Exposure to multiple stressors in the stony coral *Acropora cervicornis*: Effects on photosynthetic and respiration rates

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 15, 2018

Keywords: *Acropora cervicornis*, photosynthesis, respiration, respirometry, coral, abrasion

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Abstract

Recreational diving is an increasingly popular ecotourism activity that has allowed unprecedented amounts of direct human interaction with coral reef communities. Inadvertent contact by divers with benthic invertebrates such as delicate branching corals causes tissue abrasion that is known to impair coral growth and survival, but impacts on the underlying coral physiological processes are not well understood. In addition, global ocean temperatures are increasing due to anthropogenic climate change, but the interactive effects on reef corals of temperature stress combined with diver-caused abrasion stress remain unknown. The present study investigated impacts on the metabolic physiology of endangered branching staghorn corals *Acropora cervicornis* from both temperature and abrasion stress treatments under laboratory conditions. Using flow through respirometry, I measured net photosynthesis and respiration rates of cultured branch fragments of *A. cervicornis*. Two types of treatments were applied: (1) temperature stress, as measured at 22, 25, 28, and 30°C, and (2) abrasion stress, applied as 15% of tissue removed from fragments in contrast to non-abraded control fragments, creating 8 possible classes of the 2 treatments combined. A total of 26 coral fragments belonging to 6 genotypes were examined, with repeated measurements on some fragments, and a mixed effects linear model applied to account for effects of individual fragments. Net photosynthesis of the coral fragments under light conditions peaked at 25°C and declined at extremely low or high temperatures, while respiration increased linearly with temperature in all fragments, leading to an overall decline in the ratio of photosynthesis to respiration (P:R, a measure of energy balance)

with temperature. There was a significant interaction effect between thermal and abrasion stress, in that tissue abrasion caused significant impairment of photosynthesis rates and augmentation of respiration, especially at high temperatures. P:R was highest for both types of fragments (abraded and non-abraded) at the relatively cool temperature of 22°C. Non-abraded corals were unable to meet their metabolic energy needs (i.e.: P:R < 1) at temperatures at or above 28°C. In contrast, P:R in the abraded fragments declined to 1 (i.e.: barely meeting energy needs) at the moderate coral reef temperature of 25°C, and was substantially < 1 at all higher temperatures. We conclude that some coral colonies exposed to impacts of frequent diver visitation in the form of tissue abrasion may be unable to meet their metabolic energy demands even at moderate coral reef temperatures. Their depressed photosynthetic rates and elevated respiration at higher temperatures leads to a more highly negative energy balance than for non-abraded corals, when exposed to heat stress. Diver damage in the form of tissue abrasion thus appears to reduce coral resilience to temperature-induced bleaching events. Our results also indicate that from an energetic standpoint, warming temperatures may diminish the viability of coral asexual reproduction via fragmentation, because natural fragmentation involves tissue damage and repair processes that impair the coral's energy balance at high temperature. We recommend that reef managers mitigate the interactive effects of these two types of reef stressors by working with dive operators to incentivize recreational dive trips to corals reefs during the relatively cool winter months each year, rather than during warmer months. Altered seasonal timing of diver stress on reef corals may allow them to better withstand the temperature stress that often occurs during the relatively warm summer season.

Acknowledgments

I would like to thank Dr. Chadwick, who welcomed me into her lab and encouraged me to follow my interests. As a student from the Midwest with limited prior experience in coral biology, locating an open-minded lab that allowed me to pursue my interests in marine biology was a tough find. Although it has been challenging at times over the past two years, the experience has allowed me to grow as a scientist and as an individual. I would also like to thank my committee members, Dr. Raymond Henry and Dr. Scott Santos, for lending me their time and expertise in pursuing this endeavor. Both were instrumental in providing critical perspectives and helping with the methodology that allowed me to answer my research questions.

Thank you to the members of the Chadwick Lab, for your assistance with tank care and moral support. To Brady Holte, thank you for sharing your aquarium expertise and for always lending a listening ear whatever the problem was. I am personally thankful to the Auburn Masters Swim team, which never failed to provide an outlet and helped keep a balance between academics and competition during my time here.

And of course, I want to thank my parents for always encouraging and supporting me to follow my interests in biological sciences; without them none of this would be possible. Finally, I would like to thank the collaborators who provided coral fragments. Bill Hoffmann of the Smithsonian Marine Station (SMS) reached out and sent the first batch of coral fragments, allowing this project to materialize. These specimens were originally collected under Florida Wildlife Commission (FWC) permit #01S-612A. Amelia Moura of the Coral Restoration

Foundation (CRF), thank you for your assistance and patience. Corals obtained from the CRF had been cultured in offshore nurseries under the CRF NOAA FKNMS permit (#FKNMS-20011-159-A4). This collaboration with the CRF is part of CRF Project ID #CRF-2019-004.

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List of Abbreviations

SMS	Smithsonian Marine Station
AU	Auburn University
CRF	Coral Restoration Foundation
RTN	Rapid Tissue Necrosis
BW	Buoyant Weight
ESA	Estimated Surface Area
PSA	Precise Surface Area
TL	Total Length

Chapter I

Review of Coral Bleaching and Physiological Responses to Bleaching Events

Introduction

Often cited as the ocean's equivalent of rainforests, coral reefs around the world are home to 30 phyla of vertebrates and invertebrates (Birkeland 2015). Their propensity to support such diversity is not their only likeness to rainforests. Coral reefs are highly productive ecosystems, playing a large role in carbon sequestration (Bellewood *et al.* 2004; Lamb *et al.* 2014). Additionally, coral reefs provide indispensible services to local human communities that rely directly upon them in the form of sustenance, storm protection, and socioeconomic stability. The backbone of these critical habitats is the calcareous reef builders (Order Scleractinia). Hard-bodied corals create the massive structure of reefs by growing calcium carbonate skeletons in nutrient depleted tropical waters.

The ability to build extensive reefs in highly turbulent, shallow waters is not a simple feat. However, similar to many cnidarians, scleractinian corals harbor endosymbiont dinoflagellates of the genus *Symbiodinium* inside vacuoles within their endodermal cells. These *Symbiodinium* cells contribute a majority of their fixed carbon to the host cnidarian cells, providing more than 100% of the energy requirements for many corals (Muscatine 1990; Davies 1984; Muscatine 1973). The coral in return provides

inorganic nitrogen, phosphorus, carbon dioxide, safety, and stability to the *Symbiodinium*, in a dynamic marine environment with high planktonic turnover (Muscatine and Porter 1977; Rodrigues and Grottoli 2007; Venn *et al*. 2008; Yellowlees *et al*. 2008; Davies 1984). Aspects of this symbiotic relationship remain unresolved, with the signaling mechanisms between the coral and algal cells remaining largely unknown (Douglas 2003). The unique relationship between *Symbiodinium* and corals is only one component of the symbioses hosted by reef corals. Microbial assemblages within the outer mucus layer of the coral colony also contribute to the cycling of nutrients and energy transfer. In total, this system that makes up the backbone of modern reefs is defined as the coral holobiont (Rowher *et al.* 2002).

The growing impacts of anthropogenic climate change and coastal development have endangered coral reef ecosystems worldwide, and thus the services they provide to humans. Rising ocean temperatures, an oceanic pH decrease of 0.3-0.4 units by 2100, growing frequencies of weather anomalies such as El Nino Southern Oscillation (ENSO), terrestrial runoff, and increasingly harmful fishing practices have resulted in a sharp decline in the world's tropical reefs (Hughes *et al.* 2008; Orr *et al.* 2005; Caldiera and Wickett 2003; Hoegh-Guldberg 1999; Raymundo *et al.* 2009; Guilherme *et al*. 2013; Lamb *et al.* 2014; Douglas 2003). Additionally, growing industries and infrastructure development along coastal areas cause local instability to coral reefs via anthropogenic terrestrial runoff that sparks algal blooms, eradication of critical nursery habitats (eg, mangroves), and increased direct human-to-coral contact. More frequent and severe temperature-related bleaching episodes, paired with coral diseases, are decimating tropical reefs at an alarming rate (Hughes *et al.* 2017; Hughes 2008; Muller *et al*. 2008;

Jones *et al*. 1997). Already 30% of the world's reefs are severely damaged, and estimates predict that number will double to 60% by 2030 (Hughes *et al.* 2008; Wilkinson 2002).

This worldwide loss of corals and reefs is occurring partly through interruption of the physiological mechanisms that drive their high productivity—the symbiosis between coral and *Symbiodinium*. Thermal stress can result in a dramatic loss of the mutualistic symbiotic algae in a phenomenon known as bleaching (Douglas 2003). As ocean temperatures continue to rise, bleaching has become increasingly widespread, resulting in global-scale epidemics of bleaching on reefs in 1998, 2010, and 2015 (Hughes *et al.* 2017). Here I review the biological mechanisms that cause thermal-induced coral bleaching, and possible physiological characteristics that may enhance the resilience of the coral holobiont to bleaching stress.

What is coral bleaching?

Bleaching occurs when the coral host expels the photosynthetic algae (*Symbiodinium*) from the endoderm of the animal tissue. The *Symbiodinium* are responsible for providing to the coral some of its characteristic color hues, typically brown to yellow, via pigment molecules chlorophyll a and c_2 in addition to several carotenoids (Douglas 2003). Thus, with the expulsion of the algae, the coral host loses its color, giving it a "bleached" white appearance. Typically this occurs via severe reduction (70-90%) in algal density (Fitt *et al*. 2000). Bleaching may result from several types of environmental stressors. For instance, extreme fluctuations in temperature, high irradiance, reduced photoperiod, sedimentation, heavy metals, abnormal nutrient input,

salinity, and disease-causing pathogens all may induce bleaching responses in corals (Hoegh-Guldberg 1999; Brown *et al*. 2000; Hughes *et al*. 2008; Douglas 2003; Weiss 2010).

Of these factors, temperature is perhaps the most widely-studied with respect to coral bleaching. The overwhelmingly consistent observation in the field is that an increase of 1-3 °C above average summer surface seawater temperature (SST) results in coral bleaching (Hoegh-Guldberg & Salvat 1995; Hoegh-Guldberg *et al.* 1996; Brown *et al.* 1996; Jones *et al.* 1997; Glynn 1993; Brown 1997). An early hypothesis proposed that bleaching was an adaptive strategy, wherein corals could increase their fitness by expelling sensitive *Symbiodinium* species in exchange for more temperature resilient symbionts (Hughes *et al*. 2008). However, the current body of evidence suggests that bleaching is a stress response, often resulting in corals exhibiting increased mortality, decreased growth rates, greater susceptibility to disease, and lower fecundity (Coles and Brown 2003; Rosenberg *et al*. 2007; Weiss 2008; Hughes *et al*. 2008; Muller *et al*. 2008).

Because bleaching may serve as an umbrella term for a common symptom of many different types of environmental ailments, it is likely that there are several kinds of pathways of interaction between host and algal cells that may trigger bleaching. However, in the case of thermally-induced bleaching, the mechanism appears to initially lie within the photosynthetic pathways of the *Symbiodinium*. Damage to photosystem II (PSII) and the disruption of the Calvin cycle are prevalent among *Symbiodinium* that have been exposed to elevated temperatures (Warner *et al*. 1999; Jones *et al*. 1997; Douglas 2003). The damaging agents appear to be reactive oxygen species (ROS) that

ultimately lead to dysfunction in *Symbiodinium* photosynthesis (Brown 2002; Warner *et al*. 1999; Weis 2008; Cunning and Baker; Weiss *et al*. 2008; Smith *et al*. 2005).

Weiss (2008) proposed three mechanisms that may initiate this dysfunction: 1) the D1 protein of PSII in the thylakoid membrane becomes irreversibly damaged (Warner *et al*. 1999; Takahashi *et al*. 2004); 2) rubisco loses its function, decreasing carbon fixation (Lesser 1996; Jones *et al*. 1997; Venn *et al*. 2008); and 3) light and heat energy directly alter the electron transport train (Tchernov *et al*. 2004). Jones *et al*. (1997) suggested that initial dysfunction occurs in the Calvin cycle, potentially with rubisco (as cited above), which then results in disrupted assimilation of electrons and damage to PSII.

In all of these mechanisms, there is a resulting buildup of energy in the form of excited electrons. If the dark reactions of photosynthesis are disrupted, then carbon fixation ceases and the electrons no longer reduce $NADP^+$. Instead, excess electrons react with O_2 to produce damaging ROS, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), reactive singlet oxygen ($^1O_2$), and hydroxyl radicals ($\cdot OH$) (Jones *et al*. 1997; Lesser 2006; Tchernov *et al*. 2004; Weiss 2008). These ROS then cause further oxidative damage within the *Symbiodinium*, disrupting enzyme function, harming the thylakoids, or even causing cell apoptosis (Tchernov *et al*. 2011; Weiss 2008). Temperature increases also can cause mitochondrial damage in the coral animal host cells, via ROS production and damage to coral DNA and proteins (Dykens *et al*. 1992; Lesser and Farrell 2004).

As a result, the *Symbiodinium* become overactive and/or breakdown. The host cell then works to remove the harmful algae before the ROS begin to accumulate within the animal tissues. There are several possible mechanisms by which host coral cells expel *Symbiodinium* (Weiss 2008). *Acropora grandis* exhibited signs of exocytosis as a means

of expelling the microalgae, as indicated by intracellular calcium ion changes within the host cell cytoskeleton (Fang *et al*. 1997). *Symbiodinium* also may be digested by the coral's immune system via autophagy, or released via detachment of the entire host endodermal cell from the rest of the coral tissue (Dunn *et al*. 2007; Weiss 2008). Finally, activation of caspases provides evidence of coral cell apoptosis for expelling *Symbiodinium*, following the buildup of ROS or NO (Chipuk and Green 2008; Lorenzo *et al*. 1999; Brune *et al*. 1999). Additionally, hydrogen peroxide and reactive nitrogen species (RNS) may act as signaling molecules that activate an immunological response within the host, which then initiates pathways of algal cell elimination or coral cell apoptosis (Wiess 2008; Smith *et al*. 2005).

Physiological mechanisms in coral holobionts and their contributions to resilience

Instances of Coral Resilience to Bleaching

Although bleaching events occur on a global scale, local patterns are far from consistent. For instance, despite experiencing temperature increases, corals within an isolated lagoon in the Seychelles were protected from UV-induced bleaching when sheltered by leaf debris (Iluz *et al.* 2008). Other studies indicate that *Symbiodinium* type and abundance aid in enhancing coral temperature tolerance (Rowan *et al*.1997; Glynn *et al*. 2001; Toller *et al*. 2001; Bhagooli & Hidaka 2003; Baker *et al*. 2004). In addition, local histories of thermal stress allow some coral colonies to acclimatize to temperature

changes, making them more resilient when faced with subsequent increases (Mumby *et al*. 2001; Berkelmans and van Oppen 2006; Guest *et al*. 2012).

As corals bleach to remove their dysfunctional *Symbiodinium*, several pathways may reduce the costs associated with oxidative thermal stress, and thus affect bleaching rates. During instances in which bleaching rates vary between and within coral species, bleaching susceptibility can be identified with molecular techniques that measure such mitigating pathways. Understanding which specific physiological biomarkers are responsible for tolerance and resilience will ultimately shape future conservation and management efforts for corals. Keeping this in mind, the following section will focus on indicators of physiological variation in stress responses among corals, including variation among host species in their *Symbiodinium* assemblages, heat shock proteins (HSPs), and antioxidants.

Differences among corals in their Symbiodinium types

As it became apparent that thermal tolerances varied between and even within coral species, researchers began looking into which cellular processes might be responsible for this variation. Around the same time, advances in molecular techniques led to the discovery of vast genetic diversity within the genus *Symbiodinium*, leading to a classification of eight phylogenetic clades A-H (Baker 2003; Pochon *et al*. 2001; Coffroth *et al*. 2001). Data collected from field studies showed that coral species or even individual coral colonies may each host multiple types of *Symbiodinium* (Baker 2003;

Fabricius *et al*. 2004; Fabina *et al*. 2013; Stat *et al*. 2013; LaJuenesse *et al*. 2004; Cunning *et al*. 2015). Additionally, free-living *Symbiodinium* may be relatively diverse within the seawater or sediment surrounding coral reefs (Cunning *et al*. 2015).

Symbiodinium diversity is higher in the Caribbean than in the Indo-Pacific region; in the latter region, the vast majority of corals contain only members of clades C and D (Birkeland 2015). The physical environment also influences *Symbiodinium* abundances within corals. For instance, clade A is more abundant in Caribbean corals in shallow waters with high light levels, while clade C dominates corals of the same species located in deeper, shaded waters (LaJeunesse *et al.* 2002). With such genetic diversity and differences in algal genetic composition among coral colonies, it has become apparent that some *Symbiodinium* clades may be better suited than others to withstand thermal stress. This became clear when examination of Palauan reefs over a small area revealed that specific coral-microalgal assemblages were less prone to bleaching than were others (Fabricius *et al.* 2004). Colonies with the greatest density of clade D *Symbiodinium* were most resilient in the face of temperature-induced bleaching events (Fabricius *et al.* 2004). Other studies have shown similar findings, suggesting that clade D is more temperature resilient than clade C (Baker *et al*. 2004; Rowan 2004; Abrego *et al*. 2008; Oliver & Palumbi 2009; Glynn *et al* 2001; Tchernov *et al*. 2004).

It does not appear that such acquired resilience can be attributed to a simple increase in the density of *Symbiodinium* cells. High densities have in fact been found to make coral colonies relatively susceptible to bleaching, most likely because a high number of damaged algal cells leads to rapid production of ROS (Cunning and Baker 2013). Instead, altered proportions of clades within the coral tissue most likely lead to

increased temperature resilience (Douglas 2003). For instance, Berkelmans and van Oppen (2006) observed that adult corals could gain thermal tolerance as a result of a shift from clade C to D.

The ability of some *Symbiodinium* to tolerate thermal stress appears due to mechanisms associated with light effects, in the form of photoprotective pigments and reduction of photodamage. Resistance to photodamage overall limits the amount of ROS being produced, in addition to other harmful reactive molecules (eg, RNS). For example, coral colonies in a relatively warm location along the Australian Great Barrier Reef house *Symbiodinium* with greater carotene concentrations that those from a cooler location (Howells *et al*. 2011). The ability of carotene to halt ROS production is discussed in the antioxidant section below.

Additionally, clade D *Symbiodinium* may be relatively well adapted at diverting excess electrons into the Mehler-Ascorbate-Peroxidase (MAP) cycle, which decreases accumulation of reducing agents like ROS (reviewed in Warner *et al*. 1996). This diversion of electrons also increases the dissipation of light energy into the form of heat, further preventing reduction of the electron transport chain in PSII (reviewed in Warner *et al*. 1996). Such cellular pathways may mitigate the effects of both thermal and light stress, which often come hand in hand during bleaching, because they may occur on hot summer to fall days when both temperature and light levels are near their annual maxima, including levels of UV radiation.

The widely observed thermal tolerance of clade D does not come without a cost, however. The photosynthetic capacity of these *Symbiodinium* is reduced compared to that of other clades, and may not necessarily meet the energy requirements of stressed corals.

Coral colonies saturated with clade D symbionts exhibit a significant decrease in stored lipids available to meet metabolic needs, relative to corals with non-D *Symbiodinium* (Jones and Berkelmans 2010). Egg size and number typically decrease in these coral colonies as well, indicating a decrease in the energy allocated to reproduction in order to sustain homeostasis during stress (Jones and Berkelmans 2010). Thus, the shift in *Symbiodinium* seen in numerous studies may not be an effective long-term mechanism for increasing resilience in corals. Shifting *Symbiodinium* would then offer limited capacity with the onset of climate change, particularly as temperatures continue to rise over the next several decades. The degree of coral resilience would then depend on how quickly *Symbiodinium* can evolve and adapt to increasing temperatures.

The role of heat shock proteins

Given that some *Symbiodinium* clades appear to have greater resistance to heat stress than others, researchers are searching for which specific physiological mechanisms cause this resilience. Many have postulated that heat shock proteins (HSPs) and antioxidants play significant roles in the coral holobiont response. Both families of molecules are well conserved across a wide range of organisms, having well-documented roles in cellular stress responses and maintenance of cellular stability (reviewed in Kregel 2002; Richier *et al*. 2004). As a result, these potential biomarkers of thermal resilience have become the focus of examining coral responses to temperature increases in both field and laboratory settings.

HSPs act as chaperones and protective agents for other proteins, by maintaining processes involved with protein folding, unfolding, aggregation, degradation, and transport (Kregel 2002; Sorensen *et al*. 2003). During times of temperature stress, these molecules ensure the stability of cellular proteins that may otherwise malfunction or denature. Expression and regulation patterns of the genes associated with HSPs have been observed in coral colonies exposed to temperature increases (Brown *et al*. 2002; Rosic *et al*. 2011; Chow *et al*. 2012).

Hsp60, which plays a role in the folding system of the mitochondrial matrix, has a particular expression pattern in thermally stressed corals (Mayer 2010). Following temperature increase, corals express an initial upregulation of Hsp60 followed by a down-regulation after 36 hours (Seveso *et al*. 2014). Other HSPs (Hsp70 and Hsp90) exhibit similar trends, with increased expression in response to temperatures of 30-34 °C (Rosic *et al*. 2011; Desalvo *et al*. 2010).

Evidence of Hsp expression and production can show up within hours, and last for several days (Kregel 2002). How long such production can last is another question entirely, however. Surely, the energetics and metabolic activity of coral cells limit their cellular ability to cope with stress to a finite degree. The eventual down-regulation of the HSP genes may be an indicator that the cell can no longer cope with thermal stress (Carpenter *et al*. 2010; Seveso *et al*. 2016), or that upregulation is energetically costly and can be maintained for only a short period. Further studies into the limitations and the specific mechanisms of resilience that HSPs provide to corals in the face of bleaching will be necessary to aid in their successful conservation and management.

Possible roles of antioxidants and other molecules of tolerance

Examination of antioxidants in the coral holobiont is relatively new, as the identification of ROS in bleaching has only recently become clear. As noted above, the build up of ROS appears to play a significant role in the expulsion of *Symbiodinium* during bleaching. When ROS production exceeds a cell's antioxidant capabilities, damage to proteins, lipids, and DNA occurs (Richier *et al*. 2006). Antioxidants stabilize free radicals via transfer of electrons, and so it has been hypothesized that they play a role in bleaching resilience. Superoxide dismutases (SOD), a family of antioxidants, play a role in the stress responses in symbiotic cnidarians (Richier *et al*. 2006). Following instances of thermal stress, a number of tropical corals exhibit increased SOD abundance (Brown *et al*. 2002; Downs *et al*. 2000; Richier *et al*. 2006).

A wide variety of SODs play several types of roles in coral cells. Iron-based SOD is found in both *Symbiodinium* and host cells, suggesting that corals express this particular enzyme to combat ROS with the added metabolic activity of photosynthesis (Richier *et al*. 2006). Additional enzymes, including ascorbate peroxidase (in the MAP cycle), MnSOD, and caspase, have been found in high concentrations prior to or following bleaching events, and so may also play roles in the holobiont defense arsenal (Richier *et al*. 2006; Császár *et al*. 2009).

In addition to antioxidants, other molecules found across corals share a role in mitigating oxidation that occurs due to increased photosynthesis and thermal stress, including green fluorescent proteins (GFPs) and mycosporine-like amino acids (MAAs) (Baird *et al*. 2009). These molecules act as a natural sunscreen by absorbing UV radiation

or scattering excess light energy, reducing the amount of excited electrons to react with oxygen (Birkeland 2015). GFP-like pigments have been found to play a critical role in the way coral absorb light and heat (Lyndby *et al*. 2016).

Carotenoids are a family of pigments synthesized by *Symbiodinium* that function in light reactions and also in photoprotective roles (Rajesh *et al*. 2010). Carotenoids include carotenes and xanthophylls. β-carotene and chlorophyll are pigments that react with singlet oxygen, an ROS, thereby forming peroxide and quenching toxic ROS as well as dissipating excess energy in the photosynthetic pathway (Smith *et al*. 2005; Rajesh *et al*. 2010). Evidence additionally suggests that carotene is a precursor to antioxidants or other molecules, and may act as a natural mitigating agent in inhibiting ROS accumulation (Kiran *et al*. 2015; Howells *et al*. 2011). In phytoplankton under elevated light conditions of high PAR and UV-radiation, β-carotene levels increase (Smith *et al*. 1992). Further research into how such pigment molecules mitigate bleaching is necessary to understand the natural defenses of *Symbiodinium*, and whether these defenses are transferred to host cells.

Conclusions

With the increasingly uncertain fate of coral reefs, there is a great urgency to understand the physiological mechanisms involved in bleaching and temperature tolerance in reef-building corals. Corals may acclimatize to increased temperature through responses within either the animal host or the *Symbiodinium* endosymbionts. Local adaptations may occur to some degree in both coral animals and microalgal

symbionts, providing pockets of refuge from climate change (Brown *et al*. 2002; Douglas 2003; Weiss 2010). However, a recent analysis of the Australian Great Barrier Reef following the 2015-2016 bleaching event did not support this thermal-history-acclimation theory (Hughes *et al.* 2017). Additionally, current estimates predict seawater temperatures to rise by 2°C by the end of the century (IPCC 2007). This predicted pace of change may be too rapid for mutation and natural selection to allow corals to adequately adapt to the changing environment.

If corals worldwide cannot acclimate at the same rate as temperature change, then understanding the physiological pathways behind bleaching and thermal resilience will be paramount in supporting management decisions to enhance their survival. Knowledge about such cellular signals and molecular biomarkers can be applied towards management efforts to enhance coral reef resilience, as the state of coral reefs continue to decline. If increasingly more extreme strategies are needed, such as assisted evolution or transplantion of species (as proposed by van Oppen *et al*. 2015), researchers will need to have a firm understanding of the coral resilience mechanisms discussed here. For instance, knowledge would be required about which particular antioxidant or HSP genes could be used to bioengineer a more thermally tolerant clade C without the energetic cost of naturally occurring *Symbiodinium*. Alternatively, identifying specific genes may allow us to artificially select for coral that will be able to withstand global climate projections over the next century.

Alternative methods also may be used enhance the status of coral reefs. Careful management practices and regulation of local human activities, particularly in small coastal nations, can support the preservation of coral reefs. For instance, a managed

balance of herbivores limits the direct competition of corals with insidious macroalgae for space on limited substrate (McClanahan *et al*. 2005). Well-regulated fisheries, perhaps most notably practices used by many generations of islanders, can be sustainable while providing sustenance for growth in local communities. Overfishing has reduced levels of herbivory, and increased instances of coral disease and excess anthropogenic nutrients have ultimately limited the ability of reefs to recover from bleaching events (Hughes *et al*. 2008). As well, corals may become fractured and abraded by intensive ecotourism activities in the form of recreational diving (Krieger and Chadwick 2013; Saphier and Hoffman 2005; Uyarra *et al.* 2009), which could compound stress to corals exposed to climate change. Science-based management of reefs to limit physical damage to corals from tourism activities can make local reefs more resistant to bleaching stress.

In summary, understanding of the physiological mechanisms involved in coral bleaching, and the use of modern biotechnologies, can support the development of strategies for combating the decline of coral reefs. But such tools can only be used to a certain extent from an economic and ethical standpoint. Through further research, knowledge about the physiology of the coral holobiont should be applied towards ecophysiological perspectives to support reef management for holistic and effective strategies to maintain these diverse ecosystems.

Literature Cited

Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species- specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. Proc R Soc Lond B Biol Sci 275: 2273–2282

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: The role of the host. *Trends in Ecology and Evolution* 24:16-20

Baker AC, Jones SH, Lee TS (2004) Symbiont diversity in Arabian corals and its relation to patterns of contemporary and historical environmental stress. In: Proceedings of the International Workshop on Coral Bleaching in the Arabian Region (ed. Abu Zinad A)

Bellewood DR, Hughes TP, Folke C, Nystrom M (2004) Confronting the coral reef crisis. *Nature* 429:827-833

Berkelmans R & van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: A 'nugget of hope' for coral reefs in an era of climate change. *Proceedings: Biological Sciences* 273:2305-2312

Bhagooli R and Hidaka M (2003) Comparison of stress susceptibility of in hospite and isolated zooxanthellae among five coral species. J Exp Mar Biol Ecol 291:181-197

Birkeland C (2015) Biology Trumps Management: Feedbacks and Constraints of Life-History Traits. *Coral Reefs in the Anthropocene* (231-263). Springer

Brown BE, Dunne RP, Chansang H (1996) Coral bleaching relative to elevated seawater temperature in the Anadaman Sea (Indian Ocean) over the last 50 years. *Coral Reefs* 15:151-152

Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs* 16:129-138

Brown BE, Dunne RP, Goodson MS, Douglas AE (2000) Bleaching patterns in reef corals. *Nature* 404: 142-143

Brown BE, Downs CA, Dunne RP, Gibb SW (2002) Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. Mar Ecol Prog. Ser. 242: 119-129

Brune B, von Knethen A, Sandau KB (1999) Nitric oxide (NO): an effector of of apoptosis. *Cell Death and Differentiation* 6: 969-975

Caldiera K and Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Carpenter LW, Patterson MR, Bromage ES (2010) Water flow influences the spatiotemporal distribution of heat shock protein 70 within colonies of the scleractinian coral Montastrea annularis (Ellis and Solander, 1786) following heat stress: implications for coral bleaching. J Exp Mar Biol Ecol 387: 52-59

Chipuk JE and Green DR (2008) How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? *Trends in Cell Biology* 18:157-164

Chow AM, Beraud E, Tang DWF, *et al*. (2012) Hsp60 protein pattern in coral is altered by environmental changes in light and temperature. Computational Biochemistry and Physiology-A 161: 349-353

Coffroth MA, Santos SR, Goulet TL. (2001) Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. *Mar Ecol Prog Ser* 222:85–96

Coles SL and Brown BE (2003) Coral bleaching-capacity for acclimitixation and adaptation. *Advances in Marine Biology* 46:183-223

Császár NBM, Seneca FO, van Oppen MJH (2009) Variation in antioxidant gene expression in the scleractinian coral *Acropora millepora* under laboratory thermal stress. *Mar Ecol Prog Ser* 392:93-102

Cunning R and Baker A (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change* 3:259-262

Cunning R, Yost DM, Guarinello ML, Putnam HM, Gates RD (2015) Variability of Symbiodinium Communities in Waters, Sediments, and Corals of Thermally Distinct Reef Pools in American Samoa. PLoS ONE 10(12): e0145099. doi:10.1371/journal.pone.0145099

Davies PS (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. *Coral Reefs* 2: 181–186

Desalvo MK, Sunagawa S, Voolstra CR, Medina M (2010) Transcriptomic responses to heat stress and bleaching in the elkhorn coral *Acropora palmata. Mar Ecol Prog Ser* 402:97-113

Douglas AE (2003) Coral bleaching—how and why? *Marine Pollution Bulletin* 46:385-392

Downs CA, Mueller E, Phillips S, Fauth JE, Woodley CM (2000) A molecular biomarker system for assessing the health of coral (Montastraea faveolata) during heat stress. Marine Biotechnology 2:533–544

Dunn SR *et al*. (2007). Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: every which way you lose. *Proceedings in Biological*. Sciences 274: 3079–3085.

Dykens JA, Shick JM, Benolt C, *et al*. (1992) Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. *J Exp Biol* 168: 219-241

Fabina, N. S., Putnam, H. M., Franklin, E. C., Stat, M., & Gates, R. D. (2013). Symbiotic specificity, association patterns, and function determine community responses to global changes: Defining critical research areas for coral-symbiodinium symbioses. *Global Change Biology*, *19*(11), 3306–3316. https://doi.org/10.1111/gcb.12320

Fabricius KE, Mieog JC, Colin PL, Idip D, van Oppen JH (2004) Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Molecular Ecology* 13:2445-2458

Fang L, Huang S, Lin K (1997) High temperature induces the synthesis of heat-shock proteins and the elevation of intracellular calcium in the coral Acropora grandis. *Coral Reefs* 16:127–131

Fitt W, McFarland F, Warner M *et al.* (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnology and Oceanography* 45:677-685

Glynn PW (1993) Coral reef bleaching: ecological perspectives. *Coral Reefs* 12:1-17

Glynn PW, Mate JI, Baker AC, & Calderon MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997-1998 El Nino–southern oscillation event: spatial/temporal patterns and comparisons with the 1982– 1983 event. *Bulletin of Marine Science* 69: 79–109

Guest JR, Baird AH, Maynard JA *et al*. (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLoS ONE* doi: 10.1371/journal.pone.0033353

Guilherme Becker C, Dalziel BD, Kersch-Becker MF, *et al*. (2013) Indirect effects of human development along the coast on coral health. *Biotropica* 45:401-407

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

Hoegh-Guldberg O and Salvat B (1995) Periodic mass-bleaching and elevated sea temperatures: bleaching of outer reef slope communities in Moorea, French Polynesia. *Mar Ecol Prog Ser* 11:181-190

Hoegh-Guldberg O, Berkelmans R, Oliver J (1996) Coral bleaching: Implications for the Great Barrier Reef Marine Park. In The Great Barrier Reef, Science, Use and

Management; a National Conference: Proceedings, pp. 210–224. Great Barrier Reef Marine Park Authority Publication, Townsville, Australia

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change* 2:116-120. DOI: 10.1038/NCLIMATE1330

Hughes TP, Baird AH, Bellwood DR, *et al*. (2008) Climate change, human impacts, and the resilience of coral reefs. *Science* 301:929-933

Hughes TP *et al.* (2017) Global warming and recurrent mass bleaching of corals. *Nature* 543:373-377

Iluz D, Vago R, Chadwick NE *et al.* (2008) Seychelles lagoon provides corals with a refuge from bleaching. *Research Letters in Ecology* doi: 10.1155/2008/281038

IPCC (2007) Climate change. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), The Physical Science Basis. Contribution ofWorking Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Jones RJ, Berkelmans R, Oliver J (1997) The recurrent bleaching of corals at Magnetic Island (Australia) relative to air and seawater temperature. *Mar Ecol Prog Ser* 158: 289–292

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impair- ment of the CO2 fixations mechanism in zooxanthellae. Plant Cell Environ 21:1219–1230

Jones RJ and Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. PLoS ONE, vol. 5, no. 5, Article ID e10437

Kiran B Uday, Sushma M, Prasad K V S R G, Rao V Uma Maheshwara, Bai D Jhansi Laxmi, Nisheetha V (2015) Antioxidant and Radical Scavenging Properties of β-Carotene on Cisplatin Induced Cardiotoxicity. Cardiology and Angiology An International Journal 4(3): 98-106

Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal for Applied Physiology 92: 2177-2186

Krieger J and Chadwick N (2013) Recreational diving impacts and the use of pre-dive briefings as a management strategy on Florida coral reefs. *J Coast Conservation* 17:179-189.

Lamb JB, True JD, Piromvaragorn S, Willis BL (2014) Scuba diving damage and intensity of tourist activities increases coral disease prevalence. *Biological Conservation* 178:88-96

LaJeunesse TC, Bhagooli R, Hidaka M, Done T, deVantier L, Schmidt GW, Fitt WK, Hoegh-Guldberg O (2004) Closely related Symbiodinium spp. differ in relative dominance within coral reef host communities across environmental, latitudinal, and biogeographic gradients. Mar Ecol Prog Ser 284:147–161

LaJeunesse TC, Loh WK, van Woesik R, Hoegh-Guldberg O, Schmidt GW, Fitt WK (2002) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnology and Oceanography 48:2046–2054

Lesser MP (1996) Exposure of symbiotic dinoflagellates to elevated temperatures and ultraviolet radiation causes oxidative stress and inhibits photosynthesis. Limnology and Oceanography 41:271–283

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16:187–192

Lesser MP (2006) Oxidative stress in marine environments: Biochemistry and physiological ecology. Annual Review of Physiology 68:253–278

Lesser MP and Farrell JH (2004) Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs* 23:367-377

Lorenzo HK, Susin SA, Penninger J, Kroemer G (1999) Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. *Cell Death and Differentiation* 6:516-524

Lyndby NH, Kuhl M, Wangpraseurt D (2016) Heat generation and light scattering of green fluorescent protein-like pigments in coral tissue. *Scientific Reports* doi:10.1038/srep26599

Mayer MP (2010) Gymnastics of molecular chaperones. *Molecular Cell* 39:321-331

McClanahan TR, Maina J, Starger CJ, *et al*. (2005) Detriments to post-bleaching recovery of corals. *Coral Reefs* 24:230-246

Muller EM, Rogers CS, Spitzack AS, Woesik R (2008) Bleaching increases likelihood of disease on *Acropora palmata* (Lamarck) in Hawksnest Bay, St Jon, US Virgin Islands. *Coral Reefs* 27:191-195

Mumby PJ, Chisholm JRM, Edwards AJ, Andrefouet S, Jaubert J (2001) Cloudy weather may have saved Society Island reef corals during the 1998 ENSO event. Mar Ecol

Prog Ser 222: 209–216

Muscatine L (1973) Nutrition of corals. In Biology and Geology of Coral Reefs, Vol. II. Biology, I., Jones, O.A., and Endean, R. (eds). New York, NY, USA: Academic Press, pp. 77–115

Muscatine LM (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky S (ed) Coral reefs: ecosystems of the world, vol 25. Elsevier Science, Amsterdam, pp 75–87

Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *BioScience* 27:454-460

Oliver TA, Palumbi SR (2009) Distributions of stress-resistant coral symbionts match environmental patterns at local but not regional scales. Mar Ecol Prog Ser 378: 93-103

Orr JC, Fabry VJ, Aumont O, Bopp L, *et al*. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437:681-686

Pochon X, Pawlowski J, Zaninetti L, Rowan R (2001) High genetic diversity and relative specificity among Symbiodinium-like endosymbiotic dinoflagellates in soritid foraminiferans. *Marine Biology* 139:1069–78

Raymundo LJ, Halford AR, Maypa AP, *et al*. (2009) Functionally diverse reef-fish communities ameliorate coral disease. *Proceedings from the National Academy of Science* 106:17067-17070

Rajesh P *et al*. (2010) Photoprotective compounds from marine organisms. Journal of Industrial Microbiology & Biotechnology 37:537-558

Richier S, Sabourault C, Courtiade J *et al*. (2006) Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis. The FEBS Journal* 273:4186-4198

Rodrigues LJ and Grottoli AG (2007) Energy reserves and metabolism as indicators of coral recovery from bleaching. *Limnol and Oceanogr* 52:1874-1882

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. *Marine Ecology Prog Ser* 243:1-10.

Rosenberg E, Koren I, Reshef L *et al.* (2007) The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 5:355-362

Rosic N, Pernice M, Dove S, Dunn S, Hoegh-Guldberg O (2011) Gene expression profiles of cytosolic heat shock proteins Hsp70 and Hsp90 from symbiotic dinoflagellates in response to thermal stress: possible implications for coral bleaching. Cell Stress and Chaperones 16, 69-80

Rowan R, Knowlton N, Baker A *et al.* (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* 388:265-269

Rowan R (2004) Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* 430:742–742

Saphier AD and Hoffman TC (2005) Forecasting models to quantify three anthropogenic stresses on coral reefs from marine recreation: anchor damage, diver contact and copper emission from antifouling paint. *Mar Pollut Bull* 51:590-598.

Seveso D, Montano S, Strona G *et al* (2014) The susceptibility of corals to thermal stress by analyzing Hsp60 expression. *Marine Environmental Research* 99:69-75

Seveso D, Montano S, Stronga G, Orlandi I, Galli P (2016) Hsp60 expression profiles in the reef-building coral *Seriatopora caliendrum* subjected to heat and cold shock regimes. *Marine Environmental Research* 119:1-11

Smith D, Suggett D, Baker N (2005) Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals. *Global Change Biology* 11:1-11

Smith RC, Prezelin BB, Baker KS, *et al*. (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* 255:952-959

Sorensen JG, Kristensen TN, Loeschcke V (2003) The evolutionary and ecological role of heat shock proteins. Ecology Letters 6:1025– 1037

Stat M *et al*. (2013) The distribution of the thermally tolerant symbiont lineage (Symbiodinium clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. *Ecology and Evolution* 3:1317-1329

Takahashi S, Nakamura T, Sakamizu, *et al*. (2004) Repair machinery of symbiotic photosynthesis as the primary target of heat stress for reef-building corals. *Plant Cell Physiology* 45:251-255

Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, *et al*. (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings from the National Academy of Sciences, USA* 101:13531-13535

Tchernov D. *et al*. (2011) Apoptosis and the selective survival of host animals following thermal bleaching in zooxanthellate corals. *Proceeding from the National Academy of Sciences, USA* 108:9905–9909

Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *The Biological Bulletin* 201:348-359

Uyarra MC, Watkinson AR, Cote IM (2009) Managing dive tourism for the sustainable use of coral reefs: validating diver perceptions of attractive site features. *Environ Manag* 43:1-16.

Venn AA, Loram JE, Douglas AE (2008) Photosynthetic symbioses in animals. *Journal of Experimental Botany* 59:1069-1080

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceeding from the National Academy of Sciences, USA* 96:8007-8012

Weiss VM (2008) Cellular mechanisms of cnidarian bleachng: stress causes the collapse of symbiosis. *Journal of Experimental Biology* 211:3059-3006

Weiss VM (2010) The susceptibility and resilience of corals to thermal stress: adaptation, acclimatization or both? *Molecular Ecology* 19:1515-1517

Wilkinson C, Ed., Status of Coral Reefs of the World (Australian Institute of Marine Science, Townsville, Australia, 2002)

Yellowlees D, Rees TAV, and Leggat W (2008) Metabolic interactions between algal symbionts and invertebrate hosts. *Plant, Cell, & Environment* 31:679-694

Chapter II

Effects of tissue abrasion and thermal stress

on the metabolic processes of *Acropora cervicornis*

Introduction

Coral reefs provide indispensible services in the form of sustenance, storm protection, and socioeconomic stability to the coastal communities that rely directly upon them. They also provide additional ecosystem benefits on a global scale, in that they moderate the climate via carbon sequestration, sustain extraordinary biodiversity, and provide products and services to numerous international markets (Bellwood *et al.* 2004; Lamb *et al.* 2014). However, the growing impacts of anthropogenic climate change and coastal development endanger these ecosystems worldwide, and thus the services they provide to humans. Rising ocean temperatures, an oceanic pH decrease of 0.3-0.4 units by 2100, growing frequencies of weather anomalies such as El Nino Southern Oscillation (ENSO), terrestrial nutrient runoff, and increasingly harmful fishing practices have created a synergistically detrimental cycle for the world's tropical reefs (Hughes *et al.* 2003; Orr *et al.* 2005; Caldiera and Wickett 2003; Hoegh-Guldberg 1999; Raymundo 2009; Guilherme Becker *et al*. 2013; Lamb *et al.* 2014; Douglas 2003).

The anthropogenic loss of coral reefs worldwide can be attributed partly to a disruption of the physiological mechanisms of the calcareous reef-builders (Order

Scleractinia). Scleractinian corals create the massive blueprint of coral reefs by growing a calcium carbonate skeleton in nutrient depleted tropical waters. Changes in temperature and pH often disrupt the equilibrium of the chemical reactions involved in this process. Thermal stress can result in further damage to the coral holobiont system by causing a dramatic loss of the mutualistic endosymbiotic microalgae that provide coral colonies with most of their metabolic energy (Douglas 2003). This phenomenon, known as coral bleaching, has become increasingly widespread as ocean temperatures continue to rise above summer norms, and has manifested in global scale epidemics of bleaching on reefs during 1998, 2010, and 2015 (Hughes *et al.* 2017). During a bleaching event, oxidative stress and the buildup of free radicals cause damage to photosystem II (PSII) and its repair system in the coral's microalgae, ultimately resulting in an expulsion of the microalgal symbionts from the coral animal tissue (Brown 2002; Warner et al., 1999; Weis 2008). Recent studies have examined the related changes in coral physiological processes under elevated temperature and irradiance conditions through respirometry to measure coral photosynthetic and respiration rates (Hoogenboom et al. 2006; Anthony & Hoegh-Guldberg 2003; Lesser 1997; Schutter et al. 2008; Al-Sofyani & Floos 2013; Davies 1980; Ulstrup et al. 2011). These types of measurements provide information about the energy input and output of corals under conditions of current global climate change.

In addition to coral damage caused by rising seawater temperatures, reefs increasingly are impaired by localized activities such as industrial development along coastal areas, enhanced nutrient runoff that sparks algal blooms, eradication of critical nursery habitats such as mangroves, and increasing levels of direct human-to-coral

contact. Global ecotourism trends have generated huge diving industries that depend upon coral reefs. However, intensive diving and snorkeling operations may jeopardize the stability of coral reefs (Guzner *et al.* 2010; Lamb *et al.* 2014). Tourist behaviors may result in physical damage to sensitive coral colonies (Davis and Tisdell 1995). While this damage may be unintentional, the sediment resuspension, fragmentation, and tissue abrasion caused by diving tourists all can have detrimental impacts on coral colonies (Hawkins *et al.* 1999; Zakai and Chadwick-Furman 2002). However, the impact of such activities on physiological processes in coral colonies has not received much attention. The synergistic effects of temperature extremes in combination with tissue abrasion on reef corals remain unknown, but are expected to become increasingly common especially on shallow reefs exposed to intensive ecotourism.

Staghorn corals (*Acropora cervicornis*) historically were one of the major shallow-water stony corals in Caribbean reef systems. *A. cervicornis* and the closely related *A. palmata* (elkhorn corals) are the only 2 large branching species of corals in the Caribbean Sea, and both are known to enhance habitat complexity, biodiversity, and fish abundance on reefs (Dixson and Hay 2012; Johnson *et al.* 2011; Holbrook *et al.* 2002). Due to population declines, both *A. cervicornis* and *A. palmata* are currently listed as critically endangered under the IUCN Red List. Corals belonging to the genus *Acropora* are highly susceptible to disease, temperature-induced bleaching, and the effects of other types of damaging agents (Birkeland 2015; Aronson and Precht 2001; Roger 1990; Muller *et al.* 2008). While several studies have measured rates of photosynthesis and respiration in stony corals, few have examined these processes in *A. cerivornis*, and none have determined impacts of tissue abrasion.

The goal of the present study is to assess the interaction effects between 2 types of increasingly relevant coral stressors, tissue abrasion and temperature extremes, on colonies of *A. cervicornis*. We hypothesized that tissue abrasion acts synergistically with thermal stress to hinder the ability of corals to meet their metabolic energy demands, rendering them more susceptible to bleaching and mortality. The larger implications of our results are that physiological effects on corals from seawater temperature increases are compounded by local disturbances in the form of tissue abrasion, which render corals in areas of intensive diving tourism less resilient to global climate change.

Materials and Methods

Animal collection and culture

Fragments of scleractinian corals *Acropora cervicornis* were obtained from non-profit institutions, because this species was protected by the United States Endangered Species Act, and field collecting permits were not obtainable within the timeframe of the present study. In February 2017, 10 large fragments (each 18-28 cm in length) were shipped to Auburn University (AU) overnight in bags filled with seawater, from colonies cultured at the Smithsonian Marine Station (SMS), Fort Pierce, Florida, USA. SMS had originally collected 2 colonies of *A. cervicornis* in 2002 from 6 m depth near Fort Lauderdale, Florida, and cultured them in an indoor public aquarium tank at 26 - 27°C. Both colonies were genetically identical based on molecular analyses, and both grew rapidly in the public aquarium, allowing SMS to supply branch fragments to research institutions (William Hoffman, SMS, pers. comm.).

To obtain a wider genetic diversity of coral fragments, in March 2018, 25 small fragments (each 5-10 cm length) were shipped to AU overnight wrapped in damp paper towels (dry shipping method) from colonies cultured in offshore nurseries at 6-10 m depth (25-26°C ambient seawater temperature at nurseries during the month of shipping) by the Coral Restoration Foundation (CRF), Key Largo, Florida, USA. Dry shipping was used for this latter shipment, because it enhances coral fragment survival and is standard shipping procedure by CRF (Kayla Ripple, CRF, pers. comm.). The latter shipment included 5 fragments belonging to each of 5 genetically-distinct genotypes based on molecular analysis (genotypes B8, K1, M10, M1, and M6 (Amelia Moura, CRF, pers. comm.).

Upon arrival to AU, all coral fragments were acclimated over ~ 1 hr to the seawater temperature and chemistry conditions in laboratory culture tanks. Shipment bags, each containing 5 fragments per bag, were placed into 5-gallon buckets filled with seawater from the culture tanks, to acclimate them to ambient seawater temperature. After 30 min, the fragments were removed from the bags and submerged in each bucket of seawater for 30 min more, then transferred to the culture tanks. Acclimation to irradiance conditions in the culture tanks occurred over 1 wk, because corals are known to require several days to acclimate to changes in irradiance (Anthony & Hoegh-Guldberg 2003; Titlyanov et al. 2001). The level of photosynthetically active radiation (PAR) was measured at the upper surface of each coral inside the culture tank, using a Quantum Scalar Laboratory sensor (QSL-2101) (Biospherical Instruments Inc.). Each coral was initially exposed to a low level of PAR (80 ± 20 mmol photons m^{-2} s^{-1}, mean + SD), then the level gradually was increased over several days to a moderate PAR (180 ±

20 mmol photons m^{-2} s^{-1}), equivalent to irradiance levels averaged over the entire day at ~ 18 and 23 m depth on Florida Keys coral reefs (different from the mid-day maximal irradiance levels at this depth, see below; Lesser 2000).

Two wk after arrival and acclimation to laboratory conditions, the large fragments (>15 cm length) from SMS were divided using industrial pruning shears (Model #BP 7100D, Corona) into smaller fragments (6-15 cm length) to allow them to fit into respirometry chambers (see below). The base of each fragment was attached to a circular ceramic plug (3.12 cm diameter x 1.3 cm height, Marine Depot) using cyanoacrylate gel (Reef Glue, Seachem). In each culture tank, a platform to hold the corals was created from white plastic egg crate grating (1.5 x 1.5 cm interior squares, Plaskolite) and PVC pipes. Coral fragments attached to their ceramic bases were placed in squares of this grating, with the platform ~ 8 cm above the sand at the bottom of each culture tank (Fig. 1).

Twenty-three small coral fragments (each 5–13 cm length) were created from the initial 10 large SMS fragments, and distributed evenly between the 3 culture tanks (6-9 fragments per tank). Some SMS fragments died due to power failure and unknown causes over the course of the study year, leaving 18 SMS fragments, 11 of which were used in metabolic experiments. The remaining 8 SMS fragments were not used because they were too irregular in shape to fit into the metabolic chamber, or ----- [clarify]. In March 2018 when the 26 CRF fragments arrived, the fragments belonging to each of the 5 CRF genotypes were selected randomly for distribution into each of the 3 culture tanks, so that 8-12 coral fragments total were cultured per tank, from both SMS and CRF. The same

procedure for attaching the SMS fragments to bases and positioning them on the culture platforms, was used also for the CRF fragments.

The 3 culture tanks each consisted of a closed-system setup with a 75L culture tank connected to a 75L sump, in which the seawater was filtered by protein skimmers (Class 110 INT Skimmer, Reef Octopus) and bioballs (Coral Life; same setup as used for the long-term culture of tropical sea anemones, shrimps, and fishes in the same laboratory; Roopin and Chadwick 2009, Huebner et al. 2012, Cantrell *et al.* 2015; see also DeSalvo 2010). In addition to alternating water flow provided by the sump pumps, the water flow rate was enhanced for coral culture by attaching a small pump to the inside wall of each culture tank. This was done because the survival and growth of stony corals with small polyps is enhanced at high water flow levels, which simulates the natural conditions of the exposed reef habitats where these corals often occur; high flow augments their rates of metabolic gas diffusion, removal of waste products, and delivery of suspended food and dissolved nutrients (Sheppard et al. 2009; Roberts et al. 1992; Jokiel 1978; Borneman 2001).

Water temperatures were maintained at 26 ± 1°C because this is the optimal temperature for tropical coral growth in closed system aquaria (Seveso *et al.* 2016; Lirman *et al.,* 2011; Marshall and Clode, 2004; Precht and Aronson 2004; Hubbard 2015). Salinity was maintained at 34 ppt with Instant Ocean Sea Salt Mix (Instant Ocean), with a 30 L (20%) water change in each culture tank biweekly. Other water chemical parameters were monitored regularly and remained at levels similar to those on coral reefs. Calcium, alkalinity, and magnesium were maintained at coral reef levels

using liquid additives (calcium and alkalinity: Reef Fusion, Seachem; magnesium: Tech M Magnesium, Kent Marine).

Fluorescent T5 light fixtures (Model: 3' 6-bulb, 120V, 960020, Sunlight Supply) were installed over each culture tank and provided PAR irradiance of 180 ± 20 mmol photons m^{-2} s^{-1}, equivalent to daily average irradiance levels at ~ 18-23 m depth on Florida Keys coral reefs (Lesser 2000) as measured at the upper surface of each coral (see also above). Light fixtures were set to a 12h:12h light:dark cycle. Corals were fed weekly with a mixture of Coral Frenzy (Marine Depot) and live *Artemia* brine shrimp hatched from dried eggs (San Francisco Bay Brand). 0.5 g of dried *Artemia* eggs were cultured for 72 hours in 800 ml of seawater. After hatching they were added to 400 ml of saltwater that had been stirred with 2g of Coral Frenzy. The final food mixture consisted of both *Artemia* and Coral Frenzy in 1.2 L of seawater. Corals were fed at the end of each week, so that prior to respirometry trials near the start of each following week, they were starved for at least 48 hours. This was done to limit effects of recent heterotrophic feeding on photosynthesis and respiration rates (Ferrier-Pagès et al. 2010). During feeding, the sump pumps were turned off and the pumps were left on that were attached inside the culture tanks, so that food particles circulated throughout each culture tank and did not go into the sump. 400ml of food mixture was added to each tank (3 tanks x 400 ml = 1200 ml total food mixture). The sump pumps remained off for 40 min, allowing all corals to feed on the suspended food particles; all of the coral fragments were observed to capture and ingest food items each week.

All coral fragments arrived alive in both shipments, but mortality rates after arrival differed between the shipments. Six of the 26 fragments that arrived from the

SMS exhibited rapid tissue necrosis (RTN), or sloughing of tissue, during the first week in the laboratory. In an effort to salvage fragments that appeared to be worsening in condition, the fragments were cut with industrial shears (Model #BP 7100D, Corona) ~2 cm above the line of tissue loss. This emergency procedure worked for 3 of the fragments, which recovered and exhibited normal polyp extension within 1-2 wk. Other incidents that caused fragment mortality included temperature spikes in August 2017 (upwards of 4°C above normal culture temperature) and a power outage in December 2017, during which water flow was diminished and temperature dropped to 22°C. Of the fragments that arrived from the CRF, only 2 of the 25 fragments did not survive the shipping process. One large fragment was divided in two due to its size. No CRF fragments died between March 2018 and the end of the study in August 2018.

Most of the cultured corals consistently exhibited expanded polyps and grew, indicating healthy physiological state. After ~ 30-60 d in culture, both the soft tissues and calcareous skeletons of most corals began to grow over the epoxy and ceramic plug at the base of each fragment (Fig. 2). After skeletal growth onto the plug was observed, each fragment was used in respiration and photosynthesis trials (see below). Another parameter used to gauge coral health was color. Fragment color was recorded as the closest hue to that on a standard 6-hue color scale used for approximating the health of reef corals (CoralWatch, University of Queensland, coralwatch.org). Fragments were considered healthy if they had a color of at least D3 or C3. If an individual had a hue lighter than these values (i.e., D2), it was not used in a respirometry trial.

Trials with the respirometry setup (see description below) were conducted intermittently during November 2017 to July 2018. Fragments belonging to all 6

genotypes (1 SMS plus 5 CRF) were used in respirometry experiments. For temperature variation experiments, 11 SMS fragments were used, and of those 10 were exposed to more than one temperature; of the 14 CRF fragments used, 10 were exposed to more than one temperature. In the abrasion trials, 6 SMS fragments were used, and all were exposed to more than one temperature, while of the 11 CRF fragments used in abrasion trials, 6 were exposed to more than one temperature (see details below).

Respirometry setup

A custom-made respirometry chamber was designed to hold each coral fragment in an upright position and to accommodate the ceramic plug to which each fragment was attached (Fig 3). A hollow acrylic plexiglass cylinder (height = 23 cm, diameter = 6 cm) was attached to rubber stoppers wrapped in Parafilm at both ends. The basal stopper had a shallow depression (~ 1 cm deep) drilled in the center to serve as a holder for the ceramic plug with the coral attached. The interior volume of the stoppered chamber was 440 ml, similar to the volume of respirometry chambers used in past studies on corals (Table 1).

At a height of 2.4 cm above the bottom of the chamber, and at the same distance down from the top of the chamber, on opposite sides, small holes were drilled and a Precision Glide 18 G 1 ½ needle was inserted into each hole and glued into place. The outer end of each needle was attached to flexible silicone tubing (3.4 mm internal diameter; standard intravenous tubing). A stopcock upstream of the first needle controlled the rate of seawater flow through the chamber. Water flowed by gravity from a reservoir tank on a shelf above the chamber to an input O_2 electrode, then through the needle at the

bottom of chamber. Water exited the chamber through the top needle, then to an output O_2 electrode and a bucket on the floor. Both O_2 electrodes (Strathkelvin Instruments, Motherwell, North Lanarkshire, UK) were connected to a Cameron Instrumental OM200 Oxygen Meter, which displayed the dissolved O_2 readings in torr (modified after Szczebak et a. 2011; Fig. 3).

After each trial, the 20L water reservoir that fed into the respirometry chamber was refilled with seawater (34 ppt) mixed from Instant Ocean crystals, same as the seawater used for culture tanks (see above). The reservoir contained an air bubbler to continuously aerate the seawater. Seawater temperature inside the reservoir was altered to obtain the desired treatment temperatures in the respirometry chamber. To obtain a seawater temperature of 25-26°C in the respirometry chamber, an Aqueon adjustable glass heater (50W) was placed inside the reservoir and set to 28-29°C. Due to heat loss as the seawater traveled through the silicon tubing, the seawater reaching the chamber typically lost ~ 3-4°C. For temperature trials at 22, 28, and 30°C, the heater in the reservoir was adjusted, and the coiled tubing ran through a temperature-regulated water bath before reaching the respirometry chamber (Fig. 3).

Net Photosynthesis Rates

To measure their rates of net photosynthesis at several temperatures, coral fragments were transferred individually from the culture tanks into the respirometry chamber. Before placing each fragment in the chamber, the following variables were recorded while the fragment was still submerged in its culture tank: color, total length, and maximum diameter. Fragment color was recorded as the closest hue to that on a

standard 5-hue color scale used for approximating the health of reef corals (CoralWatch, University of Queensland, coralwatch.org). Fragment total length and maximal diameter were measured to the nearest 1 mm using flexible measuring tape, because this was more accurate than using a stiff ruler given the slightly curved shape of most fragments. Maximal diameter was measured as the widest point along the fragment long axis, which often was near the base. Any macroalgae or other organisms attached to the ceramic plug were scrubbed off using a stiff toothbrush (Colgate), to prevent non-coral organisms from affecting the measured metabolic rates. Then while still submerged in the culture tank, the ceramic plug was fitted onto the Parafilm-covered rubber stopper from the base of the respirometry chamber, and the chamber tube and top stopper were placed over the entire fragment and plug, trapping some of the tank seawater inside the chamber. This process kept the coral fragment completely submerged in seawater and prevented any shocks due to rapid change in seawater temperature or exposure to air.

With the coral fragment inside, the chamber then was attached to the respirometry setup (Fig. 3). The water flow rate was set at 30 ml min^{-1} by adjusting the stopcock position in the tubing, to gradually flush the retained seawater from the culture tank out of the chamber (~ 15 min for 440 ml chamber volume). During this initial water flushing, the coral fragment also was acclimated to the seawater temperature for its assigned trial. The water flow rate was adjusted to ~ 8-13 ml min^{-1} for the duration of ~ 2 hr incubation to measure net photosynthesis rate (after Jokiel and Coles 1977; Schutter et al. 2008; Hoogenboom et al. 2006). An adjustable LED light fixture (Model HYA05-LENS-55*3W-B, Galaxyhydro) was set up lateral to the chamber to evenly expose the long axis of the coral fragment to irradiance (Fig. 3). Irradiance level was measured inside the

chamber by filling the chamber with seawater prior to trials, and inserting the probe of a Quantum Scalar Laboratory sensor (QSL-2101, Biospherical Instruments Inc.) into the chamber. The light then was adjusted so that 350 ± 20 mmol photons m^{-2} s^{-1} reached the chamber interior (approximate irradiance during the midday maximum on Florida coral reefs at ~ 18-23 m depth) (Lesser 2000). This irradiance level was ~ 2x that in the culture tanks (see above), so that the corals were exposed to irradiance conditions that mimicked those they would experience at midday on the coral reef, and thus photosynthesize at mid-day rates.

Before each trial, both oxygen electrodes were calibrated with air-saturated seawater at the same temperature as in the chamber. After the coral fragment had acclimated for 2 hr inside the chamber with flowing seawater at the desired temperature, both the inflow and outflow oxygen levels were recorded every min for 30 min. Net photosynthesis rate then was calculated and normalized to coral surface area. In order to do this, the solubility factor of oxygen was determined for each trial at their experimental temperature based on the values from Dejours (1975). This converted the observed ΔO_2 from the O2 meter from torr to μmol L^{-1}. Then the average ΔO_2 value for the 30 min observation period was adjusted to water flow rates, and normalized to surface area of the coral fragment in order to obtain per-surface area rates similar to those reported in previous respirometry experiments on corals (Equation 1, after Anthony & Hoegh-Guldberg 2003; Lesser 2000):

Eq. 1

$$\frac{Average\ O_2\ (\mu mol\ L^{-1}) * Flow\ rate\ (ml\ min^{-1}) *\ 60min}{conversion\ factor\ 1000\ \left(\frac{ml}{L}\right) * Fragment\ Surface\ Area\ (cm^2)}$$

Respiration Rates

Coral respiration rates were measured during the daytime by covering the respirometry chamber with blackout cloth to simulate dark conditions. This process yielded accurate coral nighttime respiration rates, because previous studies have revealed no significant difference between dark respiration rates measured during the daytime vs. nighttime for stony coral fragments (Muthiga and Szmant 1987). A respiration trial was run immediately after the photosynthesis trial for each fragment, while it was still in the chamber. The light fixture adjacent to the chamber was turned off and blackout cloth was wrapped completely around the chamber (after Coles and Jokiel 1977). The coral then was allowed to acclimate to dark conditions for 2 hr, by which time the oxygen levels appeared to re-stabilize. Then the oxygen inflow and outflow measurements were recorded every min for 30 min, similar to the photosynthesis trials, and respiration rate was calculated using the same equation (Eq. 1).

At the end of each paired photosynthesis and respiration trial, seawater temperature inside the chamber was gradually returned to the culture tank temperature. The entire chamber with the fragment remaining inside was re-submerged in the culture tank, and left there for 10-20 min as the seawater within the chamber adjusted to the ambient tank temperature. This was done to minimize any temperature shocks to the corals after respirometry trials. With the respirometry chamber inside the culture tank, the coral was removed underwater. To prevent accumulation of microorganisms and biofilm that might affect future respirometry measurements, the plexiglass chamber was washed

with DI water and scrubbed thoroughly before the next trial. Effects of bacteria on respiration and photosynthesis measurements were considered to be negligible, because blank trials run in the chambers filled with seawater but lacking coral fragments showed almost no O_2 flux between inflow and outflow electrodes.

Coral size parameters

Three types of size parameters were calculated for each coral fragment: buoyant weight (BW), estimated surface area (ESA), precise surface area (PSA). BW was determined each month for a period of 4-12 mo per fragment, depending on how long each was cultured in the laboratory (after Jokiel 1978). A weighing frame made of plastic egg crate grating was attached to a paper clip using thin nylon fishing line. This apparatus was submerged in a 40-L tank filled with seawater and hung using nylon line from the under-hook of an electric balance (Pioneer PA 2201, Ohaus) placed on a frame over the tank. Each coral fragment was placed gently onto the plastic weighing frame and its BW was recorded on the electronic balance to the nearest 0.1 g while the fragment remained submerged. This process appeared to cause minimal stress to the corals, because they all re-expanded their feeding polyps and ceased to produce excess mucus within a few minutes of being returned to their culture tanks.

Immediately prior to the use of each fragment in a respirometry trial, its ESA was calculated using the equation for surface area of a cylinder shell surface, $SA = 2 \pi hr$, where SA = surface area, h = total length of the major and minor branches, and r = ½

maximal diameter as measured near the fragment base (Fig. 3). This method was used because it provides similar coral surface area estimates to those obtained using advanced 3D scanning techniques of the coral surface (Naumann et al. 2008), and past studies have used branch length and diameter measurements of *Acropora* fragments to obtain surface area estimates (Dennison and Barnes 1988). Given the limited number of specimens available for the present study, ESA was used as a proxy for PSA in estimating rates of photosynthesis and respiration, because PSA could not be calculated without sacrificing the fragments.

After each fragment had been used in respirometry trials, it was sacrificed and its PSA was obtained. A Water Pik (Waterpik Water Flosser, Ultra, Rite Aid) was used to remove all live tissue from each fragment (after Johannes and Wiebe 1969; Richier *et al.* 2003), then the cleaned coral skeletons were air-dried overnight. PSA was obtained using the tin foil method: the weight of tin foil required to completely cover each fragment was compared to the weight of tin foil required to cover an object with known surface area (after Marsh 1970). Three body size metrics were used to estimate the precise surface area of each fragment during respirometry trials (buoyant weight at time of trial, final buoyant weight, and final precise surface area), by assuming a proportional relationship between buoyant weight and surface area (Equation 2):

Eq. 2

$$\frac{Trial\ Surface\ Area\ (cm^2)}{Trial\ Buoyant\ weight\ (g)} = \frac{Final\ Surface\ Area\ (cm^2)}{Final\ Buoyant\ weight\ (g)}$$

Effects of stressors on net photosynthesis and respiration rates

To determine effects of two types of stressors (temperature variation and tissue abrasion) on coral rates of net photosynthesis and respiration, corals were subjected to stress treatments prior to respirometry trials. Coral fragments were randomly assigned to each stress treatment, with some fragments exposed to multiple treatments due to the small number of fragments available for the study. Potential confounding effects from previous stress trials were accounted for in the statistical model (see statistical analyses below).

Temperature

Net photosynthesis and respiration rates were measured at four temperatures: 22, 25, 28, and 30°C. We considered 25°C as the control temperature, because this was the temperature in the culture tanks, and is known to be the optimal temperature for tropical stony coral growth (Seveso *et al.* 2016; Lirman *et al.,* 2011; Marshall and Clode, 2004; Precht and Aronson 2004; Hubbard 2015). The treatment temperatures were selected based on the temperature range recorded on coral reefs in Florida (US DOC/NOAA/NWS/ National Data Buoy Center). The bleaching threshold for corals typically occurs at 1-2°C above summer maximum temperatures (Baker et al. 2008; Jokiel and Coles 1990;Jokiel 2004; Hughes et al. 2008). Because the culture tanks were maintained at 25-26°C (and field conditions were the same temperature in the offshore Florida nurseries where the corals were before collection, see above), 28°C was

considered to be a threshold level for bleaching, and fragments were expected to show metabolic responses indicative of a bleaching event. Additionally, the extent of impacts from recent bleaching events in combination with IPCC models suggest that temperatures may rise beyond the 1-2°C bleaching threshold, hence 30°C was added to the temperature trials (Hughes et al. 2017; Baker et al. 2008; IPCC 2007). On the other end of the spectrum, we chose 22°C to reflect common minimum temperatures in the Florida Keys during winter months (US DOC/NOAA/NWS/ National Data Buoy Center).

For the temperature stress treatments, the temperature of seawater flowing into the respirometry chamber was altered gradually, at < 0.5°C min^{-1}, based on past studies (Coles and Jokiel 1977). This temperature adjustment allowed for an acclimation period of 20-40 min to the temperatures in the stress trials (22, 28, and 30°C), in addition to the 2-hr incubation period within the respirometry chamber. The water in the chamber was checked every 15 min during temperature acclimation, by opening the top of the chamber and inserting a digital thermometer (Model: 9840, Taylor).

When the chamber seawater reached the desired treatment temperature, it was resealed by reinserting the top stopper, and the coral was allowed to acclimate to the final temperature for 2 hr before oxygen levels were recorded. The chamber seawater temperature was rechecked again at the end of recording the oxygen flux values, to ensure that the treatment temperature had been maintained. A small beaker filled with DI water, a heater, and coils of the inflow tubing leading to the chamber served as a temperature bath to maintain the treatment temperature of seawater as it flowed from the upstream reservoir into the chamber (Fig. 3). Any trial in which the chamber temperature deviated from the treatment value by > 1°C was re-run.

Tissue abrasion

To simulate the abrasion stress inflicted on corals when scuba divers and snorkelers scrape coral surfaces with their fins, hands, or other appendages (Zakai and Chadwick-Furman 2002, Krieger and Chadwick 2012), the coral fragments were abraded. To obtain preliminary information on how rates of coral healing from abrasion wounds varied with wound size, 4 randomly-selected fragments were manipulated. This provided information on the abrasion wound size to use for the stress treatments in the respirometry trials. Two types of preliminary abrasion wounds were created on the fragments: small (1.5 cm^2 surface area) and large (5 cm^2 surface area; n = 2 fragments each; approximate dimensions of each abraded area = 1 x 1.3 cm and 1.4 x 3.5 cm respectively). Abrasion wounds were created by scraping the coral surface with the blunt handle of a pair of metal forceps. Coral tissue was completely removed by scraping the coral until only the underlying white calcium carbonate skeleton was visible in the abraded area. The abraded area was along the midsection of the long axis of each fragment. Photographs of the abraded fragments were taken daily to gauge recovery time. Fragments were considered to have fully recovered when they had regained normal brown pigmentation and polyps were visible throughout the previously abraded area.

Based on results of the preliminary trials (see Results for details), *A. cervicornis* fragments were selected randomly to have approximately 15-20% of their surface area abraded (= ~ 2.5-8.4 cm^2 surface area, or wound dimensions of ~ 1 x 2.5 to 2 x 4.2 cm) to inflict enough stress to induce a physiological response, but not so much as to endanger fragment survival.

Infliction of the abrasion treatments was done within the culture tank, and the fragment then was allowed 72 hours recovery prior to respirometry trials. This allowed for any loose damaged tissue or excess mucus along the margins of the abraded area to disperse and be filtered out of the seawater in the culture tank. The dimensions of the abraded tissue area were measured to the nearest mm using calipers, and the abraded surface area (ASA) was calculated (= length x width of the wounded area). The ASA then was subtracted from ESA and PSA values when calculating photosynthesis and respiration rates, so that metabolic rates were reported per unit of live coral tissue; i.e., the calculations were corrected for the removed tissue, and measured only the oxygen fluxes of the coral surface that remained covered with living tissue.

Fragments that had been abraded were assigned randomly to temperature treatments at 22, 25, 28, and 30°C, to test for interaction effects between the two types of stressors examined (temperature and abrasion).

Statistical analyses

To test for the effect of temperature on the metabolic rates of *A. cervicornis* fragments, a repeated measures ANOVA was run using the lme function within the nlme package of the statistical program R (R Core Team 2016). This nonlinear model included a random effect for individual fragment, to account for any variability contributed by a particular fragment being tested in multiple treatments; i.e., for a fragment used in trials at both 22°C and 28°C, any confounding effects of treatment order were accounted for in the model. A repeated measures ANOVA was used to test for variation in the metabolic rates of abraded fragments among temperatures, in a similar analysis to the one above.

Subsequently, both models were combined to test for interaction between the two stress variables of temperature and tissue abrasion. All three models were used to compare differences among photosynthetic rates, respiration, and P:R ratio in the stress treatments compared to the control treatments. Results are expressed as means ± 95% CI, unless otherwise indicated.

Results

Relationships among Fragment Size Parameters

All 4 size parameters of the coral fragments (buoyant weight [BW], total length [TL], and estimated surface area [ESA] and precise surface area [PSA]) correlated significantly with each other (Fig. 4). The tightest correlation was between ESA and PSA, while the loosest correlation was between TL and BW. For each 1 cm^2 increase in ESA, there was a 0.89 ± 0.053 cm^2 increase (n = 32) in PSA (d.f. = 30; $p < 0.0001$; $r^2 = 0.974$; Fig. 4A), indicating that ESA slightly underestimated PSA, but that the two measures were highly correlated. For each 1 g increase in buoyant weight (BW), there was a 6.83 ± 0.83 cm^2 increase in PSA (d.f. = 30; $p < 0.001$; $r^2 = 0.902$; Fig. 4B). As such, the correlation between BW and PSA, while significant, does not explain as much of the variation in PSA as does the relationship between ESA and PSA. For each 1 cm increase in TL, there was a 4.25 ± 0.29 cm^2 increase in PSA (d.f. = 30; $p < 0.001$; $r^2 = 0.966$; Fig. 4C), indicating a fairly tight correlation between TL and PSA. Additionally, for each 1 cm^2 increase in ESA, there was a 0.12 ± 0.013 g increase in BW (d.f. = 30; $p < 0.001$; $r^2 = 0.92$) (Fig. 4D), indicating that ESA could be used to estimate BW for certain fragments.

Also, for every 1 cm increase in TL, there was a 0.56 ± 0.078 g increase in BW (d.f. = 30; $p < 0.01$; $r^2 = 0.876$; Fig. 4E). Therefore, ESA was a better indicator of BW than was TL for these coral fragments. These size relationships together indicate that it is possible to accurately predict the PSA of the coral fragments from their ESA measurements, and that their other relationships among size parameters are more loosely correlated.

Coral Fragment Growth and Healing Rates

The coral fragments that arrived from the Smithsonian Marine Station (SMS) in February 2017 grew rapidly during the first several months under laboratory conditions, then their growth slowed and remained at lower but steady rates for up to 10 mo (Fig. 5A-C). Most fragments grew at ~ 0.1g mo^{-1} in terms of BW. Coral fragments that arrived from the Coral Restoration Foundation (CRF) in March 2018 also exhibited growth in the culture tanks, at rates of ~ 25% mass gain over 5 mo, equivalent to ~ 0.1-0.2 g mo^{-1}, somewhat more rapid than the growth of the SMS fragments (Fig. 6A-E). Due to the irregular shape of many of the fragments, growth rates as measured by TL appeared to be an inaccurate method to assess short-term growth; the TL of some of the fragments decreased slightly among monthly measurements, even though they gained mass as recorded by BW, so their growth rates were not reported in TL.

Within 3-4 wk after arrival to the laboratory, the coral fragments produced calcareous skeleton and soft tissue that extended onto the ceramic plugs at their bases (Fig. 2). Feeding polyps remained continually expanded on ~ 75% of the 26 SMS fragments, and 100% of the 23 CRF fragments (Fig. 2). Fragments that grew rapidly

exhibited white or pale polyps at the fragment branch tip, whereas those that grew more slowly often exhibited terminal polyps that were darker in color.

The CRF fragments varied in their growth patterns among culture tanks. All fragments in culture tank #4 (n = 6) exhibited dark color (C4, D4 or darker), active polyp extension, and white branch tips throughout the duration of the study, and they grew the most of the 26 CRF fragments. Based on this observation, the other 20 CRF fragments then were transferred out of culture tanks in a separate room, and into the culture tanks (7-9 fragments total per tank x 3 tanks) in the same room as tank #4, after which they exhibited improved health (color and polyp extension) as well as growth at a rate comparable to that in tank #4. This transfer occurred prior to respiration trials, therefore we assumed no tank effect in the respirometry results, although there may have been a tank effect in the early fragment growth trends.

Preliminary observations indicated that relatively large abrasion wounds (5 cm^2 surface area) fully recovered in ~ 45 days (range = 40-47 d, n = 2 fragments, Fig. 8), while smaller abrasion wounds (1.5 cm^2) fully recovered in only ~ 32 days (31-39 days, n = 2 fragments, Fig. 9). As such, abrasion wound size for the main experiment was set at 15-20% of the coral's total surface area (~ 2.5-8.4 cm^2 surface area, or wound dimensions of ~ 1.0 x 2.5 to 2.0 x 4.2 cm). The larger wound size was used for abrasion treatments so that enough stress was inflicted to induce a detectable physiological response, while still allowing for complete wound healing within 2 mo.

Coral Respiration and Photosynthesis

Effects of temperature

There was a significant interaction effect between temperature and abrasion ($p < 0.01$) on rates of photosynthesis by the coral fragments, so the abraded vs. non-abraded fragments were analyzed separately in terms of their photosynthetic rates. Conversely, there was no significant interaction effect between temperature and abrasion on either coral respiration rates ($p = 0.70$) or their ratios of photosynthesis to respiration (P:R ratios, $p = 0.44$).

In the non-abraded (control) trials ($n = 65$), rates of photosynthesis peaked at 25°C and decreased as temperature increased or decreased (Fig. 7a; Table 2). Seawater temperature of 25°C thus was assumed to be the thermal optimum for photosynthesis in cultured fragments of *A. cervicornis*, with a net photosynthetic rate of 1.14 ± 0.08 μmol O_2 L^{-1} cm^{-2} hr^{-1}. Corals incubated at relatively low temperature (22°C) produced only 0.67 ± 0.11 μmol O_2 L^{-1} cm^{-2} hr^{-1}, representing a significant decline in oxygen production of 41% less than that at the 25°C thermal optimum ($p < 0.001$). Corals exposed to relatively high temperature (28°C) likewise produced only 0.47 ± 0.11 μmol O_2 L^{-1} cm^{-2} hr^{-1}, representing even greater decline (59%) from the rate at 25°C (significantly less; $p < 0.001$). *A. cervicornis* fragments exposed to extremely high temperature (30°C) significantly depressed their photosynthetic rates even further, to 0.21 ± 0.12 μmol O_2 L^{-1} cm^{-2} hr^{-1}, corresponding to an 82% decrease from maximal values at the 25°C thermal optimum ($p < 0.001$). The net photosynthetic rate of corals at extremely high temperature

(30°C) was significantly lower (by 69%) than that at relatively low temperature (22°C; $p < 0.001$).

In contrast to the pattern observed for coral net photosynthesis, the respiration rate increased linearly with temperature (Fig. 7b; Table 1), at a more gradual rate than changes in photosynthesis. Corals incubated at the 25°C thermal optimum for photosynthesis consumed oxygen at a rate of 0.57 ± 0.08 μmol O_2 L^{-1} cm^{-2} hr^{-1}. At the relatively low temperature of 22°C, they exhibited oxygen consumption of only 0.33 ± 0.11 μmol O_2 L^{-1} cm^{-2} hr^{-1}, significantly lower than that at 25°C (by 42%, $p < 0.05$). Respiration at the high temperature of 28°C increased to 0.72 ± 0.11 μmol O_2 L^{-1} cm^{-2} hr^{-1}, which was only a 27% increase from that at 25°C, and therefore only slightly and not significantly greater than at 25°C ($p = 0.157$). At extremely high temperature (30°C), respiration reached its highest observed value of 0.81 ± 0.12 μmol O_2 L^{-1} cm^{-2} hr^{-1}, representing a significant increase of 42% over that at the 25°C thermal optimum for photosynthesis ($p < 0.05$). As such, respiration rates at both levels of high temperature (28 and 30°C) were significantly greater than at low temperature (22°C). Corals incubated at 28°C consumed 118% more than those incubated at 22°C ($p < 0.005$), and at 30°C, they consumed 145% more than when incubated at 22°C ($p < 0.001$).

Based on the above patterns, the ratio of photosynthesis to respiration (P:R ratio) exhibited by the coral fragments decreased significantly with temperature. Corals incubated at low temperature (22°C) had P:R ratios as high as ~3:1, which decreased approximately linearly to P:R ratios < 1:1 at both of the high temperatures (28 and 30°C, Fig. 7c). The highest P:R ratio was 2.54 ± 0.39, and recorded at low temperature (22°C; Fig. 7c; Table 1). Although not significantly different, the mean was 22% higher than for

the P:R ratio at 25°C, which was only 2.08 ± 0.26 ($p = 0.243$). Fragments at 28°C further decreased their P:R ratio to 0.70, dipping below the metabolic equilibrium of P:R = 1. As such, P:R at 28°C was 66% less than at 25°C, significantly lower ($p < 0.001$). The lowest P:R ratio was exhibited by corals incubated at the highest temperature (30°C), which was only 0.30 ± 0.41. This corresponded to a significant decline of 86% from P:R at 25°C ($p < 0.001$). The P:R ratio also differed significantly between 22°C and 28°C, and between 22°C and 30°C. When incubated at 28°C, the coral fragments exhibited a P:R ratio 73% less than at 22°C ($p < 0.005$); and when at the highest examined temperature of 30°C, their P:R ratio was 88% less than that at the lowest temperature (22°C, $p < 0.001$).

Effects of Tissue Abrasion

In a pattern that contrasted with that of non-abraded control fragments, photosynthesis rates of the abraded fragments did not peak at medium temperature (25°C) but instead decreased gradually with temperature (Fig. 7a; Table 2). Fragments of *A. cervicornis* that had sustained 15% tissue abrasion exhibited net photosynthetic rates of only 0.46 ± 0.10 μmol O_2 L^{-1} cm^{-2} hr^{-1} when incubated at the moderate temperature of 25°C. This was not significantly different from the net photosynthetic rate of those incubated at the cooler temperature of 22°C, which was 0.51 ± 0.12 μmol O_2 L^{-1} cm^{-2} hr^{-1}, representing only an 11% increase in mean oxygen production ($p = 0.66$). Their net photosynthetic rates decreased further at elevated temperatures (28 and 30°C), to very low values of 0.047 ± 0.11 μmol O_2 L^{-1} cm^{-2} hr^{-1} and 0.16 ± 0.11 μmol O_2 L^{-1} cm^{-2} hr^{-1}, respectively. Thus, abraded fragments at 28°C exhibited the lowest net photosynthetic rates, with a significant decline of 90% from those at 25°C ($p < 0.005$). While corals at 30°C did not exhibit the lowest mean photosynthetic rate, there was a significant decline

of 64% from the moderate temperature of 25°C ($p < 0.05$). There was no significant difference between 28°C and 30°C, in terms of net photosynthetic rates ($p = 0.33$).

Due to these large depressions in the net photosynthetic rate of the abraded corals relative to those that had not been abraded, during some of the temperature treatments, the abraded fragments photosynthesized at significantly lower rates per unit live tissue area (that is, of their remaining non-abraded tissue) than did the non-abraded control fragments (Fig. 7a). At 25°C, the abraded fragments photosynthesized at rates that were significantly less than exhibited by non-abraded control fragments ($p < 0.05$), representing a 60% decline in oxygen production by *A. cervicornis* fragments that had suffered tissue abrasion, despite being incubated in seawater at the thermal optimum for photosynthesis of 25°C. At 28°C, the abraded corals exhibited an even larger impairment of net oxygen production, in that they produced 91% less than did the non-abraded fragments ($p < 0.01$). There was no significant difference in photosynthetic rate between corals in the abrasion and control treatments, at either 22 or 30°C ($p = 0.19$ and 0.30, respectively).

In terms of respiration rates, similar to the pattern observed for non-abraded control fragments, the abraded *A. cervicornis* fragments increased respiration linearly with temperature (Fig. 7b; Table 2). Abraded corals exposed to the lowest temperature of 22°C exhibited the lowest respiration rate of 0.39 ± 0.14 μmol O_2 L^{-1} cm^{-2} hr^{-1}, while at 25°C, abraded fragments had respiration of 0.59 ± 0.13 μmol O_2 L^{-1} cm^{-2} hr^{-1}. Thus, the colder temperature lowered mean oxygen consumption by 35%, but this difference was not significant ($p = 0.14$). On the other hand, abraded corals at 28°C exhibited greatly increased respiration rates of 0.97 ± 0.13 μmol O_2 L^{-1} cm^{-2} hr^{-1}. Compared to abrasion

trials at 25°C, respiration was significantly higher, elevated by 63% ($p < 0.01$). Even further, the highest temperature of 30°C caused the highest rate of coral respiration at 0.98 ± 0.13 μmol O_2 L^{-1} cm^{-2} hr^{-1}. The fragments with abrasion, when exposed to high temperature, respired at a mean rate that was 66% than that at 25°C ($p < 0.01$).

In terms of pairwise comparisons among temperatures, the mean respiration of abraded corals at 28°C was 151% higher than at 22°C, a significant increase in oxygen consumption ($p < 0.001$). This pattern continued as the corals exposed to 30°C respired at mean rate that was 155% more than at 22°C, corresponding to a significant increase in oxygen demand ($p < 0.001$).

When comparing abraded to non-abraded (control) coral fragments, the abraded corals respired on average more per unit tissue area than did the non-abraded corals at all incubation temperatures, and this difference increased with temperature level (Fig. 9b). The difference between control and abraded fragments was not as pronounced for respiration as it was for photosynthesis, in that only at 28°C was there a significant difference between the abrasion treatments, with abraded fragments consuming significantly more oxygen (by 35% on average) than did non-abraded fragments ($p < 0.05$). Further analysis with a multivariate model accounting for random effects by fragment did not detect any significant differences between the non-abraded fragments and those that had abrasions treatments, when tested at the same temperature (22°C, $p = 0.79$; 25°C, $p = 0.16$; and 30°C, $p = 0.45$).

Based on the above patterns, the ratio of photosynthesis to respiration (P:R ratio) exhibited by the abraded fragments decreased significantly with temperature, similarly to the overall trend for non-abraded fragments (Fig. 7c; Table 2). However, P:R was much

lower at all temperatures in abraded than non-abraded corals. For abraded corals, it was greater than 1:1 only at 22 and 25°C. The P:R ratio for abraded *A. cervicornis* fragments incubated at 25°C was only 1.11 ± 0.25, barely above the compensation point of P:R = 1. The highest average for abraded corals was at 22°C, with a P:R of 1.81 ± 0.30. This equated to a 63% increase over that at 25°C, and was significantly greater ($p < 0.05$). However, at 28°C, abraded coral fragments had a P:R well below 1, representing only 0.13 ± 0.29, in part because during some trials the corals had negative net photosynthetic rates, with overall oxygen consumption even under light conditions. This was an 89% decline in P:R, a significant decrease when compared to that for abraded corals at 25°C ($p < 0.005$). The P:R of abraded corals at 30°C was slightly higher, at 0.34 ± 0.30, but was not significantly different from that at 28°C ($p = 0.51$). Abraded fragments at 30°C demonstrated a significant decrease in P:R compared to that at 25°C, with a 70% decline ($p < 0.05$). The energy balance of abraded corals remained above compensation point only at the 2 cooler temperatures examined, and were well below compensation (P:R ~ 1:1) in terms of their metabolic energy ratios, at both of the warmer temperatures examined.

Due to the depressed photosynthesis and augmented respiration rates in abraded corals, at some temperatures these fragments exhibited significantly lower P:R ratios than did their non-abraded counterparts. At the thermal optimum of 25°C, abraded fragments had P:R values that were significantly less than those of non-abraded corals ($p < 0.005$). This represented a 47% reduction in energy availability to the holobiont when abraded vs. when not abraded. A similar trend was found for the trials at 28 °C, wherein abraded corals had a significantly lower P:R than non-abraded fragments ($p < 0.005$),

corresponding to an even larger 82% decrease in P:R ratio between the control (non-abraded) and abraded fragments. On the other hand, there was no significant difference between non-abraded and abraded fragments at either 22°C ($p = 0.682$) or 30°C ($p = 0.136$) in terms of their P:R values.

Overall, the results revealed significant effects of both temperature and tissue abrasion on the per-unit tissue area rates of all metabolic parameters examined (photosynthesis, respiration, and P:R ratio), with some interaction effects between the 2 types of stressors.

Discussion

General comments

We show here that under laboratory conditions, photosynthetic rates of the endangered branching coral *Acropora cervicornis* peak at moderate coral reef seawater temperatures (~25°C) but that respiration rates increase linearly with temperature, such that in undamaged colonies the photosynthesis to respiration ratio (P:R) falls below the energy compensation threshold level of 1:1 above ~ 28°C. In addition, our results indicate that tissue abrasion stress, such as that caused by recreational divers, compounds the impacts of temperature stress on metabolic processes, and causes these corals to fall well below energy compensation at 28 °C, which is only 2-3°C higher than their acclimated temperature, enhancing their vulnerability to the effects of global climate change. Interaction between these two types of stressors (temperature and tissue abrasion) indicates that while extreme temperatures pose a threat to the metabolic energy gain of

the coral holobiont, abrasion of the living tissue from even 15% of the colony surface significantly reduces the net photosynthetic output per unit area of remaining tissue. The level of physiological impairment due to tissue abrasion was most extreme at moderate temperature (25°C), where net photosynthetic rates were diminished to less than half (only ~ 37%) those of non-abraded corals. Abraded corals respired more per unit area of remaining tissue than did non-abraded corals, and this effect increased with temperature. Overall, abrasion stress compounded the effects of temperature stress, causing the corals to fail to meet their metabolic energy requirements at warmer temperatures. These results have implications for the local management of reef-based tourism, in that coral reefs exposed to high levels of diving tourism could be significantly more vulnerable to the effects of climate change, through the physiological impacts of non-intentional diver abrasion of coral tissues. Conversely, sustainable management of reef tourism to limit coral abrasion rates could render reefs more resilient to climate change.

Coral size parameter correlations and growth rates

The strong correlation observed here between coral surface area estimates obtained through two major methods, the tin foil method (Marsh 1970) and the geometric equation method (Naumann et al. 2009), indicate that non-invasive geometric equations can fairly accurately estimate the size of coral branches. The tin foil method, similar to several other types of coral surface area measurement techniques, requires sacrificing the coral, whereas for corals with cylindrical branches, the dimensions of the branch's cylinder shell surface ($SA = 2 \pi hr$; see details in Methods) can be measured easily with only minimal disturbance to the live coral. Naumann et al. (2009) confirmed that for branching

corals in particular, the geometric equation provides surface area approximations that are similar to advanced surface area determination techniques such as 3D scanning. Therefore, researchers who work with branch fragments of corals could effectively normalize their physiological and other data to unit surface area using this non-invasive method. The use of this size estimation technique prolongs the lifespan of laboratory specimens, allowing researchers to utilize them for additional studies and thereby reducing the number of corals that need to be harvested from wild populations on coral reefs, or grown in coral nurseries, for laboratory research. Studies that measure physiological and other processes *in situ* on coral reefs also could employ this method with limited disturbance to corals, especially for small colonies with only a few branches or relatively simple body shapes. Given the current threats to coral reefs and the governmental regulations that limit the collection especially of endangered coral species, the efficient use of specimens can be enhanced by these types of measurement methods, thereby improving the conservation of corals used in scientific research.

The correlation between precise surface area (PSA) and total length (TL) of the coral fragments was the second strongest correlation among the biometric variables. Because TL was used in the estimated surface area (ESA) calculation, the additional step of measuring maximal branch fragment diameter would be ideal for laboratory studies. However, in the field where branching corals are typically more complex and maximal branch diameter could be difficult to identify and measure, using TL as a proxy for PSA would be the next best noninvasive method. Although the other correlations among fragment size parameters were significant, the low r^2 values indicate that not much of the variation in the data can be explained by buoyant weight (BW). Thus, while BW

provided information on the growth rates of the examined laboratory fragments across time, we do not recommend using it as a proxy for PSA to normalize physiological measurements.

Observed increases in the BW of coral fragments during the present study indicated that their physiological condition supported body growth throughout several months of laboratory culture, so they were meeting their metabolic energy needs with enough surplus for growth. These corals thus were in robust health and served as reliable specimens for *ex situ* laboratory-based measurements of photosynthetic and respiration rates. The rates of both processes reported here were within the range of those reported from past studies on the metabolic rates of scleractinian corals, from both laboratory and field studies (Table 3).

Rates of net photosynthesis

Our results show that in terms of net photosynthetic rate, *A. cervicornis* performs optimally at a seawater temperature of ~ 25°C, and that although photosynthesis declined slightly at the cooler temperature examined (22°C), the decline was more pronounced at relatively high temperatures (28-30°C). Other studies also have documented that tropical coral photosynthesis peaks at moderate coral reef seawater temperatures of 25-27°C, and declines from this maximum at both cooler (22-24°C) and warmer temperatures (< 30°C) (Table 3; also Saxby et al. 2003). There thus appears to be a thermal optimum at moderate tropical seawater temperature for net photosynthesis in reef corals, which varies somewhat among coral reef regions; deviations from this optimum result in impaired

rates of coral primary productivity (Lesser 1997; Ferrier-Pagès et al. 2010; Ultstrup et al. 2011).

Because our laboratory culture tanks were maintained at 25-26°C, and the source colonies for our corals also were exposed to those temperatures (see Methods), the corals used here may have been acclimatized to that seawater temperature, leading to their exhibiting optimum photosynthesis at 25°C, as known from past studies (Coles and Brown 2003; Howells et al. 2013). However, this temperature range appears to be optimal for tropical coral culture in general, as previous work in laboratory settings has documented that many tropical corals grow most rapidly at ~ 26-27°C (Seveso et al. 2016; Clausen and Roth 1975; Jokiel and Coles 1977; Marshall and Clode 2004; Marshall and Clode, 2004; Precht and Aronson 2004; Hubbard 2015). Our observation of impaired photosynthetic output at extreme seawater temperatures parallels the known pattern of enhanced coral bleaching (expulsion of microalgae) at extreme sea surface temperature (SST; at both high and lower than average field temperatures) on coral reefs worldwide. A 1-2°C spike in summer average SST often leads to increased frequency of coral bleaching on reefs (Cook et al. 1990; Fitt et al. 1993; Podesta & Glynn, 2001; Brown, 1997; Hughes et al. 2017), and cooler temperatures also can cause bleaching on tropical reefs (Lirman et al. 2011; Hoegh-Guldberg et al. 2005).

These patterns suggest that impaired net photosynthesis of the microalgae harbored by corals is one of the physiological mechanisms related to coral colony bleaching at extreme temperatures. Stress to the cellular processes involved in coral photosynthesis has been postulated as the reason for the accumulation of reactive oxygen species (ROS) and expulsion of *Symbiodinium* that occur during bleaching (Brown 2002; Warner *et al*.

1999; Weis 2008; Cunning and Baker; Smith *et al*. 2005; Jones *et al*. 1997; Lesser 2006; Tchernov *et al*. 2004; Chipuk and Green 2008; Lorenzo *et al*. 1999; Brune *et al*. 1999; Dunn *et al*. 2007). At the cellular level, breakdown in the function of photosystem II (PSII) and its repair mechanisms at extreme temperatures (Warner *et al*. 1999; Jones *et al*. 1997; Douglas 2003; Weiss 2008; Takahashi *et al*. 2004; Venn *et al*. 2008) likely contributed to the temperature-related patterns of coral photosynthesis observed here.

Our results revealed major impairment of net photosynthesis in corals with abraded tissues, in that abraded corals incubated at 25°C exhibited photosynthesis rates that were only ~ 1/3 (37%) those of non-abraded corals at the same temperature. These findings indicate that even when these corals are not under thermal stress, tissue abrasion of only 15% of the colony surface significantly undermines the coral's ability to meet metabolic demands through photosynthesis. Additionally, corals exposed to 28°C exhibited net consumption of oxygen under daytime irradiance conditions, rather than the typical oxygen production response expected from photosynthetic organisms. Because the flow through respirometer used in these experiments measured net photosynthesis (i.e.: gross photosynthesis – daytime respiration), this would suggest that daytime coral respiration masked any photosynthetic output from the corals with abrasions at this temperature.

When exposed to abrasion stress, the coral fragments exhibited their highest mean photosynthetic rate at 22°C, but this rate was not significantly different from that at 25°C. While coral photosynthesis rates at high temperature have been well documented (Lesser et al. 1997; Ferrier-Pagès et al. 2010; Ulstrup et al. 2011), the effects of relatively low temperature are not as well understood. Differences in photosynthesis rate between the undamaged and abraded corals at both 22°C and 25°C also were not significant, but the

mean level of reduction in photosynthesis rate between abraded vs. non-abraded corals was less at 22°C than 25°C. This pattern may relate to the respiration rates of abraded corals being lower at 22°C than at 25°C, leading to lower relative values of net photosynthesis.

Respiration

The coral respiration rates observed here increased linearly with seawater temperature, with the warmest examined conditions yielding the highest oxygen consumption rates, at values similar to those reported from previous studies on coral respiration rates (Table 3). This temperature-dependent pattern has been observed in other tropical stony corals examined to date, in that respiration rate appears to increase with temperature also in *Turbinaria reniformis*, *Pocillopora damicornis* (Ultstrup et al. 2011; Abdulmohsin and Floos 2013), *Stylophora pistillata* (Ferrier-Pagès et al. 2010), *Siderastrea siderea* (Muthiga and Szmant 1987), and *Agaricia tenuifolia* (Lesser 1997). Augmented coral respiration rate during dark conditions at relatively high temperature is coupled with cellular stress responses in which corals typically exhibit initial up-regulation of heat-shock proteins (HSPs) and antioxidants, both of which require energy consumption (Seveso *et al*. 2014; Rosic *et al*. 2011; Desalvo *et al*. 2010; Mayer 2010; Richier *et al*. 2006; Brown *et al*. 2002; Downs *et al*. 2002; Császár *et al*. 2009). HSPs often act as chaperone proteins, working to sustain or repair the quaternary structure of proteins that may become compromised from denaturation at high temperatures (reviewed in Kregel 2002; Richier *et al*. 2004; Sorensen *et al*. 2003). The antioxidants produced by heat-stressed corals are molecules that can mitigate the accumulation of ROS as a result of

overactive photosynthetic pathways and the subsequent breakdown of PSII, as described above (Richier *et al*. 2006; Brown *et al*. 2002; Downs *et al*. 2002; Császár *et al*. 2009). Therefore, it is likely that the increased oxygen consumption rates at relatively high temperature observed in the present study were caused in part by a thermally induced stress response, wherein fragments of *A. cervicornis* enhanced the energetically-expensive production of HSPs and antioxidants. Further research is needed to determine patterns of correlation and cause-effect relationships between coral respiration rates and the production of HSP and antioxidants.

The observed rates of oxygen consumption between non-abraded and abraded corals differed significantly only at 28°C, indicating that at some temperatures, the effects of coral abrasion may not be manifested through changes in coral respiratory rate. However, at the warmer temperatures examined (28 and 30°C), the mean respiratory rate of abraded corals was higher than that of non-abraded fragments in contrast to no clear difference at cooler temperatures (22 and 25°C), suggesting that more extensive testing or large sample sizes may reveal an effect of temperature on differences in respiration rates between abrasion treatments. At relatively low temperatures, tissue abrasion may have less negative impact overall on coral physiology, as discussed above in terms of effects on photosynthesis rate.

The respiration rates reported here from daytime measurements of corals in darkened chambers likely are representative of nocturnal rates, because our preliminary trials revealed that dark respiration rates of *A. cervicornis* as measured in both the day and nighttime were similar, and thus that these corals did not appear to exhibit any clear circadian rhythm of respiration. This pattern is similar to that reported by Muthiga and

Szmant (1987), who found that the respiratory rates of individual colonies of *Siderastrea siderea* during daytime and nighttime were not significantly different.

Ratio of Photosynthesis to Respiration (P:R)

As described in the classic publication by Coles and Jokiel (1977), the P:R ratio is a dimensionless number that can be used as an indicator for the overall energy budget of the coral holobiont, regardless of biomass and size differences among corals. Examination of the P:R ratio thus is useful for understanding how photosynthetic organisms meet their metabolic energy demands under stress conditions. The values obtained here for P:R ratios of *A. cervicornis* fragments were consistent with those reported from previous studies on stony corals, although it is worth noting that a wide range of values have been reported in the literature (Table 3).

For non-abraded coral fragments, P:R decreased almost linearly with temperature (Fig 9B), and the corals at 22°C exhibited the highest mean value at 3.19 due to their exhibiting the lowest respiration rates, indicating that their energy needs were more than met through photosynthesis. P:R dipped below 1 at warmer temperatures (28 and 30°C) in the non-abraded fragments, revealing that their microalgae were producing less organic matter than required for cellular respiration of the coral holobiont (Coles and Jokiel 1977). These data suggest that healthy, undamaged fragments of *A. cervicornis* are unable to meet their metabolic requirements when exposed to the bleaching threshold temperatures known for coral reefs (1-2°C above mean summer SST; Cook et al. 1990; Fitt et al. 1993; Brown, 1997; Hughes et al. 2017). Previous studies have reported similar

patterns, wherein P:R ratios were reduced at elevated temperatures in other species of corals including *Agaricia tenuifolia* (Lesser 1997); *Stylophora pistillata*, *Turbinaria reniformis*, *Galaxea fascicularis* (Ferrier-Pagès et al. 2010); *Pocillopora damicornis*, *Montipora verrucosa, Porites compressa*, and *Fungia scutaria* (Coles and Jokiel 1977).

Based on the present study, the reduction in P:R ratio with temperature in stony corals appears to result from a significant decrease in photosynthetic activity as temperature increases. Because net photosynthesis declines far more rapidly than dark respiration increase with temperature, this pattern suggests that a breakdown in the photosynthetic pathway causes energy disequilibrium in corals exposed to thermal stress.

When exposed to tissue abrasion, the examined corals exhibited a similar trend in P:R ratios with temperature, but at far lower ratios than those of non-abraded corals. At relatively cool temperature (22°C), net oxygen production was greater than consumption, indicating that energy demands were being met. However, even at 25°C, the "control" temperature, mean P:R ~ 1, suggesting that even in optimal acclimated conditions, abraded fragments generated barely enough energy for tissue repair.

Of greater concern is the observed pattern at relatively warm temperatures (28 and 30°C) of damaged corals exhibiting net oxygen consumption even at high light levels, suggesting that if a colony becomes abraded prior to a rise in coral reef seawater temperature, it could be catastrophic for the colony in terms of meeting its metabolic requirements. Both types of stressors applied here place augmented energy demands on corals, in terms of repairing tissue and re-growing the underlying calcium carbonate skeleton after abrasion, and in terms of cellular responses to combat the breakdown of the photosynthetic pathway and buildup of ROS after extreme temperature stress. Due to the

significant interaction effect between these two stressors on photosynthetic rates in *A. cervicornis*, it appears from an energetic standpoint that if these corals are exposed to diver damage, they become even more vulnerable to starvation in the event of a rise in sea temperature.

Limitations of the study

Several limitations of the present study may have affected the results presented here. The flow rate (8-13 ml/min) through the respiratory chamber was relatively slow, and the setup lacked a stir bar to enhance water flow rate inside the chamber, in contrast to the conditions in some previously published studies on coral metabolic rates (Table 1). A stir bar was not used because it was not logistically possible to insert one easily in the small chamber with the coral ceramic plug attached to the chamber base. A higher water flow rate through the chamber also was not employed, because of the sensitivity of the oxygen electrodes. Water flow rate is a matter of concern in this type of study, because water flow and turbulence shape coral reef ecosystems, and as such, these variables influence growth, mortality, reproductive rates and even photosynthesis and respiration in coral species (Davies 1983; Jokiel 1978; Patterson 1985; Dennison and Barnes 1988). Methodology in studies measuring the physiological parameters of stony corals has been far from uniform. Both flow-through (or unidirectional) and static (or closed) respirometry systems have been employed to quantify the metabolic rates of corals, with the latter sometimes but not always utilizing a stir bar (Table 1). The thickness of the boundary layer that surrounds object in fluids is inversely related to water flow rate; the greater the flow, the thinner the boundary layer. In corals, an accumulation of O_2 at the

coral surface due to a thick boundary layer may result in an inhibition of photosynthetic activity (Crossland & Barnes, 1977l; Black et al., 1976; Dennison and Barnes 1988). Stir bars and increased water flow can reduce the boundary layer surrounding organisms in respirometry experiments. Dennison and Barnes (1988) found that both photosynthetic and respiratory rates of *Acropora formosa* were ~ 25% lower in unstirred trials, but suggested that protruding calices could increase turbulence along a fragment and increase the surface area available for gas exchange. Given the rugosity of branch fragments of *Acropora* spp. (Fig. 3; Fig. 10), a closer look at stirred and unstirred, as well as low flow and high flow conditions in respirometry experiments could further clarify the extent to which their rates of photosynthesis and respiration vary with these factors. However, because the metabolic rates reported here are within the range of those reported in past studies on corals of this genus (Table 3), it is unlikely that they were substantially altered by our experimental water flow conditions.

The limited number of genotypes (6) examined here, and variation among them, also potentially could inaccurately reflect patterns for the entire species of *Acropora cervicornis*. However, the present study examined the largest sample size (26 different coral fragments, 65 non-abrasion respirometry trials, and 40 abrasion trials) and genotypic diversity (6 distinct genotypes) of published respirometry studies on any coral species (Table 3). Most published studies have examined a sample size of 10 or fewer coral fragments total, and most do not describe the genotypic diversity of the fragments. Further, in previous studies it has been common practice to collect fragments from a single mother colony, thereby limiting the number of genotypes used in measurements — this may be due to difficulty in obtaining permits for field collection (Table 3). Rates of

expansion of the coral polyps and their tentacles varied among the *A. cervicornis* fragments used here for respirometry trials, and in particular, the fragments belonging to one of the genotypes exhibited low levels of polyp expansion, in which the polyps remained contracted most of the time. Corals with small polyps such as *A. cervicornis* have been suggested to exhibit more effective autotrophy than those with large polyps (Drew 1973), but more heterotrophic corals may in fact be more resilient to bleaching (Grottoli et al. 2006), suggesting a trade-off between these 2 coral types as ocean temperatures rise. We observed relatively low photosynthetic rates of the *A. cervicornis* fragments that had little polyp extension (ie: contracted polyps; Fig. 10) suggesting that this relationship may be a sign of diminished resilience to thermal stress in relation to the other genotypes examined here, which expanded their polyps more (Fig. 10). Further research is needed about how this behavioral variation relates to photosynthesis and respiration rates; if routinely contracted corals are more sensitive to stressors, then coral expansion level could provide a visual indicator in addition to overall colony color level, as to the vulnerability of coral colonies to stressors.

Broader Impacts and Conclusions

Results from the current study have several implications for the conservation management of coastal ecosystems, especially tropical coral reefs. They show that even minor tissue abrasion to reef-building coral colonies (< 15% of the colony surface) can severely limit their capacity to meet energetic demands, even more so when accompanied by the thermal stress that is affecting coral reefs worldwide. As ecotourism and recreational diving become increasingly popular, more divers than ever are visiting the

world's coral reefs (Ong and Musa 2011; Abidin and Mohamed 2014). However, both SCUBA diving and snorkeling can directly damage reef corals via enhanced sedimentation, tissue abrasion and skeletal fracture, in addition to indirectly enhancing the predation rates of corallivorous reef organisms (Hawkins and Roberts 1992; Zakai and Chadwick-Furman 2002; Guzner et al. 2010). Branching corals experience greater diver-caused damage relative to their abundance than do corals with other growth forms on reefs (Hawkins and Roberts 1992).

In extreme cases, the unsustainable practices of local dive operators, coupled with high rates of diver visitation, cause visible degradation of reef health and physical complexity (Davenport and Davenport 2006; Guzner et al. 2010; Abidin and Mohamed 2014; Lamb et al. 2014; Gil et al. 2015). Our data demonstrate how the energetic balance and vulnerability to heat stress of corals may be affected by diver-caused abrasion to coral surfaces.

Snorkeling and SCUBA diving certification courses generate ~ 1 million new recreational divers each year, due in part to increases in the affordability and accessibility of dive training courses around the world (Davenport and Davenport 2006). The development of local businesses focused on diving services may provide significant economic means for many communities in island- and developing-nations, allowing these areas to improve their livelihoods in a potentially sustainable manner. The degree of sustainability of such activities, however, depends on whether they are managed in ways that do not degrade their coral reef resource base, through the provision of adequate educational infrastructure and regulation of reef diving. The results presented here on how temperature and abrasion status impact coral P:R ratios provide scientific support for

the development of targeted reef conservation management strategies by dive operators and reef managers. At the coldest temperature examined here (22°C), abraded fragments had a net photosynthesis to respiration ratio (P:R) of ~ 1.8, indicating that during the winter months with colder water temperatures on reefs, coral fragments may be able to meet their energy demands despite tissue damage. As such, these measurements provide evidence that in colder temperatures, respiration and net photosynthetic rates in coral allow for an energy surplus that can be devoted to tissue repair. Therefore, in an effort to become more sustainable and maintain the overall health of coral reefs, dive operators and coastal managers could incentivize winter diving by tourists. While education and dive briefings may be useful for reducing damage to benthic organisms, some unintentional tissue abrasion to corals from the equipment of divers visiting coral reefs is inevitable. However, lowering the prices for dive trips during colder months and increasing them during warmer months could incentivize winter diving and lessen the impact of such damage, because damaged corals may be more able to meet their energy demands for healing at relatively low temperatures. Given the ~ 30-40 d duration to complete healing of the wounds observed here, the augmented energy demand to heal abrasion would not necessarily continue into summer periods which experience relatively high temperatures. High diver visitation rates during relatively cold conditions thus may not be as detrimental to corals as the same number of dives in warmer months. In order to fully understand the effectiveness of incentivizing the relatively cold months for recreational diving on reefs, further research is needed to on how the healing rates of coral colonies vary with temperature and among species. This type of knowledge would

inform dive operators and reef managers about how to partition rates of diver visitation, given the seasonal temperature conditions and dominant coral species on local reefs.

Our results also have implications for the design of fisheries management policies. Corallivorous organisms, such as parrotfishes and filefishes, consume coral tissue and leave abrasions that remove tissue and also damage the calcium carbonate skeleton of reef corals (Guzner et al. 2010). When apex predators such as sharks and groupers that feed on these corallivores are removed from reef ecosystems, populations of the corallivores can increase (Dulvy et al. 2004), causing a top-down effect of enhanced predation on corals, and leaving large areas of coral skeleton exposed. Our results thus provide scientific support for the conservation management of species important to coral reef fisheries such as groupers, due to potential cascade effects on coral abrasion rates and therefore coral energy budgets. Related evidence also indicates that the abundance of small invertebrate corallivores (*Drupella* snails) is enhanced at heavily-dived reef sites, because the snails are attracted to the high proportions of abraded and broken corals at those sites (Guzner et al. 2010). Our finding of significant physiological impact on corals from a single abrasion event on only 15% of the coral surface, means that even low rates of corallivory or diver-caused abrasion are likely to impair coral physiological processes, which should be taken into account when designing both fisheries and ecotourism management policies for coral reefs.

The clonal reproductive process of branching species such as *A. cervicornis* may involve fragmentation following disturbance events such as hurricanes and heavy wave action (Tunnicliffe 1981; Highsmith 1982; Harrison and Wallace 1990). A clean break along a coral branch can be classified as a type of abrasion wound, given the exposure of

the underlying calcium carbonate skeleton and the tissue regeneration needed to cover the exposed skeletal area. Therefore, given the low P:R ratios of branch fragments observed here at relatively high temperatures, fragmentation may become an energetically non-viable method of reproduction in these corals as the global climate warms, in that small broken or abraded fragments are expected to produce less oxygen than they consume. In order to determine changes in the energetic viability of coral branch fragmentation as a clonal replication mechanism, research is needed on the energetics of corals undergoing branch breakage under various temperature regimes, in comparison with alternate reproductive strategies such as the broadcast spawning of sexual propagules. Related research on the compounding physiological effects of rising global temperatures and frequent abrasion or breakage from ecotourism activities is expected to shed light on how corals will cope with projected anthropogenic impacts in the 21st century.

Literature Cited

Al-Sofyani AA & Floos YAM (2013) Effect of temperature on two reef-building corals *Pocillopora damicornis* and *P. verrucosa* in the Red Sea. *Oceanologia*, 55(4):917–935

Anthony KRN & Hoegh-Guldberg O (2003) Variation in coral photosynthesis, respiration and growth characteristics in contrasting light microhabitats: an analogue to plants in forest gaps and understoreys? Functional Ecology 17:246–259

Aronson RB & Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460:25–38

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

Bellewood DR, Hughes TP, Folke C, & Nystrom M (2004) Confronting the coral reef crisis. Nature 429:827-833

Birkeland C (2015) Biology Trumps Management: Feedbacks and Constraints of Life-History Traits. Coral Reefs in the Anthropocene (231-263) Springer

Black CC, Burris JE, & Everson RG (1976) Influence of oxygen concentration on photosynthesis in marine plants. Aust. J. Plant Physiol. Vol. 3, pp. 81-86

Borneman EH (2001) Aquarium corals: selection, husbandry, and natural history. Microcosm Ltd., Charlotte

Brown BE, Downs CA, Dunne RP, & Gibb SW (2002) Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. Mar Ecol Prog. Ser. 242: 119-129

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16:129-138

Brune B, von Knethen A, & Sandau KB (1999) Nitric oxide (NO): an effector of of apoptosis. Cell Death and Differentiation 6:969-975

Caldiera K & Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Cantrell CE, Henry RP, & Chadwick NE (2015) Nitrogen transfer in a Caribbean mutualistic network. Marine Biology, 162(12):2327–2338

Clausen C & Roth AA (1975) Effect of temperature and temperature adaptation on calcification rates in the hermatypic coral *Pocillopora damicornis*. Marine Biology 33:93-100

Chipuk JE & Green DR (2008) How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends in Cell Biology 18:157-164

Coles SL & Jokiel PL (1977) Effects of temperature on photosynthesis and respiration in hermatypic corals. Marine Biology 43:209–216

Coles SL & Brown BE (2003) Coral bleaching-capacity for acclimitization and adaptation. Advances in Marine Biology 46:183-223

Cook CB, Logan A, Ward J, Luckburst B, Berg Jr. CJ (1990) Elevated temperatures and bleaching on a high latitude coral reef – the 1988 Bermuda event. Coral Reefs 9:45–49

Crossland CJ & Barnes DJ (1977) Gas-exchange studies with the Staghorn coral *Acropora acuminata* and its zooxanthellae. Marine Biology 40:185-194

Császár NBM, Seneca FO, van Oppen MJH (2009) Variation in antioxidant gene expression in the scleractinian coral *Acropora millepora* under laboratory thermal stress. Mar Ecol Prog Ser 392:93-102

Cunning R & Baker A (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nature Climate Change 3:259-262

Davenport J, & Davenport JL (2006) The impact of tourism and personal leisure transport on coastal environments: A review. Estuarine, Coastal and Shelf Science, 67(1–2):280–292

Davies PS (1980) Respiration in some Atlantic reef corals in relation to vertical distribution and growth form. Biological Bulletin 158:187-194

Davies PS (1983) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs 2:181–186

Dejours, Pierre (1975) Principles of Respiratory Physiology. Elsevier.

Dennison WC & Barnes DJ (1988) Effect of water motion on coral photosynthesis and calcification. J. Exp. Mar. Biol. Ecol. 115:67–71

Desalvo MK, Sunagawa S, Voolstra CR, Medina M (2010) Transcriptomic responses to heat stress and bleaching in the elkhorn coral *Acropora palmata*. Mar Ecol Prog Ser 402:97-113

Davis D & Tisdell C (1996) Economic management of recreational scuba diving and environment. Journal of Environmental Management 48:229–248

Dixson DL & Hay ME (2012) Corals chemically cue mutualistic fishes to remove competing seaweeds. Science 338:804-807

Douglas AE (2003) Coral bleaching—how and why? Marine Pollution Bulletin 46:385-392

Downs CA, Mueller E, Phillips S, Fauth JE, & Woodley CM (2000) A molecular biomarker system for assessing the health of coral (*Montastraea faveolata*) during heat stress. Marine Biotechnology 2:533–544

Drew EA (1972). The biology and physiology of alga-invertebrate symbioses. II. The density of symbiotic alga cells in a number of hermatypic corals and alcyonarians from various depths. J. Exp. Mar. Biol. Ecol. 9:71-75.

Dulvy NK, Freckleton RP, & Polunin NVC (2004) Coral reef cascades and the indirect effects of predator removal by exploitation. Ecol Lett 7:410-416

Dunn SR et al. (2007) Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: every which way you lose. Proceedings in Biological. Sciences 274:3079–3085

Ferrier-Pagès C, Rottier C, Beraud E, & Levy O (2010) Journal of Experimental Marine Biology and Ecology Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress : Effect on the rates of photosynthesis. Journal of Experimental Marine Biology and Ecology, 390(2): 118–124

Fitt W, McFarland F, Warner M et al. (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnology and Oceanography 45:677-685

Gil MA, Renfro B, Figueroa-Zavala B, Penié I, & Dunton KH (2015) Rapid tourism growth and declining coral reefs in Akumal, Mexico. Marine Biology, 162(11): 2225–2233

Grottoli AG, Rodrigues LJ, & Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature, 440(7088):1186–1189

Guilherme Becker C, Dalziel BD, Kersch-Becker MF, et al. (2013) Indirect effects of human development along the coast on coral health. Biotropica 45:401-407

Guzner B, Novplansky A, Shalit O, & Chadwick NE (2010) Indirect impacts of recreational SCUBA diving: patterns of growth and predation in branching stony corals. Bulletin of Marine Science 86:727-742

Harrison PL & Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the world. 25. Coral reefs. Elsevier, Amsterdam, pp 133-207

Hawkins JP & Roberts CM (1992) Effects of recreational SCUBA diving on fore-reef slope communities of coral reefs. *Biological Conservation* 62:171–178

Hawkins JP, Roberts CM, Van't Hof T, de Meyer K, Tratalos J, Aldham C (1999) Effects of recreational scuba diving on Caribbean coral and fish communities. Conservation Biology 13:888-897

Highsmith RC (1982) Reproduction by fragmentation in corals. Mar Ecol Prog Ser 7:207–226

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

Hoegh-Guldberg O, Fine M, Skirving W, Johnstone R, Dove S, Strong A (2005) Coral bleaching following wintry weather. Limnology and Oceanography, 1, doi: 10.4319/lo.2005.50.1.0265

Holbrook SJ, Brooks AJ, Schmitt RJ (2002) Predictability of fish assemblages on coral patch reefs. Mar. Freshw. Res. 53:181–188

Hoogenboom MO, Anthony KRN, & Connolly SR (2006) Energetic cost of photoinhibition in corals, 313:1–12

Howells EJ, Berkelmans R, van Oppen MJ, Willis BL, & Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. Ecology, 94:1078-1088

Hubbard DK (2015) Reef Biology and Geology—Not just a matter of scale. In: Birkeland C (Ed) Coral Reefs in the Anthropocene. Springer, New York, pp. 43-66

Huebner LK, Dailey B, Titus BM, Khalaf M, Chadwick NE (2012) Host preference and habitat segregation among Red Sea anemonefish: effects of sea anemone traits and fish life stages. Mar. Ecol. Prog. Ser. 464:1-15

Hughes TP, Baird AH, Bellwood DR, et al. (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

Hughes TP et al. (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373-377

Hughes TP, Baird AH, Bellwood DR, et al. (2008) Climate change, human impacts, and the resilience of coral reefs. Science 301:929-933

IPCC (2007) Climate change. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), The Physical Science Basis. Contribution ofWorking Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Johannes RE & Wiebe WJ (1970) Method for determination of coral tissue biomass and composition. Limnol. Oceanogr. 15: 822-824

Johnson MK, Holbrook SJ, Schmitt RJ, & Brooks AJ (2011) Fish communities on staghorn coral: effects of habitat characteristics and resident farmerfishes. Environ. Biol. Fish 91:429–448

Jokiel PL (1978) Effects of water motion on coral reefs. J. Exp. Mar. Biol. Ecol. 35: 87-97

Jokiel PL, Maragos JE, Franzisket L (1978) Coral growth: buoyant weight technique. Monogr. Oceanogr. Methodol. 5:529-542

Jokiel PL (2004) Temperature stress and coral bleaching. In: Coral Health and Disease (eds Rosenberg E, Loya Y), pp. 401-425. Springer-Verlag, Heidelberg

Jokiel PL & Coles SL (1977) Effects of temperature on the mortality and growth of Hawaiian reef corals. Mar Biol 43(3):201–208

Jokiel PL & Coles SL (1990) Response of Hawaiian and other Indo Pacific reef corals to elevated temperatures. Coral Reefs 8:155-162

Jones RJ, Berkelmans R, & Oliver J (1997) The recurrent bleaching of corals at Magnetic Island (Australia) relative to air and seawater temperature. Mar Ecol Prog Ser 158:289–292

Jones RJ, Hoegh-Guldberg O, Larkum AWD, & Schreiber U (1998) Temperature-induced bleaching of corals begins with impair- ment of the CO2 fixations mechanism in zooxanthellae. Plant Cell Environ 21:1219–1230

Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal of Applied Physiology 92:2177-2186

Krieger J & Chadwick N (2013) Recreational diving impacts and the use of pre-dive briefings as a management strategy on Florida coral reefs. J Coast Conservation 17:179–189

Lamb JB, True JD, Piromvaragorn S, Willis BL (2014) Scuba diving damage and intensity of tourist activities increases coral disease prevalence. Biological Conservation 178:88-96

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16:187–192

Lesser MP (2000) Depth-dependent photoacclimatization to solar ultraviolet radiation in the Caribbean coral Montastraea faveolata. Marine Ecology Progress Series 192:137-151.

Lesser MP (2006) Oxidative stress in marine environments: Biochemistry and physiological ecology. Annual Review of Physiology 68:253–278

Lirman D, Schopmeyer S, Manzello D, Gramer LJ, Precht WF, Muller-Karger F, … Thanner S (2011) Severe 2010 cold-water event caused unprecedented mortality to corals of the Florida reef tract and reversed previous survivorship patterns. PLoS ONE, 6(8). https://doi.org/10.1371/journal.pone.0023047

Lorenzo HK, Susin SA, Penninger J, Kroemer G (1999). Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. Cell Death and Differentiation 6:516-524

Marsh JA (1970) Primary productivity of reef-building calcareous red algae. Ecology 51:255–263

Marshall AT & Clode P (2004) Calcification rate and the effect of temperatures in a zooxanthellate and an azooxanthellate scleractinian reef coral. Coral Reefs 23:218-224

Mayer MP (2010) Gymnastics of molecular chaperones. Molecular Cell 39:321-331

Muller EM, Rogers CS, Spitzack AS, Woesik R (2008) Bleaching increases likelihood of disease on *Acropora palmata* (Lamarck) in Hawksnest Bay, St Jon, US Virgin Islands. Coral Reefs 27:191-195

Muthiga NA & Szmant AM (1987) The effects of salinity stress on the rates of aerobic respiration and photosynthesis in the hermatypic coral *Siderastrea siderea*. *Biol. Bull.* 173:539-551.

Naumann M, Niggl W, Laforsch C, Glaser C, & Wild, C (2009) Coral surface area quantification–evaluation of established techniques by comparison with computer tomography. Coral Reefs, 28:109-117

Ong TF & Musa G (2011) An examination of recreational divers' underwater behaviour by attitude–behaviour theories. Curr. Issues Tourism 14 (8):779– 795

Orr JC, Fabry VJ, Aumont O, Bopp L, et al. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681-686

Patterson MR (1985). The effects of flow on the biology of passive suspension feeders: prey capture, feeding rate, and gas exchange in selected Cnidarians. Ph.D. thesis, Harvard University, Cambridge, Massachusetts, 342 pp.

Precht WF & Aronson RB (2004) Climate flickers and range shifts of reef corals. Frontiers in Ecology and the Environment, 2:307–314

Podesta GP & Glynn PW (2001) The 1997-98 El-Nino event in Panama and Galapogos: an update of thermal stress indices relative to coral bleaching. Bulletin of Marine Science 69:43-59

Raymundo LJ, Halford AR, Maypa AP, et al. (2009) Functionally diverse reef-fish communities ameliorate coral disease. Proceedings from the National Academy of Science 106:17067-17070

Richier S, Merle P, Furla P, & Pigozzi D (2003) Characterization of superoxide dismutases in anoxia- and hyperoxia-tolerant symbiotic cnidarians. *Biochimica et Biophysica Acta* 1621: 84–91

Richier S, Furla P, Plantivaux A, Merle P, & Allemand D (2005) Symbiosis-induced adaptation to oxidative stress. J. Exp. Biol. 208:277–285

Richier S, Sabourault C, Courtiade J et al. (2006) Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, Anemonia viridis. The FEBS Journal 273:4186-4198

Roberts HH, Wilson PA, Lugo-Fernandez A (1992). Biologic and geologic responses to physical processes: Examples from modern reef systems of the Caribbean-Atlantic region. Continental Shelf Research 12:809-834.

Rogers CS (1990) Responses of coral reefs and reef organisms to sedimentation. Marine Ecology Progress Series, 62:185–202

Rosic N, Pernice M, Dove S, Dunn S, Hoegh-Guldberg O (2011) Gene expression profiles of cytosolic heat shock proteins Hsp70 and Hsp90 from symbiotic dinoflagellates in response to thermal stress: possible implications for coral bleaching. Cell Stress and Chaperones 16:69-80

Roopin M & Chadwick NE (2009) Benefits to host sea anemones from ammonia contributions of resident anemonefish. J. Exp. Mar. Biol. Ecol. 370:27-34

Saxby T, Dennison WC, Hoegh-Guldberg O (2003) Photosynthetic responses of the coral Montipora digitata to cold temperature stress. Marine Ecology Progress Series 248:85-97

Seveso D, Montano S, Strona G et al (2014) The susceptibility of corals to thermal stress by analyzing Hsp60 expression. Marine Environmental Research 99:69-75

Seveso D, Montano S, Stronga G, Orlandi I, Galli P (2016) Hsp60 expression profiles in the reef-building coral Seriatopora caliendrum subjected to heat and cold shock regimes. Marine Environmental Research 119:1-11

Schutter M, van Velthoven B, Janse M, Osinga R, Janssen M, Wijffels R, Verreth J (2008) The effect of irradiance on long-term skeletal growth and net photosynthesis in *Galaxea fascicularis* under four light conditions. J. Exp. Mar. Biol. Ecol. 367:75-80

Sheppard RC, Davy SK, Pilling GM (2009) Biology of Habitats: The Biology of Coral Reefs. Oxford University Press, Oxford, 2009. ISBN: 978.0.19.856635.9

Smith D, Suggett D, Baker N (2005) Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals. Global Change Biology 11:1-11

Sorensen JG, Kristensen TN, Loeschcke V (2003) The evolutionary and ecological role of heat shock proteins. Ecology Letters 6:1025– 1037

Takahashi S, Nakamura T, Sakamizu, et al. (2004) Repair machinery of symbiotic photosynthesis as the primary target of heat stress for reef-building corals. Plant Cell Physiology 45:251-255

Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, et al. (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proceedings from the National Academy of Sciences, USA 101:13531-13535

Titlyanov E, Bil K, Fomina I, Titlyanov T, Leletkin V, Eden N, Malkin A, Dubinsky Z (2000) Effects of dissolved ammonium addition and host feeding with Artemia salina on photoacclimation of the hermatypic coral Stylophora pistillata. Mar Biol 137:463–472

Tchernov D, Kvitt H, Haramaty L, Bibby TS, Gorbunov MY, Rosenfeld H, Falkowski PG (2011) Apoptosis and the selective survival of host animals following thermal bleaching in zooxanthellate corals. Proceeding from the National Academy of Sciences, USA 108:9905–9909

Tunnicliffe V (1981) Breakage and propagation of the stony coral Acropora cervicornis. Proc. Natl. Acad. Sci. USA 78(4):2427–2431

Ulstrup KE, Kühl M, van Oppen MJH, Cooper TF, & Ralph PJ (2011) Variation in photosynthesis and respiration of two reef-building corals from geographically distinct populations: Variation in photosynthesis and respiration in geographically distinct populations of two reef-building coral species. Aquatic Biology 12:241-248.

Venn AA, Loram JE, Douglas AE (2008) Photosynthetic symbioses in animals. Journal of Experimental Botany 59:1069-1080

Warner ME, Fitt WK, & Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. Proceeding from the National Academy of Sciences, USA 96:8007-8012

Weiss VM (2008) Cellular mechanisms of cnidarian bleachng: stress causes the collapse of symbiosis. Journal of Experimental Biology 211:3059-3006

Zainal Abidin SZ & Mohamed B (2014) A Review of SCUBA Diving Impacts and Implication for Coral Reefs Conservation and Tourism Management. SHS Web of Conferences, 12: 01093. DOI: 10.1051/shsconf/ 20141201093

Zakai D & Chadwick-Furman NE (2002) Impacts of intensive recreational diving on reef corals at Eilat, northern Red Sea. Biological Conservation 105:179-187

Table 1. Characteristics of respirometry chambers and water flow methods used in published studies on respiration and photosynthesis rates of scleractinian corals. Shown are the coral species examined (in alphabetical order), volume of the respirometry chamber used, ambient seawater temperature in the chamber, whether the system was static (no water flow during measurements) or flow-through (water continuously flowing through the chamber during measurements), water flow rate in the chamber, whether a stir bar was used to enhance water circulation within the chamber, and the source references for published studies on each species. "-" indicates no information available.

Coral Species	Chamber Volume (L)	Temperature	Closed or Flow Through	Flow Rate	Stir Bar	Source
Acropora cervicornis	0.253	28 °C	Closed	-	N	Davies (1980)
Acropora cervicornis	0.4	22-28 ° C	Flow Through	8-13 ml/min	N	Present study

Acropora formosa	0.5	-	Closed	-	Y + N	Dennison & Barnes (1988)
Acropora palmata	0.253	28 °C	Closed	-	N	Davies (1980)
Acropora spp.	0.475	21 °C	Closed	-	N	Drew (1973)
Agaricia grahamae	0.253	28 °C	Closed	-	N	Davies (1980)
Agaricia lamarcki	0.253	28 °C	Closed	-	N	Davies (1980)
Agaricia tenuifolia	2.5	20-22 ° C	Closed	-	Y	Lesser (1997)
Agaricia undata	0.253	28 °C	Closed	-	N	Davies (1980)
Dichocoenia stokesii	0.5	22-25 ° C	Closed	-	Y	Telesnicki & Goldberg (1995)
Galaxea fascicularis	3.5	25.5-26.5 ° C	Flow Through	~ 10 cm/s	N	Schutter et al. (2008)
Galaxea fascicularis	0.7	20-21 ° C	Flow	420 ml/min	N	Al-Horani (2003)
Goniastrea spp.	0.475	21 °C	Closed	-	N	Drew (1973)
Meandrina meandrites	0.5	22-25 ° C	Closed	-	Y	Telesnicki & Goldberg (1995)
Millepora tenera	0.475	21 °C	Closed	-	N	Drew (1973)
Montastraea annularis	0.253	28 °C	Closed	-	N	Davies (1980)

Table 1, continued.

Coral Species	Chamber Volume (L)	Temperature	Closed or Flow Through	Flow Rate	Stir Bar	Source
Montastraea cavernosa	-	28 °C	Closed	3 L/min	N	Lasker (1981)
Montastraea cavernosa	0.253	28 °C	Closed	-	N	Davies (1980)
Montastraea faveolata	3.7	29-30 ° C	Closed (flushed every 60 min)	-	Y	Lesser (2000)
Montipora monasteriata	3.1	ambient	Unidirectional	5-6 cm/s	N	Anthony & Hoegh-Guldberg (2003)

Montipora verrucosa	2.5	26 ° C	Closed	-	Y	Stambler et al. (1994)
Pocillopora damicornis	2.5	26 ° C	Closed	-	Y	Stambler et al. (1994)
Pocillopora damicornis	0.11	25-35 ° C	Closed	-	N	Al-Sofyani & Floos (2013)
Pocillopora verrucosa	0.11	25-35 ° C	Closed	-	N	Al-Sofyani & Floos (2013)
Siderastrea siderea	0.68	26 ° C	Unidirectional	5.8 cm/s	Y	Vollmer & Edmunds (2000)
Turbinaria mesenterina	2.7	26.5-28 ° C	Flow Through	6 cm/s	N	Hoogenboom et al. (2006)

Table 2. Variation in the net photosynthesis rate (P), respiration rate (R), and P:R ratio of staghorn corals *Acropora cervicornis* with 2 types of tissue abrasion treatments (non-abraded control vs. abraded fragments) and 4 levels of seawater temperature (22, 25, 28, and 30°C). n = number of measurements made on coral fragments at each temperature. See text for details.

Abrasion Treatment	Temperature (°C)	Net Photosynthesis Rate (µmol O2 L^{-1} cm^{-2} h^{-1})		Respiration Rate (µmol O2 L^{-1} cm^{-2} h^{-1})		P:R Ratio		
		Mean	SE	Mean	SE	Mean	SE	n
Non-abraded (control)	22	0.671	0.113	0.330	0.110	2.543	0.390	15
	25	1.140	0.082	0.571	0.075	2.080	0.260	19
	28	0.471	0.108	0.723	0.105	0.697	0.371	18
	30	0.205	0.118	0.812	0.115	0.297	0.406	13
Abraded	22	0.507	0.115	0.386	0.136	1.814	0.303	10
	25	0.455	0.096	0.594	0.125	1.110	0.253	10
	28	0.047	0.111	0.967	0.130	0.127	0.292	10
	30	0.163	0.113	0.984	0.133	0.336	0.298	10

Table 3. Variation in net photosynthesis and respiration rates among scleractinian coral species, as reported in published respirometry studies. Shown are the coral species examined in each study, net photosynthetic and respiration rates (means + SD or SE, depending on the study), P:R ratio (photosynthesis to respiration ratio, a measure of energy balance; values are those presented in each study, or calculated here from data in

the study), seawater temperature, level of irradiance, sample size (number of corals examined), and other notes about the study, whether the respirometry chamber was deployed in the laboratory or field, original collection location of the corals used, the main questions of interest in the study, and the publication source for each study.

Coral Species	Net Photosynthesis Rate (μmol O2 L^{-1} cm^{-2} h^{-1})	Respiration Rate (μmol O2 L^{-1} cm^{-2} h^{-1})	SD or SE	P:R	Temperature	Irradiance (μmol photons m^{-2} s^{-1})	n	other notes	Field or Laboratory	Coral origin (Location)	Source
Acropora cervicornis	-	0.35 (±0.01)**	SD	N/A	28 °C	0	2	Original depth 15m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Acropora cervicornis	0.02 (±0.15)	1.49 (±0.18)	SE	0.12 (±0.29)	28 °C	350±20	10	Tissue abrasion	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora cervicornis	0.22 (±0.15)	1.60 (±0.19)	SE	0.33 (±0.30)	30 ° C	350±20	10	Tissue abrasion	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora cervicornis	0.37 (±0.16)	1.09 (±0.15)	SE	0.34 (±0.72)	30 ° C	350±20	13	-	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora cervicornis	0.56 (±0.44)	0.90 (±0.22)	SE	1.11 (±0.25)	25 ° C	350±20	10	Tissue abrasion	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora cervicornis	0.60 (±0.15)	0.66 (±0.19)	SE	1.81 (±0.30)	22 ° C	350±20	10	Tissue abrasion	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora cervicornis	0.70 (±0.15)	0.89 (±0.14)	SE	0.76 (±0.66)	28 °C	350±20	18	-	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora cervicornis	0.93 (±0.16)	0.39 (±0.14)	SE	3.21 (±0.69)	22 ° C	350±20	15	-	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora cervicornis	1.57 (±0.14)	0.69 (±0.10)	SE	2.42 (±0.47)	25 ° C	350±20	19	-	Laboratory	Ft. Lauderdale and Florida Keys (USA)	Present study
Acropora palmata	-	0.84 (±0.10)**	SD	N/A	28 °C	0	2	Original depth 5m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Agaricia grahamae	-	0.14 (±0.031)**	SD	N/A	28 °C	0	3	Original depth 80m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Agaricia lamarcki	-	0.40 (±0.08)**	SD	N/A	28 °C	0	2	Original depth 40m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Agaricia tenuifolia	2.73 (±0.54)	1.11 (±0.67)	SD	2.46 b	28-35 °C	max 1900	4	Elevated (28-35°C) temperatures	Laboratory	Carrie Bow Cay (BLZ)	Lesser (1997)
Agaricia tenuifolia	3.89 (±0.15)	0.44 (±0.13)	SD	8.88 b	28-35 °C	max 1900	4	Elevated (28-35°C) temperatures + antioxidants	Laboratory	Carrie Bow Cay (BLZ)	Lesser (1997)
Agaricia tenuifolia	4.28 (±0.17)	0.44 (±0.15)	SD	9.79 b	28-32 °C	max 1900	4	Normal (28-32°C) temperatures	Laboratory	Carrie Bow Cay (BLZ)	Lesser (1997)
Agaricia undata	-	0.25**	SD	N/A	28 °C	0	1	Original depth 66m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Fungia scutaria	-	-	SE	3.89 (±0.36)	30 ° C	(Midday seawater surface levels in Hawaii)	3-4	-	Laboratory	Enewetak, Marshall Islands	Coles and Jokiel (1977)

a (calculated from averaged daily irradiance values)
* originally reported as μmol O2 cm^{-2} min^{-1}
**originally reported as μL O2 cm^{-2} $hour^{-1}$
*** originally reported as nmol O2 cm^{-2} s^{-1}
† originally reported as nmol O2 cm^{-2} h^{-1}
†† Originally reported Pgross (or Pmax), calculation made by the equation Pgross=Pnet+Rdark
b (calculated from given values)

Table 3. (continued)

Coral Species	Net Photosynthesis Rate (μmol O2 L^{-1} cm^{-2} h^{-1})	Respiration Rate (μmol O2 L^{-1} cm^{-2} h^{-1})	SD or SE	P:R	Temperature	Irradiance (μmol photons m−2 s−1)	n	other notes	Field or Laboratory	Coral origin (Location)	Source
Galaxea fascicularis	0.54 *	-		N/A	25.5-26.5 ° C	38		-	Laboratory	did not specify	Schutter et al. (2008)
Galaxea fascicularis	0.97 (±0.15) ††	0.83 (±0.11)	SE	1.17 b	31 ° C	300 (±30)	8	31°C starved	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Galaxea fascicularis	2.43 (±0.28) ††	1.1 (±0.16)	SE	2.21 b	31 ° C	300 (±30)	8	31°C fed	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Galaxea fascicularis	2.46 (±0.24) ††	1.24 (±0.18)	SE	1.98 b	26 ° C	300 (±30)	8	26°C	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Galaxea fascicularis	4.62 *	-		N/A	25.5-26.5 ° C	410		-	Laboratory	did not specify	Schutter et al. (2008)
Montastraea annularis	-	0.46 (±0.06)**	SD	N/A	28 °C	0	3	Original depth 40m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Montastraea annularis	-	0.55 (±0.11)**	SD	N/A	28 °C	0	4	Original depth 10m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Montastraea annularis	-	0.63 (±0.15)**	SD	N/A	28 °C	0	3	Original depth 20m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Montastraea annularis	-	0.81 (±0.32)**	SD	N/A	28 °C	0	3	Original depth 2m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Montastraea faveolata	1.48 (±0.12)	0.43 (±0.12)	SD	3.44 b	29-30 ° C	242 a	6	Depth of 18m	Field	Conch Reef, Key Largo, FL (USA)	Lesser (2000)
Montastraea faveolata	1.88 (±0.14)	0.08 (±0.14)	SD	23.50 b	29-30 ° C	170 a	6	Depth of 23m	Field	Conch Reef, Key Largo, FL (USA)	Lesser (2000)
Montastraea faveolata	1.93 (±0.07)	0.11 (±0.06)	SD	17.55 b	29-30 ° C	60 a	6	Depth of 30m	Field	Conch Reef, Key Largo, FL (USA)	Lesser (2000)
Montastraea faveolata	2.12 (±0.14)	0.02 (±0.13)	SD	106.00 b	29-30 ° C	201 a	6	Depth of 10m	Field	Conch Reef, Key Largo, FL (USA)	Lesser (2000)
Montastraea faveolata	3.10 (±0.17)	0.06 (±0.16)	SD	51.67 b	29-30 ° C	682 a	6	Depth of 3m	Field	Conch Reef, Key Largo, FL (USA)	Lesser (2000)
Montastrea cavernosa	-	0.21 (±0.03)**	SD	N/A	28 °C	0	2	Original depth 40m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Montastrea cavernosa	-	0.30 (±0.07)**	SD	N/A	28 °C	0	3	Original depth 20m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Montastrea cavernosa	-	0.41 (±0.08)**	SD	N/A	28 °C	0	3	Original depth 13m	Laboratory	Discovery Bay (JAM)	Davies (1980)
Montipora monasteriata	2.31 (±0.31) ††	0.43 (±0.09)	SE	5.37 b	-	0 to 1300	7	Cave habitat, originally	Laboratory	Wistari Reef, Heron Island (AUS)	Anthony & Hoegh-Guldberg (2003)
Montipora monasteriata	2.54 (±0.40) ††	0.70 (±0.19)	SE	3.63 b	-	0 to 1300	6	Overhang habitat, originally	Laboratory	Wistari Reef, Heron Island (AUS)	Anthony & Hoegh-Guldberg (2003)
Montipora monasteriata	2.59 (±0.12) ††	1.33 (±0.13)	SE	1.95 b	-	0 to 1300	8	Open habitat, originally	Laboratory	Wistari Reef, Heron Island (AUS)	Anthony & Hoegh-Guldberg (2003)
Montipora verrucosa	-	-	SE	2.85 (±0.17)	30 ° C	(Midday seawater surface levels in Hawaii)	3-4	-	Laboratory	Kaneohe, HI (USA)	Coles and Jokiel (1977)

a (calculated from averaged daily irradiance values)
* originally reported as μmol O2 cm^{-2} min^{-1}
**originally reported as μL O2 cm^{-2} $hour^{-1}$
*** originally reported as nmol O2 cm^{-2} s^{-1}
† originally reported as nmol O2 cm^{-2} h^{-1}
†† Originally reported Pgross (or Pmax), calculation made by the equation Pgross=Pnet+Rdark
b (calculated from given values)

Table 3. (continued)

Coral Species	Net Photosynthesis Rate (μmol O2 L^{-1} cm^{-2} h^{-1})	Respiration Rate (μmol O2 L^{-1} cm^{-2} h^{-1})	SD or SE	P:R	Temperature	Irradiance (μmol photons m^{-2} s^{-1})	n	other notes	Field or Laboratory	Coral origin (Location)	Source
Montipora verrucosa	-	1.44 (±0.24)*	SD	N/A	26 ° C	0	4	ambient Ammonium (control)	Laboratory	Kaneohe, HI (USA)	Stambler et al. (1994)
Pocillopora damicornis	-	-	SE	2.21 (±0.19)	30 ° C	(Midday seawater surface levels in Hawaii)	3-4	-	Laboratory	Kaneohe, HI (USA)	Coles and Jokiel (1977)
Pocillopora damicornis	-	0.29 (±0.10)**	SD	N/A	35 ° C	-	10	-	Field	Obhur Creek, Red Sea (SAU)	Al-Sofyani & Floos (2013)
Pocillopora damicornis	-	0.29 (±0.14)**	SD	N/A	25 ° C	-	10	-	Field	Obhur Creek, Red Sea (SAU)	Al-Sofyani & Floos (2013)
Pocillopora damicornis	-	0.37 (±0.10)**	SD	N/A	30 ° C	-	10	-	Field	Obhur Creek, Red Sea (SAU)	Al-Sofyani & Floos (2013)
Pocillopora damicornis	-	2.1 (±0.42)*	SD	N/A	26 ° C	0	4	ambient Ammonium (control)	Laboratory	Kaneohe, HI (USA)	Stambler et al. (1994)
Pocillopora damicornis	0.47 (±0.07)***	1.01 (±0.25)***	SE	0.54 (±0.16)	24 °C	900	4	Originally Central GBR, winter temperatures	Laboratory	Davies Reef/Broadhurst Reef, Great Barrier Reef (AUS)	Ulstrup et al. (2011)
Pocillopora damicornis	1.30 (±0.86)***	2.30 (±1.08)***	SE	0.87 (±0.40)	24 °C	900	4	Originally Northern GBR, winter temperatures	Laboratory	Lizard Island, Great Barrier Reef (AUS)	Ulstrup et al. (2011)
Pocillopora damicornis	1.98 (±0.90)***	1.30 (±0.18)***	SE	1.79 (±0.99)	28.5 °C	900	4	Originally Central GBR, summer temperatures	Laboratory	Davies Reef/Broadhurst Reef, Great Barrier Reef (AUS)	Ulstrup et al. (2011)
Pocillopora damicornis	3.17 (±1.15)***	1.55 (±0.50)***	SE	4.36 (±1.79)	28.5 °C	900	4	Originally Northern GBR, summer temperatures	Laboratory	Lizard Island, Great Barrier Reef (AUS)	Ulstrup et al. (2011)
Pocillopora verrucosa	-	0.31 (±0.09)**	SD	N/A	25 ° C	-	10	-	Field	Obhur Creek, Red Sea (SAU)	Al-Sofyani & Floos (2013)
Pocillopora verrucosa	-	0.41 (±0.11)**	SD	N/A	35 ° C	-	10	-	Field	Obhur Creek, Red Sea (SAU)	Al-Sofyani & Floos (2013)
Pocillopora verrucosa	-	0.42 (±0.10)**	SD	N/A	30 ° C	-	10	-	Field	Obhur Creek, Red Sea (SAU)	Al-Sofyani & Floos (2013)
Porites compressa	-	-	SE	2.15 (±0.19)	30 ° C	(Midday seawater surface levels in Hawaii)	3-4	-	Laboratory	Enewetak, Marshall Islands	Coles and Jokiel (1977)
Siderastrea siderea	1.42 (±0.22)†	0.535 (±0.09)†	SD	2.66 b	22-26 ° C	100	12	32ppt	Laboratory	Offshore of St. Teresa FL (USA)	Muthiga and Szmant (1987)
Stylophora pistillata	0.31 (±0.15) ††	0.43 (±0.03)	SE	0.72 b	31 ° C	300 (±30)	8	31°C starved	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Stylophora pistillata	0.90 (±0.11) ††	0.70 (±0.06)	SE	1.29 b	31 ° C	300 (±30)	8	31°C fed	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Stylophora pistillata	1.20 (±0.18) ††	0.64 (±0.07)	SE	1.88 b	26 ° C	300 (±30)	8	26°C	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Turbinaria mesenterina	2.60 (±0.06)	0.40 (±0.10)	SE	6.50 b	26.5-28 ° C	345 ± 54	4	-	Laboratory	Nelly Bay, Magnetic Island (AUS)	Hoogenboom et al. (2006)

a (calculated from averaged daily irradiance values)
* originally reported as μmol O2 cm^{-2} min^{-1}
**originally reported as μL O2 cm^{-2} $hour^{-1}$
*** originally reported as nmol O2 cm^{-2} s^{-1}
† originally reported as nmol O2 cm^{-2} h^{-1}
†† Originally reported Pgross (or Pmax), calculation made by the equation Pgross=Pnet+Rdark
b (calculated from given values)

Table 3. (continued)

Coral Species	Net Photosynthesis Rate (µmol O2 L^{-1} cm^{-2} h^{-1})	Respiration Rate (µmol O2 L^{-1} cm^{-2} h^{-1})	SD or SE	P:R	Temperature	Irradiance (µmol photons m−2 s−1)	n	other notes	Field or Laboratory	Coral origin (Location)	Source
Turbinaria reniformis	0.67 (±0.08) ††	0.43 (±0.01)	SE	1.56 b	31 ° C	300 (±30)	8	31°C starved	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Turbinaria reniformis	0.86 (±0.29)***	0.79 (±0.18)***	SE	1.35 (±0.53)	24 °C	900	4	Originally Central GBR, winter temperatures	Laboratory	Davies Reef/Broadhurst Reef, Great Barrier Reef (AUS)	Ulstrup et al. (2011)
Turbinaria reniformis	2.23 (±0.30) ††	1.10 (±0.12)	SE	2.03 b	31 ° C	300 (±30)	8	31°C fed	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Turbinaria reniformis	2.38 (±0.36)***	1.84 (±0.40)***	SE	1.58 (±0.47)	28.5 °C	900	4	Originally Northern GBR, summer temperatures	Laboratory	Lizard Island, Great Barrier Reef (AUS)	Ulstrup et al. (2011)
Turbinaria reniformis	2.70 (±0.76)***	1.33 (±0.22)***	SE	1.89 (±0.31)	24 °C	900	4	Originally Northern GBR, winter temperatures	Laboratory	Lizard Island, Great Barrier Reef (AUS)	Ulstrup et al. (2011)
Turbinaria reniformis	3.23 (±0.51) ††	1.26 (±0.17)	SE	2.56 b	26 ° C	300 (±30)	8	26°C	Laboratory	did not specify	Ferrier-Pagès et al. (2010)
Turbinaria reniformis	5.26 (±2.23)***	3.28 (±0.432)***	SE	1.57 (±0.69)	28.5 °C	900	4	Originally Central GBR, summer temperatures	Laboratory	Davies Reef/Broadhurst Reef, Great Barrier Reef (AUS)	Ulstrup et al. (2011)

a (calculated from averaged daily irradiance values)
* originally reported as µmol O2 cm^{-2} min^{-1}
**originally reported as µL O2 cm^{-2} hour^{-1}
*** originally reported as nmol O2 cm^{-2} s^{-1}
† originally reported as nmol O2 cm^{-2} h^{-1}
†† Originally reported Pgross (or Pmax), calculation made by the equation Pgross=Pnet+Rdark
b (calculated from given values)

Figure 1. Photograph of staghorn coral *Acropora cervicornis* fragments in a culture tank at Auburn University. Fragments were glued onto ceramic plugs, inserted into plastic platforms, and cultured in 150L closed-system tank setups. Note that the coral skeleton and tissue have grown over the plugs at the bases of the fragments, which had been cultured in the laboratory for 4 to 6 weeks. For scale, the squares in the plastic grating are 1.5 cm in diameter.

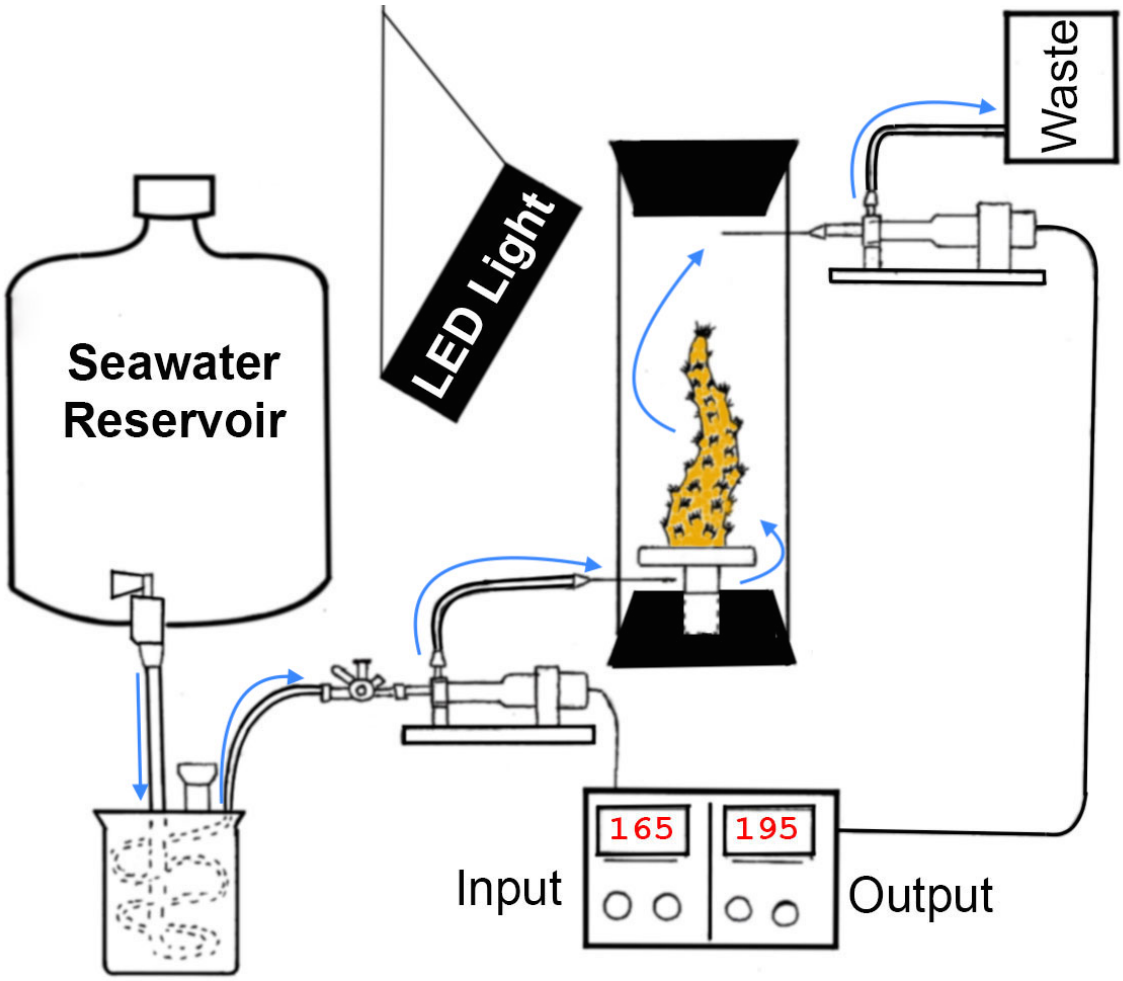

Figure 2. Diagram of the respirometry setup used to measure photosynthesis and respiration rates in *Acropora cervicornis* fragments. Two Strathkelvin Instrument O_2 electrodes were connected to a Cameron Instrumental OM200 Oxygen Meter, which displayed the dissolved O_2 readings in torr. Blue arrows show the direction of seawater flow, from a seawater reservoir to a water bath with coiled tubing, through the respirometry chamber, then out to a waste reservoir. During photosynthesis trials, an adjustable LED light fixture (Model HYA05-LENS-55*3W-B, Galaxyhydro) was positioned near the long axis of the coral. Prior to each trial, irradiance was adjusted so that the midpoint of each fragment received 350 ± 20 umol photons m^{-2} s^{-1}. During respiration trials, the respirometry chamber containing each coral was covered in blackout cloth to measure oxygen consumption under dark conditions.

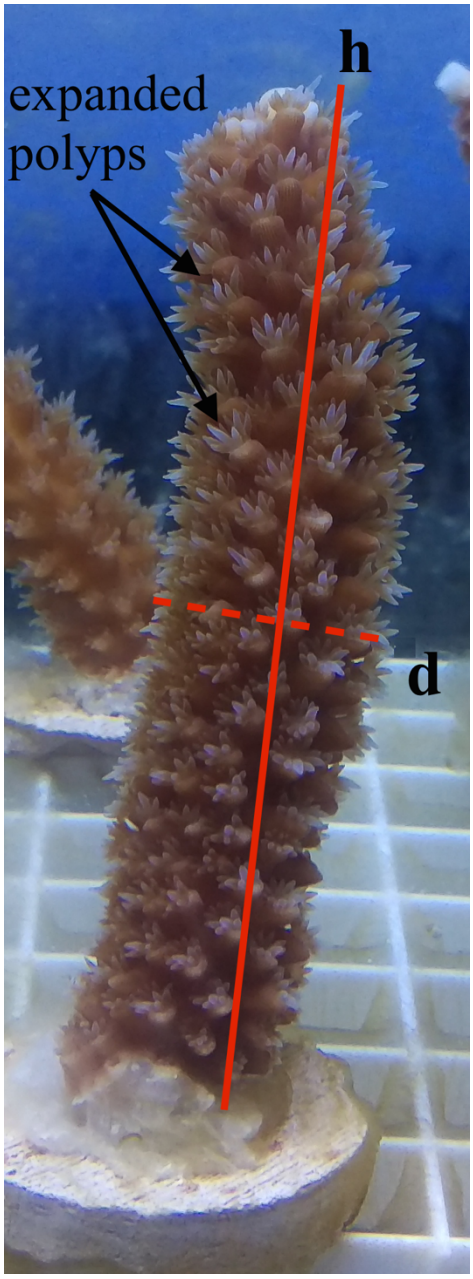

Figure 3. Photograph of a cultured fragment of staghorn coral *Acropora cervicornis*, showing the dimensions used to calculate estimated surface area (ESA). *h* = total length, *d* = maximal diameter. Note the ceramic plug at the coral base, and the plastic grating in which the plug was inserted for culture in laboratory tanks. Note also the expanded tentacles of polyps visible along the sides of the fragment. This fragment had been cultured in the laboratory for ~ 4 weeks prior to being photographed.

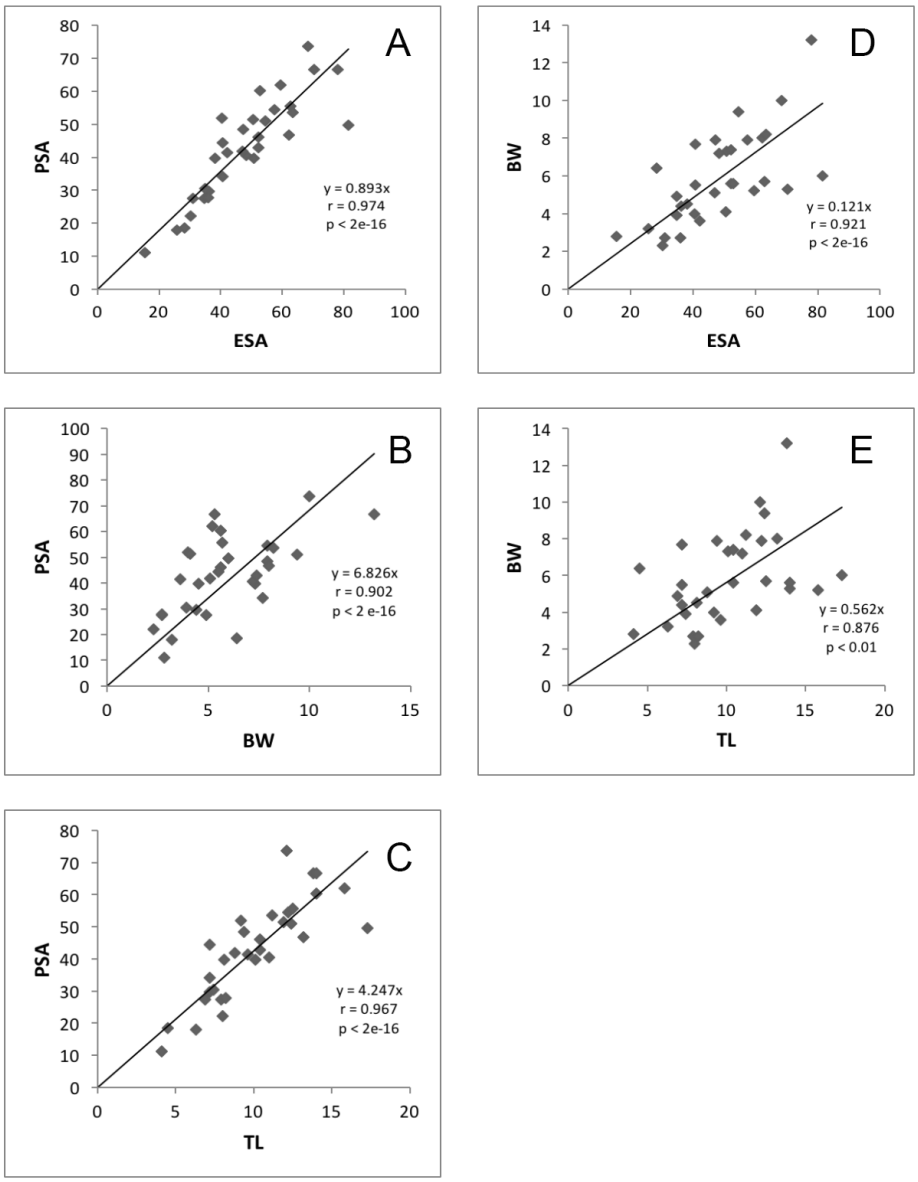

Figure 4. Relationships between sets of biometric data collected to measure the sizes of fragments of staghorn corals *Acropora cervicornis* (n = 32 fragments). Precise surface area (PSA) was determined by covering fragments (after they had been sacrificed) with tinfoil, and using an equation derived from objects with known surface areas (after Marsh 1970). Estimated surface area (ESA) was calculated using the equation for a cylinder shell surface A=2 πhr (Naumann et al. 2009), as shown in Fig. 3. Bouyant weight (BW) was measured by suspending coral fragments in a plastic frame submerged in a tank filled with seawater, with the frame hanging from an electronic balance above the tank (after Jokiel 1978). Total length (TL) was measured for each fragment, using a flexible measuring tape to allow the tape to curve slightly along the length of each fragment, some of which had a slightly curved shape (see Figs. 1 and 3).

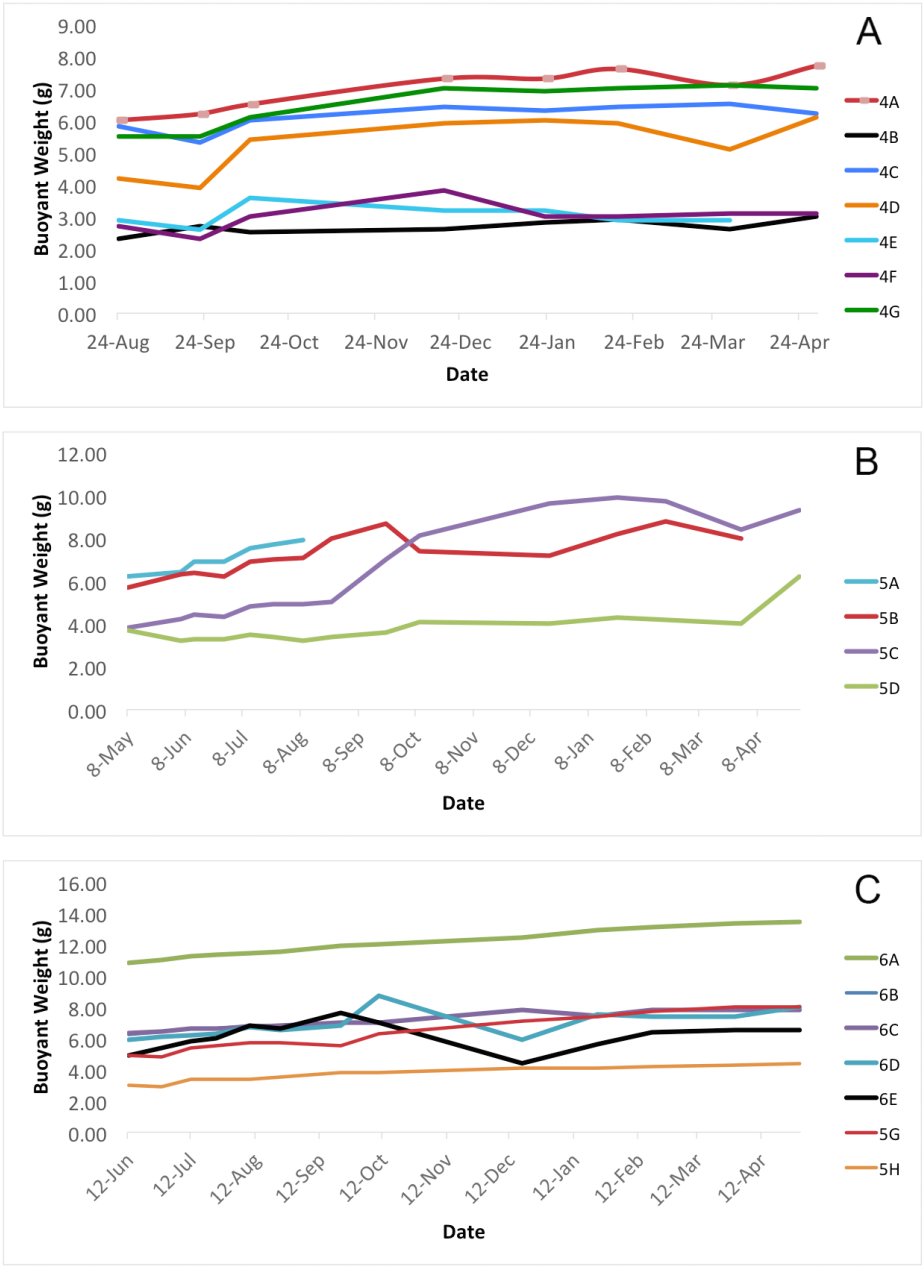

Figure 5. Growth patterns of fragments of *Acropora cervicornis* that arrived from public aquarium tanks at the Smithsonian Marine Station, Fort Pierce, Florida, USA. Growth is shown over 10 months for 4-7 coral fragments per tank, in 3 laboratory tanks (# 4, 5, and 6) at Auburn University. Note the general upward trend of coral size over time, and the slight up-and-down variation in size among measurement periods, some of it likely due to measurement error.

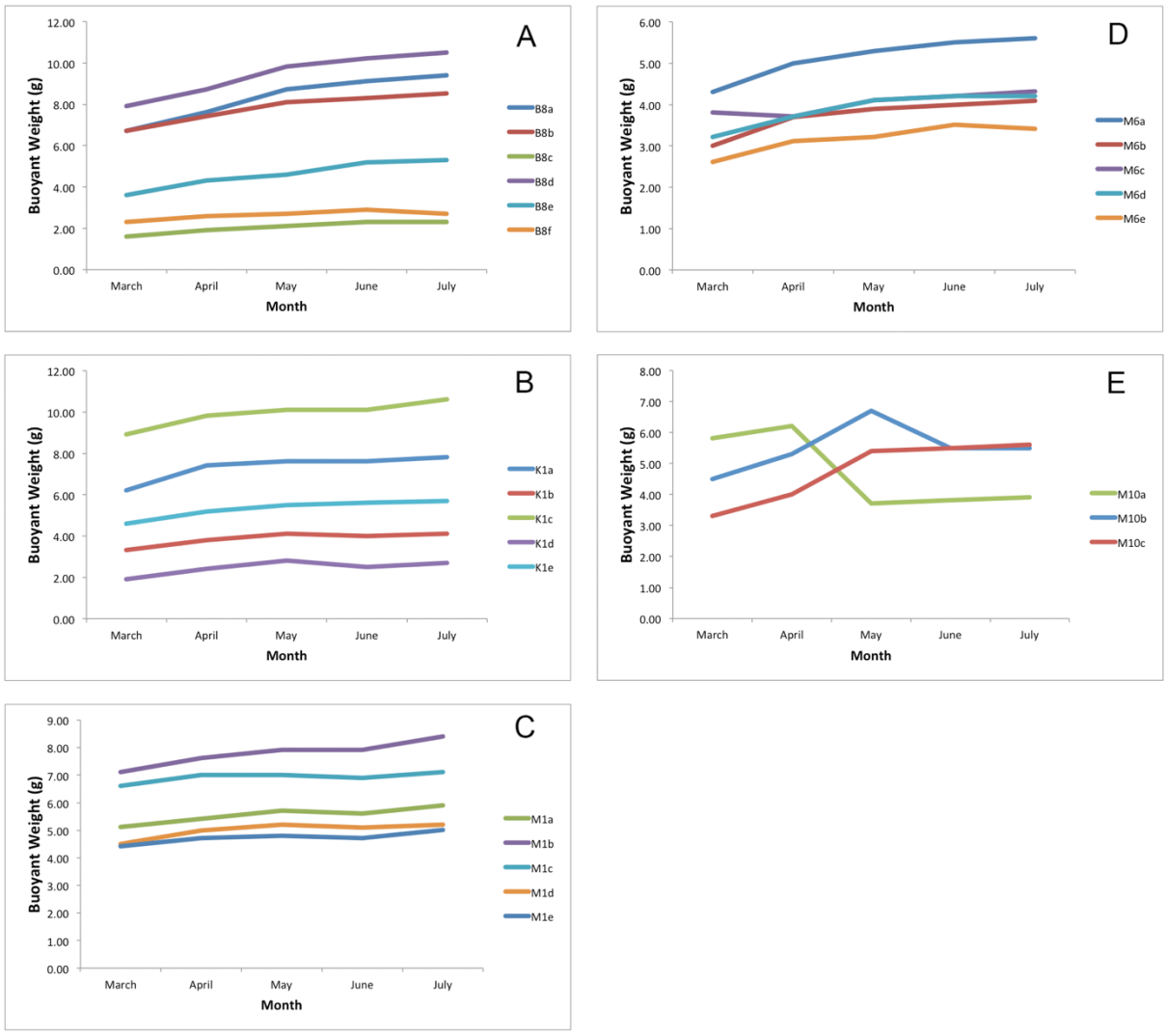

Figure 6. Growth patterns of fragments of *Acropora cervicornis* that arrived from offshore nurseries of the Coral Restoration Foundation, Key Largo, Florida, USA. Growth is shown over 4 months for 3-6 coral fragments per genotype, dispersed among in 3 laboratory tanks at Auburn University. Note the general upward trend of coral size over time, and the slight up-and-down variation in size among measurement periods, some of it likely due to measurement error. Genotype M exhibited large variation in size between some time periods, which can be attributed to an accidental fragmentation during culture. The coral genotypes included B8 (n = 6), K1 (n = 5), M10 (n = 3), M1 (n = 5), and M6 (n = 5).

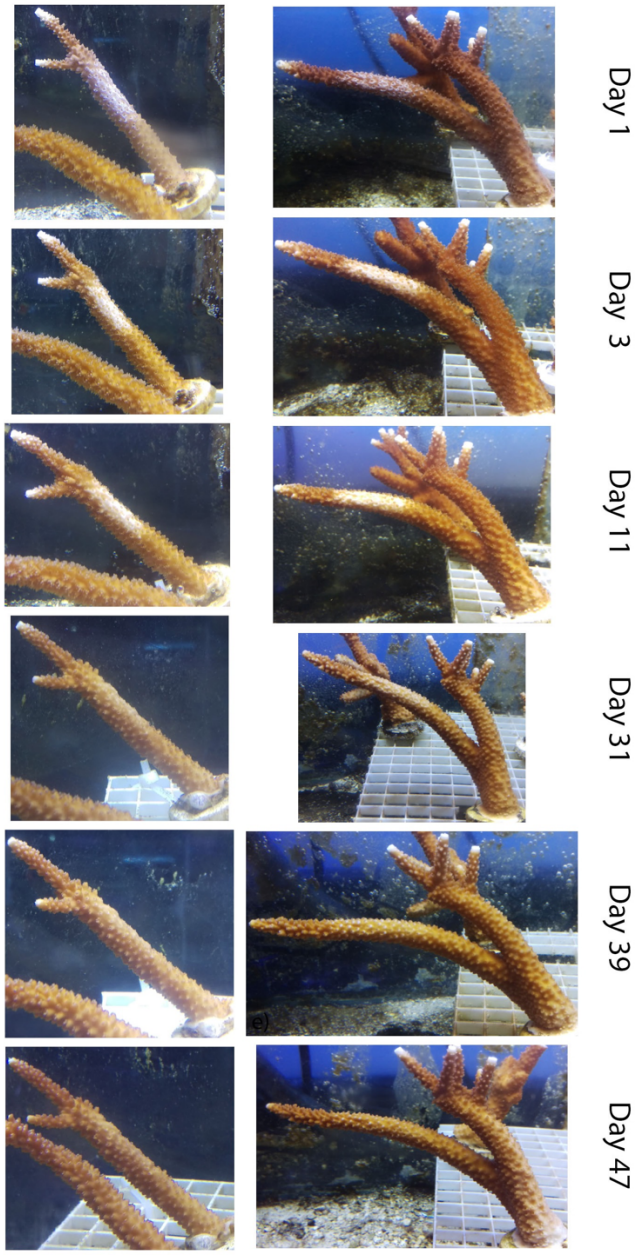

Figure 7. Recovery patterns of two fragments of *Acropora cervicornis* from abrasion wounds, in which relatively large abrasion wounds had been inflicted. Each wound consisted of ~ 5 cm^2 of live coral tissue abraded from the fragment, revealing the underlying white calcareous skeleton. The two fragments picture here had arrived from the Smithsonian Marine Station, Fort Pierce, FL, USA, and had been acclimated to culture tanks at Auburn University, Auburn, AL, USA, for 4 months before the abrasion trials began.

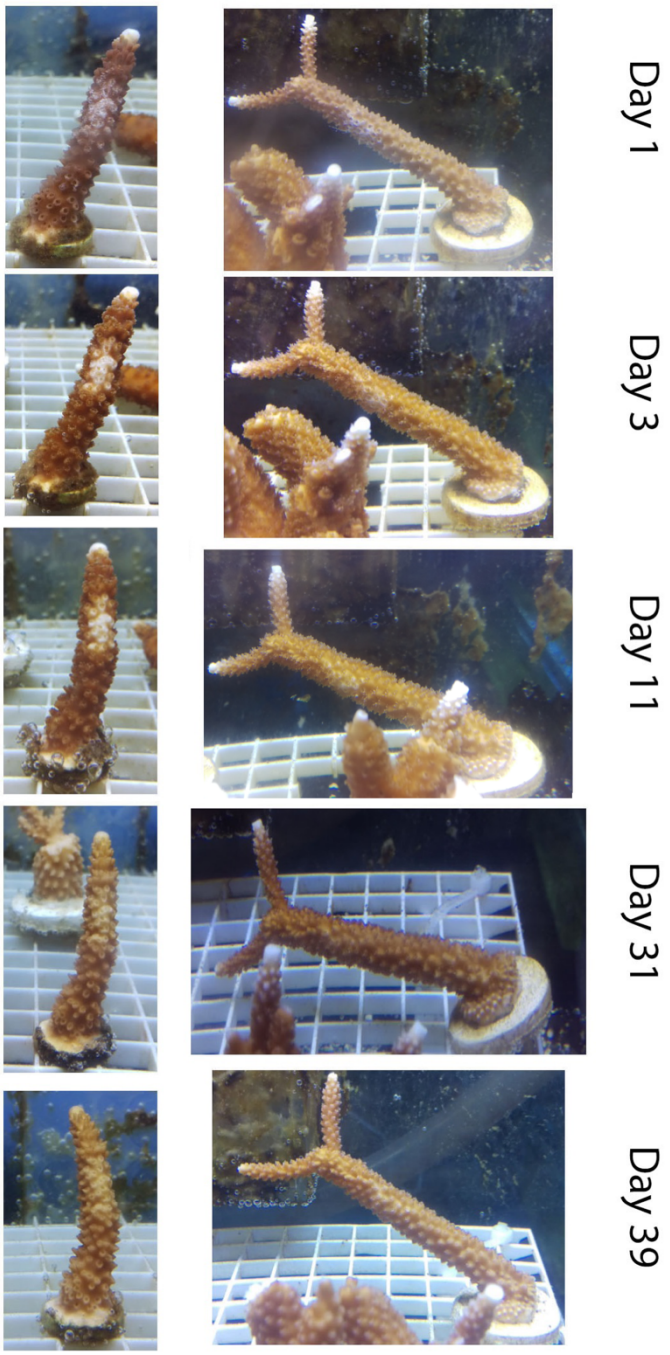

Figure 8. Recovery patterns of two fragments of *Acropora cervicornis* from abrasion wounds, in which relatively small abrasion wounds had been inflicted. Each wound consisted of ~ 1.5 cm^2 of live coral tissue abraded from the fragment, revealing the underlying white calcareous skeleton. The two fragments picture here had arrived from the Smithsonian Marine Station, Fort Pierce, FL, USA, and had been acclimated to culture tanks at Auburn University, Auburn, AL, USA, for 4 months before the abrasion trials began.

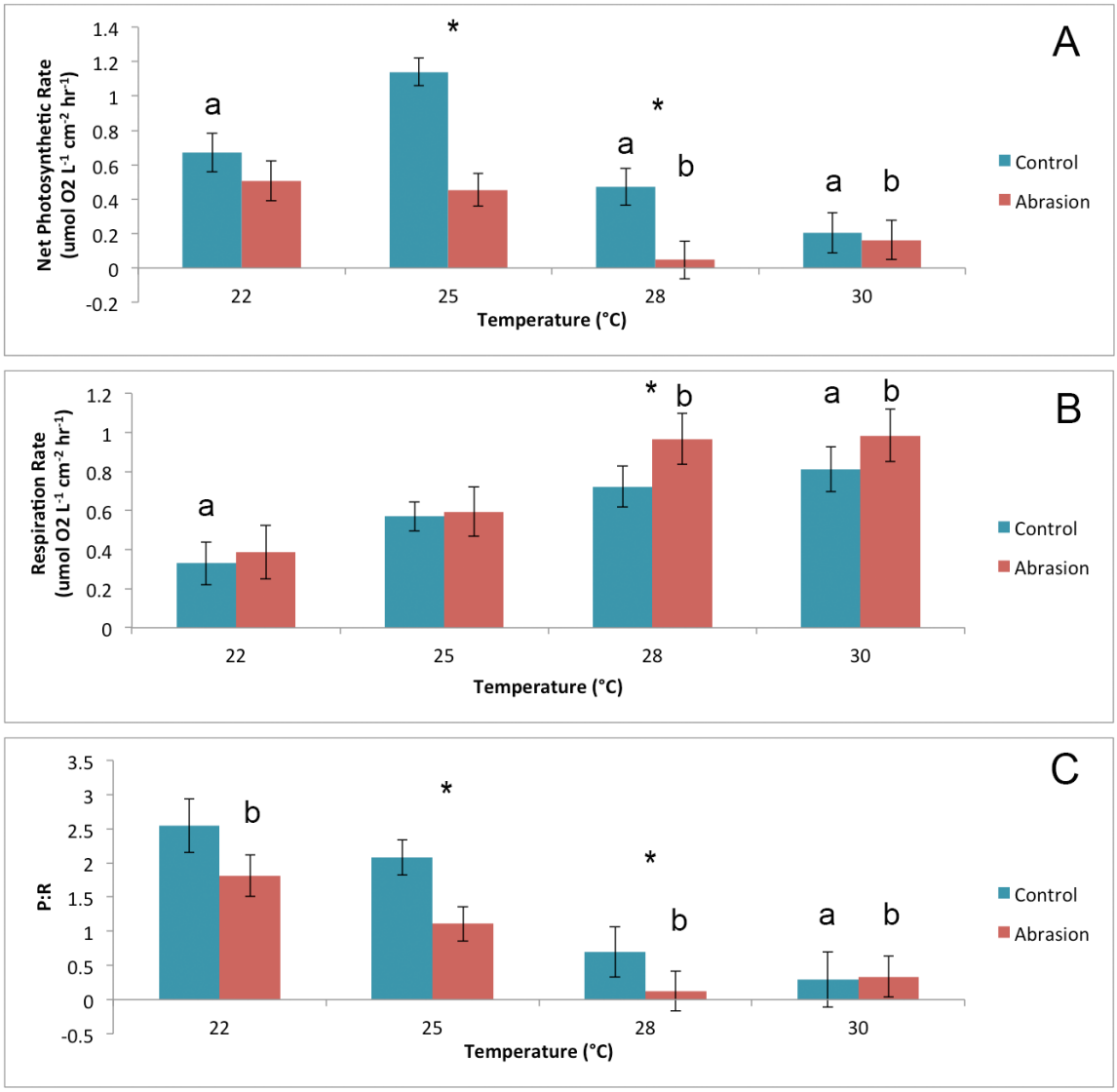

Figure 9. Variation in the (A) net photosynthesis rate, (B) respiration rate, and (C) P:R ratio (photosynthesis to respiration rate, a measure of energy balance) of *Acropora cervicornis* fragments, with seawater temperature and tissue abrasion status. Abraded fragments had ~15% of live coral tissue scraped off at least 72 h prior to being used in respirometry trials. Temperature was increased gradually after each fragment was immersed in the respirometry chamber, so that fragments acclimated over 2 h to the trial temperature, and to the irradiance level of 350 ± 20 mmol photons m^{-2} s^{-1}. 'a' and 'b' indicate values that differed significantly from those at the control temperature (25 °C), for fragments that were not abraded (n = 65 measurements) and those that were abraded (n = 40 measurements), respectively. * indicates values that differed significantly between the abraded and non-abraded fragments at each temperature. Shown are means ± 1 SE.

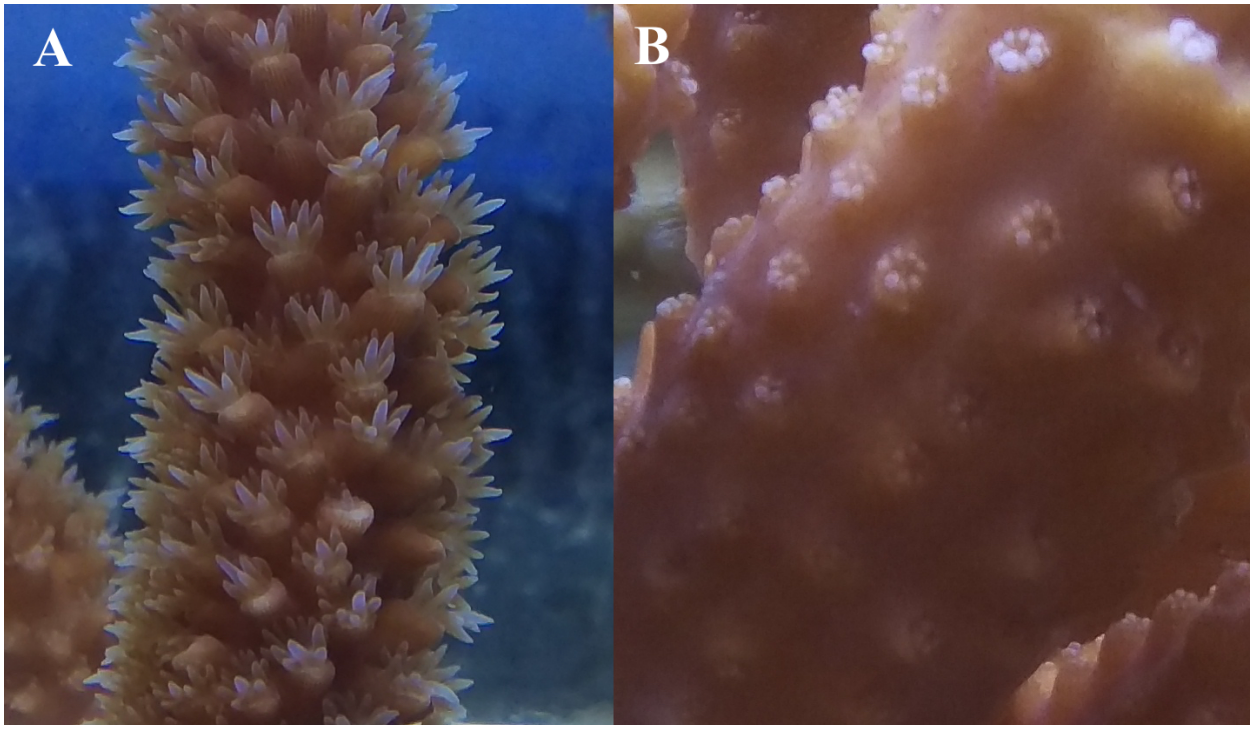

Figure 10. Variation in polyp expansion levels between fragments belonging to two different genotypes of staghorn coral *Acropora cervicornis*. Both fragments been cultured in tanks at Auburn University and exposed to the same light and temperature conditions for several mo prior to being photographed. Panel A depicts genotype B8 (fragment B8b) obtained from an offshore nursery of the Coral Restoration Foundation (CRF), whereas panel B shows fragment # 4E that belonged to a genotype obtained from the Smithsonian Marine Station (SMS). Note that polyps on the CRF fragment are much more expanded than those on the SMS fragment. See Figs. 6A and 5A for the growth rates these two fragments, respectively.