Universidade de Aveiro
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Vantagens da microfragmentação no cultivo do coral *Montipora capricornis*

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Marinha Aplicada, realizada sob a orientação científica do Doutor Rui Jorge Miranda Rocha, Diretor de Operações, de Inovação e Desenvolvimento da Riasearch; Professor Auxiliar convidado do Departamento de Biologia e membro do CESAM, Universidade de Aveiro; e da co-orientadora Doutora Andreia do Carmo Martins Rodrigues, Investigadora em Pós-Doutoramento do Departamento de Biologia e CESAM da Universidade de Aveiro.

Este trabalho é dedicado aos meus pais, pois sem eles nada disto seria possível.

o júri

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agradecimentos

São muitos aqueles que me acompanharam ao longo deste percurso e tornaram possível alcançar este momento. Nada disto seria possível sem a força de todos eles. Em primeiro lugar tenho que agradecer à minha família, o meu pai, a minha mãe, o meu irmão, por sempre acreditarem em mim e por estarem sempre presentes com o seu apoio incondicional. Aos meus orientadores, o Doutor Rui Rocha e a Doutora Andreia Rodrigues, obrigado por me apontarem no caminho certo com todo e por todo o conhecimento e experiência que adquiri com eles. Ao Davide, pela disponibilidade e paciência incalculáveis, e por estar sempre lá para me empurrar para a frente apesar dos obstáculos. À Ana Costa, por todas as dores de cabeça que teve comigo e por tudo aquilo que me ensinou no laboratório e na manutenção dos aquários. À Andreia e ao Agostinho, a melhor equipa de trabalho que alguma vez tive o prazer de pertencer. Agradeço-lhes todas as horas de limpeza aquários, de montagem de sistemas, as asneiras, as frustrações, as noitadas, as discussões e as gargalhadas. À Sílvia e à Vitória, por toda a ajuda que me deram no laboratório. Aos meus amigos, por todas dicas e conselhos e por ser com estas pessoas fantásticas que eu vivo os melhores momentos. Por fim, à Mariana, por fazer levantar todas as dúvidas, por caminhar sempre ao meu lado e por podermos olharmos para a frente juntos.

A todos um Muito Obrigado!

palavras-chave Corais, aquacultura, microfragmentação, fotobiologia, crescimento

resumo

Os recifes de coral são dos ecossistemas marinhos com maior riqueza de recursos e de uma importância vital para diversos organismos, incluindo o Homem. Deles é possível tirar partido de vários recursos, em particular para as indústrias da Pesca e Farmacêutica. Hoje em dia a maioria dos recifes de coral encontra-se em declínio devido à exploração excessiva dos seus recursos, e ao aumento dos níveis de poluição de origem antropogénica a nível global. Para combater este declínio, o aumento do conhecimento científico sobre os corais e as suas interações com o ambiente que se inserem é essencial. A Aquacultura de corais permite assim que melhor se consiga observar e estudar estes organismos, bem como a encontrar soluções que possibilitem que os mesmos possam resistir e prosperar às atuais condições de adversidade. Com este trabalho, pretendeu-se estudar o método de reprodução assexuada da espécie de coral escleratinoso *Montipora capricornis* através da fragmentação de uma colónia mãe. Tal foi realizado com recurso a diferentes utensílios de corte e também à utilização da técnica de microfragmentação. Para tal, foram executados três ensaios experimentais. No primeiro testou-se a utilização de uma ferramenta de corte elétrica contra uma ferramenta de corte convencional, onde não foram detetadas diferenças significativas em nenhum dos parâmetros avaliados. No segundo e terceiro ensaios, cada um deles focou-se no uso exclusivo de uma das ferramentas de corte usadas anteriormente para comparar o crescimento de microfragmentos contra fragmentos de tamanho maior, e em ambos os ensaios onde foram encontradas diferenças significativas no crescimento absoluto da área dos fragmentos de tamanho maior. No entanto é necessária mais investigação sobre as técnicas de cultura ex-situ apropriadas e avaliação das condições de stress da espécie *M. capricornis* para a obtenção de conclusões definitivas.

keywords Corals, aquaculture, microfragmentation, photobiology, growth

abstract

Coral reefs are among the marine ecosystems with bigger richness of resources and are of vital importance to several organisms, including Mankind. From them it's possible to benefit from various resources, in particular for the Fishing and Pharmaceutical Industries. Nowadays, the majority of coral reefs is suffering a decline due to excessive resource gathering and the rising of anthropogenic pollution's levels at a global scale. To fight such decline, it is essential we increase scientific knowledge about corals and how they interact with their environment. Therefore, coral's Aquaculture allows us to better observe and study these organisms, as well as finding solutions that make possible for them to better resist and prosper the present conditions of adversity. This work wants to study the method of asexual reproduction of the scleractinian coral *Montipora capricornis* through the fragmentation of a parent colony. This was done through the use of different cutting tools and also use of the microfragmentation technique. For this, three experimental essays were performed. The first tested the use of an electric cutting tool against a conventional cutting tool, in which no significant differences were detected on any of the observed parameters. In the second and third essays, each one of them focused on the exclusive handling of one of the cutting tools previously used in order to compare the growth of microfragments against fragments with a bigger size. Both essays found significant differences on the absolute growth of the bigger sized fragments. However, more research is necessary about the proper culture conditions ex-situ and the evaluation of stress conditions of the *M. capricornis* in order to obtain definitive conclusions.

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1. Introduction

1.1. Climate change and its consequences

Our planet has suffered many phenomena of climate change throughout the Ages. These oscillations have occurred in both intervals of millions of years as well as short intervals of tens of years. Earth's indications of climate change show that levels of Ultraviolet-B and CO_2 concentrations have been much higher than today's concentration values (Hallegraeff, 2010).

Global temperatures' regulation is vastly influenced by the ocean and its effects. The ocean is capable of absorbing 93% of the heat that is trapped by greenhouse effects and is also capable of absorbing around 25% to 30% of CO_2 emissions that are caused by human activity and that would have remained in the atmosphere, increasing global warming. Besides all these factors, the ocean can produce 50% of the oxygen available in our planet because of photosynthetic organisms like marine plants and algae (Hoegh-Guldberg et al., 2019).

Nonetheless, the ocean's capacity to aid in these important services is in jeopardy (Hoegh-Guldberg et al., 2019). Waters' temperature and acidification, caused by the excess of CO_2 dissolving into ocean waters, are interfering with marine ecosystems and disturbing services such as food availability, subsistence, and safe coastal habitation which billions of people are dependent of. (Hoegh-Guldberg et al., 2019; IPCC 2014, 2018, 2019).

Climate change impacts the biophysical environment that will eventually affect and bring consequences to other human activities, but these impacts appear to be so far away in time that they are often ignored when establishing management plans. However, these impacts can already be observed in our planets' oceans and seem to become more evident as time passes on. Over the last century, concentration of atmospheric CO_2 as seen an increase of 40% and this number is expected to keep rising, causing even more stress on marine systems by means of ocean warming and acidification (Ruckelshaus et al., 2013). Abnormalities in global temperatures and sea levels worldwide are becoming commonly and more rapidly observed and predicted (Ruckelshaus et al., 2013). These abnormalities are most troubling as they will cause a series of additional challenges for species'

environmental resilience and ocean productivity, destabilizing ecological interactions as well as coastal human communities (Ruckelshaus et al., 2013). Emissions of greenhouse gases in the past have ensured changes in the planet's climate in one way or another, despite any measure taken now or in the near future to help alleviate impacts of the past reckless human activity (Ruckelshaus et al., 2013).

Corals are stenothermic and calcifying organisms, which makes them especially delicate organisms regarding water pH and temperature variations. Studies show that carbon released into the oceans due to human activities and pollution, which results in an increase of acidity and temperature levels, is the cause for disturbances found in coral growth and metabolism causing the decay of coral reefs worldwide (Wild et al., 2011).

1.1.1 Ocean Acidification and coral calcification

The rise of oceans' acidity levels brings various repercussions to scleractinian corals as reef-builders, directly affecting their function as ecosystem engineers. Ocean acidification results from the absorption by oceanic water of the CO_2 released by human activity worldwide (Wild et al., 2011). This absorption raises CO_2 partial pressure (pCO_2) in the water column, lowers pH, raises the total dissolved CO_2 ([CO_2] and [HCO_3]) concentrations, and lowers [CO_3^{2-}] concentrations in saltwater (Wild et al., 2011). These alterations to oceans' chemistry can provoke a reaction from coral's physiological processes (Wild et al., 2011).

Maintaining seawater calcium concentration [Ca^{2+}] at constant value, a drop in [CO_3^{2-}] leads to a decline of the saturation state of aragonite (Ω_{arag}), the $CaCO_3$ polymorph generated by coral calcification. Tropical surface waters are roughly 4-fold supersaturated regarding aragonite (Hoegh-Guldberg et al. 2007). However, if the effect of ocean acidification keeps on going, these saturation values are expected to decrease (Wild et al., 2011). For effective aragonite accretion, scleractinian corals need saltwater that is super-saturated in aragonite. Reduced external Ω_{arag} in acidified saltwater prevents the necessary growth of Ω_{arag} within the internal calcifying fluid, resulting in a reduction in calcification rate (Wild et al., 2011). This loss in skeletal growth performance, which is driven by ocean acidification,

immediately leads to a decrease in scleractinian corals' engineering ability to build important reef ecosystems (Wild et al., 2011).

Another risk that comes with ocean acidification and that can gravely affect coral reefs' survival is the inhibition of sexual reproduction. This may happen by affecting sperm movement (Morita et al., 2009) or the development and growth of corals after settlement, which suggests serious disturbances in coral recruitment and competitiveness against other coral species. This unbalance can cause changes to the reef and the community's framework (Wild et al., 2011).

Even more worrying is that ocean acidification is considered the agent for coral bleaching, bringing even more destructive repercussions for coral reefs and the scleractinian corals that give them shape (Wild et al., 2011).

1.1.2. Ocean warming and coral bleaching

The magnitude and rate of physical and chemical changes in the ocean are generating a wide variety of general reactions in marine species, ecosystems, and regions. The fact that fairly minor levels of change have resulted in rather major biological consequences, with clear evidence of non-linear trends, tipping points, and other generally complicated reactions, is also noteworthy. The implications of a fast-changing ocean for species, ecosystems, and dependent communities are exemplified by coral reactions to changes in ocean conditions, notably mass coral bleaching (Hoegh-Guldberg et al., 2017).

The warm-water coral symbiosis with *Symbiodinium* is extremely sensitive to physical and chemical changes in the environment around corals. Short periods of extreme heat or cold, different periods of light incidence, as well as exposure to poisons like cyanide, can cause the impairment of the symbiosis and subsequently the loss of brown symbionts and a paling (thus "bleaching") of the coral host. The symbiosis between Scleractinian corals and *Symbiodinium* is broken during coral bleaching, although it can return if the circumstances are not too abnormal for too long. While coral tissue bleaching has been documented for at least a century, reports of bleaching at broad geographic scales were unknown in the scientific literature until 1979. However, from 1980 onwards, mass coral bleaching has

afflicted whole reefs and areas, frequently resulting in severe reef-building coral losses. The lack of scientific reports prior to 1979, as well as the close relationship between bleaching and rising sea temperatures, and also the performance of extensive laboratory and mesocosm studies, greatly backup the conclusion that mass coral bleaching and mortality are novel and caused by the exposure of warm water coral-reefs to rising sea temperatures (Hoegh-Guldberg et al., 2017).

Small (1–2°C) sea surface temperature (SST) rises over a region's long-term summer maximum can cause mass coral bleaching and death (Strong et al., 2011). The quantity of coral bleaching will grow as temperatures rise over longer periods of time, resulting in higher mortality. The amplitude and duration of temperature extremes are strongly linked to mass coral bleaching and death. These associations are used in conjunction with satellite data to determine SST anomalies in order to track the frequency and severity of mass coral bleaching and death (Strong et al., 2011). As a result, there is a large degree of certainty that the rise in mass coral bleaching and death since the early 1980s is attributable to human climate change, specifically ocean warming. Loss of symbionts from coral tissues can cause hunger, illness, reproductive failure, and a lack of competitive capacity in comparison to other species on coral reefs due to a loss of photosynthetic energy (Hoegh-Guldberg et al., 2017).

1.2. Corals

Corals are marine organisms within the phylum Cnidaria and are divided by two different classes. One of which is the Hydrozoa class, whose members are corals with large and easily breakable skeletons. The other is the class Anthozoa, containing the subclasses Octocorallia and Hexacorallia. Soft corals in general are considered octocorals as they lack the ability to produce hard skeletons and therefore can’t grow to large sizes. Besides those, gorgonian sea fans, with forms and shapes resembling trees or bushes, with flexible internal skeletons, are also included as octocorals. Lastly, Antipatharia, or "black corals," which form a tree- or stick-like structure decorated with knobs or spines, and Scleractinia, which produce the hard, calcium-based reefs most often associated with corals, and are two types of hexacorals (Roberts and Hirshfield, 2004).

The Scleractinia order is known to include polypoidal marine invertebrates inhabiting warm waters of abundant luminosity and to have the unique ability to create the frame foundation that becomes a coral reef. This ability to create and build reefs comes from the fact that scleractinian corals are able to extract from the water and then rapidly produce hard calcium carbonate ($CaCO_3$) skeletons (Stanley and Hardie, 1999). These aggregations of exoskeletons are then fused together by action of crustose coralline algae, thus laying the foundation for the edification of complex structures known as coral reefs. (Stanley, 2003; Harrison, 2011).

These reef-building organisms are also known to share a symbiotic link with zooxanthellae, unicellular dinoflagellate algae, which aggregate in large quantities in the endodermal tissues of the coral host. These photosymbionts, classified as the genus *Symbiodinium*, give corals a big advantage in relation to other heterotrophic animals, as they provide their hosts with bigger energy reserves by producing carbohydrates and lipids which are then transferred to the host's cells. These high levels of available energy provided by the zooxanthellae, besides being a great advantage for corals in ensuring their survival in more nutrient-deprived environments, are also what stimulates calcification and skeletal growth (Stanley, 2003).

Even though scleractinian corals are distributed worldwide, their presence is particularly considerable in more shallow and tropical waters. This is because access to sunlight is critical to fuel the endosymbiotic relationship shared with dinoflagellate organisms, known as zooxanthellae. This relationship provides corals with the energy required for fast calcification and therefore growth of the reef-building coral structures. These reef-building corals can also be classified as "hermatypic" corals and usually contain in their structures millions of zooxanthellae; other corals that lack this ability of fast calcification and growth, and therefore can't provide a significant contribution to reefs' formations are classified as "ahermatypic" corals which contrast greatly with hermatypic corals as ahermatypic corals have a very reduced count of zooxanthellae (Harrison, 2011).

Limitations in exploration and lack of research of deeper environments don't allow scientists to know the exact number of existing scleractinian species and their global richness overall. This coupled with the fact that some species present such variable

features that it becomes hard to establish a reliable taxonomic classification, and there is also the possibility of hybridization between some species which makes it even more difficult to determine the level of richness of these organisms (Harrison, 2011).

1.2.1. Life Cycle and Reproduction

Corals' life cycle consists of two phases, a polyp stage which is the more prevalent and when the organisms have a more sedentary lifestyle in the benthic zone, and the planula larval stage with a briefer duration where the organisms are dispersed in the pelagic zone. During the polyp stage, corals are found in their predominant form, with large tissue and skeletal growth resulting in the forms that will then serve as foundations for the coral reefs' structure. During this stage, corals also have the capacity of reproducing asexually, as well as sexually through the production of gametes which will then fertilize each other and lead to the larval stage – the planula – becoming planktonic and developing some capacity of dispersion. During this larval stage, the planula will try to find some space on the substrate to attach itself and metamorphize into the juvenile polyp, hence beginning the formation and growth of the calcium carbonate exoskeleton. Eventually, the new coral grows to become sexually reproductive, thus completing the organism's life cycle (Harrison, 2011).

In face of harsh or limiting environmental conditions, asexual reproduction ensures the creation of genotypic equivalent descendants in order to extend the survival of said genotype. On the other hand, and when exposed to favourable environmental factors, corals can shift their strategy towards sexual reproduction, resulting in recombination of genes and emergence of new genotypes that may introduce advantages in prospering the inhabited environment and outfit the species with better chances of survival. There are four different strategies of sexual reproduction that can be used by corals, which are: hermaphroditic broadcast spawners, hermaphroditic brooders, gonochoric broadcast spawners, or gonochoric brooders. Hermaphroditic corals are able to produce both male and female gametes within the polyps, in contrast with gonochoric corals which can only produce one

gender within the colony. Besides this, corals can also either disperse their gametes to the water column which classifies them as broadcast spawners, or have fecundation and planula gestation happen within polyp cells which classifies them as brothers. Despite this, classifications regarding corals' sexual reproductive strategy shouldn't be so readily made, as some species may adopt more than one method and become simultaneously hermaphroditic and gonochoric, or broadcast spawner and brooder, within the same colony (Harrison, 2011).

1.2.2. Asexual Reproduction

There are several methods of asexual reproduction that can be differentiated. Coral colonies develop through asexual budding, whereas clones of the same colony can emerge through other asexual strategies. The majority of hermatypic corals and a few ahermatypic can grow and form colonies through budding of polyps. The new developed polyps may emerge through growth and cellular division of already existing polyps or by growing through tissue gaps between already formed polyps. In this way the colony keeps renewing itself and growing larger by secreting more calcium carbonate skeleton which is then populated by the new budding polyps (Harrison, 2011).

This formation of coral colonies and subsequent growth of their structural exoskeleton and biomass increase brings a great advantage to corals, as it enables them to grow to proportions that wouldn't be possible if single polyps were to develop individually, outside of a colony structure. In this way coral colonies are able to prosper by rising above the benthic zone, gaining access to a large number of resources. This method also guaranties the subsistence of the colony as a whole in case of disease or death of some of its parts. The bigger intake of resources by the colony structure also favours sexual reproduction because the larger and older the colony becomes the more gametes it is able to produce and therefore ensure genetic dominance within the population (Harrison, 2011).

Besides all the advantages of asexual reproduction for single colony growth, corals are also able to divide asexually and generate clones of the parent colony they separated from. They are able to do so through events like separation of polyps, colony cleavage or fragmentation which may be caused by environmental

phenomena such as strong current and waves, storms, or through the action of other living organisms. Another way of asexual colony division is the development of asexually brooded planulae which can occur when there is an impediment to sexual reproduction (Harrison, 2011).

In this way, a considerable portion of a coral reef's space may be occupied by identical colony clones which can also become extensively distributed. Depending on the population or coral species in question, reproductive strategies can differ largely in their relevance of sexual or asexual methods of distribution. This goes to show the high level of adaptability shown by these organisms and the wide array of strategies available to them in order to have the most success in their survival (Harrison, 2011).

1.2.3. Aquaculture of corals

According to FAO (2014), aquaculture industry has become the food production activity with the most accentuated growth of 2012. Besides food production, aquaculture also brings other considerable benefits and interests to other anthropogenic activities. Some of those benefits include production of live feeds for animal consumption, biofuel, conservation and repopulation of damaged ecosystems, assistance to pharmaceutical research, cosmetics, nutritional and additive products, to name a few. In this way, the aquaculture industry brings new commercial importance to various aquatic resources that would have been previously considered of no value (Leal et al., 2018).

Corals have become a product that keeps becoming more and more prominent in the aquaculture market. Supply and demand keep increasing, even though a lot of it is still collected from natural habitats. Among the most favoured and with higher request are scleractinian corals, although some soft corals are not far behind. There have been many techniques for cultivating corals, many varying between onshore and offshore methods. Onshore aquaculture of corals is more technical, requiring the assistance of recirculating systems to maintain the animals. As for the offshore aquaculture, it requires a lot less intervention and investment as corals will remain exposed to the natural factors of the environment. This method is also known as mariculture and although costs are lower, the risks are higher as the corals are

subject to the conditions of the environment and can be affected by both biotic and abiotic stressors. One way of alleviating this is by suspending the produced corals in the middle of the water column, which improves access to water flow, reduces sediment build-up and lowers de risk of contact with predators (Leal et al., 2018).

On the other hand, albeit being costlier, ex-situ coral aquaculture allows for more precise control of biotic and abiotic factors like light exposure, water temperature, pH, salinity, etc. According to Osinga et al. (2011), corals even show improved healing properties after being fragmented on ex-situ conditions. Despite these advantages in ex-situ aquaculture, it should be stated that such a rigorous control of these environmental conditions may, in fact, introduce some variables, like the relation of the coral with microorganisms, that aren't as prevalent or don't even exist for corals cultivated on in-situ conditions (Leal et al., 2013; Osinga et al., 2011).

1.3. Objectives

This work will focus entirely on the maintenance and growth assessment of fragments of the hermatypic hard coral *Montipora capricornis* (Veron, 1985). *M. capricornis* is a stone coral of small polyps. The corals come in a vast variety of forms and colours, namely uniform purple, blue, or brown. Colonies are flat plates in tiers or whorls, sometimes it can be found forming columns, other times it forms encrusting or irregularly contorted laminae. Corallites are immersed. There are no tuberculae or papillae. The coenosteum is coarse in appearance. This species can be found in shallow, tropical reef environments, mostly in lagoons up to 20 metres in depth. Its distribution is commonly centered around waters of the Indo-Pacific region (Veron, 1985; Veron, 2000).

The main objective of this dissertation is to determine whether the size of the fragments of *M. capricornis* is determinant in asexual reproduction and growth of the corals ex-situ, as well as determine if microfragmentation hastens the growth of those fragments. Simultaneously, this work will assess the effect that different cutting tools / types of cut will have after fragmentation of *M. capricornis*.

2. Methodology

2.1. Recirculated System

For the entire duration of the experiments, the conditions for maintaining the coral fragments were strictly identical. The recirculated system used was composed of two tanks with 240 litres of capacity, each measuring 150cm (w) by 40cm (l) by 40cm (h); a filtration sump with 180 litres of capacity, measuring 80cm (w) by 45cm (l) by 50cm (h); and a tank for reversed osmosis (RO) water with 54 litres of capacity, measuring 30cm (w) by 30cm (l) by 60cm (h).

Water movement inside the aquariums was ensured by two pumps (Turbelle nanostream 6055, TUNZE - Germany) in each tank, one on each side. Lighting was provided by a T5 fluorescent light (Sea REEF-SPEC, 80W) placed above each aquarium, with one Red light and one Actinic light.

Water filtration occurs in the SUMP and is composed of five processes, (i) a skimmer (Deltec SC 500) which removes dissolved organic compounds (DOC) present in the water; (ii) activated carbon which adsorbs a number of dissolved contaminants that can degrade water quality; (iii) bio-balls which contain beneficial bacterium that are used in biological filtration; (iv) a Kalkwasser reactor (Kalkwassermischer km 500) used to keep water's pH value from ranging too much and maintain carbonate hardness; this component is also connected to the (v) RO water replacement system. Whenever evaporation occurs in the system, the decrease in the water level activates a sensor which then activates a pump (Deltec aquastat 1001) that will replace the evaporated water by new RO water which will ensure the system's salinity remains constant at 35 ppt.

This recirculated water system has the water performing cycle after cycle of circulation between the water tanks and the sump. The water descending from the tanks drops in one end of the SUMP where it passes by the skimmer and two thermostats (Eheim Jagger 300W) used to maintain water temperature. After that, the water passes by the activated carbon, gravel bag and bio-balls filters. It is then pumped (using a EHEIM universal 1200 L/min) into an Ultraviolet (UV) filtration device (Vecton 600, TMC), which will provide a sterilization process to the water. Finally, the water in the UV filter is directed to the chiller (Hailea Model: HC-500A),

which is set to regulate water temperature to 25°C before being set back to the sump where a pump (EHEIM universal 2400 L/min) directs the water back to the aquarium tanks.

Water exchanges are made regularly to the system and excess of algal material is scrubbed off and discarded during the water exchange. Saltwater is produced by adding synthetic salt (Coral PRO salt, Red Sea) to RO water (V2 Pure 360, TMC) which is freshwater that passes by a reversed osmosis filtration system.

2.2. The Coral Species and Fragmentation

This study evaluates the growth and development of the coral species *Montipora capricornis* after being subjected to various techniques of fragmentation. The parent colony was being kept at the ECOMARE facilities (CESAM, University of Aveiro), where it was already acclimatized to the aquariums' conditions. This facility was also the main location where the tests with the coral *M. capricornis* took place. Parameters remained stable throughout the experiment with salinity at 35 ± 1 ppt; temperature at 25 ± 1 ºC; PAR at 40 – 70 µmol m^{-2} s^{-1} (Apogee MQ-500 PAR Meter); phosphates, nitrites, and ammonia at approximately 0 ppm; and a 12:12 (12 hours light | 12 hours dark) photoperiod was used.

Figure 1 – Parent colony of *M. capricornis* used to extract all coral material required for fragmentation. ECOMARE, CESAM, University of Aveiro

Previous preparation for the experiment required the manufacture of several cement bases where the coral fragments would be fixated afterwards. These bases were prepared with using a 2:1 portion of aragonite and cement, mixed in water, and poured in small plastic cups in order to provide them their round shape. After leaving them to dry, the bases were then placed in running RO water and left there for two weeks to wash any present chemicals, and then they were left to dry once more. This type of base was chosen due to its porosity which can be beneficial for filtration purposes and also due to being able to mould in order to meet various specific requirements.

For all series of tests and fragmentation process, a few large pieces of coral were broken off the parent colony using hand applied force. Then the broken pieces were fragmented into various fragments of similar sizes. For half of those it was used a Einhell Grinding and Engraving tool (TC-MG 135 E) with a small hard disk blade attached to the tool in order to be able to cut through the hard skeleton of the animals. As for the other half, a scalpel was found not to be the ideal tool for fragmenting *M.* capricornis, as it would sometimes break the blade or be hard to cut through the coral's tissues and skeleton. So instead, a common mezzaluna kitchen knife was used as it had an ideal weight, blade length and thickness to perform the intended incisions. For both tools, the edges of the coral fragments were sliced off, leaving a square shaped fragment fixed at the centre of the cement base with an area ranging between 1,5–4,0 cm^2. Every coral fragment was then glued to a cement base using a super-glue gel (chemical base: Ethyl Cyanoacrylate) obtainable at any local drug store or super-market.

The first question that needed answering was whether the cutting tool used in the fragmentation would affect in any way the coral's health and growth rate. So, by comparing the cuts of both tools, we intend to learn if different types of cuts would result in different growth rates of the coral species and if regeneration of the coral tissues would be affected depending on the type of cut and tool that is being used for fragmentation. For this series of tests, 28 fragments were made using the electrical cutting tool (n = 28) and another 28 fragments using the mezzaluna blade (n = 28). Photos were taken to each base with fragments glued to it at the beginning

and end of the test time, and its areas were measured using the software program ImageJ 1.52a (Wayne Rasband National Institutes of Health, USA).

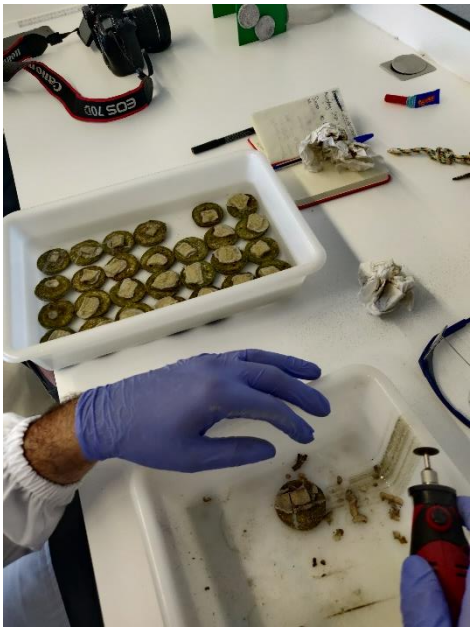

Figure 2 – Fragmentation process of *M. capricornis* using the Einhell Grinding and Engraving tool

The second series of tests involved the exclusive use of the Einhell Grinding and Engraving tool. For one test, fragments of 3–5 cm^2 each would serve as a means of comparison for the growth rates between normal sized fragments and microfragments. The other test would see individual microfragments (0,5–1,0 $cm^{2)}$ glued to separate cement bases in order to assess their growth rate in comparison to the previous test. For this series of tests, 73 regular sized fragments were made (n = 73), compared against 24 microfragments (n = 24). All the tests described in this second series had an elapsed time of two months. Photos were taken to each sample for area measurement at the beginning and end of the experiment, which had a duration of two months.

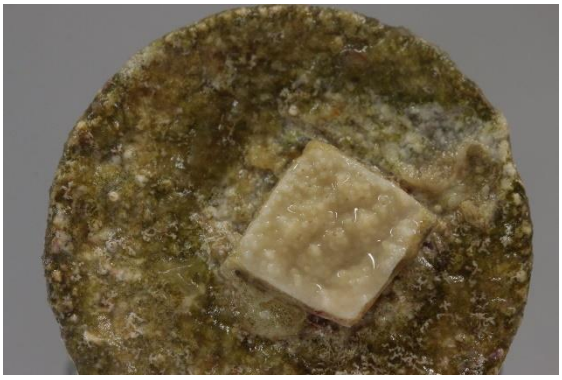

Figure 3 – Coral fragment of *M. capricornis* glued to a cement base with the Ethyl Cyanoacrylate super-glue gel

Lastly, a final test was made where fragmentation was made solely with a non-electronic blade. For that the mezzaluna kitchen knife was once again required. Fragments of regular size ranging between approximately 3–6 cm^2 were cut and glued to a cement base. At the same time, a same amount of microfragments of 0,5–1,5 cm^2 of area was also cut so that growth ratios could be compared against the regular sized fragments. For this series of tests, 30 regular sized fragments were made (n = 30), compared against 30 microfragments (n = 30). Like the previous tests, this one had a duration of two months and photos were taken to each fragment and microfragment, both at the beginning and ending of the test time and each fragment's surface area was measured.

2.3. Photobiology Analysis

Every test previously described was also accompanied by a measurement of the photosynthetic activity of zooxanthellae, and for that a Pulse-Amplitude-Modulated (PAM) fluorometer (Junior-PAM, Walz TM, Germany) was used. Measurements were taken from every fragment of every test and this was done both at the start and ending of any test time. Measurements were also taken from the parent colony before any extraction of coral body from it.

The used PAM fluorometer is characterized as a non-invasive method, of easy handling (Glud et al., 2002) and for being able to detect stress levels indirectly (Dove and Hoegh-Guldberg, 2011). The modulate and the saturated light pulses were transmitted by a 1,5 mm plastic optical fibre, pointing at the coral perpendicularly to its surface.

There are three pathways that light energy may follow when reaching photosynthetic organisms: (i) the photochemical quenching pathway, when light is used during photosynthesis, and occurs in the reaction centre of the photosystem II (PSII) through photochemical reactions; (ii) the non-photochemical quenching pathway, where energy is converted to heat and is dissipated; and (iii) fluorescence caused by the radioactive decay of the energy. The fluorometry method gives information about how much light energy is used in the first two described pathways by measuring the light energy detected through the third pathway (reviewed by William et al., 2001).

The photochemical pathway to absorb light is at its maximum when the samples are left during a certain amount of time in total darkness, causing the little amount of light energy emitted by the measuring pulse of the PAM fluorometer to be almost totally absorbed by the reaction centre of PSII, resulting in minimal fluorescence (F_0). Meanwhile, an intense pulse of light will result in saturation of the PSII reaction centre, thus resulting in maximized levels of fluorescence (F_M). The samples must be in total darkness for a long enough period in order to make sure that the PSII reaction centre be completely open and there is minimal competitiveness between the photochemical pathways (William et al., 2001). Because of this, all samples from the tests performed were left in darkness for at least 15 minutes before any measurement of photosynthetic activity.

Using the PC software WinControl V3.29 it was possible to measure F_0, a parameter associated with the quantity of chlorophyl α (Serôdio et al., 2001), and F_M. Using these two values it is possible to calculate the fluorescence yield ratio shown in equation 1, where F_V corresponds to the variable fluorescence. The potential photochemical capacity of the PSII in algae is demonstrated by this ratio (William et al., 2001).

$$\frac{F_V}{F_M} = \frac{(F_M - F_0)}{F_M}$$

2.4. Statistical analysis

The statistical differences between groups' continuous variables in each test were calculated by a Welch two sample t-test. This tests for the null hypothesis that the true difference in means is not equal to 0. The difference in mortality was

calculated by a Pearson's χ^2 test with Yates' continuity correction. Analyses were run in *R* software version 4.0 (R Core Team, 2020). Figures were built with the package *ggplot2* (Wickham, 2016). Groups with significance $\leq$ 0.05 were considered statistically different.

3. Results

For the first test, comparing the response of fragments to the cut of the electric tool or the mezzaluna blade, no differences were found. Absolute area growth (Δ Area) was similar [0,238 (SD = 0,340) cm^2 for electric tool; 0,236 (SD = 0,898) cm^2 for mezzaluna; $t_{(34.80)}$=0.009, p=0.993], as well as the specific growth rate (SGR) [0,0011 (SD = 0,0024) for electric tool; 0,0004 (SD = 0,0040) for mezzaluna; $t_{(44.64)}$=0.828, p=0.412] and F_v/F_m, [0,553 (SD = 0,015) for electric tool; 0,550 (SD = 0,020) for mezzaluna; $t_{(50.42)}$=0.577, p=0.566] (Fig.1). No mortality was registered in this test.

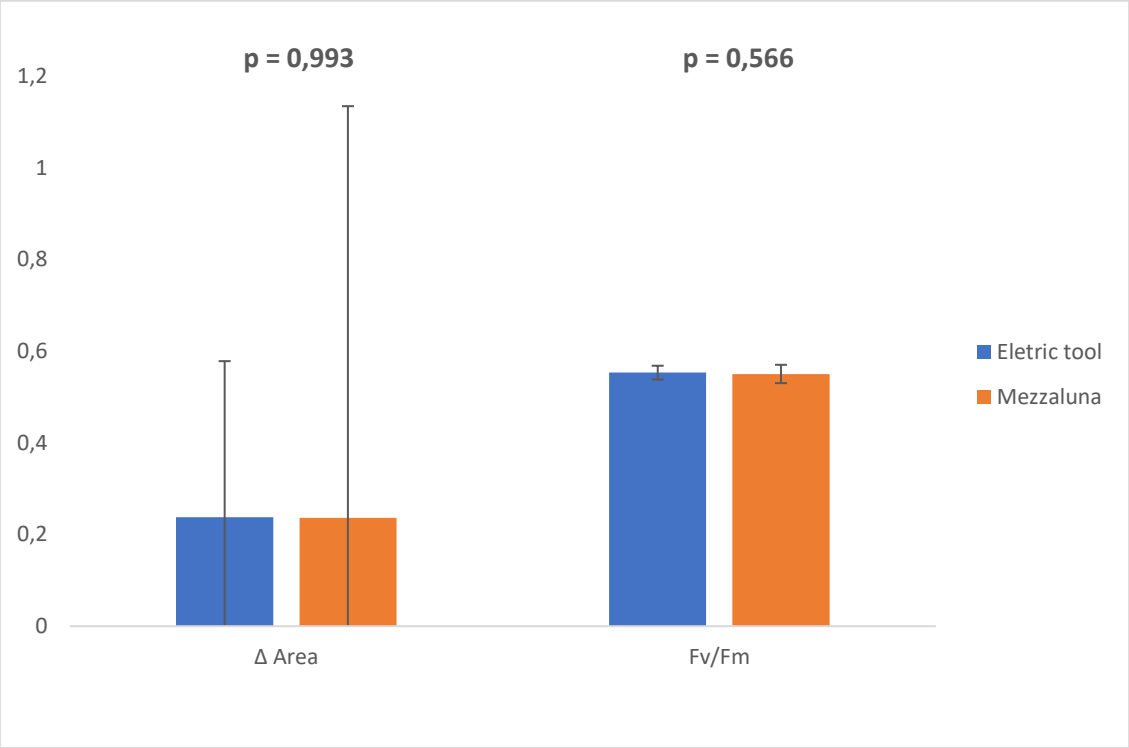

Figure 4 – Barplot containing the absolute area growth (Δ Area) and maximum photosynthetic efficiency (Fv/Fm) for fragments cut with electric tool and mezzaluna blade, showing standard deviation lines. The p-value for the t-test is shown above each variable.

When testing for the size of fragment, two different essays were performed, one with electric tool and another with mezzaluna blade. The test with electric tool showed differences in Δ Area [0,691 (SD = 1,125) cm^2 for regular fragment; 0,203 (SD = 0,343) cm^2 for microfragment; $t_{(93.73)}$=3.090, p=0.003] and F_v/F_m [0,516 (SD = 0,017) for regular fragment; 0,498 (SD = 0,031) for microfragment; $t_{(24.60)}$=2.701,

p=0.012] (Fig 2.), but not in SGR [0,0021 (SD = 0,0035) for regular fragment; 0,0033 (SD = 0,0037) for microfragment; $t_{(34.97)}$=1.344, p=0.187]. Mortality was marginally different in this test, as microfragments showed proportionally more mortality than regular sized fragments, but no statistical differences were measured [$\chi^2_{(1)}$ = 3.518, p = 0.061)].

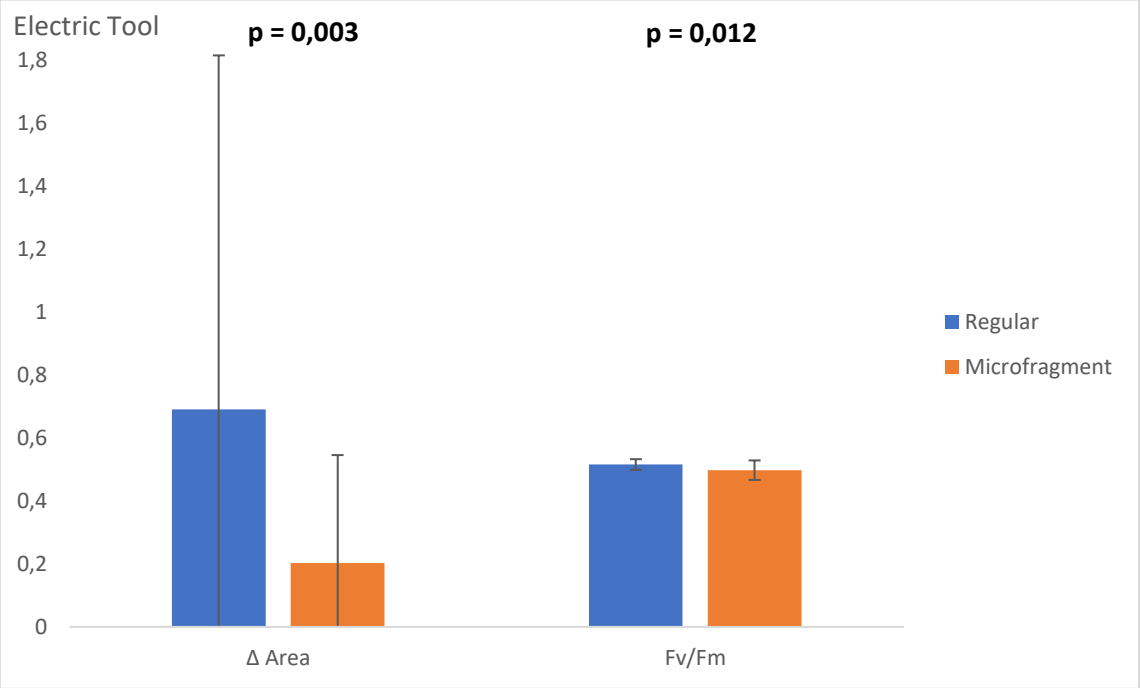

Figure 5 – Barplot containing the absolute area growth (Δ Area) and maximum photosynthetic efficiency (Fv/Fm) for fragments cut with electric tool with two different sizes, microfragments and regular, showing standard deviation lines. The p-value for the t-test is shown above each variable.

The test with the mezzaluna blade showed differences in Δ Area [1,546 (SD = 1,484) cm^2 for regular fragment; 0,535 (SD = 0,376) cm^2 for microfragment; $t_{(32.96)}$=3.609, p=0.001] but not for Fv/Fm [0,584 (SD = 0,010) cm^2 for regular fragment; 0,585 (SD = 0,009) for microfragment; $t_{(39.99)}$=0.223, p=0.825] (Fig. 3), neither SGR [0,0035 (SD = 0,0069) for regular fragment; 0,0053 (SD = 0,0048) for microfragment; $t_{(51.92)}$=1.139, p=0.260]. Mortality was not statistically different in this test, although once again microfragments showed more mortality than regular sized fragments [$\chi^2_{(1)}$ = 1,667, p = 0,197)].

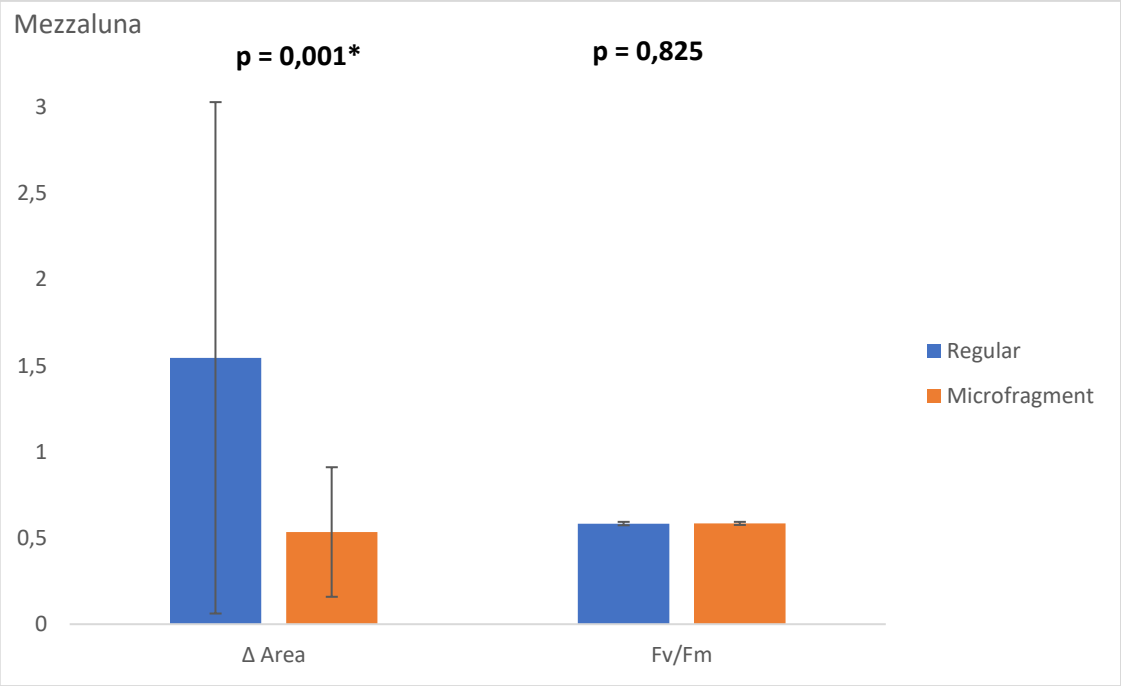

Figure 6 – Barplot containing the absolute area growth (Δ Area) and maximum photosynthetic efficiency (Fv/Fm) for fragments cut with mezzaluna blade with two different sizes, microfragments and regular, showing standard deviation lines. The p-value for the t-test is shown above each variable.

4. Discussion

An initial test to compare the use of an electrical fast spinning cutting tool and a traditional utensil blade didn't show any significant differences between both cutting types on any of the evaluated parameters. This is consistent with visual observations of the fragments. For both tests, coral fragments presented the same coloration throughout the two test months. As there was no mortality at all during the test time, and the coral fragments were kept in the same water tank at exactly the same conditions of temperature, salinity, lighting and water physico-chemical parameters, it is safe to assume that both reacted very similarly to the present conditions. Therefore, it is safe to assume that the choice of cutting tool for fragmentation is of no consequence when fragmenting and cultivating fragments of *M. capricornis* ex-situ, regarding that all other environmental parameters remain the same and no particular stressor affects one of the tested groups.

Microfragmentation using the electrical cutting tool presented a few different results. Comparing the absolute area growth, calculated by subtracting the measured fragment area on the final test day to the measured area immediately after fragmentation, of both microfragments and regular sized fragments showed significant growth differences between tests. Regular sized fragments showed more signs of growth which was also observable during the test's running time, even though we have no significant differences between specific growth rates of both tests. There is also a significant difference to the fragment's photobiology, where regular size fragments show a higher F_v/F_m value than microfragments. This might indicate that, despite environmental stressors being the same to both tests, microfragments were exposed to more stress (Rocha et al. 2013). This stress can result from the microfragments own reduced sizes (Leal et al., 2016), as smaller bodies have access to less resources and therefore can't allocate the required energy for skeletal secretion (Shu Qin et al., 2021). Although there isn't a significant comparison in mortality for this test, it is worth to note that there was a higher mortality to microfragments than regular sized fragments that could also explain higher stress level felt by the microfragments of *M. capricornis*.

The final test compared *M. capricornis* fragment sizes when fragments are made using a utensil blade, most commonly found as a kitchen tool. As in the previous test, the absolute area growth is significantly different, with microfragments showing less signs of growth than regular sized fragments. Mortality has no significant differences between both types of fragments. Despite that more deaths occurred with microfragments which might indicate that it is harder for microfragments of *M. capricornis* to access sources of energy for their survival and growth. Measurements of the F_v/F_m shows no significant differences between both tests, indicating that there were no apparent stressors affecting any one of the two tests (Rocha et al. 2013). This might explain that using a utensil blade for microfragmentation might actually cause less stress to the coral than using an electrical blade. Although more research is required to determine if this is true or not. Even though these tests were made with a steady control of all parameters, the time period of their execution is not the same. Therefore, a new challenge should be performed to assess the cutting tool used in microfragmentation of *M. capricornis* during the same time period, with the same environmental conditions for all the fragments.

Along the duration of all the tests, some fragments didn't glue as well as other to the cement bases used for their fixation, requiring additional handling, albeit brief, to glue them back to their base. This was particularly true to microfragments, which had more tendency to separate themselves from their base during aquarium cleaning and maintenance. This inability to attach themselves to their substrate may be the reason why higher mortality and stress levels were more present in microfragments (Williams and Miller, 2010).

5. Conclusion

In conclusion, no differences were found in the coral *M. capricornis*' growth when using different cutting tools during fragmentation, regarding that the size of the fragments when performing fragmentation is the same overall. When comparing microfragmentation (~ 1cm^2) against regular fragments (3-5 cm^2), tests have shown that microfragments had a worse growth performance than regular size fragments of the coral *M. capricornis*.

However, these results need more scientific confirmation to be considered true. The tests performed should have a longer duration, suggestively between 6 to 12 months as to allow for a longer acclimation of the coral after microfragmentation. In addition, the tests performed were not made at the same period, despite having the same duration, which could have introduced unknown factors that may have influenced the results. Also more size classes should be introduced to the tests in order to compare *M. capricornis* growth rate between a broader range of sizes.

6. References

FAO (2014) The State of World Fisheries and Aquaculture: Opportunities and Challenges. Food and Agriculture Organization of the United Nations, Rome.

Glud RN, Rysgaard S, Kuhl M. 2002. A laboratory study on O_2 dynamics and photosynthesis in ice algal communities: quantification by microsensors, O_2 exchange rates, ^{14}C incubations and a PAM fluorometer. *Aquatic Microbial Ecology* **27**: 301–311.

Hallegraeff, G.M. (2010), Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. Journal of Phycology, 46: 220-235. https://doi.org/10.1111/j.1529-8817.2010.00815.x

Harrison P.L. (2011) Sexual Reproduction of Scleractinian Corals. In: Dubinsky Z., Stambler N. (eds) Coral Reefs: An Ecosystem in Transition. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-0114-4_6

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., et al. (2007). Coral reefs under rapid climate change and ocean acidification. Science 318, 1737–1742. doi:10.1126/SCIENCE.1152509

Hoegh-Guldberg, O., Ortiz, J. C., & Dove, S. (2011). The future of coral reefs. *Science*, *334*(6062), 1494–1495. https://doi.org/10.1126/SCIENCE.334.6062.1494-B

Hoegh-Guldberg, O., Poloczanska, E. S., Skirving, W., Dove, S. (2017). Coral Reef Ecosystems under Climate Change and Ocean Acidification. Front. Mar. Sci. 4:158. doi:10.3389/fmars.2017.00158

Hoegh-Guldberg, O., Lovelock, C., Caldeira, K., Howard, J., Chopin, T., & Gaines, S. (2019). The ocean as a solution to climate change: Five opportunities for action.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental

Panel on Climate Change. Edited by R.K. Pachauri and L.A. Meyer. Geneva: IPCC. www.ipcc.com.

IPCC. 2018. Global Warming of 1.5°C: An IPCC Special Report on the Impacts of Global Warming of 1.5°C above Pre-Industrial Levels and Related Global GHG Emission Pathways, in the Context of Strengthenin the Global Response to the Threat of Climate Change, edited by J. B. R. Matthews. Geneva: World Meteorological Organization.

IPCC. 2019. An IPCC Special Report on the Ocean and Cyrosphere in a Changing Climate (SROCC). www.ipcc.com.

Leal, M.C., Ferrier-Pagès, C., Petersen, D. and Osinga, R. (2016), Coral aquaculture: applying scientific knowledge to ex situ production. Rev Aquacult, 8: 136-153. https://doi.org/10.1111/raq.12087

Leal, M.C., Rocha, R.J.M., Rosa, R. and Calado, R. (2018), Aquaculture of marine non-food organisms: what, why and how? Rev Aquacult, 10: 400-423. https://doi.org/10.1111/raq.12168

Leal, M. C., Calado, R., Sheridan, C., Alimonti, A., Osinga, R. (2013), Coral aquaculture to support drug discovery, Trends in Biotechnology, Volume 31, Issue 10, Pages 555-561, ISSN 0167-7799, https://doi.org/10.1016/j.tibtech. 2013.06.004

Morita, M., Suwa, R., Iguchia, A., Nakamura, M., Shimada, K., et al. (2009). Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. Zygote 18, 103–107. doi:10.1017/S0967199409990177

Osinga, R. et al. (2011) The biology and economics of coral growth. Mar. Biotechnol. 13, 658–671

Roberts, S. and Hirshfield, M. (2004), Deep-sea corals: out of sight, but no longer out of mind. Frontiers in Ecology and the Environment, 2: 123-130. https://doi.org/10.1890/1540-9295(2004)002[0123:DCOOSB]2.0.CO;2

Rocha, R.J.M., Pimentel, T., Serôdio, J., Rosa, R., Calado, R., 2013. Comparative performance of light emitting plasma (LEP) and light emitting diode (LED) in

ex situ aquaculture of scleractinian corals, Aquaculture, Volumes 402–403, Pages 38-45, ISSN 0044-8486, https://doi.org/10.1016/j.aquaculture.2013.03.028

Ruckelshaus, M., Doney, S.C., Galindo, H.M., Barry, J.P., Chan, F., Duffy, J.E., English, C.A., Gaines, S.D., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley, L.D. (2013). Securing ocean benefits for society in the face of climate change, Marine Policy, Volume 40, Pages 154-159, ISSN 0308-597X, https://doi.org/10.1016/j.marpol.2013.01.009

Serôdio, J., da Silva, J.M., Catarino, F., 2001. Use of in vivo chlorophyll a fluorescence to quantify short-term variations in the productive biomass of intertidal microphytobenthos. Marine Ecology Progress Series 218, 45–61.

Shu Qin Sam, Chin Soon Lionel Ng, Yuichi Preslie Kikuzawa, Tai Chong Toh, Wan Ting Sim & Loke Ming Chou (2021) Influence of fragment size on post transplantation growth and survival of domed scleractinian corals, Marine Biology Research, 17:4, 327-340, DOI: 10.1080/17451000.2021.1957934

Stanley, G. D. (2003). The evolution of modern corals and their early history, Earth-Science Reviews, Volume 60, Issues 3–4, Pages 195-225, ISSN 0012-8252, https://doi.org/10.1016/S0012-8252(02)00104-6

Stanley, S. M., Hardie, L. A. (1999). Hypercalcification: paleontology links plate tectonics and geochemistry to sedimentolgy. GSA Today 9 (2), 1 –7.

Strong, A. E., Liu, G., Skirving, W., and Eakin, C. M. (2011). NOAA’s Coral Reef Watch program from satellite observations. Ann. GIS 17, 83–92. doi:10.1080/19475683.2011.576266

Veron, J.E.N. (1985). New Scleractinia from Australian coral reefs. Records of the Western Australian Museum 12:147-183.

Veron, J.E.N. (2000). Corals of the World. Townsville: Australian Institute of Marine Science. Volumes 1-3. 1410pp.

Wild, C., Hoegh-Guldberg, O., Naumann, M. S., Colombo-Pallotta, M. F., Ateweberhan, M., Fitt, W. K., Iglesias-Prieto, R., Palmer, C., Bythell, J. C., Ortiz, J., Loya, Y., Woesik, R. (2011). Climate change impedes scleractinian corals as primary reef ecosystem engineers. Marine and Freshwater Research 62, 205-215. https://doi.org/10.1071/MF10254

Wickham H. (2016). Programming with ggplot2. In: ggplot2. Use R!. Springer, Cham. https://doi.org/10.1007/978-3-319-24277-4_12

William, K.F., Barbara, E.B., Mark, E.W., Richard, P.D., 2001. Coral bleaching: Interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20, 51–65. https://doi.org/10.1007/s003380100146

Williams, D.E. and Miller, M.W. (2010), Stabilization of Fragments to Enhance Asexual Recruitment in Acropora Palmata, a Threatened Caribbean Coral. Restoration Ecology, 18: 446-451. https://doi.org/10.1111/j.1526-100X.2009.00579.x