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Changes to coral health and metabolic activity under oxygen deprivation

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Scleractinian corals play a critical role in tropical marine ecosystems by providing essential structure and food for coral reef inhabitants. However, recent investigations have found an alarming increase in coral losses from a variety of stressors, which threatens the general health and resilience of tropical coastal ecosystems. The effect of oxygen deprivation on the health of Hawaiian corals based on local drivers is of particular concern. On Hawaiian reefs, the fast-growing, invasive algae *Gracilaria salicornia* overgrows coral heads, restricting water flow and light, thereby smothering corals. Field data shows hypoxic conditions (dissolved oxygen (DO₂) < 2 mg/L) occurring underneath algal mats at night, and concurrent bleaching and partial tissue loss of shaded corals. This study evaluates changes in coral health and metabolism through the replication of hypoxic conditions in a laboratory setting in order to limit the contribution of additional environmental variables to health shifts. Analyses of metabolic stress were accomplished through the quantification of lactate dehydrogenase and opine dehydrogenase activities. Through experimentation, treatment corals were observed to exhibit significant increases in alanopine and strombine dehydrogenase activities (ADH and SDH, respectively), but little to no lactate or octopine dehydrogenase activity. These findings indicate that not only is hypoxia a major source of increased coral stress, which occurs in response to invasive algae mat smothering, but provide novel insight into coral metabolic shifts in extremely low-oxygen environments and point to ADH and SDH as possible tools for hypoxia mitigation.
Changes to Coral Health and Metabolic Activity Under Oxygen Deprivation

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Abstract

Scleractinian corals play a critical role in tropical marine ecosystems by providing essential structure and food for coral reef inhabitants. However, recent investigations have found an alarming increase in coral losses from a variety of stressors, which threatens the general health and resilience of tropical coastal ecosystems. The effect of oxygen deprivation on the health of Hawaiian corals based on local drivers is of particular concern. On Hawaiian reefs, the fast-growing, invasive algae *Gracilaria salicornia* overgrows coral heads, restricting water flow and light, thereby smothering corals. Field data shows hypoxic conditions (dissolved oxygen \( \text{DO}_2 < 2 \text{ mg/L} \)) occurring underneath algal mats at night, and concurrent bleaching and partial tissue loss of shaded corals. This study evaluates changes in coral health and metabolism through the replication of hypoxic conditions in a laboratory setting in order to limit the contribution of additional environmental variables to health shifts. Analyses of metabolic stress were accomplished through the quantification of lactate dehydrogenase and opine dehydrogenase activities. Through experimentation, treatment corals were observed to exhibit significant increases in alanopine and strombine dehydrogenase activities (ADH and SDH, respectively), but little to no lactate or octopine dehydrogenase activity. These findings indicate that not only is hypoxia a major source of increased coral stress, which occurs in response to invasive algae mat smothering, but provide novel insight into coral metabolic shifts in extremely low-oxygen environments and point to ADH and SDH as possible tools for hypoxia mitigation.
Introduction

Global coral health

Coral reefs are important cultural, ecological, and economic resources, providing critical marine habitats for many invertebrates, fish, and algae species (McClanahan, Polunin & Done, 2002; Hughes et al., 2003). Their complex structure provides marine life with food, suitable habitats for growth, and protection from predators, while also acting as natural barriers that buffer adjacent coastlines from coastal erosion (McManus, 1997; White, Vogt & Arin, 2000; Cesar, Burke & Pet-soede, 2003; Bishop et al., 2011). However despite their value, coral reefs have been devastated by increased anthropogenic impacts and changing abiotic environmental factors that continue to overwhelm these ecosystems (McClanahan, Polunin & Done, 2002; Hughes et al., 2003; Fabricius, 2005; De’ath et al., 2012; Bahr, Jokiel & Rodgers, 2015).

Destructive fishing practices, terrestrial run-off and pollution, sewage effluent infusion, coupled with intensifying storms, regional warming events, and invasive species impacts, have led to major losses in coral cover, fish abundance, and decreased ability of reefs to support local human populations (Stimson, Larned & Conklin, 2001; Fox et al., 2003; Fabricius, 2005; Dailer et al., 2010). These continued pressures have resulted in mass coral bleaching events, and have the ability to disrupt spawning patterns and trigger widespread coral mortality (Downs et al., 2002; Levitan et al., 2014; Bahr, Jokiel & Rodgers, 2015; Paxton et al., 2015). As a result, mass losses of coral cover and subsequent shifts in the ecosystem balance has the potential to further reduce the abundance and diversity of fish and invertebrate species (Jones et al., 2004). Historically, mass coral mortality in combination to altered environmental conditions has led to phase shifts in coral reef structure, wherein coral reefs have shifted from a coral to an algae
dominated state (McCook, 1999; Stimson, Larned & Conklin, 2001). Although herbivores, such
as urchins and fish, can potentially limit the expansion of algae growth (Westbrook et al., 2015),
aggressive invasive algae and herbivore grazing preference for native species has had a profound
effect on coral reefs, especially in the Hawaiian Islands where such algae have been found to
overgrow coral colonies and negatively impact coral health (McCook, Jompa & Diaz-Pulido,
2001; Stimson, Larned & Conklin, 2001; Smith et al., 2004). These impacts include algal
overgrowth creating oxygen and solar radiation-poor environments for corals, as well as
induction of bleaching through photoinhibition and reduced photorespiration (Martinez, Smith &
Richmond, 2012).

Management strategies have been employed to mitigate the impacts from stressors, such
as overgrowth by invasive algae (Kittinger et al., 2013; Westbrook et al., 2015). However, these
efforts occur once a stressor has already inflicted a significant amount of damage to a reef
(Stimson, Larned & Conklin, 2001; Conklin & Smith, 2005; Wolanski, Martinez & Richmond,
2009).

Corals are often subjected to multiple stressors, and the key metric of effect has been
mortality. There is a need to develop biomarkers to detect changes in coral health pre-mortem in
order to proactively preserve coral reef environments by identifying the key stressors and
targeting not only corals under the highest stressor impacts, but also those beginning to show
molecular warnings of decline. In order to address this need, we developed biomarkers indicative
of hypoxic stress in the coral Montipora capitata, a major reef-building coral in Hawaii. It is
hoped that these biomarkers can be developed into diagnostic tools to give researchers and
managers the ability to rapidly assess changes in coral health before coral mortality occurs.
Cellular metabolic energy production in animals consists of two general pathways, aerobic and anaerobic respiration, defined by the necessary presence or absence of oxygen, respectively. The simple sugar, glucose, acts as the primary substrate for these pathways, wherein glucose initially undergoes anaerobic catabolism to be converted to pyruvate through the glycolytic pathway, producing 2 moles of adenosine tri-phosphate (ATP), the main energy source employed in cellular respiration, per mole of glucose (figure 1). Once pyruvate is formed, it is processed through the pyruvate dehydrogenase enzyme complex to provide the required substrates, such as acetyl-CoA, for the Kreb’s Cycle and Electron Transport Chain (ETC). These pathways complete the cellular degradation of glucose using oxygen as a final electron acceptor in the ETC, resulting in the formation of water and a net production of 38 moles of ATP per mole of glucose (Tielens & Van Hellemont, 1998; Nelson & Cox, 2008).

Under low oxygen conditions, organisms shift from aerobic to anaerobic metabolism of glucose, allowing for the continuation of energy production under hypoxic to anoxic conditions (Nelson & Cox, 2008). However, this reliance on anaerobic respiration is accompanied by a significant decrease in net energy production from 38 moles to 2 moles of ATP per mole of glucose (Nelson & Cox, 2008). This renders the production of lactic acid through the metabolism of pyruvate essential for maintaining energy stores necessary for high energy consumption events, which involve heavy depletion of dissolved oxygen in tissues due to increased respiration over short periods of time (Nelson & Cox, 2008).

Rather than entering the pyruvate dehydrogenase enzyme complex following glycolysis, pyruvate is instead converted to lactate by the enzyme lactate dehydrogenase (LDH) (equation
While this reaction replenishes stores of NAD$^+$, the required co-factor for catabolism of glucose molecules, it is inefficient in producing sufficient energy needed for prolonged metabolic activity (Nelson & Cox, 2008). This inadequacy can be mitigated by reduced metabolic activity (decreased expenditure of energy) and replenishment of dissolved oxygen concentrations in the tissues. Marine organisms, specifically marine invertebrates, have been shown to survive periods of environmental hypoxia (dissolved oxygen ($\text{DO}_2 \leq 2.0 \text{ mg/mL}$) for 5 or more days (Schöttler, 1982). However, this reduced capacity for ATP production puts marine invertebrates at a greater disadvantage in energy production if they must rely upon anaerobic respiration for longer periods of time (Livingstone, 1991). Furthermore, in many marine invertebrates, LDH and lactate production is not the only anaerobic metabolic pathway exploited under oxygen debt (Schöttler, 1982; Livingstone, 1991). Thus, characterization of other enzyme pathways is important to understanding the response of these organisms to oxygen absence.

Many marine invertebrates, such as crustaceans and echinoderms, have been found to rely upon LDH and lactate production as a means of maintaining metabolism during oxygen stress (de Zwaan & Putzer, 1985). However, the activities of opine dehydrogenases (OpDH) have also been discovered in many marine invertebrate tissues (Plaxton & Storey, 1982; de Zwaan & Putzer, 1985; Livingstone, 1991; Sato et al., 1993; Lee, Lee & Pan, 2011). This enzyme suite, consisting of enzymes such as, octopine dehydrogenase (ODH), alanopine dehydrogenase (ADH), and strombine dehydrogenase (SDH) (equations 2, 3, and 4), has been characterized in a wide variety of organisms and has been found in most cases, to be the favored in anaerobic respiration over LDH (Livingstone, 1991; Sato et al., 1993). In many middle and lower marine invertebrates, such as molluscs, polychaetes, cnidarians and poriferans, OpDH activities have been found to be significantly higher than that of LDH (Sato et al., 1993). Yet, the
activity of OpDH has not been shown to be as prevalent in the metabolic pathways of higher
marine invertebrates (Sato et al., 1993). Although the mechanisms by which these opine
pathways are favored over one another remain limited, environmental conditions and anatomical
and behavioral characteristics appear to influence the presence and use of these enzymes (Fields,
1983; Livingstone, 1991; Sato et al., 1993).

Many simple marine invertebrates are comprised of simple tissue and organ construction
and tend to be less mobile (Fields, 1983). Conversely, higher marine invertebrates, which rely
mainly upon LDH in anaerobic metabolism, usually have greater mobility and more evolved
organ systems (Fields, 1983). Due to their lack of mobility, many of the lower marine
invertebrates cannot escape environmental perturbations such as hypoxic environments and
hence evolve various coping mechanisms (Vaquer-Sunyer & Duarte, 2008). Many marine
Annelids have been found to be exceptionally well adapted to living in anoxic environments and
are able to survive under prolonged periods of anaerobic respiration (Schöttler, 1982). However,
many marine organisms, such as various crustaceans and cnidarians, are not well suited for living
under long-term oxygen deprivation (Schöttler, 1982; Vaquer-Sunyer & Duarte, 2008).

Information regarding the activity and presence of enzymes that characterize cellular
anaerobic respiration is limited for cnidarians. Though studies have been conducted analyzing
the presence and production of LDH and OpDHs in the sea anemone Diadumene leucolena, little
to no other published data exists describing the metabolic responses to oxygen deprivation in
corals (Ellington, 1977, 1979). Due to their distribution and lack of motility, corals are
vulnerable to hypoxia-inducing circumstances including algal overgrowth, eutrophication and
sedimentation (Martinez, Smith & Richmond, 2012). Studies have been conducted documenting
the negative impacts of oxygen deprivation on coral health (Stimson, Larned & Conklin, 2001;
Smith et al., 2006; Fabricius, 2011); however, these studies relied upon proxies analyzing long-term effects and percent coral mortality. There is a need to understand coral health on a molecular level in order to monitor changes in health to reveal negative impacts prior to mortality. In this study, we sought to determine the activity of enzymes associated with anaerobic respiration, ADH, SDH, ODH, and LDH, under prolonged oxygen deprivation, such that they may serve as proxies for characterizing hypoxic stress in coral. *M. capitata* was chosen as the model for this study due to its wide-spread distribution and observations of invasive algal overgrowth and oxygen deprivation of *M. capitata* in Kaneohe Bay, Oahu, Hawaii.

**Materials and Methods**

Collection

*Montipora capitata* nubbins were cultivated in open flow-through seawater tanks at the Kewalo Marine Laboratory under Department of Land and Natural Resources – Division of Aquatic Resources coral collection permits (Oahu, HI, USA). The Kewalo seawater system is fed by unfiltered seawater from an intake pipe 300 m offshore in 10 m deep water. Large coral colonies were fragmented into nubbins approximately 20 cm$^3$ in size (n = 157) and attached to a substrate using Gorilla Glue (Cincinnati, OH, USA). These mounted nubbins were then suspended vertically in tanks for two weeks prior to any sampling in order to allow recuperation from stress resulting from manipulation. Nubbins were inspected and cleaned biweekly of sediment and algae.

Hypoxic Exposure

Following recovery, *M. capitata* nubbins were cut from their substrate and randomly assigned to a reference (n$_{tank}$ = 5), exposure control (n$_{control}$ = 30), or treatment (n$_{treatment}$ = 30)
groups. Reference nubbins were left on their substrate to account for stress resulting from the exposure setup. All other nubbins were placed in 250 mL beakers with 200 mL of seawater. Treatment beakers were bubbled with nitrogen gas (GasPro, Oahu, HI, USA) to remove oxygen from the seawater, while control beakers were bubbled with air using Tetra Whisper 20 Gallon air bubblers (Blacksburg, VA, USA). Gas bubbling was performed continuously during exposures. Hanna (HI 9828; Woonsocket, RI, USA) Multiparameter probes and YSI (6820-C-M; Yellow Springs, OH, USA) instruments were used to measure dissolved oxygen (ppm, % saturation, and mg/L), pH, and temperature at the start and finish of 12-hour bubbling periods. After 12 hours of bubbling, beakers were returned to the open flow through tank and oxygen levels restored in order to mimic field-observed cycles of oxygen deprivation. This cycle of bubbling and ‘recuperation’ persisted for a maximum of 5 days and was conducted to simulate smothering conditions observed by Martinez, Smith, and Richmond (2012).

Beakers were sealed with Parafilm (Neenah, WI, USA) and fume hood sashes were lowered and covered with foil to block all light from entering during the treatments to inhibit the production of oxygen through photosynthesis by the symbiotic zooxanthellae. To further mimic field conditions, treatments began at sunset and concluded before sunrise. Following 3 and 6-hour and 1, 3, and 5-day bubbling cycles, coral nubbins were collected from their respective beakers \((n_{\text{treatment}}=5 \text{ and } n_{\text{control}}=5 \text{ per time period})\), gently and rapidly blotted dry, placed in 50 mL Falcon tubes, flash frozen with liquid nitrogen, and immediately transferred to a VWR 5656 -80°C freezer (Radnor, PA, USA).

Tissue Preparation of S9 Subcellular Fractions
Liquid nitrogen was used to cool ceramic mortars, pestles, and pliers before crushing of coral nubbins. Pliers were used to fragment corals into smaller pieces before grinding them into a fine powder with a chilled mortar and pestle. Approximately 500 mg of crushed coral were transferred to 2 mL microcentrifuge tubes. Samples were homogenized on ice with 700 μL of homogenizing buffer (49,500 μL 0.01 M Tris-HCl pH 8.0, 500 μL dimethyl sulfoxide, 8.7 mg phenylmethylsulfonyl fluoride) for 60 seconds using an Ultra-Turrax homogenizer.

Homogenates were centrifuged at 2,000 rcf for 5 minutes at 4°C using an Eppendorf Microcentrifuge 5415D (Hauppauge, NY, USA) to pellet coral skeletal fragments and zooxanthellae. The supernatant was transferred to new microcentrifuge tubes and spun at 10,000 rcf for 20 minutes at 4°C. The resulting supernatant, representing the S9 post-mitochondrial fraction of coral protein, was then aliquoted and stored frozen at -80°C to preserve enzyme integrity and stop biological activity until analyses were performed.

**Protein Concentration Quantification**

Protein concentration for all samples was determined using the bicinchoninic acid (BCA) assay method. Clear 96-well microplates (Greiner 65580x; Grenier Bio-One, Monroe, NC, USA) were loaded with known dilutions of bovine serum albumin (BSA) (1.0, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 mg/mL; 20 uL per well) as standards, and unknown coral samples diluted 1:5 in milliQ water (20 uL S9 fraction with 80 uL water; 20 uL per well) in triplicate wells. The developing reagent (20,000 μL BCA solution, 400 μL Cu²⁺SO₄) was made fresh prior to adding 200 uL per well. Plates were thoroughly mixed and then incubated at 37°C for 30 minutes inside a SpectraMax M5 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Samples were then read using SoftMax Pro 5.4 software (Molecular Devices, Sunnyvale, CA, USA) at λ=562 nm and protein concentrations calculated from the generated standard curve.
these assay purposes, samples were re-extracted if protein concentration fell below the minimum threshold of 1 mg/mL.

**Chemicals**

The following chemicals (analytical grade or higher) were purchased Sigma-Aldrich Chemical (St. Louis, MO, USA): L-alanine, L-arginine, glycine, and NADH. All other chemicals or reagents were purchased from VWR Scientific (Batavia, IL, USA) and were analytical grade or higher.

**Enzyme Kinetic Assays**

Activity of LDH, ADH, SDH, and ODH was determined by monitoring the loss of absorbance due to the oxidation of NADH to NAD+ as previously described by Fields et al. (1980), Fields and Hochachka (1981), Lee, Lee, and Pan (2011), and Zewe and Fromm (1965a,b). On ice, plates were loaded with sodium pyruvate, Tris-HCl buffer, L-alanine/L-arginine/glycine, and S9 protein fraction and then incubated for 3 minutes at 28°C (LDH assays employed sodium pyruvate and substituted L-alanine/L-arginine/glycine volumes with buffer). The reaction was then initiated with the addition of NADH. The plate was then mixed and read at $\lambda = 340$ nm for 30 minutes at 28°C (30 second read intervals)(Horecker & Kornberg, 1948).

Activity units were defined as nanomoles of NADH oxidized per minute per mg protein (nmols/min/mg prot). The assay conditions of opine dehydrogenases were 100 mM sodium pyruvate, 100 mM Tris-HCl (pH 7.2), 1 mM NADH, 200 mM L-alanine/glycine/L-arginine, and 10 µg S9 protein fraction, in a final reaction volume of 100 µL. LDH activity was determined in the same reaction mixture with the omission of amino acids and a corresponding increased volume of buffer. Assay controls were carried out by the substitution of substrates with buffer to
account for the endogenous levels of NADH degradation within the coral sample. Background activity levels were subtracted from observed sample activities.

Results

Visual Observations

Visual observations of tank controls found no observable physical changes over time, as tissue remained dark orange-brown in color. Polyps were regularly extended and appeared large and healthy with no mesenterial filament extrusion. Likewise, air-bubbled exposure controls showed no physical changes, including response to bubbling, over the duration of testing. Polyps remained brown in color and were fully extended. No tissue loss was observed in any of the tank or exposure controls. However, hypoxia treatment corals displayed many physical changes, worsening with the duration of exposures.

No physical signs of stress were seen in corals immediately following the first night (12 hours) of nitrogen bubbling. However, at the start of the second night of bubbling, coral samples displayed slight bleaching and color loss. Treatment polyps remained contracted during the duration of exposures. Continued treatment resulted in increased bleaching and tissue loss on coral nubbins, but no coral nubbin deaths. By the fifth cycle of testing, coral nubbins under hypoxic conditions were almost completely bleached or had lost all tissue, fitting observations of field-observed corals (Martinez, Smith & Richmond, 2012). Remaining polyps were dark brown and appeared shriveled. Due to the low amount of remaining tissue, day 5 samples were unsuitable for processing.

To note, pH and temperature did not significantly vary between treatment, control, and reference tank conditions.
**Statistical Analysis**

Statistical analyses were performed using the program Prism 5 (GraphPad Software, La Jolla, CA, USA). Through a two-way analysis-of-variance (ANOVA) using Bonferroni post-hoc tests, the differences in enzyme activity over the 4 treatment times (3 and 6 hours and 1 and 3 days) and 3 treatment types (treatment, air-bubbled controls, and tank controls) were determined. This test investigated the significance of differences between treatment times, treatment types, and differences in the interaction between treatment times and types, within each enzyme activity assay. This interaction between time and type describes whether time and type had a significant combinatory effect on changes in enzyme activity.

**Enzyme Activity**

Enzyme kinetic assays detected little to no LDH or ODH activity in coral samples. Although several samples across treatment type and time displayed small positive values for enzyme activity, these were not significant increases in activity over control values (p>0.05, CI=95%).

Conversely, significant increases in SDH activity were found to occur within treatment corals, with samples from both 1 and 3 days expressing significantly higher activity versus control corals (figure 2, p<0.05 and p<0.001, CI=95%, respectively). Further, when investigating the impact of oxygen deprivation, exposure time, and the interaction of both factors on the metabolic activity of these samples, it was found that SDH activity varied significantly with respect to treatment type (F=65.57, p<0.0001), treatment duration (F=4.944, p= 0.0082), and interaction between treatment duration and type (F=3.039, p=0.0485). Importantly, control and tank samples displayed no significant differences over 3 and 6 hour and 1 and 3 day times sets.
(p>0.05, CI=95%), indicating that bubbling within small chambers had no effect on target enzyme activity.

Alanopine dehydrogenase (ADH) activity reflected similar findings as in SDH activity assays, where activity varied significantly with treatment type (F=26.30, p= 0.0009) and time (F=3.162, p= 0.0430). However, the combinatory effect of oxygen deprivation over time had no significant effect on ADH activity (F=2.545, p= 0.0799). As in SDH activity results, ADH activity did not significantly differ between air-bubbled controls and tank references (p>0.05, CI=95%). Yet, as seen in figure 3, differences in ADH activity significantly differed between both control groups and treatment corals after 3 days of treatment (p<0.01, CI=95%). Overlap with control activity values resulted in no significant variation in ADH activity during 1 and 3 hour and 1 day exposure periods (p>0.05, CI=95%).

Discussion

Increased activity of ADH and SDH enzymes in *M. capitata* exposed to prolonged durations of hypoxic conditions (>12 hours) mimic other recorded increases in gastropod species (Dando, 1981; Fields & Hochachka, 1981; Plaxton & Storey, 1982; Eberlee, Storey & Storey, 1983; Lee, Lee & Pan, 2011), as well as the cnidarian *Diadumene leucolena* (Ellington, 1979). Though sequential significant increases in ADH and SDH activity were not found with increasing time intervals, treatment values in figures 1 and 2 demonstrate general trends of increasing activity with increasing duration of treatment. These results suggest that under prolonged intervals of anoxia, *M. capitata* becomes increasingly dependent on the activity of ADH and SDH to mediate anaerobic metabolism.
While ODH and LDH activity has been monitored in the cnidarian *Bunodosoma cavernata* and various coelenterates and poriferans, their activity was barely detectable for *M. capitata* in this study (Livingstone, 1991; Sato et al., 1993). Although it is well documented that ODH and LDH are important in the processing of glycolytic products under anaerobic conditions (Sato et al., 1993; Lee, Lee & Pan, 2011), our lack of evidence does not mean that ODH and LDH do not exist in coral. The longest hypoxic treatment employed in this study was 5 days. However, it has been found that under chronic exposure to multiple weeks of hypoxia, other pathways for anaerobic respiration can be activated, becoming a significant pathway for metabolic turnover (de Zwaan & Putzer, 1985). Additional studies propose that ADH and SDH act as the primary and anaerobic metabolic responses pathways during hypoxia, while ODH is important for chronic hypoxia exposure (de Zwaan & Putzer, 1985).

The significant positive relationship between exposure length and enzyme activity of ADH and SDH in *M. capitata* supports the implementation of these enzymes as biomarkers of exposure for oxidative stress. However, further research is necessary to provide additional evidence to confirm the presence of these enzymes through Western Immunoblotting or other molecular techniques. Furthermore, future studies monitoring the effect of long-term oxygen deprivation are required to fully understand variation between acute and chronic coral metabolic responses. Additionally, investigation of endogenous substrate and co-factor concentrations for these target enzymes within tissues through further colorimetric assays can help elucidate changes in the overall pathways and provide a better understanding of homeostatic health and subtler molecular alterations resulting from hypoxic exposure.

**Conclusions**
Our findings indicate that, *M. capitata* increasingly relies upon ADH and SDH for anaerobic catabolism under low-oxygen conditions. This demonstrates that prolonged or repeated exposure to anoxia drives corals to rely upon less efficient, anaerobic means of energy production. Under extended periods of low-oxygen exposure this may lead to energy deficits, which could result in increased susceptibility of corals to acute stress events, further leading to bleaching and tissue loss. Future efforts will focus on the application of these methods toward the analysis of oxygen deprivation on corals of different genera, elucidating possible variations in enzymatic stress response. These findings will aid in customizing the targeting of biomarkers for stress evaluation and may identify inter-species variation in preferred metabolic pathways. However, these findings currently point to ADH and SDH activity assays as suitable biomarkers for rapid analyses of oxidative stress in corals with potential adaptation for future analyses of environmental impacts on corals and coral reefs.

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Figure 1. Typical cellular respiration pathway in eukaryotic cells.

Figure 2. Strombine dehydrogenase (SDH) activity (nmols/min/mg prot) versus treatment time and type (3, 6 hours and 1, 3 days). Bars represent mean ± SD. Treatments with significantly higher SDH activity than controls are denoted in treatments marked with asterisks (*= p<0.05, ***= p<0.001).
Figure 3. Alanopine dehydrogenase (ADH) activity (nmols/min/mg prot) versus treatment time and type (3, 6 hours and 1, 3 days). Bars represent mean ± SD. The 3 day treatment with significantly higher ADH activity than controls is denoted by an asterisk (** = p<0.01).

Equation 1,2,3,4. Conversion of pyruvate to lactate by lactate dehydrogenase (LDH, 1), pyruvate and alanine to alanopine by alanopine dehydrogenase (ADH, 2), pyruvate and arginine to octopine by octopine dehydrogenase (ODH, 3), and pyruvate and glycine to strombine by strombine dehydrogenase (SDH, 4).