PHYSIOLOGICAL AND IMMUNOLOGICAL ACCLIMATION IN CORALS DIFFERING IN LIFE-HISTORY TRAITS: A FIELD EXPERIMENT

By

Fabiola Rivera Irizarry

A thesis submitted to the
DEPARTMENT OF BIOLOGY
FACULTY OF NATURAL SCIENCES
UNIVERSITY OF PUERTO RICO
RÍO PIEDRAS CAMPUS

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2020

Río Piedras, Puerto Rico

This thesis has been accepted by the faculty of the

DEPARTMENT OF BIOLOGY
FACULTY OF NATURAL SCIENCES
UNIVERSITY OF PUERTO RICO
RÍO PIEDRAS CAMPUS

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Master thesis committee:

_____
Alberto M. Sabat, Ph.D.
Advisor

_____
James Ackerman, Ph.D.

_____
Eduardo Rosa-Molinar, Ph.D.

_____
Misaki Takabayashi, Ph.D.

PHYSIOLOGICAL AND IMMUNOLOGICAL ACCLIMATION IN CORALS DIFFERING IN LIFE-HISTORY TRAITS: A FIELD EXPERIMENT

A mi hijo, mi mamá, mi tía,
mi papá, mis hermanos,
y todos los que siempre creyeron en mí

ACKNOWLEDGMENTS

First of all, I would like to thank my dear son Fabián, for being my everyday motivation to achieve this goal. Very special thanks to my beloved mother Monín Irizarry, my aunt Tita Irizarry, my father Santiago Rivera, my sister Zamarie and my brothers Christopher and Ralphy for the constant help and support throughout my graduate studies. This achievement would have not been possible if it was not for their unconditional love and support.

I am deeply grateful to my thesis committee, Alberto Sabat, Eduardo Rosa-Molinar, James Ackerman and Misaki Takabayashi, for their patience throughout the years, for helping me become a better scientist and for sticking with me until the end of my master's degree journey.

Fieldwork was possible thanks to the logistical support of the NGO Sociedad Ambiente Marino staff and volunteers. Special thanks to Alex Mercado, Samuel Suleimán, Pedro Alejandro and Gero Cabrera. Laboratory work was possible thanks to the enormous contribution of my BIOL 4990 students, specially Giannina Aponte and Yesenia Bruno, and the unconditional help from laboratory technicians Jaime S. Fonseca and Miosotis Alicea. Statistical analyses were possible thanks to Edwin Hernández's teachings.

Funding for this thesis was provided by the NSF PRCEN HRD-1137725 and the Decanato de Estudios Graduados e Investigación (DEGI) Título V/PPOHA grants. Additional funding was provided by the Department of Biology of UPR-RP.

TABLE OF CONTENTS

ABSTRACT

Corals can exhibit much variability in life-history strategies. A proposed categorization recognizes competitive, stress-tolerant and weedy species. 'Competitive' corals invest more energy towards growth, 'stress-tolerant' towards maintenance and 'weedy' towards reproduction. While competitive corals are declining due to climate change and anthropogenic stressors, stress-tolerant and weedy are thriving. We performed a reciprocal transplant experiment between a degraded and a more pristine reef, and measured the physiological (*Symbiodinium* density, chlorophyll *a* concentration, protein concentration) and immunological (oxidative stress) response of the stress-tolerant *Pseudodiploria strigosa* and *Orbicella annularis,* and the weedy *Porites astreoides*. Results indicate that the studied species can rapidly and successfully acclimate to local environmental conditions. Comparisons among species suggest that the physiological and immunological response in *P. astreoides*, *P. strigosa* and *O. annularis* are more influenced by species-specificity than by life-history strategy.

Keywords: coral; *Porites astreoides*; *Pseudodiploria strigosa*; *Orbicella annularis*; *Symbiodinium*; acclimation; physiology; immunology; oxidative stress

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Coral reefs are highly productive and biodiverse ecosystems that provide vital ecological services such as protection to coasts against wave energy, habitat to countless commercial and ecologically important species, eco-tourism and recreation (Crossland et al., 1991). A recent study by Spalding, et al. (2018) estimated an overall value of all reef-associated tourism at over $7.9 billion per year in the Caribbean, with Puerto Rico and Dominican Republic being the countries with the most visitor expenditure (over a billion dollars together) per year, directly linked to coral reefs. Despite their ecological, economic and social importance, coral reefs are experiencing a worldwide decline (Hughes et al., 2003) due to chronic and acute biological, physical and anthropogenic stressors. An example of a significant biological stressor is the increase in occurrence and severity of diseases that have been documented in the last decade (Pollock et al., 2011, Pinzón et al., 2014, Vega-Thurber et al., 2014, Lamb et al., 2014, Buerger and van Oppen, 2018, Gintert et al., 2019). Physically, Caribbean corals are susceptible to strong wave action product of hurricanes which can cause dislodgement and breakage of colonies (Edwards et al., 2011). And anthropogenically, land-dwelling sources of pollution such as run-off, discharge of used waters, dredging and poorly planned coastal development are likely to introduce pathogens (Pandey et al., 2014, Maynard et al., 2015, Haapkyla et al., 2011) and increase nutrients (Wiedenmann et al., 2012, D'Angelo and Wiedenmann, 2014), sediment and turbidity (Pollock et al., 2014) to the water column which can be all prejudicial to coral health and survival (Richmond et al., 2018).

Due to these stressors, the Caribbean region alone has suffered a reduction of approximately 80% of coral cover, resulting in the alteration of community structure and ecosystem dynamics (Gardner et al., 2003). More specifically, the loss of key herbivores mainly due to land-based pollution and

overfishing coupled with recent massive coral bleaching events due to rise in sea surface temperature have caused the shift of many coral dominated reefs to turf and macroalgae dominated ones. (Hughes et al., 2003).

The dire global state of coral reefs has led marine scientists to develop management strategies with the goal of restoring coral reef ecosystem function. Efforts such as watershed management (Bartley et al., 2014), reef restoration through coral farming (Hernández-Delgado et al., 2014, Lirman & Schopmeyer, 2016), the designation of marine protected areas (Lundquist et al., 2005), the designation of no-take zones (Hernández-Delgado et al., 2000) and local fishing regulations (Hilborn, 2016) are examples of the strategies that have been and are currently being implemented to try to stop and reverse the deterioration of coral reefs. Nevertheless, in a world of changing climate conditions and continuous anthropogenic pressure, it has become extremely difficult to manage reef corals due to their susceptibility to environmental stressors that are difficult, if not impossible, to mitigate such as light irradiance, sea surface temperature, and pH. Hence, the significant decline of coral cover worldwide demands marine scientists to develop more informed management strategies that take into consideration corals' response to environmental stress. For this reason, scientists have become interested in the study of corals' stress response at the physiological and immunological level to try to predict the fate of coral reefs in the near future (Mydlarz et al., 2009, 2010, Palmer et al., 2009, 2011a, 2011b, Palmer and Taylor-Knowles, 2011, Mydlarz and Palmer, 2011, Pinzón et al., 2014a, 2014b). In this master thesis project, I aim to contribute to these areas of research by assessing corals' response to fluctuations in light irradiance and water quality due to anthropogenic factors such as the ones mentioned above. Below, I will

describe general aspects of coral physiology, immunology, and resource allocation as an introduction to stress response dynamics.

Physiological aspects

Corals are extremely sensitive to variability in abiotic factors such as water temperature and pH. However, some degree of physiological plasticity may allow them the capacity to acclimatate and survive to environmental changes. The high dependency of these organisms on sunlight makes it particularly essential to possess light-dependent plasticity. In this sense, reef-building (scleractinian) corals possess a symbiotic relationship with the unicellular algae *Symbiodinium spp*. In such relationship, the coral provides shelter to the symbionts within the endodermal cells and in exchange, the algae supply the coral with organic compounds produced by photosynthesis. These compounds provide up to 90% of the energy required for the host's metabolic functions (Muscatine, 1974, Sumich, 1996, Lesser, 2013). Thus, any disturbance that alters *Symbiodinium spp*. functionality such as changes in light irradiance, temperature, carbon dioxide availability and nutrient concentration is likely to affect coral reproduction (Fabricius, 2005), larval settlement and survival (Babcok & Smith, 2000, Fabricius, 2005, Richmond et al., 2018), calcification (Dodge & Vaisnys, 1977), and overall fixation of nitrogen (Radecker et al., 2015) and carbon (Perry et al., 2013). For example, a major threat to corals and one of the main sources of stress is the reduction of light irradiance due to increased turbidity resulting from anthropogenic activities such as unregulated coastal development. Shading as a result of turbidity has been suggested to disturb the mutualistic relationship with *Symbiodinium spp*. by negatively affecting their photosynthetic yield as well as cell density (Phillip & Fabricius, 2003). Likewise, increases in light irradiance may also induce photoinhibition (Franklin et al., 1996, Ralph et al., 1999, Anthony and Hoegh-Guldberg,

2003). When this occurs, the overall photosynthetic capacity of the alga is reduced due to damage in photosystem II. In the photosynthetic process, the photosystems' functions are light absorption and electron transfer. The photosystem II is particularly more sensitive to light than the rest of the photosynthetic apparatus, thus plants, algae and cyanobacterium living under elevated light are continuously investing energy in reversible structural and functional adjustments in their photosynthetic apparatus (Powles, 1984, Anderson, 1986, Barber and Anderson, 1992, Neidhardt et al., 1998). Under chronic stress, however, damage to the photosystem II can become lethal and corals may be obligated to expel their symbionts, also known as coral bleaching. Nonetheless, the degree of photoinhibition and the capacity of repair in *Symbiodinium spp*. is species/clade-specific. For instance, it has been demonstrated that as a response to stress, *Symbiodinium* can photo-acclimate to down-shifts and up-shifts in light irradiance to maximize light utilization (MacIntyre et al., 2002). In this regard, there are six *Symbiodinium* clades identified in coral (A, B, C, D, F, and G) (Baker, 2003), where clade C is known to better adapt to a wide range of irradiances and temperatures and clade D is known to be thermally more resistant in comparison to others (LaJeunesse, 2018). Different species tend to possess different clade combinations (Baker, 2003, Jones et al., 2008). This difference is sometimes also seen between colonies from the same species and even within a colony. The presence of different types of *Symbiodinium* has been suggested to be the reason why corals have different photosynthetic rates (Hennige et al., 2011).

Photosynthetic yield can also depend on the amount of light irradiance available for absorption. For example, Anthony and Hoegh-Guldberg (2003) demonstrated that corals within the same reef but growing in different habitat types such as caves or open habitats differ in daily photosynthetic rates due to differences in light irradiance. As part of that study, they conducted a reciprocal transplantation that resulted in the successful acclimation and survival of colonies. And

demonstrating *Symbiodinium*'s capacity to photo-acclimate. Chlorophyll *a* concentration plays an important role in such acclimation given that in many microalgae, the rate of light absorption is largely correlated with chl *a* content (Eppley and Sloan, 1966). Indicating that the level of resilience of *Symbiodinium* cells to stress is crucial in coral survival. Nevertheless, the success of any symbiotic relationship depends on the contribution of both species involved. Hence, the coral host has a series of metabolic responses to stress which, depending on the type of environmental pressure, include up or down-regulation of heat-shock proteins (Olsen et al., 2013, Bellantuono et al., 2012), up or down-regulation of nitrogen and carbon fixation (Fabricius, 2005), immune-related responses (Mydlarz et al., 2009, Mydlarz et al., 2010, Palmer et al., 2011a, 2011b, Soderhall & Cerenius, 1998, Asokan et al., 1997, Sugumaran, 2002, Muñoz et al., 2006, Perdomo-Morales et al., 2007), among others.

Immunological aspects

Immunity is an essential component in the coral physiological response to stress and pathogens (Mydlarz et al., 2010). Coral, in particular, are similar to other invertebrates and even humans in that their immune response is cellular and humoral (Mydlarz et al., 2009, Mydlarz et al., 2010, Palmer et al., 2011a, 2011b, Soderhall & Cerenius, 1998, Asokan et al., 1997, Sugumaran, 2002, Muñoz et al., 2006, Perdomo-Morales et al., 2007) and is composed of three phases: recognition, signaling and effector cascades (Pinzón et al., 2014). Some signaling mechanisms are activated in the presence of foreign pathogens. However, other mechanisms such as effector cascades may not necessarily be exclusively active due to pathogens (Pinzón et al., 2014). For example, immune responses have also been detected in corals affected by shading and sedimentation (Sheridan et al., 2014), physical damage (Horricks et al., 2019), and UV radiation (Wall et al., 2018). Effector

cascades include the initiation of the melanin synthesis cascade through the conversion of prophenoloxidase (PPO) enzyme to its active form, phenoloxidase (Mydlarz et al., 2010, Palmer & Traylor-Knowles, 2012, Pinzón et al., 2014b). Melanin has been known to be involved in wound healing (Meszaros & Bigger, 1999), protection against pathogen invasion (Mucklow, 2004), and protection against UV radiation (Brenner & Hearing, 2008). PPO is responsible for the general coordination of immune responses, pathogen and parasite resistance, and tissue regeneration (Palmer et al., 2010).

The activation of the melanin synthesis cascade implies the production of intermediate cytotoxic radicals known as reactive oxygen species (ROS) or oxyradicals which provide antimicrobial defense in cell phagocytosis and play a key role in PPO and cellular apoptosis signaling (Nappi & Ottaviani, 2000, Mydlarz et al., 2009). The main ROS produced during these processes include superoxide anions (O_2^-) hydrogen peroxide (H_2O_2), hydroxyl radicals (^-OH), peroxyl radicals (ROO^-), alkoxyl radicals (RO^-) and peroxynitrite (HOONO) (Regoli et al., 2000). Although ROS are produced as intermediates in normal metabolism, significant increases can become harmful to coral and lead to oxidative stress which can trigger damage to biological molecules such as lipids, proteins and DNA, tissue necrosis, cellular apoptosis and eventually the expulsion of *Symbiodinium* (Lesser, 2006, 2011, Rotcher and Ostrander, 2011). Another source of harmful ROS comes from *Symbiodinium* during thermal stress (Flores-Ramírez and Liñán-Cabello, 2007, Palmer et al., 2009, Olsen et al., 2013) and increased UV radiation (Regoli et al., 2000, Smith et al., 2013). Above-average light irradiance (Regoli et al., 2000) and sea surface temperatures (Richter et al., 1990) can induce damage in the photosystems I and II resulting in the production of primary free radicals such as superoxide anions and peroxide that can diffuse to the cytoplasm

of coral cells (Flores-Ramírez and Liñán-Cabello, 2007). These harmful ROS can be experimentally identified, among other ways, through the enzymatic activity of antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Murphy et al., 2019). Antioxidants are defense mechanisms that are activated by the immune system to scavenge ROS and thus prevent oxidative stress and bleaching (Downs et al., 2002, Flores-Ramírez & Liñán-Cabello, 2007). In eukaryotes, the SOD enzyme, which contains Zinc (Zn^{+2}) or Copper (Cu^{+2}), catalyzes the dismutation of the toxic superoxide anion (O_2^-) into molecular oxygen (O_2) or the less toxic hydrogen peroxide (H_2O_2). This is done through a redox reaction where O_2^- is oxidized and Cu^{+2} is reduced. Zn/Cu SOD is available commercially as a bovine serum with a dye that turns blue when Cu^{+2} is reduced in presence of O_2^-, indicating enzymatic activity of SOD (Pinzón et al., 2014a). The resulting H_2O_2 is subsequently degraded by CAT or GPx into O_2 and water ($2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$). Therefore, SOD, CAT and GPx are crucial oxidizing agents in cellular metabolism to prevent oxidative damage.

Life-history strategies and resource allocation

The energy budget of an organism is allocated amongst growth, reproduction, maintenance and storage (Homyack, 2010). Theoretically, the energy allocated to one function cannot be used for another (Homyack, 2010). Environmental disturbances that negatively affect an individual may induce a decrease in overall budget or the relocation of energy towards maintenance, for example, causing a reduction in growth, reproduction and/or storage (Stearns, 1992, Homyack, 2010). Under stressful conditions, corals relocate energy resources to maintain immune system functions (Pinzón et al., 2014a, Mydlarz et al., 2010) and upregulate survival strategies such as melanin synthesis (Mydlarz et al., 2010) and mucus production (Sheridan et al., 2014). There is evidence

that the latter comes to the expense of other vital functions such as growth and reproduction (Pinzón et al., 2014a, 2014b, Brown & Bythell, 2005, Telesnicki & Goldberg, 1995). For instance, direct contact of coral tissue with settled sediment can induce tissue damage by abrasion (Sheridan et al., 2014). Corals respond to this type of tissue damage by activating the melanin synthesis cascade and upregulating the production of a polysaccharide mucus membrane that protects its tissue and eventually removes sediment particles (Edmunds and Davis, 1989). A study suggested that such increase of mucus production in *Porites porites,* as a response to sedimentation stress, resulted in a lower energy investment for growth (Edmunds and Davis, 1989). Certainly, these physiological costs vary among species mainly because corals are generally understood to allocate energy according to their life-history strategy (Darling et al., 2012). Such strategies are what make corals resistant or susceptible to changing environmental conditions and comprise the biological basis of resilience. For example, corals that invest less energy on maintenance are more likely to be susceptible to stress, disease and bleaching, while corals that do the opposite are recognized to be more resilient to external stressors (Palmer et al., 2010, Darling et al., 2012, Pinzón et al., 2014a, 2014b). Based on these observations, a study of hierarchical cluster analysis of 143 species, divided scleractinian corals in three main groups according to life-history strategy: competitive, stress-tolerant and weedy (Darling et al., 2012). Corals from these groups differ in many aspects, such as taxonomy, morphology and type of sexual reproduction. The selection of one strategy or the other implies a series of trade-offs that can influence energy budget, demography and population biology (Table 1). For instance, competitive corals are fast-growing branching or plating species that reproduce by broadcast spawning. These corals are capable of competing for resources by creating large canopies and shading out other species. In the Caribbean, members from this group include *Acropora palmata* and *Acropora cervicornis*. These types of corals invest

most of their energy towards growth but at the expense of maintenance (Fig. 1). As a result, they are highly susceptible to environmental changes, thus, can only be dominant under ideal water-quality conditions (Darling et al., 2012, Mercado-Molina et al., 2015, Paradis et al., 2019). The second group is characterized by longer generation times and mass spawning species such as the Caribbean *Pseudodiploria strigosa* and *Orbicella annularis*. This type of life-history strategy promotes high maintenance and energy storage which the coral can use to survive in unfavorable conditions or reproduce massively under favorable ones, but at the expense of slow growth. The third group denominated as weedy are mostly small opportunistic brooding colonies (Chornesky and Peters, 1987), such as species from the genus *Agaricia, Madrasis* and Atlantic *Porites*. These types of coral invest most of their energy towards brooding reproduction and maintenance, at the expense of small colony size. These traits make them capable of colonizing recently disturbed habitats in a timely manner, mainly because of their high investment towards brooding

Table 2. Life-history strategy trade-offs of studied species.

Life-history strategy	**Competitive**	**Stress-tolerant**	**Weedy**
Morphology	branching	domed	domed
Size	large	large	small
Growth rate	fast	slow	slow
Sexual reproduction	spawning	spawning	brooding
Reproductive cycles	1	1	9-12
Reproductive output per cycle	high	high	low
Recruitment rate	low	low	high
Tolerance to environmental disturbance	low	high	high

*Szmant, 1986, Darling et al., 2012

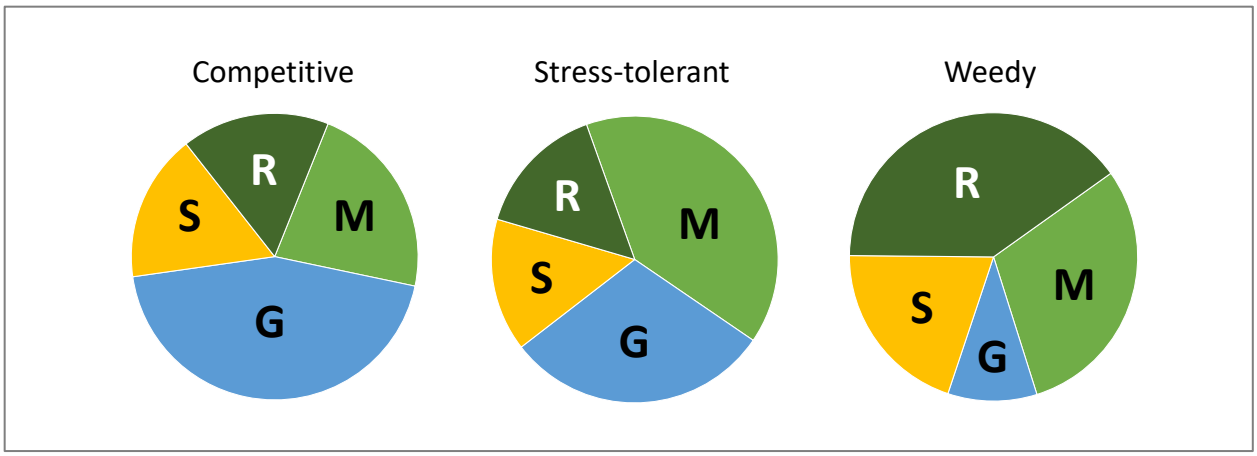

Figure 1. Estimated energy budget distribution of competitive, stress-tolerant and weedy corals based on Homyack, 2010 and Darling et al., 2012. R = reproduction, M = maintenance, G = growth and S = storage.

reproductive strategy and maintenance. In this regard, brooding reproduction is known to demand more energy than spawning (Szmant, 1986). In essence, broadcast spawning corals, release both, eggs and sperm, in a massive spawning event that typically happens once a year, external fertilization occurs and larvae develop in the water column independent of the parental coral (Szmant, 1986). While corals that brood, release sperm to the water column and retain their eggs in their gonads where internal fertilization takes place (Szmant, 1986, Harrison & Wallace, 1990, Harrison, 2010). The larva then develops within the polyp where more than 60% of the requirements for growth are obtained vertically from the mother coral (Stake & Sammarko, 2003, Zhang et al., 2009). This includes proteins, carbohydrates, lipids, endosymbionts and chlorophyll pigments (Zhang et al., 2009). Once maturity is reached, the larvae are released to settle in the substratum (Szmant, 1986, Harrison & Wallace, 1990, Harrison, 2010). This process generally occurs several times a year following lunar periodicity. Even though spawning is the most dominant mode of reproduction known among corals (Harrison, 2011), the brooding strategy increases the likelihood of larval survival and the chances of a successful settlement in appropriate substrate by spending less time in the water column and traveling shorter distances (Szmant, 1986).

This allows higher local recruitment rates in comparison to broadcast spawners (Szmant, 1986). Nevertheless, brooding corals are disposed to inbreeding due to self-fertilization and proximity to the mother coral, thereby diminishing genetic diversity (Ayre and Hughes, 2000, Nishikawa et al., 2003).

Although corals, in general, are sensitive to environmental changes, their life-history strategy coupled to their physiological plasticity will likely influence their chances of survival (Mydlarz et al., 2010). This is why, given the higher susceptibility of competitive corals to stress, scientists are anticipating alterations in future community structure by losing competitive corals and dominating stress-tolerant and weedy species (Green et al., 2008). For this reason, with this project, I seek to better understand stress response in weedy and stress-tolerant corals by assessing immune and physiological dynamics. Corals' physiological and immunological responses to different types of stress are usually assessed under controlled laboratory conditions (Gleason, 1993, Iglesias-Prieto and Trench, 1994, Phillip and Fabricius, 2003, Flores-Ramírez and Liñán-Cabello, 2006, Palmer et al., 2009, Mydlarz and Palmer, 2011, Sheridian et al., 2014, Pinzón et al., 2014a, 2014b, Hawkins et al., 2015). However, experimental studies under actual water quality conditions *in situ* are scarce and information available on physiological and immunological dynamics of corals differing in life-history strategy is also very limited. This is why, in this project, I decided on a field-based experiment where I induced stress in corals by performing a reciprocal transplantation between two reefs contrasting in water quality and turbidity, with the overall objectives of evaluating **(1) physiological adaptations of stress-tolerant and weedy corals in stressful environments** and **(2) whether life-history strategy influences physiological plasticity and immunological response to changes in light irradiance and water-quality.**

The species used as case study were: *Porites astreoides* (weedy), *Pseudodiploria strigosa* (stress-tolerant) and *Orbicella annularis* (stress-tolerant) (Fig. 2). If the response of these species to transplantation is influenced by life-history strategy, then data from *P. strigosa* (stress-tolerant)

Figure 2. Studied species. A, *Porites astreoides* (weedy); B, *Pseudodiploria strigosa* (stress-tolerant); C, *Orbicella annularis* (stress-tolerant).

and *O. annularis* (stress-tolerant) should be similar, and *P. astreoides* (weedy) should differ significantly from both species. *O. annularis* was particularly selected because although Darling et al. (2012) classify it as a stress-tolerant spawning coral alongside *P. strigosa*, there is evidence that this species is highly susceptible to bleaching (Hernández-Pacheco et al., 2012) and disease (Pinzón et al., 2014b). In fact, this species is enlisted as 'threatened' under the Endangered Species Act since 2014, while *P. astreoides* and *P. strigosa* are classified as 'least concern'. Thus, with *O. annularis* I aim to understand whether it behaves similarly to *P. strigosa* when exposed to differential light stress and contrasting water quality. This research will not only help to understand physiological and immunological responses to stress in corals differing in life-history strategy but will also allow me to comprehend the capacity of acclimation and/or physiological plasticity of corals from the same species but adapted to drastically different water conditions. To my knowledge, this is the first project in the Caribbean where evaluations of these types of stress responses are made *in situ* between two different reefs.

METHODOLOGY

Study sites

The study was performed in two fringing reefs located in Fajardo, Puerto Rico: Cayo Largo (CLA) [18°18'54.04"N, 65°34'56.50"W] and Roncador (RON) [18°20'36.15"N, 65°36'52.49"W] (Fig. 3). These sites were selected because they are similar in that both have moderate topographic relief but differ noticeably in water quality, particularly in turbidity. Cayo Largo is located more offshore, approximately 5 km from Roncador, and possesses noticeably clear waters. Roncador is located inshore and has chronic turbidity likely due to its proximity to the Fajardo River, coastal run-off, plus commercial and recreational boat traffic. The latter was observed (on-site) to drastically resuspend settled sediments every time the ferry to the island municipalities of Culebra and Vieques passed adjacent to RON, limiting greatly the visibility (Fig.4). To quantify the dissimilarities between the two reefs, light irradiance and temperature were measured every minute for two weeks on each site with Onset HOBO light/temperature data loggers (UA-002-64).

Experimental design

The objective of this research was to measure the effect of the different water quality regimes at RON and CLA on one weedy and two stress-tolerant coral species found in both reefs. I performed a reciprocal transplantation where I exposed corals from RON to CLA's water quality and vice versa, to induce stress and assess the physiological and immunological response. On each site, at a depth of 3-5 m, five healthy-looking colonies (no disease or bleaching) of similar size of *Porites astreoides* (weedy), *Pseudodiploria strigosa* (stress-tolerant) and *Orbicella annularis* (stress-tolerant) were carefully collected. Four fragments of at least $5cm^2$ were obtained from Ps=*Pseudodiploria strigosa*, Oa=*Orbicella annularis*) and colony number (1-5). Two out of the

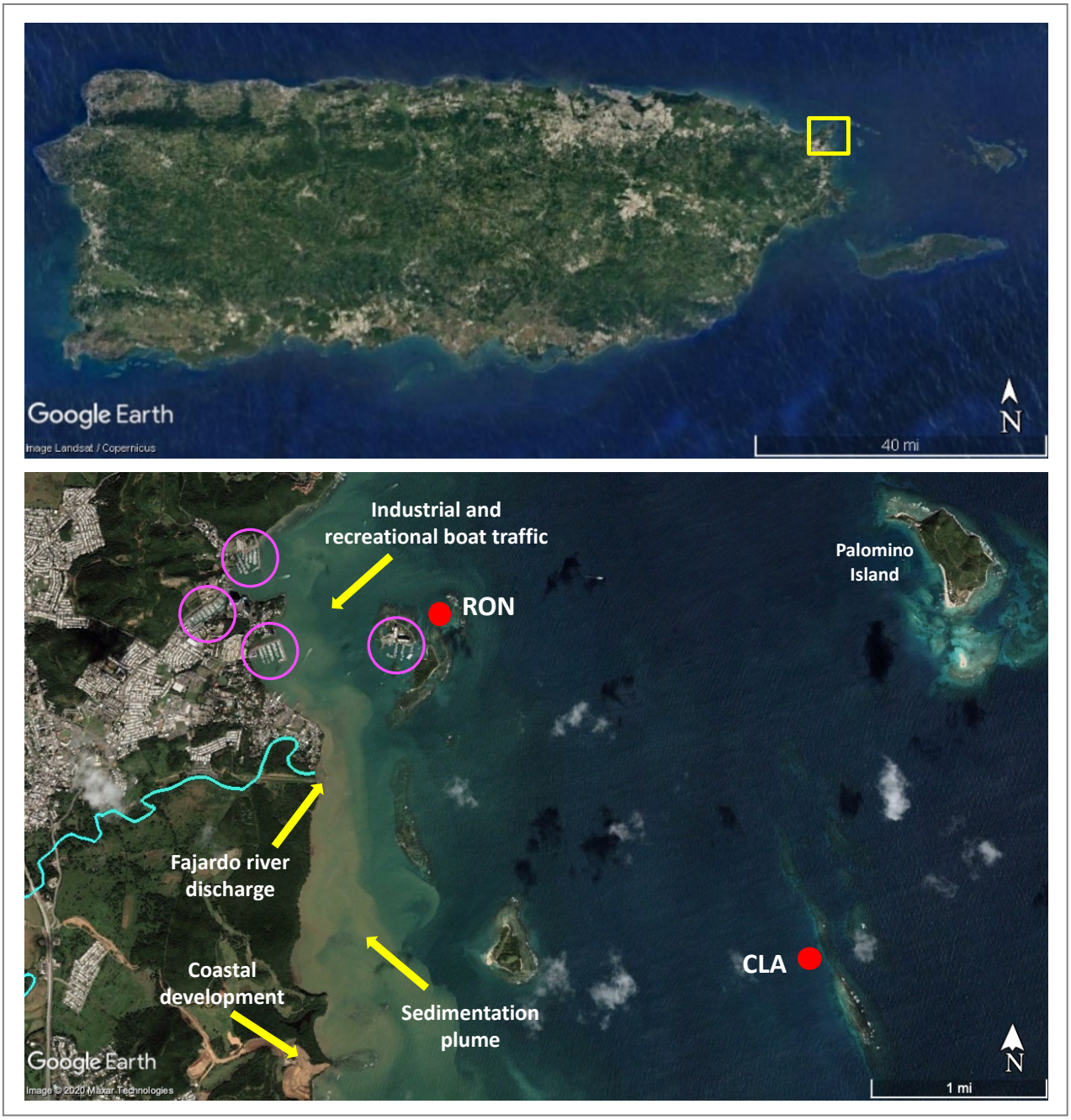

Figure 3. Study sites in Fajardo, Puerto Rico (Top). Bottom: Cayo Largo (CLA) and Roncador (RON) with anthropogenic stressors in yellow arrows and purple circles (marinas). Blue line delineates the Fajardo River path.

Figure 4. Qualitative comparison of contrasting turbidity in CLA (left) and RON (right).

four fragments remained on their reef of origin and classified as ‘control’ while the other two fragments were transplanted to the other reef and classified as ‘transplant’. On each site, a total of 60 fragments (controls and transplants) were randomly placed in two table-like structures (49 x 61 x 32 cm) (Fig. 5) separated by approximately two meters to create a block design. Fragments were left to recover from handling for seven days (Fuess et al., 2017) before sampling to avoid including the effect of fragmentation stress and thus focus on the transplantation effect. A total of three comparisons were evaluated: RON control vs CLA control (turbid vs clear), RON control vs transplant (to CLA) (turbid to clear), and CLA control vs transplant (to RON) (clear to turbid).

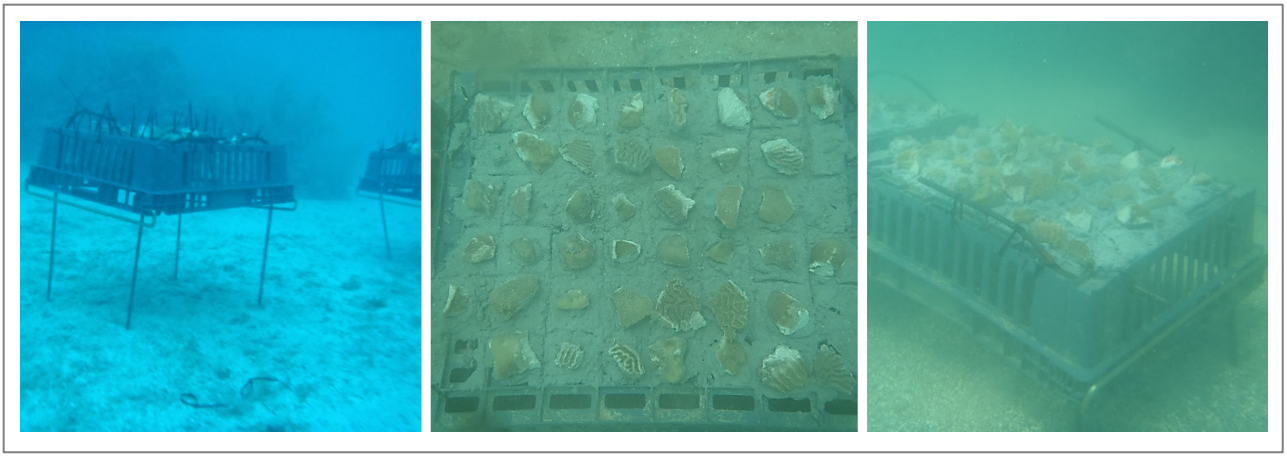

Figure 5. Coral fragments randomly placed in table-like structures.

Sample collection

To evaluate physiological and immunological response I conducted sample collections on both sites on day 7 after transplantation and again on day 28. One control and one experimental (transplant) fragment from each colony of each species were collected randomly. Sampling was carried on a 12:00pm-2:00pm timeframe to normalize for diurnal variability (Griffin et al., 2006). This was done by carefully detaching fragments from the structure and placing them in individually labeled black whirl-pak bags (Sigma-Aldrich) to protect from direct sunlight. Immediately upon collection, samples were frozen in dry ice, brought back to the laboratory and stored in -80˚C until further analysis. A total of 120 fragments (60 control and 60 experimental) were processed for protein concentration, *Symbiodinium spp.* density, chlorophyll *a* concentration and enzymatic activity of superoxide dismutase antioxidant. The remaining 30 fragments that were not sampled were transplanted back to the reef with cement.

Laboratory assays

Tissue preparation. Coral samples were thawed once and tissue was separated from the skeleton using a *Paashe artist's airbrush* (Mydlarz and Palmer, 2011) in combination with sodium

phosphate buffer (PBS, 50mM, pH=7.0) (Pinzón et al., 2014b). Once the skeleton became exposed, the fragments were photographed scale-by-side and surface area was measured with the free license software Coral Point Count with Excel extensions (Köhler and Gill, 2006). The resulting slurry was homogenized and centrifuged at 800rpf to separate coral tissue content from *Symbiodinium* cells. The coral-host tissue content (liquid phase) was used to measure variables related to the host (total protein and antioxidant activity) and the resulting pellet with *Symbiodinium* cells was used to measure cell density and chlorophyll *a* concentration.

Physiology. The physiological stress response was evaluated by measuring *Symbiodinium spp.* density, chlorophyll *a* concentration and total protein concentration. To calculate symbiont density the resulting pellet was resuspended in 5mL of filtered seawater and vortexed, this was the stock sample. A 1mL aliquot was transferred to a microtube and cells were stained and preserved with Lugol iodine solution (Scheufen et al., 2017). Density was determined by cell quantification with a Neubauer hemocytometer (LW Scientific) (n = 16 replicates) and counts were corrected for homogenate volume and coral surface area (Flores-Ramírez and Liñán-Cabello, 2006). Results are presented as No. of cells cm^{-2}. Another 1mL aliquot from the stock sample was obtained for Chl *a* concentration measurement. Samples were centrifuged one pulse at 10,000rpf, or until cells completely separated from the filtered seawater. Afterward, the cells were washed with dH_2O, centrifuged again, dried the most possible and stored at -80°C for 24 hours to break cell walls. Subsequently, 1mL of 100% acetone (Jeffrey & Humphrey, 1975) was added to samples, the pellet was crushed with a fine agitator and then stored for another 24 hours at -4°C to extract chlorophyll *a* from cells. In a 96-well microplate absorbance of samples (3 replicates) was measured at 663 and 630nm (Jeffrey & Humphrey, 1975) with a microplate spectrophotometer (Tecan Infinite 200

PRO). To calculate final Chl *a* concentration, absorbance of 100% acetone at 750nm was subtracted from processed samples (Johan et al., 2014) and I used the equations for dinoflagellates provided by Jeffrey and Humphrey (1975). Results are presented as μg of Chl *a* per cm^2. With these data, I calculated Chl *a* content (in picograms) per *Symbiodinium* cell.

A 1mL aliquot was obtained from the coral tissue (liquid phase) from the initial slurry to measure total protein concentration, which was assessed with the commercially available kit "Pierce BCA Protein Assay Kit" (Thermo Fisher Scientific) following manufacturer's instructions. Briefly, in a 96-well microplate, 25uL of a sample (3 replicates) were mixed with 200uL of bicinchoninic acid (BCA) working reagent for colorimetric detection of total protein. Absorbance was measured at 562nm in a plate reader and protein concentration of unknown samples was determined using a standard curve.

*Immunology***.** For the immunological response, another 1mL aliquot was obtained form coral tissue content and the enzymatic activity of antioxidant superoxide dismutase (SOD) was measured following Pinzón et al., 2014b. Enzymatic activity of SOD was evaluated with the commercially available kit "SOD Determination Kit" (Sigma Aldrich) (Couch et al., 2008, Pinzón et al. 2014b, Mydlarz and Palmer, 2011). This is a colorimetric assay based on the production of a water-soluble formazan dye upon the reduction of Dojindo's highly water-soluble tetrazolium salt with a superoxide anion (O^{2-}). SOD inhibits this reaction by breaking down those anions into H_2O_2 and O_2, thus, enzymatic activity is quantified by measuring the decrease in color development. Briefly, 20uL of sample solution (3 replicates) were placed in a 96-well microplate and mixed with 200uL of working reagent solution. The reaction was induced by adding 20uL of enzyme working

solution and after 20 minutes of incubation (37°C), the absorbance was read at 440nm in a plate reader. Three controls were used as part of this assay. Besides the 200uL of working reagent solution, the first control (blank 1) included 20µL of double distilled water and 20µL of enzyme working solution, the second (blank 2), included 20µL of sample and 20µL of enzyme dilution buffer, and the third (blank 3), included 20µL of double distilled water and 20µL of enzyme dilution buffer. Following manufacturer's instructions, SOD inhibition rate was then calculated with the equation:

$$\frac{(\mathrm{Abs}_{\mathrm{blank1}} - \mathrm{Abs}_{\mathrm{blank3}}) - (\mathrm{Abs}_{\mathrm{sample}} - \mathrm{Abs}_{\mathrm{blank2}})}{\mathrm{Abs}_{\mathrm{blank1}} - \mathrm{Abs}_{\mathrm{blank3}}} \text{ x } 100$$

Results were normalized to milligrams of protein and are presented here as SOD inhibition units.

Statistical analyses

The experimental design allowed me to consider three factors: site (RON, CLA), treatment (control, transplant) and species (*P. astreoides, P. strigosa* and *O. annularis*); and five dependent variables: *Symbiodinium* density, Chlorophyll *a* concentration per cm^2, Chlorophyll *a* content per *Symbiodinium* cell, coral-host protein concentration, and coral-host superoxide dismutase enzymatic activity. Using PRIMER v7 software, comparisons were made for each variable between sites, between treatments, and among species. Data were square-root transformed due to variance heterogeneity and PERMANOVA analyses were performed to determine statistical significance. This test was the best option to analyze the data since it did not comply with the premises of normality, I had a small sample size and, the loss of some samples caused us to have differences in the number of replicates analyzed. Environmental data (light and temperature) was analyzed for differences with Kolmogorov-Smirnov test. Light data in particular was assessed from 6:00am to 6:00pm.

RESULTS

Light and Temperature at RON and CLA

This experiment was conducted between August and September of 2016. Data from On-Set HOBO sensors revealed significant differences in light irradiance between RON and CLA (Kolmogorov-Smirnov, $D=0.2734$, $p<0.001$). RON was documented to receive 43.8% less light irradiance in comparison to CLA ($\bar{X}$=6,623.6 and 11,778 Lux, respectively) (Fig. 6). No significant differences were detected in temperature (Fig 7), both sites averaged 30.2ºC which is typically the highest sea surface temperature of the year in the Caribbean throughout August-September.

Symbiodinium density, chlorophyll a and protein concentration

Turbid vs Clear

After 28 days of transplantation, significant differences among control colonies from RON (turbid) and CLA (clear) were observed in *Symbiodinium* density, chlorophyll *a* concentration and protein concentration ($p<0.05$, Table 2, Fig. 8). These three variables presented considerably higher amounts at RON when compared to CLA (Table 3), with an average of 43.8% more *Symbiodinium* density, 31.7% more Chl *a* concentration, and 19.1% more protein concentration. No differences were observed in Chl *a* per cell indicating that Chl *a* concentration in each site is controlled by cell density rather than pigment content per cell. Interestingly, among species comparisons indicate that *P. astreoides, P. strigosa* and *O. annularis* have a similar density of symbiotic algae but differ in Chl *a* concentration mainly due to differences in Chl *a* per *Symbiodinium* cell (Table 4, Fig. 9). *P. astreoides* exhibited to have higher amounts of Chl *a* per cell, than the other species, while *O. annularis* consistently displayed the lowest concentrations of such pigment. Overall protein

concentration was also found to differ among species were *O. annularis* again showed to have a significantly lower concentration than the rest of the species.

From Turbid to Clear

Transplantation from RON to CLA appeared to only have an effect in Chl *a* concentration (Table 2, Fig.10). A significant 23.9% reduction was observed in corals transplanted to CLA when compared to controls from RON (Table 5). No dissimilarities were detected in symbiont density, Chl *a* per cell nor in protein concentration (Fig. 11). Significant differences among species were observed in symbiont density, Chl *a* per cell (Fig. 12), and protein concentration ($p<0.05$, Table 4, Fig. 13). *P. strigosa* resulted to be the species with the highest *Symbiodinium* density. Although *O. annularis* had lower density, Chl *a* content per cell was similar in both stress-tolerant species, but still significantly lower than *P. astreoides.* In protein concentration, *O. annularis* also demonstrated to be the species with the least protein concentration. These results are similar to the ones mentioned above among control corals from both sites.

From Clear to Turbid

Transplantation from CLA to RON also showed to have an effect in Chl *a* concentration (Table 2, Fig. 10). A significant 24.7% increase was observed in corals transplanted to RON when compared to controls from CLA. Significant differences were also observed in protein concentration, with a 19.1% increase in transplanted corals (Table 6, Fig. 11). When I evaluated pairwise comparisons among species, no dissimilarities were detected in *Symbiodinium* density nor Chl *a* concentration (Fig. 12). However, results from Chl *a* per cell and protein concentration (Fig. 13) were consistent

to those from transplanted corals from the turbid reef to the clear one, *P. astreoides* with the highest Chl *a* per cell, and *O. annularis* with the lowest protein concentration (Table 4).

SOD enzymatic activity

Turbid vs Clear

Significant differences in the enzymatic activity of the antioxidant SOD were observed between control colonies from RON and CLA ($p<0.05$, Table 3, Fig. 8). Turbid water (RON) colonies revealed 28.4% higher activity than those from CLA. Comparisons among species revealed significant differences (Table 4, Fig. 9) where *P. astreoides* and *O. annularis* expressed higher activity of SOD than *P. strigosa*.

From Turbid to Clear

Seven days after transplanting colonies from RON to CLA, a significant difference was detected in SOD activity, suggesting that increase in light irradiance had an effect in the immune response of corals adapted to turbid water (Fig. 11). Surprisingly, once transplanted to clear water, corals reduced drastically their SOD activity by 35% (Table 5). Pairwise comparisons revealed that *P. astreoides* was the species with the highest SOD activity, followed by *O. annularis* and lastly *P. strigosa* with the least activity (Fig. 13).

From Clear to Turbid

Transplanting colonies from CLA to RON resulted in a significant difference in SOD activity, suggesting that a decrease in light irradiance due to an increase in turbidity also has an effect in coral immune response ($p<0.05$, Table 2, Fig. 11). Corals subjected to lower light irradiance had

a significant increase of 34.2% of SOD activity (Table 6). Comparisons among species did not show significant differences in SOD activity, contrary to what I saw in corals transplanted from the turbid reef to the clear one (Fig. 13). However, this was mainly due to higher variance among colonies from each species. Colonies of *P. astreoides* had higher variability (CV = 44.15%) than *P. strigosa* (CV = 37.69%) and *O. annularis* (CV = 24.02%).

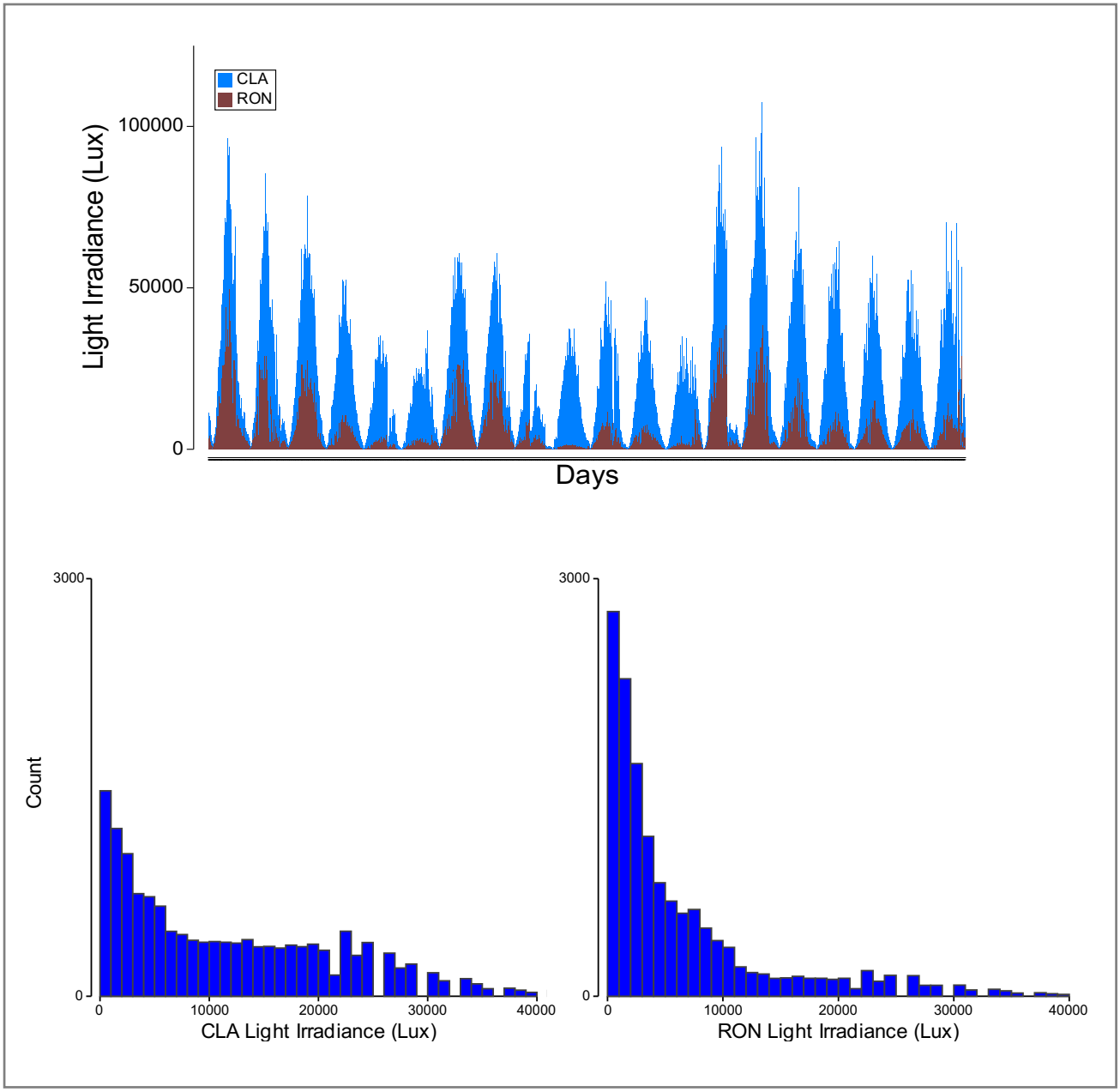

Figure 6. Light irradiance in RON and CLA during the study period. Top: 20 days raw data from 6:00am to 6:00pm. Bottom: Histogram representation of data.

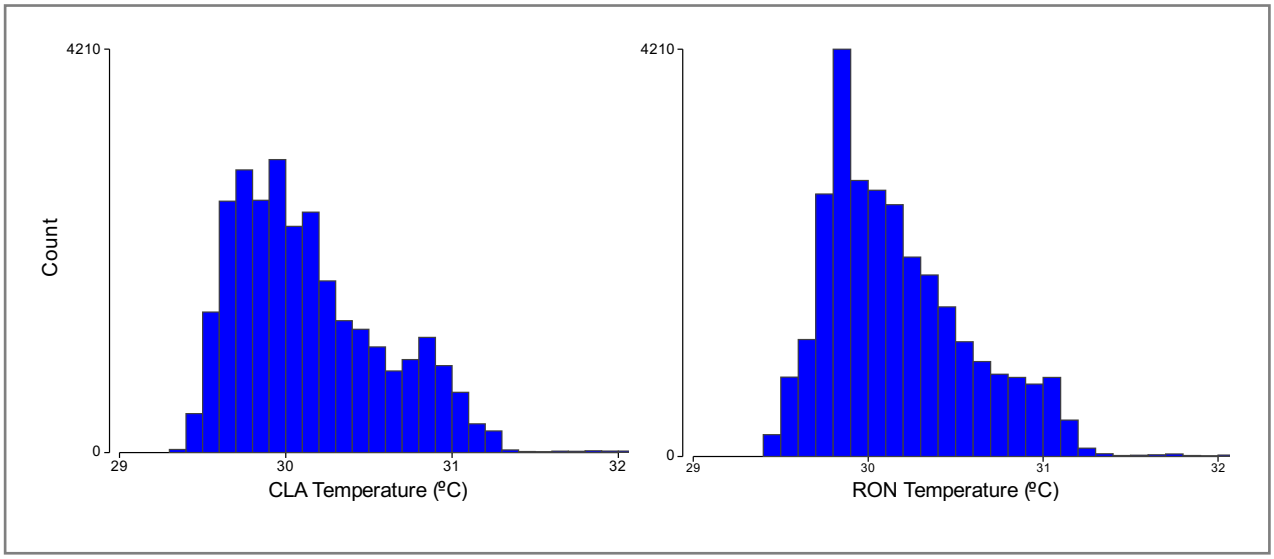

Figure 7. Temperature at RON and CLA during the study period.

Table 2. Results of PERMANOVA analyses for comparisons of physiological and immunological parameters between sites RON (turbid) and CLA (clear) and between control and transplanted colonies.

Parameter	RON vs CLA			RON to CLA			CLA to RON		
	Turbid *vs* Clear			Turbid → Clear			Clear → Turbid		
	df	*Pseudo-F*	*p*	*df*	*Pseudo-F*	*p*	*df*	*Pseudo-F*	*p*
Symbiodinium density	1,54	10.9540	**0.001**	1,54	1.2815	0.260	1,54	0.4269	0.616
Chlorophyll *a* concentration	1,53	10.8450	**0.001**	1,53	4.9670	**0.028**	1,54	4.3936	**0.037**
Chlorophyll *a* per *Symbiodinium* cell	1,53	1.1279	0.290	1,53	0.0850	0.884	1,53	1.7854	0.177
Total protein concentration*	1,53	6.8279	**0.009**	1,53	2.2250	0.131	1,54	3.6385	**0.051**
Superoxide dismutase*	1,22	7.6695	**0.010**	1,24	13.7750	**0.001**	1,22	10.6840	**0.006**

*Note that this parameter corresponds to coral-host

**Significant differences are marked as bold.

***Data was square-root transformed prior to statistical analyses due to variance heterogeinity.

Table 3. Mean ± Standard error values of parameters evaluated among control and transplanted colonies from RON (turbid) and CLA (clear).

Parameter	RON	RON to CLA	CLA	CLA to RON
	Turbid (control)	Turbid → Clear (transplant)	Clear (control)	Clear → Turbid (transplant)
Symbiodinium density (x10^5 cells cm^{-2})	8.10 ± 0.95	8.28 ± 1.51	4.55 ± 0.57	5.07 ± 0.54
Chlorophyll *a* concentration (μg cm^{-2})	2.05 ± 0.20	1.56 ± 0.13	1.40 ± 0.15	1.86 ± 0.18
Chlorophyll *a* per *Symbiodinium* cell (pg)	3.34 ± 0.56	3.04 ± 0.29	3.56 ± 0.42	4.72 ± 0.69
Total protein concentration (mg cm^{-2})*	1.10 ± 0.09	1.01 ± 0.13	0.89 ± 0.10	1.10 ± 0.11
Superoxide dismutase (SOD inhibition units mg $protein^{-1}$)*	9533 ± 919	6193 ± 660	6823 ± 633	10367 ± 853

*Note that this parameter corresponds to coral-host

Table 4. Results of PERMANOVA analyses among species from RON (turbid) and CLA (clear), and from control and transplanted colonies with pairwise comparisons.

Parameter	RON vs CLA				RON to CLA				CLA to RON			
	Turbid *vs* Clear				Turbid → Clear				Clear → Turbid			
	df	*Pseudo-F*	*p*	*Pairwise*	*df*	*Pseudo-F*	*p*	*Pairwise*	*df*	*Pseudo-F*	*p*	*Pairwise*
Symbiodinium density	2,54	2.5636	0.074	Pa = Ps = Oa	2,54	5.6373	**0.004**	Ps **>** Pa = Oa	2,54	0.6548	0.572	Pa = Ps = Oa
Chlorophyll *a* concentration	2,53	3.4716	**0.034**	Pa = Ps **>** Oa	2,53	1.9785	0.137	Pa = Ps = Oa	2,54	2.1301	0.117	Pa = Ps = Oa
Chlorophyll *a* per *Symbiodinium cell*	2,53	7.2196	**0.002**	Pa **>** Ps = Oa	2,53	8.3681	**0.001**	Pa **>** Ps = Oa	2,53	6.1150	**0.004**	Pa **>** Ps = Oa
Total protein concentration*	2,53	10.1130	**0.000**	Pa = Ps **>** Oa	2,53	4.4185	**0.012**	Pa = Ps **>** Oa	2,54	7.7342	**0.001**	Pa = Ps **>** Oa
Superoxide dismutase*	2,22	4.0181	**0.031**	Pa = Oa **>** Ps	2,24	8.8244	**0.001**	Pa **>** Oa **>** Ps	2,22	2.5483	0.099	Pa = Ps = Oa

Key to abbreviations: Pa, *Porites astreoides;* Ps, *Pseudodiploria strigosa;* Oa, *Orbicella annularis*

*Note that this parameter corresponds to coral-host

**Significant differences are marked as bold.

***Data was square-root transformed prior to statistical analyses due to variance heterogeinity.

Table 5. Mean ± Standard error values of physiological and immunological parameters evaluated among control and transplanted species from RON (turbid).

Parameter	RON			RON to CLA		
	Turbid (control)			Turbid → Clear (transplant)		
	Pa	*Ps*	*Oa*	*Pa*	*Ps*	*Oa*
Symbiodinium density (x10^5 cells cm^{-2})	5.80 ± 1.18	10.59 ± 2.27	7.90 ± 0.95	5.32 ± 1.88	14.04 ± 3.36	5.47 ± 1.29
Chlorophyll *a* concentration (μg cm^{-2})	2.22 ± 0.37	2.38 ± 0.44	1.57 ± 0.10	1.37 ± 0.21	1.91 ± 0.23	1.40 ± 0.19
Chlorophyll *a* per *Symbiodinium* cell (pg)	5.52 ± 1.58	2.54 ± 0.31	2.19 ± 0.26	3.83 ± 0.37	1.91 ± 0.31	3.40 ± 0.60
Total protein concentration (mg cm^{-2})*	1.16 ± 0.13	1.16 ± 0.22	0.84 ± 0.05	0.97 ± 0.16	1.30 ± 0.33	0.71 ± 0.11
Superoxide dismutase (SOD inhibition units mg $protein^{-1}$)*	13084 ± 1474	7158 ± 1098	8358 ± 764	7732 ± 1091	4166 ± 624	6681 ± 1146

*Note that this parameter corresponds to coral-host.

Key to species abbreviations: Pa, *Porites astreoides;* Ps, *Pseudodiploria strigosa;* Oa, *Orbicella annularis*.

Table 6. Mean ± Standard error values of physiological and immunological parameters evaluated among control and transplanted species from CLA (clear).

Parameter	CLA			CLA to RON		
	Clear (control)			Clear → Turbid (transplant)		
	Pa	*Ps*	*Oa*	*Pa*	*Ps*	*Oa*
Symbiodinium density (x10^5 cells cm^{-2})	3.72 ± 0.87	5.60 ± 1.14	4.33 ± 0.97	4.73 ± 1.11	5.30 ± 1.06	5.20 ± 0.67
Chlorophyll *a* concentration (μg cm^{-2})	1.53 ± 0.32	1.68 ± 0.27	0.99 ± 0.15	2.18 ± 0.25	1.79 ± 0.45	1.62 ± 0.20
Chlorophyll *a* per *Symbiodinium cell* (pg)	4.61 ± 0.73	2.76 ± 0.31	3.30 ± 0.96	7.00 ± 1.61	3.39 ± 0.65	3.65 ± 0.73
Total protein concentration (mg cm^{-2})*	1.20 ± 0.19	0.99 ± 0.14	0.49 ± 0.07	1.24 ± 0.23	1.24 ± 0.27	0.83 ± 0.06
Superoxide dismutase (SOD inhibition units mg $protein^{-1}$)*	7015 ± 1440	5678 ± 772	7728 ± 657	12591 ± 1571	8155 ± 1337	10354 ± 960

*Note that this parameter corresponds to coral-host.

Key to species abbreviations: Pa, *Porites astreoides;* Ps, *Pseudodiploria strigosa;* Oa, *Orbicella annularis*.

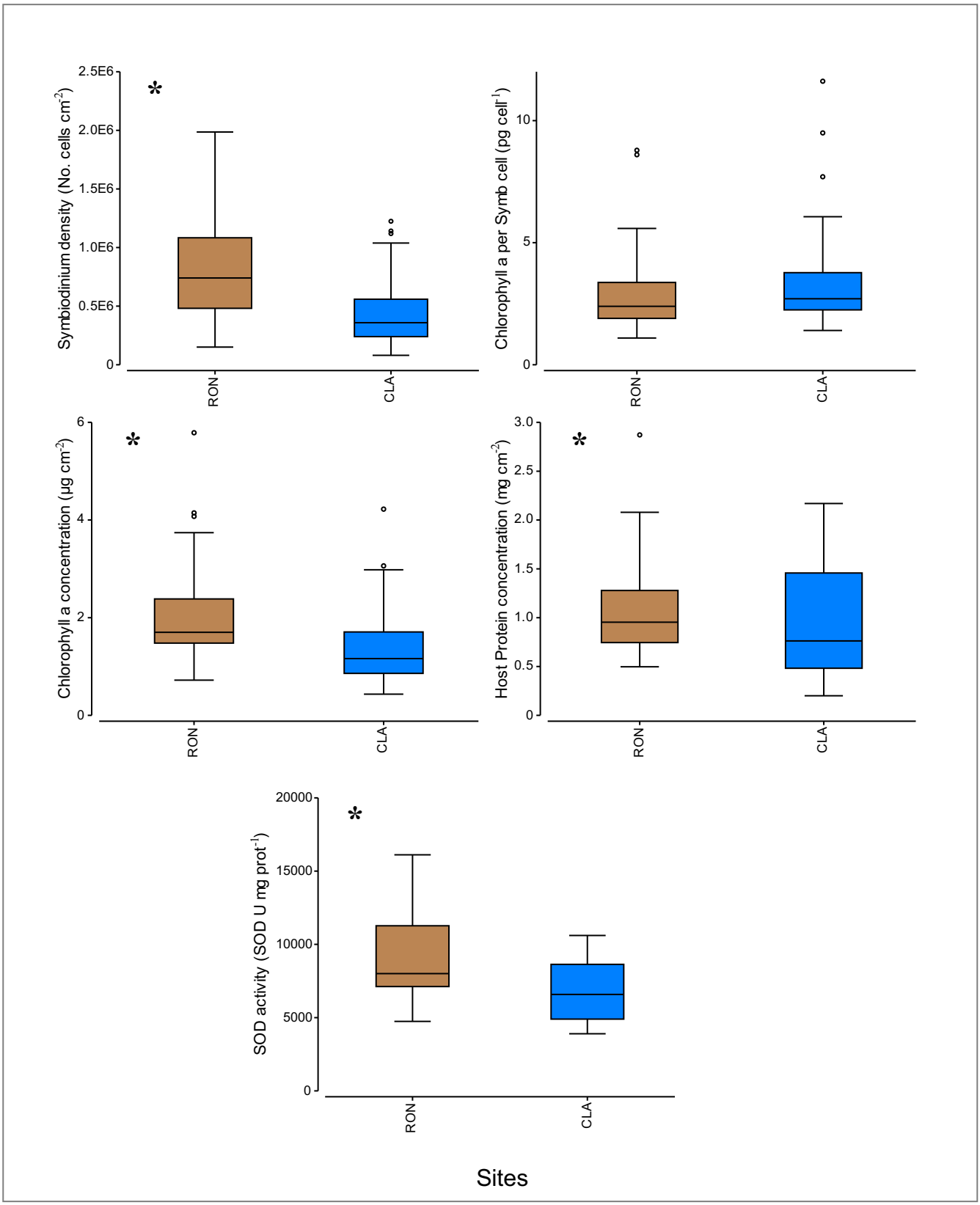

Figure 8. Comparison of physiological and immunological parameters among control corals from RON and CLA (turbid vs clear). Box represents the median (centerline), 75 percentile (top), and 25 percentile (bottom). Asterisks indicate significant differences. Open circles are outliers. Brown: RON, Blue: CLA. Note that protein concentration and SOD correspond to coral-host, not *Symbiodinium*.

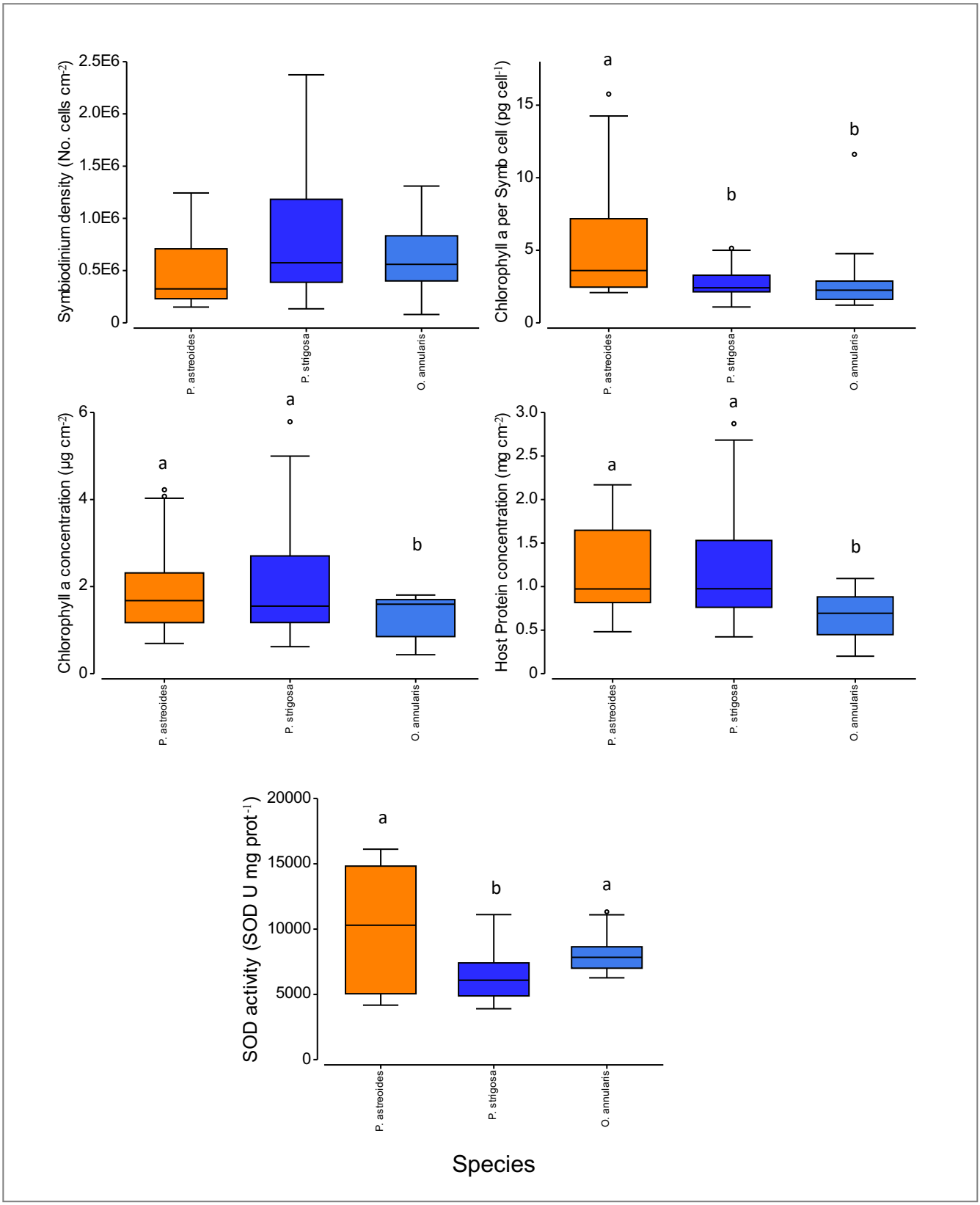

Figure 9. Comparison of physiological and immunological parameters among species from control corals from RON and CLA (turbid vs clear). Box represents the median (centerline), 75 percentile (top), and 25 percentile (bottom). Letters indicate significant differences. Orange: weedy, Blue: stress-tolerant. Open circles are outliers. Note that protein concentration and SOD correspond to coral-host, not *Symbiodinium*.

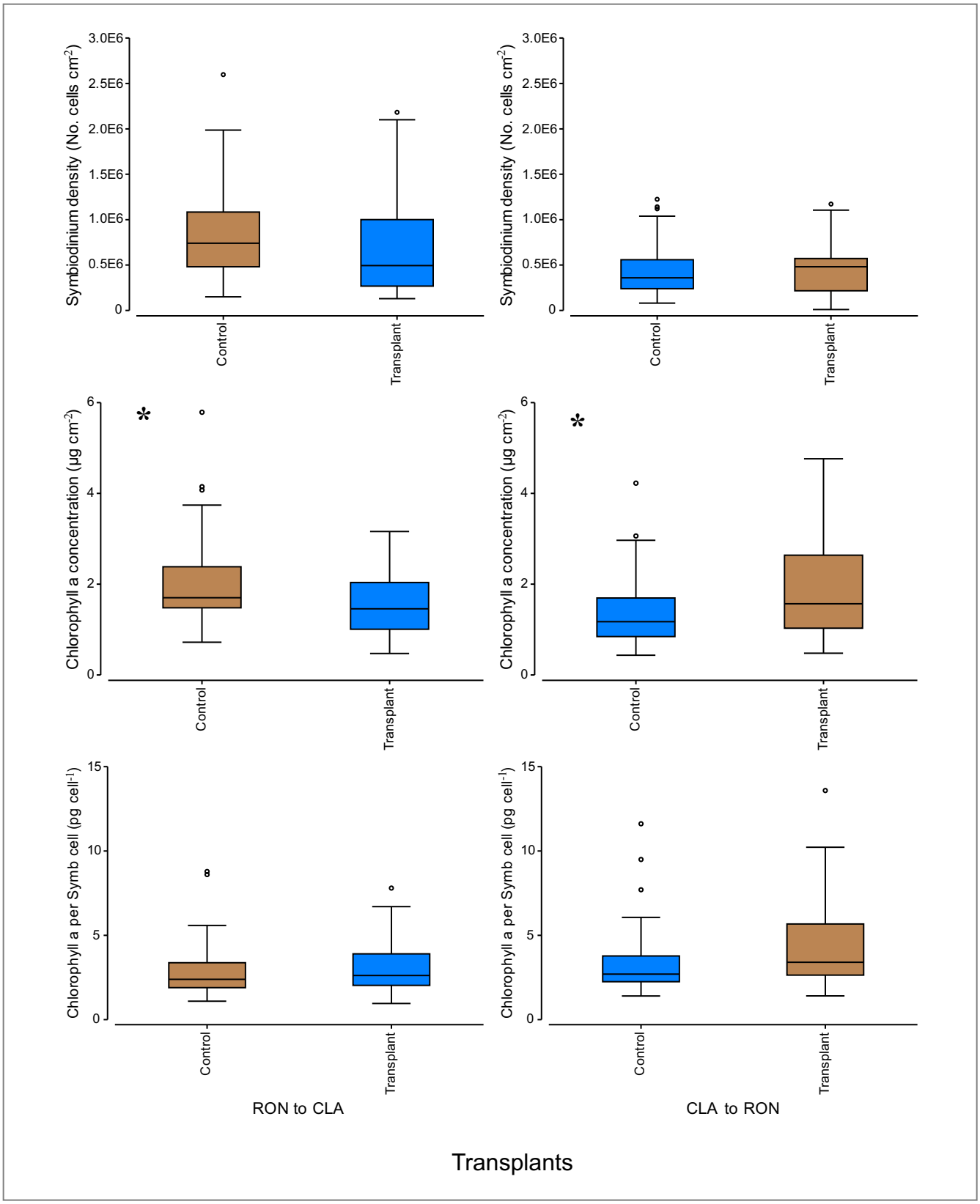

Figure 10. Comparison of physiological parameters: *Symbiodinium* density, Chlorophyll *a* concentration per cm^2 and Chlorophyll *a* per *Symbiodinium* cell, among treatments. Left: control corals from RON vs transplanted corals from RON to CLA (turbid to clear). Right: control corals from CLA vs transplanted corals from CLA to RON (clear to turbid). Box represents the median (centerline), 75 percentile (top), and 25 percentile (bottom). Open circles are outliers. Asterisks indicate significant differences. Brown: RON, Blue: CLA. Note that protein concentration and SOD corresponds to coral host, not *Symbiodinium*.

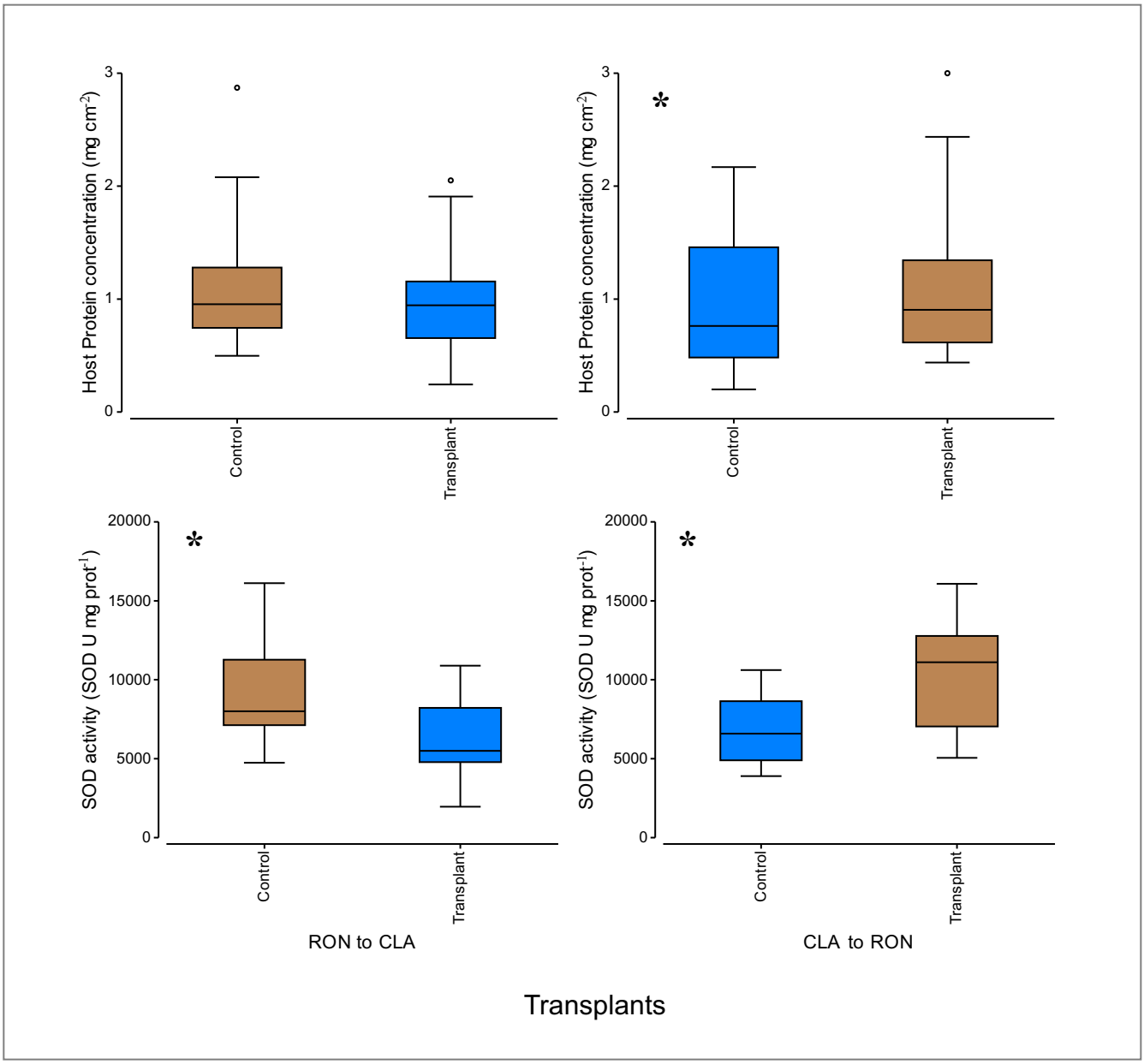

Figure 11. Comparison of physiological (Coral-host Total protein concentration) and immunological (SOD enzymatic activity) parameters among treatments. Left: control corals from RON vs transplanted corals from RON to CLA (turbid to clear). Right: control corals from CLA vs transplanted corals from CLA to RON (clear to turbid). Box represents the median (centerline), 75 percentile (top), and 25 percentile (bottom). Open circles are outliers. Asterisks indicate significant differences. Brown: RON, Blue: CLA. Note that protein concentration and SOD corresponds to coral host, not *Symbiodinium*.

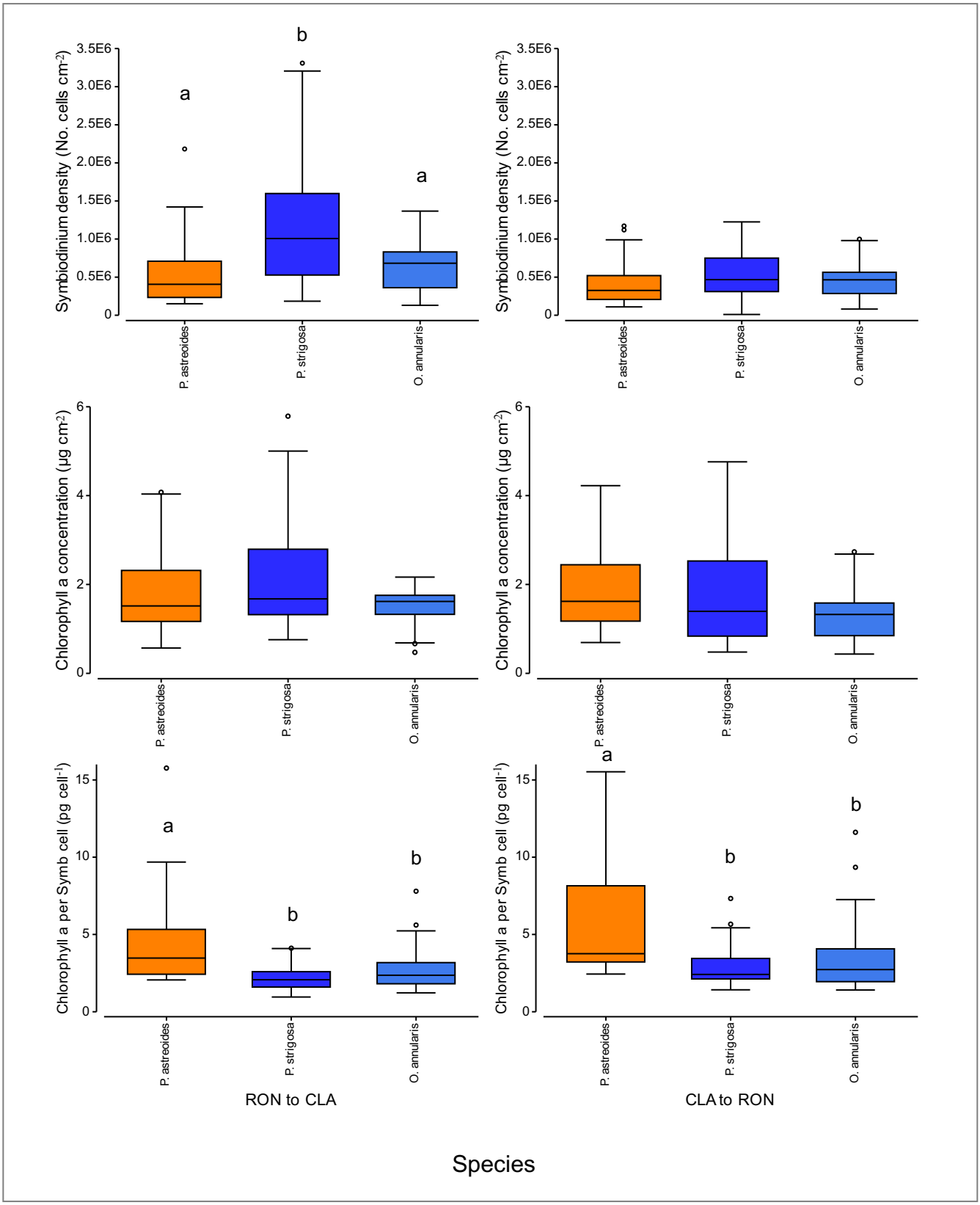

Figure 12. Comparison of physiological parameters: *Symbiodinium* density, Chlorophyll *a* concentration per cm^2 and Chlorophyll *a* per *Symbiodinium* cell, among species. Left: control corals from RON vs transplanted corals from RON to CLA (turbid to clear). Right: control corals from CLA vs transplanted corals from CLA to RON (clear to turbid). Box represents the median (centerline), 75 percentile (top), and 25 percentile (bottom). Open circles are outliers. Letters indicate significant differences. Orange: weedy, Blue: stress-tolerant. Note that protein concentration and SOD corresponds to coral host, not *Symbiodinium*.

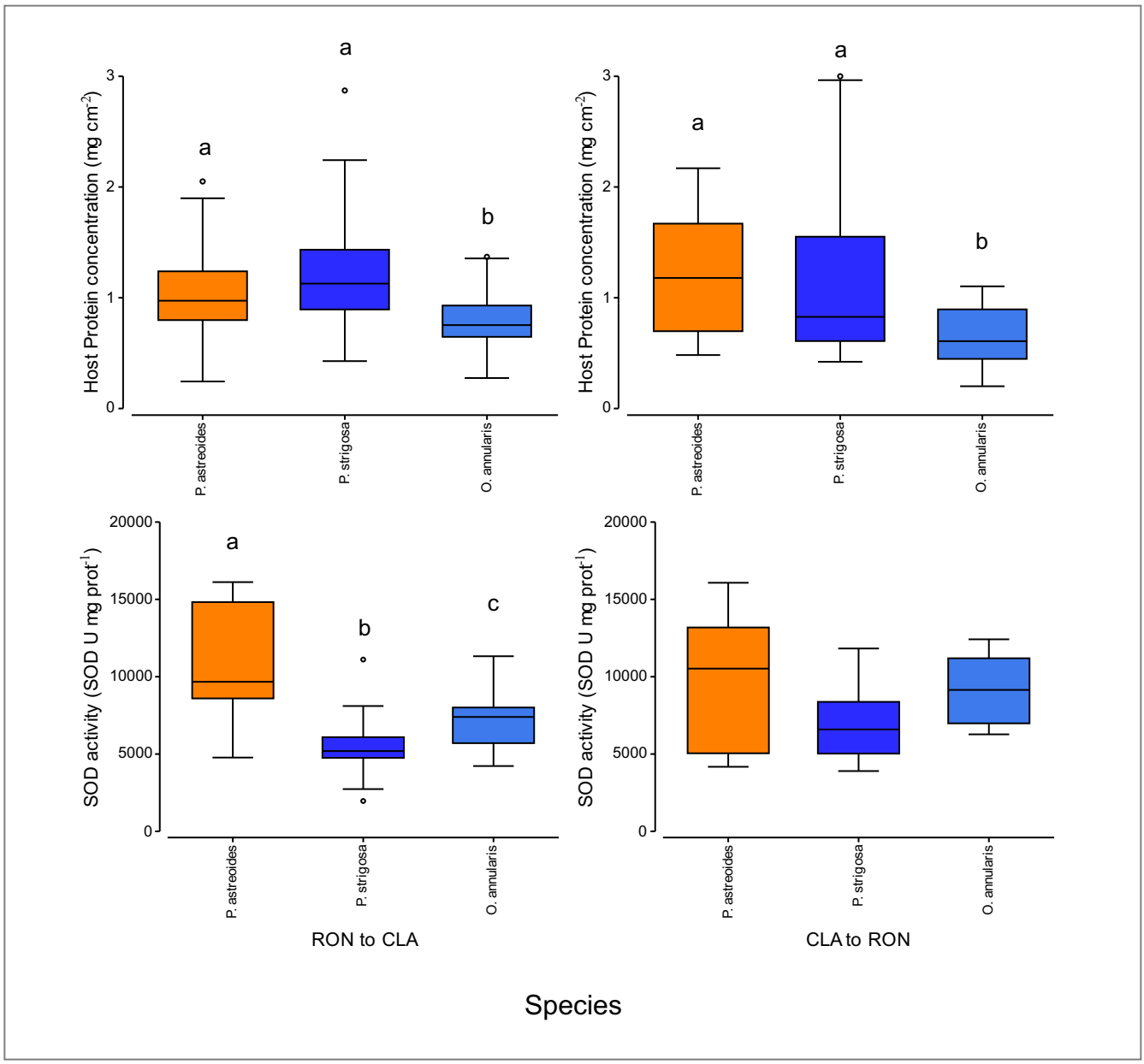

Figure 13. Comparison of physiological (Coral-host Total protein concentration) and immunological (SOD enzymatic activity) parameters among species. Left: control corals from RON vs transplanted corals from RON to CLA (turbid to clear). Right: control corals from CLA vs transplanted corals from CLA to RON (clear to turbid). Box represents the median (centerline), 75 percentile (top), and 25 percentile (bottom). Open circles are outliers. Letters indicate significant differences. Orange: weedy, Blue: stress-tolerant. Note that protein concentration and SOD corresponds to coral host, not *Symbiodinium*.

DISCUSSION

Differences in physiological parameters between RON and CLA indicate that colonies of the different species were able to adapt to local conditions and survive, grow and reproduce under different amounts of light irradiance. Major differences were observed in *Symbiodinium* density, chlorophyll *a* concentration per superficial area and protein concentration. After 28 days of transplantation, corals were capable of undergoing modifications, most prominently in chlorophyll *a* concentration per cm^2. Immunologically, differences in antioxidant activity demonstrate that water quality in RON provided a more stressful environment to controls and transplants (CLA to RON). While CLA provided a healthier environment to controls and transplants (RON to CLA) by reducing stress. Differences among species revealed that *P. astreoides* and *P. strigosa* are more similar physiologically while *P. astreoides* and *O. annularis* are more similar immunologically. These intrinsic variances suggest that physiological and immunological parameters in the studied species are influenced by species-specificity and not necessarily by life history-strategy.

Symbiodinium density and chlorophyll a

Controls

Observed differences of *Symbiodinium* density and Chl *a* concentration among colonies from the turbid (RON) and clear (CLA) reefs resemble that of plants growing in contrasting light regimes (e.g. canopy and understory) (Anthony and Hoegh-Guldberg 2003, Khalid et al., 2019). For instance, in the presence of low light, understory plants need higher chlorophyll levels than canopy plants (Augspurger et al., 2005), to harvest as much light as possible and attain the photosynthetic rate required for metabolic functions. Similarly, in this study, corals from RON exhibited higher

Chl *a* concentration per cm² than corals from CLA due to greater *Symbiodinium* density. Such differences between turbid and clear water reefs have also been reported in corals inhabiting the same reef but growing in caves versus open habitats (Anthony and Hoegh-Guldberg, 2003). My data also confirms the strong positive correlation between Chl *a* concentration per cm² and symbiont density that has been described previously in other scleractinian corals (Phillip and Fabricius, 2003, Anthony and Hoegh-Guldberg, 2003) (Fig 14). It also supports that both parameters are inversely correlated to light irradiance (Scheufen et al., 2017). I expected Chl *a* content per *Symbiodinium* cell to follow the same negative correlation (MacIntyre et al., 2002). However, such relationship was not observed in my data and neither did Anthony and Hoegh-Guldberg's (2003) study with shaded versus open-habitat corals. This suggests that the differences observed in Chl *a* concentration per cm² between sites were regulated by *Symbiodinium* density rather than pigment content per *Symbiodinium* cell.

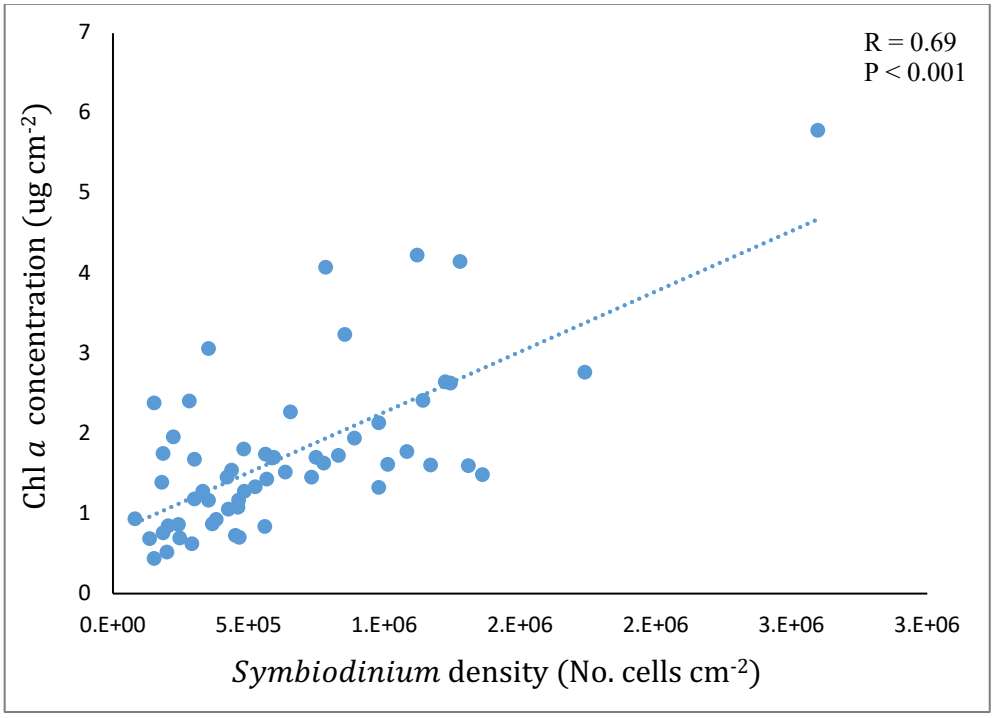

Figure 14. Pearson correlation analysis of Chlorophyll *a* concentration and *Symbiodinium* cell density of control colonies.

Colonies of the three studied species differed significantly in most physiological parameters, and these differences were consistent in both reefs. This indicates that although species behave differently within the same reef, similar tendencies can be observed among different reefs in a greater or lower scale according to water quality. Corals are known to vary light absorption by alternating symbiont density and chlorophyll concentration, together, or individually (Scheufen et al., 2017) and dependent of water quality and seasonality.

Transplants

In this study, corals transplanted from the turbid reef (RON) to clear one (CLA) significantly decreased overall Chl *a* concentration per cm^2; while corals transplanted from clear to turbid did the opposite. This suggests that colonies from both sites opted for pigment modifications rather than symbiont density shifts. These results concur with a study were corals were transplanted from open habitats to shaded areas and vice versa in the same reef (Anthony and Hoegh-Guldberg, 2003). In that study, changes in light irradiance stimulated photo-physiological modifications in Chl *a* concentration coupled with changes in photosynthetic rates with the aim of acclimation (Anthony and Hoegh-Guldberg, 2003).

Previous studies have reported that light reduction may increase symbiont density (Fabricius, 2005), and light increase may induce photoinhibition and symbiont expulsion, thereby lowering cell density (Brown, 1996, Hennige et al., 2011, Hawkins et al., 2015, Wall et al., 2018). In fact, light increase combined with high temperatures can induce significant oxidative stress in corals that can eventually lead to bleaching (Hawkins et al., 2015). Interestingly, my study was performed

during the highest sea surface temperatures of the year in the Caribbean (August-September) with an average of 30°C in both sites. This temperature is enough to induce stress and bleaching in coral if exposed continuously for numerous weeks (Glynn, 1991, Coelho et al., 2017). Results show that overall symbiont density was unaffected by either transplantation, but comparisons among species revealed than some were more affected than others when transplanted from turbid to clear. These findings will be discussed below. For the lack of difference in symbiont density in corals transplanted from clear to turbid, possible explanations include: (1) symbionts might not have enough capacity for mitotic division to increase density (Gleason, 1993); (2) sedimentation might interfere with cellular division and/or symbiont acquisition (Phillip and Fabricius, 2003); (3) it could take more time to obtain symbionts from the water column than to release them (Quigley et al., 2017); or (4) *Symbiodinium* in the studied species chose to adjust internal photo-mechanisms such as photosynthetic pigments rather than modify cell density (Anthony and Hoegh-Guldberg, 2003, Fabricius, 2005, Kuanui et al., 2020). My results suggest that the latter is likely the case.

Differences in Chl *a* concentration per cm^2 in my study are consistent with another study were corals transplanted to high light irradiance reduced overall Chl *a* (Anthony and Hoegh-Guldberg, 2003). In that study, the authors suggest that after 21 days, the transplantation effect induces changes in photosynthetic capacity, specifically subsaturation points and maximum quantum yield (F_v/F_m). In my study, I did not measure photosynthetic parameters but the significant shifts in Chl *a* concentration per cm^2 could be directly related to photo-acclimation dynamics. On the other hand, the non-significant results of Chl *a* content per *Symbiodinium* cell in this study are not consistent with a study were cultured *Symbiodinium* cells drastically reduced their photosynthetic pigments when exposed to high light and increased them in low light (Iglesias-Prieto and Trench,

1994). But there is data indicating that through other factors that reduce light such as sedimentation stress, chlorophyll contents per cell may remain unaltered (Phillip and Fabricius, 2003).

Comparisons among the three studied species indicate that although no significant differences were observed between controls and transplants from RON and CLA, transplantation had a stronger effect at the species level. The decrease in *Symbiodinium* density in colonies of *P. astreoides* (weedy) and *O. annularis* (stress-tolerant) transplanted from turbid to clear suggest that symbionts present in these two species where more sensitive to increase in light irradiance, while *P. strigosa*'s were more resistant. I did not identify *Symbiodinium* clades present within the sampled species but in future studies, I will consider this to further understand these results.

Chl *a* content per *Symbiodinium* cell appeared to be the parameter most influenced by both transplantations among species. However, regardless of the direction of transplantation *P. astreoides* was consistent in maintaining higher levels of pigment content within endosymbionts than the stress-tolerant corals. These results suggest that there are some inherent physiological differences among species that do not necessarily relate to transplantation effect nor life-history strategy.

Protein concentration

Controls

Most articles involving protein measurements in coral do not report total protein data, likely due to the ambiguity related to which proteins upshift and downshift. We, nonetheless, decided to include this data, as it may be useful as a reference to future studies. Corals from the turbid reef (RON) exhibited significantly higher amounts of total protein than those from the clear reef (CLA).

This was unexpected and differs from what Anthony and Hoegh-Guldberg (2003) saw in the pacific coral *Montipora monasteriata,* where they observed that corals from open habitats had higher protein concentration than shaded ones. However, those corals inhabited the same coral reef while ours did not. In my case, the RON and CLA sites differ in several aspects other than light regimes such as nutrients, sedimentation, contaminants, among others. One of the main causes that appear to influence turbidity in RON is sedimentation due to coastal runoff, Fajardo River discharge and industrial and recreational boat traffic. In fact, in CLA, I noticed the presence of common Caribbean corals from all clades described in Darling et al. (2012), indicating an apparently healthy reef. However, in RON, competitive corals such as *A. palmata* and *A. cervicornis* are not present (Rogers et al., 1983, Rogers, 1990, Fabricius, 2005, Erftemeijer et al., 2012). A review about the effects of terrestrial run-off in the physiology of corals presents data suggesting that moderate concentrations of nutrients and particulate organic matter can provide substantial energy and growth benefits for some corals (Fabricius, 2005). In this study, I did not consider other water quality parameters aside from turbidity, but I believe that the high algal cover observed in the turbid reef (RON) may be related to nutrient intake from the Fajardo River and thus could also be related to the high protein concentration detected. Future studies should address this hypothesis.

Comparisons among species from both sites show higher protein concentration in *P. astreoides* and *P. strigosa* in comparison to *O. annularis*. Differences in the *P. strigosa* and *O. annularis* are consistent with other studies that compare physiological parameters among several stress-tolerant species (Scheufen et al., 2017). Literature states that parameters such as protein concentration and the ones mentioned above are variable even within species with the same stress-tolerant strategy.

Other studies suggest that after massive spawning events, coral protein content may be altered (Leuzinger et al., 2003, Anthony, 2006). This study was conducted between August and September which are the months where most Caribbean scleractinian corals spawn their gametes (Szmant, 1986), thus there is a possibility this could have influenced such differences between my stress-tolerant species. Nevertheless, similarities between *P. astreoides* and *P. strigosa* were consistently observed among control corals from RON and CLA.

Transplants

Even though corals transplanted from the turbid reef to the clear one did not exhibit changes in total protein concentration, I did observe an increase in corals transplanted from clear to turbid to quantities similar to control corals from the turbid reef. Other coral transplant experiments did not identify differences in protein concentration (Anthony and Hoegh-Guldberg, 2003), and neither did light shift laboratory experiments (Gleason, 1993). Possible explanations for my results include the following: from turbid to clear (1) the studied species are capable of physiologically acclimating to high light without compromising total protein concentration (Anthony and Hoegh-Guldberg, 2003) or (2) perhaps upregulation of proteins involved in defense occurred (Smith et al., 2013) while other types of protein suffered degradation or denaturalization (Down et al., 2013) and thus no significant change in protein concentration was detected. Further experimental studies measuring different types of proteins, such as structural (e.g. for calcification), storage (e.g. lipids), enzymes (e.g. for defense), among others, are needed. In this study, I only measured antioxidant enzymes to detect immune activity which will be discussed below. As for corals transplanted from clear to turbid, perhaps (1) 28 days of light reduction might upregulate defense-related proteins (Sheridian et al., 2014) but not necessarily affect negatively all other proteins or (2) possible

nutrient intake and particulate organic matter from the mouth of the Fajardo River may have been used as a food source by corals and therefore induced the increase in protein concentration (Anthony and Fabricius, 2000, Fabricius, 2005).

Differences in protein concentration among colonies from the three studied species subjected to transplantation followed the same tendency observed among control corals from both sites. This indicates that although colonies transplanted from clear to turbid increased protein concentration, while conspecifics remained unaltered, total protein was equally affected among species.

SOD enzymatic activity

Controls

The significant differences observed in SOD enzymatic activity among control corals from RON and CLA are consistent with previous studies indicating that antioxidant activity varies according to light, temperature and regional water conditions (Downs et al., 2002, Couch et al., 2008, Palmer et al., 2010). Elevated SOD activity in control corals from RON (turbid) in comparison to controls from CLA (clear) suggest that local corals inhabiting the turbid reef were more stressed during the sampling period (Couch et al., 2008). This was expected since CLA is considered to be a relatively healthy reef while RON is considered to be an impacted one. Above-average light irradiance and sea surface temperature are generally associated with oxidative stress in coral (Downs et al., 2002). Thus, given that the temperature regime was similar in both reefs, and that RON received considerably lower light irradiance than CLA, it can be suggested that oxidative stress in RON could be related to other factors. For instance, there is evidence that high sedimentation rates can induce an immune response in corals as a result of tissue abrasion and damage-associated

molecular patterns (Sheridian et al., 2014). This activates the melanin synthesis pathway which produces ROS as bypass products, hence promoting antioxidant activity (Sheridian et al., 2014, Poquita-Du et al., 2019, Mydlarz and Palmer, 2009). Preliminary observations indicate that RON has higher sedimentation and resuspension rates than CLA but further analysis must be conducted to validate this information. Hypo-salinity (below average salinity levels) has also been linked to increased SOD activity in corals (Downs et al., 2009). Given the proximity of RON to the mouth of the Fajardo River, there is a possibility that high precipitation events on the river's watershed might decrease average salinity levels in RON. However, no significant precipitation events were documented during the study period.

Other possible sources that have been demonstrated to stimulate oxidative stress in coral are inorganic contaminants such as heavy metals (Rotchell and Ostrander, 2011, Zhou et al., 2018) and organic contaminants such as hydrocarbons (Ramos and García, 2007, Downs et al., 2011, Rotchell and Ostrander, 2011, Richmond et al., 2018, Xiang et al., 2019). Common pollutants may enter aquatic systems via anthropogenic sources such as industrial, agricultural and recreative (Woo et al., 2009). In this sense, the Fajardo River passes through the town of Fajardo which has several industries that probably discharge pollutants that likely affect the stream and eventually reach RON. However, more specific studies involving the river's water quality should be performed to validate this. Heavy metals in particular, are toxic in trace amounts, may remain permanently in the marine environment and can potentially induce severe oxidative stress in aquatic organisms (Clark, 2001, Woo et al., 2009). In fact, there is evidence of increased SOD cellular transcription in several freshwater and saltwater-adapted fish (Woo et al., 2009). Hence, if SOD levels in corals from RON are influenced by heavy metal contamination it would be very

likely that fish may also be contaminated. As for hydrocarbons, these are also hazardous pollutants that are highly resistant to degradation in marine environments (Xiang et al., 2019). Polycyclic aromatic hydrocarbons may enter the marine environment through water discharges, fuel, oil spills, and coastal tourism (Tarrant et al., 2014, Xiang et al., 2009). These have also been documented to increase SOD activity in coral (Ramos and García, 2007, Xiang et al., 2009) and other cnidarians such as anemones (Tarrant et al., 2014). Thus, possible pollutants in the river discharge and constant ferry and recreational boat traffic adjacent to RON may be potential hydrocarbon sources influencing SOD enzymatic activity in local RON corals. More specific ecotoxicological studies are needed to determine if heavy metals and hydrocarbons are more present in RON than in CLA.

Comparisons among the three studied species revealed similarities in SOD activity between *P. astreoides* (weedy) and *O. annularis* (stress-tolerant). This was unexpected given that in a study by Pinzón et al. (2014b), they demonstrated that complex corals' (mostly weedy) constitutive levels of certain immune traits involve more microbial activity, while robust (mostly stress-tolerant) display higher antioxidant activity. This would allow weedy corals to combat stress without spending resources in more energetically costly traits such as antioxidants. While stress-tolerant corals seem to invest greater amounts of energy in the immune system. However, my results indicate that a weedy species can have similar or higher antioxidant activity than some stress-tolerant, suggesting that life-history strategy may not necessarily influence constituent antioxidant activity in corals, and it is likely species-specific (Diaz et al., 2016). Other studies suggest that microorganisms present in the coral holobiont such as heterotrophic bacteria can produce and degrade ROS simultaneously with the coral (Diaz et al., 2013, Shaked and Armoza-Zvuloni, 2013). Thus, ROS in coral are likely influenced not only by the coral host and

endosymbionts, but by the community of microorganisms associated to the coral which can also be species-specific, and in some cases, it can vary even within colonies from the same species (Couch et al., 2008). This holobiont assemblage has been proposed to have the potential to extend or reduce the physiological and immunological capabilities of the coral host (Mydlarz et al., 2010, Radecker et al., 2015).

Transplants

Our twenty-eight days' post-transplantation results addressed the question of which relocation generates more oxidative stress? RON to CLA (turbid to clear) or CLA to RON (clear to turbid). The remarkable differences in light regimes between RON and CLA coupled with all other possible factors influencing water quality are ample reasons to expect immune responses in corals subjected to transplantation. Significant shifts of SOD activity in both transplantations demonstrate that changes in water conditions may generate an immune response, but the degree of this response may vary according to the water's biogeochemistry (D'Angelo and Weidenmann, 2014). The significant reduction in oxidative stress in corals transplanted from RON to CLA was an interesting finding, given that exposure to high light combined with high temperature (Hawkins, 2015) was thought to generate more stress in these shade-adapted corals (Couch et al., 2008). This result, along with the increase in SOD activity in conspecific corals transplanted from CLA to RON suggests that water quality differences between RON and CLA, rather than differences in light levels, were the cause for the observed differences in the antioxidant activity. This supports my premise that other factors present in RON such as pollutants provided a more stressful environment that generated significantly higher levels of superoxide radicals in the corals studied during the research period.

Comparisons among species subjected to transplantation revealed that SOD activity followed similar patterns in both relocations that were also similar to activity in control colonies. *P. astreoides* and *O. annularis* were more stimulated by transplantation than *P. strigosa.* Although significant differences were only observed in corals transplanted from RON to CLA, the lack of difference in corals transplanted from CLA to RON was due to high variances among colonies. These variances are indicative that colonies from the same species responded in different ways, with some colonies being more stimulated than others by the low water quality in RON. Variability in coral can be observed among species, among colonies from the same species and even within an individual colony. Nevertheless, my results indicate that colonies from *P. astreoides* had higher variability than the stress-tolerant species, which is expected for weedy species. It may also suggest that although *P. strigosa* and *O. annularis* have been documented to be more susceptible to thermal-stress and diseases (Hernández-Pacheco et al., 2012, Fuess et al., 2013, Pinzón et al., 2014a), they might be more resistant to light reduction, sedimentation stress and/or pollutants.

CONCLUSIONS

My data shows that weedy and stress-tolerant corals have physiological and immunological mechanisms to survive local water quality conditions and can acclimate to environmental changes in a relatively short time (1 month). However, more research is needed to substantiate this claim. Data were consistent in that *Symbiodinium* density and Chl *a* concentration per cm^2 are positively related and inversely proportional to light irradiance. These patterns are similar to plant photo-physiology dynamics in light regimes. Higher protein concentration in RON corals might be related to the presence of high nutrients and particulate organic matter that could be used as a heterotrophic source of nourishment in low light availability. Other potential stressors such as pollutants, sedimentation and possible pathogens appear to be providing a more stressful environment for corals inhabiting RON rather than the light factor alone.

Comparisons among species suggest that physiological dynamics in *P. astreoides*, *P. strigosa* and *O. annularis* are not influenced by life-history strategy but by species-specificity. No specific pattern that would differentiate weedy from stress-tolerant was observed in constitutive physiological parameters nor physiological responses to light shifts, and this is consistent with available literature. Physiological similarities between *P. strigosa* and *P. astreoides* in this study, explain why both these species have been classified as resilient, given similar levels of physiological plasticity and demographic traits (Edmunds, 2010). Immunological dynamics suggest differences among control and transplanted with *P. astreoides* and *O. annularis* SOD activity being more stimulated than *P. strigosa*. Although all species increased SOD when transplanted from CLA to RON (clear to turbid), the stress-tolerant species were more consistent

in their SOD activity to both transplantations, while the weedy was more variable. This variability suggests that in some cases the weedy species might become more stressed when facing water degradation while the stress-tolerant species could be more resistant to this type of environmental disturbance. Given *P. astreoides*' high population growth and prevalence in Caribbean reefs in the last decade (Green et al., 2008), it can be suggested that the three species are stress-tolerant but differ in the way they handle physiological and immunological dynamics.

Although the measured variables are very broad indicators of physiology and oxidative stress response, this study suggests that physiological plasticity and oxidative stress in corals may be influenced by water quality. However, further physiological and immunological studies should be done to support this speculation. This experiment also suggests that general categories such as 'weedy' or 'stress-tolerant' may not reflect the complex response of coral species to stress. In order to further understand differences among the studied species, further genetic and microbial studies should be done. In addition, further ecotoxicological studies are needed in RON corals, RON water quality and the Fajardo river watershed to investigate presence of possible pollutants and their source of origin. This information will allow scientists to develop management strategies that may lead to possible improvement of water quality in this reef.

LITERATURE CITED

Anderson J.M. (1986) Photo-regulation of the composition, function and structure of thylakoid membranes. *Annual Review of Plant Physiology* 37: 93–136.

Anthony, K.R.N. (2006) Enhanced energy status of corals on coastal, high-turbidity reefs. *Marine Ecology Progress Series* 319: 111-116.

Anthony K.R.N. and Fabricius K.E. (2000) Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *Journal of experimental marine biology and ecology* 252(2): 221-253.

Anthony K.R.N. and Hoegh-Guldberg O. (2003) Variation in coral photosynthesis, respiration and growth characteristics in contrasting light microhabitats: an analog to plants in forest gaps and understories? *Functional Ecology* 17: 246-259.

Asokan R., Arumugam M. and Mullainadhan P. (1997) Activation of prophenoloxidase in the plasma and hemocytes of the marine mussel *Perna viridis* Linnaeus. *Developmental & Comparative Immunology* 21(1): 1-12.

Augspurger C.K., Cheeseman J.M. and Salk C.F. (2005) Light gains and physiological capacity of understorey woody plants during phenological avoidance of canopy shade. *Functional Ecology* 19(4): 537-546.

Ayre D.J., & Hughes T.P. (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution* 54(5): 1590-1605.

Babcock R. and Smith L. (2000) Effects of sedimentation on coral settlement and survivorship. *Proceedings 9th International Coral Reef Symposium*, Bali, Indonesia 23-27.

Baker A.C. (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annual *Review of Ecology, Evolution, and Systematics* 34(1): 661-689.

Barber J. and Andersson B. (1992) Too much of a good thing: light can be bad for photosynthesis. *Trends in Biochemical Sciences* 17: 61–66.

Bartley R., Bainbridge Z.T., Lewis S.E., Kroon E.J., Wilkinson S.N., Brodie J.E. and Silburn D.M. (2014) Relating sediment impacts on coral reefs to watershed sources, processes and management: A review. *Science of the Total Environment* 468-469: 1138-1153.

Bellantuono A.J., Hoegh-Guldberg O., & Rodriguez-Lanetty M. (2012). Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B*: Biological Sciences, 279(1731): 1100-1107.

Brenner M. and Hearing V.J. (2008) The protective role of melanin against UV damage in human skin. *Photochemistry and Photobiology* 84: 539-549.

Brown B.E. and Bythell J.C. (2005) Perspective on mucus secretion in reef corals. *Marine Ecology Progress Series* 296: 291-309.

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

Buerger P. and van Oppen M.J.H. (2018) Viruses in corals: hidden drivers of coral bleaching and disease? *Microbiology Australia* 9-12.

Chornesky E.A. and Peters E.C. (1987) Sexual reproduction and colony growth in the scleractinian coral *Porites astreoides*. *Biological Bulletin* 172: 161-177.

Clark R. (2001) Marine Pollution. *Oxford University Press*, New York.

Coelho V.R., Fenner D., Caruso C., Bayles B.R., Huang Y. and Birkeland C. (2017) Shading as a mitigation tool for coral bleaching in three common Indo-Pacific species. *Journal of experimental marine biology and ecology* 497: 152-163.

Couch C.S., Mydlarz L.D., Harvell C.D. and Douglas N.L. (2008) Variation in measures of immunocompetence of sea fan coral, *Gorgonia ventalina*, in the Florida Keys. *Marine biology* 155(3): 281-292.

Crossland C.J., Hatcher B.G. and Smith S.V. (1991) Role of coral reefs in global ocean production. *Coral Reefs* 10: 55-64.

Darling E.S., Alvarez-Filip L., Oliver T.A., McClanahan T.R. and Côté I.M. (2012) Evaluating life-history strategies of reef corals from species traits. *Ecology Letters* DOI: 10.1111/j.1461-0248.2012.01861.x

D'Angelo C. and Wiedenmann J. (2014) Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival. Current Opinion in *Environmental Sustainability* 7: 82-93.

Diaz J.M., Hansel C.M., Apprill A., Brighi C., Zhang T., Weber L., McNally S. and Xun, L. (2016) Species-specific control of external superoxide levels by the coral holobiont during a natural bleaching event. *Nature communications*, 7: 13801 DOI: 10.1038/ncomms13801

Diaz J.M., Hansel C.M., Voelker B.M., Mendes C.M., Andeer P.F. and Zhang, T. (2013) Widespread production of extracellular superoxide by heterotrophic bacteria. *Science* 340(6137): 1223-1226.

Dodge R.E. and Vaisnys J.R. (1977) Coral populations and growth patterns: Responses to sedimentation and turbidity associated with dredging. *Journal of Marine Science* 35: 715-730.

Downs C.A., Fauth J.E., Halas J., Dustan P., Bemiss J. and Woodley C. (2002) Oxidative stress and seasonal coral bleaching. *Free radical biology & medicine* 33: 533-43. DOI: 10.1016/S0891-5849(02)00907-3

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

Downs C.A., McDougall K.E., Woodley C.M., Fauth J.E., Richmond R.H., et al. (2013) Heat-Stress and Light-Stress Induce Different Cellular Pathologies in the Symbiotic Dinoflagellate during Coral Bleaching. *PLoS one* 8(12): e77173. DOI:10.1371/journal.pone.0077173

Downs C.A., Woodley C.M., Fauth J.E., Knutson S., Burtscher M.M., May L.A., ... and Ostrander G.K. (2011) A survey of environmental pollutants and cellular-stress markers of *Porites astreoides* at six sites in St. John, US Virgin Islands. *Ecotoxicology* 20(8): 1914 DOI: 10.1007/s10646-011-0729-7

Edmunds, P.J. (2010) Population biology of *Porites astreoides* and *Diploria strigosa* on a shallow Caribbean reef. *Marine Ecology Progress Series* 418: 87-104.

Edmunds P.J. and Davies P.S. (1989) An energy budget for *Porites porites* (Scleractinia) growing in a stressed environment. *Coral Reefs* 8: 37–43.

Edwards H.J., Elliott I.A., Eakin C.M., Irikawa A., Madin J.S., McField M., ... & Mumby P.J. (2011) How much time can herbivore protection buy for coral reefs under realistic regimes of hurricanes and coral bleaching? *Global Change Biology* 17(6): 2033-2048.

Eppley R.W. and Sloan P.R. (1966) Growth rates of marine phytoplankton: correlation with light absorption by cell chlorophyll a. *Physiologia Plantarum* 19(1): 47-59.

Erftemeijer P.L., Riegl B., Hoeksema B W. and Todd P.A. (2012) Environmental impacts of dredging and other sediment disturbances on corals: a review. *Marine pollution bulletin* 64(9): 1737-1765.

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

Flores-Ramírez L. A. and Liñán-Cabello, M. A. (2007) Relationships among thermal stress, bleaching and oxidative damage in the hermatypic coral, *Pocillopora capitata*. *Comparative Biochemistry and Physiology Part C*: Toxicology & Pharmacology 146(1-2): 194-202.

Fuess L.E., Pinzón C. J.H., Weil E., Grinshpon R.D., Mydlarz L.D. (2017) Life or death: disease-tolerant coral species activate autophagy following immune challenge. *Proceedings of the Royal Society B* 284: 20170771. http://dx.doi.org/10.1098/rspb.2017.0771

Gardner T.A., Coté I.M., Gill J.A. and Grant A. (2003) Long-term region-wide declines in Caribbean corals. *Science* 301: 958.

Gintert B.E., Precht W.F., Fura R., Rogers K., Rice M., Precht L.L., ... and Robbart, M. L. (2019) Regional coral disease outbreak overwhelms impacts from a local dredge project. *Environmental monitoring and assessment* 191(10): 630.

Gleason D.F. (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreoides*. *Limnology and Oceanography* 38(7): 1452-1463.

Gleason D.F. (1998) Sedimentation and distributions of green and brown morphs of the Caribbean coral *Porites astreoides* Lamarck. *Journal of Experimental Marine Biology and Ecology* 230(1): 73-89.

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

Green D.H., Edmunds P.J. and Carpenter R.C. (2008) Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. *Marine Ecology Progress Series* 359: 1-10.

Griffin S.P., Bhagooli R. and Weil E. (2006) Evaluation of thermal acclimation capacity in corals with different thermal histories based on catalase concentrations and antioxidant potentials. *Comparative Biochemistry and Physiology* Part A 144: 155-162.

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

Hawkins T.D., Krueger T., Wilkinson S.P., Fisher P.L. and Davy S.K. (2015) Antioxidant responses to heat and light stress differ with habitat in a common reef coral. *Coral Reefs* 34(4): 1229-1241.

Hennige S.J., McGinley M.P., Grottoli A.G. and Warner M.E. (2011) Photoinhibition of *Symbiodinium spp*. within the reef corals *Montastraea faveolata* and *Porites astreoides*: implications for coral bleaching. *Marine Biology* 158(11): 2515-2526.

Hernández-Delgado E.A., Mercado-Molina A.E., Alejandro-Camis P.J., Candelas-Sánchez F., Fonseca-Miranda J.S., González-Ramos C.M., Guzmán-Rodríguez R., Mège P., Montañez-Acuña A.A., Olivo-Maldonado I., Otaño-Cruz A. and Suleimán-Ramos S.E. (2014) Community-Based Coral Reef Rehabilitation in a Changing Climate: Lessons Learned from Hurricanes, Extreme Rainfall, and Changing Land Use Impacts. *Open Journal of Ecology* 4: 918-944.

Hilborn R. (2016) Marine biodiversity needs more than protection. *Nature* 535: 224-226.

Homyack J.A. (2010) Evaluating habitat quality of vertebrates using conservation physiology tools. *Wildlife Research* 37: 332-342.

Horricks R.A., Herbinger C.M., Lillie B.N., Taylor, P. and Lumsden, J.S. (2019) Differential protein abundance during the first month of regeneration of the Caribbean star coral *Montastraea cavernosa*. *Coral Reefs* 38: 45–61 DOI:https://doi.org/10.1007/s00338-018-01754-9

Hughes T. P., Baird A.H., Bellwood D.R., Card M., Connolly S.R., Folke C., Grosberg R., Hoegh-Guldberg O., Jackson J.B.C., Kleypas J., Lough J.M., Marshall P., Nyström M., Palumbi S.R., Pandolfi J.M., Rosen B. & Roughgarden J. 2003. Climate change, human impacts, and the resilience of coral reefs. *Science* 301: 929-933.

Iglesias-Prieto R. and Trench R.K. (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Marine ecology progress series* 113(1): 163-175.

Johan F., Jafri M.Z., Lim H.S., & Maznah W.W. (2014). Laboratory measurement: Chlorophyll-a concentration measurement with acetone method using spectrophotometer. *Proceedings of the 2014 IEEE* 744-748.

Jones, A. M., Berkelmans, R., van Oppen, M. J., Mieog, J. C., & Sinclair, W. (2008). A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B*: Biological Sciences, 275(1641): 1359-1365.

Khalid M., Raza M., Yu H., Sun F., Zhang Y., Lu F., ... and Li W.C. (2019) Effect of shade treatments on morphology, photosynthetic and chlorophyll fluorescence characteristics of soybeans (Glycine max L. Merr.). *Applied Ecology and Environmental Research* 17(2): 2551-2569.

Kirk, J. T. (1994). Light and photosynthesis in aquatic ecosystems. *Cambridge University Press*. Cambridge.

Köhler K.E. and Gill S.M. (2006) Coral Point Count with Excel extensions (CPCe): A visual basic program for the determination of coral and substrate coverage using random point count methodology. *Computers and Geosciences* 32: 1259-1269.

Kuanui P., Chavanich S., Viyakarn V., Omori M., Fujita T. and Lin, C. (2020) Effect of light intensity on survival and photosynthetic efficiency of cultured corals of different ages. *Estuarine, Coastal and Shelf Science* 235: 106515.

LaJeunesse T., Parkinson J.E., Gabrielson P.W., Jin H., Davis J., Voolstra C.R. and Santos S.R. (2018) Systematic Revision of Symbiodiniaceae highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology* 28: 2570–2580.

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

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

Lesser M.P. (2011) Coral bleaching: Causes and mechanisms. In: Z. Dubinsky, N. Stambler (eds) Coral reefs: An ecosystem in transition. Springer, pp 405–420.

Lesser M.P. (2013) Using energetic budgets to assess the effects of environmental stress on corals: are I measuring the right things? *Coral Reefs* 32: 25–33.

Leuzinger S., Anthony K.R.N. and Willis B.L. (2003) Reproductive energy investment in corals: scaling with module size. *Oecologia* 136(4): 524-531.

Lundquist C.J. and Granek E.F. (2005) Strategies for successful marine conservation: Integrating socioeconomic, political, and scientific factors. *Conservation Biology* 19(6): 1771-1778.

MacIntyre H.L., Kana T.M., Anning T. and Geider R.J. (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. *Journal of phycology* 38(1): 17-38.

Maynard J., Van Hooidonk R., Eakin C.M., Puotinen M., Garren M., Williams G., ... and Harvell C.D. (2015) Projections of climate conditions that increase coral disease susceptibility and pathogen abundance and virulence. *Nature Climate Change* 5(7): 688-694.

Mercado-Molina A.E., Ruiz-Diaz C.P., Pérez M.E., Rodríguez-Barreras R. and Sabat A.M. (2015) Demography of the threatened coral *Acropora cervicornis*: implications for its management and conservation. *Coral Reefs* 34(4): 1113-1124.

Mucklow P.T., Vizoso D.B., Jensen K.H., Refardt D., Ebert D. (2004) Variation in phenoloxidase activity and its relation to parasite resistance within and between populations of *Daphnia magna*. *Proceedings of the Royal Society B* Biological Sciences 271: 1175–1183.

Muñoz P., Meseguer J. and Esteban M.A. (2006) Phenoloxidase activity in three commercial bivalve species. Changes due to natural infestation with *Perkinsus atlanticus*. Fish Shellfish *Immunology* 20, 12–19.

Murphy J.W., Collier A.C. and Richmond, R.H. (2019) Antioxidant enzyme cycling over reproductive lunar cycles in *Pocillopora damicornis*. *PeerJ*: 7, e7020.

Muscatine, L. (1974) Endosymbiosis of cnidarians and algae. In Coelenterate biology: reviews and new perspectives pp. 359-395. Academic Press New York.

Muscatine L. and Porter J.W. (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 27: 454-460.

Mydlarz L.D. and Palmer C.V. (2011) The presence of multiple phenoloxidases in Caribbean reef-building corals. *Comparative Biochemistry and Physiology*, Part A 159(4): 372-378.

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

Mydlarz L.D., McGinty E.S. and Harvell C.D. (2010) What are the physiological and immunological responses of coral to climate warming and disease? *The Journal of Experimental Biology* 213: 934-945.

Nappi A.J. and Ottaviani E. (2000) Cytotoxicity and cytotoxic molecules in invertebrates. *Bioessays* 22(5): 469-480.

Neidhardt J., Benemann J.R., Zhang L. and Melis, A. (1998) Photosystem-II repair and chloroplast recovery from irradiance stress: relationship between chronic photoinhibition, light-harvesting chlorophyll antenna size and photosynthetic productivity in *Dunaliella salina* (green algae). *Photosynthesis Research* 56(2): 175-184.

Nishikawa A., Katoh M. and Sakai K. (2003) Larval settlement rates and gene flow of broadcast-spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. *Marine Ecology Progress Series* 256: 87-97.

Olsen K., Ritson-Williams R., Ochrietor J.D., Paul V.J. and Ross, C. (2013) Detecting hyperthermal stress in larvae of the hermatypic coral *Porites astreoides*: the suitability of using biomarkers of oxidative stress versus heat-shock protein transcriptional expression. *Marine biology* 160(10): 2609-2618.

Palmer C.V. and Taylor-Knowles N. (2012) Towards an integrated network of coral immune mechanisms. *Proceedings of the Royal Society B* 279: 4106-4114.

Palmer C.V., Bythell J.C. and Willis B.L. (2011a) A comparative study of phenoloxidase activity in diseased and bleached colonies of the coral *Acropora millepora*. *Developmental and Comparative Immunology* 35: 1096-1099.

Palmer C.V., McGinty E.S., Cummings D.J., Smith S.M., Bartels E. and Mydlarz, L.D. (2011b) Patterns of coral ecological immunology: variation in the response of Caribbean corals to elevated temperature and a pathogen elicitor. *The Journal of Experimental Biology* 214: 4240-4249.

Palmer C.V., Modi C.K., Mydlarz L.D. (2009) Coral Fluorescent Proteins as Antioxidants. PLoS ONE 4(10): e7298. DOI: 10.1371/journal.pone.0007298

Pandey P.K., Kass P.H., Soupir M.L., Biswas S. and Singh V.P. (2014) Contamination of water resources by pathogenic bacteria. *AMB Express* 4(1): 51.

Paradis B.T., Henry R.P. and Chadwick N.E. (2019) Compound effects of thermal stress and tissue abrasion on photosynthesis and respiration in the reef-building coral *Acropora cervicornis* (Lamarck, 1816). *Journal of Experimental Marine Biology and Ecology* 521: 151222.

Perdomo-Morales R., Montero-Alejo V., Perera E., Pardo-Ruiz Z. and Alonso-Jiménez E. (2007) Phenoloxidase activity in the hemolymph of the spiny lobster *Panulirus argus*. *Fish & Shellfish Immunology* 23: 1187-1195.

Perry C.T., Murphy G.N., Kench P.S., Smithers S.G., Edinger E.N., Steneck R.S. and Mumby P. (2013) Caribbean-wide decline in carbonate production threatens coral reef growth. *Nature Communications* 4:1402 DOI: 10.1038/ncomms2409

Phillip E. and Fabricius K. (2003) Photohysiological stress in scleractinian corals in response to short-term sedimentation. *Journal of Experimental Marine Biology and Ecology* 287(1): 57-78.

Pinzón C. J.H., Beach-Letendre J., Weil E., Mydlarz L.D. (2014a) Relationship between Phylogeny and Immunity Suggests Older Caribbean Coral Lineages Are More Resistant to Disease. *PLoS ONE* 9(8): e104787. DOI:10.1371/journal.pone.0104787

Pinzón C. J.H., Dornberger L., Beach-Letendre J., Weil E. and Mydlarz L.D. (2014b) The link between immunity and life-history traits in scleractinian corals. PeerJ 2:e628; DOI: 10.7717/peerj.628

Pollock F.J., Lamb J.B., Field S.N., Heron S.F., Schaffelke B., Shedrawi G., ... & Willis B.L. (2014) Sediment and turbidity associated with offshore dredging increase coral disease prevalence on nearby reefs. *PLOS one* 9(7).

Pollock F.J., Morris P.J., Willis B.L. and Bourne D.G. (2011) The urgent need for robust coral disease diagnostics. *PLoS pathogens* 7(10).

Poquita-Du R.C., Huang D., Chou L.M., Mrinalini and Todd P.A. (2019) Short Term Exposure to Heat and Sediment Triggers Changes in Coral Gene Expression and Photo-Physiological Performance. *Frontiers in Marine Science* 6:121 DOI: 10.3389/fmars.2019.00121

Powles S.B. (1984) Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology* 35: 15–44.

Quigley K.M., Willis B.L. and Bay L.K. (2017) Heritability of the *Symbiodinium* community in vertically-and horizontally-transmitting broadcast spawning corals. *Scientific reports* 7(1): 1-14.

Radecker N., Pogoreutz C., Voolstra C.R., Widenmann J. and Wild C. (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends in microbiology* 23(8): 490-497.

Ramos R. and García, E. (2007) Induction of mixed-function oxygenase system and antioxidant enzymes in the coral *Montastraea faveolata* on acute exposure to benzo (a) pyrene. *Comparative Biochemistry and Physiology Part C*: Toxicology & Pharmacology 144(4): 348-355.

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

Richmond R.H., Tisthammer K.H. and Spies N.P. (2018) The Effects of Anthropogenic Stressors on Reproduction and Recruitment of Corals and Reef Organisms. *Frontiers in Marine Science* 5: 226. DOI: 10.3389/fmars.2018.00226

Richter M., Rühle W. and Wild A. (1990) Studies on the mechanism of photosystem II photoinhibition II. The involvement of toxic oxygen species. *Photosynthesis Research* 24(3): 237-243.

Rogers, C. S. (1990). Responses of coral reefs and reef organisms to sedimentation. *Marine ecology progress series* 62(1): 185-202.

Rogers C. S., Gilnack M. and Fitz III H. C. (1983) Monitoring of coral reefs with linear transects: a study of storm damage. *Journal of Experimental Marine Biology and Ecology* 66(3): 285-300.

Rotchell J.M. and Ostrander G.K. (2011) Molecular toxicology of corals: a review. *Journal of Toxicology and Environmental Health Part B* 14(8): 571-592.

Scheufen T., Krämer W.E., Iglesias-Prieto R. and Enríquez S. (2017) Seasonal variation modulates coral sensibility to heat-stress and explains annual changes in coral productivity. *Scientific Reports* 7(1): 1-15.

Shaked Y. and Armoza-Zvuloni R. (2013) Dynamics of hydrogen peroxide in a coral reef: sources and sinks. *Journal of Geophysical Research* 118: 1793–1801.

Sheridan C., Grosjean Ph., Leblud J., Palmer C.V., Kushmaro A. and Eeckhaut I. (2014) Sedimentation rapidly induces an immune response and depletes energy stores in hard coral. *Coral Reefs* 33: 1067-1076.

Smith E.G., D'Angelo C., Salih A. and Wiedenmann, J. (2013) Screening by coral green fluorescent protein (GFP)-like chromoproteins supports a role in photoprotection of zooxanthellae. *Coral Reefs* 32(2): 463-474.

Soderhall K. and Cerenius L. (1998) Role of prophenoloxidase activating system in invertebrate immunity. *Current Opinion in Immunology* 10: 23–28.

Soto-Santiago F.J., Mercado-Molina A.E., Reyes-Maldonado K. and Sabat A.M. (2017) Physiological Response to Thermal Stress of the Caribbean Corals *Orbicella annularis* and *Porites astreoides*. *Environmental Sciences* 11(2): 48-57.

Spalding M.D., Longley-Wood K., Acosta-Morel M., Cole A., Wood S., Haberland C. and Ferdana Z. (2018) Estimating Reef-Adjacent Tourism Value in the Caribbean. The Nature Conservancy. Retrieved from: https://oceanwealth.org

Stake J.L. and Sammarco P.W. (2003) Effects of pressure on swimming behavior in planula larvae of the coral *Porites astreoides* (Cnidaria, Scleractinia). *Journal of experimental marine biology and ecology* 288(2): 181-201.

Stearns, S. C. (1992) The Evolution of Life Histories, Oxford University Press, Oxford.

Sugumaran M. (2002) Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. *Pigment Cell Research* 15: 2–9.

Sumich J.L. (1996) An introduction to the biology of marine life, 6th ed. Brown, Dubuque, pp. 255–269.

Szmant A. (1986) Reproductive ecology of Caribbean reef corals. *Coral Reefs* 5: 43-54.

Tarrant A.M., Reitzel A.M., Kwok C.K. and Jenny M.J. (2014) Activation of the cnidarian oxidative stress response by ultraviolet radiation, polycyclic aromatic hydrocarbons and crude oil. *Journal of Experimental Biology* 217(9): 1444-1453.

Telesnicki G.J. and Goldberg W.M. (1995) Effects of turbidity on the photosynthesis and respiration of 2 South Florida Reef coral species. *Bulletin of Marine Science* 57(2): 527-539.

Torres-Pérez J.L., Armstrong R.A. and Detrés Y. (2014) Impacts of UV Radiation on Caribbean Coastal Marine Ecosystems In J.A. Radosevich, UV Radiation: Properties, Effects, and Applications Physics, Research and Technology Series (pp. 371). New York: Nova Science Publishers.

Vega Thurber R.L., Burkepile D.E., Fuchs C., Shantz A.A., McMinds R. and Zaneveld J.R. (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Global Change Biology* 20(2): 544-554.

Wall C.B., Ricci C.A., Foulds G.E., Mydlarz L.D., Gates R.D. and Putnam H.M. (2018) The effects of environmental history and thermal stress on coral physiology and immunity. *Marine biology* 165(3): 56.

Wiedenmann J., D'Angelo C., Smith E.G., Hunt A.N., Legiret F.E., Postle A.D. and Achterberg, E.P. (2013) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nature Climate Change* 3(2): 160-164.

Woo S., Yum S., Park H.S., Lee T.K. and Ryu J.C. (2009) Effects of heavy metals on antioxidants and stress-responsive gene expression in Javanese medaka (*Oryzias javanicus*). *Comparative Biochemistry and Physiology Part C*: Toxicology & Pharmacology 149(3): 289-299.

Xiang N., Jiang C., Huang W., Nordhaus I., Zhou H., Drews M. and Diao X. (2019) The impact of acute benzo (a) pyrene on antioxidant enzyme and stress-related genes in tropical stony corals (Acropora spp.). *Science of The Total Environment* 694: 133474.

Zhang T., Diaz J.M., Brighi C., Parsons R.J., McNally S., Apprill A. and Hansel, C. M. (2016) Dark production of extracellular superoxide by the coral *Porites astreoides* and representative symbionts. *Frontiers in Marine Science* 3: 232.

Zhang Y., Million W.C., Ruggeri M. and Kenkel C.D. (2019) Family matters: Variation in the physiology of brooded *Porites astreoides* larvae is driven by parent colony effects. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 238: 110562.

Zhou Z., Yu X., Tang J., Wu Y., Wang L. and Huang, B. (2018) Systemic response of the stony coral *Pocillopora damicornis* against acute cadmium stress. *Aquatic Toxicology* 194: 132-139.