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Larval settlement among different substrates in the reef-building coral *Acropora tenuis*

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Declaração de autoria de trabalho

Larval settlement among different substrates in the reef-building coral *Acropora tenuis*

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluídas.

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Abstract

Coral bleaching and tropical storms are becoming more frequent due to climate change. These events will negatively impact the composition and community structure of coral reefs. It is important to understand how settlement protocols can be improved to maximize the efficiency of restoration projects. The hypothesis tested were the following: 1) Does inoculation with coralline algae (CCA) enhances larval settlement over ceramic and coral skeleton substrate? 2) Are there differences in the settlement rate over ceramic and coral skeleton substrate either with or without coralline inoculation and, 3) Are there differences in settlement outcomes between the different zones on the ceramic tiles? For the first and second hypothesis the settlement was higher when the substrate was inoculated with CCA showing significantly differences, while the presence of coral skeleton was non-significant in facilitating settlement. For the third hypothesis, the six different orientation zones on the ceramic tiles were: (1) within the grooves, (2) top surface, (3) side of horizontal tiles and (4) within the grooves, (5) top surface or (6) side of the vertical tile. With our results showing no differences in settlement among zones, which can be related to mechanisms to reduce post-settlement mortality mainly due to grazing, algal growth, and sedimentation. Hence, we suggest that CCA should be adopted in standard settlement protocols, with no benefits shown from the use of dead coral and specific zones of the ceramic tiles.

Resumo

As alterações climáticas são uma das principais causas da degradação das populações de corais que se tem vindo a observar a nível mundial, com maior expressão nos últimos anos. O aumento da temperatura da água do mar é um dos principais fatores que contribui para esta degradação. Com incrementos registados desde os anos 80, é ainda expetável aumentos na ordem dos 3ºC até ao ano 2100. As "Ondas de Calor Marinhas" (em inglês "Marine Heatwaves") apresentam uma maior periodicidade e duração, resultando assim num aumento do período de tempo em que os corais estão sob stress térmico, levando ao seu branqueamento. Este fenómeno, se mantido durante longos períodos de tempo, pode levar à morte dos corais e é uma das maiores causas da sua mortalidade, mesmo com pequenos incrementos de temperatura que rondam os 1-2ºC. Se as populações de corais não forem expostas a estas condições durante muito tempo, há a possibilidade de recuperação total do branqueamento sofrido. Outro aspeto inerente à mortalidade dos corais é a acidificação dos oceanos. A sua maior causa está relacionada com atividades humanas, que resultam num aumento do pH da água. Esse aumento leva à dissolução do esqueleto dos corais, impactando também a sua reprodução e as fases iniciais e desenvolvimento dos corais.

Recuperar naturalmente as comunidades de corais é improvável e com cada vez menos tempo entre eventos climáticos extremos, fazê-lo implica encontrar métodos para melhorar a sua resiliência. Foi então acordado entre investigadores que a melhor forma de conservar as comunidades de corais seria através da proteção dos seus habitats, juntamente com a reabilitação das suas populações. Uma das fases do ciclo de vida dos corais que maior importância tem no processo de recuperação de populações é o recrutamento. Um recrutamento bem-sucedido provém de bons níveis de assentamento em conjunto com bons índices de sobrevivência pós-assentamento. O assentamento larvar ocorre quando as larvas desenvolvem a capacidade de selecionar o local ideal no substrato através de pistas que podem ter diferentes origens, entre as quais físicas, químicas ou microbianas. A alga coralina é uma alga que está presente no substrato bentónico dos recifes. Acredita-se que esta alga auxilie no assentamento dos corais devido às pistas químicas e microbianas que emite. Contudo, o assentamento só é otimizado caso a espécie de alga coralina tenha afinidade com essa espécie de coral, uma vez que diferentes espécies de corais têm diferentes afinidades

para diferentes espécies de alga coralina. O assentamento dos corais pode ser afetado pelo tipo de substrato disponibilizado, assim como pelos diferentes ângulos em que as larvas escolhem assentar, pois estes tem diferenças de luz, presença de algas, sedimentação e predação. As maiores causas de mortalidade pós-assentamento são a competição com algas ou com outros corais, a sedimentação e a predação, principalmente acidental. Assim sendo, os estudos relacionados com o assentamento de corais sempre foram alvo de muita atenção, tendo em conta ser um processo fundamental para alargar o conhecimento e otimizar a recuperação das populações de corais.

O presente estudo foi realizado nas instalações do Oceanário de Lisboa, entre outubro e dezembro de 2022, utilizando corais recolhidos da Grande Barreira de Corais na Austrália em setembro de 2022. Neste estudo testou-se a influência da alga coralina no melhoramento do assentamento dos corais, e as de assentamento entre peças de cerâmica e esqueleto de coral, tanto inoculados como não inoculados com alga coralina. Testou-se ainda a existência de diferenças nos resultados de assentamento entre diferentes zonas nas peças de cerâmica. As peças utilizadas têm formas diferentes, uma quadrada e outra em pirâmide. As zonas criadas em cada uma delas foram: dentro das ranhuras, na parte superior da cerâmica e na lateral da cerâmica. Para além disso, foram feitas observações para determinar a mortalidade dos pólipos primários durante o período de um mês. Para auxiliar na previsão da desova dos corais, seguiu-se o desenvolvimento das gametas dos corais, a cada duas semanas, através de observações das gonadas. Das dez colónias de *Acropora tenuis*, quatro desovaram, sendo os gametas recolhidos e colocados em recipientes de fertilização. Quatro dias após a fertilização, as larvas foram transferidas para os recipientes de assentamento. Foram utilizados dezasseis recipientes para assentamento, cada um com uma capacidade de oito litros. Em cada recipiente foram colocadas oitocentas e cinquenta larvas, utilizando aproximadamente uma larva por cm^2, sendo que o sugerido por investigadores é 0.5 a 1.5 larvas por cm^2.

Como esperado, nas peças inoculadas com alga coralina observou-se uma maior taxa de assentamento, teoricamente devido às pistas químicas e microbianas emitidas pela alga coralina. Para o fator substrato não foram observadas diferenças significativas, querendo isto dizer que ambos os tipos de substrato terão o mesmo efeito no assentamento das larvas. As diferentes zonas das peças de cerâmica também não apresentaram diferenças significativas, o que quer dizer que o assentamento não é melhorado por nenhuma zona específica. A escolha do local de assentamento está também relacionada com a tentativa das larvas reduzirem a mortalidade pós-assentamento. Cada zona apresenta certas vantagens, a ranhura pode proteger da predação, mas é muito comum a acumulação de sedimentos neste local o que pode ter o efeito contrário ao desejado e impedir o assentamento. Durante as observações dos pólipos primários, houve uma diminuição do número de pólipos entre as duas semanas e um mês. Isto indica que a mortalidade neste período foi alta, podendo esta estar relacionada com a presença de algas, sedimentação ou predação dos gastrópodes e peixes presentes nos aquários.

Conclui-se que a utilização da alga coralina é facilitadora no assentamento de larvas de coral e que deve ser utilizada sempre que possível em protocolos de assentamento. Como o tipo de material de substrato não mostrou qualquer tipo de influência nos níveis de assentamento, a escolha de substrato pode ser baseada noutros critérios como o preço, facilidade de preparação e manutenção do substrato. De seguida, é também sugerido que nenhuma das zonas das peças de cerâmica melhora o assentamento das larvas, recomendando assim que em futuros estudos estejam disponíveis várias zonas para assentamento. Por fim, as observações dos pólipos primários mostraram a diminuição do número de pólipos num mês comparando com as duas semanas. Apesar de neste estudo não terem sido estudadas diretamente as causas de mortalidade dos pólipos, é sugerido com base em estudos passados que esta mortalidade seja resultante da presença de alga, principalmente alga filamentosa e de predação acidental de peixes e gastrópodes presentes nos aquários com o intuito de controlar a quantidade de alga. Estes resultados, quando combinados com outros trabalhos já existentes, podem ajudar a melhorar os protocolos de assentamento e assim maximizar os resultados de projetos de reabilitação, possibilitando a diminuição dos valores de declínio de populações de corais.

Keywords

Crustose coralline algae; Polyps; Larval ecology; Orientation; Scleractinian corals; Settlement substrate

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List of abbreviations, acronyms and symbols

CCA- Crustose coralline algae

CITES- The Convention on International Trade in Endangered Species of Wild Fauna and Flora

OA- Ocean acidification

PVC- Polyvinyl chloride

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Chapter 1- General introduction

1.1 Climate change effects on corals

Climate change and anthropogenic disturbances are causing rapid degradation of coral reefs (Hughes et al., 2017; Jorissen et al., 2021). Ocean acidification and warming are the main threats for coral reefs related with climate change (Cornwall et al., 2019). Sea surface temperatures have been rising since the 1980s (Hughes et al., 2017), with an average of 0.07ºC rise in the past century (Dalton et al., 2020) and it is still expected to rise 2.73ºC more by 2100 (Cornwall et al., 2019), leading to a collapse of coral reefs due to severe bleaching (Hazraty-Kari et al., 2022). Marine heatwaves are becoming more frequent and longer (Oliver et al., 2018) being one of the main causes of coral mortality due to coral bleaching (Cornwall et al., 2019) , worsen when combined with high irradiance (Page et al., 2023). . Bleaching of corals happens when the temperature exceeds the physiological tolerance of the coral. Thermal stress is caused to corals under high temperatures, leading to a decline on the symbiotic zooxanthellae (*symbiodinium* spp.) and their photosynthetic pigments that are corals primary source of nutrition (Hoey et al., 2016), causing corals to lose their color. Corals that have suffered bleaching are physiologically damaged, and when bleaching happens for a long period of time it leads to high levels of coral mortality (Hughes et al., 2017). With heatwaves occurring more frequently, corals will have less time available to recover from these events and losses of coral cover and species diversity will be observed (Dalton et al., 2020). Bleaching can be induced by increasing the temperature by 3ºC to 4ºC during short periods of time (1-2 days) or by increments of 1ºC to 2ºC during longer periods of time (several weeks) (Jokiel & Coles, 1990). Scleractinian corals are usually close to their upper thermal limit and a slight increase (>1ºC) can cause large-scale mortality and habitat degradation (Hoey et al., 2016).

Ocean acidification is another major threat to the survival of corals, due to human activities. Fossil fuels burning, and deforestation have triggered an increase of carbon dioxide (CO_2) releases to the atmosphere from 280 to above 400 µatm (Figuerola et al., 2021). When combining ocean acidification and ocean warming it is expected for corals to start dissolving when CO_2 levels reach around 450ppm, which is predicted to happen as soon as 2030 and

2050 (Erez et al., 2011). It is still expected a decrease of pH levels around ~0.3 by the year 2100 (Figuerola et al., 2021). Scleractinian corals are extremely sensitive to changes in seawater chemistry and ocean acidification will intensify the effects of increasing temperatures, leading to severe bleaching for a certain temperature (Hoey et al., 2016).

Ocean acidification is characterized by the decrease of the ocean pH due to the rising dissolution of carbon dioxide (CO_2) in oceanic waters (Allemand & Osborn, 2019). The CO_2 combined with water, forms carbonic acid (equation 1) (Kroeker et al., 2013). This dissociates into bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and associated protons (H+) in each reaction (equations 2 and 3) (Erez et al., 2011). Carbonate ions are an essential part of the physical structure of corals, they form skeleton of corals through the reaction of carbonate ion with calcium, forming calcium carbonate ($CaCO_3$) (equation 4). The protons in excess released from the dissociation will react with the carbonate ion, not allowing the calcium to bond and decreasing the amount of calcium carbonate available (Allemand & Osborn, 2019).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \quad (1)$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (2)$$

$$HCO_3 \leftrightarrow CO_3^{2-} + H^+ \quad (3)$$

$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3 \quad (4)$$

Although reductions on calcification were demonstrated, the increase of CO_2 can also negatively affect reproduction, early stages of development of corals (N. S. Webster et al., 2013), the morphology of corals with the skeleton changing to a more porous and possibly fragile phenotype (Tambutté et al., 2015), and reductions in coral diversity were observed (Fabricius et al., 2011) .

After a bleaching event, if the conditions don't remain for too long, corals can recover the breakdown of the symbiosis between scleractinian corals and *symbiodinium* (Hoegh-Guldberg et al., 2017). *Acropora millepora* showed a rapid recovery after severe bleaching, which indicates this species has high resilience (Page et al., 2023). It is suggested that corals have the ability to acclimate to higher water temperatures with no bleaching and reduction of physiological activity such as photosynthesis, respiration, growth and survival rate (Hazraty-Kari et al., 2022). *Acropora* is a fast-growing branching coral, and it is more prone to die due to bleaching than slow growing corals. These are also vulnerable to other disturbances due to fragmentation caused by storms and cyclones, disease, and predation (Page et al., 2023), but with this fast growth was already observed complete recovery of *acropora* populations after seven years, although with lower abundance (Watt-Pringle et al., 2022).

Ocean acidification (OA) slows coral reef growth and reproduction slowing the recovery of damaged corals from intense events as mass bleaching (Albright & Cooley, 2019). Long-term experiments regarding OA are scarce (Comeau et al., 2019) and most studies are controlled laboratory studies (Wall et al., 2016). A 12-month study with scleractinian corals showed that corals submitted to low pH during that period, calcified and reformed colonies after being back in normal conditions, showing that these species can maintain basic life functions (Fine & Tchernov, 2007). It was also showed that corals with other available food sources than photosynthesis can increase the feeding rate and stored energy reserves to mitigate the reduction in calcification in high CO_2 ambient (Towle et al., 2015). Some corals as the massive *Porites* that are long-lived structurally simple corals can establish dominance, trough replacing fast-growing structurally complex corals at pH of 7.8, showing that even though loss of biodiversity is observed it is possible to keep coral cover (Fabricius et al., 2011). This genus can show resilience to ocean acidification through their ability to regulate the pH within their calcifying fluid, maintaining this pH higher than the seawater pH, it is still not known if they can maintain this through a coral lifetime and if more sensitive corals have the same capacity (Wall et al., 2016).

To help coral population withstand the current and future climate change events, they need to become more tolerant. It is possible to manipulate coral colonies tolerance through "assisted evolution", that consists of interventions as selective breeding or genetic engineering, to improve coral performance (DeFilippo et al., 2022). Studies show that corals can genetically adapt to local conditions, climate change events as bleaching that provoke high mortality apply selection pressure to the populations, being expected that the generation spawned by these survivors will be better adapted to recurrent climate change events (Baums et al., 2019). Three of these approaches are: (1) managed or selective breeding, that is based on sexual reproduction, as cross-breeding between different populations of the same species, between species and cross-breeding of heat tolerant colonies from the same population, (2) (Pre)conditioning, where the organisms are exposed to sublethal stress that can increase tolerance to succeeding stress exposures and, (3) Microbiome manipulation, where individual microbes, microbial communities or their hosts are manipulated to be heat tolerant (McLeod et al., 2022).

1.2 Restoration

Extreme events triggered by climate change are more frequent and more severe, leaving less time for corals to recover from these disturbances between events (Boström-Einarsson et al., 2020). Resilience is the capacity to recover from these disturbances (Birrell et al., 2008) and it must be enhanced to be able to recover coral communities. A natural recovery of coral communities is unlikely or impossible if coral resilience is low (Boström-Einarsson et al., 2020). Habitat conservation can be done by either protection if the habitat remains intact or by restoration if it is degraded (Possingham et al., 2015). A passive habitat protection approach was typically preferred than restoration (Boström-Einarsson et al., 2018), however more recently experts concluded that conservation of coral communities should include habitat protection and restoration to guarantee long-term health of reefs (Westoby et al., 2020).

Ecological restoration is the process that consists of the recovery of biodiversity and ecological function of a damaged ecosystem to levels prior of the degradation (Rey Benayas et al., 2009). A restored ecosystem can continue its development without additional assistance, as it contains enough biotic and abiotic resources (Boström-Einarsson et al., 2020). There are different restoration methods, that can be divided into asexual propagation methods, sexual propagation methods and substratum enhancement methods (Omori, 2019). Asexual propagation methods include direct transplantation and coral gardening (Boström-Einarsson et al., 2020), the last one having two phases, the nursey phase, and the transplantation phase (Bayraktarov et al., 2019). Nursey phase can happen either *in situ* and *ex situ*, with different advantages and disadvantages. *In situ* nursing is less expensive and allows for exposure to biotic and abiotic variables that are not controlled, as predation or algae growth and there are restrictions related with the colony size. *Ex situ* nursing is far more expensive than *in situ*, but the biotic and abiotic environment are highly controlled and there are no restrictions related with colony size (Barton et al., 2017). The sexual propagation method is named larval enhancement, and the substratum enhancement methods are the substratum addition, substratum stabilization and substratum enhancement with electric field or by removing macroalgae (Boström-Einarsson et al., 2020).

1.3 Settlement

Recruitment is an important phase on the process of recovering coral populations as it affects the structure and maintenance of reef communities (Lei et al., 2021). A successful recruitment contains settlement and post-settlement survival (Randall et al., 2020). It is important that corals choose to recruit into the most appropriate substratum they can find, since this phase is irreversible (Jorissen et al., 2021), and will also impact post-settlement survival (Ritson-Williams et al., 2010). When larvae are capable of settling, they use their sensory abilities to find the suitable settlement substrate, distinguishing and selecting habitats that have a positive effect on post-recruitment survival (Foster & Gilmour, 2016). Different cues induce larvae settlement (Ritson-Williams et al., 2014) and these can be chemical or microbiological (Lei et al., 2021). Physical cues are related with surface complexity (Siboni et al., 2020), light sensitivity or with color preference with a higher preference for red colors (Foster & Gilmour, 2016).

Crustose coralline algae (CCA) are calcareous red algae that cover around 30% of the benthic substrate on coral reefs (Siboni et al., 2020). CCA are thought to help induce coral larvae settlement, even though it is still not fully understood the properties that induce settlement for corals, it is known that the cues these algae emit are mainly from chemical and microbial origin (Jorissen et al., 2021). There are coral larvae with settlement preferences to specific CCA species (Sneed et al., 2014) , however there are CCA species that inhibit settlement (Lei et al., 2021) .

The type of substrate used by the larvae to settle will affect the survival of the corals (Sneed et al., 2014). Several types of substrates and materials have been used, such as coral skeleton, ceramic tiles, terracotta tiles, cement blocks, glass jars, petri dishes and PVC plates (Petersen et al., 2005). Settlement rates are also influenced by the different angles of the substrates, since the angle affects the light conditions, sedimentation accumulation and grazing (Kennedy et al., 2017). Vertically oriented tiles are expected to have high settlement outcomes because there is less sedimentation than on horizontal tiles (Salinas-de-León et al., 2011), they are also expected to settle in crevices, where they may be able to avoid grazing (Petersen et al., 2005).

1.4 Post-settlement mortality

Post-settlement mortality for scleractinian corals and other marine invertebrates is very high, around 90% (Penin et al., 2010; Wilson & Harrison, 2005). The main causes of mortality after settlement are competition, sedimentation, and predation (Penin et al., 2011). An example of a competitor is algae that with their rapid recruitment and growth can dominate the space available (F. J. Webster et al., 2015). Competition with corals occurs through various interactions, for example physical interactions as shading or abrasion or even chemical interactions that induce mortality (Box S, 2007). Sedimentation impacts corals through the reduction of recovery after disturbance events (Ricardo et al., 2017). Predation is thought to be a result of incidental grazing, this is a process meant to inhibit proliferation of macroalgae with the aim of diminishing the competition between corals and algae (Trapon et al., 2013). It has also been shown that with an increase in the size of juveniles, they will have a reduction in mortality, since they can easily overcome the main causes of predation (Wilson & Harrison, 2005).

1.5 Coral ecology

Acropora is the ecological dominant genus in the Indo-Pacific (Hughes et al., 2019). *Acropora tenuis* is commonly found in the Indo-Pacific and the Red Sea (Humanes et al., 2016) (Fig. 1.1). Tropical coral reefs support around 25% of known marine species, with approximately 1.9 million species to inhabit coral reefs and surroundings (Hoegh-Guldberg et al., 2017).

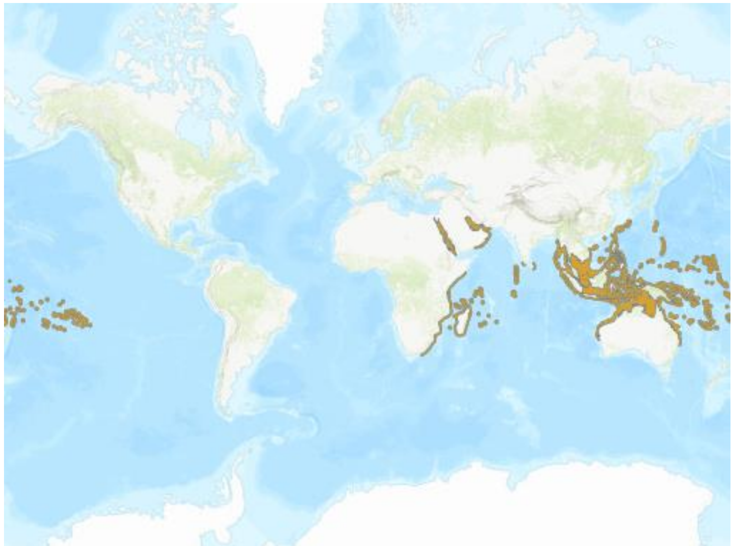

Figure 1.1- Geographic distribution of Acropora tenuis (IUCN 2023)

Scleractinian corals have a bipartite life cycle that consists in a dispersive pelagic larval phase and a sedentary juvenile to adult phase (Trapon et al., 2013). The dispersive pelagic phase is composed of cross fertilization. Around one to two hours later is possible to observe cell division and, two days after fertilization larvae can be fully developed, and ready to settle permanently on the substrate (Edwards, 2010). Meanwhile, the sedentary phase involves the settlement and metamorphose into juvenile polyp with the formation of the calcium carbonate exoskeleton. Continuous growth during a presexual juvenile phase leads to the development of a sexually reproductive adult coral (Harrison, 2011), where gametogenesis is observed. Corals become reproductively mature from 1 to 5 years after settlement, depending on the size of the colonies. (Edwards, 2010).

Most scleractinian corals are hermaphroditic broadcasters spawners (Tan et al., 2020), meaning they produce both male and female gametes within each polyp with posterior spawning of gametes from the polyps into the sea where external fertilization and development occurs (Harrison, 2011). These corals usually spawn once per year, approximately one week after the full moon in October or November (Harrison et al., 1984) in an event called mass spawning event (Kaniewska et al., 2015). This is a result of numerous corals species releasing gametes in a synchronous way during a period after the sunset, near the new moon, where there is no light (Keith et al., 2016). The time of spawning can be shaped by various cues, the sea surface temperature (SST) is suggested to be one of them even though inconsistences exist, and the lunar cycle (Baird et al., 2009). It is possible to predict spawning by breaking a branch from a colony and, by analyzing the color of the eggs, it is possible to estimate gonad maturity. These go from a white or cream color when they are not mature, to a pink or orange when they are getting ready to spawn (Omori & Iwao, 2014).

1.6 Relevancy of the study

In this study, the effects of using substrate inoculated with CCA on settlement outcomes are addressed, being also discussed the use of two different substrate types and the division of the ceramic tiles in three different zones. Ultimately, the development of primary polyps during a one-month period was analysed, to understand how the mortality evolves. The aim of this study was to understand how these three factors, impacted the settlement of coral larvae, so in the future it can be applied to improve new settlement protocols.

This study focused on the coral species *Acropora tenuis*, one of the dominant genus in the Indo-Pacific Ocean (Hughes et al., 2019). The hypothesis studied were inoculation with coralline algae enhanced larval settlement, the exposure of larvae to this algae may enhance larval settlement due to chemical and microbial cues. It was also hypothesised if there were differences in settlement outcomes between ceramic tiles and coral skeleton, to understand which type of substrate would better enhance settlement. By dividing the ceramic tiles in different zones, allowed to have various settlement zones each offering different conditions, to better understand settlement outcomes between the different zones. This was used to study the hypothesis if existed differences in settlement outcomes between the three different zones on the ceramic tiles. Finally, it was also followed the development of primary polyps during one month with focus on how mortality evolved during that period of time. Understanding how these three factors can be improved for better settlement outcomes will lead to overall improvements in settlement outcomes. Additionally, the observations made during the one-month period aimed to understand the mortality development. Even though the causes of mortality were not directly studied, it is suggested, based on previous studies with similar conditions as this one, that the main causes of mortality of the primary polyps could be an increase in quantity of turf algae and could also be due to predation of fish and gastropods added to control algae.

The results from his study provide information on how to enhance larval settlement which is one of the most challenging periods of the coral life cycle. By providing information on how to enhance settlement rates, settlement protocols will be improved, leading to development of higher quality coral restoration protocols. These combined simultaneously with habitat protection will have greater impacts on the conservation of coral communities. *Acropora* is one of the most important genus due to having the largest distribution and species richness of reef-building corals (Zayasu et al., 2016). Improving settlement on *Acropora tenuis* confers knowledge for new settlement experiments in other species from the same genus, that if successful, can decrease the current decline of coral populations.

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Chapter 2- Manuscript

Larval settlement among different substrates in the reef-building coral *Acropora tenuis*

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Keywords

Crustose coralline algae; Polyps; Larval ecology; Orientation; Scleractinian corals; Settlement substrate

Abstract

Coral bleaching and tropical storms are becoming more frequent due to climate change. These events will negatively impact the composition and community structure of coral reefs. It is important to understand how settlement protocols can be improved to maximize the efficiency of restoration projects. The hypotheses tested were the following: 1) Does inoculation with coralline algae (CCA) enhances larval settlement over ceramic and coral skeleton substrate? 2) Are there differences in the settlement rate over ceramic and coral skeleton substrate either with or without coralline inoculation and, 3) Are there differences in settlement outcomes between the different zones on the ceramic tiles? For the first and second hypothesis the settlement was higher when the substrate was inoculated with CCA showing significantly differences, while the presence of coral skeleton was non-significant in facilitating settlement. For the third hypothesis, the six different orientation zones on the ceramic tiles were: (1) within the grooves, (2) top surface, (3) side of horizontal tiles and (4) within the grooves, (5) top surface or (6) side of the vertical tile. With our results showing no differences in settlement among zones, which can be related to mechanisms to reduce post-settlement mortality mainly due to grazing, algal growth, and sedimentation. Hence, we suggest that CCA should be adopted in standard settlement protocols, with no benefits shown from the use of dead coral and specific zones of the ceramic tiles.

2.1 Introduction

Coral reefs are facing continuous and rapid degradation due to anthropogenic disturbances (Jorissen et al., 2021; Lei et al., 2021). Ocean warming and the increase in the frequency of heatwaves leads to high mortality in corals due to bleaching (Cornwall et al., 2019), and ocean acidification leads to a reduction in the calcification of corals as well as affects reproduction and the early development stages (Webster et al., 2013). Coral larval recruitment is a vital step for the maintenance and recovery of coral communities that suffer from these disturbances (Doropoulos et al., 2018; Lei et al., 2021; Ritson-Williams et al., 2010).

Corals have a bipartite life cycle that begins with a stage where larvae are found freely swimming and then, after settlement, develop and metamorphose into a benthic phase called polyp, whereby the formation of the calcium carbonate exoskeleton is initiated (Neder et al., 2019). Recruitment comprises three sequential life-history phases including larval availability and transport, larval settlement and metamorphosis, and post-settlement survival (Doropoulos et al., 2017). Settlement is the behaviour where larvae search and settle in the most suitable habitat, transitioning from the dispersal phase on the water column to the attachment on the chosen surface (Gómez-Lemos et al., 2018; Ritson-Williams et al., 2010). Settlement can be enhanced by physical cues such as surface irregularity or angle of the substrate, chemical cues related to reef substrate, and microbial biofilm cues (Foster & Gilmour, 2016; Lei et al., 2021; Tebben et al., 2015). These cues transmit information about the quality of the microhabitat that leads to corals settling or, in case the habitat is not ideal, either delays it or completely inhibits it (Jorissen et al., 2021). Crustose coralline algae (CCA) have been found to facilitate settlement and metamorphose to some species of corals due to their microbiological and chemical cues (Jorissen et al., 2021; Lei et al., 2021; Sneed et al., 2014).

Crustose coralline algae are calcareous red algae found in coral reefs worldwide, covering around 30% of the benthic substrate (Siboni et al., 2020). CCA facilitation for coral larval settlement and metamorphosis depends on the species, and some species of CCA can even have inhibitory effects on settlement (Ritson-Williams et al., 2010). Some coral species have a general settlement preference for CCA, others prefer to settle only in the presence of certain CCA species, and some can even prefer to settle in the substrate where adult corals of the same species are present and not CCA (Elmer et al., 2018; Sneed et al., 2014). Furthermore, studies have shown that CCA presence is not required for coral larval settlement and metamorphosis (Ritson-Williams et al., 2016). However, it is still not fully understood if coral settlement is facilitated by CCA or other inducers (Lei et al., 2021). We aimed to test the simultaneous effect of CCA presence and different substrate types on the species *Acropora tenuis* (Dana 1846).

The Genus *Acropora* has the largest distribution and species richness of reef-building corals (Zayasu et al., 2016). *Acropora tenuis* is a branching scleractinian coral, that grows on upper reef slopes. It has a general distribution in the Indo-Pacific (van der Ven et al., 2022), being mainly found in the Western Pacific and Red Sea (Watanabe et al., 2006). *A. tenuis* is a hermaphroditic broadcast spawner and presents an annual gametogenic cycle (Zayasu et al., 2016) releasing eggs and sperm bundles to the water column during synchronized spawning events (van der Ven et al., 2016). As for the conservation status of this species it is classified as near threatened (IUCN 2014). This species is particularly important to study since it has been used for several successful restoration projects (Zayasu et al., 2018). Aquariums can contribute to these restoration projects in various forms, they can contribute with knowledge on biology and life history of species, they have experience on conservative breeding, assisted colonization and reintroduction programs, and they are also genetic reservoirs of threatened species (da Silva et al., 2019). Several settlement aspects of this coral species remain unknown, and these are the focus of our work.

Substrate type is also a major factor tested when addressing coral settlement including coral skeleton, ceramic tiles, cement blocks, or petri dishes, where the ceramic tiles and coral skeleton were mostly preferred (Petersen et al., 2005). Settlement rates can also be influenced by the orientation angle of the settlement tiles, where some studies show a preference for vertically orientated tiles (Salinas-de-León et al., 2011). This preference is likely related to light conditions, sediment accumulation, algal overgrowth, or grazing intensity that changes accordingly with the different angles (Kennedy et al., 2017; Trapon et al., 2013). Studies were also done comparing settlement between vertical and horizontal tiles, inside and outside presence of grooves (Petersen et al., 2005), the settlement in different spatial orientations with various materials (Kennedy et al., 2017), and settlement between different orientations, inside and outside grooves and between two different materials (Ricardo et al., 2017). Even though previous studies were done comparing, type of substrate, presence of CCA and orientation, to our knowledge no study has been done yet regarding these three factors together while dividing the ceramic tiles in 3 different zones, the side of the tile, within the grooves and the top surface of the tiles.

Preliminary observations were also made of post-settlement mortality for the focus species, to understand how primary polyps mortality develops in a one-month period and what may be the causes that could had an impact in the mortality of the current study. Post-settlement mortality is usually very high with up to 99% of mortality in the first year of life (Wilson &

Harrison, 2005), usually influenced by various factors such as overgrowth of macroalgae, competition, sedimentation, grazing or predation (Trapon et al., 2013), bleaching, or disease (Craggs et al., 2019). Knowing that the post-settlement period has low survival, our aim was to understand the point in time when the settled larvae were transformed into primary polyps, and to follow the development of survival and mortality of the primary polyps one month after fertilization.

To our knowledge, no study has compared settlement outcomes of two different types of substrate, with and without inoculation of CCA, while studying the settlement behavior of larvae between six different spatial orientation in ceramic tiles. This would be more representative of the settlement habitat conditions in nature since it offers increased habitat availability.

Hence, we tested the following hypotheses: 1) Does inoculation with coralline algae enhances larval settlement over ceramic and coral skeleton substrate? 2) Are there differences in the settlement rate over ceramic and coral skeleton substrate either with or without coralline inoculation and, 3) Are there differences in settlement outcomes between the different zones on the ceramic tiles?.

The null hypotheses were: a) inoculation with coralline algae does not enhance larval settlement over ceramic or coral skeleton substrate, b) settlement rate is the same over ceramic and coral skeleton substrate, with or without coralline algae inoculation and c) settlement outcomes do not display any differences between the different zones on the ceramic tiles.

2.2 Material and methods

2.2.1) Laboratory setup and coral experimental procedures

This study was conducted at Oceanário de Lisboa facilities, Portugal in 2022, using fragments of ten adult colonies of *Acropora tenuis* (Dana, 1846). Oceanário is a “aquatic educational and research private company” active in coral research, with different corals species such as *Turbinara reniformis, Galaxea fascicularis, Acropora tenuis, Pocillopora damicarnis,* *Stylophora pistillata*, *Psammocora contigua*, *Montipora capricornis, Echinopora lamellosa* (Dias et al., 2018, 2020; Dias, Ferreira, Gouveia, Madeira, et al., 2019a, 2019b)).

The corals were collected from the Vlasoff reef in the Great Barrier Marine Parks, Australia between 19 and 26 of September 2022 with the CITES 22PTLX00091l . Besides corals, fish, crabs and gastropods were also present in the mesocosm aquarium. They are commonly used for algae growth control.

Fish and gastropods were added to the aquariums to prevent pest development such as aiptasia, diatom algae and turf algae. The fish and gastropods added were 18 *Chromis viridis*, 1 *Synchioropus splendidus*, 2 *Ecsnius bicolor*, 1 *Acreichtys tomentosus*, 1 *Chelmon rostratus*, 3 *Chrysiptera hemicyanea*, 1 *Forcipiger flavissimus*, 2 *Calcinus sp.*, 5 *Calcinus elegans*, 20 *Throchus sp.*, 5 *Throchus histrio*, 2 *Lithopoma tectum* and 1 *Turbo brunneus*.

These were fed once per day, the coral diet was composed of isochrysis, copepods, rotifers, 24h nauplii, coral V-Powder and the teleost fed regime contains mysis, calanus, artemia, cyclops, lobster eggs, and red plankton *ad libitum* once a day.

2.2.2) Environmental control and feeding regime

The system used had a volume of 830 liters. A web-based microprocessor (Neptune System, Apex) connected to the mesocosm aquariums was used to recreate the environmental conditions needed to stimulate coral spawning. The Apex software app enables regulating and monitoring some of the parameters with the greatest impact on coral spawning, such as temperature (between 23.8-27.6 ºC), salinity (33.5-35ppt), pH (8.15-8.35), the photoperiod and the lunar cycle.

Water analyses were run daily in the laboratory and both the values for salinity and pH were later compared with the Apex. Other parameters such as ammonium, nitrites, nitrates, phosphates, alkalinity, and calcium were also measured twice a week. Supplementation was provided daily with 8g of magnesium and weekly with 2 ml of iodine. These values were adjusted depending on the parameters of the water analysis. Alkatronic and dosetronic stations were used to monitor and stabilize the alkalinity, based on the dkh consumption of the system.

With the spawning activity of corals happening during night-time, there was a need to alter the Apex clocks to eight hours prior (Dias et al., 2020; Dias, Ferreira, Gouveia, & Vinagre,et al., 2019; Dias, Ferreira, Gouveia, Madeira, et al., 2019; Dias, Madeira, et al., 2019).Therefore, the spawning activity would occur during the day. With this shift, it was necessary to put blackout blinds covering the space between the two aquariums, to avoid light from the outside disrupting the coral environment.

Corals were fed once a day. During the feeding period the main pump, the mechanic filter, and the protein skimmer were stopped for a period of an hour and a half. During the year, there are three distinct feed regimes, varying in quantity, variety, and concentration of the feed, depending on their nutritional needs due to gonad development. The first period occurs between April and July, and it is the period with the lowest food concentration. From January to March and August to October is the period with the average food concentration. The third period is between November and December, it has the highest concentration of food since it corresponds to the period where the spawning takes place, meaning their nutritional needs are higher.

2.2.3) Sampling for gamete development, spawning, and gamete collection

Colonies were sampled for the presence of gametes and identification of the development stage. Sampling took place every two weeks, starting approximately one month prior to the predicted date of spawning. Samples took place on 6th October, 17th October, and 3rd November 2022. For this procedure, a single branch was removed from the inner zone of the colony, avoiding branches from the periphery, since they can be non-reproductive zones. A stereomicroscope was used for the analysis of the branches maturity and pictures were taken (Fig. 2.1).

Spawning occurred on the 15th and 16th of November, with the expected date being between the 12th and 16th of November. To ensure spawning was not missed, daily observations started on the 9th of November, the first day after the full moon, in case the colonies spawned earlier than anticipated.

During spawning observations, all lights were off as well as the main pump and the nero pump, to keep the water stationary. Two floating gamete collectors were assembled above the gravid colonies. Colonies were verified every fifteen minutes for any sign of bundle setting using red-light torches. When the lights on the aquarium were turned on and no signs of spawning were found, the collectors were disassembled, and all the pumps were turned on.

From the ten colonies of *Acropora tenuis* only four spawned. Gametes were collected from the surface of the water inside the collectors with the help of glasses and pipettes.

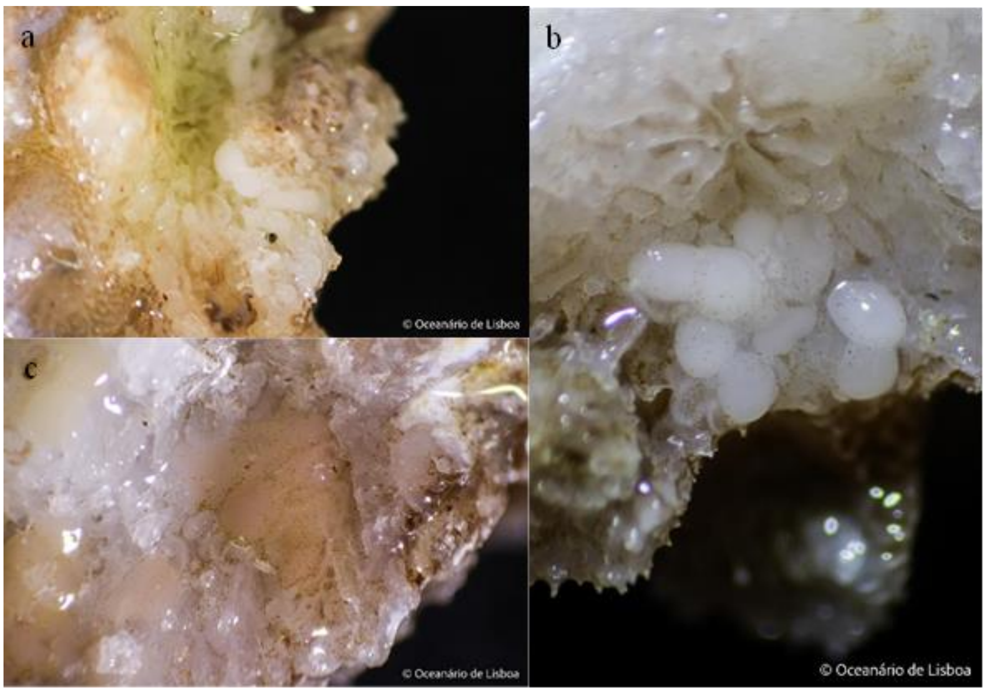

Figure 2.1- Transverse sections of a Acropora tenuis for gamete development, a) sample of 7th October, b) sample of 17th October, c) sample of 3rd November

2.2.4) Fertilization and embryogenesis

Gametes were relocated to fertilization containers, maintained at 27º C. The water was gently mixed for 1 hour to facilitate the separation of sperm and eggs and hence, enhance fertilization.

One hour after the beginning of the fertilization, embryos were transferred to the embryogenesis containers. Here, the fertilization rate was calculated using five samples from the containers. These were inside the mesocosm aquarium with temperatures averaging 27ºC. Gentle water flow was slowly introduced to each container. To assess the different embryogenic phases, five samples were collected and analyzed using a stereomicroscope.

2.2.5) Coral settlement comparison in different substrates

After four days, the larvae were transferred from the embryogenesis to the settlement containers. Larvae were tested for their settlement in response to the conditioning of substrate with coralline algae and to different types of substrate: ceramic tiles and coral skeleton. In previous studies ceramic tiles were the substrate with the higher number of settled larvae, presenting advantages as the material is cheaper, it is not required too much preparation and it is promptly available in the same sizes (Harriott & Fisk, 1987).

Sixteen 8L containers were used, with four containers per treatment, arranged in a randomized block design. A total of 850 larvae were added to each container. The number of larvae per container was calculated based on the available area for the larvae to settle, using an approximate value of one larva per cm^2. The recommended density for settlement on artificial substrata suggested in coral restoration guidelines is between 0.5-1.5 cm^2 (Omori & Iwao, 2014).

Here, were tested the hypotheses: 1) Does inoculation with coralline algae enhance larval settlement over ceramic and coral skeleton substrate? and 2) Are there differences in the settlement rate over ceramic and coral skeleton substrate either with or without coralline inoculation. Hence, four treatments were chosen based on variations of the substrate available: coral skeleton inoculated with coralline algae, coral skeleton not inoculated, ceramic tiles inoculated with coralline algae, and ceramic tiles not inoculated.

The substrate inoculated with coralline algae was previously conditioned for ~7 months (May to November) in seawater to create a layer of crustose coralline algae (CCA). The CCA present in the substrate was a mixture of two different genus *Sporolithon sp.* and *Lithophyllum sp.*.

Settlement observations were repeated on days 5 and 9 after fertilization and transfer to the settlement containers. Observations were done on each piece of substrate individually using a stereomicroscope. They consisted in taking each piece individually from the settlement containers and with the help of a stereomicroscope studying each surface of the substrate looking for settled larvae.

2.2.6) Zone selection for larval settlement on ceramic tiles

The ceramic tiles treatments included two different shapes present in the same number. A vertical (pyramid shape) and a horizontal (square shape), both containing small grooves (Fig.2.2). To assess the differences in settlement outcomes between different zones on the ceramic tile, observations were made simultaneously with the comparison in different substrates.

Larvae could settle in three different regions in both vertical and horizontal ceramic tiles: the side of the tile, within the grooves, and on the top surface of the tiles. Hence, the hypothesis tested was: “Are there differences in settlement outcomes between the different zones on the ceramic tiles?”. Settlement outcomes between the different zones were tested with an analysis of variance (ANOVA).

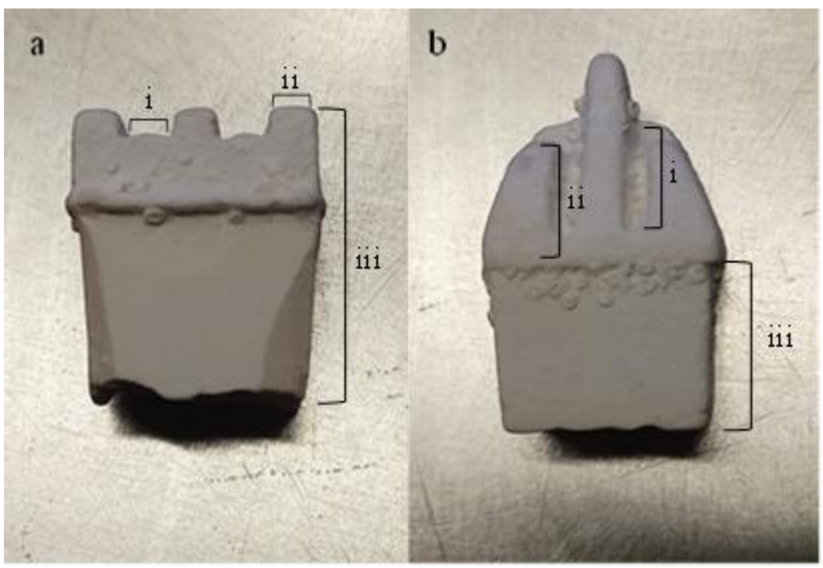

Figure 2.2- Side view of ceramic tile types. a) horizontal tile, (i) within the grooves, (ii) top surface, (iii) side b) vertical tile, (i) within the grooves, (ii) top surface, (iii) side

2.2.7) Observation of primary polyp development

To follow the development of the settled larvae to primary polyps in the different treatments, two observations were made. For the observations, each piece of the substrate was analyzed under a stereomicroscope, to assess the development of the settled larvae to primary polyps.

The first observation for the primary polyps was done sixteen days after transfer to the settlement container, and the second was done one month after.

2.2.8) Experimental design and statistical analysis

The hypothesis comparing different substrates two factors in its design including “condition”, level 1-inoculated, level 2-non-inoculated and; “substrate type”, level 1- ceramic, level 2- coral skeleton. Hence a two-way ANOVA was used for data analysis.

The settlement behaviour in the ceramic tiles was tested using a one-way ANOVA with the factor “surface”. Six different settlement surface zones were defined: (1) within the grooves, (2) top surface or (3) side of horizontal tiles and (4) within the grooves, (5) top surface or (6) side of the vertical tile.

The primary polyps settlement was tested using a two-factor ANOVA, with the factors “inoculation”, level 1 inoculated, level 2-non inoculated and; “substrate type”, level 1- ceramic, level 2- coral skeleton. All statistical analyses were carried out using GraphPad Prism 9. All ANOVA tests were preceded with tests for homogeneity of variances and correcting tests were applied if necessary.

2.3) Results

2.3.1) Coral settlement comparison in different substrates

The 5-day-old larval settlement ranged between 0.2% and 62.7% across different treatments (Fig. 2.3). The treatments with the highest settlement were ceramic inoculated, followed by skeleton inoculated. The lowest number of larvae settlement were on non-inoculated skeleton substrate and ceramic not inoculated.

The 9-day-old larvae showed significantly different rates of settlement in response to treatments with inoculated substrate (P=0.00) but there was no difference for the factor substrate type (P=0.9657). The variation of larvae settled varied between 0.2% and 67.0% across different treatments (Fig. 2.3). Settlement occurred mainly in treatments with inoculated substrate, ceramic, and coral skeleton. The lowest number of settled larvae belong to the treatments of ceramic not inoculated followed by non-inoculated skeleton. Overall, settlement was higher when compared with 5-day-old larvae, except the treatment with skeleton not inoculated substrate, where a decrease of 0.0% was observed.

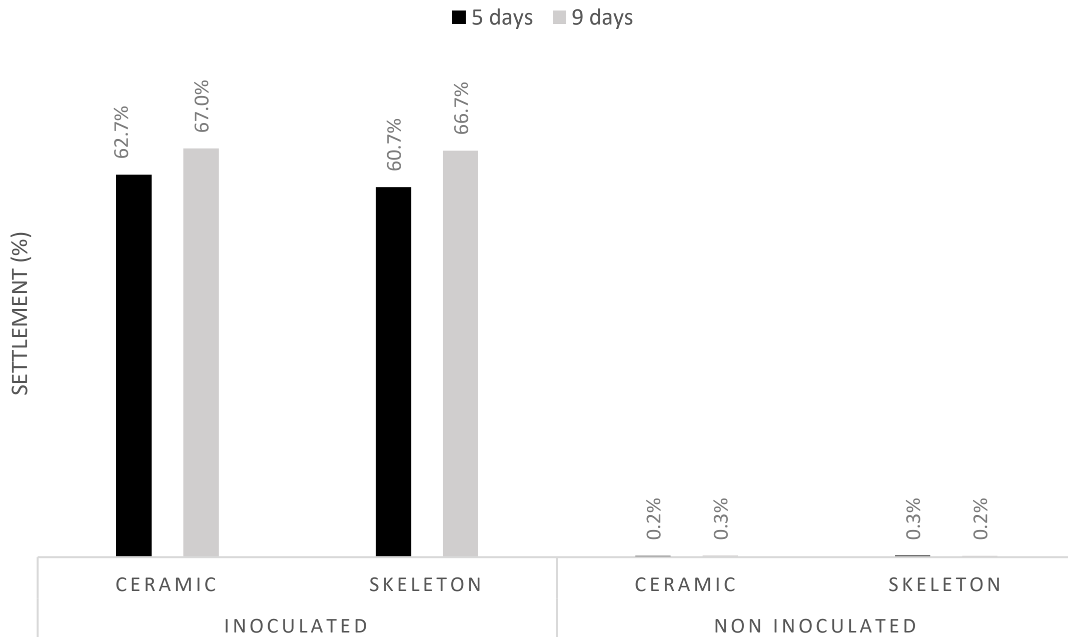

Figure 2.3- Larvae settlement response to different treatments

2.3.2) Zone selection for larval settlement on ceramic tiles

The 5-day-old larvae presented the highest settlement on the side of the horizontal substrate and on the groove from the vertical substrate (horizontal side 23.2%, vertical groove 13.4%, Fig. 2.4); followed by the surface top of the vertical (10.9%), the side of the vertical (8.4%) and the groove of the horizontal (6.7%). The surface top of the horizontal was the zone where the lowest settlement was recorded (0.4%).

The 9-day-larvae had no significant differences in settlement among the different zones (P=0.07). There were higher settlements outcomes on the side of the horizontal and the groove on the vertical ceramic tiles (23.0% and 14.8% respectively) (Fig. 2.4); followed by the surface top of the vertical (12.0%), the side of the vertical (9.1%), and the groove of the horizontal (8.2%). The lowest settlement was on the surface top of the horizontal (0.3%). Comparisons showed an increase in settlement for all the zones except on the side and the top surface of the horizontal ceramic tiles, which suffered small decreases.

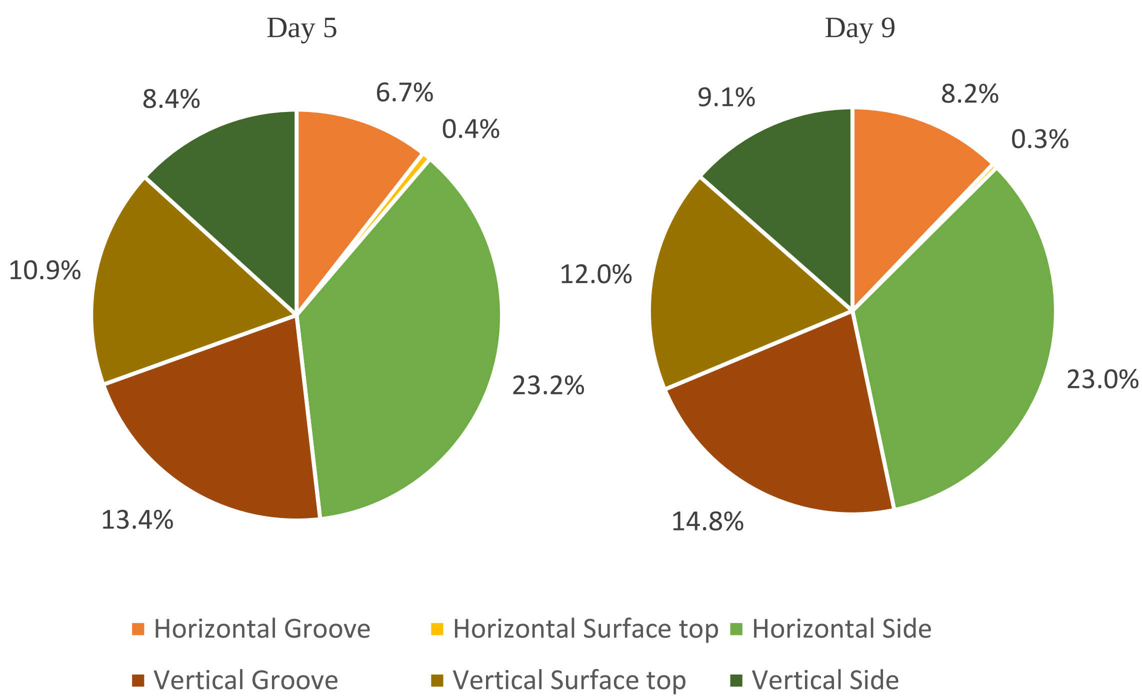

Figure 2.4- Settlement outcomes for different zones in the ceramic tiles

2.3.3) Primary polyps development

At two weeks, the presence of primary polyps ranged between 0.0% and 62.8% depending on the treatments (Fig. 2.5). Both the ceramic inoculated (62.8%) and the skeleton inoculated (61.5%) had the highest amount of primary polyps. The lowest number was on the ceramic (0.2%) and skeleton non-inoculated (0.0%).

The 1-month primary polyps had significant differences among CCA presence (P=0.0252), but there was no difference for the factor substrate type (P=0.7915). Primary polyps were mainly found on the ceramic inoculated (33.1%), followed by the skeleton inoculated (20.2%) (Fig. 2.5). Ceramic and skeleton non-inoculated had the lowest number of primary polyps (0.0%). In all four treatments, the presence of primary polyps decreased to 0.0% in the second observation.

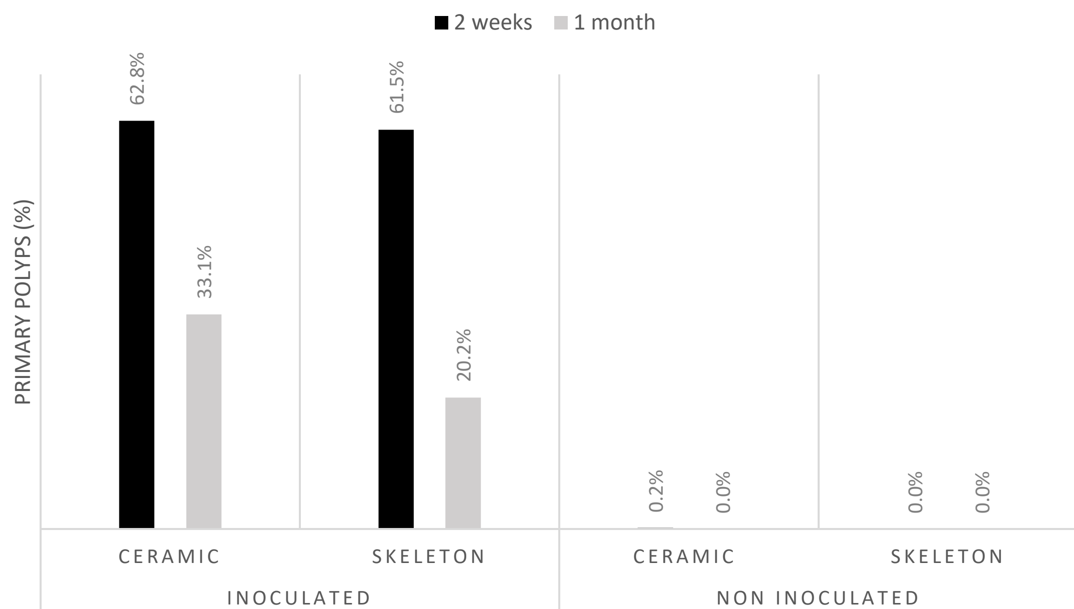

Figure 2.5 - Primary polyps in different treatments at 2 weeks and 1 month

2.4) Discussion

Overall, the results indicate a significantly higher settlement when both the substrate types were inoculated with coralline algae refuting the null hypothesis: "Inoculation with coralline algae does not enhance larval settlement over ceramic or coral skeleton substrate", since significant differences were observed. The null hypothesis "Settlement rate is the same over ceramic and coral skeleton substrate, with or without coralline algae inoculation" is confirmed since it was observed no significant difference between substrate types. Moreover, the null hypothesis "Settlement outcomes do not display any differences between the different zones on the ceramic tiles." is confirmed since no significant differences in settlement between the different zones of the ceramic tiles were found. Observations of the primary polyps development showed a decrease in the number of primary polyps between the 2 weeks and 1 month. The results will now be discussed separately.

2.4.1) Coral settlement across different substrates

Our results demonstrate significantly higher settlement outcomes when the substrates are inoculated with coralline algae for both ceramic substrates and dead coral substrates. These results suggest that coral larval settlement may be facilitated by coralline algae, which is in line with previous studies that showed that coralline algae-inducing cues can facilitate the settlement of coral larvae (Lei et al., 2021; Ritson-Williams et al., 2014; Siboni et al., 2020). There are several types of CCA cues that help induce larval settlement, CCA cues can be chemical cues (Tebben et al., 2015), microbial (Jorissen et al., 2021), and it is suggested that spectral cues can also have influence on settlement, with larvae showing higher settlement in red surfaces (Foster & Gilmour, 2016; Mason et al., 2011). However, it is also shown that only some CCA species can facilitate larval settlement, and coral larvae present different behaviour depending on the CCA species (Ritson-Williams et al., 2010).

When comparing settlement outcomes in different substrate materials, no differences were found. These results suggest that in this study, coral larval settlement was not facilitated by neither of the two substrates available. In previous studies it was shown that dead coral skeleton can attract morphologically similar coral larvae species due to physical, chemical, or bacterial cues (Norström et al., 2007), with coral larvae settling in holes with the approximate size of the larvae (Whalan et al., 2015). Because no significant differences in settlement outcomes were found between either substrates, we suggest using the ceramic tiles for *A. tenuis* due to the advantages they present regarding dead coral skeleton. The main ones being the reduction in price and in preparation of the substrate since ceramic tiles are previously available in different sizes and don´t require as much care (Harriott & Fisk, 1987).

2.4.2) Zone selection for larval settlement on ceramic tiles

The results showed no significant differences on settlement outcomes between the different zones of the ceramic tiles. However, there was a higher settlement rate on the side of the horizontal tiles. This is in agreement with previous studies (Cruz & Harrison, 2017; Goh & Lee, 2008). These choices can be related to biotic and abiotic conditions and may be a way for larvae trying to reduce post-settlement mortality through grazing, algal growth, and sedimentation (Trapon et al., 2013). On the other hand, some studies showed higher number of larvae to settling in the grooves, possibly to escape predation (Petersen et al., 2005). Nonetheless, the refuge that the grooves can provide can also inhibit settlement if sediment is present (Ricardo et al., 2017), which is not likely to happen on the side of the tiles. In addition, higher mortality was found in previous studies on horizontal surfaces also due to sedimentation, suggesting a relation between the orientation of the tiles and post-settlement survival (Davies et al., 2013). Harrington et al (2004) suggest that there is a higher preference for horizontal surfaces if these are not associated with increased sediment, algal growth, and grazing (Harrington et al., 2004). Another essential aspect is that larval settlement can change with different light conditions, depending on the coral species and their required light regime. These changes were observed in previous studies, with an increase in depth resulting in changing settlement from vertical to horizontal substrates (Carleton & Sammarco, 1987). This can be associated with the fact that in shallow water, the horizontal surfaces have a high exposure to light meaning more algae will be present (Rogers et al., 1984). As previously mentioned, coral larvae can choose substrate to reduce post-settlement mortality from algal growth, sedimentation, and grazing. These and the competition with algae on horizontal surfaces where the light is stronger, leads to, our results having the side of the tiles with the highest number of coral larvae settled.

2.4.3) Primary polyps development

In our study, there was a decrease in the number of primary polyps between the two weeks and one month observations. Various factors can impact post-settlement mortality for newly settled corals including macroalgae (Box S, 2007), sediments (Weber et al., 2012), and predation, mostly by accidental grazing (Christiansen et al., 2009). Algae can affect corals through a rise in the levels of dissolved organic carbon (DOC) which promotes microbial activity. This in turn can lead to the population growth of the algae around the coral, creating a zone of hypoxia that stresses the corals and often results in mortality (Smith et al., 2006). To help reduce algal organic matter (Penin et al., 2010; Villanueva et al., 2013), reduce competition and improve the growth and survival of juvenile and adult corals gastropods and herbivorous fish are used (Trapon, Pratchett, & Hoey, 2013). However, the use of grazers can also impact mortality in early life stages with predation being one of the main factors impacting post-settlement mortality, mainly by accidental grazing (Penin et al., 2010). In this study it was not possible identify the factor impacting mortality, however, we believe that the mortality is associated with the quantity of algae, especially the increased turf algae that the substrates had upon the 1-month observation.

2.4.4) Conclusion

In conclusion, our study has provided experimental evidence that CCA facilitates coral larvae settlement and should always be used to improve coral settlement protocols. Additionally, whether the substrate material was dead coral or ceramic tiles, had no influence on the outcomes of coral larvae settlement. With no significant difference on settlement outcomes on different substrate types, the choice of substrate can be based on the price of the material and on the level of preparation and care of the substrate. Our results also suggest no differences on settlement outcomes between the different zones where coral larvae chose to settle. It is then suggested to have various types of zones available for the larvae to settle in and not to focus only on specific ones. To conclude, the results regarding the observation of the primary polyps showed a decrease in the number of polyps in one month compared with the two weeks, which can be related to turf algae or predation.

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