GENETIC AND ENVIRONMENTAL BASIS FOR *SYMBIODINIUM* SPECIFICITY IN THE CORAL-DINOFLAGELLATE SYMBIOSIS

Thesis submitted by
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Abstract

The mutualism between scleractinian corals and the dinoflagellate genus *Symbiodinium* forms the nutritional basis for coral reef structure and growth, and contributes significantly to the physiology and resilience of the coral holobiont. Although the composition and diversity of *in hospite Symbiodinium* communities are known to vary among coral taxa, it is not yet clear what drives the formation of these communities. In particular, little is known about the full diversity of *Symbiodinium* communities in the early life stages of corals, their dynamics through time or the genetic contributions of coral hosts to these communities. Quantifying the diversity of *Symbiodinium* communities and the contribution of host genetics to their structure has broad implications for the capacity of these communities to undergo selection and therefore their adaptive potential. This thesis aims to deeply describe *Symbiodinium* communities in egg, larval, juvenile and adult life stages across a range of coral species, with a focus on quantifying this community as a continuous quantitative genetic trait to estimate host-symbiont heritabilities in corals with contrasting reproductive and symbiont transmission modes. Such knowledge is essential to evaluate the potential for adaptation and ecological rescue of coral populations though intervention strategies targeted at coral microbial communities, which have garnered substantial interest in recent years.

In order to more fully characterize *Symbiodinium* communities in corals, I used next-generation sequencing (NGS) and adapted bioinformatics tools to construct a novel pipeline that identifies and quantifies these symbionts. To convert high dimensional data into a quantitative genetic (QG) trait for heritability analysis, I adopted tools developed from mathematical ecology theory that incorporate presence/absence, abundance, sequence divergence and rarity into a single metric. Unlike conventional methods for genotyping *Symbiodinium*, this NGS method is able to detect community members at very low abundance to the type or intra-type level, which has previously only been done on a limited number of coral species and never in their early life-history stages. These methodologies were used to calculate Bayesian heritability (h^2) estimates for three coral species that represent vertical (maternal transfer) and horizontal (environmental acquisition) symbiont transmission strategies (Chapters 2 and 3).

Vertical transmission of *Symbiodinium* communities is widely assumed to have high fidelity in brooding coral species that transmit *Symbiodinium* directly from parent

to offspring. However, using brooded larvae with known parentage, I show that planulae of a vertically-transmitting, cryptic species of *Seriatopora hystrix* harbour novel diversity not found in adult colonies (Chapter 2). Moreover, the *Symbiodinium* community was found to be only 33% heritable (h^2, Bayesian narrow-sense heritability). I also found significant micro-scale spatial variation in the diversity of *Symbiodinium* communities associated with adult corals, further suggesting that substantial symbiont flexibility exists in vertically-transmitting, brooding corals across multiple life stages. These results overturn the paradigm that *Symbiodinium* communities in brooding corals are exclusively vertically transmitted, and instead suggest a new mixed-mode transmission strategy that is more in line with symbiosis models in other invertebrate groups. Results also highlight the potential for selection and adaptation of the symbiont community in corals sharing this transmission strategy, and their potential amenability to microbial intervention strategies, such as assisted evolution.

In contrast to brooding corals, *Symbiodinium* communities associated with the majority of broadcast spawning corals are acquired horizontally from the environment and are assumed to have low fidelity. However, QG heritability estimates calculated for juveniles of the broadcast spawning coral *Acropora tenuis* were greater ($h^2 = 0.36$) than expected for a horizontally transmitting corals using two methods: 1) regression-based estimation, and 2) Bayesian linear mixed model estimation (Chapter 3). In comparison, heritability of *Symbiodinium* communities in the broadcast spawning coral *Montipora digitata,* which transmits *Symbiodinium* from maternal parent to eggs, was higher ($h^2 = 0.57$), although still not as high as expected for a vertically-transmitting coral. Both *A. tenuis* and *M. digitata* contained novel core, common and rare *Symbiodinium* types not previously documented in these species. These findings suggest that coral species with contrasting *Symbiodinium* transmission strategies influence the uptake of their symbiont communities through the transmission of specific genetic architecture from one generation to the next. At the same time, the presence of novel types in juveniles and eggs underscores a degree of flexibility in symbiotic associations for these species.

The presence of novel *Symbiodinium* diversity in the three coral species studied here does not necessarily imply that they are functionally relevant to the host. To assess the ecological significance of novel community structures found in preceding chapters and test if variability among *Symbiodinium* communities translates to differences in fitness outcomes, as would be expected from a heritable trait, the fates of *A. tenuis* juveniles were monitored and compared among families. *Symbiodinium* communities

hosted by juveniles differed significantly between high- and low-surviving families for all three measures quantified: symbiont taxonomic richness, identity and relative abundance (Chapter 4). Results suggest a selective advantage associated with harbouring a specific *Symbiodinium* community, and highlight *Symbiodinium* type A3 as a potentially key symbiont partner for this early life stage in *A. tenuis*. Parental identity also significantly affected larval weight, settlement success and juvenile survival. These results link substantial heritability estimates to differential fitness outcomes in juveniles, indicating that maternal colony identity can be an important driver of population demographic processes in coral populations.

Marine sediments are one of the most important reservoirs of *Symbiodinium* diversity for uptake by immature corals, and their importance is further implicated by the substantial contributions that environmental influence had on QG heritability estimates in the three study species. However, little is known about the biogeography of free-living *Symbiodinium* across environmental gradients. Deep sequencing of symbiont communities in marine sediments collected from eight sites along a temperature and water-quality gradient revealed substantial diversity and biogeographical partitioning of *Symbiodinium* types (Chapter 5). Juveniles of *Acropora tenuis* and *Acropora millepora* exposed to sediments took up distinct communities, compared both to each other and to symbiont availability within sediments. Significant differences in photochemical efficiency, growth and survival of juveniles were also attributed to symbionts acquired over the 145 days of sediment exposure. Variability in *Symbiodinium* type distributions among these reef habitats could be attributed to significant differences in sediment size classes, total organic nitrogen, and trace metals (al and fe) among sites. Results highlight spatial variability in the distribution of *Symbiodinium* types, and demonstrate that juvenile corals are selecting a relatively small and specific community from a large diversity of available types, whilst also supporting flexibility in the relationship dependent on external environmental conditions.

In summary, this thesis presents the first comprehensive appraisal of genetic and environmental influences governing *Symbiodinium* communities across all major reproductive and symbiont transmission modes in corals. A new mixed mode model of transmission in vertically transmitting corals was discovered, as well as novel diversity across eggs, planulae, juveniles and adults in three important coral species, and also across a range of sediment habitats. Results highlight the substantial adaptive potential of the symbiont community to rescue reefs from adverse climatic changes.

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1.1 The coral holobiont

Scleractinian corals are referred to as holobionts because of the intimate nature of symbioses among corals, dinoflagellates in the genus *Symbiodinium,* bacteria and other microbial partners (Rohwer et al., 2002; Thompson et al., 2015). The endosymbiont *Symbiodinium* has long been recognised as a key partner in the holobiont, enabling corals to build reefs. Indeed, the formation and long-term survival of coral reef habitats worldwide are predicated on the obligate symbiosis between these photosynthetic dinoflagellates and their coral hosts. In particular, calcification is enhanced in the presence of photosynthetically active radiation, which is absorbed by *Symbiodinium* cells living within coral tissues and converted into metabolic products, some of which are translocated to their hosts, enabling them to produce calcareous skeletons, respire, grow and reproduce (Iluz and Dubinsky, 2015). *Symbiodinium* also produce metabolites that influence the structure of bacterial communities associated with many cnidarian species (Bourne *et al.*, 2013), further contributing to coral health and nutrition (Thurber *et al.*, 2009). Microbes (including *Symbiodinium)* also have the potential to shape the abundance and diversity of coral hosts at higher spatial scales through differential impacts on growth and mortality. For example, the early and specific acquisition of *Symbiodinium* by coral larvae and juveniles increases juvenile survivorship and growth (Graham *et al.*, 2013; Suzuki *et al.*, 2013), further highlighting the key role that symbiont communities have in coral health and survival.

1.2 Dinoflagellate symbioses and *Symbiodinium* functional diversity

S*ymbiodinium* provide essential nutrients to their hosts (e.g. photosynthetically fixed carbon in the form of glycerol, glucose, and alanine amongst others) (Muscatine *et al.*, 1984; Davy *et al.*, 2012), with recent evidence of substantial nutrient transfer and host utilization at the subcellular level elucidating the fundamental nature of this association (Kopp *et al.*, 2015; Pernice *et al.*, 2015). Nine different clades of *Symbiodinium* are currently recognized (A-I), with hundreds of distinct types within these clades (Pochon and Gates, 2010; Pochon *et al.*, 2012). New molecular tools have led to the recognition that deeper taxonomic levels beyond the clade level (i.e., the type or sub-type level) have profound effects on host health. This is not surprising given the coarse level of the clade designation, which may more appropriately describe taxonomic differences at the Order or Family level (Rowan and Powers, 1992; LaJeunesse, 2001). Type level diversity impacts both *Symbiodinium* and host physiology, and even types

within the same clade differ in a range of physiological characteristics (types distinguished based on sequence variation in ITS-2). For example, type C3 (*S. thermophilum*), A1, A20, D_{Ber06} and F2 have high thermal tolerance, but types A_{Mie09} and D_{Abr08} do not (Sawall *et al.*, 2014; Hume *et al.*, 2015; Swain *et al.*, 2016). Infectiousness and uptake in early life-history stages of corals also varies amongst types, with types A3 and D1-4 exhibiting high infectivity compared to type A2 (Kuniya *et al.*, 2015). Variability among clades and among types also exists for: carbon fixation and transfer rates (Cantin *et al.*, 2009), metabolite classes produced and their abundances (Klueter *et al.*, 2015), transcriptional profiles (Parkinson *et al.*, 2016) and bacterial associations in coral juveniles (Littman *et al.*, 2009). Clade and type identity also impacts a range of coral host traits (reviewed in: van Oppen *et al.*, 2009). Significantly, such variability can influence host responses to environmental stressors.

Large scale phylogenetic analyses, combined with biological and ecological information for clades B, C and D, and also including information at the within-type level, like C3, confirm that type and sub-type diversity are the relevant units of investigation (LaJeunesse *et al.*, 2014; Thornhill *et al.*, 2014; Parkinson *et al.*, 2015). Single base pair differences in key genotypic regions (e.g., intragenomic spacer region-2) can be the sole difference between important taxonomic entities, for example between a new thermally tolerant C3 type (*S. thermophilum*) and the ubiquitous C3 type (Hume *et al.*, 2015). Although the multicopy nature of *Symbiodinium* genomes and the presence of intragenomic variants make taxonomic assignments for distinct *Symbiodinium* sequences difficult (Figure 1.1), advances have been made in naming and elucidating the functional diversity within *Symbiodinium* (Wham *et al.*, 2011; LaJeunesse *et al.*, 2014; Thornhill *et al.*, 2014; Parkinson *et al.*, 2015). Furthermore, microsatellite and deep sequencing studies suggest that the functional level of diversity for *Symbiodinium* may be at the intra-type level (Howells *et al.* 2012; Lajeunesse *et al.* 2012; reviewed in Parkinson & Baums 2014). In this thesis, I will therefore use the following terminology: clades to represent the genus level, types to represent the species level, sub-types to represent the strain level and Operational Taxonomic Units (OTUs) to represent the genotype level. In particular, I focus on these last two key functional levels of interest.

Progress in surveying the diversity of *in hospite Symbiodinium* communities has been ongoing since at least the early 1980's (Trench *et al.*, 1981), although a majority of genotyping has only been done to the clade level. Therefore, there is a large gap in

knowledge of *Symbiodinium* diversity at the functional type or subtype levels. Only with the advent of deep sequencing, microsatellite loci and qPCR has diversity that was missed by earlier methodologies (Kennedy *et al.*, 2015) begun to be captured (Arif *et al.*, 2014; Green *et al.*, 2014; Quigley *et al.*, 2014; Thomas *et al.*, 2014; Davies *et al.*, 2016). Currently, very few studies have characterized *Symbiodinium* diversity in the early life-history stages of corals, and no studies have surveyed the functional type level of diversity. To date, the majority of studies have not quantified the comparative abundances of types within *Symbiodinium* communities, although it is now known that relative abundances of certain symbiont types and fine scale dynamics among types can predict bleaching outcomes and tolerances to stress events (Berkelmans and van Oppen 2006; Jones *et al.*, 2008; Cunning and Baker, 2014; Cunning *et al.*, 2015; Bay *et al.*, 2016). Therefore, there are considerable gaps in understanding the functional level of diversity and abundance for both the adult and juvenile life-history stages of corals.

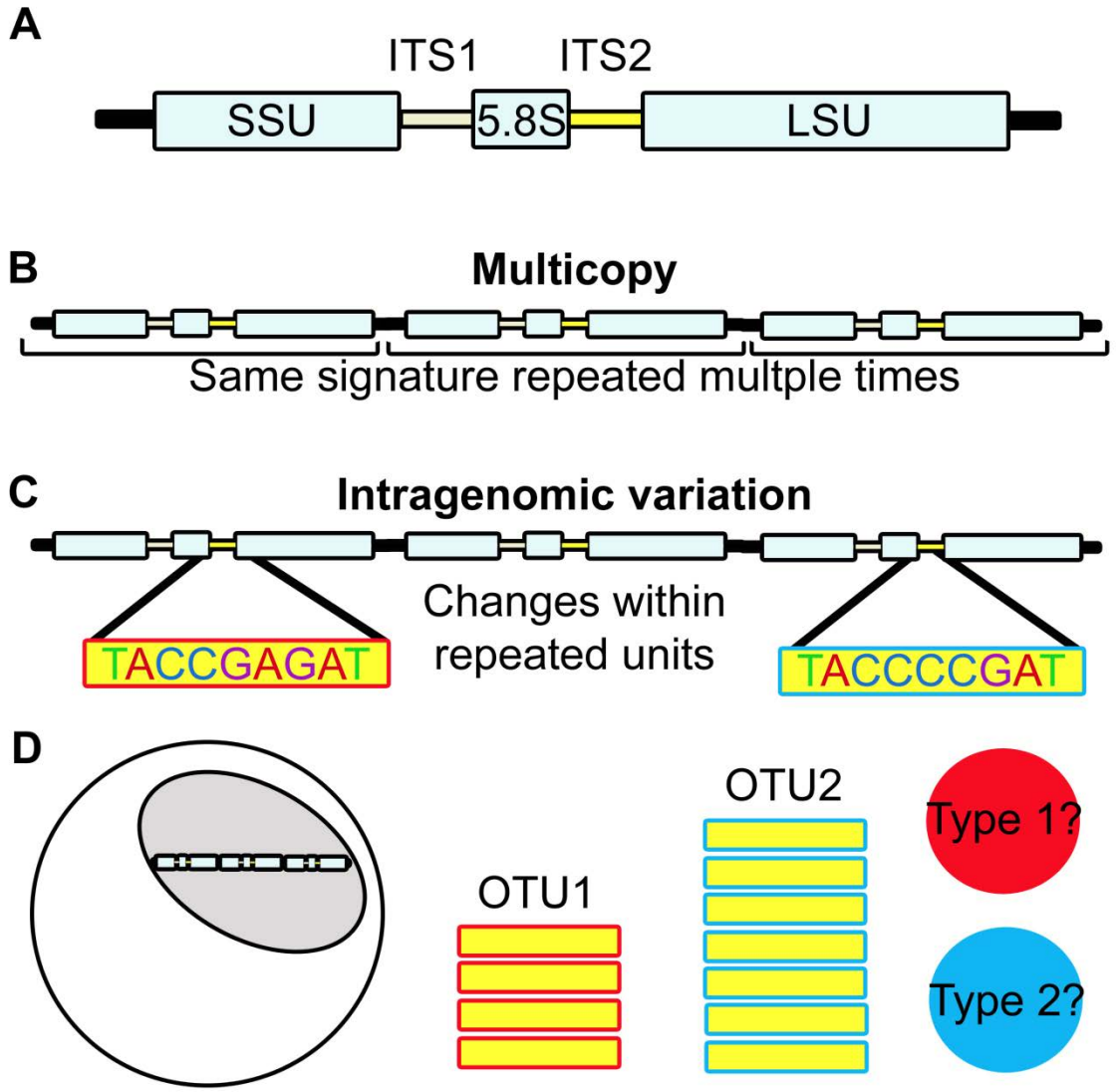

Figure 1.1 The multicopy, intragenomically variable genome of *Symbiodinium*. A) Structure of the SSU-LSU region found in the nuclear genome of Symbiodinium. Common marker genes include SSU, 5.8S and LSU genes (light blue bars), including the hypervariable internal transcribed spacer regions 1 and 2 (ITS-1 and ITS-2; grey and yellow bars). The ITS-2 region is the marker most commonly used for Symbiodinium genotyping and was used in this thesis. B) Multicopy regions are common across many Symbiodinium types and are formed when gene regions are duplicated multiple times in tandem. qPCR studies of single types suggest variation in copy number can range from 900 to over 3000 copies per cell for ITS-1 (Mieog et al. 2007). C) Intragenomic variants are formed when base pair changes, indels, transitions and transversions create variations in sequence identity from one copy to the next (ITS-2 region represented by a yellow box outlined in red compared to ITS-2 region represented by a yellow box outlined in blue). D) Genotyping a region with multicopy, intragenomic variants may result in the retrieval of multiple sequences of one identity (red ITS-2) compared to another (blue ITS-2), each with differing abundances. Therefore, although a single Symbiodinium cell was genotyped (white cell), the end result appears to be two distinct types (red Type 1 cell and blue Type 2 cell), with differing abundances.

1.3 Threats to coral reefs

Coral reef productivity enables millions of people worldwide access to critical resources, including food, income generation through tourism and fishing, and storm protection (Moberg and Folke, 1999). However, reef health is in serious decline as a consequence of increasing sea surface temperatures and acidification (Hoegh-Guldberg et al., 2007), diseases (Harvell *et al.*, 2007), bleaching caused by thermal stress, cyclones, and predation by crown-of-thorns seastars (Bruno *et al.*, 2007; Eakin *et al.*, 2010; De'ath *et al.*, 2012). Mass mortality events, selective mortality of certain species, and/or shifts to a new stable state of algal-dominated reefs (Hughes, 1994; Baker *et al.*, 2008; Mumby, 2009; Pandolfi *et al.*, 2011) are increasing in frequency. In only the last 100 years, ocean temperatures have increased by 0.74°C and pH has decreased by 0.1 unit (IPCC, 2007), causing a range of detrimental effects on coral reefs, particularly extreme and widespread bleaching events (Baker *et al.*, 2008). The early life-history stages of corals have been found to be particularly sensitive to changes in environmental parameters (i.e. water quality and temperature: (Graham *et al.*; Fabricius, 2005; Negri and Hoogenboom, 2011; Chua *et al.*, 2013; Humanes *et al.*, 2016).

In recent decades, bleaching has been estimated to account for ~10% of coral mortality on the Great Barrier Reef (GBR) and 11- 51.5% of mortality in the Caribbean (Gardner *et al.*, 2003; Wilkinson *et al.*, 2008; De'ath *et al.*, 2012), and this proportion is projected to increase as sea temperatures continue to rise globally, making these events more frequent and severe. Often, the loss of a large proportion of the *Symbiodinium* community from coral host tissues (defined as bleaching) is a precursor to host mortality, making it a key phenomenon to understand for predicting reef health (Weis, 2008; Ainsworth et al., 2011). Substantial work has been done to identify the physiological causes of bleaching, and to identify the characteristics that make certain species vulnerable and others resilient (e.g., Loya *et al.* 2001; van Woesik *et al.* 2011). The scope and frequency of bleaching is in part determined by phenotypic variation in characteristics that enable adaptation and/or acclimatisation of the coral host (Baird *et al.*, 2009a) and/or *Symbiodinium* (Buddemeier and Fautin 1993). The identity of *Symbiodinium* hosted has long been implicated as a dominant factor in bleaching risk (Glynn *et al.*, 2001; Baird *et al.*, 2009a; Abrego *et al.*, 2012; Cunning *et al.*, 2015). Recent molecular work has elucidated that bleaching caused by heat and light stress is predominantly the result of host regulated exocytosis of *Symbiodinium* cells and not host-cell apoptosis, *Symbiodinium* cell apoptosis, *Symbiodinium* degradation, or

exocytosis of the host cell surrounding the symbiont cells (symbiosome vacuole) (Bieri *et al.*, 2016). *Symbiodinium* expulsion as the primary cause of bleaching suggests that it is host derived mechanisms used to regulate symbiont density (Baird *et al.*, 2009a), especially as a majority of those cells expelled are intact and healthy (Bhagooli and Hidaka, 2004; Bieri *et al.*, 2016). Further work is needed to determine the role as well as origin (host or symbiont) of these apoptotic pathways as the formation, maintenance and breakdown will no doubt inform as to the flexibility of the symbiosis. Therefore, understanding the flexibility of the coral-*Symbiodinium* partnership, and what impact genetic and environmental constraints have on its establishment in early life-history stages is key to assessments of bleaching risk on reefs.

1.4 Coral reproduction and symbiont acquisition in the early life-history stages of corals

Scleractinian corals have two predominant modes of reproduction: broadcast spawning of eggs and sperm for external fertilization, or internal fertilization and brooding of planula larvae (Baird *et al.*, 2009b) (Figure 1.2). The majority (~63%) of corals worldwide are hermaphroditic broadcast spawners; in comparison, ~14% of species are either gonochoric or hermaphroditic brooders (Baird *et al.*, 2009b). Broadcast spawning corals release positively buoyant gamete bundles into the water column, followed by fertilization and development of larvae at the sea surface (Babcock *et al.*, 1986). Typical of marine broadcast spawning species, the initial mortality of coral gametes and larvae is very high (Graham *et al.*, 2008). Larvae that do survive are competent to settle four to five days post-fertilization (Babcock and Heyward), and depending on the species, can remain in this life-stage for up to 244 days (Graham *et al.*, 2008, 2013). Settlement of larvae can occur within a week post fertilization, triggered by chemical cues from the reef benthos (i.e., crustose coralline algae, Babcock and Heyward; Heyward and Negri, 1999; Davies *et al.*, 2013), with larvae then going on to metamorphose into juvenile corals. In brooding species, fertilization is internal, either through selfing (Brazeau *et al.*, 1998) or fertilization from sperm taken-up from neighbouring colonies (Maier, 2010; Warner *et al.*, 2016). Reproductive events for brooding species are generally more frequent than for broadcast spawners, with larval release occurring monthly compared to just one or two events per year (Jokiel *et al.*, 1985; Babcock *et al.*, 1986).

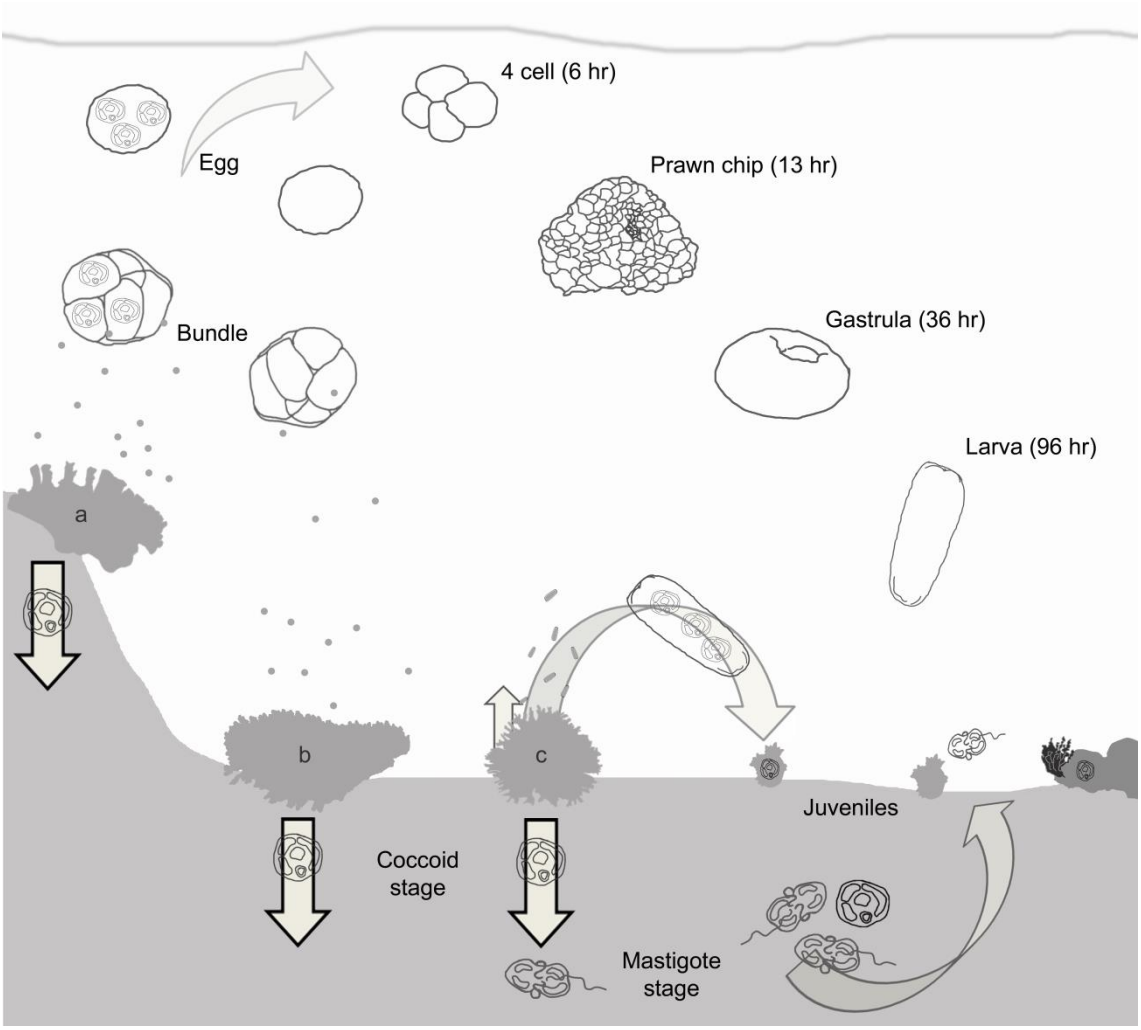

Figure 1.2 Schematic overview of patterns in the sexual reproduction of scleractinian corals (based on Ball et al. 2002; Baird et al. 2009b), modes of *Symbiodinium* transmission (Baird et al. 2009b), and aspects of the *Symbiodinium* life cycle (Freudenthal 1962; Fitt & Trench 1983; Yamashita et al. 2011; Nitschke et al. 2015). Broadcast spawning corals (**a, b**) and brooding corals (**c**) shown here either have: *Symbiodinium* transferred directly to eggs (**a**) or planulae (**c**); or, as in most broadcast spawning corals, *Symbiodinium* are acquired from the environment by larvae or juveniles (**b**). Timing is based on normal development for *A. millepora*.

Numerous studies have documented how environmental factors shape the fate of coral juveniles, including how drivers of mortality can change as the juvenile grows (Birrell *et al.*, 2005, 2008; Doropoulos *et al.*, 2012; Davies *et al.*, 2013; Trapon *et al.*, 2013). Estimates of early mortality range from 30-99.8% of coral juveniles lost within the first year (Babcock, 1985; Wilson and Harrison, 2005; Davies *et al.*, 2013; Trapon *et al.*, 2013). Indeed, interactions between growth rates and settlement density may impact juvenile mortality (Vigliola *et al.*, 2007; van Oppen *et al.*, 2014). Juvenile mortality has also been found to vary by reproductive mode (Baird *et al.*, 2009b) and among coral genera, potentially tied to species-specific differences in growth rates (Babcock and Mundy, 1996; Trapon *et al.*, 2013).

Symbiodinium are transferred to coral larvae or juveniles either horizontally (uptake from the environment) or vertically (maternally passed on to either the eggs or planulae). Horizontal transmission is generally found in species that broadcast spawn, whereas vertical transmission is more common in brooding corals (Baird *et al.*, 2009b). To facilitate infection through horizontal transmission, *Symbiodinium* types must be ubiquitous in the environment or have the ability to detect and move to coral hosts. It is not yet clear whether symbiont infection generally occurs at the larval or recruit stage, however, both life stages have the ability to establish symbioses (Schwarz *et al.*, 1999; Abrego *et al.*, 2009). Although a preliminary study documented ten times greater densities of *Symbiodinium* cells in sediments than in the water column (Littman *et al.*, 2008), the structure and composition of the free-living *Symbiodinium* community on the GBR are currently unknown. Likewise, the extent to which these communities vary in response to environmental variables such as temperature and water quality (i.e., nutrients and particle sediment size) or biogeographically, and how such variation might impact *Symbiodinium* uptake in juvenile corals are unknown.

1.5 Host reproductive strategies and symbiotic outcomes

1.5.1 Assumptions regarding symbiont transfer during maternal transmission

It is generally assumed that the fidelity of symbiont transfer is much greater for corals with vertical transmission than for corals with horizontal transmission; concomitantly, vertical transmitters typically exhibit a more conserved community of symbionts than horizontal transmitters (Baker, 2003). Symbionts of vertically-transmitting species are also thought to be more mutualistic and less parasitic (Dusi *et*

al., 2015), as shown by increases in the quantity of metabolic products shared between symbionts and coral hosts compared to horizontally-transmitting species (Kenkel et al. *in-review*). Theoretically, a vertical transmission strategy is more likely to be selected for in stable environments, or in environments with low host densities or low symbiont-host contact rates, where maternal transmission of symbionts is assumed to ensure that specific partnerships are made (Dusi *et al.*, 2015). However, the fidelity with which symbionts are transferred vertically from parental colonies to offspring and the extent to which offspring are able to acquire symbionts secondarily from environmental sources have not been tested empirically for vertically-transmitting corals, particularly not with techniques sensitive enough to distinguish type and sub-type level diversity, although partial environmental acquisition has been suspected (Padilla-Gamiño *et al.*, 2012; Byler *et al.*, 2013).

1.5.2 Assumptions regarding symbiont transfer during horizontal acquisition

Symbiodinium uptake in horizontally-transmitting species is typically assumed to be completely environmentally regulated, thus larvae or juveniles acquire only those *Symbiodinium* that are available from environmental sources (Cumbo *et al.*, 2013). Juveniles of horizontally-transmitting species do appear to be flexible in their acquisition of symbionts (Little *et al.*, 2004; Abrego *et al.*, 2009), but it is still unclear whether such communities are more diverse compared to communities that are vertically-transmitted to coral eggs (Padilla-Gamiño *et al.*, 2012), largely because of differences in methods used among studies and the paucity of genotyping data available for symbionts associated with early life-history stages of corals. Furthermore, the full diversity of *Symbiodinium* communities in the environment (i.e., water column, sediments, algal surfaces) remains unknown, although recent studies have begun to use deep sequencing technologies to address this deficit (Cunning *et al.*, 2015). However, previous coarse-level comparisons between adult colony diversity and diversity in multiple environmental sources suggest that sediments are the predominant source of symbionts for uptake by corals (Pochon *et al.*, 2010; Takabayashi, *et al.*, 2012). Further work is needed to compare *Symbiodinium* communities associated with the early life-history stages of corals to environmental sources of *Symbiodinium* (Coffroth *et al.*, 2001, 2006), particularly using methods that can detect type and intra-type diversity at both dominant and background abundances.

1.6 Nature *versus* nurture: what regulates the *Symbiodinium* community in corals?

The diversity and abundance of *Symbiodinium* types within corals are important fitness traits and major drivers of coral physiological performance and stress tolerance. Whilst it is known that *Symbiodinium* identity and abundance influence the early life-history stages of corals (Little *et al.*, 2004; Cantin *et al.*, 2009; Renegar, 2015), that coral juveniles can take up types not associated with adults (Little *et al.*, 2004; Abrego *et al.*, 2009), and that certain *Symbiodinium* types are particularly good at thriving at high temperatures (e.g. *S. thermophilium* Hume *et al.*, 2015), questions remain about how flexible immature corals are in the formation of their symbioses. Importantly, the potential for thermally-tolerant *Symbiodinium* to increase the adaptive capacity of corals by increasing their performance and tolerance to increasing ocean temperatures is dependent on whether or not juvenile or adult corals are able to establish symbiosis with such types (Kinzie *et al.*, 2001; Lewis and Coffroth, 2004; Coffroth *et al.*, 2006). Therefore understanding genetic constraints on *Symbiodinium* uptake, as well as environmental availability, is essential to evaluate the potential of *Symbiodinium* communities to enable corals to adapt or acclimate to a changing climate. For example, if corals are genetically pre-determined to take up a specific community (Poland and Coffroth, 2016), then their ability to acclimate ("switch" / "shuffle"; Buddemeier & Fautin 1993; Fautin & Buddemeier 2004) or adapt (Figure 1.3) through associations with new thermally-resilient types will be limited.

The initial establishment of symbiosis and potential for switching ultimately depend on local availability of symbionts (van Oppen et al., 2001; van Oppen et al., 2005; Finney et al., 2010; Sanders and Palumbi 2011), their infection virulence (Abrego et al., 2009), uptake availability (Manning and Gates 2008), and holobiont plasticity (Wicks et al., 2010) and cellular mechanisms of host-symbiont specificity (reviewed in: Davy *et al.*, 2012). In particular, cell surface molecules, for example, lectin (host)-glycan (symbiont) interactions, seem to play a particularly important role in recognition and incorporation for symbiosis establishment and maintenance (Wood-Charlson *et al.*, 2006; Kvennefors *et al.*, 2008; Bay *et al.*, 2011; Kuniya *et al.*, 2015). However, the regulation of key nutrients by the host in the form of inorganic carbon, phosphorus or nitrogen may also help to regulate *Symbiodinium* biomass once symbiosis has been established (reviewed in: Davy *et al.*, 2012).

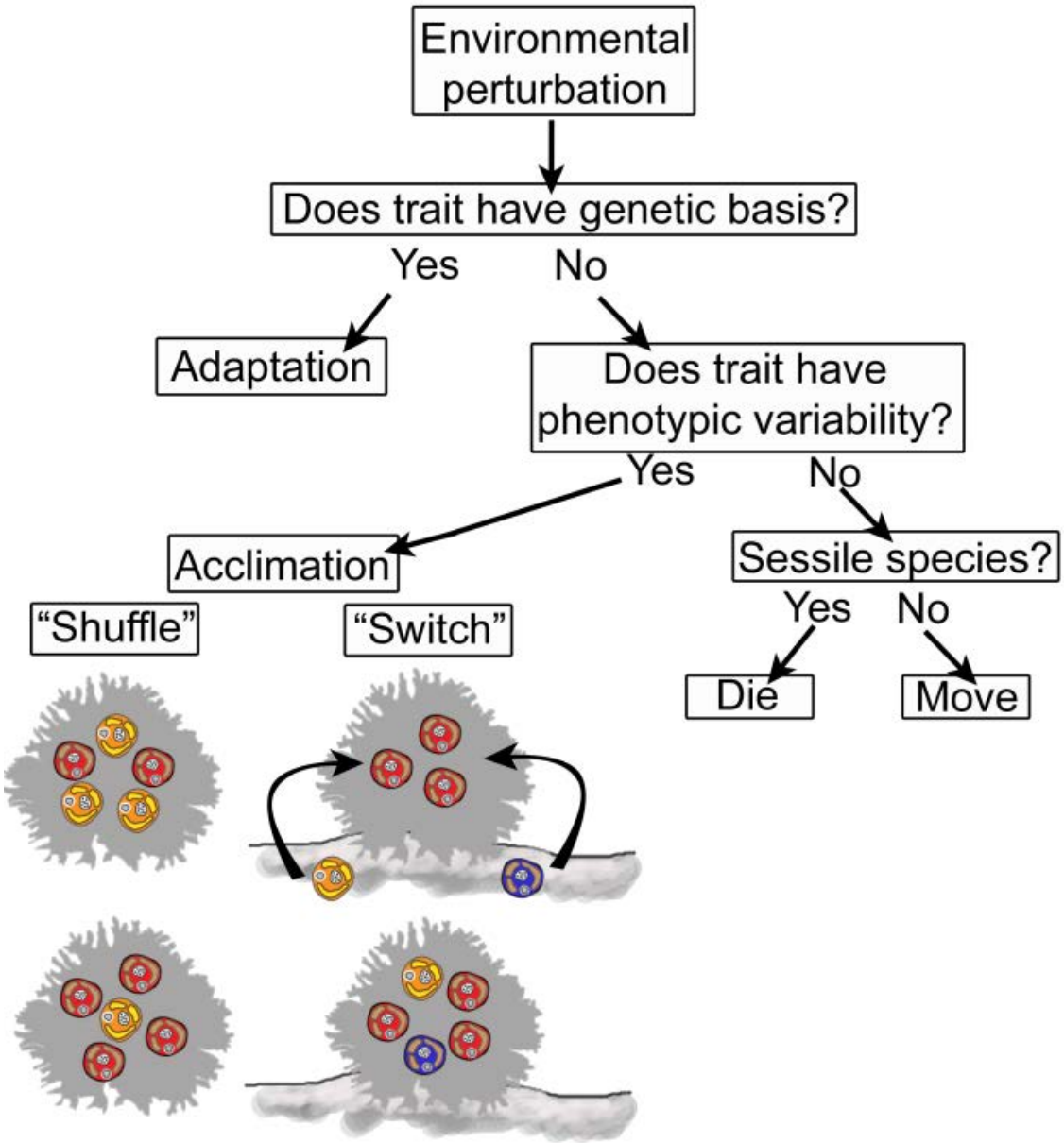

Figure 1.3 Options for the coral-*Symbiodinium* symbiosis to respond to a changing environment. Organisms have four mechanisms for responding to changes in their environment: 1) adaptation, 2) acclimation, 3) migration, and 4) death. As sessile organisms, corals have limited capacity for migration, except through larval dispersal. Numerous studies have demonstrated the capacity of corals to acclimate through shuffling their *Symbiodinium* communities (i.e., relative changes in the abundance of *in hospite* members of communities). In comparison, switching involves the uptake of new *Symbiodinium* types from environmental sources and is less well-documented (but see Lewis & Coffroth 2004; Coffroth et al. 2010; Boulotte et al. 2016). The primary goal of this PhD is to quantify the potential of corals to adapt through heritable, beneficial changes in their *Symbiodinium* communities.

The overarching goal of this thesis is to quantify factors regulating *in hospite* *Symbiodinium* communities in the early life history stages of multiple species of scleractinian corals, specifically the genetic and environmental drivers shaping these consortia. Quantitative genetic analyses and estimations of narrow-sense heritabilities enable both the genetic and environmental contributions to traits to be determined (Falconer and Mackay, 1995). Narrow-sense heritability (h^2 = additive genetic variance/ phenotypic variance) refers to the proportion of variation in a trait that is due to variation in genotypes among individuals, where a value of 1 indicates variation due entirely to genetics, and 0 indicates variation due entirely to environmental factors (Visscher *et al.*, 2008). Although traits with high heritability do not guarantee fixed phenotypes, high heritability implies that variability in the trait is predominantly controlled by the individual's genotype rather than by the environment (Visscher *et al.*, 2008). Importantly, even low levels of heritability and genetic variance suggest that populations have some capacity to respond to natural or artificial selection (evolvability), although rates of adaptation may be reduced (Lynch and Walsh, 1998).

1.7 Study aims and objectives

In this dissertation, my overarching goal is to quantify the extent to which coral hosts with a variety of reproductive modes and symbiont transmission strategies determine the structure of *Symbiodinium* communities associated with their early life-history stages. I also assess the extent to which environmental availability of different *Symbiodinium* types influences the composition of *Symbiodinium* communities established in coral juveniles. Results provide new insights into the flexibility and specificity of the coral-*Symbiodinium* symbiosis and the biological feasibility and likelihood that changes in symbiont types will enable rapid acclimation or adaptation of corals to environmental change.

Aim 1) Quantify the contribution that host genetics makes to the *Symbiodinium* community associated with larvae of a brooding, vertically-transmitting coral. Comparisons of *Symbiodinium* communities among brooded larvae with known parentage and between larvae and their parents will reveal the fidelity with which *Symbiodinium* communities are transferred in a brooding, vertically-transmitting coral. Quantifying the heritability of these *Symbiodinium* communities will provide insights into the flexibility of the symbiosis in corals

with this mode of reproduction and transmission. Knowledge of the extent to which *Symbiodinium* communities in such species are governed by host genotype *versus* the environment is an important indicator of the amenability of the community to change, especially on short time-scales required to keep up with the rapid pace of climate change.

Aim 2) Quantify the contribution that host genetics makes to the *Symbiodinium* community associated with juveniles of broadcast-spawning corals with vertical and horizontal symbiont acquisition using host-relatedness and trait-based approaches. Comparisons of *Symbiodinium* communities amongst eggs and juveniles with known parentage and amongst eggs and juveniles and their parents will reveal the fidelity with which *Symbiodinium* communities are transferred and acquired in both vertically- and horizontally-transmitting, broadcast spawning corals. Quantifying the heritability of these *Symbiodinium* communities will provide insights into the flexibility of the symbiosis in corals that share the same reproductive mode but that have contrasting symbiont transmission strategies. Comparisons amongst species with variable levels of host genotype regulation of *Symbiodinium* communities will provide estimates as to the feasibility of community change, as well as its adaptive potential as environmental conditions continue to change.

Aim 3) Identify parental and *Symbiodinium* community impacts on multiple early life-history stages of a broadcast spawning coral. Quantifying the impacts that parental identities have on larval weight, settlement and larval and juvenile survival will provide important information for the selective breeding of coral colonies for restoration efforts. Determining if symbiont communities differ significantly among juvenile families with variable survivorship provides a link between heritability estimates (genotypes), symbiont communities and juveniles survival on reefs, demonstrating that specific symbiont communities have adaptive benefits for corals.

Aim 4) Describe the *Symbiodinium* sediment community across temperature and water-quality gradients and its impact on juvenile uptake and fitness. Comparisons of the distribution of free-living and *in hospite Symbiodinium*

communities among inshore *versus* offshore sites at northern and central Great Barrier Reef latitudes will elucidate spatial variation in the *Symbiodinium* types available for uptake by coral juveniles. An evaluation of *Symbiodinium* diversity in the sediments is also fundamental to assessing the availability and distribution of thermally-tolerant species available for uptake, the degree to which spatial patterns of *in hospite* symbiont communities are a function of their availability, and how those communities impact juvenile fitness.

1.8 Thesis structure

The objectives outlined above are addressed in Chapters 2 through 5. In Chapter 2, I identify and quantify *Symbiodinium* communities and convert this high-dimensional community data into a single quantitative diversity trait. Using this method, I quantify heritability of the *Symbiodinium* community in a brooding, vertically-transmitting coral. In Chapter 3, I apply the same pipeline, diversity trait and heritability analysis to two broadcast spawning coral species with contrasting modes of symbiont transmission, either transmitting *Symbiodinium* communities vertically to eggs, or releasing azooxanthellate eggs for environmental acquisition. Building on evidence of the significant influence of parental genetics in structuring *Symbiodinium* communities in juveniles of a broadcast spawning coral (Chapter 3), in Chapter 4 I identify how maternal and paternal identity impact four early life history traits (larval weight, larval survival, larval settlement and juvenile survival) and link them to heritable differences in the *Symbiodinium* communities in juveniles. In Chapter 5, I evaluate the influence of free-living *Symbiodinium* communities in the sediments on the establishment of symbiont communities using gamete crosses among known genotypes and exposing the resulting juveniles to different *Symbiodinium* communities. I explore biogeographical partitioning of *Symbiodinium* communities across temperature and water quality gradients and evaluate if different communities result in different fitness outcomes for juveniles. Lastly, in Chapter 6, I synthesize results from the previous four Chapters and discuss the implications of these results for: intervention and conservation efforts on reefs, the potential for evolutionary rescue of coral populations, selection of colonies for restoration and artificial reef sites, and the potential resiliency of coral species with differing reproductive and symbiont transmission strategies.

Chapter 2: Heritability of the *Symbiodinium* community in a brooding, vertically-transmitting coral reveals moderate genetic regulation and mixed-mode transmission

This chapter was submitted for publication as:
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2.1 Abstract

Understanding the heritability of *Symbiodinium* communities in corals is essential to predicting how coral reefs will respond to global climate change and consequent disruptions in symbioses. The influence of genetic *versus* environmental constraints on endosymbiotic populations is fundamental to determining the host's ability to modify that community and therefore manipulate host resilience. To quantify the genetic regulation of *Symbiodinium* communities transmitted vertically, I genotyped 60 larvae of a cryptic species of *Seriatopora hystrix* (ShA), and their parent colonies (9 maternal and 45 paternal colonies) from a localised site using high throughput sequencing. Bayesian heritability (h^2) analysis revealed that 33% of variability of *Symbiodinium* communities in these larvae is genetically controlled. Importantly, *Symbiodinium* communities associated with brooded larvae were distinct from those associated with parent colonies and included novel types not found in parents. These findings indicate that obligate *Symbiodinium* communities are only partially inherited from their maternal parent, and contribute to overturning the paradigm that *Symbiodinium* communities in brooding corals are exclusively vertically transmitted. Instead, I suggest that brooding corals follow a mixed-mode strategy similar to those observed in other invertebrate symbioses. The presence of non-parental symbiont types in newly released larvae and significant spatial variation in abundant types (C120, D1, and D1a) among adults suggest flexibility in the establishment of symbiosis and that microhabitat differences may play an important role in structuring *in hospite* *Symbiodinium* diversity even in partially genetically-regulated communities. Altogether these findings imply that vertically- transmitting, brooding corals may be more resilient to environmental change than previously expected.

2.2 Introduction

Symbiosis is fundamental to life on Earth, underpinning the existence of numerous prokaryotic and eukaryotic species and shaping the physiology and health of organisms (Moya *et al.*, 2008; Gilbert *et al.*, 2012; Lewis *et al.*, 2015). Microbial symbionts also enable hosts to expand their niche breadth, facilitating their ability to move into environments otherwise unsuitable to their physiology (Goffredi *et al.*, 2007). Corals, like many other reef organisms, engage in symbiosis with photosynthetic dinoflagellates of the genus *Symbiodinium*, allowing them to thrive in oligotrophic tropical seas through the utilization of symbiont photosynthates. Similar nutritional facilitation is present in other taxa, for example, in sap-sucking insects that rely on their microbial partners to supplement their diets (Baumann, 2005) and in legumes that rely on rhizobia to fix nitrogen (Oldroyd *et al.*, 2011). Nutritional symbioses also drive diversification of host and symbiont lineages (Douglas, 1989; Brucker and Bordenstein, 2012; Oliver *et al.*, 2014), with derived eukaryotic symbionts like *Symbiodinium* having gone through multiple cycles of diversification and expansion attributed to symbiosis (Thornhill *et al.*, 2014). Importantly, these relationships also provide additional genetic variation to the holobiont on which selection may operate (Moran *et al.*, 2008; Russell *et al.*, 2012), thus facilitating coevolution between host and symbiont or between symbionts (Moran and Dunbar, 2006; Moran *et al.*, 2008; Moya *et al.*, 2008). The degree to which endosymbiotic *Symbiodinium* communities have coevolved with their coral hosts remains a subject of debate and depends to a large extent on the fidelity of *Symbiodinium* community inheritance.

Symbionts may be acquired from the environment (horizontal transmission) or passed maternally into eggs or larvae (vertical transmission), with the latter being the most prevalent mode of transmission in brooding scleractinian corals (Baird *et al.*, 2009b). Maternally-derived symbionts may encompass the transmission of one or multiple symbionts (superinfections) and, at least in well-studied insect vertical symbioses, these vertically transmitted symbionts strongly impact host reproduction, behaviour and co-evolution (Russell *et al.*, 2012; Andersen *et al.*, 2013). Transmission of insect symbionts may be exclusively vertical or may occur initially as vertical transfer followed later by horizontal transmission (Fujishima and Fujita, 1985; Sandström *et al.*, 2001; Kaltz *et al.*, 2003; Scheuring and Yu, 2012; Andersen *et al.*, 2013; Oliver *et al.*, 2014). It is not yet clear if exclusively vertical or mixed modes of transmission occur in brooding corals (Byler *et al.*, 2013). Furthermore, the fidelity of

coral and insect symbioses is variable, with some associations partaking in strict host/symbiont fidelity, whilst others are able to form symbiosis with many groups of distantly related organisms (Moya *et al.*, 2008; Fabina *et al.*, 2013). The symbioses of *Wolbachia* and *Spiroplasma* bacteria throughout *Drosophila* genera and lepidopterans, for example, are highly specific and exclude other bacterial lineages through a dynamic and mature immune response, to the extent that specific *Drosophila* species host novel and specific *Wolbachia* and/or *Spiroplasma* strains (Mateos *et al.*, 2006; Russell *et al.*, 2012). Conversely, in addition to *Wolbachia* (Alphaproteobacteria) and *Spiroplasma* (Mollicutes), other insect phyla host representatives from other groups, including the Gammaproteobacteria, *Baceteroidetes*, and Enterobacteriaceae (Mateos *et al.*, 2006; Moran *et al.*, 2008). The Formicidae host especially diverse symbiont communities (Russell *et al.*, 2012), although examples of extreme symbiont specificity have also been observed (Andersen *et al.*, 2013). Fundamental studies of the diversity of symbiont communities transmitted from parent to offspring are only beginning to be explored in corals, although a recent study suggests that *Symbiodinium* transmission dynamics may be as complex as those observed in the Arthropoda (Padilla-Gamiño *et al.*, 2012).

Although maternal transfer of *Symbiodinium* and bacteria is less well-characterized in corals than in terrestrial invertebrates (Apprill *et al.*, 2009; Padilla-Gamiño *et al.*, 2012; Sharp *et al.*, 2012; Byler *et al.*, 2013), specificity is theorized to be much greater when symbionts are transmitted vertically compared to horizontally (Douglas, 1998; Baker, 2003) (but see Padilla-Gamiño *et al.* 2012). Hosts may form strict associations with only one *Symbiodinium* type (and *vice versa*) or multiple symbionts and hosts may also associate with multiple partners (Little *et al.*, 2004; Abrego *et al.*, 2009a,b; Fabina *et al.*, 2012; Byler *et al.*, 2013; Poland and Coffroth, 2016), where different subtypes represent distinct strains. Superinfections of multiple *Symbiodinium* types of varying abundances are also common in scleractinian corals, a system with potentially similar dynamics to those seen in aphids and sharpshooter cicada superinfections (Moran *et al.*, 2008; Oliver *et al.*, 2014). However, unlike studies of insect symbiont specificity, no studies have used high throughput sequencing to examine maternally-transmitted *Symbiodinium* communities and the diversity of low abundance *Symbiodinium* types in detail, nor have any studies quantified the contribution of parental genetics to the maturation of coral-*Symbiodinium* symbioses.

It is increasingly appreciated that different *Symbiodinium* types vary in their impact on holobiont physiology because of variation in their abilities to produce and transfer photosynthates to the coral host under differing light, temperature and nutrient environments (Little *et al.*, 2004; Berkelmans and van Oppen, 2006; Reynolds *et al.*, 2008; Cantin *et al.*, 2009; Hume *et al.*, 2015; LaJeunesse *et al.*, 2015). Moreover, environmental stress may bring about shifts in the domination of different *Symbiodinium* types, with evidence that these changes can benefit the host under altered conditions (Jones *et al.*, 2008; Cunning *et al.*, 2015). This flexibility to acquire resilient types or shuffle symbionts may be genetically regulated, but it is currently not clear if *Symbiodinium* communities are, for example, controlled by heritable host immune responses similar to those that shape symbiont diversity in *Drosophila* (Mateos *et al.*, 2006). Although evidence of host-*Symbiodinium* specificity is clear (Fabina *et al.*, 2013), more broadly, it is unknown if the transfer and maintenance of symbionts is under the control of parental genetics (heritability), which could potentially limit the flexibility of the symbiont community in response to environmental change.

The narrow-sense heritability of a trait (h^2) describes the degree to which variability in that trait is explained by genetic factors. Increasingly, studies are revealing that the genetic architecture behind particular traits and pathologies is complex (Cho, 2015). For example, microbial diversity in the human gut is a complex trait under partial genetic control (Zoetendal *et al.*, 2001; Ley *et al.*, 2006; Benson *et al.*, 2010; Turnbaugh *et al.*, 2010; Campbell *et al.*, 2012), as is the abundance of microbial symbionts (Liu *et al.*, 2015). If one were to quantify the *Symbiodinium* community as a complex trait with an h^2 value of 1, variability of the community is implied to be mostly due to host genetics. Conversely, an h^2 value estimated at 0 implies no genetic basis for variability in the community, and therefore that it is not under selection and cannot evolve (no evolvability; Lynch and Walsh, 1998). Although an h^2 estimate close to 1 does not necessarily guarantee absolute genetic determination as a result of gene segregation (Visscher *et al.*, 2008), a large heritability estimate of the *Symbiodinium* community may imply that only changes in host genotypes would result in community shifts. Thus, changes in the environmental availability of *Symbiodinium* or in environmental conditions would not induce *in hospite* community shifts. Furthermore, if symbiont communities are under genetic regulation, selection can act upon the community and enable it to evolve in response to external pressures. Predicting the potential, direction and speed of such changes is important to accurately assess the

future of coral reefs under projections of climate change and other anthropogenic stressors. Understanding the relative contributions that host genetics *versus* the environment make to the composition of *Symbiodinium* communities through estimations of h^2 will improve the accuracy of such predictions, given that *Symbiodinium* types vary in their net benefit to the host and that their proportional abundance in the community directly impacts host fitness.

To explicitly describe *Symbiodinium* transfer between adults and offspring and quantify narrow-sense heritability (h^2), I employed high throughput Miseq technology to sequence individual planula larvae across a spectrum of relatedness. I took advantage of a novel parentage analysis approach to decipher the paternal identity of each larva. I also discuss the potential of these larvae to acclimate to novel environments, in light of heritability estimates and the fidelity of symbiont transfer from maternal colonies to larvae.

2.3 Materials and Methods

2.3.1 Study species and sampling design

The common, hermaphroditic coral *Seriatopora hystrix* broods sexually-produced larvae following internal fertilization of eggs by sperm from surrounding colonies (Ayre and Resing, 1986; Warner *et al.*, 2016). DNA extracts of planula larvae were selected from an earlier study that assessed sperm dispersal distances and larval parentage of a cryptic species within the *S. hystrix* species complex, hereafter referred to as ShA (Warner *et al.*, 2015, 2016). All colonies of ShA were tagged and sampled for molecular analyses within a 16 m x 16 m sampling area, with additional colonies sampled from two adjacent transects (totalling 16 m x 40 m area) within the Lizard Island lagoon (S14°41.248, E145°26.606; Warner *et al.* 2015, 2016). This previous work enabled us to examine the effect of both maternal and paternal identity on larval *Symbiodinium* communities across a full pedigree of larval relatedness. The sampling design included full-sib, half-sib, and four individuals produced by selfing (see further details on the sampling design of the Appendix A and Table S2.1).

2.3.2 *Symbiodinium* community genotyping

The *Symbiodinium* communities of adults and larvae were quantified with amplicon sequencing of the ITS-2 locus using the same DNA extractions that had been used to assign microsatellite genotypes and paternity described above. Nine maternal, 45 assigned paternal colonies (which included the nine maternal colonies) and all larvae belonging to paternity confidence categories designated as Very High, High, and Medium by Warner et al. (2016) (n = 60 larvae) were sequenced with the primers: ITS2alg-F and ITS2alg-R (Pochon *et al.*, 2001) and paired-end Illumina Miseq technology. Library preparation and sequencing were performed at the University of Texas at Austin's Genomics Sequencing and Analysis Facility (USA) using their standard protocols, including Bioanalyzer (Agilent) based DNA standardization and pooled triplicate PCR before library preparation.

Raw reads (total = 6,875,177) were analysed using the USEARCH and UPARSE pipeline (v.7; Edgar 2013) following a similar approach outlined in Chapter 2. Briefly, reads were filtered, clustered into OTUs, annotated with NCBI nt database and *Symbiodinium* specific searches (further details in Appendix A Table S2.2). Using these methods, the majority of the OTUs were re-assigned to a clade/type level, leaving only 0.03% of cleaned reads (1459 reads, 78 OTUs) that could not be classified, and which may represent new *Symbiodinium* types (Table S2.3, Figure S2.1, Appendix A).

To account for variable read-depth across all samples, sample reads were normalized using 'DESeq2' and 'Phyloseq' implemented in R (R Core Team, 2012; McMurdie and Holmes, 2013; Love *et al.*, 2014). Nonmetric multidimensional scaling (NMDS) was performed and plotted using the normalized counts matrix using 'Phyloseq', 'vegan' , and 'ggplot' (Wickham, 2009; Schloerke *et al.*, 2014). Genetic distances between OTUs were calculated in 'Ape' (Paradis *et al.*, 2004). Statistical testing of variation in OTU abundance was performed on raw reads in 'DESeq2', which incorporates variance normalization of OTU abundance, and interpreted using the Bejamini-Hochberg correction for multiple-inferences of p-adjusted alpha at 0.05. 'DESeq2' outputs are expressed in multiplicative (log2 fold) terms between or among treatments (Love *et al.*, 2014). Therefore, a log2 fold change of 3 (3 log2 fold change) would represent an increase of normalized abundance of 8 (2^3) in treatment "A" compared to treatment "B".

2.3.3 Estimating the diversity and heritability of *Symbiodinium* communities

I used a species diversity measure (D) that takes into account OTU relatedness and uses three criteria to calculate *Symbiodinium* community diversity: richness, evenness and sequence similarity among species using the following equation (Leinster and Cobbold, 2012):

$$^{q}D^{Z}_{ij}(p),$$

where "q" is a measure of the relative importance of rare species from 0 (very important) to ∞ (not important), and Z is a matrix of genetic similarities of OTUs i through j. Pairwise percent similarities between OTUs sequences were calculated in 'Ape' with a "raw" model of molecular evolution, in which the simple proportion of differing nucleotides between pairwise comparisons is calculated and no assumption is made regarding the probability of certain nucleotide changes over others. Finally, P is a matrix of normalized abundances corresponding to each sample and OTU.

Heritability of *Symbiodinium* diversity in the 60 larvae was calculated using the package 'MCMCglmm' (Hadfield, 2010) utilizing the diversity metrics described above and the coefficient of relatedness between individuals was set as a random effect. Models were run with 1.5 $x10^6$ iterations, a thinning of 50, and burn-in of 10% of the total iterations. A non-informative flat prior specification was used following an inverse gamma distribution (Wilson *et al.*, 2010). Assumptions of chain mixing, normality of posterior distributions, and autocorrelation were met. The posterior heritability was calculated by dividing the model variance attributed to relatedness by the sum of additive and residual variance. Deviance Information Criterion was used to test if adding a maternal random effect had a statistically significant effect on heritability estimates.

2.3.4 Multiple ITS-2 copies and intragenomic variation

Intragenomic variation within and between *Symbiodinium* types makes classifying type-level diversity in *Symbiodinium* based on sequence data difficult (Quigley *et al.*, 2014). To assess if multiple copies and intragenomic variation of ITS-2 genes could potentially bias abundance estimates across *Symbiodinium* types, I undertook a three-step bioinformatics approach as outlined in Chapter 2. Briefly, OTUs were firstly divided by clade and inspected for co-occurrence across samples using the tree function in 'Phyloseq' and grouped into subsets of those co-occurring OTUs.

Secondly, OTUs increasing proportionally and with high correlation coefficients were inspected. Finally, pairwise percent identities were calculated for these subsets of OTUs using the package 'Ape' (Paradis *et al.*, 2004) and correlations of variance-normalized abundances were calculated for those pairs that had greater than 85% similarity with the functions ggpairs in the package 'GGally' (Schloerke *et al.*, 2014). The diversity metric was calculated taking into account possible intragenomic variation by pooling the raw abundances of potential intragenomic variants (OTUs: 8/10, 12/22/24, 28/223, 3/6, 588/848) and heritability was calculated using the same parameters described above. As I found little evidence of intragenomic variation amongst OTUs, those results and their impact on heritability estimates are only discussed in Appendix A.

2.3.5 Colony size and spatial location of adult ShA colonies

To determine if *Symbiodinium* communities varied with colony size (as a proxy for colony age), adult colonies were divided into five size classes based on their mean diameter (Warner *et al.*, 2016): < 8 cm (n = 1 colony), 8 – <14 cm (n = 19), 14 – <20 cm (n = 13), 20 – <26 cm (n = 11), and 26 – 32 cm (n = 1). Testing for differential abundance of *Symbiodinium* OTUs was performed in the same manner as for larval communities using differential abundance testing performed in 'DESeq2'.

Sitepainter (Gonzalez *et al.*, 2012) and Inkscape (Albert *et al.*, 2013) were used to evaluate if spatial patterns were present in *Symbiodinium* communities associated with the 45 genotyped colonies of ShA across the 16 m x 40 m sampling area. Gradient Boosted Models and linear models were run in the package 'gbm' (Ridgeway, 2006) to examine the spatial distributions of the ten most abundant OTUs. Linear models were checked for assumptions of linearity, normality, and homogeneity of variance. Square-root transformations were used to correct for issues of normality or heterogeneity. Latitude and longitude coordinates were centered before fitting models. The package 'Spatstat' (Baddeley and Turner, 2005) was used to visualize spatial variability in abundances of the three most significantly heterogeneous OTUs across this spatial gradient (OTUs: 1, 3, and 6). In order to test for competitive exclusion amongst the three spatially significant OTUs, Spearman's Rho rank correlation coefficients and corresponding p-values were generated for each OTU comparison (OTU1 vs. OTU3, OTU1 vs. OTU6, OTU3 vs. OTU6) using the base 'stats' package in R.

2.4 Results

2.4.1 Distinct *Symbiodinium* communities in brooded larvae and adults

Symbiodinium communities differed between adult colonies and larvae in the brooding coral ShA (Figure 2.1A). Adult corals had similar communities to one another, whereas *Symbiodinium* communities were more variable among larvae. On average, adults contained 29.9±0.6 (SE) OTUs and larvae had 22±0.4 OTUs. Five log2 fold fewer unique OTUs were recovered from adults than from larvae (17 vs. 93 OTUs; Figure 2.2). Of the 17 unique adult OTUs, ten belonged to clade C, three were from each of clades A and D, and one was unknown. Unique to larvae were 17 OTUs from clade C, four from clade E, one from each of clades A, B, and G, and 69 of unknown type (only identified by host or host/*Symbiodinium* identifiers – see Appendix A) (Figure 2.1B). Of the unique larval OTUs, only C1-OTU136 (type followed by OTU designation) and two unknown OTUs (148 and 149) were present in larvae in abundances that differed significantly from those in adults after adjusted p-values were calculated. Although raw read counts were low, C1-OTU136 was present in some larvae from every dam, except dam 3 (although only one individual from that dam was sequenced).

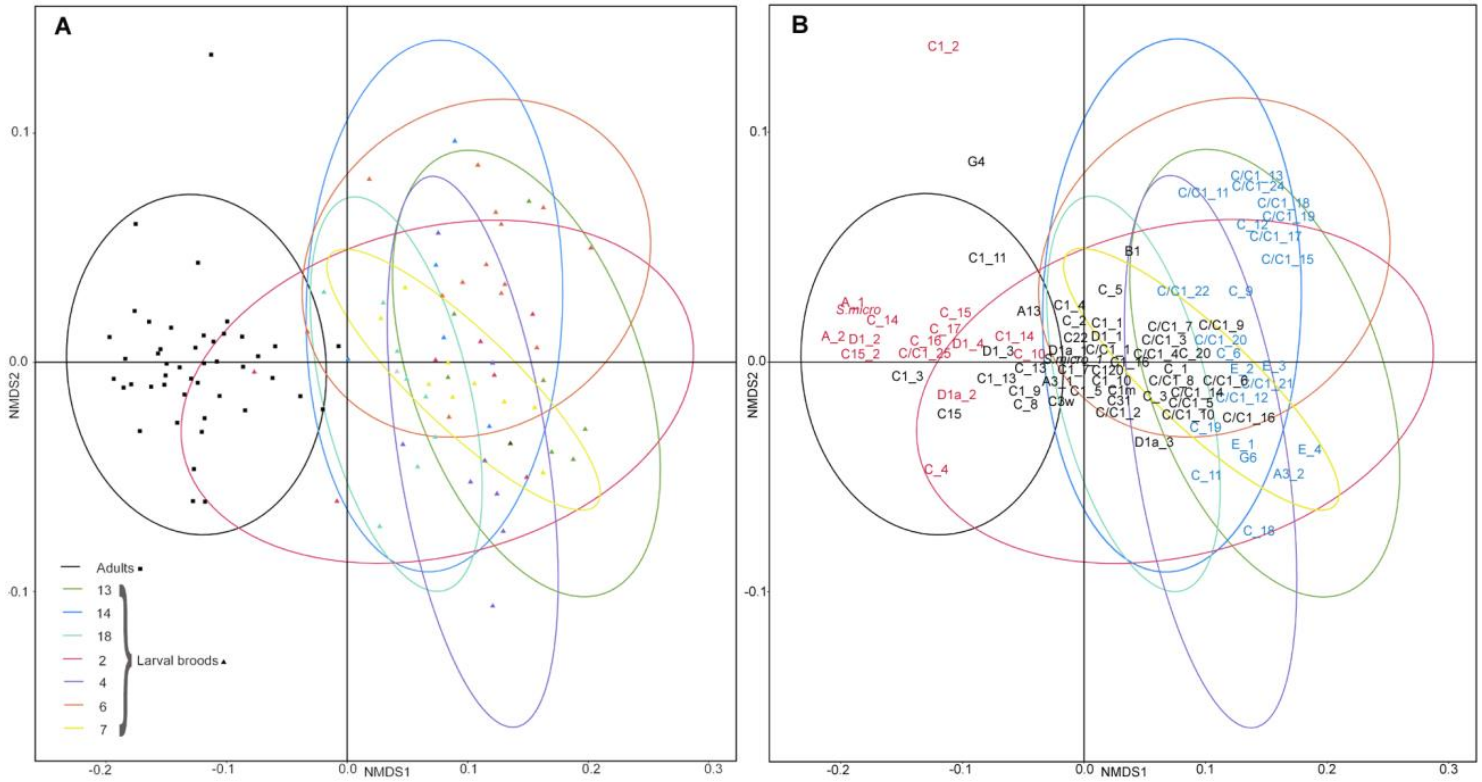

Figure 2.1 Nonmetric multidimensional scaling (NMDS) plots, based on a Bray-Curtis distance matrix of variance-normalized OTU abundances and sequence similarity between OTUs (pairwise percent identities), illustrate differences between *Symbiodinium* communities associated with adult colonies (black squares) and larvae (coloured triangles) of the brooding coral *Seriatopora hystrix* ShA. Ellipses encircling symbols of the corresponding colour represent 95% probability regions for adults and larval samples from each larval brood. **A**) Each point represents a unique adult or larval coral sample. **B**) Each OTU name represents a unique OTU, with only *Symbiodinium*-specific OTUs plotted for clarity (therefore excluding unknown OTUs attributed to the host species). OTU names have also been shorted for readability, see Appendix A Table S2.5 for full names. OTUs in red are those found uniquely in adult coral samples, those in blue are found uniquely in larval samples and those in black are shared between adult and larval samples. Samples presented in (**A**) and OTUs presented in (**B**) share the same ordination space, although they have been separated here for figure clarity. Ellipses corresponding to dams 3 and 10 are not represented, as each had only one larva per dam.

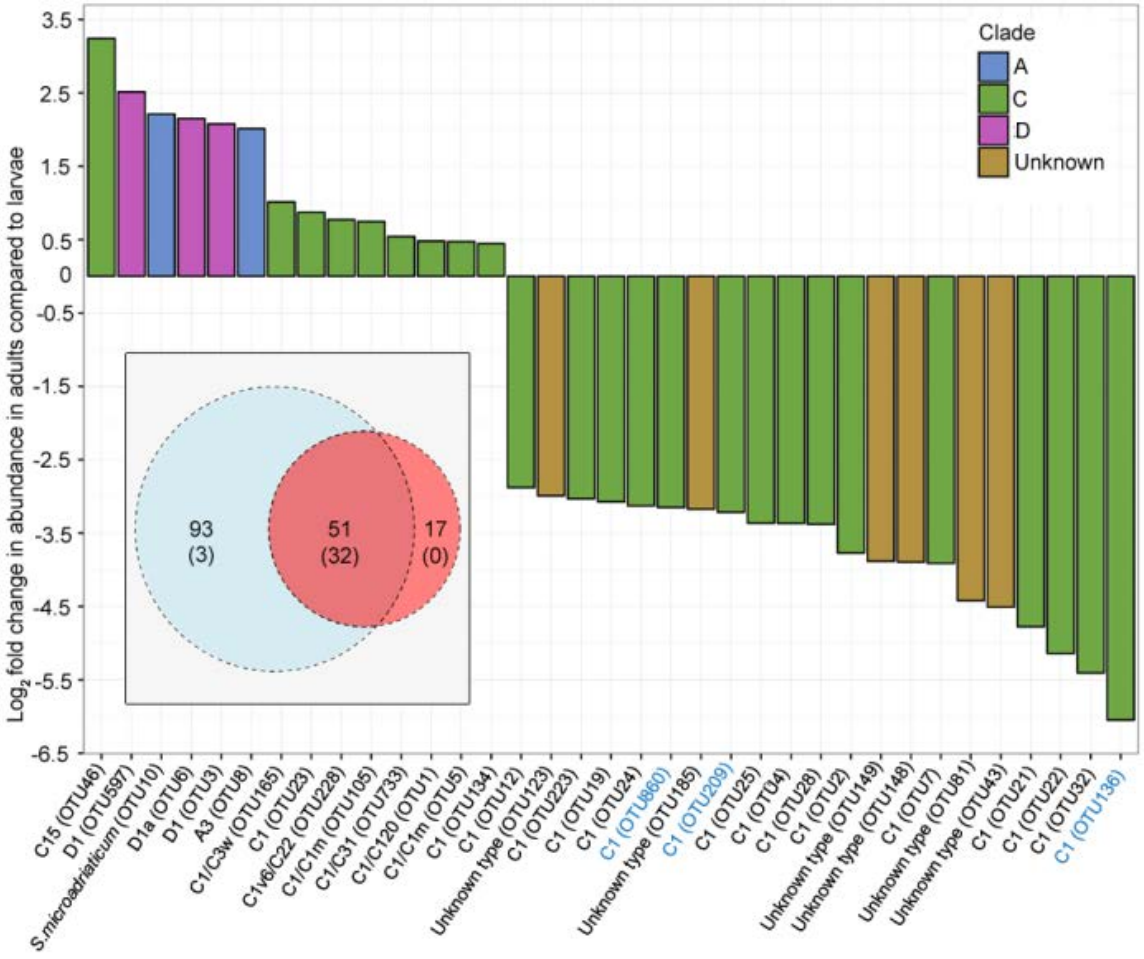

Figure 2.2 Log2 fold change in the abundance of all 35 significantly differentially abundant OTUs from adults and larvae (p-adj < 0.05), including those OTUs unique to larvae in blue text. Colours in the bar plot identify *Symbiodinium* clades (A = blue, C = green, D= purple, unknown types, only blasted to host name = brown). A positive change indicates the OTU is more abundant in adults. Inset: The venn diagram represents OTUs unique to larvae in light blue, unique to adults in light red, and shared between them in dark red. Values in parenthesis represent the number of OTUs that were significant in each of these categories after p-adjustments.

Fifty-one OTUs were shared by adult colonies and planula larvae, and the abundances of only 32 of these differed significantly between the two groups when p-adjusted (DESeq2 negative binomial generalized linear models of log2 fold adjusted p-values Benjamini-Hochberg (B-H) multiple-inference correction of alpha < 0.05, Table S2.4, Appendix A). Of these 32 OTUs, 21 were from clade C, 3 from clade D, 2 from clade A, and 6 were unknown OTUs (Figure 2.2). Adults were characterised by up to 2.5 log2 fold more D-types (D1-OTU3, D1-OTU597, D1a-OTU6), and up to 2.2 log2 fold more A-types (*microadriaticum*-OTU10 and A3-OTU8) compared to larvae. Nine of the 21 C-types had up to 3.2 log2 fold higher abundances in adults (including multiple C1 types, C120/C120a-OTU1, C1m-OTU5/105, C1v6/C22-OTU228, C15-OTU46, C31-OTU733, and C3W-OTU165) and the remaining C1 types had between 2.9 - 5.4 log2 fold lower abundances in adults. The 6 unknown OTUs had 2.9- to 4.5 log2 fold lower abundances in adults compared to larvae.

2.4.2 *Symbiodinium* community variation across maternal larval broods

Planula larvae that shared the same maternal colony generally clustered when *Symbiodinium* OTU richness, abundance, and DNA distance between OTUs were incorporated into analyses (Figure 2.1A, B). However, there were significant differences in the abundance of certain OTUs amongst larval broods (for a full description of those differences see Appendix A and Table S2.4). Larval brood 2 (larvae produced by dam 2) were characterized by larger abundances of *S. microadriaticum*-OTU10 and A3-OTU8. Broods 3, 7 and 10 had significantly less of C1-OTU2 whilst broods 4, 6, 13, 14, and 18 had significantly less or more of many C-types compared to other broods, including C120/C120a-OTU1, C1-OTU2, C1-OTU4, C1-OTU7, C1-OTU21, C1-OTU32, C1v1e -OTU44, C1m-OTU105, C1-OTU134, and C31-OTU733. Types D1-OTU3 and D1a-OTU6 also varied significantly in their abundances between larval broods, particularly broods 2, 4, and 18 vs. brood 13.

2.4.3 Heritability

Leinster and Cobbold estimates of *Symbiodinium* community diversity ranged from 2.037644477 to 2.419352824 across all 60 larvae. Notably, variance around median estimates decreased as relatedness between individual larvae increased (Figure 2.3). The posterior mean heritability of the *Symbiodinium* community in ShA larvae was 0.43 ± 0.21 SD, with a posterior mode of 0.33 (95% Bayesian credibility interval (BCI)

0.1-0.8; Figure S2.2, Appendix A). Adding maternal identity as a random effect did not improve the model (Deviance Information Criterion < 2 units) but decreased the posterior mean and mode of heritability slightly (mean = 0.37± 0.21 SD and mode = 0.19; BCI: 0.1 - 0.8).

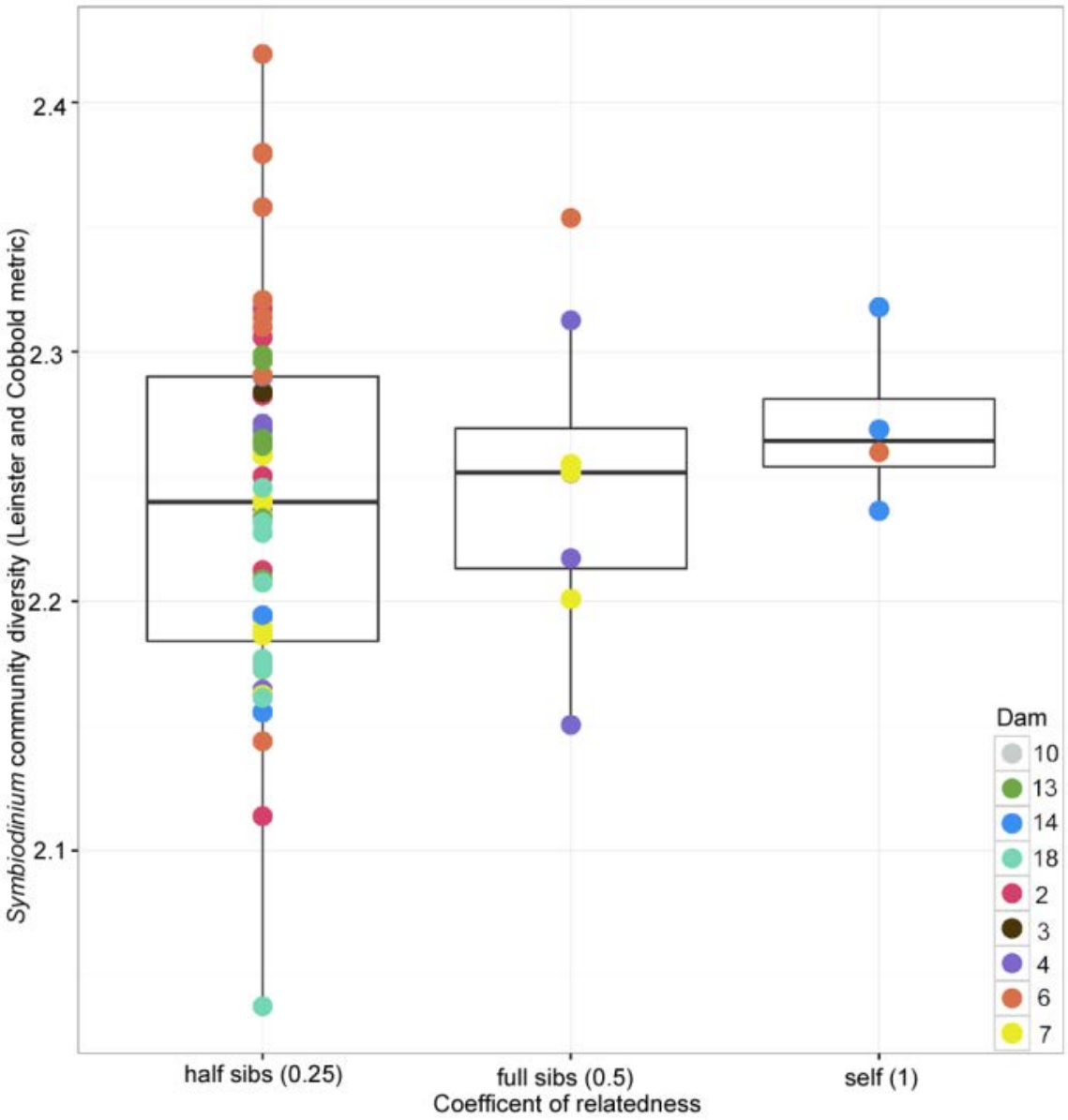

Figure 2.3 Boxplots showing medians, quartiles and minimum/maximum values of *Symbiodinium* community diversity (Leinster and Cobbold metric) in relation to individual larval relatedness (half sibs (0.25), full sibs (0.5), larvae produced from selfing (1.0)). Each larva is coloured by its respective dam.

2.4.4 Colony size, spatial distribution, and *Symbiodinium* diversity in adults

Of the 68 *Symbiodinium* OTUs found in adults, the abundance of only four OTUs differed significantly among the five size classes. Two C-types (OTUs 228 and 105) had at least 2.1- to 4.1 log2 fold (see Materials and Methods) higher abundances in the 26 – 32cm class compared to corals in each of the other four size classes (B-H adjusted p-values: $3.1x10^{-13}$ - $3.1x10^{-2}$; Table S2.4). Similarly, there was a 2.1 log2 fold lower abundance of C31-OTU733 in corals from the 8 – 14cm class compared to the single colony in the largest size class. Colonies from the 8 – 14cm class also had 3.18 log2 fold lower abundances of C1-OTU4 (B-H adjusted p-values: 0.001) compared to corals in the 14 – 20cm class.

Spatial distribution analyses of the ten most abundant OTUs in adults revealed fine-scale variation in their abundances, however distribution patterns differed significantly for only three OTUs at $p = 0.05$ (Figure 2.4). Although y-coordinates did not explicitly represent depth, an increase in y-coordinates did correspond to gradual increases in depth (personal observation, P.Warner). Abundances of *Symbiodinium* C120/C120a-OTU1 were significantly higher from x-coordinates -5 to 5 within the sampling quadrat (Gradient Boosted Model (GBM): $p = 0.019$), with a significant positive relationship with increasing y-coordinates (GBM: $p = 0.00841$). An increase in y-coordinates alone did not significantly affect C120/C120a-OTU1's abundances (GBM: $p = 0.956$), as confirmed by abundances remaining low for x-values >5, even as y-values increased. The abundance of D1-OTU3 was significantly higher in the top-right and for x-values <0 of the sampling quadrat (x and y interaction, GBM: $p = 0.0393$). D1a-OTU6 was significantly less abundant in the top left coordinates and in the inner area of the quadrat compared to the surrounding areas (GBM: $p = 0.0405$). Finally, although the variance normalized abundances of all three OTUs were significantly positively correlated overall (Spearman's rank correlation rho: OTU1 vs. OTU3 = 0.45, OTU1 vs. OTU6 = 0.42, OTU3 vs. OTU6 = 0.77, all $p < 0.004$), extremely low abundances of C120/C120a-OTU1 at x-coordinates >15 contrast markedly with high abundances of the two D-types in that same region (Figure 2.4).

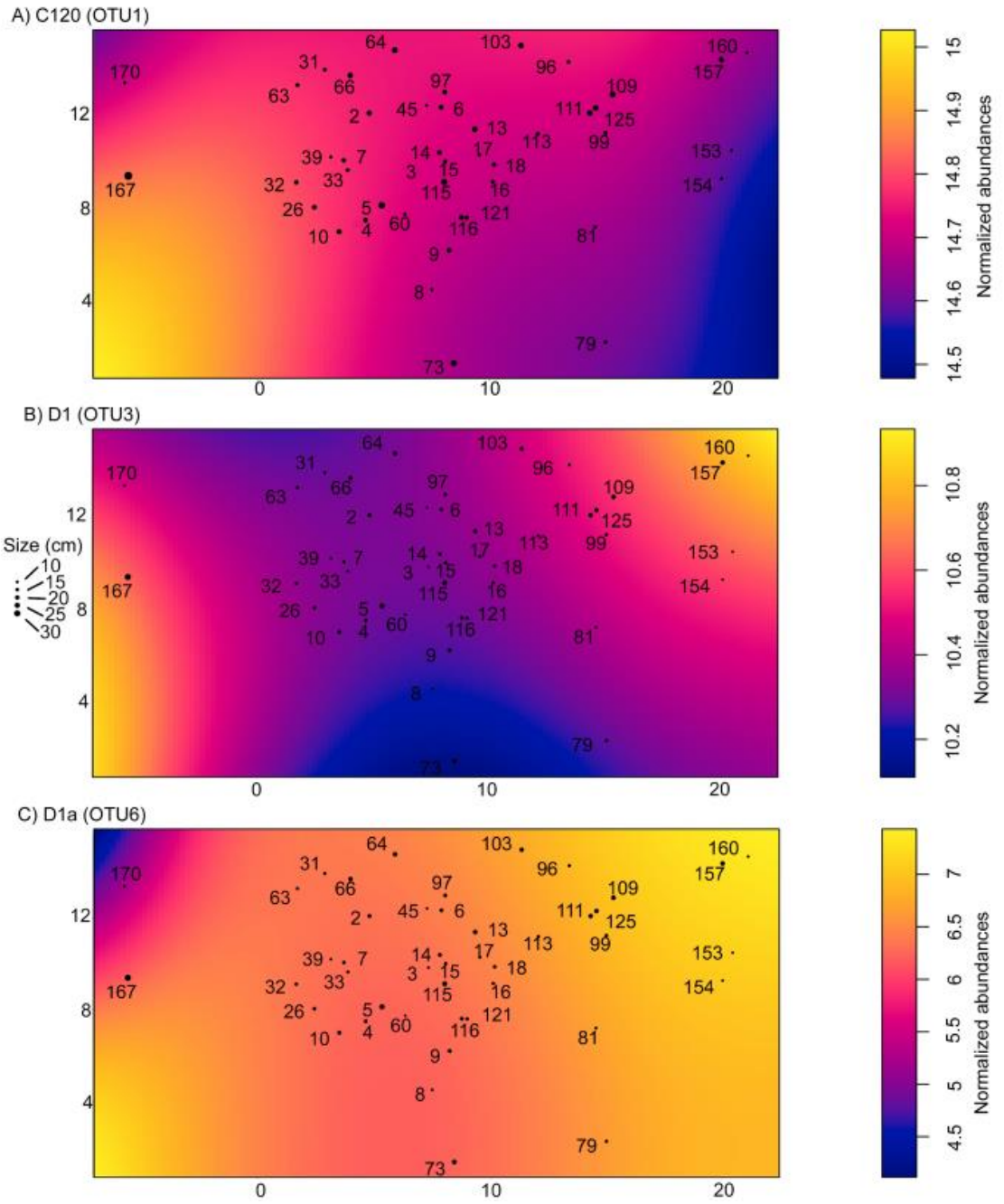

Figure 2.4 Spatial patterns in the abundances of **A**) OTU1 (C120/C120a), **B**) OTU3 (D1), and C) OTU6 (D1a), using normalized abundance data across a 16 m x 40 m sampling site (x and y grid in meters). Positions of the 45 genotyped adult colonies are denoted by numbers. Colours represent changes in the normalized abundance of each OTU across sampling site coordinates, with yellow representing the highest density and blue the lowest. Sizes of the black circles represent size classes of coral colonies in cm.

2.5 Discussion

Here I show that *Symbiodinium* communities associated with larvae of the brooding coral *Seriatopora hystrix* ShA are moderately heritable, with only 33% of variability in the diversity of these symbiont communities under genetic regulation. Much greater heritability of the *Symbiodinium* community was expected in this vertically-transmitting coral, particularly because a number of other important reproductive and fitness traits, such as fertilization success, larval heat tolerance, protein content, settlement success, settlement substrate preferences, and juvenile growth and survivorship, have high genetic heritability in corals (Meyer *et al.*, 2009; Kenkel *et al.*, 2011, 2015; Baums *et al.*, 2013; Dixon *et al.*, 2015). Additionally, availability of a full larval pedigree for this species (Warner *et al.*, 2016) provided a unique opportunity to evaluate the relative contributions of heritability and maternal environmental effects to the composition of larval *Symbiodinium* communities in a brooding coral. Model selection shows that maternal environmental effects (the effect of larvae sharing a common maternal environment) did not explain a significant amount of variability in *Symbiodinium* communities among larvae. This result and narrow-sense heritability estimates indicate that the general clustering of communities of larvae that shared a dam, even when maternal broods contained larvae from different sires, was due to inherited genotypes amongst those larvae. Although I found heritability of *Symbiodinium* communities to be moderate in ShA, the posterior distribution of the heritability estimate indicates a larger value, suggesting that genetic regulation may resolve to be greater with increased sampling effort.

2.5.1 Mixed-mode *Symbiodinium* uptake allows for local adaptation and flexibility to change

These findings suggest that the brooding coral ShA has a mixed-mode transmission strategy, in which dominant symbionts are transmitted vertically but additional background strains may be acquired from environmental sources. These findings contradict previous assumptions that maternally-transmitted symbiont communities are transferred to offspring with high fidelity in corals (Douglas, 1998; Baker, 2003; Fabina *et al.*, 2012). The limited genetic regulation of *Symbiodinium* communities found for ShA larvae has important implications for the evolvability of these communities and for the capacity of brooding corals to vary their communities in

response to changing environmental conditions. For example, if levels of heritability and genetic variance are low, then responses to natural or artificial selection (evolvability) would be limited (Visscher *et al.*, 2008). On the other hand, highly heritable symbiont communities with low genotypic variation could be equally problematic for vertically-transmitting coral populations if communities are thermally sensitive and temperatures increased anomalously. The moderate levels of genetic regulation found here suggest that this species has some capacity to respond to changing environmental conditions, thus intervention efforts to facilitate beneficial, phenotypic community changes may be possible (Visscher *et al.*, 2008). Given that assisted evolution efforts involving specific *Symbiodinium* types have been shown to be promising in horizontally-transmitting corals (Levin *et al.*, 2016), it may be that vertically-transmitting, brooding species with moderate fidelity like ShA are also good candidates for assisted *Symbiodinium* uptake.

The appreciable amount of variation in the composition of larval *Symbiodinium* communities that was not under genetic control implies that novel *Symbiodinium* types are acquired from the environment. This is evident in the retrieval of up to 93 larval-specific OTUs, potentially from uptake of non-symbiotic types residing in the maternal gastrovascular cavity. Free-living *Symbiodinium* within the maternal gastrovascular cavity may enter brooded larvae without engaging in symbiosis with the maternal colony. Alternatively, although it is unclear whether less than one day old ShA larvae are able to ingest *Symbiodinium* through their oral pore, four-day old *Fungia scutaria* larvae are able to do this (Schwarz *et al.*, 1999), ShA larvae could potentially take up non-symbiotic *Symbiodinium* residing in the surface mucus of the maternal colony. This mixed-mode transmission is consistent with other symbiotic systems, such as wild *Drosophilia hydei* populations (Oliver *et al.*, 2014), *Acromyrmex* ants (Scheuring and Yu, 2012; Andersen *et al.*, 2013), and paramecium (Fujishima and Fujita, 1985; Kaltz *et al.*, 2003). The novel diversity found in ShA larvae also mirrors the increased *Symbiodinium* diversity detected in eggs of *Montipora captiata* compared to adults (Padilla-Gamiño *et al.*, 2012), bacterial communities of larvae in the brooding coral *Porites astreoides*, and various bacterial communities associated with sponge species with supposed vertical transmission (Schmitt *et al.*, 2008; Sharp *et al.*, 2012). Such consistency across phyla suggest that it may be evolutionarily advantageous to compromise between a completely vertically- and a horizontally-acquired symbiont community, as both strategies provide distinct advantages and disadvantages. In *S.*

hystrix, vertical transmission of *Symbiodinium* that are locally-adapted to the parental environment is likely to provide benefits for a species able to self-fertilize (Sherman, 2008; Warner *et al.*, 2016) and that has highly localised larval dispersal (e.g., Underwood *et al.* 2007; van Oppen *et al.* 2008; Noreen *et al.* 2009). However, an exclusively vertically-transmitted community might become a liability if environmental conditions change or if larval dispersal distances are long, potentially resulting in disruption of the partnership and deregulation of symbiont abundances with harmful physiological effects on the host, such as bleaching in corals (Cunning *et al.*, 2015) or wasp parasitism in insects (Xie *et al.*, 2010; Oliver *et al.*, 2014). Thus, a mixed-mode strategy resulting in superinfections of multiple symbionts that confer benefits to the host (e.g., parasitoid protection in aphid hosts; Sandström *et al.* 2001; Oliver *et al.* 2014) generally provides more flexibility to adjust to variable environmental conditions. Similarly, a mixed mating strategy of selfing and outcrossing in ShA, combined with a functional nutritional symbiosis upon release, may facilitate both local and long-distance dispersal (Warner *et al.*, 2016). These findings reveal that greater diversity and flexibility in *Symbiodinium* transmission exist than previously thought, highlighting that evolvability in these corals may confer greater than expected resilience in this and similar vertically-transmitting species.

Processes other than environmental uptake by larvae may also have contributed to the different communities observed in larvae *versus* adults. Competitive exclusion theory suggests that it may be impossible to transmit an exact parental symbiont community due to intra-symbiont competition, as conditions promoting growth for some symbionts may be different in earlier life stages (Moran *et al.*, 2008). The novel diversity found in larvae may provide host benefits similar to those observed in insect symbioses with moderate heritability estimates, enabling uptake of variable symbiont types to contend with changing conditions through ontogeny (Abrego *et al.*, 2009). Specifically, *Symbiodinium* C1-OTU136 was uniquely identified in larvae, which may suggest that it represents an adaptive advantage for this life stage. Clade C types are taxonomically diverse (LaJeunesse, 2005; Thornhill *et al.*, 2014) and exhibit a range of tolerances for light and temperature, as borne out by variability in their *in hospite* distributions in adults. In a study of another pocilloporid species, *Stylophora pistillata*, types C35/a and C78 were found only in shallow colonies, type C79 in deeper colonies, and C8/a in both habitats (Sampayo *et al.*, 2008). Larval settlement and early juvenile

survival are hypothesized to be highest in cryptic areas of low-light that offer protection from predation (Maida *et al.*, 1994; Suzuki *et al.*, 2013). Optimal settlement environments differ substantially from light environments experienced by adults, potentially by as much as 10 fold (Suzuki *et al.*, 2013). Therefore, it is possible that variation in *Symbiodinium* communities between larvae and adults observed here relates to different selective pressures associated with differing light environments (Gómez-Cabrera *et al.*, 2008), as observed for sunlit tops *versus* shaded sides of adult corals (Rowan *et al.*, 1997; Kemp *et al.*, 2008).

2.5.2 The role of immunity in shaping *Symbiodinium* communities

Rather than an adaptive response, the greater variation and diversity of *Symbiodinium* communities associated with larvae may indicate that the larval immune response is not developed sufficiently to differentiate between *Symbiodinium* types most appropriate for larval physiology. If true, then the ubiquitous presence of *Symbiodinium* C1-OTU136 in larvae may be a consequence of an opportunistic type taking advantage of immature immunity. The coral immune system matures over time (Frank *et al.*, 1997; Puill-Stephan *et al.*, 2012), and the lack of a fully operational immune response is thought to explain fusion among closely-related larvae and juveniles in the first six months, leading to chimerism in juveniles and adult colonies of *Acropora millepora* (Puill-Stephan *et al.*, 2009). Once the immune response matures, it is possible that a winnowing process eliminates symbionts that are not physiologically beneficial to the coral host (Abrego *et al.*, 2009; Byler *et al.*, 2013). The immune system is an obvious mechanism by which the host could exert control over the symbiotic community by regulating the establishment of either individual *Symbiodinium* types (Bay *et al.*, 2011) or whole clades or functional units (i.e., clades or types with similar metabolic roles) (Ley *et al.*, 2006). Mechanisms of immunity that could be transmitted through parental genetics include the production of specific antimicrobial peptides (e.g., Raina *et al.* 2016) that control the abundance and diversity of communities established in offspring, as observed with bacteria in *Hydra* (Fraune *et al.*, 2010; Franzenburg *et al.*, 2013). A range of other components of both the innate and adaptive immune response have been implicated in shaping invertebrate symbiont communities, such as T-cells, Nod2, and defensins (as reviewed in Franzenburg *et al.*, 2013), some of which have been documented in *Symbiodinium* establishment in corals (Wood-Charlson *et al.*, 2006; Bay *et al.*, 2011; Davy *et al.*, 2012). Further work is needed to clarify if novel types, such as

C1-OTU136, confer adaptive benefits to early life stages or if their common and unique presence in larvae is a function of an under-developed immune response. Future studies combining *Symbiodinium* community genotyping with gene expression should provide insights into the specific inherited mechanisms that contribute to establishing coral-*Symbiodinium* symbioses and explain *Symbiodinium* community variability among and within coral species.

2.5.3 Intragenomic streamlining as an genomic consequence of strict mutualisms

The limited evidence for signatures of multicopy or intragenomic variation in *Symbiodinium* in this study is not surprising in light of recent genomic studies of the genetic architecture of mutualistic species. Genome loss, restructuring and gene transfer have been observed in many symbionts involved in stable mutualisms, within which functionality is transferred to the host and is thought to confer a level of host-control over symbiotic processes (Ochman and Moran, 2001; Moya *et al.*, 2008; Medina and Sachs, 2010). As maternally-transferred symbioses are thought to be one of the most co-evolved forms of mutualisms, a reduction in genome size (and thus a potential pruning of intragenomic variants) might be greater than expected. Alternatively, maternally-derived symbionts may lack the ability to survive in a free-living stage due to the highly dependent symbiosis, and therefore may have lost the opportunity to develop a highly multi-copy genome. Currently, only the chloroplast genome of *Symbiodinium* C3 and a draft nuclear genome of *Symbiodinium* B1 (*minutum)* exist, both of which show evidence of re-structuring and plasticity, potentially due to a symbiotic lifestyle (Shoguchi *et al.*, 2013; Barbrook *et al.*, 2014). Prokaryotic and eukaryotic gene transfer in the B1 genome has also been detected (Shoguchi *et al.*, 2013) and the chloroplast C3 genome has undergone extreme reductions (Barbrook *et al.*, 2014). Additional genomic sequencing of exclusively vertically-transmitting corals is needed to elucidate whether maternally-transferred symbionts have significantly less multi-copy genes than *Symbiodinium* acquired horizontally.

2.5.4 Microhabitat variation and winnowing shape adult *Symbiodinium* communities

The disparate *Symbiodinium* communities between larvae and adults found here further indicate that the re-shaping of the *Symbiodinium* community through ontogeny is

an important developmental process in corals. Ontogenetic variability in microbial communities (*Symbiodinium* and bacteria) appears common in both vertically- and horizontally-transmitting coral species, including *Acropora* sp. (Abrego *et al.*, 2009) and *Porites astreoides* (Sharp *et al.*, 2012). These results suggest that ShA also undergoes a winnowing period similar to that observed for *Symbiodinium* communities in *A. tenuis* (Abrego *et al.*, 2009a; b) and octocorals (Coffroth *et al.*, 2001; Poland *et al.*, 2013; Poland and Coffroth, 2016) and bacterial communities in corals (*Acropora sp.*: Littman *et al.* 2009; Lema *et al.* 2014; *Pocillopora meandrina:* Apprill *et al.* 2009), other cnidarians (*Nematostella vectensis* and *Hydra*; Franzenburg *et al.*, 2013; Mortzfeld *et al.*, 2015) and molluscs (Nyholm and McFall-Ngai, 2004). I did not detect much variation in *Symbiodinium* communities associated with corals ranging in diameter from 8 cm to >30 cm, which could represent corals across a range of ages, for example from 3-10 years (Babcock, 1991). This is likely because winnowing processes could occur much earlier in the development of the brooding coral ShA than in the development of broadcast-spawning corals (Abrego *et al.*, 2009a, b; Lema *et al.*, 2014), such as around metamorphosis or within the first year of life. The few types that did differ significantly between size classes belonged to clade C and may reflect variability in the abundance of specific *Symbiodinium* types available in the local environment during a specific spawning season. For example, the greater abundance of OTUs 105 and 228 in the largest and presumably oldest coral (26 – 32 cm) compared to all other classes (although there was only one sample in this size class) might reflect a greater abundance of these types in the environment at the time of settlement. The diversity of *Symbiodinium* in the sediments is extremely variable across both water-quality and temperature gradients (Chapter 5) and may be structured temporally as well. This study highlights the paucity of research on the development of *Symbiodinium* communities across corals with different reproductive and transmission modes and the need for further work to define the winnowing period for ShA. Identifying at what stage winnowing occurs in brooding corals will provide crucial insights into when the flexibility to associate with potentially stress-tolerant types ends and fixation of *Symbiodinium* communities begins.

The only spatial variation in *Symbiodinium* communities associated with adult corals involved three of the most abundant symbiont types, and could potentially be attributed to patterns in the spatial distribution of host corals. Gradients in *Symbiodinium* abundances detected at this study site, primarily based on differentially

abundant C120/C120a-OTU1, D1-OTU3, and D1a-OTU6, may have been structured by variable temperature and light microhabitats interacting with differing photo-physiologies among symbiont types (Baker *et al.*, 2004; LaJeunesse *et al.*, 2009). For example, vertical light gradients within individual colonies have been shown to drive symbiont community structure, with light-tolerant symbiont types dominating abundances along the topmost parts of colonies and types preferring low light environments occurring along the sides and basal regions (Rowan *et al.*, 1997; Kemp *et al.*, 2008). Significant differences in irradiance among coral tissues are thought to structure *Symbiodinium* communities at the micro scale (Wangpraseurt *et al.*, 2012, 2014), thus differences at larger (meter) scales found in this study could be important for structuring *in hospite Symbiodinium* diversity as well. Moreover, although this study examined spatial patterns in a 2-D space, lower y-coordinates also corresponded to shallower depths towards the reef flat (personal observation, P.Warner), which may have interacted with variables other than light to impact symbiont abundances. I also found that the abundances of *Symbiodinium* C120/C120a-OTU1 appeared inversely related to abundances of D1-OTU3 and D1a-OTU6 across a section of the sampling area, suggesting that competitive exclusion interacting with environmental variables may also contribute to patterns of differential abundance. More work is needed to understand how fine-scale environmental variables impact *Symbiodinium* presence and abundance at the type-level, so that population dynamics of these communities can be modelled under changing environmental conditions.

2.5.5 Conclusion

Based on novel heritability and paternity analyses, I show that contrary to expectations, *Symbiodinium* communities associated with the brooding coral ShA are under only partial host genetic regulation. Moreover, novel *Symbiodinium* types found in larvae but not in adults suggest that this species has some flexibility to associate with novel symbionts across generations. Further work is needed to follow these unique larval symbionts through juvenile ontology to adulthood in order to determine their significance for host physiology. Additionally, identifying the nature of the heritable genetic mechanisms I quantify here will provide important insights into how *Symbiodinium* communities may be targeted for intervention strategies to increase reef resilience.

Chapter 3: Heritability of the *Symbiodinium* community in vertically- and horizontally-transmitting broadcast spawning corals

3.1 Abstract

The dinoflagellate-coral partnership affects many, if not all, aspects of coral health and stress tolerance, particularly tolerance to thermal stress that causes bleaching. However, the comparative roles of host genetic *versus* environmental factors in determining the composition of this community *in hospite* are largely unknown. Here I quantify the heritability of *Symbiodinium* communities in two broadcast spawning corals with different symbiont transmission modes: *Acropora tenuis,* which has environmental acquisition, and *Montipora digitata,* which has maternal transmission of symbionts. Using high throughput sequencing of the ITS-2 region to characterize communities in parental colonies, juveniles and eggs, I describe new *Symbiodinium* types in both coral species, and previously unknown dynamics in the early life history stages of corals. After one month of natural uptake in the field, *Symbiodinium* communities associated with *A. tenuis* were dominated by A3, C1, D1, A-type CCMP828, and D1a in proportional abundances that were conserved across two years of sampling. In contrast, *M. digitata* eggs were dominated by type C15, although D1, A3.1, and two other A types (A and CCMP828) were also present. Host genetic *versus* environmental influences accounted for, on average, 29% *versus* 71% of phenotypic variation found in *Symbiodinium* communities in the horizontal transmitter *A. tenuis*. In contrast, they accounted for 62% *versus* 38%, respectively, in the vertical transmitter *M. digitata*. These Bayesian heritability (h^2) estimates for *Symbiodinium* communities highlight that, although transmission strategies differ between the two species, each strategy ensures the structure and maintenance of a specific symbiont community in juveniles and eggs. I hypothesize that heritability of recognition mechanisms ensures similar symbiont communities in offspring of both species, while the maternal provisioning of symbionts providing an additional physical mechanism for transferal in vertical transmitters. Regardless of the process, these results reveal hitherto unknown flexibility in the acquisition of *Symbiodinium* communities; and substantial heritability in both cases provides ample material for selection to produce partnerships that are locally adapted to changing environmental conditions.

3.2 Introduction

Disruptions of the endosymbiotic partnership between the dinoflagellate *Symbiodinium* and its coral host have accounted for some of the largest coral reef mortality events in the last 20 years, with global mortalities estimated at 16% in the 1998 event alone (Wilkinson *et al.*, 2008) and in 2016, up to 22% mortality on the Great Barrier Reef (Great Barrier Reef Marine Park Authority, 2016). Coral bleaching, defined as either loss of *Symbiodinium* cells from coral tissues or reduction in symbiont photosynthetic pigments, represents a threat that is increasing in both frequency and magnitude (Glynn, 1993; Hoegh-Guldberg, 1999; Eakin *et al.*, 2009; Normile, 2016). If coral reefs are to persist under climate change, corals must either disperse to new unaffected habitats, acclimate through phenotypic plasticity, and/or adapt through evolution (Charmantier *et al.*, 2014). However, a major impediment to understanding the capacity of corals to adapt to a changing climate is the lack of knowledge about the extent to which *Symbiodinium* communities associated with corals are inherited and hence subject to change as well as selection.

Bleaching sensitivity is variable within and among species (Hughes *et al.*, 2003), but the mechanisms causing this variability remain unknown. Coral hosts have a repertoire of molecular mechanisms that provide some capacity to respond to thermal stress, including heat tolerance-associated genes (Dixon *et al.*, 2015), antioxidant pathways (Jin *et al.*, 2016), and fluorescent pigments and enzymes (Salih *et al.*, 2000). However, the *Symbiodinium* community hosted by corals has long been recognized as the primary factor determining bleaching susceptibility (Glynn *et al.*, 2001; Baird *et al.*, 2009a). Although the exact role of host and symbiont in bleaching remains unclear (Brown *et al.*, 1995; Baird, *et al.*, 2009a), recent evidence showing that bleaching is primarily caused by the expulsion of *Symbiodinium* cells, rather than host-cell degradation or detachment (Bieri *et al.*, 2016), further corroborates that stress responses of the coral holobiont are driven by impacts on the photosymbiont. Host influence is however also evident (Kenkel *et al.*, 2013; Hawkins *et al.*, 2015; Krueger *et al.*, 2015) and may play an equally important role in determining bleaching susceptibility. The increased adaptation potential of *Symbiodinium,* because of their greatly reduced generation times compared to corals (van Oppen *et al.*, 2011), raises the possibility that endosymbiotic *Symbiodinium* communities could influence host adaptation to changing

climates through symbiosis via increased host niche expansion (LaJeunesse, Smith, *et al.*, 2010; Fellous *et al.*, 2011).

There are nine recognized *Symbiodinium* clades (Pochon *et al.*, 2012), which encompass substantial sequence and functional variation at the intra-clade (type) level (reviewed in Baker, 2003). Conventional technology often overlooks taxonomic resolution at the type level, leading to many studies comparing *Symbiodinium* communities at the clade and dominant level, although this trend is changing (see: Silverstein *et al.*, 2015; Wilkinson *et al.*, 2016). However, deep sequencing technologies currently available can detect type level diversity present at low abundances (Quigley *et al.*, 2014) and are now being applied to coral-*Symbiodinium* diversity (Arif *et al.*, 2014; Kenkel *et al.*, 2015; Boulotte *et al.*, 2016), although not yet for early life-history stages of corals. Therefore, there are gaps in the basic knowledge of the composition of *Symbiodinium* communities at lower, functionally relevant taxonomic levels, including those at background abundances, in the eggs and juveniles of corals.

Natural variation in the composition of coral-associated *Symbiodinium* communities exists among coral populations and species (Baker, 2003; Fabina *et al.*, 2013), with certain communities offering greater bleaching resistance compared to others (Berkelmans and van Oppen, 2006; Bay *et al.*, 2016). It is not yet known what enhances or constrains the capacity of corals to harbour stress tolerant *Symbiodinium* types and whether changes to *Symbiodinium* communities in response to environmental stressors are stochastic or deterministic (Theis *et al.*, 2016). Given the importance of *Symbiodinium* communities for bleaching susceptibility and mortality of the coral holobiont (Cunning and Baker, 2014; Cunning *et al.*, 2015), quantifying the proportional contributions of genetic and environmental factors to community formation, regulation and stress tolerance is important for understanding coral health. If the *Symbiodinium* community is heritable, changes to these communities may bring about adaptation of the holobiont as a whole. Under this scenario, *Symbiodinium* community shifts are equivalent to changes in host allele frequencies, thus opening up new avenues for artificial selection, assisted evolution and microbiome engineering (van Oppen *et al.*, 2015; Theis *et al.*, 2016).

Symbiodinium communities associated with scleractinian corals are either acquired from the environment (horizontal transfer) or passed maternally from adults to eggs or larvae (vertical transfer). Approximately 85% of scleractinian coral species broadcast spawn eggs and sperm into the environment, and of these, ~80% acquire

symbionts horizontally; the remaining ~20% acquire them vertically (Baird *et al.*, 2009b). Vertically-transmitted symbiont communities are predominantly found in brooding corals with internal fertilization (Baird *et al.*, 2009b) and are theorized to be of lower diversity and higher fidelity (Baker, 2003). Conversely, horizontal transmission has generally been assumed to result in weaker fidelity, although fidelity may be increased through the development of strong host-genotype-symbiont community associations (Douglas and Werren, 2016). Studies specifically quantifying the genetic component governing the *Symbiodinium* community established in offspring of both horizontal and vertical transmitters are needed to elucidate the potential for adaptation through symbiont community changes.

Heritability describes the genetic components of variability in a trait using analysis of co-variance among individuals with different relatedness (Lynch and Walsh, 1998). The ratio of additive genetic variance to phenotypic variance (V_A/V_P) is defined as narrow-sense heritability (h^2) (Falconer and Mackay, 1995). The degree of heritability of a trait ranges from 0 - 1, and provides information about the influence of parental genetics on variability of that trait (Falconer and Mackay, 1995). Therefore, information regarding if and how traits might change from one generation to the next can be predicted from measures of heritability, where the predicted change in offspring phenotypes are proportional to h^2 (i.e., the breeder's equation) (Visscher *et al.*, 2008). Determining the genetic contribution is particularly important for understanding the potential for adaptation and for predicting strength of selection responses (i.e, the 'evolvability' of a trait) (Houle, 1992; Charmantier *et al.*, 2014; Hedrick *et al.*, 2014).

To quantify the potential for selection of endosymbiotic *Symbiodinium* communities associated with broadcast spawning corals in response to changes in environmental conditions (i.e., climate change-induced), I characterized symbiont communities associated with adults and juveniles of the horizontal transmitter *Acropora tenuis* and with adults and eggs of the vertical transmitter *Montipora digitata* using high-throughput sequencing. Using community diversity metrics, I derived the narrow-sense heritability (h^2) of these communities and identified new *Symbiodinium* types recovered from juveniles and eggs compared to their parental colonies. Finally, I described previously unknown *Symbiodinium* community dynamics in the early life history stages of these two common coral species.

3.3 Methods

3.3.1 Horizontally-transmitting broadcast spawner

For crossing experiments, gravid colonies of the broadcast-spawning coral *Acropora tenuis* were collected in 2012 and in 2013 from the northern (Princess Charlotte Bay: 13°46'44.544"S, 143°38'26.0154"E) and central Great Barrier Reef (GBR) (Orpheus Island: 18°39'49.62"S, 146°29'47.26'E).

In 2012, nine families of larvae were produced by crossing gametes from four corals (OI: A-B, PCB: C-D) on 2 December following published methods (Quigley *et al.*, 2016, Chapter 4). The nine gamete crosses excluded self-crosses (Table S3.1). Larvae were stocked at a density of 0.5 larvae per ml in static culture vessels (one per family) and supplied with 1 µm filtered seawater in a temperature-controlled room set at 27°C (ambient seawater temperature). Water was changed one day after fertilization and every two days thereafter. For settlement 25 settlement surfaces (colour-coded glass slides) were added to each larval culture vessel six days post-fertilization, along with chips of ground and autoclaved crustose coralline algae (CCA) (*Porolithon onkodes* collected from SE Pelorus: 18°33'34.87"S, 146°30'4.87"E). The number of settled juveniles was quantified for each family, slides from each family were placed randomly within as well as across the three slide racks sealed with gutter guard mesh, and the racks affixed to star pickets above the sediments in Little Pioneer Bay (18°36'06.2"S, 146°29'19.1"E) 11 days post fertilization (13 December 2012). Slide racks were collected 29 days later (11 January 2013) after which natural infection by *Symbiodinium* had occurred as observed using light microscopy. Juveniles were sampled from each cross (n=6-240 juveniles/family, depending on survival rates), fixed in 100% ethanol and stored at -20°C until processing.

In 2013, 25 families were produced from gamete crosses among eight parental colonies: four from PCB and four from Orpheus Island (full details of colony collection, spawning, crossing and juvenile rearing in Quigley et al., 2016 (Chapter 4) (Table S3.2). Larvae were raised in three replicate cultures per family. Settlement was induced by placing autoclaved chips of CCA onto settlement surfaces, which were either glass slides, calcium carbonate plugs or the bottom of the plastic culturing vessel. Settlement surfaces with attached juveniles were deployed randomly to the same location in Little Pioneer Bay as in the previous year, 19 days post fertilization and collected 26 days

later. Samples of juveniles (1-194 juveniles per family) were preserved in 100% ethanol and stored at -20°C until processing.

3.3.2 Vertically-transmitting broadcast spawner

Thirty-two gravid colonies of *Montipora digitata,* which spawns zooxanthellate eggs, were collected from Hazard Bay (S18°38.069', E146°29.781') (dams 1-27) and Pioneer Bay (S18°36.625', E146°29.430') (dams 28-32) at Orpheus Island on the 30th March and 1st of April 2015. Colonies were placed in constant-flow, 0.5 µm filtered seawater in outdoor raceways at Orpheus Island Research Station. Egg-sperm bundles were collected from a total of nine different colonies on the 4th and 5th of April, filtered through a 100 µm mesh and rinsed three times to remove sperm from the eggs. Individual eggs and adult tissue samples were then placed in 100% ethanol and stored at -20°C until processing.

3.3.3 Deep sequencing of juveniles and eggs

The number of juveniles of *A. tenuis* sequenced from each of the 9 crosses in 2012 ranged from 2-29 individuals (average: 11.3±SE 3) (Table S3.1); a single sample from each parental colony was sequenced concurrently. In 2013, the number of *A. tenuis* juveniles sequenced from each of the 20 (of the 25) families that survived field deployment ranged from 1-21 individuals (average: 8.6±1 SE) (Table S3.2). Three samples per colony were sequenced concurrently for Orpheus parents and one sample per colony for Princess Charlotte Bay parents. For *M. digitata,* 5-12 eggs per dam were sequenced, and one sample per maternal colony sequenced concurrently.

DNA was extracted from juveniles of *A.tenuis* in 2012 and 2013 with an SDS method as described in Quigley *et al.*, 2016 (Chapter 4). For *M. digitata,* single egg extractions used the same extraction buffers and bead beating steps as above, although without the subsequent washes and precipitation steps because of the small tissue volumes of single eggs (Gloor *et al.*, 1993). Library preparation, sequencing and data analysis were performed separately for 2012 and 2013 samples of *A. tenuis* and *M. digitata,* as described in Quigley *et al.*, 2016 (Chapter 4). Briefly, the USEARCH pipeline (v. 7) (Edgar, 2013) and custom-built database of all *Symbiodinium*-specific NCBI sequences were used to classify reads (Altschul *et al.*, 1990; Camacho *et al.*, 2009), with blast hits above an E-value threshold of 0.001 removed, as they likely

represented non-specific amplification of other closely-related Dinoflagellata (Table S3.3). *Symbiodinium* OTUs listed as uncultured or *ex situ* were assigned these terms, either because their NCBI identifiers were listed as uncultured or because they were identified only by their host isolate name (e.g. *Amphisorus hemprichii*). In order to assess if *Symbiodinium* community diversity was estimated correctly with this sampling strategy, rarefaction curves and the number of unobserved OTUs were estimated using the packages '*iNext*' and '*vegan*' (Oksanen *et al.*, 2013; Hsieh *et al.*, 2016). From the juvenile samples of *A. tenuis* collected in 2012, 6/102 samples had underestimated *Symbiodinium* community diversity. These juveniles were from three families (AB, BC, and CB), and only represented 4.5-14% of the juveniles from those families. From juveniles collected in 2013, 22/172 samples were underestimated, with juveniles from 11 of the 20 families. These juveniles however were only underestimated by very few OTUs (average 2.9±0.9). All adults from 2012 and 2013 were sequenced to saturation. It is therefore unlikely that diversity measures and heritability estimates were affected substantially due to the relatively few juveniles that were under-sequenced and their overall low number of "missing" OTUs.

3.3.4 Data analysis and visualization

Fan dendrograms were constructed using a raw alignment function and neighbour joining tree algorithm from the 'ape' package (Paradis *et al.*, 2004). Sample metadata were mapped onto trees using the package 'diverstree' (FitzJohn, 2012). To aid in visualizing the phylogenetic relationships on the tree, only OTUs that were found within at least three samples were kept, reducing the total OTU count from 422 to 134 for 2012 samples and from 568 to 181 for 2013 samples, giving an overall total of 315 OTUs for *A. tenuis*. Furthermore, as *A. tenuis* data for the two years were clustered and mapped separately, it is conceivable that some OTUs were shared between the two datasets. To determine the overlap in OTUs between years, the 315 OTUs were aligned in Clustal OMEGA (Sievers *et al.*, 2011) and those that clustered together and also Blasted to the same accession number (54/315) were deemed to be the same OTU, resulting in a total of 261 distinct OTUs. In total, 80 unique OTUs were found in 2012, 127 were found in 2013, and 54 were shared between years. OTUs with a relative normalized abundance of less than 0.01% were classified as "background", whilst those with abundances greater than 0.01% were considered "principal." OTUs were further classified by ubiquity across samples, as either: "core" (OTUs found in >75% of

samples), "common" (25 -75% of samples) or "rare" (0 -25%). As far fewer OTUs were recovered from *M. digitata* samples, all 101 OTUs were used from the one year sampled, and were classified by abundance and ubiquity as described above. Differential abundance testing was performed with 'DESeq2', with Benjamini-Hochberg p-adjusted values at 0.05. Networks and heatmaps were constructed using un-weighted Unifrac distances of the normalized *Symbiodinium* abundances in eggs only, where maximum distances were set at 0.4.

3.3.5 Heritability

The *Symbiodinium* community associated with each adult, juvenile (*A. tenuis*) or egg (*M. digitata*) of the two coral species was characterized as a continuous quantitative trait, by converting community composition into a single diversity metric, as detailed in Chapter 2. Collapsing complex assemblage data into a single diversity value (local diversity measure) (Leinster and Cobbold, 2012) was necessary so that univariate heritability statistics could be applied. The Leinster and Cobbold diversity metric incorporates variance-normalized OTU abundances from linear models using negative binomial distributions, OTU sequence diversity, and OTU rarity (Leinster and Cobbold, 2012). Incorporating both abundance and diversity of *Symbiodinium* types into heritability estimates is essential, as even changes in *Symbiodinium* community abundance dynamics change the functional output of the symbiosis as a whole (Cunning *et al.*, 2015) and are important in determining coral resilience and bleaching susceptibility (Cunning and Baker, 2012, 2014; Cunning *et al.*, 2015). The metric is a superior indicator of S*ymbiodinium* community diversity because it incorporates multiple aspects of diversity instead of a single estimate of species richness.

3.3.5a Regression-based estimates of heritability

Phenotypic values of offspring can be regressed against parental midpoint (average) phenotypic values, with the slope being equal to the narrow-sense heritability of the trait of interest (Falconer and Mackay, 1995; Visscher et al., 2008). Parental midpoint values were calculated by taking the average of the dam and sire *Symbiodinium* diversities for each family and then regressed against diversity values for the offspring of each family. Precision of the heritability estimate increases when parents vary "exceptionally" in the trait of interest, therefore, inclusion of parents

representing phenotypic "extremes" increases precision (Falconer and Mackay, 1995). Here I consider biological "extremes" in *Symbiodinium* communities as being those that are dominated by either type D or C communities, given ample evidence of contrasting physiological impacts on coral hosts by different dominant *Symbiodinium* clades (i.e., differential bleaching outcomes associated with D *versus* C communities; Cunning *et al.*, 2015). Therefore, parental colonies selected for breeding crosses were dominated by C1 (families W10, W5) or had mixed communities of C1/D1 (W7), C1/D1/D1a (W11, PCB4, PCB9, PCB6, PCB8), or multiple A, C1 and D types (OI3, 4, 5, 6) (Figures S3.1, S3.2).

3.3.5b Bayesian linear mixed model estimates of heritability

Heritability estimates were derived from estimates of additive genetic variance calculated from the 'animal model,' a type of quantitative genetics mixed effects model incorporating fixed and random effects, and relatedness coefficients amongst individuals (Kruuk, 2004). The animal model was implemented using Bayesian statistics with the package 'MCMCglmm' (Hadfield, 2010). The model incorporated the diversity metric calculated for each juvenile and the pedigree coefficient of relatedness as random effects. Bayesian heritability models were run with 1.5 $x10^6$ iterations, a thinning level of 800 (*A. tenuis*) or 250 (*M. digitata*), and a burn-in of 10% of the total iterations. A non-informative flat prior specification was used, following an inverse gamma distribution (Wilson *et al.*, 2010). Assumptions of chain mixing, normality of posterior distributions and autocorrelation were met. The posterior heritability was calculated by dividing the model variance attributed to relatedness by the sum of additive and residual variance. The impact of environmental covariance (V_{EC}) was reduced by randomly placing families within the outplanting area (Falconer and Mackay, 1995). Maternal environmental effects, also assessed within these models, were not significant for either *A. tenuis* or *M. digitata,* based on Deviance Information Criteria (DIC) from Bayesian models (Wilson *et al.*, 2010). The influence of settlement surface for *A. tenuis* juveniles in 2013 was assessed using linear mixed models (fixed effect: substrate, random effect: family) in the 'nlme' package (Pinheiro *et al.* 2014), with the first principal component extracted from PCoA plots incorporating weighted unifrac distances of normalized *Symbiodinium* abundances for juveniles. Model assumptions of homogeneity of variance, normality, and linearity were met. Substrate

type did not significantly explain *Symbiodinium* community differences among samples (LME: $F_{(4)}= 1.05$, $p = 0.38$).

3.4 Results

3.4.1 *Symbiodinium* communities associated with *A. tenuis* juveniles

After one month in the field, there were clear similarities at the clade level between *Symbiodinium* communities associated with the 2012 and 2013 families of *A. tenuis* juveniles, with 54 OTUs (17.1%) shared between the two years (Figure 3.1A), including similar proportions of OTUs retrieved across the clade level (Table S3.4). In both years, the majority of OTUs were recovered from three clades (2012 vs. 2013 proportional abundances): A (41.7% vs. 28.1%), C (14% vs. 31.3%) and D (42.6% vs. 35.7%). Moreover, the number of OTUs from each of these clades was similar between years (clade A: 72 vs. 88 OTUs; clade C: 110 vs. 119; clade D: 44 vs. 54, in 2012 and 2013 respectively). *Symbiodinium* types from clades B, E, F, G, uncultured *Symbiodinium* and *ex situ* identified types were also recovered, as well as clades H and I from the 2013 families.

The 422 OTUs from 2012 *A. tenuis* juveniles could be divided roughly into 112 categories including clades A-G, uncultured and *ex situ* OTUs (Figure 3.1B). The highest diversity of OTUs belonged to uncultured and C1 types (78 and 68 OTUs, respectively), although types from clades A and D were also diverse and included A13, A3, CCMP828, D1 and D1a (Figure 3.1B). The predominant patterns characterising *Symbiodinium* communities associated with the 2012 families were the high abundance of *Symbiodinium* types A3, C1, D1, and A-type CCMP828, and the comparatively lower abundance of D1a. Substantial variation in the diversity and relative abundance of these types and others was present both within families and amongst them (Figure S3.3, Supplementary Results in Appendix B). Juveniles harboured more unique OTUs compared to adults (111 unique OTUs compared to 2 in adults), with only 21 OTUs shared between life stages. Many of the *S. microadriaticum* OTUs were only found in 2012 and there were also D, D1 and D1a OTUs that were unique to this sampling year (Figure 3.1A). A majority of all OTUs were rare (112 OTUs found in less than 25% of samples), whilst 16 were common (25-75% of samples), and 6 core types (two A3 types, CCMP828, C1, D1, D1a).

The greatest diversity of OTUs found in juveniles in 2013 belonged to C1, A3 and uncultured types (Figure 3.1B), as found in juveniles from 2012. In contrast to 2012 families, however, OTUs were retrieved from all 9 *Symbiodinium* clades (A-I), potentially due to the increased number of juveniles sequenced. In addition to the recognized *Symbiodinium* species detected in 2012 samples, *S. natans,* and *S. kawagutii* were also detected in 2013 samples, along with other well-known types C3, C33 and H1, amongst others (Figure 3.1B). The most abundant OTUs recovered belonged to C1, D1, A3, A-type CCMP828, D1a, and two other D-types (Figure S3.4). Both inter- and intra- family variation in *Symbiodinium* species composition and abundance were detected at the clade and type level, with 2013 families generally able to be divided into low-diversity and high-diversity families, although three families had juveniles with both characteristics (Figure S3.4, Supplementary Results in Appendix B, Table S3.5). A majority of OTUs were unique to juveniles (151), with far fewer OTUs unique to adults (2) or found in both life stages (28). Furthermore, the majority of OTUs were at background abundances, comprising less than 0.01% of normalized abundances (115 background OTUs vs. 57 principal OTUs). I also identified 5 core and 4 common members of *A. tenuis* early juvenile communities (Core: A3, CCMP828, C1, D1, D1a; Common: A3-OTU725, C1-OTU121, CCMP2456 and an uncultured type), with 172 OTUs identified as rare members.

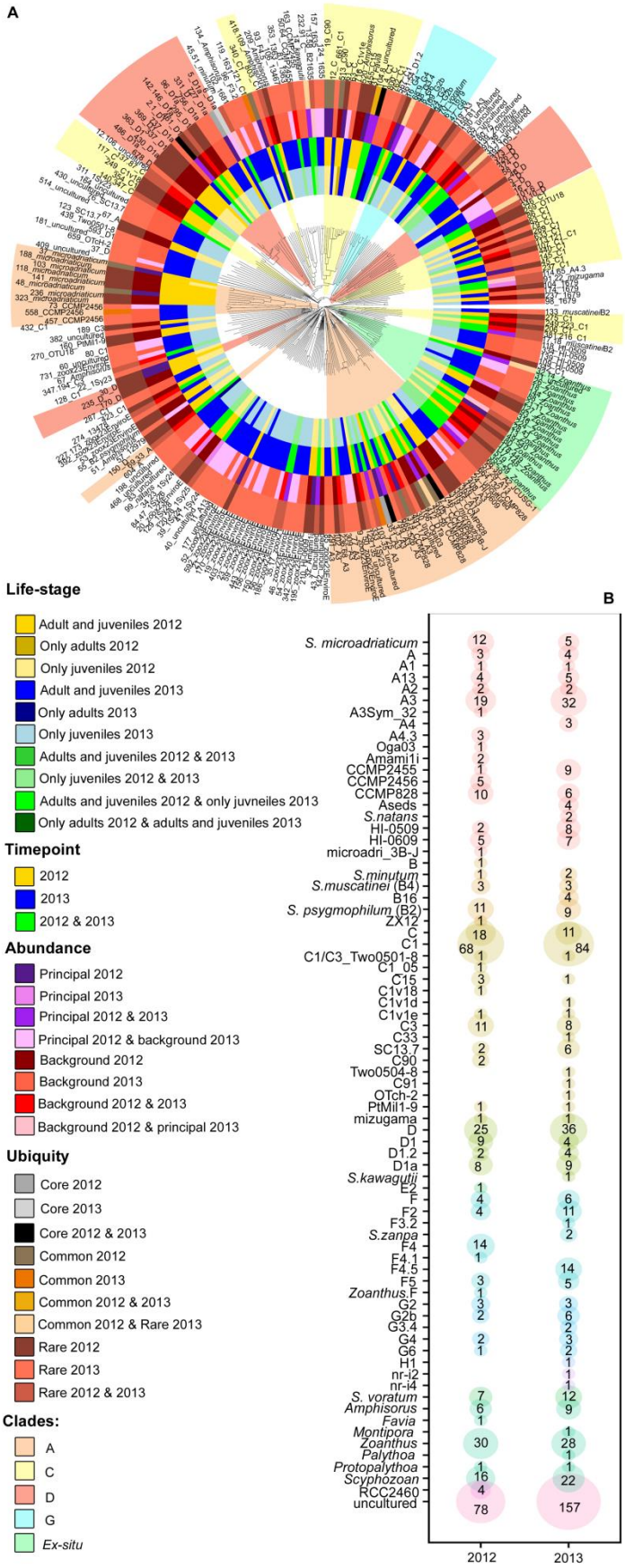
A
B
Life-stage
Adult and juveniles 2012
Only adults 2012
Only juveniles 2012
Adult and juveniles 2013
Only adults 2013
Only juveniles 2013
Adults and juveniles 2012 & 2013
Only juveniles 2012 & 2013
Adults and juveniles 2012 & only juvneiles 2013
Only adults 2012 & adults and juveniles 2013
Timepoint
2012
2013
2012 & 2013
Abundance
Principal 2012
Principal 2013
Principal 2012 & 2013
Principal 2012 & background 2013
Background 2012
Background 2013
Background 2012 & 2013
Background 2012 & principal 2013
Ubiquity
Core 2012
Core 2013
Core 2012 & 2013
Common 2012
Common 2013
Common 2012 & 2013
Common 2012 & Rare 2013
Rare 2012
Rare 2013
Rare 2012 & 2013
Clades:
A
C
D
G
Ex-situ
S. microadriaticum
A
A1
A13
A2
A3
A3Sym_32
A4
A4.3
Oga03
Amami1i
CCMP2455
CCMP2456
CCMP828
Aseds
S.natans
HI-0509
HI-0609
microadri_3B-J
B
S.minutum
S.muscatinei (B4)
B16
S. psygmophilum (B2)
ZX12
C
C1
C1/C3_Two0501-8
C1_05
C15
C1v18
C1v1d
C1v1e
C3
C33
SC13.7
C90
Two0504-8
C91
OTch-2
PtMil1-9
mizugama
D
D1
D1.2
D1a
S.kawagutii
E2
F
F2
F3.2
S.zanpa
F4
F4.1
F4.5
F5
Zoanthus.F
G2
G2b
G3.4
G4
G6
H1
nr-i2
nr-i4
S. voratum
Amphisorus
Favia
Montipora
Zoanthus
Palythoa
Protopalythoa
Scyphozoan
RCC2460
uncultured
2012
2013

Figure 3.1 A) Fan dendrogram of 261 *Symbiodinium* ITS-2 OTUs retrieved from *Acropora tenuis* juveniles and adults in 2012 and 2013. The Neighbour-Joining dendrogram was constructed using "raw" APE alignments of only those OTUs that were retrieved from 3 or more samples (134/422 OTUs in 2012 and 181/568 OTUs in 2013). Concentric circles from innermost to the outermost position represent OTUs present: 1) Life-stage, 2) Year, 3) Normalized abundance (principal: > 0.01%, background < 0.01%), 4) Ubiquity (Core: >75% of samples, Common: 25-75%, Rare: < 25%). Semi-transparent coloured triangles represent clade designation to which OTUs belong. **B**) Type level *Symbiodinium* diversity retrieved per year. Colours represent the clade designations and the size of the circles represent the number OTUs collapsed per type. Circle diameters are proportional to the number of OTUs. The first column represents OTUs sampled from juveniles in 2012 and the second represents OTUs sampled from juveniles in 2013.

3.4.2 *Symbiodinium* communities associated with *M. digitata* eggs

99.1% of the total cleaned reads belonged to C15 (OTU1) out of the 101 OTUs retrieved, with this type making up 98.8 % (±0.5 SE) and 99 % (±0.1 SE) of all reads retrieved from dams and eggs, respectively. However, the other 100 OTUs were found dispersed across eggs and adults, with on average 7 (±0.9 SE) OTUs per egg and 5.3 (±0.9 SE) OTUs per adult. The most abundant OTUs following C15 were an *ex situ Amphisorus* identified OTU (potentially another C15 type), D1, A3.1, and two other A types (A and CCMP828). The highest diversities of OTUs were retrieved from clades A (73) and C (18), whereas D and *ex situ* each had 3 OTUs represented. Adults could generally be distinguished from eggs by the unique presence of A2, A3, HA3-5, C1_8, and G3 (OTU69, 68, 119, 18, 67) (Figure 3.2A) and a greater proportional abundance of a type A symbiont (OTU4) in dams 29, 32, 7, 8 and 9 (lower: 26, 28, absent: 11, 24)(Figure S3.5). This type was only found in one egg (24r8). However, of these 5 unique adult OTUs, none were found in more than 2 adult colonies. 82 OTUs were found in eggs and not adults, of which 43 were found in 3 or more eggs, and a majority were uncultured types at background levels from the eggs of dam 29 (Figure 3.2A). Of the 14 OTUs shared between life stages, 12 were found in 3 or more samples.

Eggs from dams 24, 7, 8, and 9 were similar in their background diversity, associating with an *ex situ Amphisorus* type (OTU2), D1 (OTU3), D1a (OTU6), C1 (OTU8), A3 (OTU5) and another C15 type (OTU50) (Figure S3.5, Table S3.6). D1a was found in a total of 15 eggs, but only 1 adult (9), with a majority of these eggs from dam 9 (4 eggs). Eggs from dams 11, 26, 28 and 32 had the OTUs outlined above, as well as different A (CCMP828-OTU12, but not eggs from dam 32) and C1 types (OTU10), and their own unique types per egg family (Figure S3.5, Table S3.1). Unique types detected in eggs included B1-OTU31 in eggs from dam 11, uncultured type-OTU59 (dam 26), *S. microadriaticum*-OTU29 (dam 28), and C/D/*ex situ* types (dam 32). Whilst OTU diversity was generally low in eggs, eggs from dam 29 had very high diversity (although dam 29 diversity was low), with 4 of 12 eggs having 31-65 OTUs, 2 having 11-13 OTUs, and the remaining 6 eggs having 2-5 OTUs (Figure S3.5, Table S3.6). Furthermore, particular eggs from this dam were distinct from the other symbiont communities in eggs (Figure 3.2, Figure S3.5, Table S3.7), most likely due to the large number (n = 61) of uncultured types.

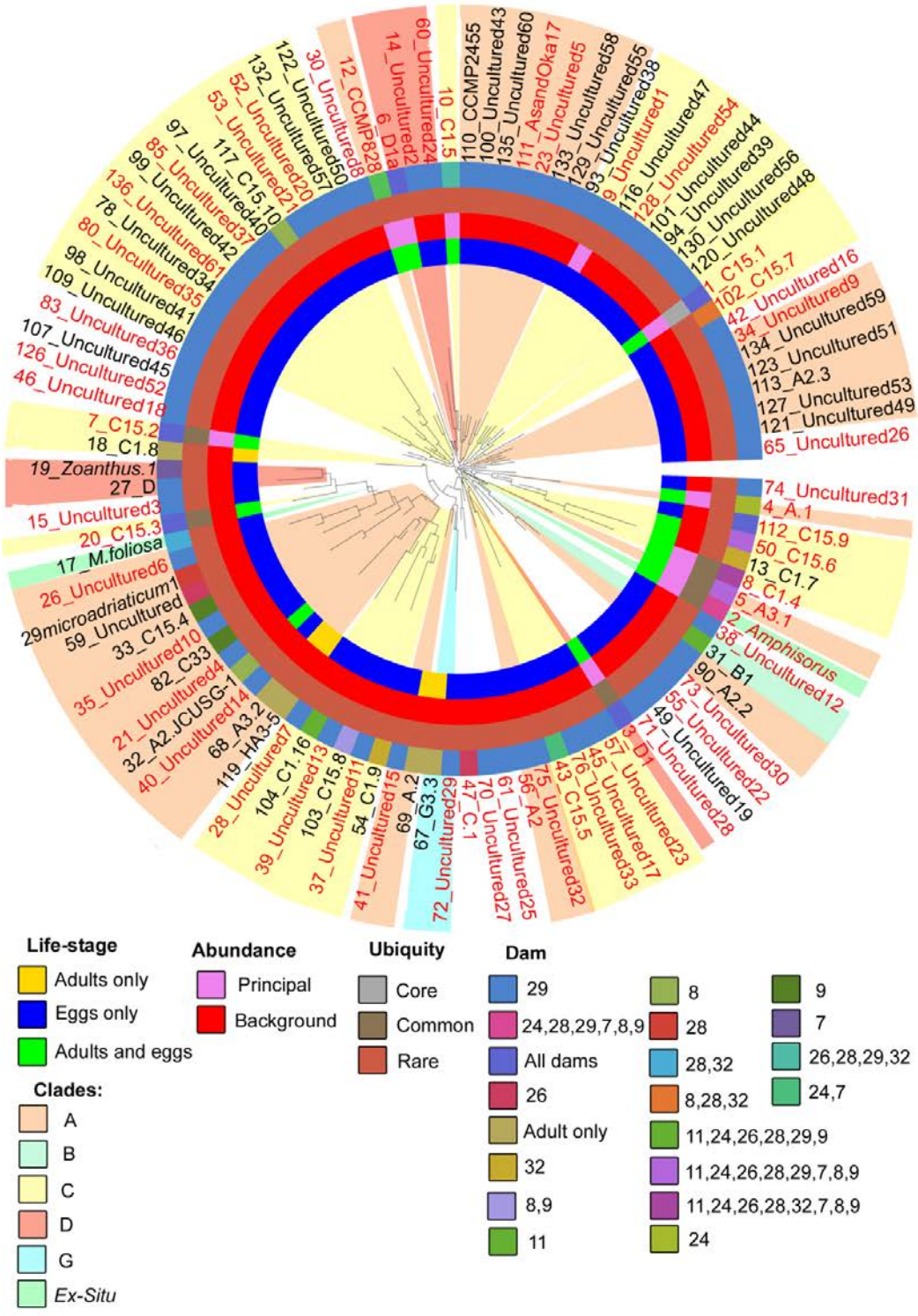

Figure 3.2 Fan dendrogram of 101 *Symbiodinium* ITS-2 OTUs retrieved from *Montipora digitata* eggs and adults. The Neighbour-Joining dendrogram was constructed using "raw" APE alignments. Concentric circles from innermost to the outermost position represent OTUs present: 1) Life-stage, 2) Normalized abundance (principal: > 0.01%, background < 0.01%), 3) Ubiquity (Core: >75% of samples, Common: 25-75%, Rare: < 25%), and 4) Dam identity. Semi-transparent coloured triangles represent clade designation to which OTUs belong.

3.4.3 Heritability of *Symbiodinium* community in *A. tenuis* juveniles and *M. digitata* eggs

3.4.3a Narrow-sense heritability estimate through regression (trait-based estimation)

Mid-parent regression estimates for the 29 *A. tenuis* families from 2012 and 2013 indicated that narrow-sense heritability (h^2) of the *Symbiodinium* community is 0.3 (Figure S3.7). Parent-offspring regression analysis of the 99 *M. digitata* eggs genotyped from 9 dams resulted in a heritability estimate of 0.156 (slope= 0.078 x 2 as a single parent) (Figure S3.8). Therefore, 30% and 16% of the measured variation in the *Symbiodinium* community in *A. tenuis* and *M. digitata*, respectively, was due to genetic differences.

3.4.3b Relatedness-based comparison

Bayesian linear mixed models, and specifically, the animal model, were used to verify heritability measurements, as they are more robust to unbalanced designs compared to regression analysis. Furthermore, the animal model utilizes all levels of relatedness between individuals in a given dataset, and not just parent-offspring comparisons, thus giving it greater power for data collected in field studies (Wilson *et al.*, 2010). The narrow sense heritability estimate (h^2) of the *Symbiodinium* community in *A. tenuis* juveniles was 0.29, with a 95% Bayesian credibility interval for the additive genetic component of 0.06-0.86. The mean heritability is 0.36 (±0.21 SD)(Table 3.1). Although the credibility interval is very large, the posterior distribution of h^2 suggests that the statistical support around 0.29 is quite high because of the high density of estimates between 0.2-0.4. Therefore, similar to estimates calculated through regression of parent and offspring phenotypes above, 29% of the variation in *Symbiodinium* community was found to be due to genetic effects. The maternal transfer of *Symbiodinium* in the broadcast spawning coral *M. digitata* had a narrow sense heritability estimate of 0.62 (0.27-0.86 95% Bayesian credibility interval), with a mean heritability of 0.57 (±0.16 SD)(Table 3.1). Furthermore, models including maternal effects arising from eggs developing in a shared maternal environment (maternal environmental effects for both *A. tenuis* and *M. digitata*) were not significantly better than those that did not include maternal effects (DIC no maternal environmental effects included < DIC maternal environmental effects included), suggesting that maternal

environmental effects did not significantly explain similarities in *Symbiodinium* diversity among eggs or among juveniles.

Table 3.1 Summary of the narrow-sense heritability methods and estimates used in this study.

Heritability estimation method:	**Trait-based (regression)**	**Relatedness-based (Bayesian)**			
	h^2 (slope)	**h^2 (mode)**	**95% Bayesian credibility interval**	**h^2 (mean)**	**± SD**
A. tenuis	0.30	0.29	0.06-0.86	0.36	0.21
M. digitata	0.16	0.62	0.27-0.86	0.57	0.16

3.5 Discussion

Quantification of the *Symbiodinium* community in two broadcast spawning corals with contrasting modes of symbiont transmission revealed that heritability of the community differs from patterns of fidelity expected based on current paradigms for vertical and horizontal transmitters. Surprisingly, mean Bayesian heritability estimates for *Symbiodinium* communities associated with larvae of *Acropora tenuis* were higher (0.29) than the low levels of fidelity expected for environmentally-acquired symbionts. Conversely, heritability estimates for *Symbiodinium* communities associated with eggs of *Montipora digitata* were lower (0.62) than the high levels of fidelity expected for vertically-transmitted symbionts.

Substantial heritability of the *Symbiodinium* community in both vertically- and horizontally-transmitting corals highlights the important role of host genetics in governing the composition of symbiont communities in corals. These results are consistent with studies that have demonstrated a similarly important role of host genetics in governing the composition of symbiotic bacterial communities in mammals, insects and cnidarians (Ley *et al.*, 2008; Benson *et al.*, 2010; Campbell *et al.*, 2012; Franzenburg *et al.*, 2013), as well as the abundance of bacteria in insects (Parkinson *et al.*, 2016) and humans (Liu *et al.*, 2015). Importantly, the partial genetic regulation of variation in the diversity of *Symbiodinium* communities found in these two coral species suggests there is potential for the symbioses in both species to evolve and adapt, contributing to the development of optimal symbiont-host partnerships in a changing environment.

These findings of 0.29-0.62 narrow-sense heritability estimates are consistent with what is known about factors regulating *Symbiodinium*-coral symbioses, which have the characteristic hallmarks of host-controlled regulation. For example, *Symbiodinium* cells are enveloped in a host-derived symbiosome, with only a few (2-8) symbiont cells per host membrane (Davy *et al.*, 2012), indicating that the host regulates *Symbiodinium* on an almost individual cell basis, facilitating overall population regulation (Parkinson *et al.*, 2016) and potentially community composition within the holobiont. Thus it is not surprising that it would be advantageous for the host's molecular architecture governing the *Symbiodinium* community to be passed from one generation to the next. Mapping the genetic loci responsible for these heritability estimates will be the next step, with further work potentially identifying mechanisms through targeted gene expression,

whole transcriptomic and QTL analysis, including knock-down trials to confirm candidate loci and putative pathways (Spor *et al.*, 2011).

3.5.1 *Symbiodinium* community in *A. tenuis*

The discovery of heritable variation (29%) of the *Symbiodinium* community in *A.tenuis* juveniles is surprising given that they acquire *Symbiodinium* exclusively from the environment. Strong temporal stability in the relative proportions and numbers of OTUs retrieved for both the principal and background *Symbiodinium* clades and types (excluding uncultured *Symbiodinium*) between the two years suggests that genetic regulation governing the *Symbiodinium* community extends to types found at very low abundance. Alternativly, as a majority of the variability in symbiont community is due to environmental sources (71%), similarities between symbiont communitites across years may also be due to the stability of the free-living *Symbidonium* populations in the seawater and sediments. One mechanism that could increase fidelity of the *in hospite* community in organisms with horizontal transmission would be the development of strong host genotype – symbiont community associations, which have been implicated through phylosymbiosis analyses (i.e, comparing symbiosis prevalence across phylogenetic relationships) in *Hydra*, wasps, and primates (Douglas and Werren, 2016). Furthermore, moderate to high heritability increase the efficacy of breeding schemes (Visscher *et al.*, 2008), which may be of particular importance as more coral restoration efforts are focused on assisted evolution of hosts, microbes and the holobiont in general. These results will aid in the development of active reef restoration methods that take advantage of the link between genetic mechanisms and desired phenotypes (Visscher *et al.*, 2008).

These results for *A. tenuis* juveniles provide the first in-depth picture of the complexity of the *Symbiodinium* community during the first month of uptake for a broadcast spawning coral. In contrast to a previous study, which suggested that month-old juveniles at this location were dominated by a clade D *Symbiodinium* type, with only low levels of C1 and A-types detected (Abrego *et al.*, 2009). Alternately, these data suggest that only 14.7- 22% of juveniles (15/102 in 2012 and 38/172 in 2013) were dominated (> 50%) by a D type *Symbiodinium*, potentially signifying temporal changes in the free-living symbiont community over time. Importantly, types C1, A3, and A-type CCMP828 also dominated communities associated with some juvenile *A. tenuis*, and no juveniles exclusively hosted a single clade or type, a result corroborated by lab

and field based experiments (Gómez-Cabrera *et al.*, 2008; Abrego *et al.*, 2009; Cumbo *et al.*, 2013; Suzuki *et al.*, 2013; Yamashita *et al.*, 2013; Kuniya *et al.*, 2015). Notably, the majority of D-dominated juveniles came from 4 of the 29 crosses (F5 (9 juveniles), F19 (5), BC (5), AD (5)), suggesting that juveniles from these genotypic crosses have a higher propensity to establish symbiont communities dominated by *Symbiodinium* D, presumably because of their genetic architecture. Microscale differences in *Symbiodinium* abundances in the sediments or water column may also have caused the increased propensity to harbour clade D symbionts in these families and highlights the need for further studies mapping the diversity of free-living *Symbiodinium*.

The significance of the many *Symbiodinium* types found in extremely low abundance for juvenile and adult physiology is currently unclear. In adult corals, fine scale dynamics (i.e. changes in relative abundance or variable diversity of only a few types within a larger community) of *Symbiodinium* communities are very important to bleaching susceptibility, recovery and physiology (Parkinson *et al.*, 2015; Cunning *et al.*, 2015). Although background types are likely to have a negligible effect compared to principal types like A3, C1, and D1 when corals are healthy, they may increase in importance when environmental conditions are sub-optimal. On the other hand, the presence of many low-abundance types may have negative fitness outcomes for coral juveniles if they reflect the inability of the coral host to maintain stable symbioses with beneficial types. This hypothesis is supported by the observation of high *Symbiodinium* diversity in juveniles from high mortality coral families, particularly in the number of "unculturable" types observed (Quigley *et al.*, 2016) (Chapter 4). These communities contrasted markedly with those of juveniles from low-mortality families, which had a very conserved and specific diversity and suggests that high-mortality families had lost the ability to regulate community diversity, leading to proliferation in the number of types present at initially low background densities and potentially opportunistic types. Such proliferation of initially low abundance types could represent dysbiosis of symbiotic communities, which is known to lead to disease in human populations (Spor *et al.*, 2011; Vangay *et al.*, 2015) and could also impact coral health outcomes.

3.5.2 *Symbiodinium* community in *M. digitata*

Although the heritable signal found for *Symbiodinium* communities associated with eggs of the broadcast spawning coral *M. digitata* was strong (62%), even greater

fidelity was expected, given that eggs acquire *Symbiodinium* communities in the maternal environment. Despite *Symbiodinium* C15 dominating symbiont communities in both eggs and dams, maternal transfer lacked precision in one dam (dam 29) in particular, whose eggs had highly variable *Symbiodinium* communities that included uncultured OTUs, similar to previous reports from this coral genus (Padilla-Gamiño *et al.*, 2012). There are many precedents for inexact maternal transfer of the symbiont community, including studies in insects showing that vertical transmission is rarely perfect (Oliver *et al.*, 2014) due to intra-symbiont competition (Moran *et al.*, 2008). Such imprecision in maternal transfer is a product of the fitness costs associated with the maintenance of superinfections (stable coexistence of multiple symbionts) and can be overcome if selection for coexistence is greater than such costs, as superinfections may provide distinct benefits in the form of different traits conferred by each symbiont (i.e., different symbionts provide different nutrients to the host)(Vautrin and Vavre, 2009). For *M. digitata,* imprecision may represent a type of bet-hedging to maximise the likelihood that some offspring will survive when eggs are dispersed away from the parental habitat and encounter environments that select for traits associated with different symbionts. This variation also highlights the potential flexibility of the *M. digitata-Symbiodinium* symbioses, which may enable the host to vary its symbiotic partnerships in response to environmental change by benefitting from new host-symbiont combinations.

Surprisingly, much of the diversity found in *M. digitata* eggs was not present in parent colonies, a result previously observed in larvae of the brooding, vertically-transmitting coral *Seriatopora hystrix* (Chapter 2) and between *A. tenuis* juveniles and adults (this study). This suggests that eggs are acquiring symbionts from sources external to the maternal transmission process or may represent incomplete sampling of *Symbiodinium* diversity within adult communities. Mixed systems involving both vertical and horizontal transmission are known (e.g. bacteria in clams; reviewed in Douglas and Werren, 2016) and have recently been demonstrated in brooding corals (Chapter 2). The cellular machinery needed for recognition of appropriate *Symbiodinium* types (Davy *et al.*, 2012) would not be developed in the egg cytoplasm, where *Symbiodinium* are present pre-fertilization (Hirose and Hidaka, 2006). Therefore, eggs exposed to transient symbionts in the dam's gastrovascular cavity or by parasitic *Symbiodinium*-containing vectors (e.g. ciliates Ulstrup *et al.,* 2007; symbiont transmission by parasitic vectors: reviewed in Moran *et al.*, 2008), may retain these

communities until recognition systems of larvae or juveniles mature. Interestingly, one type (OTU111) found in three eggs from dam 29's family were identified as a free-living A type recovered from Japanese marine sediments (EU106364, Hirose *et al.*, 2008), suggesting that these unique OTUs and potentially others found in eggs represent non-symbiotic, potentially opportunistic symbionts. Further work needed to determine the potential ecological role of these symbionts includes comparing communities in eggs to fertilized larvae to see if they are retained.

3.5.3 Heritable mechanisms

Maternal environmental effects, such as dam lipid contributions or fitness, have well known effects on the early life stages of many marine organisms (Ritson-Williams *et al.*, 2009). However, these analyses show that Bayesian models were not significantly improved with the addition of dam identity, suggesting that significant heritability estimates are attributable to genetic effects and not due to maternal environmental effects arising from eggs sharing the same dam (Wilson *et al.*, 2010), and also excluding others such as cytoplasmic inheritance (Wolf and Wade, 2009). Whilst I can only speculate about the exact mechanisms that are being inherited by offspring, likely candidates may be those involved in recognition and immunity pathways (Davy *et al.*, 2012), with cell-surface proteins playing an important role in the selection of specific *Symbiodinium* strains by coral hosts (Schwarz *et al.*, 1999; Weis *et al.*, 2001; Markell and Wood-Charlson, 2010), such as Tachylectin-2-like lectins, which have been implicated in the acquisition of A3 and a D-type in *A. tenuis* (Bay *et al.*, 2011; Fransolet *et al.*, 2012; Kuniya *et al.*, 2015). Indeed, suppression or modification of the immune response has often been implicated in the formation of the *Symbiodinium*-cnidarian partnership (Schnitzler and Weis, 2010; Davy *et al.*, 2012; Mohamed *et al.*, 2016). Although this has not yet been demonstrated in corals, human studies have shown that immune system characteristics underpin heritable components of the genome (h^2=~0.36)(Cho, 2015) and at least 151 heritable immunity traits have been characterized, including 22 cell-surface proteins (Roederer *et al.*, 2015).

Similarly, the juvenile coral may be primed to take up specific *Symbiodinium* types through the transfer of genetic machinery that results in a by-product(s) that ensures the juvenile is colonized by beneficial types and prevents colonization by unfavourable symbionts through competitive exclusion (e.g., maternal imprinting

controlled by offspring loci (Wolf and Wade, 2009). Such a bi-product may be something akin to amino acids, which have been shown to regulate the abundances of *Symbiodinium* populations (Gates *et al.*, 1995). Sugars have also been found to influence bacterial communities in corals (Lee *et al.*, 2016) and may have similar roles in regulating *Symbiodinium* communities. Trehalose, in particular, has been identified as an important chemical attractant between *Symbiodinium* and coral larvae and may help to regulate the early stages of symbiosis (Hagedorn *et al.*, 2015). Human studies also provide examples of sugars (both maternal and offspring derived) that make infant intestines less habitable for harmful bacteria, setting up conditions for preferential colonization by favourable bacteria (Lewis *et al.*, 2015). Bacterial diversity in cnidarian hosts can also be modulated through the production of antimicrobial peptides (Franzenburg *et al.*, 2013) and bacterial quorum sensing behaviour (Golberg *et al.*, 2011). Although neither of these mechanisms has been explored with respect to the regulation of *Symbiodinium* communities, similar host/symbiont bi-products may also be produced to modulate their presence and abundance.

3.5.4 Conclusion

My results provide new insights into the role of host genetics and inheritance in governing *Symbiodinium* communities in corals, which is crucial for understanding factors governing coral health and fitness, particularly under stress conditions (i.e. bleaching risk: Cunning *et al.*, 2015), and the potential for host-symbiont partnerships to evolve. I describe substantial variation in the *Symbiodinium* communities associated with individuals and families in two broadcast spawning corals, particularly in *A. tenuis* juveniles. Variability among and within families and evidence that the variation is heritable in both species supports the likelihood that adaptive change is possible in this important symbiotic community. The confirmation of heritability of the *Symbiodinium* community quantified here in *A. tenuis* and *M. digitata* also confirms that genetic mechanisms are being transmitted from parents to offspring that influence variability of the *Symbiodinium* community in juveniles and eggs. Adaptive change through heritable variation of symbionts is therefore another mechanism that corals have to contend with current and future stressors, such as climate change. These results provide hope that corals will be able to evolve and therefore limit further coral reef degradation.

Chapter 4: Maternal effects and *Symbiodinium* community composition drive differential patterns in juvenile survival in the coral *Acropora tenuis*

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4.1 Abstract

Coral endosymbionts in the dinoflagellate genus *Symbiodinium* are known to impact host physiology and have led to the evolution of reef-building, but less is known about how symbiotic communities in early life history stages and their interactions with host parental identity shape the structure of coral communities on reefs. Differentiating the roles of environmental and biological factors driving variation in population demographic processes, particularly larval settlement, early juvenile survival and the onset of symbiosis is key to understanding how coral communities are structured and to predicting how they are likely to respond to climate change. I show that maternal effects (that here include genetic and/or effects related to the maternal environment) can explain nearly 24% of variation in larval settlement success and 5-17% of variation in juvenile survival in an experimental study of the reef-building scleractinian coral, *Acropora tenuis*. After 25 days on the reef, *Symbiodinium* communities associated with juvenile corals differed significantly between high-mortality and low-mortality families based on estimates of taxonomic richness, composition, and relative abundance of taxa. These results highlight that maternal and familial effects significantly explain variation in juvenile survival and symbiont communities in a broadcast-spawning coral, with *Symbiodinium* type A3 possibly a critical symbiotic partner during this early life-stage.

4.2 Introduction

Ecological and biological processes commonly recognized to shape community composition include predation, competition and stochastic events that naturally change the abundance and diversity of species through time. However, for coral reef communities facing an increasing number of pressures that impact their productivity, health, and composition (Ban *et al.*, 2014), processes influencing the replenishment of coral populations must also be considered. For reefs to recover and contend with mounting pressures, larval settlement and juvenile survival must be at least equal to or higher than mortality rates of mature colonies. Studies to date suggest that these early life-history processes may be substantially influenced by parental genotype and condition, however, the roles of parental genetics and transgenerational effects in enhancing larval and juvenile survival have only begun to be empirically tested for corals (Császár *et al.*, 2010; Kenkel *et al.*, 2011, 2015; Baums *et al.*, 2013; Nanninga and Berumen, 2014; Dixon *et al.*, 2015). Quantification of the relative contributions of genetic and environmental impacts on larval settlement and juvenile survival is needed to predict how coral populations are likely to respond to changing selective pressures in the future. Moreover, understanding the potential of pre- and post-recruitment processes to regenerate coral populations and rates of recovery is essential to predict at-risk and sensitive areas unable to compensate for natural and human-induced disturbance (Hughes *et al.*, 2000; Baird *et al.*, 2009a; Lukoschek *et al.*, 2013).

Annual coral recruitment rates vary from 35- to 100-fold and are only partly explained by adult colony abundance and fecundity (Hughes *et al.*, 2000). Consequently, additional factors besides strict stock-recruitment relationships determine juvenile abundance on reefs. Once metamorphosed, many factors influence juvenile abundances and species composition of coral assemblages, including abiotic (e.g., environmental conditions and storms: (Mumby, 1999; Shima and Swearer, 2009) and biotic factors (e.g., parental reproductive mode: (Baird *et al.*, 2009b); accidental predation by herbivorous fish: (Doropoulos *et al.*, 2012; Trapon *et al.*, 2013); juvenile growth and intra/inter species competition (Ritson-Williams *et al.*, 2009). The timely and specific acquisition of different clades/types of *Symbiodinium*, a key dinoflagellate genus found in corals, has also been linked to juvenile survival, with clades A and D found in greater proportions in surviving juveniles of *Acropora yongei* (Suzuki *et al.*, 2013). *A. millepora* juveniles also survived better in low-light treatments when symbiont communities were composed of roughly equal proportions of C1 and D

Symbiodinium, whereas corals with C1-dominated communities survived better in high-light treatments (Abrego *et al.*, 2012). Although it is clear that environmental factors play a role in coral juvenile survival, further work is needed to elucidate and quantify the contributions of parental genotypes *versus* non-genetic maternal effects and the acquisition of symbionts on coral juvenile fates.

Maternal effects have been shown to govern variability in a range of traits among individual gametes and larvae (Visscher *et al.*, 2008) and contribute to structuring communities of marine fish and invertebrates (Nanninga and Berumen, 2014). For broadcasting corals that do not invest resources in either mating or parental care and for which gamete production is the sole reproductive investment, it is particularly critical to understand factors influencing gamete number and quality, which in turn, affect several aspects of early life-history stages (Airi *et al.*, 2014). For example, maternal provisions of lipid fulfill almost half the metabolic needs of coral larvae (Harii *et al.*, 2007, 2010) and have been shown to influence larval growth and survivorship in both fish and corals (Gagliano *et al.*, 2007; Graham *et al.*, 2013). However, the quality of maternal provisioning is dependent on the environmental conditions experienced by the mother. Variations in larval and egg morphology associated with hormone exposure within the maternal environment (McCormick, 1999) and age at maternal reproduction (Mousseau and Dingle, 1991) are further demonstrations of how non-genetic maternal effects can impact offspring fitness traits. Whilst these impacts are not due to parental genetics *per se*, they are a direct result of parental physiology impacting later generations.

Parental genotypes can contribute significantly to offspring fitness and their potential for local adaptation through selection for optimal expressions of offspring traits under local environmental conditions. In fish, maternal and paternal genetic factors are known to dictate a range of key larval traits, including size and growth, and to underpin variability in larval survival and settlement, and juvenile survivorship (Green and McCormick, 2005). In corals, the parental identity of larvae influences up to 47% of settlement success in the spawning coral *A. millepora* in aquaria (Kenkel *et al.*, 2011). Recent tank experiments with brooding corals found that 94% of the variability in juvenile survivorship and 27-30% of variability in juvenile growth in aquaria were due to familial genotypes (Kenkel *et al.*, 2015). Parental combinations also impact other early life-history traits in corals, such as protein content, affinity to settlement cues,

fertilization success and larval heat tolerance (Meyer *et al.*, 2009; Baums *et al.*, 2013; Dixon *et al.*, 2015). Lipid profile characteristics are also under substantial parental genetic regulation in human studies (Skipper, 2008; Willer *et al.*, 2008). Therefore, quantifying the impacts of parental genotype on processes governing recruitment success is key to understanding local adaptation and the potential for change, however, the impacts of parental genotype on a range of early life-history processes in corals, notably juvenile survivorship in the wild, are still unknown.

To further current understanding of the extent to which parental identities drive variation in larval survival, larval settlement and juvenile survival, this study quantified parental effects on key early life-history stages in the broadcasting coral *Acropora tenuis*. Using larvae from intra- and inter-population crosses, I determined the impacts of parental identity on larval survival, weight and settlement, and on juvenile survival in the field. I also compare *Symbiodinium* communities among families with high and low juvenile mortality and discuss potential pathways that are likely to underpin the patterns found.

4.3 Materials and Methods

4.3.1 Collection of colonies

For inter- and intra-population crosses, 14 reproductively mature colonies of *Acropora tenuis* were collected at the end of October 2013 from Wilkie Reef (13°46'44.544"S, 143°38'26.0154"E) in Princess Charlotte Bay in the far northern sector of the Great Barrier Reef. Corals were transported by boat to Orpheus Island Research Station (OIRS) and acclimated in outdoor holding tanks for 22 days under constant flow-through conditions. Ten colonies of *A. tenuis* were collected from South Orpheus on the 19th and 20th of November 2013 (18°39'49.62"S, 146°29'47.26'E) and housed at OIRS under the same flow-through conditions for one-two days prior to spawning. Colonies collected were separated by at least 5 m, and given differences in colony colour and limited success of asexual reproduction in corymbose corals, they were assumed to represent distinct genotypes.

4.3.2 Reproductive crosses and experimental design

Following signs of spawning imminence in *A. tenuis,* i.e., the appearance of egg-sperm bundles under the oral disk and polyps extended but tentacles retracted, individual colonies were isolated until gametes were released between 17:30 and 19:30 hours on the 21st November. Gamete bundles were collected for crosses among four Orpheus Island (O), and four Wilkie (W) colonies. Gametes were gently skimmed from the surface of the water, then eggs and sperm were separated by washing through a 1 µm sieve three times. The density of sperm was quantified with a Neubauer hemocytometer in triplicate replicates, and sperm mixtures then diluted to a density of approximately $1x10^6$ sperm per liter to optimize fertilization success (Willis *et al.*, 1997).

Twenty-five distinct coral families were produced by adding equal numbers of eggs from one parental colony to equal concentrations of sperm from a second parental colony and allowing eggs to fertilise for three hours (Appendix C, Table S4.1). Aliquots of developing embryos were taken from each family every hour for three hours to quantify fertilization success and cell division to the four-cell stage (Ball *et al.*, 2002). Once a majority of the fertilized eggs of all families had reached the four-cell division stage, the number of zygotes was counted in four replicate 0.5 mL samples with a light microscope in order to stock culture replicates with equal densities of developing embryos. Three culture replicates per cross were established at a density of 1.5 four-celled embryos per 2 ml (~20,475 embryos per family summed across the three replicates).

4.3.3 Larval rearing, settlement and field deployment

Each family was cultured in triplicate 9.1 L plastic containers in a temperature-controlled room set at 25° C. Two culture replicates received a constant flow of 26°C filtered seawater (FSW), whilst the third replicate was static. To maximize the chance of juveniles surviving, I chose to utilize both static and flow-through larval cultures because of variability in the success of both methods. FSW was drawn off the reef crest at OIRS and stepped through a filter system consisting of 50, 25, 10 µm filters followed by a final UV treatment. Each flow-through replicate culture had an outflow covered by a 10 µm filter and air curtain to prevent eggs and larvae from collecting on the outflow filter. Complete filtered FSW changes were undertaken on all cultures the first day post-

fertilization (pf) and on days 3, 5, 8, 10. By days 3 and 4 pf, larvae were ciliated and motile, consistent with the 96 hr stage of larval development reported for this species (Ball *et al.*, 2002). All culture replicates for Families 25 and 29 perished, despite culturing conditions identical to those of other families. Families were scored as alive or dead, in the former case (alive) based on the survival of some larvae across all three culture replicates.

Once settlement trials confirmed competent settlement behaviour (Heyward and Negri, 1999) on the sixth day post-fertilization, flow-through was turned off and a crustose coralline algae (CCA) settlement cue was provided to larvae. Chips of freshly collected CCA (*Porolithon onkodes*) from SE Pelorus (18°33'34.87"S, 146°30'4.87"E) were placed onto: 1) glass slides in culture replicate one, 2) calcium carbonate plugs positioned in a plastic stand in culture replicate two, or 3) directly onto the bottom of culture replicate vessel three. Settlement was quantified as counts of settled juveniles per culture replicate on days 9, 11, 14, and 17 pf, and then summed. Therefore settlement was quantified as the total number of settled juveniles 17 pf per family per replicate. Metamorphosed individuals (juveniles) were out-planted 19 days pf to Little Pioneer Bay, Orpheus Island (18°36'06.2"S, 146°29'19.1"E). Glass sides and calcium carbonate plugs with attached juveniles were placed in custom-built holders attached to star pickets hammered into the reef substratum. Because juveniles settled onto culture replicate 3, these vessels were also placed in the field. These settlement containers from culture replicate 3 for each family were inverted and strung randomly on horizontal metal rods attached to star-pickets. Rods were arranged so that containers were located equidistantly above coral rubble. Plastic mesh netting was affixed to the opening of each container using rubber bands to enable water exchange whilst preventing predation. In addition, the small plastic stand used to hold the calcium carbonate plugs in culture replicate 2 had high numbers of settled juveniles, therefore these plastic stands were also attached inside the containers from culture replicate 3 corresponding to their respective family. Juveniles were left in the field for 25 days, after which time the number of surviving juveniles in each family was quantified. All juveniles were subsequently preserved in 100% ethanol.

4.3.4 Larval weights

Approximately 20 swimming larvae per family were fixed in 100% ethanol 18 days pf and stored at -20°C. Two larvae per family were freeze-dried overnight and then

individually weighed to the nearest 0.0001 mg on a UMX2 ultra microbalanace (Mettler Toledo; Greifensee, Switzerland). Larval weights were combined across families to enable statistical inferences at the level of dam (n=4-8 larvae/dam) or population cross (n=10-13 larvae/population cross). Larvae could not be collected from the two families in which all larvae died (F25 and F29). Larvae of a third family (F23) were inadequately preserved for larval weight analysis. Although collecting larvae 12 days after settling had commenced may have biased calculations of mean larval weights, if larvae from a specific weight class were to settle first and become unavailable for collection, this issue would have been common to all families. Therefore any potential bias in calculating mean larval dry weight per family because of sampling time would not have affected comparisons among families.

4.3.5 Statistical design and analysis

Statistical tests were undertaken to evaluate parental effects on larval survivorship, larval weight, settlement success and juvenile survivorship. Data were partitioned in four ways: 1) population purebreds *versus* population hybrids (OO, WW vs. OW, WO); 2) population cross (OO vs. WW vs. OW vs. WO); 3) family (i.e., parental cross); and 4) maternal or paternal identity (dam, sire). Assessments of purebreds vs. hybrids and the 4 types of population crosses evaluated if local adaptation at the population level might be driving patterns at either the family or individual parent level. All three culture replicates per family were used to quantify larval survival (larvae surviving/culture). Only two culture replicates were used to quantify settlement because settlement was extremely low in all larval families in the first replicate (i.e., where settlement surfaces were glass slides). All juveniles on the calcium carbonate plugs died due to algal infestation, so only juveniles that had settled on the plastic plug stands were used from culture replicate 2. Because juveniles that had settled on the plug stands in replicate 2 and on plastic containers in replicate 3 were physically combined in the field, they were summed to quantify juvenile survival, which was measured as the number of juveniles surviving per family. All statistical tests were run in R (Team, 2013), with an alpha level at 0.05. Means are reported plus/minus their standard errors (±SE).

4.3.5a Population effects on larval survival, weight and settlement, and on juvenile mortality

Two-sided Fisher's Exact tests (R Team, 2013) were used to determine the effects of purebred *versus* hybrid lineage (n= 13 and 12 families, respectively) and specific population cross (OO vs. OW vs. WW vs. WO) (Appendix C, Table S4.3) on larval survivorship (cultures scored as alive or dead). Linear models were used to assess the impacts of lineage purity (n = 23 larvae for each purebred and hybrid comparison) and specific population cross (Appendix C, Table S4.3) on larval weights. Models were run in the R based package '*Stats*' (R Team, 2013), and the suitability of linear models was assessed with standard diagnostic plots. The package '*Multcomp*' (Bretz *et al.*, 2010) was used to extract appropriate comparisons across the population crosses and perform *post hoc* Tukey's HSD tests, adjusted for multiple comparisons using the "single-step" method. The impact of lineage purity and population cross on the number of settled individuals (n= 12 and 11 families for purebreds and hybrids, respectively; Appendix C, Table S4.3) and on the number of juveniles surviving in the field (n= 11 and 9 families for purebreds and hybrids, respectively; Appendix C, Table S4.3) was calculated using the non-parametric Kruskal-Wallis test (R Team, 2013). Tukey's *post hoc* tests of significance were generated with generalized linear negative binomial models (glms) implemented in the package '*MASS*' (Venables and Ripley, 2003).

4.3.5b Familial and parental effects on larval survival, weight, and settlement success, and on juvenile mortality

The effects of parental identity on larval survivorship (Appendix C, Table S4.3) and larval weights (Appendix C, Table S4.3) were determined using the methods described above. The familial and parental effects on settlement (Appendix C, Table S4.3) and the interactive effects of settlement number and parental identity on juvenile survival (Appendix C, Table S4.3) were assessed using glms and generalized linear mixed effects models (glmms). A Poisson distribution with observations treated as a random effect was used to model how settlement varied by dam, sire and their interaction (all random effects). The same model was used to examine the effects of each dam and sire compared to dam/sire means by treating each in turn as a fixed effect. A negative binomial mixed model was used to assess how settlement abundance (fixed effect) and parental identity (dam, sire, interaction as random effects) influenced juvenile abundance (i.e. survival) in the field. The observation-level random effect in

the first model and the negative binomial in the second model were used to account for overdispersion. The VarCorr function from the '*nlme*' package (Pinheiro *et al*. 2014) was used to assess the variability of the random effects using standard deviations. Glmms were run using the packages '*lme4*' (Bates *et al*., 2014) and '*MASS*' with Gauss-Hermite quadrature (GHQ). The addition of sire and sire-by-dam interaction accounted for no additional variability in the second glmm model and the model was refit with dam-by-settlement interaction as a fixed effect. To determine the individual effect of each dam on juvenile survivorship and her interactive effect with settlement abundance, deviation coding (each level compared to the grand mean) was specified as above, with dam, settlement and their interaction as fixed effects. Settlement as a single term was not significant, dropped and the model was refitted with the interaction term and dam as fixed effects. The packages '*ggplot2*' and '*effects*' were used to visualize model predictions (Fox, 2003; Wickham, 2009). '*Ggplot2*' was also used to calculate the R^2 value for comparison of larval weight and percent mortality. R^2 values were calculated in two ways to account for families that died at the larval culturing phase, with those families either counted as having 100% mortality or excluded from the analysis. Assumptions of homogeneity of variance, independence, non-linearity, normality and overdispersion were assessed. Model fit was compared using AICc calculated from the R package '*MuMIn*' (Bartoń, 2013)

4.3.6 *Symbiodinium* genotyping and analysis of field juveniles

Illumina sequencing of the ITS-2 locus was used to determine if differences in *Symbiodinium* communities existed among the five coral families with the highest and three families with the lowest percent mortality (Table 4.1). All juveniles from the F8 family died, but six individuals could be sampled from the third (excluded) culture replicate that also had similarly high mortality (96%).

Table 4.1 Number of individually sequenced juveniles per coral family, for families with the highest and lowest percent mortality, including the average number of mapped reads (post paired-end merging and filtering) across each family and their respective standard errors (SE), with the exception of F17, in which there was only one survivor.

Family	Mortality (%)	Number of juveniles sequenced	Average number mapped reads (±SE)
F12	0	12	77,999 ± 3,468
F4	2.7	12	68,523 ± 5,871
F1	6.9	13	56,262 ± 3,930
F14	90.16	6	140,120 ± 15,198
F28	91.6	2	45,632 ± 28,825
F18	96.9	3	148,201 ± 35,468
F17	98.9	1	175,042 ± NA
F8	100	6	24,951 ± 11,404

DNA from single juveniles was extracted using Wayne's method (Wilson *et al.*, 2002) and lysed using a FastPrep-24 matrix with 1 mm silicia spheres (MPBio) three times at 30 s and 4.0 m/s. Library preparation and paired-end Miseq sequencing (Illumina) were performed at the University of Texas at Austin's Genomics Sequencing and Analysis Facility (USA), using the following primers: ITS2alg-F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTG) and ITS2alg-R (3'-TTCGTATATTCATTCGCCTCCGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5') (Pochon *et al.*, 2001). A total of 55 samples were sequenced, with 1 – 13 juveniles per family (Table 4.1). Raw reads were analysed using the USEARCH and UPARSE pipeline (Edgar, 2013) (v. 7). Chimeric reads were filtered and reads with an Expected Error greater than 1.0 were discarded (Edgar and Flyvbjerg, 2015). Remaining reads clustered with the default 97% identity and minimum cluster size of 2 (thus eliminating all singleton reads), after which all reads were globally aligned to 99% similarity with gaps counted as nucleotide differences. After filtering and mapping, 1,848,018 reads remained (44.1% of raw reads), partitioned across 333 OTUs. OTUs were then annotated using BLAST+ run against the full non-redundant nucleotide NCBI database (Altschul *et al.*, 1990; Camacho *et al.*, 2009). OTUs that did not blast to *Symbiodinium* accessions were removed manually (4.2%, many belonging to other Dinoflagellata) with 1,769,859 *Symbiodinium* specific reads belonging to 136 OTUs remaining. The majority of reads were attributed to clades A (36.2% reads, 16 OTUs), D (29.8%, 14), C (19.6%, 15), uncultured eukaryotes (10.3%, 45) and *Symbiodinium* identified from environmental sampling ("environmental *Symbiodinium*") (2.6%, 31). Clades G (1.4%, 5), B (0.1%, 4), F (0.03%, 5), and H (0.007%, 1) were represented by far fewer reads and OTUs than those from C, D and A.

Cleaned sequence data were variance-normalized using a geometric mean and shifted log transformation implemented in the '*DESeq2*' package in R (Love *et al.*, 2014). Differential abundance analysis was performed with the same package using the contrast function to extract specific comparisons from the negative binomial generalized linear model. OTUs that were found to have significantly different abundances and which were previously identified as either "uncultured eukaryotes" or "environmental *Symbiodinium*" using the full NCBI database were re-blasted to a custom-built database of all *Symbiodinium*-specific NCBI sequences. This allowed a reclassification of

OTU19 to clade E (JN406302, Sweet, 2014) and OTU4 to clade D. Nonmetric multidimensional scaling (NMDS) was performed on variance-normalized OTU abundances using the packages '*Phyloseq*', '*vegan*' and '*ellipse*' with a Bray-Curtis dissimilarity matrix (Murdoch *et al.*, 2007; McMurdie and Holmes, 2013; Oksanen *et al.*, 2013). NMDS analysis does not assume linear relationships between underlying variables and OTUs, and the resulting distances between samples calculated are indicative of their similarity, with samples positioned closer in space being more similar (Ramette, 2007). The use of a Bray-Curtis matrix allows for incorporation of presence/absence data, as well as the abundance of OTUs. A permutational multivariate analysis of variance was used to determine if *Symbiodinium* communities differed significantly between high and low mortality families using the '*adonis*' function in '*vegan*.' The Chao1 metric describes species richness between families with differential mortality and was calculated in '*Phyloseq*'. This metric incorporates differences in library sizes between samples and is especially appropriate for datasets with many low abundance OTUs (Hughes *et al.*, 2001; Hill *et al.*, 2003).

4.3.7 Multiple ITS-2 copies and intragenomic variation

Symbiodinium genomes are notoriously large and composed of multiple-copies of intragenomic variants (Shoguchi *et al.*, 2013). Intragenomic variation within and between *Symbiodinium* types makes classifying species-level diversity in *Symbiodinium* challenging (Thornhill *et al.*, 2007; Quigley *et al.*, 2014). Currently, diversity has only been definitively determined from simple communities of a few species using single cell analyses (Wilkinson *et al.*, 2015) or approximated from serial dilutions (Mieog *et al.*, 2007; Quigley *et al.*, 2014); diversity has not been verified for complex communities made up of hundreds of OTUs. To evaluate the presence of multiple copies and/or intragenomic ITS-2 variants, I employed an approach based on three criteria. Meeting all three of the following criteria was required to identify an OTU as being multi-copy and/or an intragenomic variant: 1) co-occurrence, 2) proportionality, and 3) percent similarity. Specifically, OTUs found to occur in regularly increasing or decreasing multiples of a baseline abundance (large R^2 values) across a large number of samples might feasibly co-occur within the same cells and hence may be multiple copies of the same gene. A high percent similarity is indicative of intragenomic variants having multiple copies of the same gene according to the above pattern (Arif *et al.*, 2014), although multiple, divergent sequences may possibly co-exist within single

genomes simultaneously (LaJeunesse, *et al.*, 2010). *Symbiodinium* types that are very divergent (low similarity) but occur in similar patterns across samples and in similar proportions may conversely represent divergent types that share similar ecological niches.

The following analyses were performed on the ten OTUs that had significantly different abundances in high and low mortality families. OTUs were first separated by clade using initial Blast+ identities (C: OTU1/OTU121/ OTU162, environmental/clade E: OTU4/OTU19, *Symbiodinium*1635: OTU13/OTU124), and their co-occurrence across samples was identified using the tree function in '*Phyloseq*', with the variance-normalized read depth described above. Correlations between these OTUs were calculated with the function ggpairs in the package '*GGally*' (Schloerke *et al.*, 2014). Geometric distances and aligned pairwise similarity were calculated for these OTUs using the package '*ape'* (Paradis *et al.*, 2004) and Clustal Omega (McWilliam *et al.*, 2013) with Geneious (Kearse *et al.*, 2012), respectively.

4.4 Results

4.4.1 Population effects on larval survival, weight and settlement, and juvenile mortality

4.4.1a Larval survival

Neither lineage purity (i.e., OO, WW vs. OW, WO) nor population cross (i.e., OO vs. WW vs. OW vs. WO) significantly affected the survival of larval cultures (Fisher's exact tests: $p = 1$ for both). 92.3% of purebred lineages (12/13 purebred families had all 3 culture replicates survive) and 91.7% of hybrid lineages (11/12 hybrid families) survived this stage. All culture replicates persisted for all OO and OW families, and 83.3-85.7% of WO and WW crosses survived (5/6 and 6/7 families, respectively).

4.4.1b Larval weights

Larval weights did not differ significantly between purebred (OO, WW) and hybrid (OW, WO) larvae (Linear Model (LM): $F_{1,44} = 0.084$, $p = 0.77$), which respectively weighed 12.8±0.9 and 13.1±0.9 µg, on average. However, larval weights differed significantly among offspring of the four population crosses. Dry weights of

larvae from purebred Orpheus lineages and Orpheus dam crosses were significantly heavier than weights of larvae from crosses involving Wilkie dams (15.1±1.3 - 9.3±0.5 µg, Tukey's *post hoc* test (TPT): $p < 0.001$, Figure 4.1a). Larval weights did not differ significantly between OO and OW crosses (TPT: $p = 0.85$), nor between WO and WW crosses (TPT: $p = 0.89$) (Figure 4.1a).

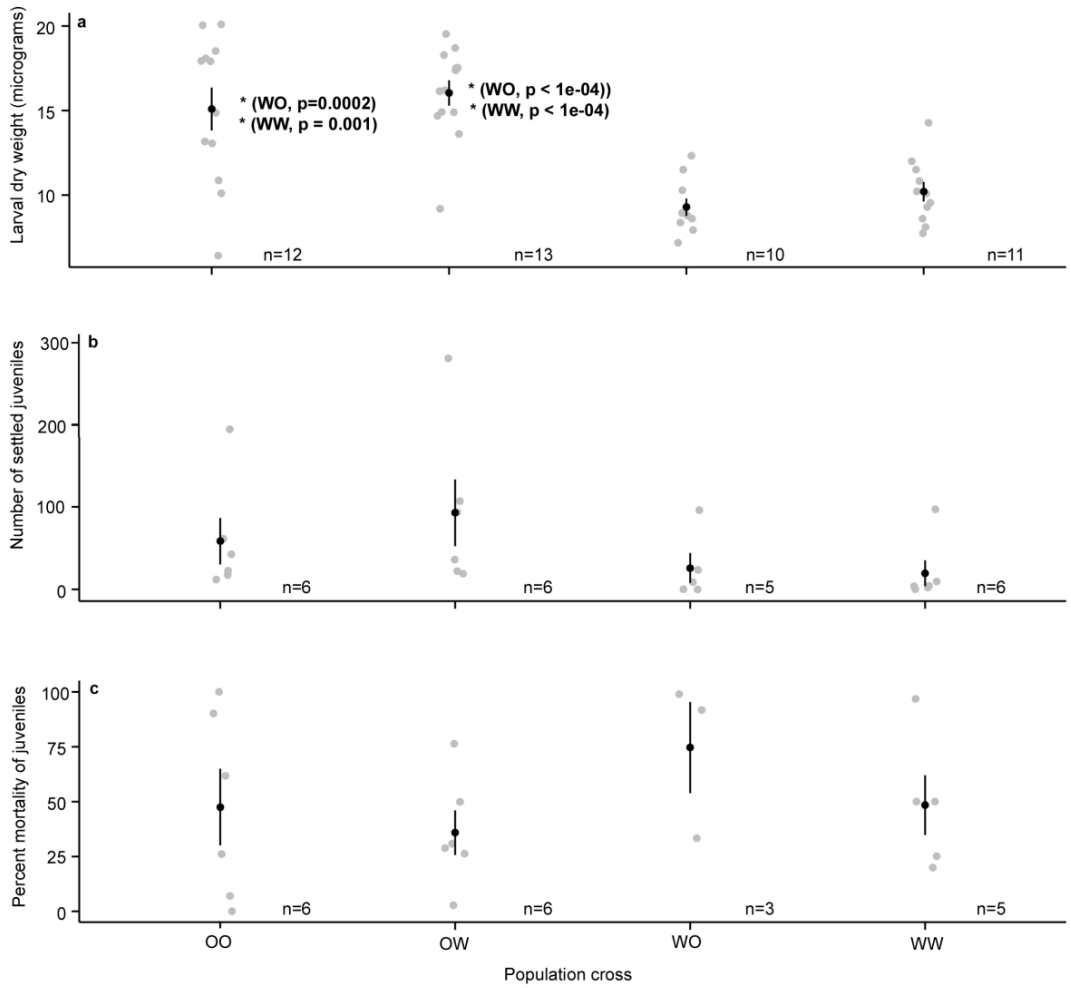

Figure 4.1 Variation among population crosses in a) mean (±SE) dry weight of larvae; b) mean (±SE) number of settled larvae; and c) mean (±SE) relative juvenile mortality (%) after 25 days of field exposure. Black circles are means; vertical bars are standard errors; light grey points are raw data. P-values indicate Tukey's *post hoc* significance test results of specific comparisons. For example, the mean larval dry weight of population cross OO was significantly greater ($p = 0.0002$) than the mean larval dry weight of cross WO. Samples sizes are indicated to the right of each population cross.

4.4.1c Settlement success

The number of settled juveniles did not vary with lineage purity (Kruskal-Wallis rank sum test (KW): $\chi^2_1 = 0.55$, $p = 0.46$), with purebred lineages having, on average, 39±16.4 settled juveniles compared to 62±24.8 settled juveniles in hybrid lineages. Whilst crosses with Orpheus dams (OO, OW) had more settlers compared to crosses with Wilkie dams, these differences were not statistically significant (GLM, TPT: p = 0.17-0.99, Figure 4.1b).

4.4.1d Field mortality

Percent mortality of juveniles after 25 days in the field was not significantly described by lineage purity (KW: $\chi^2_1 = 0.1$, $p = 0.73$) or population cross (KW: $\chi^2_3 = 2.5$, p = 0.47); 47.9±10.8% and 48.8±11% of purebreds and hybrids died respectively. Juveniles of Orpheus dams crossed with Wilkie sires suffered the lowest mortality (35.9%±10.2), whilst those from Wilkie dams crossed with Orpheus sires had the highest mortality (74.7%±20.8) (Figure 4.1c).

4.4.2 Familial and parental effects on larval survival, weight and settlement success, and on juvenile mortality

4.4.2a Larval survival

Cultures for 92% of the families had larvae surviving at the end of the rearing stage (i.e., 23 of 25 families had all 3 culture replicates survive). Dam identity significantly affected larval survivorship (Fisher's exact test: $p = 0.007$), driven by the mortality of two families from Wilkie dam W7. Sire identity had no effect on larval survivorship (Fisher's exact test: $p = 0.58$). The two families that died were sired by O5 and W11.

4.4.2b Larval weights

Mean larval weight varied significantly among dams (LM, TPT: $F_{6,39}$=8.615, respective *p-values* below) and ranged from 17.6±1.6µg (dam O6) to 9.7±0.73µg (dam W5) (Figure 4.2a). Dam O6 larvae were significantly heavier (LM, TPT: p = 0.001-0.002) compared to larvae of all three Wilkie dams but not the other Orpheus dams (LM, TPT: p = 0.35-1.0). The same pattern was true for dam O5 (LM, TPT: p = 0.004-0.007 and 0.8-1.0) and dam O4 (LM, TPT: p = 0.003-0.006, 0.7-1.0). The mean dry

weight of dam O3 larvae did not differ statistically from mean larval weights of any other dam (LM, TPT: $p = 0.15$-0.81), with O3 larvae weighing less than those of the other Orpheus dams but more than mean larval weights of the Wilkie dams. The three Wilkie dams produced the lightest larvae, which did not differ significantly in weight from each other (LM, TPT: $p = 1.0$).

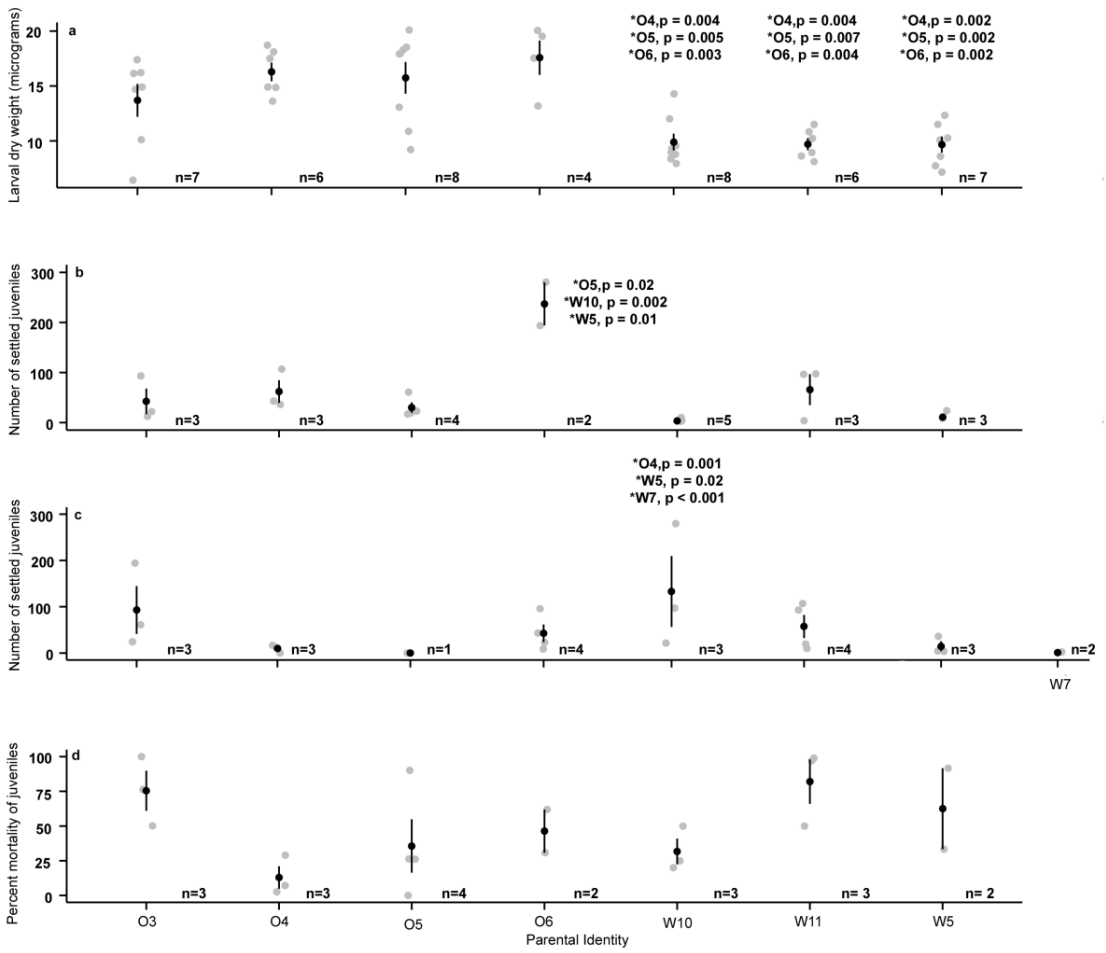

Figure 4.2 Variation among dams in **a**) mean (±SE) dry weight (µg) of larvae per dam; **b**) mean (±SE) number of settled larvae per dam; c) mean (±SE) number of settled larvae per sire; and d) mean (±SE) relative mortality (%) of juveniles per dam during 25 days of field exposure. Black circles are means; vertical bars are standard errors; light grey points are raw data. P-values indicate Tukey's *post hoc* significance test results of specific comparisons. For example, the mean larval dry weight of parental identity W10 was significantly less (p = 0.004) than the mean larval dry weight of O4. Samples sizes are indicated to the right of each parental identity.

4.4.2c Settlement success

Larvae from 86.9% of the families (i.e., 20 of 23 families) settled, with larvae of the remaining three families (F21, F26, F30) continuing to swim at day 19 pf, at which time larvae from the other families had settled and juveniles were deployed in the field. The GLMM analysis indicates that the random effects of dam, sire, their interaction and observation-level effect accounted for 61.9% of settlement variability. Of the 61.9%, maternal identity explained approximately 23.9% of the total variability in settlement, sire explained 37.9%, and the dam by sire interaction explained 9.91%. Dam O6 families had the largest number of settled juveniles, whilst dam W10 families had the lowest (Figure 4.2b). Using dam O6 as a reference, the number of larvae settling differed significantly for dam O6 in comparison to dams O5, W10 and W5 (GLMM TPT: $p = 0.002$-0.02). Alternatively, with sire W10 as a reference, larvae from this sire settled significantly less in comparison to larvae from sires O4, W5 and W7 (GLMM TPT: $p =/< 0.001$-0.02, Figure 4.2c). The comparison in larval settlement between sires W10 and O5 was not significant, as larvae from only one family with sire O5 remained by this stage of the analysis (GLMM TPT: $p = 1$).

4.4.2d Juvenile mortality on the reef

Substantial variation in juvenile mortality was detected among families, with an average mortality of 48.3% ±7.5 after 25 days in the field; only a single family (F8) suffered 100% juvenile mortality. Mortality of juveniles ranged from 12.9±8.1% for juveniles of Orpheus dams to 82±16% for juveniles of Wilkie dams (Figure 4.2d), with juveniles of dams from both locations suffering both high mortality (O3 and W11) and low mortality (O4 and W10). Initial juvenile abundance (i.e., settlement number), the combined random effects of dam/sire/dam x sire interaction, and residual variance accounted for 53.0%, 12.8% and 34.1% of the total variability in juvenile survivorship, respectively. Of the random effects, GLMM partitioned 37.9% of the variation (4.9% of the total) to maternal effects and 62.0% (7.9% of the total) was residual variance (potentially encompassing an unmeasured biological or abiotic variable). There was no effect of sire or of dam x sire interaction on juvenile mortality. With the direct maternal effects on juvenile survival (4.9%) in addition to the carry-on indirect maternal effects on juvenile survival through settlement (23.9% of 53% = 12.6%), the total maternal contribution to juvenile survivorship was 17.5% (the sum of 4.9% and 12.6%).

Once dam identity and the dam x settlement number interaction were included as fixed effects in the above model, the effect of larval settlement success on the abundance of surviving juveniles was no longer significant (GLMM TPT: $p = 0.14$), although both dam identity (O4, O5, W10, W11, GLMM TPT: $p = 4.3e^{-7}$-0.076) and the dam x settlement abundance interaction (O5, W10, GLMM TPT: $p = 0.0097$-0.015) were significant. I then re-fit the model without settlement as a single-factor (now only including dam and dam x settlement interaction). Modeled survival of juveniles from dams W10, O6, and O3 indicates that as settlement increases, predicted juvenile abundance increases, even with initially low settlement abundances (Figure 4.3).

Summary statistics indicate that this positive correlation may be predominantly due to dam identity alone in the case of dam W10 (GLMM TPT: $p = 0.005$) compared to the dam by settlement interaction (GLMM TPT: $p = 0.019$). Juveniles from dam W5 had very low survivorship regardless of settlement abundance, with no significant relationship between settlement number and dam identity on juvenile survival (GLMM TPT: $p = 0.18$). Survival of W11 juveniles showed marginal statistical evidence for an interaction with dam identity (GLMM TPT: $p = 0.076$), but modelled juvenile survival appeared to benefit little from increased settlement numbers (GLMM TPT: $p = 0.99$) (Figure 4.3). Poor survival of O5 juveniles was attributable to dam identity and dam by settlement interaction (GLMM TPT: $p = 1.8e^{-5}$, 0.02). The predicted abundance of juveniles of dams O3, O4, and O6 increased with an increase in settlement number due to the significant effect of dam by settlement interaction (GLMM TPT: $p = 1.8^{-12}$-$8.2e^{-5}$), although predicted abundance of juveniles from dam O4 increased even at low settlement values, which indicates that the significance of the interaction may be predominantly from dam identity (GLMM TPT: $p = 4.3^{-7}$). Finally, variation in juvenile survival was not well explained by larval weight for the seven dams that had surviving juveniles. This was true whether families that had failed settlement were included as 100% mortality ($R^2 = 0.328$) or excluded completely ($R^2 = 0.201$, Appendix C, Figure S4.1).

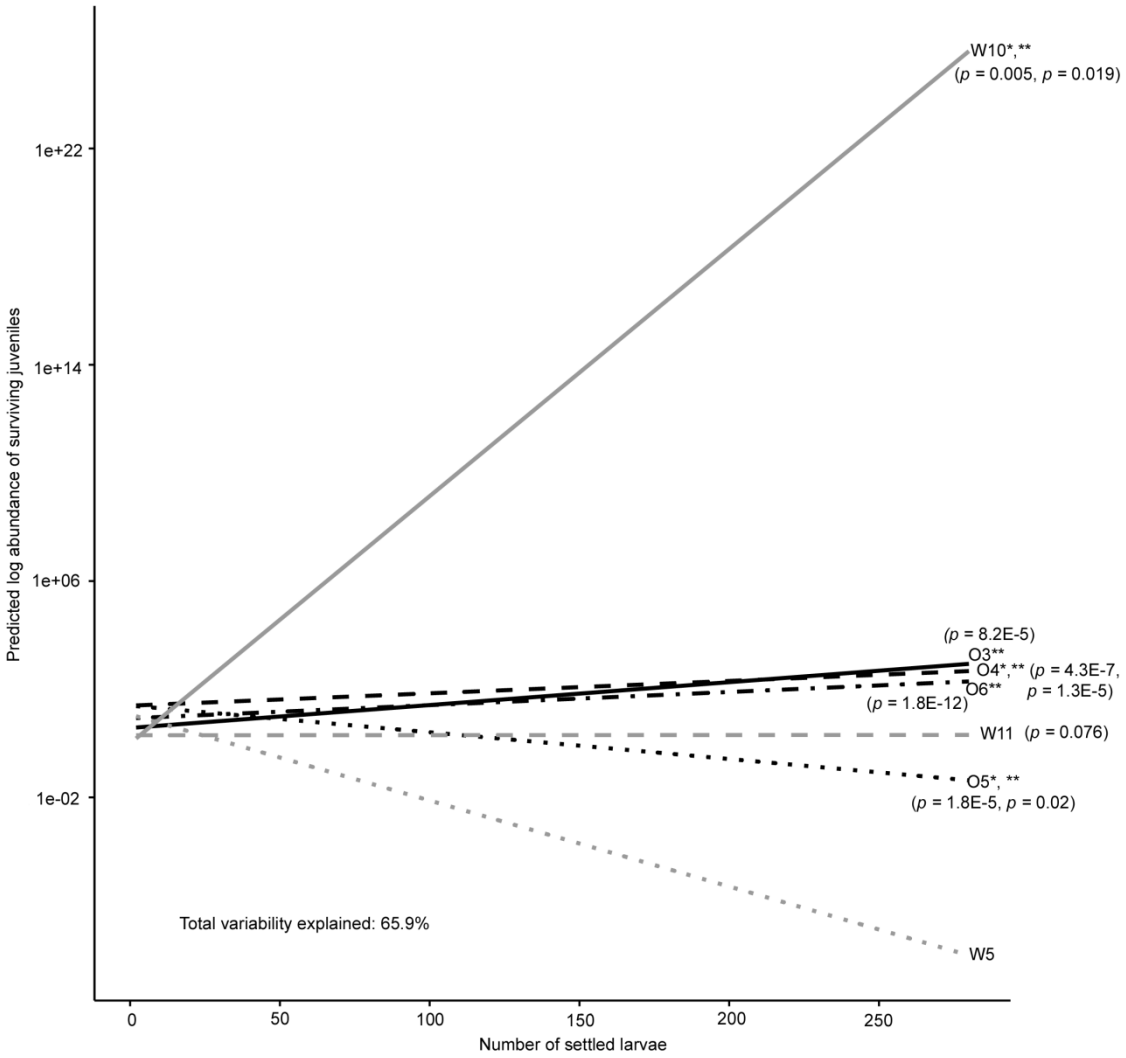

Figure 4.3 Relationship between predicted logged juvenile abundance after 25 days in the field, as explained by the number of settled larvae and dam identity based on outputs from the generalized linear model (GLM). Asterisks adjacent to dam labels signify significant effects due to dam identity and/or the dam x settlement interaction on juvenile survival derived from the generalized linear negative binomial model in which settlement abundance as a single factor has been dropped but remains as an interactive factor with dam identity. A single asterisk (*) signifies a significant effect due to dam identity, double asterisk (**) signifies a significant dam x settlement abundance effect. Note that the effect of dam W11 identity was only marginally significant ($p = 0.076$).

4.4.3 *Symbiodinium* community in families with high and low percent mortality

Illumina sequencing of the ITS-2 locus revealed 136 *Symbiodinium*-specific OTUs across the 55 samples. *Symbiodinium* communities differed significantly in overall species richness and diversity between high and low mortality families (Permutational multivariate analysis of variance; by family: R^2=0.21, df=6, p = 0.001; by mortality: R^2=0.18, df=1, p = 0.001). High mortality families had a greater number of *Symbiodinium* types present (average Chao1 ± SE: 16.4±1.7) compared to low mortality families (12.7±2.7 *Symbiodinium* types). Variability in OTU richness and abundance was greater within families of high mortality crosses (between juveniles of the same families) than within low mortality families (Bray-Curtis, NMDS, Figure 4.4). Juveniles from high mortality families differed markedly from each other, with *Symbiodinium* communities of some individuals resembling the conservative diversity of low mortality families whilst others were more variable. *Symbiodinium* communities associated with juveniles of high mortality families had significantly lower abundance of A3, D1, D1a, and two C1 *Symbiodinium* types, and greater abundance of two F and one C type, and of a clade E environmental *Symbiodinium* type compared to juveniles of low mortality families (Figure 4.5, B-H adjusted p-values = 6.15×10^{-28} - 0.039, Appendix C, Table S4.2).

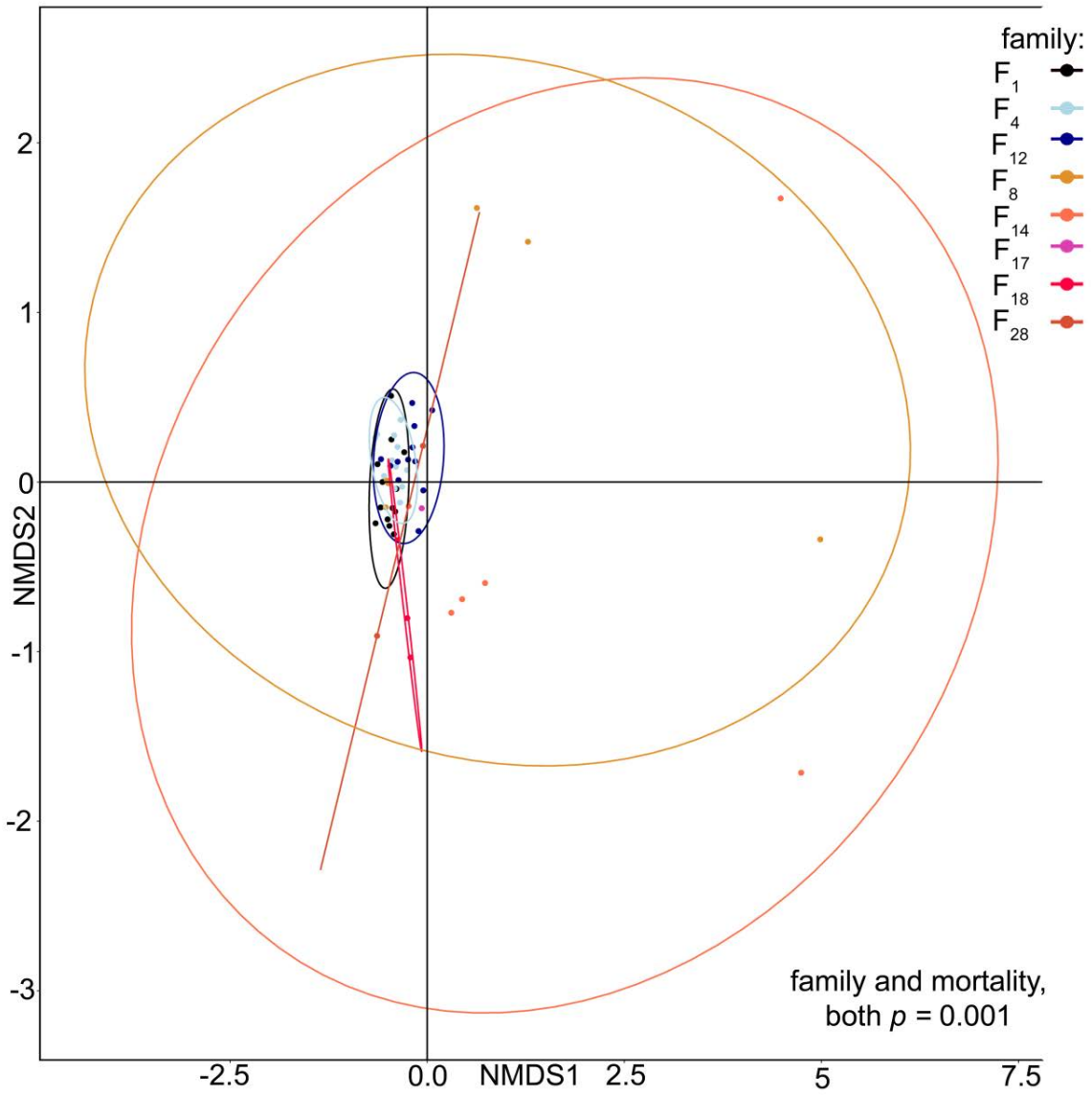

Figure 4.4 Nonmetric multidimensional scaling (NMDS) plot using a Bray-Curtis distance matrix of variance normalized OTU abundances. Families with low mortality (< 10%) are in cool colours and high mortality families (> 90%) in warm colours. Each point represents a unique juvenile, where its family is indicated by colour. Ellipses with corresponding colours represent the 95% probability region for each family. Significant effects ($p = 0.001$) of family and mortality class (high vs. low) are derived from Permutational multivariate analysis of variance.

Symbiodinium communities associated with juveniles of the three low mortality families were similar in both *Symbiodinium* OTU richness and abundance (Bray-Curtis, NMDS, Figure 4.5), however, the abundances of some *Symbiodinium* types differed significantly among low mortality families. Family 1 juveniles had 3.1 log2 fold less D1a (B-H adjusted p-values = 0.001) and 3.7 log2 fold more C1 (B-H adjusted p-values = 0.0009) compared to F4 juveniles, but the abundances of all *Symbiodinium* types were similar compared to abundances of *Symbiodinium* types associated with juveniles of Family 12. Concomitantly, *Symbiodinium* communities associated with F12 juveniles differed from those of F4 juveniles, with 3.6-fold less C1 (B-H adjusted p-values = 0.0007) and 3.3-fold more D1a (B-H adjusted p-values = 0.0007).

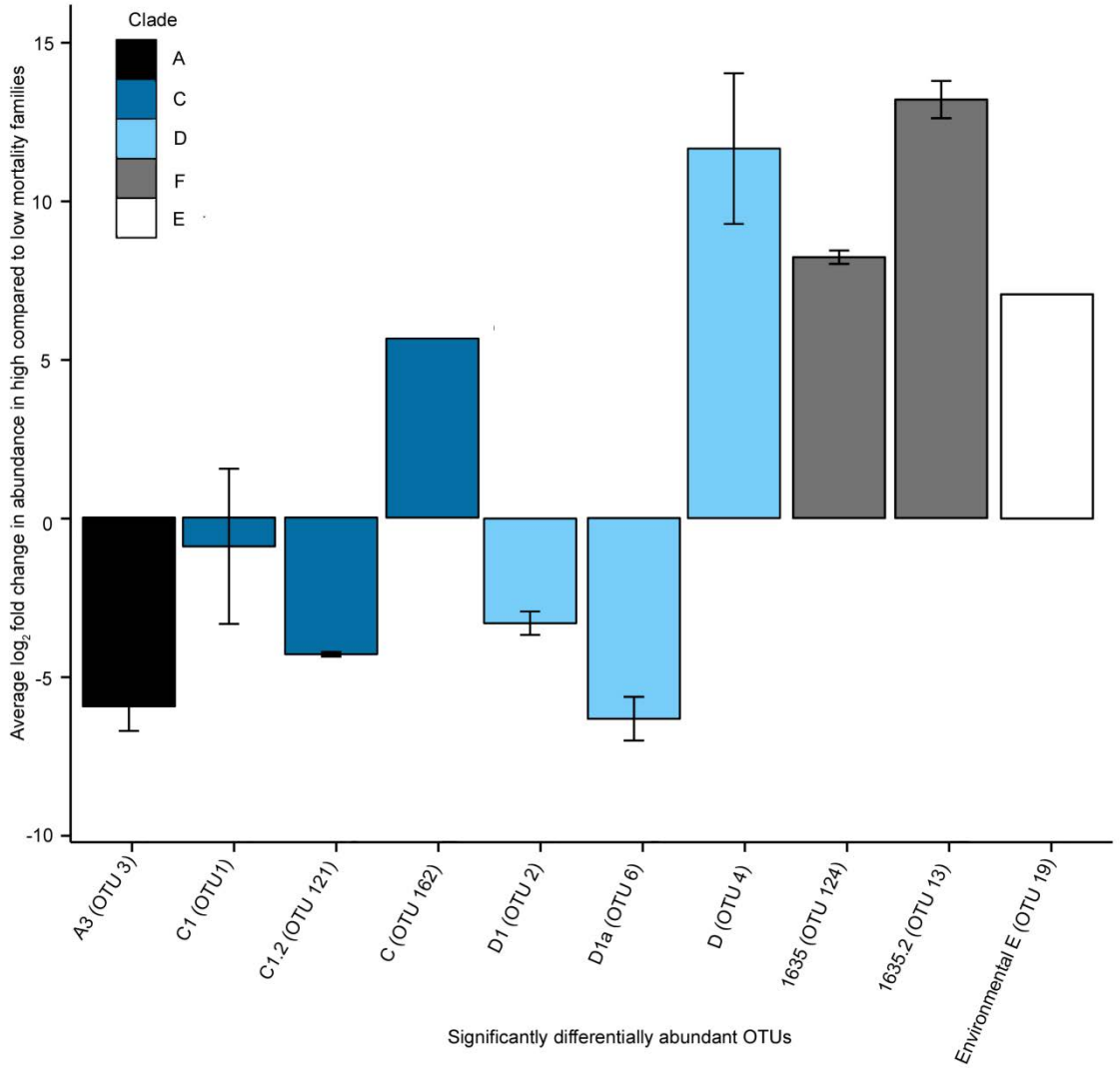

Figure 4.5 Average log2 fold changes in abundance in high (> 90%) mortality families in comparison to low (< 10%) mortality families. Log2 fold changes were calculated from negative binomial generalized linear models in DESeq2, with standard error bars representing significant Benjamini-Hochberg p-values ($p < 0.05$). Colours represent the classification of different *Symbiodinium* types in clades: A, C, D, F and environmental *Symbiodinium* (clade E).

4.4.4 Intragenomic variants

I did not detect evidence of intragenomic/multi-copy variants in the ten OTUs that distinguished high and low mortality families based on these three criteria. There was not a strong level of co-occurrence in any of the OTUs. Within clade C, OTU1 and 121 co-occurred in 42 of the 65 samples, but neither co-occurred with OTU162. OTU13 was present in four additional samples compared to OTU124. Similarly, OTU4 was found in 11 additional samples compared to OTU19. Where OTUs did co-occur, only OTU13 and 124 had a strong level of proportionality ($R^2 = 0.992$), OTU1 and 121 had moderate proportionality ($R^2 = 0.739$), and all other combinations of OTUs had very low proportionality ($R^2 = -0.0551 - 0.06$). Pairwise geometric distance analysis indicated that OTU1 and OTU121 had 57.6% pairwise similarity, whereas aligned pairwise percent similarity analysis indicated 96.2% pairwise similarity. OTU13 and OTU124 had 76.4% and 96.9% pairwise similarity, respectively, based on these two analyses. Both comparisons were characterized by multiple base pair differences and blocks of deletions.

4.5 Discussion

4.5.1 Maternal influence on density-dependent and density-independent juvenile mortality

Here I present evidence of maternal effects on the survival of early life-history stages of the coral *Acropora tenuis* during the first month of development on the reef. Maternal identity underpinned 23.9% of the variability found in larval settlement success and explained 17.5% of variability in juvenile survival. In contrast, neither paternal effects nor paternal by maternal interaction significantly impacted juvenile survivorship in the field. These results on larval settlement and juvenile survival indicates that maternal carry-on effects reach through several developmental stages in a broadcast spawning coral. Importantly, it appears that the initial number of settled juveniles is not a significant factor in predicting the number of survivors once maternal identity is taken into account. Initial settlement number was only significant when its interaction with certain dam identities was included, creating conditions of both density-dependent mortality and density-independent mortality driven by dam identity. Initial settlement numbers were not important for dams W10 and W5, with all juveniles either surviving or dying, respectively, regardless of initial settlement numbers. This pattern

was especially clear for dam W10, which produced families with very low numbers of settlers that still resulted in very high juvenile percent survival (F22, F23, F24). It is unlikely that families with high mortality died due to genetic incompatibility between parental colonies (i.e., selfing between clonemates), given patterns of both very high and very low survivorship found for families resulting from crosses involving colonies from the same population (e.g., O3xO4 and O3xO5 failed, but O4xO5 was successful).

These findings suggest that some aspect(s) of maternal identity, either at an environmental or genetic level, directly influences the fitness of early life-history stages, predisposing juveniles to either live or die. At an ecological level, this signifies that juvenile survivorship is not merely a product of fecundity of the parent colony or initial recruitment numbers, and that high recruitment does not guarantee greater survival and community representation. The maternal effects on larval settlement and juvenile survival demonstrated here provide evidence that differences in colony identity may scale up through differential survival of early life-history stages and contribute significantly to shaping heterogeneous reef communities. Maternal identity should be taken into consideration as an important factor contributing to juvenile survival and population demography, in conjunction with currently recognized factors like predation, competition and disease.

4.5.2 Impacts of *Symbiodinium* community on survival of coral juveniles

The *Symbiodinium* community acquired by juveniles of broadcast-spawning corals offers a potential link between maternal effects and juvenile survivorship. Juvenile families had different symbiont communities after less than one month of field deployment and exposure to *Symbiodinium*, representing the earliest record of symbiont specificity in a coral species with horizontal transmission yet reported, potentially due to the deep sequencing approached utilized. Microbes (both bacterial and eukaryotic) are known to heavily impact coral development and health (Cantin *et al.*, 2009; Putnam *et al.*, 2012; Thompson *et al.*, 2015; Glasl *et al.*, 2016), but few studies to date have investigated the links between parental identity (maternal or paternal effects) and *Symbiodinium* assemblage acquired by juveniles with horizontal symbiont transmission.

These results identify a potential link between maternal identity and the juvenile *Symbiodinium* community that may have an environmental or genetic basis, and propose that an inherited or transmitted mechanism, whose expression differs across

dams, leads to differential formation of *Symbiodinium* communities among families and contributes to differences in juvenile survival. Specifically, juveniles of low mortality families had a more conserved *Symbiodinium* community compared to high mortality families, consistent with greater selectivity for specific *Symbiodinium* types. Low mortality families also contained a high proportional abundance of *Symbiodinium* types A3 and D1, suggesting that these are critical symbionts for survival of *Acropora tenuis* juveniles. Increased survivorship of *Acropora yongei* juveniles in Japan was also found to be associated with a significantly greater abundance of clade A *Symbiodinium* (Suzuki *et al.*, 2013). Whilst the authors attributed high survivorship specifically to the early acquisition of symbionts at the larval stage, they acknowledge that the selective uptake of specific symbiont types could have led to differential survivorship. These data support this latter conclusion and highlight that the acquisition of *Symbiodinium* A3 is ecologically essential to early juvenile survivorship in species of *Acropora*. Alternatively, differences amongst the *Symbiodinium* communities detected here may be the result, rather than the driver, of mortality, with some unmeasured factor compromising host-symbiont regulation, leading to dysbiosis and death. Further work is needed to elucidate these alternative explanations, as well as the fitness benefits and costs of hosting particular symbiont communities.

It is currently unclear whether *Symbiodinium* uptake in early life stages of corals is specific (Suzuki *et al.*, 2013) or random (Cumbo *et al.*, 2013), however these results suggest that the high mortality experienced by certain juvenile families may be due to either the specific absence of A3 or to the non-specific uptake of a community that becomes detrimental for the juvenile coral host (Ley *et al.*, 2006). According to this latter interpretation, it is not the presence or absence of single symbionts that cause a decrease in host fitness, but the community composition and their interactions with a specific host genotype and environment that are detrimental. Non-selective community uptake may influence juvenile survival in two ways: 1) it may expose juveniles to opportunistic or sub-optimal *Symbiodinium* types, and 2) whilst the additional *Symbiodinium* types may not be opportunistic, they may occupy niche space and influence the density of other more beneficial *Symbiodinium* types. Both of these signatures can be seen in this data. I found that more diverse symbiont communities were generally associated with high mortality families and that those juveniles had seven-fold higher proportions of an environmental, potentially novel opportunistic *Symbiodinium* type (clade E OTU19). This type was only found in 1 out of 37 low

mortality juveniles vs. 9 out of 28 high mortality juveniles. Secondly, the main OTUs from the low mortality families (OTUs 1,2,3,6,121) were also at lower abundances in high mortality juveniles, with a concomitant fold-increase in certain OTUs (4,13,19,124,164). A similar increase in the abundance of non-beneficial fungi was documented in *Hydra* in the absence of the specific microbial community typically associated with healthy hosts, causing mortality in those individuals infected (Fraune *et al.*, 2015; Gilbert *et al.*, 2015). Alternatively, highly diverse symbiotic communities may also confer negative effects on the host due to increased competition amongst symbionts (reviewed in: Oliver *et al.*, 2014).

Differences in the variability of *Symbiodinium* assemblages in this study highlight the role coral associated microbes can play in shaping the abundance and diversity of reef communities at local spatial scales through differential impacts on coral juvenile growth and mortality. Further support for this hypothesis is provided by microbial-driven density-dependent mortality of juveniles through increased pathogen transmission when juvenile densities are high (Vermeij and Sandin, 2008; Vermeij *et al.*, 2009), as well as mortality of adults in zones of competitive exclusion with turf algae, which cause hypoxic zones next to coral tissue and subsequent increases in virulent bacteria (Barott *et al.*, 2011). Also, the early acquisition of *Symbiodinium* increases juvenile survivorship and growth (Graham *et al.*, 2013; Suzuki *et al.*, 2013). Evidence that maternal effects influence juvenile survival, combined with the fact that low mortality families had conserved *Symbiodinium* assemblages, provide a potential link between maternal identity and the composition of the *Symbiodinium* communities associated with juvenile corals, with some dams able to provide their offspring with mechanisms to select for beneficial communities (or exclude non-beneficial ones) whilst others do not.

The maternal effects detected here may have several underlying mechanisms that can be environmental and genetic (Wolf and Wade, 2009). Maternal environmental effects may be caused by differences in energy provision (lipid) provided to larvae, variations in egg or sperm quality due to colony age, or through epigenetic effects (reviewed in: Marshall *et al.*, 2008; Ritson-Williams *et al.*, 2009). Another potential mechanism is genetic maternal effects through heritability of maternal genes that influence the development of an appropriate immune system in coral juveniles. Cell to cell recognition and phagocytosis have been hypothesized as key precursors to the

establishment and maintenance of symbiosis with *Symbiodinium* (Davy *et al.*, 2012) and may underpin maternal genetic effects through the transmission of genes that regulate a repertoire of immune-related, cell-surface proteins adapted for certain environmental conditions, and/or symbiont types. A third possibility is that maternal identity affects juvenile survival through an unmeasured mechanism that results in dysbiosis of the symbiont community and juvenile mortality, as discussed above. These findings demonstrate that further research is needed to identify mechanisms underlying these maternal and familial effects in juveniles.

4.5.3 No evidence of local adaptation in northern and central populations of *Acropora tenuis*

I found that variation in larval survival, larval settlement success, and field survivorship of juveniles was greater among families than between the two populations. Whilst definitive statements about local adaptation of the northern and central GBR populations are not possible because juveniles were not reciprocally transplanted between the two locations, I present preliminary evidence for the lack of a significant effect of either lineage purity or population cross on percent mortality of juveniles. These results suggest that there was no fitness benefit associated with outplanting on their natal reef for Orpheus juveniles (either OO or OW), and no fitness cost associated with a non-natal grow-out location for Wilkie juveniles (either WW or WO). The lack of a strong population effect in these data suggests a lack of local adaptation, which is surprising given previous evidence of population genetic structure in corals (Underwood *et al.*, 2009; Torda *et al.*, 2013) and the benefits of being locally adapted to the parental environment (Galloway, 2005). However, the absence of locally-adapted populations within species' ranges can occur when reproductive propagules disperse widely, or if environmental variation is low and at a scale smaller than propagule dispersal (Galloway, 2005). This would be expected for many marine broadcasting species due to the high dispersal potential of currents. Indeed, the population genetic structure of *Acropora* species appears to be open in the Northern and Central sections of the GBR (van Oppen *et al.*, 2011), presumably because the longevity of *A. tenuis* propagules (up to 69 days) would enable them to disperse far from maternal colonies (Graham *et al.*, 2008). Therefore, larval dispersal may be sufficiently high between Wilkie and Orpheus populations for gene flow to lead to fairly well-mixed populations. A similar study of *Porites astreoides* also concluded that population origin had little to

no impact on juvenile survival (Kenkel *et al.*, 2015). Interestingly, southern GBR juveniles appear to be locally adapted (van Oppen *et al.*, 2014), a finding in line with microsatellite data showing that the southern GBR represents a distinct region according to population structure analyses (van Oppen *et al.*, 2011). Finally, the lack of a population effect may also be due to the directionality of the outplanting environment. Whilst other environmental factors may be equally or more important than temperature (e.g., turbidity, nutrients, currents), annual temperatures at Orpheus, where the juveniles were raised, are lower than those at Wilkie (Dixon *et al.*, 2015), therefore all juveniles were exposed to temperatures within their normal thermal ranges. In a concurrent study of the impact of heat stress on larvae from the two populations, Orpheus juveniles only exhibited lower survival when they were subjected to increased or higher temperatures found at Wilkie latitudes (Dixon *et al.*, 2015).

4.5.4 Differing reproductive modes and symbiont transmission strategies may drive the magnitude of parental effects on coral fitness

Reproductive mode is commonly assumed to be an important factor when assessing juvenile mortality risk (Ritson-Williams *et al.*, 2016), but empirical comparisons of maternal influences on the survival of juveniles originating from broadcast *versus* brooding modes are lacking. Unlike *A. tenuis*, brooding coral species have internal fertilization and gestation of planula larvae which are provisioned with *Symbiodinium* through vertical transmission. One could hypothesize that the magnitude of parental or maternal effects should be greater in brooding *versus* broadcasting corals. For example, brooded larvae 1) are exposed to the maternal environment, 2) are typically larger than larvae developed from spawned gametes, 3) acquire maternally-derived symbionts, and 4) are almost immediately competent to settle, leading to typically shorter dispersal distances from maternal colonies. Consistent with this line of reasoning, 94% of variation in juvenile survival has been attributed to parental genetics in the brooding coral, *Porites astreoides,* compared to the estimated 17.5% in this study, although both species achieved similar mortality, on average, within the first months (61% *versus* 51.7% survivorship, respectively) (Kenkel *et al.*, 2015). These results suggest that the magnitude of parental effects may be tied to modes of reproduction and symbiont transmission, highlighting crucial but understudied links between parental identity, *Symbiodinium* community composition and local adaptation.

4.5.5 Conclusion

Maternal identity impacts multiple early life stages of the broadcast-spawning coral *Acropora tenuis*. Maternal identity is particularly important in determining juvenile survivorship, but initial settlement abundance was typically de-coupled, although this varied by colony. Juveniles of low mortality families also have a distinct and conserved *Symbiodinium* community, including a *Symbiodinium* type (A3) that is potentially crucial for early juveniles, and propose that maternal identity and juvenile survivorship are linked through the transmission of environmental and/or genetic mechanisms that influence symbiont community regulation. Future studies assessing the drivers of juvenile mortality and coral reef restoration efforts should consider maternal identity and *Symbiodinium* community composition of juveniles, along with other well-known abiotic and biotic mechanisms, as being important in shaping reef landscapes.

Chapter 5: Geographic variation in free-living *Symbiodinium* communities across a temperature and water quality gradient impacts symbiont uptake and fitness in juvenile acroporid corals

5.1 Abstract

Knowledge of the diversity, distribution and abundance of the dinoflagellate genus *Symbiodinium* is essential for understanding the flexibility of the coral-*Symbiodinium* symbiosis and therefore its potential for acclimation and adaptation. The majority of corals acquire their endosymbiont *Symbiodinium* from environmental sources anew each generation. Despite this, little is known about the free-living diversity of *Symbiodinium* communities and how this affects uptake and *in hospite* communities in coral juveniles. *Symbiodinium* community diversity was determined in sediments collected from eight reefs along thermal (latitudinal) and water-quality (inshore-offshore) gradients on the Great Barrier Reef using the ITS-2 locus. Those free-living *Symbiodinium* communities in the sediments were compared to those acquired by *A. tenuis* and *A. millepora* juveniles during continual exposure to sediments (11 to 145 days) from eight reefs in experimental treatments. Communities associated with juveniles and sediments differed substantially, with the sediments harbouring 4 times more unique OTUs compared to juveniles (1125 OTUs vs. 271), moreover, only 10.6% of these OTUs were shared between juveniles and sediments. The diversity and abundance of *Symbiodinium* types differed among sediment samples from different temperature and water-quality environments. Juveniles exposed to these sediments acquired different *Symbiodinium* communities and displayed different rates of infection, mortality, and photochemical efficiencies. This study demonstrates that *Symbiodinium* uptake in juveniles of acroporid corals is selective within the constraints of environmental availability, and that the biogeography of different *Symbiodinium* types follows patterns along latitudinal and water quality environmental gradients on the Great Barrier Reef.

5.2 Introduction

The establishment of symbiosis between the dinoflagellate *Symbiodinium* and scleractinian corals is fundamental for the survival of both partners and for the formation and health of coral reefs. Over 75% of coral species establish this relationship anew each generation through the acquisition of *Symbiodinium* from environmental sources, a process termed horizontal transmission (Fadlallah, 1983; Baird *et al.*, 2009b). It remains unclear how the establishment of symbiosis is controlled, but recent studies quantifying the heritability of this community in coral larvae show that it is controlled in part by symbiont availability in the environmental pool (Chapter 2). Understanding and quantifying the contribution that environmental availability makes to the establishment of symbiosis is important to determine the flexibility of this association and whether it is open to modification. Environmental conditions on tropical reefs, including the Great Barrier Reef (GBR), are rapidly changing, with increases in mean temperatures and pCO_2 concentrations of particular concern (Donner, 2009). Furthermore, projected increases in turbidity, nitrate/nitrite levels and organic carbon range from 24%- 100% (Lønborg *et al.* 2016, *personal communication*), all of which may impact photosynthetic organisms like *Symbiodinium* and their host organisms. As climate change continues to devastate reefs worldwide, the ability of corals to expand or change their symbiotic partners to types better suited to future climate conditions may help predict species and community survival. Therefore, understanding current *Symbiodinium* availability and the environmental conditions that shape community composition of free-living populations is essential to projecting how environmental change will impact symbiont acquisition, and by extension, coral physiological flexibility.

Quantifying the contribution that environmental availability of *Symbiodinium* has on the establishment of symbiosis has been difficult, as little is known about the diversity, abundance, and spatial patterns of these free-living dinoflagellates generally beyond the clade level (e.g. Gou *et al.* 2003; Huang *et al.* 2013). Studies from the Pacific and Caribbean show that the water column, coral rubble, sediments and algal/cyanobacterial surfaces have distinct *Symbiodinium* communities (Carlos *et al.*, 1999; Gou *et al.*, 2003; Coffroth *et al.*, 2006; Hirose *et al.*, 2008; Manning and Gates, 2008; Porto *et al.*, 2008; Venera-Ponton *et al.*, 2010; Pochon *et al.*, 2010; Reimer *et al.*, 2010; Takabayashi *et al.*, 2012; Sweet, 2014; Yamashita and Koike, 2013; Huang *et al.*, 2013; Granados-Cifuentes *et al.*, 2015). For example, comparative studies between

Caribbean and Hawaiian reefs show that environmental diversity reflects the dominant clades found in corals in these regions, with the highest diversity and abundance belonging to clades A and B in both the water column and sediments in the Caribbean, whereas A and C are more prevalent on Pacific reefs (Takabayashi, *et al.*, 2012). Additional diversity has been discovered using *Symbiodinium* infection in coral larvae or juveniles as a proxy for environmental *Symbiodinium* availability (Coffroth *et al.*, 2006), with clade A recovered from coral early life-history stages exposed to sediments in Japan, and B and C in juveniles exposed to reef water (Adams *et al.*, 2009). Larval infections using sediments sourced from the northern and central GBR also identified C1, C2, and D, supporting their presence in these sediments (Cumbo *et al.*, 2013). Finally, environmental sources of *Symbiodinium* in sediments compared to the water column have been shown to significantly increase acquisition of *Symbiodinium* in larvae (Adams *et al.*, 2009). *Symbiodinium* in sediments also act as a link between symbiont populations in adults and those established in juveniles, where adult colonies seed the sediments with symbionts for subsequent juvenile uptake (Nitschke *et al.*, 2015). Furthermore, this evidence and the higher degree of overlap between adult and sediment symbiont communities compared to other environmental sources suggest that the sediments are the principal biome for *Symbiodinium* acquisition in corals. The application of deep sequencing technology has revealed new free-living diversity (Cunning *et al.*, 2015), but more studies are needed across diverse habitats to fully elucidate biogeographical patterns in the distribution and abundance of these important symbionts.

In hospite and cultured *Symbiodinium* have distinct physiological responses to thermal, irradiance and nutrient regimes at both the clade and type level (van Oppen *et al.*, 2009), but it is unknown if variable tolerances for these factors shape their free-living distributions. For example, clades A and B are generally characterized as thermally sensitive, (although A1 is thermotolerant; (Sawall *et al.*, 2014)), whilst clades D and F are more thermally tolerant (e.g. Karim *et al.* 2015). As photosynthetic organisms, biogeographic patterns of free-living *Symbiodinium* may also follow water quality gradients, as high levels of suspended sediments change light environments (Storlazzi *et al.*, 2015). Parameters such as mud content and Secchi depth correlate well with distribution patterns of *Symbiodinium in hospite*. For example, decreased mud content and increased Secchi depth anomalies correlate well with increased abundances

of types within clade C on the GBR (Cooper *et al.*, 2011) and clades C and D in the Caribbean (Toller *et al.*, 2001a, 2001b; Garren *et al.*, 2006). *Symbiodinium* have also been shown to respond to variable light microgradients created within different coral tissue layers (Wangpraseurt *et al.*, 2014). Finally, host CO_2 respiration and metabolic wastes in the forms of nitrogen (N), phosphorus (P) and carbon compounds are utilized by symbionts during photosynthesis (Iluz and Dubinsky, 2015), with utilization of these nutrients differing amongst *Symbiodinium* clades/types (e.g. greater nitrate uptake in C *versus* D (Devlin, 2015)). Both P and N are thought to be host-regulated and limiting for *in hospite Symbiodinium* (Rees, 1991) but inorganic forms are readily available in interstitial waters of carbonate sediments (Entsch *et al.*, 1983) and may therefore represent important nutrients in structuring environmental *Symbiodinium* populations and communities. It is currently unknown if gradients in temperature, light, and nutrients across reefs are important in structuring free-living *Symbiodinium* distributions.

To elucidate the distributions of free-living *Symbiodinium* populations on the GBR and examine how variations in thermal, irradiance and nutrient levels impact these populations, the *Symbiodinium* sediment community was characterized using deep sequencing across a water quality (inshore/offshore) and a temperature (north/central) gradient. Aposymbiotic juveniles of *Acropora tenuis* and *A. millepora* were exposed to these sediments and the resulting *in hospite* diversity after 11 – 145 days was characterized to examine how availability of free-living *Symbiodinium* types impacted uptake during early life-history stages. Finally, the initial infection dynamics and longer term physiological impacts on coral juvenile health and survival were also described in the context of variation in their *Symbiodinium* communities.

5.3 Materials and Methods

5.3.1 Experimental design and sample collection

5.3.1a Sediment collections

Sediments were collected from four sites along a water-quality gradient in the northern sector of the GBR in October of 2013 and in the central sector in 2014 to coincide with mass coral spawning. Wallace Islets and Wilkie Island (northern sector) and Pandora and Magnetic Island (central sector) are located inshore, where water quality is typified by fine particulate matter and high organic loads (Figure S5.1A, Figure S5.2). Sites at Great Detached and Tydeman (northern sector) and Rib and Davies (central sector) reefs are off-shore and experience fewer terrestrial inputs. At each site, surface sediments (top 10 cm) were collected from 4 m depth and maintained in bins supplied with 0.1 µm filtered flow-through seawater under shaded conditions until experimentation (one bin with three litres of sediment per site in 2013; three replicate bins, each with three litres of sediment per site in 2014). An additional three litres of sediment per site at all northern and central reefs were immediately frozen at -20°C for later analysis. In 2013, experiments occurred at Orpheus Island Research Station (OIRS), and in 2014, at the National Sea Simulator (Seasim) at the Australian Institute of Marine Sciences (AIMS). Seawater temperatures in sediment treatments at OIRS followed the natural temperature cycle on Orpheus Island reefs (Figure S5.1B). The seawater in sediment treatments at AIMS was filtered to 0.4 µm and followed the average temperatures of the four central sector reefs for that time of year during the first month of infection, and was then maintained at 27.4°C for the grow-out period (Figure S5.1A, B).

In both years, experimental treatments were covered with three layers of shade cloth so that approximately 50 µmol photons m^{-2} s^{-1} illumination was available for coral juveniles. To compare *Symbiodinium* communities before and after juveniles were exposed to central sector sediment treatments, an additional litre of sediment was frozen post-experiment (145 days post-experiment).

5.3.1b Spawning and rearing juveniles of *Acropora tenuis and Acropora millepora* for exposure to northern and central sediments

Eight colonies of *A. tenuis* from Wilkie Bay, plus eight *A. tenuis* and six *A. millepora* colonies from south Orpheus Island were spawned in November 2013 at OIRS. Spawning and gamete fertilization were performed as described in Quigley *et al.*, 2016 (Chapter 4). Briefly, eggs and sperm collected from colonies of *A. tenuis* and *A. millepora* were combined in three bulk fertilisation bins: one for *A. tenuis* Orpheus, one for *A. tenuis* Wilkie, and one for *A. millepora* Orpheus. At the 4-cell stage, embryos were washed three times and gently added to three, 500 L culture tanks (corresponding to each fertilization batch described above) connected to 27°C constant flow-through 0.1 µm filtered seawater. Terracotta tiles that had been curing in raw seawater at OIRS for 1.5 months were autoclaved (Tuttnauer, Netherlands) and added to the larval rearing tanks six days post fertilization. Tiles were strung vertically in columns of 5 into each of the culture tanks (25 tiles in each culture tank), with ground-up, autoclaved crustose coralline algae (*Porolithon onkodes*) pipetted onto the top of each tile to induce larval settlement and metamorphosis. Larvae were left to settle with low aeration and water flow for five days. Tiles with attached *A. tenuis* and *A. millepora* juveniles were randomly placed into each of the four bins with northern sediments 11 days post-fertilization. Specifically, six tiles with attached juveniles of *A. tenuis* were placed in each sediment treatment (in total: n = 24 tiles and 4,012 juveniles). The number of tiles with attached *A. millepora* juveniles placed in each sediment treatment varied (n = 2 tiles for the Wilkie sediment treatment, 7 for Wallace, 4 for Great Detach, 7 for Tydeman) in order to have roughly equivalent numbers of *A. millepora* juveniles per sediment treatment (in total: n= 903 juveniles of *A. millepora)*. All surviving juveniles from each treatment were sampled after 35 days of sediment exposure and stored in 100% ethanol at -20°C until DNA extraction (Table S5.1).

For larval rearing in 2014, four *A. tenuis* colonies from Magnetic Island and eight *A. millepora* colonies from Trunk Reef were collected and kept in filtered flow-through seawater at 27°C with natural light in the Seasim at AIMS. These species were spawned in October and November 2014, respectively. Gametes from two colonies of *A. tenuis* and from eight colonies of *A. millepora* were mixed in one bulk fertilization bin per species. Fertilization and larval rearing followed the same protocol that was used in 2013. Larvae were settled onto 6-well plates (20 larvae per well), with a single piece of autoclaved crustose coralline alga in each well. Plates containing *A. tenuis*

juveniles were placed in each of the sediment tanks for natural infection by *Symbiodinium* (3-5 replicate 6-well plates per tank). Plates containing *A. millepora* juveniles were also placed in each of the 3 replicate sediment tanks per site for natural infection by *Symbiodinium* (1-2 replicate 6-well plates per tank). Prior to placement in tanks, each well containing *A. millepora* and *A. tenuis* juveniles was photographed at 10x magnification in order to follow individual juveniles through time. Juveniles were sampled and stored in the same manner as those from the northern sector. However, unlike juveniles exposed to northern sediments that were only collected at one time point representing 35 days of exposure to sediments (Table S5.1), juveniles exposed to central sediments were sampled over 12 time points, representing 11 days of exposure to sediments until 145 days of exposure to sediments (days post exposure: d.p.e.) (Table S5.1).

5.3.2 Genotyping the *Symbiodinium* community in sediments and within juveniles

5.3.2a Filtration and concentration of sediments

To concentrate *Symbiodinium* cells prior to DNA extraction, frozen sediments were thawed, then filtered with a series of Impact vibratory sieves (500, 250, 125, and 63 µm stainless steel mesh) using filtered seawater (0.1 µm filtration) and concentrated into 5.5 L of 0.1 µm filtered seawater. To detach the *Symbiodinium* from the sediment grains, the filtration process was repeated five times. All equipment was washed and autoclaved between samples at 124°C and 200 kPa for 20 minutes (Sabac T63). To concentrate the full 5.5 L of sediment and *Symbiodinium* filtrate into a < 50 ml pellet, the slurry was centrifuged to decrease the volume following a series of steps at 4°C: 4500 rcf for 5 min (x2), 10 min (x3), 15 min (x3) and a final 30 min (x1) (Allegra X-15R: Beckman-Coulter). The pellet was frozen at -20°C until the time of extraction.

5.3.2b DNA extraction

Symbiodinium DNA was extracted from 3 replicate sediment filtrate samples per site (each 10 grams), including from samples collected both before and after experimental treatments involving sediments from the central sites in 2014 (total n = 39 sediment samples). DNA was extracted using the Mo Bio Powermax Soil DNA Isolation Kit (Carlsbad, CA) following a modified manufacturer's protocol. Custom steps included an initial digestion undertaken after the addition of solution C1 in a

rotating oven (80 rpm) at 65°C for 30 minutes as well as three, 20 s bead-beating steps (6.0 m/s) with 1mm silica spheres (MPBio) on the FastPrep-24 5G (MP Biomedicals). DNA was extracted from 59 and 176 coral juveniles from the northern and central sectors, respectively, using a SDS digestion and ethanol precipitation protocol (Wilson *et al.*, 2002) (Table S5.1). This extraction also included an equivalent bead-beating step at the lysis buffer step that consisted of three x 30 s of 1 mm silica spheres beat at 4.0 m/s.

5.3.2c Sequencing and data analysis

Next generation amplicon sequencing of the ITS-2 locus, data processing, and bioinformatics were performed as described in Quigley *et al.*, 2016 (Chapter 4). Briefly, Illumina Miseq sequencing was performed in three batches (2013 juveniles, 2014 juveniles, and sediments from both years). Raw reads from the 274 samples sequenced were analysed using the USEARCH and UPARSE pipeline (Edgar, 2013) (v. 7). Read mapping with a 97% identity threshold resulted in, on average, 39,260 (SE±1170) reads per sample. A total of 2,188 OTUs were identified with a custom *Symbiodinium* database built from all known *Symbiodinium* sequences retrieved from the complete NCBI database using Blast+ of taxon id: 2949 (Altschul *et al.*, 1990; Camacho *et al.*, 2009) (Table S5.2). OTUs were discarded if their Blast+ Expect value (E) was greater than 0.001 (De Wit *et al.*, 2012), as they likely represented non-specific amplification (i.e. other dinoflagellates), resulting in 1562 OTUs remaining.

Mapped reads were variance-normalized using the '*DESeq2*' package (v. 1.6.3) implemented in R (Love *et al.*, 2014). Abundances of *Symbiodinium* were compared among treatments using negative binomial generalized linear models in '*DESeq2*' (v. 1.6.3). Adjusted p-values were derived using the Benjamini-Hochberg Multiple-inference correction of alpha = 0.05. '*DESeq2*' outputs are expressed in multiplicative (log2 fold) terms between or among treatments (Love *et al.*, 2014). Therefore, a log2 fold change of 3 (3 log2 fold change) would represent an increase in the normalized abundance of 8 (2^3) in treatment "A" compared to treatment "B".

Seven analyses were performed to compare *Symbiodinium* communities among the different treatments and samples (temporal sampling design in Table S5.1) using '*DESeq2*' differential abundance testing. Analysis One: comparison among sample type (all juveniles or all sediment samples), cross-shelf position (inshore or offshore), and sector (northern or central sector) (type*shore*sector). Two: comparison among

sediment treatments from northern inshore vs. northern offshore vs. central inshore vs. central offshore sites (sediments: shore*sector). Within this analysis, the following comparisons were extracted: A) comparison between sediments from inshore vs. offshore sites (sediments: inshore*offshore), and B) comparison between sediments from northern vs. central sites (sediments: north*central). Three: comparison between pre- and post-experiment sediment treatments from each central reef site (sediments: reef*pre/post). Pre- and post-experimental sediments were similar in their *Symbiodinium* communities, with the only significantly differentially abundant *Symbiodinium* OTUs being those that did not vary significantly in juveniles (Supplementary Results in Appendix D). Four: comparison between juveniles (both coral species combined after exposure to sediment treatments for 27-41 days) from northern inshore vs. northern offshore vs. central inshore vs. central offshore sites (juveniles: shore*sector). Within this analysis, the following comparisons were extracted: A) comparison between juveniles exposed to sediments from inshore vs. offshore sites (juveniles: inshore*offshore) and B) comparison between juveniles exposed to sediments from northern vs. central sites (juveniles: north*central). Five: comparison between *A. millepora* and *A. tenuis* juveniles exposed to northern sediment treatments for 35 days (*A. millepora*A. tenuis* in 2013). Six: comparison between *A. millepora* and *A. tenuis* juveniles exposed to central sediment treatments for 27- 30 days (*A. millepora*A. tenuis* in 2014). Seven: comparison between *A. tenuis* juveniles exposed to central sediment treatments from 11 to 90 days (*A.tenuis* juveniles: reef*time point).

Nonmetric multidimensional scaling (NMDS) was performed on variance-normalized OTU abundances using the packages '*Phyloseq*', '*vegan*' and '*ellipse*' with a Bray-Curtis dissimilarity matrix (Murdoch *et al.*, 2007; McMurdie and Holmes, 2013; Oksanen *et al.*, 2013). NMDS analysis does not assume linear relationships between underlying variables, and distances between samples are indicative of their similarity, with closer samples more similar in their OTU diversity and abundance (Ramette, 2007). Permutational multivariate analysis of variance was used to determine if *Symbiodinium* communities differed significantly between factors using the '*adonis*' function in '*vegan*'. Correlation coefficients of variance-normalized, averaged abundances of each of the 1,562 OTUs across inshore/offshore, north/central juveniles and sediments were calculated and visualized using the package '*corrplot*' (Wei, 2013).

5.3.3 Juvenile physiological measures

5.3.3a Time to infection and survival

The number of days to infection was defined as the first day when *Symbiodinium* cells were visible throughout the whole juvenile (i.e., within the oral disc, tentacles, polyp column), as determined by microscopic observation of each juvenile. The total number of days surviving was determined by counting the total number of days an individual juvenile was observed alive based on microscopic observations.

To determine the effect of sediment source on the number of days to infection and the number of days surviving, generalized linear mixed models were fit in '*lme4*' (Bates *et al.*, 2014). These models compared inshore and offshore treatments (inshore* offshore), as well as pairwise comparisons between each central sector sediment site (Davies*Rib*Magnetic*Pandora). Within the nested experimental design, sites were replicated within the inshore and offshore categories, and shore and site were treated as fixed effects. Replicate tanks within site and replicate settlement plates within tanks were accounted for as random effects. The intercept was allowed to vary among sites, among tanks within sites, and among plates within tanks. A negative binomial distribution was used to account for over-dispersion in the time-to-infection models and a Poisson distribution was used for survival models. The overall impact of both of these fixed factors was assessed using the Likelihood Ratio Test through the 'Anova' function in the '*Car*' package (Fox *et al.*, 2010). As gradient was not significant in the survival model, it was dropped and the model re-fit. The glht function from the '*multcomp*' package was applied to extract Tukey *post hoc* tests for each site (Hothorn *et al.*, 2008). The Rib replicate 3 tank was removed, as only one of five plates survived, suggesting a specific issue with that tank. For the infection and survival models, outliers were statistically identified using the standard boxplot rule and Hampel identifier on the basis of values of plates within individual replicate tanks. Ultimately, the boxplot rule was chosen as it is not reliant on a median measure and thus has better performance in slightly asymmetric distributions. This resulted in the removal of three sub-replicate (plate) data points from the infection data (plate 25 in Pandora 1, plate 17 in Magnetic 1 and plate 27 in Davies 1) and two sub-replicate points in the survival data (plate 1 in Rib2 and plate 21 in Magnetic 1).

5.3.3b Photosynthetic measurements

Photophysiological performance of *in hospite Symbiodinium* was assessed in *A. tenuis* juveniles with Imaging Pulse Amplitude Modulated (iPAM) fluorometery and its affiliated software (Walz, Effeltrich, Germany). The iPAM allows resolution at 100 µm, thus allowing for accurate photosynthetic measures of small juvenile corals (Hill and Ulstrup, 2005). Symbiont densities were large enough to obtain reliable measurements by 41 d.p.e. The actinic light was calibrated with an Apogee quantum sensor (Model MQ-200, UT, USA) with the following settings: measuring intensity=3, saturation pulse intensity=3, gain=2. Prior to measurements, juveniles were dark adapted for 10 minutes by completely covering the plate with opaque black plastic. Measurements began at 10:00 AM and maximum potential quantum yield (ratio of maximum to variable fluorescence: Fv/Fm) was calculated from areas of interest manually drawn around individual juveniles. This measurement has been used to determine photosynthetic productivity in studies of plant physiology (Maxwell & Johnson 2000), reflects the efficiency of PSII (Krause and Weis 1991), and is a widely accepted indicator of stress in corals, with Fv/Fm yields proportional to coral health (Jones et al. 1999).

To detect differences in Fv/Fm among *A. tenuis* juveniles exposed to four central sector sediment sources, Generalized Additive Mixed Models (GAMMs) were used to account for non-linear trends over time (Wood, 2006) using the package '*mgcv*' (Wood, 2006, 2008). Penalized regression spline smoothing functions were applied to the interaction between sampling date and site, whilst site itself was also included as a fixed effect. Plate was treated as a random effect and the variance structure was allowed to vary through time using the varIdent weights argument. Temporal autocorrelation from sequential measurements were dealt with using first-order autoregressive correlation structure at the deepest level (plate) (Pinheiro and Bates, 2006). Model selection was performed with AIC and the log-likelihood ratio tests using the 'anova' function from the '*nlme*' package. A log-normal distribution was used, as Fv/Fm values are non-integer values inherently greater than zero. ACF plots and normalized residual plots *versus* fitted values conformed to assumptions of no autocorrelation and heterogeneity of variance.

In order to examine if there were any relationships between Fv/Fm measurements and normalized OTU abundances in *A. tenuis* juveniles exposed to sediments from central sector sites from day 41 to day 90, nine of the overall most

abundant OTUs in these juveniles (OTU2_*minutum*, OTU3_C1, OTU2129_C15, OTU115_C, OTU1_A3, OTU9_A13, OTU4_D1, OTU7_Zoanthus.sociatus, OTU427_C15) were selected for correlation analysis of their variance-normalized abundances and Fv/Fm values. Spearman's Rho Correlation coefficients (R^2) between each OTU's average variance-normalized abundance per time point and average Fv/Fm values per time point were calculated in the R base package 'Stats' (R Team, 2013), as this metric was found to be robust to non-normal bivariate distributions (Becker *et al.*, 1988). OTU and Fv/Fm values were averaged because multiple replicate *A. tenuis* juveniles were used per sediment site per time point. One correlation coefficient was calculated per OTU per time point per site.

5.3.4 Physical characterisation of sediments from central sites in 2014

5.3.4a Particle size distribution

To compare particle size distributions in sediments among the four central sector sites, a representative sample (from across the tank and through the depth of sediment) of approximately 28 grams was collected from each of the three replicate tanks per site (n = 12 samples). Samples were centrifuged at 3000 rpm for three minutes (Beckman-Coulter, Allegra X15R) and the pellet was washed three times in an equivalent volume of DI water to remove salts. Five grams of sediment from each sample were digested for 48 hours using 10 ml of 30% hydrogen peroxide, after which sediments were freeze-dried for 48 hours. Each sample was weighed and then sieved to achieve two fractions, corresponding to particles greater or less than 2000 µm in diameter. In all cases, the majority of the total sample weight was in the <2000 µm fraction (see Results). Samples were then submitted for laser ablation using the Mastersizer 2000 with hydro (Malvern; Worcestershire, UK) at the University of Western Australia. This analysis quantified the distribution of particles ranging from 0.02-2000 µm in size as percent volume per particle size.

To test whether the proportion of sediments in the two size fractions (i.e., greater or less than 2000 µm) differed among sites, a generalized linear model (GLM) with a binomial distribution was run using '*lme4*', and contrasts were extracted using the glht function. A generalized additive model (GAM) was constructed, and the package '*mgcv*' was used to determine if significant differences in particle size distributions were present across each site by applying a separate smoother to each site (variable coefficient models).

5.3.4b Total nitrogen, total carbon, phosphorus, calcium, aluminium and fe characterisation

Nutrient content was analysed in sediment samples frozen immediately upon collection (pre) and in samples collected after the experiment was complete (post). Approximately 15 ml of sediments were subsampled from frozen sediments and post-experiment samples were collected from each replicate tank with minimal seawater. Therefore, nutrient characterisation encompassed nutrients trapped on particles as well as in interstitial pore water. Sediment samples were dried for 5 days in an 80°C SESS oven and then ground to a fine dust for 1-2 minutes using a Rocklab Ring Mill (Auckland, NZ) and agate mortar. 5-15 µg (5 decimal point balance, Mettler AE 163) of dried, ground sediments were then digested with 32% HCl for 15 min on an 80°C hot plate. Total nitrogen and total carbon were measured using a SSM-5000A Shimadzu Solid Sample Module and TOC-L Total Organic Carbon Analyser and run with 10 standards to construct standard curves for both nitrogen and carbon.

Total nitrogen, phosphorus and calcium in sediments were compared among sites using linear models. Statistical tests of (fe and al) trace metal percentages were run using the package '*nlme*' with the weights function '*varIdent*' to allow different variance structures per site to obtain homogeneity of variance. *Post hoc* tests were run using the package '*lsmeans*' (Lenth and Hervé, 2015). Differences in total organic carbon were assessed with a generalized linear model using a Poisson distribution, with a site by treatment (pre/post experimental) interaction. Statistical differences between nutrient concentrations in pre- and post-experimental sediments were determined using generalized linear models in the package '*lme4*', with Tukey's *post hoc* tests performed using the glht function. Assumptions of homogeneity of variance, normality, and over-dispersion were met. Pre- and post-experimental sediments were similar in their nutrient characteristics (Supplementary Results in Appendix D).

5.3.4c Environmental covariates

To facilitate comparisons between the results of Cooper et al.'s (2011) study of environmental drivers of *in hospite Symbiodinium* diversity and the results found here, the following parameters were selected to compare the two communities: mud and carbonate content, average sea surface temperatures (SST) from 2000-2006, and 10 water quality measures. Mud and carbonate content were categorized as per Maxwell's

(1968) scheme for the Great Barrier Reef. Mud categories were as follows: 1) pure mud (> 80%), 2) predominately mud (60 - 80%), 3) very high mud (40 - 60%), 4) high mud (20 - 40%), 5) moderate mud (10 -20%), 6) low mud (1 -10%), 7) non-mud, and mostly sand (< 1%). Carbonate categories were as follows: 1) pure facies (> 90%), 2) high carbonate facies (80 - 90%), 3) impure facies (60 - 80%), 4) transitional facies (40 - 60%), 5) terrigenous facies (20 -40%), and 6) high terrigenous facies (< 20%). Water quality measures consisted of: total dissolved nutrients (dissolved inorganic nitrogen (DIN: NO_2, NO_3, NH_4), total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), particulate phosphorus (PP), particulate nitrogen (PN)) and irradiance (Suspended Solids (SS), Chlorophyll *a*, Secchi depth). Water quality, irradiance and temperature data were collected from datasets generated by the Australian Institute of Marine Sciences, the Great Barrier Reef Marine Park Authority, and Department of Primary Industry and Fisheries from 1976-2006, and retrieved from eAtlas (Furnas, 2003; Furnas *et al.*, 2005; Brodie *et al.*, 2007; De'ath, 2007; De'ath and Fabricius, 2008). Values for each covariate per site were extracted from interpolated modelled data using the R packages '*dismo*' (Hijmans *et al.*, 2013) and '*raster*' (Hijmans), and figures for each were created using the package '*mapping*' and a custom Queensland spatialPolygons file created by Murray Logan (Logan, 2016).

To address the highly correlated nature of many of the irradiance and water quality measures, each of the 10 measures were z-score standardized using the scale function in base R, and then summed for each site to create a Water Quality Index measure (WQI), as per methods in Cooper et al. (2011). To identify correlations between environmental covariates and specific *Symbiodinium* OTUs, subsets of the sediment samples and OTUs were used. Of the 39 sediment samples sequenced, only the pre-experimental sediments were used (n = 27 samples, comprised of: northern = 3 reps x 4 sites, central = 3 reps x 5 sites), which reduced the total number of OTUs considered (n = 1160 out of 1562 OTUs). Furthermore, 16 types of the possible 1160 OTUs were selected for Generalized Additive Model analysis (GAMS) across 7 of the 9 clades, with an emphasis on types that have known taxonomy (Guiry and Guiry, 2016). GAMS and partial plots were constructed using the packages '*mgcv*' (Wood, 2000, 2006, 2008) of variance normalized abundance data generated from the full data set (all juvenile and sediment data), where non-significant covariates and smoothers were dropped to achieve final models. Normalized abundances of OTUs identified to the same type were summed. A non-metric multidimensional scaling plot (NMDS) with

Bray-Curtis distance and environmental covariates as vectors was created using the packages '*vegan*', and '*ggplot2*'(Wickham, 2009).

5.4 Results

5.4.1 *Symbiodinium* community comparisons among sediments and juveniles of *Acropora tenuis* and *A. millepora*

5.4.1a Overall comparison across all factors and time points

When all data were combined across all samples of sediments, juveniles (both species) and time points, *Symbiodinium* communities differed significantly between juvenile corals and sediments (Figure 5.1, Table 5.1). Of the 1,562 OTUs that passed the E-value filter, 72% (1,125) were only found in sediment samples, 17.3% (271) OTUs were unique to juveniles, and 10.6% (166) were shared between juveniles and sediments (Figure 5.2C).

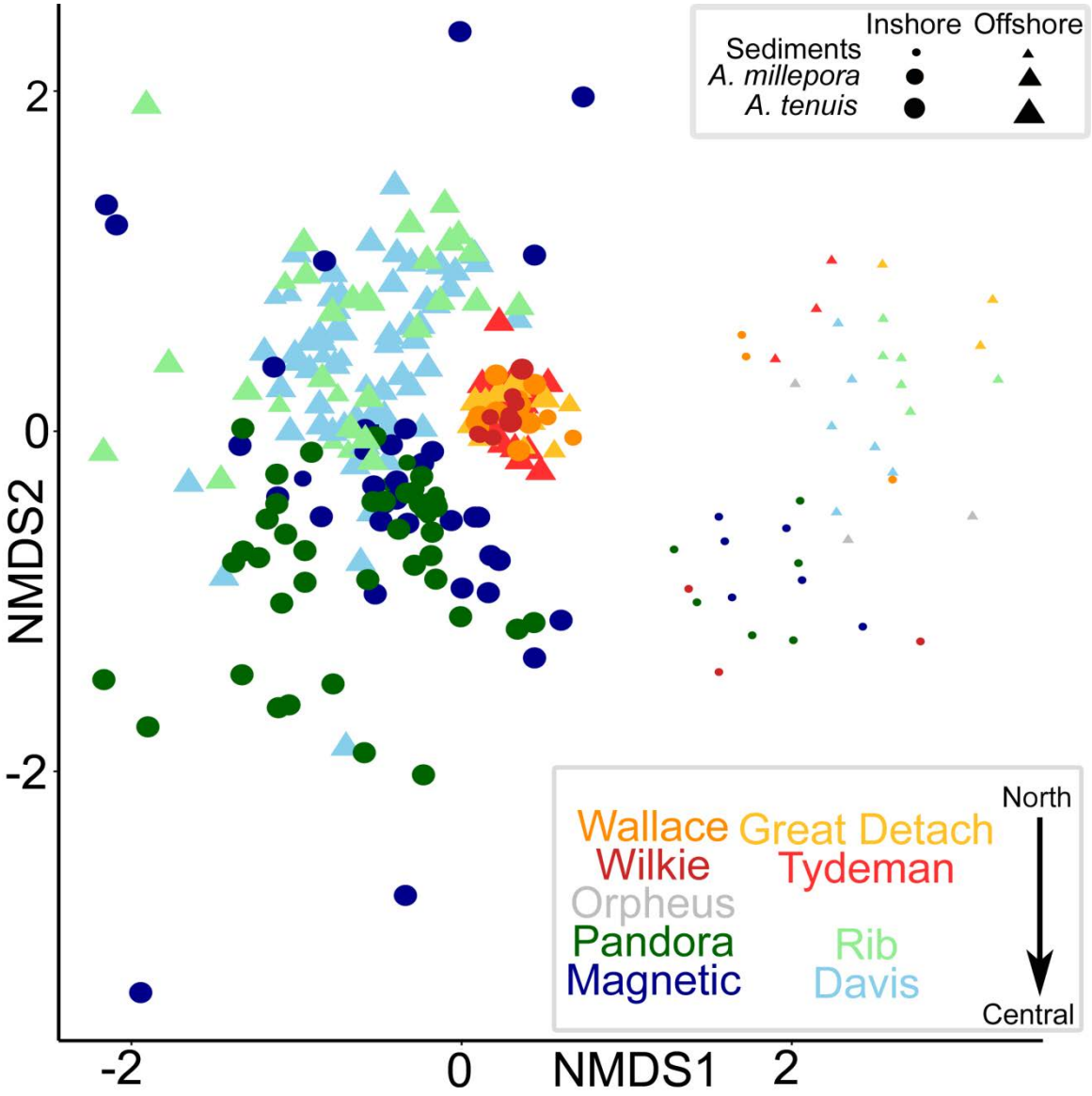

Figure 5.1 Nonmetric multidimensional scaling (NMDS) of variance-normalized abundances of *Symbiodinium* OTU's using Bray-Curtis distances. Symbols and their sizes represent: sediment samples (small circles and triangles), *A. millepora* juveniles (medium circles and triangles) and *A. tenuis* juveniles (large circles and triangles), where circles denote offshore sediment samples and juveniles exposed to offshore sediments, and triangles denote inshore sediments and juveniles exposed to them. Colours denote sector and cross-shelf origin of sediment samples, where yellow and red denote northern sediments and juveniles exposed to them; blue, green and grey colours denote central sediments and juveniles exposed to them. Orpheus samples are only represented as sediments and not juveniles.

Table 5.1 Summary of Permutational Multivariate Analysis of Variance tests to compare *Symbiodinium* community composition among juveniles and sediments. Factors are defined as follows: type (all juveniles or all sediment samples), shore (inshore or offshore) and sector (northern or central).

Factor	Df	F	R^2	*P*
Type	1	20.841	0.057	0.001
Shore	1	15.371	0.425	0.001
Sector	1	37.308	0.103	0.001
Type*Shore	1	5.226	0.014	0.001
Type*Sector	1	9.217	0.025	0.001
Shore*Sector	1	4.490	0.012	0.002
Type*Shore*Sector	1	2.610	0.007	0.004
Total	273		1.0	

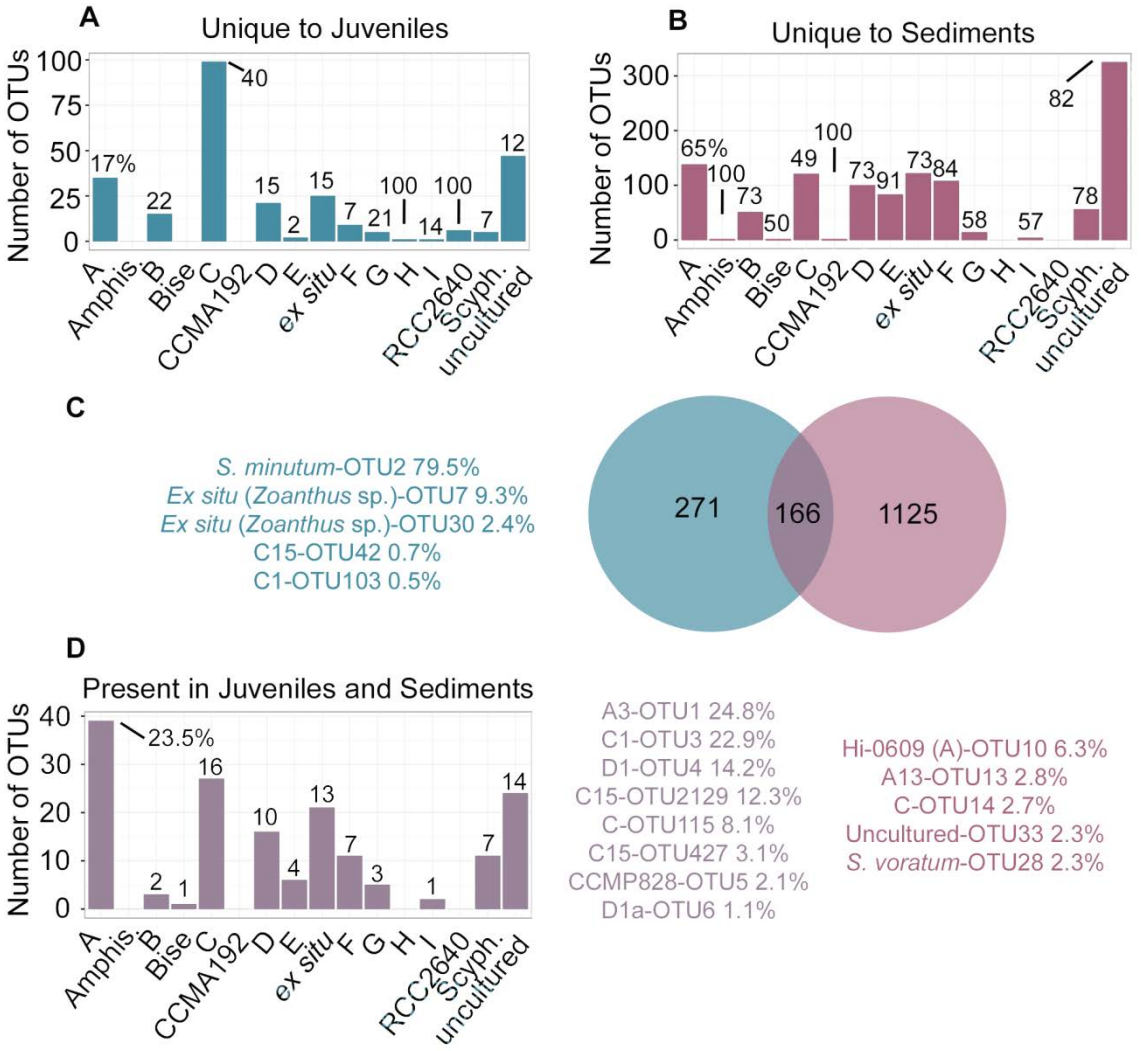

Figure 5.2 Number of *Symbiodinium* OTUs retrieved uniquely in **A**) juvenile samples, **B**) sediment samples, **D**) both juvenile and sediment samples. Values above each barplot represent the percent OTUs for that category (i.e., 35 clade A OTUs were retrieved from uniquely from juveniles samples, which represents ~17% of all clade A OTUs (total number = 212) retrieved across all categories. **C**) Venn diagram depicting the overlap in OTU diversity (i.e. the number of OTUs) between juvenile and sediment samples. OTUs listed either below or to the side shown in the same colours as the venn diagram correspond to those OTUs that make up a majority of the percent reads for each category (i.e., *S. minutum* reads made up 79.5% of all the uniquely juvenile reads). Barplots only include variables names for those categories in which reads were retrieved. Names have been abbreviated for figure clarity following their classification in NCBI. These include the following: Amphis. (unique type Fr3 extracted from host *Amphisorus* sp., accession: AJ291525), Bise (type A7 uniquely recovered from Bise Island sand in Japan, accession: EU106366), CCMA192 (unique unidentified type from clade E, accession: KJ652017), *ex situ* (diverse grouping of sequences only identified by the host name, e.g. *Zoanthus sociatus, Amphisorus hemprichii*, *Montipora foliosa*, etc., example accession: KP134474), RCC2640 (unique unidentified type belonging to clade C, accession: LN735404), *Scyphozoa* sp. (Scyph., accession: KP015090) and *Symbiodinium* classified in NCBI as uncultured (uncultured, example accession: AB849705).

5.4.1b *Symbiodinium* communities compared between juveniles and sediments

OTUs shared between juveniles and sediments predominantly belonged to clades A (39 OTUs) and C (27), as well as uncultured *Symbiodinium* (24) and *ex situ* types (21). *Symbiodinium* in clades D-H were also present in both sample types, but at lower diversities (< 40 OTUs in total) (Figure 5.2). Overall, within-clade diversity was much lower in juveniles (< 100 OTUs) than in sediments (< 300). Furthermore, the abundance of 69 OTUs differed significantly between sediment and juvenile samples (DESeq2 negative binomial generalized linear models of log2 fold adjusted p-values Benjamini-Hochberg (B-H) multiple-inference correction of alpha < 0.05, Figure 5.3), including types from clades A-G, or categorized as *ex situ* or uncultured *Symbiodinium* types. Juveniles had significantly greater abundances of clade C, in general, and of three C1 types (C15, C90, C15), specifically. Moreover, an A-type (CCMP2456: 1460) was found at 5-times greater abundance in juveniles than in sediments.

Of the three categories of comparisons used to test for similarities in the diversity and abundance of *Symbiodinium* OTUs among samples (i.e., comparisons of sediment vs. juvenile *Symbiodinium* communities, comparisons among communities in juveniles exposed to different sediment treatments, and comparisons among communities in sediments from different reefs; summarised in Figure 5.4), the diversity and abundance of *Symbiodinium* OTUs were the most dissimilar in the sediment-juvenile sample comparison (mean R^2= 0.229 ± 0.04, Figure 5.4). Within this comparison category, *Symbiodinium* communities in northern sector juveniles and sediments were the most strongly correlated (R^2=0.36-0.5; Figure 5.4), although communities in northern sector juveniles were also strongly correlated with communities in central sector inshore sediments (R^2=0.37-0.39). Interestingly, central sector juveniles and sediments were not strongly correlated (R^2 = 0.05-0.13), and central juvenile communities only slightly resembled northern sediment communities (R^2 = 0.04-0.21).

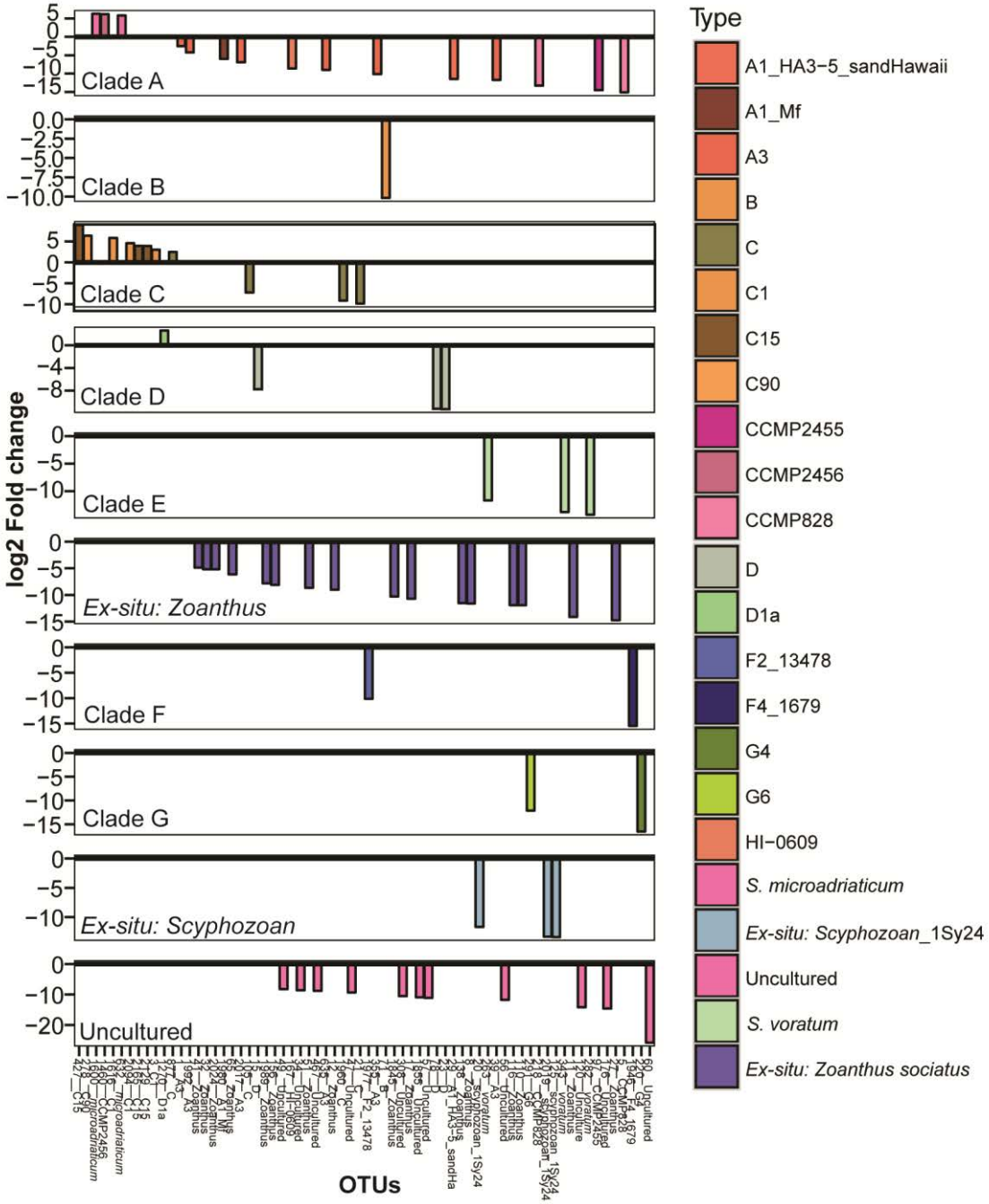

Figure 5.3 Significant log2 fold changes in normalized abundances of *Symbiodinium* OTUs in sediment-exposed juveniles compared to sediment samples. Log2 fold changes were calculated from negative binomial generalized linear models in DESeq2, where significance was determined from Benjamini-Hochberg p-values ($p < 0.05$). Colours represent the classification of different *Symbiodinium* types, and each panel represents a different clade (A, B, C, D, E, F, G) or category (uncultured and two *ex situ* categories from *Scyphozoa* or *Zoanthus* hosts). OTUs are ordered with respect to their log2 fold change, with greater log2 fold changes at the left side of the x-axis, and more negative log2 fold changes on the right. Positive values above the y = 0 line (in bold and black) denote *Symbiodinium* OTUs found in significantly greater abundances in juveniles compared to sediments. Note the different scales on the y-axis.

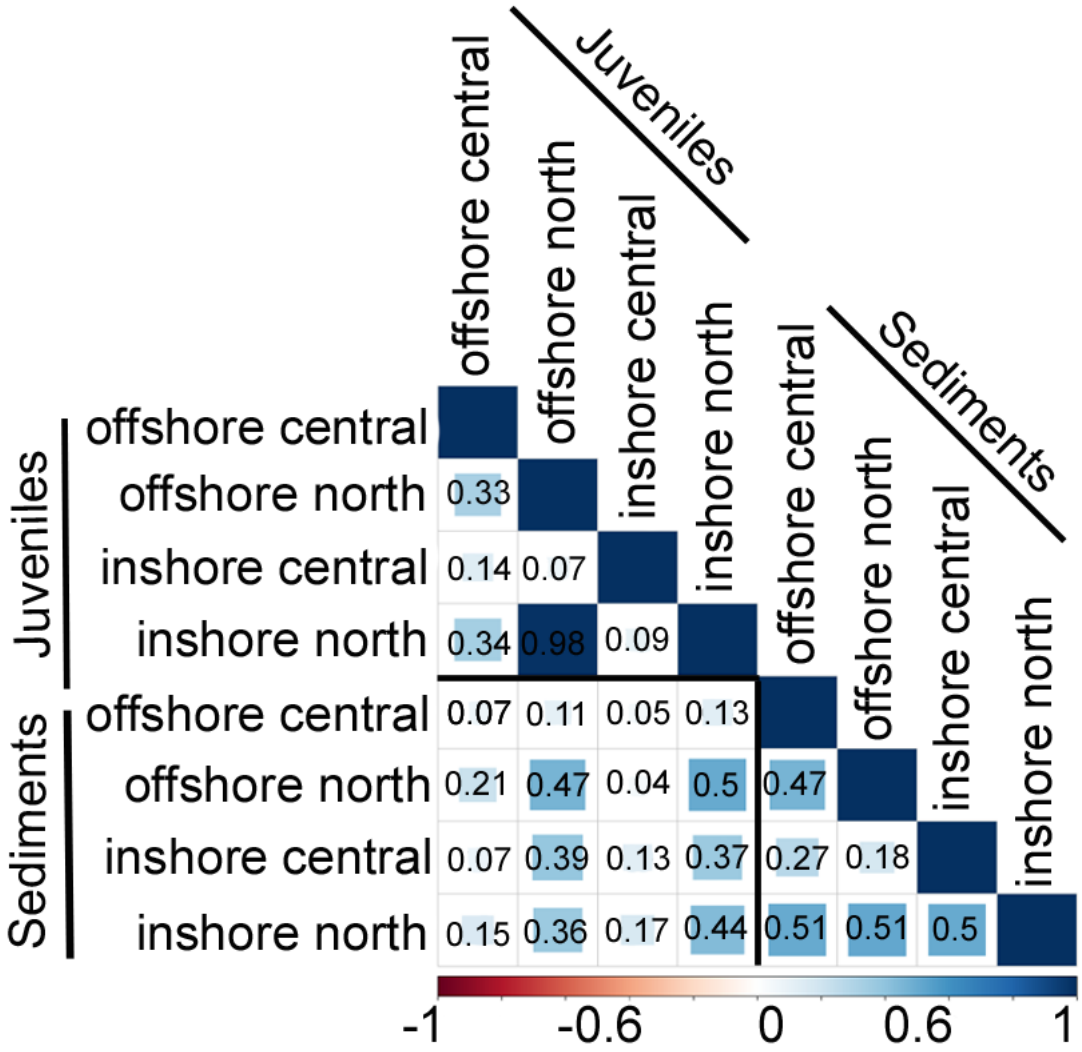

Figure 5.4 Correlation table comparing *Symbiodinium* communities (diversity and abundance) between juveniles and sediments across latitudes (northern *versus* central Great Barrier Reef) and water quality gradients (inshore *versus* offshore). Scale bar at the bottom runs from -1 in red (100% negatively correlated) to 1 in dark blue (100% positively correlated). Correlation coefficients for each comparison are in each respective box.

5.4.1c *Symbiodinium* communities compared among juveniles exposed to different sediments

No taxonomic category dominated the 271 OTUs that were recovered uniquely from juveniles. These OTUs consisted primarily of: C types (99 OTUs), uncultured *Symbiodinium* (47), A types (35), *ex situ* types (25), D types (21), and B types (15). OTUs unique to juveniles were relatively rare in abundance, comprising only 9.8% of juvenile reads. Unique juvenile OTUs predominantly belonged to clade B (*S. minutum*), with this type representing 79.5% of all reads unique to juveniles (Figure 5.2).

The diversity and abundance of *Symbiodinium* OTUs in juveniles exposed to sediments from different locations were moderately correlated (mean $R^2 = 0.33 \pm 0.14$; Figure 5.4). *Symbiodinium* communities associated with northern juveniles were highly similar at inshore and offshore sites (R^2 =0.98), however, communities in central inshore juveniles were distinct from communities in both central and northern offshore juveniles ($R^2 = 0.07$-.014).

5.4.1d Patterns in *Symbiodinium* communities within sediments

Overall patterns in sediment communities across all sites: The dominant categories or clades of *Symbiodinium* detected in reads and in OTU diversity were: *ex situ* sources (26.9% of total sediment reads, 11.1% of OTU sediment diversity), A (19.5%, 13.7%), uncultured *Symbiodinium* (18.6%, 27%) and C (11.5%, 11.5%) (Table S5.3). A majority of the unique sediment OTUs retrieved were identified as uncultured *Symbiodinium* (>300 OTUs), which represented 82% of all uncultured *Symbiodinium* OTUs across the whole dataset (Figure 5.2, Table S5.3). In the sediments, approximately 100 unique OTUs were represented from clades A, C, D, E, and F and from *ex situ* sources, along with a smaller number from clades B, I and G (Figure 5.2, Table S5.3). Greater than 91% of all clade E OTUs were retrieved exclusively from the sediments.

Of the three categories of comparisons used to test for similarities in the diversity and abundance of *Symbiodinium* OTUs among samples, *Symbiodinium* communities in sediment samples were the most strongly correlated (mean $R^2 = 0.41 \pm 0.06$, Figure 5.4). Within this comparison category, *Symbiodinium* communities within inshore, central sediments were the most dissimilar to sediment communities at other locations ($R^2 = 0.18$-0.27).

Inshore *versus* offshore sediment community comparisons: *Symbiodinium* communities in sediments differed significantly between inshore and offshore sites

(Table 5.1, Figure 5.1), with Wallace and Orpheus inshore sites falling between clusters representing these two groups. In total, the abundances of 58 OTUs differed between inshore and offshore sediments (B-H adjusted p-values < 0.05, Figure 5.5). Overall, inshore sites had higher abundances of clade D, whereas offshore sites had significantly greater abundances of uncultured *Symbiodinium* types. Interestingly, whilst only one type from clade E is currently recognized (*S. voratum*), significant differences were detected among multiple *S. voratum* OTUs (i.e. sequence variants) from inshore and offshore sediments, suggesting that ecologically relevant diversity exists at the type and sub-type level (Figure 5.5).

Inshore sediments had 5 log2 fold higher abundances of a B type and 2-3.3 log2 fold greater abundances of D1a and D1 than offshore sediments (B-H adjusted p-values < 0.05, Figure 5.5). Inshore sediments also had approximately 10 log2 fold greater abundances of uncultured type OTU234, F3.2, and A3. Offshore sites had 5-12.5 log2 fold greater abundances of 10 uncultured *Symbiodinium* types, types derived from *Scyphozoas, Amphisorus,* F2, and a diversity of A-types (A-Hi, A3, A1, CCMP828, A4.3, CCMP2455). *S. voratum* (clade E), clade C, and *ex situ* derived *Zoanthus sociatus* types also varied significantly in log2 fold abundances across inshore and offshore sites.

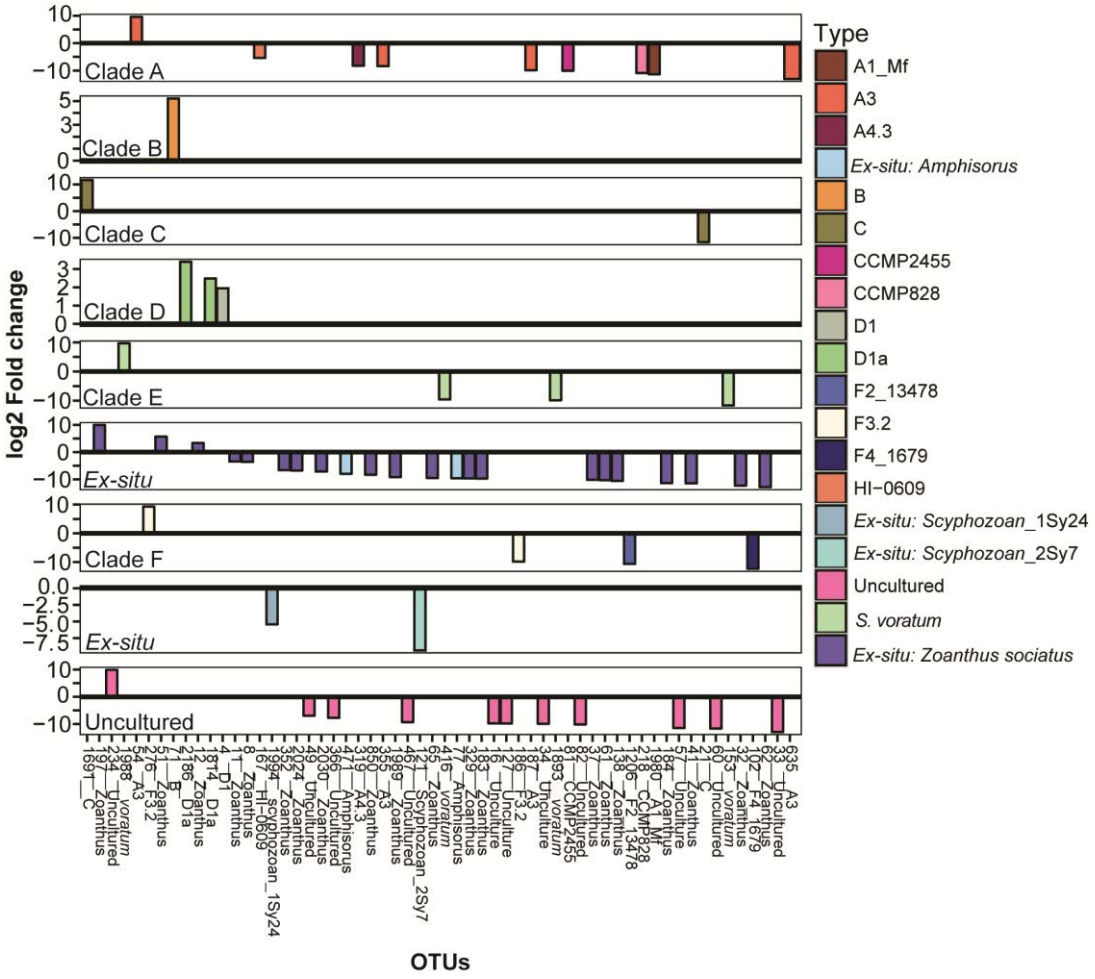

Figure 5.5 Significant log2 fold changes in normalized abundances of *Symbiodinium* OTUs in inshore compared to offshore sediments. Log2 fold changes were calculated from negative binomial generalized linear models in DESeq2, where significance was determined from Benjamini-Hochberg p-values ($p < 0.05$). Colours represent the classification of different *Symbiodinium* types, and each panel represents a different clade or category (including *ex situ* types): A, B, C, D, E, F, uncultured and three *ex situ* categories from *Scyphozoa*, *Zoanthus* or *Amphisorus* hosts. OTUs with greater log2 fold changes are at the left side of the x-axis, whereas more negative log2 fold changes are on the right. Positive values above the y = 0 line (in bold and black) denote *Symbiodinium* OTUs found in significantly greater abundances in inshore sediments. Note different scales on the y-axis.

Northern *versus* central sector sediment community comparisons: *Symbiodinium* communities within northern sector sediments differed significantly from those detected within central sector sediments (Figure 5.1, Table 5.1). The abundances of 47 OTUs differed significantly between northern and central sector sediments (B-H adjusted p-values < 0.05, Figure 5.6). Overall, *Symbiodinium* communities within clades A, C, E, and F and the uncultured category were more diverse and abundant in the northern sediments compared to central sediments (Figure 5.6). No types from clade D differed significantly in abundance between northern and central reefs.

Northern sediments had approximately 7.5-12.5 log2 fold greater abundances of 10 different A-types, including A13, A-Hi, A3, A1, CCMP828 and CCMP2455 (B-H adjusted p-values < 0.05, Figure 5.6). Northern reefs also had 5-9 log2 fold greater abundances of C and C1 types, which were not found in sediments from central reefs. There were also 10-12.5 log2 fold higher abundances of F5, F3.2, G6, and *S. voratum* in northern sediments, including many *ex situ* types from *Zoanthus*, *Scyphozoas*, *Amphisorus* and uncultured *Symbiodinium*. Central sediments had 5-6 log2 fold greater abundance of a B type and a new A type (Oku17), as well as 9 log2 fold greater A-Hi abundances. Central sediments also had 4.3-8.3 log2 fold more of three *Zoanthus ex situ* types and two uncultured *Symbiodinium* types (Figure 5.6).

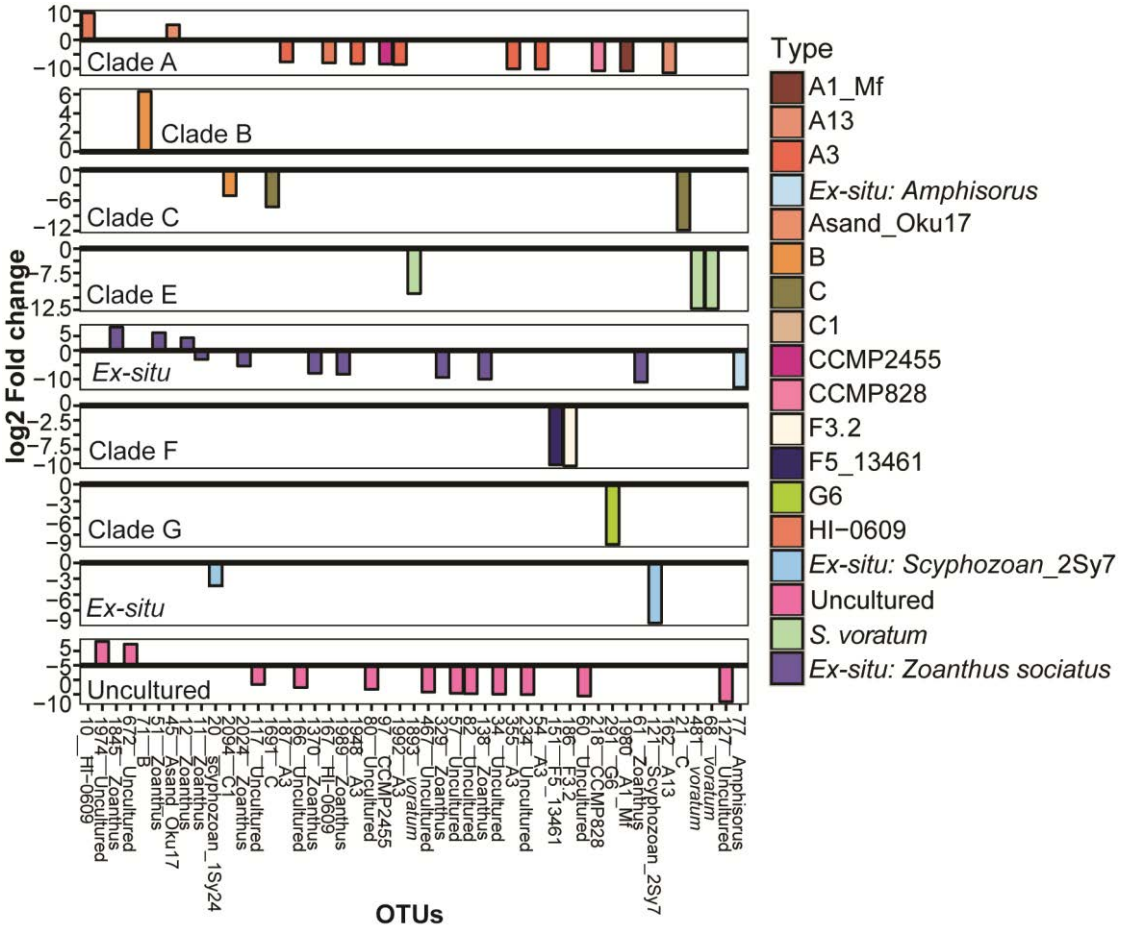

Figure 5.6 Significant log2 fold changes in normalized abundances of *Symbiodinium* OTUs in central compared to northern sector sediments. Log2 fold changes were calculated from negative binomial generalized linear models in DESeq2, where significance was determined from Benjamini-Hochberg p-values ($p < 0.05$). Colours represent the classification of different *Symbiodinium* types, and each panel represents a different clade or category (including *ex situ* types), i.e., clades A, B, C, E, F, G, and uncultured and three *ex situ* categories from *Scyphozoa*, *Zoanthus* or *Amphisorus* hosts. OTUs with greater log2 fold changes are at the left side of the x-axis, whereas more negative log2 fold changes are on the right. Positive values above the y = 0 line (in bold and black) denote *Symbiodinium* OTUs found in significantly greater abundances in central sediments.

5.4.2 Community comparisons among sediment-exposed, one month-old juveniles (27-41 d.p.e.)

5.4.2a Inshore *versus* offshore sediment treatment comparison

The early *Symbiodinium* community in juveniles differed significantly when exposed to inshore *versus* offshore sediments (Figure 5.1, Table 5.1). In particular, the abundances of 12 OTUs differed significantly in juveniles exposed to inshore *versus* offshore sediments (B-H adjusted p-values < 0.05). *Symbiodinium* communities in juveniles exposed to inshore sediments were characterized by clades B and D, and correspondingly, these clades were also at higher abundances in inshore sediments (Figure 5.7A). In contrast, juveniles in offshore sediments had a higher abundance of clade A, which was also found in greater abundance in offshore sediments.

Specifically, *S. minutum* (B1) types made up a majority of reads from juveniles exposed to inshore sediments, followed by types: A3, D1, C1, CCMP828, C15, and C. Juveniles exposed to sediments from inshore sites also had background abundances of types from ex situ *Amphisorus*, *Scyphozoa, Zoanthus,* as well as C90, CCMP2456, F5, and uncultured *Symbiodinium*. Inshore sites had 5.9-9.17 log2 fold greater abundance of D1a, 8.6 log2 fold greater D1, and 5.4 log2 fold greater CCMP2456 (B-H adjusted p-values < 0.05). Offshore sites had 5.4-7.7 log2 fold greater abundances of A3 and C15 types (B-H adjusted p-values < 0.05). Juveniles exposed to offshore sediments only had background abundances of *S. minutum*, C90, D, CCMP2456, and *Scyphozoa* derived types.

5.4.2b Northern *versus* central sector sediment treatment comparison

The early *Symbiodinium* community in juveniles differed significantly when exposed to northern *versus* central sediments. In addition, patterns in community composition at inshore *versus* offshore sites differed between sectors (Table 5.1). In particular, the abundances of 26 OTUs differed significantly in juveniles exposed to northern *versus* central sector sediments (B-H adjusted p-values < 0.05)(Figure 5.7B). Juveniles exposed to northern sediments were characterized by clades A and C, and correspondingly, these clades were also at higher abundances in northern sediments. Juveniles exposed to northern sediments also had significantly greater abundances of D1 and D1a compared to juveniles exposed to central sediments, despite northern sediments not having significantly greater abundances of clade D. Juveniles exposed to

central sediments had greater abundances of clade B, which again mirrored the greater abundances of clade B in central sediments.

Specifically, juveniles exposed to northern sediments were dominated by A3 and D1, with background abundances of C1, CCMP828, D1a, C15, C, C90 and CCMP2456. These juveniles also had 4.1-14.9 log2 fold more *S. microadriaticum*, A3, A7, CCMP2456 and CCMP828 (B-H adjusted p-values < 0.05). Juveniles exposed to northern sediments also had 2-8 log2 fold greater abundances of D1a, D1, C1, C90, and C. Juveniles exposed to sediments from the central sector were dominated by *S. minutum,* C15, C1, and C-types, including background abundances of A3, D1, A13, D1a, CCMP2456, F5, G2, *ex situ* types from *Scyphozoa, Zoanthus,* and some uncultured *Symbiodinium* types. These juveniles had 3.5-9.9 log2 fold more *S. minutum*, C, C15, and CCMP2456 compared to juveniles exposed to northern sediments.

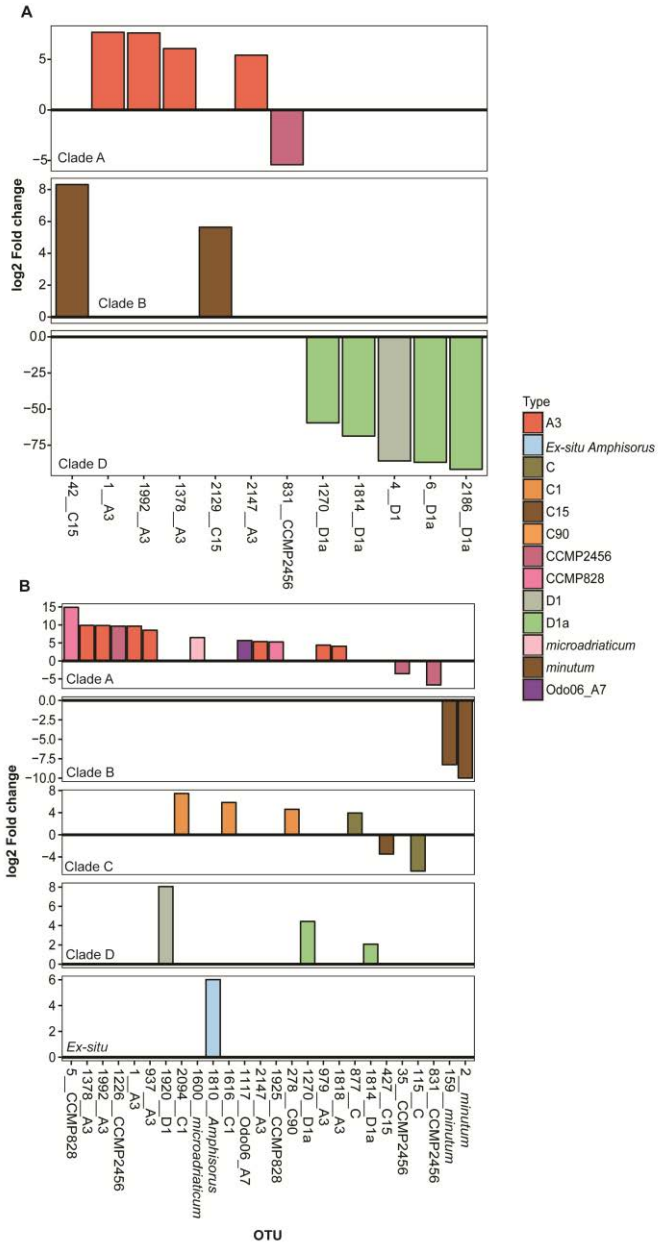

Figure 5.7 Significant log2 fold changes in normalized abundances of *Symbiodinium* OTUs in juveniles exposed to: A) inshore *versus* offshore sediments, and B) central *versus* northern sector sediments. Log2 fold changes were calculated from negative binomial generalized linear models in DESeq2, where significance was determined from Benjamini-Hochberg p-values ($p < 0.05$). Colours represent the classification of different *Symbiodinium* types, and each panel represents a different clade (including *ex situ* types). OTUs with greater log2 fold changes are at the left side of the x-axis, whereas more negative log2 fold changes are on the right. OTUs above the y = 0 line (in bold and black) are *Symbiodinium* OTUs that were found in significantly greater abundances in: **A**) inshore than in offshore sediments, and **B**) central than in northern sector sediments.

5.4.3 Temporal variation in *Symbiodinium* communities among juveniles exposed to central sector sediments

Symbiodinium communities in *A. tenuis* juveniles exposed to central sector sediments differed significantly over time (Permutational multivariate analysis of variance: $Df_{6,140}$, $F= 2.71$, $R^2= 0.09$, $p = 0.001$), by reef ($Df_{3,140}$, $F= 7.86$, $R^2= 0.13$, $p = 0.001$), and over time in a reef-dependent manner (reef*time point, $Df_{18,140}$, $F= 1.24$, $R^2= 0.13$, $p = 0.017$). At the earliest time point sampled (day 11), juveniles exposed to Davies, Magnetic and Rib sediments were all dominated by *ex situ Zoanthus sociatus* types (Figure 5.8). Juveniles exposed to Pandora sediments were uniquely dominated by type A13. Abundances of *Symbiodinium* C1, D1, C and A3 varied significantly when juveniles were exposed to sediments from different sites (B-H adjusted p-values < 0.05, Table S5.4). Large community shifts were seen 8 days later (day 19) at each site, with communities in juveniles exposed to offshore, central (Davies and Rib) sediments more closely resembling each other, whereas communities in juveniles exposed to inshore, central (Magnetic and Pandora) sediments were still distinct. Notably, the *ex situ* identified *Zoanthus sociatus* types and A13 types that were dominant in juveniles exposed to all sediment sources at the first time point were no longer present and were replaced by *S. minutum* (Pandora), D1 (Magnetic), C15 (Davies) and C15/A3 (Rib). Less dramatic shifts were seen at the third sampling time, although abundances of D1 and D1a in juveniles exposed to Magnetic and Pandora sediments decreased significantly, whilst types C1, C and *S. minutum* increased (Figure 5.8, B-H adjusted p-values < 0.05, Table S5.4). By the fourth sampling time point (day 41), C1, C, and C15 populations had increased in abundance. By the fifth time point (day 48), *Symbiodinium* communities in juveniles exposed to offshore sediments were beginning to resemble each other, particularly in their abundances of C15, A3 and C1, whereas by day 75, juveniles exposed to inshore sediments were more similar in their abundances of *S. minutum*, C1 and D1. At about three months (day 90), C1 or C15 were the dominant symbiont types in juveniles exposed to Davies, Rib and Magnetic sediments, whilst C and C1 were dominant in juveniles exposed to Pandora sediments. A more complete description of the dynamics of key *Symbiodinium* types in *A. tenuis* can be found in Appendix D.

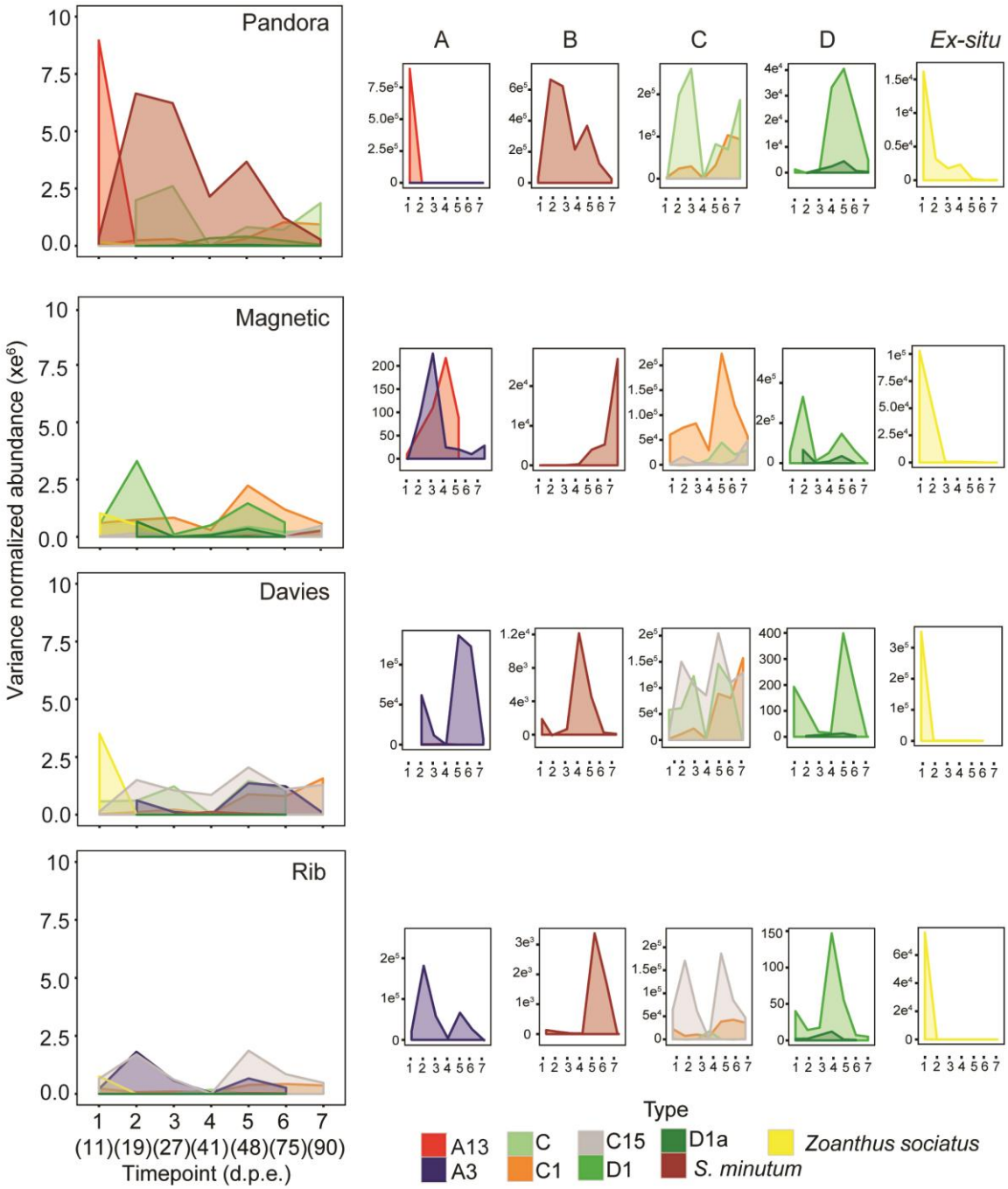

Figure 5.8 Population dynamics of key *Symbiodinium* types in *A. tenuis* juveniles exposed to central sector sediments. Abundances of *Symbiodinium* types were variance normalized. Analyses were restricted to 7 time points between day 11 and day 90 of the sediment treatment experiment (d.p.e.). The larger panels are all to the same scale, whilst the smaller panels for each site have y-axes that are scaled to best show abundance dynamics for each *Symbiodinium* type. Colours represent nine key *Symbiodinium* types, whose abundances differed significantly among time points, as well as among sites.

5.4.4 Time to infection and survival of juveniles exposed to central sector sediments

Infection was significantly more rapid in juveniles exposed to offshore sediments (19.1 days ± 0.09) than in juveniles exposed to inshore sediments (24.3 days ±2.13) (negative binomial generalized linear mixed model: $p = 0.014$), with no effect of site ($p = 0.45$) (Figure 5.9A). The cross-shelf (shore) effect was predominantly driven by significantly lower mean times to infection for juveniles exposed to offshore Rib and Davies sediments (19±0 days and 19.3±0.15 days, respectively) compared to inshore Pandora sediments (26.2 days ±4.37, $p = 0.0145$). Juveniles exposed to inshore Magnetic sediments had intermediate times to infection (22.54 days ±1.06) that were not significant once the fixed effect of gradient was dropped.

Survival of juveniles did not differ when juveniles were exposed to offshore (68.9±5.7 days) *versus* inshore (71.95±1.2) sediments (Poisson GLMM: $p = 0.179$), although significant differences did exist at the site level ($p = 0.002$) (Figure 5.9B). Juveniles exposed to Rib sediments survived significantly fewer days (51±0 days) compared to those exposed to Davies (80.8±7.9, TPH: $p = 0.003$) and Magnetic (75±0, TPH: $p = 0.03$) sediments. However, survival did not differ significantly between juveniles exposed to Rib *versus* Pandora sediments (69.1±2.1, TPH: $p = 0.1$). Survival of juveniles exposed to sediments from Davies, Magnetic and Pandora did not differ significantly (TPH: $p = 0.5$-0.9).

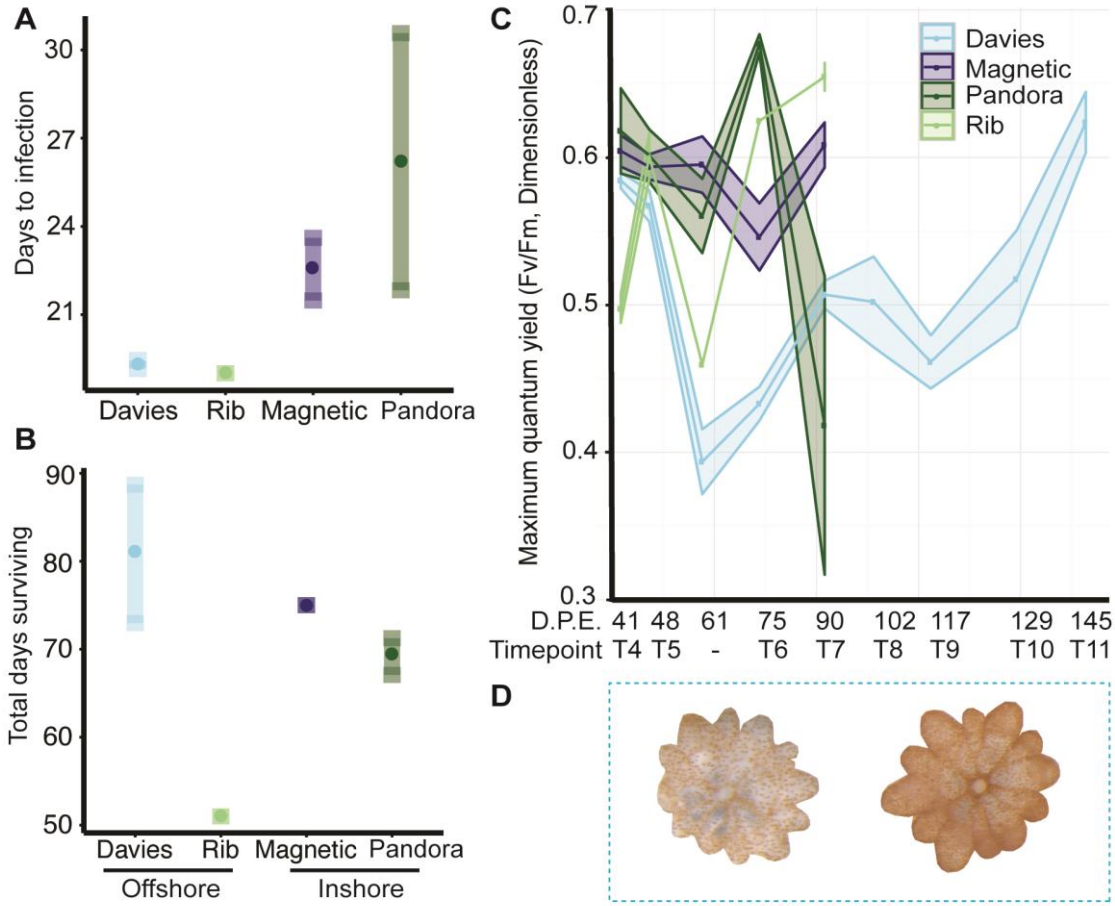

Figure 5.9 Time to infection and survival of *Acropora tenuis* juveniles exposed to sediments from central GBR sites. Inshore reefs are in darker shades and offshore sites in lighter shades (see legend). **A**) Mean time to infection ± SE per sediment treatment. Circles represent means; minimum and maximum values are located at the end of each standard error whisker. **B**) Juvenile survival in days (mean ± SE) per sediment treatment. **C**) Maximum quantum yield (Fv/Fm) of juveniles from 52 to 166 days post-fertilization. Due to low survivorship and cumulative sampling of Rib recruits, only one individual was measured at days 59 and 72, and two individuals at day 102, representing the number of days exposed to treatments. **D)** Two juveniles from the Davies sediment treatment highlight the level of variation in symbiont proliferation within sites. Both juveniles were collected at day 117.

5.4.5 Photo-physiology of juveniles exposed to sediment treatments

5.4.5a Temporal patterns in photochemical efficiency compared among juveniles exposed to central sector sediments

The photochemical efficiency (Fv/Fm) of juveniles varied significantly among sediment treatments and over time (Figure 5.9C, GAM: $p = 0.0283$, Table S5.8). Fv/Fm values for juveniles exposed to Davies sediments were initially ~0.6, but dropped sharply by day 48, and then generally increased until day 145. In comparison to juveniles exposed to Davies sediments, temporal patterns in Fv/Fm values differed significantly for juveniles exposed to sediments from the other three sites: Magnetic ($t=2.275$, $p = 0.0236$), Pandora ($t = 2.137$, $p = 0.0334$), and Rib ($t = 1.997$, $p = 0.0467$).

5.4.5b Patterns in photochemical efficiency and *Symbiodinium* community dynamics compared within and among sites

Differences in Fv/Fm yields among juveniles exposed to sediments from different sites were potentially driven by distinct *Symbiodinium* communities in these juveniles (Figure 5.9C, Figure 5.8). For example, juveniles exposed to Pandora and Magnetic sediments had the most similar Fv/Fm values (~0.6) at day 41, when juveniles in both sediment treatments had substantial populations of D1 (OTU4, Magnetic: 16,141 ± 9488, Pandora: 17,200 ± 16207). Juveniles in Davies and Rib treatments, however, had lower Fv/Fm values (closer to 0.5), and were dominated by C15 (Davies juveniles) or C1 (Rib juveniles). By day 75, all Fv/Fm values were divergent, as were the symbiont communities in juveniles (Figure 5.8). Interestingly, the greatest quantum yields for juveniles exposed to Rib sediments were recorded at day 90, when this site had its most diverse community of symbionts.

Initial and subsequent decreases in Fv/Fm in juveniles exposed to Davies sediments coincided with increases in A3 abundance (Spearman's rho $R^2= -0.74$) (Table 5.2). Fv/Fm values for juveniles exposed to Magnetic sediments were highly positively correlated with increasing abundances of *S. minutum* (Spearman's rho $R^2= 0.8$). Patterns in the Fv/Fm values of juveniles exposed to Magnetic and Pandora sediments were also highly correlated with changes in the abundance of *Symbiodinium* types C and A3 ($R^2=$ 0.8 and 1.0, -0.8 and -1). However, patterns in Fv/Fm values varied as abundances of these types differed between sediment treatments. Specifically, Fv/Fm values increased when C and A3 abundances increased in juveniles exposed to Magnetic sediments, whereas Fv/Fm values decreased when abundances of these two types increased in

juveniles exposed to Pandora sediments. Fv/Fm values in juveniles exposed to Rib sediments were most highly correlated with the abundances of types D1, C1 and C15 (R^2= 1, 0.8, 0.8).

Table 5.2 Spearman's rho R^2 correlation coefficients calculated for each of the nine most highly abundant OTUs in juveniles compared to Fv/Fm values per site. Although A13 (OTU9) was one of the most abundant types in juveniles overall, this OTU was only highly abundant in time points 1-3. Therefore, no correlation coefficients were calculated due to the zero abundance of this particular A13 OTU (OTU9) by time point 4 when Fv/Fm measurements commenced.

			Offshore				**Inshore**			
			Davies		**Rib**		**Magnetic**		**Pandora**	
Clade/Category	**OTU**	**Type**	**Fv/Fm**	**R^2**	**Fv/Fm**	**R^2**	**Fv/Fm**	**R^2**	**Fv/Fm**	**R^2**
B	2	*S. minutum*		0.41		0.2		**0.8**		-0.2
C	3	C1		-0.08		**0.8**		0.4		0.2
C	2129	C15		-0.01		**0.8**	NA	NA		-0.4
C	115	C		-0.23		0.2		**0.8**		**-0.8**
A	1	A3		**-0.74**		**-0.6**		**1.0**		**-1.0**
A	9	A13	NA	NA	NA	NA	NA	NA	NA	NA
D	4	D1		0.059		**-1.0**		0.4		0.4
Ex situ	7	*Zoanthus*		0.24		-0.25		0.1		0.4
C	427	C15		0.21		0.4		0.4		-0.4

5.4.6 Variation in *Symbiodinium* communities between sediment-exposed juveniles of *A. tenuis* and *A. millepora*

5.4.6a Northern sediment treatment compared between *A. tenuis* and *A. millepora* juveniles

Symbiodinium communities in juveniles exposed to northern sediments for 35 days did not differ between *A. tenuis* and *A. millepora* (Permutational multivariate analysis of variance: $Df_{1,58}$, F= 2.036, R^2= 0.034, p = 0.095). In these sediment treatments, juveniles of both *A. millepora* and *A. tenuis* were dominated by *Symbiodinium* A3 and D1, and to a lesser extent by D1a. The abundance of nine OTUs varied between species (B-H adjusted p-values < 0.05, Table S5.5). *A. millepora* juveniles had ~2-6.5 log2 fold greater abundances of C1 types and *S. microadriaticum*. *A. tenuis* juveniles had 3-6.9 log2 fold greater abundances of two C15 types, CCMP2456 and G4. Both species also hosted *Symbiodinium* types C90, C, CCMP828, F3.2, F4, *S. minutum*, and uncultured *Symbiodinium* at very low abundances that did not differ significantly between the two species (Table S5.5).

5.4.6b Central sediment treatment compared between *A. tenuis* and *A. millepora* juveniles

Juveniles of *Acropora millepora* and *A. tenuis* differed significantly in the symbiont communities hosted after 27 – 30 days of exposure to central sediments (Permutational multivariate analysis of variance: $Df_{1,41}$, F= 4.83, R^2= 0.107, p = 0.001). *A. millepora* juveniles were dominated by C1, C15, D and *S. minutum* types. This species also had background abundances of A3, C, D1a, *S. microadriaticum* and *Zoanthus* types. *A. tenuis* juveniles exposed to central sediments were dominated by *S. minutum*, C, C15, C1 and six A types. Background diversity in *A. tenuis* was higher compared to *A. millepora* juveniles and included types A13, CCMP2456, D, D1, F5, G2, *ex situ Scyphozoa, Zoanthus* and uncultured *Symbiodinium* OTUs. Compared to *A. millepora* juveniles, *A. tenuis* had significantly greater (2-7 log2 fold higher) abundances of *S. minutum*, C1, F5, CCMP2456 types, and 6 uncultured *Symbiodinium* (B-H adjusted p-values < 0.05, Table S5.6). *A. millepora* had significantly greater (3.6-8.3 log2 fold higher) abundances of C1, C, C15, *S. microadriaticum* types, D1a, and D1.

5.4.7 Physical characterization of central sector sediments

5.4.7a Total organic carbon, nitrogen, phosphorus, calcium, and trace metals

Nutrient profiles of sediments differed significantly among sites. Rib sediments had significantly greater total nitrogen compared to Pandora sediments, but total nitrogen did not differ significantly in any other pairwise combination of sites (Table 5.3). Within post-experimental sediments, Magnetic and Pandora sediments had significantly lower percent calcium compared to Davies sediments (Table 5.3). Conversely, post-experimental Davies sediments had significantly lower percentages of al and fe compared to Magnetic and Pandora sediments (all $p < 0.0001$). Rib post-experimental sediments also had significantly less al and fe compared to Magnetic and Pandora sediments (all $p < 0.0001$), although Rib and Davies sediments did not differ significantly (Table 5.3). A similar pattern was detected in pre-experimental sediments, with both Davies and Rib sediments having significantly lower percentages of al and fe compared to Magnetic sediments (all $p < 0.0001$), but not compared to each other ($p = 0.79$). No significant differences in percentages of carbon or phosphorus were detected among sites (Figure 5.10A, Table 5.3).

5.4.7b Sediment size classes

The percentage of particles in sediment samples that were <2000 μm in size varied only marginally among the four central sector sites, i.e., between 84.5% ± 4.3 and 88.2% ± 4.4 of total sediment weight. Correspondingly, 11.8% ± 4.4 to 15.5% ± 4.3 of total sample weights comprised particles >2000 μm. No statistical differences in the proportion of sediments in size classes larger or smaller than the 2000 μm cut-off were detected among sites (Table 5.3). Considering sediments in the < 2000 μm particle size class, and using Davies as a reference site, sediment size distributions varied significantly among sites (Table 5.3). Davies, Pandora and Magnetic had the greatest proportion of sediments within the ~700 μm range, whereas Rib sediments were predominantly ~1000 μm (Figure 5.10B). Davies and Pandora sediments had the largest range of fine particle sizes (0-250 μm), whereas Magnetic and Rib had the greatest abundance of particles from 1750-2000 μm.

Table 5.3 Summary statistics for nutrient and grain size characteristics of sediments from the central sector in 2014. Abbreviations are as follows: Tukey's *post hoc* test (TPH), Linear Model (LM), Generalized Linear Model (GLM), Generalized Least Squares (GLS), Generalized Additive Models (GAM).

Nutrient	Comparison	Method	*P* =
Total organic carbon (%)	Pre- and post	Poisson GLM: TPH	0.934
	Site	Poisson GLM: TPH	0.997-1
Total nitrogen (TN=PN+TDN) (%)	Pre- and post-experimental	LM: TPH	0.2
	Site	LM: TPH	• Rib > Pandora (**0.01**) • All other pairwise comparisons insignificant (0.07-0.88)
Total phosphorus (TP=PP+TDP) (mg/kg)	Pre- and post-experimental	LM: TPH	0.651
	Site	LM: TPH	0.1-0.99
Calcium (%)	Pre- and post-experimental	LM: TPH	**0.015 (Pre > Pre)**
	Site	LM: TPH	• Pre: 0.08-1 • Post: Magnetic, Pandora < Davies (**0.0315, 0.0541**)
Al (%)	Pre- and post-experimental	GLS	**0.0285 (Pre > Pre)**
	Site	GLS TPH	• Pre: Davies, Rib < Magnetic (< **0.0001)** • Post: Davies, Rib < Magnetic, Pandora (both < **0.0001**), no difference Rib and Davies (0.79 - 0.98)
Fe (%)	Pre- and post-experimental	GLS	**0.018 (Pre > Pre)**
	Site	GLS TPH	• Pre: Davies, Rib < Magnetic (< **0.0001)** • Post: Davies, Rib < Magnetic, Pandora (both < **0.0001**), no difference Rib and Davies (0.79 - 0.98)
Sediment particles size > 2000 µm and < 2000 µm	Site	GLM TPH	All pairwise comparisons insignificant (> 0.761)
Sediment particles size > 2000 µm and < 2000 µm	Site	GAM	**$< 2^{-16}$**
Distribution of particles < 2000 µm	Site compared to Davies	GAM	• Magnetic (p = **1.98^{-09}**) • Pandora (p = **4.89^{-10}**) • Rib (p = **0.0245**)

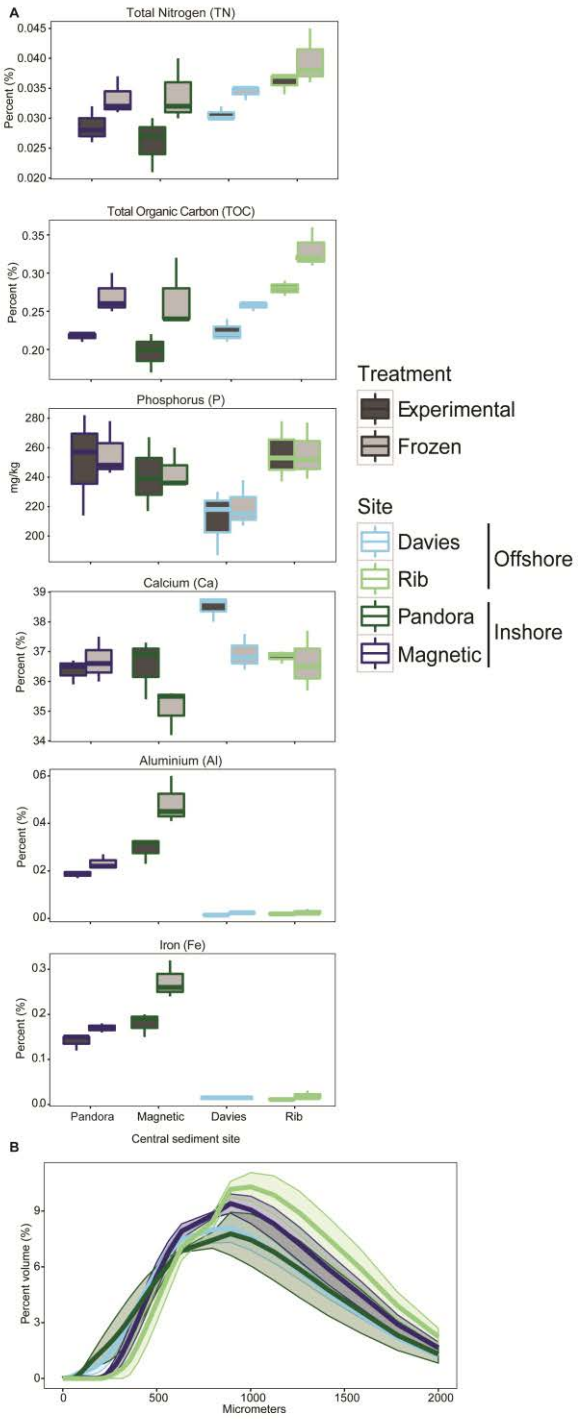

Figure 5.10 Nutrient and sediment size profiles for central sector sediments collected in 2014 at the time of initial collection (pre-) and at the end of the experiment (post-). **A)** From top to bottom: total nitrogen, total organic carbon, phosphorus, calcium, aluminium, iron. **B)** Sediment profiles in percent volume of sample per site for sediment particles less than 2000 µm in size. Treatments include frozen (pre-experimental sediments in dark grey) *versus* experimental (post-experimental in light grey). Colours correspond to each sediment site location, with inshore sites represented by dark hues and offshore sites as light hues.

5.4.8 Environmental covariates of *Symbiodinium* communities in sediments

WQI, SST, carbonate and mud content correlated with the normalized abundances of *Symbiodinium* types from clades A, B, C and D, but not types within clades E, F and G (Figure 5.11, Figure S5.2, Figure S5.3, Table S5.7). Responses of free-living *Symbiodinium* to these environmental covariates were generally linear. Types from clade A were most variable in the covariates that influenced their distributions, with *S. natans* increasing in abundance with increasing WQI and decreasing SST, and decreasing in abundance with lower carbonate and mud content. This contrasted with A3 abundances, which decreased with increasing WQI. The abundance of type A2 was significantly correlated with SST, with greater abundances at higher temperatures. B2 and B4 increased in abundance with increasing SST and sand content (low mud). C1 increased in abundance as carbonate content decreased, whereas none of the environmental covariates were particularly important in explaining the distributions of types C15, C3 or C90. Interestingly, temperature did not significantly explain D1 or D1a abundances, which significantly increased as carbonate decreased, again in contrast to *in hospite* patterns. *S. voratum* (E) distributions did increase with less carbonate. Finally, none of the environmental covariates tested here significantly explained abundances of types *S. kawaguti* (F1), G3 and G6, suggesting that other, unmeasured environmental covariates are important in explaining the distributions of these types.

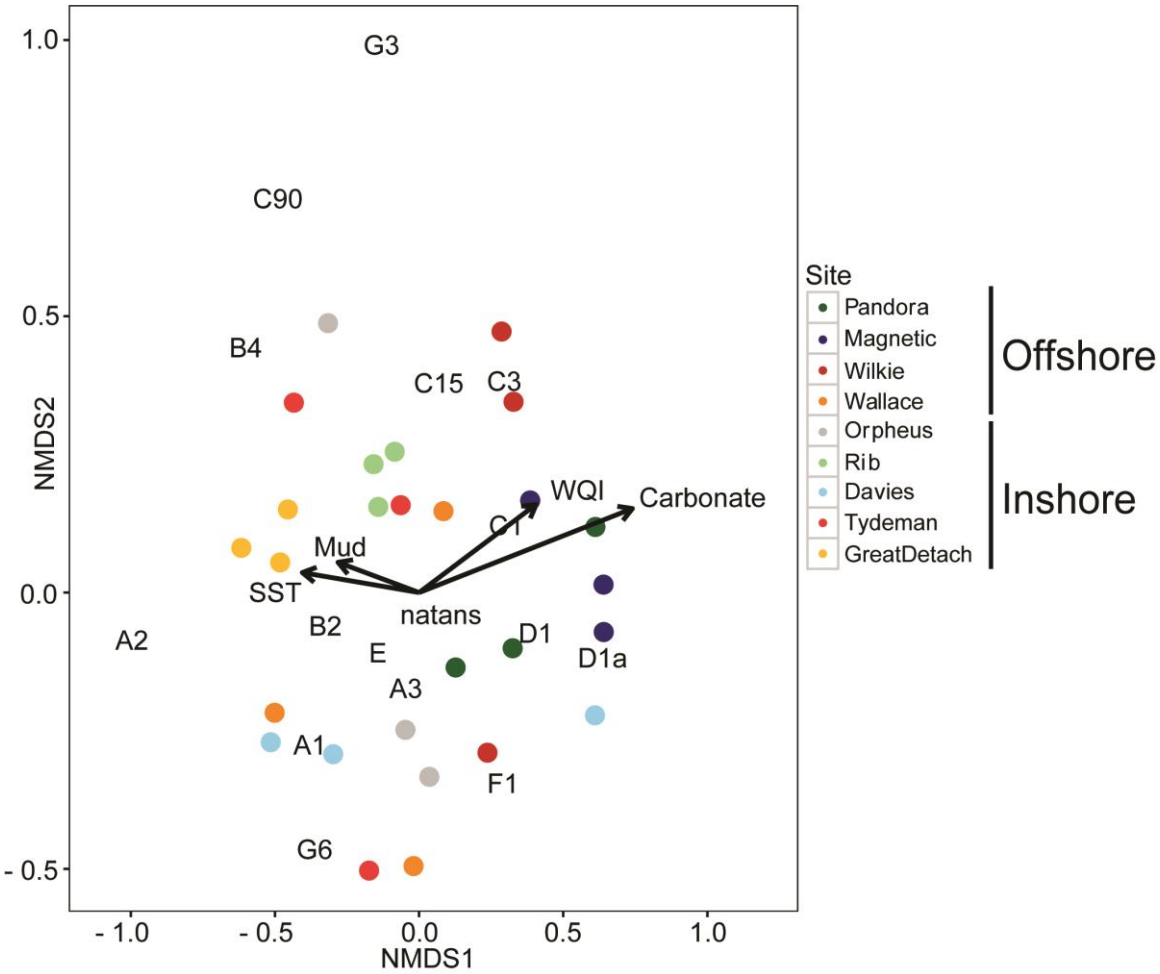

Figure 5.11 Nonmetric multidimensional scaling (NMDS) of variance-normalized *Symbiodinium* type abundances (summed OTUs that belong to the same type) in pre-experimental sediment samples using Bray-Curtis distances. Northern sediments are in yellow and red, and central sediments are in grey, blue and green colours. Inshore and offshore sediment samples are in dark and light hues, respectively. Environmental covariates (mud, carbonate, Sea Surface Temperature, and Water Quality Index) are overlaid as vectors. Large vector values for mud and carbonate represent low percent mud and carbonate (see Materials and Methods). *Symbiodinium* types in black are also overlaid onto the ordination space. As an example, high normalized abundances of types D1 and D1a correspond to high mud content (i.e. low vector values of mud).

5.5 Discussion

Comparisons between free-living *Symbiodinium* communities in sediments and endosymbiotic communities in recently settled juveniles of the horizontally-transmitting corals *Acropora tenuis* and *A. millepora* reveal a bipartite strategy governing *Symbiodinium* acquisition. Low overlap between OTUs in sediments and those taken up by juveniles indicates a degree of selectivity in symbiont acquisition. On the other hand, variation in *Symbiodinium* communities acquired by juveniles reflecting variation among sediment sources indicates that local environmental availability also plays a role, albeit lesser, in structuring *in hospite Symbiodinium* communities. Variation in the diversity and abundance of *Symbiodinium* types in sediment sources corresponded to a thermal gradient between sites in the northern and central sectors of the GBR and a water quality gradient between nearshore and offshore sites across the continental shelf, suggesting that environmental gradients influence free-living communities. Detection of previously unknown levels of *Symbiodinium* diversity in the sediments, combined with evidence that *Symbiodinium* community composition influences infection rates, survival, and photo-physiology of coral juveniles, has implications for local adaptation of both free-living *Symbiodinium* populations and symbiotic partnerships.

5.5.1 *Symbiodinium* uptake by *Acropora* juveniles is selective but varies with local availability

In combination, findings that only 10.6% of the 1,562 OTUs recovered in this dataset were shared between juveniles and sediments, and that OTUs taken up did not necessarily reflect dominant types in sediments, contradict the suggestion that acquisition of *Symbiodinium* from environmental sources is "promiscuous" in early life-history stages of corals (Cumbo *et al.*, 2013). Instead, the low overlap found between the two communities is consistent with a degree of selectivity in juvenile uptake, a conclusion supported by other studies that have compared seawater or benthic communities with communities in either adult hard corals (Pochon *et al.*, 2010; Huang *et al.*, 2013; Sweet, 2014) or juvenile octocorals/acroporids (Coffroth *et al.*, 2006; Yamashita *et al.*, 2013). Despite only a small proportion of *Symbiodinium* types being taken up out of the wide range of environmental *Symbiodinium* available, *Symbiodinium* communities in juveniles were not highly conserved among sites (with the exception of inshore/offshore northern juveniles). *In hospite* communities tended to differ with

sediment source, suggesting that local availability of *Symbiodinium* in the sediments influences *in hospite* juvenile diversity to a degree. For example, juveniles exposed to inshore sediments with higher abundances of clades B and D concomitantly had higher abundances of these two clades relative to other symbiont types. Such linkages between local sediment and *in hospite* communities at the clade level may have important implications for local adaptation of coral hosts.

Overall, patterns in *Symbiodinium* communities shared between juveniles and sediments were more similar between inshore and offshore sites than between northern and central sites (with the exception of clade C). In particular, communities acquired by juveniles exposed to inshore *versus* offshore sediments from the northern sector were highly correlated. The greater differences in community composition when juveniles were exposed to northern *versus* central sector sediments suggest that temperature (or another factor that varies with latitude) may be a more important factor than water quality in structuring *in hospite Symbiodinium* communities in juveniles, at least in relation to symbiont abundances in sediments. Interestingly, both the free-living sediment communities at the central inshore sites and the *in hospite* communities in juveniles exposed to these sediments were the most distinct of all sediment and juvenile samples examined. The parallel distinctiveness of these communities suggests a direct link between *Symbiodinium* diversity in the sediments and communities acquired by juveniles, and may reflect substantial levels of human impact on reefs around Magnetic and Orpheus Islands. However, further work is needed to elucidate how human activities affect free-living *Symbiodinium* communities, and the potential knock-on effects for coral juveniles in these areas.

5.5.2 Predisposition for uptake of specific *Symbiodinium* types

Although local availability of free-living *Symbiodinium* appears to be a factor structuring *in hospite* symbiont communities in acroporids at the clade level, correlation analyses revealed low similarity in the abundance and diversity of *Symbiodinium* between juvenile and sediment communities at the type/OTU level. Such lack of congruence between sediment communities and communities found in juveniles exposed to them suggests that a genetic component contributes to the regulation of symbiont communities. Theoretically, no change in symbiont communities in juveniles under different availability scenarios suggests that host genetics offer predominant control in regulating those communities (Cunning et al. 2015). Evidence that host

genetics plays a major role in regulating *Symbiodinium* communities in juvenile acroporids is consistent with a recent study showing a heritable component to the *Symbiodinium* community acquired by juveniles of at least one coral species (Chapter 2, Poland and Coffroth, 2016). Predisposition for acquiring certain *Symbiodinium* types is also supported by acquisition of clade C symbionts in offshore sediment treatments when their comparative availability in sediments was low, and potentially signifies the presence of recognition mechanisms that result in clade C dominating adult communities of these corals (Abrego *et al.*, 2009). A bipartite system, involving both genetic and environmental contributions to the structuring of symbiont communities, has also been observed in closely-related *Nematostella*-bacterial partnerships (Mortzfeld *et al.*, 2015). In these symbioses, core epithelial bacterial communities in *Nematostella* juveniles were conserved, but otherwise, bacterial communities in juveniles strongly resembled bacterial communities found in their local habitat. The influence of environmental symbiont availability in structuring *Symbiodinium* communities in acroporid juveniles highlights the importance of managing environmental stressors that may impact diversity and subsequent uptake.

Juvenile *in hospite Symbiodinium* communities also varied through time, although it is unclear whether temporal changes reflected ontogenetic changes associated with juvenile development or *Symbiodinium*-*Symbiodinium* dynamics. The progression of *in hospite* juvenile communities through time towards the composition of adult communities however, suggests that developmental changes are important in structuring *Symbiodinium* diversity. The lack of change in relevant symbiont availability within the sediments from the start to the end of the experiment indicates that lack of symbionts available for infection was not the underlying reason. Similar fine-scale successional patterns over short time-scales (1-2 weeks) in bacterial communities have been tied to developmental (including immunological) remodelling in *Nematostella*, amphibians and insects (Mortzfeld *et al.*, 2015). Although developmental changes associated with metamorphosis in these systems may be more extreme than changes associated with growth in *A. tenuis,* coral juveniles commence calcification to form complex external skeletons from ~20-50 days post fertilization. Skeletogenesis and developmental changes specific to corals have large impacts on gene expression, and differentiate the juvenile stage from larval and adult expression patterns (Reyes-Bermudez *et al.*, 2009). Accordingly, temporal differences and successional patterns in

Symbiodinium communities associated with *A. tenuis* juveniles may be tied to differential calcification requirements and a specific preference for clade C over clade D symbionts, as this clade provides more photosynthates to juveniles (Cantin *et al.*, 2009), and can cause 3-fold greater growth rates in juveniles (Little *et al.*, 2004).

Differences in the *Symbiodinium* communities acquired by juveniles of the two host species, *A. millepora* and *A. tenuis,* highlight different evolutionary trajectories in the development of symbioses between these two congeneric species. Although *Symbiodinium* communities did not differ significantly overall between these two species when juveniles were exposed to northern sediments, communities in these two species did differ when exposed to sediments from the central section. Therefore, although there was limited overlap between *Symbiodinium* richness in *A. tenuis* juveniles and the sediments, other species may benefit from additional *Symbiodinium* richness in sediments. One mechanism that may contribute to structuring *A. tenuis* and *A. millepora* communities is the production of species-specific peptides, which have been shown to regulate distinct bacterial communities in closely related species of *Hydra* (Franzenburg *et al.*, 2013). Finally, *A. millepora* juveniles in both the northern and central sediment treatments had 3.8-5.6 log2 fold greater abundances of *Symbiodinium* D1 and D1a than *A. tenuis*. If species-specific preferences for these types exist, and if thermally tolerant D types do provide an adaptive advantage at the juvenile life stage, then *A. tenuis* juveniles may be at a comparative disadvantage under future climate change scenarios. The substantial variability detected here across water quality and temperature gradients through time and by species highlights a degree of flexibility in the acquisition of *Symbiodinium* communities by acroporid juveniles. Such flexibility may be beneficial in environments that are prone to fluctuations, or for species that have long-distance larval dispersal. Further work is needed to determine the adaptive benefits that accrue with such flexibility in the acquisition of symbiont populations by juvenile corals.

5.5.3 High diversity in sediment *Symbiodinium* communities reflects variation in environmental parameters

Four times more unique *Symbiodinium* OTUs were recovered from the sediments than from juveniles, representing 72% (1125) of the OTU diversity detected overall, and indicating that the diversity of the free-living community is high. Whilst results of this study are consistent with previous reports of clades A and C occurring in

high abundances in Pacific sediment communities (Manning and Gates, 2008; Takabayashi, *et al.*, 2012), the majority of sediment reads detected here belonged to uncultured or *ex situ* types. This indicates that many undiscovered and presently uncharacterized types exist in the sediments and may represent a reservoir of diversity with potentially adaptive benefits for corals if algae are symbiotic. Potential sediment reservoirs of *Symbiodinium* differed among the four regions (north inshore, north offshore, central inshore, central offshore), with the most extreme differences being between the northern offshore and central inshore sediments. As discussed above, the inshore central sediments harboured the most distinct *Symbiodinium* communities found. Two other comparisons, northern inshore *versus* either northern offshore or central offshore sediments, were at most, 51% similar. These strong biogeographic patterns in *Symbiodinium* community composition have also been found within clades *in hospite* (Howells *et al.*, 2009) and related to seasonal variations in temperature, nutrients and irradiance. Furthermore, the abundance of different types was explained by different environmental covariates, in both free-living (this study), and *in hospite* (Cooper et al., 2011) *Symbiodinium* communities. For example, C1 dominates *P. verrucosa* colonies in colder, nutrient poor waters of the Arabian Gulf, whilst A1 dominates warmer, higher nutrient and more turbid waters (Sawall *et al.*, 2014). Offshore reef corals in Palau were also found to be dominated by C types, whilst warmer inshore corals harboured D1a or D1-4 (Russell *et al.*, 2016). Interestingly, these same patterns were found amongst the GBR sediment communities, with C types dominating in cooler, nutrient poor reefs (central offshore) and A types dominating in warmer, higher nutrient, turbid waters (northern inshore). Detection of C types in central offshore sediment treatments and A types in northern inshore sediment treatments in both free-living and *in hospite Symbiodinium* provides compelling evidence that environmental factors can aid in the structuring of both symbiotic and non-symbiotic communities. The diversification and partitioning of *Symbiodinium* communities in sediments as a consequence of exposure to different light and nutrient environments may reflect balancing selection that maintains high diversity of *Symbiodinium* in sediment reservoirs in light of competitive exclusion amongst symbiont types, as has been observed in diverse soil bacterial communities (Martorell *et al.*, 2015). Recent evidence that ecological factors explain significant variation in the genetic structure of clade C communities (Davies *et al.*, 2016) suggests substantial

potential for local adaptation of symbionts. These results highlight the paucity of taxonomic information currently known about this important niche and the potentially stress-tolerant species it may contain.

5.5.4 Changes in the diversity and relative abundance of dominant and background types of *Symbiodinium* impact coral juvenile physiology

Variation in juvenile survival among sediment treatments may have been driven either by differences in the composition of *Symbiodinium* communities acquired or by fine-scale changes in the relative abundances of specific types. Juveniles exposed to Davies (offshore) and Magnetic (inshore) sediments survived the longest, whilst Rib (offshore) and Pandora (inshore) juveniles survived for less time. Although community composition in coral juveniles differed substantially overall, by 90 days post exposure communities associated with juveniles in Davies (longest surviving) and Rib (shortest surviving) treatments were much more similar to each other than communities in juveniles exposed to Davies compared to either Magnetic or Pandora sediments. This lack of correspondence between *Symbiodinium* community diversity and juvenile survival suggests that other factors are causing differential mortality, such as differences in the relative abundances of certain types among sites. Changes in the relative abundances of dominant and background types can translate to large scale impacts at the colony level. For example, increases in the initial proportional abundance of clade D was sufficient to ameliorate bleaching severity in adult corals (Cunning *et al.*, 2015), and relative abundances of certain *Symbiodinium* types differed between coral juveniles in high surviving *versus* low surviving families (Chapter 4, Quigley *et al.*, 2016). In addition, the impact that the proportional abundance of a type might have on a coral juvenile may vary, depending on the nature and intensity of a stressor, with photochemical efficiency greater when higher proportions of D are present during thermal stress *versus* lower without such stress (Cunning *et al.*, 2015). Small shifts in bacterial community composition in *Nematostella* also resulted in changes to host fitness, potentially caused by symbionts eliciting differential immune responses corresponding to different selective pressures (Mortzfeld *et al.*, 2015). Therefore, changes solely in the relative abundances of types can impact host fitness and survival of juveniles. Alternatively, the early acquisition of certain types may have led to greater long-term survival of juveniles in the current study, with 6-fold greater abundances of C and D1 in juveniles exposed to Davies and Magnetic sediments at the first time point,

which corresponded with higher survival, compared to juveniles exposed to sediments from other sites, as has been documented for *Acropora yongei* juveniles (Suzuki *et al.*, 2013). Further work is needed to understand how the dynamics of mixed communities contribute to long-term juvenile survival.

Differences in the *in hospite Symbiodinium* communities and in the abundances of individual types among juveniles may also explain differences in time to infection and photosynthetic parameters (Coffroth *et al.*, 2001; Kinzie *et al.*, 2001). *Symbiodinium* infection/proliferation occurred more quickly when juveniles were exposed to offshore compared to inshore sediments, and followed clade-level differences in symbiont community diversity within sediments. Juveniles from Davies and Rib treatments were dominated by C15, A3 and C-types by day 19, whereas juveniles in Pandora and Magnetic treatments were dominated by *S. minutum*, D-types or C1/C-types. Cultured D-type *Symbiodinium* generally infect juveniles more slowly than C1 (personal observation), potentially explaining why juveniles that had greater proportional abundances of D symbionts exhibited slower *Symbiodinium* proliferation in Pandora and Magnetic treatments. Alternatively, juveniles in Rib and Davies treatments were dominated by A3, C15 and C at these early time points, which may reflect their capacity to infect and proliferate more quickly. Additionally, *S. minutum* is often an incidental isolate and not found in the host from which it was isolated (Shoguchi *et al.*, 2013; McIlroy *et al.*, 2016).

There was also high variability in Fv/Fm values among *Symbiodinium* communities in juveniles from different sediment treatments. Juveniles in Rib and Davies treatments generally had lower yields compared to juveniles in Magnetic and Pandora treatments under equivalent PAR. Variability may again be attributed to differences in *Symbiodinium* communities in juveniles among treatments, as has been found for dominant symbiont types under a range of conditions (Hoadley *et al.*, 2015), as well as across S*ymbiodinium* clades that vary in baseline photosynthetic traits in culture (A and C: ~0.4-0.5; B, D, and F: 0.6, Karim *et al.* 2015). However, other studies have not found that type abundance significantly predicts changes in Fv/Fm (C23 and C3.245: Pontasch *et al.* 2014; 18 types across clades A-D, and F: Suggett *et al.* 2015). However, type A3 was found to impact juvenile physiology, irrespective of its abundance. For example, although A3 was present at background abundances in juveniles exposed to Magnetic and Pandora sediments, its presence strongly impacted

Fv/Fm values in juveniles from both treatments. The same effect was seen when A3 dominated *Symbiodinium* communities, with large A3 populations correlating strongly with steep decreases in Fv/Fm values in the Davies and Rib treatments. The importance of numerically rare background symbionts has also been observed in *Nematostella* and sponge microbiomes (Erwin *et al.*, 2012; Mortzfeld *et al.*, 2015), and provides corroborative support that background types contribute to changes in the photo-physiological performance of juveniles.

Lower Fv/Fm values in juveniles exposed to Rib and Davies sediments may indicate that the symbionts in these sediments may normally be exposed to higher light fields (due to increased sediment particle size or decreased turbidity). For example, a greater proportion of Rib sediments were in larger particle size classes and juveniles exposed to them also had the lowest Fv/Fm values. The lower yields in juveniles exposed to offshore sediments may indicate that a lower percentage of absorbed light is being used by photosystems (or more energy being dissipated by non-photochemical quenching mechanisms). These results suggest that *Symbiodinium* types from offshore environments may be adapted to higher light environments and have lower light-capture efficiencies, lower concentrations of chlorophyll or higher RuBisCO concentrations (Iluz and Dubinsky, 2015).

Paradoxically, spikes in Fv/Fm were also observed preceding mortality events in both Rib and Magnetic treatment juveniles. Whilst this may be caused by measuring different individuals from one time point to another (due to loss through mortality and sampling), a spike in chlorophyll fluorescence similar to patterns seen in Rib and Magnetic juveniles has also been documented in single and small clusters of *Symbiodinium* cells proceeding bleaching and mortality of individual coral polyps (Shapiro *et al.*, 2016). Mortality may have also been linked with a breakdown in non-photochemical quenching processes, with communities in these three sites potentially having weaker non-photochemical quenching compared to Davies symbiont communities.

5.5.5 Conclusion

The low overlap between *Symbiodinium* communities in the sediments and *in hospite Symbiodinium* communities in juveniles exposed to those sediments suggests that symbiont uptake overall is a selective process, although local availability of *Symbiodinium* in the environment also influences *in hospite* structure to a smaller

degree. This information, paired with evidence of strong biogeographical partitioning of free-living *Symbiodinium* communities in the sediments has implications for local adaptation of symbiotic partnerships. Future studies should consider how climate change and other anthropogenic stressors may disrupt the linkages between free-living *Symbiodinium* sinks and uptake in coral juveniles, especially given that a vast majority of coral species take up *Symbiodinium* anew each generation. However, the newly discovered *Symbiodinium* diversity in the sediments presented here (i.e. uncultured types and new variants of described types) provides hope that corals will be able to utilize that diversity, either at initially low background abundances or potentially to form new partnerships that will allow them to withstand further degradation.

Chapter 6: Leveraging knowledge of genetic and environmental contributions to *Symbiodinium* community regulation in juvenile and adult corals for conservation and restoration approaches

Research presented in this thesis significantly advances current knowledge of the comparative contributions of environmental and genetic factors to the structure of *Symbiodinium* communities in the early life-history stages of corals, specifically by quantifying the extent to which *Symbiodinium* communities are inherited across a range of reproductive and symbiont transmission modes. Results also highlight that the fitness and survival of coral juveniles are at least partly governed by the specific *Symbiodinium* communities they host, with *Symbiodinium* A3, C1, D1 and D1a potentially representing keystone types (*sensu* Paine, 1995), in which these types have wide-ranging effects on their ecosystem (i.e. the coral host), even at low abundances. In this final chapter, I synthesize results from Chapters 2 to 5 to develop a new perspective on coral-symbiont transmission and explain the relevance of these findings for conservation practices, coral reef restoration, and assisted evolution initiatives. In particular, evidence of heritable genetic variation in *Symbiodinium* communities presented here establishes that adaptation through natural selection on the *Symbiodinium* community is possible, highlighting a previously undocumented mechanism that may enable corals to respond to increasing environmental pressures, particularly those due to climate change.

6.1 Coral host genetic underpinnings of the *Symbiodinium* community

The importance of the *Symbiodinium* community for coral holobiont fitness has long been recognized (Baker, 2003), however the genetic underpinnings and environmental factors that regulate its diversity and abundance are still poorly understood (Davy *et al.*, 2012). It has been suggested that the early life-history stages of corals with horizontal transmission are open to colonization by most *Symbiodinium* types associated with environmental sources (Cumbo *et al.*, 2013), whereas those with vertical transmission are controlled by maternal transmission (Baker, 2003), although these assumptions have never been formally tested. Other components of the current model for regulation of the *Symbiodinium* community include initial symbiont-host recognition, proliferation and control of *Symbiodinium,* and winnowing to an eventual stable symbiosis (reviewed in Davy *et al.* 2012). However, the specific mechanisms and the relative contributions of each partner (*Symbiodinium* and coral host) to mediate each of these events are still unclear, thus questions concerning the role, importance and mechanisms of host genetic regulation of symbiont communities remain unanswered.

Data presented in Chapters 2 and 3 demonstrate that variability in *Symbiodinium* communities among juvenile corals can be attributed, at least partly, to host genotype, whereby the strength and fidelity of associations vary with both host reproductive mode and symbiont transmission mode. Significantly, heritability estimates varied in magnitude from those expected based on host reproductive mode; broadcast-spawning, horizontally-transmitting species exhibited much greater heritability, whereas brooding, vertically-transmitting species exhibited lower heritability than expected. The demonstration of heritable additive genetic variation of *Symbiodinium* communities in corals with both reproductive and both transmission modes is novel and confirms that ample genetic variation in *Symbiodinium* communities exists upon which natural selection may work.

6.2 Maternal and paternal colony identity shape juvenile abundances on reefs

In Chapters 4 and 5, I present new evidence of the ecological impacts that parental identity and the free-living *Symbiodinium* community structure have on the survival and fitness of multiple early life-history stages of corals. In Chapter 4, the use of quantitative genetic breeding designs to produce larval families of known pedigree demonstrate that maternal and paternal identity play key roles in determining larval weight, larval survival, settlement success and juvenile survivorship in the field, and show for the first time that breeding colony identity can directly impact resulting reef structure through its impact on pre- and post-settlement larval ecology. These findings have direct implications for the maintenance and recovery of reefs, where larval availability, settlement success and juvenile survival are all key processes that dictate recovery potential (Ritson-Williams *et al.*, 2009). Furthermore, by tracking juvenile families that varied in survivorship, I also show links between post-settlement success and the *Symbiodinium* community established. Specifically, Chapter 3 quantifies the extent to which inherited mechanisms regulate the *Symbiodinium* community in *A. tenuis* juveniles, and Chapter 4 demonstrates that the maternal parent has a significant impact on juvenile survival. Taken together, these results suggest that maternal colonies transmit a genetic architecture that predisposes juveniles to establish a particular symbiont community, with some communities leading to increased and others to decreased juvenile survival (Chapter 4). Results also suggest that a highly conserved *Symbiodinium* community characteristic of high surviving juvenile families represents an optimal symbiont community in *A. tenuis* juveniles and may potentially represent a

form of local adaptation (Figure 6.1). Dysbiosis (i.e. imbalances or deregulation of the *Symbiodinium* community) of such optimal communities may take a variety of forms (Vangay *et al.*, 2015; Egan and Gardiner, 2016), including: 1) loss/reduced abundances of keystone *Symbiodinium* types (i.e. A3, C1, D1, D1a), 2) increased diversity associated with unstructured communities that are highly variable within and among coral families, 3) changes in metabolic function, and 4) increased abundances of pathobionts (potentially *Symbiodinium* from clades E and F) (Starzak *et al.*, 2014). Pathobionts represent symbionts that may become pathogens when environmental conditions select against symbiotic behaviour and, as *Symbiodinium* are sister phyla to the Apicomplexan parasitic protists, it is reasonable to hypothesise that some of the novel, uncultured *Symbiodinium* from the sediments (Chapter 5) and potentially those from clades E and F detected in juveniles from low-surviving families (Chapter 4) may represent potential pathobionts. Regardless of whether the greater diversity of *Symbiodinium* communities associated with juveniles of low-surviving families led to dysbiosis, or whether some other factor (e.g. pathogenic *Vibrio* spp. infection) lead to dysbiosis or directly increased juvenile mortality in these families, Chapter 4 demonstrates clear links between *Symbiodinium* community composition and juvenile survival.

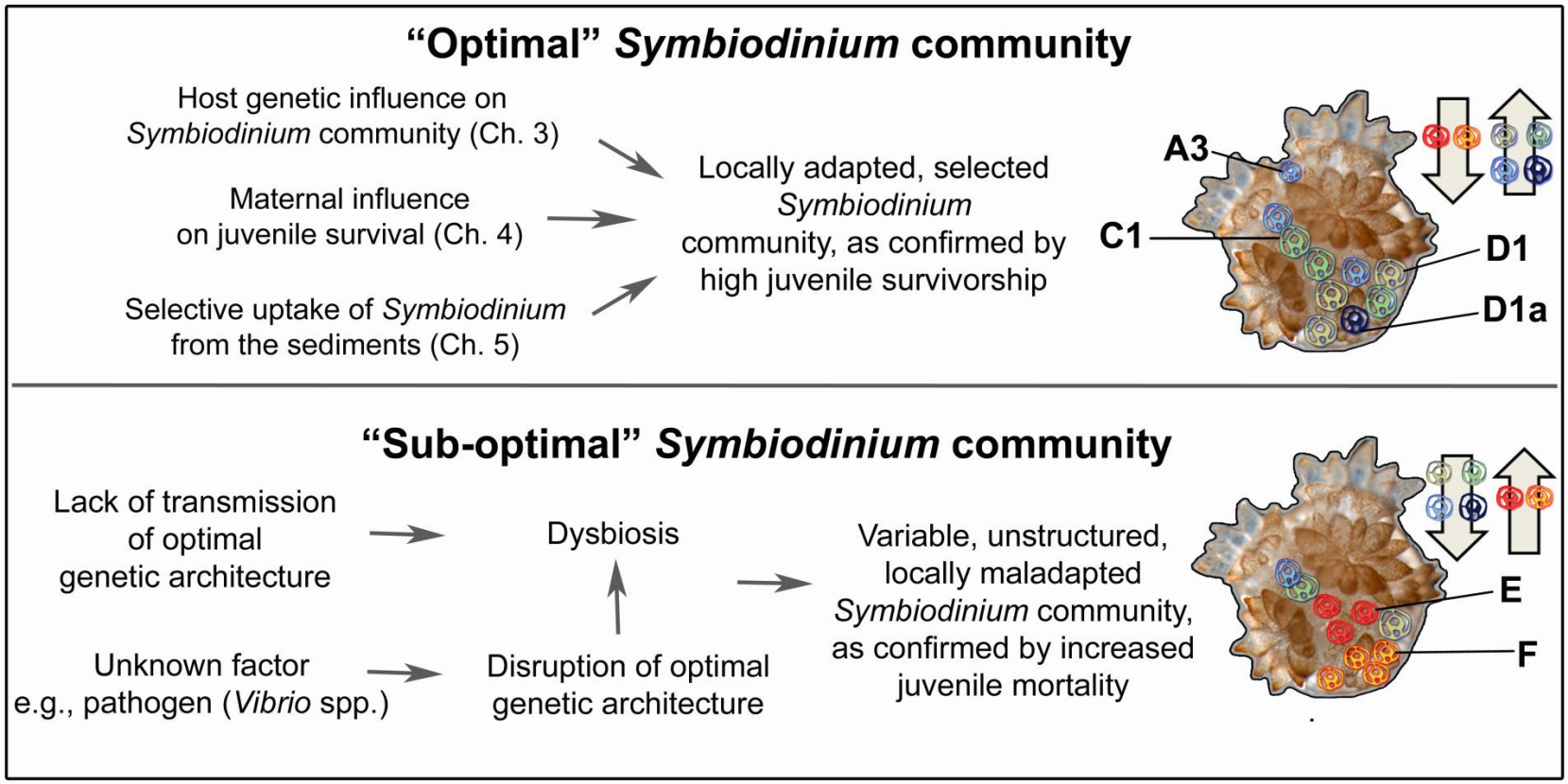

Figure 6.1. Synthesis of factors found in this thesis to contribute to the regulation of *Symbiodinium* communities in juveniles of the broadcast-spawning, horizontally-transmitting coral *Acropora tenuis,* and the consequence of loss of regulation of *Symbiodinium* communities for coral juveniles. Thick grey arrows on the right of juveniles represent increases or decreases in abundance of *Symbiodinium* types, with optimal symbiont communities having decreased abundances of potential pathobionts from clades E and F (red and orange *Symbiodinium* cells) compared to increased abundances of keystone types (blue, green and tan shaded cells representing A3, C1, D1 and D1a *Symbiodinium* types) and vice-versa for sub-optimal symbiont communities. Juvenile photo courtesy E. Howells.

6.3 A new paradigm for *Symbiodinium* transmission in vertically-transmitting corals

Chapters 2 and 3 present compelling evidence for a new mixed mode of *Symbiodinium* community transmission in corals that release either zooanthellate eggs or larvae. Previously, after surveying approximately 400 coral species, only two mechanisms, horizontal and vertical transmission, were described (Baird *et al.*, 2009b). However, in both of the "vertically-transmitting" corals studied in this thesis (*Montipora digitata* and *Seriatopora hystrix*), there was a lack of fidelity in the transmission of *Symbiodinium* communities and hence not all types were transferred from maternal colonies to offspring (either eggs or planulae). Moreover, a number of unique OTUs were found in eggs and planulae that were not detected in maternal colonies, suggesting secondary uptake from environmental sources in these early life-history stages. Although secondary uptake was suspected in earlier studies of vertically-transmitting corals (Padilla-Gamiño *et al.*, 2012; Byler *et al.*, 2013), it was not possible to detect the potential presence of low abundance types before the use of deep sequencing technology (Metzker, 2010). The confirmation of a mixed mode transmission strategy that includes both vertical and horizontal transmission in these species has important implications for acclimatization and adaptation of the host, as it suggests that uptake of novel, thermally-tolerant or stress-resistant *Symbiodinium* types is possible.

6.4 The importance of heritable variation for coral reef conservation and restoration intervention strategies

The detection of heritable regulation of the *Symbiodinium* community from Chapters 2 and 3 has important implications for intervention strategies targeting coral reef conservation and restoration through "mutualist-mediated rescue" (Pillai *et al.*, 2016), whereby rapidly evolving *Symbiodinium* communities may be able to promote the persistence of their more slowly evolving hosts if they are unable to keep pace with rates of environmental change. Increases in host niche breadth through mutualist-mediated rescue can include changes to symbiotic community diversity and abundance (mutualist-mediated ecological rescue), or alternatively, adaptive mutations in either the host or symbiont genomes that result in beneficial symbiont community traits (mutualist-mediated evolutionary rescue) (Pillai *et al.*, 2016). Accordingly, the potential

for evolution of symbiotic communities that impart greater tolerance to the holobiont could lead to changes in gene frequencies and fitness-related phenotypes of the symbionts and coral host (Carlson et al., 2014; Pillai et al., 2016; Theis et al., 2016). Such evolutionary rescue contrasts with ecological rescue, which is more akin to acclimatization (i.e. shuffling/switching) (Pillai *et al.*, 2016) and does not involve selection acting upon heritable variation (Carlson *et al.*, 2014). Although evidence has been accumulating for some time that corals can acclimatize to increased temperatures through *Symbiodinium* communities by shuffling (Berkelmans and van Oppen, 2006; Jones *et al.*, 2008; Cunning and Baker, 2014; Cunning *et al.*, 2015; Bay *et al.*, 2016) or switching (Lewis and Coffroth, 2004; Coffroth *et al.*, 2010; Boulotte *et al.*, 2016), the absence of quantitative evidence for host genetic regulation of this community has meant that the potential for selection and adaptation of *Symbiodinium* communities has been unknown. Therefore, the process of adaptive selection on heritable variation of the *in hospite Symbiodinium* community is a significant, but hitherto undocumented, mechanism by which corals may be able to respond to environmental stressors. The quantitative genetics approaches employed in Chapters 2 and 3 verify the genetic basis for host regulation of this community, raising the possibility that these communities will evolve in response to a changing climate and enable the longer-term persistence of their hosts. This gives corals one additional mechanism in their toolbox to contend with rapid environmental change.

In the event that the pace of climate change is too rapid for evolutionary rescue through naturally evolving *Symbiodinium* communities (Gonzalez *et al.*, 2012), artificial directional selection or selective breeding (Visscher *et al.*, 2008) may be an additional approach to contend with stressors caused by climate change. Artificial directional selection on symbiotic communities in coral hosts has been proposed as a promising approach to help restore degraded reefs (van Oppen *et al.*, 2015; Theis *et al.*, 2016), and has been shown to improve the health and fitness of a range of organisms (reviewed in Mueller and Sachs 2015). Microbial engineering of the *Symbiodinium* community may provide similar benefits. Efforts are currently underway on this front in coral reef conservation, with assisted evolutionary techniques being applied to both the coral host and isolated *Symbiodinium* cultures (Levin *et al.* 2016, Chakravarti et al. *in-review*). The evidence for additive genetic variance presented in this thesis suggests that, at least for efforts directed at selecting symbiont populations in the host, assisted evolution merits further research.

Estimates of heritability presented in Chapters 2 and 3 provide key numerical inputs to models that project the feasibility of natural and artificial selection, where selection strength is proportional to the product of the additive genetic variance and narrow-sense heritability (Houle, 1992; Visscher *et al.*, 2008). My results show that ample genetic variation exists to underpin artificial selection of the *Symbiodinium* community, and that in species such as *Montipora digitata*, adaptation could occur rapidly due to the large (0.62) heritability estimate in the absence of genetic constraints on evolution. Furthermore, heritability values determined in these chapters also provide key data for the construction of evolutionary rescue models (Gonzalez *et al.*, 2012). Evolutionary rescue models can be used to identify species at risk due to slow adaptive responses or to model longevity of species under different risk profiles. For example, future population densities and distributions of corals can be modelled across a range of different threat scenarios (e.g., ocean warming, acidification, nutrification) by combining estimates of larval dispersal (movement), phenotypic plasticity (acclimation), and genetic variation (adaptation) (Figure 1.3), where estimates of adaptation can now include experimentally derived values in place of hypothetical estimates.

Success of selective breeding for beneficial symbiont communities may be dependent on the availability of appropriate rescue genotypes (i.e., individuals in the population that are resilient to stress) (Gonzalez *et al.*, 2012). In this thesis, I identify two sources of rescue genotypes for use in targeted selection and adaptation practices: 1) maternal genotypes, and 2) *in hospite Symbiodinium* communities. Firstly, evidence of maternal effects, where specific dams (O4,W11) produced juvenile cohorts of high survival ("super dams", Chapter 4), suggests that targeting such dams as potential candidates in selective breeding efforts (i.e. as rescue genotypes) would produce high quality brood stock for restoring degraded reefs. Specifically, these "super dams" produced juvenile families with high survivorship, regardless of larval abundance, which suggests their brood stock would result in an improvement of early life survivorship of juvenile corals and thereby maximize conservation efforts. One possible mechanism that may explain the high juvenile survival attributed to these "super dams" is the presence of maternal genotypes that provide offspring with the genetic architecture to establish consistently-structured, *Symbiodinium* communities that include keystone types (Figure 6.1, Chapter 4). The lack of significant evidence for

maternal environmental effects on eggs, larvae and juveniles across all coral species was surprising (Chapters 2 and 3). These results thereby discount traditional maternal (environmental) effects (i.e., lipid provisioning or other maternal "factors"), and instead point toward genetic effects as the causal factor driving variability in *Symbiodinium* communities in these early life-history stages.

Secondly, in addition to identifying potential maternal rescue genotypes, selective breeding efforts should consider focusing on selecting corals with particular *Symbiodinium* communities (the host's *Symbiodinium* community, e.g., those that include the keystone types mentioned earlier) (Chapters 2, 3, 4), a strategy that has already been proposed for both *Symbiodinium* and bacterial communities in corals (van Oppen *et al.*, 2015). Selection on the genotypes of corals that exhibit a beneficial or optimal *Symbiodinium* community may result in juveniles and adults that are more resilient to stress and may open up other possibilities for symbiont community manipulations to further improve coral health and survival. Results from Chapter 4 identify one such optimal phenotype (Figure 6.1), but the high diversity of *Symbiodinium* types in sediments (Chapter 5) provides ample diversity for a range of different optimal host-symbiont phenotypes. This therefore increases the probability of finding appropriate rescue phenotypes and therefore genotypes in corals.

It is significant that phenotypic variation in both maternal colonies and symbiont communities exists in the standing stock of Great Barrier Reef corals and that new mutations are not necessarily needed to bring about directional selection, decreasing the time-frame needed for potentially large-scale positive impacts on impacted reefs. For at least some coral species on the Great Barrier Reef, effective population sizes are very large due to substantial gene flow amongst reefs (Underwood *et al.*, 2007; van Oppen *et al.*, 2011), increasing the chance for beneficial genetic variants to spread throughout populations (Gonzalez *et al.*, 2012). Furthermore, potential rescue genotypes will vary with the type of stressor, and further work should be focused on identifying host genotypes that correlate with stress-tolerant host phenotypes. Therefore, even without the creation of new host genotypes through natural or artificial mutation, results from this thesis confirm that at least two sources of genotypic variation are currently available, further highlighting the potential for evolutionary rescue of corals.

6.5 A model for *Symbiodinium* regulation and its adaptive potential in corals

Results presented in this thesis address a number of key knowledge gaps concerning the establishment and maintenance of symbiosis between corals and *Symbiodinium*, as synthesized in the following model for *Symbiodinium* community regulation in corals (Figure 6.2). The figure also summarises the substantial flexibility in symbiont transmission modes found and the adaptive potential of the *Symbiodinium* community revealed across multiple coral reproductive modes. Key points summarised in the model are designated a letter (A-F) and discussed further in the following text.

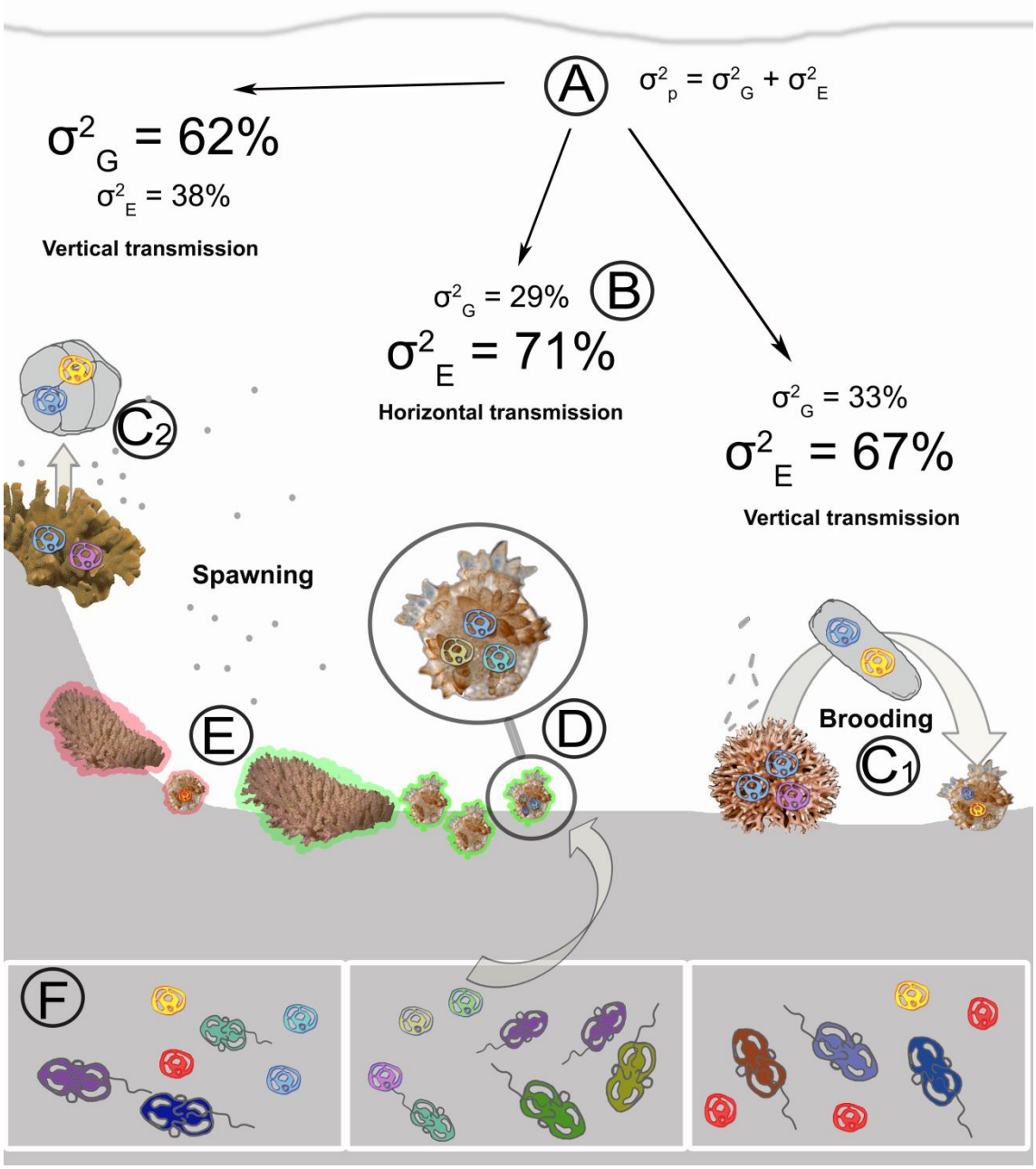

Figure 6.2 Model describing the synthesis of new information contributed by this thesis regarding the establishment of symbiosis between *Symbiodinium* and early life-history stages of corals. Spherical cells of different colours represent different *Symbiodinium* types, whilst flagellated cells represent free-living *Symbiodinium* types. Adult and juvenile colonies of *A. tenuis* outlined in green and red represent dams that produce high surviving cohorts of juveniles ("super dams," green), whilst those that produce low surviving cohorts are in red. Coral photos provided by the Australian Institute of Marine Sciences (adults) and E. Howells (juveniles). Stylized *Symbiodinium* cells adapted from T. LaJeunesse.

A. *Symbiodinium* communities in corals with both major modes of reproduction (spawning and brooding) and symbiont transmission (horizontal and vertical transmission) are regulated to some extent by heritable genetic mechanisms. This suggests that there is substantial raw genetic material for adaptation and hence for evolvability of the symbiont community in coral species representing both major reproductive and symbiont transmission modes.

B. Genetic underpinnings of the *Symbiodinium* community in spawning, horizontally-transmitting hosts like *A. tenuis* are much greater than expected, and on-par with brooding, vertically-transmitting hosts. However, heritability estimates suggest that symbiont communities in *A. tenuis* and *S. hystrix* are predominantly driven by environmental variation in *Symbiodinium* availability.

C. High environmental influence (σ^2_E), low transfer fidelity (i.e., not all maternal types represented) and mixed-mode transmission (novel types in juveniles that are not found in maternal colonies) provide evidence of flexibility in coral-*Symbiodinium* symbiosis in the brooding coral *S. hystrix* (**C1**). Such flexibility may positively impact the resilience of brooding species that are generally sensitive to stress.

Environmental influence on the *Symbiodinium* community in *M. digitata* eggs was lower than in *S. hystrix* larvae, with symbiont communities in eggs also exhibiting lower fidelity than expected and mixed-mode symbiont transmission (**C2**).

D. *A. tenuis* juveniles that hosted a specific and conserved *Symbiodinium* community made up of keystone *Symbiodinium* types (i.e. A3, C1, D1, D1a) survived better than juveniles with variable, unstructured communities (i.e. communities with high diversity including members from clades E and F and low community similarity amongst juveniles). Overall, *A. tenuis* juveniles in general exhibited five times greater type-level diversity than has been reported previously.

E. Juveniles with keystone symbiont communities and corresponding high survival originated from specific families and maternal colonies (i.e. F1, F4, F12). The significant effect of maternal identity supports the inheritance of genetic architecture that selects for symbiotic partners, with subsequent impacts on juvenile survival. For example, some maternal colonies may transmit suitable genetic architecture that selects for the optimal *Symbiodinium* community for juvenile survival (corals outlined in green), whereas other maternal colonies may transmit unsuitable architecture that results in sub-optimal, unstructured symbiotic communities and low survival of juveniles

(corals outlined in red). Variability amongst dams resulted in density-dependent and density-independent patterns in juvenile mortality caused by specific maternal identities, potentially due to differences in these transmitted recognition mechanisms. **F.** Evidence of selectivity in the acquisition of *Symbiodinium,* based on comparisons between the free-living *Symbiodinium* community in sediments and communities associated with coral juveniles, provides further corroboration of host genetic regulation of the symbiont community. Free-living *Symbiodinium* also displayed a strong biogeographical structure, with communities varying significantly across water-quality and temperature gradients. High diversity of *Symbiodinium* types in sediments may increase the probability of establishing stress-resilient *Symbiodinium* and host partnerships, thereby facilitating evolutionary and genetic rescue.

6.6 Further directions

Confirmation of additive genetic variation regulating the *Symbiodinium* community in both brooding and broadcast spawning corals is an important first step towards understanding the potential of the coral holobiont to respond to future climate conditions. Future work should: 1) identify the heritable mechanisms responsible for regulating the *Symbiodinium* community in corals, 2) determine the direction and speed of selection on the *Symbiodinium* community, 3) compare contributions of each parent to this heritable genetic variation, as well as other non-inherited epigenetic mechanisms, and 4) determine spatial and temporal variation in environmental availability of *Symbiodinium*. Identifying the heritable mechanisms will involve mapping candidate gene loci and identifying signal-recognition receptors that have already been implicated as important regulators of the *Symbiodinium*-host establishment process (Davy *et al.*, 2012). Undertaking knock-out experiments of identified receptors in conjunction with infection experiments will also be important for confirming how the structure of different receptors shapes symbiont community composition. Identifying which mechanisms are being inherited by offspring to the gene(s) level will provide important information for selective breeding experiments targeting the selection of optimal genetic complements or architecture over others.

Secondly, targeted selection experiments should also be undertaken to explore the feasibility of changing *Symbiodinium* communities to those more favourable to local habitats (e.g., the conserved *Symbiodinium* community in low-mortality juveniles found in Chapter 3). Traditional breeding programs have long utilized heritability estimates to

inform the effectiveness and speed of selection on traits, and these concepts should be applied to coral husbandry as well. Heritability of other important fitness traits in corals should also be calculated (for example, antioxidant production), which would allow for a direct comparison of traits important for coral resilience, thereby allowing for comparisons across traits, with those having larger breeding values being the best targets for directional selection programs.

Finally, I found that heritability estimates vary in magnitude between reproductive modes, however it remains unclear how much of the variability in heritability estimates are due to uni- *versus* bi-parental inheritance or to the potential influence of non-inherited mechanisms. Results from this thesis suggest there may be a relationship between the length of time elapsed between fertilization and the exposure of embryos to *Symbiodinium*, with brooding species such as *S. hystrix* exhibiting low paternal effects (and therefore weak bi-parental inheritance) because eggs may have already been colonised by *Symbiodinium* before contact with sperm. Alternatively, the spawning coral *A. tenuis* may exhibit strong paternal effects (and therefore strong bi-parental inheritance) because sperm contact with eggs occurs well before larval/juvenile exposure to *Symbiodinium*. Strong paternal effects may come about from sperm chromosomal imprinting, or paternal mRNAs and non-coding RNAs transferred with sperm to eggs (Stoeckius *et al.*, 2014). Again, understanding the mechanisms underlying *Symbiodinium* community variation may lead to novel solutions in manipulating the community.

6.7 Concluding remarks

This thesis contributes to the general understanding of the biology, ecology and genetics of *Symbiodinium* - coral symbioses across multiple ontogenetic, reproductive and symbiont transmission modes. In a broader research context, these results also contribute to understanding how symbiosis is established and maintained in basal metazoans, with implications for understanding symbiotic regulation in other, more derived lineages (e.g., insects and humans). These results also represent the first application of deep sequencing technology to the early life-history stages of corals. This application enabled the discovery of a new, complex community of *Symbiodinium,* and has opened up new research avenues concerning the influence of keystone symbionts and communities through coral ontogeny. The results presented here can be used

directly in evolutionary rescue models that will help scientists, conservationists and managers predict and ameliorate pressures from local and global environmental changes that are facing coral reefs worldwide.

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Appendices

Appendix A

2.1 Supplementary material and methods for Chapter 2

2.1.1 Study species and sampling design

Eighteen mature colonies were collected from the site one day before the predicted larval release period in November 2009. Brooding colonies were maintained in separate aquaria supplied with 1 μm filtered seawater at Lizard Island Research Station. Larvae were collected from the 18 colonies early in the morning, within 5 hours of release, every day for two weeks and preserved in 100% ethanol. Multilocus genotypes were generated from microsatellite markers for all adult colonies (including brooding dams) and larvae and used to assign paternity. Paternity assignments were then used to construct consensus confidence categories of paternity (Very High, High, Medium, Low) for each of the larvae genotyped in the current study. A full description of the site, extraction methods and parentage analysis can be found in (Warner *et al.*, 2016).

2.1.2 *Symbiodinium* community genotyping

Chimeric reads were filtered and those with an Expected Error greater than 1.0 were discarded (Edgar and Flyvbjerg, 2015). Remaining reads clustered with the default 97% identity and minimum cluster size of 2, thus eliminating all singleton reads. Sequences were globally aligned with 99% similarity, with gaps counted as differences. This resulted in 927 OTUs, consisting of 4,689,233 reads (67.5% of original data) across the 105 samples (mean per sample = 44,659±1940). OTUs were annotated through locally run BLAST+ searches against the complete non-redundant NCBI nt database. *Symbiodinium* specific reads made up a majority of the dataset with 4,638,791 (98.9% of the total cleaned reads) across 161 OTUs. OTUs that did not blast to *Symbiodinium* or host name accessions were filtered and removed, totalling 50,442 reads (1.07% of the total cleaned reads) and 766 OTUs. Of the 161 OTUs (Table S2.2), 0.03% were only identified in NCBI as the host species that symbionts were extracted from and could therefore not be identified to the type level and will thereafter be referred to as "unknowns." Blast hits were used to classify each OTU, first by clade and then, where possible, by type. To further classify to the type level, OTUs were re-blasted using Blastn with *Symbiodinium*-specific taxonomy search criteria (taxon id:

2949). Furthermore, clade-level OTUs were also aligned after manual editing with BioEdit (Hall, 1999) to known reference sequences available from the Santos Lab (http://www.auburn.edu/~santosr/sequencedatasets.htm) and from previous *Symbiodinium* phylogenetic analyses (LaJeunesse, *et al.*, 2010). Additional reference sequences were added to these resources to represent the known *Symbiodinium* diversity for the *S. hystrix* species complex (C120: HM185737, C120a: HM185738, C1aa: HM185739, C3ff: HM185740, C1mHM185741, C3nt: EF541149) (Tonk *et al.*, 2013).

Symbiodinium OTUs from clades A-G were aligned separately (Baker and Correa 2009) using Clustal Omega (Sievers *et al.*, 2011). Trees were constructed in ClustalW2 Phylogeny with default settings (Larkin *et al.*, 2007; Goujon *et al.*, 2010; McWilliam *et al.*, 2013)(Figure S2.1). All mean values are accompanied by their standard errors unless otherwise stated.

2.2 Supplementary results for Chapter 2

2.2.1 *Symbiodinium* community changes across maternal larval broods

The occurrence and abundance of OTUs in larvae that shared colony 2 as a dam did not differ significantly from those of larvae in broods from dams 3, 4 or 18. In contrast, broods from these 4 dams (2, 3, 4, 18) were associated with seven different OTUs compared to broods from dams 6, 7, 13, and 14. Larvae from dam 2 had between 1.5-2.4 log2 fold greater abundance of A3 and *S. microadriaticum* compared to broods of dams 6, 7, 13 and 14 (Table S2.4, B-H adjusted p-values: 0.04 - 4 e^{-05}). They also had almost 2 log2 fold greater abundances of D1 and D1a (OTUs 3 and 6) than dam 13 broods, 5.4 log2 fold less C-type OTU55 and 7 log2 fold more C1-OTU2 (B-H adjusted p-values: 9.6 e^{-03}- 4.8 e^{-03}). The dam 2 brood also had 2.7 log2 fold less C1-OTU32 (B-H adjusted p-values: 0.03). The composition of *Symbiodinium* communities associated with larvae from dam 3's brood was comparable to that of many other families, but did differ significantly from *Symbiodinium* communities associated with dam 4's larvae, by having 3.5-5.9 log2 fold less of two C-types (OTU2 and 44), and from dam13's larvae, by having 4.4 log2 fold less C1-OTU2 (B-H adjusted p-values: 0.0001 - 0.02). C1v1e-OTU44 distinguished dam 4's larvae from those of other dams, occurring in 5.2-7 log2 fold greater abundances in dam 4 larvae compared to broods from dams 6, 7,10, 14, and 18 (B-H adjusted p-values: 2.46 e^{-07} - 6.08 e^{-03}). There was also 1.1-1.4 log2 fold lower abundance of C31- OTU733 in larvae from dam 4 compared to larvae from dams 6 and

14, and 2-2.6 lower abundance of clade C1- OTU7 than in larvae from dams 6 and 13 (B-H adjusted p-values*:* 6.59 e^{-03} - 3.04 e^{-02}). Larvae of dam 4 did have a log2 fold more C1-OTU134 than larvae of dam 6 (B-H adjusted p-values*:* 1.13 e^{-02}). Finally, dam 13 was the most different in abundance compared to dam 4 due to varying abundances of clade C OTUs 2, 4, 21, and log2 fold increases in D1a-OTU6, D1-OTU3 and A3-OTU8 (Table S4.4). In conjunction with the differences discussed above, the dam 6 brood also had differential abundances compared to dam broods 7, 13, and 18 across seven clade C *Symbiodinium*, with each brood having log2 fold differences corresponding to unique C-types. The only exception was C1-OTU4, in which brood 6 had significantly less of this clade C type compared to either 13 or 18 (B-H adjusted p-values*:* 3.2 e^{-03} - 0.05). There were no differences compared to dam broods 10 or 14. Dam 7 broods had over 7 log2 fold greater abundances of clade C1-OTU2 (B-H adjusted p-values*:* 3.2 e^{-42}). Larvae produced from dam 10 were distinct from brood 13 with 1.4-5.9 log2 fold greater abundances two C-types (OTU1, OTU2) as well as previously mentioned differences with other broods (B-H adjusted p-values*:* 5.3 e^{-08} - 4.9 e^{-02}). Dam 13 larvae had large log2 fold differences in abundance of C types OTU2 and 4 compared to dam 14 and brood 18 due to the abundance of one A, three C, and two D types. Finally, dam 14 larvae significantly differed in abundance compared to broods 2, 4, 13, and 18 in their 5 log2 fold lower abundance of clade C1-OTU4 (B-H adjusted p-values*:* 0.008).

2.2.2 Multiple ITS-2 copies and intragenomic variation

2.2.2a Analysis of all *Symbiodinium* OTUs

The level of co-occurrence of OTUs, correlations of proportional abundance and percent pairwise similarity did not reveal evidence of ITS-2 intragenomic multicopy signatures in any of the *Symbiodinium* clades retrieved from ShA. Of the seven clade A sequences, only *S. microadriaticum*-OTU10 and A3-OTU8 co-occurred across samples in the same general pattern and had strongly correlated relative proportions (R^2=0.853). However, both blasted to distinct A types (e-values: 2.00E-155 and 1.00E-157) with a percent pairwise similarity of 49.8%. Twenty-six of the 62 C-type OTUs co-occurred and 39 of 676 comparisons had proportional correlations greater than R^2= 0.5. However, only three comparisons had R^2 values > 0.80 (OTU22xOTU12: 0.82, OTU12xOTU24: 0.83, OTU223xOTU28: 0.85) and none of these pairs had sequence similarity greater than 51.3% nucleotide identity. Three of the 7 clade D OTUs co-occurred and the abundance of D1-OTU3 was moderately correlated (R^2=0.761) with the abundance of

D1a-OTU6. But, D1 and D1a were only 58.8% similar. Only one comparison (OTU588 and OTU848) between the four clade E OTUs resulted in a large proportional correlation (R^2=0.71). However, OTU848 was only found in one of the four samples that contained OTU588 and they shared 37.1% nucleotide identity. The two clade G OTUs were not found in any of the same samples (R^2=-0.24).

2.2.2b Inspection of significant p-adjusted OTUs for intragenomic variant signature

Significantly different OTU abundances based on Bejamini-Hochberg p-adjusted values were found among the following categories: I) larvae and adults (35 OTUs), II) maternal broods (15 OTUs) and III) colony size classes (4 OTUs). To specifically confirm that none of these OTUs were multiple copies of the ITS-2 gene, I looked for co-occurrence and proportional differences between them, as described above. *S. microadriaticum*-OTU10 and A3-OTU8 were found in both categories I and II, however, for reasons explained above, I am confident that they are distinct biological entities. D1-OTU3 and D1a-OTU6 were also found in categories I and II, and D1-OTU597 found only in category I. D1-OTU3 and D1a-OTU6 co-occurred in all categories, as well as across every sample (but not always proportionally), thus making them reasonable candidates as intragenomic variants. However, recent work combining multiple markers and microsatellites has distinguished D1 as *S. glynii* and D1a as *S. trenchii,* despite vestiges of D1 ITS-2 being found within the genome of D1a (LaJeunesse, *et al.*, 2010; LaJeunesse *et al.*, 2014). Finally, D1-OTU597 was also found in 56 of 106 samples in much lower abundances than the other two D OTUs. If D1-OTU597 is an intragenomic variant of either *S. glynii* or *S. trenchii,* then it should increase proportionally with an increase in either of these OTUs; this was not the case, as shown by the R^2 correlation coefficients (D1-OTU3: 0.5, D1a-OTU6: 0.3). From clade C, there were 24 and 9 OTUs in categories I and II, respectively, totalling 25 unique OTUs (8 OTUs were found in both categories). The C OTUs 12, 22 and 24 showed signs of strong proportional increase/decrease (R^2= 0.82-0.85), but only OTUs 223 and 28 had a high correlation coefficients (R^2=0.85). The abundances of all OTUs described above differed between larvae and adults.

2.2.2c Heritability

The mean and mode of posterior heritability distribution increased by incorporating intragenomic variants (mean: 0.45 ± 0.21 SD; mode 0.38 BCI: 0.1-0.9). Even with a moderate sample size (n = 60), the majority of heritability estimates in the posterior distribution of the heritability estimate had a greater mode than the one estimated, suggesting that the most probable values of h^2 are greater than the estimate derived here (Figure S2.2).

Supporting tables for Chapter 2

Table S2.1 Pedigree information for each of the 60 ShA larvae. Each dam and sire number represents a unique colony. ShA are hermaphroditic and the nine dams were therefore also represented as sires. Grey and white boxes delineates different maternal broods.

Larval Id	Dams	Sire
2.1.6	2	115
2.1.9	2	45
2.2.11	2	79
2.2.12	2	31
2.2.2	2	111
2.2.3	2	3
2.2.6	2	16
3.1.26	3	97
4.1.6	4	81
4.2.11	4	5
4.2.8	4	9
4.2.9	4	16
4.4.14	4	26
4.4.1	4	5
4.4.2.17	4	5
4.4.3	4	103
6.1.1	6	33
6.2.12	6	64
6.2.18	6	6
6.2.20	6	10
6.2.5	6	154
6.4.12	6	18
6.4.17	6	73
6.4.1	6	60
6.5.2	6	79
6.5.4	6	160
6.6.11	6	4
6.6.19	6	64
6.6.1	6	66
6.6.20	6	97
7.1.1	7	2
7.3.18	7	2
7.3.19	7	32
7.3.24	7	79
7.3.25	7	33
7.3.3	7	3
7.3.5	7	116
7.3.7	7	2
7.3.8	7	7
10.2.12	10	113

13.2.12	13	125
13.2.3	13	60
13.2.9	13	113
13.3.10	13	167
13.3.3	13	170
13.3.4	13	109
13.3.9	13	39
14.1.10	14	14
14.1.11	14	14
14.1.13	14	14
14.1.18	14	113
14.1.23	14	8
18.1.4	18	96
18.2.12	18	13
18.2.3	18	153
18.2.4	18	121
18.2.6	18	99
18.3.10	18	63
18.3.11	18	157
18.3.5	18	17

Table S2.2 Final abundance of cleaned reads assigned to each *Symbiodinium* clade and their percent abundance and OTU number across all clades.

Clade	Reads	Percent of cleaned reads	OTUs
A	69775	1.5	7
B	47	0.001	1
C	4,393,359	94.7	62
D	173,549	3.7	7
E	106	0.002	4
G	496	0.01	2
Unknown	1459	0.03	78
Total	4,638,791	-	161

Table S2.3 A large number of clade C identified OTUs could not be identified to the type level using a general Blast search against the whole NCBI nt database. In order to increase taxonomic resolution, I re-blasted OTUs to a *Symbiodinium* specific database (taxon id: 2949) as well as aligned them using ClustalW to known standards for this species (Tonk *et al.*, 2013). I have summarized the taxonomic designations here for only those OTUs that I found to be significantly differently abundant (p-adjusted) between the comparisons of interest (adult vs. planula; between maternal broods; adult size classes). P-adjusted values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons.

OTU name	**Blast identity**	**Blast E-value for general/ *Symbiodinium* specific search**	**Accession N°**	**Phylogenetic designation**
OTU1	C1	2.00e-168	JN558041	C120, C120a
OTU2	C1	2e-33	KM041000.1/KM040971.1	C
OTU4	C1	5e-35	KM041000.1/KM040971.1	C
OTU5	C1	3.00e-166	JN558041	C1m
OTU7	C1	3e-24	KM041000.1	C
OTU12	C1	2e-34	KM041000.1	C
OTU19	C1	1e-22	KM041000.1	C
OTU21	C1	2e-39	KM041000.1	C
OTU22	C1	1e-30	KM041000.1	C
OTU23	C1	1e-156	JN558041.1	C1
OTU24	C1	2e-26	KM041000.1	C
OTU25	C1	1e-30	KM041000.1	C
OTU28	C1	4e-29	KM041000.1	C
OTU32	C1	2e-26	KM041000.1	C
OTU44	C1v1e	1e-149	HG942431.1	-
OTU46	C15	2.00e-168	JN558044	C15
OTU105	C1	1.00e-151	JN558041	C1m
OTU134	C1	6.00e-162	JN558041	-
OTU136	C1	2e-33	KM041000.1	C
OTU165	C1	1.00E-163	KM041000.1	C3w
OTU209	C	3e-06	AB294631.1	C1
OTU223	C1	4e-29	KM041000.1	C
OTU228	C1v6	5e-175	HG942433.1	C22
OTU733	C1	4.00e-113	JN558041	C31
OTU860	C1	2e-21	KM041000.1	C

Table S2.4 Summary table for differential abundance testing using DESeq2 comparing *Symbiodinium* communities: A) adults and planulae, B) larval broods differing by dam, and C) adult colony size classes. Values were derived from negative binomial models. Padj values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons.

Comparison	OTU	Identity	Basemean	Log2 fold	Padj
Adult vs. planula	OTU22	C/C1	26.1	-5.1	1.17 e -48
Adult vs. planula	OTU3	D1	848.8	2.1	5.67 e -30
Adult vs. planula	OTU2	C/C1	2671.1	-3.8	4.72 e -28
Adult vs. planula	OTU10	*microadriaticum* (A1)	137.6	2.2	4.1 e -24
Adult vs. planula	OTU8	A3	231.2	2	4.3 e -24
Adult vs. planula	OTU12	C/C1	79.2	-2.9	3.9 e -19
Adult vs. planula	OTU24	C/C1	31.7	-3.1	1.67 e -18
Adult vs. planula	OTU7	C/C1	122.4	-3.9	6.67 e -17
Adult vs. planula	OTU165	C1/C3w	68.3	1	6.67 e -17
Adult vs. planula	OTU32	C/C1	3.6	-5.4	1.74 e -16
Adult vs. planula	OTU6	D1a	75.6	2.2	3.3 e -16
Adult vs. planula	OTU25	C/C1	1.3	-3.4	1.5 e -11
Adult vs. planula	OTU136	C/C1	1.1	-6	4.58 e -11
Adult vs. planula	OTU28	C/C1	18.2	-3.4	1.07 e -10
Adult vs. planula	OTU597	D1	0.93	2.5	1.07 e -09
Adult vs. planula	OTU43	Unknown	1.8	-4.4	3.8 e -09
Adult vs. planula	OTU23	C1	19.8	0.87	5.95 e -09
Adult vs. planula	OTU105	C1/C1m	113.1	0.74	2.1 e -08
Adult vs. planula	OTU4	C/C1	846.2	-3.4	2.54 e -08
Adult vs. planula	OTU223	C/C1	10	-3	4.01 e -07

Adult vs. planula	OTU1	C1/C120, C120a	23,094.40	0.48	2.97 e -06
Adult vs. planula	OTU21	C/C1	12.3	-4.8	4.6 e -06
Adult vs. planula	OTU81	Unknown	0.6	-4.5	8.05 e -06
Adult vs. planula	OTU5	C1/C1m	169	0.47	5.5 e -05
Adult vs. planula	OTU228	C/C1v6/C22	18	0.77	8.2 e -05
Adult vs. planula	OTU19	C/C1	17.2	-3.1	2.42 e -03
Adult vs. planula	OTU149	Unknown	0.26	-3.9	2.66 e -03
Adult vs. planula	OTU123	Unknown	0.32	-2.99	2.75 e -03
Adult vs. planula	OTU733	C31/C1	67.4	0.54	5.49 e -03
Adult vs. planula	OTU148	Unknown	0.31	-3.9	1.01 e -02
Adult vs. planula	OTU209	C/C1	0.16	-3.2	1.73 e -02
Adult vs. planula	OTU860	C/C1	0.16	-3.2	2.16 e -02
Adult vs. planula	OTU185	Unknown	0.19	-3.2	2.32 e -02
Adult vs. planula	OTU46	C15	0.59	3.2	3.26 e -02
Adult vs. planula	OTU134	C1	72.9	0.44	3.43 e -02
Lv2 vs. Lv6	OTU10	*microadriaticum* (A1)	52.2	1.7	0.002
Lv2 vs. Lv6	OTU8	A3	98.1	1.5	0.01
Lv2 vs. Lv6	OTU32	C/C1	5.9	-2.8	0.03
Lv2 vs. Lv7	OTU10	*microadriaticum* (A1)	52.2	1.9	0.003
Lv2 vs. Lv13	OTU2	C/C1	4396.4	7	5.05 e -35
Lv2 vs. Lv13	OTU8	A3	98.1	2.4	4.00 e -05
Lv2 vs. Lv13	OTU10	*microadriaticum* (A1)	52.2	2.2	2.33 e -04
Lv2 vs. Lv13	OTU44	C/C1v1e	2.2	-5.5	9.61 e -04

Lv2 vs. Lv13	OTU3	D1	346.5	1.7	4.85 e -03
Lv2 vs. Lv13	OTU6	D1a	30	2	9.63 e -03
Lv2 vs. Lv14	OTU10	*microadriaticum* (A1)	52.2	2.1	0.01
Lv2 vs. Lv14	OTU8	A3	98.1	1.8	0.04
Lv3 vs. Lv4	OTU44	C/C1v1e	2.2	-5	0.006
Lv3 vs. Lv4	OTU2	C/C1	4396.4	-3.5	0.02
Lv3 vs. Lv13	OTU2	C/C1	4396.4	4.4	0.00019
Lv4 vs. Lv6	OTU44	C/C1v1e	2.2	7	2.46 e -07
Lv4 vs. Lv6	OTU134	C1	61.7	1	1.13 e -02
Lv4 vs. Lv6	OTU733	C31/C1	54.4	-1.1	1.76 e -02
Lv4 vs. Lv6	OTU7	C/C1	197.1	-2	1.90 e -02
Lv4 vs. Lv7	OTU44	C/C1v1e	2.2	5.4	0.0003
Lv4 vs. Lv10	OTU44	C/C1v1e	2.2	5.5	0.002
Lv4 vs. Lv13	OTU2	C/C1	4396.4	7.9	1.35 e -47
Lv4 vs. Lv13	OTU7	C/C1	197.1	-2.6	6.59 e -03
Lv4 vs. Lv13	OTU6	D1a	30	1.7	3.04 e -02
Lv4 vs. Lv13	OTU4	C/C1	1289.7	-3.3	4.09 e -02
Lv4 vs. Lv13	OTU3	D1	346.5	1.3	4.16 e -02
Lv4 vs. Lv13	OTU8	A3	98.1	1.2	4.97 e -02
Lv4 vs. Lv13	OTU21	C/C1	21.6	-3.7	4.97 e -02
Lv4 vs. Lv14	OTU44	C/C1v1e	2.2	5.3	0.0060767
Lv4 vs. Lv14	OTU733	C31/C1	54.4	-1.4	0.0314999
Lv4 vs. Lv18	OTU44	C/C1v1e	2.2	6.8	3.73 e -05

Lv3 vs. Lv4	OTU44	C/C1v1e	2.2	-5	0.006
Lv3 vs. Lv4	OTU2	C/C1	4396.4	-3.5	0.02
Lv3 vs. Lv13	OTU2	C/C1	4396.4	4.4	0.00019
Lv4 vs. Lv6	OTU44	C/C1v1e	2.2	7	2.46 e -07
Lv4 vs. Lv6	OTU134	C1	61.7	1	1.13 e -02
Lv4 vs. Lv6	OTU733	C31/C1	54.4	-1.1	1.76 e -02
Lv4 vs. Lv6	OTU7	C/C1	197.1	-2	1.90 e -02
Lv4 vs. Lv7	OTU44	C/C1v1e	2.2	5.4	3.09 e -04
Lv4 vs. Lv10	OTU44	C/C1v1e	2.2	5.5	2.40 e -03
Lv4 vs. Lv13	OTU2	C/C1	4396.4	7.9	1.35 e -47
Lv4 vs. Lv13	OTU7	C/C1	197.1	-2.6	6.59 e -03
Lv4 vs. Lv13	OTU6	D1a	30	1.7	3.04 e -02
Lv4 vs. Lv13	OTU4	C/C1	1289.7	-3.3	4.09 e -02
Lv4 vs. Lv13	OTU3	D1	346.5	1.3	4.16 e -02
Lv4 vs. Lv13	OTU8	A3	98.1	1.2	4.97 e -02
Lv4 vs. Lv13	OTU21	C/C1	21.6	-3.7	4.97 e -02
Lv4 vs. Lv14	OTU44	C/C1v1e	2.2	5.3	6.08 e -03
Lv4 vs. Lv14	OTU733	C31/C1	54.4	-1.4	3.15 e -02
Lv4 vs. Lv18	OTU44	C/C1v1e	2.2	6.8	3.73 e -05
Lv6 vs. Lv7	OTU1	C1/ C120, C120a	19319.9	-0.7	0.02
Lv6 vs. Lv7	OTU105	C1/C1m	85.1	0.8	0.03
Lv6 vs. Lv13	OTU2	C/C1	4396.4	6.8	2.20 e -42
Lv6 vs. Lv13	OTU44	C/C1v1e	2.2	-5.3	1.67e -03

Lv6 vs. Lv13	OTU4	C/C1	1289.7	-4.5	3.22 e -03
Lv6 vs. Lv18	OTU4	C/C1	1289.7	-3.3	0.05
Lv6 vs. Lv18	OTU32	C/C1	5.95	2.9	0.05
Lv6 vs. Lv18	OTU81	Unknown	0.96	4.5	0.05
Lv6 vs. Lv18	OTU134	C1	61.7	-0.9	0.05
Lv7 vs. Lv13	OTU2	C/C1	4396.4	7.3	3.2 e -42
Lv10 vs. Lv13	OTU2	C/C1	4396.4	5.9	5.3 e -08
Lv10 vs. Lv13	OTU1	C1/ C120, C120a	19319.9	1.4	4.9 e -02
Lv13 vs. Lv14	OTU2	C/C1	4396.4	-6.7	7.15 e -28
Lv13 vs. Lv14	OTU4	C/C1	1289.7	6.1	2.21 e -05
Lv13 vs. Lv18	OTU2	C/C1	4396.4	-7	5.96 e -38
Lv13 vs. Lv18	OTU8	A3	98.1	-1.8	4.61 e -03
Lv13 vs. Lv18	OTU44	C/C1v1e	2.2	5.1	4.61 e -03
Lv13 vs. Lv18	OTU21	C/C1	21.6	5.3	5.47 e -03
Lv13 vs. Lv18	OTU6	D1a	30	-1.9	8.59 e -03
Lv13 vs. Lv18	OTU3	D1	346.5	-1.3	3.80 e -02
Lv14 vs. Lv18	OTU4	C/C1	1289.7	-4.9	0.008
8-14 vs. 14-20	OTU4	C/C1	-	3.18	0.001
26-32 vs. < 8	OTU105	C1/C1m	-	3.8	1.8 e -06
26-32 vs. < 8	OTU228	C/C1v6/C22	-	3.4	1.1 e -02
26-32 vs. 8-14	OTU105	C1/C1m	-	4.1	3.1 e -13
26-32 vs. 8-14	OTU228	C/C1v6/C22	-	2.7	3.2 e -03
26-32 vs. 8-14	OTU733	C31/C1	-	2.1	3.1 e -02

26-32 vs. 14-20	OTU105	C1/C1m	-	3.95	1.3 e -11
26-32 vs. 14-20	OTU228	C/C1v6/C22	-	2.99	1.7 e -03
26-32 vs. 26-32	OTU105	C1/C1m	-	3.9	5.4 e -11
26-32 vs. 26-32	OTU228	C/C1v6/C22	-	2.66	1.7 e -02

Table S2.5 Summary table of the full OTU names and their abbreviations depicted in Figure 2.1. 83 of the total 161 *Symbiodinium* OTUs found in *S. hystrix* adults and larvae (excluding unidentified types) were abbreviated for clarity.

OTU name	Clade	Abbreviation
OTU45_A	A	A_1
OTU196_A	A	A_2
OTU511_A13	A	A13
OTU8_A3	A	A3_1
OTU554_A3	A	A3_2
OTU10_micro	A	*microadriaticum*_1
OTU129_micro	A	*microadriaticum*_2
OTU181_B1	B	B1
OTU24_C	C	C_1
OTU27_C	C	C_2
OTU44_C	C	C_3
OTU91_C	C	C_4
OTU193_C	C	C_5
OTU209_C	C	C_6
OTU228_C	C	C_7
OTU252_C	C	C_8
OTU285_C	C	C_9
OTU517_C	C	C_10
OTU530_C	C	C_11
OTU569_C	C	C_12
OTU571_C	C	C_13
OTU724_C	C	C_14
OTU731_C	C	C_15
OTU740_C	C	C_16
OTU743_C	C	C_17
OTU750_C	C	C_18
OTU760_C	C	C_19
OTU22_C	C	C.23
OTU23_C1	C	C1_1
OTU29_C1	C	C1_2
OTU47_C1	C	C1_3
OTU51_C1	C	C1_4
OTU58_C1	C	C1_5
OTU105_C1	C	C1_6
OTU134_C1	C	C1_7
OTU165_C1	C	C1_8
OTU437_C1	C	C1_9
OTU464_C1	C	C1_10
OTU730_C1	C	C1_11
OTU733_C1	C	C1_12
OTU736_C1	C	C1_13
OTU741_C1	C	C1_14

OTU1_C1	C	C1_15
OTU5_C1	C	C1_16
OTU46_C15	C	C15_1
OTU451_C15	C	C15_2
OTU2_C_variant	C	Cv_1
OTU53_C_variant	C	Cv_10
OTU86_C_variant	C	Cv_11
OTU136_C_variant	C	Cv_12
OTU184_C_variant	C	Cv_13
OTU223_C_variant	C	Cv_14
OTU432_C_variant	C	Cv_15
OTU562_C_variant	C	Cv_16
OTU783_C_variant	C	Cv_17
OTU833_C_variant	C	Cv_18
OTU846_C_variant	C	Cv_19
OTU4_C_variant	C	Cv_2
OTU860_C_variant	C	Cv_20
OTU881_C_variant	C	Cv_21
OTU886_C_variant	C	Cv_22
OTU169_C_variant	C	Cv_24
OTU589_C_variant	C	Cv_25
OTU7_C_variant	C	Cv_3
OTU12_C_variant	C	Cv_4
OTU19_C_variant	C	Cv_5
OTU21_C_variant	C	Cv_6
OTU25_C_variant	C	Cv_7
OTU28_C_variant	C	Cv_8
OTU32_C_variant	C	Cv_9
OTU3_D1	D	D1_1
OTU120_D1	D	D1_2
OTU597_D1	D	D1_3
OTU904_D1	D	D1_4
OTU6_D1a	D	D1a_1
OTU201_D1a	D	D1a_2
OTU803_D1a	D	D1a_3
OTU75_E	E	E_1
OTU340_E_foram	E	E_2
OTU588_E	E	E_3
OTU848_E_foram	E	E_4
OTU34_G4	G	G4
OTU200_G6	G	G6

Supporting figures for Chapter 2

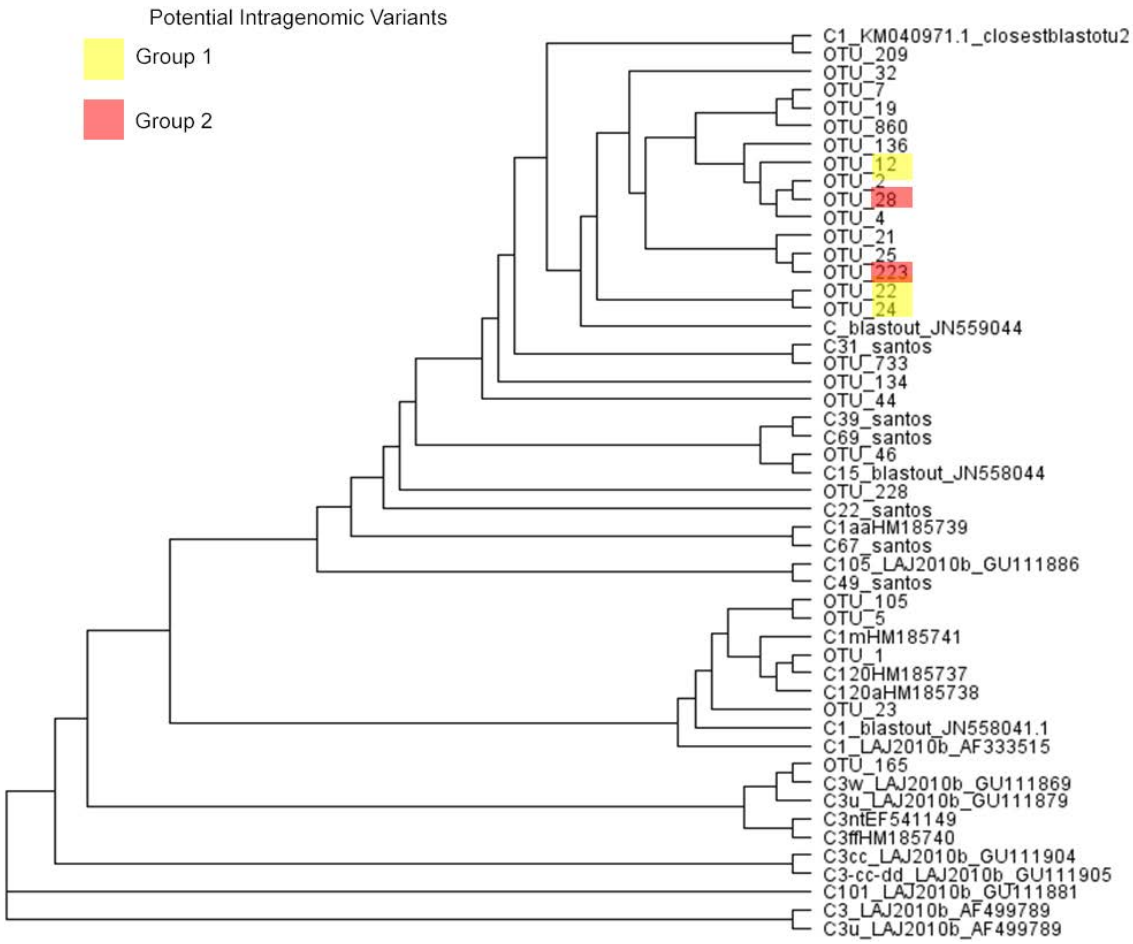

Figure S2.1 ClustalW2 Neighbour-joining cladogram showing all OTUs with significant p-adjusted (values between: 1) planulae and adults (35 OTUs), 2) between maternal broods (15 OTUs), 3) size classes (4 OTUs). Additional sequences are references of *Symbiodinium* types known to inhabit ShA or those whose genetic diversification has been investigated (tip labels without "OTU"). P-adjusted values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons.

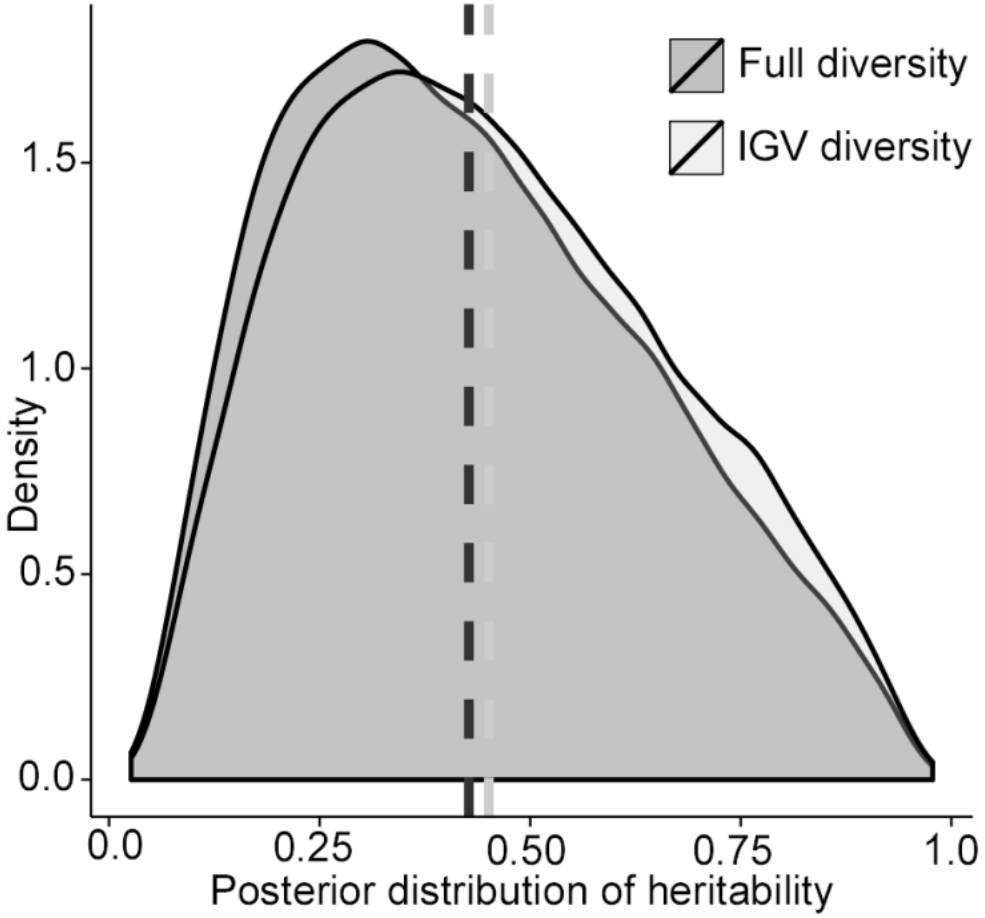

Figure S2.2 The posterior Bayesian heritability distributions using full diversity metric (dark grey) and intragenomic variants (IGV, light grey). The dashed lines represent the mean narrow-sense heritability, with the colour corresponding to the type of diversity metric used.

Appendix B

Supplementary results Chapter 3

3.1 Comparisons of *Symbiodinium* communities among 2012 families of *A. tenuis*

Juveniles from family AB had characteristically high proportions of both D1 and A3, which collectively made up ~75% of their *Symbiodinium* communities. CCMP828 made up ~30-50% of reads in 33% (4/12) AB juveniles, with concomitant decreases in proportional abundances of A3. Three of the 12 AB3/12 juveniles had large abundances of two G4 types, *Zoanthus* and *Favia ex situ* types, C90, and an F *ex situ* type. All juveniles also had a diversity of background types from clades A, B, C, D, G, *ex situ* and uncultured *Symbiodinium* types, with, on average, 25.8± 2 OTUs per sample. Juveniles from family AC followed a similar pattern of high diversity (19.8± 2.5), although whereas type C1 was never the dominant type in AB juveniles, 2/8 AC juveniles were dominated by C1. The other juveniles in this family were dominated by D1 and CCMP828, with A3, C1, an uncultured type (OTU7), or a D type (OTU6) also abundant in some juveniles. Juveniles from family AD also had many background types at very low abundance (average OTUs: 23±2), although the dominant OTUs (A3, D1 and CCMP828) were found in the same pattern as in families AB and AC. Compared to those families however, type C1 was in much lower and D1a in much higher proportional abundance in these families, respectively.

BC juveniles had, on average, 22±1.3 OTUs, with individuals dominated by combinations of: D1/CCMP828/C1/A3 and background D1a with types CCMP828, D1, C1. In total, 106 background OTUs were recovered, including 24 A types (A3, A2, *microadriaticum*, A13, A4.3), B (*S. muscatinei*), 29 C types (C1, C3, mizugama, C90), 12 D types (D1, D1a, D), 3 E (*S. voratum*), 12 *ex situ* (*Zoanthus, Scyphozoa, Amphisorus*), 3 F (F, F4), G4, 19 uncultured *Symbiodinium*, and two types identified as RCC2640. The CB family was also similar to juveniles from family BC, with on average, 26.7±1.5 OTUs per juvenile, and 216 OTUs retrieved from this family. Like BC, the CB family had a background diversity of clade A (72) and C types (110), D, E and uncultured symbiont types, including: A3, *microadriaticum,* A4.3, A2, A13, C1, C90, mizugama, C15 and C3. Family CB, however, had a greater number of different G, F and B types. CD and CA family were also very diverse, with on average 24.7±3.5 and 27.5±7.5 OTUs per juvenile respectively.

Juveniles from BA family were most similar in *Symbiodinium* diversity (17.8±1.4) and composition to AC juveniles, with C1 dominant in 2/9 juveniles (~75%) in C1 and the remaining juveniles harbouring mixed communities of D1, CCMP828, and A3 and lower abundances of D1a. D type OTU6 was also again recovered in low abundances. BD family juveniles (average OTUs: 17.8±2) were also similar to these other families, with D1 and A3 dominating *Symbiodinium* communities in conjunction with CCMP828 and C1.

3.2 Comparisons of *Symbiodinium* communities among 2013 families of *A. tenuis*

Individual juveniles from F1 were dominated by varying abundances of multiple types belonging to: A3, C1 and D1, with some individuals also having large populations of G4, D (OTU10), *S. minutum*, and A type CCMP828 (OTU7). D1a was also found in abundances ranging from ~3-15% in many juveniles (Figure S3.4), with types C3, CCMP2456, G4 and B2 also present (Table S3.5). The same pattern of the dominant type fluctuating among A3, C1 and D1 was found in families F10, F12, F2, F5, and F6. Family F28 was similar in composition to the preceding families, although with the addition of A13 (OTU11). Notably, dominant *Symbiodinium* types in F4 juveniles also varied among D1, C1, A3, D1a and CCMP828 types, although C1 was much less prevalent in this family, with only 2/12 juveniles having greater than background abundances.

Families F13, F19, F22, F23, F24, F27, and F9 had much less diversity, as juveniles from these families were dominated by fluctuating proportions of D1, C1, A3 and D1a and in two individuals, CCMP828; with very few other OTUs in background proportions compared to those found in F1,F10 and F12. Generally, families F14 and F18 were distinct from the other families, with 5/6 (F14) and 4/9 (F18) juveniles partially dominated by new D types (F14: OTU4, F18: OTUs 10/16/95), C1 and A13 (OTU11). These families had, on average, the highest number of OTUs per juvenile (F14: 34.7 ± 11.9, F18: 25.2 ± 5.3), and the greatest number of OTUs recovered across all juveniles (F14: 138 total OTUs, F18: 140)(Table S3.5). Four juveniles (14cbr1, 14cbr2, 18b.1r1N2, 18br1) were particularly diverse, with 45 - 84 OTUs detected, and uncultured *Symbiodinium* types such as Zoox23-OTU9 were at particularly high abundance and diversity in these families.

The remaining families (F8, F15, and F17) had juveniles that were similar to both the first and second group of families described. For example, families F8 and F15 had 3/6 and 4/7 individuals with the characteristic C1, D1, D1a, A3 mix, whilst the remaining juveniles strongly resembled families with high OTU diversity and were typified by *S. psygmophilum*, *S. natans, S. voratum, S. minutum*, C91, G4, HI-0609, and uncultured or *ex situ* types. F17 was similar, although additional *Symbiodinium* diversity also included A2 and sediment derived A-type *Symbiodinium* (OTU361).

3.3 Comparisons of *Symbiodinium* communities among families of *M. digitata* eggs

The proportional abundance of C15 (OTU1) was lower than the average C15 abundance in eggs in three of dam 29 eggs (91.2-93.7% C15); concomitantly, proportional abundances increased in a large diversity of other types, with types A3 (OTU5), D1 (OTU3) and two uncultured *Symbiodinium* types (OTU9 and 14) more abundant than in eggs from other dams. Eggs from dam 11 had much less of the *ex situ Amphisorus* type; instead background abundances were made up of C1, B1, two A types (A3, CCMP828), and D1, which were all types more prevalent in dam 11. The only OTUs that differed significantly (BF p-adj < 0.05) in abundance between egg families were C1_5-OTU10, uncultured-OTU9 and 14, *Amphisorus*-OTU2, and C1_4-OTU8 (Figure S3.5, Table S3.6).

Supporting tables for Chapter 3

Table S3.1 Gamete cross design for *Acropora tenuis* juveniles from spawning season 2012. Colonies A and B were collected from Orpheus Island (OI), and colonies C and D were collected from Princess Charlotte Bay (PCB). Numbers in parenthesis following each cross are the number of individual juveniles sequenced per cross. Parent colonies collected and crossed from Orpheus are annotated OI (2012) and O (2013), and colonies from Princess Charlotte Bay are PCB (2012) and W (2013).

		Sire			
		A (OI)	**B (OI)**	**C (PCB)**	**D (PCB)**
Dam	**A (OI)**	-	BA (9)	CA (2)	-
	B (OI)	AB (12)	-	CB (29)	-
	C (PCB)	AC (8)	BC (22)	-	-
	D (PCB)	AD (12)	BD (5)	CD (3)	-

Table S3.2 Summary of the 25 gamete crosses performed, of which individuals from 20 families survived the larval stage, settled as juveniles and survived in the field. Numbers in parenthesis represent the number of juveniles sequenced per family. Parent colonies collected and crossed from Orpheus are annotated OI (2012) and O (2013), and colonies from Princess Charlotte Bay are PCB (2012) and W (2013).

		Sire							
		O4	**O6**	**O3**	**O5**	**W11**	**W10**	**W7**	**W5**
Dam	**O4**		F1 (13)			F2 (12)			F4 (12)
	O6			F5 (21)			F6 (14)		
	O3	F8 (6)				F9 (8)	F10 (13)		
	O5	F12 (12)	F13 (11)	F14 (6)		F15 (7)			
	W11		F17 (5)				F18 (9)		F19 (6)
	W10	F26 (NA)			F21 (NA)	F22 (6)		F23 (1)	F24 (3)
	W7				F25 (NA)	F29 (NA)			
	W5		F27 (5)	F28 (2)				F30 (NA)	

Table S3.3 Summary of the sequencing performed for *A. tenuis* and *M. digitata* species. Samples were either juveniles (J), eggs (E), or adults (A). *Gamete crosses involved 8 parental colonies (4 from Wilkie reef in Princess Charlotte Bay and 4 from Orpheus); 3 replicates per colony were sequenced for Orpheus parents (total=12 samples), and 1 replicate per Wilkie colony (total=4 samples), totalling 16 adult samples sequenced.

Species	Year	Samples	Families (N°)	Average reads/sample ± SE	Total cleaned reads	Average cleaned reads/sample ± SE	OTU N°
A. tenuis	2012	106 (102 J, 4 A)	9	83,595± 2425	6,027,635	56,864± 2009	422
A. tenuis	2013	188 (172 J, 16 A*)	25	72,107± 4329	6,873,216	36,560± 2922	568
M. digitata	2015	108 (99 E, 9 A)	9	15,365 ± 817	1,398,184	12,946± 710	101

Table S3.4 Summary of sequencing results for *A. tenuis* for 2012 and 2013 crosses, post clean-up and E-value filters.

Year	2012			2013		
	Sum reads	Percent (%)	OTUs	Sum reads	Percent (%)	OTUs
A	2515240	41.73	72	1914858	28.1	88
B	2845	0.05	17	51591	0.75	18
C	839606	13.93	110	2154506	31.35	119
D	2566176	42.57	44	2454930	35.72	54
E	1197	0.02	8	593	0.01	12
F	8370	0.14	27	9587	0.14	39
G	20768	0.34	8	73212	1.07	16
Ex situ	15036	0.25	54	40178	0.58	62
Uncultured	58388	0.97	78	156452	2.28	157
RCC2640	9	0.00	4	0	0.00	0
H	0	0.00	0	17201	0.0001	1
I	0	0.00	0	108	0.00	2
TOTAL	6027635	100.00	422	6873216	100.00	568

Table S3.5. OTU diversity retrieved per family of *Acropora tenuis* juveniles in 2013. Bolded values below family name represent the total number of OTUs retrieved from that family.

Family (F-)/Adult (O- or W-)	Clade A	Clade C	Clade D	Clades B/*Ex situ*/G/H/Uncultured
F1 (**73**)	A3_OTU3, A3_OTU408, A3_OTU665, A3_OTU725, A3_OTU736, A3_OTU799, A3_OTU491, CCMP828_OTU7, CCMP2456_OTU73, CCMP2456_OTU457, CCMP828_OTU458, CCMP2456_OTU558, 12979_OTU638, 1631_OTU119, 65_OTU369,	C_OTU137, C_OTU232, C1_OTU1, C1_OTU113, C1_OTU237, C1_OTU326, C1_OTU370, C1_OTU597, C1_OTU549, C1_OTU559, C1_OTU567 C1_OTU661 C1_OTU743 C1_OTU776, C1_OTU121 C1_OTU803, Two0504-8_OTU269, C3_OTU637, C3_OTU347, SC13.7_OTU36	D1_OTU2, D_OTU4, D1a_OTU6, D_OTU10, D1a_OTU142, D_OTU373, D1_OTU593 D1a_OTU727 D1a_OTU756,	minutum_OTU45, psygmophilum_B2_1635_OTU13, psygmophilum_B2_1636_OTU124, Zoanthus_OTU21, Zoanthus_OTU18, Scyphozoa_medusae1Sy23_OTU22, Scyphozoa_medusae_1Sy24_OTU120, zoox23_OTU23, zoox23_OTU19, zoox23_OTU156, zoox23_OTU377, zoox23_OTU253, uncultured_OTU177, uncultured_OTU30, uncultured_OTU181, uncultured_OTU382, uncultured_OTU12, uncultured_OTU409, uncultured_OTU514, uncultured_OTU578, uncultured_OTU590, uncultured_OTU639, uncultured_OTU104, uncultured_OTU672, uncultured_OTU103, G4_OTU68, G4_OTU730, G6_OTU8, F3.2_OTU96 kawagutii_OTU14
F2 (**95**)	A3_OTU3, CCMP828_OTU7, CCMP2456_OTU73, A4_OTU165, A3_OTU238, A3_OTU362, A3_OTU404, A3_OTU408, CCMP828_OTU458, CCMP2456_OTU558, A13_OTU594, A3_OTU665, A3_OTU674, A3_OTU725, A3_OTU799	C_OTU232, C_OTU63 C1_OTU1, C1_OTU121 C1_OTU145, C1_OTU236,C1_OTU237, C1_OTU242, C1_OTU287, C1_OTU432, C1_OTU440, C1_OTU448, C1_OTU492, C3_OTU347, C1_OTU641, C1_OTU661, C1_OTU724, C1_OTU669, C1_OTU755, C1_OTU567, C1_OTU762, SC13.7_OTU76, SC13.7_OTU422	D_OTU37, D1_OTU2 D_OTU4,D_OTU5,D1D1a_OTU6, D_OTU10, , D_OTU16, D1a_OTU756, D1a_OTU142, D_OTU331, D1a_OTU748, D1a_OTU737	psygmophilum_B2_1635_OTU13, psygmophilum_B2_1636_OTU124, minutum_OTU45, muscatinei_OTU17, zoox23_OTU253, zoox23_OTU335, zoox23_OTU371, zoox23_OTU403, G2b_OTU94, G4_OTU68, zoox23_OTU443, zoox23_OTU59, zoox23_OTU85, zoox23_OTU46, kawagutii_OTU14, zoox23_OTU561, zoox23_OTU19, zoox23_OTU591,zoox23_OTU592, zoox23_OTU615 zoox23_OTU750, zoox23_OTU23, zoox23_OTU643, Zoanthus_OTU141, Zoanthus_OTU352, Zoanthus_OTU49 Zoanthus_OTU26, Scyphozoa_medusae_1Sy24_OTU595,Zoanthus_OTU18, Scyphozoa_medusae_1Sy24_ OTU84, Scyphozoa_medusae_1Sy24_OTU39, uncultured_OTU164, uncultured_OTU130, uncultured_OTU198, uncultured_OTU327, uncultured_OTU382, uncultured_OTU430, uncultured_OTU514, uncultured_OTU461, uncultured_OTU104, uncultured_OTU97, uncultured_OTU33, uncultured_OTU577, HI-0609_OTU24

F4 (**85**)	A3_OTU3, CCMP828_OTU7, Oku16_sand_OTU53, CCMP2456_OTU73, A3_OTU408, A3_OTU238, CCMP828_OTU458, CCMP2456_OTU558, A_OTU574, CCMP2456_OTU587, A3_OTU665, A3_OTU725, CCMP828_OTU746, A3_OTU799	C1_OTU1, SC13.7_OTU76, C1_OTU79, C1_OTU86 C1_OTU113, C1_OTU121, C_OTU137, C1_OTU236, C1_OTU281, C3_OTU302, C1_OTU326, C1_OTU429, C1_OTU432, C1_OTU237, C1_OTU492, C33_OTU508, C1_OTU547, C1_OTU557, C1_OTU633, C1_OTU649, SC13.7_OTU670, PtMil1-9_OTU642, OTcH-2_OTU659	D1_OTU2, D_OTU4, D1a_OTU6, D_OTU10, , D_OTU37, D_OTU101, D_OTU126, D1a_OTU142, D_OTU373, D_OTU374, D1_OTU593, D1_OTU647, D_OTU678, D1a_OTU737, D1a_OTU727, D_OTU16	G6_OTU8, uncultured_OTU12, psygmophilum_B2_1635_OTU13, Zoanthus_OTU18, uncultured_OTU27, Zoanthus_OTU28, uncultured_OTU30, uncultured_OTU33, minutum_OTU45, uncultured_OTU55, Scyphozoa_medusae_1Sy24_OTU84, uncultured_OTU103, uncultured_OTU104, zoox23_OTU156, psygmophilum_B2_1637_OTU157, uncultured_OTU177, uncultured_OTU181, zoox23_OTU195, uncultured_OTU214, zoox23_OTU227, Zoanthus_OTU352, uncultured_OTU382, voratum_OTU406, uncultured_OTU409, HI-0509_OTU427, uncultured_OTU430, uncultured_OTU455, zoox23_OTU495, 13478_OTU530, Amphisorus_OTU576, Scyphozoa_medusae_1Sy24_OTU596 kawagutii_OTU14
F5 (**116**)	A3_OTU3, CCMP828_OTU7, CCMP2455_OTU50, A2_JCUSG-1_OTU66, CCMP2456_OTU73, CCMP2457_OTU173, A3_OTU799, A3_OTU238, A3_OTU408, A3_OTU725, A3_OTU665, CCMP2456_OTU558	C1_OTU1, SC13.7_OTU36, C_OTU63, C1_OTU113, C1_OTU86, C1_OTU121, C_OTU232, C_OTU137, C1_OTU249, C1_OTU250, C_OTU140, C15_OTU340, C1_OTU79, C1_OTU237, C3_OTU347, C1_OTU432, C1/C3_Two0501-8_OTU439, C1_OTU440, C1_OTU762, C1_OTU803, C3_OTU734, C1_OTU738, C1_OTU661, C33_OTU508, C1_OTU567, CCMP2456_OTU457, CCMP828_OTU458	D1_OTU2, D_OTU5, D1a_OTU6, D_OTU10, , D_OTU16, D_OTU58, D_OTU101, D1a_OTU142, D_OTU187, D_OTU149, D_OTU331 D_OTU373, D_OTU374, D_OTU426, D1a_OTU756, D1a_OTU727, D_OTU678	kawagutii_OTU14, G6_OTU8, uncultured_OTU12, psygmophilum_B2_1635_OTU13, Zoanthus_OTU18, zoox23_OTU19, Zoanthus_OTU21, Scyphozoa_medusae1Sy23_OTU22, zoox23_OTU23, zoox23_OTU25, Zoanthus_OTU26, uncultured_OTU27, Zoanthus_OTU28, uncultured_OTU33, Scyphozoa_medusae_1Sy24_OTU34 Montipora_OTU38, uncultured_OTU42, minutum_OTU45, zoox23_OTU46, Zoanthus_OTU49, uncultured_OTU55, 1679_OTU61, voratum_OTU64, G4_OTU68, Scyphozoa_medusae_1Sy24_OTU84, F3.2_OTU96, uncultured_OTU103, uncultured_OTU104, Scyphozoa_medusae_1Sy24_OTU120, psygmophilum_B2_1636_OTU124, uncultured_OTU130, zoox23_OTU143, uncultured_OTU164, uncultured_OTU177, uncultured_OTU181, voratum_OTU216, 1679_OTU285, nr-i4_OTU301, uncultured_OTU306, Zoanthus_OTU316, Scyphozoa_medusae1Sy23_OTU333, zoox23_OTU342, 04-218-SCI.01_OTU368, uncultured_OTU382, uncultured_OTU402, zoox23_OTU403, uncultured_OTU430, psygmophilum_OTU453, Zoanthus_OTU454,

				Scyphozoa_medusae_1Sy24_OTU489, zanpa_OTU498, uncultured_OTU514, Zoanthus_OTU545, 13478_OTU548, OTcH-2_OTU659, , uncultured_OTU663, Amphisorus_OTU666, psygmophilum_OTU671, Scyphozoa_medusae_1Sy24_OTU729, uncultured_OTU754
F10 (**112**)	A2_JCUSG-1_OTU66 A2_OTU215 A3_OTU128 A3_OTU291 A3_OTU3 A3_OTU309 A3_OTU408 A3_OTU476 A3_OTU491 A3_OTU665 A3_OTU725 A3_OTU799 CCMP2455_OTU50 CCMP2456_OTU163 CCMP2456_OTU558 CCMP2456_OTU73 CCMP2456_OTU757 CCMP828_OTU458 CCMP828_OTU664 CCMP828_OTU7	C_OTU137 C1/C3_Two0501-8_OTU439 C1_OTU1 C1_OTU113 C1_OTU121 C1_OTU204 C1_OTU241 C1_OTU381 C1_OTU383 C1_OTU412 C1_OTU419 C1_OTU440 C1_OTU567 C1_OTU589 C1_OTU661 C1_OTU762 C1_OTU79 C1_OTU803 C1v1d_OTU355 SC13.7_OTU753 SC13.7_OTU76	D_OTU10 D_OTU16 D_OTU328 D_OTU373 D_OTU4 D_OTU5 D1.2_OTU361 D1_OTU2 D1a_OTU142 D1a_OTU6 D1a_OTU737	1363_OTU353 1679_OTU174 1679_OTU61 1681_OTU102 1681_OTU303 Amphisorus_OTU134 F4.5_OTU93 G4_OTU68 HI-0509_OTU109 HI-0509_OTU139 HI-0509_OTU334 HI-0509_OTU427 HI-0609_OTU24, minutum_OTU385 minutum_OTU45 psygmophilum_B2_1635_OTU13 psygmophilum_B2_1636_OTU124 psygmophilum_B2_1637_OTU157 psygmophilum_OTU330 Scyphozoa_medusae_1Sy24_OTU44 Scyphozoa_medusae_1Sy24_OTU501 Scyphozoa_medusae_1Sy24_OTU84 Scyphozoa_medusae1Sy23_OTU22 uncultured_OTU104 uncultured_OTU12 uncultured_OTU164 uncultured_OTU177 uncultured_OTU181 uncultured_OTU198 uncultured_OTU30 uncultured_OTU33 uncultured_OTU42 uncultured_OTU514 uncultured_OTU55 uncultured_OTU639 uncultured_OTU751 uncultured_OTU83 uncultured_OTU97 Zoanthus_OTU144

				Zoanthus_OTU21 Zoanthus_OTU224 Zoanthus_OTU26 Zoanthus_OTU28 Zoanthus_OTU352 Zoanthus_OTU49 zoox23_OTU155 zoox23_OTU170 zoox23_OTU186 zoox23_OTU19 zoox23_OTU195 zoox23_OTU23 zoox23_OTU272 zoox23_OTU346 zoox23_OTU438 zoox23_OTU443 zoox23_OTU59 zoox23_OTU614 zoox23_OTU731 zoox23_OTU9 kawagutii_OTU14
F6 (**107**)	A_OTU574 A_OTU69 A2_JCUSG-1_OTU66 A3_OTU110 A3_OTU3 A3_OTU408 A3_OTU665 A3_OTU725 A3_OTU799 CCMP2456_OTU457 CCMP2456_OTU558 CCMP2456_OTU73 CCMP828_OTU458 CCMP828_OTU656 CCMP828_OTU664 CCMP828_OTU7 CCMP828_OTU746	C_OTU232 C1_OTU1" C1_OTU113 C1_OTU121 C1_OTU145 C1_OTU287 C1_OTU336 C1_OTU363 C1_OTU381 C1_OTU423 C1_OTU440 C1_OTU448 C1_OTU459 C1_OTU463 C1_OTU467 C1_OTU496 C1_OTU499 C1_OTU624 C1_OTU633 C1_OTU79 C91_OTU484 SC13.7_OTU76	D_OTU16 D_OTU179 D_OTU328 D_OTU372 D_OTU4 D1_OTU2 D1a_OTU142 D1a_OTU6	1631_OTU119 kawagutii_OTU141681_OTU102 G2_OTU265 G4_OTU68 G6_OTU47 G6_OTU8 HI-0609_OTU24 HI-0609_OTU465 minutum_OTU45 Montipora_OTU38 natans_OTU99 nr-i2_OTU235 psygmophilum_B2_1635_OTU13 psygmophilum_B2_1636_OTU124 Scyphozoa_medusae_1Sy24_OTU34 Scyphozoa_medusae_1Sy24_OTU660 Scyphozoa_medusae_1Sy24_OTU84 Scyphozoa_medusae1Sy23_OTU333 Scyphozoa_medusae1Sy23_OTU653 uncultured_OTU103 uncultured_OTU104 uncultured_OTU12 uncultured_OTU130 uncultured_OTU164 uncultured_OTU177

				uncultured_OTU239 uncultured_OTU27 uncultured_OTU278 uncultured_OTU33 uncultured_OTU42 uncultured_OTU430 uncultured_OTU477 uncultured_OTU514 uncultured_OTU55 uncultured_OTU588 uncultured_OTU598 uncultured_OTU608 uncultured_OTU640 voratum_OTU70 Zoanthus_OTU178 Zoanthus_OTU21 Zoanthus_OTU224 Zoanthus_OTU234 Zoanthus_OTU26 Zoanthus_OTU28 Zoanthus_OTU298 Zoanthus_OTU32 Zoanthus_OTU352 Zoanthus_OTU49 zoox23_OTU155 zoox23_OTU19 zoox23_OTU227 zoox23_OTU228 zoox23_OTU23 zoox23_OTU25 zoox23_OTU392 zoox23_OTU59 zoox23_OTU592 zoox23_OTU85
F8 (**86**)	A2_JCUSG-1_OTU66 A3_OTU238 A3_OTU3 A3_OTU309 A3_OTU354 A3_OTU476 A3_OTU736 A4.3_OTU466 CCMP2455_OTU50	C_OTU534 C1_OTU1 C1_OTU113 C1_OTU121 C1_OTU167 C1_OTU273 C1_OTU544 C1_OTU550 C1_OTU740	D_OTU5 D_OTU575 D_OTU655 D1.2_OTU361 D1_OTU2 D1a_OTU6	13467_OTU106 13478_OTU662 1363_OTU353 1679_OTU174 1679_OTU247 1679_OTU490 1679_OTU98 Amphisorus_OTU304 Amphisorus_OTU487 kawagutii_OTU14 F4.5_OTU93

	CCMP2456_OTU163 CCMP828_OTU254 CCMP828_OTU7 minutum_OTU45	C1v1d_OTU355 C3_OTU637 C91_OTU484		G4_OTU68 G6_OTU47 G6_OTU8 HI-0509_OTU109 HI-0509_OTU118 HI-0509_OTU139 HI-0509_OTU334 HI-0609_OTU182 HI-0609_OTU24 muscatinei_OTU133 natans_OTU99 psygmophilum_B2_1635_OTU13 psygmophilum_B2_1636_OTU124 psygmophilum_B2_1637_OTU157 psygmophilum_B2_1638_OTU286 psygmophilum_B2_1639_OTU747 Scyphozoa_medusae_1Sy24_OTU84 uncultured_OTU103 uncultured_OTU104 uncultured_OTU180 uncultured_OTU197 uncultured_OTU260 uncultured_OTU30 uncultured_OTU31 uncultured_OTU33 uncultured_OTU672 uncultured_OTU742 uncultured_OTU83 uncultured_OTU97 voratum_OTU216 voratum_OTU401 voratum_OTU431 voratum_OTU70 Zoanthus_OTU158 Zoanthus_OTU178 Zoanthus_OTU224 Zoanthus_OTU28 Zoanthus_OTU32 Zoanthus_OTU49 zoox23_OTU170 zoox23_OTU186 zoox23_OTU19 zoox23_OTU195 zoox23_OTU731

F9 (**52**)	A2_OTU215 A3_OTU3 A3_OTU674 A3_OTU725 A4.3_OTU114 Amami1f_OTU787 CCMP2455_OTU50 CCMP828_OTU458 CCMP828_OTU7 CCMP828_OTU746 microadriaticum_OTU580 microadriaticum_OTU683 microadriaticum_OTU760 microadriaticum_OTU788	C_OTU140 C_OTU232 C_OTU63 C1_OTU1 C1_OTU121 C1_OTU167 C1_OTU249 C1_OTU661	D_OTU10 D_OTU101 D_OTU179 D_OTU331 D_OTU37 D_OTU4 D1_OTU2 D1a_OTU6 D1a_OTU756	1681_OTU303 Amphisorus_OTU134 B16_Z1_OTU296 G4_OTU68 HI-0609_OTU24 muscatinei_OTU17 Scyphozoa_medusae_1Sy24_OTU84 Scyphozoa_medusae1Sy23_OTU311 Scyphozoa_medusae1Sy23_OTU653 uncultured_OTU104 uncultured_OTU181 uncultured_OTU33 voratum_OTU692 voratum_OTU705 Zoanthus_OTU117 Zoanthus_OTU178 Zoanthus_OTU32 Zoanthus_OTU49 Zoanthus_OTU809 zoox23_OTU195 zoox23_OTU493
F10 (**112**)	A2_JCUSG-1_OTU66 A2_OTU215 A3_OTU128 A3_OTU291 A3_OTU3 A3_OTU309 A3_OTU408 A3_OTU476 A3_OTU491 A3_OTU665 A3_OTU725 A3_OTU799 CCMP2455_OTU50 CCMP2456_OTU163 CCMP2456_OTU558 CCMP2456_OTU73 CCMP2456_OTU757 CCMP828_OTU458 CCMP828_OTU664 CCMP828_OTU7 HI-0509_OTU109 HI-0509_OTU139	C_OTU137 C1/C3_Two0501-8_OTU439 C1_OTU1" C1_OTU113 C1_OTU121 C1_OTU204 C1_OTU241 C1_OTU381 C1_OTU383 C1_OTU412 C1_OTU419 C1_OTU440 C1_OTU567 C1_OTU589 C1_OTU661 C1_OTU762 C1_OTU79 C1_OTU803 C1v1d_OTU355 SC13.7_OTU753 SC13.7_OTU76	D_OTU10 D_OTU16 D_OTU328 D_OTU373 D_OTU4 D_OTU5 D1.2_OTU361 D1_OTU2 D1a_OTU142 D1a_OTU6 D1a_OTU737	1363_OTU353 1679_OTU174 1679_OTU61 1681_OTU102 1681_OTU303 Amphisorus_OTU134 F4.5_OTU93 G4_OTU68 kawagutii_OTU14 minutum_OTU385 minutum_OTU45 psygmophilum_B2_1635_OTU13 psygmophilum_B2_1636_OTU124 psygmophilum_B2_1637_OTU157 psygmophilum_OTU330 Scyphozoa_medusae_1Sy24_OTU44 Scyphozoa_medusae_1Sy24_OTU501 Scyphozoa_medusae_1Sy24_OTU84 Scyphozoa_medusae1Sy23_OTU22 uncultured_OTU104 uncultured_OTU12 uncultured_OTU164

	HI-0509_OTU334 HI-0509_OTU427 HI-0609_OTU24			uncultured_OTU177 uncultured_OTU181 uncultured_OTU198 uncultured_OTU30 uncultured_OTU33 uncultured_OTU42 uncultured_OTU514 uncultured_OTU55 uncultured_OTU639 uncultured_OTU751 uncultured_OTU83 uncultured_OTU97 Zoanthus_OTU144 Zoanthus_OTU21 Zoanthus_OTU224 Zoanthus_OTU26 Zoanthus_OTU28 Zoanthus_OTU352 Zoanthus_OTU49 zoox23_OTU155 zoox23_OTU170 zoox23_OTU186 zoox23_OTU19 zoox23_OTU195 zoox23_OTU23 zoox23_OTU272 zoox23_OTU346 zoox23_OTU438 zoox23_OTU443 zoox23_OTU59 zoox23_OTU614 zoox23_OTU731 zoox23_OTU9
F12 (**60**)	A2_JCUSG-1_OTU66 A3_OTU183 A3_OTU3 A3_OTU408 A3_OTU476 A3_OTU665 A3_OTU725 A3_OTU736 A3_OTU799 A4.3_OTU114	C_OTU140 C_OTU232 C_OTU63 C1/C3_Two0501-8_OTU439 C1_OTU1" C1_OTU113 C1_OTU121 C1_OTU237 C1_OTU413 C1_OTU432	D_OTU10 D_OTU16 D_OTU374 D_OTU4 D_OTU426 D_OTU456 D_OTU5 D_OTU678 D1_OTU2 D1a_OTU6	1679_OTU61 HI-0609_OTU24 Scyphozoa_medusae_1Sy24_OTU39 Scyphozoa_medusae_1Sy24_OTU390 uncultured_OTU103 uncultured_OTU104 uncultured_OTU177 uncultured_OTU206 uncultured_OTU33 uncultured_OTU430

	CCMP2456_OTU558 CCMP2456_OTU73 CCMP828_OTU458 CCMP828_OTU7 CCMP828_OTU746	C1_OTU567 C1_OTU661 C1_OTU762 C1_OTU803 C3_OTU637	D1a_OTU756	uncultured_OTU514 uncultured_OTU672 Zoanthus_OTU26 Zoanthus_OTU28 Zoanthus_OTU352 zoox21_OTU35 zoox23_OTU186 zoox23_OTU23 zoox23_OTU9
F13 (**57**)	A13_OTU57 A3_OTU3 A3_OTU408 A3_OTU665 A3_OTU725 A3_OTU779 A3_OTU799 CCMP2456_OTU558 CCMP2456_OTU73 CCMP828_OTU458 CCMP828_OTU7	04-218-SCI.01_OTU368 C_OTU140 C_OTU232 C_OTU63 C1_OTU1" C1_OTU121 C1_OTU237 C1_OTU567 C1_OTU633 C1_OTU661 C1_OTU688 C1_OTU762 C1_OTU767 C1_OTU79 C1_OTU803	D_OTU10 D_OTU16 D_OTU5 D1_OTU2 D1a_OTU6 D1a_OTU727 D1a_OTU756 D1a_OTU811	12979_OTU625 13478_OTU184 1679_OTU174 Amphisorus_OTU67 G6_OTU8 minutum_OTU45 Palythoa_OTU568 Protopalythoa_OTU395 Scyphozoa_medusae_1Sy24_OTU120 uncultured_OTU104 uncultured_OTU108 uncultured_OTU129 uncultured_OTU514 uncultured_OTU812 Zoanthus_OTU208 Zoanthus_OTU28 Zoanthus_OTU316 Zoanthus_OTU49 zoox23_OTU19 zoox23_OTU322 zoox23_OTU391 zoox23_OTU552 zoox23_OTU59
F14 (**138**)	A13_OTU11 A2_JCUSG-1_OTU66 A3_OTU111 A3_OTU128 A3_OTU159 A3_OTU191 A3_OTU238 A3_OTU291 A3_OTU3 A3_OTU309 A3_OTU339 A3_OTU476	C_OTU140 C_OTU232 C_OTU259 C_OTU63 C1_OTU1 C1_OTU121 C1_OTU249 C1_OTU315 C1_OTU381 C1_OTU451 C1_OTU528 C1_OTU610	D_OTU10 D_OTU126 D_OTU16 D_OTU313 D_OTU4 D_OTU405 D_OTU5 D_OTU58 D_OTU678 D_OTU722 D_OTU781 D1.2_OTU207	13467_OTU106 13478_OTU621 1363_OTU687 1363_OTU81 1679_OTU223 1679_OTU271 1679_OTU61 1679_OTU804 1679_OTU98 Amphisorus_OTU203 Amphisorus_OTU418 B16_OTU175

	A3_OTU482 A3_OTU71 A3_OTU725 A3_OTU77 A3_OTU782 A4.3_OTU114 A4.3_OTU466 CCMP828_OTU7 microadriaticum_OTU772	C1_OTU613 C1_OTU617 C1_OTU762 C1_OTU80 C1v1e_OTU716 C3_OTU529 C3_OTU690	D1.2_OTU277 D1.2_OTU312 D1_OTU2 D1a_OTU6	B16_OTU329 B16_Z1_OTU296 F3.2_OTU96 G2_OTU244 G2_OTU424 G2b_OTU169 G2b_OTU192 G2b_OTU94 G3.4_OTU48 G4_OTU68 G6_OTU8 HI-0509_OTU118 HI-0509_OTU138 HI-0509_OTU139 HI-0509_OTU152 HI-0509_OTU773 HI-0609_OTU210 HI-0609_OTU225 HI-0609_OTU24 mizugama_OTU91 muscatinei_OTU133 muscatinei_OTU17 natans_OTU99 Odo06_A7_OTU384 Oku03_sand_OTU205 psygmophilum_B2_1635_OTU13 Scyphozoa_medusae_1Sy24_OTU34 Scyphozoa_medusae_1Sy24_OTU41 uncultured_OTU104 uncultured_OTU115 uncultured_OTU180 uncultured_OTU190 uncultured_OTU20 uncultured_OTU246 uncultured_OTU260 uncultured_OTU290 uncultured_OTU31 uncultured_OTU33 uncultured_OTU375 uncultured_OTU40 uncultured_OTU416 uncultured_OTU43 uncultured_OTU468 uncultured_OTU502

				uncultured_OTU510 uncultured_OTU60 uncultured_OTU681 uncultured_OTU715 uncultured_OTU791 uncultured_OTU813 uncultured_OTU83 uncultured_OTU97 voratum_OTU212 voratum_OTU263 voratum_OTU264 voratum_OTU616 Zoanthus_OTU117 Zoanthus_OTU188 Zoanthus_OTU26 Zoanthus_OTU28 Zoanthus_OTU295 Zoanthus_OTU320 Zoanthus_OTU352 Zoanthus_OTU49 zoox23_OTU156 zoox23_OTU186 zoox23_OTU276 zoox23_OTU54 zoox23_OTU731 zoox23_OTU9
F15 (**131**)	A1_Mf_OTU321 A13_OTU11 A13_OTU57 A13_OTU65 A2_JCUSG-1_OTU66 A3_OTU159 A3_OTU176 A3_OTU3 A3_OTU408 A3_OTU476 A3_OTU482 A3_OTU725 A3_OTU77 A3_OTU818 CCMP2455_OTU50 CCMP2456_OTU558 CCMP2456_OTU584	C_OTU137 C_OTU140 C_OTU232 C_OTU63 C1_OTU1" C1_OTU121 C1_OTU237 C1_OTU249 C1_OTU381 C1_OTU429 C1_OTU444 C1_OTU469 C1_OTU567 C1_OTU634 C1_OTU661 C1_OTU762 C1_OTU766	D_OTU16 D_OTU4 D_OTU5 D_OTU58 D_OTU623 D_OTU629 D_OTU678 D_OTU714 D1.2_OTU207 D1_OTU2 D1_OTU89 D1a_OTU6 D1a_OTU727	13467_OTU106 13478_OTU283 13478_OTU398 1363_OTU100 1363_OTU81 1679_OTU174 1679_OTU332 1679_OTU433 1679_OTU61 Amphisorus_OTU134 Amphisorus_OTU203 Amphisorus_OTU51 Amphisorus_OTU67 B16_OTU175 F4.5_OTU603 F4.5_OTU93 G2_OTU424

	CCMP2456_OTU73 CCMP828_OTU7	C3_OTU529 SC13.7_OTU76		G2b_OTU105 G2b_OTU169 G2b_OTU94 G3.4_OTU48 G4_OTU221 G4_OTU68 G6_OTU47 G6_OTU8 H1_OTU542 HI-0509_OTU109 HI-0509_OTU118 HI-0509_OTU138 HI-0509_OTU139 HI-0609_OTU148 HI-0609_OTU182 HI-0609_OTU225 HI-0609_OTU24 mizugama_OTU91 muscatinei_OTU17 psygmophilum_OTU453 Scyphozoa_medusae_1Sy24_OTU120 Scyphozoa_medusae_1Sy24_OTU34 Scyphozoa_medusae_1Sy24_OTU41 Scyphozoa_medusae_1Sy24_OTU74 Scyphozoa_medusae_1Sy24_OTU807 Scyphozoa_medusae_1Sy24_OTU84 Scyphozoa_medusae1Sy23_OTU311 uncultured_OTU104 uncultured_OTU12 uncultured_OTU123 uncultured_OTU164 uncultured_OTU181 uncultured_OTU255 uncultured_OTU274 uncultured_OTU33 uncultured_OTU42 uncultured_OTU585 uncultured_OTU60 uncultured_OTU627 uncultured_OTU645 uncultured_OTU681 uncultured_OTU774 uncultured_OTU83

				uncultured_OTU90 uncultured_OTU97 voratum_OTU70 zanpa_OTU612 Zoanthus_OTU117 Zoanthus_OTU28 Zoanthus_OTU352 Zoanthus_OTU49 zoox23_OTU170 zoox23_OTU186 zoox23_OTU23 zoox23_OTU231 zoox23_OTU342 zoox23_OTU380 zoox23_OTU387 zoox23_OTU400 zoox23_OTU437 zoox23_OTU54 zoox23_OTU56 zoox23_OTU59
F17 (**50**)	A13_OTU11 A2_JCUSG-1_OTU66 A3_OTU3 A3_OTU665 A3_OTU725 A3_OTU799 Asand_Oku17_OTU631 CCMP2456_OTU73 CCMP828_OTU7	C_OTU162 C_OTU232 C_OTU63 C1_OTU1" C1_OTU121 C1_OTU145 C1_OTU669	D_OTU4 D_OTU5 D1_OTU2 D1a_OTU6	13478_OTU184 13478_OTU310 13478_OTU512 Amphisorus_OTU51 Amphisorus_OTU67 G6_OTU8 HI-0509_OTU139 Scyphozoa_medusae_1Sy24_OTU34 Scyphozoa_medusae_1Sy24_OTU41 Scyphozoa_medusae_1Sy24_OTU84 Scyphozoa_medusae1Sy23_OTU311 uncultured_OTU104 uncultured_OTU108 uncultured_OTU185 uncultured_OTU198 uncultured_OTU33 uncultured_OTU399 uncultured_OTU42 Zoanthus_OTU26 zoox23_OTU150 zoox23_OTU156 zoox23_OTU19 zoox23_OTU23

				zoox23_OTU248 zoox23_OTU262 zoox23_OTU380 zoox23_OTU403 zoox23_OTU551 zoox23_OTU59 zoox23_OTU750
F18 (**140**)	A13_OTU11 A13_OTU471 A2_JCUSG-1_OTU66 A3_OTU183 A3_OTU200 A3_OTU291 A3_OTU3 A3_OTU339 A4.3_OTU114 A4_OTU165 CCMP2455_OTU50 CCMP2456_OTU558 CCMP2456_OTU73 CCMP828_OTU458 CCMP828_OTU7	C_OTU137 C_OTU700 C1_OTU1 C1_OTU113 C1_OTU121 C1_OTU194 C1_OTU325 C1_OTU661 C1_OTU680 C1_OTU703 C1_OTU789 C1_OTU79 C1_OTU803 C1_OTU86 C3_OTU480	D_OTU10 D_OTU16 D_OTU349 D_OTU4 D_OTU5 D_OTU531 D_OTU58 D_OTU628 D_OTU678 D_OTU763 D_OTU764 D_OTU805 D_OTU95 D1_OTU2 D1_OTU593 D1a_OTU6	13478_OTU132 13478_OTU548 1363_OTU81 1631_OTU119 1679_OTU98 1681_OTU102 B16_OTU268 F3.2_OTU96 G2b_OTU171 G2b_OTU650 G2b_OTU94 G3.4_OTU154 G4_OTU68 G6_OTU47 G6_OTU8 HI-0609_OTU24 mizugama_OTU91 muscatinei_OTU133 muscatinei_OTU699 natans_OTU122 Scyphozoa_medusae_1Sy24_OTU220 Scyphozoa_medusae_1Sy24_OTU294 Scyphozoa_medusae_1Sy24_OTU39 Scyphozoa_medusae1Sy23_OTU125 Scyphozoa_medusae1Sy23_OTU333 uncultured_OTU103 uncultured_OTU104 uncultured_OTU12 uncultured_OTU129 uncultured_OTU146 uncultured_OTU197 uncultured_OTU199 uncultured_OTU201 uncultured_OTU213 uncultured_OTU31 uncultured_OTU33

				uncultured_OTU382 uncultured_OTU40 uncultured_OTU42 uncultured_OTU43 uncultured_OTU468 uncultured_OTU494 uncultured_OTU55 uncultured_OTU573 uncultured_OTU60 uncultured_OTU605 uncultured_OTU813 uncultured_OTU825 uncultured_OTU97 Zoanthus_OTU117 Zoanthus_OTU144 Zoanthus_OTU188 Zoanthus_OTU211 Zoanthus_OTU26 Zoanthus_OTU28 Zoanthus_OTU316 Zoanthus_OTU32 Zoanthus_OTU365 Zoanthus_OTU88 zoox21_OTU35 zoox23_OTU136 zoox23_OTU15 zoox23_OTU151 zoox23_OTU156 zoox23_OTU186 zoox23_OTU19 zoox23_OTU196 zoox23_OTU218 zoox23_OTU227 zoox23_OTU23 zoox23_OTU256 zoox23_OTU272 zoox23_OTU284 zoox23_OTU293 zoox23_OTU300 zoox23_OTU377 zoox23_OTU443 zoox23_OTU46 zoox23_OTU52

				zoox23_OTU537 zoox23_OTU54 zoox23_OTU554 zoox23_OTU56 zoox23_OTU569 zoox23_OTU59 zoox23_OTU592 zoox23_OTU72 zoox23_OTU731 zoox23_OTU75 zoox23_OTU750 zoox23_OTU78 zoox23_OTU82 zoox23_OTU9 zoox23_OTU92
F19 (**35**)	A3_OTU222 A3_OTU3 CCMP2455_OTU50 CCMP2456_OTU558 CCMP2456_OTU73 CCMP828_OTU458 CCMP828_OTU7 CCMP828_OTU746	C_OTU140 C_OTU232 C1_OTU1 C1_OTU113 C1_OTU121 C1_OTU571	D_OTU16 D_OTU4 D_OTU426 D_OTU5 D1_OTU2 D1a_OTU6 D1a_OTU821	13478_OTU452 HI-0609_OTU601 minutum_OTU45 psygmophilum_OTU606 uncultured_OTU104 uncultured_OTU129 uncultured_OTU33 uncultured_OTU539 uncultured_OTU90 Zoanthus_OTU28 zoox23_OTU23 zoox23_OTU75 zoox23_OTU9 zoox23_OTU92
F22 (**35**)	A3_OTU252 A3_OTU3 A3_OTU725 CCMP2456_OTU558 CCMP828_OTU7	C_OTU140 C_OTU153 C_OTU232 C_OTU768 C1_OTU1" C1_OTU121 C1_OTU661 C1_OTU762 C1_OTU797 C1_OTU803 SC13.7_OTU684 SC13.7_OTU76	D_OTU331 D_OTU4 D_OTU5 D1_OTU2 D1a_OTU6 D1a_OTU727 D1a_OTU756	1679_OTU98 uncultured_OTU104 uncultured_OTU43 uncultured_OTU514 uncultured_OTU607 uncultured_OTU608 Zoanthus_OTU28 Zoanthus_OTU32 Zoanthus_OTU88 zoox21_OTU35 zoox23_OTU56
F23 (**13**)	A3_OTU3 CCMP828_OTU7	C1_OTU1" C1_OTU649	D1_OTU2 D1a_OTU6	Scyphozoa_medusae_1Sy24_OTU39 Zoanthus_OTU26

				Zoanthus_OTU28 Zoanthus_OTU565 zoox21_OTU35 zoox23_OTU56 zoox23_OTU59
F24 (**34**)	A3_OTU159 A3_OTU3 A3_OTU408 A3_OTU725 A3_OTU799 CCMP2455_OTU50 CCMP828_OTU458 CCMP828_OTU664 CCMP828_OTU7 CCMP828_OTU746	C_OTU232 C1_OTU1" C1_OTU121 C1_OTU815 C1_OTU86	D_OTU16 D_OTU405 D_OTU652 D1_OTU2 D1a_OTU460 D1a_OTU6	F3.2_OTU96 G4_OTU68 Scyphozoa_medusae_1Sy24_OTU39 uncultured_OTU43 uncultured_OTU55 Zoanthus_OTU21 Zoanthus_OTU28 Zoanthus_OTU352 Zoanthus_OTU88 zoox21_OTU35 zoox23_OTU136 zoox23_OTU186 zoox23_OTU392
F27 (**33**)	A3_OTU3 A3_OTU408 A3_OTU725 CCMP2456_OTU73 CCMP828_OTU458 CCMP828_OTU7 CCMP828_OTU746	C_OTU137 C_OTU140 C_OTU232 C_OTU63 C1_OTU1" C1_OTU121 C1_OTU567 C3_OTU523	D_OTU4 D1_OTU2 D1a_OTU6	1341_OTU394 HI-0609_OTU24 minutum_OTU45 uncultured_OTU104 uncultured_OTU130 uncultured_OTU177 uncultured_OTU278 uncultured_OTU33 uncultured_OTU42 uncultured_OTU43 uncultured_OTU55 Zoanthus_OTU21 Zoanthus_OTU28 zoox23_OTU72 zoox23_OTU9
F28 (**30**)	A_OTU69, A13_OTU11, A3_OTU3, A3_OTU725, CCMP828_OTU7	C_OTU232, C1_OTU, C1_OTU121, C1_OTU282, C1_OTU345, C1_OTU428, C1_OTU544, C1_OTU86,	D_OTU16, D_OTU4, D1_OTU2, D1a_OTU6	12979_OTU625, 13478_OTU132, Amphisorus_OTU418, uncultured_OTU104, uncultured_OTU177, uncultured_OTU33, uncultured_OTU468, Zoanthus_OTU144, Zoanthus_OTU21, Zoanthus_OTU483, zoox23_OTU186,

				zoox23_OTU19, zoox23_OTU52
W10 (**10**)	A3_OTU3, CCMP828_OTU7	C1_OTU1, C_OTU232, C1_ OTU121, C_OTU63	D1_OTU2, D1a_OTU6	Zoanthus_OTU32, uncultured_OTU104
W11 (**9**)	A3_OTU3, CCMP828_OTU7	C1_OTU1, C1_OTU121, C_OTU140	D1_OTU2, D_OTU4, D1a_OTU6	uncultured_OTU104
W5 (**12**)	A3_OTU3, A13_OTU11	C1_OTU1, C1_OTU121, C_OTU232, C1_OTU762, C1_OTU803	D1_OTU2, D1a_OTU6	zoox23_OTU9, uncultured_OTU42, uncultured_OTU104
W7 (**17**)	A3_OTU3, CCMP828_OTU7, A13_OTU11	C1_OTU1, C_OTU63, C1_OTU121, C_OTU140, C_OTU232, C1_OTU567, C1_OTU661, C1v1e_OTU716, C1_OTU797	D1_OTU2 D1a_OTU6	Scyphozoa_medusae_1Sy24_OTU39, uncultured_OTU104, Amphisorus_OTU418
O3 (**20**)	A3_OTU3, CCMP828_OTU7, A_OTU69	C1_OTU1, C_OTU63, C1_OTU121, C_OTU140, C_OTU232, C1_OTU567, C1v1e_OTU716, C1_OTU762, C1_OTU803	D1_OTU2, D_OTU4, D1a_OTU6	zoox23_OTU9, uncultured_OTU55, uncultured_OTU104, Zoanthus_OTU144, Amphisorus_OTU418
O4 (**15**)	A3_OTU3, CCMP828_OTU7, A_OTU69, A_OTU604	C1_OTU1, C_OTU63, C1_OTU121, C_OTU140, C_OTU232, C_OTU800	D1_OTU2, D_OTU5, D1a_OTU6	uncultured_OTU104, Amphisorus_OTU418
O5 (**19**)	A3_OTU3, CCMP828_OTU7, A_OTU69, A3_OTU476	C1_OTU1, C_OTU63, C1_OTU121, C_OTU140, C_OTU232, C1_OTU567, C1_OTU661, C1v1e_OTU716, C_OTU800	D1_OTU2 D_OTU4, D1a_OTU6	uncultured_OTU104, Zoanthus_OTU295, Amphisorus_OTU418
O6 (**20**)	A3_OTU3, A13_OTU11, A_OTU69, A3_OTU222, A_OTU604	C1_OTU1, CCMP828_OTU7, C_OTU63, C1_OTU121, C_OTU140, C_OTU232, C1_OTU567, C_OTU800, C1_OTU803	D1_OTU2 D_OTU4, D1a_OTU6	zoox23_OTU19, uncultured_OTU104, Amphisorus_OTU418

Table S3.6 OTUs found in three or more samples are in bold, less than three are not bolded. Egg values are averages including ± SE.

Dam	Life-stage, N° OTUs	A OTUs (% ± SE)	C	D	B/*Ex situ*/G/H/Uncultured
7	Adult, 7	**A3.1_OTU5 (0.04),** A_2_OTU69 (0.039), **A_1_OTU4 (1.32)**	**C15_OTU1 (97.98)**, C1_8_OTU18 (0.53), **C15_2_OTU7 (0.039)**	**D1_OTU3 (0.039)**	
7	Eggs (n =11), 12	**A3.1_OTU5 (0.08±0.05)**	**C15_9_OTU112 (0.003±0.002), C15_OTU1 (99.1±0.1), C1_4_OTU8 (0.04±0.02), C15_3_OTU20 (0.001±0.0008), C15_6_OTU50 (0.002±0.001), C15_7_OTU102 (0.03±0.005), C15_5_OTU43* (0.004±0.003)**	**D1_OTU3 (0.2±0.05), D1a_OTU6 (0.02±0.02)**	Zoanthussociatus_1_OTU19 (0.02±0.02), **Amphisorus_OTU2 (0.5±0.05)**
8	Adult, 5	**A3.1_OTU5 (0.02), A_1_OTU4 (1.31)**	**C15_OTU1 (98.66)**, **C15_3_OTU20 (0.008)**	**D1_OTU3 (0.008)**	
8	Eggs (n = 12), 14	**A3.1_OTU5 (0.17±0.06), A2_JCUSG-1_OTU32 (0.0006±0.0006)**	**C15_OTU1 (99.1±0.1)**, **C15_9_OTU112 (0.0007±0.0007),** C15_10_OTU117 (0.002±0.002), **C1_4_OTU8 (0.02±0.01), C15_3_OTU20 (0.003±0.002), C15_6_OTU50 (0.001±0.001),** C15_8_OTU103 (0.0004±0.0004), **C15_2_OTU7 (0.04±0.001), C15_7_OTU102 (0.0007±0.0007)**	**D1_OTU3 (0.3±0.1), D1a_OTU6 (0.04±0.03)**	**Amphisorus_OTU2 (0.5±0.04)**
9	Adult, 9	**A3.1_OTU5 (0.009), A_1_OTU4 (0.32)**	**C15_OTU1 (98.8)**, **C1_4_OTU8 (0.097), C15_3_OTU20 (0.008)**	**D1_OTU3 (0.097), D1a_OTU6 (0.026)**	**Amphisorus_OTU2 (0.53),** HA3-5 _OTU119 (0.017)
9	Eggs (n = 12), 14	**A3.1_OTU5 (0.07±0.04), CCMP828_OTU 12 (0.07±0.04)**	**C15_OTU1 (98.4±0.1)**, **C15_9_OTU112 (0.002±0.0009),** C15_4_OTU33 (0.007±0.007), **C1_4_OTU8 (0.034±0.01), C15_3_OTU20 (0.003±0.001), C15_6_OTU50 (0.002±0.001),** C33_OTU82 (0.002±0.002), C15_8_OTU103 (0.0003±0.0003), **C15_2_OTU7 (0.03±0.01)**	**D1_OTU3 (0.3±0.1), D1a_OTU6 (0.1±0.05)**	**Amphisorus_OTU2 (0.6±0.06)**

32	Adult, 8	**A_1_OTU4 (4.64),** A_2_OTU69 (0.045),	C1_7_OTU13 (0.009), **C15_OTU1 (95.2)**, **C15_3_OTU20 (0.009), C1_5_OTU10 (0.06), C15_2_OTU7 (0.027)**	**D1_OTU3 (0.009)**	
32	Eggs (n =5), 12		**C15_OTU1 (99.3±0.4)**, **C1_5_OTU10 (0.1±0.1),** C1_9_OTU54 (0.01±0.01), **C15_9_OTU112 (0.002±0.002),** C1_7_OTU13 (0.2±0.2), **C15_7_OTU102 (0.002±0.002), C15_2_OTU7 (0.04±0.02) C1_4_OTU8 (0.01±0.01), C15_3_OTU20 (0.002±0.002),**	D_OTU27 (0.04±0.04), **D1_OTU3 (0.2±0.1)**	Montiporafoliosa_OTU17 (0.03±0.03)
28	Adult, 2	**A_1_OTU4 (0.02)**	**C15_OTU1 (99.97)**		
28	Eggs (n = 12), 14	**A3.1_OTU5 (0.05±0.03),** Microadriaticum_ 1__OTU29 (0.01±0.01), **CCMP828_OTU 12 (0.03±0.02)**	**C15_OTU1 (99.7±0.05)**, **C15_9_OTU112 (0.003±0.002), C1_4_OTU8 (0.007±0.006), C15_3_OTU20 (0.007±0.004), C15_6_OTU50 (0.0009±0.0009), C1_5_OTU10 (0.03±0.01), C15_7_OTU102 (0.001±0.001), C15_2_OTU7 (0.03±0.005)**	**D1_OTU3 (0.08±0.03), D1a_OTU6 (0.004±0.004)**	**Amphisorus_OTU2 (0.0006±0.00006),** Montiporafoliosa_OTU17 (0.007±0.07)
26	Adult, 7	**A_1_OTU4 (0.009),** A3.2_OTU68 (0.08), A2_OTU32 (0.25)	**C15_OTU1 (99.57), C15_6_OTU50 (0.009), C1_5_OTU10 (0.045)**		G3.3_OTU67 (0.036)
26	Eggs (n = 12), 12	**A3.1_OTU5 (0.04±0.02), CCMP828_OTU 12 (0.1±0.05)**	**C15_OTU1 (99.4±0.1)**, **C15_9_OTU112 (0.0009±0.0006), C15_3_OTU20 (0.008±0.003), C_1_OTU47 (0.001±0.001), C15_6_OTU50 (0.004±0.004), C1_5_OTU10 (0.1±0.05), C15_2_OTU7 (0.02±0.01)**	**D1_OTU3 (0.24±0.07), D1a_OTU6 (0.03±0.02)**	Uncultured_OTU59 (0.02±0.02)
24	Adult, 2		**C15_OTU1 (99.53)**		**Amphisorus_OTU2 (0.47)**
24	Eggs (n = 12), 13	**A3.1_OTU5 (0.07±0.06), CCMP828_OTU 12 (0.02±0.02), A_1_OTU4 (0.0004±0.0004)**	**C15_OTU1 (99.2±0.1)**, **C15_9_OTU112 (0.002±0.002), C1_4_OTU8 (0.02±0.007), C15_3_OTU20 (0.003±0.001), C15_6_OTU50 (0.004±0.002), C15_2_OTU7 (0.05±0.02), C15_5_OTU43* (0.004±0.002)**	**D1_OTU3 (0.2±0.06), D1a_OTU6 (0.01±0.01)**	**Amphisorus_OTU2 (0.4±0.04)**

11	Adult, 5	**CCMP828_OTU 12 (0.035)**	**C15_OTU1 (99.87), C1_4_OTU8 (0.046), C15_2_OTU7 (0.01)**	**D1_OTU3 (0.034)**	
11	Eggs (n = 11), 12	**A3.1_OTU5 (0.18±0.1), CCMP828_OTU 12 (0.05±0.03)**	**C15_OTU1 (99.6±0.09)**, **C15_9_OTU112 (0.002±0.001), C1_4_OTU8 (0.05±0.02), C15_3_OTU20 (0.004±0.002), C15_6_OTU50 (0.003±0.001),** C1_16_OTU104 (0.005±0.005), **C15_2_OTU7 (0.02±0.007)**	**D1_OTU3 (0.06±0.02), D1a_OTU6 (0.015±0.01)**	B1_OTU31 (.007±0.007)
29	Adult, 3	**A_1_OTU4 (0.73)**	**C15_OTU1 (99.3)**		**Amphisorus_OTU2 (0.008)**
29	Eggs (n =12), 77	**A2_meandrinae. 1_OTU56 (0.009±0.006),** A2_meandrinae.2 _OTU90 (0.004±0.004), **A3.1_OTU5 (0.5±0.3),** A2_meandrinae.3 _OTU113 (0.004±0.003), **Asand_Oku17_O TU111 (0.004±0.003),** CCMP2455_OTU 110 (0.003±0.003), **CCMP828_OTU 12 (0.02±0.02)**	**C15_9_OTU112 (0.001±0.0007), C15_1_OTU1 (97.7±0.9), C15_3_OTU20** (0.002±0.001), **C15_6_OTU50 (0.002±0.002), C1_5_OTU10 (0.009±0.006), C15_2_OTU7 (0.03±0.01),**	**D1_OTU3 (0.5±0.4), D1a_OTU6 (0.03±0.03)**	**Amphisorus_OTU2 (0.001±0.001),** Uncultured34_OTU78 (0.004±0.004), **Uncultured5_OTU23 (0.04±0.02), Uncultured16_OTU42 (0.015±0.01),** Uncultured56_OTU130 (0.004±0.003), Uncultured41_OTU98 (0.009±0.008), Uncultured58_OTU133 (0.004±0.003), **Uncultured31_OTU74 (0.01±0.006), Uncultured21_OTU53 (0.005±0.003), Uncultured14_OTU40 (0.016±0.01), Uncultured33_OTU76 (0.01±0.007), Uncultured20_OTU52 (0.02±0.01), Uncultured11_OTU37 (0.02±0.01), Uncultured23_OTU57 (0.02±0.01), Uncultured18_OTU46 (0.02±0.01), Uncultured10_OTU35 (0.02±0.01),**

					Uncultured32_OTU75 (0.01±0.008), Uncultured48_OTU120 (0.004±0.004), Uncultured45_OTU107 (0.003±0.002), **Uncultured4_OTU21 (0.03±0.02), Uncultured9_OTU34 (0.03±0.02), Uncultured6_OTU26 (0.02±0.01),** Uncultured47_OTU116 (0.004±0.003), Uncultured19_OTU49 (0.008±0.006), Uncultured49_OTU121 (0.004±0.004), **Uncultured35_OTU80 (0.006±0.004),** Uncultured44_OTU101 (0.005±0.004), Uncultured60_OTU135 (0.003±0.003), **Uncultured26_OTU65 (0.006±0.004), Uncultured30_OTU73 (0.01±0.008),** Uncultured55_OTU129 (0.006±0.005), **Uncultured28_OTU71 (0.014±0.01), Uncultured61_OTU136 (0.003±0.002), Uncultured27_OTU70 (0.007±0.004), Uncultured29_OTU72 (0.007±0.004),** Uncultured42_OTU99 (0.003±0.002), **Uncultured36_OTU83 (0.005±0.003), Uncultured17_OTU45**

					(0.006±0.004), Uncultured13_OTU39 (0.02±0.01), Uncultured38_OTU93 (0.003±0.002), Uncultured43_OTU100 (0.004±0.003), Uncultured39_OTU94 (0.004±0.003), **Uncultured54_OTU128 (0.008±0.01), Uncultured37_OTU85 (0.005±0.003),** Uncultured50_OTU122 (0.004±0.003), Uncultured40_OTU97 (0.003±0.003), Uncultured57_OTU132 (0.002±0.001), Uncultured59_OTU134 (0.009±0.01), **Uncultured1_OTU9 (0.4±0.2), Uncultured52_OTU126 (0.005±0.003), Uncultured25_OTU61 (0.007±0.004), Uncultured8_OTU30 (0.03±0.02), Uncultured22_OTU55 (0.02±0.01), Uncultured12_OTU38 (0.01±0.006), Uncultured15_OTU41 (0.01±0.008), Uncultured3_OTU15 (0.08±0.04),** Uncultured51_OTU123 (0.003±0.003), **Uncultured2_OTU14 (0.1±0.06),** Uncultured46_OTU109 (0.005±0.01), **Uncultured24_OTU60**

					(0.007±0.003), Uncultured7_OTU28 (0.02±0.01), Uncultured53_OTU127 (0.003±0.003)

Table S3.7 DESeq2 comparisons of different egg families. Padj values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons.

Dam identity	Dam identity	OTU name	Padj
26	28	OTU_10_C1_5	9.5e-03
26	8	OTU_10_C1_5	9.5e-03
26	9	OTU_10_C1_5	1.6e-02
24	26	OTU_10_C1_5	1.e-02
29	7	OTU_14_Uncultured2	3.2e-02
29	8	OTU_14_Uncultured2	3.3e-02
29	9	OTU_14_Uncultured2	3.3e-02
24	29	OTU_14_Uncultured2	3.1e-02
26	29	OTU_14_Uncultured2	0.03
11	29	OTU_14_Uncultured2	0.04
28	29	OTU_14_Uncultured2	0.05
24	28	OTU_2_Amphisorushemprichii	1.8e-13
24	32	OTU_2_Amphisorushemprichii	3.4e-08
32	7	OTU_2_Amphisorushemprichii	2.2e-08
11	24	OTU_2_Amphisorushemprichii	1.4e-12
11	9	OTU_2_Amphisorushemprichii	1.8e-13
28	8	OTU_2_Amphisorushemprichii	3.8e-13
28	9	OTU_2_Amphisorushemprichii	3.8e-13
32	8	OTU_2_Amphisorushemprichii	5.6e-08
11	8	OTU_2_Amphisorushemprichii	3e-12
32	9	OTU_2_Amphisorushemprichii	8e-09
24	26	OTU_2_Amphisorushemprichii	1.3e-12
26	28	OTU_2_Amphisorushemprichii	2.3e-12
26	8	OTU_2_Amphisorushemprichii	2.3e-12
26	9	OTU_2_Amphisorushemprichii	4.1e-13
24	29	OTU_2_Amphisorushemprichii	3.6e-14
29	8	OTU_2_Amphisorushemprichii	8.6e-14
29	9	OTU_2_Amphisorushemprichii	8.6e-14
29	7	OTU_2_Amphisorushemprichii	2.1e-14
24	29	OTU_8_C1_4	3.1e-02
29	8	OTU_8_C1_4	3.3e-02
29	9	OTU_8_C1_4	3.3e-02
29	7	OTU_8_C1_4	1.4e-02
11	29	OTU_8_C1_4	0.01
29	7	OTU_9_Uncultured1	3.4e-02
29	8	OTU_9_Uncultured1	3.4e-02
29	9	OTU_9_Uncultured1	3.4e-02
24	29	OTU_9_Uncultured1	3.4e-02
11	29	OTU_9_Uncultured1	0.04
26	29	OTU_9_Uncultured1	0.05
28	29	OTU_9_Uncultured1	0.05

Supporting figures for Chapter 3

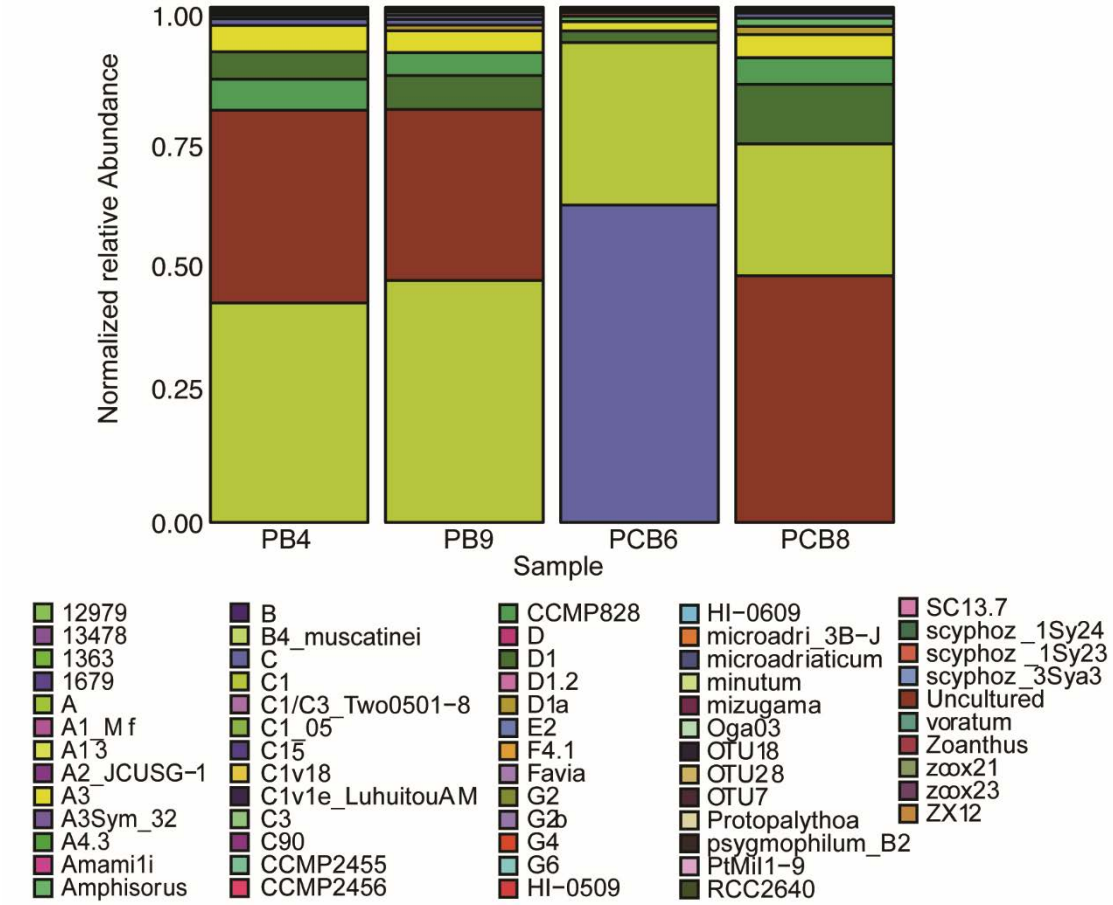

Figure S3.1 Barplot of variance-normalized abundances (scaled to 1) of *Symbiodinium* diversity associated with adult colonies of *Acropora tenuis* used in 2012 crosses. Colours represent different *Symbiodinium* types. Origins of parent colonies are: Orpheus (PB) and Princess Charlotte Bay (PC).

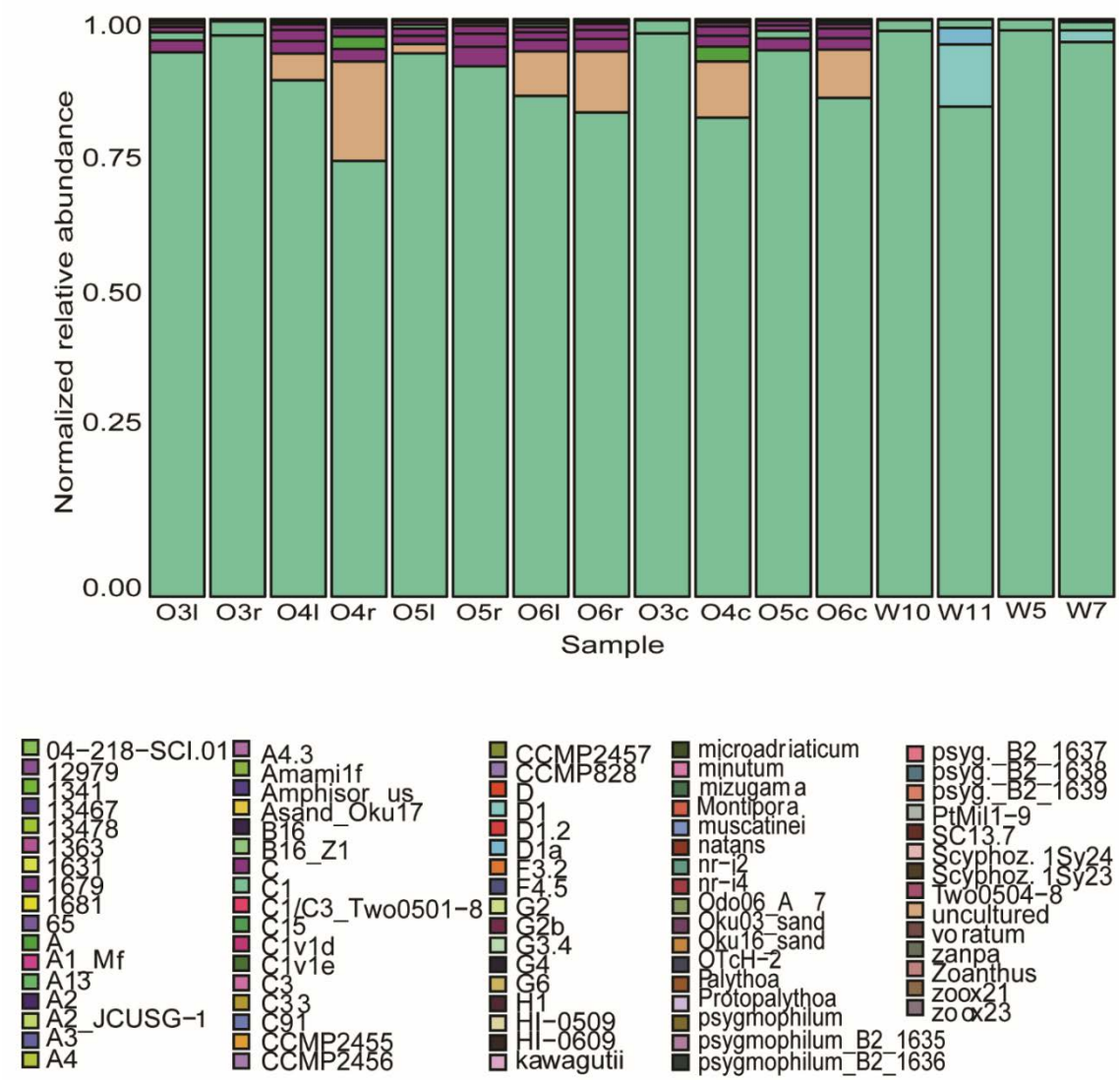

Figure S3.2 Barplot of variance-normalized abundances (scaled to 1) of *Symbiodinium* diversity associated with adult colonies of *Acropora tenuis* used in 2013 crosses. Colours represent different *Symbiodinium* types. Origins of parent colonies are: Orpheus (O) and Princess Charlotte Bay (W).

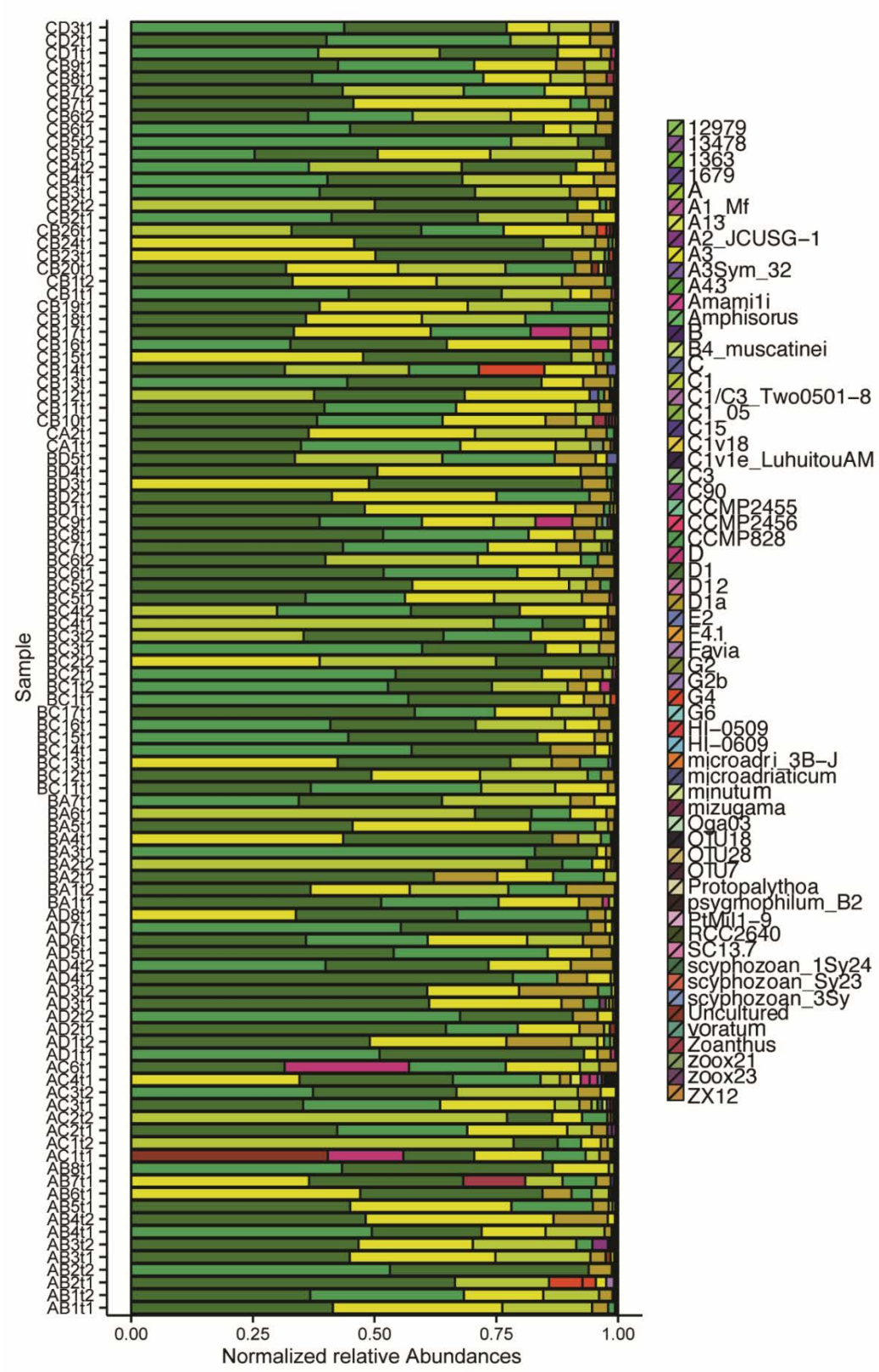

Figure S3.3 Barplot of variance-normalized abundances (scaled to 1) of *Symbiodinium* diversity associated with juveniles of *Acropora tenuis* used in 2012 crosses. Colours represent different *Symbiodinium* types.

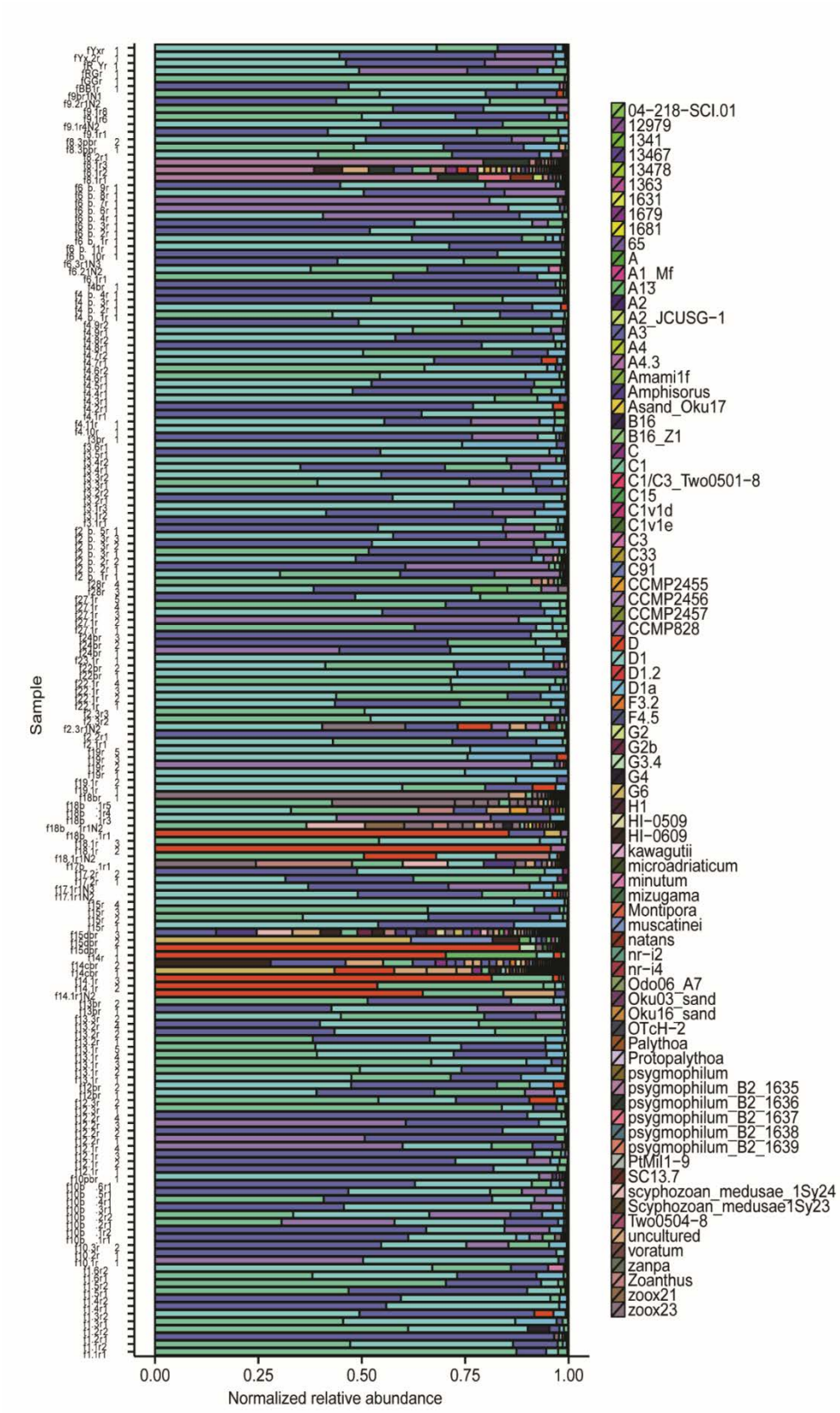

Figure S3.4 Barplot of variance-normalized abundances (scaled to 1) of *Symbiodinium* diversity associated with juveniles of *Acropora tenuis* used in 2013 crosses. Colours represent different *Symbiodinium* types.

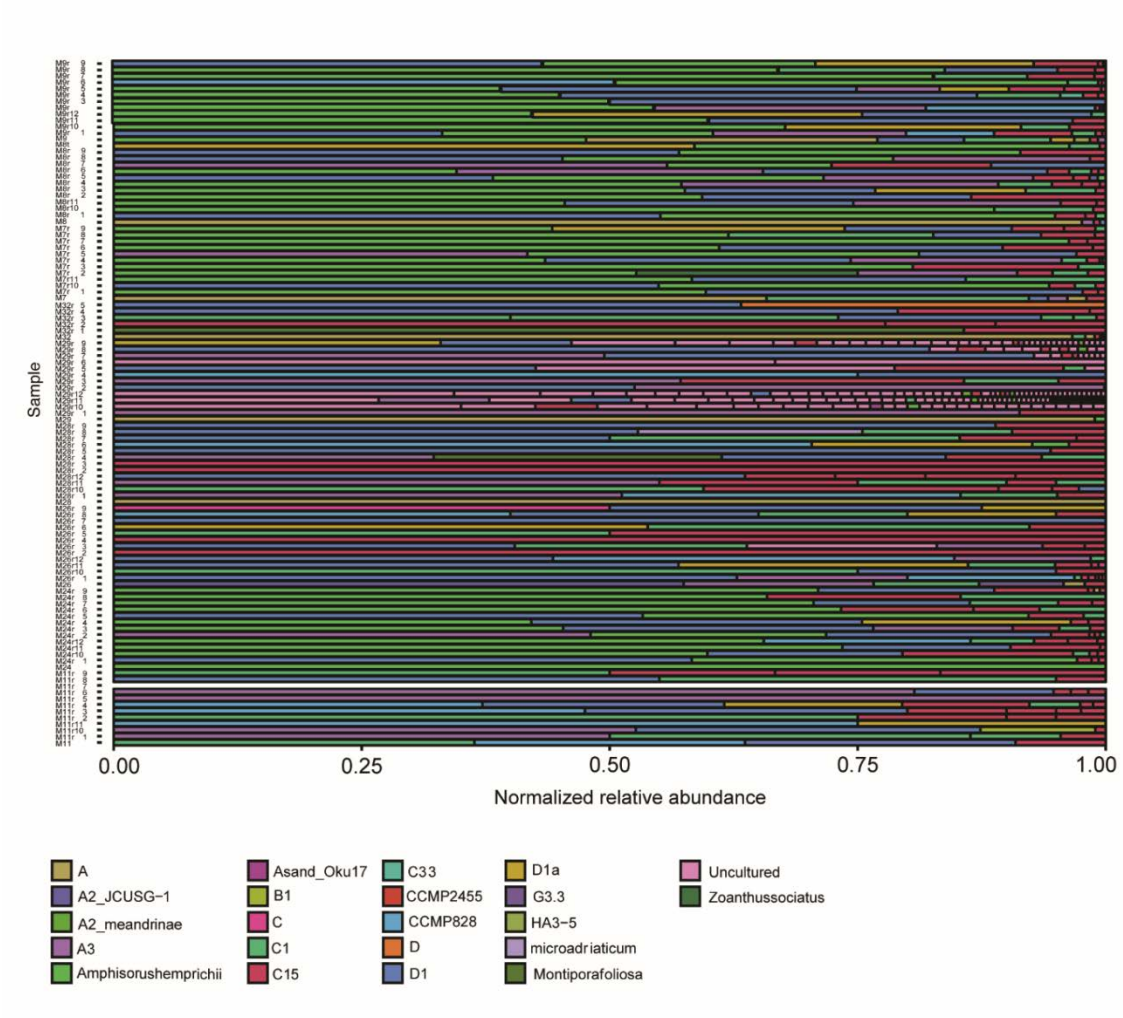

Figure S3.5 Barplot of variance-normalized abundances (scaled to 1) of *Symbiodinium* diversity associated with dams and eggs of *Montipora digitata* used in 2014. Colours represent different *Symbiodinium* types. The dominant type, C15, was excluded for clarity.

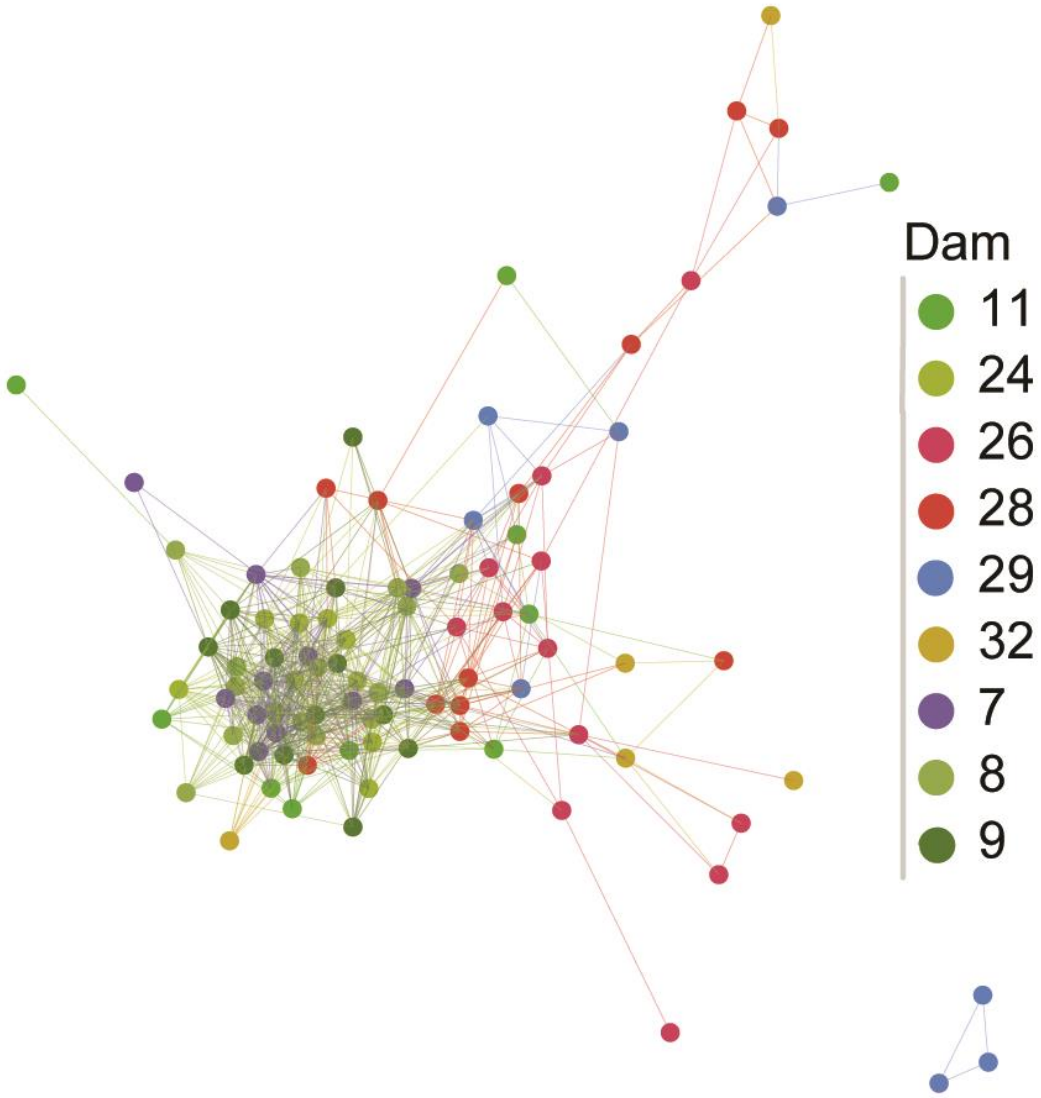

Figure S3.6 Network analysis incorporating un-weighted Unifrac distances of *Symbiodinium* diversity in *Montipora digitata* eggs. Each point represents a single egg sample. Colours correspond to dam designations from Figure 3.2.

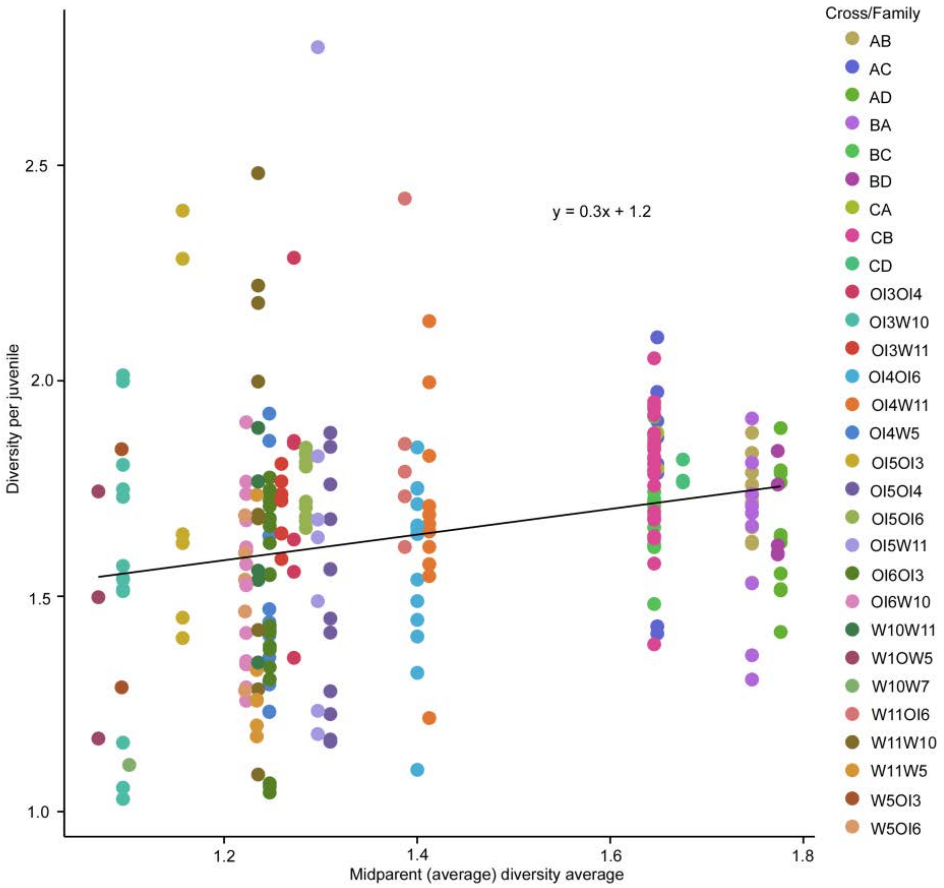

Figure S3.7 Heritability estimation, as calculated from the regression of the average *Symbiodinium* diversity metric for each *A. tenuis* juvenile against the average *Symbiodinium* diversity metric for each juvenile's dam and sire. The slope of the regression line is equivalent to the narrow-sense heritability (h^2).

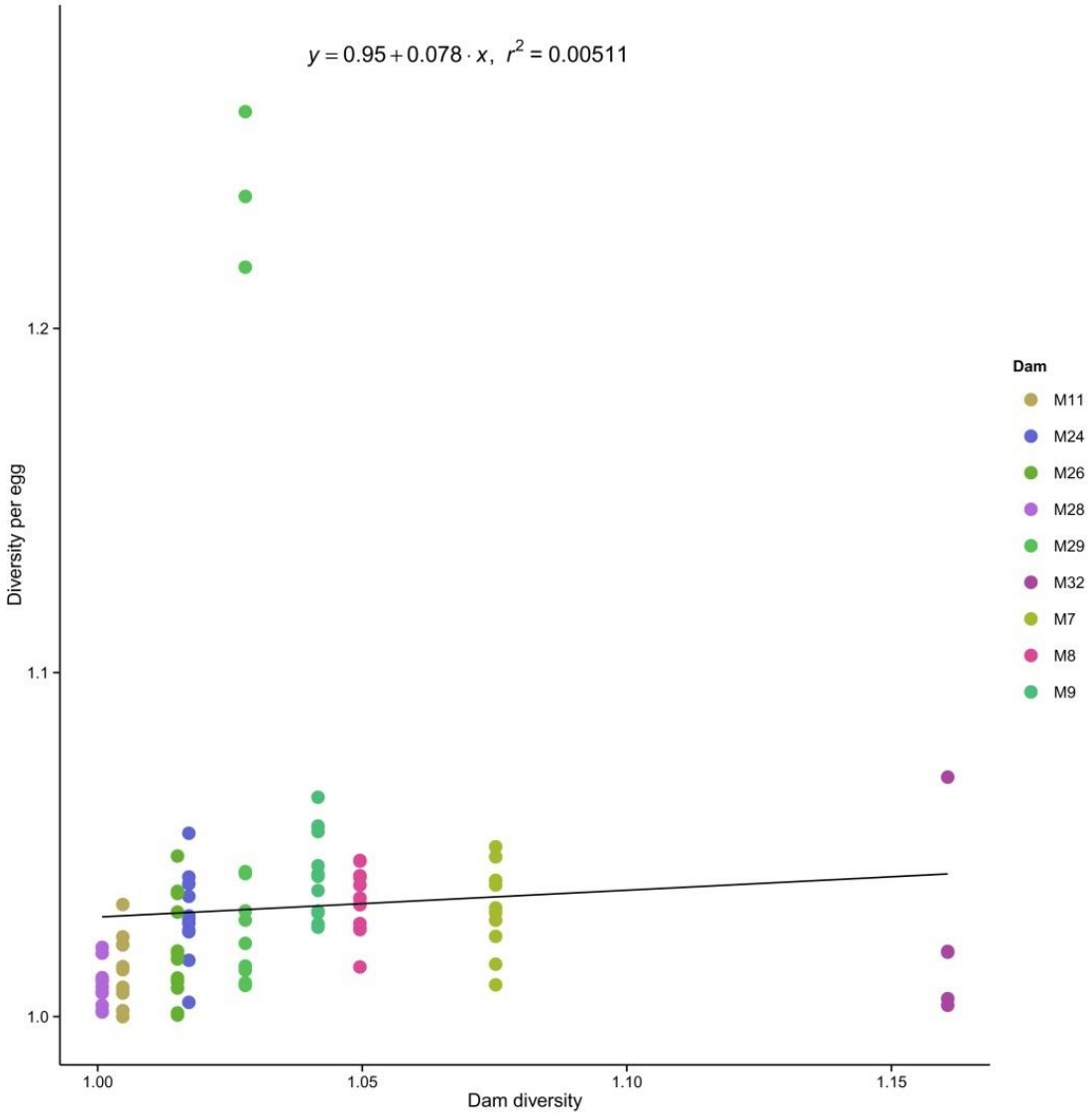

Figure S3.8 Heritability estimation, as calculated from the regression of the average *Symbiodinium* diversity metric for each *M. digitata* egg against the average *Symbiodinium* diversity metric for dams. The slope of the regression line is equivalent to the narrow-sense heritability (h^2) multiplied by 2 as only a single parent is represented on the x-axis.

Appendix C

Supporting tables for Chapter 4

Table S4.1 Summary of the 25 reproductive crosses created.

Dam	Sire							
	O4	**O6**	**O3**	**O5**	**W11**	**W10**	**W7**	**W5**
O4		F1			F2			F4
O6			F5			F6		
O3	F8				F9	F10		
O5	F12	F13	F14		F15			
W11		F17				F18		F19
W10	F26			F21	F22		F23	F24
W7				F25	F29			
W5		F27	F28				F30	

Table S4.2 Log2 fold change and corresponding adjusted p-value for comparisons of juveniles from different families. Families listed horizontally correspond to high surviving families and those listed vertically are low surviving families. Padj values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons.

	F1			**F4**			**F12**		
	Type	**Log2 fold**	**Padj**	**Type**	**Log2 fold**	**Padj**	**Type**	**Log2 fold**	**Padj**
F8	OTU_13 1635	12.79	1.5e-08	OTU_13 1635	12.43	4.3e-07	OTU_13 1635	14.35	3.6e-10
	OTU_3 A3	-3.58	1.3e-03	OTU_6 D1a	-5.17	1.2e-05	OTU_3 A3	-4.77	4.1e-06
	OTU_1 C1	-3.39	5.4e-03	OTU_2 D1	-4.17	4.4e-05	OTU_124 163	8.45	2.3e-03
	OTU_124 1635	7.80	6.6e-03	OTU_3 A3	-4.03	6.4e-04	OTU_1 C1	-3.30	3.8e-03
	OTU_2 D1	-2.14	2.8e-02	OTU_124 1635	8.42	1.3e-02	OTU_2 D1	-2.17	2.1e-02
	OTU_121 type	-4.37	2.8e-02				OTU_121 type	-4.22	2.3e-02
F14	OTU_4 Uncultured	17.50	1.4e-29	OTU_4 Uncultured	16.15	7.5e-27	OTU_4 Uncultured	17.11	6.2e-28
	OTU_6 D1a	-5.24	4.1e-07	OTU_6 D1a	-8.38	6.4e-17	OTU_3 A3	-5.39	1.0e-07
	OTU_3 A3	-4.20	5.5e-05	OTU_2 D1	-4.64	4.5e-07	OTU_6 D1a	-5.02	7.2e-07
	OTU_2 D1	-2.61	8.72E-03	OTU_3 A3	-4.66	5.7e-06	OTU_2 D1	-2.64	4.71E-03

				OTU_1 C1	3.98	4.9e-04			
F17	NA			OTU_2 D1	-4.48	0.04	NA		
	NA			OTU_6 D1a	-4.97	0.04	NA		
	NA			OTU_162 C	5.64	0.04	NA		
F18	OTU_3 A3	-8.40	2.2e-10	OTU_3 A3	-8.86	2.4e-12	OTU_3 A3	-9.59	2.1e-13
	OTU_4 Uncultured	6.95	2.6e-03	OTU_2 D1	-3.49	3.5e-03	OTU_ 4 Uncultured	6.56	7.3e-03
				OTU_4 Uncultured	5.59	3.5e-03			
				OTU_6 D1a	-3.70	4.1e-03			
F28	OTU_6 D1a	-7.16	5.8e-05	OTU_6 D1a	-10.3	1.6e-11	OTU_6 D1a	-6.94	0.0001
				OTU_19 Uncultured	7.07	9.9e-03			

Table S4.3 Sample sizes for analyses: 1) population hybrids (OO, WW vs. OW, WO); 2) population cross (OO vs. WW vs. OW vs. WO); 3) maternal or paternal identity (dam, sire) for each of the treatment comparison (larval survivorship and weight, settlement and juvenile survivorship).

Population cross	Larval survivorship (n=)		Larval weights (n=)		Settlement (n=)		Survivorship (n=)	
OO	6		12		6		6	
OW	6		13		6		6	
WO	6		10		5		3	
WW	7		11		6		5	
	Dams	**Sires**	**Dams**	**Sires**	**Dams**	**Sires**	**Dams**	**Sires**
O3	3	3	7	NA	3	3	3	3
O4	3	3	6	NA	3	3	3	2
O5	4	2	8	NA	4	1	4	0
O6	2	4	4	NA	2	4	2	4
W5	3	3	7	NA	3	3	2	3
W7	2	2	NA	NA	NA	2	NA	1
W10	5	3	8	NA	5	3	3	3
W11	3	5	6	NA	3	4	3	4

Supporting figure for Chapter 4

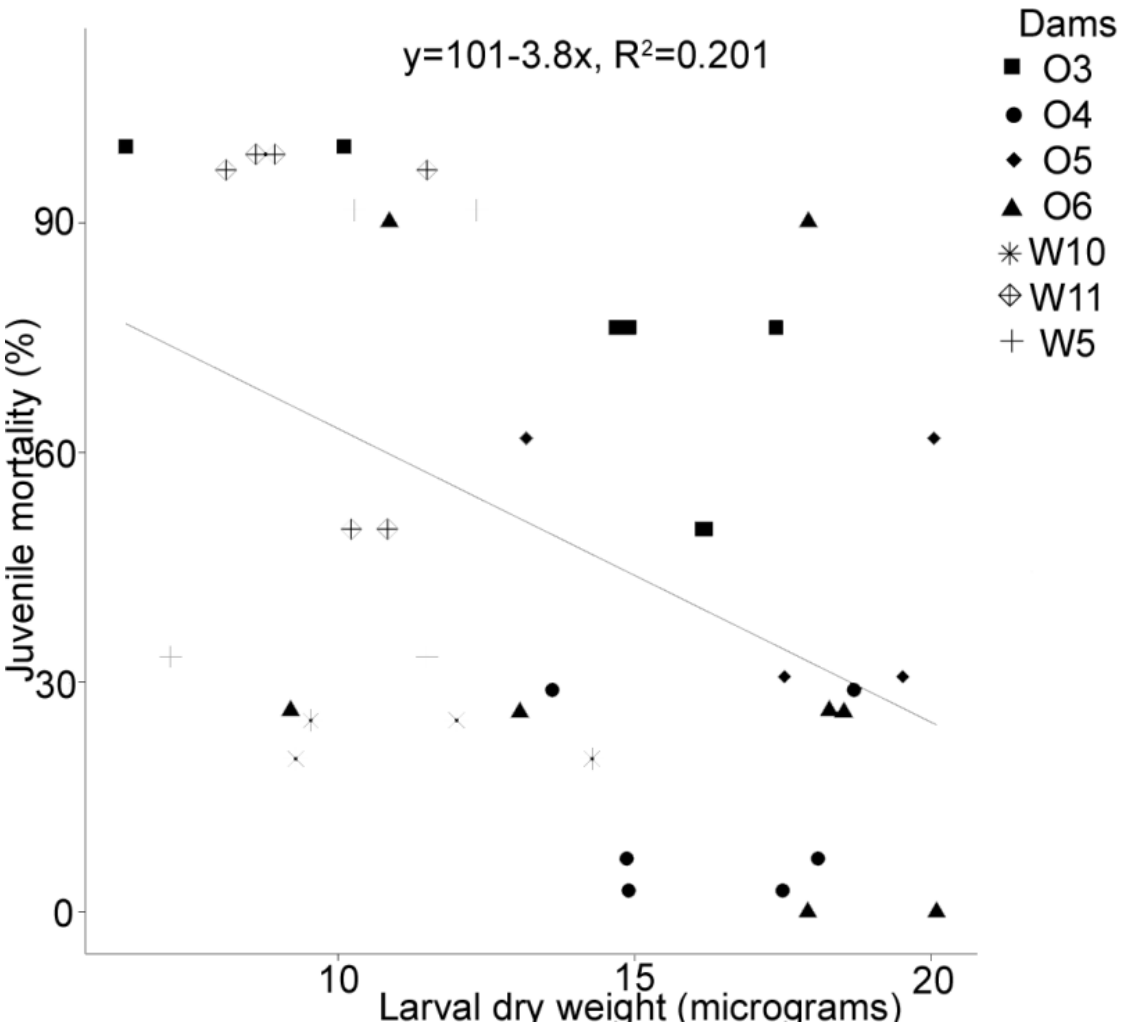

Figure S4.1 Variation in percent mortality explained by variation across larval dry weights (micrograms). Each point represents a unique larva and shapes represent dam identity.

Appendix D

Supplementary results Chapter 5

5.1 Pre- and post-experimental sediment

5.1.1 Comparison of *Symbiodinium* communities in pre- and post-experimental sediments

The sediment community changed significantly for each central sector site over the 5-month long experiment (Permutational multivariate analysis of variance: $Df_{3,23}$, F=2.10, p = 0.001). In the Magnetic sediments, only one OTU increased significantly in abundance (*Zoanthus*, OTU17, B-H adjusted p-value < 0.05) compared to the start of the experiment, whilst the remaining 31 significantly differentially abundant OTUs decreased (B-H adjusted p-values < 0.05). Of those, only OTU2129, a C15- type, had been previously identified as having significantly increased from time point 6 to 7 in Magnetic juveniles (however its overall abundance in the sediments significantly decreased). The same pattern was seen in Pandora sediments, with all but one OTU increasing in abundance (*Zoanthus*, OTU90) whilst the other 14 decreased. None of the OTUs found to be significantly different in Pandora juveniles were identified as those that changed significantly in the sediments. This was also true for Rib sediments. Only four of the 45 significantly differentially abundant OTUs in Rib sediments increased (*Zoanthus*: OTUs 8/11; *Amphisorus*: 362; G3: 1967) and none of those were found to be significantly different in juveniles. Finally, there were 37 differentially abundant OTUs in Davies sediments between time points, where the abundance of A3 decreased significantly over time almost 7-fold. The opposite was true for Davies juveniles, with A3 (OTU1) increasing significantly between time points 5-6 and 6-7.

5.1.2 Comparison of total organic carbon, nitrogen, phosphorus, calcium, and trace metals in pre- and post-experimental sediment differences

Overall the elemental composition of central sector sediments was similar both in the field and after ~6 months of experimentation in the laboratory (Figure 5.10A, Table 5.3), with no significant difference in total organic carbon content, total nitrogen, and total phosphorus between pre- and post-experimental sediments after *post hoc* tests (Figure 5.10A, Table 5.3, p = 0.2 - 0.934). However, pre- experimental sediments had

significantly greater percent: calcium ($p = 0.015$), al ($p = 0.0285$), and fe ($p = 0.018$) compared to post- experimental sediments.

5.2 Long-term symbiosis dynamics and temporal variation in *Symbiodinium* communities between central sector juveniles: differences within and between sites over time

At 11 d.p.e., juveniles exposed to Davies, Rib and Magnetic sediments also contained high abundances of different C-types that differed by sediment source: C (Davies), C1 (Magnetic), C15 and C1 (Rib). Juveniles from these three sites also had dominant uncultured *Symbiodinium* types that differed in their sequence between Magnetic and Davies/Rib. Juveniles exposed to Rib sediments were unique in having a high relative abundance of A3-type CCMP828, which was not detected in juveniles exposed to sediments from the other sites at this time-point. Interestingly the greatest number of differentially abundant OTUs were across inshore to offshore, with Rib/Davies and Magnetic/Pandora each having three differentially abundant OTUs between them. Although at background abundances, C15 was 7 log2 fold more abundant in Davies compared to Magnetic, with Davies also having 5.6 log2 fold less A3, and 6 log2 fold more C and D1 compared to Rib (B-H adjusted p-values < 0.05, Table S5.4). Magnetic also had 6 log2 fold greater abundances of D1 and C but 5.6 log2 fold less A3 compared to Pandora.

By 19 d.p.e., both Rib and Davies had C15 types in almost equal abundance compared to each other, although A3 dominated in Rib by 4 log2 fold greater abundances compared to Davies (B-H adjusted p-values < 0.05, Table S5.4). Both sites had juveniles with background abundances of C1 types, although significantly 3-5.3 log2 fold greater abundances in Davies, which also had background F2 and A13. Magnetic juveniles were heavily dominated by D1, which was at 4-5.4 log2 fold greater abundances compared to Rib and Davies juveniles. Magnetic juveniles also had abundant populations of D1a, C15 and C1 types, with significant changes in C and C1 compared to time-point 1 (B-H adjusted p-values < 0.05, Table S5.4). Magnetic also had significantly 3-5.3 log2 fold more C1 compared to Pandora. Finally, Pandora juveniles had greater dominance of four types belonging to clade B (*S. minutum*), background C and C1 populations, and a significant decrease in D1 compared to time-point 1. Pandora also had ~4 log2 fold less C, and more D1 compared to Davies and Rib. It also has 4.4 log2 fold less A3 compared to Magnetic.

By 27 d.p.e., juveniles exposed to Pandora and Rib sediments maintained the same diversity previously described. Rib juveniles increased in C1, CCMP828, D, G2, *ex situ* and uncultured types, although not significantly. Juveniles exposed to Davies sediments had less A3 than the previous sampling point and more equal populations of C, C15 and C1. Juveniles exposed to Magnetic sediments were no longer dominated by D1 (7.8 log2 fold decrease) or D1a (6.4 log2 fold decrease) (B-H adjusted p-values < 0.05, Table S5.4). The most dominant type was C1, which increased by 4.6 log2 fold from 19 d.p.e with a 7.2 log2 fold decrease in C. The greatest differences in abundance was between Davies and Pandora, with Pandora having ~4 log2 fold greater *S. minutum* and a C-type and 3.7 log2 fold lower C15. The opposite trend was true for Rib and Magnetic, with Magnetic having significantly more of these types compared to Rib. Compared to the previous time point, Pandora also had significantly less D1 but significantly more *S. minutum*, C1 and C.

No significantly differentially abundant OTUs were detected 41 d.p.e. between sites or from 27 d.p.e to 41 d.p.e, most likely due to the low level of replication for this time point. However, qualitative differences did exist between sites. For example, Pandora juveniles were still dominated by *S. minutum*, although D1 and D1a were now dominant compared to the previous C1 and C types. The emergence of a D-type was also seen in Davies/Rib and a re-emergence of D1 dominance in Magnetic juveniles such that the community looks very similar to the community seen in T2 (19 d.p.e). Magnetic juveniles also have C, C1 and C15 types seen previously as well as increased background abundances of F5, *S. minutum*, and A13. Davies are still dominated by C-types, although now almost completely by C15-types with an increase in *S. minutum* and consistently background presence of A13 at this site. Rib is again very similar to 19 and 27 d.p.e with C1 and C-types and A3 dominating (although at lower abundance) and background populations of C15, A2 and *Scyphozoa* types. Both Magnetic and Pandora have background abundances of *Zoanthus sociatus* and uncultured *Symbiodinium*.

Juveniles exposed to Rib sediments were again dominated by C15, A3 and C1 types and background *S. minutum* by 48 d.p.e. Davies communities were now very similar to Rib, with the consistently background abundances of A3 which grew in abundance to become the second most dominant type in Davies juveniles. As in Rib juveniles, C15 was the most abundant, followed by C and C1 types and background *S. minutum*. Rib did have significantly 3.2 log2 fold more A3 and 4.7 log2 fold less D1a compared to

Davies (B-H adjusted p-values < 0.05, Table S5.4). Pandora was again consistent to previous time points being dominated by *S. minutum* followed by C1, D1, D, and D1a types, with a 5.2 log2 fold decrease in C. Magnetic juveniles greatly resembled Pandora juveniles in the diversity of C, C1, D1 and D1a, although they only house background populations of *S. minutum*. C-type OTU115 did increase 7.5 log2 fold increase in Magnetic juveniles from the previous time point. The abundance of D1a was significantly 4.8 log2 fold greater and A3 was 3.3 log2 fold less in Pandora compared to Magnetic. C15 was the main differentially abundant OTU between Rib and Pandora and Magnetic and Davies with both Rib and Magnetic having ~3.5 log2 fold greater abundances.

By 75 d.p.e, Magnetic and Pandora juveniles were very similar in OTU diversity. Both were dominated by C1 types, followed by D1, C and D1a types. The only qualitative differences are the presence of C15 and *S. microadriaticum* in Magnetic juveniles. The background populations of *S. minutum* that were present at the preceding time point in Magnetic juveniles grew in abundance and were now more in line with abundances in Pandora juveniles. In regards to *S. minutum,* Pandora had 4.8 log2 fold less and Rib had 5.7 log2 fold more compared to 48 d.p.e. (B-H adjusted p-values < 0.05, Table S5.4). Alternatively, Davies and Rib diverged in community dominance, with Davies remaining very similar to previous time points in its dominance by A3 (although 7.1 log2 fold less of one type), C, C1 and C15 and with Rib now resembling Pandora and Magnetic (dominant C1 and background *S. minutum* and C). Two *S. minutum* types were in 5-6 log2 fold greater abundance in Rib compared to Magnetic and Pandora compared to Davies.

By 90 d.p.e, Rib and Davies communities are very similar in their dominance by C15 and C1 type communities with moderate A3 and background C-types and *S. minutum* and some uncultured types. Rib juveniles did have additional diversity in F5, *Scyphozoa* and *Zoanthus* types. Davies also had background C90 and CCMP2455 types not observed in Rib. *S. minutum* and a C-type were found to be at 4.3-6.1 log2 fold lower abundance in Rib compared to Davies (B-H adjusted p-values < 0.05, Table S5.4). Davies juveniles resembled the four earliest time points due to the reduction in type A3 by 4.2 log2 fold, and a 4.7 log2 fold increase in *S. minitium*. Pandora was dominated by 4-6 log2 fold more C and *S. minutum* compared to Magnetic juveniles, which were still dominated by C1 types. Pandora alternatively switched in dominance across a range of C types. Pandora juveniles also increased 4.8 log2 fold in *S. minutum*

abundance compared to the previous time point although this type did not regain its former dominance. These juveniles also decreased in D1 (6.2 log2 fold) and D1a. Magnetic juveniles saw 6.7-fold increases in *S. minutum* types, ~5.8 log2 fold increase in C15 and 4.4 log2 fold decrease in D1 abundances compared to 75 d.p.e. Overall, D1 was quite variable, with significantly greater abundances in Pandora compared to Rib, but less in Rib and Davies compared to Magnetic.

Davies juveniles survived until 145 d.p.e (four additional sampling points). No differentially abundant OTUs were detected between sampling points 90-102, 102-117, 117-129 d.p.e. in juveniles exposed to Davies sediments, however C15 was at 6.6 log2 fold greater abundances from 129-145 d.p.e.(B-H adjusted p-values < 0.05, Table S5.4). However, compared to 90 d.p.e, the overall abundance of A3 types was greater and continued to be variable until day 145. C1 and C15 types both oscillated in dominance for the remaining four time points. D1 and D1a were also detected in low abundance at 102 d.p.e. and D1 became one of the dominant types in 117 d.p.e. However, both D1 and D1a were at very low abundance in the final two time points.

Supporting tables for Chapter 5

Table S5.1 Summary of number of *A. tenuis* (not bold) and *A. millepora* (*italic* **bold**) juveniles collected at different days post exposure to sediments (d.p.e). Time points are only discussed in relation to *A. tenuis* juveniles.

D.p.e	**11**	**19**	**27**	**30**	**35**	**41**	**48**	**75**	**90**	**102**	**117**	**129**	**145**
Time point	**1**	**2**	**3**	**-**	**-**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Northern													
Great Detach	-	-	-		8, ***5***	-	-	-	-	-	-	-	-
Tydeman	-	-	-		24, ***0***	-	-	-	-	-	-	-	-
Wallace	-	-	-		9, ***4***	-	-	-	-	-	-	-	-
Wilkie	-	-	-		2, ***7***	-	-	-	-	-	-	-	-
Central													
Rib	3	5	5	***6***	-	1	7	1	4	0	0	0	0
Davies	3	6	5	***6***	-	2	14	7	6	3	3	3	4
Magnetic	4	5	5	***4***	-	3	10	3	4	0	0	0	0
Pandora	3	5	5	***6***	-	2	11	7	5	0	0	0	0

Table S5.2 Summary table of the number of raw sequencing reads, filtered reads and merged reads, with the final number of OTUs per category.

Sample type	N° samples	Raw reads	Merged/Filtered Reads	Merged reads (%) with 97% identity	N° OTUs
Juveniles	235	11,978,668	9,353,073	99.4	970
Sediments	39	1,910,315	1,480,059	97.6	1321
Total	274	13,888,983	10,833,132	99.3	2,188
E-value < 0.001	-	-	-	-	1,562

Table S5.3 Summary of read abundance and OTU diversity at the clade level across all juveniles and sediment samples compared to only those found in the sediments.

	Total				Sediments			
Clade/ category	**Reads**	**N° OTUs**	**Reads (%)**	**OTUs (%)**	**Reads**	**N° OTUs**	**Reads (%) of sediment**	**OTUs (%) of sediment**
A	2,762,200	212	26	13.6	281,909	177	19.5	13.7
Amphisorus	642	1	0.006	0.06	642	1	0.04	0.08
B	735,196	69	6.9	4.4	16,806	54	1.2	4.2
Bise	770	2	0.007	0.13	731	2	0.05	0.15
C	4,546,160	247	42.8	15.8	166,195	148	11.5	11.5
CCMA192	2150	1	0.02	0.06	2,150	1	0.15	0.08
D	1,542,579	137	14.5	8.8	128,136	116	9	8.9
E	57,975	91	0.5	5.8	57,386	89	4	6.9
Ex situ	503,089	168	4.7	10.8	388,621	143	26.9	11.1
F	64,495	128	0.6	8.2	49,724	119	3.4	9.2
G	13,036	24	0.1	1.5	9,020	19	0.6	1.5
H	5	1	4.7^{-5}	0.06	0	0	0	0
I	3,597	7	0.03	0.4	3,031	6	0.2	0.5
RCC2640	206	6	0.002	0.4	0	0	0	0
Scyphozoa	78,724	72	0.7	4.6	70,918	67	4.9	5.2
Uncultured	316,624	396	3	25.4	267,980	349	18.6	27.0
SUM	10,627,448	1,562	100	-	1,443,249	1291	-	-

Table S5.4 Summary table for differential abundance testing using DESeq2. Values were derived from negative binomial model with the following experimental design formula: Reef*Time point of only *A.tenuis* and *A.millepora* juveniles from 2014 between time points 1- 7 in which all four reefs were represented. Padj values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons (alpha < 0.05)

Time points by Reef	Comparison	OTU name and number	Basemean	Log2 fold	Padj
1	Rib vs. Davies	OTU_4__D1	6191.9	6.071784	0.0001
1	Rib vs. Davies	OTU_115__C	8595.7	6.020704	0.0001
1	Rib vs. Davies	OTU_1__A3	5188.7	-5.652263	0.005
1	Rib vs. Pandora	OTU_427__C15	1993.3	6.770046	7.7e-05
1	Davies vs. Magnetic	OTU_427__C15	1993.3	-6.819606	8e-05
1	Pandora vs. Magnetic	OTU_4__D1	6191.9	-6.09893	0.0001
1	Pandora vs. Magnetic	OTU_115__C	8595.7	-6.034413	0.0001
1	Pandora vs. Magnetic	OTU_1__A3	5188.7	5.572767	0.007
2	Rib vs. Davies	OTU_1__A3	5188.7	4.053373	0.04
2	Rib vs. Davies	OTU_3__C1	9153.4	-3.053511	0.04
2	Rib vs. Davies	OTU_1616__C1	125	-5.381309	0.04
2	Rib vs. Pandora	OTU_4__D1	6192	-5.425658	3.3e-05
2	Rib vs. Magnetic	OTU_4__D1	6192	4.200565	0.02
2	Davies vs. Pandora	OTU_4__D1	6192	-4.18538	0.02
2	Davies vs. Magnetic	OTU_4__D1	6192	5.440843	3e-05
2	Pandora vs. Magnetic	OTU_1__A3	5189	-4.019176	0.04
2	Pandora vs. Magnetic	OTU_3__C1	9153	3.030816	0.04
2	Pandora vs. Magnetic	OTU_1616__C1	125	5.348544	0.04
3	Rib vs. Magnetic	OTU_2__*minutum*	14665	-4.169964	0.02

3	Rib vs. Magnetic	OTU_115__C	8596	-4.366293	0.02
3	Rib vs. Magnetic	OTU_427__C15	1993	3.760846	0.05
3	Pandora vs. Davies	OTU_115__C	8596	4.375606	0.004
3	Pandora vs. Davies	OTU_2__*minutum*	14665	4.154799	0.004
3	Pandora vs. Davies	OTU_427__C15	1993.3	-3.745216	0.01
5	Rib vs. Davies	OTU_1__A3	5188.7	3.284569	0.03
5	Rib vs. Davies	OTU_6__D1a	284.1	-4.730965	0.03
5	Rib vs. Pandora	OTU_2129__C15	8326	3.558164	0.001
5	Davies vs. Magnetic	OTU_2129__C15	8325.761	-3.564269	0.001
5	Pandora vs. Magnetic	OTU_1__A3	5188.7	-3.262855	0.03
5	Pandora vs. Magnetic	OTU_6__D1a	284.1	4.794863	0.03
6	Rib vs. Magnetic	OTU_2__*minutum*	14665.1	5.709697	0.001
6	Rib vs. Magnetic	OTU_159__*minutum*	26.1	6.154792	0.01
6	Davies vs. Pandora	OTU_2__*minutum*	14665.0548	-5.710563	0.001
6	Davies vs. Pandora	OTU_159__*minutum*	26.1	-6.170069	0.01
7	Rib vs. Davies	OTU_115__C	8595.7	-6.133098	2e-05
7	Rib vs. Davies	OTU_2__*minutum*	14665.1	-4.337019	1.6e-02
7	Rib vs. Pandora	OTU_4__D1	6192	5.254881	0.002
7	Rib vs. Magnetic	OTU_4__D1	6192	-5.047767	0.005
7	Rib vs. Magnetic	OTU_115__C	8596	4.035173	0.05
7	Davies vs. Pandora	OTU_4__D1	6192	-5.047767	0.005
7	Davies vs. Pandora	OTU_115__C	8596	4.035173	0.05

7	Davies vs. Magnetic	OTU_4__D1	6192	-5.308002	0.002
7	Pandora vs. Magnetic	OTU_115__C	8596	6.150571	2e-05
7	Pandora vs. Magnetic	OTU_2__*minutum*	14665.1	4.342293	2e-02
Davies	5 vs. 6	OTU_1__A3	5188.7	7.136933	1.4e-06
Davies	6 vs. 7	OTU_2__*minutum*	14665.1	-4.724339	0.007
Davies	6 vs. 7	OTU_1__A3	5188.7	4.278615	0.02
Rib	5 vs. 6	OTU_2__*minutum*	14665.1	5.690951	0.03
Pandora	1 vs. 2	OTU_4__D1	6192	-5.791766	0.002
Pandora	2 vs. 3	OTU_4__D1	6192	-7.502493	9e-08
Pandora	2 vs. 3	OTU_2__*minutum*	14665.1	6.453674	9e-06
Pandora	2 vs. 3	OTU_115__C	8595.7	6.177159	2e-05
Pandora	2 vs. 3	OTU_3__C1	9153.4	3.392168	1.2e-02
Pandora	4 vs. 5	OTU_115__C	8595.7	-5.245648	0.03
Pandora	5 vs. 6	OTU_2__*minutum*	14665.1	-4.8057	0.01
Pandora	6 vs. 7	OTU_4__D1	6191.9	6.174213	0.0001
Pandora	6 vs. 7	OTU_2__*minutum*	14665.1	-4.804427	0.01
Magnetic	1 vs. 2	OTU_115__C	8595.7	-6.18168	0.001
Magnetic	1 vs. 2	OTU_1616__C1	125	6.214637	0.03
Magnetic	2 vs. 3	OTU_4__D1	6192	7.811593	5.4e-08
Magnetic	2 vs. 3	OTU_115__C	8595.7	-7.203004	2.3e-06
Magnetic	2 vs. 3	OTU_427__C15	1993.3	4.560852	1.2e-02
Magnetic	2 vs. 3	OTU_2186__D1a	267	6.388701	4e-02
Magnetic	4 vs. 5	OTU_115__C	8596	7.501243	2e-06

Magnetic	6 vs. 7	OTU_2__*minutum*	14665.1	6.796346	3e-05
Magnetic	6 vs. 7	OTU_2129__C15	8325.8	5.754242	3e-05
Magnetic	6 vs. 7	OTU_4__D1	6191.9	-4.439306	1.5e-02
Davies	10 vs. 11	OTU_2129_C15	12191.7	6.632362	0.005

Table S5.5 Summary table for differential abundance testing using DESeq2 comparing *A. tenuis* (n= 43) and *A. millepora* (n= 16) 2013 juveniles after 35 days post sediment exposure. Values were derived from negative binomial model. Padj values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons.(alpha < 0.05).

Comparison	OTUs	Basemean	Log2 fold	Padj
A.millepora vs. *A.tenuis*	OTU_1460_CCMP2456	25.9	-5.281482	1.2e-10
A.millepora vs. *A.tenuis*	OTU_2094_C1	190.9	4.056097	1.6e-05
A.millepora vs. *A.tenuis*	OTU_427_C15	87	-5.273507	3.6e-05
A.millepora vs. *A.tenuis*	OTU_2129_C15	1063.2	-3.116852	3.6e-05
A.millepora vs. *A.tenuis*	OTU_35_CCMP2456	3.6	-5.573386	4e-04
A.millepora vs. *A.tenuis*	OTU_356_G4	10.2	-6.879293	2e-03
A.millepora vs. *A.tenuis*	OTU_674_*microadriaticum*	2.2	6.508087	2.7e-03
A.millepora vs. *A.tenuis*	OTU_831_CCMP2456	1.6	-5.316148	3e-03
A.millepora vs. *A.tenuis*	OTU_3_C1	4394.8	1.802522	3.9e-03

Table S5.6 Summary table for differential abundance testing using DESeq2 comparing *A. tenuis* (n= 20) and *A. millepora* (n= 22) 2014 juveniles after 27-30 days post sediment exposure. Values were derived from negative binomial model. Padj values represent DESeq2 Bejamini-Hochberg p-adjusted values for multiple comparisons (alpha <0.05).

Comparison	OTU name and number	Basemean	Log2 fold	Padj
A.millepora vs. *A.tenuis*	OTU_2094_C1	206.8	7.326397	2.28e-15
A.millepora vs. *A.tenuis*	OTU_632_*microadriaticum*	13.8	8.254241	1.18e-07
A.millepora vs. *A.tenuis*	OTU_1616_C1	201.3	5.462061	1.18e-07
A.millepora vs. *A.tenuis*	OTU_1600_*microadriaticum*	13.3	8.127423	8.03e-07
A.millepora vs. *A.tenuis*	OTU_79_C15	17.4	6.889934	3.93e-04
A.millepora vs. *A.tenuis*	OTU_831_CCMP2456	3.9	-7.05868	1.63e-03
A.millepora vs. *A.tenuis*	OTU_4_D1	33.4	3.783966	2.29e-03
A.millepora vs. *A.tenuis*	OTU_35_CCMP2456	2.4	-6.39979	7.69e-03
A.millepora vs. *A.tenuis*	OTU_700_F5_1363	4.5	-6.91795	8.03e-03
A.millepora vs. *A.tenuis*	OTU_877_C	14.5	3.632376	9.04e-03
A.millepora vs. *A.tenuis*	OTU_2_*minutum*	1412.7	-1.9531	1.13e-02
A.millepora vs. *A.tenuis*	OTU_96_OTU18	1.8	-5.89206	2.00e-02
A.millepora vs. *A.tenuis*	OTU_1460_CCMP2456	1.7	-5.40764	2.00e-02
A.millepora vs. *A.tenuis*	OTU_275_OTU28	1.5	-5.6886	2.64e-02
A.millepora vs. *A.tenuis*	OTU_103_C1	1.8	-5.82352	2.84e-02
A.millepora vs. *A.tenuis*	OTU_985_*microadriaticum*	1.7	5.115434	3.07e-02
A.millepora vs. *A.tenuis*	OTU_1814_D1a	2.9	5.581719	3.07e-02

Table S5.7 Model outputs from Generalized Additive Models (GAMS). Each *Symbiodinium* type is fit as a single model, which may include linear and smoothing functions. To account for normality and heterogeneity of variance, log transformations (abbreviated Trans.) were used. Significant linear or smoothing terms are italicized.

Type	Factor	Trans.	edf	Ref.df	F or /t-value	*P*	Model type	R^2 (adj)	Variance explained (%)
S. natans								0.581	58.1
	WQT		1.563	1.809	13.668	*0.00013*	smoothers		
	SST		2	2	10.039	*0.00088*	smoothers		
	Mud		2	2	7.362	*0.00395*	smoothers		
	Carbonate		1	1	22.031	*0.00012*	smoothers		
A1								0.471	58.6
	WQT				5.83	0.355	lm		
	SST		1	1	16.839	*0.00048*	smoothers		
	Mud		1.741	1.933	6.969	*0.00347*	smoothers		
	Carbonate		1.926	1.994	8.4	*0.00266*	smoothers		
A2								0.166	19.9
	SST				2.488	*0.0199*	lm		
A3								0.349	40.8
	WQT	log10			-2.627	*0.0253*	lm		
B2								0.334	39.4
	SST		1	1	9.221	*0.00563*	smoothers		
	Mud		1.34	1.565	3.717	*0.02712*	smoothers		
B4								0.319	46.2
	WQT		1	1	3.203	0.088	smoothers		
	SST		1.693	1.905	3.654	0.0782	smoothers		
	Mud		1	1	4.727	*0.0413*	smoothers		
	Carbonate		1.762	1.942	1.849	0.2343	smoothers		
C1								0.175	27

	WQT				-1.646	0.1135	lm		
	Carbonate				2.613	*0.0155*	lm		
	SST				1.619	0.119	lm		
C3								-0.15	16.3
	SST				1.243	0.249	lm		
	Carbonate				-0.606	0.562	lm		
	WQT	log10			0.261	0.801	lm		
	Mud				-1.238	0.251	lm		
C15								-0.0543	10.8
	WQT				-1.238	0.229	lm		
	Carbonate				0.504	0.619	lm		
	SST				0.204	0.84	lm		
	Mud				0.882	0.387	lm		
C90								0.0616	23.2
	WQT	log10			-1.649	0.133	lm		
	Carbonate				1.284	0.231	lm		
D1								0.559	60.7
	Carbonate	log10	1.824	1.969	15.05	*2.79E-05*	smoother		
	SST				1.088	0.288	lm		
D1a								0.226	27.7
	Carbonate	log10	1.706	1.913	5.183	*0.0296*	smoother		
E								0.58	66.6
	Carbonate				-2.548	*0.032*	lm		
	WQT	log10	1.264	1.459	0.096	0.828	smoother		
F1								-0.0374	12.2
	WQT				-0.893	0.382	lm		
	Carbonate				0.391	0.7	lm		

	SST				-0.386	0.703	lm		
	Mud				0.636	0.531	lm		
G3								-0.065	9.8
	WQT				-0.978	0.339	lm		
	Carbonate	log10			1.208	0.24	lm		
	SST				0.958	0.348	lm		
	Mud	log10			0.761	0.455	lm		
G6								0.0328	18.2
	WQT				0.572	0.573	lm		
	Carbonate				-0.857	0.401	lm		
	SST				0.52	0.608	lm		
	Mud				-0.55	0.588	lm		

Table S5.8 Impact of reef site and the interaction of time and site to maximum quantum yield. Output from a generalized additive mixed effects model.

	Df	**Ref.Df**	**F**	***P***
Site	3	-	3.064	0.0283
s(Day:Davies)	-	1.984	29.849	1.72^{-12}
s(Day:Magnetic)	-	1	0.083	0.774
s(Day:Pandora)	-	1	1.426	0.233
s(Day:Rib)	-	1	1.757	0.186

Supporting figures for Chapter 5

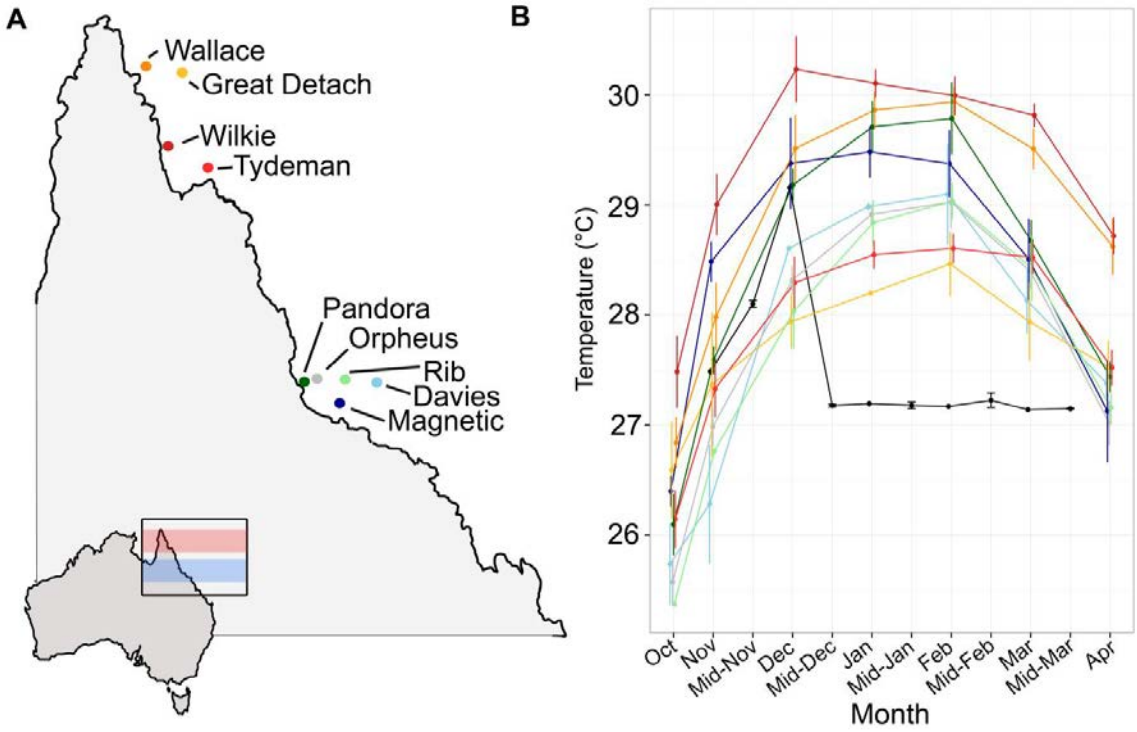

Figure S5.1 Map of sediment sampling locations along the Queensland coast in Australia (**A**). The warmer, northern sites are in red and orange colours and correspond to the red box on the inset. Central sites are in blue and green colours. Offshore sites are in lighter tones to correspond to the less turbid environments on offshore reefs and inshore reef colours are in dark tones as the water is more turbid. Panel **B** gives the long-term temperature profile of each site from October to April (around spawning period), including experimental temperature treatments for juvenile exposure to sediments in 2013 (light grey-Orpheus ambient temperature) and 2014 (black). The line colours correspond to the filled in circle colours for each site from panel A.

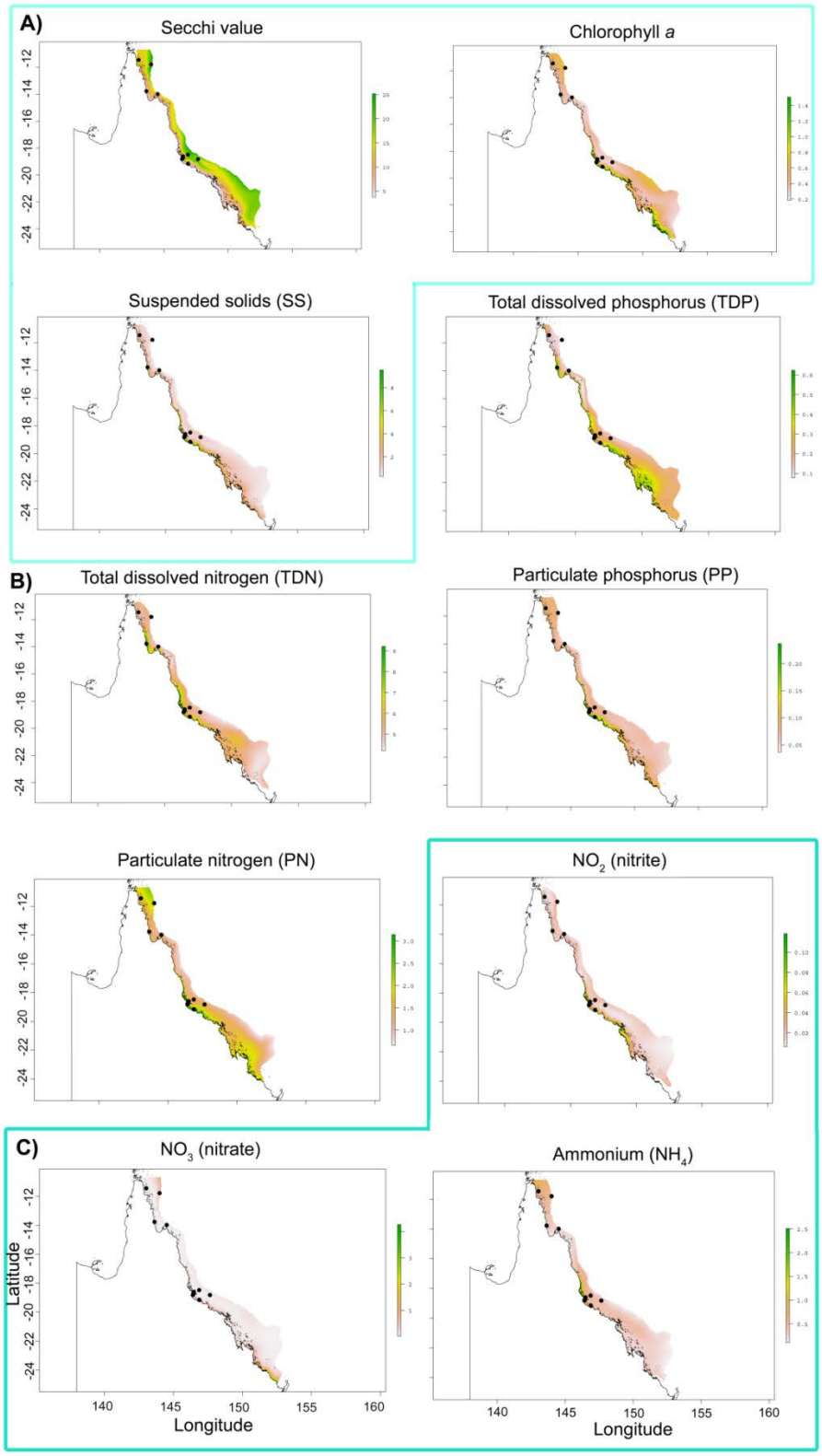

Figure S5.2 Maps showing different environmental covariates of water-quality along the Queensland coast. These ten variables were combined to create an overall water-quality index (WQI). **A)** Irradiance measures include Secchi depth, chlorophyll *a* concentration and suspended sediments whilst **B)** and **C)** are nutrient measures. Group **C)** represent dissolved inorganic nitrogen (DIN).

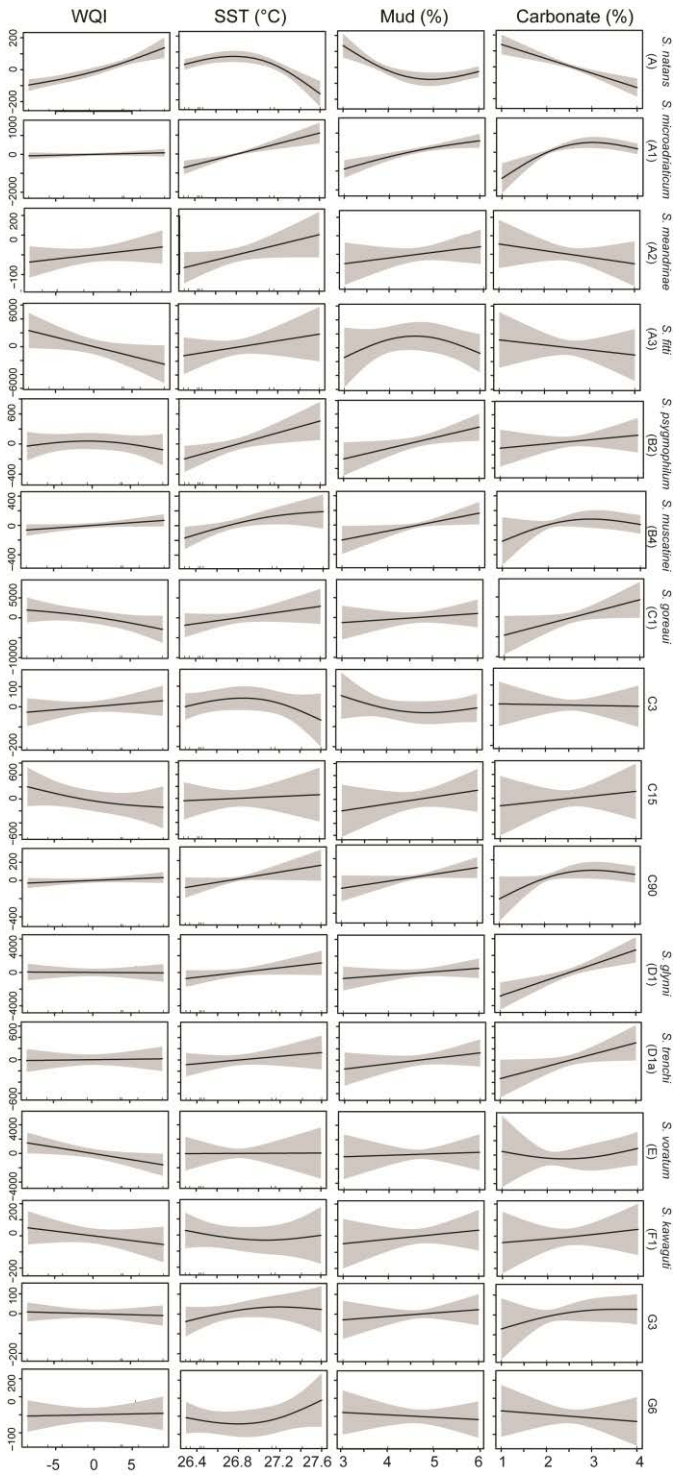

Figure S5.3 Partial plots of Generalized Additive Models along Water Quality Index (WQI), Sea Surface Temperatures (SST), mud (3 = very high mud, 6 = low mud; see Methods) and carbonate content (1 = pure carbonate, 4 = transitional carbonate). Note that the y-axis representing variance normalized *Symbiodinium* abundances varies per type. The solid line shows the modelled abundances and the grey areas are the modelled confidence limits.