A holistic approach towards understanding population dynamics of a coral reef sponge

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Statement on the contribution of others

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Abstract

Understanding processes that contribute to population maintenance is critical to the management and conservation of species. Sponges (Phylum Porifera) are an evolutionary and ecologically significant group. However, information on biological and ecological processes, such as reproduction, larval dispersal, settlement, recruitment, survival and growth that influence sponge population dynamics is surprisingly limited. This study aimed to quantify pre- and post-settlement processes that affect the demographics and distribution of *Carteriospongia foliascens* (Thorectidae, Phyllospongiinae), a common coral reef sponge widely distributed from intertidal to mesophotic zones of the Great Barrier Reef (GBR). Intertidal populations of *C. foliascens* from the inshore central GBR were selected for this population study. Extreme morphological plasticity induced by local environmental conditions is inherent in sponges, hence it is imperative to investigate hidden diversity and identify robust taxonomic units prior to ecological studies to avoid the confounding interpretation of data due to species misidentification. Taxonomic and phylogenetic assessments of the study species and closely related taxa were therefore conducted, forming an essential preamble to the study proper.

Foliose keratose sponges of the sub-family Phyllospongiinae (Dictyoceratida, Thorectidae: *Strepsichordaia*, *Phyllospongia* and *Carteriospongia*) are commonly found in intertidal and subtidal habitats of the Indo-Pacific. Lacking spicules, these sponges can be difficult to differentiate due to insufficient reliable morphological characters for species delineation. Molecular phylogenies inferred from the nuclear Internal Transcribed Spacer 2 region (ITS2) and morphometrics (19 characters; 52 character states) were used to identify evolutionarily significant units (ESUs; sensu Moritz) within foliose Phyllosponginiids collected from seven geographic locations across tropical eastern and Western Australia (Chapter 2). The ITS2 topology was congruent with the tree derived from the Bayesian inference of discrete morphological characters thereby supporting expected taxonomic relationships at the genus level, and the identification of five ESUs. However, phylogenies inferred from the ITS2 marker revealed multiple sequence clusters, some of which were characterized by distinct morphological features and specific geographic ranges. Results are discussed in light of taxonomic incongruences within this study, hidden sponge diversity and the potential role of vicariant events on present day distribution patterns. The identity of

intertidal *C. foliascens* on the inshore GBR was confirmed and facilitated further assessments of population ecology.

Sexual reproduction is integral to our understanding of population dynamics. The mode of sexuality and development, seasonality, sex ratios, gametogenesis, reproductive output and size at sexual maturity were established for *C. foliascens*, in the central GBR, over two reproductive cycles (Chapter 3). A population sexual productivity index (PoSPi) integrating key reproductive parameters was formulated to compare population larval supply over time. *C. foliascens* is reproductive all year round, gonochoric and viviparous, with larvae developing asynchronously throughout the mesohyl. The influence of environmental parameters relevant to *C. foliascens* reproduction (i.e. sea surface temperature [SST], photoperiod and rainfall) was also examined, and SST found to have the most significant effect on phenology. The reproduction of *C. foliascens* exhibited annual mono-cyclic patterns closely resembling SST fluctuations. Reproductive output was depressed at SST less than 23°C, and increased at temperatures above 23°C. Peak sperm release occurred at temperatures above 25°C, while peak larval release occurred during the annual temperature maxima (> 28°C). A two-fold increase in the maximum production of larvae (PoSPi) in *C. foliascens* occurred in the second reproductive cycle, following a depressed PoSPi in the first cycle. This reduction in PoSPi in the first reproductive cycle was associated with increases of SST by 1.25°C and rainfall by 380%, coinciding with one of the strongest La Niña events on record.

Although *C. foliascens* has been reported from the intertidal to the mesophotic, the distribution of *C. foliascens* at inshore reefs of the GBR is restricted to the intertidal with no individuals evident in adjacent subtidal habitats. The potential influence of substrate limitation, and larval pre-settlement and settlement behaviour on adult population distribution were investigated (Chapter 4). The abundance of *C. foliascens* and substrate availability was first quantified to investigate the influence of substrate limitation on adult distribution. Pre-settlement processes of larval spawning, swimming speeds, phototaxis, vertical migration, and settlement to intertidal and subtidal substrate cues were also quantified. Notably, suitable settlement substrate (coral rubble) was not limiting in subtidal habitats. *C. foliascens* released up to 765 brooded larvae sponge^{-1} day^{-1} during the day, with larvae (80 % ± 5.77) being negatively phototactic and migrating to the bottom within 40 minutes from release. Subsequently, larvae (up to 58.67 % ± 2.91) migrated to the surface after the loss of the daylight cue (nightfall), and after 34 h post-release > 98.67 % (± 0.67) of larvae had adopted a benthic habit regardless of light conditions. Intertidal and subtidal biofilms initiated similar settlement responses, inducing faster (as early 6h post-release) and

more successful metamorphosis (> 60 %) than unconditioned surfaces. *C. foliascens* has a high supply of larvae and larval behaviours that support recruitment to the subtidal. The absence of *C. foliascens* in subtidal habitats at inshore reefs is therefore proposed to be a consequence of post-settlement mortalities.

Lastly, the effect of temperature, photoperiod, rainfall and habitat on post-settlement mortality, growth, asexual reproduction (fission), larval production and recruitment were assessed over 24 months at two locations characterized by distinct hydrodynamics (wave height, Chapter 5). Location-specific differences in growth, body size and fecundity for *C. foliascens* occurred and are attributed to water movement, with a higher wave height range corresponding to higher abundances of larger, more reproductive individuals. The positive effects of hydrodynamics on growth and the production of larvae also translated to higher levels of recruitment highlighting a potential stock-recruitment relationship in this species. *C. foliascens* showed no evidence of fission, and exhibited fluctuating growth trajectories in all size classes. Decreasing variability in growth corresponded with increasing size, reflecting growth trajectories for species with indeterminate growth. These results highlight the important role of habitat for post-settlement processes, production of larvae and recruitment in sessile invertebrate species with limited larval dispersal, such as brooding sponges.

In summary, *C. foliascens* at intertidal sites on inshore reefs of the central GBR is highly reproductive, producing larvae with behaviours suggestive of low dispersal potential and endogenous recruitment. Strict intertidal distributions are linked to post-settlement processes that limit the occurrence of sponges in adjacent subtidal habitats. These subtidal regions are less than a hundred metres away from areas of high *C. foliascens* abundance. The self-recruiting and self-regulating nature of intertidal *C. foliascens* combined with the stock-recruitment relationship supported by this study, indicate the critical impact of habitat characteristics and external environmental parameters in determining recruitment levels and population structure for *C. foliascens*. Taken as a whole, this thesis represents a collection of systematic investigations that are critical for a comprehensive understanding of population dynamics in sponges, and sessile benthic invertebrates in general. The integration of datasets from all life history stages from reproduction to larval recruitment, and growth and mortality in juvenile and adult life stages, allows for realistic assessments of the vulnerability of sponge populations to predicted environmental and climatic changes.

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Chapter 1

General introduction

1.1. Understanding population dynamics of sessile marine species

The effective management and conservation of sessile marine species requires a holistic understanding of the physical, biological and ecological processes influencing population dynamics and demographics (Levin 2006; Cowen and Sponaugle 2009; Pineda et al. 2009, 2010). Most sessile marine invertebrates possess a bi-phasic lifecycle, and rely on their motile larval stages for recruitment and population maintenance (Pineda et al. 2009, 2010). The term "recruitment" is broadly defined as the addition of new individuals to populations after successfully reaching an arbitrary life stage (e.g. juveniles, Connell 1985; Caley et al. 1996). For benthic marine invertebrates, population heterogeneity exists due to variations in recruitment, which is the combined effect from two distinct lines of life history processes acting on the motile larval phase (pre-settlement processes), and sessile juvenile and adult stages (post-settlement processes) (Figure 1.1, Connell 1985; Stoner 1990; Fraschetti et al. 2003).

1.1.1. Primary limitations to recruitment

Primary limitations to recruitment (sensu Fraschetti et al. 2003) are linked to the pre-settlement processes of supply, planktonic survival, dispersal, habitat selection and settlement of motile life stages, such as larvae. These processes can shape population abundance and distribution patterns in the absence of post-settlement mortalities (Hughes et

al. 2000; Jenkins 2005; Pineda et al. 2010). The supply of larvae can be influenced by the abundance of reproductive individuals (stock population), their fecundity and the level of successful fertilization (Figure 1.1, Box 1; Pineda et al. 2009). In species possessing feeding larvae, such as mussels and barnacles, the planktonic duration of larvae can span weeks to months and result in high larval dispersal potential (Strathmann 1985). For species which disperse over wide spatial scales, the supply of larvae into populations (i.e. the actual number of larvae available for recruitment) may represent externally produced larvae arriving into the population (import of larvae) and locally produced larvae not dispersed out of the population (export of larvae) (McQuaid and Lawrie 2005). In this case, the relationship between larval production (i.e. adult abundance and fecundity) and larval supply for recruitment within populations is often de-coupled. However, the link between larval production and supply is apparent in species producing larvae that are lecithotrophic and have limited dispersal (e.g. some species of scleractinian corals and ascidians; Stoner 1992; Carlon 2002). For these organisms, small changes in the fecundity of adults can cause major reductions in recruitment (Hughes et al. 2000), highlighting the importance of sexual reproduction and larval production for population persistence in some sessile invertebrates.

Due to the restricted swimming capabilities of marine invertebrate larvae (Chia et al. 1984; Maldonado 2006; Ettinger-Epstein et al. 2008; Whalan et al. 2008a; Abdul Wahab et al. 2011), hydrodynamic processes play an important role in larval dispersal (Figure 1.1, Box 2; Underwood et al. 2009; White et al. 2010). For larvae with early competencies for settlement, or which encounter environments with long water retention times (such as bays and some reefs), endogenous recruitment (i.e. self-recruitment within natal habitats) can be a common phenomenon (Gaines and Bertness 1992; Cetina-Heredia and Connolly 2011; Figueiredo et al. 2013). Nevertheless, innate larval behaviours in response to environmental factors indirectly contribute to horizontal larval dispersal. For example, phototaxis (attraction to light) can position larvae in bodies of water of differing flow regimes through light-directed vertical migration, facilitating dispersal over larger geographic scales (Maldonado and Young 1996; dos Santos et al. 2008; Jékely et al. 2008; Whalan et al. 2008a; Morgan and Fisher 2010; Abdul Wahab et al. 2011).

Larvae occupy the plankton for a duration of time prior to reaching competency for settlement (Pineda et al. 2009). The planktonic phase exposes larvae to various mortality risks including predation, physiological stresses and dispersal to unfavourable habitats, and can influence the number of viable larvae at settlement (Figure 1.1, Box 2, Graham et al. 2008; Pineda et al. 2009). Upon reaching competency, larvae return to the benthos in search of a suitable habitat for settlement (Figure 1.1, Box 2 and 3, Harii et al. 2002; Pineda et al.

2009). For sessile invertebrates, the availability of solid and stable substrates such as coral rubble or rock is critical for primary attachment to the benthos (Figure 1.1, Box 3; Duckworth and Wolff 2011). At the benthos, larvae explore the substrate and respond to a range of physical (e.g. water flow, Larsson and Jonsson 2006, micro-topography, Maldonado and Uriz 1988 and light, Mundy and Babcock 1988; Ettinger-Epstein et al. 2008), chemical (e.g. crustose coralline algae, Negri and Heyward 1999, microbial biofilms, Whalan et al. 2008a; Abdul Wahab et al. 2011; Whalan and Webster 2014), and conspecific cues (Toonen and Pawlik 2001) contributing to settlement and metamorphosis. Upon successful metamorphosis, these new settlers form the initial seed for recruitment (Figure 1.1, Box 3).

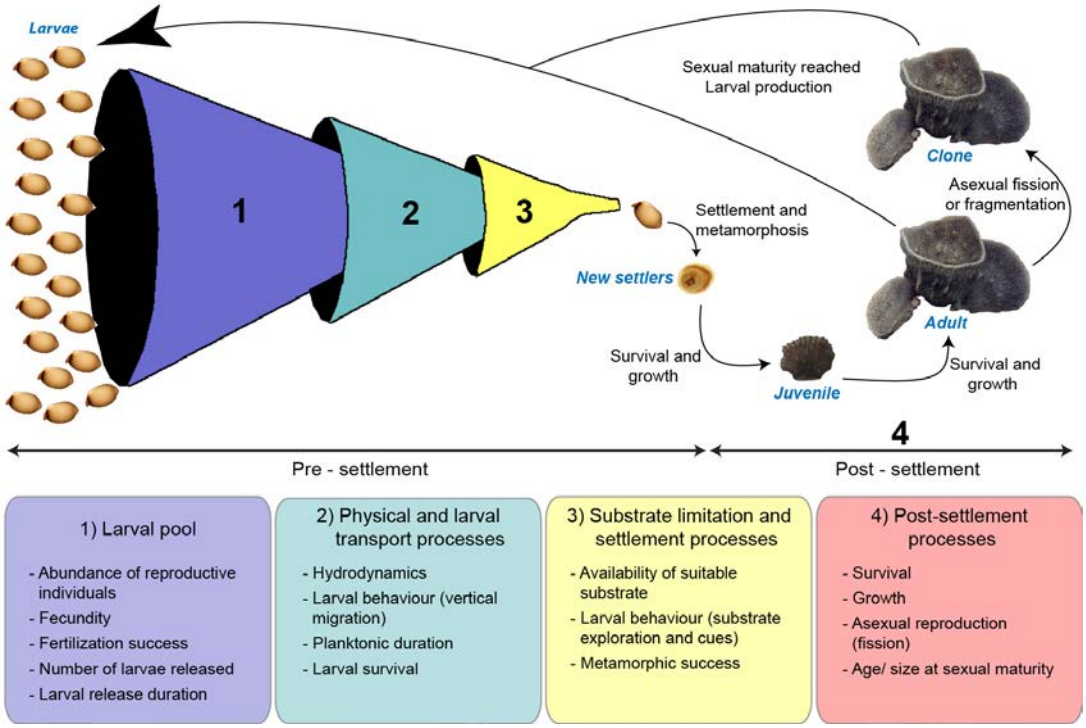

Figure 1.1: Schematic diagram of processes (pre-settlement and post-settlement) which are involved in recruitment limitation and population maintenance of sessile benthic marine invertebrates. Pre-settlement processes contributes to primary recruitment limitation and includes (Box 1) contributions to the larval pool, (Box 2) physical and larval transport processes influencing larval dispersal and (Box 3) benthic influences including substrate limitation and settlement cues. Secondary recruitment limitation (post-settlement processes, Box 4) then acts on individuals between metamorphosis and adulthood, and includes processes of survival, growth and asexual reproduction (i.e. fission). Only when individuals reach sexual maturity do they contribute back to this cycle. Modified from Pineda et al. (2009).

1.1.2. Secondary limitations to recruitment

After metamorphosis, post-settlement processes contribute to secondary limitations to recruitment (sensu Fraschetti et al. 2003), further sculpting populations (e.g. abundance, distribution and individual body size, Hunt and Scheibling 1997; Fraschetti et al. 2003).

Mortality, growth and asexual reproduction (for clonal invertebrates) influence both new settlers and existing individuals in populations. These processes are influenced by both biotic (i.e. fission, Zilberberg et al. 2006a; fragmentation, Maldonado and Uriz 1999; predation, Wulff 1995; Pawlik et al. 2013; Penin et al. 2011; competition, Aerts 2000; Vermeij and Sandin 2008; González-Rivero et al. 2012 and food availability, Frøhlich and Barthel 1997; Trussell et al. 2006) and abiotic factors (i.e. habitat surface topography, Walters and Wethey 1996; temperature, Nozawa and Harrison 2007; light quality, Thacker 2005; Erwin and Thacker 2008; Tremblay et al. 2014 and eutrophication, Fabricius 2005) (Figure 1.1, Box 4).

For some invertebrates such as sponges and coelenterates, asexual reproduction can influence population demographics through fission and fragmentation, adding new individuals (clones) to the population (Figure 1.1, Box 4; Miller and Ayre 2004; Henry and Hart 2005; Teixidó et al. 2009). For populations which rely on sexual reproduction and larval recruitment, the survival and growth of settlers (i.e. newly metamorphosed individuals) to reach recruitment size and sexual maturity is critical to population maintenance and persistence (Pineda et al. 2009). While mortality at the early post-settlement phase (e.g. juvenile) is usually very high (> 90% for marine invertebrates, Gosselin and Qian 1996; Hunt and Scheibling 1997; Wilson and Harrison 2005), size-dependent survival (where larger individuals have higher survival) in sexually derived individuals (e.g. scleractinian corals) has been demonstrated (Guest et al. 2014). Notably, individuals having higher growth rates would reach size refugia more rapidly therefore decreasing risks associated with processes of predation, indiscriminate grazing and competition (Penin et al. 2010; Dmitriew 2011).

Growth and survival of sessile benthic invertebrates are linked to environmental parameters (Figure 1.1, Box 4) (Duckworth et al. 2004; Wilson and Harrison 2005; Dmitriew 2011). Temperature plays a significant role for the growth of ectotherms. While higher ambient temperatures (within the species thermal tolerance range) generally correspond to higher growth rates for conspecifics due to associated increases in metabolic rates (Brockington and Clarke 2001; Gooding et al. 2009; Watson et al. 2014), anomalous temperatures exceeding species thermal tolerance leads to negative growth and death (De'ath et al. 2009; Cebrian et al. 2011). In addition, photoperiod and light quality are important for taxa that associate with photosynthetic symbionts, and rely on supplemental autotrophic nutrition for energetics, somatic growth and reproduction, such as scleractinian corals (Wooldridge 2010), sea anemones (Muscatine and Hand 1958; Harland and Davies 1995), tridacnid clams (Hawkins and Klumpp 1995; Jantzen et al. 2008), didemnid ascidians (López-Legentil et al. 2011) and some species of marine demosponges (Wilkinson 1983;

Erwin and Thacker 2008). Finally, sub-lethal levels of stresses associated with reduced salinity and eutrophication from terrestrial run-off can depress growth rates and reproduction, especially for species occupying intertidal zones (see review by Fabricius 2005 for a summary of stressors and associated physiological responses). Only once a size pertaining to sexual maturity is reached can individuals then contribute back to the larval pool, thus closing the population recruitment loop (Figure 1.1, Box 4).

1.2. Phylum Porifera: evolution, diversity and functional ecology

Phylum Porifera is the oldest extant metazoan group on Earth, and is a highly diverse taxa with up to 8600 species described (Philippe et al. 2009; Srivastava et al. 2010; Van Soest et al. 2012). Sponges are conspicuous components of fresh and marine aquatic environments (Hooper and Van Soest 2002), and can occur in tropical (Diaz and Rützler 2001; Hooper et al. 2002; Bannister et al. 2007), temperate (Roberts and Davis 1996; Sorokin et al. 2007) and polar regions (Dayton et al. 1974; Peters et al. 2009). Four classes exist within Porifera, Demospongiae, Hexactinellida (glass sponges), Calcarea (calcareous sponges) and the recently established Homoscleromorpha (Hooper and Van Soest 2002; Gazave et al. 2012; Van Soest et al. 2012). The Demospongiae is the most diverse class representing up to 83 % of total species, with the remaining classes forming the minority of total diversity (Hexactinellida [~ 8 %], Calcarea [~ 7 %] and Homoscleomorpha [~ 1 %]) (Van Soest et al. 2012).

Due to their abundance and wide geographic distribution, it is not surprising that sponges play significant functional roles in ecosystems (Diaz and Rützler 2001; Bell 2008; de Goeij et al. 2013). On coral reefs, the exceptional pumping capabilities of sponges underpin their ecological importance via bentho-pelagic coupling processes which contributes to carbon cycling (Reiswig 1981; Yahel et al. 2003; de Goeij et al. 2013), silica cycling (Frøhlich and Barthel 1997; Maldonado et al. 2005) and nitrogen cycling (Corredor et al. 1988; Hoffmann et al. 2009). These bentho-pelagic processes link the pelagic and benthic systems, and are critical for nutrient retention in oligotrophic systems such as coral reefs (de Goeij et al. 2013). Some of these processes, such as carbon and nitrogen cycling, are facilitated through primary and secondary production by prokaryotic symbionts such as heterotrophic bacteria and autotrophic cyanobacteria, highlighting the important roles of these associations to coral reefs (Taylor et al. 2007; Webster et al. 2012). Sponges are also important structural components of the benthos playing important roles in bio-erosion (Schönberg and Wilkinson 2001; Schönberg 2002) and reef consolidation (Wulff and Buss

1979; Wulff 1984). In addition, sponges can also harbour eukaryotic organisms, such as crustaceans, polychaete worms, molluscs and fish, demonstrating their role as important structural habitats for other co-occurring taxa (Macdonald et al. 2006; Abdo 2007; Amsler et al. 2009). Despite their ecological significance, information on sponge population dynamics are surprisingly limited.

1.3. Identifying robust taxonomical units and species diversity

Sponges are not routinely represented in management and conservation programs partly due to problematic field identification (Berman et al. 2013; Wulff 2001). Difficulties in sponge identification are attributed to the lack of reliable morphological characters for species delineation. Extreme morphological plasticity induced by environmental conditions often contributes to taxonomic ambiguity (Loh et al. 2012; Xavier et al. 2010). Multiple phenotypes within a single morphospecies can exist even in sympatry. Therefore, morphological groups can represent either independent genealogical lineages within species ranges, or extreme morphological variants within morphospecies (Andreakis et al. 2012; Freckelton et al. 2012). Molecular tools can provide greater taxonomic resolution when morphologically ambiguous specimens are encountered, however, universally suitable molecular markers for sponge barcoding that support morphological observations for delineation of robust taxonomic units and discovery of new taxa, have only recently been established (Erpenbeck and Wörheide 2007; Rua et al. 2011).

Sponge systematics and phylogenetics have traditionally relied on gross morphology and skeletal structures including spicules and spongin fibres (Hooper and Van Soest 2002; Rua et al. 2011). In recent years, molecular phylogenetics and systematics have been informative in defining spatial and temporal relationships amongst sponge evolutionarily significant units (ESUs) (Andreakis et al. 2012; Freckelton et al. 2012; Rua et al. 2011) and for establishing taxonomy in cryptic species complexes (Blanquer and Uriz 2007; Erpenbeck et al. 2012; Escobar et al. 2012; Xavier et al. 2010). Most importantly, when coupled with morphometrics, molecular phylogenetics can provide greater resolution when ambiguous taxonomic characters are encountered and help reconcile conflicting hypotheses associated with the evolutionary trajectory of morphological traits, species and natural populations, shaped over genealogical periods (Schmidt-Roach et al. 2012). For example, while some studies have questioned the reliability of traditional taxonomic characters (i.e. spicule morphology and fibre characteristics) in delineating genetically distinct lineages of sponges (McCormack et al. 2002; Paula et al. 2011), others have identified specific morphological

traits that are taxonomically informative (Borchiellini et al. 2004; Erwin and Thacker 2007; Miller et al. 2001). Due to the presence of high morphological plasticity in sponges, it is critical to conduct molecular phylogenetic investigations, in conjunction to morphological assessments, to elucidate cryptic species and identify robust taxonomic units prior to ecological studies.

1.4. *Carteriospongia foliascens*: taxonomy, ecological significance and distribution

Sponges in the family Thorectidae (Demospongiae; Dictyoceratida) comprise two subfamilies, Thorectinae and Phyllospongiinae (Cook and Bergquist 2002). On the Great Barrier Reef (GBR), thorectid sponges can represent up to 80 % of total sponge numbers and biomass (Wilkinson 1988). Phyllosponginiid sponges exhibit foliose, lamellate or folio-digitate morphologies and consist of five established genera, namely *Candidaspongia*, *Lendenfeldia*, *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* (Cook and Bergquist 2002). Species delineation within *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* is difficult as they lack spicules and have a paucity of informative skeletal characters. Furthermore, species boundaries within these genera are often confused due to the co-occurrence of multiple sympatrically distributed species with overlapping gross morphologies (Bergquist et al. 1988).

Interestingly, Phyllosponginiid sponges form close symbiotic associations with cyanobacteria (Webster et al. 2012). Two genera, *Phyllospongia* and *Carteriospongia*, are net primary producers capable of producing three times more oxygen through photosynthesis than respiration, and acquire up to 50 % of their nutrition from cyanobacterial symbionts (Wilkinson 1983, 1988). These phototrophic sponges can also release a proportion of the fixed carbon as dissolved organic carbon, contributing to coral reef nutrition (Wilkinson 1983).

Carteriospongia foliascens (Figure 1.2) is an abundant, conspicuous and widely distributed Indo-Pacific phototrophic sponge, and is found across a range of intertidal and mesophotic habitats of the Great Barrier Reef (GBR) (Wilkinson 1983,1988; Bridge et al. 2011a; author's personal observations). On the inshore reefs of the central GBR, *C. foliascens* occurs solely on the intertidal reef flat, with a complete absence of individuals in adjacent subtidal habitats (author's personal observations). At present, there is no existing information on the fundamental population biology and ecology of this species at the inshore sector of the Great Barrier Reef.

Figure 1.2: Adult *Carteriospongia foliascens*. Photograph was taken *in situ* at Juno Bay, Fantome Island, Palm Islands Group, central Great Barrier Reef.

1.5. Aims and chapter summaries

The overarching aim of this thesis is to investigate the population dynamics of an abundant GBR sponge, and determine the biological and environmental factors which influence the distribution of this species (see section 1.1, Figure 1.1). *Carteriospongia foliascens* was selected as the study species due to its wide distribution, high abundance and field accessibility (Figure 1.2). Because of the morphological plasticity in sponges (see section 1.3) rigorous phylogenetic analyses of the study species and associated taxa were conducted, prior to ecological assessments, to quantify sponge diversity in these bioregions and establish robust taxonomic units. To address the thesis aim, the thesis is divided into three segments, each focusing on a distinct life-history phase, and when integrated provide a holistic overview of population dynamics in this species. These key life-history phases are (1) reproductive biology and larval production (Figure 1.1, Box 1), (2) larval release, dispersal and settlement behaviours (Figure 1.1, Box 1, 2 and 3) and (3) post-settlement survival, growth, asexual reproduction (fission) and recruitment. The effects of environmental factors, such as temperature, photoperiod and rainfall, on these life-history phases were jointly assessed.

In Chapter 2, phylogenetic assessments of *C. foliascens* and closely related foliose taxa (sub-family Phyllospongiinae) were conducted across eastern and western tropical Australia to determine geographical distribution patterns and biogeography. This assessment used concatenated analyses of morphological and molecular taxonomic markers. Results identified hidden sponge diversity, taxonomic incongruences and the role of vicariant events influencing present day distributions of foliose Phyllospongiinae across tropical Australia. It also provided a robust taxonomic identity for intertidal *C. foliascens* for further ecological investigations in this study.

In Chapter 3, a field assessment on the effects of temperature, photoperiod and rainfall on the reproductive phenology of *C. foliascens* over two cycles (two years) was conducted. A climatic anomaly associated with one of the strongest La Niña events on record resulted in unprecedented high levels of temperature and rainfall at the study area and provided an insight into reproductive patterns under regional environmental stress.

In Chapter 4, laboratory experiments and field surveys were conducted to quantify the effects of larval release, larval behaviours and substrate availability on the distinct intertidal distribution of adult *C. foliascens* at inshore reefs of the central GBR. Larval planktonic duration, larval response to light (phototaxis) and substrate cues (biofilms) were quantified to assess larval dispersal potential, larval settlement and metamorphosis.

In Chapter 5, field monitoring of the recruitment, survival, growth, asexual reproduction (fission) and larval supply of *C. foliascens* was conducted over two years at two locations (bays) at the study area. The effects of temperature, photoperiod, rainfall and hydrodynamics on growth and larval production of *C. foliascens* between the two bays were assessed and discussed in light of recruitment patterns.

The results of this study are synthesized and discussed in Chapter 6, providing a holistic overview of key processes which regulate population dynamics for intertidal *C. foliascens* inhabiting the inshore GBR. The integration of datasets from multiple life history stages, in Chapters 3, 4 and 5, allows for a complete overview of the population dynamics of intertidal *C. foliascens*, and provides a realistic assessment of the vulnerability of populations to changing environmental conditions. In addition, this study highlights the importance of thorough taxonomic and phylogenetic assessments prior to ecological studies involving sponges and morphologically plastic taxa.

Chapter 2

Combining morphometrics with molecular taxonomy: how different are similar foliose keratose sponges from the Australian tropics?[1]

2.1. Introduction

Sponges are key residents of diverse marine habitats (Van Soest et al. 2012) and perform critical ecosystem functions (Bell 2008). Despite their ecological and evolutionary significance (Srivastava et al. 2010), sponges are rarely represented in management and conservation programs compared to other marine invertebrates, and this is partly due to problematic field identification of sponge species (Berman et al. 2013; Wulff 2001). Difficulties in sponge identification are attributed to the lack of morphological characters for species delineation and extreme morphological plasticity induced by local environmental conditions, often leading to considerable taxonomic confusion (Loh et al. 2012; Xavier et al. 2010). Multiple phenotypes within a single morphospecies can exist even in sympatry, with morphological groups representing either independent genealogical lineages within species ranges or extreme morphological variants within morphospecies (Andreakis et al. 2012; Freckelton et al. 2012). In addition, universally suitable molecular markers for sponge barcoding and to support morphological observations for delineation of robust taxonomic

[1] **Chapter 2** is adapted from Abdul Wahab MA, Fromont J, Whalan S, Webster N, Andreakis N (2014) Combining morphometrics with molecular taxonomy: how different are similar foliose keratose sponges from the Australian tropics? Mol Phylogenet Evol 73:23-39

units and discovery of new taxa, have only recently been established (Erpenbeck and Wörheide 2007; Rua et al. 2011).

Sponge systematics and phylogenetics have traditionally relied on gross morphology and skeletal structures including spicules and spongin fibres (Hooper and Van Soest 2002; Rua et al. 2011). In recent years, molecular phylogenetics and systematics have been informative in defining spatial and temporal relationships amongst sponge evolutionarily significant units (ESUs) (Andreakis et al. 2012; Freckelton et al. 2012; Rua et al. 2011) and for establishing taxonomy in cryptic species complexes (Blanquer and Uriz 2007; Erpenbeck et al. 2012; Escobar et al. 2012; Xavier et al. 2010). Most importantly, when coupled with morphometrics, molecular phylogenetics can provide greater resolution when ambiguous taxonomic characters are encountered, and help reconcile conflicting hypotheses associated with the evolutionary trajectory of morphological traits, species and natural populations, shaped over genealogical periods (Schmidt-Roach et al. 2012). For example, while some studies have questioned the reliability of traditional taxonomic characters (i.e. spicule morphology and fibre characteristics) in delineating genetically distinct lineages of sponges (McCormack et al. 2002; Paula et al. 2011), others have identified specific morphological traits that are taxonomically informative (Borchiellini et al. 2004; Erwin and Thacker 2007; Miller et al. 2001).

Sponges of the family Thorectidae (Demospongiae; Dictyoceratida) comprise two subfamilies, Thorectinae and Phyllospongiinae (Cook and Bergquist 2002). The latter exhibit foliose, lamellate or folio-digitate morphologies and consist of five established genera, namely *Candidaspongia*, *Lendenfeldia*, *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* (Cook and Bergquist 2002). Species of *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* are widespread across the Indo-Pacific region (Figure 2.1) and contribute up to 80% of sponge numbers and biomass in parts of the GBR (Wilkinson 1988). Sponge abundance, coupled with their role as significant contributors to primary productivity (through symbiotic association with cyanobacteria), highlight the potential importance of this group of sponges to coral reefs (Wilkinson 1983).

Species delineation within *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* is difficult as they lack spicules and have a paucity of informative skeletal characters. Furthermore, species boundaries within these genera are often confused due to the co-occurrence of multiple sympatrically distributed species with overlapping gross morphologies (Bergquist et al. 1988). In this study, Bayesian inference of discrete morphological features (19 characters; 52 character states) and molecular phylogenies inferred from the nuclear Internal Transcribed Spacer 2 region (ITS2) were used to identify

ESUs (sensu Moritz 1994) within foliose Phyllosponginiids (for ease of communication foliose *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* are collectively referred to as Phyllosponginiids hereafter) collected from seven geographic locations across tropical eastern and Western Australia. This study aims to 1) identify morphospecies boundaries and delineate robust taxonomic units in the study area taking into consideration previous morphological descriptions by Bergquist et al. (1988), 2) explore the levels of genetic diversity within taxonomically accepted taxa for hidden morphospecies identification and 3) assess whether the observed geographical distribution patterns of the recovered ESUs are linked to vicariance biogeography.

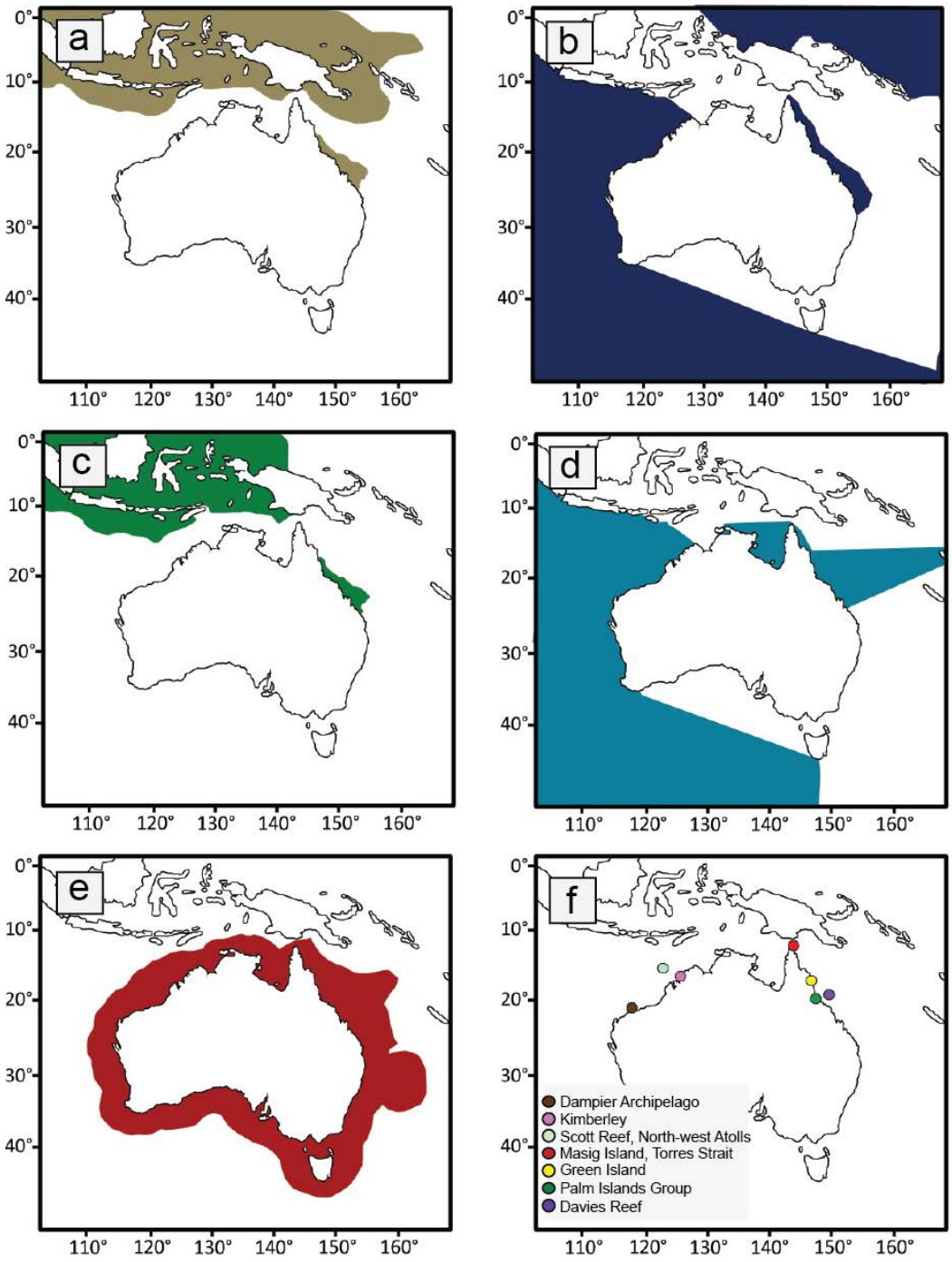

Figure 2.1: Geographic distribution of a) *Carteriospongia flabellifera*, b) *C. foliascens*, c) *Phyllospongia papyracea*, d) *P. lamellosa* and e) *Strepsichordaia lendenfeldi* across the Indo-Pacific as described on the World Porifera Database (http://www.marinespecies.org/porifera/; 14th February 2013). Additional geographic distributions for *C. foliascens* and *P. lamellosa* were consolidated from Queensland Museum species mudmaps. f) coloured dots represent sponge collection sites.

2.2. Materials and Methods

2.2.1. Sample collections

Tissue samples were collected from sponges resembling the Phyllosponginiid foliose morphology from seven geographic locations across tropical eastern and Western Australia (Table 2.1 and Figure 2.1f). 112 samples were used including 22 specimens from the Western Australian Museum (WAM) and two from the Australian Museum (AM), described in Bergquist et al. (1988) (see Table 2.1 for specimen details and geographic locations). Whole specimens were photographed in situ and tissue samples (ca. 35 cm^2) were photographed again out of water prior to preservation in 100% ethanol. Samples were stored at -20°C pending histological and genetic analyses. Additional AM specimens described in Bergquist et al. (1988) as *Carteriospongia foliascens* (AM Z3952 and AM Z4979), *C. flabellifera* (AM Z4983 and AM Z4984), *Phyllospongia papyracea* (AM Z4987 and AM Z5014), *P. lamellosa* (AM Z5016 and AM Z5017) and *Strepsichordaia lendenfeldi* (holotype, AM Z5026), were used to verify previous morphological descriptions.

2.2.2. Histological processing and quantitative microscopic analyses

Histological analyses involved a xylene clearing procedure, followed by paraffin impregnation in an automated vacuumed tissue processor (SHANDON Hypercenter XP). 90 µm sections were cut using a manual rotary microtome (Microm HM325) through manipulation of its trimming lever. Sections were oriented longitudinally from the margin of the lamellae towards the centre so that both inhalant and exhalant pinacoderm were represented (ca. 2 to 3 cm long). Sections were de-waxed by incubating overnight at 60°C followed by a one hour xylene digest. Coverslips were applied over unstained sections using DPX mountant (ThermoFisher Scientific). For quantitative assessments, relevant morphological features were identified, photographed under a dissecting (Olympus) or compound light microscope (Leica DM LB) and characters measured using ImageJ software (National Institutes of Health, USA). All observations of microscopic characters were made at ca. 1 cm from the margin of lamellae. Character measurements were factored to *a priori* identified morphogroups and analysed using pairwise permutational ANOVA (PERMANOVA) (Anderson 2005). Euclidean distance matrices, using untransformed raw data, were used for all pairwise analyses (9999 permutations). The resulting pairwise distance matrices, containing statistical information, were subsequently used in non-metric multidimensional scaling (MDS) to visualize distinct morphogroups.

2.2.3. Morphological matrix compilation

For Bayesian analysis of morphological traits, several traditional morphological characters as described in Bergquist et al. (1988), and corresponding to multiple character states (Table 2.2), were tested and numerically scored. In addition, previously described quantitative microscopic characters were coded into the matrix with statistically different values representing unique character states. Additional information on habitats at sponge collection sites was also included in the matrix (i.e. intertidal vs. subtidal).

The validity of merging ecological and behavioural characters in phylogenetic reconstruction has been previously questioned due to the susceptibility of these characters to homoplasy compared to morphological traits. This argument however lacks empirical support. It has been largely demonstrated that the same criteria used to define homology in morphological features can be used to identify informative characters related to behavioural, chemical or other ecological traits (Caetano and Machado 2013; Willmott and Freitas 2006).

2.2.4. DNA extractions, PCR amplifications and sequencing

Total genomic DNA was extracted using the Power Plant DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's protocol, with reduction of the homogenization step to 45 seconds. The standard metazoan (COI) barcoding marker (see Folmer et al. 1994 for oligo sequences) and the highly variable Internal Transcribed Spacer 2 (ITS2) (see Thacker and Starnes 2003 for oligo sequences), known to be useful for sponge taxa delineation (Erwin and Thacker 2007; Andreakis et al. 2012; Escobar et al. 2012), was PCR amplified. PCR was performed in 50 µl volumes containing approximately 10 ng DNA template, 10 µl 5x MyTaq Reaction Buffer, 0.15 µl of each primer (100 pmol mL^{-1}), 0.4 µl of bovine serum albumin (BSA; 10 mg mL^{-1}), and 0.25 µl of myTaq DNA polymerase (Bioline, London UK). The amplification protocol for COI PCR included: 95°C for 1 min; 30 cycles at 95°C for 50 s, 42°C for 50 s, and 72°C for 2 min; and a final elongation at 72°C for 10 min. The amplification protocol for ITS2 PCR included: 95°C for 1 min; 34 cycles at 95°C for 30 s, 54°C for 30 s, and 72°C for 1.5 min; and a final elongation at 72°C for 2 min. All PCR products were purified and sequenced in both directions by Macrogen Inc., Korea, using the PCR primers.

Table 2.1: Metadata of samples used in this study including previously established taxonomic status, sampling location, ID, morphotype, collection location, latitude and longitude, accession number and literature reference.

Genus	Species	Location	Sponge ID	Morphotype	Collection depth (m)	Latitude	Longitude	Accession no.	Reference
Strepsichordaia	*lendenfeldi*	Davies Reef	5	1	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef (AM Z5027 *Strepsichordaia lendenfeldi* paratype)		1	15	18° 49.024' S	147° 37.939' E		Bergquist et al. 1998; This study
		Green Island Reef B	9	1	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	5	1	10	16° 45.245' S	145° 59.240' E		This study
		Dampier Archipelago (WAM Z5755)	5	1.1	9	20° 38.990' S	116° 26.210' E		This study
		Kimberley (WAM Z54096)	12	1.1	13	15° 30.310' S	123° 36.290' E		This study
		Kimberley (WAM Z54121)	14	1.1	16	15° 20.178' S	123° 30.774' E		This study
		Kimberley (WAM Z54131)	13	1.1	16	15° 20.178' S	123° 30.774' E		This study
Carteriospongia	*flabellifera*	Green Island Reef A	84	2	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	86	2	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	82	2	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	83	2	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	89	2	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef B	17	2	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	18	2	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	15	2	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	4	2	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef A	88	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	73	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	74	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	75	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	85	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	87	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	77	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	81	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef A	76	2.1	10	16° 46.296' S	145° 59.790' E		This study
		Kimberley (WAM Z29265)	11	2.2	2	13° 54.927' S	125° 46.460' E		This study
		Kimberley (WAM Z54023)	9	2.2	0	15° 19.945' S	124° 14.58' E		This study
		Kimberley (WAM Z54029)	8	2.2	0	15° 19.945' S	124° 14.58' E		This study
		Kimberley (WAM Z54138)	10	2.2	16	15° 20.178' S	123° 30.774' E		This study
Carteriospongia	*foliascens*	Davies Reef	8	3	12	18° 49.024'S	147° 37.939'E		This study
		Davies Reef	11	3	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	3	3	12	18° 49.024' S	147° 37.939' E		This study
		Green Island Reef B	13	3	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	8	3	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	11	3	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	6	3	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	12	3	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	14	3	10	16° 45.245' S	145° 59.240' E		This study

Table 2.1 continued

Genus	Species	Location	Sponge ID	Morphotype	Collection depth (m)	Latitude	Longitude	Accession no.	Reference
Carteriospongia	*foliascens*	Green Island Reef B	16	3	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	7	3	10	16° 45.245' S	145° 59.240' E		This study
		Green Island Reef B	10	3	10	16° 45.245' S	145° 59.240' E		This study
		Fantome Island, Palm Islands Group	30	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	6	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	11	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	3	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	7	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	9	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	13	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	21	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	8	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	10	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	12	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	1	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	4	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	5	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Fantome Island, Palm Islands Group	2	3.1	0	18° 41.126' S	146° 30.731' E		This study
		Orpheus Island, Palm Islands Group	43	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	38	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	33	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	37	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	42	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	48	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	35	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	39	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	44	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	36	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	40	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	45	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	34	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	32	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Orpheus Island, Palm Islands Group	41	3.1	0	18° 35.976' S	146° 29.533' E		This study
		Scott Reef, North-west Atoll (WAM Z37657)	3	3.1	12.8	14°10.623' S	121°52.902' E		This study
		Scott Reef, North-west Atoll (WAM Z37658)	2	3.1	5.8	14°10.639' S	121°52.894' E		This study
		Scott Reef, North-west Atoll (WAM Z37667)	1	3.1	12.9	14°11.451' S	121°48.399' E		This study
		Kimberley (WAM Z54006)	2	3.2	0	15° 19.937' S	124° 6.300' E		This study
		Kimberley (WAM Z54008)	4	3.2	0	15° 19.937' S	124° 6.300' E		This study
		Kimberley (WAM Z54270)	1	3.2	0	16° 3.185' S	123° 21.532' E		This study

Table 2.1 continued

Genus	Species	Location	Sponge ID	Morphotype	Collection depth (m)	Latitude	Longitude	Accession no.	Reference
Carteriospongia	*foliascens*	Kimberley (WAM Z54282)	3	3.2	0	16° 3.185' S	123° 21.532' E		This study
		Masig Island, Torres Strait	8	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	10	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	2	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	9	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	6	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	5	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	4	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	3	3.2	0	9° 45.242' S	143° 25.100' E		This study
		Masig Island, Torres Strait	1	3.2	0	16° 45.245' S	145° 59.240' E		This study
		Masig Island, Torres Strait	7	3.2	0	16° 45.245' S	145° 59.240' E		This study
Phyllospongia	*papyracea*	Davies Reef	4	4	12	18° 49.024' S	147° 37.939' E		This study
		Green Island Reef A	34	4	10	16° 46.296' S	145° 59.790' E		This study
		Green Island Reef C	2	4	0	16° 45.435' S	145° 58.301' E		This study
		Green Island Reef C	1	4	0	16° 45.435' S	145° 58.301' E		This study
		Dampier Archipelago (WAM Z3061)	2	4.1	3.2	20° 25.730' S	116° 57.58' E		This study
		Dampier Archipelago (WAM Z3243)	1	4.1	0	20° 24.810' S	116° 50.680' E		This study
		Dampier Archipelago (WAM Z3992)	3	4.1	0	20° 25.770' S	116° 52.680' E		This study
		Dampier Archipelago (WAM Z5409)	4	4.1	0	20° 30.900' S	116° 40.220' E		This study
		Kimberley (WAM Z54004)	5	4.1	0	15° 19.937' S	124° 6.300' E		This study
		Kimberley (WAM Z54124)	7	4.1	16	15° 20.178' S	123° 30.774' E		This study
		Kimberley (WAM Z54325)	6	4.1	0	15° 52.203' S	123° 39.910' E		This study
Phyllospongia	*lamellosa*	Davies Reef	7	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	1	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	6	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	2	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	9	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	10	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	12	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	15	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	16	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	14	5	12	18° 49.024' S	147° 37.939' E		This study
		Davies Reef	13	5	12	18° 49.024' S	147° 37.939' E		This study
		John Brewer Reef (AM Z5021 *Phyllospongia lamellosa*)		5	22	18° 38.382' S	147° 2.865' E		Bergquist et al. 1988; This study
Dysidea	*granulosa*	Pago Bay, Guam				13° 25.188' N	144° 47.607' E	AF420443	Thacker and Starnes 2003
Ircinia	*oros*	Punta Santa Anna, Blannes, Spain				41° 40.350' N	2° 48.217' E	JN655183	Erwin et al. 2012
Ircinia	*variabilis*	Mar Menuda, Tossa de Mar, Spain				41° 43.217' N	2° 56.450' E	JN655198	Erwin et al. 2012

Table 2.2: Morphological characters and character states used in this study. Characters 1 to 5 are qualitative gross morphologies used to assign individuals into respective 11 *a priori* morphogroups. Characters 6 to 13 are microscopic characters that were qualitatively assessed and characters 15 to 19 are microscopic characters derived from quantitative analyses. Character 14 represents habitat that sponges were collected from.

Character		Character states				
		0	1	2	3	4
1	Growth form	Simple fan	Simple cup	Complex	—	—
2	Surface texture	Smooth	Finely conulose	Conulose	Conulose and ridged	Finely conulose and ridged
3	Conule alignment	Longitudinal	Longitudinal reticulate	Unclear	Absent	—
4	Oscule profile	Flush with surface	Both on conule and surface	—	—	—
5	Oscular arangement	Only on fan surface	Apical and on fan surface	Stellate	—	—
6	Armour consistency in pinacoderm	Even	Patchy	—	—	—
7	Primary fibre composition	Cored	Never cored	Sometimes cored	—	—
8	Primary fibre conformation	Simple	Fasiculate	Simple and forms brushes at surface	—	—
9	Mesh configuration	Regular	Irregular	—	—	—
10	Secondary fibre presence	Present	Absent	—	—	—
11	Secondary fibre composition	Always cored	Sometimes cored	Never cored	—	—
12	Tertiary fibre presence	Present	Absent	—	—	—
13	Tertiary fibre conformation	Simple	Vermiform	Both simple and vermiform	—	—
14	Habitat	Intertidal	Subtidal	—	—	—
15	Sponge fan thickness	A	B	C	—	—
16	Sand cortex depth	A	B	—	—	—
17	Primary mesh size	A	B	—	—	—
18	Primary fibre diameter	A	B	C	—	—
19	Secondary fibre diameter	A	B	C	—	—

2.2.5. Sequence alignments and model selection

Electropherograms were edited in Sequencher 4.9 (Gene Codes, Ann Abor, Michigan, USA) and sequences manually aligned and trimmed in BIOEDIT ver. 7.0.5.3 (Hall 1999) (see Table 2.1 for sequence accession numbers). For outgroup comparisons, publically available ITS2 sequences of the sponges *Dysidea granulosa* (Bergquist 1965), AF420443, *Ircinia oros* (Schmidt 1864), JN655183 and *Ircinia variabilis* (Schmidt 1862), JN655198, were downloaded from the National Centre for Biotechnology Information's website (http://www.ncbi.nlm.nih.gov/) and included in the analyses. Phylogenetic information of the ingroup sequences was assessed using g_1 statistics, a measure of the skewness in the distribution of tree-lengths among 10,000 random maximum parsimony trees (Hillis and Huelsenbeck 1992) in PAUP*4.0b10 for Windows (Swofford 2002). The significance of the $g_1$value was compared against critical values ($p = 0.01$) for four state characters given the number of distinct sequences and the number of parsimony-informative sites. Sequence diversity of the ingroup (i.e. number of haplotypes, parsimony informative characters), haplotype and nucleotide diversity within geographically predefined sampling sites were evaluated in DnaSP v5 (Librado and Rozas 2009). Pairwise comparisons among ESUs under the TN model of evolution were computed in MEGA5 (Tamura et al. 2011). Hierarchical Likelihood Ratio Tests (hLRTs) were conducted in Modeltest Version 3.7 (Posada and Crandall 1998) to identify the model of sequence evolution and parameters that best fit the alignments. Sequences produced in this study have been submitted to NCBI under accession numbers KJ174019 – KJ174055.

2.2.6. Phylogenetic analyses

Molecular phylogenies given the best-fitting model of evolution were inferred by maximum likelihood (ML) in PAUP*. Heuristic searches for ML were run in PAUP* under ten random additions and TBR branch swapping. Bootstrap support for individual clades in ML was calculated on 500 replicates using the same methods, options and constraints as used in the tree-inferences, but with all identical sequences removed (Felsenstein 1985). Bayesian posterior probability estimates of the nodes coupled with the Markov chain Monte Carlo (MCMC) algorithm was implemented in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) using model and parameters identified by Modeltest. Bayesian inference was conducted for 5,000,000 generations (two parallel runs, four chains each, starting from a random tree and sampling every 1000^{th} generation). The convergence of the parameter estimates was graphically confirmed by plotting values of likelihood against the generation time in Tracer v1.5 (Rambaut and Drummond 2007).

2.2.7. Morphological phylogeny, habitat and morphological traits reconstruction

Bayesian analyses were performed in MrBayes v3.1.2. Computations involved only the ingroup since characters for outgroups could not be retrieved from the literature. The standard discrete model (Lewis 2001) was run in MrBayes v3.1.2 on the morphological matrix (112 specimens) for 2,000,000 generations (two parallel runs, four chains each, starting from a random tree and sampling every 1000th generation). Bayesian mixed phylogenies (morphological and molecular data) were additionally computed in MrBayes v3.1.2 using the HKY+G and discrete model for the ITS2 and the morphological partition respectively for 2,000,000 generations (two parallel runs, four chains each, starting from a random tree and sampling every 1000th generation). Finally, habitat traits (intertidal vs. subtidal) and morphological characters were scored for each species and mapped on the BI molecular phylogenetic tree. Their ancestry and evolution across the phylogeny was evaluated individually using a maximum parsimony framework in Mesquite v2.75 (Maddison and Maddison 2011).

2.3. Results

2.3.1. Morphological matrix compilation and analyses

Gross morphological analyses of 112 specimens of foliose Phyllospongiinae placed individuals into 11 subtly distinct morphogroups occurring in subtidal and intertidal habitats (Figures 2.2 and 2.3). Further macro- and microscopic analyses resulted in the identification of 19 characters (a total of 52 character states) and these were scored in the morphological matrix of this study (Table 2.2 and Supplementary Table 2.1; qualitative macroscopic characters 1 to 5; qualitative microscopic characters 6 to 13). In addition, quantitative microscopic characters (Table 2.2; characters 15 to 19) were coded into the matrix with statistically different values representing unique character states. For the quantitative characters, MDS of the distance matrix from pairwise PERMANOVA identified distinct statistically supported groups used for the formulation of the character matrix (Supplementary Figure 2.1). Bayesian inference analyses of the morphological data (Supplementary Figure 2.2) placed these morphogroups into three clusters (I, II and III) corresponding to morphologies suspected at the genus level based on Bergquist et al. (1988). The aforementioned clusters represented our null hypothesis for genetic analyses.

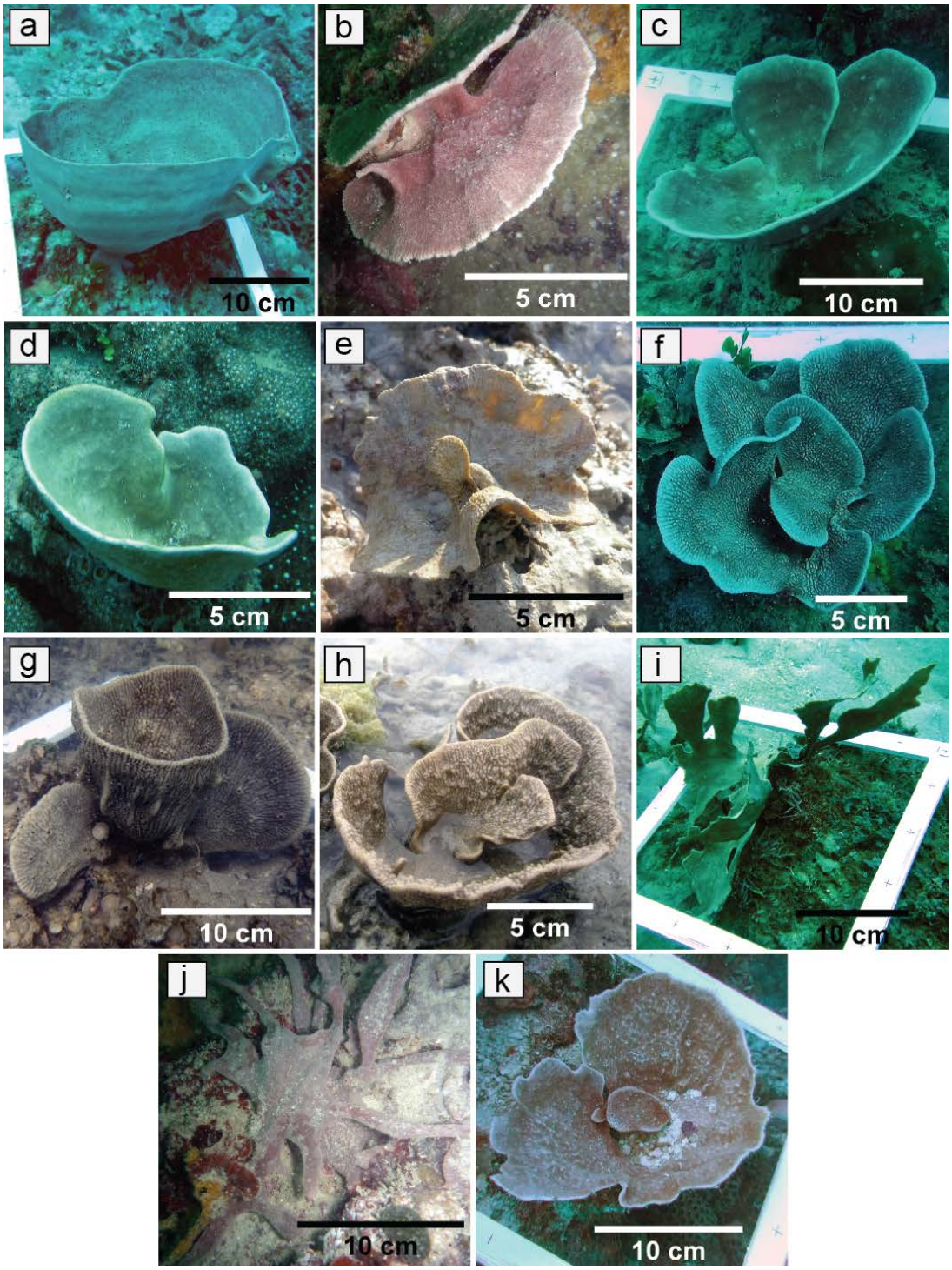

Figure 2.2: In-situ photographs of gross sponge morphologies assigned to 11 *a priori* morphotypes used in this study. a) morphotype 1; *Strepsichordaia* cf. *lendenfeldi* 1 displaying a simple cup morphology, b) morphotype 1.1; *Strepsichordaia* cf. *lendenfeldi* 2 displaying a complex double fan morphology, c) morphotype 2; subtidal *Carteriospongia* cf. *flabellifera* 1 displaying a simple fan morphology, d) morphotype 2.1; subtidal *Carteriospongia* cf. *flabellifera* 2 resembles morphotype 2 but have distinct surface morphology (see Figure 2.3), e) morphotype 2.2 ; intertidal *Carteriospongia* cf. *flabellifera* 3 exposed to air at low tide, f) morphotype 3; subtidal *Carteriospongia* cf. *foliascens* 1 displaying a complex multi-lobed fan morphology, g) morphotype 3.1; intertidal *Carteriospongia* cf. *foliascens* 2 displaying a complex cup with fans morphology, h) morphotype 3.2; intertidal *Carteriospongia* cf. *foliascens* 3 with complex morphology exposed to air at low tide, i) morphotype 4; subtidal *Phyllospongia* cf. *papyracea* 1 possess the thinnest fan and display a complex morphology, j) morphotype 4.1; subtidal *Phyllospongia* cf. *papyracea* 2 displaying a complex morphology and is thicker than morphotype 4, k) morphotype 5; subtidal *Phyllospongia* cf. *lamellosa* displaying a simple cup morphology.

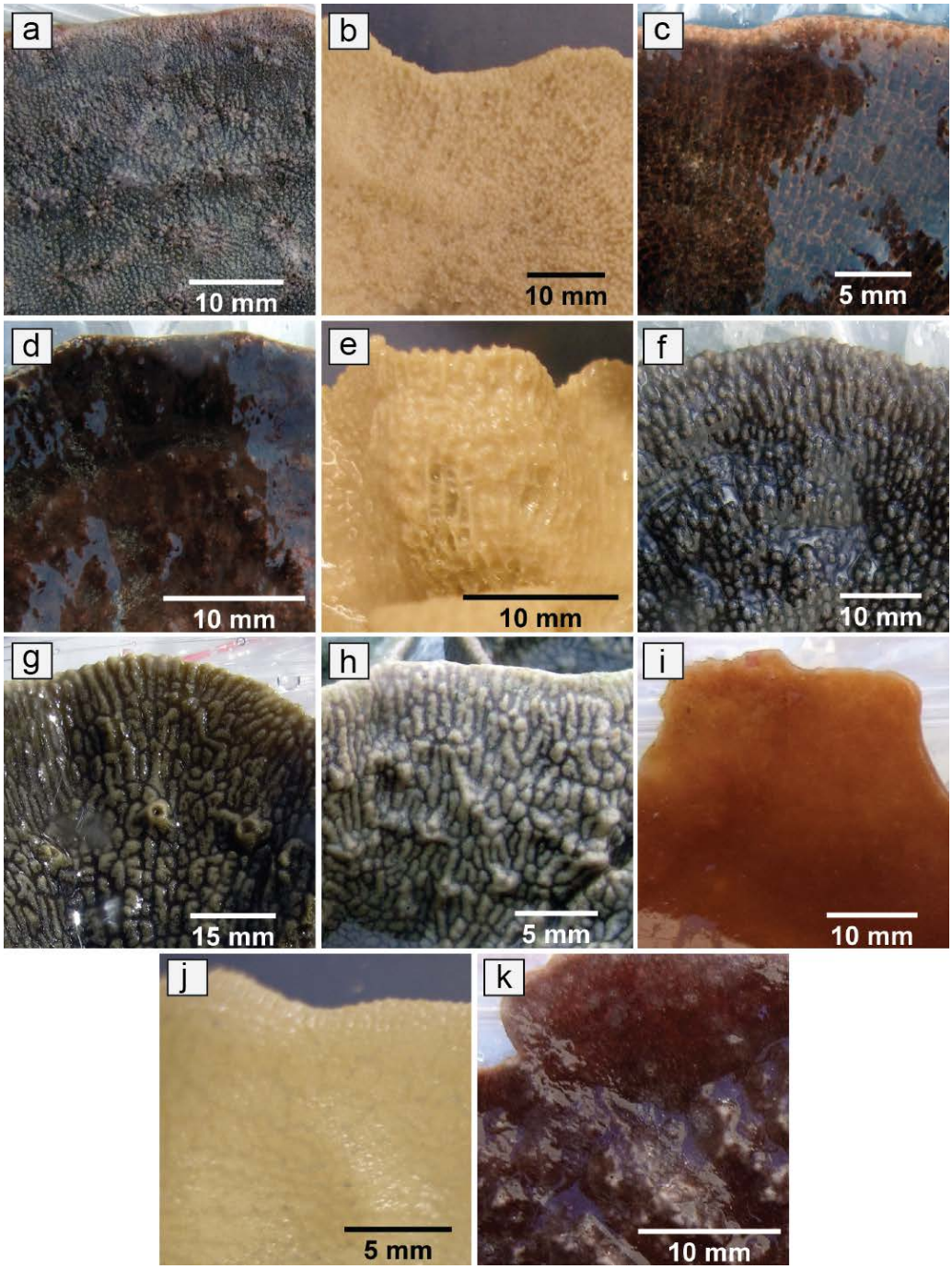

Figure 2.3: Photographs of sponge surface morphology used in conjunction with gross morphology (see Figure 2.2) to assign individuals into 11 *a priori* morphotypes used in this study. a) morphotype 1; *Strepsichordaia* cf. *lendenfeldi* 1 (fresh sample, FS hereafter) displaying a surface that is finely conulated with flushed oscules, b) morphotype 1.1; *Strepsichordaia* cf. *lendenfeldi* 2 (ethanol preserved, EP hereafter) exhibits similar surface morphology to morphotype 1 but possess raised ridges not shown here, c) morphotype 2; subtidal *Carteriospongia* cf. *flabellifera* 1 (FS) displaying distinctive longitudinal reticulation of fine conules, d) morphotype 2.1; subtidal *Carteriospongia* cf. *flabellifera* 2 (FS) lacks visible longitudinal reticulated conules seen in morphotype 2, e) morphotype 2.2 ; stronger reticulated conulation is seen in intertidal *Carteriospongia* cf. *flabellifera* 3 (EP) compared to morphotype 2 and 2.1, f) morphotype 3; subtidal *Carteriospongia* cf. *foliascens* 1 (FS) display conules that align longitudinally with no apparent reticulation and possess oscules flushed to the surface, g) morphotype 3.1; oscules are raised on conules in intertidal *Carteriospongia* cf. *foliascens* 2 (FS) with surface being more membranous than in morphotype 3, h) morphotype 3.2; intertidal *Carteriospongia* cf. *foliascens* 3 (FS) displaying similar morphology to morphotype 3.1 but having more prominent raised ridges on its surface, i) morphotype 4; subtidal *Phyllospongia* cf. *papyracea* 1 (FS) exhibiting a typically smooth morphology, j) morphotype 4.1; subtidal *Phyllospongia* cf. *papyracea* (EP) possessing very fine conules, k) morphotype 5; surface of the subtidal *Phyllospongia* cf. *lamellosa* (FS) is finely conulated with oscules raised on ridges.

2.3.2. Marker performance

The standard metazoan barcoding COI marker failed to amplify in the vast majority of the specimens following multiple attempts in variable reaction conditions. Furthermore, when successful, the PCR products delivered low quality sequencing results consisting of high levels of bacterial and/or fungal contamination. This further supports the unsuitability of the COI for barcoding in thorectid sponges (minimum success) proposed by Vargas et al. (2012) and the marker was therefore omitted from further analyses.

The ITS2 region of the nuclear ribosomal DNA (nrDNA) occurs in multiple tandem repeats located in one or more chromosomes and is known to possess a variable number of paralogous copies not yet homogenized by concerted evolution (Alvarez and Wendel 2003). These copies are known to interfere severely with phylogenetic inference especially when population-level divergence or shallow speciation events are targeted (Escobar et al. 2012; Wörheide et al. 2004). The ITS2 sequences recovered from this study showed only one ambiguous position (i.e. double peak) thus providing evidence of homogenization of excessive paralogous copies via concerted evolution for the taxonomical level in consideration. Low and/or undetected levels of intragenomic polymorphism (IGP) are not critical to delineate the operational taxonomic units (OTUs) targeted in this study (Wörheide et al. 2004).

2.3.3 Phylogenetic analyses

Final alignments consisted of 112 ITS2 ingroup sequences produced in this study and three previously published outgroup sequences. The ingroup alignment (41 unique ITS2 types) contained 633 characters, of which 10 were parsimony uninformative and 79 were parsimony informative. The length distribution of 10,000 random trees was left-skewed (g_1= - 0.56) indicating a significant amount of phylogenetic signal in the dataset. Haplotype and nucleotide diversity were variable within each of the geographically predefined sampling sites, influenced by the unequal sampling effort per site (see Table 2.3 for alignment statistics).

Cluster I was the most phylogenetically derived group in the topology and contained clades 1 to 3 (Figure 2.4). Sub- clade 1E comprised morphotypes 3, 3.1 and 3.2, in which morphotype 3 (Figure 2.2f) closely resembled AM specimens (AM Z3952 and AM Z4979) described in Bergquist et al. (1988) as *C. foliascens*. Sub-clade 1W consisting of samples from Scott Reef and the Kimberley (Western Australia) contained only morphotypes 3.1 and 3.2. Morphotype 5 grouped exclusively in clade 2 and is identified as *P. lamellosa* through

inclusion of a sequence from a previously identified specimen (AM Z5021; Bergquist et al. 1988). Sub-clade 3E included samples from the east coast of Australia (morphotype 4) resembling AM specimens examined in this study (AM Z4987 and AM Z5014), identified as *P. papyracea* in Bergquist et al. (1988). Sub-clade 3W was represented solely by morphotype 4.1 and was collected from the Dampier Archipelago and the Kimberley. Based on the overall morphology and topological positioning of the clades, the genus *Phyllospongia* was assigned to this cluster. The unexpected placement of *C. foliascens* in this cluster was noted and is discussed in later sections.

Table 2.3: Alignment statistics of geographically pre-defined sampling sites showing number of sequences (n), number of ITS2 types (h), nucleotide diversity (π), sequence diversity (Hd) and standard deviation (SD). Analyses were performed on the ingroup sequences. Missing values refer to monomorphic populations.

Sampling site	n	h	π(SD)	Hd(SD)
Davies Reef	17	7	0.027(0.009)	0.765(0.094)
Palm Islands Group	30	4	0.001(0.000)	0.342(0.072)
Green Island	32	10	0.056(0.003)	0.847(0.043)
Masig Island, Torres Strait	10	1	0.000(0.000)	0.000(0.000)
Kimberley	14	6	0.063(0.005)	0.857(0.056)
Scott Reef, North-west Atoll	3	2	0.002(0.000)	0.785(0.314)
Dampier Archipelago	5	2	0.038(0.023)	0.4(0.237)

Cluster II, recovered as sister group to Cluster I (Figure 2.4) contained clade 4. Sub-clade 4E consisting of morphotypes 2 and 2.1 collected from the east coast of Australia resembled AM specimens of *C. flabellifera* examined in this study (AM Z4983 and AM Z4984; Bergquist et al. 1988) in both gross and microscopic morphology. Sub-clade 4W consisted of samples collected from the Kimberley and was subtly distinct in morphology (morphotype 2.2) in terms of surface texture from sub-clade 4E. Distinct morphological characters (i.e. large fasciculated primary fibres and tertiary fibres associated with the primary fibres) strongly support the assignment of this cluster to the genus *Carteriospongia* (Bergquist et al. 1988).

Finally, Cluster III formed by a single clade (5), resembled a *Strepsichordaia*-like morphology, in particular, *S. lendenfeldi*. This was supported by the inclusion of the ITS2 sequence of the *S. lendenfeldi* paratype (AM Z5027; Bergquist et al. 1988) in the phylogenetic tree. Pairwise analyses of genetic distances among clades under the TN model supported our phylogenetic hypothesis for genus vs. species delineation (Supplementary Figure 2.3). A large genetic gap (0.0247 to 0.0736) between inter and intra-cluster clade comparisons separated inter-generic vs. inter-specific genetic distances.

2.3.4. Phylogeographic considerations

Haplotype and nucleotide diversity per sampling site were found to be variable, likely influenced by unequal sampling efforts (Table 2.3). Multiple clades were recovered in four out of seven locations (Davies Reef and Green Island; East Coast, the Kimberley and Dampier Archipelago; West Coast; Figure 2.6). The high levels of diversity on the east coast were always recovered in subtidal sampling sites whereas intertidal sampling sites were usually monospecific (Palm Islands and Masig Island). West coast locations showed no variability associated with subtidal or intertidal habitats.

2.3.5. Habitat and morphological traits reconstruction

Reconstructions of habitat and morphological traits over the ITS2 phylogeny identified eight out of the 19 characters to be relevant in differentiating the majority of the clades recovered (Figure 2.7, Supplementary Table 2.2). Relevant characters are those showing little or no variation within clades across the phylogeny. All other characters investigated were highly variable within clades and therefore unsuitable for species delineation.

Secondary fibre diameter was synapomorphic at the genus level with the ancestral form considered to be intermediate fibre size seen in *Strepsichordaia*, and the derived sister genera *Carteriospongia* and *Phyllospongia* evolving larger and smaller fibre diameters respectively (Figure 2.7h). Additionally, ancestral coring (i.e. inclusion of foreign debris) of secondary fibres is conserved in *Carteriospongia,* but is either reduced or lost in species of *Phyllospongia* (Figure 2.7g). Primary fibre diameter showed a similar pattern with *Carteriospongia* possessing the largest fibre size which is associated with unique fasciculate fibres in this genus (Figure 2.7e - f). *Strepsichordaia* possessed the ancestral simple primary fibre development which is conserved in *Phyllospongia.* However, clade 1 expressed a modification of these fibres to form brushes, which are aggregations of primary fibres at the surface giving rise to distinct conules (Figure 2.7a and e).

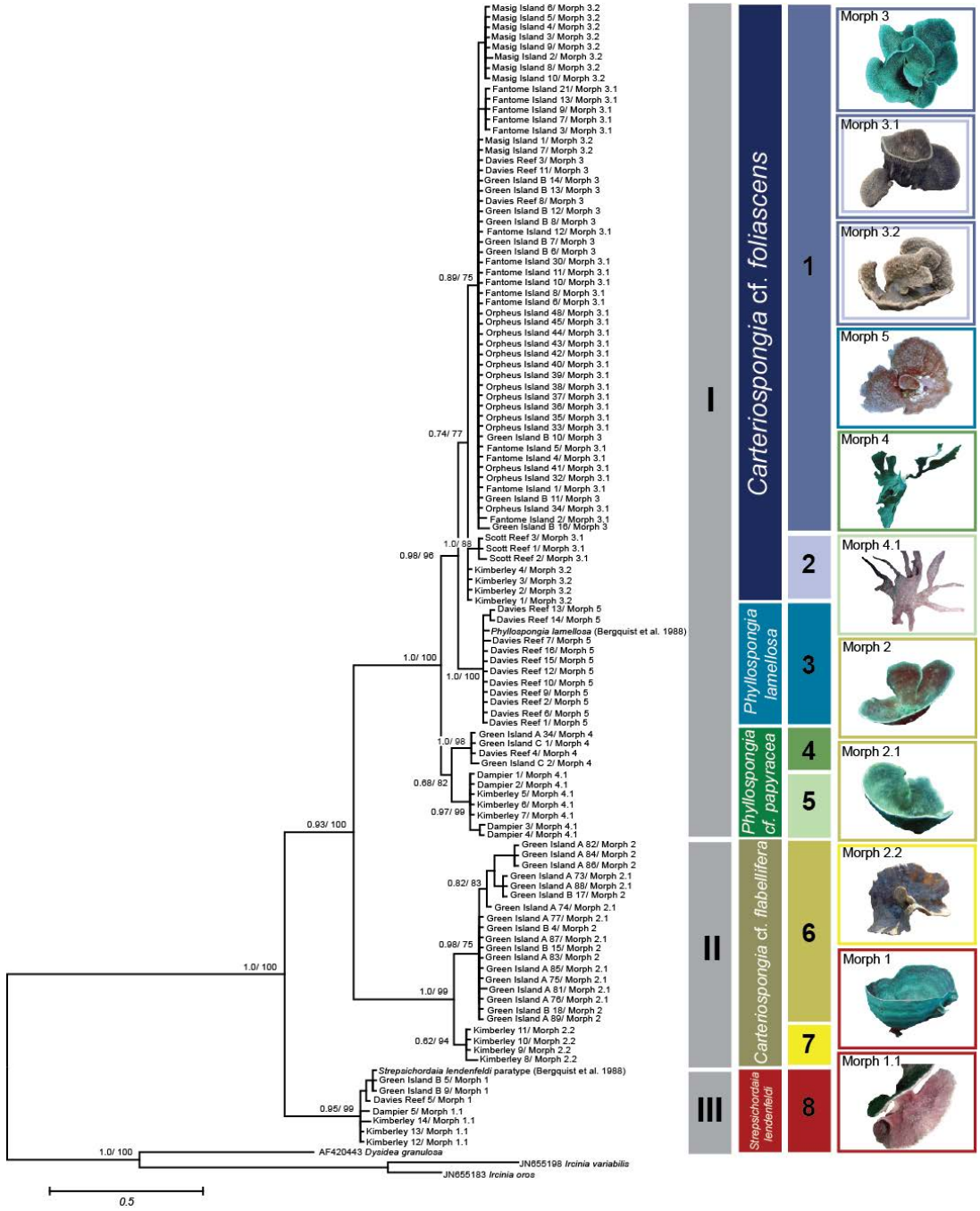

Figure 2.4: Outgroup-rooted Bayesian phylogeny inferred from the ITS2 alignment. Three main clusters corresponding to the genera *Phyllospongia* (I), *Carteriospongia* (II) and *Strepsichordaia (*III) are highlighted in grey boxes. A total of five distinct clades were recovered, corresponding to species described in Bergquist et al. (1988). Specimens from eastern (E) and Western (W) Australia are highlighted within clades. Morphotypes are represented in coloured boxes corresponding to the assigned clades.

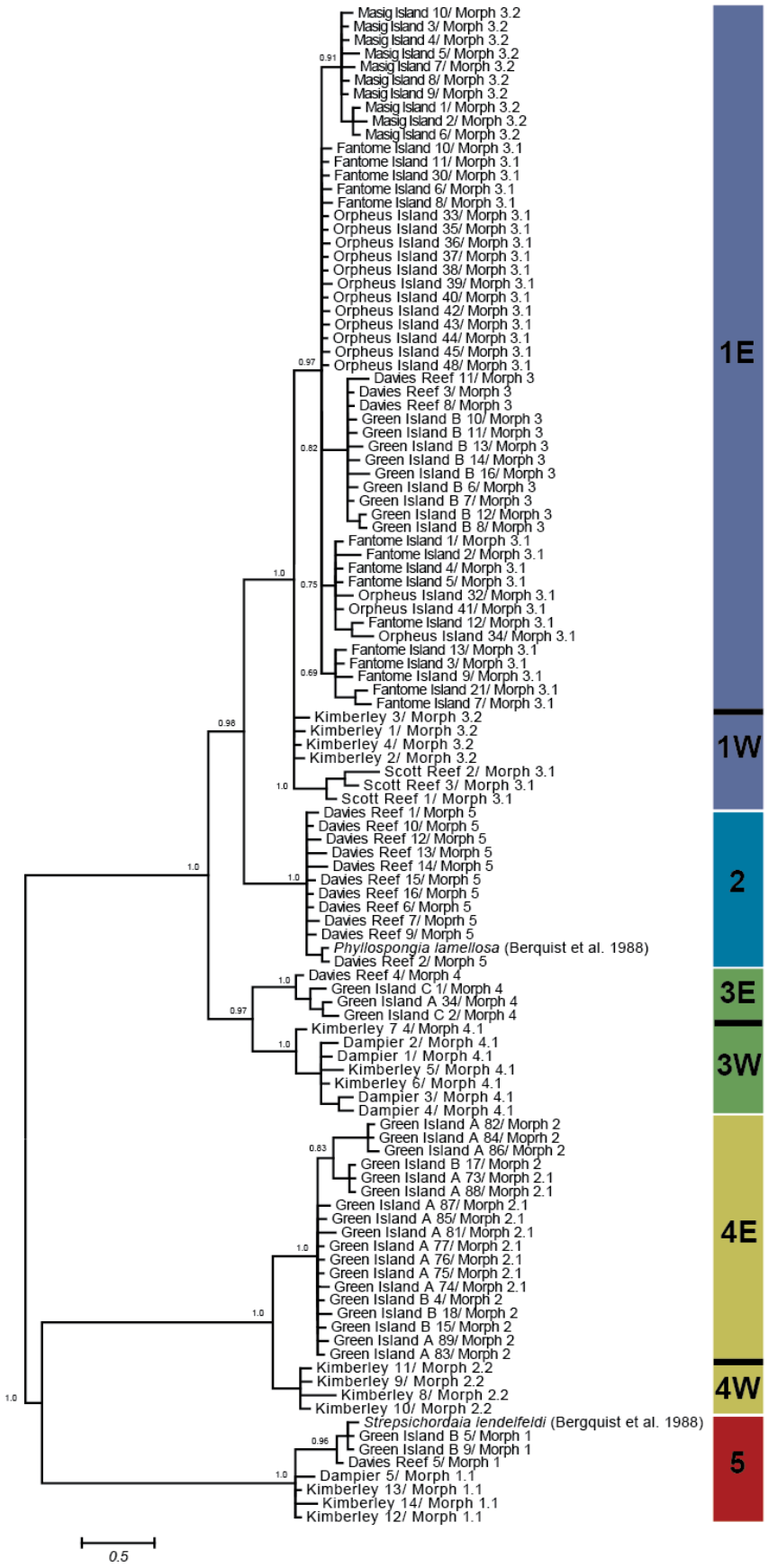

Figure 2.5: Concatenated Bayesian phylogeny using ITS2 alignment and morphological matrix without outgroups. Topology of the combined datasets is congruent to previous phylogenies based on ITS2 alignment alone (see Figure 2.4).

Specimens of *Carteriospongia* displayed distinct reticulation of fine conules which is consistent with the fasciculate nature of the primary fibres and their close association with vermiform tertiary fibres (Figure 2.7a and e; Bergquist et al. 1988). Within *Phyllospongia*, clade 3 exhibited very fine, or an absence of conules, consistent with small primary fibres and mesh sizes, and regular mesh configuration (Figure 2.7a, c and d). Mesh configuration was less regular for all other clades (Figure 2.7c). A large primary mesh size is a trait observed only in *Carteriospongia* (Figure 2.7d). Heavy surface armour (i.e. a thick sand cortex) was recovered as an ancestral trait, conserved in sub-clade 4E in *Carteriospongia*, but reduced in sub-clade 4W and within *Phyllospongia* (Figure 2.7b). All clades and sub-clades can be separated using full combinations of these eight characters except for sub-clades 1E and 1W, which did not show distinctive morphological features.

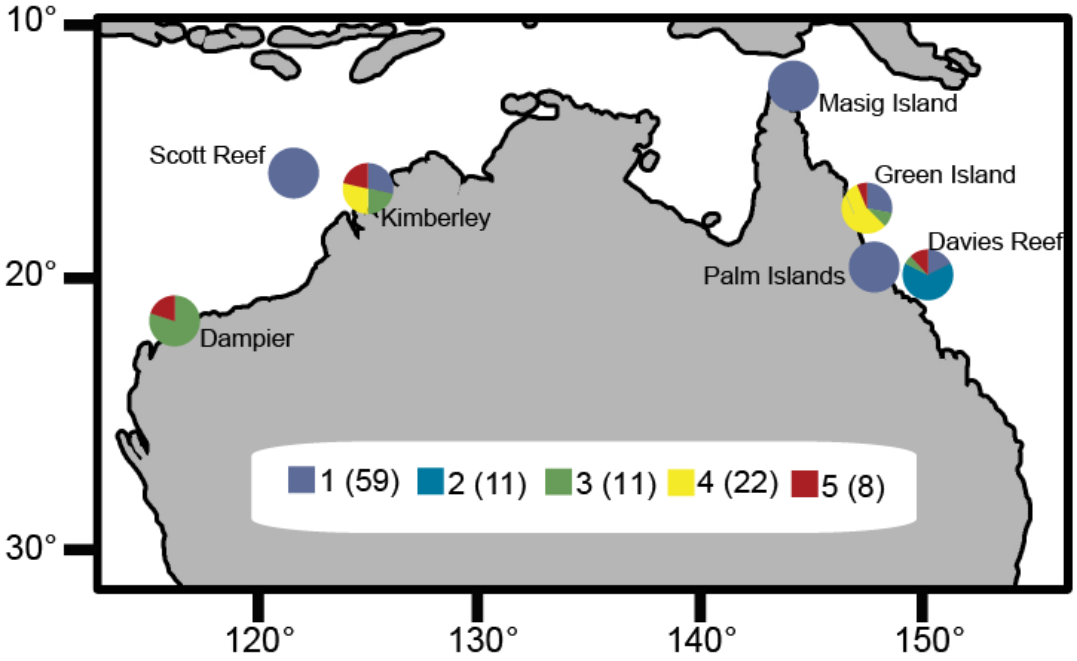

Figure 2.6: Geographical distribution of clades across tropical eastern and Western Australia. All clades occurred on both east and Western Australia, except for clade 2 (*Phyllospongia lamellosa*) which was only recovered from the east coast.

The habitat trait was variable for most clades with individuals retrieved from both intertidal and subtidal habitats (Supplementary Figure 2.4). However, it is noteworthy that sub-clades 3E, 4E and clade 5 were only recovered from the subtidal. A clear trend between habitat and fan thickness was also observed in clade 1, whereby fan morphology was thicker in intertidal specimens. However this relationship was inconsistent in all other clades (Supplementary Figure 2.4).

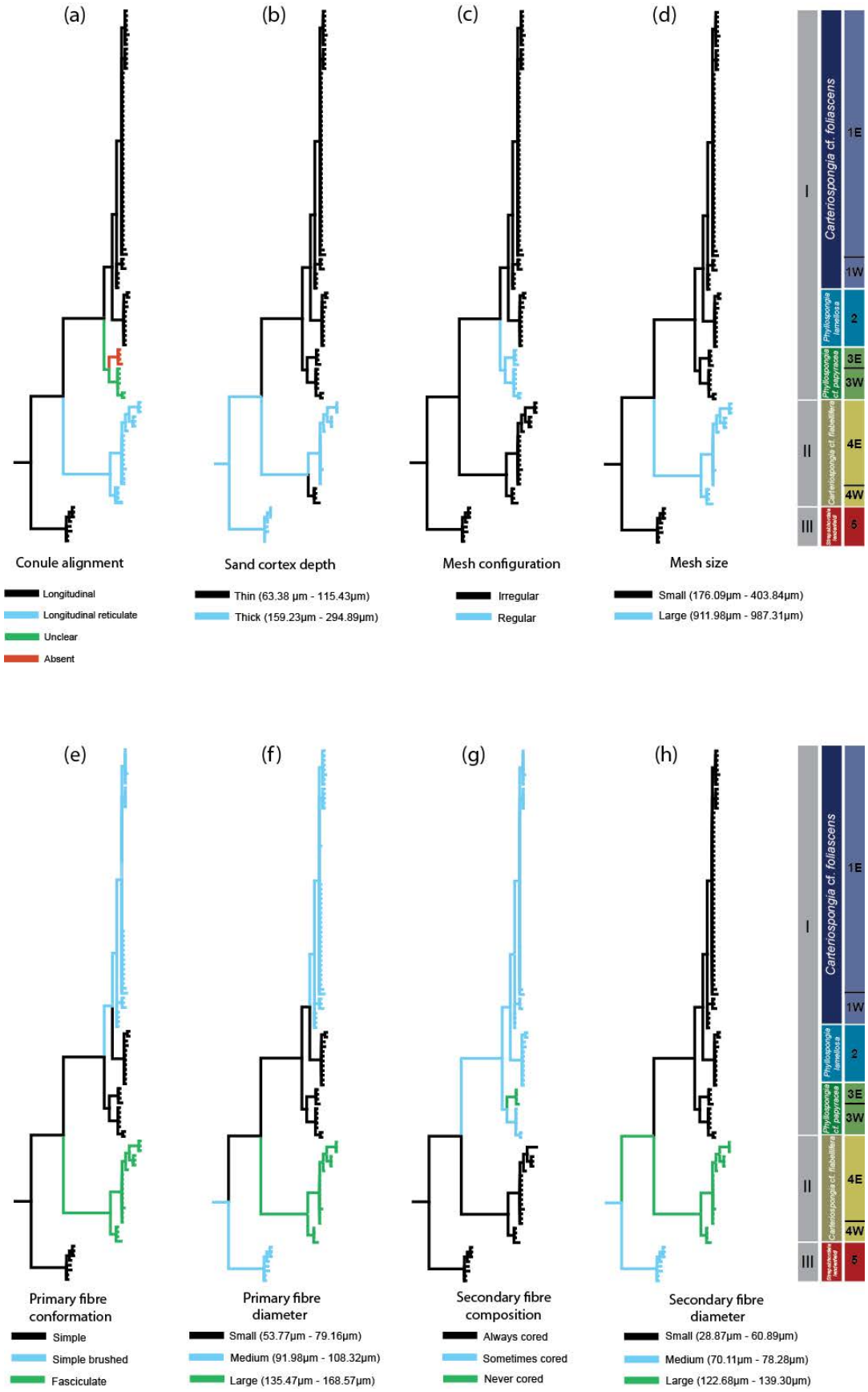

Figure 2.7: Eight most relevant morphological character reconstructions over the ITS2 Bayesian phylogeny. Clades could be separated morphologically based on combinations of these characters. Only sub-clade 1E and 1W were unable to be resolved on the basis of sole morphology.

2.4. Discussion

This study identified eight statistically well-supported clades of ITS2 sequences from specimens collected along the eastern and western coasts of tropical Australia resembling *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* foliose morphology (Figure 2.4). Molecular phylogenies were congruent with morphological differentiation at the genus level, and in some circumstances at the species level. Based on molecular data, clades 2 and 5 were identified as *Phyllospongia lamellosa* and *Strepsichordaia lendenfeldi* respectively. Clades 1, 3 and 4 on the other hand corresponded to *Carteriospongia foliascens*, *P. papyracea* and *C. flabellifera* respectively as inferred by morphological identification discussed in detail by Bergquist et al. (1988). A discrepancy in the genus assignment of a well-known taxon, *C. foliascens*, which was found to associate more closely with the genus *Phyllospongia*, is also highlighted.

Additional statistically well-supported sub-clades were recovered in this study (1W, 3W, 4W). However, their status as valid taxonomic units is somewhat compromised by the phylogenetic resolution power of the ITS2 marker (see results section 3.2). Although sub-clades can be assumed as signatures of paralogous ITS2 copies not yet homogenized by concerted evolution, their distinct geographical distribution is remarkable. Whether these correspond to valid OTUs or the result of recent population fragmentation events requires further assessment by employing additional molecular markers such as nuclear introns (Wörheide et al. 2004).

Given that clades represent assemblages of sequences characterized by strong statistical support, are reciprocally monophyletic, and possess additional characteristic geographic distributions or morphological characters in their support, they are referred to as evolutionarily significant units (ESUs), sensu Moritz (1994).

2.4.1. Morphological plasticity and implications for taxonomy

Thirty-three species distributed across five genera are presently accepted within the subfamily Phyllospongiinae (Van Soest et al. 2013). In this study, morphological and molecular species delineation supported five of the previously known foliose species discussed in detail in Bergquist et al. (1988). However, a discrepancy was observed with *C. foliascens* (ESU 1) residing within the *Phyllospongia* cluster (Cluster I; Figure 2.4 and 2.5; Supplementary Figure 2.2). Taxonomic uncertainties between *C. foliascens* and *Phyllospongia* species, in particular *P. lamellosa,* were noted by Bergquist et al. (1988) due to similarities in surface morphology and sympatric distributions of these species. This

ambiguity is confirmed in the present study by high levels of morphological plasticity encountered within *C. foliascens* from the east coast (ESU 1; i.e. three distinct morphotypes 3, 3.1 and 3.2). The three morphotypes are potential responses to environmental conditions, including light attenuation (e.g. intertidal vs. subtidal), and sedimentation (e.g. inshore vs. offshore), with these environmental conditions potentially influencing fan thickness and oscule profile (i.e. flush with the surface or raised, the latter a specific character for *P. lamellosa*). Similar morphological responses to sedimentation have been demonstrated in juvenile *Coscinoderma matthewsi* (Dictyoceratida), a sponge occurring in sympatry with *C. foliascens* (Abdul Wahab et al. 2012).

Multiple morphological similarities in skeletal organization and structure (Figure 2.7) between *C. foliascens* (ESU 1) and *P. lamellosa* (ESU 2), contradict previous reports (Bergquist et al. 1988). In addition, *C. foliascens* shared only one morphological trait (i.e. mesh configuration) with *C. flabellifera* (ESU 4), further suggesting a misplacement of this species in the genus *Carteriospongia*. Based on morphological and genetic evidence, it is proposed for the inclusion of specimens examined in this study, and those that conform to the description of *C. foliascens* (Bergquist et al. 1988) to be referred to as "*Phyllospongia* sp."

Foliose Phyllosponginiids possess symbiotic cyanobacteria and can rely on phototrophic processes to supply more than 50% of their nutrition (Webster et al. 2012; Wilkinson 1988). A thin and flattened morphology (foliose) is therefore expected for most of these sponges to maximize light capture for photosynthesis. However, conspecific sponges occupying habitats characterized by variable photic regimes can also display differing morphologies. For example, reductions in fan thickness have been observed for *C. foliascens* across intertidal, subtidal (this study) and mesophotic habitats (Bridge et al. 2011b) respectively. This morphological modification increases sponge surface area to volume ratio, and is a potential strategy to maximize light capture and optimization of photosynthesis in lowered light conditions. Morphological plasticity in response to light is well documented in other phototrophic marine taxa such as macroalgae (Talarico and Maranzana2000), seagrasses (Ralph et al. 2007) and corals (Anthony and Hoegh-Guldberg 2003b).

Despite their abundant distribution and conspicuous presence, foliose Phyllosponginiids have often been misidentified or synonymised with unrelated taxa showing similar morphological traits due to homoplasy (Cárdenas et al. 2012). This is in part due to morphological variation in response to local environmental conditions (hydrodynamics) or predation pressure (Palumbi 1984, 1986). Furthermore, due to these sponges lacking spicules, species delineation relies on other morphological characters (i.e.

fibres), some of which can be less informative (Bergquist et al. 1988). Phenotypic plasticity in sponges is common, particularly for skeletal components, which often form diagnostic characters in taxonomy (Borchiellini et al. 2004; Erpenbeck et al. 2006; Erpenbeck et al. 2012). Accordingly, a multifaceted systematic approach is critical for accurate taxonomy of Phyllosponginiids and sponges in general.

2.4.2. Phylogeny of foliose Phyllosponginiids of tropical Australia

The abrupt increase in ITS2 TN-genetic distance between inter-specific and inter-generic pairwise comparisons defines boundaries between Clusters I, II and III, i.e. the genera *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* respectively.

Strepsichordaia (Cluster III; ESU 5) represents the most basal clade in the phylogeny and is considered to be the most ancestral form amongst foliose Phyllosponginiids investigated. Specimens of ESU 5, corresponding to *S. lendenfeldi*, occur exclusively in subtidal habitats suggesting the occupation of subtidal environments to be an ancestral trait. The two derived sister groups, *Phyllospongia* (Cluster I) and *Carteriospongia* (Cluster II) consist of species inhabiting both intertidal and subtidal environments indicating that the conquest of intertidal habitats emerged synchronously in both of them. The occupation of submerged niches by *Strepsichordaia* represents a necessity for respiration, feeding and reproduction (Hooper and Van Soest 2002). On the other hand, the modern ability of *Phyllospongia* and *Carteriospongia* to cope with stressful conditions (i.e. atmospheric exposure and high irradiance) may have developed following rounds of sea level fluctuations over multiple glaciation events and periodic exposure to intertidal environmental conditions (Briggs 2000). However, *P. lamellosa* (Cluster I, ESU 2) and *C. flabellifera* (Cluster 2, ESU 4) from the east coast of Australia were recovered only from subtidal habitats suggesting that occupation of the subtidal is a polyphyletic trait that developed more than once in the genealogy of these three genera.

In foliose Phyllosponginiids, the assignment of a single phenetic trait exclusive to a particular ESU can be challenging. In this study unique phenetic traits useful for the identification of ESUs 1, 3, 4 and 5 are reported. Morphological identification of ESU 2 required the full combination of the eight most informative morphological characters primarily because of its similarity to ESU 1 (Figure 2.7). In addition, the high level of morphological differentiation between sub-clades of east and west geographical origin within ESUs remains to be interpreted. For instance, sub-clades 3E and 3W within ESU 3 originally collected under the name of *P. papyracea* and sub-clades 4E and 4W identified as

C. flabellifera could be easily differentiated using several morphological trait combinations (Figure 2.7; Supplementary Table 2.2). Sub-clades within *P. papyracea* differed in two of the morphological characters (i.e. conule alignment and secondary fibre composition; 3E having a smooth surface morphology and secondary fibres that are never cored). Sub-clades 4E and 4W within *C. flabellifera* on the other hand differed in the level of surface armour (sand cortex depth). It is currently uncertain whether sand cortex depth represents a heritable or environmentally driven character (i.e. level of natural sedimentation). In contrast, sub-clades 1E and 1W could not be differentiated solely on morphology (sharing morphotype 3.1. and 3.2) suggesting instances of cryptic genetic diversity in ESU 1 as demonstrated for other sponge species (Andreakis et al. 2012; Erwin and Thacker 2007).

2.4.3. Phylogeographic relationships

Torres Strait served as an intermittent land bridge over periodic glacial cycles of the late Pleistocene up until ~ 7,000 YBP when the sea rose to present day levels (Reeves et al. 2008; Voris 2000). These geological processes are thought to be responsible for multiple rounds of population fragmentation and speciation in several marine groups (Mirams et al.2011; Wörheide et al. 2008). Foliose Phyllosponginiids have been reported from the GBR to the Dampier Archipelago (Duckworth et al. 2008; Fromont et al. 2006; Van Soest et al. 2013; Wilkinson and Cheshire 1989). The variable levels of genetic diversity (i.e. monomorphic to highly variable geographic populations) found within sampling sites (Figure 2.6) and the complete geographical segregation of sister sub-clades (1E/1W, 3E/3W and 4E/4W) to the east and west coasts of Australia respectively suggests vicariance played a role in shaping Phyllosponginiid distribution across Torres Strait. Isolation between eastern and western taxa via historic vicariant episodes is consistent with phylogeographic patterns reported for other marine species (Andreakis et al. 2012; Mirams et al. 2011; Puckridge et al. 2013). The patterns are further confirmed by the restricted geographical range of *Phyllospongia* and *Carteriospongia* to the tropics and subtropics with discontinuities in distribution over temperate south-eastern and southern Australian bioregions (Figure 2.1a – d).

In contrast, the ancestral *S. lendenfeldi* appears to be distributed throughout Australia and occurs on both sides of Torres Strait. The present distribution of *S. lendenfeldi* suggests the possibility of a stepping-stone model for dispersal through the south of Australia (Figure 2.1e) or through secondary dispersal from the east to west of Torres Strait as proposed in other marine species (Mirams et al. 2011). Larval dispersal in *S. lendenfeldi* is currently unknown, however sponge larval competencies for other GBR dictyoceratid sponges ranges from 2 - 3 days (Abdul Wahab et al. 2011; Ettinger-Epstein et al. 2008; Whalan et al. 2008a)

and is indicative of populations having relatively low larval dispersal potential (Whalan et al. 2008b). Nevertheless, the daily dribble spawning of larvae for several weeks over variable weather and currents may aid range extension, despite short larval competencies (Whalan et al. 2008b). Therefore, the possibility for genetic mixing via larval migration from east to west being responsible for secondary contact and panmixia of reproductively undivided populations in the Holocene cannot be excluded (Mirams et al. 2011).

Several locations on the east and west coast of Australia are identified in this study as hotspots for foliose Phyllosponginiid diversity. On the east coast, biodiversity levels were higher on subtidal offshore reefs, including Green Island and Davies Reef. On the other hand, inshore, intertidal habitats were characterized by monomorphic populations. Importantly, there are clear habitat (water quality) differences between offshore and inshore GBR reefs, with turbidity decreasing with increasing distance from shore (Bannister et al. 2012; Fabricius 2005). Water quality on coral reefs can contribute to Darwinian fitness components (i.e. fecundity and oocyte size) for some sponges (Whalan et al. 2007a) and influence diversity of coral reef invertebrates (Fabricius 2005). Therefore, differences in biological diversity across habitats may reflect different adaptation potential and the capacity of positively selected genotypes to cope with distinct environmental stresses such as high sedimentation and freshwater inclusion associated with inshore environments (Fabricius 2005). For instance, while Davies Reef represents a diversity hotspot for foliose Phyllosponginiids, this was not true for the verongid sponge, *Ianthella basta,* which was represented by a single ESU at the same location (Andreakis et al. 2012). These differences could be related to distinct, species specific physiological traits and symbiotic associations in these two sponges (i.e. phototrophic for Phyllosponginiids and heterotrophic for *I. basta*) (Cheshire et al. 1997; Wilkinson 1983). Phyllosponginiids may potentially out-compete clades of *I. basta* for space in a non-turbid habitat conducive for primary producers. This hypothesis is further supported by the reverse patterns observed at the inshore Palm Islands group (which includes Orpheus Island). On the west coast, the Kimberley supported four Phyllosponginiid ESUs and also three *I. basta* ESUs, thus highlighting this region as a sponge biodiversity hotspot in north-western Australia (Keesing et al. 2011).

2.5. Conclusion

Phyllosponginiids represent an abundant, diverse and ecologically important group of foliose sponges that contribute to primary productivity critical for the maintenance and persistence of local marine biodiversity (Wilkinson 1983, 1988). In the Australian tropics, *Phyllospongia*, *Carteriospongia* and *Strepsichordaia* exhibit a number of different

morphologies previously recognized as five distinct species (Bergquist et al. 1988). Based on a combination of Bayesian inferred morphometrics and molecular systematics, multiple genetically distinct lineages were revealed, suggesting that foliose keratose sponge diversity has been largely underestimated. An enhanced understanding of diversity and species boundaries in widely distributed sponge morphospecies is critical for effective management and conservation of these ecologically important taxa.

Chapter 3

Phenology of sexual reproduction in the common coral reef sponge, *Carteriospongia foliascens*[2]

3.1. Introduction

Coral reefs are ecosystems of significant economic, cultural and ecological importance (Moberg and Folke 1999). While scleractinian corals are the most conspicuous taxa on shallow reefs, sponges (Porifera) also form significant components of coral reef communities (Diaz and Rützler 2001), and are among the key benthic taxa that contribute to reef structure (Richter et al. 2001; Bell 2008). The exceptional pumping capabilities of sponges underpin their functional role on coral reefs via bentho-pelagic coupling processes which contributes to carbon cycling (Reiswig 1981; Yahel et al. 2003; de Goeij et al. 2013), silica cycling (Frøhlich and Barthel 1997; Maldonado et al. 2005) and nitrogen cycling (Corredor et al. 1988; Hoffmann et al. 2009).

The sessile habit of sponges highlights their vulnerability to natural and anthropogenic disturbances (Przeslawski et al. 2008; Bannister et al. 2012; Bell et al. 2013). Whilst impacts to sessile coral reef communities are most noted at localized scales, predicted impacts associated with climate change may impact communities at regional scales (Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Carpenter et al. 2008). Moreover, population recovery and persistence of sessile invertebrates following environmental impacts largely

[2] **Chapter 3** is adapted from Abdul Wahab MA, de Nys R, Webster N, Whalan S (2014) Phenology of sexual reproduction in the common coral reef sponge, *Carteriospongia foliascens.* Coral Reefs 33(2):381-394

relies on the capacity of populations to supply new recruits, sexual reproduction therefore playing an obvious and pivotal role in the resilience of threatened ecosystems (Hughes et al. 2000; Colegrave 2002; Otto 2003). Despite its apparent significance, the importance of coral reef sponge reproduction to population maintenance has largely been overlooked.

On the Great Barrier Reef (GBR), foliose sponges in the subfamily Phyllosponginae can make up to 80 % of total sponge numbers and biomass (Wilkinson 1988). Interestingly, this group of sponges also form close symbiotic associations with cyanobacteria (Webster et al. 2012), with two genera *Phyllospongia* and *Carteriospongia*, being net primary producers, capable of producing three times more oxygen through photosynthesis than respiration (Wilkinson 1983). These phototrophic sponges are also capable of releasing a proportion of the fixed carbon as dissolved organic carbon, contributing to coral reef nutrition (Wilkinson 1983). Their abundance coupled with distinct functional roles, supports the ecological significance of foliose Phyllosponginiids on coral reefs.

Phenology is the study of the influences of seasonal climatic factors such as temperature, photoperiod and rainfall on periodic lifecycle events (such as reproduction) in plants and animals. This study aims to elucidate the effects of relevant natural environmental factors on sexual reproductive phenology of the common phototrophic intertidal Phyllosponginiid sponge *Carteriospongia foliascens*, and investigates the relevance of these environmental parameters to population maintenance in this species. The reproductive biology of *C. foliascens*, within the Palm Islands group was assessed over two reproductive cycles (between 2010 and 2012) to establish natural modes of sexuality (i.e. hermaphroditism or gonochorism) and development (i.e. viviparity or oviparity), seasonality, sex ratios, gametogenesis, reproductive output and size at sexual maturity. Sea surface temperature, photoperiod and monthly rainfall were also monitored to determine the relevance of these parameters to reproduction.

Fortuitously, the summer of 2010 saw record level rainfall and higher than usual sea surface temperatures over northern and eastern Australia, attributed to one of the strongest La Niña events on record (Imielska 2011; Giles 2012). As temperature contributes to phenology in other co-occurring GBR sponges (Ettinger-Epstein et al. 2007; Whalan et al. 2007b; Abdul Wahab et al. 2012), and sponges in general (Maldonado and Riesgo 2008; Longo et al. 2012; Ereskovsky et al. 2013; Mercurio et al. 2013; Zarrouk et al. 2013), the climatic anomaly experienced in this study provided a natural and large scale setting for studying the effects of elevated temperature and rainfall, on the reproduction of *C. foliascens*.

3.2. Materials and methods

3.2.1. Environmental parameters

Data for environmental parameters that potentially regulate *Carteriospongia foliascens* reproduction were sourced from readily available web databases and compiled for the period from March 2010 to June 2012. Monthly sea surface temperatures (SST) were collected from *in situ* temperature loggers (4 m depth) directly off the western shoreline of Orpheus Island (Australian Institute of Marine Science, AIMS data centre, http://data.aims.gov.au). AIMS temperature data was verified against *in situ* temperature loggers (HOBO®) deployed adjacent to sponges over the second reproductive cycle (July 2011 to June 2012). Mean monthly photoperiod was sourced from Geoscience Australia (http://www.ga.gov.au), and total monthly rainfall data from the Bureau of Meteorology (BOM, http://www.bom.gov.au). A full historical climatology record of mean monthly SST (7 years; 2003 to 2009; AIMS data centre) and mean total monthly rainfall (10 years; 2000 to 2009; BOM) was compiled to serve as baseline historical data for comparing environmental data used in this study.

3.2.2. Sample collection and preservation

Monthly samples were collected from *C. foliascens* inhabiting the intertidal reef flat of Little Pioneer Bay, Orpheus Island (18°35.976'S, 146°29.533'E), central Great Barrier Reef (GBR) between March 2010 and July 2012, except for the months from March 2011 to June 2011 due to inclement weather and logistical difficulties. Sponges were sampled to establish mode of sexuality, mode of reproduction, sex ratio, timing of gametogenesis, fecundity and size at sexual maturity. Thirty sponges were sampled each month in depths ranging from 0 m to 2 m (tide dependant), except for March 2010 and April 2010 when 25 and 15 individuals were sampled respectively. Tissue samples were collected by removing a 4 cm deep wedge from randomly selected regions of each sponge. Sponges sampled in previous months, indicated by sampling scars, were avoided. The size of each sponge sampled (maximum length, width and thickness) was also recorded.

Following field collections, 4 – 5 mm thick sections were excised from each sample, placed into a labelled histology cassette and fixed in FAACC [100 ml = 10 ml 40% formaldehyde, 5 ml glacial acetic acid, 1.3 g calcium chloride dehydrate and 85 ml tap water; (Fromont 1999)] pending histology.

3.2.3. Histological processing

Fixed samples were processed for histology using a graded ethanol dehydration and xylene clearing procedure, followed by paraffin impregnation in an automated vacuumed tissue processor (SHANDON Hypercenter XP). Tissue samples were embedded in paraffin and transverse 5 µm sections (i.e. midway along surface of sponge lamellae) cut using a manual rotary microtome (Microm HM325) to maximize surface area for microscopic assessments. Sections were de-waxed in xylene, re-hydrated and stained using Mayer's haematoxylin and Young's eosin-erythrosin (Bancroft and Gamble 2008). Cover slips were applied over stained sections using DPX mountant (Thermo Fisher Scientific).

3.2.4. Analyses of reproductive propagules

A light compound microscope (Leica DM LB), with attached digital camera (Olympus DP25), was used to identify and capture images of reproductive propagules. The image processing and analysis program ImageJ (National Institutes of Health, USA) was used to assess a reproductive output index (ROI). ROI is the proportion of the sampled section that is occupied by the sum (surface area) of all propagules present and is a validated measurement for representing reproduction in sponges (Corriero et al. 1998; Whalan et al. 2007b; Leong and Pawlik 2011; Abdul Wahab et al. 2012). Mode of sexuality and reproduction, seasonality, sex ratios and size at sexual maturity were also determined using histological samples. Female propagules were classified as oocytes, embryos and larvae (Figure 3.1a). Their large size and low numbers within each section allowed for measurement of all propagules within each sample. Spermatic cysts, which were smaller and more abundant (Figure 3.1b), were quantified by randomly sampling five fields of view from each slide (Whalan et al. 2007b; Abdul Wahab et al. 2012).

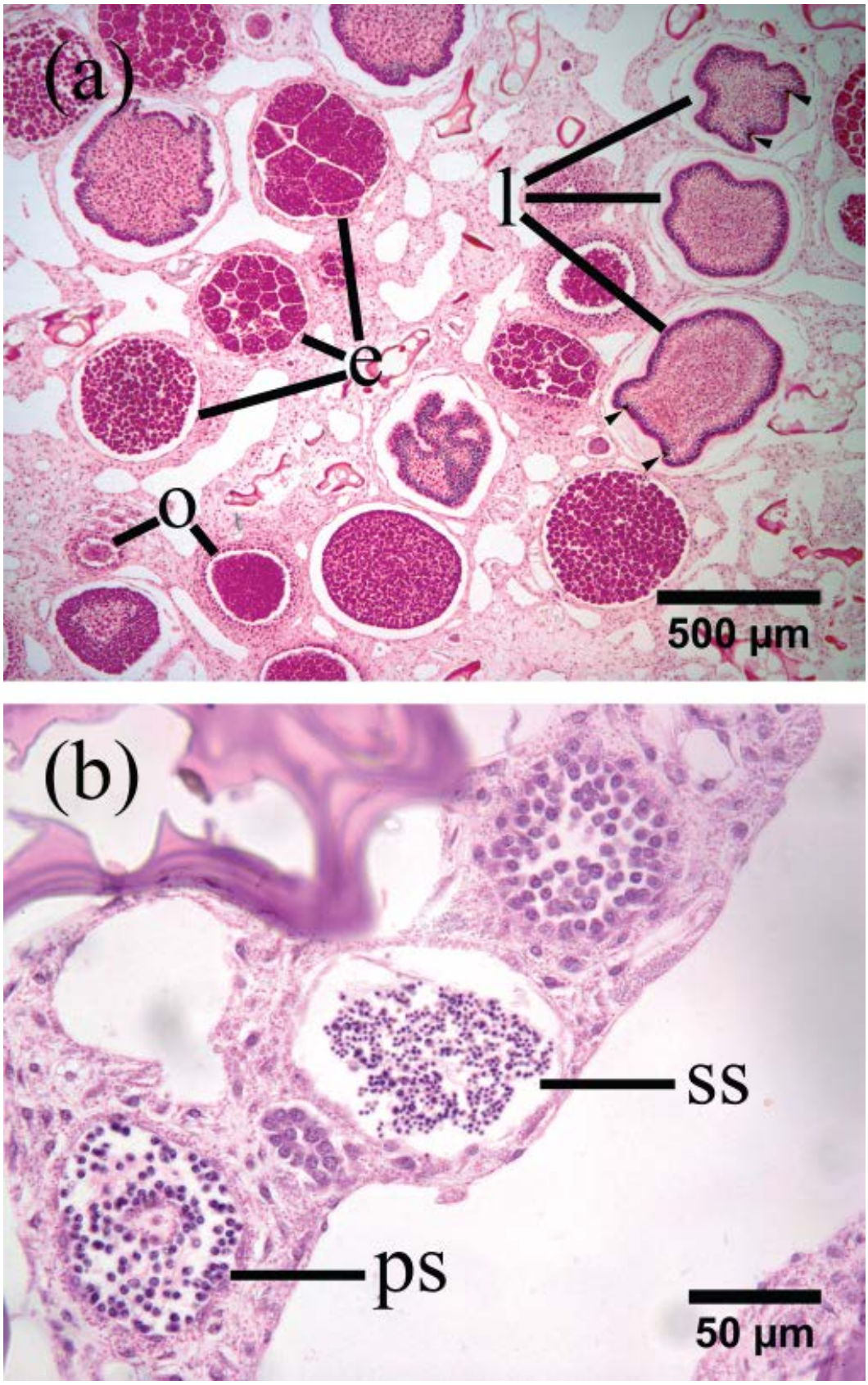

Figure 3.1: *Carteriospongia foliascens.* Photo-micrographs displaying reproductive propagules. a) oogenesis displaying asynchronous development of oocytes (o), embryos (e) and larvae (l) within an individual sponge. Arrows indicate positions of pigmented posterior rings typical of tufted parenchymella larvae. b) Spermatogenesis showing asynchronous development of primary (ps) and secondary spermatic cysts (ss) within an individual sponge and synchronous development of sperm within cysts.

The capacity for a population to produce functional larvae for dispersal and recruitment is the epitome of sexual reproductive success, and is a relevant measure for understanding population dynamics. To amalgamate information derived from reproductive parameters examined in this study (i.e. proportion reproductive, ROI and fertilization success), a population sexual productivity index (PoSPi) was formulated to aid comparison of larval productivity of the population over time. PoSPi considers 1) the proportion of the population that are female and reproductive, 2) fertilization success as represented by proportion of the population female propagules that were fertilized (i.e. embryo and larvae) and 3) population female fecundity (mean ROI):

$$\text{PoSPi} = \text{Proportion reproductive}_{\text{female}} \times \text{Proportion propagules}_{\text{fertilized}} \times \text{mean ROI}_{\text{female}} \times 100$$

For example, in a hypothetical population where all individuals are females containing only embryos and/ or larvae (proportion reproductive $_{\text{female}}$ = 1, proportion propagules $_{\text{fertilized}}$ = 1), and having a mean population ROI of 50 % (0.5), then PoSPi will be equivalent to 50. Alternatively, a population that is non-reproductive, having females possessing only oocytes or consisting of only male individuals will yield a PoSPi of zero.

3.2.5. Statistical analyses

Assumptions of normality and homoscedasticity were verified from residual plots and data transformations applied when assumptions were not met (Quinn and Keough 2002; see Supplementary Materials, Appendix). Datasets not meeting assumptions post-transformations, and those having unbalanced designs were analysed using permutational methods (PERMANOVA) (Anderson 2005). Differences in total monthly rainfall during peak *C. foliascens* reproduction (July to December), between reproductive cycles were assessed using a t-test. Pearson product moment correlation was used to define the relationship between photoperiod and sea surface temperature, and periodic regressions were used to assess cyclic patterns of reproductive parameters (proportion of population reproductive, propagule sizes, proportion fertilized propagules, ROI and PoSPi) over the two reproductive cycles investigated in this study.

Periodic regression is a powerful and robust tool for analysing cyclic data encountered in reproduction studies (Abdo et al. 2008a). It assigns an angular representation of time (θ) to each month by dividing yearly data into 360° (2π radians), which is then transformed ($\sin\theta$, $\cos\theta$, $\sin 2\theta$ and $\cos 2\theta$) and used as independent variables (regressors) for linear regression analyses (deBruyn and Meeuwig 2001). Forward stepwise regressions and Durbin-Watson tests were used to optimize periodic regression models (see Table 3.1 for

full model description) and verify effects of temporal autocorrelations in individual datasets respectively. As no significant temporal autocorrelation were detected, multiple linear regressions on the raw reproduction data using optimized models were performed (see Supplementary Materials, Appendix for more details). To assess differences between treatment factors (i.e. reproductive cycles, male and female), resulting slopes from periodic regression analyses were compared using homogeneity of slopes model (HSM) analysis, using slope coefficients as continuous variables (covariates) and treatment as a categorical variable (GLM, Statistica; Supplementary Materials, Appendix). To determine any deviation from a sex ratio of unity (1:1), a Chi square test with Yate's correction was performed using all reproductive individuals collected.

Correlations were used to examine the influence of sea surface temperature and body size on reproductive parameters (proportion reproductive, propagule sizes and ROI). To assess differences in oocyte sizes between seasons (winter: July and summer: December) a two-way ANOVA was used using a randomly selected dataset (random number generator) from each month. Differences in fertilized propagule (embryo and larvae) sizes among temperature profiles (low: June, increasing: September, high: December and decreasing: March) and reproductive cycles (cycle 1 and cycle 2) were assessed using two-way PERMANOVA. Euclidean distance matrices of raw untransformed data were used for all permutational analyses (9999 permutations).

3.3. Results

3.3.1. Environmental parameters

Mean monthly photoperiod was significantly correlated to mean monthly sea surface temperature (SST) (Pearson's $r = 0.768$, $p < 0.0001$) and did not vary between reproductive cycles (HSM: $F_{cycle(1, 15)} = 0.0$, $p > 0.5$) (Figure 3.2a; see Supplementary Materials, Appendix for detailed summary of test statistics for periodic regressions and correlations). As such, photoperiod was excluded from further analyses. SST displayed a mono-cyclic sinusoidal pattern over each reproductive season (Table 3.1). Mean total monthly rainfall between July and December (as reproduction was increasing) was significantly higher over the first reproductive cycle (Year 2010; 388.31 mm ± 118.64, mean ± SE) compared to the second reproductive cycle (Year 2011; 80.75 mm ± 48.30) (t-test: $t = 2.401$, $df = 10$, $p < 0.05$) (Figure 3.2b). Anomalous SST and rainfall patterns (compared to historic data) occurring over peak sponge reproduction (i.e. between July and December) was observed in the La

Niña year (2010), and was not pronounced in 2011 and 2012 (see Supplementary Figures 3.1 and 3.2). Mean monthly rainfall was > 300% higher from August 2010 to December 2010 (up to 933% in October 2010) when compared to mean monthly rainfall over the same period for the past 10 years (years 2000 to 2009; Supplementary Figure 3.1 and Supplementary Table 3.1). Mean SST was also higher between May 2010 to October 2010 (> 0.5°C higher; up to 2.1°C higher in September 2010) when compared to mean SST over the same period for the past 7 years (2003 to 2009; see Supplementary Figure 3.2 and Supplementary Table 3.2).

3.3.2. Patterns of reproduction

A total of 482 unique reproductive *Carteriospongia foliascens* were sampled from Little Pioneer Bay over the 28 month study period, consisting of 206 female and 276 male individuals. All remaining sponges sampled (n = 158) were non-reproductive. Based on observations from this study, female and male propagules never co-occurred in reproductive individuals sampled suggesting this species to be gonochoric. *C. foliascens* is a brooder and larvae develop asynchronously throughout the mesohyl. Sex ratio of females to males was 1:1.3 and deviated significantly from a sex ratio of unity (Chi square test with Yates correction: $\chi^2 = 9.878$, df = 1, $p < 0.005$).

Both sexes of *C. foliascens* were reproductive all year, except in March 2010 when no males were detected (Figure 3.2). In both reproductive cycles, the maximum proportion of total reproductive individuals occurred three months prior to the annual SST maxima (Figure 3.2a). Comparison of the regression slopes of the proportion of total reproductive samples between the two reproductive cycles resulted in a significant effect of cycle (Table 3.1, HSM: $F_{(1, 8)} = 5.45$, $p < 0.05$), with more reproductive individuals found in the second cycle (75.28 % ± 5.45) than in the first cycle (57.96 % ± 8.70).

Over the two cycles, male reproductive patterns were positively correlated to temperature (Pearson's r = 0.511, $p < 0.05$) and were different between cycles as explained by different regression slope coefficients (Table 3.1). The proportion of reproductive males was lower in the second reproductive cycle (July 2011: 3.3 %, 21.8°C) over the annual temperature minima (July 2010: 6.6 %, 23.0°C) (Figure 3.2a). The proportion of reproductive males increased with rising temperatures between August 2011 (20 %) and October 2011 (60 %) and fluctuated around this level over the warmer months (October 2011 to March 2012) before decreasing as temperature again approached the annual minima (June 2012: 20%, 22.9°C).

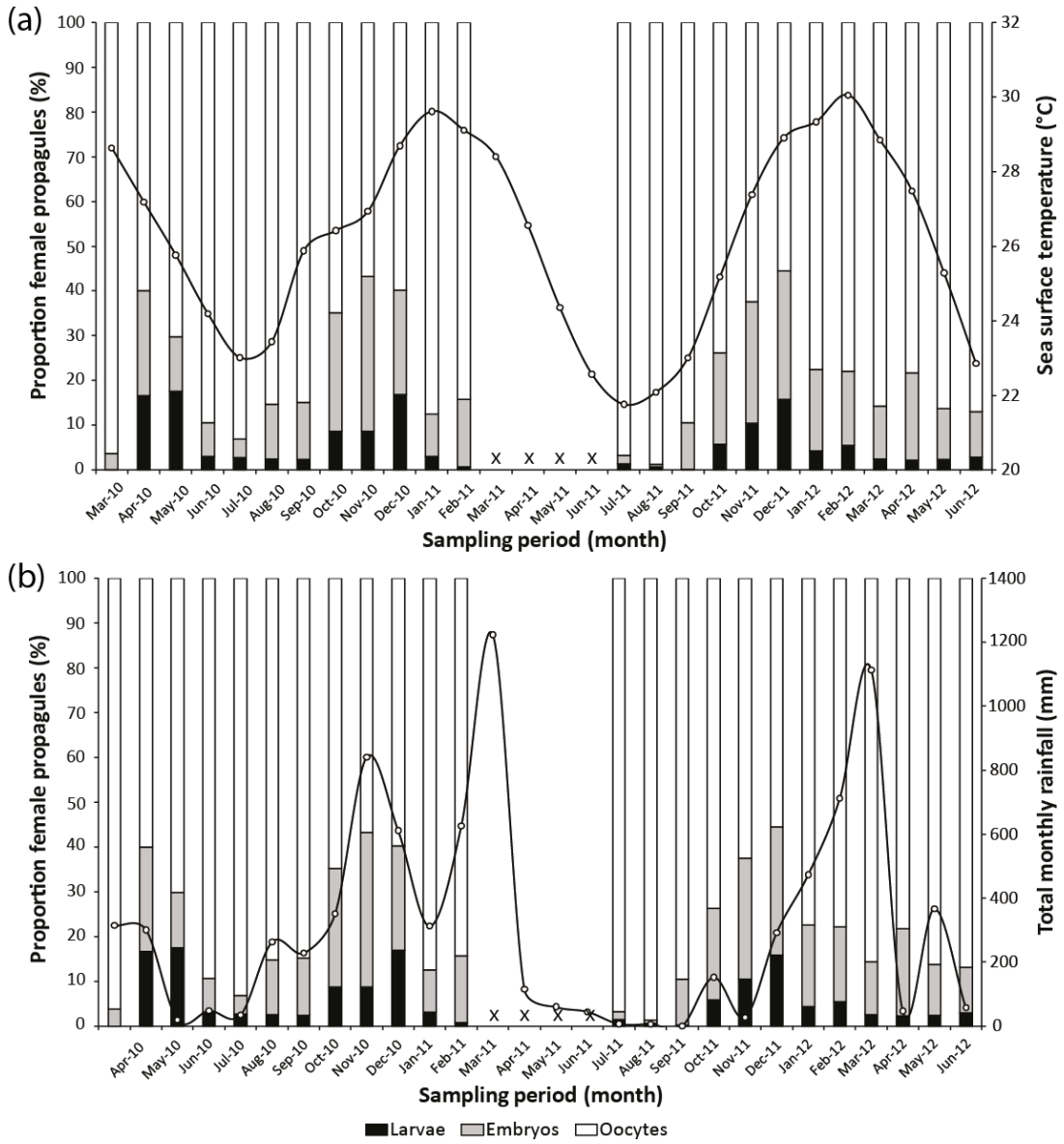

Figure 3.2: *C. foliascens.* Little Pioneer Bay, Orpheus Island. Proportion of total male and female sponges over a 28 month sampling period, considering a) mean monthly sea surface temperature (SST, °C) and b) total monthly rainfall (mm) (March 2011 to June 2011 represents missing data, x). Black dashes (—) represent total proportion of individuals that were reproductive each month.

The proportion of females that were reproductive was not significantly correlated to temperature (Pearson's r = -0.306, $p > 0.05$). The proportion of reproductive females was higher in the second cycle (July 2011: 46.7 %) compared to the first reproductive cycle over the same period (July 2010: 23.3 %) and fluctuated around this level before decreasing in June 2012 (16.7%; 22.6°C) (Figure 3.2a). The patterns of reproduction between male and female were significantly different as indicated by a significant interaction between treatment (sex) and the cos (2θ) covariate (Table 3.1, HSM: $F_{(1, 32)} = 5.13$, $p < 0.05$).

3.3.3. Gametogenesis, embryogenesis and larval development

Spermatic cysts developed asynchronously throughout the mesohyl, with spermatogenesis occurring synchronously within each spermatic cyst. Spermatic cysts were spherical with cross sectional area of 3185.40 ± 20.36 μm^2 (mean ± SE, n = 5000). Oocytes were spherical, having a distinct nucleus and were present throughout the year (Figure 3.1b and 3.3). Oocyte size fluctuated in an annual cyclic pattern (Table 3.1) and was consistent within seasons (i.e. winter or summer) between the two reproductive cycles (ANOVA: $F_{(1, 268)} = 0.169$, $p > 0.05$), but increased significantly from winter (July 2010 and 2011; 9741.16 μm^2 ± 1799.33, n = 136) to summer (December 2010 and 2011; 29294 μm^2 ± 3007.32, n = 136) (ANOVA: $F_{(1, 268)} = 31.142$, $p < 0.001$).

Embryos were spherical and comprised varying stages of cell cleavage (from two cell stage to morula, Figure 3.1b). Brooded larvae were parenchymellae characterized by a distinctive posterior pigmented ring of tufted cilia. Embryos and larvae were present in every sampling month over the two reproductive cycles, except for March 2010 and September 2011 when no larvae were detected, and August 2011 when no embryos were observed (Figure 3.3). Total proportions of post-fertilized propagules (i.e. embryos and larvae) increased with increasing temperature (Pearson' $r = 0.47$, $p < 0.05$) and was highest between October ($October_{2010}$ = 35.13 % and $October_{2011}$ = 26.26 %) and December ($December_{2010}$ = 40.26 % and $December_{2011}$ = 44.44 %) in both reproductive cycles. The higher proportions of fertilized propagules over increasing temperature (July to December) was consistent between the first and second reproductive cycle (Table 3.1, HSM: $F_{(1, 8)cycle} = 3.34$, $p > 0.05$).

Embryonic and larval sizes did not follow annual cyclic patterns (PR: $p > 0.05$). Embryonic sizes were significantly influenced by temperature profile (i.e. low: June, increasing: September, high: December and decreasing: March) and reproductive cycle (PERMANOVA: Pseudo-$F_{(3, 883)Temperature\ profile} = 2.74$, $p < 0.05$ and Pseudo-$F_{(1, 883)Cycle} = 4.70$, $p < 0.05$). For example, embryonic sizes were consistently lower over decreasing temperature profile ($Cycle1_{Decreasing}$ = 77899.17 μm^2 ± 12021.78) compared to other temperature profiles (Cycle 1_{High} = 100436.80 μm^2 ± 4430.52, $Cycle1_{Increasing}$ = 99566.09 μm^2 ± 3966.94, $Cycle1_{Low}$ = 99741.87 μm^2 ± 8093.49). Larval sizes were consistent between temperature profiles and reproductive cycles (PERMANOVA: $p > 0.05$).

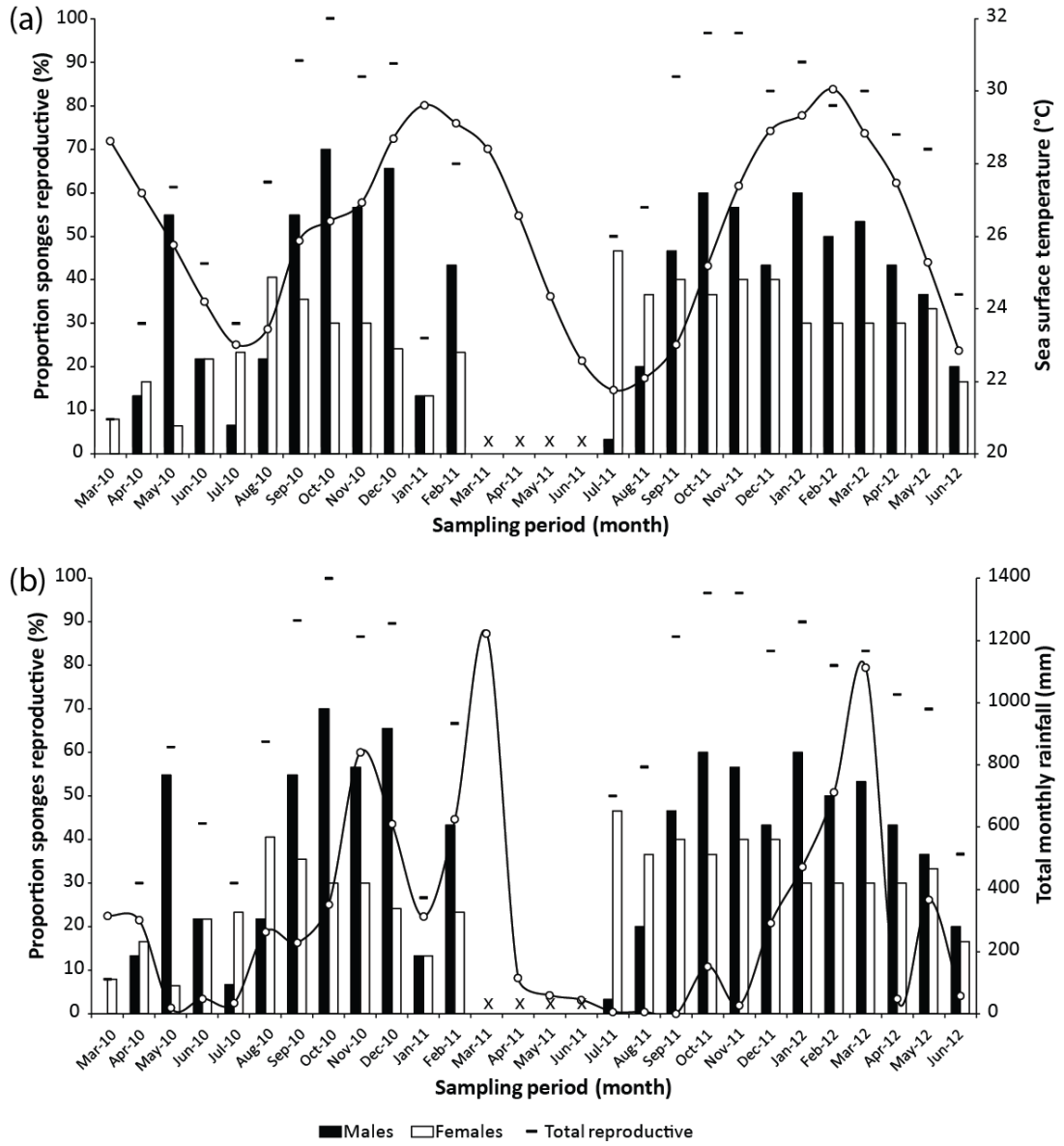

Figure 3.3: *C. foliascens*. Little Pioneer Bay, Orpheus Island. Cumulative bar graph showing proportion (%) of female propagules that were oocytes, embryos and larvae sampled over a 28 month sampling period, considering a) mean monthly sea surface temperature (SST, °C) and b) total monthly rainfall (mm) (March 2011 to June 2011 represents missing data, x).

Table 3.1: *C. foliascens*. Little Pioneer Bay, Orpheus Island. Summary table of periodic regression statistics for mean sea surface temperature (SST), proportion total reproductive, female propagule size, proportion fertilized propagules and reproductive output (ROI) assessed in this study.

$$y=B_0 + B_1\sin(\theta) + B_2\cos(\theta) + B_3\sin(2\theta) + B_4\cos(2\theta)$$

		Annual cycle			Bi-annual cycle			
		B_0	B_1	B_2	B_3	B_4	R^2	p
SST								
Over two reproductive cycles		26.21	+0.65	+3.51	N.A	N.A.	0.93	<0.0001
	p		0.0015	0.0000	N.A	N.A.		
Proportion total reproductive								
Reproductive cycle 1 (both sexes)		57.96	-30.69	+1.93	N.A.	-10.86	0.64	<0.05
	p		0.0076	0.8298	N.A.	0.2456		
Reproductive cycle 2 (both sexes)		75.28	-10.16	+18.71	N.A.	-11.67	0.90	<0.001
	p		0.0087	0.0002	N.A.	0.0043		
Male (two reproductive cycles)		38.15	-11.81	+12.73	N.A.	-11.68	0.50	<0.01
	p		0.0208	0.0136	N.A.	0.0220		
Male (reproductive cycle 1)		35.19	-19.47	N.A.	N.A.	N.A.	0.27	<0.05
	p		0.0487	N.A.	N.A.	N.A.		
Male (reproductive cycle 2)		41.11	N.A.	+19.36	N.A.	-11.67	0.86	<0.001
	p		N.A.	0.0001	N.A.	0.0039		
Female (two reproductive cycles)		28.47	-8.62	-2.41	N.A.	+0.42	0.36	<0.05
	p		0.0038	0.3714	N.A.	0.1589		
Female propagule size								
Oocyte size (reproductive cycle 2)		19667.70	-6568.79	+3968.28	N.A.	-5000.62	0.70	<0.0001
	p		0.00009	0.0080	N.A.	0.0014		
Proportion fertilized propagules								
Reproductive cycle 1		29.15	N.A.	+19.49	N.A.	N.A.	0.90	<0.001
	p		N.A.	0.0039	N.A.	N.A.		
Reproductive cycle 2		24.58	N.A.	+24.04	N.A.	N.A.	0.99	<0.0001
	p		N.A.	0.00007	N.A.	N.A.		
Reproductive output (ROI)								
Male (reproductive cycle 1)		1.33	-1.12	N.A	N.A.	-0.56	0.83	<0.001
	p		0.0002	N.A	N.A.	0.0148		
Male (reproductive cycle 2)		1.71	-1.44	N.A	N.A.	-0.92	0.80	<0.001
	p		0.0007	N.A	N.A.	0.0098		
Female (reproductive cycle 1)		1.28	-1.10	N.A	N.A.	-0.59	0.79	<0.001
	p		0.0006	N.A	N.A.	0.021		
Population sexual productivity index (PoSPi)								
Over two reproductive cycles		0.12	-0.13	+0.09	-0.08	N.A.	0.53	<0.05
	p		0.0026	0.0238	0.0443	N.A.		

y Represents the dependant variable, B_0 is the mean level of y, and B_1, B_2, B_3 and B_4 are model coefficients, and when considered together define the phase shift and amplitude. Forward stepwise regressions were conducted to optimize models prior to multiple regressions. N.A. (not applicable) refers to omitted predictor variables that did not contribute significantly to the model.

3.3.4. Reproductive output index (ROI)

Mean male ROI exhibited an annual cyclic pattern and was consistent between the two reproductive cycles (Table 3.1, HSM: $F_{(1,16)Cycle} = 2.41$, $p > 0.05$). Mean female ROI was cyclic for the first reproductive cycle (Table 3.1) but was inconsistent over the second cycle (PR: $p > 0.05$). Mean ROI for both sexes increased with increasing temperature over both reproductive cycles, with highest ROI recorded between September and December (e.g. first cycle: peak male ROI = 3.13 % ± 0.43, September 2010 and peak female ROI = 3.24 % ± 1.07, October 2010; second cycle: peak male ROI = 5.09 % ± 0.40, October 2011 and peak female ROI = 4.43 % ± 0.90, December 2011) (Figure 3.4).

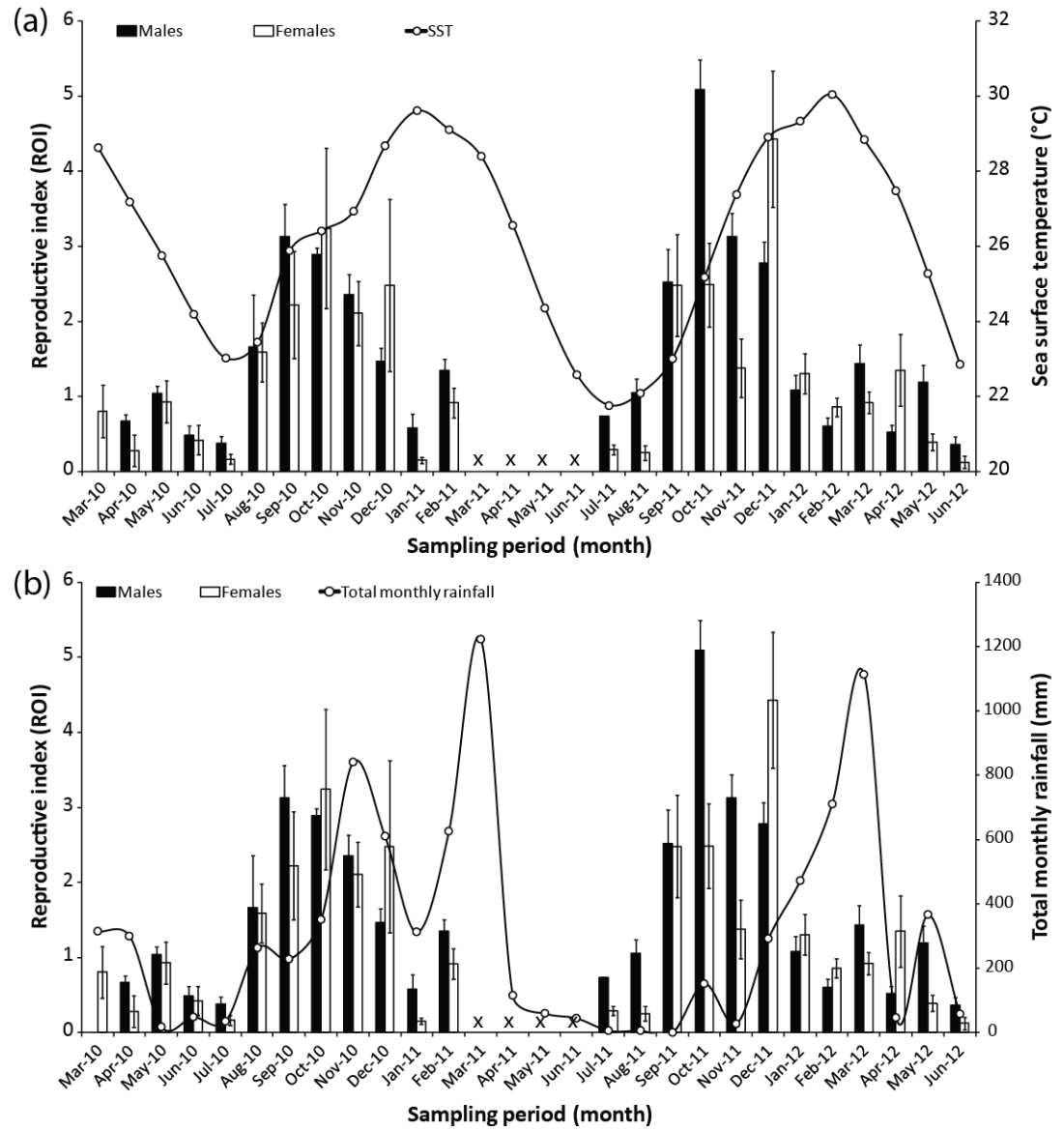

Figure 3.4: *C. foliascens*. Little Pioneer Bay, Orpheus Island. Mean male and female reproductive output index (ROI, % ± SE) over a 28 month sampling period, considering a) mean monthly sea surface temperature (SST, °C) and b) total monthly rainfall (mm) (March 2011 to June 2011 represents missing data, x).

During the second reproductive cycle, male and female ROI were lower over the annual temperature minima (August 2011: male ROI = 1.05 % ± 0.19 and female ROI = 0.25 % ± 0.10) when compared to the first reproductive cycle (August 2010: male ROI = 1.66 % ± 0.69 and female ROI = 1.59 % ± 0.39), corresponding to lower temperature in the second cycle (August 2010 = 23.4°C and August 2011 = 22.1°C) (Figure 3.4). ROI increased sharply from August 2011 to September 2011 (male ROI = 2.522 % ± 44 and female ROI = 2.48 % ± 0.68) when temperature approached 23.0°C. Over both reproductive cycles, male and female ROI decreased sharply from December to January over the annual temperature maxima (January 2011 = 29.01°C and January 2012 = 29.33°C), and ROI remained consistently low as temperature decreased towards the annual minima (< 1.44 % ± 0.25) (Figure 3.4). Female ROI was only correlated to temperature when it was increasing over low ranges (July to September: 21.8°C to 25.9°C) (Pearson's $r = 0.38$, $p < 0.01$). Similarly, male ROI was positively correlated to temperature when it increased over low ranges (Pearson's $r = 0.39$, $p < 0.01$), but was negatively correlated when temperature was increasing over high ranges (October to December: 25.2°C to 28.9°C) (Pearson's $r = -0.54$, $p < 0.001$).

3.3.5. Body size effects on reproduction

To examine the effects of sponge body size on reproduction, data from the four most reproductive months during the second reproductive cycle were used (September 2011 – December 2011). In general, sponges were skewed towards smaller classes (1 – 15 cm^3 to 106 – 120 cm^3) and sexual maturity was achieved in the smallest size class (1 – 15 cm^3) (Figure 3.5). Non-reproductive individuals were distributed over a large size range and were represented by the smallest size class (1 – 15 cm^3) to large size classes (331 – 345 cm^3). There was no effect of body size on reproductive output (ROI) in either sex, with small and large individuals displaying similar fecundity (Pearson's correlation: $p > 0.05$).

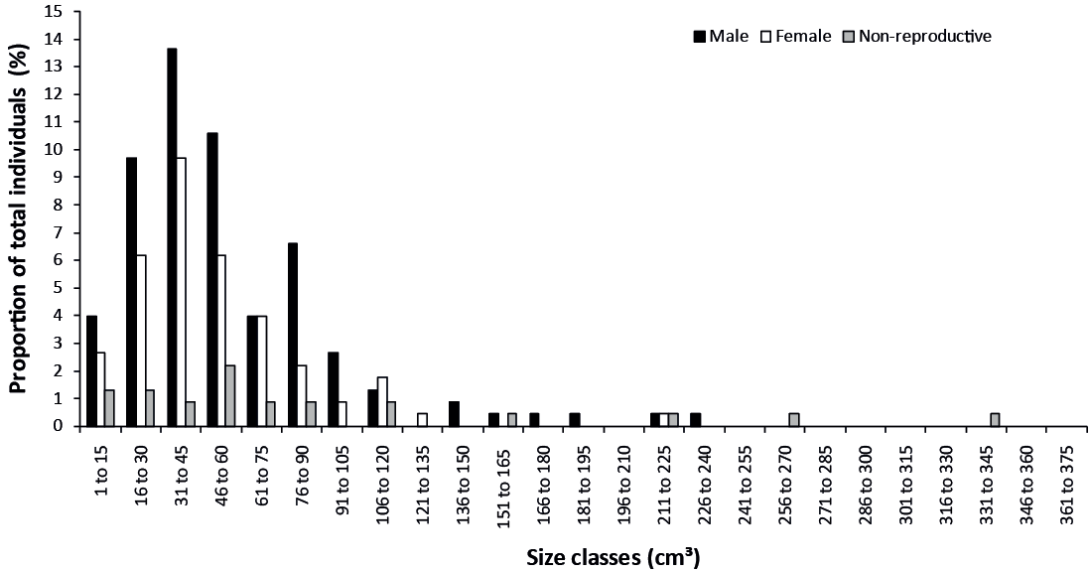

Figure 3.5: *C. foliascens*. Little Pioneer Bay, Orpheus Island. Size frequency distribution of male, female and non-reproductive sponges from the four most reproductive months of the second reproductive cycle (2011; September, October, November and December).

3.3.6. Population sexual productivity index (PoSPi)

PoSPi displayed a cyclic pattern over the two reproductive cycles and was positively correlated to increasing temperature from July to December (Pearson's $r = 0.78$, $p < 0.05$) (Figure 3.6). Maximum PoSPi was over two times higher in the second reproductive cycle (December 2011: 0.79) than in the first reproductive cycle (October 2010: 0.34), indicative of a higher ROI and proportion of reproductive females. PoSPi was three times higher when it was compared for the same period between cycles (December 2010: 0.24 and December 2011: 0.79). Lowered maximum PoSPi in the first reproductive cycle during peak *C. foliascens* reproduction, between July 2010 and December 2010, coincided with higher total monthly rainfall and higher SST minima (Figure 3.6).

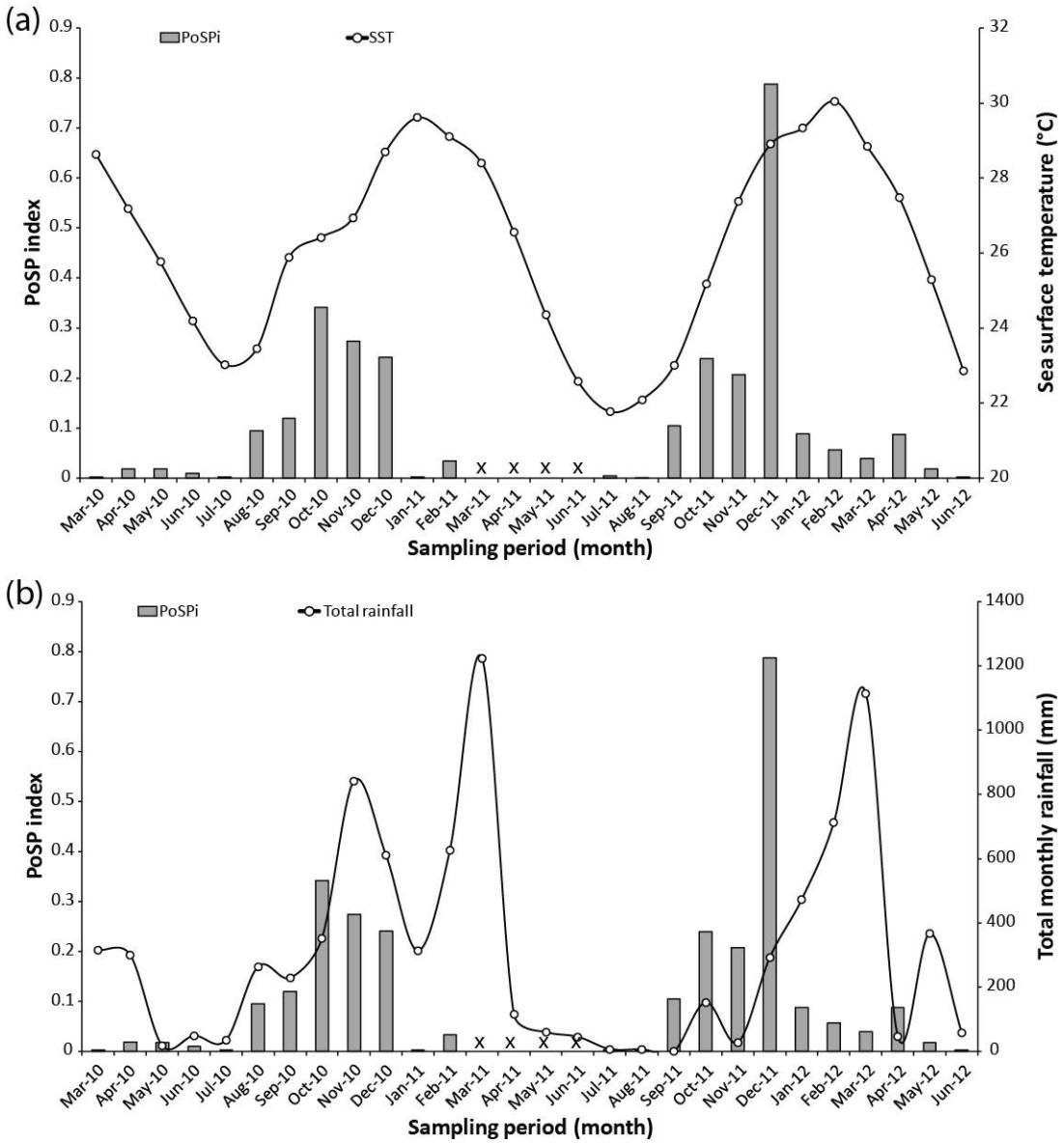

Figure 3.6: *C. foliascens*. Little Pioneer Bay, Orpheus Island. Monthly population sexual productivity index (PoSPi) over a 28 month sampling period, considering a) mean monthly sea surface temperature (SST, °C) and b) total monthly rainfall (mm) (March 2011 to June 2011 represents missing data, x). PoSPi considers 1) the proportion of the population that are female, 2) mean female population ROI and 3) proportion of female propagules that were fertilized (i.e. embryos and larvae).

3.4. Discussion

Temperature is an important environmental cue for reproduction in *Carteriospongia foliascens,* with increasing sea surface temperatures (SST) coinciding with peaks in reproductive effort. However, the maintenance of baseline reproduction throughout the year, as evidenced by production of both eggs and sperm (except for March 2010 when no male sponges were present), contrasts with other co-occurring species in the central Great Barrier Reef (Ettinger-Epstein et al. 2007; Whalan et al. 2007b; Abdul Wahab et al. 2012). It is unknown what influences year round reproduction in this species but underlying endogenous

processes may contribute to reproduction during the cooler months. Importantly, the influence of temperature on the phenology of *C. foliascens* is clearly demonstrated by annual mono-cyclic reproductive patterns closely emulating natural SST fluctuations, and having a lag offset of three months (indicated by maximum proportion of the population being reproductive and maximum ROI being reached ca. three months earlier than the annual SST maxima). This lag offset supports peak effects of SST on reproduction as it increases over lower temperature ranges immediately after the annual SST minima (between July to September), inducing sharp increases in total numbers of reproductive individuals, mean reproductive output (ROI) and mean size of oocytes.

This study confirms the importance of increasing temperature on the reproduction of co-occurring brooding sponge species (Ettinger-Epstein et al. 2007; Whalan et al. 2007b; Abdul Wahab et al. 2012), and sponges in general (Fromont and Bergquist 1994; Witte and Barthel 1994; Fromont 1999; Ereskovsky 2000; Usher et al. 2004; Abdo et al. 2008a; Longo et al. 2012; Ereskovsky et al. 2013; Mercurio et al. 2013; Zarrouk et al. 2013). While increasing SST is associated with an increase in reproductive output (ROI) in *C. foliascens* over low temperature ranges (< 25°C), the effect of SST in male sponges is reversed over higher ranges (> 25°C between October and December), whereby increasing temperature led to a decrease in reproductive output. This relates to the release of sperm and corresponds to the increasing proportions of embryo and larvae in female sponges over this period, indicating peak fertilization. Likewise, female ROI decreased sharply between December and January signalling peak release of larvae over this period.

Decreases in SST, associated with the Austral winter, also corresponds to changes in reproduction for *C. foliascens*, both in the proportion of reproductive individuals and reproductive output. Notably, the lowered mean SST minima by 1.3°C in August 2011 (22.1°C) compared to the same period in the previous year (23.4°C), is reflected in reduced male and female ROI. Interestingly, a sharp increase in ROI was initiated as SST increased above 23.0°C (between August 2011 and September 2011) complementing trends in 2010 (between July 2010 and August 2010) as SST rose to similar levels. An analogous finding has been reported for the co-occurring brooding sponge *Luffariella variabilis*, which demonstrated the initiation of spermatogenesis at temperatures above 22.5°C (Ettinger-Epstein et al. 2007) suggesting baseline temperatures (between 22.5°C to 23°C) are required for reproduction in these sponges. As *C. foliascens* is phototrophic (Wilkinson 1983, 1987) the influence of photoperiod, which co-varies with temperature, on reproduction cannot be excluded.

C. foliascens was reproductive all year round which contrasts to other co-occurring brooding sponge species that "shut-down" reproduction (i.e. complete absence of reproductive individuals) for five to seven months over decreasing and low SST (Ettinger-Epstein et al. 2007; Whalan et al. 2007b; Abdul Wahab et al. 2012). As reproduction is energetically costly (Harshman and Zera 2007), this year-round reproductive strategy may relate to their mode of phototrophic symbiosis which could provide an energetic advantage over heterotrophic species (Wilkinson 1983; Webster et al. 2012). Interestingly, other common brooding species considered "weedy" in marine habitats such as the coral *Pocillopora damicornis*, and the reef boring sponge *Thoosa mismalolli* also display year round patterns of reproduction (Richmond and Hunter 1990; Bautista-Guerrero et al. 2010). The ecological success of common and abundant species, including *C. foliascens*, could be attributed to a bet-hedging strategy of reproduction (Cohen 1966; Hopper 1999). Under this theoretical framework, producing larvae all year round would minimize the risk of total clutch mortality of larvae and juveniles when exposed to an overlapping stressful event, thus reducing variations in reproductive success and maximizing long-term fitness under variable environmental conditions (Crean and Marshall 2009).

While there were clear influences of temperature in the phenology of *C. foliascens*, this study observed an unusual climatic event in 2010 which coincided with concomitant decreases in reproduction. Within Little Pioneer Bay, a two-fold increase in maximum PoSPi was observed in the second reproductive cycle (December 2011) when compared to maximum PoSPi in the first cycle (October 2010). When PoSPi was compared between reproductive cycles over the same time period (December 2010 vs December 2011), it corresponded to a three-fold difference. Depressed PoSPi in the first reproductive cycle can be attributed to extreme weather conditions experienced on the east coast of Australia over the summer of 2010, associated with one of the strongest La Niña events on record which brought stronger trade winds and higher sea-surface temperatures to northern and eastern Australia (Imielska 2011; Giles 2012).

The La Niña year saw an increase of up to 933% in total monthly rainfall (October 2010) and 2.1°C increase in mean monthly temperature (September 2010) during period of peak *C. foliascens* reproduction (July to December) when compared to historic climatic record of the study area. Rainfall and temperature in the second reproductive cycle (2011/ 2012) did not vary greatly from historic climatic record during peak *C. foliascens* reproduction and may represent baseline levels under stable environmental conditions. Analyses of PoSPi between December 2010 and December 2011 suggests that the proportion of reproductive females (ca. 39.7% reduction) and female reproductive output (ca. 44.0%

reduction) were most affected. Fertilization success, as represented by cumulative proportion of female propagules that were embryos or larvae was reduced by less than 10%. In addition to an overall increase in rainfall and temperature, a considerable peak in total monthly rainfall in November 2010 may also account for a reduction of PoSPi in December 2010.

C. foliascens occupies inshore, intertidal habitat at Little Pioneer Bay, where salinity fluctuations from extended rainfall, and turbidity and light attenuation associated with terrigenous run-off would regularly expose sponges to physiological stress (Fabricius 2005; Cooper et al. 2007; Wolanski et al. 2008). The observed reduction in reproduction in the first reproductive cycle could be attributed to a metabolic trade-off towards increased physiological stress management during the period of increased rainfall (Uriz et al. 1995; Harshman and Zera 2007; Whalan et al. 2007a; Scott et al. 2013). Additionally, sponges have also demonstrated the ability to regulate and stop filter feeding in stressful conditions and recommence pumping when situations are more favourable (Bannister et al. 2012). This may explain sub-optimal reproduction levels over the period of high rainfall due to reduced pumping, feeding and capacity to photosynthesize, while maintaining fertilization through pumping over intermittent favourable conditions without incurring excessive mortalities.

In conclusion, while it is unclear what drives the continual reproduction of *C. foliascens* throughout the year, temperature is a critical environmental factor which clearly influences the intensity of reproduction in this species. While year to year temperature variation (1°C – 1.3°C) did not severely affect reproductive phenology, an extreme climatic event associated with unprecedented rainfall depressed the sexual productivity of the population by reducing the proportion of reproductive females and female ROI. Although PoSPi indicates that *C. foliascens* can still produce larvae during environmental perturbation, too little is known about larval, juvenile and adult stress responses to currently predict how entire populations will respond to various environmental scenarios. However, with larvae of brooding sponges having limited dispersal potential (Ettinger-Epstein et al. 2008; Whalan et al. 2008a; Abdul Wahab et al. 2011), and climate change projections predicting an accelerated increase of SST and occurrence of severe storms and rainfall (Solomon et al. 2007), intertidal sponge populations living at the edge of their ecological limits may be at risk of local extinction.

Chapter 4

Larval behaviours and their contribution to the distribution of the intertidal coral reef sponge *Carteriospongia foliascens*[3]

4.1. Introduction

The effective management and conservation of marine species requires a holistic understanding of the physical, biological and ecological processes influencing populations (Levin 2006; Cowen and Sponaugle 2009; Pineda et al. 2009). Sponges (Phylum Porifera) are abundant, diverse and play important functional roles in many aquatic ecosystems (Bell 2008; Van Soest et al. 2012). However, despite their evolutionary and ecological significance, sponge population demographics are relatively understudied (Wulff 2006; Srivastava et al. 2010). For sessile marine invertebrates such as sponges, the mobile larval phase is critical to population maintenance and biogeography (Eckman 1996; Cowen and Sponaugle 2009; Pineda et al. 2010).

Dispersal over large geographic ranges is unusual for sponges (but see Maldonado and Uriz 1999 for mechanisms facilitating large scale dispersal), with most sponge larvae exhibiting short competency periods (minutes to < 2 weeks) and restricted dispersal potential (Duran et al. 2004; Whalan et al. 2008b). Due to the restricted swimming capabilities of sponge larvae (Maldonado 2006; Ettinger-Epstein et al. 2008; Whalan et al. 2008a; Abdul

[3] **Chapter 4** is adapted from Abdul Wahab MA, de Nys R, Webster N, Whalan S (2014) Larval behaviours and their contribution to the distribution of the intertidal coral reef sponge *Carteriospongia foliascens*. PLoS ONE 9(5):e98181

Wahab et al. 2011), hydrodynamic processes are likely to play an important role in larval dispersal (Mariani et al. 2006; White et al. 2010). Nevertheless, innate larval behaviours in response to environmental factors can indirectly contribute to horizontal larval dispersal. For example, phototaxis (attraction to light) can position larvae in bodies of water of differing flow regimes through light-directed vertical migration (Wapstra and Van Soest 1987; Maldonado and Young 1996; Mariani et al. 2006; dos Santos et al. 2008; Jékely et al. 2008; Whalan et al. 2008a; Morgan and Fisher 2010; Abdul Wahab et al. 2011).

Given these properties, the adult distribution of sessile invertebrates can be strongly influenced by larval settlement and post-settlement processes. However, larval settlement also relies on the availability of suitable substrate for attachment and metamorphosis (Pineda et al. 2009). For sponges, the availability of solid and stable substrates such as coral rubble is critical for primary attachment to the benthos (Duckworth and Wolff 2011). Furthermore, the availability of substrate associated cues such as microbial biofilms, induce faster and more successful metamorphosis in sponge larvae (Whalan et al. 2008a; Abdul Wahab et al. 2011; Whalan and Webster 2014). Finally, post-settlement pressures such as predation (Wulff 1995; Pawlik et al. 2013), competition (Aerts 2000; González-Rivero et al. 2012), food availability (Frøhlich and Barthel 1997; Trussell et al. 2006) and light quality (for species possessing phototrophic symbionts) (Thacker 2005; Erwin and Thacker 2008) also influence adult population distributions and demographics.

Carteriospongia foliascens is an abundant, conspicuous and widely distributed Indo - Pacific phototrophic sponge, and is found across a range of intertidal and mesophotic habitats of the Great Barrier Reef (GBR) (Wilkinson 1983,1988; Bridge et al. 2011a; see Chapter 2 for conspecificity between intertidal populations, studied herein, and subtidal populations). *C. foliascens* is a brooding species and although reproductive all year round exhibits peak reproduction during the Austral summer from October to December (Chapter 3). Within the Palm Island region of the central GBR, *C. foliascens* occurs solely on intertidal reef flats and is absent from adjoining sub-tidal reefs (this study). To understand the potential of larval behaviours in contributing to adult distributions, this study firstly investigated patterns of larval spawning, swimming speeds, phototaxis and vertical migration. Secondly, the distribution of adult sponges and substrate availability was measured across habitats. Thirdly, the effects of biofilms (i.e. intertidal and subtidal) on larval settlement and metamorphosis were quantified.

4.2. Materials and methods

4.2.1. Study sites and benthic surveys

All field surveys and collections were conducted under the Great Barrier Reef Marine Park Authority Permit #G12/35236.1 and did not involve any endangered or protected species. The distribution and abundance of adult *Carteriospongia foliascens* were determined on the reef flats of Little Pioneer Bay, Orpheus Island (18°36.989'S, 146°29.832'E) and north Juno Bay, Fantome Island (18°41.405'S, 146°31.272'E), central Great Barrier Reef (GBR). Surveys were undertaken in January and March 2012 using belt transects (500 m x 2 m, n = 3) oriented from the shoreline edge of mangroves (shallow, intertidal) to the reef slope (deep, subtidal). Positions of sponges along transects were recorded. Water depth (cm) was recorded every 10 m along transects and adjusted relative to the tidal datum by subtracting *in situ* water level from predicted tide levels (National Tide Centre, Australian Bureau of Meteorology; http://www.bom.gov.au).

Adult *C. foliascens* are commonly found attached to coral rubble. To identify whether substrate availability was contributing to sponge distributions, additional surveys for substrate type (sand, coral rubble and live coral) were conducted at Little Pioneer Bay (Orpheus Island), Hazard Bay (Orpheus Island, 18°38.417'S, 146°29.789'E), north Juno Bay (Fantome Island) and south Juno Bay (Fantome Island, 18°41.130'S, 146°30.880'E). Belt transects (25 m x 2 m, n = 3) were oriented along three depth profiles (+100 cm, +50 cm and -100 cm) from the tidal datum (FTD). Depth profiles were selected based on the previous survey, which identified mean depths where sponges naturally occurred (+ 50 cm) and depths where sponges were absent (+ 100 cm and – 100 cm). A 1 m x 1 m quadrat, divided into a 4 x 4 grid layout, was used to systematically quantify substrate composition at 5 m intervals along transects. Area estimations for substrate type were conducted by the same observer to maintain consistency. Additionally, to quantify the realized available space for larval settlement, the proportion of coral rubble accessible for settlement (i.e. free of other macro-invertebrates and macroalgae) was recorded at + 50 cm FTD at north Juno Bay and Little Pioneer Bay in January 2012.

4.2.2. Larval release and collection

C. foliascens is a brooding sponge that spawns tufted parenchymella larvae. Although this species is reproductive throughout the year, increased reproduction occurs during the Austral summer months from October to December (Chapter 3). Fourteen reproductive sponges were

collected from Little Pioneer and south Juno Bay to provide larvae for experiments in October 2011. Sponges were maintained in aquaria receiving flow through 10 µm filtered seawater (FSW) at Orpheus Island Research Station (OIRS) until spawning. Larvae were collected from adult sponges using mesh traps following Abdul Wahab et al. (2011).

Preliminary investigations found no release of larvae at night between 1800 h and 0600 h. To quantify larval release, larval traps were placed over fourteen sponges maintained in flow-through aquaria at OIRS at 0600 h. Larvae were subsequently collected and counted at three hour intervals (0900 h, 1200 h, 1500 h and 1800 h) over seven days. Representative larval samples (n = 400) were preserved in 2.5 % glutaraldehyde (in FSW) for size measurements.

4.2.3. Larval swimming ability

Larval swimming speeds were assessed at 0, 2, 4, 6, 12, 18 and 24h post-release. A glass aquarium (50 cm x 5 cm x 5 cm) superimposed with a 1 cm x 1 cm grid on the bottom, filled with artificial seawater (ASW, 35 ppt, Tropic Marin® sea salt in distilled water) was used as a swim chamber. Larvae (n = 10) were videotaped using a Sony video camera (DCR-DVD101E) at each time period for 1 min and subsequently removed from the experiment. Larval speed ($cm.s^{-1}$) was determined by tracking larval horizontal swimming distance over time using the plugin MTrackJ in ImageJ 1.46r (National Institutes of Health, USA).

4.2.4. Pre-settlement behaviour

4.2.4.1. Behaviour of "newly released" larvae

"Newly released" larvae refer to larvae released within 1 h from adult sponges at the start of experiments. To investigate larval vertical migration behaviour in response to light (phototaxis), 1000 ml graduated cylinders (height 46 cm and diameter 6.5 cm) filled with 1000 ml of artificial seawater were used as experimental columns. Artificial seawater (ASW) was used for all experiments to exclude influences of waterborne cues on larvae. Natural daylight was used as a light source for all phototaxis experiments. Light intensities ranged between 0.2 kW m^{-2} and 276.9 kW m^{-2} between 0600 h and 1800 h daily (HOBO Pendant UA-002-64, One Temp Pty Ltd, Massachusetts).

Each experimental column was divided into two, one half covered in foil to exclude daylight and the other half uncovered for light penetration. Light treatments consisted of

natural daylight presented to 1) the top half of the experimental column (n = 3) and 2) the bottom half of the column (n = 3) following Abdul Wahab et al. (2011). Twenty larvae were introduced to the surface of each experimental column using a pipette and columns gently swirled to disperse larvae throughout the water to remove any bias of initial static water conditions and larval placement. The columns were then positioned in flow - through water baths to maintain ambient water temperature (≈ 25°C). The number of larvae in the light exposed half of each column was recorded at 0 (5 mins post-swirling), 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 min.

4.2.4.2. Behaviour of 4 h old larvae

To investigate changes in vertical migration behaviours in older larvae, the effect of partial light exposure to the top (n = 3) and bottom (n = 3) portion of each column was assessed on larvae older than 4 h (n = 50) following methods described previously. The number of larvae in the light exposed half of each column was recorded at 0, 2, 4, 6, 12, 18, 24, 30, 36, 42 and 48 h.

To examine larval vertical migration behaviour in the dark, a second experiment was designed to remove phototactic cues entirely. Experimental columns and number of larvae used (n = 50) were consistent with experiments already described. For dark treatments, daylight was excluded from experimental columns (n = 3) by covering columns entirely with aluminium foil. For light treatments, experimental columns were exposed to full daylight (n = 3). Positions of larvae in the top and bottom half of each column were recorded at 0, 2, 4, 6, 12, 18, 24, 30, 36, 42 and 48 h. Larval positions were assessed in a dark room using a red-filtered torch for the dark treatment and at night for the light treatment. Any effect of light during sampling on larval behaviour (for dark treatment and at night) was assumed to be negligible due to the low intensity and short exposure.

4.2.5. Settlement behaviour

Settlement and engagement in metamorphosis were assessed independently in all experiments. Settlement refers to a larva that has attached itself to the substrate via its anterior end and which was not dislodged with gentle agitation of the experimental chamber (e.g. swirling). Engagement in metamorphosis is defined as a distinct change in larval morphology through flattening of the posterior half to assume a hemispherical form. For ease of communication, engagement in metamorphosis is referred to as metamorphosis hereafter.

4.2.5.1. Gregariousness

The response of larvae to conspecific cues (both adult and larval derived) was tested prior to the main settlement assays. The potential effect of adult waterborne conspecific cues was first assessed by exposing larvae to 1) adult infused ASW (ai-ASW) (n = 12) and 2) sterile ASW (control; n = 12). Adult infused ASW was prepared by maintaining three palm-sized sponges in five litres of ASW without water exchange for eight hours. Aeration was supplied to avoid anoxic conditions. Sterile polypropylene jars (Sarstedt – 70 ml) were employed as experimental chambers and 40 ml of experimental ASW was used in each replicate. Larvae (up to 4 h old; n = 10) were introduced to each jar using a pipette. A 1 cm diameter hole in the lid of each jar provided gas exchange. Jars were placed into outdoor water baths to maintain natural daylight and photoperiod, and ambient water temperature. Larvae that were active, settled, metamorphosed or dead were assessed at 0, 1, 3, 5, 7, 10, 15, 25, 35 and 45 h.

To elucidate effects of larval settlement in the presence of conspecifics, a second experiment was designed to test the influence of larval density on settlement and metamorphosis. Treated, sterile, polystyrene 6-well plates (Iwaki®) filled with 10 ml of ASW were used as experimental chambers. Densities of 1, 2, 5, 10 and 20 larvae well^{-1} were selected following Abdul Wahab et al. (2011). To facilitate frequency analysis, the total number of larvae (4 h old; n = 100) used in each density treatment was kept consistent thus allowing for comparisons of solitary and multiple larvae on settlement and metamorphosis (therefore 100 wells for 1 larva well^{-1}, 50 wells for 2 larvae well^{-1}, etc.). Experimental chambers were maintained in an outdoor water bath and larval condition recorded at 0, 1, 3, 5, 7, 10, 15, 20, 25, 30, 35 and 40 h.

4.2.5.2. Effects of biofilm origin on settlement and metamorphosis

To assess larval settlement and metamorphosis to intertidal and subtidal habitats, larvae were presented with surfaces conditioned with 1) intertidal biofilms (n = 10) and 2) subtidal biofilms (n = 10). Polypropylene jars (Sarstedt – 70 ml) were placed in reefal habitats for six weeks to allow sufficient time for biofilm development. Intertidal treatments were left on the intertidal reef flat of Little Pioneer Bay (where adult *C. foliascens* occur naturally) and subtidal treatments were established at 3 m depth on an adjacent reef slope, a habitat not known to support adult populations. Fouling macro-invertebrates and filamentous macroalgae growing within experimental jars after six weeks were carefully removed using a fine brush and pair of forceps prior to the experiment, to avoid confounding effects of fouling organisms on larval settlement. Sterile jars (n = 10) were used as controls. Forty

millilitres of ASW was added to each conditioned jar using a graduated cylinder and larvae (up to 4 h old; n =10) were introduced. Jars were placed into outdoor water baths and larvae that were active, settled, metamorphosed and dead were recorded at 0, 2, 4, 6, 12, 18, 24, 30, 36 and 42 h.

4.2.6. Statistical analyses

Assumptions of normality and homoscedasticity were checked graphically (boxplot and residual plots) for each dataset before testing hypotheses, and data transformations applied when assumptions were violated (Quinn and Keough 2002). Datasets not meeting assumptions post-transformations were analysed using permutational methods (PERMANOVA) (Anderson 2005). A nested ANOVA was used on raw data to distinguish differences in the proportion of coral rubble between islands (fixed), bays (nested, random) and depth (fixed) in Statistica 10. As *C. foliascens* are only found attached to coral rubble, the proportion of substrate occupied by coral rubble across depths were assessed separately within each bay using one-way ANOVAs and Tukey's HSD tests. To evaluate larval release over time, a repeated measures PERMANOVA using the Bray Curtis resemblance matrix (9999 permutations) on raw data was employed, using day and time of day as variables. Differences in larval swimming speeds over time were assessed using a one-factor PERMANOVA (Euclidean resemblance matrix, 9999 permutations). To assess whether vertical position of larvae differed between light cue treatments, repeated measures PERMANOVA using a Euclidean resemblance matrix (9999 permutations) was performed on both ''newly released'' and 4 h old larvae logged data sets, using exposure to daylight (top or bottom exposure, or light exclusion) and time as factors. Only data from 0 h to 18 h of the experiment was used to avoid any confounding effects of reduced larval swimming after 18 h post-release on phototactic response. Gregarious settlement of larvae to adult conspecific cues was assessed using repeated measures PERMANOVA (Euclidean resemblance matrix, 9999 permutations) with water treatment type (ASW and ai-ASW) and time as variables. Homogeneity of slopes model (HSM), employing the binomial distribution and logit function was used to detect differences in settlement and metamorphosis patterns across larval conspecific densities. Only data from 20 h to 35 h, a period of accelerated larval settlement and metamorphosis, was used, with larval status frequencies as the dependent variable (i.e. dichotomous; settled/ metamorphosed and swimming/ dead), larval densities as the categorical factor and time as the continuous predictor. The Hosmer-Lemeshow (HL) test was performed to test the fit of data to the model. To evaluate differences in success of settlement and metamorphosis when exposed to biofilm types (intertidal and subtidal) as a settlement cue, the number of settled and metamorphosed larvae was first combined to

represent the proportion of larvae that attached successfully to the substrate over time. Subsequently, a repeated measures PERMANOVA (Euclidean resemblance matrix, 9999 permutations) was performed on the raw data with biofilm type and time as variables.

4.3. Results

4.3.1. Benthic surveys

4.3.1.1. Sponge distribution and substrate composition

Benthic surveys covered a depth range between 165 to – 609 cm from the tidal datum (FTD) (Table 1). *Carteriospongia foliascens* was only found attached to coral rubble and occurred over a mean depth range of 67 cm (± 11) (mean ± SE) to 42 cm (± 2) FTD at Little Pioneer Bay. The surveyed depth range for north Juno Bay transect 3 was truncated due to a gentler sloping gradient along the transect length, thus hindering detection of lower limit for *C. foliascens*. Mean *C. foliascens* depth distribution at north Juno Bay was between 74 cm (± 14, $n_{transects}$ = 3) and 28 cm (± 4, $n_{transects}$ = 2) FTD. No sponges were found in subtidal habitats (< 0 cm FTD) at either location (see Supplementary Figures 4.1 and 4.2). The maximum total number of sponges was recorded at a depth range of 40 to 49 cm FTD at Little Pioneer Bay ($n_{sponges}$ = 13) and between 30 to 39 cm FTD at north Juno Bay ($n_{sponges}$ = 46) (Supplementary Figure 4.3). *C. foliascens* was more abundant at north Juno Bay (0.19 sponge m^{-2} ± 0.01; $n_{transects}$ = 2) than at Little Pioneer Bay (0.05 sponge m^{-2} ± 0.01; $n_{transects}$ = 3), corresponding to a 282.14 % higher frequency of total sponges (Supplementary Figure 4.1, 4.2 and 4.3).

Table 4.1: *C. foliascens.* Depth distribution of adult sponges across three belt transects at Little Pioneer Bay and north Juno Bay. The surveyed depth range at each location is reflected in the last column. Depths shown in centimetres (cm) represent depth relative to the tidal datum.

Location	Belt transect number	Sponge upper limit (cm)	Sponge lower limit (cm)	Depth range of survey (cm)
Little Pioneer	1	63	39	101 to - 217
Little Pioneer	2	50	41	118 to -185
Little Pioneer	3	86	47	125 to - 193
north Juno	1	89	31	165 to - 609
north Juno	2	62	24	124 to - 198
north Juno	3	73	53	156 to 53

A survey of substrate composition was conducted in two bays at both Orpheus and Fantome Island at the mean depth profile where sponges naturally occurred (ca. +50 cm FTD), and at shallow (+100 cm FTD) and subtidal (-100 cm FTD) depth profiles where sponges were absent. Coral rubble composition was lowest at the shallowest depth profile (+100 cm FTD) and increased as water depth increased at all bays (Figure 4.1). Coral rubble was generally free of macro-invertebrates and macroalgae at both north Juno Bay (proportion bare coral rubble = 91.0 % ± 0.6) and Little Pioneer Bay (proportion bare coral rubble = 96.9 % ± 0.6) in January 2012. Live coral was absent in the shallowest depth and increased in cover from 50 cm FTD to -100 cm FTD. Alternatively, sand composition showed a reverse trend occupying a major proportion of the substrate at the shallowest depth and decreasing with increasing depth. The proportion of coral rubble was significantly different between bays (Nested ANOVA: $F_{(2, 30)} = 15.36$, $p < 0.0001$) and depths (Nested ANOVA: $F_{(2, 30)} = 133.02$, $p < 0.0001$), but not between islands (Nested ANOVA: $F_{(1, 30)} = 117.36$, $p > 0.05$). Coral rubble cover in each bay was significantly higher in subtidal depth profiles (- 100 cm FTD) compared to other depths (one-factor ANOVAs and Tukey's HSD: $p < 0.05$). The increase in coral rubble cover from shallow to deeper depths provided suitable substrata for *C. foliascens* attachment, however *C. foliascens* was absent in subtidal (+ve FTD) despite this habitat having higher coral rubble cover.

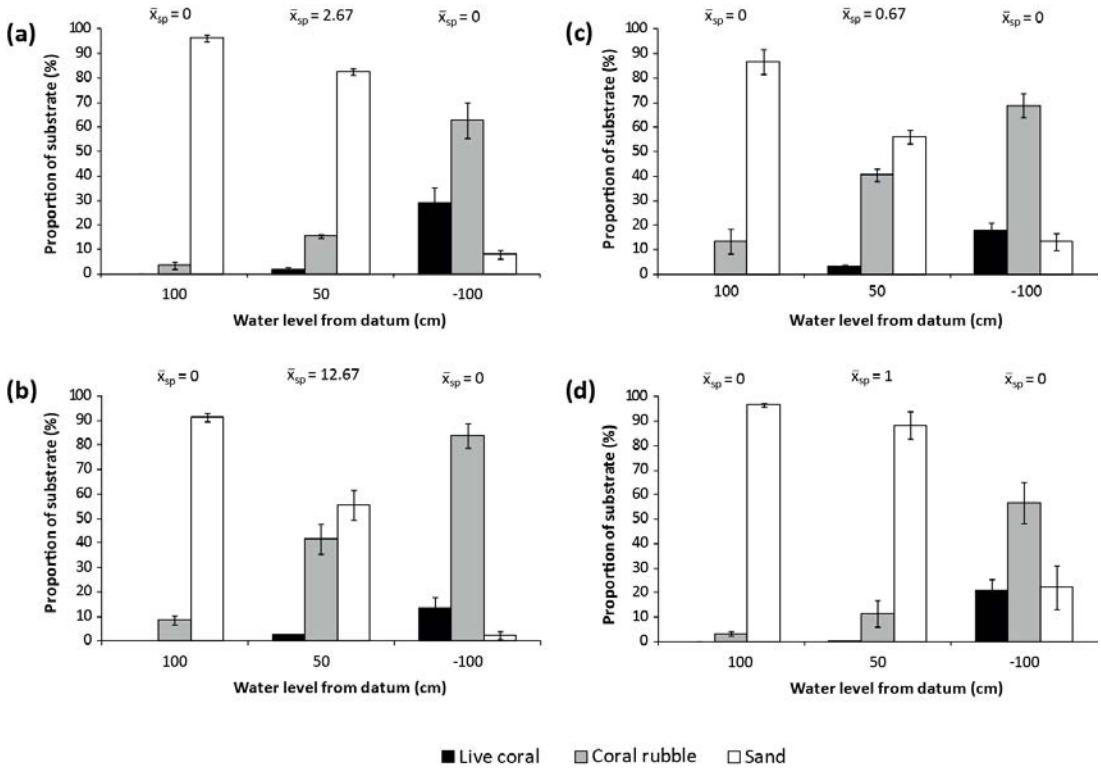

Figure 4.1: *Carteriospongia foliascens.* Substrate composition (live coral, coral rubble and sand) over three depth profiles (100, 50 and -100 cm relative to tidal datum) at (a) Little Pioneer Bay (Orpheus), (b) Hazard Bay (Orpheus), (c) north Juno Bay (Fantome) and (d) south Juno Bay (Fantome). Mean number of sponges ($\bar{x}_{sp}$ = mean number of individuals/ 50 m^2, $n_{transect} = 3$) present are highlighted over each respective depth profiles.

4.3.2. Larval release and morphological characteristics

C. foliascens is viviparous and releases negatively buoyant tufted parenchymella larvae which are cream in colour and possess a dark interior (Supplementary Figure 4.4a). Larvae are 850 µm (± 3) long, 562 µm (± 3) wide and are prolate spheroid in shape (n = 384). Sponge larvae were released between 0600 h and 1800 h, but larval release was variable with a significant interaction between day and time of day (two factor PERMANOVA: pseudo-$F_{(18,\ 332)}$ = 1.746, p < 0.001, Figure 4.2a, b). The intensity of larval release was highest between late morning and noon (0900 h to 1200 h) with sponges releasing up to 122 larvae sponge^{-1} hour^{-1} (Figure 4.2b). The cumulative frequency of larvae released by all 14 sponges over 7 days was highest between 0900 h and 1200 h ($n_{larvae\ released}$ = 4241, Figure 4.2c). Larval release decreased by 25.49 % (± 5.6) and 31.86 % (± 6.63) from the peak over early (1200 h to 1500 h; cumulative $n_{larvae\ released}$ = 2094) and late (1500 h to 1800 h; cumulative $n_{larvae\ released}$ = 1312) afternoon respectively. Larval release was lowest in the early morning (0600 h to 0900 h; maximum 60 larvae sponge^{-1} hour-^{1}; cumulative $n_{larvae\ released}$ = 1090). The mean daily larval release was 89 larvae sponge^{-1} day^{-1} ($n_{sponges}$ = 14, n_{days} = 7), with the maximum number of larvae released by an individual being 765 larvae sponge^{-1} day^{-1}. No larvae were released during the night. A total of 233 and 887 larvae were estimated to be released per square metre of substrate at Little Pioneer Bay and north Juno Bay respectively over the four most reproductive months (sponge density [sponge m^{-2}] * mean daily larval release per sponge [larvae sponge^{-1} day^{-1}] * proportion of female [0.43] sponges * days [122 days]; September to December; Chapter 3).

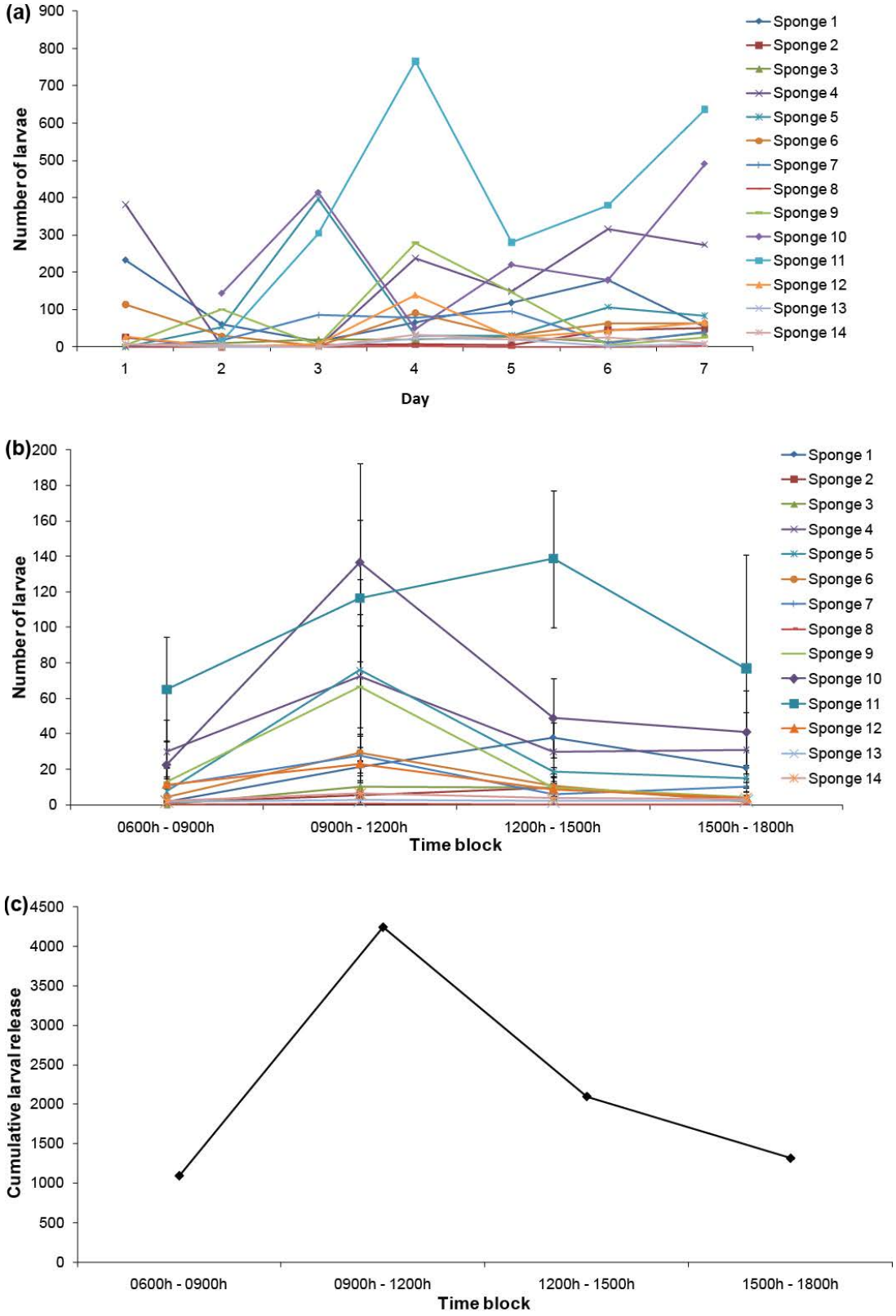

Figure 4.2: *C. foliascens*. Patterns of larval release showing (a) total daily larval release by individual sponges (n = 14) over 7 days, (b) mean larval release by individual sponges at each time block, and (c) cumulative larval release for all sponges (n = 14) at each time block over 7 days.

4.3.3. Larval swimming ability

Larval swimming speed at release was 0.37 $cm.s^{-1}$ (± 0.02) and fluctuated between 0.35 $cm.s^{-1}$ (± 0.02) and 0.43 $cm.s^{-1}$ (± 0.03) between 2 h to 12 h post-release (Figure 4.3). Pair-wise comparisons of swimming speed as larvae age showed a significant decrease in speed after 18 h post-release (0.22 $cm.s^{-1}$ ± 0.04) (PERMANOVA: pseudo-$F_{(6, 61)}$ = 8.95, p < 0.001).

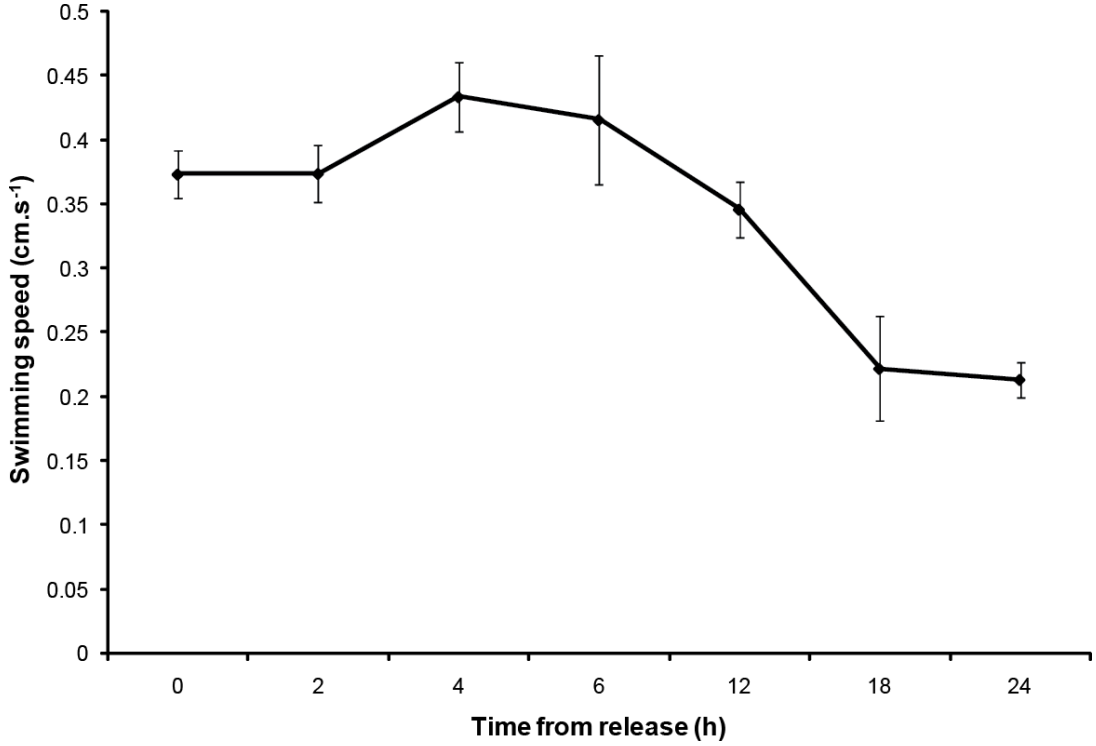

Figure 4.3: *C. foliascens*. Graph of larval swimming speeds (cm.s-1) from release (0 h) to 24 h post-release.

4.3.4. Pre-settlement behaviour

4.3.4.1. Behaviour of "newly released" larvae

Larvae were negatively phototactic within 40 min of release (Figure 4.4). When light was presented to the top half of the water column (top exposed; Figure 4.4), larvae (< 1 h old) initiated bottom migration immediately with 80% (± 5.77) occupying the darker bottom half of the experimental column at 40 min. Larvae maintained position in the darker region of the column for the rest of the experiment. When light was focused on the bottom half of the column (bottom exposed; Figure 4.4), larvae (> 96.67 % ± 1.67) maintained their position at the surface over the 240 min duration of the experiment. There was significant interaction of treatment (top/ bottom exposure) and time on vertical migration of "newly released" larvae

(two factor PERMANOVA: pseudo-$F_{(10, 44)}$ = 59.45, p < 0.001). This shows that the effect of light conditions on larval positioning in the experimental column is subject to changes over time, with an increasing effect of light conditions on larval positioning as time progressed (Figure 4.4).

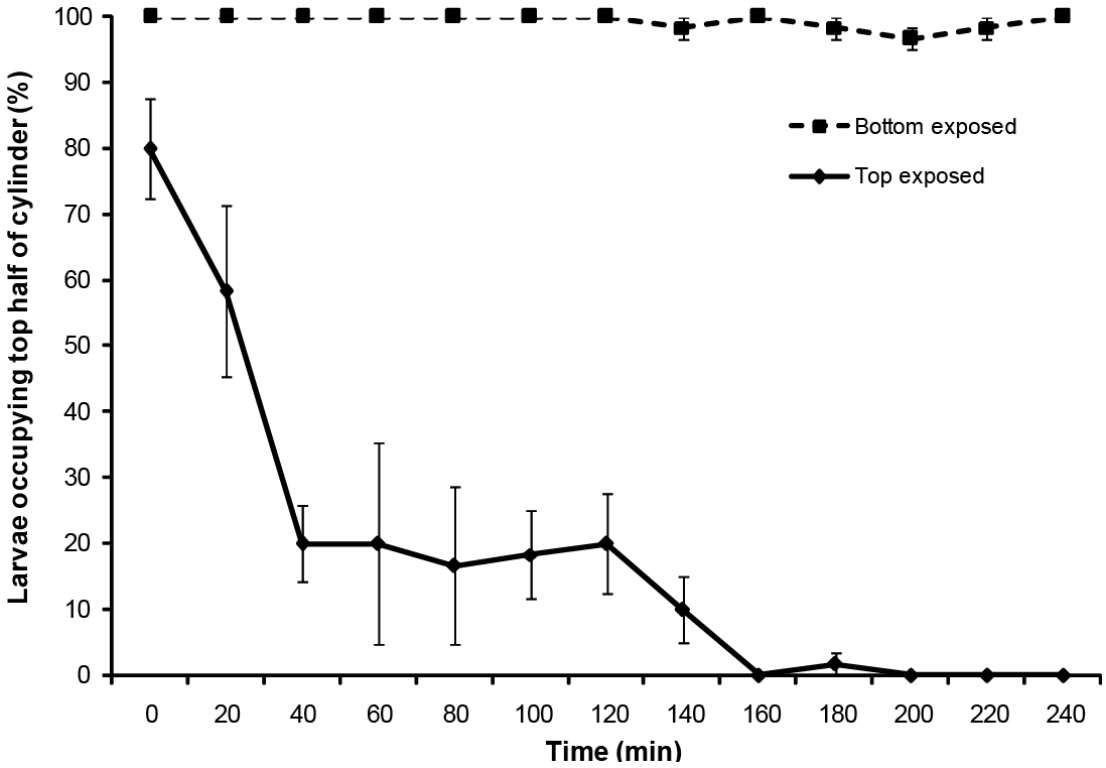

Figure 4.4: *C. foliascens.* Mean percentages (± SE, n = 3, 20 individual replicate^{-1}) of larvae (< 1 h old at start of experiment) occupying top half of the experimental water column (1000 ml graduated cylinder) when exposed to partial light over 240 minutes. Bottom exposed refers to light presented to the bottom half of the column and top exposed refers to light presented to the top half of the column.

4.3.4.2. Behaviour of 4 h old larvae

C. foliascens larvae remained negatively phototactic with age (Figure 4.5) as demonstrated by a significant interaction of treatment (top/bottom exposure) and time contributing to larval vertical migration (two way PERMANOVA: pseudo-$F_{(5,24)}$ = 25.61, p < 0.001). When the bottom half of the column was uncovered (bottom exposed), larvae (4 h old; > 80.67 % ± 2.40) maintained their position at the surface for the first 24 h of the experiment (28 h post-release) regardless of daylight conditions (day and night represented by white and black horizontal bars; Figure 4.5). Most larvae (66.67 % ± 2.91) occupied the bottom half of the column at 30 h with all larvae (100 % ± 0) positioned at the bottom of the column at 48 h. When light was focused on the top half of the column (top exposed; Figure 4.5), larvae (> 95.33 % ± 1.33) immediately migrated to the bottom and maintained this position in the dark for the first 4 h. When light was first excluded from the experiment during the night (6 h), up

to 58.67 % (± 2.91) of larvae migrated towards the exposed top half of the column before returning to the bottom at sunrise (at 18 h of experiment; Figure 4.5). By 30 h the majority of larvae (> 98.67 % ± 0.67) occupied the bottom of the column, and all larvae (100 % ± 0) had migrated to the bottom by the completion of the experiment at 42h.

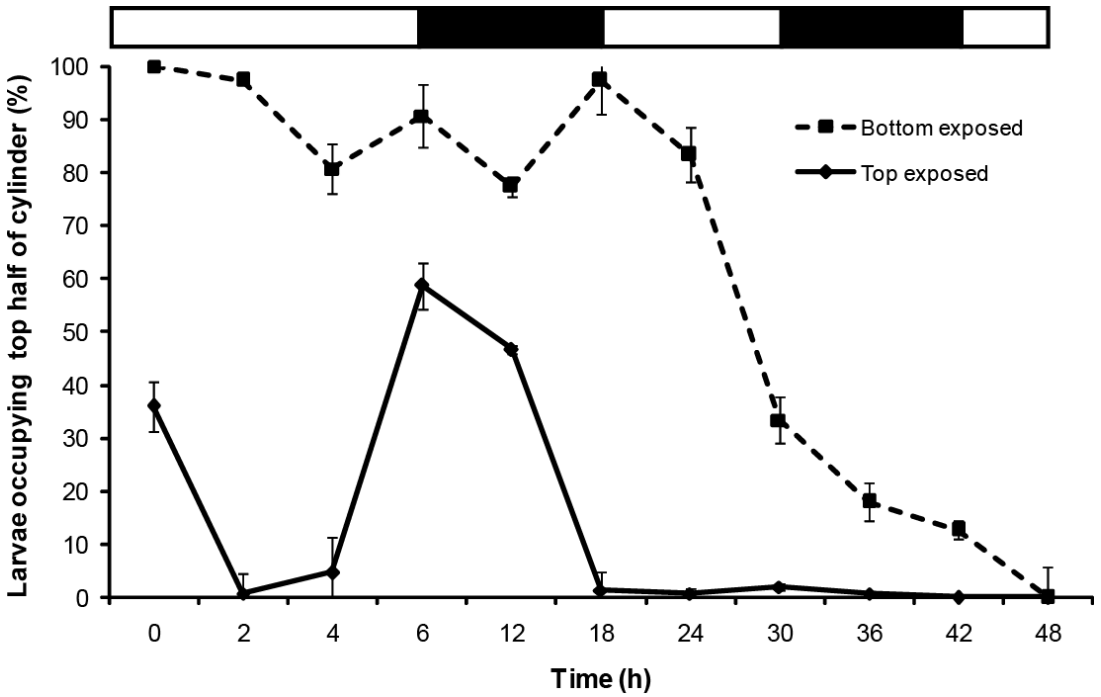

Figure 4.5: *C. foliascens.* Mean percentages (± SE, n = 3, 50 individual replicate^{-1}) of larvae (4 h old at start of experiment) occupying top half of the experimental water column (1000 ml graduated cylinder) when exposed to partial light over 48 hours. Bottom exposed refers to light presented to the bottom half of the column and top exposed refers to light presented to the top half of the column. Light and dark bars over the graph represent natural photoperiod, where light is day and dark is night.

When light was completely excluded from the water column (dark; Figure 4.6), > 78.00 % (± 7.57) of larvae (4 h old) congregated at the surface for up to 18 h (22 h post-release). Initiation of bottom migration occurred at 24 h when the majority of larvae (65.33 % ± 4.67) moved to the bottom half of the column, with completion of bottom migration achieved at 42 h (100 % ± 0) (Figure 4.6). Larvae migrated to the bottom immediately in the presence of light (light; Figure 4.6) where 93.33 % ± 3.71 of larvae occupied the bottom between 2 and 4 h. Similar to the previous experiment, larvae initiated upwards migration during the first nightfall with up to 44.67 % ± 4.67 of larvae found in the top half of the column between 6 h and 18 h (Figure 4.6). The majority of larvae (88.67 % ± 1.33) returned to the bottom of the column at sunrise (18 h) and maintained this position until bottom migration was completed at 36 h (100 % ± 0). There was a significant interaction of treatment (light/ dark) and time between the two treatment groups (two factor PERMANOVA: pseudo-$F_{(5,24)}$ = 20.81, p < 0.001). This shows that there is a changing effect

of light conditions on larval positioning in experimental columns over time, with diminishing effects of light conditions as time progressed (Figure 4.6).

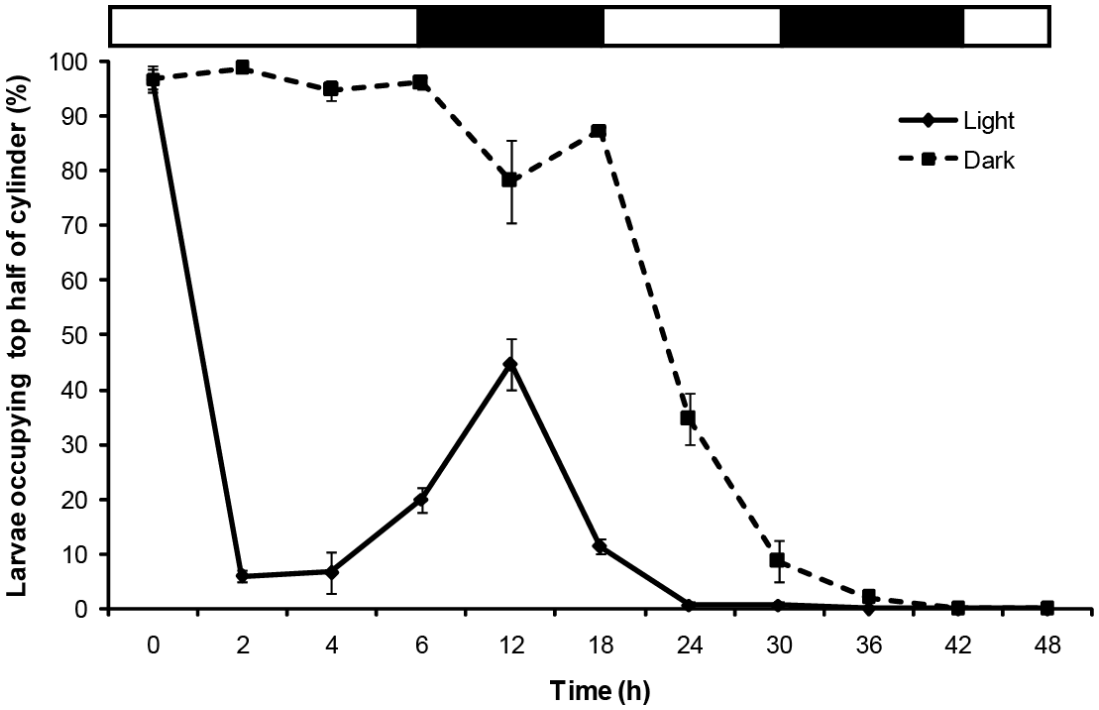

Figure 4.6: *C. foliascens.* Mean percentages (± SE, n = 3, 50 individual replicate^{-1}) of larvae (4 h old at start of experiment) occupying top half of the experimental water column (1000 ml graduated cylinder) when exposed to completely dark and light conditions over 48 hours. Light and dark bars over the graph represent natural photoperiod, where light is day and dark is night.

4.3.5. Settlement behaviour

4.3.5.1. Gregariousness

Patterns of settlement and metamorphosis in *C. foliascens* were similar when larvae were presented with artificial seawater (ASW, control) and adult conspecific seawater (ai-ASW, treatment) (two factor PERMANOVA: pseudo-$F_{(1,160)}$ = 0.63, p > 0.05). Settlement and metamorphosis occurred as early as 1 h from the start of experiment, where both the control and treatment achieved similar levels of metamorphosis (at 45 h) of 45.56 % ± 5.80 and 42.22 % ± 5.47 respectively. There was no effect of larval densities on settlement and metamorphosis, with all density treatments showing similar settlement and metamorphic trajectories over time (HSM: Wald = 7.16, df = 4, p > 0.05; HL = 10.036, p > 0.05, Figure 4.7a, b).

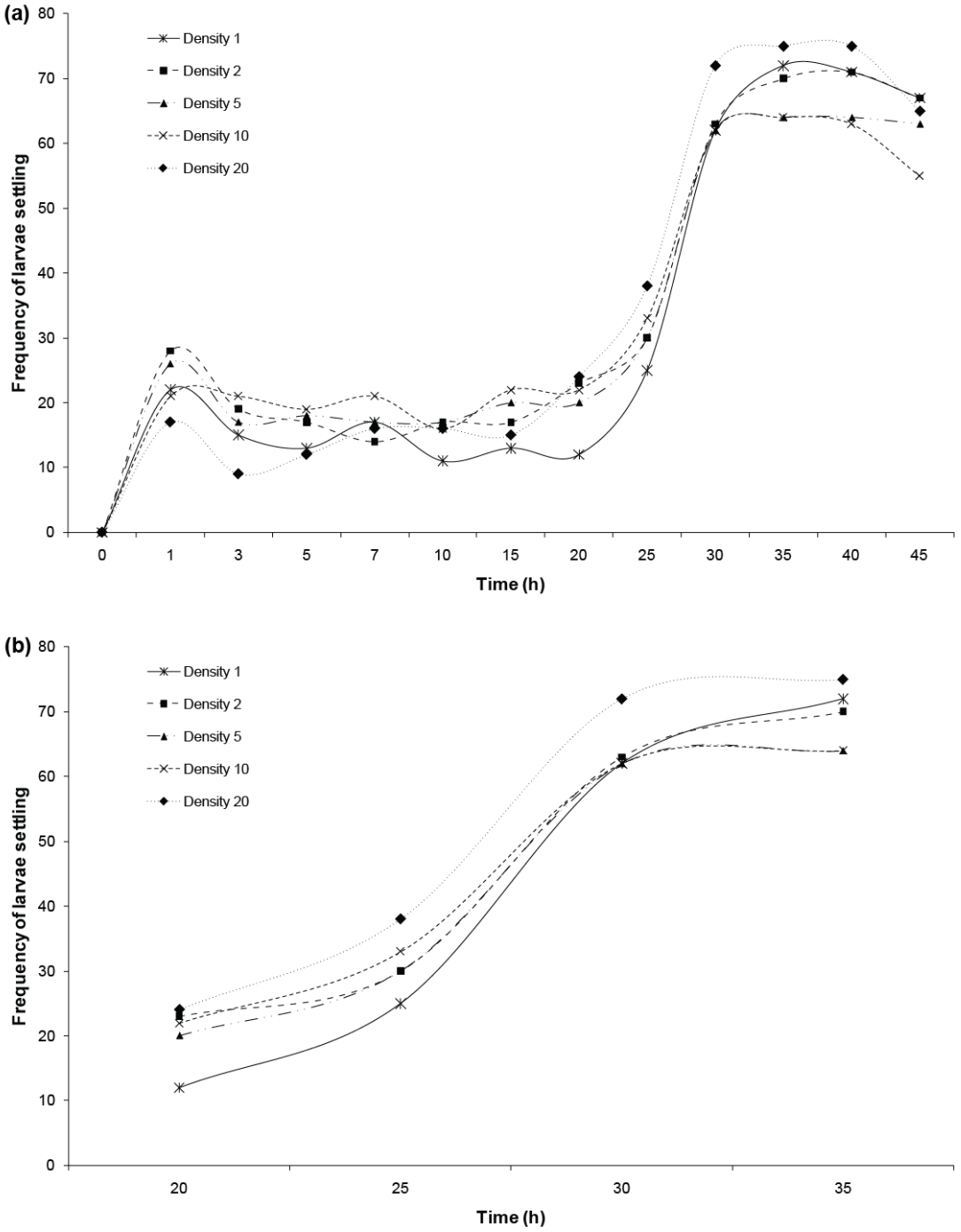

Figure 4.7: *C. foliascens.* Total frequencies of combined settlement and metamorphosis at larval densities of 1, 2, 5, 10 and 20 larvae well^{-1} over (a) the entire 45 h duration of the experiment and (b) as settlement and metamorphosis accelerated between 20 h and 35 h of the experiment.

4.3.5.2. Effects of biofilm origin on settlement and metamorphosis

Surfaces conditioned with microbial biofilms accelerated settlement and increased metamorphosis in *C. foliascens* when compared to sterile surfaces with a significant interaction of treatment and time (two factor PERMANOVA: pseudo-$F_{(18,270)}$ = 3.16, p < 0.001). The onset of larval settlement occurred at 12 h when biofilm was absent (Figure 4.8).

Metamorphosis occurred as early as 2 h with 15 % (± 0.60) and 17 % (± 0.37) settlement respectively, when intertidal and subtidal biofilms were presented to larvae. Larval metamorphosis at 42 h approached a two-fold increase when biofilms were present compared to sterile surfaces (sterile = 34 % ± 0.45, intertidal biofilm = 62 % ± 0.53 and subtidal biofilm = 63 % ± 0.70). There was no significant effect of the intertidal or subtidal origin of the biofilm on settlement and metamorphosis (pairwise PERMANOVA: t = 0.69, p > 0.05).

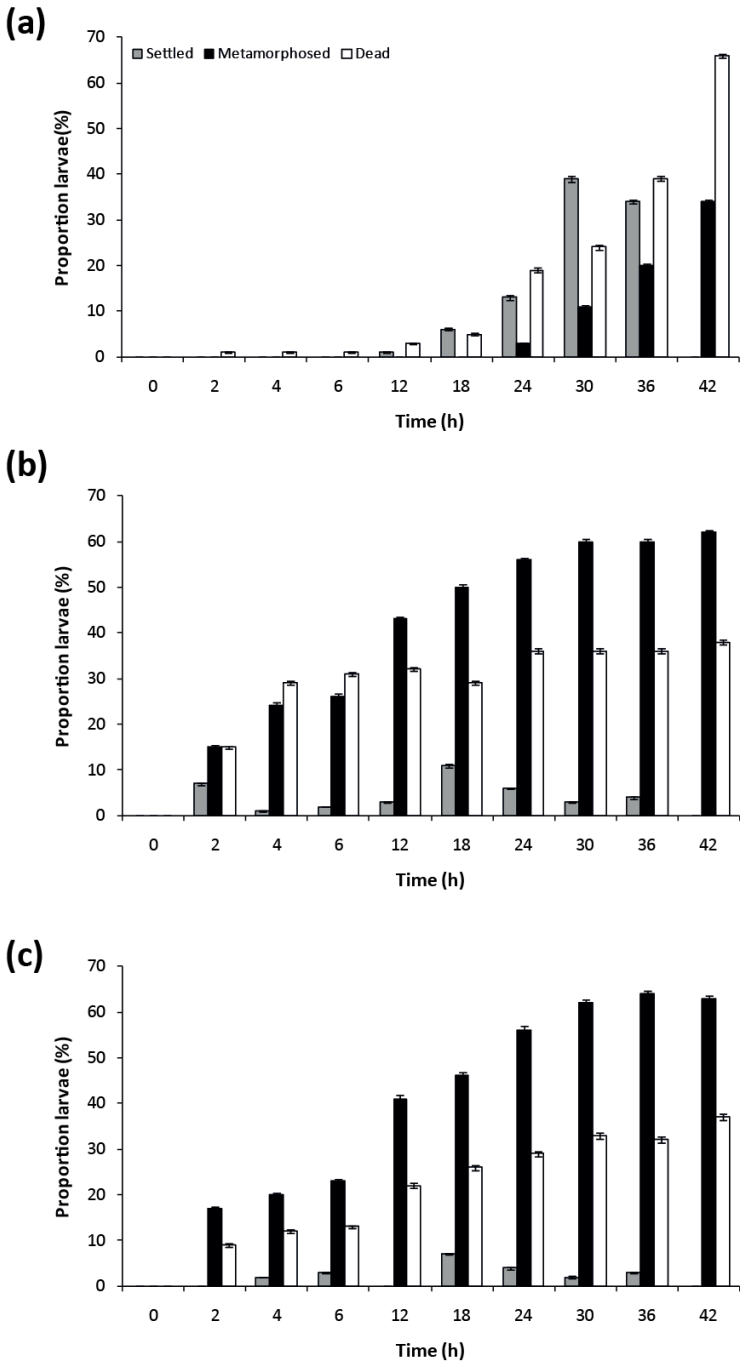

Figure 4.8: *C. foliascens*. Mean percentages (± SE, n =10 treatment^{-1}, 10 individual replicate^{-1}) of larvae (4 h old at start of experiment) that were settled, metamorphosed, or dead when presented with a surface that was (a) sterile, (b) conditioned with intertidal biofilm, and (c) conditioned with subtidal biofilm. Biofilmed surfaces were conditioned for a duration of six weeks.

4.4. Discussion

This study identifies the potential importance of larval behaviour to dispersal, recruitment and adult distributions of intertidal *Carteriospongia foliascens*. Light cues the release of larvae in *C. foliascens*, supporting the importance of light for spawning as demonstrated in other sponges (Amano 1986,1988; Ettinger-Epstein et al. 2008; Whalan et al. 2008a; Abdul Wahab et al. 2011). The number of larvae released by *C. foliascens* is low when compared to estimates of larval release for the Mediterranean sponges *Corticium candelabrum* (233 to 887 larvae m^{-2} vs 507, 000 larvae m^{-2}, Maldonado and Riesgo 2008) and *Ircinia oros* (765 larvae $sponge^{-1}$ day^{-1} vs 2350 larvae $sponge^{-1}$ day^{-1}, Maldonado 2006). In contrast, *C. foliascens* maximum larval release is similar to those reported for other co-occurring dictyoceratid Great Barrier Reef (GBR) species (i.e. *Luffariella variabilis*, 830 larvae $sponge^{-1}$ day^{-1}, Ettinger-Epstein et al. 2008; *Rhopaloeides odorabile*, 800 larvae $sponge^{-1}$ day^{-1}, Whalan et al. 2008a and *Coscinoderma matthewsi*, 595 larvae $sponge^{-1}$ day^{-1}, Abdul Wahab et al. 2011). Notably, the *C. foliascens* strategy of continued larval release over several months is unusual compared to those reported for co-occurring GBR sponge species which exhibit spawning periods of several weeks (Ettinger-Epstein et al. 2008; Whalan et al. 2008a; Abdul Wahab et al. 2011). The extended spawning period of *C. foliascens* (Chapter 3), and concomitant higher potential for larval supply, reflects patterns observed for abundant Red Sea sponge species *Niphates* sp. and *Chalinula* sp. (Ilan and Loya 1988, 1990). Year-round spawning is likely to be a factor contributing to the abundance of *C. foliascens* on the GBR where they can represent up to 80% of total sponge abundance and biomass (Wilkinson 1988; Bridge et al. 2011a, b).

Adult populations of *C. foliascens* can occur from the intertidal to mesophotic zones (Wilkinson 1988; Bridge et al. 2011a, b; Chapter 2). However, the strict depth distribution of adult *C. foliascens* to the intertidal is distinctive of inshore populations (this study). Strict day time larval release, coupled with the early onset of negative phototaxis suggests that larvae move to the benthos within 40 minutes, and may settle within hours from release as shown in other photophobic larval sponge species (e.g. *Xestospongia bocatorensis*; Collin et al. 2010). This behaviour may restrict the capacity of larvae to disperse away from natal habitats, thereby promoting conditions for endogenous recruitment of *C. foliascens*, a phenomenon also seen in scleractinian corals (Cetina-Heredia and Connolly 2011; Figueiredo et al. 2013). While larval pre-settlement behaviours, coupled with early competency for settlement, supports limited dispersal potential for this species, contradicting evidence based on laboratory results indicate larvae have the potential to disperse beyond the intertidal habitat of adults.

The ability of larvae to vertically migrate to the surface at the onset of nightfall (up to 60 % of larvae, within 8 h from release), suggests that larvae released later in the afternoon, or those that have not settled prior to nightfall, have the potential for dispersal overnight away from intertidal natal habitats and onto adjacent shallow subtidal reefs less than 100 m away. Importantly, daily spawning over several months exposes larvae to variable hydrodynamic conditions (i.e. tide changes, wind-driven surface currents) which can also facilitate larval dispersal over a range of spatial scales (Cowen et al. 2000; Whalan et al. 2008b). To date, no evidence of geotactic or barotactic mechanisms have been found in sponge larvae. However, the spiralling swimming behaviour of sponge larva, coupled with the differential weighting of larvae through the accumulation of a posterior spicule mass have been proposed to initiate responses similar to geo- or barotaxis in species possessing spicules (Warburton 1966; Maldonado et al. 1997). Being aspiculate, *C. foliascens* larval migration to the surface upon removal of a light cue, supports alternative mechanisms that initiate geo- or barotactic-like response (e.g. differential weighting of larvae through skeletal development, lipid aggregation or formation of dense proteins), which may interact with larval phototaxis in stimulating directional vertical swimming as seen in the larvae of corals, gastropods and polychaetes (Barile et al. 1994; McCarthy et al. 2002; Stake and Sammarco 2003). The eventual bottom migration of negatively buoyant larvae at 30 h post-release reflects the reduction of larval swimming vigour and increased likelihood for settlement at this time. Nevertheless, if larval dispersal is limited, the possibility of a stepping stone model of dispersal at the small geographic scale investigated is highly likely, and should translate to wider horizontal and depth distribution for *C. foliascens* (Kimura and Weiss 1964; Treml et al. 2007). The use of narrow experimental columns for vertical migration and phototaxis studies exposes larvae to static water conditions that are unrepresentative of natural hydrodynamics, and may introduce wall effects influencing larval behaviour. However, these effects can be minimized (Forward et al. 1984; Young 1995; Manuel et al. 1997).

This study shows that despite limited dispersal potential, intertidal *C. foliascens* larvae should be able to disperse to neighbouring subtidal habitats. The abundance of suitable and available settlement substrate in the subtidal (i.e. bare coral rubble), coupled with the capacity of *C. foliascens* larvae to successfully metamorphose to subtidal biofilms indicates subtidal habitats are potential recruitment environments. The absence of adult *C. foliascens* on inshore subtidal reefs of the Great Barrier Reef (GBR) potentially reflects the role that post-settlement processes have on *C. foliascens* population distributions.

C. foliascens possess symbiotic cyanobacteria, and can rely on phototrophic processes to supply more that 50% of their nutrition (Wilkinson 1983; Webster et al. 2012).

The influence of light limitation on depth distribution of phototrophic sponges, including *C. foliascens*, have been reported for populations at Davies Reef (120 km south-east offshore from the study site), with the highest abundance between 10 and 30 m, and significant reductions outside of this range (Wilkinson and Evans 1989). While reduced light penetration demarcated distributions with depth, lower numbers of sponges in shallow water was indicative of a physical disturbance (i.e. wave exposure and turbulence) that can detach sponge species having a single point of substrate attachment (e.g. stalk in *C. foliascens*) (Wilkinson and Evans 1989).

Unlike offshore locations such as Davies Reef, inshore reefs are subjected to high terrestrial run-off which can contribute to elevated levels of suspended particles (i.e. fine clay and organic particles), leading to mortalities of sponge recruits through the smothering of the aquiferous system (Maldonado et al. 2008), and reducing light penetration to the subtidal region (Fabricius 2005). In addition, associated eutrophication also accelerates phytoplankton production contributing to increased turbidity (Fabricius 2005; Bannister et al. 2012). Water quality, particularly increased turbidity at inshore environments is likely to impede photosynthesis and subsequently successful recruitment of *C. foliascens* to deeper subtidal reefs, a process reported previously in other benthic photoautotrophs such as scleractinian corals (Babcock and Mundy 1996; Anthony and Hoegh-Guldberg 2003a). While the colonization of *C. foliascens* to the subtidal may be restricted by lowered light penetration, abundance of *C. foliascens* in the intertidal at the study site may be supported by habitat characteristics (within bays) which shelter sponges from negative impacts of turbulent hydrodynamics (Wilkinson and Evan 1989). In addition, other post-settlement processes such as predation (Dayton 1989; Wulff 1995; Becerro et al. 1998; Maldonado and Uriz 1998; Pawlik et al. 2013) and space competition (Diaz and Rutzler 2001; López-Victoria et al. 2006) may also contribute to the absence of adult *C. foliascens* in the subtidal. While population genetic data and transplantation experiments would add considerably to our understanding of connectivity in *C. foliascens* populations across horizontal (i.e. inshore and offshore) and vertical distances (i.e. depths), evidence from this study suggests self-recruitment of individuals to the intertidal at inshore reefs for this species (Cowen et al. 2000; Cowen and Sponaugle 2009).

Chapter 5

The influence of habitat on post-settlement processes, larval production and recruitment in *Carteriospongia foliascens*[4]

5.1. Introduction

The term "recruitment" is broadly defined as the addition of new individuals to populations after successfully reaching an arbitrary life stage (e.g. juveniles, Connell 1985; Caley et al. 1996). The supply, survival and growth of new settlers to reach recruitment size and sexual maturity is therefore key to population maintenance and persistence (Cowen and Sponaugle 2009; Pineda et al. 2009, 2010). For marine sessile invertebrates, temporal and spatial variability associated with both pre-settlement and post-settlement stages contribute to population heterogeneity (Thorson 1966; Connell 1985; Stoner 1990; Fraschetti et al. 2003).

Primary recruitment limitation (*sensu* Fraschetti et al. 2003), involving pre-settlement processes of production, supply, survival, dispersal, habitat selection and settlement of motile life stages, such as larvae, can shape population abundance and distribution patterns (Hughes et al. 2000; Jenkins 2005; Pineda et al. 2010). Post-settlement processes subsequently contribute to secondary recruitment limitation (*sensu* Fraschetti et al. 2003), further structuring populations (e.g. abundance, distribution and individual body size) through mortality, growth and asexual reproduction. Biotic factors that could influence the mortality, growth and reproduction of sessile marine invertebrates include fission,

[4] **Chapter 5** is adapted from Abdul Wahab MA, de Nys R, Abdo D, Webster N, Whalan S (2014) The influence of habitat on post-settlement processes, larval production and recruitment in a common coral reef. J Exp Mar Biol Ecol 461:162-172

fragmentation, predation, grazing, food availability and competition (Maldonado and Uriz 1999; Zilberberg et al. 2006b; Vermeij and Sandin 2008; Penin et al. 2011; Tremblay et al. 2014). In addition, abiotic factors such as habitat surface topography, temperature, light quality and eutrophication could also influence post-settlement processes (Walters and Wethey 1996; Fabricius 2005; Nozawa and Harrison 2007; Tremblay et al. 2014). While mortality at the early post-settlement phase (e.g. juvenile) is usually very high (> 90% for marine invertebrates, Gosselin and Qian 1996, Wilson and Harrison 2005), size-dependent survival (where larger individuals have higher survival) has been demonstrated (e.g. in scleractinian corals, Penin et al. 2010; Dmitriew 2011; Guest et al. 2014).

The growth of ectotherms is significantly influenced by temperature. While higher ambient temperatures generally correspond to higher growth rates for conspecifics due to associated increases in metabolic rates (Brockington and Clarke 2001; Gooding et al. 2009; Watson et al. 2014), anomalous temperatures exceeding the thermal tolerance of a species can lead to negative growth and death (De'ath et al. 2009; Cebrian et al. 2011). In addition, photoperiod and light quality are important for taxa that associate with photosynthetic symbionts, and rely on supplemental autotrophic nutrition for energetics, somatic growth and reproduction (Muscatine and Hand 1958; Wilkinson 1983; Harland and Davies 1995; Hawkins and Klumpp 1995; Erwin and Thacker 2008; Jantzen et al. 2008; Wooldridge 2010; López-Legentil et al. 2011). For species occupying intertidal zones and inshore reefs, sub-lethal stressors, including reduced salinity and eutrophication from terrestrial run-off can lead to increased investment in physiological maintenance with concomitant effects on growth and reproduction (Fabricius 2005; Whalan et al. 2007b).

Sponges (Phylum Porifera) are conspicuous components of aquatic environments, playing significant evolutionary and functional roles (Bell 2008; Srivastava et al. 2010; de Goeij et al. 2013). Despite their ecological importance and an increased research focus to ascertain how they will respond to a changing climate (summarized in Bell et al. 2013), information on sponge population demographics and dynamics is limited. On the Great Barrier Reef (GBR), sponges in the family Thorectidae can represent up to 80 % of total sponge numbers and biomass (Wilkinson 1988). Being photosynthetic, these sponges are capable of acquiring up to 50 % of their nutrition from cyanobacterial symbionts, while releasing a proportion of the captured carbon to the environment, thereby contributing to reef nutrition (Wilkinson 1983, 1988; Webster et al. 2012). Light intensity (at different depths) is also thought to influence distribution, growth and morphology of the highly abundant Thorectidae species, *Carteriospongia foliascens* (Wilkinson and Evans 1989; Chapter 2).

Carteriospongia foliascens occurs on the intertidal reef flats of inshore sectors of the GBR. This species is reproductive all year round, and larvae potentially self-recruit to natal habitats based on relatively short larval competency periods, low dispersal potential and the capacity to settle to non-specific settlement cues (Chapter 3 and 4). This study aims to investigate processes involved in larval production and the limitation of secondary recruitment of *C. foliascens* over 24 months, by quantifying the effects of seawater temperature, photoperiod (daylight hours) and rainfall on fecundity, recruitment, growth and mortality of intertidal populations at two bays (separated by ca. 10km), within the Palm Islands group, central GBR. These two bays were selected based on differences in their size (4 km across for Juno Bay and 1 km across for Little Pioneer Bay) and hydrodynamic features (Bannister et al. 2007), while maintaining similar extrinsic environmental parameters (i.e. photoperiod and rainfall). To investigate the effects of substrate and asexual reproduction on growth dynamics in this species, changes in substrate composition and presence of fission were monitored. In addition, growth of *C. foliascens* juveniles from settlement through the first two years of life in aquaria was quantified to investigate early growth trajectories. Lastly, an index of larval productivity (population sexual productivity index; PoSPi) was quantified for Juno Bay over 12 months to investigate how habitat and demographics contribute to *C. foliascens* fecundity and larval production when compared to published PoSPi data for Little Pioneer Bay over the same period (Chapter 3).

5.2. Materials and methods

5.2.1. Study sites and sampling regime

Ten permanent quadrats (1 m x 1 m) were haphazardly established on the reef flats (ca. + 50 cm from the tidal datum) of Juno Bay, Fantome Island (18°41.405'S, 146°31.272'E), central Great Barrier Reef (GBR) and Little Pioneer Bay, Orpheus Island (18°36.989'S, 146°29.832'E) where *Carteriospongia foliascens* naturally occurred in July 2011, three months before the first sampling period in October 2011. Each quadrat consisted of 1 – 5 adult sponges at the start of the study (October 2011). Quadrats were photographed every three months over 24 months to assess changes in substrate composition, recruitment (i.e. new individuals), survival and growth in the months of January, April, July and October.

5.2.2. Environmental parameters

The study sites were selected based on differences in modelled wave energy data reported by Bannister et al. (2007), which showed a twofold increase in wave height at Juno Bay (range: 0.4 m – 0.8 m) compared to Little Pioneer Bay (range: 0 m – 0.4 m). Environmental parameters relevant to the energetics and growth of phototrophic, intertidal invertebrates were measured. Sea temperatures were monitored *in situ* using temperature loggers (HOBO®) deployed adjacent to sponges at both Juno Bay and Little Pioneer Bay over the entire duration of the study. Mean monthly photoperiod was sourced from Geoscience Australia (http://www.ga.gov.au) and total monthly rainfall data from the Bureau of Meteorology (BOM, http://www.bom.gov.au).

5.2.3. Substrate composition, adult survival and recruitment

Photographs were assessed using the image processing and analysis program ImageJ (National Institutes of Health, USA) to quantify proportion (%) of the substrate occupied by bare sand, bare coral rubble (i.e. free of other macro-invertebrates and macroalgae), *C. foliascens*, live hard coral, live soft coral, fleshy macroalgae and other sponges. The presence or absence of new individuals and existing individuals from previous sampling events were assessed at each sampling month by meticulously screening the substrata. A gridded 1 m x 1 m portable quadrat was employed to systematically record sponge position within each permanent quadrat.

5.2.4. Assessment of sponge growth

5.2.4.1. From larval settlement to 2 years old

To maximise individual survival for the assessment of growth from larval settlement to 2 year old juveniles, *C. foliascens* parenchymella larvae were settled and grown on surfaces of a High Density Polyethylene (HDPE) raceway (length – 585.0 cm, width – 71.0 cm, depth – 54.5 cm) at Orpheus Island Research Station (Chapter 4). Fourteen reproductive female sponges were collected from Little Pioneer Bay and Juno Bay in October 2011 and maintained in the raceway for six weeks. The raceway received flow-through 10 µm filtered seawater sourced 60 m off the reef crest, 480 m away from the research station. As *C. foliascens* trickle spawn during the day, over a duration of weeks to months (releasing up to ca. 800 larvae sponge^{-1} day^{-1}), and larvae are negatively phototactic and settle non-specifically to surfaces within hours from release, larvae were expected to settle unassisted

to the walls and floor of the raceway (Chapter 4). Adult sponges were removed from the raceway after six weeks. The raceway received minimal maintenance (i.e. cleaning) and received constant water flow (ca. 1440 l h^{-1}) and water exchange (ca. 63% volume h^{-1}) over the two years growth period to maintain food supply and ambient water temperature. Daylight intensity within the raceway and in the field (adjacent to adult sponges) were logged using portable light loggers (HOBO®) to compare artificial and natural light conditions *in situ*. To observe early morphological development of *C. foliascens* recruits (within a week), additional larvae (n ≈ 50) were settled onto 100 mm sterile Petri dishes to aid visualization under a dissecting microscope. Petri dishes were maintained in the same experimental raceway described previously for a week.

Two year old *C. foliascens* juveniles possessing both fan and cup morphologies were haphazardly scraped off the walls and floors of the raceway using a scalpel blade, and preserved immediately in 4% phosphate buffered formaldehyde. Volume (mm^3) of *C. foliascens* juveniles (n = 32) was quantified using water displacement in a 100 ml graduated cylinder. Distilled water, maintained at 22.25°C, was used for all volume acquisition. Marginal growth of the lamellae was predicted for sponges possessing a fan-like morphology. To test the relationship between linear marginal growth (mm) and volumetric growth (mm^3), fan perimeters (mm) of juvenile sponges (having only the fan morphology for consistency with following sections, n = 24) were quantified using image analyses of photographs.

Volume of juveniles immediately at settlement and after metamorphosis (time = 0) was assumed to be equivalent to larval volume. The volume of a single larva was calculated based on the formula for a prolate spheroid:

$$V_{larva} = \frac{4}{3} \times \pi \times \left(\frac{width}{2}\right)^2 \times length$$

where V_{larva} is the volume of a larva, *width* is the maximum distance on the larval minor axis and *length* is the maximum distance on the larval major axis. Larval dimensions for *C. foliascens* (length = 850 µm ± 3; width = 562 µm ± 3; n = 384) was inferred from Chapter 4 as larvae were sourced from the same adult sponges and time period in both studies.

The mean monthly specific growth rate of juvenile *C. foliascens* after 2 years of growth was determined by the following equation:

$$Growth\ rate = \frac{(Volume_t - Volume_{t-1})/Volume_{t-1}}{n_{month}} \times 100$$

where $Volume_t$ and $Volume_{t-1}$ are the volume of the juvenile at 2 years and at settlement (time = 0) respectively, and n_{month} is the number of months between the two sampling times (24 months).

5.2.4.2. Adult sponges

Adult sponges refer to individuals assessed during the 24 months monitoring *in situ* at Juno Bay and Little Pioneer Bay as described previously. As adult sponges can exhibit either cup or fan morphologies, only individuals possessing the common fan morphology (comprising 68% of all sponges investigated) were used for quantification of growth to allow consistency and accuracy of data interpretations.

Sponge size was determined using an underwater stereo digital camera system (Fujifilm FinePix REAL 3D W3) by capturing a series of stereo images of each sponge. Each stereo image pair were processed using PhotoMeasure software (SeaGIS; http://seagis.com.au/, verified 15 January 2014) to obtain fan perimeter measurements (linear) for each sponge (Abdo et al. 2006). The precision and accuracy of linear measurements between sampling months were verified by comparing four identical distances on a calibration frame (25 cm x 25 cm; with five equidistant points marked on each axis) photographed concurrently with each sponge at each sampling event. The mean specific growth rate of adult sponges *in situ* was determined using a similar formula described previously for 2 year old juveniles (See section 2.3.1), however in this case, volume (mm^3) is replaced by fan perimeter (mm). Asexual reproduction in the form of fission is possible in sponges and may result in the addition of new individuals into populations, therefore any potential fission was monitored by checking sponges and their immediate substrate perimeter *in situ* and in photographs.

To investigate if growth rates differed between locations and body sizes, sponges at Juno Bay and Little Pioneer Bay were categorised into four discrete size classes based on initial fan perimeter measurements. This included individuals that were very small and fell within the fan perimeter range of two year old recruits grown in aquaria (61.60 mm – 173.66

mm; < 180 mm), small (180.21 mm - 273.86 mm; < 280 mm), medium (289.21 mm - 463.03 mm; < 500 mm) and large (577.79 mm – 828.61 mm; > 500 mm).

5.2.5. Production of larvae

To compare larval production at Juno Bay to that previously reported for Little Pioneer Bay (Chapter 3), the population sexual productivity index (PoSPi) was quantified for *C. foliascens* at Juno Bay. PoSPi is a relative index of larval productivity within a population, combining relevant reproductive parameters for brooding sponges (Chapter 3). PoSPi considers (1) the proportion of the population that are female and reproductive, (2) fertilization success as represented by the proportion of female propagules that were fertilized (i.e. embryos and larvae) and (3) population female fecundity (mean reproductive output index, ROI). ROI is the proportion of the sampled section that is occupied by the sum (surface area) of all propagules present and recognized as a validated measurement of reproduction in sponges (Corriero et al. 1998; Whalan et al. 2007a). Monthly samples (n = 30) were haphazardly collected from *C. foliascens* found within a 50 m radius of permanent quadrats at Juno Bay between July 2011 to June 2012 (one reproductive cycle). Sample preservation, histological processing and analyses of reproductive propagules (i.e. proportion of population reproductive, fertilization success and female ROI) were performed following Chapter 3, Section 3.2.2 – 3.2.4. Reproductive data of *C. foliascens* from Little Pioneer Bay over the same period (July 2011 to June 2012; Chapter 3) was used to compare reproductive performance between the two bays.

5.2.6. Statistical Analyses

Assumptions of normality and homoscedasticity were checked graphically (boxplot and residual plots) for each dataset before testing hypotheses, and data transformations applied when assumptions were violated (Quinn and Keough 2002). Datasets not meeting assumptions (post-transformations), and those having unbalanced designs, were analysed using permutational methods (PERMANOVA) (Anderson 2005). Euclidean resemblance matrices with 9999 permutations of raw data were used for all permutational analyses. A t-test was used to compare mean monthly sea temperature profiles between Juno Bay and Little Pioneer Bay. Pearson's correlations were used to investigate relationships between mean monthly sea temperature and mean monthly photoperiod. To elucidate differences in substrate compositions between Juno Bay and Little Pioneer Bay, and explore drivers of variation, a principal component analysis followed by vector correlations ($p > 0.4$) was executed on the raw substrate dataset (i.e. two locations, all substrate types and all sampling

time). Repeated measures ANOVA (RANOVA, Statistica 10) was then executed to investigate the influence of location (bays, between-factor variable) and time (months, within-factor variable), on % macroalgae and % *C. foliascens*. Additionally, Pearson's correlations were used to investigate relationships between mean monthly seawater temperature, mean monthly photoperiod and total monthly rainfall to % macroalgae and % *C. foliascens* using the log transformed dataset. To investigate differences in initial *C. foliascens* densities (sponge m^{-2}) between Juno Bay and Little Pioneer Bay, a t-test was done on the October 2011 dataset. Subsequently, RANOVA was used to investigate differences in sponge densities at the start and end of the 24 month period, using location (bays, between-factor) and time (months, within-factor) as variables. Differences in survival patterns of *C. foliascens* between Juno Bay and Little Pioneer Bay over time (months) were assessed using the Cox-Mantel test. To assess differences in light conditions between sponges growing in the field and in aquaria, a one – way ANOVA (PERMANOVA) was used. The relationship between linear measurements of fan perimeter (mm) and volumetric (mm^3) measurements was assessed using a Pearson's correlation. To assess the precision and accuracy of linear measurements derived from stereo image pairs over time, a one-way ANOVA was used with time as a variable. Differences in initial sponge size (fan perimeter) between Juno Bay and Little Pioneer Bay were assessed using a t-test on the logged dataset. RANOVA (PERMANOVA) was subsequently used to assess changes in mean monthly specific growth rates (SGR) between locations (bays) and time (months). Pearson's correlations were used to define relationships between SGR and mean monthly seawater temperature, mean monthly photoperiod and total monthly rainfall. t-tests were used to compare reproductive parameters (i.e. proportion reproductive females, proportion fertilized propagules, and log PoSPi) between bays. PERMANOVA was used to compare reproductive output index (ROI) data due to an unbalanced distribution of females between bays.

5.3. Results

5.3.1. Environmental parameters

Mean monthly sea temperature was similar between bays (t-test: $t = -0.108$, $df = 48$, $p > 0.05$), therefore only temperature data from Little Pioneer Bay was used in subsequent analyses. Mean monthly sea temperature reached the annual maxima (February 2012 = 29.72°C ± 0.10 [mean ± SE]; February 2013 = 29.57°C ± 0.07) and minima (July 2012 = 21.27°C ± 0.07; July 2013 = 22.29°C ± 0.08) two months and one month after the mean monthly photoperiod maxima (December 2011 = 13.22 h ± 0.01; December 2012 = 13.22 h

± 0.00) and minima (June 2012 = 11.06 h ± 0.02; June 2013 = 11.03 h ± 0.00) respectively. Mean monthly sea temperature was significantly correlated to mean monthly photoperiod (Pearson's r = 0.7807, $p < 0.0001$). Total rainfall over the first year of the study (October 2011 to September 2012) was ca. 128 % higher than in the second year (October 2012 to September 2013), exhibiting a more intense wet season (maximum total monthly rainfall year 1 = 1113.5 mm, March 2012; maximum monthly rainfall year 2 = 450.9 mm, January 2013).

5.3.2. Substrate composition

Bare coral rubble and sand dominated the substrate across Juno Bay and Little Pioneer Bay with the mean % area fluctuating between ca. 28 % to 67%. The most dominant biotic component of the substrate was fleshy macroalgae attached to coral rubble which predominantly comprised *Padina* sp. and *Sargassum* sp. (collectively termed macroalgae hereafter), and which formed blooms (ca. 2 m high for *Sargassum* sp.) over the warmer months (October to April). Juno Bay supported a higher abundance of macroalgae compared to Little Pioneer Bay. Juno Bay achieved a maximum macroalgae substrate cover of 12.38 % ± 3.02 in October 2013 and maintained high abundances as temperature decreased after the annual maxima (April 2012 = 8.21 % ± 2.40; April 2013 = 6.35 % ± 1.94) (Figure 5.1). In contrast, Little Pioneer Bay achieved maximum macroalgae cover of 1.55 % ± 0.24 (eight fold less compared to maximum macroalgae cover at Juno Bay) in October 2012 (Figure 5.1). The next dominant biotic component of the substrate was *Carteriospongia foliascens* which achieved maximum % area of 1.44 % ± 0.29 and 0.50 % ± 0.09 at Juno Bay and Little Pioneer Bay respectively (Figure 5.1). Live coral, soft coral and other sponges formed minor components of the substrate at both locations, never exceeding 0.62 % of the substrate over the study period. Live coral was represented primarily by the massive *Goniastrea aspera* (Faviidae) and live soft coral primarily by *Lobophytum* sp. (Alcyoniidae). Other sponge species present consisted of the phototrophic *Cymbastela coralliophillia* (Axinellidae) and *Cliona orientalis* (Clionaidae).

Principal component analysis of substrate composition data, over the entire duration of the study, showed a separation of Juno Bay and Little Pioneer Bay datasets on the PC 1 axis (62.3% of total variation, 14.6% of total variation on PC 2 axis, total variation = 76.9 %; Figure 5.2), with vector correlations ($r > 0.4$) showing a potential influence of macroalgae and *C. foliascens*. There was a significant interaction of location (Juno Bay or Little Pioneer Bay) and time (months) on % macroalgae cover (RANOVA: $F_{(8,144)} = 2.06$, $p < 0.05$), and significant main effects of location (RANOVA: $F_{(1,18)} = 13.01$, $p < 0.005$) and time

(RANOVA: $F_{(8,144)} = 5.40$, $p < 0.0001$) on % *C. foliascens* cover. Macroalgae cover (%) was correlated to both temperature and photoperiod at Juno Bay, but was only correlated to photoperiod at Little Pioneer Bay (Table 5.1).

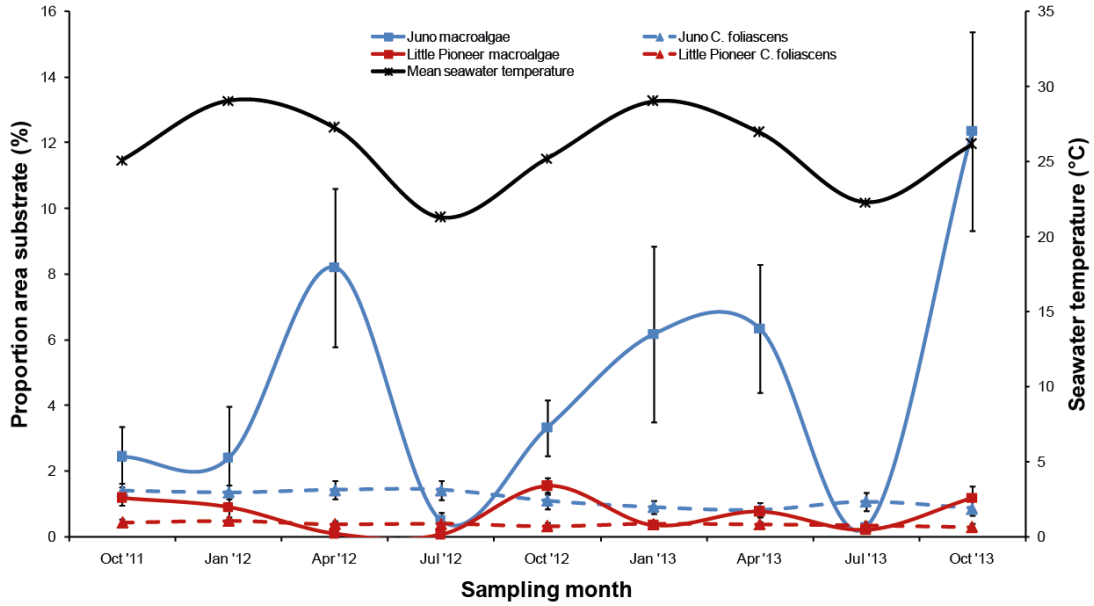

Figure 5.1: Percentage (mean % ± SE) of the substrate occupied by macroalgae and *C. foliascens* at Juno Bay and Little Pioneer Bay over the 24 month study. Red colouration represents the Little Pioneer Bay locality and blue colouration represents the Juno Bay locality. The black line represents mean seawater temperature.

Table 5.1: Summary statistics of Pearson's correlation analyses between key environmental parameters; mean monthly seawater temperature (Temperature), mean monthly daylight hours (Photoperiod) and total monthly rainfall (Rainfall), and substrate cover (log % seaweed and log % *C. foliascens*) and log mean monthly specific growth rates of different *C. foliascens* size classes (very small, small, medium and large). Values represent Pearson's r and text with asterisks represents significant results.

	Pearson's r		
Juno	**Temperature**	**Photoperiod**	**Rainfall**
Log substrate cover			
% seaweed	0.3867 *	0.2223 *	-0.1965
% *C. foliascens*	-0.054	-0.0212	0.0548
Log specific growth rates			
Very small	-0.124	-0.4692 *	0.0966
Small	-0.0540	-0.1755	-0.1550
Medium	0.1293	0.2454 *	0.2251 *
Large	0.3301 *	0.2032	0.3238 *
Little Pioneer	**Temperature**	**Photoperiod**	**Rainfall**
Log substrate cover			
% seaweed	0.158	0.3847 *	-0.1141
% *C. foliascens*	0.063	0.0581	0.1429
Log specific growth rates			
Very small	0.0290	0.3248	0.2305
Small	0.2142 *	0.1416	-0.0248
Medium	0.1809	0.3872 *	0.2048

* $p < 0.05$

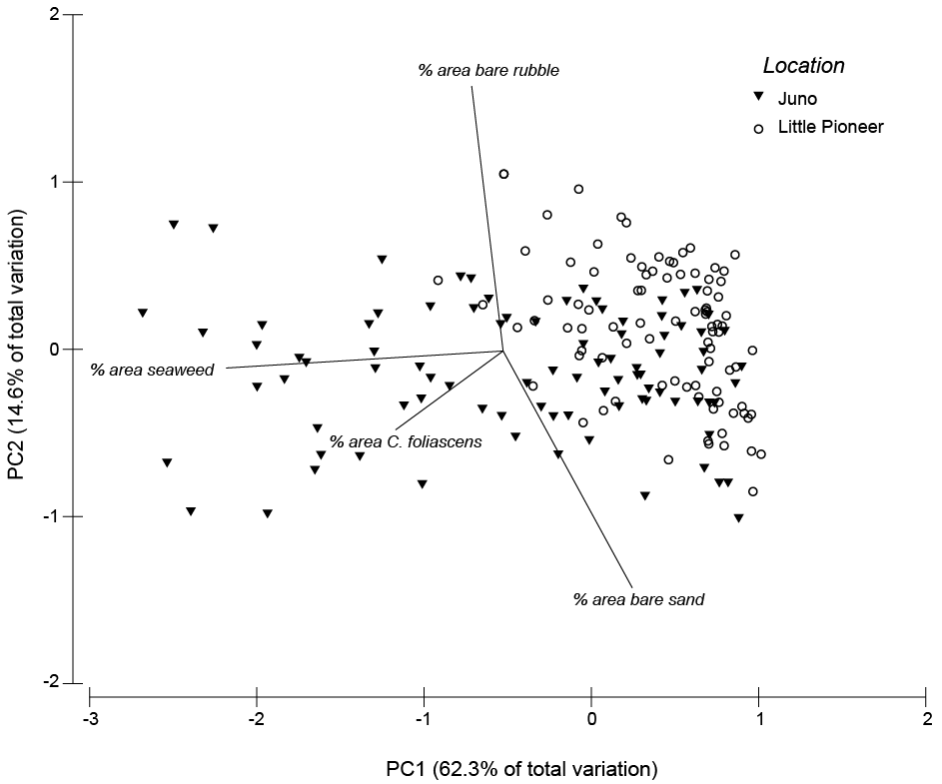

Figure 5.2: Principal component analysis of the Juno Bay and Little Pioneer Bay substrate datasets, with correlations overlay (Pearson's r > 0.4) for % area of bare rubble, bare sand, seaweed and *C. foliascens* included.

5.3.3. Adult survival and recruitment

A total of 26 and 18 adult *C. foliascens* were present at Juno Bay and Little Pioneer Bay at the start of the study (October 2011), which corresponded to sponge densities of 2.6 sponge m^{-2} ± 0.4 and 1.8 sponge m^{-2} ± 0.2 respectively. Initial sponge densities were not statistically different between locations (t test: t = -1.90, df = 18, p > 0.05). 65 % (17 out of 26; 1.7 sponge m^{-2} ± 0.2) and 56 % (10 out of 18; 1.0 sponge m^{-2} ± 0.3) of initial *C. foliascens* survived the 24 month study period at Juno Bay and Little Pioneer Bay respectively. Whilst significant effects of location (RANOVA: $F_{(1, 18)} = 5.10$, $p < 0.05$) and time (RANOVA: $F_{(1, 18)} = 20.80$, $p < 0.001$) were observed on sponge densities at the end of the study, there was no difference in the pattern of sponge mortality between locations over the 24 month period (Cox-Mantel test: statistic = - 0.4806, p > 0.05).

Six new recruits (juveniles) were recorded from Juno Bay, with two individuals surviving to the end of the monitoring period (18 and 21 months survival). Three of the four remaining recruits were detected only once (0 month survival) and one other survived for 9 months. Only one new recruit was recorded from Little Pioneer Bay which survived for 18 months.

5.3.4. Assessment of sponge growth

5.3.4.1. From larval settlement to 2 years old

Light conditions in the growing raceway did not differ to that experienced by sponges naturally *in situ* between 1000 h and 1330 h (9300 to 74 400 Lux; PERMANOVA Pseudo-$F_{(1, 305)} = 2.4324$, $p > 0.05$), but was reduced to < 2911.02 Lux ± 540.61 (up to 81.72 % light reduction) outside of this time period due to tank shading effects. One-week old juvenile *C. foliascens* grown in aquaria developed oscules and displayed distinct 3-dimensional growth, which included vertical nodular projections of the sponge body (Figure 5.3a). One year old juveniles exhibited fan or cup morphologies, dark brownish-green colouration and surface conulation (distinct bumps on the sponge body surface resulting from primary skeletal fibres projections) resembling adult sponges (Figure 5.3b, see Chapter 1 and 2 for adult sponge morphology). Two year old juveniles (Figure 5.3c) achieved a mean volume of 881.82 mm^3 ± 96.92 (n = 32), equivalent to a 627596 % increase in size when compared to volume at settlement (mean larval volume = 0.142 mm^3 ± 0.002, n = 384). Mean specific growth rate calculated from mean net volumetric size after 24 months was equivalent to 26146 % $month^{-1}$ ± 2874 (n = 32). There was a significant correlation between fan perimeter and sponge volume in two year old *C. foliascens* juveniles (n = 24, Pearson's r = 0.78, $p < 0.0001$).

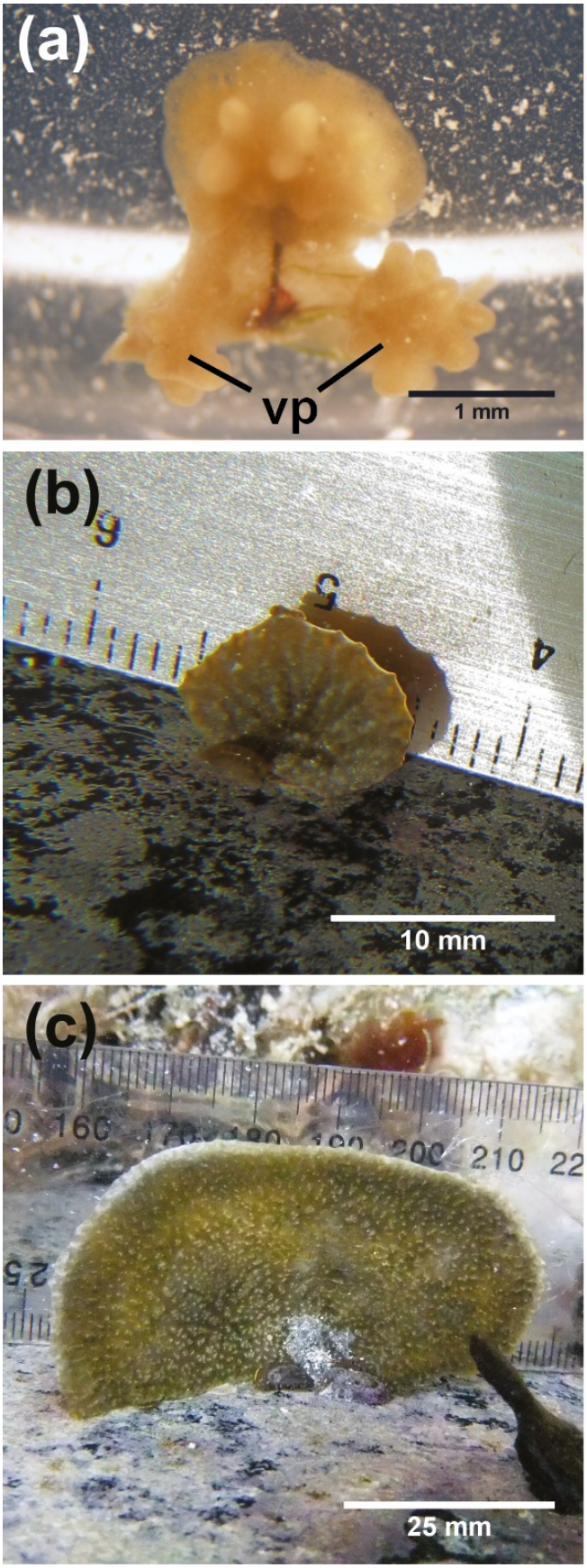

Figure 5.3: *Carteriospongia foliascens*. Photographs of *C. foliascens* grown in a flow-through aquarium showing a) a one – week old juvenile displaying vertical growth and vertical nodular projections (vp) of the sponge body, b) a one – year old recruit displaying adult fan morphology and colouration, visible to the naked eye and c) a two – year old recruit displaying the fan morphology.

5.3.4.2. Adult sponges

No evidence of fission of adult sponges was recorded over the 24 month study period. As sponge fan perimeter was significantly correlated to volume (see section 3.4.1), fan perimeter was assumed to be an accurate proxy for growth in adult sponges. Linear measurements derived from stereo imagery were consistent over 24 months (ANOVA: $F_{8,27} = 1.4$, $p > 0.05$). All sponges falling in the very small size class ($n_{Juno} = 3$, $n_{Little\ Pioneer} = 1$) were represented by new recruits (juveniles) detected over the duration of the 24 month study. A total of 13 small size ($n_{Juno} = 5$, $n_{Little\ Pioneer} = 8$), 12 medium ($n_{Juno} = 6$, $n_{Little\ Pioneer} = 6$) and 3 large ($n_{Juno} = 3$) size classed individuals were used in following analyses. There were no large sized sponges at Little Pioneer Bay. Mean sponge fan perimeter between Little Pioneer Bay (285.55 mm ± 21.70, n = 14) and Juno Bay (379.269 mm ± 47.93, n = 14) was consistent (t test: $t = 1.7112$, $df = 26$, $p > 0.05$).

C. foliascens at both Juno Bay and Little Pioneer Bay exhibited both positive and negative specific growth rates (SGR) across all size classes over the 24 month study period, and displayed high variability in SGR at each sampling event (Figure 5.4, Table 5.2). Maximum SGR was highest in the very small size class and decreased with increasing size classes, at both Juno Bay and Little Pioneer Bay (Table 5.2). When mean monthly SGR was averaged over the entire 24 month period, the Juno Bay very small, small and medium size classes, and Little Pioneer Bay very small and small size classes displayed an overall positive SGR (see Table 5.2, Overall mean SGR). The very small size class SGR at Juno Bay was tenfold higher compared to Little Pioneer Bay. The Juno Bay large size class and Little Pioneer Bay medium size class SGR fluctuated around the zero growth mark (Table 5.2). Repeated measures ANOVA detected a significant effect of time on the mean monthly SGR's for the medium and large size classes and a significant interaction of location and time in the small size class (Table 5.3). Neither location nor time influenced growth in the very small size class (Table 5.3).

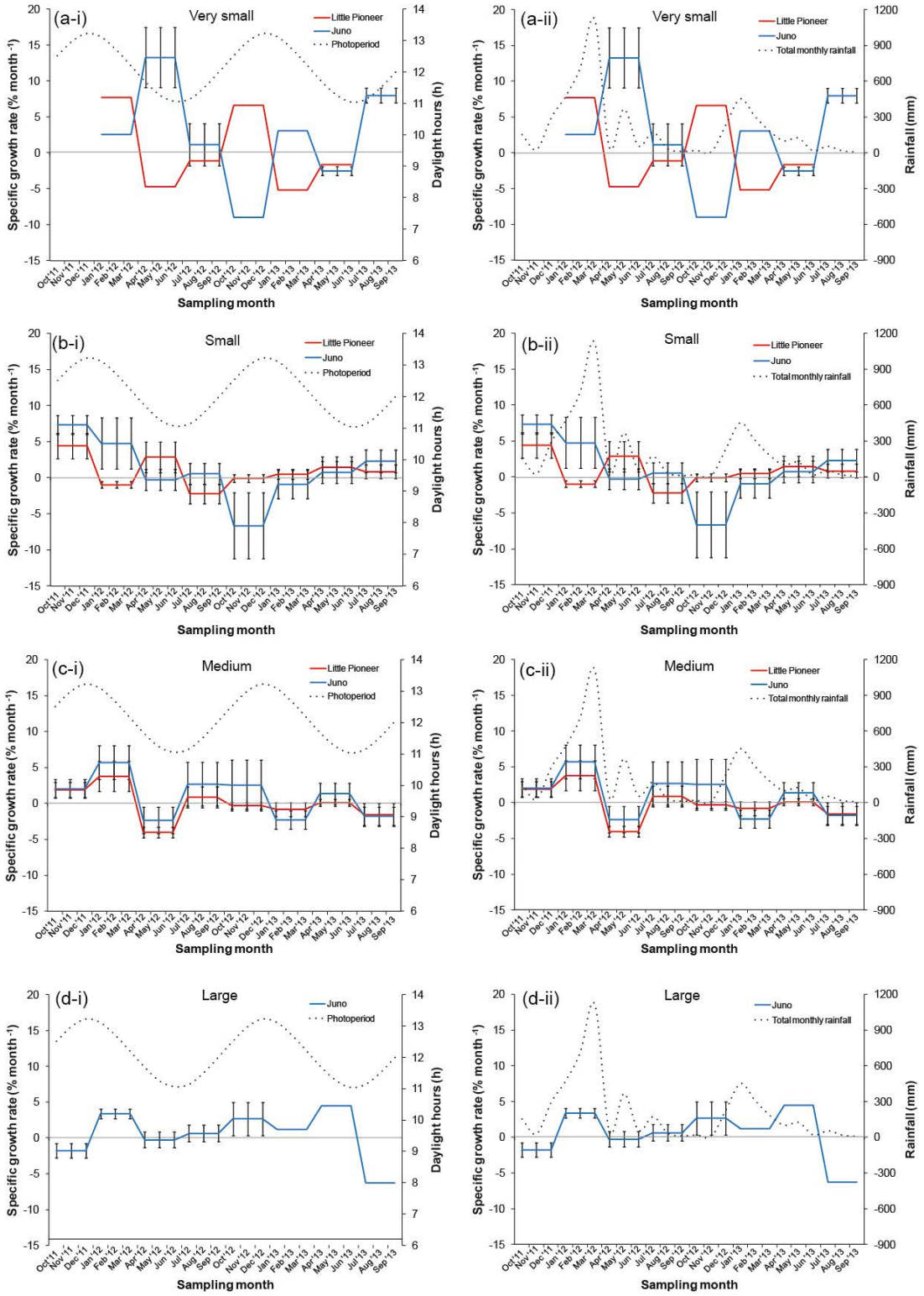

Figure 5.4: *C. foliascens*. Mean (± SE) monthly specific growth rates (SGR, % change month^{-1}) of a) very small, b) small, c) medium and d) large size classed *C. foliascens* over the 24 months study period at Juno Bay and Little Pioneer Bay. i) photoperiod and ii) rainfall which co-varies with SGR (see **Table 2**) are also plotted on the graphs to show these relationships. Red colouration represents the Little Pioneer Bay locality and blue colouration represents the Juno Bay locality. The black dotted line represents photoperiod and total monthly rainfall respectively.

Table 5.2: *C. foliascens*. Summary table showing minimum, maximum and overall (average across the total 24 months study period) mean monthly specific growth rates (SGR, % change month^{-1}) for the two locations and various sponge size class used in this study. Values represents means ± SE, and where SE is missing represent values derived from a single measurement (n = 1).

	Specific growth rates		
Juno	**Minimum mean SGR (% month -1)**	**Maximum mean SGR (% month -1)**	**Overall mean SGR (% month $^{-1}$)**
Very small	-9.03 (Oct '12 to Jan '13)	13.26 ± 4.19 (Apr '12 to Jul '12)	2.34 ± 1.48
Small	-6.65 ± 4.56 (Oct '12 to Jan '13)	7.31 ± 1.32 (Oct '11 to Jan '12)	0.96 ± 0.81
Medium	-2.36 ± 1.82 (Apr '12 to Jul '12)	5.68 ± 2.36 (Jan '12 to Apr '12)	0.99 ± 0.17
Large	-6.31 (Jul '13 to Oct '13)	4.49 (Apr '13 to Jul '13)	0.49 ± 0.66
Little Pioneer	**Minimum mean SGR (% month -1)**	**Maximum mean SGR (% month -1)**	**Overall mean SGR (% month $^{-1}$)**
Very small	-5.22 (Jan '13 to Apr '13)	7.67 (Jan '12 to Apr '12)	0.25 ± 2.27
Small	-2.26 ± 1.37 (Jul '12 to Oct '12)	4.38 ± 1.77 (Oct '11 to Jan '12)	0.82 ± 0.41
Medium	-4.05 ± 0.76 (Apr '12 to Jul '12)	3.75 ± 2.11 (Jan '12 to Apr '12)	-0.03 ± 0.11

Table 5.3: *C. foliascens*. Summary statistics of repeated measures ANOVA (PERMANOVA) on mean monthly specific growth rates (SGR, % change month^{-1}) for the very small, small, medium and large size class. These analyses considered the effects of location (bays) and time (months). Tests with asterisks represent significant results.

	Body size											
	Very small			*Small*			*Medium*			*Large*		
Source	df	Pseudo-F	p	df	Pseudo-F	p	df	Pseudo-F	p	df	Pseudo-F	p
Location	1	0.17308	0.7066	1	2.27E-02	0.8782	1	1.1654	0.2899	-	-	-
Time	6	1.1286	0.4294	7	4.3133	0.001*	7	3.7033	0.0035*	7	4.0217	0.0342*
*Location * Time*	5	2.9566	0.1069	7	2.2465	0.0448*	7	0.28577	0.9571	-	-	-
Residual	6			68			49			8		

Correlations of log mean monthly SGR with temperature, photoperiod and rainfall produced variable results for size classes at the two locations (Table 5.1, Figure 5.4). At Juno Bay, rainfall was positively correlated to mean monthly SGR for medium and large size classes (Table 5.1). Photoperiod was significantly correlated to mean monthly SGR for the very small and medium size classes at Juno Bay and the medium size class at Little Pioneer Bay, displaying a negative relationship for the very small size class at Juno Bay. Temperature was positively correlated to mean monthly SGR of the large size class at Juno Bay and small size class at Little Pioneer Bay (Table 5.1).

5.3.5. Production of larvae

A total of 288 reproductive individuals from Juno Bay were sampled from July 2011 to June 2012, equating to one reproductive cycle and consisting of 161 females and 127 males. Over the same period, 123 females and 148 males were sampled from Little Pioneer Bay, totalling 271 reproductive individuals (Chapter 3). The proportion of reproductive females was significantly higher at Juno Bay (range: 33.33% - 70.00%) than at Little Pioneer Bay (range: 16.67% - 46.67%) (Figure 5.5a, t-test: $t = 2.865$, $df = 22$, $p < 0.01$), with sponges at Juno Bay

having a higher mean proportion of fertilized propagules (Figure 5.5b, embryo and larva; Juno Bay = 24.98 % ± 4.09, Little Pioneer Bay = 14.71 % ± 2.52; t-test: t = -2.135, df = 22, p < 0.05). Female fecundity (ROI) at Juno Bay was consistently higher than at Little Pioneer Bay between July 2011 and February 2012 before fluctuating over low levels (Figure 5.5c, < 1.62 % ± 0.55) between March 2012 and June 2012. Maximum female ROI reached 6.73 %± 1.08 in November 2011 at Juno Bay and 4.43 % ± 0.90 in December 2011 at Little Pioneer Bay and was not significantly different between locations (PERMANOVA: Pseudo-$F_{1, 22}$ = 2.66, p > 0.05). Larval production (PoSPi) was consistently higher in every month of the study at Juno Bay compared to Little Pioneer Bay, (Figure 5.5d, t-test: t = 2.29, df = 22, p < 0.05; maximum PoSPi Juno Bay = 1.36, November 2011; maximum PoSPi Little Pioneer Bay = 0.79, December 2011).

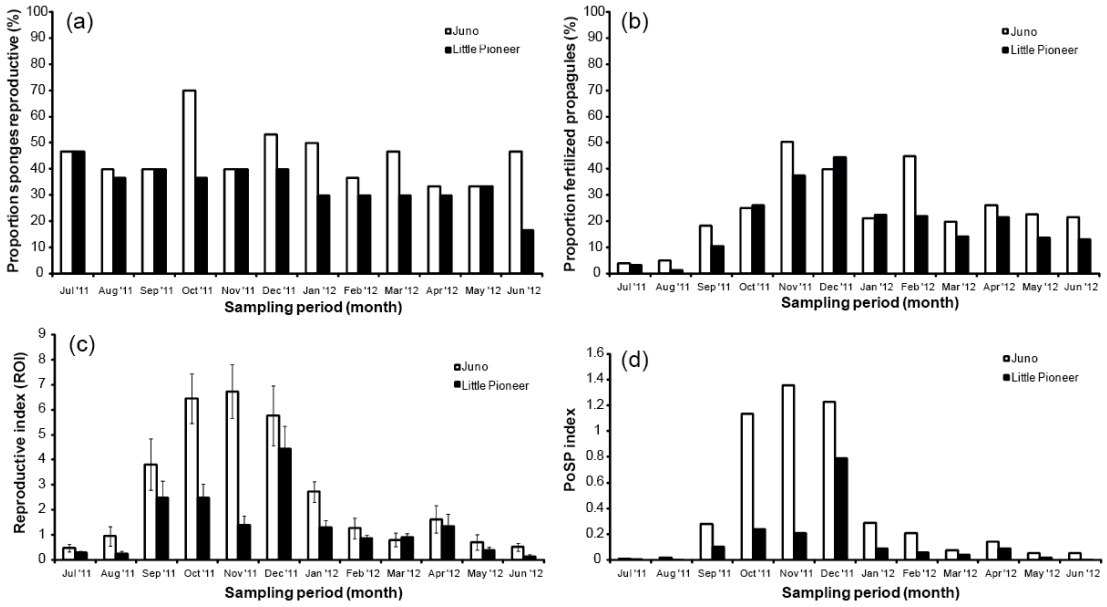

Figure 5.5: *C. foliascens*. Graphs of relevant reproductive parameters used for the formulation of the population sexual productivity index (PoSPi) at Juno and Little Pioneer Bay including (a) proportion of reproductive females, (b) fertilization success as measured by total proportion of female propagules that were embryo and larva and (c) female reproductive output index. (d) Represents graph of PoSPi between Juno Bay and Little Pioneer Bay. *C. foliascens* reproductive data for Little Pioneer Bay was taken from Chapter 3.

5.4. Discussion

Carteriospongia foliascens is a conspicuous intertidal sponge at inshore reefs of the central Great Barrier Reef (GBR) and along with seasonal macroalgae represents the primary structural sessile biota in these habitats. Growth of *C. foliascens* is primarily marginal (positive correlation between fan perimeter and volume), with mean monthly specific growth rates (SGR) of sponges *in situ* falling within the range of SGR reported for other

demosponges (summarized in Abdo et al. 2008b). SGR was highest in the first two years of growth in aquaria (26146 % ± 2874), and was between 4.8 – 4.9 orders of magnitude higher than the maximum SGR observed in the field (very small size class, Juno Bay = 13 % ± 4). While growth in aquaria may not be an accurate representation of that experienced in the wild, this is the first account of growth from settlement to recruitment size (i.e. similar size to observed recruits in the field [very small size class]), and provides a valuable insight to early growth trajectories for sponges. This period of extremely high growth in the early life stages, followed by decreasing overall mean SGR as body size (size class) increased, reflects growth trajectories demonstrated in other species possessing indeterminate growth such as the barrel sponge, *Xestospongia muta* (McMurray et al. 2008) and the red sea urchin *Strongylocentrotus franciscanus* (Ebert and Southon 2003).

Indeterminate growth exists in organisms where body size and morphology are not genetically defined (Sebens 1987). *C. foliascens* displayed fluctuating patterns of growth (positive SGR) and size reduction (negative SGR) across all size classes at both Juno Bay and Little Pioneer Bay. The variable growth rates observed for *C. foliascens* are commonly encountered in sponges (Duckworth and Battershill 2001; Garrabou and Zabala 2001; Abdo et al. 2008b; Duckworth and Wolff 2011) and other indeterminately growing taxa such as coelenterates, echinoderms and molluscs (Sebens 1987; Kozłowski 1992; Heino and Kaitala 1999). Due to this plasticity, extrinsic abiotic or biotic factors can have significant impacts on growth patterns (positive or negative), thus decoupling the relationship between age and size (i.e. size is not a good proxy for age), adding complexities and uncertainties to age predictions for *C. foliascens* and sponges in general.

Juno Bay supports a higher abundance of larger sized *C. foliascens* which also exhibit higher mean monthly SGR. This is demonstrated by tenfold higher SGR in the very small individuals at Juno Bay, and is further supported by mean overall SGR of medium sized individuals at Little Pioneer Bay fluctuating around zero % (i.e. growth asymptotes at this size class), and with Juno Bay *C. foliascens* exhibiting 0.99 % overall SGR (positive growth) for the same size, allowing individuals to reach the larger size class at Juno Bay. Being a phototrophic filter feeder (Wilkinson 1983; Webster et al. 2012), factors relevant for the governance of growth in *C. foliascens* are those involved in host metabolism and symbiotic cyanobacterial photosynthesis, analogous to that demonstrated for coral – zooxanthallae associations (Hoegh-Guldberg and Jones 1999; Anthony and Hoegh-Guldberg 2003b; Carricart-Ganivet 2004; Fabricius 2005). Temperature and photoperiod can influence holobiont energetics and function (Jones and Berkelmans 2011; Lesser 2013; Webster et al. 2011, 2013), and this is reflected in significant positive correlative relationships between

SGR and the two aforementioned environmental factors for *C. foliascens*. Interestingly, a negative relationship to photoperiod was detected in very small individuals at Juno Bay, which may be attributed to shading effects from high macroalgal abundance at this location, as seen in other phototrophic sponges (Thacker 2005; Erwin and Thacker 2008). Nevertheless, the similarities of both temperature and photoperiod at the two locations suggest other environmental processes may contribute to *C. foliascens* demographic differences.

The difference in size between Juno Bay (4 km across) and Little Pioneer Bay (1 km across) can affect water quality parameters due to variations in exposure to hydrodynamic processes (Wolanski et al. 2003; Cooper et al. 2007). Wave action is the dominant process influencing water velocity, acceleration and displacement in shallow environments (Massel 1999). Modelled wave energy data by Bannister et al. (2007), showed a twofold increase in wave height at Juno Bay (range: 0.4 m – 0.8 m) compared to Little Pioneer Bay (range: 0 m – 0.4 m). As water movement is one of the most important factors influencing the growth of sessile marine invertebrates (Palumbi 1984,1986; Kaandorp and Sloot 2001, Duckworth et al. 2004), the higher wave energy at Juno Bay may aid in diffusing the benthic boundary layer allowing for greater respiration, nutrient uptake and feeding (Fréchette et al.1989; Wolanski et al. 2003; McKee et al. 2004). In addition, the increased wave energy and corresponding hydrodynamics at Juno Bay may contribute to higher water quality (i.e. clarity and light penetration) through rapid flushing of the bay after periods of disturbances (i.e. rainfall and associated terrestrial run-off) (Fabricius 2005; Wolanski et al. 2008). The higher abundance of seasonal fleshy macroalgae (up to eightfold higher) at Juno Bay further supports this location as a superior habitat for the growth of phototropic taxa such as *C. foliascens*.

Reproduction in *C. foliascens* is achieved in the smallest size class (≤ 15 cm^3, equivalent to ca. ≤ 190 mm fan circumference; see Chapter 3). In a growing sexually mature individual, energy available for somatic growth and reproduction are different proportions of the energetic surplus after investments towards maintenance and survival, with most of this surplus invested towards reproduction when growth attenuates (Sebens 1987; Kozłowski 1992; Heino and Kaitala 1999; Harshman and Zera 2007). For *C. foliascens*, which reproduce over several months of the year, and over multiple years, this can result in fluctuations in energy investments for growth, thus supporting the variable growth patterns observed in this study (positive or negative). This is further reflected in the variability in SGR within each sampling month for all size classes, and the decreasing overall variability in SGR over the study period as growth attenuates in larger individuals. Notably, spatial

variation in fecundity exists in *C. foliascens*, with Juno Bay sponges exhibiting higher potential for larval production (PoSPi) compared to Little Pioneer Bay sponges (Chapter 3; this study). This variation may represent a higher energetic surplus at Juno Bay available for investment towards reproduction and larval production as a result of living in a more optimal environment (i.e. increased hydrodynamics).

Sessile invertebrates can have complex life stages, with some species possessing feeding larvae which could spend weeks to months in the plankton (e.g. mussels and barnacles; Strathmann 1985). For species which disperse over wide spatial scales, the supply of larvae into populations (i.e. the actual number of larvae available for recruitment) may represent externally produced larvae arriving into the population (import of larvae) and locally produced larvae not dispersed out of the population (export of larvae) (McQuaid and Lawrie 2005). In this case, the relationship between larval production (i.e. adult abundance and fecundity) and larval supply for recruitment within populations is often de-coupled. However, the link between larval production and supply is apparent in species producing larvae that are lecithotrophic and having limited dispersal (e.g. some species of scleractinian corals and ascidians; Stoner 1992; Carlon 2002). *C. foliascens* larvae display early competencies, limited dispersal potential and non - specific settlement behaviours (Chapter 4). In addition, adults occur in habitats exhibiting relatively low current magnitude (0 ms^{-1} – 0.2 ms^{-1}; Bannister et al. 2007). These characteristics when considered together support self-recruitment for *C. foliascens* populations (Gaines and Bertness 1992; Cetina-Heredia and Connolly 2011; Figueiredo et al. 2013). This is somewhat indicated by higher levels of recruitment for Juno Bay (6 individuals) compared to Little Pioneer Bay (1 individual) in this study. However, recruitment would need to be investigated at a more extensive scale to ascertain the link between adult (stock) abundance and fecundity towards recruitment in *C. foliascens* as shown in other sessile invertebrates such as scleractinian corals (Hughes et al. 2000).

Asexual reproduction can play a significant role in sponge population demography (Duran et al. 2004; Zilberberg et al. 2006b), however no evidence of fission was detected for *C. foliascens* over the 24 month monitoring period. Although population genetic data would provide more conclusive support for interpretations regarding clonal reproduction, field results from this study strongly suggest that asexual reproduction is not a significant contributor to population maintenance. Therefore, sexual reproduction and larval recruitment are the most likely processes for population replenishment in *C. foliascens* which is also the case for other sponge species (Zilberberg et al. 2006a; Mercado-Molina et al. 2011). Despite evident recruitment over the duration of the study, mortality (Juno Bay = 9 mortalities, Little

Pioneer Bay = 8 mortalities) exceeded recruitment, demonstrating low replenishment of *C. foliascens* at these two bays. However, the short monitoring duration (24 months) may overlook possible major recruitment pulses outside of the study period (prior to this study and in the future). Consecutive climatic disturbances, such the La Niña anomaly over the summer of 2010 (Imielska 2011; Giles 2012), and the passing of multiple cyclones (including the category 5 Cyclone Yasi in February 2011, www.bom.gov.au) over the study area occurred prior to the start of this study. These climatic events may have depressed reproduction in *C. foliascens* (Chapter 3), and may contribute to the low recruitment rates observed in this study through the reduction of larval production and supply, and increased mortality of planktonic larvae and newly settled individuals. While further monitoring of populations is required to fully understand population growth trajectories (i.e. replenishment rates under stable climatic conditions), higher occurrences of predicted climatic anomalies (i.e. more recurrent and intense storms) in the future may threaten recruitment dynamics in intertidal *C. foliascens* (Solomon et al. 2007).

Chapter 6

Synthesis and discussion

This study holistically addressed the biological and environmental factors that contribute to population dynamics and recruitment of the common Indo-Pacific sponge *Carteriospongia foliascens* at inshore reefs of the central Great Barrier Reef (GBR). The multi-faceted approach adopted here enabled a broad overview of the primary factors influencing *C. foliascens* populations, and thereby contributing to the effective management and conservation of this species. The major findings of this study are:

1. **Hidden sponge diversity, cryptic speciation and robust taxonomic units (Chapter 2**). High hidden diversity was discovered in previously reported "cosmopolitan" foliose sponge species in tropical Australia. The identity and evolutionary relevant morphological characteristics of *Carteriospongia foliascens* were confirmed using a combination of morphometrics and molecular phylogenetics to differentiate it from morphologically similar species.

2. **Reproductive ecology of *Carteriospongia foliascens* (Chapter 3).** Sexual maturity was reached in the smallest sized individuals. *C. foliascens* is reproductive all year round, gonochoric and viviparous, with temperature having a significant effect on reproductive phenology. Unprecedented high rainfall and temperature associated with the strongest La Niña event on record likely depressed larval productivity by reducing the number of reproductive females, fecundity and fertilization success.

3. **Larval behaviour and dispersal potential of *Carteriospongia foliascens* (Chapter 4).** Developed brooded parenchymella larvae are released only during daylight hours, are negatively phototactic, attained settlement competencies within 2 h from release and settle indiscriminately to benthic habitat cues (microbial biofilms). A short larval planktonic duration and low dispersal potential, highlights the high probability for endogenous recruitment in this species.

4. **Habitat and environmental influences on *Carteriospongia foliascens* post-settlement processes, larval production and recruitment (Chapter 5).** The growth of *C. foliascens* is highly variable across size classes and is highest in the smallest individuals. Reduced variability in growth with increasing body size reflects indeterminate growth trajectories. The influence of environmental factors such as temperature, photoperiod and rainfall are variable between size classes. Differences in habitat hydrodynamics influenced population heterogeneity, with higher wave height corresponding to higher growth rates, maximum body size and larval production. Increased larval production was associated with higher recruitment supporting a stock-recruitment relationship in this species.

The population dynamics of sessile marine invertebrates is complex, and is influenced by both biological and environmental factors (Chapter 1). Information gathered throughout this thesis are consolidated and incorporated into a schematic diagram illustrating the factors influencing the population dynamics of *C. foliascens* at inshore reefs of the GBR (Figure 6.1). Results identified variable effects of population processes at each life-history stages on adult *C. foliascens* demographics and distribution (Figure 6.1, see within Boxes for relative effects of processes). *C. foliascens* is highly reproductive, producing high numbers of larvae throughout the year (Figure 6.1, Box 1). High larval productivity is contributed to by a high abundance of reproductive individuals within populations (up to 100% reproductive in October and November), high individual fecundity (4.43 to 5.09 % ROI), high fertilization success (up to 44% $month^{-1}$) and long larval release duration (3 months to year round), allowing for a large larval pool to support recruitment.

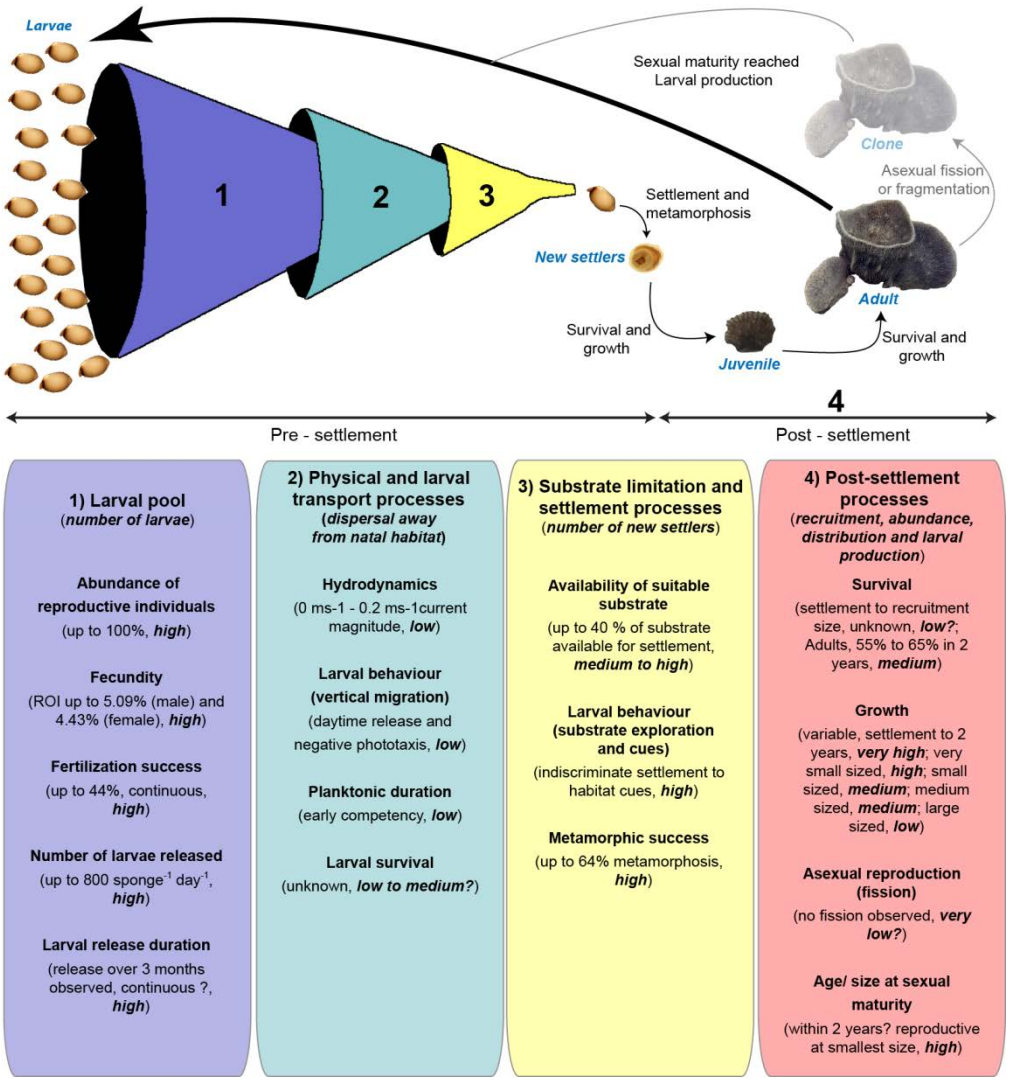

Figure 6.1: Schematic summary of relative effects of processes influencing population dynamics and demographics of intertidal *Carteriospongia foliascens* at the inshore central Great Barrier Reef. Bold italicised texts in parentheses represents relative effect size of each process to 1) number of larvae contributed to the larval pool (Box 1), 2) dispersal away from natal habitat (Box 2), 3) number of new settlers to the population (Box 3) and 4) level of recruitment, and abundance, distribution and larval production of adult sponges (Box 4). Modified from Pineda et al. (2009).

Larvae of *Carteriospongia foliascens* have a short planktonic duration (up to 46 h from release), early competency for settlement (from 6 h from release) (Figure 6.1, Box 2), indiscriminate settlement to natural habitat cues (microbial biofilms) and high metamorphic success (Figure 6.1, Box 3). These larval behaviours were found to contribute to the high potential for endogenous recruitment (Fraschetti et al. 2003; Pineda et al. 2009). Interestingly, asexual reproduction (i.e. fission) was absent within this study, highlighting the significance of sexual reproduction, larval recruitment and post-settlement processes as primary sources of adult demographic variations (Figure 6.1, Box 4). The importance of sexual reproduction for recruitment, coupled with low larval dispersal potential, also

suggests a potential stock-recruitment relationship in *C. foliascens*. This stock-recruitment relationship links larval production, larval supply and recruitment, as seen in other benthic invertebrates with restricted larval dispersal (Gaines and Bertness 1992; Stoner 1992; Carlon 2002). A stock-recruitment relationship was most evident for the Juno Bay *C. foliascens* population (Chapter 5) which had increased adult sponge density, reproductive output and recruitment.

In addition to biological processes (i.e. reproduction and larval characteristics), habitat and environmental parameters such as bay size and hydrodynamics can also influence adult mortalities, growth and larval productivity (Figure 6.1, Box 4; Chapter 5). At Juno Bay, which possessed higher hydrodynamics (i.e. wave height), *C. foliascens* exhibited larger individuals with higher larval productivity (see Chapter 5). Despite population characteristics that should translate to high levels of recruitment, such as high reproduction, low larval dispersal (retention of larvae) and high metamorphic success, recruitment levels were relatively low (1 and 6 recruits/ 10 m^2 detected in two years, Little Pioneer Bay and Juno Bay respectively; Chapter 5) highlighting the skewed influence of post-settlement processes towards population demographics and distribution in *C. foliascens* at inshore GBR habitats. Nevertheless, survival rates of larvae reaching recruitment size in the field (ca. 2 years of age) are estimated to be 0.04 % at Little Pioneer Bay (larval productivity = 2330 larvae/ 10 m^2; recruit = 1/ 10 m^2) and 0.07 % at Juno Bay (larval productivity = 8870 larvae/ 10 m^2; recruit = 6/ 10 m^2), which are comparable to low survival rates from larval to juvenile stages reported for other sessile marine invertebrate taxa (< 90 %, Gosselin and Qian 1997).

The persistence and strict distribution of *Carteriospongia foliascens* to intertidal habitats of the inshore central Great Barrier Reef (GBR) is intriguing considering the broad depth occupancy (i.e. subtidal to mesophotic) of this species at other locations on the mid- and outer-shelf GBR (Wilkinson 1983, 1988; Bridge et al. 2011a; Chapter 2). Wilkinson and Evans (1989) proposed that light reductions to deeper habitats demarcated distributions of photosynthetic sponges (including *C. foliascens*) at offshore reefs of the GBR. Under this premise, it begs the question as to whether intertidal distributions at inshore reefs of the GBR are historically coherent or reflect a response to recent increase in sedimentation (thus increased turbidity and reduced light penetration) around the study area (Wachenfeld 1997; McCulloch et al. 2003; Uthicke 2012).

Other biological factors that potentially limit the distribution of *C. foliascens* in the subtidal are predation and competition. In the Caribbean, chemically undefended sponges are susceptible to predation by species of sea stars, fishes and turtles (Meylan 1985; Wulff 1995; Pawlik et al. 2013). In addition, competitive pressures for space by other co-occurring

benthic taxa such as macroalgae, scleractinian corals and heterotropic sponges can lead to mortalities of newly settled *C. foliascens* in the subtidal (Aerts 2000; González-Rivero et al, 2012). While no direct observations of predation or competition on *C. foliascens* have been reported, future investigations, through transplantation of individuals, both newly settled and adults, to the subtidal could reveal the vulnerability of these sponges to predation and competition in these habitats.

This study observed reductions in population fecundity for *C. foliascens*, associated to anomalous La Niña precipitation and temperature inflations (Chapter 3), and proposed a depression in levels of recruitment due to stress events (e.g. cyclones) occurring over the study period (Chapter 5). In other conspicuous benthic invertebrate taxa, such as scleractinian corals, disturbances such as cyclones, diseases and overfishing of herbivorous fishes reduce recruitment, depress growth rates and induce mortalities. These effects are largely due to the increase in frequencies and intensities of coral-algal interactions, which spurred the decline of coral reef resilience and health in the Caribbean (Mumby et al. 2007). However, high reproductive output, early onset of sexual maturity (within ca. 2 years), short planktonic duration, indiscriminate larval settlement to benthic cues and fast growth in the first two years of life are characteristics which can optimize overall fitness of *C. foliascens* in stochastic environments, and reflects a bet-hedging lifestyle in this species (Wilbur and Rudolf 2006; Crean and Marshall 2009; Olofsson et al. 2009). Bet-hedging is a risk spreading strategy achieved by reducing variations in overall fitness and has been reported in bacteria (Beaumont et al. 2009), plants (Wilbur and Rudolf 2006; Childs et al. 2010), invertebrates (Hopper 1999; Menu et al. 2000; Marshall et al. 2008) and vertebrates (Einum and Fleming 2004; Nevoux et al. 2010). Reduction in the variance of fitness can be achieved by adopting physiology, reproduction or behaviour that spreads risk over time or space (Hopper 1999; Marshall et al. 2008; Crean and Marshall 2009). The year round reproduction (Figure 6.1, Box 1) coupled with early and indiscriminate larval settlement to natal habitats in *C. foliascens* (Figure 6.1, Box 2 and 3) can minimize planktonic mortalities, and promote high settlement and post-settlement fitness when environmental conditions are favourable (Graham et al. 2008, 2013; Chapter 3 and 4). Additionally, fast growth from settlement to size at sexual maturity (Figure 6.2, Box 4) reduces the probability of mortality prior to sexual reproduction (Chapter 5). Considered together, these characteristics suggest that unless entire populations are decimated under periods of stress, local and rapid recovery can be facilitated by few remaining survivors and their prolific reproductive strategy (Underwood et al. 2009).

Carteriospongia foliascens may also attribute its success in an unpredictable intertidal environment to its high potential for morphological plasticity (Chapter 2). Sponge

species exhibit morphological plasticity, modifying individual morphologies to enhance survival in differing environmental conditions (Borchiellini et al. 2004; Carballo et al. 2006; Erpenbeck et al. 2006, 2012). The morphological plasticity of *C. foliascens* in response to light conditions (e.g. fan thickness) and sedimentation (e.g. raised oscules) may be responsible for the survival of this species at inshore reefs, through the optimisation of light acquisition for symbiont photosynthesis and the reduction of smothering of aquiferous systems (Chapter 2). While only morphological differences were measured, phenotypic plasticity could also involve variation in physiology, behaviour and phenology (Stearns 1989; Nussey et al. 2007). However, these effects were not confirmed in this present study. In addition to the phenotypic plasticity of the sponge host, phenotypic response of the symbiotic cyanobacteria in *C. foliascens* could also have contributed to the overall fitness of the holobiont, presenting an interesting scope for future ecological investigations for this species (Bennet and Bogorad 1973; Campbell 1996; Stomp et al. 2008).

The systematic and holistic methodologies adopted in this thesis, which covered all life history stages of *C. foliascens*, have proven to be critical in providing a comprehensive overview of the population dynamics of this species. Taken as a whole, this thesis contributes to an improved understanding of the systematics, spatial distribution, and pre- and post-settlement processes influencing population dynamics of *C. foliascens,* and serves as a platform for future efforts into the management and conservation of this species.

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Appendix 1

Appendix to Chapter 2

Supplementary Table 2.1: Character state matrix employed in the morphological cladistic analysis in this study. Characters and character states used correspond to those described in Table 2.2.

Sponge ID	Morphotype	Characters																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AM Z5027	1	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1
Davies 5	1	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1
Green B 5	1	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1
Green B 9	1	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1
WAM Z54096	1.1	0	4	0	0	0	1	0	0	1	0	0	0	1	1	1	1	1	2	1
WAM Z54121	1.1	2	4	0	0	0	1	0	0	1	0	0	0	1	1	1	1	1	2	1
WAM Z54131	1.1	0	4	0	0	0	1	0	0	1	0	0	0	1	1	1	1	1	2	1
WAM Z5755	1.1	1	4	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1	2	1
Green A 82	2	1	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 83	2	1	2	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 84	2	1	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 86	2	2	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 89	2	2	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green B 15	2	0	2	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green B 17	2	0	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green B 18	2	0	2	1	0	1	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green B 4	2	1	1	1	0	0	1	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 73	2.1	0	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 74	2.1	1	2	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 75	2.1	1	2	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 76	2.1	1	1	1	0	0	1	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 77	2.1	1	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 81	2.1	0	1	1	0	0	1	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 85	2.1	0	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 87	2.1	1	1	1	0	1	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Green A 88	2.1	0	1	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
WAM Z29265	2.2	0	3	1	0	0	1	0	1	1	0	0	0	1	1	2	0	0	0	0
WAM Z54023	2.2	0	3	1	0	0	0	0	1	1	0	0	0	1	0	2	0	0	0	0
WAM Z54029	2.2	2	2	1	0	0	1	0	1	1	0	0	0	1	0	2	0	0	0	0
WAM Z54138	2.2	2	3	1	0	0	0	0	1	1	0	0	0	1	1	2	0	0	0	0
Davies 11	3	0	2	0	0	1	0	0	2	1	0	1	0	2	1	2	1	1	1	2
Davies 3	3	0	2	0	0	0	1	0	2	1	0	1	0	0	1	2	1	1	1	2
Davies 8	3	0	2	0	0	0	1	0	2	1	0	1	0	0	1	2	1	1	1	2
Green B 10	3	2	2	0	0	0	1	0	2	1	0	1	0	2	1	2	1	1	1	2
Green B 11	3	0	3	0	0	0	1	0	2	1	0	1	0	2	1	2	1	1	1	2
Green B 12	3	0	2	0	0	0	1	0	2	1	0	0	0	0	1	2	1	1	1	2
Green B 13	3	1	3	0	0	0	1	0	2	1	0	1	0	0	1	2	1	1	1	2
Green B 14	3	0	2	0	0	0	1	0	2	1	0	1	0	1	1	2	1	1	1	2
Green B 16	3	2	2	0	0	0	1	0	2	1	0	1	0	2	1	2	1	1	1	2
Green B 6	3	2	2	0	0	0	1	0	2	1	0	1	0	0	1	2	1	1	1	2
Green B 7	3	0	2	0	0	0	1	0	2	1	0	1	0	0	1	2	1	1	1	2

Supplementary Table 2.1 continued

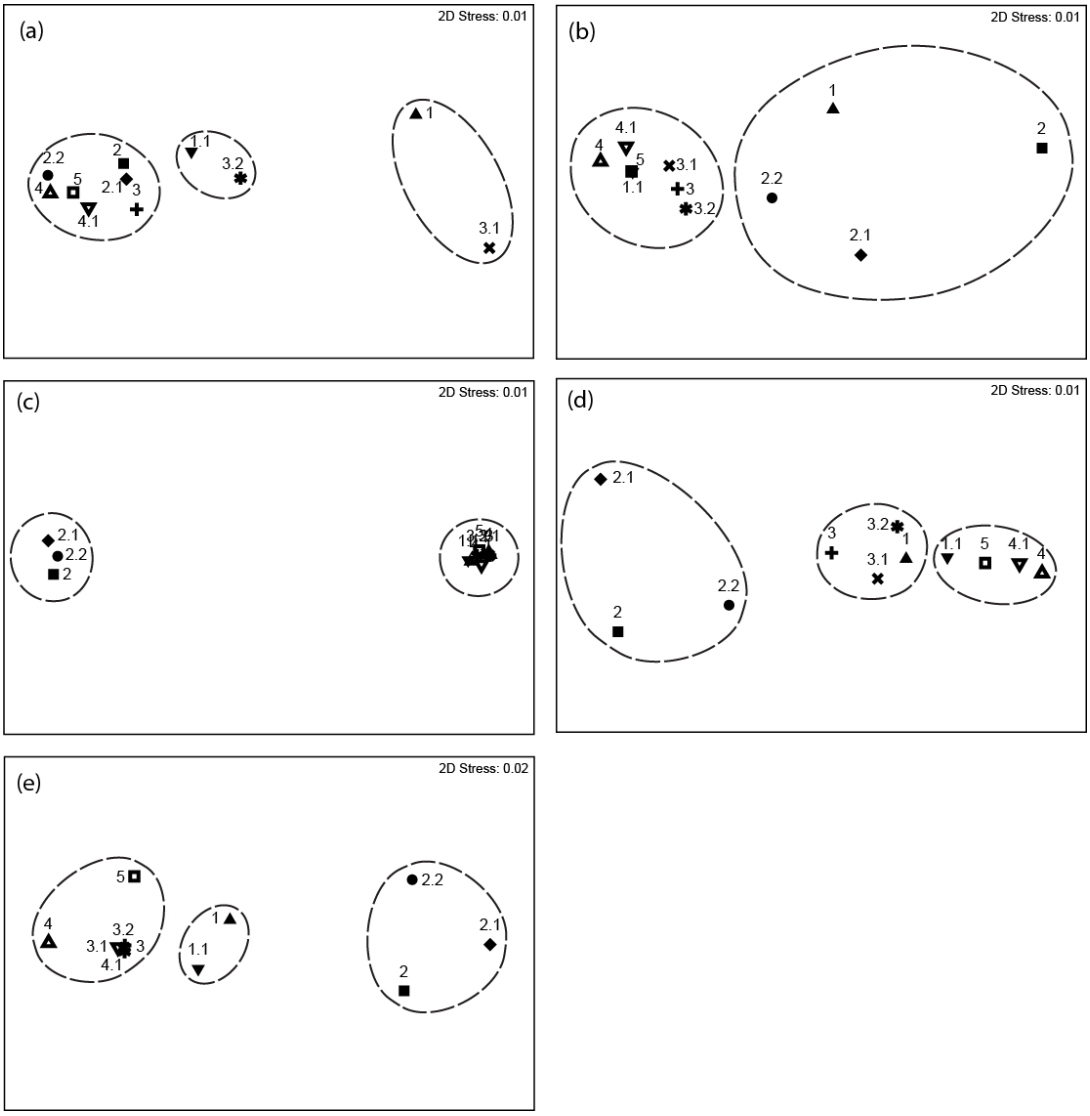
Sponge ID	Morphotype	Characters																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Green B 8	3	0	2	0	0	0	1	0	2	1	0	0	0	0	1	2	1	1	1	2
Juno 1	3.1	0	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 10	3.1	0	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 11	3.1	2	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 12	3.1	2	3	0	1	0	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 13	3.1	2	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 2	3.1	0	2	0	0	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 21	3.1	0	3	0	0	0	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 3	3.1	2	3	0	1	1	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Juno 30	3.1	1	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 4	3.1	1	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 5	3.1	0	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 6	3.1	2	3	0	1	1	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Juno 7	3.1	0	3	0	0	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Juno 8	3.1	1	3	0	1	0	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Juno 9	3.1	0	3	0	1	1	0	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 32	3.1	1	2	0	1	1	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 33	3.1	1	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Pioneer 34	3.1	2	3	0	0	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 35	3.1	0	3	0	1	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 36	3.1	1	3	0	1	1	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 37	3.1	0	3	0	1	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 38	3.1	0	3	0	1	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 39	3.1	1	3	0	1	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 40	3.1	0	3	0	1	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 41	3.1	1	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Pioneer 42	3.1	0	3	0	1	0	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Pioneer 43	3.1	1	3	0	1	1	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 44	3.1	0	3	0	1	0	1	0	2	1	0	1	0	2	0	0	1	1	1	2
Pioneer 45	3.1	0	3	0	1	0	1	0	2	1	0	1	0	0	0	0	1	1	1	2
Pioneer 48	3.1	2	3	0	1	1	1	0	2	1	0	1	0	0	0	0	1	1	1	2
WAM Z37657	3.1	0	2	0	0	1	1	0	2	1	0	1	0	0	1	0	1	1	1	2
WAM Z37658	3.1	1	2	0	0	0	0	0	2	1	0	0	0	0	1	0	1	1	1	2
WAM Z37667	3.1	0	3	0	0	0	1	0	2	1	0	1	0	2	1	0	1	1	1	2
Torres Strait 1	3.2	2	3	0	1	1	0	0	2	1	0	1	0	0	0	1	1	1	1	2
Torres Strait 10	3.2	2	3	0	1	1	1	0	2	1	0	1	0	2	0	1	1	1	1	2
Torres Strait 2	3.2	2	3	0	1	1	0	0	2	1	0	1	0	0	0	1	1	1	1	2
Torres Strait 3	3.2	2	3	0	1	1	1	0	2	1	0	1	0	0	0	1	1	1	1	2
Torres Strait 4	3.2	2	3	0	1	0	1	0	2	1	0	1	0	0	0	1	1	1	1	2
Torres Strait 5	3.2	1	3	0	1	0	1	0	2	1	0	1	0	2	0	1	1	1	1	2
Torres Strait 6	3.2	2	3	0	1	1	0	0	2	1	0	1	0	0	0	1	1	1	1	2
Torres Strait 7	3.2	2	3	0	1	1	1	0	2	1	0	0	0	0	0	1	1	1	1	2
Torres Strait 8	3.2	2	3	0	1	0	1	0	2	1	0	1	0	0	0	1	1	1	1	2
Torres Strait 9	3.2	2	3	0	1	1	1	0	2	1	0	1	0	0	0	1	1	1	1	2

Supplementary Table 2.1 continued

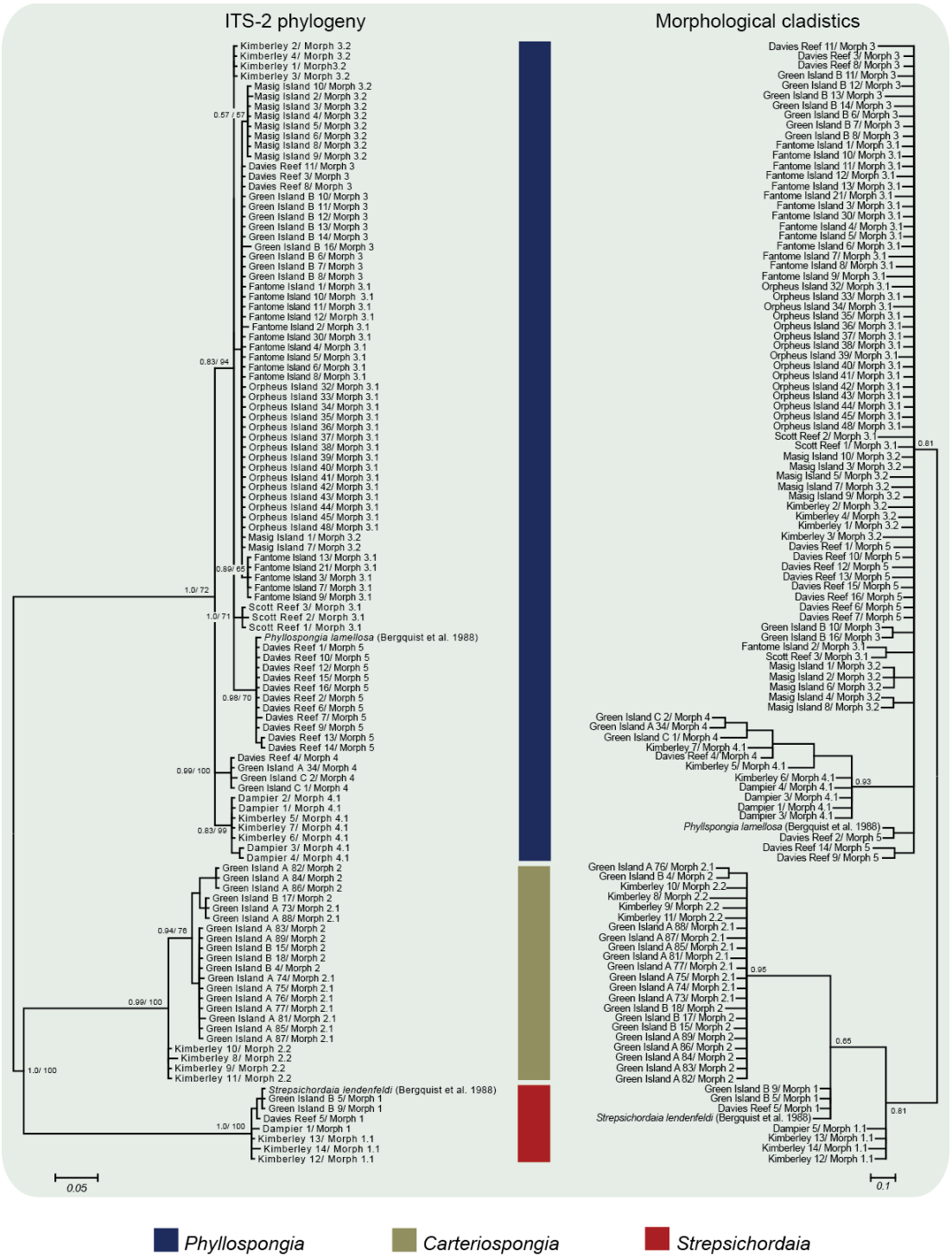
Sponge ID	Morphotype	Characters																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
WAM Z54006	3.2	0	3	0	0	1	1	0	2	1	0	1	0	2	0	1	1	1	1	2
WAM Z54008	3.2	2	3	0	0	1	1	0	2	1	0	1	0	2	0	1	1	1	1	2
WAM Z54270	3.2	2	3	0	1	1	1	0	2	1	0	1	0	2	0	1	1	1	1	2
WAM Z54282	3.2	2	3	0	0	0	1	2	2	1	0	1	0	2	0	1	1	1	1	2
Davies 4	4	2	0	3	0	0	1	2	0	0	0	1	0	2	1	2	1	1	2	2
Green A 34	4	2	0	3	0	0	1	1	0	0	0	2	0	2	1	2	1	1	2	2
Green C 2	4	2	0	3	0	0	1	1	0	0	0	2	0	2	0	2	1	1	2	2
Green C1	4	2	0	3	0	0	1	2	0	0	0	2	0	0	0	2	1	1	2	2
WAM Z3061	4.1	0	1	2	1	2	1	2	0	0	0	1	0	0	1	2	1	1	2	2
WAM Z3243	4.1	2	1	2	1	2	1	2	0	0	0	1	0	2	0	2	1	1	2	2
WAM Z3992	4.1	0	1	2	1	2	1	2	0	0	0	1	0	0	0	2	1	1	2	2
WAM Z54004	4.1	2	4	2	0	0	1	2	0	0	0	1	0	2	0	2	1	1	2	2
WAM Z5409	4.1	0	4	2	1	2	1	2	0	0	0	1	0	2	0	2	1	1	2	2
WAM Z54124	4.1	0	0	3	0	2	1	2	0	0	0	1	0	2	1	2	1	1	2	2
WAM Z54325	4.1	0	1	2	0	2	1	2	0	0	0	1	0	2	0	2	1	1	2	2
AM Z5021	5	0	1	0	1	0	1	0	0	1	0	1	0	0	1	2	1	1	2	2
Davies 1	5	0	4	0	1	1	0	0	0	1	0	1	0	0	1	2	1	1	2	2
Davies 10	5	0	4	0	1	1	1	2	0	1	0	1	0	0	1	2	1	1	2	2
Davies 12	5	2	4	0	1	1	0	2	0	1	0	1	0	0	1	2	1	1	2	2
Davies 13	5	2	4	0	1	0	1	0	0	1	0	1	0	0	1	2	1	1	2	2
Davies 14	5	1	4	0	1	1	1	2	0	1	0	1	0	2	1	2	1	1	2	2
Davies 15	5	2	4	0	1	1	1	2	0	1	0	1	0	0	1	2	1	1	2	2
Davies 16	5	0	4	0	1	1	1	0	0	1	0	1	0	0	1	2	1	1	2	2
Davies 2	5	0	1	0	1	0	1	0	0	1	0	1	0	0	1	2	1	1	2	2
Davies 6	5	0	4	0	1	1	1	2	0	1	0	1	0	0	1	2	1	1	2	2
Davies 7	5	0	4	0	1	1	1	0	0	1	0	1	0	0	1	2	1	1	2	2
Davies 9	5	1	4	0	1	1	1	2	0	1	0	1	0	0	1	2	1	1	2	2

Supplementary Table 2.2: List of significant morphological characters, summarized from Figure 2.7, for the rapid identification of foliose keratose sponge ESUs and sub-clades of the Australian tropics.

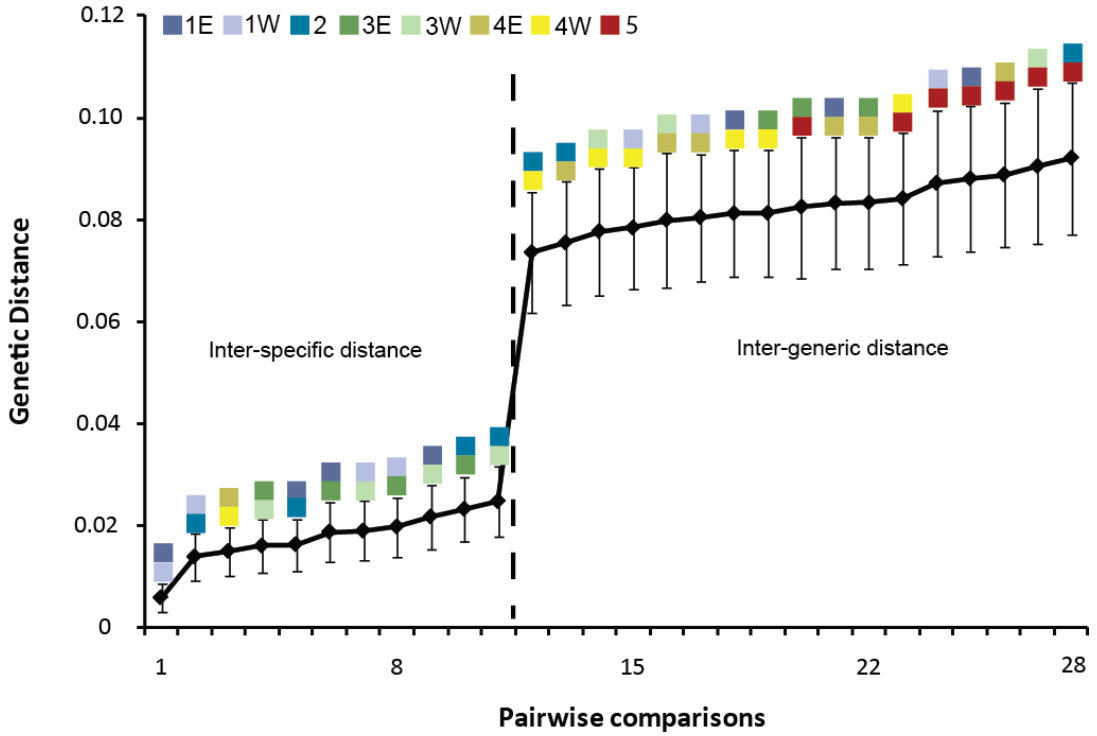
	Cluster	**I**					**II**		**III**
ID	Current taxonomy	*Carteriospongia foliascens*	*C. foliascens*	*Phyllospongia lamellosa*	*P. papyracea*	*P. papyracea*	*Carteriospongia flabellifera*	*C. flabellifera*	*Strepsichordaia lendenfeldi*
	Proposed new taxonomy	*Phyllospongia* sp.	*Phyllospongia* sp.	*P. lamellosa*	*P. papyracea*	*P. papyracea*	*Carteriospongia flabellifera*	*C. flabellifera*	*Strepsichordaia lendenfeldi*
	ESU	ESU 1	ESU 1	ESU 2	ESU 3	ESU 3	ESU 4	ESU 4	ESU 5
	Clade/ sub-clade	Sub-clade 1E	Sub-clade 1W	Clade 2	Sub-clade 3E	Sub-clade 3W	Sub-clade 4E	Sub-clade 4W	Clade 5
Morphological characters	Conule alignment	Longitudinal	Longitudinal	Longitudinal	Absent	Unclear	Longitudinal reticulate	Longitudinal reticulate	Longitudinal
	Sand cortex depth	Thin (63.38µm to 115.43µm)	Thin (63.38µm to 115.43µm)	Thin (63.38µm to 115.43µm)	Thin (63.38µm to 115.43µm)	Thin (63.38µm to 115.43µm)	Thick (159.23µm to 294.89µm)	Thin (63.38µm to 115.43µm)	Thick (159.23µm to 294.89µm)
	Mesh configuration	Irregular	Irregular	Irregular	Regular	Regular	Irregular	Irregular	Irregular
	Mesh Size	Small (176.09µm to 403.84µm)	Small (176.09µm to 403.84µm)	Small (176.09µm to 403.84µm)	Small (176.09µm to 403.84µm)	Small (176.09µm to 403.84µm)	Large (911.98µm to 987.31µm)	Large (911.98µm to 987.31µm)	Small (176.09µm to 403.84µm)
	Primary fibre configuration	Simple brushed	Simple brushed	Simple	Simple	Simple	Fasciculate	Fasciculate	Simple
	Primary fibre diameter	Medium (91.98µm to 108.32µm)	Medium (91.98µm to 108.32µm)	Small (53.77µm to 79.16µm)	Small (53.77µm to 79.16µm)	Small (53.77µm to 79.16µm)	Large (135.47µm to 168.57µm)	Large (135.47µm to 168.57µm)	Medium (91.98µm to 108.32µm)
	Secondary fibre composition	Sometimes cored	Sometimes cored	Sometimes cored	Never cored	Sometimes cored	Always cored	Always cored	Always cored
	Secondary fibre diameter	Small (28.87µm to 60.89µm)	Small (28.87µm to 60.89µm)	Small (28.87µm to 60.89µm)	Small (28.87µm to 60.89µm)	Small (28.87µm to 60.89µm)	Large (122.68µm to 139.30µm)	Large (122.68µm to 139.30µm)	Medium (122.68µm to 139.30µm)



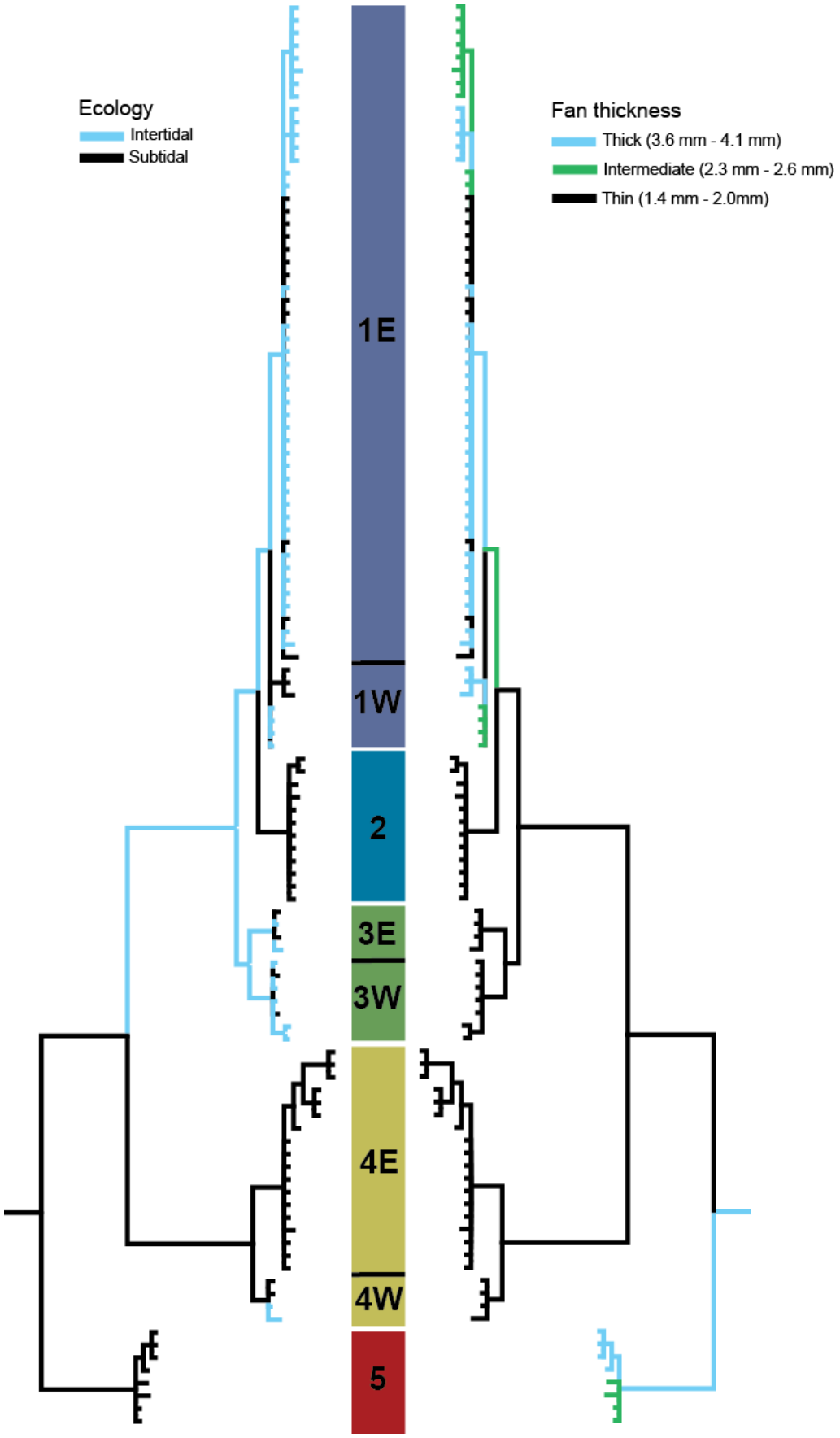
Supplementary Figure 2.1: MSDS of the 11 morphotypes with associated statistical groupings based on cluster analyses of a) fan thickness, b) sand cortex depth, c) primary fibre mesh size, d) primary fibre diameter and e) secondary fibre diameter.



Supplementary Figure 2.2: Midpoint rooted Bayesian phylogeny inferred from the ITS2 alignment and the morphological matrix. The morphological tree is congruent to the ITS2 tree at the genus level.



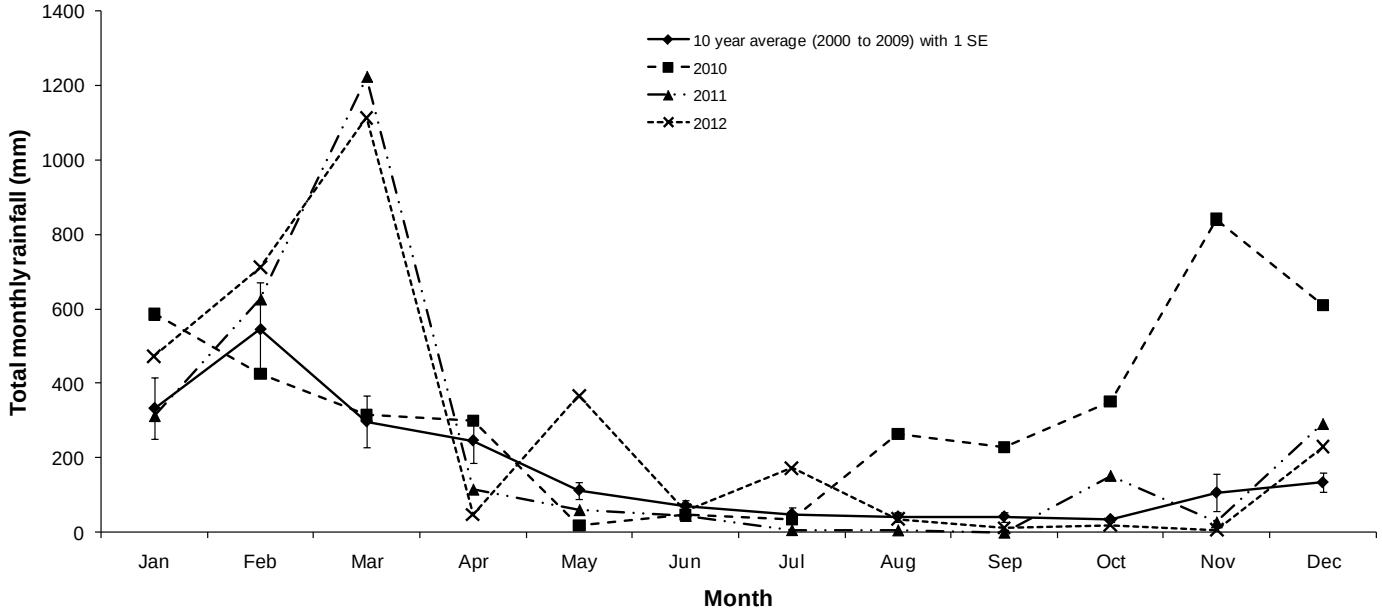
Supplementary Figure 2.3: Pairwise genetic comparisons amongst the five clades (with eastern and western sub-clades representatives included) recovered in this study supporting a genetic distance gap (dashed line) between inter-specific and inter-generic distances.



Supplementary Figure 2.4: Habitat reconstruction and corresponding fan thickness over the ITS2 Bayesian phylogeny. The middle bars represent ESUs recovered in this study. Only ESU 1 showed a clear relationship between the two characters whereby fan morphology was thicker in shallow depths.

Appendix 2

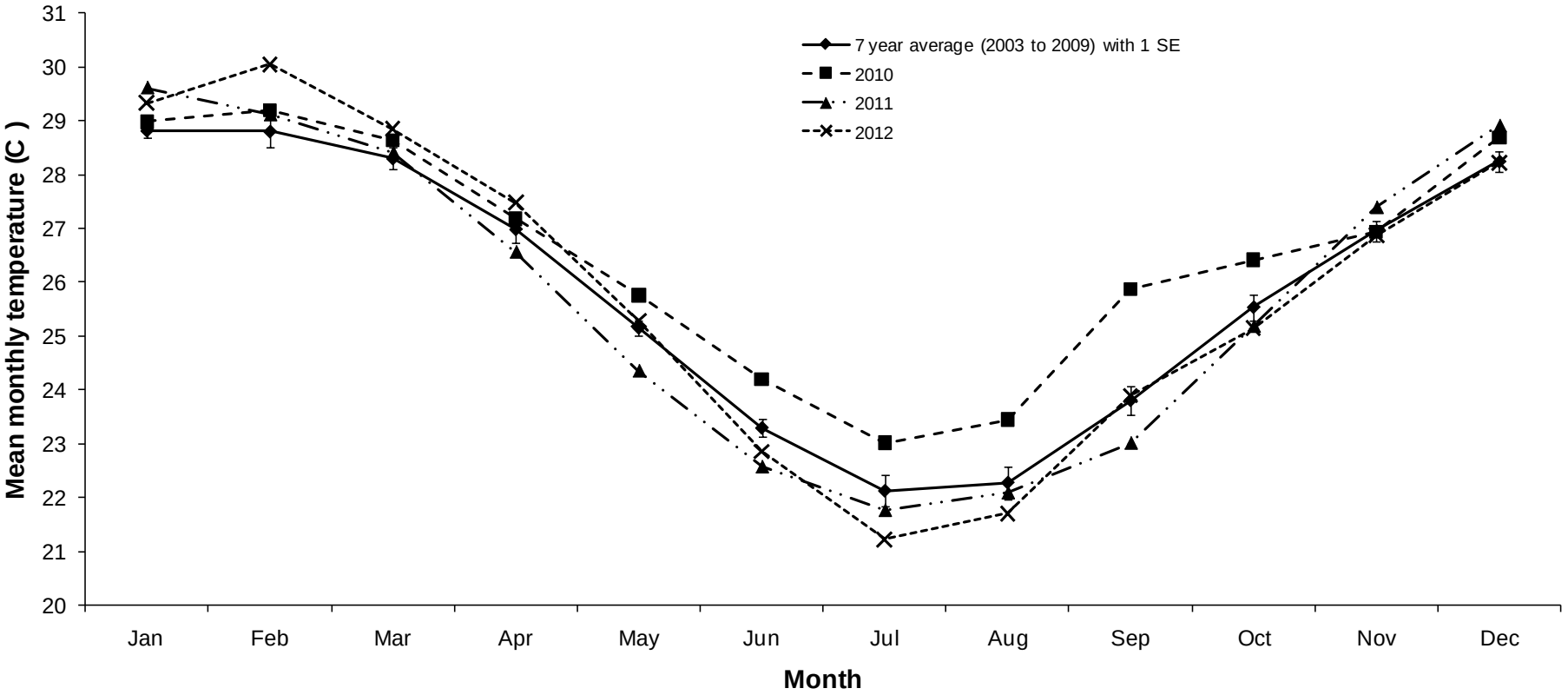
Appendix to Chapter 3



Supplementary Figure 3.1: Graph of total monthly rainfall (mm) for the study area over the period of this study (2010, 2011 and 2012). Average total monthly rainfall (± SE) at the study area over the last 10 years prior to the study (2000 to 2009) is also included to allow comparison of rainfall patterns. Total monthly rainfall between August and December 2010 was anomalously higher compared to the 10 year average. Precise increase of total monthly rainfall is summarized in Supplementary Table 3.1. Data was sourced from the Bureau of Meteorology (http://www.bom.gov.au).

Supplementary Table 3.1: Summary table of total monthly rainfall (mm) over the study area over the period of the study (2010, 2011 and 2012) and 10 year average total monthly rainfall (± SE) (2000 to 2009). % difference between each month of the study period to the 10 year average is also included. Highlighted cells (yellow) represents months whereby total monthly rainfall exceeds 300% increase when compared to the 10 year average.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
10 year average (2000 - 2009)	332.95	544.878	297.044	245.956	112.356	70.69	48.0889	41.3667	41.18	34.06	105.84	133.144
10 year SE (2000 - 2009)	82.3067	126.638	69.0899	59.2868	22.705	16.2702	17.1327	13.6242	13.5887	6.45819	50.2208	25.9139
n (months)	10	9	9	9	9	10	9	9	10	10	10	9
2010	586.1	426	314.7	300.6	18.7	48.2	34.6	263.2	228.2	351.7	869.8	611
2010 % diff from 10yr avg	76.0324	-21.817	5.94374	22.2172	-83.356	-31.815	-28.05	536.261	454.153	932.59	721.807	358.9
2011	312.8	625.7	1223.2	115.4	60.4	44.9	6.8	6.1	0	152.4	27.53	291.6
2011 % diff from 10yr avg	-6.052	14.8331	311.79	-53.081	-46.242	-36.483	-85.86	-85.254	-100	347.446	-73.989	119.01
2012	473	711.5	1113.5	47.4	366.6	57.6	172	34.9	12	18.8	6.8	230
2012 % diff from 10yr avg	42.0634	30.5797	274.86	-80.728	226.286	-18.517	257.671	-15.633	-70.86	-44.803	-93.575	72.7447



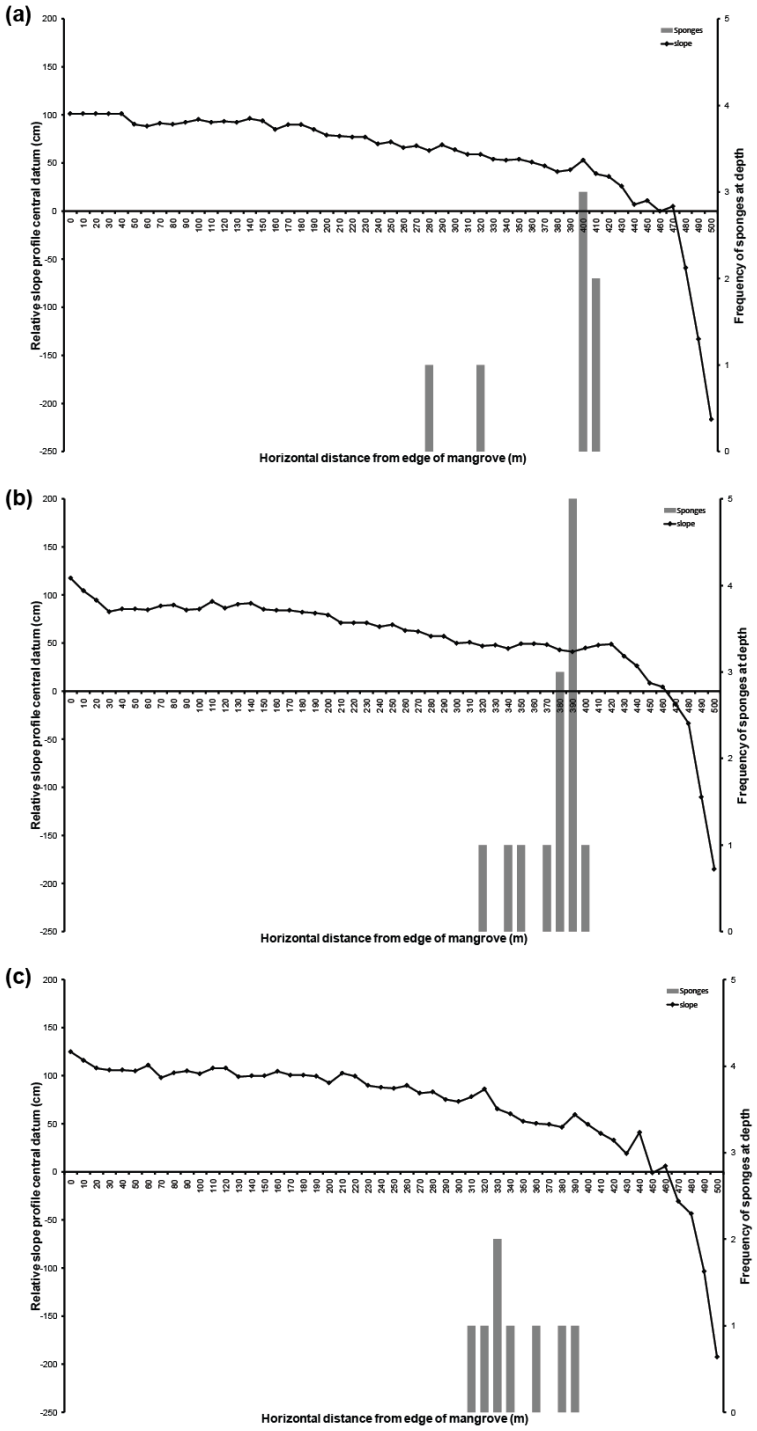
Supplementary Figure 3.2: Graph of mean monthly sea surface temperature (SST; °C) for the study area over the period of this study (2010, 2011 and 2012). Mean monthly SST (at 4 – 6m depth; ± SE) at the study area over the last 7 years prior to the study (2003 to 2009) is also included to allow comparison of SST patterns. Mean SST between May and October 2010 was anomalously higher compared to the 7 year average. Precise increase of mean SST is summarized in Supplementary Table 3.2. Data was sourced from the Australian Institute of Marine Science data centre (http://data.aims.gov.au).

Supplementary Table 3.2: Summary table of mean monthly sea surface temperature (SST; °C) over the study area over the period of the study (2010, 2011 and 2012) and 7 year average mean monthly sea surface temperature (± SE) (2003 to 2009). % difference between each month of the study period to the 7 year average is also included. Highlighted cells (yellow) represents months whereby mean monthly sea surface temperature exceeds 0.5°C difference when compared to the 7 year average.

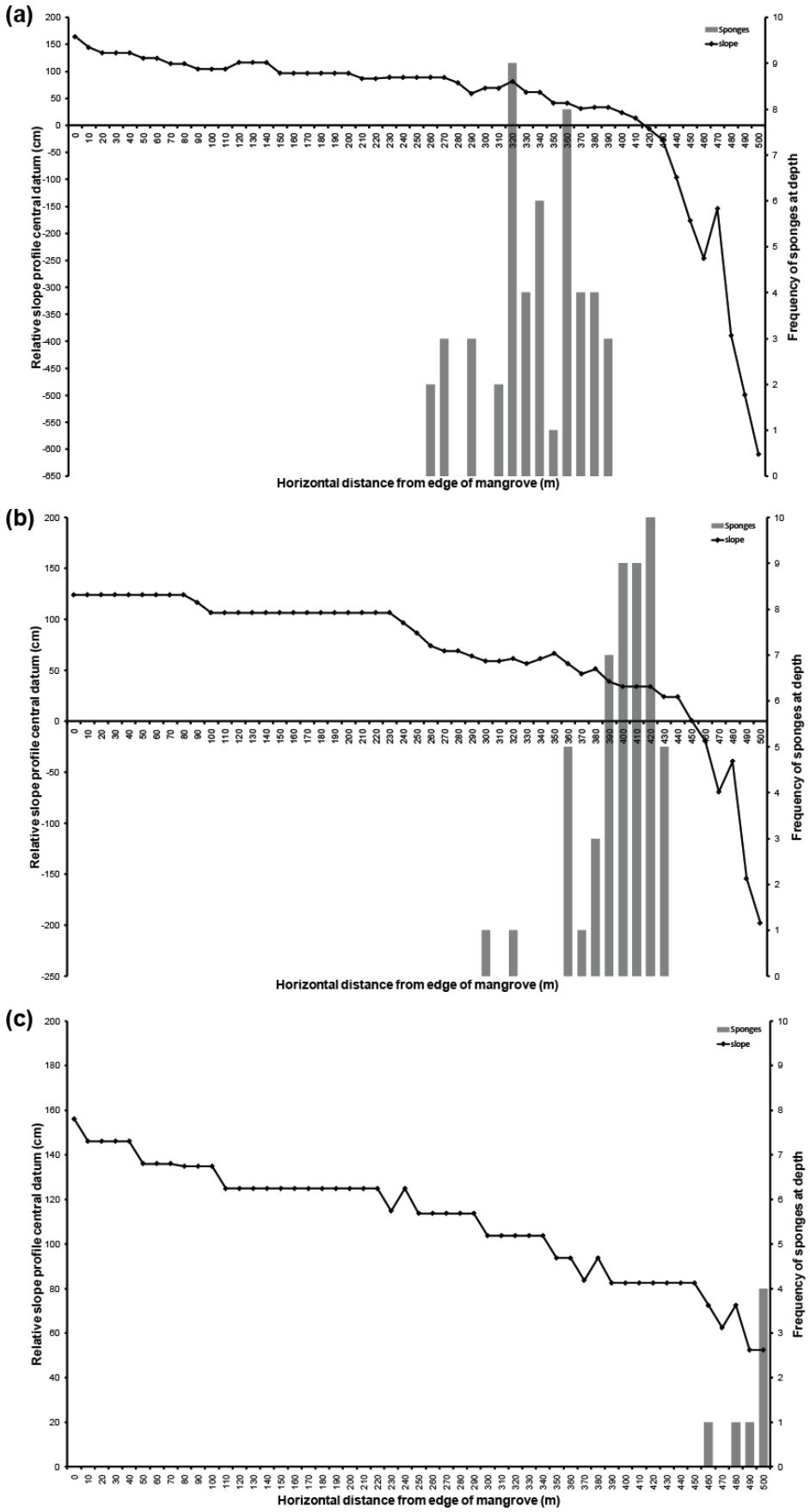
Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
7 year average (2003 to 2009)	28.819494	28.8045	28.3011	26.9846	25.1498	23.2907	22.12	22.2709	23.8027	25.5359	26.954	28.2443
7 year SE (2003 to 2009)	0.128765	0.28234	0.20856	0.24323	0.13579	0.16722	0.29132	0.29689	0.27023	0.2452	0.19808	0.18385
n	7	7	7	7	7	7	7	7	7	7	7	7
2010	28.981774	29.1911	28.63	27.18	25.761	24.197	23.017	23.446	25.878	26.417	26.933	28.687
2010 diff (C°) from 7 yr avg	0.1622799	0.38661	0.3289	0.19541	0.61121	0.90632	0.89698	1.17514	2.07534	0.88106	-0.021	0.4427
2010 % diff from 7 yr avg	0.5630907	1.3422	1.16216	0.72414	2.43028	3.89133	4.05506	5.27657	8.71893	3.45026	-0.0778	1.5674
2011	29.611	29.109	28.404	26.56	24.354	22.574	21.765	22.091	23.013	25.182	27.387	28.905
2011 diff (C°) from 7 yr avg	0.7915057	0.3045	0.1029	-0.4246	-0.7958	-0.7167	-0.355	-0.1799	-0.7897	-0.3539	0.43302	0.6607
2011 % diff from 7 yr avg	2.7464247	1.05712	0.3636	-1.5735	-3.1642	-3.0771	-1.605	-0.8076	-3.3175	-1.3861	1.60653	2.33924
2012	29.331	30.049	28.845	27.479	25.285	22.855	21.2166	21.7039	23.897	25.1461	26.8733	28.2223
2012 diff (C°) from 7 yr avg	0.5115057	1.2445	0.5439	0.49441	0.13521	-0.4357	-0.9034	-0.567	0.09431	-0.3898	-0.0807	-0.022
2012 % diff from 7 yr avg	1.7748601	4.3205	1.92185	1.83218	0.53762	-1.8706	-4.084	-2.546	0.3962	-1.5266	-0.2993	-0.0777

Appendix 3

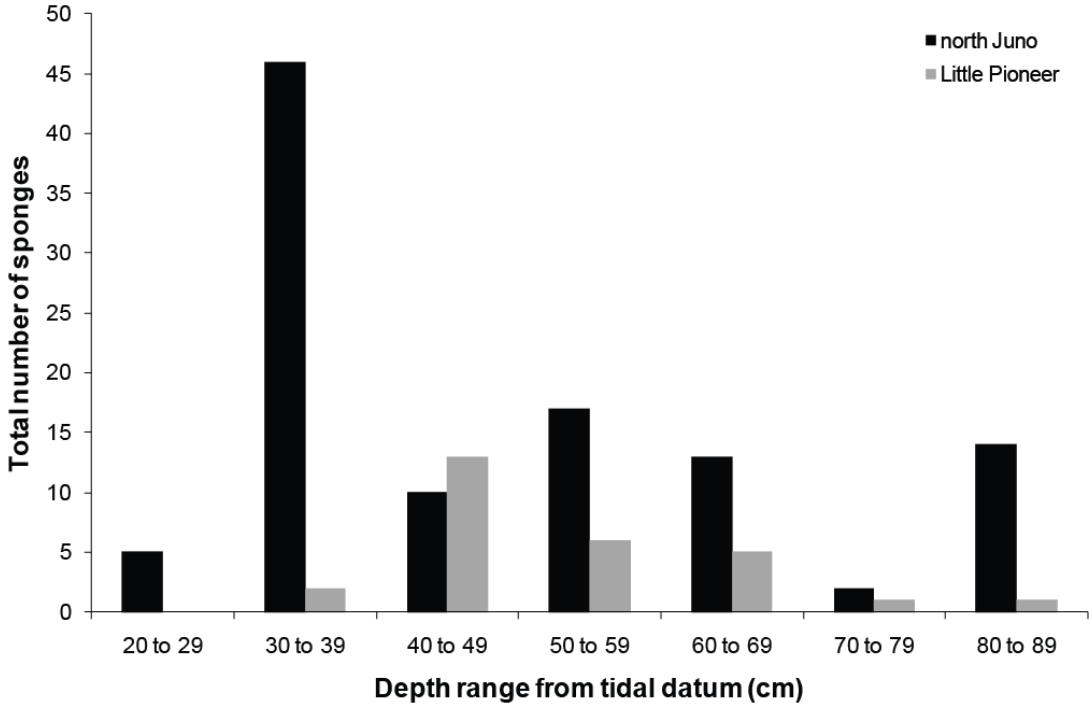
Appendix to Chapter 4



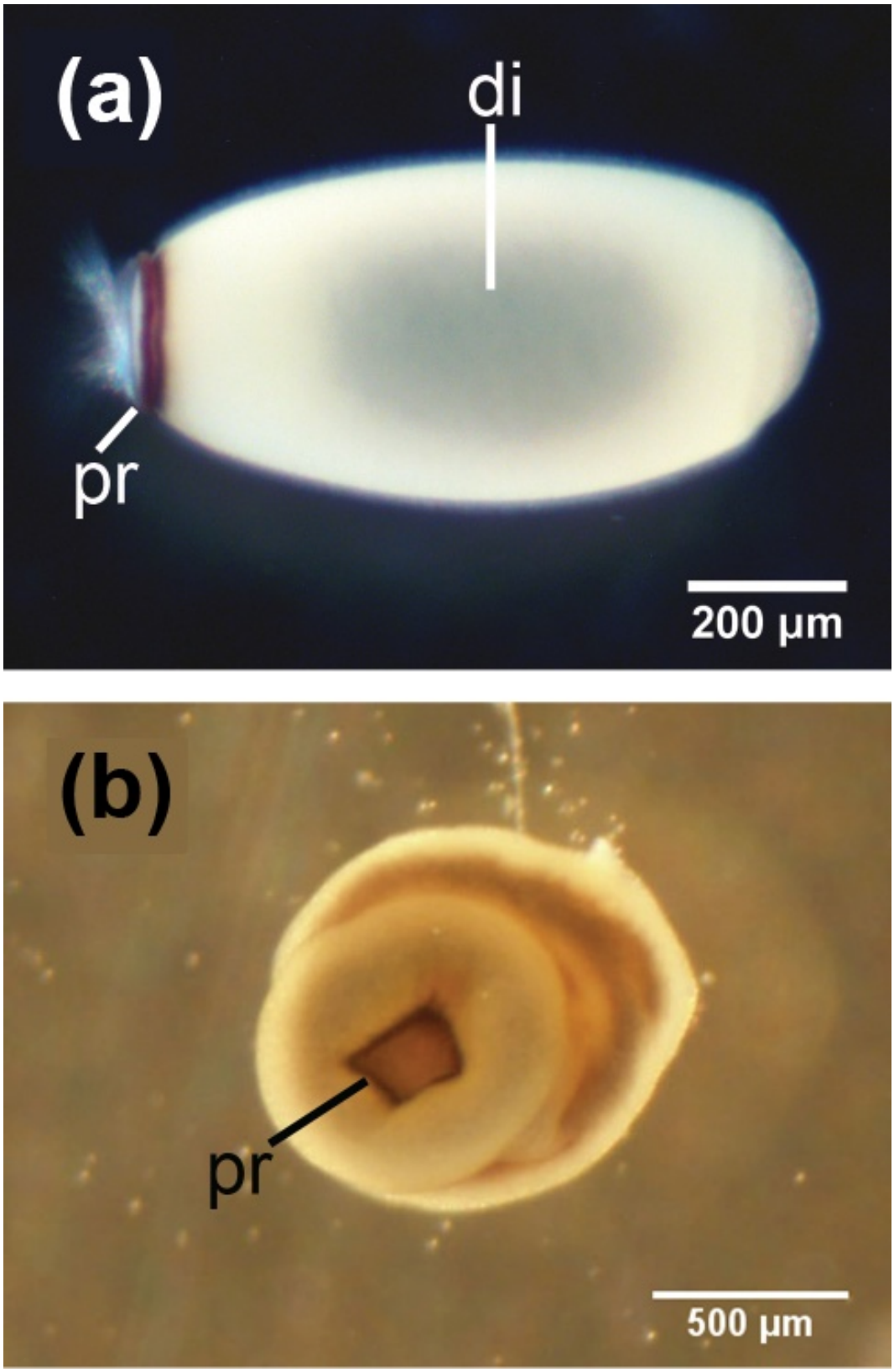
Supplementary Figure 4.1: *Carteriospongia foliascens*. Depth profile (line, cm) from the tidal datum (FTD) along a 500 m transect, and corresponding sponge abundance (grey bars, frequency) at every 10 m distance from the edge of mangroves at Little Pioneer Bay. A, B and C represents transects 1, 2 and 3 respectively.



Supplementary Figure 4.2: *C. foliascens*. Depth profile (line, cm) from the tidal datum (FTD) along a 500 m transect, and corresponding sponge abundance (grey bars, frequency) at every 10 m distance from the edge of mangroves at north Juno Bay. A, B and C represents transects 1, 2 and 3 respectively.



Supplementary Figure 4.3: *C. foliascens*. Sponge abundance (total number) at specific depth ranges for Little Pioneer Bay and north Juno Bay. Depth ranges excluded from this graph are those not supporting any sponges.



Supplementary Figure 4.4: *C. foliascens.* Micro-photographs showing (a) a typical larva with pigmented posterior ring (pr) and dark interior (di), and (b) a settled larvae with anterior end attached to the experimental substrate and undergoing metamorphosis, invagination occurs at the pigmented posterior ring (pr) and disappears when larva is engaged in metamorphosis, forming a distinct flattening of the posterior half to assume a dome-like morphology.

Appendix 4

Abdul Wahab MA, Fromont J, Whalan S, Webster N, Andreakis N (2014) Combining morphometrics with molecular taxonomy: how different are similar foliose keratose sponges from the Australian tropics? Mol Phylogenet Evol 73:23-39

Abdul Wahab MA, de Nys R, Webster N, Whalan S (2014) Phenology of sexual reproduction in the common coral reef sponge, *Carteriospongia foliascens.* Coral Reefs 1-14

Abdul Wahab MA, de Nys R, Webster N, Whalan S (2014) Larval behaviours and their contribution to the distribution of the intertidal coral reef sponge *Carteriospongia foliascens*. PLoS ONE 9(5):e98181

Abdul Wahab MA, de Nys R, Abdo D, Webster N, Whalan S (2014) The influence of habitat on post-settlement processes, larval production and recruitment in a common coral reef sponge. J Exp Mar Biol Ecol 461:162-172