UNIVERSIDADE DE LISBOA
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Vulnerability of reef-building corals towards global change

"*Documento Definitivo*"

Doutoramento em Biologia
Especialidade de Biologia Marinha e Aquacultura

Marta Andreia Duarte Dias

Tese orientada por:
Professora Doutora Catarina Vinagre
Professor Doutor Henrique Cabral

Documento especialmente elaborado para a obtenção do grau de doutor

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- Doutor José Pavão Mendes de Paula, Professor Associado com Agregação, Faculdade de Ciências da Universidade de Lisboa
- Doutora Catarina Maria Batista Vinagre, Professora Auxiliar Convidada, Faculdade de Ciências da Universidade de Lisboa (orientadora)

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Nota prévia

A presente tese apresenta artigos científicos já publicados ou submetidos para publicação (capítulos 2 a 6), de acordo com o previsto no n˚2 do artigo 25˚ do Regulamento de Estudos de Pós Graduação da Universidade de Lisboa, publicado em Diário da República 2ª série – N˚155 – 11 de Agosto de 2017. Uma vez que estes trabalhos foram realizados em colaboração, a candidata esclarece que participou integralmente na concepção dos trabalhos, obtenção de dados, análise e discussão dos resultados, bem como na redacção dos manuscritos.

A presente tese, por ser uma compilação de publicações internacionais, está redigida em Inglês. Uma lista de referências é dada no final de cada capítulo em vez de no final da tese e devido a este formato poderão haver casos de duplicação entre capítulos. Cada capítulo contém também a informação de suporte associada ao mesmo. O formato diferente de alguns capítulos reflete os requerimentos específicos dos jornais científicos aos quais os manuscritos apresentados foram submetidos.

Lisboa, setembro de 2019

Marta Dias

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ABSTRACT

Global warming is leading to large-scale coral bleaching and mass mortality, but also to increases in tropical storms' frequency and intensity. Storms allow fragmentation of reef-building corals and can lead to near-shore salinity reduction which, combined with ocean warming, will aggravate coral distress. In order to assess the susceptibility of different coral species to these environmental stressors, small fragments of nine coral species of the Indo-Pacific region were exposed to different thermal (26°C, 30°C, 32°C) and hyposaline (26°C-33psu, 30°C-33psu, 26°C-20psu, 30°C-20psu) experimental treatments for 60 days. Several parameters were assessed at different levels of biological organization: at the organism level (total and partial mortality, and coral condition based in bleaching levels), physiological level (growth rate and regeneration rate of artificially inflicted lesions), and molecular level (superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and lipid peroxidation (LPO)). Also, in order to test two different approaches to be applied in the monitoring of the effects of heat stress, some parameters were combined in integrated biomarker response indices, either in a molecular approach, approach A, using GST, CAT, LPO, and SOD, or in an approach that integrates the molecular, physiological and organism levels, approach B, using GST, CAT, LPO, SOD, partial mortality, and growth rate. Results indicate that *Pocillopora damicornis* and *Stylophora pistillata* were the most vulnerable at 30°C. *Psammocora contigua, Turbinaria reniformis*, and *Galaxea fascicularis* were the most tolerant species at 32°C. The species *P. contigua* and *G. fascicularis* were the most tolerant to low salinity (26°C-20psu). The species *G. fascicularis* was the only one capable of surviving the combined effect of high temperature and low salinity (30°C-20psu). Approach B, the most integrative approach, was considered the most adequate for evaluating the health of reef corals since it better discriminated the stress suffered by the tested species.

Keywords: global climate change, heath stress, hyposaline stress, coral bleaching, health assessment

RESUMO

Os corais construtores de recife são fundamentais para a geomorfologia, biodiversidade, produtividade e complexidade estrutural dos recifes tropicais. O sucesso ecológico dos corais construtores de recife deve-se à sua simbiose com algas dinoflageladas unicelulares (zooxantelas). Como os corais depositam carbonato de cálcio e normalmente suplementam o seu metabolismo através da provisão de compostos fotossintéticos provenientes das zooxantelas, a disrupção desta associação simbiótica sob *stress*, denominada lixiviação, resulta em reduções significativas na fotossíntese das zooxantelas acompanhadas por alterações drásticas na fisiologia resultantes na perda dos simbiontes. As respostas dos corais à lixiviação podem incluir aumento da taxa respiratória, diminuição da taxa de calcificação do esqueleto e da capacidade de regeneração do tecido, redução da taxa de crescimento e das capacidades reprodutivas, degradação do tecido e consequente morte do coral, podendo levar à perda rápida de biodiversidade do recife afetado. Os recifes de coral estão entre os ecossistemas mais vitais mas também entre os mais ameaçados globalmente. Para além das pressões locais, estes ecossistemas estão atualmente a sofrer uma pressão sem precedentes e risco de extinção devido às alterações climáticas globais que têm resultado no aumento das temperaturas à superfície e na acidificação dos oceanos. Os ecossistemas de recife de coral, embora localizados nas águas marinhas mais quentes do mundo, são considerados particularmente vulneráveis ao aquecimento global devido ao facto de muitos organismos tropicais, incluindo os corais construtores de recife e espécies associadas, viverem próximos do seu limite de tolerância térmica máximo. O aquecimento global é a principal causa da lixiviação de corais em larga escala e de mortalidade em massa em comunidades de recife de coral em todo o mundo, levando à rápida degradação dos recifes de coral. Embora atuem à escala local, outros fatores para além da temperatura elevada têm sido correlacionados com a lixiviação de corais (por exemplo, salinidade reduzida). As técnicas mais recentes de modelação do clima global indicam que as condições térmicas para a lixiviação de corais estão a tornar-se mais frequentes. O contínuo aumento das temperaturas nos oceanos tropicais irá exacerbar a severidade e a frequência do *stress* térmico sofrido pelos recifes de coral, ameaçando a persistência de recifes dominados por corais ao longo dos trópicos, havendo previsões de que locais presentemente considerados refúgios térmicos para estas espécies poderão desaparecer a meio do século. Oceanos mais quentes conduzirão a tempestades tropicais mais frequentes e intensas levando a períodos cada vez mais curtos para recuperação das comunidades de corais entre recorrências e à perspetiva

de que o dano físico experienciado pelos recifes de coral irá aumentar. Além do dano físico, a forte precipitação que acompanha as tempestades tropicais irá baixar a salinidade em áreas superficiais próximas da costa. Os efeitos da salinidade reduzida podem ser exacerbados durante episódios de temperaturas elevadas, tornando-se importante perceber o efeito conjunto destas duas variáveis ambientais. Com o previsto aumento de intensidade e frequência das tempestades tropicais também aumentará a probabilidade de reprodução assexuada por fragmentação das colónias de corais construtores de recife, podendo estes fragmentos de coral dar origem a novas colónias nos recifes degradados e desta forma contribuir para a recuperação em pequena escala dos recifes afetados. No entanto, estes fragmentos também estarão sujeitos a pressões ambientais que potencialmente condicionarão a sua sobrevivência e crescimento. O primeiro nível de resposta de um organismo a uma perturbação ambiental verifica-se a nível molecular, passando posteriormente para níveis de organização mais elevados, até haver uma clara resposta do organismo como um todo. Deste modo, o estudo de parâmetros de resposta a diferentes níveis de organização biológica irá permitir uma análise mais completa. Diferentes espécies de corais construtores de recife apresentam diferente suscetibilidade ao *stress* térmico e salino, podendo levar a alterações na composição das espécies de coral após eventos de *stress* desta natureza e até mesmo à extinção local dos corais mais sensíveis. Os principais objetivos deste trabalho foram 1) determinar a suscetibilidade de várias espécies de corais construtores de recife à exposição prolongada a temperatura elevada e salinidade reduzida, e 2) testar e otimizar duas abordagens baseadas no uso de índices de resposta integrada de biomarcadores (sigla inglesa IBR – "Integrated Biomarker Response") para avaliação da saúde dos corais construtores de recife face ao *stress* térmico, num contexto de reprodução assexuada por fragmentação. Desta forma, pequenos fragmentos de nove espécies de corais construtores de recife da região do Indo-Pacífico foram submetidos a vários tratamentos experimentais, durante 60 dias. As espécies utilizadas apresentaram diferente morfologia da colónia: ramificada (*Acropora tenuis, Pocillopora damicornis*, *Stylophora pistillata*, e *Psammocora contigua*), massiva (*Galaxea fascicularis*), placa (*Montipora capricornis* (morfotipo castanho), *Echinopora lamellosa*, e *Turbinaria reniformis*), e incrustante (*Montipora capricornis* (morfotipo verde)), esta característica inerente às espécies está ligada à sua diferente suscetibilidade relativamente às variáveis ambientais em estudo. Os tratamentos térmicos experimentais incluíram a temperatura controlo (26°C) e as temperaturas de *stress* (30°C e 32°C). Por outro lado, a experiência de efeito combinado de temperatura e salinidade incluiu os seguintes tratamentos experimentais: controlo (26°C-33psu), temperatura elevada (30°C-33psu), salinidade reduzida (26°C-20psu) e combinação de temperatura elevada com salinidade

reduzida (30°C-20psu). De modo a obter uma visão mais generalista do efeito das variáveis em estudo nos organismos testados, parâmetros de diferentes níveis de organização biológica foram estudados: nível do organismo (mortalidade total, mortalidade parcial, e condição do coral que inclui os vários níveis de lixiviação), nível fisiológico (taxa de crescimento, taxa de regeneração de lesões infligidas artificialmente no tecido dos fragmentos), e nível molecular (determinação de biomarcadores de *stress* oxidativo: peroxidação lipídica (LPO), superóxido dismutase (SOD), catalase (CAT), e glutationa S-transferase (GST)). Também, de modo a testar duas diferentes abordagens para serem aplicadas na monitorização dos efeitos do *stress* térmico em corais construtores de recife dos oceanos Indo-Pacífico, alguns parâmetros pertencentes aos vários níveis de organização biológica foram selecionados e combinados em IBR's, ou na categoria de resposta de biomarcadores de *stress* oxidativo (abordagem A: GST, CAT, LPO, e SOD) ou na categoria de resposta integrada-performance do organismo (abordagem B: GST, CAT, LPO, SOD, mortalidade parcial, e taxa de crescimento). No presente trabalho observou-se que a mortalidade aumentou com a temperatura, atingindo 100% para a maioria das espécies após os 60 dias de exposição a 32ºC, exceto para *T. reniformis, G. fascicularis,* e *P. contigua.* Estas espécies apresentaram a mortalidade parcial e lixiviação mais baixos ao longo dos tratamentos térmicos experimentais. Relativamente a *T. reniformis* e *P. contigua*, não foi observado dano oxidativo na exposição a 32ºC, embora ambas as espécies tenham apresentado uma aparência pálida. *Galaxea fascicularis* foi a única das três espécies onde fragmentos lixiviados e dano oxidativo foram observados, embora tenha apresentado alguns fragmentos com uma aparência saudável a 32ºC. A taxa de crescimento diminuiu com o aumento da temperatura, sendo mais elevada em espécies de morfologia ramificada, enquanto que a taxa de regeneração de lesões geralmente aumentou com a temperatura. Foi concluído que *T. reniformis, G. fascicularis* e *P. contigua* foram as espécies mais resistentes ao *stress* térmico. Relativamente aos resultados do efeito combinado de temperatura e salinidade, no tratamento de temperatura elevada (30°C-33psu) apenas duas espécies morreram (*P. damicornis* e *S. pistillata*), enquanto que no tratamento de salinidade reduzida (26°C-20psu) todas as espécies morreram com a exceção de duas (*P. contigua* e *G. fascicularis*) passados 60 dias. Estas duas últimas espécies apresentaram a mortalidade parcial mais reduzida, a melhor condição, e nenhuma evidência de dano oxidativo tendo-se, no entanto, verificado resposta antioxidativa. A mortalidade foi mais elevada a 30°C-20psu devido ao efeito combinado da elevada temperatura e da salinidade reduzida, atingindo 100% em oito das nove espécies de coral, com apenas *G. fascicularis* sobrevivendo a este tratamento experimental. Contudo, a mortalidade foi elevada nesta espécie, observando-se também um aumento na atividade da SOD revelador de

uma resposta antioxidativa, não tendo, no entanto, o dano oxidativo sido detetado. As taxas de crescimento diminuíram com o aumento da temperatura e a diminuição da salinidade, enquanto que as taxas de regeneração aumentaram com a temperatura atingindo um máximo a 30°C-33psu e um mínimo a 20psu. Foi concluído que *P. damicornis* e *S. pistillata* foram as espécies mais vulneráveis ao tratamento de temperatura elevada, *G. fascicularis* e *P. contigua* foram os mais tolerantes ao *stress* hipossalino, e *G. fascicularis* foi a única espécie que tolerou o efeito combinado da temperatura elevada e da salinidade reduzida. Os resultados deste trabalho também indicam que o IBR pode ser um método a ser aplicado na avaliação da saúde de corais de recife sob *stress* térmico. A abordagem B, integradora da resposta molecular, fisiológica e do organismo, foi considerada a mais adequada uma vez que refletiu melhor o *stress* diferencial sofrido pelas espécies testadas, enquanto que a abordagem A, integradora apenas de biomarcadores moleculares, não foi suficiente para discriminar a resposta da maioria das espécies testadas. Assim, a integração da resposta de parâmetros de diferentes níveis de organização será a abordagem mais adequada para a avaliação de qualidade ambiental.

Palavras-chave: alterações climáticas globais, *stress* térmico, *stress* hipossalino, lixiviação, efeitos sinergísticos

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ANOVA, analysis of variance

APX, ascorbate peroxidase

BSA, bovine serum albumin

CAT, catalase

cm, centimeter

CDNB, chloro-2,4-dinitrobenzene

CO_2, carbon dioxide

DGAV, Direcção Geral de Veterinária

e.g., for example

EDTA, ethylenediaminetetraacetic acid

ENSO, El-Niño Southern Oscillation

Eq, equation

ε, extinction coefficient

FCT, Fundação para a Ciência e Tecnologia

FELASA, Federation of European Laboratory Animal Science Associations

Fig., figure

g, grams

GPx, glutathione peroxidase

GSH, reduced glutathione

GST, glutathione S-transferase

h, hour

hsp, heat shock protein

H_2O_2, hydrogen peroxide

HO●, hydroxyl radical

IBR, integrated biomarker response

IPCC, Intergovernmental Panel on Climate Change

K, kelvin

KCl, potassium chloride

KH_2PO_4, monopotassium phosphate

KIO_4, potassium periodate

L, litre

Ln, natural logarithmic

LPO, lipid peroxidation

M, molar concentration

m, meter

MARE, Marine and Environmental Sciences Centre

MDA, malondialdehyde bis (dimethylacetal)

mg, milligrams

Min, minutes

mL, mililitre

mm, milimetres

mM, milimolar

NA, not available or not-applicable

Na_2HPO_4, disodium phosphate

NaCl, sodium chloride

NBT, nitroblue tetrazolium

nm, nanometers

$^1O_2$, singlet oxygen

O_2•-, superoxide anion radical

˚C, degree Celsius

OH, hydroxide

OOH, oxide hydroxide

OTB, oxidative theory of coral bleaching

p, p-value

PAR, photosynthetically active radiation

PBS, phosphate buffered saline

PCA, principal component analysis

PERMANOVA, permutational multivariate analysis of variance

pg, picogram

psu, practical salinity unit

RGR, relative growth rate

RNS, reactive nitrogen species

ROS, reactive oxygen species

SD, standard deviation

SDS, sodium dodecyl sulphate

SOD, superoxide dismutase

SST, sea surface temperature

TBARS, thiobarbituric acid reactive substances

XOD, xanthine oxidase

%, percentage

~, approximately

±, more or less

Δ, variation

$\mu E\ m^{-2}\ s^{-1}$, µEinsteins per m^2 per second

µg, micrograms

µL, microlitres

µmol, micromoles

CHAPTER 1

General Introduction

1.1. Coral reef ecosystems

Coral reefs are ecologically and economically important ecosystems found across the world's tropical and sub-tropical oceans (Moberg and Folke, 1999; Wild et al., 2011). These ecosystems function as important spawning, nursery, breeding and feeding areas for a multitude of organisms, being among the most productive and biologically diverse marine ecosystems on Earth (e.g. Connell, 1978; Hughes et al., 2017a). Despite covering less than 0.1% of the ocean floor, reefs host 35% of all species living in the oceans (Ormond and Roberts, 1997; Spalding et al., 2001; Knowlton et al., 2010), with approximately 95,000 described coral reef species (Reaka-Kudla, 2005). Being one of the habitats most rich in species of the world, these ecosystems are important in preserving a vast biological diversity and genetic archive for future generations (Moberg and Folke, 1999). The remarkably high habitat heterogeneity of reef systems created by the complex three-dimensional structure enables niche diversification, making possible the evolution of new species (Birkeland, 1997; Paulay, 1997). Coral reefs' complex three-dimensional structure also dissipates wave energy to protect coastlines from storms, flooding, and erosion (Heron et al., 2017; Harris et al., 2018). In terms of economic benefits, coral reefs supply many millions of people with goods and services such as seafood (e.g. fish, mussels, crustaceans, sea cucumbers, and seaweeds, Craik et al., 1990; Birkeland, 1997), recreational possibilities, coastal protection, as well as aesthetic and cultural benefits (e.g. Cesar, 1996; Done et al., 1996; Peterson and Lubchenco, 1997; Hughes et al., 2017a), with an estimated value of over USD $1 trillion globally (Costanza et al., 2014; Hoegh-Guldberg, 2015).

1.1.1. Reef-building corals and zooxanthellae symbiosis

Corals, like jellyfishes, hydras, and sea anemones, belong to the phylum Cnidaria. Corals begin life as ciliated planular larvae, which, after a period of growth, attach to a suitable substrate and remain sessile for the rest of their adult lives (Harrison and Wallace, 1990). Once attached, corals are called polyps, and can reproduce by asexual budding to form colonies. Corals can be divided into the broad categories of soft corals (e.g. sea fans and sea whips, Order Alcyonacea), and hard corals (Order Scleractinia), which lay down a calcium carbonate skeleton and are capable of building reefs (Meehan and Ostrander, 1997). Scleractinian corals are fundamental to the geomorphology, biodiversity, productivity, and structural complexity of tropical coral reef ecosystems (Hoegh-Guldberg, 2004; Idjadi and Edmunds, 2006; Hart and

Kench, 2007; Pratchett et al., 2008). The ecological success of shallow-water reef-building corals throughout tropical oligotrophic waters relies on the symbiosis between the coral animal and its unicellular dinoflagellate algae (zooxanthellae, family Symbiodiniaceae [LaJeunesse et al., 2018]; Hoegh-Guldberg et al., 2007a). Zooxanthellae live inside membrane-bound vacuoles (symbiosomes) within gastrodermal cells of the coral host in extremely high densities (10^6 cm^{-2}) (Wakefield and Kempf, 2001; Wooldridge, 2014) and selectively transfer up to 90% of their photosynthetic products (mycosporine-like amino acids, glycerol, glucose or lipids; Wang and Douglas, 1999; Shick and Dunlap, 2002; Kopp et al., 2015) across the host-symbiont barrier (Muscatine, 1990; Hoegh-Guldberg, 1999; Stanley, 2006) with the remaining percentage of photosynthetic products being used for the respiration and growth of zooxanthellae (Edmunds and Davies, 1986). Zooxanthellae also cover 30% of the host's nitrogen requirements for growth, reproduction and maintenance from dissolved nutrient uptake (Bythell, 1990). It is a crucial energy source in an otherwise clear and nutrient-poor tropical waters that powers the metabolically costly process of calcification, allowing corals to deposit huge amounts of calcium carbonate (Muscatine, 1990; Hoegh-Guldberg, 1999; Hart and Kench, 2007; Ganot et al., 2011) within the warm and sunlit subtropical and tropical waters. Furthermore, during photosynthesis, algae produce large amounts of molecular oxygen for the respiration of corals (Solayan, 2016). In return, the coral host ensures protection to the symbiont and provides a source of nutrients from its waste metabolism (carbon dioxide, ammonia, sulfates, and phosphates) essential for photosynthesis (Yellowlees et al., 2008; Shinzato et al., 2011; Davy et al., 2012). This symbiotic relationship is of extreme importance to the overall reef community. Reef-building corals provide habitat, shelter and food to many phyla of reef organisms (Jones et al., 2004; Cole et al., 2008; Rotjan and Lewis, 2008; Dubinsky and Stambler, 2010; Gibson et al., 2011), as well as mediate biological interactions among coral associated organisms (e.g., competition, Munday, 2001, Holbrook and Schmitt, 2002; predation, Coker et al., 2009), thus promoting coexistence of many species. However, due to this distinct symbiosis, stony corals are more sensitive to changes in the marine environment, and display systemic responses to environmental stress (Parkinson and Baums, 2014). Since the ability of reef-building corals to build reefs is dependent upon the presence of zooxanthellae (Muscatine and Weis, 1992; Berkelmans and Van Oppen, 2006), the disruption of this symbiotic association under stress, i.e. bleaching, can cause rapid loss of reef biodiversity (Glynn, 1993; Brown, 1997a; Hoegh-Guldberg, 1999; Hughes et al., 2003; Hoegh-Guldberg et al., 2007a) and general lowering of the skeletal calcification rate in the affected zones of the reef (Gattuso et al., 1999; Allemand et al., 2004; Colombo-Pallotta et al., 2010).

1.1.2. Factors influencing coral reefs' community structure and development

Major factors influencing coral reefs' community structure and development are: (1) coral reproductive strategies; (2) colony growth rates; (3) competitive dominance relationships; (4) predation; and (5) physical disturbance, such as storms (Maguire and Porter, 1977).

1.1.2.1. Coral reproductive strategies

Most reef-building corals reproduce both sexually and asexually (Wallace, 1985; Richmond and Hunter, 1990). Sexual reproduction is achieved by either free spawning eggs and sperm, or internally brooding larvae inside the coral polyp (Harrison, 2011). The resulting planula larvae then disperse, settle, and metamorphose to form a new coral polyp. Asexual reproduction is achieved by budding of individual coral polyps (Jackson, 1977) and vegetative fragmentation (Tunnicliffe, 1981; Highsmith, 1982; Lirman, 2000). While sexually produced larvae may establish highly dispersed and genetically diverse populations, asexual recruitment through fragmentation or fission may allow rapid expansion of a population locally and enable the propagation of well-adapted genotypes within an area (Miller and Ayre, 2004; Baums et al., 2006), or assist in the colonization of habitats unfavorable for larval settlement (Heyward and Collins, 1985). The balance between sexual and asexual reproduction within a species can be influenced by both biotic and abiotic factors. In marine environments, disturbance events (e.g. ENSO events, global warming, tropical storms, Baums et al., 2006) can dramatically alter the contribution of asexual reproduction to recruitment (Henry and Kenchington, 2004; Le Goff-Vitry et al., 2004) and affect the genotypic diversity of a species (Hunter, 1993).

1.1.2.2. Colony growth rates

Reef-building corals present a wide range of life-history strategies according with species traits (Darling et al., 2012). These life-history strategies include a) "competitive" fast-growing branching and plating species; b) "weedy" corals which are small and brood their larvae; c) "stress-tolerant" slow-growing, long-lived massive, sub-massive and encrusting species, and d) "generalist" species that display characteristics of the other three strategies (Darling et al., 2012). Competitive corals such as branching corals of both genus *Acropora* and the family Pocilloporidae dominate many Indo-Pacific reef communities in terms of substrate cover and species diversity (Cumming, 1999; Dalton and Carroll, 2011; Lenihan et al., 2011). These

corals channel resources into growth for space monopolization and reproduction rather than for maintenance of colony integrity (Wallace, 1999), being the major contributors to reef-building species richness and biomass (Aronson et al., 2002; Bellwood et al., 2004), provision of food (Pratchett, 2010), and shelter for reef dwellers (Munday, 2004; Wilson et al., 2008; Alvarez-Filip et al., 2009; Bonin, 2012). Published linear growth rates for *Acropora* and *Pocillopora* are 2.09-18.5 cm a year and 1.22-4.50 cm a year, respectively (Harriott, 1999). On the other hand, stress-tolerant corals are the long-lived and slow-growing persistent corals such as massive faviids and poritids with published linear growth rates of 0.17–1.60 cm a year and 0.13–2.21 cm a year, respectively (Babcock, 1991; Harriott, 1999; Lough et al., 1999), which allocate considerable energy towards colony maintenance (Wallace, 1999).

1.1.2.3. Competitive dominance relationships

Competition has been established as a major structuring force on coral reefs (Chadwick and Morrow, 2011), it alters demographic patterns on coral reefs in terms of population growth, decline, and turnover rates. Competition among sessile organisms is a major process on coral reefs (Done, 1992; Bak et al., 1996; Griffith, 1997; Hughes et al., 2007). Macroalgae, soft corals, ascidians, and corallimorpharians are major competitors with scleractinian corals on tropical reefs (Chadwick and Morrow, 2011). Reef-building corals can be overgrown and smothered by both soft corals (Alino et al., 1992) and corallimorpharians (Chadwick, 1991), whereas the mechanisms employed by macroalgae to compete with corals include diverse physical and chemical processes that impact all stages of the coral life cycle (Titlyanov et al., 2007). The effectiveness of competitive mechanisms in sessile organisms depends on environmental factors, especially levels of nutrients and seawater temperature, which alter their growth rates relative to scleractinian corals (Chadwick and Morrow, 2011). Intraspecific competition among stony corals is also very common on tropical reefs, it can be either direct (involving contact and aggression between adjacent colonies; Dai, 1990), or indirect. Interactions between colonies are mediated by the water column, whereas indirect competition mechanisms include allelopathy and overtopping (Baird and Hughes, 2000). Studies have revealed alteration of species diversity on reefs, and in some cases, decreased diversity, due to competitive interactions mainly among reef-building corals (e.g. Connell et al., 2004). Thus, intraspecific competition is highly important in defining coral reefs structure (Baird and Hughes, 2000).

1.1.2.4. Predation

Many observational and manipulative studies have shown the importance of predators in structuring the benthic communities and maintaining spatial heterogeneity (Glynn, 1976; McClanahan and Muthiga, 1988; Dulvy et al., 2004). Corallivorous fishes inhabiting tropical reefs include butterflyfishes (most common fish, browsers that cause damage to the tissue; Motta, 1989), and parrotfish, puffers, triggerfish, filefish, wrasses, and damselfish (excavators or scrapers with the ability to remove skeletal material along with coral tissue; Rotjan and Lewis, 2008). Damage by corallivores ranges from minor to lethal, even limited removal of tissue or skeletal structures has growth and/or fitness consequences for a scleractinian coral colony (Rotjan and Lewis, 2008). The intensity of predation can have major cascading effects on coral populations (Done et al., 2010). Population outbreaks of both crown-of-thorns starfish (*Acanthaster planci* (Linnaeus, 1758)) and muricid gastropods of the genus *Drupella* Thiele, 1925 represent one of the most significant biological disturbances on tropical coral reefs throughout the Indo-Pacific oceans (e.g. Lourey et al., 2000; Pratchett, 2005; Baine, 2006; Bruno and Selig, 2007; Pratchett et al., 2009, 2011; Osborne et al., 2011; De'ath et al., 2012). Outbreaks of these two major invertebrate corallivores have the potential to decimate coral communities, thereby altering the biological and physical structure of reef habitats (Hoeksema et al., 2013; Scott et al., 2015; Moerland et al., 2016). These two corallivores feed preferentially on fast-growing corals with high rates of recruitment, mainly *Acropora* spp., *Montipora* spp., and Pocilloporidae corals (Pratchett, 2001, 2010; Morton et al., 2002; Kayal et al., 2012; Baird et al., 2013; Tsang and Ang, 2015). Aside from being responsible for large declines in live coral cover, reduced reef resilience and recovery (e.g. Carpenter, 1997; Lam et al., 2007), selective feeding by these corallivores causes differential mortality among coral species, resulting in population regime shifts in dominance where the non-preferred coral species, usually less abundant, increase in relative abundance (e.g. De'ath and Moran 1998; Cumming, 1999, 2009; Nicolet et al., 2013). Studies have observed shifts from complex staghorn *Acropora*-dominated habitats to relatively impoverished assemblages dominated by alcyonacean soft corals at Lizard Island, Australia (e.g. Pratchett, 2010). Thus, localized population outbreaks of both *A. planci* and *Drupella* spp. can rapidly and severely reduce coral survival (e.g. Cumming, 2009).

1.1.2.5. Physical disturbances: storms

Coral reefs are routinely affected by structural disturbances (Tanner, 2017). The level at which such disasters occur may range from whole reefs during infrequent hurricanes (Stoddart, 1974) to local fragmentation of single colonies on a day to day basis (Tunnicliffe, 1981). Catastrophic events such as storms are one of the most dramatic stressors on reef communities. The effects of storms are well-documented, and can range from minimal impact to total destruction of the reef structure (Rogers, 1993; Connell, 1997; Nott and Hayne, 2001; Connell et al., 2004; Fabricius et al., 2008; De'ath et al., 2012). Storm damage is important in the dynamics of many reefs (Huston, 1985; Rogers, 1993). Effects of tropical storms may be direct, including removal of reef matrix, scouring and fragmentation (Van Woesik et al., 1991; Done, 1992), or indirect, through the near-shore salinity reduction following intense rainfall and flooding (e.g. Goreau, 1964). Survivorship among the fragments of a storm damaged coral may be high during moderate storms (Highsmith, 1982), with subsequent regeneration and growth of dispersed fragments increasing clonal biomass and spreading the risk of future mortality among independent units. Severe storm damage, however, may be followed by prolonged mortality among fragments because of their increased susceptibility to disease and predation (Knowlton et al., 1981).

1.2. Effects of global climate change in coral reef ecosystems

Coral reefs are among the most threatened global ecosystems, and among the most vital (Costanza et al., 1997; Bryant et al., 1998; Boesch et al., 2000; Reaser et al., 2000). In addition to the local stressors (e.g. pollution, dredging, overexploitation, and outbreaks of both *A. planci* and *Drupella* spp., Hughes et al., 2003; Hoegh-Guldberg et al., 2007a; Lough, 2008; Rotjan and Lewis, 2008), coral reefs are experiencing unprecedented pressure and extinction risk due to global climate change (Pandolfi et al., 2003; Hoegh-Guldberg et al., 2007b; Spalding and Brown, 2015; Hughes et al., 2017a). Rapid build-up of carbon dioxide (and other greenhouse gases) in the atmosphere has resulted in the increase in sea surface temperatures (SSTs, Parmesan, 2006; IPCC, 2014), and ocean acidification in tropical ecosystems (Parry et al., 2007; Crabbe, 2008; Doney et al., 2009). Nevertheless, recent studies have shown that the warming of tropical oceans is a much more imminent threat to coral reefs' survival than ocean acidification (e.g. Chua et al., 2013; Frieler et al., 2013). Global warming impacts on marine ecosystems have become an international concern (Wellington et al., 2001; Hughes et al.,

2018a; IPCC, 2018). Coral reef ecosystems, although located in the world's naturally warmest marine waters, are considered to be particularly vulnerable to global warming (Hughes et al., 2003; Hoegh-Guldberg et al., 2007a) due to many tropical marine organisms, including reef-building corals and associated species, living near their upper thermal tolerance limits (Goreau, 1992; Hoegh-Guldberg, 1999; Randall and Szmant, 2009; Vinagre et al., 2018, 2019). Global warming is the primary cause of coral bleaching and mass mortality in coral reef communities around the world (Hoegh-Guldberg, 1999; Lesser, 2004; Hoegh-Guldberg et al., 2007b; Hughes et al., 2017a, 2018a; Langlais et al., 2017), leading to the rapid deterioration of the world's tropical coral reefs. Although acting in a local scale, other environmental factors besides high temperature have also been correlated with coral bleaching. These include salinity fluctuations (Goreau, 1964; Nakano et al., 1997), low and high levels of illumination—especially ultraviolet radiation (Lesser and Shick, 1989a; Gleason and Wellington, 1993; Banaszakand and Trench, 1995), increased sedimentation (Stafford-Smith, 1993; Riegl and Bloomer, 1995), nutrient imbalance (Rosset et al., 2017), aerial exposure during low tide (Leggat et al., 2006; Teixeira et al., 2013), low temperatures (Steen and Muscatine, 1987; Kobluk and Lysenko, 1994), as well as combinations of these factors (e.g. Berkelmans and Oliver, 1999; Lesser and Farrell, 2004; Ainsworth et al., 2016), disease (Kushmaro et al., 1996; Rosenberg and Loya, 1999), and various anthropogenic toxicants (e.g. pesticides, Van Dam et al., 2015).

1.2.1. Climate induced changes

The natural environment of a coral consists of many physical, chemical and biological factors interacting to produce the ecological framework within which the organism must survive and reproduce (Coles and Jokiel, 1978). Coral reefs have long been considered stenotolerant ecosystems, confined by a relatively narrow range of environmental conditions (Kleypas et al., 1999). Seawater temperature and salinity are major environmental factors contributing to the survival, growth, and photosynthesis of corals (Ferrier-Pagès et al., 1999, 2007; Baird and Hughes, 2000; Chow et al., 2009). Large-scale bleaching due to increased temperature has been a major area of coral research as such events have become more frequent and are correlated with large-scale coral mortality (Hoegh-Guldberg et al., 2007b; Le Nohaïc et al., 2017; Frölicher and Laufkötter, 2018; Hughes et al., 2018b; Montefalcone et al., 2018; Stuart-Smith et al., 2018; Sully et al., 2019; Vargas-Ángel et al., 2019). However, understanding the effects of localized stressors leading to bleaching, such as reduced salinity, is critical for protection of

corals growing in marginal habitats (Mayfield and Gates, 2007). Field and laboratory evidence reveal that reef-building corals are highly sensitive to both thermal and salinity stress (Goreau, 1964; Van Woesik et al., 1995; Hoegh-Guldberg, 1999; Donner et al., 2005; Hoegh-Guldberg et al., 2007b). Corals and their symbiotic algae maintain pools of small, organic molecules within the coral cells (Gates and Edmunds, 1999). Rapid fluctuations in these biochemical pools due to environmental stress may lead to osmotic stress and ultimately the degradation of the symbiosis (Mayfield and Gates, 2007). Both heat and hyposaline conditions can change the osmotic capacity of corals (Mayfield and Gates, 2007; Wagner et al., 2010) and can, in extreme cases, lead to bleaching, although the causative agents differ (Gates et al., 1992). Coral bleaching in response to low salinity seawater after an increase in seawater temperature is the second most commonly cited cause of coral bleaching (Glynn, 1991).

1.2.1.1. Heat stress impacts on reef-building corals

Coral reefs thrive at water temperatures ranging from 17-18°C to 33-34°C (Guilcher, 1988; DeVantier et al., 2004; Chen et al., 2005; Faxneld et al., 2011; Foster et al., 2014; Tong et al., 2017; Ross et al., 2018), with the most optimum growth occurring between 25-29 °C (Jokiel and Coles, 1977; Abramovitch-Gottlib et al., 2003; Bhagooli and Hidaka, 2004; Marshall and Clode, 2004). Aside from the wide tolerance of the reefs to temperatures, the coral species composing a given reef are really stenothermal and are adapted to only the ambient water temperatures in which they grow (Wafar, 1990; Salvat and Salvat, 1992; Fan and Dai, 1999; Guest, 2005; Silverstein et al., 2011; Richards and Rosser, 2012; Turak and DeVantier, 2012; Zhao et al., 2013). Short-term exposure to heat stress leads to negative impacts in both coral host's respiration rate and symbiont's photosynthesis (Jokiel and Coles, 1990; Castillo and Helmuth, 2005). The photosynthetic machinery of the symbiotic zooxanthellae is susceptible to moderate increases in temperature, enhancing chronic photoinhibition through the degradation of photosystem II (Iglesias-Prieto et al., 1992). Long-term exposure to heat stress leads to reduced growth rates (Abramovitch-Gottlib et al., 2003; Cantin and Lough, 2014), decrease in both tissue regeneration and reproductive capacities (Hoegh-Guldberg, 2004; Diaz-Pulido et al., 2009; Albright and Mason, 2013; Levitan et al., 2014; Osborne et al., 2017), increased susceptibility to disease (Miller et al., 2009; Mydlarz et al., 2009; Burge et al., 2014) and can, in extreme cases, cause a breakdown in coral-zooxanthellae symbiosis leading to coral bleaching (Edwards et al., 2001; Liu et al., 2003; Mayfield and Gates, 2007; Adjeroud et al., 2009; Carroll et al., 2017), which may be followed by tissue degradation, and consequent death

of the affected tissue. One of the most dramatic impacts of ocean warming on coral reefs is mass coral bleaching (Glynn, 1993; Hoegh-Guldberg and Salvat, 1995; Hughes et al., 2017c; 2018a). Field and laboratory studies have shown unequivocally that sustained and anomalously high summer water temperatures are associated with mass coral bleaching events (Glynn and D'Croz, 1990; Hoegh-Guldberg, 1999, 2007b; Loya et al., 2001; Podestá and Glynn, 2001; McClanahan et al., 2009; Langlais et al., 2017; Hughes et al., 2018a). Warming-related mass bleaching events are among the greatest threats to coral reefs around the world today (Pandolfi et al., 2011; Heron et al., 2016; Donner et al., 2017; Hughes et al., 2017b,c, 2018a). Since these events can lead to mass mortality from regional to global scales, they impact both the diversity and functioning of coral reef ecosystems (Graham et al., 2007; Baker et al., 2008; Pratchett et al., 2011a). Corals are known to have adapted or acclimatized to local environmental conditions (Logan et al., 2014; Palumbi et al., 2014) with temperature thresholds for bleaching varying locally and being linked to local summertime conditions (Glynn and D' Croz, 1990). Thermal bleaching can be induced by short-term exposure (i.e. 1-2 days) at temperature elevations of 3-4 °C above the average maximum summer temperature, or by long-term exposure (i.e. several weeks) at temperature elevations of only 1-2 °C above the average maximum summer temperature (Jokiel and Coles, 1990; Glynn, 1991), but the bleaching threshold also depends on the duration and magnitude of the heat stress and on the stress history of the corals (background climate conditions, Glynn, 1996; Baker et al., 2008; Carilli et al., 2012). For instance, impacts from thermal stress have been lower at sites where short-term pulses of low-level temperature stress preceded higher thermal stress later in summer (Ainsworth et al., 2016) or that have been affected by a prior but recent thermal stress event (e.g. Thompson and Van Woesik, 2009; Heron et al., 2016). For most low-latitude 'tropical' reef systems the observed temperature threshold for bleaching lies close to 30 °C (e.g. Hoegh-Guldberg and Smith, 1989; Glynn and D'Croz, 1990; Jokiel and Coles, 1990; Brown et al., 1996; Davies et al., 1997; Mumby et al., 2001; Adjeroud et al., 2009; Guest et al., 2012). A central role for the coral host in determining upper thermal bleaching thresholds supports observations that some genera, such as *Acropora, Stylophora, Seriatopora,* and *Pocillopora*, are highly susceptible to bleaching, whereas others such as *Cyphastrea, Goniopora,* and *Porites*, are highly resistant, and this hierarchy of susceptibility is consistent over wide geographic scales (Marshall and Baird, 2000; Loya et al., 2001; McClanahan, 2004).

As stenothermic organisms, corals are particularly sensitive to warming (Brown and Suharsono, 1990; Marshall and Baird, 2000). Since the early 1980s, large-scale mass coral

bleaching events have increased in geographic extent, intensity, and frequency in response to global warming (Hoegh-Guldberg, 1999; Aronson et al. 2000; Lough, 2000; Kleypas et al., 2001; Wellington et al., 2001; Heron et al., 2016; Hughes et al., 2018a) and have contributed to the rapid degradation of coral reefs (Eakin et al., 2016; Donner et al., 2017; Hughes et al., 2017b, 2018a). Warming of tropical seas has already pushed many scleractinian coral species close to their upper thermal limit (Hoegh-Guldberg, 1999; Langdon and Atkinson, 2005; De'ath et al., 2009; Tanzil et al., 2009; Manzello, 2010). Over the past four decades, a loss of > 40% of the world's coral reefs has been observed (Burke et al., 2011). Three pan-tropical global mass bleaching events (1998, 2010, and 2015/16) associated with El Niño-Southern Oscillation (ENSO) driven warming events affected virtually all reefs in the world (Heron et al., 2016; Van Open et al., 2017; Hughes et al., 2018a). The 2015–16 El Niño event resulted in significant warming of large areas of the tropical oceans and continued bleaching of substantial areas of reef (Hughes et al., 2017b; L'Heureux et al., 2017), affecting 75% of the Indo-Pacific coral reefs (Hughes et al., 2018a). This event was unprecedented in duration and magnitude resulting in the longest and most severe global coral bleaching event on record (Eakin et al., 2016). Coral mortality was among the worst ever observed (Hughes et al., 2017b), and even remote and pristine reefs that experience minimal human degradation were severely affected (Hughes et al., 2017a).

1.2.1.2. Hyposalinity impacts on reef-building corals

Corals and other reef organisms can live in normal salinities as low as 25 psu and as high as 45 psu, even though most coral reefs occur in a more moderate salinity environment (Wolanski, 1981; Coles, 1988; Coles and Jokiel, 1992; Kleypas et al., 1999; Berkelmans et al., 2012). Osmotic stress represents a limiting physical parameter for marine organisms and especially for sessile scleractinian corals which are known to be basically stenohaline and osmoconformers, even with some species being euryhaline and withstanding significant changes in external osmolarity (Muthiga and Szmant, 1987; Coles, 1992; Manzello and Lirman, 2003; Mayfield and Gates, 2007). Since reef-building corals have a limited osmoregulation capability (Ferrier-Pagès et al., 1999; Kerswell and Jones, 2003; Manzello and Lirman, 2003), they do not possess a constant cellular osmolarity, but respond to dynamic changes in their environment by rapidly absorbing water to become iso-osmotic with their surroundings (Rankin and Davenport, 1981; Titlyanov et al., 2000; Mayfield and Gates, 2007). All cells require a stable environment to function properly. Thus, osmotic stress due to

fluctuations in cell volume and osmolyte, that is, failure of coral and algae to maintain a compatible osmotic environment, can compromise macromolecular structures and metabolic function (e.g. enzyme kinetics, Vernberg and Vernberg, 1972; Fabricius, 2005; Berkelmans et al., 2012) and lead to the production of reactive oxygen species (ROS) from increased metabolism in response to the osmotic stress (Freire et al., 2012). Therefore, major salinity changes (outside typical daily and seasonal fluctuations) can cause important cellular damage, since corals lack any developed physiological regulatory system, and can lead to the breakdown of the symbiosis (Kerswell and Jones, 2003) or even death (Hoegh-Guldberg and Smith, 1989; Mayfield and Gates, 2007; True, 2012).

The immediate reaction of corals subjected to low salinity include polyp retraction and tissue paling (Lirman and Manzelo, 2009). Short-term exposure (hours) to salinity stress can negatively influence the basal metabolic functions of the corals, inducing changes on the symbiotic algae photosynthetic efficiency (Muthiga and Szmant, 1987; Coles and Jokiel, 1992; Moberg et al., 1997; Ferrier-Pagès et al., 1999; Porter et al., 1999; Alutoin et al., 2001; Kerswell and Jones, 2003; Manzello and Lirman, 2003; Chartrand et al., 2009) and in the respiratory pathways of the coral host (Ferrier-Pagès et al., 1999; Porter et al., 1999; Alutoin et al., 2001; Faxneld et al., 2010), which in turn reduce energy levels and organic carbon (Moberg et al., 1997; Ferrier-Pagès et al., 1999; Chavanich et al., 2009). Long-term exposure to reduced salinity conditions can imply decreased growth potential (Coles, 1992) and also give rise to higher-order physiological diseases, such as gamete abnormalities and reduced viabilities, that affect fecundity, settlement success, and larval survivorship (Jokiel, 1985; Richmond, 1993; Humphrey et al., 2008; True, 2012; Scott et al., 2013) and can, in extreme cases, cause a breakdown in coral-zooxanthellae symbiosis leading to coral bleaching (Goreau, 1964; Egaña and DiSalvo, 1982; DeVantier et al., 1997; Mayfield and Gates, 2007). Acute stress during exposure to low salinity often leads to tissue damage (Van Woesik et al., 1995) and consequent coral death (Hoegh-Guldberg and Smith, 1989; Jokiel et al., 1993), followed by immediate coral's tissue sloughing. An effect that probably underlies the mass mortalities of corals after severe rainy storms or flood events (Kerswell and Jones, 2003).

Coral reefs are frequently subject to fluctuations in seawater salinity, due to precipitation, storms, freshwater runoff, periods of prolonged drought or desalination processes (Coles and Jokiel, 1992, Leichter et al., 1996; Devlin et al., 2001). Several studies investigated the response of corals to low salinity bleaching, indicating that hyposalinity is a significant

environmental factor that may impact a coral reef and influence the distribution of cnidarian species and populations (e.g. Berkelmans and Oliver, 1999; Sakami, 2000; Titlyanov et al., 2000; Rogers and Davis, 2006). Bleaching of corals in response to low salinity seawater is the second most commonly cited cause of coral bleaching (Glynn, 1991). The degree of impact by hyposaline conditions is largely determined by the degree and length of exposure (Berkelmans et al., 2012). Observations made in previous studies support previous estimates of 15 psu to 20 psu as the lower lethal salinity in reef corals (Coles and Jokiel, 1992). Coles and Jokiel (1992) stated that salinities below 15 psu sustained for more than 2 days will lead to coral mortality. In Hawaii, sudden and large drops in salinity (to 15–20 psu for 24 h) associated with heavy rains and discharge have caused coral death (Jokiel et al., 1993).

Corals in near-shore environments are particularly vulnerable to the effects of climate change (True, 2012). Bleaching and mortality of near-shore corals are often reported in association with hyposaline conditions resulting from heavy rainfall events (e.g. Egaña and DiSalvo, 1982; Van Woesik et al., 1995; Berkelmans and Oliver, 1999; Hendy et al., 2003; Lirman et al., 2008). On coral reefs, long-term exposure to hyposaline conditions resulting from heavy rainfall events is not uncommon (Devlin et al., 2001). Many studies report localized bleaching after large storms and hurricanes, torrential downpours, and river discharges that greatly lowered near-shore salinity (e.g. Goreau, 1964; Egaña and DiSalvo, 1982; Engebretson and Martin 1994; Van Woesik et al., 1995). Both Goreau (1964) and Van Woesik et al. (1995) reported striking cutoffs between discolored (bleached) and normally pigmented corals coinciding with the depth to which floodwaters penetrated, that is, only the shallow portions of the coral colonies in contact with a low salinity water layer became bleached, whereas the deeper portions remained normal. Laboratory-based studies have also confirmed that corals discolor during exposure to low-salinity seawater (Kerswell and Jones, 2003). It is believed that the expulsion of the zooxanthellae was induced by contact with water of lowered osmotic pressure on the surface of the sea (Goreau, 1964). Several reports describe salinity reductions causing massive coral and reef organism mortality (e.g. Stoddart, 1969; Bienfang, 1980; Johannes, 1980; Lewis, 1985). However, it is during major storm events that the effects of lowered salinity on coral reefs are most dramatic (Goreau, 1964; Coles and Jokiel, 1992; Jokiel et al., 1993; Van Woesik et al., 1995; Devlin et al., 2001; Blakeway, 2004; Butler et al., 2013). For example, a major reef kill in Kaneohe Bay, Oahu resulted in death without bleaching of corals to depths of 3 m (Jokiel et al., 1993). Thus, fresh water kills have a strong influence on the structure of reef coral communities.

1.2.2. Bleaching consequences

Coral bleaching is a stress response that describes the morphological changes that occur during the breakdown of the symbiotic association between the coral host and the symbiotic zooxanthellae (Glynn, 1991; DeSalvo et al., 2008). Bleaching is associated with a pronounced loss of color from affected corals due to the reduced number of zooxanthellae and/or reduced concentration of photosynthetic pigments in the zooxanthellae, the white skeleton becomes visible through the transparent coral tissue, giving the organism a "bleached" white appearance (Glynn, 1991; Baker et al., 2008; Hoegh-Guldberg, 2011; Wild et al., 2011; Hume et al., 2013). Bleaching is an obvious visual indication of significant levels of stress affecting a coral colony (Jokiel and Coles, 1990) and is fatal to the coral unless the symbiotic relationship can be quickly re-established (Hoegh-Guldberg, 2011). As healthy corals normally supplement their metabolism through the provision of photosynthetic compounds by zooxanthellae (Davy et al., 2012), bleaching culminates in significant reductions in zooxanthellae photosynthesis accompanied by drastic changes in physiology (Lesser and Shick, 1989a; Muscatine et al., 1991; Warner et al., 1996; Jones et al., 2000; Suzuki and Kawahata, 2003; Rodrigues and Grottoli, 2006). The potential physiological and ecological effects of bleaching are numerous. Although mortality might not always eventuate, responses in the coral hosts include an increase in respiration rate, declines in coral protein, lipid, and carbohydrate concentrations in the tissues, a weakening of tentacular movement (Glynn, 1991), a decrease in skeletal calcification rate (Leder et al., 1991), decrease in tissue regeneration ability (Meesters and Bak, 1993), reduced growth rates and reproductive capacities (Szmant and Gassman, 1990; Baird and Marshall, 2002; Carilli et al., 2009), increased susceptibility to disease (Harvell et al., 1999; Bruno et al., 2007; Bourne et al., 2008), tissue degradation, and consequent death of the affected tissue (Szmant and Gassman, 1990; Williams and Bunkley-Williams, 1990; Michalek-Wagner and Willis, 2001). Eventual impacts of bleaching (over years to decades) can include reduced reef rugosity, coral cover, and biodiversity (Baker et al., 2008) and perhaps local extinction of coral species (Brainard et al., 2013). Coral bleaching has become a major threat to coral reef ecosystems worldwide (Eakin et al., 2009). Coral bleaching is not always fatal, it depends on the intensity and duration of the stress (Hoegh-Guldberg, 1999). On the one hand, if the heat stress event is mild and short-lived, the symbiotic association can be re-established and their physiology may be restored (Hoegh-Guldberg et al., 2007b; Grottoli et al., 2014; Schoepf et al., 2015), with zooxanthellae populations inside the host tissues returning to pre-bleaching levels after 2–6 months depending on the coral species (Hoegh-Guldberg and Smith,

1989; Brown, 1997b; Hoegh-Guldberg and Jones, 1999) and sub-chronic impacts on coral growth and reproduction that may last for several years (Ward et al., 2002). On the other hand, if a heat stress event is sustained or particularly severe and the zooxanthellae populations are not recovered by the host, severe and/or prolonged symbiotic breakdown leads to the mortality of the coral host due to starvation (Hoegh-Guldberg, 1999; Baird and Marshall, 2002; Berkelmans et al., 2004; Oliver et al., 2009; Eakin et al., 2010, 2016). Severe bleaching can have substantial long-term impacts on coral communities, even in areas with corals tolerant to environmental extremes (Burt et al., 2011).

Severe coral bleaching events have caused mass mortality of corals in tropical regions around the globe (Goreau et al., 2000; Aronson et al., 2004; Graham et al., 2006; Baker et al., 2008). In the Indo-Pacific oceans, mass mortality of reef-building corals associated with heat stress events has occurred several times (Oliver, 1985; Brown and Suharsono, 1990; Glynn, 1990a; Glynn and De Weerdt, 1991; Brown, 1997a; Wilkinson, 2000; Glynn et al., 2001; Riegl, 2002; Hughes et al., 2018b,c; Stuart-Smith et al., 2018) with the most severe heat stress events typically associated with ocean–atmosphere phenomena, such as ENSO, that result in sustained regional elevations of ocean temperature (McPhaden, 1999; Deser et al., 2010; Eakin et al., 2016; Hughes et al., 2017b; L' Heureux et al., 2017). These stressful events lead to population collapses and consequent local extirpation (e.g. Loya et al., 2001; Hughes et al., 2003; Pandolfi et al., 2003; West and Salm, 2003; Carpenter et al., 2008; Brooker et al., 2014) contributing to the decline of coral reefs (Brown, 1997a). In recent years, episodes of mass coral bleaching have led to catastrophic loss of coral cover, with consequent phase-shifts away from coral dominance in some locations, and to persistent changes in coral community structure in many others. Widespread coral loss and associated transformation of reef habitats are jeopardizing the structure and function of coral reef ecosystems (Hughes et al., 2003; Hoegh-Guldberg et al., 2007b). On the one hand, the decline of coral cover has resulted in shifts in dominance from scleractinian corals to macroalgae imposing subsequent constraints on the replenishment and recovery capacity of coral assemblages (Done et al., 2007; Hughes et al., 2007, 2010; Cheal et al., 2010; Depczynski et al., 2013). On the other hand, other sessile organisms (e.g. sponges, ascidians or soft corals) may proliferate following extensive loss of scleractinian corals (Norström et al., 2009; Tebbett et al., 2019). These phase-shifts are reinforced by changes in ecological processes that promote the persistence of non-coral organisms and further inhibit the growth, recruitment and/or survival of corals (Hughes et al., 2010). Furthermore, persistent changes in community structure have occurred in other locations, characterized by the death of

fast-growing dominant and branching heat-susceptible coral species and their replacement by slow-growing sub-dominant and massive coral species (Hoegh-Guldberg, 1999; Adjeroud et al., 2009; Van Woesik et al., 2011; Johns et al., 2014; Eakin et al., 2016; Hughes et al., 2017b; Le Nohaïc et al., 2017), delaying reef growth (Glynn, 1991). Persistent changes in community structure towards stress-tolerant species can increase the resistance of a coral community to disturbances, so that coral cover can reach high levels in spite of frequent pulse disturbances and chronic stressors. However, the ability of even stress-tolerant corals to resist or recover is limited (Côté and Darling, 2010). Reef areas that have suffered mass mortalities eventually begin to disintegrate as physical and biological erosion outpace calcium carbonate accretion by remaining corals (Done, 1992). Loss of structural complexity from reef disintegration, combined with overgrowth by algae and lack of recruitment success of corals on damaged reefs (Glynn, 1990b; Reaka-Kudla et al., 1993; Diaz-Pulido and McCook, 2002; Gilmour et al., 2013; Eakin et al., 2016, 2017; Mullen et al., 2017), can lead to dramatically altered patterns of coral species composition and even complete restructuring of communities (Graham et al., 2006), resulting in overall decline of biodiversity, ecological services, and fisheries production in the marine tropics (Garpe et al., 2006; Graham et al., 2006, 2007, 2008; Pratchett et al., 2008; Richardson et al., 2018).

1.2.3. Predictions under future climate change scenarios

Global climate modelling and satellite observations indicate that the thermal conditions for coral bleaching are becoming more prevalent (Van Hooidonk et al., 2013; Hoegh-Guldberg et al., 2014; Liu et al., 2014, 2017; Heron et al., 2016). Average SSTs near coral reef ecosystems are predicted to further rise 1–3.7 °C over the 21st century (IPCC, 2014, 2018). Continued warming of the tropical oceans combined with projected more frequent and severe El Niño events (Power et al., 2013; Cai et al., 2014) will continue to exacerbate the severity of heat stress experienced by coral reefs (Frieler et al., 2013) with each strong El Niño event resulting in a higher level of stress than previous ones and the emergence of significant bleaching in non-El Niño years (Hughes et al., 2018a). The interval between thermal stress events has also shortened with the 0.92 °C of global warming observed to date (Van Hooidonk et al., 2013, 2014; Hoegh-Guldberg et al., 2014; Ainsworth et al., 2016; Hughes et al., 2018c), threatening the persistence of coral-dominated reefs across the tropics (Pandolfi et al., 2003; Baker et al., 2008; Frieler et al., 2013; Van Hooidonk et al., 2013, 2014, 2016; Logan et al., 2014; Gattuso et al., 2015; Heron et al., 2016; Hughes et al., 2017b, 2018a) and leading to predictions that

localities now considered to be thermal refugia could disappear by mid-century (Van Hooidonk et al., 2013). It has been suggested that, unless there is substantial thermal adaptation of coral hosts and/or their symbiotic algae (Donner et al., 2005; Baird and Maynard, 2008; Donner, 2009), mass bleaching and mortality events could occur annually on the world's coral reefs by 2050 (Nicholls et al., 2007) with a high likelihood for more globally widespread and intense bleaching events (Perry and Morgan, 2017). Modelling studies suggest that corals need to increase their thermal tolerance by about 1.5 °C to significantly delay the onset of more frequent bleaching events (Donner, 2009). Even with the aspirational Paris Agreement target of limiting global warming to 1.5 °C above pre-industrial levels, it is estimated that after 2050, 70% of the world's reefs will be at risk of severe degradation (Schleussner et al., 2016). Warmer seas are likely to drive more intense and frequent tropical storms (Emanuel, 2005; IPCC, 2014) leading to a shorter time for recovery between recurrences (Watson et al., 2001; Emanuel, 2005; Elsner et al., 2006a,b) with the prospect that the extent to which coral reefs experience physical damage may increase (Hoegh-Guldberg, 2011). Changes in global weather patterns will lead to more extreme rainfall over most of the mid-latitude land masses and over wet tropical regions (Hoegh-Guldberg et al., 2007a; IPCC, 2014). Furthermore, heavy rainfall accompanying tropical storms will lower salinity in shallow near-shore areas (Milly et al., 2002; Haapkylä et al., 2011). The rate of environmental change is likely to be considerably accelerated in the near future. Multiple synergistic environmental disturbances are likely to interact to create stressful conditions unique to the current epoch (e.g. warm water and low salinity).

1.2.4. Importance of fragmentation in corals' propagation by tropical storms

Several factors influence the success of asexual propagation by fragmentation of different coral species upon tropical storms. These include: tropical storm intensity and frequency (Lirman, 2003), the abundance of large colonies (Grosberg and Cunningham, 2001), colonies age (Schaffer, 1974), slope inclination of the substratum (Harmelin-Vivien and Laboute, 1986; Kjerfve et al., 1986), coral species morphology (Hughes, 1983), the fragment size (Highsmith et al., 1980), the type of substratum (Lirman, 2000), and the occurrence of subsequent disturbance (Rogers et al., 1982; Glynn, 1997). Tropical storms are favorable to the propagation and expansion of branching, plating, and massive coral forms by asexual reproduction of storm-generated fragments across the reef (Highsmith, 1980, 1982; Highsmith et al., 1980; Tunnicliffe, 1981; Lirman, 2000; Foster et al., 2007, 2013). During storm surge,

coral fragments may become detached from parent colonies (Richmond, 1997). Coral fragments can either regenerate lesions caused by partial colony mortality and fuse back with the original colony (Hildemann et al., 1977; Meesters and Bak, 1993) or, if the conditions are favorable, the fragments can be dispersed and survive, forming separate isogeneic colonies (Jokiel et al., 1983; Neigel and Avise, 1983). Fragment survivorship may be sufficiently high that artificially generated fragments can be successfully seeded onto degraded reefs to promote small-scale recovery (e.g. Kobayashi, 1984; Harriott and Fisk, 1988). Production of new colonies by fragmentation of established colonies is shown to be an extremely important mode of reproduction and local distribution among major reef-building corals (Highsmith, 1982). The detachment and re-distribution of hermatypic corals is an important process on reefs, fragmentation leads to their colonization of otherwise inaccessible habitats, patch reef development, reef extension, and asexual colony multiplication (Highsmith, 1980, 1982; Foster et al., 2013; Roth et al., 2013). Most importantly, it allows a species to persist when it is unable to complete its sexual reproductive life cycle (Honnay and Bossuyt, 2005).

The fragmentation of stony coral colonies appears to be a widespread and extremely important method of reproduction and distribution (Highsmith, 1980; Highsmith et al., 1980). In some areas corals may rely solely on fragmentation for persistence (Honnay and Bossuyt, 2005; Baums et al., 2006), by spreading the risk of encountering unfavorable local environments (Jackson, 1977; Highsmith, 1982). Fragmentation may be the predominant mode of reproduction for many of the major reef-building corals (Highsmith, 1982). The combination of high frequency of fragmentation and the apparent lack of sexual reproductive success has been considered as evidence of the adaptive value of fragmentation for several coral species (Bak and Engel, 1979; Highsmith et al., 1980; Bothwell, 1981; Tunnicliffe, 1981; Highsmith, 1982). Fragmentation is considered to be an adaptation to both unfavorable local environmental conditions and relatively stable habitats (Honnay and Bossuyt, 2005). The highest rates of fragmentation typically occur during stress events, beyond storms, heat stress is also responsible by high rates of fragmentation on coral reefs (Roth et al., 2013). Anomalously high water temperatures lead to coral bleaching and consequent increase in the rates of partial colony mortality (Hoegh-Guldberg, 1999). The loss of live tissue provides new substrate for bio-eroding organisms, which decreases the integrity of the skeleton and increases the probability of colony fragmentation (Glynn, 1997). Likewise, an outbreak of coral disease frequently occurs during or soon after heat stress events (Muller et al., 2008; Rogers and Muller, 2012), leading to partial colony mortality and increased rates of colony fragmentation (Glynn, 1997).

1.2.5. Recovery after disturbance

Coral community maintenance, reef growth, and recovery are highly dependent on both sexual and asexual reproductive processes (Kleypas et al., 2016; Glynn et al., 2017; Holbrook et al., 2018). Coral populations are maintained and replenished mainly by sexual larval recruitment and asexual fragmentation (e.g. Highsmith, 1982; Harrison and Wallace, 1990; Richmond, 1997). Recovery of coral populations following disturbance relies on the arrival of newly settling larval recruits (Sale et al., 2005; Cowen et al., 2006), and their successful recruitment and growth (Caley et al., 1996; Doropoulos et al., 2016), as well as on the growth and propagation of surviving coral colonies (Golbuu et al., 2007; Mumby et al., 2007; Gilmour et al., 2013; Done et al., 2015; Graham et al., 2015). Most asexual lineages depend on sporadic sexual recombination to add variation to their genomes, purge deleterious mutations, and adapt to changing environments (Kondrashov, 1988). A strategy combining asexual reproduction with low levels of sexual reproduction assemble the best of both strategies (Hurst and Peck, 1996). It is likely that both reproductive modes are effective, with one or another predominant depending on the type and severity of disturbance and the particular local conditions prevalent during recovery (Glynn et al., 2017). Predicting population recovery is complex. Recovery rates of corals depend on a number of variables, including the spatial scale of a species' distribution, life history traits such as reproduction strategies, stress tolerance, growth capacities, and competitive abilities (Darling et al., 2012; Kayal et al., 2015), how the neighboring sites fare through stress events and their capacity to supply recruits (Van Woesik et al., 2011), and the intensity (Ateweberhan et al., 2011; Riegl and Purkis, 2015), frequency, nature, and history of the disturbance (Dayton, 1971; Sousa, 1979; Adjeroud et al., 2009; Pratchett et al., 2011b; Holbrook et al., 2018; Torda et al., 2018). Furthermore, recovery rates vary among geographical locations (Baker et al., 2008; Graham et al., 2011; Ortiz et al., 2014), among reefs with different levels of conservation management (Graham et al., 2011; McClanahan, 2014) and among reefs and habitats (Golbuu et al., 2007; Osborne et al., 2011; Johns et al., 2014). For instance, reefs in the Indo-Pacific have greater recovery potential than Western Atlantic reefs (Connell, 1997; Roff and Mumby, 2012). Local practices to improve water quality (Fabricius et al., 2005; De'ath and Fabricius, 2010; Brodie et al., 2012), stabilise rubble (Fox et al., 2005), and avoid ecosystem overfishing of herbivores (Hughes et al., 2007; Doropoulos et al., 2016; Mumby et al., 2016), as well as global initiatives to reduce the ultimate burdens on reefs, such as human impacts on the environment and climate (Mora, 2008; Mora et al., 2011, 2016), all have a role to play in improve the prospects for coral recovery (Cardini

et al., 2012; Graham et al., 2015; Scheffer et al., 2015; Shlesinger and Loya, 2016; Sheppard et al., 2017; Shaver et al., 2018). Coral reefs are dynamic ecosystems and can, given time, recover from severe stress events (Sheppard et al., 2008; Graham et al., 2011; Gilmour et al., 2013), though often with modified coral composition community (Graham et al., 2006).

Regarding recovery after storms, recovery of mechanically damaged reefs to pre-disturbance levels is highly dependent on the continued growth of those colonies that remained upright after a disturbance, colonization by newly produced coral planulae, the stabilization and growth of coral fragments, and the composition of surviving coral communities (Endean, 1976; Pearson, 1981; Connell and Keough, 1985; Hughes and Tanner, 2000). Recovery time can range from a few years for reefs dominated by faster-growing branching species when asexual reproduction and regeneration are possible (Shinn, 1976) to several decades for reefs that suffer extreme damage (severe storms) that hardly any fragments of reef-building corals survive, being survival dependent upon sexual reproduction (Grigg and Maragos, 1974), and/or for reefs that are dominated by slower-growing corals (e.g. Pearson, 1981; Coles, 1984; Guzmán et al., 1991). On a regional scale, assessment of changes in coral cover following acute disturbances in locations across the Indo-Pacific revealed that the rate of return to the pre-disturbed level was related to a variety of factors, including region (Holbrook et al., 2018) and severity of disturbance (Graham et al., 2011). For instance, the time scale for recovery of coral cover to pre-disturbance values after tropical storms has varied from around a decade in areas where the substratum was not greatly damaged (i.e. physically crushed and broken) by the disturbance, to two decades where disturbances greatly altered physical structure (Connell et al., 1997; Tanner, 2017). Frequent storms do not provide sufficient time for low recruiting massive colonies to re-establish before the next storm. Moderate disturbances, on the other hand, may help maintain diversity, whereby only competitive species are affected, preventing space monopolization (Dayton, 1971; Sale, 1977; Connell, 1978; Sousa, 1979; Van Woesik, 1992).

Regarding recovery after bleaching events, once bleached, subsequent mortality or the ability of corals to recover may depend on environmental factors such as the duration and magnitude of the warm water event (Connell et al., 1997; Hughes et al., 2018b,c), background nutrient levels (Wiedenmann et al., 2013), the availability of heterotrophic food resources (Grottoli et al., 2006) or local oceanographic conditions such as upwelling (Wall et al., 2015; Barkley et al., 2018). In the Indo-Pacific, return of coral reefs to pre-disturbance coral cover after

bleaching events has been documented in several locations over the last decades. Studies from several locations including the Great Barrier Reef, Australia (Johns et al., 2014), Moorea, French Polynesia (Adjeroud et al., 2009), Okinawa, Japan (Van Woesik et al., 2011), Scott Reef, northwestern Australia (Gilmour et al., 2013), and Seychelles (Graham et al., 2015) have reported recovery from major disturbances in one to two decades. The capacity to resist and recover from future bleaching will likely be dependent on factors such as the duration between events and recovery time (Grottoli et al., 2014; Schoepf et al., 2015; Hughes et al., 2018a), acclimatization of symbiont communities and coral hosts (Middlebrook et al., 2008; Bellantuono et al., 2011; Silverstein et al., 2012; Barshis et al., 2013; Grottoli et al., 2014), recruitment and propagation of bleaching tolerant genotypes (Coles and Brown, 2003; Maynard et al., 2008; Thompson and Van Woesik, 2009; Van Woesik et al., 2011; Voolstra et al., 2011), and changes in coral community composition toward dominance by more heat-tolerant taxa (Coles and Brown, 2003; Maynard et al., 2008; Sampayo et al., 2008; Van Woesik et al., 2011). The recurrence of mass bleaching during the recovery period may prevent coral communities from achieving full recovery (Hughes et al., 2018a), outpacing the capacity of some important reef-building coral species to recover (Grottoli et al., 2014; Schoepf et al., 2015) and may preclude adaptation (Hughes et al., 2018a). While coral reefs can recover from severe bleaching, the time required for recovery is often long (Baker et al., 2008). As global temperatures have risen from 1980 to 2016, the time windows for reefs to recover from consecutive mass bleaching events have shortened from 25-30 years to just 6 years (Hughes et al., 2018a). This time interval is too short to allow a full recovery of mature coral assemblages, which generally takes 10-15 years for the short-lived and fastest growing species (Connell et al., 1997; Gilmour et al., 2013; Hughes et al., 2018a) and much longer for long-lived and slow growing species (decades to centuries, Kayanne et al., 2002; McClanahan, 2014; Glynn et al., 2015; Osborne et al., 2017). Recovery of long-lived species requires the sustained absence of another severe disturbance, which is no longer realistic while global temperatures continue to rise (Van Hooidonk et al., 2016). The time between recurrent bleaching events is likely to become even shorter as severe bleaching events are expected to occur annually by 2050 (Van Hooidonk et al., 2016).

1.2.6. Oxidative stress in reef corals

The whitening of corals is a visual indication of significant levels of stress affecting a coral colony (Jokiel and Coles, 1990), but does not indicate the causes. The first detectable response

to an environmental stressor is usually expressed as changes at cellular and biochemical level, before the physical signs of a stress are evident (Bierkens, 2000). Although the coral-algal symbiosis has many inherent benefits, hosting intracellular photosynthetic organisms may cause local hyperoxia in both host and symbiont (e.g. Dykens and Shick, 1982; Kühl et al., 1995; Richier et al., 2003; Lichtenberg et al., 2016) and promotes the generation of ROS (Dykens et al., 1992; Lesser, 2006). During both normal aerobic respiration in the coral host and photosynthesis in the symbiotic algae, potentially harmful ROS are generated by several chemical, photochemical, and biological pathways in a stepwise reduction of oxygen (Byczkowski and Gessner, 1988; Asada, 1999; Halliwell and Gutteridge, 1999; Richier et al., 2006). Nevertheless, at low levels, ROS play a key role in signal transduction of cell damage mediators and in processes such as apoptosis, autophagy, and necrosis (Fridovich, 1998; Yakovleva et al., 2009; Davy et al., 2012; Ross et al., 2013; Muller-Parker et al., 2015). Reactive oxygen species present different reactivity and membrane permeability and include: singlet oxygen ($^1O_2$), which is highly reactive and one of the main ROS produced by illuminated photosynthetic organisms as a result of electron transfer from excited chlorophyll molecules to molecular oxygen (Scandalios, 1993), hydrogen peroxide (H_2O_2), which is highly permeable across biological membranes, and superoxide anion radical (O_2•-), which is less permeable than hydrogen peroxide (Lesser, 2006). Although differing in permeability, both H_2O_2 and O_2•- have selective reactivity with biological molecules (Halliwell, 2006). Under normal conditions, cellular antioxidant defenses are activated and ROS are converted into less noxious compounds, thus preventing the harmful effects of ROS to cell macromolecules. Nevertheless, during long-term abiotic stress the production and accumulation of ROS beyond the capacity of an organism's antioxidant defenses to eliminate them, i.e. oxidative stress, can lead to damage to intracellular macromolecules such as DNA, proteins, and lipids, resulting in DNA degradation, protein oxidation, and lipid peroxidation, respectively (Lesser, 2006). The reaction of ROS with lipids, especially with unsaturated lipids of cell membranes, is considered one of the most prevailing mechanisms of cellular damage (Halliwell and Gutteridge, 1999), this is a destructive process that compromises normal cellular function. Lipid peroxidation involves three distinct steps: initiation, propagation, and termination (Yu, 1994; Halliwell and Gutteridge, 1999), ultimately forming ROO• (peroxyl radical) that then takes part in a chain reaction of peroxidation of lipids. The lipid hydroperoxide (ROOH) generated in the course of these reactions is unstable in the presence of Fe or other metal catalysts. This instability is given to the subsequent participation of ROOH in a Fenton reaction leading to the generation of RO• (alkoxy radical). Thus, in the presence of Fe, the chain reactions are propagated and

amplified. Among the degradation products of ROOH are aldehydes (malondialdehyde (MDA)) and hydrocarbons (ethane and ethylene) (Freeman and Crapo, 1982; Gutteridge and Halliwell, 1990).

To maintain a steady state of low ROS concentration, i.e. cell homeostasis, and prevent oxidative damage, several antioxidant mechanisms are present in both partners of the symbioses (Lesser and Shick, 1989b; Nii and Muscatine 1997; Richier et al., 2005; Lesser, 2006; Merle et al., 2007), mainly in chloroplasts and mitochondria, where O_2 is most active during photosynthesis and respiration, respectively (Wise, 1995; Lesser, 2006), but also in peroxisomes (Apel and Hirt, 2004) and glyoxysomes (Lesser, 2006). In biological systems, the purpose of antioxidant defenses is to eliminate singlet oxygen ($^1O_2$) at the site of production (e.g. PSII reaction centers in chloroplasts) and eliminate or reduce the flux of reduced oxygen intermediates such as $O_2\bullet-$ and H_2O_2 to prevent the production of HO● (hydroxyl radical), the most reactive form of ROS (Lesser et al., 1990; Lesser, 1997; Fridovich 1998; Asada, 1999; Halliwell and Gutteridge, 1999). These antioxidant defense mechanisms can be non-enzymatic and enzymatic. Non-enzymatic antioxidants include lipid-soluble carotenoids, retinol (vitamin A), calciferol (vitamin D), and tocopherol (vitamin E) (Edge et al., 1997), and water-soluble phycobiliproteins, ascorbate (vitamin C), glutathione (GSH), and flavonoids (Lesser, 2006). Enzymatic antioxidants include multiple isoforms of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and ascorbate peroxidase (APX) (Kurutas, 2016), the latter only present in the symbiont (Lesser et al, 1990). Cooperatively, these enzymatic antioxidants provide the first line of defense against $O_2\bullet-$ and H_2O_2, whereas carotenoids and tocopherols are proficient quenchers of $^1O_2$ (Sies, 1999; Ledford and Niyogi, 2005). Superoxide dismutase, the first enzyme involved in ROS scavenging, converts $O_2\bullet-$ to H_2O_2 close to the site of production (Lesser, 2006). Then, H_2O_2 is converted to water and oxygen by APX in the symbiont's chloroplasts and by CAT in both partners of the symbiosis (Lesser et al., 1990; Fitt et al., 2009; Lesser, 2011; McGinty et al., 2012; Krueger et al., 2014; Kurutas, 2016), and GPx uses H_2O_2 to oxidize substrates (Torres et al., 2006). While cells can employ numerous antioxidant mechanisms, the SOD–APX and SOD–CAT mechanisms are the main enzymatic antioxidant pathways in eukaryotes (Asada, 1999; Halliwell, 2006). These antioxidant enzymes limit ROS-mediated damage to intracellular macromolecules, however, they are not completely efficient at executing this task (Hayes and McLellan, 1999). Once some of the chemicals produced following the interaction of ROS with intracellular macromolecules are highly reactive, these secondary oxidation products also need to be detoxified in order to

prevent them from also damaging lipids, proteins, and DNA and ultimate lead to cell death (Dixon et al., 2010). This second line of defense against ROS is provided by enzymes such as glutathione peroxidase, glutathione S-transferase (GST), and aldehyde dehydrogenase (Hayes and McLellan, 1999; Weston et al., 2015). The enzyme GST detoxifies DNA hydroperoxides and lipid peroxides, having a central role in cell macrostructural repair (Hayes and McLellan, 1999; Limón-Pacheco and Gonsebatt, 2009). Ultimately, the detoxified metabolites generated by these enzymes are eliminated from the cell by energy-dependent efflux pumps such as the glutathione S-conjugate transporter (Hayes and McLellan, 1999).

Reactive oxygen species production is prevalent in the world's oceans and oxidative stress is an important component of the stress response in marine organisms exposed to a variety of environmental stressors (Lesser, 2012). Reef-building corals that harbor endosymbiotic algae are regularly subjected to a variety of abiotic stresses: elevated seawater temperatures, high doses of photosynthetically active radiation (PAR) and ultraviolet light, salinity fluctuations, or a combination of these factors, as they inhabit shallow-water or intertidal marine environments (Brown, 1997b). Thus, reef-building corals require robust metabolic adjustments in order to maintain homeostasis, grow, and reproduce. The potential for ROS production is high in tropical marine organisms, and antioxidant defenses are required to maintain the steady-state concentration of ROS at low levels in order to prevent oxidative stress and subsequent oxidative damage (Lesser, 2012).

Regarding the mechanisms of thermal bleaching, induction of ROS and accumulation of oxidative damage products have been correlated with heat stress in both symbiont algae and host corals (Lesser et al., 1990; Brown et al., 2002; Downs et al., 2002). The current consensus is that oxidative stress is the trigger of coral bleaching (Richier et al., 2006; Murata et al., 2007; Lesser, 2011), therefore, a unifying mechanism of coral bleaching was proposed: the 'Oxidative Theory of Coral Bleaching' (OTB). This hypothesis suggests that heat and light stress induce the bleaching cascade first by decoupling symbiont photosynthesis, leading to the excessive generation of ROS and reactive nitrogen species (RNS) in the symbiont that overwhelms its antioxidant defenses, resulting in a net increase in ROS and oxidative stress inside the symbiont cells (Perez and Weis, 2006; Dunn et al., 2007; Hawkins and Davy, 2012). ROS diffusion into the coral host tissue combined with damage and potential activation of apoptotic pathways in both partners result in symbiosis dysfunction (Weis, 2008; Hawkins et al., 2013; Paxton et al., 2013). Therefore, the OTB proposes that the expulsion of the symbionts

from the host is the final defense of corals against oxidative stress when other antioxidant defenses are overwhelmed (Gates et al., 1992; Downs et al. 2000, 2002; Putnam et al., 2017; Nielsen et al., 2018). This has been corroborated by studies that have measured increasing antioxidant activity in corals under environmental stress (increased temperature, high light, and hyposalinity, Hoegh-Guldberg, 1999), using a number of enzymatic antioxidants such as SOD, CAT, GPx, and GST to defend against the damaging effects of ROS (Lesser et al., 1990; Downs et al. 2000, 2002, 2009; Higuchi et al., 2008; Fitt et al., 2009). Coral bleaching does not always result in coral mortality. There are a number of observations both from the laboratory and the field documenting recovery from a bleaching event (Brown, 1997a; Stone et al., 1999). One hypothesis for this phenomenon, especially given the data supporting the OTB, is that the outcome for either mortality or recovery is determined by the extent of oxidative damage experienced by the coral (Downs et al., 2002).

Hyposaline conditions can induce an oxidative stress response in both the host and its algal symbionts (Downs et al., 2009) that may result in bleaching, increased damage and/or death of the organism, due to energy expenditure required to neutralize or dissipate the effects of stress and restore cellular or tissue damage (Gates and Edmunds, 1999; Morgan and Snell, 2002). In literature there is no clear consensus on the mechanisms associated with hyposaline bleaching. Some studies report impairment of symbiotic algae (Ferrier-Pagès et al., 1999; Alutoin et al., 2001; Kerswell and Jones, 2003), while others have emphasized that the coral host is the first damaged by reductions in salinity, resulting in morphological changes, tissue swelling, and necrosis (Van Woesik et al., 1995; Hoegh-Guldberg, 1999; Downs et al., 2009).

1.2.7. Different susceptibility to environmental variables among reef-building corals

There is a considerable variation in environmental tolerances among species, which can have a major influence on the community structure within sites (Brown, 1997a). It is clear that within many reefs experiencing bleaching events there are corals that retain their endosymbionts, providing interspersed patches of survival even among areas that experience widespread mortality (Page et al., 2019). A major factor influencing shifts in coral community structure is differential susceptibility of species to bleaching and their recovery capabilities following a bleaching event.

Reef-building corals are not all equally susceptible to heat stress. Coral bleaching is characteristically patchy, while bleached corals lose most of their symbiotic zooxanthellae and appear white after relatively short heat stress exposure, adjacent colonies of the same, or another, species may display normal coloration for weeks or even months living in the same conditions on the same reef (Edmunds, 1994; Hoegh-Guldberg and Salvat, 1995; Marshall and Baird, 2000; Baker et al., 2008). Different susceptibility of corals to heat stress has been linked to colony morphology (Brandt, 2009), tissue-thickness (Hoegh-Guldberg and Salvat, 1995; Loya et al., 2001), colony size (Edmunds, 2005; Shenkar et al., 2005), polyp size (Jokiel and Coles, 1974; Darling et al., 2013), respiratory rates (Jokiel and Coles, 1990), mucus production rates (Fitt et al., 2009; Wooldridge, 2009), tissue concentration of fluorescent pigments (Salih et al., 1998), heterotrophic feeding capacity (Grottoli et al., 2006; Levas et al., 2013), the capacity to transfer mass and heat (Nakamura and Van Woesik, 2001), coral species (Hoegh-Guldberg and Salvat, 1995; Marshall and Baird, 2000), species-specific physiology (Baird and Marshall 2002; Grottoli et al., 2014), coral gene expression (Barshis et al., 2013), genetic variation between coral populations from widely separated geographic regions (Coles et al., 1976; Glynn et al., 1988; Rowan and Knowlton, 1995), clade of zooxanthellae (Rowan et al., 1997; Berkelmans and Van Oppen, 2006; Cunning et al., 2016), and both cellular physiology and the strategies employed to mitigate oxidative stress (Regoli et al., 2000; Brown et al., 2002; Downs et al., 2002; Shick and Dunlap, 2002; Richier et al., 2005; Baird et al., 2009; Fitt et al., 2009; Pontasch et al., 2014). The different stress response among corals with different colony morphology is leading to different mortality and consequent shifts in coral taxa dominance, with significant impact in the dynamics and structure of coral communities (Connell, 1997; Hughes and Tanner, 2000; Edmunds, 2005). The variability of corals response to heat stress has been reported numerous times (Yamazato, 1981; Glynn, 1983, 1988, 1990a, 1993; Cortés et al., 1984; Fisk and Done, 1985; Oliver, 1985; Brown and Suharsono, 1990; Gleason, 1993; Hoegh-Guldberg and Salvat, 1995; Fujioka, 1999; Kayanne et al., 1999; Marshall and Baird, 2000; McClanahan, 2000; Wilkinson, 2000; Loya et al., 2001; Loch et al., 2002; McClanahan and Maina, 2003; McClanahan et al., 2004; Le Nohaïc et al., 2017). Fast-growing, branching coral species with high metabolic rates and thinner tissues (e.g. *Acropora, Pocillopora,* and *Stylophora*) are among the first to bleach, whereas slow-growing, massive coral species with low metabolic rates and thicker tissues (e.g. *Porites, Favia,* and *Galaxea)* are usually able to survive higher SSTs (usually another 1–2 ºC) before bleaching becomes visible (Loya et al., 2001; Darling et al., 2012; Van Woesik et al., 2012). This hierarchy of susceptibility is consistent over a wide geographic scale (McClanahan et al., 2004). For this reason, massive

corals may be more prominent on future reefs than branching corals, leading to changes in the community structure of reef-building corals and the extinction of some branching coral species.

Different tolerance of scleractinian corals to low salinity has been linked to coral species (Moberg et al., 1997; Lirman and Manzello, 2009; Faxneld et al., 2010; Berkelmans et al., 2012), colony morphology (Van Woesik et al., 1995; True, 2012), their polyp retraction response under osmotic stress (Muthiga and Szmant, 1987), mucus production rates (Van Woesik et al., 1995), and antioxidant strategies (Gardner et al., 2016). The composition of coral communities is the result from both the resilience of their constituent species exposed to recurrent and prolonged sub-lethal hyposaline conditions, and the vulnerability of species incapable of surviving such conditions. For instance, the massive coral species tend to be more tolerant to hyposaline bleaching than branching coral species (True, 2012).

1.2.8. Health assessment of reef corals

In a changing climate, ecosystems where foundation species are susceptible to the effects of elevated temperatures are thus vulnerable to major reorganization, being characterized by reduced habitat complexity and disrupted ecosystem services (Ellison et al., 2005; Hoegh-Guldberg and Bruno, 2010). The urgent need to assess the quality of marine ecosystems led to the development of several monitoring tools (Devin et al., 2014). Changes in community structure and measures of chemical contamination are frequently used to indicate ecosystem health status (Dustan and Halas, 1987; Hughes, 1994; Viarengo et al., 2000; Chase et al., 2001). Nevertheless, these represent damage manifestations rather than prognostic indices (Knap et al., 2002). Biomarkers are an example of responses that have provided valuable mechanistic information to scientists, allowing environmental managers' action before damage manifestation has occurred. Nonetheless, the multi-biomarker approaches are generally hard to interpret and produce results that are not easy to integrate in the environmental policies framework (Beliaeff and Burgeot, 2002). To fill this gap, integrative indices have been developed, and one of the most used is the Integrated Biomarker Response (IBR, Beliaeff and Burgeot, 2002). The integration of biomarkers in health indices may provide comprehensive information about the biological effects of environmental variables in marine organisms (Marigómez et al., 2013) and may thus serve as valuable tools for environmental managers (Broeg and Lehtonen, 2006; Madeira et al., 2018). This tool can be combined with morphological assessments to characterize the sub-lethal metabolic effects of general stressors

in marine organisms, as has been shown for tropical fish, crustaceans, and gastropods by Madeira et al. (2018).

1.3. Aims and thesis layout

This thesis focused on the effects of global (i.e. temperature) and localized (i.e. salinity) stressors altered by global climate change on reef coral communities of the Indo-Pacific region. Small fragments of nine Indo-Pacific reef-building coral species were submitted to several long-term heat and hyposaline treatments, as well as the combination of these environmental variables, in order to evaluate their effects at molecular, physiological, and organism levels of biological organization. Integrative indices for health assessment in reef corals under thermal stress were also tested and optimized.

This investigation is critical to understand the differential vulnerability of reef-building coral species to global climate change and can help reveal future coral species dominance and decline, and determine future biodiversity shifts on coral reef ecosystems, so that conservation efforts can be more effective.

The main aims of this thesis were to:

a) Assess the different susceptibility of reef-building coral species to long-term exposure to high temperature and hyposaline conditions, both individually and combined, by evaluating the effect of these environmental variables on parameters at different levels of biological organization: organism level (total mortality, partial mortality, and coral condition according to the bleaching level), physiological level (growth rate and regeneration rate of tissue lesions artificially inflicted to mimic predation), and molecular level (analysis of biomarkers of oxidative stress: superoxide dismutase, catalase, and glutathione S-transferase activities and lipid peroxidation levels) for 60 days in small fragments of nine reef-building coral species of the Indo-Pacific region. Assessment of which species are most vulnerable and resistant to these stressors is highly important given the future climate change scenarios, it will allow to direct conservation efforts accordingly;
b) Test and optimize two different approaches based on the use of IBR indices in order to assess the health of reef corals under long-term exposure to heat stress in a context of asexual reproduction by fragmentation. The first approach only contains biomarkers at

the molecular level (superoxide dismutase, catalase, and glutathione S-transferase activities and lipid peroxidation levels), whereas the second one contains biomarkers at molecular, physiological, and organism levels (superoxide dismutase, catalase, and glutathione S-transferase activities and lipid peroxidation levels; growth rate; partial mortality). This multi-biomarker analysis may allow the application of approaches based on adequate sets of biomarkers in the monitoring of the effects of global warming in the Indo-Pacific oceans.

This thesis comprises of 7 chapters, the first being a general view of the main themes related to coral community structure, climate change, and the structure of the present thesis; chapters 2 and 4 concern the assessment of the effects of long-term exposure to heat stress on parameters at different levels of biological organization (molecular, physiological, and organism levels) after colony fragmentation of nine reef-building coral species of the Indo-Pacific region; chapters 3 and 5 concern the assessment of the effects of long-term exposure to both low salinity and the combination of high temperature + low salinity on parameters at different levels of biological organization (molecular, physiological, and organism levels) after colony fragmentation of nine reef-building coral species of the Indo-Pacific region; chapter 6 concerns the test and optimization of two different approaches based in IBR indices for health assessment in reef corals under global warming using parameters obtained on the chapters 2 and 4 relative to seven reef-building coral species; and chapter 7 presents concluding remarks and future perspectives.

1.4. References

Abramovitch-Gottlib, L., Katoshevski, D., & Vago, R. (2003). Responses of *Stylophora pistillata* and *Millepora dichotoma* to seawater temperature elevation. Bulletin of marine science, 73(3), 745-755.

Adjeroud, M., Michonneau, F., Edmunds, P. J., Chancerelle, Y., De Loma, T. L., Penin, L., ... & Galzin, R. (2009). Recurrent disturbances, recovery trajectories, and resilience of coral assemblages on a South Central Pacific reef. Coral Reefs, 28(3), 775-780.

Ainsworth, T. D., Heron, S. F., Ortiz, J. C., Mumby, P. J., Grech, A., Ogawa, D., ... & Leggat, W. (2016). Climate change disables coral bleaching protection on the Great Barrier Reef. Science, 352(6283), 338-342.

Albright, R., & Mason, B. (2013). Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. PLoS One, 8(2), e56468.

Alino, P. M., Sammarco, P. W., & Col, J. C. (1992). Competitive strategies in soft corals (Coelenterata, Octocorallia). IV. Environmentally induced reversals in competitive superiority. Marine Ecology Progress Series, 81, 129-145.

Allemand, D., Ferrier-Pagès, C., Furla, P., Houlbrèque, F., Puverel, S., Reynaud, S., ... & Zoccola, D. (2004). Biomineralisation in reef-building corals: from molecular mechanisms to environmental control. General Palaeontology (Palaeobiochemistry), 3(6-7), 453-467.

Alutoin, S., Boberg, J., Nyström, M., & Tedengren, M. (2001). Effects of the multiple stressors copper and reduced salinity on the metabolism of the hermatypic coral *Porites lutea*. Marine Environmental Research, 52(3), 289-299.

Alvarez-Filip, Lorenzo, Nicholas K. Dulvy, Jennifer A. Gill, Isabelle M. Côté, and Andrew R. Watkinson. "Flattening of Caribbean coral reefs: region-wide declines in architectural complexity." Proceedings of the Royal Society B: Biological Sciences 276, no. 1669 (2009): 3019-3025.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology, 55, 373-399.

Aronson, R. B., Precht, W. F., Macintyre, I. G., & Murdoch, T. J. (2000). Ecosystems: Coral bleach-out in Belize. Nature, 405(6782), 36.

Aronson, R. B., Precht, W. F., Toscano, M., & Koltes, K. H. (2002). The 1998 bleaching event and its aftermath on a coral reef in Belize. Marine Biology, 141(3), 435-447.

Aronson, R. B., Macintyre, I. G., Wapnick, C. M., & O'Neill, M. W. (2004). Phase shifts, alternative states, and the unprecedented convergence of two reef systems. Ecology, 85(7), 1876-1891.

Asada, K. (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annual Review of Plant Biology, 50(1), 601-639.

Ateweberhan, M., McClanahan, T. R., Graham, N. A. J., & Sheppard, C. R. C. (2011). Episodic heterogeneous decline and recovery of coral cover in the Indian Ocean. Coral Reefs, 30(3), 739.

Babcock, R. C. (1991). Comparative demography of three species of scleractinian corals using age-and size-dependent classifications. Ecological Monographs, 61(3), 225-244.

Baine, M. S. (2006). A major outbreak of crown-of-thorns starfish in Bootless Bay, Central Province, Papua New Guinea. Coral Reefs, 25(4), 607-607.

Baird, A. H., & Hughes, T. P. (2000). Competitive dominance by tabular corals: an experimental analysis of recruitment and survival of understorey assemblages. Journal of Experimental Marine Biology and Ecology, 251(1), 117-132.

Baird, A. H., & Marshall, P. A. (2002). Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Marine Ecology Progress Series, 237, 133-141.

Baird, A. H., & Maynard, J. A. (2008). Coral adaptation in the face of climate change. Science, 320(5874), 315-316.

Baird, A. H., Bhagooli, R., Ralph, P. J., & Takahashi, S. (2009). Coral bleaching: the role of the host. Trends in Ecology & Evolution, 24(1), 16-20.

Baird, A. H., Pratchett, M. S., Hoey, A. S., Herdiana, Y., & Campbell, S. J. (2013). *Acanthaster planci* is a major cause of coral mortality in Indonesia. Coral Reefs, 32(3), 803-812.

Bak, R. P. M., Engel (1977). Coral reefs and their zonation in Netherlands Antilles. Stud. Geol. 4: 3–16. and MS Engel. 1979. Distribution, abundance and survival of juvenile hermatypic corals (Scleractinia) and the importance of life history strategies in the parent coral community. Marine Biology, 54, 341-352.

Bak, R. P. M., Lambrechts, D. Y. M., Joenje, M., Nieuwland, G., & Van Veghel, M. L. J. (1996). Long-term changes on coral reefs in booming populations of a competitive colonial ascidian. Marine Ecology Progress Series, 133, 303-306.

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine, Coastal and Shelf Science, 80(4), 435-471.

Banaszak, A. T., & Trench, R. K. (1995). Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. I. Response of the algal symbionts in culture an in hospite. Journal of Experimental Marine Biology and Ecology, 194(2), 213-232.

Barkley, H. C., Cohen, A. L., Mollica, N. R., Brainard, R. E., Rivera, H. E., DeCarlo, T. M., ... & Vargas-Ángel, B. (2018). Repeat bleaching of a central Pacific coral reef over the past six decades (1960–2016). Communications Biology, 1(1), 177.

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. Proceedings of the National Academy of Sciences, 110(4), 1387-1392.

Baums, I. B., Miller, M. W., & Hellberg, M. E. (2006). Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. Ecological Monographs, 76(4), 503-519.

Beliaeff, B., & Burgeot, T. (2002). Integrated biomarker response: a useful tool for ecological risk assessment. Environmental Toxicology and Chemistry: An International Journal, 21(6), 1316-1322.

Bellantuono, A. J., Hoegh-Guldberg, O., & Rodriguez-Lanetty, M. (2011). Resistance to thermal stress in corals without changes in symbiont composition. Proceedings of the Royal Society B: Biological Sciences, 279(1731), 1100-1107.

Bellwood, D. R., Hughes, T. P., Folke, C., & Nyström, M. (2004). Confronting the coral reef crisis. Nature, 429(6994), 827.

Berkelmans, R., & Oliver, J. K. (1999). Large-scale bleaching of corals on the Great Barrier Reef. Coral Reefs, 18(1), 55-60.

Berkelmans, R., & Van Oppen, M. J. (2006). The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope'for coral reefs in an era of climate change. Proceedings of the Royal Society B: Biological Sciences, 273(1599), 2305-2312.

Berkelmans, R., Jones, A. M., & Schaffelke, B. (2012). Salinity thresholds of *Acropora* spp. on the Great Barrier Reef. Coral Reefs, 31(4), 1103-1110.

Berkelmans, R., De'ath, G., Kininmonth, S., & Skirving, W. J. (2004). A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation, patterns, and predictions. Coral Reefs, 23(1), 74-83.

Bhagooli, R., & Hidaka, M. (2004). Photoinhibition, bleaching susceptibility and mortality in two scleractinian corals, *Platygyra ryukyuensis* and *Stylophora pistillata*, in response to

thermal and light stresses. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 137(3), 547-555.

Bienfang, P. (1980). Water quality characteristics of Honokohau Harbor: A subtropical embayment affected by groundwater intrusion. Pacific Science, 34(3), 279-291.

Bierkens, J. G. (2000). Applications and pitfalls of stress-proteins in biomonitoring. Toxicology, 153(1-3), 61-72.

Birkeland, C. (1997). Life and death of coral reefs. Springer Science & Business Media.

Blakeway, D. R. (2004). Patterns of mortality from natural and anthropogenic influences in Dampier corals: 2004 cyclone and dredging impacts. Corals of the Dampier Harbour: their survival and reproduction during the dredging programs of 2004, 65-76.

Boesch, D. F., Field, J. C., & Scavia, D. (2000). The potential consequences of climate variability and change on coastal areas and marine resources: Report of the Coastal Areas and Marine Resources Sector Team, US National Assessment of the Potential Consequences of Climate Variability and Change, US Global Change Research Program.

Bonin, M. C. (2012). Specializing on vulnerable habitat: *Acropora* selectivity among damselfish recruits and the risk of bleaching-induced habitat loss. Coral Reefs, 31(1), 287-297.

Bothwell, A. M. (1981). Fragmentation, a means of asexual reproduction and dispersal in the coral genus *Acropora* (Scleractinia: Astrocoeniida: Acroporidae)-a preliminary report. In Proceedings of the 4th International Coral Reef Symposium, Manila (Vol. 2, pp. 137-144).

Bourne, D., Iida, Y., Uthicke, S., & Smith-Keune, C. (2008). Changes in coral-associated microbial communities during a bleaching event. The ISME Journal, 2(4), 350.

Brainard, R. E., Weijerman, M., Eakin, C. M., McElhany, P., Miller, M. W., Patterson, M., ... & Birkeland, C. (2013). Incorporating climate and ocean change into extinction risk assessments for 82 coral species. Conservation Biology, 27(6), 1169-1178.

Brandt, M. E. (2009). The effect of species and colony size on the bleaching response of reef-building corals in the Florida Keys during the 2005 mass bleaching event. Coral Reefs, 28(4), 911-924.

Brodie, J. E., Kroon, F. J., Schaffelke, B., Wolanski, E. C., Lewis, S. E., Devlin, M. J., ... & Davis, A. M. (2012). Terrestrial pollutant runoff to the Great Barrier Reef: an update of issues, priorities and management responses. Marine Pollution Bulletin, 65(4-9), 81-100.

Broeg, K., & Lehtonen, K. K. (2006). Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of a multi-biomarker approach. Marine Pollution Bulletin, 53(8-9), 508-522.

Brooker, R. M., Munday, P. L., Brandl, S. J., & Jones, G. P. (2014). Local extinction of a coral reef fish explained by inflexible prey choice. Coral Reefs, 33(4), 891-896.

Brown, B. E. (1997a). Coral bleaching: causes and consequences. Coral Reefs, 16(1), S129-S138.

Brown, B. E. (1997b). Adaptations of reef corals to physical environmental stress. In Advances in Marine Biology (Vol. 31, pp. 221-299). Academic Press.

Brown, B. E., Suharsono (1990). Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. Coral Reefs, 8(4), 163-170.

Brown, B. E., Dunne, R. P., & Chansang, H. (1996). Coral bleaching relative to elevated seawater temperature in the Andaman Sea (Indian Ocean) over the last 50 years. Coral Reefs, 15(3), 151-152.

Brown, B. E., Downs, C. A., Dunne, R. P., & Gibb, S. W. (2002). Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. Marine Ecology Progress Series, 242, 119-129.

Bruno, J. F., & Selig, E. R. (2007). Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS One, 2(8), e711.

Bruno, J. F., Selig, E. R., Casey, K. S., Page, C. A., Willis, B. L., Harvell, C. D., ... & Melendy, A. M. (2007). Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biology, 5(6), e124.

Bryant, D., Burke, L., McManus, J., & Spalding, M. (1998). Reefs at risk: a map-based indicator of threats to the worlds coral reefs.

Burge, C. A., Mark Eakin, C., Friedman, C. S., Froelich, B., Hershberger, P. K., Hofmann, E. E., ... & Ford, S. E. (2014). Climate change influences on marine infectious diseases: implications for management and society. Annual Review of Marine Science, 6, 249-277.

Burke, L., Reytar, K., Spalding, M., & Perry, A. (2011). Reefs at risk revisited. ISBN 978-1-56973-762-0.

Burt, J., Al-Harthi, S., & Al-Cibahy, A. (2011). Long-term impacts of coral bleaching events on the world's warmest reefs. Marine Environmental Research, 72(4), 225-229.

Butler, I. R., Sommer, B., Zann, M., Zhao, J. X., & Pandolfi, J. M. (2013). The impacts of flooding on the high-latitude, terrigenoclastic influenced coral reefs of Hervey Bay, Queensland, Australia. Coral Reefs, 32(4), 1149-1163.

Byczkowski, J. Z., & Gessner, T. (1988). Biological role of superoxide ion-radical. International Journal of Biochemistry, 20(6), 569-580.

Bythell, J. C. (1990). Nutrient uptake in the reef-building coral *Acropora palmata* at natural environmental concentrations. Marine Ecology Progress Series, 65-69.

Cai, W., Borlace, S., Lengaigne, M., Van Rensch, P., Collins, M., Vecchi, G., ... & England, M. H. (2014). Increasing frequency of extreme El Niño events due to greenhouse warming. Nature climate change, 4(2), 111.

Caley, M. J., Carr, M. H., Hixon, M. A., Hughes, T. P., Jones, G. P., & Menge, B. A. (1996). Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics, 27(1), 477-500.

Cantin, N. E., & Lough, J. M. (2014). Surviving coral bleaching events: *Porites* growth anomalies on the Great Barrier Reef. PloS One, 9(2), e88720.

Cardini, U., Chiantore, M., Lasagna, R., Morri, C., & Bianchi, C. N. (2012). Size-structure patterns of juvenile hard corals in the Maldives. Journal of the Marine Biological Association of the United Kingdom, 92(6), 1335-1339.

Carilli, J. E., Donner, S. D., & Hartmann, A. C. (2012). Historical temperature variability affects coral response to heat stress. PLoS One, 7(3), e34418.

Carilli, J. E., Norris, R. D., Black, B. A., Walsh, S. M., & McField, M. (2009). Local stressors reduce coral resilience to bleaching. Plos One, 4(7), e6324.

Carpenter, R. C. (1997). Invertebrate predators and grazers. Life and death of coral reefs. Chapman and Hall, New York, 198-229.

Carpenter, K. E., Abrar, M., Aeby, G., Aronson, R. B., Banks, S., Bruckner, A., ... & Edgar, G. J. (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science, 321(5888), 560-563.

Carroll, A. G., Harrison, P. L., & Adjeroud, M. (2017). Susceptibility of coral assemblages to successive bleaching events at Moorea, French Polynesia. Marine and Freshwater Research, 68(4), 760-771.

Castillo, K. D., & Helmuth, B. S. T. (2005). Influence of thermal history on the response of *Montastraea annularis* to short-term temperature exposure. Marine Biology, 148(2), 261-270.

Cesar, H. (1996). Economic analysis of Indonesian coral reefs. In: Environment Department, work in progress, toward environmentally and socially sustainable development, pp 1-97.

Chadwick, N. E., & Morrow, K. M. (2011). Competition among sessile organisms on coral reefs. In Coral Reefs: an ecosystem in transition (pp. 347-371). Springer, Dordrecht.

Chartrand, K. M., Durako, M. J., & Blum, J. E. (2009). Effect of hyposalinity on the photophysiology of *Siderastrea radians*. Marine Biology, 156(8), 1691-1702.

Chase, M. E., Jones, S. H., Hennigar, P., Sowles, J., Harding, G. C. H., Freeman, K., ... & Pederson, J. (2001). Gulfwatch: Monitoring spatial and temporal patterns of trace metal and organic contaminants in the Gulf of Maine (1991–1997) with the blue mussel, *Mytilus edulis* L. Marine Pollution Bulletin, 42(6), 490-504.

Chavanich, S., Viyakarn, V., Loyjiw, T., Pattaratamrong, P., & Chankong, A. (2009). Mass bleaching of soft coral, *Sarcophyton* spp. in Thailand and the role of temperature and salinity stress. ICES Journal of Marine Science, 66(7), 1515-1519.

Cheal, A. J., MacNeil, M. A., Cripps, E., Emslie, M. J., Jonker, M., Schaffelke, B., & Sweatman, H. (2010). Coral–macroalgal phase shifts or reef resilience: links with diversity

and functional roles of herbivorous fishes on the Great Barrier Reef. Coral Reefs, 29(4), 1005-1015.

Chen, C. A., Yang, Y. W., Wei, N. V., Tsai, W. S., & Fang, L. S. (2005). Symbiont diversity in scleractinian corals from tropical reefs and subtropical non-reef communities in Taiwan. Coral Reefs, 24(1), 11-22.

Chow, A. M., Ferrier-Pagès, C., Khalouei, S., Reynaud, S., & Brown, I. R. (2009). Increased light intensity induces heat shock protein Hsp60 in coral species. Cell Stress and Chaperones, 14(5), 469-476.

Chua, C. M., Leggat, W., Moya, A., & Baird, A. H. (2013). Temperature affects the early life history stages of corals more than near future ocean acidification. Marine Ecology Progress Series, 475, 85-92.

Coker, D. J., Pratchett, M. S., & Munday, P. L. (2009). Coral bleaching and habitat degradation increase susceptibility to predation for coral-dwelling fishes. Behavioral Ecology, 20(6), 1204-1210.

Cole, A. J., Pratchett, M. S., & Jones, G. P. (2008). Diversity and functional importance of coral-feeding fishes on tropical coral reefs. Fish and Fisheries, 9(3), 286-307.

Coles, S. L. (1984). Colonization of Hawaiian reef corals on new and denuded substrata in the vicinity of a Hawaiian power station. Coral Reefs, 3(3), 123-130.

Coles, S. L. (1988). Limitation on reef coral development in the Arabian Gulf: Temperature or algal competition?. In Proceedings of the 6th International Coral Reef Symposium, 1988 (Vol. 3, pp. 211-216).

Coles, S. L. (1992). Experimental comparison of salinity tolerances of reef corals from the Arabian Gulf and Hawaii: evidence for hyperhaline adaptation. In Proceedings of the 7th International Coral Reef Symposium (Vol. 1, pp. 227-234).

Coles, S. L., & Jokiel, P. L. (1978). Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Marine Biology, 49(3), 187-195.

Coles, S. L., & Jokiel, P. L. (1992). Effects of salinity on coral reefs. Pollution in Tropical Aquatic Systems, 147-166.

Coles, S. L., & Brown, B. E. (2003). Coral bleaching—capacity for acclimatization and adaptation. In: Advances in Marine Biology, 46, 184-212.

Coles, S. L., Jokiel, P. L., & Lewis, C. R. (1976). Thermal tolerance in tropical versus subtropical Pacific reef corals. Pacific Science, 30(2), 159-166.

Colombo-Pallotta, M. F., Rodríguez-Román, A., & Iglesias-Prieto, R. (2010). Calcification in bleached and unbleached *Montastraea faveolata*: evaluating the role of oxygen and glycerol. Coral Reefs, 29(4), 899-907.

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs. Science, 199(4335), 1302-1310.

Connell, J. H. (1997). Disturbance and recovery of coral assemblages. Coral Reefs, 16(1), S101-S113.

Connell, J. H., & Keough, M. J. (1985). Disturbance and patch dynamics of subtidal marine animals on hard substrata. Natural Disturbance and Patch Dynamics, 125-151.

Connell, J. H., Hughes, T. P., & Wallace, C. C. (1997). A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecological Monographs, 67(4), 461-488.

Connell, J. H., Hughes, T. P., Wallace, C. C., Tanner, J. E., Harms, K. E., & Kerr, A. M. (2004). A long-term study of competition and diversity of corals. Ecological Monographs, 74(2), 179-210.

Cortés, J., Murillo, M. M., Guzmán, H. M., & Acuña, J. (1984). Pérdida de zooxantelas y muerte de corales y otros organismos arrecifales en el Caribe y Pacífico de Costa Rica. Revista de Biologia Tropical, 32(2), 227-231.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., ... & Raskin, R. G. (1997). The value of the world's ecosystem services and natural capital. Nature, 387(6630), 253.

Costanza, R., de Groot, R., Sutton, P., Van der Ploeg, S., Anderson, S. J., Kubiszewski, I., ... & Turner, R. K. (2014). Changes in the global value of ecosystem services. Global Environmental Change, 26, 152-158.

Côté, I. M., & Darling, E. S. (2010). Rethinking ecosystem resilience in the face of climate change. PLoS Biology, 8(7), e1000438.

Cowen, R. K., Paris, C. B., & Srinivasan, A. (2006). Scaling of connectivity in marine populations. Science, 311(5760), 522-527.

Crabbe, M. J. C. (2008). Climate change, global warming and coral reefs: Modelling the effects of temperature. Computational Biology and Chemistry, 32(5), 311-314.

Craik, W., Kenchington, R., & Kelleher, G. (1990). Coral-Reef Management. In: Dubinsky, Z. (Ed.), Ecosystems of the World 25: Coral Reefs. Elsevier, New York, pp. 453–467.

Cumming, R. L. (1999). Predation on reef-building corals: multiscale variation in the density of three corallivorous gastropods, *Drupella* spp. Coral Reefs, 18(2), 147-157.

Cumming, R. L. (2009). Population outbreaks and large aggregations of *Drupella* on the Great Barrier Reef. Great Barrier Reef Marine Park Authority.

Cunning, R., Ritson-Williams, R., & Gates, R. D. (2016). Patterns of bleaching and recovery of *Montipora capitata* in Kāne ʻohe Bay, Hawai ʻi, USA. Marine Ecology Progress Series, 551, 131-139.

Dai, C. F. (1990). Interspecific competition in Taiwanese corals with special reference to interactions between alcyonaceans and scleractinians. Marine Ecology Progress Series. Oldendorf, 60(3), 291-297.

Dalton, S. J., & Carroll, A. G. (2011). Monitoring coral health to determine coral bleaching response at high latitude eastern Australian reefs: an applied model for a changing climate. Diversity, 3(4), 592-610.

Darling, E. S., McClanahan, T. R., & Côté, I. M. (2013). Life histories predict coral community disassembly under multiple stressors. Global Change Biology, 19(6), 1930-1940.

Darling, E. S., Alvarez-Filip, L., Oliver, T. A., McClanahan, T. R., & Côté, I. M. (2012). Evaluating life-history strategies of reef corals from species traits. Ecology Letters, 15(12), 1378-1386.

Davies, J. M., Dunne, R. P., & Brown, B. E. (1997). Coral bleaching and elevated sea-water temperature in Milne Bay Province, Papua New Guinea, 1996. Marine and Freshwater Research, 48(6), 513-516.

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. Microbiology and Molecular Biology Reviews, 76(2), 229-261.

Dayton, P. K. (1971). Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs, 41(4), 351-389.

De'ath, G., & Moran, P. J. (1998). Factors affecting the behaviour of crown-of-thorns starfish (*Acanthaster planci* L.) on the Great Barrier Reef: 2: Feeding preferences. Journal of Experimental Marine Biology and Ecology, 220(1), 107-126.

De'ath, G., & Fabricius, K. (2010). Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecological Applications, 20(3), 840-850.

De'ath, G., Lough, J. M., & Fabricius, K. E. (2009). Declining coral calcification on the Great Barrier Reef. Science, 323(5910), 116-119.

De'ath, G., Fabricius, K. E., Sweatman, H., & Puotinen, M. (2012). The 27–year decline of coral cover on the Great Barrier Reef and its causes. Proceedings of the National Academy of Sciences, 109(44), 17995-17999.

Depczynski, M., Gilmour, J. P., Ridgway, T., Barnes, H., Heyward, A. J., Holmes, T. H., ... & Wilson, S. K. (2013). Bleaching, coral mortality and subsequent survivorship on a West Australian fringing reef. Coral Reefs, 32(1), 233-238..

DeSalvo, M. K., Voolstra, C. R., Sunagawa, S., Schwarz, J. A., Stillman, J. H., Coffroth, M. A., ... & Medina, M. (2008). Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. Molecular Ecology, 17(17), 3952-3971.

Deser, C., Phillips, A. S., & Alexander, M. A. (2010). Twentieth century tropical sea surface temperature trends revisited. Geophysical Research Letters, 37(10).

DeVantier, L. L., Turak, E. E., Done, T. T., & Davidson, J. J. (1997, January). The effects of cyclone Sadie on coral communities of nearshore reefs in the central Great Barrier Reef.

In Cyclone Sadie Flood Plumes in the Great Barrier Reef Lagoon: Composition and Consequences. Proceedings of a workshop held in Townsville, Queensland, Australia, 10 November 1994, at the Australian Institute of Marine Science. GBRMPA Workshop-pages: 65-88. Great Barrier Reef Marine Park Authority (GBRMPA).

DeVantier, L., De Ath, G., Klaus, R., Al-Moghrabi, S., Abdulaziz, M., Reinicke, G. B., & Cheung, C. (2004). Reef-building corals and coral communities of the Socotra Archipelago, a zoogeographiccrossroads' in the Arabian Sea. Fauna of Arabia, 20, 117-168.

Devin, S., Burgeot, T., Giambérini, L., Minguez, L., & Pain-Devin, S. (2014). The integrated biomarker response revisited: optimization to avoid misuse. Environmental Science and Pollution Research, 21(4), 2448-2454.

Devlin, M., Waterhouse, J., Taylor, J., & Brodie, J. (2001). Flood plumes in the Great Barrier Reef: spatial and temporal patterns in composition and distribution. GBRMPA research publication, 68.

Diaz-Pulido, G., McCook, L. J. (2002). The fate of bleached corals patterns and dynamics of algal recruitment. Marine Ecology Progress Series, 232, 115–128.

Diaz-Pulido, G., McCook, L. J., Dove, S., Berkelmans, R., Roff, G., Kline, D. I., ... & Hoegh-Guldberg, O. (2009). Doom and boom on a resilient reef: climate change, algal overgrowth and coral recovery. PloS One, 4(4), e5239.

Dixon, D. P., Skipsey, M., & Edwards, R. (2010). Roles for glutathione transferases in plant secondary metabolism. Phytochemistry, 71(4), 338-350.

Done, T. J. (1992). Phase shifts in coral reef communities and their ecological significance. Hydrobiologia, 247(1-3), 121-132.

Done, T., Gilmour, J., & Fisher, R. (2015). Distance decay among coral assemblages during a cycle of disturbance and recovery. Coral Reefs, 34(3), 727-738.

Done, T. T., Ogden, J. J., Wiebe, W., & Rosen, B. (1996). Biodiversity and ecosystem function of coral reefs. In Global biodiversity assessment-pages: 393-429. John Wiley & Sons.

Done, T.J., Turak, E., Wakeford, M., DeVantier, L., McDonald, A., & Fisk, D. (2007). Decadal changes in turbid-water coral communities at Pandora Reef: loss of resilience or too soon to tell?. Coral Reefs, 26(4), 789-805.

Done, T. J., DeVantier, L. M., Turak, E., Fisk, D. A., Wakeford, M., & Van Woesik, R. (2010). Coral growth on three reefs: development of recovery benchmarks using a space for time approach. Coral Reefs, 29(4), 815-833.

Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean acidification: the other CO_2 problem. Annual Review of Marine Science, 1, 169-192.

Donner, S. D. (2009). Coping with commitment: projected thermal stress on coral reefs under different future scenarios. PLoS One, 4(6), e5712.

Donner, S. D., Rickbeil, G. J., & Heron, S. F. (2017). A new, high-resolution global mass coral bleaching database. PLoS One, 12(4), e0175490.

Donner, S. D., Skirving, W. J., Little, C. M., Oppenheimer, M., & Hoegh-Guldberg, O. V. E. (2005). Global assessment of coral bleaching and required rates of adaptation under climate change. Global Change Biology, 11(12), 2251-2265.

Doropoulos, C., Roff, G., Bozec, Y. M., Zupan, M., Werminghausen, J., & Mumby, P. J. (2016). Characterizing the ecological trade-offs throughout the early ontogeny of coral recruitment. Ecological Monographs, 86(1), 20-44.

Downs, C. A., Mueller, E., Phillips, S., Fauth, J. E., & Woodley, C. M. (2000). A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. Marine Biotechnology, 2(6), 533-544.

Downs, C. A., Fauth, J. E., Halas, J. C., Dustan, P., Bemiss, J., & Woodley, C. M. (2002). Oxidative stress and seasonal coral bleaching. Free Radical Biology and Medicine, 33(4), 533-543.

Downs, C. A., Kramarsky-Winter, E., Woodley, C. M., Downs, A., Winters, G., Loya, Y., & Ostrander, G. K. (2009). Cellular pathology and histopathology of hypo-salinity exposure on the coral *Stylophora pistillata*. Science of the Total Environment, 407(17), 4838-4851.

Dubinsky, Z., & Stambler, N. (Eds.). (2010). Coral reefs: an ecosystem in transition. Springer Science & Business Media.

Dulvy, N. K., Freckleton, R. P., & Polunin, N. V. (2004). Coral reef cascades and the indirect effects of predator removal by exploitation. Ecology Letters, 7(5), 410-416.

Dunn, S. R., Schnitzler, C. E., & Weis, V. M. (2007). Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: every which way you lose. Proceedings of the Royal Society of London B: Biological Sciences, 274(1629), 3079-3085.

Dustan, P., & Halas, J. C. (1987). Changes in the reef-coral community of Carysfort Reef, Key Largo, Florida: 1974 to 1982. Coral Reefs, 6(2), 91-106.

Dykens, J. A., & Shick, J. M. (1982). Oxygen production by endosymbiotic algae controls superoxide dismutase activity in their animal host. Nature, 297(5867), 579.

Dykens, J. A., Shick, J. M., Benoit, C., Buettner, G. R., & Winston, G. W. (1992). Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. Journal of Experimental Biology, 168(1), 219-241.

Eakin, C. M., Lough, J. M., & Heron, S. F. (2009). Climate variability and change: monitoring data and evidence for increased coral bleaching stress. In Coral bleaching (pp. 41-67). Springer, Berlin, Heidelberg.

Eakin, C. M., Morgan, J. A., Heron, S. F., Smith, T. B., Liu, G., Alvarez-Filip, L., ... & Brandt, M. (2010). Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005. PloS One, 5(11), e13969.

Eakin, C. M., Liu, G., Gomez, A. M., De La Cour, J. L., Heron, S. F., Skirving, W. J., ... & Strong, A. E. (2016). Global coral bleaching 2014–2017: status and an appeal for observations. Reef Encounter, 31(1), 20-26.

Eakin, C. M., Liu, G., Gomez, A. M., De La Cour, J. L., Heron, S. F., Skirving, W. J., ... & Strong, A. E. (2017). Ding, dong, the witch is dead (?)—three years of global coral bleaching 2014–2017. Reef Encounter, 32, 33-38.

Edge, R., McGarvey, D. J., & Truscott, T. G. (1997). The carotenoids as anti-oxidants—a review. Journal of Photochemistry and Photobiology B: Biology, 41(3), 189-200.

Edmunds, P. J. (1994). Evidence that reef-wide patterns of coral bleaching may be the result of the distribution of bleaching-susceptible clones. Marine Biology, 121(1), 137-142.

Edmunds, P. J. (2005). The effect of sub-lethal increases in temperature on the growth and population trajectories of three scleractinian corals on the southern Great Barrier Reef. Oecologia, 146(3), 350-364.

Edmunds, P. J., & Davies, P. S. (1986). An energy budget for *Porites porites* (Scleractinia). Marine Biology, 92(3), 339-347.

Edwards, A. J., Clark, S., Zahir, H., Rajasuriya, A., Naseer, A., & Rubens, J. (2001). Coral bleaching and mortality on artificial and natural reefs in Maldives in 1998, sea surface temperature anomalies and initial recovery. Marine Pollution Bulletin, 42(1), 7-15.

Egaña, A. C., & DiSalvo, L. H. (1982). Mass explusion of zooxanthellae by Easter Island corals. Pacific Science, 36(1), 61-63.

Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., ... & Mohan, J. (2005). Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment, 3(9), 479-486.

Elsner, J. B., Jagger, T. H., & Tsonis, A. A. (2006a). Estimated return periods for Hurricane Katrina. Geophysical Research Letters, 33(8).

Elsner, J. B., Murnane, R. J., & Jagger, T. H. (2006b). Forecasting US hurricanes 6 months in advance. Geophysical Research Letters, 33(10).

Emanuel, K. (2005). Increasing destructiveness of tropical cyclones over the past 30 years. Nature, 436(7051), 686.

Endean, R. (1976). Destruction and recovery of coral reef communities. Biology and Geology of Coral Reefs. Vol. 3, Biology, 2, 215-254.

Engebretson, H., & Martin, K. L. (1994). Effects of decreased salinity on expulsion of zooxanthellae in the symbiotic sea anemone *Anthopleura elegantissima*. Pacific Science, 48(4), 446-457.

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Marine Pollution Bulletin, 50(2), 125-146.

Fabricius, K. E., De'ath, G., McCook, L., Turak, E., & Williams, D. M. (2005). Changes in algal, coral and fish assemblages along water quality gradients on the inshore Great Barrier Reef. Marine Pollution Bulletin, 51(1-4), 384-398.

Fabricius, K. E., De'Ath, G., Puotinen, M. L., Done, T., Cooper, T. F., & Burgess, S. C. (2008). Disturbance gradients on inshore and offshore coral reefs caused by a severe tropical cyclone. Limnology and Oceanography, 53(2), 690-704.

Fan, T. Y., & Dai, C. F. (1999). Reproductive plasticity in the reef coral *Echinopora lamellosa*. Marine Ecology Progress Series, 190, 297-301.

Faxneld, S., Jörgensen, T. L., & Tedengren, M. (2010). Effects of elevated water temperature, reduced salinity and nutrient enrichment on the metabolism of the coral *Turbinaria mesenterina*. Estuarine, Coastal and Shelf Science, 88(4), 482-487.

Faxneld, S., Jörgensen, T. L., Nguyen, N. D., Nyström, M., & Tedengren, M. (2011). Differences in physiological response to increased seawater temperature in nearshore and offshore corals in northern Vietnam. Marine Environmental Research, 71(3), 225-233.

Ferrier-Pagès, C., Gattuso, J. P., & Jaubert, J. (1999). Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellate coral *Stylophora pistillata*. Marine Ecology Progress Series, 181, 309-314.

Ferrier-Pagès, C., Richard, C., Forcioli, D., Allemand, D., Pichon, M., & Shick, J. M. (2007). Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species. The Biological Bulletin, 213(1), 76-87.

Fisk, D. D., & Done, T. T. (1985, January). Taxonomic and bathymetric patterns of bleaching in corals, Myrmidon Reef. In Proceedings of the 5th International Coral Reef Congress, Tahiti, 27 May-1 June 1985-pages: 6: 149-154.

Fitt, W. K., Gates, R. D., Hoegh-Guldberg, O., Bythell, J. C., Jatkar, A., Grottoli, A. G., ... & Iglesias-Prieto, R. (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. Journal of Experimental Marine Biology and Ecology, 373(2), 102-110.

Foster, N. L., Baums, I. B., & Mumby, P. J. (2007). Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastraea annularis*. Journal of Animal Ecology, 76(2), 384-391.

Foster, N. L., Baums, I. B., Sanchez, J. A., Paris, C. B., Chollett, I., Agudelo, C. L., ... & Mumby, P. J. (2013). Hurricane-driven patterns of clonality in an ecosystem engineer: the Caribbean coral *Montastraea annularis*. PLoS One, 8(1), e53283.

Foster, T., Short, J. A., Falter, J. L., Ross, C., & McCulloch, M. T. (2014). Reduced calcification in Western Australian corals during anomalously high summer water temperatures. Journal of Experimental Marine Biology and Ecology, 461, 133-143.

Fox, H. E., Mous, P. J., Pet, J. S., Muljadi, A. H., & Caldwell, R. L. (2005). Experimental assessment of coral reef rehabilitation following blast fishing. Conservation Biology, 19(1), 98-107.

Freeman, B. A., & Crapo, J. D. (1982). Biology of disease: free radicals and tissue injury. Laboratory investigation; a journal of technical methods and pathology, 47(5), 412-426.

Freire, C. A., Welker, A. F., Storey, J. M., Storey, K. B., & Hermes-Lima, M. (2012) Oxidative stress in estuarine and intertidal environments (temperate and tropical), in: Abele, D., Vázquez-Medina, J. P., & Zenteno-Savín, T. (Eds.), Oxidative Stress in Aquatic Ecosystems, First Edition, Blackwell Publishing Ltd, pp. 41-57.

Fridovich, I. (1998). Oxygen toxicity: a radical explanation. Journal of Experimental Biology, 201(8), 1203-1209.

Frieler, K., Meinshausen, M., Golly, A., Mengel, M., Lebek, K., Donner, S. D., & Hoegh-Guldberg, O. (2013). Limiting global warming to 2 °C is unlikely to save most coral reefs. Nature Climate Change, 3(2), 165.

Frölicher, T. L., & Laufkötter, C. (2018). Emerging risks from marine heat waves. Nature Communications, 9(1), 650.

Fujioka, Y. (1999). Mass destruction of the hermatypic corals during a bleaching event in Ishigaki Island, southwestern Japan. Journal of the Japanese Coral Reef Society, 1999(1), 41-50.

Ganot, P., Moya, A., Magnone, V., Allemand, D., Furla, P., & Sabourault, C. (2011). Adaptations to endosymbiosis in a cnidarian-dinoflagellate association: differential gene expression and specific gene duplications. PLoS Genetics, 7(7), e1002187.

Gardner, S. G., Nielsen, D. A., Laczka, O., Shimmon, R., Beltran, V. H., Ralph, P. J., & Petrou, K. (2016). Dimethylsulfoniopropionate, superoxide dismutase and glutathione as stress response indicators in three corals under short-term hyposalinity stress. Proceedings of the Royal Society B: Biological Sciences, 283(1824), 20152418.

Garpe, K. C., Yahya, S. A., Lindahl, U., & Öhman, M. C. (2006). Long-term effects of the 1998 coral bleaching event on reef fish assemblages. Marine Ecology Progress Series, 315, 237-247.

Gates, R. D., & Edmunds, P. J. (1999). The physiological mechanisms of acclimatization in tropical reef corals. The American Zoologist, 39(1), 30-43.

Gates, R. D., Baghdasarian, G., & Muscatine, L. (1992). Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. The Biological Bulletin, 182(3), 324-332.

Gattuso, J. P., Frankignoulle, M., & Smith, S. V. (1999). Measurement of community metabolism and significance in the coral reef CO_2 source-sink debate. Proceedings of the National Academy of Sciences, 96(23), 13017-13022.

Gattuso, J. P., Magnan, A., Billé, R., Cheung, W. W., Howes, E. L., Joos, F., ... & Hoegh-Guldberg, O. (2015). Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. Science, 349(6243), aac4722.

Gibson, R., Atkinson, R., Gordon, J., Smith, I., & Hughes, D. (2011). Coral-associated invertebrates: diversity, ecological importance and vulnerability to disturbance. Oceanography and Marine Biology: an annual review, 49, 43-104.

Gilmour, J. P., Smith, L. D., Heyward, A. J., Baird, A. H., & Pratchett, M. S. (2013). Recovery of an isolated coral reef system following severe disturbance. Science, 340(6128), 69-71.

Gleason, D. F., & Wellington, G. M. (1993). Ultraviolet radiation and coral bleaching. Nature, 365(6449), 836.

Gleason, M. G. (1993). Effects of disturbance on coral communities: bleaching in Moorea, French Polynesia. Coral Reefs, 12(3-4), 193-201.

Glynn, P. W. (1976). Some physical and biological determinants of coral community structure in the eastern Pacific. Ecological Monographs, 46(4), 431-456.

Glynn, P. W. (1983). Extensive 'bleaching'and death of reef corals on the Pacific coast of Panama. Environmental Conservation, 10(2), 149-154.

Glynn, P. W. (1988). Coral bleaching and mortality in the tropical eastern pacific during the 1982–83 El Nino warming event. Mass bleaching of coral reefs in Caribbean: A research strategy. NOAA's Undersea Research Program. St. Croix, US Virgin Islands Research reports, 42-45.

Glynn, P. W. (1990a). Coral mortality and disturbances to coral reefs in the tropical eastern Pacific. In Elsevier Oceanography Series (Vol. 52, pp. 55-126). Elsevier.

Glynn, P. W. (Ed.). (1990b). Global ecological consequences of the 1982-83 El Niño-southern oscillation (Vol. 52). Elsevier.

Glynn, P. W. (1991). Coral reef bleaching in the 1980s and possible connections with global warming. Trends in Ecology & Evolution, 6(6), 175-179.

Glynn, P. W. (1993). Coral reef bleaching: ecological perspectives. Coral Reefs, 12(1), 1-17.

Glynn, P. W. (1996). Coral reef bleaching: facts, hypotheses and implications. Global Change Biology, 2(6), 495-509.

Glynn, P. W. (1997). Bioerosion and coral reef growth: a dynamic balance. In Coral reefs in the Anthropocene (pp. 67-97). Springer, Dordrecht.

Glynn, P. W., & D' Croz, L. (1990). Experimental evidence for high temperature stress as the cause of El Nino-coincident coral mortality. Coral Reefs, 8(4), 181-191.

Glynn, P. W., & De Weerdt, W. H. (1991). Elimination of Two Reef-Building Hydrocorals Following the 1982-83 El Niño Warming Event. Science, 253(5015), 69-71.

Glynn, P.W., Cortés, J., Guzmán, H.M., & Richmond, R.H. (1988). El Niño (1982-83) associated coral mortality and relationship to sea surface temperature deviations in the

tropical eastern Pacific. In: Proceedings of the 6th International Coral Reef Symposium (Vol. 3. pp. 237–243).

Glynn, P. W., Mate, J. L., Baker, A. C., & Calderón, M. O. (2001). Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982–1983 event. Bulletin of Marine Science, 69(1), 79-109.

Glynn, P. W., Colley, S. B., Carpizo-Ituarte, E., & Richmond, R. H. (2017). Coral reproduction in the Eastern Pacific. In Coral Reefs of the Eastern Tropical Pacific (pp. 435-476). Springer, Dordrecht.

Glynn, P. W., Riegl, B., Purkis, S., Kerr, J. M., & Smith, T. B. (2015). Coral reef recovery in the Galápagos Islands: the northernmost islands (Darwin and Wenman). Coral Reefs, 34(2), 421-436.

Golbuu, Y., Victor, S., Penland, L., Idip, D., Emaurois, C., Okaji, K., ... & Van Woesik, R. (2007). Palau's coral reefs show differential habitat recovery following the 1998-bleaching event. Coral Reefs, 26(2), 319-332.

Goreau, T. F. (1964). Mass expulsion of zooxanthellae from Jamaican reef communities after Hurricane Flora. Science, 145(3630), 383-386.

Goreau, T. J. (1992). Bleaching and reef community change in Jamaica: 1951–1991. The American Zoologist, 32(6), 683-695.

Goreau, T., McClanahan, T., Hayes, R., & Strong, A. L. (2000). Conservation of coral reefs after the 1998 global bleaching event. Conservation Biology, 14(1), 5-15.

Graham, N. A. J., Nash, K. L., & Kool, J. T. (2011). Coral reef recovery dynamics in a changing world. Coral Reefs, 30(2), 283-294.

Graham, N. A., Jennings, S., MacNeil, M. A., Mouillot, D., & Wilson, S. K. (2015). Predicting climate-driven regime shifts versus rebound potential in coral reefs. Nature, 518(7537), 94.

Graham, N. A., Wilson, S. K., Jennings, S., Polunin, N. V., Bijoux, J. P., & Robinson, J. (2006). Dynamic fragility of oceanic coral reef ecosystems. Proceedings of the National Academy of Sciences, 103(22), 8425-8429.

Graham, N. A., Wilson, S. K., Jennings, S., Polunin, N. V., Robinson, J. A. N., Bijoux, J. P., & Daw, T. M. (2007). Lag effects in the impacts of mass coral bleaching on coral reef fish, fisheries, and ecosystems. Conservation Biology, 21(5), 1291-1300.

Graham, N. A., McClanahan, T. R., MacNeil, M. A., Wilson, S. K., Polunin, N. V., Jennings, S., ... & Bigot, L. (2008). Climate warming, marine protected areas and the ocean-scale integrity of coral reef ecosystems. PLoS One, 3(8), e3039.

Griffith, J. K. (1997). Occurrence of aggressive mechanisms during interactions between soft corals (Octocorallia: Alcyoniidae) and other corals on the Great Barrier Reef, Australia. Marine and Freshwater Research, 48(2), 129-135.

Grigg, R. W., & Maragos, J. E. (1974). Recolonization of hermatypic corals on submerged lava flows in Hawaii. Ecology, 55(2), 387-395.

Grosberg, R. K., & Cunningham, C. W. (2001). Genetic structure in the sea. From populations to communities, in edited by MD Bertness, S. Gaines & ME Hay.

Grottoli, A. G., Rodrigues, L. J., & Palardy, J. E. (2006). Heterotrophic plasticity and resilience in bleached corals. Nature, 440(7088), 1186.

Grottoli, A. G., Warner, M. E., Levas, S. J., Aschaffenburg, M. D., Schoepf, V., McGinley, M., ... & Matsui, Y. (2014). The cumulative impact of annual coral bleaching can turn some coral species winners into losers. Global Change Biology, 20(12), 3823-3833.

Guest, J. R. (2005). Reproductive patterns of scleractinian corals on Singapore's reefs (Doctoral dissertation).

Guest, J. R., Baird, A. H., Maynard, J. A., Muttaqin, E., Edwards, A. J., Campbell, S. J., ... & Chou, L. M. (2012). Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PloS One, 7(3), e33353.

Guilcher, A. (1988). Coral reef geomorphology (Vol. 1). Wiley.

Gutteridge, J. M., & Halliwell, B. (1990). The measurement and mechanism of lipid peroxidation in biological systems. Trends in Biochemical Sciences, 15(4), 129-135.

Guzmán, H. M., Jackson, J. B., & Weil, E. (1991). Short-term ecological consequences of a major oil spill on Panamanian subtidal reef corals. Coral Reefs, 10(1), 1-12.

Haapkylä, J., Unsworth, R. K., Flavell, M., Bourne, D. G., Schaffelke, B., & Willis, B. L. (2011). Seasonal rainfall and runoff promote coral disease on an inshore reef. PloS One, 6(2), e16893.

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology, 141(2), 312-322.

Halliwell, B. & Gutteridge, J. M. C. (1999). Free Radicals in Biology and Medicine. Oxford University Press, New York.

Harmelin-Vivien, M. L., & Laboute, P. (1986). Catastrophic impact of hurricanes on atoll outer reef slopes in the Tuamotu (French Polynesia). Coral Reefs, 5(2), 55-62.

Harriott, V. J. (1999). Coral growth in subtropical eastern Australia. Coral Reefs, 18(3), 281-291.

Harriott, V. J., & Fisk, D. A. (1988). Recruitment patterns of scleractinian corals: a study of three reefs. Marine and Freshwater Research, 39(4), 409-416.

Harris, D. L., Rovere, A., Casella, E., Power, H., Canavesio, R., Collin, A., ... & Parravicini, V. (2018). Coral reef structural complexity provides important coastal protection from waves under rising sea levels. Science Advances, 4(2), eaao4350.

Harrison, P. L. (2011). Sexual reproduction of scleractinian corals. In Coral reefs: an ecosystem in transition (pp. 59-85). Springer, Dordrecht.

Harrison, P. L., & Wallace, C. C. (1990). Coral reproduction. Ecosystems of the world: coral reefs. Elsevier, Amsterdam, The Netherlands, 133-208.

Hart, D. E., & Kench, P. S. (2007). Carbonate production of an emergent reef platform, Warraber Island, Torres Strait, Australia. Coral Reefs, 26(1), 53-68.

Harvell, C. D., Kim, K., Burkholder, J. M., Colwell, R. R., Epstein, P. R., Grimes, D. J., ... & Porter, J. W. (1999). Emerging marine diseases--climate links and anthropogenic factors. Science, 285(5433), 1505-1510.

Hawkins, T. D., & Davy, S. K. (2012). Nitric oxide production and tolerance differ among *Symbiodinium* types exposed to heat stress. Plant and Cell Physiology, 53(11), 1889-1898.

Hawkins, T. D., Bradley, B. J., & Davy, S. K. (2013). Nitric oxide mediates coral bleaching through an apoptotic-like cell death pathway: evidence from a model sea anemone-dinoflagellate symbiosis. The FASEB Journal, 27(12), 4790-4798.

Hayes, J. D., & McLellan, L. I. (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radical Research, 31(4), 273-300.

Hendy, E. J., Lough, J. M., & Gagan, M. K. (2003). Historical mortality in massive *Porites* from the central Great Barrier Reef, Australia: evidence for past environmental stress?. Coral Reefs, 22(3), 207-215.

Henry, L. A., & Kenchington, E. L. R. (2004). Ecological and genetic evidence for impaired sexual reproduction and induced clonality in the hydroid *Sertularia cupressina* (Cnidaria: Hydrozoa) on commercial scallop grounds in Atlantic Canada. Marine Biology, 145(6), 1107-1118.

Heron, S. F., Maynard, J. A., Van Hooidonk, R., & Eakin, C. M. (2016). Warming trends and bleaching stress of the world's coral reefs 1985–2012. Scientific Reports, 6, 38402.

Heron, S. F., Eakin, C. M., Douvere, F., Anderson, K. L., Day, J. C., Geiger, E., ... & Obura, D. O. (2017). Impacts of climate change on World Heritage coral reefs: A first global scientific assessment.

Heyward, A. J., & Collins, J. D. (1985). Fragmentation in *Montipora ramosa*: the genet and ramet concept applied to a reef coral. Coral Reefs, 4(1), 35-40.

Highsmith, R. C. (1980). Passive colonization and asexual colony multiplication in the massive coral *Porites lutea* Milne Edwards & Haime. Journal of Experimental Marine Biology and Ecology, 47(1), 55-67.

Highsmith, R. C. (1982). Reproduction by fragmentation in corals. Marine Ecology Progress Series. Oldendorf, 7(2), 207-226.

Highsmith, R. C., Riggs, A. C., & D'Antonio, C. M. (1980). Survival of hurricane-generated coral fragments and a disturbance model of reef calcification/growth rates. Oecologia, 46(3), 322-329.

Higuchi, T., Fujimura, H., Arakaki, T., & Oomori, T. (2008, July). Activities of antioxidant enzymes (SOD and CAT) in the coral *Galaxea fascicularis* against increased hydrogen peroxide concentrations in seawater. In Proceedings of the 11th International Coral Reef Symposium (pp. 7-11).

Hildemann, W. H., Raison, R. L., Cheung, G., Hull, C. J., Akaka, L., & Okamoto, J. (1977). Immunological specificity and memory in a scleractinian coral. Nature, 270(5634), 219.

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research, 50(8), 839-866.

Hoegh-Guldberg, O. (2004). Coral reefs in a century of rapid environmental change. Symbiosis, 37(1), 1-31.

Hoegh-Guldberg, O. (2011). Coral reef ecosystems and anthropogenic climate change. Regional Environmental Change, 11(1), 215-227.

Hoegh-Guldberg, O. (2015). Reviving the Ocean Economy: the case for action-2015. WWF International. Gland, Switzerland, Geneva.

Hoegh-Guldberg, O., & Smith, G. J. (1989). The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. Journal of Experimental Marine Biology and Ecology, 129(3), 279-303.

Hoegh-Guldberg, O., & Salvat, B. (1995). Periodic mass-bleaching and elevated sea temperatures: bleaching of outer reef slope communities in Moorea, French Polynesia. Marine Ecology Progress Series, 121, 181-190.

Hoegh-Guldberg, O., & Jones, R. J. (1999). Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Marine Ecology Progress Series, 183, 73-86.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. Science, 328(5985), 1523-1528.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... & Knowlton, N. (2007a). Coral reefs under rapid climate change and ocean acidification. Science, 318(5857), 1737-1742.

Hoegh-Guldberg, O., Anthony, K., Berkelmans, R., Dove, S., Fabricius, K. E., Lough, J. M., ... & Willis, B. (2007b). Vulnerability of reef-building corals on the Great Barrier Reef to climate change.

Hoegh-Guldberg, O. et al., in Climate Change 2014: Impacts, Adaptation and Vulnerability. Part B: Regional Aspects: Contribution of working group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, T. F. Stocker, Ed. (Cambridge University Press, 2013).

Hoeksema, B. W., Scott, C., & True, J. D. (2013). Dietary shift in corallivorous *Drupella* snails following a major bleaching event at Koh Tao, Gulf of Thailand. Coral Reefs, 32(2), 423-428.

Holbrook, S. J., & Schmitt, R. J. (2002). Competition for shelter space causes density-dependent predation mortality in damselfishes. Ecology, 83(10), 2855-2868.

Holbrook, S. J., Adam, T. C., Edmunds, P. J., Schmitt, R. J., Carpenter, R. C., Brooks, A. J., ... & Briggs, C. J. (2018). Recruitment drives spatial variation in recovery rates of resilient coral reefs. Scientific Reports, 8(1), 7338.

Honnay, O., & Bossuyt, B. (2005). Prolonged clonal growth: escape route or route to extinction?. Oikos, 108(2), 427-432.

Hughes, R. N. (1983). Evolutionary ecology of colonial reef-organisms, with particular reference to corals. Biological Journal of the Linnean Society, 20(1), 39-58.

Hughes, T. P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science, 265(5178), 1547-1551.

Hughes, T. P., & Tanner, J. E. (2000). Recruitment failure, life histories, and long-term decline of Caribbean corals. Ecology, 81(8), 2250-2263.

Hughes, T. P., Kerry, J. T., & Simpson, T. (2018b). Large-scale bleaching of corals on the Great Barrier Reef. Ecology, 99(2), 501-501.

Hughes, T. P., Graham, N. A., Jackson, J. B., Mumby, P. J., & Steneck, R. S. (2010). Rising to the challenge of sustaining coral reef resilience. Trends in Ecology & Evolution, 25(11), 633-642.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., ... & Lough, J. M. (2003). Climate change, human impacts, and the resilience of coral reefs. Science, 301(5635), 929-933.

Hughes, T. P., Rodrigues, M. J., Bellwood, D. R., Ceccarelli, D., Hoegh-Guldberg, O., McCook, L., ... & Willis, B. (2007). Phase shifts, herbivory, and the resilience of coral reefs to climate change. Current Biology, 17(4), 360-365.

Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B., ... & Palumbi, S. R. (2017a). Coral reefs in the Anthropocene. Nature, 546(7656), 82.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K.D., Baird, A. H., ... & Bridge, T. C. (2017b). Global warming and recurrent mass bleaching of corals. Nature, 543(7645), 373.

Hughes, T. P., Anderson, K. D., Connolly, S. R., Heron, S. F., Kerry, J. T., Lough, J. M., ... & Claar, D. C. (2018a). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science, 359(6371), 80-83.

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., ... & McWilliam, M. J. (2018c). Global warming transforms coral reef assemblages. Nature, 556(7702), 492.

Hume, B., D'angelo, C., Burt, J., Baker, A. C., Riegl, B., & Wiedenmann, J. (2013). Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: prevalence of clade C3 *Symbiodinium*, host fluorescence and ex situ temperature tolerance. Marine Pollution Bulletin, 72(2), 313-322.

Humphrey, C., Weber, M., Lott, C., Cooper, T., & Fabricius, K. (2008). Effects of suspended sediments, dissolved inorganic nutrients and salinity on fertilisation and embryo development in the coral *Acropora millepora* (Ehrenberg, 1834). Coral Reefs, 27(4), 837-850.

Hunter, C. L. (1993). Genotypic variation and clonal structure in coral populations with different disturbance histories. Evolution, 47(4), 1213-1228.

Hurst, L. D., & Peck, J. R. (1996). Recent advances in understanding of the evolution and maintenance of sex. Trends in Ecology & Evolution, 11(2), 46-52.

Huston, M. A. (1985). Patterns of species diversity on coral reefs. Annual Review of Ecology and Systematics, 16(1), 149-177.

Idjadi, J. A., & Edmunds, P. J. (2006). Scleractinian corals as facilitators for other invertebrates on a Caribbean reef. Marine Ecology Progress Series, 319, 117-127.

Iglesias-Prieto, R., Matta, J. L., Robins, W. A., & Trench, R. K. (1992). Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. Proceedings of the National Academy of Sciences, 89(21), 10302-10305.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Meyer LA (eds)]. IPCC, Geneva, Switzerland, p 151

IPCC (2018). Special Report on 1.5 degrees Celsius; Incheon, Korea, 8th October 2018.

Jackson, J. B. C. (1977). Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. The American Naturalist, 111(980), 743-767.

Johannes, R. E. (1980). Ecological significance of the submarine discharge of groundwater. Marine Ecology Progress Series, 3(4), 365-373.

Johns, K. A., Osborne, K. O., & Logan, M. (2014). Contrasting rates of coral recovery and reassembly in coral communities on the Great Barrier Reef. Coral Reefs, 33(3), 553-563.

Jokiel, P. L. (1985, May). Lunar periodicity of planula release in the reef coral *Pocillopora damicornis* in relation to various environmental factors. In Proceedings of the 5th International Coral Reef Symposium (Vol. 4, pp. 307-312).

Jokiel, P. L., & Coles, S. L. (1974). Effects of heated effluent on hermatypic corals at Kahe Point, Oahu. Pacific Science, 28, 1-18.

Jokiel, P. L., & Coles, S. L. (1977). Effects of temperature on the mortality and growth of Hawaiian reef corals. Marine Biology, 43(3), 201-208.

Jokiel, P. L., & Coles, S. L. (1990). Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs, 8(4), 155-162.

Jokiel, P. L., Hildemann, W. H., & Bigger, C. H. (1983). Clonal population structure of two sympatric species of the reef coral *Montipora*. Bulletin of Marine Science, 33(1), 181-187.

Jokiel, P. L., Ito, R. Y., & Liu, P. M. (1985). Night irradiance and synchronization of lunar release of planula larvae in the reef coral *Pocillopora damicornis*. Marine Biology, 88(2), 167-174.

Jokiel, P. L., Hunter, C. L., Taguchi, S., & Watarai, L. (1993). Ecological impact of a fresh-water "reef kill" in Kaneohe Bay, Oahu, Hawaii. Coral Reefs, 12(3-4), 177-184.

Jones, G. P., McCormick, M. I., Srinivasan, M., & Eagle, J. V. (2004). Coral decline threatens fish biodiversity in marine reserves. Proceedings of the National Academy of Sciences, 101(21), 8251-8253.

Jones, R. J., Ward, S., Amri, A. Y., & Hoegh-Guldberg, O. (2000). Changes in quantum efficiency of Photosystem II of symbiotic dinoflagellates of corals after heat stress, and of bleached corals sampled after the 1998 Great Barrier Reef mass bleaching event. Marine and Freshwater Research, 51(1), 63-71.

Kayal, M., Vercelloni, J., Wand, M. P., & Adjeroud, M. (2015). Searching for the best bet in life-strategy: A quantitative approach to individual performance and population dynamics in reef-building corals. Ecological Complexity, 23, 73-84.

Kayal, M., Vercelloni, J., De Loma, T. L., Bosserelle, P., Chancerelle, Y., Geoffroy, S., ... & Adjeroud, M. (2012). Predator crown-of-thorns starfish (*Acanthaster planci*) outbreak, mass mortality of corals, and cascading effects on reef fish and benthic communities. PloS One, 7(10), e47363.

Kayanne, H., Harii, S., Ide, Y., & Akimoto, F. (2002). Recovery of coral populations after the 1998 bleaching on Shiraho Reef, in the southern Ryukyus, NW Pacific. Marine Ecology Progress Series, 239, 93-103.

Kayanne, H., Harii, S., Yamano, H., Tamura, M., Ide, Y., & Akimoto, F. (1999). Changes in living coral coverage before and after the 1998 bleaching event on coral reef flats of Ishigaki Island, Ryukyu Islands. Journal of the Japanese Coral Reef Society, 1999(1), 73-82.

Kerswell, A. P., & Jones, R. J. (2003). Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of low-salinity bleaching. Marine Ecology Progress Series, 253, 145-154.

Kjerfve, B., Magill, K. E., Porter, J. W., & Woodley, J. D. (1986). Hindcasting of hurricane characteristics and observed storm damage on a fringing reef, Jamaica, West Indies. Journal of Marine Research, 44(1), 119-148.

Kleypas, J. A., McManus, J. W., & Menez, L. A. (1999). Environmental limits to coral reef development: where do we draw the line?. The American Zoologist, 39(1), 146-159.

Kleypas, J. A., Buddemeier, R. W., & Gattuso, J. P. (2001). The future of coral reefs in an age of global change. International Journal of Earth Sciences, 90(2), 426-437.

Kleypas, J. A., Thompson, D. M., Castruccio, F. S., Curchitser, E. N., Pinsky, M., & Watson, J. R. (2016). Larval connectivity across temperature gradients and its potential effect on heat tolerance in coral populations. Global Change Biology, 22(11), 3539-3549.

Knap, A., Dewailly, É., Furgal, C., Galvin, J., Baden, D., Bowen, R. E., ... & Moser, F. (2002). Indicators of ocean health and human health: developing a research and monitoring framework. Environmental Health Perspectives, 110(9), 839-845.

Knowlton, N., Lang, J. C., Rooney, M. C., & Clifford, P. (1981). Evidence for delayed mortality in hurricane-damaged Jamaican staghorn corals. Nature, 294(5838), 251.

Knowlton, N., Brainard, R. E., Fisher, R., Moews, M., Plaisance, L., & Caley, M. J. (2010). Coral reef biodiversity. Life in the world's oceans: diversity distribution and abundance, 65-74.

Kobayashi, A. (1984). Regeneration and regrowth of fragmented colonies of the hermatypic corals *Acropora formosa* and *Acropora nasuta*. Galaxea, 3, 13-23.

Kobluk, D. R., & Lysenko, M. A. (1994). "Ring" bleaching in southern Caribbean *Agaricia agaricites* during rapid water cooling. Bulletin of Marine Science, 54(1), 142-150.

Kondrashov, A. S. (1988). Deleterious mutations and the evolution of sexual reproduction. Nature, 336(6198), 435.

Kopp, C., Domart-Coulon, I., Escrig, S., Humbel, B. M., Hignette, M., & Meibom, A. (2015). Subcellular investigation of photosynthesis-driven carbon assimilation in the symbiotic reef coral *Pocillopora damicornis*. MBio, 6(1), e02299-14.

Krueger, T., Becker, S., Pontasch, S., Dove, S., Hoegh-Guldberg, O., Leggat, W., ... & Davy, S. K. (2014). Antioxidant plasticity and thermal sensitivity in four types of *Symbiodinium* sp. Journal of Phycology, 50(6), 1035-1047.

Kühl, M., Cohen, Y., Dalsgaard, T., & Jørgensen, B. B., Revsbech, N. P. (1995). Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. Marine Ecology Progress Series, 117, 159-172.

Kurutas, E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition Journal, 15(1), 71.

Kushmaro, A., Loya, Y., Fine, M., & Rosenberg, E. (1996). Bacterial infection and coral bleaching. Nature, 380(6573), 396.

L'Heureux, M. L., Takahashi, K., Watkins, A. B., Barnston, A. G., Becker, E. J., Di Liberto, T. E., ... & Mosquera-Vásquez, K. (2017). Observing and predicting the 2015/16 El Niño. Bulletin of the American Meteorological Society, 98(7), 1363-1382.

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., & Santos, S. R. (2018). Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Current Biology, 28(16), 2570-2580.

Lam, K., Shin, P. K. S., & Hodgson, P. (2007). Severe bioerosion caused by an outbreak of corallivorous *Drupella* and *Diadema* at Hoi Ha Wan marine park, Hong Kong. Coral Reefs, 26(4), 893-893.

Langdon, C., & Atkinson, M. J. (2005). Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. Journal of Geophysical Research: Oceans, 110(C9).

Langlais, C. E., Lenton, A., Heron, S. F., Evenhuis, C., Gupta, A. S., Brown, J. N., & Kuchinke, M. (2017). Coral bleaching pathways under the control of regional temperature variability. Nature Climate Change, 7(11), 839.

Le Goff-Vitry, M. C., Pybus, O. G., & Rogers, A. D. (2004). Genetic structure of the deep-sea coral Lophelia pertusa in the northeast Atlantic revealed by microsatellites and internal transcribed spacer sequences. Molecular Ecology, 13(3), 537-549.

Le Nohaïc, M., Ross, C. L., Cornwall, C. E., Comeau, S., Lowe, R., McCulloch, M. T., & Schoepf, V. (2017). Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia. Scientific Reports, 7(1), 14999.

Leder, J. J., Szmant, A. M., & Swart, P. K. (1991). The effect of prolonged "bleaching" on skeletal banding and stable isotopic composition in *Montastrea annularis*. Coral Reefs, 10(1), 19-27.

Ledford, H. K., & Niyogi, K. K. (2005). Singlet oxygen and photo-oxidative stress management in plants and algae. Plant, Cell & Environment, 28(8), 1037-1045.

Leggat, W., Ainsworth, T. D., Dove, S., & Hoegh-Guldberg, O. (2006). Aerial exposure influences bleaching patterns. Coral Reefs, 25(3), 452-452.

Leichter, J. J., Wing, S. R., Miller, S. L., & Denny, M. W. (1996). Pulsed delivery of subthermocline water to Conch Reef (Florida Keys) by internal tidal bores. Limnology and Oceanography, 41(7), 1490-1501.

Lenihan, H. S., Holbrook, S. J., Schmitt, R. J., & Brooks, A. J. (2011). Influence of corallivory, competition, and habitat structure on coral community shifts. Ecology, 92(10), 1959-1971.

Lesser, M. P. (1997). Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs, 16(3), 187-192.

Lesser, M. P. (2004). Experimental biology of coral reef ecosystems. Journal of Experimental Marine Biology and Ecology, 300(1-2), 217-252.

Lesser, M. P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. Annual Review of Physiology, 68, 253-278.

Lesser, M. P. (2011). Coral bleaching: causes and mechanisms. In Coral reefs: an ecosystem in transition (pp. 405-419). Springer, Dordrecht.

Lesser, M. P. (2012). Oxidative stress in tropical marine ecosystems. Oxidative stress in aquatic ecosystems, 1, 9-19.

Lesser, M. P., & Shick, J. M. (1989a). Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production,

photoinhibition, and enzymic defenses against oxygen toxicity. Marine Biology, 102(2), 243-255.

Lesser, M. P., & Shick, J. M. (1989b). Photoadaption and defenses against oxygen toxicity in zooxanthellae from natural populations of symbiotic cnidarians. Journal of Experimental Marine Biology and Ecology, 134(2), 129-141.

Lesser, M. P., & Farrell, J. H. (2004). Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs, 23(3), 367-377.

Lesser, M. P., Stochaj, W. R., Tapley, D. W., & Shick, J. M. (1990). Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs, 8(4), 225-232.

Levas, S. J., Grottoli, A. G., Hughes, A., Osburn, C. L., & Matsui, Y. (2013). Physiological and biogeochemical traits of bleaching and recovery in the mounding species of coral *Porites lobata*: implications for resilience in mounding corals. PloS One, 8(5), e63267.

Levitan, D. R., Boudreau, W., Jara, J., & Knowlton, N. (2014). Long-term reduced spawning in *Orbicella* coral species due to temperature stress. Marine Ecology Progress Series, 515, 1-10.

Lewis, J. B. (1985). Groundwater discharge onto coral reefs, Barbados (West Indies). In Proceedings of the 5th International Coral Reef Symposium (Vol. 6, pp. 477-481).

Lichtenberg, M., Larkum, A. W., & Kühl, M. (2016). Photosynthetic acclimation of *Symbiodinium* in hospite depends on vertical position in the tissue of the scleractinian coral Montastrea curta. Frontiers in Microbiology, 7, 230.

Limón-Pacheco, J., & Gonsebatt, M. E. (2009). The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 674(1-2), 137-147.

Lirman, D. (2000). Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. Journal of Experimental Marine Biology and Ecology, 251(1), 41-57.

Lirman, D. (2003). A simulation model of the population dynamics of the branching coral *Acropora palmata* Effects of storm intensity and frequency. Ecological Modelling, 161(3), 169-182.

Lirman, D., & Manzello, D. (2009). Patterns of resistance and resilience of the stress-tolerant coral *Siderastrea radians* (Pallas) to sub-optimal salinity and sediment burial. Journal of Experimental Marine Biology and Ecology, 369(1), 72-77.

Lirman, D., Deangelo, G., Serafy, J., Hazra, A., Hazra, D. S., Herlan, J., ... & Clausing, R. (2008). Seasonal changes in the abundance and distribution of submerged aquatic vegetation in a highly managed coastal lagoon. Hydrobiologia, 596(1), 105.

Liu, G., Strong, A. E., & Skirving, W. (2003). Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching. Eos, Transactions American Geophysical Union, 84(15), 137-141.

Liu, G., Heron, S., Eakin, C., Muller-Karger, F., Vega-Rodriguez, M., Guild, L., ... & Strong, A. (2014). Reef-scale thermal stress monitoring of coral ecosystems: new 5-km global products from NOAA Coral Reef Watch. Remote Sensing, 6(11), 11579-11606.

Liu, G., Skirving, W. J., Geiger, E. F., De La Cour, J. L., Marsh, B. L., Heron, S. F., ... & Eakin, C. M. (2017). NOAA Coral Reef Watch's 5km satellite coral bleaching heat stress monitoring product suite version 3 and four-month outlook version 4. Reef Encounter, 32(1), 39-45.

Loch, K., Loch, W., Schuhmacher, H., & See, W. R. (2002). Coral recruitment and regeneration on a Maldivian reef 21 months after the coral bleaching event of 1998. Marine Ecology, 23(3), 219-236.

Logan, C. A., Dunne, J. P., Eakin, C. M., & Donner, S. D. (2014). Incorporating adaptive responses into future projections of coral bleaching. Global Change Biology, 20(1), 125-139.

Lough, J. M. (2000). 1997–98: Unprecedented thermal stress to coral reefs?. Geophysical Research Letters, 27(23), 3901-3904.

Lough, J. M. (2008). 10th anniversary review: a changing climate for coral reefs. Journal of Environmental Monitoring, 10(1), 21-29.

Lough, J. M., Barnes, D. J., Devereux, M. J., Tobin, B. J., & Tobin, S. (1999). Variability in growth characteristics of massive *Porites* on the Great Barrier Reef. In CRC Reef Research Centre Technical Report (Vol. 28, p. 95).

Lourey, M. J., Ryan, D. A., & Miller, I. R. (2000). Rates of decline and recovery of coral cover on reefs impacted by, recovering from and unaffected by crown-of-thorns starfish *Acanthaster planci*: a regional perspective of the Great Barrier Reef. Marine Ecology Progress Series, 196, 179-186.

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., & Van Woesik, R. (2001). Coral bleaching: the winners and the losers. Ecology Letters, 4(2), 122-131.

Madeira, C., Mendonça, V., Leal, M. C., Flores, A. A., Cabral, H. N., Diniz, M. S., & Vinagre, C. (2018). Environmental health assessment of warming coastal ecosystems in the tropics–Application of integrative physiological indices. Science of the Total Environment, 643, 28-39.

Maguire, L. A., & Porter, J. W. (1977). A spatial model of growth and competition strategies in coral communities. Ecological Modelling, 3(4), 249-271.

Manzello, D. P. (2010). Coral growth with thermal stress and ocean acidification: lessons from the eastern tropical Pacific. Coral Reefs, 29(3), 749-758.

Manzello, D., & Lirman, D. (2003). The photosynthetic resilience of *Porites furcata* to salinity disturbance. Coral Reefs, 22(4), 537-540.

Marigómez, I., Garmendia, L., Soto, M., Orbea, A., Izagirre, U., & Cajaraville, M. P. (2013). Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill “Mussel Watch”. Ecotoxicology, 22(3), 486-505.

Marshall, A. T., & Clode, P. (2004). Calcification rate and the effect of temperature in a zooxanthellate and an azooxanthellate scleractinian reef coral. Coral Reefs, 23(2), 218-224.

Marshall, P. A., & Baird, A. H. (2000). Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs, 19(2), 155-163.

Mayfield, A. B., & Gates, R. D. (2007). Osmoregulation in anthozoan–dinoflagellate symbiosis. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(1), 1-10.

Maynard, J. A., Anthony, K. R. N., Marshall, P. A., & Masiri, I. (2008). Major bleaching events can lead to increased thermal tolerance in corals. Marine Biology, 155(2), 173-182.

McClanahan, T. R. (2000). Bleaching damage and recovery potential of Maldivian coral reefs. Marine Pollution Bulletin, 40(7), 587-597.

McClanahan, T. R. (2004). The relationship between bleaching and mortality of common corals. Marine Biology, 144(6), 1239-1245.

McClanahan, T. R. (2014). Decadal coral community reassembly on an African fringing reef. Coral Reefs, 33(4), 939-950.

McClanahan, T. R., & Muthiga, N. A. (1988). Changes in Kenyan coral reef community structure and function due to exploitation. Hydrobiologia, 166(3), 269-276.

McClanahan, T. R., & Maina, J. (2003). Response of coral assemblages to the interaction between natural temperature variation and rare warm-water events. Ecosystems, 6(6), 551-563.

McClanahan, T. R., Baird, A. H., Marshall, P. A., & Toscano, M. A. (2004). Comparing bleaching and mortality responses of hard corals between southern Kenya and the Great Barrier Reef, Australia. Marine Pollution Bulletin, 48(3-4), 327-335.

McClanahan, T. R., Weil, E., Cortés, J., Baird, A. H., & Ateweberhan, M. (2009). Consequences of coral bleaching for sessile reef organisms. In Coral bleaching (pp. 121-138). Springer, Berlin, Heidelberg.

McGinty, E. S., Pieczonka, J., & Mydlarz, L. D. (2012). Variations in reactive oxygen release and antioxidant activity in multiple *Symbiodinium* types in response to elevated temperature. Microbial Ecology, 64(4), 1000-1007.

McPhaden, M. J. (1999). Genesis and evolution of the 1997-98 El Niño. Science, 283(5404), 950-954.

Meehan, W. J., & Ostrander, G. K. (1997). Coral bleaching: a potential biomarker of environmental stress. Journal of Toxicology and Environmental Health, Part A Current Issues, 50(6), 529-552.

Meesters, E. H., & Bak, R. P. (1993). Effects of coral bleaching on tissue regeneration potential and colony survival. Marine Ecology Progress Series, 189-198.

Merle, P. L., Sabourault, C., Richier, S., Allemand, D., & Furla, P. (2007). Catalase characterization and implication in bleaching of a symbiotic sea anemone. Free Radical Biology and Medicine, 42(2), 236-246.

Michalek-Wagner, K., & Willis, B. L. (2001). Impacts of bleaching on the soft coral Lobophytum compactum. I. Fecundity, fertilization and offspring viability. Coral Reefs, 19(3), 231-239.

Middlebrook, R., Hoegh-Guldberg, O., & Leggat, W. (2008). The effect of thermal history on the susceptibility of reef-building corals to thermal stress. Journal of Experimental Biology, 211(7), 1050-1056.

Miller, J., Muller, E., Rogers, C., Waara, R., Atkinson, A., Whelan, K. R. T., ... & Witcher, B. (2009). Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. Coral Reefs, 28(4), 925.

Miller, K. J., & Ayre, D. J. (2004). The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity, 92(6), 557.

Milly, P. C. D., Wetherald, R. T., Dunne, K. A., & Delworth, T. L. (2002). Increasing risk of great floods in a changing climate. Nature, 415(6871), 514.

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems. Ecological Economics, 29(2), 215-233.

Moberg, F., Nyström, M., Kautsky, N., Tedengren, M., & Jarayabhand, P. (1997). Effects of reduced salinity on the rates of photosynthesis and respiration in the hermatypic corals *Porites lutea* and *Pocillopora damicornis*. Marine Ecology Progress Series, 157, 53-59.

Moerland, M. S., Scott, C. M., & Hoeksema, B. W. (2016). Prey selection of corallivorous muricids at Koh Tao (Gulf of Thailand) four years after a major coral bleaching event. Contributions to Zoology, 85(3), 291-309.

Montefalcone, M., Morri, C., & Bianchi, C. N. (2018). Long-term change in bioconstruction potential of Maldivian coral reefs following extreme climate anomalies. Global Change Biology, 24(12), 5629-5641.

Mora, C. (2008). A clear human footprint in the coral reefs of the Caribbean. Proceedings of the Royal Society B: Biological Sciences, 275(1636), 767-773.

Mora, C., Graham, N. A., & Nyström, M. (2016). Ecological limitations to the resilience of coral reefs. Coral Reefs, 35(4), 1271-1280.

Mora, C., Aburto-Oropeza, O., Bocos, A. A., Ayotte, P. M., Banks, S., Bauman, A. G., ... & Brooks, A. (2011). Global human footprint on the linkage between biodiversity and ecosystem functioning in reef fishes. PLoS Biology, 9(4), e1000606.

Morgan, M. B., & Snell, T. W. (2002). Characterizing stress gene expression in reef-building corals exposed to the mosquitoside dibrom. Marine Pollution Bulletin, 44(11), 1206-1218.

Morton, B., Blackmore, G., & Kwok, C. T. (2002). Corallivory and prey choice by *Drupella rugosa* (Gastropoda: Muricidae) in Hong Kong. Journal of Molluscan Studies, 68(3), 217-223.

Motta, P. J. (1989). Dentition patterns among Pacific and Western Atlantic butterflyfishes (Perciformes, Chaetodontidae): relationship to feeding ecology and evolutionary history. Environmental Biology of Fishes, 25(1-3), 159.

Mullen, A. D., Treibitz, T., Roberts, P. L., & Jaffe, J. S. (2017, April). An underwater microscope for in situ imaging of seafloor organism. In Novel Techniques in Microscopy (pp. NTu1C-1). Optical Society of America.

Muller, E. M., Rogers, C. S., Spitzack, A. S., & Van Woesik, R. (2008). Bleaching increases likelihood of disease on *Acropora palmata* (Lamarck) in Hawksnest Bay, St John, US Virgin Islands. Coral Reefs, 27(1), 191-195.

Muller-Parker, G., D'elia, C. F., & Cook, C. B. (2015). Interactions between corals and their symbiotic algae. In Coral reefs in the Anthropocene (pp. 99-116). Springer, Dordrecht.

Mumby, P. J., Hastings, A., & Edwards, H. J. (2007). Thresholds and the resilience of Caribbean coral reefs. Nature, 450(7166), 98.

Mumby, P. J., Steneck, R. S., Adjeroud, M., & Arnold, S. N. (2016). High resilience masks underlying sensitivity to algal phase shifts of Pacific coral reefs. Oikos, 125(5), 644-655.

Mumby, P., Chisholm, J., Edwards, A., Clark, C., Roark, E., Andrefouet, S., & Jaubert, J. (2001). Unprecedented bleaching-induced mortality in *Porites* spp. at Rangiroa Atoll, French Polynesia. Marine Biology, 139(1), 183-189.

Munday, P. L. (2001). Fitness consequences of habitat use and competition among coral-dwelling fishes. Oecologia, 128(4), 585-593.

Munday, P. L. (2004). Habitat loss, resource specialization, and extinction on coral reefs. Global Change Biology, 10(10), 1642-1647.

Murata, N., Takahashi, S., Nishiyama, Y., & Allakhverdiev, S. I. (2007). Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1767(6), 414-421.

Muscatine, L. (1990). The role of symbiotic algae in carbon and energy flux in reef corals. Ecosystems of the world. Coral Reefs.

Muscatine, L., & Weis, V. (1992). Productivity of zooxanthellae and biogeochemical cycles. p. 257 -271. In Falkowski, P. G., & Woodhead, A. D. (Eds.). (2013). Primary productivity and biogeochemical cycles in the sea (Vol. 43). Springer Science & Business Media.

Muscatine, L., Grossman, D., & Doino, J. (1991). Release of symbiotic algae by tropical sea anemones and corals after cold shock. Marine Ecology Progress Series. Oldendorf, 77(2), 233-243.

Muthiga, N. A., & Szmant, A. M. (1987). The effects of salinity stress on the rates of aerobic respiration and photosynthesis in the hermatypic coral *Siderastrea siderea*. The Biological Bulletin, 173(3), 539-551.

Mydlarz, L. D., Couch, C. S., Weil, E., Smith, G., & Harvell, C. D. (2009). Immune defenses of healthy, bleached and diseased *Montastraea faveolata* during a natural bleaching event. Diseases of Aquatic Organisms, 87(1-2), 67-78.

Nakamura, T., & Van Woesik, R. (2001). Water-flow rates and passive diffusion partially explain differential survival of corals during the 1998 bleaching event. Marine Ecology Progress Series, 212, 301-304.

Nakano, Y., Yamazato, K., Masuhara, H., & Ito, S. (1997). Responses of Okinawan reef-building corals to artificial high salinity. Galaxia, 13, 181-195.

Neigel, J. E., & Avise, J. C. (1983). Clonal diversity and population structure in a reef-building coral, *Acropora cervicornis*: self-recognition analysis and demographic interpretation. Evolution, 437-453.

Nicholls, R. J., Wong, P. P., Burkett, V.R., Codignotto, J. O., Hay, J. E., et al. (2007). Coastal systems and low-lying areas. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Parry ML, Canziani OF, Palutikof JP, Van der Linden PJ and Hanson CE, editors. Cambridge University Press, Cambridge. pp 315–356.

Nicolet, K. J., Hoogenboom, M. O., Gardiner, N. M., Pratchett, M. S., & Willis, B. L. (2013). The corallivorous invertebrate *Drupella* aids in transmission of brown band disease on the Great Barrier Reef. Coral Reefs, 32(2), 585-595.

Nielsen, D. A., Petrou, K., & Gates, R. D. (2018). Coral bleaching from a single cell perspective. The ISME Journal, 12(6), 1558.

Nii, C. M., & Muscatine, L. (1997). Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Carlgren, 1943): contribution of the animal to superoxide ion production at elevated temperature. The Biological Bulletin, 192(3), 444-456.

Norström, A. V., Nyström, M., Lokrantz, J., & Folke, C. (2009). Alternative states on coral reefs: beyond coral–macroalgal phase shifts. Marine Ecology Progress Series, 376, 295-306.

Nott, J., & Hayne, M. (2001). High frequency of 'super-cyclones' along the Great Barrier Reef over the past 5,000 years. Nature, 413(6855), 508.

Oliver, J. (1985). Recurrent seasonal bleaching and mortality of corals on the Great Barrier Reef. In Proceedings of the 5th International Coral Reef Symposium (Vol. 4, pp. 201-206).

Oliver, J. K., Berkelmans, R. & Eakin, C. M. (2009) in Ecological Studies: Analysis and Synthesis (eds Van Oppen, M. J. H. & Lough, J. M.) 21–39 (2009).

Ormond, R. F. G., Roberts, C. M. (1997). The biodiversity of coral reef fishes. In: Marine Biodiversity Patterns and Processes. Ormond RFG, Gage JD, Angel MV (eds). Cambridge University Press, Cambridge UK, 449p.

Ortiz, J. C., Bozec, Y. M., Wolff, N. H., Doropoulos, C., & Mumby, P. J. (2014). Global disparity in the ecological benefits of reducing carbon emissions for coral reefs. Nature Climate Change, 4(12), 1090.

Osborne, K., Dolman, A. M., Burgess, S. C., & Johns, K. A. (2011). Disturbance and the dynamics of coral cover on the Great Barrier Reef (1995–2009). PloS One, 6(3), e17516.

Osborne, K., Thompson, A. A., Cheal, A. J., Emslie, M. J., Johns, K. A., Jonker, M. J., ... & Sweatman, H. P. (2017). Delayed coral recovery in a warming ocean. Global Change Biology, 23(9), 3869-3881.

Page, C. E., Leggat, W., Heron, S. F., Choukroun, S. M., Lloyd, J., & Ainsworth, T. D. (2019). Seeking Resistance in Coral Reef Ecosystems: The Interplay of Biophysical Factors and Bleaching Resistance under a Changing Climate: The Interplay of a Reef's Biophysical Factors Can Mitigate the Coral Bleaching Response. BioEssays, 1800226.

Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N., & Bay, R. A. (2014). Mechanisms of reef coral resistance to future climate change. Science, 344(6186), 895-898.

Pandolfi, J. M., Connolly, S. R., Marshall, D. J., & Cohen, A. L. (2011). Projecting coral reef futures under global warming and ocean acidification. Science, 333(6041), 418-422.

Pandolfi, J. M., Bradbury, R. H., Sala, E., Hughes, T. P., Bjorndal, K. A., Cooke, R. G., ... & Warner, R. R. (2003). Global trajectories of the long-term decline of coral reef ecosystems. Science, 301(5635), 955-958.

Parkinson, J. E., & Baums, I. B. (2014). The extended phenotypes of marine symbioses: ecological and evolutionary consequences of intraspecific genetic diversity in coral–algal associations. Frontiers in Microbiology, 5, 445.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics, 37, 637-669.

Parry, M. L., Canziani, O. F., Palutikof, J. P., Van der Linden, P. J., Hanson, C. E. (2007) Technical Summary. Climate Change 2007: Impacts, Adaptation and Vulnerability.

Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Parry, M. L., Canziani, O. F., Palutikof, F. P., Van der Linden, P. J., Hanson, C. E., editors. Cambridge University Press, Cambridge. pp 23–78.

Paulay, G. (1997). Diversity and distribution of reef organisms. Life and death of coral reefs, 198-229.

Paxton, C. W., Davy, S. K., & Weis, V. M. (2013). Stress and death of cnidarian host cells play a role in cnidarian bleaching. Journal of Experimental Biology, 216 (15), 2813–2820.

Pearson, R. G. (1981). Recovery and recolonization of coral reefs. Marine Ecology Progress Series, 105-122.

Perez, S., & Weis, V. (2006). Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. Journal of Experimental Biology, 209(14), 2804-2810.

Perry, C. T., & Morgan, K. M. (2017). Bleaching drives collapse in reef carbonate budgets and reef growth potential on southern Maldives reefs. Scientific Reports, 7, 40581.

Peterson, C. H., Lubchenco, J. (1997). On the value of marine ecosystems to society. In: Daily, G.C. (Ed.), Nature's Services. Societal Dependence on Natural Ecosystems. Island Press, New York, pp. 177–194.

Podestá, G. P., & Glynn, P. W. (2001). The 1997–98 El Nino event in Panama and Galapagos: an update of thermal stress indices relative to coral bleaching. Bulletin of Marine Science, 69(1), 43-59.

Pontasch, S., Hill, R., Deschaseaux, E., Fisher, P. L., Davy, S. K., & Scott, A. (2014). Photochemical efficiency and antioxidant capacity in relation to *Symbiodinium* genotype and host phenotype in a symbiotic cnidarian. Marine Ecology Progress Series, 516, 195-208.

Porter, J. W., Lewis, S. K., & Porter, K. G. (1999). The effect of multiple stressors on the Florida Keys coral reef ecosystem: a landscape hypothesis and a physiological test. Limnology and Oceanography, 44(3part2), 941-949.

Power, S., Delage, F., Chung, C., Kociuba, G., & Keay, K. (2013). Robust twenty-first-century projections of El Niño and related precipitation variability. Nature, 502(7472), 541.

Pratchett, M. S. (2001). Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the western Pacific. Marine Ecology Progress Series, 214, 111-119.

Pratchett, M. S. (2005). Dynamics of an outbreak population of *Acanthaster planci* at Lizard Island, northern Great Barrier Reef (1995–1999). Coral Reefs, 24(3), 453-462.

Pratchett, M. S. (2010). Changes in coral assemblages during an outbreak of *Acanthaster planci* at Lizard Island, northern Great Barrier Reef (1995–1999). Coral Reefs, 29(3), 717-725.

Pratchett, M. S., Schenk, T. J., Baine, M., Syms, C., & Baird, A. H. (2009). Selective coral mortality associated with outbreaks of *Acanthaster planci* L. in Bootless Bay, Papua New Guinea. Marine Environmental Research, 67(4-5), 230-236.

Pratchett, M. S., Hoey, A. S., Wilson, S. K., Messmer, V., & Graham, N. A. (2011a). Changes in biodiversity and functioning of reef fish assemblages following coral bleaching and coral loss. Diversity, 3(3), 424-452.

Pratchett, M. S., Trapon, M., Berumen, M. L., & Chong-Seng, K. (2011b). Recent disturbances augment community shifts in coral assemblages in Moorea, French Polynesia. Coral Reefs, 30(1), 183-193.

Pratchett, M. S., Munday, P. L., Wilson, S. K., Graham, N. A., Cinner, J. E., Bellwood, D. R., ... & McClanahan, T. R. (2008). Effects of climate-induced coral bleaching on coral-reef fishes—ecological and economic consequences. In Oceanography and Marine Biology (pp. 257-302). CRC Press.

Putnam, H. M., Barott, K. L., Ainsworth, T. D., & Gates, R. D. (2017). The vulnerability and resilience of reef-building corals. Current Biology, 27(11), R528-R540.

Randall, C. J., & Szmant, A. M. (2009). Elevated temperature reduces survivorship and settlement of the larvae of the Caribbean scleractinian coral, Favia fragum (Esper). Coral Reefs, 28(2), 537-545.

Rankin, J. C., & Davenport, J. (1981). Animal osmoregulation. John Wiley & Sons.

Reaka-Kudla, M. L. (2005). Biodiversity of Caribbean coral reefs. Caribbean Marine Biodiversity: The Known and the Unknown, 259-276.

Reaka-Kudla, M. L., O'Connell, D. S., Regan, J. D., & Wicklund, R. I. (1993, June). Effects of temperature and UV-B on different components of coral reef communities from the Bahamas. In Proceedings of the Colloquium on Global Aspects of Coral Reefs: Health, Hazards and History (pp. 1-7).

Reaser, J. K., Pomerance, R., & Thomas, P. O. (2000). Coral bleaching and global climate change: scientific findings and policy recommendations. Conservation Biology, 14(5), 1500-1511.

Regoli, F., Cerrano, C., Chierici, E., Bompadre, S., & Bavestrello, G. (2000). Susceptibility to oxidative stress of the Mediterranean demosponge *Petrosia ficiformis*: role of endosymbionts and solar irradiance. Marine Biology, 137(3), 453-461.

Richards, Z. T., & Rosser, N. L. (2012). Abundance, distribution and new records of scleractinian corals at Barrow Island and Southern Montebello Islands, Pilbara (offshore) bioregion. Journal of the Royal Society of Western Australia, 95, 155.

Richardson, L. E., Graham, N. A., Pratchett, M. S., Eurich, J. G., & Hoey, A. S. (2018). Mass coral bleaching causes biotic homogenization of reef fish assemblages. Global Change Biology, 24(7), 3117-3129.

Richier, S., Furla, P., Plantivaux, A., Merle, P. L., & Allemand, D. (2005). Symbiosis-induced adaptation to oxidative stress. Journal of Experimental Biology, 208(2), 277-285.

Richier, S., Merle, P. L., Furla, P., Pigozzi, D., Sola, F., & Allemand, D. (2003). Characterization of superoxide dismutases in anoxia-and hyperoxia-tolerant symbiotic cnidarians. Biochimica et Biophysica Acta (BBA)-General Subjects, 1621(1), 84-91.

Richier, S., Sabourault, C., Courtiade, J., Zucchini, N., Allemand, D., & Furla, P. (2006). Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis*. The FEBS Journal, 273(18), 4186-4198.

Richmond, R. H. (1993). Coral reefs: present problems and future concerns resulting from anthropogenic disturbance. The American Zoologist, 33(6), 524-536.

Richmond, R. H. (1997). Reproduction and recruitment in corals: critical links in the persistence of reefs. Life and death of coral reefs. Chapman & Hall, New York, 175-197.

Richmond, R. H., & Hunter, C. L. (1990). Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Marine Ecology Progress Series. Oldendorf, 60(1), 185-203.

Riegl, B. (2002). Effects of the 1996 and 1998 positive sea-surface temperature anomalies on corals, coral diseases and fish in the Arabian Gulf (Dubai, UAE). Marine Biology, 140(1), 29-40.

Riegl, B., & Bloomer, J. P. (1995). Tissue damage in scleractinian and alcyonacean corals due to experimental exposure to sedimentation. Marine & Environmental Sciences Faculty Proceedings, Presentations, Speeches, Lectures. 114.

Riegl, B., & Purkis, S. (2015). Coral population dynamics across consecutive mass mortality events. Global Change Biology, 21(11), 3995-4005.

Rodrigues, L. J., & Grottoli, A. G. (2006). Calcification rate and the stable carbon, oxygen, and nitrogen isotopes in the skeleton, host tissue, and zooxanthellae of bleached and recovering Hawaiian corals. Geochimica et Cosmochimica Acta, 70(11), 2781-2789.

Roff, G., & Mumby, P. J. (2012). Global disparity in the resilience of coral reefs. Trends in Ecology & Evolution, 27(7), 404-413.

Rogers, C. S. (1993). Hurricanes and coral reefs: the intermediate disturbance hypothesis revisited. Coral Reefs, 12(3-4), 127-137.

Rogers, C. S., & Muller, E. M. (2012). Bleaching, disease and recovery in the threatened scleractinian coral *Acropora palmata* in St. John, US Virgin Islands: 2003–2010. Coral Reefs, 31(3), 807-819.

Rogers, C. S., Suchanek, T. H., & Pecora, F. A. (1982). Effects of hurricanes David and Frederic (1979) on shallow *Acropora palmata* reef communities: St. Croix, US Virgin Islands. Bulletin of Marine Science, 32(2), 532-548.

Rogers, J. E., & Davis, R. H. (2006). Application of a new micro-culturing technique to assess the effects of temperature and salinity on specific growth rates of six *Symbiodinium* isolates. Bulletin of Marine Science, 79(1), 113-126.

Rosenberg, E., & Loya, Y. (1999). Vibrio shiloi is the etiological (causative) agent of *Oculina patagonica* bleaching: General implications. Reef Encounter, 25, 8-10.

Ross, C. L., Schoepf, V., DeCarlo, T. M., & McCulloch, M. T. (2018). Mechanisms and seasonal drivers of calcification in the temperate coral *Turbinaria reniformis* at its latitudinal limits. Proceedings of the Royal Society B: Biological Sciences, 285(1879), 20180215.

Ross, C., Ritson-Williams, R., Olsen, K., & Paul, V. J. (2013). Short-term and latent post-settlement effects associated with elevated temperature and oxidative stress on larvae from the coral *Porites astreoides*. Coral Reefs, 32(1), 71-79.

Rosset, S., Wiedenmann, J., Reed, A. J., & D'Angelo, C. (2017). Phosphate deficiency promotes coral bleaching and is reflected by the ultrastructure of symbiotic dinoflagellates. Marine Pollution Bulletin, 118(1-2), 180-187.

Roth, L., Muller, E. M., & Van Woesik, R. (2013). Tracking *Acropora* fragmentation and population structure through thermal-stress events. Ecological Modelling, 263, 223-232.

Rotjan, R. D., & Lewis, S. M. (2008). Impact of coral predators on tropical reefs. Marine Ecology Progress Series, 367, 73-91.

Rowan, R., & Knowlton, N. (1995). Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proceedings of the National Academy of Sciences, 92(7), 2850-2853.

Rowan, R., Knowlton, N., Baker, A., & Jara, J. (1997). Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature, 388(6639), 265.

Sakami, T. (2000). Effects of temperature, irradiance, salinity and inorganic nitrogen concentration on coral zooxanthellae in culture. Fisheries Science, 66(6), 1006-1013.

Sale, P. F. (1977). Maintenance of high diversity in coral reef fish communities. The American Naturalist, 111(978), 337-359.

Sale, P. F., Cowen, R. K., Danilowicz, B. S., Jones, G. P., Kritzer, J. P., Lindeman, K. C., ... & Steneck, R. S. (2005). Critical science gaps impede use of no-take fishery reserves. Trends in Ecology & Evolution, 20(2), 74-80.

Salih, A., Hoegh-Guldberg, O., & Cox, G. (1998). Photoprotection of symbiotic dinoflagellates by fluorescent pigments in reef corals. In Proceedings of the Australian Coral Reef Society 75th Anniversary Conference (pp. 217-230). School of Marine Science, The University of Queensland Brisbane, Australia.

Salvat, F., & Salvat, B. (1992). Nukutipipi Atoll, Tuamotu Archipelago; geomorphology, land and marine flora and fauna and interrelationships. Atoll Research Bulletin, 357, 1–43.

Sampayo, E. M., Ridgway, T., Bongaerts, P., & Hoegh-Guldberg, O. (2008). Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proceedings of the National Academy of Sciences, 105(30), 10444-10449.

Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. Plant Physiology, 101, 7.

Schaffer, W. M. (1974). Optimal reproductive effort in fluctuating environments. The American Naturalist, 108(964), 783-790.

Scheffer, M., Barrett, S., Carpenter, S. R., Folke, C., Green, A. J., Holmgren, M., ... & Van Nes, E. H. (2015). Creating a safe operating space for iconic ecosystems. Science, 347(6228), 1317-1319.

Schleussner, C. F., Lissner, T. K., Fischer, E. M., Wohland, J., Perrette, M., Golly, A., ... & Mengel, M. (2016). Differential climate impacts for policy-relevant limits to global warming: the case of 1.5 °C and 2 °C. Earth System Dynamics, 7, 327-351.

Schoepf, V., Grottoli, A. G., Levas, S. J., Aschaffenburg, M. D., Baumann, J. H., Matsui, Y., & Warner, M. E. (2015). Annual coral bleaching and the long-term recovery capacity of coral. Proceedings of the Royal Society B: Biological Sciences, 282(1819), 20151887.

Scott, A., Harrison, P. L., & Brooks, L. O. (2013). Reduced salinity decreases the fertilization success and larval survival of two scleractinian coral species. Marine Environmental Research, 92, 10-14.

Scott, C. M., Mehrotra, R., & Urgell, P. (2015). Spawning observation of *Acanthaster planci* in the Gulf of Thailand. Marine Biodiversity, 45(4), 621-622.

Shaver, E. C., Burkepile, D. E., & Silliman, B. R. (2018). Local management actions can increase coral resilience to thermally-induced bleaching. Nature Ecology & Evolution, 2(7), 1075.

Shenkar, N., Fine, M., & Loya, Y. (2005). Size matters: bleaching dynamics of the coral *Oculina patagonica*. Marine Ecology Progress Series, 294, 181-188.

Sheppard, C. R. C., Harris, A., & Sheppard, A. L. S. (2008). Archipelago-wide coral recovery patterns since 1998 in the Chagos Archipelago, central Indian Ocean. Marine Ecology Progress Series, 362, 109-117.

Sheppard, C., Sheppard, A., Mogg, A., Bayley, D., Dempsey, A. C., Roche, R., ... & Purkins, S. (2017). Coral bleaching and mortality in the Chagos Archipelago. Atoll Research Bulletin, 613, 1-26.

Shick, J. M., & Dunlap, W. C. (2002). Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annual Review of Physiology, 64(1), 223-262.

Shinn, E. A. (1976). Coral reef recovery in Florida and the Persian Gulf. Environmental Geology, 1(4), 241.

Shinzato, C., Shoguchi, E., Kawashima, T., Hamada, M., Hisata, K., Tanaka, M., ... & Fujiyama, A. (2011). Using the *Acropora digitifera* genome to understand coral responses to environmental change. Nature, 476(7360), 320.

Shlesinger, T., & Loya, Y. (2016). Recruitment, mortality, and resilience potential of scleractinian corals at Eilat, Red Sea. Coral Reefs, 35(4), 1357-1368.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. Experimental Physiology: Translation and Integration, 82(2), 291-295.

Silverstein, R. N., Correa, A. M., & Baker, A. C. (2012). Specificity is rarely absolute in coral–algal symbiosis: implications for coral response to climate change. Proceedings of the Royal Society B: Biological Sciences, 279(1738), 2609-2618.

Silverstein, R. N., Correa, A. M., LaJeunesse, T. C., & Baker, A. C. (2011). Novel algal symbiont (*Symbiodinium* spp.) diversity in reef corals of Western Australia. Marine Ecology Progress Series, 422, 63-75.

Solayan, A. (2016). Biomonitoring of Coral Bleaching-A Glimpse on Biomarkers for the Early Detection of Oxidative Damages in Corals. Invertebrates-Experimental Models in Toxicity Screening, 101.

Sousa, W. P. (1979). Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. Ecology, 60(6), 1225-1239.

Spalding, M. D., & Brown, B. E. (2015). Warm-water coral reefs and climate change. Science, 350(6262), 769-771.

Spalding M. D., Ravilious, C., Green, E. P. (2001). World Atlas of Coral Reefs. United Nations Environment Programme, World Conservation Monitoring Centre. University of California Press: Berkeley. 416pp.

Stafford-Smith, M. G. (1993). Sediment-rejection efficiency of 22 species of Australian scleractinian corals. Marine Biology, 115(2), 229-243.

Stanley, G. D. (2006). Photosymbiosis and the evolution of modern coral reefs. Science, 312(5775), 857-858.

Steen, R. G., & Muscatine, L. (1987). Low temperature evokes rapid exocytosis of symbiotic algae by a sea anemone. The Biological Bulletin, 172(2), 246-263.

Stoddart, D. R. (1969). Ecology and morphology of recent coral reefs. Biological Reviews, 44(4), 433-498.

Stoddart, D. R. (1974). Post-hurricane changes on the British Honduras reefs: re-survey of 1972. In Proceedings of the Second International Symposium on Coral Reefs. (Vol. 2).

Stone, L., A., Rajagopalan, B., Bhasin, H., & Loya, Y. (1999). Mass coral reef bleaching: a recent outcome of increased El Niño activity?. Ecology Letters, 2(5), 325-330.

Stuart-Smith, R. D., Brown, C. J., Ceccarelli, D. M., & Edgar, G. J. (2018). Ecosystem restructuring along the Great Barrier Reef following mass coral bleaching. Nature, 560(7716), 92.

Sully, S., Burkepile, D. E., Donovan, M. K., Hodgson, G., & Van Woesik, R. (2019). A global analysis of coral bleaching over the past two decades. Nature Communications, 10, 1264.

Suzuki, A., & Kawahata, H. (2003). Carbon budget of coral reef systems: an overview of observations in fringing reefs, barrier reefs and atolls in the Indo-Pacific regions. Tellus B: Chemical and Physical Meteorology, 55(2), 428-444.

Szmant, A., & Gassman, N. J. (1990). The effects of prolonged “bleaching” on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs, 8(4), 217-224.

Tanner, J. E. (2017). Multi-decadal analysis reveals contrasting patterns of resilience and decline in coral assemblages. Coral Reefs, 36(4), 1225-1233.

Tanzil, J. T. I., Brown, B. E., Tudhope, A. W., & Dunne, R. P. (2009). Decline in skeletal growth of the coral *Porites lutea* from the Andaman Sea, South Thailand between 1984 and 2005. Coral Reefs, 28(2), 519-528.

Tebbett, S. B., Streit, R. P., & Bellwood, D. R. (2019). Expansion of a colonial ascidian following consecutive mass coral bleaching at Lizard Island, Australia. Marine Environmental Research, 144, 125-129.

Teixeira, T., Diniz, M., Calado, R., & Rosa, R. (2013). Coral physiological adaptations to air exposure: heat shock and oxidative stress responses in *Veretillum cynomorium*. Journal of Experimental Marine Biology and Ecology, 439, 35-41.

Thompson, D. M., & Van Woesik, R. (2009). Corals escape bleaching in regions that recently and historically experienced frequent thermal stress. Proceedings of the Royal Society B: Biological Sciences, 276(1669), 2893-2901.

Titlyanov, E. A., Yakovleva, I. M., & Titlyanova, T. V. (2007). Interaction between benthic algae (*Lyngbya bouillonii*, *Dictyota dichotoma*) and scleractinian coral *Porites lutea* in direct contact. Journal of Experimental Marine Biology and Ecology, 342(2), 282-291.

Titlyanov, E., Bil, K., Fomina, I., Titlyanova, T., Leletkin, V., Eden, N., ... & Dubinsky, Z. (2000). Effects of dissolved ammonium addition and host feeding with *Artemia salina* on photoacclimation of the hermatypic coral *Stylophora pistillata*. Marine Biology, 137(3), 463-472.

Tong, H., Cai, L., Zhou, G., Yuan, T., Zhang, W., Tian, R., ... & Qian, P. Y. (2017). Temperature shapes coral-algal symbiosis in the South China Sea. Scientific Reports, 7, 40118.

Torda, G., Sambrook, K., Cross, P., Sato, Y., Bourne, D. G., Lukoschek, V., ... & Willis, B. L. (2018). Decadal erosion of coral assemblages by multiple disturbances in the Palm Islands, central Great Barrier Reef. Scientific Reports, 8(1), 11885.

Torres, M. A., Jones, J. D., & Dangl, J. L. (2006). Reactive oxygen species signaling in response to pathogens. Plant Physiology, 141(2), 373-378.

True, J. D. (2012, July). Salinity as a structuring force for near shore coral communities. In Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia (Vol. 9, p. e13).

Tsang, R. H. L., & Ang, P. (2015). Cold temperature stress and predation effects on corals: their possible roles in structuring a nonreefal coral community. Coral Reefs, 34(1), 97-108.

Tunnicliffe, V. (1981). Breakage and propagation of the stony coral *Acropora cervicornis*. Proceedings of the National Academy of Sciences, 78(4), 2427-2431.

Turak, E., & DeVantier, L. (2012). Biodiversity and Conservation Priorities of Reef-building Corals in Timor Leste, with focus on the Nino Konis Santana National Marine Park.

Van Dam, J. W., Uthicke, S., Beltran, V. H., Mueller, J. F., & Negri, A. P. (2015). Combined thermal and herbicide stress in functionally diverse coral symbionts. Environmental Pollution, 204, 271-279.

Van Hooidonk, R., Maynard, J. A., & Planes, S. (2013). Temporary refugia for coral reefs in a warming world. Nature Climate Change, 3(5), 508.

Van Hooidonk, R., Maynard, J. A., Manzello, D., & Planes, S. (2014). Opposite latitudinal gradients in projected ocean acidification and bleaching impacts on coral reefs. Global Change Biology, 20(1), 103-112.

Van Hooidonk, R., Maynard, J., Tamelander, J., Gove, J., Ahmadia, G., Raymundo, L., ... & Planes, S. (2016). Local-scale projections of coral reef futures and implications of the Paris Agreement. Scientific Reports, 6, 39666.

Van Oppen, M. J., Gates, R. D., Blackall, L. L., Cantin, N., Chakravarti, L. J., Chan, W. Y., ... & Harrison, P. L. (2017). Shifting paradigms in restoration of the world's coral reefs. Global Change Biology, 23(9), 3437-3448.

Van Woesik, R. (1992). Ecology of coral assemblages on continental islands in the southern section of the Great Barrier Reef, Australia (Doctoral dissertation, James Cook University).

Van Woesik, R., Ayling, A. M., & Mapstone, B. (1991). Impact of tropical cyclone'Ivor'on the Great Barrier Reef, Australia. Journal of Coastal Research, 7(2), 551-558.

Van Woesik, R., DeVantier, L. M., & Glazebrook, J. S. (1995). Effects of Cyclone\'Joy\'on nearshore coral communities of the Great Barrier Reef. Marine Ecology Progress Series, 128, 261-270.

Van Woesik, R., Sakai, K., Ganase, A., & Loya, Y. (2011). Revisiting the winners and the losers a decade after coral bleaching. Marine Ecology Progress Series, 434, 67-76.

Van Woesik, R., Irikawa, A., Anzai, R., & Nakamura, T. (2012). Effects of coral colony morphologies on mass transfer and susceptibility to thermal stress. Coral Reefs, 31(3), 633-639.

Vargas-Ángel, B., Huntington, B., Brainard, R. E., Venegas, R., Oliver, T., Barkley, H., & Cohen, A. (2019). El Niño-associated catastrophic coral mortality at Jarvis Island, central Equatorial Pacific. Coral Reefs, 38(4), 731-741.

Vernberg, W. B., & Vernberg, F. J. (1972). The synergistic effects of temperature, salinity, and mercury on survival and metabolism of the adult fiddler crab, Uca pugilator. Fishery Bulletin, (2).

Viarengo, A., Lafaurie, M., Gabrielides, G. P., Fabbri, R., Marro, A., & Romeo, M. (2000). Critical evaluation of an intercalibration exercise undertaken in the framework of the MED POL biomonitoring program. Marine Environmental Research, 49(1), 1-18.

Vinagre, C., Dias, M., Cereja, R., Abreu-Afonso, F., Flores, A. A., & Mendonça, V. (2019). Upper thermal limits and warming safety margins of coastal marine species–Indicator baseline for future reference. Ecological Indicators, 102, 644-649.

Vinagre, C., Mendonca, V., Cereja, R., Abreu-Afonso, F., Dias, M., Mizrahi, D., & Flores, A. A. (2018). Ecological traps in shallow coastal waters—Potential effect of heat-waves in tropical and temperate organisms. PloS One, 13(2), e0192700.

Voolstra, C. R., Sunagawa, S., Matz, M. V., Bayer, T., Aranda, M., Buschiazzo, E., ... & Medina, M. (2011). Rapid evolution of coral proteins responsible for interaction with the environment. PloS One, 6(5), e20392.

Wafar, M. V. M. (1990). Global warming and coral reefs. Proceedings of Sea Level Variation, 411-432.

Wagner, D. E., Kramer, P., & Van Woesik, R. (2010). Species composition, habitat, and water quality influence coral bleaching in southern Florida. Marine Ecology Progress Series, 408, 65-78.

Wakefield, T. S., & Kempf, S. C. (2001). Development of host-and symbiont-specific monoclonal antibodies and confirmation of the origin of the symbiosome membrane in a cnidarian–dinoflagellate symbiosis. The Biological Bulletin, 200(2), 127-143.

Wall, M., Putchim, L., Schmidt, G. M., Jantzen, C., Khokiattiwong, S., & Richter, C. (2015). Large-amplitude internal waves benefit corals during thermal stress. Proceedings of the Royal Society B: Biological Sciences, 282(1799), 20140650.

Wallace, C. C. (1985). Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora*. Marine Biology, 88(3), 217-233.

Wallace, C. C. (1999). The Togian Islands: coral reefs with a unique coral fauna and an hypothesised Tethys Sea signature. Coral Reefs, 18(2), 162-162.

Wang, J. T., & Douglas, A. E. (1999). Essential amino acid synthesis and nitrogen recycling in an alga–invertebrate symbiosis. Marine Biology, 135(2), 219-222.

Ward, S., Harrison, P., & Hoegh-Guldberg, O. (2002, January). Coral bleaching reduces reproduction of scleractinian corals and increases susceptibility to future stress. In Proceedings of the 9th International Coral Reef Symposium, Bali, 23-27 October 2000, (Vol. 2, pp. 1123-1128).

Warner, M. E., Fitt, W. K., & 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 & Environment, 19(3), 291-299.

Watson R. T., and Core Writing Team, Eds., 3rd Assessment Report of the Inter-Governmental Panel on Climate Change. Climate Change 2001: Synthesis Report.

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. Journal of Experimental Biology, 211(19), 3059-3066.

Wellington, G. M., Glynn, P. W., Strong, A. E., Navarrete, S. A., Wieters, E., & Hubbard, D. (2001). Crisis on coral reefs linked to climate change. Eos, Transactions American Geophysical Union, 82(1), 1-5.

West, J. M., & Salm, R. V. (2003). Resistance and resilience to coral bleaching: implications for coral reef conservation and management. Conservation Biology, 17(4), 956-967.

Weston, A. J., Dunlap, W. C., Beltran, V. H., Starcevic, A., Hranueli, D., Ward, M., & Long, P. F. (2015). Proteomics links the redox state to calcium signaling during bleaching of the scleractinian coral *Acropora microphthalma* on exposure to high solar irradiance and thermal stress. Molecular & Cellular Proteomics, 14(3), 585-595.

Wiedenmann, J., D'Angelo, C., Smith, E. G., Hunt, A. N., Legiret, F. E., Postle, A. D., & Achterberg, E. P. (2013). Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nature Climate Change, 3(2), 160.

Wild, C., Hoegh-Guldberg, O., Naumann, M. S., Colombo-Pallotta, M. F., Ateweberhan, M., Fitt, W. K., ... & Loya, Y. (2011). Climate change impedes scleractinian corals as primary reef ecosystem engineers. Marine and Freshwater Research, 62(2), 205-215.

Wilkinson, C. C. (2000). World-wide coral reef bleaching and mortality during 1998: A global climate change warning for the new millennium?. In Seas at the millennium: an environmental evaluation-pages: 43-57.

Williams Jr, E. H., & Bunkley-Williams, L. (1990). The world-wide coral reef bleaching cycle and related sources of coral mortality. Atoll Research Bulletin, 335, 1-71.

Wilson, S. K., Burgess, S. C., Cheal, A. J., Emslie, M., Fisher, R., Miller, I., ... & Sweatman, H. P. (2008). Habitat utilization by coral reef fish: implications for specialists vs. generalists in a changing environment. Journal of Animal Ecology, 77(2), 220-228.

Wise, R. R. (1995). Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light. Photosynthesis Research, 45(2), 79-97.

Wolanski, E. E. (1981, January). Aspects of physical oceanography of the Great Barrier Reef lagoon. In The Reef and Man. Proceedings of the 4th International Coral Reef Symposium, Manila Philippines, 18-22 May 1981-pages: 1: 375-381. Marine Sciences Center, University of the Philippines.

Wooldridge, S. A. (2009). A new conceptual model for the enhanced release of mucus in symbiotic reef corals during ‘bleaching’conditions. Marine Ecology Progress Series, 396, 145-152.

Wooldridge, S. A. (2014). Differential thermal bleaching susceptibilities amongst coral taxa: re-posing the role of the host. Coral Reefs, 33(1), 15-27.

Yakovleva, I. M., Baird, A. H., Yamamoto, H. H., Bhagooli, R., Nonaka, M., & Hidaka, M. (2009). Algal symbionts increase oxidative damage and death in coral larvae at high temperatures. Marine Ecology Progress Series, 378, 105-112.

Yamazato, K. (1981). A note on the expulsion of zooxanthellae during summer, 1980 by the Okinawan reef-building corals. Sesoko Marine Science Laboratory technical report, 8, 9-18.

Yellowlees, D., Rees, T. A. V., & Leggat, W. (2008). Metabolic interactions between algal symbionts and invertebrate hosts. Plant, Cell & Environment, 31(5), 679-694.

Yu, B. P. (1994). Cellular defenses against damage from reactive oxygen species. Physiological Reviews, 74(1), 139-162.

Zhao, M. X., Yu, K. F., Shi, Q., Chen, T. R., Zhang, H. L., & Chen, T. G. (2013). Coral communities of the remote atoll reefs in the Nansha Islands, southern South China Sea. Environmental Monitoring and Assessment, 185(9), 7381-7392.

CHAPTER 2

Mortality, growth and regeneration following fragmentation of reef-forming corals under thermal stress

Dias, M., Ferreira, A., Gouveia, R., Cereja, R., & Vinagre, C. (2018). Journal of Sea Research, 141, 71-82. DOI:10.1016/j.seares.2018.08.008.

Abstract

Storms inflict damage to corals resulting in fragments that have the potential to regenerate thus contributing to the asexual reproduction of the parental colony. Extreme climatic events like these are predicted to increase in the future due to ocean warming, which is also the primary cause of coral reef bleaching and consequent coral mortality in the tropical and subtropical seas. This way it is urgent to investigate the differential effect of warming over post-fragmentation and regeneration processes among the scleractinian hermatypic coral species. This study investigated the mortality, growth and regeneration capacity of nine reef-forming coral species of the Indo-Pacific. Fragments were exposed to 26 °C, 30 °C, and 32 °C for 60 days. Half of these fragments was inflicted with one injury and the other half was used as control. Mortality, partial mortality, bleaching level, growth and regeneration of artificial injuries were assessed. Mortality increased with temperature, reaching 100% for most species after 60 days, at 32 ºC, but *Psammocora contigua* which showed remarkably lower mortality (40%) and all coral fragments of *Turbinaria reniformis* and *Galaxea fascicularis* survived the experiment. Partial mortality was lowest for *P. contigua, T. reniformis*, and *G. fascicularis* even at 32 ºC. These three coral species were also the most resistant to bleaching. Growth rates decreased with temperature, with the exception of *G. fascicularis* that maintained similar growth rates at 26 ºC and 30 ºC. Regeneration rates generally increased with temperature. It was concluded that *P. contigua, T. reniformis,* and *G. fascicularis* fragments show higher capacity to withstand higher temperatures.

Keywords: global climate change, thermal stress, coral regeneration, bleaching.

2.1. Introduction and literature review

Corals are dominant species of tropical coral reef ecosystems and have a unique and complex symbiotic relationship with dinoflagellate microalgae (zooxanthellae), contained within their gastrodermal cells (Hoegh-Guldberg, 1999). The ability of scleractinian corals to deposit calcium carbonate skeletons and to generate the physically complex reef structure is attributed to these dinoflagellates (Meehan and Ostrander, 1997).

Global climate change is leading to both rising sea surface temperatures and ocean acidification, jeopardizing coral reefs survival (Carpenter et al., 2008; Padilla-Gamiño et al., 2013). However, it has been shown by recent studies that the warming of tropical oceans is a much more imminent threat to coral reefs' survival than is ocean acidification (e.g. Chua et al., 2013; Frieler et al., 2013). The reef-building corals that undergo bleaching have reduced growth rates and reproductive capacity (Szmant and Gassman, 1990; Baird and Marshall, 2002), impaired healing (Meesters and Bak, 1993), and increased susceptibility to disease (Harvell et al., 1999). Bleaching makes the host organism white due to a loss of symbionts, which allows the underlying skeleton to be visible (Baker et al., 2008). If thermal stress is sustained, this may result in widespread coral mortality (Szmant and Gassman, 1990; Brown et al., 2002). Mass bleaching episodes have the potential to dramatically change coral community structure (Gleason, 1993; Glynn, 1993) and in severe cases cause population collapse and local extinction (Aronson et al., 2000).

An increase in the frequency and intensity of bleaching events is expected (Eakin et al., 2009; Heron et al., 2016), given that sea temperatures surrounding coral reefs are projected to increase by 1 - 3.7 ℃ by the year 2100 (IPCC, 2014). Globally, thermally induced bleaching due to climate change was predicted to occur annually in most oceans by 2040 (Hoegh-Guldberg, 1999; Crabbe, 2008; Van Hooidonk and Huber, 2009). Associated with ongoing increases of tropical sea surface temperatures (SST) are increases in the frequency and maximum intensity of categories 4 and 5 storms (0–25%), and increases in tropical cyclones rainfall rates (5–20%) (Christensen et al., 2013; IPCC, 2014).

Due to wave action (Stimson, 1978), storm surge (Randall and Eldredge, 1977), and touristic activities (e.g. diving, snorkeling, and trampling, Davenport and Davenport, 2006) coral fragments may become detached from parent colonies and disperse across the reef (Highsmith,

1982). Increased storminess should favor the ability to propagate effectively by fragmentation (Lasker, 1990). Many branching corals are routinely broken and scattered about during storms (e.g., Highsmith, 1980; Tunnicliffe, 1981). Asexual reproduction by fragmentation of plating and massive coral forms has also been noticed (Foster et al., 2007).

Fragmentation of established colonies resulting in the formation of new coral colonies is known as an extremely important asexual mode of reproduction for many of the major reef-building corals (Highsmith, 1982; Bruno, 1998). Fragmentation by corals with high growth rates results in their domination of certain reef zones (Tunnicliffe, 1981), rapid growth of reefs on which these corals are abundant (Glynn et al., 1994), and rapid recovery from disturbances (Shinn, 1976; Glynn and Fong, 2006). Fragmentation may be adaptive (Cook, 1979; Highsmith et al., 1980; Highsmith, 1982), given that a considerable number of the most successful corals have incorporated fragmentation into their life histories. Asexual reproduction is probably the main process involved in the origin of new coral reefs (Glynn, 1993).

There is interspecific variability in reef-building corals' susceptibility to increased temperature (McClanahan et al., 2007; Seveso et al., 2014). Their susceptibility depends on colony morphotype (Brandt, 2009), tissue thickness (Loya et al., 2001), colony size (Shenkar et al., 2005), the capacity to transfer mass and heat (Van Woesik and Jordán-Garza, 2011), coral species (Hoegh-Guldberg and Salvat, 1995; Marshall and Baird, 2000), genetic variation between coral populations from widely separated geographic regions (Coles et al., 1976; Glynn et al., 1988; Rowan and Knowlton, 1995), and the genetic constitution of the symbiotic microalgae (*Symbiodinium* spp.) (Rowan et al., 1997).

In many coral bleaching reports, there is noticeable variation in the extent of bleaching, as some colonies remain pigmented but adjacent ones of the same or different species undergo bleaching (Rowan et al., 1997; Baker et al., 2008; Montano et al., 2010). These different susceptibilities lead to major structural shifts in coral communities (Ostrander et al., 2000; Aronson et al., 2004), where hardier corals (i.e. massive and encrusting thick-tissued species) will eventually replace less resilient corals (i.e. branched and thin-tissued species) (Kayanne et al., 1999; Van Woesik et al., 2011).

The aim of this work was to evaluate how elevated temperatures will affect the mortality, growth and regeneration after fragmentation of an important number of reef-building corals of

the Indo-Pacific oceans. Such an evaluation is crucial to understand the differential vulnerability of reef-forming coral species to global climate change.

2.2. Materials and methods

2.2.1. Study species

This study evaluated nine Indo-specific coral species with contrasting morphologies: four branching species (*Acropora tenuis, Pocillopora damicornis, Stylophora pistillata,* and *Psammocora contigua),* three plating species, (*Montipora capricornis* brown morphotype (BM), *Turbinaria reniformis,* and *Echinopora lamellosa),* one encrusting species (*Montipora capricornis* green morphotype (GM)), and one massive species (*Galaxea fascicularis).* All coral colonies used in this study have been kept in captivity at Oceanário de Lisboa (Portugal) for several years, which gave us knowledge on their thermal history. These coral species were chosen in order to use the largest number of species available at Oceanário de Lisboa with different levels of bleaching susceptibility: severe (*A. tenuis*, *P. damicornis*, and *S. pistillata*), high (*M. capricornis*), moderate (*E. lamellosa*), and low (*T. reniformis, G. fascicularis*, and *P. contigua*) (Marshall and Baird, 2000), and different colony morphology, a characteristic that has been proven to have influence in coral species susceptibility to thermal stress (Loya et al., 2001). Coral species identification was made according to Veron (2000).

2.2.2. Acclimation conditions and experimental setup

The experiments were conducted at Oceanário de Lisboa, Portugal (www.oceanario.pt). Twenty replicate fragments were cut from each coral colony using a pincer or a pair of pliers. For the branching coral colonies the fragments were cut approximately 20-40 mm in length and the fragments for the plate, encrusting and massive corals were obtained by cutting approximately 30 mm sided squares. A single colony per coral species was targeted in order to eliminate sources of variation from other factors that affect thermal susceptibility (DeSalvo et al., 2008) such as tissue thickness (Loya et al., 2001), genetic constitution of the symbiotic microalgae (*Symbiodinium* spp.) (Rowan et al., 1997), metabolic rates (Gates and Edmunds, 1999), mucus production rates (Fitt et al., 2009), tissue concentration of fluorescent pigments (Salih et al., 1998), and thermal history (Brown et al., 2002). All fragments were placed over egg crate panels in the coral stock aquarium until acclimation to the experimental aquarium.

The live wet mass of each coral fragment was obtained by blotting it with a paper towel to remove excess seawater, then weighing it in air on an electronic balance to the nearest 0.01 g (Titlyanov et al., 2005). Each fragment was glued with epoxy putty to the top of a pre-weighed and numbered nylon expansion anchor. Placement of the fragment varied by morphology with the branching fragments in vertical position and the plating, encrusting and massive fragments placed in horizontal positions. Then, the set (coral fragment + anchor) was weighed to remove the epoxy putty weight off the calculations and placed back over egg crate panels in the coral stock aquarium.

After one day in the coral stock aquarium, the sets were acclimated 1 °C per hour above the temperature of the coral stock aquarium (25.1 ± 0.4). Coral reef-flat communities can experience temperature changes of 1 °C $hour^{-1}$ during spring tides (Berkelmans and Willis, 1999), and most of the coral species in this study colonize the reef-flat zone (Brown and Suharsono, 1990), so we used this heating rate to be similar to the conditions that most of these corals would experience in their natural environment. In order to standardize, this heating rate was applied for all the coral species. The coral fragments were placed 2 cm apart from one another and arranged by coral species. Fragments were exposed to three temperature treatments: control 26 °C (26.1± 0.2 SD), 30 °C (30.2 ± 0.5 SD) and 32 °C (32.2 ± 0.5 SD) seawater temperatures during sixty days, with the duration of one, five and seven hours of acclimation, respectively.

Ten coral fragments of each species were used as controls (undamaged) and the other ten fragments were inflicted with circular injuries designed to simulate damage by predators (Fig. SM2.1 in supplementary material), after being acclimated to the experimental aquarium. These artificial injuries were 3 mm in diameter and were done using a Dremel rotatory tool with a cutting disk. Only one injury was inflicted to each coral fragment in their middle section to avoid edge effects. After these procedures, all fragments were placed over two 40 x 40 cm egg crate panels suspended 15 cm below the water surface of the experimental aquarium.

The experimental aquarium (400 L) was fitted with a sump (280L) filled with bioballs for biological filtration in which two Fluval M300 heaters, as well as a Hailea 500 chiller controlled water temperature. For water circulation purposes, an AquaMedic OceanRunner 3500 pump

provided a turnover rate of 5 times per hour. An AquaMedic Turboflotor 5000 Shorty protein skimmer helped keeping nutrient concentrations low and increased surface water motion in the aquarium was accomplished by using an AquaClear 110 powerhead. Lighting requirements similar to the coral stock aquarium were attained by using a Litpa Jet5 floodlight with an AquaMedic 400W HQi lamp (13000K) on a 12 h light/ 12 h dark cycle. An air-stone was used in the aquarium to ensure good oxygen concentrations.

Photosynthetically Active Radiation (PAR) levels were measured with a spheric quantic sensor (LI-193SA) and a data logger (1400 model) and varied between 320-345 $\mu E.m^{-2}s^{-1}$ in the 400-700 nm waveband. Water quality parameters such as water temperature, pH and salinity were measured on a daily basis. Water samples were also weekly analyzed to determine ammonium, nitrites, nitrates, calcium concentration as well as oxygen concentration and saturation and alkalinity. These parameters were maintained as follow: pH at 8.2-8.3, salinity at 33-33.5 psu, alkalinity approximately at 100 mg l^{-1}, nitrites between 0.002 and 0.005 mg l^{-1}, nitrates between 0 and 2 mg l^{-1}, calcium concentration between 389 and 401 mg l^{-1}, oxygen saturation at 104% and oxygen concentration between 6.5 and 7.1 mg l^{-1}. Salinity was maintained with daily balanced additions of reverse osmosis freshwater and filtered artificial seawater. Whenever alkalinity levels were below 100 mg l^{-1} sodium bicarbonate was added to the system. Aquarium cleaning routines were done as required to avoid algal growth, and these included expansion anchors cleaning with a toothbrush and egg crate replacement, at least 3 times a week.

2.2.3. Analytical procedures

2.2.3.1. Mortality assessments and bleaching level

Mortality was quantified as the percentage of dead fragments and partial mortality was visually quantified by estimating the percentage of dead area in the coral fragments and put it into four classes: [0,25%[, [25,50%[, [50,75%[and [75,100%]. The bleaching level of each coral species was visually assessed according to four categories: normal, pale, bleached and dead (Jokiel and Coles, 1974). Mortality and bleaching were assessed every 20 days until the end of the experiment and always by the same person to remove observer bias.

2.2.3.2. Growth rate measurements

The coral fragments were weighted both in the first and last day of the experiment in order to calculate the growth rates for each coral fragment. The growth rate of the coral fragments was calculated using the formula 2.1:

$$\mu = ((m_1 - m_0) / (m_0 \times \Delta t)) \times 100$$

where, μ is the growth rate measured in % day^{-1}, m_0 is initial weight, m_1 is the weight at the end of the experiment, and Δt is the time between the two measurements of weight (Brinkhuis, 1985).

2.2.3.3. Regeneration rate of injuries

The size of the injury was measured immediately after their infliction and then every 20 days until the end of the experiment. Injury recovery was quantified by recording the diameter of each injury with a steel caliper calibrated in millimeters. The tissue regeneration rates (Ts) were obtained by calculating, the difference between the areas of the recovered surfaces (Ra) for any given interval (T1 and T0 in days), as follows (Cróquer et al., 2002), formula 2.2:

$$Ts = [Ra(T_1) - Ra(T_0)]/(T_1 - T_0)$$

2.2.4. Statistical analyses

A permutational multivariate analysis of variance (PERMANOVA) based on Euclidean distances (Anderson, 2001) was used to test whether coral fragments' mortality, partial mortality, growth and regeneration rates were affected by temperature and coral species. PERMANOVA was also used to test whether coral fragments' mortality, partial mortality and growth rate were affected by the presence/absence of injury. Values of the pseudo-F statistic were computed using 9999 permutations. Analyses were performed using PERMANOVA+ for PRIMER v6 (PRIMER-E Ltd., Plymouth). As PERMANOVA is based on permutations it is more robust to the assumptions of ANOVA (Anderson, 2001). Post-hoc pair-wise comparisons were then performed using PERMANOVA to compare between temperature experiments and coral species. Differences were considered significant at $p < 0.05$.

2.3. Results

The presence/absence of injury had no effect on the mortality, partial mortality and growth rate of the fragment (PERMANOVA $p > 0.05$, Table 2.1) and therefore it was not considered as a factor in further analysis, with no separation between control and injured fragments.

Table 2.1. Summary of results of PERMANOVA permutation tests applied to report the effects of temperature, coral species and presence/absence of injury in coral fragments' mortality, partial mortality, growth and regeneration rates. Significant differences are marked a bold.

	df	SS	MS	Pseudo-F	$p_{(perm)}$
		Mortality			
Temperature	2	6.9×10^{5}	3.5×10^{5}	5.98×10^{2}	**0.0001**
Coral species	8	5.4×10^{5}	6.7×10^{4}	1.17×10^{2}	**0.0001**
Injury	1	3.9×10^{2}	3.9×10^{2}	0.67	0.5537
Temperature × Coral species	16	6.3×10^{5}	4.0×10^{4}	68.43	**0.0001**
Temperature × Injury	2	3.0×10^{3}	1.5×10^{2}	2.59	**0.0193**
Coral species × Injury	8	6.8×10^{3}	8.5×10^{2}	1.50	0.0749
Temperature × Coral species × Injury	16	2.6×10^{4}	1.6×10^{3}	2.85	**0.0001**
		Partial mortality			
Temperature	2	6.6×10^{5}	3.3×10^{5}	4.80×10^{2}	**0.0001**
Coral species	8	5.3×10^{5}	6.6×10^{4}	96.10	**0.0001**
Injury	1	1.2×10^{3}	1.2×10^{3}	1.72	0.1610
Temperature × Coral species	16	5.7×10^{5}	3.6×10^{4}	51.93	**0.0001**
Temperature × Injury	2	1.9×10^{3}	9.5×10^{2}	1.37	0.2282
Coral species × Injury	8	6.3×10^{3}	7.9×10^{2}	1.14	0.2918
Temperature × Coral species × Injury	16	2.0×10^{4}	1.3×10^{3}	1.89	**0.0009**
		Growth rate			
Temperature	2	26.0	13.0	3.21×10^{2}	**0.0001**
Coral species	8	25.0	3.1	77.35	**0.0001**
Injury	1	7.9×10^{-4}	7.9×10^{-4}	1.94×10^{-2}	0.8929
Temperature × Coral species	8	11.1	1.4	34.38	**0.0001**
Temperature × Injury	2	0.3	0.1	3.36	**0.0367**
Coral species × Injury	8	0.7	8.7×10^{-2}	2.15	**0.0307**
Temperature × Coral species × Injury	8	0.5	6.3×10^{-2}	1.56	0.1376
		Regeneration rate			
Temperature	2	2.7	1.3	26.39	**0.0001**
Coral species	8	6.2	0.8	15.35	**0.0001**
Temperature × Coral species	16	4.1	0.3	5.08	**0.0001**

2.3.1. Mortality and bleaching level

2.3.1.1. Mortality

Mortality was significantly affected by the independent effects of temperature and coral species (PERMANOVA $p < 0.05$, Table 2.1). There was also a significant effect of the interactions of temperature and coral species (PERMANOVA $p < 0.05$, Table 2.1).

Coral fragments' mortality presented significant differences among temperatures (PERMANOVA $p < 0.05$, Table 2.1.), with the exception of *A. tenuis* fragments that showed similar mortality between 26 ºC and 30 ºC (PERMANOVA pair-wise $p > 0.05$, Fig. 2.1a).

The fragments of *A. tenuis* presented mortality at both 26 ºC and 32 ºC, however, at 26 ºC they presented 10% mortality and maintained it stable until the end of the experiment (Fig. 2.1a). At 30 ºC, *A. tenuis* did not show any mortality, whereas at 32 ºC it perished on the 60th day (Fig. 2.1a).

Acropora tenuis, *P. damicornis*, and *S. pistillata* had significantly greater mortality since the 20th day (15% - 25%) than all other coral species at 32 ºC (PERMANOVA pair-wise $p < 0.05$, Fig. 2.1), but did not vary among each other (PERMANOVA pair-wise $p > 0.05$, Fig. 2.1a, g and h).

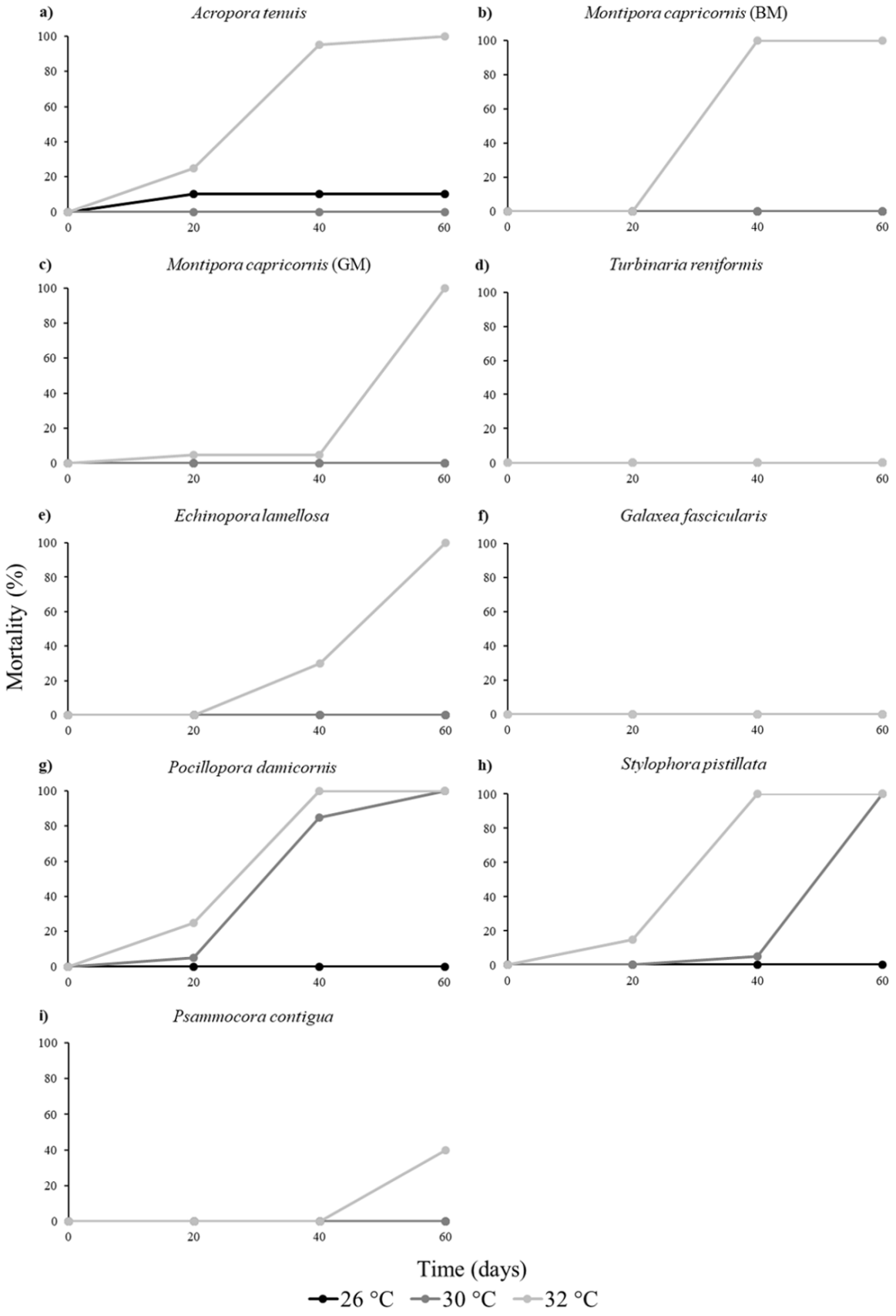

Figure 2.1. Mortality of the coral species' fragments studied throughout the three temperature experiments.

2.3.1.2. Partial mortality

Partial mortality was significantly affected by the independent effects of temperature and coral species (PERMANOVA $p < 0.05$, Table 2.1). There was also a significant effect of the interactions of temperature and coral species (PERMANOVA $p < 0.05$, Table 2.1).

Coral fragments' partial mortality presented significant differences among temperatures (PERMANOVA $p < 0.05$, Table 2.1), with the exceptions of *A. tenuis* and *T. reniformis* fragments. *A. tenuis* showed similar partial mortality between 26 ºC and 30 ºC, whereas *T. reniformis* showed it among all temperatures (PERMANOVA pair-wise $p > 0.05$, Fig. 2.2a and d).

The fragments of *A. tenuis* presented partial mortality in the [75,100] class at both 26 ºC and 32 ºC, however, at 26 ºC they presented very low percentage and maintained it stable until the end of the experiment (Fig. 2.2a). At 30 ºC, *A. tenuis* only started to show partial mortality on the 40th day, whereas at 32 ºC it started on the 20th day and with higher partial mortality (Fig. 2.2a).

Acropora tenuis, *P. damicornis*, and *S. pistillata* had significantly greater partial mortality since the 20th day (20% - 25% in the [75,100] class) than all other coral species at 32 ºC (PERMANOVA pair-wise $p < 0.05$, Fig. 2.2), but did not vary among each other (PERMANOVA pair-wise $p > 0.05$, Fig. 2.2a, g and h).

Montipora capricornis (GM) and *E. lamellosa* fragments presented intermediate levels of partial mortality when compared with the other coral species in study (PERMANOVA pair-wise $p < 0.05$, Fig. 2.2), but did not vary among each other (PERMANOVA pair-wise $p > 0.05$, Fig. 2.2c and e). These coral species presented low percentage of partial mortality in the same class (5% and 30% in the [75,100] class on the 40th day for *M. capricornis* (GM) and *E. lamellosa*, respectively, Fig. 2.2c and e).

Turbinaria reniformis and *G. fascicularis* fragments had significantly less partial mortality than all other coral species at 32 ºC (PERMANOVA pair-wise $p < 0.05$, Fig. 2.2), but did not vary between each other (PERMANOVA pair-wise $p > 0.05$, Fig. 2.2d and f). These coral species presented low percentage of partial mortality in the same class (5% and 15% in the [25,50[class on the 60th day for *T. reniformis* and *G. fascicularis*, respectively, Fig. 2.2d and f).

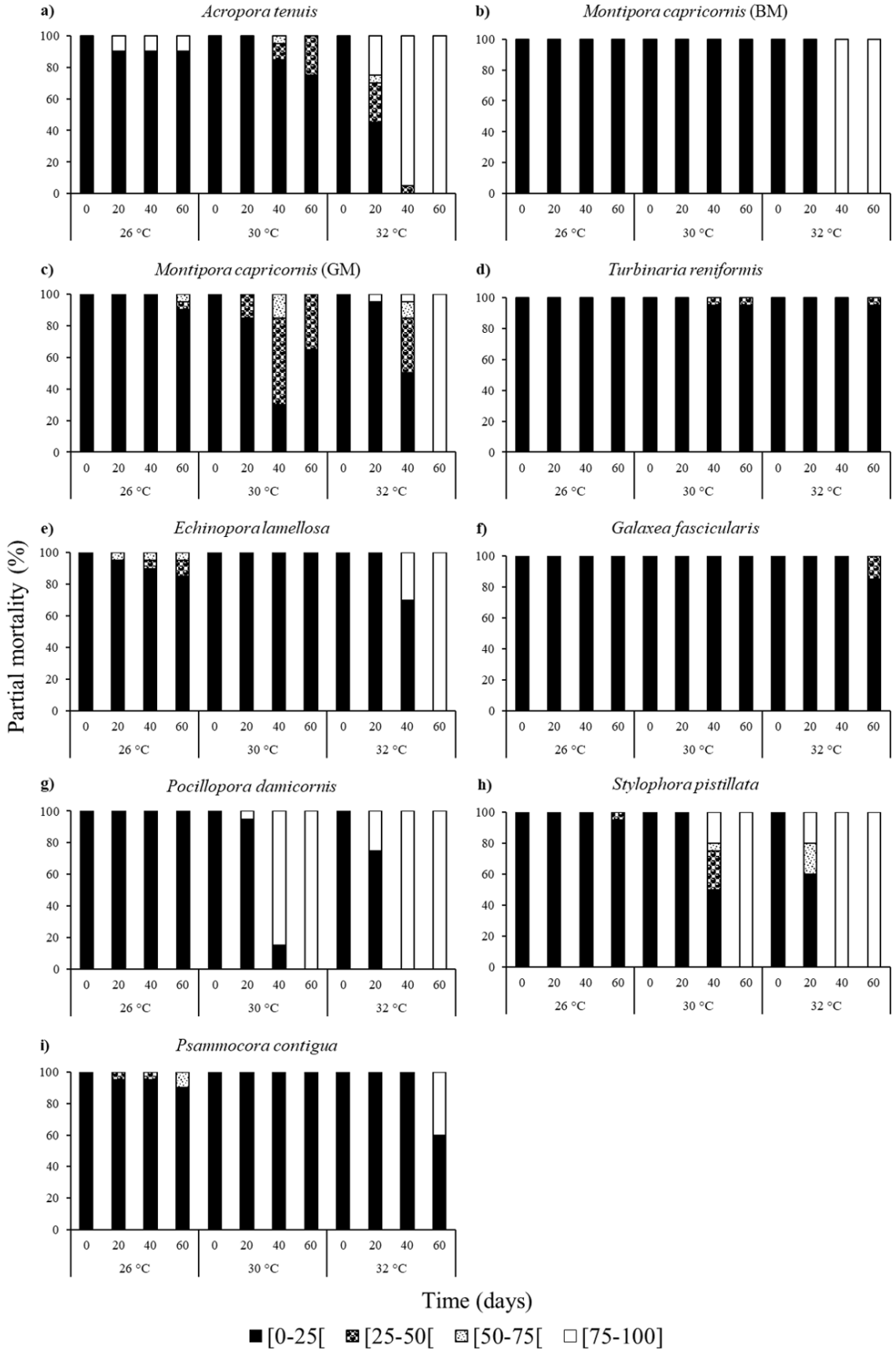

Figure 2.2. Partial mortality of the coral species' fragments studied throughout the three temperature experiments separated in percentage classes [0,25%[, [25,50%[, [50,75%[and [75,100%].

2.3.1.3. Bleaching level

At 26 ºC, almost all the coral species remained with their normal coloration throughout the 60 days experiment (Fig. 2.3). *A. tenuis* was the most sensitive species with 10% of their coral fragments dead by the 20th day of the experiment (Fig. 2.3a).

At 30 °C, the fragments of *A. tenuis*, *M. capricornis* (GM), *E. lamellosa*, and *G. fascicularis* remained normal throughout the experiment (Fig. 2.3a, c, e and f). Seventy five percent of the fragments of *M. capricornis* (BM) and *P. contigua* were pale on the 60th day of experiment, whereas the ones of *T. reniformis* were completely paled on the 40th day (Fig. 2.3b, d and i). A decrease in the percentage of bleached fragments was evident in *P. damicornis* and *S. pistillata* fragments since the 20th day (80% and 5%, respectively) due to them succumbing to mortality that reached the 100% on the 60th day of the experiment (Fig. 2.3g and h).

At 32 ºC, the coral species *A. tenuis*, *M. capricornis* (BM), *M. capricornis* (GM), *E. lamellosa*, *P. damicornis*, *S. pistillata*, and *P. contigua* increased in the bleaching level since the 20th day, whereas the coral species *G. fascicularis* only presented it since the 40th day, with all these species reaching the dead level, exception for *G. fascicularis* (Fig. 2.3a-c and e-i). The coral species *T. reniformis* only reached the pale level (Fig. 2.3d).

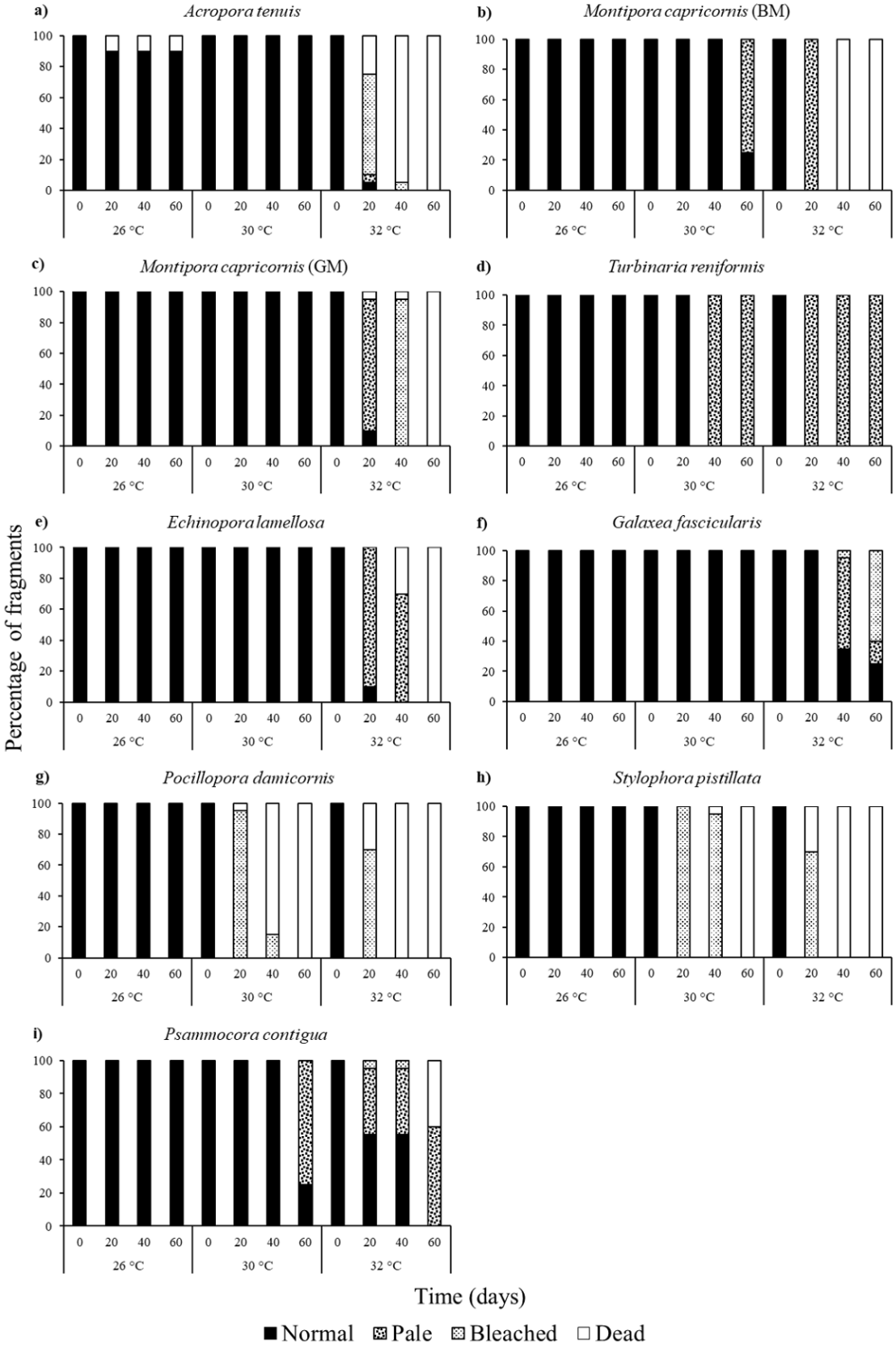

Figure 2.3. Bleaching level of the coral species' fragments studied throughout the three temperature experiments.

2.3.2. Growth rate

In Table 2.2, not available (NA) indicates that the coral fragments of a specific coral species were dead before the end of a given temperature experiment.

Temperature significantly impacted growth rates (PERMANOVA $p < 0.05$, Table 2.1.) with the highest growth rates of all coral species observed at 26 ℃, except for *G. fascicularis.* This species growth rate was similar at 26 ºC and 30 ºC (PERMANOVA pair wise $p > 0.05$; Table 2.2). Each of the other coral species showed significant intraspecific differences among temperatures (PERMANOVA pair wise $p < 0.05$), with the exception of *P. contigua* that had its growth rate impacted in the same way at 30 ºC and 32 ºC (PERMANOVA pair wise $p > 0.05$; Table 2.2).

At 26 ºC, *A. tenuis* and *P. contigua* had the highest growth rates (PERMANOVA pair wise $p < 0.05$) (1.64 ± 0.38% day^{-1} and 1.54 ± 0.32% day^{-1}, respectively; PERMANOVA pair wise $p > 0.05$), whereas *G. fascicularis* displayed the lowest growth rates (0.28 ± 0.08% day^{-1}, PERMANOVA pair wise $p < 0.05$; Table 2.2). At 30 ºC, *A. tenuis* had the highest growth rates (0.57 ± 0.12% day^{-1}, PERMANOVA pair wise $p < 0.05$), whereas *E. lamellosa* and *M. capricornis* (GM) displayed the lowest growth rates (PERMANOVA pair wise $p < 0.05$) (0.17 ± 0.06% day^{-1} and 0.14 ± 0.05% day^{-1}, respectively; PERMANOVA pair wise $p > 0.05$; Table 2.2). At 32 ºC, *P. contigua* had the highest growth rates (0.42 ± 0.10% day^{-1}, PERMANOVA pair wise $p < 0.05$), whereas *T. reniformis* displayed the lowest growth rates (0.11 ± 0.06% day^{-1}, PERMANOVA pair wise $p < 0.05$; Table 2.2).

Table 2.2. Coral fragments' average growth rate ± standard deviation in the three temperature experiments (% day^{-1}). NA – not available

Species	26 °C	30 °C	32 °C
A. tenuis	1.64 ± 0.38	0.57 ± 0.12	NA
M. capricornis (BM)	0.92 ± 0.20	0.34 ± 0.21	NA
M. capricornis (GM)	0.79 ± 0.20	0.14 ± 0.05	NA
T. reniformis	0.56 ± 0.33	0.24 ± 0.09	0.11 ± 0.06
E. lamellosa	0.51 ± 0.36	0.17 ± 0.06	NA
G. fascicularis	0.28 ± 0.08	0.28 ± 0.10	0.20 ± 0.09
P. damicornis	1.23 ± 0.23	NA	NA
S. pistillata	0.76 ± 0.19	NA	NA
P. contigua	1.54 ± 0.32	0.41 ± 0.13	0.42 ± 0.10

2.3.3. Regeneration rate

In Table SM2.1 of the supplementary material, not available (NA) was used in the time points after regeneration of all the coral fragments for a given coral species and when the coral fragments died.

Regeneration rate was significantly affected by the independent effects of temperature and coral species (PERMANOVA $p < 0.05$, Table 2.1). There was also a significant effect of the interactions of temperature and coral species (PERMANOVA $p < 0.05$, Table 2.1).

Regeneration rates of *M. capricornis* (BM), *G. fascicularis,* and *P. contigua* increased from the 26 ºC experiment (PERMANOVA pair wise $p < 0.05$, Table 2.3) to both 30 ºC and 32 ºC experiments (PERMANOVA pair wise $p > 0.05$, Table 2.3). *A. tenuis* regeneration rate only was significantly different between 26 ºC and 30 ºC, whereas *S. pistillata* showed it between 30 ºC and 32 ºC (PERMANOVA pair wise $p < 0.05$, Table 2.3). *T. reniformis* and *E. lamellosa* regeneration rates were significantly different among all temperature experiments (PERMANOVA pair wise $p < 0.05$, Table 2.3), both *M. capricornis* (GM) and *P. damicornis* regeneration rates decreased significantly at 32 ºC (PERMANOVA pair wise $p < 0.05$, Table 2.3).

At 26 ºC, the fragments of *S. pistillata* presented the highest regeneration rates (0.65 ± 0.29 mm^2 day^{-1}, PERMANOVA pair wise $p < 0.05$), whereas the lowest regeneration rate was

presented by *M. capricornis* (BM), *T. reniformis*, and *M. capricornis* (GM) fragments (PERMANOVA pair wise $p < 0.05$) (0.24 ± 0.16 mm^2 day^{-1}, 0.16 ± 0.10 mm^2 day^{-1} and 0.17 ± 0.03 mm^2 day^{-1} respectively; PERMANOVA pair wise $p > 0.05$; Table 2.3). At 30 ºC, *G. fascicularis*, *S. pistillata*, *M. capricornis* (BM), *P. contigua*, *E. lamellosa*, and *P. damicornis* had the highest regeneration rates (PERMANOVA pair wise $p < 0.05$) (0.73 ± 0.27 mm^2 day^{-1}, 0.71 ± 0.32 mm^2 day^{-1}, 0.69 ± 0.21 mm^2 day^{-1}, 0.59 ± 0.27 mm^2 day^{-1}, 0.58 ± 0.27 mm^2 day^{-1}, 0.50 ± 0.25 mm^2 day^{-1} respectively; PERMANOVA pair wise $p > 0.05$), whereas *M. capricornis* (GM) displayed the lowest regeneration rates (0.16 ± 0.07 mm^2 day^{-1}, PERMANOVA pair wise $p < 0.05$, Table 2.3). At 32 °C, the fragments of *E. lamellosa*, *G. fascicularis*, and *P. contigua* had the highest regeneration rates (PERMANOVA pair wise $p < 0.05$) (0.84 ± 0.18 mm^2 day^{-1}, 0.88 ± 0.48 mm^2 day^{-1} and 0.63 ± 0.49 mm^2 day^{-1}, respectively; PERMANOVA pair wise $p > 0.05$, Table 2.3), whereas *P. damicornis* and *M. capricornis* (GM) displayed the lowest regeneration rates (PERMANOVA pair wise $p < 0.05$) (0.13 ± 0.02 mm^2 day^{-1} and 0.09 ± 0.07 mm^2 day^{-1}, respectively; PERMANOVA pair wise $p > 0.05$, Table 2.3).

Table 2.3. Coral fragments' average regeneration rate ± standard deviation in the three temperature experiments (mm^2 day^{-1}) measured until complete regeneration or the last time point they were still alive. For more information see supplementary material.

Species	26 °C	30 °C	32 °C
A. tenuis	0.23 ± 0.09	0.42 ± 0.22	0.30 ± 0.14
M. capricornis (BM)	0.24 ± 0.16	0.69 ± 0.21	0.51 ± 0.27
M. capricornis (GM)	0.17 ± 0.03	0.16 ± 0.07	0.09 ± 0.07
T. reniformis	0.16 ± 0.10	0.35 ± 0.16	0.52 ± 0.16
E. lamellosa	0.30 ± 0.11	0.58 ± 0.27	0.84 ± 0.18
G. fascicularis	0.30 ± 0.06	0.73 ± 0.27	0.88 ± 0.48
P. damicornis	0.40 ± 0.13	0.50 ± 0.25	0.13 ± 0.02
S. pistillata	0.65 ± 0.29	0.71 ± 0.32	0.46 ± 0.13
P. contigua	0.22 ± 0.10	0.59 ± 0.27	0.63 ± 0.49

2.4. Discussion and conclusions

Acropora tenuis, *P. damicornis*, and *S. pistillata* had the highest susceptibility to thermal stress during the study, displaying more bleaching and tissue loss early in the experiment compared to other species. In field studies, branching acroporids (e.g. *Acropora* spp.) and pocilloporids (e.g. *Pocillopora damicornis* and *Stylophora pistillata)* are the taxa most sensitive to bleaching stress, usually suffering high mortality, particularly in the Indo-Pacific (Fujioka, 1999; Marshall and Baird, 2000). *T. reniformis* only presented partial mortality by the 40th day at 30 ºC and at

the end of the 32 ºC experiment, and both *G. fascicularis* and *P. contigua* only presented it at the end of the 32 ºC experiment, indicating that these coral species fragments are most resistant to thermal stress than the other coral species fragments in study. In the field, *Turbinaria*, *Galaxea*, and *Psammocora* are considered resistant to bleaching displaying low mortality when compared with other taxa, with their unbleached colonies generally surrounded by bleached colonies of other taxa (Marshall and Baird, 2000; Stimson et al., 2002; Bhagooli and Hidaka, 2003).

The discoloration evidenced by the corals in this study resulted from either the loss of their zooxanthellae, the degradation of their photosynthetic pigments, or a combination of both, with the extent of their bleaching and ultimately their mortality possibly being considered as good stress indicators (Glynn, 1993; Hoegh-Guldberg, 1999). At 30 ºC, the coral species *M. capricornis* (BM), *T. reniformis*, and *P. contigua* showed color attenuation although never reaching the bleached level, whereas *P. damicornis* and *S. pistillata* reached the bleached level and complete mortality. These findings make us consider that *M. capricornis* (BM), *T. reniformis*, and *P. contigua* fragments probably presented higher levels of stress at 30 ºC than at 26 ºC, displayed as color attenuation at 30 ºC, and that *P. damicornis* and *S. pistillata* are highly susceptible to thermal stress since they were the only ones with bleached fragments and complete mortality at 30 ºC over the course of 60 days. At 32 ºC, most of the coral species died, except for *T. reniformis, G. fascicularis,* and *P. contigua*, however, the time interval in which they reached complete mortality varied interspecifically. *T. reniformis* and *G. fascicularis* showed no mortality throughout all the experiment and only 40% of *P. contigua* fragments were dead at the end of this experiment. These findings make us consider these three species as highly tolerant to thermal stress due to their null or low mortality over the course of 60 days. The bleaching level of all coral species was highest at 32 ºC. This can be explained by the higher thermal stress present at higher temperatures (Glynn, 1984; Goreau et al., 2000).

The hierarchy of species susceptibility to thermal stress presented here: (*A. tenuis, P. damicornis, S. pistillata*) > *M. capricornis* (BM) > (*M. capricornis* (GM), *E. lamellosa)* > *P. contigua* > (*T. reniformis, G. fascicularis*) is in accordance to the ones observed in other studies with species of the Indo-Pacific (Marshall and Baird, 2000; Stimson et al., 2002).

According to our results, coral survival declined significantly at 32 °C with an increase in bleaching level and mortality with increasing exposure, although it had remained high at 26 °C

and 30 ºC. A probable explanation could be related to corals' bleaching temperature threshold, which in many regions is close to 31 °C (Glynn, 1984; Goreau et al., 2000), thus confirming the high bleaching level and mortality observed at 32 ºC. Our results are also in accordance with those of Stimson et al. (2002) who reported high mortalities for *Acropora* spp., *Pocillopora damicornis*, and *Stylophora pistillata* and low mortalities for *Turbinaria* spp., *Galaxea fascicularis*, and *Psammocora contigua* during the bleaching event of late summer 1998 in northern Okinawa.

At 32 ºC, *G. fascicularis* fragments acquired a lighter coloration than their typical red-brown coloration. This discoloration was most obvious on the coenosarc between the corallites, while individual polyps remained with their red-brown coloration. In these flat surfaces of tissue, the algae are likely to experience the cumulative effects of highest light intensities and elevated temperature, while the algae situated in the base of a polyp or in the tentacles might experience shading by the coral's tissue and skeleton or other algae (Brown et al., 1995). In the last twenty days of this experiment, the fragments of *G. fascicularis* changed to white with yellow fluorescent polyps possibly due to the presence of fluorescent pigments in the coral that enhanced its bleaching resistance (Salih et al., 2000).

Our results show that the cause of bleaching in these coral species might probably be an interaction between water temperature and the amount of time above their bleaching threshold, rather than just having temperatures above their bleaching threshold (Fitt et al., 2001; McClanahan et al., 2001). In particular, there was a pronounced difference among these coral species in the time necessary to respond to thermal stress. The most likely explanation for this phenomenon is the existence of differences in interspecific respiration rates. Faster-growing species, such as the *Acropora* spp., are generally assumed to have a higher respiration rate than the slower-growing massives, and a high correlation between respiration rate and coral susceptibility to thermal stress has long been recognized (Jokiel and Coles, 1990; Gates and Edmunds, 1999).

Another explanation for the time taken for the different coral species in this study to respond to thermal stress might be related with the fact that colony morphology also influences coral vulnerability to bleaching. Branching pocilloporids *(Stylophora pistillata, Pocillopora damicornis)* and staghorn corals (*Acropora* spp.) are cited in many studies, both in experimental work (Jokiel and Coles, 1974) and in bleaching episodes in the field (Glynn, 1983; Loya et al.,

2001), as being morphologies severely damaged by thermal stress (Brown and Howard, 1985; Hoegh-Guldberg and Salvat, 1995). These corals bleach more often than massive and encrusting growth forms (Marshall and Baird, 2000; Loya et al., 2001; McClanahan et al., 2002). However, other factors must also be considered such as tissue thickness (Loya et al., 2001), density of zooxanthellae or the weight of tissue per unit coral surface area (Stimson et al., 2002), the clade of *Symbiodinium* spp. (Magalon et al., 2007), taxa or coral phylogeny (McClanahan et al., 2004), density of fluorescent tissue pigment granules (FPG; Salih et al., 2000), and colony integration (Baird and Marshall, 2002). Previous studies have identified *Turbinaria reniformis, Galaxea fascicularis,* and *Psammacora contigua* as tolerant species to thermal stress (Yamazato, 1981, 1999; Marshall and Baird, 2000), which the results of this study support. Although *P. contigua* has branching morphology, it was reported to have high densities of zooxanthellae in comparison with the densities of other branching genera such as *Pocillopora, Acropora, Stylophora* (Stimson et al., 2002).

Growth rate has been cited as one of the best quantitative measures to test stress due to a disturbance, since this parameter integrates a variety of physiological processes (Birkeland et al., 1976; Muscatine, 1990; Pratchett et al., 2015). In the fragments of acroporid species (*Acropora tenuis* and *Montipora capricornis*) it was possible to observe the spread of the basal tissue over the epoxy putty or the bare skeleton following its contour, known as fast self-attachment after transplantation (Guest et al., 2011), whereas in the fragments of *T. reniformis, E. lamellosa,* and *G. fascicularis* no self-attachment was observed, as their growth occurred by horizontal extension of its margins. Also, the growing of several new branches in the fragments of *S. pistillata* was observed. This may happen as a way of *S. pistillata* to re-acquire the colony symmetry lost after its separation, which is a known characteristic of this species' colonies (Rinkevich, 2000).

According to our results, coral fragments' growth rates decreased with increasing temperature, which is in accordance with physiological data showing an accentuated decline in coral growth at temperatures only a few degrees above optimum levels (Berkelmans and Willis, 1999). In all temperature experiments, *A. tenuis* and *P. contigua* presented the highest growth rates, with the exception of *A. tenuis* at 32 ºC that died. Assuming that plate and massive morphologies grow slower than branched ones (Guest et al., 2011), the lower growth rates of *G. fascicularis* at 26 ºC, *M. capricornis* (GM) and *E. lamellosa* at 30 ºC and *T. reniformis* at 32 ºC can thus be justified. *G. fascicularis* was the only coral species that did not present significant growth rate

differences at 26 ºC and 30 ºC, suggesting that this coral species is resistant to thermal stress. All the other species presented significant differences at 26 ºC and 30 ºC, this probably is a result of thermal stress at 30 ºC, where their growth rates were lower (Szmant and Gassman, 1990; Fine et al., 2002). The fragments of *P. contigua* did not present significant differences at 30 ºC and 32 ºC, which means that although this coral species had shown severe visual signs of stress at 32 ºC, its growth rate was affected in the same way at these temperatures.

The ability of corals to regenerate from injuries is crucial to their survival from disturbances such as hurricanes and predators since poor regenerative ability can lead to a reduction in colony fitness (Hall, 1997). Coral fragments' regeneration rate differed with temperature. The coral species *A. tenuis, M. capricornis* (BM), *T. reniformis, E. lamellosa, G. fascicularis,* and *P. contigua* seem to benefit with the increase in temperature as their regeneration rates increased with temperature, thus confirming the assumption that an increase in water temperatures accelerates coral fragments' metabolism and, as long as their specific stress levels were not attained, their regeneration rates will be greater at higher temperatures (Coles and Jokiel, 1977; Kramarsky-Winter and Loya, 2000). Having this in mind, the coral species *M. capricornis* (GM), *P. damicornis,* and *S. pistillata* might have presented their highest stress levels at 32 ºC where they showed their lowest regeneration rates.

Hall (1997) ranked the regenerative ability of eleven different coral species according to their morphologies (arborescent > bushy > tabular > massive > submassive). Our study results were not in accordance with those of Hall (1997), probably because the experimental conditions were different. Hall's work was developed in natural environment whereas ours was developed in laboratory. In natural conditions, corals are exposed not only to changes of other environmental variables (e.g. salinity (Faxneld et al., 2010), solar radiation (Lesser and Farrell, 2004), sedimentation and turbidity (Browne, 2012), aerial exposure (Teixeira et al., 2013), water flow (Lenihan et al., 2008)), but also to competition (Baird and Hughes, 2000), predation (Rotjan and Lewis, 2008), algal over growth (Diaz-Pulido et al., 2009), and continuous colony overturning (Campbell et al., 2007)), which in the present study were controlled. Also, the fact that in this study we used small fragments instead of large colonies, the use of different coral species, the ratio diameter of injury/coral area was much higher than the one used in this study, and Hall (1997) made observations for a longer period of time which would allow for more healing time of the injured corals (74 days instead of 60 days). All these factors are known to affect corals' regeneration capacity of lesions (Denis et al., 2011).

Differential susceptibility and mortality among species can exert a major influence on coral community structure (Pratchett, 2001). According with the results obtained in this study, it is expected that the coral species *T. reniformis, G. fascicularis,* and *P. contigua* will be more resistant to high temperature and bleaching episodes and thus becoming more important in terms of coral cover. Nevertheless, it is important to have in consideration that the more fragile and thermal susceptible coral species tend to be fast-growing species (Marshall and Baird, 2000; McClanahan et al., 2004) and that in some bleaching episodes it has been observed that more resistant coral species did not increase in absolute density and cover through time because they are often slow-growing species (Van Woesik et al., 2011; Guest et al., 2016).

On the other hand, other disturbances present in their natural habitat need to be taken into account, such as predation (Pratchett, 2001), competition (Baird and Hughes, 2000) and extreme events (e.g. tropical cyclones, Fabricius et al., 2008) that, like bleaching episodes, affect with greater magnitude fast-growing coral species. As a result, mortality of thermal susceptible species, often numerically dominant, may promote the coexistence of a higher number of species unless the increase in frequency of bleaching episodes causes local eradication or prevents susceptible species from resettle (Loya et al., 2001; McClanahan and Maina, 2003). Thus, although *T. reniformis* and *G. fascicularis* are slow-growing coral species, it is expected that these resistant species will become dominant. Although *P. contigua* is a fast-growing coral species, it showed a high ability to resist to thermal stress and it is expected to increase in coral cover and subsequent dominance faster than *T. reniformis* and *G. fascicularis*. This pattern of recovery was observed in other studies (Brown and Suharsono, 1990; Baird and Marshall, 2002).

Another aspect to have into account is the ability of corals to adapt to recurrent thermal stress, which occurs through the selective removal of highly susceptible genotypes, followed by reproduction and successful recruitment of corals with higher photoprotective defense (influx of coral colonies with thermal tolerance) (Penin et al., 2013; Pratchett et al., 2013). It was observed in several studies that such adaptation may result in a reverse of the normal hierarchy of bleaching susceptible coral species (Guest et al., 2016; Carroll et al., 2017). Nevertheless, gradual adaptation to increased temperatures by coral assemblages in the Indo-Pacific can easily be undone, delayed or even stopped by other natural and anthropogenic stressors and

disturbances (e.g. *Acanthaster planci* outbreaks, Adjeroud et al., 2005) and may have altogether different selective forcing on coral population and communities (Pratchett et al., 2013).

Having in account that this study was made with colonies coming from the same mother colony, meaning low genetic variability, it is important to consider that some results may vary with those in future studies.

In conclusion, long-term exposure to 32 °C was beyond the bleaching resistance capacity of the majority of the corals. *A. tenuis, P. damicornis*, and *S. pistillata* were the most affected by thermal stress, which may result in these species becoming less important in terms of coral cover in coral reef communities subjected to successive bleaching episodes, whereas the coral species *T. reniformis, G. fascicularis*, and *P. contigua*, due to their greater ability to resist to successive bleaching episodes as to other natural and anthropogenic stressors and disturbances, are likely to show greater capacity to withstand higher temperatures and might thus become more important in terms of coral cover. Nevertheless, *P. contigua* may increase in coral cover and subsequent dominance faster than *T. reniformis* and *G. fascicularis*. This knowledge may have ecological implications in coral reef conservation and management, as well as in the understanding of coral reef alterations in the near future.

2.5. References

Adjeroud, M., Chancerelle, Y., Schrimm, M., Perez, T., Lecchini, D., Galzin, R., & Salvat, B. (2005). Detecting the effects of natural disturbances on coral assemblages in French Polynesia: A decade survey at multiple scales. Aquatic Living Resources, 18(2), 111–123.

Anderson, M. J. (2001). A new method for non-parametric analysis of variance. Austral Ecology, 26, 32–46.

Aronson, R. B., Macintyre, I. G., Wapnick, C. M., & O'Neill, M. W. (2004). Phase-shifts, alternative states, and the unprecedented convergence of two reef systems. Ecology, 85, 1876–1891.

Aronson, R. B., Precht, W. F., Macintyre, I. G., & Murdoch, T. J. T. (2000). Ecosystems: Coral bleach-out in Belize. Nature, 405, 36.

Baird, A. H., & Hughes, T. P. (2000). Competitive dominance by tabular corals. An experimental analysis of recruitment and survival of understorey assemblages. Journal of Experimental Marine Biology and Ecology, 251, 117–132.

Baird, A. H., & Marshall, P. A. (2002). Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Marine Ecology Progress Series, 237, 133–141.

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: an ecological assessment of the long-term impacts, recovery trends and future outlooks. Estuarine, Coastal and Shelf Science, 80, 435–471.

Berkelmans, R., & Willis, B. L. (1999). Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs, 18, 219-228.

Bhagooli, R., & Hidaka, M. (2003). Comparison of stress susceptibility of in hospite and isolated zooxanthellae among five coral species. Journal of Experimental Marine Biology and Ecology, 291, 181-197.

Birkeland, C., Reimer, A. A., & Young, J. R. (1976). Survey of marine communities in Panama and experiments with oil. Environmental Protection Agency, Ecological Research Series, United States.

Brandt, M. E. (2009). The effect of species and colony size on the bleaching response of reef-building corals in the Florida Keys during the 2005 mass bleaching event. Coral Reefs, 28, 911-924.

Brinkhuis, B. H. (1985). Growth patterns and rates. Handbook of physiological methods. Ecological field methods: macroalgae. NJ Cambridge University Press, pp. 461– 477.

Brown, B. E., Howard, L. S. (1985). Assessing the Effects of "Stress" on Reef Corals. Advances in Marine Biology, 22, 1-63.

Brown, B. E., & Suharsono (1990). Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. Coral Reefs, 8, 163-170.

Brown, B. E., Le Tissier, M. D. A., & Bythell, J. C. (1995). Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. Marine Biology, 122, 655-663.

Brown, B., Dunne, R., Goodson, M., & Douglas, A. (2002). Experience shapes the susceptibility of a reef coral to bleaching. Coral Reefs, 21, 119-126.

Browne, N. K. (2012). Spatial and temporal variations in coral growth on an inshore turbid reef subjected to multiple disturbances. Marine Environmental Research, 77, 71-83.

Bruno, J. F. (1998). Fragmentation in *Madracis mirabilis* (Duchassaing and Michelotti): how common is size-specific fragment survivorship in corals? Journal of Experimental Marine Biology and Ecology, 230, 169-181.

Campbell, S. J., Pratchett, M. S., Anggoro, A. W., Ardiwijaya, R. L., Fadli, N., Herdiana, Y., ... & Rudi, E. (2007). Disturbance to coral reefs in Aceh, Northern Sumatra: impacts of the Sumatra-Andaman tsunami and pre-tsunami degradation. Atoll Research Bulletin, 544, 55-78.

Carpenter, K. E., Abrar, M., Aeby, G., Aronson, R. B., Banks, S., Bruckner, A., ... & Edgar, G. J. (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science, 321(5888), 560-563.

Carroll, A. G., Harrison, P. L., & Adjeroud, M. (2017). Susceptibility of coral assemblages to successive bleaching events at Moorea, French Polynesia. Marine and Freshwater Research, 68(4), 760-771.

Christensen, J.H., Kanikicharla, K.K., Aldrian, E., An, S.-I., Cavalcanti, I.F.A., De Castro, M., … & Midgley, P.M., (eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC AR5). Cambridge, United Kingdom and New York, New York: Cambridge University Press, pp.1217-1308.

Chua, C., Leggat, W., Moya, A., & Baird, A. H. (2013). Temperature affects the early life history stages of corals more than near future ocean acidification. Marine Ecology Progress Series, 475, 85-92.

Coles, S. L., & Jokiel, P. L. (1977). Effects of temperature on photosynthesis and respiration in hermatypic corals. Marine Biology, 43, 209-216.

Coles, S. L., Jokiel, P. L., & Lewis, C. R. (1976). Thermal tolerance in tropical versus subtropical pacific reef corals. Pacific Science, 30, 159-166.

Cook, R. E., 1979. Asexual reproduction: a further consideration. The American Naturalist, 113, 769-772.

Crabbe, M. J. C. (2008). Climate change, global warming and coral reefs: Modelling the effects of temperature. Computational Biology and Chemistry, 32, 311-314.

Cróquer, A., Villamizar, E., & Noriega, N. (2002). Environmental factors affecting tissue regeneration of the reef – building coral *Montastraea annularis* (Faviidae) at Los Roques National Park, Venezuela. Revista de Biologia Tropical, 50, 1055-1065.

Davenport, J., & Davenport, J. L. (2006). The impact of tourism and personal leisure transport on coastal environments: A review. Estuarine, Coastal and Shelf Science, 67, 280-292.

Denis, V., Debreuil, J., De Palmas, S., Richard, J., Guillaume, M. M. M., & Bruggemann, J. H. (2011). Lesion regeneration capacities in populations of the massive coral *Porites lutea* at Réunion Island: environmental correlates. Marine Ecology Progress Series, 428, 105-117.

DeSalvo, M. K., Voolstra, C. R., Sunagawa, S., Schwarz, J. A., Stillman, J. H., Coffroth, M. A., … & Medina, M. (2008). Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. Molecular Ecology, 17, 3952-3971.

Diaz-Pulido, G., McCook, L. J., Dove, S., Berkelmans, R., Roff, G., Kline, D. I., … & Hoegh-Guldberg, O. (2009). Doom and boom on a resilient reef: climate change, algal overgrowth and coral recovery. PloS One, 4, e5239.

Eakin, C. M., Lough, J. M., & Heron, S. F. (2009). "Climate variability and change: monitoring data and evidence for increased coral bleaching stress," in: Van Oppen, M. J. H., Lough, J. M. (Eds.), Coral Bleaching: Patterns, Processes, Causes and Consequences, Berlin: Springer-Verlag, pp. 41–67.

Fabricius, K. E., De'ath, G., Puotinen, M. L., Done, T., Cooper, T. F., & Burgess, S. C. (2008). Disturbance gradients on inshore and offshore coral reefs caused by a severe tropical cyclone. Limnology and Oceanography, 53(2), 690-704.

Faxneld, S., Jörgensen, T. L., & Tedengren, M. (2010). Effects of elevated water temperature, reduced salinity and nutrient enrichment on the metabolism of the coral *Turbinaria mesenterina*. Estuarine, Coastal and Shelf Science, 88, 482-487.

Fine, M., Oren, U., & Loya, Y. (2002). Bleaching effect on regeneration and resource translocation in the coral *Oculina patagonica*. Marine Ecology Progress Series, 234, 119-125.

Fitt, W. K., Brown, B. E., Warner, M. E., & Dunne, R. P. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs, 20, 51-65.

Fitt, W. K., Gates, R. D., Hoegh-Guldberg, O., Bythell, J. C., Jatkar, A., Grottoli, A. G., ... & Iglesias-Prieto, R. (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. Journal of Experimental Marine Biology and Ecology, 373(2), 102-110.

Foster, N. L., Baums, I. B., & Mumby, P. J. (2007). Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastraea annularis*. Journal of Animal Ecology, 76(2), 384-391.

Frieler, K., Meinshausen, M., Golly, A., Mengel, M., Lebek, K., Donner, S. D., & Hoegh-Guldberg, O. (2013). Limiting global warming to 2 °C is unlikely to save most coral reefs. Nature Climate Change, 3(2), 165.

Fujioka, Y. (1999). Mass destruction of the hermatypic corals during a bleaching event in Ishigaki Island, southwestern Japan. Galaxea 1, 41-50.

Gates, R. D., & Edmunds, P. J. (1999). The physiological mechanisms of acclimatization in tropical reef corals. The American Zoologist, 39, 30-43.

Gleason, M. G. (1993). Effects of disturbance on coral communities: bleaching in Moorea, French Polynesia. Coral Reefs, 12, 193-201.

Glynn, P. W. (1983). Extensive "bleaching" and death of reef corals on the Pacific coast of Panama. Environmental Conservation 10(2), 149-154.

Glynn, P. W. (1984). Widespread coral mortality and the 1982/83 El Niño warming event. Environmental Conservation, 11, 133-146.

Glynn, P. W. (1993). Coral reef bleaching: ecological perspectives. Coral Reefs, 12, 1-17.

Glynn, P. W., & Fong, P. (2006). Patterns of reef coral recovery by the regrowth of surviving tissues following the 1997-98 El Niño warming and 2000, 2001 upwelling cool events in Panamá, eastern Pacific. Proceedings of the 10th International Coral Reef Symposium, 624-630.

Glynn, P. W., Cortés-Núñez, J., Guzmán-Espinal, H. M., & Richmond, R. H. (1988). El Niño (1982-83) associated coral mortality and relationship to sea surface temperature deviations in the tropical eastern Pacific. In Proceedings of the 6th International Coral Reef Symposium, Australia. (Vol. 3, pp. 237-243).

Glynn, P. W., Colley, S. B., Eakin, C. M., Smith, D. B., Cortés, J., Gassman, N. J., ... & Feingold, J. S. (1994). Reef coral reproduction in the eastern Pacific: Costa Rica, Panamá, and Galápagos Islands (Ecuador). II. Poritidae. Marine Biology, 118(2), 191-208.

Goreau, T., McClanahan, T., Hayes, R., & Strong, A. L. (2000). Conservation of coral reefs after the 1998 global bleaching event. Conservation Biology, 14(1), 5-15.

Guest, J. R., Dizon, R. M., Edwards, A. J., Franco, C., & Gomez, E. D. (2011). How quickly do fragments of coral “self-attach” after transplantation?. Restoration Ecology, 19(2), 234-242.

Guest, J. R., Low, J., Tun, K., Wilson, B., Ng, C., Raingeard, D., ... & McDougald, D. (2016). Coral community response to bleaching on a highly disturbed reef. Scientific Reports, 6, 20717.

Hall, V. R. (1997). Interspecific differences in the regeneration of artificial injuries on scleractinian corals. Journal of Experimental Marine Biology and Ecology, 212(1), 9-23.

Harvell, C. D., Kim, K., Burkholder, J. M., Colwell, R. R., Epstein, P. R., Grimes, D. J., ... & Porter, J. W. (1999). Emerging marine diseases--climate links and anthropogenic factors. Science, 285(5433), 1505-1510.

Heron, S. F., Maynard, J. A., Van Hooidonk, R., & Eakin, C. M. (2016). Warming trends and bleaching stress of the world's coral reefs 1985–2012. Scientific Reports, 6, 38402.

Highsmith, R. C. (1980). Passive colonization and asexual colony multiplication in the massive coral *Porites lutea* Milne Edwards & Haime. Journal of Experimental Marine Biology and Ecology, 47(1), 55-67.

Highsmith, R. C. (1982). Reproduction by fragmentation in corals. Marine Ecology Progress Series, 7, 207-226.

Highsmith, R. C., Riggs, A. C., & D'Antonio, C. M. (1980). Survival of hurricane-generated coral fragments and a disturbance model of reef calcification/growth rates. Oecologia, 46(3), 322-329.

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research, 50(8), 839-866.

Hoegh-Guldberg, O., & Salvat, B. (1995). Periodic mass-bleaching and elevated sea temperatures: bleaching of outer reef slope communities in Moorea, French Polynesia. Marine Ecology Progress Series, 121, 181-190.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Meyer LA (Eds.)]. IPCC, Geneva, Switzerland, p 151.

Jokiel, P. L., & Coles, S. L. (1974). Effects of heated effluent on hermatypic corals at Kahe Point, Oahu. Pacific Science, 28, 1-18.

Jokiel, P. L., & Coles, S. L. (1990). Response of Hawaiian and other Indo Pacific reef corals to elevated temperatures. Coral Reefs, 8, 155-162.

Kayanne, H., Harii, S., Yamano, H., Tamura, M., Ide, Y., & Akimoto, F. (1999). Changes in living coral coverage before and after the 1998 bleaching event on coral reef flats of Ishigaki Island, Ryukyu Islands. Galaxea, Journal of the Japanese Coral Reef Society, 1, 73-82.

Kramarsky-Winter, E., & Loya, Y. (2000). Tissue regeneration in the coral *Fungia granulosa*: the effect of extrinsic and intrinsic factors. Marine Biology, 137, 867-873.

Lasker, H. R. (1990). Clonal propagation and population dynamics of a Gorgonian coral. Ecology, 71, 1578-1589.

Lenihan, H. S., Adjeroud, M., Kotchen, M. J., Hench, J. L., & Nakamura, T. (2008). Reef structure regulates small-scale spatial variation in coral bleaching. Marine Ecology Progress Series, 370, 127–141.

Lesser, M. P., & Farrell, J. H. (2004). Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. Coral Reefs, 23, 367-377.

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., & Van Woesik, R. (2001). Coral bleaching: the winners and the losers. Ecology Letters, 4, 122-131.

Magalon, H., Flot, J.-F., & Baudry, E. (2007). Molecular identification of symbiotic dinoflagellates in Pacific corals in the genus *Pocillopora.* Coral Reefs, 26, 551-558.

Marshall, P. A., & Baird, A. H. (2000). Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs, 19, 155-163.

McClanahan, T. R., & Maina, J. (2003). Response of coral assemblages to the interaction between natural temperature variation and rare warm-water events. Ecosystems, 6, 551–563.

McClanahan, T., Maina, J., & Pet-Soede, L. (2002). Effects of the 1998 coral mortality event on Kenyan coral reefs and fisheries. Ambios, 31, 543-550.

McClanahan, T., Muthiga, N., & Mangi, S. (2001). Coral and algal changes after the 1998 coral bleaching: interaction with reef management and herbivores on Kenyan reefs. Coral Reefs, 19, 380-391.

McClanahan, T. R., Baird, A. H., Marshall, P. A., & Toscano, M. A. (2004). Comparing bleaching and mortality responses of hard corals between southern Kenya and the Great Barrier Reef, Australia. Marine Pollution Bulletin, 48, 327–335.

McClanahan, T. R., Ateweberhan, M., Graham, N. A. J., Wilson, S. K., Ruiz Sebastián, C., Guillaume, M. M. M.., Bruggemann, J. H. (2007). "Western Indian Ocean coral communities: Bleaching responses and susceptibility to extinction". Marine Ecology Progress Series, 337, 1-13.

Meehan, W. J., & Ostrander, G. K. (1997). Coral bleaching: A potential biomarker of environmental stress. Journal of Toxicology and Environmental Health, 50, 529-552.

Meesters, E. H., & Bak, R. P. M. (1993). Effects of coral bleaching on tissue regeneration potential and colony survival. Marine Ecology Progress Series, 96, 189–198.

Montano, S., Seveso, D., Galli, P., & Obura, D. O. (2010). Assessing coral bleaching and recovery with a colour reference card in Watamu Marine Park, Kenya. Hydrobiologia, 655, 99–108.

Muscatine, L. (1990). The role of symbiotic algae in carbon and energy flux in reef corals. Ecosystems of the world. Coral Reefs.

Ostrander, G. K., Armstrong, K. M., Knobbe, E. T., Gerace, D., & Scully, E. P. (2000). Rapid transition in the structure of a coral reef community: the effects of coral bleaching and physical disturbance. Proceedings of the National Academy of Sciences, 97(10), 5297-5302.

Padilla-Gamino, J. L., Bidigare, R. R., Barshis, D. J., Alamaru, A., Hédouin, L., Hernández-Pech, X., ... & Grottoli, A. G. (2013). Are all eggs created equal? A case study from the Hawaiian reef-building coral *Montipora capitata*. Coral Reefs, 32(1), 137-152.

Penin, L., Vidal-Dupiol, J., & Adjeroud, M. (2013). Response of coral assemblages to thermal stress: are bleaching intensity and spatial patterns consistent between events? Environmental Monitoring and Assessment, 185, 5031–5042.

Pratchett, M. S. (2001). Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the western Pacific. Marine Ecology Progress Series, 214, 111-119.

Pratchett, M. S., McCowan, D., Maynard, J. A., & Heron, S. F. (2013). Changes in bleaching susceptibility among corals subject to ocean warming and recurrent bleaching in Moorea, French Polynesia. PLoSOne, 8, e70443.

Pratchett, M. S., Anderson, K. D., Hoogenboom, M. O., Widman, E., Baird, A. H., Pandolfi, J. M., ... & Lough, J. M. (2015). Spatial, temporal and taxonomic variation in coral growth—implications for the structure and function of coral reef ecosystems. Oceanography and Marine Biology: An Annual Review, 53, 215-295.

Randall, R., & Eldredge, L. (1977). Effects of Typhoon Pamela on the coral reefs of Guam. Proceedings of the 3rd International Coral Reef Symposium, 2, 525-531.

Rinkevich, B. (2000). Steps towards the evaluation of coral reef restoration by using small branch fragments. Marine Biology, 136, 807-812.

Rotjan, R. D., & Lewis, S. M. (2008). Impact of coral predators on tropical reefs. Marine Ecology Progress Series, 367, 73-91.

Rowan, R., & Knowlton, N. (1995). Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proceedings of the National Academy of Sciences, 92(7), 2850-2853.

Rowan, R., Knowlton, N., Baker, A. C., & Jara, J. (1997). Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature, 388, 265-269.

Salih, A., Hoegh-Guldberg, O., & Cox, G. (1998). Photoprotection of symbiotic dinoflagellates by fluorescent pigments in reef corals. In Proceedings of the Australian Coral Reef Society

75th Anniversary Conference (pp. 217-230). School of Marine Science, The University of Queensland Brisbane, Australia.

Salih, A., Larkum, A., Cox, G., Kühl, M., & Hoegh-Guldberg, O. (2000). Fluorescent pigments in corals are photoprotective. Nature, 408, 850-853.

Seveso, D., Montano, S., Strona, G., Orlandi, I., Galli, P., & Vai, M. (2014). The susceptibility of corals to thermal stress by analyzing Hsp60 expression. Marine Environmental Research, 99, 69-75.

Shenkar, N., Fine, M., & Loya, Y. (2005). Size matters: bleaching dynamics of the coral *Oculina patagonica*. Marine Ecology Progress Series, 294, 181-188.

Shinn, E. A. (1976). Coral reef recovery in Florida and the Persian Gulf. Environmental Geology, 1(4), 241.

Stimson, J. (1978). Mode and timing of reproduction in some common hermatypic corals of Hawaii and Enewetak. Marine Biology, 48, 173-184.

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.

Szmant, A. M., & Gassman, N. J. (1990). The effect of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis.* Coral Reefs, 8, 217-224.

Teixeira, T., Diniz, M., Calado, R., Rosa, R. (2013). Coral physiological adaptations to air exposure: heat shock and oxidative stress responses in *Veretillum cynomorium*. Journal of Experimental Marine Biology and Ecology, 439, 35-41.

Titlyanov, E. A., Titlyanova, T. V., Yakovleva, I. M., Nakano, Y., & Bhagooli, R. (2005). Regeneration of artificial injuries on scleractinian corals and coral/algal competition for newly formed substrate. Journal of Experimental Marine Biology and Ecology, 323, 27–42.

Tunnicliffe, V. (1981). Breakage and propagation of the stony coral *Acropora cervicornis*. Proceedings of the National Academy of Sciences, 78(4), 2427-2431.

Van Hooidonk, R., & Huber, M. (2009). Quantifying the quality of coral bleaching predictions. Coral Reefs, 28, 579-587.

Van Woesik, R. & Jordán-Garza, A. G. (2011). Coral populations in a rapidly changing environment. Journal of Experimental Marine Biology and Ecology, 408, 11-20.

Van Woesik, R., Sakai, K., Ganase, A., & Loya, Y. (2011). Revisiting the winners and the losers a decade after coral bleaching. Marine Ecology Progress Series, 434, 67-76.

Veron, J. E. N. (2000). Corals of the World, Vol. 1, 2, 3, Mary Stafford-Smith (Ed.) Publisher: Australian Institute of Marine Science. p 489.

Yamazato, K. (1981). A note on the expulsion of zooxanthellae during summer, 1980 by the Okinawan reef-building corals. Sesoko Marine Science Laboratory technical report, 8, 9-18.

Yamazato, K. (1999). Coral bleaching in Okinawa, 1980 vs 1998. Galaxea, 1, 83-87.

2.6. Supplementary material

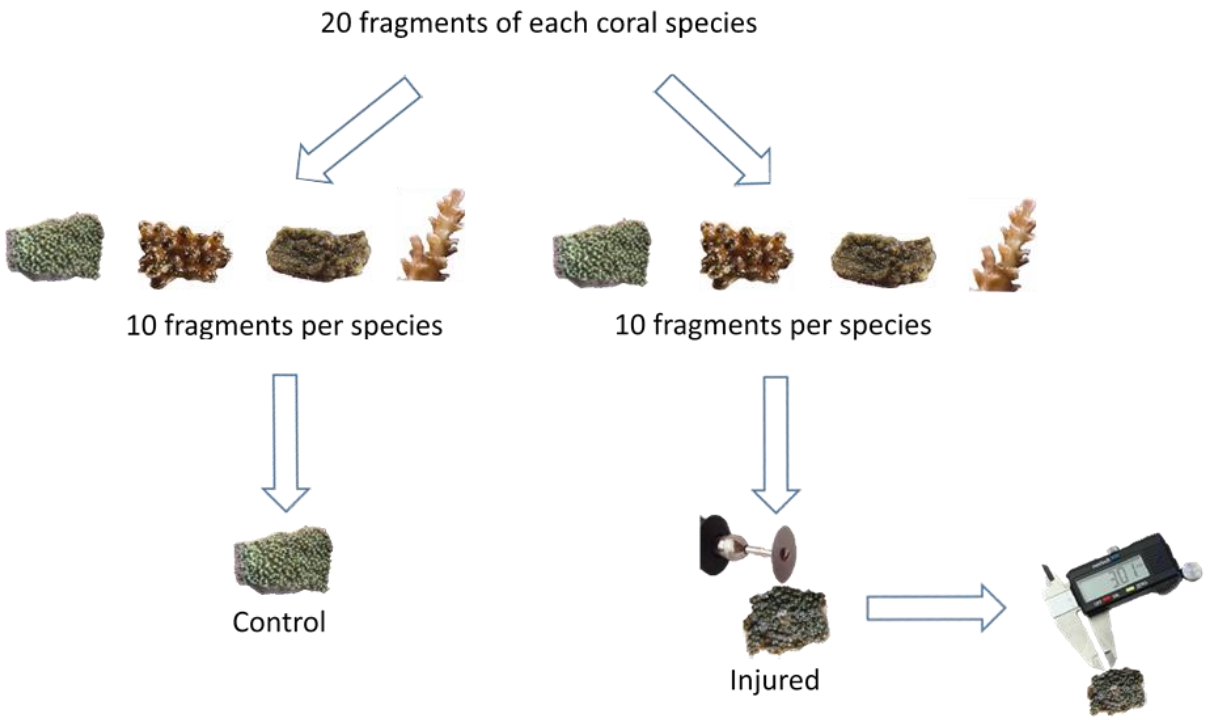

Figure SM2.1 – Separation of fragments in control and injured fragments.

Table SM2.1. Regeneration rate (RR), number of coral fragments not regenerated (NR), regenerated (R) and dead (D) of each coral species (At - Acropora tenuis; Mc B - Montipora capricornis (brown morphotype); Mc G - Montipora capricornis (green morphotype); Tr -Turbinaria reniformis; El - Echinopora lamellosa; Gf - Galaxea fascicularis; Pd - Pocillopora damicornis; Sp - Stylophora pistillata; Pc - Psammocora contigua) for period of time in days (20, 40, 60) and temperature experiment (26 °C, 30 °C, 32 °C). NA – not available.

	26 °C												30 °C												32 °C											
	20				40				60				20				40				60				20				40				60			
Species	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**	RR	**NR**	**R**	**D**
At	0.24 ± 0.09	2	6	2	0.12 ± 0.05	0	8	2	NA	0	8	2	0.43 ± 0.23	2	8	0	0.27 ± 0.11	0	10	0	NA	0	10	0	0.30 ± 0.14	5	5	0	NA	0	1	9	NA	0	0	10
Mc B	0.29 ± 0.16	8	2	0	0.14 ± 0.10	5	5	0	0.11 ± 0.05	1	9	0	0.72 ± 0.21	2	8	0	0.41 ± 0.39	0	10	0	NA	0	10	0	0.51 ± 0.27	5	5	0	NA	0	0	10	NA	0	0	10
Mc G	0.16 ± 0.07	10	0	0	0.18 ± 0.09	10	0	0	0.15 ± 0.09	3	7	0	0.24 ± 0.14	10	0	0	0.09 ± 0.05	10	0	0	0.23 ± 0.13	8	2	0	0.11 ± 0.07	10	0	0	0.08 ± 0.08	10	0	0	NA	0	0	10
Tr	0.19 ± 0.14	9	1	0	0.11 ± 0.11	6	4	0	0.09 ± 0.08	3	7	0	0.34 ± 0.28	8	2	0	0.33 ± 0.22	0	10	0	NA	0	10	0	0.52 ± 0.16	0	10	0	NA	0	10	0	NA	0	10	0
El	0.23 ± 0.14	9	1	0	0.34 ± 0.13	0	10	0	NA	0	10	0	0.58 ± 0.27	3	7	0	0.55 ± 0.71	1	9	0	0.13 ± 0.00	1	9	0	0.84 ± 0.18	0	10	0	NA	0	10	0	NA	0	0	10
Gf	0.33 ± 0.18	10	0	0	0.29 ± 0.11	3	7	0	0.16 ± 0.09	0	10	0	0.91 ± 0.46	3	7	0	0.29 ± 0.22	1	9	0	0.09 ± 0.00	0	10	0	0.96 ± 0.55	5	5	0	0.43 ± 0.22	1	9	0	0.16 ± 0.00	1	9	0
Pd	0.40 ± 0.13	0	10	0	NA	0	10	0	NA	0	10	0	0.50 ± 0.25	1	9	0	NA	0	1	9	NA	0	0	10	0.13 ± 0.02	2	4	4	NA	0	0	10	NA	0	0	10
Sp	0.65 ± 0.28	1	9	0	0.24 ± 0.00	0	10	0	NA	0	10	0	0.67 ± 0.38	2	8	0	0.54 ± 0.14	0	9	1	NA	0	0	10	0.46 ± 0.13	0	8	2	NA	0	0	10	NA	0	0	10
Pc	0.20 ± 0.13	3	7	0	0.16 ± 0.06	0	10	0	NA	0	10	0	0.64 ± 0.31	6	4	0	0.37 ± 0.16	0	10	0	NA	0	10	0	0.63 ± 0.49	0	10	0	NA	0	10	0	NA	0	5	5

CHAPTER 3

Synergistic effects of warming and lower salinity on the asexual reproduction of reef-forming corals

Dias, M., Ferreira, A., Gouveia, R., & Vinagre, C. (2019). Ecological Indicators, 98, 334-348. DOI:10.1016/j.ecolind.2018.11.011

Abstract

Tropical storms produce competent self-regenerating coral fragments capable of originating new coral colonies. These climatic events are predicted to increase in the future due to ocean warming, the main cause of coral bleaching. Estimated increases in precipitation will affect salinity at shallow reef areas which combined with ocean warming will aggravate the effects of coral bleaching. In this study the long-term effects of increased temperature (30 ºC), low salinity (20 psu) and the combination of these stressors were investigated and compared to the control treatment (26 ºC, 33 psu). Mortality, condition, growth, and regeneration ability of nine Indo-Pacific reef-forming coral species were assessed, for 60 days. The coral colonies used were kept in captivity for several years, providing information on their thermal and salinity history. Twenty replicate fragments were cut from each coral species' colony, weighed and glued to the top of a numbered expansion anchor and then acclimated to the experimental aquarium. Half of these fragments was inflicted with one circular injury in order to test its effects on mortality, condition, and growth rate and also to estimate tissue regeneration rates. The results showed that mortality rate was highest in the high temperature + low salinity treatment, reaching 100% for seven out of the nine coral species, in the first six days of experiment, 100% for *Psammocora contigua* on the 40th day and 75% for *Galaxea fascicularis* on the 60th day of experiment. Partial mortality was lowest for *P. contigua* and *G. fascicularis* in the low salinity treatment. These two coral species also presented the best coral condition. Coral fragments growth rates decreased as temperature increased and salinity decreased except for *G. fascicularis* fragments that showed similar growth rates at 26 ºC and 30 ºC for both salinities. Regeneration rates increased with temperature being highest in the high temperature treatment and lowest at 20 psu. It was concluded that *G. fascicularis* and *P. contigua* are the most resilient to low salinity. *G. fascicularis* fragments were the only ones that withstood the synergistic effects of high temperature and low salinity and are likely to become dominant through asexual reproduction in the Indo-Pacific coral reefs in a warmer ocean, especially in coastal areas prone to low salinity episodes.

Keywords: global climate change, high temperature, low salinity, synergistic effects, heat stress, hyposaline stress.

3.1. Introduction and literature review

Coral reefs are one of the largest and most diverse reservoirs of biodiversity throughout the world (Baker et al., 2008; Prada et al., 2010). Scleractinian corals are the dominant taxonomic group of tropical coral reef ecosystems, as their association with symbiotic intracellular dinoflagellate (zooxanthellae, *Symbiodinium* spp.) (Hoegh-Guldberg, 2011) is responsible for the high productivity and structural complexity of coral reefs (Bruno and Bertness, 2001; Idjadi and Edmunds, 2006). Coral reefs worldwide are among the most vulnerable ecosystems to global climate change (Hughes et al., 2003; West and Salm, 2003; Hoegh-Guldberg et al., 2007). Climate change is leading to rising sea surface temperatures, low seawater salinities, and ocean acidification (Hughes et al., 2003; Hoegh-Guldberg et al., 2007; Faxneld et al., 2010; IPCC, 2014) compromising scleractinian corals' growth and mortality (Carpenter et al., 2008; Padilla-Gamiño et al., 2013). Currently, one-third of reef-building corals experience high extinction risk from climate change (Carpenter et al., 2008).

Coral bleaching episodes are strongly associated with sustained and anomalously high sea surface temperatures (Jokiel and Coles, 1974; Hoegh-Guldberg, 1999; Loya et al., 2001; McClanahan et al., 2009). Bleaching episodes can also be triggered by a range of other environmental stress factors, including salinity (Wild et al., 2011). Localized coral bleaching events due to low seawater salinities at shallow reef areas (Kerswell and Jones, 2003), following intensive rainfall during tropical storms, have been reported throughout the world (Goreau, 1964; Coles and Jokiel, 1992; Jokiel et al., 1993; Van Woesik et al., 1995; Blakeway, 2004). These events' effects may be exacerbated during episodes of elevated seawater surface temperatures (Xiubao et al., 2009; Faxneld et al., 2010).

Coral bleaching is a stress reaction that describes the morphological changes that occur during the breakdown of the coral-zooxanthellae association (Glynn, 1991; DeSalvo et al., 2008). Upon bleaching, corals lose a considerable number of their zooxanthellae and/or photosynthetic pigments per algal cell (Glynn, 1993; Hoegh-Guldberg, 1999), giving the colony a white appearance (Brown, 1997). As healthy corals normally supplement their metabolism through the provision of photosynthetic compounds by zooxanthellae (Davy et al., 2012), bleaching

culminates in significant reductions in zooxanthellae photosynthesis accompanied by drastic changes in physiology (Lesser and Shick, 1989, Muscatine et al., 1991). Therefore, bleached corals usually decrease skeletal calcification rate (Leder et al., 1991), tissue regeneration ability (Meesters and Bak, 1993), growth rates and reproductive capacities (Szmant and Gassman, 1990; Baird and Marshall, 2002), as well as increased susceptibility to disease (Harvell et al., 1999). If a heat stress event is prolonged or particularly severe it may result in partial or complete coral mortality (Brown et al., 2002; Jones, 2008; Hoegh-Guldberg, 2011; Wooldridge, 2014). On the other hand, when corals survive the heat stress event and the stress has ended, the symbiotic association can be re-established and their physiology may be restored (Grottoli et al., 2014; Schoepf et al., 2015). Mass coral bleaching episodes are responsible for live coral cover depletion in many parts of the world (Aronson et al., 2002; Bruno and Selig, 2007; Baker et al., 2008; Burt et al., 2011), resulting in reef community structure shifts that reduce biodiversity in the marine tropics (Baker et al., 2008; Eakin et al., 2009; Norström et al., 2009; Schutte et al., 2010).

Average sea temperatures surrounding coral reefs are projected to increase by 1– 3.7 °C by the end of the century (IPCC, 2014, 2018), as are the frequency and severity of thermally induced coral bleaching events in most tropical oceans (Crabbe, 2008; IPCC, 2014, 2018). Globally, thermally induced bleaching is projected to occur annually in most oceans within 20–40 years (Crabbe, 2008; Császár et al., 2010). Warmer seas are likely to drive more intense and frequent tropical storms (hurricanes, cyclones, typhoons) (Walsh and Ryan, 2000; Hughes et al., 2003; Emanuel, 2005) with larger peak wind speeds and heavier precipitation (Emanuel 2005; IPCC 2014). The frequency and maximum intensity of categories 4 and 5 storms are also expected to increase (0–25%) besides an increase in tropical cyclones rainfall rates (5–20%) (Christensen et al., 2013; IPCC, 2014), which may lead to drastic changes in inshore salinity levels (Milly et al., 2002; Haapkylä et al., 2011).

Coral community maintenance, repopulation, and recovery are greatly dependent on both sexual and asexual reproductive processes (Glynn et al., 2017). While sexually produced larvae may establish highly dispersed and genetically diverse populations, asexual recruitment through fragmentation or fission may allow rapid expansion of a population locally and enable the propagation of well-adapted genotypes within an area (Miller and Ayre, 2004; Baums et al.,

2006), or assist in the colonization of habitats unfavorable for larval settlement (Heyward and Collins, 1985). The ratio of sexual and asexual recruitment is expected to change over the geographic range of a species, depending on the frequency of sexual recruitment, genet longevity (Coffroth and Lasker, 1998), and disturbance effects (e.g. ENSO events, global warming, tropical storms, Baums et al., 2006). Under current changes in climatic conditions and unparalleled anthropogenic disturbance to reef ecosystems, the success of sexual reproduction may be low (Hoegh-Guldberg, 2004; Albright and Mason, 2013; Levitan et al., 2014). This way, a rapidly changing environment may favor alternative asexual mechanisms of propagation.

Tropical storms are favorable to the propagation and expansion of branching coral forms by asexual reproduction of storm-generated fragments across the reef (Highsmith et al., 1980; Tunnicliffe, 1981; Highsmith, 1982; Lirman, 2000), which later re-attach to the substrate, growth and form a new colony (Foster et al., 2007). Asexual reproduction by fragmentation of plate and massive coral forms has also been noticed (Highsmith, 1980; Foster et al., 2007, 2013). Over the long term, fragmentation can decrease the risk of genotype mortality through the spatial separation of the fragments (Harper, 1977; Hughes, 1983). Given the current predictions of more frequent and intense storms it is reasonable to assume that coral asexual reproduction will increase through coral fragmentation.

Rapid coral fragments' recovery is attained when asexual reproduction and regeneration are possible (Highsmith, 1982). Following disturbance, the production of new colonies by fragmentation of existing colonies is considered to be a primary mode of reproduction and local distribution among major reef-building corals (Pearson, 1981; Tunnicliffe, 1981; Highsmith, 1982; Connell and Keough, 1985; Bruno, 1998). Asexual regeneration after fragmentation prevails in reef communities as it is observed in many coral species (Tunnicliffe, 1981; Fong and Glynn, 2000; Glynn and Fong, 2006). Fragmentation may also be adaptive, since it has been incorporated into the life history of a considerable number of the most successful scleractinian corals (Highsmith, 1982).

There is a noticeable variation in the extent of bleaching, both within and between coral colonies on a reef (Hoegh-Guldberg and Salvat, 1995; Marshall and Baird, 2000). Interspecific variability in reef-building corals susceptibility to heat stress can depend on the zooxanthellae clades (Rowan et al., 1997), colony morphology (Lang et al., 1992; Dias et al., 2018), tissue thickness (Hoegh-Guldberg, 1999; Loya et al., 2001), colony size (Edmunds, 2005; Shenkar et al., 2005), metabolic rates (Gates and Edmunds, 1999), mucus production rates (Fitt et al., 2009; Wooldridge, 2009), tissue concentration of fluorescent pigments (Salih et al., 1998), seasonality (Scheufen et al., 2017), heterotrophic feeding capacity (Grottoli et al., 2006; Levas et al., 2013), and coral species (Marshall and Baird, 2000; Dias et al., 2018). In addition, different tolerance of scleractinian corals to low salinity depends on coral species (Moberg et al., 1997; Lirman and Manzello, 2009; Faxneld et al., 2010; Berkelmans et al., 2012), colony morphology (Van Woesik et al., 1995; True, 2012), and their polyp retraction response under osmotic stress (Muthiga and Szmant, 1987). Nevertheless, not all the different descriptors proposed to explain the different susceptibility of scleractinian corals to both heat and hyposaline stressors are truly sustained by experimental evidence.

This differential tolerance of scleractinian corals to the effects of heat and hyposaline stressors may lead to dramatic changes in the community structure of coral reefs (Loya et al., 2001; True, 2012), however, few studies have been conducted on the combined effects of temperature and salinity on scleractinian corals (Coles and Jokiel, 1978; Hoegh-Guldberg and Smith, 1989; Porter et al., 1999; Xiubao et al., 2009; Faxneld et al., 2010), and only Xiubao et al. (2009) estimates their long-term effects (10 days). In addition, these studies only include one to two coral species with no contrasting morphologies.

In order to evaluate the effects of global (i.e. temperature) and localized (i.e. salinity) stressors altered by global climate change on reef coral communities, we submitted nine reef-building corals to high temperature (30 ºC), low salinity (20 psu), and their combination compared to control treatment (26 ºC, 33 psu) for 60 days and assessed how these environmental parameters affected their mortality, condition, growth, and regeneration ability. To our knowledge, this is the first study to assess the long-term effects of high temperature and low salinity on the mortality, coral condition, growth and regeneration post-fragmentation in nine common and widely distributed reef-building corals of the Indo-Pacific oceans (Veron, 1990, 2000). We

hypothesize that (i) mortality, condition, growth, and regeneration rates of the coral fragments vary as a function of species, temperature, salinity, and injury (ii) high temperature and low salinity have a synergistic negative effect on corals' mortality, condition, growth, and regeneration rates. Such an assessment is critical to understand the differential vulnerability of reef-forming coral species to these two different stressors and can help reveal future coral species dominance and decline and determine future biodiversity shifts on coral reef ecosystems.

3.2. Materials and methods

3.2.1. Coral specimens and maintenance conditions

This study investigated nine common and widely distributed Indo-Pacific coral species (Veron, 1990, 2000) with different morphologies: three plating species (*Montipora capricornis* brown morphotype (BM), *Turbinaria reniformis*, and *Echinopora lamellosa*), one massive species (*Galaxea fascicularis*), four branching species (*Acropora tenuis, Pocillopora damicornis, Stylophora pistillata*, and *Psammocora contigua)*, and one encrusting species (*Montipora capricornis* green morphotype (GM)). These coral species were chosen in order to use the largest number of species available at Oceanário de Lisboa with different colony morphology, a characteristic mentioned as having influence in coral species susceptibility to heat and hyposaline stress (Loya et al., 2001; True, 2012). Coral species identification was made according to Veron (2000). Information relative to the coral species in study and their zooxanthellae was taken from the literature and given in Tables 3.1 and 3.2. The coral colonies used in the present study have been kept in captivity in a coral stock aquarium at Oceanário de Lisboa (Portugal) for several years, which gave us information on their thermal and salinity history. Also, as all the coral colonies were subjected to the same homogeneous conditions during growth, we could investigate the species-specific differences to the experimental treatments.

Table 3.1. Characteristics of the coral species in study. Data taken from Veron, 1990, 2000; Richmond, 1997; Loya et al., 2001; LaJeunesse et al., 2004.

Coral species	Family	Colony morphology	Tissue thickness	Skeleton morphology	Reproductive mode	*Symbiodinium* clade	Depth range	Habitat
A. tenuis	Acroporidae	branching	thin	axial corallites are long and tubular; radial corallites are arranged in a neat rosette and have slightly flaring lips (0.8-1.2 mm diameter)	spawner	C, D	1-20m	Upper reef slopes, shallow subtidal habitats
M. capricornis (BM)	Acroporidae	plating	thin	corallites are immersed (0.6-1 mm diameter)	spawner	C	2-20 m	Lagoons, shallow tropical reef environments
M. capricornis (GM)	Acroporidae	encrusting	thin	corallites are immersed (0.6-1 mm diameter)	spawner	C	2-20 m	Lagoons, shallow tropical reef environments
T. reniformis	Dendrophylliidae	plating	thin	corallites are widely spaced, thick walled, immersed to conical in shape (2.8-4.2 mm diameter)	spawner	C, D	2-15 m	Fringing reefs where the water is turbid
E. lamellosa	Merulinidae	plating	thin	corallites are relatively thin walled and small (2.3-3.4 mm diameter)	spawner	C,D	1-40 m	Shallow water habitats with flat substrates
G. fascicularis	Euphylliidae	massive	thick	corallites are of mixed sizes, usually with 5.8-10.7 mm diameter with numerous septa reaching the corallite centre	spawner	C, D	1-15 m	Reef environments protected from strong wave action
P. damicornis	Pocilloporidae	branching	thin	corallites are immersed (0.8-1 mm diameter)	brooder	A, B, C, D	1-20 m	Shallow waters (exposed reef fronts, mangrove swamps)
S. pistillata	Pocilloporidae	branching	thin	corallites are immersed, conical or hooded (0.9-1.4 mm diameter)	brooder	A, C, D	1-15m	Shallow water reef environments exposed to strong wave action
P. contigua	Psammocoridae	branching	thin	corallite structures are fine and corallites are shallow (1.1-1.5 mm diameter)	spawner	C, D	0-30 m	Shallow reef environments and soft substrate

Table 3.2. Published data of zooxanthellae density and chlorophyll *a* concentration for the coral species in study. No data were found for *Montipora capricornis* (both morphotypes).

Coral species	Zooxanthellae density ($\times 10^6$ cells cm^{-2})	Chorophyll *a* $area^{-1}$ ($\mu g\ cm^{-2}$)	Cholophyll *a* $cell^{-1}$ ($pg\ cell^{-1}$)	Reference
Acropora tenuis	-	0.33-0.82	-	Abrego, 2008
	0.28-0.39	2.20-6.90	-	Tanaka et al., 2014
	-	6.98-13.97	-	Vogel, 2015
Turbinaria reniformis	0.37-0.87	-	-	Béraud et al., 2013
	0.47-0.76	-	-	Courtial et al., 2017
	0.76-1.37	-	-	Ferrier-Pagès et al., 2010
	0.59	3.30	-	Grottoli et al., 2018
	0.28 - 0.50	-	6.61 - 11.63	Hoadley et al., 2016
	0.61-0.80	3.50-4.75	-	Schoepf et al., 2013
	3.51 - 5.27	0.93 - 2.09	-	Tolosa et al., 2011
	-	1.21	-	Treignier et al., 2008
	0.91-1.60	-	-	Tremblay et al., 2015
	0.31-0.59	-	-	Ulstrup et al., 2006
	0.54-0.62	3.40-5.24	-	Zawada, 2015
Echinopora lamellosa	2.22	-	-	Li et al., 2008
	0.06 - 0.17	-	-	Titlyanov et al., 2001
Galaxea fascicularis	-	-	8.81 - 9.00	Al-Moghrabi et al., 1995
	1.24 - 3.18	-	-	Borell et al., 2008
	12.00	-	-	Brown et al., 1995
	1.80-2.91	-	-	Ferrier-Pagès et al., 2010
	0.58 - 1.00	2.33 - 4.16	4.24 - 4.56	Hidaka and Miyagi, 1999
	0.48-1.12	-	-	Higuchi et al., 2008
	2.90	-	6.75	Hii et al., 2009
	2.65-6.45	-	-	Mwaura et al., 2009
	-	1.00-4.10	-	Schutter et al., 2010
	2.58-3.17	3.66-4.21	1.54-1.81	Schutter et al., 2011
	2.60	-	-	Stimson et al., 2002
	0.10-1.02	-	-	Xiubao et al., 2009
Pocillopora damicornis	1.85-2.32	-	-	Courtial et al., 2017
	2.00	-	0.50	Glynn and D'Croz, 1990
	1.70	-	-	Glynn et al., 1992
	0.80-8.30	2.04-12.96	1.81-2.90	Hueerkamp et al., 2001
	0.31-0.51	3.00-9.00	-	Jokiel et al., 1982
	0.42-1.12	-	2.79-3.30	Jones et al., 2000
	0.53-0.79	7.44-13.00	13.21-19.92	Kinzie et al., 1984
	1.86	-	-	Li et al., 2008
	0.57-1.87	0.87-3.92	1.21-4.23	Mayfield et al., 2013
	0.35-2.33	-	-	Mwaura et al., 2009
	-	0.26	-	Nakano et al., 2009
	0.29	2.75-3.17	-	Schoepf et al., 2013
	0.53	-	-	Schrameyer et al., 2014
	0.40	-	-	Stimson and Kinzie, 1991
	1.10	-	-	Stimson et al., 2002
	1.20	-	-	Stimson, 1997
	0.73-1.10	-	-	Titlyanov and Titlyanova, 2002
	1.66	-	-	Titlyanov et al., 1996
	0.32-1.01	-	-	Ulstrup et al., 2006

Table 3.2. Published data of zooxanthellae density and chlorophyll *a* concentration for the coral species in study. No data were found for *Montipora capricornis* (both morphotypes) (continuation).

Coral species	**Zooxanthellae density ($\times 10^6$ cells cm^{-2})**	**Chorophyll a area^{-1} (µg cm^{-2})**	**Cholophyll a cell^{-1} (pg cell^{-1})**	**Reference**
Stylophora pistillata	0.83-1.02	-	-	Amir and Mohamed, 2017
	0.55-0.67	-	-	Ammar et al., 2013
	1.03-1.94	-	-	Borell et al., 2008
	-	3.60	-	Dubinsky et al., 1984
	0.06	1.82	3.00	Dubinsky et al., 1990
	1.60	3.60	2.20	Falkowski and Dubinski, 1981
	1.40-1.89	-	-	Ferrier-Pagès et al., 2010
	0.83-1.02	-	-	Fitt et al., 2009
	3.02-3.38	-	-	Godinot et al., 2011
	0.47-0.52	-	7.23-7.61	Hoegh-Guldberg and Smith, 1989
	0.22-0.74	-	-	Jimenez et al., 2012
	1.24-1.34	-	-	Jones et al., 1999
	0.18-1.01	0.70-2.46	0.88-1.60	Kerswell and Jones, 2003
	0.50	-	-	Kuroki and Van Woesik, 1999
	1.11	2.61	2.43	Muscatine et al., 1989
	1.04	3.11	2.98	Porter et al., 1984
	1.10	-	-	Stimson et al., 2002
	0.69-1.60	-	-	Titlyanov and Titlyanova, 2002
	0.68	-	-	Titlyanov et al., 1996
	1.42-1.97	-	-	Tremblay et al., 2012
	2.73-5.38	-	-	Tremblay et al., 2015
Psammocora contigua	1.09	-	-	Li et al., 2008
	1.90	-	-	Stimson et al., 2002

The coral stock aquarium system includes two connecting tanks (3500 L) where the coral collection is kept and is fitted with a degasification tower and Fiji live rock (0.5 m^3) for biological filtration. For water circulation purposes, a Hayward 1hp water circulation pump provided a turnover rate of 3.5 times per hour to the two identical tanks that hold the coral collection. An AquaMedic Turbo 5000 protein skimmer helps keeping nutrient concentrations low and increased surface water motion in the aquarium is accomplished by using two 250 L Carlson surge devices. This aquarium is also fitted with a calcium reactor (25-35 L/h). Lighting requirements are attained by using four Litpa Jet5 floodlight with an AquaMedic 400W HQi lamp (10000K) fitted on each on a 12 h light/ 12 h dark cycle. Eight air-stones are used in the aquarium to ensure good oxygen concentrations.

Photosynthetically Active Radiation (PAR) levels were measured with a spheric quantic sensor (LI-193SA) and a data logger (1400 model) and varied between 300-350 µE.$m^{-2}s^{-1}$ in the 400-700 nm waveband. Water quality parameters such as water temperature, pH and salinity are measured on a daily basis. Water samples are also weekly analyzed to determine ammonium, nitrites, nitrates, calcium concentration as well as oxygen concentration and saturation and alkalinity. The average values for the water quality parameters of the coral stock system are the following: water temperature at 25.0 °C, pH at 8.16, salinity at 33.0 psu, alkalinity at 100 mg l^{-1}, ammonia at 0.00 mg l^{-1}, nitrites at 0.003 mg l^{-1}, nitrates at 0.150 mg l^{-1}, calcium concentration at 346 mg l^{-1}, oxygen saturation at 105% and oxygen concentration at 7.1 mg l^{-1}. Calcium concentration levels are kept through daily 15L *kalkwasser* additions alongside the effluent water of the running calcium reactor aforementioned. Salinity is measured using a WTW Inolab conductivity meter Level 1 and is maintained with weekly additions of a saturated saline solution. Whenever alkalinity levels are below 100 mg l^{-1} sodium bicarbonate is added to the system. Aquarium cleaning routines are performed every week and a half and include a 15% water change, egg-crate replacement, bottom sand siphoning and cleaning of the tank walls.

3.2.2. Experimental setup

The experiments were conducted at Oceanário de Lisboa, Portugal (www.oceanario.pt).To test the effects of species on mortality, condition, growth, and regeneration rates, 20 replicate fragments were cut from each coral colony, using a pincer or a pair of pliers. For the plate, massive, and encrusting coral colonies the fragments were obtained by cutting approximately 30 mm sided squares and the fragments for the branching corals were cut approximately 20-40 mm in length. These procedures were performed in order to represent the fragmentation of the coral species colonies during a tropical storm event. Afterwards, the coral fragments were placed over egg crate panels in the coral stock aquarium until acclimation to the experimental aquarium.

The live wet mass of each coral fragment was obtained by blotting it with a paper towel to remove excess seawater, then weighing it in air on an electronic balance to the nearest 0.01 g (Titlyanov et al., 2005). Each coral fragment was glued with epoxy putty to the top of a numbered and pre-weighed nylon expansion anchor. Placement of the coral fragment differed with morphology: plate, massive, and encrusting fragments were placed in horizontal position and the branching fragments in vertical position. These procedures were performed in order to reduce to the lowest possible the dead tissue area resulting by epoxy putty application according with the coral morphology. Then, the set (anchor + coral fragment) was weighed to remove the epoxy putty weight off the calculations and placed back over egg crate panels in the coral stock aquarium to recover from the handling procedures for one day before acclimation to the experimental aquarium.

To determine the long-term response of the nine coral species to the individual and combined effects of high temperature and low salinity the coral fragments were exposed to different treatments: (a) control (26 °C, 33 psu) (26.1 ± 0.2 SD; 33.0 ± 0.1 SD), (b) high temperature (30 ºC) (30.2 ± 0.5 SD), (c) low salinity (20 psu) (20.0 ± 0.1 SD), and (d) high temperature + low salinity (30 ºC, 20 psu) (29.9 ± 0.2 SD; 20.0 ± 0.1 SD). Twenty coral fragments of each coral species were exposed to each of the different treatments for 60 days. Coral fragments were acclimated one hour per °C above the temperature of the coral stock aquarium (25 ºC). Coral reef-flat communities can experience temperature changes of 1 °C $hour^{-1}$ during spring tides (Berkelmans and Willis, 1999), and most of the coral species in this study colonize the reef-flat zone (Brown and Suharsono, 1990), so we used this heating rate to be similar to the conditions that most of these corals would experience in their natural environment. In order to standardize, this heating rate was applied for all the coral species. Also, studies have shown that salinity can drop very quickly in near-shore areas after heavy rainfalls (from 30 to 15 psu within 24 h, Jokiel et al., 1993), especially when concurrent with low tides (min to hours, Kerswell and Jones, 2003). Thus, we allowed the coral species to acclimate from 33 psu to 20 psu during five hours in both low salinity and high temperature + low salinity treatments. The coral fragments were placed 2 cm apart from one another and organized by coral species to avoid contact between fragments of the same and/or different coral species.

The experimental aquarium (400 L) was fitted with a sump (280L) filled with bioballs for biological filtration in which a Hailea 500 chiller and two Fluval M300 heaters controlled water temperature. For water circulation purposes, an AquaMedic OceanRunner 3500 pump provided a turnover rate of 5 times per hour. An AquaMedic Turboflotor 5000 Shorty protein skimmer helped keeping nutrient concentrations low and increased surface water motion in the aquarium was accomplished by using an AquaClear 110 powerhead. Lighting requirements similar to the coral stock aquarium were attained by using a Litpa Jet5 floodlight with an AquaMedic 400W HQi lamp (13000K) on a 12 h light/ 12 h dark cycle. An air-stone was used in the aquarium to ensure good oxygen concentrations.

Photosynthetically Active Radiation (PAR) levels were measured with a spheric quantic sensor (LI-193SA) and a data logger (1400 model) and varied between 320-345 $\mu E.m^{-2}s^{-1}$ in the 400-700 nm waveband. The experiments were conducted under low light conditions (320-345 $\mu E.m^{-2}s^{-1}$) in order to minimize synergistic effects with high light conditions (Fitt et al., 2001; Scheufen et al., 2017). Water quality parameters such as water temperature, pH and salinity were measured on a daily basis. Water samples were also weekly analyzed to determine ammonium, nitrites, nitrates, calcium concentration as well as oxygen concentration and saturation and alkalinity. These parameters were maintained as follow: pH at 8.3, alkalinity approximately at 100 mg l^{-1}, nitrites between 0.002 and 0.003 mg l^{-1}, nitrates between 0 and 1 mg l^{-1}, calcium concentration between 389 and 401 mg l^{-1}, oxygen saturation between 101 and 104% and oxygen concentration between 6.6 and 7.1 mg l^{-1}. Salinity was measured using a WTW Inolab conductivity meter Level 1 and it was maintained with daily balanced additions of reverse osmosis freshwater and filtered artificial seawater. Whenever alkalinity levels were below 100 mg l^{-1} sodium bicarbonate was added to the system. Aquarium cleaning routines included expansion anchors cleaning with a toothbrush and egg crate replacement, at least 3 times a week.

To test the effects of injury on mortality, condition, and growth rate of the nine coral species, ten coral fragments of each species were used as controls (undamaged) and the other ten fragments were inflicted with circular injuries designed to simulate damage by predators, after being acclimated to the experimental aquarium. These artificial injuries were 3 mm in diameter and were performed using a Dremel rotatory tool with a cutting disk. Only one injury was

inflicted in the middle section of each coral fragment to avoid edge effects. After these procedures, all fragments were placed over two 40 x 40 cm egg crate panels suspended 15 cm below the water surface of the experimental aquarium.

3.2.3. Analytical procedures

3.2.3.1. Mortality and coral condition assessments

Mortality was quantified as the percentage of dead fragments and partial mortality was visually quantified by estimating the percentage of dead area in the coral fragments and establishing four classes: [0,25%[, [25,50%[, [50,75%[, and [75,100%]. The coral condition of each coral species was visually assessed according to four categories: normal, pale, bleached and dead (Jokiel and Coles, 1974). Normal corals were defined as having their normal coloration while pale corals showed a visible decrease in pigmentation. Bleached corals were considered as totally colorless and dead corals had no tissue at all. Mortality was assessed every day of the experiment with only the relevant days indicated in the results, whereas partial mortality and coral condition were assessed every 20 days until the end of the experiment. Mortality, partial mortality, and coral condition were assessed always by the same person to remove observer bias.

3.2.3.2. Growth rate measurements

The coral fragments were weighted both in the first and last day of experiment in order to calculate the growth rate for each coral fragment. The relative growth rate (RGR), expressed as daily biomass increase, was calculated using the formula 3.1:

$$RGR = [\ln (Wf/Wi)] \,/\, [(tf - ti)] \times 100$$

where Wi is the initial weight, Wf is the final weight and tf and ti is the time interval between the starting and end date (Marinho-Soriano et al., 2006).

3.2.3.3. Regeneration rate of injuries

The size of the coral fragments' injury was measured immediately upon their infliction and every 20 days afterwards until the end of the experiment. Injury recovery was quantified through photograph analysis and the injury area was estimated using ImageJ image software. Whenever the injury was not visible, due to partial mortality or bleaching, that species fragment would not be considered for regeneration purposes. The tissue regeneration rates (Ts) were estimated after complete fragment regeneration or the last point in time each fragment was still alive. These regeneration rates were obtained by calculating the difference between the areas of the recovered surfaces (Ra) for any given interval (T_1 and T_0 in days), as follows (Cróquer et al., 2002), formula 3.2:

$$Ts = [Ra(T_1) - Ra(T_0)]/(T_1 - T_0)$$

3.2.4. Statistical analyses

A four-factor nested PERMANOVA (Anderson, 2001) was used, with Euclidean distance to calculate the similarity matrix (coral species, temperature, salinity and injury were fixed factors with temperature, salinity and injury nested in coral species) that was used to test whether coral fragments' partial mortality, growth and regeneration rates were affected by coral species, temperature, salinity, and injury (for partial mortality and growth rate) and was run for 9999 permutations. Analyses were performed using PERMANOVA+ for PRIMER v6 (PRIMER-E Ltd., Plymouth). Post-hoc pair-wise comparisons were then performed using PERMANOVA to compare between treatments and coral species. Differences were considered significant at $p < 0.05$.

3.3. Results

The factor injury had no effect on the partial mortality and growth rate of the coral fragments (PERMANOVA $p > 0.05$, Table 3.3) and therefore it was not considered as a factor in further analysis, with no separation between control and injured fragments.

Table 3.3. Summary of results of PERMANOVA permutation tests applied to report the effects of coral species, temperature, salinity, and injury in coral fragments' partial mortality, growth, and regeneration rates. Significant differences are marked a bold.

	df	SS	MS	Pseudo-F	p (perm)
	Partial mortality				
Coral species	8	2.5×10^5	3.1×10^4	68.5	**0.000**
Temperature	9	2.4×10^5	2.6×10^4	57.2	**0.000**
Salinity	9	3.9×10^6	4.3×10^5	945.1	**0.000**
Injury	9	3.8×10^3	4.3×10^2	0.9	0.525
Temperature x Salinity	9	2.5×10^5	2.7×10^4	60.1	**0.000**
Temperature x Injury	9	3.3×10^3	3.6×10^2	0.8	0.689
Salinity x Injury	9	4.9×10^3	5.4×10^2	1.2	0.262
Temperature x Salinity x Injury	9	3.3×10^3	3.7×10^2	0.8	0.684
	Growth rate				
Coral species	8	14.5	1.8	99.4	**0.000**
Temperature	7	14.5	2.1	113.3	**0.000**
Salinity	2	4.6	2.3	126.1	**0.000**
Injury	9	0.2	2.5×10^{-2}	1.4	0.194
Temperature x Salinity	1	2.2×10^{-3}	2.2×10^{-3}	0.1	0.713
Temperature x Injury	7	0.2	3.5×10^{-2}	1.9	0.074
Salinity x Injury	2	6.6×10^{-3}	3.3×10^{-3}	0.2	0.822
Temperature x Salinity x Injury	1	6.1×10^{-3}	6.1×10^{-3}	0.3	0.552
	Regeneration rate				
Coral species	8	3.7	0.5	13.0	**0.000**
Temperature	9	2.2	0.2	6.9	**0.000**
Salinity	2	0.7	0.3	9.6	**0.000**
Temperature x Salinity	2	0.4	0.2	5.6	**0.010**

3.3.1. Mortality and coral condition

3.3.1.1. Mortality

The coral species *A. tenuis* was the only one showing mortality at the control treatment (10%, Table 3.4). The fragments of *P. damicornis* and *S. pistillata* presented 100% mortality by the

60th day in the high temperature treatment (Table 3.4). In the low salinity treatment most of the coral species perished, with the exceptions of *G. fascicularis* and *P. contigua* (30% and 5%, respectively; Table 3.4). In the high temperature + low salinity treatment, *G. fascicularis* was the only survivor (survival rate of 25%, Table 3.4). In the single stressor treatments, mortality was higher in the low salinity treatment and lower in the high temperature treatment, which indicates that, in comparison with the control treatment, the decrease in salinity (-13 psu) was more detrimental to the coral species than the increase in temperature (+4 ºC). In the multiple stressors treatment, high temperature + low salinity, not only the corals species presented higher mortality percentage but also they presented it earlier in this treatment than in the single stress treatments (Table 3.4), which indicates that these two environmental variables acted synergistically.

3.3.1.2. Partial mortality

Partial mortality was significantly affected by the independent effects of coral species, temperature, and salinity (PERMANOVA $p < 0.05$, Table 3.3). There was also a significant effect of the interactions of temperature and salinity (PERMANOVA $p < 0.05$, Table 3.3).

There were significant differences in partial mortality between temperatures for most of the coral species (PERMANOVA $p < 0.05$, Table 3.3), exception of *A. tenuis* and *T. reniformis* fragments (PERMANOVA pair-wise $p > 0.05$, Table 3.5). At 30 ºC, partial mortality was in general higher than at 26 ºC, with the exception of *E. lamellosa* (Table 3.5), which indicates that the increase in temperature of 4 ºC was detrimental for most of the coral species in study.

Since the 20th day of experiment there were significant differences in partial mortality between salinities for all species (PERMANOVA $p < 0.05$, Table 3.3). At 20 psu, most coral species presented partial mortality near 100% on the 20th day, exception for *G. fascicularis* and *P. contigua* (Table 3.5). On the other hand, at 33 psu, partial mortality was relatively lower than at 20 psu, with higher partial mortality only observed in *P. damicornis* and *S. pistillata*'s fragments (Table 3.5), which indicates that the decrease in salinity of 13 psu was detrimental for all the coral species in study.

Table 3.4. Mortality rate of the nine coral species studied on specific days of the four experiments. C - control, HT - high temperature, LS - low salinity

	C									HT									LS								HT + LS								
Species	2	3	4	5	6	10	20	40	60	2	3	4	5	6	10	20	40	60	2	3	4	5	6	10	20	40	60	2	3	4	5	6	10	20	40	60
A. tenuis	0	0	10	10	10	10	10	10	10	0	0	0	0	0	0	0	0	0	20	50	65	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
M. capricornis (BM)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	40	65	90	90	100	50	70	95	95	100	100	100	100	100
M. capricornis (GM)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	10	10	15	30	75	100	100	65	100	100	100	100	100	100	100	100
T. reniformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100	100	0	25	65	100	100	100	100	100	100
E. lamellosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	60	90	100	100	100	100	100	90	100	100	100	100	100	100	100	100
G. fascicularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	15	30	30	30	0	15	40	55	55	65	70	75	75
P. damicornis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	85	100	0	0	5	85	100	100	100	100	100	0	70	100	100	100	100	100	100	100
S. pistillata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	100	0	30	50	80	85	95	95	95	100	0	25	55	90	100	100	100	100	100
P. contigua	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	0	0	0	0	0	0	85	100	100

Significant intraspecific differences were found among treatments: *G. fascicularis* and *P. contigua* at 20 psu (PERMANOVA pair-wise $p < 0.05$, Table 3.5). In the low salinity treatment they reached the class [75,100] in low percentages (30% and 15%, respectively), whilst in the high temperature + low salinity treatment they reached it in high percentages (75% and 100%, respectively; Table 3.5).

3.3.1.3. Coral condition

In the control treatment, only *A. tenuis* presented mortality (10%, Table 3.6). All the other coral species displayed their normal coloration throughout the course of the experiment (Table 3.6).

In the high temperature treatment, the fragments of *A. tenuis*, *M. capricornis* (GM), *E. lamellosa,* and *G. fascicularis* remained normal throughout the 60 days experiment (Table 3.6). 75% of the fragments of *M. capricornis* (BM) and *P. contigua* were pale on the 60th day of experiment, while *T. reniformis* fragments were completely paled on the 40th day (Table 3.6). Since the 20th day of experiment, a decrease in the percentage of bleached fragments was evident in *P. damicornis* and *S. pistillata* fragments (80% and 5%, respectively) due to them succumbing to mortality that reached 100% on the last day of the experiment (Table 3.6). These results indicate that *M. capricornis* (BM), *T. reniformis,* and *P. contigua* probably experienced some degree of heat stress in this treatment, displayed as color attenuation though never reaching the bleached condition, whereas *P. damicornis* and *S. pistillata* appear to be highly susceptible to heat stress, due to their complete mortality in this treatment.

Table 3.5. Partial mortality rate of the nine coral species studied every 20 days of the four experiments (C - control, HT - high temperature, LS - low salinity) and separated in percentage classes [0,25%[, [25,50%[, [50,75%[, and [75,100%].

		C				HT				LS				HT + LS			
Time	Species	[0,25[	[25,50[	[50,75[	[75,100]	[0,25[	[25,50[	[50,75[	[75,100]	[0,25[	[25,50[	[50,75[	[75,100]	[0,25[	[25,50[	[50,75[	[75,100]
20	*A. tenuis*	90	0	0	10	100	0	0	0	0	0	0	100	0	0	0	100
	M. capricornis (BM)	100	0	0	0	100	0	0	0	10	0	0	90	0	0	0	100
	M. capricornis (GM)	100	0	0	0	85	15	0	0	5	5	5	85	0	0	0	100
	T. reniformis	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	E. lamellosa	95	0	5	0	100	0	0	0	0	0	0	100	0	0	0	100
	G. fascicularis	100	0	0	0	100	0	0	0	20	30	20	30	0	0	30	70
	P. damicornis	100	0	0	0	95	0	0	5	0	0	0	100	0	0	0	100
	S. pistillata	100	0	0	0	100	0	0	0	5	0	0	95	0	0	0	100
	P. contigua	95	5	0	0	100	0	0	0	90	5	5	0	5	0	0	95
40	*A. tenuis*	90	0	0	10	85	10	5	0	0	0	0	100	0	0	0	100
	M. capricornis (BM)	100	0	0	0	100	0	0	0	0	0	10	90	0	0	0	100
	M. capricornis (GM)	100	0	0	0	30	55	15	0	0	0	0	100	0	0	0	100
	T. reniformis	100	0	0	0	95	5	0	0	0	0	0	100	0	0	0	100
	E. lamellosa	90	5	5	0	100	0	0	0	0	0	0	100	0	0	0	100
	G. fascicularis	100	0	0	0	100	0	0	0	0	0	70	30	0	0	25	75
	P. damicornis	100	0	0	0	15	0	0	85	0	0	0	100	0	0	0	100
	S. pistillata	100	0	0	0	50	25	5	20	0	0	5	95	0	0	0	100
	P. contigua	95	5	0	0	100	0	0	0	45	25	15	15	0	0	0	100
60	*A. tenuis*	90	0	0	10	75	25	0	0	0	0	0	100	0	0	0	100
	M. capricornis (BM)	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	M. capricornis (GM)	90	5	5	0	65	35	0	0	0	0	0	100	0	0	0	100
	T. reniformis	100	0	0	0	95	5	0	0	0	0	0	100	0	0	0	100
	E. lamellosa	85	10	5	0	100	0	0	0	0	0	0	100	0	0	0	100
	G. fascicularis	100	0	0	0	100	0	0	0	0	0	70	30	0	0	25	75
	P. damicornis	100	0	0	0	0	0	0	100	0	0	0	100	0	0	0	100
	S. pistillata	95	5	0	0	0	0	0	100	0	0	0	100	0	0	0	100
	P. contigua	90	0	10	0	100	0	0	0	45	25	15	15	0	0	0	100

In the low salinity treatment, *A. tenuis*, *T. reniformis*, *E. lamellosa*, and *P. damicornis* fragments perished in the first 20 days of experiment (Table 3.6). A decrease in the percentage of bleached fragments was evident in *M. capricornis* (BM), *M. capricornis* (GM), and *S. pistillata*. *M. capricornis* (GM) fragments started it by the 20th day with a decrease of 15%, while *M. capricornis* (BM) and *S. pistillata* started it latter, on the 40th day, with a decrease of 10% and 5%, respectively, due to them succumbing to mortality that reached the 100% on the 40th day for *M. capricornis* (GM) and on the 60th day for *M. capricornis* (BM) and *S. pistillata.* (Table 3.6). On the other hand, a decrease in the percentage of pale fragments was evident in *G. fascicularis* and *P. contigua* fragments, by the 20th day for *G. fascicularis* and the 40th day for *P. contigua* (5% and 45%, respectively), due to them becoming bleached. These two coral species were the only ones that did not reach 100% mortality (Table 3.6). These results indicate that *G. fascicularis* and *P. contigua* were the coral species most tolerant to this treatment, since these coral species only reached the dead condition in low percentages (30% and 5%, respectively).

In the high temperature + low salinity treatment, the fragments of *A. tenuis*, *M. capricornis* (both morphotypes), *T. reniformis*, *E. lamellosa*, *P. damicornis*, and *S. pistillata* presented 100% mortality in the first 20 days of experiment (Table 3.6), whilst *P. contigua* only perished on the 40th day (Table 3.6). *G. fascicularis* reached the bleached condition on the 20th day of experiment and the percentage of dead fragments reached 75% (Table 3.6). These results indicate that *G. fascicularis* was the only coral species tolerant to the full extent of this treatment, displayed by its survival rate of 25% on the 60th of experiment.

Table 3.6. Coral condition of the nine coral species studied every 20 days of the four experiments (C - control, HT - high temperature, LS - low salinity) and separated in four categories: normal, pale, bleached, and dead.

		C				HT				LS				HT + LS			
Time	Species	normal	pale	bleached	dead	normal	pale	bleached	dead	normal	pale	bleached	dead	normal	pale	bleached	dead
20	*A. tenuis*	90	0	0	10	100	0	0	0	0	0	0	100	0	0	0	100
	M. capricornis (BM)	100	0	0	0	100	0	0	0	0	0	10	90	0	0	0	100
	M. capricornis (GM)	100	0	0	0	100	0	0	0	0	10	15	75	0	0	0	100
	T. reniformis	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	E. lamellosa	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	G. fascicularis	100	0	0	0	100	0	0	0	0	5	65	30	0	0	30	70
	P. damicornis	100	0	0	0	0	0	95	5	0	0	0	100	0	0	0	100
	S. pistillata	100	0	0	0	0	0	100	0	0	0	5	95	0	0	0	100
	P. contigua	100	0	0	0	100	0	0	0	100	0	0	0	15	0	0	85
40	*A. tenuis*	90	0	0	10	100	0	0	0	0	0	0	100	0	0	0	100
	M. capricornis (BM)	100	0	0	0	100	0	0	0	0	0	10	90	0	0	0	100
	M. capricornis (GM)	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	T. reniformis	100	0	0	0	0	100	0	0	0	0	0	100	0	0	0	100
	E. lamellosa	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	G. fascicularis	100	0	0	0	100	0	0	0	0	0	70	30	0	0	25	75
	P. damicornis	100	0	0	0	0	0	15	85	0	0	0	100	0	0	0	100
	S. pistillata	100	0	0	0	0	0	95	5	0	0	5	95	0	0	0	100
	P. contigua	100	0	0	0	100	0	0	0	30	60	5	5	0	0	0	100
60	*A. tenuis*	90	0	0	10	100	0	0	0	0	0	0	100	0	0	0	100
	M. capricornis (BM)	100	0	0	0	25	75	0	0	0	0	0	100	0	0	0	100
	M. capricornis (GM)	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	T. reniformis	100	0	0	0	0	100	0	0	0	0	0	100	0	0	0	100
	E. lamellosa	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	100
	G. fascicularis	100	0	0	0	100	0	0	0	0	0	70	30	0	0	25	75
	P. damicornis	100	0	0	0	0	0	0	100	0	0	0	100	0	0	0	100
	S. pistillata	100	0	0	0	0	0	0	100	0	0	0	100	0	0	0	100
	P. contigua	100	0	0	0	25	75	0	0	20	15	60	5	0	0	0	100

3.3.2. Growth rate

Growth rate was significantly affected by the independent effects of coral species, temperature, and salinity (PERMANOVA $p < 0.05$, Table 3.3). Most coral species showed significant intraspecific differences between control and high temperature treatments (PERMANOVA pair-wise $p < 0.05$, Table 3.7), being highest in the control treatment, except for *G. fascicularis* that presented similar growth rates between these two treatments (PERMANOVA pair-wise $p > 0.05$, Table 3.7). *G. fascicularis* and *P. contigua* fragments showed significant intraspecific differences in growth rate between control and low salinity treatments, being highest in the control treatment (PERMANOVA pair-wise $p < 0.05$, Table 3.7). *G. fascicularis* fragments also presented significant intraspecific growth rate differences between high temperature and high temperature + low salinity treatments, being highest in the high temperature treatment (PERMANOVA pair-wise $p < 0.05$, Table 3.7). These results indicate that high temperature, low salinity, and high temperature + low salinity treatments affected negatively coral species' growth when compared with control treatment, displayed as growth rate decrease in these three treatments.

In the control treatment, the highest growth rates were observed in *A. tenuis* and *P. contigua* fragments (PERMANOVA pair wise $p < 0.05$) ($1.13 \pm 0.21\%$ day^{-1} and $1.08 \pm 0.17\%$ day^{-1}, respectively; PERMANOVA pair wise $p > 0.05$), whereas the lowest one was observed in *G. fascicularis* fragments ($0.26 \pm 0.07\%$ day^{-1}, PERMANOVA pair wise $p < 0.05$; Table 3.7). In the high temperature treatment, the highest growth rate was observed in *A. tenuis* fragments ($0.49 \pm 0.10\%$ day^{-1}, PERMANOVA pair wise $p < 0.05$), whereas the lowest ones were observed in *E. lamellosa* and *M. capricornis* (GM) fragments (PERMANOVA pair wise $p < 0.05$) ($0.16 \pm 0.05\%$ day^{-1} and $0.13 \pm 0.05\%$ day^{-1}, respectively; PERMANOVA pair wise $p > 0.05$; Table 3.7). In the low salinity treatment, the highest growth rate was observed in *P. contigua* fragments ($0.40 \pm 0.12\%$ day^{-1}, PERMANOVA pair wise $p < 0.05$), while the lowest one was observed in *G. fascicularis* fragments ($0.07 \pm 0.03\%$ day^{-1}, PERMANOVA pair wise $p < 0.05$; Table 3.7). These results indicate that branching corals have higher growth rates than plate, encrusting, and massive coral species.

Table 3.7. Coral species' average growth rate ± standard deviation (% day^{-1}) in the four experiments. C - control, HT - high temperature, LS - low salinity. NA – Not Available.

Species	C	HT	LS	HT + LS
A. tenuis	1.13 ± 0.21	0.49 ± 0.10	NA	NA
M. capricornis (BM)	0.73 ± 0.13	0.30 ± 0.18	NA	NA
M. capricornis (GM)	0.64 ± 0.15	0.13 ± 0.05	NA	NA
T. reniformis	0.44 ± 0.16	0.22 ± 0.08	NA	NA
E. lamellosa	0.43 ± 0.27	0.16 ± 0.05	NA	NA
G. fascicularis	0.26 ± 0.07	0.26 ± 0.09	0.07 ± 0.03	0.03 ± 0.03
P. damicornis	0.92 ± 0.14	NA	NA	NA
S. pistillata	0.62 ± 0.14	NA	NA	NA
P. contigua	1.08 ± 0.17	0.36 ± 0.10	0.40 ± 0.12	NA

3.3.3. Regeneration rate

Regeneration rate was significantly affected by the independent effects of coral species, temperature, and salinity (PERMANOVA $p < 0.05$, Table 3.3). There was also a significant effect of the interactions of temperature and salinity (PERMANOVA $p < 0.05$, Table 3.3). Almost all coral species presented significant intraspecific differences between control and high temperature treatments, with higher regeneration rates in the high temperature treatment (PERMANOVA pair-wise $p < 0.05$, Table 3.8), except for *M. capricornis* (GM), *P. damicornis*, and *S. pistillata* that presented similar regeneration rates between these two treatments (PERMANOVA pair-wise $p > 0.05$, Table 3.8). *G. fascicularis* and *P. contigua* fragments presented similar regeneration rates between low salinity and high temperature + low salinity treatments (PERMANOVA pair-wise $p > 0.05$, Table 3.8). *P. contigua* fragments presented significant intraspecific differences between high temperature and high temperature + low salinity treatments, with higher regeneration rates in the high temperature treatment (PERMANOVA pair-wise $p < 0.05$, Table 3.8). On the other hand, both *G. fascicularis* and *P. contigua* fragments presented similar intraspecific regeneration rates between control and low salinity treatments (PERMANOVA pair-wise $p > 0.05$, Table 3.8). These results indicate that regeneration rates increased with temperature, which is related with an increase in metabolism, being highest in the high temperature treatment and lowest at 20 psu as salinity decreased, probably a result of osmotic stress.

In the control treatment, *S. pistillata* fragments presented the highest regeneration rate (0.65 ± 0.29 mm^2 day^{-1}, PERMANOVA pair wise $p < 0.05$), whereas the lowest ones were presented by *M. capricornis* (BM), *T. reniformis*, and *M. capricornis* (GM) fragments (PERMANOVA pair wise $p < 0.05$) (0.24 ± 0.16 mm^2 day^{-1}, 0.16 ± 0.10 mm^2 day^{-1} and 0.17 ± 0.03 mm^2 day^{-1}, respectively; PERMANOVA pair-wise $p > 0.05$, Table 3.8). In the high temperature treatment, *G. fascicularis*, *S. pistillata*, *M. capricornis* (BM), *P. contigua*, *E. lamellosa*, and *P. damicornis* fragments had the highest regeneration rates (PERMANOVA pair wise $p < 0.05$) (0.73 ± 0.27 mm^2 day^{-1}, 0.71 ± 0.32 mm^2 day^{-1}, 0.69 ± 0.21 mm^2 day^{-1}, 0.59 ± 0.27 mm^2 day^{-1}, 0.58 ± 0.27 mm^2 day^{-1}, 0.50 ± 0.25 mm^2 day^{-1}, respectively; PERMANOVA pair-wise $p > 0.05$), whereas *M. capricornis* (GM) fragments showed the lowest regeneration rate (0.16 ± 0.07 mm^2 day^{-1}, PERMANOVA pair wise $p < 0.05$, Table 3.8). In the low salinity treatment, the highest regeneration rate was observed in *G. fascicularis* (0.26 ± 0.14 mm^2 day^{-1}, PERMANOVA pair wise $p < 0.05$) and the lowest in *P. contigua's* fragments (0.14 ± 0.05 mm^2 day^{-1}, PERMANOVA pair wise $p < 0.05$, Table 3.8). These results indicate that regeneration rate differences among these coral species do not have a pattern associated with coral morphology.

Table 3.8. Coral species' average regeneration rate ± standard deviation (mm^2 day^{-1}) in the four experiments. C - control, HT - high temperature, LS - low salinity. *Only one individual. NA – Not Available.

Species	C	HT	LS	HT + LS
A. tenuis	0.23 ± 0.09	0.42 ± 0.22	NA	NA
M. capricornis (BM)	0.24 ± 0.16	0.69 ± 0.21	NA	NA
M. capricornis (GM)	0.17 ± 0.03	0.16 ± 0.07	NA	NA
T. reniformis	0.16 ± 0.10	0.35 ± 0.16	NA	NA
E. lamellosa	0.30 ± 0.11	0.58 ± 0.27	NA	NA
G. fascicularis	0.30 ± 0.06	0.73 ± 0.27	0.26 ± 0.14	0.16 ± 0.00*
P. damicornis	0.40 ± 0.13	0.50 ± 0.25	NA	NA
S. pistillata	0.65 ± 0.29	0.71 ± 0.32	NA	NA
P. contigua	0.22 ± 0.10	0.59 ± 0.27	0.14 ± 0.05	0.18 ± 0.10

3.4. Discussion and conclusions

This study shows the differential vulnerability of reef-forming coral species towards conditions of warming and low salinity, which will negatively affect the ability of their fragments to regenerate and grow after being separated from their parent colonies, potentially leading to a major shift in tropical coral reef ecosystems' biodiversity.

3.4.1. Mortality

In the high temperature treatment, the coral species *P. damicornis* and *S. pistillata* had the highest susceptibility to heat stress during the study, displaying the worst coral condition and earlier tissue loss compared to other species. Branching pocilloporids (e.g. *Pocillopora damicornis* and *Stylophora pistillata)* are cited in many reports, both in experimental work (Jokiel and Coles, 1974; Dias et al., 2018) and in the field (Glynn, 1983; Loya et al., 2001), as being taxa severely damaged by heat stress, mainly due to their morphology (Brown and Howard, 1985; Hoegh-Guldberg and Salvat, 1995), since branching corals die more often than encrusting and massive growth forms (Marshall and Baird, 2000; McClanahan et al., 2002). However, other factors affecting coral species susceptibility to heat stress must also be considered, such as *Symbiodinium* clade (Magalon et al., 2007), tissue thickness (Loya et al., 2001), respiratory rates (Jokiel and Coles, 1990), mucus production rate (Fitt et al., 2009), tissue concentration of fluorescent pigments (FPs; Salih et al., 1998), and heterotrophic feeding capacity (Grottoli et al., 2006). According to the characteristics of the coral species described in Table 3.1, both *P. damicornis* and *S. pistillata* can harbor *Symbiodinium* clade C, which is consistent with their high susceptibility to heat stress (Rowan et al., 1997). Nevertheless, all the coral species in study can harbor *Symbiodinium* clade C so, in this case, we cannot say that *Symbiodinium* clade was a factor influencing their higher susceptibility to heat stress. Regarding all the other factors aforementioned, pocilloporids are known as having thinner tissues (Hoegh-Guldberg and Salvat, 1995), the greatest respiratory rates (Franzisket, 1970), very low mucus production (Franzisket, 1970; Fitt et al., 2009), relatively low densities of FPs (Salih et al., 1998), and a very limited heterotrophic capacity (Houlbrèque and Ferrier-Pagès, 2009; Ziegler et al., 2014) when compared with other taxa. Those inherent traits are consistent with *P.*

damicornis and *S. pistillata* higher susceptibility to heat stress (Jokiel and Coles, 1990; Salih et al., 1998; Hoegh-Guldberg et al., 1999; Grottoli et al., 2006; Fitt et al., 2009). Another aspect to have into account is the life-history strategies of the coral species in study, *P. damicornis* and *S. pistillata* are considered "weedy" corals (Darling et al., 2012), defined as small corals with brooding reproduction, fast growth rates and high population turnover (Knowlton, 2001), and weedy corals have been considered sensitive to heat stress in the Indo-Pacific (Darling et al., 2013).

Mortality and partial mortality were higher at 20 psu than at 33 psu, such reductions in salinity are known to be potentially lethal to corals, in part due to corals' limited osmoregulation capacity (Ferrier-Pagès et al., 1999; Kerswell and Jones, 2003; Manzello and Lirman, 2003). Observations in previous studies, both in experimental work (Edmondson, 1928) and in the field (Coles and Jokiel, 1992; Jokiel et al., 1993), have supported the estimates of 15 psu to 20 psu for 24 h or more as the lower lethal salinity in scleractinian corals. Many reports refer to salinity reductions causing massive coral and reef organism mortality and it is during major storm events that its effects on coral reefs are most devastating (Goreau, 1964; Van Woesik et al., 1995; Blakeway, 2004).

The mortality results obtained in the low salinity treatment indicate that the tolerance to hyposaline stress is highly species-specific. The pocilloporids *Pocillopora damicornis* and *Stylophora pistillata* and the genus *Acropora* were already known to be highly sensitive to lowered salinity (Jokiel et al., 1993; Moberg et al., 1997; Ferrier-Pagès et al., 1999; Kerswell and Jones, 2003; Blakeway, 2004). However, as all the fragments of *T. reniformis* and *E. lamellosa* perished on the 6th day of experiment, and most of the fragments of *M. capricornis* (both morphotypes) perished on the 20th day, we show that these species are also highly sensitive to lowered salinity, which was previously unknown. Most of *G. fascicularis* and *P. contigua* fragments survived for 60 days in the low salinity treatment, confirming these species exceptional tolerance to low salinity (Van Woesik, 1994; Xiubao et al., 2009).

High mortality in the high temperature-low salinity treatment indicates that long-term synergy of higher temperature and lower salinity is very detrimental to all coral species tested, leading

to the death of most of the coral fragments on the 6th day of the experiment. This synergistic effect had already been documented in other studies (Coles and Jokiel, 1978; Mayfield and Gates, 2007; Xiubao et al., 2009). Only *P. contigua* showed higher tolerance, with its fragments lasting for 40 days under these conditions. *G. fascicularis* proved to be the most resistant species to the synergy of higher temperature and lower salinity, surviving the whole 60-day experiment, albeit only with 25% of the fragments still alive by the end of the experiment. The results obtained in this treatment indicate that even short-term exposure of two days to salinities of 20 psu reduces the tolerance of several coral species to high temperature.

3.4.2. Coral condition

Understanding the variation in coral species response to heat and hyposaline stressors is necessary to accurately predict how coral reefs condition and population structure will change in a climate change scenario of high temperature and low salinity. In the control treatment almost all the coral species remained unbleached throughout the course of the experiment. In the high temperature treatment, *P. damicornis* and *S. pistillata* fragments were the only ones that reached the bleached condition and 100% mortality, which reveal that these two species are highly susceptible to heat stress.

In the low salinity treatment, four out of the nine coral species presented 100% mortality in the first 20 days of experiment without previous fragments' discoloration, *A. tenuis, T. reniformis, E. lamellosa,* and *P. damicornis*. On the other hand, the coral species *M. capricornis* (BM), *M. capricornis* (GM), *S. pistillata, G. fascicularis,* and *P. contigua* showed tissue discoloration, before complete mortality in the first three coral species. The discoloration of corals following osmotic stress is caused by the breakdown in coral-zooxanthellae symbiosis and it has been observed in other studies (Goreau, 1964; Kerswell and Jones, 2003), however, when a coral species cannot cope with the osmotic stress it perishes without previous discoloration (Hoegh-Guldberg and Smith, 1989; Jokiel et al., 1993). Coral death was followed by immediate tissue sloughing off which revealed the underlying white calcareous skeleton, although *T. reniformis* fragments became covered by thick whitish-brown slime and its coenosarc disintegrated into mucous blebs.

In the high temperature + low salinity treatment, seven out of the nine coral species showed complete mortality in the first 20 days of experiment without previous fragments' discoloration, *A. tenuis, M. capricornis* (both morphotypes), *T. reniformis, E. lamellosa, P. damicornis* and *S. pistillata*. The synergistic effects of high temperature and low salinity were more than these coral species could cope with. Nevertheless, *G. fascicularis* although presenting some percentage of bleached fragments, did not present 100% mortality.

3.4.3. Growth

Coral growth is affected by environmental parameters such as sea surface temperature and salinity (Lough and Cooper 2011), and measuring coral growth has become relevant as an indicator of possible degradation of coral reefs due to climate change (Buddemeier et al., 2004; Manzello et al., 2008; Manzello, 2010; Anthony et al., 2011). Coral fragments' growth rates decreased with increase in water temperature, this is in agreement with physiological data presenting an accentuated decline in coral growth at temperatures only a few degrees above optimum levels (Berkelmans and Willis 1999). Growth rates also decreased as salinity decreased, which is corroborated by other studies stating that scleractinian corals exposed to rapid environmental fluctuations may exhibit a limited capacity for osmoregulation (Ranklin and Davenport, 1981; Mayfield and Gates, 2007), and that the prolonged exposure to salinity stress can affect coral growth (Coles and Jokiel, 1992; Porter et al., 1999).

Our results are also in accordance with another study showing that corals' growth rates decrease if salinity changes by ± 2 psu from optimal values (Ferrier-Pagès et al., 1999). In all treatments, *A. tenuis* and *P. contigua* presented the highest growth rates, with the exception of *A. tenuis* at 20 psu and *P. contigua* in the high temperature + low salinity treatment where they died. According with Guest et al. (2011), plate and massive morphologies grow slower than branched ones. Having this in mind, the lower growth rates of *M. capricornis* (GM) and *E. lamellosa* in the high temperature treatment, and the ones of *G. fascicularis* at 26 °C for both salinities can thus be explained. *G. fascicularis* fragments were the only ones that showed similar growth rate at 26 ºC and 30 ºC for both salinities, suggesting that this coral species is resistant to this level of heat stress. All the other coral species presented their highest growth rates in the control

treatment, probably as a result of heat stress in the high temperature treatment (Szmant and Gassman, 1990; Fine et al., 2002).

3.4.4. Regeneration

Understanding how extrinsic factors such as seawater temperature and salinity limit regeneration in corals is crucial since poor regenerative ability can lead to a reduction in colony fitness (Hall, 1997). The regeneration rate of the coral species studied generally increased with temperature increment. This is expected since increase in water temperatures accelerates coral fragments' metabolism and, as long as their specific stress levels are not achieved, their regeneration rates will be greater at higher temperatures. In addition, regeneration rate decreased in hyposaline conditions, which is also expected since low salinity is known to affect regeneration due to its negative effects on coral metabolism (Jokiel et al., 1993; Moberg et al., 1997). Coral species regenerative ability could not be ranked as in Hall (1997) probably because the experimental conditions, methodology, and coral species were different. In the low salinity treatment, regeneration rate of the only two species that survived (*P. contigua* and *G. fascicularis*) was not negatively impacted compared with the control treatment, whereas in the high temperature treatment most of the coral species presented increase in regeneration rates, exception of the coral species negatively impacted by heat stress. According to Hall (1997), branching corals present the highest regenerative ability, which in this study correspond to the ones most affected by heat and hyposaline stress. Thus, the use of different stress treatments may have altered the regenerative ability of the coral species in study.

The high mortality rates registered in the high temperature + low salinity treatment deter conclusions regarding regeneration. In the only species that survived the 60 days in this treatment, *G. fascicularis*, regeneration capacity was negatively impacted compared to control treatment.

Differential susceptibility and mortality among species can exert a major influence on coral community structure (Pratchett, 2001). According with the results obtained in this study, it is

expected that the coral species *G. fascicularis* will be more resistant in a warmer ocean, especially in coastal areas prone to low salinity episodes, and thus becoming more important in terms of coral cover. Nevertheless, it is important to have in consideration that this is a slow-growing coral species and may take a long time until increase in coral cover can be observed. On the other hand, other disturbances present in their natural habitat need to be taken into account, such as competition (Baird and Hughes, 2000), predation (Pratchett, 2001), and extreme events (tropical storms, Fabricius et al., 2008) that, like heat and hyposaline stress, affect with greater magnitude fast-growing coral species (True, 2012; Dias et al., 2018). Thus, the predicted increase in temperature and both frequency and intensity of tropical storms will likely cause local eradication or prevent susceptible species from resettle and will allow *G. fascicularis'* dominance.

This study focused on the interaction of high temperature and low salinity associated with the increase in frequency and intensity of tropical storms in the Indo-Pacific oceans, however, it needs to be cautioned that other environmental variables not tested in this study can be altered by global climate change (e.g. ultraviolet radiation levels (Hoegh-Guldberg and Bruno, 2010) and pH (Manzello, 2010)) and that alterations in the environmental variables studied may also happen, such as an increase in mean daily maximum temperature (Wergen and Krug, 2010), mean salinity (Durack et al., 2013), and sea surface temperatures (SST; Collins et al., 2010). Actually, other possible combinations of the environmental variables studied can be possible in the Indo-Pacific region, such as elevated temperatures and elevated salinity, due to lack of rainfall in the Pacific region, often for months determined by the IPO and ENSO phases (Dong and Dai, 2015), or due to increased evaporation in already dry regions, such as temperate Australia, making those regions saltier (Durack et al., 2012). Thus, our study only shows one of many possible scenarios.

Resistance capacity of most corals was overcome in long-term treatments at 20 psu. The coral species *G. fascicularis* and *P. contigua* were the most resistant to salinity stress. *G. fascicularis* fragments were the only ones that withstood the synergistic effects of high temperature and low salinity. This knowledge may have ecological implications in the understanding of coral reef biodiversity changes in the near future, as well as in coral reef conservation and management.

3.5. References

Abrego, D. (2008). Temporal and environmental influences on the early establishment and maintenance of coral-*symbiodinium* symbioses (Doctoral dissertation, James Cook University), pp. 134

Albright, R., & Mason, B. (2013). Projected Near-Future Levels of Temperature and pCO_2 Reduce Coral Fertilization Success. PLoS ONE 8(2): e56468.

Al-Moghrabi, S., Allemand, D., Couret, J. M., & Jaubert, J. (1995). Fatty acids of the scleractinian coral *Galaxea fascicularis*: effect of light and feeding. Journal of Comparative Physiology B, 165(3), 183-192.

Amir, L., & Mohamed, C. A. R. (2017). A short review of coral resilience in a changing marine environment. Pollution Research, 36, 693-708.

Ammar, M. S., Obuid-AllahO, A. H., & Al-Hammady, M. A. (2013). Experimental effect of temperature and sedimentation on bleaching of the two Red Sea corals *Stylophora pistillata* and *Acropora humilis*. Nusantara Biosciences, 5, 73-83.

Anderson, M. J. (2001). A new method for non-parametric analysis of variance. Austral Ecology, 26, 32–46.

Anthony, K. R. N., Maynard, J. A., Diaz-Pulido, G., Mumby, P. J., Marshall, P. A., Cao, L., & Hoegh-Guldberg, O. (2011). Ocean acidification and warming will lower coral reef resilience. Global Change Biology, 17, 1798−1808.

Aronson, R., Precht, W., Toscano, M., & Koltes, K. (2002). The 1998 bleaching event and its aftermath on a coral reef in Belize. Marine Biology, 141, 435–447.

Baird, A. H., & Hughes, T. P. (2000). Competitive dominance by tabular corals. An experimental analysis of recruitment and survival of understorey assemblages. Journal of Experimental Marine Biology and Ecology, 251, 117–132.

Baird, A. H., & Marshall, P. A. (2002). Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Marine Ecology Progress Series, 237, 133–141.

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: an ecological assessment of the long-term impacts, recovery trends and future outlooks. Estuarine, Coastal and Shelf Science, 80, 435–471.

Baums, I. B., Miller, M. W., & Hellberg, M. E. (2006). Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. Ecological Monographs, 76(4), 503-519.

Béraud, E., Gevaert, F., Rottier, C., & Ferrier-Pagès, C. (2013). The response of the scleractinian coral *Turbinaria reniformis* to thermal stress depends on the nitrogen status of the coral holobiont. Journal of Experimental Biology, jeb-085183.

Berkelmans, R., & Willis, B. L. (1999). Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs, 18, 219-228.

Berkelmans, R., Jones, A. M., & Schaffelke, B. (2012). Salinity thresholds of *Acropora* spp. on the Great Barrier Reef. Coral Reefs, 31, 1103-1110.

Blakeway, D. R. (2004). Patterns of mortality from natural and anthropogenic influences in Dampier corals: 2004 cyclone and dredging impacts, in: Stoddart, J.A., Stoddart, S.E. (Eds.), Corals of the Dampier Harbour: Their Survival and Reproduction during the Dredging Programs of 2004, pp. 61-72.

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. Journal of Experimental Marine Biology and Ecology, 364, 116-123.

Brown, B. E. (1997). Adaptations of reef corals to physical environmental stress. Advances in Marine Biology, 31, 221-299.

Brown, B. E., & Howard, L. S. (1985). Assessing the Effects of "Stress" on Reef Corals. Advances in Marine Biology, 22, 1-63.

Brown, B. E., & Suharsono (1990). Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. Coral Reefs, 8, 163-170.

Brown, B. E., Le Tissier, M. D. A., & Bythell, J. C. (1995). Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. Marine Biology, 122, 655-663.

Brown, B., Dunne, R., Goodson, M., & Douglas, A. (2002). Experience shapes the susceptibility of a reef coral to bleaching. Coral Reefs, 21, 119-126.

Bruno, J. F. (1998). Fragmentation in *Madracis mirabilis* (Duchassaing and Michelotti): how abundant is size-specific fragment survivorship in corals? Journal of Experimental Marine Biology and Ecology, 230, 169-181.

Bruno, J. F., & Bertness, M. D. (2001). Habitat modification and facilitation in benthic marine communities, in: Bertness, M.D., Gaines, S.D., Hay, M.E. (Eds.), Marine community ecology. Sunderland: Sinauer Associates, Massachusetts, pp. 201–218.

Bruno, J. F., & Selig, E. R. (2007). Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS One, 2(8), e711.

Buddemeier, R. W., Kleypas, J. A., & Aronson, R. B. (2004). Coral reefs and global climate change: potential contributions of climate change stresses on coral reef ecosystems. Pew Center on Global Climate Change, Arlington, VA.

Burt, J., Al-Harthi, S., & Al-Cibahy, A. (2011). Long-term impacts of coral bleaching events on the world's warmest reefs. Marine Environmental Research, 72, 225-229.

Carpenter, K. E., Abrar, M., Aeby, G., Aronson, R. B., Banks, S., Bruckner, A., ... & Edgar, G. J. (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science, 321(5888), 560-563.

Christensen, J.H., Kanikicharla, K.K., Aldrian, E., An, S.-I., Cavalcanti, I.F.A., De Castro, M., … & Midgley, P.M., (eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC AR5). Cambridge, United Kingdom and New York, New York: Cambridge University Press, pp.1217-1308.

Coffroth, M. A., & Lasker, H. R. (1998). Population structure of a clonal gorgonian coral: the interplay between clonal reproduction and disturbance. Evolution, 52(2), 379-393.

Coles, S. L., & Jokiel, P. L. (1978). Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Marine Biology, 49, 187–195.

Coles, S. L., & Jokiel, P. L. (1992). Effects of salinity on coral reefs, in: Connell, D.W., Hawker, D.W. (Eds.), Pollution in Tropical Aquatic Systems. CRC Press, London, pp. 147-166.

Collins, M., An, S. I., Cai, W., Ganachaud, A., Guilyardi, E., Jin, F. F., ... & Vecchi, G. (2010). The impact of global warming on the tropical Pacific Ocean and El Niño. Nature Geoscience, 3(6), 391.

Connell, J. H., & Keough, M. J. (1985). Disturbance and patch dynamics of subtidal marine animals on hard substrata, in: Pickett, S.T.A., White, P.S. (Eds.), The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, pp. 125-151.

Courtial, L., Roberty, S., Shick, J. M., Houlbrèque, F., & Ferrier-Pagès, C. (2017). Interactive effects of ultraviolet radiation and thermal stress on two reef-building corals. Limnology and Oceanography, 62(3), 1000-1013.

Crabbe, M. J. C. (2008). Climate change, global warming and coral reefs: Modelling the effects of temperature. Computational Biology and Chemistry, 32(5), 311-314.

Cróquer, A., Villamizar, E., & Noriega, N. (2002). Environmental factors affecting tissue regeneration of the reef – building coral *Montastraea annularis* (Faviidae) at Los Roques National Park, Venezuela. Revista de Biologia Tropical, 50, 1055-1065.

Császár, N. B. M., Ralph, P. J., Frankham, R., Berkelmans, R., & Van Oppen, M. J. H. (2010). Estimating the Potential for Adaptation of Corals to Climate Warming. PLoS ONE, 5: e9751.

Darling, E. S., McClanahan, T. R., & Côté, I. M. (2013). Life histories predict coral community disassembly under multiple stressors. Global Change Biology, 19(6), 1930-1940.

Darling, E. S., Alvarez-Filip, L., Oliver, T. A., McClanahan, T. R., & Côté, I. M. (2012). Evaluating life-history strategies of reef corals from species traits. Ecology Letters, 15(12), 1378-1386.

Davy, S. K., Allemand, D., & Weis, V. M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. Microbiology and Molecular Biology Reviews, 76(2), 229-261.

DeSalvo, M. K., Voolstra, C. R., Sunagawa, S., Schwarz, J. A., Stillman, J. H., Coffroth, M. A., ... & Medina, M. (2008). Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. Molecular Ecology, 17(17), 3952-3971.

Dias, M., Ferreira, A., Gouveia, R., Cereja, R., & Vinagre, C. (2018). Mortality, growth and regeneration following fragmentation of reef-forming corals under thermal stress. Journal of Sea Research, 141, 71-82.

Dong, B., & Dai, A. (2015). The influence of the interdecadal Pacific oscillation on temperature and precipitation over the globe. Climate Dynamics, 45, 2667-2681.

Dubinsky, Z., Falkowski, P. G., Porter, J. W., & Muscatine, L. (1984). Absorption and utilization of radiant energy by light-and shade-adapted colonies of the hermatypic coral *Stylophora pistillata*. Proceedings of the Royal Society of London. Series B. Biological Sciences, 222(1227), 203-214.

Dubinsky, Z., Stambler, N., Ben-Zion, M., McCloskey, L. R., Muscatine, L., & Falkowski, P. G. (1990). The effect of external nutrient resources on the optical properties and photosynthetic efficiency of *Stylophora pistillata*. Proceedings of the Royal Society of London. B. Biological Sciences, 239(1295), 231-246.

Durack, P. J., Wijffels, S. E., & Matear, R. J. (2012). Ocean salinities reveal strong global water cycle intensification during 1950 to 2000. Science, 336, 455-458.

Durack, P. J., Wijffels, S. E., & Boyer, T. P. (2013). Long-term salinity changes and implications for the global water cycle. In International Geophysics (Vol. 103, pp. 727-757). Academic Press.

Eakin, C. M., Lough, J. M., & Heron, S. F. (2009). Climate variability and change: monitoring data and evidence for increased coral bleaching stress. In Coral bleaching (pp. 41-67). Springer, Berlin, Heidelberg.

Edmondson, C. H. (1928). The ecology of an Hawaiian coral reef. Bernice P Bishop Bulletin, 45, 1-64.

Edmunds, P. J. (2005). The effect of sub-lethal increases in temperature on the growth and population trajectories of three scleractinian corals on the southern Great Barrier Reef. Oecologia, 146(3), 350-364.

Emanuel, K. (2005). Increasing destructiveness of tropical cyclones over the past 30 years. Nature, 436, 686-688.

Fabricius, K. E., De'Ath, G., Puotinen, M. L., Done, T., Cooper, T. F., & Burgess, S. C. (2008). Disturbance gradients on inshore and offshore coral reefs caused by a severe tropical cyclone. Limnology and Oceanography, 53(2), 690-704.

Falkowski, P. G., & Dubinsky, Z. (1981). Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature, 289, 172-174.

Faxneld, S., Jörgensen, T. L., & Tedengren, M. (2010). Effects of elevated water temperature, reduced salinity and nutrient enrichment on the metabolism of the coral *Turbinaria mesenterina*. Estuarine, Coastal and Shelf Science, 88(4), 482-487.

Ferrier-Pagès, C., Gattuso, J. P., & Jaubert, J. (1999). Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellate coral *Stylophora pistillata.* Marine Ecology Progress Series, 181, 309-314.

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. Journal of Experimental Marine Biology and Ecology, 390(2), 118-124.

Fine, M., Oren, U., & Loya, Y. (2002). Bleaching effect on regeneration and resource translocation in the coral *Oculina patagonica*. Marine Ecology Progress Series, 234, 119-125.

Fitt, W. K., Brown, B. E., Warner, M. E., & Dunne, R. P. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs, 20(1), 51-65.

Fitt, W. K., Gates, R. D., Hoegh-Guldberg, O., Bythell, J. C., Jatkar, A., Grottoli, A. G., ... & Iglesias-Prieto, R. (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. Journal of Experimental Marine Biology and Ecology, 373(2), 102-110.

Fong, P., & Glynn, P. W. (2000). A regional model to predict coral population dynamics in response to El Niño–Southern Oscillation. Ecological Applications, 10(3), 842-854.

Foster, N. L., Baums, I. B., & Mumby, P. J. (2007). Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastraea annularis*. Journal of Animal Ecology, 76(2), 384-391.

Foster, N. L., Baums, I. B., Sanchez, J. A., Paris, C. B., Chollett, I., Agudelo, C. L., ... & Mumby, P. J. (2013). Hurricane-driven patterns of clonality in an ecosystem engineer: the Caribbean coral *Montastraea annularis*. PLoS One, 8(1), e53283.

Frakzisket, L. (1970). The effect of mucus on respirometry of reef corals. Internationale Revue der gesamten Hydrobiologie und Hydrographie, 55(3), 409-412.

Gates, R. D., & Edmunds, P. J. (1999). The physiological mechanisms of acclimatization in tropical reef corals. The American Zoologist, 39(1), 30-43.

Glynn, P. W. (1983). Extensive 'bleaching'and death of reef corals on the Pacific coast of Panama. Environmental Conservation, 10(2), 149-154.

Glynn, P. W. (1991). Coral reef bleaching in the 1980s and possible connections with global warming. Trends in Ecology & Evolution, 6(6), 175-179.

Glynn, P. W. (1993). Coral reef bleaching: ecological perspectives. Coral Reefs, 12(1), 1-17.

Glynn, P. W., & D'croz, L. (1990). Experimental evidence for high temperature stress as the cause of El Nino-coincident coral mortality. Coral Reefs, 8(4), 181-191.

Glynn, P. W., & Fong, P. (2006). Patterns of reef coral recovery by the regrowth of surviving tissues following the 1997-98 El Nino warming and 2000, 2001 upwelling cool events in Panamá, eastern Pacific. In Proceedings of the 10th International Coral Reef Symposium, Okinawa (Vol. 2, pp. 624-630).

Glynn, P. W., Imai, R., Sakai, K., Nakano, Y., & Yamazato, K. (1992). Experimental responses of Okinawan (Ryukyu Islands, Japan) reef corals to high sea temperature and UV radiation. Proceedings of the 7th International Coral Reef Symposium Guam, 1, 27–37.

Glynn, P. W., Colley, S. B., Carpizo-Ituarte, E., & Richmond, R. H. (2017). Coral reproduction in the Eastern Pacific, in: Glynn, P. W., Manzello, D., Enochs, I. C. (Eds.), Coral Reefs of the Eastern Tropical Pacific, Springer, Dordrecht, pp. 435-476.

Godinot, C., Houlbreque, F., Grover, R., & Ferrier-Pagès, C. (2011). Coral uptake of inorganic phosphorus and nitrogen negatively affected by simultaneous changes in temperature and pH. PLoS One, 6(9), e25024.

Goreau, T. F. (1964). Mass expulsion of zooxanthellae from Jamaican reef communities after hurricane Flora. Science, 145, 383-386.

Grottoli, A. G., Rodrigues, L. J., & Palardy, J. E. (2006). Heterotrophic plasticity and resilience in bleached corals. Nature, 440, 1186–1189.

Grottoli, A. G., Warner, M. E., Levas, S. J., Aschaffenburg, M. D., Schoepf, V., McGinley, M., ... & Matsui, Y. (2014). The cumulative impact of annual coral bleaching can turn some coral species winners into losers. Global Change Biology, 20(12), 3823-3833.

Grottoli, A. G., Martins, P. D., Wilkins, M. J., Johnston, M. D., Warner, M. E., Cai, W. J., ... & Schoepf, V. (2018). Coral physiology and microbiome dynamics under combined warming and ocean acidification. PloS One, 13(1), e0191156.

Guest, J. R., Dizon, R. M., Edwards, A. J., Franco, C., & Gomez, E. D. (2011). How quickly do fragments of coral "self-attach" after transplantation?. Restoration Ecology, 19(2), 234-242.

Haapkylä, J., Unsworth, R. K., Flavell, M., Bourne, D. G., Schaffelke, B., & Willis, B. L. (2011). Seasonal rainfall and runoff promote coral disease on an inshore reef. PloS One, 6(2), e16893.

Hall, V. R. (1997). Interspecific differences in the regeneration of artificial injuries on scleractinian corals. Journal of Experimental Marine Biology and Ecology, 212(1), 9-23.

Harper, J. L. (1977). Population Biology of Plants. Academic Press, London, 892 pp.

Harvell, C. D., Kim, K., Burkholder, J. M., Colwell, R. R., Epstein, P. R., Grimes, D. J., ... & Porter, J. W. (1999). Emerging marine diseases--climate links and anthropogenic factors. Science, 285(5433), 1505-1510.

Heyward, A. J., & Collins, J. D. (1985). Fragmentation in *Montipora ramosa*: the genet and ramet concept applied to a reef coral. Coral Reefs, 4, 35-40.

Hidaka, M., & Miyagi, A. (1999). Does enrichment by inorganic nutrients prevent bleaching in the coral *Galaxea fascicularis* exposed to high temperature? Galaxea, Journal of the Japanese Coral Reef Society, 3-7.

Highsmith, R. C. (1980). Passive colonization and asexual colony multiplication in the massive coral *Porites lutea* Milne Edwards & Haime. Journal of Experimental Marine Biology and Ecology, 47(1), 55-67.

Highsmith, R. C. (1982). Reproduction by fragmentation in corals. Marine Ecology Progress Series. Oldendorf, 7(2), 207-226.

Highsmith, R. C., Riggs, A. C., & D'Antonio, C. M. (1980). Survival of hurricane-generated coral fragments and a disturbance model of reef calcification/growth rates. Oecologia, 46(3), 322-329.

Higuchi, T., Fujimura, H., Arakaki, T., & Oomori, T. (2008, July). Activities of antioxidant enzymes (SOD and CAT) in the coral *Galaxea fascicularis* against increased hydrogen peroxide concentrations in seawater. In Proceeding of the 11th International Coral Reef Symposium.

Hii, Y. S., Bolong, A., Munafi, A., Yang, T. T., & Liew, H. C. (2009). Effect of elevated carbon dioxide on two scleractinian corals: *Porites cylindrica* (Dana, 1846) and *Galaxea fascicularis* (Linnaeus, 1767). Journal of Marine Biology, 2009.

Hoadley, K. D., Pettay, D. T., Grottoli, A. G., Cai, W. J., Melman, T. F., Levas, S., ... & Matsui, Y. (2016). High-temperature acclimation strategies within the thermally tolerant endosymbiont *Symbiodinium trenchii* and its coral host, *Turbinaria reniformis*, differ with changing pCO_2 and nutrients. Marine Biology, 163(6), 134.

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research, 50(8), 839-866.

Hoegh-Guldberg, O. (2004). Coral reefs in a century of rapid environmental change. Symbiosis, 37(1), 1-31.

Hoegh-Guldberg, O. (2011). Coral reef ecosystems and anthropogenic climate change. Regional Environmental Change, 11(1), 215-227.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. Science, 328(5985), 1523-1528.

Hoegh-Guldberg, O., & Salvat, B. (1995). Periodic mass-bleaching and elevated sea temperatures: bleaching of outer reef slope communities in Moorea, French Polynesia. Marine Ecology Progress Series, 121, 181-190.

Hoegh-Guldberg, O., & Smith, G. J. (1989). The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. Journal of Experimental Marine Biology and Ecology, 129(3), 279-303.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... & Knowlton, N. (2007). Coral reefs under rapid climate change and ocean acidification. Science, 318(5857), 1737-1742.

Houlbrèque, F., & Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. Biological Reviews, 84(1), 1-17.

Hueerkamp, C., Glynn, P. W., D'Croz, L., Maté, J. L., & Colley, S. B. (2001). Bleaching and recovery of five eastern Pacific corals in an El Niño-related temperature experiment. Bulletin of Marine Science, 69(1), 215-236.

Hughes, R. N. (1983). Evolutionary ecology of colonial reef-organisms, with particular reference to corals. Biological Journal of the Linnean Society, 20(1), 39-58.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., ... & Lough, J. M. (2003). Climate change, human impacts, and the resilience of coral reefs. Science, 301(5635), 929-933.

Idjadi, J. A., & Edmunds, P. J. (2006). Scleractinian corals as facilitators for other invertebrates on a Caribbean reef. Marine Ecology Progress Series, 319, 117-127.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Meyer LA (Eds.)]. IPCC, Geneva, Switzerland, p 151.

IPCC (2018). Special Report on 1.5 degrees Celsius; Incheon, Korea, 8th October 2018.

Jimenez, I. M., Larkum, A. W., Ralph, P. J., & Kühl, M. (2012). Thermal effects of tissue optics in symbiont-bearing reef-building corals. Limnology and Oceanography, 57(6), 1816-1825.

Jokiel, P. L., & Coles, S. L. (1974). Effects of heated effluent on hermatypic corals at Kahe Point, Oahu. Pacific Science, 28, 1-18.

Jokiel, P. L., & Coles, S. L. (1990). Response of Hawaiian and other Indo Pacific reef corals to elevated temperatures. Coral Reefs, 8, 155-162

Jokiel, P. L., & York Jr, R. H. (1982). Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. Bulletin of Marine Science, 32(1), 301-315.

Jokiel, P. L., Hunter, C. L., Taguchi, S., & Watarai, L. (1993). Ecological impact of a fresh-water "reef kill" in Kaneohe Bay, Oahu, Hawaii. Coral Reefs, 12(3-4), 177-184.

Jones, R. J. (2008). Coral bleaching, bleaching-induced mortality, and the adaptive significance of the bleaching response. Marine Biology, 154(1), 65-80.

Jones, R. J., Kildea, T., & Hoegh-Guldberg, O. (1999). PAM chlorophyll fluorometry: a new in situ technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing. Marine Pollution Bulletin, 38(10), 864-874.

Jones, R. J., Ward, S., Amri, A. Y., & Hoegh-Guldberg, O. (2000). Changes in quantum efficiency of Photosystem II of symbiotic dinoflagellates of corals after heat stress, and of bleached corals sampled after the 1998 Great Barrier Reef mass bleaching event. Marine and Freshwater Research, 51(1), 63-71.

Kerswell, A. P., & Jones, R. J. (2003). Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of low-salinity bleaching. Marine Ecology Progress Series, 253, 145-154.

Kinzie, R. A., Jokiel, P. L., & York, R. (1984). Effects of light of altered spectral composition on coral zooxanthellae associations and on zooxanthellae in vitro. Marine Biology, 78(3), 239-248.

Knowlton, N. (2001). The future of coral reefs. Proceedings of the National Academy of Sciences, 98(10), 5419-5425.

Kuroki, T., & Van Woesik, R. (1999). Changes in zooxanthellae characteristics in the coral *Stylophora pistillata* during the 1998 bleaching event. Journal of the Japanese Coral Reef Society, 1999(1), 97-101.

Lajeunesse, T. C., Bhagooli, R., Hidaka, M., DeVantier, L., Done, T., Schmidt, G. W., ... & Hoegh-Guldberg, O. (2004). Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. Marine Ecology Progress Series, 284, 147-161.

Lang, J. C., Lasker, H. R., Gladfelter, E. H., Hallock, P., Jaap, W. C., Losada, F. J., & Muller, R. G. (1992). Spatial and temporal variability during periods of "recovery" after mass bleaching on Western Atlantic coral reefs. The American Zoologist, 32(6), 696-706.

Leder, J. J., Szmant, A. M., & Swart, P. K. (1991). The effect of prolonged "bleaching" on skeletal banding and stable isotopic composition in *Montastrea annularis*. Coral Reefs, 10(1), 19-27.

Lesser, M. P., & Shick, J. M. (1989). Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. Marine Biology, 102(2), 243-255.

Levas, S. J., Grottoli, A. G., Hughes, A., Osburn, C. L., & Matsui, Y. (2013). Physiological and biogeochemical traits of bleaching and recovery in the mounding species of coral *Porites lobata*: implications for resilience in mounding corals. PloS One, 8(5), e63267.

Levitan, D. R., Boudreau, W., Jara, J., & Knowlton, N. (2014). Long-term reduced spawning in *Orbicella* coral species due to temperature stress. Marine Ecology Progress Series, 515, 1-10.

Li, S., Yu, K., Shi, Q., Chen, T., Zhao, M., & Zhao, J. (2008). Interspecies and spatial diversity in the symbiotic zooxanthellae density in corals from northern South China Sea and its relationship to coral reef bleaching. Chinese Science Bulletin, 53(2), 295-303.

Lirman, D. (2000). Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. Journal of Experimental Marine Biology and Ecology, 251(1), 41-57.

Lirman, D., & Manzello, D. (2009). Patterns of resistance and resilience of the stress-tolerant coral *Siderastrea radians* (Pallas) to sub-optimal salinity and sediment burial. Journal of Experimental Marine Biology and Ecology, 369(1), 72-77.

Lough, J. M., & Cooper, T. F. (2011). New insights from coral growth band studies in an era of rapid environmental change. Earth-Science Reviews, 108(3-4), 170-184.

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., & Van Woesik, R. (2001). Coral bleaching: the winners and the losers. Ecology Letters, 4(2), 122-131.

Magalon, H., Flot, J. F., & Baudry, E. (2007). Molecular identification of symbiotic dinoflagellates in Pacific corals in the genus *Pocillopora*. Coral Reefs, 26(3), 551-558.

Manzello, D., & Lirman, D. (2003). The photosynthetic resilience of *Porites furcata* to salinity disturbance. Coral Reefs, 22(4), 537-540.

Manzello, D. P. (2010). Coral growth with thermal stress and ocean acidification: lessons from the eastern tropical Pacific. Coral Reefs, 29(3), 749-758.

Manzello, D. P., Kleypas, J. A., Budd, D. A., Eakin, C. M., Glynn, P. W., & Langdon, C. (2008). Poorly cemented coral reefs of the eastern tropical Pacific: Possible insights into reef development in a high-CO_2 world. Proceedings of the National Academy of Sciences, 105(30), 10450-10455.

Marinho-Soriano, E., Moreira, W. S. C., & Carneiro, M. A. A. (2006). Some aspects of the growth of *Gracilaria birdiae* (Gracilariales, Rhodophyta) in an estuary in northeast Brazil. Aquaculture International, 14(4), 327-336.

Marshall, P. A., & Baird, A. H. (2000). Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs, 19(2), 155-163.

Mayfield, A. B., & Gates, R. D. (2007). Osmoregulation in anthozoan–dinoflagellate symbiosis. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(1), 1-10.

Mayfield, A. B., Fan, T. Y., & Chen, C. S. (2013). Physiological acclimation to elevated temperature in a reef-building coral from an upwelling environment. Coral Reefs, 32(4), 909-921.

McClanahan, T., Maina, J., & Pet-Soede, L. (2002). Effects of the 1998 coral morality event on Kenyan coral reefs and fisheries. AMBIO: A Journal of the Human Environment, 31(7), 543-551.

McClanahan, T. R., Weil, E., Cortés, J., Baird, A. H., & Ateweberhan, M. (2009). Consequences of coral bleaching for sessile reef organisms. In Coral bleaching (pp. 121-138). Springer, Berlin, Heidelberg.

Meesters, E. H., & Bak, R. P. (1993). Effects of coral bleaching on tissue regeneration potential and colony survival. Marine Ecology Progress Series, 189-198.

Miller, K. J., & Ayre, D. J. (2004). The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity, 92(6), 557.

Milly, P. C. D., Wetherald, R. T., Dunne, K. A., & Delworth, T. L. (2002). Increasing risk of great floods in a changing climate. Nature, 415(6871), 514.

Moberg, F., Nyström, M., Kautsky, N., Tedengren, M., & Jarayabhand, P. (1997). Effects of reduced salinity on the rates of photosynthesis and respiration in the hermatypic corals *Porites lutea* and *Pocillopora damicornis*. Marine Ecology Progress Series, 157, 53-59.

Muscatine, L., Falkowski, P. G., Dubinsky, Z., Cook, P. A., & McCloskey, L. R. (1989). The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proceedings of the Royal Society of London. B. Biological Sciences, 236(1284), 311-324.

Muscatine, L., Grossman, D., & Doino, J. (1991). Release of symbiotic algae by tropical sea anemones and corals after cold shock. Marine Ecology Progress Series. Oldendorf, 77(2), 233-243.

Muthiga, N. A., & Szmant, A. M. (1987). The effects of salinity stress on the rates of aerobic respiration and photosynthesis in the hermatypic coral *Siderastrea siderea*. The Biological Bulletin, 173(3), 539-551.

Mwaura, J., Grimsditch, G., Kilonzo, J., Amiyo, N., & Obura, D. (2009). Zooxanthellae densities are highest in summer months in equatorial corals in Kenya. Western Indian Ocean Journal of Marine Science, 8(2).

Nakano, Y., Tsuchiya, M., Rungsupa, S., & Yamazato, K. Y. K. (2009). Influence of severe freshwater flooding during the rainy season on the coral community around Khang Khao Island in the inner Gulf of Tahiland. Galaxea, Journal of Coral Reef Studies, 11, 131-138.

Norström, A. V., Nyström, M., Lokrantz, J., & Folke, C. (2009). Alternative states on coral reefs: beyond coral–macroalgal phase shifts. Marine Ecology Progress Series, 376, 295-306.

Padilla-Gamino, J. L., Bidigare, R. R., Barshis, D. J., Alamaru, A., Hédouin, L., Hernández-Pech, X., ... & Grottoli, A. G. (2013). Are all eggs created equal? A case study from the Hawaiian reef-building coral *Montipora capitata*. Coral Reefs, 32(1), 137-152.

Pearson, R. G. (1981). Recovery and recolonization of coral reefs. Marine Ecology Progress Series, 105-122.

Porter, J. W., Muscatine, L., Dubinsky, Z., & Falkowski, P. G. (1984). Primary production and photoadaptation in light-and shade-adapted colonies of the symbiotic coral, *Stylophora pistillata*. Proceedings of the Royal Society of London. Series B. Biological Sciences, 222(1227), 161-180.

Porter, J. W., Lewis, S. K., & Porter, K. G. (1999). The effect of multiple stressors on the Florida Keys coral reef ecosystem: a landscape hypothesis and a physiological test. Limnology and Oceanography, 44(3part2), 941-949.

Prada, C., Weil, E., & Yoshioka, P. M. (2010). Octocoral bleaching during unusual thermal stress. Coral Reefs, 29, 41–45.

Pratchett, M. S. (2001). Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the western Pacific. Marine Ecology Progress Series, 214, 111-119.

Ranklin, J. C., & Davenport, J. (1981). Animal osmoregulation. New York: Halsted Press.

Richmond, R. H. (1997). Reproduction and recruitment in corals: critical links in the persistence of reefs. Life and death of coral reefs. Chapman & Hall, New York, pp. 175-197

Rowan, R., Knowlton, N., Baker, A. C., & Jara, J. (1997). Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature, 388, 265-269.

Salih, A., Hoegh-Guldberg, O., & Cox, G. (1998). Photoprotection of symbiotic dinoflagellates by fluorescent pigments in reef corals. In Proceedings of the Australian Coral Reef Society 75th Anniversary Conference (pp. 217-230). School of Marine Science, The University of Queensland Brisbane, Australia.

Scheufen, T., Krämer, W. E., Iglesias-Prieto, R., & Enríquez, S. (2017). Seasonal variation modulates coral sensibility to heat-stress and explains annual changes in coral productivity. Scientific Reports, 7(1), 4937.

Schoepf, V., Grottoli, A. G., Warner, M. E., Cai, W. J., Melman, T. F., Hoadley, K. D., ... & Wang, Y. (2013). Coral energy reserves and calcification in a high-CO_2 world at two temperatures. PloS One, 8(10), e75049.

Schoepf, V., Grottoli, A. G., Levas, S. J., Aschaffenburg, M. D., Baumann, J. H., Matsui, Y., & Warner, M. E. (2015). Annual coral bleaching and the long-term recovery capacity of coral. Proceedings of the Royal Society B: Biological Sciences, 282(1819), 20151887.

Schrameyer, V., Wangpraseurt, D., Hill, R., Kühl, M., Larkum, A. W., & Ralph, P. J. (2014). Light respiratory processes and gross photosynthesis in two scleractinian corals. PLoS One, 9(10), e110814.

Schutte, V. G., Selig, E. R., & Bruno, J. F. (2010). Regional spatio-temporal trends in Caribbean coral reef benthic communities. Marine Ecology Progress Series, 402, 115-122.

Schutter, M., Crocker, J., Paijmans, A., Janse, M., Osinga, R., Verreth, A. J., & Wijffels, R. H. (2010). The effect of different flow regimes on the growth and metabolic rates of the scleractinian coral *Galaxea fascicularis*. Coral Reefs, 29(3), 737-748.

Schutter, M., Van der Ven, R. M., Janse, M., Verreth, J. A., Wijffels, R. H., & Osinga, R. (2011). Light intensity, photoperiod duration, daily light flux and coral growth of *Galaxea fascicularis* in an aquarium setting: a matter of photons?. Journal of the Marine Biological Association of the United Kingdom, 92(4), 703-712.

Shenkar, N., Fine, M., & Loya, Y. (2005). Size matters: bleaching dynamics of the coral *Oculina patagonica*. Marine Ecology Progress Series, 294, 181-188.

Stimson, J. (1997). The annual cycle of density of zooxanthellae in the tissues of field and laboratory-held *Pocillopora damicornis* (Linnaeus). Journal of Experimental Marine Biology and Ecology, 214(1-2), 35-48.

Stimson, J., & Kinzie III, R. A. (1991). The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. Journal of Experimental Marine Biology and Ecology, 153(1), 63-74.

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(4), 409-421.

Szmant, A., & Gassman, N. J. (1990). The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs, 8(4), 217-224.

Tanaka, Y., Inoue, M., Nakamura, T., Suzuki, A., & Sakai, K. (2014). Loss of zooxanthellae in a coral under high seawater temperature and nutrient enrichment. Journal of Experimental Marine Biology and Ecology, 457, 220-225.

Titlyanov, E. A., & Titlyanova, T. V. (2002). Reef-building corals—symbiotic autotrophic organisms: 2. Pathways and mechanisms of adaptation to light. Russian Journal of Marine Biology, 28(1), S16-S31.

Titlyanov, E. A., Titlyanova, T. V., Leletkin, V. A., Tsukahara, J., Van Woesik, R., & Yamazato, K. (1996). Degradation of zooxanthellae and regulation of their density in hermatypic corals. Marine Ecology Progress Series, 139, 167-178.

Titlyanov, E. A., Titlyanova, T. V., Amat, A., Yamazato, K. (2001). Morphophysiological variations of symbiotic dinoflagellates in hermatypic corals from a fringing reef at Sesoko Island. Galaxea, Journal of the Japanese Coral Reef Society, 3, 51-63.

Titlyanov, E. A., Titlyanova, T. V., Yakovleva, I. M., Nakano, Y., & Bhagooli, R. (2005). Regeneration of artificial injuries on scleractinian corals and coral/algal competition for newly formed substrate. Journal of Experimental Marine Biology and Ecology, 323(1), 27-42.

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.

Treignier, C., Grover, R., Ferrier-Pagès, C., & Tolosa, I. (2008). Effect of light and feeding on the fatty acid and sterol composition of zooxanthellae and host tissue isolated from the scleractinian coral *Turbinaria reniformis*. Limnology and Oceanography, 53, 2702-2710.

Tremblay, P., Naumann, M. S., Sikorski, S., Grover, R., & Ferrier-Pagès, C. (2012). Experimental assessment of organic carbon fluxes in the scleractinian coral *Stylophora pistillata* during a thermal and photo stress event. Marine Ecology Progress Series, 453, 63-77.

Tremblay, P., Maguer, J. F., Grover, R., & Ferrier-Pagès, C. (2015). Trophic dynamics of scleractinian corals: stable isotope evidence. Journal of Experimental Biology, 218(8), 1223-1234.

True, J. D. (2012, July). Salinity as a structuring force for near shore coral communities. In Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia (Vol. 9, p. e13).

Tunnicliffe, V. (1981). Breakage and propagation of the stony coral *Acropora cervicornis*. Proceedings of the National Academy of Sciences, 78(4), 2427-2431.

Ulstrup, K. E., Berkelmans, R., Ralph, P. J., & Van Oppen, M. J. (2006). Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. Marine Ecology Progress Series, 314, 135-148.

Van Woesik, R. (1994). Contemporary disturbances to coral communities of the Great Barrier Reef. Journal of Coastal Research, 233-252.

Van Woesik, R., De Vantier, L. M., & Glazebrook, J. S. (1995). Effects of Cyclone\'Joy\'on nearshore coral communities of the Great Barrier Reef. Marine Ecology Progress Series, 128, 261-270.

Veron, J. E. N. (1990). Checklist of the hermatypic corals of Vanuatu. Pacific Science, 44, 51-70

Veron, J. E. N. (2000). Corals of the World, vols. 1-3. Mary Stafford-Smith (Ed.) Publisher: Australian Institute of Marine Science, Townsville, Australia, p. 489.

Vogel, N. (2015). Understanding Individual and Combined Effects of Ocean Acidification, Coastal Runoff and Warming on Marine Calcifying Organisms on Tropical Coral Reefs (Doctoral dissertation, University of Bremen).

Walsh, K. J., & Ryan, B. F. (2000). Tropical cyclone intensity increase near Australia as a result of climate change. Journal of Climate, 13(16), 3029-3036.

Wergen, G., & Krug, J. (2010). Record-breaking temperatures reveal a warming climate. EPL (Europhysics Letters), 92(3), 30008.

West, J. M., & Salm, R. V. (2003). Resistance and resilience to coral bleaching: implications for coral reef conservation and management. Conservation Biology, 17(4), 956-967.

Wild, C., Hoegh-Guldberg, O., Naumann, M. S., Colombo-Pallotta, M. F., Ateweberhan, M., Fitt, W. K., ... & Loya, Y. (2011). Climate change impedes scleractinian corals as primary reef ecosystem engineers. Marine and Freshwater Research, 62(2), 205-215.

Wooldridge, S. A. (2009). A new conceptual model for the enhanced release of mucus in symbiotic reef corals during 'bleaching'conditions. Marine Ecology Progress Series, 396, 145-152.

Wooldridge, S. A. (2014). Differential thermal bleaching susceptibilities amongst coral taxa: re-posing the role of the host. Coral Reefs, 33(1), 15-27.

Xiubao, L., Hui, H., Jiansheng, L., Liangmin, H., & Junde, D. (2009). Effects of the multiple stressors high temperature and low salinity on the photosynthesis of the hermatypic coral *Galaxea fascicularis*. Acta Ecologica Sinica 29(3), 155–159.

Zawada, K. (2015). Production of Dimethyl Sulphide (DMS) by Scleractinian Coral during Aerial Exposure–Effect of Temperature and Light (Doctoral dissertation, University of Essex).

Ziegler, M., Roder, C. M., Büchel, C., & Voolstra, C. R. (2014). Limits to physiological plasticity of the coral *Pocillopora verrucosa* from the central Red Sea. Coral Reefs, 33, 1115–1.

CHAPTER 4

Long-term exposure to increasing temperatures on scleractinian coral fragments reveals oxidative stress

Dias, M., Ferreira, A., Gouveia, R., Madeira, C., Jogee, N., Cabral, H.N., Diniz, M. & Vinagre, C. (2019). Marine Environmental Research, 104758. DOI:10.1016/j.marenvres.2019.104758.

Abstract

Global warming is leading to increases in tropical storms' frequency and intensity, allowing fragmentation of reef-forming coral species, but also to coral bleaching and mortality. The first level of organism's response to an environmental perturbation occurs at the cellular level. This study investigated the long-term oxidative stress on fragments of nine Indo-Pacific reef-forming coral species exposed for 60 days to increasing temperatures (30ºC and 32ºC) and compared results with control temperature (26ºC). Coral overall condition (appearance), lipid peroxidation (LPO), catalase activity (CAT), and glutathione S-transferase (GST) were assessed. The species *Turbinaria reniformis*, *Galaxea fascicularis,* and *Psammocora contigua* were the most resistant to heat stress, presenting no oxidative damage at 30ºC. Unlike *G. fasciularis*, both *T. reniformis* and *P. contigua* showed no evidence of oxidative damage at 32ºC. All remaining species' fragments died at 32ºC. *Stylophora pistillata* and *Pocillopora damicornis* were the most susceptible species to heat stress, not resisting at 30ºC.

Keywords: climate change, biomarker, antioxidant enzymes, lipid peroxidation, catalase activity, glutathione S-transferase activity, coral condition, heat stress, heat resistance.

4.1. Introduction and literature review

Today, there is unequivocal evidence that climate change is affecting coral reef ecosystems at alarming rates (Hoegh-Guldberg et al., 2007a, b). In recent years, episodes of mass coral bleaching have dramatically changed coral reef communities in the Indo-Pacific (Khalil et al., 2013; Hughes et al., 2017, 2018; Le Nohaïc et al., 2017), often leading to shifts in reef community structure where dominant heat-susceptible coral species have been replaced by sub-dominant heat-tolerant coral species (Riegl and Purkis, 2009; Van Woesik et al., 2011).

Recent modeling suggests an increase in the frequency and intensity of bleaching events in the coming decades (Eakin et al., 2009; Frieler et al., 2013; Heron et al., 2016; Van Hooidonk et al., 2016), with sea surface temperature (SST) projected to increase by 1 to 3.7 ºC by the year 2100 (IPCC, 2014). Worldwide, mass coral bleaching events induced by warming waters were predicted to occur annually in most oceans by 2040 (Crabbe, 2008; Van Hooidonk and Huber, 2009). Increases in both frequency and maximum intensity of categories 4 and 5 storms (0–25%) are also expected as a consequence of global warming (Christensen et al., 2013).

Coral reef community recovery after disturbance is highly dependent on both asexual and sexual reproductive processes (Connell and Keough, 1985; Glynn et al., 2017). Given the predictions of increased frequency and intensity of heat stress events, the success of sexual reproduction may be low (Hoegh-Guldberg, 2004; Diaz-Pulido et al., 2009; Albright and Mason, 2013; Levitan et al., 2014; Osborne et al., 2017). Thus, asexual reproduction might be the most probable means of recovery under current changes in climatic conditions.

Tropical storms can be favorable to the propagation and expansion of scleractinian corals of varied morphologies by colony fragmentation and subsequent regeneration and growth (Highsmith, 1980; Foster et al., 2007, 2013). The production of new colonies through this asexual reproduction strategy is highly important for local distribution among major reef-building corals following disturbance (Pearson, 1981; Connell and Keough, 1985; Bruno, 1998). A great number of the most successful corals have incorporated fragmentation into their life histories (Cook, 1979; Highsmith, 1982).

Scleractinian corals consistently experience an elevated pO_2 within their tissues as an outcome of photosynthetically produced oxygen (Dykens et al., 1992; Shashar et al., 1993; Kühl et al., 1995), resulting in the production of reactive oxygen species (ROS), such as the superoxide anion radical ($\bullet O_2^-$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO•) (Asada, 1999; Richier et al., 2006). Nonetheless, during prolonged abiotic stress, the additional amount of ROS production and their accumulation results in extensive cellular damage, including cellular membrane lipid peroxidation (LPO), DNA degradation, and protein denaturation (Richier et al., 2005; Weis, 2008; Roth, 2014). Lipid peroxidation is one of the most predominant mechanisms of cellular damage (Bindoli, 1988; Halliwell and Gutteridge, 1999). This happens when ROS, particularly HO•, react with membrane lipids, leading to the disruption of the cellular membrane and the formation of lipid peroxides (Lesser, 2006).

To prevent oxidative damage, both partners of the symbiosis possess antioxidant mechanisms that maintain the steady-state concentration of ROS at low levels, having an essential role in redox homeostasis (Lesser and Shick, 1989; Nii and Muscatine, 1997). These involve both non-enzymatic mechanisms such as β-carotene, vitamin C, α-tocoferol, flavonoids, and glutathione (Meyers, 1995; Goodsell, 1996), and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione S-transferase (GST), and glutathione peroxidase (GPx) (Lesser et al., 1990; Livingstone, 1991; Rewitz et al., 2006).

Induction of ROS and accumulation of oxidative damage products have been correlated with heat stress in both symbiont algae and host corals (Lesser et al., 1990; Brown et al., 2002; Downs et al., 2002). The current consensus is that oxidative stress is the trigger of coral bleaching (Richier et al., 2006; Murata et al., 2007; Lesser, 2011), therefore, a unifying mechanism of coral bleaching was proposed: the 'Oxidative Theory of Coral Bleaching' (OTB). This hypothesis suggests that heat and light stress induce the bleaching cascade first by decoupling symbiont photosynthesis, leading to the excessive generation of ROS and reactive nitrogen species (RNS) in the symbiont that overwhelms its antioxidant defenses, resulting in a net increase in ROS and oxidative stress inside the symbiont cells (Perez and Weis, 2006; Dunn et al., 2007; Hawkins and Davy, 2012). ROS diffusion into the coral host tissue combined with damage and potential activation of apoptotic pathways in both partners result in symbiosis dysfunction (Weis, 2008; Hawkins et al., 2013; Paxton et al., 2013).

A key factor inducing shifts in coral community structure is differential susceptibility of coral species to heat stress (Marshall and Baird, 2000; Dias et al., 2018) and, among many other factors, it is related to differences in the activity of antioxidant defenses in both partners of the symbiosis (Halliwell and Gutteridge, 1999; Brown et al., 2002; Downs et al., 2002; Yakovleva et al., 2004) and the thermal tolerance of the symbiotic microalgae (*Symbiodinium* spp.) clade (Rowan et al., 1997; Kinzie et al., 2001; Magalon et al., 2007). Oxidative stress parameters such as oxidative damage of lipids and antioxidant enzyme activity have been extensively used as biomarkers of cellular stress in different taxa subjected to heat stress (Buchner et al., 1996; Abele et al., 1998; Regoli et al., 2004; Cailleaud et al., 2007; Vinagre et al., 2012, 2014; Madeira et al., 2013; Matozzo et al., 2013; Attig et al., 2014; Madeira et al., 2015, 2019; Kamyab et al., 2017; Tasselli et al., 2017; Zuo et al., 2017; Cereja et al., 2018). These oxidative stress biomarkers have also been applied to detect specific responses of corals to heat stress (Lesser et al., 1990; Downs et al. 2000), early detection of coral bleaching (Solayan, 2016), assessment of coral reefs health (Downs et al., 2005; Olsen, 2013), and identifying the susceptibility of corals towards coral bleaching (Anithajothi et al., 2014).

Dias et al. (2018, 2019) investigated the differential capability of nine reef-forming corals to grow and regenerate small fragments, mimicking a post-storm scenario in a context of higher temperatures and lower salinity. These studies identified resistant and vulnerable species to conditions that are likely to be more frequent in the future, influencing coral asexual reproduction via fragmentation. In the present study, we aim at testing the oxidative damage and antioxidant response of the same nine coral species, providing a new insight into the effects of thermal stress on coral fragments at a cellular level.

This is the first study that aims to assess the long-term effects (60 days) of heat stress on coral condition, oxidative damage (lipid peroxidation - LPO), and antioxidant enzymes activity (catalase - CAT, glutathione S-transferase – GST) post-fragmentation in nine common and widely distributed reef-building corals of the Indo-Pacific oceans (Veron, 1990, 2000). Understanding how increased temperatures will affect fragments of several coral species, at a cellular and biochemical level, is crucial to identify which species are more likely to prosper through asexual reproduction and which shall decline in the future, so that conservation efforts can be more effective.

4.2. Materials and methods

4.2.1. Coral species

This study included nine-coral species with four different morphologies: four branching species (*Acropora tenuis, Stylophora pistillata, Pocillopora damicornis*, and *Psammocora contigua*), one massive species (*Galaxea fascicularis*), three plating species, (*Montipora capricornis* brown morphotype (BM), *Turbinaria reniformis*, and *Echinopora lamellosa*), and one encrusting species (*Montipora capricornis* green morphotype (GM)). These coral species were chosen for their differences in heat stress susceptibility and colony morphology (Marshall and Baird, 2000; Loya et al., 2001). These and other characteristics of the coral species in study are given in Tables 4.1 and 4.2. Coral species were identified according to Veron (2000). The coral colonies used in the experimental treatments have been maintained in captivity in a coral stock aquarium at "Oceanário de Lisboa (Portugal)" for five years, providing their thermal history information.

4.2.2. Experimental setup

The experiments were conducted from April to November of 2014 at "Oceanário de Lisboa, Portugal" (www.oceanario.pt). To test the different coral species i) condition, ii) oxidative damage products concentration, and iii) antioxidant enzyme activity, ten replicate fragments were cut from each coral colony, using a pincer or a pair of pliers. For the branching coral colonies the fragments were cut approximately 20-40 mm in length and the fragments for the massive, plating, and encrusting were obtained by cutting approximately 30 mm sided squares. All fragments were placed over egg crate panels in the coral stock aquarium until acclimation to the experimental aquarium.

Table 4.1. Characteristics of the coral species in study.

Coral species	Family	Colony morphology	*Symbiodinium* clade	Geographic distribution	Habitat preferences	Substrate inclination	Depth range	Life history strategy	Competitive ability	Heat stress susceptibility	References
A. tenuis	Acroporidae	branching	C, D	Indo-Pacific	Outer reef flat, inner reef flat, upper reef slope, clear waters, exposed to wave action	30-45 °	1-20 m	competitive	Moderately aggressive	severe	Dai, 1990; Diaz and Madin, 2011; Fujioka, 1998; Hamilton and Brakel, 1984; LaJeunesse et al., 2004; Marshall and Baird, 2000; Mieog et al., 2007; Richards et al., 2014; Ulstrup and Van Oppen, 2003; Van Oppen et al., 2001; Veron, 2000; World Register of Marine Species
M. capricornis (BM)	Acroporidae	plating	C	Indo-West Pacific	Lagoons, shallow tropical reef environments, clear waters, protected from wave action	_	2-20 m	_	_	high	DeVantier et al., 2008; Diaz and Madin,2011; La Jeunesse et al., 2004; Marshall and Baird, 2000; Smith et al., 2009; Veron, 2000; World Register of Marine Species
M. capricornis (GM)	Acroporidae	encrusting	C	Indo-West Pacific	Lagoons, shallow tropical reef environments, clear water, protected from wave action	_	2-20 m	_	_	high	DeVantier et al., 2008; Diaz and Madin, 2011; LaJeunesse et al., 2004; Marshall and Baird, 2000; Smith et al., 2009; Veron, 2000; World Register of Marine Species
T. reniformis	Dendrophylliidae	plating	C, D	Indo-West Pacific	Lower reef slope, turbid waters, protected from wave action	5-15 °	2-15 m	_	Intermediate	low	Dai, 1990; DeVantier, 2010; Diaz and Madin, 2011; Hoeksema et al., 2014a; LaJeunesse et al., 2004; Marshall and Baird, 2000; Mieog et al., 2007; Veron, 2000; World Register of Marine Species
E. lamellosa	Merulinidae	plating	C,D	Indo-Pacific	Upper reef slope, lower reef slope, clear waters, exposed and protected from wave action	20-50 °	1-40 m	_	Moderately aggressive	moderate	Dai, 1990; DeVantier et al., 2014; Diaz and Madin, 2011; Fabricius et al., 2004; Hamilton and Brakel, 1984; LaJeunesse et al., 2004; Marshall and Baird, 2000; Sheppard, 1980; Turak and DeVantier, 2012; Veron, 2000; World Register of Marine Species

Table 4.1. Characteristics of the coral species in study (continuation).

Coral species	Family	Colony morphology	Symbiodinium clade	Geographic distribution	Habitat preferences	Substrate inclination	Depth range	Life history strategy	Competitive ability	Heat stress susceptibility	References
P. damicornis	Pocilloporidae	branching	A, B, C, D	Indo-West and eastern Pacific	Inner reef flat, clear and turbid waters, exposed and protected from wave action	5 - 60 °	1-20 m	weedy	Subordinate	severe	Dai, 1990; DeVantier, 2010; Diaz and Madin, 2011; Fabricius et al., 2004; Hamilton and Brakel, 1984; Hoeksema et al., 2014c; Kinzie et al., 2001; LaJeunesse et al., 2004; Magalon et al., 2007; Marshall and Baird, 2000; Sheppard, 1980; Veron, 2000; World Register of Marine Species
S. pistillata	Pocilloporidae	branching	A, C, D	Indo-West Pacific	Outer reef flat, inner reef flat, upper reef slope, lower reef slope; clear waters, exposed to wave action	10 - 45 °	1-15 m	weedy	Intermediate	severe	Dai, 1990; Diaz and Madin, 2011; Hamilton and Brakel, 1984; Hoeksema et al., 2014d; LaJeunesse et al., 2004; Marshall and Baird, 2000; Mieog et al., 2007; Sheppard, 1980; Turak and DeVantier, 2012; Veron, 2000; World Register of Marine Species
P. contigua	Psammocoridae	branching	C, D	Indo-West Pacific (except Hawaii and the far East)	Inner reef flat; clear and turbid waters, exposed and protected from wave action	30-45°	1-30 m	_	_	low	Diaz and Madin, 2011; Dong et al., 2009; Hamilton and Brakel, 1984; LaJeunesse et al., 2004; Marshall and Baird, 2000; Mostafavi et al., 2007; Sheppard, 1980; Sheppard et al., 2014; Veron, 2000; World Register of Marine Species

Table 4.2. Sea surface temperature (SST) range of the studied coral species according with their geographical distribution.

Coral species	Study location	Geographical distribution	SST range (°C)	Reference
Acropora tenuis	Barrow Island	Australia	23.0-29.0	Richards and Rosser, 2012
	Houtman-Abrolhos	Australia	19.8-23.5	Babcock et al., 1994
	Nansha Islands	China Sea	27.1-30.0	Zhao et al., 2013
	Yongle atoll	China Sea	24.7-29.8	Zhao et al., 2017
	Ishigaki Island	Japan	24.0-28.0	Hongo and Kayanne, 2010
	Okinotori Island	Philippine Sea	24.7-29.7	Kayanne et al., 2012
	Raffles Lighthouse	Singapore	27.3-30.1	Guest, 2005
	Southern Taiwan	Taiwan	22.5-28.2	Chen et al., 2005
	Southern Taiwan	Taiwan	20.1-28.2	Chen, 1999
	Nino Konis Santana National Marine Park	Timor Leste	25.0-28.0	Turak and DeVantier, 2012
Montipora capricornis	Houtman-Abrolhos	Australia	19.8-23.5	Babcock et al., 1994
	Kermadec Islands	New Zeland	16.0-26.0	Brook, 1999
Turbinaria reniformis	Socotra Archipelago	Arabian Sea	24.0-30.0	DeVantier et al., 2004
	Hervey Bay	Australia	22.0-26.0	DeVantier, 2010
	Barrow Island	Australia	23.0-29.0	Richards and Rosser, 2012
	Bremer Bay	Australia	17.0-21.0	Ross et al., 2018
	Dunsborough	Australia	17.0-21.0	Silverstein et al., 2011
	Lizard Island	Australia	23.9-29.4	Ulstrup et al., 2011
	Davies Reef	Australia	23.3-28.9	Ulstrup et al., 2011
	Houtman-Abrolhos	Australia	19.8-23.5	Babcock et al., 1994
	Nansha Islands	China Sea	27.1-30.0	Zhao et al., 2013
	Hirota Reef	Japan	20.2-28.3	Ikeda et al., 2006
	Okinotori Island	Philippine Sea	24.7-29.7	Kayanne et al., 2012
	Gulf of Aqaba	Red Sea	21.2-28.4	Rapuano et al., 2017
	Nino Konis Santana National Marine Park	Timor Leste	25.0-28.0	Turak and DeVantier, 2012
Echinopora lamellosa	Socotra Archipelago	Arabian Sea	24.0-30.0	DeVantier et al., 2004
	Barrow Island	Australia	23.0-29.0	Richards and Rosser, 2012
	Ningaloo Reef	Australia	22.0-27.0	Silverstein et al., 2011
	Capricorn Bunker Group	Australia	21.5-27.0	Dechnik et al., 2015
	Nansha Islands	China Sea	27.1-30.0	Zhao et al., 2013
	Yongle atoll	China Sea	24.7-29.8	Zhao et al., 2017
	Ishigaki Island	Japan	24.0-28.0	Hongo and Kayanne, 2010
	Hirota Reef	Japan	20.2-28.3	Ikeda et al., 2006
	Ricaudy reef	New Caledonia	23.0-28.0	Cabioch et al., 1999
	Okinotori Island	Philippine Sea	24.7-29.7	Kayanne et al., 2012
	Raffles Lighthouse	Singapore	27.3-30.1	Guest, 2005
	Yenliao Bay	Taiwan	18.0-28.0	Fan and Day, 1999
	Nanwan Bay	Taiwan	23.5-28.0	Fan and Day, 1999
	Penghu Archipelago	Taiwan	16.0-28.0	Chen et al., 2005
	Southern Taiwan	Taiwan	20.1-28.2	Chen, 1999
	Nino Konis Santana National Marine Park	Timor Leste	25.0-28.0	Turak and DeVantier, 2012
Galaxea fascicularis	Socotra Archipelago	Arabian Sea	24.0-30.0	DeVantier et al., 2004
	Barrow Island	Australia	23.0-29.0	Richards and Rosser, 2012
	Houtman-Abrolhos	Australia	19.8-23.5	Babcock et al., 1994
	Ningaloo Reef	Australia	22.0-27.0	Silverstein et al., 2011
	Hong Kong	China Sea	13.0-30.0	Tong et al., 2017
	Sanya	China Sea	20.0-30.0	Tong et al., 2017
	Sansha	China Sea	24.0-30.0	Tong et al., 2017
	Nansha Islands	China Sea	27.1-30.0	Zhao et al., 2013
	Hainan Island	China Sea	19.0-30.0	Zhou et al., 2011
	Yongle atoll	China Sea	24.7-29.8	Zhao et al., 2017
	Ishigaki Island	Japan	24.0-28.0	Hongo and Kayanne, 2010
	Mombasa	Kenya	25.5-29.9	Mwaura et al., 2009
	Halong Bay	Vietnam	16.0-31.5	Faxneld et al., 2011
	La Pointe-au-Sable reef	Mauritius	22.0-27.0	Cabioch et al., 1999
	Raffles Lighthouse	Singapore	27.3-30.1	Guest, 2005
	Southern Taiwan	Taiwan	22.5-28.2	Chen et al., 2005
	Nino Konis Santana National Marine Park	Timor Leste	25.0-28.0	Turak and DeVantier, 2012

Table 4.2. Sea surface temperature (SST) range of the studied coral species according with their geographical distribution (continuation).

Coral species	Study location	Geographical distribution	SST range (°C)	Reference
Pocillopora damicornis	Socotra Archipelago	Arabian Sea	24.0-30.0	DeVantier et al., 2004
	One tree island	Australia	21.4-27.4	Edmunds, 2005
	Orpheus Island	Australia	22.9-28.9	Berkelmans and Willis, 1990
	Solitary Islands	Australia	19.7-22.0	Harriott, 1999
	Hervey Bay	Australia	22.0-26.0	DeVantier, 2010
	Ningaloo Reef	Australia	22.0-27.0	Silverstein et al., 2011
	Abrolhos Island	Australia	19.0-23.0	Silverstein et al., 2011
	Rottnest Island	Australia	18.0-22.0	Silverstein et al., 2011
	Dampier	Australia	24.0-30.0	Silverstein et al., 2011
	Barrow Island	Australia	23.0-29.0	Richards and Rosser, 2012
	Lizard Island	Australia	23.9-29.4	Ulstrup et al., 2011
	Davies Reef	Australia	23.3-28.9	Ulstrup et al., 2011
	Heron Island	Australia	20.0-28.0	Connell et al., 1997
	Hall Bank	Australia	17.2-24.8	Baird and Thomson, 2018
	Capricorn Bunker Group	Australia	21.5-27.0	Dechnik et al., 2015
	Coral Bay	Australia	22.0-28.0	Foster et al., 2014
	Abrolhos Islands	Australia	20.0-25.0	Foster et al., 2014
	Marmion	Australia	17.0-24.0	Foster et al., 2014
	Nansha Islands	China Sea	27.1-30.0	Zhao et al., 2013
	Yongle atoll	China Sea	24.7-29.8	Zhao et al., 2017
	Maui	Hawaii	23.8-27.7	Piniak and Brown, 2008
	Coconut Island	Hawaii	21.0-27.0	Gates et al., 1992
	Coconut Island	Hawaii	23.0-28.0	Brahmi et al., 2012
	Mombasa	Kenya	25.5-29.9	Mwaura et al., 2009
	Kermadec Islands	New Zeland	16.0-26.0	Brook, 1999
	Okinotori Island	Philippine Sea	24.7-29.7	Kayanne et al., 2012
	La Pointe-au-Sable reef	Mauritius	22.0-27.0	Cabioch et al., 1999
	Ricaudy reef	New Caledonia	23.0-28.0	Cabioch et al., 1999
	Raffles Lighthouse	Singapore	27.3-30.1	Guest, 2005
	Southern Taiwan	Taiwan	22.5-28.2	Chen et al., 2005
	Nino Konis Santana National Marine Park	Timor Leste	25.0-28.0	Turak and DeVantier, 2012
Stylophora pistillata	Socotra Archipelago	Arabian Sea	24.0-30.0	DeVantier et al., 2004
	Barrow Island	Australia	23.0-29.0	Richards and Rosser, 2012
	Heron Island	Australia	20.0-28.0	Connell et al., 1997
	Capricorn Bunker Group	Australia	21.5-27.0	Dechnik et al., 2015
	Ningaloo Reef	Australia	22.0-27.0	Silverstein et al., 2011
	Nansha Islands	China Sea	27.1-30.0	Zhao et al., 2013
	Yongle atoll	China Sea	24.7-29.8	Zhao et al., 2017
	Hirota Reef	Japan	20.2-28.3	Ikeda et al., 2006
	La Pointe-au-Sable reef	Mauritius	22.0-27.0	Cabioch et al., 1999
	Ricaudy reef	New Caledonia	23.0-28.0	Cabioch et al., 1999
	Southern Taiwan	Taiwan	22.5-28.2	Chen et al., 2005
	Nino Konis Santana National Marine Park	Timor Leste	25.0-28.0	Turak and DeVantier, 2012
Psammocora contigua	Socotra Archipelago	Arabian Sea	24.0-30.0	DeVantier et al., 2004
	Barrow Island	Australia	23.0-29.0	Richards and Rosser, 2012
	Houtman-Abrolhos	Australia	19.8-23.5	Babcock et al., 1994
	Yongle atoll	China Sea	24.7-29.8	Zhao et al., 2017
	Nansha Islands	China Sea	27.1-30.0	Zhao et al., 2013
	Nukutipipi atoll	French Polynesia	25.0-30.0	Salvat and Salvat, 1992
	Hirota Reef	Japan	20.2-28.3	Ikeda et al., 2006
	Okinotori Island	Philippine Sea	24.7-29.7	Kayanne et al., 2012
	Nino Konis Santana National Marine Park	Timor Leste	25.0-28.0	Turak and DeVantier, 2012

Each coral fragment was glued with epoxy putty to the top of a nylon expansion anchor. Branching fragments were placed in vertical position, whereas the massive, plating, and encrusting fragments were placed in horizontal positions. The placement of the fragments varied with morphology in order to minimize the dead tissue area produced by epoxy putty application. Then, the set (anchor + coral fragment) was placed back over egg crate panels in the coral stock aquarium, to recover from the handling procedures for one day before acclimation to the experimental aquarium.

To determine the long-term response of the nine-coral species to the effect of increased temperatures, the coral fragments were exposed to different treatments: (a) control temperature (26 °C) (26.1 ± 0.2 SD) and (b) increased temperatures (30 ºC and 32 ºC) (30.2 ± 0.5 SD and 32.2 ± 0.5 SD, respectively). Ten coral fragments of each coral species were exposed to each of the different treatments for 60 days. Coral fragments were acclimated one hour per °C above the temperature of the coral stock aquarium (25 ºC). This heating rate was applied because coral reef-flat communities can experience temperature changes of 1 °C $hour^{-1}$ during spring tides (Berkelmans and Willis, 1999), and most of the coral species in this study colonize the reef-flat zone (Brown and Suharsono, 1990; Fujioka, 1998). The coral fragments were placed over two 40 x 40 cm egg crate panels suspended 15 cm below the water surface of the experimental aquarium, positioned 2 cm apart from one another, and organized by coral species to avoid contact between fragments of the same and/or different species.

The experimental aquarium (400 L) was fitted with a sump (280L) filled with bioballs for biological filtration in which two Fluval M300 heaters, as well as a Hailea 500 chiller-controlled water temperature. For water circulation purposes, an AquaMedic OceanRunner 3500 pump provided a turnover rate of 5 times per hour. An AquaMedic Turboflotor 5000 Shorty protein skimmer helped to keep nutrient concentrations low, and increased surface water motion in the aquarium was accomplished by using an AquaClear 110 powerhead. Lighting requirements similar to the coral stock aquarium were attained by using a Litpa Jet5 floodlight with an AquaMedic 400W HQi lamp (13000K) on a 12 h light/ 12 h dark cycle. An air-stone was used in the aquarium to ensure good oxygen concentrations

Photosynthetically Active Radiation (PAR) levels were measured with a spheric quantic sensor (LI-193SA) and a data logger (1400 model) and varied between 320-345 $\mu E.m^{-2}s^{-1}$ in the 400-700 nm waveband. The experimental treatments were conducted under low light conditions to minimize the synergistic effects with high light conditions (Fitt et al., 2001). Water quality parameters such as water temperature, pH and salinity were measured on a daily basis. Water samples were also weekly analyzed to determine ammonium, nitrites, nitrates, calcium concentration, alkalinity as well as oxygen concentration and saturation. These parameters were maintained as follow: pH at 8.3, salinity at 33 psu, ammonium at 0 mg l^{-1}, nitrites between 0.002 and 0.005 mg l^{-1}, nitrates between 0 and 2 mg l^{-1}, calcium concentration between 389 and

401 mg l^{-1}, alkalinity approximately at 100 mg l^{-1}, oxygen concentration between 6.5 and 7.1 mg l^{-1}, and oxygen saturation at 104%. Aquarium cleaning routines were done as required to avoid algal growth.

4.2.3. Analytical procedures

4.2.3.1. Survival, coral condition and samples storage

The number of surviving fragments of the nine-coral species was assessed every day during the 60 days of the three temperature treatments with only the relevant days indicated in the results. The coral condition of the ten fragments of each coral species per treatment was visually assessed according to four categories: normal, pale, bleached, and dead (Jokiel and Coles, 1974). Normal corals were defined as having their normal coloration while pale corals showed a visible decrease in pigmentation. Bleached corals were considered as totally colorless and dead corals had no tissue at all. Coral condition was assessed on the 60^{th} day of experiment by the same person to remove observer bias. After this procedure, six fragments of each coral species per treatment were removed from the experimental aquarium, separated from the respective anchor, and placed inside individual and identified sterilized bottles on ice-cold conditions. Nevertheless, there were combinations of temperature treatments and coral species where no fragments were taken at all due to mortality (Table 4.3). In the temperature treatments where the coral species were alive, the coral fragments in the best condition were selected since the ones presenting partial mortality would not have enough tissue for biomarker analysis. The number of fragments of each coral species used in the three biomarker analysis per treatment is given in Table SM4.1 of the supplementary material. Then, they were kept inside refrigerated boxes and transported to the laboratory where they were stored at – 80 ºC.

Table 4.3. Number of surviving fragments of the nine-coral species tested on the relevant days of the three temperature treatments.

		Days																									
Treatment	Species	1	5	7	11	16	19	20	21	26	28	29	35	36	37	38	40	41	45	46	47	48	50	52	55	59	60
26 °C	*A. tenuis*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (BM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (GM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	T. reniformis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	E. lamellosa	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	G. fascicularis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	P. damicornis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	S. pistillata	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	P. contigua	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
30 °C	*A. tenuis*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (BM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (GM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	T. reniformis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	E. lamellosa	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	G. fascicularis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	P. damicornis	10	10	10	10	9	9	9	9	9	9	9	9	8	8	3	3	2	2	2	2	1	1	1	1	0	0
	S. pistillata	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	8	8	8	8	3	2	0	0
	P. contigua	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
32 °C	*A. tenuis*	8	8	8	8	8	8	8	8	8	8	8	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	M. capricornis (BM)	10	10	10	10	10	10	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M. capricornis (GM)	10	10	10	10	10	10	9	9	9	9	9	9	9	9	9	9	9	9	9	3	3	3	3	3	3	0
	T. reniformis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	E. lamellosa	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	5	5	0	0	0	0	0	0	0	0	0
	G. fascicularis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	P. damicornis	10	10	10	10	10	9	9	8	6	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S. pistillata	10	10	10	10	10	9	9	6	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P. contigua	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	9	7	7	7	7	7

4.2.4. Protein extraction

Coral fragments were carefully rinsed with ultrapure water (mili-Q water) to remove salt water and then the excess moisture was dried with absorbent lab paper. Afterwards, all the coral fragments were smashed (1 fragment = 1 sample) with a mortar and pestle and then were placed on 5 mL plastic microtubes. The samples were then homogenized in 1 mL of phosphate buffered saline solution (0.14 M NaCl, 0.003 M KCl, 0.01 M Na_2HPO_4, 0.002 M KH_2PO_4, pH7.4) to extract cytosolic proteins, using a glass/teflon Potter Elve-jhem tissue grinder, in ice-cold conditions and mixed. The crude homogenates were then centrifuged at 4 ℃ for 15 min at 10,000 × g. The supernatant was collected, transferred to new microtubes (1.5 mL) and frozen immediately (−80 ºC).

4.2.5. Total Protein determination

For normalizing the results, the total protein content was determined through the Bradford method (Bradford, 1976). A calibration curve was obtained using bovine serum albumin (BSA) standards (0-2.0 $mg.mL^{-1}$).

4.2.6. Oxidative damage products – lipid peroxidation

The lipid peroxides assay was adapted from the thiobarbituric acid reactive substances (TBARS) protocol (Uchiyama and Mihara, 1978). In brief, five µL of each sample, already processed as previously described were added to 45 µL of 50 mM monobasic sodium phosphate buffer. Then 12.5 µL of SDS 8.1%, 93.5 µL of trichloroacetic acid (20%, pH = 3.5) and 93.5 µL of thiobarbituric acid (1%) were added to each microtube. To this mixture, 50.5 µL of Milli-Q grade ultrapure water was added. Then, the microtubes were put in a vortex for 30 s, centrifuged at 10,000 × g for 1 min, their lids were punctured with a needle and then incubated in boiling water for 10 min. To stop the reaction, they were placed on ice for a few minutes and 62.5 µL of Milli-Q grade ultrapure water was added. Then, the microtubes were placed in a

vortex and centrifuged at 10,000 × g for 1 min. Duplicates of 150 µL of the supernatant of each reaction were put into a 96-well microplate and absorbance was read at 530 nm. To quantify the lipid peroxides, an eight-point calibration curve (0–0.3 µM TBARS) was calculated using malondialdehyde bis (dimethylacetal) (MDA) standards (Merck Millipore, Portugal).

4.2.7. Enzymatic assays

4.2.7.1. Catalase activity

Catalase (CAT) activity (EC1.11.1.6) was assessed by using the peroxidatic function of catalase for determination of enzyme activity. The method is based on the reaction of catalase with methanol in the presence of hydrogen peroxide according to a method previously described by Johansson and Borg (1988) and adapted for 96-well microplate. In brief, 20 µL of sample in sample buffer (25 mM of KH_2PO_4, containing 1 mM EDTA and 0.1% BSA; pH 7.0), 30 µL methanol and 100 µL of assay buffer (100 mM KH_2PO_4, pH 7.0) were added to microplate wells. Then, 20 µL of standard (4.25 mM formaldehyde), 30 µL methanol and 100 µL of assay buffer were added to formaldehyde standard wells. To the positive control wells, were added 20 µL of diluted CAT (bovine liver CAT), 30 µL of methanol and 100 µL of assay buffer. Afterwards, the reaction was initiated by adding 20 µL of hydrogen peroxide (0.035 M H_2O_2) to the microplate wells. Then, the microplate was covered with aluminium foil and incubated during 20 min at room temperature on a shaker. Following incubation, 30 µL of potassium hydroxide (10 M KOH) was added to each microplate well to end reaction followed by adding 30 µL of Purpald chromogen (34.2 mM of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) to each well. Again, the microplate was covered and incubated on a shaker for 10 min at room temperature. Next 10 µL of potassium periodate (65.2 mM KIO_4) was added to each well and the microplate incubated for 5min on the shaker. The absorbance was read at 540 nm using a microplate reader (Benchmark, Bio-Rad, USA).

4.2.7.2. Glutathione S-transferase activity

The enzymatic assay of glutathione S-transferase (GST) activity (EC 2.5.1.18) was adapted from Habig et al. (1974), using the CDNB (1-chloro-2,4-dinitrobenzene) as the enzyme substrate, and optimized for 96-well microplates. After reading the absorbance at 340 nm GST activity was calculated using a molar extinction coefficient for CDNB of 5.3 εmM ($mM^{-1}cm^{-1}$) after correction for the microplate wells path length.

4.2.8. Statistical analyses

Three permutational univariated analysis of variance (PERMANOVA) based on Euclidean distances (Anderson, 2001) were used to test whether lipid peroxidation, catalase activity and glutathione S-transferase activity of the coral fragments were affected by temperature and coral species. Values of the pseudo-F statistic were computed with the permutation of residuals under a reduced model using 9999 permutations. Analyses were performed using PERMANOVA+ for PRIMER v6 (PRIMER-E Ltd., Plymouth). Post-hoc pair-wise comparisons were then performed using PERMANOVA to compare between temperature treatments and coral species. Significant differences were expected between both main effects and the interaction between temperature treatments and coral species. In the interaction, only the post-hoc pair-wise comparisons between different temperature treatments for the same species and between different species in the same temperature treatment were performed. Such post-hoc pair-wise comparisons were necessary for infer how temperature treatments affect each species and this way reveal the different susceptibility among coral species to heat stress. Differences were considered significant at $P < 0.05$.

4.3. Results

4.3.1. Survival

At 26 ºC, all the coral species survived throughout the experiment (Table 4.3). At 30 ºC, a great reduction in the number of surviving fragments was observed in *P. damicornis* on the 38th day and in *S. pistillata* on the 52nd day of experiment (3 fragments, Table 4.3). All the other species fragments survived throughout the total duration of the experiment (Table 4.3). At 32 °C, *P. damicornis* and *S. pistillata* were the first coral species to present a considerable reduction in the number of surviving fragments (6 and 5 fragments on the 26th day, respectively), followed by both *A. tenuis* and *M. capricornis* (BM) (4 and 0 fragments on the 35th day), *E. lamellosa* (5 fragments on the 40th day), and *M. capricornis* (GM) (3 fragments on the 47th day, Table 4.3). The species *T. reniformis* and *G. fascicularis* did not present any reduction in the number of surviving fragments, whereas *P. contigua* presented some reduction on the 50th day of experiment (7 fragments, Table 4.3)

4.3.2. Coral general condition

At 26 ºC, all the coral species maintained their normal coloration (Fig. 4.1). At 30 °C, the fragments of *A. tenuis*, *M. capricornis* (GM), *E. lamellosa*, and *G. fascicularis* maintained their normal coloration (Fig. 4.1). Seventy percent of the fragments of *M. capricornis* (BM), all the fragments of *T. reniformis*, and 60% of the fragments of *P. contigua* were pale (Fig.4.1). All the fragments of *S. pistillata* and *P. damicornis* were dead (Fig.4.1). At 32 ºC, most of the coral species were dead (*A. tenuis, M. capricornis* (both morphotypes), *E. lamellosa, S. pistillata,* and *P. damicornis*), exceptions of *P. contigua* (70% pale and 30% dead), *T. reniformis* (100% pale), and *G. fascicularis* (50% bleached, 10% pale and 40% normal) (Fig. 4.1).

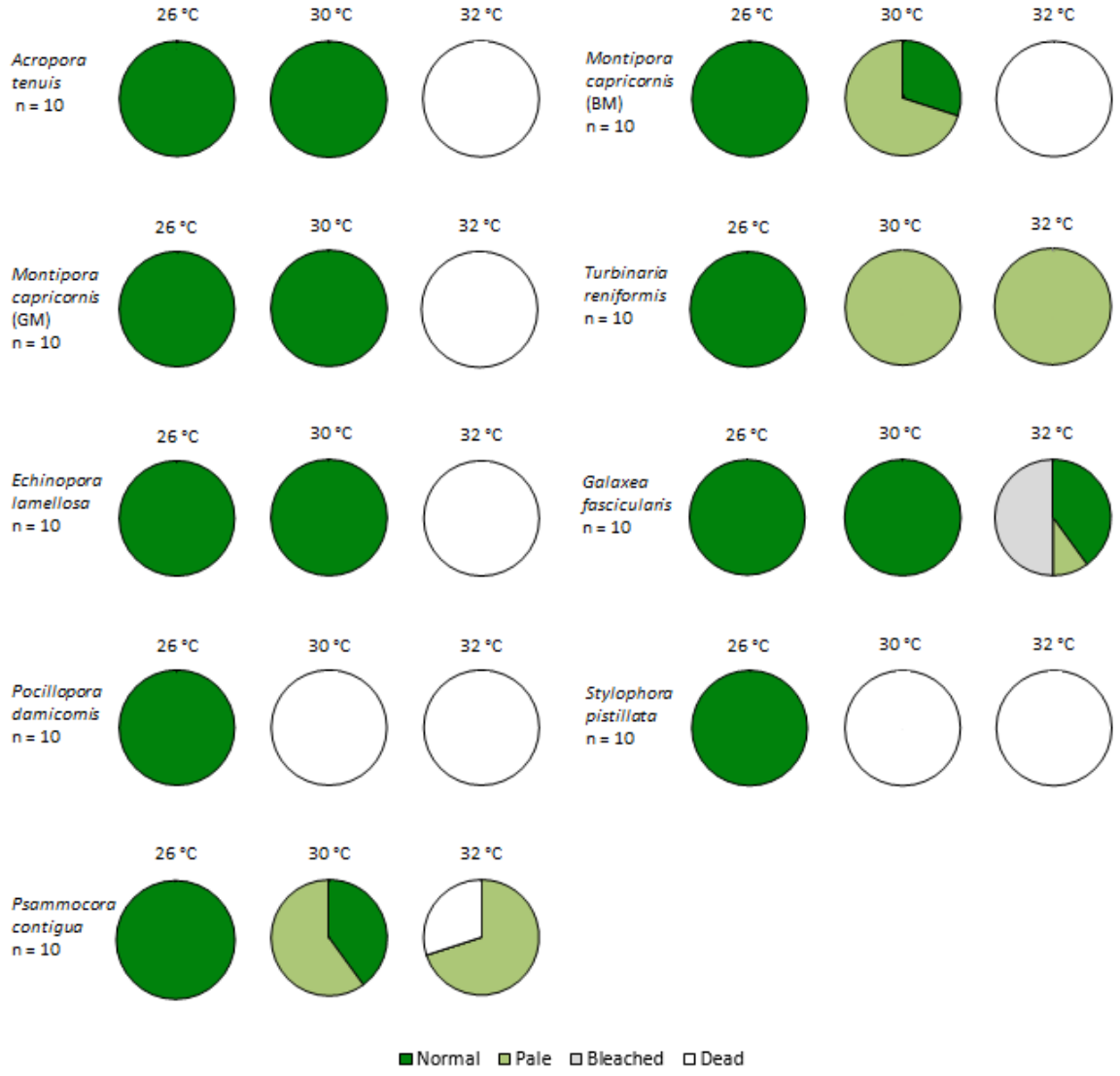

Figure 4.1. Coral condition of the nine-coral species studied in the three temperature treatments.

4.3.3. Lipid peroxidation (LPO)

Lipid peroxidation concentrations were significantly affected by the interaction of temperature and coral species (PERMANOVA $P < 0.05$, Table 4.4). Lipid peroxidation concentrations presented significant differences among temperatures (PERMANOVA pair-wise $P < 0.05$; Fig. 4.2a). *Echinopora lamellosa* fragments presented the highest LPO concentrations, whereas the fragments of *T. reniformis*, *A. tenuis* and *P. contigua* showed the lowest LPO concentrations (PERMANOVA pair-wise $P < 0.05$; Fig. 4.2a).

Table 4.4. Summary of results of PERMANOVA permutation tests applied to report the effects of coral species and temperature in coral fragments' lipid peroxidation (MDA) and both catalase (CAT) and glutathione S-transferase (GST) activities. Some combinations in the interaction temperature × species had no fragments as observed in **Table 4.3.** Significant differences are marked in bold.

	df	SS	MS	Pseudo-*F*	P(perm)	Unique perms
MDA						
Temperature	2	0.1	6.0×10^{-2}	16.0	**0.0001**	9957
Species	8	1.2	0.2	40.9	**0.0001**	9934
Temperature × Species	8	0.4	5.3×10^{-2}	14.1	**0.0001**	9941
CAT activity						
Temperature	2	0.4	0.2	16.5	**0.0001**	9953
Species	8	3.9	0.5	41.1	**0.0001**	9946
Temperature × Species	8	1.4	0.2	14.9	**0.0001**	9942
GST activity						
Temperature	2	9.6×10^{-2}	4.8×10^{-2}	15.6	**0.0001**	9946
Species	8	0.6	7.3×10^{-2}	23.6	**0.0001**	9928
Temperature × Species	8	0.2	2.5×10^{-2}	8.0	**0.0001**	9946

At 26 ºC, *M. capricornis* (GM) and *E. lamellosa* fragments had the highest LPO concentrations (PERMANOVA pair-wise P > 0.05), whereas the fragments of *M. capricornis* (BM), *T. reniformis,* and *A. tenuis* displayed the lowest LPO concentrations (PERMANOVA pair-wise P > 0.05, Table 4.5, Fig.2a). At 30 ºC, *E. lamellosa* fragments had the highest LPO concentrations (PERMANOVA pair-wise P < 0.05), whereas *A. tenuis* and *T. reniformis* fragments had the lowest LPO concentrations (PERMANOVA pair-wise P > 0.05, Table 4.5, Fig. 4.2a). At 32 ºC, *G. fascicularis* showed the highest LPO concentrations, whilst *P. contigua* presented the lowest one (PERMANOVA pair-wise P < 0.05, Table 4.5, Fig. 4.2a)

Montipora capricornis (BM), *E. lamellosa, A. tenuis* and *P. contigua* fragments had significant intraspecific differences in LPO concentrations between 26 ºC and 30 ºC (PERMANOVA pair-wise P < 0.05; Fig. 4.2a), being higher at 30 ºC in the first two coral species and higher at 26 ºC in the last two coral species. *Galaxea fascicularis* and *P. contigua* fragments also showed significant intraspecific differences in LPO concentrations between 26 ºC and 32 ºC (PERMANOVA pair-wise P < 0.05; Fig. 4.2a), being higher at 32 ºC in *G. fascicularis* and higher at 26 ºC in *P. contigua.*

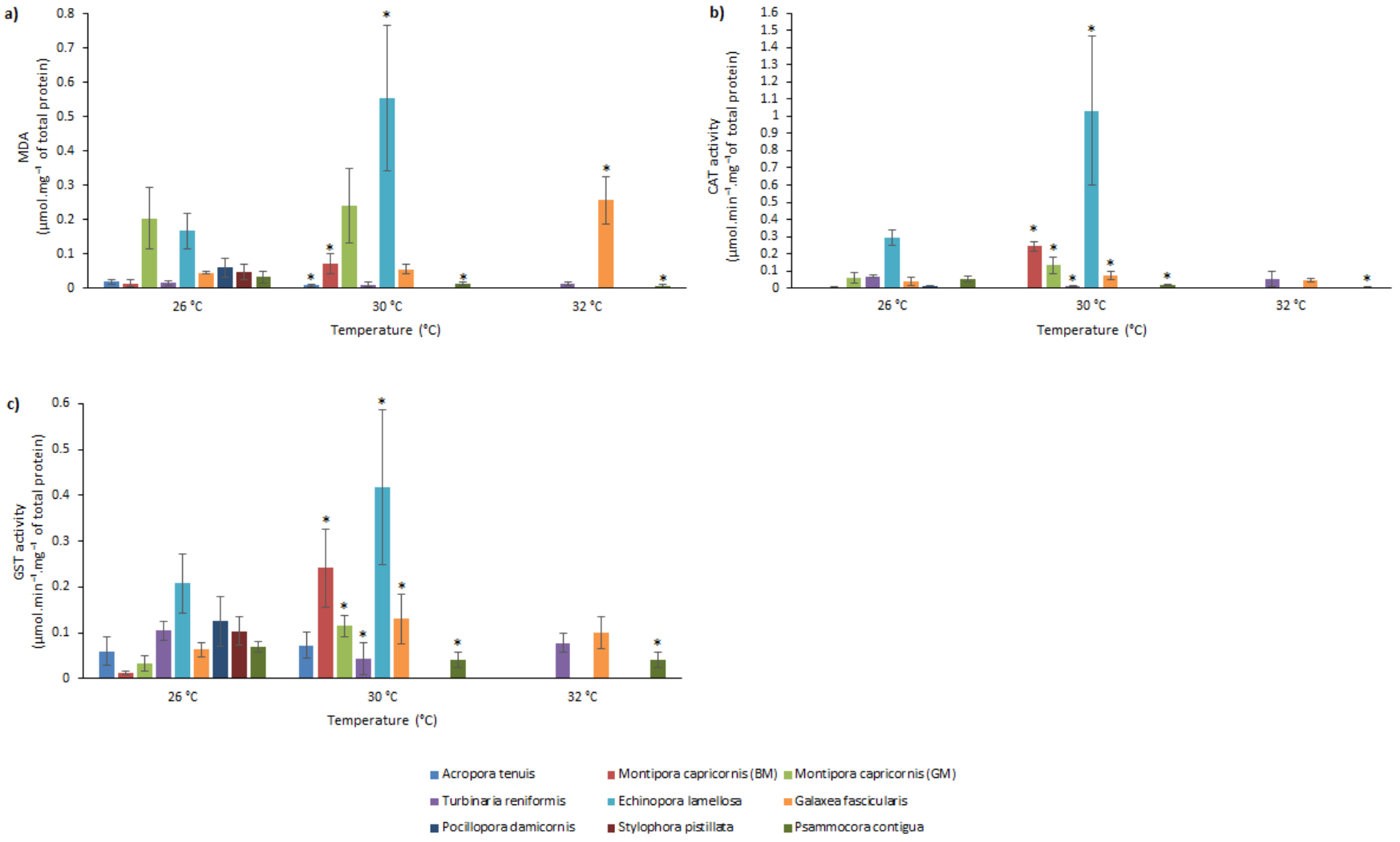

Figure 4.2. Mean ± SD of (a) MDA (µmol.mg^{-1} of total protein), (b) catalase activity (µmol.min^{-1}.mg^{-1} of total protein), and (c) glutathione S-transferase (GST) activity (µmol.min^{-1}.mg^{-1} of total protein) for the nine-coral species studied in the three temperature treatments. Asterisks (*) mark significant differences ($P < 0.05$) in relation to control temperature.

Table 4.5. Mean ± SD of MDA (µmol.mg^{-1} of total protein), catalase (CAT) activity (µmol.min^{-1}.mg^{-1} of total protein), and glutathione S-transferase (GST) activity (µmol.min^{-1}.mg^{-1} of total protein) for the nine-coral species tested in the three temperature treatments. NA – not available due to total mortality before the 60th day of experiment.

	26 °C			30 °C			32 °C		
Species	MDA	CAT activity	GST activity	MDA	CAT activity	GST activity	MDA	CAT activity	GST activity
Acropora tenuis	0.019 ± 0.006	0.003 ± 0.001	0.060 ± 0.031	0.009 ± 0.002	0.003 ± 0.000	0.073 ± 0.028	NA	NA	NA
Montipora capricornis (BM)	0.015 ± 0.010	0.007 ± 0.003	0.012 ± 0.004	0.072 ± 0.029	0.243 ± 0.030	0.241 ± 0.084	NA	NA	NA
Montipora capricornis (GM)	0.203 ± 0.089	0.061 ± 0.030	0.034 ± 0.017	0.240 ± 0.109	0.132 ± 0.050	0.115 ± 0.023	NA	NA	NA
Turbinaria reniformis	0.017 ± 0.006	0.069 ± 0.009	0.105 ± 0.020	0.011 ± 0.007	0.012 ± 0.005	0.043 ± 0.034	0.013 ± 0.005	0.052 ± 0.043	0.078 ± 0.020
Echinopora lamellosa	0.167 ± 0.050	0.294 ± 0.047	0.207 ± 0.064	0.555 ± 0.212	1.033 ± 0.436	0.419 ± 0.169	NA	NA	NA
Galaxea fascicularis	0.050 ± 0.004	0.039 ± 0.022	0.064 ± 0.015	0.056 ± 0.014	0.080 ± 0.024	0.130 ± 0.055	0.257 ± 0.068	0.046 ± 0.009	0.099 ± 0.035
Pocillopora damicornis	0.061 ± 0.028	0.014 ± 0.003	0.125 ± 0.055	NA	NA	NA	NA	NA	NA
Stylophora pistillata	0.048 ± 0.024	0.005 ± 0.001	0.104 ± 0.031	NA	NA	NA	NA	NA	NA
Psammocora contigua	0.033 ± 0.018	0.054 ± 0.019	0.070 ± 0.012	0.014 ± 0.004	0.020 ± 0.004	0.041 ± 0.017	0.007 ± 0.004	0.006 ± 0.003	0.041 ± 0.016

4.3.4. Catalase (CAT)

Catalase activity was significantly affected by the interaction of temperature and coral species (PERMANOVA $P < 0.05$, Table 4.4). Coral fragments' catalase activity presented significant differences among temperatures (PERMANOVA pair-wise $P < 0.05$), exception between 30 °C and 32 °C (PERMANOVA pair-wise $P > 0.05$; Fig. 4.2b). *Echinopora lamellosa* presented the highest catalase activity, whilst *A. tenuis* presented the lowest one (PERMANOVA pair-wise $P < 0.05$; Fig. 4.2b).

At 26 ºC and 30 ºC, the fragments of *E. lamellosa* presented the highest catalase activity, whereas the lowest catalase activity was observed in *A. tenuis* fragments (PERMANOVA pair-wise $P < 0.05$, Table 4.5, Fig. 4.2b). At 32 ºC, *T. reniformis* and *G. fascicularis* presented the highest catalase activity (PERMANOVA pair-wise $P > 0.05$), whilst *P. contigua* presented the lowest one (PERMANOVA pair-wise $P < 0.05$; Table 4.5, Fig. 4.2b).

Catalase activity of the coral species *M. capricornis* (BM), *M. capricornis* (GM), *E. lamellosa, G. fascicularis, T. reniformis,* and *P. contigua* presented significant intraspecific differences at 26 °C and 30 °C (PERMANOVA pair-wise $P < 0.05$, Fig. 4.2b), being higher at 30 ºC in the first four coral species and lower at 30 ºC in the last two coral species. The species *P. contigua* also presented significant intraspecific differences between 26 ºC and 32 ºC, being higher at 26 ºC (PERMANOVA pair-wise $P < 0.05$, Fig. 4.2b).

4.3.5. Glutathione S-transferase (GST)

Glutathione S-transferase activity was significantly affected by the interaction of temperature and coral species (PERMANOVA $P < 0.05$, Table 4.4). Coral fragments' GST activity presented significant differences among temperatures, but only between the 26 ºC and 30 ºC treatments (PERMANOVA pair-wise $P < 0.05$; Fig. 4.2c). *Echinopora lamellosa* had the

highest GST activity (PERMANOVA pair-wise P > 0.05), whereas *P. contigua* and *A. tenuis* displayed the lowest GST activity (PERMANOVA pair-wise P > 0.05; Fig. 4.2c).

At 26 ºC, *E. lamellosa* showed the highest GST activity and *M. capricornis* (BM) displayed the lowest one (PERMANOVA pair-wise P < 0.05; Table 4.5, Fig. 4.2c). At 30 ºC, the coral species *E. lamellosa* had the highest GST activity (PERMANOVA pair-wise P < 0.05), whilst *P. contigua*, *T. reniformis*, and *A. tenuis* showed the lowest GST activity (PERMANOVA pair-wise P > 0.05; Table 4.5, Fig. 4.2c). At 32 ºC, the fragments of *T. reniformis* and *G. fascicularis* presented the highest GST activity (PERMANOVA pair-wise P > 0.05), whereas the lowest GST activity was observed in *P. contigua* fragments (PERMANOVA pair-wise P < 0.05; Table 4.5, Fig. 4.2c).

Montipora capricornis (BM), *M. capricornis* (GM), *E. lamellosa*, *G. fascicularis*, *T. reniformis*, and *P. contigua* fragments showed significant intraspecific differences in GST activity between 26 ºC and 30 ºC (PERMANOVA pair-wise P < 0.05; Fig. 4.2c), being higher at 30 ºC in the first four coral species and lower at 30 ºC in the last two coral species. *Psammocora contigua* fragments also showed significant intraspecific differences in GST activity between 26 ºC and 32 ºC, being highest at 26 ºC (PERMANOVA pair-wise P < 0.05; Fig. 4.2c).

4.4. Discussion and conclusions

The results obtained in coral condition indicate that all the coral species tested are sensitive to long-term exposure to 32 °C. *Turbinaria reniformis*, *G. fascicularis*, and *P. contigua* were the species most resistant to prolonged heat stress because they were the only survivors at 32 °C, although in different conditions. Other studies have reached the same conclusion regarding the ability of these coral species to withstand heat stress both in the field (Brown and Suharsono, 1990; Stimson et al., 2002), as well as under controlled conditions (Dias et al., 2018). *Stylophora pistillata* and *P. damicornis* were the most sensitive species, since they did not tolerate long-term exposure to 30 °C, as indicated by the death of 100% of their fragments.

These coral species are considered in several studies as highly susceptible to heat stress (Stimson et al., 2002; McClanahan et al., 2004; Dias et al., 2018).

Most of the species in this study responded to elevated temperature exposure by exhibiting increased activities of the protective enzymes catalase (CAT) and glutathione S-transferase (GST). In general, antioxidant enzyme activity increased at 30 ºC (*M. capricornis* (BM), *M. capricornis* (GM), *E. lamellosa*, and *G. fascicularis*; Fig. 4.2). The induction of these antioxidant enzymes occurs as a way to counteract the effects of ROS, produced due to temperature enhanced metabolic rates (Burdon et al., 1990; Richier et al., 2006), suggested in this study as an increase in LPO concentrations. Still, the levels of lipid peroxidation increased with water temperature in some species. At 30 °C and 32 °C, the coral condition of most species worsened compared to control treatment (Fig. 4.1). This observation supports the induction of a bleaching event as a consequence of an oxidative stress period, as observed in Richier et al. (2006).

Echinopora lamellosa presented the highest oxidative damage, but also the highest antioxidant response (CAT and GST) at 30 °C. This could mean a system overcharge with an imbalance in ROS production vs destruction and subsequent tendency for ROS accumulation. Therefore, this coral species seems to have the ability to withstand long-term exposure to 30 °C, however, it did not survive at 32 °C as evidenced by its 100% mortality. This species is considered as having moderate susceptibility to heat stress (Dias et al., 2018). As a result, it was assumed it would present lower levels of oxidative damage and antioxidant response when compared with species with higher susceptibility to heat stress such as *Acropora tenuis* (Brown et al., 2002; Downs et al., 2002). Fitt et al. (2009) observed that *Porites cylindrica*, a resistant coral species to heat stress, presented higher levels of SOD and heat shock proteins (hsp) than *Stylophora pistillata*, a coral species very susceptible to heat stress. Moreover, there are other species of marine invertebrates with high resistance to thermal stress that have basal levels of hsp70 constantly elevated. This mechanism ensures their protection in environments subjected to constant temperature fluctuations (Madeira et al., 2014; Mizrahi et al., 2016).

The second species with the highest oxidative damage was *M. capricornis* (GM), this coral species evidenced antioxidant response at 30 °C (CAT and GST), although the oxidative damage in terms of LPO has not been detected. This could mean that no increase in the levels of oxidative damage products was observed with increase temperature, because the increase in antioxidant enzyme activities (CAT and GST) counterbalanced the effects of ROS avoiding cellular membrane damage. This species presented normal condition at 30 °C, which indicates great ability to control the damage at the molecular level and thus withstand long-term exposure to 30 °C.

Galaxea fascicularis was the third coral species with the highest oxidative damage, however, it only occurred at 32 °C. At 30 °C this coral species already evidenced antioxidant response, although oxidative damage has not been detected in the LPO analysis, as in the case of *M. capricornis* (GM). This is one of the most resistant species, but at 32 °C it was negatively affected by heat stress, as evidenced by 50% of its fragments being bleached. The low levels of the antioxidant enzymes at 32 °C probably reflect the exhaustion of the production system due to a great mobilization of these enzymes. This species withstood long-term exposure to 30 °C and 32 °C, but the damage evidenced in terms of LPO and its condition at 32 °C are indicative that it would hardly withstand longer periods of time at this temperature or even higher temperatures.

Psammocora contigua and *T. reniformis* presented a decrease in CAT and GST activities at 30 °C compared to the control, with *T. reniformis* presenting recovery at 32 °C. Both species are sensitive to 30 °C, they reached the pale condition, but did not die. They can survive long-term exposure to 30 °C and 32 °C, despite the evident change in terms of condition. *Turbinaria reniformis* maintained the activities of antioxidant enzymes when exposed to 32 ºC indicating that it managed oxidative stress, probably through physiological acclimation to high temperature conditions throughout the long duration of the experiment (Middlebrook et al., 2008). In the case of *T. reniformis* at 30 ºC and *P. contigua* at both 30 ºC and 32 ºC, the overall decrease of capacity of antioxidant enzymes in response to prolonged exposure to high temperature may be related to damage on ROS-sensitive enzymes, as observed by the decrease in CAT and GST activities (Hermes-Lima and Storey, 1993). Another possible explanation relates to ROS have been scavenged by fluorescent proteins. During heat-related oxidative

stress, CAT can be overwhelmed or limited (Martindale and Holbrook, 2002; Merle et al., 2007). On the other hand, fluorescent proteins are heat-resistant (Ward, 1998) and may supplement CAT during thermal oxidative stress once these proteins are also able to proficiently scavenge H_2O_2 (Palmer et al., 2009). The reduction of LPO levels in *P. contigua* fragments may suggest the occurrence of a remodeling process in the lipid composition of biological membranes (Niu and Xiang, 2018).

In both *P. contigua* and *A. tenuis* fragments the higher concentrations of LPO were observed at 26 °C. These are fast-growing coral species with higher growth rates at 26 °C than at 30 °C, and 32 ºC in the case of *P. contigua*, being the species with the highest growth rates of the nine-species studied (Dias et al., 2018). This could mean increased metabolic rates and a consequent increase in ROS production and cellular membrane damage inside their tissues at 26 ºC (Burdon et al., 1990; Richier et al., 2006).

Stylophora pistillata and *P. damicornis* are species highly sensitive to long-term exposure to 30 °C, they did not withstand heat stress even when moderate. These species presented very low CAT activity in the control treatment such as *M. capricornis* (BM) and *A. tenuis*, which can indicate low constitutive levels of this enzyme (the same does not apply for GST). In Dias et al. (2018), the hierarchy of species susceptibility to heat stress was obtained using the same experimental procedure and assessing total and partial mortality as heat stress indicators every 20 days of experiment. According to the hierarchy established in Dias et al. (2018), the species more severely affected by heat stress were *A. tenuis, S. pistillata,* and *P. damicornis*, immediately followed by *M. capricornis* (BM). Thus, the low constitutive levels of CAT could be a characteristic of these species that confers them thermal vulnerability.

The duration of heat stress events can change among years and geographic locations (Stimson et al., 2002; Liu et al., 2003; Pratchett et al., 2011). Indo-Pacific reports of heat stress events have durations between 26 days (Northwestern Hawaiian Islands in 2004; Goldberg and Wilkinson, 2004) and 184 days (Thousand Islands in 1983, Brown and Suharsono, 1990; Pulau Weh in 2010, Guest et al., 2012). On the 26th day of experiment our results show that only *P. damicornis* lost one of their fragments at 30 ºC. In addition, at 32 ºC, only *P. damicornis* and

S. pistillata would have been the most susceptible species, with the loss of four and five fragments, respectively. Thus, thermal stress duration is one of the crucial factors affecting the physiological response of coral species to heat stress. Nevertheless, a great number of studies report heat stress events with a duration around 60 days (Brown, 1997; George and John, 1999; Bruno et al., 2001; McClanahan et al., 2001; Mumby et al., 2001; Stimson et al., 2002; Adjeroud et al., 2009) or higher (Brown and Suharsono, 1990; Hoegh-Guldberg and Salvat, 1995; Cohen et al., 1997; Davies et al., 1997; Edwards et al., 2001; Riegl, 2002; Liu et al., 2003; Goldberg and Wilkinson, 2004; Penin et al., 2007; Guest et al., 2012; Riegl et al., 2012; Depczynski et al., 2013; Pratchett et al., 2013; Carroll et al., 2017; Le Nohaïc et al., 2017). So, the duration of our experiment seems adequate.

Another important factor to have into account is previous thermal history (acclimation). The colonies of the coral species in study have been subjected to the same temperature (25 ºC) for five years prior to the experimental temperature treatments, this is, without temperature variability. However, it has been shown in several studies that colonies of coral species exposed to a greater background temperature variability or prior exposure to heat stress present higher bleaching resistance (Coles and Jokiel, 1978; Castillo and Helmuth, 2005; Middlebrook et al., 2008; Oliver and Palumbi, 2011; Palumbi et al., 2014).

The heating rate is another crucial factor affecting the physiological response of the coral and its symbiotic partner (Middlebrook et al., 2010). In this study, the heating rate of 1 ºC $hour^{-1}$ was applied to mimic the temperature changes experienced by the coral species in their natural environment. The used heating rate is substantially slower than the one applied in previous studies evaluating the effect of heat stress exposure on antioxidant enzyme defenses of scleractinian corals (e.g. Yakovleva et al., 2004; Griffin et al., 2006; Higuchi et al., 2008). For instance, in Griffin et al. (2006), where an instantaneous heating rate was applied, all the *P. damicornis* nubbins died on the 5^{th} day at 32 ºC, whereas in our study all the *P. damicornis* fragments died on the 29^{th} day at 32 ºC. These different results may be related with the different heating rate, once a slower heating rate deeply delay the physiological response of the coral host and symbiotic algae (Middlebrook et al., 2010).

Branching pocilloporids, *S. pistillata* and *P. damicornis,* are one of the dominant taxa of many Indo-Pacific reef communities in terms of substrate cover and are ecologically important because their branching growth form provides habitat for other species (Cumming, 1999; Dalton and Carroll, 2011; Lenihan et al., 2011). However, these fast-growing coral species are highly susceptible to natural disturbances including extreme events (tropical storms, heat stress and ENSO events, Marshall and Baird, 2000; Bruno and Selig, 2007; Fabricius et al., 2008), coral predator outbreaks of crown-of-thorns starfish *Acanthaster planci* and *Drupella* spp. snails (Cumming, 1999; Pratchett, 2001; Bruckner et al., 2017), competition with algae (Rasher et al., 2011; Swierts and Vermeij, 2016), and diseases (Willis et al., 2004). Our results corroborate previous findings by Brown and Suharsono (1990), Marshall and Baird (2000), Stimson et al. (2002), and Dalton and Carroll (2011) that pocilloporids are highly susceptible to heat stress events. In Indo-Pacific coral communities affected by heat stress events, shifts in coral taxa dominance have often been observed (McClanahan, 2000; Adjeroud et al., 2009; Harris et al., 2014; Johns et al., 2014; Hughes et al., 2018; Torda et al., 2018). Observations of branching and plating fast-growing coral species, often more susceptible to heat stress, have been replaced by encrusting and massive slow-growing coral species, often more resistant to heat stress (Loya et al., 2001).

The coral species in study are sympatric (Veron, 1990; Fujioka, 1998), inhabiting the same geographical region (Tables 4.1 and 4.2), although living in different zones of the same reef. According to habitat preferences given in Table 4.1, both *T. reniformis* and *G. fascicularis* have preference for lower reef slope protected from the wave action, however, these coral species present different preferences in relation to water clarity and *G. fascicularis* also has preference for other reef zones. The coral species *P. damicornis*, *S pistillata,* and *P. contigua* have habitat preference for the inner reef flat zone with clear waters and exposed to wave action, overlapping their distribution on the reef. Having into account the distribution of the coral species' colonies on the reef and given the expected increases in frequency and intensity of heat stress events (Van Hooidonk et al., 2016), it might be expected that *P. contigua* would replace *P. damicornis* and *S. pistillata* on the inner reef flat zone. It would also be expected the maintenance or increase in coral coverage of *T. reniformis* on the lower reef slope with turbid water and protected from wave action. Regarding *G. fascicularis*, given that this species probably will not last longer at 32 ºC according to LPO results and that great part of thermal stress events had the duration of 60 days or more, it might decrease in coral coverage under these circumstances.

Despite these results we need to have into account that even heat-tolerant coral species have their limits and if their thermal tolerance threshold is surpassed we might observe a shift from coral dominated to algal dominated reefs with loss of functional complexity and biodiversity (Depczynski et al., 2013).

Having in account that this study was made with colonies coming from the same mother colony, meaning low genetic variability, it is important to consider that some results may vary from those in future studies.

The nine-coral species in this study responded differently to thermal stress. *Psammocora contigua, T. reniformis,* and *G. fascicularis* are heat-tolerant species, whereas *S. pistillata* and *P. damicornis* are heat-susceptible species. Oxidative damage and antioxidant enzyme activity are useful biomarkers to assess the long-term effects of heat stress in scleractinian corals.

4.5. References

Abele, D., Großpietsch, H., & Pörtner, H. O. (1998). Temporal fluctuations and spatial gradients of environmental PO_2, temperature, H_2O_2 and H_2S in its intertidal habitat trigger enzymatic antioxidant protection in the capitellid worm *Heteromastus filiformis*. Marine Ecology Progress Series, 163, 179-191.

Adjeroud, M., Michonneau, F., Edmunds, P. J., Chancerelle, Y., De Loma, T. L., Penin, L., … & Galzin, R. (2009). Recurrent disturbances, recovery trajectories, and resilience of coral assemblages on a South Central Pacific reef. Coral Reefs, 28(3), 775-780.

Albright, R., & Mason, B. (2013). Projected near-future levels of temperature and pCO_2 reduce coral fertilization success. PLoS One, 8(2), e56468.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26(1), 32-46.

Anithajothi, R., Duraikannu, K., Umagowsalya, G., & Ramakritinan, C. M. (2014). The presence of biomarker enzymes of selected scleractinian corals of Palk Bay, southeast coast of India. BioMed Research International, 2014.

Asada, K. (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annual Review of Plant Biology, 50(1), 601-639.

Attig, H., Kamel, N., Sforzini, S., Dagnino, A., Jamel, J., Boussetta, H., ... & Banni, M. (2014). Effects of thermal stress and nickel exposure on biomarkers responses in *Mytilus galloprovincialis* (Lam). Marine Environmental Research, 94, 65-71.

Baird, A. H., & Thomson, D. P. (2018). Coral reproduction at Hall Bank, a high latitude coral assemblage in Western Australia. Aquatic Biology, 27, 55-63.

Berkelmans, R., & Willis, B. L. (1999). Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs, 18(3), 219-228.

Bindoli, A. (1988) Lipid peroxidation in mitochondria. Free Radical Biology and Medicine 5, 247–261.

Babcock, R. C., Wills, B. L., & Simpson, C. J. (1994). Mass spawning of corals on a high latitude coral reef. Coral Reefs, 13(3), 161-169.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), 248-254.

Brahmi, C., Kopp, C., Domart-Coulon, I., Stolarski, J., & Meibom, A. (2012). Skeletal growth dynamics linked to trace-element composition in the scleractinian coral *Pocillopora damicornis*. Geochimica et Cosmochimica Acta, 99, 146-158.

Brook, F. J. (1999). The coastal scleractinian coral fauna of the Kermadec Islands, southwestern Pacific Ocean. Journal of the Royal Society of New Zealand, 29(4), 435-460.

Brown, B. E. (1997). Coral bleaching: causes and consequences. Coral Reefs, 16(1), S129-S138.

Brown, B. E., Suharsono (1990). Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. Coral Reefs, 8(4), 163-170.

Brown, B. E., Downs, C. A., Dunne, R. P., & Gibb, S. W. (2002). Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. Marine Ecology Progress Series, 242, 119-129.

Bruckner, A. W., Coward, G., Bimson, K., & Rattanawongwan, T. (2017). Predation by feeding aggregations of *Drupella* spp. inhibits the recovery of reefs damaged by a mass bleaching event. Coral Reefs, 36(4), 1181-1187.

Bruno, J. F. (1998). Fragmentation in *Madracis mirabilis* (Duchassaing and Michelotti): how common is size-specific fragment survivorship in corals?. Journal of Experimental Marine Biology and Ecology, 230(2), 169-181.

Bruno, J. F., & Selig, E. R. (2007). Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS One, 2(8), e711.

Bruno, J., Siddon, C., Witman, J., Colin, P., & Toscano, M. (2001). El Nino related coral bleaching in Palau, western Caroline Islands. Coral Reefs, 20(2), 127-136.

Buchner, T., Abele-Oeschger, D., & Theede, H. (1996). Aspects of antioxidant status in the polychaete *Arenicola marina*: tissue and subcellular distribution, and reaction to environmental hydrogen peroxide and elevated temperatures. Marine Ecology Progress Series, 143, 141-150.

Burdon, R. H., Gill, V., & Evans, C. R. (1990). Active oxygen species and heat shock protein induction. In Stress Proteins (pp. 19-25). Springer, Berlin, Heidelberg.

Cabioch, G., Montaggioni, L. F., Faure, G., & Ribaud-Laurenti, A. (1999). Reef coralgal assemblages as recorders of paleobathymetry and sea level changes in the Indo-Pacific province. Quaternary Science Reviews, 18(14), 1681-1695.

Cailleaud, K., Maillet, G., Budzinski, H., Souissi, S., & Forget-Leray, J. (2007). Effects of salinity and temperature on the expression of enzymatic biomarkers in *Eurytemora affinis* (Calanoida, Copepoda). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(4), 841-849.

Carroll, A. G., Harrison, P. L., & Adjeroud, M. (2017). Susceptibility of coral assemblages to successive bleaching events at Moorea, French Polynesia. Marine and Freshwater Research, 68(4), 760-771.

Castillo, K. D., & Helmuth, B. S. T. (2005). Influence of thermal history on the response of *Montastraea annularis* to short-term temperature exposure. Marine Biology, 148(2), 261-270.

Cereja, R., Mendonça, V., Dias, M., Vinagre, C., Gil, F., & Diniz, M. (2018). Physiological effects of cymothoid parasitization in the fish host *Pomatoschistus microps* (Krøyer, 1838) under increasing ocean temperatures. Ecological Indicators, 95, 176-182.

Chen, C. A. (1999). Analysis of scleractinian distribution in Taiwan indicating a pattern congruent with sea surface temperatures and currents: examples from *Acropora* and Faviidae corals. Zoological Studies-Taipei, 38, 119-129.

Chen, C. A., Yang, Y. W., Wei, N. V., Tsai, W. S., & Fang, L. S. (2005). Symbiont diversity in scleractinian corals from tropical reefs and subtropical non-reef communities in Taiwan. Coral Reefs, 24(1), 11-22.

Christensen, J.H., Kanikicharla, K.K., Aldrian, E., An, S.-I., Cavalcanti, I.F.A., De Castro, M., … & Midgley, P.M., (eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC AR5). Cambridge, United Kingdom and New York, New York: Cambridge University Press, pp.1217-1308.

Cohen, A. L., Lobel, P. S., & Tomasky, G. L. (1997). Coral bleaching on Johnston Atoll, Central Pacific Ocean. The Biological Bulletin, 193(2), 276-279.

Coles, S. L., & Jokiel, P. L. (1978). Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Marine Biology, 49(3), 187-195.

Connell, J. H., & Keough, M. J. (1985). Disturbance and patch dynamics of subtidal marine animals on hard substrata. In: Pickett STA, White PS (eds.). The ecology of natural disturbance and patch dynamics, 125-151

Connell, J. H., Hughes, T. P., & Wallace, C. C. (1997). A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecological Monographs, 67(4), 461-488.

Cook, R. E. (1979). Asexual reproduction: a further consideration. The American Naturalist, 113(5), 769-772.

Crabbe, M. J. C. (2008). Climate change, global warming and coral reefs: Modelling the effects of temperature. Computational Biology and Chemistry, 32(5), 311-314.

Cumming, R. L. (1999). Predation on reef-building corals: multiscale variation in the density of three corallivorous gastropods, *Drupella* spp. Coral Reefs, 18(2), 147-157.

Dai, C. F. (1990). Interspecific competition in Taiwanese corals with special reference to interactions between alcyonaceans and scleractinians. Marine Ecology Progress Series. Oldendorf, 60(3), 291-297.

Dalton, S. J., & Carroll, A. G. (2011). Monitoring coral health to determine coral bleaching response at high latitude eastern Australian reefs: an applied model for a changing climate. Diversity, 3(4), 592-610.

Davies, J. M., Dunne, R. P., & Brown, B. E. (1997). Coral bleaching and elevated sea-water temperature in Milne Bay Province, Papua New Guinea, 1996. Marine and Freshwater Research, 48(6), 513-516.

Dechnik, B., Webster, J. M., Davies, P. J., Braga, J. C., & Reimer, P. J. (2015). Holocene "turn-on" and evolution of the Southern Great Barrier Reef: Revisiting reef cores from the Capricorn Bunker Group. Marine Geology, 363, 174-190.

Depczynski, M., Gilmour, J. P., Ridgway, T., Barnes, H., Heyward, A. J., Holmes, T. H., ... & Wilson, S. K. (2013). Bleaching, coral mortality and subsequent survivorship on a West Australian fringing reef. Coral Reefs, 32(1), 233-238.

DeVantier, L. (2010). Reef-building corals of Hervey Bay, South-East Queensland. Baseline Survey Report to the Wildlife Preservation Society of Queensland, Fraser Coast Branch.

DeVantier, L., De Ath, G., Klaus, R., Al-Moghrabi, S., Abdulaziz, M., Reinicke, G. B., & Cheung, C. (2004). Reef-building corals and coral communities of the Socotra Archipelago, a zoogeographiccrossroads' in the Arabian Sea. Fauna of Arabia, 20, 117-168.

DeVantier, L., Hodgson, G., Huang, D., Johan, O., Licuanan, A., Obura, D., Sheppard, C., Syahrir, M. & Turak, E. (2008). *Montipora capricornis*. The IUCN Red List of Threatened Species 2008: e.T133422A3737366.

DeVantier, L., Hodgson, G., Huang, D., Johan, O., Licuanan, A., Obura, D.O., Sheppard, C., Syahrir, M. & Turak, E. (2014). *Echinopora lamellosa*. The IUCN Red List of Threatened Species 2014: e.T133088A54193471.

Dias, M., Ferreira, A., Gouveia, R., Cereja, R., & Vinagre, C. (2018). Mortality, growth and regeneration following fragmentation of reef-forming corals under thermal stress. Journal of Sea Research, 141, 71-82.

Dias, M., Ferreira, A., Gouveia, R., & Vinagre, C. (2019). Synergistic effects of warming and lower salinity on the asexual reproduction of reef-forming corals. Ecological Indicators, 98, 334-348.

Díaz, M., & Madin, J. (2011). Macroecological relationships between coral species' traits and disease potential. Coral Reefs, 30(1), 73-84.

Diaz-Pulido, G., McCook, L. J., Dove, S., Berkelmans, R., Roff, G., Kline, D. I., ... & Hoegh-Guldberg, O. (2009). Doom and boom on a resilient reef: climate change, algal overgrowth and coral recovery. PloS One, 4(4), e5239.

Dong, Z. J., Huang, H., Huang, L. M., & Li, Y. C. (2009). Diversity of symbiotic algae of the genus *Symbiodinium* in scleractinian corals of the Xisha Islands in the South China Sea. Journal of Systematics and Evolution, 47(4), 321-326.

Downs, C. A., Mueller, E., Phillips, S., Fauth, J. E., & Woodley, C. M. (2000). A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. Marine Biotechnology, 2(6), 533-544.

Downs, C. A., Fauth, J. E., Halas, J. C., Dustan, P., Bemiss, J., & Woodley, C. M. (2002). Oxidative stress and seasonal coral bleaching. Free Radical Biology and Medicine, 33(4), 533-543.

Downs, C. A., Fauth, J. E., Robinson, C. E., Curry, R., Lanzendorf, B., Halas, J. C., ... & Woodley, C. M. (2005). Cellular diagnostics and coral health: declining coral health in the Florida Keys. Marine Pollution Bulletin, 51(5-7), 558-569.

Dunn, S. R., Schnitzler, C. E., & Weis, V. M. (2007). Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: every which way you lose. Proceedings of the Royal Society of London B: Biological Sciences, 274(1629), 3079-3085.

Dykens, J. A., Shick, J. M., Benoit, C., Buettner, G. R., & Winston, G. W. (1992). Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. Journal of Experimental Biology, 168(1), 219-241.

Eakin, C. M., Lough, J. M., & Heron, S. F. (2009). Climate variability and change: monitoring data and evidence for increased coral bleaching stress. In Coral bleaching (pp. 41-67). Springer, Berlin, Heidelberg.

Edmunds, P. J. (2005). The effect of sub-lethal increases in temperature on the growth and population trajectories of three scleractinian corals on the southern Great Barrier Reef. Oecologia, 146(3), 350-364.

Edwards, A. J., Clark, S., Zahir, H., Rajasuriya, A., Naseer, A., & Rubens, J. (2001). Coral bleaching and mortality on artificial and natural reefs in Maldives in 1998, sea surface temperature anomalies and initial recovery. Marine Pollution Bulletin, 42(1), 7-15.

Fabricius, K. E., De'ath, G., Puotinen, M. L., Done, T., Cooper, T. F., & Burgess, S. C. (2008). Disturbance gradients on inshore and offshore coral reefs caused by a severe tropical cyclone. Limnology and Oceanography, 53(2), 690-704.

Fabricius, K. E., Mieog, J. C., Colin, P. L., Idip, D., & Van Oppen, M. J. H. (2004). Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. Molecular Ecology, 13(8), 2445-2458.

Fan, T. Y., & Dai, C. F. (1999). Reproductive plasticity in the reef coral *Echinopora lamellosa*. Marine Ecology Progress Series, 190, 297-301.

Faxneld, S., Jörgensen, T. L., Nguyen, N. D., Nyström, M., & Tedengren, M. (2011). Differences in physiological response to increased seawater temperature in nearshore and offshore corals in northern Vietnam. Marine Environmental Research, 71(3), 225-233.

Fitt, W. K., Brown, B. E., Warner, M. E., & Dunne, R. P. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs, 20(1), 51-65.

Fitt, W. K., Gates, R. D., Hoegh-Guldberg, O., Bythell, J. C., Jatkar, A., Grottoli, A. G., ... & Iglesias-Prieto, R. (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. Journal of Experimental Marine Biology and Ecology, 373(2), 102-110.

Foster, N. L., Baums, I. B., & Mumby, P. J. (2007). Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastraea annularis*. Journal of Animal Ecology, 76(2), 384-391.

Foster, N. L., Baums, I. B., Sanchez, J. A., Paris, C. B., Chollett, I., Agudelo, C. L., ... & Mumby, P. J. (2013). Hurricane-driven patterns of clonality in an ecosystem engineer: the Caribbean coral *Montastraea annularis*. PLoS One, 8(1), e53283.

Foster, T., Short, J. A., Falter, J. L., Ross, C., & McCulloch, M. T. (2014). Reduced calcification in Western Australian corals during anomalously high summer water temperatures. Journal of Experimental Marine Biology and Ecology, 461, 133-143.

Frieler, K., Meinshausen, M., Golly, A., Mengel, M., Lebek, K., Donner, S. D., & Hoegh-Guldberg, O. (2013). Limiting global warming to 2 ºC is unlikely to save most coral reefs. Nature Climate Change, 3(2), 165.

Fujioka, Y. (1998). Checklist of the hermatypic corals of Urasoko Bay, Ishigaki Island, southwestern Japan. Bull Nansei Natl Fish Res Inst, 31, 1-11.

Gates, R. D., Baghdasarian, G., & Muscatine, L. (1992). Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. The Biological Bulletin, 182(3), 324-332.

George, J. D., & John, D. M. (1999). High sea temperatures along the coast of Abu Dhabi (UAE), Arabian Gulf—their impact upon corals and macroalgae. Reef Encounter, 25, 21-23.

Glynn, P. W., Colley, S. B., Carpizo-Ituarte, E., & Richmond, R. H. (2017). Coral reproduction in the Eastern Pacific. In Coral Reefs of the Eastern Tropical Pacific (pp. 435-476). Springer, Dordrecht.

Goldberg, J., & Wilkinson, C. (2004). Global threats to coral reefs: coral bleaching, global climate change, disease, predator plagues and invasive species. Status of coral reefs of the world, 2004, 67-92.

Goodsell, D. S. (1996). Our Molecular Nature: The Body's Motors. Machines, and Messages.

Griffin, S. P., Bhagooli, R., & Weil, E. (2006). Evaluation of thermal acclimation capacity in corals with different thermal histories based on catalase concentrations and antioxidant potentials. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 144(2), 155-162.

Guest, J. R. (2005). Reproductive patterns of scleractinian corals on Singapore's reefs (Doctoral dissertation).

Guest, J. R., Baird, A. H., Maynard, J. A., Muttaqin, E., Edwards, A. J., Campbell, S. J., ... & Chou, L. M. (2012). Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PloS One, 7(3), e33353.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249(22), 7130-7139.

Halliwell, B. & Gutteridge, J. M. C. (1999). Free Radicals in Biology and Medicine. Oxford University Press, New York.

Hamilton, H. G., & Brakel, W. H. (1984). Structure and coral fauna of East African reefs. Bulletin of Marine Science, 34(2), 248-266.

Harriott, V. J. (1999). Coral growth in subtropical eastern Australia. Coral Reefs, 18(3), 281-291.

Harris, A., Wilson, S., Graham, N., & Sheppard, C. (2014). Scleractinian coral communities of the inner Seychelles 10 years after the 1998 mortality event. Aquatic Conservation: Marine and Freshwater Ecosystems, 24(5), 667-679.

Hawkins, T. D., & Davy, S. K. (2012). Nitric oxide production and tolerance differ among *Symbiodinium* types exposed to heat stress. Plant and Cell Physiology, 53(11), 1889-1898.

Hawkins, T. D., Bradley, B. J., & Davy, S. K. (2013). Nitric oxide mediates coral bleaching through an apoptotic-like cell death pathway: evidence from a model sea anemone-dinoflagellate symbiosis. The FASEB Journal, 27(12), 4790-4798.

Hermes-Lima, M., & Storey, K. B. (1993). In vitro oxidative inactivation of glutathione S-transferase from a freeze tolerant reptile. Molecular and Cellular Biochemistry, 124(2), 149-158.

Heron, S. F., Maynard, J. A., Van Hooidonk, R., & Eakin, C. M. (2016). Warming trends and bleaching stress of the world's coral reefs 1985–2012. Scientific Reports, 6, 38402.

Highsmith, R. C. (1980). Passive colonization and asexual colony multiplication in the massive coral *Porites lutea* Milne Edwards & Haime. Journal of Experimental Marine Biology and Ecology, 47(1), 55-67.

Highsmith, R. C. (1982). Reproduction by fragmentation in corals. Marine Ecology Progress Series. Oldendorf, 7(2), 207-226.

Higuchi, T., Fujimura, H., Arakaki, T., & Oomori, T. (2008, July). Activities of antioxidant enzymes (SOD and CAT) in the coral *Galaxea fascicularis* against increased hydrogen peroxide concentrations in seawater. In Proceedings of the 11th International Coral Reef Symposium (pp. 7-11).

Hoegh-Guldberg, O. (2004). Coral reefs in a century of rapid environmental change. Symbiosis, 37(1), 1-31.

Hoegh-Guldberg, O., & Salvat, B. (1995). Periodic mass-bleaching and elevated sea temperatures: bleaching of outer reef slope communities in Moorea, French Polynesia. Marine Ecology Progress Series, 121, 181-190.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... & Knowlton, N. (2007a). Coral reefs under rapid climate change and ocean acidification. Science, 318(5857), 1737-1742.

Hoegh-Guldberg, O., Anthony, K., Berkelmans, R., Dove, S., Fabricius, K. E., Lough, J. M., ... & Willis, B. (2007b). Vulnerability of reef-building corals on the Great Barrier Reef to climate change.

Hoeksema, B.W., Rogers, A. & Quibilan, M.C. (2014a). *Turbinaria reniformis*. The IUCN Red List of Threatened Species 2014: e.T133697A54306914.

Hoeksema, B.W., Rogers, A. & Quibilan, M.C. (2014b). *Galaxea fascicularis*. The IUCN Red List of Threatened Species 2014: e.T133662A54303932.

Hoeksema, B.W., Rogers, A. & Quibilan, M.C. (2014c). *Pocillopora damicornis*. The IUCN Red List of Threatened Species 2014: e.T133222A54216898.

Hoeksema, B.W., Rogers, A. & Quibilan, M.C. (2014d). *Stylophora pistillata*. The IUCN Red List of Threatened Species 2014: e.T133685A54306217.

Hongo, C., & Kayanne, H. (2010). Relationship between species diversity and reef growth in the Holocene at Ishigaki Island, Pacific Ocean. Sedimentary Geology, 223(1-2), 86-99.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K.D., Baird, A. H., ... & Bridge, T. C. (2017). Global warming and recurrent mass bleaching of corals. Nature, 543(7645), 373.

Hughes, T. P., Kerry, J. T., & Simpson, T. (2018). Large-scale bleaching of corals on the Great Barrier Reef. Ecology, 99(2), 501-501.

Ikeda, E., Iryu, Y., Sugihara, K., Ohba, H., & Yamada, T. (2006). Bathymetry, biota and sediments on the Hirota Reef, Tane-ga-shima–the northernmost coral reef in the Ryukyu Islands. Island Arc, 15(4), 407-419.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Meyer LA (eds)]. IPCC, Geneva, Switzerland, p 151

Johansson, L. H., & Borg, L. H. (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. Analytical Biochemistry, 174(1), 331-336.

Johns, K. A., Osborne, K. O., & Logan, M. (2014). Contrasting rates of coral recovery and reassembly in coral communities on the Great Barrier Reef. Coral Reefs, 33(3), 553-563.

Jokiel, P. L., & Coles, S. L. (1974). Effects of heated effluent on hermatypic corals at Kahe Point, Oahu. Pacific Science, 28, 1-18.

Kamyab, E., Kühnhold, H., Novais, S. C., Alves, L. M., Indriana, L., Kunzmann, A., ... & Lemos, M. F. (2017). Effects of thermal stress on the immune and oxidative stress responses of juvenile sea cucumber *Holothuria scabra*. Journal of Comparative Physiology B, 187(1), 51-61.

Kayanne, H., Hongo, C., Okaji, K., Ide, Y., Hayashibara, T., Yamamoto, H., ... & Tonegawa, M. (2012). Low species diversity of hermatypic corals on an isolated reef, Okinotorishima, in the northwestern Pacific. Galaxea, Journal of Coral Reef Studies, 14(1), 73-95.

Khalil, M. T., Cochran, J. E., & Berumen, M. L. (2013). The abundance of herbivorous fish on an inshore Red Sea reef following a mass coral bleaching event. Environmental Biology of Fishes, 96(9), 1065-1072.

Kinzie, R. A., Takayama, M., Santos, S. R., & Coffroth, M. A. (2001). The adaptive bleaching hypothesis: experimental tests of critical assumptions. The Biological Bulletin, 200(1), 51-58.

Kühl, M., Cohen, Y., Dalsgaard, T., & Jørgensen, B. B., Revsbech, N. P. (1995). Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. Marine Ecology Progress Series, 117, 159-172.

LaJeunesse, T. C., Bhagooli, R., Hidaka, M., Done, T., Schmidt, G. W., Fitt, W. K., & Hoegh-Guldberg, O. (2004). Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. Marine Ecology Progress Series, 284, 147-161.

Le Nohaïc, M., Ross, C. L., Cornwall, C. E., Comeau, S., Lowe, R., McCulloch, M. T., & Schoepf, V. (2017). Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia. Scientific Reports, 7(1), 14999.

Lenihan, H. S., Holbrook, S. J., Schmitt, R. J., & Brooks, A. J. (2011). Influence of corallivory, competition, and habitat structure on coral community shifts. Ecology, 92(10), 1959-1971.

Lesser, M. P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. Annual Review of Physiology, 68, 253-278.

Lesser, M. P. (2011). Coral bleaching: causes and mechanisms. In Coral reefs: an ecosystem in transition (pp. 405-419). Springer, Dordrecht.

Lesser, M. P., & Shick, J. M. (1989). Photoadaption and defenses against oxygen toxicity in zooxanthellae from natural populations of symbiotic cnidarians. Journal of Experimental Marine Biology and Ecology, 134(2), 129-141.

Lesser, M. P., Stochaj, W. R., Tapley, D. W., & Shick, J. M. (1990). Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs, 8(4), 225-232.

Levitan, D. R., Boudreau, W., Jara, J., & Knowlton, N. (2014). Long-term reduced spawning in *Orbicella* coral species due to temperature stress. Marine Ecology Progress Series, 515, 1-10.

Liu, G., Strong, A. E., & Skirving, W. (2003). Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching. Eos, Transactions American Geophysical Union, 84(15), 137-141.

Livingstone, D. R. (1991). Organic xenobiotic metabolism in marine invertebrates. In Advances in comparative and environmental physiology (pp. 45-185). Springer, Berlin, Heidelberg.

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., & Van Woesik, R. (2001). Coral bleaching: the winners and the losers. Ecology Letters, 4(2), 122-131.

Madeira, D., Narciso, L., Cabral, H. N., Vinagre, C., & Diniz, M. S. (2013). Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 166(2), 237-243.

Madeira, D., Narciso, L., Cabral, H. N., Diniz, M. S., & Vinagre, C. (2014). Role of thermal niche in the cellular response to thermal stress: lipid peroxidation and HSP70 expression in coastal crabs. Ecological Indicators, 36, 601-606.

Madeira, C., Madeira, D., Vinagre, C., & Diniz, M. (2015). Octocorals in a changing environment: Seasonal response of stress biomarkers in natural populations of *Veretillum cynomorium*. Journal of Sea Research, 103, 120-128.

Madeira, C., Mendonça, V., Leal, M. C., Diniz, M. S., Cabral, H. N., Flores, A. A., & Vinagre, C. 2019. Present and future invasion perspectives of an alien shrimp in South Atlantic coastal waters: an experimental assessment of functional biomarkers and thermal tolerance. Biological Invasions, 1-18.

Magalon, H., Flot, J. F., & Baudry, E. (2007). Molecular identification of symbiotic dinoflagellates in Pacific corals in the genus *Pocillopora*. Coral Reefs, 26(3), 551-558.

Marshall, P. A., & Baird, A. H. (2000). Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs, 19(2), 155-163.

Martindale, J. L., & Holbrook, N. J. (2002). Cellular response to oxidative stress: signaling for suicide and survival. Journal of Cellular Physiology, 192(1), 1-15.

Matozzo, V., Chinellato, A., Munari, M., Bressan, M., & Marin, M. G. (2013). Can the combination of decreased pH and increased temperature values induce oxidative stress in the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*?. Marine Pollution Bulletin, 72(1), 34-40.

McClanahan, T. R. (2000). Bleaching damage and recovery potential of Maldivian coral reefs. Marine Pollution Bulletin, 40(7), 587-597.

McClanahan, T., Muthiga, N., & Mangi, S. (2001). Coral and algal changes after the 1998 coral bleaching: interaction with reef management and herbivores on Kenyan reefs. Coral Reefs, 19(4), 380-391.

McClanahan, T. R., Baird, A. H., Marshall, P. A., & Toscano, M. A. (2004). Comparing bleaching and mortality responses of hard corals between southern Kenya and the Great Barrier Reef, Australia. Marine Pollution Bulletin, 48(3-4), 327-335.

Merle, P. L., Sabourault, C., Richier, S., Allemand, D., & Furla, P. (2007). Catalase characterization and implication in bleaching of a symbiotic sea anemone. Free Radical Biology and Medicine, 42(2), 236-246.

Meyers, R. A. (Ed.). (1995). Molecular biology and biotechnology: a comprehensive desk reference. John Wiley & Sons.

Middlebrook, R., Hoegh-Guldberg, O., & Leggat, W. (2008). The effect of thermal history on the susceptibility of reef-building corals to thermal stress. Journal of Experimental Biology, 211(7), 1050-1056.

Middlebrook, R., Anthony, K. R., Hoegh-Guldberg, O., & Dove, S. (2010). Heating rate and symbiont productivity are key factors determining thermal stress in the reef-building coral *Acropora formosa*. Journal of Experimental Biology, 213(7), 1026-1034.

Mieog, J. C., Van Oppen, M. J., Cantin, N. E., Stam, W. T., & Olsen, J. L. (2007). Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian

corals across the Great Barrier Reef: implications for symbiont shuffling. Coral Reefs, 26(3), 449-457.

Mizrahi, T., Goldenberg, S., Heller, J., & Arad, Z. (2016). Geographic variation in thermal tolerance and strategies of heat shock protein expression in the land snail *Theba pisana* in relation to genetic structure. Cell Stress and Chaperones, 21(2), 219-238.

Mostafavi, P. G., Fatemi, S. M. R., Shahhosseiny, M. H., Hoegh-Guldberg, O., & Loh, W. K. W. (2007). Predominance of clade D *Symbiodinium* in shallow-water reef-building corals off Kish and Larak Islands (Persian Gulf, Iran). Marine Biology, 153(1), 25-34.

Mumby, P., Chisholm, J., Edwards, A., Clark, C., Roark, E., Andrefouet, S., & Jaubert, J. (2001). Unprecedented bleaching-induced mortality in *Porites* spp. at Rangiroa Atoll, French Polynesia. Marine Biology, 139(1), 183-189.

Murata, N., Takahashi, S., Nishiyama, Y., & Allakhverdiev, S. I. (2007). Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1767(6), 414-421.

Mwaura, J., Grimsditch, G., Kilonzo, J., Amiyo, N., & Obura, D. (2009). Zooxanthellae densities are highest in summer months in equatorial corals in Kenya. Western Indian Ocean Journal of Marine Science, 8(2).

Nii, C. M., & Muscatine, L. (1997). Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Carlgren, 1943): contribution of the animal to superoxide ion production at elevated temperature. The Biological Bulletin, 192(3), 444-456.

Niu, Y., & Xiang, Y. (2018). An overview of biomembrane functions in plant responses to high-temperature stress. Frontiers in Plant Science, 9, 915.

Oliver, T. A., & Palumbi, S. R. (2011). Do fluctuating temperature environments elevate coral thermal tolerance?. Coral Reefs, 30(2), 429-440.

Olsen, K. C. (2013). The Effects of Thermal Stress and Algal Competition on the Early Life-History Stages of *Porites astreoides* and the Development of Stress-Detecting Biomarkers for Use in Scleractinian Corals.

Osborne, K., Thompson, A. A., Cheal, A. J., Emslie, M. J., Johns, K. A., Jonker, M. J., ... & Sweatman, H. P. (2017). Delayed coral recovery in a warming ocean. Global Change Biology, 23(9), 3869-3881.

Palmer, C. V., Modi, C. K., & Mydlarz, L. D. (2009). Coral fluorescent proteins as antioxidants. PloS One, 4(10), e7298.

Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N., & Bay, R. A. (2014). Mechanisms of reef coral resistance to future climate change. Science, 344(6186), 895-898.

Paxton, C. W., Davy, S. K., & Weis, V. M. (2013). Stress and death of cnidarian host cells play a role in cnidarian bleaching. Journal of Experimental Biology, 216 (15), 2813–2820.

Pearson, R. G. (1981). Recovery and recolonization of coral reefs. Marine Ecology Progress Series, 105-122.

Penin, L., Adjeroud, M., Schrimm, M., & Lenihan, H. S. (2007). High spatial variability in coral bleaching around Moorea (French Polynesia): patterns across locations and water depths. Comptes Rendus Biologies, 330(2), 171-181.

Perez, S., & Weis, V. (2006). Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. Journal of Experimental Biology, 209(14), 2804-2810.

Piniak, G. A., & Brown, E. K. (2008). Growth and mortality of coral transplants (*Pocillopora damicornis*) along a range of sediment influence in Maui, Hawai'i. Pacific Science, 62(1), 39-55.

Pratchett, M. S. (2001). Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the western Pacific. Marine Ecology Progress Series, 214, 111-119.

Pratchett, M. S., Trapon, M., Berumen, M. L., & Chong-Seng, K. (2011). Recent disturbances augment community shifts in coral assemblages in Moorea, French Polynesia. Coral Reefs, 30(1), 183-193.

Pratchett, M. S., McCowan, D., Maynard, J. A., & Heron, S. F. (2013). Changes in bleaching susceptibility among corals subject to ocean warming and recurrent bleaching in Moorea, French Polynesia. PLoS One, 8(7), e70443.

Rapuano, H., Brickner, I., Shlesinger, T., Meroz-Fine, E., Tamir, R., & Loya, Y. (2017). Reproductive strategies of the coral *Turbinaria reniformis* in the northern Gulf of Aqaba (Red Sea). Scientific Reports, 7, 42670.

Rasher, D. B., Stout, E. P., Engel, S., Kubanek, J., & Hay, M. E. (2011). Macroalgal terpenes function as allelopathic agents against reef corals. Proceedings of the National Academy of Sciences, 201108628.

Regoli, F., Cerrano, C., Chierici, E., Chiantore, M. C., & Bavestrello, G. (2004). Seasonal variability of prooxidant pressure and antioxidant adaptation to symbiosis in the Mediterranean demosponge *Petrosia ficiformis*. Marine Ecology Progress Series, 275, 129-137.

Rewitz, K. F., Styrishave, B., Løbner-Olesen, A., & Andersen, O. (2006). Marine invertebrate cytochrome P450: emerging insights from vertebrate and insect analogies. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 143(4), 363-381.

Richards, Z. T., & Rosser, N. L. (2012). Abundance, distribution and new records of scleractinian corals at Barrow Island and Southern Montebello Islands, Pilbara (offshore) bioregion. Journal of the Royal Society of Western Australia, 95, 155.

Richards, Z.T., Delbeek, J.T., Lovell, E.R., Bass, D., Aeby, G. & Reboton, C. (2014). *Acropora tenuis*. The IUCN Red List of Threatened Species 2014: e.T133020A54177216.

Richier, S., Furla, P., Plantivaux, A., Merle, P. L., & Allemand, D. (2005). Symbiosis-induced adaptation to oxidative stress. Journal of Experimental Biology, 208(2), 277-285.

Richier, S., Sabourault, C., Courtiade, J., Zucchini, N., Allemand, D., & Furla, P. (2006). Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis*. The FEBS journal, 273(18), 4186-4198.

Riegl, B. (2002). Effects of the 1996 and 1998 positive sea-surface temperature anomalies on corals, coral diseases and fish in the Arabian Gulf (Dubai, UAE). Marine Biology, 140(1), 29-40.

Riegl, B. M., & Purkis, S. J. (2009). Model of coral population response to accelerated bleaching and mass mortality in a changing climate. Ecological Modelling, 220(2), 192-208

Riegl, B. M., Purkis, S. J., Al-Cibahy, A. S., Al-Harthi, S., Grandcourt, E., Al-Sulaiti, K., ... & Abdel-Moati, A. M. (2012). Coral bleaching and mortality thresholds in the SE Gulf: highest in the world. In Coral reefs of the Gulf (pp. 95-105). Springer, Dordrecht.

Ross, C. L., Schoepf, V., DeCarlo, T. M., & McCulloch, M. T. (2018). Mechanisms and seasonal drivers of calcification in the temperate coral *Turbinaria reniformis* at its latitudinal limits. Proceedings of the Royal Society B: Biological Sciences, 285(1879), 20180215.

Roth, M. S. (2014). The engine of the reef: photobiology of the coral–algal symbiosis. Frontiers in Microbiology, 5, 422.

Rowan, R., Knowlton, N., Baker, A., & Jara, J. (1997). Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature, 388(6639), 265.

Salvat, F., & Salvat, B. (1992). Nukutipipi Atoll, Tuamotu Archipelago; geomorphology, land and marine flora and fauna and interrelationships. Atoll Research Bulletin, 357, 1–43.

Shashar, N., Cohen, Y., & Loya, Y. (1993). Extreme diel fluctuations of oxygen in diffusive boundary layers surrounding stony corals. The Biological Bulletin, 185(3), 455-461.

Sheppard, C. R. (1980). Coral fauna of Diego Garcia lagoon, following harbour construction. Marine Pollution Bulletin, 11(8), 227-230.

Sheppard, C., Turak, E. & Wood, E. (2014). *Psammocora contigua*. The IUCN Red List of Threatened Species 2014: e.T133208A54213078.

Silverstein, R. N., Correa, A. M., LaJeunesse, T. C., & Baker, A. C. (2011). Novel algal symbiont (*Symbiodinium* spp.) diversity in reef corals of Western Australia. Marine Ecology Progress Series, 422, 63-75.

Smith, R. T., Pinzon, J. H., & LaJeunesse, T. C. (2009). *Symbiodinium* (Dinophyta) diversity and stability in aquarium corals 1. Journal of Phycology, 45(5), 1030-1036.

Solayan, A. (2016). Biomonitoring of Coral Bleaching-A Glimpse on Biomarkers for the Early Detection of Oxidative Damages in Corals. In Invertebrates-Experimental Models in Toxicity Screening. InTech.

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(4), 409-421.

Swierts, T., & Vermeij, M. J. (2016). Competitive interactions between corals and turf algae depend on coral colony form. PeerJ, 4, e1984.

Tasselli, S., Ballin, F., Franchi, N., Fabbri, E., & Ballarin, L. (2017). Expression of genes involved in oxidative stress response in colonies of the ascidian *Botryllus schlosseri* exposed to various environmental conditions. Estuarine, Coastal and Shelf Science, 187, 22-27.

Tong, H., Cai, L., Zhou, G., Yuan, T., Zhang, W., Tian, R., ... & Qian, P. Y. (2017). Temperature shapes coral-algal symbiosis in the South China Sea. Scientific Reports, 7, 40118.

Torda, G., Sambrook, K., Cross, P., Sato, Y., Bourne, D. G., Lukoschek, V., ... & Willis, B. L. (2018). Decadal erosion of coral assemblages by multiple disturbances in the Palm Islands, central Great Barrier Reef. Scientific Reports, 8(1), 11885.

Turak, E., & DeVantier, L. (2012). Biodiversity and Conservation Priorities of Reef-building Corals in Timor Leste, with focus on the Nino Konis Santana National Marine Park.

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry, 86(1), 271-278.

Ulstrup, K. E., & Van Oppen, M. J. H. (2003). Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. Molecular Ecology, 12(12), 3477-3484.

Ulstrup, K. E., Kühl, M., Van Oppen, M. J., Cooper, T. F., & Ralph, P. J. (2011). Variation in photosynthesis and respiration in geographically distinct populations of two reef-building coral species. Aquatic Biology, 12(3), 241-248.

Van Hooidonk, R., & Huber, M. (2009). Quantifying the quality of coral bleaching predictions. Coral Reefs, 28(3), 579-587.

Van Hooidonk, R., Maynard, J., Tamelander, J., Gove, J., Ahmadia, G., Raymundo, L., ... & Planes, S. (2016). Local-scale projections of coral reef futures and implications of the Paris Agreement. Scientific Reports, 6, 39666.

Van Oppen, M. J., Palstra, F. P., Piquet, A. M. T., & Miller, D. J. (2001). Patterns of coral–dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host–symbiont selectivity. Proceedings of the Royal Society of London. Series B: Biological Sciences, 268(1478), 1759-1767.

Van Woesik, R., Sakai, K., Ganase, A., & Loya, Y. (2011). Revisiting the winners and the losers a decade after coral bleaching. Marine Ecology Progress Series, 434, 67-76.

Veron, J. E. N. (1990). Checklist of the hermatypic corals of Vanuatu. Pacific Science, 44, 51-70.

Veron, J. E. N. (2000). Corals of the World, vols. 1-3. Mary Stafford-Smith (Ed.) Publisher: Australian Institute of Marine Science, Townsville, Australia, p. 489.

Vinagre, C., Madeira, D., Narciso, L., Cabral, H. N., & Diniz, M. (2012). Effect of temperature on oxidative stress in fish: lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. Ecological Indicators, 23, 274-279.

Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J., & Diniz, M. S. (2014). Effect of increasing temperature in the differential activity of oxidative stress biomarkers in various tissues of the Rock goby, *Gobius paganellus*. Marine Environmental Research, 97, 10-14.

Ward, W. W. (1998). Biochemical and physical properties of green fluorescent protein. In: Chalfie M, Kain S (eds.). In Green Fluorescent Protein: Properties, Applications, and Protocols. New York: Wiley-Liss. 45–75.

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. Journal of Experimental Biology, 211(19), 3059-3066.

Willis, B. L., Page, C. A., & Dinsdale, E. A. (2004). Coral disease on the Great Barrier Reef. In Coral health and disease (pp. 69-104). Springer, Berlin, Heidelberg.

World Register of Marine Species: www.marinespecies.org. Accessed on 4 May 2019.

Yakovleva, I., Bhagooli, R., Takemura, A., & Hidaka, M. (2004). Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 139(4), 721-730.

Zhao, M. X., Yu, K. F., Shi, Q., Chen, T. R., Zhang, H. L., & Chen, T. G. (2013). Coral communities of the remote atoll reefs in the Nansha Islands, southern South China Sea. Environmental Monitoring and Assessment, 185(9), 7381-7392.

Zhao, M., Yu, K., Shi, Q., Yang, H., Riegl, B., Zhang, Q., ... & Lin, Z. (2017). Comparison of coral diversity between big and small atolls: a case study of Yongle atoll and Lingyang reef, Xisha Islands, central of South China Sea. Biodiversity and Conservation, 26(5), 1143-1159.

Zhou, G., Huang, H., Dong, Z., & Yu, Z. (2011). Temporal stability of *Symbiodinium* phylotype in scleractinian coral *Galaxea fascicularis* from a tropical fringing reef in the South China Sea. Chinese Journal of Oceanology and Limnology, 29(6), 1186.

Zuo, R., Hou, S., Wu, F., Song, J., Zhang, W., Zhao, C., & Chang, Y. (2017). Higher dietary protein increases growth performance, anti-oxidative enzymes activity and transcription of heat shock protein 70 in the juvenile sea urchin (*Strongylocentrotus intermedius*) under a heat stress. Aquaculture and Fisheries, 2(1), 18-23.

4.6. Supplementary material

Table SM4.1. Number of coral species fragments (n) used in each oxidative stress biomarker analysis (LPO – lipid peroxidation; CAT – catalase; GST- glutathione S-transferase) per experimental treatment.

	Treatments								
	26 °C			30 °C			32 °C		
Species	LPO	CAT	GST	LPO	CAT	GST	LPO	CAT	GST
Acropora tenuis	5	6	5	5	5	5	0	0	0
Montipora capricornis (BM)	5	5	5	6	6	5	0	0	0
Montipora capricornis (GM)	5	5	5	5	6	6	0	0	0
Turbinaria reniformis	6	6	5	6	5	5	5	5	5
Echinopora lamellosa	5	6	6	5	6	6	0	0	0
Galaxea fascicularis	6	5	5	5	5	5	5	5	5
Pocillopora damicornis	5	5	6	0	0	0	0	0	0
Stylophora pistillata	6	6	6	0	0	0	0	0	0
Psammocora contigua	5	6	5	5	6	5	5	5	5

CHAPTER 5

Oxidative stress on scleractinian coral fragments following exposure to high temperature and low salinity

Dias, M., Madeira, C., Jogee, N., Ferreira, A., Gouveia, R., Cabral, H.N., Diniz, M. & Vinagre, C. (2019). Ecological Indicators, 105586. DOI:10.1016/j.ecolind.2019.105586.

Abstract

Global warming is leading to both increases in frequency and intensity of tropical storms, with consequent salinity decrease at shallow reef areas, but also to mass bleaching events and mortality of reef-building corals around the world. Tropical storms can help reef-building corals to reproduce through fragmentation, allowing their expansion throughout the reefs. The combination of high temperature and low salinity may aggravate the effects of coral bleaching. Investigation of alterations at the cellular level will be useful since this is the first detectable response of organisms to changes in environmental conditions. In this study, the long-term oxidative stress induced by elevated temperature (30 ºC), low salinity (20 psu), and their combination was studied on fragments of reef-forming corals, and compared to control conditions (26 ºC, 33 psu). Determination of oxidative stress biomarkers: lipid peroxidation (LPO); superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) activities in a long-term experiment (60 days), using nine Indo-Pacific reef-forming coral species, provided useful information that was interpreted in combination with the observed general condition of these organisms (appearance: normal, pale, bleached, dead). High temperature affected the general condition of the species tested to a lower degree than did low salinity. Only two species died at high temperature, while low salinity resulted in the death of all species with the exception of two (*P. contigua* and *G. fascicularis*). Oxidative damage was detected in some species, as were antioxidant responses, at high temperature. Coral general condition was severely affected in all species in the low salinity treatment. *Galaxea fascicularis* and *Psammocora contigua* were the most resistant to salinity stress, having survived the experimental treatment. Oxidative damage was not detected in these species, but there was an antioxidant response. The high temperature + low salinity (HT+LS) treatment had synergistic effects in the condition of all species. *Galaxea fascicularis* was the only survivor in the HT+LS treatment. Mortality was high (60%) for this species, oxidative damage was not detected, but an increase in SOD activity revealed an antioxidant response.

Keywords: global climate change, oxidative stress biomarkers, heat stress, hyposaline stress, coral conservation

5.1. Introduction and literature review

Alterations in seawater temperature and salinity are two of the most important factors leading to physiological changes in scleractinian corals, as they inhabit shallow-water or intertidal marine environments (Brown, 1997a). Global climate change is pushing scleractinian corals beyond their thermal tolerance threshold leading to coral bleaching events (Hoegh-Guldberg, 1999; Carpenter et al., 2008). Recently, mass coral bleaching events have led to dramatic changes in coral species composition and community structure in the Indo – Pacific (Furby et al., 2013; Khalil et al., 2013; Hughes et al., 2017, 2018; Le Nohaïc et al., 2017). Major shifts in reef community structure from dominant branching heat-susceptible coral species to sub-dominant encrusting and massive heat-tolerant coral species have often been observed (Van Woesik et al., 2011).

Studies have shown that salinity stress can adversely affect coral growth and regeneration (Dias et al., 2019a), photosynthesis (Kuanui et al., 2015), respiration (Ferrier-Pagès et al., 1999), and can disrupt the normal cellular electrochemical processes leading to metabolic drain in corals (Vernberg and Vernberg, 1972). In extreme cases, due to corals' limited osmoregulation capability (Coles and Jokiel, 1992; Manzello and Lirman, 2003), salinity stress can cause a breakdown in coral-zooxanthellae symbiosis leading to coral bleaching and death (Goreau, 1964; Jokiel et al., 1993; Kerswell and Jones, 2003; Dias et al., 2019a). These effects may be intensified during episodes of elevated seawater surface temperatures (Faxneld et al., 2010).

Sea surface temperature (SST) anomaly events are predicted to increase in frequency and intensity over the next century (Baker et al., 2008; Cacciapaglia and Van Woesik, 2016). Global warming is expected to increase the frequency of mass bleaching events in coral reef communities around the world (Bruno et al., 2007; Crabbe, 2008). Mass bleaching events are predicted to occur annually in most tropical oceans by the year 2040 (Crabbe, 2008). As a consequence of global warming, increases in frequency and intensity of tropical storms are also expected (Emanuel, 2005; Elsner et al., 2006; Crabbe et al., 2008; Christensen et al., 2013; IPCC, 2014). Extreme precipitation events associated with tropical storms may lead to

increased risk of reduced salinity in near-shore reefs (Webster et al., 2005; Haapkylä et al., 2011; IPCC, 2014).

After disturbance, recovery of coral reef communities depends on both sexual and asexual modes of reproduction (Glynn et al., 2017). Studies have shown that heat stress can negatively affect the fertilization success and embryonic development (Negri et al., 2007), spawning (Levitan et al., 2014), survivorship and settlement of coral larvae (Randall and Szmant, 2009), as well as post-settlement survival (Nozawa and Harrison, 2007). Studies have also demonstrated that hyposaline stress can lead to a decrease in fertilization success and larval survival (Jokiel, 1985; Humphrey et al., 2008; Scott et al., 2013). Therefore, the recurrent increase in SSTs and tropical storms' frequency and intensity will likely lead to the failure of sexual means of reproduction. As a consequence, a rapidly changing environment may favor alternative asexual mechanisms of propagation.

Tropical storms can facilitate the expansion of reef-forming corals of branching, plating and massive morphologies by colony fragmentation (Highsmith et al., 1980; Highsmith, 1982; Foster et al., 2013). Fragmentation is considered a widespread and highly important asexual mean of reproduction for a great number of scleractinian coral species (Highsmith, 1980; Bruno 1998). Fragmentation can be the primary mode of population growth for species with rare sexual recruits (Tunnicliffe, 1981; Wallace, 1985) and it also allows coral species to withstand disturbance when they are incapable to complete their sexual reproductive life cycle (Honnay and Bossuyt, 2005).

Changes at cellular and biochemical level are the first detectable response of an organism to an environmental perturbation (Bierkens, 2000). Reactive oxygen species (ROS) are common by-products of both symbiotic algae photosynthesis and host aerobic cell metabolism (Byczkowski and Gessner, 1988; Asada, 1999) and these include superoxide anion radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\bullet}$) (Asada and Takahashi, 1987; Snoeijs et al., 2012). Nevertheless, during prolonged abiotic stress, e.g. heat and hyposaline stress (Lesser et al., 1990; Downs et al., 2000, 2002; Richier et al., 2006; Freire et al., 2012), the additional amount of ROS production and their accumulation results in extensive

cellular damage to both symbiotic algae and coral host. Lipid peroxidation is one of the most prevailing mechanisms of cellular damage (Halliwell and Gutteridge, 1999), it is a destructive process that compromises normal cellular function and, ultimately, leads to cell death (Lesser 1997; Perez and Weis, 2006; Olsen et al., 2013).

To prevent oxidative damage, both coral host and symbiotic algae have antioxidant mechanisms that maintain cellular ROS concentrations within a tolerable range, having a vital role in redox homeostasis (Nii and Muscatine, 1997; Richier et al., 2005). These antioxidant mechanisms can be non-enzymatic and enzymatic. Non-enzymatic mechanisms include glutathione, ascorbic acid, carotenoids, and tocopherols (Edge et al., 1997; Sies, 1997; Lesser, 2006), whereas enzymatic mechanisms include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione S-transferase (GST) (Lesser et al., 1990; Livingstone, 1991; Higuchi et al., 2008; Krueger et al., 2015). Cells employ several antioxidant strategies to detoxify ROS, but the mechanisms SOD-APX and SOD-CAT are the main enzymatic antioxidant pathways in eukaryotes (Asada, 1999; Halliwell, 2006). The enzyme SOD constitutes the first line of antioxidant defense, catalyzing the conversion of $O_2^{\bullet-}$ to water and H_2O_2 (Fridovich, 1995). Afterwards, H_2O_2 is reduced by CAT and APX to water and oxygen (Venn et al., 2008), the latter only present in the zooxanthellae (Lesser et al., 1990). The enzyme GST plays an important role in the phase II biotransformation of intermediary metabolites produced during exposure to xenobiotics by conjugating glutathione with these products, being considered a main detoxification pathway. Furthermore, GST detoxifies DNA hydroperoxides and lipid peroxides, having a central role in cell macrostructural repair (Hayes and McLellan, 1999, Limón-Pacheco and Gonsebatt, 2009).

One of the main factors inducing shifts in coral community structure is differential susceptibility of coral species to environmental stresses (Marshall and Baird, 2000; Faxneld et al., 2010; Berkelmans et al., 2012; Dias et al., 2018, 2019a,b) and, among many other factors, is related to differences in the activity of specific molecular mechanisms that prevent the generation of ROS or protect against their destructive effects (Regoli et al., 2000; Brown et al., 2002; Downs et al., 2002; Gardner et al., 2016). Oxidative stress parameters such as oxidative damage of lipids (lipid peroxidation – LPO) and antioxidant enzyme activity (superoxide dismutase – SOD, catalase – CAT, glutathione S-transferase – GST) have been widely used as

biomarkers of cellular stress in different taxa subjected to both heat and hyposaline stress (Li et al., 2008; De Zoysa et al., 2009; Freire at al., 2012; Rodrigues et al., 2012; Madeira et al., 2013; Vinagre et al., 2014; Madeira et al., 2015; Cereja et al., 2018). These oxidative stress biomarkers have also been applied in several studies evaluating the response of scleractinian corals to heat stress and bleaching (e.g. Downs et al., 2000, 2002; Yakovleva et al., 2004; Flores-Ramírez and Liñan-Cabello, 2007; Higuchi et al., 2008; Fitt et al., 2009; Krueger et al., 2015; Dias et al., 2019b). However, only two studies have been conducted on the effects of hyposaline conditions on at least one of the aforementioned oxidative stress biomarkers in scleractinian corals (Downs et al., 2009; Gardner et al., 2016). Moreover, no studies on the combined effects of high temperature and low salinity on these oxidative stress biomarkers have been made in scleractinian corals.

Dias et al. (2018, 2019a) investigated the differential ability of nine scleractinian corals to survive, grow and regenerate small fragments, mimicking a post-storm scenario in a context of higher temperatures and lower salinity. These studies identified resistant and vulnerable species to conditions that are likely to be more frequent in the future, influencing coral asexual reproduction via fragmentation. In Dias et al. (2019a), the same experimental procedure was used, but with the assessment of mortality, coral condition, growth rate, and regeneration rate as both heat and hyposaline stress indicators every 20 days of experiment. Mortality rate of the coral species was highest in the combined treatment of high temperature + low salinity, with only one species surviving this treatment (*Galaxea fascicularis)*. Only two species survived to the low salinity treatment (*Psammocora contigua*, *G. fascicularis*), presenting the lowest partial mortality and the best coral condition. In general, coral growth rates decreased as temperature increased and salinity decreased. Regeneration rates increased with temperature and decreased in the low salinity treatments. The species *Pocillopora damicornis* and *Stylophora pistillata* were the species most vulnerable, with all their fragments dead in the high temperature treatment. In the present study, we aim at testing the oxidative damage and antioxidant enzyme response of the same nine coral species, providing a new insight into the effects of heat and hyposaline stress on coral fragments at a cellular level.

5.2. Materials and methods

5.2.1. Study coral species

This study included nine common and widely distributed reef-building coral species of the Indo-Pacific region (Veron, 1990, 2000) with four different morphologies: one massive species (*Galaxea fascicularis*), one encrusting species (*Montipora capricornis* green morphotype (GM)), three plating species (*Montipora capricornis* brown morphotype (BM), *Turbinaria reniformis*, and *Echinopora lamellosa*), and four branching species (*Acropora tenuis*, *Pocillopora damicornis*, *Stylophora pistillata*, and *Psammocora contigua*). These coral species were chosen in order to use the largest number of species available at "Oceanário de Lisboa" (a large public aquarium, www.oceanario.pt) with different i) colony morphology, a characteristic mentioned as having influence in coral species susceptibility to both heat and hyposaline stress (Loya et al., 2001; True, 2012), ii) heat stress susceptibility: severe (*A. tenuis*, *P. damicornis*, and *S. pistillata*), high (*M. capricornis*), moderate (*E. lamellosa*), and low (*T. reniformis*, *G. fascicularis*, and *P. contigua*) (Marshall and Baird, 2000; Dias et al., 2018), and iii) hyposaline stress susceptibility: severe (*A. tenuis, P. damicornis, S. pistillata, M. capricornis, T. reniformis, E. lamellosa*) and low (*P. contigua* and *G. fascicularis*) (Jokiel et al., 1993; Moberg et al., 1997; Ferrier-pagès et al., 1999; Kerswell and Jones, 2003; Blakeway, 2004; Dias et al., 2019a). Coral species were identified according to Veron (2000). The coral colonies used in the experimental treatments have been maintained in captivity in a coral stock aquarium at "Oceanário de Lisboa (Portugal)" for five years, providing their thermal and salinity history information.

5.2.2. Experimental design

The experiments were conducted at "Oceanário de Lisboa, Portugal" (www.oceanario.pt). To test the long-term (60 days) effect of high temperature and hyposaline conditions, i) general condition (appearance: normal, pale, bleached, dead), ii) oxidative damage products concentration (LPO), and iii) antioxidant enzyme activity (SOD, CAT, GST), were investigated

in nine reef-forming coral species. Ten replicate fragments were cut from each coral colony, using a pincer or a pair of pliers. For the massive, encrusting and plating coral colonies the fragments were obtained by cutting approximately 30 mm sided squares and for the branching coral colonies the fragments were cut approximately 20-40 mm in length. All fragments were placed over egg crate panels in the coral stock aquarium until acclimation to the experimental aquarium.

Each coral fragment was glued with epoxy putty to the top (Ø = 1.6 cm) of a nylon expansion anchor (Ø = 1.0 cm, L = 6.0 cm). Massive, encrusting, and plating fragments were placed in horizontal position, whilst branching fragments were placed in vertical position. The placement of the fragments varied with morphology in order to minimize the dead tissue area produced by epoxy putty application. Then, the set (anchor + coral fragment) was placed back over egg crate panels in the coral stock aquarium, to recover from the handling procedures for one day before acclimation to the experimental aquarium.

To determine the long-term response of the nine-coral species to the individual and combined effects of high temperature and low salinity the coral fragments were exposed to different treatments: (a) control (26 °C, 33 psu) (26.1 ± 0.2 SD, 33.0 ± 0.1 SD), (b) high temperature (30 ºC, 33 psu) (30.2 ± 0.5 SD; 33.0 ± 0.1 SD), (c) low salinity (26 °C, 20 psu) (25.9 ± 0.3 SD; 20.0 ± 0.1 SD), and (d) high temperature + low salinity (30 ºC, 20 psu) (29.9 ± 0.2 SD; 20.0 ± 0.1 SD). Ten coral fragments of each coral species were exposed to each of the different treatments for 60 days. Coral fragments were acclimated at a rate of $1°C.hr^{-1}$ above the temperature of the coral stock aquarium (25 ºC). This heating rate was applied because coral reef-flat communities can experience temperature changes within this range during spring tides (Berkelmans and Willis, 1999), and most of the coral species in this study colonize the reef-flat zone (Brown and Suharsono, 1990; Fujioka, 1998). Moreover, studies have shown that salinity can drop very quickly in near-shore areas after heavy rainfalls (from 30 psu to 15 psu within 24 h, Jokiel et al., 1993; from 29 psu to 19 psu within 4 h, True, 2012), especially when concurrent with low tides (minutes to hours, Kerswell and Jones, 2003). Thus, the coral species were allowed to acclimate from 33 psu to 20 psu during five hours in both low salinity and high temperature + low salinity treatments. The coral fragments were placed over two 40 x 40 cm egg crate panels suspended 15 cm below the water surface of the experimental aquarium, positioned 2 cm apart

from one another, and organized by coral species to avoid contact between fragments of the same and/or different species.

The experimental aquarium (400 L) was fitted with a sump (280L) filled with bio-balls for biological filtration in which two Fluval M300 heaters, as well as a Hailea 500 chiller-controlled water temperature. For water circulation purposes, an AquaMedic OceanRunner 3500 pump provided a turnover rate of 5 times per hour. An AquaMedic Turboflotor 5000 Shorty protein skimmer helped to keep nutrient concentrations low, and increased surface water motion in the aquarium was accomplished by using an AquaClear 110 powerhead. Lighting requirements similar to the coral stock aquarium were attained by using a Litpa Jet5 floodlight with an AquaMedic 400W HQi lamp (13000K) on a 12 h light/ 12 h dark cycle. An air-stone was used in the aquarium to ensure good oxygen concentrations.

Photosynthetically Active Radiation (PAR) levels were measured with a spheric quantic sensor (LI-193SA) and a data logger (1400 model) and varied between 320-345 $\mu E.m^{-2}s^{-1}$ in the 400-700 nm waveband. The experimental treatments were conducted under low light conditions to minimize the synergistic effects with high light conditions (Fitt et al., 2001). Water quality parameters such as water temperature, salinity, and pH were measured on a daily basis. Water samples were also weekly analyzed to determine ammonium, nitrites, nitrates, calcium concentration, alkalinity as well as oxygen concentration and saturation. These parameters were maintained as follows: pH at 8.3, ammonium at 0 mg l^{-1}, nitrites between 0.002 and 0.003 mg l^{-1}, nitrates between 0 and 1.0 mg l^{-1}, calcium concentration between 389 and 401 mg l^{-1}, alkalinity approximately at 100 mg l^{-1}, oxygen concentration between 6.6 and 7.1 mg l^{-1}, and oxygen saturation between 101 and 104%. Salinity was maintained with daily balanced additions of reverse osmosis freshwater and filtered artificial seawater. Whenever alkalinity levels were below 100 mg l^{-1} sodium bicarbonate was added to the system. Aquarium cleaning routines were implemented on a weekly basis in order to minimize algal growth.

5.2.3. Survival, coral general condition and samples storage

The number of surviving fragments of the nine-coral species was assessed every day during the 60 days of the four experimental treatments with only the relevant days indicated in the results. The coral general condition was visually assessed in ten fragments of each coral species per treatment according to four categories: normal, pale, bleached, and dead (Jokiel and Coles, 1974). Normal corals were defined as having their normal coloration while pale corals showed a visible decrease in pigmentation. Bleached corals were considered as totally colorless and dead corals had no tissue at all. Coral general condition was assessed on the 60^{th} day of experiment by the same person to remove observer bias. After this procedure, six fragments of each coral species per treatment were removed from the experimental aquarium, separated from the respective anchor, and placed inside individual and identified sterilized bottles on ice-cold conditions. Nevertheless, there were combinations of experimental treatments and coral species where no fragments were taken at all due to mortality (Table 5.1). In the experimental treatments where the coral species were alive, the coral fragments with the highest amount of tissue were selected, this is, the coral fragments presenting the lowest or no partial mortality at all, since the ones presenting high partial mortality would not have enough tissue for biomarker analysis. Coral bleaching was not considered in the fragments selection since it would not have interfered with the tissue amount. The number of fragments of each coral species used in the four biomarker analysis per treatment is given in Table SM5.1 of the supplementary material. Then, they were kept inside refrigerated boxes and transported to the laboratory where they were stored at – 80 ºC.

Table 5.1. Number of surviving fragments of the nine-coral species tested on the relevant days of the four experimental treatments. C – control; HT – high temperature; LS – low salinity.

		Days																														
Treatment	Species	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16	17	21	24	25	27	33	36	38	41	43	46	48	52	55	59	60
C	*A. tenuis*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (BM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (GM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	T. reniformis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	E. lamellosa	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	G. fascicularis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	P. damicornis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	S. pistillata	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	P. contigua	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
HT	*A. tenuis*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (BM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	M. capricornis (GM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	T. reniformis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	E. lamellosa	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	G. fascicularis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	P. damicornis	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	9	9	9	9	9	9	8	3	2	2	2	1	1	1	0	0
	S. pistillata	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	8	8	3	2	0	0
	P. contigua	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
LS	*A. tenuis*	8	8	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M. capricornis (BM)	10	10	10	10	8	6	6	6	4	3	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M. capricornis (GM)	10	10	10	9	9	8	8	8	8	8	8	8	8	8	8	7	6	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	T. reniformis	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E. lamellosa	10	10	8	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G. fascicularis	10	10	10	10	10	10	10	10	10	10	10	9	8	8	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	P. damicornis	10	10	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S. pistillata	10	10	10	9	4	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
	P. contigua	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
HT + LS	*A. tenuis*	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M. capricornis (BM)	10	4	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M. capricornis (GM)	10	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	T. reniformis	10	10	10	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E. lamellosa	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	G. fascicularis	10	10	9	7	6	6	6	6	6	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	4	4	4	4
	P. damicornis	10	10	10	10	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	S. pistillata	10	10	9	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	P. contigua	10	10	10	10	10	10	10	10	10	10	10	10	10	10	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	0

5.2.4. Protein extraction

Coral fragments were carefully rinsed with ultrapure water to remove salt water and any debris and then the excess moisture was dried with absorbent lab paper. Afterwards, all the coral fragments were smashed (1 fragment = 1 sample) with a mortar and pestle and then were placed on 5 mL plastic microtubes. The samples were then homogenized in 1 mL of phosphate buffered saline solution (PBS consisting of 0.14 M NaCl, 0.003 M KCl, 0.01 M Na_2HPO_4, 0.002 M KH_2PO_4, pH 7.4) to extract cytosolic proteins, using a glass/teflon Potter Elve-jhem tissue grinder, in ice-cold conditions and mixed. The crude homogenates were then centrifuged at 4 °C for 15 min at 10,000 × g. The supernatant was collected, transferred to new microtubes (1.5 mL) and frozen immediately (−80 ºC).

5.2.5. Total Protein determination

For normalizing the results, the total protein content was determined through the Bradford method (Bradford, 1976). A calibration curve was obtained using bovine serum albumin (BSA) standards (0-2.0 $mg.mL^{-1}$).

5.2.6. Oxidative damage products – lipid peroxidation

The lipid peroxides assay was adapted from the thiobarbituric acid reactive substances (TBARS) protocol (Uchiyama and Mihara, 1978). In brief, five µL of each sample, already processed as previously described were added to 45 µL of 50 mM monobasic sodium phosphate buffer. Then 12.5 µL of SDS 8.1%, 93.5 µL of trichloroacetic acid (20%, pH = 3.5) and 93.5 µL of thiobarbituric acid (1%) were added to each microtube. To this mixture, 50.5 µL of Milli-Q grade ultrapure water was added. Then, the microtubes were put in a vortex for 30 s, centrifuged at 10,000 × g for 1 min, their lids were punctured with a needle and then incubated in boiling water for 10 min. To stop the reaction, they were placed on ice for a few minutes and 62.5 µL of Milli-Q grade ultrapure water was added. Then, the microtubes were placed in a

vortex and centrifuged at 10,000 × g for 1 min. Duplicates of 150 µL of the supernatant of each reaction were put into a 96-well microplate and absorbance was read at 530 nm. To quantify the lipid peroxides, an eight-point calibration curve (0–0.3 µM TBARS) was calculated using malondialdehyde bis (dimethylacetal) (MDA) standards (Merck Millipore, Portugal).

5.2.7. Enzymatic assays

5.2.7.1. Superoxide dismutase activity

Superoxide dismutase (SOD) activity (EC1.15.1.1) was assessed by using nitroblue tetrazolium (NBT) and xanthine oxidase (XOD) according to Sun et al. (1988). After reading the absorbance at 560 nm, SOD activity was calculated using the following equation for the % inhibition, formula 5.1:

$$((\mathrm{Abs}_{560}/\min \text{ negative control} - \mathrm{Abs}_{560}/\min \text{ sample})/(\mathrm{Abs}_{560}/\min \text{ negative control})) \times 100$$

5.2.7.2. Catalase activity

Catalase (CAT) activity (EC1.11.1.6) was assessed by using the peroxidatic function of catalase for determination of enzyme activity. The method is based on the reaction of catalase with methanol in the presence of hydrogen peroxide according to a method previously described by Johansson and Borg (1988) and adapted for 96-well microplate. In brief, 20 µL of sample in sample buffer (25 mM of KH_2PO_4, containing 1 mM EDTA and 0.1% BSA; pH 7.0), 30 µL methanol and 100 µL of assay buffer (100 mM KH_2PO_4, pH 7.0) were added to microplate wells. Then, 20 µL of standard (4.25 mM formaldehyde), 30 µL methanol and 100 µL of assay buffer were added to formaldehyde standard wells. To the positive control wells, were added 20 µL of diluted CAT (bovine liver CAT), 30 µL of methanol and 100 µL of assay buffer. Afterwards, the reaction was initiated by adding 20 µL of hydrogen peroxide (0.035 M H_2O_2) to the microplate wells. Then, the microplate was covered with aluminum foil and incubated during 20 min at room temperature under gentle agitation on a shaker. Following incubation, 30 µL of potassium hydroxide (10 M KOH) was added to each microplate well to end the

reaction, followed by adding 30 µL of Purpald chromogen (34.2 mM of 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) to each well. Again, the microplate was covered and incubated on a shaker for 10 min at room temperature. Next, 10 µL of potassium periodate (65.2 mM KIO_4) was added to each well and the microplate incubated for 5min on the shaker. The absorbance was read at 540 nm using a microplate reader (Benchmark, Bio-Rad, USA).

5.2.7.3. Glutathione S-transferase activity

The enzymatic assay of glutathione S-transferase (GST) activity (EC 2.5.1.18) was adapted from Habig et al. (1974), using the CDNB (1-chloro-2,4-dinitrobenzene) as the enzyme substrate, and optimized for 96-well microplates. After reading the absorbance at 340 nm GST activity was calculated using a molar extinction coefficient for CDNB of 5.3 εmM ($mM^{-1}cm^{-1}$) after correction for the microplate wells path length.

5.2.8. Statistical analyses

In the present study, we intended to analyze the response of each biomarker to the stress factors alone and in interaction per coral species. Interaction between the factors temperature and salinity is ecologically relevant, and analyzing their interaction was the objective of the study. Still, looking at differences between different levels of temperature, salinity or their interaction for different species would not be relevant. Thus, a three-way nested PERMANOVA was performed (Anderson, 2001). Euclidean distances were used to calculate the similarity matrix (coral species, temperature and salinity were fixed factors with temperature and salinity nested in coral species) that was used to test whether coral fragments' lipid peroxidation; superoxide dismutase, catalase and glutathione S-transferase activities were affected by coral species, temperature, and salinity. The permutation of residuals under a reduced model was selected and the model was run for 9999 permutations. Analyses were performed using PERMANOVA+ for PRIMER v6 (PRIMER-E Ltd., Plymouth). Post-hoc pair-wise comparisons were then performed using PERMANOVA to compare between treatments and coral species. Differences were considered significant at p-value < 0.05.

5.3. Results

5.3.1. Survival

In the control treatment, all the coral species fragments' survived throughout the course of the experiment (Table 5.1). In the high temperature treatment, a major decrease in the number of surviving fragments was observed in *P. damicornis* on the 38th day and in *S. pistillata* on the 52nd day of experiment (3 fragments, Table 5.1). All the other species fragments survived throughout the 60 days of the experiment (Table 5.1). In the low salinity treatment, the coral species *A. tenuis* was the first presenting the highest decrease in the number of surviving fragments (3 fragments on the 3rd day), followed by both *E. lamellosa* and *P. damicornis* (4 and 0 fragments, respectively, on the 4th day), *S. pistillata* (4 fragments on the 5th day), *T. reniformis* (0 fragments on the 6th day), *M. capricornis* (BM) (4 fragments on the 9th day), and, ultimately, by *M. capricornis* (GM) (3 fragments on the 24th day, Table 5.1). The coral species *G. fascicularis* did not present a great reduction in the number of surviving fragments, it maintained 7 surviving fragments since the 16th day until the end of the experiment (Table 5.1). *Psammocora contigua* was the only species that maintained all of its fragments alive during the 60 days of experiment (Table 5.1). In the high temperature + low salinity treatment, *A. tenuis*, *M. capricornis* (BM), *M. capricornis* (GM), and *E. lamellosa* presented the first great decrease in the number of surviving fragments (0, 4, 3 and 1 fragments, respectively, on the 2nd day), followed by *T. reniformis*, *P. damicornis,* and *S. pistillata* (0, 3 and 2 fragments, respectively, on the 5th day), *P. contigua* (2 fragments on the 16th day), and, finally, by *G. fascicularis* (4 fragments on the 33th day, Table 5.1). In the high temperature + low salinity treatment, it was observed that the majority of the tested species presented a great reduction in the number of surviving fragments earlier than in both high temperature and low salinity treatments. This response may indicate a negative synergistic effect of these two variables on the survival of the tested species (Table 5.1).

5.3.2. Coral general condition

After 60 days of the experimental trials, in control treatment, all the coral species' fragments were normal (Fig. 5.1). In the high temperature treatment, the fragments of *A. tenuis*, *M. capricornis* (GM), *E. lamellosa*, and *G. fascicularis* were normal (Fig. 5.1). Seventy percent of the fragments of *M. capricornis* (BM), all the fragments of *T. reniformis*, and 60% of the fragments of *P. contigua* were pale (Fig. 5.1). All the fragments of *P. damicornis* and *S. pistillata* were dead (Fig. 5.1). In the low salinity treatment, all the coral species' fragments were dead, exceptions of *G. fascicularis* (70% bleached and 30% dead) and *P. contigua* (10% normal, 20% pale and 70% bleached) (Fig. 5.1). In the high temperature + low salinity treatment, only the coral fragments of *G. fascicularis* were not completely dead (40% bleached and 60% dead; Fig. 5.1).

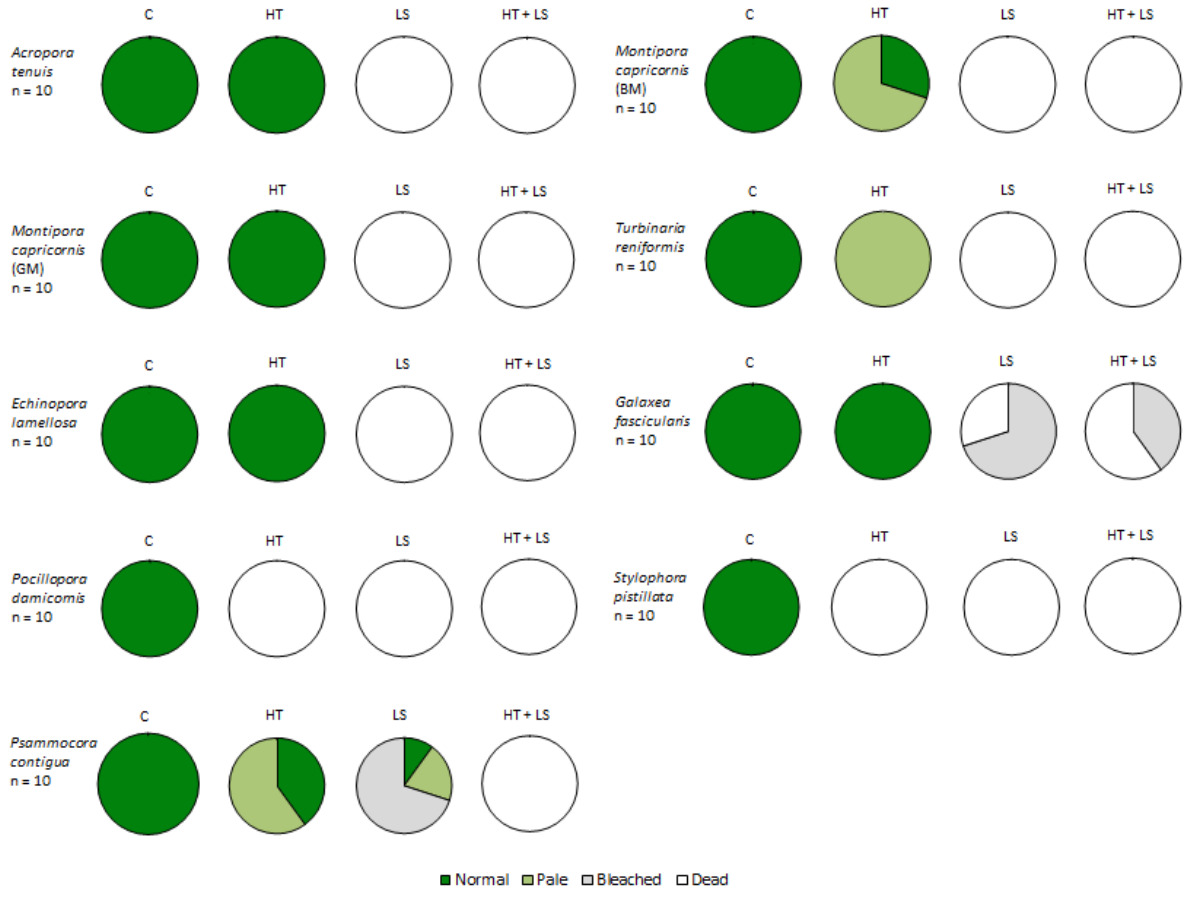

Figure 5.1. Coral general condition of the nine-coral species studied in the four experimental treatments. C - control, HT - high temperature, LS - low salinity.

5.3.3. Lipid peroxidation (LPO)

Lipid peroxidation levels were significantly affected by the independent effect of coral species and temperature (PERMANOVA p-value < 0.05, Table 5.2). In the control treatment, the fragments of *M. capricornis* (GM) and *E. lamellosa* showed the highest LPO levels (PERMANOVA pair-wise p-value < 0.05) (0.203 μmol.mg^{-1} of total protein and 0.167 μmol.mg^{-1} of total protein, respectively; Fig. 5.2a, PERMANOVA pair-wise p-value > 0.05), whilst *M. capricornis* (BM), *T. reniformis,* and *A. tenuis* fragments had the lowest LPO levels (PERMANOVA pair-wise p-value < 0.05) (0.015 μmol.mg^{-1} of total protein, 0.017 μmol.mg^{-1} of total protein, and 0.019 μmol.mg^{-1} of total protein respectively; Fig. 5.2a, PERMANOVA pair-wise p-value > 0.05). In the high temperature treatment, the fragments of *E. lamellosa* presented the highest LPO levels (0.555 μmol.mg^{-1} of total protein, Fig. 5.2a; PERMANOVA pair-wise p-value < 0.05), whilst the fragments of *A. tenuis* and *T. reniformis* displayed the lowest LPO levels (PERMANOVA pair-wise p-value < 0.05) (0.009 μmol.mg^{-1} of total protein and 0.011 μmol.mg^{-1} of total protein, respectively; Fig. 5.2a, PERMANOVA pair-wise p-value > 0.05). In the low salinity treatment, *P. contigua* fragments presented the highest LPO levels (0.051 μmol.mg^{-1} of total protein, Fig. 5.2a; PERMANOVA pair-wise p-value < 0.05) and *G. fascicularis* fragments showed the lowest LPO levels (0.017 μmol.mg^{-1} of total protein, Fig. 5.2a; PERMANOVA pair-wise p-value < 0.05). In the high temperature + low salinity treatment, *G. fascicularis* was the only coral species presenting LPO levels (0.048 μmol.mg^{-1} of total protein, Fig. 5.2a).

The fragments of *Montipora capricornis* (BM), *E. lamellosa*, *A. tenuis,* and *P. contigua* presented significant intraspecific differences in LPO levels between control and high temperature treatment (Fig. 5.2a, PERMANOVA pair-wise p-value < 0.05), being higher in the high temperature treatment in the first two coral species and higher in the control treatment in the last two coral species. The fragments of *G. fascicularis* also displayed significant intraspecific differences in LPO levels between control and low salinity treatment (Fig. 5.2a, PERMANOVA pair-wise p-value < 0.05), being higher in the control treatment.

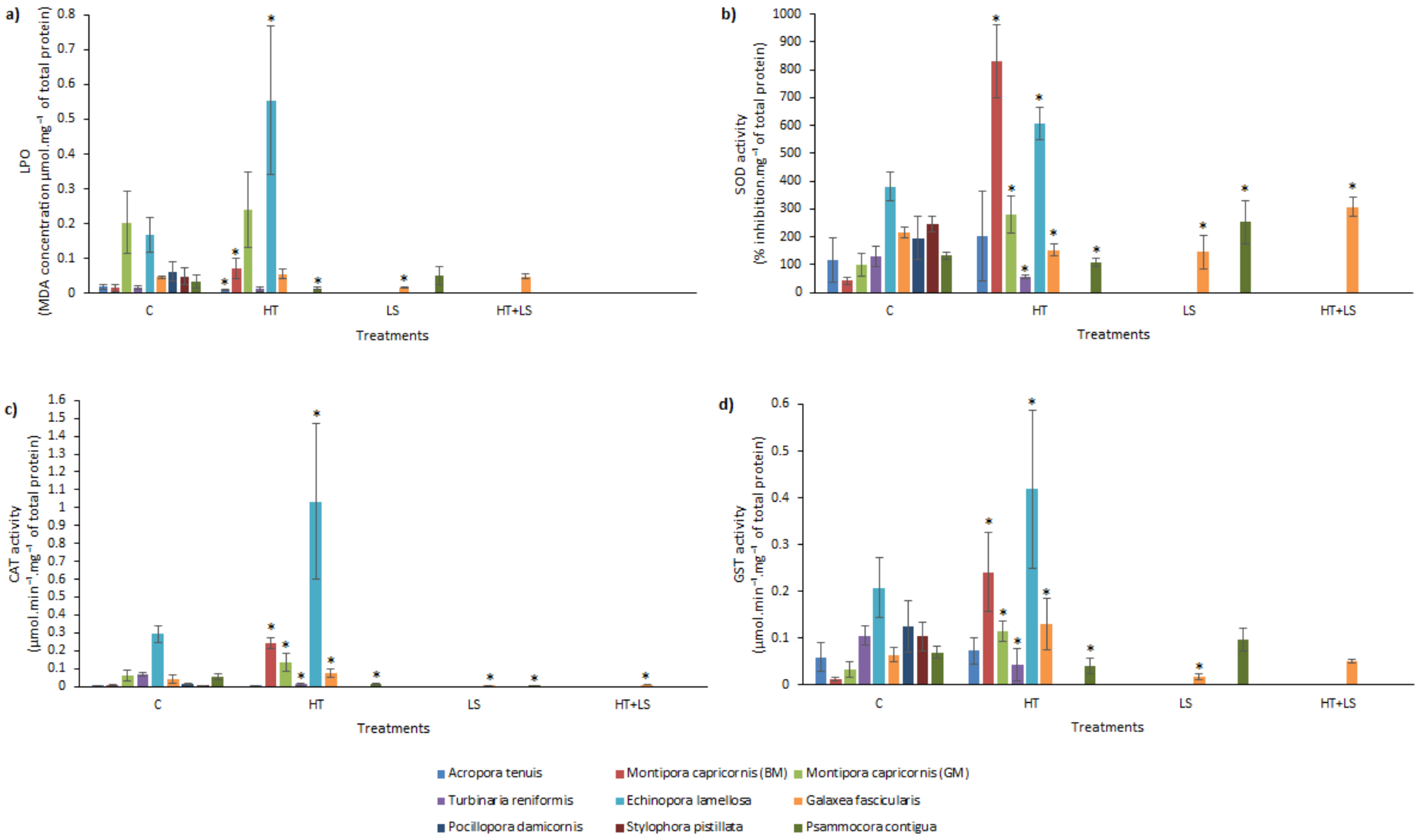

Figure 5.2. Mean ± SD of (a) LPO (MDA concentration µmol.mg^{-1} of total protein), (b) superoxide dismutase activity (% inhibition.mg^{-1} of total protein), (c) catalase activity (µmol.min^{-1}.mg^{-1} of total protein), and (d) glutathione S-transferase (GST) activity (µmol.min^{-1}.mg^{-1} of total protein) for the nine-coral species studied in the four experimental treatments. C - control, HT - high temperature, LS - low salinity. Asterisks (*) mark significant differences (p-value < 0.05) in relation to control treatment.

Table 5.2. Summary of results of PERMANOVA permutation tests applied to report the effects of coral species (Sp), temperature (Te), and salinity (Sa) in coral fragments' lipid peroxidation (LPO); superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) activities. Significant differences are marked in bold.

	df	SS	MS	Pseudo-*F*	P(perm)	Unique perms
LPO						
Sp	8	1.2	0.1	40.3	**0.0001**	9945
Te (Sp)	7	0.4	5.6×10^{-2}	15.6	**0.0001**	9950
Sa (Sp)	2	2.5×10^{-3}	1.2×10^{-3}	0.3	0.6447	9932
Te (Sp) × Sa (Sp)	1	6.1×10^{-4}	6.1×10^{-4}	0.2	0.6439	9812
SOD activity						
Sp	8	1.6×10^{6}	2.0×10^{5}	46.4	**0.0001**	9936
Te (Sp)	7	2.0×10^{6}	2.8×10^{5}	66.7	**0.0001**	9957
Sa (Sp)	2	5.3×10^{4}	2.7×10^{4}	6.3	**0.0040**	9933
Te (Sp) × Sa (Sp)	1	6.6×10^{4}	6.6×10^{4}	15.4	**0.0002**	9816
CAT activity						
Sp	8	4.2	0.5	45.0	**0.0001**	9929
Te (Sp)	7	1.8	0.3	22.3	**0.0001**	9948
Sa (Sp)	2	2.0×10^{-2}	9.9×10^{-3}	0.8	0.3902	9907
Te (Sp) × Sa (Sp)	1	1.3×10^{-3}	1.3×10^{-3}	0.1	0.5729	9547
GST activity						
Sp	8	0.6	7.5×10^{-2}	25.0	**0.0001**	9939
Te (Sp)	7	0.3	4.4×10^{-2}	14.6	**0.0001**	9937
Sa (Sp)	2	2.1×10^{-2}	1.1×10^{-2}	3.5	**0.0431**	9939
Te (Sp) × Sa (Sp)	1	1.3×10^{-3}	1.3×10^{-3}	0.4	0.4807	9808

5.3.4. Superoxide dismutase (SOD)

Superoxide dismutase activity was significantly affected by the interactive effects of temperature and salinity (PERMANOVA p-value < 0.05, Table 5.2). In the control treatment, *E. lamellosa* fragments presented the highest SOD activity (381.1% inhibition.mg^{-1} of total protein; Fig. 5.2b; PERMANOVA pair-wise p-value < 0.05), whereas the fragments of *M. capricornis* (BM) presented the lowest SOD activity (42.9% inhibition.mg^{-1} of total protein; Fig. 5.2b; PERMANOVA pair-wise p-value < 0.05). In the high temperature treatment, *M. capricornis* (BM) showed the highest SOD activity (831.4% inhibition.mg^{-1} of total protein; Fig. 5.2b; PERMANOVA pair-wise p-value < 0.05), whilst *T. reniformis* displayed the lowest SOD activity (55.3% inhibition.mg^{-1} of total protein; Fig. 5.2b; PERMANOVA pair-wise p-value < 0.05). In the low salinity treatment, *P. contigua* fragments had the highest SOD activity (253.1% inhibition.mg^{-1} of total protein; Fig. 5.2b; PERMANOVA pair-wise p-value < 0.05), whereas the fragments of *G. fascicularis* presented the lowest one (145.5% inhibition.mg^{-1} of

total protein; Fig. 5.2b; PERMANOVA pair-wise p-value < 0.05). In the high temperature + low salinity treatment only *G. fascicularis* fragments displayed SOD activity (307.9% inhibition.mg^{-1} of total protein; Fig. 5.2b).

The fragments of *M. capricornis* (BM), *M. capricornis* (GM), *E. lamellosa*, *T. reniformis*, *G. fascicularis,* and *P. contigua* presented intraspecific differences in SOD activity between control and high temperature treatment (Fig. 5.2b, PERMANOVA pair-wise p-value < 0.05), being higher in the high temperature treatment in the first three coral species and higher in the control treatment in the last three species. The fragments of *G. fascicularis* and *P. contigua* also showed significant intraspecific differences in SOD activity between control and low salinity treatment, being higher in control treatment in *G. fascicularis* and higher in low salinity treatment in *P. contigua* (Fig. 5.2b, PERMANOVA pair-wise p-value < 0.05). Moreover, *G. fascicularis* fragments presented significant intraspecific differences between control and high temperature + low salinity treatment, being higher in high temperature + low salinity treatment (Fig. 5.2b, PERMANOVA pair-wise p-value < 0.05).

5.3.5. Catalase (CAT)

Catalase activity was significantly affected by the independent effect of coral species and temperature (PERMANOVA p-value < 0.05, Table 5.2). In both the control and high temperature treatments, *Echinopora lamellosa* fragments had the highest catalase activity (PERMANOVA pair-wise p-value < 0.05) (0.294 μmol.min^{-1}.mg^{-1} of total protein and 1.033 μmol.min^{-1}.mg^{-1} of total protein, respectively; Fig. 5.2c), whilst the lowest catalase activity was detected in the fragments of *A. tenuis* in both control and high temperature treatments (PERMANOVA pair-wise p-value < 0.05) (0.003 μmol.min^{-1}.mg^{-1} of total protein in both treatments; Fig. 5.2c). In the low salinity treatment, the coral species did not present significant differences between one another (*G. fascicularis*: 0.004 μmol.min^{-1}.mg^{-1} of total protein and *P. contigua*: 0.003 μmol.min^{-1}.mg^{-1} of total protein; Fig. 5.2c, PERMANOVA pair-wise p-value > 0.05). In the high temperature + low salinity treatment, only *G. fascicularis* showed catalase activity (0.008 μmol.min^{-1}.mg^{-1} of total protein; Fig. 5.2c).

Montipora capricornis (BM), *M. capricornis* (GM), *E. lamellosa, G. fascicularis, T. reniformis,* and *P. contigua* catalase activity showed significant intraspecific differences between control and high temperature treatment (Fig. 5.2c, PERMANOVA pair-wise p-value < 0.05), being higher in the high temperature treatment in the first four coral species and higher in the control treatment in the last two coral species. The species *G. fascicularis* and *P. contigua* also displayed significant intraspecific differences between control and low salinity treatment, being higher in the control treatment (Fig. 5.2c, PERMANOVA pair-wise p-value < 0.05). Moreover, *G. fascicularis* fragments presented significant intraspecific differences between control and high temperature + low salinity treatment, being higher in control treatment (Fig. 5.2c, PERMANOVA pair-wise p-value < 0.05).

5.3.6. Glutathione S-transferase (GST)

Glutathione S-transferase activity was significantly affected by the independent effect of coral species, temperature and salinity (PERMANOVA p-value < 0.05, Table 5.2). In both the control and high temperature treatments, *Echinopora lamellosa* fragments had the highest GST activity (PERMANOVA pair-wise p-value < 0.05) (0.207 $\mu mol.min^{-1}.mg^{-1}$ of total protein and 0.419 $\mu mol.min^{-1}.mg^{-1}$ of total protein, respectively; Fig. 5.2d), whilst the lowest GST activity was detected in the fragments of *M. capricornis* (BM) in the control treatment (0.012 $\mu mol.min^{-1}.mg^{-1}$ of total protein; Fig. 5.2d, PERMANOVA pair-wise p-value < 0.05) and in the fragments of *P. contigua, T. reniformis,* and *A. tenuis* in the high temperature treatment (0.041 $\mu mol.min^{-1}.mg^{-1}$ of total protein, 0.043 $\mu mol.min^{-1}.mg^{-1}$ of total protein, and 0.073 $\mu mol.min^{-1}.mg^{-1}$ of total protein, respectively; Fig. 5.2d, PERMANOVA pair-wise p-value < 0.05). In the low salinity treatment, *P. contigua* fragments presented the highest GST activity (0.104 $\mu mol.min^{-1}.mg^{-1}$ of total protein, Fig. 5.2d, PERMANOVA pair-wise p-value < 0.05), whilst *G. fascicularis* fragments had the lowest GST activity (0.017 $\mu mol.min^{-1}.mg^{-1}$ of total protein, Fig. 5.2d, PERMANOVA pair-wise p-value < 0.05). In the high temperature + low salinity treatment only *G. fascicularis* presented GST activity (0.052 $\mu mol.min^{-1}.mg^{-1}$ of total protein, Fig. 5.2d).

The fragments of *M. capricornis* (BM), *M. capricornis* (GM), *E. lamellosa*, *G. fascicularis*, *T. reniformis*, and *P. contigua* presented significant intraspecific differences in GST activity between the control and high temperature treatment (Fig. 5.2d, PERMANOVA pair-wise p-value < 0.05), being higher in the high temperature treatment in the first four coral species and higher in the control treatment in the last two coral species. The fragments of *G. fascicularis* also showed significant intraspecific differences in GST activity between control and low salinity treatment, being higher in control treatment (Fig. 5.2d, PERMANOVA pair-wise p-value < 0.05).

5.4. Discussion and conclusions

The present study investigated the individual and combined effects of high temperature and low salinity on mortality, coral condition, oxidative damage and antioxidant enzyme activity of nine reef-forming coral species of the Indo-Pacific. The results of this experiment are in agreement with previous studies showing that long-term exposure to high temperature and low salinity individually can cause changes in coral condition (Jokiel et al., 1993; Kerswell and Jones, 2003; Dias et al., 2018, 2019a,b). Hyposaline stress proved to affect the corals in a much more severe degree than thermal stress. Only two species died at high temperature (*P. damicornis* and *S. pistillata*), while low salinity resulted in the death of all species with the exception of two (*P. contigua* and *G. fascicularis*). Furthermore, the combination of high temperature and low salinity had a negative synergistic effect on coral condition. This synergistic response had already been observed in previous studies (Coles and Jokiel, 1978; Xiubao et al., 2009; Faxneld et al., 2010; Dias et al., 2019a).

Regarding the oxidative stress biomarkers' results, long-term exposure to high temperature led to increases in the activity of the antioxidant enzymes in four species. The induction of these antioxidant enzymes occurs in order to counteract the effects of excessive ROS generated during heat stress (Burdon et al., 1990; Richier et al., 2006). However, even though cellular antioxidant enzyme defenses have been activated, the levels of lipid peroxidation increased in two species in the high temperature treatment. On the other hand, long-term exposure to low salinity led to different oxidative stress response and damage by the only two survivor species.

These results may indicate that coral species differ in their cellular physiology and the strategies applied to diminish oxidative stress.

The coral general condition observations indicate that all the coral species tested are susceptible to long-term exposure to the low salinity treatment. *Psammocora contigua* and *G. fascicularis* were the only survivors and therefore the most tolerant to prolonged hyposaline stress. Mayfield and Gates (2007) stated that while both partners of the symbiosis probably have ways of counteract hypo-osmotic stress, there is a determined threshold from which noxious physiological effects are experienced. These physiological effects include symbiotic algae loss or even death. According to our results it may seem that the threshold was surpassed for all coral species, given that even the most tolerant corals presented bleached fragments and, in the case of *G. fascicularis*, dead fragments were also observed. Moreover, given the different response of the tested coral species to protracted hyposaline conditions, this work corroborates previous studies stating that tolerance to hyposaline conditions is species-specific (Lirman and Manzello, 2009; Faxneld et al., 2010; Berkelmans et al., 2012; Dias et al., 2019a). *Psammocora contigua* and *G. fascicularis* have been cited in other studies as being highly tolerant to hyposaline conditions (Van Woesik, 1994; Xiubao et al., 2009; Dias et al., 2019a).

In the low salinity treatment, *P. contigua* was severely affected by long-term exposure to hyposaline conditions, as observed by 70% of its fragments being bleached. The increase in SOD activity observed in this species may have occurred as a way to counteract the increased ROS production resulting from the enhanced metabolic rates in consequence of osmoregulation demands (Freire et al., 2012). In the majority of biological systems, H_2O_2 scavenging is most proficiently accomplished by catalase (Munoz-Munoz et al., 2009), however, coral fluorescent proteins (FPs) are also able to proficiently scavenge H_2O_2 (Palmer et al., 2009), this may explain the *P. contigua* fragments' decrease in CAT activity in the low salinity treatment. Given that cells cannot be completely detoxified by SOD (Aguilera and Rautenberger, 2012) and that CAT activity decreased in the low salinity treatment, it is likely that FPs may have scavenged H_2O_2 preventing any cellular damage via lipid peroxidation. As the antioxidant activity is limited, bleaching, the coral's final defense mechanism against oxidative stress, was activated (Downs et al., 2002).

In the low salinity treatment, *G. fascicularis* presented a decrease in both oxidative-stress response (SOD, CAT, and GST activities) and damage (LPO levels) compared to control. This species was affected even more severely than *P. contigua* in terms of coral condition, presenting 30% of mortality. The overall decrease of capacity of antioxidant enzymes to counteract ROS during prolonged exposure to low salinity may be related to damage on ROS-sensitive enzymes, as observed by the decrease in antioxidant activity (Hermes-Lima and Storey, 1993). Another possible explanation relates to ROS have been scavenged by FPs (Bou-Abdallah et al., 2006; Palmer et al., 2009).

In the high temperature + low salinity treatment, all the coral species died except for *G. fascicularis* fragments. Even this species had its coral condition worsened compared to low salinity treatment. Thus, the coral condition results obtained in the high temperature + low salinity treatment indicate that these two environmental variables acted in synergy on the coral species in study, leading to higher bleaching and mortality when compared with the other treatments. This synergistic effect is probably the result of a strong increase in metabolic rate due to both temperature induced increase in metabolism and the costs of osmoregulation acting together. Although *G. fascicularis* presented high mortality in this treatment, the fact that a considerable proportion (40%) of its fragments survived and that there was an oxidative-stress response (SOD activity increase), suggests that this species can cope with this level of heat and hyposaline stress for 60 days.

The duration of both heat and hyposaline stress events can change among years and geographic locations (Coles and Jokiel, 1992; Stimson et al., 2002; Liu et al., 2003; Pratchett et al., 2011). Heat stress events tend to have a higher duration than reduced salinity stress events (26-184 days and 16-98 days, respectively; Brown and Suharsono, 1990; Coles and Jokiel, 1992; Van Woesik et al., 1995; Goldberg and Wilkinson, 2004; Guest et al., 2012; Butler et al., 2013). Considering our results, on the 26th day of experiment, none of the tested coral species would have been considered susceptible to the high temperature treatment. Regarding the salinity duration, on the 16th day of experiment, the species *A. tenuis, M. capricornis* (BM), *T. reniformis, E. lamellosa, P. damicornis,* and *S. pistillata* would have been considered the most vulnerable to the low salinity treatment, this is, *M. capricornis* (GM) would not have been considered vulnerable given an experiment duration of 16 days. In relation to the high

temperature + low salinity treatment, on the 26th day of experiment, the minimum number of days of a heat stress event, all the species' fragments were dead with the exception of 2 fragments of *P. contigua* and 5 fragments of *G. fascicularis*, this is, *P. contigua* would have survived to an experiment duration of 26 days. Therefore, stress duration is one of the crucial factors affecting the physiological response of coral species to both heat and hyposaline stress.

Adaptation of reef-building corals to the expected temperatures and salinities will depend on their inherent traits (e.g. ability of corals to draw on energy reserves and heterotrophic capacity, Grottoli et al., 2017) and on both magnitude and duration of the stress (D'Croz and Maté, 2002). Adaptation may occur through physiological acclimatization of both the host coral and symbiotic algae (e.g. increased production of antioxidant enzymes, Barshis et al., 2013), symbiont shuffling or shifts in the abundance of existing zooxanthellae (Jones et al., 2008; Silverstein et al., 2012), microbiome alteration (Röthig et al., 2016), and genetic adaptation (Van Woesik et al., 2011; Voolstra et al., 2011). Deviating environmental conditions can strongly impair reef-building corals' physiological performance (D' Angelo et al., 2015). For instance, natural populations of "super corals" that are tolerant of stressful conditions might arise after repeated bleaching events (McClanahan, 2017) or in more variable environments (Chui and Ang, 2017; Ruiz-Jones and Palumbi, 2017) if the duration and magnitude of the stress do not surpass those species thermal and saline threshold (Berkelmans, 2002; Depczynski et al., 2013). In our study, if the duration of the high temperature + low salinity treatment was shorter, the physiological acclimation of a high number of the tested species would probably be possible. Physiological acclimation might have been possible in a short-term experiment due to both higher number of species alive and lower level of physiological stress. Terms herein followed the terminology of Brown (1997b). Acclimation describes changes in tolerances under laboratory or other experimental conditions, generally over the short-term. Acclimatization refers to phenotypic changes, usually reversible, by an organism to stresses in the natural environment that result in the readjustment of the organism's tolerance levels, being limited by the organism's genotype.

Scleractinian corals living in near-shore reefs are especially susceptible to the effects of global climate change. As these corals live near the mainland, they are exposed to drastic changes in salinity due to heavy rainfall events associated with tropical storms (Van Woesik et al., 1995;

True, 2012). The resultant hyposaline conditions often result in bleaching and mortality of the coral species inhabiting near-shore reef environments (Goreau, 1964; Jokiel et al., 1993; Blakeway, 2004). Given that the coral species in study live in near-shore environments and that extreme precipitation events associated with tropical storms are expected to increase in the near future, most of the corals species in study won't be able to survive those conditions. Moreover, given that increases in SSTs are also expected, the combined effects of high temperature and low salinity will be extremely deleterious for all the coral species tested. Only one of the nine coral species tested could withstand the synergistic effects of high temperature and low salinity, although with considerable damage in terms of condition. *Galaxea fascicularis* is considered a "stress-tolerant" species, defined as a coral with broadcast spawning reproduction, slow-grow rates, longer generation times, large corallites and high fecundity (Darling et al., 2012). These inherent traits may provide *G. fascicularis* higher tolerance to the synergistic effects of high temperature and low salinity compared to the other coral species tested, and thus a competitive advantage over other corals in future oceans. Given the expected predictions under the global climate change scenario, major shifts from coral dominated to algal dominated communities are the most probable scenario for near-shore reefs.

This study focused on the interaction of high temperature and low salinity associated with the increase in SSTs and in tropical storms' frequency and intensity in the Indo-Pacific oceans. However, other environmental variables not tested in this study can be altered by global climate change such as ultraviolet radiation levels (Hoegh-Guldberg and Bruno, 2010), pH (Manzello, 2010), dissolved inorganic nutrient levels (Wiedenmann et al., 2013), and sediment loads (Browne, 2012). Furthermore, alterations in the environmental variables studied may also happen. Higher temperatures are a likely scenario in the future. As observed in Dias et al. (2018), long-term exposure to temperatures of 32 °C led to the death of six out of the nine coral species tested. Given the results obtained in the treatment high temperature + low salinity (30 °C + 20) of the present study, the combination of 32 °C + 20 would probably lead to the death of most of the tested species, if such conditions last 60 days.

Our study shows variability in the regulation of antioxidant enzyme activity by the coral species in study in both high temperature and low salinity treatments. *Pocillopora damicornis* and *S. pistillata* were the species most vulnerable to high temperature. The coral species *P. contigua*

and *G. fascicularis* were the most resistant to low salinity. *Galaxea fascicularis* was the only species surviving the synergistic effects of high temperature and low salinity.

5.5. References

Aguilera, J., & Rautenberger, R. (2012). Oxidative stress tolerance strategies of intertidal macroalgae, in: Abele, D., Vázquez-Medina, J. P., & Zenteno-Savín, T. (Eds.), Oxidative Stress in Aquatic Ecosystems, First Edition, Blackwell Publishing Ltd, pp. 58-71.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26(1), 32-46.

Asada, K. (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annual Review of Plant Biology, 50(1), 601-639.

Asada, K., & Takahashi, M. (1987) Production and scavenging of active oxygen in photosynthesis, in: Kyle, D. J., Osmond, C. B., & Arntzen, C. J. (Eds.), Photoinhibition. Elsevier, Amsterdam, pp. 227–287.

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine, Coastal and Shelf Science, 80(4), 435-471.

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. Proceedings of the National Academy of Sciences, 110(4), 1387-1392.

Berkelmans, R. (2002). Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. Marine Ecology Progress Series, 229, 73-82.

Berkelmans, R., & Willis, B. L. (1999). Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs, 18(3), 219-228.

Berkelmans, R., Jones, A. M., & Schaffelke, B. (2012). Salinity thresholds of *Acropora* spp. on the Great Barrier Reef. Coral Reefs, 31(4), 1103-1110.

Bierkens, J. G. (2000). Applications and pitfalls of stress-proteins in biomonitoring. Toxicology, 153(1-3), 61-72.

Blakeway, D. R. (2004). Patterns of mortality from natural and anthropogenic influences in Dampier corals: 2004 cyclone and dredging impacts, in: Stoddart, J. A., Stoddart, S. E. (Eds.), Corals of the Dampier Harbour: Their Survival and Reproduction during the Dredging Programs of 2004, pp. 61-72.

Bou-Abdallah, F., Chasteen, N. D., & Lesser, M. P. (2006). Quenching of superoxide radicals by green fluorescent protein. Biochimica et Biophysica Acta (BBA)-General Subjects, 1760(11), 1690-1695.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), 248-254.

Brown, B. E. (1997a). Coral bleaching: causes and consequences. Coral Reefs, 16(1), S129-S138.

Brown, B. E. (1997b). Adaptations of reef corals to physical environmental stress. In Advances in Marine Biology (Vol. 31, pp. 221-299). Academic Press.

Brown, B. E., Suharsono (1990). Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. Coral Reefs, 8(4), 163-170.

Brown, B. E., Downs, C. A., Dunne, R. P., & Gibb, S. W. (2002). Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. Marine Ecology Progress Series, 242, 119-129.

Browne, N. K. (2012). Spatial and temporal variations in coral growth on an inshore turbid reef subjected to multiple disturbances. Marine Environmental Research, 77, 71-83.

Bruno, J. F. (1998). Fragmentation in Madracis mirabilis (Duchassaing and Michelotti): how common is size-specific fragment survivorship in corals? Journal of Experimental Marine Biology and Ecology, 230(2), 169-181.

Bruno, J. F., Selig, E. R., Casey, K. S., Page, C. A., Willis, B. L., Harvell, C. D., (...) & Melendy, A. M. (2007). Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biology, 5(6), e124.

Burdon, R. H., Gill, V., & Evans, C. R. (1990). Active oxygen species and heat shock protein induction, in: Schlesinger, Santoro, Garaci (Eds.), Stress Proteins, Springer, Berlin, Heidelberg, pp. 19-25.

Butler, I. R., Sommer, B., Zann, M., Zhao, J. X., & Pandolfi, J. M. (2013). The impacts of flooding on the high-latitude, terrigenoclastic influenced coral reefs of Hervey Bay, Queensland, Australia. Coral Reefs, 32(4), 1149-1163.

Byczkowski, J. Z., & Gessner, T. (1988). Biological role of superoxide ion-radical. International Journal of Biochemistry, 20(6), 569-580.

Cacciapaglia, C., & Van Woesik, R. (2016). Climate-change refugia: shading reef corals by turbidity. Global Change Biology, 22(3), 1145-1154.

Carpenter, K. E., Abrar, M., Aeby, G., Aronson, R. B., Banks, S., Bruckner, A., ... & Edgar, G. J. (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science, 321(5888), 560-563.

Cereja, R., Mendonça, V., Dias, M., Vinagre, C., Gil, F., & Diniz, M. (2018). Physiological effects of cymothoid parasitization in the fish host *Pomatoschistus microps* (Krøyer, 1838) under increasing ocean temperatures. Ecological Indicators, 95, 176-182.

Christensen, J.H., Kanikicharla, K.K., Aldrian, E., An, S.-I., Cavalcanti, I.F.A., De Castro, M., (…) & Midgley, P.M., (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC AR5). Cambridge, United Kingdom and New York, New York: Cambridge University Press, pp.1217-1308.

Chui, A. P. Y., & Ang Jr, P. (2017). High tolerance to temperature and salinity change should enable scleractinian coral *Platygyra acuta* from marginal environments to persist under future climate change. PloS One, 12(6), e0179423.

Coles, S. L., & Jokiel, P. L. (1978). Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Marine Biology, 49(3), 187-195.

Coles, S. L., & Jokiel, P. L. (1992) Effects of salinity on corals, in: Connell, D. W., & Hawker, D. W. (Eds.), Pollution in tropical aquatic systems. CRC Press, Boca Raton, FL, pp. 147–166.

Crabbe, M. J. C. (2008). Climate change, global warming and coral reefs: Modelling the effects of temperature. Computational Biology and Chemistry, 32(5), 311-314.

Crabbe, M. J. C., Martinez, E., Garcia, C., Chub, J., Castro, L., & Guy, J. (2008). Growth modelling indicates hurricanes and severe storms are linked to low coral recruitment in the Caribbean. Marine Environmental Research, 65(4), 364-368.

D'angelo, C., Hume, B. C., Burt, J., Smith, E. G., Achterberg, E. P., & Wiedenmann, J. (2015). Local adaptation constrains the distribution potential of heat-tolerant *Symbiodinium* from the Persian/Arabian Gulf. The ISME Journal, 9(12), 2551.

Darling, E. S., Alvarez-Filip, L., Oliver, T. A., McClanahan, T. R., & Côté, I. M. (2012). Evaluating life-history strategies of reef corals from species traits. Ecology Letters, 15(12), 1378-1386.

D'Croz, L., & Maté, J. L. (2002). The role of water temperature and UV radiation in the recovery of the experimentally bleached coral Pocillopora damicornis from the eastern Pacific Ocean(Panama). In Proceedings of the 9th International Coral Reef Symposium, Bali, 23-27 October 2000, (Vol. 2, pp. 1111-1116).

Depczynski, M., Gilmour, J. P., Ridgway, T., Barnes, H., Heyward, A. J., Holmes, T. H., ... & Wilson, S. K. (2013). Bleaching, coral mortality and subsequent survivorship on a West Australian fringing reef. Coral Reefs, 32(1), 233-238.

De Zoysa, M., Whang, I., Lee, Y., Lee, S., Lee, J. S., & Lee, J. (2009). Transcriptional analysis of antioxidant and immune defense genes in disk abalone (*Haliotis discus discus*) during thermal, low-salinity and hypoxic stress. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 154(4), 387-395.

Dias, M., Ferreira, A., Gouveia, R., Cereja, R., & Vinagre, C. (2018). Mortality, growth and regeneration following fragmentation of reef-forming corals under thermal stress. Journal of Sea Research, 141, 71-82.

Dias, M., Ferreira, A., Gouveia, R., & Vinagre, C. (2019a). Synergistic effects of warming and lower salinity on the asexual reproduction of reef-forming corals. Ecological Indicators, 98, 334-348.

Dias, M., Ferreira, A., Gouveia, R., Madeira, C., Jogee, N., Cabral, H. N., Diniz, M., & Vinagre, C. (2019b). Long-term exposure to increasing temperatures on scleractinian coral fragments reveals oxidative stress. Marine Environmental Research, 150, 104758.

Downs, C. A., Mueller, E., Phillips, S., Fauth, J. E., & Woodley, C. M. (2000). A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. Marine Biotechnology, 2(6), 533-544.

Downs, C. A., Fauth, J. E., Halas, J. C., Dustan, P., Bemiss, J., & Woodley, C. M. (2002). Oxidative stress and seasonal coral bleaching. Free Radical Biology and Medicine, 33(4), 533-543.

Downs, C. A., Kramarsky-Winter, E., Woodley, C. M., Downs, A., Winters, G., Loya, Y., & Ostrander, G. K. (2009). Cellular pathology and histopathology of hypo-salinity exposure on the coral *Stylophora pistillata*. Science of the Total Environment, 407(17), 4838-4851.

Edge, R., McGarvey, D. J., & Truscott, T. G. (1997). The carotenoids as anti-oxidants—a review. Journal of Photochemistry and Photobiology B: Biology, 41(3), 189-200.

Elsner, J. B., Murnane, R. J., & Jagger, T. H. (2006). Forecasting US hurricanes 6 months in advance. Geophysical Research Letters, 33(10).

Emanuel, K. (2005). Increasing destructiveness of tropical cyclones over the past 30 years. Nature, 436(7051), 686.

Faxneld, S., Jörgensen, T. L., & Tedengren, M. (2010). Effects of elevated water temperature, reduced salinity and nutrient enrichment on the metabolism of the coral *Turbinaria mesenterina*. Estuarine, Coastal and Shelf Science, 88(4), 482-487.

Ferrier-Pagès, C., Gattuso, J. P., & Jaubert, J. (1999). Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellate coral *Stylophora pistillata*. Marine Ecology Progress Series, 181, 309-314.

Fitt, W. K., Brown, B. E., Warner, M. E., & Dunne, R. P. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs, 20(1), 51-65.

Fitt, W. K., Gates, R. D., Hoegh-Guldberg, O., Bythell, J. C., Jatkar, A., Grottoli, A. G., ... & Iglesias-Prieto, R. (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. Journal of Experimental Marine Biology and Ecology, 373(2), 102-110.

Flores-Ramírez, L. A., & Liñán-Cabello, M. A. (2007). Relationships among thermal stress, bleaching and oxidative damage in the hermatypic coral, *Pocillopora capitata*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 146(1-2), 194-202.

Foster, N. L., Baums, I. B., Sanchez, J. A., Paris, C. B., Chollett, I., Agudelo, C. L., (...) & Mumby, P. J. (2013). Hurricane-driven patterns of clonality in an ecosystem engineer: the Caribbean coral *Montastraea annularis*. PLoS One, 8(1), e53283.

Freire, C. A., Welker, A. F., Storey, J. M., Storey, K. B., & Hermes-Lima, M. (2012) Oxidative stress in estuarine and intertidal environments (temperate and tropical), in: Abele, D., Vázquez-Medina, J. P., & Zenteno-Savín, T. (Eds.), Oxidative Stress in Aquatic Ecosystems, First Edition, Blackwell Publishing Ltd, pp. 41-57.

Fridovich, I. (1995). Superoxide radical and superoxide dismutases. Annual Review of Biochemistry, 64(1), 97-112.

Fujioka, Y. (1998). Checklist of the hermatypic corals of Urasoko Bay, Ishigaki Island, southwestern Japan. Bull Nansei Natl Fish Res Inst, 31, 1-11.

Furby, K. A., Bouwmeester, J., & Berumen, M. L. (2013). Susceptibility of central Red Sea corals during a major bleaching event. Coral Reefs, 32(2), 505-513.

Gardner, S. G., Nielsen, D. A., Laczka, O., Shimmon, R., Beltran, V. H., Ralph, P. J., & Petrou, K. (2016). Dimethylsulfoniopropionate, superoxide dismutase and glutathione as stress response indicators in three corals under short-term hyposalinity stress. Proceedings of the Royal Society B: Biological Sciences, 283(1824), 20152418.

Glynn, P. W., Colley, S. B., Carpizo-Ituarte, E., & Richmond, R. H. (2017). Coral reproduction in the Eastern Pacific. In Coral Reefs of the Eastern Tropical Pacific (pp. 435-476). Springer, Dordrecht.

Goldberg, J., & Wilkinson, C. (2004). Global threats to coral reefs: coral bleaching, global climate change, disease, predator plagues and invasive species. Status of coral reefs of the world, 2004, 67-92.

Goreau, T. F. (1964). Mass expulsion of zooxanthellae from Jamaican reef communities after Hurricane Flora. Science, 145(3630), 383-386.

Grottoli, A. G., Tchernov, D., & Winters, G. (2017). Physiological and biogeochemical responses of super-corals to thermal stress from the Northern Gulf of Aqaba, Red Sea. Frontiers in Marine Science, 4, 215.

Guest, J. R., Baird, A. H., Maynard, J. A., Muttaqin, E., Edwards, A. J., Campbell, S. J., ... & Chou, L. M. (2012). Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PloS One, 7(3), e33353.

Haapkylä, J., Unsworth, R. K., Flavell, M., Bourne, D. G., Schaffelke, B., & Willis, B. L. (2011). Seasonal rainfall and runoff promote coral disease on an inshore reef. PloS One, 6(2), e16893.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249(22), 7130-7139.

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology, 141(2), 312-322.

Halliwell, B. & Gutteridge, J. M. C. (1999). Free Radicals in Biology and Medicine. Oxford University Press, New York.

Hayes, J. D., & McLellan, L. I. (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radical Research, 31(4), 273-300.

Hermes-Lima, M., & Storey, K. B. (1993). In vitro oxidative inactivation of glutathione S-transferase from a freeze tolerant reptile. Molecular and Cellular Biochemistry, 124(2), 149-158.

Highsmith, R. C. (1980). Passive colonization and asexual colony multiplication in the massive coral *Porites lutea* Milne Edwards & Haime. Journal of Experimental Marine Biology and Ecology, 47(1), 55-67.

Highsmith, R. C. (1982). Reproduction by fragmentation in corals. Marine Ecology Progress Series. Oldendorf, 7(2), 207-226.

Highsmith, R. C., Riggs, A. C., & D'Antonio, C. M. (1980). Survival of hurricane-generated coral fragments and a disturbance model of reef calcification/growth rates. Oecologia, 46(3), 322-329.

Higuchi, T., Fujimura, H., Arakaki, T., & Oomori, T. (2008, July). Activities of antioxidant enzymes (SOD and CAT) in the coral *Galaxea fascicularis* against increased hydrogen peroxide concentrations in seawater. In Proceedings of the 11th International Coral Reef Symposium (pp. 7-11).

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research, 50(8), 839-866.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. Science, 328(5985), 1523-1528.

Honnay, O., & Bossuyt, B. (2005). Prolonged clonal growth: escape route or route to extinction? Oikos, 108(2), 427-432.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K.D., Baird, A. H., (...) & Bridge, T. C. (2017). Global warming and recurrent mass bleaching of corals. Nature, 543(7645), 373.

Hughes, T. P., Kerry, J. T., & Simpson, T. (2018). Large-scale bleaching of corals on the Great Barrier Reef. Ecology, 99(2), 501-501.

Humphrey, C., Weber, M., Lott, C., Cooper, T., & Fabricius, K. (2008). Effects of suspended sediments, dissolved inorganic nutrients and salinity on fertilisation and embryo development in the coral *Acropora millepora* (Ehrenberg, 1834). Coral Reefs, 27(4), 837-850.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Meyer LA (eds)]. IPCC, Geneva, Switzerland, p 151

Johansson, L. H., & Borg, L. H. (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. Analytical Biochemistry, 174(1), 331-336.

Jokiel, P. L. (1985). Lunar periodicity of planula release in the reef coral *Pocillopora damicornis* in relation to various environmental factors. Proceedings of the 5th International Coral Reef Congress, 4, 307-312.

Jokiel, P. L., & Coles, S. L. (1974). Effects of heated effluent on hermatypic corals at Kahe Point, Oahu. Pacific Science, 28, 1-18.

Jokiel, P. L., Hunter, C. L., Taguchi, S., & Watarai, L. (1993). Ecological impact of a fresh-water "reef kill" in Kaneohe Bay, Oahu, Hawaii. Coral Reefs, 12(3-4), 177-184.

Jones, A. M., Berkelmans, R., Van Oppen, M. J., Mieog, J. C., & Sinclair, W. (2008). A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proceedings of the Royal Society B: Biological Sciences, 275(1641), 1359-1365.

Kerswell, A. P., & Jones, R. J. (2003). Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of 'low-salinity bleaching'. Marine Ecology Progress Series, 253, 145-154.

Khalil, M. T., Cochran, J. E., & Berumen, M. L. (2013). The abundance of herbivorous fish on an inshore Red Sea reef following a mass coral bleaching event. Environmental Biology of Fishes, 96(9), 1065-1072.

Krueger, T., Hawkins, T. D., Becker, S., Pontasch, S., Dove, S., Hoegh-Guldberg, O., (...) & Davy, S. K. (2015). Differential coral bleaching - Contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 190, 15-25.

Kuanui, P., Chavanich, S., Viyakarn, V., Omori, M., & Lin, C. (2015). Effects of temperature and salinity on survival rate of cultured corals and photosynthetic efficiency of zooxanthellae in coral tissues. Ocean Science Journal, 50(2), 263-268.

Le Nohaïc, M., Ross, C. L., Cornwall, C. E., Comeau, S., Lowe, R., McCulloch, M. T., & Schoepf, V. (2017). Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia. Scientific Reports, 7(1), 14999.

Lesser, M. P. (1997). Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs, 16(3), 187-192.

Lesser, M. P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. Annual Review of Physiology, 68, 253-278.

Lesser, M. P., Stochaj, W. R., Tapley, D. W., & Shick, J. M. (1990). Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. Coral Reefs, 8(4), 225-232.

Levitan, D. R., Boudreau, W., Jara, J., & Knowlton, N. (2014). Long-term reduced spawning in *Orbicella* coral species due to temperature stress. Marine Ecology Progress Series, 515, 1-10.

Li, E., Chen, L., Zeng, C., Yu, N., Xiong, Z., Chen, X., & Qin, J. G. (2008). Comparison of digestive and antioxidant enzymes activities, haemolymph oxyhemocyanin contents and hepatopancreas histology of white shrimp, *Litopenaeus vannamei*, at various salinities. Aquaculture, 274(1), 80-86.

Limón-Pacheco, J., & Gonsebatt, M. E. (2009). The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 674(1-2), 137-147.

Lirman, D., & Manzello, D. (2009). Patterns of resistance and resilience of the stress-tolerant coral *Siderastrea radians* (Pallas) to sub-optimal salinity and sediment burial. Journal of Experimental Marine Biology and Ecology, 369(1), 72-77.

Liu, G., Strong, A. E., & Skirving, W. (2003). Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching. Eos, Transactions American Geophysical Union, 84(15), 137-141.

Livingstone, D. R. (1991). Organic xenobiotic metabolism in marine invertebrates. In Advances in comparative and environmental physiology (pp. 45-185). Springer, Berlin, Heidelberg.

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., & Van Woesik, R. (2001). Coral bleaching: the winners and the losers. Ecology Letters, 4(2), 122-131.

Madeira, D., Narciso, L., Cabral, H. N., Vinagre, C., & Diniz, M. S. (2013). Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 166(2), 237-243.

Madeira, C., Madeira, D., Vinagre, C., & Diniz, M. (2015). Octocorals in a changing environment: Seasonal response of stress biomarkers in natural populations of *Veretillum cynomorium*. Journal of Sea Research, 103, 120-128.

Manzello, D. P. (2010). Coral growth with thermal stress and ocean acidification: lessons from the eastern tropical Pacific. Coral Reefs, 29(3), 749-758.

Manzello, D., & Lirman, D. (2003). The photosynthetic resilience of *Porites furcata* to salinity disturbance. Coral Reefs, 22(4), 537-540.

Marshall, P. A., & Baird, A. H. (2000). Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs, 19(2), 155-163.

Mayfield, A. B., & Gates, R. D. (2007). Osmoregulation in anthozoan–dinoflagellate symbiosis. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(1), 1-10.

McClanahan, T. R. (2017). Changes in coral sensitivity to thermal anomalies. Marine Ecology Progress Series, 570, 71-85.

Moberg, F., Nyström, M., Kautsky, N., Tedengren, M., & Jarayabhand, P. (1997). Effects of reduced salinity on the rates of photosynthesis and respiration in the hermatypic corals *Porites lutea* and *Pocillopora damicornis*. Marine Ecology Progress Series, 157, 53-59.

Munoz-Munoz, J. L., García-Molina, F., Varón, R., Tudela, J., García-Cánovas, F., & Rodríguez-López, J. N. (2009). Generation of hydrogen peroxide in the melanin biosynthesis pathway. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1794(7), 1017-1029.

Negri, A. P., Marshall, P. A., & Heyward, A. J. (2007). Differing effects of thermal stress on coral fertilization and early embryogenesis in four Indo Pacific species. Coral Reefs, 26(4), 759-763.

Nii, C. M., & Muscatine, L. (1997). Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Carlgren, 1943): contribution of the animal to superoxide ion production at elevated temperature. The Biological Bulletin, 192(3), 444-456.

Nozawa, Y., & Harrison, P. L. (2007). Effects of elevated temperature on larval settlement and post-settlement survival in scleractinian corals, *Acropora solitaryensis* and *Favites chinensis*. Marine Biology, 152(5), 1181-1185.

Olsen, K., Ritson-Williams, R., Ochrietor, J. D., Paul, V. J., & Ross, C. (2013). Detecting hyperthermal stress in larvae of the hermatypic coral *Porites astreoides*: the suitability of using biomarkers of oxidative stress versus heat-shock protein transcriptional expression. Marine Biology, 160(10), 2609-2618.

Palmer, C. V., Modi, C. K., & Mydlarz, L. D. (2009). Coral fluorescent proteins as antioxidants. PloS One, 4(10), e7298.

Perez, S., & Weis, V. (2006). Nitric oxide and cnidarian bleaching: an eviction notice mediates breakdown of a symbiosis. Journal of Experimental Biology, 209(14), 2804-2810.

Pratchett, M. S., Trapon, M., Berumen, M. L., & Chong-Seng, K. (2011). Recent disturbances augment community shifts in coral assemblages in Moorea, French Polynesia. Coral Reefs, 30(1), 183-193.

Randall, C. J., & Szmant, A. M. (2009). Elevated temperature reduces survivorship and settlement of the larvae of the Caribbean scleractinian coral, *Favia fragum* (Esper). Coral Reefs, 28(2), 537-545.

Regoli, F., Cerrano, C., Chierici, E., Bompadre, S., & Bavestrello, G. (2000). Susceptibility to oxidative stress of the Mediterranean demosponge *Petrosia ficiformis*: role of endosymbionts and solar irradiance. Marine Biology, 137(3), 453-461.

Richier, S., Furla, P., Plantivaux, A., Merle, P. L., & Allemand, D. (2005). Symbiosis-induced adaptation to oxidative stress. Journal of Experimental Biology, 208(2), 277-285.

Richier, S., Sabourault, C., Courtiade, J., Zucchini, N., Allemand, D., & Furla, P. (2006). Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis*. The FEBS Journal, 273(18), 4186-4198.

Rodrigues, A. P., Oliveira, P. C., Guilhermino, L., & Guimaraes, L. (2012). Effects of salinity stress on neurotransmission, energy metabolism, and anti-oxidant biomarkers of *Carcinus maenas* from two estuaries of the NW Iberian Peninsula. Marine Biology, 159(9), 2061-2074.

Röthig, T., Ochsenkühn, M. A., Roik, A., Van Der Merwe, R., & Voolstra, C. R. (2016). Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. Molecular Ecology, 25(6), 1308-1323.

Ruiz-Jones, L. J., & Palumbi, S. R. (2017). Tidal heat pulses on a reef trigger a fine-tuned transcriptional response in corals to maintain homeostasis. Science Advances, 3(3), e1601298.

Scott, A., Harrison, P. L., & Brooks, L. O. (2013). Reduced salinity decreases the fertilization success and larval survival of two scleractinian coral species. Marine Environmental Research, 92, 10-14.

Sies, H. (1999). Glutathione and its role in cellular functions. Free Radical Biology and Medicine, 27(9-10), 916-921.

Silverstein, R. N., Correa, A. M., & Baker, A. C. (2012). Specificity is rarely absolute in coral–algal symbiosis: implications for coral response to climate change. Proceedings of the Royal Society B: Biological Sciences, 279(1738), 2609-2618.

Snoeijs, P., Sylvander, P., & Häubner, N. (2012). Oxidative Stress in Aquatic Primary Producers as a Driving Force for Ecosystem Responses to Large-Scale Environmental Changes, in: Abele, D., Vázquez-Medina, J. P., & Zenteno-Savín, T. (Eds.), Oxidative Stress in Aquatic Ecosystems, First Edition, Blackwell Publishing Ltd, pp. 72-88.

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(4), 409-421.

Sun, Y. I., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. Clinical Chemistry, 34(3), 497-500.

True, J. D. (2012, July). Salinity as a structuring force for near shore coral communities. In Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia (Vol. 9, p. e13).

Tunnicliffe, V. (1981). Breakage and propagation of the stony coral *Acropora cervicornis*. Proceedings of the National Academy of Sciences, 78(4), 2427-2431.

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry, 86(1), 271-278.

Van Woesik, R. (1994). Contemporary disturbances to coral communities of the Great Barrier Reef. Journal of Coastal Research, 233-252.

Van Woesik, R., DeVantier, L. M., & Glazebrook, J. S. (1995). Effects of Cyclone\'Joy\'on nearshore coral communities of the Great Barrier Reef. Marine Ecology Progress Series, 128, 261-270.

Van Woesik, R., Sakai, K., Ganase, A., & Loya, Y. (2011). Revisiting the winners and the losers a decade after coral bleaching. Marine Ecology Progress Series, 434, 67-76.

Venn, A. A., Loram, J. E., & Douglas, A. E. (2008). Photosynthetic symbioses in animals. Journal of Experimental Botany, 59(5), 1069-1080.

Vernberg, W. B., & Vernberg, F. J. (1972). Environmental physiology of marine animals. Springer Science & Business Media.

Veron, J. E. N. (1990). Checklist of the hermatypic corals of Vanuatu. Pacific Science, 44, 51-70.

Veron, J. E. N. (2000). Corals of the World, vols. 1-3. Mary Stafford-Smith (Ed.) Publisher: Australian Institute of Marine Science, Townsville, Australia, p. 489

Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J., & Diniz, M. S. (2014). Effect of increasing temperature in the differential activity of oxidative stress biomarkers in various tissues of the Rock goby, *Gobius paganellus*. Marine Environmental Research, 97, 10-14.

Voolstra, C. R., Sunagawa, S., Matz, M. V., Bayer, T., Aranda, M., Buschiazzo, E., ... & Medina, M. (2011). Rapid evolution of coral proteins responsible for interaction with the environment. PloS One, 6(5), e20392.

Wallace, C. C. (1985). Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora*. Marine Biology, 88(3), 217-233.

Webster, P. J., Holland, G. J., Curry, J. A., & Chang, H. R. (2005). Changes in tropical cyclone number, duration, and intensity in a warming environment. Science, 309(5742), 1844-1846.

Wiedenmann, J., D'Angelo, C., Smith, E. G., Hunt, A. N., Legiret, F. E., Postle, A. D., & Achterberg, E. P. (2013). Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nature Climate Change, 3(2), 160.

Xiubao, L., Hui, H., Jiansheng, L., Liangmin, H., & Junde, D. (2009). Effects of the multiple stressors high temperature and low salinity on the photosynthesis of the hermatypic coral *Galaxea fascicularis*. Acta Ecologica Sinica, 29, 155–159.

Yakovleva, I., Bhagooli, R., Takemura, A., & Hidaka, M. (2004). Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 139(4), 721-730.

5.6. Supplementary material

Table SM5.1. Number of coral species fragments (n) used in each oxidative stress biomarker analysis (LPO – lipid peroxidation; SOD – superoxide dismutase; CAT – catalase; GST- glutathione S-transferase) per experimental treatment. C- control, HT – high temperature, LS – low salinity.

	Treatments															
	C				HT				LS				HT + LS			
Species	LPO	SOD	CAT	GST	LPO	SOD	CAT	GST	LPO	SOD	CAT	GST	LPO	SOD	CAT	GST
Acropora tenuis	5	5	6	5	5	5	5	5	0	0	0	0	0	0	0	0
Montipora capricornis (BM)	5	5	5	5	6	6	6	5	0	0	0	0	0	0	0	0
Montipora capricornis (GM)	5	6	5	5	5	6	6	6	0	0	0	0	0	0	0	0
Turbinaria reniformis	6	5	6	5	6	5	5	5	0	0	0	0	0	0	0	0
Echinopora lamellosa	5	6	6	6	5	6	6	6	0	0	0	0	0	0	0	0
Galaxea fascicularis	6	5	5	5	5	6	5	5	5	6	6	6	4	4	4	4
Pocillopora damicornis	5	6	5	6	0	0	0	0	0	0	0	0	0	0	0	0
Stylophora pistillata	6	6	6	6	0	0	0	0	0	0	0	0	0	0	0	0
Psammocora contigua	5	6	6	5	5	6	6	5	5	6	5	6	0	0	0	0

CHAPTER 6

Integrative indices for health assessment in reef corals under thermal stress

Dias, M., Madeira, C., Jogee, N., Ferreira, A., Gouveia, R., Cabral, H.N., Diniz, M. & Vinagre, C. (submitted).

Abstract

Global warming is one of the major causes of reef coral ecosystems degradation. Predictions of further rise in sea surface temperatures call for urgent action. In this study, a holistic method for bio-monitoring heat stress in reef ecosystems was tested and optimized. Long-term oxidative stress induced by elevated temperatures (30 °C and 32 ºC) was studied on fragments of reef-building corals, and compared to control conditions (26 °C). The quantification of oxidative stress biomarkers superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) activities and lipid peroxidation (LPO) levels in a long-term experiment (60 days), using seven Indo-Pacific reef-building coral species, provided useful information that was interpreted in combination with the observed partial mortality and growth rate of these organisms. These biomarkers were combined in integrated biomarker response (IBR) indices, either in a biomarker of oxidative stress response category (approach A: GST, CAT, LPO, and SOD) or in an integrated response category - organism performance (approach B: GST, CAT, LPO, SOD, partial mortality, and growth rate). The results of this study indicate that the IBRs were responsive to temperature treatment and dependent on the coral species. The approach B was the most adequate since it better reflected the stress suffered by the tested species, whereas the set of four molecular biomarkers (approach A) was not enough to explain the organismal response of most of the tested species to thermal stress conditions.

Keywords: global climate change, ocean warming, integrated biomarker response, oxidative stress biomarkers, coral performance, environment health assessment.

6.1. Introduction and literature review

Global climate change induced by anthropogenic activities has profound implications upon marine ecosystems (Lehtonen et al., 2014), as it exacerbates their degradation through increase in thermal stress due to ocean warming (Hughes et al., 2003; Hoegh-Guldberg et al., 2007). Thermal stress affects all physiological processes, ranging from protein damage, to membrane fluidity, to organ function and organism performance (Hochachka and Somero, 2002). Ultimately, it can lead to mass mortality events, an issue that has been particularly serious in many coral reefs at a worldwide level, raising public and scientific concern (Fabricius et al., 2007; Hoegh-Guldberg et al., 2007). As reef-building corals are habitat-forming species that strongly influence the macro- and micro- structure of the habitat and originate locally stable conditions for other organisms (Dayton, 1972), ecosystem functions may be severely affected by their degradation.

Most reef-building corals may not have the ability to fast adapt to the accelerating pace of climate change (Skelly et al., 2007). As a result, dramatic changes are expected in coral reef communities. In recent years, episodes of mass coral bleaching in the Indo-Pacific region have led to catastrophic loss of coral cover in some locations, and changes in coral reef communities' structure in many others (Furby et al., 2013; Khalil et al., 2013; Hughes et al., 2017, 2018; Le Nohaïc et al., 2017). These episodes result in the replacement of dominant and branching heat-susceptible coral species by sub-dominant and massive heat-tolerant coral species in some locations (Van Woesik et al., 2011), and in shifts in dominance from scleractinian corals to macroalgae or other sessile invertebrate-dominated benthic functional groups in many others (Nyström et al., 2000; Norström et al., 2009; Hughes et al., 2010; Tebbett et al., 2019). Therefore, mass bleaching events have resulted in an overall decline of biodiversity and loss of functional complexity (Baker et al., 2008; Graham et al., 2008; Pratchett et al., 2008; Ateweberhan et al., 2011).

Average sea surface temperatures (SSTs) near coral reef ecosystems are predicted to further rise 1–3.7 °C over the 21st century (IPCC, 2014, 2018). Therefore, it has been suggested that, unless there is considerable thermal adaptation of both coral hosts and their symbiotic algae

(Donner et al., 2005; Baird and Maynard, 2008; Donner, 2009), mass bleaching and mortality events could occur annually on the world's coral reefs by 2050 (Nicholls et al., 2007; Van Hooidonk and Huber, 2009). In consequence of global warming, extreme events (e.g. tropical storms) are predicted to increase in severity and frequency, leading to a shorter time for recovery between recurrences (Emanuel, 2005; Elsner et al., 2006; IPCC, 2014) with the prospect that the extent to which coral reefs experience physical damage may increase (Hoegh-Guldberg, 2011).

Coral reef community maintenance, repopulation, and recovery after disturbance are highly dependent on both sexual and asexual reproductive processes (Connell and Keough, 1985; Glynn et al., 2017). The ratio of sexual and asexual recruitment is expected to change over the geographic range of a species, depending on the frequency of sexual recruitment, genet longevity (Coffroth and Lasker, 1998), and disturbance effects (e.g. ocean warming and tropical storms, Baums et al., 2006). Studies have shown that heat stress can negatively affect the fertilization success and embryonic development (Negri et al., 2007), spawning (Levitan et al., 2014), survivorship and settlement of coral larvae (Randall and Szmant, 2009; Heyward and Negri, 2010), as well as post-settlement survival (Nozawa and Harrison, 2002, 2007). Therefore, the recurrent increases in SSTs will likely lead to the failure of sexual reproduction, being asexual propagation the most probable mean of recovery under current changes in climatic conditions.

Tropical storms are favorable to the propagation and expansion of scleractinian corals of various morphologies by asexual reproduction of storm-generated fragments through the reefs (Highsmith, 1980, 1982; Highsmith et al., 1980; Tunnicliffe, 1981; Lirman, 2000; Foster et al., 2007, 2013), which later re-attach to the substrate, growth, and form a new colony (Foster et al., 2007). Fragmentation is considered to be an adaptation to both favorable and unfavorable environmental conditions (Lirman, 2000; Honnay and Bossuyt, 2005). This asexual mode of reproduction allows coral species to persist disturbance when they cannot complete their sexual reproductive life cycle. Given the future projections of increase in tropical storms' intensity and frequency, asexual reproduction may increase through fragmentation.

In a changing climate, ecosystems where foundation species are susceptible to the effects of elevated temperatures are thus vulnerable to major reorganization, being characterized by reduced habitat complexity and disrupted ecosystem services (Ellison et al., 2005; Hoegh-Guldberg and Bruno, 2010). Nevertheless, not all reef-building corals have the same susceptibility to global warming. Recent approaches of assessing global warming impacts focus on quantifying exposure to climatic change, mostly disregarding the biological differences between species that significantly affect their vulnerability (Harley et al., 2006). The diversity of responses to environmental change among species contributing to the same ecosystem function (i.e. response diversity) is critical to resilience (Elmqvist et al., 2003). Response diversity is particularly important for ecosystem renewal and reorganization following disturbance, providing adaptive capacity in a world subjected to extreme climatic events.

The urgent need to assess the quality of marine ecosystems led to the development of several monitoring tools (Devin et al., 2014). Changes in community structure and measures of chemical contamination are frequently used to indicate ecosystem health status (Dustan and Halas, 1987; Hughes, 1994; Viarengo et al., 2000; Chase et al., 2001). Nevertheless, these represent damage manifestations rather than prognostic indices (Knap et al., 2002). Changes at simplest levels of biological complexity (e.g. molecular, cellular), which trigger effects at complex biological levels (e.g. organisms) and for which causality can be recognized, may provide prior warning of ecosystem health deterioration (Lam, 2009; Marigómez et al., 2013). Biomarkers are an example of responses at simplest levels and have provided valuable mechanistic information to scientists. Nonetheless, the multi-biomarker approaches are generally hard to interpret, and produce results that are not easy to integrate in the environmental policies framework (Beliaeff and Burgeot, 2002). To fill this gap, integrative indices have been developed, and one of the most used is the Integrated Biomarker Response (IBR) (Beliaeff and Burgeot, 2002). The IBR is a method that provides both a graphical synthesis of the different biomarker responses and a numeric value that integrates all these responses at once. The IBR is the sum of the area defined by the k biomarkers arranged in a radar diagram, following a prior step of biomarker responses standardization (Devin et al., 2014).

The integration of biomarkers in health indices may provide comprehensive information about the biological effects of environmental variables in marine organisms (Marigómez et al., 2013) and may thus serve as valuable tools for environmental managers (Broeg and Lehtonen, 2006; Madeira et al., 2018). This tool can be combined with morphological assessments to characterize the sub-lethal metabolic effects of general stressors in marine organisms. Madeira et al. (2018) defined and tested a holistic method for bio-monitoring based on a set of biomarkers in order to evaluate the effects of ocean warming in selected tropical fish, crustaceans, and gastropods. Nevertheless, no similar approach has been applied and tested in reef-building coral species. The definition of an adequate set of biomarkers may be crucial for more precise predictions of environmental health in tropical marine environments, which are urgently needed given the expected increases in SSTs and associated extreme events.

Dias et al. (2018, 2019a) investigated the long-term partial mortality, growth rate, and oxidative stress on small fragments of nine Indo-Pacific reef-forming coral species exposed for 60 days to increasing temperatures (30 °C and 32 °C) and compared results with control temperature (26 °C), mimicking a post-storm scenario in a warming of tropical oceans context. These studies identified different susceptibility among coral species to conditions that are predicted to be more frequent in the future, influencing coral asexual reproduction via fragmentation. In Dias et al. (2018), partial mortality was assessed every 20 days of experiment, whereas growth rate was assessed on the last day of experiment. Furthermore, in Dias et al. (2019a), lipid peroxidation (LPO), catalase activity (CAT), and glutathione S-transferase (GST) were also assessed on the last day of experiment. The present study used the same experimental procedure and data obtained in Dias et al. (2018, 2019a), with the exception of SOD activity data that were not included in Dias et al. (2019a) and are presented here for the first time.

The present study aims to test and optimize two different integrated biomarker approaches to be applied in the monitoring of the effects of heat stress generated by global climate change in the Indo-Pacific region. The two integrated biomarker approaches were divided in Approach A - biomarkers of oxidative stress response category: based on a set of four molecular biomarkers (GST, CAT, LPO, and SOD) and Approach B - integrated stress response category (organism performance): based on the combination of four molecular biomarkers, one biomarker at organismic level, and one biomarker at physiological level (Lam, 2009) (GST, CAT, LPO,

SOD, partial mortality, and growth rate, respectively). First, the two approaches were applied to small fragments of seven reef-building coral species of the Indo-Pacific region exposed to both control and two stress temperatures. Second, IBR index values were obtained for each species and temperature treatment combination within the two different approaches. Finally, the advantages and disadvantages of the two approaches were compared and discussed.

6.2. Materials and methods

6.2.1. Study species

This study uses the same coral species used in Dias et al. (2018, 2019a). The coral species used are *Acropora tenuis*, *Montipora capricornis* brown morphotype (BM), *Montipora capricornis* green morphotype (GM), *Echinopora lamellosa, Turbinaria reniformis, Galaxea fascicularis,* and *Psammocora contigua* that present four different colony morphologies. The species *Pocillopora damicornis* and *Stylophora pistillata* were not included in this study since they died on both heat stress treatments (30 ºC and 32 ºC), preventing any comparison with control temperature (26 ºC).

6.2.2. Experimental procedure

This study uses the same experimental procedure used in Dias et al. (2018, 2019a). In brief, ten small replicate fragments were cut from each coral species' colony. The live wet mass of each coral fragment was obtained by blotting it with a paper towel to remove excess seawater, then weighing it in air on an electronic balance to the nearest 0.01 g (Titlyanov et al., 2005). Each fragment was glued with epoxy putty to the top of a pre-weighed and numbered nylon expansion anchor, with the placement of the fragments according with their natural position in the habitat. Then, the set was weighed to remove the epoxy putty weight off the calculations and placed back over egg crate panels in the coral stock aquarium for one day before acclimation to the experimental aquarium.

To determine the long-term response of the seven coral species to the effect of increased temperatures, the coral fragments were exposed to different treatments: (a) control temperature (26 °C) and (b) increased temperatures (30 ºC and 32 ºC) for 60 days. Coral fragments were acclimated at the rate of 1 °C.h^{-1}.

6.2.3. Analytical procedures

6.2.3.1. Partial mortality assessment

Partial mortality data were obtained from Dias et al. (2018), although in this study only the data relative to the 60th day were used.

6.2.3.2. Growth rate measurements

Growth data were obtained from Dias et al. (2018), although growth rate was calculated using a different formula in the present study. The growth rate formula herein is more common for describing relative growth rates of multicellular organisms. The relative growth rate (RGR), expressed as daily biomass increase, was calculated using the formula 6.1:

$$RGR = [\ln (Wf/Wi)] \,/\, [(tf - ti)] \times 100$$

where Wi is the initial weight, Wf is the final weight and tf and ti is the time interval between the starting and end date (Marinho-Soriano et al., 2006).

6.2.3.3. Samples collection and storage

Samples collection and storage were the same of Dias et al. (2019a), however, the number of fragments of each coral species used in the four molecular biomarker analysis per treatment is

different from the one used in Dias et al. (2019a) and is given in Table SM6.1 of the supplementary material.

6.2.4. Molecular biomarker assays

All biomarker values were obtained from Dias et al. (2019a), except for SOD activity that was not calculated in the study. In Dias et al. (2019a), lipid peroxides assay (lipid peroxidation, LPO) was adapted from Uchiyama and Mihara (1978), catalase (CAT) activity was assessed according to Johansson and Borg (1988), and glutathione S-transferase (GST) activity was assessed according to Habig et al. (1974). All the molecular biomarker assays' protocols were adapted for a 96-well microplate and the data were standardized by total protein, previously determined through the Bradford method (Bradford, 1976). Superoxide dismutase (SOD) activity (EC1.15.1.1) was assessed by using nitroblue tetrazolium (NBT) and xanthine oxidase (XOD) according to Sun et al. (1988). After reading the absorbance at 560 nm, SOD activity was calculated using the following equation for the % inhibition, formula 6.2:

$$((\mathrm{Abs}_{560}/\min \text{ negative control} - \mathrm{Abs}_{560}/\min \text{ sample})/(\mathrm{Abs}_{560}/\min \text{ negative control})) \times 100$$

6.2.5. Data analysis

Only the individual coral fragments used in molecular biomarkers analysis per coral species and temperature treatment were used in data analysis (Table SM6.1 of the supplementary material). In order to integrate all results from different biomarkers (molecular, physiological, and organism levels), the integrated biomarker response (IBR) was calculated. The IBR, a simple multivariate graphic method – star plot – was calculated according to Beliaeff and Burgeot (2002) to allow visual integration of a set of early warning responses measured with biomarkers. To calculate the IBR for biomarkers, the general mean (m) and the standard deviation (s) of all data regarding a given biomarker were calculated, followed by a standardization for each situation to obtain Y, where $Y = (X - m)/s$, and X is the mean value for

the biomarker at a given species and temperature treatment interaction. Then *Z* was calculated using *Z* = -*Y* or *Z* = *Y*, in the case of a biological effect corresponding to inhibition or activation, respectively. Concerning the biological effect considered for each parameter, growth rate was assumed to decrease upon temperature increase. In a similar way, the biomarkers of oxidative stress (GST, CAT, LPO, SOD) and partial mortality were always assumed to increase with the exposure to high temperatures. The score (*S*) was calculated by $S = Z+|Min|$, where $S \geq 0$ and $|Min|$ is the absolute value for the minimum value for all calculated *Y* in a given biomarker. Note that in the cases where –Y is applied the minimum also changes, as all the distribution does. Star plots were then used to display Score results (*S*) and to calculate the IBR as follows, formula 6.3:

$IBR = \sum_{t=1}^{n} A_i$, being

$$A_i = \frac{Si}{2} \sin\beta \, (S_i \cos\beta + S_{i+1} \sin\beta)$$

and

$$\beta = \tan^{-1} \left(\frac{S_{i+1} \sin(\alpha)}{Si\text{-}Si{+}1 \cos(\alpha)}\right),$$

and where S_i and S_{i+1} are two consecutive clockwise scores (radius coordinates) of a given star plot; *Ai* corresponds to the area connecting two scores; *n* the number of biomarkers used for calculations; and $\alpha = 2\pi/n$. The IBR is obtained by summing up all the *Ai*. The IBR calculations were always performed with the same order of parameters for all species and temperature interactions: the molecular biomarkers GST, CAT, LPO and SOD, followed by partial mortality and growth rate.

Biomarkers were divided in two stress response categories:

i) Approach A - Biomarkers of oxidative stress response category: based on a set of four molecular biomarkers (GST, CAT, LPO, and SOD) and ii) Approach B - integrated stress

response category (organism performance): based on the combination of four molecular biomarkers, one biomarker at organism level, and one biomarker at physiological level (GST, CAT, LPO, SOD, partial mortality, and growth rate, respectively) - for the index calculations. To evaluate patterns in the biomarker variations, the variables were standardized (to each sample value the mean was subtracted and then divided by the standard deviation of all samples) and analyzed using a Principal Component Analysis (PCA) (Madeira et al., 2017) supported with a Group Linkage Cluster Analysis using Primer 6 from Primer-E Ltd.

The Sensibility (S) of coral species to heat stress, expressed as %, was calculated as $S = ((IBR_2 – IBR_1)/IBR_1) \times 100$ (formula 6.4); where IBR_2 is the value of IBR at the testing temperatures conditions (i.e. 30 ºC or 32 ºC) and IBR_1 is the value of IBR in the control treatment (26 °C).

Additionally, we also analyzed biomarker scores as a fitness index (E), calculated as $E = (S_1-S_2)$ (Ferreira et al., 2015), where E denotes effect and S1 and S2 stand for two different treatments. E was calculated for all possible combinations where S1 always corresponded to a lower temperature treatment than S2. Values that differed in 0.5 points from the control score were considered to be from an animal with a higher or lower fitness (higher or lower scores, respectively) (Ferreira et al., 2015).

6.3. Results

6.3.1. IBR approach A - biomarkers of oxidative stress response category

The first two principal components of all PCAs had a cumulative explained variance of at least 79% (Table 6.1). According to the PCA and cluster analysis results, all coral species presented a clear separation between the temperature groups (Fig. 6.1b-g), with the exception of *A. tenuis* (Fig. 6.1a). In the case of *M. capricornis* (BM) fragments, the groups 26 ºC and 30 ºC are well individualized along the PC1 with a strong relation with the variable CAT, being its highest values related with the 30 ºC group (Fig. 6.1b, Table 6.1). In relation to *M. capricornis* (GM),

the groups 26 ºC and 30 ºC are well individualized along the PC1 with a strong relation with the variables GST and SOD, being their highest values related with the 30 ºC group (Fig. 6.1c, Table 6.1). In *E. lamellosa* the groups 26 ºC and 30 ºC are well individualized along the PC1 with a strong relation with the variables CAT, LPO, and SOD, being their highest values related with the 30 ºC group (Fig. 6.1d, Table 6.1). In *T. reniformis* fragments, the groups 26 ºC and 30 ºC are well individualized along the PC1 with a strong relation with the variables GST, CAT, and LPO, being their highest values related with the 26 ºC group (Fig. 6.1e, Table 6.1), on the other hand, the group 32 ºC is well individualized from both 26 ºC and 30 ºC groups along the PC2 with a strong relation with the variable SOD, being its highest values related with the 32 ºC group (Fig. 6.1e, Table 6.1). In the case of *Galaxea fascicularis* fragments, the group 26 ºC is well individualized from both 30 ºC and 32 ºC groups along PC2 with a strong relation with the variable GST, being its highest values related with both 30 ºC and 32 ºC groups (Fig. 6.1f, Table 6.1), on the other hand, the groups 30 ºC and 32 ºC are well individualized along the PC1 with a strong relation with the variables LPO and SOD, being their highest values related with the 32 ºC group (Fig. 6.1f, Table 6.1); the groups 26 ºC and 32 ºC are also well individualized along the PC1 with a strong relation with the variables LPO and SOD, being their highest values related with the 32 ºC group (Fig. 6.1f, Table 6.1). In *P. contigua* the group 26 ºC is well individualized from both 30 ºC and 32 ºC groups along the PC1 with a strong relation with the variables GST and CAT, being their highest values related with the 26 ºC group (Fig. 6.1g, Table 6.1), on the other hand, the groups 30 ºC and 32 ºC are well individualized along the PC2 with a strong relation with the variable SOD, being its highest values related with the 32 ºC group (Fig. 6.1g, Table 6.1); the groups 26 ºC and 32 ºC also are well individualized along PC2 with a strong relation with the variable SOD, being its highest values related with the 32 ºC group (Fig. 6.1g, Table 6.1).

Table 6.1. Principal components analysis results of the four biomarkers of oxidative stress (GST – glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation, SOD – superoxide dismutase) in the seven Indo-Pacific coral species. PC1 and PC2 stands for axes 1 and 2 of the PCA, respectively, and the values indicated for the variables are the factor loadings for PC1 and PC2.

	A. tenuis		*M.capricornis* (BM)		*M. capricornis* (GM)		*E. lamellosa*		*T. reniformis*		*G. fascicularis*		*P. contigua*	
Parameter	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Eigenvalues	2.33	0.98	3.48	0.39	2.62	1.07	3.21	0.51	2.60	0.74	1.92	1.25	2.30	0.98
Explained variance (%)	58.4	24.4	87.0	9.8	65.4	26.6	80.1	12.8	65.1	18.6	47.9	31.3	57.4	24.5
Variables														
GST	-0.622	-0.108	-0.498	0.441	0.588	0.127	-0.455	-0.752	-0.527	0.215	0.228	-0.727	0.583	0.155
CAT	0.473	-0.141	-0.524	-0.272	0.496	-0.453	-0.523	0.418	-0.526	-0.001	0.461	-0.449	0.573	-0.330
LPO	-0.264	0.893	-0.495	0.512	0.226	0.879	-0.506	0.462	-0.537	0.437	-0.547	-0.496	0.493	-0.328
SOD	-0.566	-0.414	-0.483	-0.685	0.597	-0.081	-0.513	-0.214	-0.398	-0.873	-0.661	-0.153	0.298	0.872

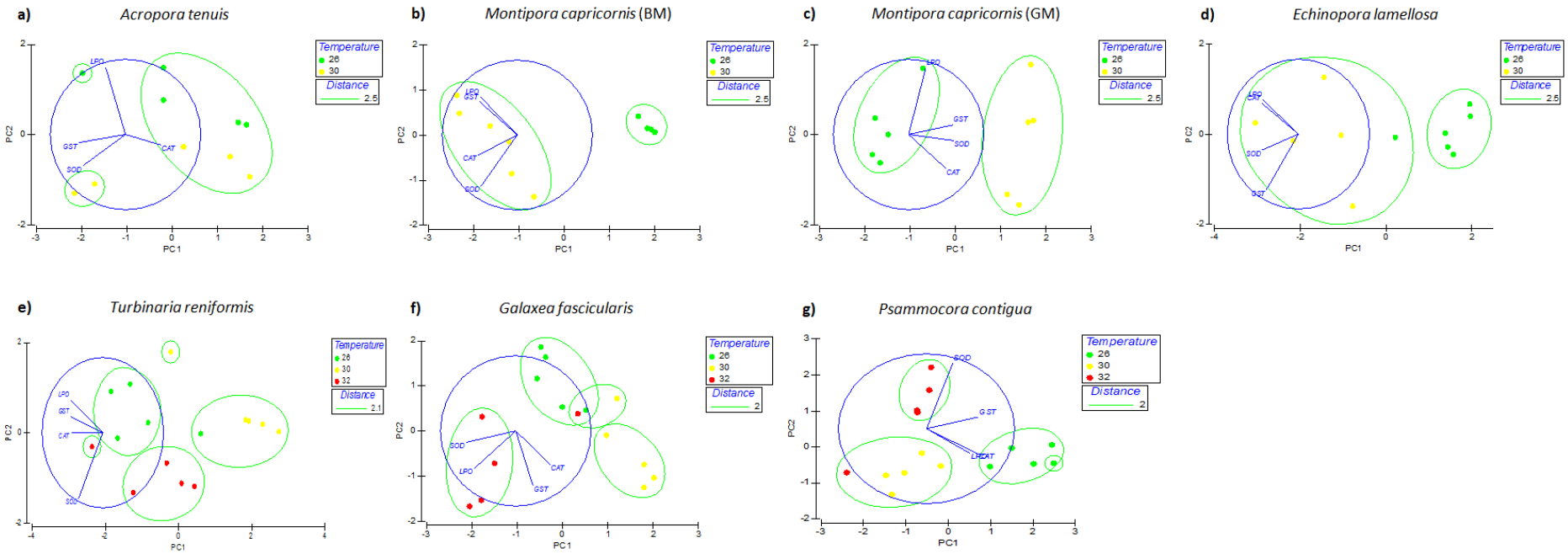

Figure 6.1. Ordination plot of the first two axes of the principal components analysis carried out to assess the effect of the three temperature treatments in the biomarkers of oxidative stress response in seven coral species of the Indo-Pacific region. Variable's factor loadings are represented in blue and relative to GST - glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation; SOD – superoxide dismutase.

In general, IBR values increased with increase in temperature (Fig. 6.2a-d and f). Exceptions to this pattern were *Turbinaria reniformis*, that presented a decrease of IBR values from 26 °C to 30 °C, but an increase in IBR values from 30 °C to 32 °C (Fig. 6.2e), and *Psammocora contigua* that presented a decrease of IBR values with increase in temperature (Fig. 6.2g). Regarding the sensibility of the coral species to heat stress, the species *M. capricornis* (BM) was the most sensitive species at 30 °C and *G. fascicularis* was the most sensitive species at 32 °C (Table 6.2).

Table 6.2. Sensibility of the seven Indo-Pacific coral species to heat stress calculated as rate of IBR variation and expressed as %. Negative percentages correspond to IBR values decrease with increase in temperature. NA – not available due to complete mortality at 32 °C.

	T(ºC)/species	*A. tenuis*	*M. capricornis* (BM)	*M. capricornis* (GM)	*E. lamellosa*	*T. reniformis*	*G. fascicularis*	*P. contigua*
Approach A	30-26	125	142798	335	370	-92	55	-72
	32-26	NA	NA	NA	NA	7	249	-67
Approach B	30-26	1209	3377	1053	233	-24	138	-52
	32-26	NA	NA	NA	NA	94	147	-51

Regarding the magnitude of differences between temperature treatments, the most responsive biomarkers to elevated temperature were GST, CAT, SOD (especially in *M. capricornis* (BM) and *E. lamellosa*, Fig. 6.2b and d) and LPO (*E. lamellosa* and *G. fascicularis*, Fig. 6.2d and f). This IBR approach proved inadequate as an environmental health index for most of the coral species. The inadequacy of this approach is given to the absence of relation between the magnitude of increase in IBR values with heat stress and both the biomarker at organism level (partial mortality) and the biomarker at physiological level (growth rate). This index based on four biomarkers of oxidative stress only proved adequate in *M. capricornis* (BM) and *M. capricornis* (GM) fragments (Fig. 6.2b and c).

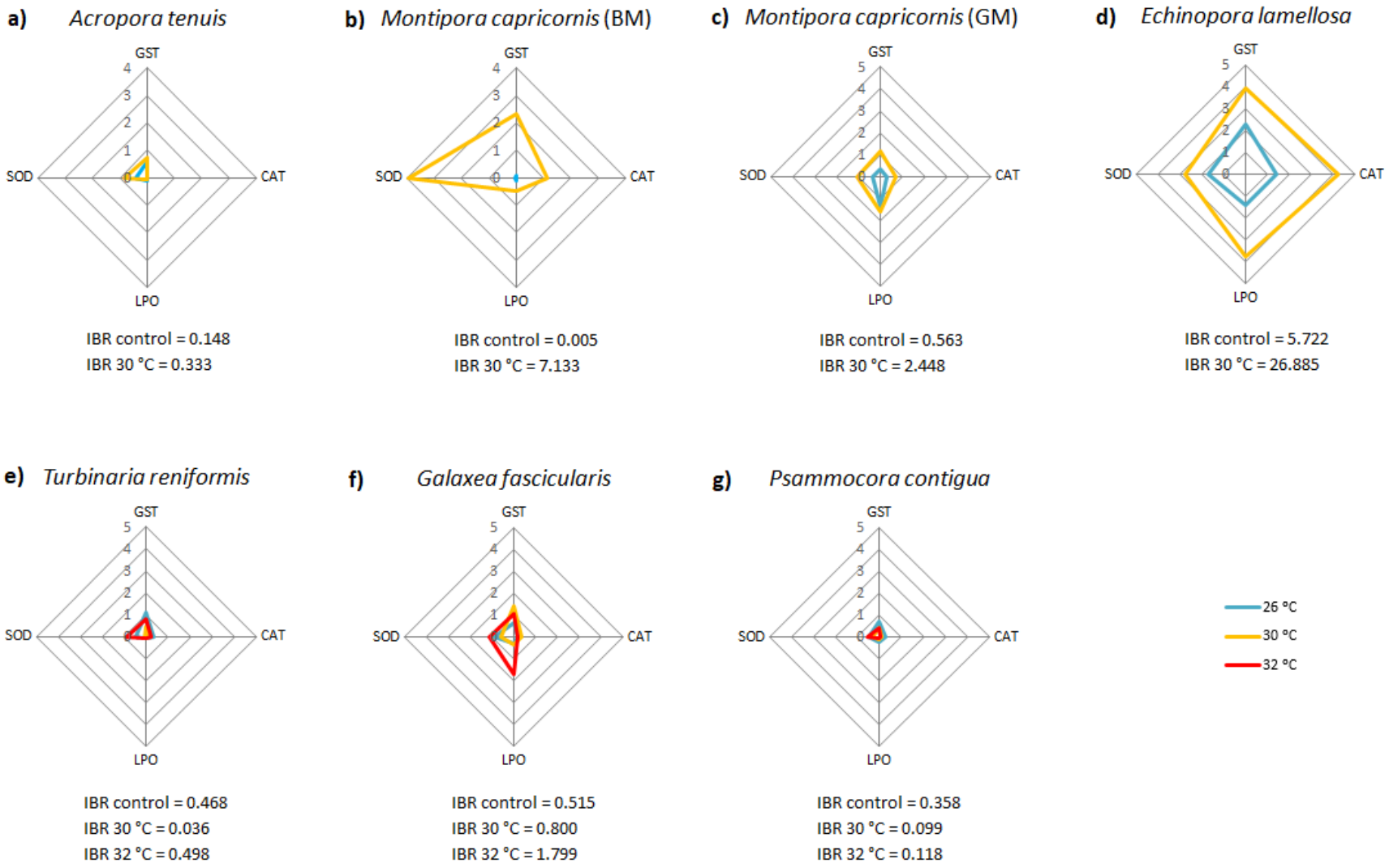

Figure 6.2. Star plots with mean scores for the seven coral species exposed to 26 °C (control) and both 30 ºC and 32 °C (stress temperatures). GST – glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation; SOD – superoxide dismutase.

6.3.2. IBR approach B - integrative stress response category (organism performance)

The first two principal components of all PCAs had a cumulative explained variance of at least 64% (Table 6.3). According to the PCA and cluster analysis results, all coral species presented a clear separation between the temperature groups (Fig. 6.3a-g). In the case of *A. tenuis*, the groups 26 ºC and 30 ºC are well individualized along the PC2 with a strong relation with the variable LPO, being its highest values related with the 26 ºC group (Fig. 6.3a, Table 6.3). In *M. capricornis* (BM) fragments, the groups 26 ºC and 30 ºC are well individualized along the PC1 and the variables most related with this differentiation were GST, CAT, and LPO, being their highest values related with the 30 ºC group. (Fig. 6.3b, Table 6.3). In *M. capricornis* (GM), the groups 26 ºC and 30 ºC are well individualized along the PC1 and the variables most related with this differentiation were GST, SOD, and growth rate, being their highest values related with the 30 ºC group (Fig. 6.3c, Table 6.3). Relative to *E. lamellosa,* the groups 26 ºC and 30 ºC are well individualized along the PC1 and the variables most related with this differentiation were CAT, LPO, and SOD, being their highest values related with the 30 ºC group (Fig. 6.3d, Table 6.3). In *T. reniformis,* the groups 26 ºC and 30 ºC are well individualized along the PC1 with a strong relation with the variable GST, being its highest values related with the 26 ºC group (Fig. 6.3e, Table 6.3), on the other hand, the group 32 ºC is well individualized from both 26 ºC and 30 ºC groups along the PC2 with a strong relation with the variables SOD and partial mortality, being their highest values related with the 32 ºC group (Fig. 6.3e, Table 6.3). In *G. fascicularis,* the group 32 ºC is well individualized from both 26 ºC and 30 ºC groups, but with a more restricted cut the groups 26 ºC and 30 ºC were also individualized (Fig. 6.3f, Table 6.3). The group 26 ºC is well individualized from both 30 ºC and 32 ºC groups along the PC2 with a strong relation with the variables GST and LPO, being their highest values related with both 30 ºC and 32 ºC groups (Fig. 6.3f, Table 6.3), on the other hand, the groups 30 ºC and 32 ºC are well individualized along the PC1 with a strong relation with the variables LPO and SOD, being their highest values related with the 32 ºC group (Fig. 6.3f, Table 6.3).

Table 6.3. Principal components analysis results of the six biomarkers relative to organism performance (GST – glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation, SOD – superoxide dismutase, PM – partial mortality, GR – growth rate) in the seven Indo-Pacific coral species. PC1 and PC2 stands for axes 1 and 2 of the PCA, respectively, and the values indicated for the variables are the factor loadings for PC1 and PC2. NA – parameter with value = 0 in the three temperature treatments.

	A. tenuis		*M.capricornis* (BM)		*M. capricornis* (GM)		*E. lamellosa*		*T. reniformis*		*G. fascicularis*		*P. contigua*	
Parameter	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Eigenvalues	2.44	2.09	4.11	1.12	4.07	1.11	3.95	0.77	2.90	1.28	1.95	1.25	3.46	1.06
Explained variance (%)	40.7	34.8	68.5	18.6	67.9	18.6	65.9	12.8	48.4	21.3	39.1	25.0	57.7	17.7
Variables														
GST	-0.555	0.277	0.468	0.128	-0.468	-0.195	0.389	0.273	0.510	-0.030	-0.213	0.720	-0.449	-0.298
CAT	0.414	-0.230	0.475	-0.092	-0.390	0.337	0.452	-0.016	0.492	-0.126	-0.472	0.451	-0.508	0.191
LPO	0.044	0.642	0.456	-0.028	-0.134	-0.891	0.454	-0.086	0.499	-0.058	0.537	0.501	-0.378	0.136
SOD	-0.554	0.150	0.424	-0.231	-0.480	-0.017	0.469	0.126	0.293	-0.652	0.635	0.163	-0.160	-0.898
PM	-0.316	-0.466	0.036	-0.908	-0.383	0.234	-0.298	0.868	-0.267	-0.576	NA	NA	-0.366	0.076
GR	0.334	0.468	-0.407	-0.312	0.483	0.005	-0.361	-0.387	0.304	0.471	0.199	-0.035	-0.487	0.208

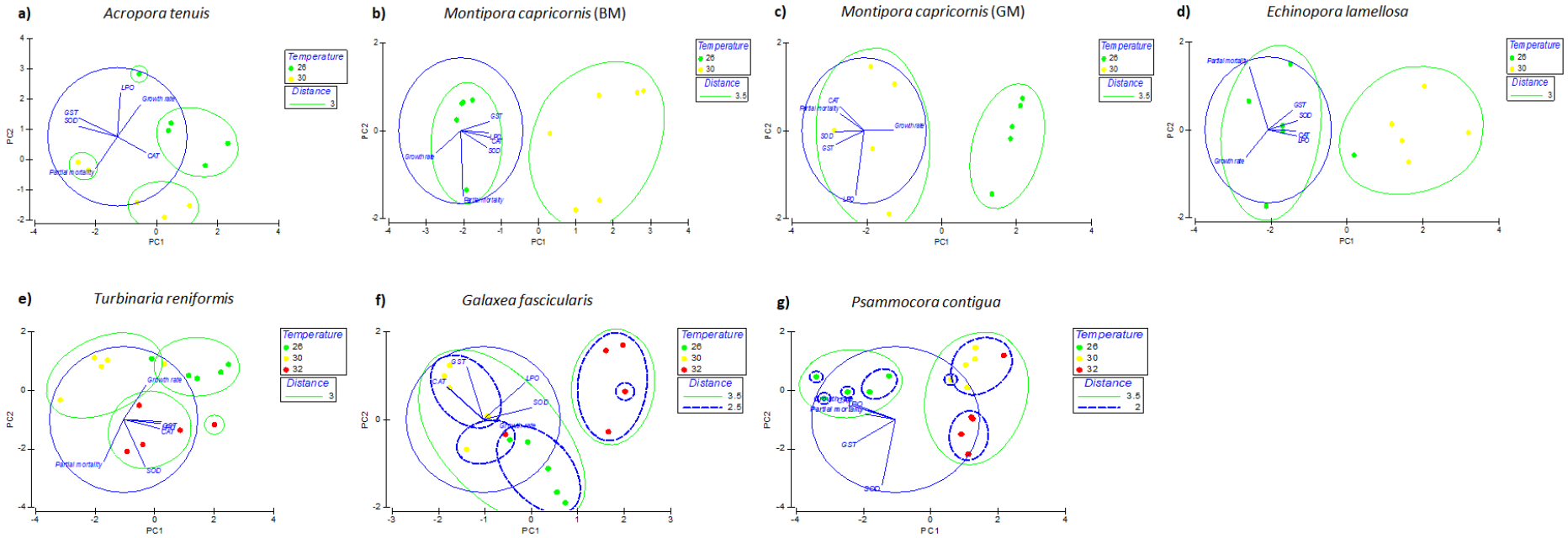

Figure 6.3. Ordination plot of the first two axes of the principal components analysis carried out to assess the effect of the three temperature treatments in the biomarkers of oxidative stress, partial mortality and growth rate response in seven coral species of the Indo-Pacific region. Variable's factor loadings are represented in blue and relative to GST - glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation; SOD – superoxide dismutase; partial mortality; and growth rate.

In *P. contigua*, the group 26 ºC is well individualized from both 30 ºC and 32 ºC groups, but with a more restricted cut it was also possible to individualize these last two groups. The group 26 ºC is well individualized from both 30 ºC and 32 ºC groups along the PC1 with a strong relation with the variable CAT, being their highest values related with the 26 ºC group (Fig. 6.3g, Table 6.3), on the other hand, the groups 30 ºC and 32 ºC are well individualized along the PC2 with a strong relation with the variable SOD, being its highest values related with the 32 ºC group (Fig. 6.3g, Table 6.3).

Star plots and index values show that increased temperature did not affect equally all tested species (Fig. 6.4). *Montipora capricornis* (BM) was the most sensitive species at 30 °C and *G. fascicularis* was the most sensitive species at 32 °C (Table 6.2).

The most responsive biomarkers according with the coral species were partial mortality and growth rate in *A. tenuis*; GST and SOD in *M. capricornis* (BM); partial mortality and growth rate in *M. capricornis* (GM); CAT and LPO in *E. lamellosa*; growth rate in *T. reniformis*; LPO in *G. fascicularis*; growth rate in *P. contigua*. The markers least influenced by increased temperature were CAT (*A. tenuis, M. capricornis* (GM), and *T. reniformis*), partial mortality (*M. capricornis* (BM), *E. lamellosa*, and *G. fascicularis*), LPO (*M. capricornis* (BM), *M. capricornis* (GM), *T. reniformis,* and *P. contigua*), growth rate (*E. lamellosa*), and SOD (*P. contigua*).

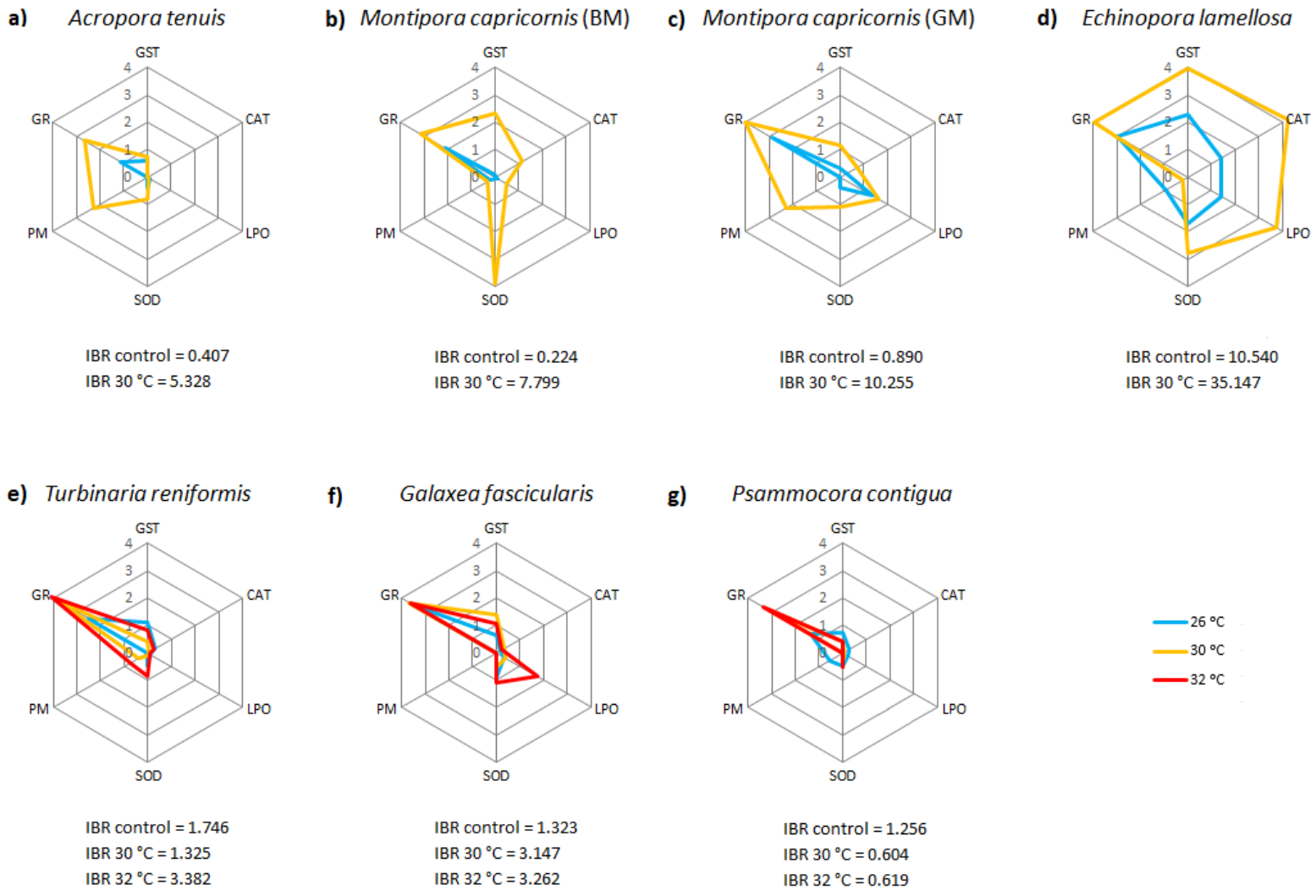

Figure 6.4. Star plots with mean scores for the seven coral species exposed to 26 °C (control) and both 30 ºC and 32 °C (stress temperatures). GST – glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation; SOD – superoxide dismutase; PM – partial mortality; GR – growth rate.

6.3.3. Fitness index

Results showed that biomarkers consistently scoring higher at both 32 ºC and 30 °C than at 26 °C were also the ones indicating deleterious effects ($E \leq -0.5$, in red in Table 6.4), namely SOD and growth rate. Biomarkers scoring higher at 32 °C than at 26 °C include growth rate, whereas the ones scoring higher at 32 °C than at 30 °C include SOD. In contrast, biomarkers indicating fluctuating effects in fitness in the long-term exposure to increased temperature were LPO and CAT for most species (Table 6.4).

In general, a decrease or maintenance in the fitness index (red filled boxes and yellow filled boxes, respectively) was observed with increase in temperature for most coral species and biomarkers. The species *E. lamellosa* and *T. reniformis* presented an increase in the fitness index (green filled boxes) in one biomarker from 26 °C to 30 °C. The *E. lamellosa* fragments presented it in partial mortality, whereas the fragments of *T. reniformis* presented it in GST (Table 6.4). The fragments of *P. contigua* presented an increase in the fitness index in partial mortality from 26 °C to 30 °C and from 26 °C to 32 °C. The most responsive biomarkers were growth rate and SOD, showing greater deleterious effects in the fitness index results (Table 6.4). All biomarkers were affected by temperature, with the exception of LPO (Table 6.5). The most responsive biomarkers were growth rate > SOD > GST > CAT > PM > LPO.

Table 6.4. Fitness index presented as a color hitmap: IBR scores were compared for biomarkers between control organisms and those exposed to both 30 °C and 32 °C. Red boxes denote deleterious effects, green denotes positive effects, whereas yellow denotes no detected effect; values are ± 0.5 (or higher) from control's score values. GST – glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation; SOD – superoxide dismutase; PM – partial mortality; GR – growth rate. NA – not available due to total mortality

	GST			CAT			LPO			SOD			PM			GR		
Species/T (°C)	26-30	26-32	30-32	26-30	26-32	30-32	26-30	26-32	30-32	26-30	26-32	30-32	26-30	26-32	30-32	26-30	26-32	30-32
A. tenuis	-0.148	NA	NA	0.000	NA	NA	0.070	NA	NA	-0.424	NA	NA	-2.249	NA	NA	-1.528	NA	NA
M. capricornis (BM)	-2.271	NA	NA	-1.128	NA	NA	-0.383	NA	NA	-3.883	NA	NA	-0.125	NA	NA	-1.037	NA	NA
M. capricornis (GM)	-0.851	NA	NA	-0.413	NA	NA	-0.249	NA	NA	-0.680	NA	NA	-2.249	NA	NA	-1.180	NA	NA
E. lamellosa	-1.681	NA	NA	-2.846	NA	NA	-2.307	NA	NA	-1.056	NA	NA	0.750	NA	NA	-1.006	NA	NA
T. reniformis	0.697	0.303	-0.394	0.261	0.074	-0.187	0.037	0.011	-0.026	0.363	-0.416	-0.779	-0.375	-0.750	-0.375	-1.271	-1.538	-0.267
G. fascicularis	-0.750	-0.399	0.351	-0.176	-0.034	0.141	-0.054	-1.413	-1.358	0.342	-0.191	-0.533	0.000	0.000	0.000	-0.083	-0.040	0.042
P. contigua	0.324	0.324	0.000	0.205	0.257	0.052	0.125	0.177	0.052	0.156	-0.034	-0.190	0.562	0.562	0.000	-1.790	-1.993	-0.204

Table 6.5. Biomarker scores (mean) for all species at different combinations of temperatures. GST – glutathione S-transferase; CAT – catalase; LPO – lipid peroxidation; SOD – superoxide dismutase; PM – partial mortality; GR – growth rate.

T(°C)/Biomarker	GST	CAT	LPO	SOD	PM	GR
26-30	-0.669	-0.585	-0.394	-0.740	-0.527	-1.128
26-32	0.076	0.099	-0.408	-0.214	-0.062	-1.191
30-32	-0.014	0.002	-0.444	-0.501	-0.125	-0.143

6.4. Discussion and conclusions

In this study, an integrated approach was used for health assessment of reef corals under thermal stress by exposing small fragments of seven common and widely distributed corals species of the Indo-Pacific oceans (Veron, 1990) to both control and two stress temperatures. Our results showed that an increase of 6 ºC in SSTs will lead to deleterious effects at all levels of biological organization for most of the species tested in this study. The health status of indicator species within ecosystems can be successfully assessed through multivariate biomarker approaches involving multiple biological and physiological measurements as previously stated by Hook et al. (2014). Therefore, we tested this methodology in coral reefs, and two comparative different approaches were applied: the first containing just the molecular biomarkers (approach A), whereas the second one contained the molecular biomarkers, one biomarker at organismic level, and one biomarker at physiological level (approach B). The chosen molecular biomarkers, glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO), were previously used in studies evaluating the effect of heat stress in reef-building coral species (Downs et al., 2000; Dias et al., 2019a,b). Also, growth rate, one of the physiological responses of an organism, has been widely used in marine invertebrates, e.g. bivalves (Widdows et al., 1982) and gastropods (Wo et al., 1999), to provide a measure of environmental quality. Overall, both IBR approaches suggest that index values were significantly affected by temperature and coral species. These results confirm the adequacy of using IBR indices in monitor physiological responses in populations of tropical reef-building corals that might be induced by heat stress events. In general, most of the tested coral species presented a clear separation between temperature treatments, with increase in both biomarkers of oxidative stress and partial mortality values and a decrease in growth rate values with increase in temperature. The increase in antioxidant enzyme activity observed in most of the coral species is expected since increase in temperature leads to increase in metabolic rates and consequent reactive oxygen species (ROS) overproduction, in order to counteract ROS negative effects, cells use a vast number of antioxidant enzymes that convert ROS into less harmful oxygen forms. Superoxide dismutase and CAT provide the first line of defense against $O_2^{\bullet-}$ and H_2O_2, respectively, whereas GST provides the second line of defense against ROS (Hayes and McLellan, 1999). The increase in LPO levels is also expected since antioxidant pathways can be overwhelmed during extreme temperature stress (Weis, 2008), and even when induction of antioxidant enzymes is observed, it may not be sufficient to avoid damage to the cellular

membrane lipids (Palmer et al., 2009). The decrease in growth rate with increase in temperature is expected since that deviations in temperature of only a few degrees from the optimum temperature lead to growth rate reduction (Berkelmans and Willis, 1999). The increase in partial mortality is given to acute stress, it may have resulted from the overwhelming of antioxidant pathways during heat stress, which may have caused localized tissue mortality (Lam, 2009). This response can be observed in the fitness index where most of corals presented a decrease or maintenance of the index.

Different coral species presented different response to heat stress at molecular, physiological, and organismic level. As observed in other studies, coral species differed in their cellular physiology and the strategies applied to diminish oxidative stress (Dias et al., 2019a,b), and in both growth rate and partial mortality according to their inherent traits (Dias et al., 2018, 2019c). For instance, *T. reniformis, E. lamellosa*, and *P. contigua* were the only species presenting increase in the fitness index in one of the biomarkers with increase in temperature.

The biomarkers growth rate and SOD were the most responsive showing greater deleterious effects in the fitness index, whereas LPO remained non-responsive to temperature increase. The increase in SOD activity may be related with this antioxidant enzyme be the first one involved in ROS scavenging (Lesser, 2012). On the other hand, growth rate is a physiological response that takes time to be perceptible (Morgan et al., 2001). Nevertheless, given that our experiment had the duration of 60 days, there was enough time for the observation of changes in growth rate. Regarding LPO non-response, it may have been that the increase in antioxidant enzyme activity was sufficient to prevent cellular damage in most of the tested species.

Most coral species presented an increase in IBR values with temperature, expected due to stress increase with temperature (Madeira et al., 2018). There were species where the two approaches were adequate (*M. capricornis* (both morphotypes)) and other species where the approach A was inadequate and the approach B was adequate (*A. tenuis, T. reniformis, E. lamellosa, G. fascicularis,* and *P. contigua)*. According with the results obtained from the tested species, the approach B was the most adequate since it better reflected the stress suffered by the tested species. On the other hand, the approach A did not have enough resolution in comparison with

the approach B, this is, the set of four molecular biomarkers (approach A) was not enough to explain the response of most of the tested species to thermal stress conditions.

In spite of some limitations, IBRs may be an effective method to be applied in the health assessment of reef corals under thermal stress. Biomarkers at molecular levels (e.g. SOD, CAT, GST, and LPO) tend to be more repeatable and predictable and are much more sensitive for identifying organism stress than whole animal responses (Smit et al., 2009), but their capability to predict significant biological effects is limited (Bartell, 2006). On the other hand, biomarkers at the physiological level (e.g. growth rate) and organismic level (e.g. partial mortality), although slower to respond and more difficult to detect, provide ‘‘integrated’’ measures of an organism’s well-being based on a range of different functional attributes and are often more ecologically relevant. Therefore, the incorporation of biomarkers of different levels of biological organization provides advantages over the application of single-marker approaches. The approach A did not express well what we could assess visually, however, this approach can be a more sensible indication of sub-lethal stress at the molecular level. The approach B is easy to interpret, however, growth rate is a factor that takes longer to react than the biomarkers of oxidative stress. Partial mortality is a sign of extreme stress, leading to the death of the organism unless acclimation is achieved or there is enough growth to surpass the loss of tissue (Guest et al., 2011). Thus, approach B is a more generalist approach, gathering different levels of biological complexity. Based on the obtained results, the use of integrated indices describing heat-induced stress as management and research tools is considered a useful approach.

Still, when applying the IBR index in field populations, it is imperative to assure a careful and adequate selection of biomarkers (Broeg and Lehtonen, 2006). The set of biomarkers used in the present study proved to be adequate for reef-building corals as this set had already responded to heat stress in Dias et al. (2019a,b). Also, ensuring that the targeted colonies are not exposed to other possible stress factors would be favored (Broeg and Lehtonen, 2006). It is also important to have in mind that these biomarkers are also known for responding to other stress factors beyond those associated with global warming, that is the case of xenobiotics (Downs et al., 2006; Rotchell and Ostrander, 2011), salinity (Dias et al., 2019b,c), allelopathy (Chadwick and Morrow, 2011; Morrow et al., 2012), and excess of solar radiation in zooxanthellae (Shick et al., 1995; Dahms and Lee, 2010). Thus, IBR index can actually be fit

to assess the reef corals' health when exposed to other factors beyond heat stress, being required further work on the response of corals to such stressors.

6.5. References

Ateweberhan, M., McClanahan, T. R., Graham, N. A. J., & Sheppard, C. R. C. (2011). Episodic heterogeneous decline and recovery of coral cover in the Indian Ocean. Coral Reefs, 30(3), 739.

Baird, A., & Maynard, J. A. (2008). Coral adaptation in the face of climate change. Science, 320(5874), 315-316.

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine, Coastal and Shelf Science, 80(4), 435-471.

Bartell, S. M. (2006). Biomarkers, bioindicators, and ecological risk assessment—a brief review and evaluation. Environmental Bioindicators, 1(1), 60-73.

Baums, I. B., Miller, M. W., & Hellberg, M. E. (2006). Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. Ecological Monographs, 76(4), 503-519.

Beliaeff, B., & Burgeot, T. (2002). Integrated biomarker response: a useful tool for ecological risk assessment. Environmental Toxicology and Chemistry: An International Journal, 21(6), 1316-1322.

Berkelmans, R., & Willis, B. L. (1999). Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs, 18(3), 219-228.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), 248-254.

Broeg, K., & Lehtonen, K. K. (2006). Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of a multi-biomarker approach. Marine Pollution Bulletin, 53(8-9), 508-522.

Chadwick, N. E., & Morrow, K. M. (2011). Competition among sessile organisms on coral reefs. In Coral Reefs: an ecosystem in transition (pp. 347-371). Springer, Dordrecht.

Chase, M. E., Jones, S. H., Hennigar, P., Sowles, J., Harding, G. C. H., Freeman, K., ... & Pederson, J. (2001). Gulfwatch: Monitoring spatial and temporal patterns of trace metal and organic contaminants in the Gulf of Maine (1991–1997) with the blue mussel, *Mytilus edulis* L. Marine Pollution Bulletin, 42(6), 490-504.

Coffroth, M. A., & Lasker, H. R. (1998). Population structure of a clonal gorgonian coral: the interplay between clonal reproduction and disturbance. Evolution, 52(2), 379-393.

Connell, J. H., & Keough, M. J. (1985). Disturbance and patch dynamics of subtidal marine animals on hard substrata. In: Pickett STA, White PS (eds.). The ecology of natural disturbance and patch dynamics, 125-151

Dahms, H. U., & Lee, J. S. (2010). UV radiation in marine ectotherms: molecular effects and responses. Aquatic Toxicology, 97(1), 3-14.

Dayton, P. K. (1972). Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. In Proceedings of the colloquium on conservation problems in Antarctica (pp. 81-96). Lawrence, Kansas, USA: Allen Press.

Devin, S., Burgeot, T., Giambérini, L., Minguez, L., & Pain-Devin, S. (2014). The integrated biomarker response revisited: optimization to avoid misuse. Environmental Science and Pollution Research, 21(4), 2448-2454.

Dias, M., Ferreira, A., Gouveia, R., Cereja, R., & Vinagre, C. (2018). Mortality, growth and regeneration following fragmentation of reef-forming corals under thermal stress. Journal of Sea Research, 141, 71-82.

Dias, M., Ferreira, A., Gouveia, R., Madeira, C., Jogee, N., Cabral, H. N., Diniz, M., & Vinagre, C. (2019a). Long-term exposure to increasing temperatures on scleractinian coral fragments reveals oxidative stress. Marine Environmental Research, 150, 104758.

Dias, M., Madeira, C., Jogee, N., Ferreira, A., Gouveia, R., Cabral, H.N., Diniz, M. & Vinagre, C. (2019b). Oxidative stress on scleractinian coral fragments following exposure to high temperature and low salinity. Ecological Indicators, 107, 105586.

Dias, M., Ferreira, A., Gouveia, R., & Vinagre, C. (2019c). Synergistic effects of warming and lower salinity on the asexual reproduction of reef-forming corals. Ecological Indicators, 98, 334-348.

Donner, S. D. (2009). Coping with commitment: projected thermal stress on coral reefs under different future scenarios. PLoS One, 4(6), e5712.

Donner, S. D., Skirving, W. J., Little, C. M., Oppenheimer, M., & Hoegh-Guldberg, O. V. E. (2005). Global assessment of coral bleaching and required rates of adaptation under climate change. Global Change Biology, 11(12), 2251-2265.

Downs, C. A., Mueller, E., Phillips, S., Fauth, J. E., & Woodley, C. M. (2000). A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. Marine Biotechnology, 2(6), 533-544.

Downs, C. A., Richmond, R. H., Mendiola, W. J., Rougée, L., & Ostrander, G. K. (2006). Cellular physiological effects of the MV Kyowa Violet fuel-oil spill on the hard coral, Porites lobata. Environmental Toxicology and Chemistry: An International Journal, 25(12), 3171-3180.

Dustan, P., & Halas, J. C. (1987). Changes in the reef-coral community of Carysfort Reef, Key Largo, Florida: 1974 to 1982. Coral Reefs, 6(2), 91-106.

Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., ... & Mohan, J. (2005). Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment, 3(9), 479-486.

Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B., & Norberg, J. (2003). Response diversity, ecosystem change, and resilience. Frontiers in Ecology and the Environment, 1(9), 488-494.

Elsner, J. B., Murnane, R. J., & Jagger, T. H. (2006). Forecasting US hurricanes 6 months in advance. Geophysical Research Letters, 33(10).

Emanuel, K. (2005). Increasing destructiveness of tropical cyclones over the past 30 years. Nature, 436(7051), 686.

Fabricius, K. E., Hoegh-Guldberg, O., Johnson, J. E., McCook, L. J., & Lough, J. M. (2007). Vulnerability of coral reefs of the Great Barrier Reef to climate change. Climate change and the Great Barrier Reef Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia, pp515–554

Ferreira, N. G., Morgado, R., Santos, M. J., Soares, A. M., & Loureiro, S. (2015). Biomarkers and energy reserves in the isopod *Porcellionides pruinosus*: the effects of long-term exposure to dimethoate. Science of the Total Environment, 502, 91-102.

Foster, N. L., Baums, I. B., & Mumby, P. J. (2007). Sexual vs. asexual reproduction in an ecosystem engineer: the massive coral *Montastraea annularis*. Journal of Animal Ecology, 76(2), 384-391.

Foster, N. L., Baums, I. B., Sanchez, J. A., Paris, C. B., Chollett, I., Agudelo, C. L., (...) & Mumby, P. J. (2013). Hurricane-driven patterns of clonality in an ecosystem engineer: the Caribbean coral *Montastraea annularis*. PLoS One, 8(1), e53283.

Furby, K. A., Bouwmeester, J., & Berumen, M. L. (2013). Susceptibility of central Red Sea corals during a major bleaching event. Coral Reefs, 32(2), 505-513.

Glynn, P. W., Colley, S. B., Carpizo-Ituarte, E., & Richmond, R. H. (2017). Coral reproduction in the Eastern Pacific. In Coral Reefs of the Eastern Tropical Pacific (pp. 435-476). Springer, Dordrecht.

Graham, N. A., McClanahan, T. R., MacNeil, M. A., Wilson, S. K., Polunin, N. V., Jennings, S., ... & Bigot, L. (2008). Climate warming, marine protected areas and the ocean-scale integrity of coral reef ecosystems. PLoS One, 3(8), e3039.

Guest, J. R., Dizon, R. M., Edwards, A. J., Franco, C., & Gomez, E. D. (2011). How quickly do fragments of coral “self-attach” after transplantation?. Restoration Ecology, 19(2), 234-242.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249(22), 7130-7139.

Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., ... & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. Ecology Letters, 9(2), 228-241.

Hayes, J. D., & McLellan, L. I. (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radical Research, 31(4), 273-300.

Heyward, A. J., & Negri, A. P. (2010). Plasticity of larval pre-competency in response to temperature: observations on multiple broadcast spawning coral species. Coral Reefs, 29(3), 631-636.

Highsmith, R. C. (1980). Passive colonization and asexual colony multiplication in the massive coral *Porites lutea* Milne Edwards & Haime. Journal of Experimental Marine Biology and Ecology, 47(1), 55-67.

Highsmith, R. C. (1982). Reproduction by fragmentation in corals. Marine Ecology Progress Series. Oldendorf, 7(2), 207-226.

Highsmith, R. C., Riggs, A. C., & D'Antonio, C. M. (1980). Survival of hurricane-generated coral fragments and a disturbance model of reef calcification/growth rates. Oecologia, 46(3), 322-329.

Hochachka, P. W., & Somero, G. N. (2002). Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press.

Hoegh-Guldberg, O. (2011). Coral reef ecosystems and anthropogenic climate change. Regional Environmental Change, 11(1), 215-227.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. Science, 328(5985), 1523-1528.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... & Knowlton, N. (2007). Coral reefs under rapid climate change and ocean acidification. Science, 318(5857), 1737-1742.

Honnay, O., & Bossuyt, B. (2005). Prolonged clonal growth: escape route or route to extinction? Oikos, 108(2), 427-432.

Hook, S. E., Gallagher, E. P., & Batley, G. E. (2014). The role of biomarkers in the assessment of aquatic ecosystem health. Integrated Environmental Assessment and Management, 10(3), 327-341.

Hughes, T. P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science, 265(5178), 1547-1551.

Hughes, T. P., Kerry, J. T., & Simpson, T. (2018). Large-scale bleaching of corals on the Great Barrier Reef. Ecology, 99(2), 501-501.

Hughes, T. P., Graham, N. A., Jackson, J. B., Mumby, P. J., & Steneck, R. S. (2010). Rising to the challenge of sustaining coral reef resilience. Trends in Ecology & Evolution, 25(11), 633-642.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., ... & Lough, J. M. (2003). Climate change, human impacts, and the resilience of coral reefs. Science, 301(5635), 929-933.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K.D., Baird, A. H., ... & Bridge, T. C. (2017). Global warming and recurrent mass bleaching of corals. Nature, 543(7645), 373.

IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Meyer LA (eds)]. IPCC, Geneva, Switzerland, p 151

IPCC (2018). Special Report on 1.5 degrees Celsius; Incheon, Korea, 8th October 2018.

Johansson, L. H., & Borg, L. H. (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. Analytical Biochemistry, 174(1), 331-336.

Khalil, M. T., Cochran, J. E., & Berumen, M. L. (2013). The abundance of herbivorous fish on an inshore Red Sea reef following a mass coral bleaching event. Environmental Biology of Fishes, 96(9), 1065-1072.

Knap, A., Dewailly, É., Furgal, C., Galvin, J., Baden, D., Bowen, R. E., ... & Moser, F. (2002). Indicators of ocean health and human health: developing a research and monitoring framework. Environmental Health Perspectives, 110(9), 839-845.

Lam, P. K. (2009). Use of biomarkers in environmental monitoring. Ocean & Coastal Management, 52(7), 348-354.

Le Nohaïc, M., Ross, C. L., Cornwall, C. E., Comeau, S., Lowe, R., McCulloch, M. T., & Schoepf, V. (2017). Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia. Scientific Reports, 7(1), 14999.

Lehtonen, K. K., Sundelin, B., Lang, T., & Strand, J. (2014). Development of tools for integrated monitoring and assessment of hazardous substances and their biological effects in the Baltic Sea. Ambio, 43(1), 69-81.

Lesser, M. P. (2012). Oxidative stress in tropical marine ecosystems. Oxidative stress in aquatic ecosystems, 1, 9-19.

Levitan, D. R., Boudreau, W., Jara, J., & Knowlton, N. (2014). Long-term reduced spawning in *Orbicella* coral species due to temperature stress. Marine Ecology Progress Series, 515, 1-10.

Lirman, D. (2000). Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. Journal of Experimental Marine Biology and Ecology, 251(1), 41-57.

Madeira, C., Mendonça, V., Leal, M. C., Flores, A. A., Cabral, H. N., Diniz, M. S., & Vinagre, C. (2018). Environmental health assessment of warming coastal ecosystems in the tropics–Application of integrative physiological indices. Science of The Total Environment, 643, 28-39.

Madeira, D., Vinagre, C., Mendonça, V., & Diniz, M. S. (2017). Seasonal changes in stress biomarkers of an exotic coastal species – *Chaetopleura angulata* (Polyplacophora)–Implications for biomonitoring. Marine Pollution Bulletin, 120(1-2), 401-408.

Marigómez, I., Garmendia, L., Soto, M., Orbea, A., Izagirre, U., & Cajaraville, M. P. (2013). Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill “Mussel Watch”. Ecotoxicology, 22(3), 486-505.

Marinho-Soriano, E., Moreira, W. S. C., & Carneiro, M. A. A. (2006). Some aspects of the growth of *Gracilaria birdiae* (Gracilariales, Rhodophyta) in an estuary in northeast Brazil. Aquaculture International, 14(4), 327-336.

Morgan, M. B., Vogelien, D. L., & Snell, T. W. (2001). Assessing coral stress responses using molecular biomarkers of gene transcription. Environmental Toxicology and Chemistry: An International Journal, 20(3), 537-543.

Morrow, K. M., Ritson-Williams, R., Ross, C., Liles, M. R., & Paul, V. J. (2012). Macroalgal extracts induce bacterial assemblage shifts and sublethal tissue stress in Caribbean corals. PLoS One, 7(9), e44859.

Negri, A. P., Marshall, P. A., & Heyward, A. J. (2007). Differing effects of thermal stress on coral fertilization and early embryogenesis in four Indo Pacific species. Coral Reefs, 26(4), 759-763.

Nicholls, R. J., Wong, P. P., Burkett, V. R., Codignotto, J. O., Hay, J. E., et al. (2007). Coastal systems and low-lying areas. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Parry ML, Canziani OF, Palutikof JP, Van der Linden PJ and Hanson CE, editors. Cambridge University Press, Cambridge. pp 315–356.

Norström, A. V., Nyström, M., Lokrantz, J., & Folke, C. (2009). Alternative states on coral reefs: beyond coral–macroalgal phase shifts. Marine Ecology Progress Series, 376, 295-306.

Nozawa, Y., & Harrison, P. L. (2002). Larval settlement patterns, dispersal potential, and the effect of temperature on settlement of larvae of the reef coral, *Platygyra daedalea*, from the Great Barrier Reef. In Proceedings of the 9th International Coral Reef Symposium (Vol. 1, pp. 409-416).

Nozawa, Y., & Harrison, P. L. (2007). Effects of elevated temperature on larval settlement and post-settlement survival in scleractinian corals, *Acropora solitaryensis* and *Favites chinensis*. Marine Biology, 152(5), 1181-1185.

Nyström, M., Folke, C., & Moberg, F. (2000). Coral reef disturbance and resilience in a human-dominated environment. Trends in Ecology & Evolution, 15(10), 413-417.

Palmer, C. V., Modi, C. K., & Mydlarz, L. D. (2009). Coral fluorescent proteins as antioxidants. PloS One, 4(10), e7298.

Pratchett, M. S., Munday, P. L., Wilson, S. K., Graham, N. A., Cinner, J. E., Bellwood, D. R., ... & McClanahan, T. R. (2008). Effects of climate-induced coral bleaching on coral-reef fishes—ecological and economic consequences. In Oceanography and Marine Biology (pp. 257-302). CRC Press.

Randall, C. J., & Szmant, A. M. (2009). Elevated temperature reduces survivorship and settlement of the larvae of the Caribbean scleractinian coral, *Favia fragum* (Esper). Coral Reefs, 28(2), 537-545.

Rotchell, J. M., & Ostrander, G. K. (2011). Molecular toxicology of corals: a review. Journal of Toxicology and Environmental Health, Part B, 14(8), 571-592.

Shick, J. M., Lesser, M. P., Dunlap, W. C., Stochaj, W. R., Chalker, B. E., & Won, J. W. (1995). Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. Marine Biology, 122(1), 41-51.

Skelly, D. K., Joseph, L. N., Possingham, H. P., Freidenburg, L. K., Farrugia, T. J., Kinnison, M. T., & Hendry, A. P. (2007). Evolutionary responses to climate change. Conservation Biology, 21(5), 1353-1355.

Smit, M. G., Bechmann, R. K., Hendriks, A. J., Skadsheim, A., Larsen, B. K., Baussant, T., ... & Sanni, S. (2009). Relating biomarkers to whole-organism effects using species sensitivity distributions: A pilot study for marine species exposed to oil. Environmental Toxicology and Chemistry: An International Journal, 28(5), 1104-1109.

Sun, Y. I., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. Clinical Chemistry, 34(3), 497-500.

Tebbett, S. B., Streit, R. P., & Bellwood, D. R. (2019). Expansion of a colonial ascidian following consecutive mass coral bleaching at Lizard Island, Australia. Marine Environmental Research, 144, 125-129.

Titlyanov, E. A., Titlyanova, T. V., Yakovleva, I. M., Nakano, Y., & Bhagooli, R. (2005). Regeneration of artificial injuries on scleractinian corals and coral/algal competition for

newly formed substrate. Journal of Experimental Marine Biology and Ecology, 323(1), 27-42.

Tunnicliffe, V. (1981). Breakage and propagation of the stony coral *Acropora cervicornis*. Proceedings of the National Academy of Sciences, 78(4), 2427-2431.

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry, 86(1), 271-278.

Van Hooidonk, R., & Huber, M. (2009). Quantifying the quality of coral bleaching predictions. Coral Reefs, 28(3), 579-587.

Van Woesik, R., Sakai, K., Ganase, A., & Loya, Y. (2011). Revisiting the winners and the losers a decade after coral bleaching. Marine Ecology Progress Series, 434, 67-76.

Veron, J. E. N. (1990). Checklist of the hermatypic corals of Vanuatu. Pacific Science, 44, 51-70.

Viarengo, A., Lafaurie, M., Gabrielides, G. P., Fabbri, R., Marro, A., & Romeo, M. (2000). Critical evaluation of an intercalibration exercise undertaken in the framework of the MED POL biomonitoring program. Marine Environmental Research, 49(1), 1-18.

Weis, V. M. (2008). Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. Journal of Experimental Biology, 211(19), 3059-3066.

Widdows, J., Bakke, T., Bayne, B. L., Donkin, P., Livingstone, D. R., Lowe, D. M., ... & Moore, S. L. (1982). Responses of *Mytilus edulis* on exposure to the water-accommodated fraction of North Sea oil. Marine Biology, 67(1), 15-31.

Wo, K. T., Lam, P. K., & Wu, R. S. (1999). A comparison of growth biomarkers for assessing sublethal effects of cadmium on a marine gastropod, *Nassarius festivus*. Marine Pollution Bulletin, 39(1-12), 165-173.

6.6. Supplementary material

Table SM6.1. Number of coral species fragments (n) used in each oxidative stress biomarker analysis (LPO – lipid peroxidation; SOD – superoxide dismutase; CAT – catalase; GST- glutathione S-transferase) per temperature treatment.

	Treatments											
	26 °C				30 °C				32 °C			
Species	LPO	SOD	CAT	GST	LPO	SOD	CAT	GST	LPO	SOD	CAT	GST
Acropora tenuis	5	5	5	5	5	5	5	5	0	0	0	0
Montipora capricornis (BM)	5	5	5	5	6	6	6	6	0	0	0	0
Montipora capricornis (GM)	5	5	5	5	5	5	5	5	0	0	0	0
Echinopora lamellosa	6	6	6	6	5	5	5	5	0	0	0	0
Turbinaria reniformis	5	5	5	5	5	5	5	5	5	5	5	5
Galaxea fascicularis	5	5	5	5	5	5	5	5	5	5	5	5
Psammocora contigua	5	5	5	5	5	5	5	5	5	5	5	5

CHAPTER 7

Final remarks and future perspectives

7.1. Concluding remarks

The present work contributed to the assessment of the vulnerability to heat and hyposaline stressors in nine reef-building coral species of the Indo-Pacific region. Such assessment was innovative due to the use of an important number of reef-building coral species, the prolonged duration of the experimental treatments, and the integration of parameters at different levels of biological organization for health status evaluation. This work also contributed to the development of two different integrated biomarker approaches to be applied in the monitoring of the effects of heat stress in reef corals of the Indo-Pacific region, with the novelty of the combination of different levels of biological organization in the second approach.

The experimental work indicated that heat stress affected more severely branching coral species than plate, incrusting, and massive species. Mortality increased with temperature, reaching 100% for most species after 60 days, at 32 °C, except for *Turbinaria reniformis*, *Galaxea fascicularis*, and *Psammocora contigua*. These same species also showed the lowest partial mortality and bleaching throughout the experimental temperature treatments. Regarding *T. reniformis* and *P. contigua*, no evidence of oxidative damage was observed at 32 °C, although both species presented a pale appearance. *Galaxea fascicularis* was the only of the three species where bleached fragments and oxidative damage were observed, although also presenting some apparently healthy fragments at 32 °C. Growth rate decreased with increase in temperature, being highest in branching species. Regeneration rates of lesions generally increased with temperature. It was concluded that *T. reniformis*, *G. fascicularis*, and *P. contigua* were the most tolerant to heat stress (Chapters 2 and 4). At 30 °C-33 psu (high temperature treatment) only two species died (*Pocillopora damicornis* and *Stylophora pistillata*), while at 26 °C-20 psu (low salinity treatment) all species died with the exception of two (*P. contigua* and *G. fascicularis*). These last two species presented the lowest partial mortality, the best coral condition, and no oxidative damage, but there was an antioxidant response. High temperature affected the condition of the tested species to a lower degree than did low salinity, with coral condition severely affected in all species in the low salinity treatment. Mortality was highest at 30 °C-20 psu due to the combined effects of high temperature and low salinity on the mortality rate, reaching 100% for eight out of the nine species, with only *G. fascicularis* surviving to this experimental treatment. Nevertheless, mortality was high for this species, an increase in SOD activity revealed an antioxidant response, however oxidative damage was not detected. Growth rates decreased as temperature increased and salinity decreased, whereas, regeneration rates

increased with temperature, reaching a maximum at 30 °C-33 psu and a minimum at 20 psu. It was concluded that *P. damicornis* and *S. pistillata* were the species most vulnerable to high temperature, *G. fascicularis* and *P. contigua* were the most tolerant to hyposaline stress, and *G. fascicularis* was the only species that withstood the combined effects of high temperature and low salinity (Chapters 3 and 5). In chapter 6, results indicate that IBR may be an effective method to be applied in the health assessment of reef corals under thermal stress. It was observed that approaches based in parameters of different levels of biological organization give a more precise idea of the impact of heat stress on the tested coral species and thus may be chosen over approaches based in parameters of only one level of biological organization.

In summary, this thesis main conclusions were:

- Different reef-building coral species present different susceptibility to both heat and hyposaline stressors, these differences may be related with their inherent traits including colony morphology.
- Response of coral species was different according to the environmental stressor at which the tested species were exposed, with hyposaline stress affecting coral species condition in a much more severe degree than heat stress;
- Only a few of the tested species could withstand long-term exposure to heat and hyposaline stressors, three species survived to heat stress, two species survived hyposaline stress and only one species survived the combined effect of heat and hyposaline stressors. In general, branching coral species were most susceptible and massive species were the most tolerant to both stressors, with the exception of the branching species *Psammocora contigua* that presented an exceptionally high tolerance to both heat and hyposaline stressors when exposed to these stressors one at the time;
- Only a few number of the tested species are likely to survive the large-scale effects of warming in tropical oceans and even a fewer number of species will withstand the synergistic effects of high temperature and low salinity in near-shore areas (without genetic adaptation or other processes not tested here);
- Asexual reproduction by fragmentation is likely to be negatively affected by the higher temperatures predicted by climate models, since all the tested parameters point to severe stress;

- In areas affected by episodic, yet prolonged, low salinity, asexual reproduction by fragmentation is likely to be severely compromised, especially if temperatures are also high during those periods;
- The use of integrated biomarker response (IBR) indices describing heat-induced stress as management and research tools was considered a useful approach. Nevertheless, when using IBR as index, the combined use of biomarkers at molecular, physiological, and organism levels is advised since together they perform a more precise assessment of the coral condition.

7.2. Future perspectives

Most of the studies assessing climate change effects on reef-building corals are based in a low number of species and mainly investigate the effects of heat stress. Nevertheless, other environmental variables will be affected by global climate change (e.g. salinity, turbidity, contamination by pollutants and pollutants mixtures, contamination by macro and microplastics) and thus their effects must also be assessed in future studies. More studies simultaneously evaluating several coral species, like the present study, are also desirable since they allow a direct comparison among different species' susceptibility when exposed to the same environmental conditions. It would also be very important to investigate cyclic conditions (e.g. cyclic temperatures and salinities that mimic tides), given that most studies, the present one included, focus only on static conditions, the application of cyclic conditions that mimic the ones found in their natural environment would allow a more adequate extrapolation of the laboratory results to their natural habitat.

Also crucial, will be to evaluate corals of the same species but from different locations, to investigate variability in environmental tolerance to present and future conditions. Stress history of the corals (background climate conditions) affect coral species' susceptibility to stressors since coral species are adapted to the environmental conditions in which they grow. Therefore, changes in environmental conditions affect coral species of different geographical locations in a different magnitude. This evaluation may allow translocation of tolerant colonies for ecological restoration in areas affected by coral loss.

A proper management of local stressors associated with anthropogenic disturbances (e.g. pollution, dredging, and overexploitation) may improve the prospects for coral recovery. Although marine reserves or no take areas may not increase ecosystem resistance to global climate change, these areas can help to accelerate recovery and successfully act as a genetic diversity source, by preserving sensitive and specialized species that cannot persist in disturbed and altered environments. Nevertheless, in order to accomplish this goal, no take areas need to be implemented in locations that could function as refuges to global climate change impacts. There are still some natural refuges (e.g. deeper reefs and colder waters). These refuges could function as source reefs, helping the repopulation of damaged reefs through larval supply. Also, species that resist extreme climate events can have traits that support a general ability to cope or adapt to new environmental conditions. Thus, a better understanding of reef-building corals inherent traits that make them naturally tolerant to disturbances might lead to innovative technologies of assisted gene flow and assisted evolution that can help to produce climate-harden corals.

ANNEXES

ANNEX 1

PhD outputs

Articles in international peer-reviewed journals

Dias, M., Ferreira, A., Gouveia, R., Cereja, R., & Vinagre, C. (2018). Mortality, growth and regeneration following fragmentation of reef-forming corals under thermal stress. Journal of Sea Research, 141, 71-82. DOI:10.1016/j.seares.2018.08.008.

Dias, M., Ferreira, A., Gouveia, R., & Vinagre, C. (2019). Synergistic effects of warming and lower salinity on the asexual reproduction of reef-forming corals. Ecological Indicators, 98, 334-348. DOI:10.1016/j.ecolind.2018.11.011

Dias, M., Ferreira, A., Gouveia, R., Madeira, C., Jogee, N., Cabral, H.N., Diniz, M. & Vinagre, C. (2019). Long-term exposure to increasing temperatures on scleractinian coral fragments reveals oxidative stress. Marine Environmental Research, 104758. DOI:10.1016/j.marenvres.2019.104758.

Dias, M., Madeira, C., Jogee, N., Ferreira, A., Gouveia, R., Cabral, H.N., Diniz, M. & Vinagre, C. (2019). Oxidative stress on scleractinian coral fragments following exposure to high temperature and low salinity. Ecological Indicators, 105586. DOI:10.1016/j.ecolind.2019.105586.

Dias, M., Madeira, C., Jogee, N., Ferreira, A., Gouveia, R., Cabral, H.N., Diniz, M. & Vinagre, C. (submitted). Integrative indices for health assessment in reef corals under thermal stress.

Posters presented in scientific meetings (national and international)

2017. Michau, Y., Madeira, C., Dias, M., Ferreira, A., Gouveia, R., Falla, J., Vinagre, C., Diniz, M.S. 2017. Long term responses to increasing temperatures in Scleractinia corals: Hsp70 and ubiquitin induction. 2nd International Congress of CiiEM – Translational research and innovation in human health sciences, Caparica, Portugal, 11-13th June, 2017.

2018. Dias, M., Ferreira, A., Gouveia, R., Cereja, R., & Vinagre, C. 2018. Individual and synergistic effects of warming and lower salinity on mortality, growth and regeneration

following fragmentation of reef-forming corals. International Meeting on Marine Research, (IMMR' 18), July 2018, Lisbon, Portugal.

2019. Madeira, C., & Dias, M., Leal, M.C., Mendonça, V., Madeira, D., Ferreira, A., Gouveia, R., Jogee, N., Flores, A.A.V., Diniz, M., Cabral, H.N., Vinagre, C. 2019. Warming in tropical marine ecosystems: can integrative physiological indices help prioritize habitats and species for conservation?, 15th European Ecological Federation Congress and 18th Portuguese Society for Ecology Meeting: Ecology Across Borders - Embedding Ecology in Sustainable Development Goals, 29th July - 2nd August 2019, Lisbon, Portugal.