# A Novel Field-Based Method of Vectoring Thermotolerant Algal Symbionts (Family Symbiodiniaceae) into Coral Colonies

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By

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# A NOVEL FIELD-BASED METHOD OF VECTORING THERMOTOLERANT ALGAL SYMBIONTS (FAMILY SYMBIODINIACEAE) INTO CORAL COLONIES

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A Novel Field-Based Method
of Vectoring Thermotolerant Algal Symbionts
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The dinoflagellate algal symbiont *Durusdinium trenchii* (Family Symbiodiniaceae) is known to confer greater thermal tolerance to its coral hosts than other common Caribbean algal symbionts and can increase in abundance on reefs following bleaching events. Adult corals can be induced to change the composition of their algal symbiont communities through controlled bleaching and recovery, but the stability and persistence of these new communities is not well understood. Recent studies have developed our understanding of the competitive dynamics that govern symbiont community shifts (and reversions) within coral hosts. This project aimed to further elucidate these interactions by tracking the spread (or loss) of different symbiont genera in colonies under natural reef conditions.

The first set of experiments, repeated in spring and in fall, tracked changes in symbiont communities as experimental cores collected from field colonies bleached and recovered under laboratory conditions, focusing on changes in dominance of *D. trenchii*. When comparing between species, a lack of initial *D. trenchii* was associated with a delay in symbiont 'shuffling' to *D. trenchii* in *Montastraea cavernosa*. Within *Orbicella faveolata* and *Siderastrea siderea*, a lack of initial *D. trenchii* was associated with reduced *D. trenchii* dominance after recovery. Initial symbiont communities were also found to shape

bleaching responses between seasonal experiment batches, with *M. cavernosa* corals collected in the fall hosting higher symbiont densities and showing greater bleaching sensitivity.

In the second part of the project these manipulated cores (and associated controls), were grafted back into their original field colonies. As implants healed back into their respective colonies we tracked symbiont community changes in these implants and also in the surrounding coral tissue. Background *D. trenchii* spread into colonies to a distance of at least a 1.25cm from 2.5cm *M. cavernosa* implants over the course of 7 months, supporting the findings of previous laboratory-based experiments, and indicating that symbionts can be transferred between implant and parent colony in the field. This transfer was accelerated by, but not dependent on, the healing of implants, suggesting a role for both endogenous (gastrovascular transfer) and exogenous (seawater transfer) mechanisms, in the migration of symbionts through coral colonies. Due to the higher thermal tolerance of *D. trenchii*, this project is not only relevant to the competitive dynamics of symbionts in wild colonies but could also inform potential interventions for increasing the bleaching resistance of massive corals using tissue implants to introduce thermotolerant symbionts.

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APPENDIX B

# Chapter 1: Introduction

## The coral climate crisis

Temperature-induced coral bleaching is an increasingly devastating problem for tropical coral reefs worldwide, as mass bleaching events become both more severe and more frequent, with roughly 75% of the world's reefs being affected in 2015-16 (Hughes *et al.*, 2018). The relentless onslaught of global and local stressors has reduced corals' capacity for recovery (Ortiz *et al.*, 2018), and driven patterns of reef degradation and decline in coral cover (De'ath *et al.*, 2012). By 2001, the combined effect of heat-induced bleaching, disease, and local stressors had already caused an 80% reduction in coral cover in the Caribbean (Gardner *et al.*, 2003). Disease and bleaching continue to raze Caribbean reefs, especially with the spread of Stony Coral Tissue Loss Disease (SCTLD, Alvarez-Filip *et al.*, 2019), and annual severe bleaching is now predicted on 90% of reefs within the next decade (van Hooidonk *et al.*, 2014). These declines foreshadow the first real-time ecosystem extinction event (Jackson, 2008): the loss of tropical coral reefs along with the ecosystem services that they provide. Most notably, reef declines bring decreases in reef structure and accretion, increasing the risk of reef submergence (Perry *et al.*, 2018) and impairing the shoreline protection afforded by reefs against storm surge and associated flooding, valued at over $4 billion USD annually (Beck *et al.*, 2018).

## Bleaching and the coral holobiont

Bleaching is the loss of algal symbionts (Family Symbiodiniaceae, (LaJeunesse *et al.*, 2018) from host gastrodermal tissues, or a reduction in their per-cell pigment

concentrations (Glynn, 1996). It is a manifestation of a dysfunctional symbiosis ("dysbiosis"), causing the coral's immune system to reject it's algae (Palmer, 2018). The production of reactive oxygen species in symbionts' chloroplasts as a result of declining photochemical efficiency under excess heat and light, is likely to play a central role in triggering the bleaching cascade and triggering the associated immune response (Weis, 2008; Cunning and Baker, 2013). While host genotype alone contributes to significant variation in bleaching susceptibility (Baird *et al.*, 2009), the importance of a holobiont-level view in evaluating traits such as thermal tolerance is becoming increasingly apparent (Kavousi, J., Denis, V., Sharp, V. *et al*., 2020); coral-associated bacteria, fungi, archaea, viruses, protists and dinoflagellates are all intimately linked (Bourne, Morrow and Webster, 2016), and provide insights into the organismal environmental history, as well as holobiont performance. Despite this, our understanding of how the entire holobiont functions is still in its infancy, as has been exemplified by the recent discovery of pervasive corallicolid eukaryotic symbionts, which seem intimately implicated in the coral-Symbiodiniaceae symbiosis (Kwong *et al.*, 2019).

**The adaptive bleaching hypothesis**

One component of the holobiont that has long been implicated in corals' bleaching phenotype are the Symbiodiniaceae that are typically lost from corals during bleaching. Different Symbiodiniaceae often confer different thermal tolerance to their hosts (Berkelmans and van Oppen, 2006), resulting in global shifts in reef symbiont communities following high temperature anomalies (Baker *et al.*, 2004). Specifically, corals hosting symbionts of the genus *Durusdinium* (formerly *Symbiodinium* clade D, LaJeunesse *et al.*, 2018) are usually less likely to bleach than those hosting other

common genera such as *Cladocopium* (Berkelmans and van Oppen, 2006; Silverstein, Cunning and Baker, 2015). Juvenile corals generally have more flexibility in their symbiotic associations (Little, van Oppen and Willis, 2004), but many adults still host multiple symbiont genera simultaneously, at least at background levels (Silverstein, Correa and Baker, 2012). These background symbionts, and symbiosis flexibility in general, potentially facilitate shifts in the symbiont community of individual colonies in response to environmental change, with bleaching providing an opportunity for new (or previously 'background') symbionts to colonize a host (Baker, 2003). In the case of thermal bleaching, colonies that expel thermally-sensitive symbionts and become colonized by thermally-tolerant symbionts such as *Durusdinium*, would then likely have a higher resistance to subsequent thermal bleaching, as first posited by the 'adaptive bleaching hypothesis' (Buddemeier and Fautin, 1993; Baker, 2001).

**Fickle or tenacious symbionts**

The longevity of shifts in corals' symbiont communities remains unclear. Field observations report 'boom-and-bust' dynamics of *Durusdinium trenchii*'s dominance on Caribbean reefs, where this symbiont becomes dominant in a wide range of host species following bleaching events but then recedes to background levels within months to a few years (Thornhill *et al.*, 2006; LaJeunesse *et al.*, 2009). The transient nature of *D. trenchii*'s dominance is also reflected in seasonal fluctuations, becoming more abundant in summer months (Thornhill *et al.*, 2006). Under laboratory conditions, *Montastraea cavernosa* hosting *Cladocopium* symbionts has been shown to bleach more readily during hight temperature stress compared to the same coral genotype hosting *Durusdinium* (Silverstein et al., 2015), and *Durusdinium* appears to be resistant to bleaching even when impaired, for example during low-temperature

stress (Silverstein, Cunning and Baker, 2017). This study implies a more tenacious (and perhaps even parasitic, Lesser, Stat and Gates, 2013) quality of *D. trenchii*, not reflected by field observations. Here, I aim to address this discrepancy by studying the effect of seasonality on symbiont shuffling (see Research Question 2.1) and the persistence of *D. trenchii* in the field in bleached and recovered corals (see Research Question 3.3).

### *Durusdinium* as a competitor

While expulsion of symbionts in response to bleaching is likely mostly under host control, recovery of endosymbionts seems contingent on symbiont dynamics. By viewing bleached host tissue as a competitive landscape over which symbionts may colonize and compete, we may better understand these dynamics (Rowan et al., 1997). Despite its thermotolerance in warm temperatures (Berkelmans and van Oppen, 2006), *Durusdinium* symbionts confer various trade-offs to their coral hosts, such as reduced growth (Jones and Berkelmans, 2010) and calcification (Pettay *et al.*, 2015), likely due to less translocation of photosynthate to the host (Cantin *et al.*, 2009; Wall *et al.*, 2020). However, these effects only seem to present under 'normal', non-stressful temperatures (Cunning, Gillette, *et al.*, 2015). These drawbacks of hosting *Durusdinium* may be manifestations of its competitive disadvantages at ambient temperatures compared to other genera.

*Durusdinium* is also less able to acquire nutrients (such as nitrates) from its host at ambient temperatures, compared to *Cladocopium* (Baker *et al.*, 2013). *Durusdinium* is also less photochemically efficient at non-stressful temperatures. In a study of three coral species (Cunning, Silverstein and Baker, 2018), *D. trenchii*'s photochemical advantage at high temperatures (compared to the dominant pre-

bleaching symbiont), predicted the extent to which *D. trenchii* became dominant during recovery; with *Montastraea cavernosa* 'shuffling' to host the most *D. trenchii*, followed by *Siderastrea siderea* and then by *Orbicella faveolata* (in which *D. trenchii* had the smallest photochemical advantage over its co-occurring symbiont). This same study showed that the photochemical disadvantage of *D. trenchii* at ambient temperatures corresponded to this same competitive hierarchy (with the greatest comparative disadvantage in *O. faveolata*), possibly relating to a combination of the host-specific photic microenvironment and the performance of the competing symbiont (Cunning, Silverstein and Baker, 2018). Here, I will repeat similar bleaching experiments in the same three coral species, to assess the repeatability of these findings and the shuffling hierarchy of *M. cavernosa*, *S. siderea* and *O. faveolata* (see Research Question 2.2). I will build on this by monitoring the persistance or loss of *D. trenchii* after shuffling.

**A numbers game**

As suggested by Lotka-Volterra population competition models (Lotka, 1920), the population density of *Durusdinium* during recovery can be understood as a function of its initial population size and its competition coefficient against a second symbiont under a particular set of environmental conditions. *Durusdinium* has been found at very low background levels across a number of coral species on the Great Barrier Reef (Mieog *et al.*, 2007) and in the Caribbean (Kennedy *et al.*, 2015), and can become dominant from as little as 0.003% pre-bleaching dominance (relative to *Cladocopium*) (Bay *et al.*, 2016). However, to ensure dominance continues to persist after 6 months of recovery, a minimum dominance of 98% *D. trenchii* to *Cladocopium* was required in *S. siderea* (Palacio, 2019). Research Question 3.2

addresses the importance of competition on the longevity of *Durusdinium* dominance in tissue grafts by monitoring the effect of *Durusdinium* dominance ratio at implantation and proximity to non-*Durusdinium* dominated tissue.

**Successional communities**

Rather than following a simple trajectory of competitive dominance over the course of recovery, it has been proposed that *Durusdinium* is an opportunist generalist capable of colonizing marginal environments, and is then displaced by successional communities (Toller, Rowan and Knowlton, 2001; LaJeunesse *et al.*, 2009). Just as reduced production (or increased absorption) of reactive oxygen species (McGinty, Pieczonka and Mydlarz, 2012) and host immune evasion or suppression may play a role in *Durusdinium*'s resistance to expulsion during bleaching, these same traits may aid it in colonisation (Merselis, Lirman and Rodriguez-Lanetty, 2018). Bleached host tissue is indeed a marginal environment even after the alleviation of thermal or light stress due to the lack of protective self-shading among symbionts, yet competition for resources such as nitrates is reduced. *Durusdinium*'s proliferation during recovery would reverse these effects (Cunning, Vaughan, *et al.*, 2015) and thus make it vulnerable to out-competition by other genera. McIlroy *et al* (McIlroy *et al.*, 2019) likened this process to the winnowing of symbiont diversity in coral recruits; they found that *D. trenchii* was prone to out-competition over time, but in the absence of competition it was the most successful colonizer, especially under high light. Further evidence of *D. trenchii*'s success as a colonizer is that *Orbicella faveolata* shuffles to *Durusdinium* dominance to a greater extent following severe bleaching compared to moderate bleaching (Cunning, Silverstein and Baker, 2015). The grafting of *Durusdinium*-dominant tissue into colonies in these experiments attempts to elucidate

the necessity of bleaching in the proliferation of *D. trenchii* by monitoring any spread from grafts into healthy tissue (Research Question 3.1).

These field-based grafting experiments are building on lab-based grafting experiments in *M. cavernosa* performed by Baker *et al* (in re-submission). Smaller, 1cm cores which had shuffled to host *D. trenchii* were grafted into 2.5cm cores hosting *Cladocopium*; *D. trenchii* spread from the small grafts at 29°C but was lost at 22°C. *D. trenchii* also spread more readily from the 2.5cm core into the 1cm core, than from the 1cm core. These results support the importance of *Durusdinium*'s competitive advantage (at higher temperatures), and of initial population size in determining the spread of *D. trenchii*'s dominance, implying that the symbiont competition dynamics between grafts are consistent with those at play during bleaching recovery. These experiments aim to address this question in three different coral species and in a field setting.

**Mechanisms of spread**

The origin of symbionts that form shuffled communities in bleaching recovery is still unclear. Since many corals do host very low, almost undetectable levels of background symbionts (Silverstein, Correa and Baker, 2012), including *Durusdinium* (Mieog *et al.*, 2007), re-colonisation of bleached tissue by a small population of residual symbionts is thought to be common (Coffroth *et al.*, 2010). However, exogenous uptake of symbionts (as seen in azooxanthellate recruits) has been demonstrated by adults during bleaching recovery (Boulotte *et al.*, 2016), although this is thought to occur on very small spatial scales due to extensive ecological compartmentalization between corals, sediment and the surrounding water (Cunning, Yost, *et al.*, 2015). Similarly, the mechanism of spread of symbionts between tissue

grafts may occur through different mechanisms; through gastrovascular transport across healed tissue, or through release from grafts and exogenous uptake by nearby polyps. Depending on how symbionts are transferred to and from grafts, the rate of transfer may depend on the rate of graft-healing, the rate of release of graft symbionts and the route & rate of gastrovascular transport.

In their *M. cavernosa* grafting experiment, Baker *et al* (in re-submission) noted a spatially discontinuous pattern of spread of *Durusdinium* and a comparatively continuous spread of *Cladocopium*: *Cladocopium* spread between grafts from the sampling points nearest the healing border, whilst *Durusdinium* spread into sampling areas 2mm and 10mm from the graft boundary at an equivalent rate. The authors suggested that this may be indicative of symbiont-specific mechanisms of spread, with *Cladocopium* spreading through gastrovascular transport and *Durusdinium* through release and exogenous oral intake. This in turn may hint at *Cladocopium*'s posited competitive dominance within fast-growing lesion-healing tissue, owing to its superior contribution to host growth rate compared to *Durusdinium (Jones and Berkelmans, 2010)*. Research Question 3.3 addresses this theory by correlating graft healing with symbiont spread.

**Symbiont manipulation as an intervention**

This study may provide insights into symbiont competition dynamics in field colonies, which in turn may further improve our ability to predict the spread of thermotolerant symbionts such as *Durusdinium trenchii*, and associated host bleaching resistance and physiological trade-offs.

This novel field trial of vectoring symbionts through tissue grafting also has obvious implications for interventions aiming to promote coral resistance and

resilience to bleaching. Whilst the manipulation of symbionts has not yet been integrated into the nursery propagation and outplanting of corals (Peixoto *et al.*, 2017), with the increase in restoration of slow-growing massive species (Boström-Einarsson *et al.*, 2020), increasing the fitness of these outplants under climate change projections must occur simultaneously. A report from the National Academy of Sciences (Committee on Interventions to Increase the Resilience of Coral Reefs, *et al.*, 2019) assessed the viability of algal symbiont manipulation as an intervention strategy, citing the lack of scalability as its main obstacle (since manipulations have only been performed in the lab), along with a lack of knowledge of the longevity of these manipulations. This tissue vectoring method may provide a more scalable alternative to manipulating corals' symbionts in the lab, providing a means to seed large, structurally important colonies with symbionts *in situ*. The longevity of these interventions over multiple years still remains to be seen, incentivizing long-term monitoring of these grafted colonies. Beyond the lasting effects within adult colonies, it is possible these interventions could have intergenerational effects if performed in brooding species given evidence that introduced symbionts can be transmitted from parent to gamete, to then colonize larvae (Quigley, Willis and Kenkel, 2019). Regardless of the lasting effects on individuals, the introduction of *D. trenchii* will likely contribute to the immediate symbiont metacommunity (Cunning, Yost, *et al.*, 2015). Furthermore, correlations have been drawn between hosting *D. trenchii* and disease resistance in multiple coral species across multiple Caribbean locations (Correa *et al.*, 2009), and there have been anecdotal reports of differential mortality from the recent Stony Coral Tissue Loss Disease in colonies hosting *Breviolum* symbionts. This may point to disease resistance benefits to introducing *D. trenchii* in addition to its bleaching resistance.

## Research Questions

- **2.1. How do seasonal changes in symbiont communities affect corals' bleaching sensitivity and propensity to switch or shuffle to *D. trenchii* dominance?**

  Hypotheses:

  - Higher symbiont densities (associated with corals during winter) and lower *D. trenchii* proportions (associated with corals during spring) will increase bleaching sensitivity.
  - This in turn will shape how easily corals can be induced to *D. trenchii* dominance, with more severe bleaching inducing a more complete and stable shuffle.

- **2.2. How does symbiont community composition change over the course of bleaching and recovery of three local massive species?**

  Hypotheses:

  - For all three coral species, *Breviolum* and *Cladocopium* symbionts become less dominant over bleaching as they are lost at a greater rate than *D. trenchii*.
  - Over recovery, almost total *D. trenchii* dominance is achieved, as these resident populations recolonize the recovering tissue.

- **3.1. How does the introduction of implants hosting a shuffled symbiont assemblage affect field colony symbiont communities?**

  Hypotheses:

- The higher the pre-implantation proportion *Durusdinium* hosted by the field colony, the less likely any increase in *D. trenchii* hosted as a result of *D. trenchii* implantation, will be detected.
- As *D. trenchii* spreads from implants and proliferates, non-*Durusdinium* symbionts are lost from colonies.

- **3.2. How does intra-colonial competition affect the loss of *Durusdinium* from implants?**

  Hypotheses:

  - *D. trenchii* is lost more readily from implants compared to corals outplanted in isolation from their parent colonies.
  - Non-*Durusdinium* symbionts invade implants faster than outplants.

- **3.3. How does the healing of implants affect the rate of symbiont transfer between implant and field colony?**

  Hypotheses:

  - The rate of change of symbiont communities between implant and field colony is correlated with rate and direction of healing.
  - The invasion of non-*Durusdinium* symbionts into implants may correlate with healing, whilst the spread of *D. trenchii* from implants may not.

# Chapter 2: Initial algal symbiont communities affect shuffling to *D. trenchii* dominance in bleaching recovery and reflect seasonal differences in bleaching sensitivity

## Overview

Seasonal differences in corals' symbiont communities are reflections of both adaptive adjustments to seasonality and relics of their recent environmental history. The bleaching sensitivity of wild colonies is most often revealed in summer when thermal stress is at its peak. This study compared the spring and fall symbiont communities of three common Caribbean massive species, *Montastraea cavernosa*, *Orbicella faveolata*, and *Siderastrea siderea*, and related these changes to corals' bleaching sensitivity. Symbiont densities were greater in October, supposedly in response to seasonally low light intensity, which resulted in higher bleaching sensitivity.

Monitoring symbiont community changes over bleaching and recovery also allowed a direct comparison of the symbiont shuffling trajectories in corals initially hosting background *D. trenchii* populations with those not, independent of season. The lack of detectable *D. trenchii* prior to bleaching incurred a delay or reduction in shuffling which may have represented either the time taken to uptake exogenous *D. trenchii* or the proliferation of extremely small (undetected) resident *D. trenchii*. These results warn of the increased bleaching sensitivity of field colonies in fall and

winter. This study also indicates the importance of background *in hospite D. trenchii* populations in the speed or extent of symbiont shuffling during bleaching recovery.

## Background

Thermal bleaching is now the biggest cause of coral death globally, and bleaching events are becoming both more frequent and more severe, with annual severe bleaching expected on 90% of the world's reefs by 2055 (van Hooidonk *et al.*, 2014; Hughes *et al.*, 2018). Bleaching is intricately linked to corals' relationships with their photosynthetic algal symbionts (family Symbiodiniaceae), affected by the thermal tolerance of both partners (Berkelmans and van Oppen, 2006; Baird *et al.*, 2009). An understanding of the population dynamics shaping the algal symbiont communities both on reefs and within coral colonies is crucial for predicting or manipulating corals' relationships with these symbionts as bleaching events escalate.

The loss of symbionts during bleaching provides an opportunity for corals to recover with shifted symbiont communities (Buddemeier and Fautin, 1993; Baker, 2003). It is common for Caribbean corals to recover from thermal bleaching with the comparatively thermally tolerant *Durusdinium trenchii (*LaJeunesse *et al., 2009)*. Independent of bleaching, *in hospite* symbiont communities go through seasonal fluctuations, with *D. trenchii* becoming more abundant in colonies during the late summer (Thornhill *et al.*, 2006).

In addition to differences in the identity of symbionts, total symbiont density shows seasonal fluctuations. A long-term multi-species Caribbean study revealed that symbiont densities, symbiont chlorophyll *a* concentrations, and symbiont PSII photosynthetic capacity is highest in the fall, all underpinned by inverse relationships

with temperature and light (Fitt *et al.*, 2000; Warner *et al.*, 2002). Higher symbiont densities have been implicated in an increased bleaching sensitivity, supposedly due to a faster build-up of heat-stress-induced reactive oxygen species (Cunning and Baker, 2014), although no link between seasonal symbiont density and bleaching sensitivity has yet been shown.

Changes in the symbiont community composition within a coral colony over bleaching and recovery may be brought about by the uptake of exogenous symbionts, or by the proliferation of very low background levels of resident symbionts, coined 'shuffling' and 'switching' respectively (Baker, 2003; Silverstein, Correa and Baker, 2012). ITS2 sampling has allowed the identification of *de novo Durusdinium* symbionts taken up by adult corals (Boulotte *et al.*, 2016), but how often this occurs on reefs compared to shuffling is not well understood, nor are the recovery implications of these two pathways. The relative importance of switching and shuffling in bleaching resilience has implications for the significance of exogenous *D. trenchii* in the reef metacommunity, and of background *in hospite D. trenchii* populations.

## Methods

### Colony selection and core collection

Initially 54 colonies of *Orbicella faveolata*, *Siderastrea siderea* and *Montastraea cavernosa* at 7.5 to 8.5 meters depth were sampled from Emerald Reef (25.406 to 25.407 N, 80.058 to 80.060 W), just North of Biscayne Bay National Park, South Florida. Of these 54 colonies, the 10 colonies (all at least 30 cm in diameter) containing the lowest proportions of *D. trenchii* (confirmed using quantitative PCR analysis) of each species (30 colonies in total), were selected for the experiment.

Selected colonies were at least 5m apart in an effort not to core colonies of the same genotype. From each colony, two 2.5cm diameter cores were drilled out using a submersible drill (Nemo Power Tools LTD) with a diamond core drill bit. Cores were drilled from the upper surfaces of colonies to avoid collecting cores with unexpected symbiont compositions as a result of intra-colonial niche partitioning (Rowan et al., 1997). The holes left in colonies were filled with a two-part epoxy putty in order to promote healing-over of the tissue. Cores were collected in March-April 2019 (hereafter referred to as the 'spring' or 'April' batch), and another set of 60 cores was collected from the same 30 colonies in October 2019 (the 'fall' or 'October' batch).

**Laboratory husbandry**

Cores were glued onto plaster plugs with cyanoacrylate 'Reef Glue Gel' (Boston Aqua Farms) and held upright in plastic racks. From each batch of 60 cores, 15 of each species were randomly assigned to the bleaching treatment, and the remaining 5 were assigned to be non-manipulated controls. This resulted in sample sizes shown in table 2.1. Racks of plugs were kept in 75-gallon, indoor, flow-through, fiber-glass tanks in the Marine Technology and Life Science Seawater (MTLSS) complex at the University of Miami's Rosenstiel School of Marine and Atmospheric Science, supplied with sand-filtered (to 10 microns) seawater from nearby Biscayne Bay. For each batch, the 45 heat-stressed cores were kept in one tank and the 15 control cores, in another, with cores being moved around in tanks with weekly cleaning to mitigate the effects of any environmental gradients across each tank. 2x 500 gph pumps were used to circulate water in each tank, and corals were kept on a 12-hour light:dark cycle using three Hydra 52HD lights (Aqua Illumination, C2 Development Inc.) at 20% intensity across all wavelengths, delivering approximately 400 lumens/$ft^2$

(equivalent to approximately 80 µmol $m^{-2}$ $s^{-1}$) across each tank. Corals were fed twice weekly with resuspended zooplankton and phytoplankton, 'Reef-Roids' (Polyp lab).

**Table 2.1. Heat stress sample sizes.** Numbers in brackets give the number of cores included in analyses, accounting for losses due to mortality over the course of heat stress and recovery.

| | **Manipulated** | | | **Non-manipulated controls** | | | **Total genets** |
|---|---|---|---|---|---|---|---|
| **Species** | **April batch** | **Oct batch** | **Total** | **April batch** | **Oct batch** | **Total** | |
| ***M. cavernosa*** | 15 | 15 | 30 | 5 | 5 | 10 | 10 |
| ***O. faveolata*** | 15 (14) | 15 (11) | 30 (25) | 5 | 5 | 10 | 10 |
| ***S. siderea*** | 15 | 15 | 30 | 5 | 5 | 10 | 10 |

## Heat stress

The tank containing control corals was kept at 27°C (+/- 1°C, daytime) throughout the duration of the heat stress experiment, for both spring and fall batches. Cores in the heat stress treatment were initially also kept at 27°C (+/- 1°C, daytime), but on day 1 of heat stress, the temperature was raised (using in-tank heaters) to 32°C (+/- 0.5°C, daytime). After 15 days under heat stress, photochemical efficiency measurements were taken using an imaging pulse-amplitude-modulated (I-PAM) fluorometer (Walz, Effeltrich, Germany). Corals are dark-adapted for 30 minutes (Warner *et al.*, 2002), then delivered a saturating pulse at 2800 lmol photons m-2 s-1 at 460 nm for 800 ms. The maximum quantum yield of symbionts' PSII (Y2) is quantified as $F_v/F_m$ (Warner, Fitt and Schmidt, 1996). I-PAM measurements were always taken in the early evening to account for any diel variation in photochemical efficiency (Warner and Berry-Lowe, 2006). After 15 days, if $F_v/F_m$ either fell below 0.25 or below 50% of the coral's pre-heat stress $F_v/F_m$, corals were placed into a glass 'recovery' aquarium

within the fiberglass tank, which was kept at 29°C (+/- 0.5°C, daytime), a warm temperature designed to promote shuffling toward *Durusdinium* dominance (Cunning, Silverstein and Baker, 2015). Corals still above these Fv/Fm thresholds were kept in heat stress and I-PAM measurements were taken every two days until Fv/Fm had declined sufficiently. These thresholds were adopted partly based on visual bleaching observations and partly based on previous observations of the same coral species kept in similar husbandry conditions (Cunning, Silverstein and Baker, 2018). After 25 (spring batch) or 22 (fall batch) days in heat stress, the temperature was raised to 33°C (+/- 0.5°C, daytime) to bleach the remaining corals. In the fall batch, the loss of four *O. faveolata* cores was due to an unidentified disease in the heat stress tank in early recovery.

During this stress-hardening process, small (~2mm) tissue samples were clipped from cores and preserved in 1% SDS in DNAB at multiple time points; 'Pre-heat stress' (cores of both treatments were sampled immediately before the start of heat stress), 'Post-heat stress' (manipulated cores were sampled when they were taken out of heat stress and control cores were sampled when heat stress ended), 'Early recovery' (manipulated cores were sampled one month after they were taken out of heat stress), and 'Pre-outplant' (all cores were sampled immediately before they were outplanted in the field for the experiment described in Chapter 3, on average one month after 'Recovery' sampling).

**DNA extraction & quantitative PCR analysis**

**Table 2.2. Ct value corrections.**

| Species | Target | Extraction efficiency | Reference | Copy number correction | Reference | Fluorescence correction | Reference |
|---|---|---|---|---|---|---|---|
| ***M. cavernosa*** | Coral | 0.982 | (Cunning, 2013) | 1 | (Silverstein, Cunning and Baker, 2015) | 3.43 | (Silverstein, Cunning and Baker, 2015) |
| | *Cladocop-ium* | 0.813 | | 50 | | 1.67 | |
| | *Durusdin-ium* | 0.813 | | 3 | | 0 | |
| ***O. faveolata*** | Coral | 0.982 | | 14 | (Cunning, Silverstein and Baker, 2015) | 5.74 | (Cunning, Silverstein and Baker, 2015) |
| | *Breviolum* | 0.813 | | 1 | | 5.41 | |
| | *Durusdin-ium* | 0.813 | | 3 | | 0 | |
| ***S. siderea*** | Coral | 0.982 | | 14 | (Cunning, 2013) | 5.74 | (Cunning, 2013) |
| | *Cladocop-ium* | 0.813 | | 23 | | 4.07 | |
| | *Durusdin-ium* | 0.813 | | 3 | | 0 | |

Aliquots of tissue samples preserved in SDS were subjected to a modified organic extraction protocol (Baker, Rowan and Knowlton, 1997) and then quantitative PCR was performed in a QuantStudio 3 machine (Applied Biosystems) using Taqman Environmental Master Mix (Applied Biosystems) and symbiont clade-specific primers and probes targeting actin genes (Cunning and Baker, 2013; Cunning, Silverstein and Baker, 2015). VIC dye was used for the *Cladocopium* probe, whilst all other probes used a FAM dye. *Cladocopium* and *Durusdinium* targets were run together in a multiplex. Symbiont cell densities were standardized against coral host cell densities, and data will be corrected for differences in DNA extraction efficiency, for differences in actin gene copy number, and for differences in dye fluorescence. Correction numbers used are shown in the table 2.2. Host copy numbers obtained

from primers and probes targeting the actin gene have been found to differ between colonies (Cunning, 2013), and copy numbers will not be calculated for this experiment. However, any resulting inaccuracies in calculations of symbiont to host ratios will only affect between-colony comparisons. Before analysis, raw qPCR data was first run through the StepOneR package (R. Cunning, 2019). Amplifications were filtered to include only those in which 2 of 2 technical replicates amplified with $C_t$ values < 40.

**Statistical analyses**

Data were analysed in RStudio (3.5.1, 2018), and plots were produced using a combination of base R and ggplot2 (H. Wickham, 2016). Symbiont to host ratio (S:H) data were log10 transformed and fitted to generalized linear models and F-statistics generated to determine statistical significance (a p-value of <0.05, <0.01, and <0.001 denoted by '*', '**' and '***' respectively). Symbiont proportion data were arcsine square-root transformed and fitted to generalized linear models with a quasi-poisson distribution, and F-statistics were generated. Paired Wilcoxon tests were used to test differences in medians shown in figure 2.1b. The 'effects' package was used to generate predictions of the amount of shuffling to *D. trenchii* shown in figures 2.5-7, and the 'emmeans' package was used to estimate marginal means between groups. For fitted models shown in figures 2.5-2.7 and supplementary figure 2.3, initial proportion *D. trenchii* was fitted as a predictive variable. However, this was not possible for *M. cavernosa* in figures 2.5 and 2.6, or for the models of corals with no initial *D. trenchii* in figure 2.7, so means plotted for these groups are simply the average observed changes in proportion *D. trenchii*.

## Results

Initial S:H (shown in figure 2.1a) was coral species-dependent ($F(2,110)=80.7$, $p=<2e^{-16}$***), but was not significantly different between batches across all three species ($F(1,109)=0.51$, $p=0.478$). The difference in initial S:H between batches was nonetheless significant in *M. cavernosa* ($F(1,36)=12.5$, $p=0.00113$**), but not in *O. faveolata* ($F(1,33)=6.48$, $p=0.809$) or *S. siderea* ($F(1,38)=0.666$, $p=0.419$). Despite small increases in median *D. trenchii* across *O. faveolata* and *S. siderea* colonies between batches shown in figure 2.1b, paired Wilcoxon tests revealed no significant differences in these medians between batches (*O. faveolata*; $V=12$, $p=0.281$. *S. siderea*; $V=2$, $p=0.093$). The effect of batch on initial proportion *D. trenchii* across all three coral species was also found to lack statistical significance ($F(1,107)=1.18$, $p=0.279$). It is worth re-stating here that the initial proportion *D. trenchii* found in these colonies does not represent a random sample of colonies; as stated under 'Core collection and colony selection', these colonies were selected from a larger subset of colonies based on their symbiont communities.

A drop in photochemical efficiency and a corresponding drop in symbiont density over heat stress (as shown in figure 2.2) is characteristic of bleaching susceptibility, whilst no drop in symbiont density despite a drop in photochemical efficiency is characteristic of bleaching resistance. Figure 2.2 shows that *M. cavernosa* corals collected in October had a lower bleaching resistance than their genotypic counterparts collected in April. When change in photochemical efficiency and batch were fitted as co-predictors of change in S:H, both variables were found to have a significant effect ($F(1,23)=15.8$, $p=0.00068$***, and $F(1,22)=60.5$, $p= 1.29e^{-07}$*** respectively). This suggests that the greater loss of symbionts per host cell in

October is indicative of increased bleaching sensitivity when both batches underwent comparable declines in photochemical efficiency.

Degree Heating Weeks (DHW), as shown in figure 2.4, was calculated as integrated time spent above maximum monthly mean (estimated as 29.5°C from nearby NOAA station FWYF1) (Liu *et al.*, 2014).

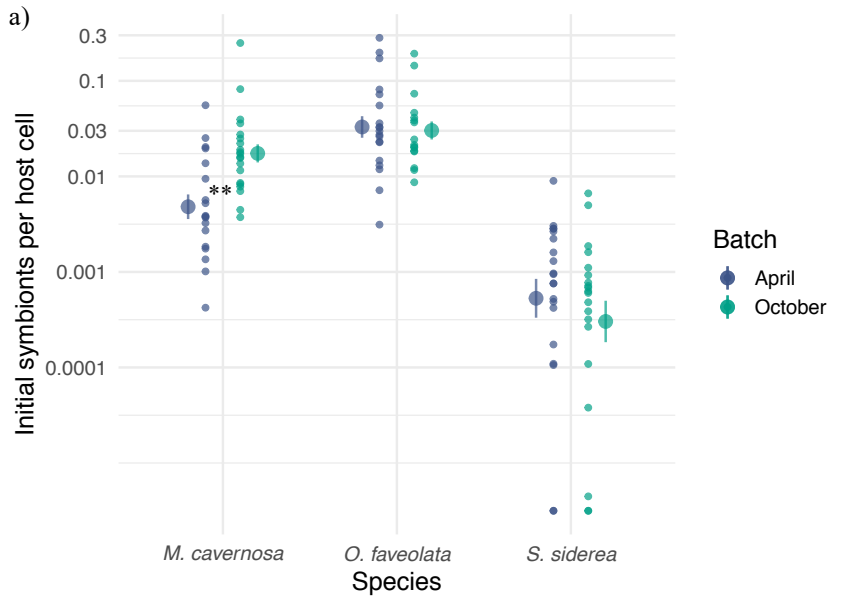



**Figure 2.1a. Seasonal differences in symbiont density.** Individuals cores are plotted with mean +- 1 standard error of S:H depicted by larger points, and '**' denoting a significant difference in mean S:H between batches of *M. cavernosa*.

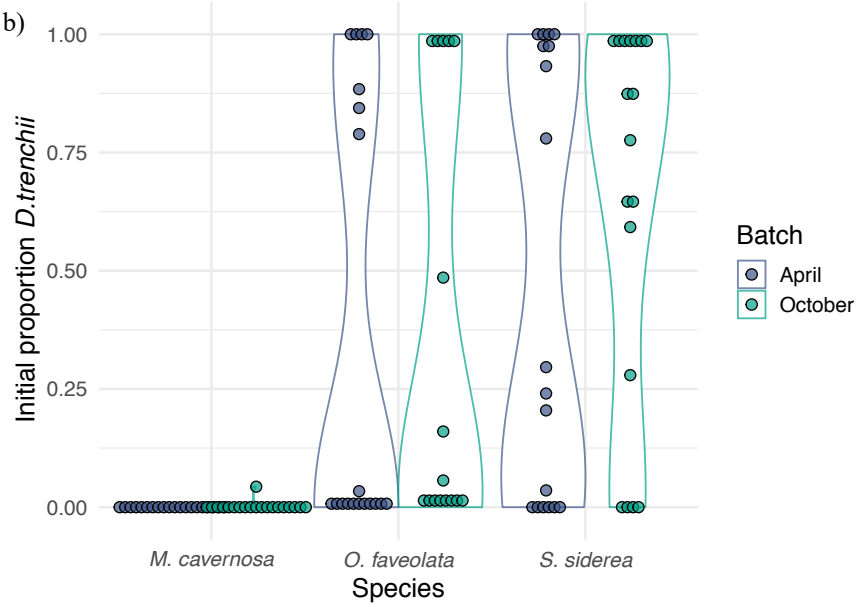



**Figure 2.1b. Seasonal differences in *D. trenchii* dominance.** A violin plot with equal maximum widths for each violin. Dots are binned into 0.033 increments in proportion.

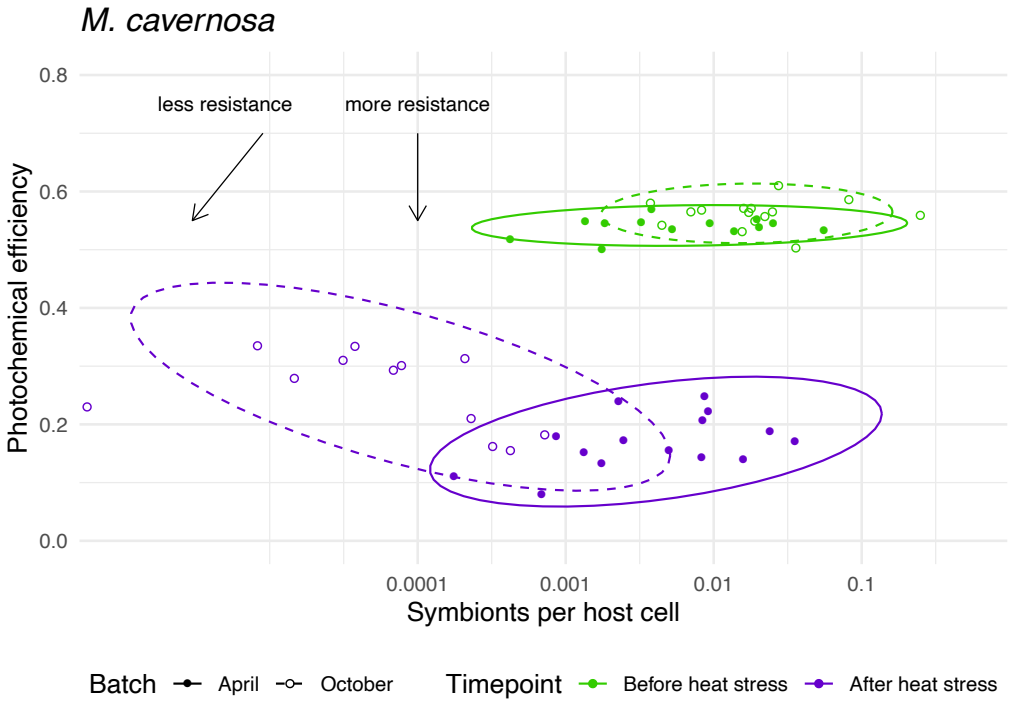
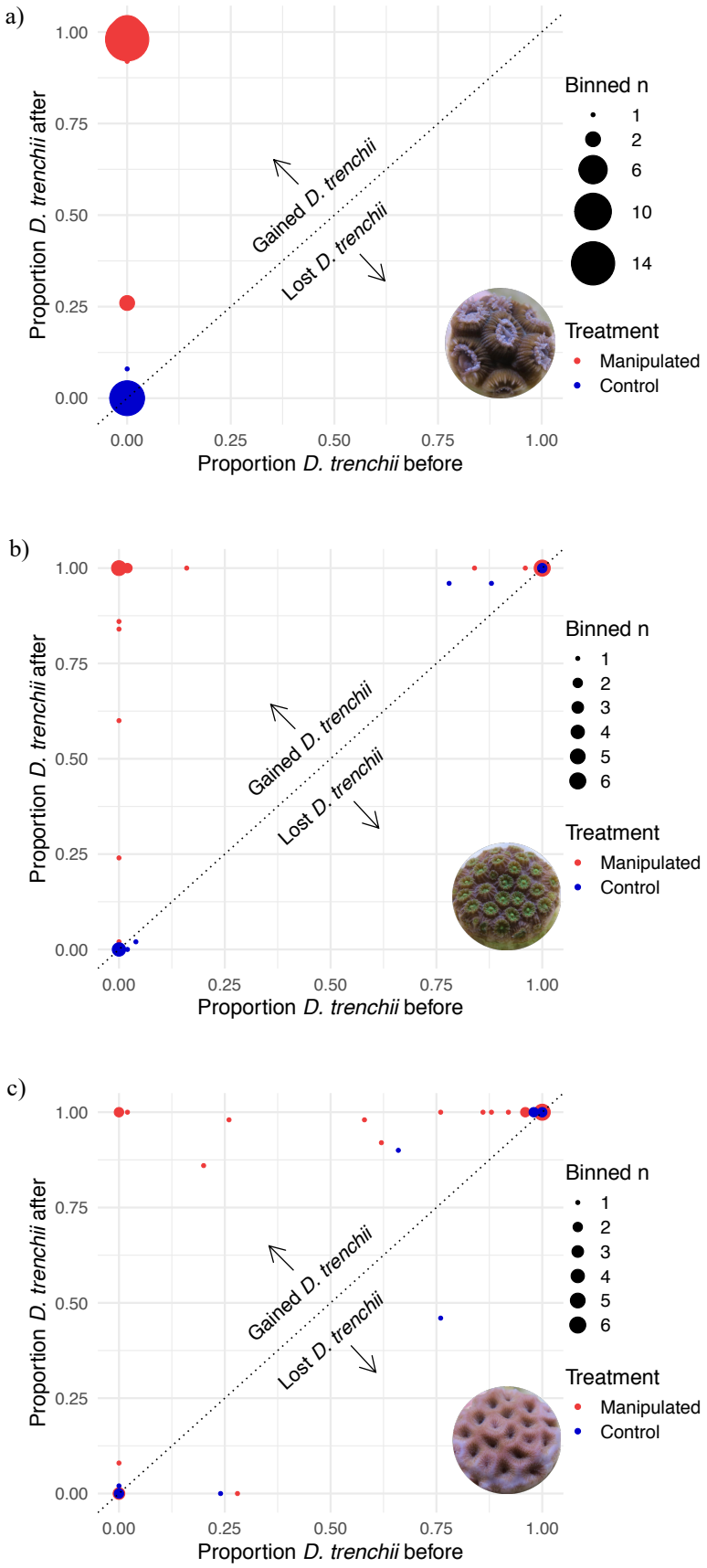



**Figure 2.2. *M. cavernosa* bleaching resistance by season.** The relationship between symbiont loss and photochemical efficiency at the start and end of heat stress for *M. cavernosa*, April and October batches. Ellipses show the 95% confidence interval of data points for each group.




**Figures 2.3a-c. Changes in *D. trenchii* dominance from heat stress.** Proportion *D. trenchii* in cores (April and October batches) before and after heat stress in *M. cavernosa* (a), *O. faveolata* (b) and *S. siderea* (c). Data are grouped into bins of width 0.02 (proportion *D. trenchii*). The tendency for grouping of data around proportions of '0' or '1' is maintained even after recovery from heat stress and symbiont shuffling.

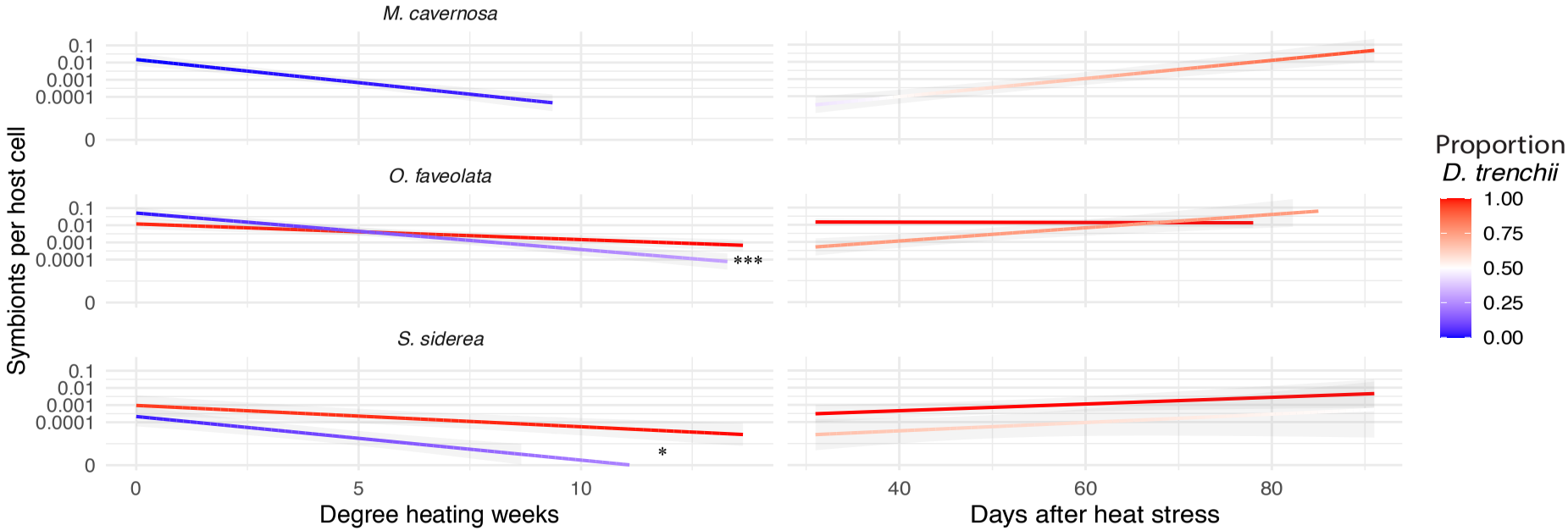



**Figure 2.4. Symbiont community changes over bleaching and recovery.** Models were fitted by species and also by initial symbiont group; lines model corals that were or were not hosting mainly *D. trenchii* before heat stress, and the colour of these lines represents changes in the average proportion *D. trenchii* hosted for that group over bleaching and recovery. Shaded areas represent 95% confidence intervals of the models. Significance stars denote differences in bleaching rates within species depending on initial symbiont group.

In figure 2.4, corals were grouped into those that hosted either <25% *D. trenchii* prior to heat stress, or those that hosted >75% *D. trenchii* prior to heat stress. Due to the tendency of corals to host a dominant symbiont genus with very low 'background' levels of other symbionts (shown by clustering of proportion data around '0' and '1' in figure 2.3), co-dominance was rare, so this excluded only 4.2% of corals from analyses. Note the absence of *M. cavernosa* corals that hosted *D. trenchii* before heat stress, as shown in figure 2.3. By grouping corals by both species and initially dominant symbiont, as in figure 2.4, it can be noted that corals initially not dominated by *D. trenchii* bleached at similar rates between species ($F(2,102)=0.80$, $p=0.45$). Within coral species, *O. faveolata* corals hosting *Breviolum* bleached significantly faster than those hosting *D. trenchii* ($F(1,44)=13.9$, $p=0.0005$***), and *S. siderea* corals hosting *Cladocopium* bleached at a faster rate than those hosting *D. trenchii* ($F(1,40)=4.190$, $p=0.047$*). Recovery rate of corals initially not dominated by *D. trenchii* was also equivalent between species ($F(2,101)=1.61$, $p=0.204$).

Figure 2.3 hints at the degree of shuffling to *D. trenchii* that occurred between these three coral species, dependent on initial proportion. Figure 2.5 quantifies the change in proportion *D. trenchii* present before and after bleaching and recovery, with fitted models based on initial proportion *D. trenchii* shown for *O. faveolata* and *S. siderea.* A generalized linear model accounting for initial proportion *D. trenchii* revealed that species had a significant effect on the amount of shuffling that occurred in bleached and recovered corals ($F(2,71)=9.99$, $p=0.00015$***).

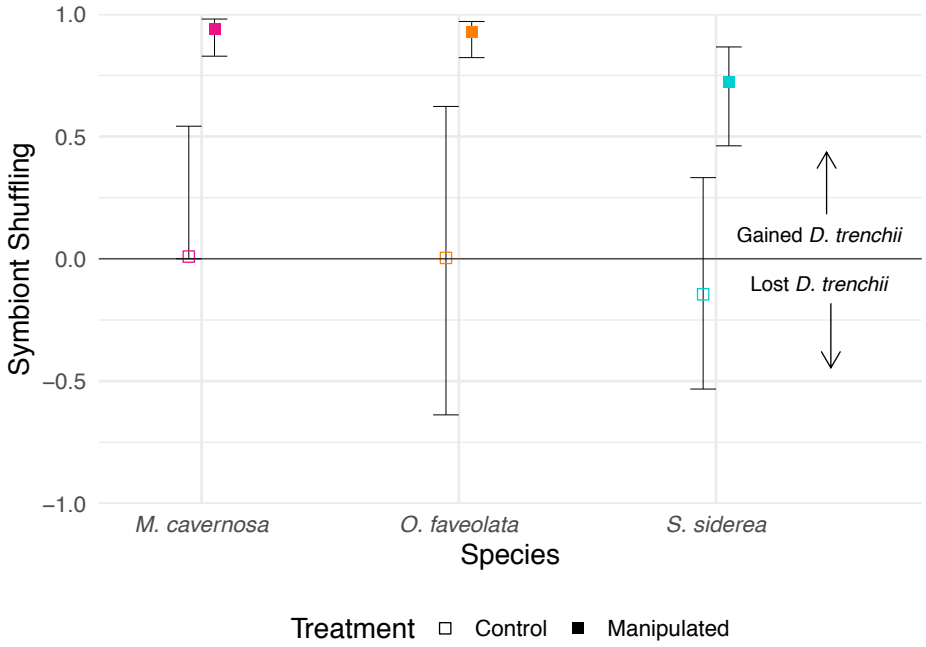



**Figure 2.5. Symbiont shuffling by treatment and species.** The amount of symbiont shuffling (change in proportion *D. trenchii* before and after heat stress and recovery), with treatment, species, (and initial proportion *D. trenchii* for *O. faveolata* and *S. siderea*) as co-predictors. Error bars represent 95% confidence intervals of *M. cavernosa* means, and of *O. faveolata* and *S. siderea* fitted means.

Also of note is the difference in timing of symbiont shuffling between coral species, as shown by the changing line colors of figure 2.4. There was a decrease in proportion *D. trenchii* between the start and end of heat stress in *O. faveolata* and *S. siderea* (as illustrated by a change from blue to purple). In comparison, only 1 of 27 *M. cavernosa* corals contained any detectable *D. trenchii* at the end of heat stress (as illustrated by the consistently blue line). Figure 2.6 further explores these differences in the timing of shuffling over bleaching recovery, showing that some shuffling had already occurred by the end of heat stress in *O. faveolata* and *S. siderea*, whilst all shuffling in *M. cavernosa* occurred later in recovery. The effect of species on the rate of shuffling over recovery was significant ($F(2,201)=11.2$, $p=2.38e^{-05}$ ***).

Figure 2.7 again models cumulative symbiont shuffling over bleaching and recovery, but *O. faveolata* and *S. siderea* corals were separated by whether or not they hosted any detectable *D. trenchii* before heat stress. The rate of symbiont shuffling

across the three timepoints did not differ significantly between *O. faveolata* corals depending of initial presence of absence of *D. trenchii* ($F(1,66)=1.54$, $p=0.220$), as shown by figure 2.7.a. However, the amount of shuffling that had occurred by 2 months of recovery, with initial proportion *D. trenchii* as a co-predictor, was significantly greater if some detectable *D. trenchii* was initially present ($F(1,20)=2.86$, $p=1.827e^{-05}$***). Similarly, initial presence or absence of *D. trenchii* did not affect the rate of shuffling in *S. siderea* ($F(1,58)=0.073$, $p=0.789$), but did increase the cumulative shuffling that had occurred 2 months into recovery ($F(1,23)=16.24$, $p=0.00056$***).

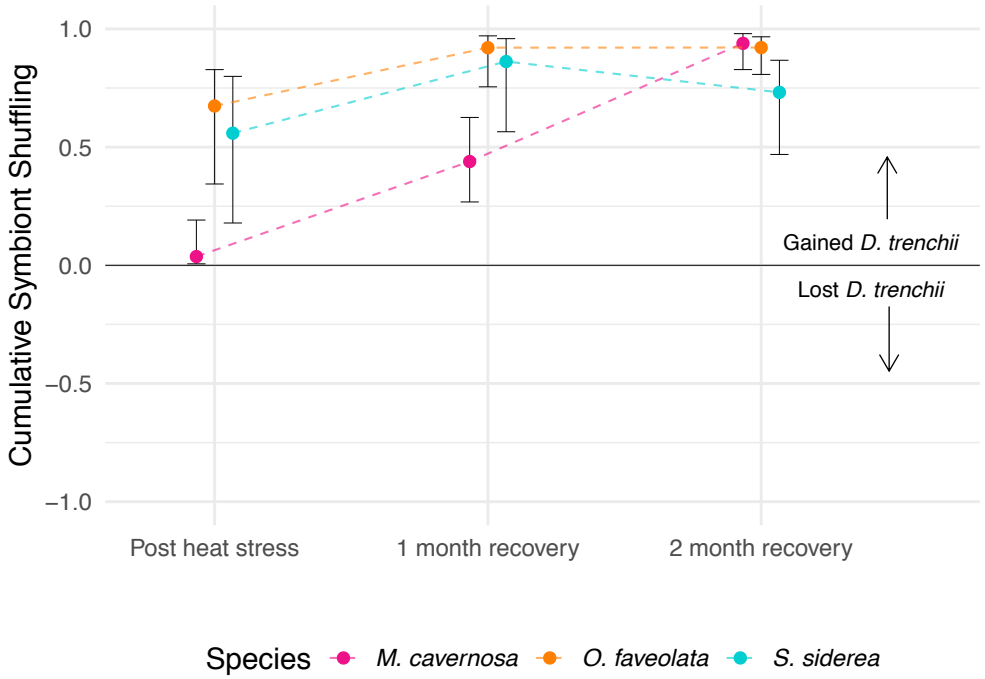



**Figure 2.6. Cumulative symbiont shuffling over bleaching and recovery.** Error bars represent 95% confidence intervals of the fitted models (and of means for *M. cavernosa*). For fitted models, the amount of symbiont shuffling to *D. trenchii* was plotted at each time point after heat stress, relative to initial proportion *D. trenchii*.

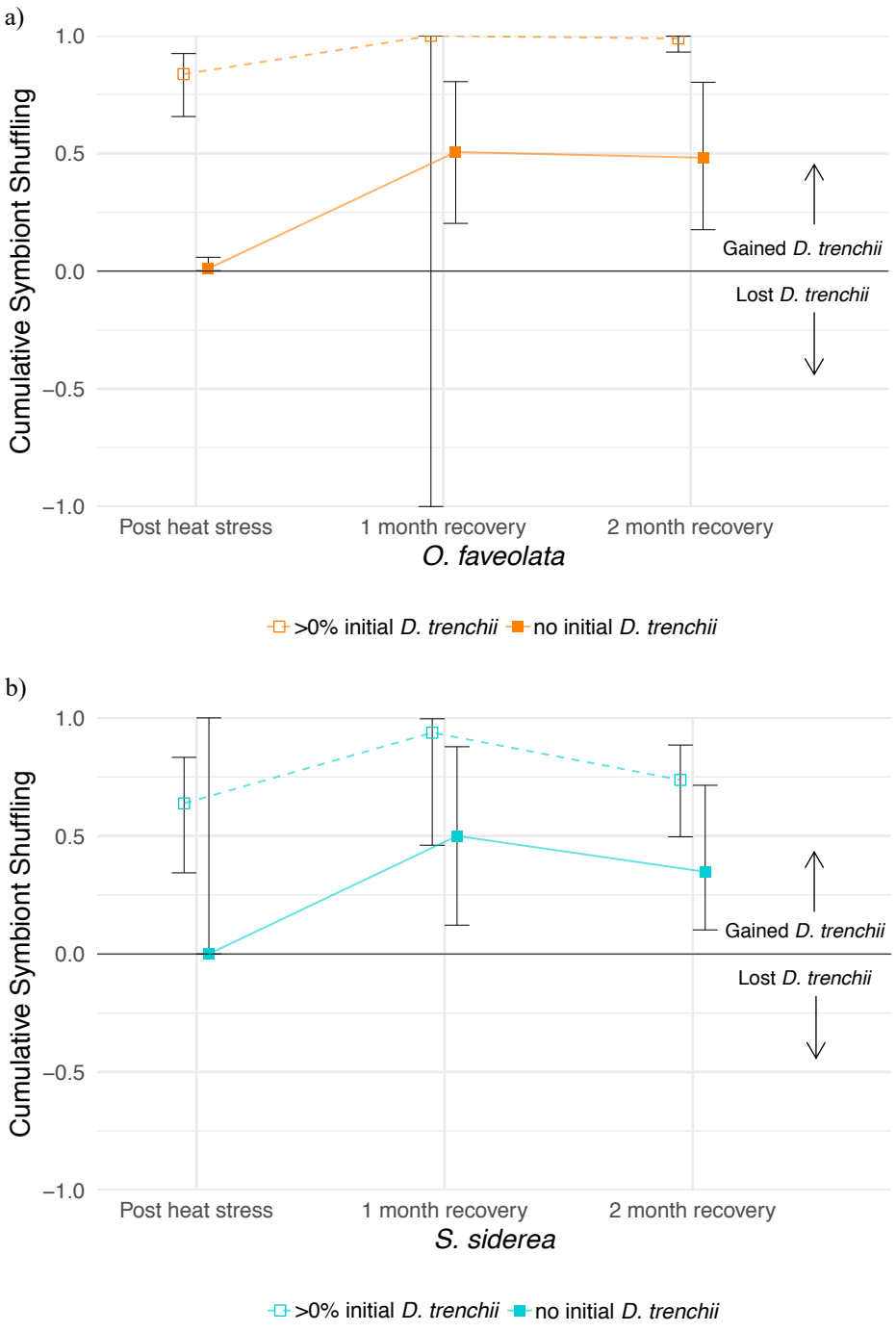



**Figure 2.7a-b. Cumulative symbiont shuffling depending on initial presence of *D. trenchii*.** *O. faveolata* (a) and *S. siderea* (b) data split by those that initially contained no detectable *D. trenchii*, and those that did. Error bars represent 95% confidence intervals of means. Fitted models based on initial proportion *D. trenchii* are shown for those corals containing any initial *D. trenchii*, whilst mean shuffling at each timepoint is shown for those corals hosting no initial *D.*

## Discussion

The seasonal increase in symbiont density observed in *M. cavernosa* between April and October shown in figure 2.1a is consistent with previous studies showing seasonal symbiont density adjustments to differences in light availability and a greater rate of host cell loss (reflected by decreases in host tissue biomass) relative to symbiont cell loss over the course of summer warming (Fitt *et al.*, 2000; Cunning and Baker, 2014). Bleaching sensitivity was also greater in *M. cavernosa* cores collected in October compared to cores collected from the same colonies in April (figure 2.2). This could be linked to a time-lagged effect of low photosynthate transfer in the winter and spring, reflected in annual lows in host tissue biomass (Fitt *et al.*, 2000). Similarly, there may well be intrinsic seasonal fluctuations in host thermal optima (Jurriaans and Hoogenboom, 2020). Very minimal paling of Scleractinia was observed at Emerald Reef between April and October 2019, so increased sensitivity due to recent bleaching is unlikely. Since symbiont density was significantly higher in October cores, it is likely that this played a role, potentially as a result of higher accumulation of reactive oxygen species during heat stress (Cunning and Baker, 2013). The more severe bleaching (greater reduction in symbiont density) in October *M. cavernosa* corals was also associated with increased proportions of *D. trenchii* in recovery (supplementary figure 2.3), supporting previous studies on the effect of bleaching severity on degree of shuffling (Cunning, Silverstein, Baker, 2015).

The host species-specific propensities for recovering with *D.* trenchii, as seen in figures 2.3 and 2.5, have previously been explained by the relative photochemical advantage of *D. trenchii* compared to competing symbionts under ambient and stressful temperatures (Cunning, Silverstein and Baker, 2018). The difference in bleaching rate in *O. faveolata* that hosted *Breviolum* compared to *D. trenchii*, was

greater than the difference in bleaching rate of *S. siderea* hosting *Cladocopium* compared to *D.* trenchii, although both differences were significant (figure 2.4). This may reflect a greater photochemical advantage under heat stress of *D. trenchii* in *O. faveolata* compared to *S. siderea* and explain the greater propensity for shuffling observed in the former. Similarly, there was perhaps a marginally greater photochemical disadvantage of hosting *D. trenchii* at non-stressful temperatures in *S. siderea* compared to *O. faveolata* (supplementary figure 2.2), which again may help explain the shuffling hierarchy between these species.

The increase in the proportion *D. trenchii* in *O. faveolata* and *S. siderea* by the end of heat stress (see figures 2.4 and 2.6) is likely indicative of the differential loss of *Breviolum* and *Cladocopium* compared to *D. trenchii*, since acquisition of novel symbionts during heat stress may be unlikely. In contrast, the proportion *D. trenchii* in *M. cavernosa* did not start to increase until after heat stress. This suggests that either background *D. trenchii* was initially at such low levels in *M. cavernosa* that it was undetectable, or that *M. cavernosa* did not 'shuffle' to *D. trenchii* dominance, but more specifically 'switched' (Baker 2003), acquiring these symbionts from external sources during recovery (Boulotte *et al.*, 2016). These qPCR methods have been shown to detect background *Durusdinium* across eight orders of magnitude (Mieog *et al.*, 2007), which supports the case of exogenous uptake of *D. trenchii* in these *M. cavernosa* corals.

We cannot confidently attribute the delay in shuffling seen in *M. cavernosa* compared to the other two species (figure 2.6) to the exogenous uptake of *D. trenchii*, because of any possible host species-specific effects on the rate of shuffling. We can however, confirm that a lack of background *D. trenchii* does not inhibit recovery with nearly 100% *D. trenchii*. It is also worth noting the contrast seen in *M. cavernosa*,

between its high propensity to host mainly *D. trenchii* in bleaching recovery, and the lack of initial *D. trenchii* found in cores (figure 2.1b). This could reflect instability in these post-recovery *D. trenchii* populations in *M. cavernosa*, and this species' tendency not to host background symbiont populations.

Comparing the changes in symbiont communities within a species depending on the initial presence or absence of *D. trenchii* (figure 2.7), showed that more shuffling occurred in corals initially hosting at least background populations of *D. trenchii*. This, together with the delayed shuffling seen in *M. cavernosa*, suggests that bleaching recovery with dominant *D. trenchii* populations, whilst not dependent on initial background populations, is increased or accelerated by their presence.

Opportunities for continued research connected to this study include using ITS2 MiSeq analysis of ITS2 variants (LaJeunesse, 2002) to allow greater resolution of the origin of shuffled communities. In addition, fluorescent tagging of introduced symbionts would allow researchers to monitor community changes *in vivo*. Fluorescence microscopy is widely used to visualize symbionts within coral tissue, and can even be used to track the uptake, proliferation and spread of Symbiodiniaceae in aposymbiotic *Exaiptasia* (Tivey, Coleman and Weis, 2019). In order to be able to track the movements of a specific population of symbionts however, the insertion of a fluorescent marker or stain into these symbionts that is distinguishable above background host fluorescence will be necessary.

# Chapter 3: Introduction of *Durusdinium trenchii* vectored into coral colonies through tissue implants: A field study

## Overview

The thermally tolerant Caribbean algal symbiont, *Durusdinium trenchii*, is seasonally abundant on reefs, gaining transient dominance across coral species after thermal bleaching events. The fluctuations between *D. trenchii* and competing symbiont genera are thought to be shaped by *Durusdinium*'s success as a hardy opportunist, capable of colonizing host tissue after a disturbance. This study, building on previous laboratory experiments, aimed to test the ability of *D. trenchii* to infiltrate massive field colonies independent of a bleaching disturbance, through the introduction of *D. trenchii* dominated tissue implants. Background levels of *D. trenchii* spread from implants into the surrounding tissue in *M. cavernosa*, displacing resident symbionts. In addition, *D. trenchii* remained dominant in implants for at least 7 months, despite the invasion of other symbionts from the surrounding tissue, even throughout the winter months. This study also provides potential insight into the largely uncharted mechanisms of symbiont transport within colonies. Symbiont exchange to and from implants was aided by, but not dependent on, the healing of implants with surrounding tissue, suggesting a role for both gastrovascular transport, and the release and re-uptake of symbionts by nearby polyps, in symbiont migration. These results provide a proof-of-concept for the vectoring of symbionts into field colonies using tissue implants. This has ramifications not only for the symbiont competition dynamics that shape *D. trenchii*'s abundance on Caribbean reefs, but also provides an

intervention mechanism by which colonies' symbiont communities may be altered *in situ* to increase their resistance or resilience to the stressors of a changing ocean.

## Background

Thermal bleaching is now the biggest cause of coral death globally, and bleaching events are becoming both more frequent and more severe, with annual severe bleaching expected on 90% of the world's reefs by 2055 (van Hooidonk *et al.*, 2014; Hughes *et al.*, 2018). Bleaching is intricately linked to corals' relationships with their photosynthetic algal symbionts (family Symbiodiniaceae), affected by the thermal tolerance of both partners (Berkelmans and van Oppen, 2006; Baird *et al.*, 2009). An understanding of the flexibility and stability of these symbioses within coral colonies is crucial for predicting or manipulating corals' relationships with these symbionts as bleaching events escalate.

The loss of symbionts during bleaching provides an opportunity for corals to recover with shifted symbiont communities (Buddemeier and Fautin, 1993; Baker, 2003). It is common for Caribbean corals to recover from thermal bleaching with the comparatively thermally tolerant *Durusdinium trenchii* (LaJeunesse et al., 2009), and under laboratory conditions corals can be induced to host *D. trenchii* by this method (Cunning, Silverstein and Baker, 2018). Independent of bleaching, *in hospite* symbiont communities go through seasonal fluctuations, with *D. trenchii* becoming more abundant in colonies during the late summer (Thornhill *et al.*, 2006). However, in a matter of several months to two or three years after bleaching events or particularly warm summers, abundant *in hospite D. trenchii* populations have receded completely or to background levels (Thornhill *et al.*, 2006; LaJeunesse *et al.*, 2009). It has been suggested that these dynamics reflect *D. trenchii*'s role as a generalist

opportunist, capable of colonising bleached tissue, which is then outcompeted by successional communities (Toller, Rowan and Knowlton, 2001; LaJeunesse *et al.*, 2009).

Under laboratory conditions, *D. trenchii* can infiltrate larger areas of healthy *Montastraea cavernosa* tissue via tissue implants (Baker *et al.*, in re-submission), which casts doubts on the role of *D. trenchii* as solely an opportunistic coloniser in marginal environments. This implantation method, if it were successful in a field setting, would provide a mechanism by which to 'seed' colonies with certain introduced symbionts. The urgent need for scalable interventions to better equip corals to survive a warming ocean has raised questions over the effectiveness and longevity of symbiont manipulation techniques such as this in a field setting (Committee on Interventions to Increase the Resilience of Coral Reefs, *et al.*, 2019). This is the first known field trial of seeding corals with algal symbionts and expands on the previous laboratory experiments of Baker *et al.* (in re-submission) by experimenting on three common Caribbean species.

The mechanisms by which symbionts migrate through colonies and may spread from tissue grafts in these aforementioned interventions, are not well understood. Studies have confirmed the migration of Symbiodiniaceae through gastrovascular canals in octocorals (Parrin *et al.*, 2016), but in hexacorals our understanding culminates in the identification of symbionts in the gastrovascular canals from histological samples (Gladfelter, 1983). Monitoring the spread of symbionts coinciding with the healing-in of implants would shed light on the importance of gastrovascular transport for symbiont migration. Exogenous uptake of symbionts on very small spatial scales (due to extensive ecological compartmentalisation between corals, sediment and the surrounding water) has also been observed (Cunning, Yost,

*et al.*, 2015), so this mechanism could also conceivably play a role in symbiont seeding from tissue grafts.

## Methods

### Core collection and laboratory manipulation

Two 2.5cm diameter cores were drilled out using a submersible drill (Nemo Power Tools LTD) with a diamond core drill bit, from 10 colonies each of *Orbicella faveolata*, *Siderastrea siderea* and *Montastraea cavernosa* at 7.5 to 8.5 m depth from Emerald Reef (25°40.640' to 25°40.725' N, 80°05.800' to 80°06.030' W), just North of Biscayne Bay National Park, South Florida. Selected colonies were all at least 30cm in diameter and were at least 5 m apart in an effort not to core colonies of the same genotype. Cores were collected at least 15 cm apart on colonies and were drilled from the upper surfaces of colonies to avoid collecting cores with unexpected symbiont compositions as a result of intra-colonial niche partitioning (Rowan et al., 1997). The holes left in colonies were filled with a two-part epoxy putty in order to promote tissue healing.

The 60 cores collected in April 2019 were maintained in 75-gallon indoor tanks as described in Chapter 2. Before any experimental manipulation, cores were randomly assigned to two laboratory treatments; manipulated colonies whose symbiont communities were changed through controlled bleaching and recovery (n=15 per species), or non-manipulated controls (n=5 per species), as illustrated in Figure 3.1a. Cores were either maintained at 27°C, or heat-stressed at 32°C and then 33°C until their photochemical efficiency had reduced by 50% and then recovered at 29°C to promote the retention of *D. trenchii*, as detailed in the methods section of

chapter 2. Cores were taken back out into the field in August 2019, on average 2 months after they came out of heat stress.

**Implantation**

Two outplanting treatments were employed: 15 cores of each species were returned to the holes from which they were originally extracted (temporary epoxy plugs were removed beforehand), and the remaining 5 were outplanted on small mounds of epoxy next to their original colonies, to control for the influence of the surrounding self tissue on retention of *D. trenchii* in cores. Of the laboratory-manipulated cores, 5 cores of each species were randomly assigned to this 'isolated outplanting' field treatment, as illustrated by Figure 3.1a. For implantation, the epoxy that had filled the core holes was removed using a drill, then the cores inserted and secured using small amounts of epoxy putty. Historically, the seasonal maximum temperature at Emerald Reef is approximately 31°C in August at the time of summer outplanting, and the seasonal minimum of approximately 24°C is reached in February at winter outplanting (NOAA historical sea surface temperature, from nearby Fowey Rocks). A HOBO data logger was deployed at time of outplanting at Emerald Reef to track water temperature.

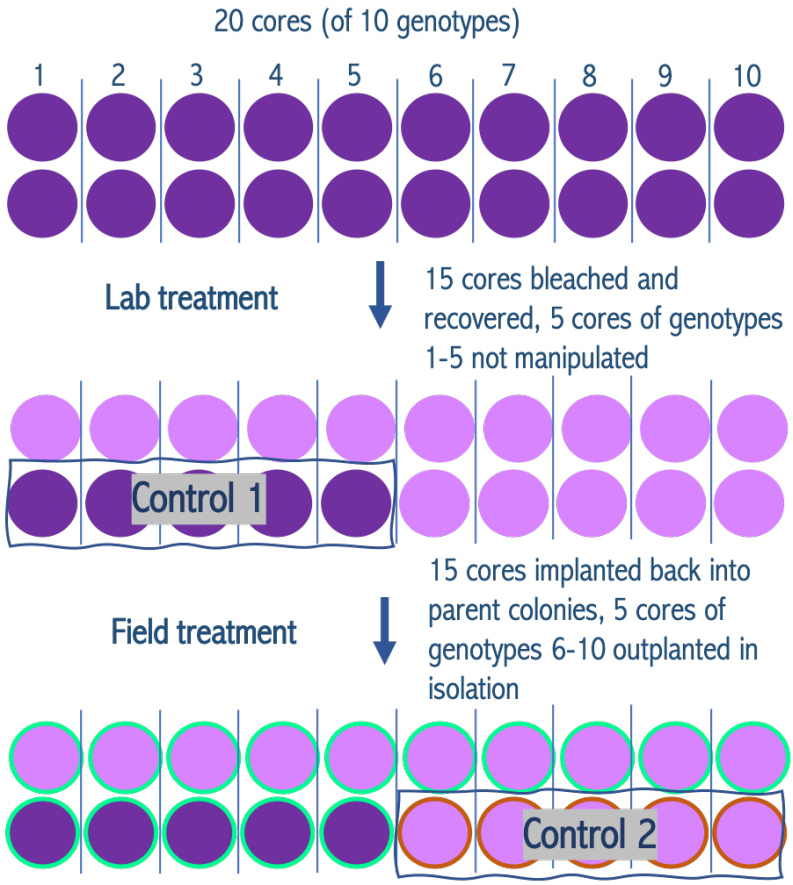



**Figure 3.1a. Treatment allocation, repeated for each species.**

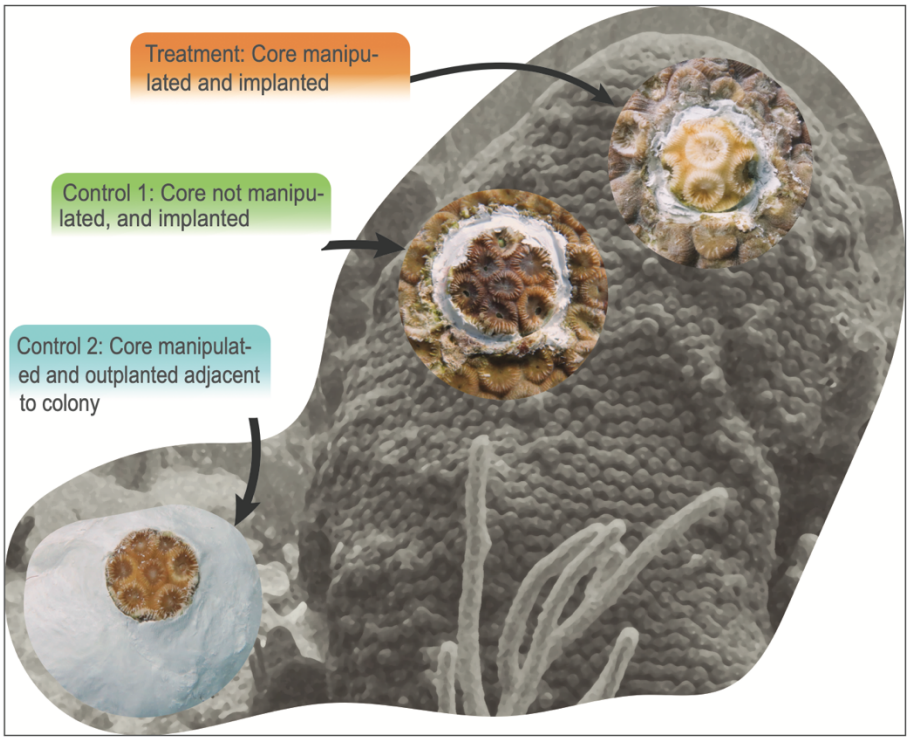



**Figure 3.1b. Illustration of field implantation and outplanting methods.**

**Colony tissue sampling**

Manipulated and non-manipulated cores and their respective colonies were sampled at time of outplanting, and then approximately every seven weeks thereafter for a total of 28 weeks. Small (~2mm) tissue samples were removed using nail clippers and preserved in 1% SDS in DNAB. DNA extraction and quantitative PCR analysis followed the protocols described in Chapter 2. For each sampling time-point, one sample was taken from near the center of each core (both implanted and isolated), and 8 samples were taken along 'mini-transects' on colonies, radiating out from implanted cores. Figure 3.2 shows that four samples were taken 1.25cm from the edge of the implant site and another four taken 2.5cm from the implant. These distances were chosen with the aim of capturing the leading edge of any symbiont spread, based on a previous laboratory tissue-grafting study, (Baker *et al.*, in re-submission), which documented the spread of symbionts by 2.5 cm from smaller (1cm) cores over an 8-month period. 13 weeks after implantation, some of the implants had started to heal back into their colonies such that there were points at which implant tissue had visibly joined with surrounding tissue. Healing was, in most cases, not uniform around the circumference of implants. Mini-transects sampling was adjusted in response to implant healing.

Implants that had not healed back into colonies were sampled along the four cardinal directions (as shown in the top left-hand panel of Figure 3.2), and these axes were rotated clockwise by 10° every time these colonies were sampled (as illustrated in the top right-hand panel of Figure 3.2) to avoid re-sampling scarred tissue. This variation in colony sampling location between timepoints also helped to capture the potential mosaic distribution of different symbiont genera across colonies (and particularly *O. faveolata*, (Kemp *et al.*, 2014, 2015). In cases where implants had

started to heal (as in the *S. siderea* implant pictured in the bottom left-hand panel of Figure 3.2), the mini-transects were rotated in order to sample tissue immediately adjacent to points of healing. In the example in Figure 3.2, the sampling transect was shifted so that 'West' samples were taken close to where the implant had started to heal.

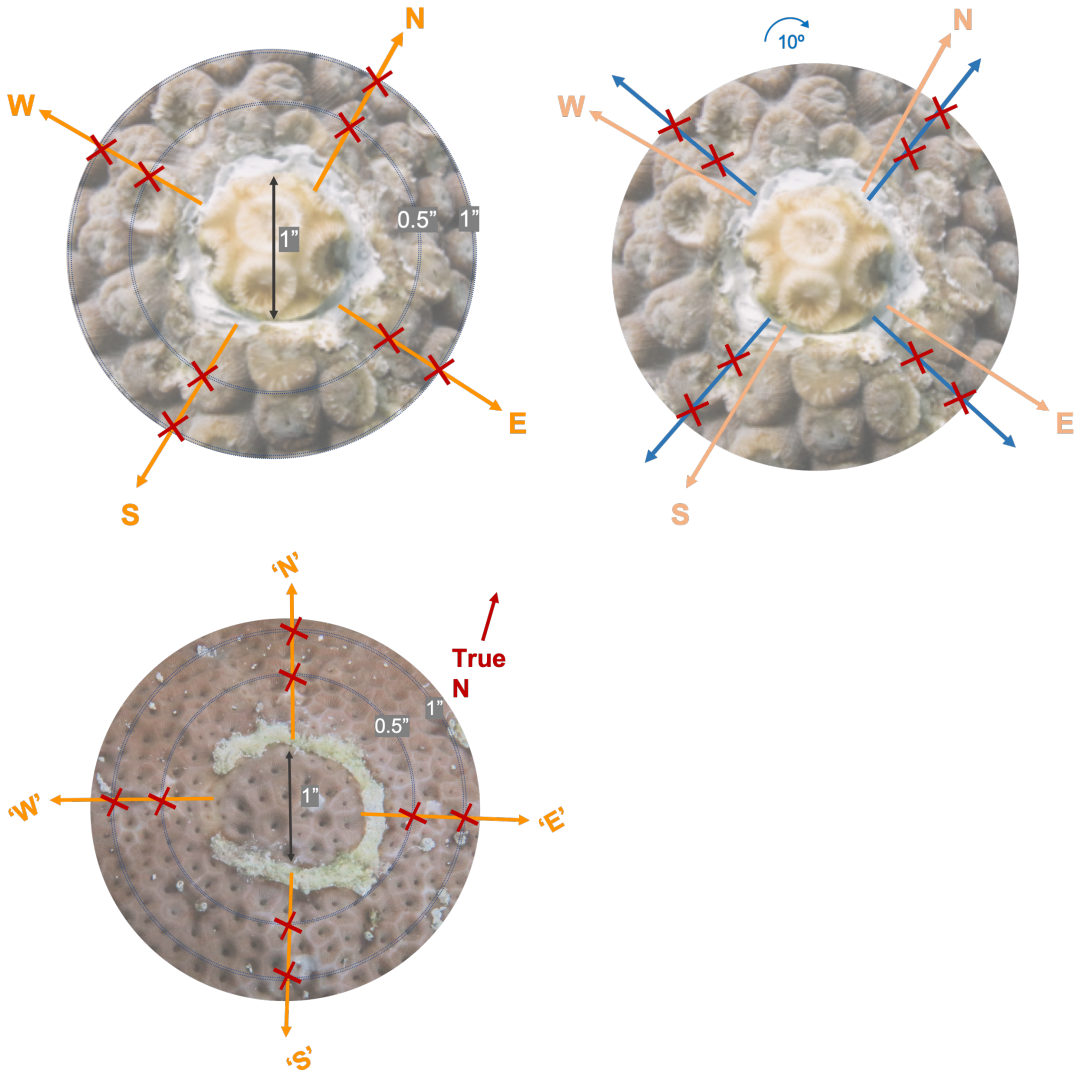



**Figure 3.2. Illustration of 'mini-transect' sampling methods.** Red 'X's indicate points at which tissue samples were taken around implants.

**Statistical analyses**

Data were analysed in RStudio (3.5.1, 2018), and plots were produced using a combination of base R and ggplot2 (H. Wickham, 2016). Symbiont to host ratio (S:H) data were log10 transformed and fitted to generalized linear models and F-statistics generated to determine statistical significance (a p-value of <0.05, <0.01, and <0.001 denoted by '*', '**' and '***' respectively). Symbiont proportion data were arcsine square-root transformed and fitted to generalized linear models with a quasi-poisson distribution, and again F-statistics were generated. The absolute abundance of a certain symbiont genus per host cell was estimated from the ratio of that symbiont to host generated by qPCR and was also log10 transformed. For models incorporating data from 4 samples at 1.25 cm or 2.5 cm from implant margins, the implant around which samples were taken was fitted as a random effect in a mixed model (to avoid pseudoreplication associated with incorporating multiple data-points from the same implant), produced using the 'lme4' package. F-tests based on Satterthwaite's method were performed on these mixed models to assess statistical significance of variables, using the package 'lmerTest'.

## Results

The degree to which laboratory manipulation of cores induced symbiont community change in favor of *D. trenchii* varied by coral species, as described in Chapter 2. In light of this, only implants that switched from <60% *D. trenchii* before heat stress to >90% *D. trenchii* after recovery (together with their respective unmanipulated and outplanted genotypic controls) were included in the analyses presented here. Of the 10 manipulated and implanted cores of each species, these thresholds included 9 *M.*

*cavernosa* implants, 4 *O. faveolata* implants and 4 *S. siderea* implants, generating a total of 17 manipulated implants, 7 non-manipulated implants and 9 manipulated outplants.

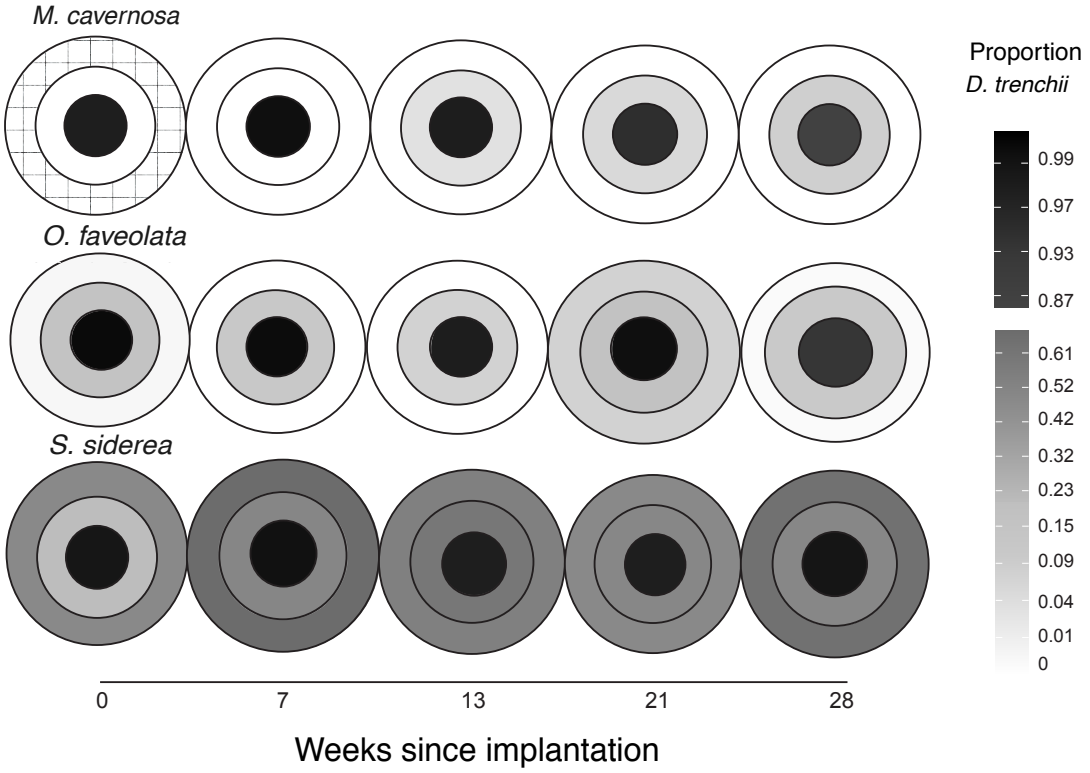



**Figure 3.3. Pictogram of proportion *D. trenchii* changes.** Circles (from smallest to largest) represent 2.5cm implants, 1.25cm sampling zone surrounding implants and 2.5cm sampling zones surrounding implants. Proportions were arcsine square-root transformed and fitted to an arcsine square-root colour scale, with equivalent untransformed proportion values provided. The hashed 2.5cm sampling zone at implantation for *M. cavernosa* represents missing data.

The manipulated cores retained most of their *D. trenchii* dominance after implantation, as shown in figure 3.3 and figure 3.7, although the proportion in *M. cavernosa* cores decreased significantly over time ($F(1,41)=7.02$, $p=0.011$*) from 98% to 86% over 28 weeks. However, there was no significant change in the proportion of *D. trenchii* in *O. faveolata* ($F(1,17)=2.66$, $p=0.121$, from 99.8% to 93%) or *S. siderea* cores ($F(1,17)=0.123$, $p=0.730$, remained at 99%). Similarly, figure 3.3

and 3.4 show the proportion of *D. trenchii* detected 1.25 cm from implant margins, which increased significantly over time in *M. cavernosa* (F(1,163)=8.91,p=0.0033**, from 0 to 7.8%), but not in *O. faveolata* (F(1,70)=0.218,p=0.642, from 16% to 10%) or *S. siderea* (F(1,71)=2.34, p=0.131, from 20% to 49%). The lack of a detectable effect in *O. faveolata* (and to a lesser extent in *S. siderea*) may be explained by greater variation in the change in the proportion of *D. trenchii* between colonies (F(3,67)=4.33, p=0.0075**, and F(3,68)=2.42, p=0.073, for *O. faveolata* and *S. siderea*, respectively), in comparison to *M. cavernosa* (F(8,155)=0.90, p=0.515). At 2.5 cm, there were no significant changes over time in any of the three species (*M. cavernosa*(F(1,94)=0, p=1), *O. faveolata* (F(1,39)=0.00215, p=0.963), *S. siderea* (F(1,59)=0.508, p=0.479)), as shown in figure 3.4.

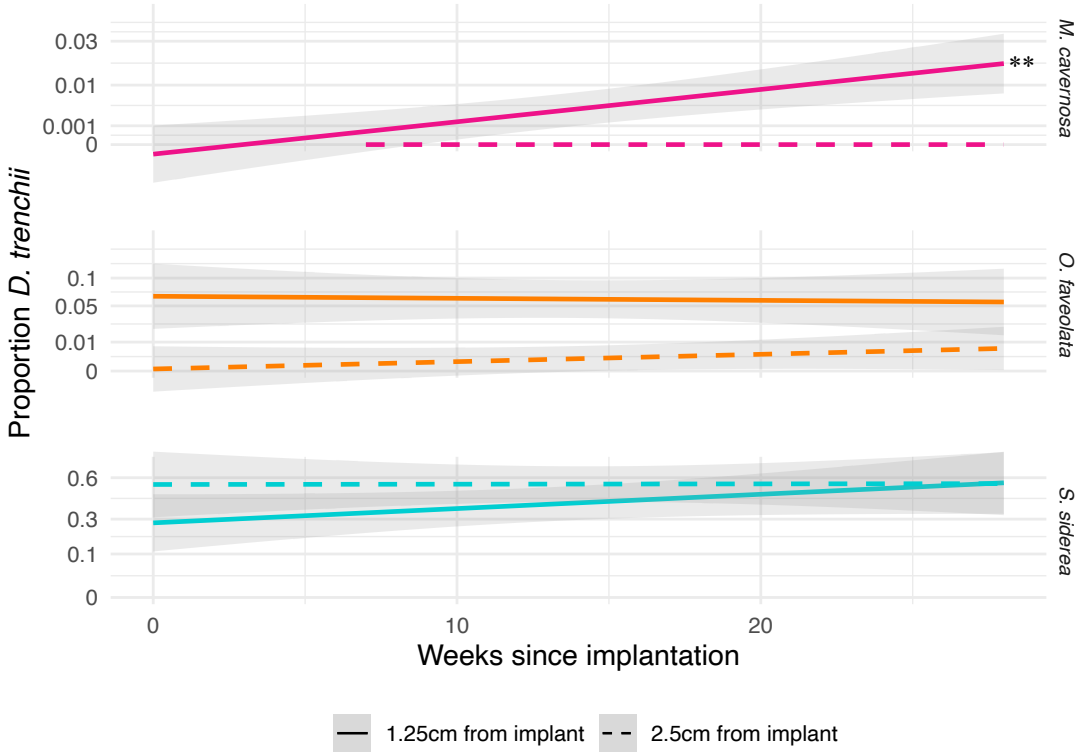



**Figure 3.4. Change in *D. trenchii* dominance over time along 'mini-transects'.** Shaded areas around fitted lines represent the 95% confidence intervals of the models. Arcsine square-root transformed data were plotted on an arcsine square-root scale, but equivalent untransformed proportion axis labels are provided for ease of interpretation. Note the scale differs between facets. The annotation '**' indicates a significant change in proportion *D. trenchii* at 1.25cm in *M. cavernosa*.

Comparing the effect of implanting manipulated cores with the effect of implanting non-manipulated cores into the same colonies reveals the effect of laboratory manipulation of symbiont communities, controlling for the implantation process. Figure 3.5 shows that the influence of implant treatment on the proportion *D. trenchii* 1.25 cm around manipulated and control implants was significant in *M. cavernosa* ($F(1,8)=22.8$, $p=0.0014$**), with higher proportions of *D. trenchii* detected in tissue surrounding manipulated implants. In contrast, the apparent decrease in the proportion of *D. trenchii* surrounding control implants was not statistically significant ($F(1,97)=3.5$, $p=0.063$), indicating that implantation by itself had no effect. Differences in the rates of change between treatment were not significant for *O. faveolata* ($F(1,41)=0.00039$, $p=0.984$) or for *S. siderea* ($F(1,41)=0.0048$, $p=0.945$), echoing the lack of significant effect shown in figure 3.4 for these species.

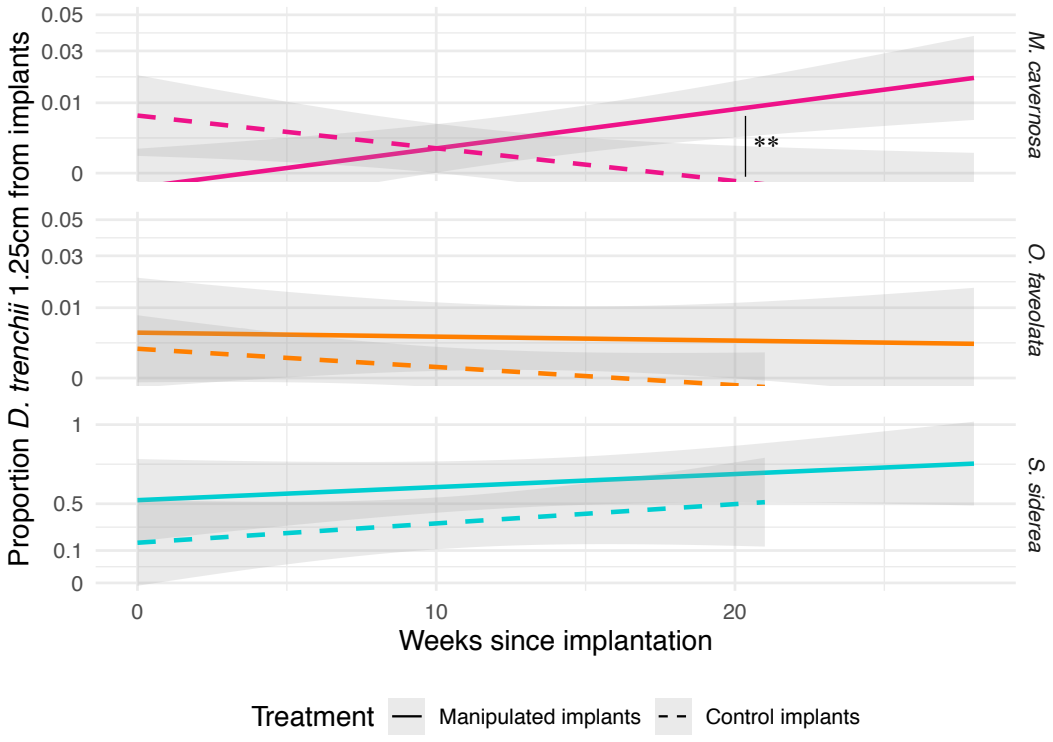



**Figure 3.5. Changes in dominance in *D. trenchii* over time around implants manipulated to host *D. trenchii* compared to non-manipulated controls.** Note *S. siderea* models are shown on a different scale with higher proportions. Only genotypic controls of manipulated cores are included here. Shaded areas represent 95% confidence intervals around fitted models. The '**' in the *M. cavernosa* top panel denotes a significant effect of core treatment on the surrounding proportion *D. trenchii*.

In order to distinguish changes in proportion *D. trenchii* caused by the loss of resident symbiont genera, from changes in proportion caused by the invasion of *D. trenchii*, figure 3.6 shows the absolute abundances of symbionts surrounding manipulated implants. The change in abundance of *D. trenchii* over time was significantly different from that of *Cladocopium* in *M. cavernosa* ($F(1,330)=94.9$, $p<0.0001$***. Independently, both the increase in *D. trenchii* abundance and the decrease in *Cladocopium* abundance were found to be significant ($F(1,161)=18.3$, $p<0.001$*** and $F(1,161)=114.6$, $p<0.001$***, respectively), suggesting that both of these effects contributed to the increase in the proportion of *D. trenchii* shown in figure 3.4. Change over time between *D. trenchii* and *Breviolum* spp. in *O. faveolata* was not different ($F(1,141)=0.072$, $p=0.788$), nor was that between the abundance of *D. trenchii* and of *Cladocopium* spp. in *S. siderea* ($F(1,144)=1.787$, $p=0.185$).

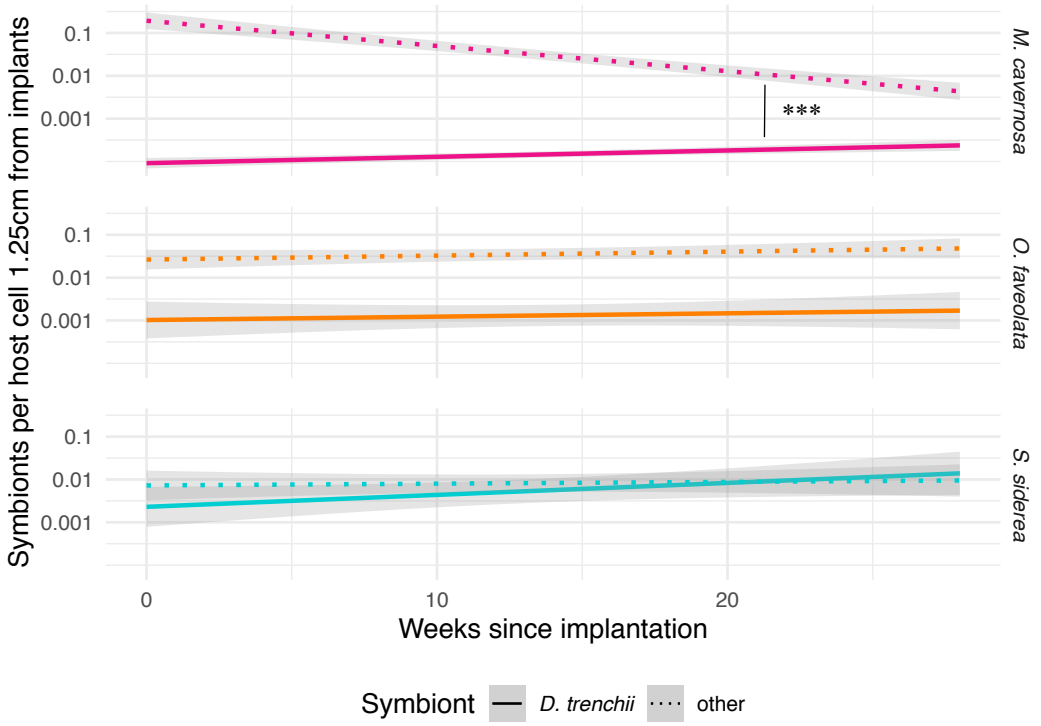



**Figure 3.6. Changes in abundance of *D. trenchii* compared to competing symbionts over time around implants.** The amount of *D. trenchii* per host cell was plotted against the amount of Cladocopium spp (for *M. cavernosa* and *S. siderea*) or *Breviolum* spp (for *O. faveolata*). Shaded areas represent 95% confidence intervals around fitted lines. In the top *M. cavernosa* panel, '***' indicates the significantly different changes in *D. trenchii* and *Cladocopium* abundance over time.

Figure 3.7 illustrates that the effect of outplanting or implantation on the change in *D. trenchii* dominance in cores has a significant effect across all species ($F(1,111)=11.08$, $p=0.0012$**), and that this effect differs between species: For *S. siderea* ($F(1,22)=15.25$, $p=0.00076$***), the decrease in proportion is greater in implants than in outplants, but this does not hold for *M. cavernosa* ($F(1,54)=2.05$, $p=0.158$) and *O. faveolata* ($F(1,29)=2.01$,$p=0.167$). These decreases in the dominance of *D. trenchii* in implants shown in the top panel of figure 3.7 could be attributed to either greater increases in the abundance of competing symbionts relative to *D. trenchii* in implants, greater decreases in *D. trenchii* abundance, or to a combination of the two. In contrast to the changes in proportion *D. trenchii* between implants and outplants, the changes in abundance of *D. trenchii* were not significantly different between implants and outplants across all three species ($F(1,73)=0.49$, $p=0.486$). This lack of effect holds true for each species individually; *M. cavernosa* ($F(1,31)=0.147$, $p=0.704$), *O. faveolata* ($F(1,24)=0.058$, $p=0.811$), *S. siderea* ($F(1,12)=3.537$, $p=0.085$). The effect of implanting or outplanting on the change in *Breviolum* or *Cladocopium* abundance is also insignificant across all species ($F(1,73)=2.616$, $p=0.111$), and for individual species; *M. cavernosa* ($F(1,31)=0.314$, $p=0.579$), *O. faveolata* ($F(1,24)=0.730$, $p=0.401$), *S. siderea* ($F(1,12)=1.44$, $p=0.253$). The lack of significance of core treatment on both the change in *D. trenchii* abundance and on competing symbiont abundance independently, suggests that the significant differences in proportion *D. trenchii* are derived from a combination of these two smaller effects.

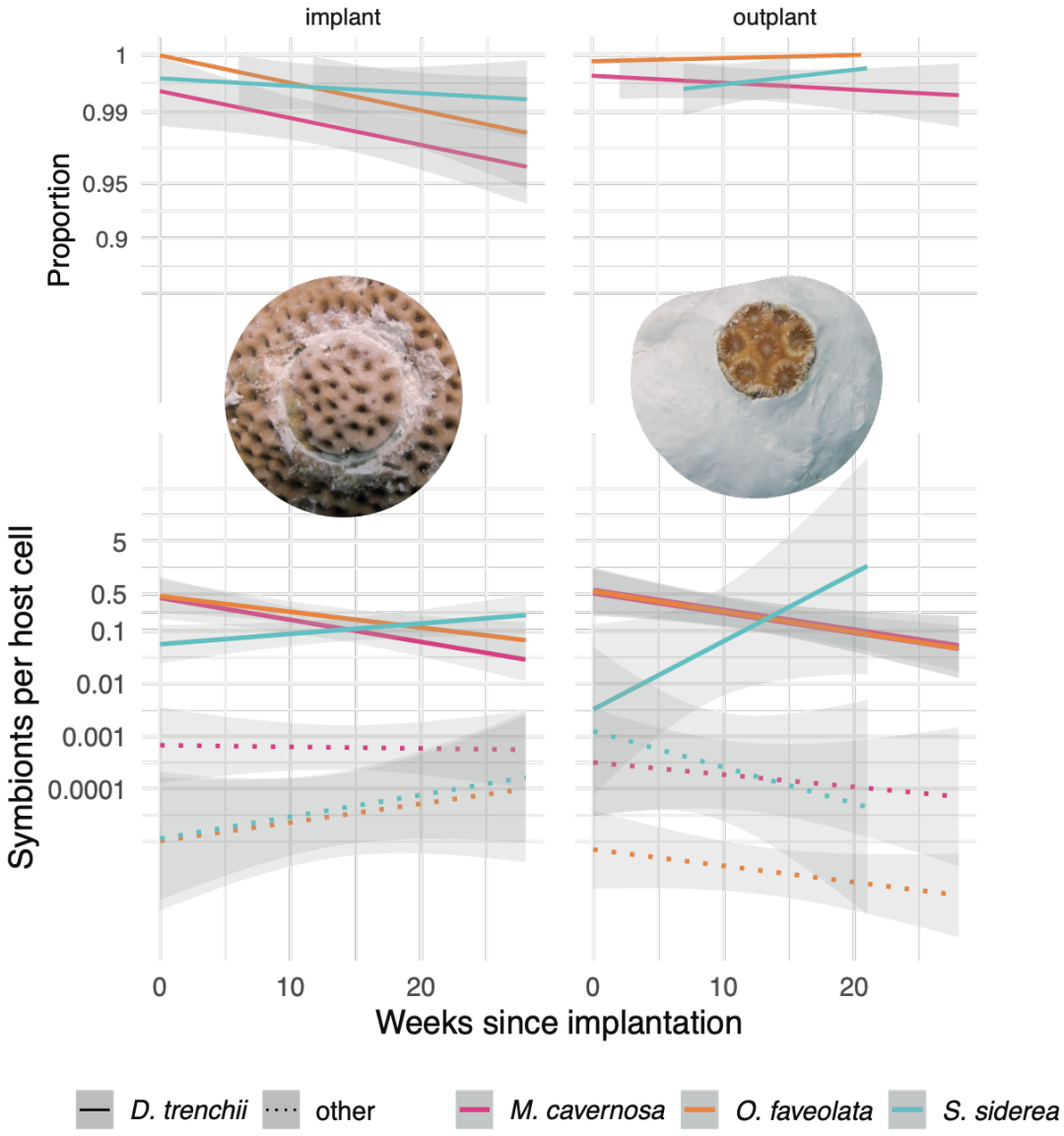



**Figure 3.7. Changes in proportion and abundance of symbionts in outplants compared to implants.** Shaded areas represent 95% confidence intervals around fitted lines.

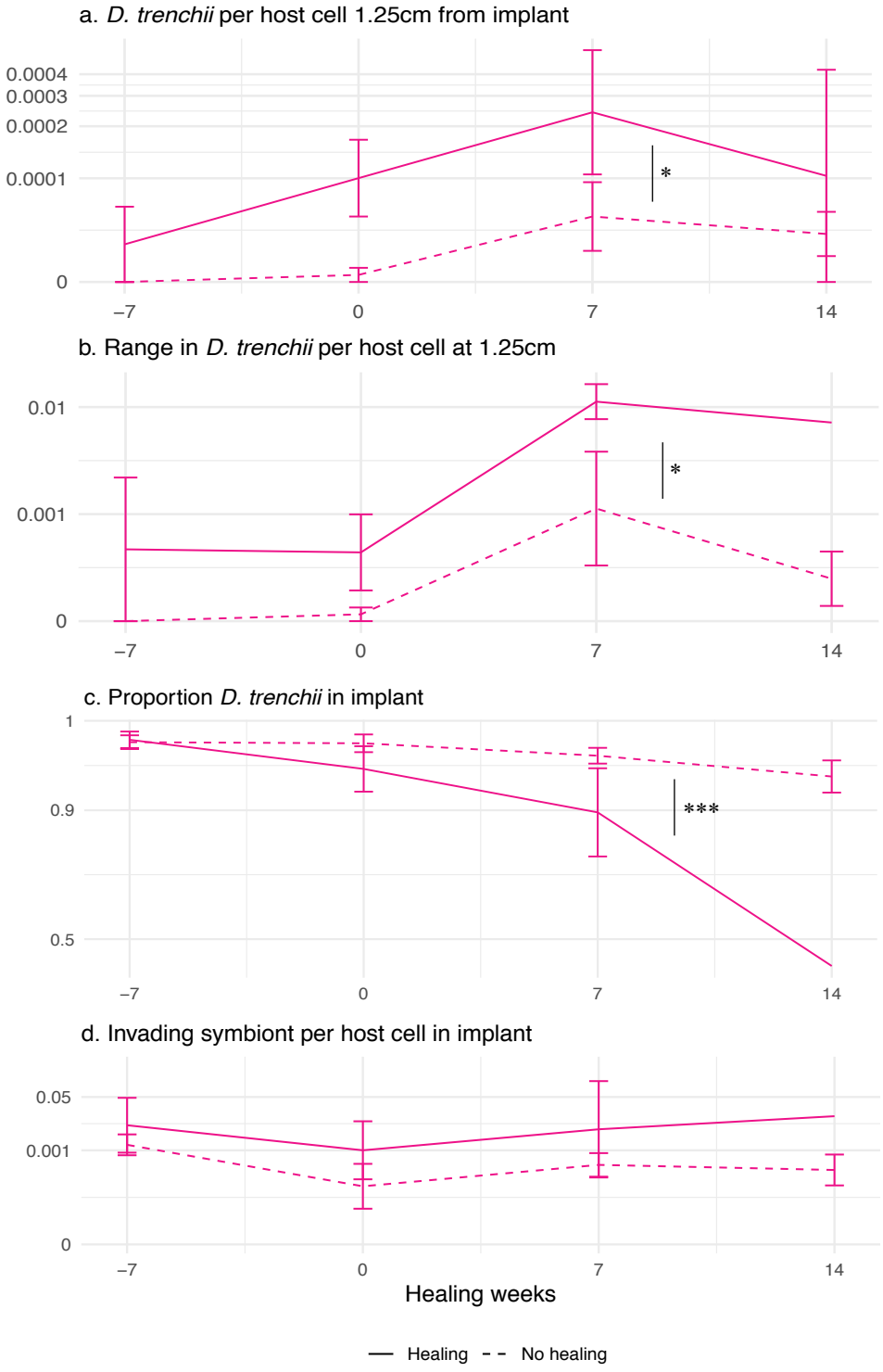
Figures 3.8a-d show the effects of healing on symbiont transfer to and from implants. These data are plotted against the time at which any healing (visible joining of tissue) was observed, rather than time since implantation as in previous plots. Unhealed implants were plotted against the average healing time-frame of healed implants. Healing occurred at a faster rate in *O. faveolata* and *S. siderea* (see supplementary figure 3.2), such that by 28 weeks after implantation, only one implant of each species had not healed, limiting any conclusions based on the effect of healing

on symbiont spread. Because of this, together with a larger effect of implantation treatment, only *M. cavernosa* data are shown here (see Supplementary Figures 3.3a-d for corresponding *O. faveolata* and *S. siderea* plots).

Between healed and unhealed implants, healing significantly increased the total amount of *D. trenchii* that spread from implants ($F(1,7)=11.9$, $p=0.0108$*), but the rate of spread was similar regardless of healing ($F(1,122)=0.855$,$p=0.357$), as shown in figure 3.8a. Healing also generated a wider range in *D. trenchii* abundance across 4 sampling points at 1.25cm surrounding each *M. cavernosa* implant ($F(1,7)=6.24$, $p=0.0411$*). Since all *M. cavernosa* implants which exhibited any healing were all only partially healed (not healed around their entire circumference), as shown in supplementary figure 3.2, it can be inferred that partial implant healing created a spatial inequality in *D. trenchii* around the implant.

Healing affected *M. cavernosa* implant symbiont communities in addition to those symbionts in the surrounding tissue. Healing caused a significantly greater decrease in the proportion *D. trenchii* in implants, and the rate of decrease was also greater ($F(1,30)=16.4$, $p=0.00035$***, and $F(1,29)=24.3$, $p=3.05e-05$***). However, this cannot be attributed exclusively to the invasion of *Cladocopium* into implants, since whilst healing apparently facilitated more *Cladocopium* to enter implants (as seen in Figure 3.8d), this effect was not statistically significant ($F(1,29)=3.44$, $p=0.074$).

**Figure 3.8a. The effect of healing on the spread of *D. trenchii* from *M. cavernosa* implants.** The '*' indicates a significant effect of healing on the proportion *D. trenchii* 1.25cm from implants.
**Figure 3.8b. The effect of healing on unevenness in *D. trenchii* abundance surrounding *M. cavernosa* implants.** the '*' indicates a significant effect of healing on the range of *D. trenchii* detected across 4 sampling locations.
**Figure 3.8c. The effect of healing on the proportion *D. trenchii* in *M. cavernosa* implants.** '***' indicates a significant effect of healing on decrease in proportion *D. trenchii*.
**Figure 3.8d. The effect of healing on the spread of competing symbionts into *M. cavernosa* implants.**


a. D. trenchii per host cell 1.25cm from implant
0.0004
0.0003
0.0002
0.0001
0
*
−7
0
7
14
b. Range in D. trenchii per host cell at 1.25cm
0.01
0.001
0
*
−7
0
7
14
c. Proportion D. trenchii in implant
1
0.9
0.5
***
−7
0
7
14
d. Invading symbiont per host cell in implant
0.05
0.001
0
−7
0
7
14
Healing weeks
Healing
No healing

## Discussion

The statistical significance of *M. cavernosa* core treatment on the surrounding proportion *D. trenchii* (as seen in figure 3.5) is strong evidence that *D. trenchii* can be vectored into *M. cavernosa* colonies by the implantation of *D. trenchii*-dominant cores. The same cannot be claimed however, for *O. faveolata* or *S. siderea*, which raises interesting questions about the symbiont community structures of these three species.

The increase in proportion *D. trenchii* detected around implants of *M. cavernosa* was from 0 to <8% after 28 weeks (figure 3.3 and 3.4). The proportion of *D. trenchii* in field colonies was already higher than this at implantation in *O. faveolata* and *S. siderea*. It is therefore possible that these small background increases in dominance would have been swamped by resident *D. trenchii* and left undetected in *O. faveolata* and *S. siderea*. In addition to consistently more *D. trenchii* detected at implantation, these species also typically host more mottled symbiont communities both within and between individual colonies (Rowan and Knowlton, 1995; Kemp *et al.*, 2015), as exemplified in this study, which could again increase the noise-to-signal ratio of implant treatment. There is also an argument to make concerning the background *D. trenchii* niche space across these three species, when considering the results shown in figure 3.4. Certain *Durusdinium* and *Cladocopium* ITS2 types have been shown to exclude each other from co-occurring at background levels (Wepfer *et al.*, 2020). The detection of *D. trenchii* in *O. faveolata* and *S. siderea* colonies at implantation indicates that these background niches were already occupied, and therefore may have been less receptive to invasion from implant symbionts. This begs the questions of whether an implantation study such as this would see a larger effect if conducted on reefs subjected to less disturbance (in the form of bleaching and disease

for example), than those of Florida, which may well have smaller initial *in hospite D. trenchii* populations.

It is also worth noting here the higher proportion *D. trenchii* found 1.25cm from the core extraction site at time of implantation, compared to at 2.5cm in *O. faveolata* (figure 3.4). This may hint at an effect of injury from drilling and lesion healing on the proportion *D. trenchii*, but this is hard to reconcile with knowledge of *D. trenchii* as a comparatively poor competitor in terms of growth rate and the photosynthate transfer requirements of fast-growing lesion tissue (Jones and Berkelmans, 2010; Wall *et al.*, 2020). Under competitive circumstances, background *D. trenchii* has been shown to divert metabolic processes away from growth and towards storage, in comparison to *Cladocopium (McIlroy, Wong and Baker, 2020)*. In contrast, the apparent decrease in *D. trenchii* in surrounding tissue after implanting unmanipulated *M. cavernosa* cores (figure 3.5), whilst not statistically significant, may hint at an inverse relationship between implantation-related lesion recovery and maintenance of *D. trenchii*.

Symbiont densities in implants were higher after bleaching and recovery (as is typical (Cunning and Baker, 2013; Kemp *et al.*, 2014)), hence all implants and outplants underwent some symbiont loss back to densities comparable to the field colonies after implantation (figure 3.7). However, the decrease in proportion *D. trenchii* in implants was comparably faster than from outplants (and was further accelerated by healing in *M. cavernosa*, figure 3.8c), which could be seen as an equilibration of symbiont populations between implant and parent colony. On the other hand, the increase in *D. trenchii* abundance around manipulated *M. cavernosa* implants despite a decrease in total symbiont density over time (attributed mainly to

the loss of *Cladocopium* symbionts, figure 3.6), suggests the competitive displacement of *Cladocopium* by *D. trenchii*.

Implant healing also shaped the spread of *D. trenchii* from *M. cavernosa* implants. More *D. trenchii* was detected surrounding healed implants than non-healed ones (figure 3.8a), and presumably at points of healing, given the higher ranges in proportion *D. trenchii* across partially healed implant sites (figure 3.8b). Whilst healing increased the amount of *D. trenchii* that spread from *M. cavernosa* implants, this spread was not dependent on healing, as evident from spread out of unhealed implants (figure 3.8a). This implies that *D. trenchii* may have spread from implants by both gastrovascular spread (as previously only shown in octocorals but assumed in hexacorals) (Parrin *et al.*, 2016), and by release (Stimson and Kinzie, 1991) and re-uptake (Coffroth *et al.*, 2010) by nearby polyps. The metacommunity effects of exogenous symbiont uptake seem to be highly spatially structured (Cunning, Yost, *et al.*, 2015), so the effects of D. trenchii release from implants on the wider reef community is uncertain.

The rate of healing in these three coral species was slowest in *M. cavernosa* (supplementary figure 3.2), as has been previously reported in studies tracking the soft tissue growth of small fragments of *M. cavernosa* and *O. faveolata* (Page, Muller and Vaughan, 2018). This may inform future implantation experiments to adjust the implant diameter by species; perhaps using larger grafts for larger-polyped species such as *M. cavernosa*, containing more in-tact polyps to fuel the healing of polyps damaged by coring. It is also conceivable that those implants dominated by *D. trenchii* healed in more slowly due to the slower host growth endowed by these symbionts (Jones and Berkelmans, 2010), but the margin of epoxy left between implants and the surrounding tissue was so variable between colonies (due to the

precision of coring during implantation) it seems likely that this, together with host species, would have had a far more substantial affect the rate of healing. It may be that rapid healing of *O. faveolata* and perhaps also *S. siderea* implants may have resulted in symbiont exchange that occurred at a much faster rate than in *M. cavernosa* and was subsequently missed by the sampling timeline of this experiment. This could provide yet another explanation for the lack of effect of implant treatment seen in *O. faveolata* and *S. siderea*.

This study was a proof-of-concept of the vectoring of symbionts into field colonies. To evaluate whether this method may have a role in intervention strategies, the crucial question is whether the presence of background symbionts can have an ecologically important role on reefs. For example, by hosting background levels of *D. trenchii*, corals may circumvent the growth disadvantages associated with *D. trenchii* dominance but increase the holobiont's fundamental niche and provide the opportunity for colonies to shuffle its symbionts to recover from bleaching (Correa and Baker, 2011). The continued monitoring of these colonies over the coming years will be important in addressing this. On the other hand, there is evidence that the presence of background symbionts may introduce competitive instability into holobionts, posing an energetic cost to the host (Kenkel and Bay, 2018). Results from chapter 2 suggest that whilst background *D. trenchii* populations may not be necessary for bleaching recovery with mainly *D. trenchii*, these background populations may accelerate or increase symbiont shuffling, which could conceivably have implications for bleaching resilience. The vectoring of background symbionts into colonies is also not limited to *D. trenchii* and could theoretically be paired with symbionts artificially selected to confer advantages at background levels (Buerger *et al.*, 2020).

These results inspire optimism about symbiont vectoring as an intervention or restoration technique because they reduce the concerns about the scalability of symbiont manipulation interventions raised in the National Academy of Sciences Interim Report on Interventions to Increase the Resilience of Coral Reefs (2019), by negating the need to apply a treatment to all outplants or entire colonies. Uncertainty surrounding the longevity of symbiont manipulation interventions was also raised in this report. In light of these concerns, it is worth noting that background *D. trenchii* that spread from *M. cavernosa* symbionts did so over the winter months when average daily temperatures dropped below 24°C (see supplementary figure 3.1). Despite *D. trenchii*'s competitive disadvantage at these temperatures, it was not diluted and outcompeted by the millions of resident symbionts in the colony tissue, supporting results seen under laboratory conditions (Silverstein, Cunning and Baker, 2017). In the absence of disturbance, symbiont communities in these study colonies showed surprisingly low levels of dynamism; given the slow exchange of implant symbionts, low levels of exchange and limited distance of spread (with effects absent at 2.5cm from implants), which actually speaks for the potential longevity of this method of intervention. Beyond any lasting effects within adult colonies, it is possible these interventions could have intergenerational effects if performed in brooding species given evidence that introduced symbionts can be transmitted from parent to gamete, to then colonise larvae (Quigley, Willis and Kenkel, 2019).

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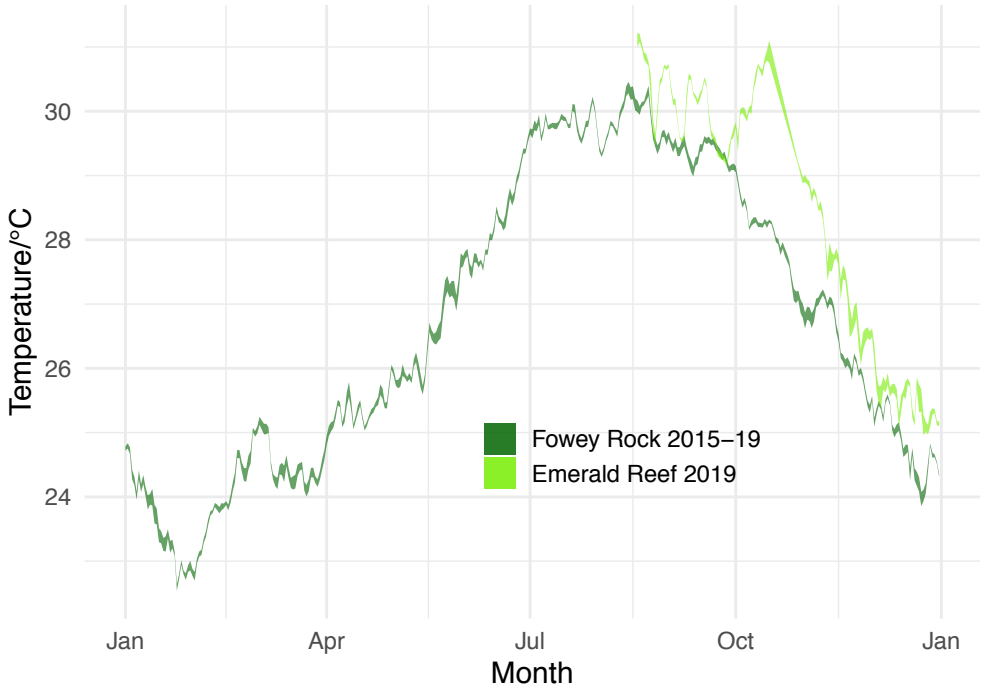
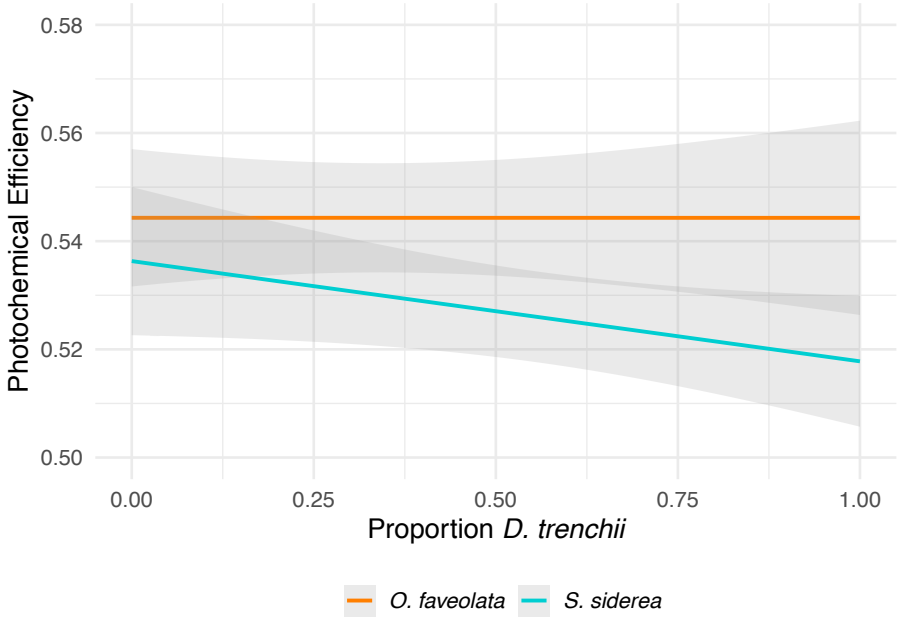
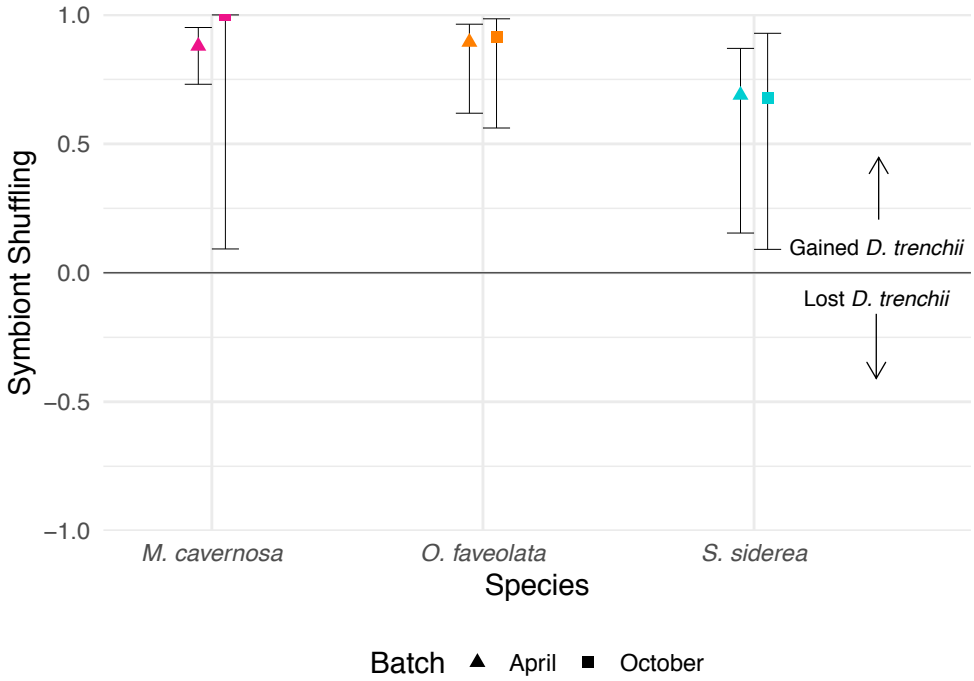
# Appendix A: Supplementary Figures for Chapter 2




**Supplementary Figure 2.1. Reef temperatures.** Temperature data was available from a Hobo logger at Emerald Reef from August to December of 2019, but water temperature data available from NOAA taken at Fowey Rocks from 2015 to 2019, approximately 9km to the South, show reasonable agreement.

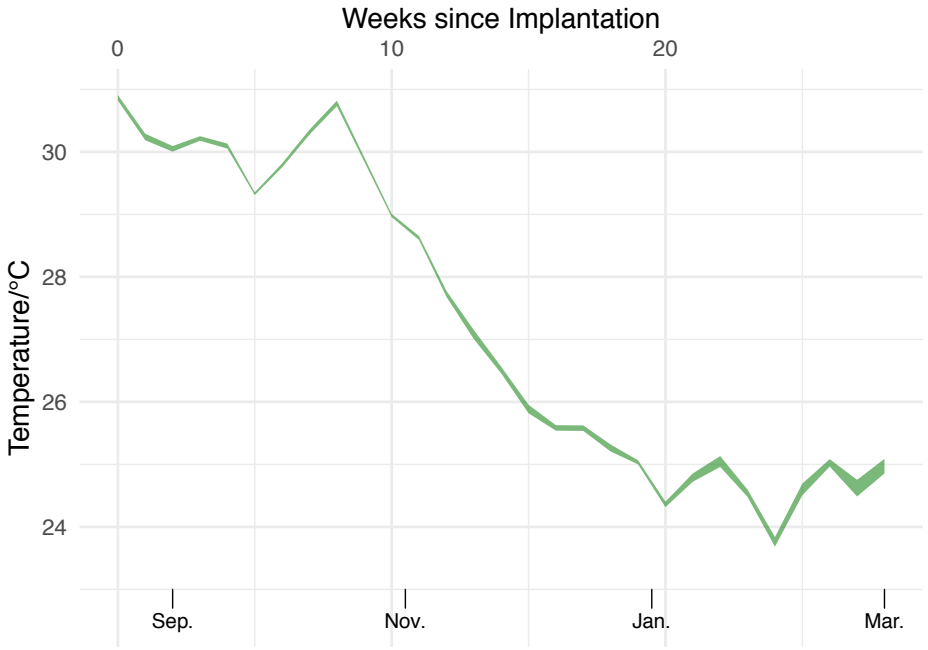



**Supplementary Figure 2.2. Initial photochemical advantage of *D. trenchii*.** The initial $F_v/F_m$ of colonies as a function of the proportion *D. trenchii*. Shaded areas represent 95% confidence intervals of generalized linear models. The relationship between photochemical efficiency and proportion *D. trenchii* does not differ significantly between these two species, ($F(1,47)=1.48$, $p=0.230$), although the relationship between photochemical efficiency and proportion *D. trenchii* is only marginally insignificant in *S. siderea* ($F(1,24)=3.46$, $p=0.075$).

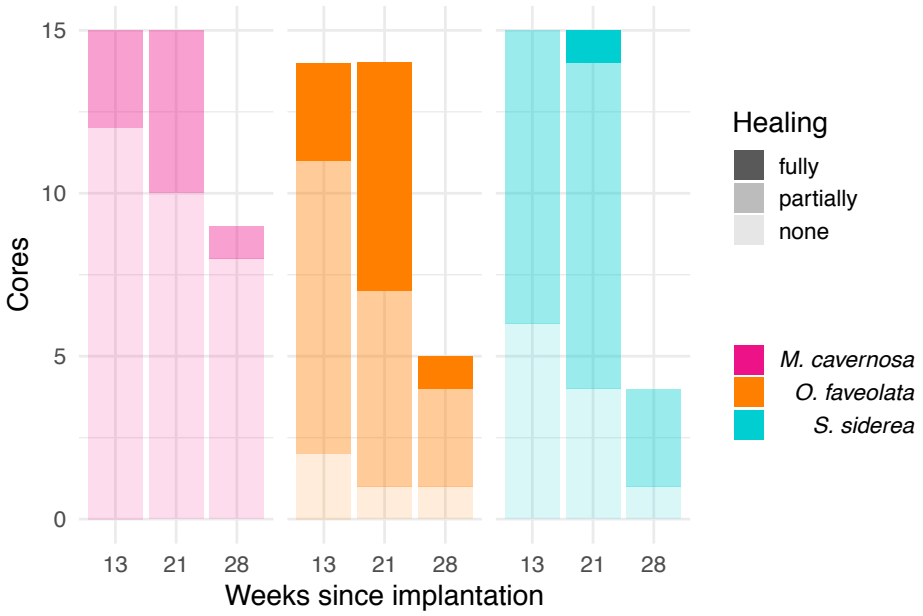



**Supplementary Figure 2.3. Symbiont shuffling between batches.** Fitted models of shuffling to *D. trenchii* are shown for *O. faveolata* and *S. siderea*, relative to initial proportion *D. trenchii*. Error bars represent 95% confidence intervals of means. The effect of batch on shuffling was not significant for *O. faveolata* ($F(1,19)=0.17$, $p=0.681$) or for *S. siderea* ($F(1,22)=0.0009$, $p=0.977$). The degree of shuffling was, however, greater in October than in April for *M. cavernosa* ($F(1,26)=6.67$, $p=0.016$*), associated with more severe bleaching in October.

# Appendix B: Supplementary Figures for Chapter 3

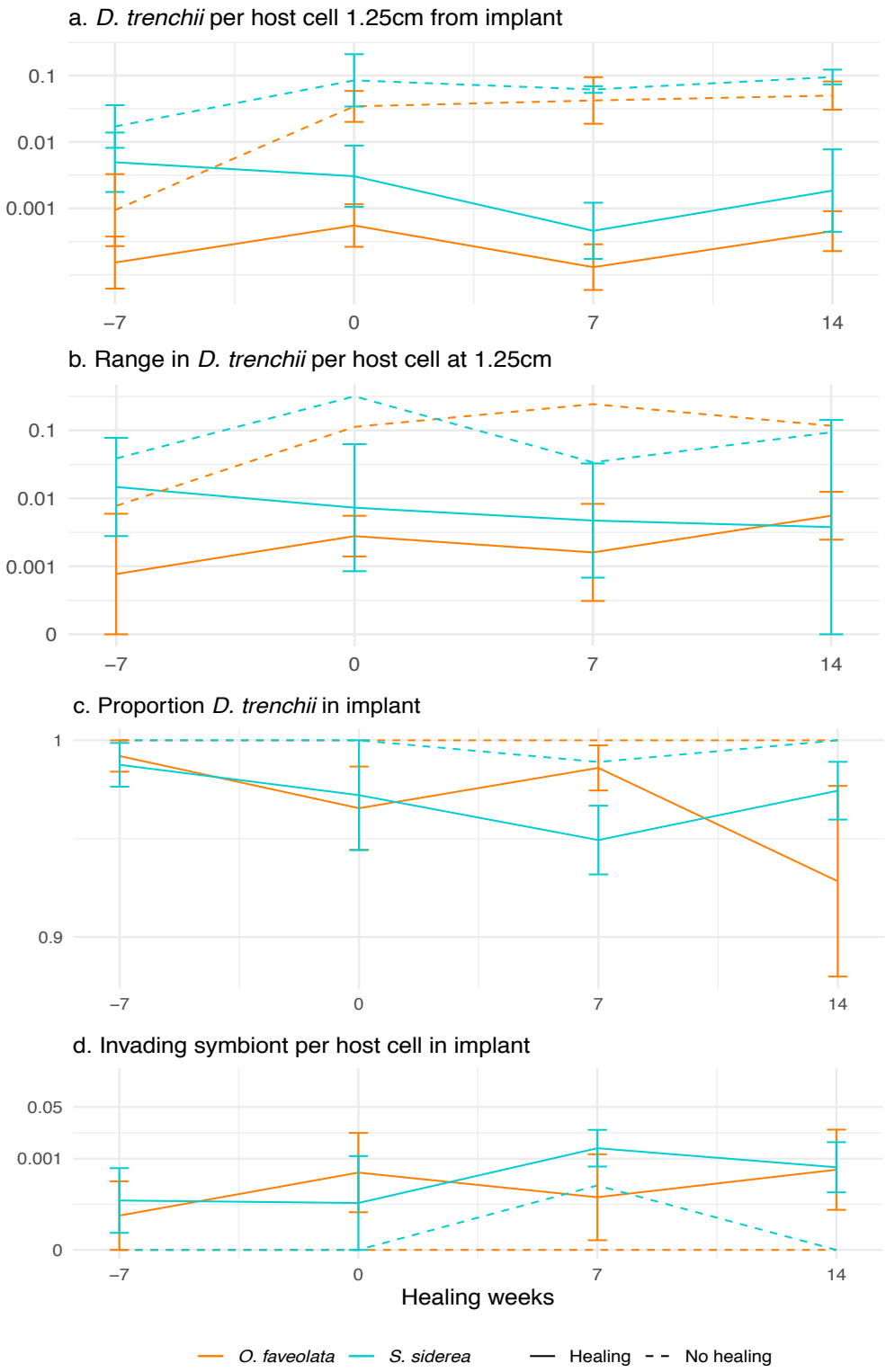



**Supplementary Figure 3.1. Reef temperature from outplanting.** Mean +- SE temperature at Emerald reef in 2019-20. Data were acquired from a HOBO logger deployed at the reef at time out outplanting.




**Supplementary Figure 3.2. Number of implants that had healed back into colonies either partially or fully over time.** This illustrates the number of cores that had healed with the surrounding tissue after implantation. Only the 17 of the 30 laboratory-manipulated implants that were deemed to have switched to *D. trenchii* dominance, were monitored at 28 weeks after implantation, hence the smaller sample size.

**Supplementary Figure 3.3a.**
Healing did not have a significant effect on the spread of *D. trenchii* from *O. faveolata* or *S. siderea* implants ($F(1,2)=8.011$, $p=0.106$, and $F(1,2)=0.753$, $p=0.477$ respectively).
**Supplementary figure 3.3b.**
The effect of healing on the range of proportion *D. trenchii* across 4 sampling points at 1.25cm surrounding each implant was not significant for *O. faveolata* ($F(1,2)=4.31$, $p=0.174$) or for *S. siderea* ($F(1,2)=0.309$, $p=0.634$).
**Supplementary figure 3.3c.**
The effect of healing in *O. faveolata* and *S. siderea* on the decrease in proportion *D. trenchii* in implants was not statistically significant ($F(1,12)=0.79$, $p=0.39$, and $F(1,11)=1.38$, $p=0.27$ respectively).
**Supplementary figure 3.3d.**
Healing did not have a significant effect on the amount of *Breviolum* found in *O. faveolata* implants ($F(1,12)=3.70$,$p=0.081$), nor on the amount of *Cladocopium* found in *S. siderea* implants ($F(1,11)=2.89$,$p=0.120$).


a. D. trenchii per host cell 1.25cm from implant
0.1
0.01
0.001
−7
0
7
14
b. Range in D. trenchii per host cell at 1.25cm
0.1
0.01
0.001
0
−7
0
7
14
c. Proportion D. trenchii in implant
1
0.9
−7
0
7
14
d. Invading symbiont per host cell in implant
0.05
0.001
0
−7
0
7
14
Healing weeks
O. faveolata
S. siderea
Healing
No healing