UNIVERSITY OF CALIFORNIA SAN DIEGO

Effect of reef location and light levels on the energy metabolism of two Caribbean coral species

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

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Professor Ron Burton
Professor Jennifer Smith

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The Thesis of Samantha Noël is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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2022

DEDICATION

To my mother, Christina Noël, who fostered my creativity and individuality, and to my sister, Jordyn Noël, to whom I owe my compassionate and selfless nature.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
Acetyl-CoA	Acetyl coenzyme-A
NAD^+/NADH	Nicotinamide adenine dinucleotide
LDH	Lactate dehydrogenase
OpDH	Opine dehydrogenase
SDH	Strombine dehydrogenase
ADH	Alanopine dehydrogenase
TCA	Tricarboxylic acid cycle
CS	Citrate synthase
MDH	Malate dehydrogenase
$FADH_2$	Flavin adenine dinucleotide
mMDH	Mitochondrial malate dehydrogenase
cMDH	Cytoplasmic malate dehydrogenase
ETC	Electron transport chain
DOC	Dissolved organic carbon
PAR	Photosynthetically active radaition
CHB	Coral homogenization bbuffer
DTNB	5,5-dithio-bis(2-nitrobenzoic acid)
BSA	Bovine serum albumin

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ABSTRACT OF THE THESIS

Effect of reef location and light levels on the energy metabolism of two Caribbean coral species

by

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Master of Science in Marine Biology

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Professor Martin Tresguerres, Chair

Anthropogenic climate change is forecasted to increase in both severity and frequency, making it important now more than ever to be able to understand how coral will fare in the changing environment. Yet a prevalent gap in knowledge on coral physiology prevents accurate determination of coral health and energetics on the reef. The goal of my thesis was to investigate coral energy metabolism in two Caribbean coral species, *Porites astreoides* and *Orbicella franksi.* To this end, I analyzed coral samples that had been collected during expedition to the Smithsonian Tropical Research Institute (STRI; Bocas del Toro, Panama) in 2015 and 2016. One set of coral samples had been collected from 3 m and 8 m depth from the Punta Caracol reef and exposed to comparable light intensities in STRI's experimental

aquarium for 10 days. A second set of samples had been collected from Punta Caracol and Eric's Reef at ~6.0 m depth after a one-year reciprocal transplant experiment. Punta Caracol is nestled inside the turbid Bahia del Almirante lagoon – with large influxes of terrestrial runoff – whereas Eric's Reef is facing the open ocean – experiencing higher seawater mixing. At Scripps Institution of Oceanography, I measured the enzymatic activity of malate dehydrogenase (MDH), citrate synthase (CS), lactate dehydrogenase (LDH), strombine dehydrogenase (SDH), and alanopine dehydrogenase (ADH) as proxies for maximum aerobic and fermentative metabolic capacity in coral. My results suggest differences in the metabolic regimes between coral collected from different depths, generally favoring higher fermentative activity in coral from 8 m. Short-term exposure to alternative light intensities did not induce significant effects to enzyme activity, suggesting the lack of an adaptive response, or that such was not needed. Corals from the transplant experiments demonstrated differences in fermentative pathway preferences whereby enzyme activity was primarily dependent on the destination reef; however, the responses were species-specific and, in many cases, opposite each other. These diverse responses after long-term acclimation to reefs with different environmental characteristics exemplifies the importance of characterizing coral energy metabolic regulation as it pertains to better assessing coral vulnerability and resilience in a changing environment.

INTRODUCTION

Over the past century, coral reef biodiversity and health have been on the decline due to increasingly prevalent anthropogenic stressors to local, regional, and global reef systems[1-4]. Coastal pollution, sea surface warming, and ocean acidification are among the myriad of events endangering the productivity of reef ecosystems[5,6]. In turn, this negatively impacts the benefits that coral reefs can provide to coastal populations[7,8]. Anthropogenic climate change is forecasted to increase in both severity and frequency, making it important now more than ever to be able to predict how coral will fare in the changing environment[9]. To do so, it is essential to continue to improve our foundational understanding of basic coral biology, such as the mechanisms utilized for maintaining energy production in response to environmental variability on the reef, so that more accurate assessments of coral resilience and susceptibility to climate change can be made.

Coral biology

Coral are members of the phylum Cnidaria, in the Anthozoan class[10]. Like all cnidarians, coral have two distinct tissue layers, the ectoderm and endoderm. Following the larval stage, subdivision of the ectoderm and endoderm result in oral and aboral tissue layers such that oral ectodermal cells form the epidermis tissue that remains in contact with the seawater, while aboral ectodermal cells form the calicoblastic tissue that builds the skeleton underneath[11,12]. The endodermis turns into the oral and aboral gastrodermis tissue surrounding the internal gastrovascular cavity. The gastrodermis exchanges nutrients and metabolites with the fluid in the cavity and helps digest food obtained *via* the polyp's mouth. Therefore, polyp size directly determines the feeding strategies of different coral species[13,14]. In addition to prey, dinoflagellate

algae reach the gastrovascular cavity and can be incorporated into some of the gastrodermal cells, resulting in the photosymbiotic relationship that is essential for coral.

Speciation of the Cnidarian phylum over the last 500 million years has culminated in a diverse spectrum of coral species[15]. Reef-building stony coral in the Scleractinian order form the complex calcium carbonate reef systems found in oligotrophic tropical areas. Molecular analysis of 16S mitochondrial RNA suggests that Scleractinian coral are grouped into two monophyletic clades, robusta and complexa[16,17]. There are many similarities but also important differences between the two clades[18,19], including varying calcium carbonate biomineralization characteristics [20,21], and polyp asexual growth modes[12,22]. Additional diversification within the families of each clade is extensive and likely account for the evolutionary success of reef-forming corals[23,24]. Widespread differences in species-specific morphology is highly dependent on various environmental factors, including hydrodynamic variability in water motion[25,26], local temperature ranges, and solar radiation[27], that dictate coral energy production, growth, and distribution on the reef[28].

Coral symbiosis

Homeostasis of the endosymbiosis with dinoflagellate algae of the *Symbiodiniaceae* family is key to coral reefs[29,30]. Following acquisition through the polyp mouth, the algae are phagocytosed into gastrodermal cells where they remain surrounded by a host-derived symbiosomal membrane[31]. Photosynthates, including amino acids and algal-derived sugars, are translocated to the coral host for energy production, growth, and reproduction[32-34]. To maintain algal production of photosynthates, the coral provides their endosymbionts with protection and required substrates for photosynthesis, such as carbon dioxide and nitrogen compounds[35-37]. In

addition, photosynthetic oxygen produced by the algal symbiont diffuses out of the symbiosome, potentially oxygenating the host tissue to support aerobic metabolism, and continuing out of the organism into the environment[38,39].

Aerobic and fermentative metabolism

The production of energy is fundamental for life. The principal molecule for storing and transferring energy in the cell is adenosine triphosphate (ATP), which can be produced using three primary pathways: phosphagen mobilization, fermentation, and aerobic respiration[40,41]. Phosphagens serve as a type of energy reserve that can donate a high energy phosphate to adenosine diphosphate (ADP) for rapid ATP formation[42]. This does not require oxygen and is typically used for processes that require quick burst of energy such as muscle contractions[43]. The other two pathways start with core glycolysis in the cytoplasm and involves a series of reduction-oxidation (RedOx) reactions catalyzed by various enzymes. Core glycolysis does not require oxygen and involves both an energy investment and energy production phase[44]. Initially, 2 ATPs must be invested to convert one sugar molecule into two glyceraldehyde phosphates. Oxidation of the resulting two 3-carbon sugars into two pyruvate molecules produces 4 ATPs, for a net production of two ATPs per glucose. There are three possible fates of the resulting pyruvates: oxidation into endpoints via fermentation, transport into the mitochondria for oxidation into acetyl coenzyme A (acetyl-CoA) for use in aerobic respiration, and oxidation to oxaloacetate to maintain $NADH/NAD^+$ ratios between the cytoplasm and mitochondria via the malate-aspartate shuttle[45] (Fig. 1).

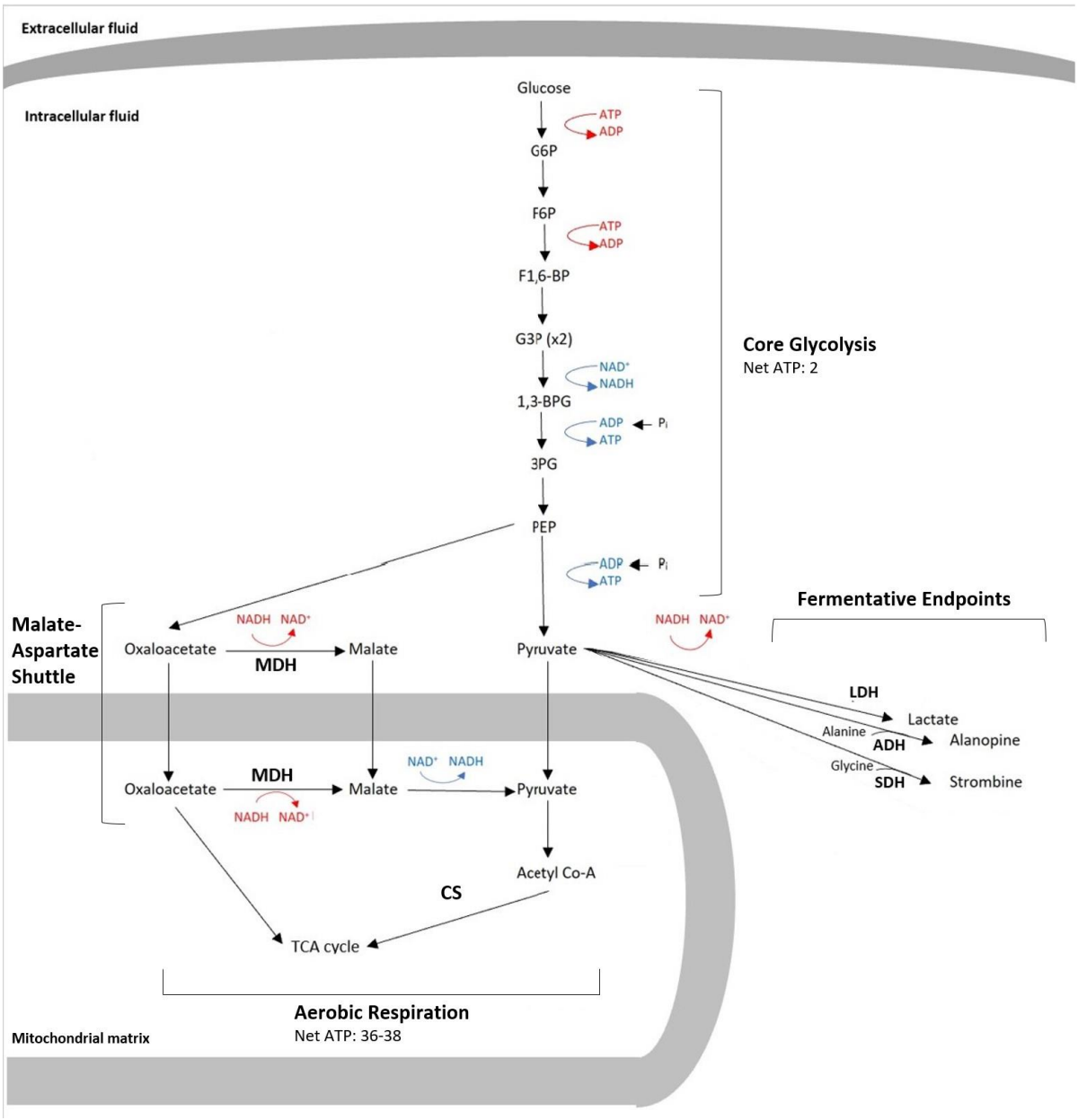

Figure 1: Summary of energy metabolic reactions studied in this thesis Core glycolysis utilizes a series of redox reactions to catabolize sugar molecules (i.e. glucose), resulting in production of two pyruvates and 2 ATP molecules. Pyruvate can undergo two fates: (1) pyruvate can be reduced into different fermentative endpoints in reactions that also regenerate NAD^+ that can be used to sustain core glycolysis during anaerobic conditions via alternative endpoints and oxaloacetate production or (2) can be shuttled into the mitochondria and oxidized to acetyl CoA for use in the TCA cycle. The terminal dehydrogenase enzymes lactate dehydrogenase (LDH), alanopine dehydrogenase (ADH), and strombine dehydrogenase (SDH) convert pyruvate into lactate, alanopine, and strombine in fermentative endpoint production. Malate dehydrogenase (MDH) catalyzes conversion of oxaloacetate and malate in the cytoplasm and mitochondria using the malate-aspartate shuttle. Citrate synthase (CS) converts acetyl CoA to citrate and is the rate-limiting enzyme in the TCA cycle aspect of aerobic respiration. This diagram is a simplified version of these pathways and is modified from Hochachka and Somero 2002[46].

Oxidation of pyruvate into fermentative endpoints continues to be oxygen-independent, and although it yields a lower net ATP production compared to that of aerobic respiration, it serves to regenerate the NAD^+ that is essential for core glycolysis, which can then continue to produce ATP during low-oxygen hypoxic or anoxic conditions[47]. The oxidation of reduced nicotinamide adenine dinucleotide (NADH) into NAD^+ is coupled to the reduction of pyruvate into endpoints utilizing terminal dehydrogenases[48]. For example, the most common terminal dehydrogenase in mammals, lactate dehydrogenase (LDH), catalyzes the reduction of pyruvate into lactate and regenerates NAD^+ during oxygen limitation. However, other animals and especially invertebrates, utilize various opine dehydrogenases (OpDHs) as the terminal dehydrogenase, which instead produce various opines as the end products (as well as NAD^+)[49-52]. Opine production differs from that of lactate in that it involves the condensation of pyruvate with an amino acid[53]. Compared to that of LDH, the condensation of amino acids catalyzed by OpDHs helps maintain a lower NADH/NAD^+ ratio in the cytoplasm which favors higher NADH oxidation rates to bolster NADH availability for RedOx reactions in various other cellular pathways[54]. There are a several OpDHs, each requiring a different amino acid. For example, strombine dehydrogenase (SDH) utilizes glycine and alanopine dehydrogenase (ADH) utilizes alanine[55,56]. OpDHs are generally found to be substrate specific[57-59], however, amino acid non-specific dehydrogenases have been observed and indicate the potential to produce various opine end-products depending on substrate availability[60,61]. The factors that determine which OpDH is utilized as the terminal dehydrogenase are not completely understood[50], however amino acid cellular pools are certainly an important component[51].

A common misconception about fermentation is that it can only take place during times of oxygen limitation and that it is not sustainable for extended periods of time because it does not produce enough ATP. However, under certain circumstances, fermentation can also occur during aerobic conditions and even be preferentially used over aerobic respiration. This type of "aerobic glycolysis", first discovered in cancer cells by Otto Warburg[62], is termed the Warburg effect and is known to be advantageous for rapid cell proliferation when glucose is present in excess amounts[63-66]. The underlying reason is that while fermentative ATP production is less efficient than aerobic respiration, it takes place at much faster rates, and thus cells that have a sufficient glucose supply can obtain more energy more rapidly. Aerobic glycolysis also requires the regeneration of NAD^+ in the terminal fermentative step, which in mammals is generally catalyzed by LDH[63]. Very recently, this phenomenon was also observed in the coral *A. yongei* kept in experimental aquaria[67]. However, instead of LDH, the corals seemed to use SDH in the terminal fermentative step. Indeed, these corals produce more strombine during the day than at night, even though oxygen levels were much higher during the day and approached hypoxia at night. During the day, photosynthesis by the algae may have produced an excess of sugars available to the coral and thus maintenance of high SDH activity during this period would have allowed to coral to produce more ATP more rapidly via fermentation. This study suggests that aerobic glycolysis is tightly coupled to sugar availability in coral and can be accomplished via OpDH activity.

Production of ATP via highly efficient aerobic respiration can only occur when there is enough oxygen available in the cell. Pyruvate from core glycolysis is transported into the mitochondria and converted to Acetyl-CoA, a rate-limiting molecule in the tricarboxylic acid (TCA) cycle, that is condensed with oxaloacetate in a reaction catalyzed by the enzyme citrate

synthase (CS)[68]. Since CS is a reaction-limiting enzyme, CS activity serves as a proxy for aerobic capacity. Following a series of enzymatic reactions, oxaloacetate is ultimately regenerated following the oxidation of malate by the enzyme malate dehydrogenase (MDH)[69,70]. Only 1 net ATP is produced during this cycle, however, the electrons that are stripped away from the TCA cycle intermediate molecules are shuttled into NADH and flavine adenine dinucleotide ($FADH_{2)}$, which carry them to the oxidative phosphorylation and chemiosmosis steps of aerobic respiration[71,72]. Additional NADH can be shunted into the mitochondria by the coordinated effort of mitochondrial MDH (mMDH) and cytoplasmic MDH (cMDH) which make up the malate-aspartate shuttle[73,74]. Because MDH is utilized in aspects of both anaerobic metabolism in the cytoplasm and aerobic metabolism in the mitochondria, it can serve as a proxy for overall metabolic capacity. Reduction of pyruvate-derived oxaloacetate by cMDH and oxidation of malate in the TCA cycle by mMDH can further regulate the cytoplasmic redox-state to favor aerobic ATP production[75]. The bulk of this ATP production occurs during oxidative phosphorylation using a series of membrane-bound transporting protein complexes that make up the electron transport chain (ETC). Via shuttling of electrons donated by NADH and $FADH_2$ out of the mitochondrial matrix, the redox reactions in the ETC create a proton concentration gradient that powers the synthesis of ATP via the membrane-bound ATP-synthase enzyme[76]. Overall, aerobic respiration produces 36-38 ATPs from each molecule of glucose.

Coral energy metabolism

Both fermentative and aerobic ATP production rely on substrates availability, especially glucose. By hosting dense populations of endosymbiotic algae, coral benefit from a large flux of glucose that is produced during algal photosynthesis and translocated to the coral host cells and

the rest of the colony. Given its absolute dependence on light, photosynthetic production of glucose, amino acids, and oxygen can only happen during the day[77]. During this period, algal oxygen production can exceed oxygen consumption by coral respiration, resulting in hyperoxia (>6.8 mg O_2 L^{-1}) in coral tissues and the surrounding water. Photosynthetic oxygen production is not possible at night and therefore net cellular respiration can deplete dissolved oxygen in and around coral tissues to potentially cause hypoxia (<2.8mg O_2 L^{-1}) or even anoxia[78]. In addition to diel changes in light, environmental factors such as depth, water turbidity, and shading due to sedimentation can influence light availability on the reef[79-82], with important, yet not completely characterized, effects on photosynthesis and thus aerobic and fermentative pathways.

A decrease in oxygen availability during periods of light limitation will ultimately impact the corals' ability to maintain aerobic respiration. Therefore, reliance on ATP production from core glycolysis and fermentative endpoints can increase. Coral have been found to utilize LDH and various OpDHs to accomplish anaerobic ATP production[49]. Exposure *Montipora capitata* to hypoxia via nitrogen bubbling induced increases in SDH activity after one day and in ADH activity after three days, indicating potential shifts in fermentation pathways related to the duration of oxygen limitation[83]. On the other hand, *A. yongei* analyzed through a diel cycle demonstrated robust SDH activity during both day and night, and no ADH activity was detected. But despite generally constant SDH activity, peaks in both strombine and alanopine production occurred when oxygen was depleted with the onset of dusk, and again at dawn when photosynthetic production of oxygen and glucose was restored. This indicates that, in addition to enzyme amount, substrate availability can have a huge effect on fermentative activity. Moreover, the relatively constant activities of CS and MDH also remained constant throughout the diel cycle, despite presumed changes in aerobic and anaerobic respiration rates resulting from

changes in photosynthetic oxygen and glucose production. Thus, total enzymatic activity is a useful proxy for maximum metabolic energy production capacity in coral, rather than instantaneous activity. This can be useful for studies that attempt to characterize energy metabolism in corals collected from the field, as total enzymatic activity is less likely to be affected by light at the time of sampling (e.g., due to clouding, day/night, etc.). Characterization of coral fermentation up to this point has created a key framework to understanding coral energy production under controlled conditions, which now can be used to assess metabolic regulation in variable reef environments.

Characterizing coral energy metabolism in the reef

To build a more comprehensive understanding of coral energy metabolism in their natural reef environments, a combined field and laboratory study was conducted led by Prof. Tresguerres and Dr. Kline, in collaboration with Prof. Andreas Andersson, Dr. Lauren Linsmayer, and Dr. Trevor Hamilton. The reefs in Bocas del Toro (Panama) provide a unique setting to explore coral metabolism in the wild due to the presence of reefs with diverse environmental conditions and proximity to the Smithsonian Tropical Research Institute's (STRI) Bocas del Toro Research Station. Punta Caracol reef is in the Bahia Almirante lagoon on the West side of Isla Colon and is connected to the open ocean by two waterways, Boca de Drago and Canal de Bocas del Toro. In contrast, Eric's Reef is located facing the open ocean, East of Isla Bastimentos (Fig. 2). However, these two reef sites share many of the same coral species, including *Acropora cervicornis, Porites asteroids,* and *Orbicella franksi,* which were selected to study potential environmental-, species-, and population-specific differences in energy metabolism.

Figure 2: Bocos Del Toro Archipelago, Panama. Experimental reef locations are shown – Punta Caracol in blue and Eric's Reef in pink. The Bahia del Almirante lagoon has two primary waterways, Boca de Drago, and Canal de Bocas del Toro. The Smithsonian Tropical Research Institute (STRI) Bocas del Toro Research Station is denoted by the white star. Image is modified from Google Maps satellite imagery.

Additionally, various environmental conditions were measured around the time of coral sampling, including spot measurements of ammonia, nitrite, nitrate, phosphate, and silicate concentrations [collectively referred to as dissolved inorganic nutrients (DINsC)] and dissolved organic carbon (DOC), and near-continuous measurements of salinity, temperature, pH, and photosynthetically active radiation (PAR) levels. *A priori*, Tresguerres, Kline and Andersson expected higher DINsC and DOC in Punta Caracol due to its proximity to populated and active agricultural coastal areas, as well as higher salinity, temperature, and pH swings due to its more limited connectivity with the open ocean, together with lower PAR levels resulting from higher

turbidity due to the muddy nature of the sediments. Although large differences in ammonia, nitrate, and DOC between the two sites were measured in November/December 2015, these were not significantly different due to high variability in the measurements (Fig. 3).

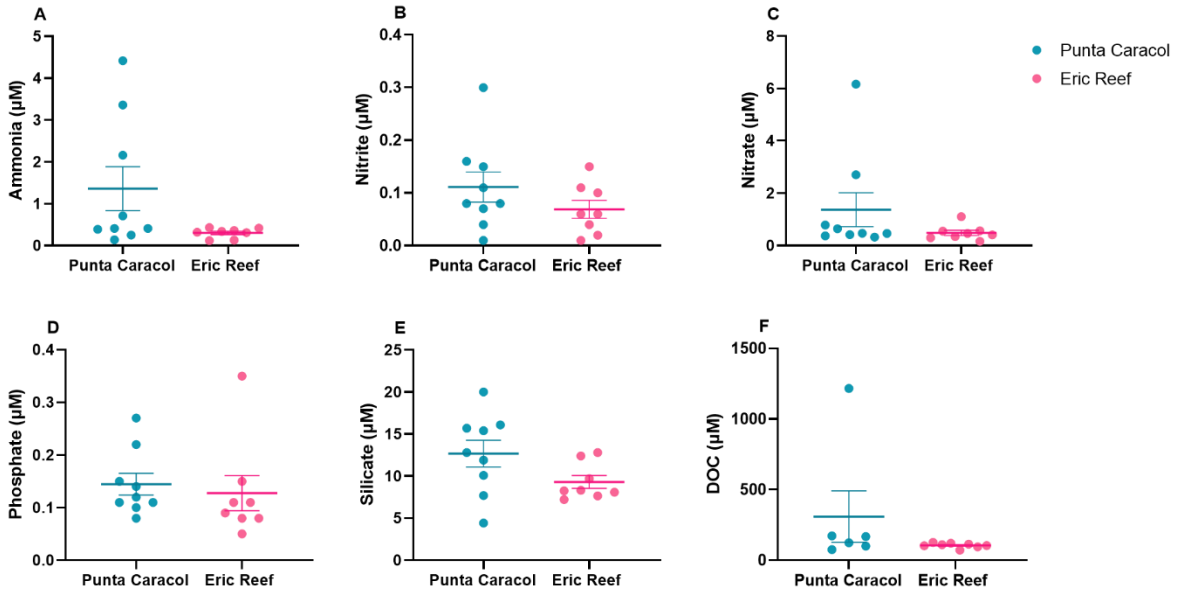

Figure 3: Dissolved nutrients and carbon in Punta Caracol Concentrations of dissolved inorganic (A) ammonia, (B) nitrite (C) nitrate, (D) phosphate, (E) silicate, and (D) organic carbon (DOC) measured over a two-week period in November/December of 2015 in Punta Caracol and Eric's Reef. Data obtained from M. Tresguerres and D. Kline.

On the other hand, average salinity (34.4 ± 0.8 ppt), temperature (~30 ºC), and pH (~7.95 pH units) at 3 m and 8 m depth measured during a ten-day in November 2015 were similar in both reefs, as were the diel ranges of temperature (0.5 ± 0.1 ºC) and pH (0.06 ± 0 .01 pH units[84]. In Punta Caracol, the average maximum daily PAR levels (measured every 30 seconds between 11 am and 1 pm) was 348.4 ± 100.5 µmol m^{-2} sec^{-1} at 3 m and 120.3 ± 93.9 µmol m^{-2} sec^{-1} at 7.8m. Unfortunately, PAR data for Eric's Reef at 3m is not available due to sensor malfunction, but average maximum daily PAR levels at 8.7 m were 94.4 ± 56.6 µmol m^{-2} sec^{-1} (i.e., comparable, and slightly lower than in Punta Caracol at 7.8 m).

Some of the *A. cervicornis* and *P. astreoides* fragments collected in 2015 were analyzed as part of the MS thesis of Cameron Hassibi in the Tresguerres laboratory[85]. That study revealed that both coral species collected in Punta Caracol generally had more robust LDH, SDH, and ADH activities than those collected in Eric's Reef. Moreover, the ratio of cumulative fermentative enzyme activity to CS activity (a proxy for relative capacity for fermentation over aerobic respiration) (Ferm:AR), was several-fold higher in corals from Punta Caracol compared to those from Eric's Reef. Altogether, this indicated higher fermentative capacity in *A. cervicornis* and *P. astreoides* colonies from Punta Caracol. In addition, *P. astreoides* from 3 m at Eric's Reef had higher CS activity and lower Ferm:AR compared to the same species at 8 .0 m in Eric's Reef, and at 3 and 8 m in Punta Caracol, which indicated higher reliance on aerobic respiration. Finally, MDH activity of *A. cervicornis* was higher in Punta Caracol than in Eric's Reef fragments regardless of depth, but MDH activity of *P. astreoides* was higher in Eric's Reef than in Punta Caracol, and especially high in Eric's Reef at 8 m.

While these results suggested important effects of local conditions on coral energy metabolism, identifying the specific underlying factors and environmental- versus population-effects is not possible from field studies alone. Moreover, the environmental measurements failed to identify any clear differences in DINsC, DOC, salinity, temperature, or pH between the two reef sites, and while PAR diminished with depth as expected, there were no clear differences between the two sites, either. Thus, to study the effect of light, *P. astreoides* and *O. franksi* fragments were collected from 3 m and 8 m depth in Punta Caracol and held in an experimental aquarium for nine days (Fig. 4a) under light intensities (Fig. 4b, 4c) that matched light levels at those depths following a factorial design. Experiments with *A. cervicornis* were also started;

however, this species did not perform well in the aquaria and showed clear signs of bleaching and tissue necrosis leading to termination of the experiment after just three days. The *A. cervicornis* fragments were sampled but they were not analyzed.

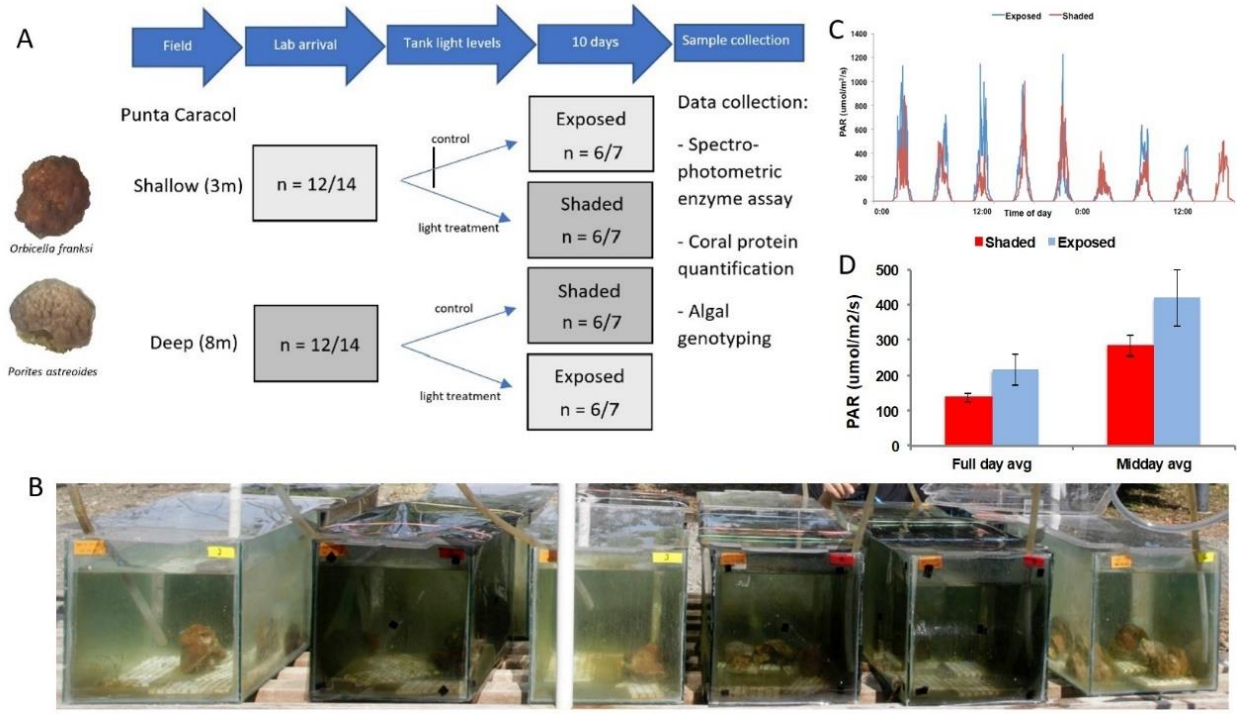

Figure 4: Light level experiment with *Porites astreoides* and *Orbicella franksi*. (A) Summary of experimental design. (B) Fragments collected from Punta Caracol at 3 m and 8 m depth were transported to STRI-Bocas del Toro station and randomly distributed in tanks with high-light ("exposed") and low-light ("shaded") using neutral density filters for nine days. Notice the darker shade of the 2nd, 4th, and 5th tanks (photo credit: M. Tresguerres & D. Kline). (B) PAR levels in the tanks during the experiment. (D) Full-day average and maximum (11am-1 pm) average PAR levels during the experiment. Data obtained from M. Tresguerres and D. Kline.

To attempt to dissect environmental versus population effects, other *P. astreoides* and *O. franksi* fragments were collected from 6 m depth at Punta Caracol and Eric's Reef and collected after a one-year reciprocal transplant experiment between the two locations (Fig. 5). A similar experiment was attempted with *A. cervicornis* at 3 m; unfortunately, all fragments transplanted into Punta Caracol were found dead after the one-year period, and those transplanted into Eric's Reef were not found. However, the frame that held the coral fragments was found ~30 m away

from the original site, suggesting the transplanted corals had been stolen or perhaps destroyed during a storm.

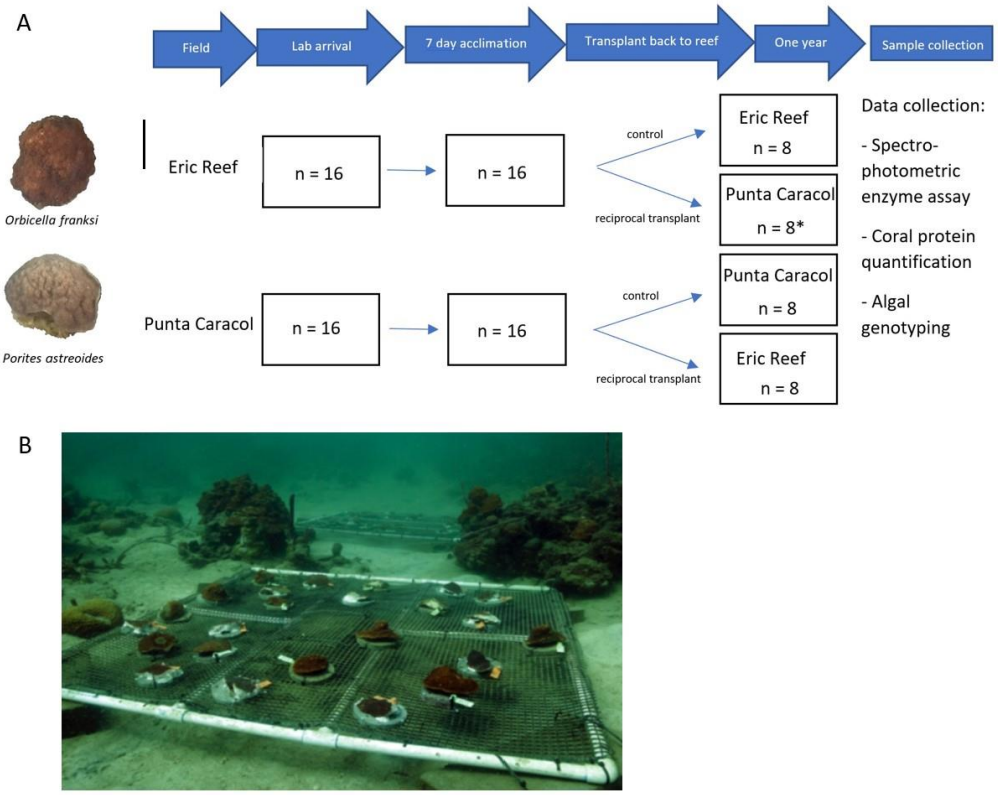

Figure 5: One-year reciprocal transplant experiment with *Porites astreoides* and *Orbicella franksi* from Punta Caracol and Eric's Reef (6 m depth). (A) Experimental design. See Methods for further details. (B) Image of the PVC frames holding *P. astreoides* and *O. franksi* during the reciprocal transplant experiment (these two frames are from Punta Caracol). Photo credit: M. Tresguerres & D. Kline.

In my thesis, I analyzed MDH, CS, LDH, SDH, and ADH activities in *P. astreoides* and *O. franksi* from the light levels and the reciprocal transplant experiments to gain further understanding about coral energy metabolism in the field. Aquarium exposure to high-light or low-light levels for nine days did not significantly affect enzymatic activity; however, coral collected from 8 m generally had higher fermentative activity. This suggested a lack of acclimation response to light levels during the nine-day period, or that such a response was not needed. In contrast, one-year reciprocal transplantation between Punta Caracol and Eric's Reef

resulted in complex patterns in enzyme activity patterns showing differential responses between *P. astreoides* and *O. franksi* in the two destination reefs. Specifically, one-year acclimation of *P. astreoides* in Punta Caracol resulted in higher MDH and SDH activities than in Eric's Reef, indicating higher overall metabolic capacity and reliance in strombine as the fermentative end-product and suggesting a relative overabundance of heterotrophic feeding on preys with high glycine content in Punta Caracol. On the other hand, *O. franksi* demonstrated generally higher CS activity regardless of the source and destination reef, while the activities of fermentative enzymes remained more or less unchanged. This indicates that *O. franksi* more heavily relied on aerobic respiration relative to *P. astreoides*, which perhaps could be related to photosynthate production by the symbiotic algae. This variation in coral responses to light levels and reef sites found in my thesis demonstrate the importance of continued characterization of coral metabolic regulation to better gauge coral susceptibility and resilience in a changing environment.

METHODS

Coral Sampling

In 2015, fragments from *P. astreoides* and *O. franksi* coral fragments were collected from 3 m and 8 m depth in Punta Caracol and Eric's Reef by snorkel, and taken to a boat, one by one, where they were placed into buckets containing seawater. Fragments were transported to the Smithsonian Tropical Research Institute (STRI) where they were glued to cement tiles and placed in an outdoor experimental aquarium supplied with flowing seawater from Bahia del Almirante. Tanks were either left exposed to the natural incident light or were "shaded" using neutral density filters to mimic comparable light intensities found at 3 m and 8 m depth. In exposed aquaria the average diel PAR was ~210 μmol m^{-2} sec^{-1} and in shaded aquaria it was ~130 μmol m^{-2} sec^{-1} (Fig. 4c). Of the fragments collected from 3 m in Punta Caracol, half were placed in high-light exposed tanks and half were placed in low-light shaded tanks. The same was done for fragments collected from 8 m depth. At the end of the nine-day period, fragments were removed and immediately frozen and stored in liquid N_2, and eventually at -80°C until analysis.

Reciprocal Transplant Experiment

P. astreoides and *O. franksi* fragments were collected in 2015 from both Punta Caracol and Eric's Reef at 6 m depth utilizing the same collection method discussed above. Fragments were transferred to STRI where they were glued to cement tiles and placed in an outdoor aquarium with flow-through seawater for seven days to allow for recovery. Half of the coral fragments from Punta Caracol were transferred back to 6 m depth at Punta Caracol, while the other half were transplanted to 6 m depth at Eric's Reef, the reciprocal reef location. The same placement of control and reciprocal-transplant fragments was done for coral collected from

Eric's Reef. One year after, in November 2016, fragments were sampled by snorkel as described above and preserved at -80 ºC until analysis. Three of the *P. astreoides* fragments transplanted from Eric's Reef and into Punta Caracol were preyed on by parrotfish and were discarded.

Tissue Homogenization

The order in which samples from all four experiments were prepared was determined using a random number generator. Reagents were stored at 20 °C prior to use and kept burrowed under ice for each experimental preparation. A Paasche model airbrush kit was sanitized prior to use using a solution of 70% ethanol and deionized water. The airbrush reservoir was filled with 20 mL of coral homogenization buffer (CHB) consisting of a 100 mM Tris buffer at pH 7.5. Using the airbrush loaded with CHB, the coral tissue was removed from the skeletons. Resulting crude homogenate of coral tissue and CHB was collected on ice in a 50 mL Falcon tube for a final volume of 9 mL. Crude homogenate was then sonicated while on dry ice at a maximum power level of 4 for 30 seconds with 10 seconds rest in between. To separate the endosymbiotic algae from the coral cells, crude homogenate was centrifuged at 500 rcf for 15 minutes at 4 °C. Following centrifugation, supernatant was collected and transferred to several aliquots for a final volume of 500 µL per aliquot. The aliquots of zooxanthellae-free coral homogenates were then flash frozen on dry ice prior to storage at -80 ºC.

NADH absorbance-based enzymatic kinetic assays

Activity of MDH, LDH, ADH, and SDH was determined based on changes in light absorbance due to the oxidation of NADH to NAD^+. Citrate synthase activity was measured by the changes in absorbance of 2-nitro-5-thiobenzoic acid following the reaction of 5,5-dithio-bis-

(2-nitrobenzoic acid) (DTNB) with a sulfhydryl thiol. Enzyme kinetic assays were optimized for corals by Linsmayer and Tresguerres, and Hassibi[86,85]. Enzyme assay solutions were thawed on ice prior to each individual assay. Malate dehydrogenase assay solution was 80 mM imidazole buffer (pH 7.0), 0.15 mM NADH, 100 mM KCl, and 0.3 mM oxaloacetate. Citrate synthase assay solution contained 80 mM Tris buffer (pH 8.0), 2.0 mM $MgCl_2$, 0.1 mM DTNB, 0.1 mM acetyl-CoA and 0.5 mM oxaloacetate. Lactate dehydrogenase assay solution contained 50 mM imidazole buffer (pH 7.0), 0.15 mM NADH, 100 mM KCl, 1.0 mM sodium pyruvate. Alanopine dehydrogenase assay solution contained of 100 mM imidazole buffer (pH 7.0), 0.2 mM NADH, 3 mM sodium pyruvate, 200 mM L-alanine. Strombine dehydrogenase assay solution contained 100 mM Tris-HCl (pH 7.0), 100 mM glycine, 0.3 mM sodium pyruvate, and 0.3 mM NADH. Spectrometric assays were done in a SpectraMax iD3 at a running temperature of 27 °C. Each well was filled with 150 µL of the corresponding enzyme assay solution and 10 µL of coral crude homogenate, or 10 µL of coral homogenization buffer as a control. Enzymes CS and LDH were ran for 15 minutes and read at a light wavelength of 412 nm and 340 nm respectively[87]. The enzymes MDH, SDH and ADH were ran for 30 minutes and read at a light wavelength of 340 nm. Enzymatic activity is reported as nmol of substrate/mg protein/min.

Protein quantification

Protein concentrations of each crude homogenate was determined using the Bradford protein assay method (Bio-Rad Laboratories, Inc., Bradford Assay). A serial dilution (0 g/L, 0.210 mg/mL, 0.420 mg/mL, 0.5775 mg/mL, 0.735 mg/mL, and 0.945 mg/mL) of bovine serum albumin (BSA) was prepared and 10 µL of each BSA concentration was loaded into corresponding wells in triplicate. Bradford dye concentrate was brought to room temperature and

properly diluted by adding 40 µL of dye to each well for a final volume of 200 µL. Using an iMark machine, the 96-well plate was read at 595 nm as well as at a ratio of 595 nm over 45 nm (Ernst & Zor 2010). Only assays with an r^2 value greater than 0.95 was used in calculating the final protein concentration.

Computation and Statistical Analysis

Computation of maximum enzymatic activity from the spectrophotometric assay and standardization with Bradford protein concentrations was done using Excel (Microsoft, Excel 2019). Enzymatic activity (nmol substrate mg $protein^{-1}min^{-1}$) was computed as shown in Eq. (1) where A is the background absorbance subtracted from the average of the three largest changes in absolute absorbance slope, b is the path length (cm), k_{NADH} is the extinction coefficient of NADH ($M^{-1}cm^{-1}$), $[p]$ is the concentration of coral protein added (mg/mL), and the volume of enzyme assay solution and coral homogenate are V_{AS} and V_{CH} respectfully.

$$\text{Enzyme activity} = \left(\frac{(A)}{(b)(k_{NADH})([p])}\right)\left(\frac{(V_{AS})}{(V_{CH})}\right)$$

Statistical analysis of the resulting enzyme activity was done using the Prism 8 program (GraphPad Software). The ROUT method for identifying multiple outliers in a dataset was performed with a maximum False Discovery Rate (Q) of 1%. Outlying values were removed from the data sets prior to statistical analysis. Graphical representation of the full datasets containing outlying values is displayed in the appendix. The parametric two-way analysis of variance (ANOVA) package was utilized to confirm if the criteria of equal variance, normality, and large and equal sample sizes were met. Due to natural predation on the reef in the reciprocal transplantation of *P. astreoides*, there was unequal sample sizes for that experiment. Additionally, the criteria for normality and equal variance were not met in some of the

experimental groups that exhibited changes in spectrophotometric absorbance less than that of the background absorbance. Due to these deviations from the criteria, all data was transformed using y= log(y+1), y=sqrt(y), or y= log(y). Parametric two-way ANOVA using Tukey Post-hoc test was deployed on transformed data to determine differences in mean enzyme activity maximums between each main effect and the interaction effect across all assays. Results are given as mean with standard error of the mean. Treatment groups (Fig. 7.d, 7.e, 8.d, 9.b) failed to meet the requirements of normality and heteroscedasticity following transformation, however, visual evaluation for skewness in the QQ plots of each treatment group revealed that the data sets appear to come from normal distributions. Therefore, parametric two-way ANOVA analysis for differences in mean enzyme activity maximums was utilized for these treatment groups. For each sample, the summation of LDH, ADH, and SDH enzyme activity was divided by the CS enzyme activity maximum to find the fermentative to aerobic activity (Ferm:AR) ratios. Specimen that had LDH, ADH, SDH or CS activity that was a significant outlier in the treatment group was removed and not included in calculating average Ferm:AR. Parametric two-way ANOVA was deployed on transformed Ferm:AR to determine differences between treatment groups.

RESULTS

Light level experiment

Enzymatic activities of *P. astreoides* collected from 3 m and 8 m depth in Punta Caracol and held under high-light and low-light aquaria levels are shown in Figure 4. The first obvious finding was a significant reduction in MDH, CS, LDH, SDH, and ADH activities in *P. astreoides* kept in the aquaria compared to those freshly collected from the field[85] (see Fig. S6.1). This indicates a decrease of both aerobic and fermentative capacity, and while the exact causes are unknown, they might be related to prey availability. This observation has important implications for making conclusions from aquarium experiments and their implications for corals in the reef.

In the aquarium experiment, MDH activity was similar among the groups and ranged between ~90 and ~150 nmol mg protein^{-1} min^{-1} (Fig. 6a), suggesting similar overall metabolic capacity. *P. astreoides* collected from 3 m (1.57 ± 0.42 nmol mg protein^{-1} min^{-1}) had significantly higher CS activity than those from 8 m (0.54 ± 0.32 nmol mg protein^{-1} min^{-1}) and was not affected by the light treatment in the aquaria (Fig. 6b). In contrast, LDH activity was over two times higher in *P. astreoides* collected from 8 m (22.55 ± 0.4.57 nmol mg protein^{-1} min^{-1}) compared to those from 3 m (8.51 ± 1.81 nmol mg protein^{-1} min^{-1}), especially in coral from 8 m placed in low light (29.14 ± 5.5 nmol mg protein^{-1} min^{-1}) that have nearly double LDH activity than 8 m coral placed in high light (15.96 ± 3.64 nmol mg protein^{-1} min^{-1}) in coral collected from 3 m and held under low light). SDH activity was higher in *P. astreoides* collected from 8 m and was not affected by the light level treatments (Fig. 6d, 6e): average SDH activity was two times higher in fragments collected from 8 m (11.71 ± 1.07 nmol mg protein^{-1} min^{-1}) compared to 3 m (5.27 ± 1.47 nmol mg protein^{-1} min^{-1}). Similarly, ADH activity was nearly double in *P.*

astreoides from 8 m (8.49 ± 2.57 nmol mg protein^{-1} min^{-1}) compared to those collected from 3 m (3.07 ± 0.85 nmol mg protein^{-1} min^{-1}) and was not affected by tank light treatments.

The Ferm:AR ratios of *P. astreoides* collected from 8 m (60.77 ± 1.01 nmol mg protein^{-1} min^{-1}) was fivefold higher than for fragments from 3 m (13.79 ± 7.16 nmol mg protein^{-1} min^{-1}) (Fig. 6f), indicating greater capacity for fermentation over aerobic respiration. On the other hand, Ferm:AR ratios were not affected by the tank light treatments.

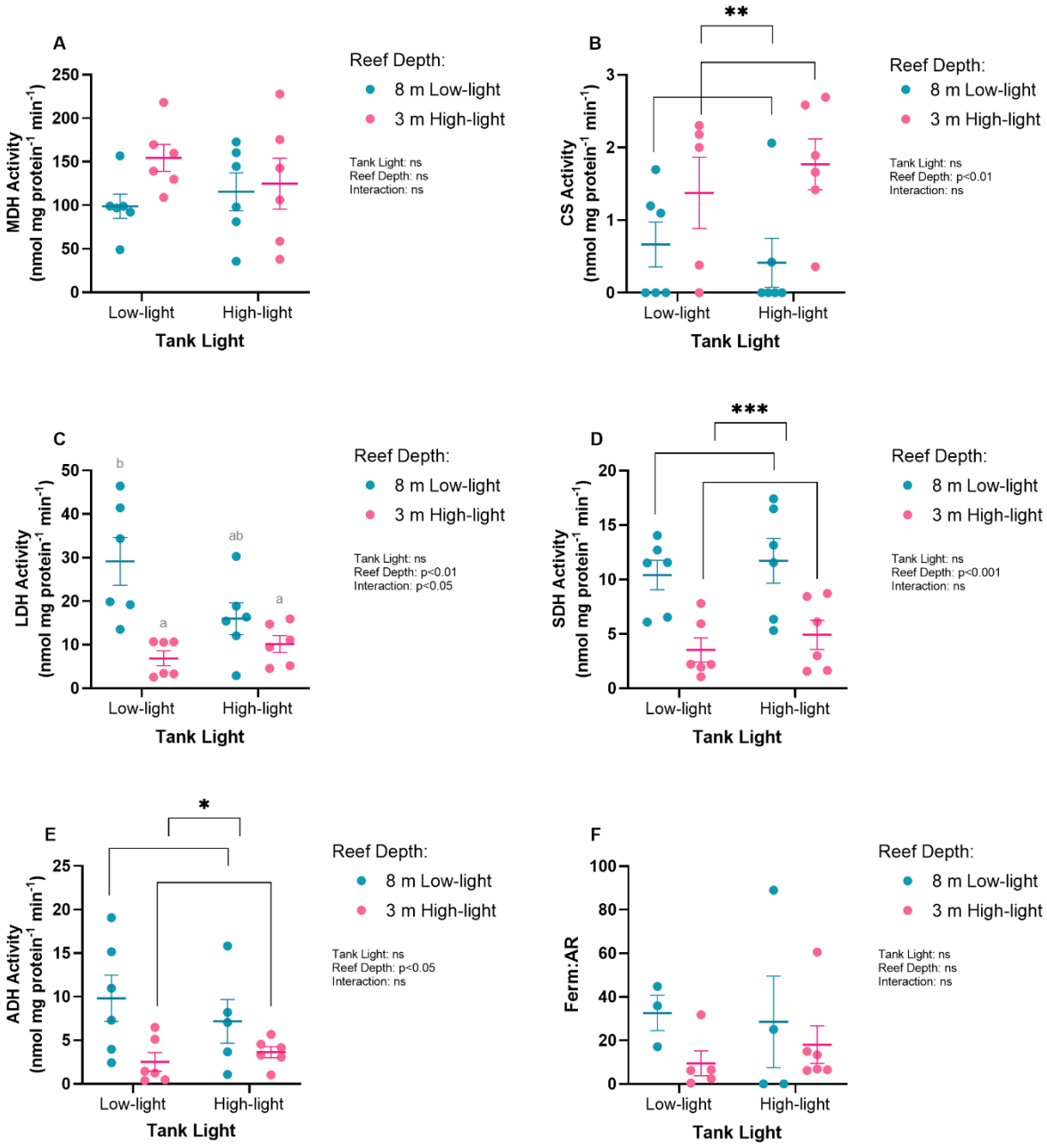

Figure 6: Metabolic enzyme activity in *Porites astreoides* collected at 3 m and 8 m from Punta Caracol in 2015. (A) Malate dehydrogenase (MDH), (B) citrate synthase (CS), (C) lactate dehydrogenase (LDH), (D) strombine dehydrogenase (SDH), (E) alanopine dehydrogenase (ADH). Field fragments were collected from Punta Caracol at low-light 8 m depth and high-light 3 m depth and immediately frozen for analysis. Aquarium fragments were collected from deep 8 m and shallow 3 m depth and placed in outdoor tanks with neutral density filters to mimic 8 m high-light and 3 m low-light levels for nine days. (F) the ratio of combined LDH, SDH, and ADH activity over CS activity (Ferm:AR) representing the relative capacity of fermentation over aerobic respiration. Significant main effects are shown by brackets and significant interaction effect shown by letters (i.e. a, b).

Enzymatic activity of *O. franksi* collected at 8 m and 3 m depth from Punta Caracol and placed in low-light and high-light tanks is shown in Figure 7. Unlike *P. astreoides,* comparison between *O. franksi* frozen immediately after collection in the field and *O. franksi* collected and placed in aquaria cannot be drawn as the samples have not been analyzed yet. *O. franksi* collected and placed in light level treatments demonstrated a large variability in the activities of all enzymes that confounded statistical analyses; however, some clear trends were observed. Average MDH activity ranged from ~20 to ~250 nmol mg protein^{-1} min^{-1}, and while no significant differences were detected among the treatments, there was a trend of higher MDH activity in coral exposed to high light regardless of collection depth (Fig. 7a). CS activity was higher in *O. franksi* collected from 8m and especially in those exposed to high light (402.86 ± 167.39 nmol mg protein^{-1} min^{-1}) (Fig. 7b). However, even the coral collected from 8 m and exposed to low light had higher CS activity (76.75 ± 23.74 nmol mg protein^{-1} min^{-1}) compared to those collected from 3 m (25.14 ± 39 nmol mg protein^{-1} min^{-1}). In contrast, LDH activity was almost thirteen times higher in *O. franksi* collected from 3 m (216.65 ± 89.81 nmol mg protein^{-1} min^{-1}) compared to 8 m (16.87 ± 3.77 nmol mg protein^{-1} min^{-1}) with an additional interaction effect of light level, whereby activity was tenfold higher in 3 coral at high light (396.85 ± 172.28 nmol mg protein^{-1} min^{-1}) compared to 3 m coral at low light (36.448 ± 13.35 nmol mg protein^{-1} min^{-1}) (Fig. 7c). SDH activity was ~nine-times higher in coral exposed to low light; however, many specimens had non-detectable SDH activity resulting in lack of statistically significant differences (Fig. 7d). ADH activity varied widely between the treatment groups, with much higher activity in *O. franksi* collected from 8 m (52.78 ± 9.96 nmol mg protein^{-1} min^{-1} versus 12.90 ± 2.38 nmol mg protein^{-1} min^{-1} at 3m) and even higher differences between coral (from

both collection depths combined) placed in high-light (51.79 ± 10.46 nmol mg protein^{-1} min^{-1}) than in low-light (13.89 ± 1.88 nmol mg protein^{-1} min^{-1}) (Fig. 7e).

The Ferm:AR ratio in *O. franksi* did not vary significantly between reef depth and light treatment groups, however, there was twentyfold higher combined Ferm:AR in coral collected from 3 m (14.28 ± 9.14 nmol mg protein^{-1} min^{-1}) compared to coral from 8 m (1.15 ± 0.59 nmol mg protein^{-1} min^{-1}) with the highest Ferm:AR in coral that were collected from 3 m and placed under high-light levels (26.22 ± 17.69 nmol mg protein^{-1} min^{-1}) (Fig. 7f).

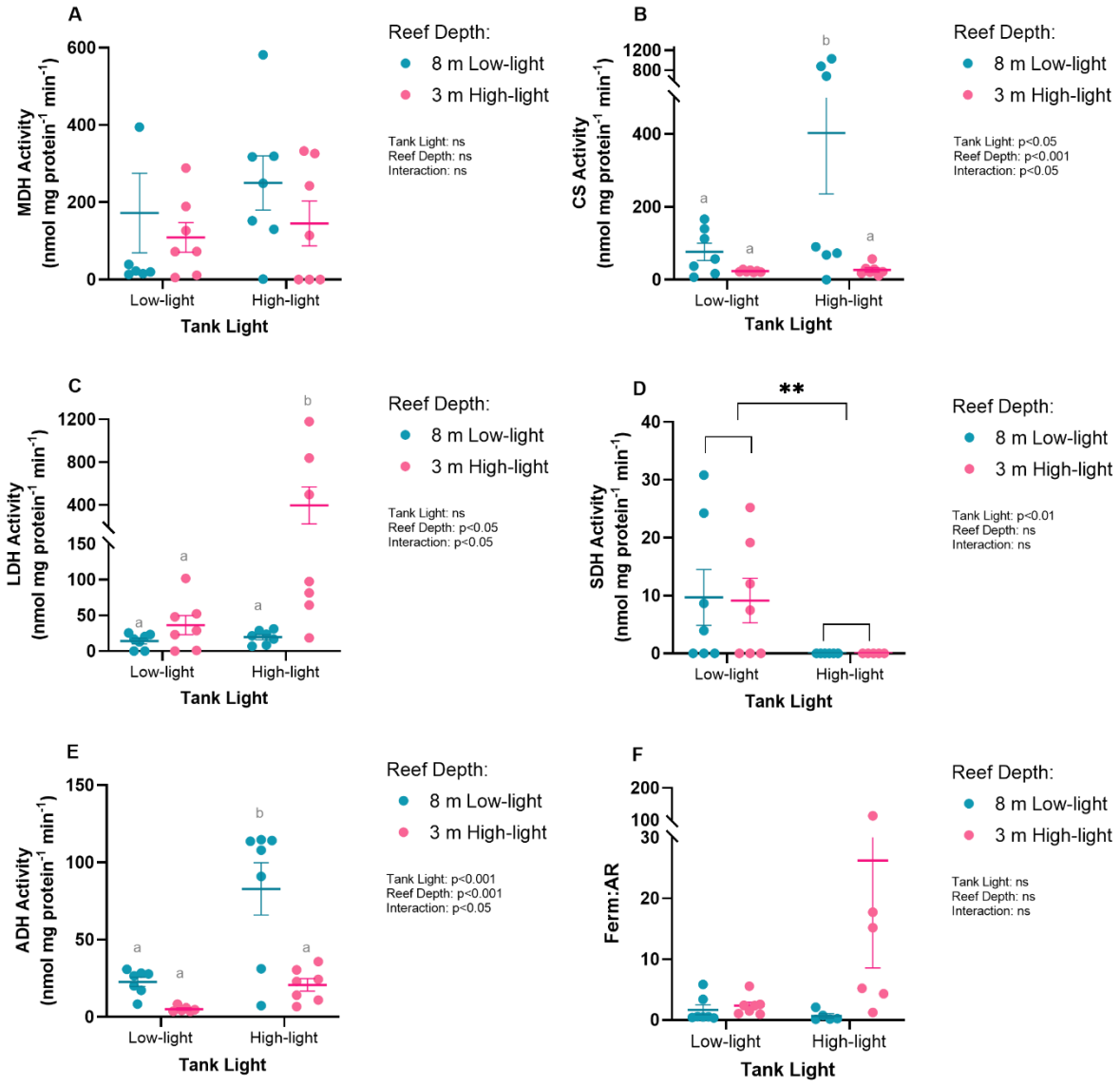

Figure 7: Metabolic enzyme activity in *Orbicella franksi* collected at 3 m and 8 m from Punta Caracol in 2015. (A) Malate dehydrogenase (MDH), (B) citrate synthase (CS), (C) lactate dehydrogenase (LDH), (D) strombine dehydrogenase (SDH), (E) alanopine dehydrogenase (ADH). Aquarium fragments were collected from deep 8 m and shallow 3 m depth and placed in outdoor tanks with neutral density filters to mimic 8 m high-light and 3 m low-light levels for nine days. (F) the ratio of combined LDH, SDH, and ADH activity over CS activity (Ferm:AR) representing the relative capacity of fermentation over aerobic respiration. Significant main effects are shown by brackets and significant interaction effect shown by letters (i.e. a, b).

Reciprocal Transplant

The enzymatic activities of *P. astreoides* transplanted between Eric's Reef and Punta Caracol are shown in Figure 8. There was extensive variability in *P. astreoides* across transplant groups, whereby source reef and destination reef affected enzymatic activity to varying levels. MDH activity was affected by both the source reef ($p<0.001$) and destination reef ($p<0.05$) (Fig. 8a), whereby activity in coral originating from Eric's Reef was ~50% higher than those from Punta Caracol (162.49 ± 30.31 nmol mg protein^{-1} min^{-1} versus 107.85 ± 7.72 nmol mg protein^{-1} min^{-1}), and coral transplanted into Punta Caracol (227.32 ± 48.04 nmol mg protein^{-1} min^{-1}) had almost four-fold higher activity than coral transplanted into Eric Reef (42.71 ± 14.02 nmol mg protein^{-1} min^{-1}). *P. astreoides* CS activity was not significantly affected by source reef or destination reef, with averages ranging between ~20 to ~130 nmol mg protein^{-1} min^{-1}; however, CS activity in coral collected from Eric Reef and placed back into Eric Reef (132.57 ± 61.28 nmol mg protein^{-1} min^{-1}) was at least four times higher than in the other groups (which ranged from ~20 to ~ 30 nmol mg protein^{-1} min^{-1}) (Fig. 8b).

LDH activity was primarily affected by source reef such that coral originating from Punta Caracol had rates of 6.07 ± 2.07 nmol mg protein^{-1} min^{-1}, and those from Eric's Reef had nearly undetectable LDH activity (Fig. 8c), except for three specimens that were identified as outliers and removed from analysis (Fig. S8c) Moreover, LDH activity in *P astreoides* collected in Punta Caracol and transplanted back into the same location (8.51 ± 2.71 nmol mg protein^{-1} min^{-1})was roughly double than in those transplanted into Eric's Reef (3.63 ± 1.39 nmol mg protein^{-1} min^{-1}). SDH activity was very robust in *P. astreoides* transplanted into Punta Caracol, while it was not detected in those transplanted into Eric's Reef (Fig. 8d). Among corals transplanted into Punta Caracol, those originally sourced from Eric's Reef had nearly two times higher SDH activity

than those originating from Punta Caracol (609.84 ± 193.41 vs. 315.82 ± 93.25 nmol mg protein^{-1} min^{-1}, respectively). Of note, both SDH activity rates are extremely high. ADH activity in *P. astreoides* exhibited high variability within the transplant groups, resulting in not significantly differences due to source reef, destination reef, or the interaction of the two. However, trends indicate ~20% greater ADH activity in *P. astreoides* placed into Eric's Reef (86.84 ± 30.36 nmol mg protein^{-1} min^{-1}) over that of Punta Caracol (37.33 ± 18.91 nmol mg protein^{-1} min^{-1}), with three fold higher activity in coral collected from Punta Caracol and placed into Eric's Reef (123.26 ± 34.61 nmol mg protein^{-1} min^{-1}) (Fig. 8e).

Multiple comparison analysis of *P. astreoides* Ferm:AR ratios demonstrated significantly higher values in corals from both source reefs and transplanted into Punta Caracol (22.42 ± 4.56 nmol mg protein^{-1} min^{-1}) than those transplanted into Eric's Reef (3.778 ± 1.58 nmol mg protein^{-1} min^{-1}) (Fig. 8f). This indicates higher relative reliance on fermentation vs. aerobic capacity in *P. astreoides* fragments transplanted into Punta Caracol over those placed in Eric's Reef.

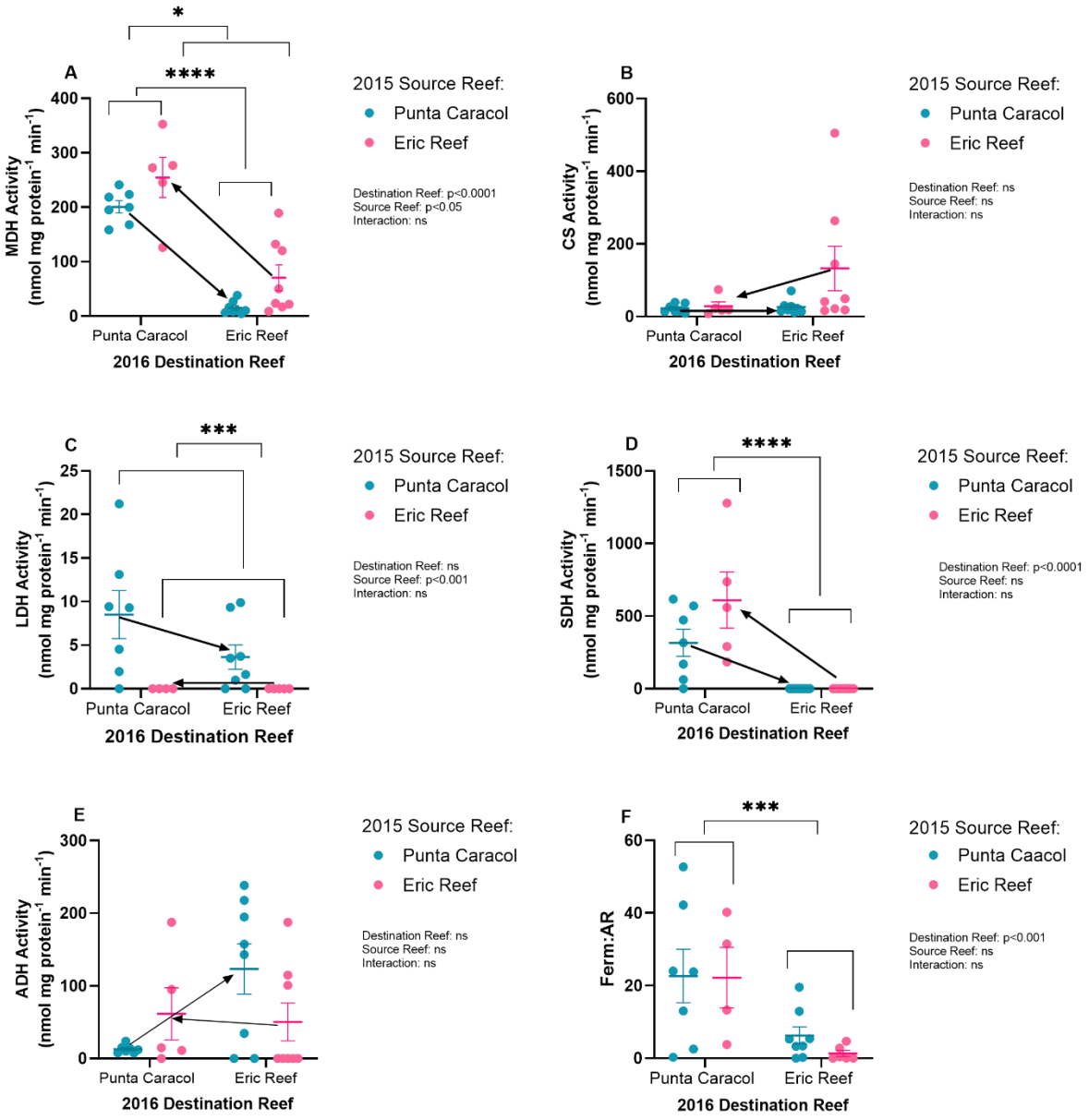

Figure 8: Metabolic enzyme activities in *Porites astreoides* collected at Punta Caracol and Eric's Reef, and reciprocally transplanted between those sites. (A) Malate dehydrogenase (MDH), (B) citrate synthase (CS), (C) lactate dehydrogenase (LDH), (D) strombine dehydrogenase (SDH), (E) alanopine dehydrogenase (ADH). (F) the ratio of combined LDH, SDH, and ADH activity over CS activity (Ferm:AR) representing the relative capacity of fermentation over aerobic respiration. Corals were collected in 2015 from their source reef location at 6m with reciprocal and control transplants placed at 6 m in the destination reef location and collected one year later in 2016. Significant main effects are shown by brackets and significant interaction effect shown by letters (i.e. a, b).

The enzymatic activity of *O. franksi* transplanted between Punta Caracol and Eric's Reef are shown in Figure 9. The most noticeable trends were high variability within each transplant group and thus limited significant differences found, and that many of the *O. franksi* responses were in opposite directions compared *P. astreoides*. Average MDH activity in *O. franksi* ranged from ~50 to 150 nmol mg protein^{-1} min^{-1}; MDH activity was not significantly affected by source reef, destination reef, or the interaction of the two (Fig. 9a). CS activity was approximately four times higher in samples originating from Punta Caracol (137.1 ± 68.75 nmol mg protein^{-1} min^{-1}) compared to those originating from Eric's Reef (35.29 ± 11.327 nmol mg protein^{-1} min^{-1}) and there was a trend for higher CS activity in corals transplanted into Punta Caracol. However, there were no significant differences between groups (Fig. 9b). LDH activity was similar across all transplant groups, ranging from ~1 to ~2 nmol mg protein^{-1} min^{-1} (Fig. 9c). SDH activity in *O. franksi* was more than 200 times higher in corals transplanted into Eric's Reef (0.437 ± 0.21 nmol mg protein^{-1} min^{-1}) compared to those transplanted to Punta Caracol (0.002 ± 0.0004 nmol mg protein^{-1} min^{-1}) (Fig. 9d). ADH activity was also affected by the destination reef, but in the opposite direction: coral transplanted into Punta Caracol had three-fold higher activity (12.71 ± 4.51 nmol mg protein^{-1} min^{-1}) compared to those transplanted into Eric's Reef (4.02 ± 1.23 nmol mg protein^{-1} min^{-1}) (Fig. 9e).

The Ferm:AR: higher in ratios in *O. franksi* fragments was not significantly different between the transplant groups, however, overall trends demonstrated that *O. franksi* transplanted from Punta Caracol into Eric's Reef had slightly higher Ferm:AR ratios (0.8 ± 0.62 nmol mg protein^{-1} min^{-1}) compared to other transplant groups (which ranged ~0.2 to ~0.5 nmol mg protein^{-1} min^{-1}).

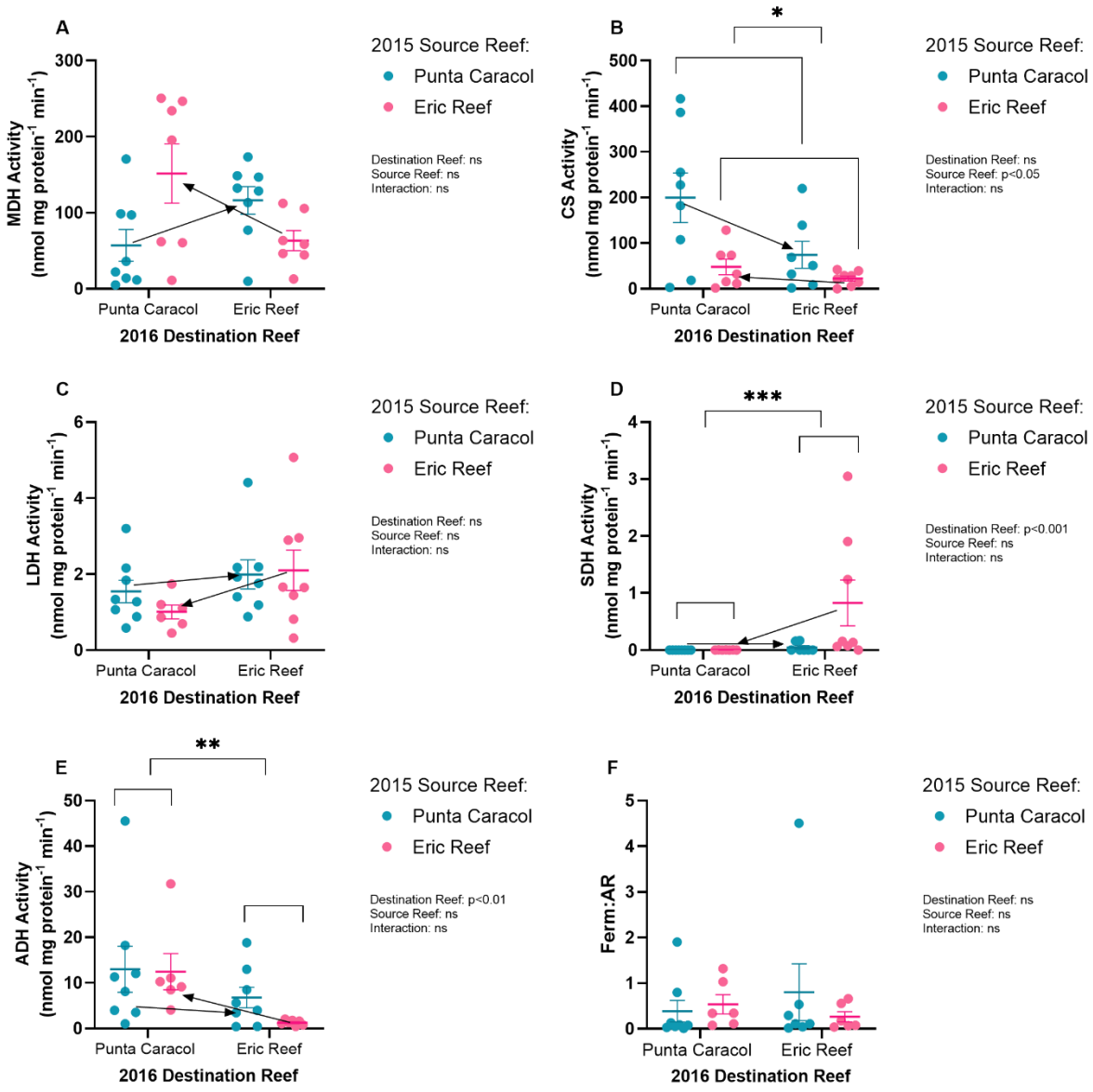

Figure 9: Metabolic enzyme activities in *O. franksi* collected at Punta Caracol and Eric's Reef, and reciprocally transplanted between those sites. (A) Malate dehydrogenase (MDH), (B) citrate synthase (CS), (C) lactate dehydrogenase (LDH), (D) strombine dehydrogenase (SDH), (E) alanopine dehydrogenase (ADH). (F) the ratio of combined LDH, SDH, and ADH activity over CS activity (Ferm:AR) representing the relative capacity of fermentation over aerobic respiration. Corals were collected in 2015 from their source reef location at 6m with reciprocal and control transplants placed at 6 m in the destination reef location and collected one year later in 2016. Significant main effects are shown by brackets and significant interaction effect shown by letters (i.e. a, b).

DISCUSSION

My Thesis provides evidence for flexibility of energy metabolic pathway utilization in two Caribbean coral species following short-term exposure to light levels found at 3 m and 8 m depth, as well as species-specific responses to long-term transplantation between distinct reef locations in Bocas del Toro, Panama. The following metabolic concepts are essential for interpreting the results of this study: (a) each enzyme activity assay is a proxy for maximum capacity of the underlying energy metabolic pathway and likely reflects responses to average medium- or long-term conditions rather than to instantaneous fluctuations[67]; (b) MDH activity is a proxy for overall energy metabolic capacity due to its dual role in the TCA cycle and the mitochondrial NADH/NAD^+ shuttle; (c) CS activity is a proxy for aerobic metabolic capacity due to its key role in the TCA cycle, in which it is a rate-limiting enzyme; (d) LDH, SDH, and ADH are alternative terminal dehydrogenases in the fermentation pathway, which allows to generate ATP anaerobically. However, fermentation can be an indication of hypoxia exposure or of an overabundance of photosynthetically produced sugars fueling aerobic glycolysis (the Warburg effect)[62,67]; (e) Ferm:AR ratio is a proxy for fermentative capacity relative to aerobic respiration capacity for each coral, and it does not provide information about absolute rates between specimens, treatments, or reef sites. For example, a given coral can have a higher Ferm:AR ratio than another coral, but also have higher aerobic metabolic capacity; (f) while a greater Ferm:AR ratio indicates higher fermentative capacity over aerobic respiration, this could indicate acclimation to hypoxia or a more robust Warburg effect [following (d)]; (g) LDH directly catalyzes the oxidation of pyruvate for NAD^+ regeneration; however, SDH and ADH additionally require glycine and alanine, respectively. Thus, SDH and ADH usage and abundance may depend on amino acid availability in each environment; and (h) OpDHs (such as

SDH and ADH) can maintain a cytosolic NADH/NAD^+ ratio that is more favorable for glycolytic ATP production compared to LDH[54]. Thus, the activity rates of the different terminal dehydrogenases are not necessarily directly comparable to each other in terms of ATP production efficiency.

With this in mind, the significant reduction in MDH, CS, LDH, SDH, and ADH activities in *P. astreoides* kept in the aquarium compared to those freshly collected from the field indicate a decrease in both aerobic and fermentative capacity[85] (Fig. S6.1). A potential reason is that the zooplankton quality or abundance in the seawater supplied to the STRI aquarium is not comparable to that in Punta Caracol, either due to natural zooplankton variations throughout Bahia del Almirante or to seawater filtration in the STRI aquarium system that is essential to prevent clogging and ensure water quality. In any case, this can have important implications for our interpretation of aquarium experiments and their relevance for their real world. If such important differences in coral energy metabolism were observed in an aquarium that supplies flowing seawater from an area directly adjacent to natural coral reefs, what is the effect in aquaria that use artificial seawater, or heated seawater from temperate oceans? Can some of the negative effects of ocean acidification, hypoxia, or warming -to name a few commonly studied stressors- be due to inadequate energy budgets resulting from non-realistic aquarium conditions?

Nonetheless, my results from the light level experiment in STRI-Bocas del Toro provide interesting insights about coral energy metabolic responses. For *P. astreoides*, the lack of significant differences in MDH activity throughout the groups indicates similar overall metabolic capacity (Fig. 6a). But, as described below, this is achieved through different combinations of aerobic and fermentative pathways contributions in the *P. astreoides* collected from the two depths and exposed to the two light levels. For example, *P. astreoides* collected from 3 m

demonstrated significantly higher CS activity compared to the those collected from 8 m (Fig. 6b), indicating higher aerobic respiration capacity. Since all tanks received the same seawater from the same source, at comparable flow rates, and each tank additionally had a recirculating mini-pump, oxygen levels can assume to have been saturated in all tanks. However, it is possible that differences in symbiotic algal photosynthesis induced higher oxygen concentrations in the coral tissues and the diffusive boundary layer of corals exposed to the higher light level thus favoring aerobic respiration. If this supposition was true, the lower CS activity in *P. astreoides* sourced from 8 m and exposed to high light could potentially be due to the presence of less efficient algal strains, or algal strains that cannot or did not have enough time to acclimate to the higher light level, coupled to a lack of free-living *Symbiodiniaceae* algae in the flowing sweater that could sustain algal-shuffling[88,89]. Ongoing genotyping of the symbiotic algal strains will help provide answers to these questions. Consistent with the idea of lower oxygen availability in the microenvironment of *P. astreoides* collected from 8 m, their LDH, SDH, and ADH activities were generally higher than in *P. astreoides* collected from 8 m at both light levels (Fig. 6c-6e), which in turn determined higher Ferm:AR ratios (Fig. 6f).

Interspecific enzymatic activity variability tended to be more variable in *O. franksi* than in *P. astreoides*. The underlying reasons are unknown, but possible causes include more complex colony morphology potentially resulting in more heterogeneous microenvironments and ensuing heterogeneous and variable physiologies, and the presence of multiple algal strains. Unlike *P. astreoides* that had similar MDH activity among groups in the light level experiment, MDH activity in *O. franksi* showed a trend for higher capacity in coral exposed to high light regardless of collection depth, and it was especially low in *O. franksi* from 8 m and exposed to low light (Fig. 7a). The potential effects of algal strains described for *P. astreoides* in the previous

paragraphs also apply to these *O. franksi* results**.** Moreover, shuffling of algal strain composition has been suggested to optimize photosynthesis in relation to depth-dependent light zonation in *Orbicella sp.*[90,89]. The higher CS activity in *Orbicella sp.* collected from 8 m (Fig. 7b) may be explained by differences in algal strain-dependent photosynthetic oxygen production. However, it is important to note that this higher CS activity was driven by very high values in three specimens.

Similarly, algal strain composition and performance could alter photosynthate production rate potentially inducing favorable conditions for aerobic glycolysis. Indeed, *O. franksi* collected from 3 m had significantly higher LDH activity, especially when in the high-light treatment (Fig. 7c), which suggests the potential use of LDH as the terminal dehydrogenase for aerobic glycolysis. The response to light levels was different for SDH and ADH in *O. franksi*, such that robust SDH activity was only observed during low light conditions, while ADH activity was the opposite (Fig. 7d, 7e). Strikingly, *O. franksi* exposed to high light had nearly undetectable SDH activity but this was very robust in the majority of *O. franksi* exposed to low light, regardless of the collection depth. This suggests differential availability of glycine between the light treatments, potentially due to adjustments to coral protein synthesis or transport of amino acids to the algal symbiont to support photosynthesis at the high light level. Oxygen availability (i.e. potential hypoxia in the low light level) is another factor that could help explain the SDH activity patterns. On the other hand, elevated ADH activity in *O. franksi* collected from 8 m and exposed to high light conditions could indicate a role in aerobic glycolysis. However, it is unclear why 8 m *O. franksi* would rely on ADH, but 3 m *O. franksi* would rely on LDH for the same purpose under identical and controlled aquarium conditions. In any case, this high propensity for

fermentation via LDH was captured by the significantly higher Ferm:AR ratio in coral from 3 m placed under high light (Fig. 7f).

Comparisons between *O. franksi and P. astreoides* in the light level experiment reveal interspecific differences in aerobic and fermentative capacity that are potentially due to variations in substrate availability and utilization and, again, algal strains. Moreover, *O. franksi* demonstrated "collection depth"- and "light treatment"-specific variability that was not observed in *P. astreoides*. Although it is tempting to associate the lack of a given response to light level changes with decreased acclimation potential and vulnerability, all coral fragments appeared equally healthy at the time of sampling (Tresguerres, *pers. comm.*) so any potential effects on fitness are not readily apparent. Two additional experimental considerations are worth mentioning: (1) the length of the experiment (nine days) may not have been enough to allow for full acclimation responses to a change in light intensity, and (2) although the aquarium setup created high and low light level conditions (maximum average PAR 400 ± 100 and ~280 ± 40 μmol m^{-2} sec^{-1}, respectively; Fig. 4d), these did not completely replicate the light conditions at 3 m and 8 m in Punta Caracol around the same time (maximum average PAR 348.4 ± 100.5 μmol m^{-2} sec^{-1} and 120.3 ± 93.9 μmol m^{-2} sec^{-1}, respectively) (full-day average PAR in the aquarium experiments were 200 ± 40 and 130 ± 10 μmol m^{-2} sec^{-1} in the high and low light level treatments but have not been yet calculated for Punta Caracol). However, this discrepancy between aquarium and field values could be expected to exacerbate the differences between the light treatments, if any.

The goal of the reciprocal transplant experiment with *P. astreoides* and *O. franksi* between Punta Caracol and Eric's Reef was to attempt to differentiate potential environmental and population effects on energy metabolic activity. Due to their respective proximities to coastal

towns and the open ocean (Fig. 2), seawater conditions in Punta Caracol and Eric's Reef could differ from each other on a daily, weekly, monthly, seasonal, annual, or unpredictable basis. Recent efforts have begun to characterize these reefs with relatively high temporal and spatial resolution over periods of a few weeks at the time during 2015 and 2016, which overlapped with the time of collection of the corals used in my thesis. Some of these results have been published[84] and revealed similar average salinity (34.4 ± 0.8 ppt), temperature (~30 ºC), and pH (~7.95 pH units), as well as similar diel variations in those parameters, in Punta Caracol and Eric's Reef in November/December 2015. During the same period, Punta Caracol exhibited ~2-3 times higher average ammonia, nitrate and DO concentrations (Fig. 3) than Eric's Reef, yet variability in the values lead no statistically significant differences.

A further look at changes in nutrient concentrations during the three-week sampling period revealed a drastic spike in DINsC and DOC at Punta Caracol around November 28th-December 3rd, potentially caused by rainfall leading to increased terrestrial runoff (Fig. 10). Although the corals used in my thesis were sampled before that spike, this opens the possibility that corals in Punta Caracol may tune their energy metabolism to these spikes and/or resulting changes in bacteria, phytoplankton, and zooplankton growth, as well as oxygen, that may occur. This has important implications for field studies that attempt to discern effects of environmental change on coral biology over more dynamic and short-term adjustments. Therefore, measurements of seawater parameters should be done through the year and with higher spatial resolution to fully capture variability on the reefs and more accurately assess potential impacts to coral energy metabolism and other biological aspects.

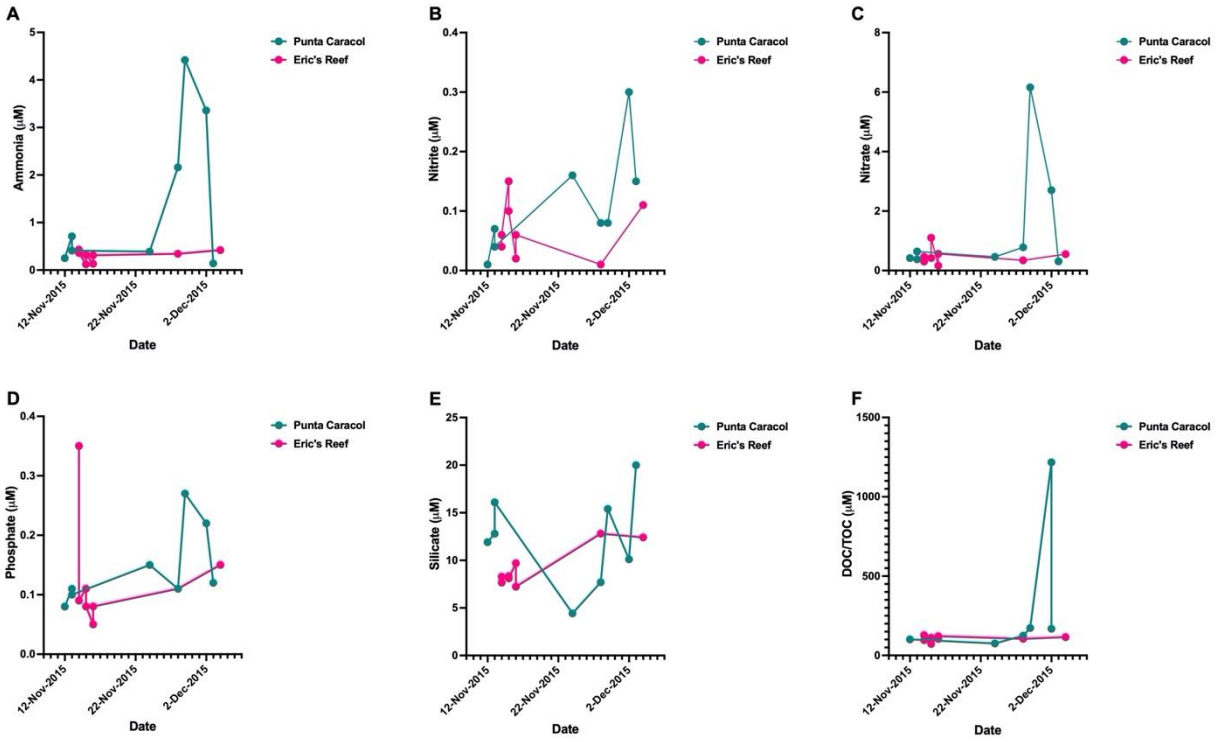

Figure 10. Time-series of dissolved nutrients and carbon in Punta Caracol. Concentrations of dissolved inorganic (A) ammonia, (B) nitrite (C) nitrate, (D) phosphate, (E) silicate, and (D) organic carbon (DOC) measured over a two-week period in November/December of 2015 in Punta Caracol and Eric's Reef. Data obtained from M. Tresguerres & D. Kline.

Energy metabolic activity in *P. astreoides* was influenced by both environmental and population effects following transplantation between Punta Caracol and Eric's Reef, with variable responses in fermentation and aerobic activity. Comparison of these results with *P. astreoides* collected from both reefs in 2015[85] shows the approximate change in general metabolic capacity, as denoted by MDH activity, at each reef, such that MDH activity was significantly higher at Eric's Reef in 2015 (i.e, prior to transplantation) and then significantly higher at Punta Caracol in 2016 (i.e., following transplantation) (Fig. S6.1). This change is helpful in assessing adjustments to general metabolic capacity due to long-term environmental effects at each reef site, however, aerobic and fermentative activity in transplanted *P. astreoides* exhibited additional population-effects related to the collection reef site. MDH activity was

significantly higher in coral originating from Eric's Reef compared to *P. astreoides* from Punta Caracol, a trend demonstrated in CS activity as well, which was nearly four times higher in coral originating from Eric's Reef and transplanted back into Eric's Reef (Fig. 8b). This points to potential differentiation in the algal populations hosted by *P. astreoides* at each reef that influenced both the amount of photosynthates available for general metabolic capacity (i.e., MDH activity), and the oxygen microenvironment available for successful aerobic respiration (i.e., CS activity).

In a similar vein, LDH activity in *P. astreoides* was impacted by population-specific effects such that significantly higher activity was found in coral sourced from Punta Caracol, with near-zero LDH values in coral sourced from Eric's Reef. Differences in symbiont populations between *P. astreoides* collected from each reef site may explain the elevated LDH activity in coral originating from Punta Caracol. However, the presence of environmental effects unique to each reef (i.e., planktonic composition, DINsC and DOC concentrations, or ranges in hypoxia-inducing events) further acted upon population-effects, such that coral collected from Punta Caracol and placed back into Punta Caracol had higher LDH activity compared to those transplanted to Eric's Reef (Fig. 8c). Differences in SDH and ADH activity in *P. astreoides* transplanted into Punta Caracol and Eric's Reef suggest that amino acid availability, as a required substrate in the OpDH pathways, influenced fermentation pathways and rates. Amino acids can be acquired by coral in several ways, including uptake of dissolved free amino acids (DFAAs) from seawater, catabolism of heterogeneously ingested prey, and as a photosynthetic byproduct from their endosymbiotic *Symbiodiniaceae*[34]. Quantified differences in stable isotopic signatures between zooplankton, coral, and endosymbiont algae across sixteen globally distributed reefs indicate that heterotrophic ingestion of zooplankton increases as a function of

chlorophyll a, an indicator of primary production on the reef. However, these spatial gradients in productivity related only to heterotrophic incorporation of carbon, not nitrogen, into the coral tissues[91]. Although heterotrophic feeding is typically the major nitrogen source for coral[92], DFAAs uptake can contribute up to 24% of coral's nitrogen needs[93], providing key nitrogen compounds required for both coral and algal protein synthesis. A potential role of symbiotic nitrogen-fixing cyanobacteria[94] cannot be ruled out; however, to my knowledge their contribution to coral nitrogen budget has never been quantified. Interestingly, dissolved inorganic nitrogen concentrations (ammonia, nitrite, nitrate) varied between the two reefs (Fig 3, 11), as do planktonic community composition and diversity between offshore (i.e. Eric's Reef) and inshore (i.e., Punta Caracol) reefs[95]. Differences between the two sites appear to be consistent, as demonstrated by elevated SDH activity in both *P. astreoides* collected from Punta Caracol in 2015[85] and coral transplanted into Punta Caracol and collected in 2016 (Fig. 8d), however, significantly higher SDH values in 2016 indicate variation in environmental factors from year to year.

Interspecific differences are evident when comparing the metabolic activity of *O. franksi* from the reciprocal transplant experiment with that of *P. astreoides* discussed above. In contrast to *P. astreoides*, overall metabolic capacity appeared to be similar across *O. franksi* transplant groups, as denoted by no significant differences in MDH activity (Fig 9a), however contributions of aerobic and fermentation activity to this overall capacity varied between destination sites. Specifically, CS activity was generally higher in *O. franksi* transplanted into Punta Caracol, and especially so for coral originating from Punta Caracol compared to those from Eric Reef (Fig. 9b). Some of the factors that affect light levels, and therefore photosynthetic oxygen production that affects aerobic respiration, include suspended particulate matter in the water column and

shading by sedimentation or benthic algal growth. Unfortunately, the PAR data around the time of collection of the coral transplants in 2016 has not been analyzed yet; however, the fact that *P. astreoides* had the opposite trend for CS [i.e. higher activity in Eric Reef (Fig. 8b)] points out yet again to potential differential photosynthetic efficiency of the endosymbiotic algal strains between the two species in the two reef locations.

Generally similar LDH activity in *O. franksi* across transplant groups (Fig. 9c), coupled by significant differences in SDH and ADH values at each destination reef (Fig. 9d, 9e), suggest that differences in amino acid availability for OpDH activity, as previously discussed in transplanted *P. astreoides* colonies, similarly affected transplanted *O. franksi*. However, species-specific differences in SDH and ADH utilization between the two reef sites (i.e. higher SDH in *P. astreoides* at Punta Caracol (Fig. 8d), but higher ADH activity in *O. franksi* at Punta Caracol (Fig. 9d)) suggest that species-specific variation in heterotrophic strategies and amino acid incorporation influenced fermentative pathway activity. Tracing of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in Hawaiian coral found that ingested organic matter and zooplankton provided up to 49% of the carbon and nitrogen found in the coral tissue of healthy *Pocillopora sp.* and *Montipora sp.*, but only 28% in *Porites sp.*[96]. Polyp size and distribution on the colony can directly dictate the feeding strategies available to the coral and thus influence the level of heterotrophic compensation between species[14]. *O. franksi* colonies generally have low density and distribution of polyps that are ~2.5-4 mm in diameter[97], whereas *P. astreoides* colonies have a higher density of smaller polyps[88]. Thus, morphological differences between the *P. astreoides* and *O. franksi* could impact heterotrophic ingestion of the varying organic plankton[95] and inorganic nutrients found in Punta Caracol and Eric's Reef (Fig. 3) and ultimately influence the carbon and nitrogen pools available for synthesis of the amino acids for OpDH pathways.

It is important to note, however, that assimilation of carbon and nitrogen into free amino acids is not accomplished by the coral alone. Endosymbiotic *Symbiodiniaceae* synthesize amino acids, linked to photosynthesis, that are translocated to the coral host[98-100]. While it has recently been suggested that metabolite pools (i.e., sugars, carbohydrates, amino acids, fatty acids, and organic acids) in the coral and symbiont are unaffected by symbiont species composition and abundance[101], the extent to which heterotrophic nitrogen and carbon are diverted from the coral to the algae for metabolite production is not completely understood, can be very variable, and potentially species-specific. A recent study analyzing isotopic signatures of amino acids in *Stylophora pistillata* showed that most of the ingested heterotrophic nitrogen was traced back to symbiont derived amino acids over that of coral amino acids[102], indicating that the symbiont was the primary benefactor of heterotrophy. Additionally, similar isotopic carbon signatures found in the amino acids of both coral host and symbiont suggest that photosynthetically derived carbon was the primary source of carbon for coral amino acid synthesis[102]. This suggest that transport of heterotrophic nitrogen to the algae is prioritized and could be used as a strategy to bolster algal sugar production and availability for coral amino acid and protein synthesis. The tracing of trophic interactions via isotopic analysis during coral symbiosis combined with the enzymatic assays used in my Thesis could aid in identify the extent to which heterotrophic compensation influences synthesis of the specific amino acids required for OpDH fermentative activity.

The Ferm:AR activity ratios in transplanted *O. franksi* and *P. astreoides* demonstrated a clear species-specific response to transplantation between differing reef locations. *P. astreoides* had significantly higher Ferm:AR ratios after being transplanted to Punta Caracol for one year (Fig. 8f), but *O. franksi* had much lower Ferm:AR ratio indicating higher capacity for aerobic respiration over fermentation across all transplant treatments (although they additionally

experienced important destination reef-dependent changes in fermentative activity) (Fig. 9f). This variability in the maximum fermentative and aerobic capacity between the two species under the same ecological conditions illustrates the diverse manners in which coral may adjust their energy metabolism on the reef. Full understanding of the mechanisms utilized in coral regulation of substrate availability and utilization for energy production is needed to assist in better assessing competitive advantages between coral species and distinct populations in the future.

Coral morphologies and skeletal structures have been found to enhance surface area and light refraction across the colony, which can improve delivery of light energy to the algae for photosynthesis[103]. In addition to clade-specific differences in skeletal biomineralization, differences in colony skeletal arrangement and properties could result in different utilization of light for photosynthesis and thus metabolite availability between *P. astreoides* and *O. franksi*. Additionally, *Symbiodiniaceae* species can adjust their capacity for light harvesting by altering the number and ratio of photosynthetic pigments in their chloroplasts[104]. These differences could lead to further variation in the rate at which photosynthetic oxygen and sugars are provided to the coral, subsequently affecting aerobic and fermentative energy metabolism. Genotyping of the algal-containing fraction from the coral samples utilized in this study are currently being processed and will provide key insight into the symbiont characteristics that may influence energy metabolic capacity.

CONCLUSION AND FUTURE DIRECTIONS

In this Thesis, I provide evidence demonstrating maintenance of energy metabolic pathway regimes in two Caribbean coral species following short-term exposure to different light levels, as well as species-specific acclimation of energy metabolism to long-term transplantation between two reef sites in Bocas del Toro, Panama. There was indication that fermentative enzyme capacity of LDH, SDH, and ADH were primarily affected by the source reef depth. Analysis of fermentative activity revealed that both *P. astreoides* and *O. franksi* can utilize LDH and OpDHs during high-light conditions, suggesting the use of aerobic glycolysis to produce ATP, regenerate NAD^+, and provide glycolytic intermediates for cell proliferation and maintenance. An influence of environmental effects, as opposed to population effects, on fermentative and aerobic capacity following the one-year transplant experiment was evident in destination reef-specific preferential use of OpDH pathways. Overall, my Thesis exemplifies how enzymatic assays can be used as a tool to understand metabolic energy strategies and capacity in coral and to dissect the dynamic relationship between light, nutrients and energy production on the reef.

To build a more holistic understanding of coral energy metabolism, future research would benefit from a detailed characterization of heterotrophic and photo-autotrophic production of sugars, glycerol and amino acids. This approach may be best accomplished using compound-specific isotope analysis to track the exchange of metabolic intermediates and substrates between the seawater, zooplankton, the coral tissue, and endosymbiotic *Symbiodiniaceae.* Additional characterization of the carrier-mediated transport of nitrogen-rich amino acids across the symbiosomal membrane during stress and recovery could illuminate coral regulation of, and reliance upon, amino acid exchange for energy production. Characterization of coral metabolic

physiology should continue to be applied to natural coral populations via multi-reef sampling at differing depths in order to capture accurate information of species-specific energy regulation in the environment. In addition to day-of-sampling measurements of nutrient and DOC concentrations in the water, sampling of planktonic communities at each reef, as well as ingested food content in the coral polyp mouth, would be beneficial. Advancements in these topics will allow for a more accurate understanding of basic coral energetics and provide new information and solutions for adaptive coral reef conservation and management.

An ever-increasing myriad of anthropogenic forces threaten the stability of earth ecosystems, endangering countless global resources, including the vast marine biodiversity that coral reefs host. Reefs are subjected to both local and global shifts in ecological conditions, including coastal eutrophication and sedimentation that can limit coral access to light and food sources, as well as shifts in sea surface temperature and pH that can drastically impact the energy requirements of shallow coral populations. Reef sites in Bocas del Toro, Panama are no stranger to such environmental changes and the results in this study highlight the need to develop accurate methodologies to assess coral metabolic capacity and regulation such that more effective strategies to aid in reef conservation can be employed.

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Martin, Noël, Samantha K., Hassibi, Cameron M., Linsmayer, Lauren B., Hamilton, Trevor J., Andersson, Andres J., Kline, David I. The thesis author was the primary author of the Thesis.

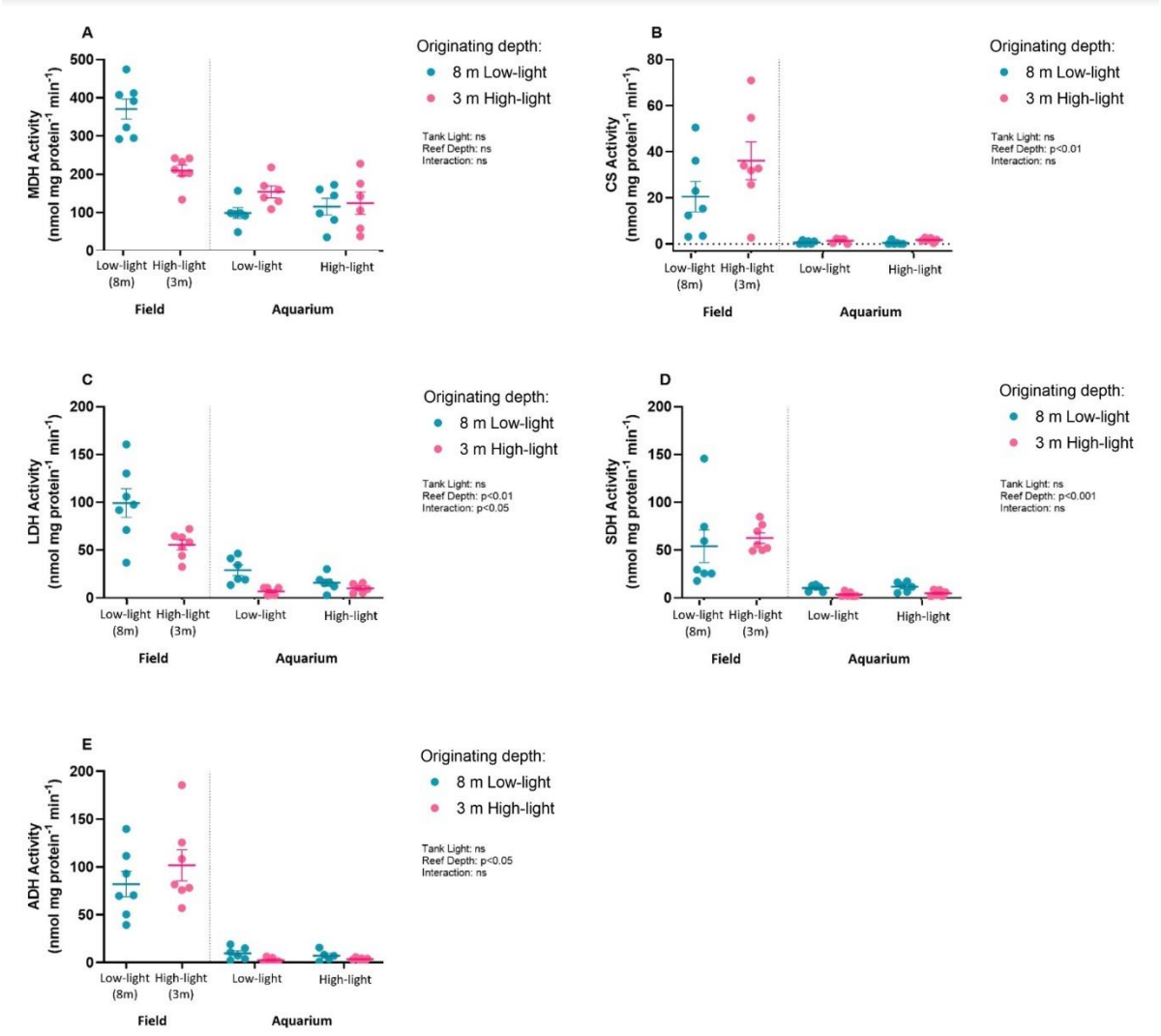

Figure S6.1: Metabolic activity in *Porites astreoides* collected at 3 m and 8 m from Punta Caracol and frozen immediately (Field: left of the dotted line) compared to those placed in low-light and high-light outdoor aquariums (Aquarium: right of the dotted line). Outlying data points are included.

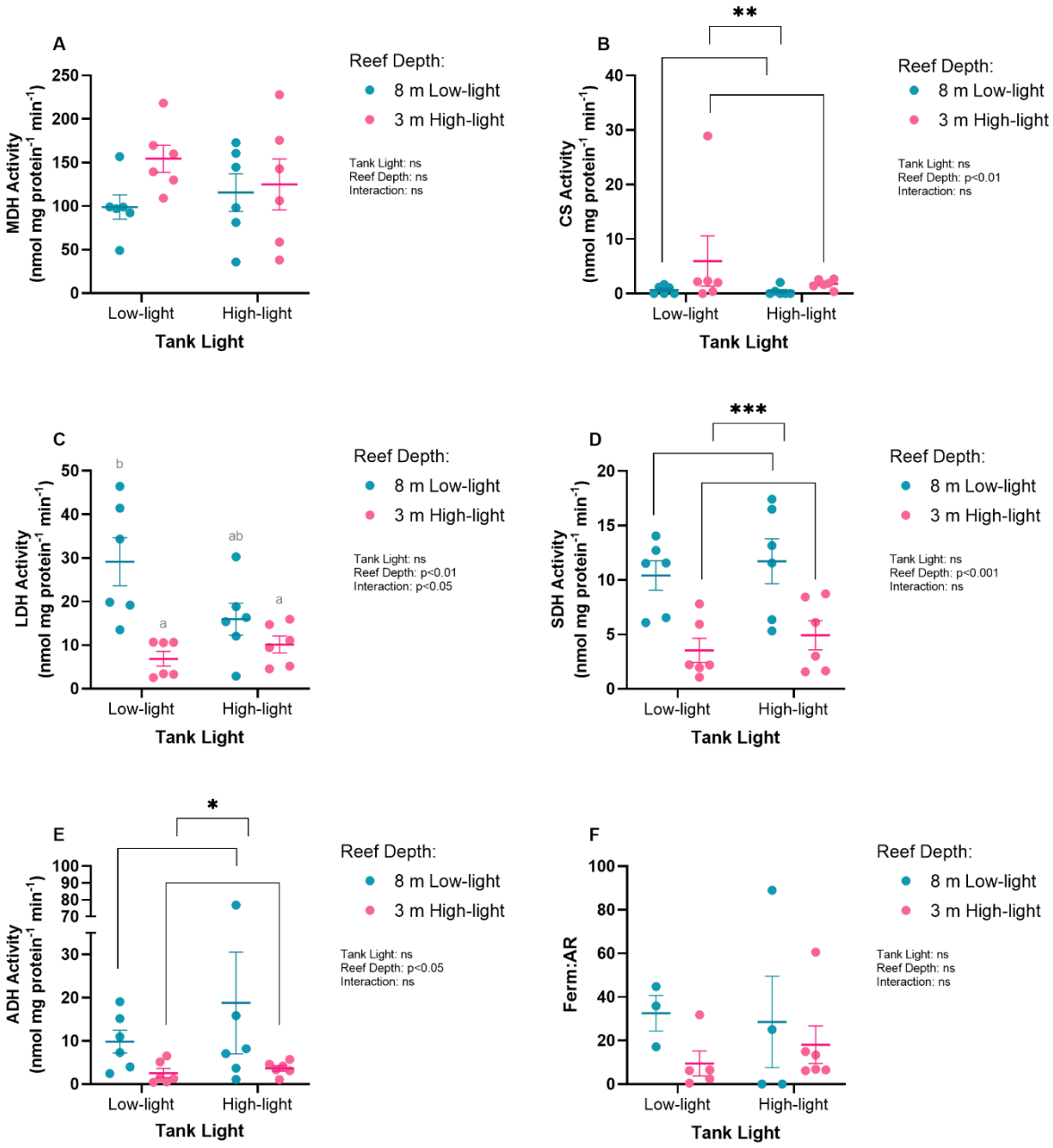

Figure S6.2: Metabolic activity in *Porites astreoides* collected at 3 m and 8 m from Punta Caracol and placed in low-light and high-light aquaria, including outliers.

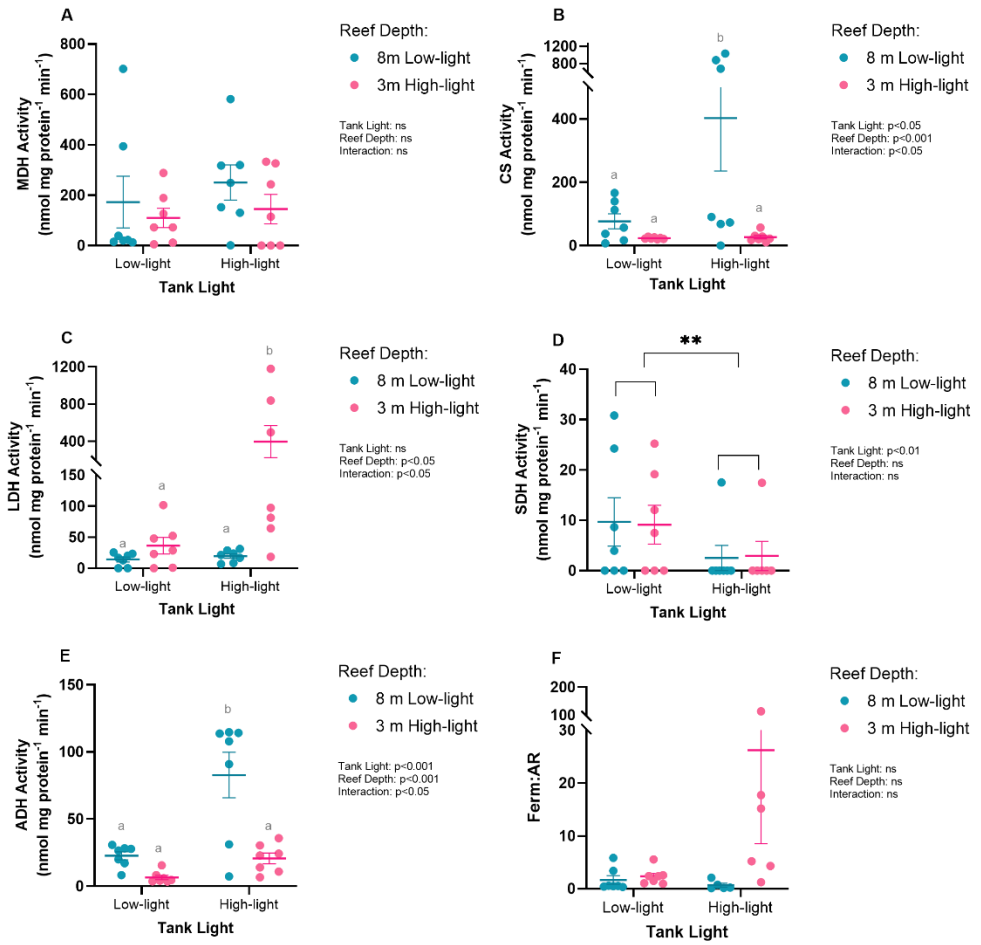

Figure S7: Metabolic activity in *Orbicella franksi* collected at 3 m and 8 m from Punta Caracol and placed in low-light and high-light aquaria, including outliers.

Table S1: Fermentation activity over aerobic activity (Ferm:AR) ratios for *P. astreoides* and *O. franksi* collected at 3 m and 8 m from Punta Caracol and placed in low-light and high-light aquaria. Ratios include outliers.

Coral Species	Reef Depth	Tank Light	LDH + SDH + ADH (nmol mg protein^{-1} min^{-1})	CS Activity (nmol mg protein^{-1} min^{-1})	Enzyme Activity Ratio
Porites astreoides	Low-light (8m)	Low-light	16.5 ± 2.9	0.66 ± 0.31	24.8 ± 9.5 (ab)
		High-light	15.5 ± 3.9	0.41 ± 0.33	37.4 ± 11.8 (b)
	High-light (3m)	High-light	6.2 ± 1.0	1.7 ± 0.34	3.5 ± 2.9 (a)
		Low-light	4.3 ± 0.8	5.9 ± 4.6	0.72 ± 0.18 (a)
Orbicella franksi	Low-light (8m)	Low-light	15.5 ± 2.5	76.8 ± 23.7	0.2 ± 0.1 (b)
		High-light	34.9 ± 9.5	402.9 ± 167.4	0.086 ± 0.056 (b)
	High-light (3m)	High-light	140.0 ± 68.2	26.7 ± 5.6	5.2 ± 12.1 (a)
		Low-light	17.4 ± 5.4	23.5 ± 1.2	0.74 ± 4.6 (ab)

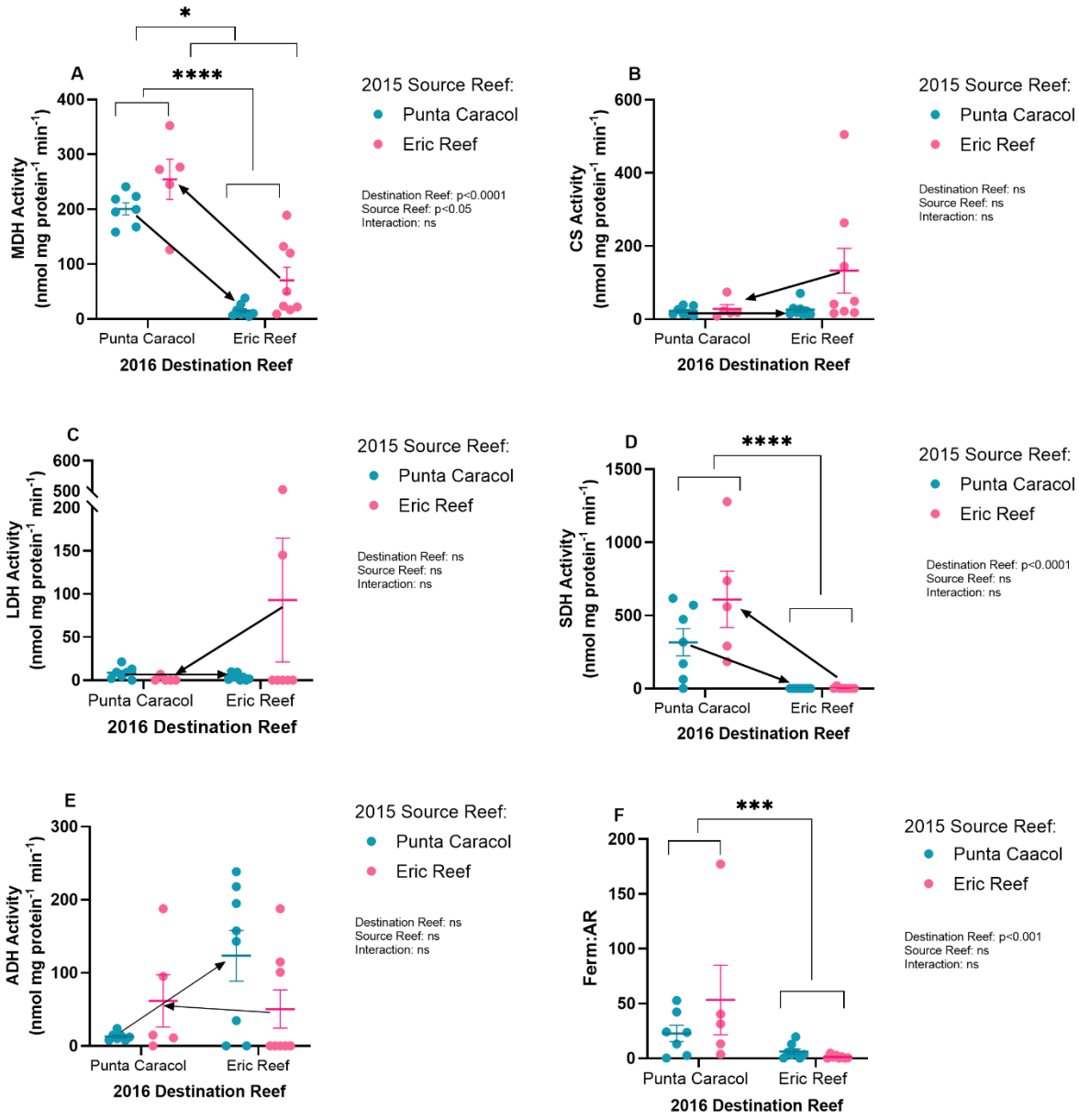

Figure S8: Metabolic enzyme activities in *Porites astreoides* collected at Punta Caracol and Eric's Reef, and reciprocally transplanted between those sites, including outliers.

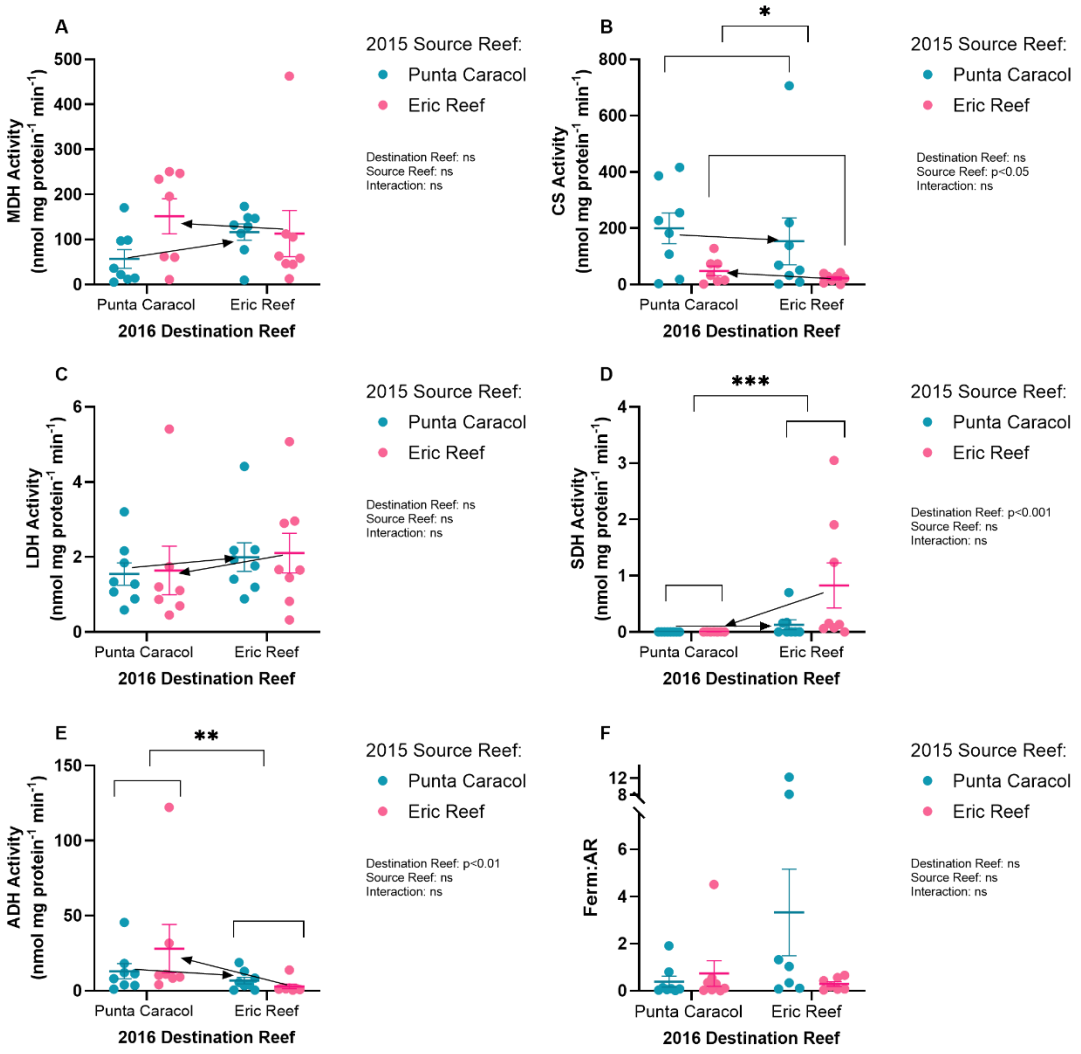

Figure S9: Metabolic enzyme activities in *Orbicella franksi* collected at Punta Caracol and Eric's Reef, and reciprocally transplanted between those sites, including outliers.

Table S2: Fermentation activity over aerobic activity (Ferm:AR) ratios for *P. astreoides* and *O. franksi* transplanted to Eric Reef and Punta Caracol. Ratios include outliers.

Coral Species	Original Reef	Destination Reef	LDH + SDH + ADH (nmol mg protein^{-1} min^{-1})	CS Activity (nmol mg protein^{-1} min^{-1})	Enzyme Activity Ratio
Porites astreoides	Punta Caracol	Punta Caracol	112.4 ± 43.6	22.2 ± 4.6	5.1 ± 9.5 (cb)
		Eric Reef	42.3 ± 16.3	26.2 ± 6.8	1.6 ± 2.4 (ab)
	Eric Reef	Eric Reef	44.7 ± 22.8	132.6 ± 61.3	0.33 ± 0.37 (a)
		Punta Caracol	224.3 ± 95.0	28.3 ± 11.7	7.9 ± 8.1 (c)
Orbicella franksi	Punta Caracol	Punta Caracol	5.3 ± 1.9	199.6 ± 54.4	0.02 ± 0.03
		Eric Reef	11.4 ± 5.8	48.1 ± 17.3	0.24 ± 0.33
	Eric Reef	Eric Reef	1.9 ± 0.57	22.5 ± 5.4	0.08 ± 0.1
		Punta Caracol	2.9 ± 0.94	153.5 ± 83.1	0.02 ± 0.01

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