AN ABSTRACT OF THE THESIS OF

Daniel M Mele for the degree of Master of Science in Marine and Environmental Sciences presented May 2021

Title: A comparison of micro-fragmenting propagation techniques for the endangered stony coral species, *Acropora palmata*

Abstract Approved

Abstract:

At the forefront of coral restoration techniques is the coral gardening approach, which utilizes a nursery phase where corals are propagated before outplanting to a degraded reef. *Ex-situ* water table nurseries have opened the door for the use of micro-fragmentation, where corals are cut into just a few centimeter pieces, as these small fragments can be grown in a highly controlled environment that is conducive for health and growth. Other techniques involve directly transplanting coral fragments to a degraded reef, thus bypassing the nursery phase.
However, these techniques have mainly been practiced with larger coral fragments. Few studies have examined the efficacy of using micro-fragmentation techniques commonly used in an *ex-situ* nursery with direct outplanting. Here, we compare direct outplant micro-fragments of *Acropora palmata* with two phases of water table grown micro-fragments 1) nursery phase and 2) outplanting phase, over two sequential 12-week studies. The 2 studies' fragments were collected from 16 distinct *A. palmata* colonies split between two locations; Stumpy Bay, and Fortuna Bay, U.S. Virgin Islands. Study 1 consisted of 16 direct outplant arrays (array = 5 micro-fragments) compared against 15 water table grown arrays. Study 2 involved outplanting the water table arrays from Study 1 and comparing them to an additional subset of 15 direct outplants arrays. Each array of direct outplants had a matching array of water table micro-fragments originating from the same parent colony. Both Study 1 and Study 2 showed a clear trend favoring water table arrays over direct outplant arrays. Water Table arrays consistently outperformed direct outplant arrays concerning growth and survival (WT = 100% survival in Study 1 and 97% survival in Study 2). However, direct outplants still performed exceptionally well, showing 91% survival in Study 1 and 85% survival in Study 2.

A COMPARISON OF MICRO-FRAGMENTING PROPAGATION TECHNIQUES FOR THE ENDANGERED STONY CORAL SPECIES, ACROPORA PALMATA

by Daniel M Mele

A THESIS

Submitted to the

University of the Virgin Islands

In partial fulfilment of
the requirements for the degree of

Master of Science

Presented April 20 , 2021
Commencement May 2021

Master of Science thesis of Daniel M Mele presented on April 20, 2021

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I understand that my thesis will become part of the permanent collection of the University of the Virgin Islands Library. My signature below authorized release of my thesis to any reader upon request.

Daniel M Mele

ACKNOWLEDGEMENTS

Firstly, thank you to Marilyn for being my advisor and giving me the freedom and support to design my own master's thesis. I appreciate the positive working environment you created in the Brandt lab, which undoubtedly helped motivate me to work through my master's degree's most challenging moments. Thank you to Dave for not only being on my committee but giving me my first opportunity to work in coral restoration at the Mote Marine Laboratory. I am sure I would not be where I am today without that experience. Thank you to Lisa for being on my committee and entrusting me to accurately represent coral restoration techniques you have been practicing for years. To my fieldworkers, Brad Arrington, Kathryn Cobleigh, and Adam Glahn, thank you for coming out with me every week for six months straight to help collect my data. Thank you to the Department of Planning & Natural Resources (DPNR) for funding my project. Thank you to the Lana Vento Charitable Trust for also helping to fund my project. Thank you to my girlfriend Caroline for understanding when I had to spend night after night working. The biggest thank you to Mom, Dad, and my brother Tristan, who have always supported my work. A special thank you to my Mom, who helped guide me in my statistics for my thesis. I would also like to thank my grandparents. They have helped open doors for me to help get me to where I am now.

AUTHOR CONTRIBUTION

This experiment was designed and conducted by Dan Mele with the help with Dr. Marilyn Brandt, Lisa Carne, and Dr. David Vaughan. Dr. Brandt, Lisa and Dr. Vaughan provided helpful details on the experimental design and guidance of best practices. Dr. Brandt assisted with statistical analysis, and data interpretation. Edit suggestions were given by Dr. Brandt, Lisa Carne, and Dr. Vaughan.

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A COMPARISON OF MICRO-FRAGMENTING PROPAGATION TECHNIQUES FOR THE ENDANGERED STONY CORAL SPECIES, ACROPORA PALMATA

Chapter 1: Introduction

Background

Coral reefs have been slowly degrading for nearly a century. However, the most significant changes have occurred in the last four decades, as recurrent coral bleaching events, emergent diseases, and a combination of anthropogenic stressors have exacerbated the decline in coral reef health (Pandolfi et al., 2003; Hughes et al., 2017). While the degradation of coral reefs has increased globally, the Western Atlantic and the Caribbean have experienced the most significant impacts of coral loss and the least signs of recovery (Baker et al., 2008; Hughes et al., 2017).

In response to coral reefs' ongoing degradation, a global effort in coral restoration projects has ensued (Boström-Einarsson et al., 2020; Schmidt-Roach et al., 2020). These restoration efforts now take place in over 56 countries with the majority in the United States, Philippines, Indonesia, and Thailand (Boström-Einarsson et al., 2020;). The popularity of coal restoration has involved a wide range of groups from the world's largest conservation organizations to citizen science and volunteer projects (Fox et al., 2005; Hesley et al., 2017; Schrack et al. 2012).

Past and Emerging Techniques in Coral Reef Restoration

Past Techniques

Early coral restoration efforts were primarily focused on transplanting corals to reefs damaged by ship groundings and development (Harriott and Fisk, 1988; Yusuf, 2014). Transplantation techniques involved harvesting corals from a donor reef and relocating them to a degraded site. While the benefits of this method may lead to an immediate increase in coral cover at the degraded site, it also results in a loss of coral cover from the donor reef (Clark and Edwards, 1995; Edwards and Clark, 1999). Other potential disadvantages of the transplantation methodology are reduced growth and

reproduction rates of transplants, dislodgment, and high mortality amongst transplanted corals, which can lead to an overall loss of coral cover between the donor and transplantation reef (Edwards and Clark, 1999; Garrison and Ward G., 2012). Over the last two decades the reasons for coral restoration have shifted to a response to a multitude of stressors, including hurricanes, bleaching, blast fishing, and degraded water quality to name a few (Boström-Einarsson et al., 2020; Shaish, 2010; Williams, 2019). The rapid growth of coral restoration projects has led to the development of newer and more successful techniques (Fox et al., 2005; Lirman and Schopmeyer, 2016; Omori, 2019).

Coral Gardening

In the last decade, coral gardening has become the forefront method for restoration efforts (Boström-Einarsson et al., 2020; Lirman and Schopmeyer, 2016; Omori, 2019). Using a combination of propagation techniques involving fragmentation and sexual recruitment, coral restoration practitioners can exponentially increase the amount of living tissue in a nursery while causing little to no damage to existing reefs (Forsman et al., 2015; Lohr et al., 2015; Monty et al., 2006).

The majority of coral gardening projects focus on fast-growing branching corals, as these species utilize fragmentation as a natural form of asexual reproduction (Boström-Einarsson et al., 2020; Lirman and Schopmeyer, 2016). The fragmentation process occurs most notably from storms and wave surge (Lirman, 2000a). While storm-generated fragments generally experience high mortality due to tumbling and sediment burial, fragments in a nursery can be secured and kept free of most environmental stressors, leading to high survival rates (Lirman, 2000a; Monty et al., 2006; Riskand Edinger, 2011; Shaish et al., 2008). Fragmentation techniques were initially practiced in *in-situ* nurseries. However, recent advancements in coral nursery design have expanded to land-based facilities, which utilize a water table system (Bartlett, 2013; Forsman et al., 2015; Johnson et al., 2011).

Water Table Nurseries

Water table nurseries present a unique set of advantages and challenges. Being able to control the abiotic parameters such as light, temperature, and water flow allows

aquarists to create an ideal environment for coral growth (Bartlet, 2013; Leal et al., 2016). Additionally, corals grown in water table nurseries can do so in the absence of corallivores and algal overgrowth, known factors that inhibit coral growth and can cause mortality (Craggs et al., 2019; Toh et al., 2013; Leal et al., 2016). The benefits of using a water table nursery have allowed for the proliferation of techniques involving sexual recruitment, fragmentation, and micro-fragmentation (Forsman et al., 2015; Ng et al., 2012; Osinga et al., 2012). With these advancements, in particular, techniques involving sexual recruitment and micro-fragmentation have exploded in popularity and opened the door for fragmenting corals into much smaller pieces than typically used in an *in-situ* nursery (Forsman et al., 2015; Johnson et al., 2011).

Micro-fragmentation is a process where corals are fragmented into small ~ 1cm x ~1cm pieces. These fragments' small size maximizes the total area of new growth and increases growth rates multifold times faster than typically measured in wild colonies (Forsman et al., 2006; Forsman et al., 2015; Lirman and Schopmeyer, 2016). This technique also makes it possible to create many fragments from a relatively small piece of coral (Shafir et al., 2001). Micro-fragmentation also allows for the fusion of fragments of identical genotypes. Fragments can be outplanted in arrays, which have the potential to fuse into a single colony, thus in theory, reducing the time for a colony to reach sexual maturity compared to outplanting a single small fragment (Forsman et al., 2015; Page et al., 2018).

Some of the same advantages for using an *ex-situ* water table nursery are also some of its disadvantages. If abiotic/biotic factors aren't rigorously managed, coral cultures can suffer high mortality (Bartlet, 2013). Additionally, water table systems can be costly to build and maintain, as aquaculture practices require a diverse set of skilled personnel (Bayraktarov et al., 2019; Spurgeon, 2001). Many Caribbean coral restoration projects are in the vicinity of hurricane impacts, making water table nurseries at risk for system failure (Personal Observation: Hurricanes Irma/2017 and Dorian 2019). While overall, the advantages of water table nurseries can be an effective method for coral restoration efforts, cost should be considered when implementing these approaches (De Groot et al., 2013; Spurgeon, 2001; Tortolero-Langarica, 2020).

Direct Outplanting

In recent years new methods of direct outplanting have emerged as an alternative technique. These techniques involve collecting, micro-fragmenting, and outplanting corals *in-situ* during the same day. This technique combines older direct transplantation techniques with the newer micro-fragmentation approaches (Plucer-Rosario and Randall, 1987; Tortolero-Langarica et al., 2020). These techniques take advantage of the rapid growth rates achieved through micro-fragmentation while causing minimal damage to the donor colony. Direct outplanting also dramatically reduces the overall operational costs by bypassing the nursery phase (Forrester et al., 2019, Tortolero-Langarica, 2020). Direct outplanting provides other benefits as it requires less highly trained personnel and can take advantage of citizen science programs that have shown to be very effective at performing the outplanting phase of coral restoration projects (Forrester et al., 2014; Hesley et al., 2017). These projects also provide an opportunity for the public to be engaged in science and become stewards of their natural resources (McKinley et al., 2017).

Researchers in Belize have shown success with bypassing the nursery stage and directly outplanting coral micro-fragments (www.fragmentsofhope.org/). Their methods utilize slightly larger micro-fragments of ~5 cm x ~ 5 cm yet still retain outplanting in arrays, leading to the fusion of multiple fragments. Fragments are relocated to a donor site that is of similar environmental parameters. By outplanting the fragments at a similar site, they should already be adapted to the environmental conditions at that site, thus eliminating the stress of transplanting coral fragments to a different reef with different environmental conditions (Forrester et al., 2012). These techniques also result in minimal loss of coral from the donor reef.

Despite the potential benefits of direct outplanting, multiple possible drawbacks should be considered. Fragmenting from a parent colony leaves an open wound that may be more susceptible to disease or predation from *Coralliophila abbreviata* (Bright et al., 2016, Knowlton et al., 1990). In addition, each direct outplant fragment has a fresh wound around its entire perimeter, thus leaving the fragments susceptible to the same ailments. Previous research has also noted reproductive failure for four years among *Acropora palmata* colonies following severe breakage (Lirman, 2000a). Direct

outplanting methods focus on gathering <5% from a parent colony. However, the collection process is imperfect as larger branches may inadvertently break when attempting to fragment a small branch (Personal Observation).

Currently, there is minimal published literature on these methods of direct outplanting. Furthermore, there is no literature regarding a comparison of direct outplant micro-fragments with water table grown micro-fragments. This study aims to compare and quantify direct micro-fragment outplants with water table grown micro-fragments of *Acropora palmata* by assessing their survival, growth, and health over two consecutive 12 week experiments (Study 1 and Study 2).

Species of Interest

Acropora palmata populations have dramatically declined in the U.S. Virgin Islands over the past four decades (Grober-Dunsmore et al., 2006). Anecdotally, *A. palmata* appears to show signs of new growth at several locations off the island of St. Thomas, USVI. For these reasons and *A. palmata's* natural tendency for fragmentation, this species was chosen to be the primary focus of this study.

While in recent years, coral restoration efforts have expanded to include a wider variety of species, the majority of restoration efforts are focused on the branching *Acropora* corals (Boström-Einarsson et al., 2020). Specifically, *Acropora palmata* is an essential species for Caribbean coral reefs in providing reef structure and habitat. *Acropora palmata* forms mostly monotypic zones, with the *palmata* zone existing closest to shore (Goreau, 1959). beginning in the 1970s, *A. palmata*, population have suffered losses as high as 90% that can be associated with the spread of white band disease (Aronson and Precht, 2001; Bruckner et al., 2002; Mayor et al., 2006). Major hurricanes have also worsened population declines, and stifled their recovery (Bruckner et al., 2002; Edmunds and Witman, 1991).

The *A. palmata* zone creates a structurally complex habitat for a wide range of marine organisms, including economically important fish species and invertebrates. (Lemoine and Valentine, 2012; Lirman, 1999). In some cases, hurricanes may assist in asexual reproduction by fragmenting portions of colonies that may settle and begin growing, adding new structure to the reef (Fongand Lirman, 1995). However, this process is imperfect, with most storm-generated fragments experiencing high mortality within the

first-month post fragmentation (Lirman and Fong, 1997; Lirman, 2000a). Survivability of fragmentation is also highly dependent on the type of substratum the fragment settles on (Lirman and Fong, 1997). These studies aim to replicate *A. palmatas* natural process of fragmentation, while applying specific techniques to increase the likeliness of survival post fragmentation

Chapter 2: Objectives and Hypothesis

Study 1 Objective: To determine if growth and survival varies between ex-situ nursery grown micro-fragments and direct outplant micro-fragments of *Acropora palmata*.

> **H_1:** Given the absence of predation, temperature fluctuations, disease and other stressors, *Acropora palmata* micro-fragments will experience greater growth and survival over the course of twelve weeks in an *ex-situ* nursery than when directly outplanted.

Study 2 Objective: To determine if directly outplanting coral micro-fragments of Acropora palmata is a viable method for coral restoration compared to ex-situ nursery grown micro-fragment outplants by showing equal or superior growth and survival.

> **H_2:** Given that they are acclimated to the outplanting location conditions, growth and survival over the course of twelve weeks of direct micro-fragment coral outplants will be equal or greater to that of nursery grown coral micro-fragments.

Chapter 3: Methods

3.1. Study Sites

The restoration study sites, Fortuna Bay and Stumpy Bay, were located in the western region of St. Thomas, U.S. Virgin Islands (Figure 1). These sites were chosen based on recent observations of the presence of apparently healthy *Acropora palmata* colonies, indicating that these were favorable locations for conspecific outplants' survival. Accessibility was also considered during the site selection process, as both

Fortuna and Stumpy Bay were reachable by boat or shore diving. This study's ex-situ component was conducted at the University of the Virgin Islands (UVI) water table coral nursery.

Figure 1. A map of the study sites, Fortuna Bay, and Stumpy Bay and the UVI water table nursery on the island of St. Thomas, U.S. Virgin islands.

3.2. Experimental Design

The study consisted of two sequential experiments to test the effectiveness of direct outplanting techniques against two stages (nursery and outplanting) of an *ex-situ* water table coral nursery. Study 1 tested H_1 and compared growth and survival of micro-fragments kept within the water tables (WT) to direct outplants (DO). Study 2 tested H_2 and compared growth and survival of outplants of micro-fragments from the WT and DO. The WT micro-fragment outplants in Study 2 were the same micro-fragments used in the water table portion of Study 1. The start of Study 1 began on 04/22/2020 (Earth Day) and ran for 12 weeks. Study 2 began eleven days after the conclusion of Study 1 on 08/04/2020 and ran for another 12 weeks. In both studies, measurements were made weekly.

3.3 Study 1: Water Table Nursery Fragments vs Direct Outplants

3.3.1. Coral Collection

One month before the start of the study, a single branch or portion of a branch was collected from eight tagged colonies of *A. palmata* (Fig. 2, S-1: Parent Colonies). The depths of each colony ranged from 1-3 meters. Collected branches constituted <%5 of the entire parent colony and were collected using a chisel and hammer. The branches were wrapped in bubble wrap and placed in separate plastic ziplock bags to minimize the damage of branches bumping into each other. The branches were transported to the UVI water table nursery in 5-gallon buckets of seawater. During this initial collection stage, the collected branches experienced 100% mortality in the water tables within the first week of collection. This was likely due to the collected *A. palmata* branches experiencing a shock from excessive UV light during the period of bringing the corals out of the water and into buckets and not supplying adequate shade in the water tables. Following the initial collection period, a second subset of branches was gathered from the same colonies and brought into the UVI water table nursery. During the second collection process, great care was given to keep the branches completely shaded during every stage of collection. Once brought back to the UVI water table nursery, two shade cloths were placed over the tables.

Over the course of a month, the branches in the water tables were slowly acclimated to increased levels of light by removing the shades for portions of the day until they were removed entirely. Each branch was placed propped up on an angle to ensure the branches' bottoms would receive similar light input as the tops.

3.3.2. Direct Outplant Fragmentation

On 04/22/2020 (Fig. 3, Fortuna Bay) and 05/03/2020 (Fig. 4, Stumpy Bay), additional branches from the same tagged *A. palmata* colonies used for the water table collection phase were collected and fragmented onshore using a Gryphon Diamond Blade Frag Saw. Each branch was fragmented into five ~3 x ~5 cm pieces (Fig. 2, S-1: Micro-fragment) for a total of 80 fragments between both locations. A few branches had

leftover fragments, which were secured back on the reef using A-788 Splash Zone epoxy. The same epoxy was used to attach each experimental micro-fragment to cement pucks made from a mixture of white portland cement and beach sand. The micro-fragments were given a 30 minute time period for the epoxy to harden before outplanting. During this period, they were kept shaded and given frequent water changes. Each set of five micro-fragments were outplanted in arrays within close vicinity to their respective parent colony (Fig. 2, S-1: Direct Outplants). This was done to more accurately represent the natural process of storm-generated fragments which tend to settle close to the parent colony (Irwin et al., 2017; Rogers et al., 1982).micro-Fragments in the arrays were placed ~3 cm apart, and were attached to the substrate using A-788 Splash Zone epoxy. To ensure a strong attachment to the substrate, algae was removed using a wire brush, prior to placing the micro-fragments. The outplant depth of arrays ranged from 1-4 meters.

3.3.3. Water Table Fragmentation

On 05/08/2020 after completing the direct outplants, the branches located in the UVI water tables were fragmented. One day before fragmenting the water table branches, a single branch belonging to the parent colony, Stumpy Bay - 2732, died from an unknown rapid tissue necrosis disease. For this reason, there were only 15 arrays and a total of 75 micro-fragments during the water table portion of Experiment 1. The same techniques and materials used for fragmenting and securing the DO were also used for WT fragments. Exceptions were that rather than securing the pucks to a substrate; micro-fragments were placed in their arrays of 5 relating to the same parent colony on egg crate racks (Fig 2. S-1: *ex-situ* Nursery). Each array was randomly assigned to one of three identical water tables, with five arrays per water table. Each week, the arrays in the three water tables were moved to their adjacent table to reduce the possibility of a specific water table advantage.

The UVI water table nursery consists of a flow-through system into 170 liter water tables. The seawater is pulled from Brewers Bay and passes through multiple cartridge filters, a UV sterilizer, and a chiller to maintain stable water temperatures. Each table consisted of a circulation pump and 20-30 *Astraea spp.* snails to help reduce the occurrence of algae blooms. Each table was siphoned 2-3 times per week to remove

particulates that entered through the system and snail excrement and other nuisance algae.

3.4. Study 2: Water Table Nursery Fragment Outplants vs Direct Outplants

3.4.1. Direct Outplant Fragmentation

The start of Study 2 began on 08/04/2020 for Fortuna Bay (Fig. 3) and 08/05/2020 for Stumpy Bay(Fig. 4) and ran for three months. The direct outplanting protocols were the same as those outlined in Study 1. The only exception was that a second subset of direct outplants was not created for Stumpy Bay - 2732 due to the water table branch of Stumpy Bay - 2732 experiencing 100% mortality prior to the start of Study 1. There were a total of 15 arrays with 75 fragments (Fig. 2, S-2: Direct Outplants).

3.4.2. Water Table Outplanting

The fragments grown at the UVI water table nursery for Study 1 were outplanted to their respective locations from which they were collected. Outplanting occurred on the same day as the second subset of direct outplants and used the same attachment techniques to the substrate. Water table arrays were outplanted directly adjacent to the second subset of direct outplants, including the same depth, orientation, and spacing to ensure each pair of arrays experienced similar environmental parameters. Similar to Study 1, outplanted arrays were placed within close vicinity to their respective parent colony. The outplant depth of arrays ranged from 1-4 meters. There were a total of 15 arrays with 75 fragments (Fig. 2, S-2: *ex-situ* Nursery Outplants).

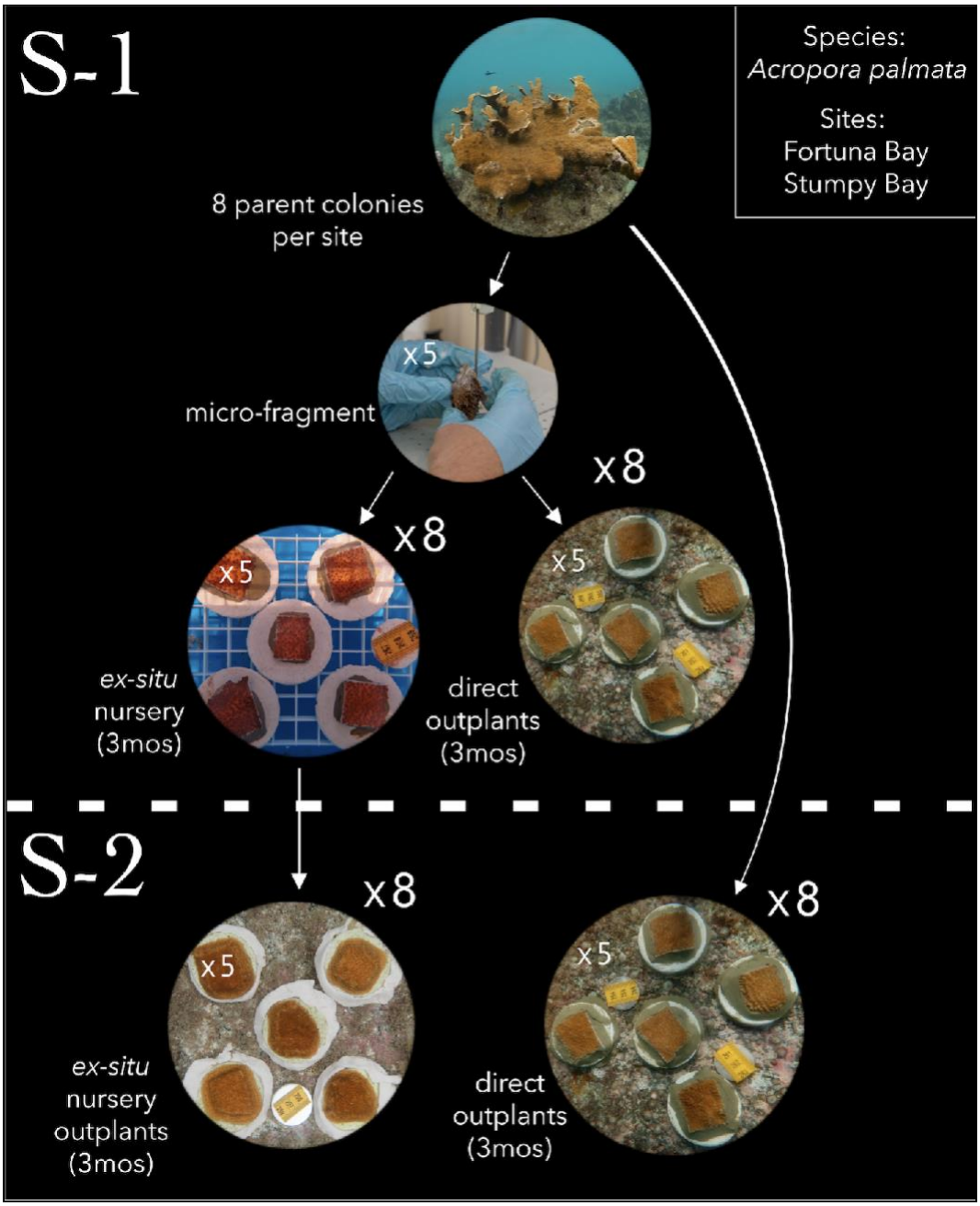


Figure 2. Schematic showing how the study was conducted. S-1 = initial coral collection phase where five micro-fragments from each of eight donor colonies are brought into an *ex-situ* nursery and five are directly outplanted. S-2 = five *ex-situ* grown micro-fragments are outplanted at the same time as the second subset of five direct outplants from the same donor colony.

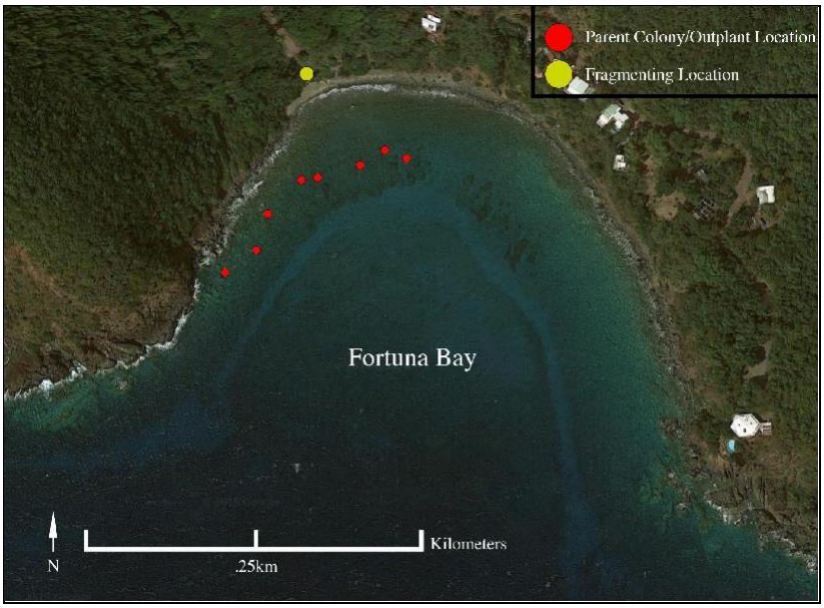


Figure 3. Map of Fortuna Bay showing the general location of each parent colony and outplant location for studies 1 and 2. Fragmenting location was included to indicate how far fragments had to travel from the point of fragmentation to being put back in the water.

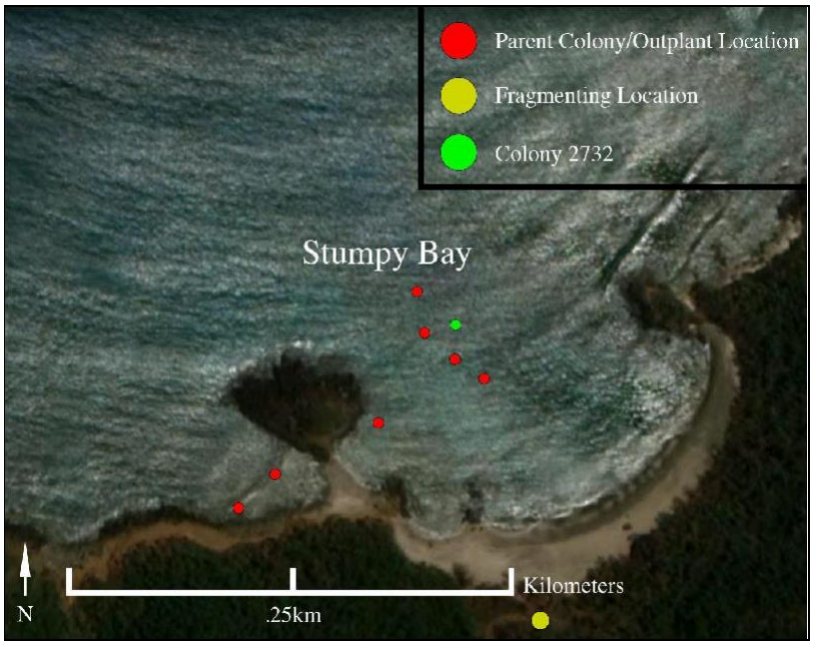


Figure 4. Map of Fortuna Bay showing the general location of each parent colony and outplant location for studies 1 and 2. Fragmenting location was included to indicate how far fragments had to travel from the point of fragmentation to being put back in the water. The green circle represents colony 2732, which was absent in study 2.

3.5. Monitoring

DO, WT grown fragments, and WT outplants from both studies 1 and 2 received the same monitoring protocols. Monitoring occurred weekly for 12 weeks after fragmentation/outplanting. Each array and parent colony was given a health assessment by recording bleaching/paling, disease, and recent/old mortality using the same protocols devised by Smith, T.B., et al. (2008) for the Territorial Coral Reef Monitoring Program (TCRMP). Survival was recorded amongst individual fragments.

Growth was defined as horizontal tissue extension from the perimeter of the individual fragment. To record growth, top-down photographs using a PVC framer were taken using a Canon PowerShot G7X MarkII in an Ikelite underwater housing (Fig 5). A scale bar was placed in the frame (Fig. 6) to allow for image analysis. Images were run through ImageJ Fiji Version 2.1.0/1.53c. While algae clearing was not part of the weekly monitoring protocols, occasionally algae had to be plucked around the edge of the coral to create an image without algae obstructing the view of the coral.

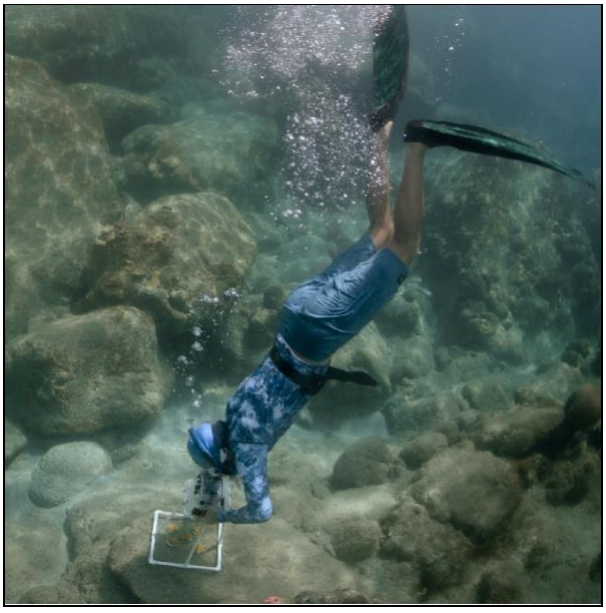


Figure 5. PVC framer and underwater camera for taking top down photographs of experimental arrays.

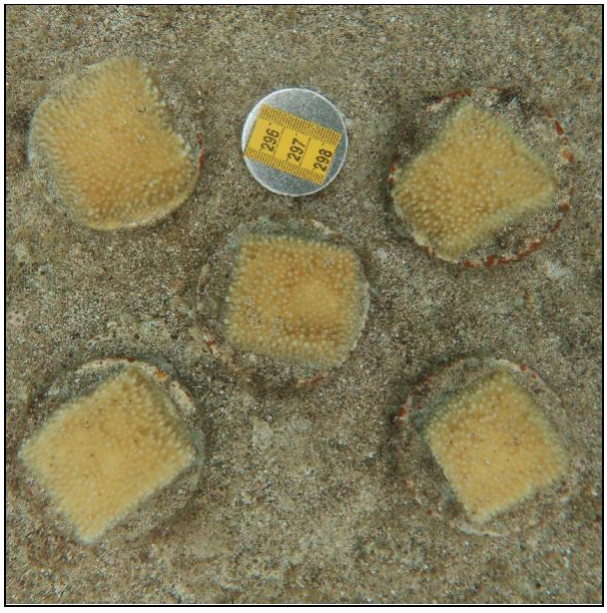


Figure 6. Example of an experimental array with scale bar.

In addition to monitoring the study micro-fragments, the donor colonies were also monitored each week for 12 weeks, and again at eleven months, applying the same health assessment protocols. The wounds from where the donor branches were collected were tracked and photographed to monitor their stages of recovery.

3.6. Statistical Analysis

All statistical analyses and graphs for Study 1 and Study 2 were performed using JMP version 14.2.0. The same statistical tests were used for both Study 1 and Study 2, except for when specifically noted. Statistical tests were used to measure the effect of technique on growth (change from baseline) for arrays and micro-fragments separately. Baseline was defined as Week 0.

To understand the impact the variability of baseline sizes of micro-fragments and arrays had on growth, a growth at Week 12 by baseline graph was constructed. Additionally a Pearson's test was run to determine if the correlation between growth and baseline was significant.

To test how technique impacted growth at Week 12 of fragments and arrays, an analysis of covariance (ANCOVA) was run with baseline as a covariate and location as a random effect. The initial model included interaction terms for technique by location and for technique by baseline. A second ANCOVA model was run excluding interactions if the interactions were not significant.

Two arrays were sampled from each parent colony, assigning these two arrays to either direct outplant or to water table. This sampling suggests that a matched paired analysis to compare techniques may be performed. A paired t-test was run on 15 matched pairs in each study. In each study, one array was missing a match and was therefore excluded from the analysis.

To examine growth week by week over the full course of the study, a repeated measures analysis was run to compare techniques for arrays and micro-fragments. Following a repeated measures analysis, a post hoc Tukey HSD was run to determine which specific weeks were significantly different between direct outplant and water table.

Growth for micro-fragments was examined analyzing all micro-fragments, including those that died anytime during the studies. In addition, growth of

micro-fragments that survived the full study were examined, excluding all that died during the study.

To measure survival, only fragments were used because all arrays survived based on at least one micro-fragment surviving within an array. A Fisher's Exact Test was run with micro-fragments being scored as 0 = dead and 1 = alive.

A chi-squared analysis was run to determine if there were significant differences in bleaching and paling between direct outplant micro-fragments cut from the bottom or the tops of branches. Fragments were ranked as either no apparent paling/bleaching = 0, paling = 1, bleached = 2.

Chapter 4: Results

4.1. Study 1

4.1.1. Comparison of Baseline Measurements for Arrays and Micro-fragments

Comparisons of baseline sizes at the start of the study (Table 1) for both arrays and fragments indicate that direct outplant (DO) sizes were significantly larger than water table (WT) sizes as seen by the 95% confidence intervals for the technique difference not including zero.

Table 1. Study 1 mean array and micro-fragment size (cm^2) at baseline (Week 0) by Water Table (WT) and Direct Outplants (DO).

	WT Mean (SD)	**DO Mean (SD)**	**WT-DO (95% CI)**
Array	47.3 (3.5) (n = 15)	67.6 (10.4) (n = 16)	-20.3 (95% CI -26.1, -14.6)
Fragment	9.5 (1.1) (n = 75)	13.5 (2.9) (n = 80)	-4.1 (95% CI -4.8, -3.4)

Looking at baseline by location (Figure 7), the greatest difference between techniques was seen at the Stumpy Bay location with a mean technique difference of about 28 cm^2 for arrays and 6 cm^2 for micro-fragments. While at Fortuna Bay the differences were about 12 cm^2 and 2 cm^2 respectively.

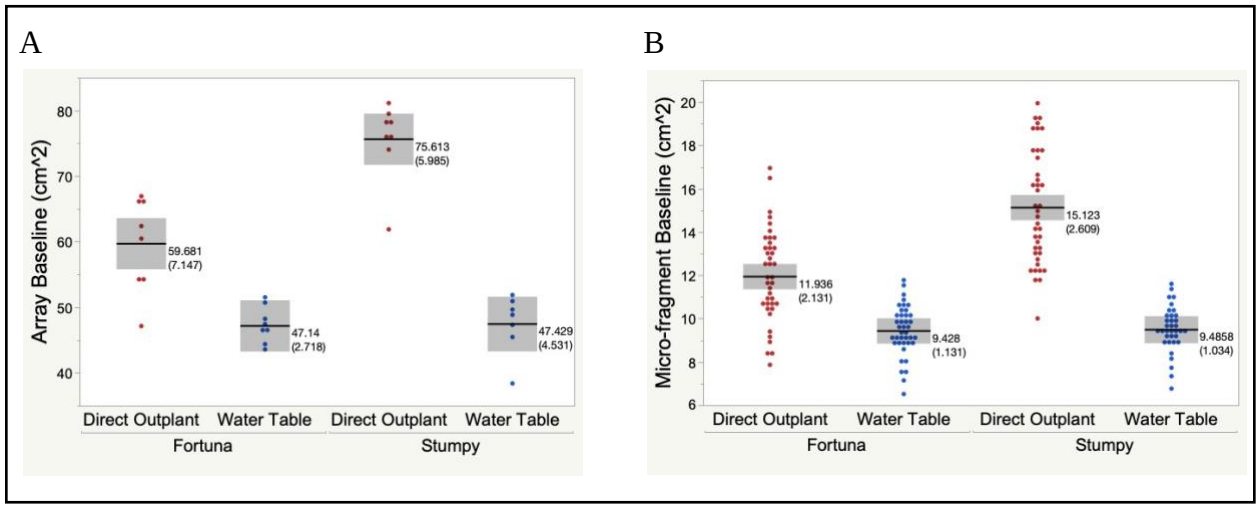


Figure 7. Scatterplot with means and standard deviations of baseline sizes (cm^2) by location and technique of (A) arrays and (B) micro-fragments.

Due to the variability in baseline size, a growth (change from baseline at Week 12) by baseline graph was constructed to determine if the variability in baseline size was correlated with growth and survival. As shown in Figure 8, no meaningful correlation (Pearson's Correlation Coefficient, r) was seen between baseline size and growth among arrays (Fig 8 (A) Fortuna: r = - 0.3, *P* = 0.2; Stumpy: r = -0.03, *P* = 0.9) nor among micro-fragments (Fig 8 (B) Fortuna: r = -0.2, *P* = 0.1; Stumpy: r = -0.05, *P* = 0.7). Despite the variability in baseline sizes of arrays and micro-fragments not suggesting an impact on growth, baseline was added as a covariate in further statistical analyses.

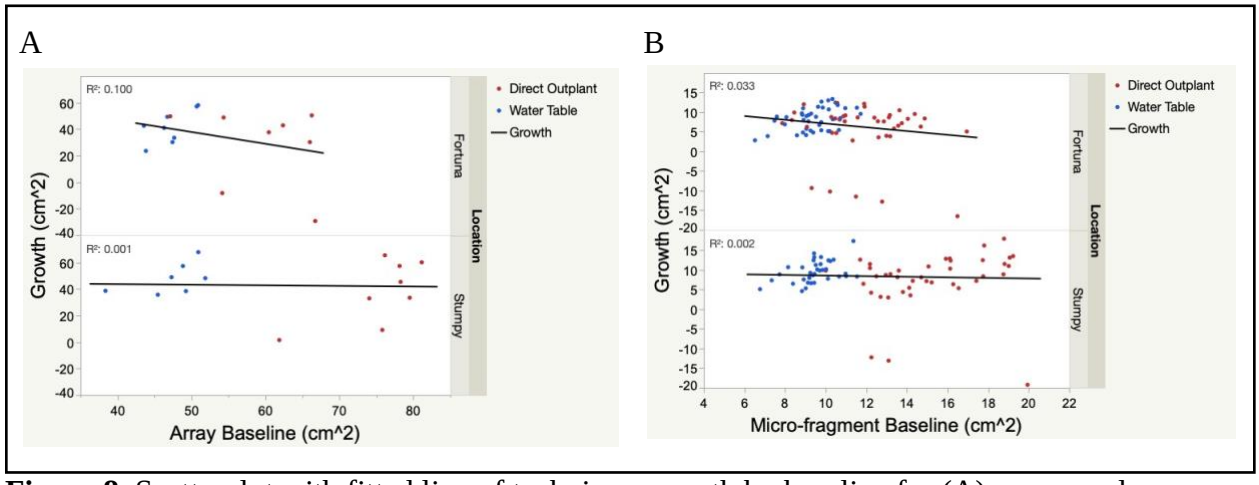


Figure 8. Scatterplot with fitted line of technique growth by baseline for (A) arrays and (B) micro-fragments

4.1.2. Survival of Micro-fragments at Week 12

Only micro-fragments were analysed for survival. All arrays were considered alive at the end of the study because in each array at least one micro-fragment was alive. At the end of 12 weeks, survival was significantly different between DO micro-fragments and WT grown micro-fragments ($P = 0.014$, Fisher's Exact Test). DO showed a 91.25% survival while WT micro-fragments showed 100% survival (Table 2) at the end of the 12 week study. Seven of 80 DO micro-fragments died and 0 of 75 WT micro-fragments died.

Table 2. Survival of direct outplant and water table fragments at week 12.

	Water Table N (%)	**Direct Outplant N (%)**
Dead	0 (0%)	7 (8.75%)
Alive	75 (100%)	73 (91.25%)
Total N	75	80

4.1.3. Comparison of Growth at Week 12 for Arrays and Micro-fragments

Average growth among all micro-fragments was 7.6 $cm^2 \pm$ 0.7cm^2 (SEM) and was fairly consistent among micro-fragments and arrays (Fig. 9).

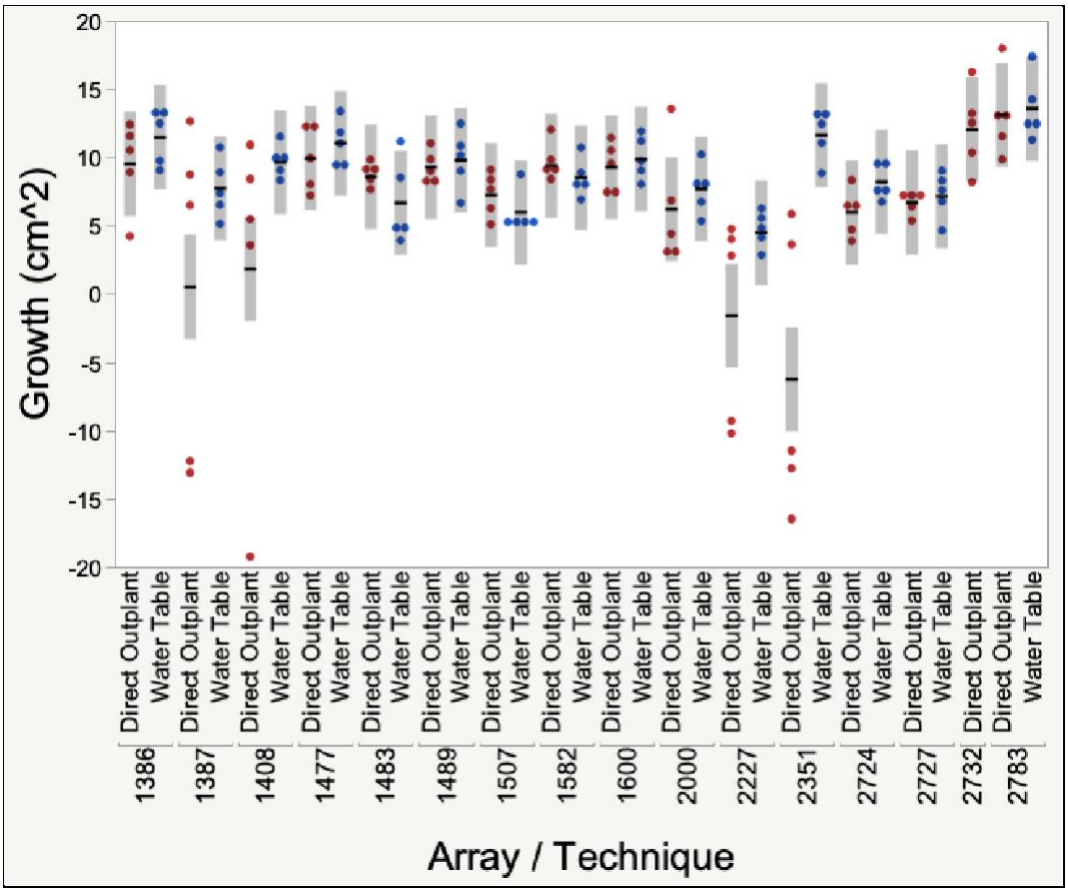


Figure 9. Scatterplot of growth at week 12 by array and technique. Each point represents an individual micro-fragment.

At the end of the study, Week 12, the mean growth (change from baseline) for WT arrays and micro-fragments was greater than for DO (Figs. 10 and 11). DO arrays had a mean growth over 12 weeks of 32.9 cm^2, while WT arrays had a mean growth of 44.6 cm^2. DO micro-fragments had a mean growth over 12 weeks of 6.3 cm^2, while WT micro-fragments had a mean growth of 8.8 cm^2. The figures on the following page illustrate that loss of coral tissue was seen for DO technique and not seen for the WT technique.

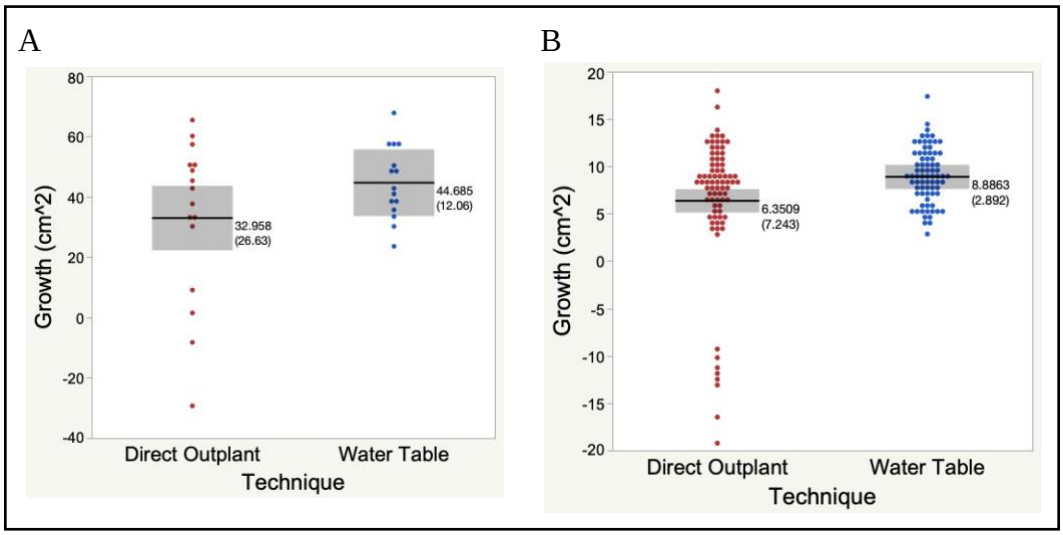


Figure 10. Scatterplot of growth at week 12 by technique for (A) arrays and (B) micro-fragments

Looking at the results at Week 12 by location shows again greater growth by WT technique than DO technique regardless of location (Fig. 11). Using an ANCOVA model with baseline as a covariate and location as a factor, the change from baseline at week 12 for arrays was not statistically significant between the the two groups (ANCOVA: F = 3.3, *P* = 0.079) while for micro-fragments, a significant difference was seen between DO and WT (ANCOVA: F = 10.4, *P* = 0.001). Tests for interactions of techniques with baseline or location showed no significant interactions. Location did not affect growth (ANCOVA: F = 0.08, *P* = 0.776).

A second model was run excluding interactions. For arrays, the results were essentially the same with no statistically significant difference between techniques (ANCOVA: F = 3.1 , *P* = 0.089), but favoring the WT technique. For micro-fragments a significant difference between DO and WT micro-fragments was seen (ANCOVA: F = 8.5, *P* = 0.004). Defining location as a random effect did not change the results for technique (Micro-fragments: ANCOVA: F = 8.3 *P* = 0.0038). Both array and micro-fragment data showed more growth in water tables than for DO by the end of 12 weeks.

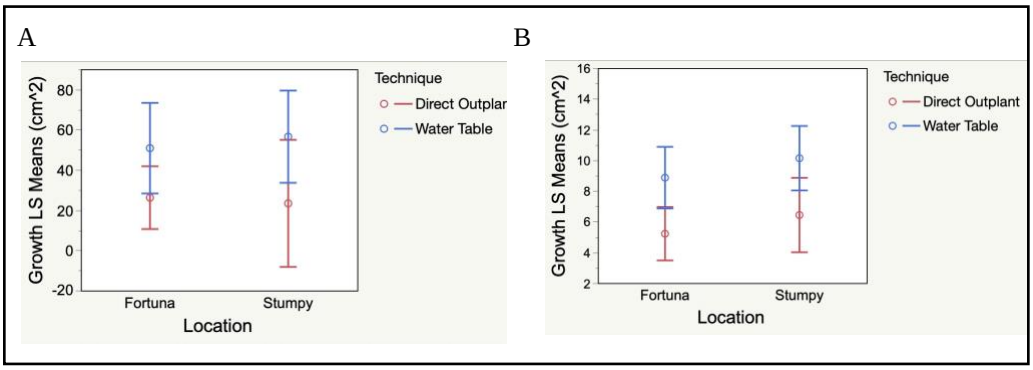


Figure 11. Least square means with confidence limits for technique by location for (A) arrays and (B) micro-fragments.

A matched analysis was done for 15 paired arrays; excluding Stumpy DO array 2732 due to the loss of the water table matching array prior to the start of the study. A comparison of DO arrays with their matching WT arrays showed no statistically significant difference between the two techniques (Fig 12. $P = 0.06$, Paired T-test). Despite not showing significance, the results clearly favor WT grown micro-fragments compared to DO with a mean difference by array of 13.5 cm^2 (95% CI -0.7, +27.7).

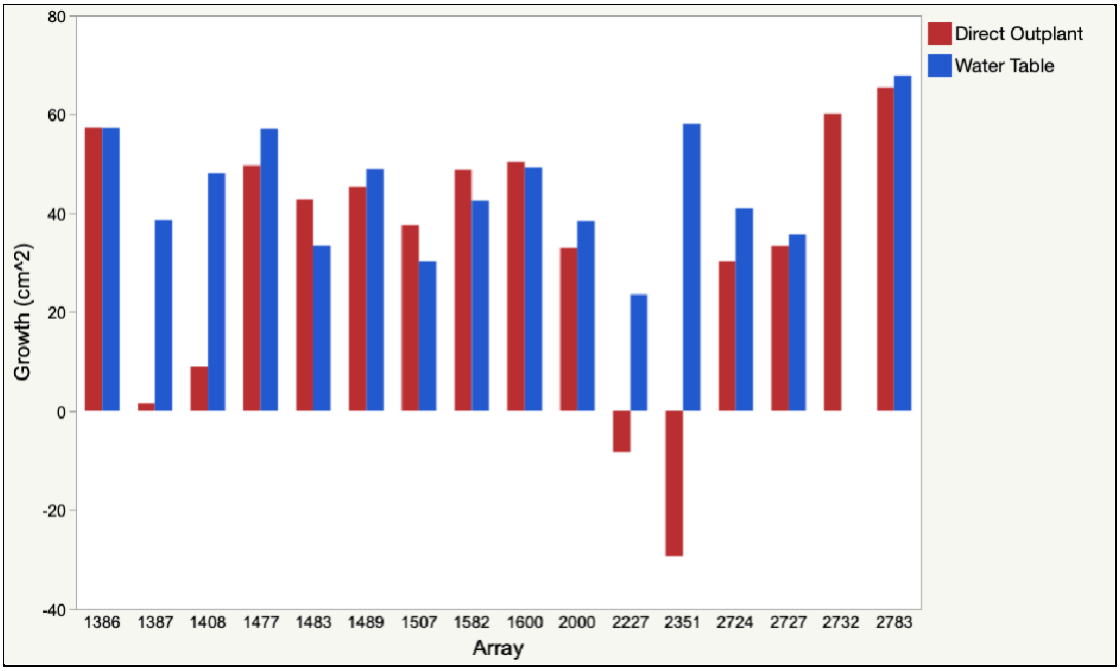


Figure 12. Bar graph of growth for matched pairs of direct outplant and water table arrays at Week 12.

4.1.4. Comparison of Array and Micro-fragment Growth by Technique over 12 Weeks

A repeated measures (RM) analysis of arrays and micro-fragments was performed to examine the change from baseline over the full twelve weeks of the study (Fig. 13). For arrays, this powerful analysis shows a significant difference ($P = 0.0006$, RM) between the techniques, with WT showing a greater change at each week and no significant interaction for technique by week. For micro-fragments, again a greater change at each week for WT was seen; however the interaction of technique by week is statistically significant ($P < 0.0001$) while the technique effect is not ($P = 0.079$). Pairwise comparisons Tukey HSD showed the only significant technique difference at week 12 in the micro-fragments analysis.

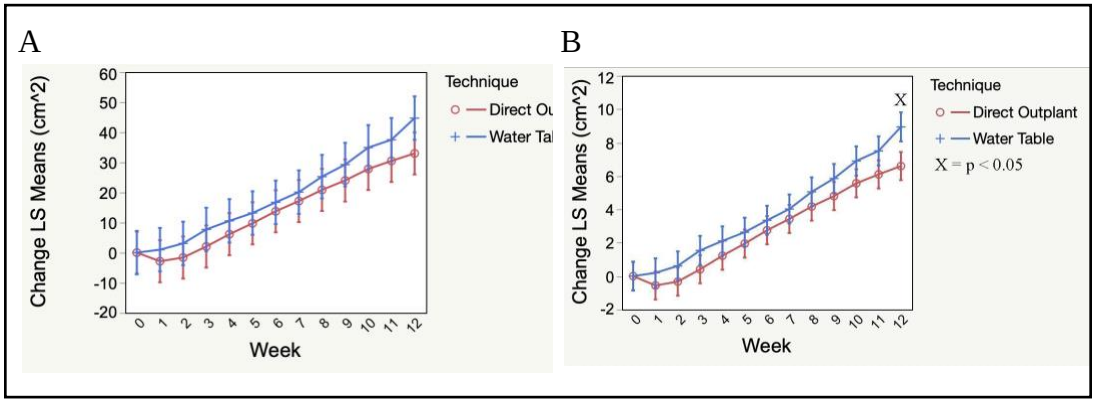


Figure 13. Repeated measure least squares means for technique and week for (A) arrays and (B) micro-fragments. X's indicate weeks where techniques were statistically significantly different.

Observed array growth data over the course of 12 weeks (Fig. 14) illustrates high variability in growth among DO micro-fragments compared to WT micro-fragments. The fitted line for DO is consistently below the WT line due to tissue loss for several arrays. The fitted line for WT arrays suggest the growth rates increase from week 7 through week 12.

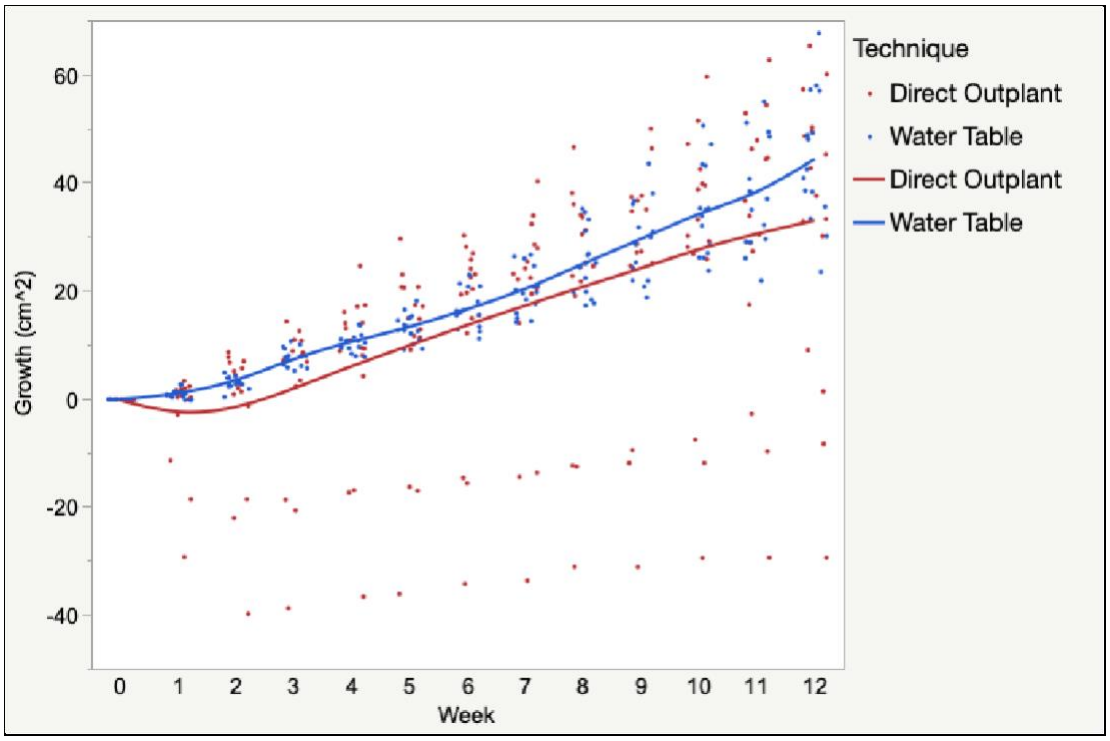


Figure 14. Raw array growth data with week by week fitted lines.

4.1.4. Comparison of Micro-fragment Growth by Technique Excluding Dead Micro-fragments

A total of 7 micro-fragments (0 WT and 7 DO) died during Study2. To assess maximum potential growth, analyses were performed excluding these 7 micro-fragments.

A repeated measures (RM) analysis of surviving micro-fragments was performed to examine the change from baseline over the full twelve weeks of the study (Fig 15). For these micro-fragments, this powerful analysis shows a significant difference ($P = 0.0001$, RM) between the techniques in favor of surviving DO, with no significant interaction for technique by week.

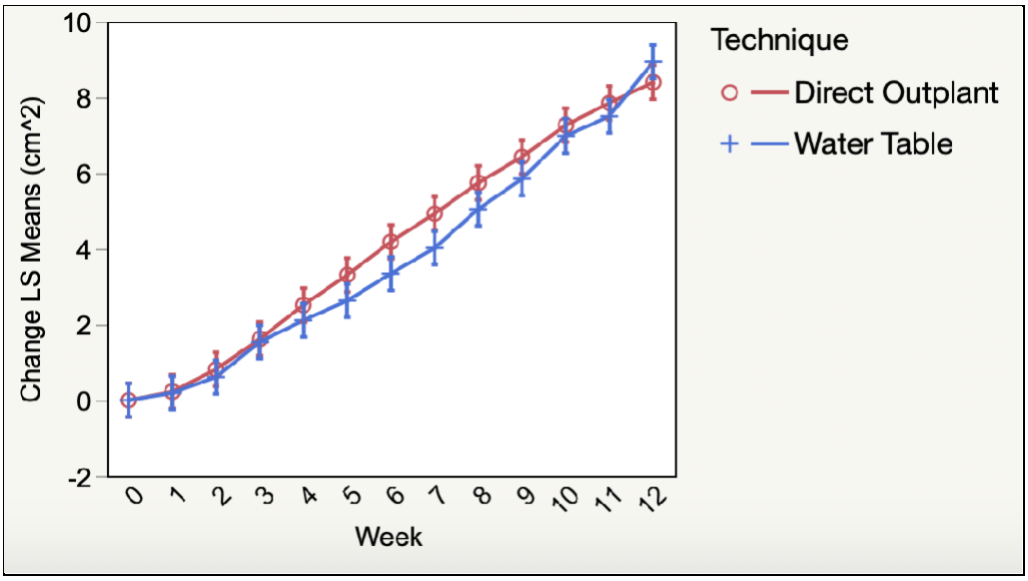


Figure 15. Repeated measures least squares means for surviving micro-fragments.

At the end of the study, Week 12, the mean growth (change from baseline) for surviving WT micro-fragments was greater than for DO (Fig 16). Surviving DO micro-fragments had a mean growth of 8.5 cm^2, while surviving WT micro-fragments had a mean growth of 8.8 cm^2.

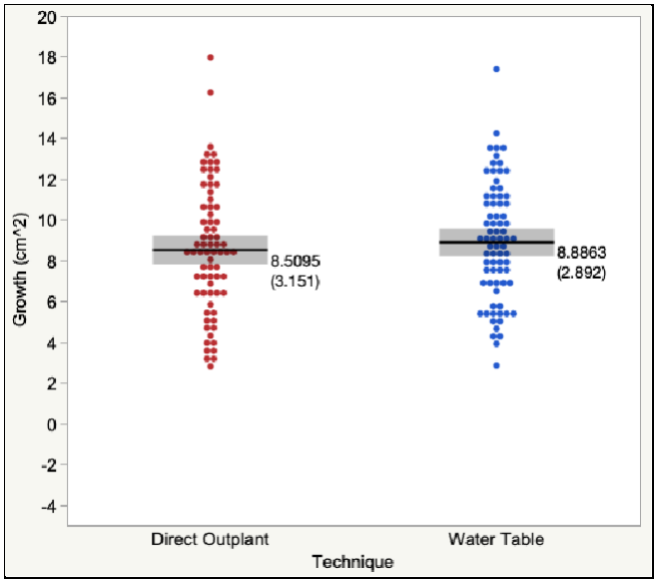


Figure 16. Scatterplot of growth at week 12 by technique for surviving micro-fragments.

Looking at the surviving micro-fragment results at Week 12 by location shows greater growth by WT technique than DO technique regardless of location (Fig 17). Using an ANCOVA model with baseline as a covariate and location as a factor, the

change from baseline at week 12 for micro-fragments was statistically significant between the two groups (ANCOVA: F = 9, *P* = 0.003).

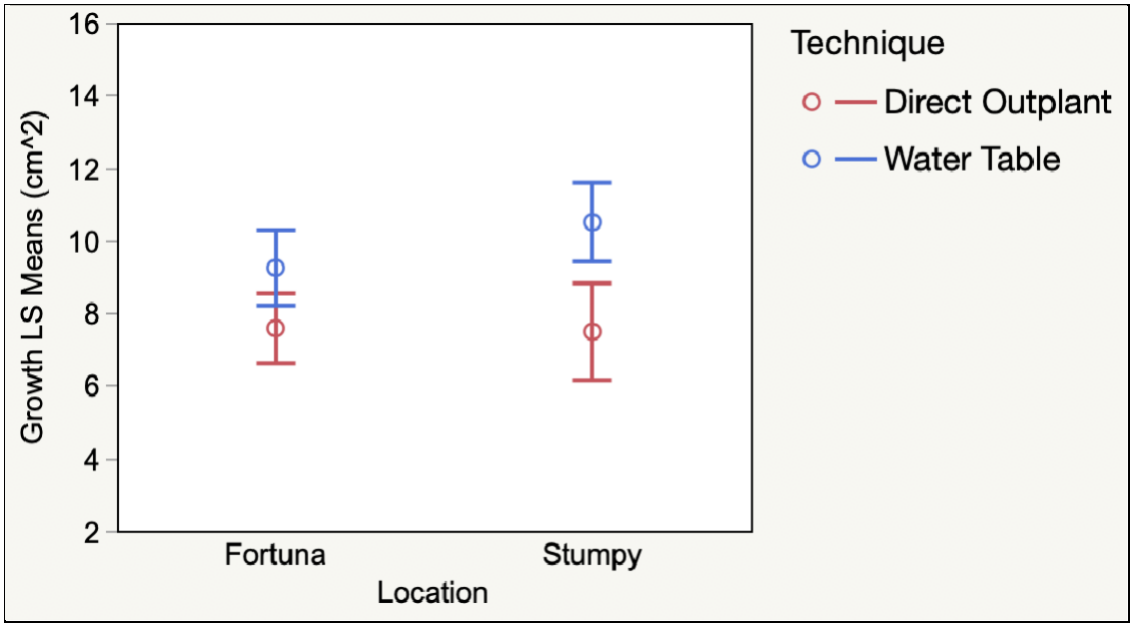


Figure 17. Micro-fragment least square means with confidence limits for technique by location, excluding dead micro-fragments.

4.1.5. Bleaching Susceptibility Due to Direct Outplant Fragmenting

During the study, DO micro-fragments that were cut from the bottom of a branch had a higher tendency to bleach or show paling. Of the 80 DO, 49 micro-fragments were cut from the tops of branches, and 31 were cut from the bottoms. Of bottom micro-fragments, 55% bleached during the study, 39% showed paling and only 6% had no apparent bleaching or paling. Of top micro-fragments, 14% bleached during the study, 2% showed paling and 84% had no apparent bleaching or paling. A Pearson's Chi-squared test showed a significant difference ($P < 0.001$) between tops and bottoms regarding bleaching and paling (Table 3).

Surprisingly, more deaths were recorded for tops (5 dead) than bottoms (2 dead). Of the dead micro-fragments, only one top and one bottom were attributed to bleaching.

Table 3. Bleaching, paling and mortality results for bottoms and tops of direct outplant micro-fragments.

	Bottoms N=31	**Tops N=49**
Bleaching	17 (55%)	7 (14%)
Paling	12 (39%)	1 (2%)
No Apparent Paling/Bleaching	2 (6%)	41 (84%)
Deaths	2 (6%, 1 bleached)	5 (10%, 1 bleached)

4.1.6 Parent Colony Lesion Recovery

All parent colonies showed wound recovery at the point of fragmentation within 2 weeks of direct outplanting. However, the time until regrowth covered the point of fragmentation varied based on wound shape and size. The shortest time period to regrowth (Median: 8 weeks) over the point of fragmentation was seen in thin shaped wounds where there was a relatively small diameter between live tissue margins. For ovular shaped wounds, the median time until regrowth covered the point of fragmentation was greater than 12 weeks (12+ indicates regrowth occurred between 12 weeks and 11

months post fragmentation). The slowest wound regrowth was seen in circular shaped wounds, as these had the largest diameter between live tissue margins, with two circular wounds at Fortuna Bay healing after the 12 week monitoring time period, and two at Stumpy Bay which have not yet regrown over the point of fragmentation.

Table 4. Parent colony wound shape and time to healing and regrowth. 12+ = Total wound area was reskinned after the 12 month experimental time period but before the 11 month monitoring.

	First Growth (weeks)	Wound Regrowth (weeks)	Wound Shape
Fortuna Bay			
1477	2	8	Thin
1483	2	12+	Ovular
1507	2	12+	Ovular
1582	2	12+	Ovular
1600	2	12+	Circular
2227	2	12+	Thin
2351	2	12	Ovular
2724	2	12+	Circular
Stumpy Bay			
1386	2	7	Thin
1387	2	Not Healed	Circular
1489	2	12+	Ovular
2000	2	12+	Thin
2727	2	8	Thin
2732	2	Not Healed	Circular
2783	2	8	Thin
1408	2	12+	Ovular

4.2. Study 2

4.2.1. Comparison of Baseline Measurements for Arrays and Micro-fragments

Comparisons of baseline sizes at the start of the study (Table 1) for both arrays and micro-fragments indicate that WT sizes were significantly larger than DO sizes. This variability was likely due to the larger WT micro-fragment size seen at the end of study 1 where end growth ranged from 67.4 cm^2 - 139.9 cm^2 for arrays, and 10.9 cm^2 - 31.3 cm^2 for micro-fragments.

Table 5. Mean array and micro-fragment size (cm^2) at baseline (Week 0) for Water Table (WT) and Direct Outplant (DO) arrays and fragments.

	WT Mean (SD)	**DO Mean (SD)**	**WT-DO (95% CI)**
Array	97 (18.6) (n = 15)	77.5 (11.7) (n = 15)	+9.5 (95% CI 7.8,31.1)
Fragment	19.3 (4.4) (n = 75)	15.7 (3.1) (n = 75)	+3.6 (95% CI 2.5, 4.9)

Looking at baseline by location (Figure 18) shows the difference between techniques was similar at both Fortuna Bay and Stumpy Bay.

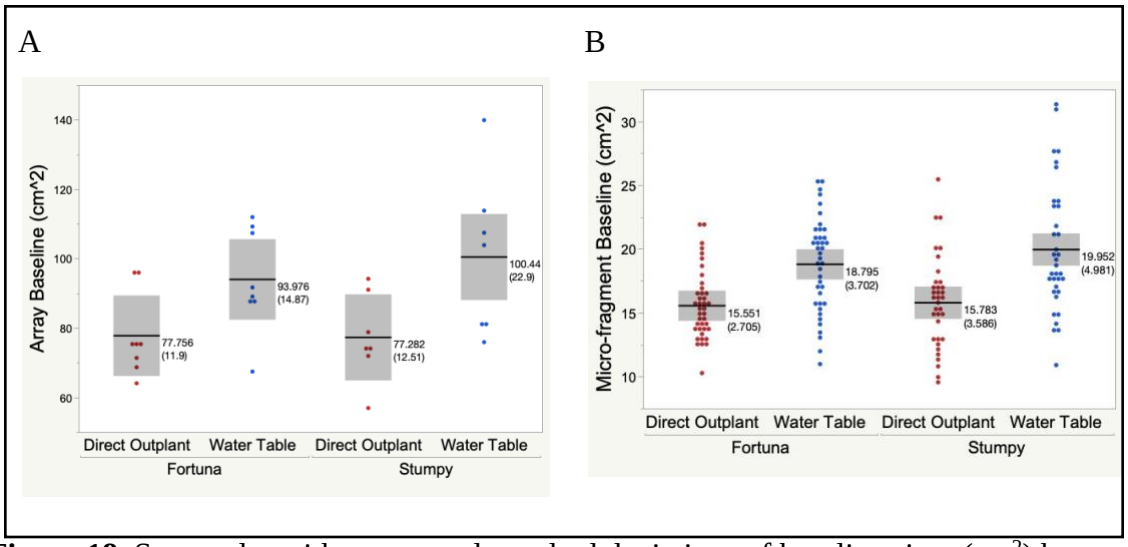


Figure 18. Scatterplot with means and standard deviations of baseline sizes (cm^2) by location and technique of (A) arrays and (B) micro-fragments.

Due to the variability in baseline size, a growth (change from baseline at Week 12) by baseline graph was constructed to determine if the variability in baseline size was correlated with growth and survival. As shown in Fig. 19, a strong correlation (Pearson's Correlation Coefficient, r) was seen between baseline size and growth among arrays at the Fortuna Bay location, but no meaningful correlation was seen at Stumpy Bay (Fig 19. Fortuna: r = 0.8, P = 0.0004; Stumpy: r = 0.3, P = 0.3). A moderate correlation was seen among fragments at Fortuna Bay, but no meaningful correlation was seen at Stumpy Bay (Fig 19. Fortuna: r = 0.5, $P < 0.0001$; Stumpy: r = 0.1, P = 0.5). To account for the

variability in baseline sizes of arrays and micro-fragments, baseline was added as a covariate in further statistical analyses.

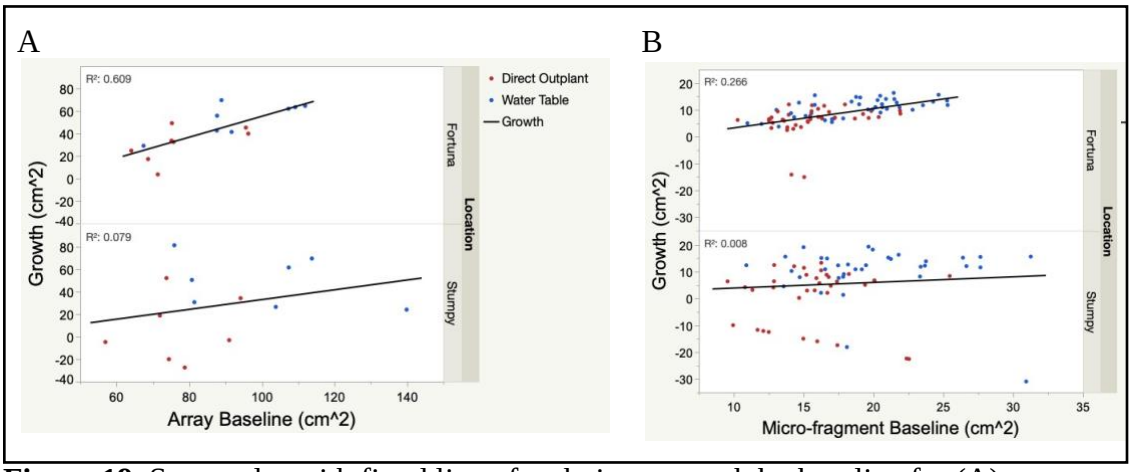


Figure 19. Scatterplot with fitted line of technique growth by baseline for (A) arrays and (B) micro-fragments.

4.2.2. Survival of Micro-fragments and Arrays at Week 12

Only micro-fragments were analysed for survival. All arrays were considered alive at the end of the study because in each array at least one micro-fragment was alive. At the end of 12 weeks, survival was significantly greater for WT micro-fragments compared to DO micro-fragments ($P = 0.02$, Fisher's Exact Test). DO showed a 85.3% survival while WT micro-fragments showed 97.3% survival (Table 6) at the end of the 12 week study. Eleven of 75 DO micro-fragments died and 2 of 75 WT micro-fragments died.

Table 6. Survival of direct outplant and water table fragments at week 12.

	Water Table N (%)	**Direct Outplant N (%)**
Dead	2 (2.7%)	11 (14.7%)
Alive	73 (97.3%)	64 (85.3%)
Total N	75	75

4.2.3. Comparison of Growth at Week 12 for Arrays and Micro-fragments

Average growth among all fragments was 7.1cm^2 ± 0.5cm^2 (SEM) and was fairly consistent among fragments and arrays (Fig. 20).

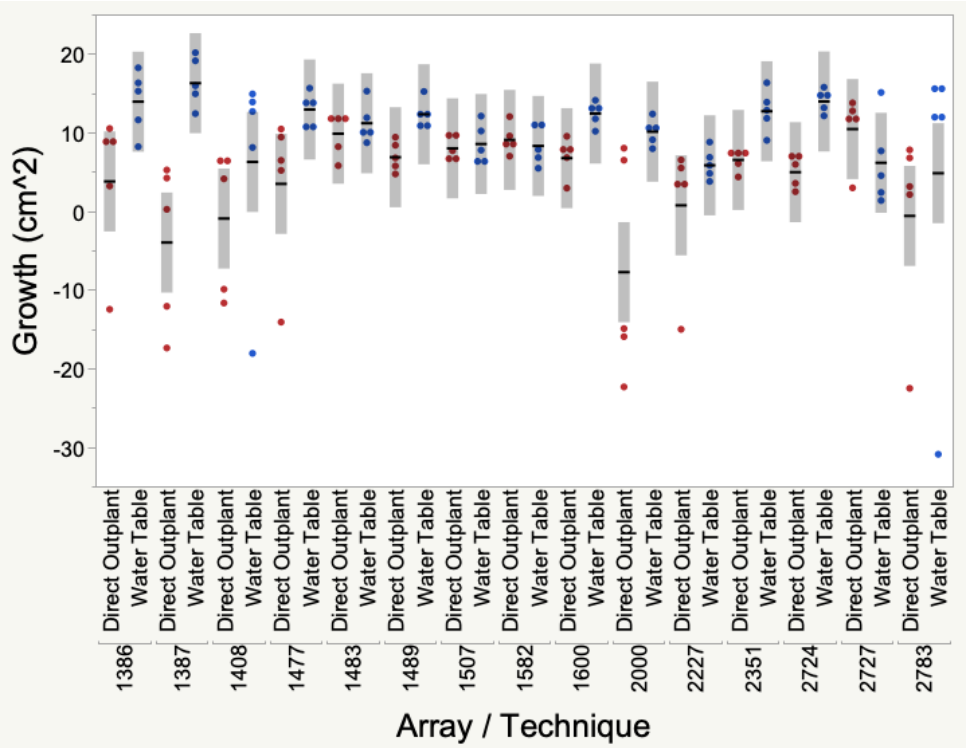


Figure 20. Scatterplot of growth at week 12 by array and technique. Each point represents an individual micro-fragment.

At the end of the study, Week 12, the mean growth (change from baseline) for water table arrays and fragments was greater than for DO (Figure 21). DO arrays had a mean growth over 12 weeks of 19.7 cm^2, while WT arrays had a mean growth of 51.5 cm^2. DO micro-fragments had a mean growth over 12 weeks of 3.8 cm^2, while WT micro-fragments had a mean growth of 10.4 cm^2. The figures below illustrate that loss of coral tissue was mostly seen for the DO technique. Only two WT micro-fragments experienced tissue loss throughout the study.

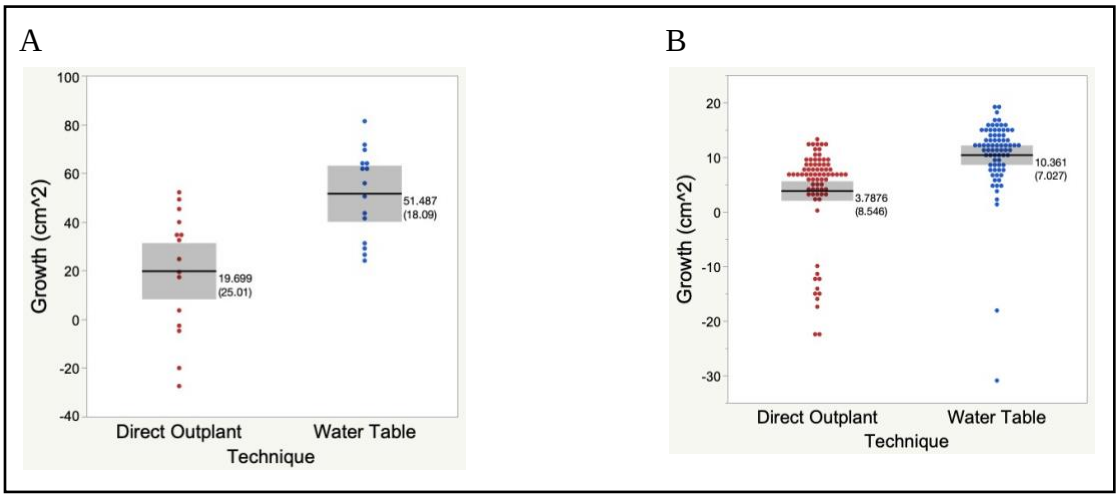


Figure 21. Scatterplot of growth at week 12 by technique for (A) arrays and (B) micro-fragments.

Looking at the results at Week 12 by location shows again greater growth by WT technique than DO technique regardless of location (Figure 22). Using an ANCOVA model with baseline as a covariate and location as a factor, the change from baseline at week 12 for arrays and micro-fragments was statistically significant between the the two groups (ANCOVA; Arrays: F = 8.1, P = 0.009, Micro-fragments: F = 21.3, P = <0.0001) Tests for interactions of techniques with baseline or location showed no significant interactions. It was noted that, for arrays and micro-fragments, Stumpy Bay showed the biggest difference between techniques, however the technique by location effect was not significant (ANCOVA; Arrays: F = 1.5, P = 0.23, Micro-fragments: F = 2.8, P = 0.09)

A second model was run excluding interactions. For arrays and micro-fragments, the results were essentially the same showing statistically significant differences between techniques (ANCOVA; Arrays: F = 9.2, P = 0.005, Micro-fragments: F = 19.9, P < 0.000) and location (Arrays: F = 3.5, P = 0.07, Micro-fragments: F = 5.4, P = 0.02). Defining location as a random effect did not change the results for technique for both arrays and fragments. Both analyses favored WT arrays and micro-fragments over DO.

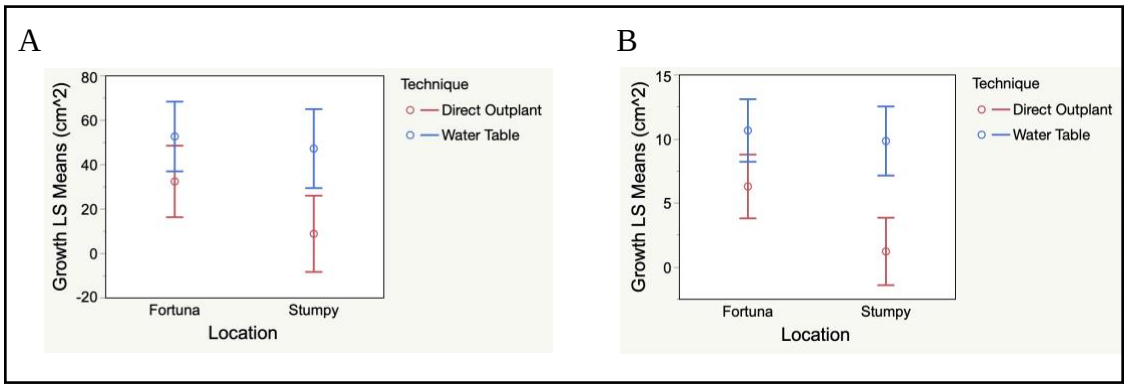


Figure 22. Least square means with confidence limits for technique by location for (A) arrays and for (B) micro-fragments.

A matched analysis was done for 15 paired arrays. A comparison of DO arrays with their matching WT arrays showed a statistically significant difference between the two techniques (Fig. 23, $P = 0.001$, Paired T-test), in favor of WT arrays. WT grown fragments showed considerably more growth compared to DO with a mean difference by array of 31.8 cm^2 (95% CI +14.7, +48.9).

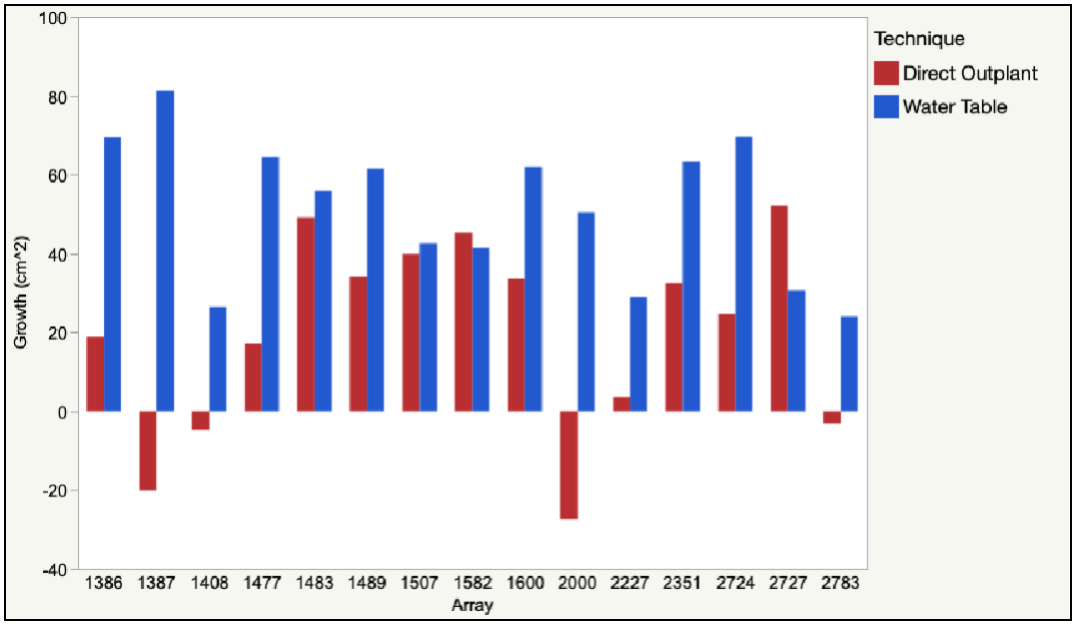


Figure 23. Bar graph illustrating direct outplant array and water table array growth at week 12 for each matched pair.

4.2.4. Comparison of Array and Micro-fragment Growth by Technique over 12Weeks

A repeated measures analysis of arrays and micro-fragments was performed to examine the change from baseline over the full twelve weeks of the study (Figure 24). This powerful analysis shows a highly significant difference between the techniques with WT showing a greater change at each week for arrays (P = <0.0001, Repeated Measures) and for micro-fragments (P = <0.0001, Repeated Measures). There was also a significant interaction for technique by week for both arrays (P = 0.0005, Repeated Measures) and for micro-fragments (P = <0.0001, Repeated Measures). There was no data for weeks 6, 7, and 11at Stumpy Bay as monitoring was not possible due to inclement weather. The exclusion of those Stumpy Bay values increases the means for weeks 6, 7, and 11. Pairwise comparisons Tukey HSD showed a significant technique difference at several weeks as indicated in the graphs below.

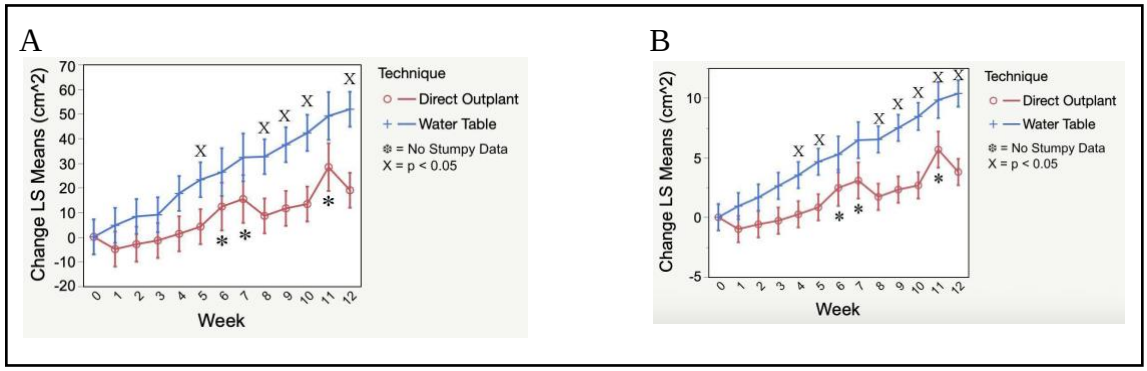


Figure 24. Repeated measure least squares means by techniques and week for (A) arrays and (B) micro-fragments. Letters indicate weeks where the technique effect was statistically significant. X's indicate weeks where technique was statistically significantly different. * indicate weeks at the Stumpy Bay location where no data was collected. due to inclement weather.

Observed array growth data over the course of 12 weeks (Fig. 25 on next page) illustrates high variability in growth among direct outplant fragments compared to WT micro-fragments. The fitted line for DO is consistently below the WT line due to tissue loss for several arrays. The fitted line for water table arrays suggest the growth rates increase from week 3 through week 12.

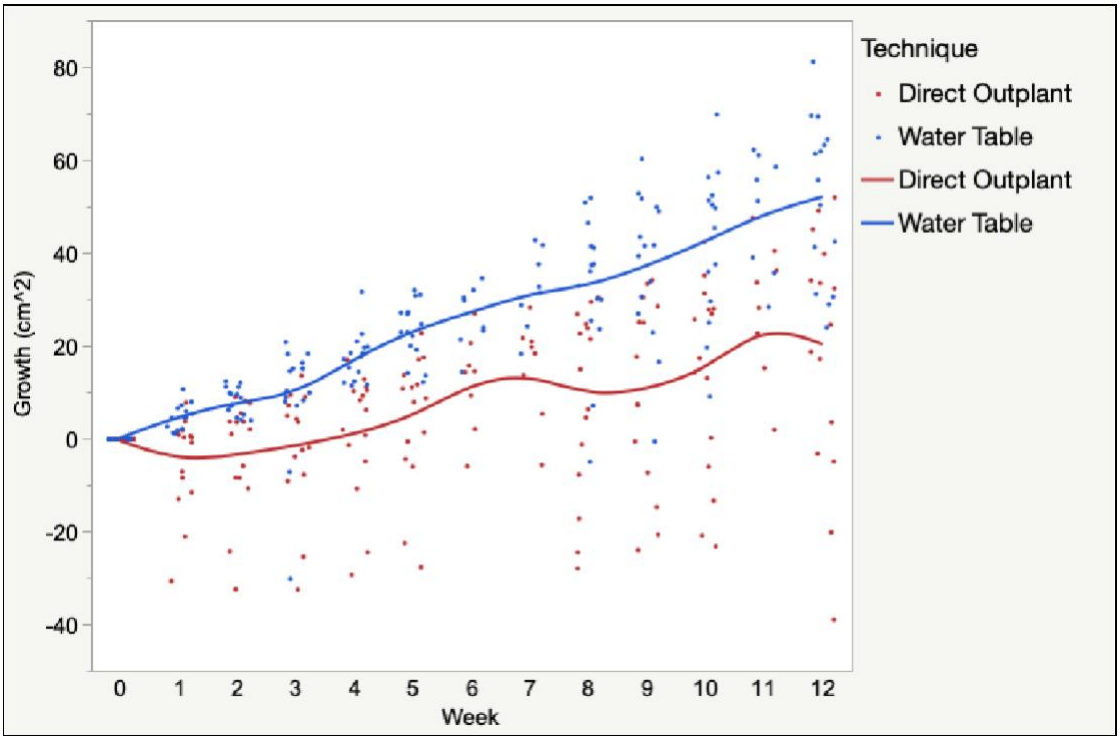


Figure 25. Raw array growth data with week by week fitted line

4.2.4. Comparison of Micro-fragment Growth by Technique Excluding Dead Micro-fragments

A total of 13 micro-fragments (2 WT and 11 DO) died during Study2. To assess maximum potential growth, analyses were performed excluding these 13 micro-fragments.

A repeated measures analysis of surviving micro-fragments was performed to examine the change from baseline over the full twelve weeks of the study (Fig 26). This powerful analysis shows a highly significant difference between the techniques with WT showing a greater change at each week for surviving micro-fragments ($P < 0.0001$, Repeated Measures). There was also a significant interaction for technique by week for surviving micro-fragments ($P < 0.0001$, Repeated Measures) There was no data for weeks 6, 7, and 11at Stumpy Bay as monitoring was not possible due to inclement weather. The exclusion of those Stumpy Bay values increases the means for weeks 6, 7, and 11. Pairwise comparisons Tukey HSD showed a significant technique difference at several weeks as indicated in the graphs below.

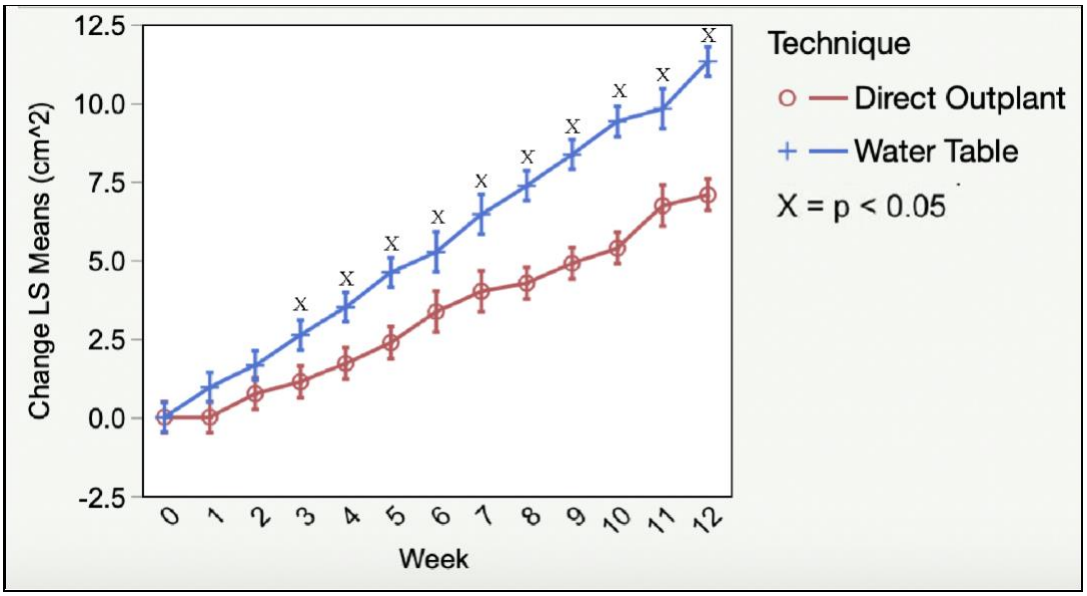


Figure 26. Repeated measure least squares means for micro-fragments by techniques and week, excluding dead micro-fragments. X's indicate weeks where technique was statistically significantly different.

At the end of the study, Week 12, the mean growth (change from baseline) for surviving water table micro-fragments was greater than for DO. Surviving DO micro-fragments had a mean growth over 12 weeks of 7 cm^2, while WT arrays had a mean growth of 11.3 cm^2.

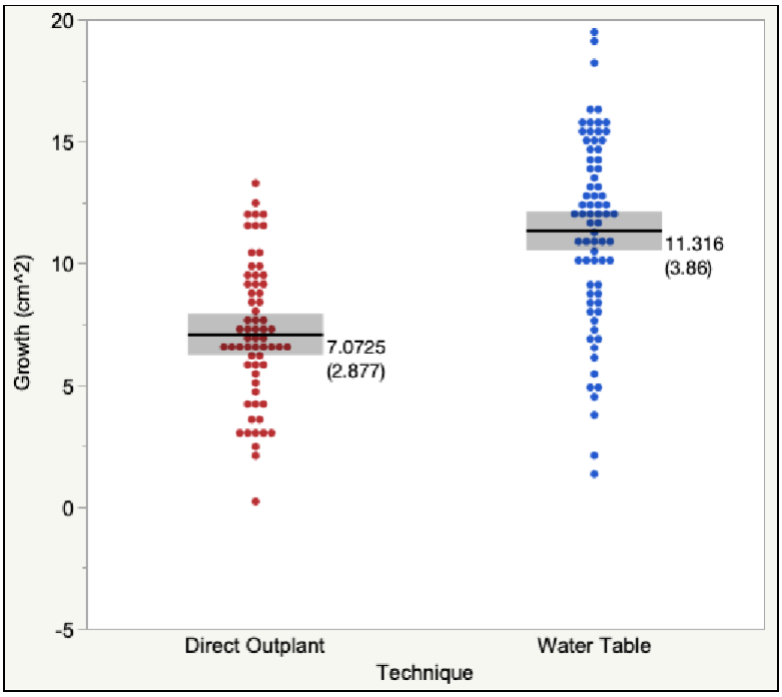


Figure 27. Scatterplot of growth at week 12 by technique for micro-fragments, excluding dead micro-fragments.

Looking at the surviving micro-fragment results at Week 12 by location shows again greater growth by WT technique than DO technique regardless of location (Fig. 28). Using an ANCOVA model with baseline as a covariate and location as a factor, the change from baseline at week 12 for surviving micro-fragments was statistically significantly different between the technique two groups (ANCOVA; F = 24.7, *P* < 0.0001).

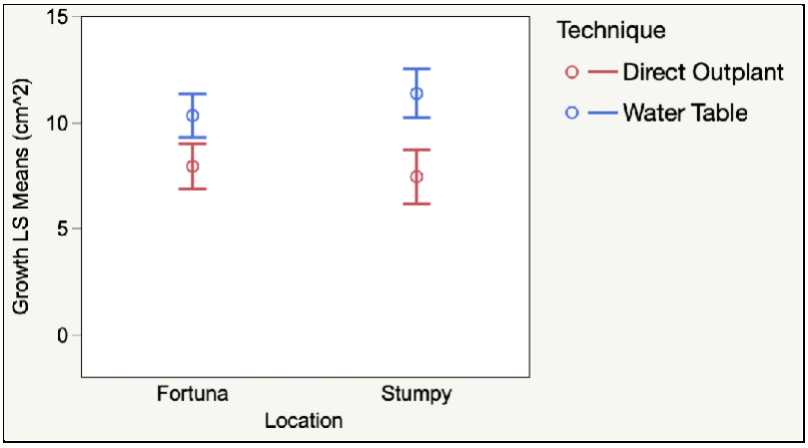


Figure 28. Micro-fragment least square means with confidence limits for technique by location, excluding dead micro-fragments.

4.2.5. Bleaching Susceptibility Due to Direct Outplant Fragmenting

Similar to Study 1, it was noticed that DO fragments that were cut from the bottom of a branch had a higher tendency to bleach or show paling. Of the 75 DO, 51 micro-fragments were cut from the tops of branches, and 24 were cut from the bottoms. 71% of bottom micro-fragments bleached during the study, 17% showed paling and only 12% had no apparent bleaching or paling. 2% of top micro-fragments bleached during the study, 2% showed paling and 96% had no apparent bleaching or paling. A Pearson's Chi-squared test showed a significant difference ($P < 0.001$) between tops and bottoms regarding bleaching and paling (Table 6).

Of the 6 bottoms that died during the study, 5 could be attributed to bleaching. Of the 5 tops that died during the study, 0 could be attributed to bleaching.

Table 7. Bleaching, paling and mortality results for bottoms and tops of direct outplant micro-fragments.

	Bottoms N=24	**Tops N=51**
Bleaching	17 (71%)	1 (2%)
Paling	4 (17%)	1 (2%)
No Apparent Bleaching/Paling	3 (12%)	49 (96%)
Deaths	6 (25%, 5 bleached)	5 (9.8%, 0 bleached)

4.2.6 Parent Colony Lesion Recovery

Similar to Study 1, all parent colonies showed wound recovery at the location of fragmentation within 2 weeks of direct outplanting, but the time until regrowth covering the point of fragmentation varied based on wound shape and size. The shortest time period to regrowth (Median: 12 weeks) over the point of fragmentation was seen in thin shaped wounds where there was a relatively small diameter between live tissue margins. Similar to Study 1, the slowest wound regrowth was seen in circular shaped wounds, as these had the largest diameter between live tissue margins. Wound regrowth labeled as 12+ indicates regrowth occurred between 12 weeks and 11 months post fragmentation.

Table 8. Parent colony wound shape and time to healing and regrowth. 12+ = Total wound area was reskinned after the 12 month experimental time period but before the 11 month monitoring.

	First Growth (weeks)	Wound Regrowth (weeks)	Wound Shape
Fortuna Bay			
1477	2	12+	Circular
1483	2	11	Thin
1507	2	10	Thin
1582	2	12	Thin
1600	2	12+	Thin
2227	2	12+	Thin
2351	2	Not Healed	Circular
2724	2	11	Thin
Stumpy Bay			
1386	2	12	Thin
1387	2	Not Healed	Circular
1489	2	5	Thin (2 branches)
2000	2	12+	Thin
2727	2	Not Healed	Circular
2783	2	12+	Circular
1408	2	9	Thin

Chapter 5: Discussion

Coral restoration has become widely popular over the last two decades in response to the rapid degradation of coral reefs (Boström-Einarsson et al., 2020; Wilkson, 2004). Methodologies involving coral gardening have become the most practiced model for coral restoration (Schmidt-Roach et al., 2020). While the majority of coral restoration studies have been focused on *in-situ* nursery techniques, far fewer studies have tested the value of an *ex-situ* water table nursery (Boström-Einarsson et al., 2020; Schmidt-Roach et al., 2020). Direct outplanting using micro-fragments also remains understudied. Our study was the first to compare water table (WT) grown micro-fragments with direct outplant (DO) micro-fragments at two stages (nursery and outplanting) of the restoration process.

5.1. Baseline Sizes of Arrays and Fragments

At the start of Study 1 and Study 2, mean micro-fragment and array baseline sizes significantly differed between WT and DO micro-fragments (Table. 9). While the size

was larger for DO in Study 1, the opposite was seen for Study 2. The difference seen for WT in Study 2 was likely due to the larger growth of WT fragments at the conclusion of Study 1. Also, branch morphology may have played a role in the variability in baseline sizes, as the shape and size of branches collected dictates the shape and size of fragments that are able to be cut.

The variability in baselines among micro-fragments and arrays was not significantly correlated with growth in Study 1 with r ranging from -0.05 to -0.3. These results are similar to Forrester et al. 2012, who also found no significant difference in growth between fragments of statistically significantly different sizes. Study 2, however did show a strong correlation between baseline and growth (Fig. 20) among arrays at the Fortuna Bay location (r = 0.8).

Table 9. Mean array and fragment size (cm^2) at baseline (Week 0) for Water Table (WT) and Direct Outplant (DO) arrays and fragments for Study 1 and Study 2.

	WT Mean (SD)	**DO Mean (SD)**	**WT-DO (95% CI)**
Study 1			
Fragment	9.5 (1.1) (n=75)	13.5 (2.9) (n=80)	-4.1 (95% CI -4.8, -3.4)
Array	47.3 (3.5) (n=15)	67.6 (10.4) (n=16)	-20.3 (95% CI -26.1, -14.6)
Study 2			
Fragment	19.3 (4.4) (n = 75)	15.7 (3.1) (n = 75)	+3.6 (95% CI 2.5, 4.9)
Array	97 (18.6) (n = 15)	77.5 (11.7) (n = 15)	+9.5 (95% CI 7.8,31.1)

5.2. Survival of Arrays and Fragments

All arrays survived to the conclusion of the study and were still alive at eleven months. Both WT and DO micro-fragments also performed exceptionally well concerning survival. While WT micro-fragments did show statistically significantly higher survival than DO micro-fragments for both studies (Table. 10), Despite this difference, DO micro-fragments showed higher survival than is typically reported for

coral restoration projects (60-70% survival, Boström-Einarsson et al. 2020) as well as similar direct outplanting studies (58-61% survival, Tortolero-Langarica et al. 2020) .

Initial tissue loss and mortality amongst coral outplants tends to occur in the first couple months due to predation, and transplant shock (Forrester et al. 2012; Page et al. 2018). This trend was true for Study 1, in which all seven DO mortalities occurred in the first two weeks (Fortuna Bay: 5, Stumpy Bay: 2). Besides two micro-fragments that their mortality could be attributed to bleaching, all other dead micro-fragments had no living tissue at the first monitoring session, and therefore the reason for death could not be determined. Despite not being able to determine mortality, previous research has shown predation to be most abundant on coral outplants during the first few weeks after outplanting (Page et al., 2018). It's entirely possible that the fragments that died from unknown causes were due to predation (Baums et al., 2003). More mortality was recorded amongst micro-fragments in Study 2 than Study 1. However, the trend was similar where nearly all micro-fragments whose mortality could not be attributed to bleaching; the cause of death was unknown, as the micro-fragments were completely dead at the first monitoring session. One micro-fragment was seen with partial mortality at the first monitoring session and appeared to suffer from rapid tissue loss, causing its total mortality by the second monitoring session. Due to the outplants' size, diagnosing their cause of death can be very difficult.

The actual survival of Study 2 DO fragments would have been slightly higher; however, two DO micro-fragments died due to dislodgement during a strong swell, and one DO micro-fragment died due to damage during the outplanting process. Previous studies (Garrison & Gregg. 2008; Garrison & Gregg. 2012; Tortolero-Langarica et al. 2020) have also reported dislodgement due to high wave energy as a significant factor in outplant mortality. Under non-experimental protocols, DO micro-fragments would be attached directly to the reef and not an intermediary puck to match WT micro-fragments. Examining the time point of when the DO micro-fragments grew out onto the puck and when dislodgement occurred, the micro-fragments would likely have been solidified to the substrate by this point and would not have been as easily dislodged as a puck attached with epoxy. Guest et al. 2011 found variability in the time period to self attachment for three species of *Acropora*, however, all showed self attachment times faster than the point

of puck dislodgement in our study. If these micro-fragments are excluded, the survival would have been 91% for DO micro-fragments for Study 2. The two WT micro-fragments that died during Study 2 were also due to dislodgement. However, securing water table micro-fragments to cement pucks in the land-based nursery is part of the methodology (Page et al. 2018), regardless of being in an experimental setting or not.

Prior to the start of the study, the loss of branch Stumpy - 2732 a day before fragmenting highlights the challenges of keeping corals alive in a water table nursery.

Unlike many other outplant studies (Koval et al., 2020; Page et al., 2019), the micro-fragments in this study experienced minimal predation. There were no visible bite marks from corallivore fishes as seen in Page et al., 2019, but the occasional *Corallophila abbreviata* was seen on or near fragments, however, none led to significant tissue loss or mortality.

Time of season may have had an influence on the survival of micro-fragments as sea surface temperatures (SST) during Study 1 (Mean U.S. Virgin Islands = 28.4C) were cooler than during Study 2 (Mean U.S. Virgin Islands = 29.3C). Throughout Study 1, SST remained below the bleaching threshold SST, however, for Study 2, the bleaching threshold STT was crossed, resulting in a Bleaching Warning and Alert Level 1 (https://coralreefwatch.noaa.gov/) during the months of September and October. The elevated temperatures seen during Study 2 may have contributed to the increase in mortality than seen during study 1.

Table 10. Survival analysis for water table (WT) and direct outplant (DO) over 12 weeks.

	WT	DO	WT-DO	p-value
Study 1 Survived	75/75 (100%)	73/80 (91%)	9%	0.01
Study 2 Survived	73/75 (97%)	64/75 (85%)	12%	0.03

5.3. Growth

Consistently for each week of Study 1 and Study 2, WT arrays and micro-fragments experienced more growth than DO arrays and micro-fragments. Repeated measures analyses showed highly significant ($p<0.006$) results for WT over DO arrays for both studies. In both studies, after an early loss of tissue for DO, the trajectory of growth for both techniques are parallel for several weeks. By about Week 7 or 8,

growth rate for WT increases over DO as illustrated by the graph of raw data for Study 1 below (Fig. 29). This graph also illustrates the greater variability observed for the DO technique compared to the WT technique which was seen in both studies.

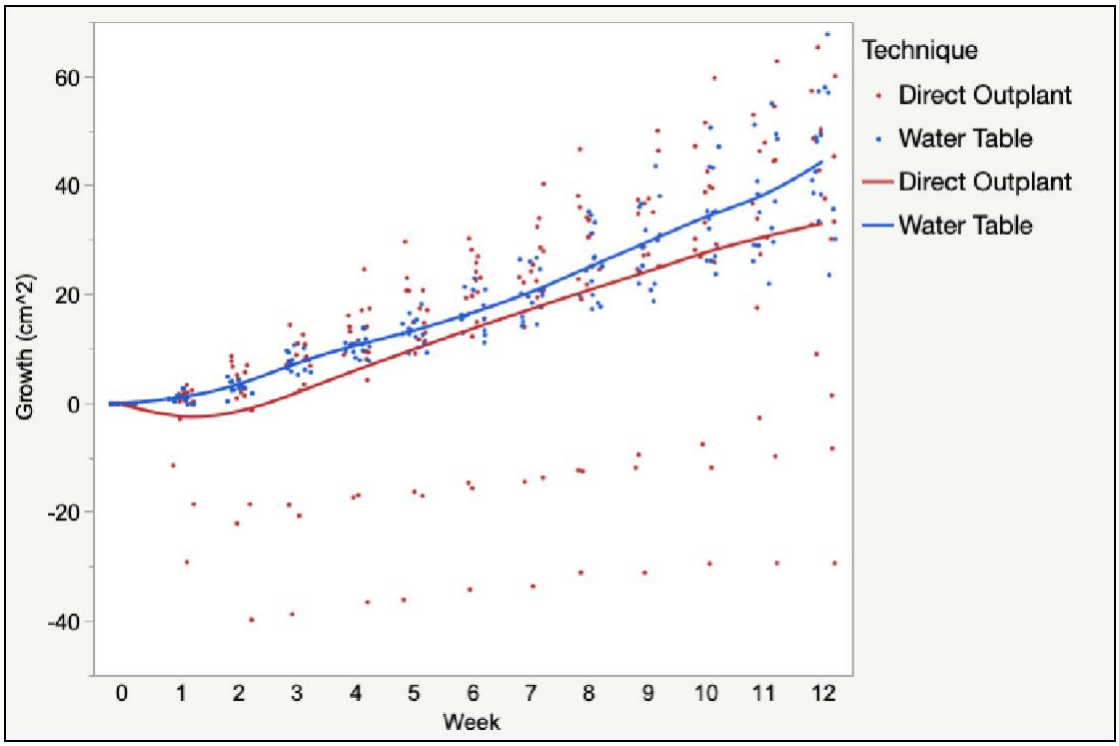


Figure 29. Study 1 raw array growth data with week by week fitted line.

Week 12 results are summarized in Table 11 below and clearly show more growth for the WT technique than the DO technique by the end of each study. These findings were expected for Study 1 as environmental conditions were controllable in the water tables to provide an optimal environment for coral growth (Leal, 2016). As a consequence of the latter, DO micro-fragments showed a loss of tissue early in the study that had a significant impact on analyses. When dead fragments were removed from the Study 1 data, the mean technique difference at Week 12 changed from WT - DO = 2.5cm^2 to WT - DO = 0.4cm^2. This indicates that dead micro-fragments decreased overall mean growth of DO however, WT and surviving DO micro-fragments had similar growth rates.

Table 11. Week 12 growth cm^2(Week 12-Week 0) by Study and Technique

	WT Mean (SD)	**DO Mean (SD)**	**WT-DO (95% CI)**	**p-value**
Study 1				
FRAG	+8.9 (2.9)	+6.4 (7.2)	+2.5 (+0.8, +4.3)	0.004[1]
FRAG No deaths	+8.9 (2.9)	+8.5 (3.2)	+0.4 (-0.6, +1.4)	0.003[1]
ARRAY	+44.7 (12.1)	+32.9 (26.6)	+11.7 (-3.6, +27)	<0.09[2]
Study 2				
FRAG	+10.3 (7.0)	+3.8 (8.6)	+6.6 (+4.1, +9.1)	<0.0001[3]
FRAG No deaths	+11.3 (3.9)	+7.1 (2.9)	+4.2 (+3.1, +5.4)	<0.0001[3]
ARRAY	+51.5 (18.1)	+19.7 (25)	+31.8 (+15.4, +48.2)	<0.008[4]

1 - Study 1 2-way ANCOVA with baseline as a covariate and with location as a factor

2 - Study 1 Matched pairs test p=0.06 and ANCOVA with baseline as a covariate and with location as a factor p=0.09

3 - Study 2 2-way ANCOVA with baseline as a covariate and with location as a factor

4 - Study 2 Matched pairs test p=0.004 and ANCOVA with baseline as a covariate and with location as a factor p=0.008

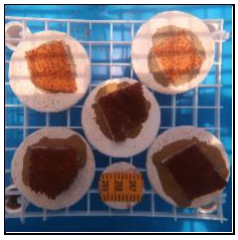
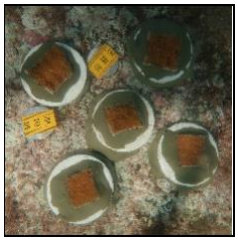
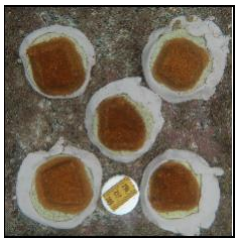
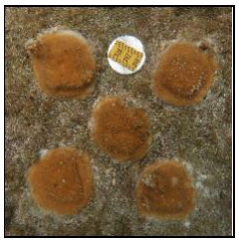
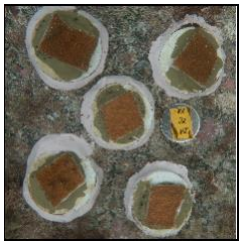
In Study 2 a larger technique effect was seen for WT over DO compared to Study 1(Table 11). One potential advantage the WT micro-fragments may have had over the DO micro-fragments in Study 2 is that WT micro-fragments had already skinned out over much of the cement puck. This meant the margin was easily identifiable through top down photographs. In contrast, DO micro-fragments had to grow down the edge of the

micro-fragment before horizontal growth was easily recorded (Table 12. Study 2 Fragmentation Day).To measure the maximum growth potential of both WT and DO micro-fragments, those micro-fragments that died during the study were removed from certain analyses. For Study 1, the repeated measures analysis showed surviving DO micro-fragments experiencing more growth than surviving WT micro-fragments overall for the 12 week study period. However, for Study 2, the opposite was seen, where WT micro-fragments experienced significantly higher growth than DO micro-fragments throughout the 12 week study period.

When looking at growth at week 12, WT micro-fragments had higher growth than DO micro-frgaments for both Study 1 and Study 2. However, the difference was minimal (Table 11; Study 1: 0.4cm^2. Study 2: 4.2cm^2).

These results suggest that the difference in the maximum potential growth between techniques over the course of 12 weeks is minimal when excluding deaths. Future studies should minimize the underlying reasons for mortality amongst DO micro-fragments, thus raising the overall growth for these techniques.

Table 12. Experimental colony Fortuna-1477 at fragmentation day, 12 weeks, and 11 months.

	Fragmentation Day	**12 Weeks**	**11 Months**
Study 1 Water Table Fortuna: 1477			
Study 1 Direct Outplant Fortuna: 1477			
Study 2 Water Table Fortuna: 1477			
Study 2 Direct Outplant Fortuna: 1477			

Comparing growth rates to other studies involving *Acropora spp.* was challenging, as most studies measure growth through linear extension (Garrison and Ward, 2012; Lirman, 2000a), while in our study, growth was measured in change of area in cm^2 due to the small size of micro-fragments. Using similar techniques to ours, Forsman et al. 2015 found an increase in growth of 329% and 154% in *Orbicella*

faveolata and *Pseudodiploria clivosa* respectively, over four months in a water table nursery. In contrast our study found an increase in growth of 95% and 49% for the total WT and DO tissue in Study 1 over three months. Study 2 found an increase in growth of 54% and 24% for the total WT and DO tissue. These findings support the use of a water table nursery in areas where there are few sources of donor colonies, and rapid increase in tissue growth is the primary goal. The low cost quick solution of direct outplanting may be more beneficial in areas of moderate coral cover where there are more donor colonies to harvest from and less risk of damaging the few remaining colonies in areas of low populations.

5.4. Bleaching of Direct Outplants

One unforeseen observation during the study was the disproportionate amount of bleaching and paling recorded on micro-fragments cut from the bottom (about 90%) of branches compared to the top (<20%) (Table 13). This trend was likely due to increased light hitting the bottom micro-fragments when they were epoxied to the reef. It has been well documented that excess UV light, especially a rapid increase over a short time, as in this study, can trigger corals to bleach (Hoegh-Guldberg et al. 1989; Osinga et al. 2008). However, very little mortality during Study 1 and 2 could be directly attributed to bleaching (of the 34 bottoms that bleached in both studies, only 6 died). In both studies, the majority of bottom micro-fragments that bleached fully recovered within the first four weeks. Future direct outplanting studies involving *A. palmata* should consider orienting micro-fragments cut from the bottom of branches in a way that minimizes the amount of UV light on the coral tissue.

Table 13. Bleaching and paling percentages among micro-fragment bottoms and tops.

		Bottoms	**Tops**
Study 1	**Bleached**	17/31 (55%)	7/49 (14%)
	Paling	12/31 (39%	1/49 (2%)
Study 2	**Bleached**	17/24 (71%)	1/51 (2%)
	Paling	4/24 (17%)	1/51 (2%)

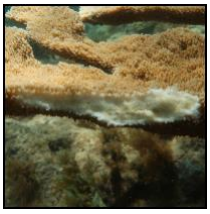
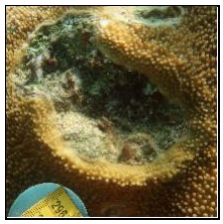
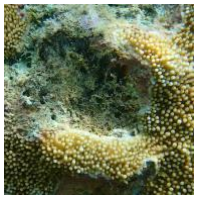
5.5. Impacts on Donor Colonies

Due to direct outplanting techniques relying on harvesting from a donor colony, understanding the impacts on the donor *A. palmata* colonies following branch collection was an important aspect of this study. For all 16 colonies the wound margin caused from branch fragmentation was healed by the second week of monitoring. A wound was considered healed when the damaged tissue margin showed new polyp growth. Lirman, 2000b, also saw the same trend of the wound margin healing and new polyp formation occurring at two weeks post-fragmentation.

The results showed an interesting relationship between shape and wound regrowth (when new coral tissue has reskinned the branch breakage point) time (Figure 31). Wounds that had the thinnest margin between live tissue showed the fastest time until regrowth covered the entire wound (min: 7 weeks, median: 11.5 weeks, max: 12+ weeks). In contrast circular wounds had the slowest times to reskin the entire wound area as the diameter between the live tissue margins was greater than the other wound shapes. Five of nine circular shaped wounds did not reskin over the entire wound area, with the other four reskinning by the eleven month check up. Previous studies (Oren. 1997, Lirman et al), have found similar results with respect to wound shape and recovery times.

Previous studies (Bright et al. 2016) have found significant interactions between *A. palmata* lesions and increased predation and disease prevalence. In this study, there was no indication that fragmentation had any lasting impacts on the donor colony. There were occasional recordings of *Corallophila abbreviata*, but no increasing number of snails was seen shortly after fragmentation through the end of the study.

Table 14. Photos of wound healing by parent colony shape and study time

Thin	 ***Week 1*** Open Wound	 ***Week 2*** New Growth	 ***Week 8*** Wound Regrowth	 ***11 months*** Wound Regrowth
Ovular	 ***Week 1*** Open Wound	 ***Week 2*** New Growth	 ***Week 12*** Partial Regrowth	 ***11 months*** Wound Regrowth
Circular	 ***Week 1*** Open Wound	 ***Week 2*** New Growth	 ***Week 12*** Partial Regrowth	 ***11 months*** Partial Regrowth

5.6. General Implications

Due to WT micro-fragments and arrays experiencing greater growth and survival at the end of Study 1 compared to DO, we accept the hypothesis that predicted WT micro-fragments and arrays would outperform DO micro-fragments and arrays. For Study 2, the expectation under the hypothesis was that DO arrays and micro-fragments would experience greater growth and survival than those arrays that were outplanted from the water tables. We reject the hypothesis due to WT micro-fragments and arrays experiencing greater growth and survival than DO micro-fragments and arrays. Overall both studies showed the benefit of including time in a water table before outplanting.

Depending on the goals of a coral restoration project, each technique has particular advantages. In areas of low coral cover, a land-based nursery may be a better option for propagating coral due to the absence of most environmental stressors. Direct outplanting may be more practical in areas of moderate coral cover where many parent colonies can be sampled, thus leaving a lower percentage of the total live tissue on a given reef intact.

Operational costs should also be considered when deciding if a water table nursery or direct outplanting will be a project's primary technique for coral restoration. In recent years the cost and scalability of building a water table nursery has become more feasible (CoralVita, 2019; Plant a Million Corals), however the ongoing maintenance and the need for trained personnel can be an expensive endeavor (Bartlett. 2013). By using direct outplanting and bypassing the nursery phase, large numbers of corals can be outplanted in a single day using trained volunteers, thus greatly reducing operational costs as demonstrated by Hesley et al., 2017.

While this study looks at *A. palmata*, one of the more commonly practiced species in coral restoration, a recent study by dela Cruz et al., 2015 demonstrated that direct outplanting can also be beneficial for slow growing massive coral species. Their study showed that despite *ex-situ* nursery fragments showing enhanced growth and survivorship, the nursery phase did not improve fragment success post-transplantation. In contrast, our study indicates there may be an advantage to a water table based nursery period, as there was increased growth, and 100% survival in the nursery, and the only water table micro-fragment mortalities post-transplantation were a result of dislodgement.

Techniques involving direct outplanting of micro-fragments are in their infancy, as there are few publications describing these methods. For this study, the researcher had 5 years experience with propagating and outplanting in a water table nursery setting, but it was the researcher’s first experience with direct outplanting. The lack of experience with direct outplanting may have influenced the results. For example, the most stressful time period during direct outplanting is the process of transporting fragments to and from the shore to be fragmented where they are experiencing rapid fluctuations in light, temperature and water flow. Future direct outplanting studies should consider techniques

that would allow for micro-fragments to be cut underwater, thus bypassing the land-based component.

Coral restoration efforts like these may be short-term solutions to mitigating some of the harmful effects climate change is having on coral reefs (Boström-Einarsson et al., 2020). However, a global effort in reducing the negative effects of climate change would likely have the biggest impact in preserving coral reefs, and other vulnerable ecosystems (Hoegh-Guldberg, 2007.

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Appendix A - Tukey HSD

Study 1 Micro-fragments Tukey HSD

Level																												Least Sq Mean
Water Table,12	A																											8.936947
Water Table,11		B																										7.499093
Water Table,10		B	C	D																								6.896714
Direct Outplant,12		B	C		E																							6.591625
Direct Outplant,11		B	C		E	F																						6.093363
Water Table,9			C	D	E		G																					5.848493
Direct Outplant,10		B	C	D	E	F		H																				5.559038
Water Table,8					E	F	G		I																			5.047773
Direct Outplant,9				D			G	H		J																		4.793863
Direct Outplant,8							G		I	J	K																	4.166900
Water Table,7					F			H	I			L	M															4.020373
Direct Outplant,7									I		K	L		N		P												3.425275
Water Table,6								H		J		L	M		O	P												3.341840
Direct Outplant,6												L	M	N		P	Q		S									2.746438
Water Table,5										J	K				O	P		R	S									2.630667
Water Table,4											K			N					R	S	T		V					2.109480
Direct Outplant,5													M		O		Q			S		U	V					1.947513
Water Table,3														N			Q				T		V	W				1.538493
Direct Outplant,4															O			R				U	V		X			1.222713
Water Table,2																	Q					U		W	X	Y		0.611907
Direct Outplant,3																		R		T				W	X	Y		0.406550
Water Table,1																						U			X	Y		0.194520
Water Table,0																						U			X	Y		2.2454e-13
Direct Outplant,0																					T			W		Y		1.0852e-13
Direct Outplant,2																								W		Y		-0.327137
Direct Outplant,1																								W		Y		-0.562012

Study 2 Micro-fragments Tukey HSD

Level																Least Sq Mean
Water Table,12	A															10.36065
Water Table,11	A	B														9.81560
Water Table,10	A	B	C													8.44101
Water Table,9	A	B	C	D												7.48352
Water Table,8		B	C	D	E											6.51136
Water Table,7		B	C	D	E	F										6.45355
Direct Outplant,11			C	D	E	F	G									5.66588
Water Table,6			C	D	E	F	G									5.25768
Water Table,5				D	E	F	G									4.64320
Direct Outplant,12					E	F	G	H								3.78756
Water Table,4						F	G	H	I							3.52969
Direct Outplant,7					E	F	G	H	I	J	K					3.07608
Direct Outplant,10							G	H	I	J						2.66773
Water Table,3							G	H	I	J	K					2.62515
Direct Outplant,6						F	G	H	I	J	K	L	M			2.46033
Direct Outplant,9							G	H	I	J	K	L				2.31427
Direct Outplant,8								H	I	J	K	L	M			1.70611
Water Table,2								H	I	J	K	L	M			1.65757
Water Table,1								H	I	J	K	L	M			0.93752
Direct Outplant,5									I	J	K	L	M			0.83484
Direct Outplant,4										J	K	L	M			0.25047
Water Table,0										J	K	L	M			1.5543e-15
Direct Outplant,0										J	K	L	M			4.4409e-16
Direct Outplant,3											K	L	M			-0.28588
Direct Outplant,2												L	M			-0.59132
Direct Outplant,1													M			-0.99139

Study 2 Micro-fragments Tukey HSD (Dead micro-fragments omitted)

Level														Least Sq Mean
Water Table,12	A													11.316192
Water Table,11		B												9.815600
Water Table,10		B												9.410353
Water Table,9		B	C											8.360233
Water Table,8			C	D										7.361438
Direct Outplant,12			C	D										7.074875
Direct Outplant,11				D	E									6.731711
Water Table,7				D	E									6.453550
Direct Outplant,10					E	F								5.378828
Water Table,6					E	F								5.257675
Direct Outplant,9						F	G							4.896500
Water Table,5						F	G	H						4.604384
Direct Outplant,8						F	G	H						4.261156
Direct Outplant,7						F	G	H	I					4.005605
Water Table,4								H	I	J				3.500397
Direct Outplant,6							G	H	I	J				3.357447
Water Table,3									I	J	K			2.613583
Direct Outplant,5										J	K	L		2.373563
Direct Outplant,4											K	L	M	1.710484
Water Table,2											K	L	M	1.648137
Direct Outplant,3												L	M	N 1.124406
Water Table,1													M	N 0.952205
Direct Outplant,2													M	N 0.749531
Direct Outplant,1														N 0.000953
Direct Outplant,0														N 6.6613e-16
Water Table,0														N 6.6613e-16

Study 2 Array Tukey HSD

Level														Least Sq Mean
Water Table,12	A													51.80327
Water Table,11	A	B												49.07800
Water Table,10	A	B	C											42.20507
Water Table,9	A	B	C	D										37.41760
Water Table,8		B	C	D	E									32.55680
Water Table,7	A	B	C	D	E	F								32.26775
Direct Outplant,11		B	C	D	E	F	G							28.32938
Water Table,6		B	C	D	E	F	G	H	I					26.28837
Water Table,5			C	D	E	F	G	H						23.21600
Direct Outplant,12				D	E	F	G	H	I	J				18.93780
Water Table,4					E	F	G	H	I	J	K			17.64847
Direct Outplant,7				D	E	F	G	H	I	J	K	L		15.38038
Direct Outplant,10						F	G	H	I	J	K	L		13.33867
Direct Outplant,6					E	F	G	H	I	J	K	L		12.30162
Direct Outplant,9						F	G	H	I	J	K	L		11.57133
Water Table,3							G	H	I	J	K	L		9.00133
Direct Outplant,8							G	H	I	J	K	L		8.53053
Water Table,2							G	H	I	J	K	L		8.28787
Water Table,1								H	I	J	K	L		4.68760
Direct Outplant,5									I	J	K	L		4.17420
Direct Outplant,4										J	K	L		1.25233
Water Table,0											K	L		8.8818e-15
Direct Outplant,0											K	L		7.1054e-15
Direct Outplant,3												L		-1.42940
Direct Outplant,2												L		-2.95660
Direct Outplant,1												L		-4.95693