THE INFLUENCE OF HETEROTROPHY ON THE RESILIENCE OF HARD CORAL *POCILLOPORA DAMICORNIS* TO THERMAL STRESS AND BLEACHING

by

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ABSTRACT

Global warming from anthropogenic greenhouse gas emissions causes temperature increases in aquatic ecosystems. The rise in environmental temperatures places sensitive organisms under thermal stress. Reef-building corals are a critically important group of animals that provide many ecosystem services for coral reef ecology and the economy and are at a high risk of loss from thermal stress. Thermal stress causes corals to lose their colour, i.e. become bleached, resulting from the loss of symbiont zooxanthellae. This diminishes the energetic benefits that zooxanthellae provide to corals leading to a decline in coral health and high mortality rates. However, corals are also predators and can thus draw nutrients from zooplankton prey to supplement their nutritional requirements. This study investigated whether heterotrophic feeding can ameliorate the effects of thermal stress on coral physiology by providing an alternative energy source to zooxanthellar photosynthesis. Fragmented *Pocillopora damicornis* coral colonies were exposed to daily maximum temperatures of up to 31°C while being either starved or fed. During the experimental period coral nubbins were monitored for changes in polyp extension, oxygen consumption rate, feeding rate, colour, chlorophyll *a* content, zooxanthellae density, antioxidant potentials and DNA integrity during stress and after a short recovery period. It was found that, as expected, coral polyp extension, oxygen consumption rate, colour health, chlorophyll *a* content, zooxanthellae density and DNA integrity were all adversely affected by thermal stress. This indicted that all these measurements were viable biomarkers for assessing the negative effects of thermal stress on coral health. Coral colour, oxygen consumption rate, chlorophyll *a* content, lipid content, antioxidant potential and DNA integrity were all significantly improved by feeding. These results indicate that feeding does play a role in improving overall coral health and supports the physiological processes in coral tissue during and after thermal stress. The conclusions from this study also have great significance for coral reef ecology and management as predictions of reef resilience can be made from zooplankton ecology and boosting zooplankton availability to corals may be considered to mitigate the harmful effects of thermal stress and bleaching.

PREFACE

The experimental work described in this dissertation was carried out in the School of Life Sciences, University of KwaZulu-Natal, Durban, from January 2011 to April 2013, under the supervision of Dr David Glassom and co-supervision of Dr Dalene Vosloo.

This study represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

This thesis is the written in the style and format of the Marine Ecology Progress Series journal.

DECLARATION - PLAGIARISM

I, Yanasivan Kisten declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	– Analysis of Variance
BCA	– Bicinchoninic Acid
FRAP	– Ferric Reducing/Antioxidant Potential
FSW	– Filtered Sea Water
KLMA	– Kenny's Low Melting Point Agarose
MSE	– Mean Squared Error
ROS	– Reactive Oxygen Species
SD	– Standard Deviation
SE	– Standard Error of the Mean
SST	– Sea Surface Temperature
TC	– Temperature Controls
TE	– Temperature Exposed
TPTZ	– 2,4,6-triperidyl-s-triazine
OTM	– Olive Tail Moment

CHAPTER 1: INTRODUCTION

1.1. Background

Anthropogenic sources of air pollution have caused many adverse changes to the Earth's climate since the industrial revolution (Harley et al. 2006, Doney et al. 2012). Global warming is one of the best known and most threatening examples of climate change and refers to the rapid and continual rise in atmospheric and sea surface temperatures (SST) caused by excessive greenhouse gas emissions (Harley et al. 2006, Doney et al. 2012). Increased temperatures result in thermal stress to many physical and ecological systems and are detrimental to organisms, biological processes and ecosystems that rely on stable physical conditions and cannot adapt to the rapid change (Walther et al. 2002, Harley et al. 2006, Doney et al. 2012).

Global warming and SST rise are particularly detrimental to marine ecosystems (Walther et al. 2002, Harley et al. 2006, Doney et al. 2012). For example, polar regions are susceptible to the loss of ecosystems that rely on polar cap ice which is melting from increased SST (Doney et al. 2012). Thermal tolerance ranges of animals at the polar regions are narrow making them vulnerable to increasing SST (Pörtner 2006). Tropical reefs are similarly at risk from SST rises due to increased incidence of tropical storms, decreased oxygen availability from reduced oxygen solubility and increased acidity from excess dissolved carbon dioxide (Walther et al. 2002, Baker et al. 2008, Lough 2008, Doney et al. 2012). Tropical organisms such as corals also have narrow thermal tolerance ranges which result in mass coral bleaching events from increased SST (Glynn 1993, Baker et al. 2008). Coral bleaching became prevalent on tropical reefs during the early 1980's due to the El Niño warming event and has since increased in distribution and intensity due to rising SSTs (Goreau & Hayes 1994, Brown 1997, Baker et al. 2008, Lough 2008). Reefs that have been bleached are at a high risk of mass coral mortality and therefore risk the loss of valuable keystone species and ecosystem engineers resulting in the major loss of ecosystem functioning (Moberg & Folke 1999, Harley et al. 2006, Knowlton 2008, Doney et al. 2012).

Coral reef bleaching is of particular concern because it results in the loss of highly productive, biodiverse and economically important ecosystems and the services they provide (Moberg & Folke 1999, Hoegh-Guldberg et al. 2007, Baker et al. 2008). Coral reefs protect the coast from waves, filter nutrients from terrestrial run-off and provide sand for beaches (Moberg & Folke 1999, Baker et al. 2008, Knowlton 2008). Corals reefs support high biodiversity by providing habitats for ecologically important species and providing nursery areas for the vulnerable life stages of species that migrate to other ecosystems (Moberg & Folke 1999, Baker et al. 2008). The species occupying corals reefs are also economically important contributing to fisheries and pharmaceutical industries (Moberg & Folke 1999, Hoegh-Guldberg et al. 2007, Baker et al. 2008). Coral reefs have aesthetic qualities that provide recreation for divers and tourism for coastal economies (Moberg & Folke 1999, Baker et al. 2008). Management and conservation of coral reefs is thus a priority for authorities and a thorough knowledge of coral physiology, coral reef ecology and coral bleaching is required for effective management and conservation strategies (Moberg & Folke 1999, Reaser et al. 2000, Nicholls & Lowe 2004, Baker et al. 2008).

The success of coral reef ecosystems is largely due to hermatypic hard corals for their high productivity and contribution to reef structure and habitat (Brown 1997, Douglas 2003). These corals may expel their resident algal symbionts under thermal stress, a phenomenon known as bleaching due to the loss of colour (Gates et al. 1992, Brown 1997, Douglas 2003). Bleached corals are deprived of the energetic benefits received from symbionts leading to decreased growth and reproductive capability, and increased disease susceptibility and mortality (Brown 1997, Abramovitch-Gottlib et al. 2003, Douglas 2003, Bruno et al. 2007). However, corals are also carnivores and are able to supplement their energetic requirements by heterotrophy which may increase their resilience to the negative effects of thermal stress and bleaching (Borell et al. 2008, Palardy et al. 2008, Connolly et al. 2012). The main aim of this study was to investigate the influence of heterotrophy on the resilience of corals to thermal stress and bleaching by using an experimental approach to test the effects of thermal stress and feeding on the physiological responses and health of corals.

1.2. The coral-algal symbiosis

Coral reef ecosystems result from the proliferation of hermatypic, or reef-building, corals which are cnidarians of the order Scleractinia (Muscatine & Porter 1977, Brusca & Brusca 2003, Lough 2008). During growth, hermatypic corals deposit skeletons of calcium carbonate which form the building blocks of coral reefs (Muscatine & Porter 1977, Shafir et al. 2006, Lough 2008). The success of hermatypic corals is due to the mutualistic symbiosis between metazoan coral polyps and unicellular algae known as zooxanthellae, the photosynthetic pigments of which may give some corals their colourful appearance (Muscatine & Porter 1977, Muller-Parker & D'Elia 1997, Baker et al. 2008, Lough 2008).

A hermatypic coral is essentially a colony of genetically identical polyps living in mutualism with zooxanthellae on a substrate of calcium carbonate deposited by the polyps during growth (Muscatine & Porter 1977, Muller-Parker & D'Elia 1997, Brickner et al. 2006). Each polyp is housed in a calcium carbonate tube, the corallum, and may be connected to adjacent polyps via the coenosarc and a confluent gastrovascular system which enables the transfer of nutrients or molecular signals to adjacent polyps increasing their scope for growth and repair (Muller-Parker & D'Elia 1997, Brickner et al. 2006, Palardy et al. 2008, Rosenberg et al. 2008). Polyps are attached to the substrate via a posterior pedal disc, and contain an outer epidermis and an inner gut cavity lined by a gastrodermis which is enclosed by an anterior mouth surrounded by tentacles (Muller-Parker & D'Elia 1997, Brusca & Brusca 2003). Polyp tentacles contain stinging nematocyst cells and are used to capture, immobilise and ingest zooplankton into the gut cavity or for defence from predators (Brusca & Brusca 2003, Houlbrèque & Ferrier-Pagès 2009). Polyps are also able to ingest particulate matter and absorb inorganic nutrients directly from the water column (Houlbrèque & Ferrier-Pagès 2009, Ferrier-Pagès et al. 2010). However, corals are highly dependent on acquiring nutrients from zooxanthellae which may contribute up to 100 % of the daily energetic requirements of some species (Muscatine & Porter 1977, Muscatine et al. 1981, Grottoli et al. 2004, Borell et al. 2008, Palardy et al. 2008).

Zooxanthellae are dinoflagellate protists of the genus *Symbiodinium* and are symbionts to various hosts of cnidarians such as corals, anemones, seas fans and jellyfish (Rowan 1998, Baker 2003). Zooxanthellae are also hosted by members of other classes of animals including molluscs, worms, sponges, other protists and various other taxa (Rowan 1998, Baker 2003). The genus *Symbiodinium* is divided into eight clades based on genetic differences (A-H) and is further divided into various subclades or strains (Baker 2003, Little et al. 2004, Coffroth & Santos 2005, Goulet 2006). Hermatypic corals usually contain zooxanthellae of clades A-D and F (Baker 2003, Coffroth & Santos 2005). The combination of populations of zooxanthellae clades resident in a coral colony depends on the species of the host, parental transmission, regional location, and the environmental conditions such as light intensity, temperature, pH and nutrients that are optimal to specific clades (Muller-Parker & D'Elia 1997, Rowan et al. 1997, Baker 2003, Karako-Lampert et al. 2005).

The coral-zooxanthellae symbiosis is a result of co-evolution between heterotrophs and autotrophs in a nutrient poor environment of tropical shallow water reefs (Muscatine & Porter 1977, Stanley Jr & Swart 1995, Furla et al. 2005). Coral polyps have evolved to aid optimal zooxanthellar photosynthesis and zooxanthellae have evolved to aid optimal coral growth for mutual benefit (Furla et al. 2005). Zooxanthellae are housed mainly in the polyp's gastrodermis and are able to transfer nutrients through the gastrovascular system of the coral (Titlyanov et al. 1996, Muller-Parker & D'Elia 1997, Furla et al. 2005). Zooxanthellae supplement polyp metabolism and growth with organic nutrients including glucose, fatty acids, amino acids and carbonate ions produced from photosynthesis (Pearse & Muscatine 1971, Porter 1976, Muller-Parker & D'Elia 1997, Papina et al. 2003, Borell et al. 2008). Zooxanthellae also allow for rapid coral skeletal growth via light-enhanced calcification (Pearse & Muscatine 1971, Muller-Parker & D'Elia 1997, Moberg & Folke 1999, Lough 2008). In return, coral polyps provide zooxanthellae with protection from the environment and a constant supply of inorganic nutrients such as carbon dioxide and ammonia required for photosynthesis (Muscatine & Porter 1977, Muller-Parker & D'Elia 1997, Bhagooli & Hidaka 2003).

1.3. Coral bleaching: causes, consequences and resilience

1.3.1. Definition and mechanism of coral bleaching

Coral bleaching is the result of a stress induced breakdown of the symbiosis between coral polyps and zooxanthellae (Brown 1997, Lesser 1997, Douglas 2003). The term coral bleaching is derived from the white appearance of corals following the loss of pigmented zooxanthellae which are expelled due to stress revealing the calcium carbonate skeleton through the colourless coral tissue (Brown 1997, Douglas 2003). The primary cause of widespread coral bleaching is a combination of high light intensity and high SST resulting from the onset of global warming (Brown 1997, Douglas 2003, Flores-Ramírez & Liñán-Cabello 2007, Baker et al. 2008). Some corals are particularly susceptible to increased temperatures because they live close to their upper thermal limit (Lesser 1997, Harley et al. 2006, Hoegh-Guldberg et al. 2007). Coral bleaching generally occurs when environmental temperatures exceed the mean seasonal maximum SST by 1-2°C (Brown 1997, Douglas 2003, Jokiel 2004, Baker et al. 2008). This can be monitored by satellite using a degree heating week method which indicates the accumulation of thermal stress on reefs over the maximum seasonal temperature for the past twelve weeks (Jokiel 2004, McClanahan et al. 2007).

Bleaching responses to high temperatures and light intensities are initially due to damage to zooxanthellae rather than coral tissue, particularly damage to lipid structures in the thylakoid membranes of zooxanthellae chloroplasts (Lesser 1996, Lesser 1997, Flores-Ramírez & Liñán-Cabello 2007, Baker et al. 2008). The structures affected include the oxygen-evolving complex which is the reaction centre of the Photosystem II of photosynthesis (Lesser 1996, Bhagooli & Hidaka 2004, Flores-Ramírez & Liñán-Cabello 2007). This damage may result in irreversible, chronic photo-inhibition if the rate of structure damage exceeds the rate of repair and decreases photosynthetic capacity for extended periods compared to dynamic photoinhibition where recovery is achieved much quicker (Lesser 1996, Lesser 1997, Gorbunov et al. 2001, Bhagooli & Hidaka 2004, Flores-Ramírez & Liñán-Cabello 2007). This photoinhibition results in the excessive production of reactive oxygen species, or ROS, due to the reaction of increased excitation energy produced by Photosystem I and oxygen produced by a

damaged Photosystem II (Downs et al. 2002, Bhagooli & Hidaka 2004, Tchernov et al. 2004, Flores-Ramírez & Liñán-Cabello 2007). ROS are damaging to zooxanthellae lipid structures further producing ROS in a positive feedback loop (Flores-Ramírez & Liñán-Cabello 2007). Host coral cells are also affected by ROS causing damage to DNA, protein and lipid structures (Downs et al. 2002, Douglas 2003, Flores-Ramírez & Liñán-Cabello 2007). If unmitigated, ROS build-up leads to zooxanthellae degradation or expulsion of zooxanthellae from polyp tissues to prevent further cellular damage causing bleaching (Lesser 1996, Downs et al. 2002, Douglas 2003, Flores-Ramírez & Liñán-Cabello 2007).

Coral bleaching can also be triggered at high ultraviolet radiation alone, low temperatures, low salinities and in response to disease (Muscatine et al. 1991, Van Woesik et al. 1995, Brown 1997, Douglas 2003, Saxby et al. 2003, Rosenberg et al. 2008). Similar to high light and temperature bleaching, the mechanism of bleaching at low temperatures is a result of the breakdown of photosystem II (Saxby et al. 2003, Hoegh-Guldberg et al. 2005). The mechanism of bleaching from low salinity has been suggested to result from hypo-osmotic damage to coral cells or enzymatic and membrane breakdown in zooxanthellae, which in turn affect photosynthetic capacity (Van Woesik et al. 1995, Moberg et al. 1997, Kerswell & Jones 2003). Bleaching from disease can have various causes depending on the pathogen, including the production of photosynthesis inhibiting molecules by bacteria leading to zooxanthellae expulsion, or viral and bacterial infection that result in zooxanthellae mortality (Brown 1997, Cervino et al. 2004, Rosenberg et al. 2008).

1.3.2. Susceptibility of corals to bleaching

Coral colonies show differential degrees of bleaching susceptibility within and between species, as well as within and between areas on a reef depending on individual genetics, placement on the reef, regional location and history of exposure to environmental conditions (Brown 1997, Rowan et al. 1997, Rowan 2004). Different growth forms of corals play a role in determining bleaching susceptibility (Marshall & Baird 2000, Loya et al. 2001, Guest et al. 2012). Branching corals such as *Acropora* and *Pocillopora* spp. are at high risk of bleaching because they rely more on energy from zooxanthellar

photosynthesis than heterotrophy and contain high zooxanthellae densities (Coles & Jokiel 1977, Loya et al. 2001, Guest et al. 2012). Massive corals such as *Favia* and *Favites* spp. rely more on heterotrophic feeding than zooxanthellar photosynthesis, contain low zooxanthellae densities and may thus be more resilient to bleaching (Coles & Jokiel 1977, Loya et al. 2001, Guest et al. 2012). However, recent research suggests that continual bleaching events may favour faster growing branching corals over slow growing massive corals (Guest et al. 2012). Although branching corals are susceptible to thermal stress at first, their fast-growing life history strategy promotes rapid evolution of adaptations to thermal stress while massive corals become less resilient with repeated occurrences (Guest et al. 2012). This was shown by Guest et al. (2012) who investigated changes in bleaching susceptibility in some Indonesian coral communities between the 1998 and 2010 warming events.

Similarly, the genetics of different zooxanthellae clades present in coral tissue also play a role in determining the bleaching susceptibility of coral colonies (Buddemeier & Fautin 1993, Rowan et al. 1997, Rowan 2004, Sampayo et al. 2008). For example, of the eight Zooxanthellae clades, it has been shown that the *Symbiodinium C* clade is one of the more susceptible to thermal stress and photoinhibition and *Symbiodinium D* is one of the more resistant clades (Rowan 2004, Sampayo et al. 2008). Zooxanthellae clade community structures within coral tissue may differ within areas on a colony, between colonies and between areas on a reef depending on parental and environmental transmission of zooxanthellae into coral tissue (Buddemeier & Fautin 1993, Loh et al. 1998, Baker 2003, Coffroth & Santos 2005, Gaither & Rowan 2010). Zooxanthellae clade communities in coral tissues organise according to the environmental conditions present because coral colonies are sessile and are not expected to experience conditions different to those of their current location (Baker 2003, Lewis & Coffroth 2004, Coffroth & Santos 2005, Coffroth et al. 2006, Gaither & Rowan 2010). Similarly, different areas on a colony such as exposed higher positioned branch tips experience different environmental conditions than lower positioned sheltered areas resulting in different zooxanthellae clade communities (Loh et al. 1998, Coffroth & Santos 2005). This acclimatization increases their susceptibility to environmental changes such as

increased temperatures (Baker 2003, Lewis & Coffroth 2004, Coffroth et al. 2006, Gaither & Rowan 2010).

Regional and topographical location on the reef is also a determining factor for thermal stress and bleaching susceptibility (Obura 2005, Fabricius 2006, Cooper & Ulstrup 2009). The topography of reefs allows for many different levels of shading, temperatures, water circulation and food availability (Obura 2005, Fabricius 2006, Cooper & Ulstrup 2009). Reefs that extend deeper than others or are positioned on coastlines or near structures that provide shading have a decreased risk of thermal stress during periods of increased temperature (Rowan et al. 1997, Obura 2005, Baker et al. 2008). Reef architecture also allows for shading in microenvironments as larger overhanging structures such as plate corals can shade the smaller underlying organisms (Obura 2005, Baker et al. 2008). Reefs that are subject to strong currents or positioned near upwelling zones may also be at a lower risk because warmer water can be cooled or exported out of the system before it induces stress (Obura 2005, Baker et al. 2008, Cooper & Ulstrup 2009). These currents also regulate the presence of nutrients and zooplankton prey needed to supplement the energetic requirements of corals under thermal stress (Borell et al. 2008). Coral location also relates to past experience as routine variations in environment variables and previous stress experiences at specific locations allow corals and zooxanthellae tissue communities to acclimatize to change (Brown et al. 2002, Obura 2005, Baker et al. 2008, Cooper & Ulstrup 2009). Corals routinely exposed to environmental change such as corals in shallow reefs and rock pools have been shown to be more resilient to thermal stress and bleaching (Obura 2005, Baker et al. 2008).

1.3.3. Ecological consequences of coral bleaching

Following bleaching, the energetic costs of stress responses and the loss of transferred energy supply from expelled zooxanthellae lead to an energy deficiency in coral cells (Brown 1997, Douglas 2003, Bruno et al. 2007). Due to this deficiency, bleached corals have reduced growth and reproduction which impacts negatively on coral populations (Szmant et al. 1990, Baird & Marshall 2002, Baker et al. 2008). Bleached corals also have less capacity for defensive responses, healing injuries and immune responses

which make them prone to disease and ultimately mortality and subsequent population decline (Glynn 1985b, Baird & Marshall 2002, Bruno et al. 2007, Baker et al. 2008, Lough 2008).

Losses of coral colonies and populations have negative impacts on the coral reef communities and ecosystems as a whole (Baker et al. 2008, Lough 2008). Symbiont crustaceans and fish associated with coral colonies are also at high risk of mortality following coral death because of the lack of food particles trapped in coral mucus and the lack of protection from predators (Glynn et al. 1985, Glynn & D'croz 1990, Glynn 1993). Coral predators and parasites that depend on corals are also at risk of mortality following coral death (Glynn 1985a, 1993, Pratchett et al. 2004). Consequently, the loss of corals has direct negative impacts on invertebrate and fish populations that depend on corals for food, protection and habitat (Glynn 1993). Coral mortality allows for macroalgal growth over coral skeletons, and combined with other anthropological impacts such as overfishing and pollution it may lead to an ecological shift from coral dominated reefs to macroalgal dominated reefs and thus a major loss of biodiversity (Hughes 1994, Harley et al. 2006, Hughes et al. 2007). The loss of coral populations and overall biodiversity results in a major loss in ecosystem functions such as coastal protection, water filtration, fisheries and tourism potential (Moberg & Folke 1999, Baker et al. 2008, Knowlton 2008).

1.3.4. Recovery from coral bleaching

Coral resilience to bleaching is determined by the strength and efficiency of the physiological responses initiated by zooxanthellae and polyp cells to prevent and repair damage (Downs et al. 2000, D'Croz & Maté 2004, McClanahan et al. 2004, Baird et al. 2009), and the extent to which coral polyps depend on energy transfer from zooxanthellae compared to heterotrophy (Marshall & Baird 2000, McClanahan et al. 2004, Grottoli et al. 2006, Guest et al. 2012). These responses are usually species specific thus coral resilience is dependent on genetics and effective gene regulation during and following thermal stress (Grottoli et al. 2006, Bellantuono et al. 2012, Guest et al. 2012). Recovery of a coral community from bleaching is similarly dependant on

adaptations and zooxanthellae dependence but also depends on the health of the ecosystem as a whole (Obura 2005, Hughes et al. 2007).

Recovery from bleaching is largely dependent on the coral's ability to replace the nutrients lost from expelled zooxanthellae by heterotrophic feeding to fulfil their energetic requirements (Grottoli et al. 2006, Borell et al. 2008, Palardy et al. 2008). Bleached corals may survive using glycogen and lipid reserves and increasing their predation and nutrient absorption effort (Grottoli et al. 2006, Ferrier-Pagès et al. 2010, Imbs & Yakovleva 2012). Healthy zooplankton populations are needed for coral feeding and subsequent recovery (Ferrier-Pagès et al. 2003, Borell et al. 2008, Palardy et al. 2008). Some species such as *Colpophyllia natans* and *Montastraea faveolata* may recover from bleaching by feeding on adjacent algal turfs by extension of their mesenterial filaments (Marhaver 2011). The presence of algae may therefore be important to recovering corals following bleaching but must be regulated by herbivores to prevent the inhibition of coral growth by algal acquisition of substrate created by dead coral tissue (Hughes et al. 2007, Marhaver 2011). Coral skeletons of species such as *Oculina patagonica* contain endolithic algae that may provide nutrients to bleached coral tissue (Fine & Loya 2002). Coral associates such as crustaceans and herbivorous fish are essential to coral bleaching recovery by preventing excessive predation and opportunistic algal overgrowth on corals respectively (Obura 2005, Stewart et al. 2006, Hughes et al. 2007).

Management tools for the conservation of coral reefs include the maintenance of healthy coral ecosystems and the protection of areas less susceptible to coral bleaching ensuring that adequate reseeding areas are available (Reaser et al. 2000, Nicholls & Lowe 2004, Yeemin et al. 2006, Baker et al. 2008). Strategies to alleviate coral bleaching and prevent mortality include: reef shading, feeding, electrical stimulation, zooxanthellae inoculation, and inducing artificial upwelling (Reaser et al. 2000, Nicholls & Lowe 2004, Yeemin et al. 2006, Baker et al. 2008).

1.4. Biomarkers of thermal, oxidative and bleaching stress in corals

Corals colonies are sessile and cannot escape from elements causing stress but may only respond to stress by limited changes in polyp behaviour, activating cellular stress response mechanisms and expelling zooxanthellae (Glynn & D'croz 1990, Downs et al. 2000, Gochfeld 2004, Ferrier-Pagès et al. 2010). These responses can be quantified and are useful as biomarkers of coral stress from increased temperatures. This study investigated several biomarkers of coral stress including polyp extension, feeding rates, oxygen consumption rates, colour, zooxanthellae density, chlorophyll *a* content, lipid content, antioxidant potential and DNA damage to give a holistic view of coral physiology at control conditions and under stress.

Prior to bleaching, coral polyps are under thermal stress from the environment and oxidative stress from zooxanthellar ROS production resulting in damage to the molecular structures in coral cells (Lesser 1996, Lesser 1997, Flores-Ramírez & Liñán-Cabello 2007). Corals cannot move away from environmental stress but may decrease their exposure to stress by retracting their polyps (Glynn & D'croz 1990, Salih et al. 2000, Gochfeld 2004). Consequently, investigating behavioural responses such as polyp retraction or extension is a useful non-invasive method of assessing coral health especially as it may indicate stress early and prior to excessive damage and recovery thereafter.

If coral polyps still cannot cope with the oxidative stress, zooxanthellae cells are detached and expelled to prevent further cellular damage (Gates et al. 1992, Brown 1997). Bleaching is usually quantified by the measurement of chlorophyll content and zooxanthellae density in harvested coral tissue (Muscatine et al. 1991, Downs et al. 2002, Visram & Douglas 2007). The degree of bleaching can also be determined using non-invasive tools such as colour measurement using reference cards (Siebeck et al. 2006).

Corals under stress are at an energy deficit from energetically costly stress responses or the loss of energy transfers from expelled zooxanthellae (Brown 1997, Douglas 2003). Coral may be able to offset this energy deficiency by utilizing glycogen and lipid stores

and actively increasing their feeding and nutrient absorption effort from other sources (Grottoli et al. 2004, Palardy et al. 2008, Ferrier-Pagès et al. 2010, Imbs & Yakovleva 2012). Investigating lipid content in coral tissues and feeding rate can therefore be used to assess the energetic state of corals under thermal stress and their potential resilience to thermal stress and bleaching.

Increased temperatures increase coral metabolic rates and cellular responses to thermal and oxidative stress which are energetically costly and increases coral respiration and oxygen consumption (Coles & Jokiel 1977, Lesser 1996, Edmunds 2005, Flores-Ramírez & Liñán-Cabello 2007). This increase in respiration further increases ROS particles produced from mitochondrial electron transport leakage resulting in further oxidative stress (Lesser 1996, Lesser 1997). Measuring oxygen consumption can thus be used as a semi-invasive tool to assess the metabolic state of corals under thermal stress.

If unmitigated, thermal and oxidative stress cause damage to protein, lipid and DNA structures in coral cells (Lesser 1996, Lesser 1997, Flores-Ramírez & Liñán-Cabello 2007). Heat-shock proteins and enzymatic repair mechanisms such as lipid biosynthesis are responsible for repairing damaged protein and lipid structures (Feder & Hofmann 1999, Downs et al. 2000, Flores-Ramírez & Liñán-Cabello 2007, Baird et al. 2009, Imbs & Yakovleva 2012) and DNA can be repaired via mechanisms such a photoreactivation and nucleotide incision repair (Baruch et al. 2005, Reef et al. 2009a, Rosic & Dove 2011). Antioxidants such as ascorbic acid, catalase and superoxide dismutase are produced to scavenge ROS particles and prevent cellular damage (Dunlap & Yamamoto 1995, Griffin & Bhagooli 2004, Yakovleva et al. 2004, Baird et al. 2009). Investigating repair mechanisms by measuring the antioxidant potential of corals cells can be used to assess the health and resilience of corals to thermal stress. If repair mechanisms are inadequate excessive damage to coral cells occurs thus assessing the damage to molecular structures such as DNA is important.

1.5. Study premise, aims and objectives

Rising atmospheric and sea surface temperatures cause thermal stress in corals leading to coral bleaching (Lesser 1997, Baker et al. 2008). The loss of symbionts during bleaching translates to a loss in symbiont energy transfers and may lead to mortality (Brown 1997, Baker et al. 2008). Despite their dependence on zooxanthellae, corals are also able to feed heterotrophically (Grottoli et al. 2006, Houlbrèque & Ferrier-Pagès 2009). Consequently, a natural response of corals to bleaching would be to turn to heterotrophy to supplement their energetic requirements (Grottoli et al. 2006, Houlbrèque & Ferrier-Pagès 2009). Heterotrophy may therefore play a role in determining the resilience of corals to thermal stress and bleaching and their rate of recovery. The main aim of this study was to investigate the role played by heterotrophy in contributing to the resilience of corals to thermal stress and bleaching and the ability of corals to recover from bleaching. The overall hypothesis for this study is that feeding plays a role in coral resilience by providing the energy and nutrients required for corals to respond to thermal stress and bleaching and maintain basal metabolism and overall health.

This study assessed the behaviour, physiology, tissue composition and cellular responses of corals that were experimentally exposed to high temperatures followed by a short recovery period with or without the presence of food. Coral health was monitored over time using non-invasive techniques such as measuring polyp extension, colour, feeding rates and oxygen consumption rates for the duration of the study. Changes in coral tissue composition were investigated by harvesting coral tissue and measuring zooxanthellae density and chlorophyll *a* content as a proxy for bleaching responses and lipid content as a proxy for energy reserve status. Similarly, invasive techniques were used to investigate intracellular physiological responses by harvesting coral tissue and measuring total antioxidant potential and DNA damage as a proxy for oxidative stress responses and damage respectively.

Although some studies have focused on aspects of coral physiology and feeding such as chlorophyll, zooxanthellae, photosynthetic efficiency and respiration under thermal stress and following recovery (Hueerkamp et al. 2001, Grottoli et al. 2004, Grottoli et

al. 2006, Ulstrup et al. 2006, Borell et al. 2008, Palardy et al. 2008, Ferrier-Pagès et al. 2010). Studies assessing the influence of heterotrophy on biochemical endpoints such as tissue lipid content are few but have been looked at recently (Imbs & Yakovleva 2012) and antioxidant capacity and DNA integrity have not yet been worked on to my knowledge. Also, to my knowledge, no single study has investigated the effect of thermal stress on this many levels of coral organisation, from behavioural to cellular. Consequently, this is the first research to investigate the influence of heterotrophy on the resilience of corals to thermal stress and bleaching using an integrated approach assessing biomarker responses from behavioural down to cellular level.

Pocillopora damicornis was chosen as the subject species due to ease of access and abundance on the KwaZulu-Natal coast, large global distribution range and the range of coral bleaching research already done on this species (Coles & Jokiel 1977, Baker et al. 2008, Cooper & Ulstrup 2009, Branch et al. 2010). *Pocillopora damicornis* is also a fast growing branching coral and so is less dependent on heterotrophy than massive corals (Loya et al. 2001, Grottoli et al. 2006). Consequently, if heterotrophy is shown to benefit recovery of a branching coral it may have more profound implications for other growth forms. Investigating thermal stress on corals from the KwaZulu-Natal coast also gives insight into the resilience of corals in temperate/sub-tropical zones which are regarded as marginal areas and are subjected to more variability in conditions than tropical and sub-tropical zones (Celliers & Schleyer 2002, McClanahan et al. 2007, Baker et al. 2008). These corals are also present in rock pools which are subject to further variability so results may have more serious implications for corals acclimatised to more stable conditions. There is also evidence that some coral distribution ranges may expand into and within higher latitudes to adapt to global warming (Baker et al. 2008, Doney et al. 2012). Consequently, communities currently occupying these areas may change to accommodate these corals and may therefore be prone to bleaching as SSTs continue to increase in the future (Muller-Parker & D'Elia 1997, Baker et al. 2008, Doney et al. 2009, Doney et al. 2012).

Global warming and other climatic changes are still an imminent threat to coral reefs. For example, according to the IPCC (2007), sea surface temperatures on the South

African east coast have been projected to rise by at least 0.5-1°C by 2029. Thus, coral bleaching and the decline of coral reefs is still a major threat to biodiversity and the economy. Knowledge regarding coral resilience to perturbation by investigation coral physiology during and following perturbation is therefore important for informed decision making for coral reef management authorities. Management authorities must be ready to implement mitigation measures should the need arise. Thus, it is also important to provide information for the most ecologically and economically viable options for management which was a possible outcome for this study.

CHAPTER 2: MATERIALS AND METHODS

2.1. Specimen collection and maintenance

Specimens of *Pocillopora damicornis* were collected from rock pools at Park Rynie (30° 19′ 0″ S, 30° 44′ 0″ E), on the South Coast of KwaZulu-Natal, South Africa in May 2012. Twenty four colonies were removed from the substrate using a hammer and chisel and transported in buckets with seawater to a shaded and air-conditioned glasshouse at the University of KwaZulu-Natal, Westville campus. Specimens were then randomly assigned to twelve aquaria for acclimation. The twelve aquaria were divided among four recirculating systems. Each system consisted of three 150 L aquaria (400×420×950 mm) connected to a central sump recirculating via King 6 submersible pump with a flow rate of 8500 $L.hr^{-1}$ (RESUN®, Shenzhen, China). Each aquarium was fitted with a power head submersible pump (REAL, Krugersdorp, South Africa) with a flow rate of 2800 $L.hr^{-1}$ to provide water motion and circulation for specimen maintenance.

Water quality was maintained by 300 Watt aquarium heaters (REAL, Krugersdorp, South Africa), a SA-2013 protein skimmer (WEIPRO®, Zongshan, China), 100 µ nylon mesh filters and live-rock biofilters. Water changes of 20 % fresh FSW (10 µm), cotton filter changes, one teaspoon of calcium hydroxide addition per system, and water quality tests were done weekly and tanks were cleaned of debris and algae monthly. Specimens were maintained at sunlight shaded by 60 % black shade cloth, temperature at 26°C monitored twice a day using aquarium thermometers, salinity at 35 ppt monitored daily using a Master refractometer (ATAGO®, Tokyo, Japan), pH at 8.2, ammonia, nitrite, nitrate and phosphate at 0 ppm and alkalinity at 1.7-2.8 $meq.L^{-1}$ monitored weekly using a Marine Lab aquarium test kit (RED SEA, Houston, TX, USA). After a week of acclimation the coral colonies were fragmented into nubbins of *ca*. 3 cm using wire cutters (n = 6-18 per colony). Nubbins were randomly assigned to the twelve aquaria, attached to plastic pegs cable-tied to plastic trays and left to acclimate for a further two weeks before experiments began.

2.2. Experimental setup and sampling procedure

For the duration of the experiment, specimens in six of the twelve tanks were assigned as temperature controls (TC) and specimens in the remaining six tanks were assigned as exposed to increased temperatures (TE) treatment (Fig. 2.1). TC specimens were maintained at constant temperatures of 25.86 ± 0.53°C (mean ± SD) (Table 2.1). Temperature for specimens in the TE treatment was raised by 1.53 ± 0.88°C (mean ± SD) daily for three days to a maximum temperature of 30.23 ± 0.71°C (mean ± SD) (Table 2.1). Maximum temperatures were maintained for a further three days and thereafter decreased by 1.36 ± 1.07°C (mean ± SD) daily to 25.75 ± 0.56°C (mean ± SD)(Table 2.1). These temperature change rates were selected to simulate natural bleaching events (Siebeck et al. 2006) and based on pilot temperature tolerance experiments which showed that 32°C was the thermal limit at which 100 % of coral nubbins died after one day of exposure (n = 6, Table 2.2). Temperature increases of 2°C daily showed more distinguishable coral colour changes than 1°C increments (personal observation).

Three of the six tanks for each of the TC and TE treatments were assigned as starved treatments and the remaining three were assigned as fed treatments (Fig. 2.1). Corals in the starved treatment received no additional nutrients while those in the fed treatment were supplied daily with live freshly hatched *Artemia salina* nauplii at a concentration of 1626 ± 737 (mean ± SD) individuals.L^{-1}. Corals are usually nocturnal predators in the wild so experimental nubbins were fed at night. Pilot observations on the feeding of nine coral nubbins using a magnifying glass, showed that excessive water motion from the aquarium pump reduced feeding to 0.04 ± 0.1 *A. salina* individuals.$nubbin^{-1}$.min^{-1} (mean ± SD) compared with 1.16 ± 0.05 individuals.$nubbin^{-1}$.min^{-1} (mean ± SD) when the pumps were turned off. Recirculation was therefore stopped during feeding. Nauplii were added and corals were allowed to feed for three h. Aquarium pumps in each tank were switched on briefly every ten min to allow for aeration and even mixing of nauplii.

During the experimental period, coral polyp extension and colour as a proxy for health were measured every two days by the same observer (Table 2.1). Polyp extension was determined visually as the number of nubbins in each tank with their polyps fully

extended at midday. Polyp extension was not taken at night due to poor visibility, and polyp retraction due to prey capture. Coral colour was determined by visually matching each nubbin colour with a CoralWatch colour reference card developed by Siebeck et al. (2006) which assigns coral colour on a scale of 0 to 6, from bleached to healthy respectively. The colour score for each nubbin was expressed as the average of the darkest and lightest coloured polyps on the nubbin.

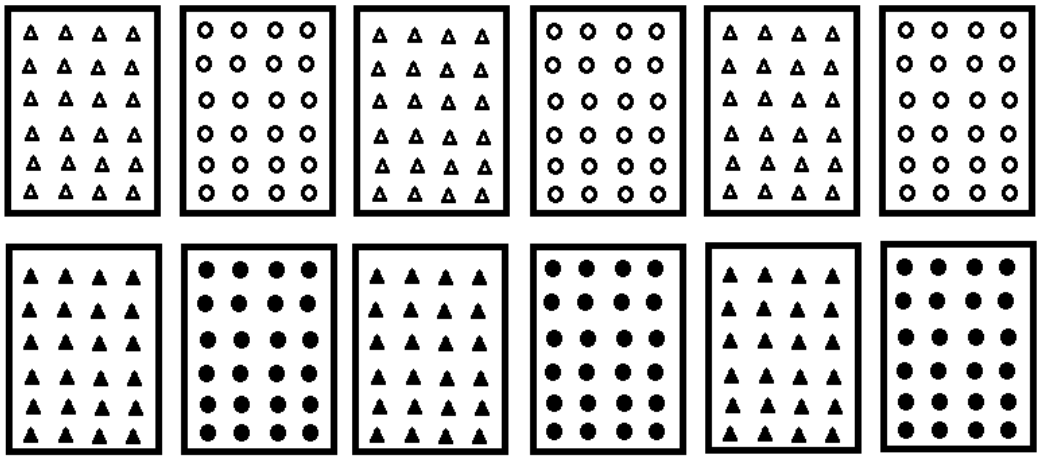

Fig. 2.1. Diagrammatic representation of the experimental setup. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved.

Dark oxygen consumption rates of three corals from each tank were measured at the start of the experiment (day 0, Table 2.1), at maximum temperature exposure (day 4, Table 2.1), at the end of exposure (day 9, Table 2.1) and after a recovery period at control temperatures (day 16, Table 2.1). Oxygen concentrations in containers with nubbins were measured using a YSI Model 52 dissolved oxygen meter (YSI, Yellow Springs, OH, USA) at the start of incubation. Containers were then sealed with caps and petroleum jelly under water and completely covered in foil to prevent light exposure and left to incubate in tanks for 90 min. A second measurement was then taken with the exact start and end times noted. Oxygen consumption rates expressed as $mg^{-1}O_2.cm^{-2}.min^{-1}$ were calculated from the depletion of oxygen concentration by each nubbin divided by time and nubbin surface area, as detailed in section 2.6.

Table 2.1. Sampling schedule, associated temperatures (°C, mean ± SD) and weather conditions for each day during the experiment. For the exposure, columns with ↑ symbols represents the days, 0-2, during which temperature was increased by 2°C daily from 26°C, ▽ symbols represents days, 3-6, at maximum exposure set at 32°C, ↓ symbols represents the days, 7-9, during which the temperature was decreased to control temperatures set at 26°C, ■ symbols represents the recovery period at control temperatures. Exposed treatments were accompanied by control treatments, in which temperatures were not altered and all treatments and controls comprised of sub-treatments where animals were either fed or starved.

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Exposure	↑	↑	↑	▽	▽	▽	▽	↓	↓	↓	■	■	■	■	■	■	■	■	■
set temp.	26	28	30	32	32	32	32	30	28	26	26	26	26	26	26	26	26	26	26
Exposure	25.5	27.4	28.3	30.1	30.3	30.5	30.1	29.7	28.4	26	25.8	26.3	25.8	25.5	25.8	25.5	25.5	25.9	25.5
actual temp.	± 0.7	± 0.7	± 1.4	± 1.1	± 0.3	± 0.5	± 0.7	± 0.5	± 0.6	± 0.3	± 0.3	± 0.7	± 0.3	± 0.5	± 0.3	± 0.5	± 0.5	± 0.5	± 0.5
Control	25.9	26.3	26	26	26	25.9	25.9	25.9	26	25.5	25.4	26.1	26.3	26	25.5	25.5	25.8	26	25.5
actual temp.	± 0.7	± 0.5	± 0.8	± 1.4	± 1.6	± 1.6	± 1.5	± 1.5	± 0.8	± 0.5	± 0.6	± 0.9	± 0.8	± 0.5	± 0.5	± 0.5	± 0.3	± 0.5	± 0.5
Weather[a]	CLR	CLR	CLR	CLR	CLR	CLR	CL	CL	OVC	CLR	CLR	CLR	OVC	CLR	CLR	CLR	OVC	CLR	CLR
Colour[b]	×		×		×		×		×		×		×		×		×		×
Polyp ext.[c]	×		×		×		×		×		×		×		×		×		×
O_2 cons.[d]	×				×					×							×		
Feeding[e]	×				×					×							×		
Tis. ext. frz[f]						×												×	
Tis. ext. liv[g]							×												×

[a] Weather recorded for the day, CLR denotes clear/sunny conditions, CL denotes cloudy/partly cloudy conditions and OVC denotes fully overcast conditions.

[b] Colour health scores taken for each coral nubbin (Siebeck et al. 2006).

[c] Polyp extension recorded during the day for each coral nubbin.

[d] Oxygen consumption measured for three coral nubbin in each tank

[e] Feeding rate measured as food concentration depletion over a 90 min period.

[f] Coral nubbins frozen for later analyses.

[g] Extraction of live tissue from coral nubbins for analyses.

Q_{10} values were calculated for oxygen consumption rates using the equation: $Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$, where R_2 is the rate on day 4 at maximum temperatures (T_2), R_1 is the rate on days 0, 9 and 16 at control temperatures (T_1).Q_{10} values between day 0 and 4 represented the rate of increase of oxygen consumption rate as temperature increased during exposure whilst Q_{10} values between day 4 and 9 and day 4 and 16 represented the rate of decrease of oxygen consumption rate as temperature decreases during recovery.

On the same days that oxygen consumption rates were measured, feeding rates in each tank were also determined at night. Three water samples of 10 mL were taken per tank at the start of feeding and again 90 min later. Nauplii that were dead and stuck to algae were not taken into account. Water samples were taken after pumps were turned on for 5 min to allow for even distribution of nauplii. *A. salina* nauplii were counted and feeding rates were calculated as the depletion of *A. salina* concentration per nubbin per min.

At maximum water temperature (day 5), six nubbins from each tank were removed, covered in aluminium foil, flash-frozen in liquid nitrogen and stored at −80°C for analyses of total lipid content, total protein concentration and antioxidant potentials (Table 2.1). On day 6, three nubbins were removed and immediately processed for zooxanthellae density, chlorophyll *a* content and DNA damage (Table 2.1). Sampling was repeated on days 17 and 18, after temperatures had been decreased and a recovery period of 8 days had been allowed (Table 2.1).

Table 2.2. Mean ± SD coral colour health scores (Siebeck et al. 2006) of four coral nubbins at different temperature regimes. Scores range from 0 to 6, from dead at 0 increasing to best health at 6.

Day	1	2	3	4	5
26°C static	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0
32°C static	4 ± 0	3 ± 2	0 ± 0	0 ± 0	0 ± 0
26°C + 2°C daily	4 ± 0	4 ± 0	4 ± 0	3.5 ± 0.6	0.5 ± 1

2.3. Tissue analyses for zooxanthellae density, chlorophyll a content and total lipid content

Fresh coral tissue was extracted from the skeleton following Johannes and Wiebe (1970). An Aquaflosser dental water pik (AQUAPULSE, Cape Town, South Africa) filled with FSW (GF/F) was used to remove coral tissue into a re-sealable plastic storage bag. The resultant slurry was filtered through a 180 µm nylon mesh to remove mucus and homogenized in a beaker with an Ultra-Turrax® stirrer homogenizer (IKA®, Staufen, Germany). The total volume of the resultant homogenate was recorded and it was subsequently divided into three aliquots each of: 10 mL into plastic bottles for zooxanthellae density counts, 15 mL into 15 mL centrifuge tubes for chlorophyll *a* content measurement, and 300 µL micropipetted into 1.5 mL microcentrifuge tubes for DNA damage measurement. Extraction of coral tissue for lipid content was performed according to the same protocol on frozen coral nubbins.

To determine the zooxanthellae density of samples, 10 mL of coral tissue homogenate was fixed in 4 % formalin in FSW and zooxanthellae were counted in a Fusch-Rosenthall counting chamber (MARIENFELD, Lauda-Königshofen, Germany) under a light microscope at 100 times magnification. Eight replicate counts were performed per sample and resultant cell concentrations were divided by slurry volume and nubbin surface area, as detailed in section 2.6, to obtain zooxanthellae densities in cells.cm^{-2}.

To determine the chlorophyll *a* content of samples, 15 mL of coral tissue homogenate was centrifuged at 4400 rpm for 5 min, the supernatant was discarded and the pellet was re-suspended in 15 mL of 99 % acetone. Samples were then kept in the dark in a freezer for 24 h and centrifuged again at 4400 rpm for 5 min, and analysed using a Trilogy® flourometer fitted with a chlorophyll *a* acidification module (TURNER DESIGNS, Sunnyvale, CA, USA). Three readings were taken per sample and resultant chlorophyll *a* concentrations were divided by slurry volume and nubbin surface area, as detailed in section 2.6, to obtain chlorophyll *a* content in µg.cm^{-2}.

Total lipids were extracted following Bligh and Dyer (1959). A 50 mL aliquot of homogenate was centrifuged at 4400 rpm for 5 min, the resultant supernatant was

discarded and the pellet was re-suspended in 6 mL of 1:2 (v/v) $CHCl_3$:CH_3OH. The sample was then homogenised and 2 mL of $CHCl_3$ was added, homogenised again and 2 mL of distilled water was added. The sample was then homogenised and then centrifuged at 1000 rpm for 5 min to separate the sample into an aqueous top layer and an organic bottom layer containing the extracted lipids. The bottom layer of the sample was removed using a Pasteur pipette, placed into a pre-weighed aluminium foil weighing boat, left to dry overnight and weighed again. The difference in the final weight of the boat with lipid and the initial weight of the empty boat was an estimation of total lipids in the sample which was divided by slurry volume and nubbin surface area, as detailed in section 2.6, to obtain lipid content in $mg.cm^{-2}$.

2.4. Antioxidant potential analyses

Coral pieces of *ca*. 1 g were broken from stored frozen nubbins and placed into sterile 2 mL microcentrifuge tubes. One millilitre of 10 $mmol.L^{-1}$ Tris-HCl (pH 7.5) was added to the tubes which were then placed in a TissueLyzer LT (QIAGEN, Hilden, Germany) to separate tissue from the skeleton. Samples were centrifuged at 10,000 rpm for 10 min to remove zooxanthellae, cellular and skeletal debris. The resultant supernatant was removed and placed in sterile 1.5 mL microcentrifuge tubes, 40 µL of which was used immediately for total protein analysis. The remaining supernatant was stored at -80°C after the addition of 5 µL of a protease inhibitor cocktail (Sigma P8340) to prevent protein degradation in stored samples.

Protein concentrations of samples were determined using a Pierce BCA total protein assay kit (THERMO SCIENTIFIC, Waltham, MA, USA) and performed to manufacturer's specifications. The kit is based on the bicinchoninic acid (BCA) method of protein determination involving the reduction of Cu^{+2} to Cu^{+1} in the presence of bicinchoninic acid (Smith et al. 1985). Triplicates of 10 µL of each coral protein sample and a standard series of bovine serum albumin of concentrations 0, 25, 125, 250, 500, 750, 1000, 1500 and 2000 $\mu g.mL^{-1}$ were added to a 300 µL 96 well microtiter plate. Thereafter, 200 µL of working reagent, comprising 50 parts reagent A and 1 part Reagent B was then added to each well. The plate was incubated at 37°C for 30 min,

cooled to room temperature and shaken before the absorbances for each well were read at 562 nm using a PowerWave XS multiwell plate reader (BIOTEK, Winooski, VT, USA). Protein concentrations in $\mu g.mL^{-1}$ were calculated comparing the absorbance of the sample to a standard curve generated from the absorbances of the standard albumin series.

Total antioxidant potential of samples were determined using the ferric reducing/antioxidant potential (FRAP) assay adapted from Griffin and Bhagooli (2004). The FRAP assay compares the intensity of the colour change from the reduction of ferric tripyridyltriazine (Fe^{+3}-TPTZ) to ferrous tripyridyltriazine (Fe^{+2}-TPTZ) by antioxidants to a standard series of Fe^{+2} (Griffin & Bhagooli 2004). Three replicates of 20 µL of protein samples and a standard series of Fe^{+2} from $FeSO_2.7H_2O$ (0, 25, 50, 75, 100, 150, 200, 500, 1000 $\mu mol.L^{-1}$) were added to a 300 µL 96 well microtiter plate. FRAP working reagent was freshly prepared by mixing 300 $mmol.L^{-1}$ acetate buffer (pH 3.6, prepared in 1.6 % glacial acetic acid), 10 $mmol.L^{-1}$ TPTZ in 40 $mmol.L^{-1}$ HCl solution and 20 $mmol.L^{-1}$ $FeCl_3.6H_2O$ in a 10:1:1 ratio respectively and 150 µL FRAP reagent was thereafter added to each well. The plate was then incubated at 37°C for 20 min in a PowerWave XS multiwell plate reader which was also used to read the cooled plate absorbance at 600 nm (BIOTEK, Winooski, VT, USA). FRAP values were then calculated by comparing sample absorbance to a standard curve of the Fe^{2+} and divided by the amount of protein previously determined for the sample to obtain the antioxidant potential in $\mu mol.L^{-1}.\mu g^{-1}$.

A trial analysis with coral samples was performed to ascertain that a 20 min incubation, as suggested by Berker et al. (2007), was adequate for the complete potential reduction of Fe^{3+} to Fe^{2+}. This was facilitated by tracking the reduction for 30 min, using the kinetic function of the KC4™ data analysis software package (BIOTEK, Winooski, VT, USA) installed with the multiwell plate reader. It was confirmed that a plateau was reached within 20 min.

2.5. DNA integrity analysis

DNA integrity was measured using a single cell gel electrophoresis assay, also referred to as the Comet assay, following Baruch et al. (2005). Microcentrifuge tubes containing 300 µL coral cell suspensions were centrifuged at 14000 rpm for two min. The resultant supernatant was discarded and the pellet was re-suspended in 200 µL of 0.65 % low melting point agarose (Sigma A9414) in Kenny's salt solution (KLMA) containing 0.4 $mol.L^{-1}$ NaCl, 9.0 mmol μL^{-1} KCl, 7.0 $mmol.L^{-1}$ K_2HPO_4 and 2.0 $mmol.L^{-1}$ $NaHCO_3$ (Steinert et al. 1998, Baruch et al. 2005). KLMA was maintained at 35°C in a water bath. Frosted microscope slides with two clear viewing windows were pre-coated with 1.0 % high melting point agarose (Sigma A9539) in 0.1 $mol.L^{-1}$ EDTA (pH 7.5) at 85°C. Each of the viewing windows was covered with 50 µL of Cell-KLMA suspension then dried and covered again with 50 µL of KLMA and finally kept on ice until solidified.

Slides containing cells were then immersed in a lysis buffer containing 2.5 $mol.L^{-1}$ NaCl, 0.1 $mol.L^{-1}$ EDTA, 1.0 % Triton X-100, 1.0 % DMSO (pH 7.5) and left overnight in the dark at 4°C for coral cell lysis (Baruch et al. 2005). After removal from lysis buffer, slides were rinsed with distilled water for 5 min then soaked in electrophoresis buffer containing 0.3 $mol.L^{-1}$ NaOH and 1.0 $mmol.L^{-1}$ EDTA, for 20 min in a horizontal gel electrophoresis apparatus to allow for DNA unwinding . Electrophoresis was then performed at 20 V and 300 mA for 20 min. Slides were removed, rinsed with distilled water for 5 min and neutralised in fresh ice cold 0.8 $mol.L^{-1}$ Tris-HCl buffer (pH 7.5). They were then rinsed with distilled water for 5 min, stained in 0.01 $mmol.L^{-1}$ EtBr, rinsed with distilled water again and kept moist at 4°C in the dark until image analysis. Fifty comets per slide were photographed under a fluorescent microscope (Nikon Eclipse E400 microscope with a Nikon E5400 camera, Tokyo, Japan) at 200× magnification (Fig. 2.2). Comet images were analysed using the comet scoring software CASP 1.2.3b (CASPLab.com, 2010, Wrocław, Poland) to determine the percentage of tail DNA and olive tail moment (OTM) which is the product of the difference of comet head and tail and the tail size fraction, thus, OTM = (mean tail length - mean head length) × (Mean Tail DNA % / 100).

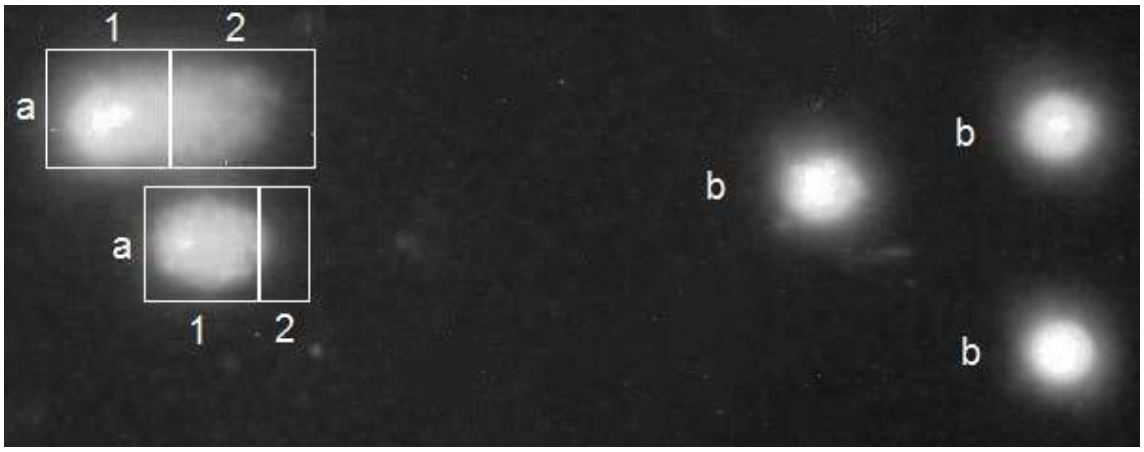

Fig. 2.2. Comet image at 200 times magnification showing the DNA integrity of coral cells exposed to elevated temperatures. Letter a) represents comets of damaged DNA with boxes 1) showing the comet head of undamaged DNA and 2) showing the comet tail of broken DNA strands migrated under electrophoresis. Letter b) represents comets of undamaged DNA with no comet tails.

2.6. Coral surface area measurement

Oxygen consumption rates, zooxanthellae densities, chlorophyll *a* content, and total lipid content measurements were standardised to coral surface area in cm^2 which was determined using the paraffin wax method (Stimson & Kinzie 1991, Naumann et al. 2009). Coral skeletons free of tissue were dried and dipped in paraffin wax melted at 80°C on a heating block. Wax covered nubbins were dried for five min then weighed. This first dip was to smooth over the porosity of the skeleton which may absorb the wax resulting in an inaccurate surface area measurement. Nubbins were dipped in wax again, dried for five min and weighed again. The difference in mass between the second and first coat of wax was used to estimate coral surface area by comparison to a wax weight to surface area calibration curve. The calibration curve was determined from similarly dipping a series of shapes of known surface areas in wax to determine the wax weight and performing a regression analysis of wax weight vs. surface area. Four wooden blocks and four glass spheres were used for surface area calibration (2.27, 6.62, 7.39, 20.7, 25.35, 37.03, 54.0, 73.74 cm^2). The resultant calibration equation determined is as follows: coral surface area = (0.007 – wax weight) / 0.0158, (r^2 = 0.99).

2.7. Statistical analyses

Repeated measures two-factor nested ANOVAs were used to compare polyp extension, colour, oxygen consumption and feeding rates (one-factor) between nubbins in the different feeding treatments (excluding feeding rates), nested within temperature treatments, sampled every two days for polyp extension and colour and on days 0, 4, 9 and 16 for oxygen consumption and feeding rates. Repeated measures ANOVA assumption of sphericity was tested using Mauchly's sphericity tests. Where assumptions were not met ($p < 0.05$), the Greenhouse-Geisser adjustment was performed. Two-factor nested ANOVAs were used to compare Q_{10} values between starved and fed corals. Three-factor nested ANOVAs were used to compare zooxanthellae densities, chlorophyll *a* content, chlorophyll *a* content per zooxanthella, lipid content, DNA damage and antioxidant potentials between feeding treatments nested within temperature treatments which was further nested within time point sampled. Bonferroni post-hoc tests were performed to identify statistical differences between all tested groups. Nested ANOVA assumptions of residual normality and homoscedasticity were tested using one-sample Kolmogorov-Smirnov and Levene's tests respectively ($p > 0.05$). All statistical analyses were performed using STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA).

CHAPTER 3: RESULTS

3.1. The influence of increased temperatures and feeding on coral health over time

3.1.1. Coral polyp extension

The percentage of coral nubbins with extended polyps was significantly lower overall (main effects) for those corals that were exposed to high temperatures (TE) than those kept at constant control temperatures (TC) ($n = 60$, $df = 1$, $F = 61.91$, $p < 0.005$). Overall polyp extension percentages changed significantly over time ($n = 12$, $df = 9$, $F = 10.03$, $p < 0.005$), decreasing during exposure (Table 3.1, Fig. 3.1). Overall polyp extension percentages did not differ significantly between starved and fed corals ($n = 60$, $df = 1$, $F = 0.72$, $p = 0.4$).

Significantly lower polyp extension percentages were found for TE corals during the exposure period on day 2 at 30°C, day 4 at 32°C, day 6 at 30°C, day 8 at 28°C, and during the recovery period on days 10, 12, 16 and 18 at 26°C (Table 3.1, Fig. 3.1). Polyp extension for starved and fed corals did not differ at any point in time (Table 3.1, Fig 3). Polyp extension did not differ significantly between starved and fed corals nested within TE and TC treatments at any time (Table 3.1, Fig. 3.1).

In summary, corals that were exposed to high temperatures retracted their polyps during exposure and several days after the exposure ended. Feeding did not influence this response.

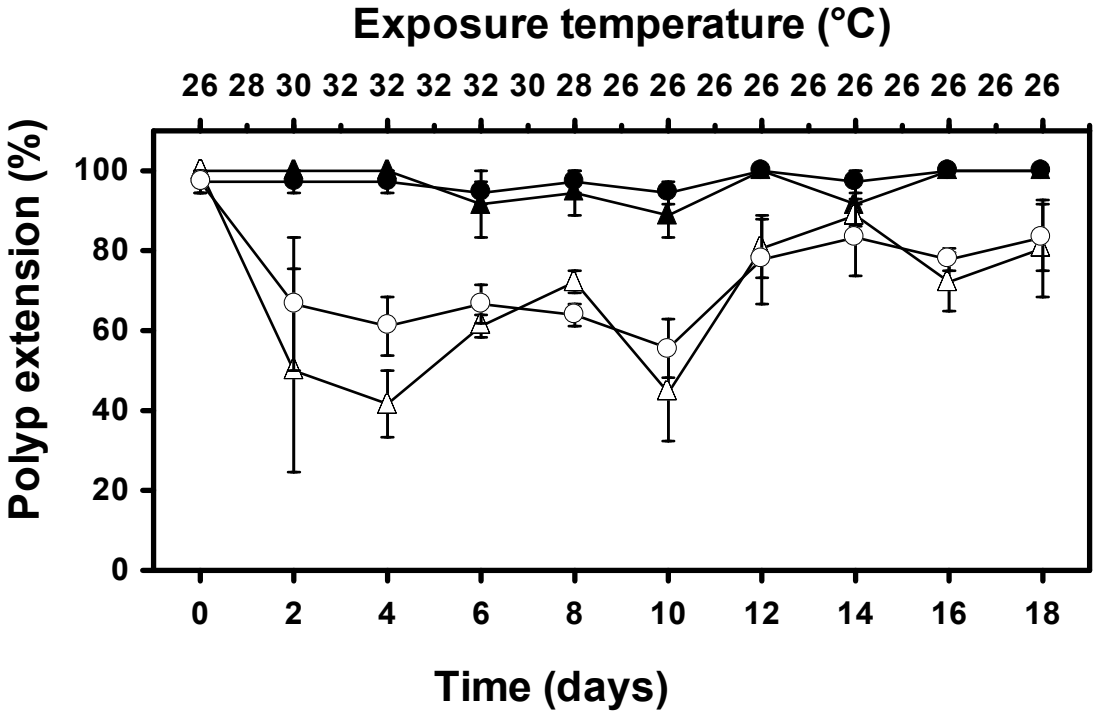

Fig. 3.1. Time series of percentage of coral nubbins (mean ± SE, n = 3) with extended polyps observed every two days. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C indicated on the top axis, empty triangles (△) denote corals that were similarly exposed but starved.

3.1.2. Coral colour health score

Coral health colour scores were significantly lower overall for TE corals than TC corals ($n = 720$, $df = 1$, $F = 89.84$, $p < 0.005$), and for starved corals than fed corals ($n = 720$, $df = 1$, $F = 13.55$, $p < 0.005$). Coral colour scores also changed significantly over time ($n = 144$, $df = 1$, $F = 54.05$, $p < 0.005$), particularly decreasing from day 4 at 32°C onwards to day 18 (Table 3.1, Fig. 3.2).

Starved TE corals had lower colour scores than fed TE corals, both having lower colour scores than starved and fed corals in the TC treatment (Table 3.1, Fig. 3.2). This effect was particularly apparent on day 4 at 32°C during exposure and on days 10, 12, 16 and 18 during the recovery period at 26°C (Table 3.1, Fig. 3.2).

In summary, coral colour health scores declined for coral exposed to high temperatures. Colour scores declined further for the starved corals than the fed corals.

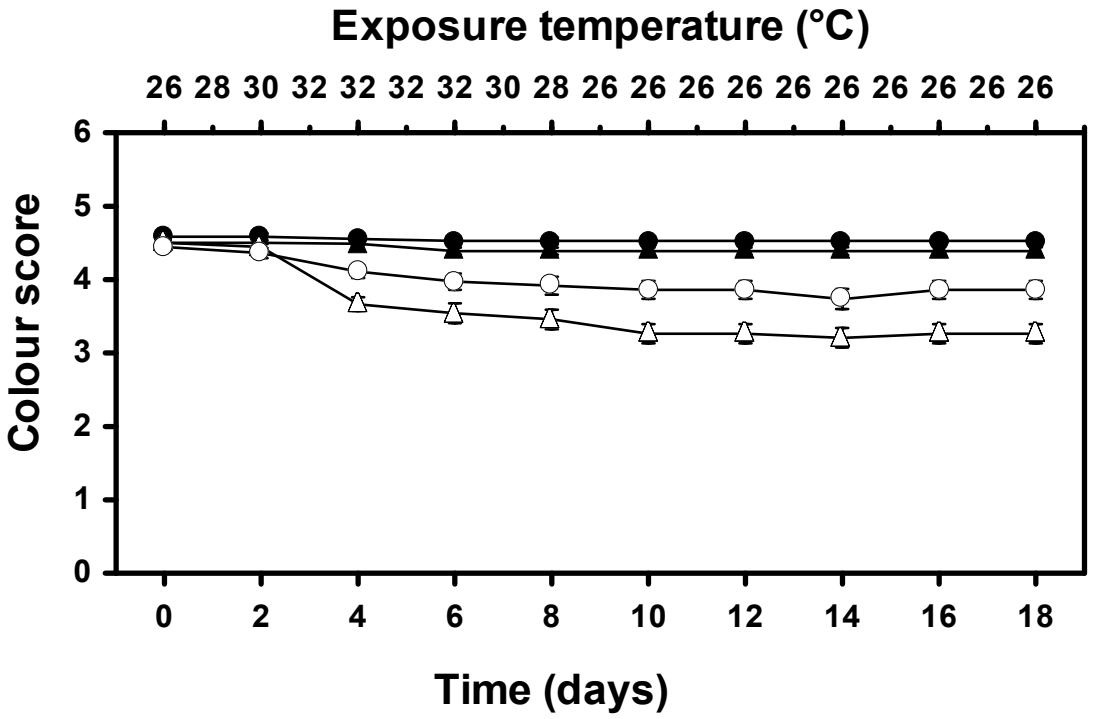

Fig. 3.2. Time series of coral health colour scores (mean ± SE, n = 36) for coral nubbins taken every two days. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved.

3.1.3. Coral oxygen consumption rates

Oxygen consumption rates were significantly higher overall for TE corals than TC corals ($n = 72$, $df = 1$, $F = 6.1$, $p = 0.02$), but did not differ between starved and fed corals ($n = 72$, $df = 1$, $F = 1.65$, $p = 0.51$). Oxygen consumption rates differed significantly over time ($n = 36$, $df = 3$, $F = 26.67$, $p < 0.005$) and were highest on day 4 and lowest on day 16 (Table 3.1, Fig. 3.3).

Table 3.1. ANOVA indicating differences in coral polyp extension, colour and oxygen consumption rates between feeding treatments of starved and fed corals nested within temperature treatments of control corals incubated at 26°C and thermally exposed corals incubated at 26-32°C sampled every two days for 19 days with associated temperatures. For exposure tanks, columns with ↑ symbols represents the days, 0-2, during which temperature was increased from 26°C by 2°C daily, ▽ symbols represents days, 3-6, maximum exposure at 32°C, ↓ symbols represents the days (7-9) during which the temperature was decreased to control temperatures at 26°C, ■ symbols represents the recovery period at constant control temperatures of 26°C. Statistics represent a two-factor, repeated measures ANOVA and asterisks (*) indicate significant differences with p-values less than 0.05. Day 'All' represents the ANOVA model including a time factor of the repeated measures, df = 9 for polyp extension and colour analyses, df = 3 for oxygen consumption analyses.

Day				2		4		6		8		9		10	
Exposure tanks treatment				↑		▽		▽		↓		■		■	
Exposure temperatures				30°C		32°C		32°C		28°C		26°C		26°C	
Statistic		n	df	F	p	F	p	F	p	F	p	F	p	F	p
Polyp extension	Model	120	3	16.5	< 0.005*	22.27	< 0.005*	13.12	< 0.005*	7.49	< 0.005*			13.12	< 0.005*
	Feeding	60	1	1.35	0.25	1.91	0.07<	1.51	0.52	0.21	0.65			1.51	0.22
	Temperature	60	1	45.49	< 0.005*	61.49	0.005*	37.68	< 0.005*	21.15	< 0.005*			37.68	< 0.005*
	Feed(Temp)	30	1	2.65	0.11	3.4	0.17	0.17	0.83	0.85	0.36			0.17	0.68
Colour	Model	1440	3	4.03	0.01*	32.74	< 0.005*	36.51	< 0.005*	25.36	< 0.005*			36.51	< 0.005*
	Feeding	720	1	< 0.005	1	12.95	< 0.005*	14.98	0.003*	9.57	0.002*			14.98	< 0.005*
	Temperature	720	1	8.88	0.003*	78.35	< 0.005*	88.74	< 0.005*	63.76	< 0.005*			88.74	< 0.005*
	Feed(Temp)	360	1	3.20	0.08	6.90	< 0.005*	5.81	0.12	2.74	0.1			5.81	0.02*
Oxygen consumption	Model	144	3			5.98	0.002*					1.47	0.24		
	Feeding	72	1			5.3	0.03*					0.01	0.91		
	Temperature	72	1			10.74	< 0.005*					3	0.09		
	Feed(Temp)	36	1			0.10	0.75					1.45	0.234		

Table 3.1 continued

Day		12				14		16		18		All			
Exposure tanks treatment		■				■		■		■					
Exposure temperatures		26°C				26°C		26°C		26°C					
Statistic		n	df	F	p	F	p	F	p	F	p	n	df	F	p
Polyp extension	Model	120	3	6.2	< 0.005*	1.37	0.25	8	< 0.005*	5.2	0.002*	12	9	14.33	< 0.005*
	Feeding	60	1	0.08	0.78	< 0.005	1	0.29	0.59	0.09	0.76	6	9	0.8	0.6
	Temperature	60	1	18.4	< 0.005*	2.85	0.09	23.43	< 0.005*	15.44	< 0.005*	6	9	7.25	< 0.005*
	Feed(Temp)	30	1	0.08	0.78	1.27	0.26	0.29	0.59	0.09	0.76	3	9	1.57	0.13
Colour	Model	1440	3	36.51	< 0.005*	35.57	< 0.005*	36.11	< 0.005*	36.11	< 0.005*	144	9	68.80	< 0.005*
	Feeding	720	1	14.98	< 0.005*	10.57	0.001*	14.82	< 0.005*	14.82	< 0.005*	72	9	7.51	< 0.005*
	Temperature	720	1	88.75	< 0.005*	92.54	< 0.005*	87.78	< 0.005*	87.78	< 0.005*	72	9	35.77	< 0.005*
	Feed(Temp)	360	1	5.81	0.02*	3.6	0.06	5.75	0.02*	5.75	0.02*	36	9	5.38	< 0.005*
Oxygen consumption	Model	144	3					1.82	0.16			36	3	20.52	< 0.005*
	Feeding	72	1					1.44	0.24			18	3	2.68	0.08
	Temperature	72	1					0.79	0.38			18	3	2.91	0.06
	Feed(Temp)	36	1					3.89	0.06			9	3	0.92	0.41

Oxygen consumption rates for TE corals were higher than TC corals on day 4 at 32°C (Table 3.1, Fig. 3.3), but did not differ significantly on days 9 and 16 at 26°C (Table 3.1, Fig. 3.3). Oxygen consumption rates for fed corals were higher than starved corals on day 4 (Table 3.1, Fig. 3.3), but did not differ on days 9 and 16 (Table 3.1, Fig. 3.3). Oxygen consumption rates for starved and fed corals nested within temperature treatments did not differ significantly on any day (Table 3.1, Fig. 3.3).

Q_{10} values for consumption rates of corals differed significantly overall when compared between combinations of day 4 and 0, day 4 and 9 and day 4 and 16 (n = 18, df = 2, F = 8.16, p = 0.001) but did not differ overall between starved and fed corals (n = 18, df = 2, F = 8.16, p = 0.193). Q_{10} values did not differ significantly between starved and fed corals nested within the different time combinations (n = 9, df = 5, F = 2.35, p = 0.11, Table 3.2).

In summary, oxygen consumption rates of corals increased during thermal exposure and for feeding but only on day 4 during exposure. The degree of oxygen consumption rate increase to temperature increases did not differ between starved and fed corals.

Table 3.2. Q_{10} values for coral oxygen consumption rates (R_1, R_2) taken on days 0, 4, 9, and 16 with related mean temperatures observed (T_1, T_2). $Q_{10} = (R_2/R_1)^{10/(T2-T1)}$. No significant differences (n = 9, df = 5, F = 2.354, p = 0.108) found between Q_{10} values for a two-factor nested ANOVA (Model: n = 9, df = 5, F = 4.66, p = 0.002).

R_2 / R_1	Feeding treatment	Mean $T_2 - T_1$ (°C)	Q_{10} (mean ± SD)
Day 4 / Day 0	Starved	29.7 − 25.4	1.32 ± 0.56
	Fed	29.8 – 25.6	2.56 ± 0.58
Day 4 / Day 9	Starved	29.7 – 26.0	-1.29 ± 0.64
	Fed	29.8 – 26.4	-1.51 ± 0.57
Day 4 / Day 16	Starved	29.7 − 25.4	-2.54 ± 1.11
	Fed	29.8 – 25.6	-2.82 ± 1.12

3.1.4. Coral feeding rates

Coral feeding rates did not differ significantly between thermally exposed and control corals (n = 36, df = 1, F = 0.33, p = 0.59), between sampling days (n = 18, df = 3, F =

0.02, p = 0.99) nor between exposed and control corals within sampling days (n = 9, df = 3, F = 0.26, p = 0.85).

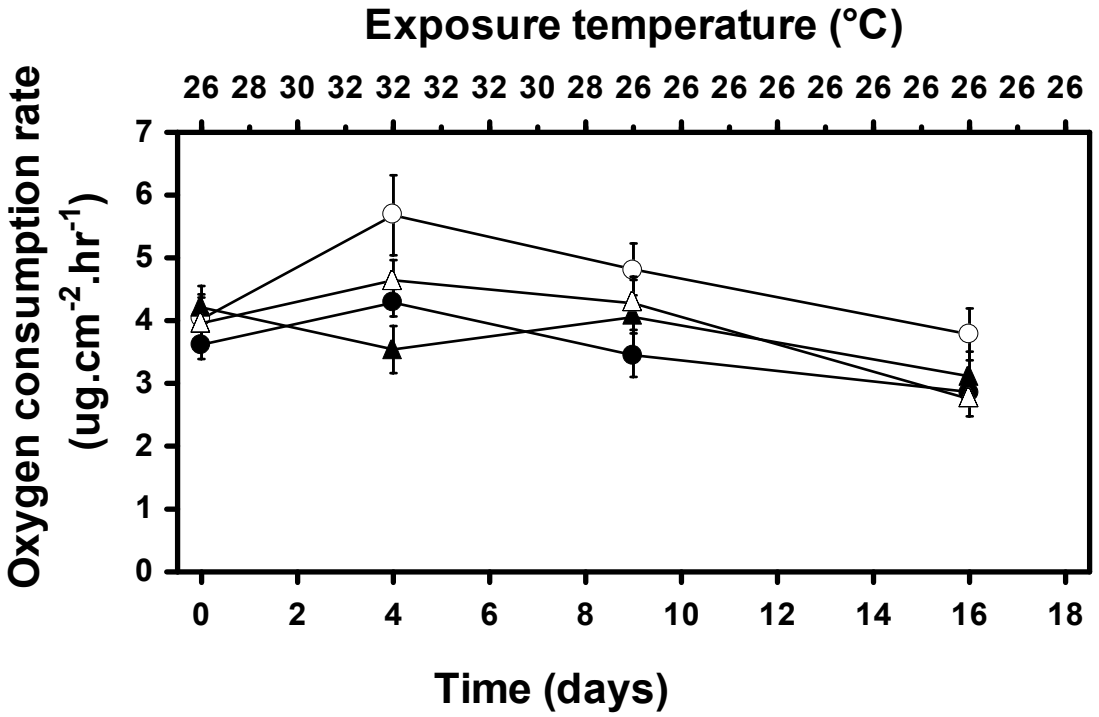

Fig. 3.3. Time series of coral oxygen consumption rates (mean ± SE, n = 9) expressed as micrograms O_2 consumed per cm^{-2} of coral surface area per h. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved.

3.2. The influence of increased temperature exposure and feeding on coral tissue composition

3.2.1. Coral zooxanthellae density

Zooxanthellae densities were significantly lower overall for TE corals than TC corals but did not differ between starved and fed corals or between corals sampled at exposure or recovery (Table 3.3).

Zooxanthellae densities did not differ significantly between feeding treatments nested within temperature treatments and sampling times (Table 3.3, Fig. 3.4).

In summary, zooxanthellae density in coral tissue was lower at high temperatures but was not influenced by feeding.

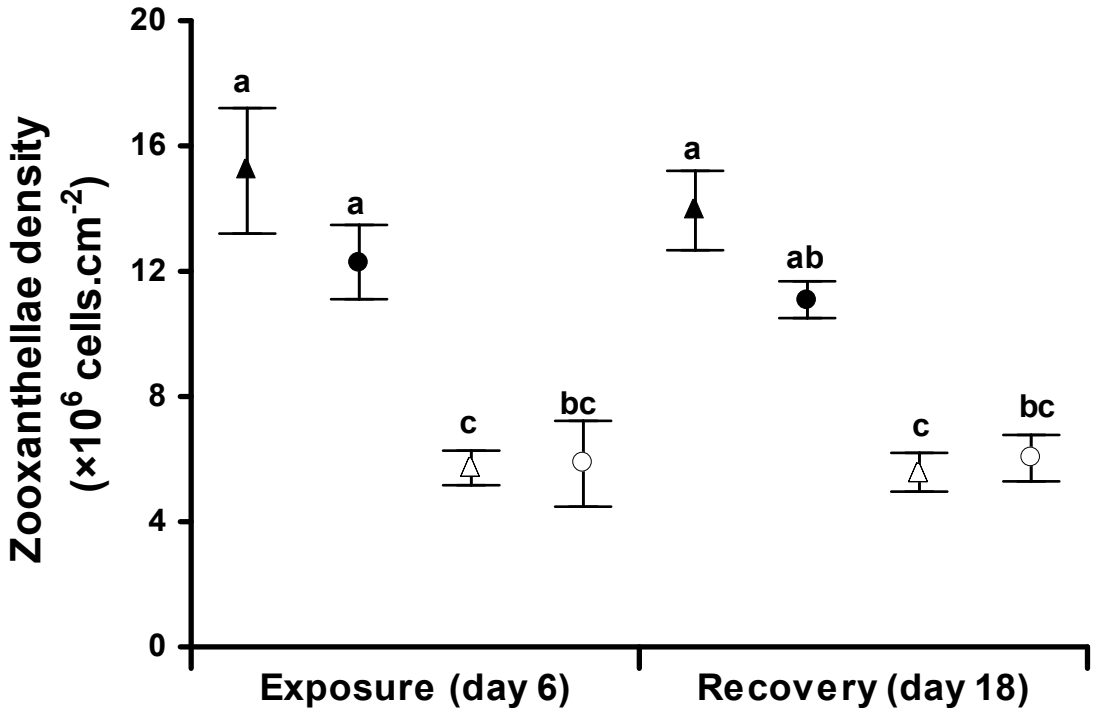

Fig. 3.4. Zooxanthellae densities (mean ± SE, n = 9) expressed as number of cells per cm^{-2} of coral surface area. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved. Letters above bars (a, b, c) indicate significant differences according to Bonferroni post-hoc tests for a three-factor nested ANOVA (n = 9, df = 64, MSE = 11.83, $p < 0.05$).

Table 3.3. ANOVAs indicating differences in zooxanthellae density, chlorophyll *a* content, chlorophyll *a* per zooxanthella, lipid content, olive tail moment, percentage tail DNA, and antioxidant potentials between corals that were starved and fed (Feeding), and between corals exposed to increasing temperatures up to 32°C and kept at constant 26°C (Temperature), and between corals sampled during exposure and at recovery (Time). Statistics represent a three-factor, nested ANOVA and asterisks (*) indicate significant differences with p-values less than 0.05.

Variable	Test	Statistic			
		n	df	F	p
Zooxanthellae density	Model	72	7	12.79	< 0.005*
	Time	36	1	0.56	0.46
	Temperature	36	1	81.95	< 0.005*
	Feeding	36	1	2.55	0.12
	Feeding(Temperature)	18	1	3.84	0.05
	Feeding(Temperature×Time)	9	3	0.21	0.89
Chlorophyll *a* content	Model	72	7	5.75	< 0.005*
	Time	36	1	4.32	0.04*
	Temperature	36	1	30.61	< 0.005*
	Feeding	36	1	0.98	0.33
	Feeding(Temperature)	18	1	0.09	0.76
	Feeding(Temperature×Time)	9	3	1.33	0.27
Chlorophyll *a* content per zooxanthella	Model	72	7	4.33	< 0.005*
	Time	36	1	8.75	< 0.005*
	Temperature	36	1	9.71	< 0.005*
	Feeding	36	1	0.11	0.74
	Feeding(Temperature)	18	1	< 0.005	0.97
	Feeding(Temperature×Time)	9	3	3.62	0.02*
Lipid content	Model	72	7	3.13	0.01*
	Time	36	1	9.66	< 0.005*
	Temperature	36	1	2.33	0.13
	Feeding	36	1	0.79	0.38
	Feeding(Temperature)	18	1	0.03	0.86
	Feeding(Temperature×Time)	9	3	2.63	0.06
Olive tail moment	Model	72	7	4.65	< 0.005*
	Time	36	1	6.12	0.02*
	Temperature	36	1	3.78	0.06
	Feeding	36	1	4.69	0.04*
	Feeding(Temperature)	18	1	3.47	0.07
	Feeding(Temperature×Time)	9	3	4.18	0.01*

Table 3.3. continued.

Variable	Test	Statistic			
		n	df	F	p
Tail DNA %	Model	72	7	5.79	< 0.005*
	Time	36	1	7.61	0.01*
	Temperature	36	1	4.57	0.04*
	Feeding	36	1	6.11	0.02*
	Feeding(Temperature)	18	1	4.34	0.04*
	Feeding(Temperature*Time)	9	3	5.15	< 0.005*
Antioxidant potential	Model	72	7	3.44	< 0.005*
	Time	36	1	4.61	0.04*
	Temperature	36	1	9.71	< 0.005*
	Feeding	36	1	3.08	0.09
	Feeding(Temperature)	18	1	4.86	0.03*
	Feeding(Temperature*Time)	9	3	1.1	0.36

3.2.2 .Coral chlorophyll a content

Chlorophyll *a* content was significantly lower overall for TE corals than TC corals and for corals sampled during recovery than at exposure but did not differ between starved and fed corals (Table 3.3). Chlorophyll *a* content per zooxanthella was significantly higher overall for TE corals than TC corals and during exposure than recovery sampling points but did not differ between starved and fed corals (Table 3.3).

Chlorophyll *a* content did not differ significantly between starved and fed corals nested within temperature treatments and time sampled (Table 3.3, Fig. 3.5). Chlorophyll *a* per zooxanthella was higher for exposed and fed corals during exposure but did not differ significantly during recovery (Table 3.3, Fig. 3.5). Chlorophyll *a* per zooxanthella for starved and fed corals nested within temperature treatments did not differ significantly at any time point (Table 3.3, Fig. 3.5).

In summary, chlorophyll *a* content was lower for thermally exposed corals but was not influenced by feeding. Chlorophyll *a* content per zooxanthella has higher for corals that were exposed but was lower after recovery and was also not influenced by feeding.

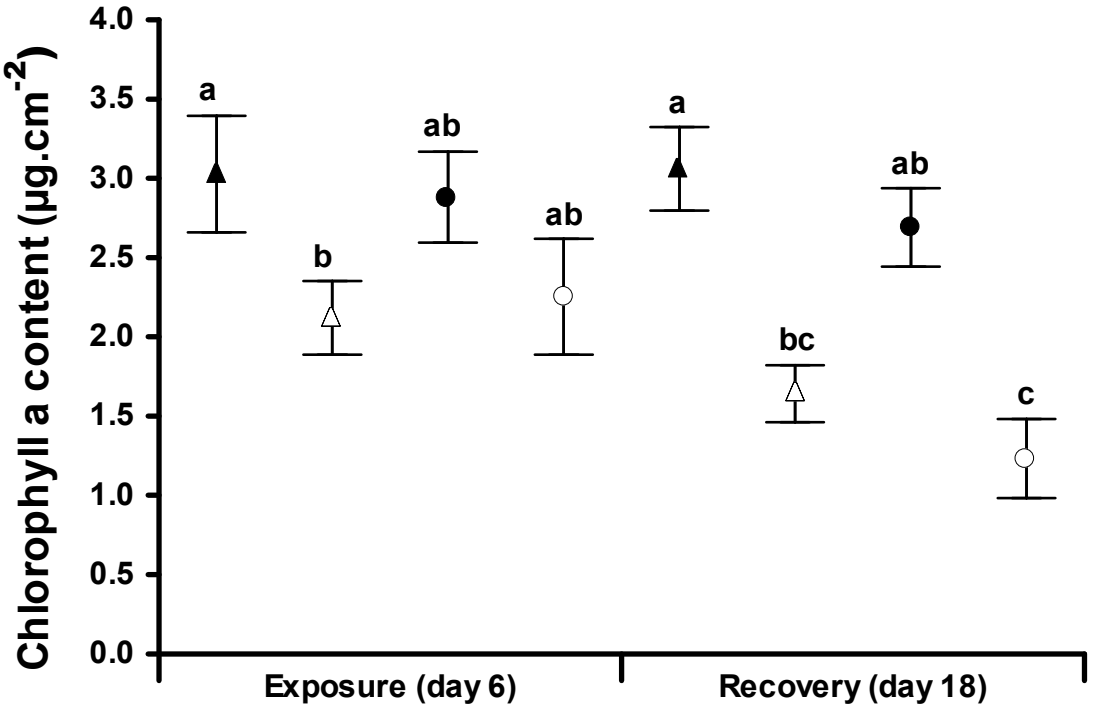

Fig. 3.5. Coral tissue chlorophyll *a* content (mean ± SE, n = 9) expressed as micrograms of chlorophyll *a* per cm^{-2} of coral surface area. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved. Letters above bars (a, b, c) indicate significant differences according to Bonferroni post-hoc tests for a three-factor nested ANOVA (n = 9, df = 61, MSE < 0.005, $p < 0.05$).

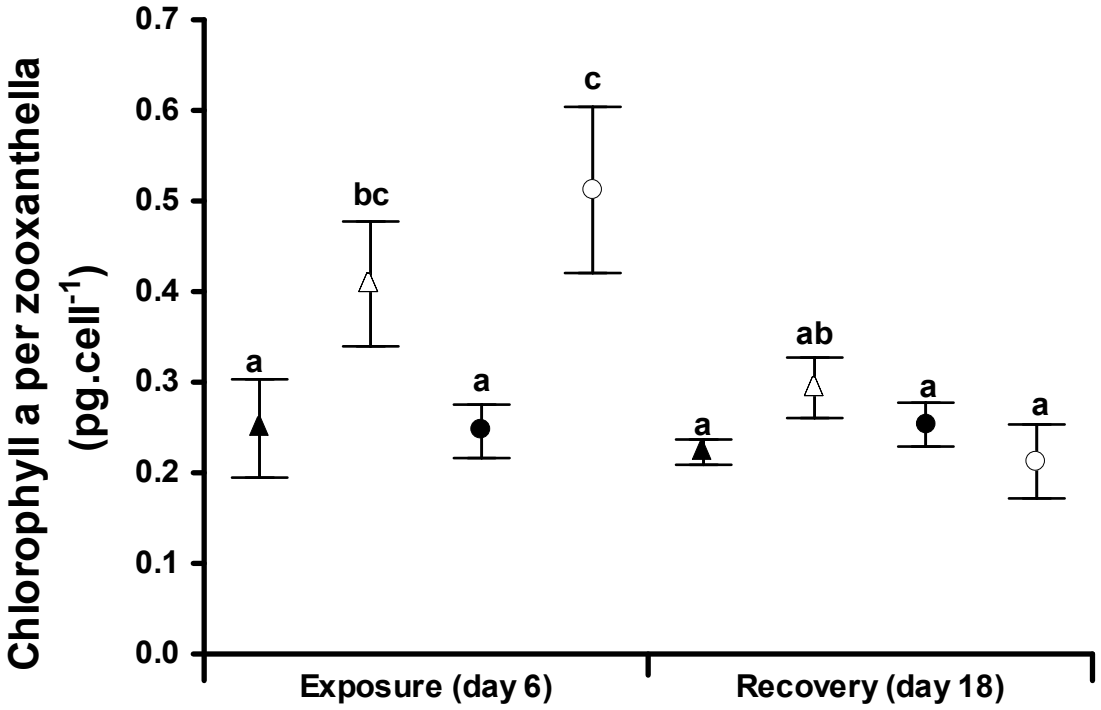

Fig 3.6. Coral tissue chlorophyll *a* content (mean ± SE, n = 9) in picograms per cell. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved. Letters above bars (a, b, c) indicate significant differences according to Bonferroni post-hoc tests for a three-factor nested ANOVA (n = 9, df = 61, MSE < 0.005, $p < 0.05$).

3.2.3. Coral total lipid content

Coral lipid content was significantly higher overall during exposure than recovery time points but did not differ between TC and TE corals or starved and fed corals (Table 3.3).

Lipid content did not differ significantly between starved and fed corals nested within temperature treatments and time point sampled (Table 3.3, Fig. 3.7). However, Bonferroni post-hoc analyses showed that starved TE corals sampled during the recovery had significantly lower lipid content than fed TC corals and starved TE corals sampled during exposure (n = 9, df = 57, MSE < 0.005, $p < 0.05$, Fig. 3.7).

In summary, lipid content of corals did not differ during thermal exposure, exposed corals showed lower lipid content during recovery particularly for corals that were starved compared to fed corals.

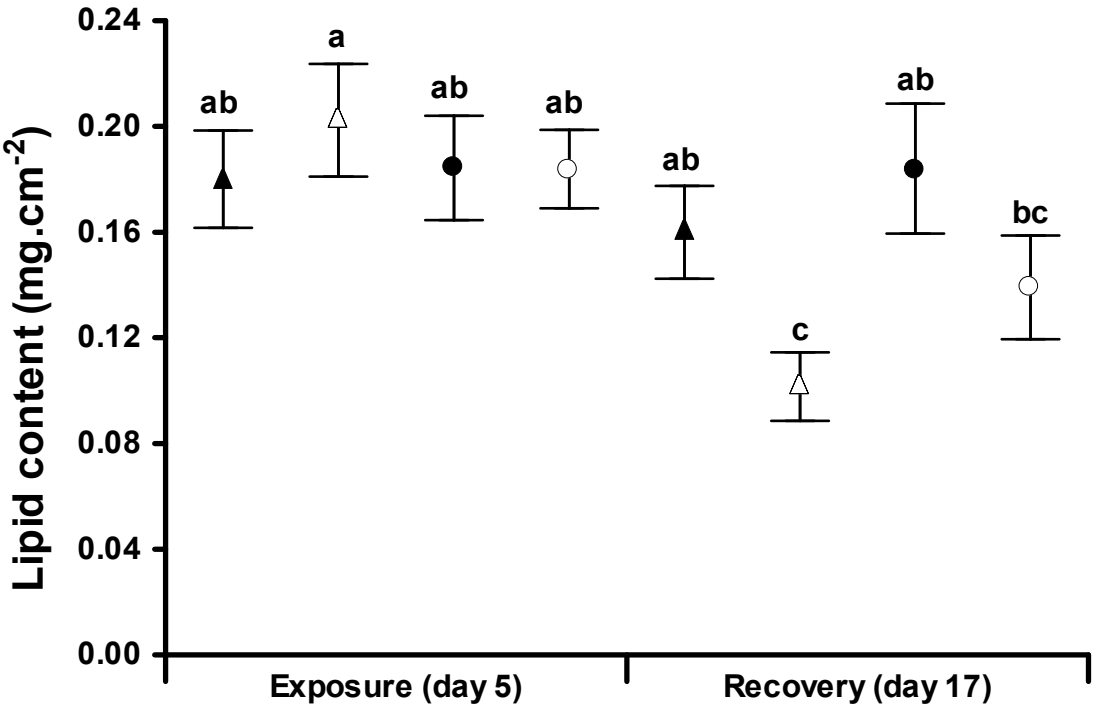

Fig. 3.7. Coral tissue lipid content (mean ± SE, n = 9) expressed as milligrams of lipid per cm^{-2} of coral surface area. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved. Letters above bars (a, b, c) indicate significant differences according to Bonferroni post-hoc tests for a three-factor nested ANOVA (n = 9, df = 57, MSE < 0.005, $p < 0.05$).

3.3. Cellular responses of coral cells from increased temperature exposure and feeding

3.3.1. Coral tissue antioxidant potential

FRAP values were significantly lower overall for TE corals than TC corals and higher during exposure than during recovery but did not differ between starved and fed corals (Table 3.3).

Corals that were exposed and starved had significantly lower FRAP values than corals that were fed when the time factor was removed (Table 3.3). FRAP values did not differ between starved and fed corals nested within temperature treatment and time points sampled (Table 3.3, Fig. 3.8).

In summary, the presence of antioxidants was lower for corals that were thermally exposed and starved.

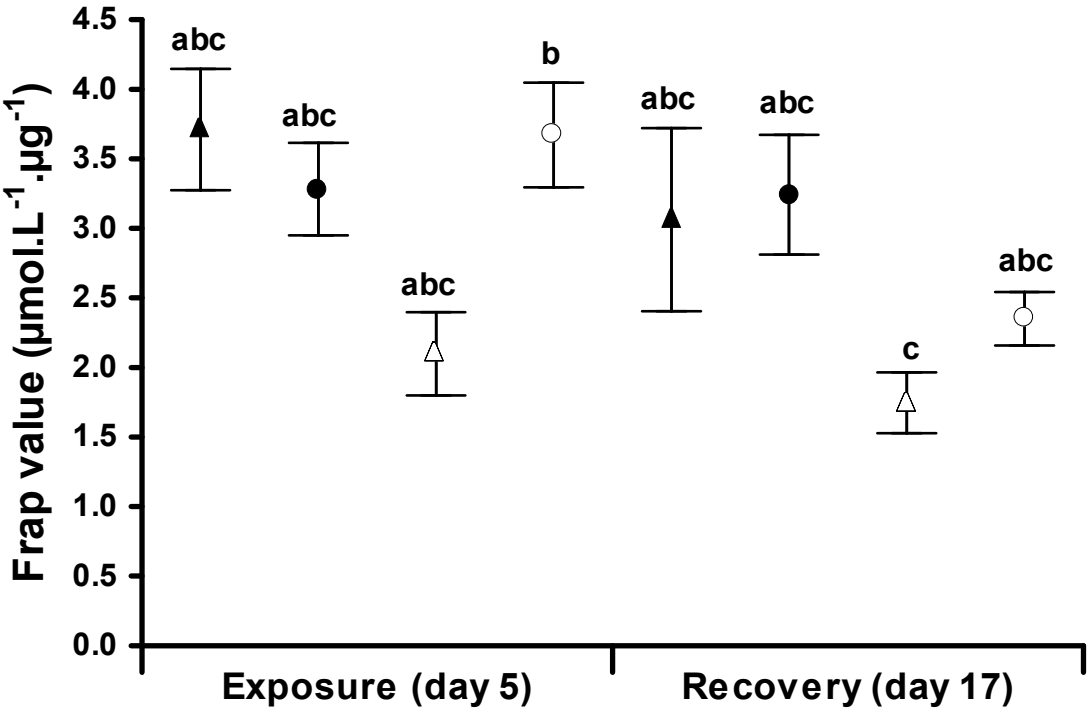

Fig. 3.8. FRAP values (mean ± SE, n = 9) indicating antioxidant potential for coral tissue expressed as micromolar Fe^{+3} per microgram of total protein. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved. Letters above bars (a, b, c) indicate significant differences according to Bonferroni post-hoc tests for a three-factor nested ANOVA (n = 9, df = 48, MSE = 1.03, $p < 0.05$).

3.3.2. Coral cell DNA integrity

Olive tail moment (OTM) was significantly higher overall for starved corals than fed corals and during exposure than during recovery, but did not differ between TE corals and TC corals (Table 3.3). Similarly, percentage of tail DNA was significantly higher overall for TE corals than TC corals, for starved corals than fed corals and during exposure than during recovery (Table 3.3). Data for percentage tail DNA was not represented graphically because it followed the same trend as OTM.

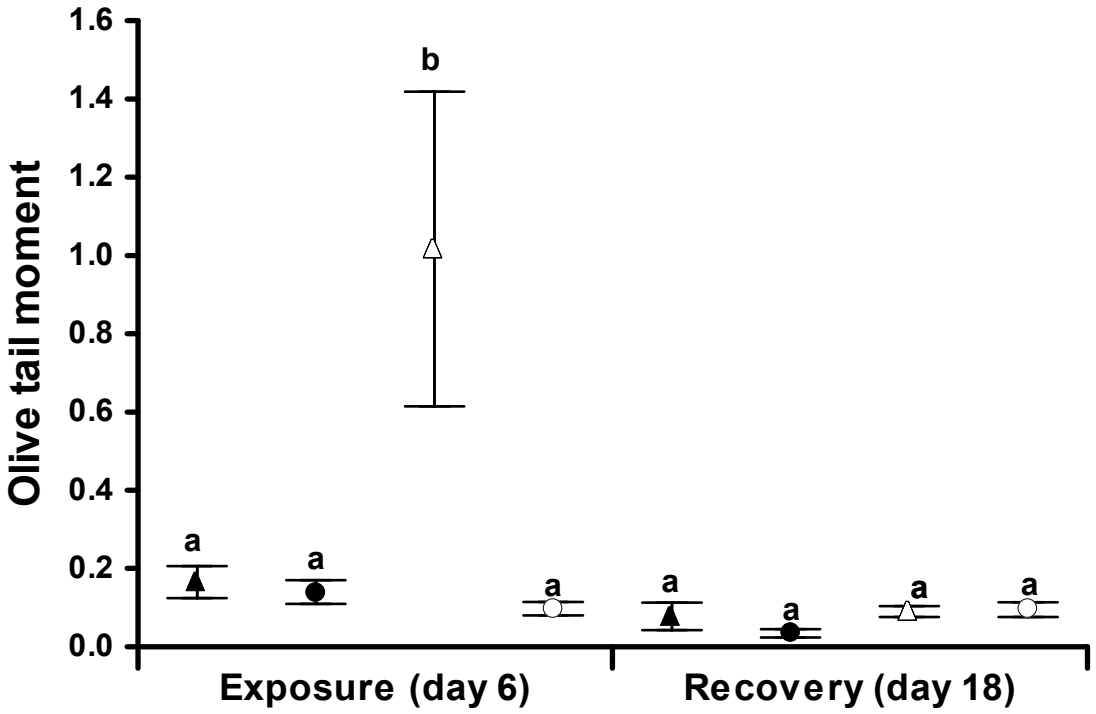

Fig. 3.9. Olive tail moment (mean ± SE, n = 9) in arbitrary units of comet assays for coral cells. Filled circles (●) denote nubbins that were fed and kept at 26°C, filled triangles (▲) denote nubbins that were starved and kept at 26°C, empty circles (○) denote corals that were fed and exposed to temperature increases up to 32°C, empty triangles (△) denote corals that were similarly exposed but starved. Letters above bars (a, b) indicate significant differences according to Bonferroni post-hoc tests for a three-factor nested ANOVA (n = 9, df = 37, MSE = 1.14, $p < 0.05$).

Starved TE showed a significantly higher OTM than all the remaining treatment groups (n = 9, df = 3, MSE = 1.34, F = 4.18, p = 0.01, Fig. 3.9). OTMs for corals in all the remaining treatment groups did not differ significantly (n = 9, df = 37, MSE = 1.34, $p > 0.05$, Fig. 3.9). Starved TE showed significantly higher tail DNA percentages during

exposure than all the remaining treatment groups ($n = 9$, $df = 3$, $MSE = 1.14$, $F = 5.15$, $p < 0.005$). Tail DNA percentages for corals in all the remaining treatment groups did not differ significantly ($n = 9$, $df = 37$, $MSE = 1.14$, $p > 0.05$).

In summary, DNA damage was higher during thermal stress but only for corals that were starved. Thermally exposed corals that were fed showed baseline levels of DNA integrity.

3.4. Overall summary of results

Coral nubbins exposed to increased temperatures retracted their polyps, decreased in colour score and increased their rate of oxygen consumption. Corals that were fed showed higher colour scores and oxygen consumption rates than the starved corals during exposure but did not differ in polyp extension.

Zooxanthellae density, chlorophyll *a* content and lipid content were lower in tissues of corals exposed to high temperatures. Chlorophyll *a* per zooxanthellae cell was higher in exposed corals during exposure than controls but decreased during the recovery phase. Feeding did not influence zooxanthellae density and chlorophyll *a* content during exposure. Lipid content for starved and exposed corals showed lower lipid content compared to other treatment groups during the recovery phase.

Corals with exposure to high temperatures showed higher tail DNA percentages and lower antioxidant potentials in their cells. Fed and exposed corals showed lower levels of tail DNA percentage and OTM and higher antioxidant potential compared to starved and exposed corals.

CHAPTER 4: DISCUSSION

This study aimed to investigate the influence of heterotrophy on coral resilience and tested whether feeding on *Artemia salina* nauplii influences the effects of thermal stress and bleaching on *Pocillopora damicornis* corals by potentially fulfilling the energetic requirements for repairing damage from thermal stress and the loss of nutrient transfers from expelled zooxanthellae. The results of this study confirmed the findings of previous research indicating that exposure to increased temperatures indeed had significant negative effects on coral physiology (Lesser 1996, Lesser 1997, Baird & Marshall 2002, Bhagooli & Hidaka 2004, Jokiel 2004, Edmunds 2005, Nesa & Hidaka 2008), and tissue composition (Glynn et al. 1985, Hoegh-Guldberg & Smith 1989, Szmant et al. 1990, Gates et al. 1992, Grottoli et al. 2004, Borell et al. 2008). Also, feeding on *Artemia salina* nauplii significantly improved the negative stress-induced effects on some aspects of the coral physiology and tissue composition of *Pocillopora damicornis,* supporting the hypothesis that heterotrophy plays a significant role in determining coral bleaching resilience (Grottoli et al. 2006, Borell et al. 2008, Palardy et al. 2008, Tolosa et al. 2011).

4.1. Behavioural responses of corals to thermal stress and feeding

The sessile nature of corals limits their behavioural responses to stress (Glynn & D'croz 1990, Gochfeld 2004). Polyp retraction into the corallum is a behavioural response to avoid predation by corallivores and decrease exposure to stress as shown by the decreased polyp extension during high temperatures in this study (Fig. 3.1) and observed on reefs (Glynn & D'croz 1990, Salih et al. 2000, Gochfeld 2004). During retraction, the oral disk and tentacles cover the underlying tissues creating a ‘polyp plug’ encased in the calcium carbonate corallum (Salih et al. 1997, Salih et al. 2000). Coral tissue in the oral disk and tentacles contain fluorescent pigments and ultraviolet radiation protective compounds such as mycosporine-like amino acids (Dunlap & Shick 1998, Salih et al. 2000, Bhagooli & Hidaka 2003). The calcium carbonate skeleton of corals is highly reflective and reduces also the amount of ultraviolet radiation transmitted to coral tissues (Salih et al. 2000, Reef et al. 2009b). Consequently, the

polyp plug and corallum both decrease the amount of light reaching coral tissues containing zooxanthellae which limits the synergistic effects of oxidative damage from ultraviolet radiation and elevated temperature (Lesser 1996, Salih et al. 1997, Salih et al. 2000, Reef et al. 2009b). The increase in polyp extension following temperature decreases (Fig. 3.1) indicated that the damage by ultraviolet radiation and thermal stress was significantly reduced (Salih et al. 1997, Salih et al. 2000).

In the current study, feeding did not influence the polyp retraction response during high temperatures and the recovery period at ambient temperatures (Fig. 3.1). Corals usually feed at night and polyp extension was measured during the day indicating that corals chose to retract their polyps during the day to deal with stress irrespective of being fed and could extend their polyps at night to feed without danger of exposure to UV radiation (Muscatine & Porter 1977, Salih et al. 2000). Increasing feeding effort is another behavioural response initiated by corals to fulfil the energetic requirements to ameliorate thermal, oxidative and bleaching stress (Palardy et al. 2008, Ferrier-Pagès et al. 2010). However, changes were not seen between feeding rates of corals experimentally exposed and unexposed to high temperatures in this study (section 3.1.4). It has previously been demonstrated that changes in feeding effort in response to bleaching are highly variable among specific taxa and environmental conditions such as depth, water flow, light intensity and food availability (Grottoli et al. 2006, Palardy et al. 2008, Houlbrèque & Ferrier-Pagès 2009, Ferrier-Pagès et al. 2010). For example, increases in feeding effort following thermal stress are known for *Montipora capitata, Turbinaria reniformis* and *Galaxea fascicularis* but not for *Porites lobata* and *Porites compressa* and decreased for *Stylophora pistillata* (Grottoli et al. 2006, Palardy et al. 2008, Ferrier-Pagès et al. 2010). The similarity in feeding rates between *Pocillopora damicornis* nubbins exposed to high temperatures and controls in this study may therefore be the result of high food availability, light availability and water flow from aquarium pumps which negates the need to expend energy to actively increase feeding effort (Grottoli et al. 2006, Palardy et al. 2008, Ferrier-Pagès et al. 2010).

4.2. Cellular responses of corals to thermal stress and feeding

Oxygen consumption rates were higher (Fig. 3.3), antioxidant potentials were lower (Fig. 3.8) and DNA damage was higher (Fig. 3.9) in corals exposed to elevated temperatures. This result confirmed that thermal stress leads to oxidative stress which causes damage to molecular structures within coral cells eliciting energetically costly stress-reducing responses and repair mechanisms (Lesser 1996, Downs et al. 2000, Lesser & Farrell 2004). Damage to DNA and other molecular structures results from the excessive production of ROS particles by coral cells and by the photoinhibition of zooxanthellar photosynthesis under thermal stress (Lesser 1996, Mitchelmore & Hyatt 2004, Baruch et al. 2005, Nesa & Hidaka 2008). The antioxidant potential and DNA damage of coral cells in this study confirmed that the corals were indeed stressed by elevated temperatures causing oxidative damage on a cellular level (Fig. 3.8 and 3.9). The onset of thermal and oxidative stress elicits physiological responses such as antioxidant and heat-shock protein production to reduce stress and either prevent or repair damage (Feder & Hofmann 1999, Downs et al. 2000). These responses are energetically costly and result in increased metabolic rates and the increased oxygen consumption rates which were seen in this (Fig. 3.3) and other studies (Coles & Jokiel 1977, Edmunds 2005, Borell et al. 2008). If energetic requirements are not fulfilled or if the stress is too intense, protection and repair mechanisms may prove to be inadequate and stress-induced damage occurs and/or persists (Griffin & Bhagooli 2004, Borell et al. 2008, Palardy et al. 2008). This may explain the decreased antioxidant potentials (Fig. 3.8) and increase in DNA damage (Fig. 3.9) in thermally exposed corals in this study. Thus, the question of the role of feeding in supplementing energetic requirements in physiological responses arises.

Oxygen consumption rates were higher for corals exposed to elevated temperatures than controls in this (Fig. 3.3) and other studies (Coles & Jokiel 1977, Edmunds 2005, Borell et al. 2008), which confirmed that increased temperature increases metabolic rates and cellular responses to thermal stress are energetically demanding resulting in the increased metabolic rates and consumption of oxygen to produce ATP. Rapidly increasing ATP production has the potential to produce ROS if mitochondrial electron transport is compromised and there is a mismatch in energy supply and demand to

mitigate ROS build-up (Lesser 1997, Downs et al. 2002, Baird et al. 2009). Consequently, increased metabolic activity leads to increased ROS production resulting in further damage as seen by the decreased antioxidants and increased DNA damage for thermally exposed corals seen in this and other studies (Lesser 1997, Downs et al. 2000, Downs et al. 2002, Griffin & Bhagooli 2004, Lesser & Farrell 2004, Nesa & Hidaka 2008, Baird et al. 2009).

Oxygen consumption rates were also higher for fed corals during exposure on day 4 irrespective of thermal exposure (Fig. 3.3) which indicated that an additional nutrient supply further increased the metabolic rates of exposed *Pocillopora damicornis* (Sorokin 1981, Borell et al. 2008, Palardy et al. 2008). This result indicated that starved corals could not increase their oxygen consumption rates to the levels of the fed corals which maintained a higher metabolic rate to respond to thermal stress (Ferrier-Pagès et al. 2003, Edmunds 2005, Borell et al. 2008, Houlbrèque & Ferrier-Pagès 2009, Ferrier-Pagès et al. 2010). Due to the variation in the data, this trend was not supported by the oxygen consumption rate data comparing corals in feeding treatments nested within temperature treatments (Table 3.1) or for the Q_{10} data which compared the degree of oxygen consumption rate change per 10°C change in temperature (Edmunds 2005, Doney et al. 2012) between fed and starved corals (Table 3.2). Q_{10} was at 1.32 ± 0.56 (mean ± SD) between days 0 and 4 for starved corals and 2.56 ± 0.58 (mean ± SD) for fed corals and have previously shown to be 2 for healthy *Pocillopora damicornis* (Coles & Jokiel 1977, Edmunds 2005). This indicates qualitatively that fed corals have increased capability to increase their metabolic rates to deal with stress compared with starved corals (Edmunds 2005, Doney et al. 2012). The increase in oxygen consumption from feeding on day 4 also partially supported the hypothesis that feeding provides more energy for repair mechanisms and cellular responses to thermal stress (Grottoli et al. 2006, Borell et al. 2008, Palardy et al. 2008), which also explains the lower DNA damage and antioxidant potentials for fed and thermally exposed *Pocillopora damicornis* in this study (Fig. 3.8 and 3.9). In summary, the results of this study indicated that increased temperature and feeding increases oxygen consumption in corals. However, starved corals are at a mismatch of energy supply and demand and

may therefore produce further ROS and are less capable energetically to respond to thermal and oxidative stress compared with corals that are fed.

The hypothesis that feeding provides energy for response mechanisms is further supported by the higher antioxidant potentials in corals exposed to elevated temperature and fed compared to exposed and starved even though the overall antioxidant potential of exposed corals were lower (Fig. 3.8). No previous research regarding the effect of feeding on the stress induced antioxidant response in corals was found. The decline in antioxidants at higher temperatures is an indication that coral responses cannot keep up with the increasing damage (Griffin & Bhagooli 2004, Liñán-Cabello et al. 2010). Griffin and Bhagooli (2004) were the first to measure antioxidant potentials in corals using the FRAP assay and investigated the antioxidant response of *Pocillopora damicornis* exposed to temperatures of 28, 29, 30 and 31°C for 3 h from controls temperatures of 27°C. Compared with the control, it was found that antioxidant potentials increased with temperature and peaked at 28°C but declined at higher temperatures until antioxidant potentials were equal to the control at 31°C (Griffin & Bhagooli 2004). This trend indicates that if temperature stress exceeds a certain threshold the ability of corals to respond via production of antioxidants diminishes (Griffin & Bhagooli 2004). A similar trend was seen by Liñán-Cabello et al. (2010) who showed initial increase in mycosporine-like amino acids after four h of ultraviolet radiation stress but declined for subsequent h of stress. The antioxidant potentials for exposed corals in this study were lower than the controls (Fig. 3.8) which agreed with the trend seen by Griffin and Bhagooli (2004) considering that that the temperatures in this study exceeded 31°C for longer than 3 h (Table 2.1).

The reduced antioxidant potential for starved corals compared with fed corals exposed to high temperatures seen in this study indicated that starved corals are at a higher risk of sustaining damage from oxidative stress (Fig. 3.8). This is further supported by the higher DNA damage in corals that were exposed and starved than exposed and fed corals and equal to those that were not exposed which indicated that heterotrophy significantly improved rates of DNA repair under thermal stress (Fig. 3.9). DNA integrity in corals have also been shown to be negatively affected by ultraviolet

radiation exposure (Baruch et al. 2005, Reef et al. 2009b), direct hydrogen peroxide exposure (Mitchelmore & Hyatt 2004), and heavy metal exposure (Schwarz et al. 2013), amongst others indicating oxidative damage to coral cells. No previous research regarding the effect of feeding on stress induced DNA damage in corals was found. However, a similar result was seen by Nesa and Hidaka (2008) who investigated the influence of antioxidants on the DNA damage of coral cells and showed that the presence of mannitol significantly reduced the DNA damage to baseline levels in *Pavona divaricata* exposed to elevated temperatures. Thus, it can be inferred that feeding on *Artemia salina* nauplii by corals provides the energy required for the action of DNA repair mechanisms such as photoreactivation or the production of antioxidants or other stress reducing compounds such as mycosporine-like amino acids (Feder & Hofmann 1999, Downs et al. 2000, Nesa & Hidaka 2008, Palardy et al. 2008, Baird et al. 2009, Houlbrèque & Ferrier-Pagès 2009).

4.3. Effect of thermal stress and feeding on coral tissue composition

Bleaching is usually quantified by the loss of zooxanthellae cell density and chlorophyll content in coral tissue (Muscatine et al. 1991, Downs et al. 2002, Visram & Douglas 2007). In this study, zooxanthella density (Fig. 3.4) was lower for corals exposed to elevated temperature, but did not recover following the end of exposure and was not influenced by feeding. Chlorophyll *a* content (Fig. 3.5) did not change during exposure but was lower following the end of exposure and was also not influenced by feeding. This led to the result that chlorophyll *a* content per zooxanthella was higher for exposed and fed corals during exposure and decreased during recovery (Fig. 3.6). These results confirmed that exposure to elevated temperature induces bleaching by the expulsion of zooxanthellae but chlorophyll content in the remaining zooxanthellae increases, particularly for fed corals (Hoegh-Guldberg & Smith 1989, Glynn & D'croz 1990, Gates et al. 1992, Downs et al. 2002, Abramovitch-Gottlib et al. 2003). Similar results were demonstrated for bleached *Stylophora pistillata* and *Seriatopora hystrix* corals which showed similar chlorophyll levels to healthy corals but lower zooxanthellae densities (Hoegh-Guldberg & Smith 1989). Hoegh-Guldberg and Smith (1989) attributed this mismatch in changes between zooxanthellae density and chlorophyll content to the type

of stress, linking zooxanthellae expulsion to thermal stress and loss of chlorophyll content rather to UV radiation stress. This conclusion supports the results of this study because although temperatures were elevated the sunlight intensity was reduced by 60 % shade-cloth. Lower light intensities and different spectral compositions have also been shown to increase chlorophyll content in some corals (Porter et al. 1984, Glynn & D'croz 1990, Titlyanov et al. 2001), however chlorophyll per zooxanthella did not increase for control temperature corals in this study (Fig. 3.6). This result also indicated that chlorophyll *a* content per zooxanthellae increased for corals exposed to high temperatures which was also seen by Hoegh-Guldberg and Smith (1989), Brown et al. (2002), Abramovitch-Gottlib et al. (2003) and has been attributed to a compensation for the loss of photosynthetic capability or carbon dioxide removal from bleaching.

Increased total chlorophyll content has also been shown for corals in other studies that were fed on zooplankton (Ferrier-Pagès et al. 2003, Ferrier-Pagès et al. 2010, Connolly et al. 2012). In a similar experiment to this study, Connolly et al. (2012) showed rapid recovery of chlorophyll and protein concentrations in *Acropora intermedia* exposed to temperatures of 31°C from controls of 27°C and fed on rotifers. Tolosa et al. (2011) similarly showed that feeding on *Artemia salina* reduced the loss of zooxanthellae density, chlorophyll and protein content in *Turbinaria remiformis* compared with starved corals at elevated temperature. This rapid recovery was not seen in this study for zooxanthellae density, chlorophyll content or colour score which indicates that recovery rates from bleaching may be species specific (Grottoli et al. 2006), for example *Turbinaria remiformis* has been shown to be slightly more resilient to increased temperature than *Pocillopora damicornis* (Ulstrup et al. 2006). Alternatively, the stress may have been more pronounced in this study because control temperatures were lower at 26°C and exposure temperatures have exceeded 31°C at times (Table 2.1). However, colour scores (Fig. 3.2) for exposed and fed corals where higher than exposed and starved corals, probably due to increased chlorophyll pigments per zooxanthella (Fig. 3.6), which indicated that feeding still played a role in increasing the bleaching resilience of corals in this and other studies (Ferrier-Pagès et al. 2003, Grottoli et al. 2006, Houlbrèque & Ferrier-Pagès 2009, Tolosa et al. 2011). The colour score system on the Coral Watch Health Chart was developed by Siebeck et al. (2006) using the

photographic brightness and saturation of four typical coral colour hues and is representative of both zooxanthellae density and chlorophyll *a* content as a non-invasive tool for measuring the health of corals in the field but have also been used for experimental assessments (Frisch et al. 2007, Montano et al. 2010). The results of this study suggest that the Coral Watch Health Chart is a viable means of measuring bleaching but is not sensitive to the relative change in zooxanthellae density and chlorophyll content in determining coral colour.

In this study, total lipid content for corals exposed to elevated temperatures were similar to the controls during exposure but declined during the recovery period (Fig. 3.7). This result indicated that, at the time of sampling on day 5 (Table 2.1), corals had sufficient glycogen reserves to provide the energy needed for responding to thermal stress (Flores-Ramírez & Liñán-Cabello 2007, Borell et al. 2008). However, following the exposure period, energy from carbohydrates and that obtained from zooxanthellae may have been depleted resulting in the dependence on lipid reserves for metabolism (Glynn et al. 1985, Grottoli et al. 2004, Borell et al. 2008, Imbs & Yakovleva 2012). In other studies, similar results were also seen for *Pocillopora damicorni*s, *Stylophora pistillata*, *Acropora intermedia*, *Montipora digitata*, *Porites compressa* but not for *Galaxea fascicularis* and *Montipora verrucosa* indicating species specific differences in lipid reserve utilization and zooxanthellae dependence (Grottoli et al. 2004, Borell et al. 2008, Imbs & Yakovleva 2012). Lipid peroxidation from production of ROS under thermal stress is also damaging to lipid structures in cells thereby reducing lipid content or resulting in cell mortality (Downs et al. 2002, Flores-Ramírez & Liñán-Cabello 2007), and may have contributed to a decrease in lipid content in the current study. Although peroxidation was not measured in this study, the decrease in lipid content (Fig. 3.7) could have resulted in some oxidative damage to lipids. Future work may thus benefit from a more detailed study of lipid peroxidation and substrate utilization to assess the energetic state of corals during thermal stress and following bleaching.

Feeding reduced the loss of lipids in corals following elevated temperatures compared to starved corals in this study (Fig. 3.7) and also by Borell et al. (2008) who similarly showed that feeding on *Artemia salin*a significantly reduced the decline in lipid content

of *Stylophora pistillata* following exposure to elevated temperature but not for *Galaxea fascicularis*. Imbs and Yakovleva (2012) recently showed that following bleaching, *Acropora intermedia* and *Montipora digitata* lost percentages of polyunsaturated fatty acids proportional to the percentage of zooxanthellae loss confirming that that these compounds are provided by zooxanthellae (Papina et al. 2003). Polyunsaturated fatty acids are required for coral metabolism and stress responses and therefore may become depleted under thermal stress (Papina et al. 2003, Tolosa et al. 2011, Imbs & Yakovleva 2012). Considering that feeding decreases coral lipid loss from stress, e.g. this study and Borell et al. (2008), and zooxanthellae provide polyunsaturated fatty acids to corals (Papina et al. 2003), it can be inferred that feeding on *Artemia salina* either provides some of the polyunsaturated fatty acids lost by bleached corals or provides the energy required to produce polyunsaturated fatty acids (Tolosa et al. 2011). This further supported the hypothesis that heterotrophy provides significant nutrients for stress responses and metabolism following the loss of energetic transfers from zooxanthellae resulting in a greater scope for resilience (Grottoli et al. 2006, Borell et al. 2008, Palardy et al. 2008, Tolosa et al. 2011).

4.4. The overall role of heterotrophy in coral resilience to thermal stress and bleaching

Overall, this study demonstrated that exposure to high temperatures negatively affects *Pocillopora damicornis* at various levels of organisation and confirmed the hypothesis that heterotrophic feeding improves coral health and resilience when exposed to high temperatures. From a behavioural standpoint, under thermal stress *Pocillopora damicornis* retracted their polyps during the day to reduce exposure (Fig. 3.1) but did not change their feeding effort at night (section 3.1.4). Physiologically, it was found that that the oxygen consumption rates of *Pocillopora damicornis* increased at high temperatures (Fig. 3.3) as a result of increased metabolic rates to fuel energetically costly stress response mechanisms such as antioxidant production (Fig. 3.8). It was inferred that lipid reserves were eventually used to offset the energy deficit caused by stress (Fig. 3.7). However, lack of available energy led to a decline in antioxidant production (Fig. 3.8) and an increase in oxidative damage of DNA (Fig. 3.9).

Consequently, zooxanthellae are expelled to prevent further damage which resulted in a decline of zooxanthellae densities (Fig. 3.4), chlorophyll content (Fig. 3.5) and apparent colour (Fig. 3.2), i.e. corals became bleached. Bleached corals may lose the nutrition, and consequently the energy, they would have obtained from expelled zooxanthellae and may depend on lipid reserves which resulted in the decline in lipid content during the recovery period (Fig. 3.7). It can be noted here that to my knowledge no other studies have attempted to follow this multi-level approach to explain the mechanism coral bleaching.

Heterotrophy played a significant role in ameliorating the negative effects of thermal stress in the physiology of *Pocillopora damicornis* but did not influence all levels of organization. The availability of *Artemia salina* prey did not influence coral polyp extension or feeding rates (Fig. 3.1, section 3.1.4). The availability of food had no influence on zooxanthellae density (Fig. 3.4) or chlorophyll content (Fig. 3.5) but slightly improved levels of lipid content in the recovery phase (Fig. 3.7). This indicated that feeding reduced the energy depletion caused by thermal stress and bleaching. Higher oxygen consumption rates were found for exposed and for fed corals which indicated higher energy availability for stress responses compared to starved corals (Fig. 3.3). Consequently, antioxidant potentials for starved corals were lower (Fig. 3.8) and resulted in higher oxidative damage of DNA (Fig. 3.9). In summary, this study demonstrates that feeding on *Artemia salina* aids in maintaining a healthy energy status of *Pocillopora damicornis* during thermal stress and aids in the recovery from a depleted energy status following bleaching. Therefore, heterotrophy does add to the resilience of *Pocillopora damicornis* to thermal stress and bleaching.

4.5. Significance for coral reefs

Pocillopora damicornis specimens in this study were collected from rock pools situated at high latitude which are usually subject to higher environmental variability compared to tropical reefs (Celliers & Schleyer 2002, Obura 2005, McClanahan et al. 2007). Thus, specimens in this study may have been more resilient to temperature changes than corals in tropical reefs due to environmental experience and because *Pocillopora*

damicornis are also regarded as one of the more resilient species (Brown et al. 2002, Obura 2005, McClanahan et al. 2007). *Pocillopora damicornis* is a branching coral which usually depend less on heterotrophy and more on zooxanthellae compared with other growth forms such as massive corals (Coles & Jokiel 1977, Loya et al. 2001, Grottoli et al. 2006, Guest et al. 2012). Thus, the influence of feeding on coral resilience to thermal stress may be more pronounced for massive corals and other growth forms that depend more on heterotrophy making interpretation of this study robust (Coles & Jokiel 1977, Loya et al. 2001, Grottoli et al. 2006, Guest et al. 2012). For example, the results of this study indicated that coral assemblages may still contain branching corals following bleaching events which were previously regarded as more susceptible to bleaching and that corals that can shift to rely on heterotrophy for their energetic requirements may survive and recover from thermal stress and bleaching (Grottoli et al. 2006, Palardy et al. 2008, Connolly et al. 2012, Guest et al. 2012).

The role played by heterotrophy in determining coral susceptibility to thermal stress and recovery from bleaching, as demonstrated by this and other studies, has ecological consequences and significance for corals reefs (Ferrier-Pagès et al. 2003, Grottoli et al. 2006, Palardy et al. 2008, Ferrier-Pagès et al. 2010). The ability of corals to switch to heterotrophy to supplement the energy required for stress responses and metabolic processes following bleaching allows corals to survive thermal anomalies (Grottoli et al. 2006, Palardy et al. 2008, Connolly et al. 2012). Feeding allows corals to recover quicker from bleaching and promotes growth and reproduction in healthy corals allowing populations to recover and grow (Baird & Marshall 2002, Ferrier-Pagès et al. 2003, Cox 2007, Rodolfo-Metalpa et al. 2008, Séré et al. 2010, Connolly et al. 2012).

Heterotrophy as a contributor to stress resilience of these corals is highly dependent on a healthy reef community as adequate zooplankton populations are required to provide the nutrients lost by expelled symbionts (Yahel et al. 2005, Grottoli et al. 2006, Palardy et al. 2008, Connolly et al. 2012). Therefore, trophic interactions influence the resilience of coral reefs to thermal anomalies because adequate zooplankton prey and adequate top-down control of zooplanktivores are required to maintain healthy zooplankton populations for coral feeding (Yahel et al. 1998, Yahel et al. 2005, Grottoli et al. 2006,

Baker et al. 2008, Knowlton 2008, Alldredge & King 2009). In addition, coral associates such as symbiont crustaceans, corallivore predators, and herbivorous fish are required to protect corals that have been weakened following thermal stress or prevent opportunistic algal growth over dead coral skeletons (Glynn 1985a, 1993, Obura 2005, Stewart et al. 2006, Hughes et al. 2007, Baker et al. 2008). Consequently, other anthropogenic impacts such as overfishing and eutrophication and can severely reduce the resilience of coral reefs resulting in the loss of coral reefs following bleaching (Glynn 1993, Hughes 1994, Hughes et al. 2007, Knowlton 2008).

Maintaining healthy and resilient coral reefs thus requires control of various anthropogenic stressors by coral reef managers. The results of this study promote an ecosystem approach to coral reef management and conservation which maintains the overall health and resilience of the coral reef due to healthy zooplankton and coral associate populations. The results of this study also suggest that coral reefs with healthy zooplankton populations may be viable candidates for marine protected areas due to the prospect of rapid recovery and subsequent reproductive capability providing reseeding areas for reefs that are more susceptible to the negative effects of thermal anomalies (Reaser et al. 2000, Obura 2005, Cox 2007, Connolly et al. 2012). Alternatively, marine protected areas can be selected to aid zooplankton recovery if zooplankton depletion is of anthropogenic cause such as overfishing, pollution and climate change (Reaser et al. 2000, Hughes et al. 2007, Doney et al. 2012). Managers can also choose to boost resilience or mitigate the harmful effects of thermal stress and bleaching by increasing the zooplankton availability of afflicted reefs (Baker et al. 2008). Baker et al. (2008) suggested that nightly illumination of coral reefs may attract zooplankton increasing their availability to corals. Zooplankton availability may also be boosted by increasing water motion, controlled nutrient input and zooplanktivore removal (Baker et al. 2008, Nicolle et al. 2011).

4.6. Significance to coral research, limitations and scope for future work

The results of this study have significance for research regarding coral physiology, thermal and oxidative stress and bleaching but have various limitations that may be

addressed in future work. In particular, no other studies have attempted to demonstrate the mechanisms of thermal stress and coral bleaching experimentally by integrating coral behaviour, coral physiology, tissue composition and cellular responses. Thus, an integrated picture of coral responses to thermal stress was portrayed and various predictions can be made from the results.

Changes in polyp behaviour have rarely been used in the literature as a marker for coral stress but have been shown in this study to be sensitive to thermal stress even prior to maximum exposure (Fig. 3.1), and may provide an early warning of unfavourable conditions. This may be an interesting aspect of investigation for future work. Also, polyp extension and retraction was only noted during the day and data for night-time polyp extension may have provided further insight into this response. Feeding rates did not change between treatments in this study. It is possible that the accuracy of the feeding effort measurement was compromised due to low sample size (n = 9), *Artemia salina* nauplii attaching to pieces of algae and other items in the tanks, nauplii death was not accounted for, and the size and orientation of nubbins and total number of polyps in tanks was not accounted for leading to high variation in the data (personal observation).

To my knowledge, this is the first study to investigate the influence of feeding on the antioxidant potential (Fig. 3.8) and DNA integrity (Fig. 3.9) in corals under thermal stress, supporting the hypothesis that heterotrophy contributes to coral resilience. However, antioxidant potentials in exposed corals declined further than controls and may have benefited from earlier sampling at lower temperatures, similar to the sampling regime in Griffin and Bhagooli (2004), to show that antioxidants initially increase in response to oxidative stress and decline when energy is not available to sustain responses. Oxygen consumption rates in this study agreed with previous research regarding coral respiration under elevated temperature. However, oxygen consumption rates were only measured in the dark this study may have benefited from an additional measurement at sunlight to infer photosynthetic rates (Borell et al. 2008). However, light intensities varied from day to day depending on weather conditions and at different positions in the tanks and may have affected this result (Table 2.1, personal

observation). Controlled lighting may have decreased the variation seen in this study but at the loss of the influence of natural light fluctuations as occurs in the wild.

Feeding was shown to improve the apparent colour of corals exposed to thermal stress which declined compared to controls demonstrating the Coral Watch Health Chart (Siebeck et al. 2006) was a viable method of measuring coral bleaching in the lab but is not sensitive to the relative contribution of zooxanthella density and chlorophyll content in determining coral colour. This hypothesis was further supported by the increased chlorophyll *a* content per zooxanthella for fed corals under thermal stress (Fig. 3.6). However, zooxanthellae (Fig. 3.4), chlorophyll *a* content (Fig. 3.5) and lipid content (Fig. 3.7) for corals exposed to elevated temperatures did not recover for either the starved or fed corals during the recovery period set for this experiment and may have benefited by a longer recovery period and/or lower exposure temperatures e.g. Tolosa et al. (2011). Also, lipid content results for the stress period may have benefited from a later sampling than day 5 to allow for further depletion of lipids under stress to indicate a difference to the controls (Fig. 3.7). Although protein concentration was determined in samples extracted to quantify antioxidant potential per protein concentration (Fig. 3.8), direct protein content in coral tissue could not be determined. Quantifying total proteins and the presence of certain proteins such as heat-shock proteins may have given further insight into substrate utilization and cellular responses in corals under thermal stress.

The results of this study demonstrated that feeding on *Artemia salina* significantly decreased the negative effects of elevated temperatures on the health of *Pocillopora damicornis*. If the energy status of an animal is compromised with exposure to high temperature, as in this study, survival, growth and reproduction are affected (Baird & Marshall 2002). Heterotrophic feeding provides the energy needed to drive stress responses, protection and repair processes needed at high temperatures and may in addition also provide sufficient energy for growth and reproduction (Baird & Marshall 2002, Ferrier-Pagès et al. 2003, Houlbrèque & Ferrier-Pagès 2009). I suggest that heterotrophy be considered as a tool of determining coral reef resilience and for amelioration of coral reef bleaching. Zooplankton populations should therefore be considered when assessing the resilience of coral reefs. The viability of this tool can

thus be tested in future work using mesochosm experiments or assessing the zooplankton population of reefs with differential degrees of bleaching damage. Another consequence of climate change is ocean acidification from increase carbon dioxide solubility in the marine environment (Doney et al. 2009). Interactions between thermal stress and acid stress seem to be a future crisis for coral reefs and may also warrant future research.

4.7. Conclusion

There is no sign of global warming slowing down and coral reefs are still at risk of thermal stress and resultant bleaching and mortality (IPCC 2007). Consequently, this study investigated heterotrophy in providing the nutritional requirements for coral metabolism and stress responses that are lost when algal symbionts are expelled under thermal stress. It was found that corals that were fed were less susceptible to damage from elevated temperature in physiological responses and tissue composition such as DNA damage and antioxidant potentials which have not been tested before, oxygen consumption, chlorophyll *a* content per zooxanthella, colour and lipid content. However, polyp behaviour, zooxanthellae density and total chlorophyll *a* content of corals exposed to elevated temperature were unaffected by feeding. Therefore, the results of this study supported the hypothesis that heterotrophy contributes to the resilience of corals to thermal stress and bleaching which has also been shown by Grottoli et al. (2006), Palardy et al. (2008) and more recently Connolly et al. (2012). This suggests that zooplankton populations play a large role in determining coral reef resilience and should be considered by coral reef managers for the conservation of coral reefs in a warming world (Reaser et al. 2000, Obura 2005).

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