Bet- hedging reproduction strategies in the massive Caribbean coral *Favia fragum*

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ABSTRACT

Bet- hedging has long been recognised as an adaptive mechanism in unpredictable environments, yet is often overlooked as an evolutionary strategy in corals to adapt to environmental conditions associated with climate change. Most environments vary spatially and temporally over the lifespan of corals, and corals use cues from their natal environments as "predictors" of the environment they will release their offspring into and may optimise phenotypes to maximise fitness to changes in the maternal environment. However, when conditions fluctuate in unpredictably or environmental cues are otherwise lost, producing a single phenotype could cause population bottlenecks. Prolonged summer periods of high sea surface temperatures combined with anthropogenic impacts are inhibiting environmental cues that have previously secured reproductive success in tropical scleractinian corals and enabled them to fine-tune their reproductive patterns to time periods that optimize external fertilization success, appropriate larval dispersal and efficient coral recruitment.

This study tested the ability of the brooding species *Favia fragum* to "hedge their bets" on reproductive strategies in the presence/ absence of lunar cues, a cue known to play an important role in synchronizing reproductive output. Results illustrated *F. fragum* exhibited dynamic bet-hedging, tailoring larval output and producing a range of larval phenotypes and thus exhibiting plasticity dependent on their environment. In the absence of lunar cues, corals varied the timing of planulae release as well as within-clutch size variation promoting dispersal and retention to their natal environment. Lunar stimulated corals concentrated release to 3-6 days, typically produced a single phenotype aiding retention and re-seeding local reef populations. Reproductive flexibility will enable coral to strategize larval/ gamete release that optimise recruitment success within unstable environmental conditions. The use of such strategies in other brooding species and broadcast spawners should be a focus of further investigation to evaluate the potential for corals to populate environments beyond the familiar and persist future unpredictability.

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LIST OF ABBREVIATIONS

ANM After New Moon

ASW Artificial Seawater

CCA Crustose Coralline Algae

CNM Constant New Moon

EPIR Enhanced Post- Illumination Dark Respiration

FPs Fluorescent Proteins

GFP Green Fluorescent Proteins

GBR Great Barrier Reef

LSCM Laser Scanning Confocal Microscopy

LSFM Light Sheet Fluorescence Microscopy

SPIM Selective Plane Illumination Microscopy

MAA Mycosporin- like amino acids

MRL Mean Resultant Length

PLD Planktonic Larvae Duration

ROS Reactive Oxygen Species

SST Sea Surface Temperature

UV Ultraviolet

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1.0 INTRODUCTION

Life originated from the ocean and at present is host to 14% of all known species (Tessmar-Raible et al., 2011). The most distinctive characteristic of the marine environment is that it is governed by a range of environmental cycles including diel photoperiod, lunar phases, tidal cycles, seasons; all influenced by the periodic re-occurrence of the sun and moon (Tessmar-Raible et al., 2011). These cycles occur across multiple time-scales from daily light/ dark cycles to monthly lunar/ semi- lunar and seasonal cycles. Marine organisms have adapted over millions of years to these rhythms and depend on their predictability to coordinate their own biological cycles. The need to anticipate and prepare for periodic changes in the environment is evident by an almost universal presence of molecular timekeeping mechanisms in both unicellular and multicellular organisms (Sorek et al., 2014).

Coral reefs are among the most productive ecosystems in the world, supporting ca. 25% of all marine organisms (Buddemeier et al., 2004). Scleractinian corals are distinguished by their ability to continuously secrete a hard calcium carbonate skeleton, it is this feature that enables scleractinian coral to build reefs thus providing the characteristic complex 3D framework (Harrison, 2011). Tropical scleractinian corals owe most of their success to the tightly coupled symbiosis with their endo-symbiotic algae, primarily *Symbiodinium* (Stat et al., 2006). The mutualistic symbiosis of coral host and algae has enabled the persistence of corals in shallow oligotrophic waters for over 200 million years (Hoegh-Guldberg, 1999). In it hypothesized that optimization of metabolic synchronization and coordination between processes in the host and the symbiont may have driven the evolution of an internal clock (Reitzel et al., 2013).

Increases in oceanic temperatures driven by elevated levels of atmospheric CO_2 is a major driver of mass bleaching/ mortality events across all geographical regions which along with other natural and anthropogenic factors (Frieler et al., 2012), has led to the loss of ca. 19% of the world's reefs over the past 50 years (Wilkinson, 2008). Unseasonably high summer temperatures, remaining 1-2^{o}C

above the norm over extended periods is the main driver of mass coral bleaching with effects observed on global scales (Hoegh-Guldberg and Ridgway, 2016; Carroll et al., 2016). Resistance and ability to recover rapidly from environmental disturbances either anthropogenic or natural, largely dictates the current status and likely future of the worlds reefs (Schoepf et al., 2015). The rate of increase in atmospheric CO_2 level up to a current value of more than 400ppm (Stap et al., 2016), is 10 times greater than any previous natural occurring rises (Kump et al. 2009). The rate of change appears to be beyond the adaptive capacity of scleractinian corals however, coral itself encompasses other organisms including bacteria, archaea, fungi and dinoflagellates to form the coral holobiont (Rosenberg et al., 2007). The composition of the holobiont differs vastly across environments changes in community structure may act as a source of coral adaptation within the reproductive lifetime and elevate resilience of the host to environmental stressors (Reshef et al., 2006). To what extent microbial communities can contribute is at the forefront of current research, corals may still be driven to seek refuge at higher latitude reef systems or migrate vertically (increasing depth) in to the mesophotic zone.

The extensive generation times of most coral species (5- 100 years) indicates most species will fail to adapt in time (Frieler et al., 2015). This combined with variable periods of larval production (Van Woesik, 2009; Norstrom and Sandstrom, 2010) and planktonic larval duration (PLD) averaging just a few days to weeks (Szmant- Froelich and Meadows, 2006) offers a very small escape window. However, as the only stage capable of mobility, the planktonic larval stage is fundamental to species survival. Hence, of all the life stages in scleractinian corals, the larval stage is suggested to adhere to the biggest evolutionary adaptations (Kenkel et al., 2011; Muir et al., 2015).

1.1 Reproduction in scleractinian corals

1.1.1 Sexual reproduction

Scleractinian corals have a simple structure with a primitive nervous system and basic anatomical features; yet exhibit diverse and complex reproductive strategies. Most are categorised into four main patterns of sexual reproduction: hermaphroditic broadcast spawners, hermaphroditic brooders, gonochoristic broadcast spawners, and gonochoristic brooders (Harrison and Wallace, 1990; Richmond and Hunter, 1990). Hermaphroditic describes corals in which both sexes develop whereas sexes are separate in gonochoric corals (Harrison, 2011). Hermaphroditism is assumed to be advantageous over gonochorism when the probability of locating members of the opposite sex is impeded and conditions favour self- fertilization. This is reflected throughout reef-building corals, with 68% of those assessed confirmed as hermaphroditic (Harrison and Wallace, 1990). Corals are then further grouped into two main reproductive modes, 82.7% practice broadcast spawning and only 14.3% brooders, 3% exhibit both modes (Harrison, 2011). Broadcast spawners release buoyant gametes into the water column where external fertilization takes place. Eggs are often packed together with sperm in bundles insuring simultaneous arrival of both gametes to the surface of the ocean. Fertilization is followed by an obligatory development period of ca. 3 days before larvae reach competency to settle (Edmunds and Bruno, 2008). During this phase, larvae mature and have been known to partake in horizontal transmission of algal symbionts initiating the start of their symbiotic relationship (Edmunds et al., 2011). In brooding corals fertilization is internal and larvae are released several days to weeks after (Harrison and Wallace, 1990). Larvae are generally competent to settle almost immediately after release and characteristically aggregate in patterns to form small colonies (Edmunds and Bruno, 2008).

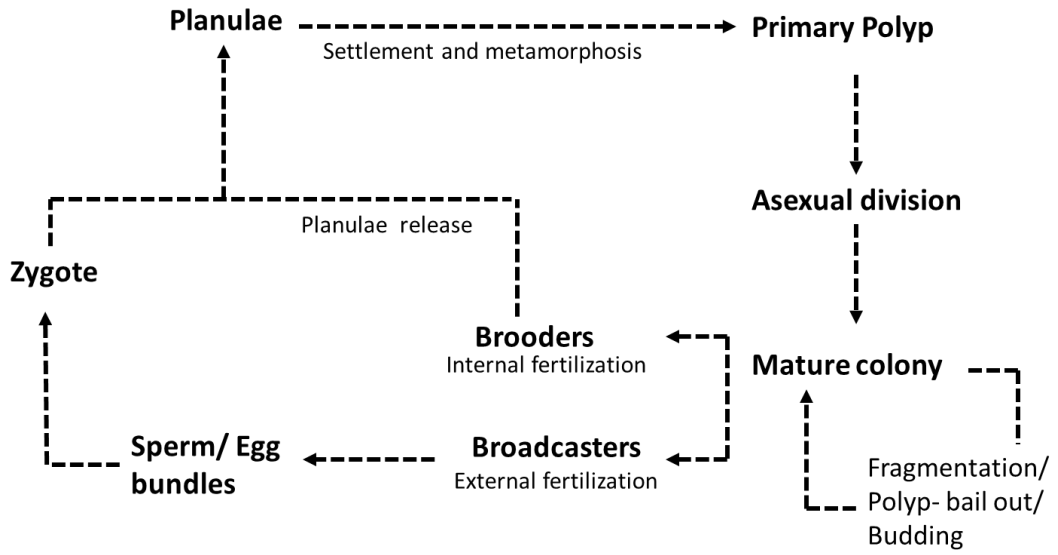

Figure 1a. A summary of coral reproduction patterns in both brooding and broadcasting corals (adapted from Vermeij et al., 2009)

Reproductive traits are generally preserved within species and genera however, exceptions do exist and not all coral species can be classified within the four aforementioned patterns. At least 13 species have been observed to exhibit mixed patterns and reproductive modes (Harrison, 2011). *Galaxea fascicularis* and *Galaxea astreata* were first observed to be simultaneous hermaphroditic, however later research revealed populations of female colonies that spawned pink eggs and hermaphroditic colonies that released sperm and white eggs (Harrison, 1988).

1.1.2 Asexual reproduction

Most scleractinian corals can produce both sexually and asexually (Combosch and Vollmer, 2013). Asexual reproduction produces genetically identical units via processes including fragmentation, partial mortality, polyp bailout or asexual production of larvae. Physical breakage of large coral colonies via storm and wave action causes small parts to fragment off and be dispersed along the reef (Lirman, 2000). Partial mortality is often associated with disease by which parts of the colony are killed leaving a patch work of live coral remaining (Aronson and Precht, 2001). Both processes result in the proliferation of smaller sexually immature colonies of identical genotypes,

impairing sexual reproduction and reducing genetic diversity (Lirman, 2000). Predicted increases in storm frequencies could cause fragmentation to become more prolific and diminish the abundance of sexually reproductive coral colonies, further impeding larval supply to degraded reefs (Wilkinson, 2008).

The occurrence of asexual production of larvae has been associated with environmental conditions that cannot support sexual reproduction (Brazeau et al., 1998), but most species investing in asexual reproduction require some sexual recombination to maintain genetic diversity (Combosch and Vollmer, 2013). Interchanging between the two modes is suggested to continue throughout the adult life (Barnes and Hughes, 1999). Patterns and factors underpinning the production of parthenogenetic larvae are poorly defined (Combosch and Vollmer, 2013). Few studies have described self- fertilisation in scleractinian corals, parthenogenesis was first documented in *Pocillopora damicornis* (Stoddart, 1983). Brazeau et al (1998) described high rates of self-fertilisation in *Favia fragum* and *Porites astreoides* under natural conditions in which both species typically inhabit sites with high turbidity hindering cross fertilization. High rates of "selfing" combined with natal philopatry may enhance local adaptation, local speciation and potentially lead to changes that will enable colonies to survive new environments (Gleason et al., 2001). However, extensive self- seeding may also lead to genetic uniformity increasing vulnerability to environmental stressors (Szmant- Froelich and Meadows, 2006).

1.1.3 Bet- hedging reproductive strategies

Trends in reproductive mode appear to be much less systematic than previously thought, both broadcast spawning and brooding has been observed within families, genera and between species from different geographical locations. *Pocillopora damicornis* in Western Australia exhibit both modes of reproduction (Ward, 1992), as does *Goniastrea aspera* in Okinawa, Japan (Sakai, 1997). This implies that reproduction has a high level of plasticity (Harrison, 1985) and reproductive

strategies may adhere to significant changes to optimise population persistence as environmental conditions continue to change.

Bet- hedging has long been recognised as an adaptive mechanism in unpredictable environments. Most environments vary spatially and temporally over the lifespan of corals, and corals use cues from their natal environments as "predictors" of the environment they will release their offspring into and may optimise phenotypes to maximise fitness to changes in the maternal environment (Bishop, 2006). However, when conditions fluctuate in unpredictably or environmental cues are otherwise lost, producing a single phenotype (regardless of cues or not) could cause population bottlenecks. Hence, corals should "hedge their bets" by producing a range of phenotypes and thus exhibit plasticity dependent on their environment. Edmunds et al (2011) suggested peak larval release of brooding Pocilloporid species, which correspond with warmest annual temperatures, correspond to times when the metabolic rates of larvae would be greatest (as indicated by maximum respiration) to shorten dispersal and stimulate local coral recruitment within these favourable habitats. Larvae released in periods of colder water would have longer dispersal stages due to reduced metabolic rates, contributing to reef connectivity by settling in reefs away from their native reef. Evidence of reproductive bet- hedging in corals is rare but should be a focus of future studies when addressing the coral resilience question particularly as it concerns environmental change and uncertainty.

Figure 1b. Bet-hedging strategies under predictable/ unpredictable environment, producing a single mean size phenotype when environment can be anticipated versus producing a range of phenotypes in unstable environmmental conditions to insure reproductive success in both scenarios.

1.2 Ultimate and proximate cues driving reproductive patterns

Most coral reproduction exhibits some degree of synchronicity (Szmant- Froelich et al., 1985), variations in timing and synchrony cause reproduction patterns to rage from temporal isolation to fine- tuned mass spawning events (Gilmour et al., 2016). On the GBR, ca. 40 species of scleractinian corals release gametes simultaneously in one night and ca. 130 species spawn over a few weeks in austral late spring (Harrison et al., 1984). This 'mass spawning' event is unique to the GBR, the scale and magnitude of synchronicity is not replicated in any other reef system (Gilmour et al., 2016). Other regions demonstrate a reduced degree of synchronicity between species, months and seasons (Shlesinger and Loya, 1985). For example, spawning patterns in Puerto Rico exhibit strong synchronisation within species, but species spawn at different times over a period of 3 months (Szmant- Froelich, 1986). Brooding corals show varying degrees of periodicity, from weak synchronicity with a monthly peak but continuous release (Fan et al., 2002) to strict synchronicity fine- tuned to a few days in a month (Johnson, 1992).

There are a multitude of mechanisms and evolutionary factors controlling the timing and synchronisation which result in the reproductive patterns exhibited by both broadcast spawners and brooders. Ultimate and proximate cues play an intricate role to fine-tune the timing of gametogenesis, spawning and release of planulae. Ultimate factors result from evolutionary selective pressures that govern species- specific responses that will enhance their survival and increase their fitness such as increased fertilization success or predator avoidance. Proximate cues describe environmental mechanisms including temperature, tidal and diel cycles, and food abundance (Harrison and Wallace, 1990). Most reproductive patterns are associated with a plethora of environmental cues that act in synergy but differ in time scale. There have been few studies that have isolated individual environmental cues associated with spawning; hence individual roles on reproductive patterns are not well understood. The relationship between proximate environmental cues and endogenous circadian rhythms also remains unknown. Sea surface temperature, lunar and diel cycles are considered the most influential cues driving the month, day and hour of spawning (Babcock et al., 1986).

1.2.1 Temperature

Temperature is often cited as the primary factor for regulating or synchronizing reproductive cycles in corals (Babcock et al., 1986). Particularly in broadcast spawning species, spawning typically occurs during the summer months when sea surface temperatures are at their maximum, where increases in temperatures have been directly linked with gamete development and maturation (Harrison and Wallace, 1990). On the GBR delays in temperature rises have been directly linked with delays in gametogenesis and hence spawning time, where offshore reefs experience increasing temperatures one month later than inshore localities. Here offshore communities were observed spawning one month later than those of inshore sites (Babcock et al., 1986). Similar spawning patterns were recorded in Taiwan where coral reefs in the south experience warmer and reduced annual temperature variations and spawn 1-2 months earlier than corals in the north (Dai et al. 1992). Laboratory studies have previously isolated and manipulated temperature. Hunter (1988) found spawning ceased in colonies of *Montipora verrucosa* and *Montipora dilatata* when exposed to constant winter temperatures. In contrast, brooding species appear to less sensitive to temperature variation when it comes to their reproductive cycles. Many typically release larvae over multiple months and in some cases year- round (Szmant- Froelich et al., 1985).

It is suggested that rather than the absolute temperature, it is the annual temperature range that exerts that greatest influence in regulating the longitudinal patters of the reproductive period and spawning synchrony (Richmond and Hunter, 1990). In the Red Sea and Arabian Gulf, variations in temperature regimes are the predominant cue driving reproductive cycles. Fadlallah and Lindo (1988) noted significant differences in the length and timing of reproduction in *Stylophora pistillata* between reefs at the same latitude in the Arabian Gulf and Red Sea. In the central parts of the Red Sea, temperature range is relatively narrow (25-31^{o}C) compared to the Gulf where variation is at the global extreme (15-35^{o}C). *S. pistillata* exhibited two peaks in planulation in the Red Sea, compared to the Gulf where the same species had a short gametogenesis cycle and a brief planulation period.

However, reefs across similar latitudes in Western and Eastern Australia experience similar temperature regimes yet there is a 6-month lapse between spawning times (Simpson, 1991).

Temperature must act at some level to regulate reproduction however, significant variations in gamete/ larval release across similar temperature regimes suggests other factors contribute to underpin reproductive patterns across all coral species.

1.2.2 Tidal regimes

The connection between land and sea represents a boundary subjected to some of the most extreme fluctuations in environmental conditions. Daily rising and falling tides are superimposed with the light/ dark diel cycle and cause huge variations in salinity, temperature, food availability, predation rates, hydrostatic pressure, water current and oxygen levels (Tessmar- Raible et al., 2011). Hence, tidal influence may act as a co- factor to influence reproductive patterns. The most probable explanation for regulation larval/ gamete released with tidal cycles is to reduce gamete dilution, optimize fertilization rates or increase dispersal and larval settlement rates (Babcock et al., 1986).

The gravitation forces of the sun and moon influence tides and coastal areas can experience two high/ low tides (semidiurnal) or one high/ low (diurnal) per day. Semidiurnal tidal cycles have an approximate 12.4- hour period/ half a lunar day and diurnal approximately 24.8 hour (Naylor, 2015). However, other factors also influence tides so that some locations can experience four tides/ day and others will lack a tidal regime (McDowall, 1969). Every 14.8 days the effects of the moon and the sun alternately amplify and oppose each other causing spring and neap tides (Sverdrup et al, 1942). Correlations between tidal and reproductive cycles appears to vary between geographical locations. Mass spawning events such as those on the GBR occur during neap low tides (Babcock et al., 1986), when tidal movement is reduced allowing external fertilisation of released gamete bundles to occur successfully (Gilmour et al., 2016) and local retention to re- populate local reefs (Szmant- Froelich and Meadows, 2006). However, in Southern Taiwan and Japan spawning occurs on high tides during

spring and neap tides (Dai et al., 1992). Coral populations inhabiting high latitudinal reefs in Houtman Abrolhos spawn at spring tides to enhance dispersal and connectivity between reefs (Babcock et al., 1994). Tidal effect likely acts synergistically with the lunar cycle and the degree to which it drives reproductive patterns appears to be directly connected to reef location.

1.2.3 Light

Light is one of the most fundamental cyclic events, it is predictable indicator and translates environmental information on daily, lunar and seasonal time- scales. The role of moonlight in regulating gamete/ larval release has been demonstrated both *in situ* and within laboratory experiments. Under artificial light regimes, the timing of larval release in *Pocillopora damicornis* and gamete release in *Montipora verrucosa* was altered, suggesting lunar cycles are used to entrain reproductive rhythms (Jokiel et al., 1985). On the GBR multi-specific synchronous broadcast spawning occurs between full-and third quarter-moon (Harrison et al., 1984). In Japan, recorded spawning patterns across the years indicated consistencies in timings between individual species (Hayashibara et al., 1993). It remains unclear what specifically induces these organisms to spawn on a certain night of the lunar cycle. Documentation of broadcast spawning is rare around the new moon however, a few species have been observed releasing gametes in Hawaii, South Africa and the Red Sea (Willis et al., 1985; Hunter, 1988; Shlesinger and Loya, 1985). Hence, light as well as other factors that include temperature and tidal cycles may act synergistically or antagonistically in advancing or delaying spawning patterns.

1.3 Circadian rhythms in scleractinian corals

Scleractinian corals depend on environmental cycles to regulate multiple physiological processes including tentacle retraction-expansion (Sebens and DeRiemer, 1977; Hendricks et al., 2012),

calcification (Gutner-Hoch et al., 2016) and reproduction (Chalker, 1977; Hoadley et al., 2011; Sorek and Levy, 2014). Linking of the endogenous clock with environmental conditions provides clear advantages and enables corals to anticipate, prepare and optimise their metabolic, behavioural and physiological states for upcoming changes in ambient conditions of light/dark cycles (Brady et al., 2009), light spectra (Gorbunov and Falkowski, 2002), ambient temperature (Mangubhai and Harrison, 2009) and nutrient availability (van Woesik, 2009).

Circadian clocks are internal systems that enable organisms to regulate patterns of activity simultaneous with their external environmental conditions (Edmunds, 1988). These rhythms are typically described as free-running systems with a periodicity of around 24 hours in the absence of external cues or under continuous stimulation (Sorek et al., 2014). There are 3 primary mechanisms involved in maintaining the clock: i. the input pathway which identifies environmental cues (such as light, temperature) ii. the central oscillator based on transcriptional or translational loops of positive/ negative feedback and iii. The output pathway which coordinates the expression of genes involved in circadian- controlled processes.

1.3.1 Detection mechanisms in coral

In terms of anatomical features, corals are simple organisms that lack specialised visual structures such as ocelli/ pigment rings (Strader et al., 2015), the mechanisms enabling them to detect light has stimulated multiple investigations (Levy et al., 2007; D'Angelo et al., 2008; Mason et al., 2012). The identification of blue- sensing cryptochromes and long-wave sensitive opsins in *Acropora* suggest these as a likely mechanism for light detection (Levy et al., 2007). Crytochromes are DNA photolyase- like photoreceptor proteins and are assumed to partake significantly in enabling corals to adapt and coordinate with external light levels. A recent study also suggests gamete release in corals is triggered by a protein similar to melanopsin (Kaniewska et al., 2015). In mammals melanopsin has been shown to be critical in synchronizing circadian rhythms with the diel light/dark cycle (Provencio

et al., 1998). Melanopsin is also capable of catalysing the activation of G- proteins. G- proteins are known to assist in the transmission of signals across cell membranes to appropriate locations in response to changes in light conditions (Brown and Robinson, 2004). Specific classes of G- proteins have also been stimulated by opsins in response to light in coral larvae (Mason et al., 2012). Kaniewska et al (2015) observed an up- regulation of processes linked to a G- protein activation cascade in *Acropora millepora* when exposed to moonlight.

1.3.2 Molecular involvement in driving reproductive patterns

The central oscillator is responsible for generating and maintaining rhythm. Most circadian clocks rely on core molecular components composed of regular positive and negative feedback loops (Reitzel et al., 2013). Technological advances in sequencing have accelerated the availability of genomic and transcriptomic databases, consequently genes involved in maintaining the circadian clock have been identified and their expression patterns in response to external cues investigated (Levy et al., 2007). CLOCK and CYCLE transcriptional activators are critical core clock components; these two positive elements appear to be almost universal components of bilaterian circadian clocks along with other positive and negative components including PERIOD, TIMELESS and CRYPTOCHROME (Lin and Todo, 2005; Hoadley et al., 2011; Hardin et al., 1990). Studies on *Nematostella vectensis, Favia fragum* and *Acropora millepora* have all confirmed the expression of CLOCK, CYCLE, CRY1 and CRY2 transcription factors (Reitzel et al., 2010; Brady et al., 2011; Hoadley et al., 2011). Of these genes, CLOCK, CRY1 and CRY2 have all been shown to undergo rhythmic expression. Specifically, CRY2 has been implicated to play a role in spawning as its' expression was upregulated in *A. millepora* during a full moon spawning event (Levy et al., 2007). Currently, there is no evidence to suggest these genes are directly involved in entraining coral behaviour. However, expression of CLOCK and the two CRY variants have also been shown to undergo rhythmic expression in insect and mammalian model organisms. The role of these clock gene orthologs have been implicated in short and long term reproductive processes (Dolatshad, 2005);

Dolatshad et al., 2009) and may be important in the synchronization of diel and monthly behaviour of scleractinian coral (Hoadley et al., 2011).

Molecular mechanisms mediating synchronisation of gametogenesis and gamete/ planulae release require further investigation (Reitzel et al., 2013). Supporting the presence of a weak circadian clock, Levy et al (2007) indicated CRY1 and CRY2 clock genes could not sustain circadian rhythm under constant darkness. Contrastingly, Hoadley et al (2011) found no association with planulation patterns and upregulation of any clock genes. It remains unclear whether reproduction patterns are the result of entrained circadian behaviour or a direct response to environmental cues.

1.3.3 Evolution of circadian clock

Three main theories have been proposed in relation to the driving forces behind the evolution of circadian clocks. The first suggests clocks evolved to ensure DNA replication occurred only during darkness to minimize UV damage. Evidence to corroborate this hypothesis is linked to the presence of blue- light sensitive cryptochromes which have been identified in different taxonomic groups including insects (Zhu et al., 2008), plants (Somers, 1998) and cnidarians (Levy et al., 2007). Photo-sensitive cryptochromes are thought to have evolved from photolyases, these enzymes require blue light to repair DNA damaged by UV. Alternatively, Edgar et al (2012) suggest they may have arisen during the Great Oxidation Event 2.5 billion years ago. The third hypothesis states that the circadian clocks arose during the symbiotic fusion between a prokaryote and an archaebacterium that resulted in the first eukaryotic organism (DeCoursey, 2003). To achieve optimization in a symbiotic relationship, both partners must synchronize metabolic processes and coordinate cell cycles, hence the evolution of an internal pacemaker.

1.4 Larval recruitment

One of the growing concerns is coral's high level of dependency on recruitment to persist through periods of continued disturbance and taking in to account the susceptibility of the early- life stages to environmental conditions. Recruitment itself encompasses a plethora of critical factors including successful reproduction (Harrison and Wallace, 1990), viability of pelagic larvae (Harrison, 2006), larval dispersal, successful settlement and post- settlement survival (Richmond, 1997).

1.4.1 Dispersal

Recent advances in our understanding of larval dispersal has begun to question earlier theories suggesting that reef communities represented open populations with substantial larval exchange between populations (Levin, 2006). Early studies assumed that due to size and motility limitation, once in the water column larvae were dispersed as passive particles and exuded no influence on their ultimate place of settlement (Caley et al., 1996). However, recent studies suggest populations may be much more closed with frequent larval retention within the natal reef (Cowen, 2002; Levin, 2006). Adding to this changing paradigm is the increasing recognition of larval behaviour and the ability of individuals to influence their own dispersal potential. Incontrovertibly, oceanic currents play a pivotal role in connectivity between coral populations, coral larvae have weak motility abilities and likely adopt the role of passive particles for at least part of their dispersal process. However, different hydrodynamics exist at varying depths in the water column providing larvae an opportunity to vertically distribute (Tay et al., 2011). In the process of transportation by surface currents, larvae develop locomotion abilities and combined with a loss of buoyancy (via the depletion of energy reserves) may be able to change their position within the water column, increasing the probability of encountering suitable sites (Raimondi and Morse, 2000). Effect on dispersal have

been shown to be significant when models incorporate the behavioural ability of larvae to vertically migrate in the water column (Levin, 2006).

Advantages of dispersal must outweigh disadvantages to make it a viable stage in the life-history of coral. The ability to disperse larvae into the water column opens the door to escape incongruous conditions, re- populate neighbouring reefs and colonise disturbed or novel habitats (Ritson- Williams et al., 2009), but the overriding factor driving dispersal is the potential to spread beneficial alleles and increase genetic variability. Palumbi (2003) concluded that genetic structure and dispersal distance were correlated and transport on intermediate scales (100kms) had the greatest influence on genetic structure. However, there are also major trade-offs associated with this pelagic stage including transportation to less hospitable habitats, predation and a reduced ability to fine- tune adaptations to local conditions (Isomura and Nishihira, 2001). Therefore, not all larvae are produced equally within or between species or even within the same cohort. Richmond (1987) indicated planulae size was positively correlated with dispersal potential, with larger larvae dispersing further than smaller which may remain for up to 103 days in the water column.

1.4.2 Settlement

Coral larvae use a hierarchy of cues to enhance the probability of transportation to settlement sites most probably due to the complexity involved in the settlement process and the high specificity of a species optimum site requirements (Grasso et al., 2011). The degree of influence an environmental cue has on coral larval behaviour is dependent on the spatial scale over which they act and their reliability as an indicator of reef structure (Gleason and Hofmann, 2011). Initial cues to impact on larvae are those that act at the largest spatial scales, coarse cues including ocean currents, light, water temperature, and changes in hydrostatic pressure all direct larvae towards the reef (Price, 2010). Precise cues including water soluble/ insoluble chemicals, sedimentation and reef topography allow for refined selection of final settlement sites and initiating metamorphosis (Gleason and

Hofmann, 2011). The decreasing distance towards settlement points is correlated with an increase in the amount of environmental cues influencing larval behaviour (see Figure 2).

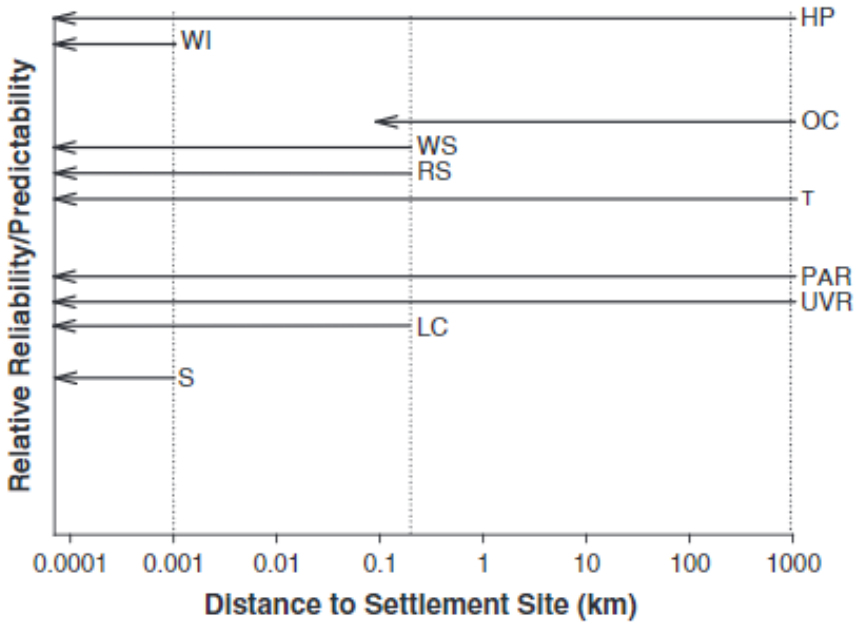

Figure 2. A model illustrating coarse and precise environmental cues based on estimated spatial scales and indicator strength of reef settlement sites. Abbreviations of abiotic and biotic cues: HP = hydrostatic pressure, WI = water insoluble chemicals, OC = ocean currents, WS = water soluble chemicals, RS = reef sounds, T = temperature, PAR = photosynthetically active radiation, UVR = ultraviolet radiation, LC = local currents, S = sedimentation (Vermeij et al., 2010; Gleason and Hofmann, 2011).

Questions remain unanswered as to how larvae acquire the ability to determine reef quality. Particularly for brooded species, fully competent larvae may imprint on their natal reef and acquire an accurate estimate of habitat quality before being dispersed into the water column (Price, 2010). It may also be detrimental to population connectivity causing more larvae to be retained in their natal reef, although imprinting has never been investigated in coral larvae (Huijbers et al., 2012). Brooded larvae are also equipped with zooxanthellae transmitted from their parents that may provide habitat

information and play a role in settlement choice (Kenkel et al., 2011)), whether this is significant to directly influence larval behaviour requires further research. For broadcast species larvae are fertilized whilst dispersing impeding any opportunity to imprint on their natal reef, therefore it is critical they develop the ability to use cues indicative of quality novel habitats (Huijbers et al., 2012). Vermeij et al (2010) suggested coral larval could distinguish reef sounds, Dixon et al (2014) also suggested larvae could differentiate between algal and coral dominated reefs via chemical cues. A recent study found ex-hospite zooxanthellae influenced CCA selectivity in aposymbiotic coral larvae and may be a huge contributing factor in the larval settlement process (Winkler et al., 2015); free-living *Symbiodinium* species aggregate on CCA which provide a source of metabolic waste products. The presence of *Symbiodinium* types differs dependent on their energetic requirements and the release rate of metabolites by CCA species hence, larvae dependent on horizontal transmission following settlement may select CCA sites based on the presence of symbionts.

Upon contact with the substrate, larvae exhibit a "sniffing dog" behaviour, exploring the substrate on a millimetre scale (Hadfield et al., 2001). Their aboral end contacts the surface multiple times until they encounter a suitable spot to complete metamorphosis or abandon the location and resume vertical swimming back into the water column to be dispersed to further settlement sites (Koehl and Hadfield, 2004). Larvae mark the beginning of their sessile life by attaching their aboral end to the surface and entering metamorphosis by flattening the aboral- oral axis and undergoing extensive tissue remodelling to form a primary polyp (Vandermeulen, 1975; Grasso et al., 2011). They must produce a pharynx which opens to the gastrovascular cavity and form a functional mouth complete with tentacles and secure formation of the primary mesenteries (Harrison and Wallace, 1990).

Metamorphosis is induced by receptors that detect chemical signals and trigger a signal-transduction process (Tran and Hadfield, 2013). The cells that detect these cues are typically concentrated in the anterior poles of many invertebrate larvae (Hadfield et al, 2000). The collective term for these columnar cells is the apical sensory organ, the cells are surrounded by microvilli which

twist together to form an apical tuft. In cnidarian larvae some bear an apical tuft while in others it appears absent, including coral larvae (Chia and Koss, 1979).

Sensory cells in the aboral epidermis have a single flagellum surrounded by multiple microvilli (Tran and Hadfield, 2012) and it has been postulated that they may be used in chemoreception based on evidence that larvae settle on the aboral end (Vandermeulen, 1974). This was recently supported when the aboral region of *Montipora capitata* larvae was removed and they failed to metamorphose to known inducing cues. Tran and Hadfield (2013) removed aboral and oral regions of coral larvae, assuming sensory cells are localised in those regions, and concluded that the distribution of sensory cells for chemoreception is variable between coral species; interestingly larvae of *Pocillopora damicornis* could metamorphose even after the removal of the aboral end. These larvae most likely sense cues along their sides and rotate whilst in the water column enabling them to best detect a chemical gradient (Hadfield et al, 2000). They also noted that an artificial inducer (Caesium Chloride) resulted in a metamorphic response at the oral and aboral region in both *P. damicornis* and *M. capitata* suggesting that the oral portion of larvae retain the ability to metamorphose but are instead deprived of signal which originates in sensory cells in aboral end. It would seem therefore that internal signalling in conjunction with chemoreception of external cues is necessary to mediate metamorphosis (Tran and Hadfield, 2013).

1.5 Larval development using LSFM

Presumably due to the experimental difficulties and constraints associated with studying mesoscopic sized larvae on natural reef systems, large knowledge gaps remain in our understanding of developmental and behavioural processes of coral pelagic larval stage all the way through to the transition in to sessile benthic life form. These gaps limit our understanding of the adaptive potential of this life stage and its influence on driving future vertical/ horizontal migration patterns of shallow water species to seek refuge from deleterious natal conditions. The necessity to understand early life

history stages is driving research to exploit techniques developed in other scientific fields and manipulate them to answer new biological questions.

Although the morphological structure of scleractinian corals can extend for metres, most chemical and biological processes occur within the tissue/ cellular layers of a few mm thickness. Developments in old and emerging microscopy techniques including histology (Downs et al., 2009), electron scanning microscopy (Tchernov et al., 2004), theta confocal microscopy (Greger et al., 2007) and nanoscale secondary ion mass spectrometry (Pernice et al., 2012) have proved pivotal in unravelling crucial insights into the organisation of tissue and *Symbiodinium* at a cellular and sub-cellular level. As a result, knowledge of coral physiology has undergone significant advancements.

A major limitation of microscopy is the need to fix samples prior to imaging, hence real time accounts of biological process in vivo are limited to the time frame at which fixation occurred. The fixation process itself can also result in cell/ tissue disruption (Shapiro et al., 2016). The development of miniature model systems of cell/ tissue cultures has been used as an alternative approach to further understand biological mechanisms underpinning symbiosis and calcification (Helman et al., 2008; Mass et al., 2012). In addition, dependency on high irradiance to retain images causes immediate physiological stress to corals, to the extent that limits even short- term investigations. High light levels excite fluorophores to produce reactive oxygen species (ROS) (Wright et al., 2002) which react with components that are easily oxidised (such as proteins, nucleic acids and lipids), reducing the fluorescence signal and resulting in photobleaching (Song et al., 1996) and phototoxicity (Salih et al., 2000).

The development of light sheet- selective plane illumination microscopy (LS- SPIM) combines a high sensitive low light approach with fast speed optical sectioning (Laissue et al., unpubl.). Exposure time is minimised and each focal plane is illuminated once, reducing the amount of photobleaching/ toxicity so the physiological state of the specimen is uncompromised and even the most sensitive specimens can be imaged over long- term (repetitive) experiments. An overriding advantage of LS- SPIM is the ability to understand cell interactions within a native multicellular environment and capture real- time biological events. Traditionally, cells are cultured on substrates or

within a media that is representative of their environment within their organism. The movement of cells themselves and their interactions with neighbouring cells defines their behaviour and functional role, thus it is essential to study at subcellular level within natural setting (environment) to truly understand cellular behaviour. Improvements in imaging apparatus has progressed to the point that is now possible to capture quantitative data at a single- cell resolution (Huisken and Stainier, 2009). These studies opened the gateway to understanding biological processes in 5D (3D over time and wavelength) as they unfold.

Auto-fluorescence in corals makes them as ideal candidates for fluorescence microscopy, and GFP- like proteins are among the most abundant proteins in corals (Oswald et al., 2007), accounting for up to 14% of the total protein content in some species (Treibitz et al., 2015). Despite their prevalence, the precise function of fluorescent proteins (FPs) within corals remains ambiguous, although it is thought they are involved in some of the most significant mechanisms used by corals to acclimate and interact with their environment (Kenkel et al., 2011). Chlorophyll a within zooxanthellae (Warner et al., 2010) also contributes to fluorescence, its excitation range partially overlaps with that of GFP's enabling simultaneous imaging of GFP and chlorophyll- a with a single excitation source (Treibitz et al., 2015). LS- SPIM could provide key insight into the developmental processes in early life stages of coral.

1.6 Thesis Aims and Objectives

The main aim of the study was to examine reproductive traits in *Favia fragum* and to understand how varying environmental conditions influence larval strategies.

Specific objectives were to:

1. Determine the effect of lunar cues on the reproductive strategy employed by *Favia fragum*
2. Determine whether reproductive traits of *F. fragum* change when faced with different environmental cues.
3. Determine whether *F. fragum* produce greater variability in larval phenotype in the absence of lunar cues.
4. Determine whether differences in larval strategies influence other key life-history traits including timing of settlement and metamorphosis

It is critical to identify whether spawning and larval release is a direct response to light or a result of entrained circadian behaviour. Unable to adapt, corals may seek refuge in subtropical areas at higher latitudes or transfer deeper into the mesophotic zone. Either way, their reproductive ability may be compromised as they are exposed to exogenous conditions and cues that are not synchronous to their own internal clocks. Depleted light levels and weakened lunar signals could causes serious disruptions to reproductive patterns and subsequent implications for other early life stage processes. The aim of this study was to assess the ability of corals to utilise a bet-hedging reproductive strategy when key environmental cues are altered through assessing the daily rate of larval release over consecutive months in the presence and absence of cues. In the hierarchy of cues associated with coral reproduction lunar cues are most likely the major driver of spawning and planulation patterns and were therefore selected as the cue to manipulate and test the impacts on reproduction.

Few studies have manipulated environments on long term scales, and variance in offspring numbers and phenotypes (e.g. size) across multiple planulation events are often neglected in ecological studies. Therefore, within this study we assess the effects of lunar cues (presence and

absence) on the reproductive strategies of a coral species using larval reproductive output, timing, and larval size, settlement and metamorphic development, to determine reproductive bet-hedging employed by the common Caribbean brooding coral *Favia fragum*

1.7 Favia fragum

Favia fragum has been investigated in a number of studies focusing on reproductive strategies (Szmant- Froelich et al., 1985; Hoadley et al., 2011); this brooding coral has extended planulation periods with multiple reproductive events, making it ideal for lab- based longitudinal studies. *F. fragum* readily reproduces under laboratory conditions, despite the complexity of cues involved in coral reproduction, and is an appropriate model representative of a simultaneous hermaphroditic brooding species.

Favia fragum is a member of the Faviidae family which constitutes the second largest family of corals in terms of number of species (Veron, 1995). *F. fragum* is widespread throughout Western Atlantic reef systems extending from the Caribbean to Bermuda (Veron, 2000). Colonies reach sexual maturity at small sizes and brood embryos to a planula larval stage. Parental colonies invest on vertical transmission of its symbionts, hence larvae released in the water column are assumed to have a short planktonic larval duration. They have also observed to be simultaneous hermaphrodites meaning the production of sperm and eggs overlaps giving way to self-fertilization (Brazeau et al., 1998). Szmant- Froelich (1985) was the first to describe continual monthly planulation throughout the year in *F. fragum* making it ideal research in to reproductive cycles and strategies.

One possible outcome of altering coral communities is the potential for local increases in the number of scleractinian corals that display resistance to long- term repeated disturbances. In some Caribbean locations, for example Bermuda, *F. fragum* is already subjected to large fluctuations in environmental conditions, where temperatures can differ by 10-15^{o}C between seasons (annual lows of 15.5- 19^{o}C and summer highs of 29- 30.5^{o}C). Its ability to exist under extremes have been identified

as key players in future coral reef communities (Green et al., 2008; Camp et al., 2016) however, future persistence will depend on its' reproductive biology as well as physiological tolerance.

2.0 METHODS AND MATERIALS

2.1 Experiment overview

Multiple environmental factors including temperature, tidal regimes and solar insolation, either in synergy or in succession at different time scales, have been implicated in regulating planulae release in brooding corals. *In situ* and laboratory measurements of planulation patterns in *Favia fragum* demonstrate that this species is capable of brooding larvae all year round suggesting no inherent temperature limitations on the planulation cycle. Therefore, patterns must be explained by alternative environmental cycles that occur monthly. The lunar cycle offers stable and predictable cues and has previously been implicated to induce reproduction of both brooding and broadcast spawning coral species (Harrison and Wallace, 1990; Szmant- Froelich et al., 1985). This study isolated lunar irradiance as the sole factor to examine planulation patterns in *Favia fragum*. Reproductively active colonies were placed into separate light treatments, one group was placed under a 12:12 light: dark (constant new moon), the other exposed to an artificial induced lunar cycle. Daily reproductive output, larval size and settlement rates were measured over a 10 month- period.

2.2 Experimental set-up

Six colonies of gravid *Favia fragum* were collected from the Horniman Museum (London, UK) and transported to the Coral Reef Research Unit (University of Essex, UK) where all experiments described in this thesis were conducted. No approval from an ethics committee was required as scleractinian corals are exempted from legislation concerning the use of animals for scientific purposes in the European Union (Directive 2010/63/EU). Previous isolation and observations of larval release conducted at the Horniman Museum confirmed all colonies were of reproductively active. Coral fecundity, defined by number of larvae produced by colony polyps, has a

positive correlation with colony size. Larger colonies typically contain more larva producing polyps and have the capacity to direct more energy towards reproduction, whereas smaller colonies may invest more resources into growth to optimise survival (Babcock,1991). For this reason, *F. fragum* colonies of approximately equal size were selected to insure similar reproductive potential across all six individuals.

Corals were maintained in a 1700 L closed aquaria system. Aquarium water consisted of reverse osmosis water mixed with a commercial synthetic sea salt (H_2Ocean pro$^+$ reef salt). All artificial sea water (ASW) was prepared in a separate mixing container made up to a salinity of 35 ppt and allowed to mix for 24 hours, enabling the pH to stabilize before being added to the aquaria system. Water was circulated through a microbial sand sump, packed with established live rock, and a protein skimmer to remove dissolved organic compounds, before being distributed between 3 separate tanks. A UV sterilizer also formed part of the filtration system but was only implemented occasionally to remove potentially harmful bacteria and unwanted free- floating algae. As the UV sterilizer does not distinguish between bacteria and beneficial planktonic organisms, it was only turned on during daylight hours to reduce the chance of eliminating those organisms that are advantageous. Water parameters were tested weekly using API colour change test kits (NT Laboratories, Ltd., Chorleywood, UK). Temperature was monitored daily, manually using a digital hand held thermometer (± 0.2^{o}C; E.T.I Ltd, Worthing, UK) and electronically using an automated data logger (HOBO, Onset Computer Corporation, MA, USA). The logger was placed centrally in the tank and set to measure both temperature and light at 30 minute intervals for the duration of the experiment. Salinity was measured daily with a TMC V_2 refractometer (Tropical Marine Centre, Chorleywood, UK). Weekly 30% water changes were performed to maintain parameters (Table 1) and additional sodium carbonate, sodium hydrogen carbonate and calcium chloride were added to sustain alkalinity and calcium levels when required. Day- time light intensity was measured using a biospherical PAR sensor- LI-250A (LI- COR Bioscience Ltd, Cambridge, UK) and maintained at 200+/- 50 umol photons m^{-2} s^{-1} using 4 54 W metal halide lamps (Growth Technology Ltd., Somerset, UK). Water pumps were provided to mimic flow current (Eheim 400, Tropical Marine Centre, Chorleywood, UK).

Table 1. Chemical breakdown of synthetic salt (D-D The Aquarium Solution Ltd, UK) used to produce artificial seawater and water parameters tested weekly, average measurements over length of experiment (10 months).

Parameters	**H_2Ocean pro$^+$ reef salt**	**Measurements**
Temperature	-	26.8 ± 0.5 ^{O}C
Salinity	-	35 ± 0.5 ppt
pH	8.3 (8.2-8.4)	8.2 ± 0.2
Nitrate (NO_3^-)	-	10 ± 5 mgL^{-1}
Nitrite (NO_2^-)	-	< 0.01 mgL^{-1}
Ammonia (NH_4)	-	< 0.01 mgL^{-1}
Phosphate (PO_3)	-	< 0.25 mgL^{-1}
Magnesium	1340 (1330-1380)	1340 ± 50 mgL^{-1}
Calcium	440 (430-460)	420 ± 40 ppm
Alkalinity	10.5 (10-11)	2.7± 0.5 mEq
Chloride	19550 (19960-20130)	-
Potassium	410 (380-420)	-

Prior to their arrival, aquaria parameters were adjusted to closely replicate those at the Horniman Museum (temperature 27^{o}C, salinity 35 ppt, light 300 µmol photons m^{-2} s^{-1}) to minimize stress on the corals and reduce the degree of physiological adjustments. During acclimatization corals re- allocate nutrients and energy supplies as a consequence of amending their physiology to new environmental conditions. Measurements of larval production during this period would be impeded by these re- adjustments hence corals were given two weeks to acclimate to the aquaria before experiments began. The light regime was set on a 12: 12 light dark cycle throughout the acclimation period and for the first 3 months. Each colony was placed in the tank and rotated daily to reduce any tank effect (due to slight differences in light intensity and water flow). Corals were target fed on a diet of krill, mysis and brine shrimp (2ml per coral) 3 times per week.

2.3 Larval production rates

To assess daily and monthly planulation patterns, every night the six *F. fragum* colonies were isolated in 1L glass jars submerged with only the lid above water level to maintain water temperature; lids were removed to allow for gas exchanged overnight. Each morning the jars were inspected for planulae which were individually counted and carefully removed from the jars with Pasteur pipettes.

2.4 Larval size measurements

Freshly released larvae were selected randomly and measured for length and width using a dissecting microscope and stage- micrometer (10 per jar, subject to numbers released per day) Transference from tub to slide combined with exposure to bright illumination from the microscope resulted in short- term stress and some larvae recoiled and spun continuously in a circle, making measurements inaccurate. Hence only measurements with clear longitudinal view of the aboral and oral end were considered for analysis. The length of the larva was measured as the longest distance from the oral to aboral end and the width of the larva was measured at the widest part of the larva perpendicular to the length. The area of a larva was calculated as the area of an ellipse:

$A = \pi ab$

where a is ½ length and b is ½ width.

2.5 Settlement rates

In this experiment settlement rate was defined as the percentage of larvae that had settled within the following pre- determined time intervals: 12h, 24h, 48h, 72h, 96h, 120+. Time 0 was defined as the point when larvae were collected and allocated into tubs (immediately during the

morning following planulation and hence assessments of settlement should be considered minimum times depending on the time of night individual larvae were produced). Slides were pre-treated in the aquarium for 2 months prior to the start of the experiment to enable bacterial biofilms and crustose coralline algae (CCA) to establish; both of which are known settlement inducers in a multitude of coral species (Negri et al., 2001; Tebben et al., 2015). Slides were placed into 5 x 5 cm tubs and ca. 20 larvae from each treatment group were placed into each tub. Larvae were pooled from all 3 colonies/ treatment due to the low numbers being produced, particularly under the lunar light cue. Light intensity, temperature and all other variables were kept the same as those in the main aquaria system. 100% water changes were completed daily until all larvae were settled. In this study larvae were considered settled if they could not be dislodged by pipetting a gentle stream of water directly at them (Miller and Mundy, 2003). Settled recruits were then transferred to wracks in the main experiment aquaria, elevated above the tank floor to reduce post- settlement mortality (through sedimentation, and predation or erosion from *Turbo* and *Stomatella* snails).

2.6 Larval production under different treatments

Two 75L tanks were set- up adjacent to each other, all 6 colonies were separated equally and randomly assigned to the two replicate tanks. The tanks were linked into the main system in order to maintain stable conditions throughout the duration of the experiment. For the remaining experiments, the filtration system was altered to improve water quality and reduce fluctuations in parameters significant to coral health including alkalinity, calcium and nitrate levels. ASW was filtered first through an algal tank (containing *Chaetomorpha*, commonly used in aquariums due to its efficient removal of phosphates and nitrates) and then passed through a protein skimmer before returning to the main system. Using ASW instead of filtered seawater offers a controlled and standardised supply of elements necessary for coral including calcium and magnesium, as corals continue to grow and lay down their calcium carbonate skeleton these elements get depleted and may limit coral growth and reproductive potential. The only opportunity to replenish these elements is to perform regular water

changes which thereby maintained and kept key elements. Nutrient limitation was avoided throughout the study by using a Triton system (TRITON GmbH, Dusseldorf, Germany) which ensured water was replete of magnesium, potassium, fluorine, boron, calcium, strontium and carbonate. Three solutions of TRITON Elementz were supplied to the main system at 200 ml/ hour using a dosing pump. Preliminary tests for carbonate hardness determined the volume of solution to raise the alkalinity to 8 KH degrees, this was then adjusted accordingly to maintain alkalinity throughout.

Tanks were set to one of two nocturnal light regimes: one tank remained in constant new moon (CNM), the other was exposed to an artificially illumination system that simulated daily sunset, sunrise and a monthly lunar cycle (Figure 3). The CNM treatment tank was covered with cardboard during the 12 hours of darkness to eradicate any potential light pollution, a HOBO sensor confirmed 0 light irradiance throughout the experiment. Colonies were rotated around the tanks daily to account for any discrepancies in light intensity and water flow. Jars were inspected daily for larvae and larval counts recorded per colony for 3 consecutive months. Colonies were then swapped between light treatments for a further 2 months and then reverted to their original light treatments for a final month (Table 2). This was done to reduce bias that could result from the order in which the light treated is provided. Larval counts were continually measured throughout all experiments.

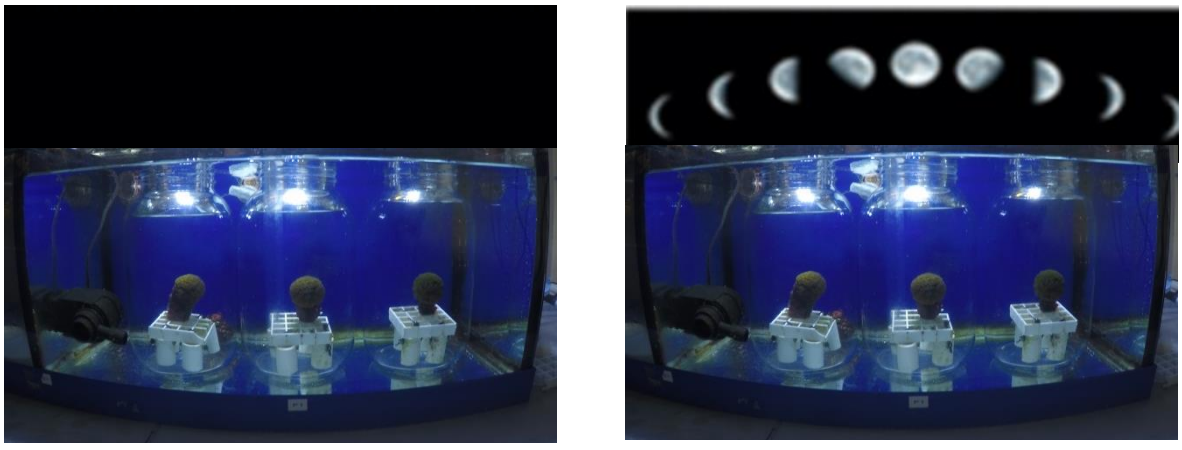

Figure 3. Nocturnal light treatments, colonies subjected to constant new moon for 12 hours (left) or exposed to an artificial lunar cycle (right). Individual colonies were isolated in single jars at night for larval collection.

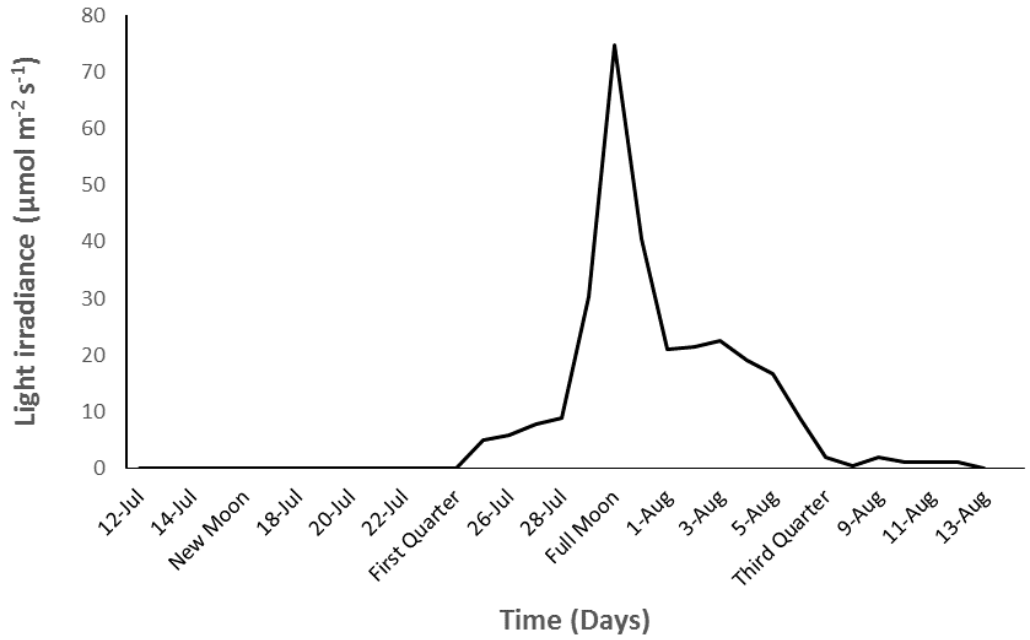

Figure 4. Light intensity of LED simulated lunar cycle over one month, measured every 30 minutes using HOBO sensor loggers.

Table 2. Light treatments of F. fragum *colonies over the 10-month period, either constant new moon ("CNM"), or artificial monthly lunar cycle ("lunar").*

MONTH	**COLONY 1, 4, 6**	**COLONY 2, 3, 5**
MARCH	CNM	CNM
APRIL	CNM	CNM
MAY	CNM	CNM
JUNE	CNM	CNM
JULY	Lunar	CNM
AUGUST	Lunar	CNM
SEPTEMBER	Lunar	CNM
OCTOBER	CNM	Lunar
NOVEMBER	CNM	Lunar
DECEMBER	Lunar	CNM

The aquaria lights used for the duration of the light manipulation experiments differed from those used in the previous 3- month experiment. These lights were selected specifically so they could be programmed to produce a lunar cycle using an AI MKII Controller (Aquaillumination, Ames, IA). Light intensity remained at 250 µmol photons m^{-1} s^{-1}, however the light spectrum during daylight hours was altered (Figure 5). A spectroradiometer was used to measure the light spectrum of each lights, U-3000 spectrophotometer fitted with an integrating sphere to minimize the scattering (ø60, Hitachi High-Technologies, Berkshire, UK).

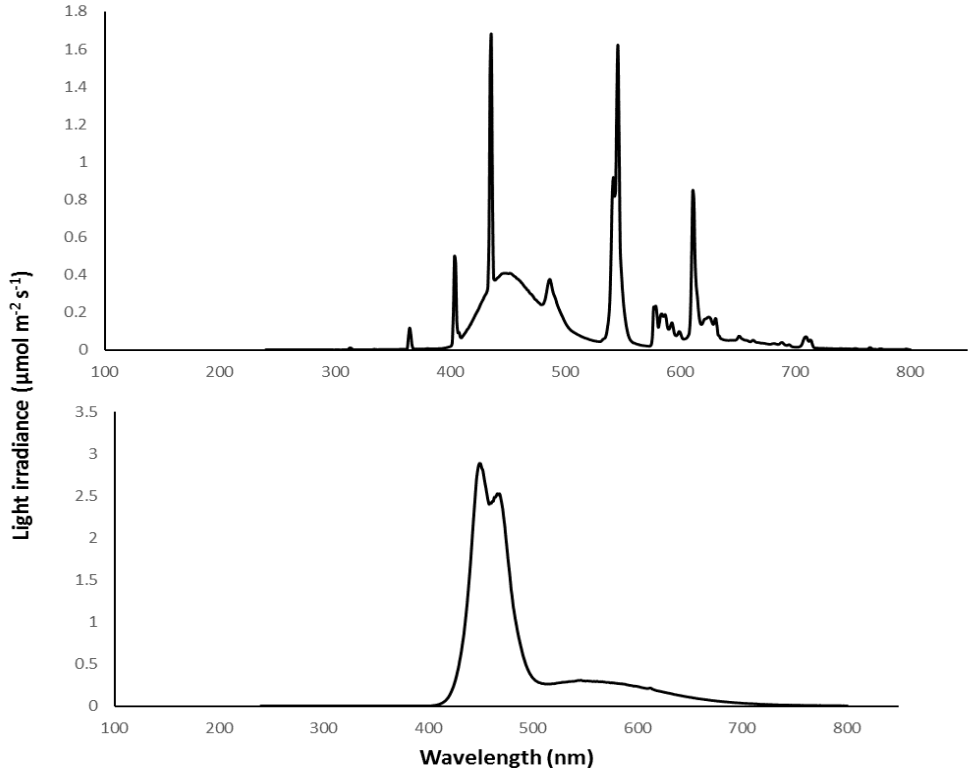

Figure 5. Day- time light spectrum of T5 Halide bulbs (top) used during first 3 months, LED lights used during nocturnal light manipulation experiments (bottom).

2.7 Metabolic rate

Respiration and photosynthesis measurements of coral colonies were taken using a standard respirometer chamber and optode technology (FOXY-R, 1.58mm diameter, Ocean Optics, Dunedin, FL, USA). The use of oxygen probes provides a non- invasive and accurate method to measure coral metabolism. Preliminary tests were carried out to establish acclimation and incubation times and volume of the vessel required for respirometry measurements. Conditions in the respiratory mesocosms were set to replicate those in the holding tanks (Temp 27.1^{o}C, salinity 35ppt); it was established that the time needed to acclimate to the mesocosms would only need to account for the stress induced during transportation.

Each oxygen electrode was calibrated before any measurements on coral respiration or photosynthesis as per manufacturer instructions (Ocean Optics). RO water was saturated with nitrogen gas and probes were left to stabilize to 0% O_2, i.e. the maximum fluorescence yield, and a tau (τ) value was set. In this experiment, tau represents fluorescence lifetime, i.e. the time taken for a molecule to remain in an excited state before returning to ground state. A second tau value was set at 20.9% O_2 in air saturated RO water, calibration was conducted at the measurement temperature (27.1^{o}C). The oxygen probes were checked once per week throughout the 3 months of metabolic measurements, re- calibration was performed when required using the procedure as above.

Before initiating coral measurements, each microcosm was filled with freshly made artificial seawater (made with RO water and H_2Ocean pro^{+} reef salt). Water was heated and maintained at 27.1^{o}C using interconnecting water jackets attached via silicon tubes to a re- circulating water bath to buffer temperature fluctuations. Oxygen concentration (µmol) was measured using an optical oxygen electrode connected to a Neofox phase fluorimeter using bifurcated optical fibres and 21-02 splice bushing. A Neofox TP thermistor was also used to continuously measure temperature and used for real- time temperature compensation of oxygen readings. Neofox optode technology uses an encapsulated Ruthenium compound to measure fluorescence (defined as τ) which is converted to oxygen values using an algorithm (provided by the manufacturer). Dependence on oxygen quenching

of fluorescence means the optodes do not consume oxygen during measurements and is unaffected by flow speed, making them suitable candidates for coral metabolic investigations (Chu et al., 2011). According to the manufacturer, drift specifications at 0% oxygen is 0.003%/ hr and at 20% oxygen 0.0015%/ hr. Replicate light equipment (4- tube T5 lightwave) was used to insure light intensity (250 µmol photons $m^{-2}s^{-1}$) and light spectrum was consistent with that in the aquarium.

Control measurements were taken for 15 minutes prior to coral measurements to confirm that the probes remained calibrated and to enable the water to reach experimental temperature. Individual colonies were transferred from the captive aquaria to experimental chambers with minimal handling time, inverted and placed into separate 500ml microcosms. The microcosms were fitted with lids designed to exclude air and eliminate gas exchange whilst accommodating both the oxygen and temperature probes. Each chamber was placed over a magnetic stirrer (Fischer Scientific Ltd, Loughborough, UK) with a magnetic stir bar at the base to maintain water movement throughout. All corals were acclimated for 15 minutes before the start of each experiment. Corals acclimate to new conditions via adjustments to their physiology through re-distribution of energy and nutrients (Levy et al., 2004). During this period measurements of metabolic processes would represent this re-allocation and not reflect true rates of oxygen consumption or production, hence time 0 was defined as the time point immediately after the acclimation period. Oxygen concentration (µmol) was automatically logged every 10 seconds using Neofox Viewer v2.20 (Ocean Optics, USA) and recorded for 30 minutes. Immediately following light measurements, the microcosms were covered with opaque plastic bags and cardboard and 30 minutes of dark measurements were recorded to calculate enhanced post- illumination dark respiration (EPIR). Corals were immediately transferred back to the main aquaria system and each microcosm was rinsed and refilled with fresh ASW in preparation for measurements on replica coral samples. Corals were randomly assigned to mesocosms to average out any artefacts from bench location.

Between incubations, resting periods were incorporated (lasting 48 hours) to minimise any stress related artefacts resulting from the analysis. Measurements were taken at the same time of day for every repeat. Photosynthesis and respiration measurements were normalised to weight using the

buoyant weight method (Jokiel et al., 1978), all four corals used were of a similar size and maintained under the same aquaria conditions therefore it was assumed that they would have similar zooxanthellae densities and genotypes.

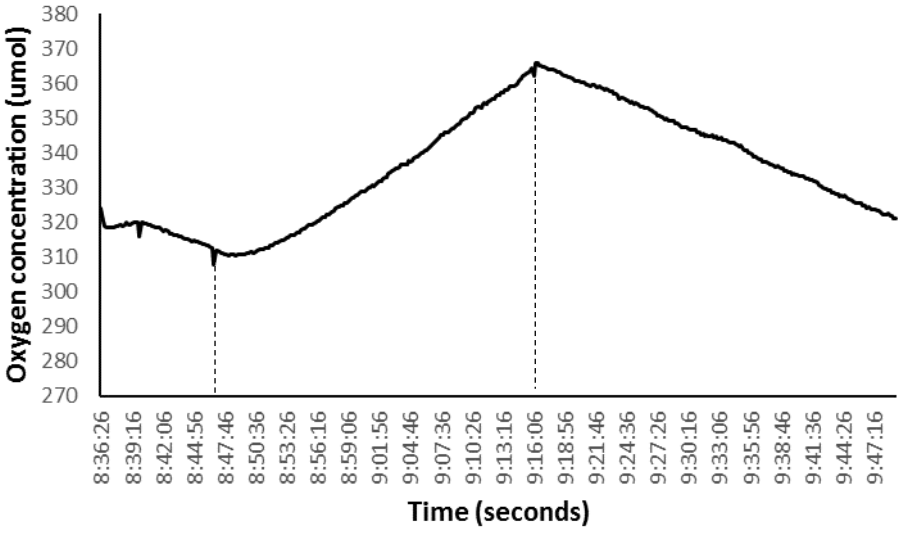

Figure 6. Graph illustrating oxygen drift during photosynthesis and EPIR measurements, dotted lines indicate start points for measurements of photosynthesis (15 mins after acclimation period) and EPIR (corals covered in black bags).

Oxygen evolution calculations

Oxygen evolution was calculated using the following equations, where $_{(F)}$ is final measurement and $_{(I)}$ is the initial measurement. Time (hours) remained constant for each experiment. End point analysis was deemed appropriate as there was very little variability within any single time point and changes were linear over the time scale used (see Fig. 6). If high variability was encountered than regression analysis using all time measured would have been used and the resulting regression coefficient considered the rate of oxygen change.

$$Net\ photosynthesis\ rate\ (P_N) = \frac{\left(\ Oxygen_{(F)} - Oxygen_{(I)}\ \right)}{Time}$$

$$Light\ Enhanced\ Dark\ Respiration\ (R_D) = \frac{\left(\ Respiration_{(F)} - Respiration_{(I)}\ \right)}{Time}$$

$$Gross\ Photosynthesis\ (P_G) = \ P_N + \ R_D$$

2.7.1 Buoyant weight technique

After determining the changes in oxygen concentration, respiration and photosynthesis rates were standardized using the buoyancy weight method (as described in Jokiel et al., 1978). This technique follows Archimedes' principle, which states "the weight of an object in air is equal to the object's weight in a liquid medium plus the weight of the liquid displaced by the object". To obtain values for volume and weight, each coral colony was independently placed on a small metal platform submerged in 2L of ASW maintained at 27ºC. The platform was suspended from the underside of the scales and housed in a plastic storage container to restrict air flow and enable the scales to stabilize before being auto- zeroed. The density of seawater is dependent on salinity and temperature; both were accounted for to determine to density of ASW. Settlement plugs were also weighed individually and accounted for in the final calculations. Water displacement was used to determine the volume (V_A) of the aragonite skeleton. Each coral was gently dried with a paper towel and submerged in 500mL of ASW in a 1L container after which the displaced water was transferred and measured in a graduated cylinder and expressed in ml. The total dry weight (W_D) was calculated using measured buoyant weight (W_W), volume of coral skeleton and density of ASW (D_{SW}), assuming $V_A \cdot D_{SW}$ is equal to the weight of displaced seawater:

$$Weight_D = \ Weight_W + (\ Volume_A \cdot Density_{SW})$$

Several assumptions are attached to this method of measuring buoyant weight. The first is that it assumes the density of tissue and mucus is equal to the density of seawater. Jokiel and Morrissey (1986) removed the mucus and tissue of *Pocillopora damicornis* and concluded near neutral buoyancy

in seawater. In addition, it assumes the liquid filled spaces within the calcareous skeleton are the same density as seawater. It also depends on the skeletal structure of the coral being completely aragonite; aragonite has a density three times that of seawater and in *P. damicornis*, at least, accounts for 99.9% of the skeletal material (Jokiel et al., 1986). The percentage of aragonite making up the skeleton of *Favia fragum* was not calculated but assumed to be close to that of *P. damicornis*. Lastly, it doesn't account for the weight of burrowing and cryptic fauna that may affect the buoyant weight of the coral. *F. fragum* however, is a massive colonial coral and its morphology offers little to no habitable crevices for macro- organisms. Also, in other studies macro- organisms eg in *Montipora*, accounted for less than 0.16% of the buoyant weight, an insignificant value (Jokiel et al., 1978).

2.8 Larval development using Light Sheet Fluorescence Microscopy (LSFM)

Live imaging of scleractinian corals has been achieved at the University of Essex through modifications of light- sheet fluorescence microscopy (LSFM). The design aimed to diminish phototoxic impacts opening up the opportunity for long term repeated measures based on the following (Laissue et al., unpubl.).

1. The organism remains in a consistent physiological state ensuring fluorescence is not influenced during imaging.
2. Three spatial dimensions is acquired for full structural information, proximal taxonomic identification of scleractinian corals has been based on their calcareous exoskeleton for years and continues to provide information on their function and values (Gutiérrez-Heredia et al., 2015).
3. The skeleton, tissue and zooxanthellae can be optically separated and most importantly the organism is completely unharmed.

Preliminary measurements were performed prior to experimental testing and adjustments were made (e.g. light intensity) accordingly to ensure corals were not physiologically stressed during

imaging. Visible signs of coral stress can be observed based on their reaction upon initial exposure to the light sheet. High light illumination used in laser- scanning confocal microscopy (LSCM) commonly induces tentacular retraction to protect from phototoxic effects (personal observation). Upon exposure of the light sheet, multiple corals tested maintained tentacle expansion, presenting no visible response to illumination levels. *Favia fragum* typically retract their tentacles during daylight hours however, reduced light levels during imaging triggered temporary expansion, the only retraction witnessed occurred during initial imaging and was attributed to transportation stress.

Images were obtained in 5D (x, y, z- axes, time and wavelength) as the specimen was mechanically manoeuvred through the light sheet; movement in the x- axis provided focussed images across the width of the sample. The y- axis positioned the sample in the focal plane of the detecting objective whilst the z-axis enabled optical sectioning that were stacked together to form a 3D image. Image acquisition was repeated under emission filters consistent with the emitted wavelengths of GFP and chl-a and used to identify and differentiate coral host tissue from symbionts.

Image acquisition

A single beam (488nm wavelength) was emitted via a 20mW continuous- wave solid state frequency- converted diode laser (FCD488-020, JDS Uniphase Corporation, CA, USA) passed through a beam expander and reflected onto a rotating mirror (AN8248NSB, Panasonic Corporation, Osaka, Japan). The mirror created a uniform wavefront, the brushless motor was powered by a 375W linear DC variable voltage supply. During acquisition, between 6 to 10V were used resulting in 0.7ms to 1.1ms pulses. The beam was then evenly split via a beam splitter combined with a prism and each beam was passed through identical cylindrical lenses and recombined at right angles. The light sheet generated optical sections of 28 µm thickness. Vessels were customised to size to accommodate live imaging within the light sheet set-up, built with optical plastic and made water-tight with silicone gel. The vessel was filled with ASW and the slide placed on a 45% angle to reduce shadowing and maximise 3D information. Slides were individually stepped through the light sheet using a z-motor, the z-axis was limited by the 25mm travel of the motorized translation stage (Thorlabs Inc., Newton, New Jersey, USA).

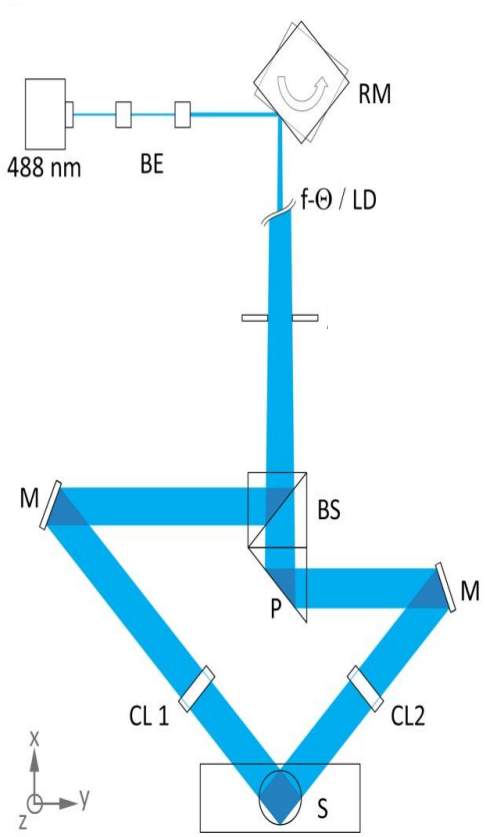

Figure 7. Schematic of light sheet set- up: A laser beam (488 nm) is passed through a beam expander (BE) and reflected on to a rotating mirror (RM). A beam splitter (BS) combined with a prism (P) divided the beam into 2, each reflected onto a mirror (M) and passed through a cylindrical lens (CL1/ CL2) and recombined at a right angle to form a light sheet (LS). The sample (S) was manoeuvred on a motorised z stage through the LS.

Images were acquired using a SZX16 stereomicroscope (Olympus KeyMed, Southend-on-Sea, UK) with a 1x Plan Apochromatic objective and a Luca-R DL-604M-#VP electron multiplication (EM)- CCD camera (Andor Technology, Belfast, Northern Ireland). This quantitative digital camera was able to detect single protons and is ideal for high- speed, low-light fluorescence microscopy (Huisken and Stainier, 2009). The camera mount was modified to allow emission filters to be inserted. Chlorophyll- a (Chl-a) emits a primary peak at ~625nm and secondary peak at ~730nm (Mazel, 1997), FPs exhibit typical emission peaks of 482- 609 nm (D'Angelo et al., 2008). GFPs excitation range partially overlaps with chlorophyll-a enabling simultaneous imaging of GFP and Chl-a within a

single excitation source (Treibitz et al., 2015). Chl-a used a chromatic reflector at 665 nm and a long pass emission filter at 664 nm, GFP used a reflector at 505 nm and a single band emission filter at 535/40 nm (Chroma Technology Corp., VT, USA). Exposure time (600 ms) and z-step size (20 µm) settings were kept identical for all acquired datasets; magnification was adjusted according to the sample size (typically x6/ x8). The top and bottom boundaries of the stage were set for individual image acquisition; focus was adjusted to achieve maximum 3D information.

Slides were kept in ASW in an incubator at 27^{o}C prior to imaging, during acquisition slides were exposed to room temperature for no longer than 5-10 minutes before being placed back into the temperature controlled aquaria. Samples were given 24 hours to recover from any stress incurred during transportation before being imaged the following day.

2.9 Data analysis

All statistical analyses were conducted using R (R Core Development Team, 2013). Prior to analysis, data was tested for normality and equal variance using Shapiro- Wilk's test for normality and Levene's test for equal variance and visually via histograms and qq- plots. No data was transformed instead non- parametric versions of analytical tests were preferred. Data that violated normality were analysed primarily via general linear models, using date and light conditions as fixed factors. Estimated means were compared using post-hoc contrasts with Bonferroni adjustment at 95% confidence levels.

To determine differences in larval counts between and within light treatment groups and phases of the moon (FM, FQ, NM, TQ), general linear models (GLM) with quasi- poisson distribution were used. Due to several days of 'zero' observations, larval counts did not meet the assumptions of parametric statistics and instead data was often over- dispersed. To determine the feasibility of quasi- models and meet the assumptions, data was tested for over- dispersion prior to GLM analysis, checking the variance was proportional to the mean (an assumption of quasi- distribution). When data

satisfied the assumptions, quasi models were preferred and predicted values were adjusted to account for over- dispersion. Larval count data was normalised to coral biomass, corals were weighed monthly using buoyant weight method. When significance was encountered ($p < 0.05$) multi- comparison post-hoc analysis was performed to distinguish which months/ groups were responsible for the differences. An independent t- test was used to determine whether corals under lunar/ non- lunar light produced more larvae overall.

Circular statistics was preferred over ANOVA (or non- parametric equivalent Kruskal- Wallis) for analysis of circadian rhythmicity because the ANOVA only evaluates whether means are significantly different. Circular statistics are appropriate when data have distributions with no true 0 and where any designation of high/ low values is purely arbitrary (eg, hours of the day). Data were normalised to the total number of larvae released by the colony for the entire lunar cycle. Lunar day 0 corresponded to the start day of each month. Moore's modification of Rayleigh's test was used to test that the data was distributed uniformly during the time periods, i.e. lunar days and months. Circular statistic tests were used to calculate the temporal concentration of larvae released, i.e. whether the number of larvae released per day were concentrated (r is close to 1) or dispersed (r is close to 0) throughout the lunar cycle.

To distinguish differences between the size of larvae produced between treatments and within lunar phases and differences in time periods of settlement rates, one -way ANOVA was performed (or non- parametric Welch t- test), followed by post- hoc analysis when appropriate.

Differences in photosynthetic rates were determined using univariate one- way analysis of variance (ANOVA; F; $\alpha = 0.05$) or non- parametric equivalent. Tukey's honest significant difference (HSD) test (t; $\alpha = 0.05$) was used for post- hoc comparison of means. To assess correlation between larval production and metabolic rate, Spearman's rank correlation was performed. Images of metamorphic development were processed using Image J.

3.0 RESULTS

3.1 Larval production patterns of **Favia fragum** *across 10 lunar cycles in the presence and absence of lunar light*

3.1.1 Comparison of larval output between two light treatments

Maintaining *Favia fragum* in a constant new moon (CNM) phase for 4 months illustrated a decline in larval output by 93%. The average number of larvae produced reduced from 172 ± 37 to 12 ± 5 larvae colony^{-1} month^{-1} (mean ± SE), larval output ceased mid- way through the last month and no larvae were released following cessation for the remaining 14 days.

All colonies placed under lunar light began to release larvae 4 days post- exposure. After the first month total larval output increased by 9.3- fold, mean larval output across all 3 colonies increased from 19 ± 9 to 177 ± 45 larvae colony^{-1} month^{-1}. The following two months larval output fluctuated falling by 51% then rising by 66% in the third month. Similarly, colonies that remained in CNM increased larval output however, the number of larvae produced was significantly higher (increasing by 64-fold). Larval output remained high for the following two months, showing a slight increase with each month (26% and 10% respectively). The first 4 months were excluded from analysis due to problems encountered with tank acclimation post-transportation and spikes in nutrient concentrations with the tank due to equipment failure. Statistical analysis confirmed colonies under CNM released significantly more larvae than colonies subjected to lunar ($t(20) = 3.121$, $p<0.05$). On average, colonies under new moon released 2.7 x more larvae than those under artificial lunar cycles (469 ± 91 compared to 172 ± 26 larvae colony^{-1} month^{-1} respectively), contradicting predicted outcomes.

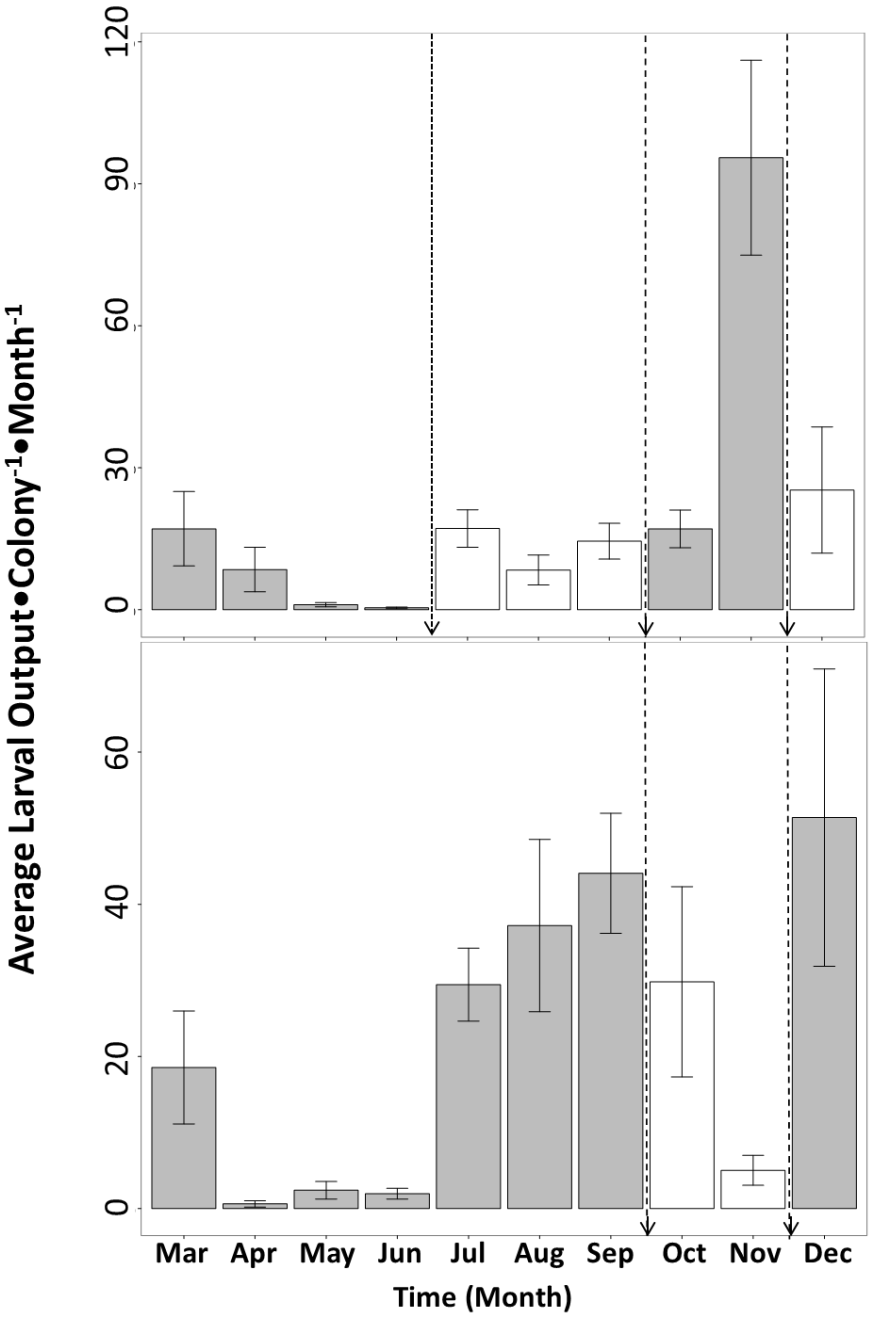

Figure 8. Monthly larval output across 10- month experimental period compared between light treatments: constant new moon (grey bars) and lunar (white bars), dotted lines indicate where colonies were switched between tanks and exposed to opposite light treatments. Two groups of equal replicates were subjected to different treatment patterns, group 1 (top) exposed to longer lunar periods, group 2 (bottom) longer period of new moon. Larval counts were normalised to coral colony biomass (n= 3), bars represent group (mean ± SE presented)

3.1.2 Comparisons of larval output within the treatment groups

Group 1

Larval production was highly variable across the months (GLM with quasi- poisson errors, *df* = 9, *n* = 3, *f* = 20.145, $p < 0.05$). Significance in variability was explained by the transition between light treatments, post hoc analysis between repeated monthly measurements indicated larval output in November was significantly higher than the other months (see Table 4), averaging 33 ± 5 larvae colony^{-1} day^{-1} (mean ± SE). Total larval release increased by 46- fold from October to November, one month after colonies were switched from lunar to CNM. A lag effect on larval release was noted following the removal of lunar light (Sep- Oct) however, larval output was still significantly higher in October (increased by 22%) than September. Similarly, the second switch also illustrated significant differences however, larval output dropped by 74% when colonies were reverted from CNM to lunar. Unlike the transition from lunar to CNM in which increased rates of larval output were delayed for 20- 30 days, declines in the rates of larval release occurred after immediate exposure to lunar. Despite the decline, average daily release remained high (10 ± 4 larvae colony^{-1} day^{-1}). No significant differences occurred between the 3 months under lunar light despite fluctuations.

*Table 3. Summary of GLM with quasi-poisson error in Group 1, fixed factors included Month and Coral Colony, *represents statistical difference at 5%.*

Treatment	Variable	Estimate	St. Error	*T*	*P*
CNM	Intercept	1.0415	0.3748	2.779	0.00559 *
	Mar	0.6992	0.4612	1.516	0.12992
	May	-2.0448	1.1075	-1.846	0.06521
	Jun	-1.5159	0.8834	-1.716	0.08656
Lunar	Jul	0.7138	0.4551	1.568	0.11715
	Aug	0.5302	0.5276	1.005	0.31516
	Sep	0.5611	0.4727	1.187	0.23561
CNM	Oct	0.9139	0.4562	2.003	0.04546 *
	Nov	2.4624	0.3905	6.306	$4.67e^{-10}$ *
Lunar	Dec	1.3055	0.4315	3.025	0.00256 *

*Table 4. Post hoc comparisons between Monthly lunar cycles within Group 1 using Tukey HSD test, *represents statistical difference at 5% in Tukey test.*

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean	5.701	2.833	0.366	0.622	5.784	4.814	4.966	7.066	33.244	10.453
SE (±)	1.627	1.432	0.161	0.193	1.161	1.387	0.937	1.316	4.738	3.847
Mar	-	-	-	-	-	-	-	-	-	-
Apr	0.860	-	-	-	-	-	-	-	-	-
May	0.205	0.659	-	-	-	-	-	-	-	-
Jun	0.173	0.747	1.000	-	-	-	-	-	-	-
Jul	1.000	0.834	0.197	0.163	-	-	-	-	-	-
Aug	1.000	0989	0.325	0.329	1.000	-	-	-	-	-
Sep	1.000	0.967	0.276	0.258	1.000	1.000	-	-	-	-
Oct	0.999	0.546	0.126	0.889	0.999	0.997	0.995	-	-	-
Nov	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	-	-
Dec	0.716	0.060	0.041*	0.018*	0.715	0.685	0.494	0.971	<0.001*	-

Group 2

Analysis confirmed significant differences between the months in group 2 (GLM with quasi-poisson errors, $df = 9$, $n = 3$, $f = 42.701$, $p < 0.05$). Larval output patterns were comparable to those of group 1, pairwise post- hoc testing indicated the variance was attributed to the months following a switch in light treatment. The greatest decrease in larval production occurred in November, larval output reduced by 84%, declines coincided with a switch from CNM to lunar and resulted in the lowest daily average rate (2 ± 1 larvae colony^{-1} day^{-1}). However, synonymous with group 2 patterns, a lag in larval output decline of 20- 30 days occurred, the month immediately after the switch indicated insignificant reductions (decrease by 27% in October). The second switch illustrated a significant incline in larval output, larval release rate was 10.6 x higher in December compared to November after colonies were switched from lunar to CNM (averaging 21 ± 6 larvae colony^{-1} day^{-1}).

Differences between the months of CNM were also significant, June to July represented the largest increase in larval output, production was 25- fold higher in July than June despite remaining under synonymous conditions. Output continued to steadily increase after July, a reversal of earlier planuation patterns in which larval production plunged in April, output declined by 96% one month after treatment under CNM. Therefore, corals exhibit a high degree in variability and adjust their reproductive output dependent on the treatment, releasing high numbers under CNM and reducing production under lunar.

*Table 5. Summary of GLM with quasi-poisson error in Group 2, fixed factors included Month and Coral Colony, *represents statistical difference at 5%.*

Treatment	Variable	Estimate	St. Error	*T*	*P*
CNM	Intercept	-1.5041	1.4395	-1.045	0.29638
	Mar	3.3260	1.4660	2.269	0.02354 *
	May	0.8961	1.7082	0.525	0.60001
	Jun	-0.2231	2.1592	-0.103	0.91771
CNM	Jul	3.7882	1.4551	2.603	0.00940 *
	Aug	4.5652	1.4519	3.144	0.00172 *
	Sep	4.1503	1.4508	2.861	0.00433 *
Lunar	Oct	4.0303	1.4547	2.771	0.00572 *
	Nov	2.0149	1.5324	1.315	0.18893
CNM	Dec	4.5600	1.4485	3.148	0.00170 *

*Table 6. Post hoc comparisons using Tukey HSD test between months within Group 2, *represents statistical difference at 5% in Tukey test.*

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean	6.183	0.222	0.544	0.177	9.817	21.351	14.100	36.959	1.666	21.240
SE (±)	2.117	0.100	0.287	0.064	1.395	5.647	2.369	4.267	0.615	5.495
Mar	-	-	-	-	-	-	-	-	-	-
Apr	0.806	-	-	-	-	-	-	-	-	-
May	0.852	1.000	-	-	-	-	-	-	-	-
Jun	0.799	1.000	1.000	-	-	-	-	-	-	-
Jul	0.990	0.156	0.193	0.150	-	-	-	-	-	-
Aug	<0.01*	<0.01*	<0.01*	<0.01*	0.119	-	-	-	-	-
Sep	0.435	<0.01*	<0.01*	<0.01*	0.967	0.743	-	-	-	-
Oct	0.794	0.03*	0.04*	0.03*	0.999	0.529	1.000	-	-	-
Nov	0.959	1.000	1.000	1.000	0.366	<0.01*	0.015*	0.096	-	-
Dec	<0.01*	<0.01*	<0.01*	<0.01*	0.058	1.000	0.644	0.412	<0.01*	-

3.1.3 Larval output between colonies within treatment groups

Group 1

Larval output was highly variable across the colonies (GLM with quasi- poisson errors, df = 2, n = 3, f = 5.111, $p < 0.05$) despite being of similar age and size. Across the entire 10-month period, C1's contribution to total larval output was significantly higher than the other two, accounting for 46% of all larvae released with an average monthly release of 293 ± 23 larvae month^{-1}. The other colonies showed similar production rates.

Group 2

Similarly, in group 2 there was high variability between the colonies (GLM with quasi-poisson errors, df = 2, n = 3, f = 55.553, $p < 0.05$). 58% of the total number of larvae released over 10 months was attained from C5, with a mean monthly total of 387 ± 21 larvae month^{-1}. No significant differences were found between C2 and C3.

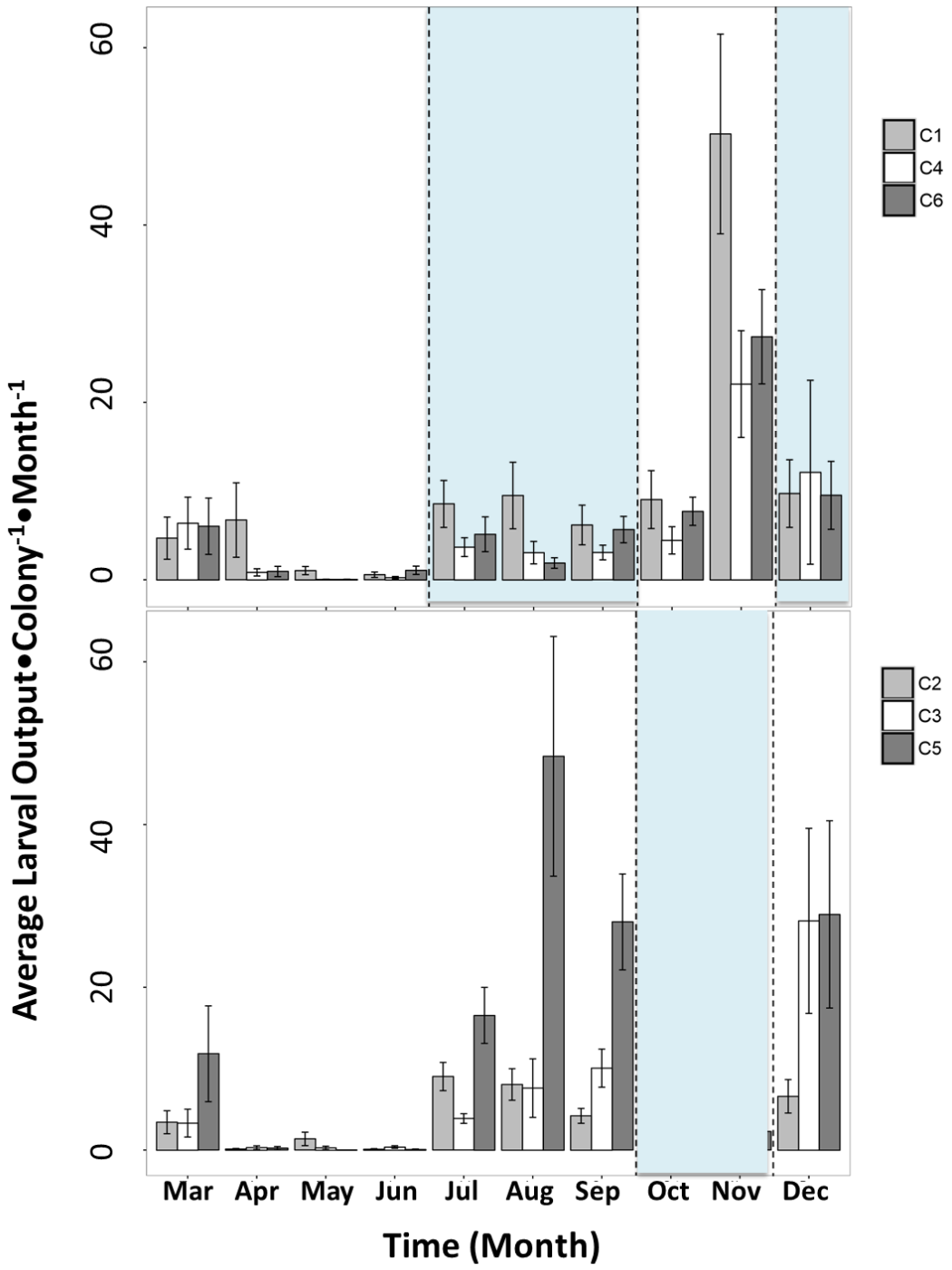

Figure 9. Larval output between colonies within treatment groups: Group 1 (top) and Group 2 (bottom). Dotted lines indicate points colonies were switched between tanks. Blue shaded areas indicate periods under lunar illumination, white areas are periods under CNM, (means ± SE are presented)

3.2 Larval output between lunar phases

3.2.1 Distribution of larval release between treatments

It was hypothesized that corals exposed to lunar cues would have peaked production around a specific lunar phase, corals in a CNM phase would lose synchronicity and larval release would be uniform across the month.

Coral subjected to lunar treatment consistently released larvae in clustered periods demonstrating peaked planulation patterns (Moore- Rayleigh, $p < 0.05$), supported by r values close to 1 in most months (see Table 7). Peaked planulation typically lasted between 3 to 6 days and accounted for ca. 50% of the total larval release over the whole month. In between planulation peaks larvae were produced at low rates, larvae were released 59 ± 7% of days per month. Time periods between peaked planulation (taken from the end day of peaked larval release to the start of the preceding peak) varied from month to month (ranging from 9- 33 days).

Corals under CNM produced larvae on 88 ± 7% of days throughout the month, indicating constant production. However, periods of peaked planulation were also present ($p < 0.05$) in most months. Synonymous with lunar corals, larvae released over peaked times contributed to ca. 50% of total larvae released and occurred over 3 to 6 days. Time periods between peaked planulation events were also highly variable, ranging from 14- 42 days. Although distributions of daily larval release across the month were insufficient to meet the assumptions of uniformity, mean resultant length (r) suggested releases rates were dispersed. Unlike lunar corals that had a clearly defined period of peaked larval release each month, daily larval release in CNM corals was highly variable with no fixed phase of peaked production as evidence by r values close to 0 (see Table 8).

When switched from CNM to lunar, the % of days larvae were released dropped (from 80% to 51%), where as those switched from lunar to CNM increased the amount of days they released larvae (from 37% to 72%). Corals switched to lunar treatment immediately returned to peaked planulation patterns, the 5-day period of peaked release accounted for 83% of total larval output.

Corals switched to CNM retained peaked planulation patterns, associated with lunar synchronicity, but daily larval release became more variable with time as demonstrated by reduced r values (see Table 8). In both cases when corals were switched between treatments, results suggest lunar light has an immediate impact on planulation patterns, transforming dispersed larval production to peaked larval production over a few days. There appears to be a lag effect of CNM on larval release as corals retain peaked production patterns even after 2 months of no lunar cues.

*Table 7. Moore- Rayleigh test for uniformity of planulation distribution patterns within a lunar month for corals under lunar stimulation, r = mean resultant length (measure of temporal concentration of larvae released), z = test for circular uniformity, p = degree of significance against uniform distribution within one lunar cycle (significance level 0.05), bold highlighted= significant values, *corals swapped between treatments*

Month	Peak day	Peak duration	Lunar Phase (+/- days)	Z	P	r
July	7	5 – 11	FQ - 1	0.271	**0.016**	0.287
August	25	24 – 27	FM - 4	0.617	**<0.001**	0.620
September	14	12 – 15	NM + 1	0.156	0.114	0.195
October*	19	18 – 22	FQ - 1	0.521	**<0.001**	0.552
November	19	17 – 20	FQ	0.540	**<0.001**	0.565
December*	7	-	FM - 4	0.579	**<0.001**	0.600

*Table 8. Moore- Rayleigh test for uniformity of planulation distribution patterns within a lunar month for corals under CNM, r = mean resultant length (measure of temporal concentration of larvae released), z = test for circular uniformity, p = degree of significance against uniform distribution within one lunar cycle (significance level 0.05), bold highlighted= significant values, *corals swapped between treatments*

Month	Peak day	Peak duration	Lunar Phase (+/- days)	Z	P	r
July	26	23 -28	FQ + 2	0.204	0.055	0.217
August	30	25 – 31	FM + 1	0.405	**<0.001**	0.409
September	16	14 – 18	NM + 1	0.044	0.368	0.108
October*	30	28 – 30	FM + 3	0.252	**0.023**	0.252
November	4	1 – 6	TQ + 1	0.231	**0.037**	0.320
December*	24	23 – 25	FM - 1	0.364	**0.002**	0.401

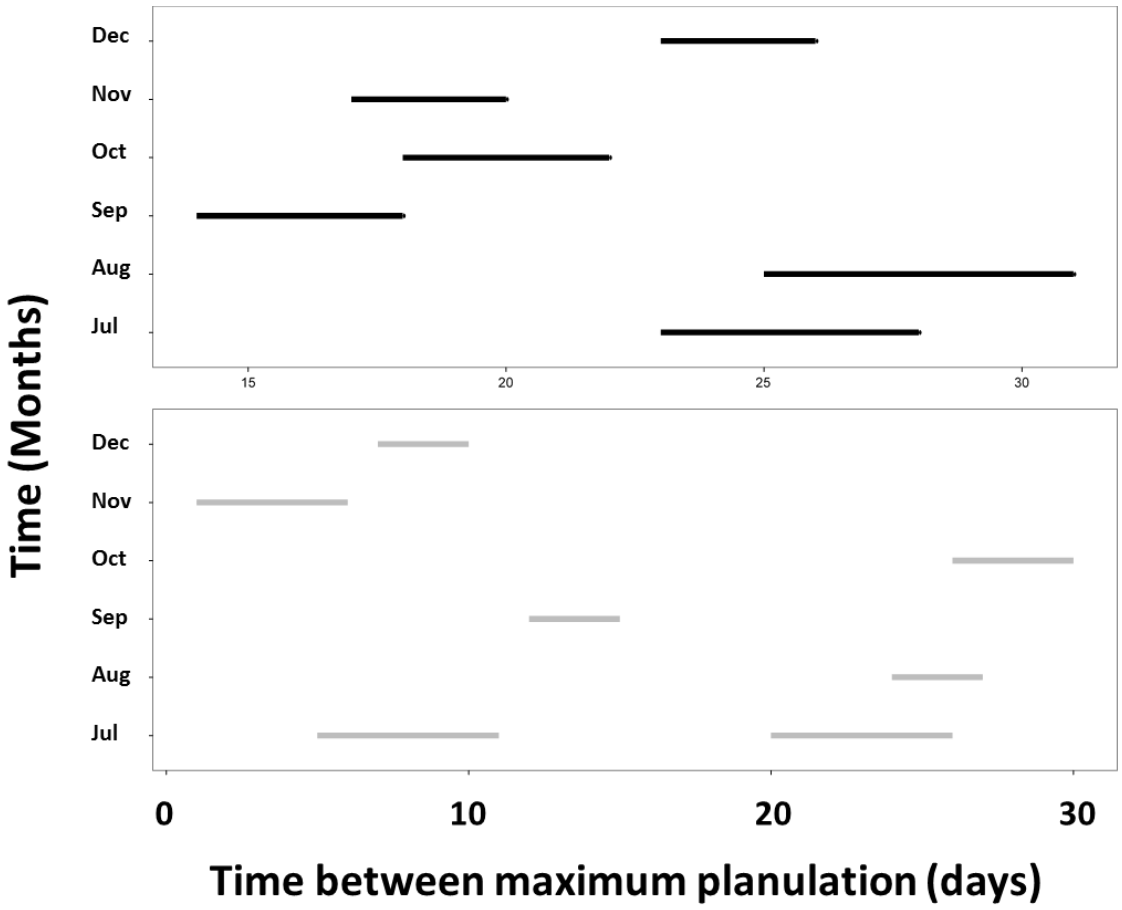

Figure 10. Timeline representing periods of maximum planulation across one month in corals under CNM (black) or lunar light (grey), lines represent the days of peak larval release, gaps indicate time periods between peak planulation events.

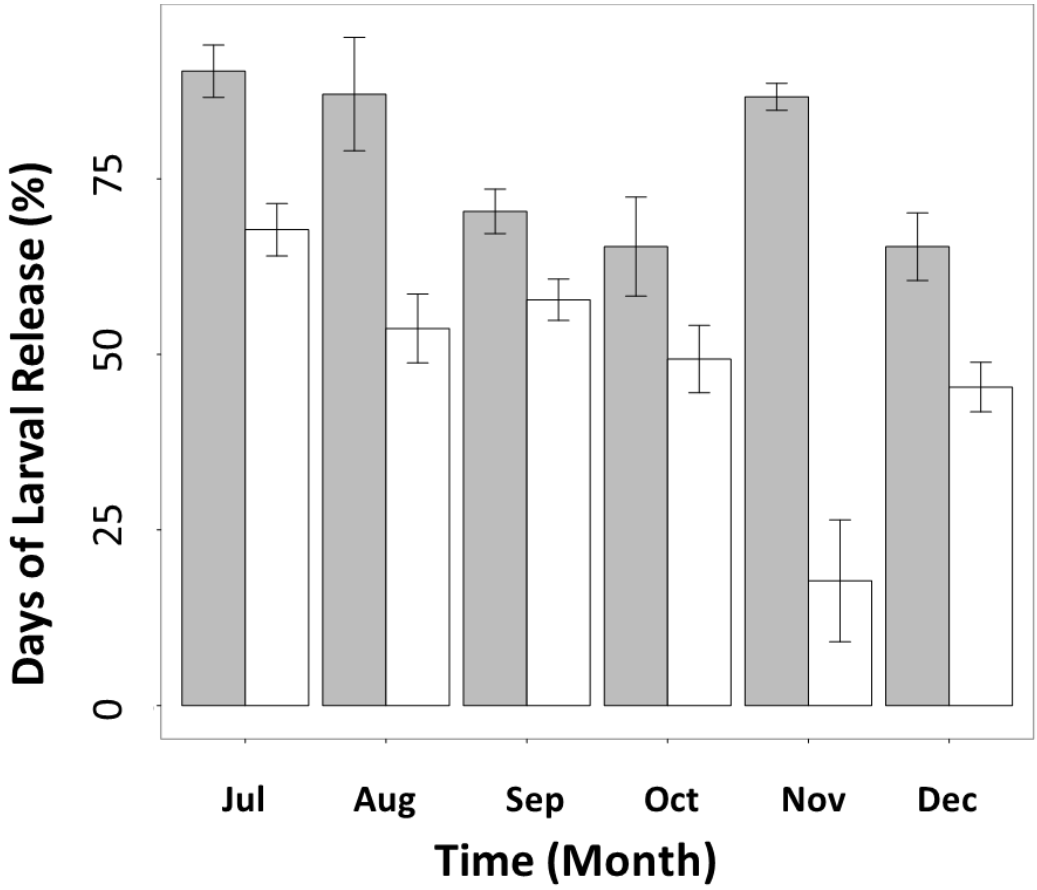

Figure 11. Percentage of days' corals released larvae per month in either CNM (grey) or lunar light (white), means ± SE presented, n = 3.

3.2.2 Synchronisation with lunar phases

Synchrony in larval release among planulating colonies under lunar influence was not associated with any lunar phase, the timing of peaked planulation events varied between the months and no significant relationship was found between maximal larval output and lunar phase. Similarly, there no significant relationship between larval production and lunar phases for corals subjected to the CNM treatment.

Despite no significant differences (largely due to variability), in both treatments average larval release was higher at full moon (CNM: 201 ± 117, Lunar: 89 ± 65 colony^{-1} day^{-1}) than new moon (CNM: 123 ± 44, Lunar: 66 ± 44 colony^{-1} day^{-1}).

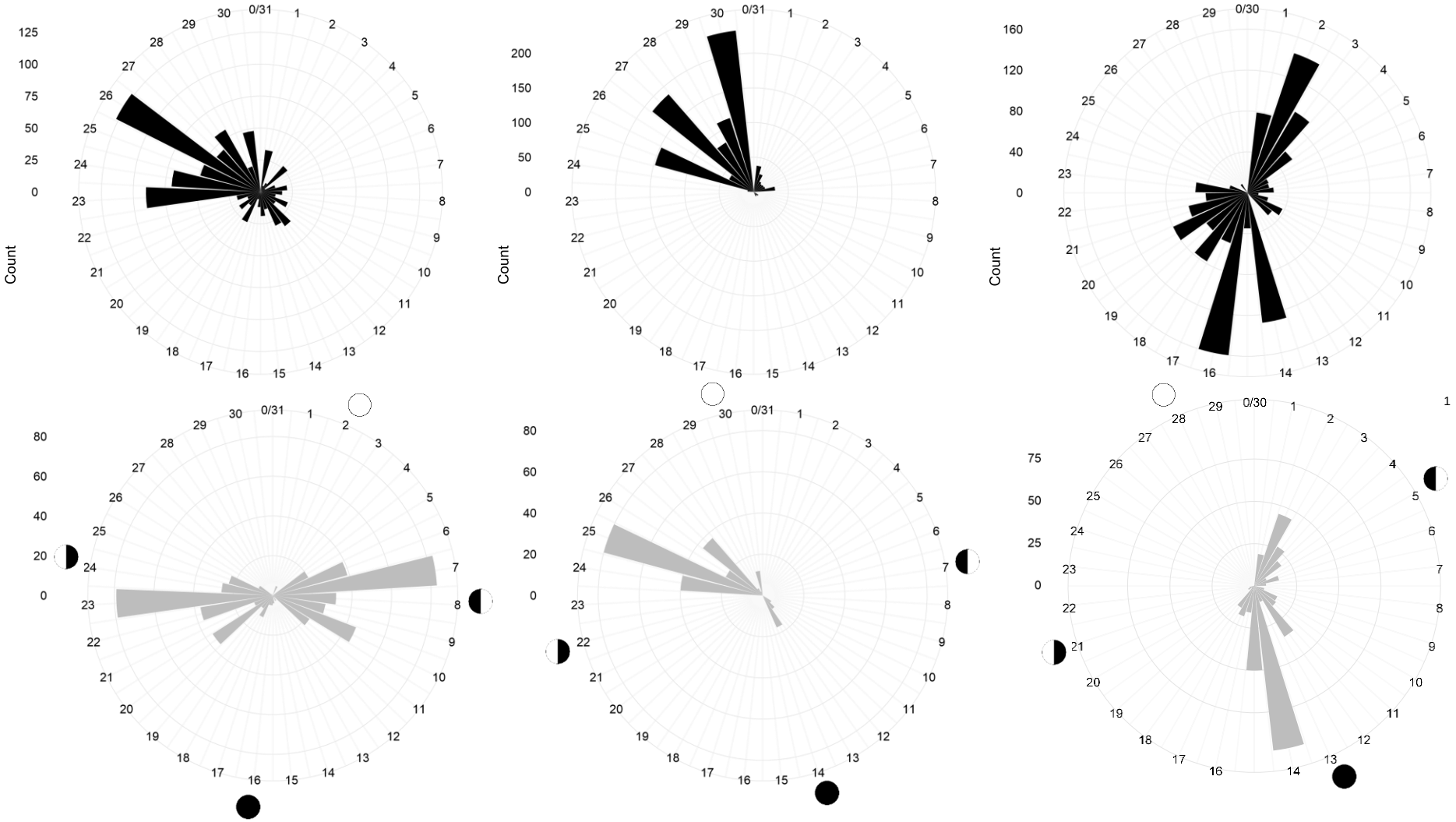

Figure 12a. Patterns of planulation periodicity in F. fragum *under CNM (black) and lunar (grey), showing patterns of larvae released in each treatment group (n = 3) observed over 3 lunar cycles (from left to right: Jul, Aug, Sep) where numbers around the circumference represent lunar days (July full moon= day 2 + 29.53 days thereafter), small circles on outskirts of circumference represent phases of moon (Clockwise from white filled circle: Full Moon, Third Quarter, New Moon, First Quarter), inner circles represent the scale as indicated by scale bars for each month & treatment.*

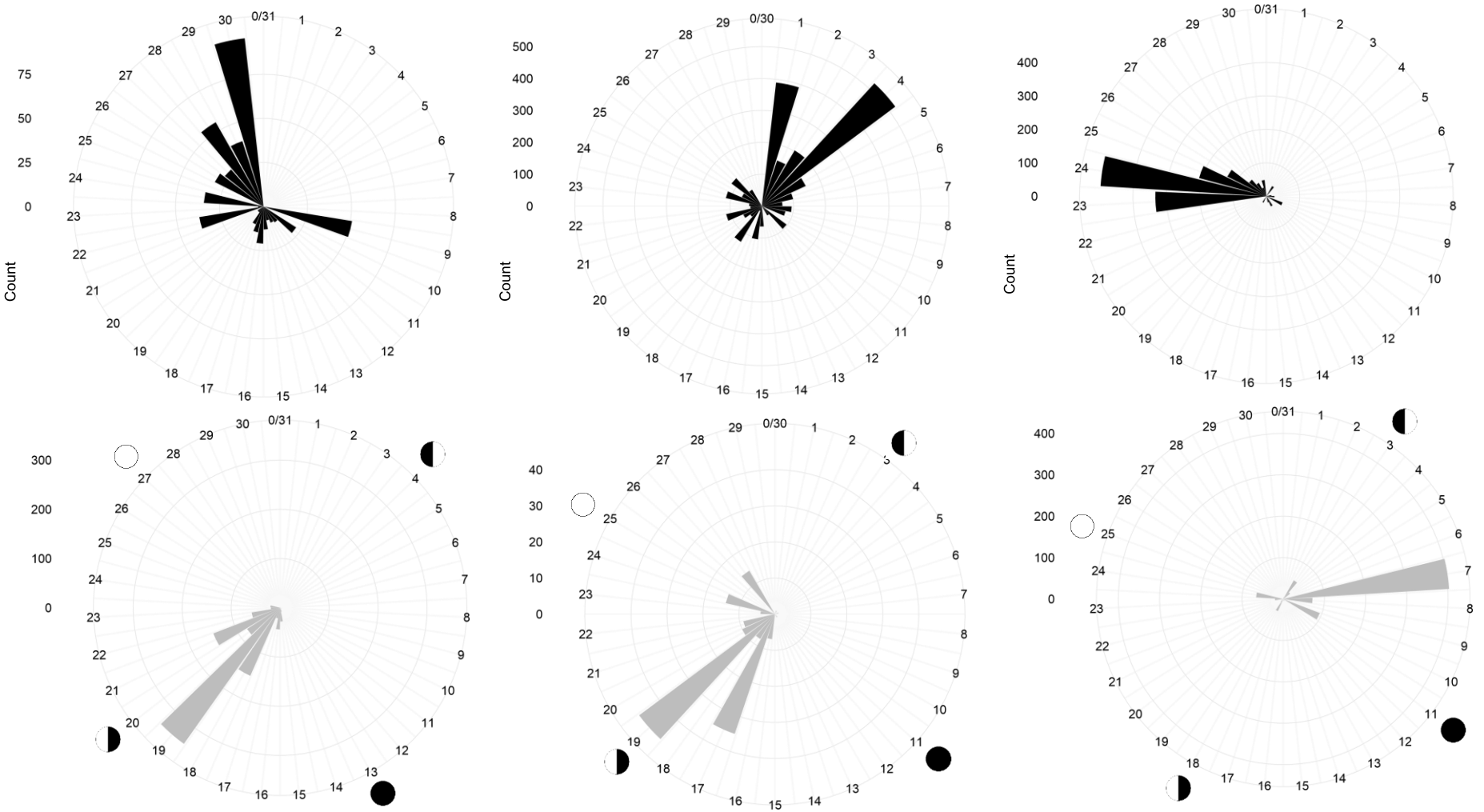

Figure 12b. Patterns of planulation periodicity in F. fragum *under CNM (black) and lunar (grey) (n = 3), showing pattern of larvae released in each treatment group observed over 3 lunar cycles (from left to right: Oct^{s}, Nov, Dec^{s}, s = corals switched between treatments) where numbers around the circumference represent lunar days (July full moon= day 2 + 29.53 days thereafter), small circles on outskirts of circumference represent phases of moon (Clockwise from white filled circle: Full Moon, Third Quarter, New Moon, First Quarter), inner circles represent the scale as indicated by scale bars for each month & treatment.*

3.3 Variations in larval sizes

3.3.1 Larval size differences between and within treatment groups

In addition to significant differences in larval output between the two treatment groups, significant differences occurred between the average size of larvae produced under different treatments (Welsh t- test: $t(1250) = 26.895$, $p < 0.001$). Larval sizes averaged across 6 lunar cycles were significantly larger (ca. 32%) in corals subjected to lunar light compared to CNM, (CNM: 0.67 ± 0.03 mm (mean ± SE), n = 586, Lunar: 0.89 ± 0.01 mm, n = 537 respectively).

Despite some variation in larval size between daily planulation events, mean larval size did not differ across the months that corals were subjected to CNM nor did they differ over time when corals were subjected to lunar treatment. However, the range in sizes was much more apparent in CNM corals (see Figure 13: ranging from 0.1 to 0.99 mm). Changes in mean larval size were immediately evident when corals were switched from CNM to lunar. Average size measurements increased by 2-folds in the first month (from 0.46 ± 0.03 to 0.86 ± 0.03 mm) and 3-folds in the second month. Similarly, corals transferred from lunar to CNM exhibited a decrease in mean larval sizes (from 0.98 ± 0.01 to 0.65 ± 0.02 mm).

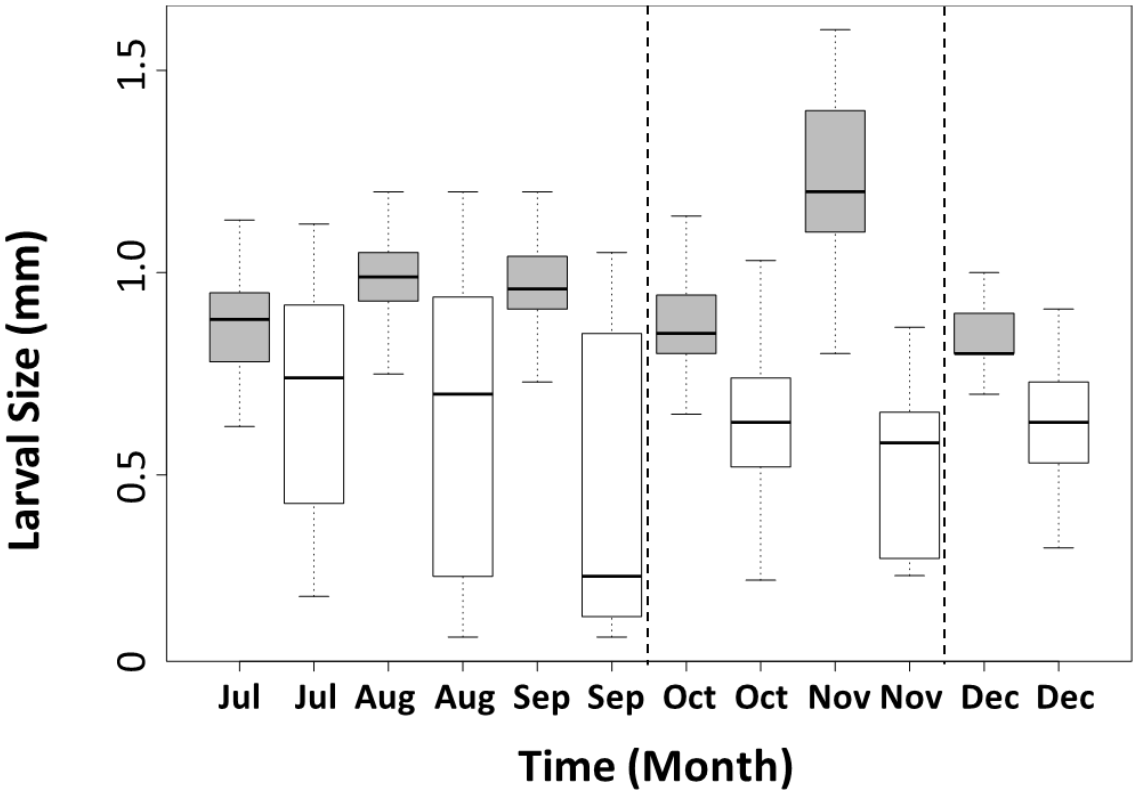

Figure 13. Distribution of larval sizes within and between corals under CNM (white) or lunar (grey), dotted lines indicate where colonies were swapped between treatments.

3.3.2 Size variance between lunar phases

No significant differences in larval size were found between phases, but mean larval size was lowest at full moon and was 40% higher at new moon (0.72 ± 0.01 and 1.01 ± 0.02 respectively). Similarly, mean larval size of CNM corals did not significantly differ between the phases.

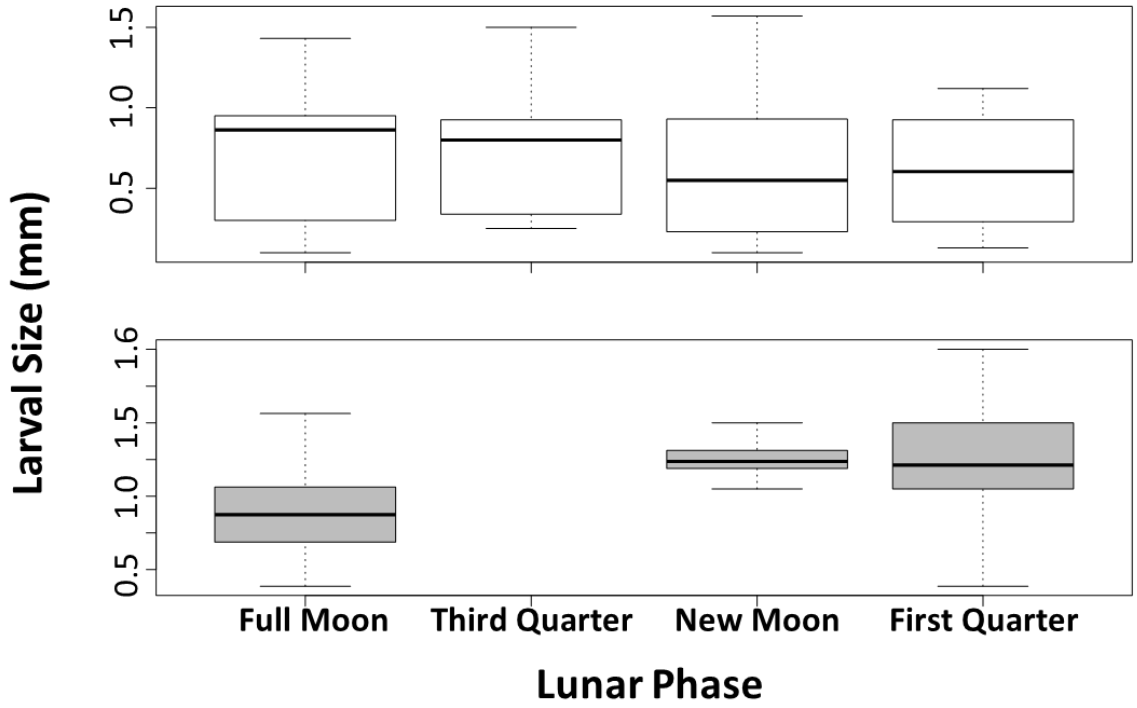

Figure 14. Size distribution of larvae produced at different phases of the moon (+/-3 days) between larvae produced by corals under CNM (top- white) and lunar (bottom- grey).

3.4 Larval settlement between treatments

3.4.1 Settlement under different light treatments

Analysis indicated no significant differences when comparing larval settlement between the two treatments due to the high variability in CNM corals however, the percentage settled within the first 12 hours is almost double in lunar corals when compared to CNM corals. Between the time periods settlement of larvae released under lunar light was significantly higher within 24 hours of release (Kruskal- Wallis: df = 6, $p < 0.05$), 60 ± 7% (mean ± SE) within 12 hours, 30 ± 4% within 24 hours. In comparison, the time periods taken for larvae produced under CNM to settle was much more variable, although a significant proportion also settled within the first 24 hours of release (df = 6, $p < 0.05$), 33 ± 4% within 12h, 38 ± 4% within 24h respectively.

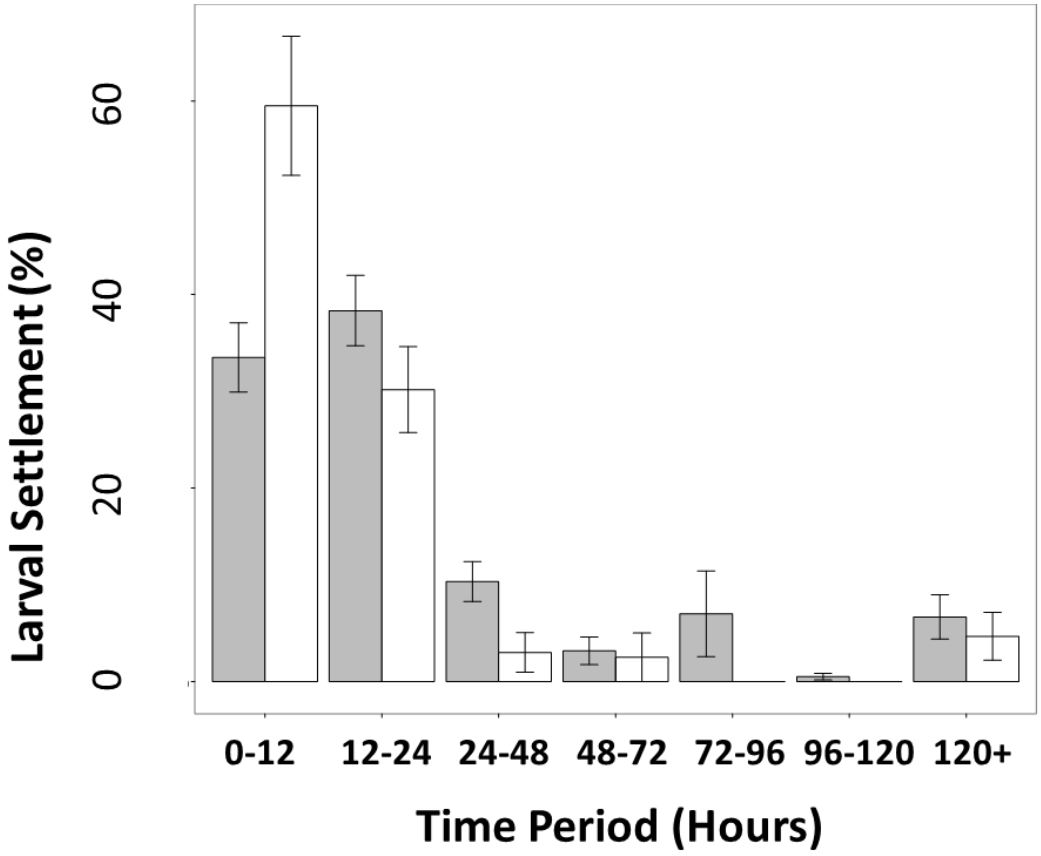

Figure 15. Percentage settlement over 6+ days between larvae released via corals under lunar (white) or CNM (grey) conditions, means ± SE presented, n = 20.

3.4.2 The effects of larval size on rates of settlement

Significant differences were found when comparing larval settlement between small (>0.6mm) and large (<0.6mm) larvae (Kruskal-Wallis: df = 6, $p < 0.05$). ca. 93% of large larvae settled within 12 to 24 hours of release, whereas small larvae had extended planktonic periods, typically over 6+ days. Only 14 ± 3% of small larvae had settled after 12 hours of release and 64 ± 10% of larvae remained unsettled 6 days post- release, some small larvae remained unsettled for > 30 days.

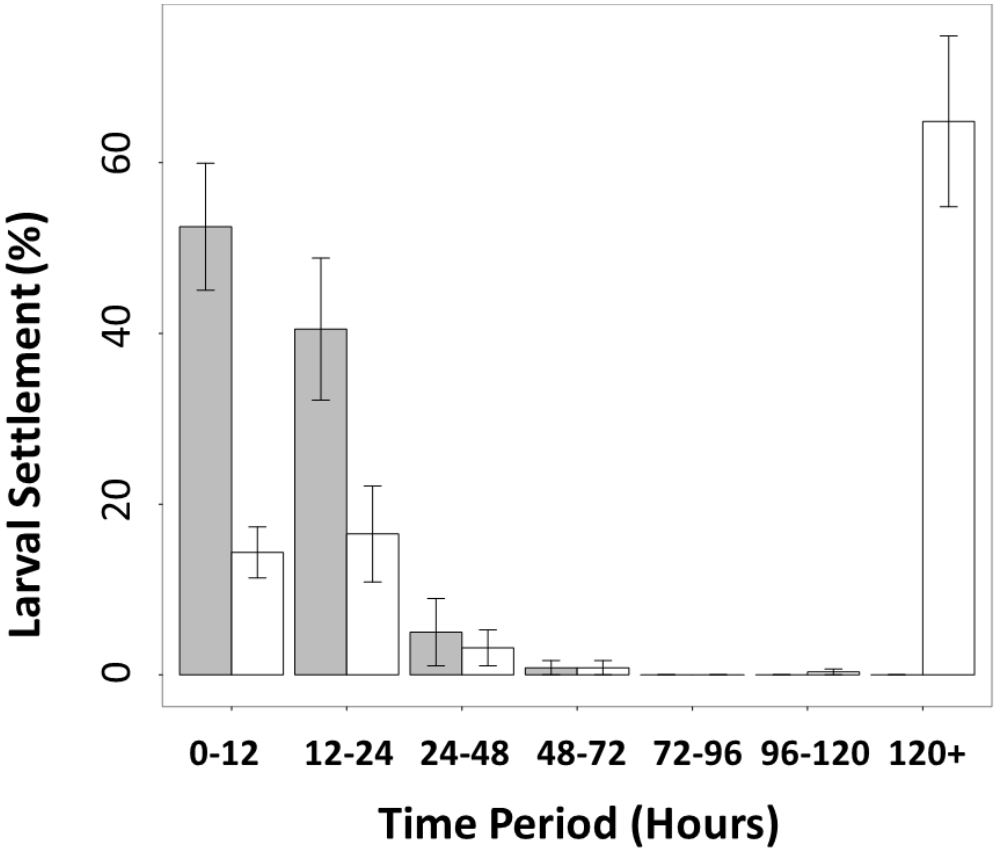

Figure 16. Percentage of larvae settled over 6+ days between two different sizes large (grey, >0.6 mm) and small (white, <0.6 mm), means ± SE presented.

3.5 Metabolic rate between treatments

3.5.1 Patterns of metabolic rate under different treatments

Previous results indicated substantial differences in larval numbers released between corals under alternate nocturnal light regimes, therefore comparative analysis was performed to identify differences in metabolic rate between the two treatment groups. Results from an independent t- test identified significant differences between the metabolic rate of corals under lunar stimulation and those under CNM ($t(168) = 3.326$, $p < 0.05$).

Metabolic rate showed a steady sequential increase in corals (Figure 17) under CNM exhibiting a 2-fold average increase from July to September (0.057 ± 0.01 to 0.11 ± 0.01 mg O_2 g^{-1} h^{-1}, mean ± SE), larval output also increased by 1.4% (July: 304 ± 114, September: 424 ± 215 larvae $colony^{-1}$ $month^{-1}$). The metabolic rate of lunar exposed corals fluctuated between the months, decreasing from July to August before inclining to reach their highest average rates in the final month (0.08 ± 0.01 mg O_2 g^{-1} h^{-1}). Larval counts also fluctuated, declining in August and with only a slight increase in September (see Figure 8). Significant changes were identified between months in corals under lunar light (Kruskal- Wallis, df = 2, $p < 0.05$), but post-hoc pairwise comparisons suggested the significance lied only between August and September (Dunn's test, $p < 0.05$). Differences also occurred in corals in CNM treatment (ANOVA, df = 2, f = 6.824, $p < 0.05$), further analysis revealed significance was only between September and July (Tukey's test, $p < 0.05$).

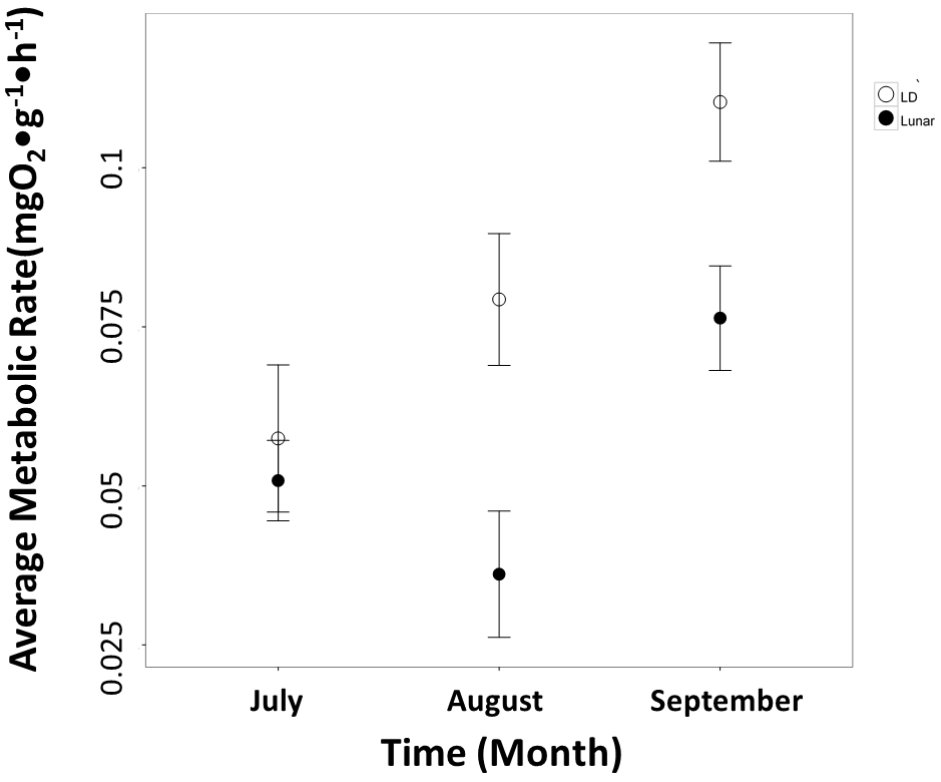

Figure 17. Comparisons between the metabolic rate of corals under lunar simulation (black circles) with corals under constant new moon (white circles) over a 3- month period, means ± SE presented, n = 2.

3.5.2 Correlation between larval production and metabolic rate

To assess whether increases in metabolic rate were associated with increases in larval production, rank correlation tests were performed. Results concluded opposite correlative patterns between the two treatment groups. A positive correlation with metabolism (r_s = 0.399, df = 82, $p < 0.05$) occurred in corals under CNM however, a negative correlation was identified in corals under lunar simulation (r_s = -0.252, df = 82, $p < 0.05$).

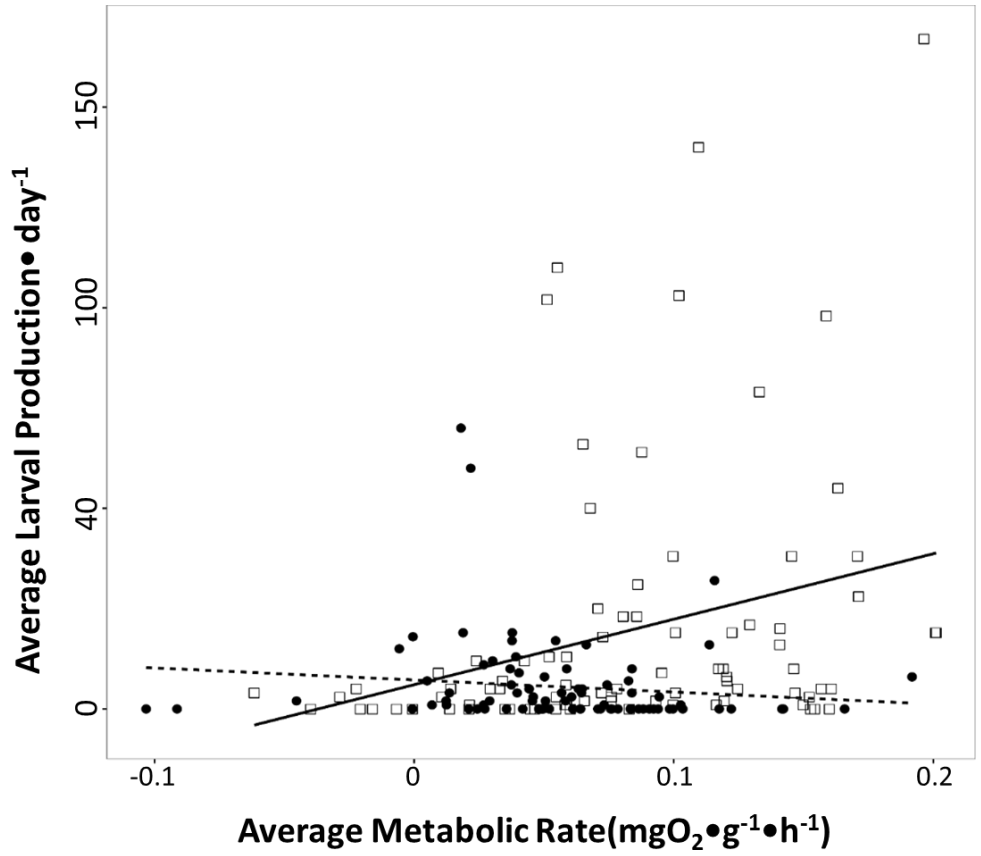

Figure 18. Correlation of average daily larval counts against metabolic rates for each treatment group, lunar (black circles, dotted line) and CNM (white squares, solid line) over 3 months.

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Both groups had similar metabolic rates in July despite CNM corals releasing almost double the number of larvae than lunar corals (304 ± 114 to 177 ± 45 larvae colony^{-1} month^{-1}), suggesting other factors may have interfered with metabolism. A threshold may exist on the number of larvae

needed to be produced before energy supplies are depleted enough to cause a noticeable effect on metabolism, as evidence by an increase in metabolic rate in synchronicity with further increases in larval output.

3.6 Metamorphic and post- settlement development in **Favia fragum**

3.6.1 Using LSFM to develop a timeline of metamorphic stages

Upon deciding on their final settlement site, coral larvae invert themselves to a position where their aboral axis is in full contact with the substrate and the oral axis extended towards the water column in a typical 'bowling pin' shape (Figure 19 1hps). This positioning prepares larvae for the onset of extensive tissue reshuffling and the formation of the primary polyp.

The first initial days of metamorphism appear to indicate the largest physical changes. Within the first day of settlement six primary mesenteries (vertical partitions) become visible (Figure 19 1dps), the mesenteries are the primary gastrodermal structures comprising the gastrodermis and mesoglea and are responsible for the aptly termed "pumpkin stage" as described by Hirose et al (2008). Secondary mesenteries form between each individual primary mesentery (Figure 19 2dps) subsequent to the organisation of gastrodermal cells. Although present from the start, the oral cavity increases in circumference and becomes more apparent throughout post- settlement. The formation of tentacular bumps at the top of each mesentery become detectable within 3 days of metamorphism (Figure 19 3dps), these form a circle surrounding the oral cavity and continue to develop until full tentacular extension is visible 6/7days post- settlement (Figure 19 7dps). Zooxanthellae are visible in every stage of the metamorphic process due to maternal infection pre- larval release and potential acquisition during the planktonic period (Hagedorn et al., 2015). Distribution of zooxanthellae appears even throughout the mesenteries and around the oral cavity (Figure 19 1-3dps) and is concentrated within the tentacles as they develop (Figure 19 6-7dps).

Larvae began to secrete a calcium carbonate exoskeleton within the first few days of settlement (Figure 19 1dps, 2dps). The exoskeleton is often termed the corallite consists of four fundamental components: basal plate, epitheca, septa and dissepiments (Todd, 2008). The septa grow upwards from the basal plate in multiples of six and are identifiable as the blade- like vertical panels that separate the mesenteries (Figure 19 1dps).

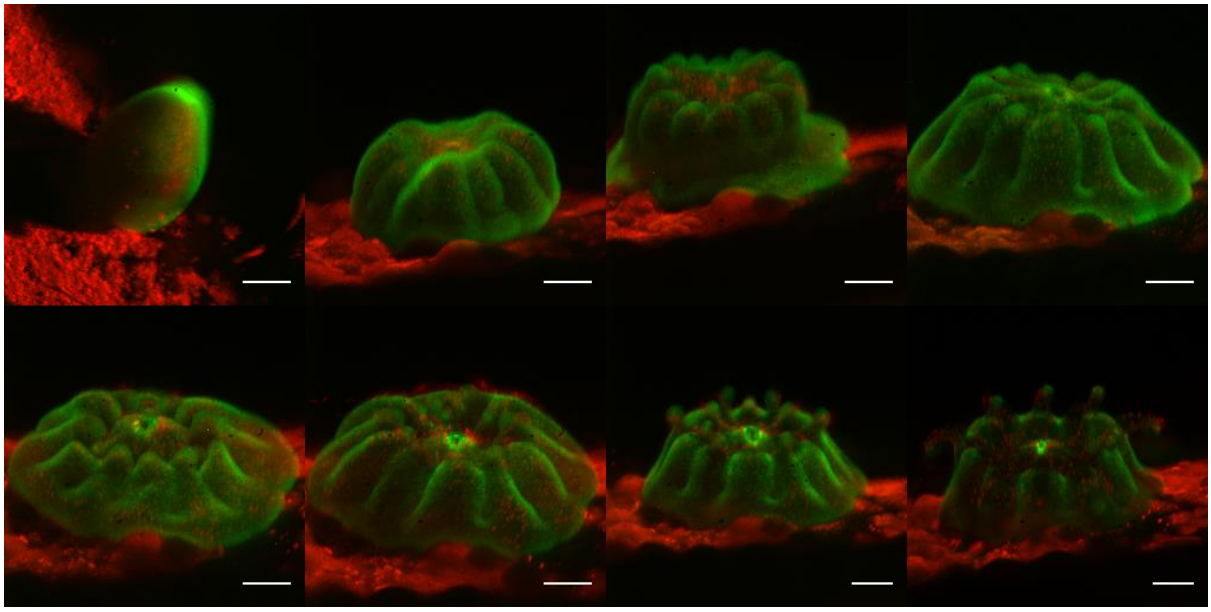

Figure 19. Transition from larva to primary polyp through metamorphic development stages in Favia fragum, *from 1 – 7 days post- settlement (dps), imaged using LSFM. GFP (green) and zooxanthellae (red) distribution. Scale bar 0.25mm.*

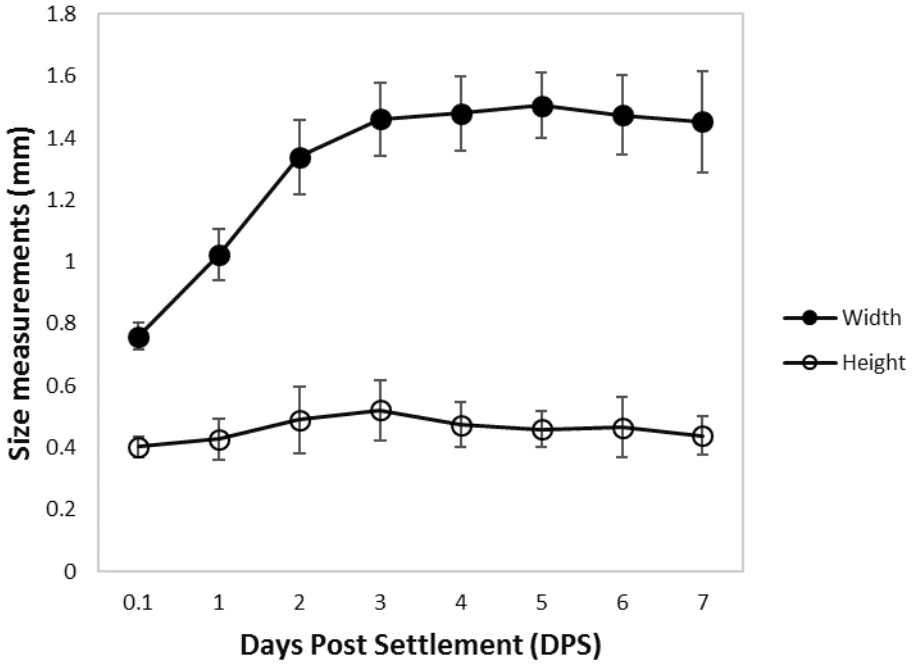

Figure 20. Average height and width measurements during 7 days of metamorphic development, Width measured across base of polyp, height measured from base to top of mesentery.

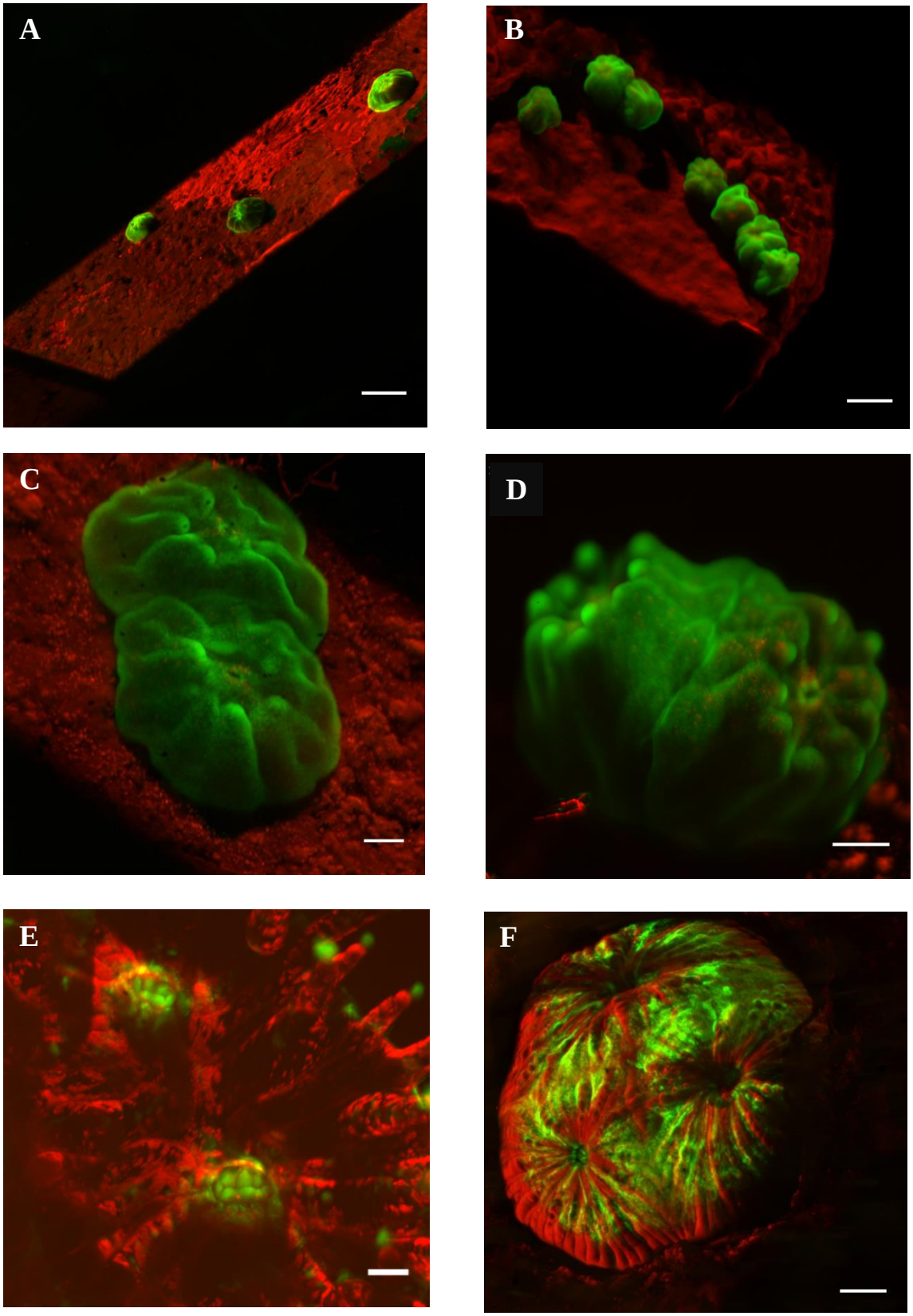

Figure 21. Both Isolated settlement (A) v aggregated settlement (B) behaviour, new recruits settled in close proximity to conspecifics (C) and in some cases led to allogeneic fusion to form a new novel entity (D). Primary polyps were dividing within 3 months of settlement (E), observed visually with the development of two oral cavities (F). All images taken using LSFM, Scale: 0.25mm

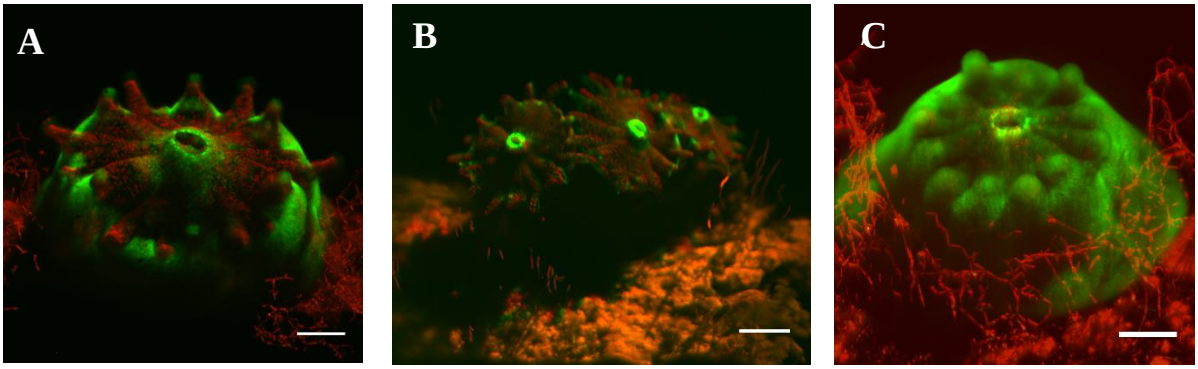

Figure 22. Newly settled polyp pre- (A) and post- (B) subjection to high nitrates early on in their development causing a rapid increase in internal Symbiodinium density, and temporary concentration of GFP abundance around the oral cavity during the stress period. Isolated primary polyp surrounded by algal mat within a days of settlement. Scale:0.25 mm.

Fluorescent patterning

During the early stages through metamorphosis fluorescence was uniform with equal distribution of GFP's across the polyp. Colony edges where the calcium carbonate skeleton is continuously laid down contained high densities of FPs as well as the tentacle tips and around the oral disk which upon retraction are suggested to form a "sun-screening polyp plug". Further development and asexual division resulted in complementery fluorescences with concentrated patterns of GFP's around specific anatomical features including the oral cavity and between the mesenteries (Figure 21 E and F).

Behavioural settlement patterns

Larvae often aggregated together in close proximity to conspecifics (Figure 21 B), isolated settlement was rare. Larvae were also observed settling in direct contact to others and in some cases led to fusion to form a single entity (Figure 21 C and D).

Over a 6 month period < 1% of settled larvae survived and began to divide to form a colony of interconnecting polyps, irrespective of size or treatment. Most settled recruits died within 14 days of settling as a result of overgrowth from surround algae (Figure 22 C). A 7 day period of nitrate contamination (ca. 80 ppm) led to visible colouration darkening indicating rapid increases in internal zooxanthellae densities (Figure 22 A and B) followed by mass mortality.

4.0 DISCUSSION

4.1 Larval output in **Favia fragum** *with and without lunar cues*

The inability to detect lunar cues is suggested to cause serious disruption to coral reproductive patterns (Kaniewska et al., 2015). Larval output in *Favia fragum* under CNM (absent of lunar cues) was significantly higher than production in the presence of lunar cues, contradicting the predicted reduction in larval output driven by the loss of lunar stimulus (cue). However, larvae were released on an almost daily occurrence over 3 monthly reproductive cycles under CNM indicated complete loss of synchronisation to lunar periodicity. Differences in larval output between the two treatments support the strategies of bet-hedging; when the environment in which offspring are released cannot be anticipated within-clutch variation increases. In this study, corals increased the number of days larvae were released as a strategy to increase reproductive success. Most brooding corals time their larval production by some phase of the lunar cycle (Stephenson, 1933). The degree of synchronicity is not synonymous among species, and the scale of synchronicity appears to vary from a) no pattern (Harriott, 1983) to b) weak periodicity with low continuous levels of larval release and a monthly peak (Fan et al., 2002) and to c) tight synchrony with peak production occurring over just a few days each month (Johnson, 1992). Light is depicted as the overarching cue for regulating cyclic reproduction events in both broadcast and brooding species and maintaining the internal circadian rhythm (Boch et al., 2011; Kaniewska et al., 2015).

Previous studies simulating specific lunar phases over extended periods noted shifts in spawning and planulation timing (eg Jokiel et al., 1985; Hunter, 1988). Jokiel et al (1985) noted a complete loss in synchronicity when colonies of *Pocillopora damicornis* were maintained under CNM and under constant full moon. Synonymous with this study, planulae were continuously released. Others suggest planulation patterns in brooding corals may be indirectly dependent on lunar phase by driving gametogenesis and fertilisation as opposed to a direct cue for larval release (Szmant- Froelich et al., 1985; Fan et al., 2002; Zakai et al., 2006). This may explain why larval release is dispersed over

the month rather than the tight synchronization (ie over a few days), observed in broadcast spawners (Harrison and Wallace, 1990).

Over three lunar cycles (Jul- Sep), CNM corals released larvae almost daily, although number released fluctuated. Continuous larval release throughout the month in the absence of environmental rhythms may be achievable if conditions remain stable and optimal for reproduction. In equatorial regions it is suggested that marine organisms have protracted reproductive periods (Pearse, 1974). Synchronicity and seasonal spawning deteriorates as a result of reduced variability in environmental conditions, including temperature and tidal cycles that are closely associated with influencing the onset of spawning events. In Kenya broadcast spawning species of *Acropora* and Faviids have extended spawning seasons and release gametes over 5 to 7 months. Proximate cues were not detected as spawning occurred regardless of lunar phases, during spring and neap tides and across several temperature regimes (Mangubhai and Harrison, 2009). Within our study laboratory conditions were stabile for the entire duration of the experiment, reflecting similar conditions as those reported by Mangubhai and Harrison (2009) but very different to those experienced by our test species *F fragum in situ*.

4.1.1 The effect of reproductive mode on reproductive patterns

Continued reproduction in the absence of environmental stimuli may be attributed to the type of reproductive mode exhibited by *F. fragum*. Broadcasting spawners appear to exhibit a higher specificity for environmental conditions to induce the production of gametes in comparison to brooding species, likely due to their limited frequency and short spawning periods (1-2 per annum over 1-7 days, see Harrison, 2011). Brooding species appear to have a reduced specificity for environmental conditions that cue the production of larvae, possibly due to the characteristics associated with brooding that allow for flexibility such as internal fertilisation and vertical transmission of symbionts.

In addition to mode, the production of larvae via asexual reproduction may have also influenced the fecundity of *F. fragum* under CNM and contributed to the resultant daily release of planulae observed in this study in the absence of lunar cues. Most corals reproduce asexually and sexually (Harrison, 2011) however, the factors driving each type are not well defined. Production of asexually derived larvae has previously been documented in *F. fragum*, Brazeau et al (1998) noted a 34% self- fertilisation rate; later attributed to isolated geographic reef locations and turbid habitat conditions reducing the ability to detect lunar cues (Goodbody- Gringley et al, 2010). Gleason et al (2001) also noted *F. fragum* exhibited high rates of parthenogenesis under natural conditions. The evolutionary advantages of parthenogenesis are unknown, thus the drivers that promote the production of genetically identical larvae are also unknown. Microsatellite genotyping in *P. damicornis* indicated ca. 87% of larvae were produced parthenogenetically, irrespective of parental genotype, habitat or lunar day (Combosch and Vollmer, 2013). Few studies have assessed the factors driving the production of parthenogenetic larvae that is also unknown whether the laboratory aquaria conditions where favourable for asexual or sexual reproduction. Larvae produced in this study were not genotyped therefore, whether larvae were produced parthenogenetically could not be assessed nor whether the conditions within the aquaria promoted sexual or asexual reproduction. Furthermore, although parthenogenesis may allow for continuous larval release and the production of genetically identical larvae, corals under CNM still produced large variations in larval phenotype with potential implications on dispersal and developmental processes.

4.2 Production synchronicity with lunar patterns

Corals under lunar light produced lower numbers of larvae per month and peak production occurred 3-6 days (per month). Brooding corals, including *Favia fragum*, pre-set their patterns of planulae release based on predictable periods of the lunar cycle. Szmant- Froelich (1985) was the first to describe continual monthly planulation throughout the year in *F. fragum*. Studies on the production rates of *F. fragum* are generally concordant with slight variation dependent on location within Atlantic

waters. In Puerto Rico (Goodbody-Gringley and Putron, 2009) and the US Virgin Islands (Carlon, 2002) planulation occurred throughout the year with peak production 8-11 days after new moon (ANM). In Bermuda planulae were produced 3-4 days post ANM continuing for 16-19 days with peak rates between 6 and 12 days (Goodbody- Gringley and Putron, 2009) but planulation ceased during winter months between January and April.

Factors driving corals to fine-tune release timings to a specific month, day and time are still under investigations. Previous explanations include reduced predation rates; this is often associated with brooding corals that release large conspicuous planulae and are thus more susceptible (Fabricius and Metzner, 2004). Close to new moon lunar illumination is at its lowest for predators that rely on visual detection (Morgan, 1995), hence the risk to larval mortality is reduced. Tidal cycles are closely coupled with the lunar cycle; varying tidal phases have huge implications on the dispersal potential of newly released gametes/ larvae. Spring tides promote long- distance dispersal whereas neap tides facilitate local retention (Goodbody- Gringley and Putron, 2009). In broadcast spawning corals the ultimate environmental driver is assumed to be related to optimising external fertilization of gametes released into the water column. Brooding species secure successful fertilisation before releasing larvae thus the factors driving planulation patterns are less clear cut. However, most studies illustrate peaked production in *F. fragum* occurs 1-2 weeks ANM during the neap tide facilitating local retention (Carlon, 2002, Goodbody-Gringley and Putron, 2009). Transference from CNM to lunar caused corals to tighten larval release and concentrate mass larval output to a few days suggesting advantages must be present to drive synchronicity between larval release and lunar periodicity.

Corals under lunar cues concentrated larval release to just a few days within the lunar cycle exhibiting some form of synchronicity. However, larvae were also released sporadically at lower numbers throughout the month and the timing of peaked planulation differentiated each month and was not associated to a single lunar phase. In the field, *F. fragum* has been found to release larvae all year round (Szmant- Froelich et al., 1985) despite experiencing seasonal and diurnal fluctuations (Goodbody- Gringley and Putron, 2009) therefore, continuous larval release may be a species specific

trait with reduced sensitivity to environmental cues and is enhanced under stable optimal conditions such as those in the aquaria in this study.

In this study, in the absence of cues the cyclic release of larvae weakened and became asynchronous. In their "free-running" state *F. fragum* exhibited a lottery mechanism releasing larvae continually throughout the month. On numerous occasions, corals were observed releasing larvae during daylight hours; predominantly when corals were placed within the respiration chambers and subjected to 30 minutes of darkness. Short exposure to darkness induced corals to expand their tentacles facilitating sporadic larval release however, only a few were released and no previous evidence of larval production during daylight hours has been observed suggesting this is not likely replicated in natural reefs. In addition to irregular time periods of planulation, corals were also observed expanding their tentacles in response to food addition during the day. These behavioural events suggest corals are able to deviate from their natural rhythm and acclimate to a new photocycle.

Manipulating daily light photoperiods resulted in altered spawning release times in broadcasting species (Brady et al., 2009). Previous studies mimicking phases of the lunar cycle and prolonging the period in which they would naturally occur also demonstrated a shift in the spawning/ planulation times (Jokiel et al., 1985). Others have proposed that the time of gamete/ larval release is a direct response to light as opposed to regulated by an internal circadian clock (Brady et al., 2009). This study partly supported the results of Brady et al (2009) as corals maintained under a simulated lunar cycle expressed stronger larval release patterns confined over a few days of the month and could fine-tune release timings on a daily scale. However, *F. fragum* can maintain planulation over a 7-month period in the absence of lunar stimuli indicating larval release is not an obligatory response to lunar light.

4.3 Fluctuating planulation patterns under constant new moon

Lowest larval release occurred during June coinciding with a complete breakdown of circadian rhythmicity. Continual declines in reproductive output and weakened circadian rhythms during the first 4 months (Mar- Jun) were synonymous across all six colonies and initially attributed to the lack of lunar cues. As anticipated after 3 months under CNM, introduction of mimicked lunar phases triggered immediate larval release in corals placed under the new treatment, suggesting *F. fragum* are obligated to rely on lunar stimulation to initiate larval release. However, corals that remained under CNM also began to release large amounts of larvae despite continual deprivation of lunar cues for a further 3 months suggesting other factors were likely responsible for the initial 3-month decline.

4.3.1 Influence of light pollution in reproduction

Several factors were highlighted as potential artificial influences on the variation in planulation patterns observed between the first 3 months and the months that followed. Although significant steps were taken to ensure that conditions were maintained consistently over the length of the study, there were multiple limitations of the experimental set-up in this initial phase. Light pollution from background sources was a major concern, the position of the tank within the aquarium exposed it to several external light sources including a 24- hour security light located directly above the tank. In addition, artificial light from adjacent laboratories did not consistently match the photoperiod of the tank lights and could have caused significant light pollution during the initial 4-month period. However, early implications of external light interference were detected within the first experimental weeks so and measures were taken to minimise possible light contamination and maintain irradiance levels at 0 µmol photons m^{-2} s^{-1} over 12 hours. Previous studies that examined

implications of prolonged night time cloud cover on larval production as a cause of changed periodicity (Jokiel et al., 1985). A recent study suggested artificial skyglow (light pollution from urbanised areas) may interfere with synchronized spawning events on natural reef systems by masking lunar cues (Aubrecht et al., 2008). Davies et al (2014) suggested coral larvae became disorientated under light treatments mimicking skyglow and selected sub-optimal settlement sites. In addition, Kaniewska et al (2015) exposed *Acorpora millepora* to light conditions mimicking urban light pollution and found disruptions to cellular signalling processes which in turn prevented coral spawning.

Although light pollution may cause serious interruptions to synchronisation patterns in broadcast spawners, there is a lack of evidence to suggest larval output would endure the same level of disruption in brooders.

4.3.2 Influence of high nitrates on reproduction

A rapid decline in larval release was observed in March with numbers remaining very low over the following 3 months. Regular water testing carried out during mid- March noted higher nitrate levels than had previously been recorded (ca. 20 ppm), measuring ca. 80 ppm. Although this value is only an approximate concentration (API test kits used are limited to colour scales), concentrations were substantially greater than would naturally occur in reef systems in the Caribbean (see Bruno et al., 2003) and high enough to significantly interrupt reproduction. Cessation of planulae production occurred in parallel to elevated nitrate levels, signs of reproductive recovery were not observed until 4 weeks following the return to previous nitrate concentrations (ca. 20 ppm). During the month following the nitrate event (April) planulation occurred over just 4 days; the total number of larvae produced post- nitrate episode was 4-fold lower than the amount produced pre- high nitrates.

Previous studies illustrated prolonged exposure to elevated nitrate levels (20 ppm) resulted in the production of smaller and fewer eggs in both *Acropora longicyathus* and *A. aspera* (Ward and

Harrison, 2000). In polluted water *Porites porites* was found to release fewer larvae and the male: female colony sex ratio was skewed 2:1 (Harrison and Ward, 2001). Others observed reduced egg size in *Montipora annularis* and failed planulation in *P. damicornis* after 4 months of exposure to increased ammonia levels (Cox and Ward, 2002). In this study nitrate contamination was detected early and *F. fragum* were subjected to short exposure of high nitrates (ca. 7 days) before returning to normal levels (20 ppm) via a series of 10% water changes to enable corals to re-adjust their physiology and prevent a significant large drop in nitrate concentration which may cause further stress to corals.

Phosphate and nitrogen are key ingredients in many biological molecules including DNA, RNA and proteins (Hylkemaa et al., 2015). In oligotrophic waters these nutrients are universally low, concentrations of phosphate and inorganic forms of nitrogen (ammonia and nitrate) typically fall well below 0.5 µm/L (Furnas, 1991). The establishment and preservation of coral reefs in nutrient poor conditions is accredited to the tight association between coral and endosymbiotic algae (LaJeunesse et al., 2010). The unique symbiosis has for years been modelled as the epitome of a shared beneficial partnership, both with equivalent gains with the coral host providing protection and compounds required for photosynthesis to *Symbiodinium* and in return photosynthetic products being translocated to coral tissue (Baker, 1999). However, emerging research suggests that the symbiosis is based on equal exploitation in which either side, contingent on specific environmental conditions, can switch from beneficial to parasitic (Wooldridge, 2014; Wooldridge, 2016).

An environment with enriched nitrates is suggested to tip the exploitation scale in favour of *Symbiodinium* with increased nutrient supply linked to enhanced photosynthetic rates, zooxanthellae density (Falkowski et al., 1993) and reduced calcification rates in the host coral (Marubini and Davies, 1996). No longer nutrient limited, zooxanthellae may retain photosynthates to invest in their own growth and development, reducing the amount of fixed carbon available to the coral host for biological processes including calcification and reproduction. Alternatively, corals and *Symbiodinium* use the same dissolved organic carbon sources (CO_2, HCO_3^-) for calcification and photosynthesis which may lead to competition between *Symbiodinium* and the host, in favour of *Symbiodinium* as

their densities increase (Marubini and Davies, 1996). Both theories result in an exponential growth in *Symbiodinium* above optimum population resulting in increased self- shading, light limitation and competition within and/or between *Symbiodinium* clades and negatively impacting photosynthetic rates. *In situ* and laboratory experiments have illustrated unanimous results, all of which concluded adverse consequences for corals when nutrient concentrations are increased (Muscatine et al., 1989; Dubinksy and Jokiel, 1994).

Early indication of nutrient stress was initially detected in other species housed in the same aquaria used for this study, *Pocillopora damicornis* fragments along with Acroporid species were among the first corals to exhibit physical signs of stress. *P. damicornis* polyps, which typically remain expanded during daylight hours, darkened in colour indicating a rapid increase in zooxanthellae density inside the coral host tissue. Weaker individuals began to initiate polyp bail- out, an extreme response strategy to stress with the motive to escape incongruent localised conditions and disperse to more hospitable locations (Gleason and Hofmann, 2011). Despite disruptions to larval production, no behavioural/ physical indication of stress was observed in colonies of *F. fragum*. These corals are typically categorized amongst the more robust scleractinian species due to their preferred location on the back reefs and reef flats (Szmant-Froelich et al., 1985) where corals are regularly exposed to turbid waters and experience the largest fluctuations in diurnal environmental conditions (Goodbody-Gringley and Putron, 2009). Thus, corals able to populate these areas have likely evolved a greater resistance to acute changes and can adjust their physiology and reproductive strategies to account for variations.

Nevertheless, coral reproduction has a low stress tolerance and is often the first process to cease upon exposure to unfavourable conditions and the last process to return following the return to stable environmental settings (Ward and Harrison, 2000). In a state of stress, corals switch to survival mode and re-direct energy resources away from reproduction to repair mechanisms (Harrison and Ward, 2001). Similar to results reported by Cox and Ward (2002) within this study failed planulation occurred and levels of larval release did not fully recover until 3 months following a return to environmental norms. The decline in larval output is therefore attributed to high nitrate levels possibly

combined with light pollution, which collectively acted as antagonistic factors destabilizing reproduction patterns by driving changes in resource partitioning and interfering with circadian rhythms respectively. These results, although very preliminary, may serve to further demonstrate the potential impacts of environmental degradation on coral resilience.

4.3.3 Influence of spectral composition on reproduction

Rapid inclines in reproductive output from all six corals were observed from June to July at which point corals were separated into the two treatments (lunar and CNM); larval release remained high during August and September. Within the first few days of July corals in both treatments began to release small amounts of larvae on a daily basis, corals under lunar exhibited peaked production rates 7 days ANM for 10 days. As anticipated, exposure to lunar induced larval production in corals however, factors influencing a rapid increase in larval output in corals remaining under CNM were unknown. Although corals were transferred to smaller tanks at the beginning of the light treatment experiments and at a different bench location, all parameters were kept consistent with the previous tank and received the same ASW supply and filtration system. Initially, it was suggested tank location may have been the contributing factor to increased reproductive output. Both treatment tanks were adjacent to each other, leading to the possibility that light contamination from the artificial lunar system may have kick- started reproduction by providing corals under CNM with weak lunar cues. However, data collected from the HOBO logger confirmed light intensity remained at 0 µmol photons m^{-2} s^{-1} throughout the entire 3 months, discarding potential lunar light contamination.

An alternative explanation may be linked to the variation in spectral composition between the two light sources during daylight hours. The lighting system was changed from T5 fluorescent tubes to an A1 LED lighting system that could replicate lunar light . Although both light sources had identical light intensities, results from the spectroradiometer indicated the light spectrum between tank lights during the first 4 months and the lights used for the remaining experimental months had

noticeable differences in their spectral quality. The light spectrum of the T5 tubes used in the initial months peaked in the blue (436 nm) and green (546 nm) spectrum, with a third peak in the red (611 nm). T5 fluorescent tubes use discharge mercury vapours with multiple peaks at different wavelengths and phosphors covering the bulbs to convert radiation to narrow bands of visible light resulting in a discrete spectrum (natural sunlight has a continuous spectrum (Riddle, 2009)). In comparison LED was shifted towards the blue spectra with only two significant peaks (452nm and 469 nm). Comparisons between T5 halide lights and LED's illustrated significant differences in growth rates in *Acropora formosa* and *Styllophora pistillata*, with colonies maintained under LED exhibiting 99% and 18% increase in growth relative to conspecifics under T5 illumination (Rocha et al., 2013).

Identification of multiple photoreceptors within scleractinian corals suggests varying wave bands within visible light are involved in sustaining clock harmonisation with the external environment, with predictable changes in spectral composition from sunrise to sunset (Roenneberg and Deng, 1997). In higher plants, red and blue have been highlighted as the primary wavelengths influencing clock synchronisation (Sorek and Levy, 2012). Given the presence of internal photosynthetic algae, similar mechanisms are suggested to exist in corals (Sorek and Levy, 2014). Results from previous studies suggest light can have wave-length specific effects on coral (Kinzie et al., 1984; Hennige et al., 2009; Szabo et al., 2014) influencing growth rates, protein content and the photochemical performance of its symbionts (Rocha et al., 2013; Kuhl et al., 1995). However, not every wavelength is equally utilised in physiological processes within scleractinian corals; red light has recently been linked to inhibitory effects and promoting increased tissue necrosis and mortality (Wijgerde et al., 2014). In contrast, blue light has been implicated to enhance multiple processes within corals including reproduction (Hoadley et al., 2011), calcification (Cohen et al., 2016), zooxanthellae density (Rocha et al., 2013), production of fluorescent proteins (D'Angelo et al., 2008) and protection against zinc-induced ROS (Wijgerde et al., 2014).

Corals can detect lunar photon fluxes using blue- sensing cryptochromes and long-wave sensitive opsins (Levy et al., 2007). Cryptochromes have been implicated to play a role in entrained cnidarian behaviour (Dolatshad et al., 2009) and up-regulation may act as a trigger for coral spawning

(Gorbunov and Falkowski, 2002; Levy et al., 2007). Other studies suggest blue light can delay spawning times; Levy et al (2007) found corals isolated under blue light experienced shifts in spawning time relative to control corals. Kanieskwa et al (2015) also found coral spawning was delayed by 6-8 hours *in A. millepora* when subjected to blue light. However, they concluded detection of blue light was essential for gamete/ larval release. It is possible the increase in availability of blue light triggered larval output in corals under CNM.

Concurrent with its coral host, *Symbiodinium* also exhibit varying responses to different wavelengths. Over millions of years, marine photosynthetic organisms have evolved mechanisms to enhance utilization of light, particularly of blue and violet wavelengths which penetrate best in the marine environment (Riddle, 2009). Zooxanthellae are primitive Pyrrophyta algae, their photosynthetic pigments are mainly chlorophyll a and c and carotenoid pigments (peridinine, xanthins) with strong absorption in the blue-green spectrum (Leletkin and Popova, 2005).

Wang et al (2008) identified pure blue light and a mixture of blue, red and infrared promoted normal zooxanthellae reproductive cycles. Blue light was more effective than mixed wavelengths, but not significantly. The study also identified that blue light effectively promoted cell cycles and growth of *Symbiodinium* clade B, a clade commonly associated with wild populations of *F. fragum* (Savage et al 2002; Finney et al., 2010). It is possible in this study transference to lights with increased blue light enhanced the growth rate of *Symbiodinium* in the host corals and resultantly increased rates of photosynthates translocated to the host. Hence, promoting increased reproductive output by increasing energy supplies. Measurements of oxygen production increased by 2.5- fold from June to July suggesting an increase in photosynthetic activity from the symbionts and suggesting this is the likely the cause of larval release in corals under CNM.

4.4 'The other circadian clock' – response of **Symbiodinium** *with and without lunar cues*

Circadian rhythms in scleractinian corals are complicated by the presence of two circadian clocks, the hosts and its internal symbionts (Sorek et al., 2014). The duet between each clock requires synchronization of metabolic processes including algal photosynthesis and coral calcification, as well as other parallel processes. Circadian periodicity in photosynthesis, growth and fluorescence has been assessed in both free- living *Symbiodinium* (Hastings, 2007) and within scleractinian corals (Sorek and Levy, 2012). Moreover, tight integration between major physiological processes of *Symbiodinium* and their coral hosts have also been identified. Coral calcification rates oscillate simultaneously with changes in the carbonate chemistry associated with symbiont photosynthesis (Tambutte et al., 2015) and activity rates of antioxidant enzymes were also found to mimic photosynthetic rates (Allemand et al., 2011).

Under 72 hours of constant illumination *Symbiodinium* (clade B), recently isolated from their host coral *Euphyllia glabrescens*, maintained natural reproductive phases for approximately 12 hours. Further analysis revealed the symbionts contained an abnormal number of chromosomes and exhibited abnormal division (Wang et al., 2008). As a mechanism of regulating internal symbiont population numbers, some scleractinian coral species expel symbionts cell entering the M phase (mitosis) in their reproductive cycle. Therefore, disruptions to the circadian rhythm of the symbionts could cause the host coral to unintentionally expel an excessive number of zooxanthellae leading to cascading consequences for the coral including severe disruptions to reproduction, depleted energy supplies, bleaching and death (Wang et al., 2008). It is unknown whether lunar periodicity has similar effects on the circadian rhythms of the coral's symbiont. Differentiating and isolating the cues that govern each clock is vital to develop further understanding of the symbiosis and the influence that each partner exhibits over one another. Whether the host and symbiont's clocks interact or run independently is unclear.

4.5 Bet- hedging reproductive strategies: larval size and settlement

4.5.1 Larval size

Despite total monthly release numbers that were double the amount produced by lunar corals, larvae produced under CNM showed the greatest size variations and on average were significantly smaller. In contrast the size of larvae produced under lunar treatment were always similar. Previous studies across multiple scleractinian corals species suggested larvae are not produced equally across the planulation period, within the same cohort or even on the same day of release (Edmunds et al., 2001; Isomura and Nishihara, 2001; Cumbo et al, 2013). Variation in larval size, at least within-brood variations, likely stems from unequal distributions of energy investments from the maternal polyp. Micro- environmental conditions including light intensity (Salih et al., 2000) and oxygen concentration (Cumbo et al., 2013) differ depending on the location of the polyp within the colony and act as contributing factors to varying larval size.

Previous studies typically conclude that selection favours uniform production with equal clutch sizes (Einum and Fleming, 2004). Bet-hedging theories define strategies that organisms develop in an attempt to optimize their fitness in variable and unpredictable environments (Olofsson et al., 2009); hence specialization dependent on specific environmental conditions should be avoided. Traditionally, bet- hedging has been implicated in the trade- off between parental survival and reproduction as well as offspring quantity vs quality. Production patterns of varying clutch and offspring sizes within both treatment groups suggests, to varying degrees, that both groups exhibit bet- hedging strategies. Corals within a lunar cycle exhibit cyclic monthly production with significant peaks at particular times of the month, this strategy is often referred to as conservative or ‘play it safe’ aimed to minimise risks (Bishop, 2006). Corals under CNM appear to invest in a lottery mechanism, consistently producing varying amounts of larvae with large size variance both within and between clutches.

Adaptive bet-hedging strategies have been described under similar studies, Cumbo et al (2012) noted larvae released close to peak release were larger than those produced earlier/ later. Edmunds et al (2011) suggested brooding Pocilloporid species correspond peak larval release to maximised larval respiration rates with the intent that larvae released in warmer seas would have shorter planktonic durations due to enhanced metabolic rates, consequently settling close distances to parental colonies. Larvae released in periods of colder water would have longer dispersal stages due to reduced metabolic rates, contributing to reef connectivity by settling in reefs away from their source. Evidence of this strategy exists in Pocilloporids in the Red Sea, larvae released early March dispersed over much greater distances than those released in late June when sea temperatures are at their highest (Edmunds et al., 2011).

Corals switched between treatments adjusted their reproductive strategy between either lottery or conservative. Investing in several reproductive strategies with similar output success is characterised as diversified bet- hedging. Demonstrating flexibility in reproductive strategies with rapid adjustment periods is crucial in environments that are unpredictable. Identifying whether corals can adjust their strategy may prove pivotal as global and local factors continue to influence environmental conditions forcing predictable cycles to become unpredictable (Frieler et al., 2015). However, whether flexibility in reproductive strategies is an isolated incident or widespread in other brooding and broadcasting species requires further investigations over long-term scales.

It also remains unclear to define whether variation, at least within the same clutch, is a haphazard result of development or a consequence of an adaptive bet- hedging strategy. The challenges of inducing coral reproduction under laboratory conditions and maintaining a regular reproductive pattern has led to a lack of long- term investigations, hence results from previous studies are loosely comparable. Studies commonly combine larvae released over consecutive lunar days to obtain necessary replicates (Putnam et al., 2008; Edmunds et al., 2011, Cumbo et al., 2012). Variations in larvae produced between those days are therefore overlooked yet could have significant consequences for the planktonic stage and subsequent settlement and post- settlement survival. Cumbo et al (2013) found *P. damicornis* larvae released on varying days of the lunar cycle exhibited

different physiological response when exposed to elevated temperature and pCO_2 rates; larvae produced on specific days exhibited increased sensitivity to temperature and depression at high pCO_2.

4.5.2 Larval settlement

Variations in reproductive output including quantity and quality of larvae released has consequences that cascade through every life history stage. In their natal reef environments, differences in larval sizes is often translated to differential survival and settlement success (Cumbo et al., 2012). In our study, the PLD of smaller larvae extended beyond the experimental period, often remaining unsettled for >30 days. Larvae produced under lunar conditions settled within hours of release. Others also noted rapid settlement in *F. fragum*, Peterson et al (2007) noted 40% of larvae released settled within 12h. Producing a range of phenotypes within a single planulation event increases the chance of some being suitable for natal reefs, whilst others can disperse to further reefs (Putnam et al., 2008; Szmant- Froelich and Meadows, 2006).

Even with the advantage of controlling external conditions, the mortality rate of newly settled corals was high. Only a few recruits over the course of this investigation progressed to primary polyp division, marking the first steps towards building a colony. Coral larvae can enhance their own survival ability by aggregating to other conspecifics, this key behavioural trait was a common observation. Successful settlement of individual larvae initiated mass settlement in remaining free-swimming larvae, which selected settlement sites within millimetres of the initiator. Despite idealistic growing conditions and adaptive behavioural traits to increase survival potential post- settlement, < 1% of settled larvae survived. Percentage survival is comparable to wild rates in which Wilson and Harrison (2005) noted in three corals species at Solitary Island 0.2 – 6% of coral recruits survived the first year, similarly earlier studies noted 94% mortality of coral recruits in the same reef location (Fairfull and Harriott, 1999).

4.6 Alternate modes of asexual reproduction

Alternate reproductive strategies were also observed in *F. fragum*, although all six colonies experienced synonymous tank conditions, only one colony exhibited polyp expulsion. It is important to define the differences between polyp expulsion and polyp- bail out. Polyp- bail out is a response associated with health- compromised corals, this process occurred instantaneously during acute high nitrate levels and is potentially a 'last- resort' strategy to escape deleterious conditions and salvage genetic diversity by releasing polyps from their calices to re- attach away from the parent colony. Polyp expulsion occurs in physiologically healthy corals, individual polyps still attached to their calices are elevated from the colony on extended calcareous stalks, the duration of this process was over 5 weeks before the polyp detached and was allowed to settle on a pre-treated CCA slide. Detached polyps are at an advanced stage of development compared to sexually and asexually derived larvae, they begin their benthic life at a juvenile stage by-passing the risks involved with larval dispersal, settlement and metamorphosis and thus reach sexual maturity at a faster rate. Observations in situ have only been observed in coral species isolated in physically challenging environments to sustain population existence in that area (Kramarsky- Winter et al., 1997). Increases in chronic disturbances may cause corals to increase their dependence on asexual reproductive strategies including polyp expulsion, with the chance of increasing genotypes with improved resiliency outweighing the loss of genetic diversity.

Massive and encrusting corals, including *F. fragum*, contribute to reef structure in areas which are exposed to chronic disturbance from high rates of wave action and sedimentation increasing the levels of turbidity and reducing irradiance levels (Harrison and Wallace, 1990). Native recruitment success is likely jeopardized as freshly released larvae/ gametes are quickly dispersed and new recruits are dislodged or asphyxiated by sediment. In order to maintain localised population numbers in physically unstable environments, some corals adopt other reproductive strategies. Asexual reproduction via polyp expulsion has previously been observed in *Favia favus* and *Oculina*

patagonica off the coast of Israel, rates of polyp expulsion were most prominent during summer and autumn months when sea surface temperatures were highest (Kramarsky- Winter et al., 1997).

Alternatively, increased budding has also been associated with periods of favourable environmental conditions. An increase in temperature combined with increased supply of zooplankton promoted higher budding rates in the temperate coral *Cladocora caespitosa* (Rodolfo- Metalpa et al., 2007). In the present study one colony of *F. fragum* initiated signs of budding (i.e. individual polyps were lifted and extending away from the main colony) throughout the experiment. The first visible signs appeared shortly after the increase in nitrate concentrations within the aquaria system, budding also occurred three months later, when tank conditions had remained at constant levels. Therefore, it was not possible to distinguish the underlying factors that induced budding. It is also unclear why only one coral exhibited budding, all six corals experienced replicate conditions including equivalent heterotrophic feeding. Further studies are required to understand the drivers and advantages of budding.

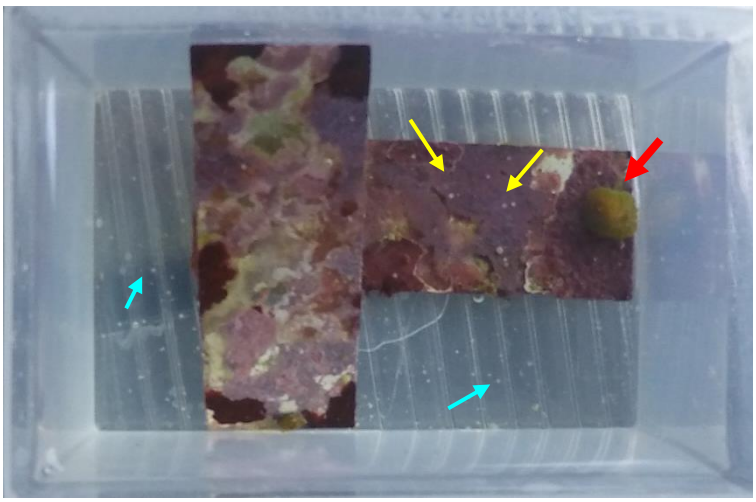

Figure 23. Polyp expelled from main colony re-attached to CCA slide and began secreting calcium carbonate skeleton (red arrow), new recruits readily settle on CCA slide (yellow arrows) and haphazardly on the tub floor (blue arrows).

4.7 Metabolic implications of reproduction

Comparative analysis of oxygen evolution indicated metabolic fluctuations related to reproductive output, an increase in the number of larvae released resulted in higher metabolic rates. Increases in metabolic rate were a direct response to larval production, irrespective of light treatment. Further analysis indicated significant increases in metabolic rate were only present during peaked larval production, i.e. when large amounts were released, suggesting either significant rates of change require a minimal number of larvae before there is a compromise on coral metabolism or the sensitivity of the testing was low so only detectable when there were significant changes. In addition, it was assumed that corals would increase their metabolic rate pre- larval release; however, results suggest that the corals instead compensate for the loss of energy supply distributed to the larvae post-larval release. Therefore, it would appear that corals up- regulate their metabolism as indicated by increases in respiration and photosynthetic rate post production to restore energy supplies.

At the time of larval production reserved lipids are allocated to larvae and this depleted from the coral host (Norstrom and Sandstrom, 2010). The general assumption is that energy sources are partitioned between different biological functions including reproduction, growth and regeneration. Most studies suggest there is a trade- off between reproduction and other processes (Isomura and Nishihara, 2001; Bishop, 2006; Cumbo et al., 2013). However, there is conflicting evidence that suggests reproduction may also be independent of energy constraints. For example, growth rates in female colonies of *Porites astreoides* continually increased even during the onset of reproduction and larval release (Rinkevich et al., 2016). Muscatine et al (1984) observed carbon translocated in multiple shallow water scleractinian corals was > 140% of their daily energetic needs. This study suggested increases in metabolism only occurred above a certain threshold of larval release. Corals under CNM continuously produced larvae and maintained a stable metabolic rate, increasing only during days post- large quantities of larval production. Pressure on metabolic processes in corals under natural conditions is likely significantly enhanced due to fluctuating parameters and irregular

external food supplements. The metabolic demand under stable laboratory conditions is therefore likely underestimated.

Although using respirometer chambers to measure oxygen evolution has continued to be the principal method both in laboratory and field studies, measurements are not representative of the complete metabolic processes within coral productivity (Chisholm and Gattuso, 1991). Studies suggest the evolution of oxygen is not synonymous with the rate of carbon fixation, this method is therefore limited as it cannot distinguish between oxygen production, consumption and/or carbon fixation (Schrameyer et al., 2014). Further measurements involving C^{14} isotopes would be needed to determine more precise measurements.

4.8 Larval metamorphosis

Metamorphosis is a critical process in corals linking one life stage to the next and defining the start point of a sessile benthic existence. Alterations to an advanced light sheet fluorescence microscopy technique has enabled real time identification and quantification of stages critical to a successful transition from larva to primary polyp, and has given further insight into the close interaction between larval conspecifics when determining settlement sites. Light sheet fluorescence microscopy (LSFM) was used to construct a timeline and distinguish the development of key anatomical features (outlined in results section 3.5).

The complex process associated with settlement and metamorphosis is likely driven by the high level of specificity required in selecting settlement sites that optimize post- settlement survival and accommodate the photosynthetic demands of the coral host's associated symbionts. The internal mechanism that mediates metamorphosis is likely similar across Cnidarian groups (Grasso et al., 2011). In *A. elegantissima* and *H. echinata* neuropeptides of the LWamide family are released upon receiving appropriate settlement cues and are suggested to be involved in initiating metamorphosis (Muller and Leitz, 2002). Others suggest metamorphosis is linked to metabolic processes, a threshold

exists in which depletion of energy reserves induces spontaneous metamorphosis (desperate larva hypothesis, Bishop et al., 2006). The metamorphic process in marine invertebrates occurs at a much faster rate in comparison to terrestrial organisms that undergo similar metamorphic transformations (Hadfield et al., 2001), attributed to the possibility that brooded larvae are primed for settlement and this pre- established competence allows for quick transitions (Grasso et al., 2011). The time period for *F. fragum* larvae from permanent attachment to a heterotrophic primary polyp was approximately 7 days. The developmental time-frame of anatomical features is concordant with the rate observed in *Acropora bobilis* and *A. microphthalma* (Hirose et al., 2008).

4.8.1 The effects of larval size on rates of metamorphosis

Despite CNM corals typically producing smaller larvae with significantly longer planktonic periods, no difference in the rate of metamorphic development was observed between larvae produced by lunar induced corals or corals maintained under constant new moon. Size appeared to have no effect on the rate of development, rates of metamorphosis of smaller larvae was similar to larger larvae and the same development processes were observed in both size categories. Even in natural planulation events, larvae are often unequally distributed in size even from the same parental colony (Roth et al., 2013). Larvae of *Seriatopora hystrix* varied in size with smaller individuals hosting lower abundances of symbionts. Despite having lower photosynthetic rates, symbiont numbers rapidly increased and both larvae with originally low or high densities development at similar rates (Roth et al., 2013). Longer planktonic periods often result in depleted lipid reserves needed for metamorphosis, the presence of zooxanthellae may counteract the reduced amounts of lipid quantities between larger and smaller larvae by supplementing energy stores (Graham, 2012). Hence, enabling small larvae to metamorphose at the same rate.

4.8.2 Larvae- algae symbiosis

The obvious disadvantage of pre- established symbiosis is the risk of transportation to environments in which the associated *Symbiodinium* clade is not optimal causing knock-on effects to the host larva. However, unlike adults that have formed mature associations with their symbiont communities, earlier life stages are assumed to lack or have a weaker *Symbiodinium* specificity (Coffroth et al., 2001). In previous cases, recently settled recruits were found to host a wider variety of symbiont populations than their adult conspecifics (Coffroth et al., 2001; Little et al., 2004). Furthermore, a number of studies found that juvenile corals change their dominant *Symbiodinium* clade dependent of the environment and growth stage (e.g. see Little et al., 2004). The dynamics of the symbiotic relationship at the larval or juvenile stage may therefore be different than the well documented mature colony associations however, generally studies are lacking with regards to the role of symbionts in developmental stages of juvenile corals and the importance of different drivers in changes in *Symbiodinium* density and assemblage structure are largely unknown .

Recent research indicated that larvae brooded from *P. damicornis* obtain significantly less nutrition from their symbionts than in adult associations. Differences were attributed to substantially lower symbiont densities, lower basal metabolic rates and reduced translocation rates in larvae (Kopp et al., 2016). Comparisons of symbiont density of larvae indicated that the average *Symbiodinium* density within the gastrodermis amounted to only 12.5% of the density in the gastrodermis of the coenosarc region in an adult coral (Kopp et al., 2016): only a small region at the boundary of the mesoglea and epiderm was identified to contain dinoflagellate densities comparable to those found in adult corals. Conversely, studies on 3- week old *P. damicornis* larvae indicated that ca.70% of photosynthetically fixed carbon was translocated to the host larval tissue (Gaither and Rowan, 2010), concurrent with rates recorded in adult corals (Muscatine et al., 1981). Therefore, the early stages of larval development appear to be heavily dependent on maternally derived resources. However the benefits of vertical transmission/ early establishment of symbiosis, may come more important further in to larval development and aid in extending planktonic periods, and thus increase dispersal ranges

and reef connectivity. The transition from larval to adult is metabolically costly and is critical to developmental and metamorphosis. Increased translocation rate enhances the amount of nutrition available for metabolic processes fundamental to larval survival and recruitment in to a mature ancestral polyp. The external environmental conditions at the time of release may have serious consequences on this process and should be a focus of future studies if recruitment is to play a significant role in recovering coral populations.

4.8.3 Patterns of GFPs in larvae to understand their function/s

Due to their abundance and ability to survive under experimental conditions coral larvae act as ideal models for studying the function of green fluorescent proteins (GFPs) and underpinning genetic mechanisms (Kenkel et al., 2011). GFPs were present throughout larval development in *F. fragum* however, varying forms of fluorescent patterning occurred during post-settlement growth. Two prominent patterns of fluorescence have been described in corals: uniform fluorescence in which GFPs are distributed equally across the coral polyp with no specificity and complementary fluorescence in which GFPs are targeted specifically to anatomical regions (Gruber et al., 2008). In this study, larvae transitioned from uniform to complementary in parallel with asexual division of the primary polyp.

Re-distributing GFPs during juvenile development indicates multiple functionality. Gruber et al (2008) suggested the majority of corals exhibit complementary fluorescence, with the highest abundance distributed to features most vulnerable to light exposure. Salih et al (2000) found tissue regions with dense populations of GFPs coincided with areas of high cell division and reproductive organs. Colony edges where the calcium carbonate skeleton is continuously laid down contained high densities of GFPs as well as the tentacle tips and areas around the oral disk which upon retraction may form a “sun-screening polyp plug” (Salih et al., 2000). Many other studies support this research concluding that GFPs are concentrated around specific areas particularly the oral cavity (Gruber et al.,

2008, Salih et al., 2000) but also in "growing areas" including branching tips (Palmer et al., 2008), the growing edge of the colony base (D'Angelo et al., 2008) as well as in regions that have recently been damaged and are undergoing repair (Palmer et al., 2009).

The distribution of GFPs has previously led researchers to propose their photo-protective function. Consequently, GFPs are most commonly associated with stabilizing the internal light microenvironment within the coral tissue, suggesting that expression may be associated with zooxanthellae distribution within the gastrodermal cells. In low light conditions or shade- adapted corals they may enhance the availability to zooxanthellae by acting as channels to direct light (Verkhusha and Lukyanov, 2004) or via they may transform incoming light to concentrate wavelengths more suitable for photosynthesis (Dove et al., 2000). Hence, GFPs are distributed between or below dinoflagellates. In high light conditions, conducive with shallow reef environments, GFPs may act in synergy with UV shielding mycosporin- like amino acids (MAAs) as photo-protectants. In high light adapted corals GFPs are localised above zooxanthellae dissipating excess light energy via fluorescence and scattering (Salih et al., 2000).

In this study, images of larvae at the beginning of their metamorphic transformation indicated enhanced expression at the oral end. Comparable morphological patterning was observed in *Favities pentagona* also displaying increased amounts of GFPs around the mouth in adult corals (Gruber et al., 2008). GFPs concentrated in this area have previously been associated with the establishment of the cnidarian- *Symbiodinium* symbiosis in azooxanthellate larvae (Hagedorn et al., 2015). The 'beacon hypothesis' suggests free-living zooxanthellae are directed by GFPs generated around the mouthparts of larvae (Hagedorn et al., 2015). Dispersing larvae heavily deplete maternally derived energy stores during the planktonic period and the metamorphic process, therefore a critical step is to generate a rapid association with *Symbiodinium* (Norstrom and Sandstrom, 2010). The concentration of free living *Symbiodinium* achieves the greatest densities in reef sediments (1000- 4000 cells/ml), numbers in the water column are substantially diluted (80 cells/ml) (Takabayashi et al., 2012). It is possible most larvae commit to horizontal transmission upon interaction with surface sites and have developed a mechanism to enhance the onset of their symbiotic relationship. Increasing the abundance of GFPs

around the oral cavity, the site at which zooxanthellae enter the host, enhances the signal strength to attract and direct dinoflagellates towards the coral (Haegdorn et al., 2015). Results from this study appear to support this theory.

In larvae, such as *F. fragum*, symbiosis is already well established before planulation. For larvae that disperse and settle at neighbouring reefs, away from parental colonies, where conditions may be inimical for host and associated symbionts, GFPs may assist in horizontal transfer and switching of symbionts to establish a symbiotic assemblage stemming from free-living symbionts that are able to survive in these new environments. Further, underdeveloped recognition systems and/ or during the early stages of symbiosis larvae are "promiscuous", acquiring multiple *Symbiodinium* types (Rodriguez- Lanetty et al., 2004; Dunn and Weis, 2009; Cumbo et al., 2013). *Acropora millepora* and *A. tenuis* larvae acquired up to four different *Symbiodinium* types upon exposure to sediments containing 6 different clades (Cumbo et al., 2013). Associating with different *Symbiodinium* clades enables juvenile corals to form symbiosis distinguishable from their parent colonies and to optimise physiological processes require symbionts best adapted to surrounding environment.

4.8.4 Aggregated settlement patterns

Throughout settlement experiments, *F. fragum* larvae commonly aggregated together on one location of the CCA slides. Given the opportunity coral larvae will amass together and settle within close proximity of each other. The allogeneic histocompatibility immune response of adult corals appears to be supressed in early life stages and instead of rejecting one another, conspecific recruits settle together to form aggregated colonies (Rinkevich, 2004). Gregarious behaviour is not an isolated characteristic of *F. frgaum* and has previously been described in other scleractinian coral species (Hidaka, 1985; Amar et al., 2008), as well as soft corals (Barki et al., 2002) and sponges (Ilan and Loya, 1990). Factors driving this early life behaviour have been attributed to enhanced inter-specific competition, increased growth and post- settlement survival rate, reduced time scale to achieve

reproductive size, increased genetic repertoire and expression of heterosis, securing potential mates for future sexual reproduction (Rivera and Goodbody-Gringley, 2014).

Fusion of juvenile coral recruits in nature has previously been observed (Sammarco, 1982). Frank et al (1997) observed frequent aggregation spats of *S. pistillata*. They noted upon encounter, conspecific larvae either fused together and established a chimera or failed to fuse. The same coral species, under laboratory settings, also exhibited high rates of fusion with 61% of interactions resulting in successful fusion (Amar et al., 2008). Aggregation rates were not a focus of this study, therefore no measurements were recorded. However, accumulation of recruits to one settlement site was regularly observed, both with fusion and non-fusion (see Fig. 21). Larvae rarely settled in isolated spots and those that settled first appeared to act as a beacon for other larvae, indicating favourable settlement sites. However, the frequency of cumulative settlement in laboratory settings is not representative of natural occurrence rates. The 5 x 5 cm larval settlement tubs may have exaggerate this behavioural trait by forcing groups of larvae to remain in close contact and providing minimized settlement areas. Gregarious behaviour on natural reef substrate likely occurs at much lower frequency due to multiple physical barriers, such as ocean currents (Gleason and Hofmann, 2011), limiting encounters with conspecifics (Koehl and Hadfield, 2004). Despite this, it appears that larvae have maintained this evolutionary tactic and utilize it opportunistically, selectively choosing to fuse with individuals of similar genetics to itself or at least settle close by.

An immediate advantage of close settlement is an enhanced defensive capability (Rivera and Goodbody- Gringley, 2014). Newly settled corals notoriously get outcompeted by other benthic organisms for prime reef space due to their weak ability to compete and slow growth rates (Arnold and Steneck, 2011). This was evident even under laboratory conditions; new recruits would be surrounded by algae or smothered by a cyanobacteria mat within a few days of settlement. Accumulating into groups acts as a ‘safety in numbers’ mechanism to increase the chance of post-settlement survival. Corals that invest in allogeneic fusion have an added advantage by fast forwarding their rate of growth at this earliest and most vulnerable stage of colony development. Brickner et al (2006) indicated solitary polyps may provide metabolites to neighbouring injured

polyps despite a connective tissue bridge, transferred through mucus or living cells during contact between expanded polyps. Rinkevich and Loya (1983) previously suggested isolated allogeneic colonies of *Stylophora pistillata* could translocate photosynthates between individuals however, they suggested cells transferred derived from competition between con-specifics. Hence resource sharing may occur between aggregated recruits to further enhance the benefits of this behavioural characteristic and prolong survivorship. Previous settlement studies found corals remaining smaller than 3mm in diameter for 2 to 3 months possess only a 20% chance of survival (Rylaarsdam, 1983) therefore, aggregate behaviour would be highly selected.

The benefits attached to fusing with conspecifics may be amplified under current and future environmental conditions. Cumulating the genetic variability between two individuals to form a novel organism has the potential to heighten its physiological capabilities to adjust and tolerate fluctuating conditions. Recently, Rinkevich et al (2016) found chimeric derived colonies asexually produce larvae with genetic combinations more diverse than that of sexually produced larvae. Further, chimeric derived larvae were able to alter their somatic constituents and customised their genetic components in response to unfavourable external conditions. In this experiment larvae from all 3 colonies per treatment were combined and pooled into the same tub. Therefore, it is unknown whether larvae from different colonies fused or only those from the same colony.

5.0 CONCLUSION

Restricted long generation times in most scleractinian corals limit their ability to adapt their physiology to changes in ocean temperatures and acidification within the necessary time-frame (Frieler et al., 2015; Stap et al., 2016); flexibility in reproductive patterns that enable corals to strategize larval/ gamete release that optimize recruitment success within unpredictable environmental conditions may be a vital element in the future persistence of corals. Corals rely on external environmental conditions to predict the environment that larvae will be exposed to however, when conditions undergo rapid changes corals may dependent on bet-hedging reproductive strategies. Anthropogenic impacts are reducing sub-cryptic settlement sites and inhibiting settlement cues reducing reproductive success (Halpern et al., 2008).

Favia fragum exhibited dynamic bet-hedging strategies and adjusted reproductive patterns rapidly in response to exposure/ absent lunar cues. Under CNM, corals distributed larval output across the whole month with high variation in larval sizes. Under lunar treatment, corals exhibited peaked larval production with little variation in larval sizes. Under conditions where lunar cues with absent, the production of a range of larval phenotypes may increase the chance of some individuals recruiting successfully by varying dispersal time. Under predictable lunar periodicity, corals optimize survival and successful recruitment by producing one mean phenotype of high quality which ensures reproductive success. *F. fragum* produced large larvae with short PLD supporting retention and re-population of local reefs.

Life- history traits can significantly influence reproductive strategies, *F. frgaum* exhibits several characteristics that may enhance persistence to future oceanic conditions. The ability of larval recruits to adhere to aggregate settlement, enhancing survival by increasing inter- specific competitive ability and, for those that form genetic chimeras, a reduction in time needed to achieve sexual maturity which may prove critical for future reef resilience (Birell et al., 2008). Despite variations in

larval size, the metamorphic rate between size classes was the same indicating the benefits and disadvantages associated with different sizes occurs relate to the pelagic and thus dispersal phase.

Species survival has long been dependent on the ability to successfully recruit as a method to safeguard their genetic variability and maintain population demography. Predictable environmental cycles, particularly lunar cycles, have enabled corals to entrain their reproductive patterns to release larvae/ gametes during the specific lunar phases thus optimizing external fertilization success, appropriate larval dispersal and efficient coral recruitment. The ability of *F. fragum* to bet- hedge as it concerns reproductive strategies has important consequence for the future persistence of corals in changing environmental conditions. Further research is needed to determine whether bet- hedging reproduction is prevalent in scleractinian corals or restricted to certain species or reproductive modes.

6.0 REFERENCES

Allemand, D., Tambutté, É., Zoccola, D., & Tambutté, S. (2011) Coral calcification, cells to reefs. *In Coral reefs: an ecosystem in transition*, Springer Netherlands, 119-150.

Amar, K.-O., Chadwick, N. E. & Rinkevich, B. (2008) Coral kin aggregations exhibit mixed allogeneic reactions and enhanced fitness during early ontogeny. *BMC Evolutionary Biology*, **8**, 126.

Arnold, S. N., & Steneck, R. S. (2011) Settling into an increasingly hostile world: the rapidly closing "recruitment window" for corals. *PLoS ONE*, **6**, e28681.

Aronson, R. B., & Precht, W. F. (2001) White-band disease and the changing face of Caribbean coral reefs. *In The ecology and etiology of newly emerging marine diseases* Springer Netherlands, 25-38.

Aubrecht, C., Elvidge, C.D., Longcore, T., Rich, C., Safran, J., Strong, A.E., Eakin, C.M., Baugh, K.E., Tuttle, B.T., Howard, A.T. & Erwin, E.H. (2008) A global inventory of coral reef stressors based on satellite observed nighttime lights. *Geocarto International*, **23**, 467-479.

Babcock, R. C., Bull, G. D., Harrison, P. L., Heyward, A. J., Oliver, J. K., Wallace, C. C. & Willis, B. L. (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Marine Biology*, **90**, 379-394.

Babcock, R. C. (1991) Comparative demography of Three species of Scleractinian Corals using age- and size-dependent classifications. *Ecological Monographs*, **61**, 225–244.

Babcock, R. C., Wills, B. L., & Simpson, C. J. (1994) Mass spawning of corals on a high latitude coral reef. *Coral Reefs*, **13**, 161-169.

Baker, A. C. (1999) The symbiosis ecology of reef-building corals.

Barki, Y., Gateño, D., Graur, D. & Rinkevich, B. (2002) Soft-coral natural chimerism: A window in ontogeny allows the creation of entities comprised of incongruous parts. *Marine Ecology Progress Series*, **231**, 91–99.

Barnes, R. S. K. & Hughes, R. N. (1999) An Introduction to Marine Ecology; Third edition. Oxford, UK: Blackwell Science Ltd,117-141.

Birrell, C., Mccook, L., Willis, B. & Diaz-Pulido, G. (2008) Effects Of Benthic Algae On The Replenishment Of Corals And The Implications For The Resilience Of Coral Reefs, *Oceanography and Marine Biology - An Annual Review*, 25- 63.

Bishop, C. D., Huggett, M. J., Heyland, A., Hodin, J., & Brandhorst, B. P. (2006) Interspecific variation in metamorphic competence in marine invertebrates: The significance for comparative investigations into the timing of metamorphosis. *Integrative and Comparative Biology*, **46**, 662–682.

Boch, C. A., Ananthasubramaniam, B., Sweeney, A. M., Doyle, F. J. & Morse, D. E. (2011) Effects of light dynamics on coral spawning synchrony. *The Biological Bulletin*, **220**, 161–173.

Brady, A. K., Hilton, J. D., & Vize, P. D. (2009) Coral spawn timing is a direct response to solar light cycles and is not an entrained circadian response. *Coral Reefs*, **28**, 677– 680.

Brady, A. K., Snyder, K. A. & Vize, P. D. (2011) Circadian cycles of gene expression in the coral, *Acropora millepora*. *PLoS ONE*, **6**, e25072.

Brazeau, D.A., Gleason, D.F. & Morgan, M.E. (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: Evidence from molecular markers. *Journal of Experimental Marine Biology and Ecology*, **231**, 225–238.

Brickner, I., Oren, U., Frank, U. & Loya, Y. (2006) Energy integration between the solitary polyps of the clonal coral *Lobophyllia corymbosa*. *Journal of Experimental Biology*, **209**, 1690–1695.

Brown, R. L., & Robinson, P. R. (2004) Melanopsin—Shedding light on the elusive Circadian Photopigment. *Chronobiology International*, **21**, 189–204.

Bruno, J. F., Petes, L. E., Drew Harvell, C., & Hettinger, A. (2003) Nutrient enrichment can increase the severity of coral diseases. *Ecology letters*, **6**, 1056-1061.

Buddemeier, R.W., Baker, A.C., Fautin, D.G. & Jacobs, R.J. (2004) The Adaptive Hypothesis of Bleaching, *Coral Health and Disease*, 427–444.

Camp, E., Suggett, D. J., Gendron, G., Jompa, J., Manfrino, C. M. & Smith, D. J. (2016) Mangrove and seagrass beds provide alternate biogeochemical services 1 for corals threatened by climate change. *Frontiers in Marine Science*, **3**.

Carlon, D. B. (2002) Production and supply of larvae as determinants of zonation in a brooding tropical coral. *Journal of Experimental Marine Biology and Ecology*, **268**, 33–46.

Carroll, A., Harrison, P., & Adjeroud, M. (2016) Susceptibility of coral assemblages to successive bleaching events at Moorea, French Polynesia. *Marine and Freshwater Research*.

Caley, M. J., Carr, M. H., Hixon, M. A., Hughes, T. P., Jones, G. P. & Menge, B. A. (1996) Recruitment and the local dynamics of open marine populations. *Annual Review Ecological System*, **27**, 477–500.

Chia, F. S. & Koss, R. (1979) Fine structural studies of the nervous system and the apical organ in the planula larva of the sea anemone *Anthopleura elegantissima*. *Journal of Morphology*, **160**, 275–297.

Chisholm, J. R. & Gattuso, J. P. (1991) Validation of the alkalinity anomaly technique for investigating calcification of photosynthesis in coral reef communities. *Limnology and Oceanography*, **36**, 1232-1239.

Chu, C. S., Lo, Y. L. & Sung, T. W. (2011) Review on recent developments of fluorescent oxygen and carbon dioxide optical fiber sensors. *Photonic Sensors*, **1**, 234-250.

Coffroth, M., Santos, S. & Goulet, T. (2001) Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. *Marine Ecology Progress Series*, **222**, 85–96.

Cohen, I., Dubinsky, Z. & Erez, J. (2016) Light enhanced calcification in Hermatypic Corals: New insights from light spectral responses. *Frontiers in Marine Science*, **2**.

Combosch, D. J., & Vollmer, S. V. (2013) Mixed asexual and sexual reproduction in the Indo-Pacific reef coral *Pocillopora damicornis*. *Ecology and Evolution*, **3**, 3379-3387.

Cox, E. F. & Ward, S. (2002) Impact of elevated ammonium on reproduction in two Hawaiian scleractinian corals with different life history patterns. *Marine Pollution Bulletin*, **44**, 1230–1235.

Cowen, R. K. (2002) Larval Dispersal and Retention and Consequences for Population Connectivity. *Coral Reef Fishes: dynamics and diversity in a complex ecosystem*, 149.

Cumbo, V. R., Baird, A. H. & van Oppen, M. J. H. (2012) The promiscuous larvae: Flexibility in the establishment of symbiosis in corals. *Coral Reefs*, **32**, 111–120.

Cumbo, V. R., Fan, T. Y. & Edmunds, P. J. (2013) Effects of exposure duration on the response of *Pocillopora damicornis* larvae to elevated temperature and high pCO2. *Journal of Experimental Marine Biology and Ecology*. **439**, 100–107.

D'Angelo, C., Denzel, A., Vogt, A., Matz, M., Oswald, F., Salih, A. & Wiedenmann, J. (2008) Blue light regulation of host pigment in reef-building corals. *Marine Ecology Progress Series*, **364**, 97–106.

Dai, C. F., Soong, K. & Fan, T. Y. (1992) Sexual reproduction of corals in northern and southern Taiwan. *Proceedings of the 7th International Coral Reef Symposium*, Guam, **1**, 448-455.

Davies, T. W., Duffy, J. P., Bennie, J. & Gaston, K. J. (2014) The nature, extent, and ecological implications of marine light pollution. *Frontiers in Ecology and the Environment*, **12**, 347–355.

DeCoursey, P. J. (2003) The behavioral ecology and evolution of biological timing systems. In: Dunlap JC, Loros JJ, DeCoursey PJ, editors. *Chronobiology: biological timekeeping*. Sunderland (MA): Sinauer Associates, 58-6.

Dolatshad, H. (2005) Developmental and reproductive performance in circadian mutant mice. *Human Reproduction*, **21**, 68–79.

Dolatshad, H., Davis, F. C. & Johnson, M. H. (2009) Circadian clock genes in reproductive tissues and the developing conceptus. *Reproduction, Fertility and Development*, **21**, 1.

Dove, S. G., Hoegh-Guldberg, O. & Ranganathan, S. (2000) Major colour patterns of reef-building corals are due to a family of GFP-like proteins. *Coral Reefs*, **19**, 197–204.

Downs, C. A., Kramarsky-Winter, E., Woodley, C. M., Downs, A., Winters, G., Loya, Y., & Ostrander, G. K. (2009) Cellular pathology and histopathology of hypo-salinity exposure on the coral *Stylophora pistillata*. *Science of The Total Environment*, **407**, 4838–4851.

Dubinsky, Z. & Jokiel, P.L. (1994) Ratio of energy and nutrient fluxes regulates symbiosis between zooxanthellae and corals. *Pacific Science*, **48**, 313-324.

Dunn, S. R. & Weis, V. M. (2009) Apoptosis as a post-phagocytic winnowing mechanism in a coral-dinoflagellate mutualism. *Environmental Microbiology*, **11**, 268–276.

Edgar, R. S., Green, E. W., Zhao, Y., van Ooijen, G., Olmedo, M., Qin, X., Xu, Y., Pan, M., Valekunja, U. K., Feeney, K. A. & Maywood, E. S. (2012) Peroxiredoxins are conserved markers of circadian rhythms. *Nature*, **485**, 459-464.

Edmunds, L. N. (1988) *Cellular and molecular bases of biological clocks: Models and mechanisms for circadian timekeeping*. New York: Springer-Verlag Berlin and Heidelberg GmbH & Co. K.

Edmunds, P., Gates, R. & Gleason, D. (2001) The biology of larvae from the reef coral *Porites astreoides*, and their response to temperature disturbances. *Marine Biology*, **139,** 981-989.

Edmunds, A. P. J., & Bruno, T. E. J. (2008) Biology of early life stage of tropical reef corals. Available at: http://www.eoearth.org/view/article/150659 (Accessed: 28 May 2015).

Edmunds, P., Cumbo, V., & Fan, T. (2011) Effects of temperature on the respiration of brooded larvae from tropical reef corals. *Journal of Experimental Biology*, **214**, 2783–2790.

Einum, S. & Fleming, I. A. (2004) Does within-population variation in egg size reduce intraspecific competition in Atlantic salmon, *Salmo salar*? *Functional Ecology*, **18**, 110–115.

Fabricius, K. E. & Metzner, J. (2004) Scleractinian walls of mouths: predation on coral larvae by corals. *Coral Reefs*, **23**, 245-248.

Fadlallah, Y. H. & Lindo, R. T. (1988) Contrasting cycles of reproduction in *Stylophora pistillata* from the Red Sea and the Arabian Gulf, with emphasis on temperature. *Proceedings of the 6th International Coral Reef Symposium*, **3**, 225- 230.

Fairfull, S. J. L., & Harriott, V. J. (1999). Succession, space and coral recruitment in a subtropical fouling community. *Marine and Freshwater Research*, **50**, 235-242.

Falkowski, P. G., Dubinsky, Z., Muscatine, L. & McCloskey, L. (1993) Population control in symbiotic Corals. *BioScience*, **43,** 606–611.

Fan, T. Y., Li, J. J., Le, S. X., & Fang, L. S. (2002) Lunar Periodicity of Larval Release by Pocilloporid Corals in Southern Taiwan. *Zoological Studies*, **41**, 288–294.

Finney, J. C., Pettay, T., Sampayo, E. M., Warner, M. E., Oxenford, H. & LaJeunesse, T. C. (2010) The relative significance of host-habitat, depth, and geography on the ecology, endemism and speciation of coral endosymbionts. *Microbial Ecology*, **60**, 250–63.

Frank, U., Oren, U., Loya, Y. & Rinkevich, B. (1997) Alloimmune maturation in the coral *Stylophora pistillata* is achieved through three distinctive stages, 4- month post-metamorphosis. *Proceedings of the Royal Society of London*, **264**, 99–104.

Frieler, K., Meinshausen, M., Golly, A., Mengel, M., Lebek, K., Donner, S. D. & Hoegh-Guldberg, O. (2012) Limiting global warming to 2 °C is unlikely to save most coral reefs. *Nature Climate Change*, **3**, 165–170.

Furnas, M. J. (1991) Net in situ growth rates of phytoplankton in an oligotrophic, tropical shelf ecosystem. *Limnology and Oceanography*, **36**, 13–29.

Gaither, M. R. & Rowan, R. (2010) Zooxanthellar symbiosis in planula larvae of the coral *Pocillopora damicornis*. *Journal of Experimental Marine Biology and Ecology*, **386**, 45–53.

Gilmour, J., Speed, C. W. & Babcock, R. (2016) Coral reproduction in Western Australia. *PeerJ*, **4**, e2010.

Gleason, D., Brazeau, D. & Munfus, D. (2001) Can self- fertilizing coral species be used to enhance restoration of Caribbean reefs? *Bulletin of Marine Science*, **69**, 933- 943.

Gleason, D. F. & Hofmann, D. K. (2011) Coral larvae: From gametes to recruits. *Journal of Experimental Marine Biology and Ecology*, **408**, 42–57.

Gorbunov, M. Y., & Falkowski, P. G. (2002) Photoreceptors in the cnidarian hosts allow symbiotic corals to sense blue moonlight. *Limnology and Oceanography*, **47**, 309-315.

Goodbody-Gringley, G. & de Putron, S. J. (2009) Planulation patterns of the brooding coral *Favia fragum* (Esper) in Bermuda. *Coral Reefs*, **28**, 959–963.

Goodbody-Gringley, G., Vollmer, S. V., Woollacott, R. M., & Giribet, G. (2010) Limited gene flow in the brooding coral *Favia fragum* (Esper, 1797). *Marine biology*, **157**, 2591-2602.

Graham, E.M. (2012) The energetics of scleractinian coral larvae and implications for dispersal. PHD thesis. James Cook University. Available at: http://researchonline.jcu.edu.au/29583/ (Accessed: 28 May 2015).

Grasso, L. C., Negri, A. P., Fôret, S., Saint, R., Hayward, D. C., Miller, D. J. & Ball, E. E. (2011) The biology of coral metamorphosis: Molecular responses of larvae to inducers of settlement and metamorphosis. *Developmental Biology*, **353**, 411–419.

Green, D., Edmunds, P. & Carpenter, R. (2008) Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. *Marine Ecology Progress Series*, **359**, 1–10.

Greger, K., Swoger, J., & Stelzer, E. H. K. (2007) Basic building units and properties of a fluorescence single plane illumination microscope. *Review of Scientific Instruments*, **78**, e023705.

Gruber, D. F., Kao, H.-T., Janoschka, S., Tsai, J. & Pieribone, V. A. (2008) Patterns of fluorescent protein expression in Scleractinian Corals. *The Biological Bulletin*, **215**, 143–154.

Gutiérrez-Heredia, L., D'Helft, C. & Reynaud, E. G. (2015) Simple methods for interactive 3D modeling, measurements, and digital databases of coral skeletons. *Limnology and Oceanography: Methods*, **13**, e10017.

Gutner-Hoch, E., Schneider, K., Stolarski, J., Domart-Coulon, I., Yam, R., Meibom, A., & Levy, O. (2016) Evidence for rhythmicity pacemaker in the calcification process of Scleractinian coral. *Scientific reports*, **6**.

Hadfield, M. G., Meleshkevitch, E. A. & Boudko, D. Y. (2000) The Apical Sensory Organ of a Gastropod Veliger Is a Receptor for Settlement Cues, *Biological Bulletin*, **198**.

Hadfield, M. G., Carpizo-Ituarte, E. J., del Carmen, K., & Nedved, B. T. (2001) Metamorphic competence, a Major Adaptive Convergence in Marine Invertebrate larvae. *American Zoologist*, **41**, 1123–1131.

Hagedorn, M., Pan, R., Cox, E.F., Hollingsworth, L., Krupp, D., Lewis, T.D., Leong, J.C., Mazur, P., Rall, W.F., MacFarlane, D.R. & Fahy, G. (2006) Coral larvae conservation: physiology and reproduction. *Cryobiology*, **52**, 33-47.

Hagedorn, M., Carter, V., Zuchowicz, N., Phillips, M., Penfield, C., Shamenek, B., Vallen, E.A., Kleinhans, F.W., Peterson, K., White, M. & Yancey, P.H. (2015) Trehalose is a chemical attractant in the establishment of coral symbiosis. *PloS ONE*, **10**, e0117087.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E. & Fujita, R. (2008) A global map of human impact on marine ecosystems. *Science*, **319**, 948-952.

Hardin, P. E., Hall, J. C. & Rosbash, M. (1990) Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. *Nature*, **343**, 536–540.

Harriott, V. J. (1983) Reproductive ecology of four scleratinian species at lizard island, great barrier reef. *Coral Reefs*, **2**, 9–18.

Harrison, P. & Ward, S. (2001) Elevated levels of nitrogen and phosphorus reduce fertilisation success of gametes from scleractinian reef corals. *Marine Biology*, **139**, 1057-1068.

Harrison, P. L. (2011) Sexual Reproduction of Scleractinian Corals. *Coral Reefs: An Ecosystem in Transition*, Springer Netherlands, 59- 85.

Harrison, P. L., Babcock, R. C., Bull, G. D., Oliver, J. K., Wallace, C. C., & Willis, B. L. (1984) Mass Spawning in Tropical Reef Corals. *Science*, **223**, 1186–1189.

Harrison, P.L. (1985) Sexual characteristics of scleractinian corals: systematic and evolutionary implications. *Proceedings of the 5th International Coral Reef Congress*, Tahiti, **4**, 337-342.

Harrison, P.L. (1988) Pseudo-gynodioecy: an unusual breeding system in the scleractinian coral *Galaxea fascicularis*. *Proceedings of the 6th International Coral Reef Symposium*, Townsville, **2**, 699-705.

Harrison, P. L., & Wallace, C. C. (1990) Reproduction, dispersal and recruitment of scleractinian corals. In Z. Dubinsky (Ed.), *Coral Reef Ecosystems, Ecosystems of the World*. Amsterdam: Elsevier Science Publishers.

Hastings, J. W. (2007) The Gonyaulax clock at 50: Translational control of Circadian expression. *Cold Spring Harbor Symposia on Quantitative Biology*, **72**, 141–144.

Hayashibara, T., Shimoike, K., Kimura, T., Hosaka, S., Heyward, A., Harrison, P., Kudo, K. & Omori, M. (1993) Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Marine Ecology Progress Series*, **101**, 253-262.

Helman, Y., Natale, F., Sherrell, R. M., LaVigne, M., Starovoytov, V., Gorbunov, M. Y. & Falkowski, P. G. (2007) Extracellular matrix production and calcium carbonate precipitation by coral cells in vitro. *Proceedings of the National Academy of Sciences*, **105**, 54–58.

Hendricks, W. D., Byrum, C. A., & Meyer-Bernstein, E. L. (2012) Characterization of Circadian behavior in the starlet sea Anemone, *Nematostella vectensis*. *PLoS ONE*, **7**, e46843.

Hennige, S. J., Suggett, D. J., Warner, M. E., McDougall, K. E. & Smith, D. J. (2009) Photobiology of *Symbiodinium* revisited: Bio-physical and bio-optical signatures. *Coral Reefs*, **28**, 179–195.

Hidaka, M. (1985) Tissue compatibility between colonies and between newly settled larvae of *Pocillopora damicornis*. *Coral Reefs*, **4**, 111–116.

Hoadley, K. D., Szmant, A. M., & Pyott, S. J. (2011) Circadian clock gene expression in the coral *Favia fragum* over Diel and lunar reproductive cycles. *PLoS ONE*, **6**, e19755.

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, **50**.

Hoegh-Guldberg, O., & Ridgway, T. (2016) Coral bleaching hits great barrier reef as global temperatures soar. *Green Left Weekly*, **1090**, 10.

Huijbers, C. M., Nagelkerken, I., Lössbroek, P. A. C., Schulten, I. E., Siegenthaler, A., Holderied, M. W. & Simpson, S.D. (2012) A test of the senses: Fish select novel habitats by responding to multiple cues. *Ecology*, **93**, 46–55.

Huisken, J. & Stainier, D. Y. R. (2009) Selective plane illumination microscopy techniques in developmental biology. *Development*, **136**, 1963–1975.

Hunter, C. L. (1988) Environmental cues controlling spawning in two Hawaiian corals, *Montipora verrucosa* and *M. dilatata*. *Proceedings of the 6th International Coral Reef Symposium*, Townsville, **2**, 727-732.

Hylkema, A., Wijgerde, T. & Osinga, R. (2015) Decreased growth of *Stylophora pistillata* with nutrient-driven elevated zooxanthellae density is largely explained by DIC limitation. *Advanced Aquarist*, **14**.

Ilan, M. & Loya, Y. (1990) Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. From the Red Sea. *Marine Biology*, **105**, 25–31.

Isomura, N., & Nishihira, M. (2001) Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. *Coral Reefs*, **20**, 309–315.

Johnson, K. (1992) Synchronous planulation of *Manicina areolata* (Scleractinia) with lunar periodicity. *Marine Ecology Progress Series*, **87**, 265–273.

Jokiel, P. L., Maragos, J. E. & Franzisket, L. (1978) Coral growth: buoyant weight technique. *Coral reefs: research methods*. UNESCO, Paris, 529-541.

Jokiel, P. L., Ito, R. Y., & Liu, P. M. (1985) Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*. *Marine Biology*, **88**, 167–174.

Jokiel, P. L. & Morrissey, J. I. (1986) Influence of size on primary production in the reef coral *Pocillopora damicornis* and the macroalga *Acanthophora spicifera*. *Marine Biology*, **91**, 15-26.

Kaniewska, P., Alon, S., Karako-Lampert, S., Hoegh-Guldberg, O. & Levy, O. (2015) Signalling cascades and the importance of moonlight in coral broadcast mass spawning. *eLife*, **4**.

Kenkel, C. D., Traylor, M. R., Wiedenmann, J., Salih, A. & Matz, M. V. (2011) Fluorescence of coral larvae predicts their settlement response to crustose coralline algae and reflects stress. *Proceedings of the Royal Society B*, **278**, 2691–2697.

Kinzie, R. A., Jokiel, P. L. & York, R. (1984) Effects of light of altered spectral composition on coral zooxanthellae associations and on zooxanthellae in vitro. *Marine Biology*, **78**, 239–248.

Koehl, M. A. R. & Hadfield, M. G. (2004) Soluble settlement cue in slowly moving water within coral reefs induces larval adhesion to surfaces. *Journal of Marine Systems*, **49**, 75–88.

Kopp, C., Domart-Coulon, I., Barthelemy, D. & Meibom, A. (2016) Nutritional input from dinoflagellate symbionts in reef-building corals is minimal during planula larval life stage. *Science Advances*, **2**, e1500681–e1500681.

Kramarsky-Winter, E., Fine, M. & Loya, Y. (1997) Coral polyp expulsion. *Nature*, **387**, 137–137.

Kühl, M., Cohen, Y., Dalsgaard, T., Jørgensen, B. & Revsbech, N. (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. *Marine Ecology Progress Series*, **117**, 159–172.

Kump, L., Bralower, T., & Ridgwell, A. (2009) Ocean Acidification in deep time. *Oceanography*, **22**, 94–107.

Laissue, P. P, Stephenson, S. Yan, G., Chen, Q., Craggs, J. & Smith, D. J (unpubl.) Swept light sheet for live, long-term microscopy of large samples at minimal phototoxicity.

LaJeunesse, T.C., Smith, R., Walther, M., Pinzón, J., Pettay, D.T., McGinley, M., Aschaffenburg, M., Medina-Rosas, P., Cupul-Magaña, A.L., Pérez, A.L. & Reyes-Bonilla, H. (2010) Host–symbiont recombination versus natural selection in the response of coral–dinoflagellate symbioses to environmental disturbance. *Proceedings of the Royal Society of London*, p.rspb20100385.

Leletkin, V.A. & Popova, L.I. (2005) Light absorption by carotenoid peridinin in zooxanthellae cell and setting down of hermatypic coral to depth. *Zhurnal obshcheĭ biologii*, **66**, 251-7.

Levin, L. A. (2006) Recent progress in understanding larval dispersal: New directions and digressions. Available at: http://dx.doi.org/10.1093/icb%2Ficj024 (Accessed: 28 May 2015).

Levy, O., Dubinsky, Z., Schneider, K., Achituv, Y., Zakai, D. & Gorbunov, M. Y. (2004) Diurnal hysteresis in coral photosynthesis. *Marine Ecology Progress Series*, **268**, 105-117.

Levy, O., Appelbaum, L., Leggat, W., Gothlif, Y., Hayward, D. C., Miller, D. J. & Hoegh-Guldberg, O. (2007) Light-responsive Cryptochromes from a simple Multicellular animal, the coral *Acropora millepora*. *Science*, **318**, 467–470.

Lin, C. & Todo, T. (2005) The Cryptochromes. *Genome Biology*, **6**, 220.

Lirman, D. (2000) Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. *Journal of Experimental Marine Biology and Ecology*, **251**, 41-57.

Little, A. F., van Oppen, M. & Willis, B. (2004) Flexibility in Algal Endosymbioses shapes growth in reef Corals. *Science*, **304**, 1492–1494.

Mangubhai, S., & Harrison, P. (2009) Extended breeding seasons and asynchronous spawning among equatorial reef corals in Kenya. *Marine Ecology Progress Series*, **374**, 305–310.

Marubini, F. & Davies, P. S. (1996) Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. *Marine Biology*, **127**, 319–328.

Mason, B., Schmale, M., Gibbs, P., Miller, M. W., Wang, Q., Levay, K. & Slepak, V. Z. (2012) Evidence for multiple Phototransduction pathways in a reef-building coral. *PLoS ONE*, **7**, e50371.

Mass, T., Drake, J. L., Haramaty, L., Rosenthal, Y., Schofield, O. M. E., Sherrell, R. M. & Falkowski, P. G. (2012) Aragonite Precipitation by "Proto-Polyps" in coral cell cultures. *PLoS ONE*, **7**, e35049.

Mazel, D. (1997) Ecological identity: Becoming a reflective environmentalist. *Interdisciplinary Studies in Literature and Environment*, **4**, 140–142.

McDowall, D. (1969) Summary. Scale effects in models of shallow tidal water. *Proceedings of the Institution of Civil Engineers*, **43**, 90.

Miller, K. & Mundy, C. (2003) Rapid settlement in broadcast spawning corals: implications for larval dispersal. *Coral Reefs*, **22**, 99-106.

Morgan, S.G. (1995) *The timing of larval release*. In: McEdward LR (ed) The ecology of marine invertebrate larvae. CRC Press, Boca Raton, FL, 157–191.

Muir, P., Wallace, C., Bridge, T. C. L., & Bongaerts, P. (2015) Diverse Staghorn coral fauna on the Mesophotic reefs of north-east Australia. *PLoS ONE*, **10**, e0117933.

Muscatine, L., Falkowski, P. G., Dubinsky, Z., Cook, P. A. & McCloskey, L. R. (1989) The effect of external nutrient resources on the population dynamics of Zooxanthellae in a reef coral. *Proceedings of the Royal Society*, **236**, 311–324.

Muscatine, L., Falkowski, P. G., Porter, J. W. & Dubinsky, Z. (1984) Fate of Photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proceedings of the Royal Society*, **222**, 181–202.

Muscatine, L., R. McCloskey, L. & E. Marian, R. (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration1. *Limnology and Oceanography*, **26**, 601–611.

Müller, W. A. & Leitz, T. (2002) Metamorphosis in the Cnidaria. *Canadian Journal of Zoology*, **80**, 1755–1771.

Naylor, E. (2015) Book review: Annual, lunar, and tidal clocks: Patterns and mechanisms of nature's enigmatic rhythms (ed. By H. Numata & B. Helm). *Journal of Biological Rhythms*, **30**, 355–356.

Negri, A., Webster, N., Hill, R. & Heyward, A. (2001) Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. *Marine Ecology Progress Series*, **223**, 121–131.

Norström, A.V. and Sandström, M. (2010) Lipid content of *Favia fragum* larvae: changes during planulation. *Coral Reefs*, **29**, 793–795.

Olofsson, H., Ripa, J. & Jonzen, N. (2009) Bet-hedging as an evolutionary game: The trade-off between egg size and number. *Proceedings of the Royal Society*, **276**, 2963–2969.

Oswald, F., Schmitt, F., Leutenegger, A., Ivanchenko, S., D'Angelo, C., Salih, A., Maslakova, S., Bulina, M., Schirmbeck, R., Nienhaus, G. U. & Matz, M. V. (2007) Contributions of host and symbiont pigments to the coloration of reef corals. *FEBS Journal*, **274**, 1102–1122.

Pearse, J.S. (1974) Reproductive patterns of tropical reef animals: three species of sea urchins. *Proceedings of the 2nd International Coral Reef Symposium*, Brisbane, **1**, 235-240.

Palmer, C. V., Mydlarz, L. D. & Willis, B. L. (2008) Evidence of an inflammatory-like response in non-normally pigmented tissues of two scleractinian corals. *Proceedings of the Royal Society*, **275**, 2687–2693.

Palmer, C. V., Roth, M. S. & Gates, R. D. (2009) Red fluorescent protein responsible for Pigmentation in Trematode-Infected *Porites compressa* Tissues. *The Biological Bulletin*, **216**, 68–74.

Palumbi, S. R. (2003) Population genetics, Demographic connectivity and the design of Marine Reserves. *Ecological Applications*, **13**,146–158.

Pernice, M., Meibom, A., Van Den Heuvel, A., Kopp, C., Domart-Coulon, I., Hoegh-Guldberg, O. & Dove, S. (2012) A single-cell view of ammonium assimilation in coral–dinoflagellate symbiosis. *The ISME Journal*, **6**, 1314–1324.

Petersen, D., Laterveer, M. & Visser, G. (2007) Sexual recruitment of the corals *Favia fragum* and *Agaricia humilis* in a 30^{m3} exhibit aquarium: Species-specific limitations and implications on reproductive ecology. *Zoo Biology*, **26**, 75–91.

Price, N. (2010) Habitat selection, facilitation, and biotic settlement cues affect distribution and performance of coral recruits in French Polynesia., *Oecologia*, **163**, 747–758.

Provencio, I., Jiang, G., De Grip, W. J., Hayes, W. P. & Rollag, M. D. (1998) Melanopsin: An opsin in melanophores, brain, and eye. *Proceedings of the National Academy of Sciences*, **95**, 340–345.

Putnam, H. M., Edmunds, P. J. & Fan, T.-Y. (2008) Effect of Temperature on the Settlement Choice and Photophysiology of Larvae from the Reef Coral *Stylophora pistillata*. *The Biological Bulletin*, **215**, 135-142.

Raimondi, P.T. & Morse, A.N.C. (2000) The Consequences of Complex Larval Behavior in a Coral, *Ecology*, **81**, 3193-3211.

Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I. & Rosenberg, E. (2006) The coral probiotic hypothesis. *Environmental Microbiology*, **8**, 2068–2073.

Reitzel, A. M., Behrendt, L. & Tarrant, A. M. (2010) Light Entrained rhythmic gene expression in the sea Anemone *Nematostella vectensis*: The evolution of the animal Circadian clock. *PLoS ONE*, **5**, e12805.

Reitzel, A. M., Tarrant, A. M., & Levy, O. (2013) Circadian clocks in the Cnidaria: Environmental Entrainment, molecular regulation, and Organismal outputs. *Integrative and Comparative Biology*, **53**, 118-130.

Richmond, R. (1987) Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. *Marine Biology*, **93**,527-533.

Richmond, R., & Hunter, C. (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Marine Ecology Progress Series*, **60**, 185–203.

Richmond, R. H. (1997) Reproduction and recruitment in corals: Critical links in the persistence of reefs. In: Birkeland C (ed) Life and death of coral reefs. Chapman & Hall, New York, 175-197.

Riddle, D. (2009) Environmental stability: Nature v. Aquarium: Photosynthetically Active Radiation, Ultraviolet Radiation, Dissolved Oxygen, and ORP, part 2. *Advanced Aquarist*, **8**.

Rinkevich, B. & Loya, Y. (1983) Oriented translocation of energy in grafted reef corals. *Coral Reefs*, **1**, 243-247.

Rinkevich, B. (2004) Allorecognition and xenorecognition in reef corals: a decade of interactions. In *Coelenterate Biology 2003*. Springer Netherlands, 443- 450.

Rinkevich, B., Shaish, L., Douek, J. & Ben-Shlomo, R. (2016) Venturing in coral larval chimerism: A compact functional domain with fostered genotypic diversity. *Scientific Reports*, **6**, 19493.

Ritson-Williams, R., Arnold, S., Fogarty, N., Steneck, R. S., Vermeij, M. & Paul, V. J. (2009) New perspectives on ecological mechanisms affecting coral recruitment on reefs. *Smithsonian Contributions to the Marine Sciences*, **38**, 437–457.

Rivera, H. E., & Goodbody-Gringley, G. (2014) Aggregation and cnidae development as early defensive strategies in *Favia fragum* and *Porites astreoides*. *Coral Reefs*, **33**, 1079-1084.

Rocha, R. J. M., Pimentel, T., Serôdio, J., Rosa, R. & Calado, R. (2013) Comparative performance of light emitting plasma (LEP) and light emitting diode (LED) in ex situ aquaculture of scleractinian corals. *Aquaculture*, **402**, 38–45.

Rodolfo-Metalpa, R., Peirano, A., Houlbrèque, F., Abbate, M. & Ferrier-Pagès, C. (2007) Effects of temperature, light and heterotrophy on the growth rate and budding of the temperate coral *Cladocora caespitosa. Coral Reefs*, **27**, 17–25.

Rodriguez-Lanetty, M., Krupp, D. & Weis, V. (2004) Distinct ITS types of Symbiodinium in Clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis. *Marine Ecology Progress Series*, **275**, 97–102.

Roenneberg, T. & Deng, T.-S. (1997) Photobiology of the Gonyaulax circadian system. I. Different phase response curves for red and blue light. *Planta*, **202**, 494–501.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R. & Zilber-Rosenberg, I. (2007) The role of microorganisms in coral health, disease and evolution. *Nature Review Microbiology*, **5**, 355–362.

Roth, M. S., Fan, T.-Y. & Deheyn, D. D. (2013) Life history changes in coral fluorescence and the effects of light intensity on larval physiology and settlement in *Seriatopora hystrix*. *PLoS ONE*, **8**, e59476.

Rylaarsdam, K. (1983) Life histories and abundance patterns of colonial corals on Jamaican reefs. *Marine Ecology Progress Series*, **13**, 249–260.

Sakai, K. (1997) Gametogenesis, spawning and planula brooding by the reef coral *Goniastrea aspera* (Scleractinia) in Okinawa, Japan. *Marine Ecology Progress Series*, **151**, 67-72.

Salih, A., Larkum, A., Cox, G., Kühl, M., & Hoegh-Guldberg, O. (2000) Fluorescent pigments in corals are photoprotective. *Nature*, **408**, 850–853.

Sammarco, P. W. (1982) Echinoid grazing as a structuring force in coral communities: Whole reef manipulations. *Journal of Experimental Marine Biology and Ecology*, **61**, 31–55.

Savage, A. M., Goodson, M. S., Visram, S., Trapido-Rosenthal, H., Wiedenmann, J. & Douglas, A. E. (2002) Molecular diversity of symbiotic algae at the latitudinal margins of their distribution: dinoflagellates of the genus *Symbiodinium* in corals and sea anemones. *Marine Ecology Progress Series*, **244**, 17-2.

Shlesinger, Y. & Loya, Y. (1985) Coral community reproductive patterns: Red Sea versus the Great Barrier Reef. *Science*, **228**, 1333-1335.

Schoepf, V., Grottoli, A. G., Levas, S. J., Aschaffenburg, M. D., Baumann, J. H., Matsui, Y., & Warner, M. E. (2015) Annual coral bleaching and the long-term recovery capacity of coral. *Proceedings Royal Society Biology*, **282** , 1887

Schrameyer, V., Wangpraseurt, D., Hill, R., Kühl, M., Larkum, A. W. & Ralph, P. J. (2014) Light respiratory processes and gross photosynthesis in two scleractinian corals. *PloS ONE*, **9**, e110814.

Sebens, K. P. & DeRiemer, K. (1977) Diel cycles of expansion and contraction in coral reef anthozoans. *Marine Biology*, **43**, 247–256.

Shapiro, O. H., Kramarsky-Winter, E., Gavish, A. R., Stocker, R., & Vardi, A. (2016) A coral-on-a-chip microfluidic platform enabling live-imaging microscopy of reef-building corals. *Nature Communications*, **7**, 10860.

Simpson, C. J. (1991) Mass spawning of corals on Western Australian reefs and comparisons with the Great Barrier Reef. *Journal of the Royal Society of Western Australia*, **74**, 85-91.

Somers, D. E. (1998) Phytochromes and Cryptochromes in the Entrainment of the Arabidopsis Circadian clock. *Science*, **282**, 1488–1490.

Song, L., Varma, C. A., Verhoeven, J. W. & Tanke, H. J. (1996) Influence of the triplet excited state on the photobleaching kinetics of fluorescein in microscopy. *Biophysical Journal*, **70**, 2959–2968.

Sorek, M., Díaz-Almeyda, E. M., Medina, M., & Levy, O. (2014). Circadian clocks in symbiotic corals: The duet between Symbiodinium algae and their coral host. *Marine Genomics*, **14**, 47–57.

Sorek, M. & Levy, O. (2012) Influence of the quantity and quality of light on Photosynthetic periodicity in coral Endosymbiotic algae. *PLoS ONE*, **7**, e43264.

Sorek, M., & Levy, O. (2014) Coral Spawning Behavior and Timing. In *Annual, Lunar, and Tidal Clocks*. Springer Japan, 81-97.

Stap, L. B., de Boer, B., Ziegler, M., Bintanja, R., Lourens, L. J., & van de Wal, R. S. (2016) CO 2 over the past 5 million years: Continuous simulation and new δ 11 B-based proxy data. *Earth and Planetary Science Letters*, **439**, 1-10.

Stat, M., Carter, D., & Hoegh-Guldberg, O. (2006) The evolutionary history of Symbiodinium and scleractinian hosts—symbiosis, diversity, and the effect of climate change. *Perspectives in Plant Ecology, Evolution and Systematics*, **8**, 23-43.

Stephenson, T. A. (1933) Lunar periodicity in reproduction. *Nature*, **131**, 622–622.

Stoddart, J. A. (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. *Marine. Biology*, **76**, 279–284.

Strader, M. E., Davies, S. W. & Matz, M. V. (2015) Differential responses of coral larvae to the colour of ambient light guide them to suitable settlement microhabitat. *Royal Society Open Science*, **2**, 150358.

Sverdrup, H. U., Johnson, M. W., & Fleming, R. H. (1942) The Oceans: Their physics, chemistry, and general biology (Vol. 7). New York: Prentice-Hall.

Szabó, M., Wangpraseurt, D., Tamburic, B., Larkum, A.W., Schreiber, U., Suggett, D.J., Kühl, M. & Ralph, P.J. (2014) Effective light absorption and absolute electron transport rates in the coral *Pocillopora damicornis*. *Plant Physiology and Biochemistry*, **83**, 159-167.

Szmant-Froelich, A. M., Reutter M. & Riggs, L. (1985) Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. *Bulletin of Marine Science*, **37**, 880-892.

Szmant-Froelich, A. (1985) The effect of colony size on the reproductive ability of the Caribbean coral *Montastrea annularis* (Ellis and Solander). *Proceedings of the 5th International Coral Reef Symposium* ,**4**, 295-300.

Szmant- Froelich, A. M. (1986) Reproductive ecology of Caribbean reef corals. *Coral Reefs*, **5**, 43–53.

Szmant- Froelich, A. & Meadows, M. (2006) Developmental changes in coral larval buoyancy and vertical swimming behaviour: Implications for dispersal and connectivity. *Proceedings of 10th International Coral Reef Symposium*, 431- 437.

Takabayashi, M., Adams, L. M., Pochon, X. & Gates, R. D. (2011) Genetic diversity of free-living *Symbiodinium* in surface water and sediment of Hawai'i and Florida. *Coral Reefs*, **31**, 157–167.

Tambutté, E., Venn, A.A., Holcomb, M., Segonds, N., Techer, N., Zoccola, D., Allemand, D. & Tambutté, S. (2015) Morphological plasticity of the coral skeleton under CO2-driven seawater acidification. *Nature communications*, **6**.

Tay, Y. C., Guest, J. R., Chou, L. M. & Todd, P. A. (2011) Vertical distribution and settlement competencies in broadcast spawning coral larvae: Implications for dispersal models. *Journal of Experimental Marine Biology and Ecology*, **409**, 324–330.

Tchernov, D., Gorbunov, M. Y., de Vargas, C., Narayan Yadav, S., Milligan, A. J., Haggblom, M. & Falkowski, P. G. (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences*, **101**, 13531–13535.

Tebben, J., Motti, C.A., Siboni, N., Tapiolas, D.M., Negri, A.P., Schupp, P.J., Kitamura, M., Hatta, M., Steinberg, P.D. & Harder, T. (2015) Chemical mediation of coral larval settlement by crustose coralline algae. *Scientific reports*, **5**.

Tessmar- Raible, K., Laboratories, M. P. F., V., Raible, F., & Arboleda, E. (2011). Another place, another timer: Marine species and the rhythms of life. *BioEssays*, **33**, 165–172.

Tran, C. & Hadfield, M. G. (2012) Are G-protein-coupled receptors involved in mediating larval settlement and metamorphosis of coral planulae?. *Biological Bulletin*, **222**, 128– 136.

Tran, C. & Hadfield, M. G. (2013) Localization of sensory mechanisms utilized by coral planulae to detect settlement cues. *Invertebrate Biology*, **132**, 195–206.

Treibitz, T., Neal, B. P., Kline, D. I., Beijbom, O., Roberts, P. L. D., Mitchell, B. G. & Kriegman, D. (2015) Wide field-of-view fluorescence imaging of coral reefs. *Scientific Reports*, **5**, 7694.

Vandermeulen, J. H. (1974) Studies on reef corals. II. Fine structure of planktonic planula larva of *Pocillopora damicornis*, with emphasis on the aboral epidermis, *Marine Biology*, **27**, 239–249.

Vandermeulen, J. H. (1975) Studies on reef corals. III. Fine structural changes of calicoblast cells in *Pocillopora damicornis* during settling and calcification. *Marine Biology*, **31**, 69–77.

Van Woesik, R. (2009) Calm before the spawn: global coral spawning patterns are explained by regional wind fields. *Proceedings of the Royal Society of London,*, rspb20091524.

Verkhusha, V. V. & Lukyanov, K. A. (2004) The molecular properties and applications of Anthozoa fluorescent proteins and chromoproteins. *Nature Biotechnology*, **22**, 289–296.

Vermeij, M.J.A., Smith, J.E., Smith, C.M., Thurber, V.R. & Sandin, S.A. (2009) Survival and settlement success of coral planulae: independent and synergistic effects of macroalgae and microbes. *Oecologia*, 325–336.

Vermeij, M. J., Marhaver, K. L., Huijbers, C. M., Nagelkerken, I., & Simpson, S. D. (2010) Coral larvae move toward reef sounds. *PloS ONE*, **5**, e10660.

Veron, J. E. N. (1995) Corals in space and time: the biogeography and evolution of the Scleractinia. Cornell University Press.

Veron, J.E.N. (2000) Reef Evolution, *Science*, **287**, 811- 812.

Wang, L.H., Liu, Y.H., Ju, Y.M., Hsiao, Y.Y., Fang, L.S. & Chen, C.S. (2008) Cell cycle propagation is driven by light–dark stimulation in a cultured symbiotic dinoflagellate isolated from corals. *Coral Reefs*, **27**, 823-835.

Ward, S. (1992) Evidence for broadcast spawning as well as brooding in the scleractinian coral *Pocillopora damicornis*. *Marine Biology*, **112**, 641–646.

Ward, S. & Harrison, P. (2000) Changes in gametogenesis and fecundity of acroporid corals that were exposed to elevated nitrogen and phosphorus during the ENCORE experiment. *Journal of Experimental Marine Biology and Ecology*, **246**, 179-221.

Warner, M. E., M. P. Lesser & P. J. Ralph, (2010) Chlorophyll Fluorescence in Reef Building Corals. In Suggett, D. J., O. Prášil & M. A. Borowitzka (eds), Chlorophyll a fluorescence in aquatic sciences: methods and applications. developments in applied phycology, Vol. 4. Springer, The Netherlands: 209–222.

Wijgerde, T., van Melis, A., Silva, C. I. F., Leal, M. C., Vogels, L., Mutter, C. & Osinga, R. (2014) Red light Represses the Photophysiology of the Scleractinian coral *Stylophora pistillata*. *PLoS ONE*, **9**, e92781.

Wilkinson, C. (2008) Status of coral reefs of the world: 2008. *Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville, Australia*, 296.

Willis, B. L., Babcock, R. C., Harrison, P. L., Oliver, J. K. & Wallace, C. C. (1985) Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. *Proceedings of the 5th International Coral Reef Congress*, Tahiti, **4**, 343-348.

Winkler, N. S., Pandolfi, J. M. & Sampayo, E. M. (2015) *Symbiodinium* identity alters the temperature-dependent settlement behaviour of *Acropora millepora* coral larvae before the onset of symbiosis. *Proceedings of the Royal Society of London* , **282**, 2014-2260.

Wilson, J., & Harrison, P. (2005) Post-settlement mortality and growth of newly settled reef corals in a subtropical environment. *Coral Reefs*, **24**, 418-421.

Wooldridge, S. A. (2014) Assessing coral health and resilience in a warming ocean: Why looks can be deceptive. *BioEssays*, **36**, 1041–1049.

Wooldridge, S. A. (2016) Excess seawater nutrients, enlarged algal symbiont densities and bleaching sensitive reef locations: 1. Identifying thresholds of concern for the great barrier reef, Australia. *Marine Pollution Bulletin*.

Wright, A., Bubb, W. A., Hawkins, C. L. & Davies, M. J. (2002) Singlet Oxygen–mediated protein oxidation: Evidence for the formation of Reactive side chain Peroxides on tyrosine Residues. *Photochemistry and Photobiology*, **76**, 35.

Zakai, D., Dubinsky, Z., Avishai, A., Caaras, T. & Chadwick, N. (2006) Lunar periodicity of planula release in the reef-building coral *Stylophora pistillata*. *Marine Ecology Progress Series*, **311**, 93–102.

Zhu, H., Sauman, I., Yuan, Q., Casselman, A., Emery-Le, M., Emery, P. & Reppert, S. M. (2008) Cryptochromes define a novel Circadian clock mechanism in monarch butterflies that may underlie sun compass navigation. *PLoS Biology*, **6**, e4.

7.0 APPENDIX

1. Data from HOBO loggers: light intensity and temperature measurements in lunar treatment tank (top) recorded for 6 months, and CNM treatment tank (bottom) recorded for 3 months