Micro-fragmentation as a Community-Based Coral Restoration Technique in a Developing Country

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

The need for restoration of coral reefs is increasing rapidly due to anthropogenic impacts and climate change. A variety of restoration methodologies are used, with micro-fragmentation showing promising results for increasing coral growth rates. Published literature on *Acropora* restoration has high variation in the size of utilized fragments, and the source of the fragments is often unknown, creating potentially unreliable results. The measuring methods are also inconsistent, and often inaccessible, making it hard to compare the findings between studies. This study aims to determine if micro-fragmentation increases the growth rate of *Acropora*, using specified fragment sizes and determine if current restoration and monitoring methodologies can be applied in remote communities. Seventy-two *Acropora* fragments were harvested from three wild mother colonies and cut into three size categories (2cm, 3cm and 5cm). Thirty-six fragments were placed at an ocean culture site, and thirty-six were placed at an aquarium culture site. Total Linear Extension and health were measured every two weeks to determine the effect of size and culture method on total linear extension, growth rates, relative growth, health and mortality rates. A cost analysis was also completed on the used methodology, and a proposed improved methodology based on findings from this study, to determine if it is applicable in a developing country. Overall, the ocean-based culture method showed higher growth, health and survival, with the 5cm fragments showing the greatest growth. Both the used and proposed improved aquaria culture method proved to be cheaper than the ocean-based culture method. It was determined that micro-fragmentation does not increase the growth rate or survivorship of *Acropora*, and growth is more successful using the ocean culture, unless adequate water quality controls can be implemented for the aquarium method.

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Introduction

Coral Biology

Corals are sessile marine animals, which can occur in both warm and cold water, depending on their species. The highest diversity and abundance of coral occurs within the Coral Triangle, which extends from the Philippines in the north, to the Malay Peninsula in the west and New Guinea in the east (Gaither et al., 2011). Corals fall into the phylum Cnidaria and subphylum Anthozoa (Allemand & Furla, 2018). They can further be divided into hard corals (Scleractinian) and soft corals (Alcyonacea) (Coker et al., 2014). They are composed of a colony of polyps, which have a basic structure consisting of tentacles, a mouth and a stomach. The polyp's tentacles and outer epidermis contain stinging cells, called nematocysts, which aid in the capture of food (Goreau et al., 1979). In hard corals, the polyps are connected by soft tissue called mesoglea and form a thin layer of tissue over a calcium carbonate skeleton. The calcium carbonate skeleton extends vertically and laterally and creates the foundation for tropical reefs and provides a substrate for other organisms to attach to and reside on (Chabanet et al., 1997; Schmitt & Holbrook, 2002). Soft corals lack this calcium carbonate structure, so the polyps are connected by mesoglea and rigid spine structures called sclerites (Bemert & Ormond, 2016). Polyps have an endosymbiotic relationship with dinoflagellates (*Symbiodinium* spp.), commonly known as zooxanthellae (Roth, 2014). Zooxanthellae are photosynthetic organisms that provide over 90% of the organic carbon needed for the survival of the host colony (Osinga et al., 2011; Quigley et al., 2018). This endosymbiotic relationship occurs in both Scleractinian corals and other organisms (e.g., clams) that are vital for the growth and function of coral reefs (Stewart et al., 2008).

Ecosystem Services of Coral Reefs

Tropical reef ecosystems are the most diverse marine ecosystems globally and provide invaluable ecosystem services (Rotjan & Lewis, 2008; Chaudhary et al., 2023). They provide resources to 25% of all marine organisms during at least one life phase (Tortolero-Langarica et al., 2020; McFarland, 2021b). Tropical reefs are one of the few ecosystems which create their own substrate, with most of it being produced by coral, in the form of its calcium carbonate skeleton (Sheppard et al., 2017). Tropical reefs provide a habitat for organisms ranging from microscopic algae to megafauna such as sharks. This can be during the juvenile stages, or for their entire lifetime (McFarland, 2021b). The loss or degradation of these ecosystems will significantly decrease flora and fauna populations, which can lead to the collapse of the food web (Smith et al., 2011).

The ecosystem services provided by tropical reefs can be split into three main categories: provisioning, regulating and cultural (Sato et al., 2020; Everard, 2022). Provisioning services are defined as products or benefits obtained from ecosystems and their processes (Millennium Ecosystem Assessment, 2003). Benefits include necessities such as food, fresh water and medicine. Regulating services include regulating the impacts of climate change, reducing coastal erosion and pollination (Everard, 2022). Cultural services can be defined as non-material benefits that enrich human lives (Millennium Ecosystem Assessment, 2003). This includes aesthetic benefits, cultural benefits, recreation and tourism (Everard, 2022).

Fishing and tourism fall into these categories and provide significant income to local economies (Woodhead et al., 2019). Marine ecosystem services contribute approximately 63% of all global ecosystem services. It is estimated that the economic value of ecosystem services provided by coastal ecosystems, is five times that of terrestrial ecosystems of the same size, further emphasising the need for restoration and conservation efforts (Sato et al., 2020).

Threats to Coral Reefs

Coral has always been subject to natural stressors, such as natural disasters, predation, and erosion. Previously, if there was sufficient time between the stressor events, coral would be able to recover naturally. Increased anthropogenic pressure on coral ecosystems has increased the frequency of stressor events, which is resulting in reduced ecosystem recovery (Eakin et al., 2018). These pressures include things such as unsustainable fishing practices, changed land use and pollution (Eakin et al., 2018; Lough & van Oppen, 2018; Oliver et al., 2018). Global climate change has become the greatest risk to tropical reefs, specifically increased severity of natural disasters, increased sea water temperatures and increased ocean acidification (Eakin et al., 2018; Oliver et al., 2018; McFarland, 2021a). The most pressing issue is rising sea surface temperatures, which has been shown to directly increase the frequency of mass bleaching events (Eakin et al., 2018; Foo & Asner, 2020). Temperature thresholds of coral are dependent on species, and their location (Lough, 2012). Coral most commonly occurs in locations with sea temperatures ranging from 18 to 31ºC but have been seen to survive extremes of 16 to 34 ºC (Gattuso & Buddemeier, 2002). If corals are unable to recover from stressor events, they usually become dominated by algae, which results in the ecosystem undergoing a phase shift. This has detrimental effects on individuals reliant on the ecosystem (Bennett et al., 2015). It often follows reduced or insufficient grazing by herbivorous fish. The altered available food sources encourage different species to inhabit the reef and increases pressure on the original inhabitants. Eventually the new inhabitants will dominate the ecosystem and a phase shift will have occurred (McManus & Polsenberg, 2004).

Coral Stress Responses

Coral bleaching is one of the best-known stress responses of coral. It occurs when the zooxanthellae are expelled from the polyps. This causes them to lose their colour revealing

the white calcium carbonate skeleton of the corals (Lough & van Oppen, 2018). Bleaching events are becoming more frequent and occurring on larger scales, due to the impacts of climate change and other anthropogenic pressures (Lough & van Oppen, 2018; Hannah et al., 2019). Mucus production occurs continually to aid in feeding, pathogen protection, sediment clearing and UV protection (Shnit-Orland & Kushmaro, 2009; Wright et al., 2019). Although the amount of mucus produced does not change when coral is under stress, the content of the mucus changes. Heat stressed corals release mucus with increased levels of protein and lipids. This can significantly reduce the colonies energy stores and increase predation (Wright et al., 2019).

Restoration efforts

Many restoration efforts have been attempted to mitigate the various anthropogenic impacts on the marine environment. These methods include transplantation and the deployment of artificial reefs (Ignasi et al., 2018). They are often harder to create and implement than land-based restoration methodologies, as they need to be applicable in a marine environment. They are often met with criticism due to their success often being small in scale, with many complications. These complications include low survival rates, high costs and location specificity. A key contributor to these low survival rates is the inability to remove the stressors that caused the initial degradation. This highlights the importance of mitigating human impacts and preventing the advancement of climate change (Eakin et al., 2018; Foo & Asner, 2020; McFarland, 2021a).

The focus of marine restoration efforts has been on the transplantation of habitat forming species such as coral and seagrass, which are able to reproduce asexually. This increases the chances of survivorship as the organism has passed the juvenile stage (Ignasi et al., 2018). Half of all living coral around the world has been lost since the 1950s. This large-scale loss has led to more time and resources to be allocated to the research and restoration of tropical

reefs (Eddy et al., 2021). These coral restoration projects are currently small scale, short term and focused on fast growing branching corals with varying rates of success. The small scale and short duration of these projects makes the long-term success of methodologies unclear. These small-scale projects are occurring globally, with a focus on techniques using coral fragmentation or the transplant of coral fragments (Boström-Einarsson et al., 2020). The three commonly occurring techniques are larval enhancement, artificial reefs and coral gardening (Lindahl, 2003; Suzuki et al., 2020; Ramm et al., 2021).

Larval Enhancement

Larval enhancement includes the collection of coral gametes from the marine environment, or from aquaculture facilities (dela Cruz & Harrison, 2017). The gametes are then exposed to varying conditions thought to encourage the fertilization of the eggs, whilst still ensuring genetic diversity (dela Cruz & Harrison, 2017; Suzuki et al., 2020). This technique has also been found to result in significantly higher survival rates for the juvenile life stages in a variety of coral species, when compared to naturally occurring spawning processes (dela Cruz & Harrison, 2020; Suzuki et al., 2020). This approach is currently more expensive than fragmentation techniques, both in-situ and ex-situ, due to initial setup costs. The benefits of this methodology, such as increased genetic diversity, need to be considered when choosing the appropriate restoration technique. If this methodology is further developed, it may be applicable as a long-term option for remote communities due to lower maintenance and monitoring costs, when compared to other methodologies (Abrina & Bennett, 2021).

Artificial Reefs

Artificial reefs can currently be found in 71 countries, on all continents, except Antarctica (Ramm et al., 2021). They can be broadly categorised into intentional and unintentional artificial reefs. Intentional reefs are structures that were installed to be used as a reef. Unintentional reefs are structures installed with an alternate purpose (e.g., harbours) or

structures that were not intentionally installed (e.g., shipwrecks), that end up as a habitat for organisms (Lima et al., 2019). Originally, the focus of artificial reefs has been to restore fish stocks and therefore improve fisheries yields, rather than protecting the ecosystem itself. This can be problematic as an artificial reef is often not a true representation of the naturally occurring ecosystem, as it is altered to provide optimal conditions for the fisheries target species (Higgins et al., 2022; Knoester et al., 2023). Coral growth is often slow on artificial reefs, unless the reefs are utilized with additional restoration techniques such as coral gardening and coral transplantation. Due to this, and the cost of the infrastructure itself, it has been determined to be the most expensive restoration technique (Bayraktarov et al., 2019).

Coral Gardening

Coral farms are commonly used for coral gardening as they can be used to produce large amounts of coral in a short period of time. The process involves harvesting fragments of coral from a parent colony and putting them in a nursery with ideal growth conditions. Once the fragments reach a specified size, they are then out planted onto reefs. The harvested fragments can also be used as a parent colony to minimise the amount of coral taken from the natural environment (Papke et al., 2021). The methodology has been used globally and has shown high levels of success when the conditions at the transplant site are favourable (Precht, 2006; Dehnert et al., 2023). Much like artificial reefs and many other restoration methods, the collection of fragments and/or micro-fragments is required (Precht, 2006). Although collection of fragments is a commonly used method due to its immediate provision of material to grow and transplant, it has flaws. This includes the limited number of colonies that are suitable for harvesting, the high labour costs of collection, transplantation and monitoring and potentially reduced genetic diversity (Harriott & Fisk, 1988; Afiq-Rosli et al., 2019).

Micro-fragmentation

Coral micro-fragmentation involves cutting sections of corals into small portions to stimulate rapid growth. It has shown success in increasing the growth rate of a variety of slow-growing species, such as Porites. The growth is stimulated by a short-term disruption of calcium homeostasis and a range of electron transport genes. This occurs as a stress response to fragmentation (Lock et al., 2022). It is often used to rapidly grow coral to a suitable size to then be transplanted on the restoration site (Lock et al., 2022). Harvesting fragments from a select few parent colonies may reduce initial genetic diversity, but the rapid increase in the colonies size when utilizing micro-fragmentation helps to combat this. Initially, it was believed that polyp age determined coral spawning ability, but micro-fragmentation research has determined that it is dependent on the size of the colony (Okubo et al., 2009). Consequently, coral reefs being restored using micro-fragments have an increased spawning capacity, aiding in increased genetic diversity.

The ideal size of fragments has not been determined and is likely to differ between species and site (Knapp et al., 2022). To determine the ideal size to use for restoration, target species and location needs to be known to ensure ineffective methods are not repeated. The size to be classified as a micro-fragment is unclear and varies from study to study, although majority of studies have focused on fragments between one and five centimetres in length or between 1 and 5 cm^2 surface area (Page et al., 2018; Koch et al., 2021; Knapp et al., 2022; Sutthacheep, 2023). For this literature review, micro-fragmentation will be defined as fragments no larger than 5 centimetres in length or 5 cm^2 in surface area.

Whilst micro-fragmentation has been extensively researched for slow-growing massive corals, such as *Porites spp.,* research into its benefits on other growth forms is limited (Mostrales et al., 2022). Its use for *Acropora spp.* has been limited, due to the high success rates of growing larger fragments of *Acropora spp.* (Lindahl, 2003; Tortolero-Langarica et al.,

2020; Woesik et al., 2021). The studies that have found micro-fragmentation to be successful for *Acropora* species have found conflicting results of the ideal fragment sizes (Herlan & Lirman, 2008; Lirman et al., 2010; Boch & Morse, 2012). These conflicting results may also be due to the variation in initial fragment sizes used in studies and the environmental conditions at the study location.

Acropora species

Acropora (Oken, 1815) is the largest genus of coral containing approximately 180 species, with over half of these species being classified by the International Union for Conservation of Nature (IUCN) as at an elevated level of threat (Richards et al., 2013; Mercado-Molina et al., 2020). The genus is named after the axial polyp, which produces radial polyps along the branches. This mode of growth is the key characteristic that allows for the diverse branching variations observed in this genus, including arborescent, tabular, digitate and corymbose growth forms (Wallace, 2011; Muko et al., 2013). *Acropora* species are some of the fastest growing coral species, although growth rate is highly variable between species and can be drastically reduced if environmental conditions are outside the optimal range of the species (Gladfelter et al., 1978; Crabbe & Smith, 2005). *Acropora* species can be found throughout the tropical and sub-tropical regions. Generally, they are found in shallow reefs with high energy levels and clear water, consequently resulting in high oxygen levels. The turbulent zones they occur in makes then vulnerable to physical damage and sedimentation, which causes their population and coral cover to vary drastically (Wallace, 1999). Their habitat range is expanding poleward due to increasing sea surface temperatures (SST) caused by global climate change (Yamano et al., 2011). This may result in phase shifts of ecosystems due to increased competitor pressures.

Acropora species represent approximately 25% of all coral species found within the Coral Triangle and is the most abundantly occurring coral species in Indonesia (Santodomingo et

al., 2015). Eighty-three species of *Acropora* were recorded in Indonesia by Wallace and Wolstenholme in 1998, but no detailed site descriptions or updated species counts have been published since (Runtukahu et al., 2007). Indonesia is located at the centre of biodiversity for many organisms, including coral. This is caused by a variety of factors including the Indonesian Throughflow current and the isolation of the region during the Miocene to present times (Wallace & Muir, 2005). The high levels of anthropogenic damage caused to reefs in Indonesia can cause detrimental effects to the surrounding marine ecosystem if they are not mitigated (Edinger et al., 2000). Recent changes in species composition on Indonesian reefs have been found to be due to the varying tolerance limits of species. The main determinant of this changing composition was found to be the presence of land-based pollution. The key factor controlling the species composition was originally geographical location (Edinger et al., 2000; Wallace & Muir, 2005).

Acropora Species Restoration and Monitoring

Restoration of *Acropora* populations has been attempted throughout the tropics, with varying success. A variety of methodologies have been used with varying fragment sizes, units of measurement and study periods. Fragments of opportunity have been used to determine the effects of time and fragment size on the growth of a variety of *Acropora* species (Tortolero-Langarica et al., 2020). Fragments of opportunity are fragments that are not connected to a colony, due to physical damage. This physical damage is usually due to anthropogenic causes or weather events. These fragments are collected and then directly transplanted (Tortolero-Langarica et al., 2020). This is the main reason for the big variety of fragment sizes used for *Acropora spp*. restoration. Throughout the literature, studied fragment sizes range from 2.5 cm to 34 cm in length (Bowden-Kerby, 2001; Lindahl, 2003; Lirman et al., 2010; Boch & Morse, 2012).

A variety of measures are used to determine fragment growth, with the most common being linear growth (Bowden-Kerby, 2001; Lirman et al., 2010; Forrester et al., 2014; Huntington & Miller, 2014; Million et al., 2021). There is no standard unit of measure for growth, so comparisons between studies can be unreliable. Other studies use surface area, 3D growth (also known as Total Linear Extension), maximum length, minimum length, 3D Photogrammetry, volume and weight (Bowden-Kerby, 2001; Boch & Morse, 2012; Forrester et al., 2014; Huntington & Miller, 2014; Million et al., 2021). These measurements have been taken at a variety of different intervals, with most studies lasting 12 months (Bowden-Kerby, 2001; Herlan & Lirman, 2008; Lirman et al., 2010; Forrester et al., 2014; Million et al., 2021).

The most common findings amongst the restoration research are mortality is highest directly after transplantation, initial health is correlated with growth and survival rates, fragments with a nursery growth stage have higher survival rates, and larger fragment sizes have a significantly higher growth rate (Bowden-Kerby, 2001; Lindahl, 2003; Lirman et al., 2010; Forrester et al., 2014; Tortolero-Langarica et al., 2020; Woesik et al., 2021)

Acropora Restoration Limitations

There has been minimal comparison between fragment sizes to determine the most effective size to use for restoration. This is likely leading to the unnecessary harvesting of corals for restoration efforts (Lindahl, 2003; Lirman et al., 2014). This lack of consistency with sizes may be due to the use of 'fragments of opportunity'. It may also be due to the broad variety of growth metrics used within studies which make it difficult to determine the ideal sizes. Knowing the ideal size of fragments for each target species is vital to ensure efficient restoration methodologies.

Micro-fragmentation as a restoration technique of *Acropora* species has the potential to allow a greater increase of coral cover, whilst minimising the amount of coral that is required for harvesting. It can also allow for fragments of opportunity to be sub-fragmented, resulting in more coral available for transplanting. Studies have shown successful growth of *Acropora* from a range of fragment sizes (i.e., 0.5 cm to 5 cm), though few studies have compared the growth rates of each size to determine the most effective (Boch & Morse, 2012; Papke et al., 2021; Mostrales et al., 2022). Determining the ideal size fragment will be dependent on the aim of the restoration program (Ignasi et al., 2018). If the aim is to provide the maximum amount of coral cover possible, in a short period of time, then the size that produces the largest increase in volume, mass or length would be optimal. If the aim is to produce a large number of colonies, whilst minimising the amount of coral that needs to be harvested, the fragment that has the highest relative growth would be optimal (Ignasi et al., 2018).

The branching growth form of *Acropora* makes measuring growth difficult. A variety of methodologies have been trialled, but there is no standard measure used in restoration or monitoring that can be found within the available literature. This lack of standardisation limits the comparability between studies. Some commonly used measures include weight, volume, surface area, photogrammetry and 3D modelling and total linear extension.

Weight has been used in a variety of *Acropora* studies, both involving micro-fragmentation and fragmentation. Boch and Morse (2012) transplanted fragments and recorded their initial weight and survivorship over 18 months, then compared it with the final weight after 18 months. Specially designed push mounts and Tygon tubing was used to ensure the fragments could be removed with minimal stress and handling. The push mounts were used to attach the tubing and fragments to the reef. They were 3.9 cm in length and 1.2 cm in diameter. Holes of approximately 1.2 cm in diameter were drilled into the reef for the push mounts to be inserted into. Approximately 2 cm of the push mount was left exposed for the Tygon tubing to be

attached to (Boch and Morse, 2012). Tygon tubing is a brand of thermoplastic tubing, that is malleable enough to be securely attached to push mounts and coral fragments using tie wraps, whilst being non-toxic and strong enough to withstand ocean conditions (Jiang et al., 2015).

This methodology limits the data collected as only two measurements of fragment weight were recorded, one at the beginning of the study and one 18 months later at the completion of the study. Lindahl (2003) also used weight as a growth measure, but attached fragments to rope using cable ties, making it more cost effective. Boch and Morse (2012) had low mortality with their methodology, and Lindahl (2003) had high mortality. Ross et al. (2015) took ten weight measurements over the span of two years. The study had high mortality due to a mass bleaching event mid-way through the study, but other mortality was not mentioned. Using weight to determine coral growth involves increased handling of fragments, especially if multiple measurements are taken. The increased handling of fragments may cause excess stress increasing mortality and decreasing growth (van Oppen & Lough, 2018).

Volume was used as a measure of growth by Huntington & Miller (2013), who found a strong correlation between ellipsoid volume and TLE. Volume was determined using maximum length, perpendicular width and height. The minimal number of measurements required for this method make it ideal for use in research with small budgets or in community run projects. The study looked at the relationship between the volume of four shapes (rectangle, sphere, cylinder and ellipsoid). It is believed from this research that the ideal shape used to determine volume will vary between both species and location. This means that to use this methodology at any other sites, regression analysis between the four volume measurements and TLE need to be completed. This is time consuming and may not be possible before commencing research.

Surface area is commonly used to measure the growth of micro-fragments of massive and encrusting corals. This is done with high accuracy due to the simple growth forms (Tortolero-Langarica et al., 2020; Lock et al., 2022). Due to the complex growth form of *Acropora,* it is not a practical measure of growth, without the use of specialised software. Forrester et al. (2013) used surface area to measure the growth of *Acropora palmata* fragments. Two methods were used depending on the complexity of the fragments shape. If it was not a complex fragment, an image was taken, and surface area was determined using a program called ImageJ. If the growth form was complex, the surface area then became an estimate using the maximum length (L), width (W) and height (H) of the fragment and the following equation: $[(L + W + H)/3]^2$. This significantly reduces reliability and can lead to highly inaccurate measures of surface area.

Studies by Lange et al., (2020) and Million et al., (2021), have found that the methodology with the highest accuracy is thought to be Photogrammetry and 3D modelling, with precision of less than two millimetres. There is a variety of software available for 3D modelling including Agisoft Metashape, Capturing Reality and Blender. Costs for these programs vary, with the most used software, Agisoft Metashape, requiring a onetime payment of up to USD3499. Capturing Reality and Blender are free to use programs, making them more applicable to community-based restoration project (Irschick et al., 2022). The broad range of data collected using this method (i.e., volume, total linear extension, height, width, surface area) makes it comparable to studies with other measuring techniques.

For short term growth monitoring, linear extension is a simple method to determine growth. It involves the measurement from the base of the coral fragment to the tip. This can be done whilst underwater, or from a scale in photographs. It can become problematic if the study is over a longer period and the fragment begins to branch. To combat this, studies have either kept measuring linear extension and counting the number of new branches forming or begun

measuring total linear extension. Simply counting the new branches that are forming prevents the measurements from becoming over complicated and more time consuming, whilst still acknowledging the changing morphology of the fragment. Other studies also measured new branches and used the sum of all lengths to show growth, which is called total linear extension, 3D growth, or tissue extension. This method can significantly increase the time required measuring coral but ensures that a true representation of growth is provided (Bowden-Kerby, 2001; Johnson et al., 2011). These methodologies ensure that the collected data is comparable with data collected from studies using more expensive methods, such as 3D modelling. Its high accuracy, when compared with Photogrammetry and 3D modelling make it an ideal standard measure of short-term branching coral growth.

Pseudoreplication is a limitation of the published research on *Acropora* restoration. This is when non-independent data points are treated as independent. This increases the chance of inaccurate statistical findings being found (Jordan, 2018). Pseudoreplication occurs in coral research when fragments are collected from the same colony but are analysed as independent samples. This means that findings may be skewed due to the limited genetic makeup of the sampled colony. For example, some colonies have genes making them more tolerant to temperature extremes, but this is not representative of the whole species (Quigley et al., 2020). Out of the papers on coral restoration mentioned in this study only few specified that samples were taken from various colonies. Some also used just one colony, which may provide an inaccurate representation of the population. The studies that used fragments of opportunity did not state the origin colony, therefore results from these studies may not be reliable.

Indonesia specific restoration attempts

Previous studies have found that up to 86% of coral reefs in Indonesian waters are facing high levels of threat, which can lead to the degradation of the reef ecosystem. The threats include pollution, unsustainable fishing practices, changed land use, invasive species (e.g., Crown of Thorns Starfish), disease, tourism and climate change (Eakin et al., 2018; Lough & van Oppen, 2018; Oliver et al., 2018; Foo & Asner, 2020; McFarland, 2021a; Boakes et al., 2022).

Indonesia is the world's second largest plastic polluter, due to the recent increased demand for single use plastics and the absence of appropriate waste management facilities (Shuker & Cadman, 2018), resulting in plastic waste being burnt or dumped into drains or bodies of water (Boakes et al., 2022). This can not only impact coral directly, through entanglement and increased disease, but indirectly through the mortality of fauna within the ecosystem (Lamb et al., 2018).

Marine ecosystems throughout Indonesia are also suffering from eutrophication, due to enrichment from plant nutrients from land runoff and water-based pollution. The enrichment is caused mainly by nitrogen and phosphorus and stimulates primary production. The increase in primary production includes the growth of algae and increase occurrence and severity of algal blooms (Karydis & Kitsiou, 2019). The changed conditions of the environment can affect food webs and water quality. Eutrophication also causes a decrease in disease resistance, accelerates erosion and reduces reproductive success (Bell, 1992; Duprey et al., 2016). Its effects have been rapidly increasing due to urbanization and the implementation of sewerage systems (Suwarno et al., 2014).

Fishing is the main form of income for the coastal population throughout Indonesia. This has resulted in the use of a variety of fishing methodologies, which are often harmful to coral reef ecosystems (Ferse et al., 2014). This includes the use of dynamite, potassium cyanide, inshore trawling, and overfishing (Boakes et al., 2022).

Increased coastal development and land use changes have a significant impact on coral reefs. The increased demand for land results in the removal of natural occurring ecosystems which provide various ecosystem services, such as sediment stabilization. These changes consequently result in increased surface run off and pollution (Baum et al., 2015). The changes in land use along the coastline also alters water drainage and stores. This results in increased water running off land, and into the sea. This water also carries the now unstabilised sediments into the ocean. The effect of this is significantly increased during monsoon season (Schill & Jensen, 2000). Although coral can recover from low amounts of sedimentation, the sedimentation rate has increased and has significantly increased the presence of disease, and mortality. It also contributes to the previously stated eutrophication and can increase the growth of algae (Bartley et al., 2014).

On a government scale, Marine Protected Areas (MPAs) can be implemented to provide protection to the area and mitigate a variety of impacts. Though MPAs are increasingly being established, the lack of management and regulation has resulted in less than 15% of management objectives being reached (Burke et al., 2011). The first deployment of artificial reefs in Indonesia was in Jakarta Bay in 1989, and they are now found all over Indonesia in varying forms. The success of these artificial reefs varies depending on the location, likely due to the varying conditions altering the settlement and survival of organisms (Ampou et al., 2019; Puspasari et al., 2020).

There has been varying success in restoration projects globally, due to a variety of reasons. Some research has been unsuccessful due to severe weather events, high initial mortality and fragment dislodgements (Boström-Einarsson et al., 2020; Tortolero-Langarica et al., 2020). High success has been shown in studies that occur in a sheltered location and use a nursery table instead of direct transplantation (Boström-Einarsson et al., 2020; Tortolero-Langarica et al., 2020). Although these studies have been successful, the limited long-term monitoring five to twelve months, means that only initial success is reported (Bowden-Kerby, 2001; Forrester et al., 2014). The short-term monitoring of restoration efforts does not account for mortality that may occur after the completion of the study and consequently does not provide a true indication of reef restoration.

Research Site Restoration History

Bali is an Indonesian province, with some of the highest marine species richness in the Asia-pacific commonly known for its appeal to tourists. Although tourism provides significant economic benefits to Bali, it is often criticised for its impacts on the environment (Boakes et al., 2022). Tourism increases the demand for land, puts pressure on the waste management systems and increases stress on ecosystems through increased human presence and contact. The main problems within Bali are pollution, unsustainable fishing practises and coastal development. The pollution occurs in multiple forms, such as plastic waste and sewerage (Boakes et al., 2022).

The threats to Bali vary around the island due to varying land uses and populations. Due to this, restoration efforts are generally location specific. Artificial reefs in Bali, paired with transplantation of coral have shown high success in survival and growth of a variety of species of coral, as well as the benefit of higher initial coral cover (ENDO et al., 2013; Onaka et al., 2013). The transplanted corals on Balinese artificial reefs were obtained through

various methodologies and were of varying sizes, which limits the ability for direct comparison and may be the reason for varying success. The success is also dependent on location and weather conditions (Boström-Einarsson et al., 2020; Knapp et al., 2022).

During the COVID-19 Pandemic, an increased focus was put on conserving and protecting the tropical reefs of Indonesia. The Indonesia Ministry of Marine Affairs and Fisheries dedicated IDR 111.2 billion (Approximately AUD 11.2 million) to the National Economic Recovery Fund in 2020, for use by the Indonesia Coral Reef Garden program in Bali. This fund was used to build 50 hectares of coral plantations throughout Bali and allowed for the development of new transplant techniques (Wicaksana, 2020).

Les Village, the location of the present study, began its coral restoration efforts in 2003. Originally, a portion of the village's income came from the ornamental fish trade. Ornamental fishing was initially done on a small scale, with minimal impact to the ecosystem. The success of this trade became well known, which resulted in an increase in ornamental fishermen. To remain competitive and increase their catch, fishermen began using potassium cyanide. This would be doused on an area that fish were residing, to stun them and make them easier to catch (M. Merta, Personal Communication, May 2023). This method was utilized from 1980 to 2000. Around 1990, the reef had become so degraded that fishermen were having to travel outside of Bali to supply the demands of the ornamental fish trade.

In 2000, members of Telapak, a forestry non-governmental organisation, visited Les Village, and taught them a more sustainable methodology, called barrier net fishing (E. Kwee, Personal Communiction, October 2023). This resulted in the formation of a fishermen organisation which aimed to use more sustainable practises and help the reef recover. By 2002, the fishermen of Les Village were no longer using potassium cyanide and had all

adopted the new methodology. In 2003, the fishermen group began constructing artificial reef structures to experiment with restoration methodologies. These projects were partially self-funded, and partially funded by an 'adopt a coral' program. In 2012, an organisation called Sea Communities was formed, with the aim of assisting in restoration and providing an alternative form of income for the community through ecotourism (M. Merta, Personal Communication, May 2023). This organisation is still operating and has significantly expanded the village's research capacity, and income from tourism and other sources.

Gaps in Research

There are three main problems within this field of research, which can lead to uncertainty when interpreting results. These include high variation in fragment sizes, inaccessible and inconsistent measuring techniques, and possible pseudoreplication of data. In this study, variation in fragment sizes will be addressed by clearly defining the term micro-fragmentation, using definitions from the literature on similar studies, using different species. An easily accessible measuring method that has shown accuracy and provides the most used growth measures will be trialled to ensure the research can be easily compared to other literature. Pseudoreplication will be avoided by ensuring samples are taken from three different parent colonies.

Aims

In this study, fragments were harvested from three parent colonies. The harvested fragments were cut into two and three centimetre lengths, to simulate micro-fragments and five centimetre lengths to act as control. Half were transplanted onto a coral table and half were placed in an aquarium. The health and growth of these fragments were then monitored fortnightly, for four months. There are two overall aims of this research: (1) Determine if micro-fragmentation increases the growth rate of *Acropora* species, (2) Determine if current

restoration and monitoring methodologies can be applied in remote communities. Each aim will be addressed in a separate section.

Aim one will be completed by monitoring the growth of each fragment and determining the fragment with the highest health. Aim two will be completed by determining the most cost-effective growth culture method, including monitoring and maintenance costs, the ideal culture method for overall growth and an accurate monitoring technique that requires minimal training. It is expected that larger fragments maintain higher levels of health, whilst smaller fragments have a faster growth rate. It is hypothesised that the mid-size (3 cm) fragment, grown in the ocean will be the ideal size for reef restoration and have the largest comparative size increase. It is also hypothesized that the aquarium culture method will be most cost effective and that the monitoring methodologies will be applicable.

Methods

Study Site

The study was conducted in Les Village, northern Bali (Figure 1). The village's local reef has had significant coral cover loss, due to anthropogenic impacts, such as unsustainable fishing practices. There are currently two coral restoration projects occurring in the village, by the Lini Foundation and Sea Communities. This has resulted in numerous artificial reef structures being deployed at 3 to 15 m depth. The area experiences strong currents and high levels of sedimentation, emphasising the need for these artificial structures. Fragments were transplanted to two main locations, coral tables and an aquarium. The coral tables (in-situ) are located approximately 50 m from shore, in 8 m of water. The aquarium (ex-situ) is located in a powered shed at a Sea Communities employee's house.

Figure 1. Map showing Bali, Indonesia, the location of the study site (Red Marker) for Acropora micro-fragmentation, Coral Table (Blue Marker), Aquaria (Green Marker) and Weather Station (Orange Marker) (Google Earth Version 7.3, 2021).

Coral Processing

Collection

Coral was collected from three naturally growing *Acropora* colonies. The species of the mother colonies was not determined as the study is focused on the *Acropora* genius, not individual species. The colonies used for harvesting were advised by Made Merta to ensure that they were not transplants from previous experiments, within 50 meters of the transplant table and at a similar depth as the table (7 meters). This was done to minimise the difference in water conditions between the natural growth site, and the table site. Parent colony A was located at 4 m depth and had a health score of five to six, according to the CoralWatch Coral Health Chart measurements. Parent colony B was located at 3.7 m depth with a health score of four to six. Parent colony C was located at 8.4 m with a health score of four to six. These colonies were chosen due to their depth, health and large size. Health score was determined

using CoralWatch coral health cards (CoralWatch, 2023), and involved matching the colours on the card to the lightest and darkest colour on the colony and recording the matching colour code. If both lightest and darkest colour codes were in the top three CoralWatch health codes, it was deemed healthy enough to be harvested from. The colony needed to be large enough that enough fragments could be harvested, whilst following the recommended guidelines of not harvesting more than 10% of the colony (Schopmeyer et al., 2017).

Fragments were harvested using wire cutters to cut off apical sections of the coral. The harvested fragments were cut at approximately 6 cm maximum, to minimise unnecessary harvesting. The harvested fragments were chosen with minimal branching to reduce complexity in measuring and transplantation. Fragments were collected from varying locations on the parent colony, to limit the stress on a single area. They were then placed into a basket, keeping the different colonies separate and immediately taken to shore. Once on shore, they were split into three separate containers filled with fresh sea water. Fragments were kept in the bowls for as long as possible, and constantly covered in ocean water when attached to the table. The ocean fragments were out of the water for two hours, and aquarium fragments for three hours. At no point did the fragments dry out completely. Gloves were worn throughout the process.

Micro-Fragmentation

Harvested fragments were cut into fragments of 3, 4 and 6 cm using a diamond blade band saw. The target micro-fragment sizes were 2 and 3 cm with 5 cm control fragments. They were cut with an extra centimetre to ensure that they could be secured to the substrate. Any excess branches that would limit the ability to attach the fragment to the substrate were also removed.

Transplantation

Ocean

Fragments were transplanted on pre-made substrate blocks and attached to tables, which were located at 7 meters depth (Figure 2). The tables were cleared of any algae growth and checked for damage the day before transplantation occurred. The substrate blocks are made of calcium carbonate, cement and sand. They were shaped to an appropriate length to be fastened onto the table with zip ties through small holes at either end of the substrate. A large indent was made in the centre of the substrate for the coral to be placed in (Figure 3). Coral was attached to the substrate using Eka Glue (patent pending), which is a non-toxic glue composed of white cement, black cement and Lem Fox wood glue (M. Merta, Personal Communication, May 2023). The fragments were then measured again with a ruler to ensure that their size was within 0.5 cm of the target fragment size of 2, 3 and 5 cm. If they exceeded this range, they were shortened or swapped for a different fragment.

Figure 2. Image of coral tables in Les Village, northern Bali, used for securing the coral substrate used in this study and community projects. Image provided by Mike Van Keulen.

Figure 3. Substrate design used for this study and community projects in Les Village. The centre indent is used for fragment attachment and sticks make holes for attachment to the coral table.

Aquaria

Coral fragments were attached using Eka Glue to the same substrate as used for the ocean fragments. This substrate was not attached to a table. The fragments were then measured again with a ruler to ensure that their size was within 0.5 cm of the target fragment size. If they exceeded this range, they were shortened or swapped for a different fragment. Eighteen fragments were placed in each of two 160L aquaria which were filled with ocean water and had constant water flow and filtration.

Culture Methods

Ocean

Coral was transplanted onto tables along the coast shown by the blue marker in Figure 1. The tables are 1 m wide and 3 m in length and have a rope top, so fragments can be secured. They are located at 6 m depth surrounded by naturally occurring reef.

Aquaria

Two 160L aquaria were used for the ex-situ culture method. Each aquarium was 100 cm long, 40 cm wide and 40cm deep. The aquarium was filled to approximately 30 cm deep. The aquaria were filled with sea water pumped directly from the ocean, and coral rubble, two days before the transplant occurred to allow for the filter to stabilize (24/05/2023). Both aquaria used the same filter and water supply. The filter used was a Bubble Magus Bio Pellet Reactor, with Pur N-Bio pellets. The filter ran constantly during the experiment providing constant waterflow for the fragments. These filter removes excess nitrate, phosphate, algae and cyanobacteria. No chemicals were added. The aquaria receive artificial light from a 50 W AUDALUX LED Flood Light, which produced 5,000 lm of blue light. These lights were turned on four days after the transplant were added, to allow them to recover from the transplant process. They were then left on for the duration of the experiment. This

methodology was adapted from the recommendations of a local coral export company (L. Andari, Personal Communication, May 2023).

Data Collection

Growth

Growth was measured fortnightly, using linear extension (LE), and measuring the length of new branches, where possible. LE was determined by placing callipers next to the fragment and taking a photo. The photo was taken from the same orientation each time. This photo was then analysed using ImageJ to determine linear extension in centimetres to the nearest millimetre. Scale was set using the callipers as a reference. The measurement was taken from the centre of the lowest visible point of the central branch, to the very tip of the central branch, using a straight-line measurement (Figure 4). Any new branch growth was counted and recorded. New branch growth was measured with callipers whilst diving. To be counted as a branch, the total length must exceed 0.5 cm and have more than five visible polyps. This data was then transferred into an Excel spreadsheet.

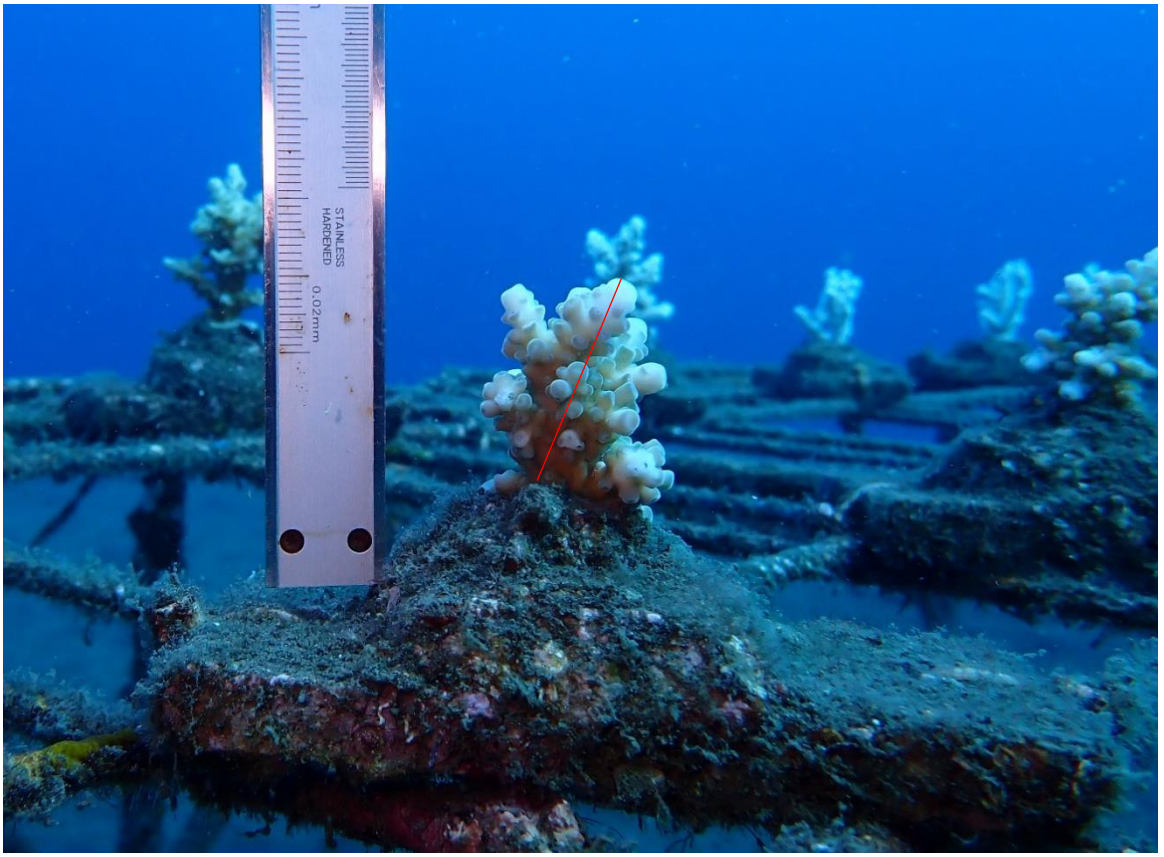


Figure 4. Measurement Photo Taken in Week 2, of a 3 cm Acropora Fragment harvested in Les Village, northern Bali, from Mother Colony A, showing Callipers used for Scale. Red Line Indicates Linear Extention Measurement.

Health

Health data was collected fortnightly using the CoralWatch coral health card, developed by the University of Queensland (Marshall, 2012). The darkest and lightest colours of the coral were matched to the colour on the card and recorded. The colour of the apical portion of the fragments and branches was disregarded, following the CoralWatch health card guidelines (CoralWatch, 2023). This was completed at the same time as the growth measurements, and a photo was also taken in case clarification was needed. This was then recorded in an Excel spreadsheet. It was also used to determine mortality; if a fragment's highest health score was equal to 1, or the fragment was covered in algae, it was recorded as dead and growth measurements were no longer taken.

Weather

Weather data was collected using a Holman Aspect Wi-Fi Analyst Weather Station, recording temperature, wind speed, wind direction and rainfall. The weather station was set up at Segara Lestari Homestay following the device guidelines, to ensure there would be no inaccuracies due to surrounding infrastructure (Figure 1). The collected data was automatically uploaded to the Weather Underground website, which stored data for the duration of the experiment (Weather Underground, 2014). At the end of the study period, the data was downloaded and input into an Excel spreadsheet.

Water Conditions

HOBO MX temperature and light loggers were used to measure water temperature (°C) and light levels (lx) every ten minutes. One logger was placed in the centre of the coral table and the other was placed in the centre of one of the aquaria. The aquaria logger was checked daily, and the ocean logger was checked weekly. Any sediment build up was cleared off during the checks. The data was transmitted at the end of the study via Bluetooth to a mobile phone using the HOBO connect app. This data was then transferred to an Excel spreadsheet. Linear regression analyses were completed on maximum, minimum and mean water temperature and air temperature to determine any correlation.

The aquaria pH and salinity were checked weekly. If values were outside the recommended range (pH = 8 to 8.5, salinity = 33 to 35 ppt) the water was changed, or the aquarium was topped up with fresh seawater and then levels were checked again to ensure it was back within the ideal range. The ideal ranges were determined from Borneman (2008) and Bartlett (2013), with slight variation due to the lack of detail provided by the used water quality tests. These levels were also recorded in an Excel spreadsheet. These were the only water quality measurements taken for the aquaria as there was no access to equipment for other measures.

Site Maintenance

Ocean

The fragments were checked after any periods of strong currents or wind to ensure there was no sediment buildup or damage to the table. If there was any sediment buildup it was removed by gently fanning the coral. Any algal growth was cleaned off the substrate monthly. The zip-ties holding the substrate to the table were checked during each dive to ensure the fragments were secure.

Aquaria

The aquaria were checked weekly to ensure the filter was running correctly and there was no algal build up. If there was algal build up the aquaria was cleaned, and water conditions were checked. If necessary, the water was changed to stabilize conditions. Approximately 50% of the water was also changed fortnightly after measurements were taken. Bio-pellets were replaced as necessary.

Data Analysis

Significance and interactions between factors, except for water conditions, were determined using Primer 7 with the PERMANOVA add on. Mean and Standard Error were determined using Microsoft Excel (Version 2307). Graphs and tables were created in Microsoft Excel (Version 2307).

Overall Growth

Total Linear Extension (TLE) (cm)

TLE was determined for each fragment using the following formula:

$$Total\ Extension + Combined\ Branch\ Length$$

If there was fragment mortality, the TLE became 0. For initial TLE, the data was square root transformed and a Bray-Curtis similarity matrix was created. A 2-factor PERMANOVA was then run to test for significant differences between culture method, and fragment size. A Pairwise PERMANOVA was then completed to determine significant interactions. For TLE per measurement period, the data was Log(X + 1) transformed and a Euclidean distance resemblance matrix was created. A 2-factor PERMANOVA was then run to test for significant differences between culture method and fragment size. A Pairwise PERMANOVA was then completed to determine significant interactions. The mean and standard error for each fragment size and culture method were determined using Microsoft Excel and plotted as a line graph.

Increase in TLE (cm)

Two measures of increase in TLE were calculated: increase using maximum TLE and increase using final TLE. This provided total maximum increase and total final increase. Increase in TLE of each fragment was determined in Microsoft Excel using the following equation:

$$Maximum\ or\ Final\ TLE - \ Inital\ TLE$$

The determined increases were Log(X + 1) transformed and a Euclidean distance resemblance matrix was created. A 2-factor PERMANOVA was then run to test for significant differences between increase type, culture method and fragment size. A Pairwise PERMANOVA was then completed to determine significant interactions. This was this was then used to determine the annual growth rate for each fragment. Mean and standard error of maximum and final increase in TLE were determined using Microsoft Excel.

Relative Growth

Final relative growth and relative growth per measurement period were determined. Two measurements of final relative growth were determined, one using the final increase in TLE and one using the maximum increase in TLE, so the effects of grazing events could be analysed. Relative growth was determined for each measuring period and overall.

Relative growth for each fragment was determined in Microsoft Excel using the following equation:

$$\left(\frac{Growth\ (cm)}{Initial\ Fragment\ Size}\right) * 100$$

The data was then Log(X + 1) transformed and a Euclidean distance resemblance matrix was created. A 2-factor PERMANOVA was then run to test for significant differences between culture method and fragment size for each measure of relative growth. A Pairwise PERMANOVA was then completed to determine significant interactions. The mean and standard error of relative growth for each fragment size were determined using Microsoft Excel.

Growth Rate

Growth rates (cm per year) were determined using both final and maximum increase in TLE in Microsoft Excel using the following equation:

$$\text{Growth rate} = \frac{Increase\ TLE}{TIME\ ELAPSED}$$

Any negative values were changed to 0. For maximum growth rates, time elapsed was the time taken for the fragment to reach the maximum TLE. The data was then Log(X + 1) transformed and a Euclidean distance resemblance matrix was created. A 2-factor PERMANOVA was then run to test for significant differences between culture method and fragment size. A Pairwise PERMANOVA was then completed to determine significant

interactions. The mean and standard error of the growth rate for each fragment size and overall was determined using Microsoft Excel.

Number of Branches

The initial number of branches, and number of branches per measurement period were determined. The number of branches on each fragment were then Log(X + 1) transformed and a Euclidean distance resemblance matrix was created. A 2-factor PERMANOVA was then run to test for significant differences between culture method and fragment size. A Pairwise PERMANOVA was then completed to determine significant interactions. The Mean and standard error of number of branches for each fragment size, per culture method es determined using Microsoft Excel.

Effects of Predation

Predation of the fragments by fish was observed in the ocean treatment. To examine the impacts of predation, final relative growth and maximum relative growth were determined, and Log(X + 1) transformed, and a Euclidean distance resemblance matrix was created. An ANOVA was then run to test for significant differences between maximum and final increase in TLE and maximum and final relative increase in TLE of all fragments and the effected fragments. The percentage loss of TLE due to predation was determined in Microsoft Excel using fragment TLE before and after the grazing event.

Coral Fragment Health

Health was measured by creating a health score, which was determined by adding the fragment's highest and lowest health score measured using the CoralWatch health card. The higher the score, the higher the fragments overall health. A Euclidean distance resemblance matrix was created and then a 2-factor PERMANOVA was then run to test for significance between culture method, and fragment size. A Pairwise PERMANOVA was then completed

to determine significant interactions. The mean and standard error of health, for each fragment size were determined using Microsoft.

Mortality Rate (%)

Mortality rate was determined as a percentage of total fragments. This was done for culture method and fragment size throughout the study. A Euclidean distance resemblance matrix was created. A 2-factor PERMANOVA was then run to test for significant differences between culture method and fragment size.

Weather

Mean (± standard error), maximum and minimum weekly temperatures (ºC) and total rainfall (mm) were determined for each measurement period and overall, using Microsoft Excel.

Water conditions

Mean (± standard error), maximum and minimum water temperatures (ºC) and light (lx) were determined for each measurement period and overall, using Microsoft Excel. An ANOVA compared all factors to identify significant differences between the two culture methods. A regression analysis was conducted comparing each water condition measure to the corresponding weather measures.

Results

Seventy-two *Acropora* fragments, of three size categories, were split between two culture methodologies to determine significant between growth, health and mortality. Overall, the 5 cm fragments had the highest success with higher growth, health and lower mortality. The ocean culture method produced higher growth and lower mortality, when compared to the aquarium culture method that had 100% mortality by week 10.

Overall Growth

Total Linear Extension (TLE)

Initial

The initial TLE of fragments varied between size and culture method, with the 5 cm fragments having the highest initial TLE for both culture methods (Table 1). Significant differences were also found between all factors, with significant interactions occurring between size and culture method. Fragment size showed the highest estimates of components of variation (11.193%) (Table 2). The interaction between the initial TLE of each fragment size per culture method was significant ($F(2) = 9.548$, $p = 0.001$) (Table 3).

Table 1. Mean Total Linear Extension ± Standard error (cm) of Acropora Fragments for each measurement period (Weeks), sorted by Fragment Size and culture method (n = 72).

		Ocean			Aquaria		
		2 cm	3 cm	5 cm	2 cm	3 cm	5 cm
Week	0	4.28 ± 0.364	5.62 ± 0.543	8.37 ± 0.693	2.61 ± 0.160	5.38 ± 0.713	9.16 ± 0.933
	2	4.80 ± 0.416	5.79 ± 0.585	10.36 ± 1.304	2.50 ± 0.129	6.22 ± 0.826	10.11 ± 1.121
	4	5.46 ± 0.581	7.07 ± 0.681	12.13 ± 1.441	2.17 ± 0.206	5.28 ± 1.065	9.18 ± 0.961
	6	5.17 ± 0.431	7.96 ± 0.779	14.62 ± 2.331	1.58 ± 0.335	4.23 ± 1.004	7.43 ± 1.568
	8	5.01 ± 0.460	7.26 ± 0.690	16.22 ± 2.464	0.00 ± 0.00	0.00 ± 0.00	2.13 ± 1.399
	10	5.30 ± 0.626	8.36 ± 0.690	17.00 ± 2.451	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	12	5.64 ± 0.763	8.85 ± 0.752	19.45 ± 2.607	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	14	4.13 ± 0.738	7.81 ± 1.162	17.14 ± 2.993	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	16	4.29 ± 0.758	8.47 ± 1.124	18.09 ± 3.507	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 2. Source of variance table for 2-factor (fragments size, culture method) PERMANOVA of Initial Total Linear Extension of Acropora fragments (cm) (n = 72) showing degrees of freedom (df), pseudo-F (F), P-values (p) and square root estimates of components of variation (%) (ECV).

	df	F	p	ECV
Size	2	75.580	0.001	11.193
Culture method	1	7.555	0.008	2.709
SizexCultureMethod	2	6.605	0.002	4.340

Results of a pairwise PERMANOVA showed significant differences between all sizes at both sites (Table 3). Significant differences were also determined for the initial TLE of 2cm fragments at each site (t = 4.612, p = 0.002).

Table 3. Results of a Pairwise PERMANOVA for interactions of Initial Total Linear Extension (cm) of Acropora Fragments (n = 72) between size and culture method, showing T-Values (t), P-Values (p), and Average Similarity (%).

Pairs of Level Factor		Interaction	t	p	Average Similarity
Size	Ocean	2cm/3cm	2.159	0.043	2.158
		2cm/5cm	6.081	0.001	3.360
		3cm/5cm	4.095	0.001	2.566
	Aquaria	2cm/3cm	5.774	0.001	1.847
		2cm/5cm	15.899	0.001	2.677
		3cm/5cm	4.712	0.001	2.083
Culture Method		2 cm	4.612	0.002	87.257
		3 cm	0.541	0.624	89.772
		5 cm	0.792	0.428	89.883

Per Measurement Period

TLE varied for each fragment size throughout the study, with the 5 cm fragments having the highest mean TLE for both the ocean-based culture method (18.09 ± 3.51 cm; mean ± standard error) and the aquaria-based culture method (2.13 ± 1.40 cm) (Figure 5 & Figure 6). All ocean fragments showed an overall increase in mean TLE, whereas all aquaria fragments showed an overall decrease (Table 1). There were significant differences in TLE between fragment size (F(2) = 0.15.994, p = 0.001) and culture method (F(1) = 229.76, p = 0.001), with culture method having the highest estimate of component of variation (3.2902%). There were also significant interactions between both factors (Table 4). TLE for each fragment size, for each culture method can be seen numerically in Appendix A and Appendix B.

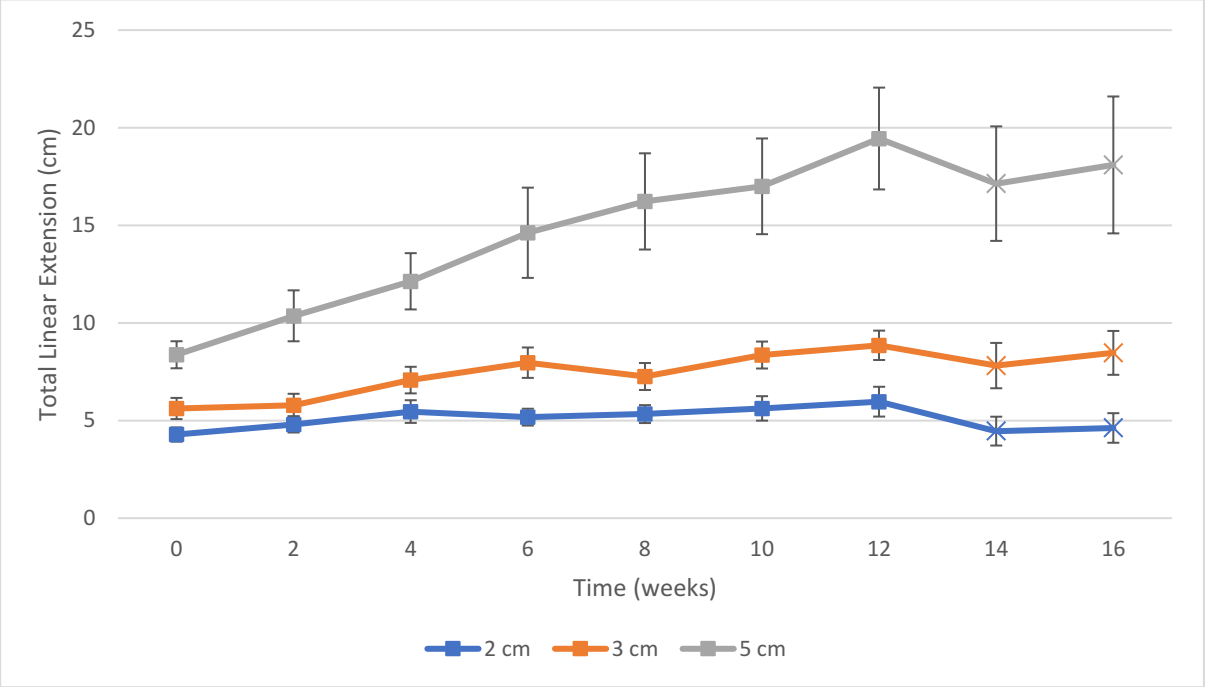


Figure 5. Mean and standard error of Total Linear Extension (cm) of ocean Acropora fragments (n = 36) per measurement period, shown by fragment size (2 cm, 3 cm and 5 cm). Predation Events are indicated by the X markers.

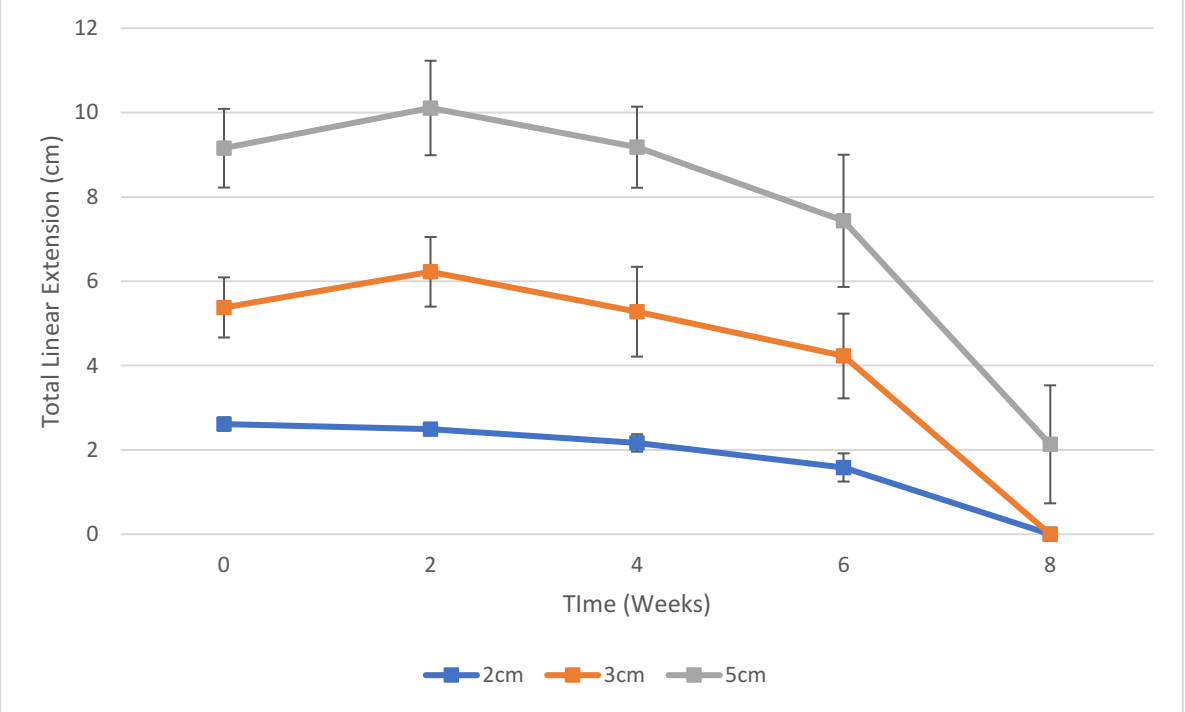


Figure 6. Mean and standard error of Total Linear Extension (cm) per measurement period of Aquaria Acropora Fragments (n = 36), shown by fragment size (2 cm, 3 cm and 5 cm).

Table 4. Results of 2-factor (fragment size, culture method) PERMANOVA for Total Linear Extension of Acropora fragments (cm), per measurement period (weeks) (n = 72) showing degrees of freedom (df), pseudo-F (F), P-values (p) and square root estimates of components of variation (ECV)

	df	F	p	ECV
Size	2	15.994	0.001	1.0316
Culture Method	1	229.76	0.001	3.2902
SizexCultureMethod	2	6.6035	0.001	0.8919

The difference in TLE per measurement period between 2 cm and 5cm (t = 4.2527, p = 0.001) and 3cm and 5cm (t = 2.5919, p = 0.004) ocean fragments were significant. Significant differences in TLE per measurement period between all aquaria fragment sizes were also found (Table 6). There were also significant differences in TLE per measurement period between culture methods for each fragment size (Table 5).

Table 5. Results of a Pairwise PERMANOVA for interactions of Total Linear Extension of Acropora Fragments (cm), per measurement period (weeks) (n = 72), showing T-values (t), P-Values (p) and Average Similarity (%) for factors size and culture method.

Pairs of Level Factor		Interaction	t	p	Average Similarity
Size	Ocean	2cm/3cm	1.768	0.067	2.158
		2cm/5cm	4.523	0.001	3.360
		3cm/5cm	2.592	0.004	2.566
	Aquaria	2cm/3cm	3.365	0.001	1.847
		2cm/5cm	5.60	0.001	2.677
		3cm/5cm	3.137	0.001	2.083
Culture Method		2 cm	6.524	0.001	3.842
		3 cm	9.025	0.001	4.961
		5 cm	10.949	0.001	6.288

The 5 cm ocean fragments maintained the highest relative growth, for the duration of the research, whereas the 3 cm fragments had the highest growth in the aquaria, except for in week 8. Grazing occurred on the ocean fragments, which resulted in a decrease in relative growth for all fragment sizes during week 16 and 18. The difference in relative growth between fragment size and culture method were significant (Table 6). Significant interactions

were also found between fragment size and culture method (Table 6). Relative growth for each fragment size, for each culture method can be seen numerically in Appendix C and Appendix D.

Table 6. Results of 2-factor (fragment size, culture method) PERMANOVA for relative growth (%) of Acropora fragments per measurement period (n = 72), determined by increase in TLE, showing degrees of freedom (df), pseudo-F (F), P-values (P) and square root estimates of components of variation (ECV).

	df	F	P	ECV
Size	2	2.4361	0.038	0.86749
Culture Method	1	78.485	0.001	5.2027
SizexCultureMethod	2	2.1458	0.053	1.0958

A Pairwise PERMANOVA between fragment size showed a significant interaction between the 2 cm and 5 cm fragments (t = 1.224, p = 0.008) but no other fragment sizes (Table 7). The interaction between culture method and size showed significant differences between the 2 cm and 5 cm (t = 1.883, p = 0.45) ocean fragments, and between the 2 cm and 3 cm (t = 2.951, p = 0.003) and 2 cm and 5 cm (t = 1.978, p = 0.023) aquaria fragments. Significant differences were found between culture methodology for each site (Table 8).

Table 7. Results of a Pairwise PERMANOVA for interactions between relative growth of Acropora fragments (%), per period, (n = 72), showing T-Values (t), P-values (P) and Average similarity.

Pairs of Level Factor	Interaction	t	p	Average Similarity
Size	2 cm/3 cm	1.224	0.193	6.670
	2 cm/5 cm	2.082	0.008	7.002
	3 cm/5 cm	1.286	0.153	7.191

Table 8. Results of a Pairwise PERMANOVA for interactions of Relative Growth (%) Per Measurement Period, between Acropora fragment size and culture method, Showing T-Values (t), P-Values (P) and Average Distance for Factors Size and Site

Pairs of Level Factor		Interaction	t	p	Average Similarity
Size	Ocean	2cm/3cm	0.730	0.666	7.144
		2cm/5cm	1.883	0.045	7.149
		3cm/5cm	1.414	0.108	7.057
	Aquaria	2cm/3cm	2.951	0.003	3.252
		2cm/5cm	1.978	0.023	2.612
		3cm/5cm	1.127	0.275	2.896
Culture Method		2 cm	4.183	0.001	7.688
		3 cm	4.475	0.001	8.516
		5 cm	7.308	0.001	10.368

Increase in TLE (cm)

Maximum Increase in TLE (cm)

Maximum increase in TLE was higher in the coral table fragments (8.14 ± 1.23 cm) than the aquaria fragments (0.79 ± 0.17 cm). It was highest in the 5 cm fragments followed by the 3 cm fragments and then the 2 cm fragments. Averages for the increase in TLE per culture method and size can be seen in Table 7. The difference of maximum increase in TLE was found to be significant between culture method ($F(1) = 111.100$, $p = 0.001$) and size ($F(2) = 12.283$, $p = 0.001$), with culture method having the highest estimate of components of variation (1.010%). There were also significant interactions found between fragment size and culture method (Table 10).

Table 9. Mean and standard error of maximum increase in Total Linear Extension (cm) (n = 72), for each Acropora fragment size (2 cm, 3 cm and 5 cm), by culture method and overall.

		Culture Method		
		Ocean	Aquaria	Overall
Fragment Size (cm)	2	2.98 ± 0.53	0.11 ± 0.06	1.54 ± 0.39
	3	5.51 ± 0.94	1.05 ± 0.23	3.28 ± 0.67
	5	15.93 ± 2.16	1.23 ± 0.36	8.58 ± 1.86
	Overall	8.14 ± 1.23	0.79 ± 0.17	4.47 ± 0.76

Table 10. Results of 2-factor (fragment size, culture method) PERMANOVA for maximum increase in Total Linear Extension (cm) of Acropora fragments (n = 72) showing degrees freedom (df), pseudo-F (F), P-values (p) and square root estimates of components of variation (ECV)

	df	F	p	ECV
Size	2	39.835	0.001	0.512
Culture Method	1	227.540	0.001	1.010
SizexCultureMethod	2	10.914	0.001	0.366

The interaction between size and culture method showed a significant difference between all fragment sizes on the coral table and between both 2 cm and 3 cm (t = 5.361, p = 0.001) and 2 cm and 5 cm (t = 3.057, p = 0.001) aquaria fragments (Table 11). The difference between all fragment sizes, for both culture methods was also significant (Table 11).

Table 11. Results of a Pairwise PERMANOVA for interactions of maximum increase in Total Linear Extension (cm) of Acropora fragments (n = 72) between size and culture method, showing T-values (t), P-Values (p) and Average Similarity (%) for factors size and culture method.

Pairs of Level Factor		Interaction	t	p	Average Similarity
Size	Ocean	2cm/3cm	2.348	0.030	0.762
		2cm/5cm	8.473	0.001	1.499
		3cm/5cm	4.742	0.001	1.068
	Aquaria	2cm/3cm	5.361	0.001	0.580
		2cm/5cm	5.057	0.001	0.616
		3cm/5cm	0.225	0.817	0.503
Culture Method		2 cm	9.151	0.001	1.173
		3 cm	5.643	0.001	1.127
		5 cm	12.215	0.001	2.057

The ocean fragments had the higher mean maximum relative growth (129.40 ± 14.33%, when compared to aquaria fragments (12.61 ± 2.34%). The 5 cm fragments showed the highest maximum relative growth (103.54 ± 21.66%), when compared to other fragment sizes. All ocean fragment sizes had significantly higher maximum increase in relative growth (F(1) = 127.950, p = 0.001) (Table 12). Significant differences were found between all factors, with a significant interaction between size and culture method (Table 13).

Table 12. Mean (± standard error) total relative increase (%), for each culture method (n = 36) and fragment size (n = 12), determined by the final increase in TLE.

		Culture Method		
		Ocean	Aquaria	Total
Fragment Size (cm)	2	73.07 ± 13.17	4.86 ± 2.67	38.96 ± 9.68
	3	120.38 ± 25.57	20.65 ± 4.80	70.51 ± 16.52
	5	194.75 ±21.92	12.32 ± 2.97	103.54 ± 21.66
	Total	129.40 ± 14.33	12.61 ± 2.34	71.00 ± 10.12

Table 13. Results of 2-factor (fragment size, culture method) PERMANOVA for relative growth (%) of Acropora fragments (n = 72), determined by maximum increase in TLE, showing degrees of freedom (df), pseudo-F (F), P-values (P) and square root estimates of components of variation (ECV).

	df	F	P	ECV
Size	2	14.998	0.001	0.722
Culture Method	1	127.950	0.001	1.775
SizexCultureMethod	2	3.672	0.033	0.446

Results of Pairwise PERMANOVAs found significant differences between the maximum increase in relative growth of the 2 cm and 5 cm fragments and 3 cm and 5 cm fragments for each culture method (Table 14). Significant differences were found for all fragment sizes, between culture methods (Table 14).

Table 14. Results of a Pairwise PERMANOVA for interactions of maximum increase in relative growth (%), between size and culture method, showing P-values (P) and average Similarity for factors size and culture method.

Pairs of Level Factor		Interaction	P	Average Similarity
Size	Ocean	2cm/3cm	0.194	1.2752
		2cm/5cm	0.001	1.4158
		3cm/5cm	0.018	1.0204
	Aquaria	2cm/3cm	0.001	2.091
		2cm/5cm	0.008	1.7571
		3cm/5cm	0.17	1.0638
Culture Method		2 cm	0.001	3.123
		3 cm	0.001	3.123
		5 cm	0.001	2.9342

Final Increase in TLE (cm)

The ocean fragments showed the highest overall final increase in TLE (4.66 ± 1.23 cm) when compared to the aquaria fragments (0.31 ± 0.13 cm). The 5 cm fragments showed the highest increase for both the ocean (9.89 ± 2.92 cm) and aquaria (0.31 ± 0.13 cm) culture method, followed by the 3 cm fragments and then the 2 cm fragments (Table 15). The differences between fragment size ($F(2) = 14.900$, $p = 0.001$) and culture method ($F(1) = 39.533$, $p = 0.001$) were all significant (Table 16). Significant interactions were also found between size and culture method ($F(2) = 6.129$, $p = 0.003$).

Table 15. Mean and standard error of final increase in Total Linear Extension (cm) (n = 72), for each Acropora fragment size (2 cm, 3 cm and 5 cm), by culture method and overall.

		Culture Method		
		Ocean	Aquaria	Total
Fragment Size (cm)	2	0.97 ± 0.43	0.037 ± 0.03	0.50 ± 0.24
	3	3.12 ± 1.17	0.39 ± 0.18	1.76 ± 0.65
	5	9.89 ± 2.92	0.52 ± 0.32	5.21 ± 1.75
	Total	4.66 ± 1.23	0.31 ± 0.13	2.49 ± 0.67

Table 16. Results of 2-factor (fragment size, culture method) PERMANOVA for final increase in Total Linear Extension (cm) of Acropora fragments (n = 72) showing degrees of freedom (df), pseudo-F (F), P-values (P) and square root estimates of components of variation (%) (ECV).

	df	F	P	ECV
Size	2	11.948	0.001	0.376
Culture Method	1	48.895	0.001	0.622
SizexCultureMethod	2	6.1287	0.003	0.364

The difference in final increase in TLE was significant between 2 cm and 5 cm ($t = 4.594$, $p = 0.001$) and 3 cm and 5 cm ($t = 4.48$, $p = 0.023$) ocean fragment sizes, but not between any aquaria fragment sizes (Table 17). The difference between fragment sizes for each culture method were all significant (Table 17).

Table 17. Results of a Pairwise PERMANOVA for interactions of final increase in Total Linear Extension of Acropora fragments (cm) (n = 72) between size and culture method, showing T-Values (t), P-Values (P) and average Similarity for factors size and culture method.

Pairs of Level Factor		Interaction	t	p	Average Similarity
Size	Ocean	2cm/3cm	2.091	0.059	0.441
		2cm/5cm	4.594	0.001	0.905
		3cm/5cm	2.498	0.023	0.835
	Aquaria	2cm/3cm	2.108	0.051	0.130
		2cm/5cm	1.746	0.099	0.150
		3cm/5cm	0.108	0.904	0.209
Culture Method		2 cm	3.057	0.005	0.465
		3 cm	3.158	0.012	0.914
		5 cm	5.261	0.001	1.671

The 5 cm fragments had the highest mean relative growth overall of 54.67 ± 15.77%. For the ocean fragments the 5 cm fragment had the greatest mean relative growth of 104.29 ± 24.01%, whereas the 3 cm fragments had the highest increase for the aquaria fragments (9.31 ± 5.00%) (Table 18). Final relative growth showed significant differences between size and culture method with an interaction between both factors (Table 19). Culture method showed the highest significant difference (P = 0.001, ECV = 5.2027), followed by size (P = 0.028 ECV = 0.86749).

Table 18. Total relative increase (%), for each culture method (n = 36) and fragment size (n = 12), determined by the final increase in TLE.

		Culture Method		
		Ocean	Aquaria	Total
Fragment Size (cm)	2	23.310 ± 10.210	1.140 ± 1.228	12.360 ± 5.610
	3	75.047 ± 28.825	9.312 ± 5.002	42.179 ± 16.093
	5	104.291 ± 24.007	5.059 ± 2.910	54.675 ± 15.772
	Total	67.549 ± 14.110	5.260 ± 2.048	

Table 19. Results of 2-factor (fragment size, culture method) PERMANOVA for relative growth (%) of Acropora fragments (n = 72), determined by final increase in TLE, showing degrees of freedom (df), pseudo-F (F), P-values (p) and square root estimates of components of variation (ECV).

	df	F	p	ECV
Size	2	2.4361	0.028	0.86749
Culture Method	1	78.485	0.001	5.2027
SizexCultureMethod	2	2.1458	0.048	1.0958

The difference in final increase in relative growth was only significant between the 2 cm and 5 cm ocean fragments. For the aquaria fragments, a significant difference was found between the 2 cm and 3 cm and 2 cm and 5 cm fragments (Table 20). A significant difference was found between culture methods for all fragment sizes (Table 20).

Table 20. Results of a Pairwise PERMANOVA for interactions of final increase in relative growth (%), between size and site, showing P-values (P) and average similarity for factors size and site.

Pairs of Level Factor		Interaction	p	Average Similarity
Size	Ocean	2cm/3cm	0.652	7.1436
		2cm/5cm	0.026	7.1492
		3cm/5cm	0.109	7.0566
	Aquaria	2cm/3cm	0.04	3.2523
		2cm/5cm	0.015	2.3121
		3cm/5cm	0.253	2.8957
Culture Method		2 cm	0.001	7.688
		3 cm	0.001	8.5155
		5 cm	0.001	10.368

Growth Rate (cm per month)

Maximum Increase in TLE

Overall, the ocean (2.095 ± 0.310 cm/month) and 5 cm fragments (2.428 ± 0.441 cm/month) had the highest maximum growth rates. For all fragment sizes, the ocean fragments had higher mean growth rates (Table 21). Significant differences were found between all factors ($p = <0.05$) (Table 22). Significant interactions between fragment size and culture method ($F(2) = 0.402$, $p = 0.001$) were also found. The interaction between culture method and size

showed significance between all ocean fragments and between the 2 cm and 3 cm (t = 5.409, p = 0.001), and 2 cm and 5 cm (t = 4.352, p = 0.001) aquaria fragments (Table 23).

Table 21. Mean growth rate ± standard error (cm per month), using maximum increase in TLE for each Acropora fragment size, sorted by culture method.

		Culture Method		
		Ocean	Aquaria	Total
Fragment Size (cm)	2	0.806 ± 0.144	0.107 ± 0.058	0.457 ± 0.105
	3	1.407 ± 0.226	0.810 ± 0.140	1.108 ± 3.511
	5	4.073 ± 0.536	0.784 ± 0.201	2.428 ± 0.441
	Total	2.095 ± 0.310	0.567 ± 0.100	1.331 ± 0.186

Table 22. Results of 2-factor (fragment size, culture method) PERMANOVA for maximum growth rate (cm/month) of Acropora fragments (n = 72) showing degrees of freedom (df), pseudo-F (F), P-values (P) and square root estimates of components of variation (ECV).

	df	F	P	ECV
Size	2	20.696	0.001	0.376
Culture Method	1	37.909	0.001	0.421
SizexCultureMethod	2	4.094	0.024	0.211

Table 23. Results of a Pairwise PERMANOVA for interactions of maximum growth rate (cm/month) (n = 72) between size and culture method, showing, T-values (t), P-values (P) and average similarity for factors size and culture method.

Pairs of Level Factor		Interaction	t	P	Average Similarity
Size	Ocean	2cm/3cm	2.571	0.018	0.424
		2cm/5cm	8.678	0.001	1.019
		3cm/5cm	5.507	0.005	0.777
	Aquaria	2cm/3cm	5.409	0.001	0.491
		2cm/5cm	4.352	0.001	0.459
		3cm/5cm	0.370	0.697	0..359
Culture Method		2 cm	6.299	0.001	0.492
		3 cm	2.298	0.035	0.141
		5 cm	7.851	0.001	1.058

Final Increase in TLE

Overall, the ocean (1.202 ± 0.315cm/month) and 5 cm fragments (1.444 ± 0.448 cm/month) had the highest maximum growth rates. Mean growth rates for all fragment sizes can be seen in Table 24. Growth rate (cm/month), using the final TLE showed significant differences between all factors (p = <0.05). P-Values and ECV for all factors can be seen in Table 25.

Table 24. Mean growth rate ± standard error (cm per month), using final increase in TLE for each Acropora fragment size, sorted by culture method.

		Culture Method		
		Ocean	Aquaria	Total
Fragment Size (cm)	2	0.243 ± 0.108	0.025 ± 0.017	0.134 ± 0.059
	3	0.800 ± 0.290	0.263 ± 0.117	0.531 ± 0.165
	5	2.563 ± 0.742	0.326 ± 0.210	1.444 ± 0.448
	Total	1.202 ± 0.315	0.205 ± 0.083	0.703 ± 0.173

Table 25. Results of 2-factor (fragment size, culture method) PERMANOVA for final growth rate (cm per month) of Acropora fragments (n = 72) showing degrees of freedom (df), pseudo-F (F), P-values (P) and square root estimates of components of variation (ECV).

	df	F	P	ECV
Size	2	14.9	0.001	0.226
Site	1	39.533	0.001	0.307
SizexCultureMethod	2	8.3227	0.001	0.232

Significant interactions between fragment size and culture method (F(2) = 8.323, p = 0.001) were also found. The interaction between culture method and size showed significance between all ocean fragments and but only the 2 cm and 3cm aquaria fragments (Table 26).

Table 26. Results of a Pairwise PERMANOVA for interactions of final growth rate (cm per month) (n = 72) between size and culture method, showing T-Values (t), P-values (P) and average similarity for factors size and culture method.

Pairs of Level Factor		Interaction	t	P	Average Similarity
Size	Ocean	2cm/3cm	2.586	0.018	0.448
		2cm/5cm	5.338	0.001	0.923
		3cm/5cm	3.183	0.01	0.848
	Aquaria	2cm/3cm	2.102	0.048	0.130
		2cm/5cm	1.611	0.122	0.150
		3cm/5cm	0.037	0.981	0.209
Culture Method		2 cm	2.604	0.019	0.184
		3 cm	2.244	0.039	0.455
		5 cm	4.534	0.001	0.928

Number of Branches

Initial Number of Branches

The 5 cm aquaria fragments had the highest initial mean number of branches (2.750 ± 0.657), followed by the 3 cm ocean fragments (1.917 ± 0.299) and 2 cm (1.917 ± 0.399) ocean fragments (Table 27). Significant differences between the initial number of branches were found between fragment sizes ($F(2) = 3.641$, $p = 0.027$), with a significant interaction occurring between size and culture method ($F(2) = 3.749$, $p = 0.029$) (Table 28). Results of a Pairwise PERMANOVA found no significance between any ocean fragment size, with significance only being found between the 2 cm and 3 cm ($t = 2.775$, $p = 0.015$) and 2cm and 5 cm ($t = 3.348$, $p = 0.004$) aquaria fragments. Significant differences were found between the initial number of branches on 2 cm fragments, for each site ($t = 4.190$, $p = 0.002$) (Table 29).

Table 27. Mean (± Standard Error) of Number of Branches, sorted by Culture Method and Acropora Fragment Size (n = 12)

		Ocean			Aquaria		
		2	3	5	2	3	5
Week	0	1.750 ± 0.267	1.917 ± 0.299	1.917 ± 0.399	0.417 ± 0.142	1.750 ± 0.458	2.750 ± 0.657
	2	2.833 ± 0.350	2.333 ± 0.379	4.250 ± 0.929	0.167 ± 0.108	2.667 ± 0.660	3.667 ± 0.915
	4	2.500 ± 0.250	2.500 ± 0.382	5.083 ± 1.102	0.167 ± 0.108	2.667 ± 0.670	3.500 ± 0.786
	6	2.750 ± 0.336	3.833 ± 0.599	7.000 ± 1.514	0.167 ±0 .108	2.500 ± 0.559	4.000 ± 0.833
	8	2.667 ± 0.397	3.167 ± 0.622	8.167 ± 1.806	0.000	0.000	1.250 ± 0.826
	10	2.333 ± 0.379	3.167 ± 0.644	7.333 ± 1.534	0.000	0.000	0.000
	12	2.417 ± 0.478	3.333 ± 0.680	9.000 ± 1.791	0.000	0.000	0.000
	14	2.000 ± 0.707	3.667 ± 0.995	6.250 ± 1.599	0.000	0.000	0.000
	16	2.083 ± 0.520	3.750 ± 0.951	6.167 ± 1.821	0.000	0.000	0.000

Table 28. Results of 2-factor (fragment size, culture method) PERMANOVA for initial number of branches on each Acropora fragment, per measurement period (n = 72) showing degrees of freedom (df), pseudo-F (F), P-values (P) and square root estimates of components of variation (ECV).

	df	F	P	ECV
Size	2	3.641	0.027	0.185
Culture method	1	2.141	0.153	0.099
SizexCultureMethod	2	3.789	0.029	0.267

Table 29. Results of a Pairwise PERMANOVA for interactions of the initial number of branches, for each measurement period (n = 72) between size and culture method, showing T-Values (t), P-values (P) and average similarity for factors size and culture method.

Pairs of Level Factor		Interaction	t	P	Average Similarity
Size	Ocean	2cm/3cm	0.308	0.817	0.424
		2cm/5cm	0.097	0.924	0.551
		3cm/5cm	0.339	0.747	0.552
	Aquaria	2cm/3cm	2.775	0.015	0.707
		2cm/5cm	3.348	0.004	1.016
		3cm/5cm	1.012	0.314	0.846
Culture Method		2 cm	4.190	0.002	0.703
		3 cm	0.680	0.519	0.552
		5 cm	0.754	0.472	0.808

Per measurement period

For the duration of the study, 5 cm ocean fragments had the highest mean number of branches (Table 27). The 2 cm aquaria fragments had the lowest mean number of branches until week six. In week 8 both the 2cm and 3cm fragments had 0 recorded branches due to mortality. The same occurred for the 5 cm fragments in week 10 (Table 27). Significance was found for the number of branches per measurement period between fragment size ($F(2) = 4.807$, $p = 0.001$) and culture method ($F(1) = 49.988$, $p = 0.001$), with a significant interaction occurring between the two factors ($F(2) = 3.049$, $p = 0.016$). Results of a Pairwise PERMANOVA showed no significance between ocean fragments, but a significant difference between 2 cm and 3cm ($t = 4.395$, $p = 0.001$) and 2 cm and 5 cm ($t = 4.021$, $p = 0.001$) aquaria fragments. The number of branches for each fragment size, per measurement period, was significant between culture methods (Table 31).

Table 30. Results of 2-factor (fragment size, culture method) PERMANOVA for number of branches on each Acropora fragment, per measurement period (n = 72) showing degrees of freedom (df), pseudo-F (F), P-values (P) and square root estimates of components of variation (ECV).

	df	F	P	ECV
Size	2	4.807	0.001	0.736
Culture Method	1	49.988	0.001	2.155
SizexCultureMethod	2	3.049	0.016	0.763

Table 31. Results of a Pairwise PERMANOVA for interactions of number of branches, for each measurement period (n = 72) between size and culture method, showing T-Values (t), P-values (P) and average similarity for factors size and culture method.

Pairs of Level Factor		Interaction	t	P	Average Similarity
Size	Ocean	2cm/3cm	1.081	0.313	2.147
		2cm/5cm	1.644	0.069	3.486
		3cm/5cm	1.073	0.298	3.335
	Aquaria	2cm/3cm	4.395	0.001	1.989
		2cm/5cm	4.021	0.001	2.638
		3cm/5cm	0.985	0.360	2.022
Culture Method		2 cm	6.611	0.001	3.359
		3 cm	4.714	0.001	3.427
		5 cm	3.439	0.001	4.860

Effects of Predation

Periodic predation of fragments was observed by unspecified fish species. Predation occurred on a total of 12 ocean fragments, four 2 cm fragments, two 3 cm fragments and six 5 cm fragments. It resulted in a 64.37% reduction in TLE of the affected 2 cm fragments, 52.51% reduction in 3 cm fragments and 64.97% reduction in 5 cm fragments. For ocean fragments affected by predation, there was a significant difference between the two measures for the 2 cm ($F(1, 6) = 20.707$, $p = 0.004$) and 5 cm ($F(1, 10) = 6.540$, $p = 0.028$) fragments. Significant differences were found between total relative increase using final TLE and maximum TLE ($F(1,142) = 7.078$, $P = 0.009$) and for culture method (Ocean – $F(1, 70) = 8.982$, $p = 0.004$, Aquaria – $F(1, 70) = 5.418$, $p = 0.023$). For ocean fragments, there was a significant difference between the two measures for the 2 cm ($F(1, 22) = 8.170$, $p = 0.009$) and 5 cm ($F(1, 22) = 7.094$, $p = 0.014$) fragments, which were the fragment sizes with a higher recorded occurrence of predation. This means the impacts of predation events and loss in TLE due to death prior to measurement had a significant negative impact on total relative growth. Records of merged branches and predation events can be found in Appendix E and Appendix F.

Coral Fragment Health

The 5 cm ocean fragments had the highest overall mean health score (8.83 ±0.19), followed by the 3 cm fragments (8.29 ± 0.24) and then the 2 cm fragments (8.07 ± 0.25). Ocean fragments showed a higher mean overall health score, for all fragment sizes, when compared to the aquaria fragments. The fragment with the highest score varied throughout the study (Table 32). A significant difference between health and all factors was determined (Table 33). Pairwise results for interactions between sizes found the difference between health scores to only be significant between the 2 cm and 5 cm fragments ($t = 2.935$, $p = 0.001$) and the 3 cm and 5 cm fragments ($t = 2.985$, $p = 0.001$) (Table 34).

Table 32. Mean health score ± standard error for each Acropora fragment size (n = 12), sorted by fragment measurement period (weeks) and culture method.

		Ocean			Aquaria		
		2cm	3cm	5cm	2cm	3cm	5cm
Week	0	10.25 ± .443	10.67 ± .371	11.17 ± .249	10.50 ±0.344	11.00 ±0.213	11.50 ±0.415
	2	9.33 ± 0.446	10.08 ± .246	11.17 ± .216	7.25 ± 1.055	9.17 ± 0.880	11.25 ±0.297
	4	8.83 ± 0.789	10.58 ± .226	10.00 ± .358	5.92 ± 0.416	6.67 ± 0.609	7.17 ± 0.595
	6	8.83 ± 0.865	9.33 ± 0.332	10.00 ±0.348	5.25 ± 0.542	4.25 ± 0.641	4.50 ± 0.617
	8	6.50 ± 0.618	6.42 ± 0.619	7.42 ± 0.186	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.668
	10	7.50 ± 0.731	7.50 ± 0.711	7.75 ± 0.366	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	12	7.83 ± 0.715	7.00 ± 0.657	8.00 ± 0.213	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	14	6.92 ± 0.671	6.25 ± 0.601	7.17 ± 0.297	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	16	6.67 ± 0.593	6.75 ± 0.629	6.83 ± 0.649	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Overall	8.07 ± 0.25	8.29 ± 0.24	8.83 ±0.19	3.21 ± 0.40	3.45 ± 0.43	3.94 ± 0.46

Table 33. Results of 2-factor (fragment size, culture method) PERMANOVA for health of Acropora fragments (n = 72), showing degrees of freedom (df), pseudo-F (F), P-values (p) and square root estimates of components of variation (ECV).

	df	F	p	ECV
Size	2	5.334	0.001	4.4486
Culture Method	1	285.43	0.001	29.427
SizexCultureMethod	2	0.530	0.738	-2.0726

Table 34. Results of a Pairwise PERMANOVA for interactions between the health scores of Acropora fragments (n = 72), showing T-Values (t) P-values (p) and average similarity (%).

Pairs of Level Factor	Interaction	t	p	Average Similarity
Size	2 cm/ 3 cm	1.320	0.173	69.164
	2 cm/5 cm	2.935	0.001	69.389
	3 cm/5 cm	2.685	0.001	72.543

Mortality Rate (%)

Aquaria fragments experienced 100% mortality by week 10 of the study and ocean fragments experienced 5.56% mortality by the end of the study. Mortality rate by culture method, per sampling time and overall can be seen in Table 35. A significant difference for mortality was found between culture method ($F(1) = 389.510$, $p = 0.001$) (Table 36). Individual mortality can be seen in Appendix E and Appendix F.

Table 35. Mortality rate (%) of Acropora Fragments, for each culture method (n = 36) and fragment size (n = 12).

		Culture Method		
		Ocean	Aquaria	Total
Sampling time (weeks)	2	0	0	0
	4	2.778	0	1.389
	6	2.778	0	1.389
	8	5.556	94.444	50
	10	5.556	100	52.778
	12	5.556	100	52.778
	14	5.556	100	52.778
	16	8.333	100	54.167
	Total	8.333	100	54.167

Table 36. Results of 2-factor (fragment size, culture method) PERMANOVA for Acropora fragment mortality (n = 72), showing degrees of freedom (df), pseudo-F (F), P-values (p) and square root estimates of components of variation (ECV).

	df	F	p	ECV
Size	2	0.977	0.410	-9.63E-03
Culture Method	1	389.510	0.001	1.016
SizexCultureMethod	2	0.419	0.804	-6.81E-02

Weather

During the study, a maximum air temperature of 39º, minimum temperature of 20.7ºC, and mean temperature of 27.09 ± 0.11ºC was recorded. A total of 13.46 mm of rainfall was also recorded. Weekly maximum, minimum and mean temperatures and total rainfall can be seen in Table 37.

Table 37. Maximum, Minimum, Mean (± Standard Error) Temperature (ºC) and Total Rainfall (mm) in Les Village, northern Bali, For Each Week of The Study

Week	Maximum Temperature (ºC)	Minimum Temperature (ºC)	Mean (± SE) Temperature (ºC)	Total Rainfall (mm)
1	37.7	22.1	27.30 ± 0.181	3.81
2	39	24.1	28.13 ± 0.202	0
3	38.9	22.7	27.69 ± 0.262	0
4	33.5	22.8	26.71 ± 0.222	0
5	34.1	22.8	27.23 ± 0.246	0
6	36.4	23.8	27.64 ± 0.158	6.6
7	35.6	21.7	27.37 ± 0.161	0
8	36.7	21	27.31 ± 0.158	0
9	34.8	21.6	26.56 ± 0.172	0
10	32.1	21.5	26.43 ± 0.126	0
11	32.4	21.1	26.29 ± 0.173	0
12	35.4	21.6	26.56 ± 0.245	0
13	34.4	22.3	26.63 ± 0.148	0
14	33.9	21.5	27.87 ± 1.273	0
15	36.1	20.7	26.69 ± 0.306	0
16	38.4	21.7	27.09 ± 0.186	3.05

Water conditions

Temperature

During the study, the ocean reached a minimum temperature of 27.67ºC and the aquarium reached a lower temperature of 23.81ºC. The maximum temperature of the ocean was 30.71ºC, which was higher than the highest aquaria temperature of 29.34ºC. Weekly maximum, minimum and mean temperatures can be seen in Table 38. Significant differences were found between the weekly minimum, maximum and mean temperature water temperatures for each culture method (Table 39). Results of a regression analysis found that

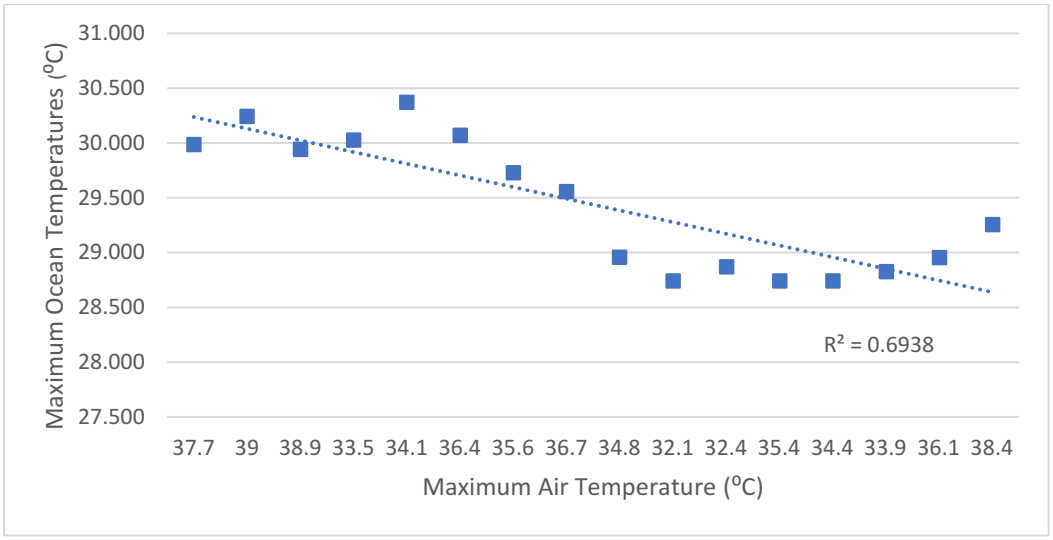
69.38% of the change in maximum ocean water temperatures can be attributed to the change in air temperature (Figure 7a). It also found that 72.72% of the change in minimum ocean temperature can be attributed to the change in minimum air temperature, and 87.53% of the change in average ocean temperature can be attributed to the change in average air temperature (Figure 7b & Figure 7c). The correlation between changes in maximum, minimum and mean aquaria water temperatures and maximum, minimum and mean air temperatures were low (14.92%, 6.99% and 0.06% respectively) (Figure 8).

Table 38. Maximum, Minimum and Mean (± Standard Error) of Weekly Water Temperatures (ºC) for Each Culture Method (Ocean, Aquaria), in Les Village, northern Bali.

Week	Ocean			Aquaria		
	Maximum Temperature (ºC)	Minimum Temperature (ºC)	Mean Temperature (ºC)	Maximum Temperature (ºC)	Minimum Temperature (ºC)	Mean Temperature (ºC)
1	29.985	28.569	29.275 ± 0.011	28.054	24.665	26.342 ± 0.028
2	30.242	28.698	29.371 ± 0.424	28.955	26.252	27.615 ± 0.022
3	29.942	28.612	29.155 ± 0.009	29.341	25.223	27.433 ± 0.031
4	30.028	28.569	29.245 ± 0.010	28.269	24.408	26.534 ± 0.029
5	30.371	28.869	29.297 ± 0.008	28.698	24.708	26.789 ± 0.030
6	30.070	28.526	29.059 ± 0.009	28.998	26.510	27.923 ± 0.020
7	29.727	28.698	29.163 ± 0.006	29.341	24.965	27.413 ± 0.032
8	29.556	27.668	28.759 ± 0.008	28.740	23.807	26.313 ± 0.038
9	28.955	27.754	28.407 ± 0.006			
10	28.740	27.968	28.358 ± 0.005			
11	28.869	27.496	28.198 ± 0.008			
12	28.740	27.496	28.161 ± 0.008			
13	28.740	27.496	27.912 ± 0.007			
14	28.826	27.840	28.236 ± 0.006			
15	28.955	27.325	27.974 ± 0.008			
16	29.255	27.668	28.137 ± 0.007			

Table 39. Results of an ANOVA testing for significant differences in minimum, maximum and mean temperature (ºC) between culture method, showing degrees of freedom (df), F-value (F) and P-value (p).

		df	F	p
Measure	Minimum	1	99.138	0.000
	Maximum	1	40.124	0.000
	Mean	1	84.065	0.000



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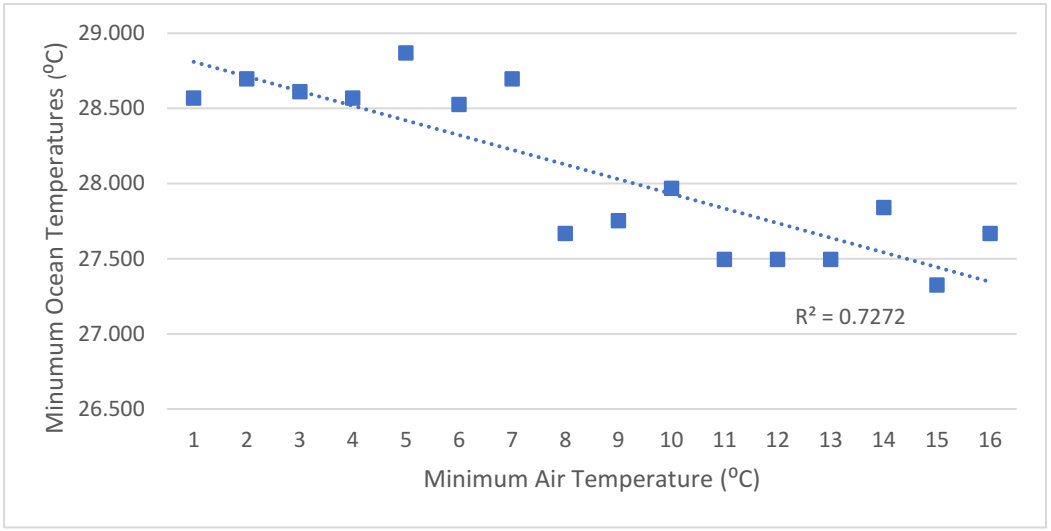

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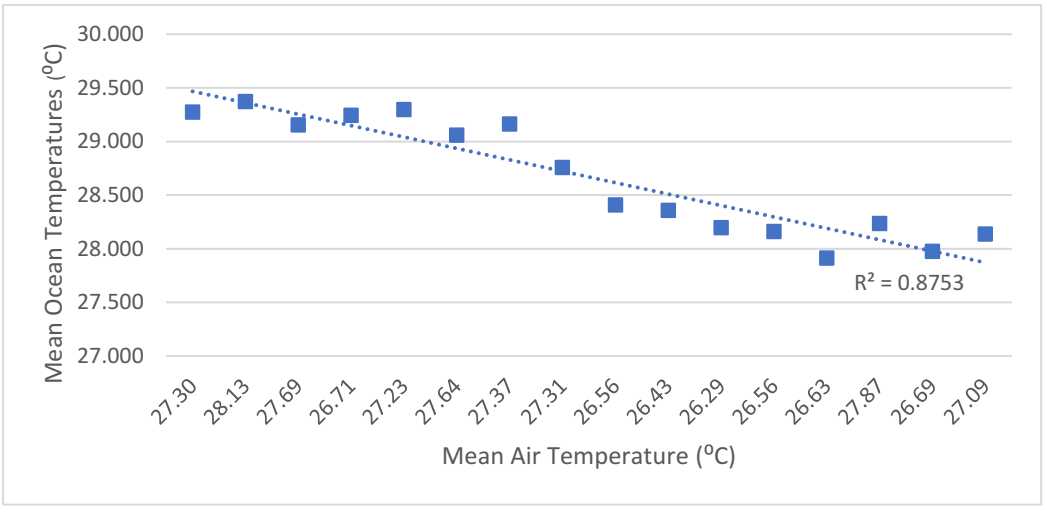
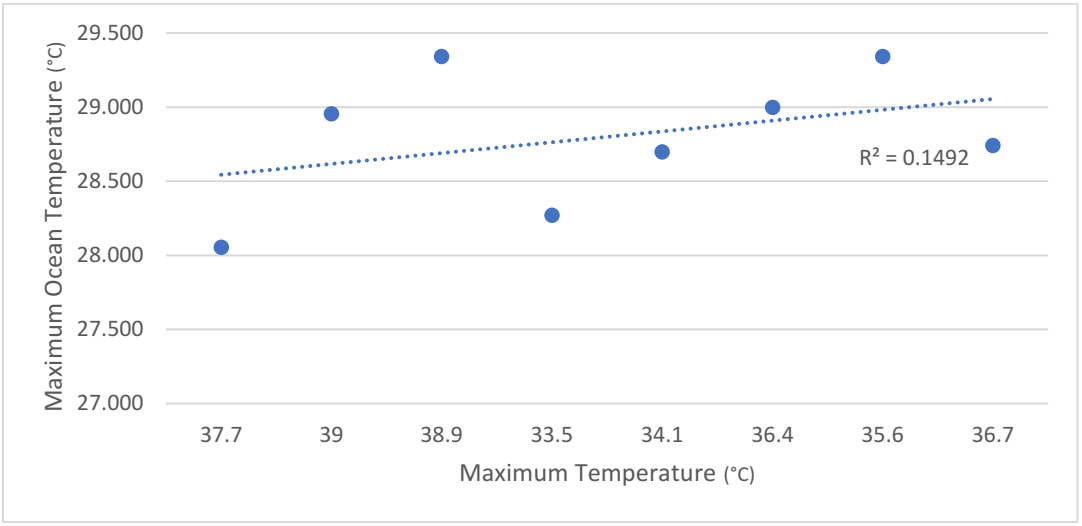


C

Figure 7. Linear Regression Analysis, with linear trendline and R^2 Values of A) Maximum Ocean Water Temperatures (°C) and Maximum Air temperatures (°C) per measurement period B) Minimum Ocean Temperatures and Minimum Air Temperatures (°C) per measurement period, C) Mean Ocean Temperatures (°C) and Mean Air Temperatures (°C) per measurement period, in Les Village, northern Bali.



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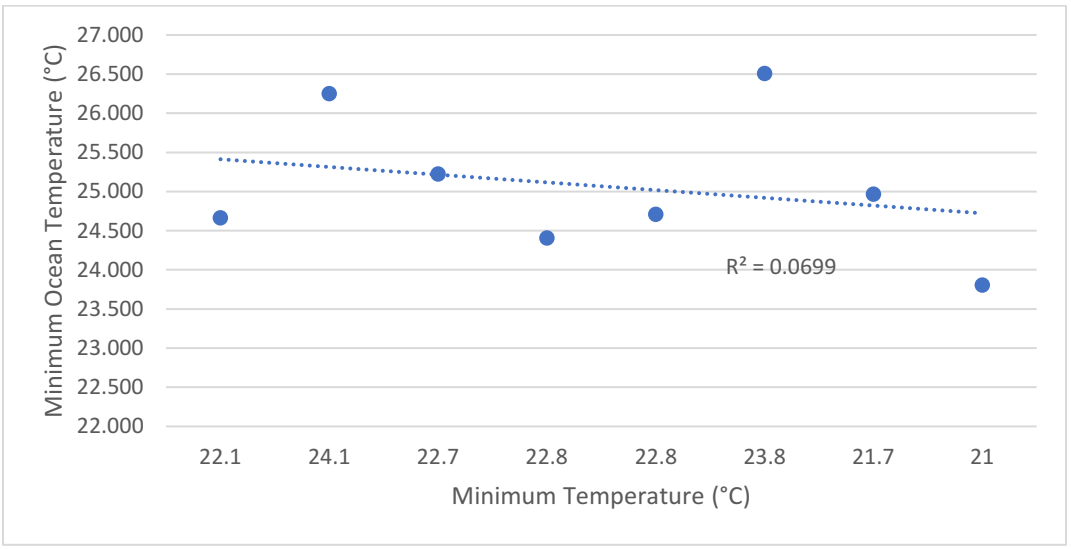

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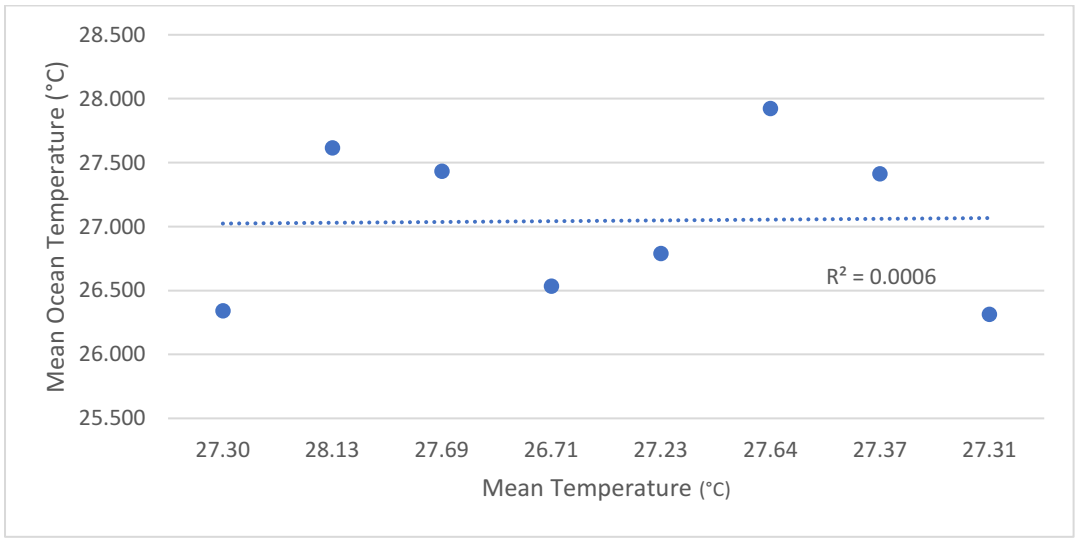

C

Figure 8. Linear Regression Analysis, with linear trendline and R^2 Values of A) Maximum Aquaria Temperatures (°C) and Maximum Air temperatures (°C), per measurement period B) Minimum Aquaria Temperatures (°C)and Minimum Air Temperatures (°C), per measurement period, C) Mean Aquaria Temperatures (°C) and Mean Air Temperatures (°C) per measurement period, in Les Village, northern Bali.

Light

During the study, both culture methods received a minimum light level of 0 Lux. The maximum light level in the ocean was 51281.92 Lux, which was higher than the aquaria highest light level of 16563.2 Lux. Weekly maximum, minimum and mean light levels can be seen in Table 40. Significant differences were found between the weekly minimum, maximum and mean light levels for each culture method (Table 41).

Table 40. Maximum, minimum and mean (± standard error) of weekly water light (lx) for each culture method (Ocean, Aquaria).

	Ocean			Aquaria		
Week	Maximum	Minimum	Mean (± Standard Error)	Maximum	Minimum	Mean (± Standard Error)
1	42168.320	0.000	6668.114 ± 338.536	5224.960	0.000	465.522 ± 16.210
2	29286.400	0.000	4511.234 ± 201.261	3060.480	482.240	664.119 ± 3.946
3	34150.400	0.000	4992.786 ± 233.101	3459.840	4.610	577.837 ± 5.780
4	24514.560	0.000	3698.010 ± 167.988	16563.200	53.080	532.151 ± 16.893
5	51281.920	0.000	6667.220 ± 306.084	738.880	131.080	427.789 ± 7.621
6	32614.400	0.000	4154.394 ± 205.911	724.480	496.480	599.834 ± 1.278
7	44441.600	0.000	6099.658 ± 289.985	712.960	104.600	522.239 ± 4.069
8	33617.920	0.000	5253.516 ± 236.585	392.800	27.380	108.608 ± 3.884
9	29030.400	0.000	4999.094 ± 233.107			
10	24207.360	0.000	3014.467 ± 144.353			
11	28334.080	0.000	4229.372 ± 207.543			
12	25446.400	0.000	3574.363 ± 169.852			
13	26951.680	0.000	2863.367 ± 159.412			
14	8663.040	0.000	1095.200 ± 55.196			
15	25180.160	0.000	1798.610 ± 96.947			
16	24432.640	0.000	2335.535 ± 128.442			
Overall	51281.92	0.000		16563.2	0.000	

Table 41. Results of an ANOVA testing for significant differences in minimum, maximum and mean light (Lx) between culture method, showing degrees of freedom (df), F-value (F) and P-value (P).

		df	F	P
Measure	Minimum	1	4.930	0.043
	Maximum	1	80.253	0.000
	Average	1	138.929	0.000

Aquaria pH and Salinity

The aquaria pH levels remained within the ideal ranges (8 – 8.5), except for after the week 4 water change, where pH increased to 9. Salinity was lower than the ideal ranges (33-35ppm), for nine of the 13 salinity measurements (Table 42).

Table 42. Aquaria pH and Salinity (ppm), showing measurement date, week and notes on significant events.

Date	Week	pH	Salinity	Notes
26-May	0	8.5	31	
2-Jun	1	8.5	31	
9-Jun	2	8.5	31	
16-Jun	3	8.5	33	
23-Jun	4	9	32	
25-Jun				Algal Bloom
26-Jun	4	8.5	31	Additional Water Change
27-Jun	4	8	35	
29-Jun	4	8.5	35	
30-Jun	5	8.5	35	
1-Jul	5	8.5	31	
7-Jul	6	8.5	31	
14-Jul	7	8.5	31	
21-Jul	8	8.5	31	

Discussion

During the study, 5 cm fragments showed the greatest growth and growth rates for both culture methods. The ocean-based fragments experienced an overall increase in TLE and low mortality. Unfortunately, due to the aquaria having water quality issues, all fragments died by

week ten. These findings resulted in 5 cm fragments cultivated in an ocean environment, may be determined as the ideal methodology for *Acropora* restoration.

Overall Growth

Total Linear Extension (cm)

TLE is a commonly used metric to measure branching coral growth, as it provides a three-dimensional growth measure. It also provides measurements required to determine growth rates and relative growth. *Acropora* species have been the focus of many restoration attempts due to their high growth rates, high survivorship, frequency of natural fragmentation, and ease of manual fragmentation (Young et al., 2012). Significance between initial TLE of each fragment size was determined to ensure any determined in growth where not due to variation in the initial TLE of fragments. The initial TLE of each fragment size were significantly different, for both the ocean and aquaria culture methods. This means any significance between growth measures and health, can be attributed to the difference in fragment size. A significant difference was found between the initial TLE of 2 cm fragments, between culture methods, due to the significantly higher number of branches on the ocean fragments. The significantly higher initial TLE of the 2 cm aquaria fragments reduces the reliability of comparisons of the 2 cm fragments, between sites.

All recorded increases in TLE were found to have significant differences between culture methods. This suggests that the ocean culture method is more effective for the growth of *Acropora* fragments. Unlike other coral species, *Acropora* growth occurs through the production of new branches and extension of the apical ends of fragments (Lirman et al., 2014). This means that the initial number of branches on fragments, can affect increases in TLE. There was a significant difference between the initial number of branches on 2 cm fragments between culture methods, with the ocean fragments having more. *Acropora* growth

occurring from the apex of branches can explain why the ocean 2 cm fragments had a greater increase in all growth measures, despite having a lower initial TLE (Lirman et al., 2014).

For the ocean culture method, significant differences were found between the 2cm and 5 cm fragments, and 3cm and 5cm fragments for measures of TLE per measurement period and using final increase in TLE. Significance was found between all fragment sizes for the maximum increase in TLE. Significance was only found between 2 cm and 5cm and 3cm and 5cm fragments when using maximum and final relative growth. Measures of relative growth per measurement period only showed significant differences between the 2 cm and 5 cm fragments. This significant difference may indicate that 3 cm fragments can be used to achieve the same relative increase as 5cm fragments, but the lack of a significant difference between 2 cm and 3 cm fragments indicate there may be another factor contributing to this significance.

For the aquaria culture method significant differences were found between all fragment sizes for TLE per measurement period. Increase in TLE of aquaria fragments only showed significant differences between 2 cm and 3cm and 2cm and 5cm when using maximum increase and showed no significant differences when using final increase. This significance may be due to the significant difference in the initial number of branches between the 2 cm and 3 cm and 2 cm and 5 cm fragments. The lack of a significant difference between final increase in TLE implies that in an aquarium setting, if water conditions can be controlled, 3cm fragments can be used to achieve the same increase in size as 5cm fragments. This is supported by the significant differences recorded for relative growth.

For the aquaria fragments, significant differences were found between the 2 and 3cm and 2 and 5cm, for all measures of relative growth. This implies that in an ex-situ setting, either 3cm or 5cm fragments can be used to achieve the same amount of relative growth. The

significant difference between 2cm and 3cm fragments that was observed using the aquaria culture method, but not the ocean culture method, should be taken into consideration when determining the ideal fragment size for new project.

The ability to use smaller fragments, to achieve the same relative growth may prove beneficial in some restoration projects. For projects which do not require a rapid increase in coral cover or have limited access to healthy mother stock, less coral would need to be harvested to achieve the same increase over time. This helps reduce stress on the naturally occurring coral.

Growth rates

Significant differences were found between the maximum and final growth rates of all fragment sizes between sites, with the ocean fragments being higher. This is likely due to the high initial mortality of aquaria fragments and overall reduction in TLE, which resulted in 64% of fragments having a growth rate of 0 cm/month. The high mortality that occurred in aquaria fragments can be attributed to inadequate water quality measures being available. To ensure the significant differences of growth rates between culture methods are an accurate representation, the study needs to be replicated with increased water quality controls, to ensure the same measurement periods for each culture method.

The 5 cm ocean fragments had the highest mean growth rates for both maximum (48.87 cm/year) and final (30.75 cm/year). The 3 cm fragments had the next highest mean maximum and final growth rates, for the ocean fragments, of 16.884 cm/year and 9.603 cm/year respectively. The 2 cm ocean fragments had the lowest maximum (9.672 cm/year) and final growth rates (2.912 cm/year). These growth rates fall within the published ranges of growth rates for *Acropora* species, which range from 0.71cm per year to 15.8cm per year (Yap & Gomez, 1984; Crabbe & Smith, 2005; Bak et al., 2009; Lirman et al., 2014).

The 5 cm fragments had the highest mean maximum (9.406 cm/year) and final (3.915 cm/year) growth rates out of the aquaria fragments. The 3 cm fragments had the second highest mean maximum and final growth rates of 9.717 cm/year and 3.151 cm/year respectively. The lowest mean maximum (1.293 cm/year) and final (0.296 cm/year) growth rates for the aquaria method and overall were determined for the 2 cm fragments. All aquaria growth rates fragments fell within the published growth rates for *Acropora* species, except the mean final growth rate for the 2 cm fragments. The lack of studies on 2 cm *Acropora* fragments is likely the cause of this studies growth rate not being within the published range (Yap & Gomez, 1984; Crabbe & Smith, 2005; Bak et al., 2009; Lirman et al., 2014).

Coral growth rates vary between species, season and throughout the literature due to the variety of growth metrics used. Growth rates of *Acropor*a species ranges from approximately 0.71cm per year to 15.8cm per year (Yap & Gomez, 1984; Crabbe & Smith, 2005; Bak et al., 2009; Lirman et al., 2014). These rates were determined using whole colonies, or fragments of an unspecified size. Lirman et al (2014), studied the growth of *Acroproa cerviconis*, using fragments that ranged from <5cm to 30cm and found a significant correlation between initial TLE and growth rates. The determined growth rate ranged from 0.9 to 9.8cm per year, for each centimetre of the fragment, with larger fragments showing higher growth. The maximum growth rates determined in this study, which ranged from 1.293 to 48.875 cm per year, align with the findings of Lirman et al (2014), and provides evidence that ocean-based culture methods, on larger fragments is more successful for rapid growth.

Another factor that may have contributed to growth rates being on the lower end of the published ranges is the way *Acropora* species skeletons grow. In massive and encrusting species, skeletal growth is observed around the edge of the colony and consequently, the area fragments are cut (Knapp et al., 2022). This reduction in size of the colony, creates an

increased edge area for growth to occur, in comparison to the fragment size. *Acropora* species growth occurs though extension of the apical ends and production of new branches, meaning that creating fragments significantly reduces the area available for new growth (Lirman et al., 2014). This supports the findings of a higher growth rate, being correlated with a larger fragment size.

Effects of Predation

Grazing by herbivorous fish is vital to ensure the maintained health of tropical reefs, as they help to remove algae and allow corals to grow unimpeded. However, grazing of the coral animal can negatively impact corals if it results in significant loss of skeleton (Rotjan & Lewis, 2008). During this study, predation caused the loss of up to 65% of the skeleton of affected fragments. Predation impact was recorded in weeks 12 and 14 after a noticeable increase in the number of fish visiting the coral table, and surrounding areas. Significant grazing and predation has been reported in a variety of studies (Schopmeyer et al., 2017; Cano et al., 2021). No direct attempt was made to prevent predation on fragments in this study, as there has been no record of it happening on previous fragments at the study site. Other studies have implemented grazing and predation prevention methodologies, which include partial and total grazer exclusion using cages and nets (Korzen et al., 2011). The majority of studies found that long-term herbivore exclusion increases the mortality rate and slows down coral productivity (Ladd & Shantz, 2020). All mortality of ocean fragments within these studies was caused by algal growth, therefore grazer exclusion is likely to increase mortality at the study site. The main exception to this is when grazer populations are higher than they would be in a healthy ecosystem (Cano et al., 2021). Increased survival rates have been found when predator exclusion is implemented for the first two weeks to three months after transplantation, as it allows time for fragments to grow large enough to prevent stress related mortality and regrow tissue rapidly. Though if predation occurs repeatedly, or

leaves a large bite scar, the predation can often lead to mortality, no matter the fragment size (Raker et al., 2023).

Health and Mortality

All fragments showed an overall decline in health throughout the duration of the study, with no specific fragment size or culture method having the highest health for the duration of the study. 5 cm fragments had the highest mean overall health score for both the ocean and aquaria culture method of 8.83 ± 0.19 and 3.94 ± 0.46, respectively. 3 cm fragments had the second highest mean overall health score for both the ocean (8.29 ± 0.24) and aquaria (3.45 ± 0.43) culture method, and the 2 cm fragments had the lowest of 8.07 ± 0.25 and 3.21 ± 0.40 respectively.

Specific health measures are not commonly recorded in studies, with more focus being on damage to the fragment, the presence of disease or the percentage of the fragment that has bleached (Seguin et al., 2008; Hein et al., 2017). It is likely that the decline in health is due to the stress of the transplantation process as reduced growth did not occur at the same time as reduced health scores and could be explained by either predation or the merging of branches. This claim is supported by findings from other coral transplantation studies that found transplanted fragments exhibited reduced pigmentation for up to a year after transplantation with no significant reduction in growth rates (Forrester et al., 2012). This reduced pigmentation could impact survival of fragments if a stressor event was to occur. It is likely due to the loss of zooxanthellae, which results in coral having weaker resistance to disease and stressor events (Quigley et al., 2018).

All ocean mortality occurred in the first eight weeks and can be attributed to algae growth. This is a commonly reported issue for new coral transplants due to weakened tissue caused

by transplantation stress (Yap, 2004). After the first mortality due to algae, the coral tables were cleaned more frequently to reduce possible mortality.

The significantly higher mortality in the aquaria can be attributed to an algal bloom. Algal blooms are often caused by increased inorganic nutrients, such as nitrogen and phosphorus (Sarkar, 2018). Unfortunately, water testing equipment for these parameters was not available, so they could not be monitored and corrected. When the bloom was noticed, a water change was completed, and the tanks were cleaned. This resulted in a reduction of pH from 9 to 8.5, levels returning to within normal ranges. To prevent this from happening in future studies, the water used to complete the water change should be held in a water tank before being put in the aquaria. This will allow for water quality to be checked before coming into contact with the fragments. It may also be beneficial to include herbivorous fish in the aquaria, to remove any algal growth that does occur. These additions and their prices are discussed further in Applicability to Remote Communities.

It is recommended that when fragments are harvested from wild stock for cultivation in an aquarium, the temperature of the aquarium remains within the harvest site's temperature range. The significant differences between mean, maximum and minimum temperatures for each culture method indicate that this did not occur. All aquaria measurements were lower than the corresponding ocean measurements. At some points during the study, the maximum aquaria temperature was lower than the ocean minimum temperature. Although the low temperatures would not have been the cause of the algal bloom and were still within recommended rages, the fragments may have had lower resistance and an impeded ability to recover (Borneman, 2008; Bartlett, 2013; Sarkar, 2018). If aquaria water temperature falls below the minimum recommended temperature range, the addition of a water heater would be recommended. It has previously been reported by Sea Communities that water temperatures in previous aquariums have exceeded recommended levels, so a water cooler may be required

in the hotter months. This will vary between study sites, so water temperatures should be monitored regularly, with alterations made when necessary.

The significant differences in light availability between culture methods are likely not a contributor to the aquaria fragments mortality. Corals can adjust to different light levels, without mortality, but can show a reduction in health during the adjustment period (Osinga et al., 2011; Hoogenboom et al., 2012). The reduction in light availability, after the algal bloom in week four, can be attributed to algal growth on the HOBO data logger. This regularly covered the sensor and potentially made the light recordings inaccurate. To prevent this from occurring in future studies, aquaria light loggers should be cleaned daily.

Conclusion

The need for coral restoration is increasingly acknowledged throughout the world. This is due to increased awareness of the impacts of climate change and anthropogenic activities on marine ecosystems (Tortolero-Langarica et al., 2020). Natural recovery of reef ecosystems is a slow process, with increasingly frequent reoccurrence of stressor events reducing the ability of ecosystems to recover. Coral restoration provides reef ecosystems with increased resilience and the ability to recover from stressor events, especially when growth is rapid through the use of methodologies such as micro-fragmentation. Fragments that are used in restoration efforts, are sourced from corals that have survived previous stressor events, which can help improve the genetic robustness of the corals within the ecosystem (van Oppen et al., 2015). The use of micro-fragmentation on certain coral species has shown an increase in growth rate when used in specific environments. It has also shown a reduced mortality rate when coral fusion is incorporated into the methodology (Forsman et al., 2015). Coral fusion occurs when fragments from the same colony are placed in close proximity, with no fragments from other colonies between them. As the coral colony on one fragment grows and reaches the colony from another fragment, they will join, significantly increasing the colony size. The increased

colony size increases resilience, growth rates and spawning capacity (Raymundo & Maypa, 2004).

The findings of this study determined that micro-fragmentation provides no benefit for in-situ growth of *Acropora* and that an ocean-based culture method is more successful, when trying to achieve rapid increases in TLE. 5 cm ocean fragments showed higher growth rates, when compared to the 5 cm aquaria fragments and other fragment sizes. To ensure that these findings are not due to short term changes in growth, they need to be confirmed with more long-term monitoring.

The lack of a significant difference in proportional growth between 3 cm and 5 cm fragments, per measurement period, indicates that 3 cm fragments can be used to achieve the same increase in initial relative growth, as 5 cm fragments. To determine its long-term applicability, a longer study needs to be completed, to ensure it is not due to stress from the micro-fragmentation process. The initial reduced health is common in micro-fragmented corals, but long-term monitoring should be implemented to ensure they recover to their initial health levels (Forrester et al., 2012).

Micro-fragmentation may prove beneficial for aquaria coral growth, with no significant difference being found for the maximum increase in TLE or any proportional growth measures between 3 cm and 5 cm fragments. No benefit was found with the use of 2cm fragments. The high mortality of the aquaria fragments limits the accuracy of these findings. It is recommended that monitoring of growth of 3 cm and 5 cm fragments is repeated, with adequate water quality control, to increase survivorship and consequently the number of growth measurements collected.

A key component of coral restorations success, for all species, is the survivorship and fusion of transplanted fragments. This is not commonly measured or reported (Bowden-Kerby,

2001; Lirman et al., 2010; Forrester et al., 2014). If fragments are transplanted in an easily identifiable area, health and mortality can be easily monitored using the same methodology as this study. High survival and significant growth in other species that have been restored using micro-fragmentation is due to fusion. This occurs when multiple fragments from the same colony are placed on the same substrate, which allows them to join and have a significantly increased size (Sutthacheep, 2023). This methodology was not trialled but has been observed on different fragments in the area. It has potential to increase transplant survival due to the rapid increase in colony size.

Another potential future research topic is the growth of *Acropora* on artificial substrate. Although calcium carbonate growth does not occur from the base of *Acropora*, the fragments were observed growing down onto the substrate during this study. It appeared to be occurring at a more rapid rate on the smaller fragments. This was not recorded or analysed statistically, but resulting in the point linear extension was measured from, being further down the substrate each measurement period. The use of substrate as an artificial skeleton could be beneficial to reef restoration, reducing the energy required for colonies to increase their size, and increasing fragment strength.

Applicability to Remote Communities

Majority of published literature focuses on the cost-effectiveness or success rate of restoration and rarely consider both factors. This results in a disconnect between state-of-the-art methodologies, and the methodologies that are applicable in developing countries (Bayraktarov et al., 2019; Tortolero-Langarica et al., 2020). A direct comparison of the success of methodologies and costs in this study will be discussed, with recommendations to improve the methodology.

Methods

Cost Analysis

The cost analysis was completed by determining the overall cost of both methodologies. Two overall costs were determined:

1. The cost of this research,
2. The cost including any additional equipment that may improve the results of the methodology if it is replicated.

Improvements to the used methodology were determined by finding the causes and potential solutions for the problems encountered during the study (i.e., algal bloom, low aquaria growth) within published literature. All aquarium equipment was purchased online, from Bali Reef Aquarium as it is a local store that delivered to the area. Any additional costs for aquarium improvements will be determined using their pricing, unless otherwise specified.

Ideal Culture Method

Information regarding the ideal culture method was determined by reviewing methodologies throughout the literature and recording any problems encountered throughout the study. These were then compared with the following success factors: mortality, health, growth, setup and monitoring requirements and cost.

Results

Cultivation Methodologies

Used Methodology

Ocean Cultivation

The total cost to use this methodology for the ocean-based culture method is AUD 6,122 for the first year and AUD 2,573 for subsequent years. This includes the collection and transplantation of 36 coral fragments, one coral table, the associated diving costs (fragment collection and fortnightly monitoring) and one-time equipment purchases. Pricing for subsequent years only included maintenance and monitoring costs. A breakdown of these expenses can be seen in Table 43.

Table 43. Outline of costs, in Australian Dollars of used methodology for 36 coral fragments, using the ocean culture method, categorised by use.

Activity/Items	Cost Per Item/Use/Time	Cost Per Annum
Measuring/Monitoring		
Callipers	30.00	30.00
Coral Health Card	5.00	5.00
Camera	1200.00	1200.00
Dive Equipment		
Tank	5.50	528.00
BCD/Mask/Regulator/Fins	25.00	1200.00
Staff/Guide (2 dives)	35.00	840.00
HOBO Temperature/Light Logger	180.00	180.00
Fragments		
Diving Equipment		
Tank	5.50	11.00
BCD/Mask/Regulator/Fins	25.00	50.00
Staff/Guide	35.00	35.00
Pak Eka Glue	0.20	7.20
Substrate	0.30	10.80
Tie Wraps	5.00	5.00
Diamond band saw	1500.00	1500.00
Table	500.00	500.00
Cleaning tools		
Wire brushes	10.00	20.00
Total First Year		**6,122**
Excluding SCUBA equipment rental		**4,872**
Total Subsequent Years		**2,573**
Excluding SCUBA equipment rental		**1,373**

Aquaria Cultivation

The total cost to use this methodology for the aquaria culture method is AUD 5,074 for the first year and AUD 1,703 for subsequent years. This includes the collection and transplantation of 36 coral fragments, one aquarium, the associated diving costs for fragment collection and one time equipment purchases. Pricing for subsequent years only included maintenance and monitoring costs. A breakdown of these expenses can be seen in Table 44.

Table 44. Outline of costs, in Australian Dollars of used methodology for 36 coral fragments, using the aquaria culture method, categorised by use.

Activity/Items	Cost Per Item/Use/Time	Cost Per Annum
Aquaria Equipment		
Aquaria	80	80
Lamp	40	80
Filter	200	200
Bio Pellets	70	70
Electricity/Generator	35 (Monthly)	420
Measuring/Monitoring		
Callipers	30	30
Coral Health Card	5	5
Camera (Optional)	1,200	1,200
Staff Maintenance Wage	100 (Monthly)	1,200
HOBO Temperature/Light Logger	180	180
Water Quality Tests		
Salinity	12.5	12.5
pH	13	13
Fragments		
Diving Equipment		
Tank	5.5	5.5
BCD/Mask/Regulator/Fins	25	25
Staff/guide	35	35
Pak Eka Glue	0.2	7.2
Substrate	0.3	10.8
Diamond band saw	1,500	1,500
Total First Year		**5,074**
Total Subsequent Years		**1,703**

Comparison

The cost analysis of used methodologies found the aquaria culture method to be AUD 1,048 cheaper to set up, and AUD 870 per annum cheaper for continual monitoring. The main contributor to the higher cost for the ocean-based methodology is the ongoing costs for the SCUBA diving equipment required for monitoring the fragments. This cost could be reduced by AUD 1,250 for the first year, and AUD 1,200 in subsequent years if the rental of diving equipment was not required. This would then make the ocean methodology AUD 252 cheaper to set up than the aquaria methodology and AUD 330 cheaper for ongoing annual monitoring.

If SCUBA equipment rental is not required, the price difference between methodologies would be minimal, making them both applicable.

Improved Methodology

Ocean Cultivation

No improvements to improve growth were determined for the ocean methodology. The optional use of a camera frame and 3D photogrammetry, to increase measurement accuracy would result in an AUD 33 increase in the cost of the first year. The total increased costs would be AUD 6,155 including SCUBA equipment rental or AUD 4,905 excluding SCUBA equipment rental.

Aquaria Cultivation

The total cost to use the improved methodology for the aquaria culture method is AUD 5,259 for the first year and AUD 1,888 for subsequent years. This includes the collection and transplantation of 36 coral fragments, one aquarium, the associated diving costs for fragment collection and one time equipment purchases. Pricing for subsequent years only included maintenance and monitoring costs. If a camera frame and 3D photogrammetry is implemented, the first-year cost is increased to AUD 5,292. A breakdown of these expenses can be seen in Table 45.

Table 45. Outline of costs, in Australian Dollars of improved methodology for aquaria culture method categorised by use.

Activity/Items	Cost Per Item/Use/Time	Cost Per Annum
Aquaria Equipment		
Aquaria	80	80
Lamp	40	80
Filter	200	200
Bio Pellets	70	70
Electricity/Generator	35 (per month)	420
Coral Food	25	25
Measuring/Monitoring		
Callipers	30	30
Coral Health Card	5	5
Camera	1,200	1,200
Staff Maintenance Wage	100 (per month)	1200
HOBO Temperature/Light Logger	180	180
Water Quality Tests		
Salinity	12.5	12.5
pH	13	13
Nitrate	25	22
Calcium	25	20
Alkalinity	13	13
Magnesium	62	62
Phosphate	30	25
Nitrite	18	18
Fragments		
Diving Equipment		
Tank	5.5	5.5
BCD/Mask/Regulator/Fins	25	25
Staff/guide	35	35
Pak Eka Glue	0.2	7.2
Substrate	0.3	10.8
Diamond band saw	1,500	1,500
Optional Extras		
Camera Frame		
PVC Pipe	10	10
Glue	7	7
Camera Mount	16	16
Total First Year		5,259
Total First Year (Camera Frame)		5,292
Total Subsequent Years		1,888

Ideal Culture method

Due to the success factors shown in Table 46, the ocean cultivation was determined to be the ideal method for *Acropora* species fragments. The significantly higher mortality rate of the aquaria fragments (100%), when compared to the ocean fragments (8.3%), is the main factor contributing to the ocean culture methods higher success. The high aquaria mortality

followed a rapid decline in fragment health and resulted in significantly lower growth than the ocean fragments. Set up requirements were met for both methods, but monitoring requirements were not met for the aquaria methodology, as inadequate water condition tests were available. The lower cost of the aquaria methodology would result in it being the ideal culture method, if the other limitations can be removed.

Table 46. Success Factors used for Determining Culture Method Applicability, with Findings from Research.

		Culture Method	
		Ocean	Aquaria
Success Factors	Mortality	8.3%	100%
	Health	Overall decline	Rapid decline
	Growth	Overall increase (4.66cm ± 1.23)	Significantly lower than ocean fragments (0.31 ± 0.13)
	Setup/monitoring requirements	SCUBA certification Two staff/volunteers	Appropriate space for aquaria No access to required water quality measures
	Cost	AUD 6,122	AUD 5,074

Discussion

During this study, the ocean cultivation methodologies showed higher success, when compared to the aquaria methodology. The aquaria methodologies were found to be cheaper, for both the used and improved methodology, when rental SCUBA equipment was required. The aquaria methodology requires increased water condition monitoring measures, to prevent algal blooms and increase growth rates. Additional improvements, to increase measurement reliability and potentially increase growth were also determined.

Cultivation Methodologies – Improvements and Costs

Both the used and improved aquaria culture methods have a lower cost than the ocean culture methods. The aquaria culture method requires additional water condition testing, to prevent algal blooms and enhance growth. Additional optional measures have also been

recommended, such as the use of 3D photogrammetry, for both methodologies to increase monitoring accuracy and survivorship.

Aquaria Cultivation

To decrease aquaria mortality and increase growth additional water conditions need to be measured and the corals need to be fed. The costs of adding water temperature controls and fish to the aquaria were also determined, but not included in the final pricing. The additional water measures include nitrate, calcium, alkalinity, magnesium, phosphate and nitrite. The monitoring of nitrogen and phosphorus is important to prevent algal blooms. Increased levels of these inorganic nutrients are the main cause of algal blooms and can significantly reduce coral calcification rates (Borneman, 2008). Identifying increased levels quickly, allows for modifications or water changes to be completed to prevent the occurrence of an algal bloom, or reduced calcification rates.

Calcium levels need to be maintained to ensure calcification of coral skeletons can occur. In aquarium environments, calcium often needs to be added to maintain coral growth rates (Borneman, 2008). Monitoring alkalinity is essential as it directly influences pH levels and can limit calcification if it is not within recommended ranges (Borneman, 2008). Magnesium levels directly influence alkalinity, so ensuring magnesium is within the correct range can help maintain alkalinity and consequently pH levels (Borneman, 2008; Bartlett, 2013). Excess levels of nitrate can cause the aquaria to become toxic to corals. Excess levels are rare, but monitoring is highly recommended if possible (Borneman, 2008).

Recommended ranges for these measures, adapted from Borneman (2008) and Bartlett (2013) can be seen in Table 47. The addition of the testing kits for these measures, increased the annual cost by AUD 160. Ensuring the coral fragments receive adequate nutrients will increase coral growth, which can be achieved using coral food (AUD 25) (Leal et al., 2016).

Table 47. Recommended Nutrient Levels in Parts Per Million (ppm) or Carbonate Hardness (KH) for Saltwater Aquaria containing Coral, Adapted from Borneman (2008) and Barlett (2013).

Measure	Recommended range
Nitrate	<0.2ppm
Calcium	380-450ppm
Alkalinity	8-10KH
Magnesium	1250-1350ppm
Phosphate	<0.03ppm
Nitrite	<0.2ppm

If required, temperature can be regulated using aquaria heaters (AUD 40) and chillers (AUD 710), which add AUD 750 to the initial set up costs. Although the temperature differences throughout between culture methods were significantly different, it only fell below the minimum recommended temperature once (Borneman, 2008; Bartlett, 2013). It has been reported by Sea Communities that bleaching occurs during the hottest periods of the year, so temperature needs to be regularly monitored. If temperature is found to go outside of recommended ranges, heaters or coolers will then need to be purchased.

Adding herbivorous fish to the aquaria can be beneficial to reduce algae growth within the aquaria and on the fragments through grazing. This has been utilized in previous Sea Communities studies and has been found to significantly reduce algae growth on and around coral fragments (Knoester et al., 2023). There is no cost involved in the collection of fish at the study site and they can be taken directly from the reef by community members, or researchers if they have the correct permits. Care needs to be taken if this methodology is replicated elsewhere as regulations may be different. The only additional cost would be for fish food, which costs AUD 21 per annum.

The total cost to use the improved methodology for the aquaria culture method is AUD 5,259 for the first year and AUD 1,888 for subsequent years. This includes the collection and transplantation of 36 coral fragments, one aquarium, the associated diving costs (fragment

collection) and one time equipment purchases. The subsequent years pricing only included maintenance and monitoring costs.

Both Cultivation Methodologies

Two possible optional methods to improve measurement accuracy were recommended for both culture methods, 3D photogrammetry and a camera frame. To reduce costs, a free to use software such as Capturing Reality or Blender could be used (Irschick et al., 2022). For accurate measures to be determined using these programs, photos need to be taken with a 60% overlap with two visible markers with a known distance between them. The Agisoft Metashape user manual advised that this could be a ruler, meaning the initial cost of callipers or a ruler, must still be included (Agisoft Metashape, 2023). Callipers are recommended as the markings on rulers fade rapidly and need frequent replacement (M. Merta, Personal Communication, May 2023). It is also highly recommended that a camera frame is also used to increase measurement accuracy.

Commonly used camera frames are made from PVC pipe, with a camera mount attached (Neufeld & Fundakowski, 2023). This ensures the orientation of photos and distance the photos are taken from the fragments remain consistent. Pricing for frame materials was determined using prices from Bunnings Warehouse website, as local hardware stores do not have readily available price guide (Bunnings Warehouse, 2023). Pricing for the camera mount was determined from Hop Market, a Jakarta based shop, that offers Bali wide delivery (Tokopedia, 2023). There were no local providers found that could supply the required clamp. If a camera frame is constructed, and used for measurements, it will increase the first-year costs for both culture methods by AUD 33 (total revised cost: Ocean AUD 6,155, Aquaria = AUD 5,292).

Comparison

The improved aquaria culture method is AUD 863 cheaper than the ocean culture method for the first year, and AUD 685 cheaper for subsequent years. If rental SCUBA equipment is not required for ocean cultivation, this methodology would be AUD 387 in the first year, and AUD 515 cheaper in subsequent years.

Ideal Culture Method

The ideal culture method, determined by the use methodology is the ocean culture method with 3 to 5 cm fragments. This can be contributed mainly to the high initial mortality of the aquaria fragments, due to a lack of water quality measures, which have been discussed in *Cultivation Methodologies – Improvements and Costs*. Overall, the lower cost of the aquaria means it is potentially more suitable for use, if the previously stated improvements can be implemented and the rental of dive equipment is required.

Monitoring Method

Three main monitoring methods are needed to prove the success of coral fragmentation: growth, health and mortality. Ideally these would be both pre- and post-transplant, to ensure transplantation is providing benefits to the reef ecosystem. Growth can be recorded in one of two ways, manually, using TLE, or with 3D photometry which can provide a larger variety of growth measures including TLE and surface area. The used manual monitoring method was found to be appliable in remote communities, with minimal training necessary. Its low cost is a benefit, with the possibility of altering the measuring methodology to not require a camera if the expense is not manageable. If a camera will be used, it is recommended that a camera frame also be used, to ensure consistency between measurement photographs and prevent inaccuracy. The use of 3D photogrammetry can significantly improve accuracy, and reduce the time required to take measurements. Its use does require some training to use the 3D modelling software, potentially limiting its applicability.

Health and mortality can be determined using CoralWatch health card easily and at a low cost. The criteria for fragments being determined dead must be predetermined and kept consistent, to ensure bleached, but alive corals are not included in the mortality counts.

The lack of monitoring after fragments have been transplanted, does not allow for the long-term success of restoration projects to be determined. Ideally, at least health and mortality of transplanted fragments should be monitored. Growth measurements of transplanted fragments would provide beneficial insight to any impacts the fragmentation and transplantation may have, but depending on the measurement methodology used, measurements may become increasingly inaccurate as the fragment grows (Huntington & Miller, 2014). When discussing the reasonings behind not monitoring transplanted fragments, I was informed that unless fragments were transplanted onto artificial structures, it was difficult to identify specific fragments. Various attempts to label the transplants were made, which included plastic and metal tags. These tags were either rapidly lost or grown over. A suitable transplanted fragment identification method needs to be developed, factoring in location-specific complications and previous attempts.

Conclusion

The used methodologies were successfully applied in a remote community, with the ocean-based culture method showing higher success. Improvements were determined and are also applicable at the research site. The cost-effective research methodology is dependent on if SCUBA equipment rental is required. If equipment rental is not required, the ocean cultivation methods is cheaper and removes the need for land-based facilities and frequent maintenance. If equipment rental is required and the recommended improvements can be implemented, the aquaria-based cultivation method is a cheaper alternative. The improved aquaria methodology has not been trialled and so the success of fragment growth is unknown. It is recommended that the method be trialled before any large-scale experiments are set up.

Although the used monitoring technique is time consuming, it can be used with minimal training and provides growth measures that are comparable with other literature. Less time-consuming methodologies, using 3D photogrammetry can be implemented at a minimal additional cost, reducing the time required for monitoring and potentially increasing accuracy.

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Appendix

Appendix A. Total Linear Extension (cm) of ocean fragments, showing Mother colony (A, B, C), Fragment size (2, 3, 5) and replicate number (1, 2, 3, 4) (n=36), per measurement period (week)

Colony	Size	Replicate	Week 0	2	4	6	8	10	12	14	16
A	2	1	4.764	7.744	6.689	3.9					
A	2	2	4.446	6.226	7.621	5.408	6.553	6.504	7.126	7.339	9.392
A	2	3	2.982	4.982	3.4	4.384	5.307	4.819	5.456	6.767	5.416
A	2	4	7.432	5.828	5.84	7.838	6.705	7.253	7.129	10.037	10.025
A	3	1	4.765	5.576	5.566	6.235	5.694				
A	3	2	6.039	6.819	8.806	11.202	13.147	13.336	14.507	18.034	18.578
A	3	3	3.515	5.729	4.444	6.534	7.907	7.599	9.71	11.18	12.088
A	3	4	2.969	3.403	3.137	3.602	4.978	5.592	5.829	8.336	11.171
A	5	1	7.433	7.169	11.284	11.645	14.76	18.576	20.27		
A	5	2	9.628	11.72	13.477	17.686	20.518	20.985	22.811	21.562	27.747
A	5	3	14.008	18.474	18.229	31.836	34.892	35.133	36.769	44.436	50.318
A	5	4	8.049	10.907	11.939	13.217	16.883	19.901	23.924	21.171	19.388
B	2	2	2.106	3.13	2.881	4.186	3.142	3.132	3.46	3.447	2.732
B	2	3	3.105	2.354	2.269	3.424	3.483	2.176	1.626	1.562	1.775
B	2	4	4.684	4.805	5.677	5.333	4.745	6.413	5.766	5.523	5.602
B	3	1	6.757	5.448	5.522	13.802	6.529	5.859	5.351	3.852	5.838
B	3	2	6.641	5.995	7.25	7.1	4.895	5.457	6.774	6.38	8.68
B	3	3	9.548	8.14	9.809	7.988	7.749	10.379	8.44	2.996	8.946
B	3	4	7.944	11.021	11.567	10.915	10.04	10.481	8.979	4.398	8.657
B	5	1	8.881	14.033	16.501	22.696	20.949	25.817	27.754	6.099	20.064
B	5	2	8.632	9.58	11.632	11.353	11.236	11.218	15.625	6.163	18.175
B	5	3	8.961	12.359	13.593	15.816	18.659	19.777	20.699	9.551	24.317
B	5	4	10.789	17.816	22.282	24.899	27.519	22.03	29.78	8.403	10.366

C	2	1	4.24	5.256	7.882	5.383	6.211	7.225	8.281	1.943	3.918
C	2	2	4.896	5.13	7.692	7.553	7.719	9.424	11.067	5.415	5.025
C	2	3	4.841	4.338	5.334	4.906	6.721	6.035	6.202	0.988	1.187
C	2	4	3.844	5.015	7.363	6.807	6.657	7.87	8.957	3.948	3.83
C	3	1	5.249	3.834	5.612	6.341	7.043	7.747	8.7	5.144	3.743
C	3	2	3.035	3.985	7.528	5.933	6.762	8.353	9.612	6.979	5.221
C	3	3	5.401	5.013	9.485	9.077	8.289	9.566	11.156	10.433	5.141
C	3	4	5.535	4.467	6.146	6.838	4.037	10.203	11.495	10.348	7.836
C	5	1	6.483	5.341	5.768	5.211	9.49	5.96	7.099	16.31	5.216
C	5	2	7.818	6.699	10.027	9.234	6.18	10.246	11.728	10.333	7.467
C	5	3	4.854	5.246	5.681	5.582	7.155	5.645	7.845	20.836	6.201
C	5	4	4.898	5.031	5.179	6.252	6.448	8.682	9.049	20.494	7.588

Appendix B. Total Linear Extension (cm) of aquaria fragments, showing Mother colony (A, B, C), Fragment size (2, 3, 5) and replicate number (1, 2, 3, 4) (n=36), per measurement period (week)

Colony	Size	Replicate	Week 0	2	4	6	8
A	2	1	2.064	2.092	2.079	2.063	
A	2	2	2.455	2.43	2.235	2.104	
A	2	3	2.62	2.39	2.344	2.463	
A	2	4	1.915	2.018	2.09	2.225	
A	3	1	12.684	14.28	14.713	11.373	
A	3	2	5.874	6.488	5.504	6.803	
A	3	3	6.456	9.168	7.864	6.683	
A	3	4	4.694	6.226	6.011	5.062	
A	5	1	9.618	9.981	8.286	10.311	
A	5	2	13.238	16.847	12.011	13.282	
A	5	3	10.942	13.548	11.626	11.322	14.942
A	5	4	11.637	13.352	12.146	11.269	10.645
B	2	1	3.298	2.97	2.88	2.965	
B	2	2	3.981	3.44	3.44		
B	2	3	2.178	1.96	1.96		
B	2	4	2.965	2.004	2.145	2.142	
B	3	1	4.696	4.973	4.813	4.362	
B	3	2	3.012	4.223	4.558	4.866	
B	3	3	4.908	4.504	4.992	4.369	
B	3	4	5.86	6.708	7.294	7.209	
B	5	1	14.166	14.813	15.219	13.197	
B	5	2	11.144	11.292	10.65	9.98	
B	5	3	7.241	7.63	8.255	7.856	
B	5	4	11.078	11.448	11.363	11.977	
C	2	1	2.271	2.99	2.39		
C	2	2	2.644	2.519	2.203		
C	2	3	2.309	2.371	2.418		

C	2	4	2.647	2.762	2.155
C	3	1	4.714	4.98	3.247
C	3	2	3.13	3.597	2.661
C	3	3	2.96	3.518	2.821
C	3	4	5.562	6.021	4.764
C	5	1	5.057	5.575	5.557
C	5	2	5.122	5.202	5.021
C	5	3	5.158	6.077	4.945
C	5	4	5.469	5.556	5.073

Appendix C. Relative growth (%) of ocean fragments, showing Mother colony (A, B, C), Fragment size (2, 3, 5) and replicate number (1, 2, 3, 4) (n=36), per measurement period (week)

Colony	Size	Replicate	Week 2	4	6	8	10	12	14	16
A	2	1	62.552	40.407	0	0	0	0	0	0
A	2	2	40.036	71.413	21.637	47.391	46.289	60.279	65.07	111.246
A	2	3	67.069	14.017	47.015	77.968	61.603	82.964	126.928	81.623
A	2	4	0	0	5.463	0	0	0	35.051	34.89
B	2	1	0	0	0	0	0	0	0	0
B	2	2	48.623	36.8	98.765	49.193	48.718	64.293	63.675	29.725
B	2	3	0	0	10.274	12.174	0	0	0	0
B	2	4	2.583	21.2	13.856	1.302	36.913	23.1	17.912	19.599
C	2	1	23.962	85.896	26.958	46.486	70.401	95.307	0	0
C	2	2	4.78	57.108	54.269	57.659	92.484	126.042	10.6	2.635
C	2	3	0	10.184	1.343	38.835	24.664	28.114	0	0
C	2	4	30.463	91.545	77.081	73.179	104.735	133.013	2.706	0
A	3	1	17.02	16.81	30.85	19.496	0	0	0	0
A	3	2	12.916	45.819	85.494	117.702	120.831	140.222	198.626	207.634
A	3	3	62.987	26.43	85.889	124.95	116.188	176.245	218.065	243.898
A	3	4	14.618	5.658	21.32	67.666	88.346	96.329	180.768	276.255
B	3	1	0	0	104.262	0	0	0	0	0
B	3	2	0	9.17	6.912	0	0	2.003	0	30.703
B	3	3	0	2.734	0	0	8.703	0	0	0
B	3	4	38.734	45.607	37.399	26.385	31.936	13.029	0	8.975
C	3	1	0	6.916	20.804	34.178	47.59	65.746	0	0
C	3	2	31.302	148.04	95.486	122.801	175.222	216.705	129.951	72.026
C	3	3	0	75.616	68.061	53.472	77.115	106.554	93.168	0
C	3	4	0	11.039	23.541	0	84.336	107.678	86.956	41.572
A	5	1	0	51.81	56.666	98.574	149.913	172.703	0	0
A	5	2	21.728	39.977	83.693	113.108	117.958	136.924	123.951	188.191

A	5	3	31.882	30.133	127.27	149.086	150.807	162.486	217.219	259.209
A	5	4	35.508	48.329	64.207	109.753	147.248	197.23	163.027	140.875
B	5	1	58.011	85.801	155.557	135.886	190.699	212.51	0	125.921
B	5	2	10.982	34.754	31.522	30.167	29.958	81.013	0	110.554
B	5	3	37.92	51.691	76.498	108.225	120.701	130.99	6.584	171.365
B	5	4	65.131	106.525	130.781	155.065	104.19	176.022	0	0
C	5	1	0	0	0	46.383	0	9.502	151.581	0
C	5	2	0	28.255	18.112	0	31.057	50.013	32.169	0
C	5	3	8.076	17.037	14.998	47.404	16.296	61.619	329.254	27.75
C	5	4	2.715	5.737	27.644	31.646	77.256	84.749	318.416	54.92

Appendix D. Relative growth (%) of aquaria fragments, showing Mother colony (A, B, C), Fragment size (2, 3, 5) and replicate number (1, 2, 3, 4) (n=36), per measurement period (week)

Colony	Size	Replicate	Week 2	4	6	8
A	2	1	1.357	0.727	0	0
A	2	2	0	0	0	0
A	2	3	0	0	0	0
A	2	4	5.379	9.138	16.188	0
B	2	1	0	0	0	0
B	2	2	0	0	0	0
B	2	3	0	0	0	0
B	2	4	0	0	0	0
C	2	1	31.66	5.24	0	0
C	2	2	0	0	0	0
C	2	3	2.685	4.721	0	0
C	2	4	4.345	0	0	0
A	3	1	12.583	15.997	0	0
A	3	2	10.453	0	15.815	0
A	3	3	42.007	21.809	3.516	0
A	3	4	32.637	28.057	7.84	0
B	3	1	5.899	2.491	0	0
B	3	2	40.206	51.328	61.554	0
B	3	3	0	1.711	0	0
B	3	4	14.471	24.471	23.02	0
C	3	1	5.643	0	0	0
C	3	2	14.92	0	0	0
C	3	3	18.851	0	0	0
C	3	4	8.252	0	0	0
A	5	1	3.774	0	7.205	0
A	5	2	27.262	0	0.332	0

A	5	3	23.816	6.251	3.473	36.556
A	5	4	14.737	4.374	0	0
B	5	1	4.567	7.433	0	0
B	5	2	1.328	0	0	0
B	5	3	5.372	14.004	8.493	0
B	5	4	3.34	2.573	8.115	0
C	5	1	10.243	9.887	0	0
C	5	2	1.562	0	0	0
C	5	3	17.817	0	0	0
C	5	4	1.591	0	0	0

*Appendix E. Ocean Fragment Notes, showing Mother colony (A, B, C), Fragment size (2, 3, 5) and replicate number (1, 2, 3, 4) (n=36), per measurement period (week). Fragments that moved during deployment, consequently exceeding ± 0.5cm of fragment size re indicated by *. F = branches fused together*

Colony	Size	Replicate	Week 2	4	6	8	10	12	14	16
A	2	1		Algal growth	Died - Algae					
A	2	2					1F to main			2F
A	2	3			1F to substrate	2F	1F to substrate	1F to substrate		
A	2	4			Algae growth		2F	1F to substrate		
A	3	1*			Bottom 1.7cm algae	Died - Algae				
A	3	2								
A	3	3*					2F		2F	
A	3	4								
A	5	1						Algae on fragment base	3.1 cm Algae	Died - Algae
A	5	2							Slight Grazing	
A	5	3								
A	5	4*							Slight Grazing	
B	2	1*						0.7cm bleached	All F to main	
B	2	2								
B	2	3		2F		2F		all branches F		
B	2	4*			2F	2F	2F	2F		
B	3	1								
B	3	2		2F			2F			
B	3	3	2F		2F	2F to main				

B	3	4		2F	2F		3F	3F to main	3 F to main	
B	5	1								
B	5	2	2F			2F to main				2F
B	5	3					2F			
B	5	4						2F		
C	2	1							Grazing	
C	2	2							Grazing	
C	2	3							Grazing	
C	2	4							Grazing	
C	3	1							Grazing	
C	3	2*								
C	3	3				1 branch broken		end polyp grazed		
C	3	4								
C	5	1*							Grazing	
C	5	2*							Grazing	
C	5	3							Grazing	
C	5	4							Grazing	

*Appendix F. Ocean Fragment Notes, showing Mother colony (A, B, C), Fragment size (2, 3, 5) and replicate number (1, 2, 3, 4) (n=36), per measurement period (week). With * indicating the occurrence of an algae bloom.*

Colony	Size	Replicate	Week 0	2	4*	6	8	10
A	2	1			Died			
A	2	2				Died		
A	2	3				Died		
A	2	4				Died		
A	3	1				Died		
A	3	2				Died		
A	3	3				Died		
A	3	4				Died		
A	5	1				Died		
A	5	2				Died		
A	5	3					Died	
A	5	4					Died	
B	2	1	Bleached 3/6		Died			
B	2	2		Died				
B	2	3	Bleached 3/6	Died				
B	2	4				Died		
B	3	1				Died		
B	3	2				Died		
B	3	3				Died		
B	3	4				Died		
B	5	1				Died		
B	5	2				Died		
B	5	3				Died		
B	5	4				Died		
C	2	1	bleached 6/6	Died				
C	2	2	bleached 6/6	Died				

C	2	3	bleached 6/6	Died	
C	2	4	bleached 6/6	Died	
C	3	1	bleached 6/6	Died	
C	3	2	bleached 6/6	Died	
C	3	3			Died
C	3	4	bleached 6/6	Died	
C	5	1			Died
C	5	2			Died
C	5	3			Died
C	5	4			Died