668		

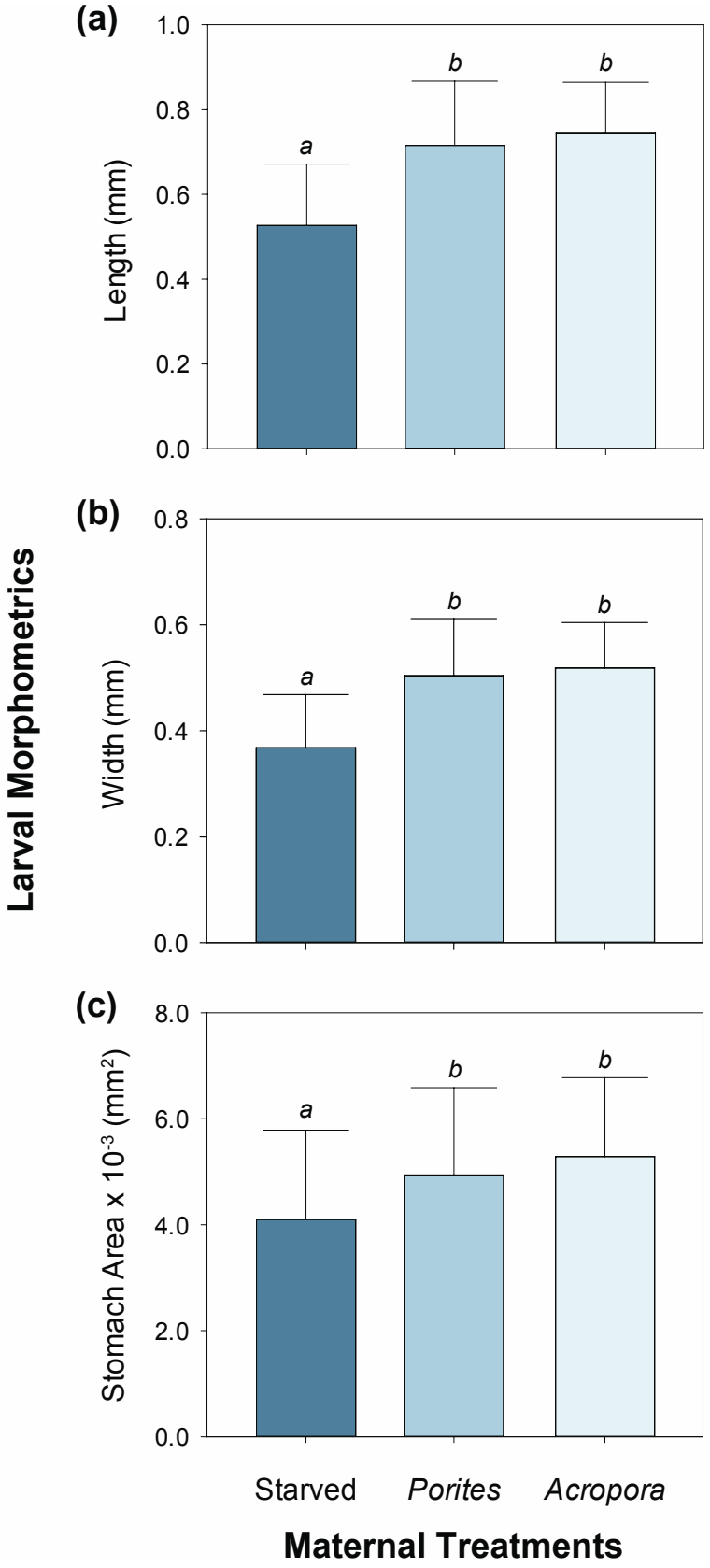

Figure 7.4. Morphometrics of larvae from females under different nutritional treatments. Image analysis measurements of (**a**) length, (**b**) width, and (**c**) stomach area (n=10). Error bars are + 1 SD and different letters are significantly different based on Tukey's *post hoc* test.

Maternal nutrition had no significant effect on daily survival rates (**Figure 7.5**), which remained above 60% across all levels after eight days of rearing. The highest mortality across all treatments was recorded four days after fertilization; starved females, in particular, decreased by an average of 8% at day four. Conversely, maternal nutrition had a significant effect on the proportion of larvae that developed normally (pseudo-$F_{2,18}$ = 8.192, p_{perm} = 0.011; **Figure 7.6a**). Among the surviving larvae after eight days, there was a higher proportion of normally developing larvae in the *Acropora*-(96 ± 2 %) and *Porites*-fed (94 ± 3 %) treatments compared to starved (68 ± 15 %) treatments. This pattern was even more apparent when the proportion of larvae that reached the late bipinnaria / early brachiolaria stage was compared (pseudo-$F_{2,18}$ = 177.720, p_{perm} = 0.004; **Figure 7.6b).** The proportion that reached the late bipinnaria / early brachiolaria stage was ten times higher in the *Acropora* treatment (50 ± 6 %) and nine times higher in the *Porites* treatment (44 ± 5 %) compared to the starved treatment (5 ± 2 %). Overall, the proportion of larvae that progressed to a new developmental stage was significantly different between treatments (G = 339.555, df = 6, $p < 0.001$; **Figure 7.7**). All pairwise comparisons were significant at Benjamini-Hochberg corrected alpha levels (**Figure 7.7**). There was a higher proportion of abnormal (31%) and early bipinnaria (43%) larvae in the starved treatment compared to the fed treatments. Larvae under the *Acropora* treatment were in relatively advanced stages with 32% at advanced bipinnaria and 50% at late bipinnaria / early brachiolaria stage. All normal larval stages were represented in the *Porites* treatment consisting of 22% at early bipinnaria, 29% at advanced bipinnaria, and 44% at late bipinnaria / early brachiolaria stage.

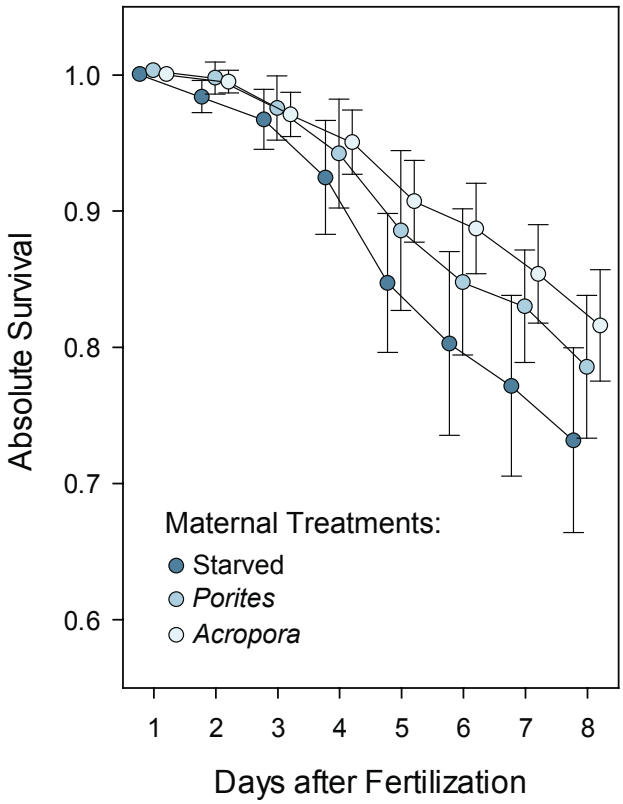

Figure 7.5 Daily survival rates of larvae reared for eight days. Data points are mean values ± 1SD of pooled proportions of surviving larvae from all females and rearing jars under each maternal nutrition treatment (n = 9).

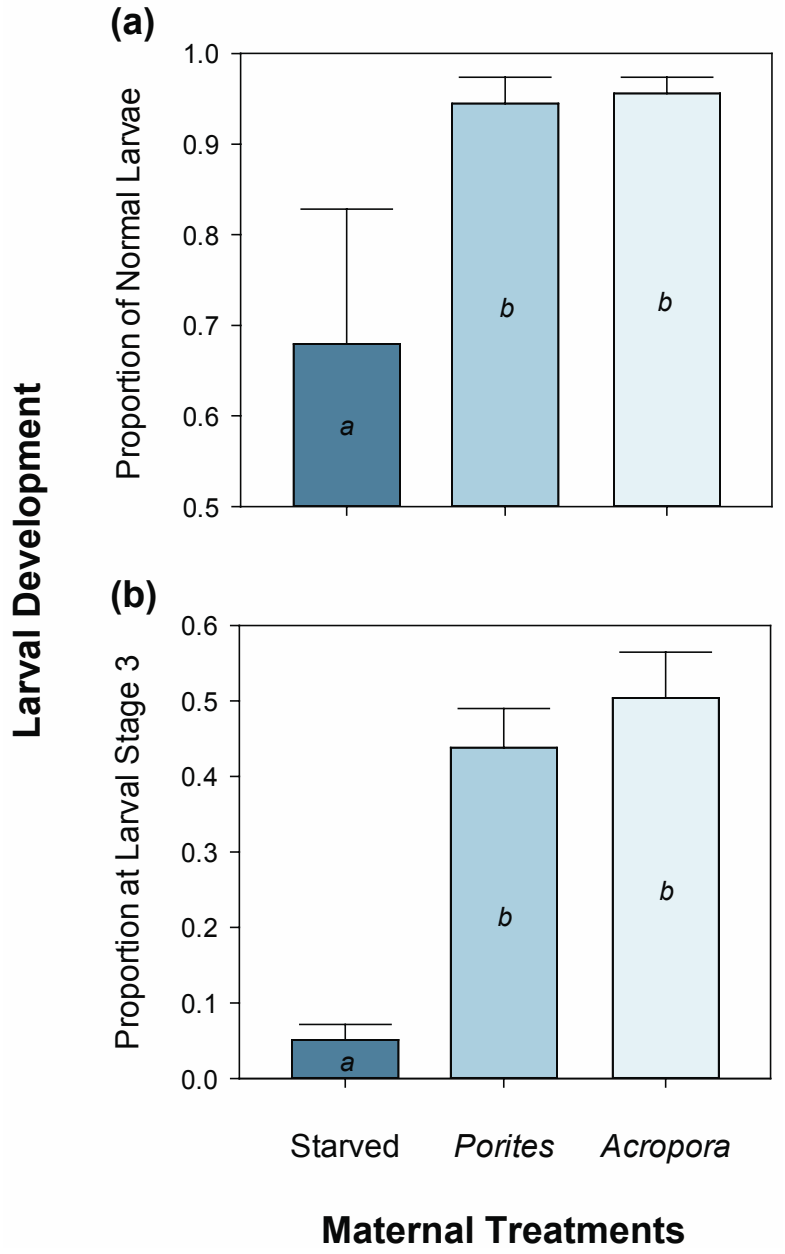

Figure 7.6 Proportion of (**a**) normal larvae and (**b**) late bipinnaria / early brachiolaria larvae at day eight. Error bars represent + 1SD and n = 9 for each maternal treatment. Different letters are significantly different based on Tukey's *post hoc* tests.

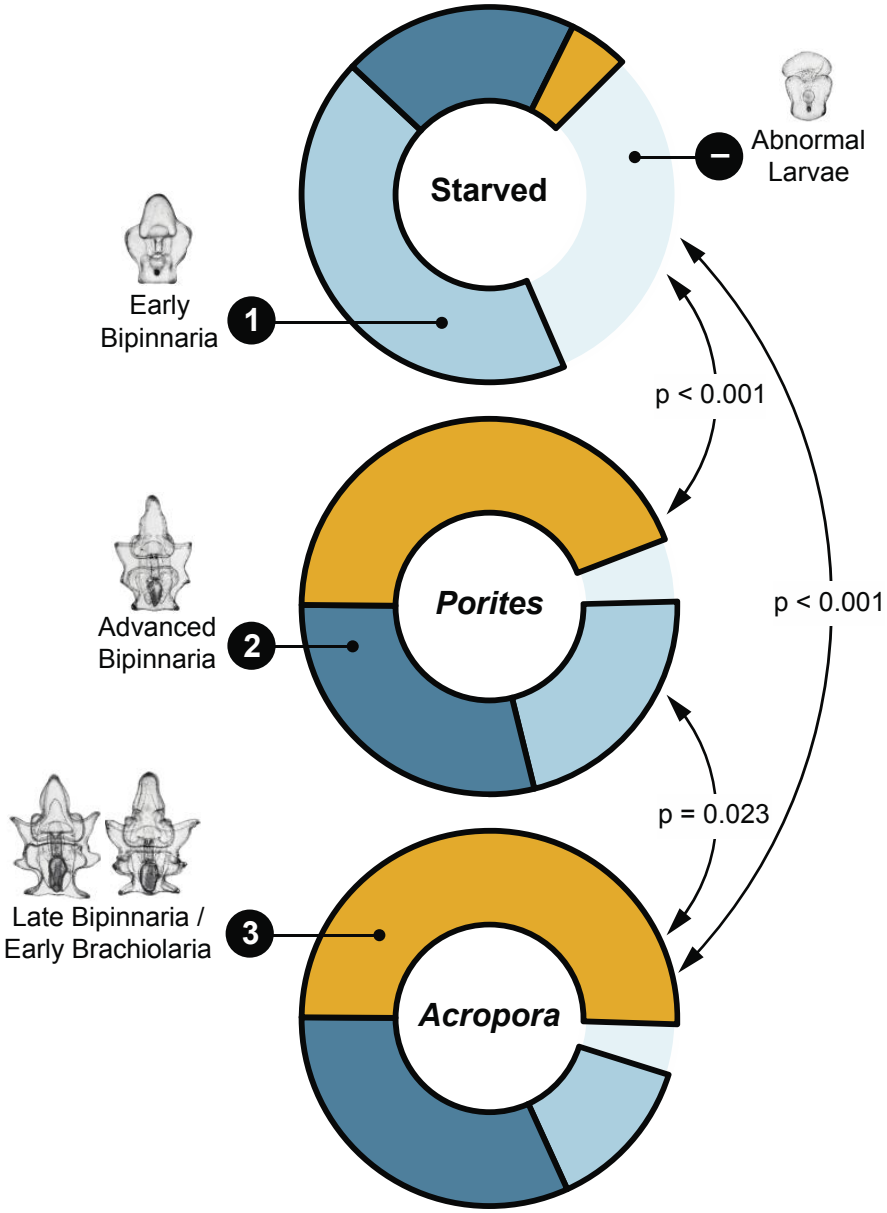

Figure 7.7 Proportion of larvae under 4 development categories: (**1**) early bipinnaria, (**2**) advanced bipinnaria, (**3**) late bipinnaria / early brachiolaria, and (-) abnormal larvae. Arrows and p-values represent *post hoc* G-test pairwise comparisons with Benjamini-Hochberg-corrected significance levels.

7.4 Discussion

Average daily coral consumption crown-of-thorns starfish in this study falls within feeding rates observed in the field during summer months on the Great Barrier Reef (Keesing and Lucas 1992). Consistent with field observations (De'ath and Moran 1998b; Pratchett et al. 2009a), consumption rates on *A. abrotanoides* was significantly higher compared to feeding rates on *P. rus*. One possible explanation for marked feeding preferences among corallivorous organisms (given that the order of preferences is not always consistent with energetic value of the different corals, e.g., Keesing (1990) is that preferred corals are those upon which feeding is most efficient, e.g. Cole and Pratchett (2011). Accordingly, I found that the consumption rates of crown-of-thorns starfish on *A. abrotanoides* (based on estimated area of live coral consumed each day) were 50% higher compared to similar sized starfish feeding on *P. rus*. This is because crown-of-thorns starfish can digest the tissues of *Acropora* much more efficiently than tissues of *Porites* (Keesing 1990). Moreover, *Acropora* corals tend to have much deeper tissue layers than *Porites*, owing to their perforate skeleton (Hughes 1987). Given that *Acropora* corals have higher energetic content and greater tissue depth compared to *Porites*, and can also feed at greater rates on *Acropora*, it is expected that crown-of-thorns starfish on *Acropora* would be in much better nutritional condition.

Despite differences in diet and food intake, no differences were apparent in the physical appearance of female crown-of-thorns starfish that were starved for 60 days versus individuals maintained on exclusive diets of *A. abrotanoides* or *P. rus*. This reflects the considerable resilience of starfish to shortages in prey (Pearson and Endean 1969). However, differences in prey intake did appear to have a significant effect on the physiological condition of crown-of-thorns starfish, with flow-on effects for individual reproductive capacity. Most notably, the starfish that were fed (even if on

sub-optimal coral prey, *P. rus*) tended to increase in weight over the course of the study, whereas starved individuals consistently lost weight. Fed individuals also had heavier gonads and pyloric caeca compared to starved individuals, even when standardized for overall body weight, as shown for other echinoderms (George 1996). Xu and Barker (1990b) suggest that the pyloric caeca act as nutrient reservoirs to support reproductive and maintenance activities under conditions of nutritional stress.

Increases in gonad weight of well-fed echinoderms can be attributed to increased oocyte size and/or higher maternal fecundity (Venable 1992). Similarly, the starfish, *L. epichlora*, had higher fecundity and produced bigger oocytes at sites with increased food availability (George 1994b). Bertram and Strathmann (1998) found that gonad volume increased as a function of oocyte size in *S. droebachiensis*. The weight of *A.planci* gonads also partly reflects increased fecundity, whereby Conand (1985) estimated that *A.planci* consistently produce 90,190 oocytes per gram of ovary. Our study clearly shows that individual starfish feeding on *A. abrotanoides* had proportionally larger gonads, and produced larger oocytes (diameter and volume) compared to *Porites*-fed and starved females. Oocytes from crown-of-thorns starfish that were fed with *A. abrotanoides* were also more uniform and more spherical in shape compared to oocytes from starved females, which is generally reflective of higher oocyte quality, as well as leading to increased rates of fertilization and larval development (Ayukai et al. 1996).

It is also noteworthy that average oocyte diameter measurements in this study were relatively bigger than previously reported measurements for crown-of-thorns starfish (**Table 7.2**). Since the mean oocyte diameter of starved treatments (0.22 ± 0.04 mm) in this study was still marginally higher than measurements from other localities, this variation may largely be from the source population and only partially due to the

experimental manipulation of diet. More importantly, the biochemical and energetic composition of oocytes warrant further investigation because these measurements show that oocytes of crown-of-thorns starfish are relatively bigger compared to oocytes from echinoderms that are obligate planktotrophs (Byrne 2001). Yolk-rich planktotrophic larvae of the Antarctic starfish, *Porania antarctica* have been found to differentiate to brachiolaria stage and increase in length even in the absence of particulate food (Bosch et al. 1991). This may help explain extreme population fluctuations in crown-of-thorns starfish compared to other asteroids with similar planktonic life histories.

For crown-of-thorns starfish, fertilization rates were not significantly different among treatments and were consistently high across all females regardless of differences in nutritional conditions, oocyte size and shape. Similarly, no significant variation in fertilization success was observed when the sea urchin, *S. droebachiensis*, was fed with artificial diets containing different levels of dietary protein and additives (de Jong-Westman et al. 1995). However, Levitan (1996) has shown that for the sea urchin *Strongylocentrotus franciscanus*, fertilization rates were higher on larger eggs, but only under sperm-limiting conditions. Variation in oocyte shape may also result in constrained or arrested development. For example, experiments in which echinoderm oocytes were artificially deformed resulted in abnormal cleavage patterns (Rappaport and Rappaport 1994). Conversely, Podolsky and Strathmann (1996) suggested that varying oocyte shape could provide a mechanism to increase oocyte-spermatozoa collisions without increasing oocyte volume. Our results warrant further investigation into whether any oocyte size and shape has an effect on fertilization rates at lower spermatozoa concentrations and whether the effect of environmental conditions could swamp the influence of gamete traits. High spermatozoa concentrations were used in this study to evaluate whether low maternal investment reduces fertilization rates.

Spawning in turbulent water conditions, lack of synchrony, and low proximity to other spawning individuals could potentially limit spermatozoa concentrations and oocyte traits may be under intense selection to increase fertilization rates in the field.

Females that were fed (regardless of whether they were fed with *A. abrotanoides* or *P. rus*) produced larger larvae with larger stomachs compared to starved females. Maternally derived energetic lipids, particularly triglycerides, fuel larval development in planktotrophic starfish (Prowse et al. 2008). High feeding rates by crown-of-thorns starfish on a lipid-rich food source, such as corals (Patton et al. 1977; Stimson 1987), may allow excess resources for gametogenesis and provide increased maternal provisioning of lipids as a buffer against unfavorable nutritional conditions during the planktonic larval stage (Byrne et al. 2008a). For instance, differences in oocyte triglyceride levels were still observable at the bipinnaria stage for the planktotrophic starfish, *Meridiastra mortenseni*, indicating flow-on effects for larval fitness (Prowse et al. 2008). The same major energetic lipid class (i.e. triglycerides) sequestered by feeding echinoderm larvae to support early juveniles is also provided by the female parent to fuel early development (Byrne et al. 2008b). Hence, additional energetic reserves allow larvae to withstand prolonged periods of starvation (George et al. 1990) and also may be used to produce larger larvae with morphologies that improve feeding effectiveness (Wolfe et al. 2015b). These early larval stage metrics are useful indicators since larger larvae usually progress much faster to advanced stages and have higher survival later in development even at unfavorable food conditions for larvae (George 1999). Although exogenous food supply may still be necessary to complete metamorphosis, faster growth reduces planktonic larval duration and exposure to larval predators (Sinervo and McEdward 1988).

Overall, survival rates were high across all treatments (>60%). Abnormal larvae continue swimming in the water column and remain alive for extended periods, but do not develop further (Fabricius et al. 2010). The proportion of stunted or deformed larvae from starved females was significantly higher and most larvae under this treatment remained at the early bipinarria stage after eight days. Even at starved larval conditions, as is the case in this study, a large proportion of larvae from fed females progressed from early bipinnaria to late bipinnaria / early brachiolaria stage. This implies that any surplus of maternally derived energetic lipids may support development even at conditions when exogenous food resources are limited. In assessing the role of exogenous food availability on larvae from these different conditions of maternal diet, the patchy nature of planktonic food resources must be considered (Boidron-Métairon 1995). At low phytoplankton levels, developing larvae of crown-of-thorns starfish may also exploit other sources of food to supplement endogenous nutrient reserves (Olson 1987). When planktonic food is abundant, larvae of *S. droebachiensis* adults from nutritionally rich habitats have been shown to metamorphose sooner than larvae from adults collected from habitats with low food availability (Meidel et al. 1999).

In summary, crown-of-thorns starfish given almost limitless access to *A. abrotanoides*, which is among the most preferred coral prey, increased in weight and had heavier gonads compared to starved females. *Acropora*-fed females also produced larger oocytes compared to *Porites*-fed and starved females. Fed starfish produced bigger larvae with larger stomachs and had a higher frequency of normally developing larvae. Females on *Acropora* diet also produced larvae that progressed to more advanced stages faster compared to *Porites*-fed starfish, which progressed faster than starved starfish. These results show that the influence of maternal diet on oocyte

characteristics was carried over to early larval stages, affecting both larval size and development. Variability at these earlier stages of development has been known to persist even after metamorphosis and impact juvenile quality (George 1994b; Podolsky and Moran 2006). This has significant implications for the reproductive capacity of female starfish living in reef habitats with varying coral community structure and abundance. Importantly, the local abundance of preferred prey (e.g., *Acropora*) could have an important impact on the overall reproductive capacity and resistance to larval starvation in the absence of phytoplankton blooms. Dense aggregations of brachiolaria-stage larvae of crown-of-thorns starfish were detected in areas where phytoplankton concentrations were low (Suzuki et al. 2016), suggesting that apart from larval nutrition provided by nutrient-rich waters, other factors may play a role in larval survival and development. Future studies need to carefully consider the nutritional condition of females from which oocytes are collected in looking at the effect of larval nutrition on development. Future work should also measure the biochemical composition of oocytes from females under different nutritional states and also contrast the magnitude of the effects of maternal nutrition (endogenous) and larval nutrition (exogenous) on larval vitality and morphometry to see if these differences disappear through compensation or persist throughout development.

Chapter 8

Interactive effects of endogenous and exogenous nutrition on larval development for crown of thorns starfish[7]

8.1 Introduction

Marked and acute increases in the local abundance of the coral-eating crown-of-thorns starfish, *Acanthaster planci* s. l., often termed "outbreaks", contribute significantly to global declines in coral cover (Bellwood et al. 2004; Hughes et al. 2010; Pratchett et al. 2014) and are a central focus of ongoing research and management to secure the future of coral reef ecosystems (Hoey et al. 2016; Westcott et al. 2016). Effective long-term management of crown-of-thorns starfish outbreaks is fundamentally dependent upon identifying the ultimate cause(s) of changes in key demographic properties that potentially differentiate outbreak and non-outbreak populations (Moore 1990). However, given the exceptional fecundity and reproductive potential of crown-of-thorns starfish (Babcock et al. 2016b), it has been suggested that very subtle changes in recruitment rates could be sufficient to initiate outbreaks (Uthicke et al. 2009), especially if primary outbreaks represent the accumulation of individuals over several successive recruitment events (e.g., Pratchett 2005). Conversely, step-changes in developmental rates and survivorship of crown-of-thorns starfish larvae have been reported across relatively moderate gradients in chlorophyll concentrations, such that periodic influxes or concentrations of nutrients (e.g., during major flood events (Brodie

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et al. 2005; Wooldridge and Brodie 2015), upwelling (Houk and Raubani 2010), or from oceanographic features such as chlorophyll fronts (Houk 2007)) may be an important precursor to crown-of-thorns starfish outbreaks (Fabricius et al. 2010; Wolfe et al. 2015a; Pratchett et al. 2017).

For planktotrophic larvae, the energy required for survival, growth, and development can be derived from two sources: parental investment in the oocyte and nutrient acquisition by pelagic larvae from the external environment (McEdward 1997; Bertram and Strathmann 1998; Byrne et al. 2008b). There is a clear dissociation between the adult and larval nutritional environments of crown-of-thorns starfish and the factors influencing the abundance of adult food (coral) and larval food (microalgae) may be quite different. The importance of exogenous food, acquired through the filter feeding activity of larvae, to complete development is well established for crown-of-thorns starfish (Lucas 1982; Okaji et al. 1997; Fabricius et al. 2010; Uthicke et al. 2015; Wolfe et al. 2015a; Pratchett et al. 2017). The "larval-starvation hypothesis" is predicated on the notion that normally low levels of nutrients in near-reef environments would generally constrain growth and development of crown-of-thorns starfish larvae and the release from starvation during periods of nutrient-induced phytoplankton blooms significantly enhances larval survival and recruitment success leading to population outbreaks (Birkeland 1982; Lucas 1982). Fabricius et al. (2010) reported dramatic increases in survival and competency of crown-of-thorns starfish larvae with every doubling of chlorophyll concentration (a proxy for phytoplankton abundance) above 0.25 $\mu g \cdot L^{-1}$. In addition, Uthicke et al. (2015) also demonstrated that algal food concentration has a strong influence on larval development, with temperature as a modulator. Apart from exogenous food availability, the condition of crown-of-thorns starfish larvae (at least during early larval stages) will also be partly influenced by the

nutritional condition of females during spawning, whereby well-fed females produce larger and faster developing larvae (Caballes et al. 2016). The question is whether the effects of maternal provisioning are sufficient to offset potential limitations on larval growth and survivorship when larvae are exposed to low levels of food in the environment?

The nutritional condition, and therefore fitness, of adult crown-of-thorns starfish is clearly dependent on availability of coral prey (Lucas 1984), and may also vary with differences in the local availability of different types of corals (Caballes et al. 2016). It is well known that adult crown-of-thorns starfish have very specific prey preferences, generally feeding on *Acropora* and *Montipora* corals to the exclusion of all other coral genera when available (Pratchett et al. 2014). Although crown-of-thorns starfish will eat virtually all scleractinian corals and can cause comprehensive depletion of corals during severe population outbreaks, there is often serial depletion of different coral genera (e.g., Kayal et al. 2012), whereby less preferred coral prey are generally consumed only after other more preferred corals are locally depleted. While it is yet to be effectively shown, strong feeding preferences by crown-of-thorns starfish likely reflect the variation in the nutritional content and/or food value of different coral prey (Ormond et al. 1976). If so, it can be inferred that the nutritional condition of crown-of-thorns starfish would be maximized when feeding on generally preferred corals such *Acropora*, while condition is likely to decline after preferred coral prey have been locally depleted, such that feeding is restricted to less preferred corals (e.g., *Porites*). In Guam, Caballes et al. (2016) showed that crown-of-thorns starfish maintained for 8-weeks on a diet of *Acropora abrotanoides* gained weight and produced bigger oocytes, compared to conspecifics that were maintained on a diet of *Porites rus* or starved.

Moreover, differences in the diet and nutritional condition of females had a significant bearing on the quality and quantity of their progeny (Caballes et al. 2016).

Tropical reef waters are typically oligotrophic in the absence of flood or upwelling events (Lucas 1982; Revelante and Gilmartin 1982). Under conditions of scarce exogenous food, echinoid larvae have been known to respond through adaptive changes in shape and by increasing the size of feeding structures to improve the efficiency of food capture (McEdward 1986). For example, pluteus larvae of sea urchins use ciliated bands on feeding arm rods to capture food, hence developing longer arm rods can improve clearance rates of particulate food (Hart and Strathmann 1994). For asteroid larvae, arms do not elongate until the later stages; in lieu of this, asteroids modify larval shape to maintain high clearance rates at food-limiting conditions. Bipinnaria larvae of the sand starfish, *Luidia foliolata*, maintained at high food concentration had pointed anterodorsal and posterodorsal arms, whereas larvae at low food levels had rounded arms, which was associated with high clearance rates (George 1994a). Wolfe et al. (2015b) documented phenotypic plasticity in 7-day old crown-of-thorns starfish larvae in response to a range of algal food concentrations. However, the effect of adult diet and corresponding maternal investment on how larvae respond to variable conditions of exogenous food availability is unknown. This has important implications in understanding how crown-of-thorns starfish larvae thrive even in oligotrophic conditions.

The purpose of this study was to evaluate the individual, additive, and interactive effects of endogenous ("Maternal") and exogenous ("Larval") nutrition on larval vitality and morphology. Previous studies (Caballes et al. 2016) have shown that there are significant maternal effects on the quality of crown-of-thorns starfish larvae, but it is unknown whether significant levels of endogenous nutrients could offset potential

limitations associated with low availability of planktonic food (e.g., Lucas 1982). Maternal nutrition could affect larval planktonic duration by affecting larval growth rates, but effects of exogenous food (phytoplankton) could overwhelm maternal effects (Bertram and Strathmann 1998). This study aims to determine whether the effects of maternal provisioning disappear through compensation or persist throughout development under different conditions of food availability for larvae. The gonad index and size of oocytes from females fed with *Acropora*, mixed diet (*Acropora*, *Pocillopora*, *Porites*), *Porites*, and starved females were compared as a proxy for maternal provisioning and oocyte quality. The effects of endogenous and exogenous nutrition on (1) absolute survival; (2) development and competency; and (3) growth and morphology of larvae were specifically addressed in this paper.

8.2 Methods

8.2.1. Collection and maintenance of specimens

Adult individuals of the Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) were collected on 26 October 2015 from Eyrie Reef (14.705660° S, 145.379154° E), located 8 km west of Lizard Island in the northern Great Barrier Reef (GBR), Australia. Starfish were transported to the Lizard Island Research Station and placed in 1000-L oval tanks with continuous flow of fresh seawater (27.15 ± 0.97 °C; 35.46 ± 0.07 psu; pH 8.17 ± 0.01). Sex was determined by examining contents drawn from gonads along the arm junction using a syringe with a large-bore biopsy needle (Caballes and Pratchett 2014). Twelve female starfish were allowed to acclimatize to ambient aquarium conditions for three days, without food prior to being assigned to one of four different feeding treatments (described below). A fresh batch of male starfish was collected from Eyrie Reef on 29 November 2015 and gravid males were placed in 1000-L oval tanks with flow-through seawater and maintained on a mix of *Acropora intermedia*, *Porites cylindrica*, and *Pocillopora damicornis* corals for three days. Coral fragments used for experimental feeding treatments were collected from within the Lizard Island lagoon (14.697030° S, 145.451410° E) and allowed to acclimatize in plastic aquaria for 24 h (GBRMPA Permit No. G15/38002.1). Coral infauna (e.g., *Trapezia* crabs) were physically removed from all coral fragments so as not to deter feeding by crown-of-thorns starfish (Pratchett 2001).

8.2.2. Maternal feeding treatments

Twelve intact female starfish of approximately similar size (diameter = 338 ± 8 mm; wet weight = 1633 ± 91 g) were randomly split into four groups and each group of three starfish placed in 300-L plastic aquaria with flow through seawater. All females were nearing reproductive maturity based on microscopic examination of oocytes drawn from starfish using the biopsy procedure described in the previous section. Oogenesis of crown-of-thorns starfish from the GBR is usually most active between September and November, while some have been observed to rapidly complete oogenesis within a month (i.e., between November and December) (Lucas 1973). Starfish were assigned to one of four different "Maternal" feeding treatments (n = 3) for 30 days: (i) *Acropora* (fed with *Acropora intermedia*); (ii) Mixed (fed with *Acropora intermedia*, *Porites cylindrica*, and *Pocillopora damicornis*); (iii) *Porites* (fed with *Porites cylindrica*); and (iv) Starved (no food provided, only dead coral skeletons). Supply of coral food for fed treatments was replenished as soon as the piece of coral provided had been completely consumed. Sample size (n = 3) was kept low to limit the amount of coral fed ad libitum to starfish. Wet weight of each starfish, prior to and 30 days after feeding treatments, was measured. Gonads and pyloric caeca were also weighed after feeding treatments to calculate the gonad index (GSI) and the pyloric caeca index (PCI) for each individual. The average weight of gonads and pyloric caeca from three arms was multiplied with the total number of arms of each starfish to estimate the total gonad or pyloric caeca weight. GSI and PCI were expressed as the ratio of gonad or pyloric caeca weight to the total weight of the starfish (Conand 1985).

8.2.3. Spawning induction and oocyte metrics

Gonad lobes were dissected from the twelve females and ovaries were rinsed in 0.2-µm filtered seawater (FSW) to remove loose oocytes. Ovary lobes were treated in 10^{-5} M 1-methyladenine to induce ovulation. Released oocytes were transferred into containers with filtered seawater and wet mounted on glass slides for microscopic examination. Diameters (D_{oocyte}) of the long and short axes of 100 randomly selected mature oocytes (have undergone germinal vesicle breakdown) from each treatment were measured using image analysis of micrometer-scaled photographs of oocytes in Image J (Schneider et al. 2012). Oocyte volume (V_{oocyte}) was calculated using the formula for an oblate spheroid:

$$V_{oocyte} = 4/3 \times \pi \times a^2 \times b, \quad (8.1)$$

where ***a*** is the radius of the major axis (long axis) and ***b*** is the radius of the minor axis (short axis).

8.2.4. Fertilization and larval rearing

Oocytes from each female were placed in separate 1-L beakers with FSW kept at 28 °C. Approximately 200 oocytes from each female were transferred into triplicate 250-mL beakers using a glass pipette. Spermatozoa were collected from the testes of five males and checked for motility under a microscope. Roughly equal amounts of spermatozoa from each male were combined and counted using a haemocytometer. Oocytes were fertilized with spermatozoa diluted to achieve a spermatozoa-to-oocyte ratio of 100:1.

Fertilized eggs were pooled for each maternal diet treatment group as variation among females had previously been found to be minimal (Caballes et al. 2016). Zygotes

from each group were separately reared in round acrylic containers equipped with stirrers. After 48 h, 100 actively swimming bipinnaria larvae with fully formed mouth, stomach, and anus were siphoned into separate plastic culture bottles with 150 mL FSW. Algal food was prepared from a mixture of sterile cultures of *Dunaliella tertiolecta* at 30% (strain CS-175) and *Chaetoceros muelleri* at 70% (strain CS-176). crown-of-thorns starfish have been previously reared to settlement using *D. tertiolecta* (Lucas 1982) and *C. muelleri* (Caballes et al., unpublished manuscript) individually and as a mixture (Uthicke et al. 2015). Sampling of phytoplankton communities in the GBR has shown *Chaetoceros* to be one of the most dominant microalgae taxa during flood events (Devlin et al. 2013), hence I used a higher proportion of *C. muelleri* in this study. Final cell densities were quantified using a haemocytometer. Each group of larvae was assigned to three exogenous ("Larval") nutrition treatments: (i) "High Food" (fed twice daily at 10^4 cells·mL^{-1}) (ii) "Low Food" (fed twice daily at 10^3 cells·mL^{-1}) and iii) "Starved" (no algal food, 0.2-µm FSW). There were six replicate culture bottles for each of the 12 combinations of endogenous and exogenous nutrition treatments (total of 72 culture bottles). Each culture bottle was connected to an air hose set at one bubble per second to prevent larvae from settling on the bottom. Water changes with fresh FSW were performed daily. Surviving larvae in each culture bottle were counted every four days for 16 days during water changes. At 4 and 10 days after the start of feeding, 10 normally developing larvae from each bottle were immediately photographed using a camera mounted on a microscope. Maximum length, maximum width, posterior width, ciliated band length, and gut area were measured using ImageJ (**Figure 8.1**). At day 4, 8, and 16 after the start of feeding, all surviving larvae were categorized into the following developmental stages: **(1) early bipinnaria**—gut fully formed, preoral and anal lobes present, coelomic pouches below or close to mouth; **(2) advanced**

bipinnaria—coelomic pouches fuse as axohydrocoel above the mouth, anterodorsal and posterolateral arms start to form; **(3) early brachiolaria**—brachiolar arms start to appear as stump-like projections from the anteroventral surface of the larvae, anterior extension of axohydrocoel, anterodorsal, posterolateral, and posterodorsal arms start to elongate, preoral arms start to form; **(4) mid-late brachiolaria**—brachiolar arms prominent, starfish rudiment developing in the posterior region of larvae, postoral arms form, and other larval arms more elongated; and **(5) abnormal**—stunted and deformed larvae (Yamaguchi 1973a; Byrne and Barker 1991; Caballes et al. 2016).

8.2.5. Statistical analyses

Statistical comparison of oocyte diameter and volume was made using a two-factor mixed model hierarchical analysis of variance (ANOVA) with "Maternal Nutrition" as a fixed effect (four levels) and "Female" (three levels, random) nested within "Maternal Nutrition". No departures from normality and homogeneity of variance were detected for all data. A post hoc Tukey's test was used for pairwise comparisons of fixed factor means. A generalized linear model (GLM) with quasibinomial errors and logit link function was used to analyze the effect of maternal nutrition and larval feeding treatments (fixed categorical predictors) on the proportion of surviving and normally developing larvae and percentage of larvae at the brachiolaria stage (response variables). Treatments with zero variance (e.g., 0% larvae at the mid-late brachiolaria stage across all replicates for treatment with no algal food) were excluded from this analysis. Pairwise post hoc tests were subsequently performed using the Tukey's method in "lsmeans" function in R (R Core Team 2016). The frequency distribution of larvae under different developmental stages was analyzed as a contingency table using log-linear models with log link and Poisson error terms (Agresti

1996) to examine larval progression in relation to "Maternal" and "Larval" nutrition treatments. "Developmental Stage" was considered as a response variable so all models included the interaction between "Maternal" and "Larval" nutrition (Quinn and Keough 2002). Degrees of freedom (*df*) were calculated and deviance statistics (χ^2) were used to compare models in R (R Core Team 2016). Pairwise comparisons were done using G-test of independence with correction for false discovery rate (Benjamini and Hochberg 1995). Data for measurements of morphological traits were analyzed using two-way ANOVA testing for the main and interactive effects of "Maternal" and "Larval" nutrition treatments. Data were log-transformed when assumptions of normality or homogeneity of variance were not met. Significant tests were followed by post hoc Tukey's test for pairwise comparisons within fixed effects. Principal component analysis (PCA), implemented using the "vegan" package in R, was used to visualize the effect of "Maternal" and "Larval" nutrition treatments on larval morphology. Morphometric data were log-transformed and the average per replicate culture bottle was used to avoid pseudoreplication. Further morphometric comparisons were performed using permutational multivariate ANOVA (PERMANOVA) on 9999 permutations under a reduced model (Anderson 2001). Morphological traits were log-transformed and Euclidean distances were used to generate a resemblance matrix in PRIMER v.6 (Primer-E Ltd., Plymouth, UK). Means and standard deviation (± SD) were calculated for all data in each treatment. All statistical analyses were performed in R, unless stated otherwise, and p-values (p) below 0.05 were considered statistically significant in all tests.

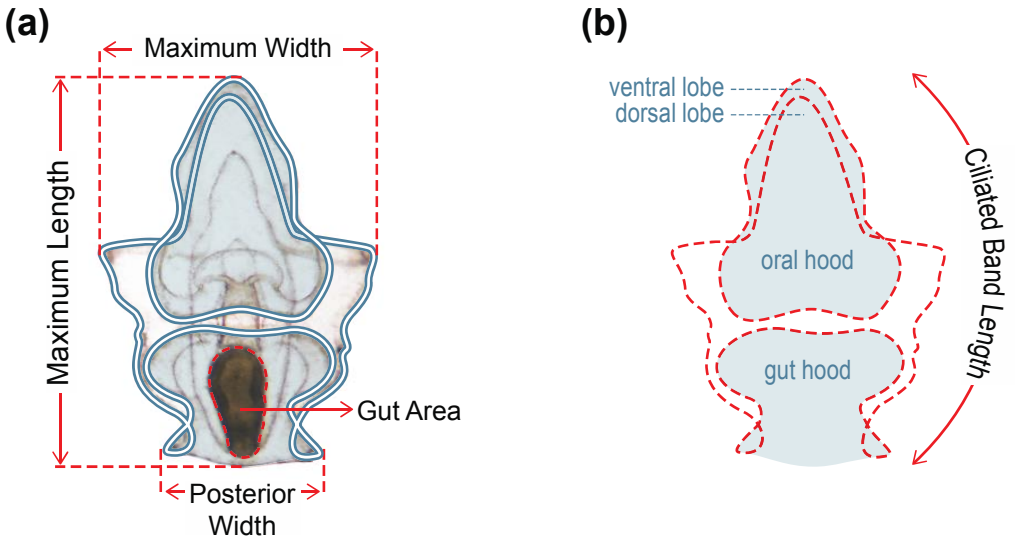

Figure 8.1. Morphometric measurements of larvae taken four and ten days after commencement of feeding: (**a**) size of morphological features; (**b**) Ciliated band length = sum of the traced perimeter measurements of the oral hood and ventral lobe, gut hood, larval sides, and dorsal lobe (red dashed outline).

8.3. Results

8.3.1. Maternal and oocyte metrics

Initial diameter ($F_{3,8}$ = 0.19, p = 0.9011) and weight ($F_{3,8}$ = 0.21, p = 0.8898) of female starfish used under the four maternal nutrition treatments were not significantly different. Weight change after 30 days was also not significantly different between maternal diet treatments ($F_{3,8}$ = 0.47, p = 0.7130). Maternal diet had a significant effect on pyloric caeca indices (PCI; $F_{3,8}$ = 4.60, p = 0.0374), mainly due to females under the mixed diet treatment having significantly higher PCI than starved starfish. The gonadosomatic index (GSI) of females given *Acropora* (24.1 ± 1.7% SD, standard deviation in all instances hereafter) and mixed (24.2 ± 1.4%) diets were significantly higher than *Porites*-fed (13.2 ± 1.6%) and starved (11.2 ± 2.5%) starfish ($F_{3,8}$ = 14.29, p = 0.0014).

Variation in oocyte diameter (D) and volume (V) was consistent with patterns for GSI between treatments (**Figure 8.2**). The diameter and volume of oocytes from starfish placed under *Acropora* (D = 0.22 ± 0.01 mm; V = 0.0051 ± 0.0009 mm^3) and mixed (D = 0.22 ± 0.02 mm; V = 0.0051 ± 0.0010 mm^3) diet treatments were significantly larger compared to *Porites*-fed (D = 0.19 ± 0.02 mm; V = 0.0033 ± 0.0008 mm^3) and starved (D = 0.19 ± 0.01 mm; V = 0.0031 ± 0.0006 mm^3) females. Maternal diet treatments accounted for 60% and 63% of the variation in oocyte diameter and volume, respectively (**Appendix E – Table E1**). There was a significant difference in oocyte size among females within treatments, but this only accounted for 5% of the variation (**Appendix E – Table E1**).

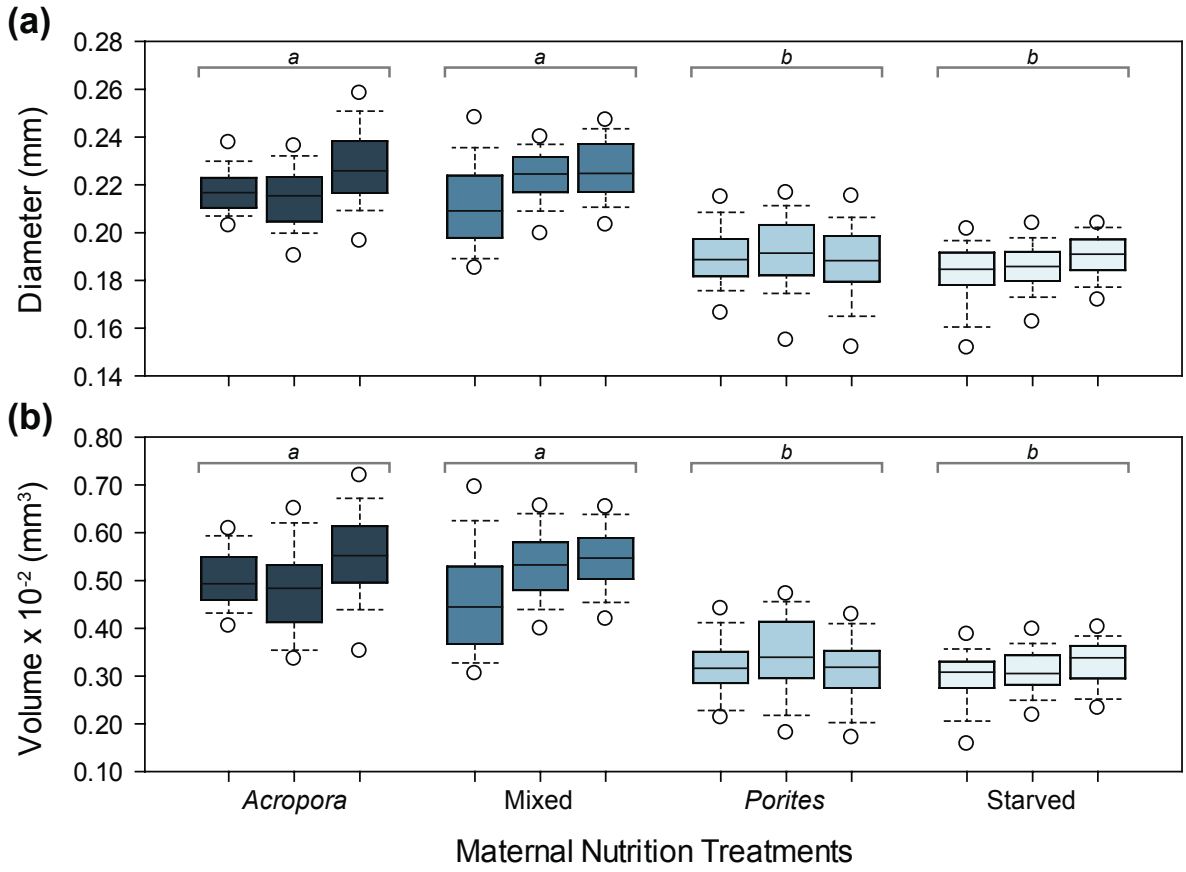

Figure 8.2. Size of oocytes from female starfish under different maternal nutrition treatments: (**a**) oocyte diameter; (**b**) oocyte volume. Boxplots with different letters above are significantly different.

8.3.2. Larval survival

Absolute survival of larvae (without taking into account normal development and larval stage) was high (>85%) across all treatments at day 4 (**Figure 8.3**) and no significant differences were found between maternal nutrition and between larval diet treatments (GLM, Table 8.1). At day 8, maternal effects were significant, with a higher proportion (>70%) of surviving larvae from females that were fed (*Acropora*, Mixed, *Porites*) compared to the starved treatment (56.9 ± 13.1%) (**Figure 8.3**). At 12 and 16 days after the onset of larval ability to feed, maternal and larval nutrition treatments had a significant additive effect on larval survival. At day 12, survival was >60% for larvae from females on a coral diet, while survival was only 48.4 ± 14.2% for larvae from unfed starfish. Survival was also >60% for larvae provided with exogenous food, while only 52 ± 15.6% of starved larvae survived at day 12. At the end of the experiment (day 16), survival was almost twice as high for larvae from maternally fed treatments (*Acropora* = 50.1 ± 14.6%, Mixed = 49.4 ± 15.7%, *Porites* = 45.3 ± 19.0%) compared to those from the starved treatment (37.7 ± 17.1%). Larvae that were fed with microalgae also had a higher survival rate (High: 55.9 ± 13.1%; Low: 53.8 ± 10.7%) compared to larvae with no food (28.4 ± 10.5%) at day 16 (**Figure 8.3**).

Table 8.1. Analysis of deviance for binomial generalized linear models (GLMs) testing the effects of maternal nutrition and larval feeding treatments on the proportion of surviving larvae at 4, 8, 12, and 16 days after the onset of the ability of larvae to feed. Hereafter, Maternal Diet: **Acr** = *Acropora*, **Mix** = mixed diet, **Por** = *Porites*, **Stv** = starved; Algal Food Concentration (cells·mL^{-1}): **Hi** = 10^4, **Lo** = 10^3, **No** = 0.

Source	***df***	χ^2	***P***	***Post Hoc***
Day 4				
Maternal Nutrition	3	2.58	0.9349	
Larval Nutrition	2	2.12	0.8396	
Maternal Nutrition x Larval Nutrition	6	4.71	0.9927	
Day 8				
Maternal Nutrition	3	140.00	**0.0001**	Acr = Mix = Por > Stv
Larval Nutrition	2	27.92	0.1281	
Maternal Nutrition x Larval Nutrition	6	15.63	0.8901	
Day 12				
Maternal Nutrition	3	98.03	**0.0035**	Acr = Mix = Por > Stv
Larval Nutrition	2	60.24	**0.0152**	Hi = Lo > No
Maternal Nutrition x Larval Nutrition	6	20.05	0.8349	
Day 16				
Maternal Nutrition	3	61.00	**0.0072**	Acr = Mix = Por > Stv
Larval Nutrition	2	435.23	**<0.0001**	Hi = Lo > No
Maternal Nutrition x Larval Nutrition	6	5.81	0.9794	

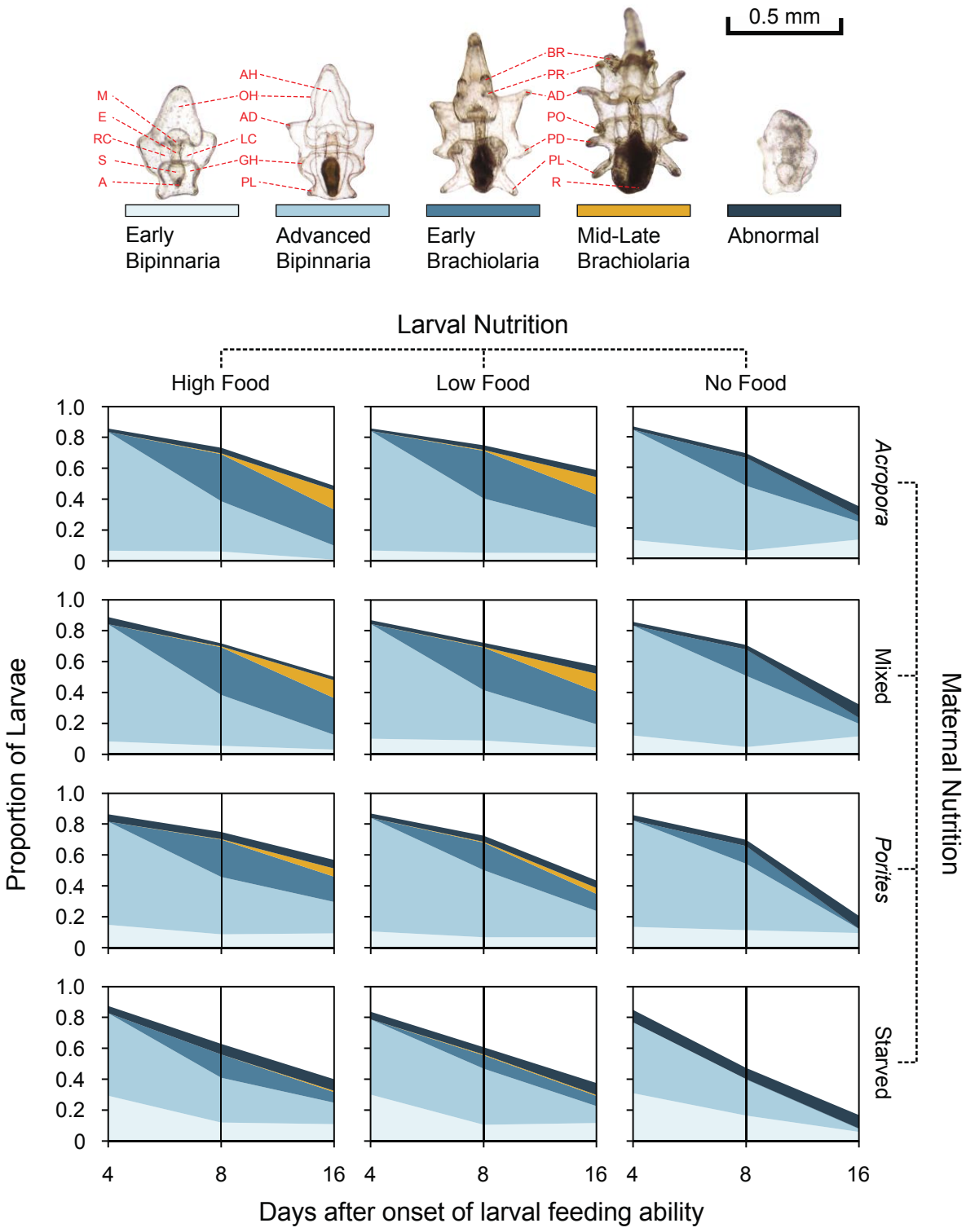

Figure 8.3. Larval survival and progression of larval development at 4, 8, and 16 days. Morphological traits used in scoring larvae are: mouth (**M**), esophagus (**E**), right coelomic pouch (**RC**), stomach (**S**), anus (**A**), axohydrocoel (**AH**), oral hood (**OH**), anterodorsal arm (**AD**), left coelomic pouch (**LC**), gut hood (**GH**), posterolateral arm (**PL**), brachiolar arm (**BR**), preoral arm (**PR**), anterodorsal arm (**AD**), postoral arm (**PO**), posterodorsal arm (**PD**), posterolateral arm (**PL**), and rudiment (**R**).

8.3.3. Larval development

At day four after the onset of larval feeding ability, the majority of the larvae across all treatments were at the advanced bipinnaria stage. The distribution of larvae among the different developmental stages was dependent on maternal nutrition (Table 8.2), with a higher proportion of larvae that were still at early bipinnaria under the starved treatment (35.7 ± 18.1%) compared to larvae from starfish placed on *Acropora* (9.9 ± 4.9%), mixed (11.8 ± 4.6%), and *Porites* (15.4 ± 10.5%) coral diet (**Figure 8.3**). At day 8, larval development was dependent on maternal and larval nutrition treatments (Table 8.2). The stage of development was bimodal (**Figure 8.3**), with the majority of larvae remaining at advanced bipinnaria and the main driver of variation was the proportion of larvae at the brachiolaria stage (Maternal Nutrition: *Acropora* = 36.9 ± 9.9%, Mixed = 35.2 ± 10.0%, *Porites* = 24.1 ± 10.8%, Starved = 13.4 ± 13.4%; Larval Nutrition: High = 35.3 ± 11.0%, Low = 29.9 ± 13.8%, No Food = 16.9 ± 12.1%). At day 16, maternal provisioning and larval diet had a significant additive effect on the developmental progression of larvae (Table 8.2). The developmental stage frequency distribution of larvae from starfish on *Acropora* and mixed diets were significantly different from *Porites*-fed and starved treatments (**Figure 8.3**). Retrogression of larvae from brachiolaria or advanced bipinnaria back to early bipinnaria was evident for larvae from starved females and for starved larvae from *Porites*-fed females (**Figure 8.3**).

Results of statistical analyses of normal development and larval competency are summarized in **Appendix E – Table E2**. At Day 8, maternal diet had a significant effect on the proportion of normally developing larvae (**Figure 8.4a**) and the proportion of larvae reaching the brachiolaria stage (**Figure 8.4c**). Under maternal treatments that were fed with coral, the proportion of normally developing larvae (*Acropora* = 95.7 ± 3.2%, Mixed = 96.4 ± 2.7%, *Porites* = 94.1 ± 3.8%) was significantly higher compared

to those from the starved treatment (88.9 ± 11.1%). The proportion of larvae from starfish on *Acropora* and mixed diets that reached the brachiolaria stage was 1.5 times higher compared to *Porites*-fed treatments and 2.7 times higher compared to starved treatments. At this point, larval nutrition did not have a significant effect on the proportion of normally developing larvae, but the proportion of larvae reaching the brachiolaria stage was twice as high for treatments provided with algal food compared to starved larvae (**Figure 8.4c**). At 16 days after the onset of larval feeding capability, maternal provisioning and larval diet had a significant additive effect on the proportion of larvae that developed normally (**Figure 8.4b**). The proportion of normally developing larvae from crown-of-thorns starfish on *Acropora* and mixed diets was 8% higher compared to *Porites*-fed treatments and 18% higher compared to starved treatments. Fed larvae were also 1.4 times more likely to undergo normal development compared to starved larvae (**Figure 8.4b**). Maternal condition also had a strong influence on the proportion of larvae that reached the mid-to-late brachiolaria stage after 16 days. Treatments on *Acropora* and mixed diets were 2.3 and 13.7 times more likely to reach competency compared to *Porites*-fed and starved treatments, respectively. None of the unfed larvae reached the mid-to-late brachiolaria stage at 16 days and were excluded in the analysis due to zero variance. There was no significant difference in the proportion of larvae at the mid-to-late brachiolaria stage between high and low algal food treatments.

Table 8.2. Analysis of deviance for log-linear models testing complete and conditional dependence of larval development on maternal provisioning and larval diet at 4, 8, and 16 days.

Source	*df*	χ^2	*P*	*POST HOC*
Day 4				
Maternal Nutrition	36	82.80	**<0.0001**	Acr = Mix = Por ≠ Stv
Larval Nutrition	32	7.34	0.9663	
Maternal Nutrition x Larval Nutrition	24	4.94	1.0000	
Day 8				
Maternal Nutrition	36	79.62	**<0.0001**	Acr = Mix ≠ Por = Stv
Larval Nutrition[1]	32	51.55	**0.0157**	
Maternal Nutrition x Larval Nutrition	24	22.05	0.5763	
Day 16				
Maternal Nutrition	36	103.49	**<0.0001**	Acr = Mix ≠ Por = Stv
Larval Nutrition	32	143.85	**<0.0001**	Hi = Lo ≠ No
Maternal Nutrition x Larval Nutrition	24	12.38	0.9753	

[1] Power not sufficient to show significant differences in post hoc pairwise comparisons.

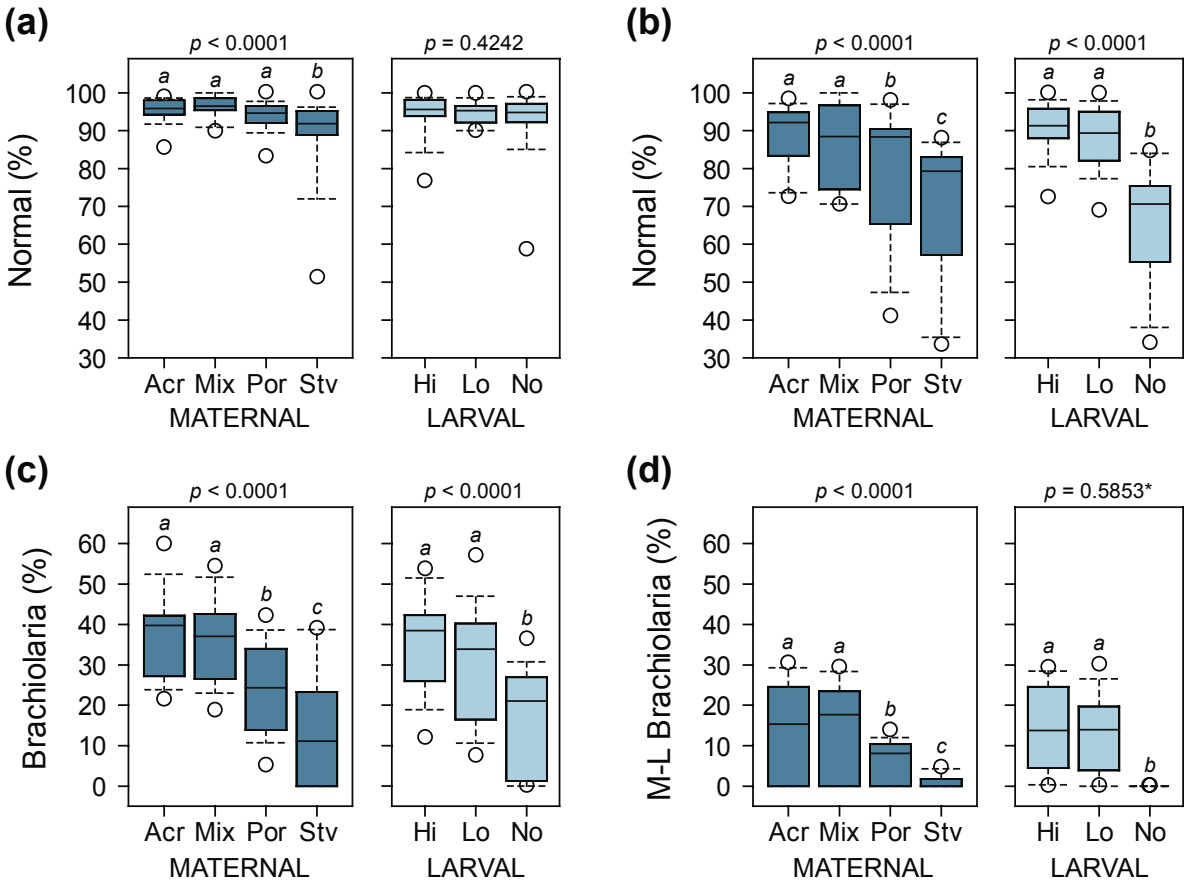

Figure 8.4. Proportion of normally developing larvae at 8 days (**a**) and 16 days (**b**) after the onset of the ability of larvae to feed, and proportion of larvae at the brachiolaria stage after 8 days (**c**) and larvae at the mid-to-late (M-L) brachiolaria stage after 16 days (**d**). *p*-values are from overall binomial GLMs and different letters are significantly different based on post hoc pairwise comparisons (* *p*-value for comparison between high and low algal food treatments only; starved larvae not included in analysis due to zero variance).

8.3.4. Larval growth and morphometry

Maternal diet had a significant effect on initial size across all morphological traits at four days after the onset of larval feeding ability (**Appendix E – Table E3**). Larval diet did not influence larval growth at this stage. Variation in maximum larval length, maximum width, posterior width, ciliated band length, and gut area was mainly due to maternal treatments (**Figure 8.5**). In particular, variation in larval size was driven by differences in growth rates, which was consistently higher for coral-fed treatments compared to larvae from starved starfish. Among the coral-fed treatments, larvae from starfish on *Acropora* and mixed diets were bigger compared to *Porites*-fed treatments. Patterns of variation in maximum width and posterior width were consistent with differences in ciliated band length and gut area, respectively, suggesting proportional growth of feeding structures and stomach with overall larval size (**Figure 8.5**).

At 10 days after the onset of larval feeding ability, there was a significant interaction in the effects of maternal and larval diet on larval size (**Appendix E – Table E4**). Initial differences in larval size due to maternal nutritional condition persisted at this stage, while larval diet also had a significant effect on larval growth, i.e., fed larvae were longer and wider compared to starved larvae (**Figure 8.6a–c**). Larvae under low algal food concentration had disproportionately longer ciliated bands (**Figure 8.6d**) in relation to maximum length and width (**Figure 8.6e,f**). The influence of maternal effects on gut area was reduced, while the effect of larval nutrition was more pronounced at this stage (**Figure 8.6g**).

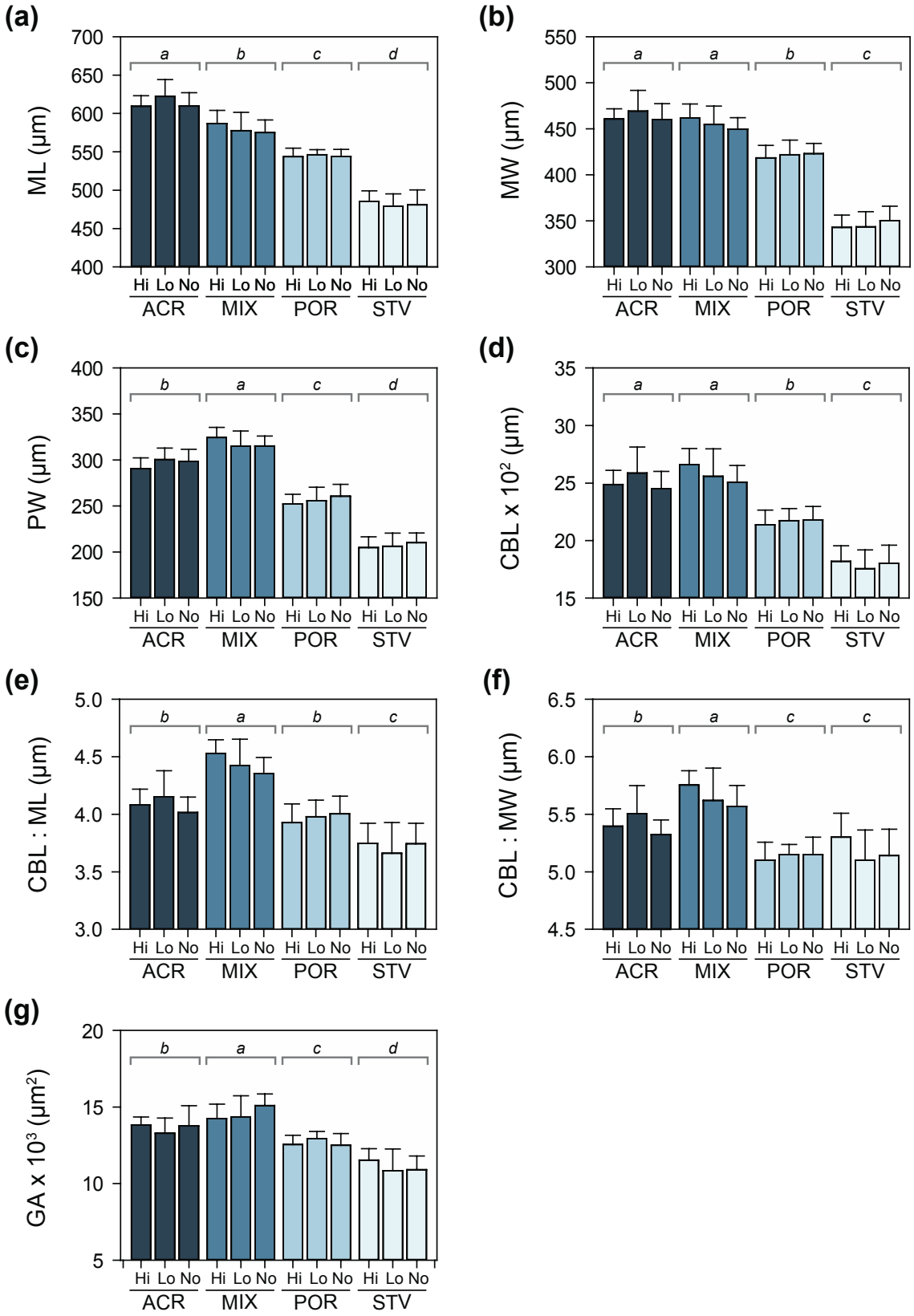

Figure 8.5. Morphometric measurements of larvae (± SD) at Day 4: (**a**) maximum length (**ML**), (**b**) maximum width (**MW**), (**c**) posterior width (**PW**), (**d**) ciliated band length (**CBL**), (**e**) ratio of **CBL** to **ML**, (**f**) ratio of **CBL** to **MW**, and (**g**) gut area (**GA**). Letters above bars denote significant differences as determined by Tukey's post hoc tests following two-way ANOVA.

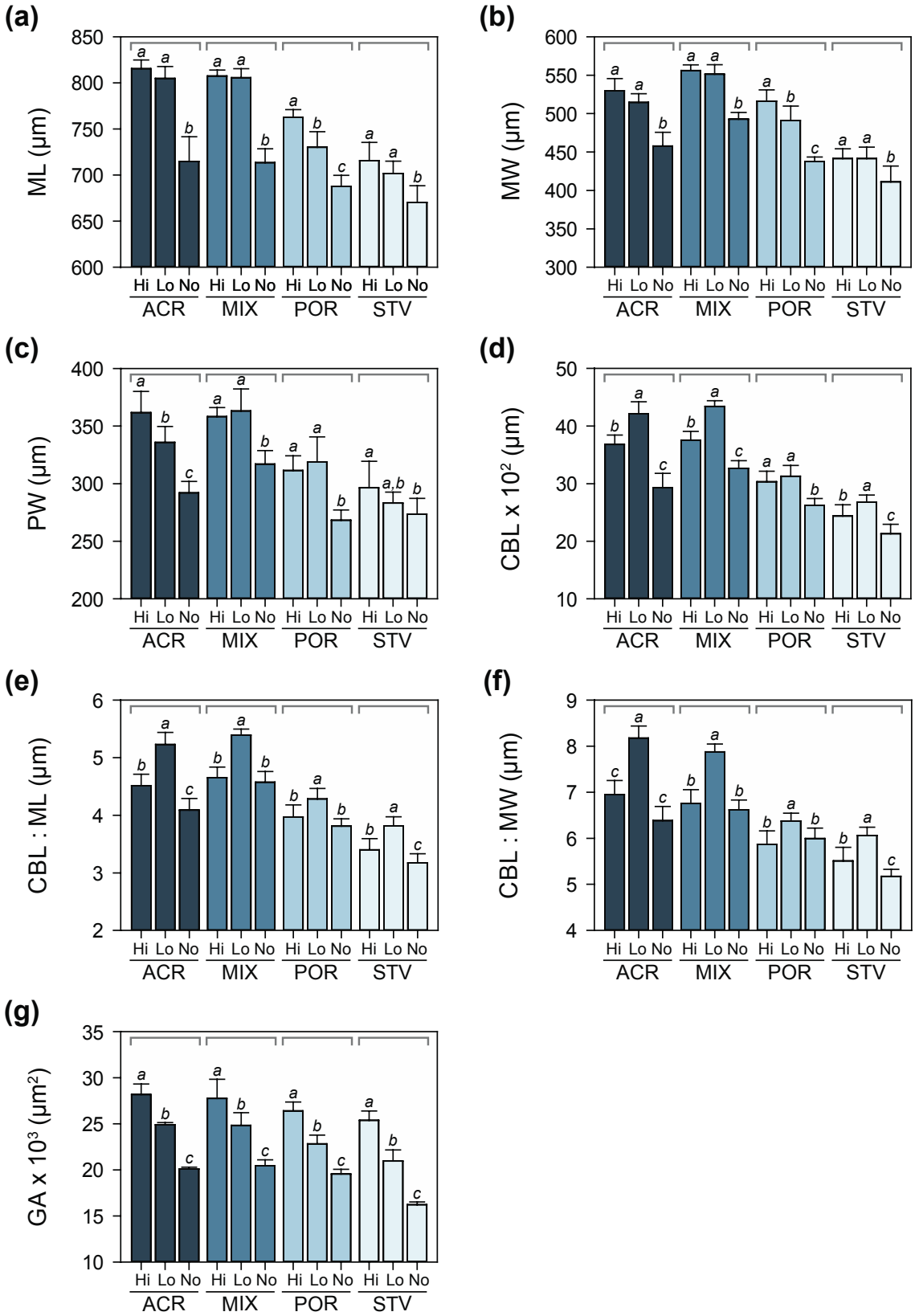

Figure 8.6. Morphometric measurements of larvae (± SD) at Day 10: (**a**) maximum length (**ML**), (**b**) maximum width (**MW**), (**c**) posterior width (**PW**), (**d**) ciliated band length (**CBL**), (**e**) ratio of **CBL** to **ML,** (**f**) ratio of **CBL** to **MW**, and (**g**) gut area (**GA**). Different letters above bars indicate significant differences based on Tukey's post hoc tests following two-way ANOVA.

Consistent with individual measurements of morphological traits, maternal diet explained 66.2% of the variation in overall larval allometry at four days after the onset of larval feeding ability (Table D5). Larvae from females under *Acropora* and mixed diets were generally longer and wider. At this stage, ciliated band length and gut area coincided with larval growth (i.e., increase in larval length and width), irrespective of changes in larval morphology (**Figure 8.7a**).

At day 10, endogenous and exogenous nutrition explained 37.5% and 38.9% of the variation in larval morphology, respectively (Table D5). The effects of these factors were interactive and mostly driven by differences in ciliated band length and gut area (**Figure 8.7b**). Ciliated bands were disproportionately longer in relation to maximum length and maximum width, which indicates phenotypic change in response to the concentration of algal food. Gut area also varied with algal food concentration, i.e., larvae under high food concentration treatments had larger stomachs compared to low food and starved treatments.

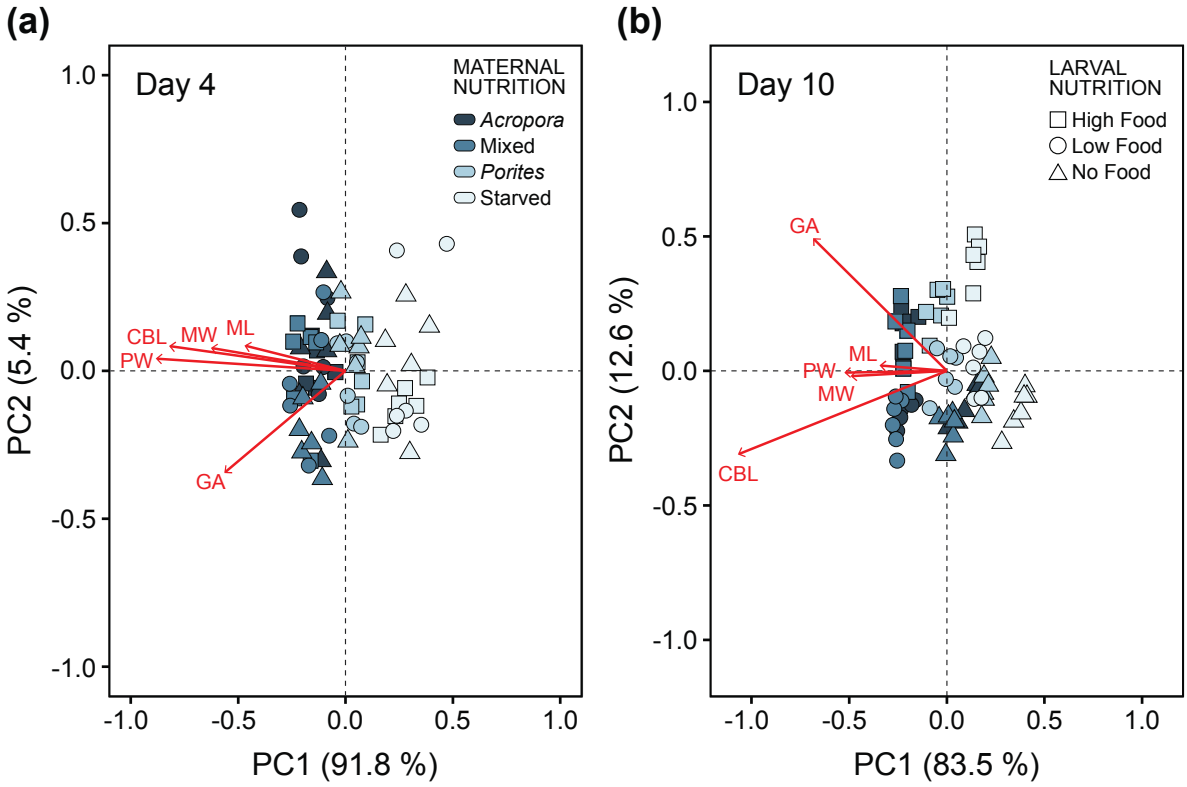

Figure 8.7. Principal component analysis (PCA) plot of morphological traits measured to analyze similarities in larval morphology at (**a**) four days and (**b**) ten days after the onset of feeding ability. Red vectors are morphometric measurements of maximum length (**ML**), maximum width (**MW**), posterior width (**PW**), ciliated band length (**CBL**), and gut area (**GA**). Maternal nutrition treatments are indicated by different colors and larval feeding treatments are indicated by symbols. Individual data points are average values from 10 replicate larvae per experimental bottle.

8.4. Discussion

This study shows that the inferred nutritional condition, and thereby the diet, of crown-of-thorns starfish has a significant and lasting effect on larval vitality. Notably, crown-of-thorns starfish fed on either an exclusive diet of *Acropora* or a mixed diet in which *Acropora* represented ~33% of available prey produced larger gonads and oocytes, which were correlated with larval growth and morphology, as well as rates of larval survival, development, and competency. The influence of endogenous nutrition was most apparent in the earlier stages of development (i.e., bipinnaria stage) while the significance of exogenous nutrition was manifested in later stages (i.e., advanced bipinnaria to the brachiolaria stage). My results suggest that the quantity and quality of coral food rations to female starfish differed sufficiently to affect reproductive investment in crown-of-thorns starfish, as evidenced by significant variations in oocyte diameter and volume. This is consistent with a previous study by Caballes et al. (2016), which showed that crown-of-thorns starfish fed ad libitum with *Acropora* for two months during peak oogenesis produced significantly larger oocytes compared to oocytes from *Porites*-fed and starved females. Intraspecific variation in oocyte size in many echinoderms is often mediated by differences in food quality and quantity (George 1996). Within species, larger oocytes are generally associated with specimens collected from field sites with abundant food and with animals under high food treatments in laboratory studies (George 1994b, 1996; Bertram and Strathmann 1998). Using the gonad index and oocyte size as an index for maternal investment, the present study demonstrates that maternal effects persist through to later stages of development and affect how larvae respond to varying conditions of food availability in the external environment.

Neither endogenous provisioning to the egg or exogenous food availability influenced initial larval survival (all above 85% at day 4), but larvae from fed adults, regardless of diet, generally showed faster growth and development compared to those from starved adults. This suggests that early larval success is significantly influenced by maternal condition. The influence of adult diet and nutritional condition on initial growth and development has been previously shown for crown-of-thorns starfish (Caballes et al. 2016) and other echinoderms (de Jong-Westman et al. 1995; George 1996, 1999; Byrne et al. 2008b). After the digestive tract differentiates (usually two days after fertilization), crown-of-thorns starfish larvae enter a facultative feeding period where they are capable of feeding, but do not necessarily require food for growth and development due to existing maternal reserves (McEdward 1997). Starved larvae have been shown to survive for long periods and develop all the way to early brachiolaria (Lucas 1982; Caballes et al. 2016). The onset of the ability to feed occurs at around the same stage across several species with planktotrophic larvae, but the onset of the need to feed and the duration of the facultative feeding period vary dramatically according to oocyte size and quality (Herrera et al. 1996), and hence maternal diet. In the present study, the influence of maternal effects on survival and development remained strong at eight days after the onset of the ability to feed. This stage is normally the transition phase from bipinnaria to brachiolaria larval form. Continued development at this stage suggests that maternal reserves can buffer the effects of low food availability or starvation. Rate of survival, normal development, and larval progression was higher in larvae from coral-fed adults (*Acropora*, mixed, *Porites* diet) compared to starved treatments. Moreover, the proportion of larvae that progressed to the brachiolaria stage was higher in starfish on *Acropora* and mixed diet compared to *Porites*-fed treatments. Differences in the inferred nutritional condition between adult

starfish on *Acropora* and mixed diet versus *Porites*-fed females was most likely influenced by variable consumption rates between coral species. In the present study, estimated coral consumption was higher for *Acropora intermedia* and *Pocillopora damicornis* compared to *Porites cylindrica*. Similarly, when coral food was rationed ad libitum to crown-of-thorns starfish for 60 days, consumption rates of *Acropora abrotanoides* were significantly higher compared to *Porites rus* (Caballes et al. 2016). Efficiency in feeding and digestion was significantly higher on acroporid and pocilloporid corals compared to poritids (Keesing 1990). This is in accordance with crown-of-thorns starfish feeding preferences observed in the field and in controlled laboratory assays, where the frequency of predation and predation rates on *Porites* was consistently lower compared to *Acropora* and *Pocillopora* corals (reviewed by Pratchett et al. 2014). General models of optimal diet theory would predict that crown-of-thorns starfish would prefer to feed on corals with the highest nutritional value to maximize energetic return (Ormond et al. 1976). Nutritional analyses of corals showed that the energetic and protein contents of acroporid and pocilloporid corals were marginally higher compared to poritids (Keesing 1990).

Variation in larval survival, development, and competency at the 16 days after onset of feeding ability was mainly driven by exogenous food availability. High mortality rates at day 16 were documented for starved larvae and none of the larvae reached the mid-to-late brachiolaria stage. Maternal effects on initial larval vitality in the earlier stages persisted in the later stages. Even when provided with a high concentration of algal food, very few larvae from starved females progressed to the mid-to-late brachiolaria stage. However, larvae from *Porites*-fed females were able to partially compensate for these initial differences by feeding in the plankton. The facultative feeding period of crown-of-thorns starfish larvae appears to exceed 10 days

after fertilization, and potentially longer for larvae from well-fed starfish. The assumption that the length of the facultative feeding period is correlated with oocyte size (McEdward 1997) is supported by my results. The size of crown-of-thorns starfish oocytes is relatively bigger compared to other tropical planktotrophic asteroids (Caballes et al. 2016) and other echinoderms, which may allow storage of surplus nutrients essential for larval growth (Byrne et al. 2008b; Prowse et al. 2008). The success of crown-of-thorns starfish in exploiting a lipid-rich food resource (i.e., scleractinian corals (Stimson 1987)) more so than any other reef organism also allows reproductively mature females to allocate energetic and structural resources directly towards the oocyte and indirectly to the juvenile (e.g., in the forcipulate starfish, *Pisaster ochraceous*; George 1994b). Maternal provisioning of surplus nutritional reserves to the oocyte may allow larvae to withstand prolonged periods of starvation (George et al. 1990) and produce larger larvae with structures that improve feeding efficiency in food-limited environments (Wolfe et al. 2015b).

Larvae from starved females were shorter, narrower, and had smaller stomachs compared to those from fed starfish, while female crown-of-thorns starfish on *Acropora* and mixed diets produced bigger larvae with larger gut areas compared to *Porites*-fed treatments. At this stage, ciliated band length was proportional to overall larval size. In a previous study, Caballes et al. (2016) reported that four-day old larvae from crown-of-thorns starfish fed with *Acropora* or *Porites* were bigger in terms of length, width and stomach area compared to those from starved starfish. Divergence in initial larval size and form was mainly driven by maternal diet treatments. The influence of exogenous nutrients is negligible at this stage (4 days after the onset of the ability of larvae to feed) due to available maternal reserves. While maternal provisions were still present, Byrne

et al. (2008b) did not observe a significant size difference between fed and unfed larvae of the echinoid, *Tripneustus gratilla*.

The onset of phenotypic response of larvae comes in later, influenced by the synergistic effects of endogenous and exogenous nutrition. Initial differences in larval size and development rate influenced by endogenous maternal reserves were carried over to later stages. Continuous supply of high concentrations of algal food for 16 days did not compensate for initial deficiencies of larvae from starved and *Porites*-fed females. Likewise, growth in larvae from starfish on *Acropora* and mixed diets was stunted in the absence of particulate food. Supplemental storage lipids from maternal provisions may be important in allocating resources for larval growth and for building larval feeding structures. Exceptionally high feeding rates on a lipid-rich food source such as hermatypic corals (Patton et al. 1983; Harland et al. 1993; Yamashiro et al. 1999) uniquely predisposes crown-of-thorns starfish to increased maternal reserves. Lipid levels in corals are higher than most marine invertebrates (Giese 1966). Maternally derived energetic lipids, particularly triglycerides, fuel early development in echinoderms (Prowse et al. 2008). Moreover, elevated levels of lipids during egg production in corals (Arai et al. 1993) coincides with oogenesis in crown-of-thorns starfish in the GBR, hence an increase in the amount of lipids in crown-of-thorns starfish diet prior to spawning. Although the proportion of triglycerides in *A. intermedia* (Imbs and Yakovleva 2012), *P. damicornis and P. cylindrica* (Yamashiro et al. 1999) were almost identical, variable consumption rates on these species could drive differences in maternal provisioning. Byrne et al. (2008a) suggest that the presence of triglycerides later in development may be a bet-hedging strategy to maintain a buffer against uncertain food supply for larvae. My results support this proposed strategy, i.e., the degree of allometric elongation of ciliated bands in relation to larval size was more

pronounced among larvae from starfish on *Acropora* and mixed diets compared to larvae from starfish under poor maternal nutritive conditions.

Enhancement of feeding capacity is set by the total length of the ciliated band, which requires complementary increases in body size or changes in larval shape to maximize the length of ciliated bands (McEdward 1986; Hart and Strathmann 1994). George (1994a, 1999) demonstrated that the bipinnaria larvae of asteroids are capable of changing the size of their feeding structures in response to the amount of available algal food. For crown-of-thorns starfish larvae, allometric growth of the ciliated band relative to body size can be achieved by increasing the length and width of the larval body coupled with allometric development of bigger oral and gut hoods. Larval crown-of-thorns starfish in starved and low food condition had longer ciliated bands relative to body size (Wolfe et al. 2015b). Few studies have proposed reliable cues that stimulate these changes in the size of larval feeding structures. Shilling (1995) found that echinoderms respond morphologically to organic compounds in the environment that may indicate the availability of dissolved and particulate nutrients. Larvae may also respond morphologically upon detection of chemical and physical cues from algal cells (Miner 2007).

Given that larval size and shape influence feeding capability, changes in larval morphology will have important functional consequences. In this study, phenotypic plasticity aided by maternal provisions and in response to the environmental nutritive regime may explain the differential success in survival, growth, and development of larvae. Plasticity in larval development has been shown to reduce pelagic larval duration (Hart and Strathmann 1994), which consequently increases survival by reducing exposure to planktonic predators (Sinervo and McEdward 1988) and by reducing the probability of advective loss from adult habitat (Strathmann 1978). The

ability of larvae from well-fed females to modify feeding structures in response to oligotrophic conditions, which is typical for reef waters, may help explain reported outbreaks in locations where the likelihood of elevated phytoplankton levels induced by terrestrial runoff is low (Lane 2011; Kayal et al. 2012; Roche et al. 2015; Suzuki et al. 2016).

8.5. Conclusions

Maternal diet had strong effects on larval survival, development, and growth at the earlier stages. Ciliated band length was proportional to larval growth at this stage. The effect of exogenous diet becomes more pronounced at the later stages, presumably when maternal provisions have been exhausted. Under low algal food conditions, larvae compensate by increasing the length of ciliated feeding bands in relation to larval size, which improves food capture and feeding efficiency. However, the effects of endogenous nutrition persist through to the later stages of larval development, as larvae from starved females did not possess supplemental maternal reserves to develop longer ciliated bands in response to low-food conditions. Resilience of crown-of-thorns starfish larvae from starvation and food-limiting conditions is influenced, in part, by the availability of surplus maternal reserves in the earlier stages of development and then later through compensatory morphological plasticity to improve the efficiency of food capture. Although acquisition of particulate food may still be necessary to fuel larval growth for successful metamorphosis, initial advantages or deficiencies in larval survival, growth, and development are carried over in later stages. Phenotypic plasticity influenced by endogenous provisions and in response to exogenous food availability

may be an important strategy in boosting the reproductive success of crown-of-thorns starfish, leading to population outbreaks.

Chapter 9

General Discussion

Extreme variability in adult abundance is common among marine organisms, particularly among broadcast spawners with planktonic larvae (Uthicke et al. 2009). However, few marine organisms show changes in abundance of the magnitude, or rate, exhibited by crown-of-thorns starfish (Chesher 1969; Zann et al. 1990). Given the life-history characteristics of crown-of-thorns starfish, it is almost harder to explain the persistence of low-density populations than it is to explain outbreaks (Pratchett et al. 2014 – **Chapter 2**). Most notably, crown-of-thorns starfish have exceptional fecundity (Babcock, Milton, et al. 2016), which is equal or greater than the individual fecundity of any other marine invertebrates (Thorson 1950). As such, it is very likely that *Acanthaster* sp. populations will fluctuate periodically through the effects of random exogeneous (environmental or biotic) variation on reproductive success or larval survival.

Small changes in proportional fertilization, development and larval survival for *Acanthaster* sp. can have a profound influence on absolute levels of population replenishment. Lucas (1986) estimated that for a female starfish that produces 100 million eggs during its reproductive life, only 0.000002% reproductive success is required to maintain turnover and maintain local populations, while very moderate increases in reproductive success (0.001%) will increase larval settlement and adult abundance 100-fold. Thus, the most prominent hypotheses that attempt to explain the cause of population outbreaks attribute variability in adult populations to factors

affecting the reproductive biology and early life history stages of crown-of-thorns starfish (Caballes and Pratchett 2014 – **Chapter 3**).

The early life stages and processes in the life cycle of crown-of-thorns starfish are highly subject to the vagaries of local environmental conditions. This thesis examined the sensitivity and responses of these early life stages and processes (i.e. gametogenesis, spawning, fertilization, embryonic development) to a suite of environmental variables to establish thresholds and infer potential effects on the population dynamics of crown-of-thorns starfish (**Figure 9.1**). Environmental variables influencing planktotrophic larvae have also been thought to be largely independent from those influencing benthic adults (Uthicke et al. 2009). This thesis also established a link between the nutritional condition of adults and the survival and development of larvae.

9.1 Implications for recruitment success in crown-of-thorns starfish

Despite the high reproductive potential of crown-of-thorns starfish, the spatiotemporal patchiness of populations suggests that multiple factors, acting on different stages in its life cycle, must occur in concert to initiate primary outbreaks (**Figure 9.1**, Wooldridge and Brodie 2015). Crown-of-thorns starfish in the GBR have a seasonal reproductive cycle, where gametogenesis commences around late August to early September and culminates with spawning in December. Based on surveys and intensive sampling of crown-of-thorns starfish in the GBR from 2013 to 2015, temporal patterns in the relative frequency of gonad maturation stages, were generally consistent with predicted austral summer breeding season in the GBR. However, analysis of gonadosomatic indices (*GSI*), oocyte size frequency, and gonad histology revealed marked interannual variation in reproductive timing and output (**Chapter 4)**. This variability was correlated with seawater temperature where larger increments (~2°C)

approaching peak summer temperatures between November and December 2014 resulted in the release of almost all mature gametes; as opposed to the previous year where incremental rise in temperature was more gradual and starfish appeared to spawn repeatedly, but to a lesser extent, until late in the spawning season. Synchronized gametogenesis and spawning will be more advantageous, particularly for aggregated individuals, as this increases the amount of gamete released and significantly improves fertilization rates (Babcock and Mundy 1992a; Babcock et al. 1994).

Inter-annual variability in reproductive timing and output among crown-of-thorns starfish (**Chapter 4**) indicates that specific cues are involved in inducing and synchronizing gamete release. Explicit testing of potential spawning cues revealed that abrupt temperature change and high concentrations of certain species of phytoplankton (e.g. *Skeletonema*) may be important in inducing gamete release in more sensitive males (Caballes & Pratchett 2017 – **Chapter 5**). Pheromones associated with sperm released by these sensitive male starfish subsequently synchronized spawning with other starfish. Environmental cues for spawning may be more important in populations from low-latitude locations where there are no distinct reproductive cycles (Pratchett et al. 2014 – **Chapter 2**). Given the immediate temporal linkage between the timing of spawning and fertilization events, variability in the extent and synchronicity of gamete release will significantly influence reproductive success. As fertilization immediately follows spawning, existing environmental conditions during gamete release could potentially limit fertilization rates and early development even when sperm-to-egg ratios are optimal. Rates of fertilization, cleavage, and gastrulation were highest at ambient environmental conditions (temperature, salinity, pH) during the spawning season (Caballes et al. 2017a – **Chapter 6**), which suggests that spawning is timed so that conditions are optimal for reproductive success.

Effects of temperature and pH on fertilization and early development of *A.* cf. *solaris* mostly corresponded with the sensitivity of sperm to these stressors, while response to salinity was largely due to detrimental effects on osmotic balance in eggs (Caballes et al. 2017a – **Chapter 6**). Water-soluble compounds associated with eggs also enhanced sperm activity, particularly in environmental conditions where sperm motility was initially limited, which again highlights the importance of aggregations in improving reproductive success. Moreover, the effects of these environmental factors may also counteract one another; for example, the spawning season of crown-of-thorns starfish usually coincide with peak summer temperatures, as well as high precipitation; and while fertilization and embryonic development may be robust to high temperatures (up to 34°C), the proportion of embryos progressing to subsequent larval stages may be significantly lower when salinities drop (below 25 psu) during heavy rainfall events that result in high freshwater discharge from rivers (Caballes et al. 2017a – **Chapter 6**, Allen et al. 2017). These results have important implications on geographical range expansion and potential for long-range dispersal of crown-of-thorns starfish. Gametes, fertilization, and embryonic development were robust to temperature, salinity, and pH levels that are outside the range found at the normal geographical limits of adult distribution (Caballes et al. 2017a – **Chapter 6**). Bipinnaria larvae are also tolerant to a wide range of temperature and salinity levels (Henderson 1969; Habe et al. 1989). Gametes, embryos, or bipinnaria larvae swept into less favorable environmental conditions may proceed with normal development during transport to distant reef sites with conditions appropriate for later stages (e.g. brachiolaria larvae) that may be less resistant to environmental variation (Henderson and Lucas 1971; Habe et al. 1989).

In Moran's seminal review on the *Acanthaster* phenomenon (Moran 1986), he clearly articulated that crown-of-thorns starfish and their coral prey are "intimately

linked and should not be studied in isolation". However, previous studies have mostly considered unidirectional effects of crown-of-thorns starfish on the abundance and composition of prey (mostly, scleractinian) corals (reviewed in Pratchett et al. 2014 – **Chapter 2**). Birkeland and Lucas (1990) have alluded to the effect of the nutritional status of adult starfish on gonad quality, but this is the first study that explicitly tested the effect of food availability and recent feeding history of individual starfish on egg quality and subsequent larval development. Despite the dissociation between the adult and larval nutritional environments of crown-of-thorns starfish, a clear link between the nutritional condition of adults and larval fitness was demonstrated. Starfish fed *ad libitum* with preferred *Acropora* corals produced larger eggs and bigger larvae that progressed to more advanced developmental stages prior to commencement of larval feeding (Caballes et al. 2016 – **Chapter 7**). The effects endogenous maternal provisioning also influenced later stages of development by modulating compensatory morphological plasticity in larvae to improve food capture in response to low food conditions in the plankton (Caballes et al. 2017b – **Chapter 8).**

From an evolutionary perspective, these results imply that the eggs of crown-of-thorns starfish may belong to an intermediate size class characterized by extended facultative feeding periods (McEdward 1997). Larvae from starfish maintained on a diet, which consisted of a significant proportion of *Acropora* corals, were able to develop to advanced bipinnaria and early brachiolaria stages in the absence of particulate food (Caballes et al. 2016 – **Chapter 7**; Caballes et al. 2017b – **Chapter 8**). Although the nutritional condition of gamete sources was not stated, Lucas (1984) was also able to rear starved crown-of-thorns larvae to brachiolaria stage. Crown-of-thorns starfish eggs are relatively bigger in size compared to other tropical asteroids with similar planktotrophic life histories (Table 2 in Caballes et al. 2016 – **Chapter 7**).

Nevertheless, crown-of-thorns starfish larvae are obligately planktotrophic as there is no evidence that it can complete metamorphosis in the absence of exogenous food. (Fabricius et al. 2010; Wolfe et al. 2015a; Pratchett et al. 2017). However, the ability of crown-of-thorns starfish to utilize maternal reserves as buffers against nutritionally poor larval environments, may partly explain higher recruitment rates in this species compared to other echinoderms with high fecundity and planktotrophic developmental mode.

From an ecological perspective, these results show that the reproductive potential of crown-of-thorns starfish is conditional upon their recent feeding history (Caballes et al. 2016 – **Chapter 7**; Caballes et al. 2017b – **Chapter 8**), highlighting important feedbacks that may contribute to coupled oscillations in abundance of crown-of-thorns starfish and their preferred coral prey (mainly, *Acropora* spp.). Because food quality (coral community structure) and quantity (coral abundance) varies widely among reef locations and habitats, local variation in maternal nutritional condition is likely to modulate reproductive success, and hence population size (**Figure 9.1**). It is tempting to speculate that abundant food supply (i.e. high *Acropora* spp. cover) may be an important pre-requisite for initiating outbreaks. There have been four documented major waves of outbreaks in the GBR, commencing in 1962, 1979, 1993, and 2010 (Pratchett et al. 2014 – **Chapter 2**). The period between outbreaks (10-20 years) corresponds with the estimated amount of time it takes for coral populations to recover after being impacted by crown-of thorns starfish outbreaks (Seymour and Bradbury 1999; Lourey et al. 2000, Colgan 1987). Coral spatial-temporal simulation models also show that the maximum frequency of the outbreak waves was controlled by the rate of coral recovery, with slower rates resulting in lower outbreak frequencies (Fabricius et al. 2010). However, these results have been taken to suggest that low coral cover prevents the

establishment of outbreaks because there is no food available for adult starfish. Here, I propose an alternative or supplemental interpretation of the close association of coral and crown-of-thorns starfish population dynamics. Apart from providing food for adult starfish, high abundance of preferred coral species could improve the nutritional condition of reproductively mature starfish, and consequently improve its reproductive potential. As demonstrated by these results, well-fed females produce bigger larvae that develop faster to shorten planktonic larval duration and reduce predation risk (Sinervo and McEdward 1988), or resist starvation, thereby increasing potential for long-range dispersal (Reitzel et al. 2005). Small changes in survival rates as a result of this strategy may result in pronounced increases in recruitment success, leading to population outbreaks. It will also be interesting to monitor the recovery of corals from the current bleaching event and assess whether this will prolong the interval between the next wave of outbreaks in the GBR.

The tolerance of early life history stages and process to a suite of environmental stressors and the plasticity in reproductive behavior and larval morphology add to a growing list of traits (Caballes and Pratchett 2014 – **Chapter 3**; Pratchett et al. 2014 – **Chapter 2**; Babcock et al. 2016b; Wolfe et al. 2017) that predispose crown-of-thorns starfish to primary outbreaks, as well as traits that increase the likelihood of secondary outbreaks. Taken together, these results demonstrate that variable sensitivity of early life history stages and processes to environmental factors can have flow-on effects that disproportionately impact recruitment success and population replenishment in crown-of-thorns starfish (**Figure 9.1**). The cumulative effects of environmental variables on the success of different stages and processes in the life cycle of crown-of-thorns starfish ultimately dictate the available number of larvae that settle and recruit on reefs.

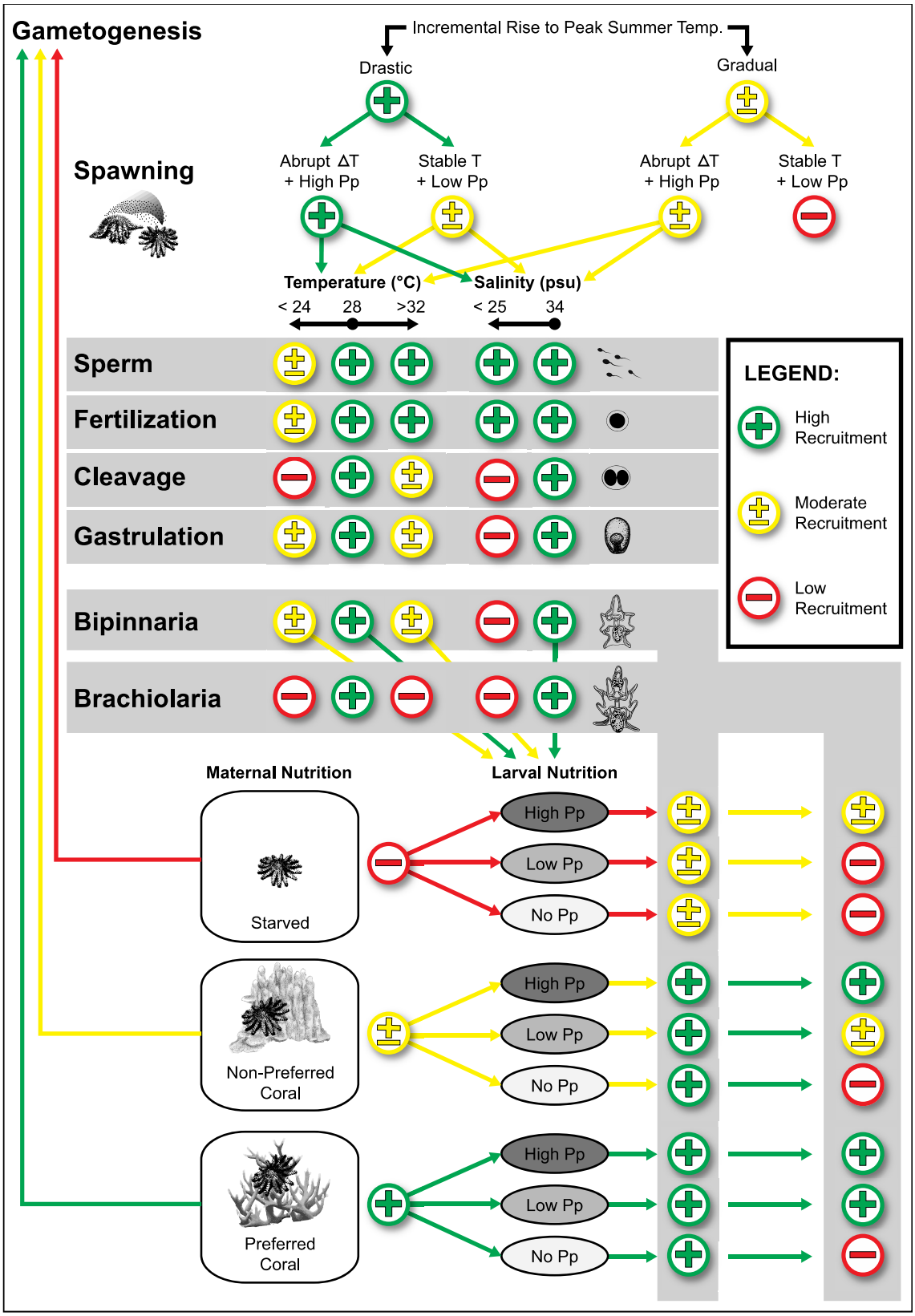

Figure 9.1 Schematic diagram of summarized stage-specific responses to environmental variables, with predicted recruitment rates based on experiments from **Chapter 4** to **Chapter 8**. Data for temperature and salinity effects on bipinnaria and brachiolaria larvae from Pratchett et al. (2014) and Caballes and Pratchett (2014). (△T = change in temperature; Pp = phytoplankton).

9.2 Implications for future research

This body of work and other recent research on crown-of-thorns starfish (e.g., Wooldridge and Brodie 2015; Babcock et al. 2016b; Kamya et al. 2016; Nakajima et al. 2016; Sparks et al. 2016; Allen et al. 2017; Mellin et al. 2017; Pratchett et al. 2017; Wolfe et al. 2017) have significantly improved our understanding of this enigmatic species and how early life history stages respond to environmental variability. There are however lingering questions that need to be addressed in order to fully understand and directly address the proximal causes of primary outbreaks. The logical next step is to determine what factors drive variability in settlement rates and juvenile growth and survival. Further fine scale monitoring and more frequent sampling of crown-of-thorns starfish within the "initiation box" is needed to clearly establish correlations between patterns in gametogenic activity and interannual environmental variability (Pratchett et al. 2014). Finescale variation in sex ratios and the presence of large females may also play a role in triggering gamete relesease in aggregated starfish. Spawning synchronicity is considered to be the most fundamental constraint on the fertilization success of broadcast spawning, gonochoric species (Babcock et al. 1986; Levitan 1995; Olive 1995), therefore investigating the underlying mechanisms of the neurohormonal response in crown-of-thorns starfish to environmental cues may shed light on possible strategies to disrupt spawning synchronicity and limit reproductive success. More studies on whether crown-of-thorns starfish aggregate to spawn and what drives these aggregations are warranted. Recent work by Hall et al. (2017), which identified genes involved in conspecific communication among crown-of-thorns starfish, may help improve biocontrol strategies by disrupting these biochemical "lines of communication" particularly in aggregations during the spawning season. Further studies on the role of phytoplankton in spawning induction are also warranted; in particular, testing different

concentrations to see if spawning response is dose-dependent. Lipid (lipid class) and protein composition of eggs from starfish on different diets or from populations collected from reefs with different levels of *Acropora* cover will also be important to unequivocally support the link between adult nutritional condition and maternal provisioning to the egg. Building upon the results of **Chapter 8** (Caballes et al. 2017b) regarding the potential for larval phenotypic plasticity in crown-of-thorns starfish, the morphological response of larvae to environmental stressors (e.g temperature, salinity, pH) and its potential implications to larval survival needs to be investigated (e.g. Pia et al. 2012).

As mentioned above, the next logical step is to determine the factors that influence settlement and recruitment rates. For example, it is not known whether recruitment is limited by the density of competent larvae or by the availability of suitable microhabitats. This will help us better understand the source-sink dynamics of populations, which is essential in modelling connectivity. Despite substantial advances in crown-of-thorns starfish research over the past three decades, some questions pertaining to early life history stages and larval ecology listed by Moran (1986) in his review of this phenomenon are still partly or entirely unanswered (Pratchett et al. In prep). Although it is now widely recognized that crown-of-thorns starfish is a complex of four different species (Vogler et al. 2008; Haszprunar et al. 2017), the question of whether key demographic traits (e.g., feeding rates, growth rates, fecundity) vary among putative species, which potentially contributes to geographic variation in incidence and severity of outbreaks, needs to be prioritized in future work. Furthermore, field and laboratory experiments need to be designed to address the following important questions: i) Is larval survival more dependent on the diversity rather than density of algal species?; ii) Is there a positive correlation between larval density, recruitment

density, and adult density?; and iii) Where do larvae occur in the water column? Does their position vary throughout their planktonic period? What factors are responsible for determining their position?

9.3 Management implications

Of the many disturbances (e.g. climate induced coral bleaching, increasing prevalence of coral disease, increasing severity of tropical storms) contributing to degradation of coral reef ecosystems in the Indo-Pacific, outbreaks of crown-of-thorns starfish are considered to be the most amenable to direct and immediate intervention (e.g., De'ath et al. 2012). De'ath et al. (2012) argued that preventing or containing crown-of-thorns starfish outbreaks may be the most feasible and effective strategy to reduce and/ or reverse widespread declines in live coral cover, thereby improving the capacity of reef systems to cope with inevitable threats due to sustained and ongoing climate change as well as other more direct anthropogenic disturbances. The principal objective of control efforts should be to reduce or mitigate coral mortality, rather than necessarily eradicate crown-of-thorns starfish (Westcott et al. 2016). Past control efforts (reviewed by Birkeland and Lucas 1990) were successful when i) there was adequate warning of an approaching outbreak; ii) small aggregations were in readily accessible locations (e.g., shallow reef environments); and iii) when response was rapid and controls were undertaken repeatedly and regularly. In the previous sections, I have emphasized the importance of aggregations (even small aggregations) in precipitating outbreaks by improving synchronicity and fertilization rates. Early detection of these aggregations and rapid response, ideally months prior to the breeding season, will be

essential in effectively limiting reproductive success (Bos et al. 2013; Dumas et al. 2016).

The efficiency of control methods has greatly improved since the introduction of the single-shot injection method (Rivera-Posada et al. 2014), such that the government-funded control programs currently kill up to 50,000 crown-of-thorns starfish per month. However, these approaches remain costly and labour intensive, and are unlikely to be feasible on the scale of entire reef systems. It is necessary therefore, to explore the potential of timely and spatially explicit control activities to contain or prevent outbreaks. Hock et al. (2014) proposed a novel method to manage crown-of-thorns starfish outbreaks based on connectivity models that identify the intervention locations with the highest probability of limiting population expansion by selectively targeting local populations most likely to expand their future range. It is important to realize that these models are only as good as the data and assumptions on which they are based and will ultimately depend on accurate estimation of fundamental biological parameters. Key aspects of reproduction and recruitment in crown-of-thorns starfish are necessary to populate connectivity models and improve predictions used to develop targeted control strategies that mitigate coral mortality. Maternal effects data provided here can be integrated into these connectivity models to revise the classification of "source" and "sink" reefs. For example, the probability of a "source" reef reseeding larvae may diminish over time as the cover of preferred coral species in that reef is progressively depleted.

Although past and ongoing control programs have no doubt mitigated coral mortality, these efforts are directed towards the vehicle of the problem and not the root cause. Long-term and permanent solutions need to address the ultimate causes of outbreaks. Support for the hypotheses seeking to explain the initiation of outbreaks

remains equivocal and this debate will certainly continue. Subscribing to a single hypothesis may be oversimplifying the problem and management strategies that target a single potential cause may risk neglecting other factors that operate simultaneously (Babcock et al. 2016a). As demonstrated here, the *Acanthaster* phenomenon involves multiple factors involving different stages in the life cycle of crown-of-thorns starfish. Inherent complexity in the population dynamics of crown-of-thorns starfish warrants careful exploration using stage-based demographic models and spatially explicit population models (Morello et al. 2014), but these models will further necessitate increased research into the demography and behavior of crown-of-thorns starfish, especially during non-outbreak and pre-outbreak periods. Emerging threats posed by climate change (Hughes et al. 2017) provide a renewed imperative to mitigate all sources of coral mortality (including crown-of-thorns starfish outbreaks) and reverse the ongoing degradation of coral reef ecosystems, in order to maximize adaptive capacity and resilience.

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APPENDIX A

Chapter 1 – Overarching Map of Sampling Locations

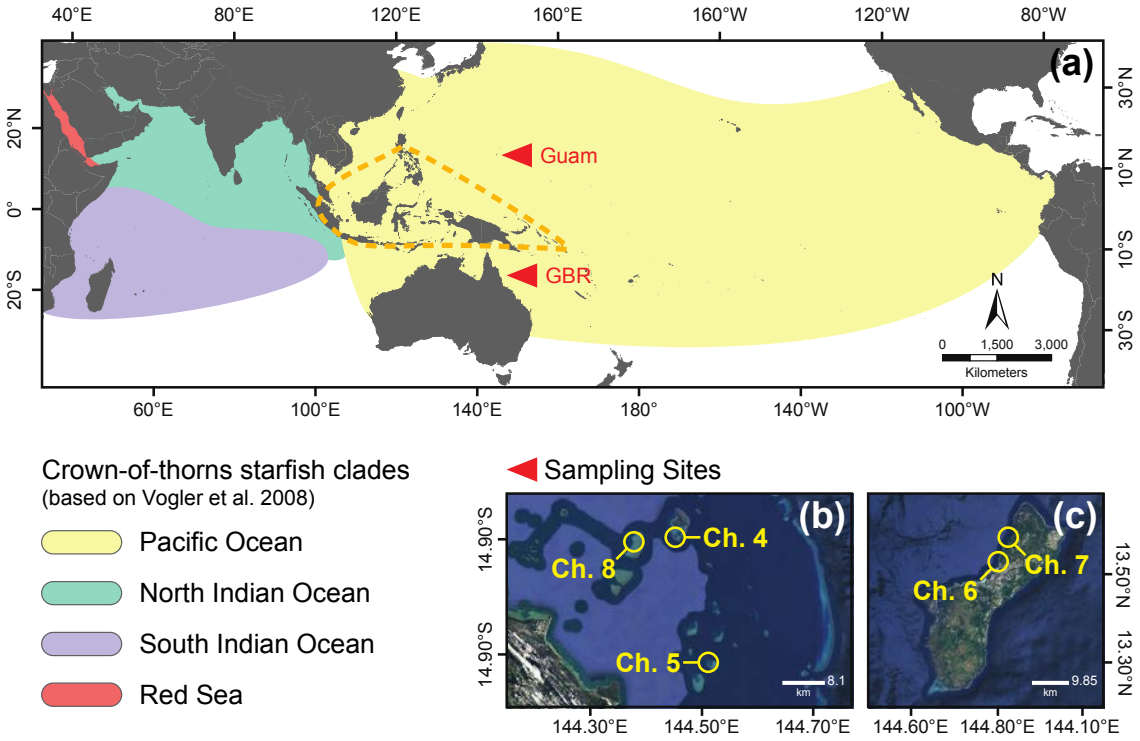

Figure A1. Map of sampling sites: **(a)** geographic distribution of putative species* of crown-of-thorns starfish (*Acanthaster* spp.) based on clades identified by Vogler et al. (2008) (dashed orange line represents rough delineation of the Coral Triangle); **(b)** sampling sites in northern Great Barrier Reef, Australia for **Chapter 4** (between Palfrey and South Reefs within the Lizard Island lagoon), **Chapter 5** (Unnamed Reef 14-133), and **Chapter 8** (Eyrie Reef); **(c)** sampling sites in northwest Guam, Micronesia for **Chapter 6** (Puntan dos Amantes) and **Chapter 7** (Ague Point). * Note: All crown-of-thorns starfish were collected within the known range of the Pacific 'species', *Acanthaster* cf. *solaris*, as suggested by Haszprunar and Spies (2014) and Haszprunar et al. (2017).

APPENDIX B
Chapter 4 – Supplementary Information

Table B1. Mean diameter (± SE) and mean wet weight (± SE) of crown-of-thorns starfish samples collected in the GBR; values in parentheses are sample sizes (N) for each reef. Numbers (No.) shown here correspond to reef numbers in Figure 1.

No.	Reef	Code	Diameter (mm)	Weight (g)	Date Collected
	Lizard Island Section				
1	Lizard Island*	14-116	273 ± 2 (872)	708 ± 16 (644)	Sep-Dec 2013; Jan-Apr, Aug-Dec 2014; Jan-May 2015
2	MacGillivray Reef*	14-114	276 ± 4 (336)	774 ± 28 (305)	Oct 2013
3	Eagle Island Reef*	14-188	358 ± 12 (19)	1772 ± 152 (19)	Nov 2015
4	N. Direction Island*	14-143	298 ± 4 (305)	900 ± 32 (294)	Oct 2013
5	Martin Reef*	14-123	354 ± 17 (19)	1876 ± 226 (19)	Nov 2015
6	Ribbon Reef No.10*	14-146	36 ± 2 (20)		Nov 2014
7	S. Direction Island*	14-147	301 ± 5 (259)	934 ± 33 (244)	Oct 2013
8	Unnamed Reef 14-133*	14-133	286 ± 19 (13)	1088 ± 203 (13)	Nov 2015
9	Two Islands Reef*	15-002	329 ± 7 (20)		Mar 2014
10	Mackay Reefs*	15-024	298 ± 16 (20)		Mar 2014
11	Forrester Reef*	15-009	332 ± 11 (20)		Mar 2014
12	Startle Reef*	15-028	280 ± 10 (30)		Nov 2012; Mar 2014
13	Lark Reef*	15-033	316 ± 11 (20)		Mar 2014
	Cooktown Section				
14	Boulder Reef*	15-012	330 ± 14 (20)		Nov 2014
15	Unnamed Reef 15-044*	15-044	331 ± 13 (20)		Nov 2014
16	Unnamed Reef 15-072*	15-072	35 ± 2 (20)		Nov 2014
17	Emily Reef*	15-082	250 ± 3 (348)	635 ± 18 (348)	Feb 2014
18	Irene Reef*	15-084	304 ± 15 (10)		Jan 2013
19	Endeavour Reef*	15-089	274 ± 3 (350)	741 ± 21 (340)	Nov 2012; Feb 2014
20	Pickersgill Reef*	15-093	277 ± 3 (322)	771 ± 19 (321)	Feb 2014
21	Morning Reef*	15-098	217 ± 24 (10)		Oct 2012
22	Spitfire Reef*	16-012A	294 ± 3 (300)		Sep 2013
23	Unnamed Reef 16-018A*	16-018A	166 ± 26 (10)		Nov 2012
24	Undine Reef B*	16-020B	317 ± 10 (30)		Mar, Sep 2014
25	Rudder Reef*	16-023	379 ± 23 (10)		Sep 2014
26	Chinaman Reef*	16-024	317 ± 8 (20)		Mar 2014
27	Opal Reef*	16-025	27 ± 5 (3)		Oct 2014
28	Tongue Reef*	16-026	182 ± 35 (16)		Oct, Nov 2014
	Cairns Section				
29	Low Isles Reef*	16-028	314 ± 14 (9)		Oct 2014
30	Batt Reef*	16-029	283 ± 8 (30)		Mar, Oct 2014
31	Norman Reef*	16-030	32 ± 2 (16)		Nov 2014
32	Michaelmas Reef*	16-060	319 ± 37 (5)	1343 ± 396 (5)	Sep 2014

33	Vlasoff Reef*	16-044B	301 ± 19 (21)		Mar, Sep 2014
34	Arlington Reef*	16-064	309 ± 2 (401)	1037 ± 21 (334)	Aug 2013; Mar, Sep, Oct 2014
35	Green Island*	16-049	177 ± 17 (29)		Mar, Oct 2014
36	Thetford Reef	16-068	324 ± 18 (10)		Sep 2014
37	Moore Reef	16-071	228 ± 22 (10)		Dec 2014
38	Elford Reef	16-073	329 ± 6 (99)	1292 ± 57 (89)	Sep 2014; Mar 2015
39	Briggs Reef	16-074	96 ± 20 (28)		Oct 2014; Mar 2015
40	Sudbury Reef	17-001A	349 ± 26 (10)		Mar 2015
41	Stevens Reef	17-005	330 ± 20 (3)		Nov 2015
42	Maori Reef	17-006	318 ± 9 (37)		Nov 2015
43	Coates Reef	17-011	273 ± 57 (3)		Nov 2015
44	Hedley Reef	17-014	279 ± 3 (331)	886 ± 30 (328)	Sep 2014; Nov 2015
45	McCulloch Reef	17-016	263 ± 4 (312)	786 ± 30 (308)	Sep 2014; Nov 2015
46	Noreaster Reef	17-062	364 ± 15 (27)		Nov 2015
47	Taylor Reef	17-064	283 ± 36 (7)		Oct, Nov 2015
	Townsville Section				
48	Otter Reef	18-018	213 ± 18 (3)		Oct 2015
49	Trunk Reef	18-027	263 ± 11 (42)	871 ± 90 (42)	Nov 2014
50	Bramble Reef	18-029	244 ± 7 (75)	615 ± 48 (75)	Nov 2014
51	Rib Reef	18-032	252 ± 10 (43)	560 ± 65 (36)	Nov 2014; Oct 2015
52	Centipede Reef	18-088	380 ± 50 (4)	2488 ± 868 (4)	Nov 2014
53	Davies Reef	18-096	335 ± 63 (3)	1752 ± 646 (3)	Nov 2014
	Swains Section				
54	Sweetlip Reef	22-140	366 ± 20 (6)	1759 ± 369 (6)	May 2015
55	Dicks Reef	22-141	375 ± 5 (94)	1577 ± 52 (94)	May 2015

* Reefs within the "outbreak initiation zone" as defined by AIMS and shown in **Figure 4.1**

APPENDIX C
Chapter 5 – Supplementary Information

Table C1 Odds ratios and confidence intervals of pairwise comparisons between treatments for each spawning experiment. FSW = 0.2-□m filtered seawater; LS-FSW = low salinity filtered seawater; NE-FSW = nutrient-enriched filtered seawater; PP = combination of three phytoplankton species.

Source	Odds Ratio	95% CI	*P*
(a) Temperature			
Male			
28°C vs 28°C→30°C	3.667	(0.118 – 113.736)	1.000
28°C vs 26°C→30°C	121.000	(2.017 – 7259.723)	**0.008**
28°C→30°C vs 26°C→30°C	33.000	(1.064 – 1023.620)	**0.048**
Female			
28°C vs 28°C→30°C	1.000	(0.017 – 59.998)	1.000
28°C vs 26°C→30°C	3.667	(0.118 – 113.736)	1.000
28°C→30°C vs 26°C→30°C	3.667	(0.118 – 113.736)	1.000
(b) Water Quality			
Male			
FSW vs LS-FSW	1.923	(0.197 – 18.812)	1.000
FSW vs NE-FSW	1.000	(0.084 – 11.932)	1.000
LS-FSW vs NE-FSW	1.923	(0.197 – 18.812)	1.000
Female			
FSW vs LS-FSW	1.000	(0.018 – 56.466)	1.000
FSW vs NE-FSW	1.000	(0.018 – 56.466)	1.000
LS-FSW vs NE-FSW	1.000	(0.018 – 56.466)	1.000
(c) Phytoplankton			
Male			
Control vs *Dunaliella*	1.000	(0.084 – 11.932)	1.000
Control vs *Skeletonema*	7.857	(0.865 – 71.385)	0.119
Control vs *Chaetoceros*	1.923	(0.197 – 18.812)	1.000
Dunaliella vs *Skeletonema*	7.857	(0.865 – 71.385)	0.119
Dunaliella vs *Chaetoceros*	1.923	(0.197 – 18.812)	1.000
Skeletonema vs *Chaetoceros*	4.086	(0.564 – 29.617)	0.315
Female			
Control vs *Dunaliella*	1.000	(0.018 – 56.466)	1.000
Control vs *Skeletonema*	1.000	(0.018 – 56.466)	1.000
Control vs *Chaetoceros*	1.000	(0.018 – 56.466)	1.000
*Dunaliella*l vs *Skeletonema*	1.000	(0.018 – 56.466)	1.000
Dunaliella vs *Chaetoceros*	1.000	(0.018 – 56.466)	1.000
Skeletonema vs *Chaetoceros*	1.000	(0.018 – 56.466)	1.000
(d) Gamete			

Male			
Control vs Sperm	44.200	(1.795 – 1088.207)	**0.007**
Control vs Egg	3.400	(0.120 – 96.706)	1.000
Sperm vs Egg	13.000	(1.329 – 127.168)	**0.041**
Female			
Control vs Sperm	10.818	(0.463 – 252.804)	0.200
Control vs Egg	3.400	(0.120 – 96.706)	1.000
Sperm vs Egg	3.182	(0.350 – 28.908)	0.569
(e) Sperm and PP			
Male			
Control vs Sperm	26.714	(1.143 – 624.270)	**0.026**
Control vs Sperm + PP	26.714	(1.143 – 624.270)	**0.026**
Sperm vs Sperm + PP	1.000	(0.150 – 6.655)	1.000
Female			
Control vs Sperm	10.818	(0.463 – 252.804)	0.200
Control vs Sperm + PP	6.538	(0.266 – 160.977)	0.467
Sperm vs Sperm + PP	1.655	(0.228 – 11.994)	1.000

* Fishers Exact Test p-value of pairwise comparisons

APPENDIX D
Chapter 6 – Supplementary Information

Table D1. Results of statistical analyses on the effects of temperature, salinity, and pH on sperm behavior, fertilization, and early development.

Source	*df*	Statistic (F, χ^2)	*P*
Temperature			
Sperm Speed[1]			
temperature	8	85.96	**< 0.0001**
egg extract	1	13.16	**0.0005**
temperature × egg extract	8	0.76	0.6353
Sperm Motility[2]			
temperature	8	1233.07	**< 0.0001**
egg extract	1	31.34	**0.0008**
temperature × egg extract	8	32.79	0.1612
Fertilization[2]	8	1316.20	**< 0.0001**
Cleavage[2]	7	521.09	**< 0.0001**
Gastrulation[2]	7	632.82	**< 0.0001**
Salinity			
Sperm Speed[1]			
salinity	7	5.83	**< 0.0001**
egg extract	1	2.93	**0.0918**
salinity × egg extract	7	0.31	0.9449
Sperm Motility[2]			
salinity	7	525.43	**< 0.0001**
egg extract	1	16.42	**0.0682**
salinity × egg extract	7	9.62	0.9626
Fertilization[2]	7	597.86	**< 0.0001**
Cleavage[2]	5	369.59	**< 0.0001**
Gastrulation[2]	5	504.40	**< 0.0001**
pH			
Sperm Speed[1]			
pH	4	28.57	**< 0.0001**
egg extract	1	3.85	**0.0568**
pH × egg extract	4	0.74	0.5706
Sperm Motility[2]			
pH	4	669.24	**< 0.0001**
egg extract	1	38.11	**0.0033**
pH × egg extract	4	18.05	0.3943
Fertilization[2]	4	234.28	**< 0.0001**
Cleavage[2]	4	95.37	**< 0.0001**
Gastrulation[2]	4	213.24	**< 0.0001**

[1] Two-way Analysis of Variance (ANOVA): *F* value
[2] Analysis of Deviance for generalized linear models (GLM): χ^2 value

APPENDIX E
Chapter 8

Table E1. Results of mixed model hierarchical ANOVA for diameter and volume of oocytes from female starfish under four maternal diet treatments: **Acr** = *Acropora*, **Mix** = mixed diet, **Por** = *Porites*, **Stv** = starved.

Source	*df*	*F*	*P*	ECV[1]	Post Hoc
Oocyte Diameter					
Maternal Nutrition	3	34.88	<0.0001	60%	Acr = Mix > Por = Stv
Female (Maternal Nutrition)	8	14.82	<0.0001	5%	
Error	1188			36%	
Oocyte Volume					
Maternal Nutrition	3	40.17	<0.0001	63%	Acr = Mix > Por = Stv
Female (Maternal Nutrition)	8	15.15	<0.0001	5%	
Error	1188			32%	

[1] ECV = estimates of components of variation

Table E2. Analysis of deviance for binomial generalized linear models (GLMs) testing the effects of maternal nutrition and larval feeding treatments on the proportion of normally developing larvae and larvae at brachiolaria stage after 8 days; and normally developing and larvae at mid-to-late brachiolaria stage after 16 days. Maternal Diet: **Acr** = *Acropora*, **Mix** = mixed diet, **Por** = *Porites*, **Stv** = starved; Algal Food Concentration (cells ml^{-1}): **Hi** = 10^4, **Lo** = 10^3, **No** = 0.

Source	*df*	χ^2	*P*	Post Hoc
Day 8				
% Normal				
Maternal Nutrition	3	57.82	0.0001	Acr = Mix = Por > Stv
Larval Nutrition	2	4.63	0.4242	
Maternal Nutrition x Larval Nutrition	6	6.38	0.8834	
% Brachiolaria				
Maternal Nutrition	3	201.75	<0.0001	Acr = Mix > Por > Stv
Larval Nutrition	2	137.35	<0.0001	Hi = Lo > No
Day 16				
% Normal				
Maternal Nutrition	3	69.38	<0.0001	Acr = Mix > Por > Stv
Larval Nutrition	2	172.19	<0.0001	Hi = Lo > No
Maternal Nutrition x Larval Nutrition	6	6.66	0.6557	
% Mid-Late Brachiolaria				
Maternal Nutrition	3	133.90	<0.0001	Acr = Mix > Por > Stv
Larval Nutrition	1	1.14	0.5853	Hi = Lo; No = 0

Table E3. Results of two-way ANOVA testing the main and interactive effects of maternal nutrition and larval feeding treatments on different morphometric measurements taken 4 days after the onset of larval feeding. Maternal Diet: **Acr** = *Acropora*, **Mix** = mixed diet, **Por** = *Porites*, **Stv** = starved; Algal Food Concentration (cells ml^{-1}): **Hi** = 10^4, **Lo** = 10^3, **No** = 0.

Source	*df*	*F*	*P*	Post Hoc
Maximum Length (ML)				
Maternal Nutrition	3	212.72	<0.0001	Acr>Mix>Por>Stv
Larval Nutrition	2	0.45	0.6415	
Maternal x Larval Nutrition	6	0.62	0.7116	
Maximum Width (MW)				
Maternal Nutrition	3	211.44	<0.0001	Acr=Mix>Por>Stv
Larval Nutrition	2	0.07	0.9333	
Maternal x Larval Nutrition	6	0.68	0.6686	
Posterior Width (PW)				
Maternal Nutrition	3	267.41	<0.0001	Mix>Acr>Por>Stv
Larval Nutrition	2	0.36	0.6978	
Maternal x Larval Nutrition	6	0.89	0.5089	
Ciliated Band Length (CBL)				
Maternal Nutrition	3	93.62	<0.0001	Mix=Acr>Por>Stv
Larval Nutrition	2	0.46	0.6327	
Maternal x Larval Nutrition	6	0.87	0.5254	
CBL : ML				
Maternal Nutrition	3	50.76	<0.0001	Mix>Acr=Por>Stv
Larval Nutrition	2	0.32	0.7299	
Maternal x Larval Nutrition	6	0.92	0.4866	
CBL : MW				
Maternal Nutrition	3	26.95	<0.0001	Mix>Acr>Por=Stv
Larval Nutrition	2	1.40	0.2544	
Maternal x Larval Nutrition	6	1.12	0.3600	
Gut Area				
Maternal Nutrition	3	43.66	<0.0001	Mix>Acr>Por>Stv
Larval Nutrition	2	0.36	0.6993	
Maternal x Larval Nutrition	6	1.00	0.4319	

Table E4. Results of two-way ANOVA testing the main and interactive effects of maternal nutrition (**Acr** = *Acropora*, **Mix** = mixed diet, **Por** = *Porites*, **Stv** = starved) and larval feeding (**Hi** = 10^4, **Lo** = 10^3, **No** = 0 cells ml^{-1}) treatments on different morphometric measurements taken at day 10 after onset of larval feeding ability.

Source	*df*	*F*	*P*	Post Hoc
Maximum Length (ML)				
Maternal Nutrition	3	125.45	<0.0001	Hi: Acr = Mix > Por > Stv; Lo: Mix = Acr > Por > Stv; No: Acr = Mix > Por = Stv
Larval Nutrition	2	184.04	<0.0001	Acr: Hi = Lo > No; Mix: Hi = Lo > No; Por: Hi > Lo > No; Stv: Hi = Lo > No
Maternal x Larval Nutrition	6	7.80	<0.0001	
Maximum Width (MW)				
Maternal Nutrition	3	164.86	<0.0001	Hi: Mix > Acr = Por > Stv; Lo: Mix > Acr > Por > Stv; No: Mix > Acr =Por > Stv
Larval Nutrition	2	127.38	<0.0001	Acr: Hi = Lo > No; Mix: Hi = Lo > No; Por: Hi > Lo > No; Stv: Hi = Lo > No
Maternal x Larval Nutrition	6	3.80	0.0028	
Posterior Width (PW)				
Maternal Nutrition	3	62.92	<0.0001	Hi: Acr =Mix > Por = Stv; Lo: Mix > Acr = Por > Stv; No: Mix > (Acr = Stv, Acr > Por, Stv = Por)
Larval Nutrition	2	60.62	<0.0001	Acr: Hi > Lo > No; Mix: Lo = Hi >No; Por: Lo = Hi > No; Stv: Hi = Lo, Hi > No, Lo = No
Maternal x Larval Nutrition	6	4.31	0.0011	
Ciliated Band Length (CBL)				
Maternal Nutrition	3	235.85	<0.0001	Hi: Mix = Acr > Por > Stv; Lo: Mix = Acr > Por > Stv; No: Mix > Acr > Por > Stv
Larval Nutrition	2	133.23	<0.0001	Acr: Lo > Hi > No; Mix: Lo > Hi >No; Por: Lo = Hi > No; Stv: Lo > Hi >No
Maternal x Larval Nutrition	6	3.53	0.0046	
CBL : ML				
Maternal Nutrition	3	224.28	<0.0001	Hi: Mix = Acr > Por > Stv; Lo: Mix = Acr > Por > Stv; No: Mix > Acr > Por > Stv
Larval Nutrition	2	106.62	<0.0001	Acr: Lo > Hi > No; Mix: Lo > Hi = No; Por: Lo > Hi = No; Stv: Lo > Hi > No
Maternal x Larval Nutrition	6	2.86	0.0162	
CBL : MW				
Maternal Nutrition	3	175.38	<0.0001	Hi: Acr = Mix > Por > Stv; Lo: Acr = Mix > Por > Stv; No: Mix = Acr > Por > Stv
Larval Nutrition	2	114.34	<0.0001	Acr: Lo > Hi > No; Mix: Lo > Hi =No; Por: Lo > No =Hi; Stv: Lo > Hi > No
Maternal x Larval Nutrition	6	6.54	<0.0001	
Gut Area				
Maternal Nutrition	3	62.51	<0.0001	Hi: Acr = Mix > Stv, Acr > Por = Stv, Mix = Por;

Larval Nutrition	2	425.75	<0.0001	Lo: Acr = Mix > Por > Stv; No: Mix = Acr = Por > Stv Acr: Lo > Hi > No; Mix: Lo > Hi > No; Por: Lo > Hi > No; Stv: Lo > Hi > No
Maternal x Larval Nutrition	6	4.57	0.0007	

Table E5. Results of permutational multivariate ANOVA (PERMANOVA) testing the main and interactive effects of maternal diet and larval feeding treatments on larval morphology. Maternal Diet: **Acr** = *Acropora*, **Mix** = mixed diet, **Por** = *Porites*, **Stv** = starved; Algal Food Concentration (cells ml^{-1}): **Hi** = 10^4, **Lo** = 10^3, **No** = 0.

Source	*df*	pseudo-*F*	*P*(perm)	ECV[1]	Post Hoc
Day 4					
Maternal Nutrition	3	123.91	<0.0001	66.2%	Acr ≠ Mix ≠ Por ≠ Stv
Larval Nutrition	2	0.37	0.8525	4.1%	
Maternal x Larval Nutrition	6	0.82	0.6503	4.4%	
Residual	60			25.3%	
Day 10					
Maternal Nutrition	3	141.63	<0.0001	37.5%	Hi: Acr = Mix ≠ Por ≠ Stv Lo: Acr ≠ Mix ≠ Por ≠ Stv No: Acr ≠ Mix ≠ Por ≠ Stv
Larval Nutrition	2	175.23	<0.0001	38.9%	Acr: Hi ≠ Lo ≠ No Mix: Hi ≠ Lo ≠ No Por: Hi ≠ Lo ≠ No Stv: Hi ≠ Lo ≠ No
Maternal x Larval Nutrition	6	3.92	<0.0001	9.7%	
Residual	60			13.9%	

[1] ECV = estimates of components of variation.

APPENDIX F
Publications arising from this thesis

2017 **Caballes CF**, Pratchett MS. Environmental and biological cues for spawning in the crown-of-thorns starfish, *Acanthaster* spp. *PLoS One* 12: e0173964.

2017 **Caballes CF**, Pratchett MS, Buck ACE. Interactive effects of endogenous and exogenous nutrition on larval development for crown-of thorns starfish. *Diversity* 9: 15.

2017 **Caballes CF**, Pratchett MS, Raymundo ML, Rivera-Posada JA. Environmental tipping points for sperm motility, fertilisation, and early development in the crown-of-thorns starfish. *Diversity* 9: 10.

2016 **Caballes CF**, Pratchett MS, Kerr AM, Rivera-Posada JA. The role of maternal nutrition on oocyte size and quality, with respect to early larval development in the coral-eating starfish, *Acanthaster planci*. *PLoS One* 11: e0158007.

2014 **Caballes CF**, Pratchett MS. Reproductive biology and early life history of the crown-of-thorns starfish, *Acanthaster planci*. In: Whitmore E (ed) *Echinoderms: Ecology, Habitats, and Reproductive Biology*, pp. 102-146. Nova Publishers, New York, USA.

2014 Pratchett MS, **Caballes CF**, Rivera-Posada JA, Sweatman HPA. Limits to understanding and managing outbreaks of crown-of-thorns starfish (*Acanthaster* spp.). *Oceanography and Marine Biology: An Annual Review* 52: 133-200.

APPENDIX G
Other publications during candidature
(front matter of publications embedded in subsequent pages)

2017 Pratchett MS, Dworjanyn SA, Mos B, **Caballes CF**, Thompson CA, Blowes S. Larval survivorship and settlement of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) at varying chlorophyll concentrations. *Diversity* 9(1): 2.

2017 Cowan ZL, Ling SD, Dworjanyn SA, **Caballes CF**, Pratchett MS. Interspecific variation in potential importance of planktivorous damselfishes as predators of *Acanthaster* sp. eggs. *Coral Reefs* 36: 653–661.

2016 Tusso S, Morcinek K, Vogler C, Schupp PJ, **Caballes CF**, Vargas S, Wörheide G. Genetic structure of the crown-of-thorns seastar in the Pacific Ocean, with focus on Guam. *PeerJ* 4: e1970.

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

2016 Cowan ZL, Dworjanyn SA, **Caballes CF**, Pratchett MS. Predation on crown-of-thorns starfish larvae. *Coral Reefs* 35: 1253–1262.

2014 Rivera-Posada JA, **Caballes CF**, Pratchett MS. Size-related variation in arm damage frequency in the crown-of-thorns sea star, *Acanthaster planci*. *Journal of Coastal Life Medicine* 2(3): 187–195.

2014 Rivera-Posada JA, Pratchett MS, Aguilar C, Grand A, **Caballes CF**. 2014. Bile and its application as novel method for controlling *Acanthaster planci* outbreaks on the Great Barrier Reef. *Ocean & Coastal Management* 102: 383–390.

2013 Rivera-Posada JA, **Caballes CF**, Pratchett MS. Lethal doses of oxbile, peptones and thiosulfate-citrate-bile-sucrose agar (TCBS) for *Acanthaster planci*; exploring alternative population control options. *Marine Pollution Bulletin* 75: 133–139.

diversity

MDPI

Communication

Larval Survivorship and Settlement of Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*) at Varying Algal Cell Densities

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Abstract: The dispersal potential of crown-of-thorns starfish (CoTS) larvae is important in understanding both the initiation and spread of population outbreaks, and is fundamentally dependent upon how long larvae can persist while still retaining the capacity to settle. This study quantified variation in larval survivorship and settlement rates for CoTS maintained at three different densities of a single-celled flagellate phytoplankton, *Proteomonas sulcata* (1×10^3, 1×10^4, and 1×10^5 cells/mL). Based on the *larval starvation hypothesis*, we expected that low to moderate levels of phytoplankton prey would significantly constrain both survival and settlement. CoTS larvae were successfully maintained for up to 50 days post-fertilization, but larval survival differed significantly between treatments. Survival was greatest at intermediate food levels (1×10^4 cells/mL), and lowest at high (1×10^5 cells/mL) food levels. Rates of settlement were also highest at intermediate food levels and peaked at 22 days post-fertilization. Peak settlement was delayed at low food levels, probably reflective of delayed development, but there was no evidence of accelerated development at high chlorophyll concentrations. CoTS larvae were recorded to settle 17–43 days post-fertilization, but under optimum conditions with intermediate algal cell densities, peak settlement occurred at 22 days post-fertilization. Natural fluctuations in nutrient concentrations and food availability may affect the number of CoTS that effectively settle, but seem unlikely to influence dispersal dynamics.

Keywords: *Acanthaster*; coral reefs; food limitation; larval competency; planktonic larval duration (PLD)

1. Introduction

Sessile and benthic marine invertebrates are fundamentally dependent on the larval phase of their lifecycle for dispersal away from natal reefs, which is important for enabling colonization of new habitats, recolonization following population depletion, and genetic exchange among sub-populations [1,2]. Despite the short larval duration of most marine organisms (days to months), larvae may be dispersed over great distances [2]. Importantly, ecologically and evolutionarily significant levels of genetic exchange occur over very large (even oceanic) scales (e.g., [3]). There is however, evidence for some species that most of the larvae (up to 60%) settling on a given reef are of local origin [4], implying that most larvae may not even travel beyond the confines of a single reef. Ultimately, there may be two distinct modes (short versus long, retention versus dispersal,

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REPORT

Interspecific variation in potential importance of planktivorous damselfishes as predators of *Acanthaster* sp. eggs

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Received: 13 September 2016 / Accepted: 3 February 2017

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 (≤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.

Keywords *Acanthaster* · Chemical defence · Coral reefs · Predation · Saponins

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Genetic structure of the crown-of-thorns seastar in the Pacific Ocean, with focus on Guam

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ABSTRACT

Population outbreaks of the corallivorous crown-of-thorns seastar (COTS), *Acanthaster 'planci' L.*, are among the most important biological disturbances of tropical coral reefs. Over the past 50 years, several devastating outbreaks have been documented around Guam, an island in the western Pacific Ocean. Previous analyses have shown that in the Pacific Ocean, COTS larval dispersal may be geographically restricted to certain regions. Here, we assess the genetic structure of Pacific COTS populations and compared samples from around Guam with a number of distant localities in the Pacific Ocean, and focused on determining the degree of genetic structure among populations previously considered to be isolated. Using microsatellites, we document substantial genetic structure between 14 localities from different geographical regions in the Pacific Ocean. Populations from the 14 locations sampled were found to be structured in three significantly differentiated groups: (1) all locations immediately around Guam, as well as Kingman Reef and Swains Island; (2) Japan, Philippines, GBR and Vanuatu; and (3) Johnston Atoll, which was significantly different from all other localities. The lack of genetic differentiation between Guam and extremely distant populations from Kingman Reef and Swains Island suggests potential long-distance dispersal of COTS in the Pacific.

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Additional Information and Declarations can be found on page 16

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Subjects Biogeography, Evolutionary Studies, Marine Biology
Keywords *Acanthaster 'planci'*, Microsatellites, Pacific, Genetic structure, Crown-of-thorns seastar, COTS

INTRODUCTION

The crown-of-thorns seastar (COTS), *Acanthaster 'planci,'* is a specialised coral predator and one of the most important biological threats to coral reefs throughout the Indo-Pacific (*Pratchett et al., 2014*). It has a complicated taxonomic history; although initially considered a single widespread Indo-Pacific species (reviewed in *Haszprunar & Spies, 2014*), recent

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diversity

MDPI

Article

Benthic Predators Influence Microhabitat Preferences and Settlement Success of Crown-of-Thorns Starfish (*Acanthaster* cf. *solaris*)

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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.

Keywords: behaviour; coral reefs; predation; resilience

1. 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 [1]. Reef-based predators include both planktivorous fishes and sessile invertebrates (e.g., corals) that intercept larvae as they swim towards benthic habitats [2,3], as well as infaunal invertebrate predators that will feed on starfish that settle to specific microhabitats [4]. Both pre- and post-settlement mortality play important roles in structuring populations of marine organisms (e.g., [5]), but predation rates are generally highest ($\geq$30% day^{-1}) immediately after settlement (reviewed by Gosselin and Qian [6]). 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., [7,8]). Moreover, there will be strong selection for settling larvae to choose microhabitats that minimise predation risk [9], either by avoiding habitats with high abundance of potential predators or preferentially settling in complex microhabitats that provide greater refuge from predators.

Coral Reefs
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REPORT

Predation on crown-of-thorns starfish larvae by damselfishes

Zara-Louise Cowan[1] · Symon A. Dworjanyn[2] · Ciemon F. Caballes[1] · Morgan S. Pratchett[1]

Received: 12 April 2016 / Accepted: 4 August 2016

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 damselfish, testing: (1) the relationship between predator size and predation rate, both within and among fish species; (2) consumption rates on larvae of *Acanthaster* sp. versus larvae of a common, co-occurring coral reef asteroid *Linckia laevigata*; (3) maximal feeding rates upon both *Acanthaster* sp. and *L. laevigata*; and (4) functional responses of planktivorous fishes to increasing densities of *Acanthaster* sp. Consumption rates of crown-of-thorns larvae by damselfishes were 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.

Keywords Predation · Functional response · Chemical defence · *Acanthaster* · Larvae · Damselfish

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Size–related variation in arm damage frequency in the crown–of–thorns sea star, *Acanthaster planci*

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PEER REVIEW

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Comments

This is an important line of study that significantly adds to a long line of COTS focused research. It succeeds in answering several key questions concerning the natural predator control of COTS in the wild. Additionally it provides insight into predatory mechanisms not observed previously. Both lines of inquiry clearly answer previous questions and point to logical next lines of questioning (a hallmark of quality scientific inquiry) and practical solutions to the bigger questions their work addresses.

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ABSTRACT

Objective: To examine variation in the frequency of arm damage in different sizes of *Acanthaster planci* (*A. planci*), assess how this damage is inflicted by fish predators, and infer the potential role of predation in population regulation.

Methods: Diameters of *A. planci* collected from three sites in the Philippines were measured and arm damage frequency and severity was assessed. Frequency of arm damage was compared between sizes. Feeding behavior of fish predators was also observed in the laboratory.

Results: This study demonstrates that sublethal predation by triggerfishes on *A. planci* result in extensive arm damage. Overall, 60% of *A. planci* sampled across all sites had sublethal injuries. The frequency of individuals with missing or regenerating arms was highest in medium–sized young adults (11–20 cm), which coincides with the phase where *A. planci* shift from cryptic to exposed daytime feeding.

Conclusions: The high incidence of arm damage within intermediate–sized sea stars indicates that predators exercise some level of regulation on *A. planci* populations at a local scale. Identification and protection of putative predators that target the most vulnerable life history stages of *A. planci* are essential in developing population control strategies and reverse sustained declines in coral cover.

KEYWORDS

Acanthaster planci outbreaks, Sublethal predation, Arm damage, Population regulation

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Bile salts and the single-shot lethal injection method for killing crown-of-thorns sea stars (*Acanthaster planci*)

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ABSTRACT

Given the threat posed by population outbreaks of *Acanthaster planci* to coral reefs throughout the Indo-Pacific, significant investment is being made to reduce the number of sea stars and their effects on coral assemblages, both through ongoing direct control programs and indirectly, through targeted improvements in water quality and fisheries management. In Australia, bile salts have recently replaced sodium bisulfate as the chemical used to inject, and thereby quickly and efficiently kill, individual sea stars. This study reports on results of experimental studies conducted prior operationalizing bile salts for widespread use on Australia's Great Barrier Reef, both to optimize doses of bile salts and further examine potential side-effects of administering low doses of bile salts into individual sea stars when found at high concentrations. This study showed that injecting *A. planci* with 10 mL of 8 g l^{-1} Bile Salts No. 3 or 12 g l^{-1} of Oxgall solution into the base of the arm with a new gun adapted with a 16 Gauge x1/2" needle is the most rapid and effective way to kill individual *A. planci*, which were up to 42 cm in diameter. No immediate flow-on effects on reef fish, corals, and other benthic invertebrates were observed in laboratory experiments and field surveys. Efficient control measures using bile derivatives can offer immediate relief from ongoing COTS predation, and when done in conjunction with improved land use practices that reduce nutrient input and establishment of protected areas to protect predator species, can offer benefits for the resilience of reef ecosystems.

1. Introduction

Population outbreaks of the crown-of-thorns sea star (COTS), *Acanthaster planci*, remain one of the major causes of coral loss and habitat degradation on coral reefs throughout the Indo-Pacific (Grand et al., 2014). On Australia's Great Barrier Reef (GBR), for example, outbreaks of *A. planci* are reported to be one of the major contributors to sustained and ongoing declines in live coral cover (De'ath et al., 2012). There are also renewed and ongoing outbreaks of COTS on many other reefs throughout the Indo-Pacific (Rivera and Pratchett, 2012), which are causing widespread and often very significant levels of coral loss. Despite significant investment in addressing both declining water quality and over-fishing, effective management of COTS outbreaks is limited by equivocal understanding of the proximal causes of outbreaks in different times and places (Pratchett et al., 2014); given uncertainty about the proximal causes of outbreaks, the most immediate solution (if only a stop gap measure) is to directly control outbreak populations, through hand collections of individual sea stars or *in situ* injections of toxic substances. The feasibility and effectiveness of large-scale (e.g., reef-wide) control programs has been continually questioned (e.g., Kenchington and Pearson, 1982) because it not clear that measures required to effectively protect small patches of reefs can be achieved simply by scaling up effort (e.g., number of diver hours) in proportion to reef area. There remain however; concerted efforts to kill and/or collect COTS in many locations throughout the Indo-Pacific (Pratchett et al., 2014). Logically, the quicker and the more COTS are killed in a given reef with an outbreak population, the fewer corals will be damaged (Birkeland and Lucas, 1990) and there will be reduced likelihood of successful fertilization once

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Lethal doses of oxbile, peptones and thiosulfate-citrate-bile-sucrose agar (TCBS) for *Acanthaster planci*; exploring alternative population control options

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ABSTRACT

Effective control of outbreaks of *Acanthaster planci* represents the most immediate and practical intervention to reverse sustained declines in coral cover on reefs in the Indo-Pacific. This study explored the minimum doses of oxbile, oxgall, and thiosulfate-citrate-bile-sucrose agar (TCBS) that result in reliable and comprehensive mortality when injected into adult *A. planci*. The minimum doses required to induce 100% mortality among starfish ($n = 10$) were 4 g l^{-1} of oxbile, 8 g l^{-1} of oxgall and 22 g l^{-1} of TCBS. Moreover, there was no evidence of unintended side effects for other coral reef organisms (e.g., scleractinian corals, echinoderms and fishes) when using oxbile, oxgall, or TCBS at minimum doses. The effectiveness of peptones in killing crown-of-thorns starfish was also tested, but inconsistency in the results revealed that these proteins are unreliable.

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