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Processes and patterns in larval settlement of crown-of-thorns starfish (*Acanthaster* cf. *solaris*)

by

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Abstract

Population irruptions of the corallivorous crown-of-thorns starfish (COTS; *Acanthaster* spp.) are renowned for their recurring contributions to coral mortality and reef degradation throughout their tropical Indo-Pacific range, thereby threatening the structure and function of these important ecosystems. In contrast to other disturbances responsible for the plight of Anthropocene coral reefs, population irruptions of COTS are relatively amenable to localised management efforts. It is, therefore, imperative to capitalise on the opportunity to conserve coral reefs by advancing our understanding of this phenomenon and improving the ecological underpinning of COTS population management.

Because of extraordinary biological traits underpinning the success of this destructive coral predator, marked fluctuations in the abundance of adult COTS can arise from minor changes in key demographic rates, particularly during the inherently vulnerable early life-history stages. For population irruptions to initiate and spread, large numbers of larvae must successfully complete their planktonic-benthic transition into benthic juveniles (i.e., larval settlement, including metamorphosis). Despite the importance of larval settlement to the population replenishment and dynamics of COTS, our knowledge of the factors governing this early life-history transition is still highly fragmented. The overarching objective of the research presented in this thesis was, therefore, to improve understanding of the fundamental processes and patterns in larval settlement of COTS, and to assess their ecological implications for recruitment success and population dynamics, which has a critical influence on their ecological effects on coral reefs.

Before undertaking independent research focused on COTS, a review of the processes and patterns in echinoderm settlement revealed marked complexity and specificity in the settlement behaviour and requirements of many ecologically important groups. The larvae of

most echinoderms, including keystone sea urchins and starfishes, do not settle randomly in space in time; specific environmental cues, often associated with conspecifics and/or live substrata, are essential for the initiation of settlement and subsequent metamorphosis, contributing to high spatiotemporal variability in concomitant settlement patterns.

For COTS, experimental settlement assays demonstrated that a wide range of coralline algae species induce their larvae to settle. However, the relative capacity of different coralline algae varied substantially, ranging from > 90% in the alga *Melyvonnea* cf. *madagascariensis* to < 2% in three other coralline algae species. Importantly, I found that many algae species that are prevalent in shallow reef habitats induced high settlement rates, which challenges the prevailing hypothesis that COTS larvae predominantly settle in deep water. Considering the spatial distribution of the coralline algae used in settlement assays, this research also propounds the hypothesis that the local abundance of algae species with highly inductive settlement cues may be influencing broader-scale recruitment patterns of COTS, with a focus on Australia's Great Barrier Reef (GBR).

Another persistent information gap was addressed in experiments exploring the potential for COTS larvae to settle in response to cues associated with conspecific juveniles and adults. While such gregarious settlement behaviour has been purported to play a role in the population replenishment of COTS, like for many other marine benthic invertebrates, these experiments demonstrated that their larvae are generally not induced to settle by a wide range of potential surface-bound and waterborne conspecific cues. Moreover, I found no evidence that the settlement success facilitated by coralline algae was inhibited or enhanced by the addition of conspecific cues. While larval behaviour and movements may yet be affected by conspecific cues during their planktonic phase, these results suggest that the settlement induction, and thereby recruitment success, of COTS is specifically dependent on cues associated with coralline algae, as opposed to gregarious cues.

Results from experimental studies were complemented by a field study of larval settlement providing unprecedented insights into *in situ* settlement patterns, previously unexplored due to methodological challenges in sampling this life-history transition. Here, I introduced an innovative method to overcome these challenges, using artificial settlement collectors and a molecular-based approach, specifically droplet digital polymerase chain reaction based on taxon-specific mitochondrial DNA primers. This work demonstrated the utility of the new method and provided evidence for large variability in settlement across temporal and spatial scales on the GBR. Notably, this research also corroborated results from the coralline algae experiment, documenting that larvae readily settle in shallow reef environments, evidenced by DNA-based detections. While the innovative sampling protocol provides an opportunity to resolve other persistent information gaps relating to the biology and ecology of COTS (e.g., stock-recruitment relationship), it also has significant application as an early warning tool. Detections of elevated settlement could herald new and/or renewed population irruptions years before increases in adult densities are apparent, greatly increasing opportunities for effective suppression of COTS densities, which is critical for reducing ongoing coral loss and reef degradation.

Overall, this thesis revealed and challenged some of the fundamental processes thought to govern the settlement stage of crown-of-thorns starfish. In particular, the finding of strong settlement cue selectivity substantially advanced our understanding of where cues associated with specific reef substrata may facilitate high settlement success across multiple spatial scales. Within this operating space, this thesis also demonstrated that the discussion surrounding factors contributing to recruitment success and population fluctuations in this coral predator (e.g., larval supply) may require extension to settlement-related factors. While previously overlooked, factors such as larval settlement behaviour and the distribution of apt settlement substratum may ultimately dictate the number of larvae that recruit into adult populations, and consequently explain some of the marked spatial heterogeneity in the incidence and severity of population irruptions.

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Chapter 1: General Introduction

The corallivorous crown-of-thorns starfish (COTS; *Acanthaster* spp.) is endemic to tropical coral reef ecosystems and is one of the most well-known and extensively studied coral reef invertebrates (Moran 1986). Most critically, this keystone echinoderm is notorious for its propensity to undergo major population irruptions which present a perennial threat to already imperilled coral reefs throughout the Indian and Pacific oceans (Moran 1986, Birkeland and Lucas 1990, Pratchett et al. 2014). Following early life stages as planktonic larvae and benthic herbivorous juveniles, COTS undergo an ontogenetic dietary shift and thereafter feed predominantly on reef-building hard (Order Scleractinia) corals (Wilmes et al. 2020a). As corallivores, now featuring the characteristic spines across the aboral surface, these starfish feed extraorally by engulfing corals with their eversible stomach and digesting the coral tissue externally (Birkeland 1989). As adults, COTS also display most of the other traits they are best known for, including fast growth (Birkeland and Lucas 1990), large body size (Birkeland 1989), high fecundity (Pratchett et al. 2021a), and high rates of feeding (Keesing and Lucas 1992). Collectively, these traits contribute to the enormous destructive potential of COTS.

At low densities (<10 individuals ha^{-1}), the impact of COTS on coral abundance and reef health is negligible, but elevated densities of large adults cause widespread coral mortality and rapidly decimate live coral cover on reefs (e.g., Chesher 1969, Kayal et al. 2012, Baird et al. 2013). Concomitantly, large adult populations can also reduce coral diversity due to strong feeding preferences for some coral taxa, in particular branching Acroporidae corals (Pratchett 2007, 2010). Because corals are the foundation of coral reef ecosystems, substantial coral loss and changes in coral assemblages as a result of this coral predator have ecosystem-wide consequences (Hart et al. 1996, Pratchett et al. 2020), including the population decline and collapse of obligate coral feeding or dwelling species (Pratchett et al. 2012, Kayal et al. 2012). These destructive effects also increasingly interact with a myriad of other global and localised stressors (Mellin et al. 2019, Castro-Sanguino et al. 2021), threatening ecosystem structure and function. Thus, improving our understanding of COTS and developing effective strategies to manage COTS population irruptions must be a paramount priority for the scientists and managers tackling the plight of Anthropocene coral reefs.

Despite their reputation as a marine pest (e.g., Pratchett and Cumming 2019), reef-associated COTS are native throughout their geographical range. While previously regarded as a single species, there are at least four strongly diverged mitochondrial clades (and now recognised reef-associated species), largely restricted to different regions of the Indo Pacific (Vogler et al. 2008, Wörheide et al. 2022). This Indo-Pacific '*Acanthaster planci*' species complex includes a northern Indian Ocean species (*Acanthaster planci*), a southern Indian Ocean species (*Acanthaster mauritiensis*), a Red Sea species (*Acanthaster benziei*) and a (western) Pacific species (*Acanthaster* cf. *solaris*) (Wörheide et al. 2022). While the terms "crown-of-thorns starfish" or "*Acanthaster* spp." were used in some sections of this thesis when referring to the entire '*Acanthaster planci*' species complex (excluding the deep-water species *Acanthaster brevispinus*), subsequent sections of the thesis (**Chapters 2, 3, 4, 5, 6**) predominantly refer to the (western) Pacific species *Acanthaster* cf. *solaris* (**Figure 1.1**). Moreover, this species was also the study species for all laboratory- and field-based research conducted (**Chapters 3, 4, 5).** While the species complex is considered a globally significant driver of reef degradation, all four species have been linked to widespread coral mortality within their respective geographic ranges (Pratchett et al. 2017a), including reefs sheltered from other anthropogenic stressors (Endean and Chesher 1973, Roche et al. 2015).

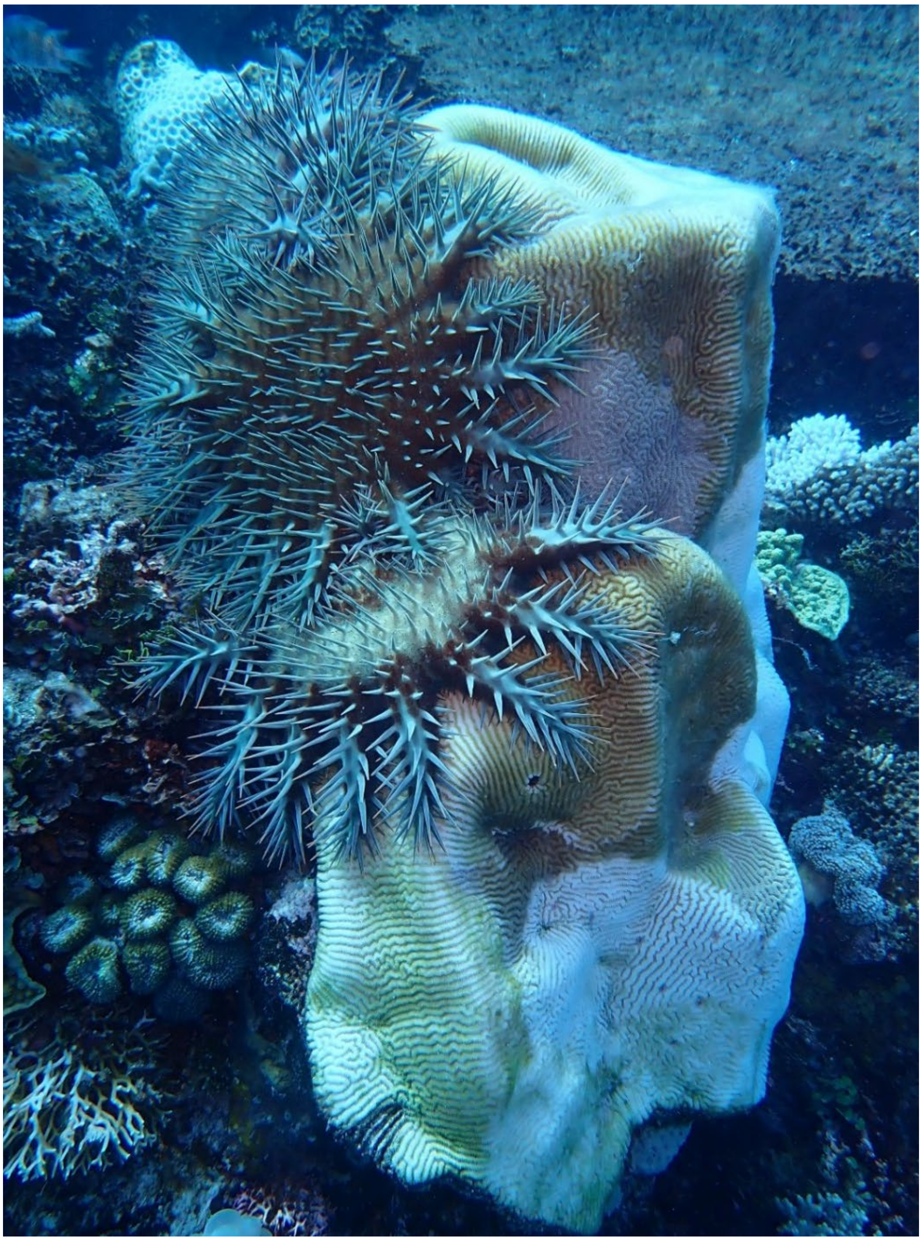

Figure 1.1 Four adult western Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) feeding on a coral colony of the genus *Leptoria* at Wilson Reef (Great Barrier Reef, Australia) in February 2021.

On the world's largest coral reef system, Australia's Great Barrier Reef (GBR), recurrent population irruptions of the western Pacific COTS (*Acanthaster* cf. *solaris*) have been extensively documented for over 50 years (Endean 1973, Reichelt et al. 1990). They represent one of the main causes of the ongoing coral decline throughout the GBR and occur alongside other major disturbances (Osborne et al. 2011, De'ath et al. 2012, Mellin et al. 2019), such as mass coral-bleaching episodes (Hughes et al. 2017) and severe tropical storms (Cheal et al. 2017). Four distinct waves of COTS population irruptions have been documented on the GBR since the 1960s, occurring at intervals of 14-17 years (Babcock et al. 2020). These distinct population irruptions exhibit comparable spatiotemporal patterns, showing a progressive southward spread at rate of approximately 1° of latitude every three years, following a primary outbreak initiation in the northern GBR (Pratchett et al. 2014). Prompted by each wave of population irruptions since the 1960's, manual population control programs have been implemented to mitigate the effects of elevated COTS densities on the GBR, more recently involving lethal injections *in situ* (Pratchett et al. 2019, Westcott et al. 2020). However, both COTS research and population control efforts have generally lagged behind the initiation and spread of population irruptions (Babcock et al. 2020). Importantly, a fifth wave of destructive population irruptions looks to be already underway in the northern and far-northern GBR (Pratchett et al. 2022), which underlines the urgent need for innovative research to improve our understanding of COTS and mitigate, if not prevent, new and renewed population irruptions.

In contrast to most other major disturbances that cause extensive coral loss (e.g., climate induced mass-bleaching), population irruptions of COTS appear to be amenable to localised management efforts (e.g., Westcott et al. 2020). However, long-term and large-scale management of COTS is conditional upon determining the proximal cause(s) of population irruptions (Pratchett et al. 2014). Two of the most prominent hypotheses put forward to explain this phenomenon implicate anthropogenic influences on COTS population. The ‘larval starvation hypothesis’ attributes uncommonly high survival rates of planktotrophic larvae (and concomitant recruitment pulses) to elevated nutrient levels because of terrestrial runoff following heavy rainfall events (Birkeland 1982, Brodie et al. 2005, Fabricius et al. 2010). Then, the ‘predator removal hypothesis’ posits that the removal of predators (through overfishing and/or habitat degradation) releases juvenile and/or adult COTS from the predation pressure that otherwise regulated their abundance (Sweatman 2008). In contrast, a third hypothesis (‘natural causes hypothesis’) argues that marked population fluctuations are a natural consequence of their biological traits, including their complex, feeding larval stages and enormously high fecundity (Vine 1973). While it is likely that predator removal and enhanced larval survival both contribute to the success of COTS (Pratchett et al. 2014), their remarkable reproductive potential and other opportunistic traits probably underpin much of its success across its geographical range and predispose them to population fluctuations (reviewed by Deaker and Byrne 2022).

Given the immense reproductive potential of COTS (Babcock et al. 2016, Pratchett et al. 2021a), relatively minor changes in key demographic rates (e.g., increased larval survival or settlement success; Wilmes et al. 2018) could readily cause population irruptions. The bipartite life history of COTS epitomises complexity characteristic of many benthic marine invertebrates and comprises a relatively short but complex planktonic larval phase, and benthic juvenile and adult phases (Doll et al. 2022 – **Chapter 2**). Like other marine benthic invertebrates, the early life-history stages of

COTS are inherently vulnerable to population mortality bottlenecks (Wilmes et al. 2018, Deaker and Byrne 2022). Towards the end of their planktonic larval phase, a cascade of behavioural and physiological processes culminates in the attachment of COTS larvae onto the substratum, and subsequent metamorphosis to commence its benthic life (= larval settlement; Doll et al. 2022 – **Chapter 2**). Because larval settlement success inherently affects recruitment patterns and population dynamics, this planktonic-benthic transition, and the factors governing it, may play a key role in the initiation and spread of COTS population irruptions (Doll et al. 2023a – **Chapter 3**). Previous accounts of population irruptions have attributed them to either single mass-settlement events (e.g., Zann et al. 1987) or progressive accumulation of recruits over successive years of elevated recruitment (e.g., Pratchett 2005). Clearly, this highlights that an improved understanding of settlement processes and patterns is indispensable in order to identify the factors promoting spatiotemporal heterogeneity in adult COTS densities.

Despite the important role of early life-history processes to recruitment success and the incidence of population irruptions for COTS, progress in resolving the persistent information gaps surrounding them has been highly fragmented (Pratchett et al. 2017a, 2021b). Among the list of priority research questions published by Moran (1986), approximately two thirds of questions surrounding the settlement and early post-settlement stages remain unresolved, while most questions pertaining to COTS larvae and adults have now been resolved (Pratchett et al. 2017a). The study of larval settlement was recently again confirmed as one of the foremost research themes to better understand and manage COTS and their destructive impact on coral reefs (Pratchett et al. 2021b). Notably, most of the settlement-related research questions highlighted in this horizon scan were concerned with either spatial settlement patterns, particularly in relation to depth or inter-reef differences, or the role of reef substrata and settlement cues (associated with coralline algae and conspecifics) in shaping the settlement phase (Pratchett et al. 2021b).

Major information gaps pertaining to the planktonic-benthic transition of COTS persist, including research questions considered critical to improve the management of this notorious coral predator (Pratchett et al. 2021b). The overarching goal of the research presented in this thesis was, therefore, to advance our understanding of the processes and patterns in larval settlement, and to assess their potential ecological implications for COTS recruitment success and the incidence of their destructive population irruptions. The objective of the chapters therein was to, individually and/or conjointly, fill priority gaps in our knowledge surrounding the settlement stage.

In **Chapter 2**, I compiled and analysed extensive literature on the processes and patterns in the larval settlement of all echinoderms, synthesising foundational research and recent advances across a wide range of marine systems. In this phylum-wide review, I outlined the complex mechanisms and processes taking place during settlement, including a detailed analysis of settlement behaviour and the various mechanisms of settlement induction. Following a critical assessment of methodological challenges and advances in sampling settlement in the field, I synthesised observations of spatial and temporal patterns in echinoderm settlement from across the globe, allowing for comparisons among echinoderm classes and species, various marine habitats, locations and climate zones.

Chapter 2 provided important new insights into the settlement of COTS and other ecologically important echinoderms, such as sea urchins, but was also fundamental to inform the approaches and methods for the following chapters and analyse the broader ecological implications of the results presented therein. The subsequent chapters (**Chapters 3-5**) focused on the western Pacific crown-of-thorns starfish (COTS; *Acanthaster* cf. *solaris*) and employed a wide range of approaches and techniques. However, they generally follow the same natural progression that transitions from questions surrounding the factors that shape the larval settlement phase (**Chapters 3, 4**) to an assessment of concomitant patterns of settlement in the field (**Chapter 5**).

In **Chapters 3 and 4**, I sought to identify the key environmental cues governing COTS settlement induction. Plasticity in settlement behaviour is widespread among echinoderms (**Chapter 2**), whereby settlement-stage larvae of some species can settle in response to a diversity of environmental stimuli, often including a wide range of gregarious or associative cues (e.g., Dworjanyn and Pirozzi 2008). While settlement-stage COTS larvae may also respond to gregarious cues (associated with conspecifics; Cowan et al. 2016a), the predominant settlement cues for COTS in the wild are presumably associated with coralline algae. However, it remains unclear whether the capacity to induce high COTS settlement rates varies among coralline algae species.

Hence, in **Chapter 3**, I conducted laboratory-based assays to study the relative capacity of 15 common coralline algae species from the GBR to induce COTS settlement. More specifically, the objective of this chapter was to test the hypothesis that a particular coralline algae species (*Lithothamnion* cf. *proliferum*), surmised to be the predominant COTS settlement cue, promoted higher settlement success than the other algae. As part of this chapter, I was further able to critically evaluate the deep-water recruitment hypothesis for COTS (Johnson et al. 1991) by assessing whether coralline algae species that occur in deep and/or shallow reef environments play important roles in settlement induction. The inclusion of larger-scale algal field-distribution data in the interpretation of my experimental results also allowed me to better evaluate the potential ecological consequences of coralline algae assemblages for the recruitment patterns of this coral predator.

Following up, in **Chapter 4**, I explored the potential for gregarious settlement behaviour in COTS larvae, which would be an ecologically significant avenue for population replenishment and the proliferation of COTS densities on reefs (see discussions in Pratchett et al. 2017a, Deaker and Byrne 2022). To resolve this information gap, I conducted laboratory experiments documenting whether larvae settled in response to the presence of conspecifics and to cues associated with conspecifics. Because the specific nature of gregarious settlement cues varies greatly among benthic marine invertebrates (reviewed by Burke 1986, Pawlik 1992), these experiments tested a range of surface-bound and waterborne cues associated with herbivorous juveniles of different ages, corallivorous juveniles and adults. In addition, this chapter explored the potential interaction between conspecific cues and the coralline algae-mediated induction of COTS settlement.

Chapter 5 shifted focus from settlement processes and cues to the study of *in situ* settlement patterns of COTS. Challenges to detecting and surveying early post-settlement juveniles *in situ*, due to their microscopic size and cryptic nature (Zann et al. 1987), had previously constrained the study of COTS settlement patterns. In this chapter, I introduce an innovative method to record COTS settlement, using purpose-built artificial settlement collectors and droplet digital polymerase chain reaction (ddPCR) based on COTS-specific mitochondrial DNA primers. The objective of this chapter was to demonstrate the utility of this method and explore spatial and temporal settlement patterns on the GBR. Specifically, I assessed whether settlement varied temporally, within a reproductive summer period and between years, and spatially, along latitudinal and depth gradients. Notably, this field-based study of spatial settlement patterns complements the laboratory-based approach in **Chapter 3**, particularly to evaluate central ecological assumptions underpinning the aforementioned deep-water recruitment hypothesis.

Finally, in **Chapter 6**, I consolidated the insights gained from all preceding chapters and interpreted them in the context of spatiotemporal heterogeneity in recruitment success and the incidence of population irruptions. Moreover, the significance and implications of these findings for the ecological underpinning of COTS management were highlighted, with a focus on the GBR. Lastly, I outlined some of the key future research avenues that capitalise on new methodological advances and results presented in this thesis.

Chapter 2: Processes and patterns in larval settlement of echinoderms[1]

Abstract

Echinoderms are a common component of benthic marine ecosystems, with many being ecologically and/or economically important. Like many marine organisms, most echinoderms have a bipartite life history with a planktonic larval phase and a benthic adult phase. The transition between these phases (i.e., settlement) is complex and comprises a cascade of events including the location, exploration and selection of suitable benthic habitat, and metamorphosis to adapt from a pelagic to a benthic lifestyle. This review provides a comprehensive synthesis of the various processes involved in the settlement phase across all five extant classes of echinoderms. Central to the review is a detailed assessment of settlement behaviour and the diverse mechanisms of settlement induction. Most echinoderms, including keystone sea urchins, starfishes and sea cucumbers, do not settle indiscriminately; specific environmental conditions or cues are often necessary for settlement to occur, resulting in marked spatial and temporal variability in settlement rates. Fluctuations in settlement, in turn, lead to major changes in the local abundance of echinoderms and often have profound ecological consequences, due to the pivotal role that many echinoderms play in ecosystem functioning. Given important knowledge gaps persist, this review also explores

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[1] Published as:

Doll, P.C., Caballes, C.F., Hoey, A.S., Uthicke, S., Ling, S.D. & Pratchett, M.S. 2022. Larval settlement in echinoderms: a review of processes and patterns. *Oceanography and Marine Biology: An Annual Review* 60, 433-494. (https://doi.org/10.1201/9781003288602-9) opportunities for future research to advance our understanding of this critical early life-history phase.

2.1 Introduction

Echinoderms (phylum Echinodermata) are commonly observed across all benthic marine habitats, ranging from exposed rocky shores and tropical coral reefs to the sandy floor of deep-sea habitats (Byrne 1990, Keesing et al. 1993, Sumida et al. 2000). There are approximately 7000 extant species (Pawson 2007) across five classes (Byrne and O’Hara 2017): Echinoidea (sea urchins, sand dollars and heart urchins), Asteroidea (starfishes or seastars and sea daisies), Ophiuroidea (brittle stars, basket stars and snake stars), Holothuroidea (sea cucumbers) and Crinoidea (feather stars and sea lilies). Echinoderms are economically important with extensive commercial fisheries for sea cucumbers (Holothuroidea) and sea urchins (Echinoidea), which are increasingly supplemented by aquaculture (Brown and Eddy 2015). Echinoderms are also ecologically important. Sea cucumbers, for example, make important contributions to nutrient cycling and energy transfer (Purcell et al. 2016). Other echinoderms have been shown to be important ecosystem engineers (Lessios et al. 1984, Carpenter 1985) and/or keystone species (Paine 1969, Hughes et al. 1985, Ling et al. 2015, Byrne et al. 2016, Menge et al. 2016).

Large fluctuations in population density are a salient characteristic of many echinoderms (Uthicke et al. 2009) and can have important consequences for ecosystem structure and function (Chesher 1969, Birkeland 1989, Estes et al. 2011, Ling et al. 2015). For example, the proliferation of sea urchins and subsequent overgrazing in many temperate reef systems has contributed to loss of kelp forest and macroalgal beds (Ling et al. 2015). Conversely, population collapse in keystone predator starfish species across the Northeast Pacific (Menge et al. 2016, Schultz et al. 2016, Harvell et al. 2019, Kay et al. 2019) has triggered trophic cascades and changed shallow water seascapes. On tropical reefs in the Caribbean, population collapse of the sea urchin *Diadema* contributed to shifts from coral- to algal-dominated habitats (Lessios et al. 1984, Carpenter 1985, Hughes et al. 1985). Periodic population irruptions of coral-feeding crown-of-thorns starfish (*Acanthaster* spp.) have also been a major contributor to sustained coral loss and reef degradation on many Indo-Pacific reefs (Bruno and Selig 2007, Kayal et al. 2012, Mellin et al. 2019). Ecological studies of echinoderms are, therefore, often focused on understanding causes of population fluctuations (Ling et al. 2019, Glockner-Fagetti and Phillips 2020, Caballes et al. 2021), which are inextricably linked to life-history processes that affect larval ecology and patterns of settlement or recruitment (e.g., Ebert 1983, Uthicke et al. 2009, Metaxas 2013).

Echinoderms exhibit considerable life-history diversity (Byrne et al. 1999, McEdward and Miner 2001, 2007, Byrne and O’Hara 2017, Ebert 2021a), but are generally gonochoric, and reproduce sexually by broadcast-spawning gametes that are fertilised externally. Accordingly, most echinoderms have a planktonic larval phase, which lasts from days to months (Strathmann 1987, Hadfield et al. 2001). Larval development is characterised by a series of distinct developmental stages (Byrne 2013, Byrne and O’Hara 2017). Many echinoderms have lecithotrophic larvae (McEdward and Miner 2001, 2007, Uthicke et al. 2009), meaning that they do not or cannot feed, and larval duration is constrained by initial energy reserves. Most of the best known and extensively studied echinoderm species (e.g., *Acanthaster* spp., *Diadema* spp.) are, however, planktotrophic (Uthicke et al. 2009), and this capacity to feed means that larvae may remain in the plankton for much longer (*cf*. lecithotrophic larvae) and potentially disperse much further (Emlet 1995). More importantly, egg size and energy content (= maternal provisioning) are significantly higher in species with lecithotrophic larvae (McEdward and Chia 1991, Falkner et al. 2015), which consequently constrains fecundity. Echinoderms with planktotrophic larvae have potentially much higher reproductive capacity (Uthicke et al. 2009), but the extent to which the reproductive capacity is realised depends on exogenous food availability for larvae.

Despite the wide range of biotic (e.g., predation and starvation) and abiotic factors (e.g., dispersal to unfavourable habitats) that may constrain larval development and survival (Rumrill 1990, Lamare and Barker 1999, Cowan et al. 2016a, 2020), population replenishment and dynamics of echinoderms (like all marine species with a dispersive larval phase) is also conditional on settlement success and early post-settlement survival (Balch and Scheibling 2001). After completing larval development, echinoderms undergo metamorphosis, wherein they rapidly develop morphologies that are adapted to their benthic adult habit (*sensu* Hadfield et al. 2001). This planktonic-benthic transition, hereafter referred to as settlement (*sensu* Scheltema 1974), comprises a cascade of events that often occur rapidly, but can have far-reaching consequences for the population dynamics and distribution of echinoderms, and the ecosystems in which they play important roles. To assess these potential consequences, a comprehensive understanding of the environmental conditions and cues necessary for echinoderm larvae to settle, and how they translate to concomitant settlement patterns in the field is needed.

Past reviews of settlement induction and metamorphosis in marine invertebrate larvae have largely focused on ascidians, cnidarians, corals, hydroids, molluscs and/or polychaetes (e.g., Burke 1983a, 1986, Pawlik 1992, Rodríguez et al. 1993, Wieczorek and Todd 1998, Hadfield and Paul 2001, Hadfield 2011). Despite extensive primary research on settlement in echinoderms (e.g., Birkeland et al. 1971, Cameron and Hinegardner 1974, Highsmith 1982), there has been limited synthesis or review of this work. Strathmann (1978) and Pearce (1997) reviewed settlement induction in echinoderm larvae. Meanwhile, Balch and Scheibling (2001) provided a comprehensive synthesis of patterns of echinoderm recruitment, building on the initial review of recruitment patterns by Ebert (1983). General patterns of settlement are also briefly summarised in more recent syntheses focusing on the larval ecology of echinoids (Metaxas 2020) and asteroids (Metaxas 2013). However, a considerable body of research from the past two decades has substantially advanced our understanding of the processes and patterns of settlement in echinoderms. It is therefore timely to provide a comprehensive, phylum-wide review covering all aspects of this critical and complex early life-history transition.

The aim of this review is to synthesise foundational research and recent advances in the study of the various processes and patterns in echinoderm settlement. Importantly, this review provides a detailed overview of published work on larval settlement across a wide range of marine habitats and environments. It starts with a summary of the complex mechanisms and processes taking place during this planktonic-benthic transition. Central to this review is a detailed synthesis of settlement behaviour and the various mechanisms of settlement induction, including both natural and artificial chemical cues. I then critically assess predominant sampling techniques used to measure settlement *in situ*, followed by a review of spatial and temporal settlement patterns, which allows for comparisons among echinoderm classes and species across various marine habitats, locations and climate zones. The final section of this review highlights persistent and critical information gaps and outlines future directions for research pertaining to this important early life-history transition.

2.2 Larval settlement: the planktonic-benthic transition

2.2.1 Terminology

While often used interchangeably to describe planktonic-benthic transitions, settlement and metamorphosis are distinct processes for echinoderms. Scheltema (1974) defined settlement as a behavioural response of planktonic larvae resulting in the termination of their pelagic larval stage and assumption of a benthic life. As such, settlement typically refers to the descent from the water column and contact with the substratum. However, such substratum contact is reversible (Burke 1983a, Crisp 1984, Pawlik 1992, Hadfield and Paul 2001), whereby larvae may abort their benthic habit and resume swimming to seek out alternative settlement substrates or habitats (**Figure 2.1**). By contrast, metamorphosis is considered an irreversible morphogenic event, which includes the loss of larva-specific and emergence of juvenile-specific structures (**Figures 2.2, 2.3**; Hadfield and Paul 2001). Moreover, metamorphosis may occur before or after the contact with the substratum, depending on the species (**Figure 2.1**).

For the purpose of this review, settlement will be used to refer to the more general definition of the transition from the planktonic larval to the benthic, post-metamorphic stage, as used in many other marine taxa (Hadfield 1986, Pawlik 1992, Rodríguez et al. 1993). In many echinoderms, this settlement phase may include various behavioural components (*sensu* Crisp 1984). This review only uses the morphogenic term ‘metamorphosis’ in specific reference to the physiological and morphological changes involved in this event, which appears necessary given some echinoderms metamorphose in the plankton prior to contacting any substratum (Domanski 1984, Hendler 1991, McEdward and Miner 2001, Selvakumaraswamy and Byrne 2004), and numerous studies specifically report metamorphic responses of echinoderm larvae (e.g., Pearce and Scheibling 1991, Yazaki and Harashima 1994, Naidenko 1996, Matsuura et al. 2009).

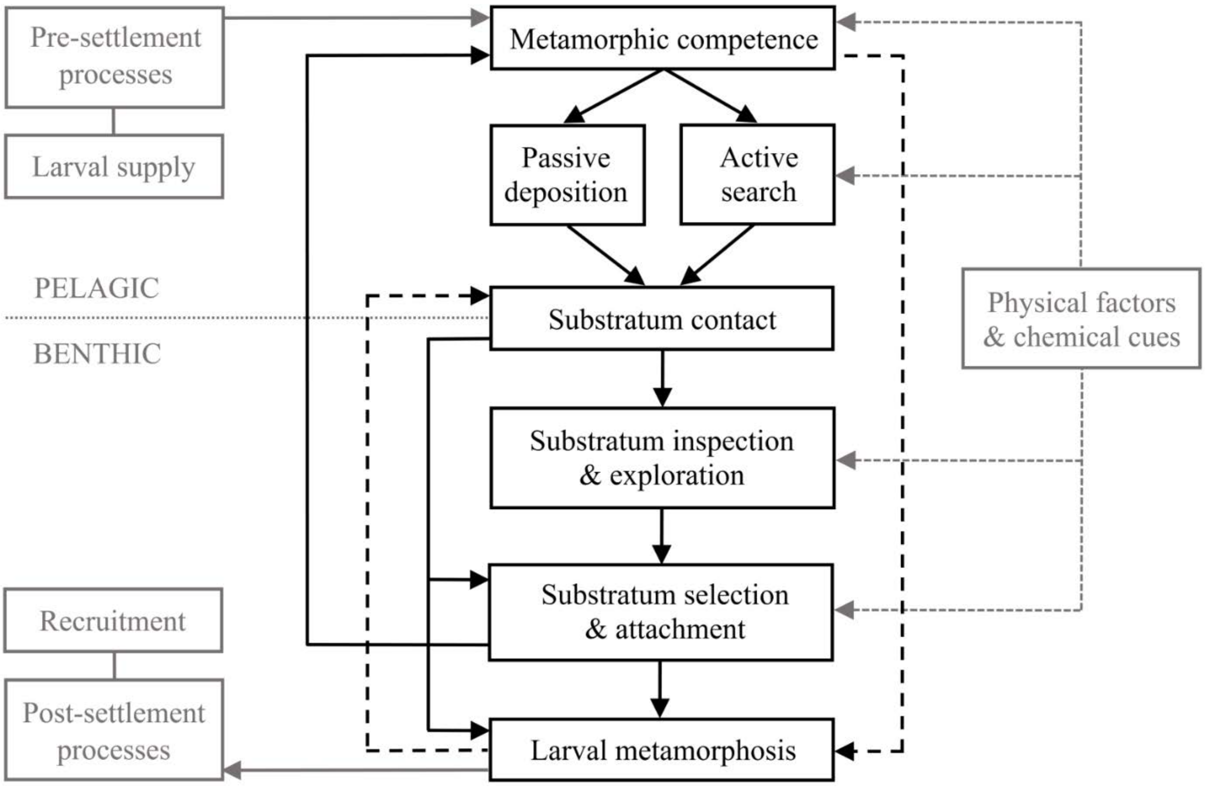

Figure 2.1 Key stages and processes in the settlement of echinoderms with planktonic larvae. Some echinoderms metamorphose in the plankton prior to contacting the substratum (dashed black arrows).

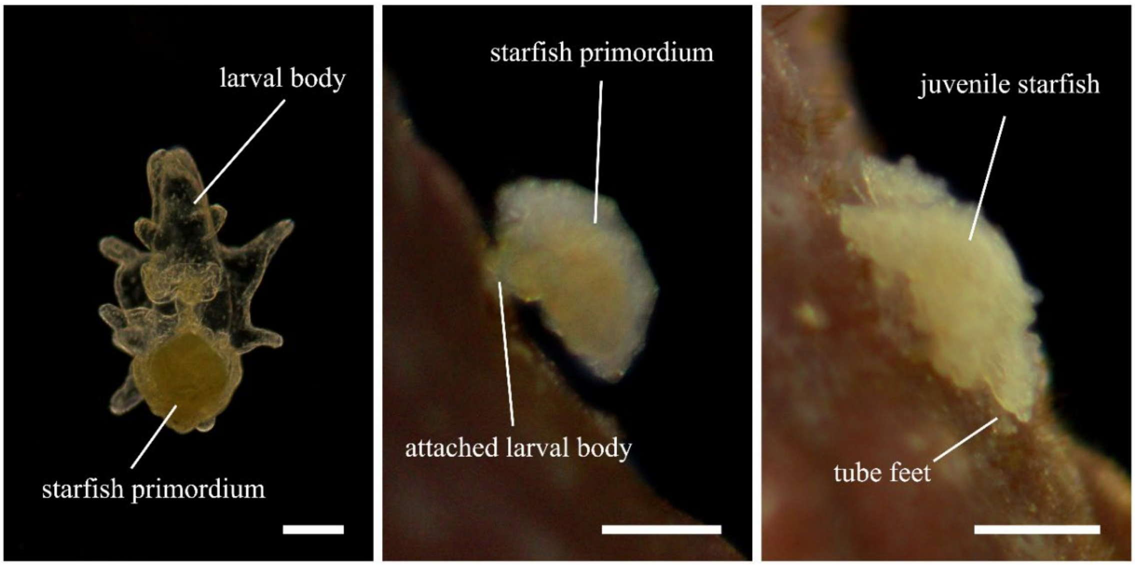

Figure 2.2 Larval development through metamorphosis in the Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*), an ecologically important asteroid with a pelagic planktotrophic larval stage and complex larval morphogenesis: (A) brachiolaria larva; (B) metamorphosing larva absorbing the larval body; (C) post-metamorphic juvenile. Scale = 0.25 mm. Photographs by C.F. Caballes.

2.2.2 Pre-settlement processes

A range of abiotic and biotic factors may influence echinoderm larvae prior to settlement. Crucially, mortality is thought to be very high during the sometimes highly prolonged planktonic larval stage (Cameron et al. 1985, Bosch et al. 1987, Pratchett et al. 2017b, Ebert 2021b). With a few exceptions (Rumrill 1987, López et al. 1998, Lamare and Barker 1999), most estimates of larval mortality in echinoderms come from controlled laboratory studies, such that larval mortality may be even higher in natural environments, due to increased environmental fluctuations, limited prey availability and potentially high rates of predation (Pearse and Cameron 1991, Ebert 2021b).

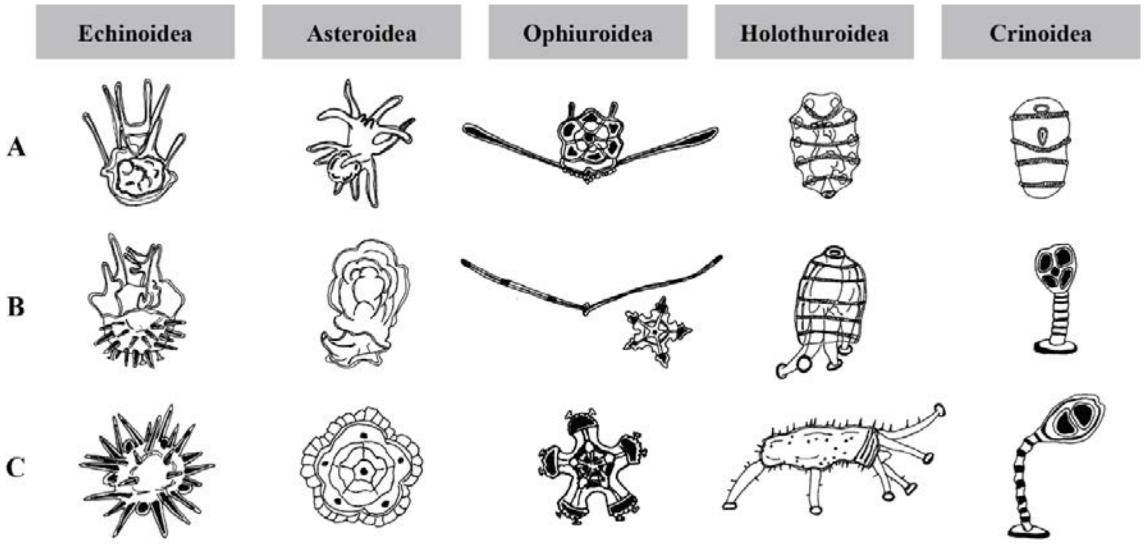

Figure 2.3 Echinoderm larval development through metamorphosis, as exemplified by an echinoid (*Tripneustes gratilla*), asteroid (*Acanthaster* cf. *solaris*), ophiuroid (*Ophiothrix exigua*), holothuroid (*Holothuria scabra*) and crinoid (*Antedon serrata*): (A) late-stage larva; (B) settlement and metamorphosis; (C) post-metamorphic juvenile. Illustrations by C.F. Caballes; based on Caballes and Pratchett (2014), Kitazawa et al. (2015), Toha et al. (2017), Yamakawa et al. (2020) and Nontunha et al. (2021).

The principal abiotic factors affecting echinoderm larvae during their planktonic life stage are thought to be advection, temperature and salinity (reviewed by Balch and Scheibling 2001). Most notably, the dispersal and distribution of planktonic larvae depends on advective transport, which in turn is driven by large-scale oceanographic features and currents, and local hydrodynamics (Ebert 1983, Pearse and Cameron 1991). Given their generally limited swimming capacity, larval dispersal models often assume that echinoderm larvae are passive particles (Montgomery et al. 2017), although swimming is critical in determining larval distribution and settlement patterns at relatively small spatial scales. The important role of hydrodynamic forces in determining large-scale (e.g., inter-reef) patterns of species’ distributions and population connectivity has been demonstrated for the Pacific crown-of-thorns starfish *Acanthaster* cf. *solaris* (Black and Moran 1991; Hock et al. 2014) and the echinoid *Centrostephanus rodgersii* (Banks et al. 2007). However, detailed biophysical models which incorporate species-specific knowledge of larval biology and behaviour (e.g. Bode et al. 2019) are yet to be developed for any echinoderm species.

Changes in key physical variables such as temperature and salinity may also influence larval development (Byrne et al. 2009, Li et al. 2011a, Privitera et al. 2011, Kamya et al. 2014), survival (Lucas 1973) and planktonic larval duration (Thorson 1950, Agatsuma et al. 1998). For example, larvae of the echinoid *Dendraster excentricus* take three times as long to reach the six-armed larval stage at 12°C compared to 22°C (McEdward 1984). Numerous studies attribute fluctuations in reproductive behaviour and settlement rates of echinoderms to changes in temperature (Balch et al. 1999, Hernández et al. 2010, Sotelo-Casas et al. 2016, Glockner-Fagetti and Phillips 2020, Okamoto et al. 2020, Caballes et al. 2021), although these patterns may be conflated by simultaneous changes in a range of environmental conditions. In some echinoids, temperature changes have explicitly been linked to population irruptions and range extensions (Hart and Scheibling 1988, Ling et al. 2008, 2009).

In terms of biotic factors, predation and starvation in the pelagic developmental habitat have long been recognised as key factors contributing to high levels of mortality among planktotrophic larvae across a range of marine taxa (Thorson 1950, Birkeland 1982, Young and Chia 1987). For echinoderms, laboratory experiments have demonstrated the importance of predators in regulating larval densities (Rumrill and Chia 1985, Cowan et al. 2016b, 2020). At the same time, food availability and potential starvation inherently constrain the condition, size and survival of planktotrophic echinoderm larvae that are reliant on exogenous nutrition to complete development and facilitate settlement (Basch and Pearse 1995). Larval starvation has been viewed as less important than predation in directly limiting larval supply (Pearse and Cameron 1991); however, for several echinoderm species, there is experimental evidence that larval nutrition influences the quality and quantity of planktotrophic larvae (Fenaux et al. 1994, Fabricius et al. 2010). Spatiotemporal variation in settlement rates of echinoderms has been attributed to differences in phytoplankton availability in the weeks or months preceding settlement (Hernández et al. 2010, García-Sanz et al. 2014, Glockner-Fagetti and Phillips 2020, Okamoto et al. 2020), suggesting that food availability may exert significant effects on planktotrophic echinoderm larvae. The qualitative effect of starvation on the condition of echinoderm larvae prior to and during settlement was confirmed by Basch and Pearse (1995), who demonstrated a strong effect on larval size, development, swimming and substrate searching behaviour of the asteroid *Asterina miniata*. Likewise, Pratchett et al. (2017) reported delays in peak settlement of *Acanthaster* cf. *solaris* at low food levels, which are likely reflective of delays in larval development. Thus, even if direct mortality due to starvation is unlikely under natural conditions, prolonged planktonic existence may increase the risk of predation and thus lead to higher total mortality, or advection to less suitable settlement sites (Thorson 1950). Conversely, a shortened planktonic phase, due to early metamorphosis of less developed echinoderm larvae in response to settlement cues, may also increase total mortality within a cohort of echinoderms (Mos and Dworjanyn 2016).

While a range of abiotic and biotic factors can influence the abundance and distribution of larval echinoderms, and thus the temporal and spatial variation in the supply of competent larvae, the individual roles and combined effect of these processes on larval settlement remain unclear. Further adding complexity to this puzzle, larval cloning has been described for some echinoderms (Balser 1998, Allen et al. 2019, Hart et al. 2021). If supported by environmental conditions, cloning has the potential to influence dispersal distance of planktonic larval stages and increase the number of larvae reaching metamorphic competence (Allen et al. 2019), which, in turn, may influence rates of settlement and recruitment (Hart et al. 2021).

Species with complex, planktonic, feeding larvae are common across all echinoderm classes, except for crinoids (McEdward and Miner 2001, Uthicke et al. 2009). However, all five classes also possess lecithotrophic larval types in both planktonic and benthic developmental habitats (McEdward and Miner 2001, Raff and Byrne 2006, Byrne and O'Hara 2017), with benthic development and brooding documented in some echinoids (Emlet et al. 1987), crinoids (Lahaye and Jangoux 1985), holothuroids (McEuen 1986) and ophiuroids (Ebert 1983). Importantly, the dispersal, condition and survival of planktonic echinoderm larvae appears to be governed by a suite of abiotic and biotic variables, which will, in turn, affect spatiotemporal patterns of settlement (Basch and Pearse 1995). When analysing the drivers of settlement patterns, however, the capability for long-distance dispersal also needs to be considered, especially for species with prolonged planktonic phases, as observed in asteroids of the genus *Luidia* which are known to spend >1 year in the water column as exceptionally large post-larvae (Wilson 1978, Domanski 1984).

2.2.3 Metamorphic competence and substratum search

Metamorphic competence is the developmental capacity of larvae to undergo complete morphogenesis that is necessary for settlement including metamorphosis (Hadfield et al. 2001). Such competence is conditional upon complete larval development, which requires a minimum prescribed, but species-specific, pre-competency period (Sutherby et al. 2012). Larval developmental rates and the minimum pre-competency period are, however, influenced by nutritional resources and temperature (Lucas 1982, Hadfield et al. 2001). In general, most planktotrophic larvae in the pelagic habitat attain competence after a few weeks to several months. For example, planktotrophic larvae of the echinoid *Strongylocentrotus purpuratus* acquire metamorphic competence after 4–6 weeks post-fertilisation (Strathmann 1987). Lecithotrophic echinoderm larvae may acquire competence within days (Pawlik 1992, Hadfield et al. 2001). Another echinoid, *Heliocidaris erythrogramma*, for example, has a highly reduced larva, which develops from large eggs and attains metamorphic competence within 3–6 days (Byrne et al. 2001).

While metamorphic (or settlement) competence is conditional upon larval development, metamorphosis itself is often induced by specific environmental conditions or cues (as discussed later). Importantly, echinoderm larvae may attain competence long before they actually settle, deferring metamorphosis until settlement opportunities present (Birkeland et al. 1971, Hadfield et al. 2001, Pratchett et al. 2017b). Extended competency periods are particularly pronounced for planktotrophic larvae (Hadfield et al. 2001), with larvae remaining in the water column until the presence of suitable habitat is signalled by abiotic or biotic cues, as opposed to immediate metamorphosis in a potentially unsuitable habitat. For example, short-term exposure to turbulence signalling proximity to reefs can cause echinoid larvae to immediately transition from a pre-competent to a competent state (Gaylord et al. 2013, Hodin et al. 2015, 2018, Ferner et al. 2019), which allows them to respond to chemical cues before and after they contact the substratum (Hodin et al. 2020). Similarly, histamine, a compound associated with various marine algae, has been shown to modulate metamorphic competence in *Strongylocentrotus purpuratus* (Sutherby et al. 2012). At the same time, this organic compound is hypothesised to maintain the developmental priming of competence once larvae are morphologically competent by inhibiting settlement in the absence of suitable cues (Sutherby et al. 2012). More specifically, histamine achieves the maintenance of competency in echinoid larvae by inhibiting caspase-mediated apoptosis (Sutherby et al. 2012), and thus suppressing the destruction of larval-specific structures necessary to settle and metamorphose until a suitable settlement site is found.

Throughout this competency period, larvae continue to live a functional planktonic life until it culminates in either passive deposition onto the substratum, or active selection of a suitable habitat/substratum (**Figure 2.1**). Likewise, larvae are thought to delay settlement in response to cues indicative of an unsuitable settlement site (Hadfield and Paul 2001), although inhibitory cues have rarely been explored in echinoderm studies. All ophiuroids and some groups of asteroids and holothuroids metamorphose in the plankton and then often settle indiscriminately (Wilson 1978, Domanski 1984, Hendler 1991, Komatsu et al. 2000, McEdward and Miner 2001, Selvakumaraswamy and Byrne 2004, Morgan and Jangoux 2005). However, other echinoderm larvae with a planktonic larval phase generally require physical or chemical settlement cues and subsequent contact with the substratum in order to metamorphose

(Hadfield and Paul 2001). Once a larva has the ability to discriminate cues, substratum choice appears to be primarily limited by the availability of suitable substrata, and the availability and concentration of pertinent cues in the water column. Waterborne chemical cues originating from conspecifics (Pearce and Scheibling 1990a, Dworjanyn and Pirozzi 2008) and various algal substrata (Williamson et al. 2000, Swanson et al. 2004) have been demonstrated to initiate settlement and subsequent metamorphosis in echinoderm taxa. Such cues can be highly specific (**Table 2.1**) and are mostly detected in relatively close proximity to their source organisms.

Passive transport is generally thought to be the major determinant in the dispersal of echinoderm larvae over broad spatial scales. Echinoid larvae are hypothesised to also use environmental signposts such as turbulence to narrow down potential areas of settlement at relatively localised scales (Gaylord et al. 2013, Hodin et al. 2020). Short-term behavioural responses of echinoid larvae to such signposts include the cessation of swimming, which facilitates sinking and contact with the substratum (Ferner et al. 2019). Although direct evidence of active vertical movement is sparse, the extremely fast sinking and substratum attachment in response to inductive settlement cues (**Tables 2.1, 2.2**) suggests that most competent echinoderm larvae do not randomly or passively sink to the bottom. Rapid sinking may also be a response to the high densities of planktivorous invertebrates and fish that associate with the substratum (Tegner and Dayton 1981, Balch and Scheibling 2001).

Other key physical variables with the potential to substantially alter settlement patterns are temperature and pH (Mos et al. 2011, Li et al. 2011b). Settlement pulses in echinoids, holothuroids and ophiuroids have been linked to temperature peaks and fluctuations (Balch et al. 1999, García-Sanz et al. 2014, Sotelo-Casas et al. 2016, Glockner-Fagetti and Phillips 2020). For example, larval settlement of the echinoid *Strongylocentrotus purpuratus* in southern California was orders of magnitude lower during warm, El Niño conditions (Okamoto et al. 2020), while settlement of *Diadema africanum* was found to be high during warm years in the Canary Islands (Hernández et al. 2010). However, these links could be driven by other factors, such as spawning induced by temperature change (Caballes and Pratchett 2017, Caballes et al. 2021), and need to be explicitly tested in field and laboratory experiments. In a laboratory study, settlement of the echinoid *Tripneustes gratilla* was unaffected by temperatures between 24 and 30°C but was substantially reduced at 33°C (Mos et al. 2011). Likewise, warmer temperatures reduced settlement rates in the holothuroid *Apostichopus japonicus* (Li et al. 2011b) and negatively affected development in settlement-stage individuals of the echinoid *Heliocidaris erythrogramma* (Byrne et al. 2011). Moreover, reduced settlement rates at lowered pH have been demonstrated in some echinoids (García et al. 2015), asteroids (e.g., Uthicke et al. 2013) and holothuroids (e.g., Li et al. 2011b). Low pH negatively affected morphological traits, but not settlement rates in the echinoid *Centrostephanus rodgersii*; however, high pH reduced settlement rates considerably (Mos et al. 2020). Conversely, the echinoids *Evechinus chloroticus* (Espinel-Velasco et al. 2020) and *Pseudechinus huttoni* (Houlihan et al. 2020) appeared unaffected by changes in pH. Settlement was delayed at reduced pH in the echinoid *Paracentrotus lividus* (García et al. 2015), but not in *Arbacia lixula* (Wangensteen et al. 2013) and *Heliocidaris erythrogramma* (Byrne et al. 2011). Espinel-Velasco et al. (2018) and Nelson et al. (2020) argued that decreases in seawater pH may affect microbial communities and thus also indirectly alter the settlement of echinoderms that respond to cues associated with biofilms. On balance, both passive processes and active vertical migration in response to environmental cues appear to play significant, but highly variable roles between the attainment of competence and the first contact with the substratum in planktonic echinoderm larvae.

2.2.4 Substratum contact, exploration and selection

Once a competent larva has found its way to the substratum, contact is established and attachment of the larva to the surface of the substratum occurs (Yamaguchi 1973, Hamel and Mercier 1996, Gosselin and Jangoux 1998). For many sessile marine invertebrates, attachment to a substratum may already represent the initiation of metamorphosis into their juvenile form (Hadfield and Paul 2001). Conversely, many motile species, including echinoderms, can detach and reattach to substrata while delaying metamorphosis and exploring the suitability of various settlement sites (Crisp 1984, Hadfield and Paul 2001, Scheibling and Robinson 2008, Vellutini and Migotto 2010). Competent larvae of the crown-of-thorns starfish *Acanthaster* cf. *solaris* (**Figure 2.2**) have been observed to drift downwards and flex the anterior body dorsally to orient their brachiolar arms towards the substratum in an attempt to test the suitability of substratum surfaces for settlement (Yamaguchi 1973). Similarly, the asteroid *Patiriella regularis* swims along the substratum surface and exhibits searching behaviour using its median brachium (Byrne and Barker 1991). Echinoid larvae (*Paracentrotus lividus*) interrupt the beatings of their epaulette cilia to reach the substratum, which is then tested by primary podia (Gosselin and Jangoux 1998, Flammang et al. 1998). Without appropriate cues, the larvae fold back their podia and ascend back into the water column via ciliary beating of the epaulettes (Gosselin and Jangoux 1998). In crinoids, the adhesive pit serves to attach the larval body to the substratum by developing the attachment disc of a juvenile (McEdward and Miner 2001). Upon first substratum contact with its tentacles, the holothuroid *Cucumaria frondosa* spent up to 40 hours searching the substratum surface for a suitable settlement site using its ambulacral podia (Hamel and Mercier 1996).

In most cases, specificity in echinoderm settlement is mediated by chemical cues associated with the biogenic substrate (Pawlik 1992, Hadfield and Paul 2001, Dworjanyn and Pirozzi 2008). Evidence of the inductive capacity of cues associated with conspecifics (e.g., Pearce and Scheibling 1990a), prey (e.g., Birkeland et al. 1971), algae (e.g., Williamson et al. 2020) and biofilms (Hugget et al. 2006) is highly abundant for echinoderms (**Table 2.1**). When such cues signal the suitability of a site, the cascade of settlement events, comprising initial adhesion (Crisp 1984) and metamorphic morphogenesis of echinoderm larvae, can occur rapidly (Juinio-Meñez and Bangi 2010, Swanson et al. 2012). If, however, no inductive cue is present, the explorative period may be prolonged (Hamel and Mercier 1996), and competent larvae may swim off in a broad-scale search for other surfaces. As a result, a larva may risk being exposed to benthic and sometimes planktonic predators during this period (Tegner and Dayton 1981, Cowan et al. 2016a,b), but the trade-off is an increased chance of settlement and post-metamorphic success.

Although their importance appears to be secondary to chemical cues, physical factors, such as light intensity and the physical properties associated with the substratum, may also play a significant role in settlement induction once a competent larva has contacted the substratum surface (Young and Chia 1984, Privitera et al. 2011). More specifically, sedimentation, the contour, microtopography, texture, exposure and the thermal capacity of a substratum may affect where competent larvae choose to settle, depending on taxon-specific habitat requirements and cue responsiveness (Young and Chia 1984, Walters and Wethey 1991, Hamel and Mercier 1996). For example, fine sediments have been identified as a potential inhibitor of settlement for *Evechinus chloroticus* (Phillips and Shima 2006, Walker 2007), while larvae of the crown-of-thorns starfish *Acanthaster* cf. *solaris* are thought to favour particular substrata because their rough texture and depressions suit their metamorphosing larvae (Lucas 1974, Ormond and Campbell 1974). Other asteroids (Barker 1977, Barker and Nichols 1983, Metaxas et al. 2008) and holothuroids (Hamel and Mercier 1996) predominantly settle on the underside of rocks and rubble. This pattern of substratum selectivity may be linked to light exposure, as settlers of the holothuroid *Cucumaria frondosa* not only showed strong selectivity for the undersides of the physical substratum but were highly sensitive to light (Hamel and Mercier 1996). When exposed to light, settlement of *C. frondosa* larvae was highest on rocky substrates with crevices, relatively high on gravel and smooth rocky substrates and substantially lower on mud, sand and glass (Hamel and Mercier 1996). Largely photopositive during their exploration and substratum selection, these larvae were observed to rapidly change their initial site of substratum contact and move to light sheltered areas on the substratum (Hamel and Mercier 1996). Similarly, *Patiriella regularis* asteroids settle on the undersides of substrata and appear to be light sensitive during the settlement phase (Byrne and Barker 1991). Given the generally high prevalence of light sensitivity across this phylum (Byrne and O'Hara 2017), light availability and intensity are likely also to play an underappreciated role in the substratum search and selection by other echinoderm groups.

2.2.5 Larval metamorphosis

Upon reception of metamorphic cues, larval metamorphosis, a complex network of cellular processes which involve the transformation and reorganisation of most tissues and cells, is induced (**Figure 2.3**, Pearse and Cameron 1991, Morgan and Jangoux 2005). All ophiuroids, and some asteroids, holothurians and echinoids are able to metamorphose in the water column and sink to the substratum in their juvenile form (Wilson 1978, Domanski 1984, Hendler 1991, McEdward and Miner 2001, Morgan and Jangoux 2005, Selvakumaraswamy and Byrne 2006). However, crinoids and most asteroid, echinoid and holothuroid groups, including the ecologically and economically important taxa discussed in this review, generally attach to a substratum prior to metamorphosis (Pearse and Cameron 1991, McEdward and Miner 2001). Metamorphosis in echinoderms begins with a larva initiating the degeneration of larva-specific characters and ends once all juvenile characters have emerged and the juvenile is fully functioning in its (usually benthic) habitat (*sensu* Hadfield et al. 2001). In general, this includes a transformation of the fundamental bilateral symmetry of echinoderm larvae into a juvenile form with a radial symmetry (**Figures 2.2, 2.3**; McEdward and Miner 2001). The numerous morphogenic events occurring during this period have been documented for some species, including echinoids (e.g., Mazur and Miller 1971), asteroids (e.g., Caballes and Pratchett 2014) and ophiuroids (e.g., Morgan and Jangoux 2005), and have been reviewed in detail for Echinoidea (e.g., Burke 1983a, Pearse and Cameron 1991). For example, competent (late brachiolaria) larvae of the asteroid *Acanthaster* cf. *solaris* attach to the substratum surface and metamorphose into their juvenile form by absorbing the larval body into the asteroid (**Figure 2.2**). Similarly, most echinoids must undergo substantial physiological changes concomitant with the transition from a pelagic and planktotrophic larva to a benthic and herbivorous juvenile (**Figure 2.3**).

Neural mechanisms for the induction of echinoderm metamorphosis have been supported by multiple lines of evidence (Sutherby et al. 2012, Caballes and Pratchett 2014). Specifically, neurons in the apical organ of competent echinoid larvae are suggested to detect exogenous metamorphic cues and translate that information into behavioural and physiological responses (Burke 1979, 1983b, Brandhorst and Bishop 2001). However, little is known about the causal relationships among the various cellular processes occurring during metamorphic transformation. In echinoids, inductive cues are likely transduced by epithelial cells via Ca^{2+}-dependent K^+ conductance, triggering the involution of the larval epithelium (Emlet 1988, Pearse and Cameron 1991). Few superficial organs have been characterised in echinoid larvae; however, cues for the nerve cells may be received through sensory cells on the surface of the podia of the echinoid rudiment (Burke 1980, Smith et al. 2008, Ullrich-Lüter et al. 2011, Valero-Garcia et al. 2016, Marconi et al. 2019). At the same time, the eversion of the urchin rudiment causes the musculoepithelial cells to contract, which alters its shape and everts the rudiment (Pearse and Cameron 1991, Vellutini and Migotto 2010). Once the larval epithelium is fully collapsed, the adult epithelium fuses on the aboral surface (Pearse and Cameron 1991, Vellutini and Migotto 2010). The enclosed larval cells then lose their attachments and degenerate (Cameron 1975, Chia and Burke 1978). Some tissues documented to break down soon after the start of metamorphosis include the epidermis, oesophagus, epaulettes, larval mouth and the ciliary bands, while the skeletal mesenchyme and larval gut are transformed into adult tissues (Chia and Burke 1978, Burke 1983a). Similar transformations occur in other echinoderm larvae, including many asteroids, echinoids and ophiuroids, which see adult rudiments unfolded and the larval body involuted into what will become the aboral surface of the juvenile body (**Figure 2.3**; Burke 1983a, Hadfield et al. 2001). In crinoids, the vestibule and associated ventral structures rotate towards the posterior end of the larvae; however, metamorphic transformations do not include a distinct adult rudiment oriented orthogonally to the larval plane of symmetry (**Figure 2.3**; McEdward and Miner 2001). Altogether, the complex network of these metamorphic processes, which are variable among taxa, transforms competent echinoderm larvae into fully functioning juveniles, which, in most cases, are able to live, move and feed in their benthic habitat.

2.2.6 Post-settlement processes

Newly settled echinoderms are generally subject to very high mortality within the first weeks to months of their benthic life stage (Gosselin and Qian 1997, Hunt and Scheibling 1997, Balch and Scheibling 2001). As such, our understanding of post-settlement processes is critical to determine how patterns established at settlement are altered, and the consequent effects on adult populations (Underwood and Fairweather 1989). Recruitment to juvenile and adult populations is highly variable in echinoderms, both temporally and spatially (reviewed by Ebert 1983, Balch and Scheibling 2001, Ebert 2021b), and plays a vital role in the dynamics of echinoderm populations and, in the case of ecologically important taxa, may substantially alter marine ecosystems (McClanahan 1988, Bonaviri et al. 2012, Pratchett et al. 2014). Settlement rates do not always explain patterns of distribution and abundance of conspecific juveniles and adults (Loosanoff 1964, Rowley 1989, Vermeij et al. 2010, Williams et al. 2010, 2011, Rogers and Lorenzen 2016), and adult population dynamics may be driven by early post-settlement processes, including high mortality rates soon after settlement. In the case of *Diadema antillarum* in the southern Caribbean Sea, for example, unknown sources of high post-settlement mortality may have prevented a recovery of the local adult population following the 1983 die-off, despite a substantial recovery of settlement rates (Vermeij et al. 2010).

Most early post-settlement mortality in echinoderms has been attributed to various invertebrate and fish predators (Sala and Zabala 1996, Sala et al. 1998, Hereu et al. 2005, Pederson and Johnson 2006, Clemente et al. 2009, Bonaviri et al. 2012, Cowan et al. 2017, Keesing et al. 2018). Small, recently emerged size classes are often most vulnerable to predation (McClanahan and Muthiga 1989, Pederson and Johnson 2006, Clemente et al. 2007, Ling and Johnson 2009), such as the size-dependent predation by rock lobsters on the kelp overgrazing echinoids *Heliocidaris erythrogramma* and *Centrostephanus rodgersii* (Pederson and Johnson 2006, Ling et al. 2009). Other factors that may contribute to the loss of post-settlement echinoderms from populations include starvation (Keesing and Halford 1992), disease (Hughes et al. 1985, Lessios 1988), abiotic disturbances (Cameron and Fankboner 1989), space competition (Hamel and Mercier 1996) and migration (Young and Chia 1982, Hamel and Mercier 1996), although movement over large distances is rare for small post-settlement juveniles (Rowley 1989). Of all post-settlement processes, mortality is generally viewed as having the greatest effect on recruitment to juvenile populations, but determining the relative contributions of these processes in the field has proven to be challenging (Andrew and Choat 1985, Balch and Scheibling 2001). Few studies have assessed post-settlement mortality of echinoderms under field conditions (but see Andrew and Choat

1985, Rowley 1990, Sewell and Watson 1993, Ling and Johnson 2012). Keesing et al. (2018) demonstrated the effect of predation on small post-settlement juveniles of *Acanthaster* cf. *solaris* in a caging experiment and developed a model of size- and age-dependent mortality to calculate a settlement rate threshold that leads to destructive population irruptions of this corallivorous asteroid. Quantitative estimates of both settlement rates and early post-settlement mortality may not only improve our understanding of the supply-side ecology of echinoderms (*sensu* Lewin 1986), but also help resolve priority research questions pertaining to these processes in ecologically or economically important species.

2.3 Settlement behaviour

Many echinoderm larvae that are competent to settle and metamorphose respond to chemical cues indicating the suitability of a substratum and environment for their benthic life-history stage. Settlement cues originate from a wide range of sources, and their characterisation appears critical to our understanding of spatiotemporal settlement patterns, and thus the supply-side ecology of echinoderm taxa (Hadfield and Paul 2001). Moreover, identifying mechanisms of settlement induction has applicability in the successful and efficient settlement of echinoderms in aquaculture settings (reviewed by Hodin et al. 2019). Chemical cues identified to induce echinoderm settlement generally fall under one of four categories: gregarious cues, associative cues (e.g., prey), biofilm-associated cues and neurotransmitter or ion-associated cues (**Tables 2.1, 2.2**). These cues, associated with various biotic sources, can be surface bound or waterborne cues and may thus act before the substratum is physically contacted by the larva or once this contact has been established (**Figure 2.1**). Initially, only surface-bound, non-polar settlement cues were considered capable of inducing larval settlement (Pawlik 1992), because the inductive effectiveness of waterborne cues was assumed to be hindered by rapid dilution and dispersion in the surrounding seawater, inhibiting larvae from reaching the appropriate substratum (Chia et al. 1984). More recently, numerous studies have shown that water-soluble cues associated with conspecifics or live substrata can function as *in situ* inducers of larval settlement and metamorphosis in echinoderms (e.g., Williamson et al. 2000, Swanson et al. 2004, Dworjanyn and Pirozzi 2008). In most echinoderms, a single inducer is expected to trigger the entire settlement process, including the initial substratum attachment and larval metamorphosis (e.g., Burke 1984, Pearce and Scheibling 1990b). However, in cases where the inductive compound has not been fully characterised, or only conditioned water treatments have been demonstrated to induce both settlement and metamorphosis, multiple chemical compounds may be present and affecting the two processes (Hadfield and Paul 2001).

Echinoid larvae settle readily in response to a wide range of natural chemical cues, including conspecifics, seagrasses, fleshy and coralline algae, and biofilms (**Table 2.1**). Echinoid larvae often respond to a suite of cues as opposed to a single chemical signal. Apart from a few species known to respond to cues associated with conspecifics (Highsmith 1982, Highsmith and Emlet 1986), chemical cue specificity is relatively low in echinoids. Likewise, chemical cues appear to be required for the induction of settlement and metamorphosis in asteroids, although this has only been demonstrated for few asteroid species of ecological importance (Birkeland et al. 1971, Barker 1977, Johnson et al. 1991, Johnson and Sutton 1994). In these studies, asteroids exhibited associative settlement in response to cues associated with polychaetes (Birkeland et al.

1971, Bryan 2004) and coralline algae with bacterial films (Johnson et al. 1991, Johnson and Sutton 1994). Few studies have examined possible settlement and metamorphic cues in holothuroids, ophiuroids and crinoids; however, some species of these echinoderm classes appear to exhibit gregarious settlement (**Table 2.1**). Holothuroids will settle without stimulation, but the presence of periphytic diatoms (Ito and Kitamura 1997, Agudo 2007), seagrass (Mercier et al. 2000) and macroalgal surfaces (Agudo 2007) can increase settlement and metamorphic success. More so than other echinoderms, competent ophiuroid larvae metamorphose spontaneously in the plankton and settle naturally in the absence of specific exogenous cues (Hendler 1991, Selvakumaraswamy and Byrne 2006, Hodin et al. 2019).

2.3.1 Gregarious settlement

Gregarious settlement, the settlement of larvae in response to cues from conspecific individuals, has been well documented in many benthic marine invertebrates (reviewed by Burke 1986). Sessile marine benthic invertebrates demonstrating chemically specific gregarious settlement have been studied extensively, and many of the chemical cues they respond to have been fully or partially characterised (Pawlik 1992, Hadfield and Paul 2001). Although the vast majority of echinoderms are motile, their locomotory capacities are often limited to relatively small spatial scales and some species are even considered effectively sessile (Young and Chia 1982). Gregarious settlement can thus be of high ecological importance. The presence of conspecifics likely indicates species-specific habitat suitability and may support post-settlement growth and survival due to prey availability, and suitable environmental conditions (Slattery 1997). In addition, population aggregations can potentially reduce mortality rates via predator swamping (Highsmith 1982, McCallum 1987) and enhance fertilisation rates and reproductive success (Crisp 1979, Babcock et al. 1994). At the same time, conspecific aggregations could increase competition for resources, especially for species with narrow ecological niches (Crisp 1979, Slattery 1997). Settlement and metamorphosis in response to conspecifics has been demonstrated in all classes of echinoderms except asteroids (**Table 2.1**). In contrast to many sessile marine invertebrate taxa, the specific chemical stimuli triggering these responses in echinoderms have not been characterised, except for one irregular echinoid (Highsmith 1982, Burke 1984).

Larvae of the sand dollar *Dendraster excentricus* preferentially settled and metamorphosed on sand from beds of adult conspecifics (Highsmith 1982, Burke 1984, Highsmith and Emlet 1986). The chemical cue produced by adults, which was extracted from sand that has been isolated for 7 weeks, effectively induced metamorphosis in *D. excentricus* (Highsmith 1982). Larval settlement responses to sand treated with proteolytic enzymes and sand in dialysis tubing indicated that the responsible chemical cue is a small peptide with low molecular mass (Highsmith 1982). This characterisation was confirmed by Burke (1984), who isolated a purified 980 Da peptide that induced metamorphosis from sand extracts using both gel permeation and liquid chromatography. However, even for purified inducers like this peptide, we do not have any information about its amino acid composition and structure, and thus the reason for its specificity in settlement response is unclear. Peptide pheromone signalling may be driving the settlement in response to substrata or water conditioned with conspecifics in other irregular (Pearce and Scheibling 1990a, Takeda 2008) or regular echinoids (e.g., Dworjanyn and Pirozzi 2008), but their chemical inducers have yet to be characterised. The sand dollar *Scaphechinus mirabilis* metamorphosed faster in adult-conditioned sand (Takeda 2008). Likewise, larvae of the sand dollar *Echinarachnius parma* showed a comparably high responsiveness to a water-soluble cue associated with sand conditioned with adult conspecifics in aquaria (Highsmith and Emlet 1986, Pearce and Scheibling 1990a). In the field, such specialised responses likely result in strongly preferential settlement of larvae in or near established sand dollar beds, demonstrating that settlement can occur onto populations of motile echinoderms that live in dense aggregations (Burke 1984). Small proportions of larvae of *E. parma* and *Dendraster excentricus* settled on non-conditioned substrata (Highsmith 1982, Burke 1984, Pearce and Scheibling 1990a) and newly settled *D. excentricus* have been observed outside of adult sand dollar beds in the field (Cameron and Rumrill 1982). Unlike in some sessile species (Nelson 1979), adult aggregations of these species can therefore not entirely be attributed to gregarious settlement of their larvae. However, the gregarious settlement of *D. excentricus*, *Scaphechinus mirabilis* and *Echinarachnius parma* is generally assumed to be contributing to an aggregated pattern in the distribution of adult populations.

In general, cue specificity is not high in echinoid settlement (Chia et al. 1984), and the sand dollar species mentioned above are assumed to be an exception to this rule. Accordingly, no evidence of gregarious settlement was found for the echinoid *Heliocidaris erythrogramma* (Huggett et al. 2006). Likewise, adult urchins, adult-conditioned seawater, their faecal matter and conspecific settlers did not induce larval metamorphosis in the echinoid *Strongylocentrotus droebachiensis* (Pearce and Scheibling 1991). A regular echinoid, *Tripneustes gratilla*, has been induced to settle in response to conspecific chemical cues (Dworjanyn and Pirozzi 2008). Larvae settled at high rates in the presence of conspecific juveniles and their faeces. Settlement of this echinoid, however, was also readily induced by cues associated with numerous types of seagrasses, algae, coralline algae and, specifically, bacterial biofilms (Dworjanyn and Pirozzi 2008). These results were corroborated in a second study, which reported settlement induction of *T. gratilla* larvae by adult conspecifics, however, at significantly lower rates than in response to macroalgal cues (Mos et al. 2011). This suggests that even echinoid species found to be highly responsive to conspecific cues probably settle in response to a wide range of biotic chemical cues in nature.

Evidence of gregarious settlement in holothuroids, crinoids, ophiuroids and asteroids is highly fragmented. Competent larvae of the holothuroid *Psolus chitonoides* preferred to settle in response to conspecifics in experimental studies (Young and Chia 1982). Field experiments reporting high settlement either directly on top or around the sole of conspecific adults substantiated the hypothesis of gregarious settlement in this holothuroid (Young and Chia 1982). Likewise, larval crinoids settling gregariously in culture (Mladenov and Chia 1983, Obuchi et al. 2010) are supported by reports of crinoid settlers found on top or associated with the cirri of conspecific adults (Mladenov and Chia 1983), suggesting that gregarious settlement in crinoids may be a common and overlooked phenomenon. Preferential settlement in the proximity of conspecific adults has also been reported in the ophiuroid *Ophiothrix fragilis* (Warner 1971, Morgan and Jangoux 2004, 2005). Larval metamorphosis in ophiuroids begins spontaneously in the plankton; however, the presence of adult conspecifics clearly enhances the loss of two posterolateral rods and larval settlement (Morgan and Jangoux 2005). The loss of their posterolateral rods near a brittle star bed increases the likelihood of larvae settling in a suitable habitat (Morgan and Jangoux 2005), and hooked spines on settlers without their posterolateral rods may help them cling directly onto adults (Morgan and Jangoux 2004). However, in cases where the habitat or dietary requirements of settlers and conspecific adults differ, larval echinoderms may instead settle in response to chemical cues associated with prey or substratum they require during their early post-settlement stage. In the case of motile echinoderms, these juveniles may later migrate into habitat utilised by conspecific adults. For example, larvae of the asteroid *Mediaster aequalis* settled in response to tubes of a polychaete worm that juveniles were frequently observed on, while the presence of juvenile *M. aequalis* failed to trigger a settlement response (Birkeland et al. 1971). Spines and tube feet of conspecifics did not affect settlement rates in larvae of *Acanthaster* cf. *solaris* (Henderson and Lucas 1971), although analyses of settlement preferences based on static choice chambers suggest that larvae may be attracted to adult conspecifics (Cowan et al. 2016a).

2.3.2 Associative settlement

Associative settlement, or settlement onto heterospecific plant or animal species (Crisp 1974), is seemingly widespread among echinoderms. Associative settlement can be broadly categorised based on predatory, herbivorous, parasitic and other non-parasitic associations between species (Pawlik 1992). In the case of echinoderms, this phenomenon most commonly refers to species responding to chemical cues associated with suitable habitat (Williamson et al. 2000, Ling et al. 2019) and potential prey species of herbivorous juveniles or adults (Rowley 1989, Pearce and Scheibling 1990b, 1991, Johnson et al. 1991).

Numerous species of echinoids, asteroids and holothurians have been found to settle and metamorphose in response to seagrass, coralline red algae, and red, green and brown fleshy algae (**Table 2.1**). However, the relative capacity of these substrata to induce settlement in echinoderm larvae varies greatly. For example, studies testing for differential settlement rates of echinoids in response to these substratum types reported that species of coralline red algae and red macroalgae generally induced the highest percentage of larval settlement, while the inductive capacity of brown algae, green algae and seagrasses was relatively low (Pearce and Scheibling 1991, Dworjanyn and Pirozzi 2008, Swanson et al. 2012). Moreover, chemical cue specificity is highly variable within and among echinoderm classes (**Table 2.1**). The echinoid *Tripneustes gratilla* was induced to settle by all 11 macroalgae tested including 6 brown algae, 1 green algae, 2 red algae and 3 coralline algae (Dworjanyn and Pirozzi 2008). In contrast, settlement in the asteroid *Mediaster aequalis* is induced predominantly by a specific chemical cue associated with the polychaete *Phyllochaetopterus prolifica* (Birkeland et al. 1971, Bryan 2004). In many cases, differences in the inductive capacity of natural substrata, as well as cue specificity of settlers, are likely linked to the presence or absence of specific chemical compounds in these substrata or animal prey. Purified compounds that induce associative settlement in echinoids have been isolated from red algae (Williamson et al. 2000, Swanson et al. 2004, 2006, 2012), coralline red algae (Kitamura et al. 1993, 1994) and endophytic microalgae (Taniguchi et al. 1994, Takahashi et al. 2002).

Naturally occurring settlement cues associated with red algae have been demonstrated for several ecologically important echinoid species (Rowley 1989, Pearce and Scheibling 1991, Williamson et al. 2000, Dworjanyn and Pirozzi 2008, Privitera et al. 2011), and chemical constituents of red algae that induce settlement and metamorphosis have recently been identified (Swanson et al. 2004, 2006, 2012). Newly settled echinoids of the species *Holopneustes purpurascens* are predominantly found on the red algae *Delisea pulchra* (Williamson et al. 2000, Swanson et al. 2006), although adult densities in the canopy of *D. pulchra* and the brown algae *Ecklonia radiata* are similar (Williamson et al. 2000). This pattern is hypothesised to be a response to a chemical cue produced by *Delisea pulchra*, as competent *Holopneustes purpurascens* larvae metamorphosed to cues associated with *Delisea pulchra*, but not the brown algae *Ecklonia radiata* (Williamson et al. 2000). The metamorphic cue was isolated and characterised by nuclear magnetic resonance spectroscopy as a water-soluble floridoside-isethionic acid complex (Williamson et al. 2000). Swanson et al. (2004) corrected this finding and identified the chemical identity of this cue produced by *Delisea pulchra* as histamine, by isolating the cue from the polar extract via cation-exchange chromatography. Corroborating this characterisation, the histamine content found in *D. pulchra* was reported to be one or more orders of magnitude higher than in the brown algae *Ecklonia radiata* and four other common species of fleshy and coralline algae (Swanson et al. 2004). Histamine exposure also appears to modulate metamorphic competence in other echinoids (Sutherby et al. 2012). With the first quantitative *in situ* measurements of a natural settlement cue in the habitat of a marine organism, Swanson et al. (2006) showed that seawater surrounding *Delisea pulchra* contained far higher concentrations of histamine than for all other fleshy and coralline algae tested. The effectiveness of histamine as a metamorphic cue was demonstrated further for the lecithotrophic larvae of the echinoids *Holopneustes inflatus* and *Heliocidaris erythrogramma*, and the planktotrophic larvae of *Centrostephanus rodgersii* (Swanson et al. 2012). Conversely, histamine had a weak or no inductive effect on larvae of *Heliocidaris tuberculata* and *Tripneustes gratilla* (Swanson et al. 2012). A lower specificity in the metamorphic cues of these two habitat-generalist herbivores may be due to a relatively low restriction in their habitat and prey selection as juveniles and adults (Swanson et al. 2012). In contrast, dissolved histamine that originates from red algae on other plants likely serves as an exogenous cue for competent larvae of more habitat-specialised species such as *Holopneustes purpurascens* and *H. inflatus*, signalling the presence of a suitable habitat in which to settle (Swanson et al. 2012). Cues associated with histamine certainly have a role in settlement and metamorphic processes of echinoderms, which may extend past this group of ecologically important echinoids.

Settlement and metamorphic responses to natural chemical cues in other echinoids and asteroids have been described for numerous species of coralline red algae (**Table 2.1**). Both articulate and encrusting assemblages of coralline red algae may be comparably or even more inductive to some larvae than fleshy red macroalgae (Dworjanyn and Pirozzi 2008, Swanson et al. 2012). For example, encrusting coralline species of the genus *Lithothamnion* induce settlement and metamorphosis in competent *Acanthaster* cf. *solaris* larvae (Johnson et al. 1991, Johnson and Sutton 1994), and the echinoids *Strongylocentrotus purpuratus* (Rowley 1989) and *S. droebachiensis* (Pearce and Scheibling 1990b, 1991). Similarly, many echinoid species settle and metamorphose in response to cues associated with the articulated corallines *Amphiroa anceps* (Williamson et al. 2000, Swanson et al. 2006, 2012, Dworjanyn and Pirozzi 2008) and *Corallina* spp. (Lamare and Barker 2001, Pearce and Scheibling 1990b, 1991, Kitamura et al. 1992, Mos et al. 2011). Purified compounds responsible for the induction of settlement and metamorphosis in two commercially important echinoid species, *Pseudocentrotus depressus* and *Anthocidaris crassispina*, have previously been characterised from lipophilic extracts of the coralline red alga *Corallina pilulifera* (Kitamura et al. 1992, 1993, 1994). Silica gel column chromatography demonstrated that simple lipids including free fatty acids were the inducers of this settlement response (Kitamura et al. 1992). Further chemical analyses of a mixture of free fatty acids detected in *C. pilulifera* revealed that larvae of both echinoids only underwent settlement and metamorphosis in response to eicosapentaenoic acid and arachidonic acid (Kitamura et al. 1993). Responses of both echinoid larvae to a wide range of saturated and unsaturated fatty acids were highly specific and suggested that only highly unsaturated fatty acids (dihomo-γ-linolenic acid and eicosatrienoic acid) triggered strong metamorphic larval responses (Kitamura et al. 1994), which appears to be a similar induction mechanism for other motile marine invertebrates (Pawlik and Faulkner 1986).

Different chemical compounds isolated from extracts of the endophytic green microalgae *Ulvella lens* have been identified to induce larval settlement and metamorphosis in the echinoids *Strongylocentrotus nudus* (Taniguchi et al. 1994, Takahashi et al. 2002) and *S. intermedius* (Takahashi et al. 2002). The compound dibromomethane, which was found in seawater in which *Ulvella lens* and three coralline algae species were cultured, is the principal inducer of settlement and metamorphic responses in *Strongylocentrotus nudus* (Taniguchi et al. 1994). Further investigating chemical cues associated with *Ulvella lens,* Takahashi et al. (2002) reported that settlement and metamorphic responses of *Strongylocentrotus nudus* and *S. intermedius* were only triggered by relatively high concentrations of dibromomethane, and a polyunsaturated fatty acid (eicosapentaenoic acid) did not induce settlement at all. Instead, several active compounds isolated from *Ulvella lens* extracts were identified as glycoglycerolipids (Takahashi et al. 2002), which are a class of cell wall-associated compounds abundant in marine algae (Tebben et al. 2015). These findings underline the importance of assessing taxa-specific inducers to gain an accurate understanding of the chemical cues driving the mechanisms and patterns of larval settlement in echinoderms.

2.3.3 Biofilm-induced settlement

Biofilms are complex and dense consortia of microorganisms, which accumulate on the surfaces of most marine substrata (Dobretsov 2010). Marine biofilms usually consist of various species of bacteria, *Archaea* and unicellular organisms such as diatoms (Rahim and Kitamura 2004, Dobretsov 2010) and commonly include secreted extracellular substances in which the cells of these component organisms are situated (Hadfield 2011). The reasons why competent larvae choose to settle on biofilms are not always clear. Biofilms may be a possible food source for some newly settled echinoderms (de la Uz et al. 2013), while in some cases, settlement is thought to occur in response to cues simply signalling the presence of a nontoxic surface, which accumulated a biofilm and microorganisms over time (Hadfield 2011). Most research on biofilm-induced settlement in echinoderms has focused on cues associated with biofilm bacteria (**Table 2.1**); however, echinoderm larvae have also been induced to settle by various diatoms (Ito and Kitamura 1997, Rahim and Kitamura 2004, Agudo 2007, Mos et al. 2011), which are single-celled algae commonly found in marine biofilm assemblages (Dobretsov 2010). Among echinoderm classes, the inductive capacities of marine biofilm consortia have been demonstrated in echinoids (e.g., Lamare and Barker 2001, Rahim and Kitamura 2004), asteroids (e.g., Barker 1977, Johnson and Sutton 1994) and holothuroids (e.g., Ito and Kitamura 1997).

Early reports of settlement induction in echinoderms by marine biofilms (e.g., Cameron and Hinegardner 1974, Barker 1977) have raised the question whether many of the natural chemical cues of echinoderm settlement attributed to marine algae and other live substrata may, in fact, be produced by marine bacteria and unicellular eukaryotes on the surfaces of these substrata. Differential inductive capacity of naturally occurring algal substrata with and without biofilms, as well as isolated cues from the biofilms and their compounds, has been investigated for echinoderm larvae (Pearce and Scheibling 1990b, Johnson et al. 1991, Johnson and Sutton 1994, Swanson et al. 2006, Dworjanyn and Pirozzi 2008, Mos et al. 2011). It appears that in some cases, only the biofilm is required to induce settlement (Swanson et al. 2006, Dworjanyn and Pirozzi 2008), while others demonstrated that the algal substratum, but not the biofilm (Pearce and Scheibling 1990b) or both (Johnson and Sutton 1994) were essential to produce the inductive response.

Antibacterial treatment of the coralline red algae *Amphiroa anceps* substantially reduced larval settlement rates of the echinoid *Holopneustes purpurascens* (Swanson et al. 2006). Here, a settlement cue from the coralline algae is proposed to be bacteria-derived histamine, which is released into the surrounding seawater. Two isolated bacterial strains, *Photobacterium phosphoreum* and *Thalassomonas viridans*, cultured from the surfaces of the corallines induced settlement and contained histamine, while no histamine was detected in coralline extracts (Swanson et al. 2006). Further supporting the hypothesis of Steinberg et al. (2001) that generalist herbivorous echinoids metamorphose in response to biofilms, Dworjanyn and Pirozzi (2008) demonstrated the necessity of bacterial film growing on algal surfaces in producing an inductive compound. The removal of bacteria from the surface of three macroalgae significantly inhibited settlement of *Tripneustes gratilla* larvae in response to these macroalgae (Dworjanyn and Pirozzi 2008), which demonstrates the larvae did not respond to cues originating from the algae per se. The echinoid *T. gratilla* appears to have very little specificity in its settlement cues and likely settles on a wide range of surfaces with bacterial films present. Conversely, larvae of the same echinoid species were induced to settle at substantially higher rates by the addition of macroalgae-conditioned seawater to biofilm treatments (Mos et al. 2011), which indicates that compounds associated with the alga itself may at least contribute to settlement induction. Bacteria-derived settlement cues as inducers of echinoid settlement were also not supported by Pearce and Scheibling (1990b). Here, antibiotic treatment of the coralline red algae *Lithothamnion glaciale* and subsequent reduction of surface bacteria by two orders of magnitude did not affect the capacity of *L. glaciale* to induce metamorphosis in the echinoid *Strongylocentrotus droebachiensis* (Pearce and Scheibling 1990b).

Substratum selection by crown-of-thorns starfish (*Acanthaster* cf. *solaris*) may also be influenced by chemical cues produced by epiphytic bacteria (Johnson et al. 1991, Johnson and Sutton 1994). Larval settlement and metamorphosis of this asteroid is inhibited in the absence of bacteria on highly inductive shards of the coralline alga *Lithothamnium pseudosorum* (likely *Lithothamnion* cf. *proliferum*), with settlement rates reduced to low levels when the coralline alga was treated with antibiotics (Johnson et al. 1991). Likewise, Yamaguchi (1973) reported that larval settlement of *Acanthaster* cf. *solaris* was not induced by bleached coralline algae. Moreover, settlers of this asteroid were only observed in sections of the coralline algal substratum with high densities of epiphytic bacteria, but not on the sections with little or no bacterial film cover (Johnson et al. 1991, Johnson and Sutton 1994). However, when isolated from soluble algal compounds, surface bacteria failed to induce settlement in *Acanthaster* cf. *solaris* larvae (Johnson and Sutton 1994). This demonstrates that either chemical compounds from both the coralline algae and the bacteria are essential to induce settlement and metamorphosis, or, more likely, the bacteria require the substratum from the alga to produce the inductive compound (Johnson and Sutton 1994).

Among a range of other marine bacteria identified to induce echinoderm settlement (**Table 2.1**), the bacterial genus *Pseudoalteromonas*, and especially the bacterium *P. luteoviolacea*, has been recognised as strongly inductive and important to biofilm-associated settlement (Hadfield 2011). Huggett et al. (2006) isolated 250 bacterial strains from coralline algae and tested their capacity to induce settlement in the echinoid *Heliocidaris erythrogramma*. Many strains of bacteria induced substantially higher settlement rates than the corallines, and *Pseudoalteromonas* dominated highly inductive strains (Huggett et al. 2006). This culture-based result was supported by an assessment of larval responses to characterised biofilms in the field, which reported the highest metamorphic rates for the highly inductive *P. luteoviolacea* (Huggett et al. 2006). Other bacterial genera represented in highly inductive strains were *Photobacterium, Shewanella* and *Vibrio*; however, the same genera were also abundant in low inducers (Huggett et al. 2006). This indicates that the inductive capacity of marine bacteria is species-specific, and the settlement response of echinoderm larvae to bacterial films is likely determined by its specific bacterial composition. Altogether, this body of research suggests that the role of biofilms as cues in the settlement behaviour and metamorphosis of echinoderm larvae may be much more complex and widespread than previously suspected and certainly warrants further research.

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
Gregarious settlement cues			
Peptide	*Echinoidea*	*Dendraster excentricus*	*Highsmith (1982), Burke (1984), Highsmith and Emlet (1986)*
Not characterised	*Echinoidea*	*Echinarachnius parma, Scaphechinus mirabilis, Tripneustes gratilla*	*Highsmith and Emlet (1986), Pearce and Scheibling (1990a), Takeda (2008), Dworjanyn and Pirozzi (2008), Mos et al. (2011)*
	Ophiuroidea	*Ophiothrix fragilis*	*Warner (1971), Morgan and Jangoux (2004), Morgan and Jangoux (2005)*
	Holothuroidea	*Molpadia intermedia, Psolus chitonoides*	*Young and Chia (1982), McEuen and Chia (1985)*
	Crinoidea	*Antedon bifida, Florometra serratissima, Dorometra sesokonis*	*Mladenov and Chia (1983), Lahaye and Jangoux (1985), Obuchi et al. (2010)*
Associative settlement cues			
Seagrass			
Enhalus acoroides	*Holothuroidea*	*Holothuria scabra*	*Mercier et al. (2000), Agudo (2007)*
Halophila ovalis	*Echinoidea*	*Holopneustes purpurascens, H. inflatus*	*Swanson et al. (2012)*

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
Posidonia australis	*Echinoidea*	*Holopneustes purpurascens, H. inflatus*	*Swanson et al. (2012)*
Posidonia oceanica	*Echinoidea*	*Paracentrotus lividus*	*Privitera et al. (2011)*
Thalassia hemprichii	*Holothuroidea*	*Holothuria scabra*	*Mercier et al. (2000), Agudo (2007)*
Zostera capricorni	*Echinoidea*	*Holopneustes purpurascens, H. inflatus*	*Swanson et al. (2012)*
Red algae			
Chondrus crispus	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Delisea pulchra	*Echinoidea*	*Holopneustes purpurascens*	*Williamson et al. (2000), Swanson et al. (2004, 2006)*
Carbohydrate		*Holopneustes purpurascens*	*Williamson et al. (2000)*
Floridoside-isethionic acid			
Histamine		*Holopneustes purpurascens, H. inflatus, Heliocidaris erythrogramma, Centrostephanus rodgersii*	*Swanson et al. (2004, 2006, 2012)*
Laurencia rigida	*Echinoidea*	*Holopneustes purpurascens*	*Williamson et al. (2000)*
Laurencia obtusa, L. rigada, Laurencia sp.	*Echinoidea*	*Centrostephanus rodgersii, Tripneustes gratilla*	*Dworjanyn and Pirozzi (2008), Swanson et al. (2012)*
Mastocarpus stellatus	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Palmaria palmata	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
Polysiphonia harveyi, P. lanosa	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Solieria robusta	*Echinoidea*	*Holopneustes purpurascens*	*Williamson et al. (2000)*
Red algal turf	*Echinoidea*	*Strongylocentrotus purpuratus, Paracentrotus lividus*	*Rowley (1989), Privitera et al. (2011)*
Coralline red algae			
Amphiroa anceps	*Echinoidea*	*Centrostephanus rodgersii, Holopneustes purpurascens, H. inflatus, Heliocidaris erythrogramma, Tripneustes gratilla*	*Williamson et al. (2000), Huggett et al. (2006)* *Swanson et al. (2006, 2012), Dworjanyn and Pirozzi (2008)*
Corallina elongata	*Echinoidea*	*Arbacia lixula, Echinocardium cordatum, Paracentrotus lividus*	*Nunes and Jangoux (2008), Privitera et al. (2011)*
Corallina pilulifera	*Echinoidea*	*Pseudocentrotus depressus, Anthocidaris crassispina*	*Kitamura et al. (1992, 1993, 1994)*
Polyunsaturated fatty acids			*Kitamura et al. (1993, 1994)*
Eicosapentaenoic acid			*Kitamura et al. (1993)*
Arachidonic acid			*Kitamura et al. (1993)*
Dihomo-γ-linolenic acid			*Kitamura et al. (1994)*
Eicosatrienoic acid			*Kitamura et al. (1994)*

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
Corallina officinalis	*Echinoidea*	*Centrostephanus rodgersii, Heliocidaris erythrogramma, Holopneustes purpurascens, Strongylocentrotus droebachiensis, Tripneustes gratilla*	*Pearce and Scheibling (1990b, 1991), Williamson et al. (2000), Huggett et al. (2006), Dworjanyn and Pirozzi (2008), Mos et al. (2011), Swanson et al. (2012)*
Corallina sp.	*Echinoidea*	*Evechinus chloroticus*	*Lamare and Barker (2001)*
Lithothamnion californicum	*Echinoidea*	*Strongylocentrotus purpuratus*	*Rowley (1989)*
Lithothamnion glaciale	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1990b, 1991)*
Lithothamnion sp. (likely *L.* cf. *proliferum*)	*Asteroidea*	*Acanthaster cf. solaris*	*Johnson et al. (1991), Johnson and Sutton (1994), Uthicke et al. (2018)*
Lithophyllum incrustans	*Echinoidea*	*Arbacia lixula, Paracentrotus lividus*	*Privitera et al. (2011)*
Lithophyllum yessoense	*Echinoidea*	*Strongylocentrotus nudus*	*Taniguchi et al. (1994)*
Dibromomethane			*Taniguchi et al. (1994)*
Mesophyllum insigne	*Asteroidea*	*Stichaster australis*	*Barker (1977)*
Neogoniolithon fosliei	*Asteroidea*	*Acanthaster cf. solaris*	*Johnson et al. (1991)*
Phymatolithon laevigatum, R. rugulosum	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1990b)*
Coralline red algae spp.	*Echinoidea*	*Echinometra mathaei, E. oblonga, E.* spp.*, Strongylocentrotus droebachiensis, Tripneustes gratilla*	*Rahman and Uehara (2001), Dworjanyn and Pirozzi (2008), Scheibling and Robinson (2008)*
Non-coralline crustose red algae			

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
Hildenbrandia rubra	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Brown algae			
Dictyota dichotoma	*Echinoidea*	*Centrostephanus rodgersii, Tripneustes gratilla*	*Dworjanyn and Pirozzi (2008)* *Swanson et al. (2012)*
Dilophus marginatus	*Echinoidea*	*Centrostephanus rodgersii, Tripneustes gratilla*	*Dworjanyn and Pirozzi (2008), Swanson et al. (2012)*
Ecklonia radiata	*Echinoidea*	*Heliocidaris erythrogramma, Holopneustes purpurascens, Tripneustes gratilla*	*Huggett et al. (2006), Swanson et al. (2006), Dworjanyn and Pirozzi (2008)*
Fucus distichus	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Homeostrichus olsenii	*Echinoidea*	*Holopneustes purpurascens, Tripneustes gratilla*	*Swanson et al. (2006), Dworjanyn and Pirozzi (2008)*
Laminaria digitata and *L. longicruris*	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Pylaiella littoralis	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Sargassum linearifolium	*Echinoidea*	*Centrostephanus rodgersii, Heliocidaris erythrogramma, Tripneustes gratilla*	*Huggett et al. (2006), Dworjanyn and Pirozzi (2008), Mos et al. (2011), Swanson et al. (2012)*
Sargassum vestitum	*Echinoidea*	*Heliocidaris erythrogramma*	*Huggett et al. (2006)*
Sargassum sp.	*Echinoidea*	*Tripneustes gratilla*	*Juinio-Meñez and Bangi (2010)*
	Holothuroidea	*Holothuria scabra*	*Agudo (2007)*

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
Stypocaulon scoparium	*Echinoidea*	*Paracentrotus lividus*	*Privitera et al. (2011)*
Zonaria angustata	*Echinoidea*	*Tripneustes gratilla*	*Dworjanyn and Pirozzi (2008)*
Green algae			
Codium fragile	*Echinoidea*	*Heliocidaris erythrogramma*	*Huggett et al. (2006)*
Enteromorpha linza	*Echinoidea*	*Paracentrotus lividus*	*Gosselin and Jangoux (1996)*
Enteromorpha sp.	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Spongomorpha aeruginosa	*Echinoidea*	*Strongylocentrotus droebachiensis*	*Pearce and Scheibling (1991)*
Ulva lactuca	*Echinoidea*	*Tripneustes gratilla*	*Dworjanyn and Pirozzi (2008)*
Endophytic microalgae			
Ulvella lens	*Echinoidea*	*Apostichopus japonicas, Strongylocentrotus nudus*	*Taniguchi et al. (1994), Takahashi et al. (2002), Matsuura et al. (2009)*
Dibromomethane	*Echinoidea*	*Strongylocentrotus nudus*	*Taniguchi et al. (1994)*
Glycoglycerolipids	*Echinoidea*	*Strongylocentrotus intermedius, S. nudus*	*Takahashi et al. (2002)*
Polychaete			
Phyllochaetopterus prolifica	*Asteroidea*	*Mediaster aequalis*	*Birkeland et al. (1971), Bryan (2004)*
Biofilm-associated cues			
Bacteria			

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
Photobacterium phosphoreum	*Echinoidea*	*Holopneustes purpurascens*	*Swanson et al. (2006)*
Photobacterium spp.	*Echinoidea*	*Heliocidaris erythrogramma*	*Huggett et al. (2006)*
Pseudoalteromonas luteoviolacea, other *Pseudoalteromonas* spp.	*Echinoidea*	*Heliocidaris erythrogramma*	*Huggett et al. (2006)*
Shewanella spp.	*Echinoidea*	*Heliocidaris erythrogramma*	*Huggett et al. (2006)*
Thalassomonas viridans	*Echinoidea*	*Holopneustes purpurascens*	*Swanson et al. (2006)*
Vibrio spp.	*Echinoidea*	*Heliocidaris erythrogramma*	*Huggett et al. (2006)*
Other/unspecified bacteria	*Echinoidea*	*Arachnoides placenta, Arbacia punctulata, Heliocidaris erythrogramma, Lytechinus pictus, Tripneustes gratilla*	*Cameron and Hinegardner (1974), Huggett et al. (2006), Dworjanyn and Pirozzi (2008), Mos et al. (2011)*
	Asteroidea	*Acanthaster cf. solaris*	*Johnson et al. (1991), Johnson and Sutton (1994)*
Periphytic diatoms			
Achnanthes sp.	*Holothuroidea*	*Apostichopus japonicus*	*Ito and Kitamura (1997)*
Amphora sp.	*Holothuroidea*	*Apostichopus japonicus*	*Ito and Kitamura (1997)*
Navicula sp.	*Holothuroidea*	*Holothuria scabra, Apostichopus japonicus*	*Ito and Kitamura (1997), Agudo (2007)*
Nitzschia spp.	*Echinoidea*	*Tripneustes gratilla*	*Mos et al. (2011)*

Table 2.1 Summary of natural chemical cues known to induce larval settlement and metamorphosis in echinoderm classes and species.

Chemical stimulus	Class	Species	Reference
	Holothuroidea	*Holothuria scabra, Apostichopus japonicus*	*Ito and Kitamura (1997), Agudo (2007)*
Platymonas sp.	*Holothuroidea*	*Holothuria scabra*	*Agudo (2007)*
Multiple species (*predominantly Navicula, Amphora, Achnanthes and Nitzschia* spp.)	*Echinoidea*	*Anthocidaris crassispina, Pseudocentrotus depressus*	*Rahim and Kitamura (2004)*
Unspecified biofilm	*Echinoidea*	*Anthocidaris crassispina, Evechinus chloroticus, Pseudocentrotus depressus, Tripneustes gratilla*	*Lamare and Barker (2001), Rahim and Kitamura (2004), Mos et al. (2011)*
	Asteroidea	*Stichaster australis, Coscinasterias calamaria*	*Barker (1977)*

2.3.4 Neurotransmitter- and ion-induced settlement

Aside from the natural chemical cues associated with conspecifics, prey species and biofilms, various ‘artificial’ compounds have been identified to trigger larval settlement in echinoderms (**Table 2.2**). These inducers include numerous neurotransmitters (e.g., dopamine), neurotransmitter precursors (e.g., L-DOPA) and ions (e.g., potassium), which support the role of neuronal receptors in initiating a settlement response (Rodriguez et al. 1993). Although these neuroactive compounds and ions may not always act as predominant cues of settlement in nature, their study furthers our understanding of chemoreception, neural control and the mechanisms driving larval response (Slattery 1997). Investigating and understanding their effects is of considerable interest, particularly their potential to improve settlement success and survival rates in echinoderm aquaculture (Slattery 1997, Nontunha et al. 2021).

2.3.4.1 Amino acid derivatives

Several amino acid derivatives have been shown to effectively induce settlement and metamorphosis in echinoderms, including glutamine (Yazaki and Harashima 1994, Naidenko 1996), gamma aminobutyric acid (GABA; Pearce and Scheibling 1991, Rahman and Uehara 2001, Bryan 2004, Sun et al. 2014, Nontunha et al. 2021), glutamic acid (Naidenko 1996) and monosodium glutamate (Nontunha et al. 2021). Glutamine is an essential amino acid and the precursor of GABA, an inhibitory neurotransmitter. Levels of GABA are enhanced by glutamic acid, which, in turn, is often used in the form of its sodium salt, monosodium glutamate (Nontunha et al. 2021). Induction of metamorphosis by glutamine has been observed in the echinoid species *Pseudocentrotus depressus* (Yazaki and Harashima 1994), *Scaphechinus mirabilis* and *Strongylocentrotus intermedius* (Naidenko 1996). Glutamic acid was also shown to induce metamorphosis in some echinoid species (*Scaphechinus mirabilis* and *Strongylocentrotus intermedius*; Naidenko 1996), but not all (*Pseudocentrotus depressus*; Yazaki and Harashima 1994) and had a toxic effect on *Scaphechinus mirabilis* and *Strongylocentrotus intermedius*, which was possibly caused by a decrease in seawater pH from 8.4 to 7.5 at a glutamic acid concentration of 100 µg/mL (Naidenko 1996). Monosodium glutamate (MSG), a glutamic acid derivative, induced larvae of the holothuroid *Holothuria scabra* to settle at high rates, and MSG was also shown to be the least toxic in comparison with other neurotransmitters tested (Nontunha et al. 2021). Another neuroactive compound, serotonin, which is a derivative of tryptophan, failed to trigger any settlement and metamorphosis response in the Japanese holothuroid *Apostichopus japonicus* (Sun et al. 2014, Matsuura et al. 2009) and the Pacific sand dollar *Dendraster excentricus* (Burke 1983b).

GABA is an inhibitory neurotransmitter, which can produce depolarisation of cells capable of activating metamorphosis (Baloun and Morse 1984). Induction by this endogenous chemical cue is known to occur in a wide range of benthic marine invertebrates (Slattery 1997) and has thus been extensively tested for numerous sea echinoids, asteroids and holothuroids (**Table 2.2**), although results are variable both among and within echinoderm classes. Five echinoid species, including *Echinometra mathaei*, *E. oblonga* and *Strongylocentrotus droebachiensis*, have been found to metamorphose in response to GABA (Pearce and Scheibling 1990b, Rahman and Uehara 2001). On the other hand, no effect at all was observed in *Dendraster excentricus* (Burke 1983b) and the echinoid *Pseudocentrotus depressus* (Yazaki and Harashima 1994). Likewise, bioassays with GABA induced settlement and metamorphosis in larvae of the asteroid *Mediaster aequalis* (Bryan 2004), but not the crown-of-thorns starfish, *Acanthaster* cf. *solaris* (Johnson et al. 1991). Holothurians have successfully been induced by a wide range of neurotransmitters (**Table 2.2**), including GABA (Sun et al. 2014, Nontunha et al. 2021). GABA induced high rates of settlement in *Holothuria scabra* larvae, while a pair of studies found this compound to trigger larval settlement behaviour (Sun et al. 2014), but not metamorphosis (Yazaki and Harashima 1994) in *Apostichopus japonicus*. The highly variable results encountered in the response of echinoderm larvae to GABA may explain why this neurotransmitter has not been generally used as an inducer of settlement and metamorphosis in the aquaculture of commercially important echinoderm taxa.

2.3.4.2 Tyrosine derivatives

Catecholamines (dopamine, epinephrine and norepinephrine) and their precursor levodopa (L-DOPA) are tyrosine derivatives with numerous biological functions, such as structural proteins, hormones and neurotransmitters. As in many other marine benthic invertebrates, these tyrosine derivatives are reliable inducers of larval settlement in echinoderms (**Table 2.2**). Epinephrine and norepinephrine are similar tyrosine derivatives that act as neurotransmitters and hormones. Epinephrine has been shown to initiate metamorphosis, but not settlement, in the Japanese holothuroid *Apostichopus japonicus,* while norepinephrine initiates both settlement behaviour and metamorphosis (Matsuura et al. 2009, Sun et al. 2014). Conversely, neither compound was found to be an effective inducer of larval metamorphosis in the echinoid *Dendraster excentricus* (Burke 1983b). Dopamine and L-DOPA, which increases dopamine neurotransmission, have been identified as the most efficient chemical cues to induce settlement of *Apostichopus japonicus* (Matsuura et al. 2009, Sun et al. 2014), *Holothuria scabra* (Nontunha et al. 2021) and the sand dollar *Dendraster excentricus* (Burke 1983b). These findings suggest that dopamine acts as the chemical transmitter during settlement induction in various echinoderm taxa. This endogenous chemical cue is thought to either directly stimulate larval tissues containing the effectors of metamorphosis, or indirectly trigger it by inducing the release of substances stimulating these tissues

(Burke 1983b). The interference and inhibition of metamorphosis by reserpine (Burke 1983b), a compound known to deplete catecholamines, further supports this conclusion.

2.3.4.3 Choline derivatives

The neurotransmitter acetylcholine is the sole choline derivative documented to induce effective larval settlement in an echinoderm, the holothuroid *Apostichopus japonicus* (Sun et al. 2014). This neuroactive compound, however, failed to induce larval metamorphosis in the sand dollar *Dendraster excentricus* (Burke 1983b). Choline, a precursor to acetylcholine, is a bound constitute of membranes surrounding cells. In contrast to acetylcholine, this compound had no significant inductive effect on *Apostichopus japonicus* larvae (Sun et al. 2014); however, it has been suggested to have an indirect effect on holothuroid settlement by participating as precursors in acetylcholine biosynthesis rather than by acting directly on the cholinergic receptors (Rodriguez et al. 1993). Like some catecholamine derivatives, choline derivatives are thus generally unlikely to directly induce echinoderm settlement and metamorphosis in nature by acting directly on cholinergic receptors, but instead more likely act as precursors in the synthesis and release of neurotransmitters (Hirata and Hadfield 1986, Slattery 1997).

2.3.4.3 Ions

In addition to neurotransmitters and their precursors, some ions can stimulate larval settlement in echinoderms (**Table 2.2**). The sensory bases of induction indicate that the nervous system plays a significant role in the cascade of events comprising benthic marine invertebrate settlement and metamorphosis (Burke 1983b). The successful transmission of neural signals relies on the electrical potential across cell membranes, which, in turn, is affected by the permeability of these membranes to ions (Rodriguez et al. 1993). Hence, any ions or compounds that affect ionic transport through cell membranes can trigger inductive responses, and their potential effects on echinoderm larvae should be assessed.

The highly inductive effects of potassium ions (K^+) on echinoderm settlement have been documented, particularly for echinoids (**Table 2.2**). At least 15 echinoid species from seven families have been induced to settle and metamorphose at variable potassium chloride concentrations within 1 hour of exposure (Hodin et al. 2019). More broadly, the inductive effects of potassium have also been observed in the holothuroid *Apostichopus japonicus* (Sun et al. 2014). Increased exogenous K^+ likely activates settlement responses through a depolarisation of externally accessible cells, which are directly involved in the recognition of natural inducers (Yool et al. 1986). Tetraethyl ammonium, a compound that selectively occludes potassium channels, inhibits metamorphosis (Rodriguez et al. 1993), which suggests that potassium could act through this channel in echinoderm larvae. Conversely, no settlement was induced by high concentrations of potassium in larvae of the crown-of-thorns starfish *Acanthaster* cf. *solaris* (Johnson et al. 1991).

Other ions that may trigger settlement and metamorphosis in echinoderms are calcium (Ca^{2+}), ammonium (NH_4^+) and magnesium (Mg^{2+}). Calcium has been implicated in the transmission or modulation of signals in the echinoid *Lytechinus variegatus* (Cameron et al. 1989) and other marine invertebrate larvae (Yool et al. 1986, Yu et al. 2008). Sun et al. (2014) reported inductive effects of Ca^{2+} on the settlement behaviour of *Apostichopus japonicus* larvae; however, no obvious effect on settlement success was found. Larvae of this holothuroid have also been induced to settle in response to ammonium ions (NH_4^+), although ammonium was concluded to be a relatively ineffective inducer in comparison with potassium (Sun et al. 2014). Magnesium ions appear to have no effect on *A. japonicus* larvae (Sun et al. 2014), which corroborates previous conclusions in other benthic marine invertebrates (Baloun and Morse 1984, Yool et al. 1986). Conversely, an inhibitory effect of Mg^{2+} was documented in the echinoid *Lytechinus variegatus* (Cameron et al. 1989). Here, excess magnesium interferes with echinoid metamorphosis in the presence of inductive biofilms by competing with Ca^{2+} for binding molecules involved with the release of neurotransmitters (Cameron et al. 1989). Similar inhibitory effects of magnesium may be found in *Apostichopus japonicus* or other echinoderm larvae if tested in the presence of metamorphically active biofilms.

Multiple lines of evidence support the notion that ionic fluxes play a role in the induction of larval settlement and metamorphosis (Cameron et al. 1989). Because of their inductive capacity, some of these ions, especially potassium, are more commonly utilised as agents in the cultivation of numerous benthic marine invertebrates, replacing the use of neurotransmitters such as GABA or L-DOPA (Rodriguez et al. 1993).

Table 2.2 Summary of ‘artificial’ chemical cues (i.e., neurotransmitters and ions) known to induce larval settlement and metamorphosis in echinoderm classes and species.

Compound	Class	Species	Reference
Amino acid derivatives			
Glutamine	Echinoidea	*Scaphechinus mirabilis*, *Strongylocentrotus intermedius*, *Pseudocentrotus depressus*	Yazaki and Harashima (1994), Naidenko (1996)
γ-Amino butyric acid (GABA)	Echinoidea	*Echinometra mathaei*, *E. oblonga*, *E.* spp., *Strongylocentrotus droebachiensis*	Pearce and Scheibling (1990a), Rahman and Uehara (2001)
	Asteroidea	*Mediaster aequalis*	Bryan (2004)
	Holothuroidea	*Apostichopus japonicas*	Sun et al. (2014)
		Holothuria scabra	Nontunha et al. (2021)
Glutamic acid	Echinoidea	*Scaphechinus mirabilis*, *Strongylocentrotus intermedius*	Naidenko (1996)
Monosodium glutamate	Holothuroidea	*Holothuria scabra*	Nontunha et al. (2021)
Tyrosine derivatives			
Dopamine	Echinoidea	*Dendraster excentricus*	Burke (1983b)
	Holothuroidea	*Apostichopus japonicus*, *Holothuria scabra*	Matsuura et al. (2009), Sun et al. (2014), Nontunha et al. (2021)
L-3,4-Dihydroxyphenylalanine (L-DOPA)	Echinoidea	*Dendraster excentricus*	Burke (1983b)
	Holothuroidea	*Apostichopus japonicas*	Matsuura et al. (2009), Sun et al. (2014)
		Holothuria scabra	Nontunha et al. (2021)

Table 2.2 Summary of 'artificial' chemical cues (i.e., neurotransmitters and ions) known to induce larval settlement and metamorphosis in echinoderm classes and species.

Compound	Class	Species	Reference
Epinephrine	Holothuroidea	*Apostichopus japonicus*	Matsuura et al. (2009)
Norepinephrine	Holothuroidea	*Apostichopus japonicus*	Matsuura et al. (2009)
			Sun et al. (2014)
Choline derivative			
Acetylcholine	Holothuroidea	*Apostichopus japonicus*	Sun et al. (2014)
Ions			
K^+	Echinoidea	*Clypeaster rosaceus*, *Colobocentrotus atratus*, *Dendraster excentricus*, *Diadema antillarum*, *Echinarachnius parma*, *Echinometra lucunter*, *E. viridis*, *Heterocentrotus mamallatus*, *Leodia sexiesperforata*, *Lytechinus* variegatus, *Mellita tenuis*, *Mesocentrotus franciscanus*, *Strongylocentrotus droebachiensis*, *S. fragilis*, *S. pallidus*, *S. purpuratus*	Cameron et al. (1989), Pearce and Scheibling (1994), Carpizo-Ituarte et al. (2002), Heyland et al. (2004, 2006), Heyland and Hodin (2004), Gaylord et al. (2013), Hodin et al. (2015, 2019)
	Holothuroidea	*Apostichopus japonicus*	Sun et al. (2014)
Ca^{2+}	Echinoidea	*Lytechinus variegatus*	Cameron et al. (1989)
NH_4^+	Holothuroidea	*Apostichopus japonicus*	Sun et al. (2014)

2.4 Sampling methods for the study of settlement patterns

A variety of sampling methods have been trialled and used to study echinoderm settlement patterns (**Figure 2.4**); however, there are no standard or established sampling techniques used among or even within echinoderm classes (**Tables 3.3, 3.4, 3.5, 3.6**). The extremely small size and often cryptic nature of recently settled echinoderms has greatly constrained progress in the study of this important life-history stage in their natural environments. In the past, inter-annual settlement patterns of some species have commonly been inferred based on the distribution and abundance of juveniles or adults (e.g., Zann et al. 1987). However, as demonstrated for the asteroids *Asterias forbesi* (Loosanoff 1964) and *Acanthaster* cf. *solaris* (Keesing and Halford 1992), and the echinoid *Strongylocentrotus purpuratus* (Rowley 1989), high larval settlement rates do not always give rise to high rates of recruitment to juvenile and adult populations. Conventional field-based studies usually only detect newly settled individuals that have had time to grow (e.g., Wilmes et al. 2020b), such that it is very likely that differential post-settlement survival will have already modified patterns of abundance established at settlement. Visual underwater searches within quadrats or transect belts have reported densities of settlers soon after predicted settlement for echinoids (Hunte and Younglao 1988, Agatsuma et al. 1998), asteroids (Wilmes et al. 2020b) and holothuroids (Hamel and Mercier 1996). Densities of recently settled ophiuroids have mostly been studied using various grab samplers, including mouse-trap samplers, Van Veen grab samplers, sled samplers and sediment traps (Gage and Tyler 1981a,b, Muus 1981, O'Connor et al. 1983, Künitzer 1989, Sumida et al. 2000). These techniques usually limit ophiuroid studies to few and short sampling intervals (**Table 2.5**); however, the logistical difficulties of sampling such cryptic settlers, particularly in deep-sea habitats, do not allow extensive sampling designs as seen in some shallow-water echinoderm taxa (e.g., Ebert et al. 1994). Conventional underwater surveys, inferences based on adult densities or back-calculations based on size distributions (e.g., Yokoyama and Amaral 2011) almost invariably lack data on early post-settlement processes, including mortality rates, to effectively represent settlement patterns (Keesing et al. 2018). Mortality may be very high during this period due to individuals adapting to the new benthic habitat and exposure to a new suite of predators (Wilmes et al. 2018), necessitating novel approaches of sampling individuals during or soon after settlement.

To address the inherent limitations in recording settlement in natural substrates and habitats, various artificial settlement collectors have been employed (Loosanoff 1964, Bak 1985, Harrold et al. 1991, Keesing et al. 1993, Ebert et al. 1994, Lamare and Barker 2001, Uthicke et al. 2019). It is not clear how rates of settlement and early post-settlement survival differ between these artificial collectors and natural substrata, such that the collectors only allow relative comparisons of settlement potential, as opposed to absolute rates of larval settlement (Keesing et al. 1993). Early deployment, prior to anticipated settlement, to allow for extensive pre-conditioning and colonisation of the collectors could mitigate, but not resolve this limitation. If absolute rates of settlement had to be obtained (e.g., for stock-recruitment modelling), experimental assays comparing settlement rates and early post-settlement mortality on natural and conditioned artificial substrata could provide an indication of differential inductive capacity and survival. Potential differences could be then applied when calculating settlement rates for a reef area based on settler densities found in artificial collectors. In any case, these artificial collectors facilitate separation of settlement from post-settlement processes to produce a more accurate census of settlers and have thus been widely used in recent decades. More than two-thirds of the 53 studies on echinoderm settlement patterns summarised here used some form of artificial settlement collector (**Figure 2.4**), and some of these studies compared the relative efficiency of multiple collector designs for quantitative assessments of echinoderm settlement rates (Lamare and Barker 2001, Hereu et al. 2004, García-Sanz et al. 2012, Balsalobre et al. 2016). In general, these artificial samplers are designed to maximise microhabitat complexity and surface area, which is favourable for settlement and metamorphosis (Hunte and Younglao 1988, Harrold et al. 1991, Keesing et al. 1993). Moreover, these methods minimise disturbance to the benthos and can be easily deployed and retrieved for variable sampling durations and intervals.

Artificial turf panels, scrubbing brushes and some other similar collector designs are commonly used to assess spatiotemporal settlement patterns in echinoids (**Figure 2.4**), but have also been shown to facilitate settlement of ophiuroids, asteroids and holothuroids in the field (Wing et al. 1995a, Balch et al. 1999, Balch and Scheibling 2000, Jennings and Hunt 2010). Both types of sampling devices are usually suspended vertically in the water column, either from overhanging ledges from shore, or anchored to the substratum. Harrold et al. (1991) examined *Strongylocentrotus purpuratus* settlement using pieces of PVC pipe, which were filled with either high surface area plastic matrix or articulated coralline algae and suspended 1 m above the substratum. Higher densities of settlers were recorded in the designs using the plastic matrix, although this may have been due to a higher number of predators in the coralline algal collectors (Harrold et al. 1991). This design was later modified by Miller and Emlet (1997) and Balch et al. (1998) who lined PVC pipes with plastic turf, which is an effective settlement surface for echinoids (Harris et al. 1994) and has frequently been used in subsequent studies (Balch and Scheibling 2000, Lambert and Harris 2000, Jennings and Hunt 2010). Balch and Scheibling (2000) demonstrated the utility of suspending collectors in the water column for mitigating the potential effects of early post-settlement mortality due to predation, as substantially more ophiuroid settlers were collected in the high (2.3 m off bottom) than in the low (0.2 m) collectors.

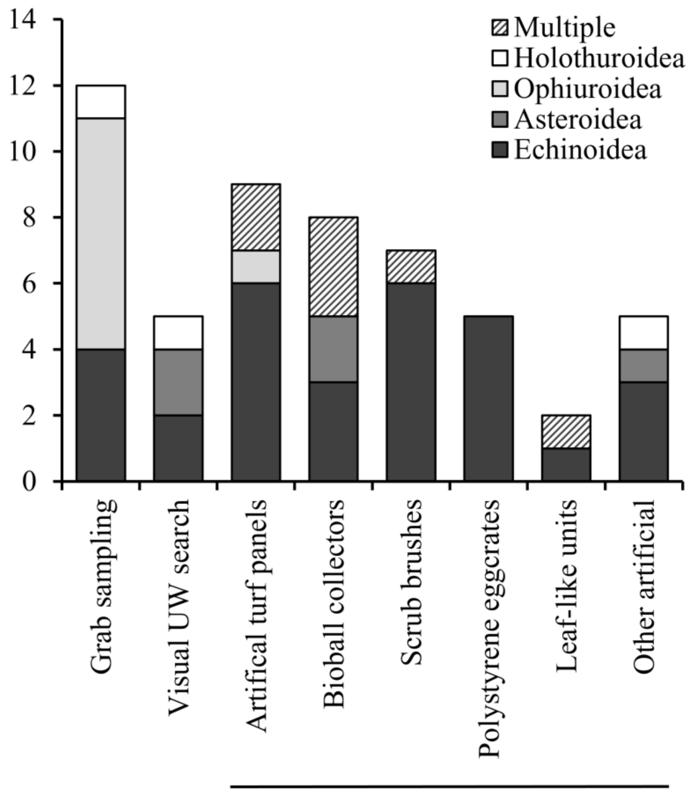

Figure 2.4 Frequency distribution of sampling methods used in 53 studies that assessed spatial and/or temporal patterns of larval settlement in one or multiple classes of echinoderms. The horizontal line indicates the sampling methods utilising artificial settlement collectors.

Scrubbing brushes with nylon bristles were first used by Ebert et al. (1994) to document settlement rates of the echinoids *Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus*. Due to their ease of use and cost-effectiveness, scrubbing brushes are still widely used (e.g., Schroeter et al. 1996, Okamoto et al. 2020). Studying *Evechinus chloroticus* settlement in New Zealand, Lamare and Barker (2001) compared scrubbing brush samplers (Ebert et al. 1994), AstroTurf panels (Ebert et al. 1991) and PVC pipes filled with plastic matrix (Harrold et al. 1991) and found the unstandardised mean number of settlers recovered from the PVC pipe design to be three- to fivefold higher than from the other two sampler designs. Conversely, wooden scrubbing brushes with vegetal bristles recorded considerably higher numbers of settlers than oyster shells, artificial grass and plastic matrix in trials measuring *Paracentrotus lividus* settlement in the Western Mediterranean Sea (Hereu et al. 2004). These comparative assessments reveal that it is probably not viable to compare settlement rates measured using contrasting methods, although most methods in and of themselves will provide insights into settlement patterns.

The settlement collectors designed by Keesing et al. (1993) consisted of a net bag containing 100 plastic aquarium filter bioballs, which provide an extremely high surface area, and successfully sampled settlers of numerous species of asteroids, ophiuroids, crinoids and echinoids on Australia’s Great Barrier Reef. Laboratory trials demonstrated that competent larvae of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) readily settle on plastic surfaces, which had been pre-conditioned in seawater aquaria (Keesing et al. 1993). Aquarium filter bioballs have since been incorporated in various modified versions of these collectors to efficiently sample settlement in a diverse range of habitats and different echinoderm species (Hernández et al. 2006, 2010, Clemente et al. 2009, Uthicke et al. 2019, Glockner-Fagetti and Phillips 2020, Doll et al. 2021 – **Chapter 5**). The suitability of this method to sample settlement rates in a wide range of echinoderms was corroborated by quantitative comparisons of multiple collector designs (Balsalobre et al. 2016); nylon nets containing plastic bioballs showed higher reproducibility and efficiency in sampling settlement than vertically oriented scrubbing brushes with vegetal bristles, or horizontal triangular mats of coconut fibre. Other artificial settlement collectors shown to effectively sample echinoid and ophiuroid species include settlement plates consisting of polystyrene egg crates and plexiglass (Bak 1985, Miller et al. 2009, Vermeij et al. 2010, Rogers and Lorenzen 2016), and various designs of leaf-like units consisting of plastic mesh frames and plastic raffia (García-Sanz et al. 2012, 2014).

The biggest constraint to using artificial settlement collector methods is the post-collection process time required to remove, sort and identify newly settled echinoderms, which are often microscopic (Keesing et al. 1993). Moreover, reliable visual identification of newly settled individuals may not be possible, especially if large numbers of sympatric species exist within sampling regions (Keesing et al. 1993). Innovative genetic analyses (e.g., eDNA) have emerged as a promising tool to address these constraints and further the utility of artificial settlement collectors to advance the understanding of settlement patterns (Uthicke et al. 2019, Doll et al. 2021 – **Chapter 5**). Doll et al. (2021 – **Chapter 5**) successfully detected newly settled *Acanthaster* cf. *solaris* in settlement collectors using taxon-specific mtDNA primers and standard polymerase chain reaction (PCR) and validated the utility of this molecular-based approach in assessing spatiotemporal settlement patterns of this asteroid. If developed as a quantitative sampling tool, this method would be of particular utility for large-scale, high replicate studies of settlement for ecologically important taxa, and echinoderm groups for which reliable visual identification is not possible.

Although artificial settlement collectors cannot be regarded as providing absolute numbers of echinoderm settlers, they certainly provide comparable estimates of spatial and temporal variation in settlement and can complement *in situ* sampling of older juveniles in natural habitats. Simultaneous sampling using both these approaches will allow for improved discrimination between settlement and post-settlement processes (Harrold et al. 1991). Overall, artificial settlement collectors provide a relatively non-destructive sampling method and standardised index to study spatial and temporal settlement patterns for most echinoderm groups. Genetic methods are also likely to reduce processing time and greatly enhance capacity to assess spatiotemporal variability in settlement.

2.5 Spatial and temporal patterns of settlement

2.5.1 Echinoidea

Marked seasonality in settlement has been documented in many echinoid taxa, and this pattern appears to be consistent among different habitat types and climate zones (**Table 2.3**). Species of the genera *Strongylocentrotus* and *Mesocentrotus*, commonly studied along the California coast (Ebert et al. 1994, Wing et al. 1995a, Miller and Emlet 1997, Okamoto et al. 2020) and in the western Atlantic (Balch and Scheibling 2000, Lambert and Harris 2000, Jennings and Hunt 2010), predominantly settle between April and July each year. Along the California coast, unimodal settlement peaks or primary settlement pulses occurred in June (Miller and Emlet 1997, Okamoto et al.

2020) and July (Wing et al. 1995a). This timing coincides with warmer (or increasing) water temperatures, suggesting that temperature-induced spawning, as observed in other echinoderms (e.g., Caballes et al. 2021), may be governing this pattern. In contrast, Harrold et al. (1991) reported two distinct peaks in the settlement of *Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus* in Monterey Bay, occurring from April through July and December through February, respectively. Ebert et al. (1994) recorded multiple annual settlement pulses, however, all between February and July each year. As part of a 27-year study, Okamoto et al. (2020) demonstrated annual settlement peaks during April and May and strong seasonal settlement fluctuations, which were highly synchronous among sites within Southern California locations. Here, temporal patterns of larval settlement were linked to fluctuations in ocean temperature and climate, which may impact pre-settlement processes and larval supply in *S. purpuratus* (Okamoto et al. 2020). Strong intra-annual variability in settlement rates was further reported for *Evechinus chloroticus* in New Zealand (Lamare and Barker 2001, Walker 2007, Glockner-Fagetti and Phillips 2020). Likewise, major settlement peaks of the echinoid *Paracentrotus lividus* only occurred between February and April on the Canary Islands (García-Sanz et al. 2014) and between April and June in the Western Mediterranean Sea (Hereu et al. 2004, Tomas et al. 2004). On tropical and subtropical reefs, the echinoid *Diadema antillarum* settles continuously, but in low numbers throughout the year (Bak 1985, Miller et al. 2009, Williams et al. 2010), with substantial settlement pulses detected in the spring and autumn months in Curaçao (Bak 1985, Vermeij et al. 2010), and in July (Williams et al. 2010) or August (Millet et al. 2009) in Puerto Rico. Settlement of *D. africanum* follows a clear unimodal pattern, with a main settlement peak between August and October (Hernández et al. 2006, 2010, García-Sanz et al.

2014), which is probably linked to highly seasonal spawning events in the months prior (Hernández et al. 2006). This strong seasonal settlement pattern is likely directly or indirectly driven by temperature; however, settlement rates may also be negatively affected by reduced food availability in the months prior to settlement (Hernández et al. 2010).

While the timing of annual peaks in the settlement of most echinoids is relatively consistent among years, inter-annual variability in the magnitude of these pulses is generally high (**Table 2.3**). Settlement of *Strongylocentrotus nudus* occurred annually at relatively low densities off the north-west coast of Japan, except for a strong settlement pulse in 1984 and some smaller pulses in 1990–1992 and 1995 (Agatsuma et al. 1998). Highly variable settlement among years was also documented for *Mesocentrotus franciscanus and Strongylocentrotus purpuratus* off the California coast (Miller and Emlet 1997, Okamoto et al. 2020), and *S. droebachiensis* in Nova Scotia (Balch and Scheibling 2000) and Bocabec Cove, Canada (Jennings and Hunt 2010). Similarly, Tomas et al. (2004) and Hereu et al. (2004) reported substantial inter-annual differences in settlement of *Paracentrotus lividus* in the Mediterranean Sea by one and more than two orders of magnitude, respectively. Conversely, settlement rates of *Diadema antillarum* were relatively similar among years in the southern Caribbean Sea, although rates were only monitored over three consecutive years (Bak 1985). High inter-annual variability in *D. africanum* settlement at Canary Islands locations between 2002 and 2008 was likely driven by temperature or food availability during the planktonic larval phase (Hernández et al. 2010). Likewise, Glockner-Fagetti and Phillips (2020) suggested that settlement pulses, which varied considerably among years, were correlated with seawater temperature and chlorophyll concentration. These field studies did not explicitly demonstrate the causative nature of these links; however, the potential effects of starvation (Byrne et al. 2008) and temperature (McEdward 1984, Privitera et al. 2011) on the condition of echinoderm larvae prior and during settlement indicate that these processes may, in fact, be driving temporal settlement patterns.

A range of abiotic factors, including hydrodynamic forces, appear to be driving spatial differences in settlement rates of echinoids, particularly at large scales. Jennings and Hunt (2010) described highly variable settlement of *Strongylocentrotus droebachiensis* among sampling locations. As highest settler densities occurred at the site closest to the larger body of water, larvae transported to the area by currents would reach this site first and settle if competent and stimulated by an inductive cue (Jennings and Hunt 2010). The geographical position in relation to hydrodynamic processes may be an important factor in explaining larger-scale spatial variation in the settlement of other *Strongylocentrotus* species (Wing et al. 1995b, Agatsuma et al. 1998), and echinoids more broadly (Lamare and Barker 2001, Balsalobre et al. 2016). For example, the settlement of *S. droebachiensis* in the Gulf of Maine was one and three orders of magnitude higher than on Nova Scotia’s Atlantic Coast and the Bay of Fundy, respectively, indicating substantial differences in larval supply to these locations (Balch et al. 1998). Synchrony in settlement pulses of *S. purpuratus* among geographical subunits off the California coast may be governed by physical processes affecting larval supply at regional scales (Ebert et al. 1994, Okamoto et al. 2020), although *Mesocentrotus franciscanus* did not form such geographic subunits with respect to coherence in settlement pulses (Ebert et al. 1994). At a smaller spatial scale, Clemente et al. (2009) found similar numbers of *Diadema africanum* settlers among sampling locations and sites in the Canary Islands and concluded that larval supply is unlikely to be limiting *D. africanum* settlement at any of the sampled locations. Settler densities of echinoid species, including *Mespilia globulus* and *Echinometra* sp., were generally high across ten sites at Davies Reef on Australia’s Great Barrier Reef, although settlement was considerably greater at the front reef sites than on the back reef sites (Keesing et al. 1993). In contrast, settlement rates of *Diadema antillarum* in the southern Caribbean Sea were higher in sheltered lagoons and back reefs than in fore reef habitats (Rogers and Lorenzen 2016). Comparing settlement rates of *Evechinus chloroticus* in the Hauraki Gulf, New Zealand, Walker (2007) found no settlers at a sheltered, high-sediment location, while settler densities were high, although spatiotemporally variable, at a wave-exposed, low-sediment location.

Larval supply and the exposure to physical factors thus appear to also play a role in determining settlement patterns at smaller spatial scales. However, with decreasing spatial scale, taxon-specific chemical cues have been shown to also commonly play an important role in the processes surrounding larval settlement and thus relative rates of settlement at varying sites, habitats and depths. Hereu et al. (2004) found settlement rates of *Paracentrotus lividus* to relate to wave exposure and currents, but not substratum type or adult densities, suggesting settlement patterns were not driven by chemical stimuli. Conversely, settlement of *P. lividus* was found to be generally higher on vertical rock wall habitats than in seagrass meadows at the same location in the Western Mediterranean Sea (Tomas 2004). García-Sanz et al. (2014) reported the same species to preferentially settle on soft substrata including seagrass, as opposed to hard substrata such as barrens and macroalgal beds, in the Canary Islands. Competent *P. lividus* larvae readily settle in response to chemical cues associated with various substrata (**Table 2.1**), including seagrass, brown macroalgae and coralline red algae

(Privitera et al. 2011). Thus, the presence of chemical stimuli associated with inductive substrata may be driving these contrasting patterns at different locations.

Settler densities of *Strongylocentrotus droebachiensis* were higher in barren grounds than in adjacent seagrass beds (Balch et al. 1998). Larvae of this species respond to numerous types of coralline algae and red, brown and green fleshy algal species (Rowley 1989, Pearce and Scheibling 1990b, 1991). The main food source of *Strongylocentrotus* spp. is brown macroalgae, with occasional omnivory (Tegner 2001, Scheibling and Hatcher 2007). Competent *S. droebachiensis* larvae are likely induced to settle by a chemical cue associated with their preferred prey or conspecifics present in the urchin barrens, but not the adjacent seagrass beds. Similarly, the relatively high densities of *Diadema africanum* in urchin barren habitats, compared to macroalgal beds (Hernández et al. 2010, García-Sanz et al. 2014) and/or seagrass meadows (García-Sanz et al. 2014), may be driven by gregarious settlement cues, which have been demonstrated in other echinoid species (Highsmith 1982, Burke 1984, Highsmith and Emlet 1986, Pearce and Scheibling 1990a, Dworjanyn and Pirozzi 2008, Mos et al. 2011, Ling et al. 2019). Differential settlement of echinoids along depth gradients has only been assessed in two studies. Larvae of both *Paracentrotus lividus* and *Strongylocentrotus droebachiensis* preferentially settled at shallow depths, with decreased settlement rates reported below 10 m (Harris et al. 1994, Hereu et al. 2004). In the case of *P. lividus*, this pattern is likely governed by physical processes or cues affecting larval movement or behaviour, as the results of this study suggest that the rocky substratum, although colonised by a rich algal assemblage, is not a cue for its settlement (Hereu et al. 2004).

2.5.2 Asteroidea

Available evidence suggests the settlement of asteroids is also highly variable within and among years (**Table 2.4**), although much of the research to date has focused on two genera, the corallivorous crown-of-thorns starfish (*Acanthaster* spp.) on coral reefs (Zann et al. 1987, Keesing et al. 1993, Uthicke et al. 2019, Wilmes et al. 2020b, Doll et al. 2021 – **Chapter 5**), and asteroids of the genus *Asterias* in North America (Loosanoff 1964, Balch and Scheibling 2000, Jennings and Hunt 2010). Loosanoff (1964) monitored settlement rates of the temperate asteroid *Asterias forbesi* using shells inside collectors at Long Island Sound for bi-weekly intervals from 1937 to 1961. Settlement of *A. forbesi* predominantly occurred between early July and early September for a mean duration of 7 weeks; however, both the annual timing and the duration of this settlement period varied among years (Loosanoff 1964). This high inter-annual variability of settlement in this asteroid was further confirmed by Jennings and Hunt (2010) off Canada’s Atlantic Coast. Here, settlement of *A. forbesi* and *A. rubens* peaked around the same time in 2005, from early August to early September, but settler densities were considerably lower in the last year (Jennings and Hunt 2010). Likewise, Balch and Scheibling (2000) reported generally high, but variable settlement among years for *A. forbesi* and *A. vulgaris* in the same region. Temporal trends, such as the highly variable settlement among sampling years of *Coscinasterias muricata* in New Zealand, are likely governed by the timing of pre-settlement processes and variable larval supply, as settlement pulses of *C. muricata* were positively correlated with larval food availability and seawater temperature (Glockner-Fagetti and Phillips 2020). Similar biotic and abiotic factors may be driving inter-annual differences in settlement observed in other asteroid species, such as *Stichaster australis* in New Zealand (Barker

1979). In Fiji, Zann et al. (1987) observed a major settlement pulse of *Acanthaster* cf. *solaris* in only one of the nine sampling years from 1979 to 1987. The low number of settlers recorded in most years was suggested to represent ‘normal’ settlement events, while spikes in the abundance of settlers in some years may give rise irruptive population growth (Zann et al. 1987).

Temporal settlement variability within (Uthicke et al. 2019) and among years (Doll et al. 2021 – **Chapter 5**) has only recently been explicitly demonstrated for crown-of-thorns starfish. However, the marked temporal patterns and propensity to undergo major population irruptions displayed by *Acanthaster* cf. *solaris* (Pratchett et al. 2014) are most likely linked to differential rates of larval supply (Birkeland 1982, Brodie et al. 2005), settlement and subsequent early post-settlement processes. It is unclear whether strong pulses and inter-annual differences in the settlement of both *A.* cf. *solaris* and *Asterias* spp. are driven by adult population density fluctuations of these species (Uthicke et al. 2009), or whether it is, in fact, settlement patterns that drive these adult population fluctuations in the first place.

Settlement patterns of both *Acanthaster* cf. *solaris* and *Asterias* spp. are also highly variable among regions and locations throughout their geographical ranges (Loosanoff 1964, Jennings and Hunt 2010, Doll et al. 2021 – **Chapter 5**). In the case of *Acanthaster* cf. *solaris*, spatial patterns of larval settlement on the Great Barrier Reef likely depend on spatiotemporal availability of larvae, driven by the latitudinal progression of their population irruptions, with regional estimates of settlement coinciding with densities of adult asteroids (Doll et al. 2021 – **Chapter 5**). Settlement of asteroid species is also highly variable at smaller spatial scales. Settlement of *Coscinasterias muricata* was high at two sites inside the Wellington Harbour, New

Zealand, but completely absent at the three sites on the southern coast and the mouth of the harbour (Glockner-Fagetti and Phillips 2020). At this localised scale, such contrast in settlement success may be driven by substratum-related cues, as shell cover was exclusively found in the harbour and correlated with echinoderm settlement pulses overall (Glockner-Fagetti and Phillips 2020).

Similarly, physical or chemical cues acting at the site or habitat scale may also play a role in the smaller-scale settlement patterns displayed by these asteroids (Keesing et al. 1993, Balch and Scheibling 2000, Wilmes et al. 2020b). For example, densities of juvenile *Acanthaster* cf. *solaris* (approx. 6 months post-settlement) were positively related to the cover of rubble, and more commonly observed in fore reef as opposed to back reef habitats (Keesing et al. 1993, Wilmes et al. 2020b). Settlement of competent *A.* cf. *solaris* larvae in response to cues from some crustose coralline algae species (preferred settlement substratum and juvenile prey; Wilmes et al. 2020a) and associated biofilms are well established (Johnson et al. 1991, Johnson and Sutton 1994). Thus, the availability of rubble beds encrusted with certain species of crustose coralline algae appears to govern the settlement patterns of this species within reefs. The high occurrence of coral rubble supporting epilithic crustose coralline algae at the base of reef slopes in deep water has led to the hypothesis that *A.* cf. *solaris* larvae predominantly settle on these deep-water habitats (Johnson et al. 1991). However, both their preferred substratum and settlers are also commonly found in shallow water environments in other studies (Wilmes et al. 2020b, Doll et al. 2021 – **Chapter 5**). Doll et al. (2021 – **Chapter 5**) found no difference in settlement among three depth ranges sampled between 4 and 12 m, and records of newly settled *A.* cf. *solaris* across a wide range of depths (1–18 m) (Wilmes et al. 2020b) support the conclusion that *A.* cf.

solaris readily settle in shallow water environments. Similarly, settlement rates of *Asterias forbesi* were remarkably similar among three shallow water depths (3–9 m) assessed at any given year (Loosanoff 1964). These studies suggest that it is the presence of particular reef substrata and associated chemical cues, rather than depth, that is driving reef-scale settlement patterns of *Acanthaster* cf. *solaris* and other asteroids showing strong substratum selectivity.

2.5.3 Ophiuroidea

Compared to other echinoderm classes, the magnitude and timing of ophiuroid settlement appears to be relatively consistent (**Table 2.5**). Studies on *Ophiocten gracilis* (Gage and Tyler 1981a, Sumida et al. 2000) and *Ophiura ljungmani* (Gage and Tyler 1981b) indicate that settlement in these deep-sea ophiuroids occurs annually at the Rockall Trough in the East Atlantic. Highest densities are generally recorded during early summer in May and June, across a wide range of deep-sea sampling depths (Gage and Tyler 1981a, Sumida et al. 2000). Spatial settlement patterns remain largely unexplored; however, a range of physical processes affecting larval supply and advection in deep-sea environments may generally be driving recruitment in these species (Ebert 1983). Ophiuroid larvae can randomly metamorphose in the plankton and then settle indiscriminately (Hendler 1991, McEdward and Miner 2001, Morgan and Jangoux 2005, Selvakumaraswamy and Byrne 2006), which suggests that chemical cues associated with the substratum are unlikely to play an important role, especially given that such deep-sea habitats are characterised by relatively uniform soft substrata (Gage and Tyler 1981a,b).

Consistent annual settlement seasons have also been observed in ophiuroid species at relatively shallow depths in the North Sea and North-East Atlantic Ocean (Muus 1981, O'Connor et al. 1983, Künitzer 1989). Settlement of *Amphiura filiformis* in Øresund, Denmark, occurred annually between August and December and peaked during a short 2- to 6-week window in September to November (Muus 1981). The seasonal settlement pattern displayed by *A. filiformis* was consistent among temperate locations, as settlement in the North Sea, German Bight and off the Irish coast occurred and peaked during the same months (O'Connor et al. 1983, Künitzer 1989). Similarly, larval settlement of *Ophiopholis aculeata* and *Ophiura* spp. was observed in Nova Scotia, Canada, from July to early August each year (Balch and Scheibling 2000). Here, spatial differences in settler densities between sites appear to be driven by variable larval supply due to differences in hydrodynamic conditions, although settlement rates were also higher in urchin barren grounds than in seagrass beds (Balch and Scheibling 2000). Importantly, spatiotemporal settlement patterns and recruitment may also be influenced by cloning of ophiuroid larvae, if environmental conditions support planktonic existence and dispersal (Balser 1998, Hart et al. 2021). Settlement of *Ophiothrix fragilis* in the Western Mediterranean Sea took place in several patches and peaked in June (Turon et al. 2000). This ophiuroid species is thought to settle gregariously at times (Warner 1971, Morgan and Jangoux 2004, Morgan and Jangoux 2005), which may enhance the chance of settlement by competent larvae in suitable habitats; however, this link has not been demonstrated in field-based studies.

2.5.4 Holothuroidea

While there has been extensive research on settlement induction (and cues) for sea cucumbers (discussed above), as necessary for aquaculture, there have been few field-based studies that examine settlement patterns or early life-history processes (**Table 2.6**). Settlement of *Australostichopus mollis* was only detected at one of five sites in the Wellington region in New Zealand during two sampling years (Glockner-Fagetti and Phillips 2020). Settler densities of holothuroids in Bocabec Cove, Canada, including *Psolus fabricii* and *Cucumaria frondosa*, varied considerably between and within sampling years (Jennings and Hunt 2010). However, settlement patterns did not vary spatially, as high pulses occurred in July 2005 at multiple sites, likely driven by currents differentially affecting larval supply at different points in time (Jennings and Hunt 2010). Newly settled *Cucumaria frondosa* were predominantly found at shallow depths (0–20 m) along a rocky subtidal depth gradient, while settlers were rare and absent in intermediate (20–40 m) and deeper waters (40–60 m), respectively (Hamel and Mercier 1996). The highest number of newly settled individuals was recorded in areas characterised by high cover of gravel, small rocks and boulders, and high light availability (Hamel and Mercier 1996).

In complimentary experimental studies, Hamel and Mercier (1996) demonstrated strong selectivity for the undersurfaces of rocks and rubble, which may be linked to the high light sensitivity displayed by *C. frondosa* during the settlement phase. Largely photopositive during their substratum search and selection, competent larvae likely react to not only physical cues associated with the substratum, but also light intensity. In laboratory studies, other holothuroid species have also been induced to metamorphose in response to cues associated with seagrass (Mercier et al. 2000, Agudo

2007), brown algae (Agudo 2007), periphytic diatoms associated with biofilms (Ito and Kitamura 1997, Agudo 2007) and conspecifics (Young and Chia 1982); however, the relevance of these results has yet to be tested in their natural environments.

2.5.5 Crinoidea

In contrast to the other echinoderm classes, many groups of crinoid species do not have complex planktotrophic larvae, with lecithotrophic larval types in both pelagic and benthic developmental habitats being more common (Lahaye and Jangoux 1985, McEdward and Miner 2001). Keesing et al. (1993) recorded low numbers of crinoid settlers at Davies Reef on Australia's Great Barrier Reef between November 1991 and February 1992. A dozen crinoid larvae settled at the front reef sites, while only three settlers were found at the back reef sites (Keesing et al. 1993). *Antedon bifida* doliolaria appear to settle selectively and gregariously in aquaria and the field, with larvae most observed near or within adult colonies (Lahaye and Jangoux 1985). Larvae of the planktotrophic crinoid *Florometra serratissima* and the external brooder *Dorometra sesokonis* have also previously been shown to metamorphose in response to conspecific cues (Mladenov and Chia 1983, Obuchi et al. 2010). While there have been no observations of gregarious settlement by these two species in the field, the strong gregarious settlement responses of *Florometra serratissima* in culture suggest that gregarious settlement may play an important role in the formation and maintenance of adult aggregations of *F. serratissima* and many other crinoid species with a planktonic larval stage (Mladenov and Chia 1983).

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Arbacia lixula	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Settlement rates were relatively low and varied among seasons, as all newly settled echinoids were recorded between January and May.	Monthly; Feb 2009–Feb 2010	Plastic cushion-shaped leaf-like units	García-Sanz et al. (2014)
Arbacia lixula	Canary Islands, and Costa Brava, Spain (subtropical)	Gently sloping rocky barren bottom; 5–10 m	The plastic bioball collectors reported the highest settler densities. No settlers were found at the Mediterranean site (Costa Brava). At the eastern Atlantic site (Canary Is.), a considerably higher number of settlers was recorded in the collectors deployed for 28 days than in those deployed for 14 days.	Deployments in Feb (Canary Is.) and Jun 2012 (Costa Brava); two retrievals from both locations 14 and 28 days post-deployment	Plastic bioballs in nylon nets; vertical scrub brushes with vegetal bristles; horizontal triangular mats of coconut fibre	Balsalobre et al. (2016)
Dendraster excentricus	Monterey Bay, the USA (subtropical)	Inshore sand dollar bed; 8–15 m	Settlement occurred at all sampling times during the summer months of 1980, and settler densities were relatively constant throughout this time period.	Fortnightly; Jul–Oct 1980	Eckman grab samples and cylindrical cores	Cameron and Rumrill (1982)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Diadema africanum	Canary Islands, Spain (subtropical)	Rocky subtidal zone; 5–6 m	Substantial intra-annual variation in settlement observed at both sites. The primary peak between August and October may be related to spawning intensity (April–June), while a secondary peak in January/February suggests that factors other than spawning intensity may also be driving local settlement patterns.	Monthly (with exceptions); Apr 2002–Apr 2003	Plastic bioballs inside net bags	Hernández et al. (2006)
Diadema africanum	Canary Islands, Spain (subtropical)	Rocky subtidal zone; 5–10 m	Settlement was observed at all sites within three survey locations (MPA; fished; and heavily fished). The number of settlers found was similar between sites and locations, which suggests larval supply is unlikely to be limiting recruitment in any of the three locations.	Single deployment; Sep–Oct 2006	Plastic bioballs inside net bags	Clemente et al. (2009)
Diadema africanum	Canary Islands, Spain (subtropical)	Urchin barrens, and macroalgal beds; 6–10 m	Single annual peak in settlement during August to October, which was consistent among years and locations; however, inter-annual variability in settlement was high at the Abades and Boca Cangrejo locations. Higher densities of settlers were found in the barren areas than in the macroalgal beds. Strong spatiotemporal settlement variability likely driven by temperature; however, settlement rates were also negatively correlated with food availability 2 months pre-settlement.	Monthly; Apr 2002–Apr 2008 (Abades), May 2002–Apr 2003 (Masca), Dec 2003–Apr 2008 (Boca Cangrejo) and Oct 2006 (El Hierro)	Plastic bioballs inside net bags	Hernández et al. (2010)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Diadema africanum	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Settlement followed a clear unimodal pattern with a main peak occurring in September and October and few newly settled echinoids observed in the months following. Densities were greater in urchin barrens and sandy patches than in the other habitats.	Monthly; Feb 2009–Feb 2010	Plastic cushion-shaped leaf-like units	García-Sanz et al. (2014)
Diadema antillarum	Curaçao, southern Caribbean Sea (tropical)	Coral reef; 8 m	Settlement occurred continuously and peaked in the spring and autumn months. Settlement rates were relatively similar among years but differed between sites.	Fortnightly with exceptions; 1982–1984	Settlement plates consisting of two polystyrene egg crates and plexiglass in-between	Bak (1985)
Diadema antillarum	Barbados, eastern Caribbean Sea (tropical)	Coral reef	Low settlement was detected between June and August and peaked in July. Settlers were always found within the front third of the reef (offshore) on rugose substrata and aggregated with adults.	Monthly; Oct 1984–Dec 1985	Visual UW searches for settlers	Hunte and Younglao (1988)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Diadema antillarum	Florida Keys, the USA (subtropical); Puerto Rico (tropical)	Variable habitats (fore reef, back reef, drowned reef and bank reef) and sampling depths; 3–19 m	While settlers were detected in low numbers at all locations, only one of the Puerto Rico sites recorded a substantial settlement pulse. These results corroborate the hypothesis of low larval supply (and settlement) limiting the recovery of this species in the Florida Keys.	Monthly; Aug 2005–Jul 2006 (Florida Keys), and Jun 2006–Oct 2006 (Puerto Rico)	Settlement plates consisting of two polystyrene egg crates and plexiglass in-between	Miller et al. (2009)
Diadema antillarum	La Parguera, Puerto Rico (tropical)	Coral reef; 19–6 m (shelf-edge), 10–5 m (mid-shelf) and 2–3 m (inshore)	Substantially higher settlement at shelf-edge reef (275 recruits) compared to mid-shelf (two recruits) and inshore reefs (0 recruits). Unimodal settlement peak in July, with low settler densities from October to June.	Monthly; Sep 2005–Sep 2006	Artificial turf on horizontal and vertical plates	Williams et al. (2010)
Diadema antillarum	Curaçao, southern Caribbean Sea (tropical)	Coral reef; 3–5 m	Settlement peaked in early June, similar to peaks in 1982 and 1983 (historic data comparison). Mean settlement rates in 2005 were > two times lower compared to pre-*D. antillarum* die-off numbers (1982–83), but substantially higher than those measured after the die-off (1984). No spatial variation among sites around the island.	Fortnightly; Mar–Jul 2005	Settlement plates consisting of two polystyrene egg crates and Formica sheets in-between	Vermeij et al. (2010)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Diadema antillarum	La Parguera, Puerto Rico (tropical)	Coral reef; 3–17 m (depending on water depth)	Highest settlement at 9 m, intermediate at 6 and 12 m, and lowest at 3 and 15 m. Substantially higher settlement at two shelf-edge reefs (217 of 220 settlers), with little and no settlement detections at mid- and inner-shelf reefs. Settlement peaked in September but was also relatively high in July.	Monthly; Feb–Apr and Jul–Sep 2008	Artificial turf on vertical plates	Williams et al. (2011)
Diadema antillarum	Curaçao, southern Caribbean Sea (tropical)	Variable habitats (fore reef, sheltered lagoon or back reef)	Settlement was variable in space. Significantly higher settlement rates were observed in sheltered habitats (lagoon and back reef) than in fore reef habitats.	Monthly; May/Jun–Aug 2010	Settlement plates consisting of two polystyrene egg crates and plastic sheets in-between	Rogers and Lorenzen (2016)
Diadema antillarum	Xcalak, Mexico (tropical)	Coral reef lagoon and fore reef zone; <1, 2.5, 3 and 7 m	Sampled a total of five settlers during the 11-month period, all of which settled in the fore reef habitat at 7 m (4 in August and 1 in December).	Monthly; May 2014–Apr 2015	Settlement plates consisting of polystyrene egg crates and acrylic sheets in-between	Maldonado-Sánchez et al. (2019)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Evechinus chloroticus	South Island, New Zealand (temperate)	Subtidal and moderately sloping rocky reefs, and bolder fields; 12 m	Substantial spatiotemporal variability was reported as settlement was highly variable between years and distinct peaks in settlement were recorded at both locations. Settlement was generally higher in Doubtful Sound than in Tory Channel, and a major settlement pulse occurred in Doubtful Sound between August and November 1992. In contrast, settlement at Tory Channel peaked in March 1993.	Every 1–2 months; Nov 1991–Oct/Nov 1994	PVC pipe containing sheets of light diffuser panels	Lamare and Barker 2001
Evechinus chloroticus	Hauraki Gulf, New Zealand (subtropical)	Urchin barrens and shallow mixed-algal assemblages; 4–5 m	No settlers were found at the sheltered, high-sediment location (Little Manly), while spatiotemporal variability in settlement was evident among sites and sampling months at the wave-exposed, low-sediment location (Leigh).	Nov 2003–Mar 2004	Underwater suction sampling and sieving	Walker (2007)
Evechinus chloroticus	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Highly variable settlement among sampling years and locations. Settlers were most abundant in 2019 at Shelly Bay, and smaller pulses of settlement occurred at Beaker Bay (2018, 2019) and Kau Bay (2018). Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually, Nov 2016–Apr 2017; Nov 2017–Apr 2018; Dec 2018–Feb 2019	Plastic bioballs inside nylon nets	Glockner-Fagetti and Phillips (2020)
Mesocentrotus franciscanus	Northern and southern California, the USA (subtropical)	Not stated, but likely variable	Marked seasonality in settlement as most settlement occurred during one or multiple settlement pulses between February and July each year. Settlement was highly variable among years, and spatial settlement clusters did not form contiguous geographic subunits.	Weekly; Feb/Mar 1990–Jul 1993 at most sites, with shorter periods at others	Scrub brushes with nylon bristles	Ebert et al. (1994)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Mesocentrotus franciscanus	Southern Oregon, the USA (temperate)	18 m (Port Oxford) and 22 m (Gregory Point)	Settlement occurred from April to August at both locations. Settler densities were variable between locations and sampling years but were most consistently high in June. Settlement pulses were stronger in 1995 and generally associated with warm water events characterised by a mixed water column and northward wind stress.	Deployments ranging from 9 to 35 days; Feb to Aug in 1994 and 1995	AstroTurf inside PVC pipe	Miller and Emlet (1997)
Paracentrotus lividus	Tossa de Mar, Spain (temperate)	Vertical wall occupied by seaweeds; 3, 6 and 9 m	Settler densities (<2 mm) varied substantially within and among years. Unimodal peaks in settlement were observed during the each of the four recruitment seasons (May–August), but peak densities were much higher in 1992 and 1995, compared to 1993 and 1994.	Fortnightly; Jun 1992–Jul 1993, Jun 1994–Dec 1995	Substrate collection using 20 × 20 cm^2	López et al. (1998)
Paracentrotus lividus	Medes Islands Marine Reserve, Spain (temperate)	Rocky bottoms colonised by a rich algal assemblage; 5, 10, 15 and 20 m	Reported single annual settlement peak within 3 weeks (May–June) and substantial inter-annual differences in settlement by more than two orders of magnitude. Preferential settlement at shallow depths (5 and 10 m). Spatial variation was found at all scales, and settlement was related to wave exposure and currents, but not to substratum type, level of protection and adult densities, suggesting physical processes may be driving these variable settlement patters.	Weekly (spring–late summer) or every other week (rest of the year); Mar 1998–Oct 2000	Scrub brushes with vegetal bristles	Hereu et al. (2004)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Paracentrotus lividus	Medes Islands Marine Reserve, Spain (temperate)	Seagrass meadow, and vertical rock wall; 3, 5 (and 10 m at seagrass meadow only) in Apr 2000–July 2001; then 5 m only	Major settlement peak in April/May and minor peak in October/November. Inter-annual variability in settlement was high (~one order of magnitude). Settlement was generally higher in the vertical rock wall habitat than in the seagrass meadow.	Weekly or fortnightly (Apr 2000–Jul 2001); additional sampling in autumn–winter 2002 and early summer 2003	Scrub brushes with vegetal bristles	Tomas et al. (2004)
Paracentrotus lividus	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Densities of newly settled echinoids differed substantially among the four habitats surveyed, with collectors deployed in sandy patches reporting greater numbers than in the other habitats.	Single deployment for 4 weeks from early Aug to early Sep 2008	(A) Plastic leaf-like units; (B) cushion-shaped, leaf-like units; and (C) cushion-shaped units	García-Sanz et al. (2012)
Paracentrotus lividus	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Almost all newly settled echinoids were recorded during a main peak between February and April. During this peak, abundances of settlers differed substantially among the four habitats surveyed, with soft substrata (sandy patches and seagrass) reporting greater densities than hard substrata (barrens and macroalgal beds).	Monthly; Feb 2009–Feb 2010	Plastic cushion-shaped, leaf-like units	García-Sanz et al. (2014)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Paracentrotus lividus	Canary Islands, and Costa Brava, Spain (temperate)	Gently sloping rocky barren bottom; 5–10 m	The plastic bioball collectors reported the highest settler densities. No significant effect of sampling time was reported as the number of settlers did not differ between collectors deployed for 14 and 28 days at both locations.	Deployments in Feb (Canary Is.) and Jun 2012 (Costa Brava); retrieval from both locations 14- and 28-days post-deployment	Plastic bioballs in nylon nets; vertical scrub brushes with vegetal bristles; horizontal triangular mats of coconut fibre	Balsalobre et al. (2016)
Strongylocentrotus droebachiensis	Isles of Shoals, the USA (temperate)	Kelp beds and barren grounds; 0–30 m	Annual settlement pulses were substantial, and settlement decreased with depth below 9 m.	Single deployments in Jun–Jul 1990, 1992, and 1993	Plastic turf	Harris et al. (1994)
Strongylocentrotus droebachiensis	Gulf of Maine, the USA; Bay of Fundy and Nova Scotia, Canada (temperate)	Kelp beds, silt and barren grounds; 5–30 m	Settlement in the Gulf of Maine was three orders of magnitude higher than in the Bay of Fundy and one order of magnitude higher than on Nova Scotia’s Atlantic Coast. Spatial differences in settlement among sites within these regions were within the same order of magnitude. Settler densities were higher in barren grounds than in adjacent kelp beds.	Fortnightly to bi-monthly sampling intervals; May–Sep 1994	Plastic turf and scrub brushes	Balch et al. (1998)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Strongylocentrotus droebachiensis	Nova Scotia, Canada (temperate)	Kelp beds and urchin barren grounds; 5–10 m	Nearly all settlement occurred in July each year, but few settlers were reported up to October. Settlement was very low in 1992 and 1993, and the only major settlement occurred in early July 1994. Settlement rates were quite similar between kelp and barren sites.	Fortnightly; Jun–Nov 1992, May–Nov 1993 and Jun–Oct 1994	AstroTurf inside PVC pipe	Balch and Scheibling (2000)
Strongylocentrotus droebachiensis	Gulf of Maine, the USA (temperate)	7 m	Settlement peaked in mid-June, relatively high throughout June and early July, and low to absent from mid-July to early September. No differential settlement was reported during the peak in settlement among six substrate types deployed; however, settlers preferentially settled on coralline algae encrusted substratum during the remainder of the sampling period.	Weekly; 48-hour deployments in Jun–mid-Jul; 1-week deployments in late Jul–early Sep	AstroTurf plexiglass panels	Lambert and Harris (2000)
Strongylocentrotus droebachiensis	Bocabec Cove, Canada (temperate)	Rocky subtidal zone	Highly variable settlement among years, intra-annual sampling periods and sites. Highest density of settlers in mid-July 2005 at the site closest to the larger body of water, Passamaquoddy Bay. If transported to the area by currents, larvae would likely reach this site first and, if competent, settle.	Fortnightly; Jun–Oct 2004; Jun–Oct 2005	AstroTurf inside PVC pipe	Jennings and Hunt (2010)
Strongylocentrotus purpuratus	Northern and southern California, the USA (subtropical)	Not stated, but likely variable	Settlement pulses were strongly seasonal (spring); however, there was a strong spatiotemporal variability in the number of settlers. Sites in northern, central and southern California were shown to form geographic subunits with respect to coherence in settlement pulses.	Weekly; Feb/Mar 1990–Jul 1993 with exceptions	Scrub brushes with nylon bristles	Ebert et al. (1994)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Strongylocentrotus purpuratus	Southern Oregon, the USA (temperate)	18 m (Port Oxford) and 22 m (Gregory Point)	Settlement occurred but was generally low from April to August at both locations. Settler densities peaked at Gregory Point in June 1995, while settlement at Port Oxford was highest in late July–early August 1994 and March–April 1995. Settlement pulses were generally associated with warm-water events characterised by a mixed water column and northward wind stress.	Deployments ranging from 9 to 35 days; Feb–Aug in 1994 and 1996	AstroTurf inside PVC pipe	Miller and Emlet (1997)
Strongylocentrotus purpuratus	Southern and northern California, the USA (subtropical)	Shallow subtidal and intertidal rocky habitats	Settlement varied greatly, both inter- and intra-annually, at all three locations over 27 years. Following a seasonal pattern with peak settlement during April–May, settlement fluctuations were highly synchronous among sites within Southern California locations. Large spatiotemporal settlement patterns were related to differential responses to fluctuations in ocean temperature and climate.	Weekly (1990–2003) and fortnightly (2003–2016)	Scrub brushes with nylon bristles	Okamoto et al. (2020)
Strongylocentrotus purpuratus and *Mesocentrotus franciscanus*	Santa Barbara, the USA (subtropical)	Urchin barren grounds and kelp beds	Newly settled echinoids were found in low numbers from April to July in 1984 and 1985, and in April 1986. In early May 1986, many settlers of both species were present in high numbers on both foliose red algal turf and crustose coralline algae substrata.	Irregular sampling frequency; 1984–1986, mostly during summer	Collection of reef shale pieces	Rowley (1989)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Strongylocentrotus purpuratus and *Mesocentrotus franciscanus*	Monterey Bay, the USA (subtropical)	Kelp forest; 10 m	Two distinct peaks in settlement occurred from December through February and April through July.	Monthly; Jul 1988–Jul 1989	Plastic matrix or articulated coralline algae inside a PVC pipe	Harrold et al. (1991)
Strongylocentrotus purpuratus and *Mesocentrotus franciscanus*	Northern California, the USA (subtropical)	Rocky habitat; 10–12 m	The total seasonal settlement rate varied substantially among the locations sampled. While very few settlers were recorded at Duxbury Reef, Bodega Head and Salt Point, while settlement was markedly higher at Point Reyes.	Weekly; Apr–early Sep 1993	Scrub brushes with polypropylene bristles	Wing et al. (1995b)
Strongylocentrotus purpuratus and *Mesocentrotus franciscanus*	Northern California, the USA (subtropical)	Rocky habitat; 5–20 m	Settlement occurred at low levels from late April to late July, and synchronous peaks were reported at both locations in mid-July.	Weekly; Apr–early Sep 1992	Scrub brushes with polypropylene bristles	Wing et al. (1995a)
Strongylocentrotus purpuratus and *Mesocentrotus franciscanus*	Southern California, the USA (subtropical)	Kelp forest, inshore and offshore habitats; 12–15 m	Settlement was generally low (0–10 settlers per collector) across locations in both years, and there was no evidence of lower settlement rates of either species at sites situated under the kelp canopy as opposed to offshore sites.	Weekly (5–20 days); Mar–May 1991, Jan 1992 and Apr–Jul 1992	Scrub brushes with nylon bristles	Schroeter et al. (1996)
Strongylocentrotus nudus	Hokkaido, Japan (temperate)	Rocky substratum; 1–9 m	Settlement annually occurred at generally low densities between 1980 and 1995, except for a strong settlement pulse recorded in 1984. Relatively high densities of newly settled echinoids were also found in 1990–1992 and 1995. Settler densities varied between the two sampling locations Okushiri Island and Shimamaki.	Annually, Aug or Sep from 1980 to 1995, excluding 1981 and 1983	Underwater visual quadrat survey	Agatsuma et al. (1998)

Table 2.3 Summary of studies assessing spatial and temporal settlement patterns in echinoids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Echinoid spp. (i.a. *Mespilia globulus* and *Echinometra* sp.)	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Numbers of echinoid settlers were generally high at all sites, although settlement was significantly greater at the front reef sites than on the back reef sites.	Single deployment for 2 months from Nov 1991 to Jan 1992	Plastic bioballs inside net bags	Keesing et al. (1993)

Table 2.4 Summary of studies assessing spatial and temporal settlement patterns in asteroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Acanthaster cf. *solaris*	Suva Barrier Reef, Fiji (tropical)	Coral reef; shallow depth (crest)	A major settlement pulse only occurred in one (1984) of the nine sampling years (1979–1987). The low number of newly settled asteroids recorded in most years is likely the ‘normal’ amount of settlement success, while unusually high settlement of larvae in some years may give rise to population irruptions.	Monthly to annually; 1979–1987	Quadrat searches for newly settled individuals	Zann et al. (1987)
Acanthaster cf. *solaris*	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Low numbers of settlers were found in the artificial collectors during the summer sampling period (late November to late February). Settlement was only observed at two front reef sites, while no settlers were recorded at any of the back reef sites.	Single deployment for 2 months from Nov 1991 to Jan 1992	Plastic bioballs inside net bags	Keesing et al. (1993)

Table 2.4 Summary of studies assessing spatial and temporal settlement patterns in asteroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Acanthaster cf. *solaris*	Moore Reef, Australia (tropical)	Coral reef; 2–9 m	Settlement occurred between October 2016 and February 2017. The settlement pulse was stronger between December and March than between October and February.	Oct 2016–Feb 2017, Dec 2016–Mar 2017	Plastic bioballs inside leaf baskets	Uthicke et al. (2019)
Acanthaster cf. *solaris*	Central Great Barrier Reef, Australia (tropical)	Coral reef; 1–18 m	Densities of settlement-stage individuals were highest at intermediate depths (8–14 m) and increased and decreased with the availability of rubble and coral substrata, respectively. At the reef scale, settler density was greatest in south-western and northern fore reef habitats.	May and Jun 2017	Underwater visual quadrat survey	Wilmes et al. (2020)
Acanthaster cf. *solaris*	Northern and central Great Barrier Reef, Australia (tropical)	Coral reef; 4–12 m	Settlement varied considerably among sampling periods and peaked between October 2016 and January 2017. Reports substantial latitudinal variation as collectors deployed in the central GBR were > ten times more likely to record settlers than in the northern GBR. No preferential settlement was detected among depths, suggesting these larvae readily settle in shallow reef environments.	Four sampling periods: Oct 2016–Jan 2017; Dec 2016–Mar 2017; Nov 2017–Feb 2018; and Nov 2019–Feb 2020	Plastic bioballs inside leaf baskets	Doll et al. (2021 – **Chapter 5**)
Asterias forbesi	Long Island Sound, the USA (temperate)	3–9 m	Settlement mostly occurred between early July and early September for an average period of 52 days, but the timing and length of the settlement period varied between years. Settlement was also highly variable among the three areas and ten nested sites; however, settler densities were remarkably similar among the three depths studied (3, 6 and 9 m) at any given year.	Twice per week; 1937–1961	Shells inside collectors	Loosanoff (1964)

Table 2.4 Summary of studies assessing spatial and temporal settlement patterns in asteroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Asterias forbesi and *A. vulgaris*	Nova Scotia, Canada (temperate)	Kelp bed and urchin barren ground; 5–10 m	Settlement was generally high, but variable among years and occurred from late July to early October. Higher densities of settlers were observed in and above kelp beds than in the urchin barrens.	Fortnightly; Jun–Nov 1992, May–Nov 1993 and Jun–Oct 1994	AstroTurf inside PVC pipe	Balch and Scheibling (2000)
A. forbesi and *A. rubens*	Bocabec Cove, Canada (temperate)	Rocky subtidal zone	Highly variable settlement among years, intra-annual sampling periods and sites. Settlement peaked from early August to early September 2005. Settler densities were lower in 2004 and peaked later. Highest densities for both years were recorded at the site closest to the larger body of water, Passamaquoddy Bay. If transported to the area by currents, larvae would likely reach this site first and, if competent, settle.	Fortnightly; Jun–Oct 2004, Jun–Oct 2005	AstroTurf inside PVC pipe	Jennings and Hunt (2010)
Choriaster granulatus	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Low numbers of settlers were recorded at front and back reef sites during the summer sampling period (late November to late February).	Single deployment for 2 months from Nov 1991 to Jan 1993	Plastic bioballs inside net bags	Keesing et al. (1993)
Coscinasterias muricata	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Highly variable settlement among sampling years and locations. Settlers were highly abundant in 2019 at two locations (Kau Bay and Shelly Bay), and a smaller pulse of settlement occurred in 2017 at the same sites. Overall echinoderm settlement pulses were positively correlated with seawater temperature, chlorophyll and shell cover (exclusively found at these Harbour sites).	Annually; Nov 2016–Apr 2017, Nov 2017–Apr 2018, Dec 2018–Feb 2019	Plastic bioballs inside nylon net	Glockner-Fagetti and Phillips (2020)

Table 2.4 Summary of studies assessing spatial and temporal settlement patterns in asteroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Culcita novaeguineae	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	Low numbers of settlers were found in the artificial collectors during the summer sampling period (late November to late February). Settlement was only observed at one back reef site and two front reef sites.	Single deployment for 2 months from Nov 1991 to Jan 1994	Plastic bioballs inside net bags	Keesing et al. (1993)
Asteroid sp.	Northern California, the USA (subtropical)	Rocky habitat; 5–20 m	Settlement occurred at low levels from early April to late July and peaked in mid-July at the Bodega Marine Laboratory location. At Salt Point, a strong, synchronous settlement pulse was recorded in mid-late July, but settlement was absent before and after.	Weekly; Apr–early Sep 1992	Scrub brushes with polypropylene bristles	Wing et al. (1995a)
Asteroid sp. A	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Inter-annual and spatial variation in settlement observed. Settlement occurred in 2018 and 2019 at Princess Bay and Breaker Bay. Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually; Nov 2016–Apr 2017, Nov 2017–Apr 2018, Dec 2018–Feb 2020	Plastic bioballs inside nylon net	Glockner-Fagetti and Phillips (2020)
Asteroid sp. B	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Little settlement occurred in 2019 at Princess Bay. Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually; Nov 2016–Apr 2017, Nov 2017–Apr 2018, Dec 2018–Feb 2021	Plastic bioballs inside nylon net	Glockner-Fagetti and Phillips (2020)

Table 2.5 Summary of studies assessing spatial and temporal settlement patterns in ophiuroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Amphiura chiajei	Øresund, Denmark (temperate)	Muddy sand; 27 m	Settlers were observed in relatively small numbers, and the settlement period was found to start in early November in 1964, which was 3 months later than in *Amphiura filiformis*.	Fortnightly; Oct 1963–Oct 1966	Mouse-trap sampler	Muus (1981)
Amphiura filiformis	Øresund, Denmark (temperate)	Muddy sand; 27 m	Settlement occurred annually between August and December and peaked during a 2- to 6-week period in September to November.	Fortnightly; Oct 1963–Oct 1965	Mouse-trap sampler	Muus 1981
Amphiura filiformis	Galway Bay, Ireland (temperate)	Silty sand; 20 m	Little settlement over the 8-year period as only approximately 5% of the population were newly settled individuals (<4 mm). Settlement likely peaked in the autumn months from September to November.	3–10 monthly samples; 1974–1976	Van Veen grab and diver-operated suction sampling	O'Connor et al. (1983)
Amphiura filiformis	German Bight and North Sea (temperate)	Muddy sand; 38 and 54 m	Suggests the annual settlement season begins in July to August and the timing of settlement likely progresses from SW to NE during the summer and autumn months.	Every Mar/Apr in 1983, 1984 and 1986 to 1988; also in Jun, Jul/Aug, and Nov in 1983 and 1987, and in Dec 1987	Van Veen grab and Reineck box sampling	Künitzer (1989)
Ophiocten gracilis	Rockall Trough, NE Atlantic (temperate)	Silty sand; 600–1200, 2200 and 2900 m	Highest densities of settlers were recorded during sampling at depths of approximately 2900 m in May and July 1975, June 1978 and May 1980.	Sampling every 2–7 months; May 1975–Sep 1980	Epibenthic sled sampling	Gage and Tyler (1981a)

Table 2.5 Summary of studies assessing spatial and temporal settlement patterns in ophiuroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Ophiocten gracilis	Rockall Trough, NE Atlantic (temperate)	1000 m and 1400 m	Settlement occurred in both traps (1000 m; 1400 m) during mid-May.	May–Jul 1996	Sediment traps	Sumida et al. (2000)
Ophioderma longicaudum	Canary Islands, Spain (subtropical)	Four subtidal habitats: seagrass meadows, urchin barrens, macroalgal beds and sandy patches; 5–10 m	Densities of newly settled ophiuroids differed substantially among the four habitats surveyed, with collectors deployed in macroalgal beds reporting greater numbers than in the other habitats.	Single deployment for 4 weeks from early Aug to early Sep 2008	(A) plastic leaf-like units; (B) cushion-shaped, leaf-like units; and (C) cushion-shaped units	García-Sanz et al. (2012)
Ophionereis reticulata	Baleeiro Isthmus, Brazil (subtropical)	Intertidal rock boulder habitat	Analysis of recruitment patterns suggests intense settlement pulses in February and from September to November.	Jan 2001–Dec 2002	-	Yokoyama and Amaral (2011)
Ophiopholis aculeata and *Ophiura* spp.	Nova Scotia, Canada (temperate)	Kelp bed and urchin barren ground; 5–10 m	Within the annual 2-week settlement period, a major settlement pulse occurred over a 3-day interval, and settlement declined over the following 6 days. This settlement pulse was preceded by rapid temperature and salinity fluctuations and related to a change in current direction.	Three-day intervals during 2-week settlement period in Jul/Aug 1993	AstroTurf inside PVC pipe	Balch et al. (1999)

Table 2.5 Summary of studies assessing spatial and temporal settlement patterns in ophiuroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Ophiopholis aculeata and *Ophiura* spp.	Nova Scotia, Canada (temperate)	Kelp bed and urchin barren ground; 5–10 m	Settlement was observed from July to early August each year. Spatial differences in settlement between sites were likely related to variable larval supply due to local differences in hydrodynamic conditions. Settlement rates were higher in the barrens than in kelp beds, and substantially more settlers were collected in the high (2.3 m off bottom) than in the low (0.2 m) collectors.	Fortnightly; Jun–Nov 1992, May–Nov 1993 and Jun–Oct 1994	AstroTurf inside PVC pipe	Balch and Scheibling (2000)
Ophiothrix fragilis	Blanes, Spain (temperate)	Subvertical rocky walls and sandy bottom; 8–12 m	Compared the pattern found after settlement had ceased (July 1997 survey) with that found while settlement was still under way (June 1999 survey). Settlement occurred in multiple batches and peaked in June.	July 1997 and June 1999	Grab sampling of paired sponge-turf samples	Turon et al. (2000)
Ophiura ljungmani	Rockall Trough, NE Atlantic (temperate)	Silty sand; 2260–2925 m	Settlement occurs annually in summer, but the timing and magnitude of settlement likely varies among years.	Irregular sampling frequency; Jan 1979–Sept 1980	Epibenthic sled sampling	Gage and Tyler (1981b)
Ophiuroid species	Central Great Barrier Reef, Australia (tropical)	Coral reef; 15 m	High numbers of ophiuroid settlers were sampled at both the front reef and back reef locations. While no difference in settlement rates was observed between these two locations, settlement was patchy, with variable rates at the scale of hundreds of metres.	Single deployment for two months from Nov 1991 to Jan 1992	Plastic bioballs inside net bags	Keesing et al. (1993)

Table 2.5 Summary of studies assessing spatial and temporal settlement patterns in ophiuroids.

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Ophiuroid species	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Settlers were recorded continuously from year to year; however, significant spatial variation was recorded. Settlement occurred during all three sampling years at Princess Bay and Beaker Bay, and little settlement occurred in 2017 at Moa Point. These sites had relatively high macroalgal cover.	Annually; Nov 2016–Apr 2017; Nov 2017–Apr 2018; Dec 2018–Feb 2019	Plastic bioballs inside nylon net	Glockner-Fagetti and Phillips (2020)

Table 2.6 Summary of studies assessing spatial and temporal settlement patterns in holothuroids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Australostichopus mollis	Wellington region, New Zealand (temperate)	Rocky reef; 4–7 m	Settlement occurred only in 2017 and 2019 at Shelly Bay. Overall echinoderm settlement pulses were positively correlated with shell cover, seawater temperature and chlorophyll.	Annually; Nov 2016–Apr 2017; Nov 2017–Apr 2018; Dec 2018–Feb 2019	Plastic bioballs inside nylon net	Glockner-Fagetti and Phillips (2020)
Cucumaria frondosa	St. Lawrence Estuary, Canada (temperate)	Rocky subtidal zone; 0–60 m	Newly settled individuals were predominantly found at shallow depth between 0 and 20 m, rare between 20 and 40 m and virtually absent below 40 m. The highest densities of settlers were recorded in areas with high cover of gravel, small rocks and boulders, and also high light availability.	Seasonally; at the beginning of each season from spring 1992 to winter 1994	Belt transect surveys	Hamel and Mercier (1996)

Table 2.6 Summary of studies assessing spatial and temporal settlement patterns in holothuroids

Species	Location (climate)	Habitat; depth	Patterns observed	Sampling frequency; dates	Methodology	Reference
Leptosynapta clarki	Vancouver Island, Canada (temperate)	Mid-intertidal mudflat	Unimodal settlement likely occurred in both years around February to March prior to annual recruitment pulses in April to May.	Bi-monthly; May 1990–Aug 1991	Sediment coring	Sewell (1994)
Pentamera chierchia and *Neothyone gibber*	Marietas Islands, Mexico (tropical)	Coral reef with sandy and rocky bottom areas	Recruitment density presented seasonal changes, with peaks for both species in May to June, and low recruitment for the rest of the year. This peak is suggested to be due to a settlement event following the March–April spawning time. Thus, a pulse in settlement likely occurred in April or May.	Bi-monthly; Mar 2011–Feb 2012	Coral skeletal structures as settlement collectors	Sotelo-Casas et al. (2016)
Holothuroid spp. (mostly *Psolus fabricii*, and few *Cucumaria frondosa*)	Bocabec Cove, Canada (temperate)	Rocky subtidal zone	Settler densities varied substantially between and within years; however, no spatial variation in settlement was reported. Only one settler was found during 2004, while high settlement pulses were detected in July 2005 at multiple sites.	Fortnightly; Jun–Oct 2004; Jun–Oct 2005	AstroTurf inside PVC pipe	Jennings and Hunt (2010)

2.6 Future directions in research

There is a suite of environmental stimuli that induce settlement of echinoderm larvae, although the specificity and relative importance of these cues vary greatly among species and classes. Environmental cues are thought to become progressively more important at smaller spatial scales and play a critical role in settlement processes once a planktonic larva has attained metamorphic competence. Physical factors have been demonstrated to induce, inhibit or affect the processes occurring during this phase; however, chemical cues in particular have received considerable attention in echinoderm studies. Despite decades of extensive research effort, specific chemical compounds from environmental inducers of echinoderm settlement have not been explicitly identified, with a few exceptions (e.g., four polyunsaturated fatty acids in Kitamura et al. 1993, 1994; histamine in Swanson et al. 2004). In contrast to some other marine invertebrate taxa (reviewed by Hadfield and Paul 2001), there have only been very few attempts to fully or partially characterise the various chemical cues proven to induce echinoderm settlement. As echinoderm larvae can settle in response to low molecular-weight, water-soluble chemical cues, these cues need to not only be fully characterised, but also quantified (i.e., relevant concentrations) in the habitat of the species. The challenges in carrying out such purifications and characterisations are large, and often complicated by the polarity of molecules and low concentrations of compounds (Hadfield and Paul 2001) but can be overcome using new methodologies. By isolating the chemical cue produced by an alga from the polar extract by cation-exchange chromatography, Swanson et al. (2004) presented evidence that histamine, an invertebrate neurotransmitter, is an important natural inducer of echinoid settlement, correcting the characterisation in a previous study (Williamson et al. 2000). Larvae perceived low concentrations of histamine and responded rapidly to the inducer, and the characterisation was further corroborated by analyses of histamine concentrations in various algae and in the seawater surrounding the most inductive alga

(Swanson et al. 2004, 2006, 2012). Similarly, the physiology and settlement ecology of many other echinoderms could be linked, if chemical cues are fully characterised.

The roles played by biofilms as cues in echinoderm settlement are much more widespread and complex than formerly thought. When tested in isolation from the substratum, many studies have identified biofilms, and associated bacteria or diatoms, as the ‘true’ inducer of settlement in echinoderms (e.g., Huggett et al. 2006). Previous settlement assays that did not differentiate biofilms from the underlying substrata may thus be of limited use in drawing conclusions about echinoderm settlement behaviour in the field. At the same time, the specific chemical components of biofilms, which serve as cues for settlement in larvae, are largely unknown. Portions of complex protein-lipopolysaccharides, which make up much of the extracellular polymeric substances secreted by microorganisms, have been suggested as a cue (Hadfield 2011); however, such evidence is generally weak, especially for water-soluble cues. Many past studies on the effects of biofilms tested single-taxon cultures of bacteria or other microorganisms in their assays, which does not allow generalisations about larval responses under natural conditions, but nevertheless progresses the identification of specific chemical components that may be important inducers or inhibitors of echinoderm settlement. The development of new *omic* techniques certainly presents an opportunity to characterise microbes in biofilms and generally enhance our understanding of specific receptors and genes responsible for settlement processes in echinoderms (reviewed by Dobretsov and Rittschof 2020). To gain a greater understanding of the ecological and developmental processes surrounding settlement, it is critical not only to test how widespread and relatively important biofilm-induced and bacteria-mediated settlement is among echinoderm taxa, but also to identify the specific chemical components of biofilms which serve as cues. More generally, research on chemosensory-mediated settlement behaviour could also facilitate our ability to address ecological issues, such as population irruptions of the corallivorous crown-of-thorns starfish (Pratchett et al. 2014). While there is likely a multitude of semiochemicals detectable by echinoderm larvae, the characterisation of the few that regulate their behaviour, such as the movement towards or away from the source, could inform the management of such a complex issue (Hall et al. 2017, Motti et al. 2018). Different echinoderm species are likely to respond to different suites of distinct chemical or biochemical cues, but early work has demonstrated that there are many common features to inductive and inhibitory factors.

Further descriptive studies of larval responses to single physical or chemical cues in nature are unlikely to improve our general understanding of the mechanisms driving these processes. There have been numerous studies on the role of physical factors in settlement behaviour (e.g., turbulence), and even more on the induction of settlement by chemical cues, commonly associated with substrata or conspecifics. A considerable shortcoming of these studies has been the highly unnatural conditions in which these effects were tested, as echinoderm larvae do not encounter these environmental cues in isolation in the field. The relevance and potential applications of these cues in the natural environment thus remain largely unknown. Specifically, most studies tested these isolated cues in small containers and in the absence of flow. Larval settlement in echinoids is enhanced by turbulence through a competence shift and short-term behavioural responses facilitating substratum contact (Gaylord et al. 2013, Ferner et al. 2019). Hydrodynamic processes may play an underappreciated role in the induction of echinoderm settlement at the habitat scale and should thus be integrated into settlement assays. Chemical cues associated with substrata and conspecifics could be tested under more realistic flow regimes, and additionally, other physical factors such as light intensity could be considered. Concomitant efforts towards advancing our ability to track larval movements in the field would complement this research. Testing a suite of environmental cues is a non-trivial challenge, but natural conditions must be replicated as closely as possible when studying settlement in the laboratory. This endeavour is worth the effort for a more realistic and accurate understanding of the complex processes in larval settlement of echinoderms.

The ability to link information on settlement processes and induction to patterns of settlement and recruitment observed in the field has been constrained by challenges to effective and accurate sampling of echinoderm settlers, due to their extremely small size and cryptic nature. To address the limitations of conventional sampling techniques, various artificial settlement collector designs have been developed and successfully used in recent decades. The number of echinoderm studies documenting spatial and temporal settlement patterns in the field has thus markedly increased, although the majority of studies are focused on a few echinoid, asteroid and holothuroid species of ecological or economic importance. In theory, artificial collectors facilitate separation of settlement from post-settlement processes. However, this separation depends on how frequently collectors are deployed and retrieved, and in the case of unimodal annual settlement pulses, the timing of retrieval with respect to the settlement event. Because echinoderm settlers are subject to various early post-settlement processes including high mortality during the first days and weeks of their benthic life stage (Hunt and Scheibling 1997, Balch and Scheibling 2001), field studies with relatively long deployment periods are unlikely to accurately describe settlement rates, but rather relative rates of recruitment. Nevertheless, further assessments of spatiotemporal settlement patterns at appropriate scales appear critical to our understanding of the supply-side ecology of echinoderms, especially if such assessments occur in conjunction with quantitative descriptions of abiotic (e.g., temperature and hydrodynamics) and biotic factors (e.g., chlorophyll, availability of settlement substrata or conspecific aggregations) acting at the same scales. Some echinoderm studies have reported correlation of spatiotemporal settlement patterns with environmental variables, including temperature, chlorophyll concentrations and currents. However, most of these studies have failed to establish causative links with appropriate field or laboratory experiments. To explain the roles of settlement cues and processes in the natural environment, future studies on settlement patterns should thus not only aim to quantify pertinent environmental variables, but also attempt to resolve critical information gaps surrounding the links between these factors and settlement patterns observed in the field.

In brief, I argue that to advance our understanding of echinoderm settlement, the following four directions in research should be prioritised in future studies: (1) detailed characterisation of specific chemical compounds inducing echinoderm settlement; (2) examination of biofilm-induced and bacteria-mediated settlement; (3) settlement assays testing suites of abiotic and biotic cues, replicating natural conditions as closely as possible; and (4) *in situ* quantification of settlement rates and pertinent environmental variables, with complementary experimental studies to establish causative links.

2.7 Concluding remarks

Our review of larval settlement in echinoderms has highlighted considerable spatial and temporal variability in settlement patterns across all classes (see also Balch and Scheibling 2001), although variability is much less apparent for ophiuroids. It has long been known that echinoderm larvae do not settle randomly in space and time (Ebert 1983), yet the processes and mechanisms that influence settlement patterns are highly complex and poorly understood. Settlement patterns, and the factors that influence them, are nonetheless fundamental to the population dynamics, distribution and abundance of echinoderms. This review highlights the wide range of abiotic and biotic factors involved in the movement (locomotion and dispersal) and distribution of echinoderm larvae prior to settlement, and complex interactions between larvae and their environment throughout the settlement process. Important settlement cues are often associated with particular substrata or organisms that occur in benthic habitats. However, the specificity and the relative importance of settlement cues vary among classes, whereby echinoids appear to have much more generalised settlement cues, at least compared to asteroids. Further research is clearly warranted to better understand settlement in echinoderms, especially for species that are ecologically or economically important. Critically, marked fluctuations in settlement success, and thereby local abundance, of ecologically important echinoderms can have broad-scale impacts on the structure and functioning of marine ecosystems.

Chapter 3: Settlement cue selectivity by crown-of-thorns starfish larvae[2]

Abstract

Population irruptions of crown-of-thorns starfish (COTS) cause extensive degradation of coral reefs, threatening the structure and function of these important ecosystems. For population irruptions to initiate and spread, large numbers of planktonic larvae have to successfully transition into their benthic life-history stage (i.e., settlement), whereby larval behaviour and the presence of settlement cues may shape spatial patterns of recruitment and adult densities. This study demonstrates that a wide range of coralline algae species induce COTS larvae to settle, however, the capacity to promote settlement success varied manyfold among algal species, ranging from > 90% in *Melyvonnea* cf. *madagascariensis* to < 2% in *Lithophyllum* cf. *kotschyanum* and two *Porolithon* species at 24 hours. Because many coralline algae species that promote high settlement success are prevalent in shallow reef habitats, the findings of this study challenge the hypothesis that COTS larvae predominantly settle in deep water. Considering both larval behaviour and algal ecology, this study highlights the ecological significance of coralline algae communities in driving recruitment patterns of COTS. More specifically, the local abundance of highly inductive coralline algae (especially, *Melyvonnea* cf. *madagascariensis*) may explain some of the marked spatial heterogeneity of COTS populations and the incidence of population irruptions.

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3.1 Introduction

Environmental cues play pivotal roles in the regulation of animal life histories, particularly in the timing and completion of major life-history transitions (Shipley et al. 2020, Oestreich et al. 2022). Response mechanisms to environmental cues permit animals to orchestrate these transitions and optimise survival at transition points which are characterised by high mortality (Low and Pärt 2009, Visser and Gienapp 2019). For many animals with complex life cycles, survival rates are particularly low at ontogenetic boundaries between early life-history stages (Sullivan 1989, Gaillard et al. 1998, Low and Pärt 2009). Consequently, the presence of apt environmental cues, combined with the capability of animals to respond to them during early ontogeny, can drastically change the dynamics of populations (Gaillard et al. 1997, Sæther et al. 2000, Ozgul et al. 2010).

The transition from larval to juvenile stages, which in most animals includes some form of metamorphosis (Truman and Riddiford 1999, Suzuki et al. 2013, Nagamine et al. 2016), is largely governed by environmental cues (Laudet 2011). Groups of insects, fishes, amphibians and marine invertebrates have evolved neural and hormonal mechanisms translating abiotic and biotic cues to navigate this inherently vulnerable phase (Denver 1997, Doherty et al. 2004, Lowe et al. 2021). Most benthic marine invertebrates display bipartite life histories with a highly specialised larval settlement stage (Rodríguez et al. 1993). This irreversible planktonic-benthic transition (i.e., settlement) can be a major population bottleneck (Underwood and Fairweather 1989, Hunt and Scheibling 1997). However, response to environmental inputs (e.g., chemical cues, associated with conspecifics or benthic substrata) may result in the settlement of larvae in locations that confer higher likelihood of survival and recruitment (Pawlik 1992, Hadfield and Paul 2001, Doll et al. 2022 – **Chapter 2**). The larval decision of where and when to settle is thus of fundamental importance.

Population irruptions of crown-of-thorns starfish (*Acanthaster* spp., COTS) remain a significant driver of coral loss and reef degradation (Pratchett et al. 2021b), which are increasingly compounded by climatic disturbance (Mellin et al. 2019, Castro-Sanguino et al. 2021). For population irruptions to occur and spread among coral reefs, large numbers of planktonic larvae must successfully transition into the benthic juvenile stage (Pratchett et al. 2014, Deaker and Byrne 2022). In contrast to other echinoderm larvae that metamorphose during their planktonic stage (Doll et al. 2022 – **Chapter 2**), COTS metamorphosis is initiated after substratum contact (Caballes and Pratchett 2014). Because of exceptionally high mortality rates in early juvenile COTS (Keesing and Halford 1992, Keesing et al. 2018) and limited adult movement behaviour (Pratchett et al. 2017c, Ling et al. 2020), settlement rates are likely to be the foremost constraint on local abundance and the incidence of population irruptions (MacNeil et al. 2017, Wilmes et al. 2018, Doll et al. 2021 – **Chapter 5**). Larval settlement of COTS in the wild is presumably induced by coralline algae and their associated microbial communities (Johnson and Sutton 1994), however, it is unclear whether all or only some coralline algae have the capacity to induce high settlement rates (Pratchett et al. 2021b). The alga *Lithothamnion* cf. *proliferum* was so far surmised to be the predominant settlement cue, which gave rise to the hypothesis that COTS mostly settle in deep, inter-reef habitats (Johnson et al. 1991). Recent advances in the taxonomy and contrasting ecology of different coralline algae species do, however, necessitate a renewed exploration of settlement induction.

Here, I assess the relative capacity of a diversity of coralline algae to induce COTS settlement in order to test the hypothesis that the alga *Lithothamnion* cf. *proliferum* promotes higher settlement rates than the other species. Notably, this research facilitates a critical evaluation of the deep-water recruitment hypothesis (Johnson et al. 1991) by considering whether other algal species that play important roles in COTS settlement induction occur in deep and/or shallow reef habitats. The integration of larger-scale algal field-distribution data in the interpretation of the experimental results further enables us to better understand the ecological consequences of coralline algae assemblages for the recruitment patterns and ecological impact of this nuisance starfish.

3.2 Materials and methods

To obtain settlement-stage western Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*, COTS) for experimental assays, larvae were reared at the Australian Institute of Marine Science (AIMS) National Sea Simulator (**Appendix A**), following Uthicke et al. (2018). Larval development was microscopically examined until metamorphic competency was determined 14 days post-fertilisation. Experimental treatments included 14 living coralline algae species and 1 living Peyssonneliaceae alga (collectively referred to as coralline algae in this study; **Table 3.1**, **Appendix A**) with relatively high abundance on Australia’s Great Barrier Reef (GBR), and structural control (sterile aragonite) and filtered seawater (FSW) control treatments. A diversity of coralline algae was collected from two central GBR locations (**Appendix A**) and identified based on morpho-anatomical features and molecular sequencing (**Appendix A**, Jeong et al. 2022), and cut into replicate 5 x 5 mm live chips for use in experiments.

To test the effects of different coralline algae species on the settlement response of competent COTS larvae, 12 replicate settlement assays were conducted for each of the 17 experimental treatments. I used six-well cell culture plates and fully randomised the distribution of all replicate assays among the 204 wells. After adding 10 mL FSW and a single chip of one of 15 different algal species or sterile aragonite to the wells, I carefully introduced approximately 10 competent COTS larvae per well using glass pipettes. All well plates were kept in a temperature-controlled room (28 °C) matching the light conditions during larval rearing (12-12, light-dark). Using stereo microscopes, larval settlement was scored 24 and 48 hours after larvae were introduced. For each replicate well, I recorded the number of competent late-brachiolaria larvae remaining in the water column (= swimming) and the number of individuals which had successfully attached to the treatment chip or well bottom and commenced or completed metamorphic transformation into a juvenile with radial symmetry (= settled, **Figure 3.1**).

Statistical analyses were performed using R software (version 4.1.3., R Core Team 2022). To compare the responses of COTS larvae to coralline algae and control treatments, I considered the proportion of settled postlarvae and swimming larvae in each assay well. The effects of treatments and time (24, 48 hours) on this proportional data frame were modelled using a binomial generalised linear model with a logit link (*stats* package, R Core Team 2022). This model was fitted with the bias-reduction method *brglmFit* (Firth 1993, Kosmidis and Firth 2009) from the *brglm2* package (Kosmidis et al. 2020) to avoid data separation due to outcomes with only zeros in the control treatments. Model assumptions were evaluated based on inspection of diagnostic plots and figures were generated using the *ggplot2* package (Wickham 2016). I calculated estimated marginal means, confidence intervals and post hoc comparisons using the *emmeans* package (Lenth 2022) and the *cld* function from the *multcomp* package (Hothorn et al. 2008). To account for multiple comparisons and control for the false discovery rate, p-values (alpha = 0.05) were adjusted using the *BY* correction method following the Benjamini-Yekutieli Procedure (Benjamini and Yekutieli 2001).

Table 3.1 Ecological information on the 15 coralline algae species analysed in settlement assays. Relative abundance along the GBR shelf is categorised as rare (< 20%), moderate (20-70%) and common (> 70%), largely calculated based on total abundance data reported in Dean et al. (2015) (e.g., species abundance in 'outer' reefs divided by the species abundance across all three shelf positions). Taxonomic, morpho-anatomical and collection information are provided in **Appendix A**.

			relative abundance (GBR shelf)			
species	habitat	irradiance level	inner	mid	outer	source (GBR abundance)
Melyvonnea cf. *madagascariensis*	shallow - deep reef	low - mid	rare	common	rare	Dean et al. (2015)
Neogoniolithon fosliei	crest, shallow reef	high	rare	rare	common	Dean et al. (2015)
Adeylithon bosencei	shallow - deep reef	low - high	rare	rare	common	Steneck 1982, G.D.-P. pers. obs.
Hydrolithon cf. *reinboldii*	shallow - deep reef	mid	moderate	rare	moderate	Dean et al. (2015)
Lithophyllum cf. *insipidum*	crest, shallow reef	mid - high	rare	moderate	moderate	Dean et al. (2015)
Lithothamnion cf. *proliferum*	crevices, caves	low	rare	common	rare	Dean et al. (2015)
Titanoderma cf. *tessellatum*	shallow - deep reef	low - mid	rare	moderate	moderate	Dean et al. (2015)
Amphiroa foliacea	shallow - mid reef	mid - high	rare	moderate	moderate	G.D.-P. pers. obs.
Sporolithon sp.	crevices, caves	low	rare	rare	common	Dean et al. (2015)
Ramicrusta sp.	crevices, caves	low	rare	moderate	moderate	G.D.-P. pers. obs.
Lithophyllum cf. *pygmaeum*	crest, shallow reef	mid - high	rare	moderate	moderate	Dean et al. (2015)
Porolithon sp. A	reef crest	high	common	rare	rare	G.D.-P. pers. obs.
Porolithon sp. B	reef crest	high	rare	moderate	moderate	Dean et al. (2015)
Lithophyllum cf. *kotschyanum*	reef crest	mid - high	rare	moderate	moderate	Dean et al. (2015)
Porolithon sp. C	reef crest	high	rare	moderate	moderate	Dean et al. (2015)

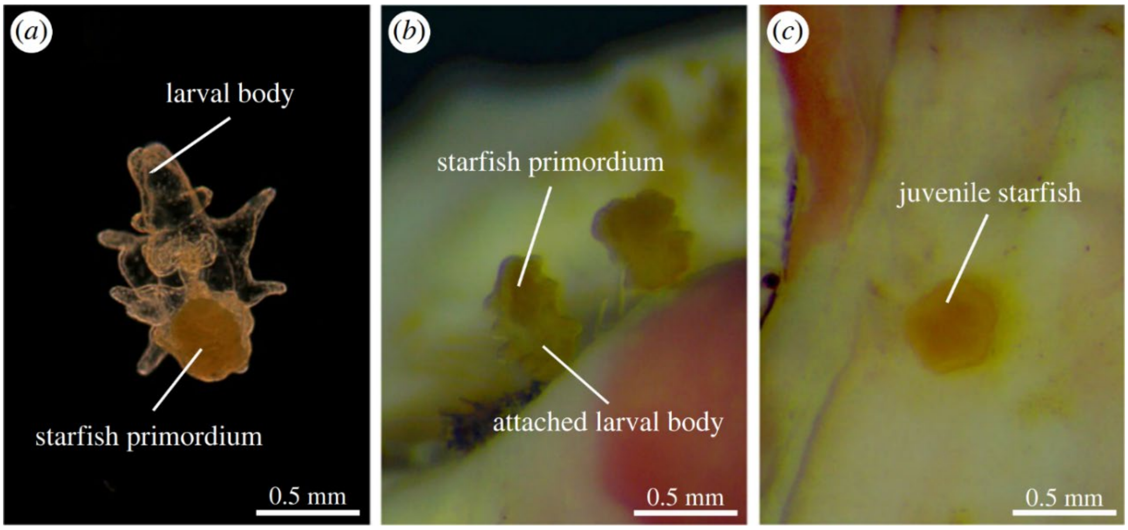

Figure 3.1 Larval development through metamorphosis in crown-of-thorns starfish: (*a*) brachiolaria larva; (*b*) metamorphosing larva absorbing the larval body; (*c*) post-metamorphic juvenile (photographs by Ciemon Caballes and Peter Doll).

3.3 Results

Whilst larval settlement was induced in all coralline algae treatments (**Table 3.1**, **Figure 3.2*a***), no larvae settled in both controls and settlement success differed substantially among the 17 treatments (**Figure 3.2*a***, $F_{16,391} = 7.97$, $P < 0.001$). Highest settlement rates (mean ± s.e.: 90.7% ± 2.9 at 24 h; 98.3% ± 1.1 at 48 h) were recorded in the presence of *Melyvonnea* cf. *madagascariensis*, whilst limited settlement was recorded (< 2% at 24 h; < 10% at 48 h) for two *Porolithon* species and *Lithophyllum* cf. *kotschyanum*. Settlement rates were relatively high (30-60% at 24 h) for seven coralline algae treatments, with an evident hierarchy in larval settlement responses to cues associated with different coralline species (**Figure 3.2*a***). Settlement rates differed significantly between scoring times ($F_{1,390} = 62.97$, $P < 0.001$), although there was no interaction with treatments (**Figure 3.2*b***, $F_{16,374} = 0.52$, $P = 0.939$), reflecting consistent differences in settlement rates among different algal species.

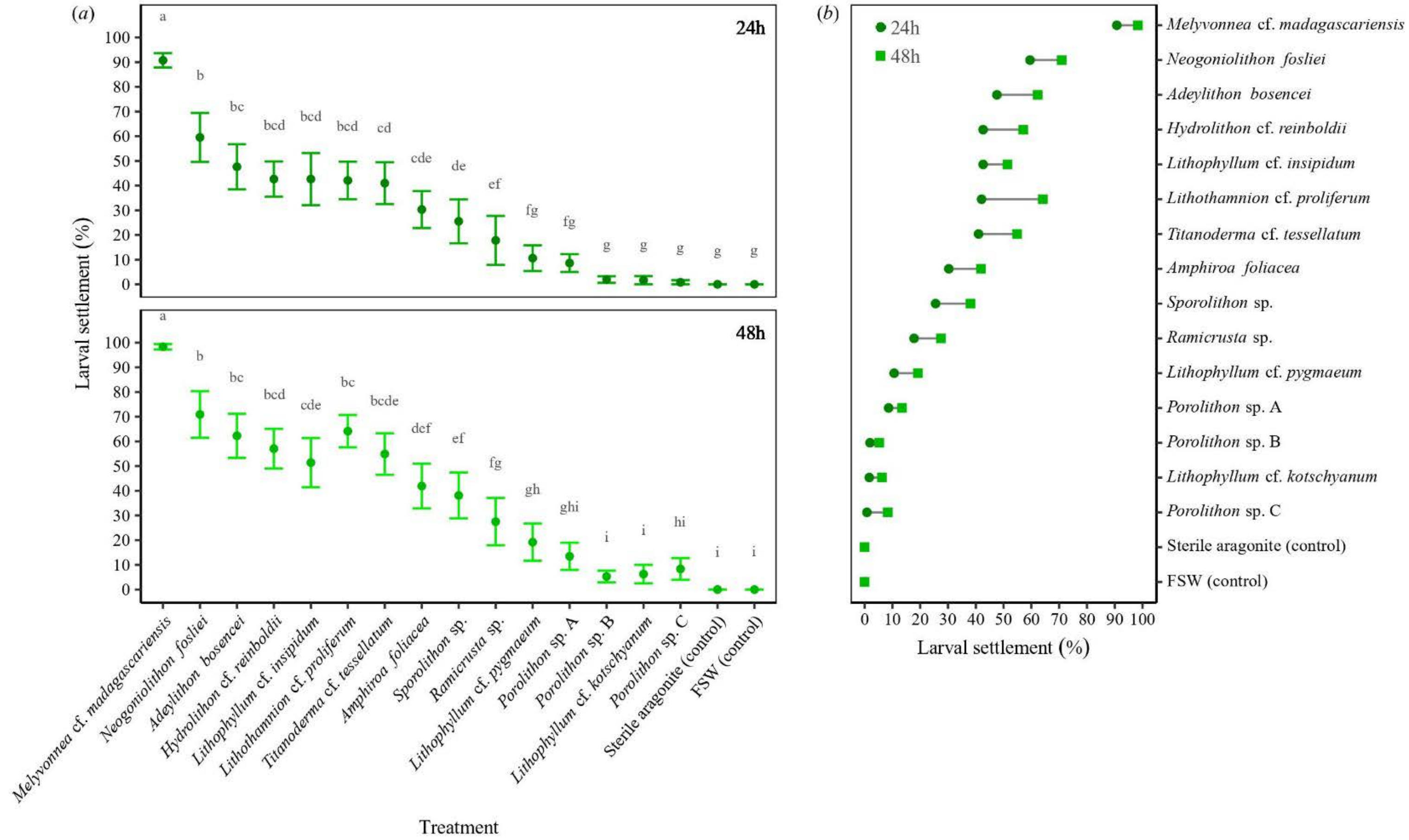

Figure 3.2 (*a*) Settlement cue responses of crown-of-thorns starfish larvae to 15 coralline algae species at 24 and 48 hours (means ± standard error, $n = 12$). Letters denote statistical differences among treatments, with treatment means not sharing any letter being significantly different (Benjamini-Yekutieli-adjusted $P < 0.05$). (*b*) Differences between the mean settlement rates at 24 and 48 hours after experiment commencement. FSW = filtered seawater.

3.4 Discussion

Settlement cues and inducers are critically important in regulating the abundance of many different benthic marine invertebrates (Hadfield and Paul 2001), yet there is very limited understanding of the factors that influence settlement rates of COTS (Pratchett et al. 2021b). The results of this study demonstrate that a wide range of crustose and articulated (geniculate) coralline algae induce COTS settlement, though there were marked differences in settlement rates associated with different algal species. Although it remains to be resolved whether such differences are driven by algal compounds and/or microbial communities (but see Johnson and Sutton 1994), this study corroborates the role of chemical cues in governing benthic habitat selection by planktonic COTS larvae. Like other echinoderm groups (Doll et al. 2022 – **Chapter 2**), COTS larvae display active searching and testing behaviour (Henderson and Lucas 1971, Yamaguchi 1973) to detect chemical cues that presumably signal the presence of preferred early-juvenile food (i.e., coralline algae, Deaker et al. 2020, Wilmes et al. 2020a) or the absence of toxic surfaces and coral polyps that can injure juveniles (Chesher 1969). Physical microhabitat characteristics (**Appendix A**) and the irradiance conditions that algal species occur in (**Table 3.1**) do not appear to be primary factors in COTS settlement induction, however, in the presence of a suitable settlement cue, larvae likely select low light microhabitats within the reef matrix for shelter during metamorphosis (Caballes and Pratchett 2014).

Importantly, the results of this study indicate that COTS larvae do not require cues associated with the coralline alga *Lithothamnion* cf. *proliferum* or other deep-water species for settlement induction. Many algal species that are prevalent at moderate and shallow depths (**Table 3.1**) induced high rates of settlement in this study, including species that primarily occur in shallow reef environments (e.g., *Neogoniolithon fosliei* and *Adeylithon bosencei*). Most notably, the alga *Melyvonnea* cf. *madagascariensis,* which is widely distributed across the depth continuum, promoted considerably higher settlement success than *Lithothamnion* cf. *proliferum* (likely *Lithothamnium pseudosorum* in Johnson et al. 1991). In part because the latter species was previously considered to be the gold standard for COTS settlement induction (Johnson and Sutton 1994) and assumed to be relatively rare at moderate and shallow depths (Johnson et al. 1991), the deep-water recruitment hypothesis proposed that COTS larvae predominantly settle in deep (> 30 m), inter-reef habitats (Johnson et al. 1991). However, this species can be abundant in crevices, caves and overhangs in shallow water (G Diaz-Pulido, pers. obs.). The findings of this further challenge this hypothesis by demonstrating a shallow-water prevalence of suitable settlement cues, which is supported by recorded settlement (Doll et al. 2021 – **Chapter 5**) and newly settled juveniles (Wilmes et al. 2020b) at a range of shallow to intermediate depths (1-18 m).

Apparent selectivity for particular coralline algae by settling COTS larvae has potential importance for understanding population dynamics, because settlement delay and settlement in adverse environments can negatively impact recruitment success (Rodríguez et al. 1993, Pechenik 1990). Marine invertebrate larvae with a specialised settlement stage generally respond to environmental cues to maximise recruitment rates (Pawlik 1992), but limited plasticity in settlement behaviour and narrow habitat requirements imply dependence on encountering suitable habitat at small spatiotemporal scales. There is laboratory-based evidence that COTS larvae can extend their competency periods in the absence of environmental cues (Pratchett et al. 2017b), and the results of this study show that some larvae temporarily defer settlement even in the presence of settlement cues. However, delayed settlement will increase exposure to predators (Cowan et al. 2017, 2020) and can reduce growth and subsequent survival in early juveniles (Rodríguez et al. 1993, Pechenik 1990). Specificity in substratum selection may decrease during the competency period and larvae are more likely to eventually settle in adverse environments (Meyer et al. 2018), increasing susceptibility of juveniles to benthic predation (Wilmes et al. 2018, Cowan et al. 2016a) and starvation (Wilmes et al. 2018, Yamaguchi 1974). Elevated mortality rates before and after settlement may consequently limit recruitment success in habitats with limited settlement cues.

More specifically, the availability of suitable settlement habitat may play a critical role in explaining inter-reef differences in the abundance of COTS and the incidence of population irruptions. The findings of this study indicate that specific algal species (e.g., *Melyvonnea* cf. *madagascariensis*) may be particularly important for promoting or regulating natural settlement rates of COTS. Population irruptions degrading the GBR predominantly occur on reefs in the mid-section of the continental shelf (Sweatman 2008), raising questions pertaining to potential drivers of low adult densities on inshore reefs (Pratchett et al. 2021b). Limited inter-reef connectivity may constrain larval supply in some locations, yet many inshore reefs have been highlighted as significant larval sinks (Hock et al. 2014), pre-disposing them to COTS infestations. Food limitation of planktotrophic larvae is also unlikely to disproportionately inhibit recruitment on inshore reefs given the relatively high concentrations of large phytoplankton in these locations (Wooldridge and Brodie 2015). Instead, the low overall abundance of coralline substrata on inshore reefs (Fabricius and De'ath 2001, Dean et al. 2015, Diaz-Pulido et al. 2016) likely contributes to reduced settlement success in these locations. Moreover, the coralline algae species inducing relatively high settlement rates in this study, except *Hydrolithon* cf. *reinboldii*, are absent or relatively rare in most inshore reefs (**Table 3.1**; Steneck 1982, Ringeltaube and Harvey 2000, Dean et al. 2015). I hypothesise, therefore, that low abundance of coralline algae, particularly of species promoting high settlement rates, poses a significant constraint for COTS recruitment on many inshore reefs. However, in situ studies of settlement rates and coralline algae assemblages across this shelf gradient are required to substantiate this hypothesis.

Notably, the combination of limited larval supply and scarcity of suitable settlement habitat would greatly reduce localised risk of COTS population irruptions and associated coral loss.

In the face of ever-increasing threats to coral reefs, it appears essential to consider all mechanisms that determine recruitment success in this keystone coral predator, both from a theoretical (e.g., life-history theory) and applied perspective (e.g., population control). Whilst previously overlooked in the discussion of factors contributing to the incidence of COTS population irruptions, larval settlement behaviour and settlement cue availability emerge as factors capable of explaining some of the marked spatial heterogeneity in recruitment and adult population sizes.

Chapter 4: Effects of conspecific cues on crown-of-thorns starfish settlement[3]

Abstract

Population irruptions of crown-of-thorns starfish (COTS; *Acanthaster* spp.) remain a major cause of coral reef degradation throughout the Pacific and Indian Oceans and are inherently modulated by larval settlement and recruitment success. Gregarious larval settlement, as exhibited by many other ecologically important marine invertebrates, can catalyse population growth and replenishment. However, whether conspecific cues induce or influence the settlement of COTS larvae remains a critical information gap. This experimental study examined the induction of COTS settlement in response to a range of conspecific cues associated with early- and late-stage herbivorous juveniles, corallivorous juveniles and adults. Competent COTS larvae were generally not induced to settle by the presence of conspecifics or cues associated with conspecifics, while the settlement success of COTS in the presence of coralline algae was not inhibited or enhanced by adding conspecific conditioned seawater. Rather than being reinforced by gregarious settlement, the recruitment of COTS populations appears dependent on associative settlement cues (i.e., coralline algae and/or associated microbial communities) signalling suitable benthic habitat.

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[3] Published as:

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(https://doi.org/10.1038/s41598-023-44422-x)

4.1 Introduction

Cues signaling the presence of conspecific animals can be an effective indicator of habitat suitability, which can be used as an adaptive strategy to form aggregations that confer density-dependent benefits to members (Stamps 1988, Courchamp et al. 1999, Danchin et al. 1998, Farrell et al. 2012). The ability of conspecifics to respond to these cues appears particularly important at major ontogenetic transition points when naïve animals arrive in unfamiliar environments (Muller, 1998). While pertinent information may be gathered from various inputs, including tactile stimuli, it is often chemical signals (i.e., semiochemicals) that elicit salient developmental, physiological and behavioural responses by conspecific individuals (reviewed by Buxton et al., 2020).

Conspecific cues have been implicated in the larval settlement behaviour of many marine invertebrates with bipartite life histories (reviewed by Burke, 1986, Pawlik, 1992), including ecologically important echinoderms (Highsmith 1982, Young and Chia 1982, Burke 1984, Pearce and Scheibling, 1990, Dworjanyn and Pirozzi, 2008). During their planktonic-benthic transition phase (i.e., larval settlement), conspecific cue-mediated (or gregarious) settlement presents an avenue for planktonic larvae to select favourable benthic habitat and thereby enhance survival probability at and after this ontogenetic boundary (Doll et al. 2022 – **Chapter 2**). In most cases, the settlement response (including metamorphosis) is induced by surface-bound and/or waterborne chemical cues, which derive from conspecific juveniles or adults (Burke 1986). For example, sea urchin and sand dollar larvae settle in the isolated presence of conspecific juveniles or adults, their faeces, and conspecific-conditioned water or surfaces (e.g., Pearce and Scheibling 1990a, Dworjanyn and Pirozzi 2008). Because this gregarious settlement behaviour commonly manifests in strongly patterned recruitment (Toonen and Pawlik 1994, Zimmer and Butman 2000), it can have profound effects on the oftentimes irruptive population dynamics of echinoderms and other benthic marine invertebrates (Pawlik 1992, Doll et al. 2022 **– Chapter 2**).

In the case of the corallivorous crown-of-thorns starfish (COTS), conspecific cue-mediated settlement would play a key role in the localised proliferation of COTS on coral reefs. Gregarious recruitment mechanisms as documented for other marine invertebrates could result in positive feedback loops that reinforce population replenishment and rapid population growth of COTS (Uthicke et al. 2009), exacerbating their pervasive threat to the structure and function of coral reef ecosystems. While the ability of adult COTS to respond to waterborne chemical cues derived from conspecifics is well established (Beach et al. 1975, Caballes and Pratchett 2017, Hall et al. 2017, Motti et al. 2018), evidence of cue-mediated behaviour prior or during larval settlement is highly fragmented. Attraction of COTS larvae to conspecific cues was initially hypothesised based on the relatively high abundance of late-stage juveniles at a reef within the adult outbreak area (Chesher 1969). This hypothesis was only recently corroborated in a static choice chamber experiment, which documented horizontal movement of late-stage COTS larvae towards adult conspecifics (Cowan et al. 2016a) and has since been considered as an avenue for COTS population replenishment (Pratchett et al. 2017a, Wilmes et al. 2018, Deaker and Byrne 2022). However, whether the ecologically fundamental process of larval settlement is induced by conspecifics remains a critical information gap for COTS (Pratchett et al. 2021b).

The main objective of this study was thus to test whether COTS larvae settle in response to the presence of conspecifics and to cues associated with conspecifics. Considering the diversity of gregarious cues known to induce marine invertebrate settlement (reviewed by Burke 1986, Pawlik 1992), I examined the specific nature of the potential gregarious cue by assessing surface-bound and waterborne cues associated with early- and late-stage herbivorous juveniles, corallivorous juveniles and adults, representing the different life history-stages that COTS larvae may encounter in the natural environment. Given that COTS settlement is already known to be induced by a wide range of coralline algae-associated cues (Doll et al. 2023a – **Chapter 3**), this study also assessed the interactions between conspecific cues and coralline algae-mediated settlement induction. Importantly, filling these gaps in our understanding of the chemical cues that trigger COTS settlement could improve the ecological underpinning of current culling efforts and/or aid the development of innovative population control methods using semiochemicals (Motti et al. 2018).

4.2 Materials and methods

4.2.1. Spawning and larval rearing

Adult western Pacific crown-of-thorns starfish (COTS, *Acanthaster* cf. *solaris*) were collected from mid-shelf reefs in the central Great Barrier Reef (GBR) and held in flow-through unfiltered seawater tanks (26.5 ± 0.5 °C) at the Australian Institute of Marine Science National Sea Simulator. COTS were sexed upon collection using a hypodermic needle (Caballes et al. 2017), with males and females transported and kept separately in the flow-through tanks. The water temperature used throughout the subsequent rearing and experiment processes of both experiments (28 ± 0.5 °C) represents summer temperatures (COTS reproductive season) in the collection area.

COTS were spawned on November 24th, 2021, for Experiment 1 (juvenile conspecific cues) and November 7th, 2022, for Experiment 2 (adult conspecific cues). For each spawning, a small number of ovary lobes was obtained from six female COTS by making small incisions at the proximal ends of their arms. The ovary lobes were then rinsed with filtered seawater (fsw) through a 500 µm mesh to remove any loose eggs. To induce the maturation and release of the remaining eggs, the ovary lobes were placed into beakers containing 200 mL fsw with 1-methyladenine (treatment concentration: 10-5 M) for 60 minutes. Approximately 20 minutes prior to the completion of the maturation step, sperm was obtained from the arms of six male COTS and 2 µL of dry sperm from each male was mixed in 15 mL fsw. After the eggs were rinsed through a 500 µm mesh to remove any unshed eggs or connective tissues, the number of eggs per mL was counted in the stock solution. Part of the sperm stock solution (1 mL) was then added to the egg stock solution and more than 80% successful fertilisation was observed using a stereo microscope. The fertilised embryos were then divided between two 70 L vats with low air line setting at a density of approximately 10 embryos ml^{-1}.

Twenty-four hours post-fertilisation, 100% water exchanges were conducted to remove any undeveloped embryos and dead larvae. At 48 hours post-fertilisation, the water exchange process was repeated to concentrate healthy larvae and larval stages were scored using stereo microscopes. The larvae were then moved into 16 L flow-through culture cones, stocked at approximately 1 larvae mL^{-1}. At 5 days post-fertilisation, larvae started feeding on Dunaliella sp. (CSIRO CS-353; Australian National Algae Culture Collection Strain List 2022) and Isochrysis sp. (CSIRO CS-177) stock cultures. All rearing tanks were supplied with the algal feeds (1200-1500 cells per ml) via automatically dosed treatment tanks with controlled chlorophyll concentrations (Uthicke et al. 2018). Full water changes were carried out three times a week, whereby healthy larvae were siphoned into holding buckets while rearing cones, tubes and air lines were thoroughly cleaned. I regularly examined larval development using stereo microscopes throughout the rearing process and determined larvae competent to settle once they reached the late-brachiolaria stage with a well-developed rudiment. Metamorphic competency was further corroborated by 24-hour trials using a coralline alga known to induce settlement (*Lithothamnion* cf. *proliferum*) and, for both experimental larval batches, competency was confirmed at 14 days post-fertilization (December 9th, 2021, and November 21st, 2022). Because multiple culture cones were required for the experiments, larvae from the different cones were thoroughly mixed before allocation to treatments.

4.2.2 Experimental treatments

Experiment 1 (juvenile conspecific cues) and 2 (adult conspecific cues) consisted of nine and five experimental treatments, respectively (**Table 4.1**), including positive (treatments 1.7 and 2.4; Doll et al. 2023a – **Chapter 3**) and negative controls (treatments 1.9 and 2.5). To obtain 1-month old juveniles for Experiment 1 (treatments 1.1 and 1.4), COTS were spawned on November 9th, 2021, and larvae were reared following the aforementioned protocol. Settlement was facilitated using the coralline alga *Lithothamnion* cf. *proliferum* and post-metamorphic juveniles were raised on a mixed coralline algae diet at the National Sea Simulator for two weeks. To obtain 1-year old juveniles (treatments 1.2, 1.3, 1.5, 1.6 and 1.8), COTS larvae were reared following the same protocol and settled in early December 2020. The herbivorous juveniles were raised on a mixed coralline algae diet for 12 months and a subset of this juvenile cohort was transitioned to a coral diet (*Acropora* spp.) six weeks prior to experiment commencement (treatments 1.3 and 1.6). At the start of the experiment, the mean sizes of the 1-year-old herbivorous and corallivorous juveniles were 0.84 cm (± 0.04 se) and 1.19 cm (± 0.04 se), respectively. The conditioned fsw treatments (1.4 - 1.6) were obtained by placing five individuals from each juvenile cohort (1.1 - 1.3) in glass aquaria filled with 810 mL fsw for 48 hours. Pieces of *Lithothamnion* cf. *proliferum* (treatments 1.7, 1.8 and 2.4) were identified based on morpho-anatomical features and molecular sequencing (Doll et al. 2023a – **Chapter 3**) and cut into replicate 0.5 x 0.5 cm chips prior to experiment commencement.

Table 4.1 Treatments used in settlement assays with crown-of-thorns starfish larvae (*n* = 12 independent and randomised wells for each treatment).

Treatment	Description
Experiment 1	
1.1 1-m (alg)	1-month old herbivorous juvenile (coralline algae spp.)
1.2 1-y (alg)	1-year old herbivorous juvenile (coralline algae spp.)
1.3 1-y (cor)	1 year-old corallivorous juvenile (*Acropora* spp.)
1.4 csw 1-m (alg)	fsw conditioned with 1-month old herbivorous juveniles (1.1)
1.5 csw 1-y (alg)	fsw conditioned with 1-year old herbivorous juveniles (1.2)
1.6 csw 1-y (cor)	fsw conditioned with 1-year old corallivorous juveniles (1.3)
1.7 *L. pro*	0.5 x 0.5 cm live coralline algae chip (*Lithothamnion* cf. *proliferum*) (positive control)
1.8 *L. pro* + csw	herbivorous 1-y-o juvenile conditioned fsw (1.5) added to coralline algae chip (1.7)
1.9 fsw	filtered seawater (negative control)
Experiment 2	
2.1 tube foot	adult tube foot obtained from single 20 cm Ø male
2.2 spine	adult spine piece (1.5 cm length) obtained from single 20 cm Ø male
2.3 csw	conditioned seawater from 1,200 L flow-through tank containing 12 males (15-35 cm Ø)
2.4 *L. pro*	0.5 x 0.5 cm live coralline algae chip (*Lithothamnion* cf. *proliferum*) (positive control)
2.5 fsw	filtered seawater (negative control)

4.2.3. Settlement assays and analysis

To test whether the presence of conspecifics and conspecific cues induce COTS larvae to settle, 12 replicate settlement assays were conducted for each of the experimental treatments. Using six-well cell culture plates, I fully randomised the distribution of all replicate assays among the 108 and 60 wells for the juvenile and adult conspecific cue experiments, respectively. Upon adding 10 mL fsw and the treatments, I carefully pipetted approximately 10 competent COTS larvae to each well. Settlement success was scored 24 hours after the larvae were introduced using stereo microscopes. For each replicate assay, I recorded the number of swimming late-brachiolaria larvae and the number of individuals which attached to the treatment or well bottom and commenced or completed metamorphic transformation into a juvenile with radial symmetry (= settled).

Statistical analyses were performed and figures were computed using R software (v. 4.1.3, R Core Team 2022). Mean settlement success for each treatment was calculated and plotted based on the settlement rates obtained from replicate assays (*n* = 12 per treatment), considering the proportion of swimming larvae and settled postlarvae. To determine whether settlement success in the presence of coralline algae (treatment 1.7) may be inhibited or enhanced by adding conspecific conditioned seawater (treatment 1.8), a two-sample *t* test was performed using the *stats* package (R Core Team 2022). Due to the absence of settlement success across treatments not containing coralline algae, these data were not formally analysed. Figures were generated using the *ggplot2* package (Wickham 2016).

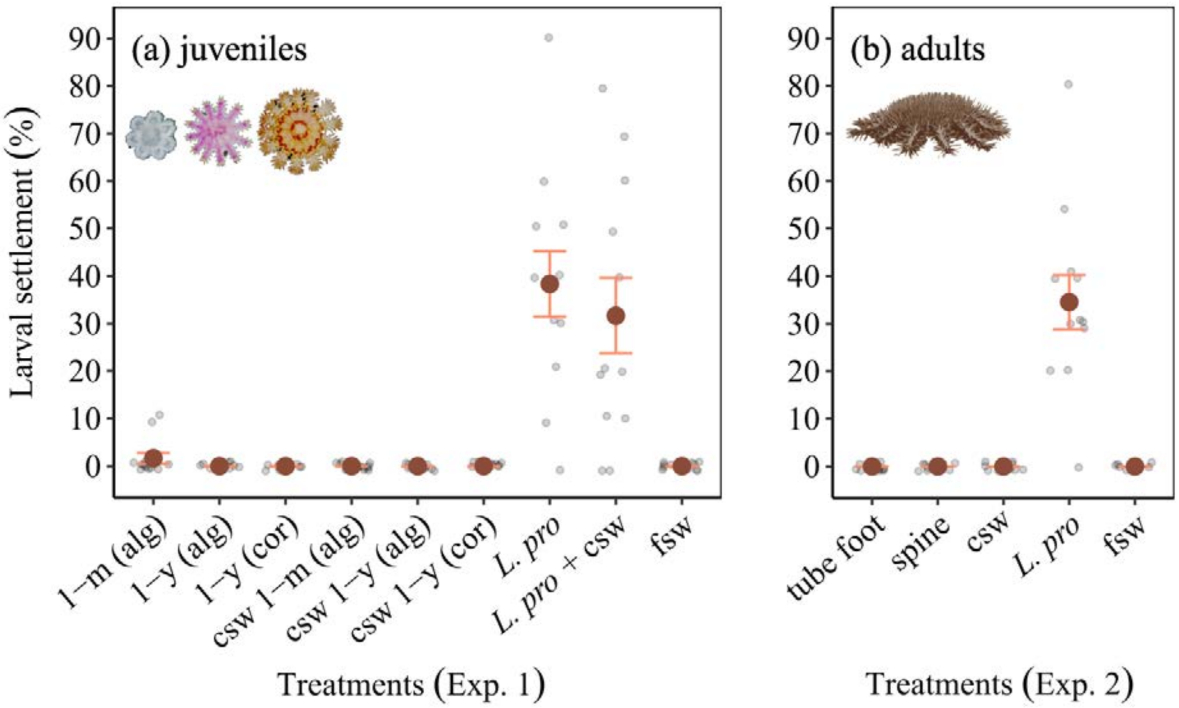

Figure 4.1 Settlement success of crown-of-thorns starfish larvae (means ± se, *n* = 12) for each treatment, calculated based on replicate assay settlement rates (grey points). Descriptions of the experimental treatments are listed in **Table 4.1**.

4.3 Results and discussion

Establishing whether COTS larvae settle gregariously advances our understanding of ecological mechanisms that drive population growth and replenishment in this keystone coral predator. Here, I found no evidence of COTS settlement induction in the presence of conspecifics or by cues associated with conspecifics (**Figure 4.1**). While two individuals had commenced metamorphosis in the presence of 1-month-old herbivorous juveniles (1.67 % settlement ± 1.12 se; *n* = 120 larvae), this likely denotes spontaneous settlement in the absence of a suitable settlement cue, which has previously been observed in small proportions of late-stage COTS larvae (Wolfe et al. 2015, Pratchett et al. 2017b). No larvae settled across all other juvenile or adult conspecific treatments (**Figure 4.1**), which is in direct contrast to the hypothesis suggesting that COTS settlement may be mediated by conspecific cues (Chesher 1969, Pratchett et al. 2017a, Wilmes et al. 2018, Deaker and Byrne 2022), which was largely based on documented larval movement towards adult conspecifics in a static choice chamber experiment (Cowan et al. 2016a). While some degree of conspecific chemo-attraction of settling COTS larvae towards conspecifics is conceivable (Motti et al. 2018) and may influence the movement of planktonic larvae prior to settlement, the results of this study suggest that COTS settlement induction and concomitant spatiotemporal settlement patterns must be largely governed by other environmental inputs, such as coralline algae-associated cues (Doll et al. 2023a – **Chapter 3**).

The absence of conspecific-cue mediated settlement in COTS stands in contrast to the conspicuous mechanisms and patterns of gregarious settlement in other echinoderms (reviewed by Doll et al. 2022 – **Chapter 2**) and many ecologically important marine invertebrate taxa (Scheltema et al. 1981, Suer and Phillips 1983, Jensen and Morse 1984, Toonen and Pawlik 1996, Dreanno et al. 2006). Chemical cues released by conspecifics have been shown to induce larval settlement responses in sea urchins (Dworjanyn and Pirozzi 2008, Mos et al. 2011), sand dollars (Highsmith 1982, Burke 1984, Highsmith and Emlet 1986, Takeda 2008), sea cucumbers (Young and Chia 1982, McEuen and Chia 1985), brittle stars (Warner 1971, Morgan and Jangoux 2004, 2005), and feather stars (Mladenov and Chia 1983, Lahaye and Jangoux 1985, Obuchi et al. 2010). However, the identification of specific chemical compounds triggering this behaviour has proven elusive in most cases (Doll et al. 2022 – **Chapter 2**), except for a small peptide responsible for settlement induction in the sand dollar *Dendraster excentricus* (Highsmith 1982, Burke 1984, Highsmith and Emlet 1986). Independent of this gregariousness, plasticity in settlement behaviour is generally quite prevalent among echinoderms (Pawlik 1992, Doll et al. 2022 – **Chapter 2**), contrasting the seemingly high cue specificity displayed by COTS larvae (Johnson and Sutton 1994, Doll et al. 2023a **– Chapter 3**).

Notably, the results of this study also provided no evidence that the addition of conspecific conditioned seawater inhibits or enhances settlement success in the presence of a known settlement cue, the coralline alga *Lithothamnion* cf. *proliferum* (**Figure 4.1**; two-sample *t* test, *t* = 0.64, df = 22, *P* = 0.532). Mean settlement success for wells containing *L*. cf. *proliferum* (38.33 % ± 6.83 se) and the combination of *L*. cf. *proliferum* and seawater conditioned with conspecific juveniles (31.67 % ± 7.96 se) was comparably high and similar to previously reported COTS settlement rates in the presence of this coralline alga (Doll et al. 2023a **–** C**hapter 3**). Planktonic COTS larvae are unlikely to encounter isolated environmental stimuli in the field but are instead often exposed to a suite of waterborne chemical cues with the potential to promote or inhibit settlement (Doll et al. 2022 – **Chapter 2**). On coral reefs, the same rubble environments in which herbivorous COTS juveniles are usually observed also provide large amounts of the food source (i.e., coralline algae) required for this life-history stage (Wilmes et al. 2018, 2020, Doll et al. 2021 **– Chapter 5**). The results of the present study indicate that the selection of rubble habitats by settlement-stage COTS larvae is not affected by the presence of conspecific juveniles inhabiting these reef environments.

Although the prevalence of gregariousness in benthic marine invertebrate communities indicates that the benefits may outweigh the costs (Pawlik 1992), the lack of gregarious settlement behaviour displayed in this study is not necessarily disadvantageous for COTS, at least at the individual organism level. There are clear trade-offs between potential advantages (e.g., favourable habitat, including food availability) and disadvantages (e.g., competition for food) of this phenomenon for members of resulting aggregations (Burke

1986, Pawlik and Faulkner 1986, Pawlik 1992, Slattery 1997). On balance, the presence of conspecifics usually signals suitable environmental conditions, however, planktonic larvae may obtain some of the same benefits by responding to associative cues, originating from heterospecific organisms such as juvenile food sources (Crisp 1974), without the potential costs associated with conspecific aggregations. Considering the relatively narrow ecological niche of COTS during their herbivorous juvenile stage (Wilmes et al. 2020a), settlement in the proximity of juveniles would likely be maladaptive, since limited distribution of individuals and concomitant food competition for coralline algae may inhibit their early post-settlement growth and fitness (Pawlik 1992). It is not surprising that settling COTS larvae also appear insensitive to the presence of larger coral-feeding individuals, given marked differences in their diet and habitat requirements (Zann et al. 1987).

The relative importance and influence of conspecific settlement cues among echinoderms and other marine invertebrates is clearly taxon-specific, and, warrants further research for ecologically important groups. The results of this study suggest that COTS larvae require associative cues originating from coralline algae (and/or microbial communities), rather than gregarious cues, for the successful transition to their benthic juvenile stage. COTS larvae may be attracted to chemical cues released by conspecifics and move towards them during their planktonic phase (Cowan et al. 2016a), however, an algae-derived cue appears necessary to induce the cascade of behavioural events associated with settlement (Doll et al. 2022 – **Chapter 2**). In any case, the absence of salient gregarious settlement behaviour as we know it from other benthic marine invertebrates represents good news for coral reefs featuring high adult COTS densities, because it diminishes the likelihood of high self-recruitment and positive feedback loops that reinforce population growth and replenishment.

Chapter 5: DNA-based detection and patterns of crown-of-thorns starfish settlement[4]

Abstract

Population irruptions of the western Pacific crown-of-thorns starfish (COTS; *Acanthaster* cf. *solaris*) are a perennial threat to coral reefs and may be initiated by fluctuations in reproductive or settlement success. However, the processes dictating their early life-history, particularly larval settlement, remain poorly understood given limitations in sampling larvae and newly settled juveniles in the field. Here, I introduce an innovative method to measure COTS settlement using artificial settlement collectors and droplet digital polymerase chain reaction (ddPCR) based on COTS specific mtDNA primers. This study demonstrated the utility of this method and explored temporal and spatial patterns of COTS settlement on the Great Barrier Reef (GBR) from 2016 to 2020. Settlement varied considerably among sampling periods at Rib Reef and peaked between October 2016 and January 2017. The results of this study further suggest that COTS larvae readily settle in shallow reef environments, with no preferential settlement detected among depths tested (4-12 m). Substantial variation among GBR regions was revealed in 2019-20, as collectors deployed on reefs in the central GBR were > 10 times as likely to record newly settled COTS as reefs in the northern GBR near Lizard Island. The trends reported here add to our understanding of this critical life-history stage, however, further method validation and

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[4] Published as:

Doll, P.C., Messmer, V., Uthicke, S., Doyle, J.R., Caballes, C.F. & Pratchett, M.S. 2021. DNA-based detection and patterns of larval settlement of the corallivorous crown-of-thorns sea star (*Acanthaster* sp.). *The Biological Bulletin* 241, 271-285. (https://doi.org/10.1086/717539) larger-scale studies are needed to address pertinent information gaps such as the stock-recruitment dynamics of this species. Most importantly, fluctuations in COTS settlement can now be detected using this sampling protocol, which demonstrates its utility in heralding new and renewed population irruptions of this destructive starfish.

5.1 Introduction

Crown-of-thorns starfish (COTS; *Acanthaster* spp.) are major contributors to coral loss and coral reef degradation on Australia's Great Barrier Reef (GBR) as a result of their propensity to undergo major population irruptions (Pearson 1972, Endean and Stablum 1975, Pratchett et al. 2014, Mellin et al. 2019). Understanding the supply-side ecology of COTS and the drivers of their population irruptions has thus been a perennial research activity since the 1960s (reviewed by Moran 1986, Pratchett et al. 2017a). The exceptional reproductive potential of this taxon suggests that even small changes in key demographic rates could lead to population irruptions (Uthicke et al. 2009, Babcock et al. 2016). Population irruptions of COTS have been attributed to single mass-recruitment events (Zann et al. 1987), or progressive accumulation of starfish over successive years of elevated recruitment (Kenchington 1977, Stump 1996, Pratchett 2005). Sudden versus progressive increases in population size highlight the critical importance of assessing demographic processes that influence population replenishment. Our ability to predict, detect, understand and manage irruptions, however, has largely been constrained by persistent knowledge gaps surrounding the early life-history of COTS (Caballes and Pratchett 2014, Wilmes et al. 2018, Pratchett et al. 2021b).

Challenges of studying COTS larvae and juveniles in the field, due to their small size and cryptic nature (Zann et al. 1987, Doherty and Davidson 1988), have greatly constrained progression in addressing pertinent information gaps surrounding these life stages. More recently, COTS larvae have been detected and quantified in plankton samples using molecular techniques (Doyle et al. 2017). Wilmes et al. (2016, 2020a) demonstrated that 6+ month old starfish can be effectively sampled in their natural environments, using fine scale visual sampling in appropriate habitats. However, substantial progress has yet to be made in the detection and assessment of COTS settlement. The larval settlement phase, which begins with the onset of a behavioural search for suitable substratum and includes the attachment to the substratum triggering metamorphosis and subsequent commencement of the benthic life stage (Rodriguez et al. 1993, Tomas et al. 2004), plays a crucial role in determining the population and community structure of starfishes (Balch and Scheibling 2001). Artificial settlement collectors have previously been deployed on the GBR to measure settlement rates of echinoderms, including COTS (Keesing et al. 1993). Furthermore, progress made in DNA-based detection of the larval (Uthicke et al. 2015, 2019, Doyle et al. 2017) and post-settlement stages (Uthicke et al. 2019, Doyle and Uthicke 2021, Kwong et al. 2021) provides an opportunity to assess COTS settlement in the field and explore spatiotemporal patterns.

Marked spatiotemporal variation in the abundance of COTS is almost certainly linked to differential rates of settlement and recruitment (Zann et al. 1987, Uthicke et al. 2009). However, studies have made inferences about inter-annual settlement patterns based on the densities and distribution patterns of 6+ month old juveniles or adults (e.g., Zann et al. 1987). On the GBR, Doherty and Davidson (1988) observed more than one order of magnitude of variation in COTS recruitment density over three years. Likewise, Zann et al. (1987, 1990) reported highly variable inter-annual recruitment over 13 consecutive years in Fiji, with peak recruitment three orders of magnitude greater than the previous and following years. These studies concluded that outbreak populations of COTS were driven by the heavy recruitment events observed, which, in turn, were attributed to high rates of larval starfish settlement

(Zann et al. 1987, 1990; Doherty and Davidson 1988). However, high rates of larval settlement do not necessarily give rise to heavy recruitment into juvenile and adult starfish populations (Ebert 1983, Keesing and Halford 1992, Lang et al. 2021), especially if juveniles experience high rates of mortality (Keesing et al. 2018) and remain as juveniles for unknown periods of time (Deaker et al. 2020). Loosanoff (1964) monitored settlement rates of the starfish *Asterias forbesi* by measuring settler densities within a few days of settlement over 25 years. Here, inter-annual settlement rates fluctuated up to four orders of magnitude, however, no relationship between larval settlement rates and adult population densities in subsequent years was observed (Loosanoff 1964). This further highlights the necessity to develop techniques measuring natural rates of COTS settlement or newly settled juveniles, as opposed to recruitment to older juvenile and adult populations.

Variation in settlement rates likely also exists at multiple spatial scales, but questions regarding spatial settlement patterns are mostly unresolved for COTS (Pratchett et al. 2017a). The extent to which COTS settlement patterns vary along depth gradients on coral reefs is regularly questioned because of its relevance to the irruptive growth displayed by *Acanthaster* populations. The deep-water recruitment hypothesis proposed by Johnson et al. (1991) suggests that COTS larvae predominantly settle in relatively deep water (20-40 m), and only start ascending the reef slope after their ontogenetic diet shift from coralline algae to coral as 6+ month old juveniles (Yamaguchi 1974, Wilmes et al. 2020a). Conversely, earlier reports suggested larvae readily settle in shallow reef environments (Chesher 1969, Endean 1973), and such high densities of newly settled starfish have yet to be observed at the depth range described by Johnson et al. (1991).

At larger spatial scales, substantial variation in settlement rates among reefs and locations have been described for a wide range of other starfish taxa (e.g., Balch and Scheibling 2000, Jennings and Hunt 2010, Glockner-Fagetti and Phillips 2020). On the GBR, we expect similar trends for COTS settlers as the presence and quantity of the physical, biological and chemical factors dictating their settlement preferences are highly variable across such gradients (Johnson et al. 1991, Cowan et al. 2016a, Pratchett et al. 2017b, Wilmes et al. 2020b). However, to establish whether settlement preferences determine spatial settlement patterns in the field, the limitations to effective sampling of COTS settlers along pertinent spatial gradients must be overcome.

If reliable measurements of settlement patterns in the field are obtained across appropriate spatiotemporal scales, it will allow us to fill persistent information gaps related to this early life-history stage, but also further our understanding of the various links between larval supply, settlement rates, recruitment and adult population densities. Our inability to explore these links to date has prevented us from testing the assumptions of hypotheses attempting to explain primary population irruptions, such as that larval supply is a generally limiting factor, and irruptions likely occur because of increased larval survival (Pratchett et al. 2014). It is essential to include settlement rates as a variable in demographic models (Underwood and Fairweather 1989) and assessing the stock-recruitment relationships of this species is paramount to testing the effectiveness of the current population regulation solely based on the culling of adult starfish. Moreover, the efficacy of culling activities may be further refined if new methods to monitor larval densities and settlement rates are integrated into an effective early warning system for new and renewed population irruptions of this destructive starfish.

Here, I introduce an innovative method to record larval settlement of the western Pacific COTS, *Acanthaster* cf. *solaris*, using purpose-built artificial settlement collectors and genetic analyses. The purpose of this study was to confirm that newly settled COTS can be detected using this method, and to explore preliminary temporal and spatial patterns in COTS settlement on the GBR. Of particular interest to this study was whether: (1) settlement varied within a reproductive summer period, and among years; (2) settlement would be recorded at shallow depths; and (3) settlement varied between GBR regions. To explore temporal trends in larval settlement, larval settlement collectors were deployed at Rib Reef in the central GBR over four sampling periods from 2016 to 2020. The extent to which larvae settle in shallow reef environments was assessed via collector deployments across a range of depths (4-12 m) during the same sampling periods. To test whether settlement varied at larger spatial scale, reefs located in the northern and central regions of the GBR were sampled during the 2019-20 reproductive season.

5.2 Materials and Methods

5.2.1 Sampling methodology

For the purpose of this study, settlement is defined as the appearance of newly settled COTS (*Acanthaster* cf. *solaris*) in artificial settlement collectors during a sampling period (*sensu* Hernández et al., 2006). Settlement collectors were made up of two leaf baskets filled with approximately 60-70 aquarium filter Ovi-Flow bio balls (Aquasonic, Wauchope, Australia; **Figure 5.1**). This method was a modification from previously designed samplers (Keesing et al. 1993, Hernández et al. 2006), which have been shown to be efficient collectors of newly settled echinoderms. Bio balls have a very high surface-to-volume ratio, which is favorable for settlement and metamorphosis (Hunte and Younglao 1988). Keesing et al. (1993) showed that larvae settled readily on sea water conditioned plastic surfaces in laboratory trials. Leaf basket assemblies were filled with bio balls to the maximum extent to keep the individual bio balls immobile inside the collector. During deployment, each collector was secured to the substrate with a 400 mm long stainless-steel stake and a 600 mm long piece of wire rope, allowing the positively buoyant apparatus to float approximately 1 m above the substrate (**Figure 5.1**).

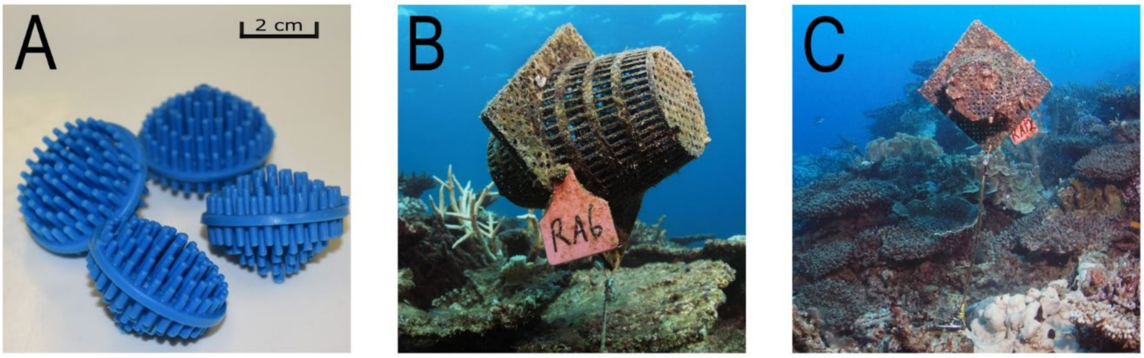

Figure 5.1 Artificial collector method utilised to measure crown-of-thorns starfish (*Acanthaster* cf. *solaris*) settlement. Photographs of the bio balls (A; Aquasonic Ovi-Flow balls) used, a larval settlement collector in the field (B), and a larval settlement collector secured to the substrate using stainless steel stakes and wire rope (C).

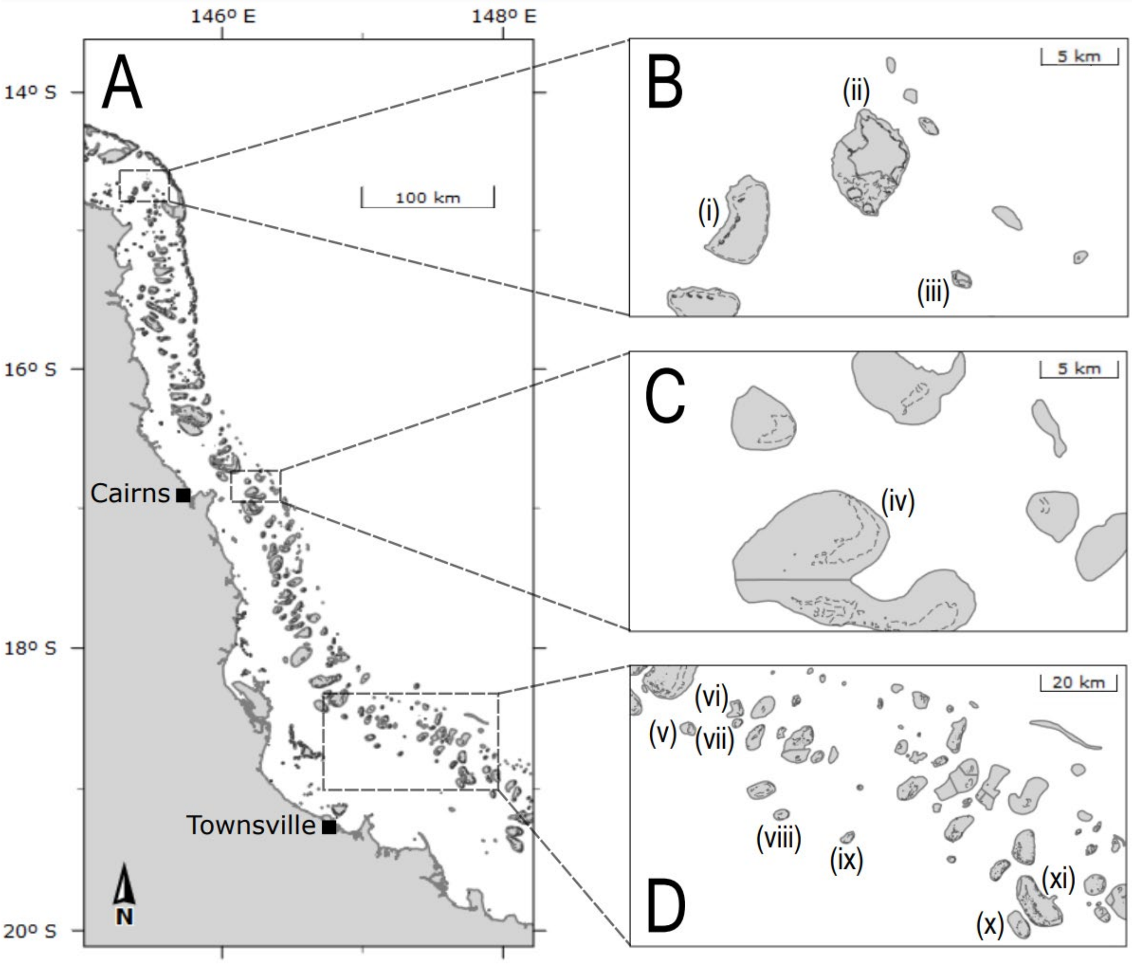

Figure 5.2 Maps indicating the field sample locations and reefs along the Great Barrier Reef (GBR) at which crown-of-thorns starfish (*Acanthaster* cf. *solaris*) settlement collectors were deployed. (A) The three sampling locations in the northern and central GBR. (B) Reefs sampled in the northern GBR near Lizard Island: (i) Eyrie Reef, (ii) North Reef, (iii) North Direction Island. (C) Reef sampled in the northern GBR near Cairns: (iv) Moore Reef. (D) Reefs sampled in the central GBR: (v) Rib Reef, (vi) Kelso Reef, (vii) Little Kelso Reef, (viii) Lodestone Reef, (ix) Keeper Reef, (x) Little Broadhurst Reef, (xi) Big Broadhurst Reef.

Table 5.1 Number of replicate larval settlement collectors deployed and retrieved for each sampling period and reef, and number and percentage of collectors in which crown-of-thorns starfish (*Acanthaster* cf. *solaris*) settlement was detected.

Sampling period	Sampling reef	Collectors deployed (No.)	Collectors retrieved (No.)	Collectors with newly settled COTS (No.)	Settlement detection (%)
October 2016 - January 2017	Rib Reef [a]	27	18	13	72.2
	Moore Reef [b]	27	27	4	14.8
December 2016 - March 2017	Rib Reef [a]	27	22	4	18.2
	Moore Reef [b]	27	27	4	14.8
November 2017 - February 2018	Rib Reef [a]	27	23	4	17.4
	Lodestone Reef [a]	27	21	7	33.3
October/November 2019 – January/February 2020	Eyrie Reef [c]	5	5	0	0
	North Reef [c]	5	3	0	0
	North Direction Island [c]	5	5	1	20.0
	Rib Reef [a]	6	6	1	16.7
	Kelso Reef [a]	6	6	3	50.0
	Little Kelso Reef [a]	6	6	2	33.3
	Lodestone Reef [a]	6	6	4	66.7
	Keeper Reef [a]	6	6	4	66.7
	Little Broadhurst Reef [a]	6	6	5	83.3
	Big Broadhurst Reef [a]	6	6	4	66.7

[a] Sampling reefs located in the central Great Barrier Reef near Townsville

[b] Sampling reefs located in the northern Great Barrier Reef near Cairns

[c] Sampling reefs located in the northern Great Barrier Reef near Lizard Island

A total of 219 larval settlement collectors were deployed on the GBR (**Figure 5.2**) during four sampling periods between October 2016 and February 2020 (**Table 5.1**). Sampling periods chosen (approximately 2.5 months) coincided with the COTS summer reproduction and spawning season (Uthicke et al. 2019), with collectors deployed prior and retrieved after the predicted spawning. It was not feasible to deploy settlement collectors for shorter periods in this study, however, the caveat of post-settlement mortality prior to collector retrieval must be considered when interpreting the results presented hereafter. All larval settlement collectors were deployed at sites with relatively steep reef slopes defined by a high cover of coral rubble encrusted with coralline algae, which is where COTS juveniles are typically observed (Wilmes et al. 2020b).

During the 2016-17 and 2017-18 sampling periods, three replicate collectors were deployed within three different depth ranges (4-6 m; 7-9 m; 10-12 m) at three sites per reef, totaling 27 collector deployments per reef. Collectors were deployed on Moore Reef and Rib Reef (**Figure 5.2**) in both October and December 2016, and retrieved in January and March 2017, respectively (**Table 5.1**). Additional sets of 27 collectors were deployed at Rib Reef and Lodestone Reef in November 2017 and retrieved in February 2018 (**Table 5.1**; **Figure 5.2**). 57 larval settlement collectors were deployed in October and November 2019 and retrieved in January and February 2020. Here, sampling sites were Rib Reef (6 collectors deployed), Kelso Reef (6), Little Kelso Reef (6), Lodestone Reef (6), Keeper Reef (6), Little Broadhurst Reef (6) and Big Broadhurst Reef (6) in the central GBR, and North Reef (5), North Direction Island (5) and Eyrie Reef (5) in the northern GBR (**Figure 5.2**).

5.2.2 Sample preparation

Prior to retrieval, each collector was covered in a fine mesh cotton bag. Upon retrieval, samples inside the sealed cotton bags were held in sea water flow-through tubs until processing. Each individual collector was processed inside a 20-liter bucket, which was modified with an approximately 100 x 100 mm sized drainage window fitted with fine mesh (120 μm) to contain any early post-settlement starfish (> 1000 μm in size). The cotton bag, leaf baskets and individual bio balls inside each collector were hosed with high-pressure sea water, as commonly done in echinoderm settlement studies (Balsalobre et al. 2016, Glockner-Fagetti and Phillips 2020). As some newly settled starfish may not be dislodged using the pressurised water, any remaining organic material was removed using fine tweezers to maximise removal efficiency without breaking up the entire apparatus in ethanol. Upon removal of all material, the remaining sea water was drained from the bucket through the fine mesh window. The sample was transferred into a 600 mL jar and preserved in 100% ethanol, with a material to ethanol ratio of at least 1:10, and stored in the freezer at -20 °C until further analyses. In preparation for DNA extraction, each sample was drained of ethanol and placed in a vacuum freeze dryer (Dynavac) for 12 to 36 hours. Dry samples were homogenised by blitzing them individually in a high-speed blender (NutriBullet) until a fine powder was produced. Blender cups and blades were soaked for 30 minutes in a bleach solution (10% NaOCl), thoroughly rinsed and dried between each sample. The homogenous powder was transferred into 15 mL or 50 mL Falcon Conical Centrifuge Tubes (Thermo Fisher Scientific, Waltham, MA), depending on its sample volume and weighed to the nearest 0.005 g.

5.2.3 DNA extraction

The following protocol resulted in the successful amplification of COTS DNA amongst the sample and was subsequently used. This extraction protocol presents a modified version of the protocol described in Wilson et al. (2002). DNA was extracted from a small subsample (0.025-0.050 g) in a 2 mL Eppendorf tube, which was subsequently weighed to the nearest 0.001 g. Wayne's Grinding Buffer was prepared with 100 mM Tris pH9, 100 mM EDTA pH8, 1% SDS, 100 mM NaCl and Milli-Q water, and 750 μL of Wayne's Grinding Buffer was added to each sample. The samples were vortexed and incubated at 65 °C for 60 minutes. Following incubation, the samples were placed in crushed ice to cool down, and 187.5 μL of 5 M KOAc was added to each sample. Samples were vortexed again, placed on ice for 10 minutes and spun at maximum speed (16100 rcf) in a bench top centrifuge at room temperature for 20 minutes. The supernatant was transferred to a new Eppendorf tube. Isopropanol (600 μL) was added to each tube to precipitate the DNA and the resulting solution was gently mixed and allowed to stand at room temperature for 5 minutes. Samples were spun at maximum speed and room temperature for 15 minutes. The supernatant was carefully decanted and 600 μL of 70% EtOH was added to each tube. The sample was mixed gently and spun at maximum speed and room temperature for 5 minutes. The supernatant was removed again, and this step was repeated a second time. Following this, the supernatant was removed with extreme care using a pipette. The DNA pellet was air-dried for 5 minutes and resuspended in 150 μL of 10 mM Tris pH9. Given the large sample volumes, I could not use the DNA extraction methods originally applied for larval and adult DNA (Uthicke et al. 2015, 2018a, 2019; Doyle et al., 2017; Doyle and Uthicke, 2021).

5.2.4 Analysis via droplet digital PCR (ddPCR)

Standard polymerase chain reactions (PCR) were run in 2017 and 2018 as described in Uthicke et al. (2019), using 10 μL assay volumes containing 1 μL DNA template, 5 μL AmpliTaq gold 360 x 2 master mix and 400 nM forward and reverse COTS COI gene primers (COTS-COI_F_1321, COTS-COI_R_1446; Doyle et al., 2017). These primers are specific for all *Acanthaster* species except *A. brevispinus*, however, of the species detected only the western Pacific COTS (*Acanthaster* cf. *solaris*) exists in the study area. Using standard PCRs, COTS DNA was clearly detected in some settlement collector samples as evidenced by strong bands on 2% agarose gel, with weak bands detected for some samples categorised as potential detections. After trialing quantitative PCR (qPCR), which proved difficult to successfully run due to its high sensitivity to inhibitors, ddPCR was trialed, which showed a greater resistance to inhibitors and provides relatively low limits of quantification (LOQ) (Uthicke et al., 2018a). A preliminary trial of 16 samples using ddPCR produced positive COTS DNA detections in congruence with the results from standard PCRs. Hence, ddPCR analyses were completed for all 193 larval settlement collectors retrieved between 2017 and 2020. Twenty-five μL assay volumes of PCR mix contained 5 μL DNA template,12.5 μL 2x ddPCR Supermix for Probes (no dUTP) (Bio-Rad, cat. no. 1863005), 400 nM forward and reverse COTS COI gene primers (F-primer: COTS-COI_F_1321; R-primer: COTS-COI_R_1446) and 100 nM hydrolysis probe (5’ FAM-CTATCTCATCCATAGGCAGCAC; Doyle et al. 2017). Droplet digital PCR was conducted using the Bio-Rad QX200 droplet generator and reader systems.

Twenty microlitres of the prepared PCR mix was pipetted into the appropriate well of a Droplet Generator DG8 cartridge (Bio-Rad, cat. no. 1864008), with 70 μL of Droplet Generation Oil for Probes (Bio-Rad, cat. no. 1863005) pipetted into the oil chambers of the cartridge. Cartridges were covered with a DG8 Gasket (Bio-Rad, cat. no. 1863009) and droplets were generated using a QX200 Droplet Generator (Bio-Rad, cat. no. 1864002). The gasket was discarded, and the generated 8 droplets were carefully pipetted into a semi-skirted 96-Well PCR Plate (Bio-Rad, cat. no. 12001925). Upon completing these steps for twelve sets of samples, the PCR plate was sealed with a pierceable foil heat seal (Bio-Rad, cat. no. 1814040) in a PX1 PCR Plate Sealer (Bio-Rad) and placed into a C1000 Touch Thermal Cycler (Bio-Rad). The following PCR protocol was run, with volume set to 40 μL and lid heating set to 105 °C: 95 °C for 10 min, one cycle; 95 °C for 30 s; 60 °C for 1 min, forty cycles; 98 °C for 10 min, one cycle; and infinite hold at 10 °C. PCR droplets were counted using a QX200 Droplet Reader (Bio-Rad, cat. no. 1864003), ddPCR Droplet Reader Oil (Bio-Rad, cat. no. 1863004) and the droplet reading software QuantaSoft. Two sets of negative (Tris) and positive controls were analyzed using the ddPCR method described.

5.2.5 Statistical analyses

All modelling was carried out in a Bayesian framework using R version 4.0.3 (R Core Team, 2020) because of the unbalanced sampling design, and the small and variable sample sizes. As the mtDNA copy numbers obtained using the modified extraction method did not allow us to report exact settler numbers based on previous analyses using a different extraction technique (Doyle et al. 2017), predicted probabilities were estimated from a binomial distribution. Larval settlement detections were determined using a conservative threshold (8 copies 20 μL^{-1}) based on the LOQ (8 copies per well at the upper 95% confidence interval; Uthicke et al., 2018a). Trends in COTS settlement detection probability over time and across depths were modelled via a Bayesian generalised linear model (GLM) and the stan_glm function (rstanarm package; Goodrich et al. 2020), using all samples collected at Rib Reef from 2017 to 2020 (*n* = 69 collectors). Generic weakly informative normal priors were specified for both the intercept term and the coefficients in *Q*-space. A total of 40,000 Gibbs sampling iterations were performed across four chains with a warmup of 18,000 and thinning rate of 20. Trends in settlement detection probability across sampled reefs, regions and depths were modelled via a Bayesian generalised linear mixed model (GLMM) and the stan_glmer function (rstanarm package, Goodrich et al. 2020), using all samples collected from 10 reefs nested in two regions on the GBR in 2019/20 ($n = 55$). Here, non-informative priors were used, and 40,000 Gibbs sampling iterations were performed across four chains with a warmup of 18,000 and thinning rate of 20. Models were assessed using MCMC plotting functions including trace plots, autocorrelation plots, rhat histograms and neff histograms in the R packages bayesplot (Gabry and Mahr 2021) and coda (Plummer et al. 2006). Residual diagnostics were checked using DHARMa (Hartig 2016). All violin plots were produced using the package ggplot2 (Wickham 2016).

5.3 Results

A total of 193 of the 219 (88.13%) larval settlement collectors deployed over the duration of this study were retrieved (**Table 5.1**). The only two collectors lost during the 2019-2020 sampling period (North Reef) were both deployed at shallow depth. Likewise, collectors lost during the 2016-2017 and 2017-2018 sampling periods were mostly those deployed at shallow (63%) and intermediate depths (29%).

Out of the 193 settlement collectors retrieved and analyzed in this study, COTS settlement was recorded in 60 (31.09%) (**Table 5.1**; **Appendix B**). During three sampling periods from 2016 to 2018, settlement was recorded at all three sampling reefs, which were located in the central (near Townsville) and northern regions (near Cairns) of the GBR (**Table 5.1**). During the 2019-20 sampling period, newly settled COTS were recorded at all seven reefs in the central GBR, however, only one of the three sampling reefs in the northern GBR (near Lizard Island) recorded settlement (**Table 5.1**).

Considerable temporal variation in settlement was observed at Rib Reef (**Table 5.1**; **Figure 5.3A**). Among all sampling periods, the predicted probability of COTS settlement detection was highest between October 2016 and January 2017 (median = 64%; CI_{95} = [43; 81]). Settlement was predicted to be lower between December 2016 and March 2017 (20%, CI_{95} = [9; 38]), and during the 2017-18 (24%, CI_{95} = [12; 40]) and 2019-2020 (20%, CI_{95} = [5; 50]) sampling periods (**Figure 5.3A**).

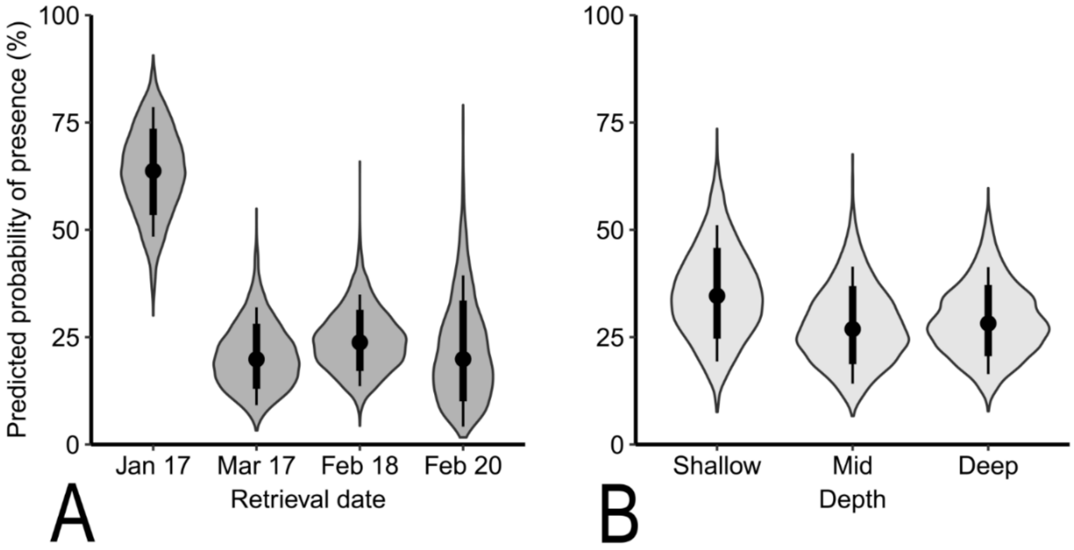

Figure 5.3 Crown-of-thorns starfish (*Acanthaster* cf. *solaris*) settlement patterns at Rib Reef (central Great Barrier Reef). Violin plots illustrate the predicted probability of settler DNA presence (%) in larval settlement collectors deployed (A) during four sampling periods (October 2016–January 2017; December 2016–March 2017; November 2017–February 2018; October/November 2019–January/February 2020) and (B) across three depth ranges (shallow = 4–6 m; mid = 7–9 m; deep = 10–12 m) during the same sampling periods. The median (dot), interquartile range (thick line), and 1.5 times the interquartile range (thin line) are indicated inside each violin plot. The violin plot outlines indicate kernel probability density; that is, the width of the grey area represents the proportion of the data located there.

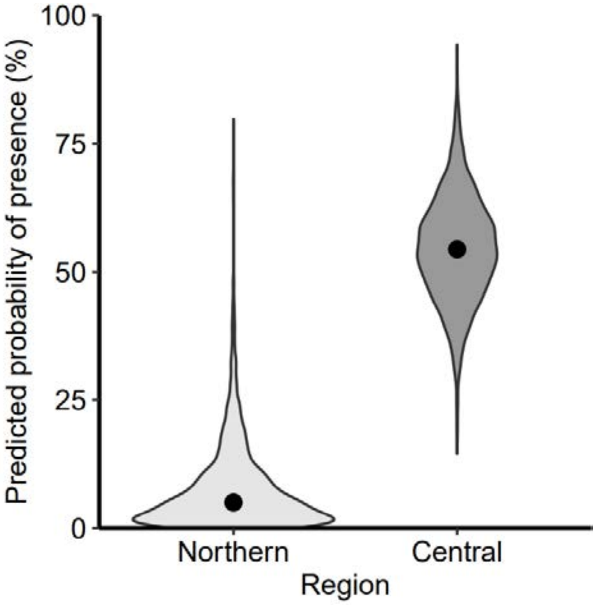

Figure 5.4 Violin plot comparing the predicted probability of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) settler DNA presence (%) in larval settlement collectors deployed at reefs in the northern (near Lizard Island) and central regions of the Great Barrier Reef from October/November 2019 to January/February 2020. The median is indicated as a dot inside each violin plot, and the violin plot outlines indicate kernel probability density.

Sampling depth showed no effect on larval settlement, with the proportion of collectors containing newly settled COTS broadly similar among shallow (8 of 19 collectors; 42.1%), intermediate (6 of 23; 26.1%) and deep collectors (8 of 27; 29.6%) at Rib Reef over the same time periods. In the model, collectors at shallow depth reported a settlement probability of 35% (CI_{95} = [17; 59]) to contain DNA of newly settled COTS, which was comparable to collectors deployed in intermediate (27%; CI_{95} = [13; 49]) and deep water (28%; CI_{95} = [17; 48]) (**Figure 5.3B**).

Substantial latitudinal variation in COTS settlement was observed during the 2019-20 sampling period (**Table 5.1**; **Figure 5.4**). The model predicted collectors on reefs located in the central region of the GBR (54%; CI_{95} = [35; 75]) to be > 10 times as probable to record larval settlement as collectors on reefs in the northern GBR near Lizard Island (5%; CI_{95} = [0; 24]) (**Figure 5.4**).

5.4 Discussion

This study demonstrates the utility of artificial settlement collectors combined with genetic analyses to explore spatial and temporal settlement patterns in COTS. Artificial collectors have been used widely to assess larval settlement of echinoderms (e.g., Hereu et al. 2004, Jennings and Hunt 2010, García-Sanz et al. 2014, Balsalobre et al. 2016), including COTS (Keesing et al. 1993). However, the artificial settlement collectors used in this present study differ from settlement sampling devices used in past studies (e.g., Kessing et al. 1993). Both Keesing et al. (1993) and Hernández et al. (2006) used net bags to contain the bio balls and anchored collectors to the substrate using plastic ropes (Hernández et al. 2006) or ropes attached to a heavy weight (Keesing et al. 1993). Here, I make use of hard plastic leaf baskets, stainless-steel stakes and wire rope to minimise dislodgements and damage to collectors and maximise retrieval rates across all reef environments. Nonetheless, it is not known how rates of settlement (and early post-settlement survival) vary between artificial settlement collectors and natural substrata, such that artificial settlement collectors only facilitate relative comparisons of settlement potential, rather than absolute measures of larval settlement (Keesing et al. 1993). In this study, settlement collectors were deployed for relatively long time periods to allow for extensive colonization and pre-conditioning of settlement substrata prior to settlement, but it remains to be tested whether larval starfish are more or less likely to settle within these artificial settlement collectors, compared to nearby natural substrata.

The biggest constraint in using artificial settlement collectors for measuring settlement rates of echinoderms has been the amount of time required to manually sort samples under a stereomicroscope, as necessary to detect and distinguish microscopic settlers (Keesing et al. 1993). Based on recent progress made in the detection of starfish larvae in plankton samples (Ward et al. 2008, Uthicke et al. 2015, Doyle et al. 2017), and the DNA-based detection of other benthic invertebrates in sediments (Wei et al. 2018), this method uses ddPCR to detect DNA of newly settled COTS, which is expected to improve detectability, but certainly increases efficiency in processing samples. To minimise the potential for environmental DNA contamination, a conservative threshold was set regarding the presence or absence of COTS settlement for each collector. Small quantities of waterborne DNA originating from adults or perished individuals would thus not be mistaken for newly settled starfish, however, contamination by large amounts of COTS DNA, such as newly settled COTS in the stomach of a predator residing in the artificial collector, cannot be ruled out. To validate this method and quantify settlers in future studies, control samples measuring COTS DNA outside the settlement season, and the mtDNA numbers of individual settlers at different stages of development need to be analysed using this method. The mtDNA copy numbers per individual for each larval stage determined by Doyle et al. (2017) cannot be compared to the settlement collector data because a different DNA extraction method had to be utilised. To further validate the link between DNA and the presence of newly settled COTS inside the artificial collectors, control samples with known mtDNA numbers may be spiked with newly settled juveniles and analyzed using this protocol. This molecular method will be of particular utility if large numbers of collectors were to be deployed as part of large-scale settlement studies or population monitoring efforts. It also provides an opportunity to study settlement and recruitment in other groups such as brittle stars (Ophiuroidea), for which reliable visual identification of newly settled individuals in collected samples was not possible because of the large number of sympatric species on coral reefs (Keesing et al. 1993).

The results of this study suggest that there was COTS settlement during all four sampling periods, however, considerable variation was recorded within and among the reproductive periods. An implicit assumption of this study was that spawning occurred within a relatively short time window in December to January each summer (*sensu* Uthicke et al. 2015, 2019, Caballes et al. 2021) and larvae settled onto reef substratum within about one month thereafter (*sensu* Keesing and Halford 1992, Pratchett et al. 2017b), suggesting that sampled individuals were fewer than two months old. Settlement of COTS larvae is unlikely within the first two weeks (Olson 1987) and more than four weeks after fertilization (Pratchett et al. 2017b, but see Allen et al. 2019, Hart et al. 2021), with peak settlement occurring approximately three weeks post-fertilization (Pratchett et al. 2017b). The short time spent in the plankton pre-settlement and high variability in larval abundance detected in plankton samples within one summer reproductive period (Uthicke et al. 2019) suggest that COTS settlement rates may also vary among temporally staggered deployment periods within the same summer period. At Rib Reef (central GBR), which was experiencing outbreak densities of adult COTS during the sampling years (Australian Institute of Marine Science (AIMS) 2015), predicted settlement was higher between October to January than December to March (2016-17). This result could be due to a strong settlement pulse early in the summer or reduced survival rates of newly settled starfish from December to March as they likely spent longer time periods in collectors prior to retrieval. Conversely, Uthicke et al. (2019) reported settlement to be greater between December to March than October to February (2016-17) at Moore Reef (northern GBR). This highlights the large spatiotemporal variability often observed in the early life history processes of echinoderms (Lamare and Barker 2001, Tomas et al. 2004, Hernández et al. 2010, García-Sanz et al. 2014). Settlement at Rib Reef decreased over the duration of this study (2016-20), which matches previous reports of inter-annual settlement variation in COTS (Zann et al. 1987, 1990, Doherty and Davidson 1988) and other echinoderms (Loosanoff 1964, Balch and Scheibling 2000, Hereu et al. 2004, Glockner-Fagetti and Phillips 2020). Given that this temporal trend is limited to one reef and a relatively short time period, inter-annual settlement patterns need to be assessed more widely to further our understanding of the various processes regulating this life-history stage.

The extent to which COTS larvae settle in shallow versus deep-water environments on coral reefs has been a controversial research question for decades (Pratchett et al. 2017a, 2021b). Along a depth gradient of 4 to 12 m at Rib Reef, no difference in settlement was predicted among the three depth ranges therein. These results suggest that COTS larvae readily settle in shallow reef environments. Recent records of newly settled COTS across a wide range of depths (1-18 m) support this conclusion, although mean density of early post-settlement COTS was highest between 8 to 14 m (Wilmes et al. 2020b). The apparent lack of preferential settlement at particular depths found in this study corroborates early reports by Chesher (1969) and Endean (1973). Johnson et al. (1991) hypothesised COTS larvae to predominantly settle at the base of rubble slopes in deep water, which was primarily linked to the high occurrence of the crustose coralline algae (CCA) *Lithothamnium pseudosorum* (likely *Lithothamnion* cf. *proliferum*) in these areas. However, this species of CCA can be readily found in low-light environments such as caves or overhangs in shallow reef environments (S. Uthicke pers. obs.). The induction of settlement and metamorphosis by bacteria occurring on the surfaces of *L. pseudosorum* and other CCA is well established (Johnson et al. 1991, Johnson and Sutton 1994), and *L. pseudosorum* collected from shallow depths have been routinely used to induce settlement in larval COTS experiments (e.g., Uthicke et al. 2013). In this study, settlement collectors were deployed in coral rubble environments with high cover of CCA across all depths (4-12 m), including shallow reef environments, which suggests that it is the presence of particular reef substrata and associated chemical cues, rather than depth, that is primarily driving small-scale settlement patterns on coral reefs (Wilmes et al. 2020b, Doll et al. 2023a – **Chapter 3**).

At a larger spatial scale, the analysis of settlement across 10 reefs in 2019-20 found substantial variation between the northern (near Lizard Island) and central regions of the GBR (near Townsville). Collectors deployed in the central GBR were more than 10 times as likely to report larval settlement as reefs in the northern region. High settlement variation among reefs and locations has been reported for various other Asteroidea (Jennings and Hunt 2010, Glockner-Fagetti and Phillips 2020), however this is the first direct evidence of such trend for COTS on the GBR. The latitudinal difference in settlement between the northern and central GBR highlighted by this new data is clearly reflected in the distribution of adult starfish, with the northern GBR being in a post-outbreak state, and the central GBR experiencing relatively high densities of adults on many reefs (Australian Institute of Marine Science (AIMS) 2015, Pratchett et al. 2020). High rates of self-recruitment by COTS have been hypothesised because of their chemoattraction to aggregations of conspecific adults (Chesher 1969, Cowan et al. 2016a) and some limited insights on larval dispersal versus retention rates (Black et al. 1995, Vanhatalo et al. 2017). In addition, various other physical and chemical factors, such as the presence or absence of rubble environments at particular reefs and regions may be dictating strong spatial patterns of COTS settlement. The substantial settlement variation between areas with high and low adult population densities demonstrates that unresolved questions pertaining to the self-recruitment of COTS need to be investigated at various spatial scales to further our understanding of the dynamics of their population irruptions on the GBR.

The temporal and spatial settlement trends described in this study have advanced our understanding of the processes dictating the early life history of COTS. Despite the important role of early life history processes to the irruptive dynamics of starfish populations (Uthicke et al. 2009), settlement patterns of COTS and the implications thereof have remained unexplored due the limitations to sampling them (Doherty and Davidson 1988). Recent progress allows us to investigate such patterns and provides an opportunity to add settlement as a variable in demographic models and assess stock-recruitment dynamics for this boom-and-bust species. For example, estimates of larval settlement using this method may be used to test the larval settlement threshold of 5 m^{-2} required to reach destructive outbreak densities of adult COTS (10 ha^{-1}) based on a model of size- and age-dependent mortality (Keesing et al. 2018). Notably, exploring links between larval supply (e.g., Uthicke et al. 2019), settlement, juvenile abundance (e.g., Wilmes et al. 2020b) and adult densities also allows us to test the effectiveness of the current management approaches, mainly focused on the culling of adult starfish (Westcott et al. 2020).

Most importantly, this new method has application to the early detection of new and renewed population irruptions of COTS. To date, population irruptions only became apparent when high adult densities are detected on reefs (Zann et al. 1987, Pratchett et al. 2014). Annual deployments of larval settlement collectors across a hierarchy of spatial scales could detect localised fluctuations and sharp increases in settlement rates, and thus herald new and renewed population irruptions before they are evident in adult densities. In conjunction with annual monitoring of larval supply (Uthicke et al. 2019) and recruitment (Wilmes et al 2020a), this innovative method may progress the development of a systematic early warning system for COTS population irruptions, and thus potentially reduce the perennial threat of this destructive starfish to coral reefs.

Chapter 6: General Discussion

Many ecologically important marine invertebrates, including keystone echinoderms, display bipartite life histories in which planktonic larvae transition to benthic juveniles (i.e., larval settlement; Scheltema 1974, Hadfield 1986). Importantly, this early life-history transition is complex and dynamic, comprising a cascade of processes that often occur rapidly, but can have profound consequences for recruitment success and adult distribution (Pawlik 1992, Rodríguez et al. 1993, Doll et al. 2022 – **Chapter 2**). Despite the inherent importance of larval settlement to population replenishment and dynamics, the factors and processes governing this life history transition are still poorly understood, largely driven by methodological challenges, for many ecologically important taxa (Doll et al. 2022 – **Chapter 2**), including the corallivorous crown-of-thorns starfish (COTS; Pratchett et al. 2021b). The planktonic larvae of COTS, like many other echinoderms (Doll et al. 2022 – **Chapter 2**), do not randomly settle in space and time (Henderson and Lucas 1971, Zann et al. 1987, Doll et al. 2021, 2023a – **Chapters 3, 5**). If we accept that COTS larvae can choose where (or when) to settle and thereby commence their benthic life stage, this inevitably raises two general (but critical) research questions: where do they settle preferentially and why do they choose to settle in specific habitats? (Pratchett et al. 2017a, 2021). This thesis provides empirical work towards answering these questions by integrating a wide range of approaches and techniques. While **Chapters 3** and **4** addressed key knowledge gaps surrounding the environmental factors or cues that shape COTS settlement, **Chapter 5** provided unprecedented insights into the *in situ* settlement patterns of COTS. In this **Chapter 6**, I consolidated the insights gained from all preceding chapters, outlined how they advance (or challenge) our understanding of COTS settlement, and interpreted them in the context of spatiotemporal heterogeneity in recruitment and adult abundance.

6.1 Key processes and patterns in crown-of-thorns starfish settlement

While the movement of planktonic COTS larvae over broad spatial scales is presumed to be generally passive (Caballes and Pratchett 2014), larval behaviour and the availability of cues signalling benthic habitat suitability play an increasingly important role with decreasing distance to the reef substratum (Yamaguchi 1973, Caballes and Pratchett 2014, Doll et al. 2022 – **Chapter 2**). Once COTS larvae have reached settlement competency, they can move downward towards the substratum and orient their brachiolar arms against the substratum surface to test its suitability for attachment and metamorphosis (**Figure 2.2**). COTS larvae appear to have relatively specialised settlement requirements, with only particular live substrata inducing settlement responses (Henderson and Lucas 1971, Johnson et al. 1991, Doll et al. 2023a – **Chapter 3**). Moreover, the results from settlement assays conducted in **Chapters 3** and **4** corroborated the prevailing notion that a settlement cue, originating from live coralline algae and/or associated surface microbes, is obligatory for the induction of COTS settlement, at least during the initial stages of their competency period (Henderson and Lucas 1971, Johnson et al. 1991). In contrast to many other echinoderms (reviewed by Doll et al. 2022 – **Chapter 2**), COTS settlement does not appear to be mediated by cues associated with conspecific juveniles or adults (Doll et al. 2023b – **Chapter 4**). Conspecific-cue mediated settlement was largely purported for COTS based on documented larval movement towards adult conspecifics in a static choice chamber setting (Cowan et al. 2016a), however, the cascade of behavioural and physiological events associated with COTS settlement appears to be largely governed by algae-associated cues (Doll et al. 2023a, b – **Chapters 3, 4**).

While the induction of COTS settlement by coralline algae was first reported in the 1970s (e.g., Yamaguchi 1973), the research presented in this thesis significantly advanced our knowledge of the relative importance of different coralline algae species as settlement inducers (Doll et al. 2023a – **Chapter 3**). Experimental assays using 15 algal species revealed marked settlement cue selectivity by COTS larvae, including several species that induced less than 2 % of larvae to settle (Doll et al. 2023a – **Chapter 3**). Importantly, only some coralline algae, especially the alga *Melyvonnea* cf. *madagascariensis*, promote high settlement rates in COTS (Doll et al. 2023a – **Chapter 3**). This particular algal species induced much higher rates of settlement than even *Lithothamnium pseudosorum* (now likely *Lithothamnion* cf. *proliferum*), which was previously suggested to be the foremost settlement cue for COTS (Johnson et al. 1991). This demonstrates that COTS may be more selective in terms of their preferred settlement substrata than previously thought, which may denote narrow habitat and/or dietary requirements during their herbivorous early juvenile stage. Critically, the distribution or abundance of these specific algal species that readily induce COTS settlement, may be fundamental in understanding spatial patterns in the incidence of population irruptions (see Sections 6.2, 6.4).

Contrary to the suggestions of the deep-water recruitment hypothesis (Johnson et al. 1991), multiple lines of new evidence presented in this thesis indicate that COTS larvae readily settle in shallow reef environments (Doll et al. 2021, 2023a – **Chapters 3, 5**). The prevailing hypothesis postulated that COTS larvae predominantly settle in deep (> 30 m) habitats, partly underpinned by the assumption that an algal species known to induce COTS settlement, *Lithothamnium pseudosorum* (now likely *Lithothamnion* cf. *proliferum*), was relatively scarce at shallow and intermediate depths (Johnson et al. 1991). However, this alga is not necessarily a deep-water species, but rather a relatively sciaphilic alga that occurs in crevices, caves and overhangs across the reef depth continuum (Doll et al. 2023a – **Chapter 3**). Moreover, COTS settlement in the field is not exclusively, nor likely predominantly, induced by this algal species, or any other deep-water algal species (Doll et al. 2023a – **Chapter 3**). A range of other coralline algae, capable of promoting equally high or even higher settlement success than *Lithothamnion* cf. *proliferum*, are prevalent in the shallow habitats of coral reefs (Doll et al. 2023a – **Chapter 3**). These findings are supported by documented patterns of *in situ* settlement across a range of shallow and intermediate depths (Doll et al. 2021 – **Chapter 5**). Because the artificial settlement collectors were not deployed in deep water (> 30 m), however, the possibility that some (or many) larvae settle at depths beyond the limits of this study cannot be ruled out (Wilmes et al. 2020b, Doll et al. 2021 – **Chapter 5**). Nevertheless, this laboratory- and field-documented prevalence of apt settlement cues and larval settlement in shallow reef environments challenges the ecological underpinning of one of the prevailing paradigms pertaining to the settlement of COTS larvae.

6.2 Implications for recruitment success and the incidence of population irruptions

Substantial variability in adult abundance is a salient characteristic of many echinoderms, especially among broadcast spawners with complex, feeding planktonic larval and benthic juvenile life stages (Uthicke et al. 2009, Doll et al. 2022 – **Chapter 2**). Considering the intricate life history and opportunistic traits of COTS (Deaker and Byrne 2022), it is not surprising that this starfish epitomises extreme spatiotemporal heterogeneity in adult abundance. As such, large periodic population fluctuations could be caused by small changes to their early life-history parameters, such as elevated larval survival or settlement success (Lucas 1982, Brodie et al. 2005, Wilmes et al. 2018). The mechanisms and processes governing the early life-history phases and transitions of COTS, in turn, are most likely subject to strong spatiotemporal variability in a suite of environmental factors (Caballes and Pratchett 2014, 2017, Caballes et al. 2017, Doll et al. 2023a - **Chapter 3**). Clearly, for adult COTS populations to reach outbreak-level densities in a given place and time, one or multiple recruitment cohorts must have benefitted from favourable conditions during most, if not all, early life-history phases and transitions.

The settlement and early post-settlement stage of COTS, like for other echinoderms with bipartite life histories (Doll et al. 2022 – **Chapter 2**), is inherently vulnerable to population mortality bottlenecks (Wilmes et al. 2018, Deaker and Byrne 2022). Over the course of this planktonic-benthic transition, naïve COTS larvae are presented with the challenges of an unfamiliar benthic environment, new dietary requirements (i.e., coralline algae) and a new suite of predators (Cowan et al. 2017). Because newly settled COTS are subject to extremely high mortality, primarily linked to predation, during their first days and weeks as herbivorous juveniles, localised settlement rates must surpass a significant threshold for cohorts to eventually reach outbreak-level adult densities (Keesing et al. 2018). While patterns established at settlement may be altered along the way, high settlement success certainly increases the likelihood of localised recruitment pulses and subsequent proliferation in the abundance of adults (Zann et al. 1987, 1990).

In particular, the apparent settlement cue selectivity displayed by COTS larvae (Doll et al. 2023a – **Chapter 3**) has important implications for their recruitment and population dynamics, because larval settlement in marginal habitats or a delay of settlement can substantially elevate mortality rates prior or after settlement, and thus reduce the strength of the recruitment cohort (Pechenik 1990, Rodríguez et al. 1993). The research presented in this thesis posits that only the cues associated with some coralline algae species may be promoting ecologically significant settlement success of COTS in the field (Doll et al. 2023a – **Chapter 3**). The habitat requirements of settlement-stage COTS may thus be quite specialised, in contrast to many other echinoderms that settle in response to cues associated with a large diversity of live substrata (Doll et al. 2022 – **Chapter 2**). This is further supported by their inability to utilise chemical cues associated with conspecific juveniles to navigate this life-history transition (Doll et al. 2023b – **Chapter 4**). In order to realise the recruitment success usually associated with preferential settlement habitat (Pawlik 1992), competent COTS larvae must not only encounter apt benthic habitat (or associated chemical cues) during a short time window, but also at limited spatial scales.

While COTS larvae can defer settlement in the absence of suitable settlement cues (Pratchett et al. 2017b), a prolonged planktonic phase exposes larvae to continued high daily mortality rates (Rumrill 1990, Cowan et al. 2017, 2020) and may also result in reduced growth and survival during their early post-settlement life stage (Pechenik 1990, Rodríguez et al. 1993). If, however, competent COTS larvae are eventually forced to settle in non-preferred or marginal habitat, they are likely to encounter adverse environmental conditions, including relatively high exposure to benthic predators and/or low food availability (Cowan et al. 2017, Wilmes et al. 2018). As a result, larval and/or juvenile cohorts may be experiencing elevated mortality rates in locations with little or no suitable settlement substratum. This, in turn, will likely minimise the contribution of these cohorts to the local adult population (Keesing et al. 2018). Where larvae chose to commence their benthic life history clearly matters a lot to the incidence and severity of COTS population irruptions, independent of other factors that can contribute to the spatiotemporal heterogeneity of COTS abundance (e.g., larval advection). A small cohort of competent COTS larvae reaching and selecting favourable benthic habitat will likely contribute more to recruitment success than a large cohort of larvae settling in unfavourable habitat.

Notably, the results presented in **Chapter 4** (Doll et al. 2023b) of this thesis also challenge the notion that the (self-) recruitment of COTS populations may be reinforced by gregarious settlement (Chesher 1969, Pratchett et al. 2017a, Wilmes et al. 2018, Deaker and Byrne 2022). Especially the secondary phase of population irruptions, during which localised adult densities can remain high for many years until hard coral cover is significantly reduced

(Pratchett et al. 2014), has been linked to the idea that adults may provide cues for COTS settlement. Instead, it appears more likely that high rates of settlement, and thus recruitment success, mostly rely on larval responses to algae-associated cues (Doll et al. 2023a – **Chapter 3**). Taken together, these results suggests that COTS recruitment may be particularly limited on reefs with low cover of settlement-promoting coralline algae species. I hypothesise, therefore, that the scarcity of apt settlement habitat may contribute to relatively low risk of COTS population irruptions on such reefs, or even entire geographic regions.

6.3 Management implications

Scientists and managers are faced with the seemingly insurmountable challenge of mitigating, if not preventing, the detrimental effects of COTS population irruptions on Anthropocene coral reefs, which are increasingly compounded by climatic disturbance (Mellin et al. 2019). Contrary to other major disturbances responsible for ongoing and widespread coral loss, population irruptions of COTS are relatively amenable to direct intervention, predominantly through manual removal or *in situ* culling of adult starfish (Westcott et al. 2016). Moreover, indirect pathways to coral reef conservation exist through an improved knowledge of the root cause(s) of population irruptions, or at least the factors contributing to their severity. Notably, the objective of both direct and indirect population management approaches would not necessarily have to be the complete eradication of COTS from coral reefs, but rather the mitigation of population irruptions and associated coral mortality (Westcott et al. 2016, Babcock et al. 2020).

The settlement cues COTS larvae employ to navigate their planktonic-benthic transition may be the key to an innovative control approach. The settlement processes and cues of other marine invertebrates have been used to develop novel strategies to control benthic adult populations (Motti et al. 2018). For example, an inhibitory cue (norepinephrine) reduces settlement and subsequent invasions of a fouling organism, the bryozoan *Bugala neritina* (Price et al. 2017). However, inhibitory cues have not been specifically tested for COTS, although chemical cues associated with benthic invertebrates known to predate on early juvenile COTS (Cowan et al. 2017) may regulate larval behaviour, such as the movement away from the source of the cue (Motti et al. 2018). Other echinoderm larvae can detect and respond to a myriad of chemical cues, including isolated neurotransmitters and ions (reviewed by Doll et al. 2022 – **Chapter 2**). The potential scientific value of characterising and isolating chemical cues, particularly compounds shown to inhibit echinoderm settlement (Doll et al. 2022 – **Chapter 2**), is therefore not limited to fundamental research but could be applied towards a management strategy based on the chemosensory-mediated inhibition of COTS settlement.

Despite improvements in the efficiency and effectiveness of current population control efforts, the manual culling of adult starfish is arguably too labour- and cost-intensive to contain COTS densities throughout large geographic areas, such as the GBR (Pratchett and Cumming 2019, Babcock et al. 2020). As a result, the effective management of this phenomenon necessitates innovative research to improve the prediction and/or early detection of localised population irruptions. For example, the marked inter-reef differences in the incidence and severity of COTS population irruptions, specifically on the GBR, may be linked to the prevalence (or lack) of suitable settlement and recruitment habitat in some locations (Doll et al. 2021, 2023a – **Chapters 3, 5**). New insights into the spatial distribution of apt settlement substrata (and associated cues) presented in this thesis (Doll et al. 2023a – **Chapter 3**) may thus have potential application towards the prediction of which reefs, or even regions, may be at high (or low) risk of experiencing strong recruitment pulses. Importantly, reefs without suitable settlement habitat or cues may be very unlikely to ever support high densities of COTS, thereby effectively reducing the area of concern, greatly increasing the viability and effectiveness of monitoring and management. Such insights, in turn, may increase opportunities to effectively suppress adult densities in the infancy of population irruptions.

The innovative sampling protocol introduced in **Chapter 5** (Doll et al. 2021) of this thesis also has significant application for the early detection of new and renewed population irruptions of COTS. The survey of spatiotemporal trends in settlement rates, using artificial settlement collectors, droplet digital PCR and COTS-specific mitochondrial primers, has the potential to provide a reliable measure of recruitment strength (Doll et al. 2021, 2022 – **Chapters 2, 5**), particularly if used in conjunction with complementary monitoring of larval supply (Uthicke et al. 2019) and juvenile densities (Wilmes et al. 2020b). New and renewed population irruptions of COTS currently only become apparent once localised adult abundances are already relatively high, at which point population control efforts may be unfeasible. However, detections of elevated settlement could herald recruitment pulses multiple years before increases in adult abundance are noticed, thereby aiding the effective suppression of COTS densities during the early stages of a population irruption, which is paramount for mitigating coral mortality and reef degradation.

6.4 Avenues for future research

We are only just beginning to understand the specific nature of the settlement cues that COTS and other echinoderms use to navigate the inherently vulnerable life-history transition from planktonic larva to benthic juvenile (Doll et al. 2022 – **Chapter 2**). Chemical cues produced by biofilms (in particular, epiphytic bacteria), may play an underappreciated role in explaining some of the marked differences in settlement rates among coralline algae species (Doll et al. 2023a – **Chapter 3**). Isolating coralline algae species (if not specimens) and their associated microbial communities from field collection to experiment commencement would improve our ability to infer field settlement patterns based on experimental assay results. The antibiotic treatment of the coralline alga *Lithothamnium pseudosorum* (Johnson et al. 1991; now likely *Lithothamnion* cf. *proliferum* in Doll et al. 2023a – **Chapter 3**) significantly reduced settlement of COTS, indicating that the cue may be produced by epiphytic bacteria (Johnson et al. 1991). However, surface bacteria failed to induce settlement in isolation, thus both algal compounds and microbial communities may play significant roles in the mediation of COTS settlement (Johnson and Sutton 1994). Clearly, testing the relative effects of antibiotic treatment for a range of different algal species may establish to what extent compounds produced by the algae and/or surface microbes are driving the large variability in inductive capacity detected among algal species (Doll et al. 2023a – **Chapter 3**).

Importantly, to gain an improved understanding of the ecological and developmental mechanisms underpinning this life-history transition, it should not only be tested how prevalent biofilm- or bacteria-mediated settlement is, but also what specific components of biofilms, or what specific bacteria (or bacterial groups), are responsible for inducing settlement (Doll et al. 2022 – **Chapter 2**). For example, a network analysis, combining settlement assay results with microbial community data for the algal species tested, could identify specific bacteria or bacterial groups promoting (or inhibiting) settlement success in COTS larvae. Likewise, the identification of other isolated chemical cues that may potentially inhibit COTS settlement (e.g., neurotransmitters such as norepinephrine) would stimulate further research into the potential application of such cues towards chemosensory-based population control efforts (Motti et al. 2018).

The innovative settlement sampling method introduced in this thesis provides an opportunity to resolve other critical information gaps relating to the biology and ecology of COTS (Pratchett et al. 2021b, Doll et al. 2021 – **Chapter 5**). Deployment of artificial settlement collectors in deep water (20 – 30m) will provide additional insight into the potential settlement of COTS larvae in deep reef habitats (Johnson et al. 1991, Wilmes et al. 2020b, Doll et al. 2021 – **Chapter 5**). Notably, future field-based studies of settlement must attempt to significantly shorted deployment periods to mimise the effect of post-settlement mortality, an important challenge highlighted in **Chapter 2** (Doll et al. 2022). Based on the findings of **Chapter 3** (Doll et al. 2023a), I denote that the local abundance (and community composition) of coralline algae may be an underappreciated factor in explaining marked inter-reef differences in COTS abundance. This hypothesis could be substantiated with *in situ* surveys of COTS settlement (e.g., Doll et al. 2021 – **Chapter 5**) and coralline algae assemblages (e.g., Dean et al. 2015) across spatial scales of interest. Specifically, on the GBR, some reefs or geographic areas (e.g., mid-shelf reefs) are considerably more likely to experience high COTS densities (Sweatman 2008). Some of this spatial heterogeneity could be explained by future field surveys if differences in coralline algae cover and/or the abundance of highly inductive coralline algae (e.g., *Melyvonnea* cf. *madagascariensis*) correlate with patterns of settlement and adult abundance. Importantly, exploring the potential links between adult densities (Pratchett et al. 2022), larval supply (Uthicke et al. 2019), settlement (Doll et al. 2021 – **Chapter 5**), and thereby the stock-recruitment dynamics of COTS (Pratchett et al. 2021b), will also further our knowledge of population build-up and retention at the scale of reefs or reef clusters, and assess the effectiveness of the current management focus on culling adults (Westcott et al. 2020).

The intricate story of planktonic COTS larvae finding a new home for their benthic juvenile life is still not fully resolved. However, research presented in this thesis contributes towards an improved understanding of this immensely important life-history transition and should stimulate new conceptual perspectives or innovative approaches to resolve other priority knowledge gaps in the future. Advancing our understanding of the key early life-history processes of COTS, and thereby improving the ecological underpinning of their management, presents an important and unprecedented opportunity to mitigate the perennial threat of their population irruptions to already imperilled coral reefs.

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Appendix A: Supplementary information for Chapter 3 (Doll et al. 2023a)

Spawning and rearing methodology

Adult western Pacific crown-of-thorns starfish (*Acanthaster* cf. solaris, COTS) were collected from mid-shelf reefs in the central Great Barrier Reef (GBR) and transported to holding tanks at the Australian Institute of Marine Science (AIMS) National Sea Simulator in October 2021. These flow-through holding tanks were supplied with unfiltered seawater (~ 28 °C) and food (i.e., coral) was periodically supplied. This seawater temperature (28 °C ± 0.5 °C), used throughout the holding, larval rearing and experiment process, represents temperatures in the source area of the COTS collected during the reproductive season (November – January).

COTS were spawned on November 1st, 2021, following an established protocol. Small incisions were made at the proximal ends of the arms of six female COTS to obtain a small number of ovary lobes from each individual and place them in a 200 mL filtered seawater (FSW) beaker (~ 28 °C). Ovary lobes were washed with FSW through a 500 µm mesh to remove any loose eggs. The ovary lobes in FSW were transported to a temperature-controlled Sea Simulator room (28 °C) for fertilisation. The ovary lobes of each female were placed into beakers with 200 mL FSW and one vile of 10^{-5} M 1-Methyl adenine (= egg stock solution) to induce the maturation and release of the eggs. This maturation step during which mature eggs sink to the bottom took approximately 1 hour. About 20 minutes prior to the completion of this maturation step, incisions were made into the arms of six male COTS and scraped a small quantity of sperm from each male into the wells of a 6-well cell culture plate. The eggs were rinsed through a 500 µm mesh to remove any unshed eggs or connective tissues and the number of eggs per mL was counted in the stock solution. The dry sperm of the males was mixed (2 µL from each male in ~ 15 mL FSW), and 1 mL of this sperm stock solution was added to the egg stock solution. Successful fertilisation (presence of a fertilisation envelope) of > 80% was confirmed using a stereo microscope. Embryos were divided between two 70 L vats (low air line setting).

At 24 hours post-fertilisation, the air lines were removed for 20 minutes, and water changes were conducted for both vats. This water change removed any undeveloped embryos and dead larvae that sank to the bottom of the vats once air lines were removed, while healthy larvae with phototactic tendencies swam to the surface. Larval stages were scored using 6-well cell culture plates and stereo microscopes. At 48 hours post-fertilisation, the process (24 h) was repeated to concentrate healthy larvae. Then, 20 flow-through rearing cones (16 L volume, 14 L FSW working volume each) were stocked with larvae at a density of approximately 1 larvae mL^{-1}. Larvae were reared under these conditions for the next 72 hours, after which larvae started feeding. The algal feed was cultured by trained AIMS Sea Simulator staff and included stock cultures of *Dunaliella* sp. and *T-Isocrysis* sp., each contributing about half of the chlorophyll a concentration. Each rearing cone was supplied via automatically dosed treatment tanks with controlled chlorophyll concentration. Full water changes were carried out three times a week. All healthy larvae from each cone were siphoned into a holding bucket with a 20 µm mesh net, while the rearing cones (including tubes and air lines) were thoroughly cleaned and filled with FSW prior to returning the larvae. I regularly examined larval development using a stereo microscope throughout the rearing duration. Larvae were determined competent to settle once they reached the late-brachiolaria stage with a well-developed rudiment (14 days post-fertilisation). Metamorphic competency was also confirmed by settling a subset of larvae in on pieces of *Lithothamnion* cf. *proliferum* over a 24-hour period immediately prior to experiment commencement.

Taxonomic, morpho-anatomical and collection information

While the alga *Ramicrusta* sp. (Peyssonneliales) belongs to a different taxonomic subclass (Rhodymeniophycidae) than the 14 coralline species (Corallinophycidae), I collectively refer to the 15 algal species as coralline algae throughout this manuscript. * See Peña et al. (2020) for the most recent phylogeny of the Corallinophycidae [1].

species	**family ***	**collection site**	**growth habit**	**key morpho-anatomical characteristics used for species identification**
Melyvonnea cf. *madagascariensis*	Mesophyllumaceae	Davies Reef	branching	surface with protuberances; multiporate conceptacles; coaxial hypothallus and cell fusions [2,3]
Neogoniolithon cf. *fosliei*	Corallinaceae	Davies Reef	encrusting	very large conceptacles; skin chicken like surface; individual trichocytes; cell fusions [2,4]
Adeylithon bosencei	Lithophyllaceae	Davies Reef	branching	cylindrical branches; strongly tessellate surface; trichocytes in loosely defined fields; cell fusions [5]
Hydrolithon cf. *reinboldii*	Lithophyllaceae	Magnetic Island	encrusting	knobby protuberances; strongly tessellate surface; dimerous hypothallus; cell fusions [2,4,6]
Lithophyllum cf. *insipidum*	Lithophyllaceae	Magnetic Island	encrusting	dimerous thallus; strongly tessellate surface; secondary pit connections [2,7]
Lithothamnion cf. *proliferum*	Hapalidiaceae	Davies Reef	branching	lobed branches; very smooth surface; multiporate conceptacles; cell fusions [4,8,9]
Titanoderma cf. *tessellatum*	Lithophyllaceae	Davies Reef	encrusting	concentric whorls; large green-brown conceptacles; secondary pit connections ([2] as *Tenarea tesselatum*, [4])
Amphiroa cf. *foliacea*	Lithophyllaceae	Davies Reef	branching	articulated (geniculate); cylindrical to flattened branches; secondary pit connections [10]
Sporolithon sp.	Sporolithaceae	Davies Reef	encrusting	thick thallus; smooth surface; presence of sori [2]
Ramicrusta sp.	Peyssonneliaceae	Davies Reef	encrusting	thick thallus; only partially calcified (surface soft)
Lithophyllum cf. *pygmaeum*	Lithophyllaceae	Davies Reef	branching	pointy to round branches; slightly tessellate surface; secondary pit connections ([4], [9] as *Lithophyllum tamiense*)
Porolithon sp. A	Lithophyllaceae	Magnetic Island	encrusting	conspicuous yellow-green conceptacles; trichocytes in well-defined fields; cell fusions
Porolithon sp. B	Lithophyllaceae	Davies Reef	encrusting	‘orange’ species in *Porolithon* cf. *onkodes* complex; trichocytes in well-defined fields; cell fusions
Lithophyllum cf. *kotschyanum*	Lithophyllaceae	Davies Reef	branching	thick robust branches; cylindrical to flattened; slightly tessellate surface; secondary pit connections [4,6]
Porolithon sp. C	Lithophyllaceae	Davies Reef	encrusting	‘chalky’ species in *Porolithon* cf. *onkodes* complex; trichocytes in well-defined fields; cell fusions

Molecular-based method for algal species identification

DNA molecular methods were used for taxonomic confirmation of the experimental algal species. For DNA extraction and polymerase chain reaction (PCR) amplification, samples were carefully cleaned with brush and forceps under a dissecting microscope to remove epiphytes and endophytes, and collected by scraping the thallus surface with a single edge razor blade. Genomic DNA was extracted using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Each PCR tube contained a 30 μl mixture of 4 μL genomic DNA, 1 μl of 10 pmol forward + 1 μl of reverse primers, 8.6 μl distilled water, and 15.4 μL the HelixAmp Ready–2x–Go Series (NanoHelix, Daejeon, Korea). Amplifications and sequencing of the field-collected material used two primer sets either *psb*AF1/*psb*AR2 or *psb*AF1/*psb*A600R [11] for *psb*A, and three primer sets F762/R*rbc*Start or F993/R*rbc*Start or F1150/R*rbc*Start [12]. The PCR amplification consisted of an initial denaturation for 2 min at 95°C, followed by 40 cycles of 20 sec denaturation at 95°C, annealing for 40 sec at 40°C and extension for 1 min at 72°C. The final extension step was carried out at 72°C for 5 min. These primers were also used in cycle sequencing.

Sequencing of specimens was performed by Macrogen (Seoul, South Korea). Sequences were deposited in GenBank (see table below). Sequences generated in the present study and others obtained from GenBank were aligned with Clustal W [13] and manually corrected using MEGA7 [14]. PartitionFinder 2 [15] was used to determine the best partition scheme and model of evolution as implemented by RAxML. Maximum likelihood (ML) analysis was conducted under the GTR+G+I model of sequence evolution with 1,000 bootstrap replicates using RAxMLGUI v1.5 [16-18]. Bayesian analysis was performed using MrBayes 3.1.2 [19,20]. Markov chain Monte Carlo runs were carried out for two million generations, each with one cold chain and three heated chains, using the GTR+Γ+I evolutionary model, with sampling and printing occurring every 1,000 generations. Summary trees were generated using a burn-in of 25%.

GenBank Accession numbers for *psbA* and *rbcL* sequences

species	psbA GenBank accession No.	rbcL GenBank accession No.
Melyvonnea cf. *madagascariensis*	OP830452	OP830467
Neogoniolithon fosliei	OP830450	OP830465
Adeylithon bosencei	OP830454	OP830469
Hydrolithon cf. *reinboldii*	OP830457	OP830472
Lithophyllum cf. *insipidum*	OP830456	OP830471
Lithothamnion cf. *proliferum*	OP830448	OP830463
Titanoderma cf. *tessellatum*	OP830447	OP830462
Amphiroa foliacea	OP830453	OP830468
Sporolithon sp.	OP830455	OP830470
Ramicrusta sp.	OP830458	OP830473
Lithophyllum cf. *pygmaeum*	OP830449	OP830464
Porolithon sp. A	OP830446	OP830461
Porolithon sp. B	OP830444	OP830459
Lithophyllum cf. *kotschyanum*	OP830451	OP830466
Porolithon sp. C	OP830445	OP830460

References (**Appendix A** – Doll et al. 2023a)

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Appendix B: Supplementary information for Chapter 5 (Doll et al. 2021)

Larval settlement detections across sampling periods and sites

Sample	Sampling reef	Latitude	Longitude	Sampling period	Depth	COTS settlement
1	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Shallow	YES
2	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Shallow	YES
3	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Shallow	YES
4	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Mid	YES
5	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Mid	YES
6	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Mid	YES
7	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Deep	YES
8	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Deep	YES
9	Rib Reef	-18.49141	146.87051	Oct 16 - Jan 17	Deep	YES
10	Rib Reef	-18.49258	146.87442	Oct 16 - Jan 17	Shallow	YES
11	Rib Reef	-18.49258	146.87442	Oct 16 - Jan 17	Shallow	YES
12	Rib Reef	-18.49258	146.87442	Oct 16 - Jan 17	Deep	NO
13	Rib Reef	-18.49258	146.87442	Oct 16 - Jan 17	Deep	YES
14	Rib Reef	-18.49258	146.87442	Oct 16 - Jan 17	Deep	YES
15	Rib Reef	-18.47157	146.87440	Oct 16 - Jan 17	Shallow	NO
16	Rib Reef	-18.47157	146.87440	Oct 16 - Jan 17	Shallow	NO
17	Rib Reef	-18.47157	146.87440	Oct 16 - Jan 17	Mid	NO
18	Rib Reef	-18.47157	146.87440	Oct 16 - Jan 17	Deep	NO
19	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Shallow	NO
20	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Shallow	NO
21	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Shallow	NO
22	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Mid	NO
23	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Mid	NO
24	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Mid	NO
25	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Deep	NO
26	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Deep	NO
27	Moore Reef	-16.89336	146.19093	Oct 16 - Jan 17	Deep	NO
28	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Shallow	NO

29	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Shallow	YES
30	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Shallow	NO
31	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Mid	NO
32	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Mid	NO
33	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Mid	YES
34	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Deep	NO
35	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Deep	NO
36	Moore Reef	-16.88682	146.19227	Oct 16 - Jan 17	Deep	NO
37	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Shallow	NO
38	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Shallow	NO
39	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Shallow	NO
40	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Mid	YES
41	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Mid	YES
42	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Mid	NO
43	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Deep	NO
44	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Deep	NO
45	Moore Reef	-16.84700	146.21800	Oct 16 - Jan 17	Deep	NO
46	Rib Reef	-18.49141	146.87051	Dec 16 - Mar 17	Shallow	YES
47	Rib Reef	-18.49141	146.87051	Dec 16 - Mar 17	Mid	NO
48	Rib Reef	-18.49141	146.87051	Dec 16 - Mar 17	Mid	YES
49	Rib Reef	-18.49141	146.87051	Dec 16 - Mar 17	Mid	YES
50	Rib Reef	-18.49141	146.87051	Dec 16 - Mar 17	Deep	NO
51	Rib Reef	-18.49141	146.87051	Dec 16 - Mar 17	Deep	YES
52	Rib Reef	-18.49141	146.87051	Dec 16 - Mar 17	Deep	NO
53	Rib Reef	-18.49258	146.87442	Dec 16 - Mar 17	Shallow	NO
54	Rib Reef	-18.49258	146.87442	Dec 16 - Mar 17	Mid	NO
55	Rib Reef	-18.49258	146.87442	Dec 16 - Mar 17	Mid	NO
56	Rib Reef	-18.49258	146.87442	Dec 16 - Mar 17	Deep	NO
57	Rib Reef	-18.49258	146.87442	Dec 16 - Mar 17	Deep	NO
58	Rib Reef	-18.49258	146.87442	Dec 16 - Mar 17	Deep	NO
59	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Shallow	NO
60	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Shallow	NO
61	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Shallow	NO

62	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Mid	NO
63	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Mid	NO
64	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Mid	NO
65	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Deep	NO
66	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Deep	NO
67	Rib Reef	-18.47157	146.87440	Dec 16 - Mar 17	Deep	NO
68	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Shallow	NO
69	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Shallow	NO
70	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Shallow	NO
71	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Mid	NO
72	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Mid	NO
73	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Mid	NO
74	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Deep	YES
75	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Deep	YES
76	Moore Reef	-16.89336	146.19093	Dec 16 - Mar 17	Deep	NO
77	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Shallow	NO
78	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Shallow	YES
79	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Shallow	NO
80	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Mid	NO
81	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Mid	NO
82	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Mid	NO
83	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Deep	NO
84	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Deep	NO
85	Moore Reef	-16.88682	146.19227	Dec 16 - Mar 17	Deep	YES
86	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Shallow	NO
87	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Shallow	NO
88	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Shallow	NO
89	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Mid	NO
90	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Mid	NO
91	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Mid	NO
92	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Deep	NO
93	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Deep	NO
94	Moore Reef	-16.84700	146.21800	Dec 16 - Mar 17	Deep	NO

95	Rib Reef	-18.49141	146.87051	Nov 17 - Feb 18	Shallow	NO
96	Rib Reef	-18.49141	146.87051	Nov 17 - Feb 18	Mid	NO
97	Rib Reef	-18.49141	146.87051	Nov 17 - Feb 18	Mid	NO
98	Rib Reef	-18.49141	146.87051	Nov 17 - Feb 18	Mid	NO
99	Rib Reef	-18.49141	146.87051	Nov 17 - Feb 18	Deep	YES
100	Rib Reef	-18.49141	146.87051	Nov 17 - Feb 18	Deep	NO
101	Rib Reef	-18.49141	146.87051	Nov 17 - Feb 18	Deep	NO
102	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Shallow	NO
103	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Shallow	NO
104	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Mid	YES
105	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Mid	NO
106	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Mid	YES
107	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Deep	NO
108	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Deep	NO
109	Rib Reef	-18.49258	146.87442	Nov 17 - Feb 18	Deep	NO
110	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Shallow	NO
111	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Shallow	YES
112	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Mid	NO
113	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Mid	NO
114	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Mid	NO
115	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Deep	NO
116	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Deep	NO
117	Rib Reef	-18.47157	146.87440	Nov 17 - Feb 18	Deep	NO
118	Lodestone Reef	-18.68239	147.11136	Nov 17 - Feb 18	Shallow	YES
119	Lodestone Reef	-18.68239	147.11136	Nov 17 - Feb 18	Mid	NO
120	Lodestone Reef	-18.68239	147.11136	Nov 17 - Feb 18	Mid	NO
121	Lodestone Reef	-18.68239	147.11136	Nov 17 - Feb 18	Deep	NO
122	Lodestone Reef	-18.68239	147.11136	Nov 17 - Feb 18	Deep	NO
123	Lodestone Reef	-18.68239	147.11136	Nov 17 - Feb 18	Deep	NO
124	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Shallow	YES
125	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Shallow	YES
126	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Mid	NO
127	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Mid	YES

128	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Mid	NO
129	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Deep	NO
130	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Deep	YES
131	Lodestone Reef	-18.69978	147.09572	Nov 17 - Feb 18	Deep	NO
132	Lodestone Reef	-18.70094	147.09839	Nov 17 - Feb 18	Shallow	NO
133	Lodestone Reef	-18.70094	147.09839	Nov 17 - Feb 18	Mid	NO
134	Lodestone Reef	-18.70094	147.09839	Nov 17 - Feb 18	Mid	NO
135	Lodestone Reef	-18.70094	147.09839	Nov 17 - Feb 18	Mid	NO
136	Lodestone Reef	-18.70094	147.09839	Nov 17 - Feb 18	Deep	YES
137	Lodestone Reef	-18.70094	147.09839	Nov 17 - Feb 18	Deep	YES
138	Lodestone Reef	-18.70094	147.09839	Nov 17 - Feb 18	Deep	NO
139	Rib Reef	-18.49141	146.86965	Nov 19 - Jan 20	Shallow	NO
140	Rib Reef	-18.49141	146.86965	Nov 19 - Jan 20	Mid	YES
141	Rib Reef	-18.49141	146.86965	Nov 19 - Jan 20	Mid	NO
142	Rib Reef	-18.49141	146.86965	Nov 19 - Jan 20	Deep	NO
143	Rib Reef	-18.49141	146.86965	Nov 19 - Jan 20	Deep	NO
144	Rib Reef	-18.49141	146.86965	Nov 19 - Jan 20	Deep	NO
145	Kelso Reef	-18.44848	146.99525	Nov 19 - Jan 20	Shallow	NO
146	Kelso Reef	-18.44848	146.99525	Nov 19 - Jan 20	Mid	NO
147	Kelso Reef	-18.44848	146.99525	Nov 19 - Jan 20	Mid	YES
148	Kelso Reef	-18.44848	146.99525	Nov 19 - Jan 20	Mid	YES
149	Kelso Reef	-18.44848	146.99525	Nov 19 - Jan 20	Deep	NO
150	Kelso Reef	-18.44848	146.99525	Nov 19 - Jan 20	Deep	YES
151	Little Kelso Reef	-18.47627	146.98575	Nov 19 - Jan 20	Shallow	NO
152	Little Kelso Reef	-18.47627	146.98575	Nov 19 - Jan 20	Mid	NO
153	Little Kelso Reef	-18.47627	146.98575	Nov 19 - Jan 20	Mid	NO
154	Little Kelso Reef	-18.47627	146.98575	Nov 19 - Jan 20	Mid	YES
155	Little Kelso Reef	-18.47627	146.98575	Nov 19 - Jan 20	Deep	YES
156	Little Kelso Reef	-18.47627	146.98575	Nov 19 - Jan 20	Deep	NO
157	Lodestone Reef	-18.69690	147.11576	Nov 19 - Jan 20	Shallow	YES
158	Lodestone Reef	-18.69690	147.11576	Nov 19 - Jan 20	Mid	YES
159	Lodestone Reef	-18.69690	147.11576	Nov 19 - Jan 20	Mid	YES
160	Lodestone Reef	-18.69690	147.11576	Nov 19 - Jan 20	Deep	NO

161	Lodestone Reef	-18.69690	147.11576	Nov 19 - Jan 20	Deep	NO
162	Lodestone Reef	-18.69690	147.11576	Nov 19 - Jan 20	Deep	YES
163	Keeper Reef	-18.74255	147.26536	Nov 19 - Jan 20	Shallow	NO
164	Keeper Reef	-18.74255	147.26536	Nov 19 - Jan 20	Mid	YES
165	Keeper Reef	-18.74255	147.26536	Nov 19 - Jan 20	Mid	YES
166	Keeper Reef	-18.74255	147.26536	Nov 19 - Jan 20	Deep	YES
167	Keeper Reef	-18.74255	147.26536	Nov 19 - Jan 20	Deep	NO
168	Keeper Reef	-18.74255	147.26536	Nov 19 - Jan 20	Deep	YES
169	Big Broadhurst Reef	-18.94652	147.72639	Nov 19 - Jan 20	Shallow	YES
170	Big Broadhurst Reef	-18.94652	147.72639	Nov 19 - Jan 20	Mid	NO
171	Big Broadhurst Reef	-18.94652	147.72639	Nov 19 - Jan 20	Mid	YES
172	Big Broadhurst Reef	-18.94652	147.72639	Nov 19 - Jan 20	Mid	NO
173	Big Broadhurst Reef	-18.94652	147.72639	Nov 19 - Jan 20	Deep	YES
174	Big Broadhurst Reef	-18.94652	147.72639	Nov 19 - Jan 20	Deep	YES
175	Little Broadhurst Reef	-18.95682	147.68991	Nov 19 - Jan 20	Shallow	YES
176	Little Broadhurst Reef	-18.95682	147.68991	Nov 19 - Jan 20	Mid	YES
177	Little Broadhurst Reef	-18.95682	147.68991	Nov 19 - Jan 20	Mid	YES
178	Little Broadhurst Reef	-18.95682	147.68991	Nov 19 - Jan 20	Deep	NO
179	Little Broadhurst Reef	-18.95682	147.68991	Nov 19 - Jan 20	Deep	YES
180	Little Broadhurst Reef	-18.95682	147.68991	Nov 19 - Jan 20	Deep	YES
181	North Direction Is.	-14.74960	145.51380	Oct 19 - Feb 20	Shallow	NO
182	North Direction Is.	-14.74960	145.51380	Oct 19 - Feb 20	Mid	NO
183	North Direction Is.	-14.74960	145.51380	Oct 19 - Feb 20	Mid	NO
184	North Direction Is.	-14.74960	145.51380	Oct 19 - Feb 20	Mid	NO
185	North Direction Is.	-14.74960	145.51380	Oct 19 - Feb 20	Deep	YES
186	Eyrie Reef	-14.73390	145.37893	Oct 19 - Feb 20	Mid	NO
187	Eyrie Reef	-14.73390	145.37893	Oct 19 - Feb 20	Deep	NO
188	Eyrie Reef	-14.73390	145.37893	Oct 19 - Feb 20	Deep	NO
189	Eyrie Reef	-14.73390	145.37893	Oct 19 - Feb 20	Deep	NO
190	Eyrie Reef	-14.73390	145.37893	Oct 19 - Feb 20	Deep	NO
191	North Reef	-14.64487	145.45445	Oct 19 - Feb 20	Mid	NO
192	North Reef	-14.64487	145.45445	Oct 19 - Feb 20	Mid	NO
193	North Reef	-14.64487	145.45445	Oct 19 - Feb 20	Mid	NO

Latitude and longitude geographic coordinates of sampling sites are provided in Decimal Degrees (DD).

Appendix C: List of publications arising from this thesis (Chapters 2 to 5).

1. **Doll, P.C.**, Caballes, C.F., Hoey, A.S., Uthicke, S., Ling, S.D. & Pratchett, M.S. 2022. Larval settlement in echinoderms: a review of processes and patterns. *Oceanography and Marine Biology: An Annual Review* 60, 433-494. (Chapter 2) (https://doi.org/10.1201/9781003288602-9)

2. **Doll, P.C.**, Uthicke, S., Caballes, C.F., Diaz-Pulido, G., Abdul Wahab, M.A., Lang, B.J., Jeong, S.Y. & Pratchett, M.S. 2023a. Settlement cue selectivity by larvae of the destructive crown-of-thorns starfish. *Biology Letters* 19, 20220399. (Chapter 3) (https://doi.org/10.1098/rsbl.2022.0399)

3. **Doll, P.C.**, Uthicke, S., Caballes, C.F., Patel, F., Gomez Cabrera, M., Lang, B.J. & Pratchett, M.S. 2023b. Induction of crown-of-thorns starfish settlement is not mediated by conspecific cues. *Scientific Reports* 13, 17119. (Chapter 4) (https://doi.org/10.1038/s41598-023-44422-x)

4. **Doll, P.C.**, Messmer, V., Uthicke, S., Doyle, J.R., Caballes, C.F. & Pratchett, M.S. 2021. DNA-based detection and patterns of larval settlement of the corallivorous crown-of-thorns sea star (*Acanthaster* sp.) *The Biological Bulletin* 241, 271-285. (Chapter 5) (https://doi.org/10.1086/717539)

Appendix D: List of publications during candidature not arising from this thesis.

5. Thompson, C.A., Hoey, A.S., Montanari, S.R., Messmer, V., **Doll, P.C.** & Pratchett, M.S..2021. Territoriality and condition of Chevron Butterflyfish (*Chaetodon trifascialis*) with varying coral cover on the Great Barrier Reef, Australia. *Environmental Biology of Fishes* 104, 53-69.

6. **Doll, P.C.**, Munday, P.L., Bonin, M.C. & Jones, G.P. 2021. Habitat specialisation and overlap in coral reef gobies of the genus *Eviota* (Teleostei: Gobiidae). *Marine Ecology Progress Series* 677, 81-94.

7. Lang, B.J., Donelson, J.M., Caballes, C.F., **Doll, P.C.** & Pratchett, M.S. 2021. Metabolic responses of Pacific crown-of-thorns sea stars (*Acathaster* sp.) to Acute Warming. *The Biological Bulletin* 241, 347-358.

8. Hoey, A.S., Harrison, H.B., McClure, E.C., Burn, D., Barnett, A., Cresswell, B., **Doll, P.C.**, Galbraith, G. & Pratchett, M.S. 2021. Coral Sea Marine Park Coral Reef Health Survey 2021. Report prepared for Parks Australia (107 pp).

9. Pratchett, M.S., Caballes, C.F., Cvitanovic, C., Raymundo, M.L., Babcock, R.C. … **Doll, P.C.** et al. 2021. Knowledge gaps in the biology, ecology and management of the Pacific crown-of-thorns sea star, *Acanthaster* sp., on Australia’s Great Barrier Reef. *The Biological Bulletin* 241, 330-346.

10. Lang, B.J., Donelson, J.M., Caballes, C.F., Uthicke, S., **Doll, P.C.** & Pratchett, M.S. 2022. Effects of elevated temperature on the performance and survival of pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*). *Marine Biology* 169, 43. (https://doi.org/10.1007/s00227-022-04027-w)

11. Pratchett, M.S., Caballes, C.F., Burn, D., **Doll, P.C.**, Chandler, J.F., Doyle, J.R. & Uthicke, S. 2022. Scooter-assisted large area diver-based (SALAD) visual surveys to test for renewed outbreaks of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) in the northern Great Barrier Reef. A report to the Australian Government by the COTS Control Innovation Program (32 pp).

12. Lang, B.J., Uthicke, S., Caballes, C.F., **Doll, P.C.**, Donelson, J.M. & Pratchett, M.S. 2023. Impacts of ocean warming on the settlement success and post-settlement survival of crown-of-thorns starfish (*Acanthaster* cf. *solaris*). *Coral Reefs* 42 (1), 143-155.

13. **Doll, P.C.** 2023. Microscopic starfish and how to find them: Developing a molecular-based approach to measure crown-of-thorns-starfish settlement rates. *Reef in Review* 52, 48-49.

14. Chandler, J.F., Burn, D., Caballes, C.F., **Doll, P.C.**, Kwong, S.L.T., Lang, B.J., Pacey, K.I. & Pratchett, M.S. 2023. ncreasing densities of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) at Lizard Island, northern Great Barrier Reef, resolved using a novel survey method. *Scientific Reports* 13, 19306. (https://doi.org/10.1038/s41598-023-46749-x)

15. Pratchett, M.S., Chandler J.F., Choukroun S.M., **Doll, P.C.**, Lang, B.J., Kwong, S., Chen, C.C.M., Emslie, M.J., Caballes C.F., Uthicke S. & Matthews, S.A. 2024. Biophysical processes involved in the initiation and spread of population irruptions of crown-of-thorns starfish on the Great Barrier Reef (p. 290-305). *Oceanographic Processes of Coral Reefs. Physical and Biological Links in the Great Barrier Reef* (2nd edition). E Wolanski & M Kingsford (eds). CRC Press, Boca Raton, Florida. (htttps://doi.org/10.1201/9781003320425-23)

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Taylor & Francis

LARVAL SETTLEMENT IN ECHINODERMS: A REVIEW OF PROCESSES AND PATTERNS

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Abstract Echinoderms are a common component of benthic marine ecosystems, with many being ecologically and/or economically important. Like many marine organisms, most echinoderms have a bipartite life history with a planktonic larval phase and a benthic adult phase. The transition between these phases (i.e. settlement) is complex and comprises a cascade of events including the location, exploration and selection of suitable benthic habitat, and metamorphosis to adapt from a pelagic to a benthic lifestyle. This review provides a comprehensive synthesis of the various processes involved in the settlement phase across all five extant classes of echinoderms. Central to the review is a detailed assessment of settlement behaviour and the diverse mechanisms of settlement induction. Most echinoderms, including keystone sea urchins, starfishes and sea cucumbers, do not settle indiscriminately; specific environmental conditions or cues are often necessary for settlement to occur, resulting in marked spatial and temporal variability in settlement rates. Fluctuations in settlement, in turn, lead to major changes in the local abundance of echinoderms and often have profound ecological consequences, due to the pivotal role that many echinoderms play in ecosystem functioning. Given important knowledge gaps persist, this review also explores opportunities for future research to advance our understanding of this critical early life-history phase.

Keywords: Marine Ecology; Environmental Cues; Settlement Induction; Larval Behaviour; Metamorphosis; Recruitment; Marine Benthic Invertebrates

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Research

Marine biology

Settlement cue selectivity by larvae of the destructive crown-of-thorns starfish

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Population irruptions of crown-of-thorns starfish (COTS) cause extensive degradation of coral reefs, threatening the structure and function of these important ecosystems. For population irruptions to initiate and spread, large numbers of planktonic larvae have to successfully transition into their benthic life-history stage (i.e. settlement), whereby larval behaviour and the presence of settlement cues may shape spatial patterns of recruitment and adult densities. Our results demonstrate that a wide range of coralline algae species induce COTS larvae to settle; however, the capacity to promote settlement success varied manyfold among algal species, ranging from greater than 90% in *Melyvonnea* cf. *madagascariensis* to less than 2% in *Lithophyllum* cf. *kotschyanum* and two *Porolithon* species at 24 h. Because many coralline algae species that promote high settlement success are prevalent in shallow reef habitats, our findings challenge the hypothesis that COTS larvae predominantly settle in deep water. Considering both larval behaviour and algal ecology, this study highlights the ecological significance of coralline algae communities in driving recruitment patterns of COTS. More specifically, the local abundance of highly inductive coralline algae (especially, *Melyvonnea* cf. *madagascariensis*) may explain some of the marked spatial heterogeneity of COTS populations and the incidence of population irruptions.

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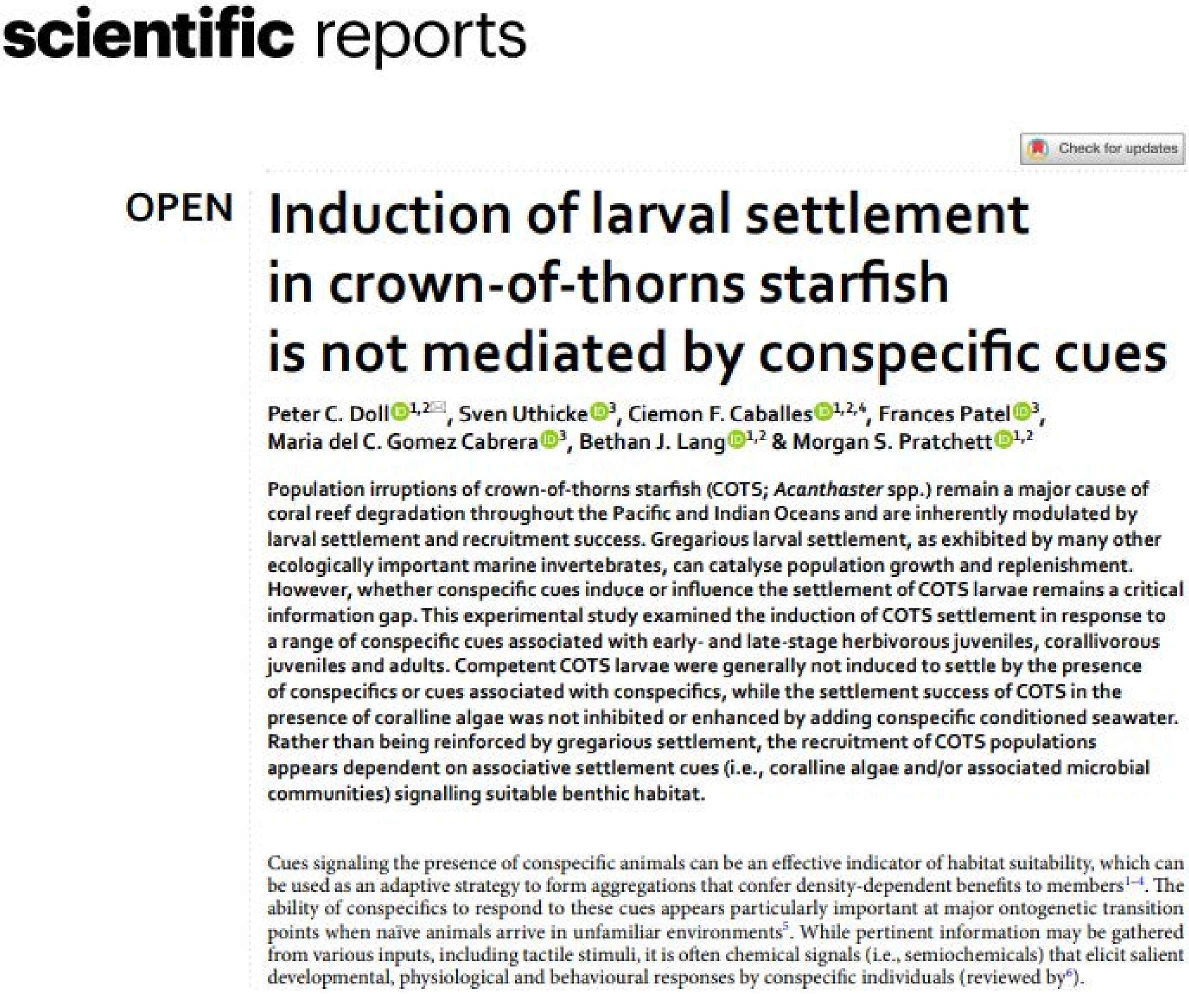

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Induction of larval settlement in crown-of-thorns starfish is not mediated by conspecific cues

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Population irruptions of crown-of-thorns starfish (COTS; *Acanthaster* spp.) remain a major cause of coral reef degradation throughout the Pacific and Indian Oceans and are inherently modulated by larval settlement and recruitment success. Gregarious larval settlement, as exhibited by many other ecologically important marine invertebrates, can catalyse population growth and replenishment. However, whether conspecific cues induce or influence the settlement of COTS larvae remains a critical information gap. This experimental study examined the induction of COTS settlement in response to a range of conspecific cues associated with early- and late-stage herbivorous juveniles, corallivorous juveniles and adults. Competent COTS larvae were generally not induced to settle by the presence of conspecifics or cues associated with conspecifics, while the settlement success of COTS in the presence of coralline algae was not inhibited or enhanced by adding conspecific conditioned seawater. Rather than being reinforced by gregarious settlement, the recruitment of COTS populations appears dependent on associative settlement cues (i.e., coralline algae and/or associated microbial communities) signalling suitable benthic habitat.

Cues signaling the presence of conspecific animals can be an effective indicator of habitat suitability, which can be used as an adaptive strategy to form aggregations that confer density-dependent benefits to members[1–4]. The ability of conspecifics to respond to these cues appears particularly important at major ontogenetic transition points when naïve animals arrive in unfamiliar environments[5]. While pertinent information may be gathered from various inputs, including tactile stimuli, it is often chemical signals (i.e., semiochemicals) that elicit salient developmental, physiological and behavioural responses by conspecific individuals (reviewed by[6]).

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DNA-Based Detection and Patterns of Larval Settlement of the Corallivorous Crown-of-Thorns Sea Star (*Acanthaster* sp.)

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Abstract. Population irruptions of the western Pacific crown-of-thorns sea star (*Acanthaster* sp.) are a perennial threat to coral reefs and may be initiated by fluctuations in reproductive or settlement success. However, the processes dictating their early life history, particularly larval settlement, remain poorly understood given limitations in sampling larvae and newly settled juveniles in the field. Here, we introduce an innovative method to measure crown-of-thorns sea star settlement, using artificial settlement collectors and droplet digital polymerase chain reaction based on crown-of-thorns sea star-specific mitochondrial DNA primers. This study demonstrated the utility of this method and explored temporal and spatial patterns of crown-of-thorns sea star settlement on the Great Barrier Reef from 2016 to 2020. Settlement varied considerably between sampling periods at Rib Reef and peaked between October 2016 and January 2017. Our results further suggest that crown-of-thorns sea star larvae readily settle in shallow reef environments, with no preferential settlement detected between depths tested (4–12 m). Substantial variation between Great Barrier Reef regions was revealed in 2019–2020, because collectors deployed on reefs in the central Great Barrier Reef were >10 times as likely to record newly settled crown-of-thorns sea stars as reefs in the northern Great Barrier Reef near Lizard Island. The trends reported here add to our understanding of this critical life-history stage; however, further method validation and larger-scale studies are needed to address pertinent information gaps, such as the stock-recruitment dynamics of this species. Most importantly, fluctuations in crown-of-thorns sea star settlement can now be detected using this sampling protocol, which demonstrates its utility in heralding new and renewed population irruptions of this destructive sea star.

Introduction

Crown-of-thorns sea stars (COTS; *Acanthaster* sp.) are major contributors to coral loss and coral reef degradation on Australia's Great Barrier Reef (GBR) as a result of their propensity to undergo major population irruptions (Pearson, 1972; Endean and Stablum, 1975; Pratchett *et al.*, 2014; Mellin *et al.*, 2019). Understanding the supply-side ecology of COTS and the drivers of their population irruptions has, thus, been a perennial research activity since the 1960s (reviewed in Moran, 1986; Pratchett *et al.*, 2017a). The exceptional reproductive potential of this taxon suggests that even small changes in key demographic rates could lead to population irruptions (Uthicke *et al.*, 2009; Babcock *et al.*, 2016). Population irruptions of COTS have been attributed to single mass-recruitment events (Zann *et al.*, 1987) or the progressive accumulation of sea stars over successive years of elevated recruitment (Kenchington, 1977; Stump, 1996; Pratchett, 2005). Sudden *versus* progressive increases in population size highlight the critical importance of assessing demographic processes that influence popu-

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Territoriality and condition of chevron butterflyfish (*Chaetodon trifascialis*) with varying coral cover on the great barrier reef, Australia

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Stefano R. Montanari · Vanessa Messmer ·
Peter C. Doll · Morgan S. Pratchett

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Abstract The chevron butterflyfish, *Chaetodon trifascialis*, is among the most specialised coral-feeding fish, and while it is known to be very susceptible to extensive depletion of its preferred coral prey (tabular *Acropora* spp.), their specific responses to changing coral cover are poorly understood. The purpose of this study was to test for variation in territorial behaviour and condition of *C. trifascialis* relative to spatial variation in coral cover across four mid-shelf reefs on the Great Barrier Reef. Explicit consideration was also given to the territorial arrangement and interactions among sympatric individuals, with a view to better understanding the sociality of this species. Variation in overall coral cover (which ranged from 26.5–73.4% among sites) as well as cover of tabular *Acropora* (13.3–44.8%) had limited effect on the territoriality or body condition of *C. trifascialis*. Rather, individual variation in territoriality was attributable to differences in gender and size of fish. Male *C. trifascialis* were generally larger and also had larger territories than female counterparts. They also interacted with conspecifics (and congenerics) much more than females. Taken together, these results support previous assertions that *C. trifascialis* is haremic. There was, however, limited evidence of male territories encompassing the territories of >1 female. While the sociality of *C. trifascialis* is clearly atypical of *Chaetodon* butterflyfishes, more work is needed to understand their reproductive biology as well as their behavioural responses to changing coral cover.

Keywords Behaviour · Chaetodontidae · Coral reefs · Competition · Sociality

Introduction

Marine ecosystems are significantly degraded throughout the world, owing to increasing anthropogenic pressures on species and habitats (Roberts et al. 2002;

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Habitat specialisation and overlap in coral reef gobies of the genus *Eviota* (Teleostei: Gobiidae)

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ABSTRACT: On coral reefs, many small coral-associated fishes exhibit high levels of habitat specialisation, which can contribute to their susceptibility to habitat loss. However, high levels of habitat partitioning may buffer communities from the loss of particular habitat types. This study provides a quantitative evaluation of habitat specialisation, substratum selectivity and habitat overlap of 9 *Eviota* (Gobiidae) species in Kimbe Bay, Papua New Guinea. All but 1 locally common *Eviota* species were strongly associated with scleractinian coral substrata, and species ranged from habitat generalists to obligate coral specialists with some of the most extreme fine-scale patterns of habitat specialisation known for coral reef fishes. Patterns of substratum selectivity varied greatly within the genus, but many species showed distinct preferences for particular corals, most notably the scleractinian genera *Acropora* and *Porites*. Most species exhibited low habitat overlap and partitioned habitat on a fine spatial scale, but there were notable exceptions. Two *Acropora* coral specialists and some species with strong preference for massive *Porites* coral exhibited high overlap. Overall, the local abundance of *Eviota* species varied in relation to the degree of habitat specialisation, with the most generalised species more abundant than the habitat specialists. Habitat structure and species-specific differences in habitat specialisation, substratum selectivity and habitat overlap are likely to be key drivers explaining the distribution and abundance of *Eviota* species and the local community structure. Most importantly, many coral-dependent and less abundant species of *Eviota* may be vulnerable to habitat loss as a result of the ongoing degradation of coral reefs.

KEY WORDS: Habitat specialisation · Cryptobenthic reef fish · Niche breadth · Live coral · Selectivity · Habitat overlap · Coral reef

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Metabolic Responses of Pacific Crown-of-Thorns Sea Stars (*Acanthaster* sp.) to Acute Warming

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Abstract. Climate change and population irruptions of crown-of-thorns sea stars (*Acanthaster* sp.) are two of the most pervasive threats to coral reefs. Yet there has been little consideration regarding the synergies between ocean warming and the coral-feeding sub-adult and adult stages of this asteroid. Here we explored the thermosensitivity of the aforementioned life stages by assessing physiological responses to acute warming. Thermal sensitivity was assessed based on the maximal activity of enzymes involved in aerobic (citrate synthase) and anaerobic (lactate dehydrogenase) metabolic pathways, as well as the standard metabolic rate of sub-adult and adult sea stars. In both life stages, citrate synthase activity declined with increasing temperature from 15 °C to 40 °C, with negligible activity occurring >35 °C. On the other hand, lactate dehydrogenase activity increased with temperature from 20 °C to 45 °C, indicating a greater reliance on anaerobic metabolism in a warmer environment. The standard metabolic rate of sub-adult sea stars increased with temperature throughout the testing range (24 °C to 36 °C). Adult sea stars exhibited evidence of thermal stress, with metabolic depression occurring from 33 °C. Here, we demonstrate that crown-of-thorns sea stars are sensitive to warming but that adults, and especially sub-adults, may have some resilience to short-term marine heatwaves in the near future.

Introduction

Environmental temperature is one of the most important abiotic factors influencing marine ectotherms (Munday *et al.*, 2008, 2009; Nguyen *et al.*, 2011; Schulte, 2015; Hoey *et al.*, 2016; Pinsky *et al.*, 2019). In these animals, which include sea stars and other echinoderms, the rate of cellular processes and, consequently, their physiology are inherently linked to the ambient thermal environment (Lawrence, 1984; Lagerspetz and Vainio, 2006; Nilsson *et al.*, 2009; Donelson *et al.*, 2010; Hofmann and Todgham, 2010; Byrne, 2011; Uthicke *et al.*, 2014; Caballes *et al.*, 2017). Because of the important role that temperature plays in the function and performance of these animals, elevated temperatures may have a major influence on their abundance and distribution (Lawrence, 1984; Schulte *et al.*, 2011; Ling, 2013). Critically, sustained ocean warming is expected in the coming decades as a result of climate change (Helmuth *et al.*, 2014; Hoegh-Guldberg *et al.*, 2018; IPCC, 2021), which will be further compounded by the increasing incidence and severity of marine heatwaves (Lynch *et al.*, 2014; Frölicher and Laufkötter, 2018; Hoegh-Guldberg *et al.*, 2018; IPCC, 2021).

In general, rates of cellular processes accelerate as temperature rises, following the Arrhenius law (Clarke and Fraser, 2004;

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Knowledge Gaps in the Biology, Ecology, and Management of the Pacific Crown-of-Thorns Sea Star, *Acanthaster* sp., on Australia's Great Barrier Reef

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Abstract. Crown-of-thorns sea stars (*Acanthaster* sp.) are among the most studied coral reef organisms, owing to their propensity to undergo major population irruptions, which contribute to significant coral loss and reef degradation throughout the Indo-Pacific. However, there are still important knowledge

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Abbreviations: COTS, crown-of-thorns sea stars (*Acanthaster* sp.); eDNA, environmental DNA; GBR, Great Barrier Reef.

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ORIGINAL PAPER

Effects of elevated temperature on the performance and survival of pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)

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Abstract

Population irruptions of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) have caused substantial damage to coral reefs, but it is largely unknown how this asteroid will fare in a warmer ocean. We exposed these starfish to one of four thermal treatments, with final temperatures of 26 °C (control, annual average), 28 °C (summer average), 30 °C (summer maximum) and 32 °C (predicted summer maximum by 2100). We measured the righting time, movement rate, standard metabolic rate and probability of survival of the crown-of-thorns starfish at various timepoints over ~60 days. We found that while temperature did not affect righting time, it did significantly affect movement rate. The movement rate of starfish increased across the 26 to 30 °C range, with those at 28 °C and 30 °C moving 18 and 27% faster than those at the control temperature. Similarly, the standard metabolic rate of starfish increased from 26 to 30 °C, with metabolism 100% and 260% faster at 28 °C and 30 °C compared to those at the 26 °C control. At 32 °C, individual starfish exhibited a 14% slower movement rate, a 33% slower metabolic rate, and also exhibited a fourfold lower probability of survival than those at 30 °C. These results indicate that 32 °C is above the thermal optimum of crown-of-thorns starfish, suggesting that prolonged exposure to temperatures that are expected to be regularly exceeded under near-future climate change may be detrimental to this species.

Keywords Climate change · Coral reefs · Behaviour · Movement · Metabolic rate · Mortality

Coral Reefs
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REPORT

Impacts of ocean warming on the settlement success and post-settlement survival of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)

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Sven Uthicke[3] · **Peter C. Doll**[1,2,3] ·
Jennifer M. Donelson[1] · **Morgan S. Pratchett**[1]

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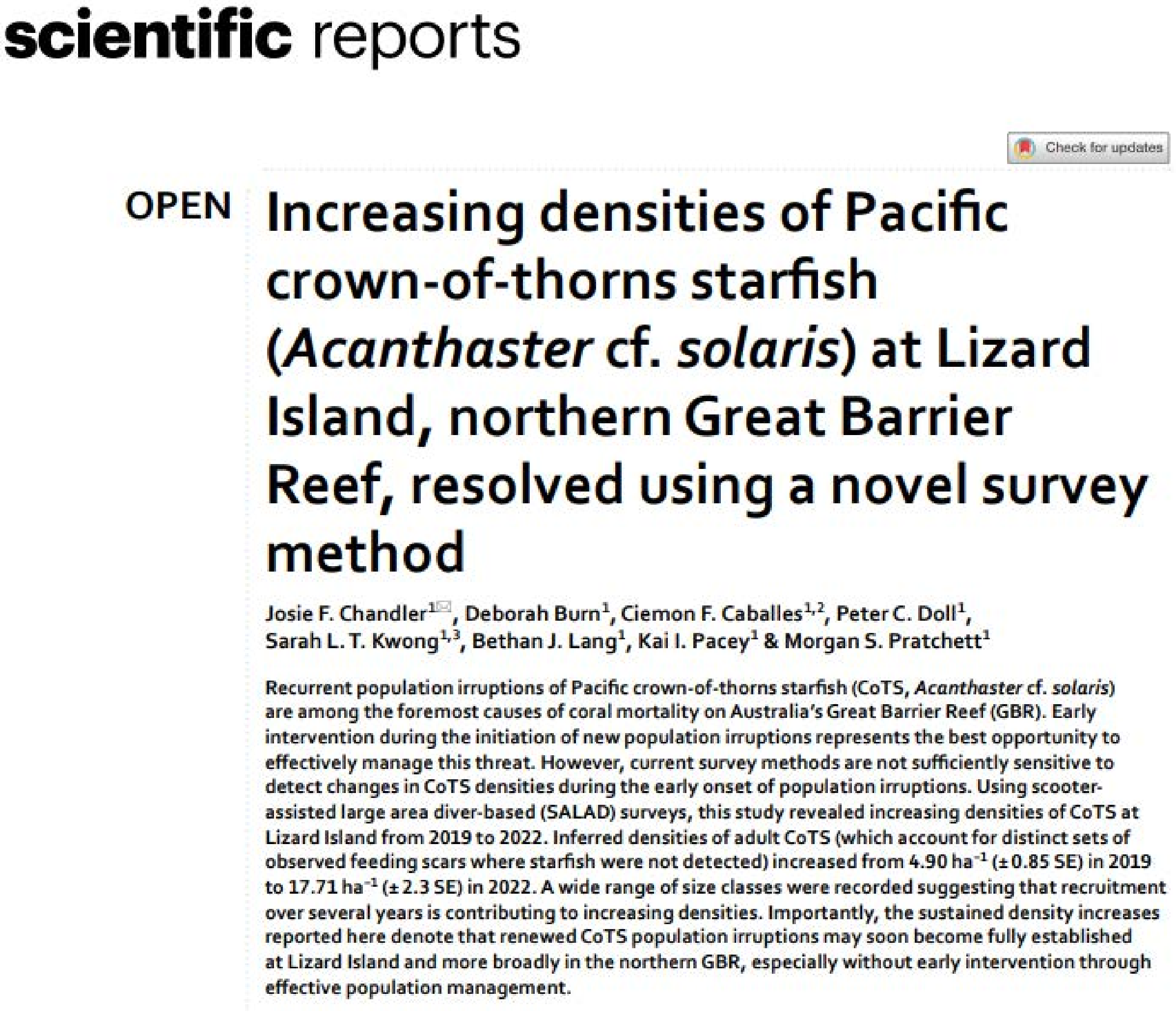
Abstract Ocean warming and population irruptions of crown-of-thorns starfish (CoTS; *Acanthaster* cf. *solaris*) are two of the greatest threats to coral reefs. As such, there is significant interest in understanding how CoTS may be directly impacted by rising ocean temperatures. Settlement of planktonic larvae and subsequent metamorphosis is purported to be a major population bottleneck in marine invertebrates, yet it is unknown how ocean warming will impact these processes in CoTS. Herein, the effect of temperature (28 °C ambient, 30 °C, 32 °C, 34 °C) on the settlement success, metamorphic success, and post-settlement survival of this corallivore was explored. While larval settlement was robust to elevated temperature, with at least 94% of larvae settling after 48 h across all temperatures, it was observed that settlement success was lower on substrate that had been pre-treated ≥ 32 °C. Metamorphic success was also significantly constrained at temperatures ≥ 32 °C. At 32 °C and 34 °C metamorphic success was 16% and 63% lower than at ambient temperature, respectively. Significant adverse effects of warming on post-settlement survival were observed at even cooler temperatures, with 10% lower survival at 30 °C compared to at ambient temperature, and at 34 °C, survival was 34% lower. Substantial reductions in metamorphic success and early post-settlement survival at elevated temperatures, as well as negative impacts of warming on the settlement substrate and its capacity to induce settlement, may present a bottleneck for recruitment in a warmer ocean.

Keywords Temperature · Metamorphosis · Larvae · Coralline algae · Settlement cue · Great Barrier Reef

Introduction

Climate change is a growing threat to marine ecosystems, and coral reefs are particularly vulnerable (Hoegh-Guldberg et al. 2017; Hughes et al. 2017; IPCC 2019; Mellin et al. 2019; Pratchett et al. 2021a). Ocean warming and the higher prevalence and intensity of marine heatwaves are



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Increasing densities of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) at Lizard Island, northern Great Barrier Reef, resolved using a novel survey method

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Sarah L. T. Kwong[1,3], Bethan J. Lang[1], Kai I. Pacey[1] & Morgan S. Pratchett[1]

Recurrent population irruptions of Pacific crown-of-thorns starfish (CoTS, *Acanthaster* cf. *solaris*) are among the foremost causes of coral mortality on Australia's Great Barrier Reef (GBR). Early intervention during the initiation of new population irruptions represents the best opportunity to effectively manage this threat. However, current survey methods are not sufficiently sensitive to detect changes in CoTS densities during the early onset of population irruptions. Using scooter-assisted large area diver-based (SALAD) surveys, this study revealed increasing densities of CoTS at Lizard Island from 2019 to 2022. Inferred densities of adult CoTS (which account for distinct sets of observed feeding scars where starfish were not detected) increased from 4.90 ha^{-1} (± 0.85 SE) in 2019 to 17.71 ha^{-1} (± 2.3 SE) in 2022. A wide range of size classes were recorded suggesting that recruitment over several years is contributing to increasing densities. Importantly, the sustained density increases reported here denote that renewed CoTS population irruptions may soon become fully established at Lizard Island and more broadly in the northern GBR, especially without early intervention through effective population management.

20 Biophysical Processes Involved in the Initiation and Spread of Population Irruptions of Crown-of-Thorns Starfish on the Great Barrier Reef

Morgan S. Pratchett, Josie F. Chandler, Severine M. Choukroun, Peter C. Doll, Bethan J. Lang, Sarah Kwong, Carla C.M. Chen, Mike J. Emslie, Ciemon F. Caballes, Sven Uthicke, and Sam A. Matthews

20.1. INTRODUCTION

Population irruptions (also referred to as outbreaks; Birkeland 1982) of the western Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) remain one of the foremost causes of coral mortality on Australia's Great Barrier Reef (De'ath et al. 2012; Mellin et al. 2019; Pratchett et al. 2021a) and have contributed to coral loss and reef degradation at many locations throughout the Indo-West Pacific (Pratchett et al. 2014; Figure 1). During population irruptions, densities of crown-of-thorns starfish (CoTS) may exceed 10,000 starfish per km^2, and given their large size and very effective mode of feeding, often cause extensive coral loss (e.g., Chesher 1969; Kayal et al. 2012). During the most recent population irruptions of CoTS at Moorea in French Polynesia, for example, the cover of hard corals (order Scleractinia) declined from > 40% in 2005 to < 5% in 2010, with disproportionate effects on coral taxa (e.g., *Acropora* spp.) that are known to be highly preferred prey species of CoTS (Kayal et al. 2012). Although coral loss was partly compounded by other disturbances (e.g., cyclones), spatio-temporal variation in coral loss was strongly correlated with the size and abundance of CoTS (Kayal et al. 2012).

Population irruptions of CoTS have been reported from the Red Sea (Ormond et al. 1973) to Hawaii (Kenyon and Aeby 2009) but tend to be most pronounced and have the greatest ecological impacts within the western and central Pacific (e.g., Japan, Australia, Fiji, and French Polynesia). This area represents the geographic range of the western Pacific CoTS, *Acanthaster* cf. *solaris* (Haszprunar et al. 2017). This species is morphologically and genetically distinct from the three different species of CoTS (including *Acanthaster planci* (Linnaeus, 1958)) that occur in the Indian Ocean (Haszprunar et al. 2017). It is also possible that there is a distinct eastern Pacific species, nominally, of Pacific CoTS species (Haszprunar et al. 2017). Regional differences in the occurrence and ecological impacts of population irruptions of *Acanthaster* spp. may relate to inter-specific differences in the biology or behaviour of different CoTS species (Pratchett et al. 2017a), though this is yet to be tested.

Although the ecological effects and broader ecosystems consequences of population irruptions of CoTS are well-documented (Pratchett et al. 2017a), considerable uncertainty remains about what causes or initiates population irruptions (e.g., Babcock et al. 2016a). Several alternative, not necessarily mutually exclusive, hypotheses have been proposed to account for population irruptions of CoTS (Table 1). These alternative hypotheses have been extensively discussed and debated (e.g., Birkeland and Lucas 1990; Babcock et al. 2016a), though this discourse (and much of the corresponding research) has largely focussed on biological processes (e.g., changes in reproductive output and larval survival of CoTS) or intrinsic bio-physical processes (*sensu* Treml et al. 2015), such as the availability of prey that directly affects growth and survival during successive life stages. Conversely, very limited consideration has been given to the environmental or habitat characteristics (but see Wooldridge and Brodie 2015) or extrinsic biophysical process (*sensu* Treml et al. 2015). The majority of research into the biology and ecology of CoTS has also been conducted in the western Pacific and on *A.* cf. *solaris* (Pratchett et al. 2017a), including extensive research conducted on Australia's Great Barrier Reef (GBR). Recurrent population irruptions of CoTS recorded on the GBR appear to be somewhat unique in the global context (Pratchett et al. 2014) but may nonetheless provide a very important case study for improved understanding of the biological and physical processes that contribute to the initiation or occur-