POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Can heterotrophic feeding improve coral thermal stress response? – The case study of *Palythoa sp.*

Andreia Filipa Carvalho Da Silva

2021

POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Can heterotrophic feeding improve coral thermal stress response? – The case study of *Palythoa sp.*

Andreia Filipa Carvalho Da Silva

Dissertação para obtenção do Grau de Mestre em Aquacultura

Dissertação realizada sob a orientação do Doutor Rui Rocha, professor auxiliar convidado do Departamento de Biologia da Universidade de Aveiro e da especialista Teresa Baptista, Professora Adjunta na Escola Superior de Turismo e Tecnologia do Mar

2021

Can heterotrophic feeding improve coral thermal stress response? – The case study of *Palythoa sp*.

Agradecimentos

A elaboração desta dissertação foi de todo desafiante. Desafio este que não teria acontecido se o Doutor Rui Rocha não me tivesse acolhido (mais uma vez) na sua equipa. Obrigada por me ter dado esta oportunidade, ter acompanhado neste percurso e confiar nas minhas capacidades. Agradecer também à professora Teresa Baptista por me ter acompanhado ao longo destes anos em Peniche, tanto como professora como orientadora.

Não podia deixar de agradecer à equipa, a preciosa ajuda da Sílvia e da Andreia Rodrigues na elaboração dos Biomarcadores; aos meus colegas de laboratório, Agostinho e Zé Diogo por terem aguentado o barco durante os períodos mais adversos e pelo companheirismo durante este ano e meio; ao Davide pela preciosa ajuda na estatística e pela habilidade em destabilizar a equipa; à Ana Costa por me ter ajudado sempre, por me ter aturado, por todos os cafés, por nunca ter duvidado das minhas capacidades quando eu mesma o fazia, ter exigido que eu desse sempre o meu melhor, mas acima de tudo por se ter tornado uma amiga. Obrigada por todos os momentos, todas as gargalhadas, fizeram com que esta estadia em Aveiro se tornasse melhor.

Às minhas colegas de casa, à Liliana, à Matilde, à Vera e à Ana, obrigada por todos os crepes, por me "obrigarem" a gostar de sushi, a todas as conversas até às tantas, e acima de tudo por ficarem contentes com as minhas conquistas.

Agradecer também o apoio das pessoas que estiveram presentes nesta etapa, à Catarina e à Inês por partilharmos o desespero e pela motivação mútua. À Cláudia pela amizade, por estar lá sempre e por todas memórias e bons momentos que construímos ao longo destes anos. À minha bestie de todo o sempre, Rita, obrigada por estares sempre presente, pelo apoio e pelas palavras certas no momento certo. À Amendoim, à Pity, à Cris, e a todas as outras pessoas que observaram de perto este meu percurso um obrigada.

A todas as pessoas que não mencionei em cima e que contribuíram de alguma maneira para o meu percurso académico um grande obrigada. Um obrigada a Peniche, a cidade que me acolheu durante muito tempo e ao curso BMB que me trouxe muitos bons momentos. Obrigada a todas as pessoas que conheci nas minhas estadias na Berlenga, saía de lá sempre com o coração cheio e com uma vontade gigante de voltar.

À minha família, a parte mais importante deste percurso pois sem o apoio incondicional deles nada disto seria possível. Aos meus pais, obrigada por todo o carinho, amor e por apoiarem os meus sonhos e acompanharem os meus desafios. Ao meu irmão que ainda é pequeno para saber o quão gosto dele e o quão me deixa irritada ao mesmo tempo. À minha madrinha e ao meu tio, obrigada por terem acompanhado de perto este percurso. Espero que vos tenha deixado orgulhosa e que o continue a fazer.

Assim me despeço de um ciclo onde fui muito feliz e com muito orgulho deste caminho.

Resumo

Os efeitos das alterações climáticas têm vindo a ser observados em todo o mundo, designadamente nos recifes de coral, o que pode impactar irremediavelmente a biodiversidade e os serviços ecossistémicos dos oceanos. Quando estão sob *stress,* os corais fotossintéticos podem perder os seus endossimbiontes (dinoflagelados do género *Symbiodinium*, usualmente designados como zooxantelas), sendo este processo classificado como branqueamento. O aumento da temperatura nas águas manifesta-se de duas maneiras, na água da superfície do mar e sob a forma de ondas de calor. A frequência das ondas de calor tem vindo a aumentar ao longo das últimas décadas, aumentando também a frequência com que os eventos de branqueamento de corais ocorrem. Como consequência, as espécies de corais mais suscetíveis a estes eventos sofrem danos irreversíveis, uma vez que, com a perda dos endossimbiontes fotossintéticos, ficam privadas de parte substancial da sua nutrição, o que pode levar à sua degradação e, em casos mais extremos, à sua morte. Para além da nutrição autotrófica, constituída pelos produtos da fotossíntese, os corais fotossintéticos têm também a capacidade de se alimentar heterotroficamente, sendo por isso classificados como animais mixotróficos. Segundo alguns estudos, esta estratégia de alimentação pode contribuir para tornar os corais mais resilientes ao *stress* térmico. Neste sentido, no presente trabalho pretendeu-se avaliar a resposta ao *stress* térmico de mini colónias de *Palythoa sp.* (subclasse Hexacorallia; ordem Zoantharia) após 4 meses de cultivo sem (NF) e com (F) fornecimento de alimento (dieta microencapsulada, anteriormente otimizada para a espécie). Após este período, procedeu-se ao teste de *stress* térmico: dois grupos (25NF e 25F) permaneceram com a temperatura de cultivo (25±1°C), enquanto outros dois grupos (30NF e 30F) foram sujeitos a uma subida gradual da temperatura da água, durante 24h até atingir os 30±1°C. Esta temperatura foi mantida durante oito dias. Corais alimentados foram comparados com corais não alimentados de modo a avaliar o efeito da dieta num cenário de *stress*. Foi efetuada uma avaliação dos parâmetros de fotobiologia, biomarcadores de *stress* térmico e oxidativo, dano oxidativo e reservas energéticas. Durante o ensaio não houve registo de mortalidade. Apesar dos corais não alimentados não se mostrarem significativamente mais suscetíveis ao *stress*, os corais alimentados apresentam um melhor estado metabólico. Este facto pode ser crucial no período de recuperação após eventos de *stress*. Assim, sugere-se que o alimento tem um efeito positivo na resistência dos corais ao aumento da temperatura da água, como consequência das ondas de calor, quando previamente bem suplementados.

Palavras-Chave: *Zoantharia*; Suplementação; *Stress* térmico; Reservas energéticas; Balanço energético; *Stress* oxidativo

Abstract

The effects of climate change have been observed all over the world, specifically on coral reefs, which may have an irreparable impact on biodiversity and ecosystem services in the oceans. When they are under stress, the photosynthetic corals can lose their endosymbionts, (dinoflagellates from the genus *Symbiodinium,* usually known as zooxanthellae) being this process described as mass coral bleaching. Climate change have been associated with the rise of sea surface temperature and the occurrence of marine heatwaves. The frequency at which marine heatwaves occur has increased over the last few decades, resulting in a higher frequency at which coral bleaching events occurs. As a consequence, coral species most susceptible to these events suffer irreversible damage, as zooxanthellae represents a substantial part of their nutrition, which can lead to coral degradation and, in more extreme cases, to their death, shifting the structure of coral reefs. However, in addition to autotrophic nutrition, provided by the products of photosynthesis, photosynthetic corals can also feed by heterotrophy, and are consequently classified as mixotrophic animals. According with several studies, this feeding strategy can contribute to corals resilience to stress. In this context, the aim of this study was to evaluate the response to heat stress of mini colonies of *Palythoa sp*. (subclass Hexacorallia; order Zoantharia) after four months of culture without (NF) and with (F) feed supply. Immediately after this period the thermal stress test was performed: two groups (25NF and 25F) remained with the culture temperature (25±1°C) while other two groups (30NF and 30F) were exposed to a gradual water temperature increase during 24 hours until reaching 30±1°C. This temperature was maintained during eight days. Fed corals were compared with corals without feed supply, in order to evaluate the diet effect in a scenario of thermal stress. To support this experimental assay, an evaluation of photobiology parameters, oxidative and thermal stress biomarkers, oxidative damage, and energy reserves was carried out. No mortality was recorded during the experimental assay. Although not supplemented corals did not show higher susceptibility to thermal stress, fed corals exhibited a better metabolic state. This can be crucial to the recovery period after a stress event. In this way, it is suggested that feed supply can have a positive effect on the resilience of corals when they are exposed to an increase in water temperature, as a consequence of marine heatwaves, when properly supplemented in advance.

Keywords: *Zoantharian* corals; Feed supply; Heat stress; Energy reserves; Energetic balance; Oxidative stress.

Table of contents

List of figures

List of tables

1. Introduction

1.1 Importance of Coral Reefs

Coral reefs are one of the most biologically productive and highly biodiverse ecosystems in the world, providing harbour for approximately 25 percent of all known marine species (Burke *et al.,* 2011): around 800 species of reef-building corals (stony corals) and 600 species of soft corals were recorded as well as 4,000 coral reef-associated fish species (Spalding *et al.,* 2001) and an uncountable species of sponges, marine worms, sea-urchins, molluscs, crabs, shrimps, and other crustaceans, sea-anemones and sea-fans (Hovland, 2008). This high biodiversity has been highly prospected in the last years, including corals that produce bioactive natural compounds with potential for new drugs against cancer, bacterial infections, viruses, or other diseases (Rocha *et al.*, 2011). These ecosystems provide valuable benefits: are important livelihoods for coastal communities, representing a nursery for relevant commercial species; are an attraction for tourism and divers from all around the world; generate sand for the beaches and protect the coastlines from full force storms (Burke *et al.,* 2011). Nevertheless, they are one of the world´s most threatened ecosystems (Hughes *et al.,* 2003; Spalding *et al.,* 2001). The IUCN Red List elaborates a Red List Index ("IUCN Red List of Threatened Species," 2020) revealing that coral species are moving towards an increased extinction risk faster than any another group of animals. The Great Barrier Reef serves as an example: it is the most extensive coral reef, covering the east coast of Australia for more than 2,000 km and 145 km in-deep (Lalli and Parsons, 1993). However, between 1985 and 2012, it suffered a decrease in coral cover of nearly 40 percent due to some critical climate events (Great Barrier Reef Marine Park Authority, 2016).

1.2 Corals' Biology and Ecology

1.2.1. Classification and anatomy

According to Spalding *et al.* (2001), corals are simple organisms formed by polyps (represented in figure 1) which are comprised of a simple tubular body with a ring of tentacles around the mouth (Burke *et al.,* 2011). These organisms belong to the Phylum Cnidaria, one of the most famous animals' marine ornamental trade like sea anemones, sea fans and jellyfishes (Calado *et al.,* 2017). This group has some common features such as radial symmetry and planula and polyp life stage. All corals belong to the Anthozoa class and most of them are included in the Scleractinia and Alcyonacea orders (Figure 2). Scleractinian corals, or most commonly referred to as stony corals, have the capability of building a calcareous skeleton and are usually know as reef-building corals (Calado *et al.,* 2017). Alcyonacean corals, usually known as soft corals, have spiny skeleton sclerites instead of a calcareous skeleton (Calado *et al.,* 2017), to confer stability to the colony. Other coral species are included in Zoantharia order, the third-largest from the Hexacorallia subclass, performing an important role in the reef ecosystem (Rocha *et al.,* 2020). They are described as colonial species that do not secrete a skeleton but instead incorporate sediments for their protection or support (Spalding *et al.,* 2001).

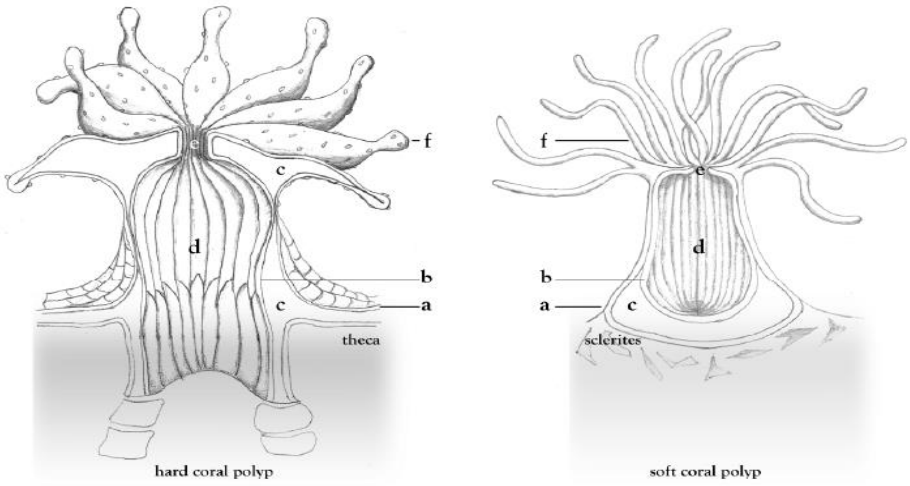

Figure 1- Illustration of hard and soft coral polyp. a-epidermis or ectoderm; b-gastrodermis or endoderm; c-mesoglea; d- gastrovascular cavity; e-mouth; f-tentacles. This illustration was a courtesy of Rui Rocha.

Also, it is possible to define the corals concerning the presence of photosynthetic dinoflagellates from the genus *Symbiodinium*, known as zooxanthellae, in their tissue (J.Veron, 2000). A great number of corals are covered by zooxanthellae, commonly called symbiotic, and the ones without zooxanthellae are called asymbiotic (Calado *et al.,* 2017).

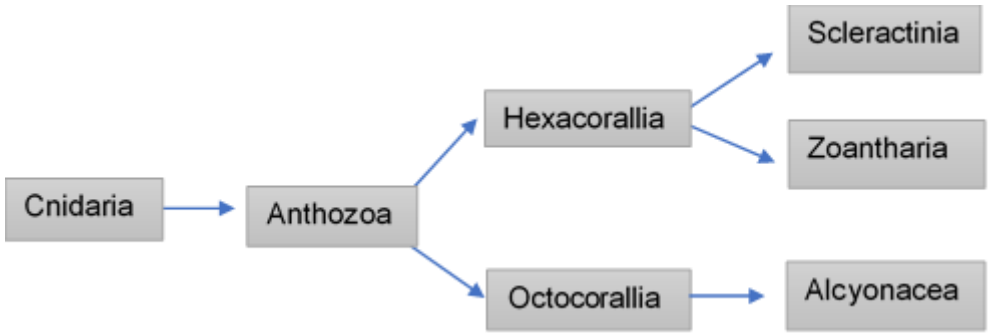

Figure 2- Diagram of coral classification, according to the following sequence: Phylum → Class → Subclass → Order.

1.2.2 Habitat

Corals comprehend a lot of species, some of them adapted to live in very different environments than tropical ones, being found in all oceans and at all depths (Spalding *et al.,* 2001). There are specimens living in extreme water temperatures (*e.g.* Carreiro-Silva *et al.,* 2017) or even with high suspend sediments (Albert *et al.,* 2015). However, tropical coral reefs harvest a considerable amount of all coral species (Dias *et al.,* 2018) and this is why they are usually associated to shallow marine habitats (Spalding *et al.,* 2001). Tropical corals are distributed in tropical and sub-tropical zones and tolerate temperature variation between 23°C and 29°C and salinity levels between 32 to 35, according to the geographic region. Photosynthetic tropical corals are restricted to the euphotic zone due to the presence of zooxanthellae. Generally, tropical corals are absent in turbid waters because highly suspended sediment levels can be harmful affecting the fecundity, juvenile growth, recruitment survival (reviewed by Fabricius, 2005 and Jones *et al.* 2016) and photosynthetic activity (Browne *et al*., 2014).

1.2.3 Reproduction

Corals can reproduce sexually and asexually. Concerning the sexually reproduction, corals can be classified as gonochoric or hermaphroditic (Jones *et al.* 2015 and Twan *et al.* 2006). Most coral species are simultaneous hermaphrodites and have an annual cycle of reproduction culminating in an annual broadcast spawning during one or a few consecutive nights (Baird *et al.,* 2009) to increase the chances of fertilization (Twan *et al.,* 2006). Important factors such as water temperature, usually when it reach its annual maximum, and the lunar annual cycle, are crucial for the spawning (Calado *et al.,* 2017). This mode of reproduction provides to these sessile organisms the capability to recruit into new habitats and improve population recovery to disturbances (Howells *et al.,* 2016) and, as a consequence, the creation of genetic links between different reefs (Twan *et al.,* 2006). The most usual asexual reproduction is by fragmentation where coral fragments can land in appropriate substrates, growing and producing new colonies (Twan *et al.,* 2006). In nature, this fragmentation is provided by storms, wave action, fish predation or other source of physical action (Richmond, 1997). Asexual reproduction reduce genetic variation, which is a negative factor in the natural environment leading to an increased population's vulnerability to disturbances (Richmond, 1997), however it can be a good factor to isolate specific characteristics in the laboratory. It is also the most usual and easier reproduction method, with a fast recovery rate (Hughes, 1983).

1.2.4 Mixotrophic feeding

Photosynthetic tropical corals are mixotrophic organisms having two different feeding patterns, obtaining nutrients from autotrophy and heterotrophy pathways. The greater part of food supply (30 - 90%) is provided by photosynthesis (autotrophy), performed by zooxanthellae. The polyp and the zooxanthellae, when in a perfect symbiosis, live in a win-win situation where dinoflagellates give to the polyp host vital substances such as organic compounds and carbohydrates (Titlyanov and Titlyanova, 2002) allowing them to grow in oligotrophic waters, were nutrients are very limited (Hovland, 2008). In addition, the algae expel oxygen (O_2) from photosynthesis, during the light period, using the carbon dioxide (CO_2) expelled by the polyps (Hovland, 2008). The host not only provides shelter to zooxanthellae (Patton, 1976) but also with inorganic nitrogen and phosphate for biosynthesis where they are transformed into organic compounds and transferred to the host (reviewed by Furla *et al.* (2005)). These organic compounds facilitate, for example, the release of particulate and dissolved organic matter in form of mucus by the corals (Falkowski *et al.,* 1984). Mucus is used

as a defence mechanism against stressful conditions (*e.g.* Teai *et al.,* 1998 and Niggl *et al.,* 2009) and plays an important role in heterotrophic feeding (*e.g.* Goldberg, 2002).

A range of 10 to 40% of the total heterotrophic feed comes from predation (Titlyanov and Titlyanova, 2002) using coral tentacles (Hovland, 2008). This opportunistic exogenous feeding through several trophic paths (Nahon *et al.,* 2013) also provides important nutrients like nitrogen, phosphorus and alternative forms of lipids that are not obtained through autotrophic pathway (Houlbrèque and Ferrier-Pagès, 2009). Some studies reveal that corals are highly benefited when heterotrophic feeding is supplied with an appropriate diet (*e.g.* Conlan *et al.,* 2019), which could depend on species to species. Also, previous research carried out by our team (data not published yet) proves that different soft coral species have specific optimal growth diet. Some of the main factors to an optimal growth diet, depending on morphology and nutritional requirements of corals, are the size of the prey and zooplankton class (*e.g.* Palardy *et al.,* 2008). Quantity can also be a key factor: Costa *et al.* (2016) showed a lower growth rate of *Sarcophyton* cf. *glaucum* when over-fed with rotifers, compared with unfed corals.

1.3 Reef Threats

Coral reefs are facing different types of local and global-level threats (reviewed by Burke *et al.* (2011). The first ones include direct human activities near the coral reefs (Burke *et al.,* 2011), like destructive and overfishing practices, marine-based pollution and coastal development (Albright and Cooley, 2019; Burke *et al.,* 2011; Calado *et al.,* 2017). The second one affects the reef environment indirectly by the cumulative impact of human activities on world climate and the chemical of the oceans such as (i) thermal stress, leading to coral bleaching, (ii) ocean acidification, slowing coral growth (Burke *et al.,* 2011) and decreasing the reproduction rate (Albright and Cooley, 2019), (iii) and the increased frequency of storms (Albright and Cooley, 2019).

The organisms can adapt to these environmental changes to a certain level (IPCC, 2018). Climate changes are restructuring the marine and coastal ecosystems (Cramer *et al.,* 2018), being related since early in coral reefs ecosystem (Dustan and Halas, 1987), inducing phase-shifts in reef composition (Cruz *et al.,* 2015) and biodiversity loss (Cramer *et al.,* 2018).

1.3.1. Water temperature rise and coral response

Climate change effects includes sea surface temperature (SST) variations and occasional marine heatwaves (MHWs) events, which have increased in frequency over the years (Leggat *et al.,* 2019). The SST increase is the average gradual increase in surface temperature due to climate changes of anthropogenic or natural causes (Marshall and Schuttenberg, 2006). The definition of MHWs is not consensual but the most recent was described by Hobday *et al.* (2016), which claims the occurrence of a MHWs when the water temperature is above the 90th percentile for historical conditions (last 30 years), at a particular geographical area, during at least five consecutive days. Several MHWs have been reported: El Niño during 2009/10 in the Central Pacific, and La Niña event in late 2010 (Dalton *et al.,* 2020), the El Niño event in Sydney Harbour during 2015/16 (Goyen *et al.,* 2019), among others (*e.g.* Oliver *et al.,* 2018 and Smale *et al.,* 2019), being also highly correlated with mass coral bleaching events (Hughes *et al.*, 2018).

Climate change, mainly water temperature rise, has been strongly studied in hard corals (*e.g.* Dias *et al.,* 2018; Dias *et al.,* 2019a; Dias *et al.,* 2019b; Grottoli *et al.,* 2018; Paradis *et al.,* 2019; Torrents *et al.,* 2008). However, the same is not verified for soft corals, despite the abundance and importance they represent for the reefs (Strychar *et al.,* 2005). Only a few studies have been carried out in this direction (*e.g.* Kemp *et al.,* 2006 and Sikorskaya *et al.,* 2020) leading to a lack of information in this area.

The rise of water temperature induces several stress responses by the corals. This stress causes photoinhibition in photosynthesis which consequently increase the reactive oxygen species (ROS) concentration in form of *e.g.* superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) (reviewed by Lesser, (2006)). The production of ROS beyond what the organism can tolerate is called oxidative stress (for more detail please see section 2.7- Antioxidant mechanisms) and leads to cellular damage (Lesser, 2006), including cellular membrane lipid peroxidation (LPO) (Weis, 2008). Under normal conditions, both coral host and zooxanthellae have mechanisms (antioxidants defences) that control the amount of ROS present in their organisms. With great increase of ROS concentration, zooxanthellae are unable to respond (reviewed by Smith *et al.* (2005)). This leads to a reduction in the symbiont density and/or their pigments (Downs *et al.,* 2002; Weis, 2008), resulting in white coral tissue, the phenomenon called coral bleaching (William *et al.,* 2001). Bessell-Browne *et al.* (2014) support this theory with their study with *Cascinaraea marshae,* where it is suggested a decrease in the density of

symbiotic cells and consequent decrease in the amount of chlorophyll with the intensification of coral bleaching. These growing bleaching events (Hoegh-Guldberg *et al.,* 2007) can reach over large distances, affecting hundreds of kilometres, denominated as mass bleaching events (Marshall and Schuttenberg, 2006). Coral reefs become more vulnerable to other threats, as pathogens (Banin *et al.,* 2003) and even incapable of recovery from stress events (reviewed by Carballo-Bolaños *et al.,* 2019).

Coral's susceptibility to bleaching events has been primarily explained by coral morphology (Loya *et al.,* 2001), biochemical composition and metabolism (Rodrigues and Grottoli, 2007) and the different zooxanthellae clades (Abrego *et al.,* 2008 and Rowan, 2004) or densities (Stimson *et al.,* 2002). Dias *et al.* (2018) have demonstrated that different species are more susceptible to thermal stress than others, with temperature and exposure time being the main factors in coral bleaching. Temperature variation and exposure are correlated and essential to determine the timing and severity of the bleaching response. A small temperature increase can cause bleaching over a longer time while a high temperature increase can cause bleaching with shorter exposure time (Marshall and Schuttenberg, 2006).

Nevertheless, if the stress conditions reduce, zooxanthellae can recover or colonize again the bleached coral tissue and enhance the chances of survival (Marshall and Schuttenberg, 2006). Otherwise, if the stress continues, the coral will be covered with filamentous algae and consequently die (illustrated in figure 3). A better understanding of the host-symbiont interactions which regulate the physiology of the holobiont coral-algae endosymbiosis would provide new knowledge about how coral reefs may respond to environmental change (Abrego *et al*., 2008).

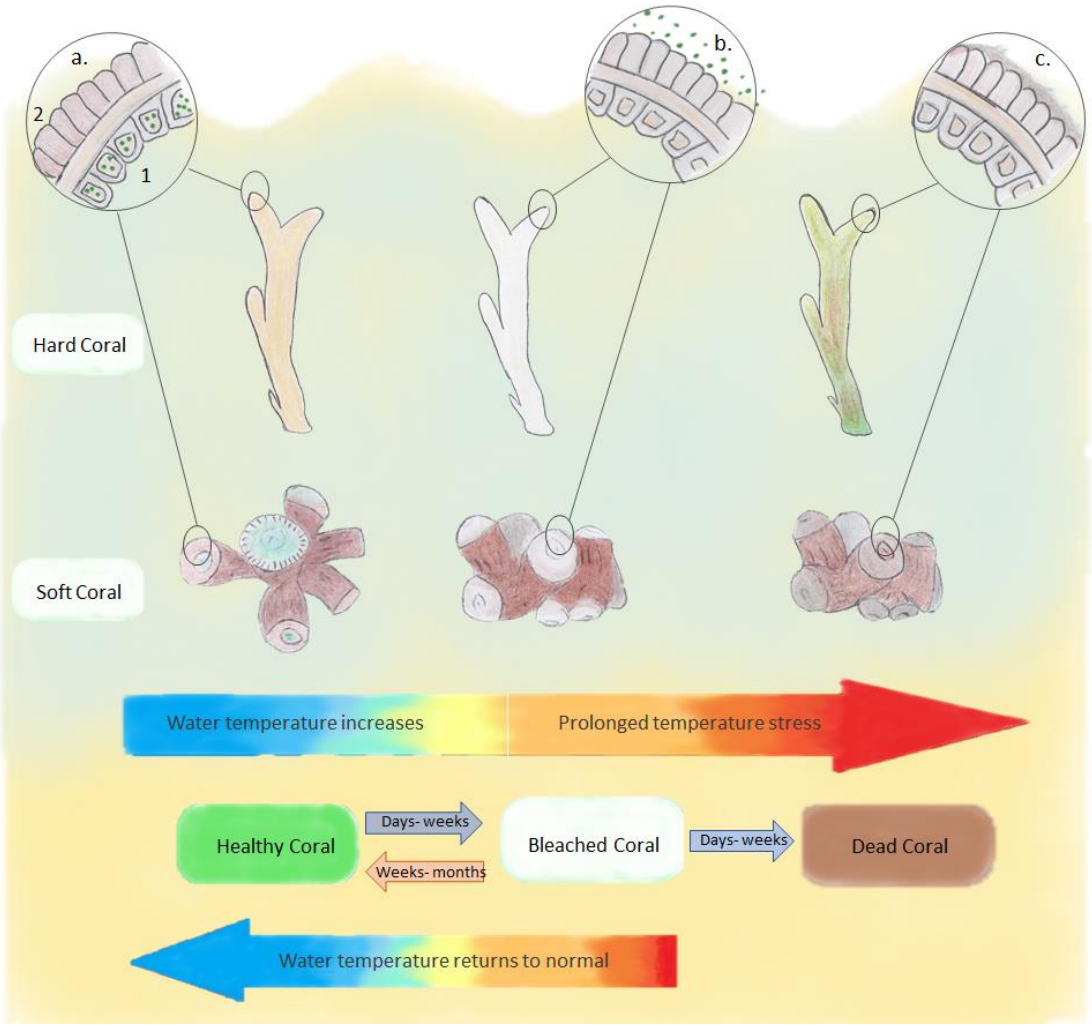

Figure 3 - Illustration of coral bleaching and coral dead. (a.) In a healthy coral the zooxanthellae live in symbiose with coral; when water temperature increase (b.) corals expel the zooxanthellae from their cellular (1) to extracellular (2), if the stress continues (c.) the prejudicial algae settle in coral tissue leading to their dead.

1.3.1.2. Feeding as a resource of the bleaching corals

Some recent studies have been done to evaluate if heterotrophic feeding enhance corals' resistance to climate events (*e.g.* Hadjioannou *et al. (*2019)). As a consequence of zooxanthellae absence in coral tissue due to bleaching, photosynthetic activity declines on bleached and recovering corals, forcing the need to find alternatives to the metabolic requirements. Thus, stored energies and heterotrophic feeding play an important role in bleaching corals (Grottoli *et al.,* 2006). As energy resources are limited, organisms that can increase heterotrophic input of fixed carbon during bleaching events must have an ecology fitted for long-term survival (Grottoli *et al.,* 2006). The time between the severe bleaching event

and the beginning of a high mortality event is influenced by two factors: (i) the lipidic storage before the beginning of coral bleaching, and (ii) the capability to acquire energy, *i.e.* carbon, from heterotrophic feeding (Anthony *et al.,* 2009). A research made by Bessell-Browne *et al.* (2014) suggests that bleached corals have higher heterotrophic levels supporting long-term survival while bleached. Following recovery, although, heterotrophic feeding levels appear to decrease, indicating an active change to the usual feeding strategy (Bessell-Browne *et al.,* 2014), demonstrating feeding plasticity according to needs. Additionally, Towle and colleagues (2015) report that fed corals, in contrast to the unfed corals, can maintain growth rates when exposed to climate change scenarios. However, an experimental assessment conducted by Ferrier-Pagès and her team (2010) shows that when in thermal stress, different species have distinct heterotrophic feeding rates. For example, *Turbinaria reniformis* and *Galaxea fascicularis* have a higher heterotrophic feeding rate when exposed to thermal stress, meanwhile, *Stylophora pistillata* has a higher heterotrophic feeding rate when not exposed to thermal stress. However, it should be noted that these three species have different metabolisms and physiological characteristics that may influence these different behaviours. Other studies have been demonstrated different behaviours relative to their heterotrophic capacity and recovery time from the photoautotrophic system (*e.g.* Hughes and Grottoli, (2013)), suggesting that each species reacts in distinct ways. Further research is needed to better understand what promotes the increment in host feeding adjusted according to each specie's physical and behavioural characteristics.

1.3.2 Coral aquaculture

Several strategies have been developed to promote coral health and coral resistance to stress conditions. Establishment of marine protected areas (MPA's) (Christie and White, (2007)), control of herbivory populations (Ledlie *et al.,* 2007), restoration of coral reefs (Montseny *et al.,* 2019) and repopulations of damaged coral reefs and evolving communities in conservation activities (Bambridge *et al.,* 2019). Key for the success of coral reefs repopulations are the selection of species more resistant to environmental stress (reviewed by Van Oppen *et al.,* 2015) and aquaculture (Spalding *et al.,* 2001). Coral aquaculture can be performed *ex situ* (Forsman *et al.* (2006) and Rocha *et al.,* (2013b)) or *in situ* (Bongiorni *et al.,* 2011 and Linden *et al.,* 2019), aiming natural compounds production for cosmetic and pharmaceutical industries (Leal *et al.,* 2018); biomass culture for the aquarium trade; and reef restoration campaigns. *In situ* propagation can be operated in fixed or/and suspended coral

nurseries usually located in a protected area with appropriated conditions which benefits from the natural environmental (reviewed by Leal *et al.,* 2018). This method is less expensive (without feed and light input) however organisms are exposed to uncontrolled biotic and abiotic factors when compared with *ex situ* method. The major risks of *ex situ* propagation are power failure and disease outbreaks being expensive due to the high costs, mostly with electricity (reviewed by Barton *et al.,* 2017) since the use of recirculated systems (Rocha *et al.,* 2015).

Some research has been conducted in order to optimize coral growth *ex situ*. Such studies focus on different kinds of sediment (Lirman, 2000), different fragmentation types, orientation (Soong and Chen, 2003) and size (Forsman *et al.,* 2006), adequate water flow (Sebens *et al.,* 2003), quality (reviewed by Borneman, 2008) and water movement (Forsman *et al.,* 2012), the effect of different light source (Rocha *et al.,* 2013b) and light intensity (Rocha *et al.,* 2013a), and nutritional assays (Conlan *et al.,* 2019). An optimal growth diet represents an added value for aquaculture and some studies have been done to find the optimal diet for some coral species. Most of them in scleractinian corals (*e.g.* Conlan *et al.* (2019); Houlbrèque and Ferrier-Pagès, (2009); Tagliafico *et al.* (2018)) meanwhile, in soft corals few researches have been carried out in this direction (Costa *et al.,* 2016).

The optimized coral growth is a key factor for aquaculture since this provides higher growth rates being able to decrease the pressure on natural stocks by decreasing the need to collect colonies from the natural environment for other purposes (Leal *et al*., 2013; Lecaillon, 2004), since the capacity of restocking natural stocks are in decline (*e.g.* Bongiorni *et al.* (2011)).

1.4 Study objective

This study aimed to evaluate if heterotrophic feed supply increases (or not) coral resilience facing thermal stress events. For this, feed (experimental microencapsulated diet previously optimized for this species) and non-feed *Palythoa sp.* colonies were exposed to a five degrees water temperature rise during eight days. To our knowledge, this is the first study where optimal nutrition and heat stress were combined in Zoantharia.

2. Methodology

2.1 Coral species collection and fragmentation

This study evaluates an Indo-pacific coral specie, *Palythoa sp.*. The mother-colonies were collected from their natural environment, at Batam, Indonesia. After arriving in our laboratories, colonies were stock in recirculated systems (see section “Recirculated System and experimental design”) for adaptation to laboratory conditions. Parameters remained stable, within the following values: salinity at 35 ± 1; temperature at 25 ± 1 °C; Photosynthetically Active Radiation (PAR) of 60 - 90 µmol m^{-2} s^{-1} (Apogee MQ-500 PAR Meter); phosphates, nitrites, nitrates and ammonia approximately 0 ppm (table i). A 12:12 (light : dark) photoperiod was used.

Table i- Mean ± Standard deviation of the measured values.

Aquarium	Phosphates	Nitrates	Calcium (ppm)	Magnesium (ppm)	KH (dKH)	Salinity	pH	Temperature	Dissolved oxygen
1	0 ± 0	0 ± 0	475± 0	1440 ± 80	6,5 ± 1,9	36,2 ± 0,2	8,0 ± 0	26,1 ± 0,3	8,3 ± 0
2	0 ± 0	0 ± 0	466,7 ± 27,5	1430 ± 78,1	8,3 ± 1,7	36,2 ± 0,1	8,1 ± 0	30,7 ± 0,3	7,7 ± 0,2

After one month of acclimatation, coral fragmentation was proceeded with a scalpel, a spatula, and a tweezer. *Palythoa sp.* colonies were fragmented, cutting the coenenchyma tissue, into mini colonies of three polyps and glued with n-butyl-cyanoacrylate to the base. After, mini colonies were stocked into an identical system for two months to allow cicatrisation and recovery.

The bases for coral fragments (as the ones that are represented in figure 4) were previously handmade with cement and aragonite, left in osmosis water for two weeks to wash any present chemical and then left to dry. This type of base was used because it can work as a biological filter, due to its porosity, and it is also possible to adapt according to the required features (more porous, thinner, perforated, etc.).

Figure 4- Example of a coral base handmade in laboratory.
Photo provided by Davide Silva

2.2 Recirculated System

The acclimatation and experimental recirculated systems were identical. The systems were composed of two tanks with a capacity of 240 litres (150cm × 40cm × 40cm) each one, a filtration sump with a capacity of 180 litres (80cm × 45cm × 50cm) and a tank with reverse osmosis (R.O.) water with a capacity of 54 litres (30cm × 30cm × 60cm) (figure 5).

The water movement inside the aquarium was provided by two circulation pumps (Turbelle nanostream 6055, TUNZE - Germany), one on each side and a T5 fluorescent light system (Sea REEF-SPEC, 2 x 80W) was placed above the aquarium with one red and one actinic light.

The filtration system was located in the sump and it is composed by five components, (i) a protein skimmer (Deltec SC 500) which removes the dissolved organic compounds (DOC) from water; (ii) active carbon which does chemical filtration by adsorbing dissolved contaminants that can negatively affect the water quality; (iii) bio-balls which make biological filtration through nitrifying bacteria and a (iv) Kalkwasser reactor (Kalkwassermischer km 500)

used to maintain the pH and the carbonate hardness; and (v) a R.O. water replacement system (Deltec aquastat 1001).

The water in the system was circulated through the skimmer and two heaters with thermostats (Eheim Jagger 300W). After that, it passed by the active carbon, sand and bio-balls. Next, the water was pumped (EHEIM universal 1200 l/min) into the Ultraviolet (UV) filtration (Vecton 600, TMC), which led to a disinfection process of the water. In the end, the water was moved from the UV to the chiller (Hailea Model: HC-500A), previously set for 25 °C, and then the water was ready to go back to the aquarium by another pump (EHEIM universal 2400 l/min). In addition, an osmosis water supply system was used, to avoid a salinity increase when evaporation occurs. The water level sensor (Deltec Aquastat 1001) detects when the level decreases, activating the osmosis water pump (Rena Flow 400) which delivers osmosis water in the sump. The saltwater was made with synthetic salt (Coral PRO salt, Red Sea) and with osmosis water, which is freshwater that passes by an osmosis reverse filtration system (V2 Pure 360, TMC).

Figure 5- Illustration of the aquarium system composed of two aquariums and with the filtration system located in the sump.

2.3 Diets and feeding

The microencapsulated diet was produced by SPAROS I&D, situated in Olhão, Portugal. The mixtures of squid and fish meal diet were homogenised and subjected to extrusion (diameter: < 100 µm), using an extruder (pilot scale) with double screw (Clextral BC45; Clextral, France). A total of 49 mini colonies were fed with a previously tested optimal growth diet (data not published yet), composed by squid and fish meal (SFM), in one recirculated system. Other 49 mini colonies were kept in another system without external feed supply. Note that, as previously referred, corals have diverse nutrition, meaning that they are not fasting in this treatment. Corals were rotated within experimental tanks, to avoid any positional effects. The supplied diet was provided two times per week, for four months. Before feeding, the circulation pumps and the water inlet valve were switched off and 117 mg (0.585 mg L^{-1}) were provided to fed corals, with a Pasteur pipette. Feed intake was ensured by observing the polyps' mouth closure (Reimer, 1971). After 30 minutes of supply, the water inlet valve was turned on.

Table ii- Chemistry of squid and fish meal diet.

Diet / Compound	SFM
Humidity (%)	10.0
Protein (%MS)	60.0
Fat (%MS)	12.0
Ashes (%MS)	-
Fibre (%MS)	2.6
Energy (MJ Kg-1 MS)	23.3

2.4 Temperature challenge

Immediately before the temperature rise, it was necessary to put in the same aquarium both fed and unfed corals. To ensure that both groups were under the same conditions the feeding was ceased during the temperature challenge. Coral fragments were exposed to a gradual water temperature rise for 24 hours until reaching the maximum temperature (30 ± 1ºC) and were maintained for eight days (figure 6). This temperature was chosen concerning some MHWs extreme events already recorded and reported by Reed *et al.* (2016) and their duration was chosen in relation to the most common frequency of MHWs events (Oliver *et al.*, 2018).

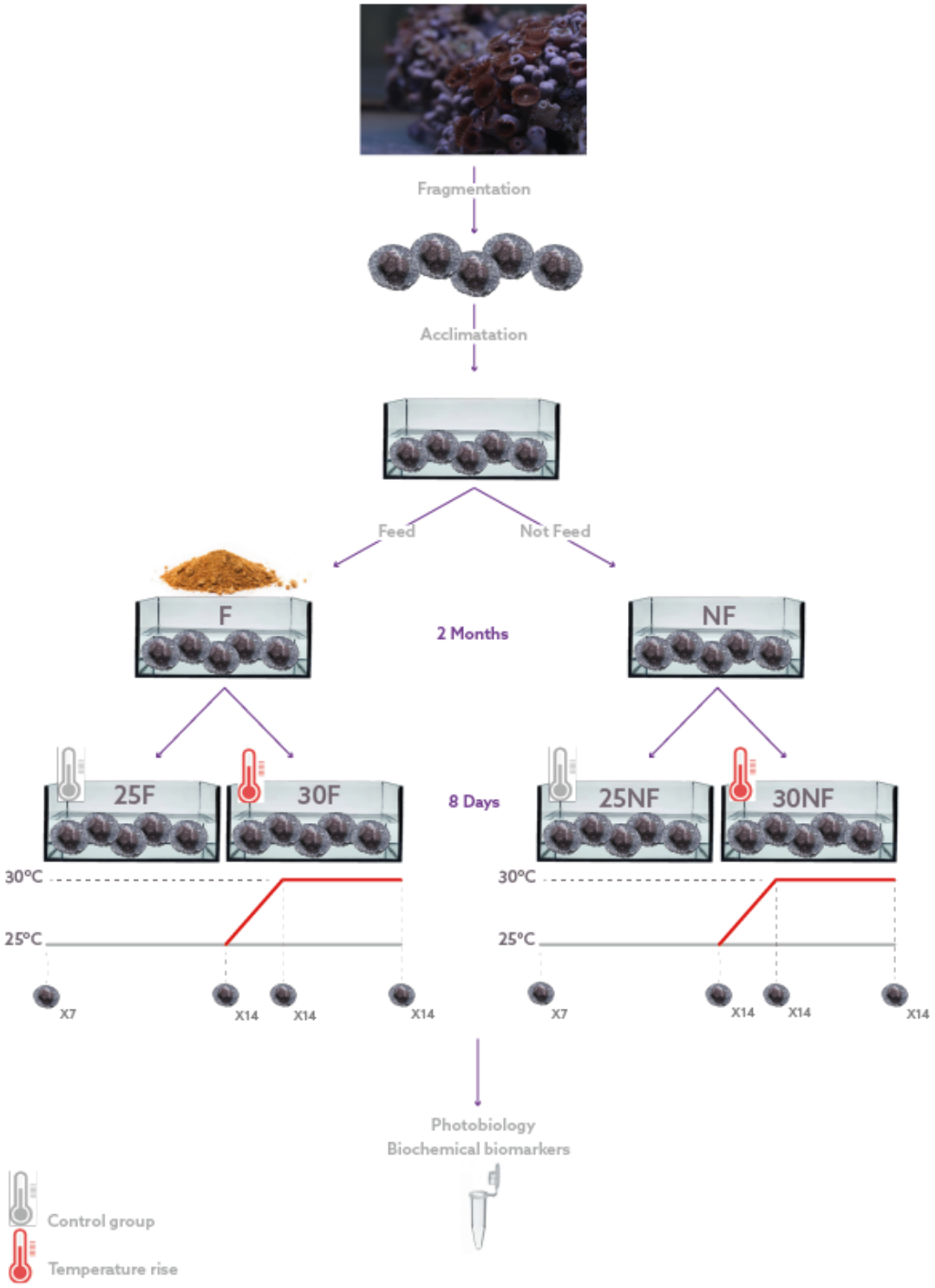

Figure 6- Representative scheme of the experimental design. First a fragmentation was carried out from mother colonies, then acclimation was performed for four months. After this period half of the fragments were fed (F) with a squid and fish meal-based diet and another half was maintained without any supplementation (NF). Subsequently, a temperature increase was performed on not-fed (30NF) and fed (30F) corals, consisting of a 24-hour temperature increase that remained stable for eight days. A control group with not-fed (25NF) and fed (25F) corals was always maintained at a constant temperature of 25ºC. Sampling for photobiology and biomarker analysis was carried out before temperature increase (Ti) where seven fragments of fed (F) and unfed (NF) corals were taken; at the time of temperature increase (T0) where seven fragments from each treatment (25NF, 25F, 30NF, 30F) were sampled; one day after temperature remained at 30ºC where seven fragments from each treatment were sampled; and eight days (T8) after temperature remained stable where seven fragments from each treatment were also sampled.

-

For this, 49 corals were submitted to the temperature rise and 49 remain with the common temperature (control). Four sampling points were performed during the challenge, the first one immediately before the temperature rise (T_i), second sampling point when the temperature reached the 30ºC (T_0), third sampling point one day after the temperature reached the 30ºC (T_1), and the last sampling point eight days after the temperatures reached the 30ºC (T_8). Analysis of oxidative stress biomarkers and photobiology analysis were performed in each sample. No mortality was observed during the temperature challenge.

2.5 Photobiology Analysis

The photosynthetic activity of zooxanthellae was measure by Pulse-Amplitude-Modulated (PAM) fluorometer (Junior-PAM, Walz TM, Germany), recognized for being a non-invasive method, easy to handle (Schreiber, 2016) that can indirectly detect stress (Dove and Hoegh-Guldberg, 2011). A 1.5 mm plastic optical fibre placed perpendicular to the coral, was used to transmit the modulate and the saturated light pulses. Light energy that reaches photosynthetic organisms can follow three pathways: (i) photosynthesis, through photochemical reactions in the reaction centre of photosystem II (PSII) (photochemical quenching pathway); (ii) dissipation of energy in form of heat (non-photochemical quenching pathway); and (iii) radioactive decay of the energy via fluorescence. The last pathway is the light energy measured by fluorometry method, which provides the information regarding the amount of light energy used in the first two described pathways (reviewed by William *et al.* (2001)).

When coral fragments are dark adapted the ability of the photochemical pathways to absorb light energy is maximized. The measuring light pulse of the PAM fluorometer will be almost completely absorbed by the opened reaction centres of PSII, thus the fluorescence is minimal (F_o) while a saturation light pulse will cause the PSII reaction centres' to close, and the maximum fluorescence (F_m) is measured. In this study, the samples were exposed to a dark period of at least 15 minutes before the measure (dark adapted fluorescent). The dark period must be long enough to ensure that the PSII reaction centres are completely open and competitive events between the photochemical paths are minimized (William *et al.,* 2001).

To calculate the fluorescence yield ratio, presented in the following equation, the measure of F_o, a parameter reported being related with the amount of chlorophyll *a* (Serôdio *et al.,* 2001) and F_m was taken. This ratio indicates the potential photochemical capacity of the PSII in the zooxanthellae (William *et al.,* 2001), where F_v is the variable fluorescence.

$$F_v/F_m = (F_m - F_0) \ / \ F_m$$

2.6 Biochemical biomarkers

Oxidative and thermal stress biomarkers, as mentioned above, maintain the ROS concentration in tolerable levels for the cells (reviewed in Smith *et al.,* (2005)). These antioxidant mechanisms can be non-enzymatic (*e.g.* Total Glutathione (TG) and Heat Shock Protein (HSP)) and enzymatic (*e.g.* Catalase (CAT), and Glutathione S-Transferase (GST)). Also, it is possible to observe cellular damage through the rupture of the cell membrane and the formation of lipid peroxides referred to as lipid peroxidation (LPO). In addition, total lipid, protein and carbohydrate content were assessed for testing the general metabolic activity of the organism.

2.6.1 Sample preparation

Frozen corals were individually smashed in a ceramic mortar and a pestle with the help of liquid nitrogen and homogenized on ice through an ultrasound equipment, sonicator, (pulsed mode of 10% for 30 s, 250 Sonifier, Branson Ultrasonics) with 1400 µL of ultra-pure water. From each homogenate sample one aliquot of 200 µL was taken for the determination of lipid peroxidation with the addition of 4 % butylated hydroxytoluene (BHT) in methanol, three aliquots were taken for the analysis the cellular energy allocated (CEA) who allows the quantification of lipids (250 µL), sugar and protein contents (250 µL), and for the energy consumption which is measured by electron transport system (ETS) (250 µL). The remaining homogenate was diluted with 0.2 M K-phosphate buffer, pH 7.4, and centrifuged for 15 min at 10,000 g (4 ºC). An aliquot of 100 µL for the post-mitochondrial supernatant (PMS) was used to assess catalase, glutathione S-transferase and total glutathione activities. The PMS was divided into microtubes and stored in −80 °C until further analyses.

All biomarkers determinations were performed spectrophotometrically adapted to micro-assays set up in 96 well flat bottom plates (Rodrigues *et al*., 2017, 2015), with the Microplate reader MultiSkan Spectrum (Thermo Fisher Scientific, USA).

2.6.2 Oxidative stress biomarkers

As stated above the PMS fraction allows us to calculate CAT and GST activity. In addition it was possible to calculate the protein concentration of PMS according to the Bradford method (Marion M Bradford, 1976), using bovine γ-globulin as a standard. Catalase activity was determined by measuring decomposition of the substrate H_2O_2 at 240 nm (Clairborne, 1985). Glutathione-S-transferase activity was determined following the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm (Habig *et al.*, 1974). Endogenous lipid peroxidation (LPO) was determined by measuring thiobarbituric acid-reactive substances (TBARS) at 535 nm (Bird and Draper, 1984). Protein carbonylation was measured by the quantification of carbonyl groups based on the reaction of 2,4-dinitrophenylhydrazine (DNPH) with carbonyl groups, according to the DNPH alkaline method described by Mesquita *et al.* (2014). The amount of carbonyl groups was quantified spectrophotometrically at 450 nm (22,308 $mM^{-1}cm^{-1}$extinction coefficient) and results were expressed in nmol carbonyl per mg protein.

2.6.3. Heat shock proteins (HSP70)

HSP70/HSC70 content was assessed by ELISA, adapted from Rosa *et al.* (2014). The sample was added to a 96 well microplate and allowed to incubate overnight at 4°C. The next day, the microplates were washed (3×) in 0.05% PBS-Tween-20. A blocking solution (1% BSA, Sigma-Aldrich) was added to each well and left to incubate at room temperature for 2 h. Microplates were washed (3×) with 0.05% PBS-Tween-20 and 5 µg ml^{-1} primary antibody was added to each well for overnight incubation at 4ºC (1º Anti-HSP70 mouse mAB (C92F3A-5) Millipore). This procedure detects 72 and 73 kDa proteins, which corresponds to the molecular mass of inducible HSP70 and HSC70. The non-linked antibodies were removed by washing the microplates again, which were then incubated overnight at 4°C with 1 µg ml^{-1} of the secondary antibody, anti-mouse IgC (2º Anti-mouse IgC (fab specific) Sigma). After another wash, substrate p-nitrophenyl phosphate was added to each well and incubated for 30 min at room temperature. Then, a stop solution (3 mol l^{-1} NaOH) was added to each well and the

absorbance was read at 405 nm in a 96 well microplate reader (Benchmark, Bio-Rad, Hercules, CA, USA). The amount of HSP70/HSC70 in the samples was calculated from a curve of absorbance based on serial dilutions of purified HSP70 active protein standard (HSP70 protein Millipore) to a range from 0 to 2000 ng ml^{-1}.

2.6.4. Cellular energy allocation

The energy consumption (ETS) and the energy available (lipids, protein and carbohydrates) were assessed following the method of De Coen and Janssen (De Coen and Janssen, 1997) with a few modifications for microplate reading (Rodrigues *et al.*, 2015). The final CEA value is calculated as: CEA = Ea / Ec (Verslycke *et al.*, 2004).

Energy available (Ea)

Total lipid content was separated by centrifuged (1000g during 5 minutes to 4°C) homogenate sample with the addition of chloroform, methanol and pure water in a 2:2:1 proportion. Then the organic phase of each sample was separated for a glass tube and 500 µL of H_2SO_4 (95 - 98%) was added and incubated at 200°C for 15 minutes. After cooling down ultra-pure water was added (700 µL) to each tube and the absorbance was measured in the microplate at 350 nm using tripalmitin as a lipid standard.

For the aliquot of carbohydrate and protein was added 98 µL of TCA to 250 µL of homogenate sample and centrifuged at 1000 g during 10 minutes to 4°C. Then the supernatant (used for carbohydrates) and the pellet (used for proteins) were separate. To the supernatant (100 µL) was added 200 µL of phenol (5%) and 800 µL of H_2SO_4 (95-98%). After an incubation period of 30 minutes at 20°C the absorbance was read to 492 nm using glucose as a standard. For protein quantification was added to the pellet 500 µL of NaOH, incubated (30 min at 60°C) and neutralized with 250 µL of HCl. Total protein content was then quantified following Bradford's (Bradford, 1976) method at 592nm and with bovine serum albumin as standard. Fractions of energy available are converted into energetic equivalent values using the corresponding energy of combustion: 39500 mJ g^{-1} lipid, 17500 mJ g^{-1} glycogen, 24000 mJ g^{-1} protein (Gnaiger, 1983).

Energy consumption (Ec)

Electron transport system activity was measured using the INT (Iodonitrotetrazolium) reduction assay in which ETS was measured as the rate of INT reduction in the presence of the nonionic detergent Triton X-100, with the absorbance read at 490 nm. Cellular oxygen consumption rate is calculated based on the stoichiometrical relationship in which for 2 µmol of formazan formed, 1 µmol of oxygen is consumed. Ec value is obtained by the conversion to energetic values using the specific oxyenthalpic equivalent for an average lipid, protein and carbohydrate mixture of 480 kJ/ mol O2 (Gnaiger, 1983).

2.7 Statistical Analyses

All the obtained results are presented graphically in boxplots. Statistical analyses of *Fv/Fm*, CAT, GST, LPO, HSP70, proteins, carbohydrates, lipids, Ea, Ec and CEA were performed using R program version 4.0.2.

After conducting a test of data normality and since we were unable to achieve normality, we proceeded to a permutational multivariate analysis of variance (PERMANOVA). The null hypothesis of this test is that the metric centroid does not differ between groups (in our case, Diet, Temperature and Time). PERMANOVA was conducted with *adonis()* function in Vegan package. Since this test is sensitive to data dispersion and could be confused within-groups variation with among-groups variation, we performed an analysis of multivariate homogeneity (PERMDISP) with the *betadisper()* function to test if groups differed in their dispersion. The null hypothesis of this test is that the average within-group dispersion is the same in all groups. For these two tests mentioned above the number of permutations was set to 9999 and a *p-value* of > 0.05 was considered significant.

After PERMANOVA, the Kruskall Wallis test followed by a post-hoc Conover test was conducted were conducted for the significant PERMANOVA results to reveal the source of those differences. Conover reports the results among multiple pairwise comparisons after a Kruskal-Wallis test for stochastic dominance among groups. The null hypothesis for each

pairwise comparison is that the probability of observing a randomly selected value from the first group that is larger than a randomly selected value from the second group equals one half. Bonferroni corrections were conducted for the Conover test to control the family wise error rate, with an adjusted *p-value*, preserving the alpha value at 0.05.

3. Results

PERMANOVA results (table iii) showed significant differences in factor diet (p-value < 0.001), in factor time (p-value < 0.001) and in the interaction between time and diet (p-value < 0.01). Permanova, is sensitive to the dispersion of distances between points in the same groups. To ensure that the statistical test results were not being influenced by this condition, dispersion tests were performed for each factor. No statistically significant differences were detected in diet, temperature, and time factors (p-value = 0.350, p-value = 0.341, p-value = 0.09 respectively) for dispersion analyses.

Table iii- Results of PERMANOVA test for all the variables.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Time	3	105.76	35.252	3.6074	0.10120	0.0001	***
Diet	1	41.92	41.917	4.2893	0.04011	0.0005	***
Temperature	1	8.38	8.378	0.8573	0.00802	0.5400	
Time:Diet	3	63.41	21.137	2.1629	0.06068	0.0024	**
Time:Temperature	3	17.41	5.802	0.5937	0.01666	0.9399	
Diet:Temperature	1	3.38	3.383	0.3462	0.00324	0.9609	
Time:Diet:Temperature	3	22.96	7.654	0.7833	0.02197	0.7518	
Residuals	80	781.79	9.772		0.74812		
Total	95	1045.00			1.00000		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.1 Photobiology Analyses

The statistical results for photosynthetic analyses are represented in figure 7. Significant differences for *F*v/*F*m were just detected in the control group (25NF) between the first assessment (Ti) and the final assessment (T8) (p-value <0,05) and between Ti and the third assessment (T1) (p-value < 0,05). Also, statistic differences were reported between Ti and T1 (p-value < 0,001) in stressed corals which were not fed (30NF). Besides the statistical differences, all treatments showed a decrease pattern in the *F*v/*F*m measures with an exception in both fed corals groups which show a recovery in the last sample time.

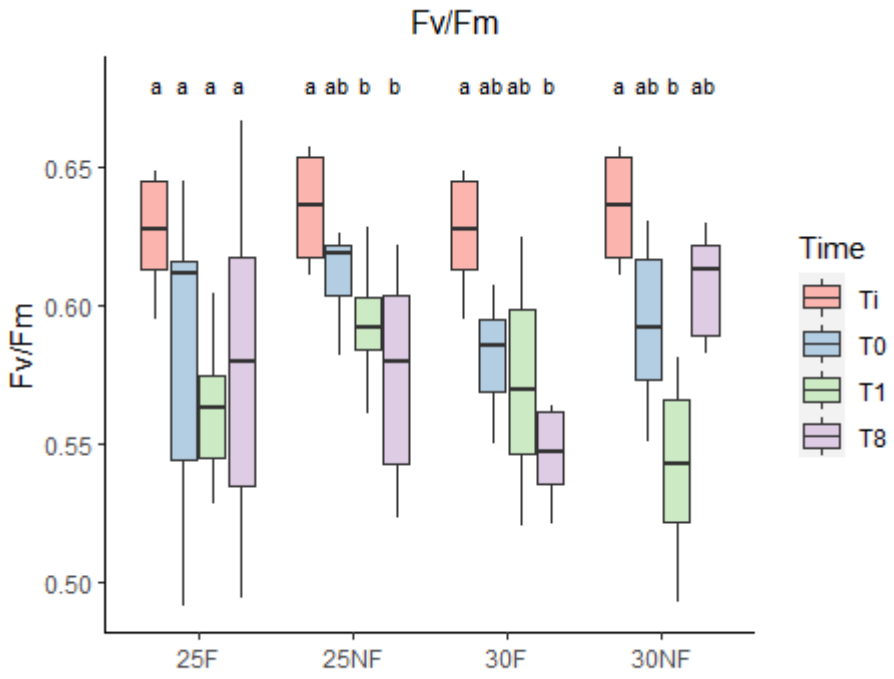

Figure 7- Graphical illustration of the photobiology for the four different groups: 25F- 25°C with food supply; 25NF - 25°C without food supply; 30F - 30°C with food supply; 30NF - 30°C without food supply. Also, different sampling points are represented as time: Ti – Before temperature rise; T0 – when temperature reaches their maximum; T1 – one day after being exposed to maximum temperature; T8- eight days after being exposed to maximum temperature. Different colours represent the four sampling points. Significant statistical differences (p < 0.05) are represented as letters (a, b and ab) when significant differences are reported within each treatment.

3.2 Oxidative stress

Catalase activity (figure 8. A) shows a significant difference at T8 between the groups heated fed (30F) and heated not-fed with a p-value < 0,05. Even though there are no additional statistical differences, it is possible to observe two different patterns, one for corals who were subjected to a feed supply and another one in the corals who did not. It should also be highlighted that in stressed corals the fed ones start with higher activity of catalase than the not-fed corals. Also, the group of corals who were fed shows more oscillations than the opposite group, especially in the corals who were exposed to thermal stress.

For the GST activity (figure 8. B) differences were found between the first and the third sampling point (p-value < 0,05) and between Ti and the last sampling point (p-value < 0,05) both in the control not fed group (25NF). Besides these statistical differences it can be observed that in opposite to catalase activity, corals without a feed supply had higher GST activity than the opposite group. In addition, corals under higher temperatures showed an increase pattern regarding to GST activity, with statistical differences between the first and the third sampling point (p-value < 0,05). In the absence of additional statistical differences, it should be noted that in all study groups GST activity is tending to increase.

Significant differences for HSP70 (figure 8. C) were found at T0 between fed and not fed corals who were exposed to a temperature rise (p-value < 0,05). Despite these differences, fed corals group present higher HSP70 concentration than not-fed corals at the beginning of the experimental essay. Also, in the heated corals, both of the different groups show different patterns, with the fed corals increasing their HSP70 content at T0, and then decreasing until T8. In the not-fed corals their HSP70 concentration decreases between Ti and T0 increasing in T1 and then decreasing briefly in T8. Unheated corals also decrease their HSP70 concentration especially the not-fed corals, despite this reduction, during all sampling points the fed corals present higher HSP70 activity than not-fed corals.

No statistically significant differences were detected for the LPO (figure 8. D) however, it is possible to observe that fed corals showed higher values at the beginning of the assay than not-fed corals. Fed corals in the heated treatment decrease their values of lipid peroxidation in the other sample points, in opposite to this, in the not-fed corals, the LPO values decrease in T0 and then increase until T8. In contrast, corals in control group which have not been

supplemented increase slightly until the values become constant in T1 and T8. Otherwise, supplemented corals showed a decrease between the first and the second sampling point and then a major LPO activity until the end of the experiment. This variable is the one which presents the major dispersion representing a greater variability in data.

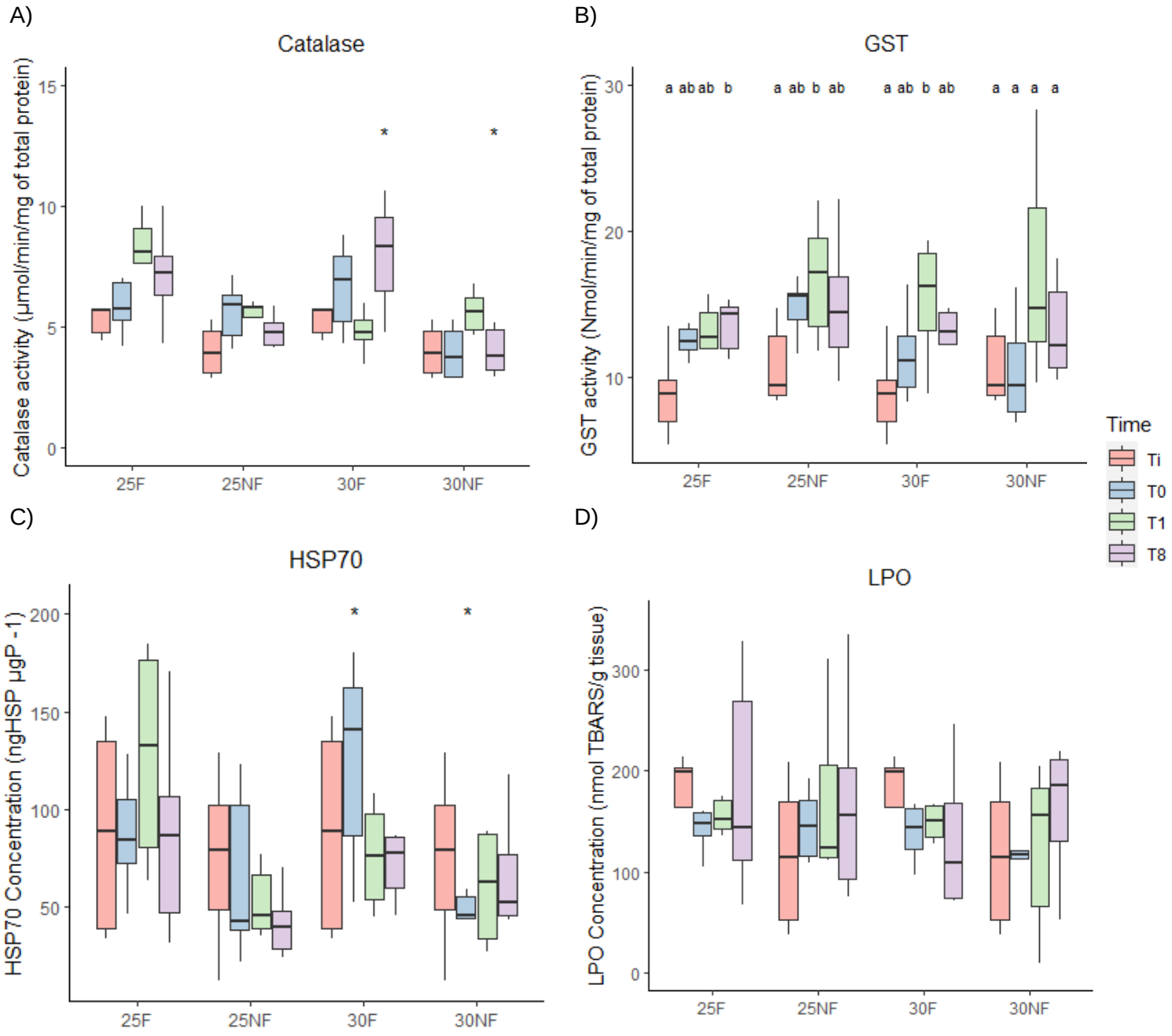

Figure 8- Graphical illustration of the analysis of Catalase activity (A), GST activity (B), HSP70 concentration (C) and of LPO concentration (D) for the four different groups: 25F - 25°C with feed supply; 25NF - 25°C without feed supply; 30F - 30°C with feed supply; 30NF - 30°C without feed supply. Also, different sampling points are represented as time: Ti – Before temperature rise; T0 – when the temperature reaches their maximum; T1 – one day after being exposed to maximum temperature; T8- eight days after being exposed to maximum temperature. Significant differences are represented as asterisks (*) when significant differences ($p < 0.05$) are reported at the same time point between treatments and as letters (a, b and ab) when significant differences are reported within each treatment.

3.3 Cellular Energy Allocation

Not-supplemented corals contained more lipid content (figure 9. A) than supplemented corals did at the beginning of the experiment (Ti), however in stressed corals, at T0, fed corals (30F) increased their lipid content while not-fed corals (30NF) decreased, showing statistically significant differences (p-value < 0,05). Nevertheless, not-fed corals (30NF) recover their lipid content increasing in T1 and then decreasing again in T8. In the control groups, oscillations of this variable also occur, especially in the not-fed corals (25NF), however no additional statistically significant differences were detected in any treatment.

The protein content in all treatments showed a tendency to reduce their concentration. This reduction is more visible in not supplemented corals (both control and temperature rise groups). In contrast with the other variables, this one showed similar concentration in all groups at the beginning of the experiment. The greater difference in stressed corals is that fed corals (30F) just significantly reduce their protein content in the last assessment, being this statistically different from the first assessment (p-value < 0,05), from T0 (p-value < 0,05) and from T1 (p-value < 0,05). Although there are no additional statistically significant differences, a reduction is observed in not-supplemented corals. This reduction is more gradual with a slight recovery in the final sample point. In addition, a considerable reduction in the not supplemented control group can be seen in figure 9. B, especially after sample point Ti.

Carbohydrates do not report statistically significant differences, but a tendency to reduce their content is observed in all treatments. As observed in figure 9. C, the greatest reduction is represented in stressed corals, which have been supplemented (30F), at T0, with a recovery in the next sampling points. The remaining treatments do not show very strong oscillations, presenting very similar values between sampling points of the same treatment.

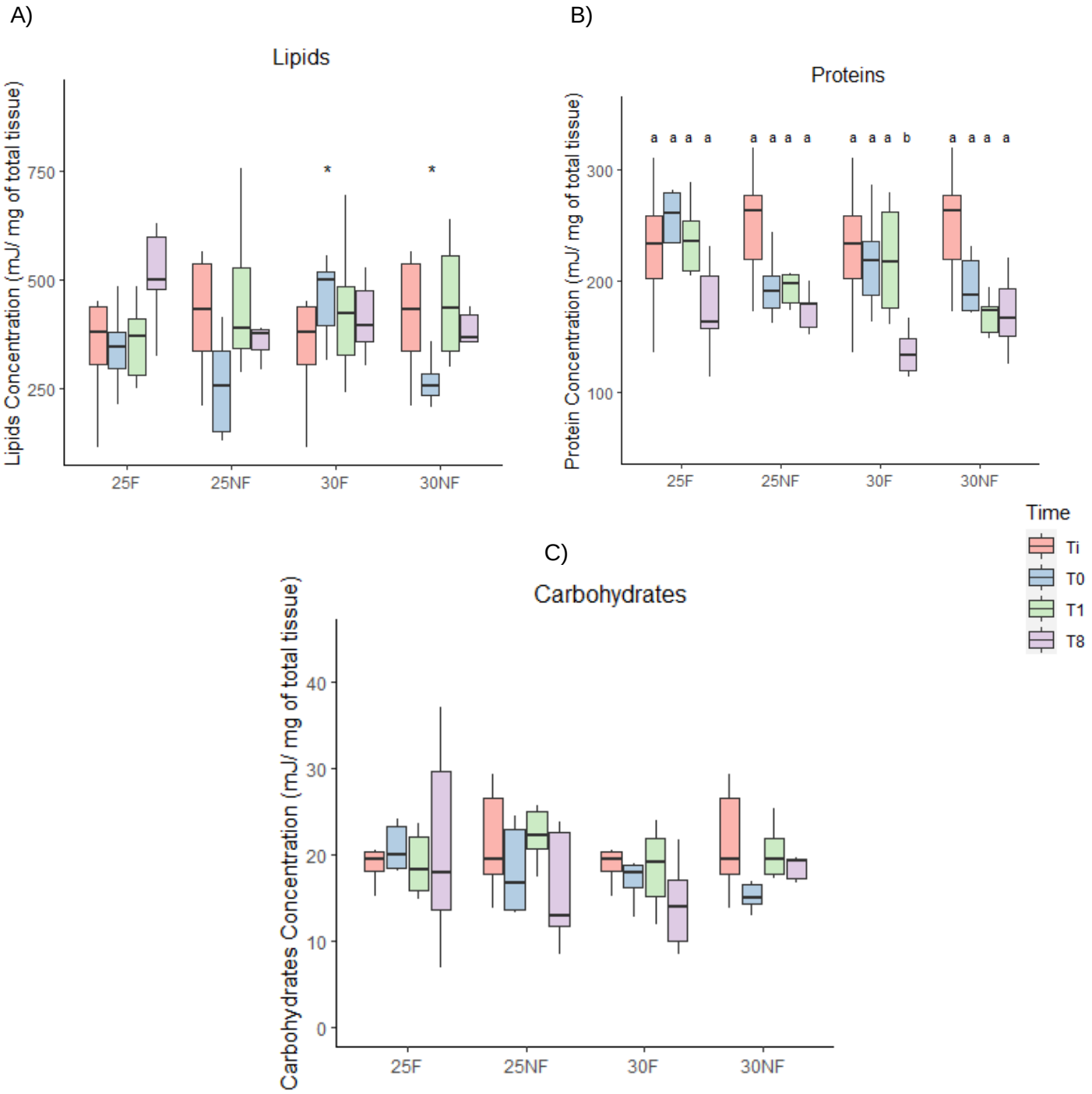

Figure 9- Graphical illustration of the analysis of lipids concentration (A), proteins concentration (B) and carbohydrates concentration (C) for the four different groups: 25F - 25°C with feed supply; 25NF - 25°C without feed supply; 30F - 30°C with feed supply; 30NF - 30°C without feed supply. Also, different sampling points are represented as time: Ti – Before temperature rise; T0 – when the temperature reaches their maximum; T1 – one day after being exposed to maximum temperature; T8- eight days after being exposed to maximum temperature. Significant differences ($p < 0.05$) are represented as (i) asterisks (*) when significant differences are reported at the same time point between treatments and as (ii) letters (a, b and ab) when significant differences are reported within each treatment.

Energy available is another parameter in this study, however, it does not show any statistically significant difference. Although some oscillations are observed in figure 10.A, the initial energy available is similar in both not-fed and fed corals, and even between stressed and control groups, the energy available is similar. Highlighting only a slight decrease which occurs in the heated not-fed corals (30NF) between the first and the second assessment which is then followed by a recovery that extends until the end of the assay. Our data variability is clearly demonstrated by the biggest standard error in some treatments, especially at Ti and T1.

The energy consumption (Fig. 10.B) was only significantly lower between corals from the same treatment (30F) at T8, when compared to all other sampling points (Ti $p < 0,05$; T0 $p < 0,05$; and T1 $p < 0,01$). When looking for differences between fed and not fed corals, within the 30º C of temperature, they are only present for the T8 sampling. Corals without any feed supplement add higher energy consumption after eight days under high temperatures than the fed ones ($p < 0.05$).

Cellular energy allocation (CEA) exhibits different patterns regarding different treatments (figure 10.C). Corals that have not been supplemented present a higher CEA when compared with supplemented corals at the beginning of the experiment. In the control fed group, the CEA decreases since Ti with an increment in CEA in the last sampling point however in the not-fed corals this increment does not occur. Corals that were exposed to thermal stress without feed supply started with a statistically significant reduction between the first and the second sampling point (p-value < 0,05). Corals that were exposed to thermal stress and with feed supply presented statistical differences between the second and the last assessment with a p-value < 0,05. Conover test also reported a significant difference in the stressed corals group in the last sampling point between not-fed and fed corals (p-value < 0,05), having the latter the highest value.

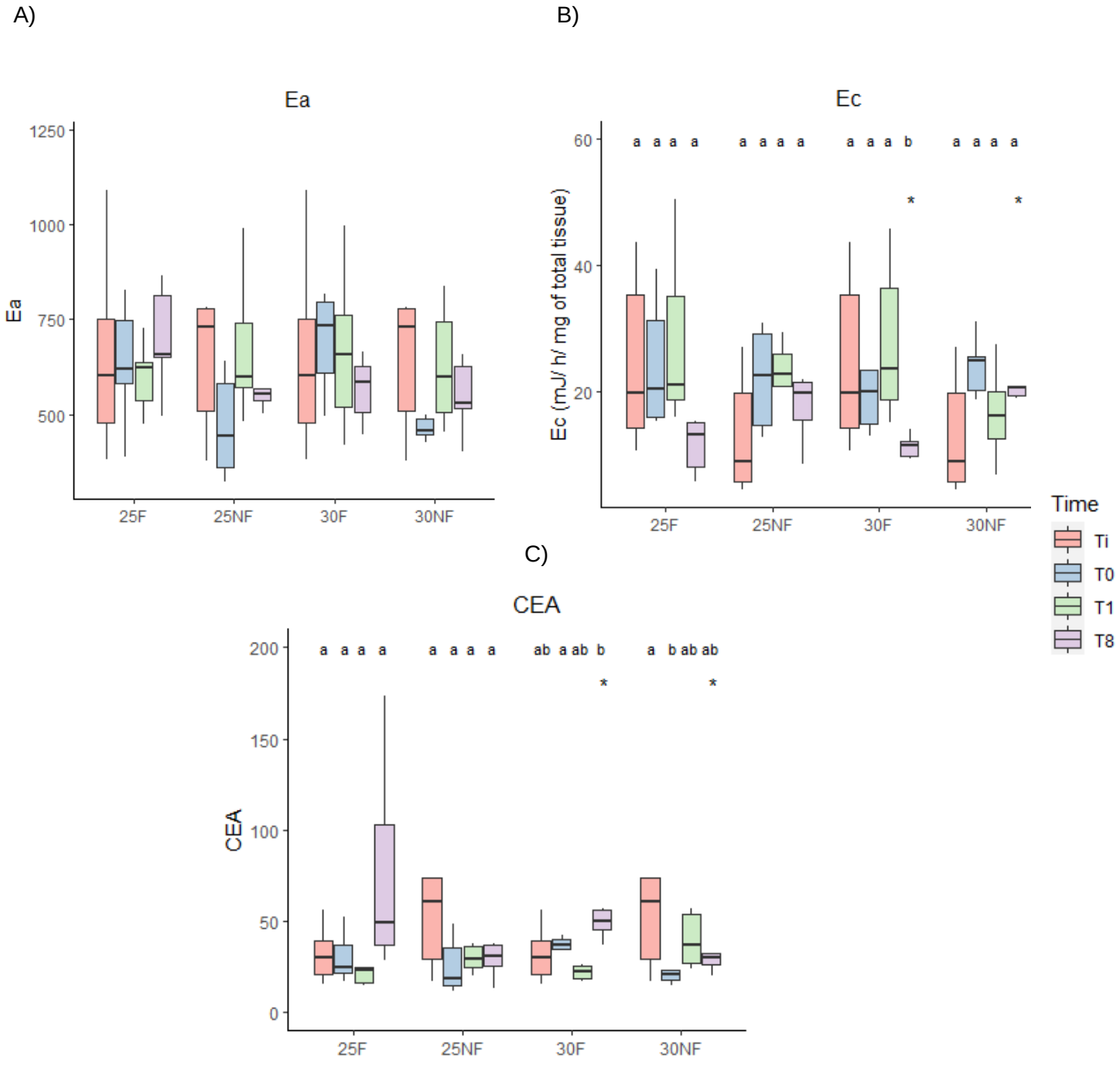

Figure 10- Graphical illustration of the analysis of energy available (Ea) (A), energy consumption (Ec) (B) and cellular energy allocation (C) for the four different groups: 25F - 25°C with feed supply; 25NF - 25°C without feed supply; 30F - 30°C with feed supply; 30NF - 30°C without feed supply. Also, different sampling points are represented as time: Ti – Before temperature rise; T0 – when the temperature reaches their maximum; T1 – one day after being exposed to maximum temperature; T8- eight days after being exposed to maximum temperature. Significant differences are represented as (i) asterisks (*) when significant differences ($p < 0.05$) are reported at the same time point between treatments and as (ii) letters (a, b and ab) when significant differences are reported within each treatment.

4. Discussion

Optimal microencapsulated diet as a heterotrophic feed supply to *Palythoa sp*. in an *ex situ* culture, seems to affect positively the coral fitness when they are under thermal stress. Antioxidant defences as catalase, GST activity and even the HSP70 concentration increased faster when corals have been fed prior to the stress event. Also, LPO levels in all corals groups under study remained unchanged. In addition, feed supplementation seems to positively affect the lipids content. Ferrier-Pagès *et al*. (2010) also concluded that feed supply provides protection against cellular damage in three different species of reef-building corals, and Borell and their team (2008) reported an increase of lipid and protein content in two species of hard corals when supplied with heterotrophic feed and exposed to a heat treatment.

Photobiology is related to zooxanthellae density in the host (Warner *et al*., 1996). These organisms are divided into different species, commonly called clades (reviewed by Pochon *et al*., 2006). Different clades have different characteristics, for example, clade *D* has a higher tolerance to thermal stress (Rowan, 2004). Despite the fact that, in this study, the zooxanthellae clade associated with the coral colonies was not identified, Burnett (2002) reported that *Palythoa sp.* is associated with clade *C* and clade *D* in Indonesia. Also, Reimer and Todd in 2009 reported that three different species of the genera *Palythoa* in Singapura are associated with clade *D*. Although this studies were performed with colonies collected from the natural habitat, it can explain the data observed in the present study since the *Fv/Fm* measures exhibit normal values for corals which have not been exposed to any stress and acclimated in the dark for 15 minutes as reported by Hoegh-Guldberg and Jones (1999). In supplemented corals exposed to higher temperatures, a statistically decrease between the first and the last sampling were visible, whereas in the not supplemented corals this occurs earlier, between the first and the third sampling point. This can suggest a positive effect in fed supply once that in unfed corals the photosystems seem to be affected more earlier than in the fed ones. In line with this, Borell and Bischof (2008) also indicates that fed corals, of the species *S. pistillata*, have the capacity to dissipate excess energy caused by heat, thus preventing damage and inactivation of photosystems II, reducing the production of reactive oxygen species.

Antioxidant enzymes activity is affected by several factors, as temperature fluctuations (Dias *et al*., 2020) or exposure to pollutants (Rocha *et al*., 2020). Dias *et al.* (2020) observed, in hard coral species, that the activity of antioxidant enzymes (e.g. CAT and GST) usually increases with the rise of the water temperature, however, this can vary from species to

species. Catalase belongs to the first line of defence against ROS since it decomposes the hydrogen peroxide (H_2O_2) into molecular oxygen (O_2) and water (H_2O) neutralizing the toxic action of H_2O_2 (Ighodaro and Akinloye, 2018). It is expected that when a stressing agent begins to impact cells, catalase activity will be one of the first mechanisms to increase in order to control damage by excess ROS. To the authors' knowledge, there is no other research evaluating the effect of catalase activity on corals, especially in *Palythoa* genus, with heterotrophic feed supplementation and thermal stress. It is known that catalase activity differs throughout the day time (Levy *et al*., 2006) and has different reactions to external factors as pollutants (Rocha *et al*., 2020). In addition, Levy *et al*., (2006) made a modulation of catalase behaviour suggesting that before any period of acclimatization defence mechanisms in the species *Plerogyra sinuosa* trigger the catalase activity. The fact that catalase activity at the end of the assay is significantly higher in fed corals than in unfed can suggest a possible acclimatation by corals which have been feed. Also this behaviour can indicates that feed supply can enhance the capacity of corals to synthesis antioxidant compound as Borell and Bischof (2008) speculate in the species *S. pistillata.*

A second line of defence, in which the Glutathione S-Transferase (GST) is included, is activated in order to detoxify noxious metabolites from the first detoxification pathway (Hayes and McLellan, 1999). Also, GST activity is demonstrated to increase as temperature increases in some species*, e.g. Montipora capricornis* (Dias *et al*., 2019b). Since GST is activated after catalase (Hayes and McLellan, 1999), it was expected that GST activity increases along with catalase, however that just happens in fed corals**.** As expected, it was observed an increment of GST activity after temperature increases. In fed corals a significantly increase in GST activity were detected between the initial and the third sampling point. This increment also occurs in unfed corals however is not statistically significant. Despite this, the significant increase in the corals which have been feed suggests a positive effect of heterotrophic feed supply, increasing the activity of this antioxidant mechanism for counterbalance the ROS effect.

Additionally, another mechanism which regulates the stress response is the HSP70, which recognize and bond proteins that have a non-native conformation, like denatured ones (Feder and Hofmann, 1999), due to the exposure to high temperatures, for example (Neurath *et al*., 1944), preventing cellular damage. The HSP70 have a variety of families based on their molecular weight and the homology of the sequence, with the HSP70 being mainly associated with the regulation of thermal stress, reduction of denatured proteins, among others functions (Feder and Hofmann, 1999). HSP70 are an important biological biomarker with a fast response

to the environmental stress. This response is triggered by the presence of non-native protein conformations, above of a certain level, in the cells (reviewed by Sørensen *et al*. (2003)). As occurs in other species for example in *Porites cylindrica*, *S. pistillata* (Fitt *F*., 2009) and in *Acropora grandis* (Fang *et al*., 1997) the HSP70 concentration increases with the temperature rise. However, this increment only occurs in fed corals since these have significantly higher values than not supplemented corals at T0. After the temperature remains stable, corals which have been supplemented have a non-significant decrease. This decrease, besides the non-significance, can be related with a possible acclimation by corals due to thermal stress. Sørensen *et al*., (2003) reports in their review article that a population exposed to a continuous stress, without fluctuations, reduce their HSP70 production since the cost of their expression is exceeds their benefits. It is possible to suggest that diet supplementation can have a positive effect on the regulation of HSP concentration by helping the coral to achieve a balance so there is no unnecessary energy cost in HSP expression.

When the antioxidant defences cannot cope with the overproduction of ROS, this accumulates in the cells and protein, lipid and DNA damage can occur (Hayes and McLellan, 1999). Even though there are no statistically significant differences in the LPO levels, they slightly decrease during the time, in the fed corals exposed to heat stress. This reduction is followed by an increase in catalase and GST activity. Also, Dias *et al*., (2019b) reports this pattern in *G. fascicularis*, a thermo tolerant species, when exposed to a long-term (60 days) temperature rise. This evidence can suggest that the antioxidant mechanisms acted efficiently in order to counterbalance the effect of the ROS in corals that have been exposed to thermal stress. In addition, Fitt *et al*.,(2009) demonstrates that during a short-term (five days) test where *P. cylindrica* were exposed to heat, the species did not showed any cellular damage.

Other indicator of coral health is the energy consumption and energy available (lipid, proteins and carbohydrates). Few researches mention the specific biochemical composition of corals, and even less when nutrition and thermal stress relations are the study object (Borell *et al*., 2008 and Erica K. Towle *et al*., 2015). Smith *et al*., (2005) observed that in *Porites compressa* the recovery of the energy reserves is related with the recovery of the zooxanthellae density, however, *Montipora capitata* and their energy reserves recovery are related with the increase of heterotrophic feed for a long-term recovery period. Lipid content represents the greater part of energetic reserves and they can be obtained by the zooxanthellae and from the heterotrophic feeding (Teece *et al*., 2011). In cases of thermal stress, coral survival is affected by their lipids reserves at the beginning of the stress (Anthony

et al., 2009), being, in this case, their content source mainly associated to heterotrophic via (Teece *et al*., 2011). In the present study feed supplement in *Palythoa sp.* did not affect the lipids content, however at the moment that temperature rise a statistical decrease was observed in unfed corals when compared with fed ones. In concordance with that, Tolosa and their team (2011) found evidence that lipid concentration also increases in fed corals when compared with starved corals from the species *T. reniformis* that have been exposed to thermal stress. This fact suggests that lipid production increases to support respiration and building tissue reserves (Tolosa *et al.*, 2011), providing a better coral fitness.

A study made by Borell and their team (2008) suggests a positive effect between proteins concentration and heterotrophic feed, showing a higher reduction of proteins concentration in starved corals than in fed corals after 15 days exposure to high temperatures in species *S. pistillata*. In accordance with that, Tolosa *et al*. (2011) show a higher protein concentration in fed corals who have been exposed to thermal stress than in unfed corals. These previous studies support the results of the present study suggesting a positive effect in protein concentration and heterotrophic feed supply during heat stress. The decline observed at last sampling point in fed corals which have been exposed to thermal stress can be explained by the fact that during the thermal stress the feed supply was ceased. Suggesting that they already used all available proteins and have not an available source of protein in the water, even with the non-filtered water.

As lipids and proteins, carbohydrates are also an important source of energy from photosynthesis by zooxanthellae. During photosynthesis, glucose, a source of carbohydrate, is produced through Calvin cycle and carried over to the host (Smith *et al*., 2005). Carbohydrates in the species *Palythoa sp*. does not vary significantly between different groups and over time. This was expected since the photobiology parameter reveals few significant differences. Besides it is known that carbohydrates have seasonal variations, presenting higher quantities during the winter-spring being this related with the among/quality of the feed available (Rossi *et al*., 2005).

Corals allocate their energy in reproduction, activity, tissue growth, respiration and feeding (Rossi *et al*., 2005) having a positive input of energy through heterotrophic and autotrophic patterns and also it is possible to observe shifts between their energy allocation when exposed to a stressor (Leuzinger *et al*., 2012). The energy available (Ea) is influenced

through the among of proteins, lipids, and carbohydrates. Observing these data, and even though that non statistically differences were detected, it is important to emphasise that the unfed corals exposed to heat stress follow the same pattern that lipids content. Strengthening the importance of lipids in this variable. In addition, the group of corals which have been fed, during the entire experimental test presents constant values when compared with the opposite group. This could be due to the extra proteins and nutrients that feed supply provides to coral, leaving them with more reserves to spend when exposed to a stressor. Once that corals depend on the energy stored before the stress begins, especially when photosynthesis and/or heterotrophic ways are not possible (Smith *et al*., 2005).

On the other hand, energy consumption in supplemented corals which were exposed to thermal stress decreases significantly in the last assessment of the study. Also, at this point they are statistically different from stressed unfed corals, being the energy consumption from the last ones higher than in the stressed fed corals. Fed corals exposed to stress, on the eighth day, decreases their values of energy consumption, when compared to the others sampling points in the same treatment. This can mean that the supplemented corals on the eighth day are moving towards a metabolic equilibrium, contrarily to unfed ones.

The energetic balance, represented by CEA, follows the energy consumption pattern, which was expected since the CEA is calculated in function of the Ec and the Ea. The energetic balance in unfed corals, decreases significantly at the moment thermal stress begins, while fed corals do not experience significant changes. On the eighth day, not supplemented corals have a statistically lower energy balance than supplemented corals due to their higher energy consumption. In other words, the fed corals seem to improve their fitness on the eighth day compared to when the temperature rises. Besides, seems to be a shift from their homeostasis state in corals which was subjected to a feed supplementation founding a balance with the new condition that they are exposed to.

Concluding, despite some research citied were performed under different laboratory conditions and with different temperature triggers it is visible that heterotrophic feed supply have a positive impact on energetic reserves, especially in lipids and protein concentration. Consequently, energetic reserves positively influence the metabolic balance which has a positive effect on corals' resilience when they are exposed to high temperatures. With this in mind, as fed corals had a better metabolic balance than the ones not fed, during the heat event, it is suggested that feed supply may help corals maintaining homeostasis during stress.

Still, the lack of considerable oxidative stress and absence of cellular damage, together with healthy photobiology capacity through this study can indicate that we are looking at a thermo-tolerant species, as already Graham and Sanders in 2016 reported. That is why, the population of zoanthids, such as the genera *Palythoa* is increasing in areas where they are not very frequent such as the Canary Islands (González-Delgado *et al*., 2018) and in the Southwest Atlantic (Cruz *et al*., 2016).

Further studies, in order to corroborate this work need to be done, especially in concern to energetic reserves and balance, since the lake of this subject difficulties the comparison between other investigation studies. Also, work in standardise the laboratory condition, especially regarding the feed supply it needs to be done, facilitating the development of new studies and the comparison between them. Other species can be included in this kind of works, since there is a lack of information especially in soft corals groups in order to better understand the effect of occasional marine heatwaves in the shifts of coral reefs.

5. Acknowledgements

This work is part of project ORNAFEEDS-17913, supported by Portugal and the European Union through FEDER, COMPETE 2020 and CRESC Algarve 2020, in the framework of Portugal 2020.

6. Attachments

Table iv- Register of the water parameters during the period of thermal stress.

Date	Aquarium	Phosphates	Nitrates	Calcium (ppm)	Magnesium (ppm)	KH (dKH)	Salinity	pH	Temperature	Dissolved Oxigen
03.06.2020	1	0	0	475	1360	-	36,2	8,022	25,8	8,30
	2	0	0	485	1480	-	36,0	8,118	25,9	8,26
04.06.2020	1	-	-	-	-	8,7	36,4	8,034	26,4	8,20
	2	-	-	-	-	10,2	36,1	8,120	30,6	7,56
05.06.2020	1	-	-	-	-	-	36,3	8,104	26,1	8,27
	2	-	-	-	-	-	36,1	7,990	30,5	7,62
06.06.2020	1	-	-	-	-	-	36,3	8,025	26,3	8,20
	2	-	-	-	-	-	36,3	8,127	31,0	7,52
07.06.2020	1	0	0	475	1520	5,6	36,4	8,014	26,3	8,24
	2	0	0	480	1340	7,14	36,3	8,125	31,1	7,55
08.06.2020	1	-	-	-	-	-	36,3	7,999	26,4	8,27
	2	-	-	-	-	-	36,3	8,114	30,8	7,57
09.06.2020	1	-	-	-	-	-	35,8	8,005	25,6	8,28
	2	-	-	-	-	-	36,2	8,114	30,6	7,67
10.06.2020	1	-	-	-	-	-	36,2	8,005	26,3	8,29
	2	-	-	-	-	-	36,1	8,123	30,6	7,62
11.06.2020	1	0	0	475	1440	5,3	36,2	7,971	26,0	8,30
	2	0	0	435	1470	7,6	36,1	8,098	30,5	7,62

7.Bibliography

Abrego, D., Ulstrup, K.E., Willis, B.L., Van Oppen, M.J.H., 2008. Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. Proc. R. Soc. B Biol. Sci. 275, 2273–2282. https://doi.org/10.1098/rspb.2008.0180

Albert, S., Fisher, P.L., Gibbes, B., Grinham, A., 2015. Corals persisting in naturally turbid waters adjacent to a pristine catchment in Solomon Islands. Mar. Pollut. Bull. 94, 299–306. https://doi.org/10.1016/j.marpolbul.2015.01.031

Albright, R., Cooley, S., 2019. A review of interventions proposed to abate impacts of ocean acidification on coral reefs. Reg. Stud. Mar. Sci. 29, 100612. https://doi.org/10.1016/j.rsma.2019.100612

Anthony, K.R.N., Hoogenboom, M.O., Maynard, J.A., Grottoli, A.G., Middlebrook, R., 2009. Energetics approach to predicting mortality risk from environmental stress: A case study of coral bleaching. Funct. Ecol. 23, 539–550. https://doi.org/10.1111/j.1365-2435.2008.01531.x

Baird, A.H., Guest, J.R., Willis, B.L., 2009. Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals. https://doi.org/10.1146/annurev.ecolsys.110308.120220

Bambridge, T., Chlous, F., D'Arcy, P., Claudet, J., Pascal, N., Reynaud, S., Rodolfo-Metalpa, R., Tambutté, S., Thomassin, A., Virto, L.R., 2019. Society-based solutions to coral reef threats in french pacific territories. Reg. Stud. Mar. Sci. 29, 100667. https://doi.org/10.1016/j.rsma.2019.100667

Banin, E., Vassilakos, D., Orr, E., Martinez, R.J., Rosenberg, E., 2003. Superoxide dismutase is a virulence factor produced by the coral bleaching pathogen </i>*Vibrio shiloi*. Curr. Microbiol. 46, 418–422. https://doi.org/10.1007/s00284-002-3912-5

Barton, J.A., Willis, B.L., Hutson, K.S., 2017. Coral propagation: a review of techniques for ornamental trade and reef restoration. Rev. Aquac. 9, 238–256. https://doi.org/10.1111/raq.12135

Bessell-Browne, P., Stat, M., Thomson, D., Clode, P.L., 2014. *Coscinaraea marshae* corals that have survived prolonged bleaching exhibit signs of increased heterotrophic feeding. Coral Reefs 33, 795–804. https://doi.org/10.1007/s00338-014-1156-z

Bird, R.P., Draper, H.H., 1984. Comparative studies on different methods of malonaldehyde determination. Methods Enzymol. 105, 299–305.

Bongiorni, L., Giovanelli, D., Rinkevich, B., Pusceddu, A., Chou, L.M., Danovaro, R., 2011. First step in the restoration of a highly degraded coral reef (Singapore) by *in situ* coral intensive farming. Aquaculture 322–323, 191–200. https://doi.org/10.1016/j.aquaculture.2011.09.024

Borell, E.M., Bischof, K., 2008. Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress. Oecologia 157, 593–601. https://doi.org/10.1007/s00442-008-1102-2

Borell, E.M., Yuliantri, A.R., Bischof, K., Richter, C., 2008. The effect of heterotrophy on photosynthesis and tissue composition of two scleractinian corals under elevated temperature. J. Exp. Mar. Bio. Ecol. 364, 116–123. https://doi.org/10.1016/j.jembe.2008.07.033

Borneman, E.H., 2008. Chapter 1 Introduction to the husbandry of corals in aquariums : A review. Adv. Coral Husb. Public Aquariums.

Bradford, Marion M, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Browne, N.K., Precht, E., Last, K.S., Todd, P.A., 2014. Photo-physiological costs associated with acute sediment stress events in three near-shore turbid water corals. Mar. Ecol. Prog. Ser. 502, 129–143. https://doi.org/10.3354/meps10714

Burke, L., Reytar, K., Spalding, M., Perry, A., 2011. Reefs at Risk Revisited, Defenders. World Resources Institute- Washington. DC, USA.

Burnett, W.J., 2002. Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. Mar. Ecol. Prog. Ser. 234, 105–109. https://doi.org/10.3354/meps234105

Calado, R., Olivotto, I., Planas, O.M., G., J.H., 2017. Marine Ornamental species Aquaculture, John Wiley. ed, Wiley Blackwell. Wiley Blackwell.

Carballo-Bolaños, R., Soto, D., Chen, C.A., 2019. Thermal Stress and Resilience of Corals in a Climate-Changing World. J. Mar. Sci. Eng. 8, 15. https://doi.org/10.3390/jmse8010015

Carreiro-Silva, M., Ocaña, O., Stankovic, D., Sampaio, Í., Porteiro, F.M., Fabri, M.C., Stefanni, S., 2017. Zoantharians (Hexacorallia: Zoantharia) associated with cold-water corals in the

azores region: New species and associations in the deep sea. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00088

Christie, P., White, A.T., 2007. Best practices for improved governance of coral reef marine protected areas. Coral Reefs 26, 1047–1056. https://doi.org/10.1007/s00338-007-0235-9

Clairborne, A., 1985. Catalase activity, in: Greenwald, R.A. (Ed.), CRC Handbook of Methods in Oxygen Radical Research. CRC Press, Boca Raton, pp. 283–284.

Conlan, J.A., Humphrey, C.A., Severati, A., Parrish, C.C., Francis, D.S., 2019. Elucidating an optimal diet for captive *Acropora* corals. Aquaculture 513, 734420. https://doi.org/10.1016/j.aquaculture.2019.734420

Costa, A.P.L., Calado, R., Marques, B., Lillebø, A.I., Serôdio, J., Soares, A.M.V.M., Serrão, E.A., Rocha, R.J.M., 2016. The effect of mixotrophy in the *ex situ* culture of the soft coral *Sarcophyton cf. glaucum*. Aquaculture 452, 151–159. https://doi.org/10.1016/j.aquaculture.2015.10.032

Cramer, W., Guiot, J., Fader, M., Garrabou, J., Gattuso, J.P., Iglesias, A., Lange, M.A., Lionello, P., Llasat, M.C., Paz, S., Peñuelas, J., Snoussi, M., Toreti, A., Tsimplis, M.N., Xoplaki, E., 2018. Climate change and interconnected risks to sustainable development in the Mediterranean. Nat. Clim. Chang. 8, 972–980. https://doi.org/10.1038/s41558-018-0299-2

Cruz, I.C.S., de Kikuchi, R.K.P., Longo, L.L., Creed, J.C., 2015. Evidence of a phase shift to Epizoanthus gabrieli Carlgreen, 1951 (Order Zoanthidea) and loss of coral cover on reefs in the Southwest Atlantic. Mar. Ecol. 36, 318–325. https://doi.org/10.1111/maec.12141

Cruz, I.C.S., Meira, V.H., de Kikuchi, R.K.P., Creed, J.C., 2016. The role of competition in the phase shift to dominance of the zoanthid *Palythoa cf. variabilis* on coral reefs. Mar. Environ. Res. 115, 28–35. https://doi.org/10.1016/j.marenvres.2016.01.008

Dalton, S.J., Carroll, A.G., Sampayo, E., Roff, G., Harrison, P.L., Entwistle, K., Huang, Z., Salih, A., Diamond, S.L., 2020. Successive marine heatwaves cause disproportionate coral bleaching during a fast phase transition from El Niño to La Niña. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.136951

De Coen, W.M., Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed *Daphnia populations*. J. Aquat. Ecosyst. Stress Recover. 6, 43–55.

Dias, M., Ferreira, A., Gouveia, R., Cereja, R., Vinagre, C., 2018. Mortality, growth and

regeneration following fragmentation of reef-forming corals under thermal stress. J. Sea Res. 141, 71–82. https://doi.org/10.1016/j.seares.2018.08.008

Dias, Marta, Ferreira, A., Gouveia, R., Vinagre, C., 2019. Synergistic effects of warming and lower salinity on the asexual reproduction of reef-forming corals. Ecol. Indic. 98, 334–348. https://doi.org/10.1016/j.ecolind.2018.11.011

Dias, M., Madeira, C., Jogee, N., Ferreira, A., Gouveia, R., Cabral, H., Diniz, M., Vinagre, C., 2020. Integrative indices for health assessment in reef corals under thermal stress. Ecol. Indic. 113. https://doi.org/10.1016/j.ecolind.2020.106230

Dias, Marta;, Madeira, C., Jogee, N., Ferreira, A., Gouveia, R., Cabral, H., Diniz, M., Vinagre, C., 2019. Oxidative stress on scleractinian coral fragments following exposure to high temperature and low salinity. Ecol. Indic. 107. https://doi.org/10.1016/j.ecolind.2019.105586

Dove, S.G., Hoegh-Guldberg, O., 2011. The cell physiology of coral bleaching 55–71. https://doi.org/10.1029/61ce05

Downs, C.A., Fauth, J.E., Halas, J.C., Dustan, P., Bemiss, J., Woodley, C.M., 2002. Oxidative stress and seasonal coral bleaching. Free Radic. Biol. Med. 33, 533–543. https://doi.org/10.1016/S0891-5849(02)00907-3

Dustan, P., Halas, J.C., 1987. Changes in the reef-coral community of Carysfort reef, Key Largo, Florida: 1974 to 1982. Coral Reefs 6, 91–106. https://doi.org/10.1007/BF00301378

Fabricius, K.E., 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. Mar. Pollut. Bull. 50, 125–146. https://doi.org/10.1016/j.marpolbul.2004.11.028

Falkowski, P.G., Dubinsky, Z., Muscatine, L., Porter, J.W., 1984. Bioenergetics Symbiotic Coral. Bioscience 34, 705–709.

Fang, L.-S., Huang, S.-P., Lin, K.-L., 1997. High temperature induces the synthesis of heat-shock proteins and the elevation of intracellular calcium in the coral *Acropora grandis* . Coral Reefs 127–131.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology, Annual Review of Physiology. https://doi.org/10.1146/annurev.physiol.61.1.243

Ferrier-Pagès, C., Rottier, C., Beraud, E., Levy, O., 2010. Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the

rates of photosynthesis. J. Exp. Mar. Bio. Ecol. 390, 118–124. https://doi.org/10.1016/j.jembe.2010.05.007

Fitt, W.K., Gates, R.D., Hoegh-Guldberg, O., Bythell, J.C., Jatkar, A., Grottoli, A.G., Gomez, M., Fisher, P., Lajuenesse, T.C., Pantos, O., Iglesias-Prieto, R., Franklin, D.J., Rodrigues, L.J., Torregiani, J.M., Van Woesik, R., Lesser, M.P., 2009. Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. https://doi.org/10.1016/j.jembe.2009.03.011

Forsman, Z.H., Kimokeo, B.K., Bird, C.E., Hunter, C.L., Toonen, R.J., 2012. Coral farming: Effects of light, water motion and artificial foods. J. Mar. Biol. Assoc. United Kingdom 92, 721–729. https://doi.org/10.1017/S0025315411001500

Forsman, Z.H., Rinkevich, B., Hunter, C.L., 2006. Investigating fragment size for culturing reef-building corals (*Porites lobata* and *P. compressa*) in *ex situ* nurseries. Aquaculture 261, 89–97. https://doi.org/10.1016/j.aquaculture.2006.06.040

Furla, P., Allemand, D., Shick, J.M., Ferrier-Pagès, C., Richier, S., Plantivaux, A., Merle, P.L., Tambutté, S., 2005. The symbiotic anthozoan: A physiological chimera between alga and animal. Integr. Comp. Biol. 45, 595–604. https://doi.org/10.1093/icb/45.4.595

Gnaiger, E., 1983. Calculation of Energetic and Biochemical Equivalents of Respiratory Oxygen Consumption, in: Gnaiger, E., Forstner, H. (Eds.), Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 337–345. https://doi.org/10.1007/978-3-642-81863-9_30

Goldberg, W.M., 2002. Feeding behavior, epidermal structure and mucus cytochemistry of the scleractinian *Mycetophyllia reesi*, a coral without tentacles. Tissue Cell 34, 232–245. https://doi.org/10.1016/S0040-8166(02)00009-5

González-Delgado, S., López, C., Brito, A., Clemente, S., 2018. Marine community effects of two colonial zoanthids in intertidal habitats of the Canary Islands. Reg. Stud. Mar. Sci. 23, 23–31. https://doi.org/10.1016/j.rsma.2018.03.006

Goyen, S., Camp, E.F., Fujise, L., Lloyd, A., Nitschke, M.R., LaJeunensse, T., Kahlke, T., Ralph, P.J., Suggett, D., 2019. Mass coral bleaching of *P. versipora* in Sydney Harbour driven by the 2015–2016 heatwave. Coral Reefs 38, 815–830. https://doi.org/10.1007/s00338-019-01797-6

Graham, E.R., Sanders, R.W., 2016. Species-specific photosynthetic responses of symbiotic zoanthids to thermal stress and ocean acidification. Mar. Ecol. 37, 442–458.

https://doi.org/10.1111/maec.12291

Great Barrier Reef Marine Park Authority, 2016. Interim report: 2016 coral bleaching event on the Great Barrier Reef.

Grottoli, A.G., Rodrigues, L.J., Palardy, J.E., 2006. Heterotrophic plasticity and resilience in bleached corals. Nature 440, 1186–1189. https://doi.org/10.1038/nature04565

Grottoli, A.G., Wilkins, M.J., Johnston, M.D., Levas, S., Schoepf, V., Dalcin, M.P., Wilkins, M.J., Warner, M.E., Cai, W.-J., Hoadley, K.D., Pettay, D.T., Melman, T.F., 2018. Coral physiology and microbiome dynamics under combined warming and ocean acidification. PLoS One 13, e0191156.

Habig, W.H.H., Pabst, M.J.J., Jacoby, W.B., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hadjioannou, L., Jimenez, C., Rottier, C., Sfenthourakis, S., Ferrier-pagès, C., 2019. Response of the temperate scleractinian coral *Cladocora caespitosa* to high temperature and long-term nutrient enrichment. Nat. -scientific reports 1–11. https://doi.org/10.1038/s41598-019-50716-w

Hayes, J.D., McLellan, L.I., 1999. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic. Res. 31, 273–300. https://doi.org/10.1080/10715769900300851

Hobday, A.J., Alexander, L. V., Perkins, S.E., Smale, D.A., Straub, S.C., Oliver, E.C.J., Benthuysen, J.A., Burrows, M.T., Donat, M.G., Feng, M., Holbrook, N.J., Moore, P.J., Scannell, H.A., Sen Gupta, A., Wernberg, T., 2016. A hierarchical approach to defining marine heatwaves. Prog. Oceanogr. 141, 227–238. https://doi.org/10.1016/j.pocean.2015.12.014

Hoegh-Guldberg, O., Jones, R.J., 1999. Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar. Ecol. Prog. Ser. 183, 73–86. https://doi.org/10.3354/meps183073

Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.., Edwards, A.J., Caldeira, K., Knowlton, N., Eakin, C.M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R.H., Dubi, A., Hatziolos, M.E., 2007. Change and Ocean Acidification. Science (80-.). 318, 1737–1742. https://doi.org/10.1126/science.1152509

Houlbrèque, F., Ferrier-Pagès, C., 2009. Heterotrophy in tropical scleractinian corals. Biol. Rev. 84, 1–17. https://doi.org/10.1111/j.1469-185X.2008.00058.x

Hovland, M., 2008. Deep-water Coral Reefs, Unique Biodiversity Hot-Spots. https://doi.org/10.1007/978-1-4020-8460-7

Howells, E.J., Ketchum, R.N., Bauman, A.G., Mustafa, Y., Watkins, K.D., Burt, J.A., 2016. Species-specific trends in the reproductive output of corals across environmental gradients and bleaching histories. Mar. Pollut. Bull. 105, 532–539. https://doi.org/10.1016/j.marpolbul.2015.11.034

Hughes, A.D., Grottoli, A.G., 2013. Heterotrophic compensation: A possible mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0081172

HUGHES, R.N., 1983. Evolutionary ecology of colonial reef-organisms, with particular reference to corals. Biol. J. Linn. Soc. 20, 39–58. https://doi.org/10.1111/j.1095-8312.1983.tb01588.x

Hughes, T.P., Anderson, K.D., Connolly, S.R., Heron, S.F., Kerry, J.T., Lough, J.M., Baird, A.H., Baum, J.K., Berumen, M.L., Bridge, T.C., Claar, D.C., Eakin, C.M., Gilmour, J.P., Graham, N.A.J., Harrison, H., Hobbs, J.P.A., Hoey, A.S., Hoogenboom, M., Lowe, R.J., McCulloch, M.T., Pandolfi, J.M., Pratchett, M., Schoepf, V., Torda, G., Wilson, S.K., 2018. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science (80-.). 359, 80–83. https://doi.org/10.1126/science.aan8048

Hughes, T.P., Baird, A.H., Bellwood, D.R., Card, M., Connolly, S.R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J.B.C., Kleypas, J., Lough, J.M., Marshall, P., Nystrom, M., Palumbi, S.R., Pandolfi, J.M., Rosen, B., Roughgarden, J., 2003. Climate Change, Human Impacts, and the Resilience of Coral Reefs. Science (80-.). 301, 929–934. https://doi.org/10.1126/science.1085046

Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 54, 287–293. https://doi.org/10.1016/j.ajme.2017.09.001

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 greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, A Companion to Applied Ethics. https://doi.org/10.1002/9780470996621.ch50

IUCN Red List of Threatened Species [WWW Document], 2020. https://doi.org/10.5860/choice.49-6872

J.Veron, 2000. Corals of the world. Australian Institute of Marine Science, Townsville, Australia.

Jones, R., Bessell-Browne, P., Fisher, R., Klonowski, W., Slivkoff, M., 2016. Assessing the impacts of sediments from dredging on corals. Mar. Pollut. Bull. 102, 9–29. https://doi.org/10.1016/j.marpolbul.2015.10.049

Jones, R., Ricardo, G.F., Negri, A.P., 2015. Effects of sediments on the reproductive cycle of corals. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2015.08.021

Kemp, D.W., Cook, C.B., LaJeunesse, T.C., Brooks, W.R., 2006. A comparison of the thermal bleaching responses of the zoanthid *Palythoa caribaeorum* from three geographically different regions in south Florida. J. Exp. Mar. Bio. Ecol. https://doi.org/10.1016/j.jembe.2006.03.017

Lalli, C.M., Parsons, T.R., 1993. Biological oceanography: an introduction. https://doi.org/10.1016/0022-0981(96)02604-4

Leal, M.C., Calado, R., Sheridan, C., Alimonti, A., Osinga, R., 2013. Coral aquaculture to support drug discovery. Trends Biotechnol. 31, 555–561. https://doi.org/10.1016/j.tibtech.2013.06.004

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

Lecaillon, G., 2004. The “C.A.R.E.” (collect by artificial reef eco-friendly) system as a method of producing farmed marine animals for the aquarium market: an alternative solution to collection in the wild. Live reef fish Inf. Bull. 12, 17–20.

Ledlie, M.H., Graham, N.A.J., Bythell, J.C., Wilson, S.K., Jennings, S., Polunin, N.V.C., Hardcastle, J., 2007. Phase shifts and the role of herbivory in the resilience of coral reefs. Coral Reefs 26, 641–653. https://doi.org/10.1007/s00338-007-0230-1

Leggat, W.P., Camp, E.F., Suggett, D.J., Heron, S.F., Fordyce, A.J., Gardner, S., Deakin, L., Turner, M., Beeching, L.J., Kuzhiumparambil, U., Eakin, C.M., Ainsworth, T.D., 2019. Rapid Coral Decay Is Associated with Marine Heatwave Mortality Events on Reefs. Curr. Biol. 29, 2723-2730.e4. https://doi.org/10.1016/j.cub.2019.06.077

Lesser, M.P., 2006. Oxidative Stress In Marine Environments: Biochemistry and Physiological Ecology. Annu. Rev. Physiol. 68, 253–278. https://doi.org/10.1146/annurev.physiol.68.040104.110001

Leuzinger, S., Willis, B.L., Anthony, K.R.N., 2012. Energy allocation in a reef coral under varying resource availability. Mar. Biol. 159, 177–186. https://doi.org/10.1007/s00227-011-1797-1

Levy, O., Achituv, Y., Yacobi, Y.Z., Dubinsky, Z., Stambler, N., 2006. Diel "tuning" of coral metabolism: Physiological responses to light cues. J. Exp. Biol. 209, 273–283. https://doi.org/10.1242/jeb.01983

Linden, B., Vermeij, M.J.A., Rinkevich, B., 2019. The coral settlement box: A simple device to produce coral stock from brooded coral larvae entirely *in situ*. Ecol. Eng. 132, 115–119. https://doi.org/10.1016/j.ecoleng.2019.04.012

Lirman, D., 2000. Fragmentation in the branching coral *Acropora palmata* (Lamarck): Growth, survivorship, and reproduction of colonies and fragments. J. Exp. Mar. Bio. Ecol. 251, 41–57. https://doi.org/10.1016/S0022-0981(00)00205-7

Loya, Y., Sakai, K., Sambali, H., Woesik van, R., 2001. Coral bleaching : the winners and the losers, Ecology Letters.

Marshall, P., Schuttenberg, H., 2006. A Reef Manager's Guide to Coral Bleaching., Great Barrier Reef Marine Park Authority. Townsville, Australia.

Mesquita, C.S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., Marcos, J.C., 2014. Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Anal. Biochem. 458, 69–71. https://doi.org/10.1016/j.ab.2014.04.034

Montseny, M., Linares, C., Viladrich, N., Olariaga, A., Carreras, M., Palomeras, N., Gracias, N., Istenič, K., Garcia, R., Ambroso, S., Santín, A., Grinyó, J., Gili, J.M., Gori, A., 2019. First attempts towards the restoration of gorgonian populations on the Mediterranean continental shelf. Aquat. Conserv. Mar. Freshw. Ecosyst. 29, 1278–1284. https://doi.org/10.1002/aqc.3118

Nahon, S., Richoux, N.B., Kolasinski, J., Desmalades, M., Pages, F.F., 2013. Spatial and Temporal Variations in Stable Carbon (δ 13 C) and Nitrogen (δ 15 N) Isotopic Composition of Symbiotic Scleractinian Corals. PLoS One 8, 81247. https://doi.org/10.1371/journal.pone.0081247

Neurath, H., Greenstein, J.P., Putnam, F.W., Erickson, J.O., 1944. The chemistry of protein denaturation. Chem. Rev. 34, 157–265. https://doi.org/10.1021/cr60108a003

Niggl, W., Glas, M., Laforsch, C., Mayr, C., Wild, C., 2009. First evidence of coral bleaching stimulating organic matter release by reef corals. Proc. 11th Int. Coral Reef Symp. Ft.

Lauderdale, Florida, 7-11 July 2008, Sess. 19 7–11.

Oliver, E.C.J., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L. V., Benthuysen, J.A., Feng, M., Sen Gupta, A., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Straub, S.C., Wernberg, T., 2018. Longer and more frequent marine heatwaves over the past century. Nat. Commun. 9, 1–12. https://doi.org/10.1038/s41467-018-03732-9

Palardy, J.E., Rodrigues, L.J., Grottoli, A.G., 2008. The importance of zooplankton to the daily metabolic carbon requirements of healthy and bleached corals at two depths. J. Exp. Mar. Bio. Ecol. 367, 180–188. https://doi.org/10.1016/j.jembe.2008.09.015

Paradis, B.T., Henry, R.P., Chadwick, N.E., 2019. Compound effects of thermal stress and tissue abrasion on photosynthesis and respiration in the reef-building coral *Acropora cervicornis* (Lamarck, 1816). J. Exp. Mar. Bio. Ecol. 521, 151222. https://doi.org/10.1016/j.jembe.2019.151222

Patton, W.K., 1976. Animal Associates of Living Reef Corals, in: Biology and Geology of Coral Reefs V3: Biology 2. p. 25. https://doi.org/10.1016/b978-0-12-395527-2.50008-1

Pochon, X., Montoya-Burgos, J.I., Stadelmann, B., Pawlowski, J., 2006. Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. Mol. Phylogenet. Evol. 38, 20–30. https://doi.org/10.1016/j.ympev.2005.04.028

Reed, D., Washburn, L., Rassweiler, A., Miller, R., Bell, T., Harrer, S., 2016. Extreme warming challenges sentinel status of kelp forests as indicators of climate change. Nat. Commun. 7. https://doi.org/10.1038/ncomms13757

Reimer, A., 1971. Feeding Behavior in the Hawaiian Zoanthids *Palythoa* and *Zoanthus* 512–520.

Reimer, J., Todd, P., 2009. Preliminary molecular examination of zooxanthellate zoanthid (Hexacorallia, Zoantharia) and associated zooxanthellae (*Symbiodinium spp.*) diversity in Singapore. Raffles Bull. Zool. 22, 103–120.

Richmond, R., 1997. Reproduction and Recruitment in Corals: Critical Links in the Persistence of Reefs., in: Life and Death of Coral Reefs. p. 176. https://doi.org/10.1007/978-1-4615-5995-5

Rocha, J., Peixe, L., Gomes, N.C.M., Calado, R., 2011. Cnidarians as a source of new marine bioactive compounds - An overview of the last decade and future steps for bioprospecting. Mar. Drugs 9, 1860–1886. https://doi.org/10.3390/md9101860

Rocha, R.J.M., Bontas, B., Cartaxana, P., Leal, M.C., Ferreira, J.M., Rosa, R., Serôdio, J., Calado, R., 2015. Development of a Standardized Modular System for Experimental Coral Culture. J. World Aquac. Soc. 46, 235–251. https://doi.org/10.1111/jwas.12186

Rocha, R.J.M., Calado, R., Cartaxana, P., Furtado, J., Serôdio, J., 2013a. Photobiology and growth of leather coral *Sarcophyton cf. glaucum* fragments stocked under low light in a recirculated system. Aquaculture 414–415, 235–242. https://doi.org/10.1016/j.aquaculture.2013.08.018

Rocha, R.J.M., Pimentel, T., Serôdio, J., Rosa, R., Calado, R., 2013b. Comparative performance of light emitting plasma (LEP) and light emitting diode (LED) in *ex situ* aquaculture of scleractinian corals. Aquaculture 402–403, 38–45. https://doi.org/10.1016/j.aquaculture.2013.03.028

Rocha, R.J.M., Rodrigues, A.C.M., Campos, D., Cícero, L.H., Costa, A.P.L., Silva, D.A.M., Oliveira, M., Soares, A.M.V.M., Patrício Silva, A.L., 2020. Do microplastics affect the zoanthid *Zoanthus sociatus*? Sci. Total Environ. 713, 136659. https://doi.org/10.1016/j.scitotenv.2020.136659

Rodrigues, A.C.M., Gravato, C., Quintaneiro, C., Bordalo, M.D., Barata, C., Soares, A.M.V.M., Pestana, J.L.T., 2017. Energetic costs and biochemical biomarkers associated with esfenvalerate exposure in *Sericostoma vittatum*. Chemosphere 189, 445–453. https://doi.org/10.1016/j.chemosphere.2017.09.057

Rodrigues, A.C.M., Gravato, C., Quintaneiro, C., Golovko, O., Žlábek, V., Barata, C., Soares, A.M.V.M., Pestana, J.L.T., 2015. Life history and biochemical effects of chlorantraniliprole on *Chironomus riparius*. Sci. Total Environ. 508, 506–513. https://doi.org/10.1016/j.scitotenv.2014.12.021

Rodrigues, L.J., Grottoli, A.G., 2007. Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnol. Oceanogr. 52, 1874–1882. https://doi.org/10.4319/lo.2007.52.5.1874

Rosa, R., Trubenbach, K., Pimentel, M.S., Boavida-Portugal, J., Faleiro, F., Baptista, M., Dionisio, G., Calado, R., Portner, H.O., Repolho, T., 2014. Differential impacts of ocean acidification and warming on winter and summer progeny of a coastal squid (*Loligo vulgaris*). J. Exp. Biol. 217, 518–525. https://doi.org/10.1242/jeb.096081

Rossi, S., Snyder, M.J., Gili, J.M., 2005. Protein, carbohydrate, lipid concentrations and HSP 70-HSP 90 (stress protein) expression over an annual cycle: Useful tools to detect feeding constraints in a benthic suspension feeder. Helgol. Mar. Res. 60, 7–17.

https://doi.org/10.1007/s10152-005-0009-0

Rowan, R., 2004. Coral bleaching: Thermal adaptation in reef coral symbionts. Nature 430, 2004–2004.

Sebens, K.P., Helmuth, B., Carrington, E., Agius, B., 2003. Effects of water flow on growth and energetics of the scleractinian coral *Agaricia tenuifolia* in Belize. Coral Reefs 22, 35–47. https://doi.org/10.1007/s00338-003-0277-6

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. Mar. Ecol. Prog. Ser. 218, 45–61. https://doi.org/10.3354/meps218045

Sikorskaya, T. V., Ermolenko, E. V., Imbs, A.B., 2020. Effect of experimental thermal stress on lipidomes of the soft coral *Sinularia sp.* and its symbiotic dinoflagellates. J. Exp. Mar. Bio. Ecol. 524. https://doi.org/10.1016/j.jembe.2019.151295

Smale, D.A., Wernberg, T., Oliver, E.C.J., Thomsen, M., Harvey, B.P., Straub, S.C., Burrows, M.T., Alexander, L. V., Benthuysen, J.A., Donat, M.G., Feng, M., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Sen Gupta, A., Payne, B.L., Moore, P.J., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat. Clim. Chang. 9, 306–312. https://doi.org/10.1038/s41558-019-0412-1

Smith, D.J., Suggett, D.J., Baker, N.R., 2005. Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals? Glob. Chang. Biol. 11, 1–11. https://doi.org/10.1111/j.1529-8817.2003.00895.x

Soong, K., Chen, T. an, 2003. Coral transplantation: Regeneration and growth of *Acropora* fragments in a nursery. Restor. Ecol. 11, 62–71. https://doi.org/10.1046/j.1526-100X.2003.00100.x

Sørensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6, 1025–1037. https://doi.org/10.1046/j.1461-0248.2003.00528.x

Spalding, M., Corinna, R., Edmund P., G., 2001. World Atlas of Coral Reefs. Prepared at the UNEP World Conservation Monotoring Center, University of California Press, Barkeley U.S.A.

Stimson, J., Sakai, K., Sembali, H., 2002. Interspecific comparison of the symbiotic relationship in corals with high and low rates of bleaching-induced mortality. Coral Reefs 21, 409–421. https://doi.org/10.1007/s00338-002-0264-3

Strychar, K.B., Coates, M., Sammarco, P.W., Piva, T.J., Scott, P.T., 2005. Loss of Symbiodinium from bleached soft corals *Sarcophyton ehrenbergi*, *Sinularia sp.* and *Xenia sp.* J. Exp. Mar. Bio. Ecol. https://doi.org/10.1016/j.jembe.2004.12.039

Tagliafico, A., Rangel, S., Kelaher, B., Christidis, L., 2018. Optimizing heterotrophic feeding rates of three commercially important scleractinian corals. Aquaculture 483, 96–101. https://doi.org/10.1016/j.aquaculture.2017.10.013

Teai, T., Drollet, J.H., Bianchini, J.P., Cambon, A., Martin, P.M.V., 1998. Occurrence of ultraviolet radiation-absorbing mycosporine-like amino acids in coral mucus and whole corals of french polynesia. Mar. Freshw. Res. 49, 127–132. https://doi.org/10.1071/MF97051

Teece, M.A., Estes, B., Gelsleichter, E., Lirman, D., 2011. Heterotrophic and autotrophic assimilation of fatty acids by two scleractinian corals, *Montastraea faveolata* and *Porites astreoides*. Limnol. Oceanogr. 56, 1285–1296. https://doi.org/10.4319/lo.2011.56.4.1285

Titlyanov, E.A., Titlyanova, T. V., 2002. Reef-building corals - Symbiotic autotrophic organisms: 1. General structure, feeding pattern, and light-dependent distribution in the shelf. Russ. J. Mar. Biol. 28, S1–S15. https://doi.org/10.1023/A:1021836204655

Tolosa, I., Treignier, C., Grover, R., Ferrier-Pagès, C., 2011. Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral *Turbinaria reniformis*. Coral Reefs 30, 763–774. https://doi.org/10.1007/s00338-011-0753-3

Torrents, O., Tambutté, E., Caminiti, N., Garrabou, J., 2008. Upper thermal thresholds of shallow vs. deep populations of the precious Mediterranean red coral *Corallium rubrum* (L.): Assessing the potential effects of warming in the NW Mediterranean. J. Exp. Mar. Bio. Ecol. https://doi.org/10.1016/j.jembe.2007.12.006

Towle, Erica K, Enochs, I.C., Langdon, C., 2015. Threatened Caribbean Coral Is Able to Mitigate the Adverse Effects of Ocean Acidification on Calcification by Increasing Feeding Rate. https://doi.org/10.1371/journal.pone.0123394

Towle, Erica K., Enochs, I.C., Langdon, C., 2015. Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate. PLoS One 10, 1–17. https://doi.org/10.1371/journal.pone.0123394

Twan, W.H., Hwang, J.S., Lee, Y.H., Wu, H.F., Tung, Y.H., Chang, C.F., 2006. Hormones and reproduction in scleractinian corals. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. https://doi.org/10.1016/j.cbpa.2006.01.011

Van Oppen, M.J.H., Oliver, J.K., Putnam, H.M., Gates, R.D., 2015. Building coral reef resilience through assisted evolution. Proc. Natl. Acad. Sci. U. S. A. 112, 2307–2313. https://doi.org/10.1073/pnas.1422301112

Verslycke, T., Ghekiere, A., Janssen, C.R., 2004. Seasonal and spatial patterns in cellular energy allocation in the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) of the Scheldt estuary (The Netherlands). J. Exp. Mar. Bio. Ecol. 306, 245–267. https://doi.org/10.1016/j.jembe.2004.01.014

Warner, M.E., Fitt, W.., Schmidt, G.W., 1996. The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: a novel approach. Plant, Cell Environ. 19, 291–299.

Weis, V.M., 2008. Cellular mechanisms of Cnidarian bleaching: Stress causes the collapse of symbiosis. J. Exp. Biol. 211, 3059–3066. https://doi.org/10.1242/jeb.009597

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