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Applications to aid the recovery of threatened coral populations

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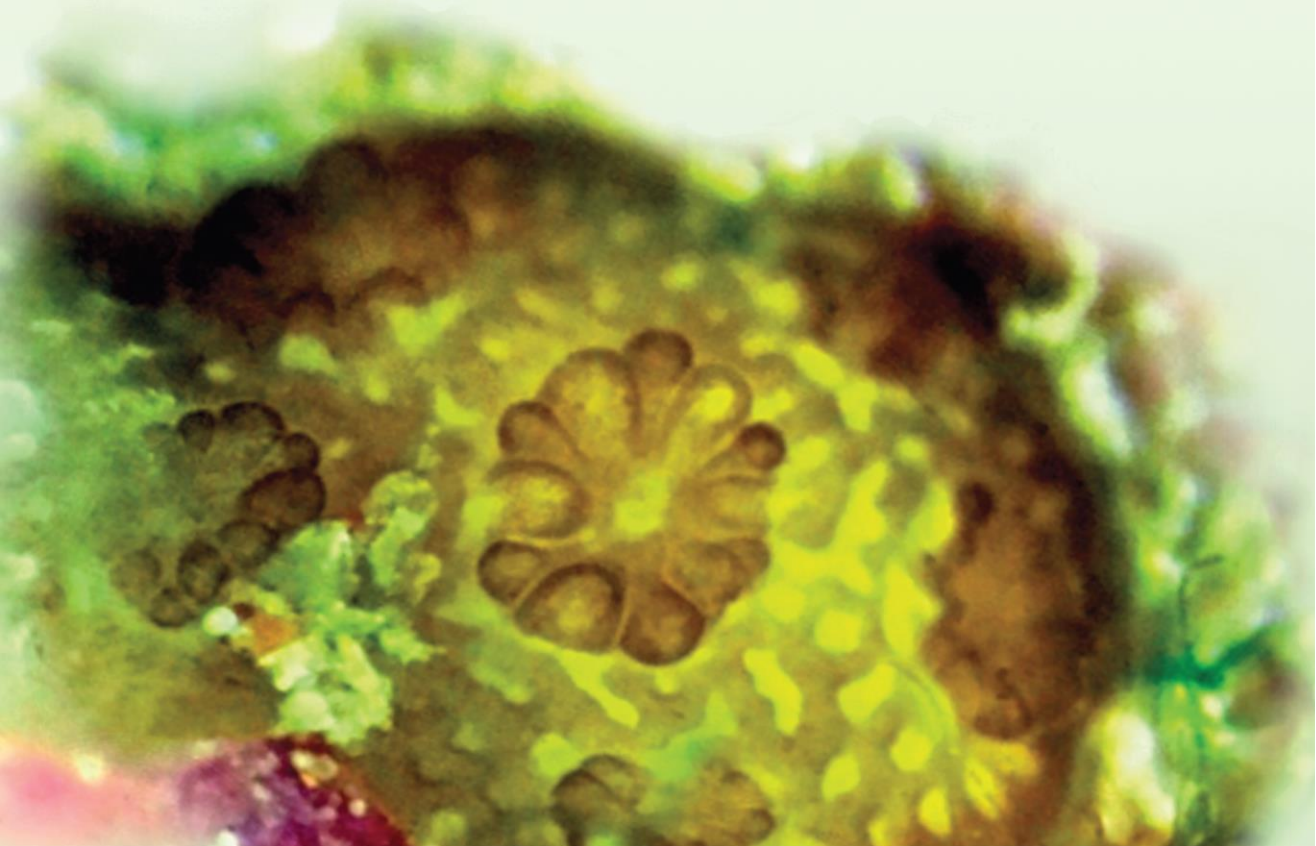
Valérie F Chamberland



Environmental Drivers of Recruitment Success in Caribbean Corals

Applications to Aid the Recovery of Threatened Coral Populations

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Applications to Aid the Recovery of Threatened Coral Populations

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Valérie Francine Chamberland

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Faculteit der Natuurwetenschappen, Wiskunde en Informatica

To my sister

Contents

Chapter 1

Introduction and thesis outline

Valérie F Chamberland

Introduction

With the increasing pressure of humankind on nature, adequately preserving and managing populations of threatened species is crucial to halt the loss of biodiversity that currently occurs at an unprecedented rate (Cardinale et al. 2012). To design proper nature protection policies, it is essential to understand the environmental conditions under which the populations of different species will persist or decline in a given habitat (Begon et al. 2006). Essentially, populations grow through the arrival of new individuals, either via birth or immigration, and shrink through death or emigration (Caley et al. 1996). Only if these four processes are well-documented, will it be possible to predict changes in population size through time and space, which in turn can guide conservation and management decisions.

Populations of most marine species are demographically open, whereby their replenishment largely depends on immigrating planktonic larvae arriving from elsewhere (Caley et al. 1996; Hixon et al. 2002). The successful establishment of these larvae in a local population, i.e., *larval recruitment*, is therefore a pivotal process determining the long-term fate of especially degrading marine populations (Caley et al. 1996; Hixon et al. 2002; Wen et al. 2013). Predicting larval recruitment rates of marine benthic species however represents a formidable challenge. Marine organisms possess complex life cycles, whereby the majority of species develop from planktonic larvae with drastically different body shapes, behaviours, needs and habitats compared to their adult growth form (Giangrande et al. 1994; Marshall and Morgan 2011). Some species produce few, well-nurtured offspring that leave the parent once fully developed, while others produce millions of unfertilized eggs that, after external fertilization, spend days to months in the water column before settling to the benthos (Giangrande et al. 1994; Marshall and Morgan 2011). Several taxa even display triphasic life cycles and undergo multiple metamorphoses, alternating between benthic and pelagic forms (Boero et al. 1997). In addition, monitoring marine larvae in the plankton is challenging due to their small size and because they may disperse over large distances (i.e., tens to hundreds of km) before recruiting. As a consequence, tracking populations of marine species can be difficult, impeding our ability to effectively predict their growth or decline, and thus to adequately manage them.

Chances of successful recruitment are small even in healthy ecosystems. During their planktonic phase, marine larvae are at risk of predation (Bailey and Houde 1989; Allen 2008) and starvation (Olson and Olson 1989), they might encounter abiotic conditions outside their range of physiological tolerance (e.g., temperature, available dissolved oxygen, pH, salinity) (Bashevkin and Pechenik 2015; Allen et al. 2017), and may be transported to inadequate habitats (e.g., open ocean) by currents (Pineda et al. 2007). In the hours to months following settlement, risks of

mortality remain high as young settlers are extremely vulnerable to predation, competition for food and space, developmental anomalies, and changes in abiotic conditions at their recruitment site (Gosselin and Qian 1997). As a consequence, mortality rates in most marine species are highest during their earliest life stages and often exceed 95% in their first year (type III mortality, reviewed in Rumrill 1990). Thus, in addition to being difficult to track due to their small size, dispersal over large distances, and complex life cycle, marine larvae are susceptible to variability in their environment, resulting in large fluctuations in recruitment rates through time. The challenge of including recruitment rates in the long-term dynamics of marine populations has long been recognized as *The Recruitment Problem* (e.g., Bakun 1985; Cohen et al. 1988; Bailey and Houde 1989; Roughgarden et al. 1991).

Historically, fisheries studies have provided the foundations of theoretical ecology on recruitment dynamics for marine species (e.g., Hjort 1914; Ricker 1954; Cushing 1975). These studies were complimented by the important work of Gunnar Thorson on intertidal and subtidal marine invertebrates (e.g., Thorson 1936, 1950, 1966), and later by the work of several ecologists on the population dynamics of intertidal barnacles and gastropods (e.g., Roughgarden 1975; Fairweather 1988; Roughgarden et al. 1988; Underwood and Fairweather 1989; Gaines and Bertness 1992). More recently, much work on larval recruitment dynamics is applied to the design and implementation of marine reserves aimed at preserving threatened populations of marine fishes and invertebrates (e.g., Shanks et al. 2003; Planes et al. 2009; Harrison et al. 2012). Such studies increasingly use advanced technologies such as molecular tools (Heyden et al. 2014) and simulation models (Kerr et al. 2010; Paris et al. 2013). Nonetheless, predicting changes in recruitment rates in degrading marine populations under threat from global change remains an arduous task, as the susceptibility of marine organisms to these natural and anthropogenic threats during their earliest life stages remains poorly documented in many taxa.

The processes affecting larval recruitment success in scleractinian corals, and their implications for the maintenance and recovery of one of Earth's oldest and most biodiverse ecosystems, the coral reef, are considered in this thesis.

The importance of successful recruitment to the maintenance and recovery of coral populations

Coral reefs are amongst the most productive ecosystems on Earth. They provide habitat to 35% of all marine species (Reaka-Kudla 1997; Knowlton et al. 2010) and food, livelihoods and coastal protection to at least 500 million people (Wilkinson 2008). The latest estimates suggest that coral reefs on average provide $352,250 USD ha^{-1} annually through fisheries, coastal protection, tourism and as a source of new medicines (de Groot et al. 2012). Yet, over one third of reef-building coral species (*Scleractinia*) are currently threatened by extinction (IUCN 2017) due to habitat destruction, overexploitation and anthropogenically-driven climate change (Carpenter et al. 2008).

The Caribbean region is no exception. In the past four decades, the average coral cover in the Caribbean has rapidly decreased, from 35% in the 1970s to 16% at present (Jackson et al. 2014). Severe overfishing of herbivorous parrotfishes in the early to mid-20^{th} century followed by the mass mortality of herbivorous sea urchin *Diadema antillarum* in 1983-84, resulted in a decrease in grazing activity, increased algal growth, and as a consequence in increased coral mortality. In addition, the die-off of formerly dominant acroporid corals in the late 1970s caused a sharp decrease in coral cover in shallow reefs. More recently, extreme warming events (e.g., 2005, 2010) and ongoing habitat degradation continue to threaten Caribbean coral populations.

Along with the decline of adult Caribbean coral communities, the abundance of coral recruits decreased dramatically over the past three decades (Hughes and Tanner 2000; Vermeij et al. 2011). Larval recruits are not only important to replenish coral populations, but also maintain genetic variation in otherwise inbreeding populations. Many coral species reproduce asexually, whereby colonies break into fragments that attach to the seafloor and grow into new individuals genetically identical to the parent colony (Highsmith 1982). In contrast, larvae are generated through sexual reproduction (figure 1.1), whereby genetic recombination ensures the formation of new genetic varieties that increase the genetic diversity in recipient populations (Baums 2008). Populations harbouring high genetic diversity prove more resilient to acute disturbances in their environment (Reusch et al. 2005), and have a greater potential of adapting to the conditions associated with climate change and habitat degradation (Baums 2008).

Sexual reproduction in corals is however highly influenced by natural and anthropogenic sources of stress. Amongst others, increases in sea surface temperature, ultraviolet radiation, sedimentation, pollution and diseases can reduce fecundity, limit fertilization success, and impair pre- and post-settlement survival in

corals (reviewed by Richmond 1997 and Harrison 2011). Coral reef degradation results in significant reductions in corals' reproductive output (Hartmann 2014), and in some cases, to the complete failure of larval recruitment (Williams et al. 2008). Declines in recruitment success in Caribbean corals likely contribute to further population decline, lowered genetic diversity (Baums et al. 2006; Foster et al. 2013), and consequently slow or even prevent the maintenance and recovery of imperilled coral populations.

The life cycle of scleractinian corals

Sexual reproduction and larval development

Like many other marine invertebrates, scleractinian corals have a biphasic life cycle, consisting of a short free-swimming larval stage followed by a sessile benthic stage (Harrison 2011) (figure 1.1). There are three modes of larval development in corals. In brooding species, fertilization occurs internally within the maternal polyp where the embryo develops before it is released as a motile planula larva (figure 1.2*a*). In broadcast spawning species, fertilization occurs externally in the water column after gametes are released by parental polyps (figure 1.2*b*-*e*) (Harrison 2011). A third group of species uses intratentacular fertilization, whereby eggs are held in tentacles until they are fertilized by sperm released by male colonies (figure 1.2*f*). These species thus release embryos rather than planulae or eggs (Vermeij et al. 2010; Marhaver et al. 2015). Brooders generally form smaller-sized colonies than broadcast spawning species, provision their larvae with larger lipid resources and disperse over shorter distances (Szmant 1986; Harrison and Wallace 1990; Ritson-Williams et al. 2009). In brooding species, algal symbionts are usually transferred vertically from the mother's gastrodermal cells to oocytes or larvae, whereas most broadcast spawning species acquire algal symbionts horizontally from the environment (Hirose et al. 2001). Most brooders have multiple reproductive cycles per year, releasing larvae up to 12 months per year (Szmant 1986). In contrast, broadcast spawners typically have one gametogenic cycle per year (Szmant 1986; Harrison and Wallace 1990), which, in the Caribbean, ends in the late summer or early autumn (August-October) during one synchronous spawning event. Coral species can be further classified into hermaphroditic species that produce both male and female gametes that are released simultaneously in sperm-egg bundles or as larvae (figure 1.2*b*,*c*), and gonochoric species that form colonies that are either male (figure 1.2*d*) or female (figure 1.2*e*,*f*) and release sperm or eggs, respectively (reviewed in Harrison 2011).

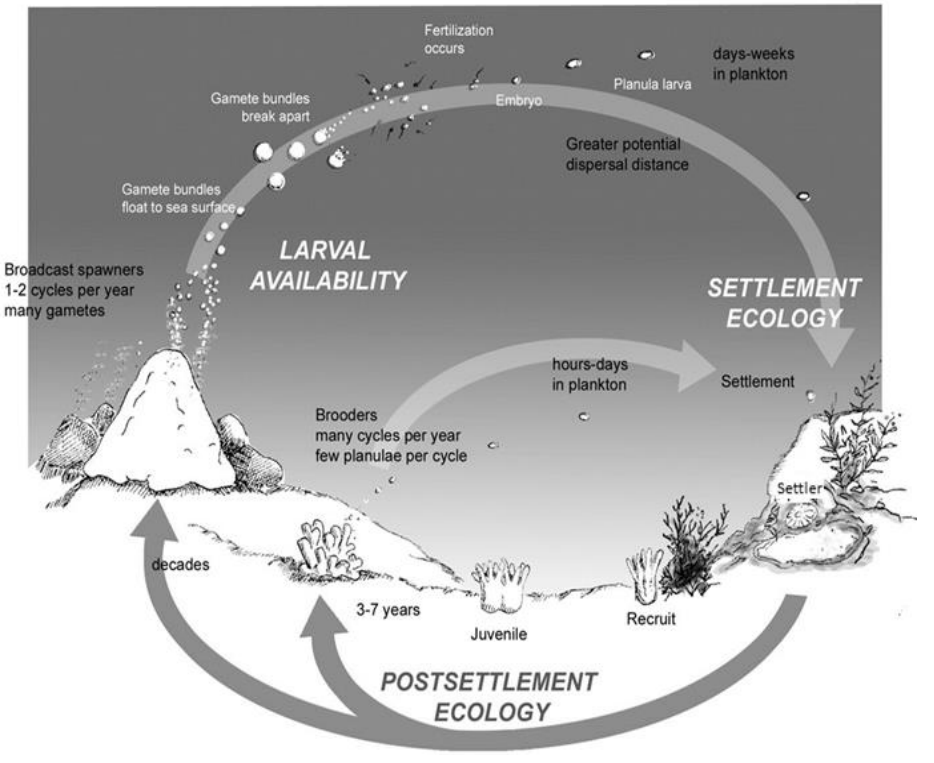


Figure 1.1. The life cycle of corals. Coral recruitment involves three sequential phases. Larval availability depends on successful gamete production and fertilization, embryogenesis and subsequent larval survival. Larval settlement requires that open space is available, competitors occur in low abundance and that positive settlement cues are present. Lastly, recruitment results from successful post-settlement survival and growth (from Ritson-Williams et al. 2009).

Larval dispersal, settlement and metamorphosis

Corals produce non-feeding (i.e., lecitotrophic) larvae that disperse for hours to weeks in the plankton and must settle and metamorphose into their feeding benthic form before depleting their energetic resources (Harii et al. 2002; 2007). Settlement occurs when a larva moves from the water column and attaches to the benthos. Subsequent metamorphosis involves a developmental process during which a larva undergoes a series of morphological and physiological transformations from their larval form into a primary polyp or settler (Harrison and Wallace 1990) (figure 1.1). Substrate selection for settlement by coral larvae depends on a myriad of factors such as light availability (Mundy and Babcock 1998), substrate colouration (Mason et al. 2011) and micro-topography (Nozawa 2008; Brandl et al. 2014), but is

foremost driven by the presence of positive and negative chemical cues. Positive cues that trigger settlement are generally released by other organisms that indicate appropriate habitats for survival and growth (Ritson-Williams et al. 2009). Crustose coralline algae (CCA) and associated bacteria are for example known to induce settlement and metamorphosis in corals (Negri et al. 2001; Ritson-Williams et al. 2010, 2016). Without such positive cues, larvae may fail to settle and metamorphose, and consequently, to successfully recruit.

When conditions favourable for settlement are encountered, coral larvae attach to the substratum by secreting a calcareous exoskeleton, and metamorphose into a benthic polyp (i.e., settler) (figure 1.1) with a mouth and tentacles (Richmond 1997).

Post-settlement survival and growth

The successful transition of a free-swimming coral larva to a benthic primary polyp does not guarantee its place within a coral reef community. Most settlers die shortly after settlement (> 90% mortality rate in the first weeks to months) because their small size renders them vulnerable to predation and competition (Vermeij and Sandin 2008; Penin et al. 2010; Martinez and Abelson 2013). Survival rates of newly settled corals increase substantially with increasing colony size, and early post-settlement growth therefore is a critical process contributing to successful recruitment (Vermeij and Sandin 2008; Penin et al. 2010). In sum, true recruitment in corals depends on the combined outcome of settlement, post-settlement survival and post-settlement growth.

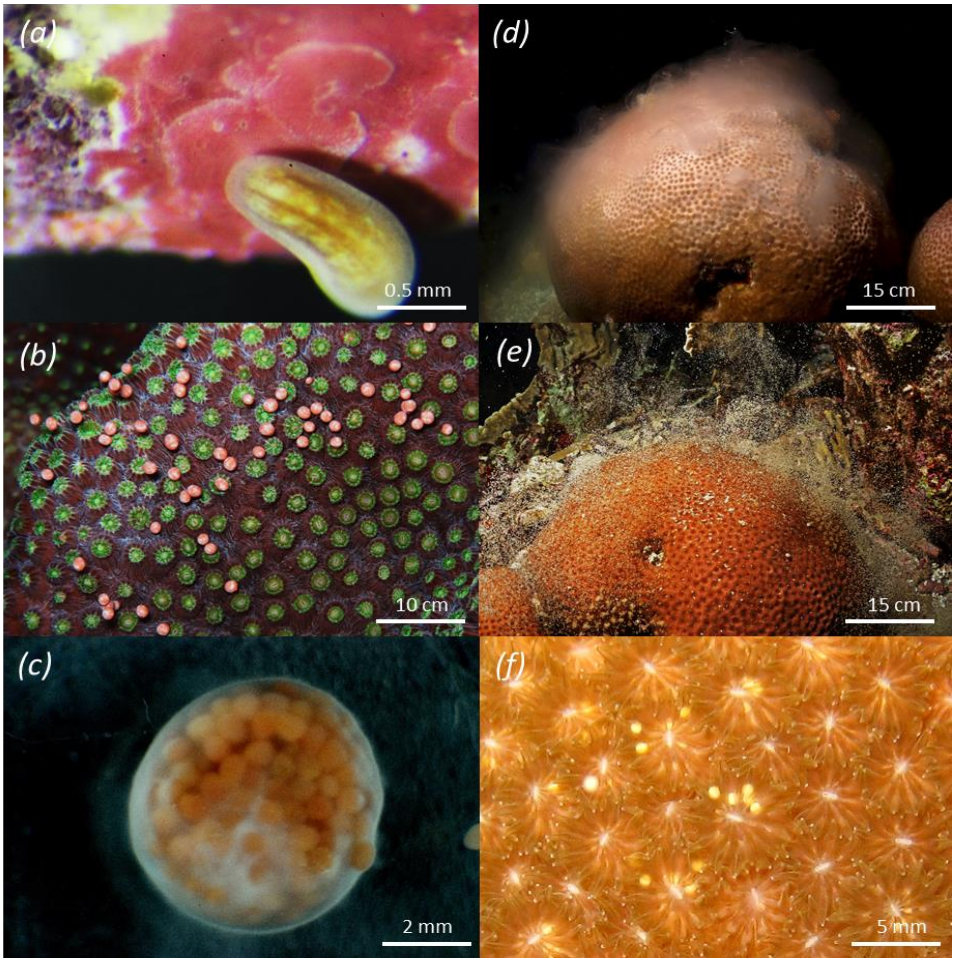


Figure 1.2. Modes of larval development and sexual systems in corals. *(a)* In brooding species, fertilization and subsequent larval development occur internally in the maternal polyp, and released larvae are fully developed and motile. (*b-e)* Broadcast spawning species release male and female gametes in the water column where fertilization and larval development occur. *(f)* In a few species, eggs are retained in the tentacles and exposed to ambient seawater until fertilized, and are released as embryos. (*a-c*) In hermaphroditic corals, polyps have both male and female functions and release *(a)* larvae or (c) sperm-egg bundles. (*d-f*) In gonochoric corals, *(d)* male colonies release sperm and *(e-f)* female colonies release eggs. Photos by *(a)* S Snowden, *(b)* E Muller, *(c)* R Villaverde, *(d,e)* B Mueller and *(f)* MJA Vermeij.

Studying the earliest life stages of corals: state of the art

Sexual reproduction in corals has been studied for over 200 years (Cavolini c. 1790, cited in Lacaze-Duthiers 1897). Early studies focused on brooding species, whereas most documentation on broadcast spawning species dates from the early 1980s following the discovery of mass coral spawning events on the Great Barrier Reef (Harrison and Wallace 1990). In the Caribbean, most research on sexual reproduction in corals also began in the 1980s (Fadlallah 1983; Szmant 1986) and has since then continued to grow, with reports on previously undocumented spawning times for several species published only recently (e.g., Vermeij et al. 2010; Weil and Vargas 2010; Muller and Vermeij 2011; Marhaver et al. 2015). Nonetheless, detailed information on species' early life-histories, from larvae/gamete release to early post-settlement development, is only available for less than ~50% of all Caribbean coral species.

Where available, data is often sparse and mostly concern timing and mode of reproduction (e.g., Fadlallah 1983; Szmant 1986; Alvarado et al. 2004; Weil and Vargas 2010; Levitan et al. 2011; Fogarty et al. 2012), and to a smaller extent larval development, dispersal and settlement (e.g., Vermeij et al. 2009; Mason et al. 2011; Marhaver et al. 2015). Even less is known about species-specific characteristics driving the survival and growth of newly settled corals (but see Szmant and Miller 2006; Ritson-Williams et al. 2010, 2016). As a consequence, the conditions under which coral offspring may overcome the severe risks of mortality inherent to their earliest life phase and successfully recruit into the adult community, remain poorly known.

These data limitations are primarily linked to the technical constraints associated with studying naturally recruiting corals in the field. Monitoring the fate of coral larvae in the plankton is practically impossible due to their small size ($\leq$ 1 mm in diameter (Ø)), lack of characteristics allowing species level identification, and because they may disperse tens to hundreds of km before settling. Recent settlers are also difficult to find and identify to the species level on a reef due to their small size ($\leq$ 2 mm Ø). By the time they reach sizes large-enough to be detected ($\geq$ 1 cm Ø), coral settlers typically have already survived for more than one year, and thereby have already overcome more critical earlier life stages (Vermeij and Sandin 2008; Martinez and Abelson 2013). Corals that recently settled on a reef are subject to a myriad of (a)biotic factors, and their complex interactions (Ritson-Williams et al. 2009) make it difficult to isolate individual effects of contributing parameters in the field. In addition, the conditions experienced by a larva during its pelagic phase can have important fitness consequences during its subsequent benthic stage so that the physiological state of a coral settler likely not only reflects the effects of processes ongoing at its recruitment site (Pechenik et al. 1998; Pechenik 2006; Vermeij et al.

2009; Hartmann et al. 2013). Early life stages should therefore ideally be studied successively, rather than in isolation (Pechenik 2006). Studying aforementioned aspects of the earliest life stages in corals is nearly impossible *in situ* and, as a consequence, is often studied under controlled experimental or laboratory conditions.

Techniques to rear coral offspring *ex situ* from wild-caught gametes/larvae are relatively recent and increasingly more successful (Omori 2005; Petersen et al. 2005; Vermeij et al. 2006; Omori et al. 2008; Nakamura et al. 2011), and have enabled the study of the earliest life stages in a growing number of Caribbean coral species (e.g., *Montastraea faveolata:* Vermeij et al. 2009, *Porites astreoides, Favia fragum, Agaricia agaricites, Acropora* spp., and *Pseudodiploria strigosa*: Ritson-Williams et al. 2010, 2016, *Dendrogyra cylindrus*: Marhaver et al. 2015). Rearing young corals under laboratory conditions further allows to empirically test the influence of environmental factors individually or in combination, and across successive life stages. Thus, as *ex situ* larval rearing techniques improve, so does our knowledge on the environmental factors that drive recruitment success in corals.

Propagating sexual coral recruits to aid the recovery of threatened Caribbean coral populations

> "In the past 30 years, the percent cover of live coral has decreased on a global scale, raising the question: How can we increase the number of corals in these ecosystems for recovery?"
> *- Ritson-Williams et al. (2009)*

A common restoration approach is the asexual propagation of fragments that are cultured in coral nurseries before they are outplanted to degraded reef areas (Quinn and Kojis 2006; Johnson et al. 2011; Nedimyer et al. 2011). While this approach has been observed to locally increase coral cover as well fish abundances (Cabaitan et al. 2008), it does not allow the formation of new genotypes through genetic recombination, which may limit the adaptation potential of restored populations (Baums, 2008). In addition, because slow-growing massive forms suffer high mortality rates following fragmentation (Shaish et al. 2008), the gardening approach is almost exclusively applied to fast-growing species with branching and plate-like growth forms such as *Acropora* spp.

Following the development of *ex situ* larval rearing techniques, propagating sexual coral recruits for restoration purposes became increasingly used to assist the recovery of threatened coral populations (Petersen et al. 2006, 2008; Baria et al. 2010; Nakamura et al. 2011; Villanueva et al. 2012; Guest et al. 2014). Methods using sexual coral recruits for coral restoration aim to increase coral cover and promote genetic diversity in recipient populations (Edwards 2010). After gametes are collected from wild populations and fertilised *ex situ*, coral larvae are typically settled on artificial settlement substrates or "settlement tiles" that are then outplanted to the reef. While the success of sexual coral propagation techniques has improved over recent years, mortality rates among newly settled corals remains extremely high due to processes affecting post-settlement survival and growth (Edwards 2010) and as a consequence, this technique is currently only applied at small experimental scales ($\leq$ 1 hectare). Studies addressing the (species-specific) processes that drive recruitment success are therefore needed to further overcome this bottleneck, and to potentially allow this technique to be implemented at meaningful ecological scales.

Thesis objectives and outline

This thesis aims to expand our understanding of the ecological processes affecting recruitment in Caribbean corals, with a particular focus on identifying the conditions under which recruitment will be successful. This is investigated through a series of *in situ* observational studies as well as *ex situ* and *in situ* manipulative experiments. Findings collected during these studies are then used to improve techniques using sexually produced larvae for reef restoration purposes.

Part 1. Environmental drivers of recruitment success in Caribbean corals

Current studies on the early life stages of reef-building Caribbean corals foremost concern threatened species such as the *Montastraea* species complex and *Acropora* spp. (e.g., Vermeij et al. 2006; Erwin et al. 2008; Erwin and Szmant 2010; Ritson-Williams et al. 2010, 2016; Voolstra et al. 2011; Miller 2014). Much less is known on species with weedy and stress-tolerant life-histories which appear better at coping with environmental degradation (Darling et al. 2012), and could thereby represent ideal candidate species for reef restoration. Members of the subfamily Faviinae are conspicuous and abundant reef-building species throughout the Caribbean region (Weil and Vargas 2010). Despite their high local abundance and higher current recruitment rates compared to the mid-1970s (Vermeij et al. 2011), information on their reproductive biology and earliest life stages is limited. In **Chapter 2**, we describe the reproductive biology and early life ecology of the grooved brain coral *Diploria labyrinthiformis* (Linnaeus, 1758), and investigate this species' ability to recruit under elevated nutrient concentrations.

In **Chapter 3**, we examine the implications of variable symbiont inheritance on the performance of brooded coral larvae exposed to physiological (heat stress) and ecological (absence of positive settlement cues) stress. Maternally inherited algal symbionts can fulfil up to 70% of a larva's energy demand (Gaither and Rowan 2010; Harii et al. 2010; Kopp et al. 2016). High symbiont densities in non-feeding coral larvae could therefore potentially enable the search for high-quality settlement habitats for longer periods of time before energetic resources are depleted. Coral-algal symbiotic relationships are however extremely susceptible to temperature anomalies associated with climate change, and under heat stress, can lead to severe oxidative damage and mortality of the coral host. We therefore investigate the potential costs and benefits of this symbiotic relationship during the larval stage of a brooding coral.

Settlement of free-swimming coral larvae is inhibited by chemicals and bacteria produced or promoted by turf- and macroalgae (Kuffner et al. 2006; Vermeij et al. 2009), whereas chemicals produced by certain species of CCA and their associated bacteria promote coral settlement (Negri et al. 2001; Ritson-Williams et al. 2010,

2016). In **Chapter 4** we quantify the interactive effects of two common causes of reef degradation, overfishing of herbivorous fishes and nutrient pollution, on the dynamics of benthic algal succession. We then examine how the resulting algal communities affect the settlement success of coral larvae.

Part 2. Propagating sexual coral recruits to aid the recovery of threatened Caribbean coral populations

Healthy populations of the elkhorn coral (*Acropora palmata*) fulfil important ecological functions on shallow Caribbean reefs. They provide habitat for a variety of reef organisms as well as coastal protection from incoming storms, and they significantly contribute to gross community calcification and nitrogen fixation on shallow reefs (reviewed in Harborne et al. 2006). During the mid to late 1970s, a Caribbean-wide outbreak of white-band disease (WBD) reduced the abundance of *A. palmata* by more than 95% (reviewed in Jackson et al. 2014). Since then, a lack of recovery prompted restoration initiatives throughout the Caribbean. In **Chapter 5**, we describe the first successful rearing, outplanting and long-term (2.5 yr) survival of *A. palmata* recruits reared from wild-caught gametes. We further test whether the cost-effectiveness of restoration efforts for this critically endangered species could be improved by shortening *ex situ* grow-out periods in land-based nurseries before outplanting settlers on the reef.

Chapter 6 reports on the long-term success of *A. palmata* propagation techniques developed in chapter 5, whereby four-year-old laboratory-reared recruits reached sexual maturity in the field, and were observed spawning synchronously with the native population at their outplanting site.

Using sexual recruits for restoration purposes is currently too expensive to be applied on large scales. The expenses associated with outplanting corals account for a large fraction of these costs because current outplanting techniques require tedious handling of binding materials (e.g., cable-ties, epoxy, nails) underwater, and are therefore incredibly time consuming. In **Chapter 7**, we design and test two tetrapod-shaped artificial settlement substrates for coral larvae that can be outplanted rapidly by simply wedging them in reef crevices without the use of binding materials. Based on their common use in coastal defences to dissipate wave energy, tetrahedral shapes are expected to have limited movement once deployed on the reef. These new settlement substrates may therefore enable effective outplanting of sexual coral recruits at low costs.

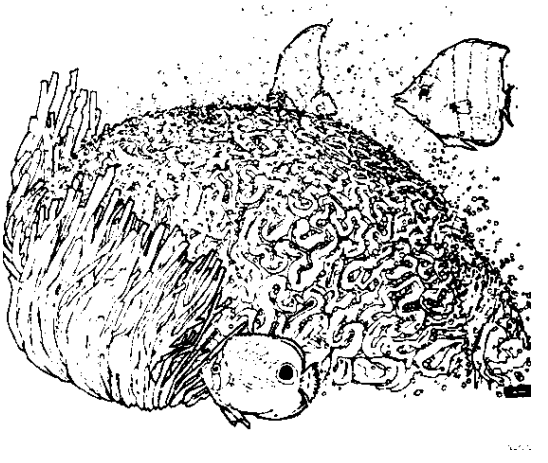
Part 3. Synthesis and future directions.

Chapter 8 discusses the main findings from previous chapters, and provides directions for future research to further improve our understanding of the recruitment dynamics of Caribbean corals, and ultimately to upscale the application of sexual coral restoration techniques to meaningful ecological scales.

Chapter 2

The reproductive biology and early life ecology of a common Caribbean brain coral, *Diploria labyrinthiformis* (Scleractinia: Faviinae)

Valérie F Chamberland,
Skylar Snowden, Kristen L
Marhaver, Dirk Petersen,
Mark JA Vermeij



Drawing by A Pilon

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Abstract

Despite the fact that most of the severe demographic bottlenecks in coral populations occur during their earliest life stages, information on the reproductive biology and early life-history traits of many coral species is limited, and often inferred from adult traits only. This study reports on several atypical aspects of the reproductive biology and early life ecology of the grooved brain coral *Diploria labyrinthiformis* (Linnaeus 1758), a conspicuous reef-building species on Caribbean reefs. The timing of gamete release of *D. labyrinthiformis* was monitored in Curaçao over eight consecutive months, and embryogenesis, larval behaviour and settlement rates were observed and quantified. We further studied growth and symbiont acquisition in juvenile *D. labyrinthiformis* for 3.5 years, and compared settler survival under ambient and nutrient-enriched conditions *in situ*. Notably, *D. labyrinthiformis* reproduced during daylight hours in six consecutive monthly spawning events between May and September 2013, with a peak in June. This is the largest number of reproductive events per year ever observed in a broadcast spawning Caribbean coral species. In settlement experiments, *D. labyrinthiformis* larvae swam to the bottom of culture containers 13 h after spawning and rapidly settled when provided with settlement cues (42% within 14 h). After five months, the survival and growth rates of settled juveniles were 3.7 and 1.9 times higher, respectively, for settlers that acquired zooxanthellae within one month after settlement, compared to those that acquired symbionts later on. Nutrient enrichment increased settler survival four-fold, but only for settlers that had acquired symbionts within one month after settlement. With at least six reproductive events per year, a short planktonic larval phase, high settlement rates, and a positive response to nutrient enrichment, the broadcast spawning species *D. labyrinthiformis* displays a range of reproductive and early life-history traits that are more often associated with brooding coral species, illustrating that classical divisions of coral species by reproductive mode alone do not always reflect the true biology and ecology of their earliest life stages.

Introduction

Shifts in the taxonomic composition of coral communities in response to local and global threats have occurred on coral reefs worldwide, whereby historically dominant reef-building species have been replaced by more opportunistic and stress-tolerant species (Loya et al. 2001; Pratchett et al. 2011; Darling et al. 2012). For instance, non-framework-building coral species (e.g., *Siderastrea radians*, *Porites astreoides* and *Agaricia agaricites*) are now abundant on many Caribbean reefs formerly dominated by Acroporids and the *Montastraea* species complex (Aronson et al. 2004; Darling et al. 2012). Species traits and life-history characteristics have been used to predict corals' responses to environmental change (Loya et al. 2001; Darling et al. 2012), but the mechanisms that allow certain coral taxa to cope with conditions on present-day reefs are not yet fully understood. For example, small-sized brooding corals with fast growth rates and short generation times are often the first coral species to colonize newly available space and are capable of dominating in degraded environments. In contrast, long-lived broadcast spawning species are predicted to dominate in undisturbed areas due to their sensitivity to environmental stressors (Darling et al. 2012). Such classifications based on life-history are derived from species-specific characteristics that, for the most part, arise well after a coral has successfully survived through its earliest life stages (e.g., Loya et al. 2001; Darling et al. 2012). Yet most of the severe demographic bottlenecks in coral populations occur during these earliest life stages (Vermeij and Sandin 2008; Doropoulos et al. 2016), whose species-specific dynamics are therefore likely to underlie ongoing changes in coral community composition on reefs.

Shifts in species composition are often reinforced by concordant changes in recruitment patterns. For example, the absolute abundance of coral juveniles on Curaçao has decreased by more than 50% over the last three decades, but individual changes in abundances differed greatly among species (Vermeij et al. 2011). Juveniles of *Acropora* spp. have virtually disappeared from Curaçaoan reefs, while weedy and stress-tolerant species such as *Siderastrea* spp., *Madracis* spp, *Montastraea cavernosa*, *Stephanocoenia intersepta*, and *Pseudodiploria* spp. (Darling et al. 2012) recruit in greater numbers than they did three decades ago (Vermeij et al. 2011). Such species either produce more offspring at present, and/or possess early life-history characteristics that allow them to better cope with current conditions on Caribbean reefs.

Differences in early life-history traits also determine coral species' ability to successfully recruit to the same habitat. For example, larvae of the brooding species *Agaricia humilis* are 55% less likely to settle under thermal stress and under reduced salinity, whereas larvae of the broadcast spawning species *Montastraea faveolata* do not experience similar negative effects under those same stressful conditions (Hartmann et al. 2013). Settling larvae of different coral species also display species-specific preferences for distinct crustose coralline algae (CCA) (Heyward and Negri 1999), surface orientations and light levels (Babcock and Mundy 1996; Baird et al. 2003). While such behaviours can potentially explain changes in coral community composition through time, little information exists on the earliest life stages of many Caribbean coral species. Detailed information on species reproductive characteristics (e.g., reproductive mode, timing of gametogenesis, and fecundity) is available for only ~50% of all Caribbean coral species (e.g., Fadlallah 1983; Szmant 1986). Even less is known about ecological processes that are important during these species' early life stages, from larval development, to survival and behaviour, to settlement, post-settlement survival, and settler growth. Such information would contribute to a better understanding of how species-specific behaviours in early life stages contribute to the changing composition of Caribbean coral communities.

Members of the subfamily Faviinae are conspicuous and abundant reef-building species throughout the Caribbean region (Weil and Vargas 2010). Not much is known about their reproductive biology and earliest life stages, despite their high local abundance and higher current recruitment rates compared to the mid-1970s (Vermeij et al. 2011). Here we describe the reproductive biology and early life ecology of the grooved brain coral *Diploria labyrinthiformis* (Linnaeus, 1758). This species is a simultaneous hermaphrodite and one of only two known Caribbean broadcast spawning species to reproduce during the spring rather than in autumn (Alvarado et al. 2004; Weil and Vargas 2010; Muller and Vermeij 2011). Information on the earliest life stages of *D. labyrinthiformis* does not yet exist, with the exception of one study comparing settlement rates of larvae on diseased versus healthy CCAs (Quéré and Nugues 2015). To describe this species' reproductive biology, we monitored the timing of gamete release of a *D. labyrinthiformis* population *in situ* on Curaçao for eight consecutive months in 2013. We further collected and cross-fertilized gametes to document this species' embryogenesis, larval behaviour and settlement rates. Lastly, we described its early post-settlement biology in terms of settler survival, growth, onset of symbiosis, and development up to the age of 3.5 yr. Eutrophication is a factor contributing to coral reef degradation (Fabricius 2005), but the direct effects of increased nutrient availability

on a coral's physiology are not always negative and can vary among coral taxa (Shantz and Burkepile 2014). Because *D. labyrinthiformis* is increasing in abundance on eutrophic reefs on Curaçao (Vermeij et al. 2007), we assessed this species' early post-settlement survival and growth rates *in situ* under ambient and enriched nutrient conditions for a period of five months.

Materials and methods

Study site

This study was carried out on the leeward coast of the island of Curaçao (12°N, 69°W) in the southern Caribbean Sea. Monitoring for spawning activity and gamete collection were carried out at the Holiday Beach reef (12°6'25"N, 68°56'54"W) where *D. labyrinthiformis* is abundant.

Monitoring of the timing of reproduction

We documented the timing of gamete release of 40 *D. labyrinthiformis* colonies between 4 and 9 m depth along a ~200 m-long transect parallel to shore. Only colonies larger than 100 cm^2 were included to ensure all colonies had reached sexual maturity (Weil and Vargas 2010). Each colony was numbered with a cattle ear tag fixed to the adjacent substrate. Between April and October 2013, all 40 *D. labyrinthiformis* colonies were monitored daily from 1 h before sunset until sunset starting 9 days after the full moon (AFM) until 14 days AFM. Only 30 colonies were monitored in July. For each colony, we recorded the occurrence of gamete release and the time at which it began and ended.

On days when spawning occurred, schools of butterflyfishes (*Chaetodon capistratus* and *C. striatus*) were observed swimming from one *D. labyrinthiformis* colony to another and feeding on the gamete bundles they released (Muller and Vermeij 2011). In several instances, butterflyfishes were observed feeding on or picking at a colony's surface while no gametes were visible to divers, suggesting that they could detect the presence of gamete bundles inside the polyps and perhaps fed on them before they were released. To determine whether this behaviour indeed coincided with subsequent spawning of *D. labyrinthiformis*, we tracked the number of butterflyfishes that swarmed around colonies and either fed on released gametes or on the colony's surface, and noted the time at which each behaviour started and stopped. We further took video footage of schools of butterflyfishes feeding on the

bundles released by spawning colonies. From frame-by-frame analyses of this footage, we approximated visually the proportion of released bundles that were eaten before they were no longer preyed on by butterflyfishes.

Collection of gametes and larval rearing

Twelve days AFM in May 2012, we collected egg-sperm bundles from 11 haphazardly chosen *D. labyrinthiformis* colonies between 5 and 12 m depth. Spawning occurred from 45 to 15 min before sunset and egg-sperm bundles were collected by "tenting" colonies with coned-shaped nets made of plastic tarps. Bundles were collected in plastic, removable 50-mL Falcon tubes placed at the top of the nets and transported to the laboratory within 1 h of collection. Gametes from all colonies were pooled in a 2-L plastic bowl (Sterilite, MA, USA) and sperm density was adjusted to ~10^6 cells mL^{-1} by adding 0.7-µm-filtered seawater (Whatman GF/F, GE Life Sciences, PA, USA) following Hagedorn et al. (2009). Once egg-sperm bundles broke apart, fertilization was allowed to take place for 90 min after which the embryos were rinsed three times with filtered seawater in a 1-L plastic fat separator (Scandicrafts Cuisine International, USA) to remove excess sperm. This was done by pouring out seawater that contained sperm through the spout of the fat separator after positively buoyant eggs had concentrated at the surface. The embryos were then kept in filtered seawater in closed clear 2-L polystyrene containers (Dart Container Corporation, MI, USA). Following Vermeij et al. (2006), larvae density was kept low ($\leq$ 1 larva mL^{-1}) and the water in the containers was exchanged daily (~50%) to prevent the build-up of microbial communities that feed on substances released (mainly lipids) by deteriorating unfertilized eggs and/or dying larvae. The larval culture was kept at ~28°C, which was similar to the daily average sea surface temperature (SST) in May 2012 (NOAA Coral Reef Watch 2012).

Documentation of embryogenesis and larval behaviour

Immediately after fertilization, subsamples of 40 embryos were placed in six individual standard Petri dishes (10 cm diameter (Ø)) containing 40 mL of filtered sweater to document embryogenesis and larvae behaviour. Embryogenesis was documented by photographing the embryos/larvae under a dissecting microscope at various developmental stages as defined by Okubo et al. (2013). The behaviour of developing embryos and later larvae was assessed two to three times a day until day 2 after spawning (AS), and once on days 3, 4, and 6 AS by recording the number of individuals that were (1) alive, (2) floating at the surface, (3) swimming in the water column, (4) swimming on the bottom, (5) lying on the bottom, or (6) had settled. To

induce settlement and metamorphosis, a small fragment (~0.25 cm^2) of CCA (*Hydrolithon boergesenii*) was placed in the centre of each Petri dish 89 h AS. The CCA species *H. boergesenii* is known to promote settlement of *D. labyrinthiformis* larvae (Quéré and Nugues 2015).

Settlement of larvae

Three days AS, all larvae not used for documenting embryogenesis and larval behaviour were transported to the Curaçao Sea Aquarium where they were reared and settled in a land-based nursery system. This system consisted of five flow-through aquaria (215 × 69 × 64 cm, L × H × W; acrylic) which were continuously supplied with offshore seawater from the nearby reef (see chapter 6 for a detailed description of this system). Approximately 10,000 larvae were transferred to two settlement containers, each consisting of a plastic container (36 × 31 × 24 cm, L × H × W; Sterilite, MA, USA) filled with ~23 L of 50-µm-filtered seawater and containing 75 ceramic pottery tripods for settlement surfaces (kiln stilts, 6 cm Ø; Carl Jaeger Tonindustriebedarf GmbH, Germany; Supplementary Material, figure S2.1*a,b).* Tripods were previously conditioned for three months in the aquarium system to allow for the development of biofilms that help induce larval settlement and metamorphosis (Heyward and Negri 1999). Filtering the seawater through a 50-µm mesh ensured the removal of large debris and sediments while allowing smaller zooxanthellae cells (5–10 µm) that naturally occurred in the seawater to pass through. The settlement containers were partially submerged in a larger aquarium to maintain constant water temperatures (~28°C) and water inside each container was refreshed daily (~75%) with filtered seawater to maintain water quality. Water movement inside each container was created by two airlifts placed at opposite corners of the containers. A subsample of five tripods per container was inspected daily and photographed under a dissecting microscope to track settlement and metamorphosis. On day 7 AS, very few larvae remained in the water column and all the tripods with settlers were transferred to a flow-through culture system to allow for settler development and growth.

Documentation of post-settlement survival and growth

To document the survival and growth of *D. labyrinthiformis* settlers under natural conditions, we outplanted 18 tripods each harbouring 53 ± 21 (mean ± SD) 1-month-old settlers to a reef next to the Curaçao Sea Aquarium (12°04'59"N, 68°53'44"W). Prior to outplanting, the location and size (number of polyps) of each settler were recorded under a dissecting microscope. The presence of symbionts in their tissue (absent, low density, high density) was also estimated based on tissue

coloration. Clusters consisting of more than one settler were excluded from the growth analyses because fusion of two or more individuals would influence growth and survival estimates (Raymundo and Maypa 2004). The tripods were secured on the top of three plastic disks (30 cm Ø) with cable ties and transported to the reef (figure S2.1*c*). Each disk had a central 1-cm-diameter opening and was fixed to the reef at 5–6 m depth by fitting it over a steel bar (1 cm Ø; 65 cm length) that was fixed vertically into the reef (figure S2.1*c*). The disks with the tripods were brought back to the laboratory after 1, 2 and 5 months to quantify the survival and growth of each juvenile as described above. After 2 and 5 months, each juvenile's maximum diameter was also measured. After the two first surveys, the tripods were immediately returned to their original location on the reef. Because only seven tripods still harboured at least one live *D. labyrinthiformis* after 5 months, the latter were not returned to the reef but were instead kept in our aquarium facilities and observed for 3 yrs.

Assessment of post-settlement survival and growth under nutrient enriched conditions

An additional 18 tripods with *D. labyrinthiformis* settlers were mapped and outplanted similar as described above. One slow release fertilizer spike (9% total N, 12% available P; Jobes Rose Fertilizer Spike, TX, USA) was placed at the centre of each disk approximately 10 cm away from the tripods (figure S2.1*d*) to recreate nutrient-enriched conditions as in Thacker et al. (2001), who measured 10- and 5-fold increases in N and P, respectively. The fertilizer spikes were replaced every 3 to 4 weeks to ensure continuous nutrient enrichment and spikes had never completely dissolved before they were replaced. The nutrient enrichment plots were located ≥10 m away from the ambient (un-enriched) plots to avoid cross-contamination. Settler survival and growth rates were assessed as described above and compared with that of settlers grown under natural conditions.

Statistical analysis

Because increases in sea surface temperature (SST) are known to trigger gamete release in corals (van Woesik et al. 2006; Keith et al. 2016), a regression analysis was used to test whether the occurrence of spawning in *D. labyrinthiformis* could be predicted based on SSTs recorded on Curaçao in 2013. The proportion of colonies that released gametes during each monthly monitoring period (n = 8) was regressed against the average increase in SST during the previous month. A maximum likelihood (ML) approach was used to test for differences in (1) survival rates (i.e., the proportion of recruits alive at each time point relative to the initial

population) between recruits grown in ambient and nutrient enriched conditions, (2) survival rates between settlers that acquired symbionts within or later than one month after they settled, and (3) random effects among disks. We used a binomial distribution to estimate the most likely probability of survival in each treatment and their interactions at each time point. A null model with equal survival probabilities across all treatments (one-parameter model) was compared to models with unequal survival probabilities between treatment groupings (two- or three-parameter models). The best-fit values of each model were estimated based on the summed log likelihood of each model and the best combination of treatments was determined using Akaike's information criterion. Significant differences between the best fit-model and all other models with equal numbers of parameters were assessed with a post hoc comparison based on the assumption of equal Bayesian prior expectations. See Hilborn and Mangel (1997) for details on this statistical approach. After assumptions of normality and homogeneity were confirmed, two-way ANOVA followed by Tukey's post hoc pairwise comparisons tested for differences in growth rates (1) between recruits grown in ambient and nutrient enriched conditions, and (2) between settlers that acquired symbionts within or later than one month after they settled.

Results

Timing of reproduction

Of the 40 monitored *D. labyrinthiformis* colonies, 67.5% released gametes during one or more of six monthly spawning events between May and September 2013 (figure 2.1*a*). Individual colonies reproduced for a maximum of two consecutive months. A reproductive peak occurred in the spring (May–July) during which 50.0% of the population spawned, whereas 17.5% of the population spawned in late summer–early autumn (August–September) (figure 2.1*a*). Colonies that spawned during the spring did not spawn later in the year, and vice versa. The number of colonies that spawned each month was not correlated with the average increase in SST the month before (Regression analysis, $p = 0.35$; figure 2.1*a*). Spawning always occurred between 10 and 13 days AFM and peaked on days 11 and 12 AFM (80% of observations, $n = 59$; figure 2.1*a*). Gamete release (figure 2.2*a*) occurred between 52 and 2 min before sunset and sperm-egg bundles were often released in pulses. Typically, one section of the colony spawned for 5 ± 5 min

(mean ± SD) after which all gamete release stopped, then resumed after 3 to 20 min. This resulted in 1 to 3 spawning pulses per colony per day.

When spawning occurred, schools of butterflyfishes (2–50 individuals) moved from one *D. labyrinthiformis* colony to another. They remained around each colony and inspected its surface for several seconds before moving to another colony. In some cases, they started feeding on the colony's surface while no gametes were visible to divers. In 67% (n = 33) of cases where larger schools of butterflyfishes (≥ 25 individuals) started feeding on a colony's surface, gamete release occurred within 30 min, suggesting that butterflyfishes were either eating gamete bundles from inside the polyps or immediately after they were released. After release, most gamete bundles were eaten by butterflyfishes with, in most instances, less than ~10% of the bundles escaping predation.

Embryogenesis and larval development

Gamete bundles broke apart ~45 min after they were released (figure 2.2*b*). Eighty minutes AS, 95% of all eggs were fertilized and underwent their first holoblastic cleavage (figure 2.2*c*), followed by a second cleavage 30 min later (figure 2.2*d*). Cell divisions progressed quickly and at 3 h AS, embryos reached an 8- to 32-cell blastomere stage. While the first two cleavages were symmetrical, cell divisions thereafter yielded unequally-sized cells, causing the developing embryos to become irregularly shaped. Between the 2- and 16-cell stages, one third of the embryos broke apart (36 ± 13%, n = 16 pictures of 11 to 56 embryos) however the

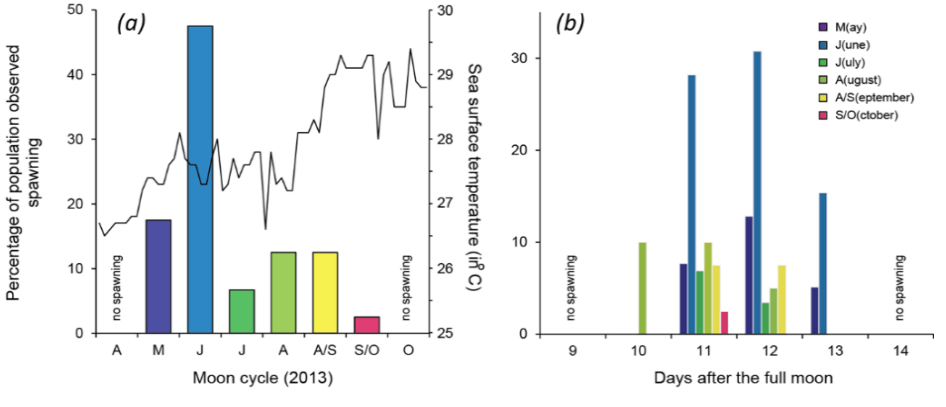

Figure 2.1. Proportion of a *Diploria labyrinthiformis* population observed spawning *(a)* each month (bars) relative to sea surface temperatures (line), and *(b)* each day relative to the lunar cycle (n = 40 colonies).

resulting cells (or clusters of cells) remained viable (figure 2.2*e*). Embryo breakage was not always symmetrical, generating smaller embryos that comprised 1 to 8 cells. Size variation in developing embryos increased at this point, but increased mortality resulting from embryo breakage was not observed (figure 2.3). Five hours AS, embryos had developed into the morula or "prawn-chip" stage (64 to 256 cells) in which embryos were flattened, but kept their irregularly-shaped appearance (figure 2.2*f*). Six hours AS, embryos obtained a concave-convex bowl shape (i.e., blastula) corresponding to the onset of gastrulation (figure 2.2*g*). Embryos became rounded again at 10 h AS and ~10% (n = 60) displayed small nodules protruding from one or two sides of their surface (figure 2.2*h*).

Larval behaviour and settlement

Ten hours AS embryos started moving for the first time; 1% of them were observed spinning at the water surface, indicating their transition from an embryo to a larval. Only 1 h later 95% of the larvae showed the same behaviour. At this point, larvae were ball-shaped and measured 308 µm ± 84 (n = 56) in diameter. Variation in larva size was large as a consequence of earlier embryo breakage observed between 2 and 5 h AS. Larvae subsequently developed into pear-shaped individuals (figure 2.2*i*) that moved away from the surface. By 13 h AS, 60% of the larvae were lying or swimming on the bottom of the rearing containers, both in the main culture and in the petri dish replicates (figure 2.3). Larvae were first observed settling at 103 h AS (i.e., 14 h after they were provided with settlement cues), with 43 ± 7% (mean ± SE) of all larvae having completed metamorphosis at that time (figure 2.2*j*, 2.3). After six days, 86 ± 3% of the initial number of larvae were alive, of which 64 ± 5% had settled (figure 2.3). The large majority of *D. labyrinthiformis* larvae settled on the undersides of the tripods (94.5 ± 8.0%, mean ± SD, n = 18 tripods). One month after settlement, polyps had fully developed tentacles and 45% (n = 1893) of all settlers had acquired zooxanthellae (figure 2.2*k*).

Post-settlement growth and development

At the time they were introduced to the reef, the 1-month-old juveniles were single polyps that measured < 1 mm in diameter. Five months later, recruit size varied considerably and ranged between 0.2 and 6.0 mm (n = 106; figure 2.2*l*). Between the ages of 3 and 6 months, primary polyps increased in diameter at an average rate of 0.21 ± 0.03 mm month^{-1} (mean ± SE, n = 106).

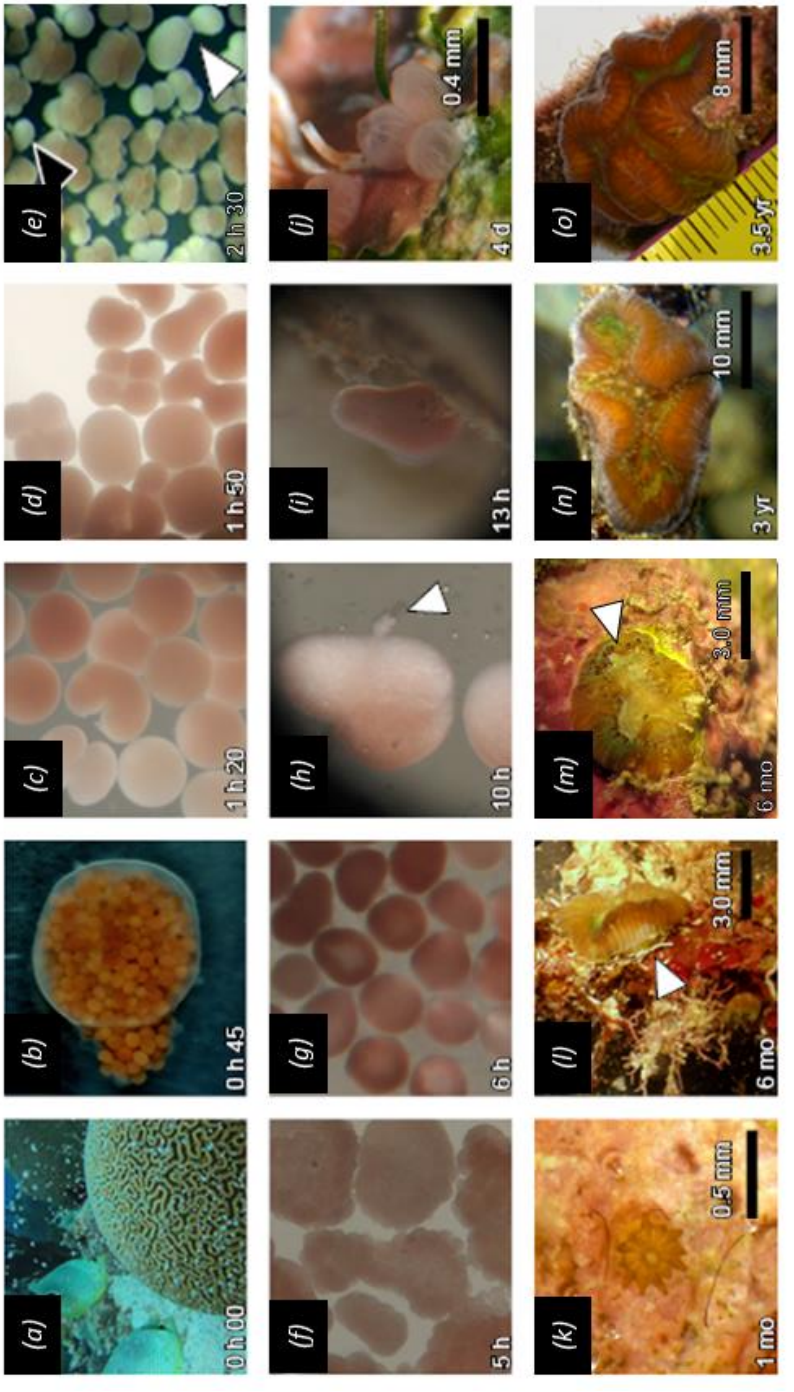
(a)
0 h 00
(b)
0 h 45
(c)
1 h 20
(d)
1 h 50
(e)
2 h 30
(f)
5 h
(g)
6 h
(h)
10 h
(i)
13 h
(j)
0.4 mm
4 d
(k)
0.5 mm
1 mo
(l)
3.0 mm
6 mo
(m)
3.0 mm
6 mo
(n)
10 mm
3 yr
(o)
8 mm
3.5 yr

Figure 2.2. Embryogenesis, larval behaviour and settlement, and early post-settlement development of *Diploria labyrinthiformis*. Colonies released sperm-egg bundles *(a)* between 57 and 2 min before sunset and between 10 and 13 days after the full moon (AFM) from May through September. Sperm-egg bundles broke apart *(b)* ca. 45 min after spawning (AS). The first holoblastic cleavage *(c)* occurred 80 min AS, followed by a second holoblastic cleavage 30 min later, resulting in a four-blastomere stage zygote *(d)*. At that point many embryos broke into smaller embryos *(e)* that remained viable. The *white arrowhead* points an ongoing embryo breakage, and the *black arrowhead* shows a single-celled embryo resulting from breakage. After 3 h, embryos were at the 8- to 16-blastomere stage and after 5 h they were at the 64-256 cell prawn-chip stage *(f)*. Six hours AS, embryos became bowl-shaped *(g)* indicating the onset of gastrulation. Embryos became rounded again after 12 h *(h)*. At that stage ca. 10% of embryos displayed small nodules protruding from their lateral sides (shown by *arrowhead*). After 13 h, embryos had fully developed into pear-shaped, motile larvae *(i)*. *(j)* 42% of larvae had settled on crustose coralline algae 14 h after they were provided with settlement cues. One-month-old settlers had fully developed tentacles and 45% had acquired zooxanthellae *(k)*. Six-month-old settlers displayed variable sizes *(l)* and grew in a plate-like form and were slightly elevated above the substrate (shown by arrowhead). The first polyp division resulted in a smaller lateral polyp *(m)* (shown by the *arrowhead*). Three-year-old colonies had divided into 2 to 4 polyps and displayed a grooved shape typical of brain corals *(n)*. A 3.5 year old recruit reached 3 cm in size and had 8 polyps *(o)*. Photos by *(a, c–j)* S Snowden, *(b)* R Villaverde, and *(k–o)* VF Chamberland.

Six-month-old recruits formed large corallites with a thick tissue layer that started folding into the groove-like pattern typical of brain corals. Recruits grew upward in a plate-like shape with their edges elevated above the substratum (figure 2.2*l*). At the age of six months, 100 of 106 surviving recruits still consisted of a single polyp, and the rest had divided into two-polyp colonies. The division of the first polyp was always asymmetrical and generated a colony with a large central polyp and a smaller lateral polyp (figure 2.2*m*). A three-year-old recruit that survived in our aquarium facilities reached a maximum diameter of 2.5 cm and consisted of four polyps (figure 2.2*n*). Polyp division rates increased rapidly thereafter; the same recruit doubled in polyp number during the following 6 months (figure 2.2*o*).

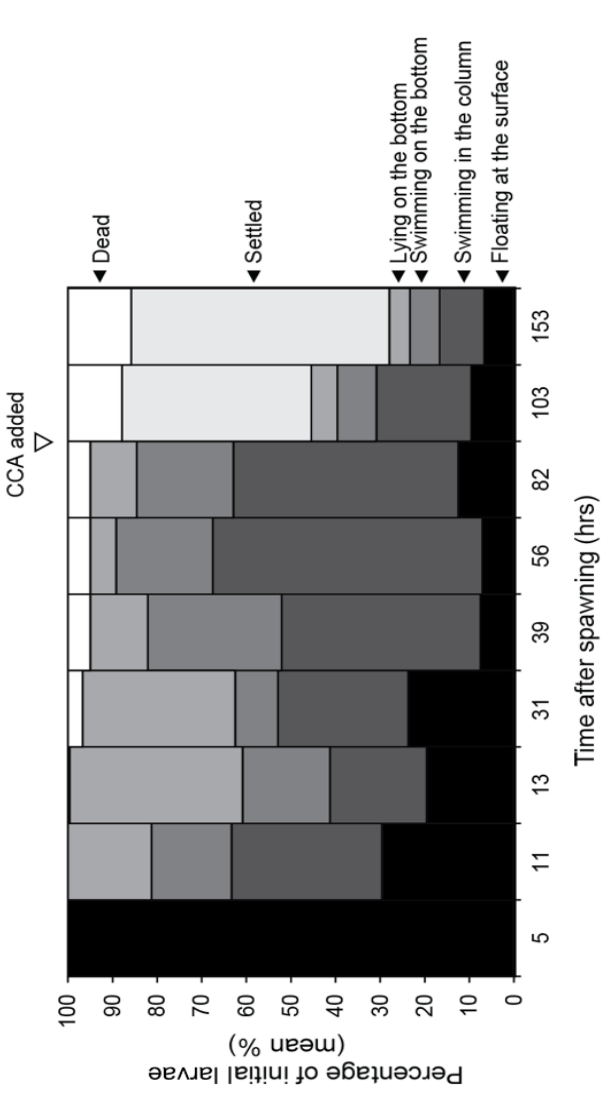

Figure 2.3. Survival, behaviour, and settlement of *Diploria labyrinthiformis* embryos and larvae during the first six days following spawning, and before and after addition of crustose coralline algae to promote settlement. *Bars* represent the percentage of the initial number of embryos (n = 40) displaying each behaviour, averaged across replicates (n = 6).

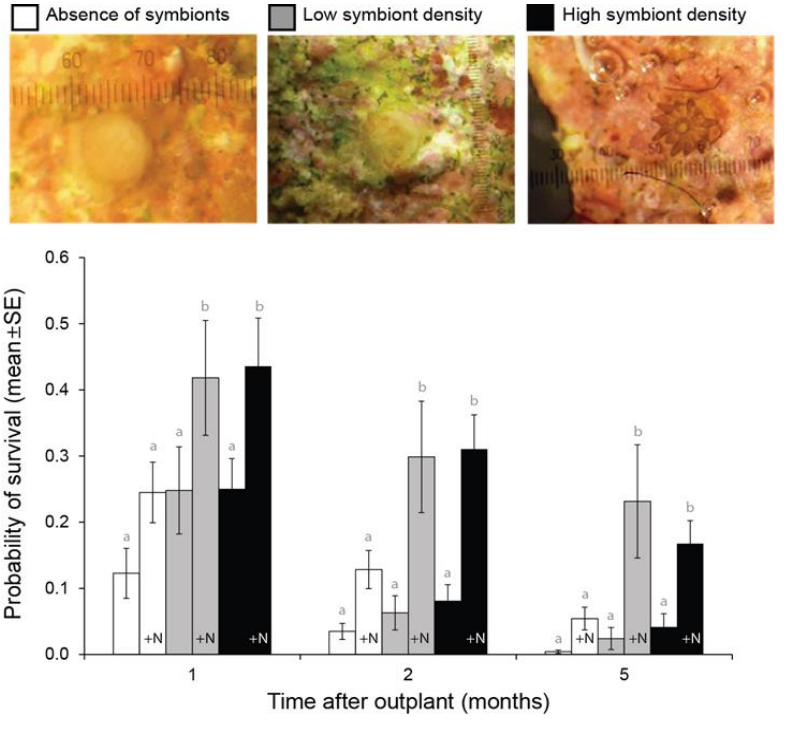

Figure 2.4. Positive and interactive effect of early symbiont acquisition and nutrient enrichment on the survivorship of *Diploria labyrinthiformis* two-week-old settlers that lacked symbionts (*clear bars*), or had acquired low (*grey bars*) or high symbiont (*black bars*) densities. *Letters above bars* indicate significantly different groups within each time point as determined by a maximum likelihood analysis with $p < 0.05$. Photos by VF Chamberland.

Post-settlement ecology: onset of symbiosis and nutrient enrichment

When settlers were transferred to the reef, symbiont densities estimated from tissue coloration varied considerably among individuals; settlers either lacked symbionts altogether (54 ± 18%) or contained low (13 ± 9%) or high densities of zooxanthellae (34 ± 36%) (mean ± SD, n = 36 tiles; figure 2.4). One month later, only 1.6% (n = 124) of the settlers still did not harbour symbionts. Settlers that acquired high densities of symbionts within one month following settlement had grown into 1.6- and 1.9-fold larger polyps 2 and 5 months after they were outplanted, respectively (two-way ANOVA, 2 months: $F_{2,225} = 15.03$, $p < 0.01$, 5 months: $F_{2,104} = 7.71$ $p < 0.01$), and were 3.7 times likelier to survive to the age of 6 months (11.4 ± 2.5%, mean ± SE) than those that lacked zooxanthellae at the time they were outplanted (2.9 ± 0.9%) (ML, two-parameter model: [absence] ≠ [low density = high density], $p < 0.01$; figure 2.4). When exposed to elevated nutrient

concentrations, recruit survival increased four-fold, but only in recruits that had acquired zooxanthellae within one month following settlement (ML, two-parameter model: [ambient] = [+N]* [absence] ≠ [+N]* [low density, high density], $p < 0.05$; figure 2.4). Nutrient enrichment did not affect settler growth rates (two-way ANOVA, 2 months: $F_{1,225} = 2.35$, $p = 0.127$, 5 months: $F_{1,104} = 0.74$, $p = 0.391$).

Discussion

Caribbean broadcast-spawning coral species typically have one gametogenic cycle per year, which, in the northern hemisphere, ends in the late summer or early autumn (August–October) during one synchronous spawning event (Fadlallah 1983; Szmant 1986). In contrast, *D. labyrinthiformis* on Curaçao released gametes during six consecutive months, with a peak in the spring and smaller reproductive events in the late summer to early autumn (figure 2.1*a*). This is the first report of a Caribbean broadcasting species with six spawning events in one year. Biannual spawning, whereby conspecifics spawn in the spring and autumn, occurs in several Indo-Pacific species. In Australia, 31% of *Acropora* species reproduce during both seasons (Gilmour et al. 2016). And similar to these *Acropora* species, *D. labyrinthiformis* colonies on Curaçao also spawned either in the spring or in the late summer–early autumn, but not both. Such reproductive isolation between sympatric conspecifics can result in genetic divergence and has been proposed as a mechanism for speciation in corals (Dai et al. 2000; Rosser 2015).

Spreading reproductive investments throughout the year has been proposed as an evolutionary strategy to escape stressors that occur either randomly or seasonally. Alvarado et al. (2004) suggested that spring spawning by *D. labyrinthiformis* in Colombia carries adaptive advantages due to the higher availability of suitable surfaces for settlement during winter and spring when algal cover is lower than at other periods of the year. Furthermore, weaker current and tide regimes during this period could contribute to higher fertilization rates. A link between spawning times and seasonal environmental factors was also proposed for corals in Taiwan (Dai et al. 2000) where offspring produced during a later reproductive peak would avoid high mortality caused by typhoons, heavy rainfall and bleaching episodes. In the Caribbean, *D. labyrinthiformis* is the only known broadcast-spawning species to spread reproductive investments over multiple spawning events within one year, equivalent to Caribbean brooding species which typically release larvae year-round (Szmant 1986). Brooding life histories are generally associated with short-lived

species that produce small numbers of offspring per brood. Szmant (1986) hypothesized that releasing larvae over multiple cycles per year rather than all at once can at least in part offset the small brood size and short life spans in brooding species. *Diploria labyrinthiformis* could have adopted this bet-hedging strategy to optimize its overall fitness by spreading its reproductive output through time to avoid occasional circumstances that could result in the complete loss of one year's reproductive investment. It remains unclear why reproduction over many months is atypical of Caribbean broadcast-spawning species, while it is common in Caribbean brooding and Indo-Pacific coral taxa.

The timing of gamete release by *D. labyrinthiformis* differs among locations throughout the Caribbean. In Bonaire, an island 60 km east of Curaçao, this species' reproductive timing is similar to that reported here (E Muller, pers. comm.). However, *D. labyrinthiformis* reproduces during the late summer in Mexico (S Snowden, pers. obs.), while in Puerto Rico (Weil and Vargas 2010) and Colombia (Alvarado et al. 2004) it spawns only during a single spawning event in the spring. These differences in reproductive timing, in terms of one versus multiple spawning events per year and the season(s) during which spawning occurs, are not related to latitude, a phenomenon observed in Western Australia where the occurrence of biannual spawning decreases towards higher latitudes (Rosser 2013). Although seasonal increases in sea temperature are known to trigger gamete release in corals (van Woesik et al. 2006; Keith et al. 2016), the reproductive peak of *D. labyrinthiformis* on Curaçao did not correlate with increases in SST in 2013 (NOAA Coral Reef Watch 2013; figure 2.1*a*). Thus, spatial differences in the reproductive timing of *D. labyrinthiformis* could be the result of other environmental cues such as photoperiod (Babcock et al. 1994), regional wind fields (van Woesik 2010), monthly rainfall (Mendes and Woodley 2002), or of internal rhythm inherited from ancestral populations (Rosser 2013).

Most corals reproduce during the night to reduce predation on gametes by diurnal plankton feeders (Westneat and Resing 1988). In contrast, several brooding sponges, ascidians, gorgonians and bryozoans release distasteful or chemically defended larvae during the day to reduce the predation risks associated with daylight spawning (Lindquist and Hay 1996). While it is unclear whether egg-sperm bundles produced by *D. labyrinthiformis* possess some form of chemical defence, they were clearly palatable to butterflyfishes. Predation by butterflyfishes dramatically reduced the number of intact gametes (~90%) reaching the surface of the water column and this appeared to be a major impediment to larva production in *D. labyrinthiformis*.

Embryogenesis in *D. labyrinthiformis* followed the general sequence of development described for other coral species (Okubo et al. 2013), with the exception that a third of the developing embryos broke apart during the first cell divisions, resulting in large numbers of smaller-sized embryos. Embryo breakage was first described for *A. millepora* in response to hydrodynamic disturbance (Heyward and Negri 2012). Resulting *A. millepora* embryos remained viable and developed into normal, although smaller, larvae and settlers. *D. labyrinthiformis* larvae generated through fragmentation also showed no signs of abnormal development and remained viable, albeit smaller than larvae that developed from non-fragmented embryos. The production of planktonic clones has been suggested as a mechanism to increase reproductive output once the critical step of fertilization is successfully accomplished (Heyward and Negri 2012). With only 3–31% of the *D. labyrinthiformis* population releasing gametes on each spawning day, and with most egg-sperm bundles being consumed by butterflyfishes upon release, embryo breakage could in part offset the reduced fertilization success associated with daytime and asynchronous spawning in this species.

Embryos of *D. labyrinthiformis* developed rapidly; after 13 h, the majority of motile larvae were negatively buoyant and 60% of the larvae were already lying or swimming on the bottom (figure 2.3). Furthermore, almost half of the larvae settled within 14 h after being provided with settlement cues (figure 2.3). Short planktonic phases and rapid settlement are traits normally associated with a brooding reproductive strategy (Harrison and Wallace 1990; Carlon and Olson 1993) and this suggests a limitation on the dispersal potential of *D. labyrinthiformis* larvae relative to other Caribbean broadcasting species (Miller and Mundy 2003). There is increasing evidence that not all broadcast-spawned larvae disperse as far as previously assumed, and that subtle species-specific differences in the duration of embryogenesis and larval behaviour can contribute to observed differences in adult distributions (Miller and Mundy 2003; Szmant and Meadows 2006; Tay et al. 2011). Larvae of the Caribbean broadcasting species *O. faveolata* develop similarly to those of *D. labyrinthiformis* (i.e., time to motility as short as 15 h AS), but spend between 50 and 75 h in the water column before they initiate settlement (Szmant and Meadows 2006). In contrast, *D. labyrinthiformis* likely has a much smaller average dispersal distance compared to *M. faveolata* as it remains planktonic for a much shorter period of time (13 h). At the other extreme, embryogenesis in *A. palmata* lasts much longer than in the aforementioned species (3.75 days) and motile larvae remain in the water column for at least 5 d and for up to 20 d before moving to the bottom for settlement (Baums et al. 2005). These differences in planktonic duration and larval behaviour clearly have important consequences for a species'

dispersal potential. Describing all coral species with external fertilization simply as broadcast spawners ignores these subtle but potentially crucial differences. A more refined classification of species based on reproductive, developmental, and early life-history would allow for a better understanding and prediction of the composition and connectivity of coral populations.

Diploria labyrinthiformis recruits had growth rates similar to those reported for other Caribbean brain corals, (≤ 2-yr-old *Colpophyllia natans:* 0.2–0.3 mm month^{-1}; and *Pseudodiploria strigosa*: 0.4 mm month^{-1}; van Moorsel 1988). *Diploria labyrinthiformis* recruits grew in a plate-like shape that was partially elevated above the substrate (figure 2.2*l*). A similar growth form was described for *Agaricia agaricites* and *C. natans* by van Moorsel (1985, 1988) who found that this growth strategy reduced competitive interactions with neighbouring organisms such as filamentous algae compared to coral juveniles that grew encrusted over the substrate. While rapid linear expansion allows recruits to quickly occupy space on the reef, three-dimensional rather than two-dimensional growth could allow young recruits to escape competition when they are most vulnerable due to their small size (Vermeij and Sandin 2008; Doropoulos et al. 2016).

Eutrophication generally impedes coral recruitment as algal growth limits space available for settlement, and increases post-settlement mortality through algal overgrowth and promotion of microbial growth resulting in anoxia (Hunte and Wittenberg 1992; Fabricius 2005; Smith et al. 2006). In light of this, four-fold greater survival of *D. labyrinthiformis* settlers under elevated nutrient concentrations relative to ambient conditions (figure 2.4) is somewhat surprising. However, 95% of *D. labyrinthiformis* larvae settled on the undersides of the tripods where the benthic community was dominated by CCA and not altered by nutrient enrichment over the course of the experiment (figure S2.2), in contrast to the community that grew on the topsides of the tripods which became dominated by algal turfs (figure S2.1*e,f*). Interestingly, the positive effect of nutrients on *D. labyrinthiformis* was dependent on the timing of zooxanthellae uptake by the settlers (figure 2.4). Settlers that acquired symbionts early in life were 3.7 times likelier to survive to the age of six months and grew twice as large as those that initiated symbiosis later in life. Nutrient enrichment promotes zooxanthellae cell growth and subsequent carbon translocation to the coral host (Tanaka et al. 2006), and a similar influence of nutrient enrichment on the growth of symbiotic vs aposymbiotic settlers was described for *Acropora digitifera* (Tanaka et al. 2013). *Acropora digitifera* settlers containing zooxanthellae that were provided with nutrients had increased growth rates and were better able to compete with benthic microalgae compared to

settlers that lacked zooxanthellae. Thus, these and our findings illustrate that nutrient enrichment does not necessarily coincide with negative consequences for coral recruitment, and highlight the importance of the onset of symbiosis early in life in corals.

Several aspects of the reproductive biology and early life ecology of *D. labyrinthiformis* described in this study are atypical of Caribbean broadcast-spawning species. While biannual reproduction of sympatric conspecifics in broadcast spawners has so far only been described for Indo-Pacific species, this phenomenon occurs in Caribbean species as well. With many reproductive events per year and a short embryogenic phase followed by rapid settlement, *D. labyrinthiformis* displays traits that are normally associated with brooding species. Our findings therefore show that early life-history characteristics are not necessarily more similar within than between classically divided coral groupings such as brooders and spawners, but that a gradual continuum likely exists with "classical" broadcast spawning and brooding species on either end.

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Supplementary material

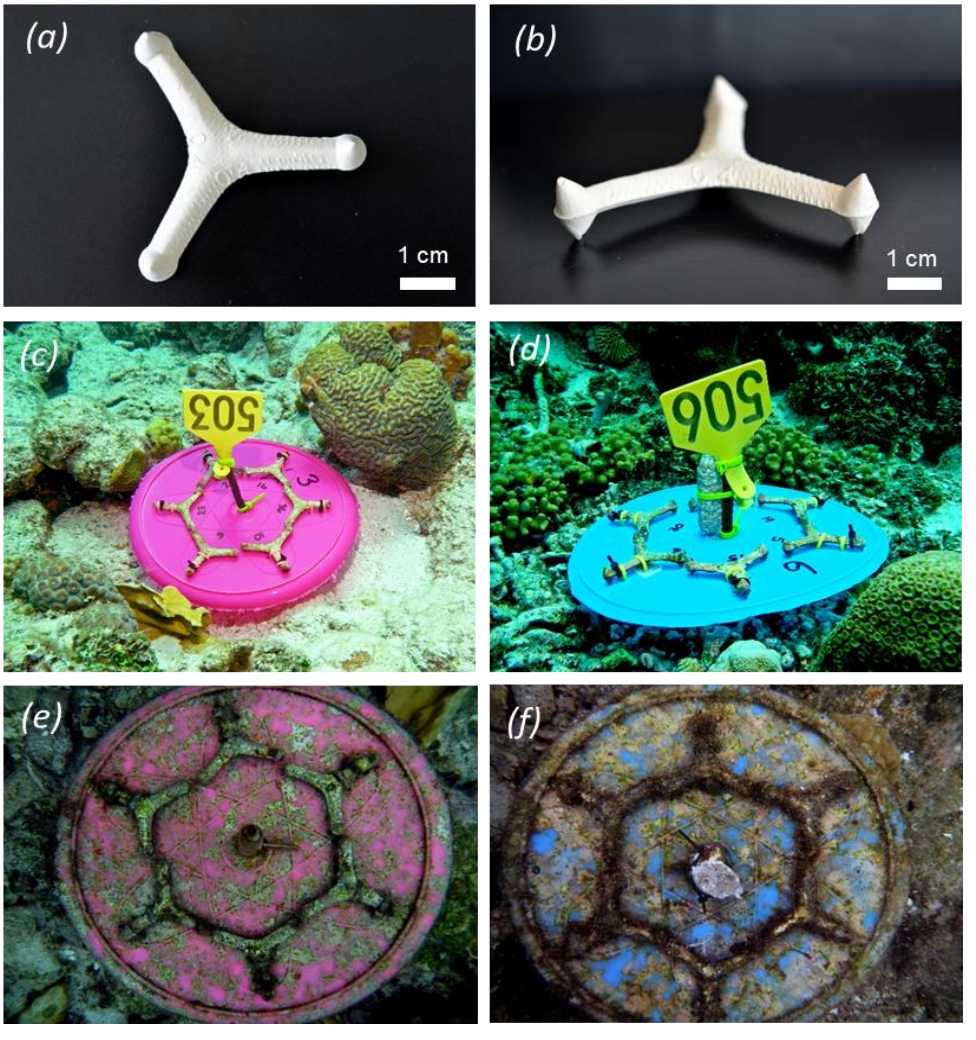

Figure S2.1. Artificial substrates used for settlement of *Diploria labyrinthiformis* larvae and to outplant the settled juveniles. A tripod from *(a)* a top view and *(b)* a side view. The plastic disks on which the tripods were attached with cable-ties were fitted over steel bars previously fixed vertically into the reef structure and were *(c)* kept under ambient conditions and *(b)* exposed to nutrient enrichment recreated by a fertilizer spike placed at the centre of the disks. The benthic community that developed on the topsides of the tripods after five months was mostly dominated *(e)* by crustose coralline algae in the ambient treatment, and *(f)* by algal turfs in the nutrient enriched treatment. Photos by *(a,b,d,f)* VF Chamberland and *(c,d)* B Mueller.

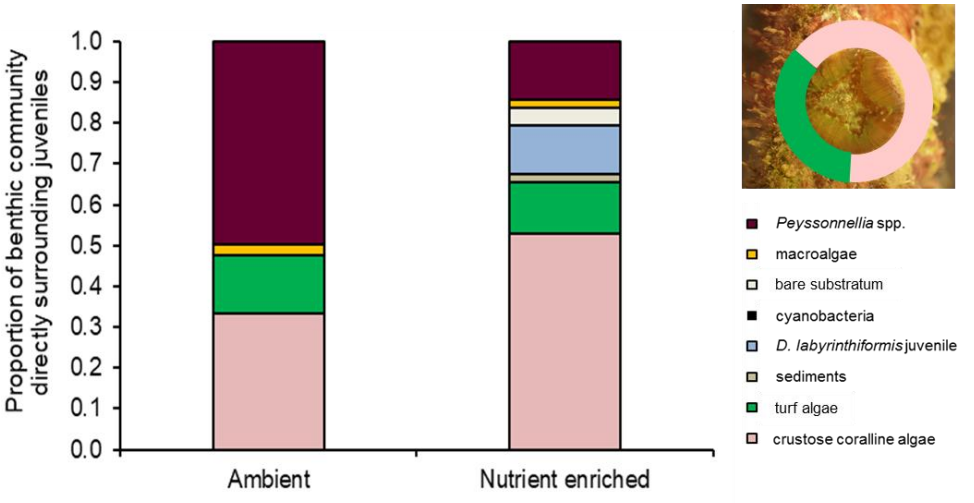

Figure S2.2. Benthic community that directly surrounded each *Diploria labyrinthiformis* juvenile after they were reared *in situ* in ambient and nutrient enriched conditions for five months. The benthic community surrounding the juveniles was quantified under a dissecting microscope and expressed as the proportion of the juvenile's diameter in contact with different benthic groups. 95% of larvae settled on the undersides of the tripods, and one month after outplanting, all live juveniles were found on the undersides of the tripods where the benthic community was not altered by increased nutrient availability (one-way ANOSIM, $R = 0.0075$, $p = 0.13$). The undersides of the tripods were dominated by crustose coralline algae and *Peyssonnellia* spp., and nutrient enrichment did not promote the growth of algal turfs nor macroalgae on the undersides of the tripods. A lager number of juveniles survived under nutrient enriched conditions and therefore grew more often in close proximity with conspecifics that settled on the same tripod.

Chapter 3

Costs and benefits of maternally inherited algal symbionts in coral larvae

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Abstract

Many marine invertebrates provide their offspring with symbionts. Yet, the consequences of maternally inherited symbionts on larval fitness remain largely unexplored. In the stony coral *Favia fragum* (Esper 1797), mothers produce larvae with highly variable amounts of endosymbiotic algae and we examined the implications of this variation in symbiont density on the performance of *F. fragum* larvae under different environmental scenarios. High symbiont densities prolonged the period that larvae actively swam and searched for suitable settlement habitats. Thermal stress reduced survival and settlement success in *F. fragum* larvae, whereby larvae with high symbiont densities suffered more from non-lethal stress and were five times more likely to die compared to larvae with low symbiont densities. These results show that maternally inherited algal symbionts can be either beneficial or harmful to coral larvae depending on the environmental conditions at hand, and suggest that *F. fragum* mothers use a bet-hedging strategy to minimise risks associated with spatio-temporal variability in their offspring's environment.

Introduction

Maternally inherited symbionts such as bacteria, viruses, protists, fungi, algae and cyanobacteria, are widespread in multicellular organisms. They are important modulators of their hosts' phenotypes as they can provide energetic resources (Muscatine and Porter 1977; Cavanaugh 1994) and protection from diseases, predators and pathogens (Haine 2008). Within-host symbiont density can further influence the hosts' phenotypes by regulating physiological processes such as rates of energy acquisition (Hoogenboom et al. 2010), and susceptibility to thermal stress (Cunning and Baker 2012). While symbiotic associations generally benefit both host and symbiont, they often represent a delicate balance that breaks down when environmental conditions change (Childress and Fisher 1992; Downs et al. 2002; Weldon et al. 2013). Yet, even in well studied host-symbiont systems (e.g., in insects), the degree to which presumed benefits from maternally inherited symbionts (and variability therein) change in response to varying environmental conditions is not well known (Hosokawa et al. 2007; Corbin et al. 2017).

Endosymbiosis is common in marine phyla such as the Porifera (Lee et al. 2001), Mollusca (Cheng 1967), Cnidaria (Muller-Parker and Davy 2001; Baker 2003), Annelida, Nematoda and Tunicata (Dubilier et al. 2008) and is found in a wide variety of marine ecosystems, such as hydrothermal vents, cold seep habitats (Dubilier et al. 2008), coral reefs (Baker 2003) and temperate waters (Sutton and Hoegh-Guldberg 1990; Muller-Parker and Davy 2001). Members of aforementioned taxa associate with various endosymbionts, such as methane- and sulfur-oxidizing bacteria (Dubilier et al. 2008), photosynthesizing single-celled algae (Venn et al. 2008), and nitrogen-fixing and photosynthesizing cyanobacteria (Carpenter 2002). These endosymbiotic partners provide their hosts with energetic resources that they are unable to obtain themselves, especially in nutrient-poor or extreme environments (Cavanaugh 1994).

A subset of species that form obligate symbioses as adults transfer those symbionts vertically to their offspring (e.g., corals: Hirose et al. 2001, sponges: Usher et al. 2001; Ereskovsky et al. 2005, ascidians : Kojima and Hirose 2012, bivalves: Krueger et al. 1996), whereby the amount of symbionts that each offspring receives, and thus the potential benefit to larval fitness, can vary greatly (Usher et al. 2001; Gaither and Rowan 2010; Roth et al. 2013). Large variation in symbiont densities among individual offspring produced by the same mother (Isomura and Nishihira 2001; Gaither and Rowan 2010; Roth et al. 2013) therefore suggests a "bet-hedging strategy" typical of mothers facing uncertainty regarding the future

environment their offspring will experience (Crean and Marshall 2009). Furthermore, symbiotic relationships in marine invertebrates are often beneficial only within a narrow range of (a)biotic conditions. Changing environmental conditions can therefore, in addition to variable symbiont provisioning, affect the fitness benefits a host receives from their endosymbionts (e.g., Mollusca, Annelida and Nematoda: Childress and Fisher 1992, Cnidaria: Downs et al. 2002; Venn et al. 2008, Porifera: Venn et al. 2008; Webster et al. 2008; Cebrian et al. 2011).

Most tropical stony coral species live in obligate symbiosis with endosymbiotic algae (*Symbiodinium* spp.). The corals provide shelter and CO_2 produced during respiration, and in turn receive photosynthates that facilitate their survival in nutrient-poor, tropical waters (Muscatine and Porter 1977). In some species, algal symbionts are transferred directly from mothers to planula larvae during embryogenesis (Hirose et al. 2001), and can fulfil up to 70% of a larva's energy demand depending on symbiont density and the availability of other resources such as lipids (Gaither and Rowan 2010; Harii et al. 2010; Kopp et al. 2016). Coral-algal symbiotic relationships are, however, extremely susceptible to temperature anomalies associated with climate change. Under high temperature and/or solar radiation, algal symbionts produce excessive amounts of reactive oxygen species causing corals to expel their symbionts to prevent tissue damage, resulting in "coral bleaching" (Downs et al. 2002). During prolonged warmer periods, corals become deprived of the energetic resources provided by their now missing endosymbionts and die (Hoegh-Guldberg et al. 2007; Baker et al. 2008). This illustrates that the presence of *Symbiodinium* can provide corals with necessary energetic resources, but that such benefit only occurs under particular environmental conditions. Yet, most research has centred on algal symbionts in adult corals. How coral larvae provided with different densities of algal symbionts will perform in different environments is still a major open question.

Here we used the stony coral *Favia fragum* (Esper 1797) to investigate the implications of variable maternal provisioning of algal symbionts on offspring performance under different environmental conditions. We first determined if trade-offs existed between a mother's symbiont investments and the size and number of offspring she produced. Secondly, we reared larvae provided with different symbiont densities under physiological (heat stress) and environmental (lack of positive settlement cues) stressors to determine under what conditions differential symbiont provisioning results in offspring fitness costs or benefits. We predicted that the benefits of endosymbiosis during the larval phase of this marine invertebrate are context-dependent, whereby larvae harbouring larger numbers of symbionts

benefit from additional energetic resources, but are also more sensitive to higher temperatures relative to larvae provided with smaller numbers of symbionts.

Methods

Study species

Favia fragum is a conspicuous coral species occurring in a wide range of shallow marine habitats (Carlon and Budd 2002) in the Western Atlantic. It releases symbiotic planula larvae between 6 and 16 days after new moon (ANM) with maximum release during day 11 ANM (Szmant-Froelich et al. 1985). *F. fragum* produces lecithotrophic larvae capable of settling immediately after release when positive settlement cues are present (Carlon and Olson 1993), and must settle before energetic resources required for settlement and metamorphosis are depleted.

Collection and rearing of *F. fragum* larvae

This study was carried out on the Caribbean island of Curaçao (12ºN, 69ºW), located 60 km north off Venezuela. Nineteen healthy (i.e., no signs of bleaching, disease or tissue discolouration) *F. fragum* colonies of similar sizes (13.9 cm^2 ± 3.3, mean ± SD) were collected five days ANM in June 2013 between 3 and 6 m depth at the Curaçao Sea Aquarium reef (12°04'59"N, 68°53'44"W) and placed individually in 1-L beakers. Each beaker was constantly supplied with 100-µm-filtered seawater to create an overflow into a semi-submerged larval collection cup with a 150-µm mesh filter bottom. Because *F. fragum* larvae are positively buoyant, the overflow caused larvae from each colony to concentrate in a separate collection cup. Both the beakers and the collection cups were partially submerged in a flow-through aquarium system at temperatures similar to natural seawater (~28°C, NOAA Coral Reef Watch 2013) for the duration of the experiment. Larvae produced by each mother during the night were collected the following morning between 7:00 and 8:00 AM and placed in a 500-mL polystyrene deli container (DART, MI, USA) filled with 300 mL of 0.7-µm-filtered seawater (Whatman GF/F, GE Life Sciences, PA), and kept at 28°C and exposed to ~12 h light natural light cycles until they were used in one of the following experiments.

Variation in size and symbiont content in *F. fragum* offspring

To determine the variability in size and symbiont content in *F. fragum* offspring, larvae produced by each colony were collected and counted daily throughout one

reproductive cycle (6-18 ANM). From the total number of larvae released by each mother each day, 20 randomly selected larvae were stored in 0.5 mL of 3.7%-formalin-filtered seawater in 1.5-mL tubes (Eppendorf, NY, USA). If a colony released less than 20 larvae in a day, the entire daily brood was sampled. To compare the size of all sampled larvae, the longitudinal and transversal axes were measured with a scale bar under a dissecting microscope and larval volume was calculated assuming the volume of a spheroid (van Moorsel 1983).

To quantify the number of symbionts contained per larva, each larva was first homogenized using a Teflon PTFE pestle (3.2-mm diameter flat tip cone; Bel-Art SP Scienceware, CA, USA). Symbiotic cells were isolated from the larval tissue by spinning the sample at 6708g for 30 min in a mini centrifuge (Eppendorf, NY, USA). Pellets were re-suspended in 30 µL of filtered seawater with 3.7% formalin and this resuspension was brought onto the counting chamber of a hemocytometer (Bright-Line; Hausser Scientific, PA, USA) to count the number of algal symbionts under a fluorescence microscope (Orthoplan; Leica, Germany). For each larva, we counted four 1 x 1 mm^2 squares with a 0.1 mm height of the hemocytometer (i.e., four 0.1 µL aliquots per larva). The four counts were averaged and converted to the total number of symbionts per larva and to the number of symbiotic cells per mm^3 of larval tissue (symbiont density).

Larval colour as proxy for symbiont density

Because larvae vary in size, symbiont density (i.e., the number of symbionts per mm^3 of larva) appears more suitable than total symbiont number to study physiological processes (Cunning and Baker 2012). *F. fragum* larvae show large variation in colouration, reflecting differences in symbiont density (Gaither and Rowan 2010; Roth et al. 2013). To ensure that differences in larval coloration could be used as a proxy for symbiont density, we assessed the density of symbionts following the methods described above in 30 beige, 30 brown and 30 dark-brown larvae released 10 days ANM. Because the number of algal symbionts per larva can change through time via cell proliferation or mortality (Gaither and Rowan 2010), 30 additional larvae belonging to each coloration group were kept in 0.7-µm-filtered seawater for ten days after which their symbiont density was reassessed to determine whether a larva's initial assignment to a coloration group could change during the course of the experiments.

Experiment 1: Effects of an extended pelagic phase

To test if larvae can delay settlement (i.e., extend their pelagic phase) when they contain higher symbiont densities, larvae from each colouration group were reared in seawater with and without settlement cues to experimentally shorten or lengthen their pelagic phase, respectively. On the day they were released, eight larvae were placed in individual 350-mL polystyrene cups (n = 6; Darnel Inc., NC, USA) containing 250 mL of 0.7-µm-filtered seawater and immediately provided with clay pottery tripods as settlement substrates (kiln stilts, 6 cm diameter; Carl Jaeger Tonindustriebedarf GmbH, Germany). These tripods were pre-conditioned in a flow through aquarium for three months prior to the experiment to allow the colonization by crustose coralline algae (CCA) known to induce larval settlement in *F. fragum* (Randall and Szmant 2009). In the second treatment, larvae of each colouration group were placed in identical cups as above (n = 6) but with 250 mL of 0.22-µm-filtered seawater (EMD Millepore, MA, USA) which delays the development of microbial communities that trigger settlement, causing larvae to not settle and remain pelagic (Vermeij et al. 2009). Seawater in both treatments was exchanged daily (~75%) and kept at 28°C.

Larval survival and behaviour were assessed daily between 8:00 and 9:00 AM for the duration of the experiment. For each surviving larva, we noted whether it lay motionless (1) at the surface or (2) on the bottom, moved around in (3) the water column, or (4) on the bottom, or (5) had settled. After five days, 15-40% of the larvae forced to remain pelagic stopped swimming and had become inactive. Therefore, the experiment was ended to ensure that a sufficient number of larvae with an extended pelagic phase survived to assess their ability to settle once provided with positive cues (see below).

Experiment 2: Effects of heat stress

To investigate if *F. fragum* larvae provisioned with different symbiont densities have different survival rates and behaviour under elevated temperatures, larvae from each colour group were reared at the average monthly mean seawater temperature in June 2013 (28.5° ± 0.3°C, mean ± SD) and at elevated temperatures (31.1° ± 0.2°C) corresponding to the coral bleaching threshold on Curaçao (NOAA Coral Reef Watch 2013). Six 35-L plastic containers (Sterilite, MA, USA) were filled with 25 L of seawater (n = 3 containers per temperature treatment) and the water temperature inside each container was regulated using a 300-W submersible water heater (Aquatop, CA, USA). The water temperature was monitored every five minutes with a temperature logger (HOBO Pro V2; Onset Computer Corporation,

MA, USA). Two water pumps placed at the opposite corners of each container ensured mixing of the water inside each container. Larvae (n = 8) were added to 15-mL conical polystyrene tubes (BD Biosciences, CA, USA) containing 14 mL of 0.7-µm-filtered seawater. Nine tubes with larvae were assigned to each of six experimental treatments (symbiont density (3) × temperature (2)).

Larval behaviour was assessed daily between 8:00 and 9:00 AM for the duration of the experiment by removing the tubes from the baths and examining the larvae with a dim light. Behavioural categories were scored as described above. Once a replicate was scored, the tube lid was removed for five seconds to allow gas exchange, closed, and randomly reassigned to a water bath of the same experimental treatment. In a similar set up, Hartmann et al. (2013) found that larval respiration did not lead to significant reductions in dissolved oxygen concentrations, and that larvae were therefore unlikely to suffer from low-oxygen stress (Hartmann et al. 2013). On day 8, less than 20% of the larvae containing high symbiont densities reared under elevated temperature were still alive. We therefore ended the experiment to ensure enough larvae remained alive in all treatments to assess if their ability to subsequently settle depended on their prior exposure to heat stress (see below).

Quantification of sub-lethal stress

Pulse amplitude modulated (PAM) fluorometry was used to determine whether *F. fragum* larvae with different symbiont densities experienced different levels of sub-lethal stress when exposed to elevated temperatures or when forced to extend their pelagic phase. The effective quantum yield ($\Delta F/F_m'$), which is a measure of the photochemical efficiency of Photosystem II of the endosymbiotic algae under ambient light, was used as a proxy for ‘‘sub-lethal stress” (Phillip and Fabricius 2003; Fai et al. 2007) and measured using a diving PAM (Walz, GmbH, Germany) fitted with a 1.5-mm-diameter mini fibre-optic cable. Measurements were performed at 9:00 AM in a dim room. Individual larvae were suspended in a small volume of seawater directly in front of the sensor and the effective quantum yield of four individual larvae was measured three times and averaged per larva. Following the methods of Putnam *et al.* (Putnam et al. 2008), the measuring light of the PAM was set to a constant intensity of “8” in “burst mode” and the gain was set to “12” to obtain F_t values between 400 and 800. Measurements were taken at day 0, 3 and 5 during experiment 1, and at day 0, 4 and 8 during experiment 2. After each time point, larvae were removed from the experiments to avoid potential stress effects caused by repetitive handling in later PAM measurements.

Latent effects on settlement success

To test if stress experienced by a larva during its pelagic phase affected its ability to settle, larvae from experiments 1 and 2 were provided with settlement substrates and subsequent settlement rates were quantified. On day 8, surviving larvae from the heat stress experiment were transferred to individual 1-L polystyrene containers (DART, MI, USA) that contained 500 mL of 0.7-µm-filtered seawater kept at 28°C, and one clay tripod as described above serving as a settlement surface. This could be replicated 9 times for larvae reared under ambient temperatures (3 to 8 larvae per container), and 7 to 8 times (2 to 6 larvae per container) for those previously exposed to elevated temperature. Settlement rates of larvae with high symbiont densities exposed to heat stress could not be assessed due to total mortality in six (out of nine) replicates during experiment 2. Survival and settlement rates were recorded daily thereafter. After day 12, larvae that had not settled no longer changed their behaviour nor settled, and the experiment was stopped at day 15. For larvae that were forced to extend their pelagic phase, one tripod was added to each replicate (n = 6) on day 5 similar to above after larval behaviour was scored, and survival and settlement rates were recorded daily until day 13, since no changes in settlement were observed after day 10.

Statistical analysis

To test for differences in larval size and in symbiont provisions within and among individual broods, we used Welch's F-tests for unequal variances followed by Bonferroni's *post-hoc* multiple comparisons because data did not meet the assumption of homoscedasticity (Levene's test, $p < 0.05$). Differences in symbiont densities among the three larval colouration groups were assessed similarly. Regression analyses were used to test if brood size (i.e., number of larvae released by a mother throughout a planulation cycle) influenced (i) the average number and density of symbionts allocated to each larva, (ii) average larval size, and (iii) if larval symbiont density varied independently of larval size. Because fecundity in clonal organisms is partly determined by the number of gravid modules in a colony (polyps in corals) (Hall and Hughes 1996), we standardized fecundity among individual *F. fragum* colonies as the number of larvae released per polyp during one planulation cycle.

Differences in larval survival and settlement between treatments for experiments 1 and 2 were compared using Kaplan-Meier (K-M) analysis followed by *post-hoc* tests in case of significant main effects. Survival curves were compared using K-M log rank tests that for each group calculate the chi-square value for each "event

time*"*, and subsequently sum the results to generate a single chi-square value used to determine if survival curves of different experimental groups differ. Death and settlement were defined as "events" whereas larvae for which event time could not be determined were included as "censored", i.e., were alive at the end of the experiment and did not die (survival analyses) or died or did not settle (settlement analyses).

In experiment 1, a maximum likelihood (ML) approach (Hilborn and Mangel 1997) was used to detect differences in larval settlement and swimming activity among treatments at experimental time points (i.e., at day 5 when CCA were added, day 13 when the experiment was ended and at day 9 as an intermediate time point between aforementioned time points). Based on a binomial error distribution, we calculated the probability of larval settlement or inactivity in each treatment at each time point. A series of models with different combinations of parameters reflecting the experimental treatments was built to identify treatment groups with significantly different means from one another. A null model with equal probabilities across all treatments (1-parameter model) was compared to models with unequal probabilities of larval settlement or inactivity between treatment groupings (2 to 6-parameter models). The best-fit values of each model were estimated and the best combination of treatment groupings was selected with Akaike's information criterion (AIC). Significant differences between the best fit-model and all other models with equal numbers of parameters were assessed with a *post-hoc* comparison based on the assumption of equal Bayesian prior expectations and with a likelihood ratio test (LRT). We compared settlement rates at day 5 among larval colouration groups (symbiont density) reared with and without CCA. Differences in settlement were expressed as the proportion of larvae that settled relative to the initial number of larvae. Differences in larval settlement success were assessed similarly, but expressed as the proportion of larvae that settled relative to the total number of larvae that were alive on the day they were provided with settlement cues. The proportion of inactive larvae was calculated as the proportion of larvae that were motionless relative to the number of larvae that were still alive and had not settled, and compared among larval colour groups for each time point after delayed and immediate addition of settlement cues. Finally, differences in effective quantum yield (experiments 1 and 2) were assessed with a two-way ANOVA followed by Tukey's *post-hoc* tests.

Results

Larval coloration as a proxy for symbiont density

Beige, brown and dark brown larvae differed significantly in symbiont density (Welch's F-test, $F_{50.3} = 798.7$, $p < 0.001$) (figure 3.1*a*). Beige larvae had low symbiont densities (mean ± SD ($n = 30$): $2.3\times10^4 \pm 0.3$ cells·mm^{-3}), brown larvae had intermediate symbiont densities ($3.6\times10^4 \pm 0.8$ cells·mm^{-3}), and dark-brown larvae had high symbiont densities ($4.7\times10^4 \pm 0.2$ cells·mm^{-3}). In all three colour groups, the symbiont density remained unchanged after 10 days (Welch's F-test, beige: $F_{52.5} = 0.5$, $p = 0.47$, brown: $F_{57.7} = 0.3$, $p = 0.59$, dark brown: $F_{56.2} = 0.6$, $p = 0.45$) (figure 3.1*a*). Of all sampled larvae ($n = 2318$), 36% hosted low symbiont densities, 33% hosted intermediate symbiont densities and 31% hosted high symbiont densities. Larval sizes were similar among the three colour groups (Welch's F-test, $F_{57.0} = 1.3$, $p = 0.29$) (figure 3.1*b*), which could therefore be used as a proxy for symbiont density in experiments 1 and 2, independently of larval size.

F. fragum fecundity and variation in larval size and symbiont content

F. fragum mothers produced different amounts of larvae ranging from 0.3 to 53.3 larvae·polyp^{-1} released by one colony throughout the planulation cycle. Larval release commenced 6 days ANM and all colonies stopped producing larvae 19 days ANM. Maximum larval release occurred between 11 and 12 days ANM (11 ANM: 0.69 ± 0.81, 12 ANM: 0.59 ± 0.47, mean larva·polyp^{-1} ± SD, $n = 19$). Variability in larval size and total symbiont number per larva was high within broods produced by each mother during one reproductive cycle (coefficient of variation (CV) ± SE: 70.5% ± 2.2 and 72.5% ± 2.7, respectively) (figure 3.2*a*,*b*). Variability in symbiont density was lower (CV: 28.2% ± 1.5) (figure 3.2*c*). Overall, larval sizes ranged from 0.014 to 0.339 mm^3 and their numbers of symbiotic cells ranged from 375 to 20475 cells·larva^{-1}, whereas symbiont density of larvae varied from 1.5×10^4 to 6.0×10^4 cells·mm^{-3} ($n = 2318$).

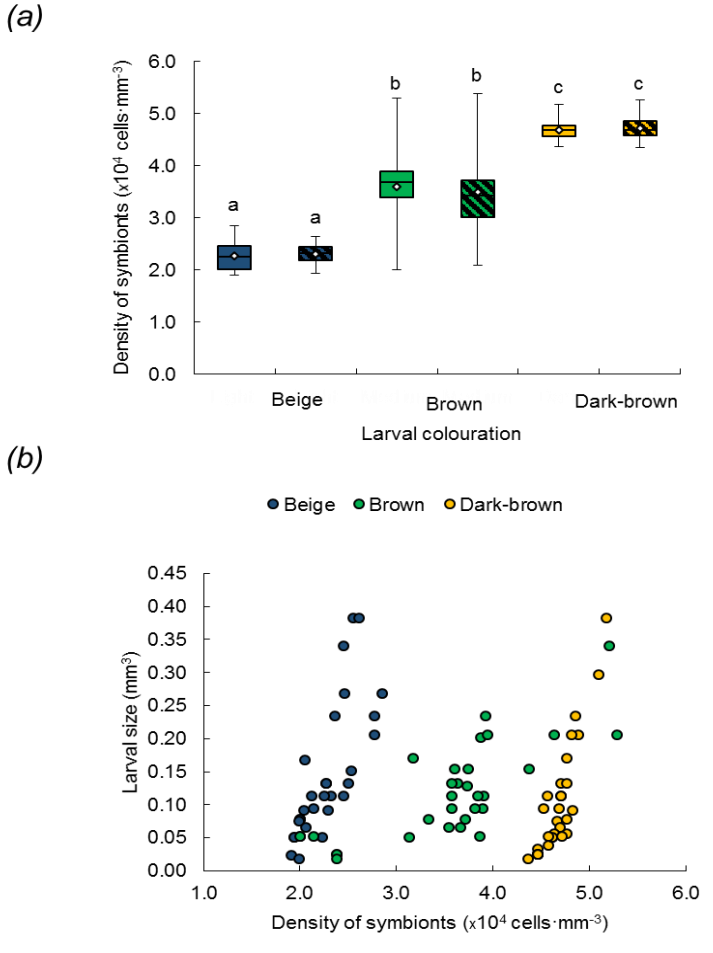


Figure 3.1. *(a)* Symbiont density in larvae sorted in three colour groups on the day they were released (*open box plots*) and ten days later (*hatched box plots*). *Upper boundaries* are 3rd quartiles (Q3), *middle boundaries* are medians, *lower boundaries* are 1st quartiles (Q1), and *white markers* are the means. *Whiskers* show the maximum and minimum values within the upper and lower limits calculated as Q3+1.5*IQR and Q1-1.5*IQR, where IQR is the interquartile range. *Bars* that do not share the same letter are significantly different, as determined with Welch's F-test followed by Bonferroni's pairwise comparisons. *(b)* Distribution of symbiont density and larval size in each of the three colour groups.

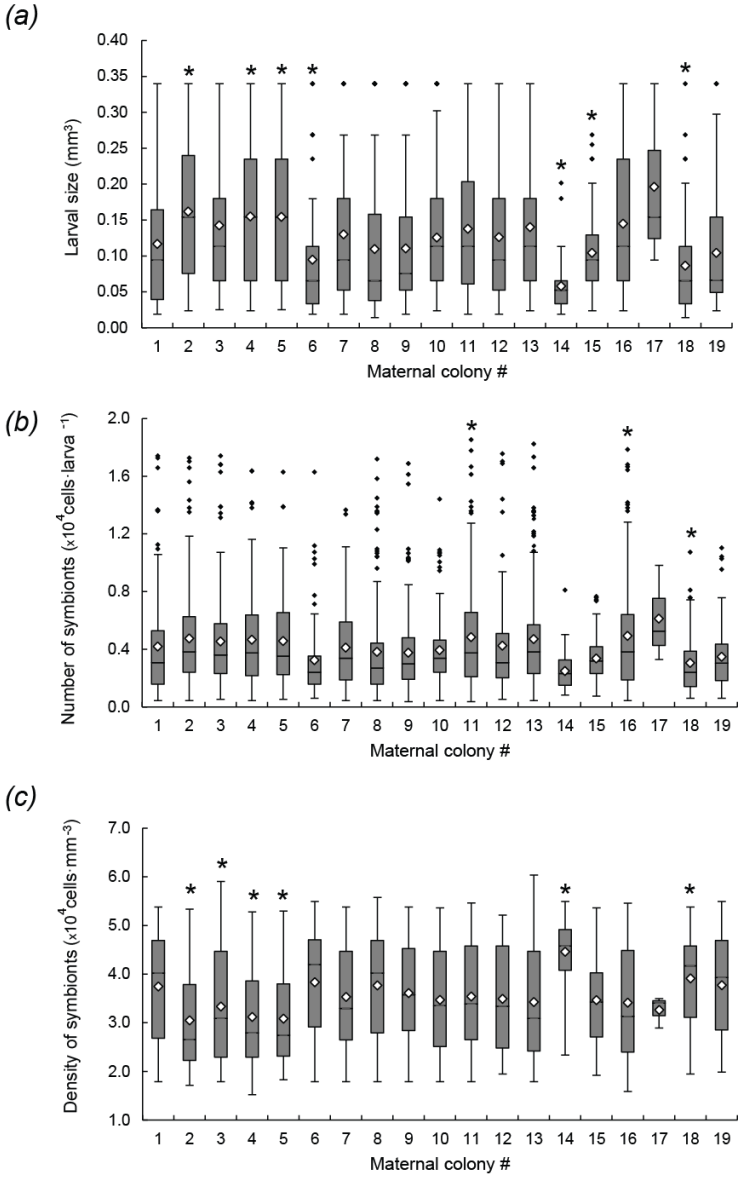


Figure 3.2. Box plots showing maternal investment in terms of *(a)* larval size, *(b)* number of symbionts per larva, and *(c)* symbiont density. *The box plots* have the same structure as in figure S1. In *(a)*, *top whiskers* for several mothers have the same maximum value due to the 0.1 mm increments of the ruler used to measure larvae under the microscope. *Black markers* are outliers. *Asterisks* denote mothers that provisioned their offspring differently than others as determined with Welch's F-test of unequal variances followed by Bonferroni's pairwise comparisons. Sample sizes vary according to the total number of larvae sampled per mother throughout their entire reproductive cycle (n = 3-184).

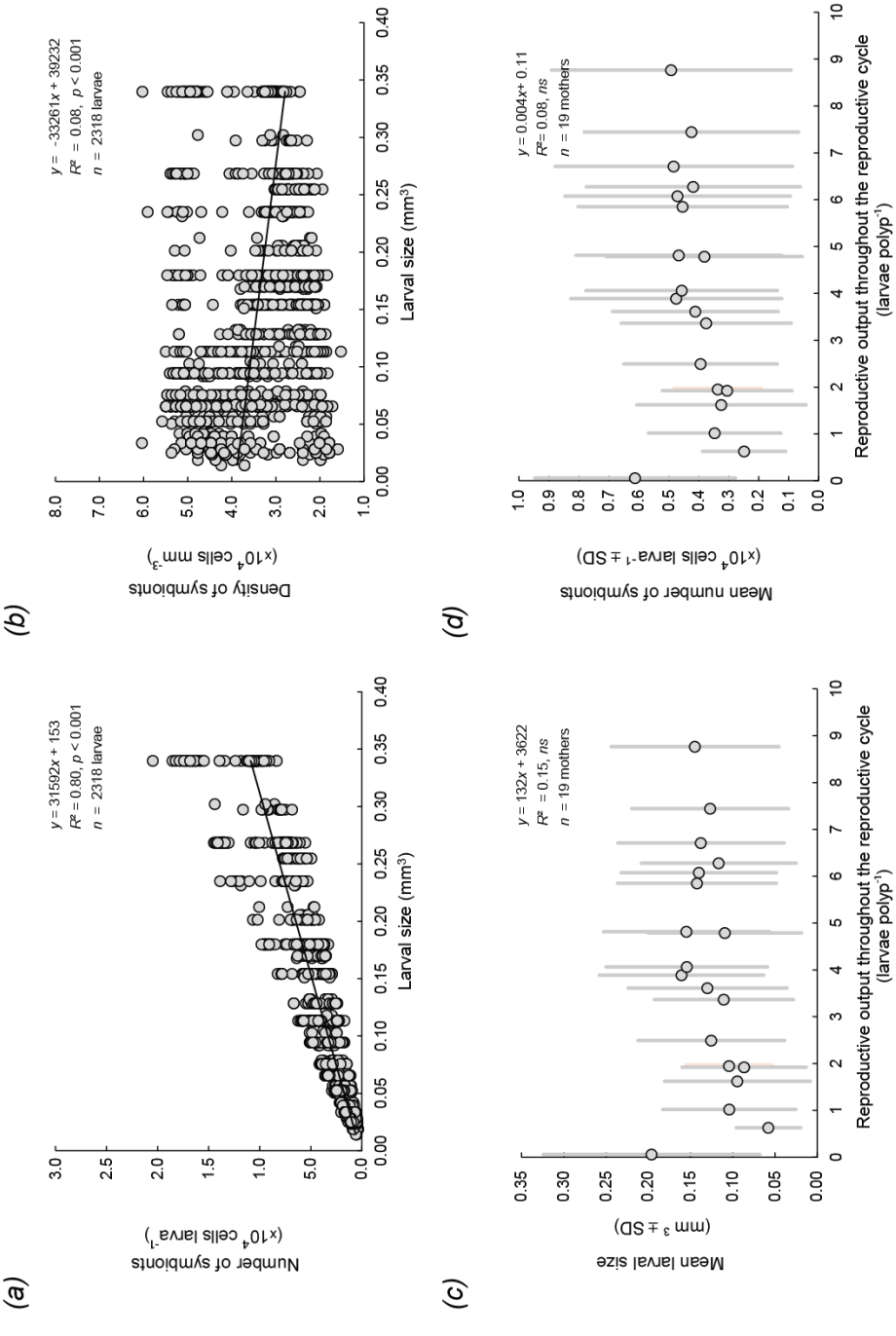
The total number of symbionts provided to individual larvae increased with larval size (R^2 = 0.8, n = 2318, p < 0.001; figure 3.3*a*). However, larval size explained only 8% of the variation in symbiont density (R^2 = 0.08, n = 2318, p < 0.001; figure 3.3*b*). Larval size, symbiont number and symbiont density showed large variation among mothers and within broods of the same mother (figure 3.2). No trade-offs existed between the total number of larvae produced by each mother during a reproductive cycle and larval size (R^2 = 0.15, n = 19, p = 0.23; figure 3.3*c*) or the number of symbionts per larva (R^2 = 0.08, n = 19, p = 0.10; figure 3.3*d*).

Effects of an extended pelagic phase

When settlement cues were provided to larvae immediately after release, those with low and intermediate symbiont densities settled faster and ultimately in higher numbers than larvae with high symbiont densities (K-M log rank test, χ^2_2 = 5.0, p < 0.05; figure 3.4*a*, table S3.1 in supplementary materials) (day 13: ML, three-parameter model, p < 0.05; table S3.2). Non-settled larvae with high symbiont densities remained more active (swimming) towards the end of our experiment compared to larvae with low symbiont densities (day 10 to 12: ML, two-parameter model, p < 0.05; table S3.3) of which 67% had become inactive by day 10 (figure 3.4*b*).

The withholding of CCA as settlement cues forced *F. fragum* larvae to delay settlement and extend their pelagic phase. After 5 days, only 4% of all larvae without settlement cues had settled whereas 54% of all larvae with settlement cues had settled by that time (day 5: ML, three-parameter model, p < 0.05; figure 3.4*a*, table S3.2). Delaying settlement slightly reduced larval mortality (by 1.5%) over the entire experimental period (K-M log rank test, χ^2_1 = 4.1, p < 0.05). After two days without settlement cues, larvae containing low symbiont densities became 2 to 15 fold less active than those with high symbiont densities (day 3 to 5: ML, two-parameter model, p < 0.05; figure 3.2*c*, table S3.3). When provided with settlement cues, low symbiont density larvae gradually resumed swimming and were equally active as those with intermediate and high symbiont densities by day 7 (day 7 to 13: ML, null-model; figure 3.4*c*).

Figure 3.3. Analysis of *(a)* number of algal symbionts and *(b)* symbiont density in coral larvae of different sizes, and investigation of possible trade-offs between the total number of larvae produced by each mother during a reproductive cycle and *(c)* mean larval size and *(d)* the mean number of symbionts per larva.


(a)
y = 31592x + 153
R² = 0.80, p < 0.001
n = 2318 larvae
Number of symbionts
(x10⁴ cells larva⁻¹)
Larval size (mm³)
(b)
y = -33261x + 39232
R² = 0.08, p < 0.001
n = 2318 larvae
Density of symbionts
(x10⁴ cells mm⁻³)
Larval size (mm³)
(c)
y = 132x + 3622
R² = 0.15, ns
n = 19 mothers
Mean larval size
(mm ³ ± SD)
Reproductive output throughout the reproductive cycle
(larvae polyp⁻¹)
(d)
y = 0.004x + 0.11
R²= 0.08, ns
n = 19 mothers
Mean number of symbionts
(x10⁴ cells larva⁻¹ ± SD)
Reproductive output throughout the reproductive cycle
(larvae polyp⁻¹)

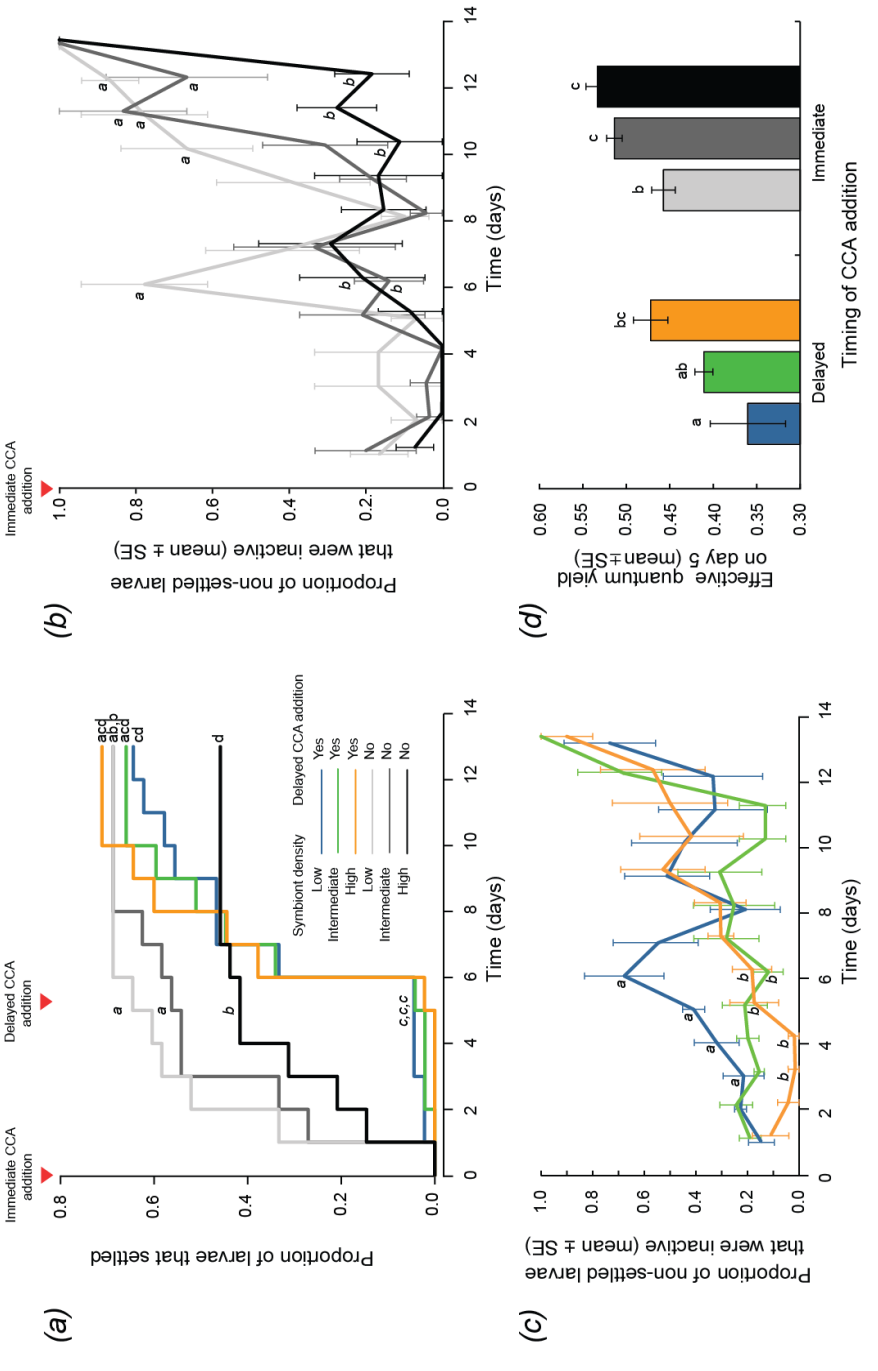

(a)
Proportion of larvae that settled
Time (days)
Immediate CCA addition
Delayed CCA addition
Symbiont density
Delayed CCA addition
Low Yes
Intermediate Yes
High Yes
Low No
Intermediate No
High No
acd
abcb
acd
cd
a
a
b
d
c,c,c
(b)
Proportion of non-settled larvae that were inactive (mean ± SE)
Time (days)
Immediate CCA addition
(c)
Proportion of non-settled larvae that were inactive (mean ± SE)
Time (days)
(d)
Effective quantum yield on day 5 (mean±SE)
Delayed
Immediate
Timing of CCA addition
a
ab
bc
b
c
c

Figure 3.4. Effects of symbiont density on activity and settlement of coral larvae. *(a)* Settlement rates of coral larvae with different symbiont densities when crustose coralline algae (CCA) were provided either immediately upon release or delayed to day 5 (n = 6 replicates). *(b)* Proportion of non-settled larvae that ceased to swim when provided with settlement cues immediately upon release. *(c)* Proportion of non-settled larvae that ceased to swim when forced to extend their pelagic phase. *(d)* Effective quantum yield of symbionts at day 5 in non-settled coral larvae that were provided with CCA either immediately upon release or at day 5 (n = 4 larvae per treatment). In *(a)* and *(d)*, significantly different treatment groups are shown by *non-italicized letters*, as tested with Kaplan-Meier's analysis and a two-way ANOVA, respectively. *Italicized letters* in *(a)*, *(b)* and *(c)* indicate significant differences at individual time points, as tested with a maximum likelihood approach, and are exclusively shown for treatment groups that differed.

The algal symbionts of non-settled larvae forced to delay settlement were more stressed at day 5 than the symbionts of non-settled larvae provided with settlement substrates immediately after release (two-way ANOVA, $F_{1,18}$ =44.2, $p < 0.001$; figure 3.4*d*, table S3.4). Furthermore, sub-lethal stress was higher in larvae with low symbiont densities than in larvae with high symbiont densities (two-way ANOVA, $F_{2,18} = 17.6$, $p < 0.001$; figure 3.4*d*, table S3.4).

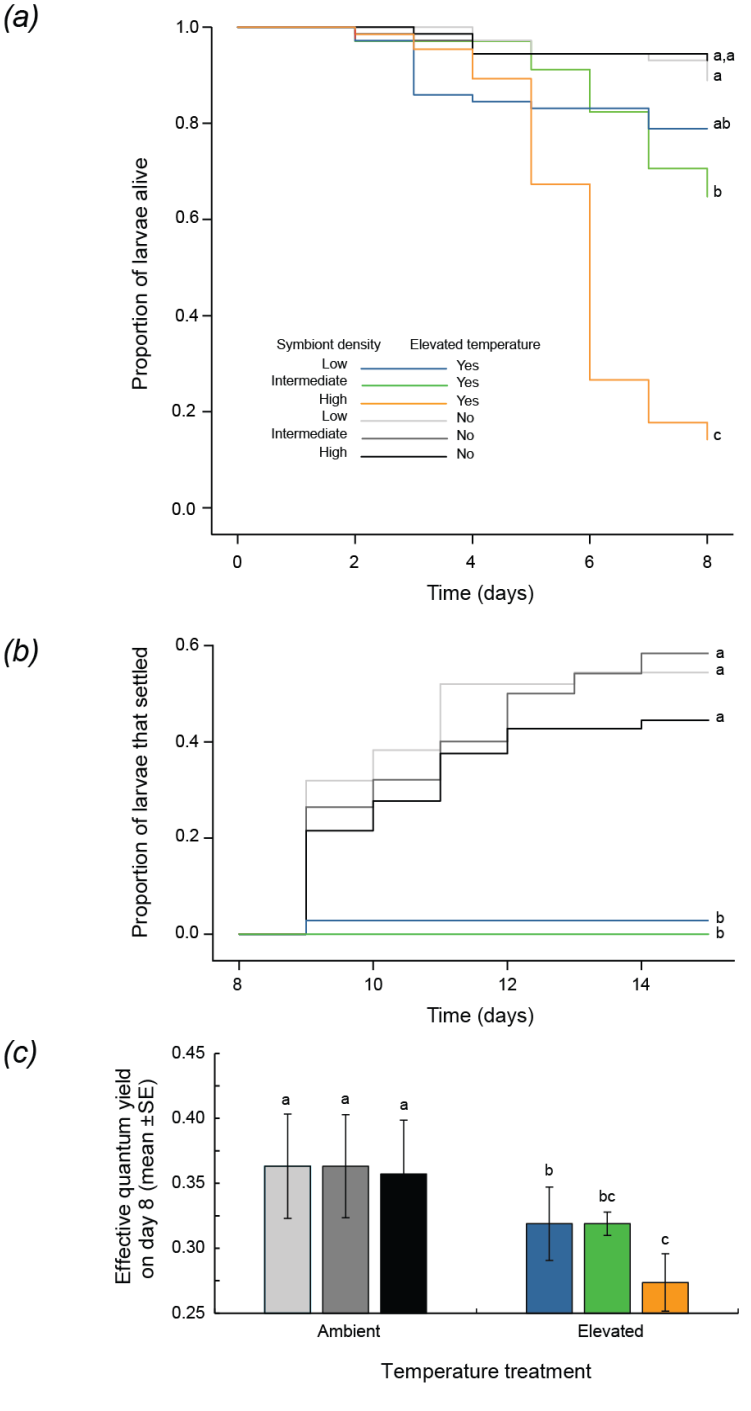
We did not detect latent effects in the form of reduced settlement success caused by an extended pelagic phase in *F. fragum* larvae, independent of differences in symbiont density. When larvae initially reared without CCA as settlement cues were provided with CCA on day 5, they initiated settlement within 24 h and, by day 9, had settled in equal numbers as larvae provided with settlement cues immediately after birth (day 9: ML, null model; figure 3.4*a*, table S3.2).

Effects of heat stress

Elevated temperature reduced survival of *F. fragum* larvae and larvae containing high symbiont densities were five times less likely to survive under elevated temperature compared to larvae with low symbiont densities (K-M log rank test, $\chi^2_1 = 45.5$, $p < 0.001$; figure 3.5*a*, table S3.5). Elevated temperature also inhibited settlement of coral larvae independent of symbiont density (K-M log rank test, $\chi^2_1 = 14.9$, $p < 0.001$; figure 3.5*b*, table S3.6). In addition, physiological stress levels (indicated by lower effective quantum yield of the algal symbionts) was higher in larvae kept at elevated temperatures, particularly if the larvae contained high symbiont densities (two-way ANOVA, $F_{2,11} = 4.2$, $p < 0.05$; figure 3.5*c*, table S3.7). Thus, while the direct negative effects of thermal stress on *F. fragum* larvae were

more severe for larvae hosting high symbiont densities (figure 3.5*a,c*), latent effects caused by thermal stress resulted in low settlement rates for all *F. fragum* larvae, regardless of the density of symbionts they contained (figure 3.5*b*).

Figure 3.5. Effects of symbiont density on coral larvae exposed to heat stress. *(a)* Survival rates of larvae with different symbiont densities at ambient versus elevated temperature, plotted throughout their pelagic phase (n = 9 replicates). *(b)* Settlement rates of larvae after they were relieved from heat stress and provided with CCA as settlement cues at day 8 (ambient: n = 9 replicates with 3 to 8 larvae, elevated: n = 7 to 8 replicates with 2 to 6 larvae). Note that the treatment combining elevated temperature and high symbiont densities is excluded because too many larvae had died in this treatment during the preceding experiment. *(c)* Effective quantum yield of symbionts at day 8 in non-settled coral larvae exposed to ambient versus elevated temperature (n = 4 larvae per treatment). *Lines* and *bars* that do not share the same letter are significantly different, as tested with *(a,b)* Kaplan-Meier analyses and *(c)* a two-way ANOVA.


(a)
Proportion of larvae alive
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
Time (days)
a,a
a
ab
b
c
Symbiont density
Elevated temperature
Low
Intermediate
High
Low
Intermediate
High
Yes
Yes
Yes
No
No
No
(b)
Proportion of larvae that settled
0.6
0.4
0.2
0.0
8
10
12
14
Time (days)
a
a
a
b
b
(c)
Effective quantum yield on day 8 (mean ±SE)
0.45
0.40
0.35
0.30
0.25
a
a
a
b
bc
c
Ambient
Elevated
Temperature treatment

Discussion

Differential provisioning of symbionts by mothers could be of fundamental importance for their offspring's fitness since symbionts can provide their larval host with energy resources. We used the brooding coral *F. fragum* as a model species to examine the ecological consequences of potential trade-offs in larval characteristics that result from variable symbiont provisioning. We found that larger per-offspring symbiont investments by coral mothers incurred both costs and benefits depending on the environmental context experienced by the offspring.

Absence of trade-offs between brood size and per-offspring investment

Studies on maternal provisioning often focus on a possible trade-off between the number of offspring a mother produces and her per-offspring resource allocation, whereby a larger reproductive output results in smaller per-offspring investments (Smith and Fretwell 1974). Negative relationships between offspring number and size are indeed widespread in both plants and animals (e.g., reptiles: Ford and Seigel 1989, insects: Fox et al. 1997, plants: Aarssen and Jordan 2001, fish: Williams 2001), but there is increasing evidence that this trade-off is not as ubiquitous as previously assumed. In many taxa no correlation between offspring number and size exists, and in some cases positive correlations were even observed (Venable 1992; Bernardo 1996). In our study, we also found no correlation between the number of larvae produced by *F. fragum* mothers and their per-offspring allocation of resources, neither in terms of larval size nor symbiont number (figure 3.3*c,d*). Hence, our results provide additional evidence supporting that mothers are not always bound to a compromise between total reproductive output and per-offspring investments.

Larger symbiont provisions allow for prolonged larval activity

Forcing *F. fragum* offspring to extend their pelagic phase did not reduce their survival or ability to settle (figure 3.4*a*). Within a week, however, it led to physiological stress and inactivity in larvae with low symbiont densities (figure 3.4*c,d*). In contrast, larvae with intermediate and high symbiont densities maintained a higher photosynthetic efficiency, possibly resulting from benefits associated with self-shading among algal symbionts (Roth 2014), and swam continuously during the first week (figure 3.4*c,d*). Active swimming represents the largest energetic cost during a marine invertebrate's larval stage (Marshall et al. 2003a), and in symbiotic coral larvae, this energy may be derived from lipid catabolism (Harii et al. 2007), photosynthetic products supplied by their

endosymbionts (Harii et al. 2010), or both. Large lipid reserves, for example, allow ascidian larvae to search for high-quality settlement habitats for a longer period of time compared to smaller conspecifics (Marshall and Keough 2003). By ceasing to swim, *F. fragum* larvae with low symbiont densities, and therefore likely less energetic resources, could reduce their energy demand to avoid exhaustion and mortality at the cost of encountering favourable habitats for settlement. In environments where positive settlement cues are scarce, coral larvae with small symbiont provisions are therefore expected to be less successful in finding optimal habitats for settlement and post-settlement survival. When provided with settlement cues immediately upon release, larvae with high symbiont densities swam longer (figure 3.4*b*) and settled at lower rates than those with lower symbiont densities (figure 3.4*a*). Lower immediate rates of settlement coupled with active swimming and general lower physiological stress in larvae with high symbiont densities (figure 3.4*d)* suggests that larger symbiont provisions enables larger dispersal distances and promote population connectivity (Goodbody-Gringley et al. 2010), but only when settlement cues were present. When settlement substrates were initially withheld such "dispersal behaviour" disappeared (figure 3.4*a*). By delaying settlement, larvae risk passing their competency period and, while alive, can no longer settle and metamorphose (Nishikawa and Sakai 2005). Larvae in other species have often been observed to separate out in a group that settles relatively quick after release and another group of larvae that settles much later (Wilson and Harrison 1998). Such phenomenon would be in agreement with our aforementioned suggestion that these larvae actively explore habitats outside their native range, though it is not possible, within the context of this study, to definitively confirm this scenario as we did not test if non-settled larvae had retained their ability to settle and metamorphose at the end of our experiment.

High symbiont densities can become harmful to the host

Heat stress exerted direct negative effects on *F. fragum* larvae (figure 3.5), in particular in larvae that hosted higher densities of symbionts, as they had a lower photosynthetic efficiency and survival rate (figure 3.5*a*,*c*). When larvae were relieved of thermal stress, latent effects were widespread. Nearly all *F. fragum* larvae that had been exposed to heat stress failed to settle, regardless of symbiont density (figure 3.5*b*). Hartman *et al.* (Hartmann et al. 2013) observed similar patterns in *Agaricia humilis,* another Caribbean brooding coral that produces symbiotic larvae. In this species, large larval size (indicative of larger lipid reserves) did not counteract the negative latent effects of heat stress, resulting in low settlement success equivalent to that of smaller larvae. In contrast, aposymbiotic

larvae produced by broadcast spawning coral species suffered no or only small direct and latent effects from exposure to heat stress compared to symbiotic larvae (Yakovleva et al. 2009; Hartmann et al. 2013). Heat stress is largely mediated via the algal symbionts which produce excessive amounts of reactive oxygen species at elevated temperatures (Downs et al. 2002). Thus, despite benefits in terms of energy provisioning, harbouring symbiotic algae lowers the ability of coral larvae to tolerate higher temperatures, whereby larval susceptibility to heat stress increases with symbiont density.

Mixed benefits of symbiont provisioning in different environments

Our finding that maternally inherited symbionts can be both beneficial and harmful to larvae contrasts with the common, almost invariably positive effects of larger maternal lipid provisioning (i.e., larger sizes) on the performance of marine invertebrate offspring (Marshall and Keough 2008). Larger sized, non-feeding marine invertebrate larvae typically can search for suitable settlement surfaces for longer periods of time, show increased post-metamorphic survival and growth, are stronger competitors for space, attain sexual maturity faster, and are more fecund than smaller conspecifics (e.g., marine snails: Moran and Emlet 2001, bryozoans: Marshall et al. 2003a; Marshall and Keough 2008, ascidians: Marshall et al. 2006). While the aforementioned benefits appear straightforward when considered in terms of lipid provisions alone, marine invertebrate larvae that also contain symbionts could experience physiological stress, as found in this study, due to altered symbiont performance under certain environmental conditions (e.g., Mollusca, Annelida and Nematoda: Childress and Fisher 1992, Cnidaria: Downs et al. 2002, Porifera: Webster et al. 2008; Cebrian et al. 2011). For example, we hypothesize that in an environment where *F. fragum* larvae risk depleting their energy reserves before they settle due to e.g., a scarcity of suitable settlement habitats, smaller larvae are at a disadvantage due to limited energetic reserves that can be derived from lipid catabolism. If they contain high symbiont densities, they can compensate for this lack of resources by using energy derived from algal photosynthates resulting in equal settlement success as larger larvae that host low symbiont densities. However, during warmer periods, larvae harbouring high symbiont densities, regardless of size, are prone to oxidative damage so that large larvae with high symbiont densities can be expected to perform worse than smaller larvae provisioned with low symbiont densities. In this scenario, the benefits of larger maternal lipid contributions could be enhanced by large symbiont provisions, but also offset by the disadvantages associated with high symbiont densities under certain environmental contexts. Future studies in which larvae are not only separated according to

symbiont density (this study), but also according to size are needed to conclusively confirm this hypothesis.

Bet-hedging as a strategy in variable environments

In marine invertebrate larvae, size variability is highest for lecithotrophic species with internal fertilization (Marshall and Keough 2008). Offspring size and symbiont content in *F. fragum* was remarkably variable (figure 3.2*a,b*), greatly exceeding the variability found in many other invertebrates, including many coral species (Marshall and Keough 2008; Gaither and Rowan 2010). As a result, *F. fragum* larvae of all sizes could contain all ranges of symbiont densities (figure 3.3*b*), thus generating a diversified pool of phenotypes mixing these two traits. The dynamic bet-hedging theory predicts that such variability in offspring phenotypes benefits populations or species growing in fluctuating and unpredictable environments (Crean and Marshall 2009). *F. fragum* occurs in tidal pools, shallow reefs and seagrass beds, which are known for high spatial and temporal fluctuations in (a)biotic parameters (e.g., light, habitat availability, temperature, distance between conspecifics, water-movement) (Carlon and Budd 2002). Therefore, we hypothesize that the extreme variability in maternal provisioning in *F. fragum* is indeed a response to the high level of unpredictability of this species' environment. Because large symbiont provisions can come with both costs and benefits depending on the environmental context, producing larvae with a wide range of symbiont densities prevents the complete loss of one reproductive cycle investment in the advent of harsh environmental conditions. Thus, *F. fragum* mothers appear to minimize risks associated with spatio-temporal uncertainty in their offspring's environment by producing larvae of all possible combinations of sizes and symbiont densities, whereby each combination can benefit or negatively impact larval performance depending on the environmental conditions at hand. In addition to gradual adaptation over multiple generations, variation in maternal symbiont provisions could act as another pathway by which corals prove capable of responding to environmental stressors such as environmental degradation and global warming.

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Supplementary material

Table S3.1. Summary of Kaplan-Meier analysis of differences in settlement of larvae containing different densities of symbionts in response to immediate and delayed additions of settlement cues: *post-hoc* results (pairwise Log Rank (Mantel-Cox) comparisons). Significant results are underlined.

			χ2	*p*	χ2	*p*	χ2	*p*	χ2	*p*	χ2	*p*
Withholding of settlement cues?	*Symbiont density*		II		III		IV		V		VI	
Yes	Low	I	3.88	0.049	0.04	0.835	5.96	0.015	0.44	0.506	0.28	0.599
No	Low	II			3.81	0.051	0.28	0.596	2.79	0.095	4.96	0.026
Yes	Medium	III					5.97	0.015	0.22	0.643	0.35	0.553
No	Medium	IV							4.76	0.029	6.68	0.010
Yes	High	V									0.78	0.378
No	High	VI										

Table S3.2. Summary of maximum likelihood analyses output of differences in settlement success of larvae containing different densities of symbionts in response to immediate and delayed additions of settlement cues (extended pelagic phase). The best-fit model was identified based on Akaike's information criterion (AIC) score and thereafter compared with other models with an equal number of parameters using a likelihood ratio test (LRT).

Model type	ML	df	AIC	LRT against best-fit model	Parameters
Larval settlement at day 5					
Null model	94.03	0	96.04	n.a.	[All variables]
Two-parameter model	42.11	1	46.11	p = 0.09 (not different from best-fit)	[Immediate], [Delayed]
Three-parameter model	92.10	2	98.10	n.a.	[Low], [Intermediate], [High]
Three-parameter model	39.84	2	45.84	best-fit	[Immediate*(Low+ Intermediate)], [Immediate*High], [Delayed]
Six-parameter model	39.27	5	51.27	n.a.	[Immediate*Low], [Immediate*Intermediate], [Immediate*High], [Delayed*Low], [Delayed*Intermediate], [Delayed*High]
Larval settlement at day 9					
Null model	58.34	0	60.34	best-fit	[All variables]
Two-parameter model	58.27	1	62.27	n.a.	[Immediate], [Delayed]
Two-parameter model	56.59	1	60.59	n.a.	[Immediate*(Low+ Intermediate)+Delayed], [Immediate*High]
Three-parameter model	57.45	2	63.45	n.a.	[Low], [Intermediate], [High]
Three-parameter model	55.64	2	61.64	n.a.	[Immediate*(Low+Intermediate)], [Immediate*High], [Delayed]
Six-parameter model	55.12	5	67.12	n.a.	[Immediate*Low], [Immediate*Intermediate], [Immediate*High], [Delayed*Low], [Delayed*Intermediate], [Delayed*High]
Larval settlement at day 13					
Null model	65.98	0	67.98	n.a.	[All variables]
Two-parameter model	65.63	1	69.63	p = 0.07 (not different from best-fit)	[Immediate], [Delayed]
Two-parameter model	63.09	1	67.09	best-fit	[Immediate*(Low+ Intermediate)+Delayed], [Immediate*High]
Three-parameter model	65.15	2	71.15	n.a.	[Low], [Intermediate], [High]
Six-parameter model	62.82	5	74.82	n.a.	[Immediate*Low], [Immediate*Intermediate], [Immediate*High], [Delayed*Low], [Delayed*Intermediate], [Delayed*High]

Table S3.3. Summary of maximum likelihood analyses output of differences in swimming behaviour of larvae containing different densities of symbionts, in response to immediate and delayed additions of settlement cues (extended pelagic phase). The best-fit model was identified based on Akaike's information criterion (AIC) score and thereafter compared with other models with an equal number of parameters using a likelihood ratio test (LRT). Output is shown exclusively for time points at which the null model was rejected.

Model type	ML	df	AIC	LRT against best-fit model	Parameters
Immediate CCA addition					
Larval inactivity at day 6					
Null model	32.771	0	34.771	n.a.	[All groups]
Two-parameter model	30.625	1	34.625	$p < 0.01$	[Low + Intermediate], [High]
Two-parameter model	25.674	1	29.674	best-fit	[Low], [Intermediate + High]
Two-parameter model	30.819	1	34.819	$p < 0.01$	[Low + High], [Intermediate]
Three-parameter model	25.564	2	31.564	n.a.	[Low], [Intermediate], [High]
Larval inactivity at day 10					
Null model	24.228	0	26.228	n.a.	[All groups]
Two-parameter model	20.638	1	24.638	$p = 0.09$ (not different from best-fit model)	[Low + Intermediate], [High]
Two-parameter model	18.391	1	22.391	best-fit	[Low], [Intermediate + High]
Two-parameter model	23.974	1	27.974	$p < 0.01$	[Low + High], [Intermediate]
Three-parameter model	17.915	2	23.915	n.a.	[Low], [Intermediate], [High]
Larval inactivity at day 11					
Null model	26.478	0	28.478	n.a.	[All groups]
Two-parameter model	20.127	1	24.127	best-fit	[Low + Intermediate], [High]
Two-parameter model	25.637	1	29.637	$p < 0.01$	[Low], [Intermediate + High]
Three-parameter model	19.696	2	25.696	n.a.	[Low], [Intermediate], [High]

Table S3.3.Continued.

Model type	ML	df	AIC	LRT against best-fit model	Parameters
Larval inactivity at day 12					
Null model	25.21	0	27.21	n.a.	[All groups]
Two-parameter model	18.30	1	22.30	best-fit	[Low + Intermediate], [High]
Two-parameter model	22.036	1	26.036	$p < 0.05$	[Low], [Intermediate + High]
Two-parameter model	23.841	1	27.841	$p < 0.01$	[Low + High], [Intermediate]
Three-parameter model	18.036	2	24.036	n.a.	[Low], [Intermediate], [High]
Delayed CCA addition					
Larval inactivity at day 3					
Null model	22.91	0	24.91	n.a.	[All groups]
Two-parameter model	18.66	1	22.66	best-fit	[Low + Intermediate], [High]
Two-parameter model	21.09	1	25.09	$p = 0.08$ (not different from best-fit model)	[Low], [Intermediate + High]
Two-parameter model	47.88	1	51.88	$p < 0.05$	[Low + High], [Intermediate]
Three-parameter model	18.41	2	24.41	n.a.	[Low], [Intermediate], [High]
Larval inactivity at day 4					
Null model	30.01	0	32.01	n.a.	[All groups]
Two-parameter model	23.418	1	27.418	best-fit	[Low + Intermediate], [High]
Two-parameter model	25.645	1	29.645	$p = 0.1$ (not different from best-fit model)	[Low], [Intermediate + High]
Two-parameter model	30.001	1	34.001	$p < 0.01$	[Low + High], [Intermediate]
Three-parameter model	22.332	2	28.332	n.a.	[Low], [Intermediate], [High]
Larval inactivity at day 5					
Null model	33.163	0	35.163	n.a.	[All groups]
Two-parameter model	31.875	1	35.875	$p = 0.09$ (not different from best-fit model)	[Low + Intermediate], [High]
Two-parameter model	29.55	1	33.55	best-fit	[Low], [Intermediate + High]
Three-parameter model	29.514	2	35.514	n.a.	[Low], [Intermediate], [High]
Larval inactivity at day 6					
Null model	38.532	0	40.532	n.a.	[All groups]
Two-parameter model	36.483	1	40.483	$p < 0.001$	[Low + Intermediate], [High]
Two-parameter model	24.873	1	28.873	best-fit	[Low], [Intermediate + High]
Two-parameter model	32.3	1	36.3	$p < 0.01$	[Low + High], [Intermediate]
Three-parameter model	24.451	2	30.451	n.a.	[Low], [Intermediate], [High]

Table S3.4. Summary of two-way ANOVA analysis on effective quantum yield data of larvae containing different densities of symbionts in response to immediate and delayed additions of settlement cues on day 5, and subsequent *post-hoc* tests (Tuckey). Significant results are underlined.

Two-way ANOVA: main effects

Source	SS	df	MS	F	Sig.
Withholding settlement cue (WSC)	0.043	1	0.043	44.210	0.000
Symbiont density (SD)	0.034	2	0.017	17.600	0.000
WSC x SD	0.002	2	0.001	1.160	0.336
Error	0.017	18	0.001		

Two-way ANOVA: *post-hoc* comparisons

Withholding of settlement cues?	*Symbiont density*		II	III	IV	V	VI
Yes	Low	I	0.218	0.001	0.004	0.000	0.000
Yes	Medium	II		0.122	0.363	0.003	0.001
Yes	High	III			0.982	0.486	0.149
No	Low	IV				0.181	0.041
No	Medium	V					0.966
No	High	VI					

Table S3.5. Summary of Kaplan-Meier analysis of differences in survival of larvae containing different densities of symbionts in response to ambient and elevated seawater temperatures: *post-hoc* results (pairwise Log Rank (Mantel-Cox) comparisons). Significant results are underlined.

Temperature	*Symbiont density*		χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
			II		III		IV		V		VI	
Ambient	Low	I	1.30	0.254	0.69	0.406	3.03	0.082	11.46	0.001	80.44	0.000
Ambient	Medium	II			0.10	0.749	7.39	0.007	17.99	0.000	84.75	0.000
Ambient	High	III					6.14	0.013	16.17	0.000	83.69	0.000
Elevated	Low	IV							2.408	0.121	45.48	0.000
Elevated	Medium	V									42.18	0.000
Elevated	High	VI										

Table S3.6. Summary of Kaplan-Meier analysis of differences in settlement of larvae containing different densities of symbionts in response to ambient and elevated seawater temperatures: *post-hoc* results (pairwise Log Rank (Mantel-Cox) comparisons). Significant results are underlined. Note that the treatment combining elevated temperature and high symbiont densities is excluded because too many larvae had died in this treatment during the preceding experiment.

	Symbiont		χ2	*p*	χ2	*p*	χ2	*p*	χ2	*p*
Temperature	*density*		II		III		IV		V	
Ambient	Low	I	0.001	0.974	1.338	0.247	20.28	0.000	20.35	0.000
Ambient	Medium	II			1.573	0.210	22.13	0.000	21.74	0.000
Ambient	High	III					14.87	0.000	15.28	0.000
Elevated	Low	IV							0.871	0.351
Elevated	Medium	V								
Elevated	High	VI								

Table S3.7. Summary of two-way ANOVA analysis on effective quantum yield data of larvae containing different densities of symbionts exposed to ambient and elevated temperatures on day 8, and subsequent *post-hoc* tests (Tuckey). Significant results are underlined.

Two-way ANOVA : main effects

Source	SS	df	MS	F	Sig.
Temperature (TEMP)	0.023	1	0.023	10.590	0.004
Symbiont density (SD)	0.008	2	0.004	4.170	0.050
TEMP x SD	0.001	2	0.001	0.322	0.729
Error	0.077	18	0.002		

Two-way ANOVA : *post-hoc* results

Temperature	*Symbiont density*		II	III	IV	V	VI
Ambient	Low	I	0.830	0.711	0.404	0.182	0.013
Ambient	Medium	II		0.999	0.972	0.793	0.140
Ambient	High	III			0.994	0.8936	0.206
Elevated	Low	IV				0.944	0.445
Elevated	Medium	V					0.753
Elevated	High	VI					

Chapter 4

Light, nutrients and herbivory affect early algal succession and coral settlement rates on a Caribbean reef

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This chapter is in preparation for submission

Abstract

On coral reefs, opportunities for successful settlement of coral larvae are of key importance for the long-term survival of coral populations. With increasing eutrophication of coastal waters and overfishing of herbivores however, open space is often rapidly occupied by fleshy algae that may diminish opportunities for coral settlement. In this study, grazing pressure and nutrient concentrations were manipulated *in situ* to investigate how these factors affect algal community succession in light-exposed and cryptic habitats. Coral settlement in response to these communities was then studied *ex situ* with larvae of the brooding coral *Favia fragum* (Esper, 1797). Algal communities harboured four times more turf algae in light-exposed habitats than in cryptic habitats, and four times less crustose coralline algae (CCA) and remaining open space. On light-exposed surfaces, reduced herbivory resulted in a 1.8-fold increase in turf algae cover and, in combination with nutrient enrichment, also promoted macroalgal growth. In contrast, in cryptic habitats, reduced herbivory led to a 1.5-fold decrease in CCA cover and increased the amount of bare substratum. Settlement rates of coral larvae were similar in all nutrient-herbivory treatments when larvae were offered a single algal community, and therefore had no choice. Thus, settlement surfaces were always available to coral larvae, even in communities dominated by fleshy algae. When provided the choice, however, *F. fragum* larvae preferred settling in cryptic habitats dominated by CCA and bare substratum. Furthermore, on light-exposed surfaces, larvae also preferentially settled in microhabitats with bare substratum while avoiding turf algae. Herbivory and nutrient levels influenced larval preferences for specific algal communities, but those preferences differed depending on successional stage: while algal communities grown under nutrient enrichment and/or reduced herbivory were most conducive to coral settlement at primary successional stages, communities grown under natural reef conditions were preferred at intermediate and advanced successional stages. Our findings highlight how light exposure, reduced herbivory and nutrient enrichment affect algal community succession, with a negative effect of turf algae but positive effects of CCA and open space on coral settlement.

Introduction

Anthropogenically impacted reefs often experience higher than natural nutrient concentrations, and decreased grazing of benthic algae due to the overfishing of herbivorous fishes (Bellwood et al. 2004). Overfishing weakens the relative contribution of top-down regulation as a structuring factor in communities of primary producers, whereas nutrient enrichment increases the relative importance of bottom-up regulation (Hay 1981; Walker and Ormond 1982). In coral reef communities where space to grow or settle is generally limiting, reef building organisms (corals and crustose coralline algae (CCA)) have to compete with fleshy algae (macroalgae and turf algae) for this limiting resource (Barott et al. 2012). Nutrients and herbivores are important drivers of the outcome of this competition whereby fleshy algae generally outcompete reef building organisms on overfished reefs, and the abundance of macroalgae further increases in eutrophied waters (Smith et al. 2010).

The Relative Dominance Model (Littler and Littler 1984; Littler et al. 2006) has arguably been the most successful in explaining how the relative abundance of calcifying corals and algae, and fleshy macroalgae and turf algae changes within coral reef communities under varying levels of herbivory (top-down regulation) and nutrient availability (bottom-up regulation). Under heavy grazing by herbivores, the abundance of calcifying benthic organisms increases, and the relative abundance of CCA further increases at high nutrient levels (figure 4.1). Under reduced grazing levels, the abundance of all fleshy algal species increases, and the relative abundance of macroalgae versus turf algae increases with nutrient enrichment (Wanders 1977; Burkepile and Hay 2006, 2008). Numerous studies have linked reduced herbivory and increased nutrient concentrations to phase shifts, whereby coral communities shift from coral- to fleshy algae-dominated systems, thus supporting the predictions of the Relative Dominance Model (Hughes 1994; Bellwood et al. 2004; Hoegh-Guldberg et al. 2007).

Algae are generally the first to colonize space that opens up within reef communities due to storms, hurricanes and (accidental) predation, and subsequently (≥100 days) develop into late-successional communities (Wanders 1977; Steneck and Dethier 1994; McClanahan 1997; Fricke et al. 2011). During the earliest stages of algal community succession (< 40 days), ephemeral species belonging to the *Chlorophyta* and crustose coralline species can temporarily dominate, after which the composition of developing algal communities often shifts to that predicted by the Relative Dominance Model (Wanders 1977; Hixon and Brostoff 1996; Smith et

al. 2001; Fricke et al. 2011) depending on environmental factors such as light availability, hydrodynamics, upwelling, and seasonal changes in temperature (McClanahan 1997; Roth et al. 2015; Doropoulos et al. 2016). In late successional communities, CCA dominate cryptic environments that receive less light than exposed reef surfaces preferred by most fleshy algae (Adey and Vassar 1975; Dethier 1994; Kendrick 1991; Mallela 2007; Steneck and Dethier 1994; Wanders 1977). Corals generally settle near the edges (< 1.5 cm) in cryptic habitats from which little growth brings them to full light required for colony growth (Arnold et al. 2010).

The proportion of settling coral larvae that will successfully recruit on the benthos depends on the abundance and composition of local algal communities (Birrell et al. 2005, 2008; Doropoulos et al. 2016). Turf algae and macroalgae produce specific chemicals and promote pathogenic microorganisms contributing to higher mortality rates and reduced settlement success of coral larvae (Kuffner et al. 2006; Vermeij et al. 2009). Furthermore, these fleshy algae pre-empt space and can overgrow recently settled corals, further reducing recruitment success. In contrast to fleshy algae, CCA or CCA-associated bacteria produce chemicals that induce settlement and metamorphosis in coral larvae (Morse and Morse 1996; Negri et al. 2001; Webster et al. 2004; Hadfield 2011). Coral larvae actively search for suitable settlement locations on the benthos (Morse et al. 1994; Morse and Morse 1996; Raimondi and Morse 2000; Baird et al. 2003; Harrington et al. 2004; Vermeij 2006) and prefer open space or areas dominated by certain CCA species, and avoid settling in habitats dominated by fleshy algae (e.g., Arnold et al. 2010; Arnold and Steneck 2011; Birkeland 1977; Ritson-Williams et al. 2016, 2009; Vermeij 2006).

Assuming that settling coral larvae search for habitats characterized by open space or covered by CCA, potentially suitable habitats for successful larval recruitment include (1) recently opened space, (2) early successional algal communities that are still dominated by CCA, (3) CCA-dominated cryptic habitats and (4) CCA-dominated late successional algal communities that formed in response to increased nutrient availability and high grazing regimes as predicted by the Relative Dominance Model. However, experimental studies linking the interactive effects of herbivory and nutrient levels on algal community succession with settlement opportunities for coral larvae on these algal communities are rare. This study therefore aimed to quantify the effects of herbivory and nutrient availability on the successional dynamics of dominant Caribbean algal groups in both cryptic and light-exposed habitats. Subsequently, we tested how the resulting algal communities affected recruitment success of a common Caribbean coral

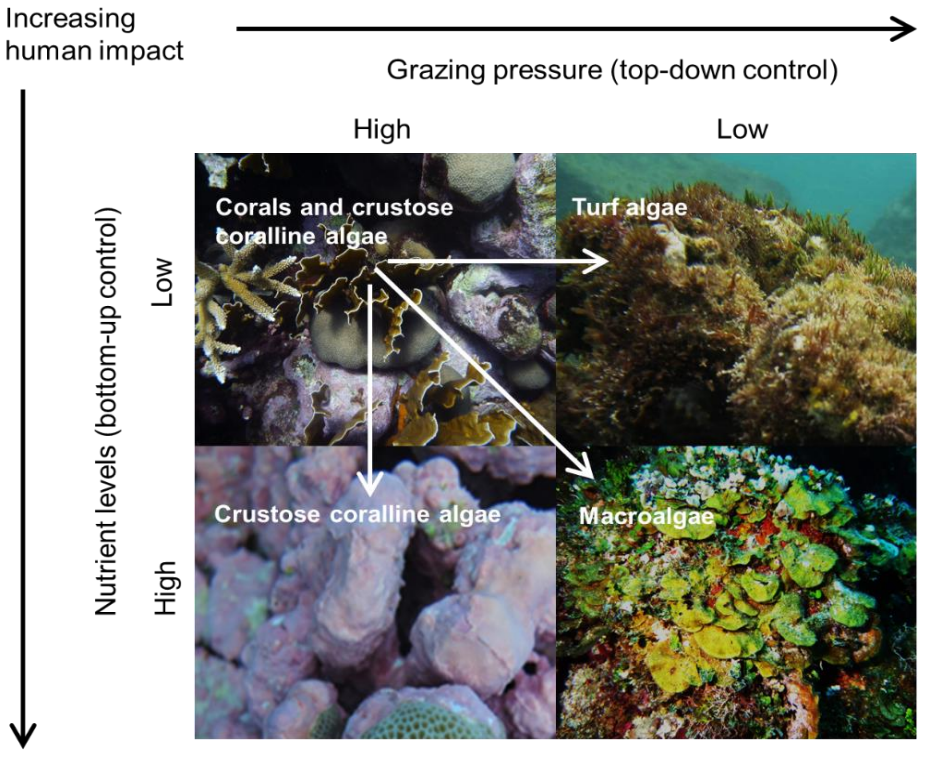


Figure 4.1. Schematic representation of the Relative Dominance Model, adapted from Littler et al. (2006). Under high grazing activity, hard corals and crustose coralline algae (CCA) are predicted to dominate, whereas CCA are expected to be at a competitive advantage when nutrient levels are high. Under reduced herbivory, turf algae are predicted to dominate. Nutrient enrichment in combination with reduced herbivory is expected to lead to higher abundances of macroalgae. Photos by VF Chamberland, MJA Vermeij, C Carruthers and J Flower.

species. Specifically, we sought to answer the following questions: (i) How does decreased herbivory and nutrient enrichment, separately and interactively, alter the composition of algal communities in light-exposed and cryptic habitats developing on bare substrates? (ii) To what degree does the resulting relative abundance of major functional algal groups (turf algae, macroalgae and CCA) influence the settlement success of coral larvae? (iii) Can varying levels of herbivory, nutrient availability and light exposure indirectly shape coral recruitment by influencing the relative abundance of dominant algal groups known to facilitate or impede coral settlement? To address the aforementioned questions, artificial settlement tiles were

conditioned *in situ* under different herbivory and nutrient levels, and the resulting development of benthic algal communities on the tiles' light-exposed uppersides and cryptic undersides was monitored. Algal communities grown under different nutrient and herbivory levels at the same successional stage, or grown under the same environmental conditions but at different successional stages, were used in choice experiments with settling larvae of the brooding coral *Favia fragum* (Esper 1797) to quantify their habitat preferences and settlement success.

Materials and methods

Study site

This study was carried out between December 2013 and March 2014 on Curaçao (12ºN, 69ºW), a Southern Caribbean island located 60 km north off Venezuela. The experiment was conducted at the Sea Aquarium reef (12°04'59"N, 68°53'45"W), a relatively healthy site characterized by intermediate coral cover (28%), low turf algae (23%) and macroalgae (12%) cover, and a decent herbivorous fish community (54 g m^{-2}) (Blue Halo, Waitt Institute, unpub. data) (figure 4.2*a*,*b*).

Experimental design

The effects of nutrients and herbivory on the succession of benthic algal communities were studied using a randomized factorial design. Twelve 80 × 80 cm patches of coral rubble were selected as experimental plots along a 100-m-long transect between 7 and 10 m depth, avoiding sandy areas and well-developed algal and coral communities. Three experimental plots were randomly assigned to each of four nutrient-herbivory treatments: (1) natural reef conditions ($N^{-}H^{+}$), (2) nutrient enrichment ($N^{+}H^{+}$), (3) herbivory reduction ($N^{-}H^{-}$) or (4) both ($N^{+}H^{-}$). One of the $N^{+}H^{+}$ plots was however excluded during the study because territorial threespot damselfish (*Stegastes planifrons*) created a territory in the plot, resulting in an abnormally high abundance of turf algae and visible sediment trapping, known to severely impact settlement success in corals (Birrell et al. 2005; Arnold et al. 2010).

Herbivory by large-bodied fish and invertebrates was reduced by covering relevant experimental plots with cages (80 × 80 × 30 cm, L × W × H) made of chicken wire (mesh size Ø 4 cm) and PVC tubing (Ø 2.5 cm). Cages were secured to the reef framework by hammering 90-cm-long steel rods through the opened,

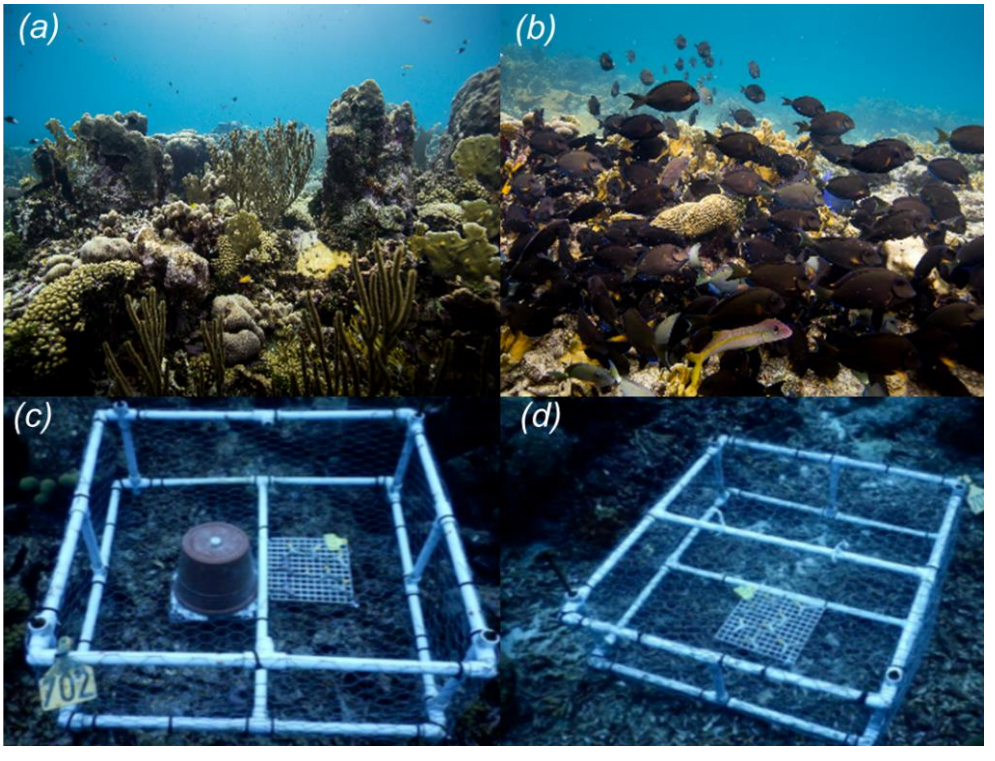


Figure 4.2. Impression of the study site and experimental plots at the Curaçao Sea Aquarium reef. *(a)* The benthic community is characterized by intermediate coral cover and low algal abundance. *(b)* Large schools of blue tangs (*Acanthurus* spp.) were often observed grazing at the site. Examples of experimental plots with *(c)* opened cages and nutrient enrichment and *(d)* and closed cages without nutrient enrichment. Photos by *(a,b)* P Selvaggio and *(c,d)* L Röpke.

upright, PVC pipes in each corner of the cages. To control for caging artefacts, similar cages were installed at all other experimental plots but with an open roof to allow herbivore access (Connell 1997) (figure 4.2*c,d*). Cages were cleaned bi-weekly for the duration of the experiment.

Unglazed Ø 20 cm terracotta pots were filled with 1.6 kg of turf fertilizer (24 % soluble nitrogen and 25 % soluble reactive phosphorous; SCOTTS ®, OH, USA) to locally double the availability of nutrients following Smith et al. (2001). Pots were inverted and glued to Plexiglas plates and the pots' drainage hole was closed with a plastic cork to ensure slow diffusion of nutrients through the porous terracotta. The pots were placed at the centre of each nutrient enrichment (N^+) plot and refilled monthly (figure 4.2*c*).

Clay tripod settlement tiles (kiln stilts, Ø 6 cm; Carl Jaeger Tonindustriebedarf GmbH, Germany; figure 4.3) were used as artificial substrates to track the development of algal communities in response to the factors described above. The upperside of these tripods served as light-exposed habitat, whereas their underside served as cryptic habitat. The thin (0.7 cm Ø) "arms" of these tiles allow herbivores to reach cryptic habitats (*sensu* Arnold et al. 2010) so that the influence of herbivory on cryptic habitats could be tracked as well. Ten tiles were zip-tied to plastic grids (egg crate, 30 × 30 cm; cell size: 1.1 × 1.1 × 0.9 cm, L × W × H) at a 2 to 3 cm distance from one another and placed in each experimental plot for 15, 30 or 90 days, resulting in 360 individual settlement tiles that were harvested simultaneously at the beginning of each coral settlement experiment. The three time points were chosen to represent algal communities at primary (15 days), intermediate (30 days) and advanced (90 days) stages of early succession, as previously established by Fricke (2011) for Curaçaoan reef communities.

To quantify the algal community composition on each tile at various time points, tiles were transported to a shaded, onshore flow-through aquarium system where the top- and undersides of all tiles were photographed at high resolution (Powershot SX200 IS; Canon, USA) (figure 4.3). Pictures were then analysed by overlying each side of each tile with 40 to 50 random points and scoring the algal group underneath each point using Coral Point Count with Excel extensions v4.1 (CPCe; Kohler and Gill 2006). Five benthic classes were distinguished: (1) bare substratum, (2) CCA, (3) encrusting algae other than CCA, (4) turf algae, and (5) macroalgae. Following Littler and Littler (2011a), filamentous algae that lacked structural characteristics were identified as turf algae. Algae with distinct structural attributes (e.g. dichotomous division for *Dictyota* sp. or thin lettuce-like appearance for *Ulva* sp.) were defined as macroalgae (Littler and Littler 2011b). Calcifying macroalgae such as *Halimeda* spp. were never observed growing on the tiles.

Coral larvae rearing

Favia fragum is a small, dome-shaped brooding stony coral that occurs in a wide range of shallow habitats in the Western Atlantic (Carlon and Budd 2002; Darling et al. 2012). It releases non-feeding planula larvae between 6 and 16 days after new moon (ANM) with a maximum release at day 11 ANM (Szmant-Froelich et al. 1985). When positive settlement cues are present, *F. fragum* larvae are capable of settling immediately after release (Carlon and Olson 1993).

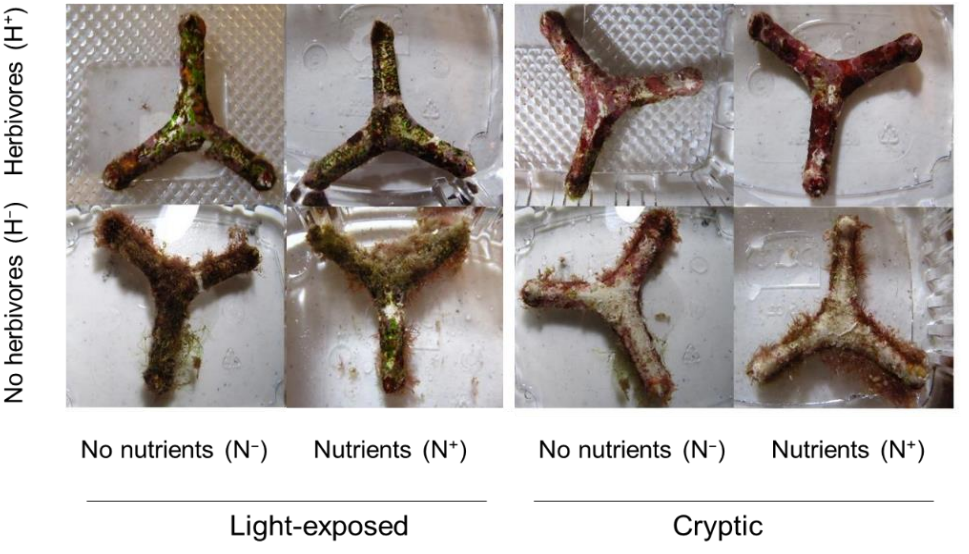


Figure 4.3. Examples of substrate tiles conditioned under each experimental treatment for 90 days and photographed for subsequent benthic community assessment with CpCe software. Photos by CGB Grupstra and L Linssen.

Eighty *F. fragum* colonies were collected at the study site between 3 and 6 m depth two days before the onset of their planulation cycle in March 2014, and kept in a flow-through aquarium system (215 × 69 × 64 cm, L × H × W; acrylic) continuously supplied with natural seawater (~2300 L hr^{-1}) from the nearby reef (see Chapter 6 for details on this system). Two hours before sunset between 9 and 11 days ANM, colonies were placed overnight in one 180-L plastic-coated Styrofoam cool box (Princeware Glacier, UK) containing ~150 L of 100-µm-filtered seawater. Every morning between 8:00 and 9:00, colonies were removed from the cool box and all larvae released during the previous night were collected using glass transfer pipettes. Larvae released on different days were kept separately in 500-mL clear polystyrene deli containers (DART, MI, USA) that contained ~350 mL of 4.0-µm-filtered seawater (Whatman, GE Life Sciences, PA, USA) until they were assigned to an experiment. Rearing coral larvae in filtered seawater slows the development of microbial communities that feed on substances released by dying larvae (mainly lipids) and delays the formation of biofilms that induce larval metamorphosis (Vermeij et al. 2009). Water in all containers was exchanged daily (~75%) and larval density was kept low (< 1 larva mL^{-1}) to avoid density dependent mortality (Vermeij et al. 2006). All containers were kept in the laboratory at 27°C, which corresponded to the sea surface temperature on the reef in March 2014

(NOAA Coral Reef Watch 2014). After the experiment was ended, parental colonies were epoxied (marine epoxy putty; Star Brite, USA) to the reef at their original location.

Coral settlement experiments

Two choice experiments were performed in which larvae were provided with tiles harbouring either algal communities from different successional stages of the same nutrient-herbivory treatment (experiment 1), or algal communities from different nutrient-herbivory treatments of the same successional stage (experiment 2). An additional no-choice experiment (experiment 3) was conducted where larvae were offered single tiles harbouring one of the 12 distinct algal communities (*4 nutrient-herbivory treatments* × *3 successional stages*). All experiments were conducted with ≤ 48-hr-old larvae in the deli containers described above. Algal communities can be kept successfully under these experimental conditions (Vermeij et al. 2009) and did not change, at least not visually, during the time they were kept in the containers.

Experiment 1: choice among algal communities from different successional stages

This experiment tested whether settling larvae prefer algal communities at primary (15 days), intermediate (30 days) or advanced (90 days) stages of early succession. Three tiles from different successional stages of the same nutrient-herbivory treatment (N^-H^+, N^+H^+, N^-H^- or N^+H^-) were placed together in a deli container to which 30 two-day-old larvae were added. This was replicated 6 to 10 times for each of the nutrient-herbivory treatments. Larvae were given 48 hrs to settle after which the tiles were inspected under a dissecting microscope and the number of settlers and their location on the tiles (i.e., upperside or underside) were recorded.

Experiment 2: choice among algal communities from different nutrient-herbivory treatments

The experimental approach in this experiment was similar to that of experiment 1, but larvae were offered four tiles harbouring algal communities of the different nutrient-herbivory treatments (N^-H^+, N^+H^+, N^-H^- and N^+H^-) at the same successional stage. This choice experiment was replicated 8 to 10 times for each of the three successional stages (15, 30 or 90 days). Forty *F. fragum* larvae were added to each deli container and given 48 hrs to settle, after which the number of settlers and their location on the tiles were assessed as described above.

Experiment 3: no choice among algal communities

This no-choice experiment aimed at comparing settlement rates of larvae in each of the 12 distinct algal communities (*4 nutrient-herbivory treatments* × *3 successional stages*). Ten two-day old *F. fragum* larvae were placed in individual deli containers with one tile harbouring one of the 12 algal communities. This was replicated three to 12 times depending on the number of tiles available in the experimental plots. Containers were checked daily and the number of settlers and their location on the tiles were recorded as described above. The experiment was ended after eight days.

Settlement preferences for microhabitats

The algal community composition in a 1-mm-wide band directly surrounding each settler was quantified in each experiment described above to assess which microhabitats were selected by settling *F. fragum* larvae. The dominant benthic class (i.e., bare substratum, CCA, encrusting algae other than CCA, turf algae or macroalgae) in each 5% increment of the 1-mm-wide band around each settler was noted, resulting in 20 measurements to describe the microhabitat surrounding each settler.

Data analysis

Algal community succession

Non-metric multidimensional scaling (NMDS) was used to visualize the successional trajectories of algal communities. The percent cover of the five benthic classes (i.e., bare substratum, CCA, encrusting algae other than CCA, turf algae and macroalgae) were used as response variables. Euclidian distances between each pair of samples were calculated after standardization of the data to construct a distance matrix. A redundancy analysis (RDA) was performed to quantify the amount of variability in algal community composition in cryptic and light-exposed habitats that could be explained by the fixed factors under study (i.e., nutrients, herbivory and successional stage).

Generalized linear mixed-effects modelling (GLMM) was performed to identify which factors significantly influenced the abundance of the five benthic classes. Because succession trajectories in light-exposed and cryptic habitats differed substantially, the GLMM analyses were conducted separately for these two habitat types. Successional stage (s), herbivory (h) and nutrients (n) were included as fixed

factors, whereas measurements within the same experimental plots were incorporated as repeated measures. A gamma-distributed error model with a log-link function was used. Subsequently, Akaike's information criterion (AIC) was used to determine the model with most support from the data among each of the following candidate models: 1) ~successional stage, 2) ~successional stage + herbivory, 3) ~successional stage + herbivory + nutrients and 4) ~successional stage + herbivory × nutrients. For each benthic class, the model with the lowest AIC was retained for further interpretation. Pearson's residuals for each selected model were visually examined for normality and homoscedasticity. The significance of each of the models' terms was determined based on 95% confidence intervals and associated z-score.

Coral settlement experiments

In the choice experiments 1 and 2, settlement rates on a given algal community were expressed as the proportion of settled *F. fragum* larvae that had selected this algal community across all replicates after 48 hrs. In experiment 1, larvae were provided with the choice among six algal communities (*3 successional stages* × *2 habitat types*), whereas in experiment 2, larvae could choose among eight algal communities (*4 nutrient-herbivory treatments* × *2 habitat types*). Following Ritson-Williams et al. (2010), the number of larvae available to settle in each algal community was assumed to not be limited because total settlement never reached 100% in all replicates by the end of the experiment. Measurements on individual tiles were therefore considered independent. Chi-square goodness of fit tests were performed to determine whether larvae settled evenly among the six or eight choices of algal communities, or if they preferred or avoided settling in distinct communities. In case of significant effects, exact binomial tests were performed for individual algal communities at a time to identify which of the six or eight communities were either preferred or avoided by settling larvae.

In the no-choice experiment 3, settlement rates were expressed as the proportion of initial *F. fragum* larvae that survived and subsequently settled in each of the 12 algal communities (*4 nutrient-herbivory treatments* × *3 successional stages*) after eight days. Data were homoscedastic (Levene's test, $F_{11,88} = 1.866$, $p = 0.06$) and differences in settlement rates among treatments were therefore assessed with a two-tailed, three-way ANOVA, with successional stage (s), herbivory (h) and nutrients (n) as fixed factors. Bonferroni's *post-hoc* tests were performed to identify settlement preferences for distinct algal communities. Subsequently, preferences for either cryptic or light-exposed habitats were determined with exact binomial tests.

For each of the 12 algal communities, the proportion of larvae that selected either cryptic or light-exposed habitats (observed proportion) was compared to the expected proportion of 0.5 assuming random settlement behaviour.

To determine if settling *F. fragum* larvae either searched for specific microhabitats within algal communities, or settled randomly within those communities, chi-square goodness of fit tests were performed to determine if the composition of the local algal communities in a 1 mm band surrounding the settlers (observed composition) corresponded to that of the overall community composition that developed on the tiles (expected composition). To do so, the composition of the community directly surrounding settlers was averaged over all replicates for each of the 12 possible treatments (*4 nutrient-herbivory treatments* × *3 successional stages*) and the two habitat types (light-exposed and cryptic). Exact binomial tests were subsequently performed to identify which of the five benthic classes (i.e., bare substratum, CCA, encrusting algae other than CCA, turf algae and macroalgae) were either preferred or avoided by *F. fragum* larvae in light-exposed and cryptic habitats.

Bonferroni-corrected *p*-values were used for all of the above statistical analyses to adjust for multiple hypothesis testing.

Results

Algal community succession in light-exposed and cryptic habitats

The composition of algal communities that developed in light-exposed and cryptic habitats differed substantially (NMDS and RDA, figure 4.4). Turf algae dominated on the light-exposed uppersides of the tiles already during the primary stage of succession (15 days), whereas the cryptic undersides of the tiles had remained mostly bare after 15 days, after which they slowly became dominated by CCA during later successional stages (figure 4.5). Across all treatments after 90 days, turf algae were on average five times more abundant than CCA in light-exposed habitats, whereas CCA were on average four times more abundant than turf algae in cryptic habitats (figure 4.5). Encrusting algae other than CCA were 2.3-fold more abundant in light-exposed habitats than in cryptic habitats (figure 4.5). Macroalgae (foremost *Dictyota* spp.) were the slowest colonizers, resulting in a low average cover of 2% and 3% on cryptic and light-exposed surfaces respectively after 90 days (figure 4.5). Finally, 26% of cryptic surfaces had remained bare after 90

days, whereas only 6% of light-exposed surfaces still consisted of bare substratum at that time (figure 4.5).

Algal community succession in response to herbivory and nutrients

Overall, 30% of the variation in community composition could be explained by the factors under study (RDA, figure 4.4*b*). In both light-exposed and cryptic habitats, successional stage and herbivory were the main drivers of differences in algal communities through time, whereas nutrient levels did not always have a significant effect (GLMM, table 4.1) (figure 4.5).

Abundances of all benthic classes on light-exposed surfaces depended on herbivory, with the exception of CCA (GLMM, table 4.1). After 90 days, reduced herbivory had resulted in a 1.8-fold increase in turf algal cover and a 2.7-fold decrease in the cover of encrusting algae other than CCA in comparison to natural reef conditions (figure 4.5). The cover of bare substratum was 2.5-fold lower in communities grown under reduced herbivory and 1.7-fold lower in communities exposed to nutrient enrichment than in communities grown under natural reef conditions (figure 4.5).

Nutrient enrichment significantly increased the abundance of macroalgae by 12.5-fold, but only under reduced herbivory conditions (GLMM, table 4.1) (figure 4.5). Thus, within the context of our study, reduced herbivory had a larger impact on light-exposed algal communities than nutrient enrichment. Herbivores influenced algal community succession in cryptic habitats as well (GLMM, table 4.1). The presence of large-bodied herbivores facilitated the growth of CCA by 1.5-fold and repressed turf algae growth by 1.6-fold in comparison with communities grown under reduced herbivory conditions (figure 4.5). Grazing by herbivores further resulted in a 1.6-fold reduction in remaining uncolonized, bare substratum after 90 days (figure 4.5). Nutrient enrichment affected communities that developed on the undersides of the tiles to a lesser extent than herbivory, leading to a 1.2-fold increase in cover of encrusting algae other than CCA, and to a 1.3 decrease in turf algal abundance compared to communities grown under ambient nutrient levels (figure 4.5). In sum, similar to light-exposed habitats, herbivory had a greater effect on algal communities in cryptic habitats than nutrient enrichment. The effect of herbivory reduction however differed between the two habitat types: it led to more turf algae and less bare substratum in light-exposed habitats, whereas it led to less CCA cover and more bare substratum in cryptic habitats.

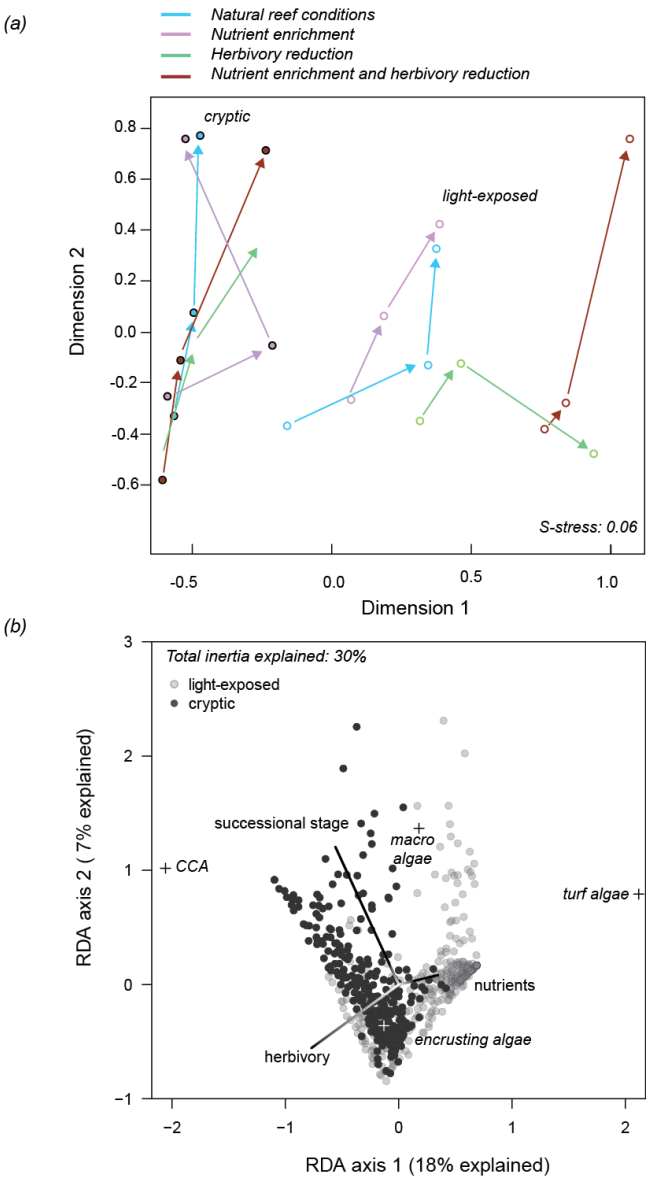


Figure 4.4. *(a)* Non-metric multidimensional scaling (NMDS) of algal communities in light-exposed (*clear circles*) and cryptic (*filled circles*) habitats, and grown under different herbivory and nutrient levels. *Arrows* show the temporal trajectories of communities from 15 to 30 and 90 days. *(b)* Redundancy analysis (RDA) triplot of algal community composition in cryptic and light-exposed habitats as function of successional stage, herbivory and nutrient levels. Together, these factors explained 30% of the total variation in community composition.

Table 4.1. Generalized linear mixed effect models (GLMM) best describing changes in percent cover of each benthic class (i.e., bare substratum, crustose coralline algae (CCA), encrusting algae other than CCA, turf algae and macroalgae) in response to successional stage (S: 15, 30, 90 days), herbivory (H: H^+, H^-) and nutrients (N: N^-, N^+). Observations within the same experimental plots were included as repeated measures and models were constructed based on a gamma error distribution with a log-link function. Models that best fit the data were selected according to Akaike's information criterion (AIC). The factorial effects of herbivory reduction (H^-) and nutrient enrichment (N^+) as well as their interaction (H^-N^+) on the percent cover of each functional group are shown at the right end of the table, for light-exposed and cryptic habitats separately. Significant terms are underlined and were determined based on 95% confidence intervals and associated z-scores (shown in table S4.1).

Model		Factorial effects		
light-exposed habitats		H^-	N^+	H^-N^+
bare substratum	$= \underline{3.84} - \underline{0.61}S30 - \underline{1.74}S90 - \underline{0.60}H^- - \underline{0.39}N^+$	<u>0.55</u>	<u>0.67</u>	-
cca	$= \underline{1.48} + \underline{0.84}S30 + \underline{0.87}S90$	-	-	-
encrusting algae	$= \underline{2.48} - 0.29S30 + 0.24S90 - \underline{0.93}H^- - 0.46N^+$	<u>0.40</u>	0.63	-
turf algae	$= \underline{3.02} + 0.22S30 + 0.05S90 + \underline{1.07}H^-$	<u>2.93</u>	-	-
macroalgae	$= \underline{-2.27} + 0.15S30 + \underline{1.38}S90 + \underline{1.15}H^- - 0.36N^+ + \underline{1.85}H^-N^+$	<u>3.14</u>	0.70	<u>13.94</u>
cryptic habitats				
bare substratum	$= \underline{4.03} - \underline{0.33}S30 - \underline{1.10}S90 + \underline{0.35}H^-$	<u>1.42</u>	-	-
cca	$= \underline{2.38} + \underline{0.97}S30 + \underline{1.70}S90 - \underline{0.40}H^-$	<u>0.67</u>	-	-
encrusting algae	$= \underline{2.80} + 0.05S30 - \underline{0.37}S90 - 0.17H^- - \underline{0.37}N^+ - \underline{0.57}H^-N^+$	0.84	<u>1.45</u>	<u>0.69</u>
turf algae	$= \underline{1.42} + 0.36S30 + \underline{0.73}S90 + \underline{0.59}H^- + \underline{0.58}N^+ - \underline{0.61}H^-N^+$	<u>1.80</u>	<u>1.78</u>	<u>1.73</u>
macroalgae	$= \underline{-2.18} + 0.30S30 + \underline{1.87}S90 + \underline{1.39}H^-$	<u>4.02</u>	-	-

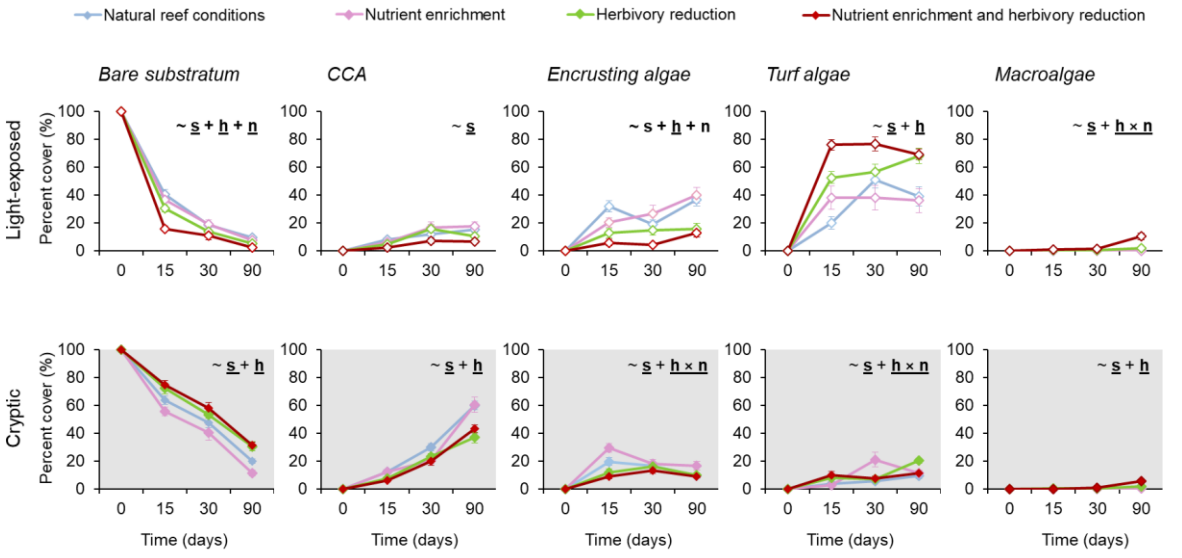


Figure 4.5. Percent cover of the five dominant benthic functional groups (i.e., bare substratum, CCA, encrusting algae other than CCA, turf algae and macroalgae) through time in light-exposed and cryptic habitats, and when exposed to different herbivory and nutrient levels. Error bars are standard errors (n = 20-34 tiles per nutrient-herbivory treatment × successional stage combination). The factors that most influenced the growth of each functional group were determined with a generalized linear mixed modelling (GLMM) approach. Models that best explained the data are shown for each functional group (s = successional stage, h = herbivory, n = nutrients). Significant terms (underlined) of each model were determined based on 95% confidence intervals and associated z-score.

Coral settlement experiments

Favia fragum larvae settled on tiles harbouring algal communities grown under all nutrient-herbivory treatments ($N^{-}H^{+}$, $N^{+}H^{+}$, $N^{-}H^{-}$, and $N^{+}H^{-}$) and all successional stages (15, 30, and 90 days) across all three experiments (figure 4.6).

Experiment 1: choice among algal communities from different successional stages

When provided with the choice among algal communities from different successional stages grown under natural levels of herbivory, *F. fragum* larvae preferred settling in communities at intermediate and late stages of succession (30 and 90 days) in cryptic habitats, in which they settled 1.6 to 3 times more than expected under random settlement behaviour (Chi-square test, $N^{-}H^{+}$, $\chi^2_5 = 50.7$, $p < 0.013$, $N^{+}H^{+}$, $\chi^2_5 = 46.3$, $p < 0.013$, table S4.2) (figure 4.*6a*). When provided with algal communities grown under reduced herbivory conditions, settling larvae did not display preferences for specific successional stages, nor for a specific habitat type (table S4.2; figure 4.*6a*).

Experiment 2: choice among algal communities from different nutrient-herbivory treatments

When provided with algal communities from the primary stage of succession (15 days), *F. fragum* larvae preferred settling in algal communities from cryptic habitats with reduced herbivory and/or nutrient enrichment, where they were found in 1.7 to 2.2-fold higher abundances than expected if settlement was random (Chi-square test, 15 days, $\chi^2_7 = 107.1$, $p < 0.017$, table S4.3) (figure 4.6*b*). Conversely, settlers were 2.2 to 3.6 times less abundant in light-exposed algal communities than expected, with the exception of communities grown under nutrient enriched conditions (figure 4.6*b*).

When offered algal communities from the intermediate stage of succession (30 days), *F. fragum* larvae settled 1.8 to 1.9 times more than expected in cryptic habitats with natural levels of herbivory, whereas they avoided light-exposed habitats with reduced herbivory (Chi-square test, 30 days, $\chi^2_7 = 37.7$, $p < 0.017$, table S4.3) (figure 4.6*b*).

Lastly, when provided with the choice among algal communities at advanced successional stages (90 days), coral larvae displayed preferences exclusively for cryptic habitats under natural reef conditions, where they settled 2.6-fold more than expected (Chi-square test, 90 days, $\chi^2_7 = 79.4$, $p < 0.017$, table S4.3) (figure 4.6*b*).

In contrast, larvae avoided algal communities that developed in light-exposed habitats under nutrient enrichment alone, and in cryptic habitats under both reduced herbivory and nutrient enrichment (figure 4.6*b*).

In sum, these results indicate that *F. fragum* larvae displayed preferences for algal communities that developed in cryptic habitats, while avoiding algal communities in light-exposed habitats. Larval preferences for specific algal communities were also influenced by herbivory and nutrient levels, but those preferences differed depending on successional stage: while communities grown under altered levels of nutrients and/or herbivory were most conducive to coral settlement at primary successional stages, communities grown under ambient reef conditions were preferred at intermediate and advanced successional stages.

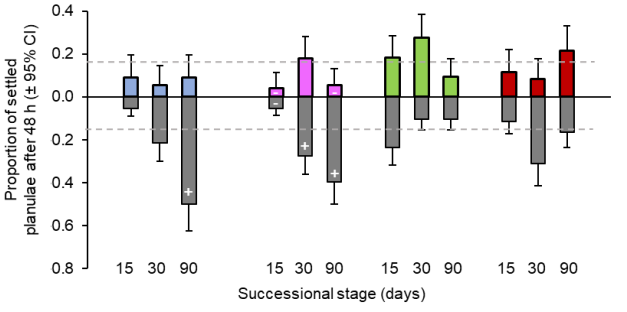
No-choice experiment 3: single algal communities

When *F. fragum* larvae were provided with single algal communities at a time, their settlement rates were not influenced by herbivory or nutrient levels (three-way ANOVA, Nutrients, $F_{1,88} = 0.5$, $p = 0.501$, Herbivory, $F_{1,88} = 3.1$, $p = 0.08$, table S4.4) (figure 4.6*c*). However, overall settlement rates were slightly but significantly higher in the advanced stage of algal succession (90 days) than in the primary stage (15 days) (three-way ANOVA, Successional stage, $F_{2,88} = 3.9$, $p < 0.025$, table S4.4) (figure 4.6*c*). In these experiments, coral larvae did not show a significant preference for cryptic or light-exposed habitats (table S4.5).

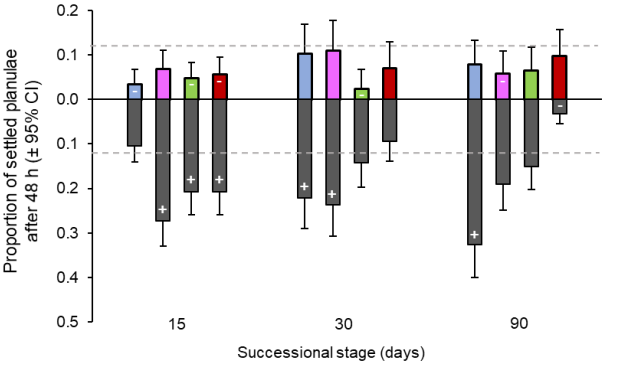
Settlement preferences for microhabitats within algal communities

Across all treatments and successional stages, settled larvae were foremost surrounded by bare substratum (55% ± 1) and CCA (26% ± 1) ($n = 1182$ settlers, figure 4.7). Less than 4% of all larvae settled in direct contact with turf algae, and only one settler was found next to a macroalga (figure 4.7). In light-exposed habitats, the algal composition in the immediate neighbourhood of settlers differed markedly from the overall algal community composition (table S4.6, figure 4.7). While turf algae were dominant in light-exposed habitats (53% ± 2, $n = 337$ tiles), they were avoided by settling *F. fragum* larvae (5% ± 1, $n = 470$ settlers) (Exact binomial test, $p < 0.005$, table S4.7) (figure 4.7). Larvae preferentially settled on or next to bare substratum (48% ± 2), which was not very abundant in light-exposed habitats (17% ± 1) (Exact binomial test, $p < 0.005$, table S4.7) (figure 4.7).

(a) Experiment 1: choice among successional stages



(b) Experiment 2: choice among nutrient-herbivory treatments



(c) Experiment 3: no choice among algal communities

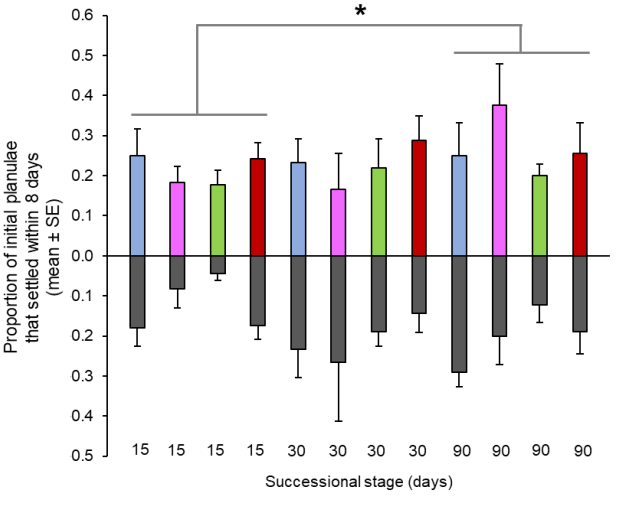


Figure 4.6. Settlement preferences of *Favia fragum* larvae for algal communities grown under different nutrient and herbivory levels and at different successional stages. *(a)* Proportion of settled larvae that selected algal communities grown under the same nutrient-herbivory treatment, but at different successional stages (n = 6-10 replicates for a total of 56-76 settlers). *(b)* Proportion of settled larvae that selected algal communities at the same successional stage, but grown under different nutrient-herbivory treatments (n = 8-10 replicates for a total of 127-231 settlers). *(c)* Proportion of initial larvae that survived and settled when provided with a single algal community at a time (n = 3-12 replicates). In *(a)* and *(b)*, *error bars* are 95% confidence intervals calculated with Clopper-Pearson's exact method, and *dashed lines* indicate the expected even settler distribution under random settlement behaviour. *White (+)* and *(–)* signs respectively denote significant deviations from random settlement as determined with chi-square goodness of fit tests, followed by exact binomial tests with Bonferroni-corrected p-values. In *(c)*, *asterisks* denotes significant treatment groupings as determined with a three-way ANOVA followed by Bonferroni's *post-hoc* comparisons.

In cryptic habitats, the algal composition in the immediate neighbourhood of *F. fragum* settlers differed from the overall community in only three out of the 12 treatment combinations (table S4.6, figure 4.7). Bare substratum and CCA were preferred microhabitats for settlement, accounted for 49% ± 1.4 and 26% ± 1.2 of the cryptic surfaces on average (n = 337 tiles), and were equally abundant in the immediate neighbourhood of the settlers (Exact binomial test, bare substratum, 60% ± 1.4, p = 0.107, CCA: 28% ± 1.4, p = 0.200, n = 718 settlers, table S4.7) (figure 4.7). In sum, these results confirm the settlement-inhibiting effects of turf algae, and the settlement-facilitating role of bare substratum and CCA, and thereby illustrate the role of light availability as an indirect factor affecting settlement processes in corals.

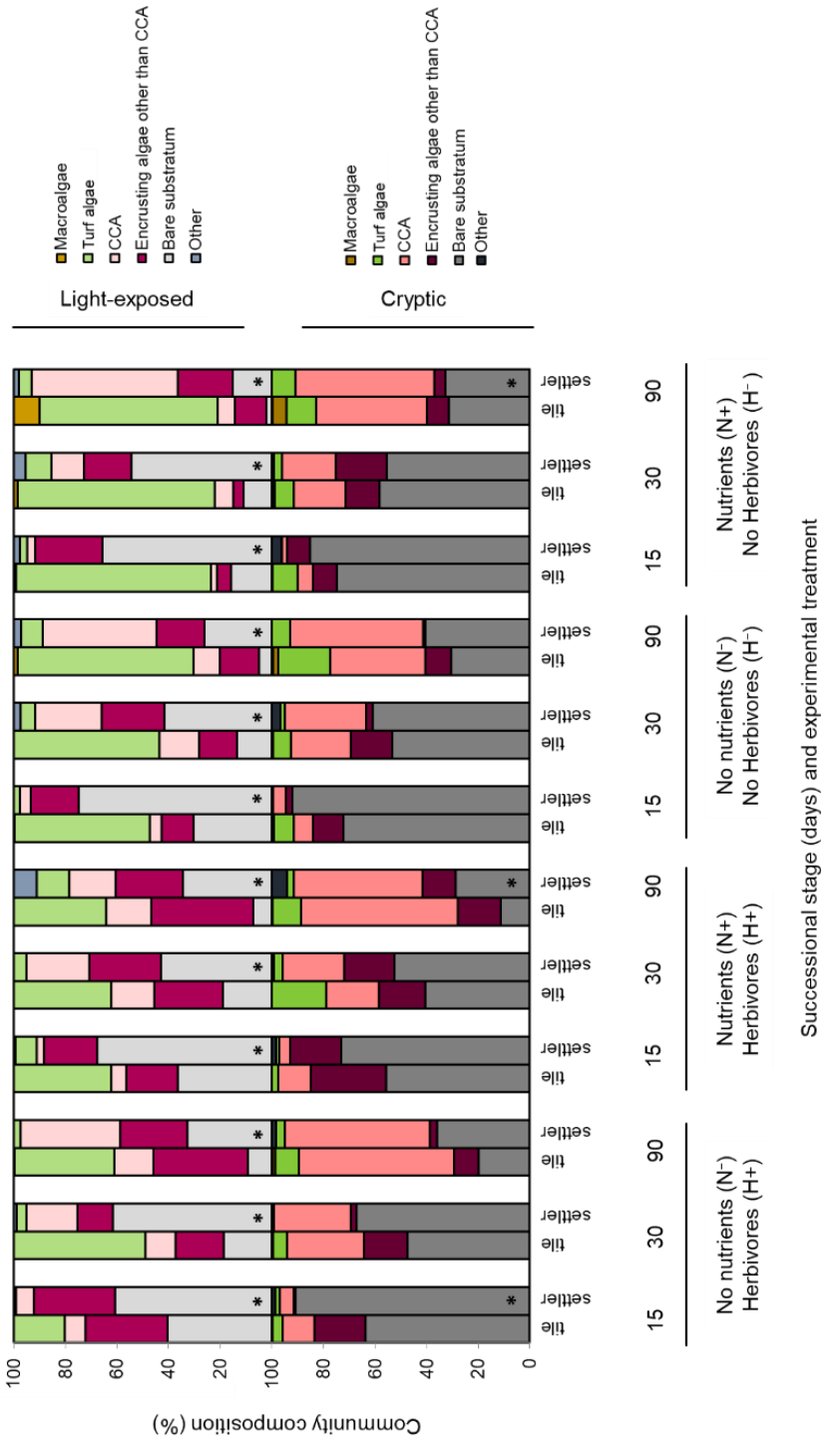

Macroalgae
Turf algae
CCA
Encrusting algae other than CCA
Bare substratum
Other
Light-exposed
Cryptic
Community composition (%)
100
80
60
40
20
0
tile
settler
*
15
30
90
No nutrients (N⁻)
Herbivores (H+)
Nutrients (N+)
Herbivores (H+)
No nutrients (N⁻)
No Herbivores (H⁻)
Nutrients (N+)
No Herbivores (H⁻)
Successional stage (days) and experimental treatment

Figure 4.7. Settlement preferences of *Favia fragum* larvae for microhabitats within algal communities grown under different levels of nutrients and herbivory for 15, 30 and 90 days. The algal composition directly surrounding settlers (1 mm band around settlers) is compared to the overall composition of algal communities on the light-exposed (upperside) and cryptic (underside) surfaces of the tiles. *Asterisks* denote micro-communities around settlers that differed significantly from the overall community composition on the tiles, as determined with chi-square analyses with Bonferroni correction for multiple hypothesis testing ($p < 0.0021$).

Discussion

On shallow tropical coral reefs, light intensity, nutrient availability and grazing pressure by herbivores are three environmental factors that influence competitive interactions among marine algae and sessile invertebrates, and as a consequence also play a role in determining the outcome of successional dynamics within such communities (Jackson 1977; van den Hoek et al. 1978; Jackson and Winston 1982; Littler and Littler 1984; Huston 1985; Smith et al. 2001; Littler et al. 2006). This study shows that the composition of algal communities that developed on artificial settlement substrates during early succession stages (three months) depended foremost on light exposure of these communities. Nutrient levels and herbivory also contributed, albeit to a lesser degree, to the formation of different algal communities (figure 4.4*a*,*b*, 4.5). These algal communities in turn influenced settlement of the Caribbean brooding coral *Favia fragum*, both in terms of settlement rates and spatial distribution of settlers within these algal communities.

Differences in algal succession between light-exposed and cryptic habitats

On coral reefs, dark cryptic environments (e.g., crevices, under-hangs, caves) are predominantly colonized by sponges and encrusting calcareous organisms (i.e., bryozoans, CCA, serpulid worms, foraminifera, bivalves) (Jackson 1977; Jackson and Winston 1982; Day 1983; Vermeij 2006; Mallela 2007), whereas zooxanthellate corals and fleshy marine algae such as frondose macroalgae and filamentous turfs are most abundant on exposed, sunlit surfaces (Day 1983; Jackson 1977; van den Hoek and Breeman 1978; Vermeij 2006). The algal communities that developed on the substrate tiles' cryptic and light-exposed surfaces during the course of this study also differed markedly (figure 4.4*a*). Open space on the light-exposed surfaces was rapidly colonized with only 30% of the substratum remaining bare after 15 days. Conversely, cryptic environments were colonized much more slowly, where open space still comprised 68% of the surface area after 15 days. The algal composition

of light-exposed and cryptic habitats differed especially in the abundance of CCA and turf algae (figure 4.4*b*). After three months, CCA covered 40-60% of the cryptic habitats, whereas turf algae dominated in light-exposed communities where they covered 40-80% of the surface (figure 4.5). These differences in colonization rates and algal assemblages between the tiles' upper surfaces and undersides were likely driven by the distinct light requirements and growth rates of CCA and turf algae. Turf algae are well adapted to high light and capable of rapid expansion through vegetative reproduction, except when heavily grazed by herbivores (Wanders 1977; Carpenter 1985; Steneck and Dethier 1994; Airoldi 1998; Fricke et al. 2014). CCA on the other hand are sensitive to high irradiance, can grow under low-light conditions, and have slower growth rates than turf algae (Adey and Vassar 1975; van den Hoek et al. 1978; Leukart 1994). Given these characteristics, turf algae typically outcompete CCA under high-light conditions on degrading reefs, whereas CCA more successfully colonize newly opened space in low-light environments (Adey and Vassar 1975; Wanders 1977; Littler and Littler 1984; Kendrick 1991; Dethier 1994; Steneck and Dethier 1994; Littler et al. 2006; Mallela 2007).

Influence of reduced herbivory and nutrient enrichment on algal succession

Nutrient and herbivory manipulations both affected the composition of algal communities. In light-exposed habitats, reductions in grazing rates resulted in 1.8 times higher abundances of turf algae compared to communities grown under ambient grazing pressure (figure 4.5). Nutrient enrichment also increased the abundance of macroalgae, but only when large-bodied herbivores were excluded (figure 4.5). These observations thus partially support the predictions of the Relative Dominance Model (Littler & Littler 1984; figure 4.1). This conceptual model, however, also predicts that CCA dominate the benthos under high nutrient and herbivory regimes (figure 4.1). While Smith et al. (2010) and Littler et al. (2006) indeed found significant increases in CCA growth under nutrient enrichment, followed by the dominance of CCA within 12 to 24 months, CCA cover in the current study was not affected by nutrient enrichment (figure 4.5). This could in part be explained by the fact that the algal communities studied on Curaçao were much younger (three months) than those studied by Smith et al. (2010) (12 months) and Littler et al. (2006) (24 months) in Hawaii and Belize respectively. In Hawaii, CCA cover in all treatments also remained below 20% under fertilized conditions during the first six months, but increased to over 60% cover between seven and 12 months. Our study was ended after three months, and while the predicted positive effects of nutrients on CCA growth were not observed here, it cannot be excluded that these effects would have manifested themselves during later successional stages.

Grazing by herbivores can facilitate recruitment and growth of CCA by reducing the abundance of turf algae that would otherwise often outcompete CCA (Steneck 1977; Wanders 1977). In this study, the facilitating role of herbivores on CCA growth was observed on the cryptic undersides of the tiles, where the presence of grazers repressed turf algae by 1.6-fold and resulted in a 1.5-fold higher abundance of CCA. Grazing on light-exposed surfaces also reduced the cover of turf algae, but did not result in increased cover by CCA. Contrary to the predictions of the Relative Dominance Model (Littler & Littler, 1984; figure 4.1), the abundance of turf algae in light-exposed communities in the current study was high (38%) even in the presence of large herbivores. Smith et al. (2010) also found relatively high abundances of turf algae during the earliest stages of algal community succession under high grazing activity, but that turfs were gradually replaced by CCA in older (≥ seven-month-old) algal communities. Thus, at early successional stages, turf algae may dominate on the light-exposed surfaces due to the slower growth rates of coralline species. In sum, grazing by large-bodied herbivores appeared more important than nutrient enrichment in driving early algal succession, confirming similar findings of Belliveau and Paul (2002) who also found that herbivores were primarily responsible for shaping early algal community succession (five weeks) in Guam.

Settling corals select algal communities in cryptic habitats

On Caribbean reefs, coral larvae generally prefer settlement habitats dominated by living or dead calcifying organisms (i.e., CCA or open substratum, Ritson-Williams et al. 2016). CCA and associated microorganisms, including most common species of CCA on Curaçao (Quéré and Nugues 2015), often produce chemicals that induce coral settlement and metamorphosis (e.g., Heyward and Negri 1999; Harrington et al. 2004; Ritson-Williams et al. 2016). Fleshy algae, in contrast, inhibit settlement through space pre-emption, overgrowth, silt trapping, oxygen depletion, and through the production of allelopathic compounds (Fabricius 2005; Ritson-Williams et al. 2009; Vermeij et al. 2009). Sub-cryptic and cryptic low-light environments on coral reefs are typically preferred by settling coral larvae relative to light-exposed surfaces (Harriott and Fisk 1987; Carlon 2001; Vermeij 2006; Arnold et al. 2010) because they act as better refuges for coral setters compared to light-exposed surfaces that are often colonized by fleshy algae (Babcock and Davies, 1991; Babcock and Mundy, 1996; Fabricius, 2005; Miller, 2014; Smith et al., 2001). In the current study, *F. fragum* larvae displayed clear settlement preferences for cryptic habitats (figure 4.6*a*,*b*) where bare substratum and CCA remained abundant (≥ 60% combined) despite altered nutrient and herbivory levels.

In light-exposed habitats, algal communities in contrast became rapidly dominated by turf algae, in particular under reduced herbivory conditions (figure 4.5). Thus, these results highlight the important role of cryptic habitats as refuge for settling coral larvae in degrading reef communities, where fleshy algae dominate on light-exposed surfaces.

Within these cryptic environments, larvae preferentially settled in algal communities grown under nutrient enrichment and/or reduced herbivory during the primary stage of succession (15 days), but shifted their preference to algal communities that developed under natural levels of herbivory at later successional stages (30 and 90 days; figure 4.6*b*). This shift in larval preference is likely associated with the observed successional changes in algal community composition. At the primary stage of succession, cryptic habitats were still mainly comprised of uncolonized substratum with sparse CCA cover (figure 4.5). At later stages of succession, however, CCA were most abundant in algal communities grown under natural levels of herbivory. By promoting CCA growth (Steneck 1977; Wanders 1977), and creating open space due to surface biting (especially by parrotfish species, Mumby 2009), herbivores constantly create benthic conditions conducive to coral settlement. Reduced herbivory in combination with nutrient enrichment, in contrast, promotes the growth of macroalgae and turf algae that are unfavourable to settling coral larvae (Kuffner et al. 2006). When at an advanced successional stage (90 days), such algal communities were avoided by settling larvae (figure 4.6*b*). Thus, in addition to light exposure, herbivory and nutrients also indirectly affected settlement success of *F. fragum* larvae.

Corals forced to settle in sub-optimal algal communities successfully search for CCA and open space

When *F. fragum* larvae were provided with single algal communities at a time, settlement success no longer depended on the composition of the different algal communities that had developed on the tiles. In the no-choice experiment, larvae settled equally on tiles conditioned under all nutrient-herbivory treatments (figure 4.6*c*). Apparently, all experimental benthic communities still harboured suitable settlement surfaces despite the structuring effects of herbivory and nutrient availability on the composition of these early successional communities. *F. fragum* larvae almost invariably settled next to or onto bare substratum or CCA and avoided turf algae, despite that the latter were abundant in most algal communities (figure 4.5, 4.7). These results confirm that CCA and open space are required for successful settlement by coral larvae (e.g., Ritson-Williams et al. 2009; Arnold et al. 2010;

Arnold and Steneck 2011), and that settling larvae avoid turf algae (Kuffner et al. 2006; Vermeij 2006; Vermeij and Sandin 2008; Arnold et al. 2010; Arnold and Steneck 2011). These results further suggest that when larvae are forced to settle in habitats dominated by unfavourable algal groups such as turf algae, they can still be successful as long as preferred habitats are still present at very small spatial scales (mm). It is however important to note that coral species differ in settlement behaviour resulting in species-specific settlement patterns (Bak and Engel 1979; Ritson-Williams et al. 2010, 2016), and that these findings for *F. fragum*, a species with a 'stress-tolerant' life history (Darling et al. 2012), are likely applicable only to species with a similar life-history strategy.

Consequences of coral settlement in sub-optimal algal communities

While this study shows that coral reefs experiencing chronic nutrient pollution and overfishing of herbivores may still harbour suitable microhabitats for settling coral larvae, the subsequent survival and growth of coral settlers in such environments is likely to be compromised. Early post-settlement survival and growth in corals are highly dependent on interactions with surrounding benthic communities (Vermeij 2006; Arnold et al. 2010). In light-exposed communities dominated by fleshy algae, newly settled corals can be negatively affected by algal overgrowth, silt trapping, oxygen depletion, and by allelopathic compounds produced by fleshy algae and the microbial communities they harbour (Fabricius 2005; Ritson-Williams et al. 2009; Vermeij et al. 2009). Thus, coral larvae that successfully settle in algal communities dominated by turf algae and macroalgae are likely to suffer from high post-settlement mortality as those benthic communities age towards later successional stages, and competitive interactions strengthen.

In conclusion, recruitment in scleractinian corals is known to vary at small spatial and temporal scales (e.g., Bak and Engel 1979; Maida et al. 1994; Dunstan and Johhson 1998; Vermeij 2006) and to be affected by a myriad of (a)biotic factors (reviewed in Ritson-Williams et al. 2009). This study provided new insights into how light exposure, nutrient availability and grazing activity by herbivores can structure algal communities, and thereby affect patterns of larval settlement at fine scales on Caribbean coral reefs.

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Supplementary material

Table S4.1. Statistical output for the generalized linear mixed effect models (GLMM) best describing changes in percent cover of each benthic class (i.e., bare substratum, CCA, encrusting algae other than CCA, turf algae and macroalgae in response to successional stage (S: 15, 30, 90 days), herbivory (H: H^+, H^-) and nutrients (N: N^-, N^+). Observations within the same experimental plots were included as repeated measures and models were constructed based on a gamma error distribution with a log-link function. Models that best fit the data were selected according to Akaike's information criterion (AIC). Significant terms are underlined as determined by 95% confidence intervals (CI) and associated *z*-score.

			95% CI				
	model	mean	lower bound	upper bound	SE	*z*-score	*p*-value
light-exposed habitats							
bare substratum	~ s + h + n						
	Intercept	3.8	3.5	4.1	0.2	25.5	<u><0.001</u>
	S30	−0.6	−0.9	−0.3	0.1	4.5	<u><0.001</u>
	S90	−1.7	−2.0	−1.5	0.1	12.6	<u><0.001</u>
	H^-	−0.6	−0.9	−0.3	0.2	3.9	<u><0.001</u>
	H^-N^+	−0.4	−0.7	−0.1	0.2	2.6	<u><0.001</u>
cca	~ s						
	Intercept	1.5	1.1	1.9	0.2	7.3	<u><0.001</u>
	S30	0.8	0.5	1.2	0.2	4.6	<u><0.001</u>
	S90	0.9	0.5	1.2	0.2	4.8	<u><0.001</u>
encrusting algae	~ s + h + n						
	Intercept	3.4	2.9	4.0	0.3	12.4	<u><0.001</u>
	S30	−0.3	−0.7	0.2	0.2	1.3	0.208
	S90	0.2	−0.2	0.7	0.2	1.1	0.283
	H^-	−0.9	−1.5	−0.4	0.3	3.2	<u>0.001</u>
	N^+	−0.5	−1.0	0.1	0.3	1.6	0.117
turf algae	~ s + h						
	Intercept	3.0	2.3	3.8	0.4	8.1	<u><0.001</u>
	S30	0.2	−0.1	0.5	0.1	1.6	0.110
	S90	0.1	−0.2	0.3	0.1	0.4	0.735
	H^-	1.1	0.1	2.0	0.5	2.2	<u>0.030</u>
macroalgae	~ s + h × n						
	Intercept	−2.3	−2.7	−1.9	0.2	10.9	<u><0.001</u>
	S30	0.2	−0.2	0.5	0.2	0.9	0.372
	S90	1.4	1.0	1.7	0.2	8.0	<u><0.001</u>
	H^-	1.2	0.6	1.7	0.3	4.3	<u><0.001</u>
	N^+	−0.4	−0.9	0.2	0.3	1.2	0.222
	H^-N^+	1.9	1.1	2.6	0.4	4.6	<u><0.001</u>

Table S4.1. Continued.

			95% CI				
	model	mean	lower bound	upper bound	SE	*z*-score	*p*-value
cryptic habitats							
bare substratum	~ s + h						
	Intercept	4.0	3.8	4.3	0.1	35.1	<0.001
	S30	−0.3	−0.5	−0.2	0.1	5.0	<0.001
	S90	−1.1	−1.2	−1.0	0.1	16.0	<0.001
	H^-	0.4	0.1	0.6	0.1	2.4	0.018
cca	~ s + h						
	Intercept	2.4	2.1	2.7	0.1	17.6	<0.001
	S30	1.0	0.8	1.2	0.1	8.8	<0.001
	S90	1.7	1.5	1.9	0.1	15.5	<0.001
	H^-	−0.4	−0.7	−0.1	0.2	2.4	0.016
encrusting algae	~ s + h × n						
	Intercept	2.8	2.6	3.1	0.1	21.5	<0.001
	S30	0.1	−0.2	0.3	0.1	0.4	0.730
	S90	−0.4	−0.6	−0.1	0.1	2.7	0.007
	H^-	−0.2	−0.5	0.1	0.2	1.1	0.270
	N^+	0.4	0.0	0.7	0.2	2.2	0.028
	H^-N^+	−0.6	−1.0	−0.1	0.2	2.5	0.012
turf algae	~ s + h × n						
	Intercept	1.4	1.1	1.8	0.2	7.7	<0.001
	S30	0.4	0.0	0.7	0.2	1.9	0.059
	S90	0.7	0.4	1.1	0.2	3.9	<0.001
	H^-	0.6	0.2	1.0	0.2	2.9	0.004
	N^+	0.6	0.1	1.0	0.2	2.5	0.012
	H^-N^+	−0.6	−1.2	0.0	0.3	2.0	0.050
macroalgae	~ s + h						
	Intercept	−2.2	−2.7	−1.7	0.3	8.5	<0.001
	S30	0.3	−0.1	0.7	0.2	1.7	0.097
	S90	1.9	1.5	2.3	0.2	9.5	<0.001
	H^-	1.4	0.8	2.0	0.3	4.3	<0.001

Table S4.2. Statistical output for experiment 1 in which settling *Favia fragum* larvae where provided with the choice among algal communities grown under the same nutrient-herbivory treatment, but at different successional stages. Chi-square goodness of fit tests were performed to compare the overall distribution of settlers among the six possible algal communities (*3 successional stages* × *2 habitat types*) to the expected even distribution of 1:1:1:1:1:1 assuming random settlement behaviour. In case of significant effects (*), exact binomial tests were performed for each of the six individual algal communities to identify which were either preferred (+) or avoided (−) by settling larvae. Significant *p*-values are underlined and ns stands for non-significant.

		Natural reef conditions					Nutrient enrichment					Herbivory reduction					Nutrient enrichment & herbivory reduction				
		n	χ^2	df	*p*-value	sign.	*n*	χ^2	df	*p*-value	sign.	*n*	χ^2	df	*p*-value	sign.	*n*	χ^2	df	*p*-value	sign.
Overall distribution [a]		56	50.7	5	<u>< 0.001</u>	*	73	46.3	5	<u>< 0.001</u>	*	76	13.8	5	0.017	ns	61	13.1	5	0.023	ns
Post-hoc [b]																					
Habitat type	Successional stage	*n*	obs.	exp.	*p*-value	sign.	*n*	obs.	exp.	*p*-value	sign.	*n*	obs.	exp.	*p*-value	sign.	*n*	obs.	exp.	*p*-value	sign.
light-exposed	15 days	56	5	9.3	0.120	ns	73	3	12.2	<u>0.003</u>	(−)	76	14	12.7	n.a.	n.a.	61	7	10.2	n.a.	n.a.
	30 days	56	3	9.3	0.023	ns	73	13	12.2	<u>0.007</u>	(−)	76	21	12.7	n.a.	n.a.	61	5	10.2	n.a.	n.a.
	90 days	56	5	9.3	0.120	ns	73	4	12.2	<u>< 0.001</u>	(−)	76	7	12.7	n.a.	n.a.	61	13	10.2	n.a.	n.a.
cryptic	15 days	56	3	9.3	0.008	ns	73	4	12.2	<u>0.001</u>	(−)	76	18	12.7	n.a.	n.a.	61	7	10.2	n.a.	n.a.
	30 days	56	12	9.3	0.084	ns	73	20	12.2	0.117	ns	76	8	12.7	n.a.	n.a.	61	19	10.2	n.a.	n.a.
	90 days	56	28	9.3	<u>< 0.001</u>	(+)	73	29	12.2	<u>0.003</u>	(+)	76	8	12.7	n.a.	n.a.	61	10	10.2	n.a.	n.a.

[a]Bonferroni-corrected *p*-value of 0.013 to correct for multiple hypothesis testing (*n* = 4 tests)
[b]Bonferroni-corrected *p*-value of 0.008 to correct for multiple hypothesis testing (*n* = 6 tests)

Table S4.3. Statistical output for experiment 2 in which settling *Favia fragum* larvae were provided with the choice among algal communities at the same successional stage, but grown under different nutrient-herbivory treatment. Chi-square goodness of fit tests were used to compare the overall distribution of settlers among the eight possible algal communities (*4 nutrient-herbivory treatments × 2 habitat types*) to the expected even distribution of 1:1:1:1:1:1:1:1 assuming random settlement behaviour. In case of significant effects (*), exact binomial tests were performed for each of the eight individual algal communities to identify which were either preferred (+) or avoided (−) by settling larvae. Significant *p*-values are underlined and ns stands for non-significant.

		15 days					30 days					90 days				
		n	χ^2	df	*p*-value	sign.	*n*	χ^2	df	*p*-value	sign.	*n*	χ^2	df	*p*-value	sign.
Overall distribution [a]		231	107.1	7	< 0.001	*	127	37.2	7	< 0.001	*	153	79.4	7	< 0.001	*
Post-hoc [b]																
Habitat type	Nutrient-herbivory treatment	*n*	obs.	exp.	*p*-value	sign.	*n*	obs.	exp.	*p*-value	sign.	*n*	obs.	exp.	*p*-value	sign.
light-exposed	N^-H^+	183	8	28.9	< 0.001	(−)	127	13	15.9	0.085	ns	153	12	19.1	0.021	ns
	N^+H^+	183	16	28.9	0.006	ns	127	14	15.9	0.099	ns	153	9	19.1	0.003	(−)
	N^-H^-	183	11	28.9	< 0.001	(−)	127	3	15.9	< 0.001	(−)	153	10	19.1	0.007	ns
	N^+H^-	183	13	28.9	< 0.001	(−)	127	9	15.9	0.019	ns	153	15	19.1	0.063	ns
cryptic	N^-H^+	183	24	28.9	0.074	ns	127	28	15.9	< 0.001	(+)	153	50	19.1	< 0.001	(+)
	N^+H^+	183	63	28.9	< 0.001	(+)	127	30	15.9	< 0.001	(+)	153	29	19.1	0.006	ns
	N^-H^-	183	48	28.9	< 0.001	(+)	127	18	15.9	0.086	ns	153	23	19.1	0.059	ns
	N^+H^-	183	48	28.9	< 0.001	(+)	127	12	15.9	0.067	ns	153	5	19.1	< 0.001	(−)

[a] Bonferroni-corrected *p*-value of 0.017 to correct for multiple hypothesis testing (*n* = 3 tests)

[b] Bonferroni-corrected *p*-value of 0.006 to correct for multiple hypothesis testing (*n* = 8 tests)

Table S4.4. Statistical output for experiment 3 in which settling *Favia fragum* larvae were provided with one of the 12 possible algal communities (*4 nutrient-herbivory treatments* × *3 successional stages*) at a time and allowed to settle for 8 days. A three-way ANOVA was performed to test the effects of successional stage, nutrients and herbivory as well as their interactive effects. In case of significant effects (*), Bonferroni's *post hoc* pairwise comparisons were performed to determine treatment groupings. Significant *p*-values are underlined and ns stands for non-significant.

Three-way ANOVA

Source	SS	df	MS	*F*	*p* -value	sign.
Intercept	145700	1	145700.0	353.1	< 0.001	*
Nutrients	188	1	188.3	0.5	0.501	ns
Herbivory	1268	1	1268.1	3.1	0.083	ns
Successional stage	3182	2	1590.9	3.9	0.025	*
Nutrients × Herbivory	1490	1	1490.1	3.6	0.061	ns
Nutrients × Successional stage	258	2	129.0	0.3	0.732	ns
Herbivory × Successional stage	1005	2	502.4	1.2	0.301	ns
Nutrients × Herbivory × Successional	1066	2	532.8	1.3	0.280	ns
Error	36315	88	412.7			

Bonferonni's *post hoc* comparisons

Successional stage		mean difference	SE	*p* -value	sign.
15 days	30 days	−10.12	5.4	0.1823	ns
	90 days	−13.7	5.2	0.0291	*
30 days	15 days	10.2	5.4	0.1823	ns
	90 days	−3.5	5.6	1.0000	ns
90 days	15 days	13.7	5.2	0.0291	*
	30 days	3.5	5.6	1.0000	ns

Table S4.5. Statistical output for experiment 3 in which settling *Favia fragum* larvae were provided with one of the 12 possible algal communities (*4 nutrient-herbivory treatments* × *3 successional stages*) at a time and allow to settle for 8 days. Exact binomial tests were performed to determine if larvae preferred settling in light-exposed or cryptic habitats based on the expected distribution of 1:1 assuming random settlement behaviour. Significant *p*-values are underlined and ns stands for non-significant.

Nutrient-herbivory treatment	Successional stage	*n*	obs. cryptic	obs. light-exposed	exp.	*p*-value[a]	sign.
N^-H^+	15 days	43	18	25	22	0.0692	ns
	30 days	42	21	21	21	0.1224	ns
	90 days	54	29	25	27	0.0934	ns
N^+H^+	15 days	16	5	11	8	0.0667	ns
	30 days	13	8	5	7	0.1571	ns
	90 days	23	8	15	12	0.0584	ns
N^-H^-	15 days	20	4	16	10	0.0046	ns
	30 days	41	19	22	21	0.1113	ns
	90 days	29	11	18	15	0.0644	ns
N^+H^-	15 days	50	21	29	25	0.0598	ns
	30 days	39	13	26	20	0.0148	ns
	90 days	40	17	23	20	0.0807	ns

Table S4.6. Statistical output for chi-square goodness of fit tests performed to compare the composition of algal communities directly surrounding (1 mm) *Favia fragum* settlers (observed composition) to that of the overall algal community composition that developed on the light-exposed and cryptic surfaces of the tiles (expected composition) for each of the nutrient-herbivory treatments and successional stages. A Bonferroni-corrected *p*-value of 0.0021 was used to correct for multiple hypothesis testing (n = 24 tests). Significant *p*-values are underlined.

		Natural reef conditions			Nutrient enrichment			Herbivory reduction			Nutrient enrichment & herbivory reduction		
Habitat type	Successional stage	*n*	χ^2	*p* -value	*n*	χ^2	*p* -value	*n*	χ^2	*p* -value	*n*	χ^2	*p* -value
light-exposed	15 days	38	28.26	<u>≤0.0001</u>	30	51.97	<u>≤0.0001</u>	41	116.92	<u>≤0.0001</u>	50	306.06	<u>≤0.0001</u>
	30 days	37	150.48	<u>≤0.0001</u>	32	63.69	<u>≤0.0001</u>	46	116.43	<u>≤0.0001</u>	40	287.19	<u>≤0.0001</u>
	90 days	42	132.79	<u>≤0.0001</u>	28	123.18	<u>≤0.0001</u>	35	259.25	<u>≤0.0001</u>	51	546.47	<u>≤0.0001</u>
cryptic	15 days	45	34.89	<u>≤0.0001</u>	72	14.59	0.100	70	21.38	0.010	76	13.59	0.14
	30 days	61	24.23	0.004	58	18.82	0.027	45	19.41	0.020	44	5.95	0.75
	90 days	107	23.05	0.006	66	37.00	<u>0.001</u>	42	27.84	<u>0.001</u>	32	11.14	0.27

Table S4.7. Statistical output for exact binomial tests performed to compare the relative cover of each of the five benthic classes (i.e., bare substratum, CCA, encrusting algae other than CCA, turf algae, macroalgae) directly surrounding (1 mm) *Favia fragum* settlers (observed cover) to that of the overall algal community composition that developed on the light-exposed and cryptic surfaces of the tiles (expected cover). n = 20 measurements, reflecting the 10% increments scored along the perimeter of each settler to assess the composition of their surrounding benthic community. A Bonferroni-corrected p-value of 0.005 was used to correct for multiple hypothesis testing (n = 10 tests). Significant p-values are underlined and ns stands for non-significant, whereas (+) and (−) signs denote whether specific benthic classes were preferred (+) or avoided (−) by settling larvae.

Habitat type	Benthic class	*n*	exp. %	obs. %	*p* -value	sign.
light-exposed	bare substratum	20	17.31	47.77	0.003	(+)
	CCA		9.65	22.22	0.083	ns
	encrusting algae other than CCA		18.93	22.76	0.217	ns
	turf algae		52.84	5.27	<0.001	(−)
	macroalgae		1.26	0.00	0.776	ns
cryptic	bare substratum	20	48.61	60.03	0.107	ns
	CCA		26.37	26.40	0.200	ns
	encrusting algae other than CCA		14.46	8.17	0.239	ns
	turf algae		9.45	2.48	0.137	ns
	macroalgae		0.93	0.04	0.829	ns

Chapter 5

Restoration of critically endangered elkhorn coral (*Acropora palmata*) populations using larvae reared from wild-caught gametes

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Abstract

Elkhorn coral (*Acropora palmata,* Lamarck 1816) populations provide important ecological functions on shallow Caribbean reefs, much of which was lost when a disease reduced their abundance by more than 95% beginning in the mid-1970s. Since then, a lack of significant recovery has prompted rehabilitation initiatives throughout the Caribbean. Here, we report the first successful outplanting and long-term survival of *A. palmata* settlers reared from gametes collected in the field. *A. palmata* larvae were settled on clay substrates (substrate units) and either outplanted on the reef two weeks after settlement or kept in a land-based nursery. After 2.5 years, the survival rate of *A. palmata* settlers outplanted two weeks after settlement was 6.8 times higher (3.4%) than that of settlers kept in a land-based nursery (0.5%). Furthermore, 32% of the substrate units on the reef still harboured one or more well-developed recruit compared to 3% for substrate units kept in the nursery. In addition to increasing survival, outplanting *A. palmata* settlers shortly after settlement reduced the costs to produce at least one 2.5-year-old *A. palmata* individual from $325 to $13 USD. Thus, this study not only highlights the first successful long-term rearing of this critically endangered coral species, but our results also show that early outplanting of sexually reared coral settlers can be more cost-effective than the common approach of nursery rearing for restoration efforts aimed at rehabilitating coral populations.

Introduction

Caribbean coral communities were historically dominated by elkhorn corals (*Acropora palmata*, Lamarck 1816) between depths of 0 to 5 m, where this species was so abundant that shallow Caribbean reef habitats were historically described as the "palmata zone" (Goreau 1959; Bak 1975). Well-developed *A. palmata* populations contribute to important ecological processes and services of Caribbean coral reefs such as habitat provisioning for a variety of reef organisms, coastal protection, gross community calcification, and nitrogen fixation (Nagelkerken 1974; Gladfelter et al. 1978; Harborne et al. 2006). During the mid to late 1970s, a Caribbean-wide outbreak of white-band disease (WBD) reduced the abundance of *A. palmata* by more than 95% (Gladfelter et al. 1978; Aronson and Precht 2001; Acropora Biological Review Team 2005; Jackson et al. 2014). After this period, populations that survived the disease were subsequently impacted by hurricanes (Goreau 1992), coral bleaching (Williams and Bunkley-Williams 1990), algal overgrowth (McClanahan and Muthiga 1998), and predation by corallivorous snails and damselfish (Williams and Miller 2012). The species' decline without indications of recovery and current lack of recruitment (van Moorsel 1989; Vermeij et al. 2011) resulted in its listing as "critically endangered" on the Red List of Threatened Species in 2008 (IUCN 2013).

Lower coral species diversity in the Caribbean compared to the Indo-Pacific (Veron 2000; Spalding et al. 2001; Roberts et al. 2002; Miloslavich et al. 2010) limits functional redundancy in this region, thus the probability that the local regional species pool harbours species capable of replacing others that have declined in abundance is quite low in the Caribbean (Fonseca and Ganade 2001; Bellwood et al. 2004). This is particularly true for the Caribbean coral genus *Acropora* which consists of only three species. In contrast, Indo-Pacific acroporids comprise more than one hundred species (Veron 2000), making it unsurprising that the local decline of one or a few Indo-Pacific *Acropora* species is generally followed by rapid colonization and regrowth of others (Kojis and Quinn 2001). In contrast, no Caribbean coral species has filled the habitat in which *A. palmata* was formerly abundant (Nagelkerken and Nagelkerken 2004). Since no other Caribbean coral species resembles *A. palmata* morphologically or ecologically, the return of *A. palmata* itself appears the only pathway by which shallow Caribbean reefs could regain their former composition and function.

To assist the recovery of *A. palmata* populations, restoration efforts were initiated throughout the Caribbean region using asexual propagation or "coral

gardening" approaches whereby fragments are cultured from donor colonies in nurseries before they are outplanted on the reef (Young et al. 2012). While asexual propagation of Caribbean acroporids has been successful (e.g., Quinn and Kojis 2006; Johnson et al. 2011; Nedimyer et al. 2011), it requires that fragments are harvested from otherwise healthy colonies. It also limits the formation of new genotypes through genetic recombination, which may hamper the generation of genotypes better adapted to the altered environmental conditions on modern-day Caribbean reefs (Reusch et al. 2005; Baums 2008). Using sexually- instead of asexually-produced offspring avoids these issues. Moreover, the use of eggs rather than fragments could yield a much larger number of individuals that can be reared for restoration efforts. Attempts to raise sexual recruits of *A. palmata* in closed-system aquaria has proven partially successful (Petersen et al. 2008), but has only led to the outplanting of a small number of individuals and lacks evidence of significant long-term survival (Szmant and Miller 2006; Miller 2014; AM Szmant and MW Miller, pers. comm.). Presently, successful rearing and outplanting of large numbers of sexually produced larvae followed by long-term survival (> 6 months) has only been reported for three Indo-Pacific acroporid species (Omori et al. 2008; Nakamura et al. 2011; Villanueva et al. 2012; Guest et al. 2014), whereas studies achieving similar success for any Caribbean coral species do not exist.

Reared coral larvae are generally settled onto artificial substrates and kept in land-based or ocean nurseries for several months to years before they are outplanted (Nakamura et al. 2011; Baria et al. 2012; Villanueva et al. 2012; Guest et al. 2014). Land-based nurseries are generally assumed to offer stable and more protected environments for coral settlers relative to actual reef environments due to reduced fish predation, algal competition, and sedimentation (Nakamura et al. 2011). Because increased size corresponds to lower mortality in recently settled corals (Vermeij and Sandin 2008), extended grow-out periods are expected to increase the success of restoration efforts by allowing settlers to grow before they are outplanted on a reef. Alternatively, outplanting coral offspring soon after settlement might select for genotypes capable of acclimatizing to the conditions at the outplant site. Exposing recently settled corals to moderate stress conditions could also lead to increased tolerance to more severe stress conditions experienced later in life (van Oppen et al. 2015), a process equivalent to "hardening" in plants (Beck et al. 2004). Keeping settlers in nurseries for long periods of time or by outplanting them soon after settlement are consequently two different approaches that each have specific advantages for restoration purposes.

Reduced nursery periods would also aid to making restoration efforts more economically viable. Large scale restoration efforts are currently extremely expensive due to the high costs associated with nursery maintenance and the outplanting of artificial substrates by hand. The costs to rear and outplant one artificial substrate containing at least one coral recruit ("recruit-substrate unit", RSU) currently range from $5.40 USD (Villanueva et al. 2012) to $163 USD (Nakamura et al. 2011). Such estimates only include expenditures until the initial outplant of the RSUs and assume that all outplanted RSU will become one adult colony. Because not all outplanted recruits will survive, a much larger number of RSUs is needed to repopulate an area, which further increases the costs of any restoration effort. Reducing the time that settlers spend in a nursery could lower the costs associated with restoration efforts, but it is currently not known whether early outplanting of settled coral larvae represents a more effective and cost-efficient restoration approach compared to traditional nursery-based methods.

Here we describe the first successful rearing, outplanting and long-term (2.5 yr) survival of *A. palmata* recruits that were reared from gametes collected in the field. We tested whether the effectiveness of restoration efforts for this critically endangered Caribbean coral species could be improved by shortening *ex situ* grow-out periods to two weeks before outplanting settlers on the reef and determined whether post-settlement survival, colony growth and cost-effectiveness was significantly different from those of recruits reared in land-based nurseries over a 2.5 year period.

Material and Methods

Study location and nursery set-up

This study was carried out on the island of Curaçao (12ºN, 69ºW) in the Southern Caribbean. Reproductively active *A. palmata* populations are abundant at our study site near the Curaçao Sea Aquarium (12°04'59"N, 68°53'44"W) (figure 5.1*a*). In 2010, a land-based facility to rear corals and their larvae was built at this site, consisting of five individual, flow-through aquaria (215 × 69 × 64 cm, L × H × W; acrylic). Two centrifugal pumps (1.5 HP; Hayward Super-pumps, NJ, USA) pumped seawater through a 100-m-long polypropylene pipe (Ø 10 cm) with an off-shore intake at 7 m depth through each aquarium at a rate of ~ 2,300 L hr^{-1}. The continuous pumping of seawater ensured that the water temperature in the five aquaria followed natural fluctuations in sea surface temperatures (SST). Seawater

entering each aquarium was first filtered through a bag filter (200 µm; Pentair Aquatic Eco-Systems Inc., NC, USA) to reduce the accumulation of sediments and debris in the tanks. Filters were cleaned every two days. Additional water movement in each aquarium was provided by a recirculation pump (~ 1,823 L hr^{-1}, SHE 2.9; Sweetwater High-Efficiency Pump, FL, USA,). All tanks were placed under a roof of UV-permeable acrylic (70% UV transmission, Solacryl SUVT; Spartech Polycast, USA) to expose corals to natural light/dark cycles. Every week, sediments and algae were manually removed from the aquaria throughout the study period. Algal growth was also suppressed by juvenile doctorfish (*Acanthurus chirurgus*, < 5 cm total length, ~10 per aquarium) and blue-legged hermit crabs (*Clibanarius tricolor*, ~100 per aquarium). Visual assays at the beginning of the study confirmed that these species did not interact with or fed on coral recruits.

Gamete collection, larval rearing and settlement

Acropora palmata is a hermaphroditic broadcasting coral species that releases gametes once or twice a year in the fall (Szmant 1986; figure 5.1*b*). Three days after the full moon in August 2012, we collected egg-sperm bundles from four colonies between depths of 1 to 5 m. The colonies spawned 3.5 hrs after sunset and gamete bundles were collected using cone-shaped nylon nets, in which the floating gamete bundles concentrated at the top of the net into an inverted removable 50-mL polypropylene conical centrifuge tube (Falcon; Fisher Scientific, USA). Collected bundles were immediately transported to the lab. After bundles had broken apart, we mixed sperm and eggs from all colonies in one 2.0-L plastic bowl (Sterilite, MA, USA). We added filtered seawater (GF/F) to obtain a sperm concentration of ~10^6 cells mL^{-1} following Hagedorn et al. (2009). Fertilization was allowed to take place for 1.5 hrs after which we rinsed eggs and embryos twice over a 100-µm plankton mesh with filtered seawater (GF/F) to remove excess sperm. We then transferred the embryos to specially-designed kreisels that were used as larval rearing devices (Hagedorn et al. 2009; figure 5.1*c*). These kreisels consisted of 18-L heavy-walled polyethylene drum funnels (Bel-Art SP Scienceware, CA, USA) with four 13.0 × 4.5 cm nylon screen mesh (240 µm) covered openings at their undersides. The kreisels were placed at the surface of the aquaria described above and stable temperatures (28-29°C) and salinities (~35 ppt) within each kreisel were ensured through continuous water exchange with the aquarium water. An adjustable upward-directed water flow was generated from the bottom centre of the kreisels by a submergible water pump (700 gph, model 7, Magnetic Drive Pump; Danner Manufacturing, NY, USA) to ensure that developing embryos were distributed evenly throughout the kreisels. Four adjustable water spigots were mounted around

the side of each kreisel to create a rotating water flow that prevented developing embryos from sticking to the kreisel walls. Each kreisel contained 15 L of seawater and water inside each was refreshed at approximately 2 L min^{-1}. During the early stages of embryo development till the end of gastrulation water flow was kept lower (1 L min^{-1}) to maintain embryo integrity (Heyward and Negri 2012). Embryo density in the kreisels was kept low (600-700 embryos L^{-1}) to prevent the build-up of bacteria that thrive on the substances released (mainly lipids) by dying embryos and larvae. The percentage of successfully fertilized eggs was determined 3h after fertilization by quantifying the proportion of eggs going through cell divisions. Three days post-fertilization, we transferred all larvae to four plastic containers (36 ×31 × 24 cm, L × W × H; Sterilite, MA, USA) to allow larvae to settle. Each container contained ~23 L of filtered seawater (50 µm) and 80 clay pottery tripods (kiln stilts, Ø 6 cm, Carl Jaeger Tonindustriebedarf GmbH, Germany; figure 5.1*d*). The tripods provide different surface orientations for settlement, and their low physical profile (0.8 cm height) ensures that growing recruits can attach to the reef substrate early on. The tripods had been cured for two months in the aquarium system to allow the development of biofilms known to induce larval settlement in corals (Ritson-Williams et al. 2010). Water inside the settlement bins was exchanged daily (75%) to maintain water quality and kept at 28-29°C by partly submerging the bins inside the culture aquaria. Airlifts placed at opposite corners of the containers ensured water movement inside the settlement containers. Approximately 2,500 larvae were added to each settlement bin and allowed to settle for five days after which we sub-sampled 80 tripods and assessed settlement rates under a blue light (Nightsea, MA, USA). All tripods with ≥ 1 settler (henceforth described as a recruit-substrate unit or RSU) were placed inside one of the large flow-through aquaria to allow further development.

Outplanting experiment

To assess the effect of shortened grow-out periods, we compared the survival and growth of settlers transferred to the reef at the age of two weeks (figure 5.1*e*) to that of settlers raised in the land-based nursery over a period of 2.5 years. To minimize potential confounding effects of density dependent processes (Suzuki et al. 2012; Edwards et al. 2015), we only used RSUs with roughly similar settler densities (mean settler density per substrate unit = 11.1 ± 4.4 SD). We recorded the exact location of each settler on 60 RSUs of which 30 were randomly assigned to the reef and 30 to the land-based nursery. This allowed us to track the initial settlers during subsequent surveys and identify *A. palmata* recruits that could have recruited naturally to the RSUs after the initial outplant. We transported the RSUs to the reef

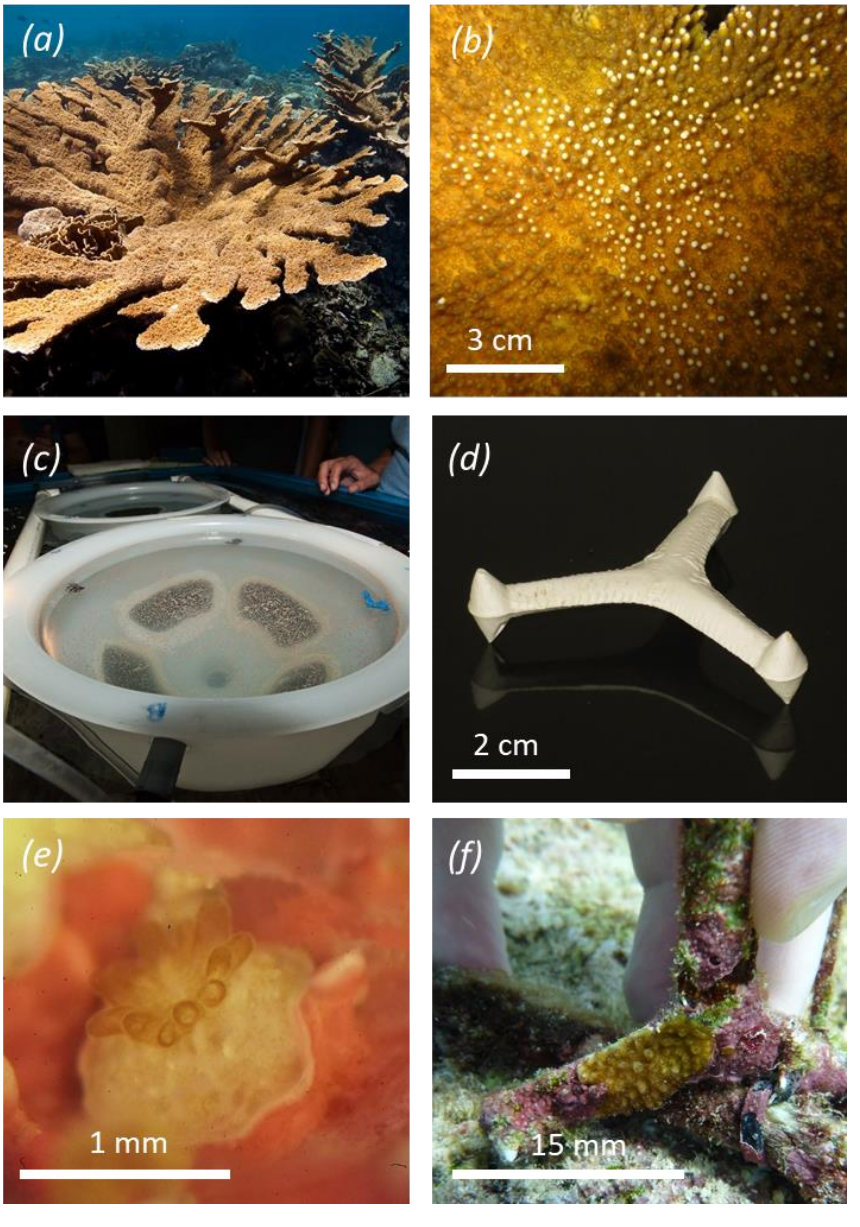

Figure 5.1. *(a)* Healthy *Acropora palmata* population at the Curaçao Sea Aquarium reef. *(b) A. palmata* colony releasing sperm-egg bundles. *(c)* The kreisel system in which *A. palmata* embryos developed. *(d)* Tripod made of clay for larval settlement. *(e)* Two-week-old *A. palmata* settler and *(f)* one-year-old *A. palmata* recruit outplanted to the reef two weeks post-settlement. Photos by *(a,c)* P Selvaggio, *(b,d,e)* VF Chamberland and *(f)* D Petersen.

in Ziploc bags and attached them with zip-ties at 50 cm intervals to three pre-installed nylon ropes (5 m) at approximately 2.5 m water depth. The tripods were attached so that the side facing up during the settlement experiment was also facing upwards on the reef. Tripods were not permanently fixed to the reef so that they could be returned to the laboratory to assess the survival rates of all known settlers after one month. Hereto, we carefully detached the tripods by cutting the zip-ties and quantified the number of surviving *A. palmata* settlers *ex situ* under a dissecting microscope, after which all RSUs were returned to their original locations on the reef. The RSUs kept in the land-based nursery were assessed similarly. We quantified the proportion of live settlers after 6, 11, 17, and 31 months *in situ* because settlers had grown sufficiently large (> 3 mm) to be counted by the naked eye. In addition to recruit survivorship, we calculated the proportion of substrate units that still harboured ≥ 1 settler (i.e., represented a RSU) through time for both treatments. We also calculated the proportion of the total number of settlers that died between surveys. Recruit size was measured after 17 and 31 months by photographing individual colonies in planar view against a ruler for scale so that their size could be measured using the imaging software ImageJ (Abramoff et al. 2004).

Cost-effectiveness analysis

The cost-effectiveness of the two restoration methodologies was calculated following Edwards (2010). These costs pertained to (1) nursery maintenance, (2) larval rearing and settling and (3) RSU outplanting and monitoring (table 5.1). The costs to build our land-based nursery facility amounted to US$52,100.00 (table S5.1 in supplementary material) but were not included in the cost-effectiveness analysis. Even simple flow-through aquarium systems such as the one we used are not essential for mass culture of coral larvae. Equal larval rearing success has been achieved using low-technology equipment such as plastic containers filled with static filtered-seawater (Edwards 2010; Villanueva et al. 2012; Guest et al. 2014). If the bottom of each aquarium would have been maximally covered, the maximum number of RSUs that could be produced in our experimental setup was 4,000. We used this number in our cost-effectiveness calculations assuming that the full capacity of our nursery system would normally be used to support large scale restoration efforts. We calculated the fraction of the initial 4,000 RSUs that would remain after 1, 6, 11, 17, and 31 months assuming similar survival rates observed for the RSUs that were outplanted to the reef and those kept in the nursery system.

The cost-calculations for rearing and settling larvae were identical for both treatments. For RSUs kept in the nursery for 2.5 years, the monthly maintenance costs of the nursery were included in the costs to produce one RSU. For RSUs that were outplanted on the reef two weeks after settlement, only one month of nursery maintenance was included to account for its use during initial larval rearing and settling. All 4,000 RSUs were simultaneously outplanted two weeks after settlement resulting in a fixed one-time cost. The cost of outplanting one nursery-raised RSU at 1, 6, 11, 17 and 31 months was set to $1/4{,}000^{th}$ of the costs to outplant 4,000 RSUs to the reef at once. This underestimates the true costs of outplanting the increasingly smaller number of available RSUs through time, but is assumed to suffice as an estimate within the context of our study. Finally, we performed a cost breakdown analysis to identify which elements contributed most to the total production costs for 2.5-year-old RSUs reared on the reef and in the nursery.

Statistical analysis

We used a maximum likelihood (ML) approach to test if settler survival rates differed between the two treatments. We assumed that the probability of survivorship for individuals that settled on the same tile was a function of experimental conditions, but that survivorship of each individual was independent of the fate of neighbours on the same substrate unit. As such, we assumed that tile-specific survivorship (i.e., proportion of surviving settlers relative to the starting density) was binomially distributed. We assumed that survivorship within each treatment was determined by a single parameter, s_x, such that the proportion of settlers surviving at the end of the experiment was binomially distributed around the expectation $n_{j,x}s_x$, where n_{jx} is the initial density on tile j exposed to treatment x. The best-fit values of all distinct parameter combinations were estimated and the best combination of parameters was selected using Akaike's Information Criteria (when the number of parameters was different) and based on an assumption of equal Bayesian prior expectations (when the number of parameters was the same). See Hilborn and Mangel (1997) or Vermeij and Sandin (2008)for more details on this statistical approach.

Differences in the probability that one substrate unit still harbours at least one settler through time (i.e., proportion of remaining RSUs) between the reef and the nursery were tested with Fisher's exact test. Because settler mortality rates (i.e., the average proportion of settlers dying per month) and size data did not meet the assumption of a normal distribution (Shapiro-Wilk, $p < 0.05$), we tested for differences in growth and mortality rates between corals reared *in situ* and those

kept in the nursery using a one-way non-parametric analysis of variance (PERMANOVA, Anderson 2001) in PAST 1.97 (Hammer et al. 2001).

Table 5.1. Overview of the monetary costs to produce 4,000 recruit-substrate units (RSUs) separated by the costs for nursery maintenance costs, larval rearing and outplanting/monitoring.

Item	Specifications	Quantity	Cost per item [a]	Total cost [a]	Percent
Nursery maintenance costs (per year)					
Labor[b]		*(hours)*			
maintenance	1 aquarist, 2 hrs/day, 365 days	$730.00	$10.50	$7,665.00	60%
repairs	1 construction worker, 2 hrs/mo, 12 mo	$24.00	$8.75	$210.00	2%
Utilities					
electricity & (fresh) water				$5,000.00	21%
			total	$12,875.00	100%
			cost per month	*$1,072.92*	
Larval rearing costs					
Labor		*(hours)*			
gamete collection	4 divers, 3h/day, 4 days	$48.00	$10.50	$504.00	6%
rearing work	2 aquarists, 8h/day, 10 days	$160.00	$10.50	$1,680.00	19%
Materials		*$0.00*			
gamete collection	SCUBA gear	$4.00	$400.00	$1,600.00	18%
gamete collection	SCUBA air tanks	$16.00	$5.00	$80.00	1%
gamete collection	nylon nets	$30.00	$15.00	$450.00	5%
larval culture	kreisels	$20.00	$80.00	$1,600.00	18%
settlement substrates	tripods	$4,000.00	$0.60	$2,400.00	27%
consumables	various (e.g., pipettes, filter bags, plastic containers)	1 (order)	$500.00	$500.00	6%
			total	$8,814.00	100%
Outplanting[c] and Monitoring[d]					
Labor		*(hours)*			
rope installation	2 divers, 3 dives/day, 6h/day, 9 days	$108.00	$10.50	$1,134.00	18%
RSU outplant	2 divers, 3 dives/day, 6h/day, 14 days	$168.00	$10.50	$1,764.00	28%
monitoring	2 divers, 3 dives/day, 6h/day, 6 days	$72.00	$10.50	$756.00	12%
Materials		*(number)*			
rope installation	SCUBA air tanks	$50.00	$5.00	$250.00	4%
rope installation	1000 m nylon rope	$1.00	$500.00	$500.00	8%
rope installation	U-shaped stainless steal nails	$3,000.00	$0.10	$300.00	5%
RSU outplant	SCUBA air tanks	$80.00	$5.00	$400.00	6%
RSU outplant	cable-ties	$4,000.00	$0.25	$1,000.00	16%
monitoring	SCUBA air tanks	$36.00	$5.00	$180.00	3%
			total	$6,284.00	100%

[a] Costs are in US dollars.

[b] Wages are based on standard Curaçaoan allowances at the time the project was started in 2010 (Curaçao GDP per capita is $15,000 (Central Inteligence Agency 2015)

[c] Assuming that one diver secures 20 m of rope to the reef substrate, or outplants 50 RSUs per 1h dive

[d] Assuming that 10% of the outplants are monitored after 6, 12, and 18 months, and that one diver monitors 50 outplants per 1h dive.

Results

Outplanting experiment

Eighty percent of the collected eggs were fertilized, resulting in a total of approximately 10,000 *A. palmata* larvae three days after spawning occurred. Approximately 4,000 larvae were placed in the settlement containers and settled on 320 tripods at an average density of 12.5 ± 9.7 (mean ± SD) settlers per tripod (range = 1-41, n = 80 tripods). Settlement most commonly occurred on the undersides of the tripods (79.6% ± 18.4, n = 80 tripods). During the first month after outplanting, the settlers returned to the reef at the age of two weeks showed 15% lower survival than those kept in the nursery (ML, $p < 0.05$), but their survival was 3-9% higher for each subsequent time point (ML, $p < 0.05$; figure 5.2*a*). As a result, the average survival of *A. palmata* settlers after 31 months was 6.8 times higher for settlers grown on the reef (3.4% ± 1.3, mean ± SE) than for those kept in the nursery (0.5% ± 0.5). Outplanting recently settled larvae also resulted in 10 times more RSUs that could be used for restoration purposes (Fisher's exact test, $p < 0.05$; figure 5.2*b*), as 32% of the tripods on the reef still harboured at least one recruit after 31 months versus only 3% in the land-based nursery.

Mortality rates in our land-based nursery gradually decreased through time, whereas mortality rates of settlers on the reef increased or decreased between subsequent surveys (figure 5.2*c*). The highest mortality rate for settlers returned to the reef at the age of two-weeks occurred during the first month after outplanting, during which 34.2% ± 4.2 (mean ± SE) of the settlers died (figure 5.2*c*). The survival rate of larvae that had settled on the undersides of the tripods was twice as high during this month (72.0% ± 5.1) than of individuals that had settled on the topsides of the tripods (35.2% ± 7.9) (ML, two-parameter model, $p < 0.05$). Mortality rates of outplanted settlers remained high during the following five months after which 15.3% ± 2.5 of the settlers were still alive (figure 5.2*a,c*). Mortality rates rapidly decreased thereafter and remained low (average monthly mortality rate: 2.8% ± 1.2; figure 5.2*c*) until settlers were 11-month-old. Mortality rates increased between 11 and 17 months during which 8.6% ± 1.6 of the remaining settlers died every month on average (figure 5.2*a,c*). After 17 months mortality rates declined again and remained low until the end of the study (figure 5.2*c*).

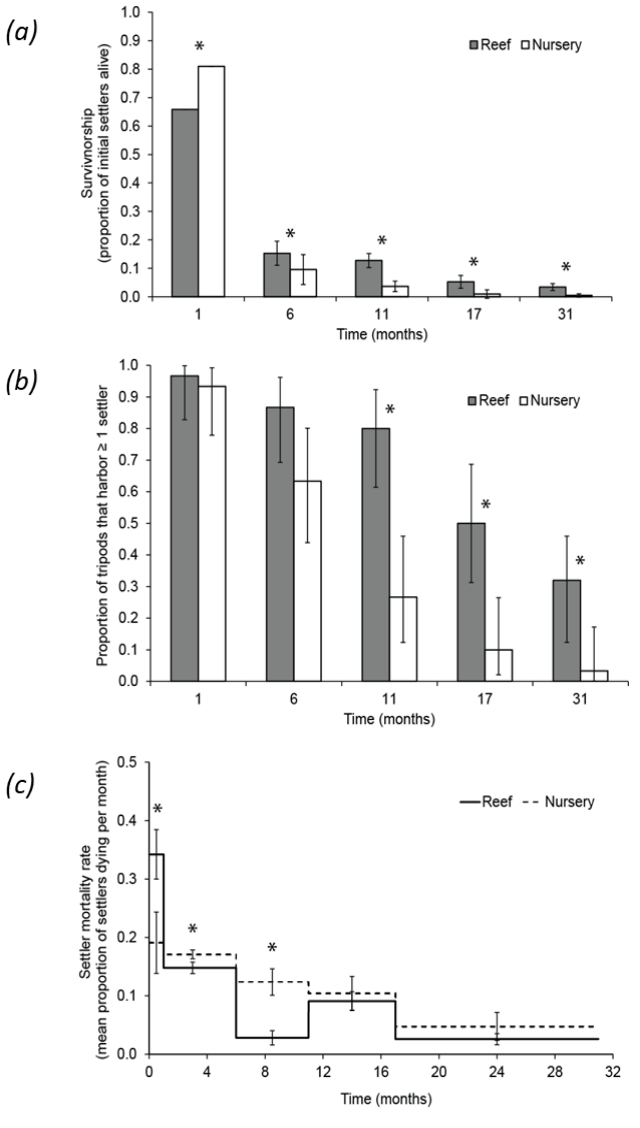

Figure 5.2. Proportion of *(a) A. palmata* settlers alive and *(b)* of substrate units harbouring at least one live settler (RSU) on the reef and in the land-based nursery after 1, 6, 11, 17, and 31 months. *(c)* Settler mortality rates on the reef and in the nursery throughout the 31 months study period. The proportion of settlers that died between surveys was standardized to a monthly mortality rate. *Error bars* in *(a,c)* are standard error and in *(b)* are 95% confidence intervals (Clopper-Pearson's exact method). *Asterisks* indicate statistically different groups as determined with a maximum likelihood in *(a)* Fisher's exact test of independence in *(b)* and with a PERMANOVA in *(c)*, with $p < 0.05$ and $n = 30$ substrate units.

Settlers in the nursery experienced equal or higher mortality rates compared to outplanted settlers, with the exception of the first month after settlement when mortality rates of settlers kept in the nursery were 1.8 times lower than those of outplanted settlers (PERMANOVA, $p < 0.01$). Mortality rates of 6 to 11-month-old settlers were 4.4 times higher in the nursery than on the reef (figure 5.2*c*), which resulted in a threefold reduction in both the number of live settlers and number of remaining RSUs in the nursery compared to the reef after 11 months (figure 5.2*a*,*b*). Higher mortality in the nursery during the first year of this study thus caused the resulting lower number of surviving settlers and RSUs in this treatment after 11 months until the end of the study, despite the fact that mortality rates on the reef and in the nursery were similar after 11 months.

The size of settlers outplanted to the reef and those kept in the nursery did not differ after 17 and 31 months (PERMANOVA, 17 mo, $p = 0.81$, 31 mo, $p = 0.61$). After 31 months, settlers on the reef measured 16.7 $cm^2 \pm 20.1$, mean ± SD, $n = 9$) whereas the only individual remaining in the nursery measured 12.9 cm^2. After 31 months, 6 out of the 9 remaining colonies on the reef had started forming upright branches that were 2.9 cm ± 1.4 ($n = 6$) tall on average. After 17 months, two out of 17 settlers growing on tripods had attached to the reef substrate and this number increased to three out of nine after 31 months. Because tripods were attached to a rope rather than directly attached to the substrate, most of the tripods were not entirely in contact with the reef substrate and could move, albeit slightly, on the rope, especially during storms. This likely also explained the loss of five tripods that had detached from the ropes between 17 and 31 months. None of the recruits attached to the aquarium surface in the nursery because the tripods had to be regularly moved for aquarium maintenance.

Cost-effectiveness analysis

At maximum capacity our system holds 4,000 RSUs, of which 133 (3.3%) would still have ≥ 1 settler after 31 months based on the data from our experiment (table 5.2). The number of remaining RSUs on the reef exceeded that in the land-based nursery almost 10-fold (32.0%) at the end of the 2.5 year study period (1280; table 5.2). Combining these data with the costs overviewed in table 5.1, a 2.5-year-old RSU reared on the reef cost $13 USD, whereas rearing one RSU in the nursery for the same period of time cost $325 (table 5.2). The nearly 30-fold higher costs to produce one nursery reared RSU mainly resulted from the costs associated with operating the nursery system, which accounted for 79% of the total costs of producing RSUs in the nursery (table 5.2). Larval rearing and outplanting contributed 55% and 39% to the total costs per RSU for RSUs that were returned to

the reef two weeks after settlement (table 5.2). Purchasing tripods accounted for almost one third of the rearing costs (27%), while manually outplanting 4,000 RSUs (i.e., air tank rental and labour) accounted for 57% of the total outplanting costs (table 5.1).

Table 5.2. Comparison of the cost-effectiveness and total cost breakdown for RSUs outplanted to the reef two weeks after settlement versus RSUs kept in the nursery for extended periods of time.

	Time (in months)	0	1	6	11	17	31
Nursery							
	remaining RSUs	100%	93%	63%	27%	10%	3%
	cost per RSU[a]	$3.50	$3.90	$7.40	$21.30	$71.30	$324.60
Reef							
	remaining RSUs	100%	97%	87%	80%	50%	32%
	cost per RSU[a]	$4.00	$4.20	$4.70	$5.10	$8.10	$12.60

Cost breakdown[a] (for a 31 mo old RSU)	Nursery maintenance	Larval rearing	Outplanting & monitoring
Nursery	79%	20%	1%
Reef	7%	55%	39%

[a] Costs are in US dollars

Discussion

This is the first study to report successful outplanting followed by long-term survival and growth of *Acropora palmata* settlers reared from gametes. Our results show that *A. palmata* larvae can be settled and outplanted, and that post-settlement survival rates were sufficiently high that, if produced in greater numbers, RSUs can be used for restoration purposes. One third of the outplanted RSUs harboured more than one juvenile colony after 2.5 years (figure 5.2*b*) despite high settler mortality during the first six months (86%; figure 5.2*a*). While seemingly high, mortality estimates for *A. palmata* in this experiment were similar to mortality rates for presumably more robust Indo-Pacific *Acropora* species (Edwards et al. 2010; Guest et al. 2014) and exceeded natural recruitment of *A. palmata* which currently approaches zero relative to historical baselines (van Moorsel 1989; Vermeij et al. 2011).

The geometry of the tripods (figure 5.1*d*), which allowed for settlement on undersides, appears to be an important element contributing to the long-term survival of the outplanted *A. palmata* settlers. The undersides of artificial settlement substrates are slowly colonized by cryptic communities that act as better refuges for coral settlers during the initial successional stages of these artificial substrates (Raimondi and Morse 2000; Vermeij 2006) compared to the upper surfaces. These more exposed upper surfaces are rapidly colonized by turf algae (Fricke et al. 2011) known to impair survival and growth in young corals (Babcock and Davies 1991; Babcock and Mundy 1996; Smith et al. 2001; Fabricius 2005; Miller 2014). Cryptic habitats such as the tripods' undersides represent only a relatively small proportion of the total surface provided by settlement substrates in previous studies, and/or settlers preferring this habitat were killed when those substrates were attached to the reef (Petersen et al. 2005; Nakamura et al. 2011; Villanueva et al. 2012; Guest et al. 2014; Miller 2014). In contrast, in this study the thin legs of the tripod substrates allowed older settlers on the cryptic undersides of the tripods to grow onto the exposed upper surfaces where their survival and growth benefitted from higher light levels (Maida et al. 1994; Babcock and Mundy 1996; Miller 2014). These observations illustrate the importance of including cryptic microhabitats into the design of artificial settlement substrates used for restoration purposes.

Acropora palmata recruits on the reef suffered unexpectedly high mortality sometime between 11 and 17 months post-outplanting (figure 5.2*a*,*b*), most likely caused by high wave action. Because tripods were attached to a rope instead of permanently secured to the reef, growing recruits could not attach to the reef substrate as most tripods were not in full contact with the reef framework (figure 5.1*f*) and moved back and forth during high wave action. The importance of firmly stabilizing outplanted recruits is confirmed by our observations from a simultaneously conducted experiment, whereby one-year-old *A. palmata* recruits were permanently stabilized onto an artificial breakwater consisting of large limestone boulders with epoxy (Star Brite, FL, USA). Here, all recruits had overgrown the tripods after only six months, and after 1.5 year colonies had higher growth (~20 times) and survival rates (67% after 2.5 years, $n = 9$) compared to equally old settlers used in this study (VF Chamberland and D Petersen, unpub. data; figure 5.3). Combined, these observations indicate that securing RSUs to the reef substrate would have improved the effectiveness of our current study.

Land-based nurseries are generally assumed to offer stable and more protected environments for coral settlers relative to actual reef environments due to reduced fish predation, algal competition, and sedimentation (Nakamura et al. 2011).

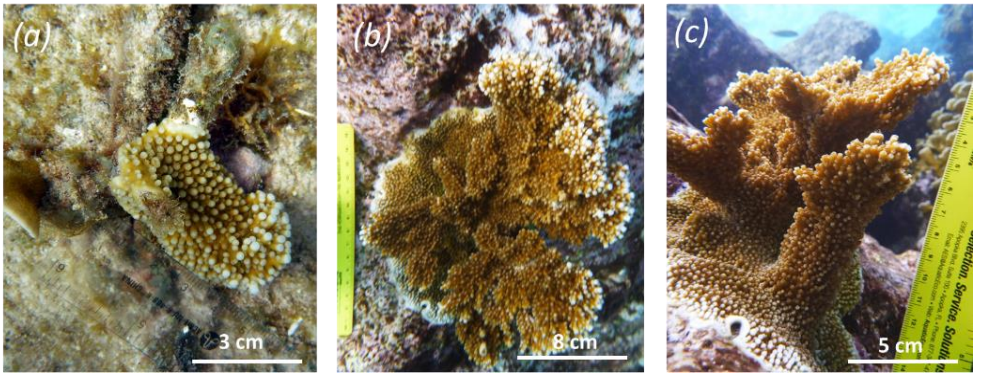

Figure 5.3. Juvenile *A. palmata* colonies outplanted at the Curaçao Sea Aquarium reef: *(a)* example of a 2.5-year-old colony that was attached to a nylon rope in this study was ~20 times smaller than *(b,c)* equally old colonies that were secured to an artificial breakwater with epoxy. Photos by VF Chamberland.

Nevertheless, *A. palmata* settlers that were transferred to the reef shortly after settlement survived 6.8 times better compared to settlers that were kept in our land-based rearing facility. Petersen et al. (2006) also found that the success of rearing *Acropora* recruits in aquaria is highly unpredictable due to the occurrence of unforeseen and uncontrollable changes in (a)biotic factors. Diseases, predators and parasites can reach unnaturally high abundances in confined aquarium environments when their natural enemies or environmental controls are absent (Petersen et al. 2006; Borneman 2008). In our nursery tanks, *A. palmata* recruits suffered from unexpected forms of predation and competition. A sudden ciliate infection caused ~25% of one-year-old colonies to die within 48h and the number of anemones and colonial hydroids occasionally explosively increased resulting in overgrowth and subsequent mortality of settlers in our nursery tanks (VF Chamberland, pers. obs.). Additionally, the trade winds that normally cooled our nursery ceased to blow in October 2012 coinciding with the annual maximum in seawater temperature (29.5°C; NOAA Coral Reef Watch 2012). This caused water temperatures inside the nurseries to rise to 31°C. While this temperature is not directly lethal for *A. palmata* (Desalvo et al. 2010; Polato et al. 2013) settlers did experience suboptimal temperature conditions for one month. Lastly, *A. palmata* typically occurs in areas with strong wave action (Bak 1975) and the growth rate of *Acropora* increases with increased water flow (Nakamura and Yamasaki 2005). Rearing species such as *A. palmata* that require highly hydrodynamic environments may therefore be challenging in aquarium systems where high wave action is difficult or impossible to generate. In sum, the combined effects of unforeseen stressors (diseases, competition, and physical factors) likely explain the lower survival rates observed

in our land-based nursery compared to that of settlers that were immediately outplanted to the reef. Extended grow-out periods in land-based nursery facilities therefore does not guarantee that more coral recruits can be reared for restoration purposes compared to much cheaper *in situ* approaches.

Our cost-benefit analysis demonstrated that rearing coral offspring in land-based facilities over extended periods of time was expensive and consequently economically unviable. Keeping one RSU in our nursery for 2.5 years cost $325, of which 79% covered the operational costs of the nursery (table 5.2). These estimates are in the same order of magnitude as those reported by Nakamura et al. (2011) who calculated that producing one 10-mo-old RSU reared in an outdoor nursery cost $163 (*A. tenuis*). Shortening grow-out periods from 2.5 years to two weeks significantly lowered the costs of producing *A. palmata* RSUs. After 2.5 years, a RSU on the reef cost $13 compared to $325 for those kept in the nursery. The cost breakdown analysis showed that the cost-effectiveness of restoration methods could be further improved by using cheaper settlement substrates and more efficient outplanting methodologies. In the end, the cost at which restoration efforts can be considered affordable is extremely subjective. The estimated costs to restore one hectare of degraded reef are extremely variable and range from US$ 13,000 to transplant fragments in Tanzania to millions of US$ per ha to restore a ship grounding site in the Florida Keys (Spurgeon 2001). The resources that one nation or organization is willing to allocate to restoration and the spatial extent of planned restoration efforts will ultimately define the economic viability of restoration practices on a case-by-case basis (Spurgeon 2001).

Shortening grow-out periods for *A. palmata* settlers shortly after settlement not only reduced costs but also yielded 10 times more RSUs that could be used for restoration, though we stress that such findings will depend on the quality of both the aquarium system and reef chosen for outplanting. Nevertheless, exposing two-week-old *A. palmata* settlers to natural reef conditions at our study site did not negatively impact their survival and increased the long-term success of this restoration approach (Ritson-Williams et al. 2010; Miller 2014). While post-settlement survival was relatively high in our study, the factors determining post-settlement survival differ across space (Vermeij 2006) so that the success of restoration efforts can be expected to differ among locations. To illustrate, less than 10 out of several hundred *A. palmata* settlers that were outplanted in the Florida Keys shortly after settlement survived beyond one year (MW Miller and AM Szmant, pers. comm.). Two of these settlers reached the age of seven years before they died due to a disease outbreak. While the aforementioned studies did not

employ a fundamentally different approaches from the one used here, local reef conditions are likely an important determinant of local restoration success. Reef communities on Curaçao are less degraded than those in the Florida Keys (Jackson et al. 2014) which likely explains why, despite the use of largely similar techniques and approaches, survival of outplanted *A. palmata* reared from sexually produced larvae on Curaçao is higher than in the Florida Keys.

Conclusions

Our findings show that the rearing of sexually produced larvae is possible for Caribbean coral species despite the fact that previous attempts to raise and outplant settlers of the critically endangered *A. palmata* colonies have been effectively unsuccessful. The combination of novel rearing techniques (kreisels and settlement substrates providing a variety of microhabitats), outplanting of recently settled individuals and suitable conditions at our experimental site likely underlie the results obtained in this study. While encouraging, outplanting sexual coral recruits will not "restore a reef" by itself and requires that other causes of degradation are minimized at the restoration site prior to outplanting (Mumby and Steneck 2008). Nonetheless, our findings, combined with other case studies (Omori et al. 2008; Nakamura et al. 2011; Villanueva et al. 2012; Guest et al. 2014) show that if applied on larger sales and in combination with other management tools such as fishing quotas, coastal protection, and pollution regulations, sexual coral propagation or "assisted recruitment" could contribute to restoring tropical reef communities in the future.

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Supplementary material

Table S5.1. Costs for the construction (materials and labour) of the land-based nursery at the Curaçao Sea Aquarium.

Item	Specifications	Quantity	Cost per item[a]	Total[a]
Materials		*(number)*		
culture tanks[b]	acrylic tanks, 240 x 90 x 65 cm	5	$2,700.00	$13,500.00
circulation pumps[b]	15,000-20,000 l/ hr	7	$1,100.00	$7,700.00
plumbing	pipework and valves	1	$4,000.00	$4,000.00
electrical installation	control box and wiring	1	$5,000.00	$5,000.00
roof[b]	acrylic permeable to UV light, 100 m^2	100	$80.00	$8,000.00
roof	wooden timbering	1	$2,500.00	$2,500.00
building works	concrete base for tanks and pavement	1	$4,000.00	$4,000.00
Labour[c]		*(hours)*		
preparation	4 construction workers, 3 days	96	$8.75	$840.00
building work	4 construction workers, 10 days	320	$8.75	$2,800.00
roof installation	4 construction workers, 5 days	160	$8.75	$1,400.00
tank installation and plumbing	4 technicians, 5 days	160	$10.50	$1,680.00
electrical installation	2 technicians, 2 days	64	$10.50	$672.00
			total	$52,092.00

[a] Costs are in US dollars.

[b] Costs include shipping from the USA.

[c] Wages are based on standard Curaçaoan allowances at the time the project was started in 2010 (Curaçaoan GDP per capita is $15,000 (Central Inteligence Agency 2015) and one work day is 8h.

Chapter 6

Four-year-old Caribbean *Acropora* colonies reared from field-collected gametes are sexually mature

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Rehabilitating populations of Caribbean coral species that have declined in recent decades has become a management priority throughout the region, stimulating the development of new methodologies to artificially reseed degraded reefs. Rearing larvae of ecologically important coral species appears a particularly attractive method to aid the recovery of degraded populations because genetic recombination could yield new genotypes better capable of coping with the altered conditions on modern Caribbean reefs. Well-developed elkhorn coral (*Acropora palmata*) populations form dense thickets that contribute to the maintenance of healthy and productive reefs by providing shelter to a variety of other reef organisms (Gladfelter and Gladfelter 1978). After more than 95% of *A. palmata* populations were decimated by a disease beginning in the mid-1970s, this species was listed as critically endangered under the Red List of Threatened Species (IUCN 2013) and restoration efforts were initiated throughout the region to assist its recovery (Young et al. 2012). In 2011, we collected gametes from eight *A. palmata* colonies *in situ* on Curaçao, which were subsequently cross-fertilized to generate larvae. Competent larvae were settled on clay tiles (figure 6.1*a*) and reared in a flow-through land-based nursery for one year (figure 6.1*b,c*), after which they were outplanted to a breakwater at 2-5 m depth (figure 6.1*d*) (refer to chapter 6 for details on methodology). Seven out of nine outplanted colonies survived and continued to grow *in situ* (figure 6.1*d,e*), reaching a size of 30-40 cm in diameter and 20-30 cm height after 4 years (figure 6.1*f*). On September 8th and 10th 2015, 9 and 11 days after the full moon, two colonies were observed releasing gametes between 155-175 min after sunset (figure 6.1*g,h*). This is the first time that an endangered Caribbean *Acropora* coral species was raised from larvae and grown to sexual maturity in the field. Indeed only one other study has documented age and colony size at reproductive onset in a Indo-Pacific broadcast spawning scleractinian coral reared from larvae (Baria et al. 2012). The relatively short time until onset of spawning (≤ 4 yrs) observed for *A. palmata* shows that recovery of degraded coral populations by enhancing natural recruitment rates may be practicable if outplanted colonies are able to rapidly contribute to the natural pool of larvae.

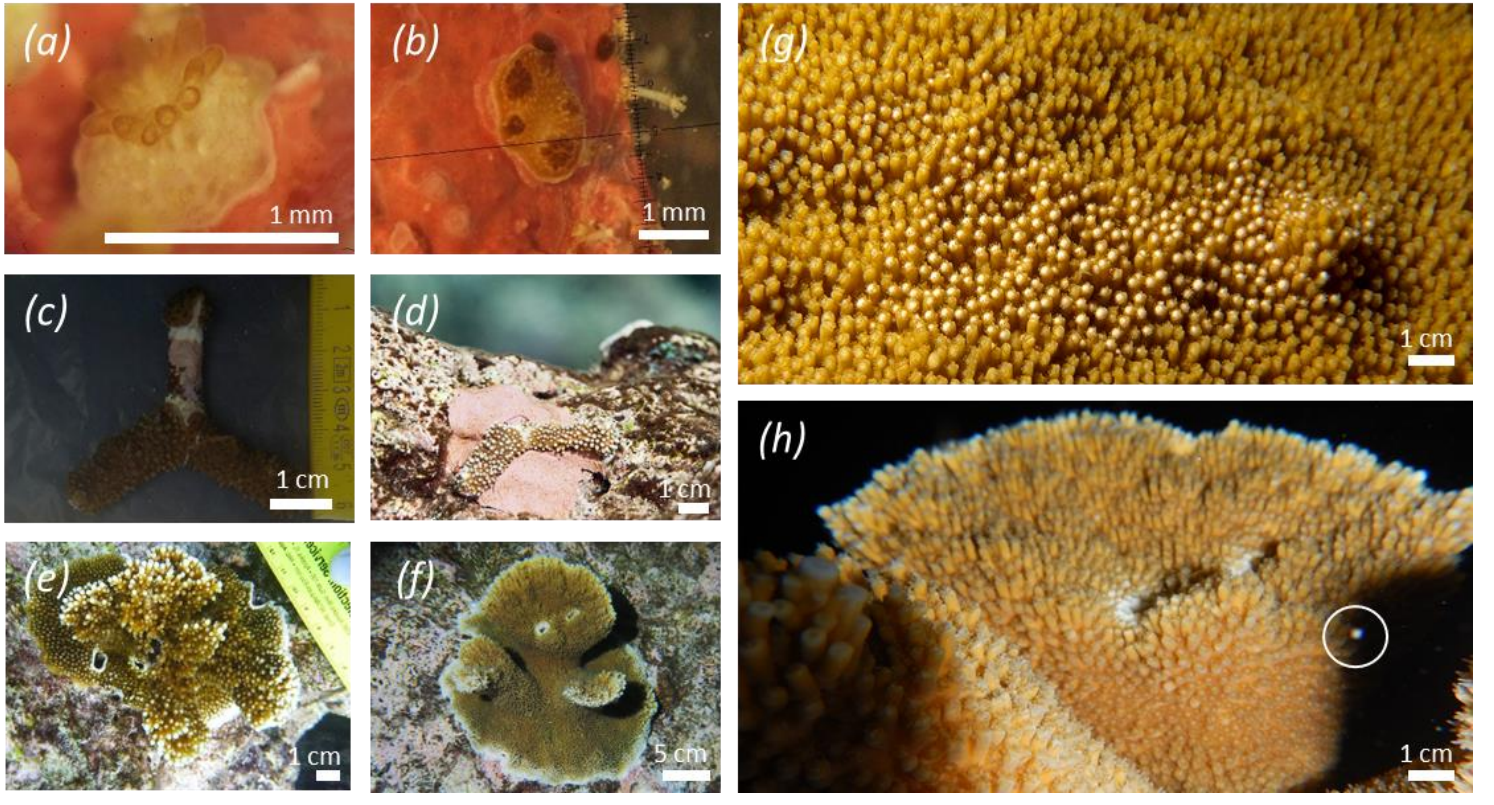

Figure 6.1. *(a) Acropora palmata* recruit (two-week-old), *(b)* Same recruit after one month. *(c)* Same recruit over-growing a tile with two other recruits (one-year-old). *(d)* Recruits directly after outplanting (one-year-old). *(e)* Recruits fused into one colony after 2.5 years. *(f)* Same colony after 4 years. *(g)* Detail of polyps with sperm-egg bundles. *(h)* Gamete bundle floating to the surface after release. Photos by *(a-f)* VF Chamberland and *(g-h)* S Snowden.

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Chapter 7

New seeding approach reduces costs and time to outplant sexually propagated corals for reef restoration

Valérie F Chamberland, Dirk Petersen, James R Guest, Udo Petersen, Mike Brittsan, Mark JA Vermeij

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Abstract

Outplanting sexually propagated corals for restoration is a recent technique aimed at increasing coral cover on degraded reefs, while preserving genetic variation within recipient populations. Manually attaching substrates with recently settled corals to the reef using binding materials is, however, time-consuming and expensive, limiting its use to small spatial scales. We present a novel approach whereby young corals are 'seeded' to the reef without the need of manual attachment. We tested two tetrapod-shaped concrete substrates (7.9 and 9.8 cm in diameter) on which coral larvae were settled. The tetrapods were deployed by simply wedging them in reef crevices, in 1.5 to 7% of the time required for traditional outplanting techniques. Seeding was most effective in reefs with moderate to highly complex topographic complexities, where tetrapods became rapidly lodged in crevices or cemented to the benthos by encrusting organisms. After one year, average recruit survival was 9.6% and 67% of tetrapods still harboured at least one coral colony, and overall, the seeding approach resulted in a 5 to 18 fold reduction in outplanting costs compared to methods requiring binding materials. While we suggest improvements to the current tetrapod designs, this novel approach represents a substantial reduction in costs and time required to introduce sexually propagated corals to reefs, and could enable larger scale reef restoration.

Introduction

The loss of ecological functions and ecosystem services provided by coral reefs worldwide has prompted conservation and management efforts to promote their recovery by addressing local causes of decline (Mumby and Steneck 2008). These measures can be 'passive' whereby natural recovery is facilitated through human intervention (e.g., implementation of fishing quotas, pollution regulation) (Sale 2008), or take the form of 'active' measures whereby humans directly manipulate the dynamics of degraded reef ecosystems (e.g., coral propagation, artificial reefs, removal of invasive species) (Rinkevich 2008). Because many coral reefs are assumed to no longer recover naturally from anthropogenic stressors (Mumby and Steneck 2008), active restoration approaches are increasingly considered, in conjunction with passive management interventions, to rehabilitate degraded reef communities.

Outplanting corals into degraded areas is a common active restoration approach aimed at increasing coral cover and structural complexity (Edwards 2010). Corals for outplanting are typically clonal asexual fragments or naturally dislodged "fragments of opportunity" of extant colonies (Shafir et al. 2006). Fragments are often grown-out in coral nurseries prior to outplanting and, when outplanted, have been observed to locally increase the abundance and diversity of fish (Cabaitan et al. 2008) as well as the number of naturally settling corals (Montoya-Maya et al. 2016). However, the use of clonally produced fragments also results in limited genetic diversity within recipient populations, and thus may reduce their potential to adapt to changing environmental conditions (Baums 2008). In contrast, the use of sexually produced corals, whereby genetic recombination ensures the formation of new genetic varieties, preserves genetic variation within outplanted corals during restoration efforts (Edwards 2010). Consequently, the use of sexually produced corals can complement more commonly used clonal approaches and provide the possibility for genetic adaptation to climate change.

Following gamete collection and ex situ fertilization, sexually produced coral larvae are generally settled on artificial settlement substrates ("settlement tiles") (Petersen et al. 2005), that are either directly outplanted to the reef (this thesis, chapter 5; Baria et al. 2010), or kept in land- or ocean-based nurseries (Baria et al. 2012; Villanueva et al. 2012; Guest et al. 2014) where coral settlers are grown to sizes (generally > 1 cm^2) that make them less vulnerable to predation and competition (Vermeij and Sandin 2008; Penin et al. 2010). The success of sexual coral propagation techniques has improved over recent years. While large numbers

of outplanted corals regularly survive past the age of one year (this thesis, chapter 5 and 6; Omori et al. 2008; Nakamura et al. 2011; Guest et al. 2014) and outplanted corals have reached sexual maturity in a few occasions (this thesis, chapter 6; Iwao et al. 2010; Baria et al. 2012), mortality among newly settled corals remains extremely high (i.e., type III survivorship) compared to restoration approaches using clonal fragments. Typically less than 5% of all cultured settlers survive for more than one year (Edwards 2010), and high (natural) levels of post-settlement mortality therefore greatly reduce the effectiveness of restoration methods using sexually produced larvae.

The high costs of both asexual and sexual restoration approaches limit their application to spatial scales (< 1 hectare) that are generally too small to re-establish ecological functions of degraded reef systems (Edwards and Gomez 2007; Bayraktarov et al. 2016). The process of outplanting of artificial substrates with settled corals to the reef typically accounts for 30% of the total restoration costs when individual corals or substrates are manually secured using binding materials (e.g., cable-ties, epoxy, nails). Gamete collection, larval rearing and larval settlement combined in contrast typically account for less than 50% of costs (Edwards 2010). Current techniques require tedious handling of binding materials underwater and are therefore incredibly time consuming. For example, previous studies found that between 4 and 20 min were needed to outplant a single substrate with coral settlers to the reef (this thesis, chapter 5; Villanueva et al. 2012; Guest et al. 2014). Restoration efforts using sexually propagated corals would especially benefit from new technologies that enable cheap and fast outplanting and increased settler survival.

In this study we tested the efficiency of outplanting three-week-old coral settlers using novel tetrapod-shaped substrates for coral settlement (figure 7.1) that can be outplanted by simply wedging them into natural crevices, without the need of binding materials. Tetrahedral shapes are commonly used in coastal defences to dissipate water movement and wave energy. Their "spikey" shape makes them relatively stable substrates once placed on the benthos (Fabião et al. 2013). Two different tetrapod-shaped substrates were designed: Type I (figure 7.1*a-b*, *e-f*) with thin conical-shaped pods, and Type II (figure 7.1*c-d*, *g-h*) with thicker triangular-shaped pods. Thinner and pointier conical pods were assumed to enhance the probability of the tetrapods to become attached or stuck to the reef. Thinner pods could also have, however, poorer structural strength, causing them to be more vulnerable to breakage. We therefore tested the two designs to quantify potential trade-offs between thicker (less breakage) and thinner (faster attachment) pods.

We hypothesized that the success of aforementioned 'seeding' approach would depend on the structural complexity of the habitat in which tetrapods were introduced. On shallow coastal reefs the attenuation of wave energy is largest on structurally complex landscapes (Monismith 2007; Huang et al. 2012). Complex reef topographies also contain a larger number of crevices, fissures and holes in which tetrapods can be wedged (Wilson et al. 2007). We therefore expected that a larger proportion of tetrapods would be retained in highly complex topographies than on reefs with low or sparse relief. To test this hypothesis we assessed if the movement of the tetrapod-shaped substrates, even if not secured with binding materials, would be low enough that they would become rapidly attached or stabilized within the reef framework in areas with low to high levels of structural complexity. Settler survival and growth were followed for one year after outplanting. Lastly, we compared the cost-effectiveness of this new approach relative to existing outplanting methods.

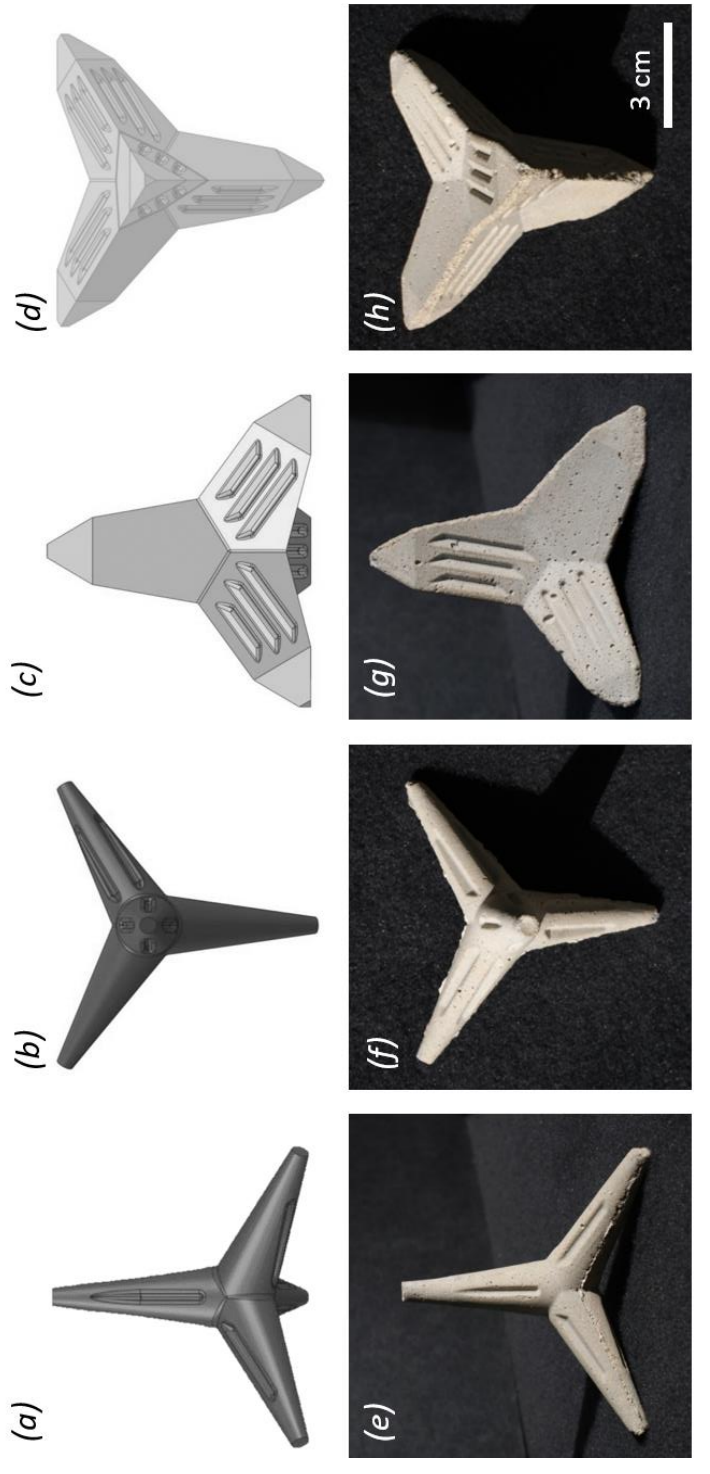

Figure 7.1. Tetrapod-shaped substrates for coral larval settlement. Computer-aided-designs (CADs) for Type I: *(a)* side view and *(b)* top view, and for Type II: *(c)* side view and (d) top view. Tetrapods before they were conditioned in a flow-through aquarium system: Type I: *(e)* Side view, and *(f)* top view, Type II: *(g)* side view, and *(h)* top view. Photos by D Petersen.

Materials and Methods

Design and production of tetrapod-shaped substrates for coral settlement

Both tetrapod types consisted of four pods positioned in tetrahedron angles (109.47°) relative to each other (figure 7.1). Tetrapod Type I (figure 7.1*a-b*, *e-f*) had thin conical-shaped pods, whereas tetrapod Type II (figure 7.1*c-d*, *g-h*) had thicker triangular-shaped pods. The tips of the pods of both tetrapod types narrowed toward their ends to increase the probability that they would get stuck in crevices and thus increase overall attachment success. Because the availability of microhabitats on artificial substrates promotes larval settlement (Petersen et al. 2005) and post-settlement survival (Nozawa 2008; Brandl et al. 2014), grooves were incorporated on each of the four pods of both designs (figure 7.1) (Type I: 3 grooves per pod, 27.5 × 2.4 × 1.3 mm, Type II: 6 grooves per pod, 28.7 × 2.4 × 1.6 mm, L × W × D, supplementary material, table S7.1). Tetrapods needed to be large enough to reduce their chance of falling into deeper reef crevices unsuitable for coral growth, but small enough that they could be easily handled during the rearing phase where larvae are settled on the tetrapods, and during the outplanting itself. The sizes and weights of both tetrapods varied, albeit minimally (Type I: Ø 7.9 cm, 51.1 g, Type II: Ø 9.8 cm, 85.6 g, table S7.1). Because coral settlers on each settlement substrate theoretically can grow into a single coral colony after successful outplanting, the use of smaller-sized substrates harbouring small numbers of settlers is more effective for restoration efforts than fewer, larger-sized substrates harbouring numerous settlers (Petersen et al. 2005; Suzuki et al. 2012). Smaller-sized substrates furthermore allow young corals to rapidly overgrow the artificial substrate and attach to underlying reef substratum which enhances their growth and survival (this thesis, chapter 5).

The tetrapods were designed using three-dimensional Computer-Aided Design software (3D-CAD; SolidWorks, Massachusetts, USA), and made of concrete. Moulds were made from polyurethane and manufactured with a multi-axis-milling machine (VTC 800/30 SR; Mazak, Germany). The tetrapods were casted in concrete between March and July 2013 and made from a homogenous mixture of 2 parts Portland cement, 4 parts river sand and 1 part water. This mixture could be easily poured in the moulds and dried rapidly. Biodegradable vegetable oil was sprayed into the moulds prior to pouring to prevent the concrete from sticking to the moulds' sides. The concrete was allowed to dry for 24 h before the tetrapods were extracted from the moulds.

Rearing and settlement of coral larvae

Experiments were conducted on Curaçao (12ºN, 69ºW), a Caribbean island located 60 km north off Venezuela. The tetrapods (n = 80 of each type) were incubated in a flow-through seawater aquarium system for six months to wash out potentially toxic and alkaline agents from the cement mixture and allow the development of biofilms that induce coral settlement and metamorphosis (Ritson-Williams et al. 2016). The aquarium system consisted of five flow-through aquaria (215 × 64 × 69 cm, L × W × H; acrylic) that were continuously supplied with natural seawater (~2300 L h^{-1}) from a nearby reef. See chapter 5 in this thesis for a detailed description of this system.

Favia fragum (Esper 1797) releases planula larvae 6 to16 days after the new moon throughout the year (Szmant-Froelich et al. 1985). Fifty adult *F. fragum* colonies were collected from the Curaçao Sea Aquarium reef (12° 4'59"N, 68°53'44"W) two days before the onset of their planulation cycle in March 2014 and kept in the aforementioned flow-through system. Between days 6 and 10 after new moon and one hour before sunset, colonies were placed overnight in two 70-L plastic cool boxes (Princeware Glacier, UK) containing ~60 L of 50-µm-filtered seawater. Every morning (between 7:00 and 8:00), all larvae released during the preceding night were collected using glass pipettes and distributed randomly among eight plastic containers (36 × 31 × 24 cm, L × W × H; Sterilite, MA, USA) filled with ~23 L of 50-µm-filtered seawater, larvae collected during previous nights and 10 Type I and 10 Type II tetrapods. The parent colonies were then removed from the cool boxes and returned to the flow-through system. All collected larvae were divided among the eight containers resulting in a total of ~600 coral larvae per container. Containers with coral larvae were partially submerged in the flow-through system to maintain natural seawater temperatures (28-29°C) and water inside the containers was exchanged daily (~75%) to maintain water quality. Two airlifts were placed in the opposite corners of each container to generate water movement and prevent the formation of stagnant water in between the tetrapods. Larvae were left in the containers for five days to settle after which all tetrapods were transferred to the flow-through system.

Larval settlement rates on each tetrapod were assessed immediately before outplanting using a blue light (Night Sea, MA, USA) that causes settled larvae to fluoresce. To determine if settlement preferences differed between the two tetrapod designs, different surface orientations (Topside, Underside) and microhabitat type (Grooved, Flat), the position of each settler on each tetrapod was mapped.

Settlement rates were calculated as the number of settlers per cm^2 of available surface area per tetrapod type, surface orientation and microhabitat type. Until they were seeded to the reef, all tetrapods with ≥ 1 live coral settler (i.e., henceforth referred to as 'seeding units', SUs) were hung ~50 cm below the water surface using 27.2-kg strength fishing line tied to PVC frames placed on top of the flow-through aquaria.

Seeding of SUs on the reef

Three weeks after *F. fragum* larvae had settled, SUs were seeded at the Curaçao Sea Aquarium reef, a relatively healthy reef approximately 100 meters from our rearing facility. Tetrapods were seeded within a 150 × 10 m area parallel to the coast at depths between 4 and 6 m and individually placed in a habitat of Low, Medium, or High structural complexity. These different habitat types occurred interspersed as small patches (2-10 m in width) within the outplanting area. Assignments to structural categories were made visually following Wilson et al. (2007). Low, Medium, or High structural complexity corresponded, respectively, to low and sparse relief, moderately complex, and very complex with numerous fissures and caves (figure 7.2*a*-c). To facilitate the search for tetrapods at each survey, individual outplant locations where one SU of each type was seeded were marked with numbered plastic tags that were fixed to the reef with cable ties (figure 7.2*d*). Tetrapods were outplanted at least 3 m from one another to avoid their potential misidentification due to their dispersal during the course of the experiment.

Ten SUs of each tetrapod type were seeded at the three levels of structural complexity. Only SUs with similar overall settler densities were used (Type I: 0.29 (± 0.11SD), Type II: 0.24 (± 0.08SD), mean number of settlers per cm^2) to minimize potential confounding effects of density dependent processes (Suzuki et al. 2012). SUs were transported from the aquaria to the reef while hanging from the same PVC frames (1 × 1 m; figure 7.2*e*) used during the initial rearing phase. Tetrapods were then seeded by a diver who cut each SU from the PVC frame and wedged them into crevices of the reef framework. Large (>10 cm Ø) and deep (>30 cm depth) crevices were avoided to reduce the chance of tetrapods being lost into the reef framework. One SU of each tetrapod type was seeded in close proximity (≤ 30 cm) to each tag (figure 7.2*d*), after which an overview-photograph (Lumix DMC-TS2, Panasonic) of the area (~1.5 × 2 m) was taken in planar view (figure S7.1) to document the surrounding benthos and the location of each tetrapod relative to each tag.

Monitoring of tetrapod dispersal and coral settler survival and growth

Tetrapod dispersal was monitored 1.5 week, 3 and 6 months after outplanting. Settlers' survival and growth rates were monitored after 3, 6 and 12 months. At each time point, the area around each tag was carefully searched for the SUs. If a SU was not found within 3 m of a tag, it was considered lost and excluded from the survivorship analysis. To calculate the dispersal of each SU through time, an overview-photograph of each outplant location was taken in planar view (~1.5 × 2.0 m) for each time point so the position of each SU could be tracked through time using natural landmarks and the tags for scale (figure S7.1). The distance between the positions of the tetrapods through time was determined using ImageJ (Schneider et al. 2012) (figure S7.1). During each survey, by gently trying to move each tetrapod, we assessed whether it had become "attached" (i.e. stuck in or cemented to the reef framework) or whether it was laying loose on the reef substratum (i.e., "non-attached"). Each tetrapod was then detached from the reef and a high resolution photograph was taken of each of its four sides (Lumix DMC-TS2, Panasonic; figure 7.2*f*) after which it was carefully returned to its original position on the reef. This may have caused the detachment of some of the already attached tetrapods, but lifting each tetrapod was necessary as surviving settlers were often found on their undersides.

To quantify survival rates of coral settlers on each tetrapod type in the three levels of topographic complexity, the number of live *F. fragum* on each tetrapod was assessed on the photographs during each time point and compared to the map overviewing the distribution of initial settlers. Settler size (in surface area in mm^2 and number of polyps) was also quantified from photographs after 6 and 12 months using ImageJ. Lastly, we calculated the proportion of outplanted SUs that could be found and still harboured ≥1 settler (i.e., still represented a SU) through time for all treatments, henceforth referred to as 'SU yield'. SU yield serves as a measure of success to compare the effectiveness of different restoration approaches, assuming that a single coral colony can theoretically grow to adulthood per outplanted SU (Edwards 2010).

Figure 7.2. Seeding and monitoring of seeding units (SUs) in different levels of structural complexity. The two tetrapod types with coral settlers were seeded in reef areas with *(a)* Low, *(b)* Medium, and *(c)* High levels of structural complexity. *(d)* Outplant locations were marked with numbered plastic tags and SUs were *(e)* transported to the reef on 1 × 1 m PVC frames by a SCUBA diver and *(d)* wedged into crevices in the reef framework less than 30 cm away from their respective tag (tetrapods are shown by the *white arrows*). *(f)* At each survey, a picture of each of the tetrapod's four sides was taken to assess settler (shown by *white circles*) survival and growth. Photos by *(a-e)* VF Chamberland and *(f)* MT Chamberland.

One week before the last survey (t = 12 months), a storm caused major breakage of *Millepora* spp. and *Acropora palmata* colonies within the study area. A total of nine tags (out of 30) could no longer be located and were likely buried under scattered *Millepora* and *Acropora* fragments or had detached. Tetrapods associated with these tags were excluded from the analysis at this time point. Dispersal distances for all tetrapods could not be measured because most natural landmarks had also been covered or were no longer present.

Cost-effectiveness of seeding sexually propagated corals

The costs of seeding the two tetrapod types was calculated following Edwards (2010), and compared to the few existing studies that quantified costs associated to outplanting techniques for sexually propagated corals. The latter studies included restoration approaches that (1) tied other types of substrates to a rope previously nailed on the reef (this thesis, chapter 5) (2) epoxied substrates to the reef (this thesis, chapter 6), and (3) secured substrates in holes previously drilled in the reef framework (Villanueva et al. 2012; Guest et al. 2014). Only the cost-effectiveness of the actual outplanting was compared and costs associated with gamete collection, larval rearing, nursery construction and maintenance were not considered. The cost-analysis therefore only included expenses related to (i) the production or purchasing of settlement substrates, (ii) materials needed to secure the substrates to the reef (e.g., cable-ties, nails, pneumatic drills, epoxy), (iii) air tanks for SCUBA divers, and, if needed, pneumatic drills, and lastly, (iv) labour required to carry out the outplant (table S7.2). Reusable items such as SCUBA and snorkelling gear were assumed to have a three year life span so their cost was divided by three to calculate their costs for one outplanting effort per year (Edwards 2010). To standardize between studies, pneumatic drills were assumed to consume one air tank per dive, and we used a ratio of one diver handling a drill per team of three divers. We did not include costs related to boat usage as this expenditure is highly dependent on local conditions such as fuel prices and distance to the restoration site.

Labour was expressed in terms of person-hour and converted to US dollars based on the median worldwide GDP at the time the work was carried out (Central Intelligence Agency 2014) (i.e., \$6.63 h^{-1}), and only included the time required to carry out the outplant itself and costs to reach outplanting sites or prepare for dives were not considered. The time needed for divers to wedge one SU into the reef was calculated from video footage taken during outplanting and was measured as the time from when a diver first held a SU in his hand ready to seed it until the SU was wedged in the reef. The time required to outplant substrates using other outplanting

techniques than seeding was taken from above-mentioned studies. To compare the total costs of the different restoration approaches, the costs to restore one hectare of reef with 10,000 SUs with 10 persons was calculated for each method and its effectiveness expressed as SU yield after one year. Because settler mortality is highest during the first year after outplanting (Vermeij and Sandin 2008), the SU yield after one year was assumed to be an adequate metric to evaluate the long-term success of sexual coral restoration efforts.

Data analysis

To compare settlement preferences between the tetrapod designs (Type I, Type II), surface orientations (Topside, Underside) and microhabitats (Grooved, Flat) Welch's F-test for unequal variances (Ruxton 2006) was used followed by Tukey's post-hoc HSD tests because data did not meet the assumption of homoscedasticity. Differences in settler survival among tetrapod types, microhabitats and levels of structural complexity were compared with Kaplan-Meier's survival analysis (Kaplan and Meier 1958) followed by log-rank (Mantel-Cox) pairwise comparisons. Fisher's exact test of independence was used to test for differences in attachment rates of the tetrapods on the reef, as well as differences in the proportion of tetrapods still harbouring at least one coral individual through time (i.e., SU yield). One-way ANOVAs were used to assess potential differences in settler growth, whereas differences in dispersal rates were tested with repeated measures ANOVAs. All analyses were performed in SPSS 24.0 (IBM Corp. 2016). Statistical values for ANOVAs and post-hoc pairwise comparisons (Supplementary tables S7.3-S7.10) as well as all data generated during this study (Supplementary data set) are available upon request.

Results

Settlement preferences of *F. fragum* larvae

An average of 70% (± 6SE, n = 8 settlement containers) of *F. fragum* larvae settled on either tetrapod design. This resulted in 60 Type I and 64 Type II SUs that, immediately after larvae settled, harboured an average of 21.2 (± 1.2SE) and 28.0 (± 1.7SE) settlers, respectively (table 7.1). *F. fragum* larvae settled in slightly higher densities (number of settlers per cm^2) on Type I than on Type II tetrapods (Welch's F-test: $F_{1,116}$ = 18.36, p < 0.001) (table 7.1). Larvae settled foremost on the

undersides of tetrapod Type II (Welch's F-test: $F_{1,49} = 11.7$, $p = 0.001$), but did not discriminate between surface orientations on Type I tetrapods (Welch's F-test: $F_{1,49} = 0.38$, $p = 0.54$) (figure 7.3*a*). For both tetrapod types, settlement rates inside grooves were 2.4 (Type I) and 2.9 (Type II) times higher than on flat surfaces (Welch's F-test: Type I, $F_{1,33} = 22.1$, $p < 0.001$, Type II, $F_{1,38} = 31.7$, $p < 0.001$) (figure 7.3*b*).

Table 7.1. Density of *Favia fragum* settlers on the two substrate designs.

Substrate design	*n*	Surface area available for settlement (cm^2)	Number of settlers per tetrapod (mean ± SE)	Number of settlers per cm^2 (mean ± SE)
Type I	60	74.3	21.2 ± 1.2	0.30 ± 0.02
Type II	64	129.9	28.0 ± 1.7	0.23 ± 0.01

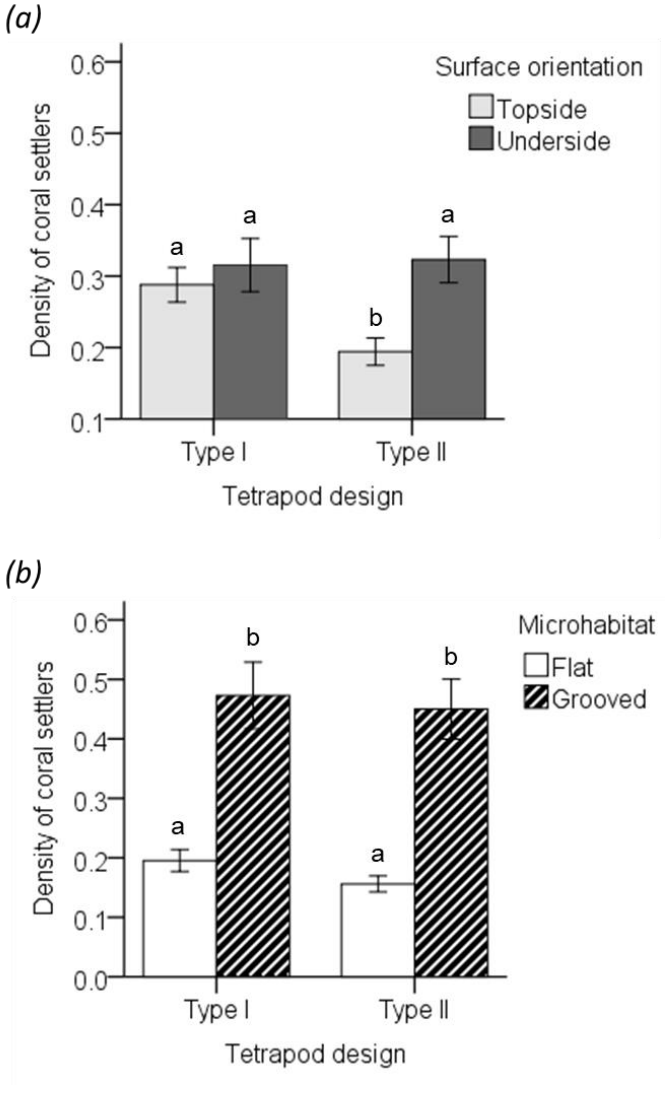

Figure 7.3. Settlement preferences of coral larvae. Comparison of *Favia fragum* settler densities between *(a)* surface orientations and *(b)* microhabitats on the two tetrapod designs. *Error bars* represent standard errors and *letters above bars* indicate statistically distinct groups as determined by Welch's F-test of unequal variances with $p < 0.05$, and with $n = 30$ and 32 independent replicates per surface orientation/microhabitat type for tetrapod Type I and II, respectively.

Tetrapod dispersal and attachment rates on the reef

Tetrapods dispersed most during the first two weeks after outplanting with an average of 6.0 cm per week (± 1.5SE, n = 57), after which they moved less than 2.0 cm per week (table 7.2) (one-way RM ANOVA: $F_{1,39}$ = 8.6, p = 0.006, table S7.3). After the first two weeks and during the subsequent 5.5 months, 50% of the tetrapods never moved. After one year 76% of the tetrapods could be recovered of which 84% were either firmly lodged in crevices and/or cemented to the reef framework by encrusting benthic organisms (table 7.3; figure 7.4).

Despite their different shapes, there were no differences in the distance that tetrapod Type I and II dispersed during the first six months of the experiment (two-way RM ANOVA: $F_{1,38}$ = 0.07, p = 0.79, table S7.4) (table 7.2). After 1 year, the relocation success of both tetrapod Type I and II was similar across all three levels of reef complexity (Type I, 81%, lower 95% confidence limit (LCL) = 60%, upper 95% confidence limit (UCL) = 92%, Type II, 70%, LCL = 46%, UCL = 88%, respectively (Fisher's exact test: p = 0.33) (table 7.3). The two tetrapod designs were also equally likely to become stabilized within the reef framework, and after one year 94% (Type I, LCL = 73%, UCL = 99%) and 71% (Type II, LCL = 45%, UCL = 88%) of tetrapods were attached to the reef (Fisher's exact test, p = 0.11) (table 7.3; figure 7.4). While the two designs proved equally effective in promoting the stabilization of the tetrapods on the reef, it is worth noting that the thinner pods of Type I were more fragile, causing them to break often during production (~ 10%, VF Chamberland, pers. obs.) and while being handled in the field (~ 10%, VF Chamberland, pers. obs.).

All tetrapods that could be recovered within high complexity habitats were attached to the reef after six months. Attachment success was lower (63%, LCL = 35%, UCL = 85%) in low complexity habitats (Fisher's exact test: $p < 0.01$) (table 7.3). After one year, recovery rates for tetrapods placed in low complexity habitats were 25% lower (64%, LCL = 31%, UCL = 89%) compared to high complexity habitats (89%, LCL = 65, UCL = 99%) (table 7.3). Tetrapods in low complexity habitats dispersed 3.4 and 4.7 times farther than in Medium and Highly complex habitats respectively during the first two weeks following the outplant (Tukey's HSD test: p = 0.002, table S7.5), resulting in a total dispersal distance averaging 60 cm (± 14SE) after six months compared to 8 cm (± 3SE), respectively (table 7.2).

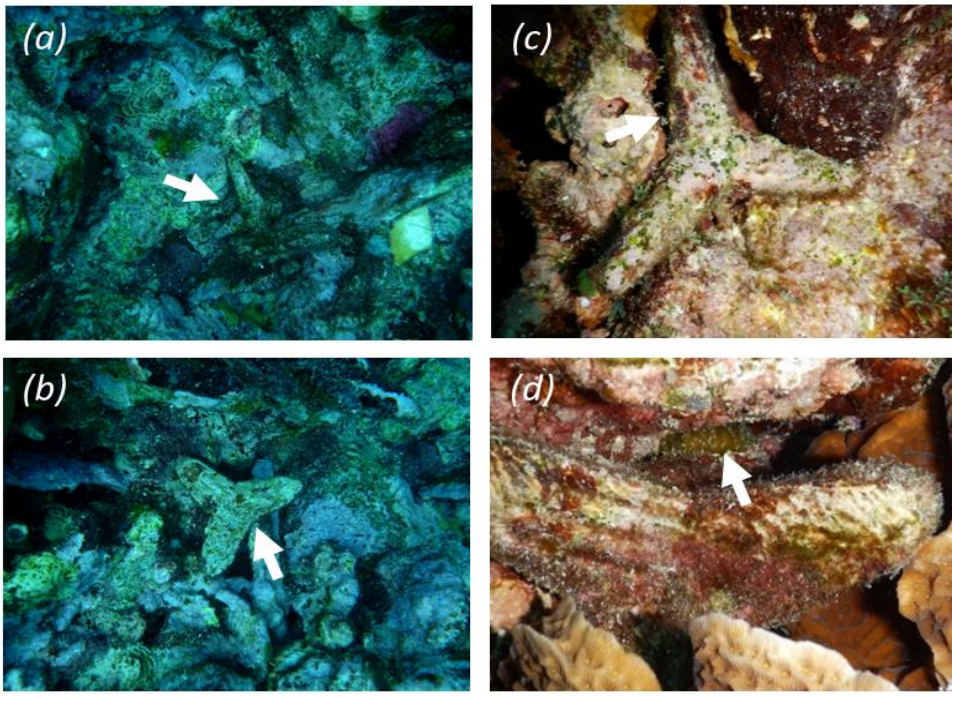

Figure 7.4. Example of the two tetrapod designs six months after they were seeded to the reef. After 6 months, 75% of the Type I *(a)* and Type II *(b)* tetrapods were firmly lodged in crevices and/or had become cemented to the reef framework by encrusting benthic organisms such as sponges, crustose coralline algae and hydrocorals, and were hardly distinguishable from the reef framework. In *(a)* and *(b)*, *white arrows* show the tetrapods in the reef framework. *(c)* and *(d)* are close-up pictures of six-month-old *Favia fragum* colonies (indicated by *white arrows*) growing on both tetrapod designs. Photos by VF Chamberland.

Survival and growth of coral settlers

After one year, an average of 9.6% of initial *F. fragum* settlers (n = 31 substrates) had survived and grown to an average size of 30.2 mm^2 (± 2.8SE, n = 60 settlers). At that point, 62% of live individuals had completed at least one polyp division and consisted of 2 to 7 polyps. Settler survival (figure 7.5*a*) on Type II tetrapods was similar (9.8%, n = 14 substrates) to that on Type I tetrapods (9.4%, n = 11 substrates) (K-M, $\chi^2_1 = 0.00$, $p = 0.99$). Growth was also equal between the two designs (one-Way ANOVA: 6 months, $F_{1,188} = 0.006$, $p = 0.94$, 12 months, $F_{1,58} = 0.02$, $p = 0.89$, table S7.6). On both tetrapod Type I and II, and across all levels of structural complexity, larvae that had settled inside grooves showed a 1.8 fold higher survival rate after one year compared to those that settled on flat surfaces (K-M, $\chi^2_1 = 7.4$, $p = 0.007$) (figure 7.5*b*), suggesting that the grooves served as sheltered microhabitats for newly settled corals. While survival rates of coral settlers on tetrapod Type I were unaffected by the distance that SUs had moved during the study period, 21.3% and 26.6% of the variation in settler survival rates on Type II tetrapods could be linked to the latter's total dispersal after respectively 3 and 6 months (Regression analysis, 3 months: $p = 0.047$, 6 months: $p = 0.020$; figure S7.2). Coral settlers on Type II tetrapods appeared therefore more vulnerable to mechanical damage as tetrapods dispersed across the reef.

The topography of the outplanting sites significantly affected the survival of *F. fragum* settlers as they were 8.7 and 5.2 times less likely to survive in areas with Low structural complexity compared to those seeded in Medium and Highly complex reefs after one year (K-M, $\chi^2_2 = 13.8$, $p = 0.001$, table S7.7) (figure 7.5*c*). The five one-year-old individuals that were still alive in Low complexity areas had however grown to equal sizes as those in Medium and High complexity reefs (Welch's F-test, $F_{2,20} = 0.53$, $p = 0.59$, table S7.8).

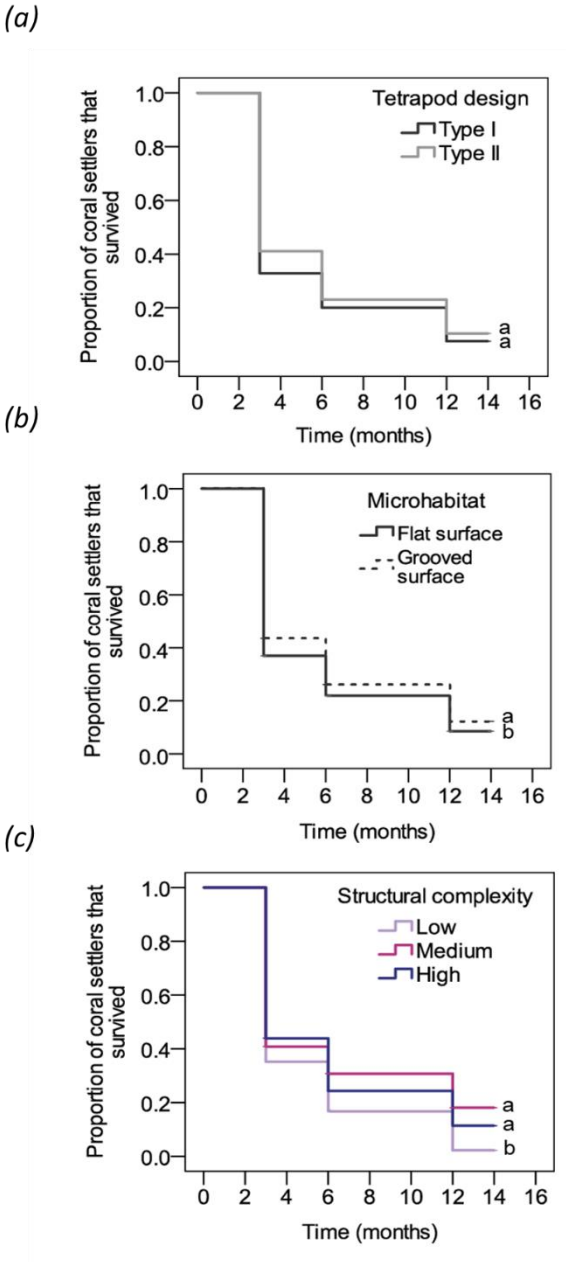

Figure 7.5. Survival of coral settlers. Proportion of initial *Favia fragum* settlers that survived through time *(a)* on Type I and II tetrapods, *(b)* inside grooves and on flat surfaces, and *(c)* that were seeded in Low, Medium and High levels of habitat complexity. *Letters next to bars* indicate statistically different groups ($p < 0.05$) as determined with a Kaplan-Meier analysis followed by pairwise log-rank (Mantel-Cox) comparisons.

Table 7.2. Dispersal of the two substrate designs seeded in three levels of reef structural complexity.

	Net dispersal rate (cm week-1)									Total dispersal (cm)		
	0 to 2 weeks			2 to 12 weeks			12 to 24 weeks			24 weeks		
Substrate type	$\bar{x}$	*SE*	*n*	$\bar{x}$	*SE*	*n*	$\bar{x}$	*SE*	*n*	$\bar{x}$	*SE*	*n*
Type I	6.3	2.5	29	1.3	0.5	19	0.6	0.2	25	32.4	8.9	25
Type II	5.6	1.5	28	1.5	0.6	21	0.3	0.1	23	32.2	8.9	23
Structural complexity												
Low	12.1	3.9	18	2.2	0.8	14	0.8	0.2	16	60.3	14.0	16
Medium	3.6	1.4	20	2.1	0.8	12	0.3	0.2	15	30.0	8.9	15
High	2.7	1.1	19	0.1	0.1	14	0.2	0.1	17	8.1	2.8	17
Overall	6.0	1.5	57	1.4	0.4	40	0.4	0.1	48	32.3	6	48

Table 7.3. Status of the two substrate designs seeded in three levels of reef structural complexity through time.

	Recovery rate (% of outplant locations)								Attachment rate (% of recovered substrates)							
	2 weeks		3 months		6 months		1 year		2 weeks		3 months		6 months		1 year	
Substrate type	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
Type I	100	29	79	24	83	30	81	21	90	29	84	19	76	25	94	17
Type II	96	28	87	23	83	29	70	20	70	27	80	20	78	23	71	14
Structural complexity																
Low	100	17	74	19	84	19	64	11	47	17	57	14	63	16	57	7
Medium	100	20	86	14	80	20	67	12	90	20	92	12	67	15	75	8
High	95	20	93	14	85	20	89	18	100	19	100	13	100	17	100	16
Overall	98	57	83	47	83	59	76	41	80	56	82	39	77	48	84	31

SU yield

Overall, 56% of initial SUs still harboured at least one *F. fragum* individual after one year and SU yield was similar between both tetrapod designs (Fisher's exact test, 3 months: $p = 0.69$, 6 months: $p = 1.00$, 12 months, $p = 1.00$, table S7.9) (figure 7.6*a*). The SU yield after one year was however 2.5 fold lower in habitats with Low structural complexity (27%, LCL = 6%, UCL = 60%) compared to Medium and High complexity reefs combined (67%, LCI = 47%, UCL = 83%) (Fisher's exact test: $p = 0.046$, table S7.10) (figure 7.6*b*). The effectiveness of the seeding approach was therefore reduced in areas with low relief, and traditional outplanting techniques using binding materials likely represent a more effective strategy in such habitats (table 7.4). However, except for low complexity areas, seeding SUs resulted in similar SU yields after one year 67%) compared to non-seeding restoration techniques using some form of binding materials (range: 25% to 70%, table 7.4).

Cost-effectiveness of seeding sexually propagated corals

Outplanting 10,000 SUs using binding materials requires 690 to 3200 person-hours, whereas 'seeding' the same number of SUs in reef crevices could be achieved in 48 person-hours (table 7.4). Because SUs could be outplanted rapidly (8.6 seconds per SU (± 0.5SE, $n = 59$) and without purchasing binding materials, seeding 10,000 SUs cost $7,000 USD compared to $22,000-$45,000 USD if coral settlers were outplanted using other techniques (table 7.4). When accounting for SU loss and settler mortality during the first year following the outplanting, remaining SUs each cost $1.00 USD (Medium and High complexity habitats) to $2.50 USD (Low complexity habitats), excluding expenses for larval rearing (table 7.4).

Table 7.4. Cost-effectiveness of different outplanting techniques.

Source	Coral species	Substrate design	Outplanting approach	Outplanting materials	Nursery phase	Reef structural complexity	Person-hour per hectare[a,b]	Cost per hectare[c]	Settler survival after one year (%)	SU yield after 1 year (%)
Current study	*Favia fragum*	tetrapod	seeding	none	3 weeks	Low	48	6800	2.1	27
						Medium	48	6800	15.1	67
						High	48	6800	10.1	67
Chamberland et al. (2016)	*Acropora palmata*	tripod	transplanting	epoxy	1 year	n.a.	1667	33400	n.a.	70
Chamberland et al. (2015)	*Acropora palmata*	tripod	transplanting	cable-tie, rope, nails	2 weeks	n.a.	690	22200	12.7	27
Guest et al. (2014)	*Acropora millepora*	plug-in	transplanting	drill, epoxy	7 months	n.a.	3200	45200	n.a.	25
					14 months	n.a.	3200	45200	n.a.	35
					19 months	n.a.	3200	45200	n.a.	43
Villanueva et al. (2012)	*Acropora valida*	tox	transplanting	epoxy	6 months	n.a.	1086	25100	n.a.	n/a

[a] Assuming 10000 Seeding Units (SUs) are needed to restore one hectare of reef

[b] Assuming 10 persons are needed to restore one hectare of reef

[c] Costs are in US dollars.

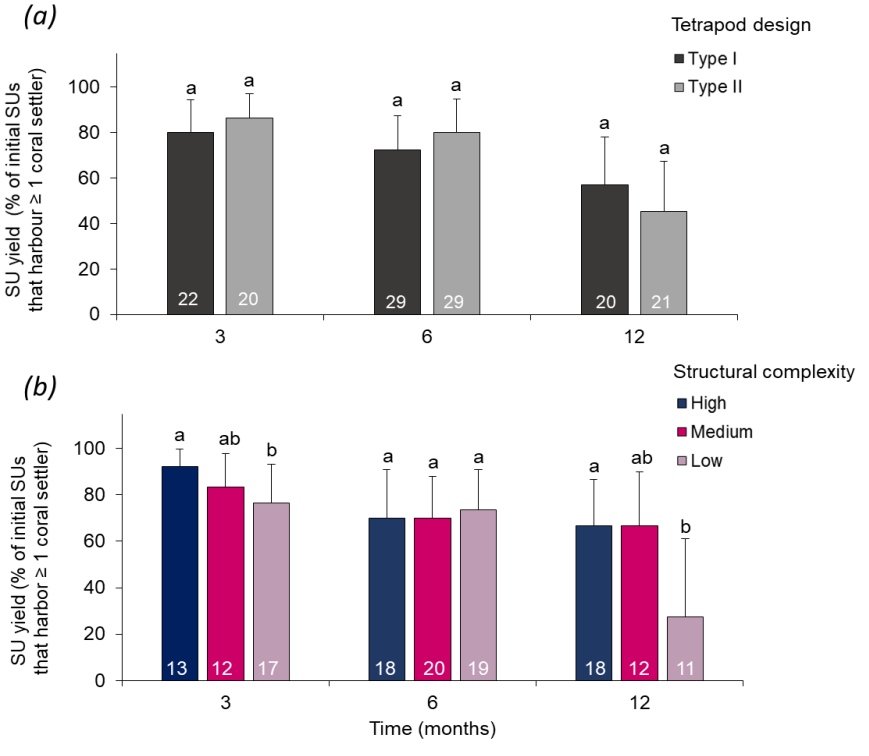

Figure 7.6. Seeding unit (SU) yield. Proportion of initial SUs that could be recovered through time and that still harboured at least one live *Favia fragum* individual between *(a)* Type I and II tetrapods and *(b)* Low, Medium and High levels of structural complexity. *Error bars* are 95% confidence intervals as determined with Clopper-Pearson's exact method. *Letters above bars* indicate significantly different groups as determined with Fisher's exact test. *Numbers within bars* indicate sample sizes and are the number of outplant locations that were monitored. The latter increases between 3 and 6 months because not all outplant locations could be monitored at t = 3 months due to logistical constraints.

Discussion

Coral restoration can only be an effective management tool if it is cost-effective and can be applied at scales similar to the processes that cause their decline (Mumby and Steneck 2008; Edwards 2010). Current practices for restoring degraded reefs are generally expensive and labour intensive, making them unviable management options for restoration across larger spatial scales (i.e., > 1 hectare). In this study we examined the possibility to improve the cost-effectiveness of outplanting sexually propagated corals by reducing the labour required to manually outplant them on the reef. We tested two tetrapod-shaped substrates for coral settlement, tetrapod Type I and II (figure 7.1), which were designed to be deployed without the need for attachment or binding materials and still become permanently attached at their outplant location. These tetrapods with coral settlers were outplanted by simply wedging them in crevices in the reef framework, which only took 1.5 to 7% of the time required to outplant sexually produced corals using traditional outplanting methods (table 7.4). While tetrapods moved around (6 cm $week^{-1}$) during the first two weeks after outplanting, they rapidly became stuck thereafter (table 7.2, 7.4). One year after they were seeded onto the reef, 76% of tetrapods could still be recovered across all three levels of reef structural complexity, where they had either become firmly lodged in crevices and/or cemented to the reef framework by encrusting benthic organisms (figure 7.4, table 7.3). Our findings therefore suggest that seeding SUs in habitats with medium to high structural complexity substantially reduces costs and time required to reintroduce corals to degraded reefs, with long-term results similar to studies whereby SUs are manually secured to the benthos (table 7.4).

Effectiveness of seeding sexually propagated corals

Theoretically, only one remaining live and healthy coral colony per outplanted SU is required to eventually yield a successful restoration outcome (Edwards 2010). The proportion of initial SUs harbouring at least one coral individual through time therefore serves as a measure to compare the effectiveness of different restoration techniques. In the current study, the SU yield in reefs with moderate to high topographic complexities was 1.5 fold higher than the median effectiveness of earlier outplanting efforts (45%, table 7.4), but much less effective on reefs with low levels of structural complexity. In such areas, tetrapods dispersed easily (table 7.2), increasing the probability that coral settlers became abraded or crushed, and often remained unattached until the end of the experiment (table 7.3). Combined, this resulted in a 5 to 9-fold increase in settler mortality (figure 7.5*c*) and 2.4 times

lower SU yield (figure 7.6*b*) relative to areas with higher levels of structural complexity. Thus, seeding the tetrapods may not be successful in areas exposed to high wave energy or with low structural complexity unless their design is improved to promote attachment in such areas. Securing the SUs with binding materials, such as epoxy, likely represents a more effective approach than seeding current tetrapod designs.

Cost-effectiveness of seeding sexually propagated corals

Overall, the new tetrapod-shaped substrates could be outplanted efficiently with low costs for labour and materials, enabling 10,000 SUs to be seeded in one hectare of reef within 48 h at a cost of $7,000 USD (table 7.4). This represented a 5 to 18 fold reduction in costs of the actual outplanting process compared to traditional outplanting techniques. The production of the tetrapods themselves accounted for a large fraction of the production cost for a single one-year-old SU ($0.50 USD) (table S7.2), indicating that the cost-effectiveness of this new technique could be further improved if tetrapods would be produced industrially or at lower costs. Because the outplanting phase normally incurs a large proportion of the costs associated with coral restoration activities (~30%) (Edwards 2010), the 'seeding' of SUs, if combined with other economical but effective larval rearing techniques, could significantly reduce the costs of restoring degraded reef systems. Under such scenario, costs of reef restoration would become more comparable to the costs of existing mangrove and salt marsh restoration programs (< $10,000 USD per hectare) (Bayraktarov et al. 2016), allowing the application of coral restoration across much larger scales.

Optimization of the tetrapod designs

While the tested tetrapod designs reduced the amount of labour and costs during the outplanting phase, they were not optimal for coral settler survival and growth. For example, the average survival of *F. fragum* settlers was only 9.6% after one year, and very low compared to the 42% survival reported for *F. fragum* settlers settled on CCA chips in Belize (Ritson-Williams et al. 2016). While the tetrapods were successfully colonized by thin CCA communities that facilitate larval settlement and metamorphosis, their light-exposed upper surfaces became rapidly overgrown by algal turfs once outplanted on the reef (figure S7.3), which likely contributed to the high mortality rates of *F. fragum* settlers during the first three months following the outplant (Arnold et al. 2010). Because algal propagules and spores easily adhere to the porous texture of concrete structures (Sakai et al. 1998),

producing the tetrapods (including microstructures such as grooves) from non-porous materials such as glass or glazed ceramics, rather than from concrete, could prevent the formation of turf algal communities on the tetrapods, and subsequently enhance the survival and growth of settled corals.

Favia fragum larvae preferentially settled inside the tetrapods' grooves where they experienced lower post-settlement mortality rates. Grooves provide spatial refuges from incidental grazing by herbivorous fishes and urchins of newly settled corals (Nozawa 2008; Brandl et al. 2014), and should therefore always be considered in settlement substrate designs to enhance settler survival (Petersen et al. 2005). Grooved surfaces accounted for less than a third of the current tetrapods' total surface area (table S7.1), and future designs could likely be improved by increasing the amount of these microhabitats.

Because coral individuals that remain as single polyps past the age of one year often no longer enter the two or more polyp stage, the survival of one-polyp settlers per se is not indicative of effective recruitment (Vermeij and Sandin 2008). Here, 62% of one-year-old *F. fragum* individuals formed two- to seven-polyp colonies (figure S7.3), and most small-sized and one-polyp settlers were found on the cryptic undersides of the tetrapods (figure S7.3), where growth is repressed by low light availability (Maida et al. 1994; Babcock and Mundy 1996). Corals that settle on the undersides of artificial settlement substrates should be able to rapidly grow into light-exposed areas, where they will benefit from higher light levels (Babcock and Mundy 1996). Sub-cryptic surfaces (e.g., vertical walls, horizontal holes or crevices on the upward facing parts of settlement substrates), rather than fully cryptic surfaces such as the undersides of the tested tetrapods, would likely represent better microhabitats to be included in future tetrapod designs to allow a certain degree of protection to new settlers, without compromising their chances to grow into light-exposed areas.

Conclusions

Sexually propagating corals to restore depauperate coral populations has thus far been a time consuming, technically challenging and an expensive undertaking (Edwards 2010), and as a result has only been applied on small scales ($\leq$ 2,000 SUs per restoration site). By avoiding the need for outplanting corals using binding materials, the seeding approach allows the deployment of large numbers of young

corals in a very short amount of time and at low cost. This technique was most effective in reefs with moderate to high topographic complexity, where tetrapods rapidly became stabilized within the reef framework and resulted in a high SU yield relative to traditional outplanting methods. While we acknowledge that improvements can still be made in future tetrapod designs to optimize the survival and growth of coral settlers, this novel approach nonetheless represents a next step towards large-scale restoration using sexually propagated corals.

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Supplementary material

Supplementary figures

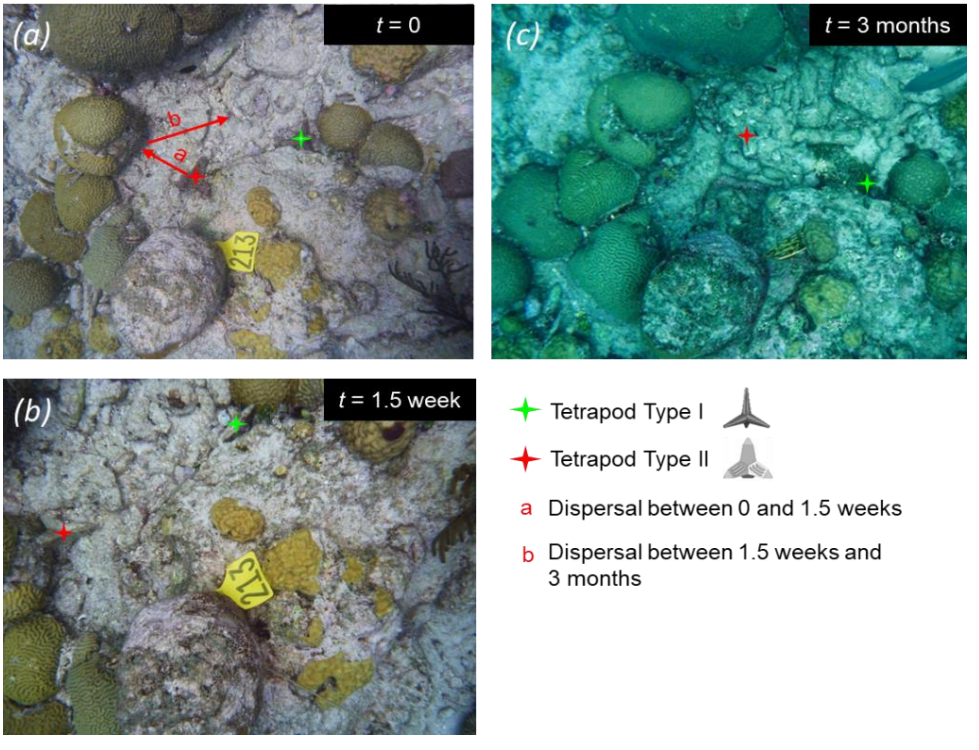

Figure S7.1. Example of photo-analysis of tetrapod dispersal for a period of three months. Type I (*green markers*) and Type II (*red markers*) tetrapods were located in overview pictures of each outplant location taken after *(a)* t = 0, *(b)* t = 1.5 week, and *(c)* t = 3 months. The tags and natural landmarks served as references to help compare the position of each tetrapod through time, while the length of the tags served as a scale to measure the distance between the previous and current position (indicated by *arrows of corresponding colours*) of the tetrapods ImageJ. In this example, tetrapod Type I stayed in place during the first three months of the experiment, while tetrapod Type II dispersed for a total distance of $a + b$. Photos by VF Chamberland.

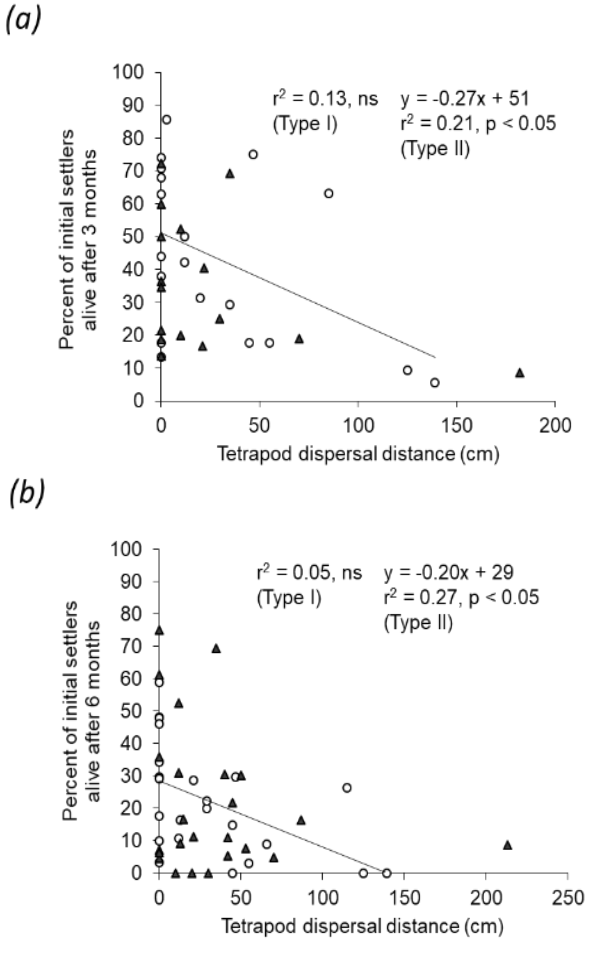

Figure S7.2. Effect of tetrapod dispersal on the survival of coral settlers. Regression analysis with dispersal distance as predictor variable and with *Favia fragum* settler survival as response variable after *(a)* 3 and *(b)* 6 months on tetrapod Type I (*filled triangles*) and Type II (*clear circles*).

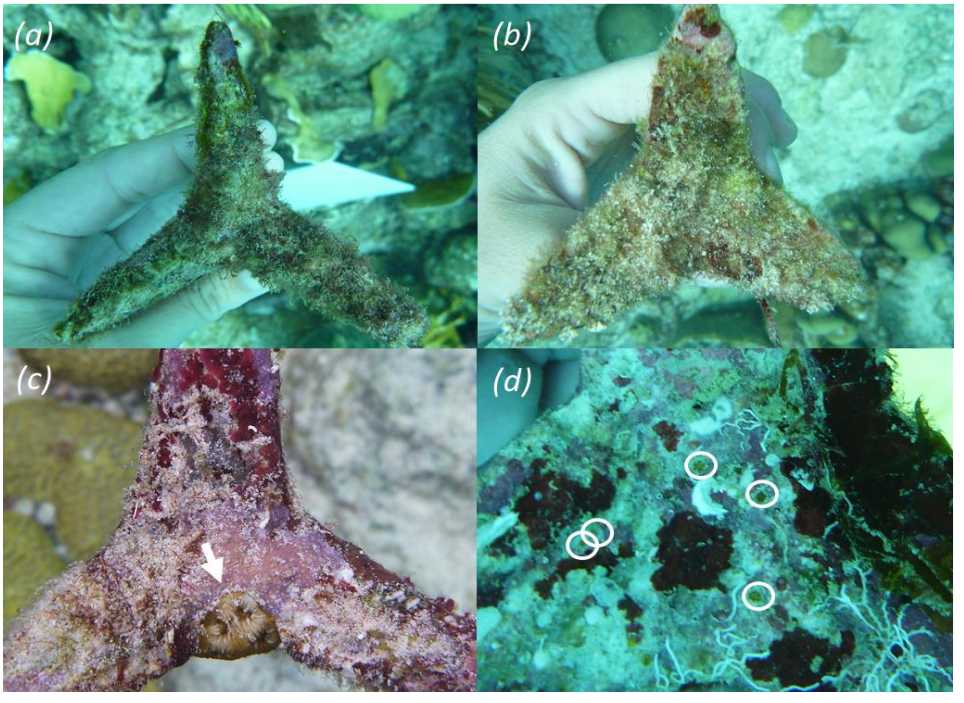

Figure S7.3. Examples of benthic communities and of coral settlers that grew on the tetrapods. Three months after they were deployed on the reef, the tetrapod's light-exposed surfaces on both *(a)* Type I and *(b)* Type II designs had become overgrown by communities of turf algae. *(c) Favia fragum* settlers that survived on the light-exposed surfaces of the tetrapods typically formed multi-polyp colonies (shown by *white arrow*) and reached larger sizes than those located on the *(d)* cryptic undersides of the tetrapods. The latter often still consisted of small, single-polyp individuals (shown by *white circles*) after one year. Photos by VF Chamberland.

Supplementary tables

(tables S7.3-S7.10 are available upon request)

Table S7.1. Dimensions of Type I and Type II tetrapods.

Tetrapod design	Dimensions[a] (mm)			Weight (g)	Total surface area[a] ($cm^{2)}$	Dimensions of grooves[a] (mm)			Surface area of grooves[ab] (cm2)
	L	*W*	*H*			*L*	*W*	*D*	
Type I	87.2	75.8	72.8	55.1	74.3	27.5	2.1	1.3	22.4
Type II	90.8	78.9	74.4	85.6	129.9	28.7	2.4	1.6	33.4

L, length; W, width; H, height; D, depth; SD, standard deviation.
[a] values measured with computer aided design (CAD) (manufactoring tolerance of tiles = 1 mm).
[b] total surface area of all grooves per substrate tile.

Table S7.2. Cost-calculation to outplant 10,000 seeding units (SUs) using different outplanting techniques.

Source		Quantity	Cost per item[a]	Total cost[a]
Current study	*Labour*[b]			
	person-hour per SU	0.005		
	person-hour per hectare	48	$6.63	$316.77
	Materials[c]			
	tetrapod	10000	$0.50	$5,000.00
	SCUBA gear[d]	10	$133.33	$1,333.33
	SCUBA air tank[e]	48	$3.00	$143.33
			total per hectare	$6,793.43
			total per SU	$0.68
Chamberland et al. 2016	*Labour*[b]			
	person-hour per SU	0.167		
	person-hour per hectare	1667	$6.63	$11,050.00
	Materials[c]			
	tripod	10000	$0.60	$6,000.00
	SCUBA gear[d]	10	$133.33	$1,333.33
	SCUBA air tank[e]	1667	$3.00	$5,000.00
	epoxy putty	1000	$10.00	$10,000.00
			total per hectare	$33,383.33
			total per SU	$3.34
Chamberland et al. 2015	*Labour*[b]			
	person-hour per SU	0.069		
	person-hour per hectare	690	$6.63	$4,574.70
	Materials[c]			
	tripod	10000	$0.60	$6,000.00
	SCUBA gear[d]	10	$133.33	$1,333.33
	SCUBA air tank[e]	690	$3.00	$2,070.00
	rope	10	$500.00	$5,000.00
	nails	7500	$0.10	$750.00
	cable ties	10000	$0.25	$2,500.00
			total per hectare	$22,228.03
			total per SU	$2.22

[a] Costs are in US dollars.

[b] Wages are based on the average worldwide GDP (median GDP per capita is $6.63 h^{-1}, Central Intelligence Agency 2014)

[c] Costs for materials were listed as stated in the studies or averaged among studies if they differed from one another

[d] Assuming a three year life span and one outplanting effort per year.

[e] Assuming 1 h of active outplanting work per air tank.

[f] Assuming a ratio of one SCUBA diver operating a pneumatic drill for three divers securing substrates to the reef.

[g] Assuming one air tank per hour to operate the pneumatic drill.

Table S7.2. Continued.

Source		Quantity	Cost per item[a]	Total cost[a]
Guest et al. 2013	*Labour*[b]			
	person-hour per SU	0.320		
	person-hour per hectare	3200	$6.63	$21,216.00
	Materials[c]			
	coral plug-in	10000	$0.03	$300.00
	SCUBA gear[d]	10	$133.33	$1,333.33
	SCUBA air tank (diver)[e]	3200	$3.00	$9,600.00
	pneumatic drill[f]	2.5	$135.00	$337.50
	SCUBA air tank (drill)[g]	800	$3.00	$2,400.00
	epoxy putty	1000	$10.00	$10,000.00
			total per hectare	$45,186.83
			total per SU	$4.52
Villanueva et al. 2012	*Labour*[b]			
	person-hour per SU	0.109		
	person-hour per hectare	1086	$6.63	$7,200.00
	Materials[c]			
	tox	10000	$0.22	$2,200.00
	SCUBA gear[d]	10	$133.33	$1,333.33
	SCUBA air tank (diver)[e]	1086	$3.00	$3,257.92
	pneumatic drill[f]	2.5	$135.00	$337.50
	SCUBA air tank (drill)[g]	271	$3.00	$814.48
	epoxy putty	1000	$10.00	$10,000.00
			total per hectare	$25,143.23
			total per SU	$2.51

[a] Costs are in US dollars.

[b] Wages are based on the average worldwide GDP (median GDP per capita is $6.63 h^{-1}, Central Intelligence Agency 2014)

[c] Costs for materials were listed as stated in the studies or averaged among studies if they differed from one another

[d] Assuming a three year life span and one outplanting effort per year.

[e] Assuming 1 h of active outplanting work per air tank.

[f] Assuming a ratio of one SCUBA diver operating a pneumatic drill for three divers securing substrates to the reef.

[g] Assuming one air tank per hour to operate the pneumatic drill.

Chapter 8

Synthesis and future directions

Valérie F Chamberland

This chapter continues the discussion on findings from previous chapters, and provides directions for future research to further improve our understanding of the recruitment dynamics of Caribbean corals. Ultimately, this knowledge may enable the application of sexual coral restoration techniques on much larger scales than currently feasible, and thereby allow the recovery of threatened coral populations at meaningful ecological scales.

Asynchronous spawning in sympatric populations: an under-appreciated phenomenon in Caribbean corals?

Genetic isolation within populations can lead to the subdivision of lineages and ultimately to the emergence of new species. In particular, genetic differences may arise when pre- or post-zygotic barriers prevent groups of individuals from exchanging genes (Bickford et al. 2007). In sympatric populations, reproductive asynchrony, such as observed in *Diploria labyrinthiformis* (this thesis, chapter 2) can prevent gametes from cross-fertilizing. This may result in limited gene flow between subpopulations that spawn at different times, potentially leading to a genetic divergence between those subpopulations. Reproductive barriers between closely related species have already been observed in species spawning at different seasons within years (Dai et al. 2000; Ohki et al. 2015; Rosser 2015) or at different hours within a day (Levitan et al. 2004).

Conspecifics that reproduce in the spring and in autumn have been documented in the Indo-Pacific (Baird et al. 2011; Rosser 2013; Gilmour et al. 2016). In many instances, populations spawning in the spring and autumn were genetically different and found to be comprised of cryptic species (e.g., Dai et al. 2000; Ohki et al. 2015; Rosser 2015). In the Caribbean region, spawning of most spawning species predominantly occurs in the fall (August-October) (Fadlallah 1983; Szmant 1986). *D. labyrinthiformis* is the first Caribbean broadcast spawning species for which biannual reproductive cycles were reported (this thesis, chapter 2). Gamete release in this population occurred monthly between May and October, with a first peak in gamete release in May-June and a second one in August-September. Colonies that spawned in the spring did not spawn in autumn and vice-versa, and occasional observations over multiple years (2013-2017) confirmed that this pattern is consistent through time. While genetic evidence is needed to confirm if these two sympatric cohorts are indeed genetically divergent, our observations that spring and autumn spawners never cross suggest that these two cohorts could eventually form

cryptic species, that are morphologically indistinguishable but with different reproductive seasons.

I hypothesise that asynchronous spawning in sympatric populations, such as that observed in *D. labyrinthiformis*, is likely a more common phenomenon in Caribbean species. For example, *Colpophyllia natans*, a conspicuous brain coral species, has been observed spawning in the summer in Puerto Rico and in the Flower Garden Banks (Steiner 1995; Hagman et al. 1998). Yet, in Puerto Rico, colonies contained mature gametes in the spring and in autumn, thereby suggesting bimodal reproductive seasonality in this species as well (E Weil, pers. comm.). Because coral spawning monitoring generally focusses on the well-known coral spawning period (August-October), spawning events of species like *C. natans* outside this period could be easily missed. *Pseudodiploria strigosa*, another Caribbean broadcast spawning brain coral, released gametes 40 min after sunset in August 2016 in Curaçao, whereas it spawned between 225 and 260 min after sunset in September and October in previous years. After gametes are released in the water column, probabilities of successful fertilization decrease rapidly through time as a consequence of gamete dilution and short competency periods, so that differential spawning in species like *P. strigosa* greatly reduces the degree of genetic mixing between these cohorts (Levitan et al. 2004). Similarly, species boundaries between *Montastraea franksi* and two closely related species (*M. annularis* and *M. faveolata*) are maintained by only a two hour difference in spawning times (Levitan et al. 2004). It is therefore likely that *P. strigosa* individuals that spawn shortly after sunset do not cross with those that reproduce later at night, and that they could as a consequence be or become genetically isolated.

While anecdotal, these observations provide sufficient grounds to further investigate differences in spawning times between sympatric conspecifics, as a factor affecting the evolutionary dynamics and (cryptic) species formation of Caribbean coral species.

Early life-history traits provide insights on the mechanisms leading to shifts in coral community composition

> "In ecology and evolutionary research, too often patterns are sought that are overly general —trying to unify all data under a single schema—and this is true of life-history generalizations."- *Bernardo, Joseph (1996)*

Strong shifts in species composition in response to local and global threats have been reported for coral reefs worldwide. In the Caribbean, many reefs historically dominated by reef-building species such as *Acropora* spp. and *Montastraea* spp. are currently dominated by non-framework-building species with opportunistic and stress-resistant life-history strategies (Aronson et al. 2004; Darling et al. 2012). Darling et al. (2012) recently proposed a trait-based classification for coral species to predict how different taxa may respond to environmental disturbances, and which species are more successful in coping with conditions on present-day reefs. This classification builds on 11 traits characterizing (sub)adult corals that already successfully survived through their earliest life stages (e.g., colony growth form, corallite diameter, colony size, generation time, skeletal density).

Similar to the adult traits compiled by Darling et al. (2012), early life-history traits are remarkably variable among coral species. Szmant (1986) for example reported large variation in egg size (50-600 µm in diameter) among nine Caribbean species. The duration of a larva's planktonic phase can last from hours in *Favia fragum* (Carlon and Olson 1993) to several weeks in *Acropora palmata* (Baums et al. 2005) and *Montastraea faveolata* (Szmant and Meadows 2006). Some species need specific CCA cues for settlement, while others can settle on recently formed biofilms (Ritson-Williams et al. 2016). Despite such differences among species, groupings of Caribbean coral species based on early life characteristics are extremely broad and focus foremost on extremes rather than on the gradual continuum that exists in early life-history characteristics. The most classical example of the broad division applied to Caribbean corals is their division based on reproductive mode in brooding and spawning species. As described in chapter 2, *Diploria labyrinthiformis* exemplifies a broadcast spawning species with early life-history traits that resemble those typically associated with brooders (e.g., multiple reproductive cycles per year, short planktonic phase, and high survival and

settlement rates). Early life-history characteristics are thus not necessarily similar among brooding and spawning species, but further studies focussing on the dynamics of the earliest life stages of corals are needed to elucidate the range of strategies employed by young corals, and to see whether potential groupings, other than the classical brooder/spawner division, exist. Below, I propose additional traits for such more nuanced classification of species based on their reproductive and early life-history characteristics. Because mortality rates in coral populations are highest during their earliest life stages, classifications combining early life and adult traits are expected to provide new insights on the mechanisms influencing species composition in coral communities as a whole.

Reproductive traits

In the species classification proposed by Darling et al. (2012), two reproductive traits are included: ***development mode*** (brooding vs spawning) and ***fecundity***. Fecundity in this case is exclusively defined as the number of eggs per polyp/mesentery at a given time and thus ignores the ***number of reproductive cycles per year*** needed to accurately predict a species' total annual reproductive output. Species can have one to up to 12 reproductive cycles per year, which need to be considered when calculating total reproductive output, i.e., the potential number of offspring that can settle at a location.

Embryogenesis and larval development

The duration of embryogenesis, i.e., larval development after eggs have become fertilized in the water column, is highly variable among broadcast spawning coral species. Once fertilized, eggs in some species can complete embryogenesis within < 24 h (e.g., *M. cavernosa, M. faveolata, S. siderea, D. labyrinthiformis*) whereas others produce embryos that require three to four days to fully develop into a motile larva (e.g., *A. palmata*: Baums et al. 2005). Developing embryos generally remain at the water surface where they are transported by currents, but also subjected to predation, ultraviolet radiation, elevated temperatures, and reduced salinity from rainfall events (Rumrill 1990). While species with long embryogenic development have greater dispersal potential resulting in increased population connectivity, they also face a higher risk of mortality during their planktonic phase. Thus, ***time to motility*** whereby larvae start swimming, and ***time to negative buoyancy*** whereby larvae move away from the surface towards the benthos, both influence the probability of a larva surviving. They are therefore important, but currently largely neglected, transitions in a larva's life with consequences for the recruitment dynamics of species.

Competency period and settlement preferences

Corals produce non-feeding (i.e., lecithotrophic) larvae that must settle and metamorphose before they exhaust their energy resources (Harii et al. 2002). Large differences in competency period have been reported both among brooding (e.g., Harii et al. 2002) and broadcast spawning species of the Indo-Pacific region (e.g., Wilson and Harrison 1998), but much less is known for Caribbean species. A larva's ***competency period*** is the period during which it starts searching for settlement surfaces, until it is no longer capable of settling due to energy depletion (Harii et al. 2002). This period can span from a few days to months depending on species (e.g., Wilson and Harrison 1998; Harii et al. 2002; Harrison 2006). Competency periods can also vary among conspecifics as a result of variable maternal provisioning in terms of nutritional resources such as lipids (i.e., ***larval size***: Isomura and Nishihira 2001) and algal symbionts (i.e., ***symbiont density***: this thesis, chapter 3). On the one hand, larvae with long competency periods benefit from a longer time-window to search for optimal settlement habitats which will contribute to higher post-settlement survival. Long competency periods are thus particularly advantageous for larvae with very specific ***settlement preferences***, and when preferred settlement cues are scarce in the environment. On the other hand, larvae with extended competency periods face high risks of mortality while dispersing in the water column. In chapter 3, for example, we found that *F. fragum* larvae provided with high densities of algal symbionts by their mothers were capable of searching for settlement substrates for longer periods of time, but were also more susceptible to temperature anomalies. Thus, extended competency periods can come with both costs and benefits that will in turn influence a larva's chances of successfully recruiting in the adult population. In *F. fragum*, mothers appear to bet-hedge the risks associated with an either short (i.e., failure to settle in optimal habitats) or long competency period (i.e., high mortality risks during the pelagic phase) by producing offspring with a range of competency periods.

Post-settlement growth strategies

In this thesis, early post-settlement growth was investigated for three species, i.e., the brooding species *F. fragum* (chapter 7) and the broadcast spawning species *D. labyrinthiformis* (chapter 2) and *A. palmata* (chapter 5 and 6). These three species have different ***post-settlement growth strategies*** that do not overlap with the classical division in brooders and spawning species as the main groups sharing similar life-history strategies. *A. palmata* settlers initiated polyp divisions shortly after they settled (two weeks), whereas polyp divisions in *D. labyrinthiformis*

occurred much later at the age of three to 12 months. Polyp divisions in *A. palmata* did not result in large increases in colony size as new polyps were small (figure 8.1*a*,*b*). In contrast, *D. labyrinthiformis* polyps grew to at least threefold their initial size before initiating their first polyp division (figure 8.1*a*,*c*). *F. fragum* displayed an intermediate strategy whereby settlers appeared to spread investments between polyp growth and division resulting in intermediate-sized colonies with large variation in polyp sizes (figure 8.1*a*,*d*). These observations suggest that a gradient exists in early growth strategies. At one end of the gradient, newly settled corals invest in rapid polyp divisions resulting in colonies with many small polyps, whereas at the other end new settlers may invest in initial polyp growth resulting in colonies with few, but larger polyps.

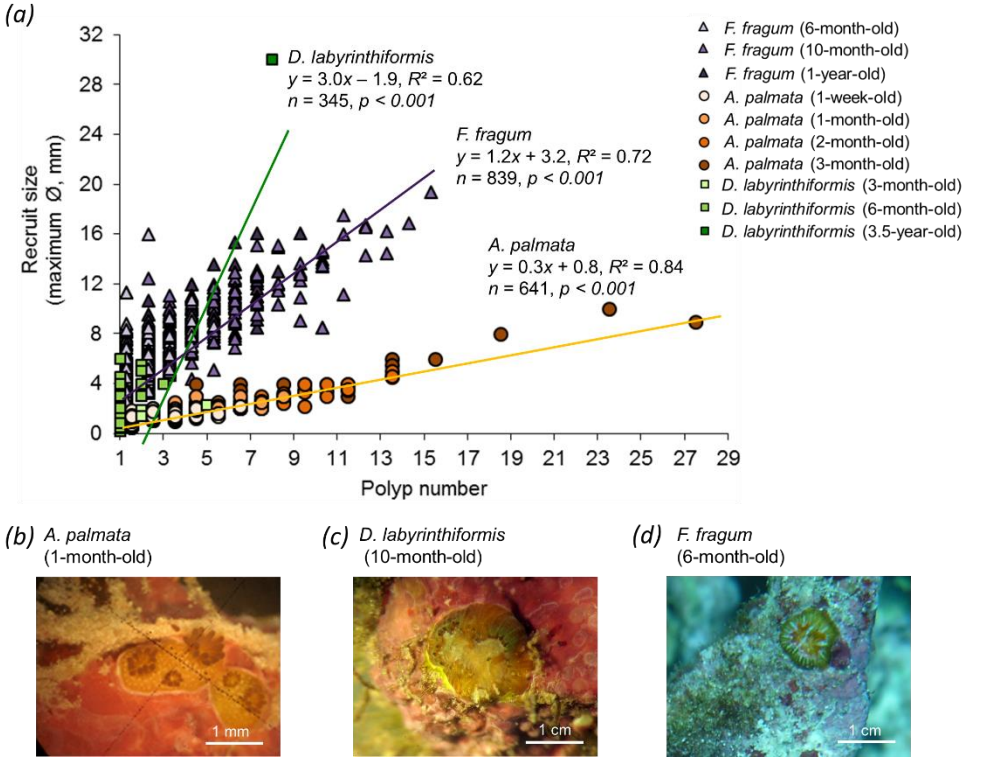

Figure 8.1. Contrasting early growth strategies in the three Caribbean species *Acropora palmata*, *Diploria labyrinthiformis* and *Favia fragum*. *(a)* Linear regression between recruit size (maximum diameter) and number of polyps. Examples of first polyp divisions in *(b) A. palmata*, *(c) D. labyrinthiformis*, and *(d) F. fragum*. This data was extracted from chapters 2, 5 and 7 of this thesis. Photos by *(b, c)* VF Chamberland and *(d)* MT Chamberland.

While it is unclear how these contrasting growth strategies may influence recruitment success in corals, growth patterns have been shown to be of fundamental importance in benthic invertebrate species' ability to compete with other sessile benthic organisms. Jackson (1977) for example found that colonial organisms (i.e., those consisting of multiple modules, such as polyps in corals) are less susceptible to overgrowth by other organisms as the death of one module (e.g., polyps, zooids, ramets) does not result in the mortality of the entire organism. Here I suggest that during the first months following settlement, *D. labyrinthiformis* displays a growth pattern similar to that of solitary organisms by investing in rapid growth of a single polyp which reduces mortality probabilities as mortality risks reduce with increasing size (Vermeij and Sandin 2008). In contrast, *A. palmata* rapidly divides in multiple polyps and forms a colonial organism early in life avoiding total mortality by spreading mortality risks among individual polyps similar to the strategy of colonial organisms described by Jackson (1977).

Further studies on coral species' early life traits will provide invaluable insights on the factors contributing to their successful recruitment, and could as a consequence allow us to better predict their probability of persistence or decline in the face of ongoing environmental change.

Three research directions to improve sexual coral restoration efforts

I. Inhibiting the recruitment of fleshy algae on artificial settlement substrates

While current restoration techniques using sexually reared recruits are increasingly more successful and cost-effective (this thesis, chapters 5, 6 and 7; Nakamura et al. 2011; Baria et al. 2012; Villanueva et al. 2012; Guest et al. 2014), recruit mortality rates remain extremely high after outplanting with less than 5% of all cultured settlers surviving for more than one year (Edwards 2010). These high rates of post-settlement mortality greatly reduce the effectiveness of outplanting young corals in terms of effort and costs, which in turn limits its application to small spatial scales.

A major cause of mortality among newly settled corals after outplanting results from strong competition with neighbouring sessile organisms (Vermeij 2006; Vermeij and Sandin 2008; Doropoulos et al. 2016). Conditioning artificial substrates under controlled conditions (e.g., low light, low sedimentation, strong water movement) ensures their colonization by CCA communities that facilitate larval settlement and post-settlement survival (this thesis, chapters 5 and 7). However, once they are outplanted, these substrates become rapidly overgrown by algal communities (mainly turf algae; figure S7.3) that severely compromise the health and survival of neighbouring coral settlers through overgrowth, silt trapping, oxygen depletion, and the release of allelopathic compounds (Fabricius 2005; Ritson-Williams et al. 2009; Vermeij et al. 2009). A critical step towards overcoming such bottlenecks in the life of recently settled corals is to prevent or at least delay the recruitment and growth of fleshy algae on these settlement substrates. The longer young corals can grow in the absence of neighbouring algae, the higher the probability they will reach larger size classes that are less susceptible to competition from neighbouring organisms (Arnold et al. 2010; Arnold and Steneck 2011) and thereby increase their probability of survival (Vermeij and Sandin 2008; Arnold et al. 2010; Doropoulos et al. 2016). Future restoration efforts should hence not only focus on the facilitation of coral settlement and survival but also on the prevention of algal settlement and survival.

Recruitment and growth in marine fleshy algae is facilitated on substrates with a porous micro-topography (i.e., texture) such as concrete and limestone, to which algal propagules and spores easily adhere (Sakai et al. 1998; Callow and Callow 2000) (figure 8.2*a*). In contrast, fleshy algae hardly recruit on smooth surfaces such as glass (figure 8.2*b*) to which they cannot attach as easily as to porous substrates.

Thus, as mentioned in chapter 7, producing settlement substrates from smooth materials such as glass or glazed ceramics, rather than from concrete, could prevent the formation of algal turf communities on these substrates, and subsequently enhance the survival and growth of settled corals that appear less sensitive to variations in substrate texture for settlement.

Several studies on the processes influencing fouling of immersed structures have found that a surface's colour, in addition to its texture, orientation and chemical composition, influences the development of certain micro- and macro-fouling communities (e.g., Swain et al. 2006; Dobretsov et al. 2013). During early community succession, black surfaces generally attract a greater diversity and abundance of sessile organisms (e.g., bivalves, ascidians, algae) compared to white surfaces (Swain et al. 2006; Guenther et al. 2009; Dobretsov et al. 2013). Similarly, settling coral larvae display preferences for substrate colouration, and settle in higher numbers on red surfaces than on other colours (Mason et al. 2011). Thus, many sessile benthic organisms appear to use spectral cues for small-scale habitat selection during settlement. Therefore, physical properties such as coloration should be considered when designing artificial settlement substrates for coral larvae. For instance, while white surfaces are not preferred by settling corals, they might represent a suitable substrate on the long term. Algae settle and grow slower on white surfaces compared to darker (red) surfaces, which could result in lower mortality rates of recently outplanted coral settlers on such surfaces. Similarly, identifying additional natural or synthetic anti-fouling compounds (e.g., algaecides,

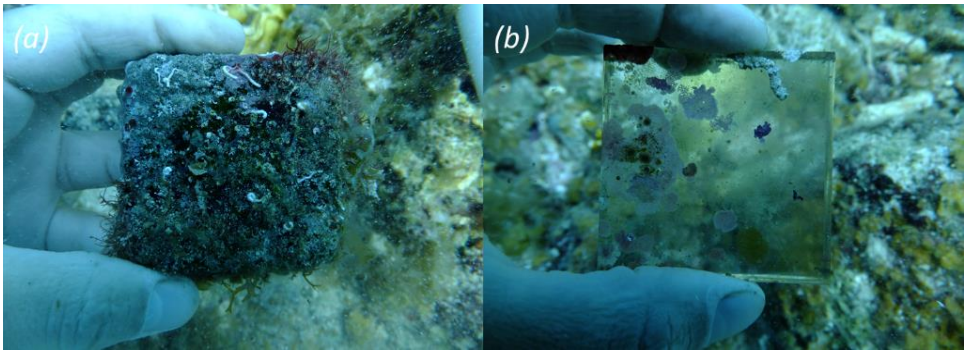

Figure 8.2. Algal communities that developed on *(a)* concrete and *(b)* glass substrates conditioned on a reef dominated by fleshy algae for a period of three months. Concrete substrates became heavily fouled by communities of turf algae whereas glass substrates were slowly colonized by thin biofilms and CCA. Photos by E Chappel and N Hurtado.

polymers, chemical compounds, microbial communities) that reduce algal growth without compromising the health of coral settlers could further increase the effectiveness of sexual coral restoration techniques.

II. The novel ecosystem concept and sexual coral restoration efforts

> "Ecological restoration efforts should aim to conserve and restore historical ecosystems where viable, while simultaneously preparing to design or steer emerging novel ecosystems to ensure maintenance of ecological goods and services"
> - *Jackson & Hobbs (2009)*

As mentioned in the previous section of this discussion, significant shifts in species composition on coral reef communities have occurred on reefs around the world. On shallow Caribbean reefs, formerly dominant *Acropora palmata* populations (figure 8.3*a*) suffered a massive die-off in the 1970s caused by a disease outbreak (Aronson and Precht 2001). The recovery of these populations has since then been hindered by increasing human impacts such as climate change, decreasing water quality, and increasing competition with turf algae and macroalgae resulting from nutrient pollution and overexploitation of parrotfishes (Acropora Biological Review Team 2005). On Curaçao, many of these shallow reefs became dominated by brain coral species (figure 8.3*b*) after the die-off of *A. palmata* populations (Nagelkerken and Nagelkerken 2004). Several of these reefs are located in front of densely populated parts of the island, where they are exposed to frequent anthropogenic impacts including overfishing, coastal development, nutrient pollution from faulty sewage treatment facilities, and chemical pollutants from a nearby oil-refinery (VF Chamberland and MJA Vermeij, pers. obs.). Despite of aforementioned stressors, some of the reefs dominated by brain corals still have a high cover of corals (~30%) and support a large number and high diversity of other marine organisms (figure 8.3*b*; VF Chamberland and MJA Vermeij, pers. obs.). Such ecosystems have recently been described as "*novel ecosystems"*. Per definition, a novel ecosystem is an ecosystem that

> "*(…) has species compositions and relative abundances that have not occurred previously within a given biome. The key characteristics are (1) novelty: new species combinations, with the potential for changes in ecosystem functioning; and (2) human agency: ecosystems that are the result of deliberate or inadvertent human action, but do not depend on continued human intervention for their maintenance."* (Hobbs et al. 2006).

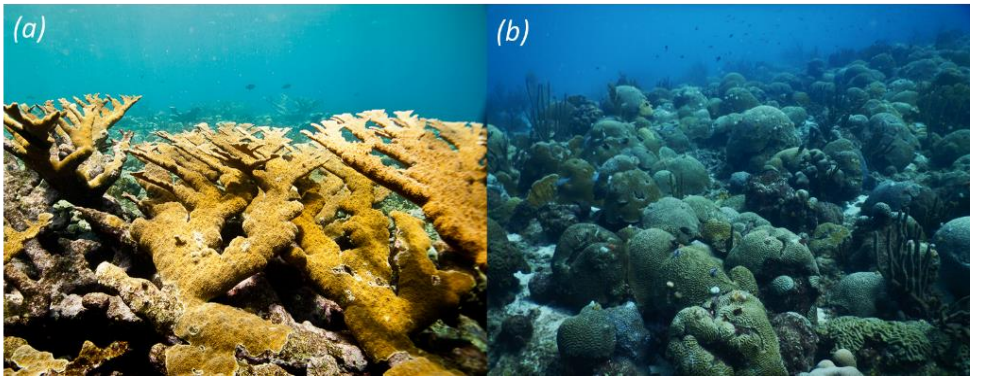

Figure 8.3. *(a)* A reef dominated by branching *Acropora palmata*, which was widespread on most shallow Caribbean reefs three decades ago. *(b)* A reef dominated by massive brain coral species, a novel ecosystem that has replaced many of the former *Acropora* reefs. Photos by *(a)* P Selvaggio and *(b)* VF Chamberland.

I propose to apply this concept for reef restoration, whereby coral species targeted for restoration in degraded areas must be selected for their natural ability to recruit, grow and reproduce in impacted or degraded areas, rather than insisting on the return of once dominant species that may no longer be capable of thriving under the conditions typifying modern reefs.

Larval rearing and outplanting efforts in the Caribbean region foremost focus on threatened species such as the critically endangered Acroporids and endangered species of the *Montastraea* complex (this thesis, chapters 5 and 6; Vermeij et al. 2006; Erwin et al. 2008; Erwin and Szmant 2010; Ritson-Williams et al. 2010, 2016; Voolstra et al. 2011; Miller 2014). While such efforts may be successful in reefs experiencing limited human impact (figure 8.4), they are likely to result in a waste of restoration funds if implemented on degraded reefs where these species appear unable to recruit naturally (even if only in small numbers).

In chapters 5 and 6, we for example reported the successful rearing and long-term survival of sexual recruits of critically endangered *A. palmata* outplanted at the Sea Aquarium reef. This reef location is in a relatively pristine state and supports a healthy and well-developed *A. palmata* population (VF Chamberland, pers. obs.). The effectiveness of outplanting *A. palmata* recruits on highly impacted reefs has on the other hand not yet been assessed. It is therefore still unclear if *A. palmata* populations could be restored to their historic abundance in degraded areas where they are currently largely absent. In contrast, brain coral species recruit in higher numbers than they did three decades ago (Vermeij et al. 2011), and often dominate

in areas experiencing chronic human impact (VF Chamberland and MJA Vermeij, pers. obs.). Thus, despite the fact that brain corals might not represent historically abundant species at depths formerly dominated by Acroporids, brain coral species and stress-resistant species are likely better candidates for reef restoration efforts aimed at increasing coral cover. Approaches focussing on restoring historically dominant species could fail after which such reefs could become dominated by algae. Outplanting resistant, rather than historically abundant, coral species in degraded reef areas may therefore result in higher coral growth and survival, thus increasing the success of restoration efforts (figure 8.4).

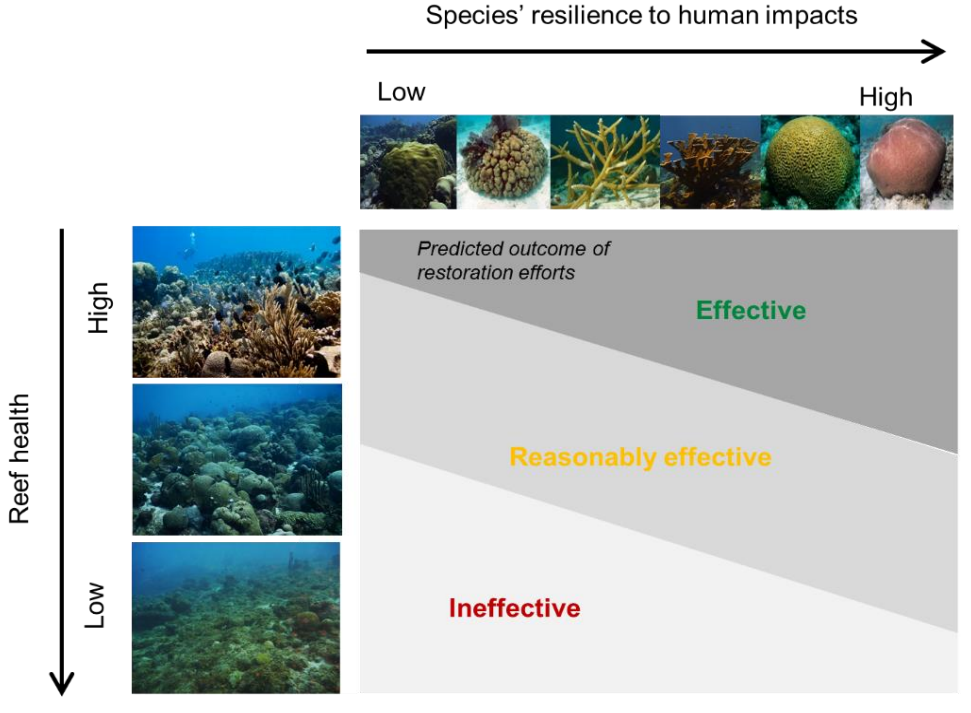

Figure 8.4. Schematic representation of the predicted outcome of restoration efforts in a gradient of reef health, based on species' resilience to human impacts. Outplanting coral species highly susceptible to anthropogenic stressors on degraded reefs is likely to be ineffective. On such reefs, outplanting stress-tolerant species is likely to result in better investments of restoration funds. The classification of species' resilience to human impacts is based on Darling et al. (2012).

III. Advancing technologies to enable mass rearing and outplanting of sexual recruits

Early post-settlement bottlenecks are undoubtedly the most pressing issue to be addressed by researchers in this field in order to improve the effectiveness of restoration approaches that make use of young corals. The fact that we are only beginning to understand some of the complexity of the ecological and biological processes affecting recently settled corals, often leads to scepticism as to whether coral restoration using sexually produced coral larvae is even possible. Based on recent progress however, such as that presented in this thesis as well as the work of many colleagues in the field (Nakamura et al. 2011; Baria et al. 2012; Villanueva et al. 2012; Guest et al. 2014), I argue that we are only but a few steps away from effectively rearing coral recruits from wild-caught gametes and subsequently ensure their long-term survival and growth after outplanting.

Hence, to conclude this thesis, I propose examples of technological advancements that, once solutions to improve post-settlement survival in recently settled corals have been found, will enable the mass rearing and outplanting of sexual recruits at much larger scales than currently feasible.

In situ larval rearing and settlement

Larval rearing is typically conducted *ex situ* either in closed containers containing small volumes of filtered seawater (this thesis, chapter 2), or in aquarium systems in which flow-through devices are partially submerged (e.g., kreisels: this thesis, chapter 5). Similarly, coral larvae are either settled in flow-through or closed systems in which artificial substrates are spread on the bottom. The scale at which we can rear and settle coral larvae is therefore often limited by the space in which these activities take place (i.e., laboratory bench and aquarium surfaces) and the labour associated with the maintenance of larval cultures in such systems (e.g., frequent water changes and removal of biofilms on containers/kreisels, constant monitoring of water quality). It is therefore virtually impossible to use *ex situ* techniques to produce more than a few hundred SUs (Seeding Units: this thesis, chapter 7) at a time. To address this issue, SECORE International is currently developing *in situ* floating devices, the "*larval rearing pools"*, in which hundreds of thousands of coral larvae can be reared simultaneously, and subsequently settled on up to a thousand substrates per pool. After gametes are fertilized *ex situ*, developing embryos are transferred directly into these pools where they complete their development and settle on substrates previously placed inside the pools. Because the pools contain large volumes of water (4320 L, 1.6 × 1.5 × 1.8 m, L ×

W × H) and are flow-through, these floating devices require minimal maintenance compared to *ex situ* larval rearing. While these larval rearing pools are still at an early testing phase, they have great potential for the mass rearing and settlement of coral larvae at lower costs per larva in terms of money and labour.

Automated outplanting of sexual coral recruits

In chapter 7, we described new artificial substrates that, due to their tetrahedral shape, can be outplanted efficiently by wedging them in reef crevices without the need for binding materials such as epoxy and nails. This approach significantly reduces the cost and time required for the outplanting phase of larval propagation efforts. Nonetheless, this technique still requires that each substrate is handled by a scuba diver, which, if translated to large scale restoration (thousands of SUs), remains a time-consuming and costly process. A similar issue arose on the Great Barrier Reef when breakouts of corallivorous crown-of-thorns starfish decimated already imperilled coral populations (Kayal et al. 2012). Colossal efforts to eradicate the starfish by scuba divers were unsuccessful as locating and removing these starfish is incredibly time consuming. Recently, a robot specifically designed to eradicate crown-of-thorns by navigating on reefs and injecting them with fatal doses of bile salts, has proven successful in controlling the exploding populations of this pest (Pultarova 2015). With the advent of increasingly sophisticated technologies, it is therefore not unforeseeable that SUs could be effectively outplanted by similar underwater robots in the near future.

Thus, while we address the post-settlement bottleneck by conducting small scale and detailed experiments, we should not preclude ourselves of thinking beyond this limitation, and start advancing technological solutions to assist the recovery of coral reefs at scales that were never considered before.

Summary

Environmental Drivers of Recruitment Success in Caribbean Corals: Applications to Aid the Recovery of Threatened Coral Populations

Survival and successful establishment of coral larvae, i.e., *larval recruitment,* is a key process determining the long-term fate of coral reefs. Yet, little is known about the factors driving the recruitment success of coral larvae. This thesis aims to gain a better understanding of the environmental processes affecting recruitment success in Caribbean corals, to identify the conditions under which recruitment can be successful, and to use this information in restoration efforts aimed at increasing larval recruitment in threatened coral communities.

We first investigated the reproductive biology and early life ecology of the brain coral *Diploria labyrinthiformis* (Linnaeus 1758), an abundant reef-building species throughout the Caribbean region. *D. labyrinthiformis* is the only known coral that spawns monthly during daylight hours from May to September, resulting in the highest number of reproductive events per year ever observed for a Caribbean broadcast spawning coral species. Our results show that *D. labyrinthiformis* larvae developed rapidly and settled in high numbers when provided with settlement cues. The survival and growth of settlers increased when they acquired algal symbionts early in life, and when provided with nutrients. *D. labyrinthiformis'* multiple reproductive events per year, its short planktonic larval phase, high settlement rates, and its positive response to nutrient enrichment show that this species is an ideal candidate for reef restoration purposes.

In contrast to broadcast spawning species, most brooding coral species provide their larvae with varying densities of algal symbionts. To explore how algal symbionts affect larval fitness, we studied the performance of larvae of the stony coral *Favia fragum* (Esper 1797) with different symbiont densities under different environmental conditions. Larvae with high symbiont densities were more active and searched for suitable settlement habitats for longer periods of time. However, such larvae also suffered (non-lethal) stress and increased mortality compared to larvae with low symbiont densities when seawater temperature increased. Consequently, maternal inheritance of large numbers of algal symbionts can be beneficial, but also detrimental to coral larvae depending on environmental conditions. The mixed benefits of maternal symbiont provisioning suggest that *F. fragum* mothers use a bet-hedging strategy to improve the recruitment success of their offspring in an environment with high spatio-temporal variability such as coral reefs.

Many coral reefs have been overgrown by turf- and macroalgae due to nutrient enrichment and the loss of key herbivores. How algal communities that develop under nutrient enrichment and reduced herbivory influence settlement success of coral larvae is however not well known. We therefore manipulated grazing pressure and nutrient levels *in situ* to investigate how the resulting early succession of algal communities affect settlement of *F. fragum* larvae in light-exposed and cryptic habitats. Reduced herbivory resulted in a twofold increase in the abundance of turf algae, and nutrient enrichment further promoted macroalgal growth under low grazing pressure. When provided the choice, *F. fragum* larvae preferred settling in cryptic habitats dominated by CCA and bare substratum. Furthermore, on light-exposed surfaces, larvae also preferentially settled in microhabitats with bare substratum while avoiding turf algae. Herbivory, successional stage and nutrient levels collectively influenced what algal communities were available to settling larvae: while algal communities grown under nutrient enrichment and/or reduced herbivory were most conducive to coral settlement at primary successional stages, communities grown under natural reef conditions were preferred at intermediate and advanced successional stages. When not provided with the choice however, larvae settled at similar rates in algal communities of all nutrient-herbivory treatments, indicating that settlement surfaces were still available within sub-optimal communities. These findings show that the availability of small-scale patches with CCA and open space is of key importance for the successful settlement of coral larvae during early algal succession, even when exposed to increased nutrient availability and reduced herbivory.

The use of sexual coral recruits for restoration purposes is being applied in a growing number of locations around the world to increase the number of individuals in recipient populations and their genetic diversity. Restoration efforts in the Caribbean foremost focus on aiding the recovery of critically endangered elkhorn corals (*Acropora palmata,* Lamarck 1816). This species provided various important ecological services within shallow Caribbean reef communities, but a disease reduced their abundance by > 95% in the mid-1970s to early 1980s. To aid this species' recovery, we developed several techniques to rear and settle *A. palmata* larvae. *A. palmata* settlers reared from gametes collected in the field survived for long periods of time (3.4% survival after 2.5 years) and some colonies spawned in synchrony with the native population at their outplanting site at the age of four years. This is the first time that an endangered Caribbean *Acropora* coral species was raised from gametes and subsequently grown to sexual maturity in the field.

While restoration techniques using sexually reared recruits are increasingly more successful, they remain expensive which limits their use to spatial scales much smaller than those at which reefs degrade. Current outplanting methods require tedious handling of binding materials (e.g., cable-ties, epoxy, nails) underwater, which is time consuming and accounts for a large fraction of restoration costs. To minimize these costs, we designed and tested two tetrapod-shaped artificial settlement substrates that can be outplanted rapidly by simply wedging them in reef crevices. Because of their tetrahedral shape, the tetrapods became rapidly lodged in crevices or cemented to the benthos by encrusting organisms. Overall, this new "seeding" approach resulted in a much faster outplanting rate and a 5 to 18-fold reduction in outplanting costs compared to traditional methods using binding materials. While our tetrapod designs can still be improved, this new "seeding" approach shows how relatively simple technical solutions can result in substantial reductions in costs and time required to outplant young corals to reefs, and thus facilitate restoration of degraded reefs across larger spatial scales.

This thesis has shown how studies on the early life-history traits of coral species improve our understanding of the factors determining their successful recruitment, and demonstrates that such findings can be used to assist the recovery of imperiled coral populations.

Samenvatting

Sturende Factoren voor de Vestigingskansen van Koralen: Toepassingen voor het Herstel van Bedreigde Koraalpopulaties in de Caraïben

De overleving en succesvolle vestiging van koraallarven op het rif bepalen in belangrijke mate of koraalriffen op termijn kunnen voortbestaan. Helaas is er weinig informatie beschikbaar over welke factoren hierbij een rol spelen. Dit proefschrift richt zich daarom op een beter begrip van de natuurlijke omstandigheden die van belang zijn voor koraallarven om zich succesvol op koraalriffen te kunnen vestigen. Deze informatie kan gebruikt worden ter verbetering van methodes om koraalpopulaties te herstellen, bijvoorbeeld door het aantal larven dat zich op koraalriffen vestigt te vergroten.

Allereerst werd de voortplanting en de ecologie van de vroegste levensstadia van het gegroefd hersenkoraal *Diploria labyrinthiformis* (Linnaeus 1758) bestudeerd. Dit is een veelvoorkomende soort die bijdraagt aan de vorming van koraalriffen in het Caribisch gebied. *D. labyrinthiformis* is de enige koraalsoort die maandelijks van mei tot en met augustus gedurende de dag eieren en zaad uitstoot, resulterend in de langste reproduktieve periode die ooit is waargenomen voor Caribische koraalsoorten die kuit schieten. De larven van *D. labyrinthiformis* ontwikkelden zich snel en vestigden zich na korte tijd op de bodem na toevoeging van natuurlijke stoffen die de vestiging van koraallarven stimuleren. Het tijdig opnemen van symbiotische algen en toevoeging van nutriënten zorgden ervoor dat gevestigde koraallarven sneller groeiden en beter overleefden. Omdat *D. labyrinthiformis* meerdere malen per jaar kuit schiet, larven slechts een korte periode in de waterkolom doorbrengen en zich daarna in grote aantallen op de bodem vestigen, en de toevoeging van nutriënten positief bijdraagt aan de overlevingskansen, is deze soort een ideale kandidaat voor koraal herstel projecten waarin getracht wordt het aantal koralen op beschadigde riffen te vergroten.

In tegenstelling tot koraalsoorten die kuit schieten, voorzien broedende koraalsoorten die larven uitstoten hun nakomelingen direct van symbiotische algen. Hierbij kan het aantal symbiotische algen per larve enorm verschillen. We onderzochten hoe het aantal symbionten per larve het functioneren van een larf beïnvloedt, door larven van het golfbal koraal *Favia fragum* (Esper 1797) met verschillende symbiont dichtheden bloot te stellen aan verschillende omgevingsfactoren. Larven met veel symbionten waren aktiever en zochten langer naar geschikte lokaties om zich te vestigen. Echter, deze larven ondervonden ook meer (sublethale) stress en vertoonden een hogere sterfte als de temperatuur van het

zeewater werd verhoogd in vergelijking met larven met weinig symbionten. Deze resultaten tonen aan dat het aantal symbionten dat een koraallarf van zijn moeder ontvangt zowel een positief als een negatief effect kan hebben, afhankelijk van de omgeving waarin de larf zich bevindt. De omgevingsafhankelijke voordelen van veel danwel weinig symbionten suggereren dat *F. fragum* moeders de overlevingskansen van hun nakomelingen trachten te verhogen middels een zogenaamde "bet-hedging" strategie. Door larven te produceren met een grote variatie aan symbiont dichtheden wedden ze op meerdere paarden tegelijk, om zo in te spelen op de natuurlijke variatie in tijd en ruimte die koraalriffen kenmerkt.

Door overbevissing van herbivore vissen en een verhoogde hoeveelheid nutriënten worden veel koraalriffen momenteel overwoekerd door turf- en macroalgen. Over de manier waarop dergelijke algen-gemeenschappen de vestiging van koraallarven beïnvloeden is niet veel bekend. Daarom werd gedurende drie maanden de hoeveelheid nutriënten en licht alswel de mate van begrazing gemanipuleerd in veldexperimenten, waarna de vestiging van *F. fragum* larven in de resulterende algengemeenschappen werd bestudeerd. Een afname in begrazing resulteerde in een verdubbeling van het aantal turfalgen. Na toevoeging van nutriënten nam ook de hoeveelheid macroalgen toe, mits de begrazing laag was. Wanneer larven geen keus hadden, bleek dat ze zich konden vestigen in elk van de verschillende algengemeenschappen, ongeacht het nutriënten en/of begrazings regime waaronder de aangeboden algengemeenschap was ontstaan. Hieruit blijkt dat geschikte vestigingsplekken altijd aanwezig waren, zelfs in algengemeenschappen die waren opgekweekt onder voor koralen onnatuurlijke omstandigheden. Als larven wel de keus hadden tussen verschillende algengemeenschappen, dan vestigden ze zich het liefst in cryptische lokaties gedomineerd door korstvormende kalkwieren en open substraat. In aan licht blootgestelde lokaties vestigden larven zich ook voornamelijk in microhabitats gedomineerd door open substraat en vermeden ze lokaties waarin turf algen voorkwamen. De mate van herbivorie, het successie stadium en de hoeveelheid nutriënten beinvloeden gezamenlijk in welke algengemeenschappen koraallarven zich vestigen: in de vroegste stadia van successie vestigden larven zich het liefst in algengemeenschappen die onder verhoogde nutriënt en/ of onder een verminderde mate van herbivorie waren opgegroeid. In latere successie stadia vestigden larven zich voornamelijk in algengemeenschappen die onder natuurlijke omstandigheden waren opgegroeid. Deze resultaten tonen aan dat de beschikbaarheid van lokale plekken met korstvormende kalkwieren en open ruimte van groot belang is voor de succesvolle vestiging van koraallarven, zelfs bij onnatuurlijk hoge hoeveelheden nutriënten en een lage begrazingsdruk.

Wereldwijd wordt meer en meer gebruik gemaakt van koraallarven voor het herstel van koraalriffen, om zo de omvang van bedreigde koraalpopulaties te

vergroten én hun genetische variatie te verhogen. Rifherstel werkzaamheden in het Caribisch Gebied richten zich voornamelijk op het herstel van de ernstig bedreigde elandsgewei koralen (*Acropora palmata*, Lamarck 1816). Deze soort speelde een belangrijke ecologische rol in ondiepe Caribische rifgemeenschappen totdat tussen mid- jaren 70 en begin jaren 80 hun aantal door een ziekte enorm afnam. Om het herstel van deze soort te faciliteren, ontwikkelden we een aantal technieken voor het opkweken van *A. palmata* larven. Larven werden opgekweekt uit in het veld verzamelde eieren en zaad. Deze gekweekte larven bleven lang in leven (overlevingspercentage van 3.4% na 2.5 jaar) en na vier jaar stootten de opgekweekte kolonies na hun uitzetting op het rif zelf eieren en zaad uit tegelijk met hun in het wild opgegroeide soortgenoten. Dit is de eerste keer dat een bedreigde Caribische koraalsoort succesvol werd opgekweekt vanaf eieren en zaad tot aan het volwassen levensstadium.

Het herstellen van koraalpopulaties middels het gebruik van zelf gekweekte koraallarven wordt steeds vaker toegepast, maar blijft duur. Hierdoor is de schaal waarop deze hersteltechnieken kunnen worden uitgevoerd vaak kleiner dan de schaal waarop koraalriffen achteruit gaan. De huidige technieken zijn vooral duur omdat opgekweekte koraallarven uiteindelijk handmatig met behulp van bijvoorbeeld kabelbinders, epoxy of spijkers op het rif bevestigd moeten worden. Dit is een bewerkelijke fase, die verantwoordelijk is voor een groot deel van de kosten voor herstelwerkzaamheden. Om deze kosten te verminderen, ontwierpen we twee tetraëder-vormige kunstmatige substraten waarop koraallarven zich kunnen vestigen waarna deze “tetrapods” op het rif kunnen worden geplaatst door ze simpelweg in gaten op het rif te duwen. De tetrapoden bleven vanzelf vast zitten op het rif, in de gaten waarin ze waren geplaatst en door overgroei door (kalkvormende) organismen. Met deze nieuwe methode kunnen per tijdseenheid veel meer koralen worden uitgezet en de kosten voor het uitplanten van koralen bleken 5 tot 18 keer lager in vergelijking met de traditionele methoden. Hoewel verdere verbeteringen mogelijk zijn, toont deze nieuwe “zaai methode” aan dat relatief kleine technische oplossingen kunnen leiden tot een substantiële afname in de benodigde tijd en kosten die gepaard gaan met het uitzetten van jonge koralen op riffen. Hierdoor kan de schaal waarop het koraal herstel wordt uitgevoerd worden vergroot.

Dit proefschrift laat zien hoe studies naar de eigenschappen van koralen tijdens hun vroegste levensstadia bijdragen aan een beter begrip van de factoren die leiden tot de succesvolle vestiging van koraallarven op het rif. Deze nieuwe kennis is van grote waarde voor de verbetering van methoden voor het aktief herstel van bedreigde koraalpopulaties.

Resúmen

Faktornan Ambiental ku ta Dirigí Eksito di Rekrutashon di Koral Karibense: Aplikashonnan pa Yuda Rekuperashon di Populashonnan di Koral Menasá

Koral por multipliká tantu seksualmente ku larva i asekualmente dor di pida kibrá ku ta sigui krese. Pasobra e manera seksual ta trese variashon genétiko ku tin e oportunidat di adaptashon, sobrebibensia i establesimentu di larva di koral ta un proseso yabi ku ta determiná destino di ref di koral riba terminá largu. Tòg tiki ta konosé tokante e faktornan ku ta dirigí e rekrutashon eksitoso di larva di koral. E tesis aki ta trata di haña mihó komprenshon di e prosesonan ambiental afektando éksito di rekrutashon pa koral karibense, di identifiká e kondishonnan bou di kual rekrutashon por ta eksitoso i di usa e informashon aki restourashon di ref dor di amplia rekrutashon larval den komunidatnan di koral bou di menasa.

Larva di koral por originá dor di bruimentu paden di e koral despues di fertilisashon i ta saka e larvanan den awa. Diferente sorto di koral ta tira keit den awa tantu webu komo spèrma i ku fertilisashon na superfisie di awa ku desaroyo di larva. Lógiko ku e tiramentu di di keit tin ku sosodé sinkronisá pa e sorto konserní.

Pa bon funshonamentu di koral di ref nan tin den nan tehido alganan uniselular simbiótikamente ku ta yuda nan tantu ku alimentashon komo di krea e ambiente pa traha nan skelèt di kalki ademas e ta duna nan koló: e asina yamá zoksantela.

Promé nos a investigá biologia reproduktivo i ekologia di bida tempran di e koral di sesu *Diploria labyrinthiformis* (Linnaeus, 1758), un sorto abundante di koral di ref ront Karibe. *D. labyrinthiformis* ta e úniko koral konosí di tira keit mensualmente durante dia di Mei te Sèptèmber, resultando den e kantidat mas altu di evento reproduktivo pa aña, opservá na un sorto di koral Karibense ku ta tira keit. Nos resultadonan ta mustra ku larva di *D. labyrinthiformis* ta desaroyá rápidamente i ta establesé na gran kantidat ora nan ta haña siñalnan di establesimentu. Sobrebibensia i kresementu di esnan establesé a oumentá ora nan a haña simbionta algal tempran den nan bida i ora nan ta haña ko'i kome. E evento reproduktivo múltiple pa aña, e fase planktóniko kòrtiku, e kantidat di establesimentu altu i su reakshon positivo na enrikesimentu di nutriente ta mustra ku e sorto aki ta un kandidato ideal pa a propósito di restourashon di ref.

Otro ku di sorto ku ta tira keit, mayoria di sorto di koral ku ta brui pa saka larvanan direkto ta proveé nan larva ku un densidat variá di simbionta algal dor di kual e kantidat di simbionta por varia hopi. Pa eksplorá kon simbionta algal ta influensia e funshonamentu di un larva, nos a studia larva di koral di balchi *Favia fragum* (Esper, 1797) ku diferente densidat di simbionta bou di diferente kondishon ambiental. Larva ku hopi simbionta tabata mas aktivo i a buska un bon lugá di establesé pa mas tempu. Pero larva asina tambe a haña mas strès i ta muri mas tantu ku subida di temperatura di awa, kompará ku larva ku ménos simbionta. Ta asina ku herensia maternal di hopi simbionta por ta benefisioso i malu pa larva di koral dependiendo di kondishon ambiental. E benefisio miksto di oferta di mama di simbionta ta sugerí ku mama di *F. fragum* ta usa un strategia di "bet-hedging" dor di duna un variashon grandi di densidat di simbionta na nan larva pa asina amplia e éksito di rekrutamentu di nan junan den un ambiente di ref ku hopi variashon den tempu i espasio.

Hopi ref di koral ta keda kubrí dor di alga denso fini i lima grandi dor di enrikesimentu di nutriente i pèrdida di hèrbívoro krusial. Kon komunidatnan di alga ekstenso ta influensiá establesimentu eksitoso di larva di koral sinembargo no ta bon konosí. Ta p'esei nos a manipulá preshon di komementu di lima i nivel di nutriente *in* situ pa investigá kon e suseshon di lima i alga ta afektá establesimentu di larva di *F. fragum* den habitat ku hopi lus i esnan skur. Redukshon di komementu ta amplia e kantidat di alga fini dos biaha i mas nutriente ademas a promové kresementu di lima grandi bou di situashon di menos komementu. Si nan por a skohe larva di *F. fragum* a preferá habitat skur i skondí dominá pa alga di kaska koralina i sustrato sunú. Ademas riba superfisie ku lus, larva tambe a preferá di establesé den lugá sunú evitando e alga fini.

Si nan no por a skohe larva a establesé igualmente den komunidat di alga di tur tratamentu di komedó di alga i nutriente, indikando ku ainda tin superfisie pa establesé dentro di komunidatnan menos bon. Esei ta mustra ku pida ku alga di kaska koralina i sustrato sunú ta di sumo importansia pa establesimentu eksitoso di larva di koral asta den kaso di hopi nutriente i poko komementu.

Uso di rekrutnan seksual pa propósito di restourashon ta keda apliká mas i mas na diferente lokalidat ront mundu pa amplia e kantidat di individual ku diversidat genétiko. Esfuerso di restourashon den Karibe ta dirigí pa yuda rekuperashon di e Koral di Palma, *Acropora palmata* (Lamarck, 1816) ku ta krítikamente menasá. E sorto aki ta proveé diferente servisio ekológiko importante den komunidat plat den

Karibe, pero un malesa a redusí nan abundansia pa mas di 95% di medio di añanan 70 te kuminsamentu di añanan 80. Pa yuda su rekuperashon nos a desaroyá diferente téknika pa kria i establesé larva di *A. palmata*. Kolonia di *A. palmata* kriá di webu fertilisá kolektá den naturalesa a sobrebibí pa basta tempu i algun di e kolonia a tira keit sinkronisá ku esnan nativo na e lugá kaminda nan a keda poné ora nan tabatin kuater aña. Esaki ta e promé bia ku un sorto di koral *Acropora* tabata kriá di webu fertilisá i despues a krese te madures seksual.

Miéntras téknika di restourashon usando rekrutnan seksualmente kriá ta birando mas i mas eksitoso, nan ta keda karu loke ta limitá nan uso na un eskala muchu mas chikitu ku e degradashon di ref. E métodonan ku ta wòrdu usá pa pone nan riba ref ta rekerí uso di manera di fiha nan bou di awa, loke ta tuma tempu i ta tuma un gran parti di e gastu di restourashon. Pa minimalisá e gastu nos a deseñá i purba dos sustrato artifisial den forma di tetrapot (4 pia) ku por pone riba ref fásilmente primi nan den buraku chikitu den ref. Organismo di kaska rápidamente a fiha nan den fondo. En general e moda di “sembramentu” nobo a hasi e ponementu i fihamentu 5 te 18 bia mas rápido. Miéntras ainda nan por keda mehorá e método relativamente simpel por resultá den un redukshon di gastu tempu i asina fasilitá restourashon di ref degradá den un area muchu mas grandi.

E tesis aki a mustra kon estudio di karakter di bida hoben di sorto di koral lo mehorá nos komprenshon di faktor ku ta determiná rekrutashon eksitoso i ta demostrá ku resultado asina por keda usá pa yuda rekuperashon di populashon di koral menasá.

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Author contributions

Chapter 2: VFC, DP and MJAV conceived the project and designed the experiment. VFC, SS, KLM and MJAV carried out the field work. VFC analysed the data. VFC and MJAV wrote the manuscript. All authors gave final approval for publication.

Chapter 3: VFC, KRWL, ACH and MJAV conceptualized the project. VFC and KRWL performed sample collection, experiments and analyses. VFC, KRWL, ACH, JH and MJAV wrote the manuscript. All authors gave final approval for publication.

Chapter 4: MJAV and VFC conceptualized and designed the project. CGBG and LL performed the experiment and processed data. EEvL, VFC and CGBG conducted the data analyses. VFC, MJAV, CGBG and JH wrote the manuscript.

Chapter 5: VFC, MB and DP conceived the project. VFC, MJAV, MB, MC, MS, SS, AS and DP carried out the field work, VFC conducted the data analysis, VFC and MJAV wrote the manuscript. All authors gave final approval for publication.

Chapter 6: VFC and DP conceptualized the project. VFC, DP, KRWL, SS, BM and MJAV conducted the field work. MJAV and VFC wrote the manuscript. All authors gave final approval for publication.

Chapter 7: DP, MB, VFC, MJAV, JRG, and UP conceived and designed the study. UP, DP, VFC and MB designed the tetrapods. VFC and MJAV carried out the experiments and collected the data. VFC analysed the data. VFC, DP, MJAV and JRG interpreted the data and wrote the manuscript, assisted by MB and UP. All authors gave final approval for submission.

Curriculum vitae

Valérie Francine Chamberland was born on 24 October 1983 in Québec City, Canada. Between 2005 and 2008, Valérie attended the Université du Québec à Rimouski where she pursued a bachelor's degree in marine biology and oceanography. She completed one year of her undergraduate studies at the Université des Antilles et de la Guyane in Guadeloupe, during which she first experienced diving on a coral reef and strengthened her passion for coral reef ecology. After she graduated with a B.Sc., she enrolled in a master program at the Université de Perpignan via Domitia in France in 2009. As part of her M.Sc. study, she conducted an internship at the CARMABI Marine Research Station on Curaçao in 2010-2011, where she documented changes in coral and fish abundances on shallow reefs following the die-off of Caribbean elkhorn and fire corals in the early 1980s. After she obtained her M.Sc. degree, Valérie worked on Curaçao for one year, during which she wrote proposals to help protect five of the island's wetlands under the International RAMSAR Convention. She further assisted CARMABI staff with work on coral reproduction and carried out a large-scale effort to relocate critically endangered elkhorn corals to prevent their destruction due to coastal development. In 2012 she started her doctoral studies at the department of Freshwater and Marine Ecology (FAME), Institute for Biodiversity and Ecosystem Dynamics (IBED) at the University of Amsterdam (UvA), in joint collaboration with SECORE International and CARMABI. Her thesis investigated the environmental factors affecting corals during their earliest life stages, with the aim of optimizing techniques that use coral recruits for reef restoration. Valérie currently works for SECORE International as a Research Scientist and is based on Curaçao at CARMABI.

(photo by P Selvaggio)

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(photo by P Selvaggio)

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While at times, pursuing a doctorate degree can feel as a rather personal and lonely journey, one can undoubtedly only triumph in this endeavour with the contribution of many others' helping hands and share of cleverness, time, guidance and candour. This PhD dissertation is only a few pages away from coming to an end, and it is with the following words that I will attempt to express my gratitude to the many of you who have wholeheartedly taken part in this journey.

✶

Klein Sint-Michiel, Curaçao
December 21, 2017

It was a calm night of August 2011 when I first witnessed coral spawning. Below the surface of Spaanse Water, colonies of the critically endangered elkhorn coral were synchronously releasing their pink gamete bundles in the water column, the latter slowly floating their way up to the surface, in an intricate spectacle that resembled an upside-down snow storm. I was a quiet observer. But in my vicinity was a buzzing team of scientists, swarming around reproductive colonies with nets to collect their gametes. The SECORE crew was fortuitous with a good catch that year. They collected hundreds of thousands of eggs and sperm cells from multiple elkhorn coral colonies. Once fertilized, these eggs would become the next generation of coral offspring to be reared for reef restoration purposes. With their treasured collection in hand, all divers sped back to shore in a hurry to mix gametes belonging to different parental colonies, and allow fertilization to take place.

As I stood on shore, I watched with relentless attention the scientists working with coral gametes under the moonlight, and wished that one day, that would too, be my work...

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It is about a half year later that I met Dr. Dirk Petersen and Mike Brittsan, respectively Executive Director and Chairman of the Board of Directors at SECORE International. They were hiring, and I was being interviewed. The selected candidate would study the earliest life stages of corals, with the aim of improving reef restoration techniques using sexually reared coral offspring.

Since August 2011, I had completed a Master's program at the University of Perpignan, and had been working on Curaçao with researchers at the CARMABI Marine Research Station, conducting work on coral reproduction and larval rearing. The vibrant "coral larvae team" composed of Dr. Mark Vermeij, Dr. Kristen Marhaver, and PhD candidate Aaron Hartmann, had taken me under their wings to teach me larval rearing work, generously sharing their secret recipes for keeping young corals alive, as well as a ton of their insightful views on ecological theories underpinning coral recruitment success. Day after day I studied the complex reproductive biology of corals, hoping for an opportunity to secure a position in this line of investigation. And there was my chance, a half year later, to pursue a PhD degree in this field.

From that point onward, things moved very fast and before I knew it, I had been hired by Dirk and Mike at SECORE, was appointed as a PhD candidate at the University of Amsterdam under the supervision of promotor Prof. dr. Jef Huisman and co-promotor Dr. Mark Vermeij, and was generously offered a warm place to work at CARMABI.

During the five years that followed, a multitude of inspiring characters crossed my path. Many of them evolved into trustworthy friends, promising students or role models, while others simply remained their exceptionally hilarious, comforting or generous selves. To all of them, I dedicate the following pages.

⋆

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I valued most, you always provided me with the academic oversight I needed to pursue these questions. You never held back on providing the feedback needed for me to improve as a scientist, but also never shied away at celebrating my achievements. You taught me an infinite amount of "science stuffs", but above all, you taught me the true value of natural history studies, and the priceless merit of a good observer's eye. I have absolute admiration for your talent as a naturalist and ecologist. Working with you is an utmost privilege.

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SECORE International

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Part of the SECORE team are also our Board of Directors, Science Advisory Board, Zoo and Aquarium Advisory Board, and more recently our Administration, Public Relations, Project and Workshop, Research and Restoration, Legal Advice and Media and Communication teams. Some of you have closely followed my journey with SECORE since 2012, and I thank each one of you for contributing in one way or another to my career. For those of you who have just recently joined the team, I look forward to keep moving SECORE ahead all together.

I would further like to take this opportunity to hand out a special thanks to two of SECORE's long-term supporters at the Green Foundation and the Clyde and Connie Woodburn Foundation.

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✶

The CARMABI Foundation

Founded in 1955, the CARMABI Foundation is the longest established marine station in the Caribbean. A tremendous amount of pioneering work on coral reefs has been carried out at CARMABI. I cannot help but mentioning that it is at CARMABI that Prof. dr. Rolf Bak, in the 70's, started the now longest standing time series documenting changes in reef communities in the whole world. Thus, 60 years after CARMABI was established, when I push open the squeaky doors of the "old lab", I cannot do otherwise than reminisce about the number of scientific mysteries that were unravelled within these very same walls, on these very same benches. Such a powerful incentive to continue investigating the complex nature of coral reefs!

Most importantly, to me, there is no CARMABI staff, there is only a CARMABI family:

Board of Directors of CARMABI. I would like to extend my gratefulness to CARMABI's Board of Directors for supporting my work on Curaçao, and for allowing me to be based at the station for these past 7 years. It is an honour to count CARMABI as one of my affiliations.

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There are a many more people at CARMABI that give their all every day to make this island a better place, whether it is through education, terrestrial research, park management or public outreach. I sincerely admire your commitment to our island.

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The Curaçao Sea Aquarium

While based on Curaçao, I also worked at the Curaçao Sea Aquarium. There, I was provided with exceptional aquarium facilities to conduct my work on larval propagation, I was granted access to one of Curaçao's most astonishing coral reefs, and most importantly, I worked alongside a precious team of people.

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⋆

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There are several other persons who are intrinsically part of my journey as a PhD candidate. Many of those persons I have also met at CARMABI:

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⋆

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My Gentlemen Club of Curaçao

There is a group of men on Curaçao whom I value tremendously, and I have secretly named them ‘My Gentlemen Club of Curaçao’. Together these gentlemen carved out a place for me on the island where I can not only thrive as a scientist, but also participate in nature conservation matters, an invaluable addition to my professional profile.

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Local Dive Operators

Conducting research on marine life comes with a ton of behind the scenes logistics, including SCUBA diving. Two local dive operators have been of much support to this thesis:

Jeroen ‘Chris’ Blokzeijl, Lisette Keus. The Diveshop is not only a place where to dive, but also a place where to be. The two of you have a contagious appreciation of life, and the many “bucket bucks” I have spent while sharing time with you were certainly well invested. You have now moved up the ladder, with recently inaugurating your own bar. I look forward to sharing more stories and drinks with you at “The Wetlab”.

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⋆

The Boka Snek

I have no words to portray how the community I live in, the community of Boka Sami, has made me feel at home on the island, giving me the at times much needed strength to persevere with my career. The only way one could grasp the nature and extent of this feeling, is to join my neighbours at the Boka Snek on a Sunday evening…

⋆

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I could not decide in which category to acknowledge this very special person. So, I created this section just for her:

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⋆

My most precious of all

The story I just wrote began in August 2011, when I first witnessed coral spawning and dreamt of working on coral recruitment. But well before that dive, well before I settled on Curaçao, and well before I even imagined I would become a marine biologist, there was more. There was my family.

Not to make those of you who are still reading any jealous, but I certainly have the absolute best family in the Universe. I probably also have the largest one of all people I know, with my mother being the last of 13 children, aunt of 23 nieces and nephews, and with the latter having 22 children themselves. Not omitting my father's, step-mother's and step-father's family, I could round this number up to about 100 family members. To all you 100 (more or less!), I say *Merci* for your encouraging and supportive words. I did notice that many of you doubled the flow of reassuring words during these past few stressful months. You certainly helped me through the finish line!

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Francine Gignac. You are 'The One' from whom I inherited 'Francine' as middle name, and it makes me proud to carry my godmother's name (it also sounds really classy!). You are simply the loveliest person I know. Thank you for being you!

Mélanie Chamberland. My sister, you are truly the most difficult person to acknowledge, as I cannot decide where to even begin. Bear with me along this attempt… You and I are so different, yet so alike. We live so far away, yet are so close. It wouldn't be a surprise if, despite the thousands of kilometres that separate us, our brains are connected through some sort of telepathical channel. We think the same, speak the same, laugh the same, all of that in a synchrony with the precision of milliseconds! Every moment spent together is a cherished one. You are truly my favourite person on this planet, and hence why this thesis is dedicated to you. It will be an absolute honour to have you stand next to me, before my defence committee on February 9th 2018.

I cannot end this thesis otherwise than by acknowledging my father.

René 'Ti-Pop' Chamberland. February 9th 2018 will mark five years since you left us. It will also be the day I will stand before my doctorate committee to defend this thesis. While Mel will be standing by my side, Linda, Maman, Dan, Ma Tante Francine and 'La Francine à Linda' will be in the audience, I suspect you will be doing something along the lines of "floating somewhere above us", bearing a large smile on your face. It will be an honour to defend, and to celebrate my work on this sacred day. If, despite your warm support and that of the rest of the family, I do get very nervous during my defence, I will think of that sentence you once told me as I was again leaving home for a foreign country: "I am proud, and somewhat jealous of you, because you have the guts to pursue the career of your dreams".

To all of you, *Merci*, *Thank you*, *Dankjewel*, *Masha danki*!

Larval recruitment is a key process determining the long-term fate of coral reefs. Yet, little is known about the factors driving the recruitment success of coral larvae. The work presented in this thesis aims to expand our understanding of the ecological processes affecting recruitment success in Caribbean corals, with a particular focus on identifying the conditions under which recruitment will be successful. Findings collected during the work presented in this thesis were then used to improve techniques using sexually produced larvae for reef restoration purposes.