

NOVA SOUTHEASTERN UNIVERSITY OCEANOGRAPHIC
CENTER

PORITES ASTREOIDES LARVAL RESPONSE TO ACUTE SALINITY STRESS

By

Ana María González Angel

Submitted to the Faculty of
Nova Southeastern University Oceanographic Center
in partial fulfillment of the requirements for
the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

July 2013

Thesis of Ana María González Angel

Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science:
Marine Biology

Nova Southeastern University
Oceanographic Center

July 2013

Approved:

Thesis Committee

Major Professor : _____
Patricia Blackwelder, Ph.D.

Committee Member : _____
Jose Lopez, Ph.D.

Committee Member : _____
Sara Edge, Ph.D.

Committee Member : _____
Esther C. Peters, Ph.D.

Abstract

Coral reef biodiversity is threatened by rapidly changing anthropogenic activities and natural perturbations, leading to massive ecological and economic consequences ranging from the loss of fisheries to coastal erosion. It is necessary to understand corals responses to environmental changes in order to determine management programs on appropriate spatial and temporal scales to address these issues. Coral larvae are the product of sexual reproduction, have the potential to recruit to new areas, and are fundamental in maintaining genetic diversity. These larvae are subjected to variations in local environmental conditions until they settle, inducing specific larval molecular response patterns. One factor that influences coral health is salinity. Low salinities can alter cell homeostasis creating stress in cells. In the natural environment larvae may be exposed to low salinities due to heavy rainfall or run-off. This study investigated larvae responses to low salinity and characterized gene expression in the reef-building coral *Porites astreoides* using a coral stress-focused microarray. Nine batches of 250+ larvae from three different colonies were collected and immediately exposed in an acute hyposalinity experiment. Samples from two treatments of 25 and 30 ppt, and a control at 35 ppt were used in this study. After experimental exposure these samples were stored in RNAlater® and molecular analysis was performed. The RNA from the samples was extracted, purified and hybridized to a coral stress-focused microarray. Statistical analysis indicates 72 genes were differentially expressed across treatments ($p<0.003$, analysis of variance). The hierarchical cluster analysis groups together the larvae exposed to salinities of 30 and 35 ppt indicating both treatments induced similar patterns of gene expression. Larvae responses to 30 ppt are minimal, suggesting larvae can tolerate acute exposures to 30 ppt salinity levels. In contrast, the lower salinity (25 ppt) induced a strong response in both the coral and zooxanthellae. The coral larvae up-regulated stress response genes and down-regulated genes associated with normal cell functioning. Additionally, the zooxanthellae down-regulated genes associated with photosynthesis. These results suggest larvae may be vulnerable to bleaching, which may affect the ability of larvae to successfully undergo metamorphosis and survive at low salinities. However, this has yet to be confirmed with complementary techniques. Long-term studies are recommended to examine the effects of hyposalinity on larvae at different time scales and life history stages.

Keywords: *Porites astreoides*, larvae, gene expression profiling, microarray, salinity

Acknowledgements

I thank my committee members for supporting me. During this process they were mentors to me, especially Dr. Blackwelder, Dr. Edge, and Dr. Peters. I had the opportunity of working very closely with the three of them at different stages of my master's degree, and I am very thankful for the time they invested teaching me a little bit of each one's area of expertise. I also have to thank the Link foundation for funding me and providing me this research experience; Dr. Edge for providing the guidance and resources necessary to develop this project through the HBOI-FAU Specialty License Plate Fund; Lisa Cohen for teaching me the lab skills necessary to conduct this project; my committee members for comments in the paper; and Ben Mason for providing the samples (that were collected under Florida Keys National Marine Sanctuary permit FKNMS-2006-009 and funded by MOTE POR-2005B-15 grant to Drs. Peter Glynn and Ben Mason). I have to thank all the people who supported me directly or indirectly during my masters. First, I have to thank my parents who have always believed in me and supported me while I had difficult financial situations. I have to thank my friends from Colombia and here. Art&ficios and Anasi are two groups very close to my heart, thank you for keeping in touch. Friends I made here made life better while I've been far away from home: Jigu, Felipe, Jaz, the Bears and Britt, Keri, RinRin, Diane, fellow students, and my "Polombians". Jenny and Lisa hosted me when I visited HBOI a couple times. Thank you, you are wonderful hosts. Finally, since I couldn't afford having a car during my studies, I have to thank all the people that gave me rides in the past two years: Joyce, Cameron, Alexandra, Jazmin, Keri, Renae, Mauricio, Brittnee, Peggy and many, many more, even my committee members gave me rides! Thank you all!

Table of Contents

Abstract.....	3
Acknowledgements.....	4
Introduction.....	6
Materials and Methods.....	16
Results.....	26
General Trends.....	27
Gene Expression Profiles.....	31
Discussion.....	34
Conclusions.....	43
References.....	45
Appendix.....	52

Introduction

Coral reefs are among the most diverse and productive ecosystems on earth and are vulnerable to anthropogenic and natural stressors (Raymundo et al., 2008). Stressors include overfishing, water pollution, sedimentation, habitat degradation, elevated water temperatures and changes in salinity (Sala & Knowlton, 2006; Downs et al., 2009; Hughes et al., 2010). In order to establish adequate mitigation programs, such as formulating scientific policies and establishing marine protected areas, it is necessary to understand how coral reef organisms respond to environmental changes. Hence, studies involving ecological processes that affect coral reef populations are necessary. Major ecological processes include reproduction, recruitment, post-settlement mortality, coral growth, fragmentation, and mortality (van Woesik and Jordán-Garza 2011). This study incorporates an ecological question on coral recruitment with one molecular technique to study the response of coral larvae to reduced salinities.

Scleractinian corals reproduce either sexually by broadcast spawning or brooding and/or asexually by budding, coral polyp expulsion, and fragmentation (Harrison 2011). Some benefits of asexual mechanisms are apparent, for example, fragmentation allows colonization of areas not suitable for larvae like soft-bottom areas (Lirman 2000). Similarly, budding allows colonies to occupy new areas and reposition themselves by creating planula-like balls that settle, attach and develop new corals from old colonies (Kramarsky-Winter and Loya 1996). Also, new propagules originated by coral polyp expulsion develop young colonies rapidly

without larval development, settlement, and metamorphosis costs (Kramarsky-Winter et al., 1996). Despite advantages of asexual reproduction mechanisms, only planula larvae have the potential to maintain genetic diversity (Edmunds et al., 2001; Harrison 2011). Additionally, due to the planktonic condition of the larval stage, dispersal is achieved to new and denuded areas (Babcock and Heyward, 1986). “Regardless of their reproductive mode, planulae must contend with a varied range of environmental conditions before settling” (Vermeij et al., 2006). Fluctuations in physical, chemical, and biological conditions such as changes in salinity, temperature, hydrostatic pressure and sedimentation (Gleason and Hofmann 2011); and additional sources of mortality, such as predation and bacterial infection, may also affect larval chances of developing into adult coral polyps (Graham et al., 2008). It has been documented that larval success in advancing to further life stages is influenced by variability in these environmental parameters (Richier et al., 2008; Rodriguez-Lanetty et al., 2009; van Woesik and Jordán-Garza, 2011). After overcoming these planktonic conditions, larvae must settle and undergo a complex metamorphosis on an appropriate substrate, founding a juvenile coral colony (Grasso et al., 2011). Larvae settlement is ecologically important since it may eventually impact coral reef demographics (Edmunds et al., 2001).

In recent times coral larvae have been subjected to more severe environmental factors than in the pre-industrial world and projected models indicate additional environmental degradation will occur (Anlauf et al., 2010; Orr et al., 2005; Hoegh-Guldberg et al., 2007). For instance, ocean temperatures have increased by as much as 1.5 °C since the 19th century (Edmunds et al., 2005) and global temperatures may rise by at least 2 °C by 2050–2100 (Hoegh-Guldberg et al., 2007). Rising temperatures are

expected to increase evaporation and consequent precipitation in some places, which is already typical of some tropical and subtropical areas (Sekercioglu 2010). For instance, in the northern Atlantic Florida Keys, summers are typically long and characterized by high precipitation. The area has a tropical maritime climate consisting of two well-defined seasons: long wet summers from May to October and mild dry winters from November to April (NOAA, 1995). Localized convective storms and intense low-pressure systems (in the form of tropical storms and hurricanes) are integral climate components of this area (NOAA, 1995). For example, the Florida Keys suffered 5 major hurricanes within 5 months in 2005 (Wilkinson and Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre 2008). Most rainfall occurs during the summer with precipitation peaking in June, although tropical disturbances are also common in November. Precipitation associated with tropical depressions and hurricanes ranges from 13 cm to 26 cm but may exceed 50 cm (reviewed in NOAA, 1995). Intensive heavy rainfalls causes reduced salinity, and coastal areas may also experience water quality decline by nutrient and sedimentation input (Faxneld et al., 2010). Additionally, salinity can remain reduced for longer periods of time (hours to weeks) under concurring low tides and heavy rainfalls (Kerswell and Jones, 2003).

“Heavy precipitation reduces salinity, increases terrestrial runoff, and overloads wastewater treatment facilities. Thus, precipitation events may expose corals to osmotic stress, sedimentation, xenobiotics and other factors affecting coral health” (Edge 2007). These conditions are becoming more severe as climate changes and natural and anthropogenic disturbances become more frequent. Predictions suggest that the impact of these combinations will be synergistic, resulting in the loss of

biodiversity (Veron 2011). However, predictions about the future of coral reefs are generally based on computing models, which are particularly difficult to create since there are multiple stressors and climate factors impinging on reefs (Wilkinson and Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre 2008). Stress is a parameter or event that has the potential to negatively impact the health and/or fitness of an organism (Thurber and Correa 2011) and alter gene expression profiles. The stress factor examined in this study was reduced salinity in a local population of *Porites astreoides*.

Porites spp. is an important structural component of coral reefs worldwide and are among the most ecologically important corals in terms of abundance, global distribution, and variety of habitats occupied (Forsman et al., 2009). *Porites astreoides* is a common reef-building coral in the tropical western Atlantic, the Gulf of Mexico and the Caribbean Sea (Fauth et al., 2006). *Porites astreoides* inhabits all reef environments (Veron 2000) including very shallow waters (≤ 2 m) (Gleason 1993). This species is a typical small-sized weedy or opportunistic brooder coral, and it is characteristic of reef zones frequently impacted by storms, low tidal exposures or other forms of physical disturbance (Szmant 1986). *P. astreoides* releases mature swimming larvae from April to September (Chornesky and Peters, 1987; McGuire 1998) typically coinciding with the rainy season in the northern Atlantic. Moreover, the reproductive season consists of several reproductive cycles and larval release is correlated with the water temperature and the moon cycle (Chornesky and Peters, 1987; McGuire, 1998).

As mentioned above, coral larvae are exposed to environmental factors while in their planktonic stage. One of those environmental factors is low salinity. Salinity is typically measured in the Practical Salinity Units (PSU). The Practical Salinity Unit (PSU) is a dimensionless unit established by UNESCO in 1978 to measure the concentration of dissolved salts in water (UNESCO et al. 1982, NASA 2013). The Practical Salinity scale was established to improve the standardization of the previous salinity scale, which was based on the relation of conductivity-chlorinity of seawater (UNESCO et al. 1982). According to the previous scale, the salinity of standard seawater (from the North Atlantic ocean) was equal to 35 parts per thousand, which was then established by UNESCO in 1978 as equivalent to 35 in the practical salinity scale (UNESCO et al. 1982). Regardless of their mode of reproduction, brooded larvae and released gametes may experience salinity changes in the sea surface. “Variations in salinity due to large-scale hydrodynamic processes or rainfall are likely to be a potential natural stressor during the larval phase, as cnidarians are not capable of osmoregulation” (Guldberg and Smith 1989, reviewed in (Vermeij et al., 2006)). Moreover, “salinities as low as 28 PSU do occur near the water surface after heavy rainfall (M. J. A. Vermeij pers. obs.) and occasional salinity differences up to 2 PSU across 7 m of depth have been observed for the Florida Keys (Porter et al., 1999) indicating that, depending on local reef topography, lower salinities can even occur at the bottom” (Vermeij et al., 2006).

Depressed salinity may affect gametes from mass spawning species, their larvae, and larvae from brooding species since they tend to be highly buoyant and remain near to the surface for prolonged periods of time (Aranda et al., 2011). There is no specific data, however, on the duration of the planktonic larval stage and dispersal of *Porites*

astreoides (Pichon 2011). But it is known that the brooded larvae of *Porites astreoides* contain great amounts lipids that are held in the developing gastrodermis (Patricia Blackwelder, personal communication). Lipid concentration is important since planula larvae primarily depend on stored lipid for their nutrition (Grasso et al., 2008) and because the amounts of stored lipid are related to buoyancy. For instance, a study including eggs and larvae from the broadcast-spawning species *Acropora palmata*, *Montastraea annularis* and *M. franksi* reported they contained a high concentration of lipids (60–70% by weight) and float in surface waters for 3–4 days following spawning (Wellington and Fitt 2003). Note that *Montastraea annularis* and *M. franksi* were recently renamed *Orbicella annularis* and *O. franksi* (Budd et al., 2012).

Porites astreoides larvae can be exposed to depressed salinities mostly under two conditions. Larvae coming from corals on inshore reefs experience freshwater intrusion chronically. This is possible given the near shore ubiquitous distribution of this species in the north Atlantic, the Gulf of Mexico and the Caribbean. And second, released larvae can encounter acute to moderate hyposalinity conditions at the water's surface during heavy rains, especially if they concur with low tides (Jokiel et al. 1993; Kerswell and Jones 2003). Moreover, this species has been reported in very shallow waters where salinity changes can be more dramatic than at deeper waters.

Salinity fluctuations can alter homeostasis. Homeostasis is the essential regulation by an organism of the composition of its internal environment (Wilkins and Wilkins, 2003). Homeostasis involves many processes that vary depending on the organism. One of these processes is osmotic regulation. Maintaining the total amount of

intracellular solutes within some narrow range is essential for the normal functioning of cells (Kirshner 1991). Most marine invertebrates keep the osmotic concentration of their bodies equal or close to concentrations in the ocean. For example, corals are stenohaline and osmoconformers (Chartrand et al., 2009). “Osmoconformers change their internal osmotic concentration when exposed to changing osmotic concentrations, thus remaining isosmotic with the medium. [...] However, although most marine organisms are osmoconformers this does not mean that their body fluids have the same solute composition as the seawater. On the contrary, they maintain their internal salts concentration that are out of equilibrium with the environment, and this requires extensive regulation” (Schmidt-Nielsen 1997). Hence, cell membranes are permeable to water but impermeable to solutes. Consequently, changes in the extracellular concentrations will induce cell volume changes (Schmidt-Nielsen 1997). For example, lowered salinities will induce water uptake increasing cellular volume and altering cytoskeletal organization. This is known as cell swelling. Cell swelling can induce karyolysis, a state of nuclear swelling and loss of chromatin (Galloway et al., 2005). Previous studies of *Montastraea faveolata* larvae have reported that hyposalinity increased pre and post-settlement mortality and altered larval behavior and substrate choice upon settlement (Vermeij et al., 2006). Hyposalinity is also associated with bleaching and mortality in corals (Downs et al., 2009; Berkelmans and Oliver 1999).

The objective of this study was to characterize the gene expression profiles of *Porites astreoides* larvae exposed to depressed salinities in order to assess how this factor affects larvae molecular pathways; and, consequently their potential to develop into

further life stages. This was possible by using the technique of microarrays, which principle is closely associated with the Central Dogma of Molecular Biology.

The Central Dogma of Molecular Biology is a fundamental principle in molecular biology (Niwa and Slack 2007) and states that DNA codes for amino acids or proteins indirectly through a process including transcription to RNA, which is then translated to the amino acid product (Figure 1) (Crick 1970). Transcription of RNA from one strand of DNA is the first step in gene expression (Hartl and Jones 2005). However, gene expression can also be regulated at the level of RNA processing, alternate patterns of splicing, transcript or mRNA stability or epigenetics (Hartl and Jones, 2005). In this study, the technique of microarrays was used to study changes in gene expression profiles between treatments. The technique of microarrays has advanced greatly by combining improvements in genomics, recombinant DNA technology, optics, fluid flow systems, and high-speed data acquisition and processing (Prescott et al., 2002).

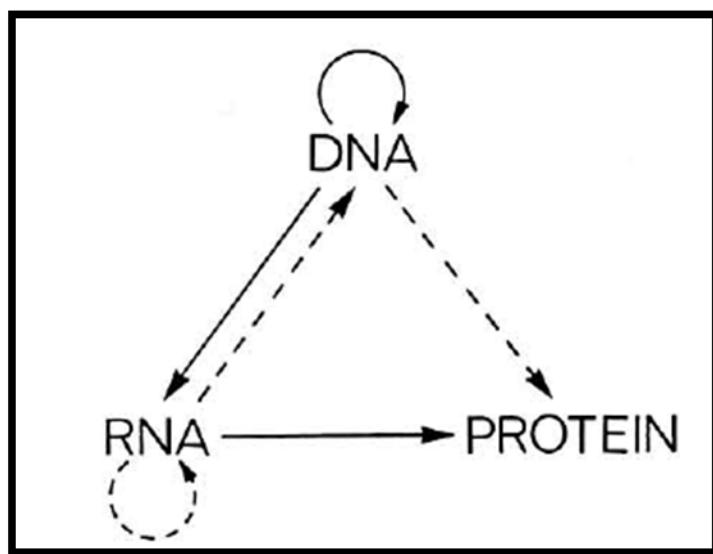


Figure 1: Central Dogma of Molecular Biology. “Solid arrows show general transfers and dotted arrows show special transfers”. Image and quoted text were obtained from (Crick 1970).

Microarrays make possible to follow expression of thousands of genes and study global gene regulation (Prescott et al., 2002) simultaneously in parallel samples (Saccone & Pesole 2003). Therefore, genome-wide patterns of gene expression (Hartl and Jones 2005) can be assessed. The expression of a gene is dictated in part by the integration of cellular and environmental signals controlling the transcription activity and protein regulation (Saccone and Pesole 2003). Hence, this technique has been widely used to study environmental effects on gene expression. Other applications of the microarray technique include comparative genomics among species or populations and identification of fast-evolving genes (Forêt et al., 2007).

The gene expression profiles obtained with the technique of microarray are qualitative and quantitative. First, the qualitative information depends on what genes are spotted onto the microarray. This greatly depends on the bioinformatics' resources (i.e. gene sequences) available when the microarray chips are designed and the research question intended to answer. Second, the quantitative information obtained depends on the relative amounts of gene expression detected. This is associated with the physiological state of each gene. The physiological state of a gene can fall in one of three categories: repressed, basal or induced. During gene repression the transcription machinery is off and cannot access the DNA promoter whereas in basal state the gene is accessible although there is no active transcription. Finally, when a gene is induced the transcription machinery is activated (Saccone and Pesole 2003). These physiological states will be addressed here as down-, up- and neutral gene regulation. Up-regulation and down-regulation can also be referred to as over- and under-expression, respectively. In this study, a coral stress-focused microarray was used. Dr.

Sara Edge from the Marine Genomics Laboratory at Harbor Branch Oceanographic Institute – Florida Atlantic University designed the microarray. The available custom-made microarray was printed by Agilent and contained 1,368 coral genes and 327 *Symbiodinium* sp. gene probes that represent 100 holobiont biomolecular pathways related to stress and regulatory pathways.

In this study, recently released larvae from *Porites astreoides* were acutely exposed to reduced salinity conditions and their gene expression profiles were characterized by the technique of microarrays. The question intended to answer was: how do larvae from *Porites astreoides* respond to acute low salinity exposures at a molecular level? Interestingly, Balm (1999) stated “stress tends to occur during unpredictable perturbations of the environment and not as result of the predictable changes that occur during the course of, for example, a day, tide changes or the seasons.” Therefore, considering the overlap between the reproductive season of *Porites astreoides* and the rainy season in the north Atlantic the null hypothesis tested in this study was that *P. astreoides* larvae exposed to lower salinity seawater have the same gene expression profile as larvae not exposed to lower salinity seawater. If the null hypothesis was supported by the data this would indicate that larvae do not respond to low salinities and may be adapted to depressed salinities of 30 and 25 ppt. Since larvae may encounter these conditions naturally in the field during every reproductive season, perhaps they are preprogrammed with some adaptive mechanisms to withstand hyposalinity. Also, up-regulation of normal developmental associated pathways would be expected in all the larvae with consistent expression of pathways associated with, for example, growth and metabolism. Note that these assumptions

would be similar for the zooxanthellae that would be expected to consistently up-regulate photosynthesis and normal cell functioning.

On the contrary, the alternate hypothesis stated that low salinities would induce different gene expression profiles. In this case each treatment was expected to promote a unique gene expression profile. Down-regulation of normal regulatory and developmental pathways of the treatments compared to the control were expected. Furthermore, up-regulation of stress-associated pathways in the coral and zooxanthellae were expected as well.

Materials and Methods

To examine the effects of low salinity on *Porites astreoides* larvae the following experiment was performed. Dr. Ben Mason conducted the larvae collection and exposures.

Three different colonies of *Porites astreoides* were collected at Little Grecian Reef, Key Largo, in the Florida Reef Tract under the Florida Keys National Marine Sanctuary permit FKNMS-2006-009. Colonies were collected in June 21 2006 prior to the full moon (June 25) and kept in outdoor mesocosms covered by shade cloth at the facilities of the Rosenstiel School of Marine and Atmospheric Science (RSMAS), University of Miami. Water was sourced from Bear Cut (adjacent to RSMAS) and pumped through the mesocosms. Colonies were not fed but had access to food (plankton) living within the mesocosm and pumped in with water. Salinity averaged 36 ppt, and hypo- or hypersalinity within the mesocosms as a result of rain or

evaporation was not an issue as the turnover rate was high within the mesocosms (Ben Mason, personal communication). Coral colonies were kept until they released the larvae and were returned to their original site within a week after spawning. Colonies and larvae were maintained at 25 °C.

Nine batches of >250 newly released larvae were immediately exposed to a low salinity exposure experiment under three salinity conditions. The experiments were conducted in plastic solo cups housed within 10-gallon aquaria containing about 6 cm of water on a lab bench (to serve as a water bath and stabilize the temperature). Larvae were kept indoors. Light conditions ranged within 10 to 30 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ¹. The controls were maintained at 35 parts per thousand (ppt) and two treatments were maintained at 30 ppt and 25 ppt. “Seawater contains 35% salts; and, although it varies somewhat with geographical location the relative amounts of dissolved ions remain very nearly constant” (Shmidt-Nielsen, 1997). Therefore the control was established at 35 ppt. Additionally, the low salinities selected are within the range expected after a heavy rainfall (Jokiel et al. 1993). Different containers were used per treatment and sample (Figure 2). Each batch of 250 larvae was regarded as one sample. Three colony batches were obtained per treatment, each one from a different parental colony. Larvae were collected twenty-four hours after exposure and fixed in *RNAlater®* (QIAGEN). Nine samples total were collected for this study and maintained at -80 °C until RNA processing.



Figure 2: Experimental setting. Note that parallel studies were performed including larvae from other species, which explains why there are so many containers in the picture. In this study only the larvae from *Porites astreoides* that were exposed to a low salinity were included. Courtesy of Ben Mason.

Members of Dr. Edge's lab contributed to initial RNA extractions in 2006, 2008, and 2010. Samples preserved in RNAlater® (QIAGEN) were transferred to new 2 mL tubes filled with TRIzol (TRIzol®, Invitrogen™, NY, USA) because the RNA extraction protocol followed was the TRIzol extraction method (Sara Edge, personal communication). In 2012 the best samples per colony and treatment were selected to proceed with the microarray analysis for this study.

The available, custom-made microarray designed by Dr. Edge and printed by Agilent Technologies (unpublished) consists of 1,368 coral and 327 *Symbiodinium* sp. gene probes replicated three times each, along with positive and negative controls. The microarray contains 100 holobiont biomolecular pathways related to stress and regulatory pathways including information for apoptosis, cytochromes, xenobiotic response (pesticides, dioxin, PAH's), DNA repair, inflammation, wound healing, cell signaling (receptors, activators), heat shock elements, housekeeping (which encode essential metabolic enzymes or cellular components and are expressed constitutively

at relatively low levels at the cells (Hartl & Jones 2005)), kinases, oxidative stress & redox homeostasis, cytochrome P450s, peroxisome proliferator response, phosphatases, transcription factors, respiration, and metabolism. Additionally, the array included genes specific to *Symbiodinium* spp. These genes were involved, for example, in normal cell functioning pathways like photosynthesis (which were used as a proxy for zooxanthellae health), cell respiration and DNA replication.

Samples preserved in TRIzol® (Invitrogen, Carlsbad, CA, USA) were thawed at room temperature. RNA was isolated from larvae tissue using a standard phenol-chloroform protocol (Chomczynski and Sacchi 1987) and purified using lithium chloride precipitation. If the process had to be interrupted, samples were frozen at -80 °C before adding the lithium chloride. Samples were always thawed on ice unless otherwise stated.

Since in this technique expressed RNA is used as a template to quantify gene expression, great RNA quality is mandatory and only intact RNA can be used. RNA is a thermodynamically stable molecule but it is rapidly digested in the presence of the nearly ubiquitous RNase enzymes (Schroeder et al., 2006). During RNase activity, the 28S band disappears faster than the 18S band in gels and electropherograms allowing the detection of a beginning degradation (Schroeder et al., 2006). Good quality RNA appears in electropherograms and gels mostly as two distinct bands corresponding to 18S and 28S ribosomal RNA. In contrast, degraded RNA appears as multiple and/or faint bands (Figure 3, right hand side image).

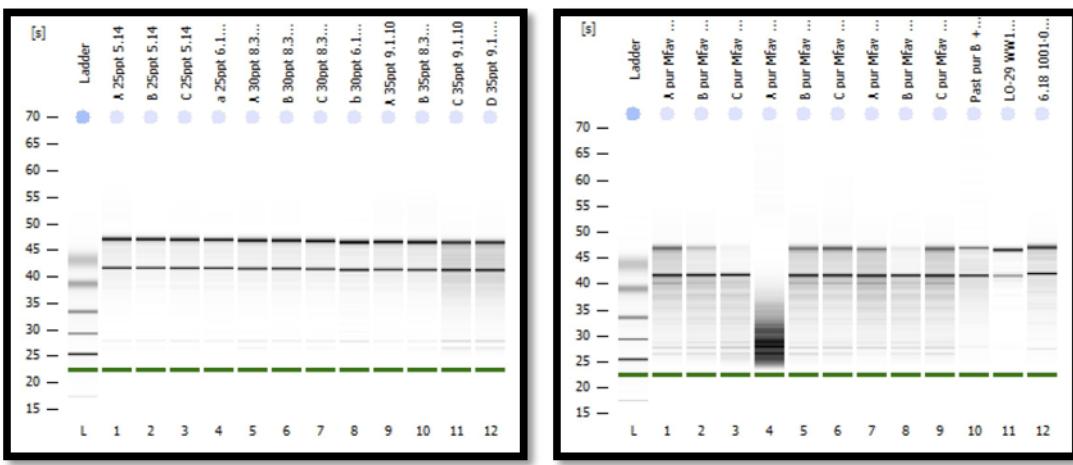


Figure 3: Electropherogram examples obtained using the 2100 Bioanalyzer instrument from Agilent Technologies. The Y-axis corresponds to the molecular weight and the X-axis corresponds to the location of the sample in the chip. Sample names are at the top. The ladder is a reference column. Some of the samples analyzed in the left-hand image were actually used in this study. The image to the left is a good example of expected bands for good quality RNA showing distinct bands typical from the 18S and 28S RNA subunits. In contrast, the image to the right shows multiple samples with degraded RNA (samples 2, 3, 4, 8, 10 and 11).

RNA was quantified with a Nanodrop Spectrophotometer (Thermo Scientific) and quality was assessed using gel electrophoresis (1% agarose) and analyzed with the 2100 Bioanalyzer Instrument (Agilent Technologies). If these procedures were not scheduled to be performed on the same day, samples were frozen at -80 °C in between RNA quality assessments. In addition, RIN values (RNA Integrity Number; Agilent Technologies Bioanalyzer 2100) were measured as indicators of RNA integrity. “RIN can be correlated with the outcome of downstream experiments, i.e. by performing this quality assessment step users can prevent themselves from erroneous results and loss of money and resources” (Schroeder et al., 2006). RIN values range from 0 to 10, 0 representing totally degraded RNA and 10 representing perfectly intact RNA. In this study most samples RIN values ranged between 8.80 and 9.40; and, only one sample had a RIN value below 8.80, RIN = 7. Only samples with high quality (260/280 and 260/230 ratio values greater than 2, RIN values greater than 7) and high quantity of

RNA (at least 200 ng/ μ L) were labeled. Labeling with Cyanine 3 was followed according to the manufacturer's protocol (Low Input Quick Amp Labeling One Color Kit, Agilent Technologies). Samples were diluted to obtain an initial quantity of 200 nanograms of total RNA for labeling and frozen at -80 °C. Reagents and samples were thawed on ice. After preparing the spike-in control RNA (RNA Spike-In Kit, One Color by Agilent) and labeling reaction, cDNA was synthesized in a water bath for 2.5 hours at 40 °C (2 μ L of 5x 1st strand buffer, 1 μ L of 0.1 M DTT, 0.5 μ L of 10 mM dNTP, 1.2 μ L of Affinity Script RNase Block mix were added per sample). cDNA served as a template for cRNA synthesis. To each sample was added 0.75 μ L Nuclease-free water, 3.2 μ L 5X Transcription Buffer, 0.6 μ L 0.1M DTT, 1 μ L NTP mix, 0.21 μ L T7 RNA Polymerase Blend and 0.25 μ L Cyanine 3-CTP. The reaction occurred in a covered water bath at 40 °C for 2.5 hours.

Cyanine 3 is a photoreactive compound that binds to cRNA molecules and fluoresces on laser excitation. Measurement of the fluorescent intensities is compared between spots on the microarray chip and used to quantify levels of gene expression. Labeled cRNA was purified using spin columns following the manufacturer's protocol (RNeasy MinElute kit, Qiagen). Sample quality was assessed with the Nanodrop Spectrophotometer (Thermo Scientific). If needed, samples were stored frozen at -80 °C after the cDNA synthesis, cRNA synthesis and amplification or cRNA purification steps (Agilent Technologies Inc. 2010).

Labeled samples were hybridized to replicate microarrays according to the manufacturer's protocol (One-Color, Low Input Quick Amp Labeling Kit, Agilent TechnologiesTM). A gasket slide with 8 separate o-rings was used to separate replicate

microarrays on a chip (Figure 5). The gasket slide was placed in a microarray chamber and loaded well by well with a mixture of 600 ng of Cy-3 labeled sample (the volume varied depending on sample concentration), 5 µg of Blocking agent, 1µL of Fragmentation Buffer and enough nuclease free water to complete a total volume of 40 µL.

A microarray chip with 8 replicate arrays was placed on top of the gasket slide with the active side of the chip in direct contact with labeled samples. A clamp was used to tighten the assembly of the gasket chamber and the microarray chip. The hybridization was performed in a Hybridization Oven, manufactured for Agilent Technologies by Sheldon Manufacturers, Inc. (Cornelius, OR, USA), at a rotating speed of 10 rpm at 65 °C for approximately 17 hours. “Hybridization is the process in which two complementary sequences find each other and lock together” (Figure 4) (Suárez et al., 2009). These two complementary sequences correspond to an immobilized target DNA on the chip, and the mobile cDNA obtained from the sample. After hybridization occurred, Agilent Gene Expression wash buffers 1 and 2, with 0.005% Triton X-102 (10%) were added, per instructions from the protocol mentioned above, to reduce the possibility of array wash artifacts (Agilent, Catalogue: 5188-5327). Consequently, the gasket chamber was opened and the microarray chip and gasket slide were placed in a 50 mL tube containing wash buffer 1 (Agilent). The chip and the gasket slide were separated and the chip was washed and rinsed in two different steps: rinsing in wash buffer 1 at room temperature for 1 minute followed by rinsing in wash buffer 2 at approximately 37 °C for 1 minute. Immediately after washing, chips were scanned using a Gene Pix Professional 4200A scanner (Axon Instruments, Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 525 nm,

which excites the Cy3 dye of the labeled samples. Images of each microarray chip, with 8 arrays, were obtained using GenePix Pro 7 software (Molecular Devices, Sunnyvale, CA, USA). Given that each microarray contains eight chips, only eight samples can be hybridized at the time. Therefore, a total of two microarray chips were necessary to conduct this experiment. In the first microarray hybridization eight samples were used and in the second one the last sample was hybridized onto a chip that was shared with samples from a different experiment.

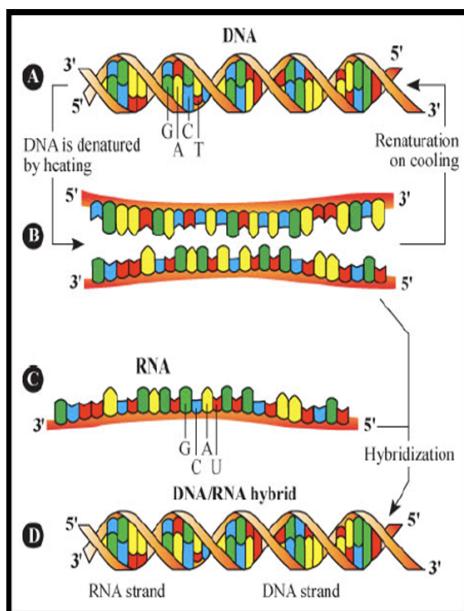


Figure 4: Hybridization of nucleic acids. In the case of microarrays cRNA is labeled with Cyanine 3. Image obtained from (Suárez et al., 2009).

Saturated images were avoided. A saturated image contains saturated pixels that appear very bright white. “A saturated pixel is not an accurate measurement of the signal from the pixel, so it is imperative to avoid saturation” (Molecular Devices Corp. 2005). Non-saturated images were obtained and saved as Target Image File Format (.tiff). In order to determine the gene expression levels, the data were extracted from the images by aligning a grid that corresponds to the identity and

location of each spot on the arrays. The hybridization intensity is represented by the amount of fluorescent emission, which gives an estimate of the relative amounts of the different transcripts that are represented (Allison et al. 2006) (Figure 5, right). GenePix Pro 7 software was used to align and extract the data in each array. Each array contains 15744 spots or features consisting of the replicate gene probes and positive/negative controls. The targeted known sequences are called probes and each one is spotted three to five times; each one called a feature. It was expected that the intensity of hybridization (which is measured by reading the Cy3 associated with the sample) was similar in the replicate features corresponding to one probe. In order to reduce noise, negative controls were removed from the final analysis. Features were averaged in order to evaluate the relative expression of each probe resulting in a dataset of 4178 probes. Statistical analysis of probe expression levels between samples was analyzed using JMP Genomics 6.0 (SAS Institute). The data were log2 transformed and normalized with Loess Normalization (Edge and Voss 2010; Edge et al. 2013). The distribution of the variability among the variables chip, array, hybridization date, treatment, and colony was assessed as part of the quality control (QC). Principle component analysis of the variability was also included. Most of the variability was explained by the array (31%) (8 of out 9 chips came from the same array) and the treatments (26%). Additionally, an analysis of variance (ANOVA) (F-test) was performed. ANOVA was done to test if there were differences in the gene expression of a total of nine samples.

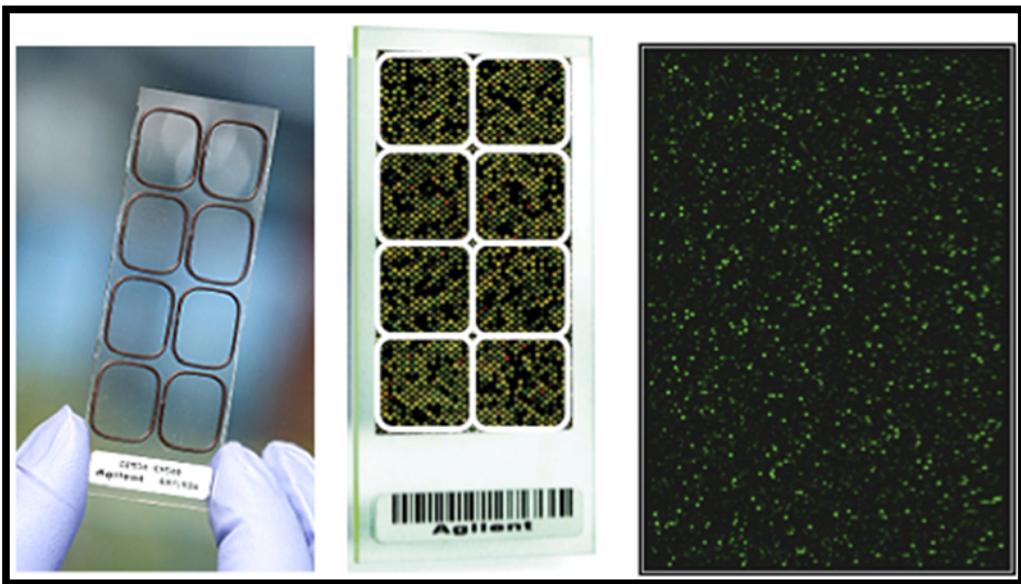


Figure 5: Microarray setting. The image to the left-hand side is a slide containing eight o-rings where the samples were deposited (Obtained from Agilent). The image to the middle represents a microarray slide containing eight arrays (Obtained from Agilent). The image to the right is one scanned microarray block showing differential gene expression (not all the spots are equally bright indicating some sequences are more or less expressed). This block includes 15744 spots, which contain empty spots, positive and negative controls and targeted sequences. Each gene sequence (probe) appears three to five times (features) per chip.

In an attempt to examine this species' gene expression profiles, available adult samples collected by Andrew Mason (no relation to Ben Mason) were initially included in this study. Eight adult samples were collected in June 14, 15, 16, and 17, 2012 in the Virgin Islands. Samples were collected at depths ranging from 0.6 to 17 meters. Salinity in the field ranged between 28.32 and 34.52 PSU at the surface, and between 28.33 to 34.61 PSU at the bottom. Temperature ranged from 28.6 to 33.1 °C at the surface, and 28.5 to 32.9 °C at the bottom. Samples were preserved in TRIzol (TRIzol®, Invitrogen™, NY, USA) and shipped to the Marine Genomics Lab in Harbor Branch Oceanographic Institute – Florida Atlantic University where they were processed. Samples were processed with three different RNA extraction protocols. Some samples were not preserved in enough TRIzol (the preservative did not cover

the sample completely). Samples released a lot of mucus making the solution very turbid. The first RNA extraction protocol followed was the same one followed to extract RNA from the larvae. The second protocol consisted of a pellet extraction of RNA followed by DNA digestion (QIAGEN, catalog number 79254) and the QIAGEN column purification (MinElute, Qiagen). The third protocol was a combination of the previous two (Mikhail Matz, personal communication). Extraction of good quality RNA was not possible since the pellets were heavily pigmented. Trying to remove the pigment to purify the RNA resulted in loss of RNA (Sara Edge, personal communication). Hence, RNA yields were very low indicating poor quality and low quantities of RNA (data not shown). This may have occurred given that adult hard coral material has relatively small amounts of living tissue per sample sometimes making even large-scale extractions yield little RNA (Forêt et al. 2007). These samples were not included in this study.

Results

In this study, the analysis of variance (ANOVA) indicated that 72 genes were differentially expressed by the batches of larvae exposed to the different salinity levels and gene regulation was statistically significant ($p < 0.003$) between treatments. A detailed list of genes is in Table 1 in the Appendix. In this study, general trends were analyzed in order to look for commonalities in the expressed genes with a general perspective. Expressed genes were also analyzed in a section of gene expression profiles, which were specific to the profiles treatments induced.

General trends

Note that genes were grouped within functions and functions were grouped within functional categories. Studying general trends allow the determination of what were the most common genes expressed during this experiment by comparing their corresponding functions or functional categories. All the samples were included and the results are shown in Figure 6. Figure 6 has two bar charts; both were obtained by including all the genes that were significantly expressed in this experiment in all the samples at the same time. The bar chart at the left-hand side shows the functional categories that were significantly expressed between the treatments. Similarly, the bar chart at right-hand side shows the functions that were more expressed. The most over-expressed functional category was Normal Cell Function (Figure 6, left) and the most over-expressed function was cellular signaling (Figure 6, right).

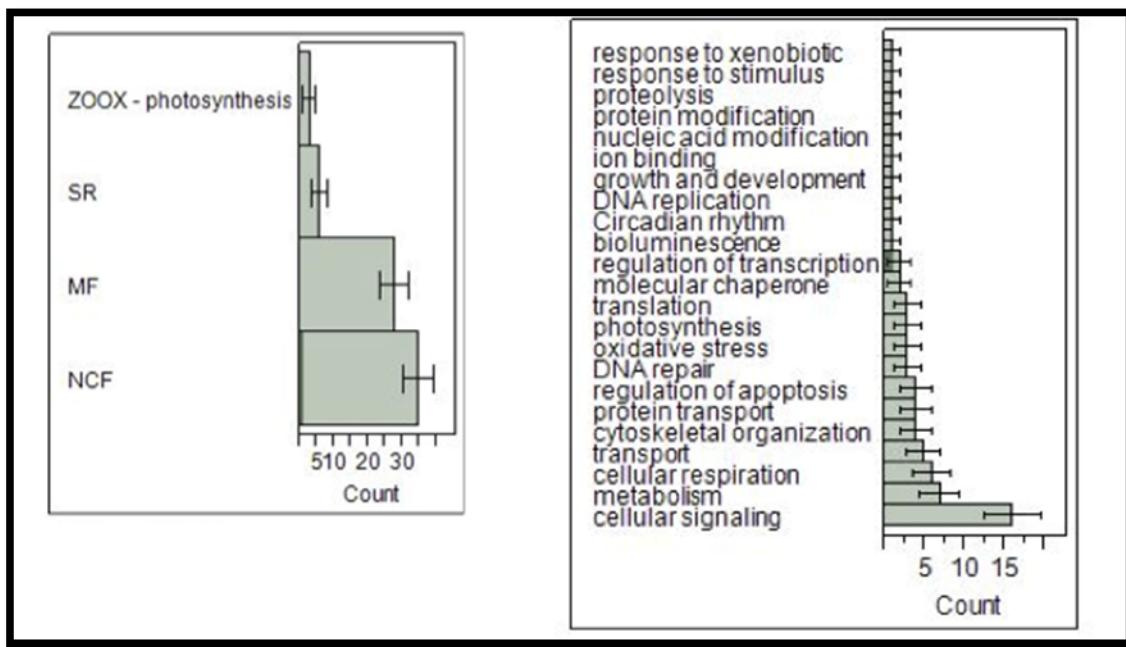


Figure 6: Bar charts including the 72 significantly expressed genes grouped by functional category (left) and function (right) showing general expression trends. ZOOX: Zooxanthellae photosynthesis, SR: Stress response, MF: Multifunctional, NCF: Normal Cellular Functions.

“Hierarchical cluster analysis graphically presents gene expression in a tree diagram, (dendrogram). Hierarchical clusters are often accompanied by heatmaps that consist of small cells, each consisting of a color, which represent relative expression values. Heatmaps are often generated from hierarchical cluster analyses of both samples and genes. Heatmaps offer a quick overview of clusters of genes that show similar expression values” (Allison et al. 2006).

Results indicate that larvae exposed to the lower salinity were less similar than the other two treatments as shown in the dendrogram (Figure 7). In other words, based on sample and gene expression patterns, the gene expression profile induced by the lowest salinity is different than the mild salinity and the control. A more detailed heatmap showing the clusters and labeled by function is available in the appendix (Figure 1).

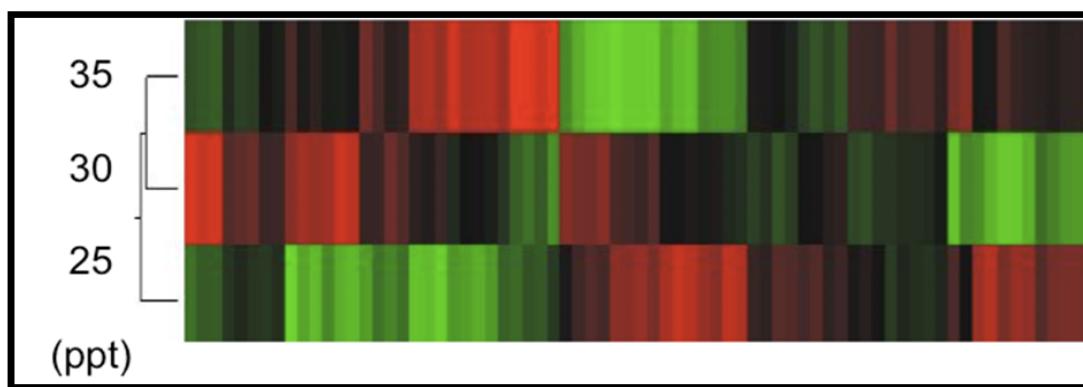


Figure 7: Heatmap indicating gene expression profiles for the salinity treatments. Hierarchical clustering of the Standardized Least Square Mean for each treatment used was performed using the Fast Ward method. Colors indicate relative expression of the 72 genes that were differentially expressed. Green, red and black indicate down-, up-, and neutral gene regulation, respectively. Different shades of color indicate relative amount of expression. Expression levels ranged from -1.767 to 1.8066.

Figure 8 compares the relative expression of each treatment by functional category. A strong stress response was observed in larvae exposed to the lowest salinity treatment (25 ppt). This is evident by the up-regulation of genes in the stress response functional group. Also, zooxanthellae photosynthesis and normal cell function were down-regulated. Interestingly, the medium salinity-treated larvae (30 ppt) did not show a stress response but rather a similar profile to the control. This suggests larvae can tolerate acute exposures to 30 ppt hyposalinity conditions, but requires further investigation.

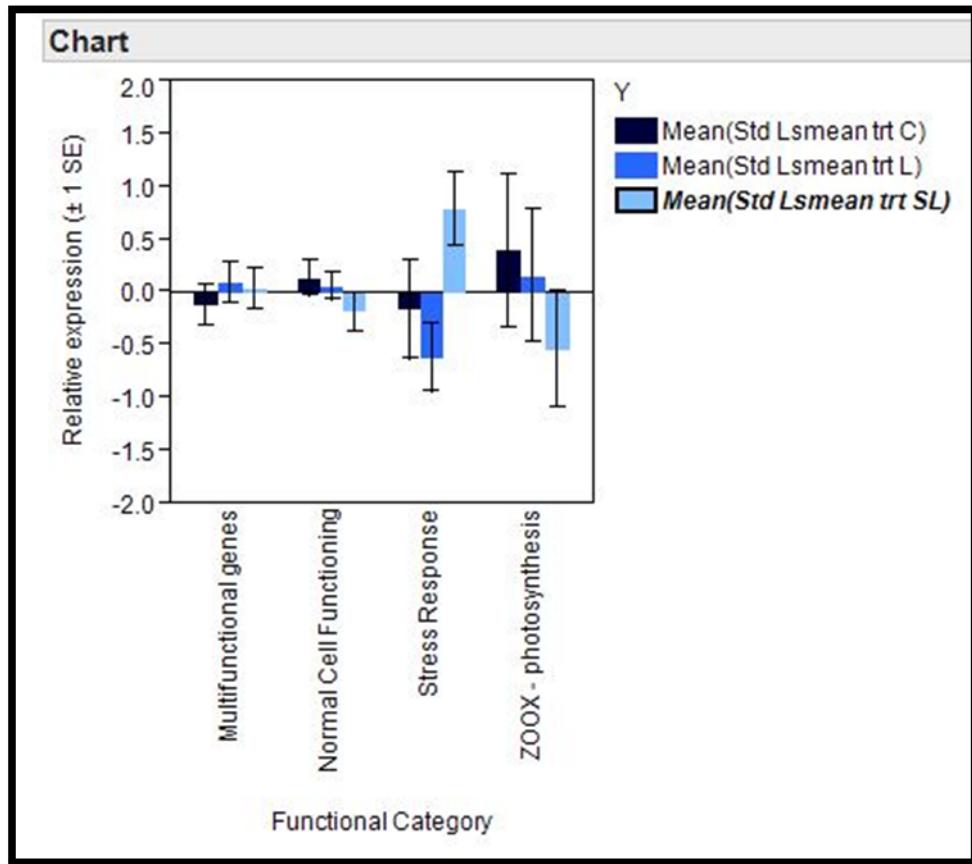


Figure 8: Bar charts showing differential gene expression of treatment groups by functional categories. Only significantly expressed genes were included (72) and both coral and zooxanthellae are represented. C: control (35 ppt), L: low salinity (30ppt), SL: lowest salinity (25 ppt). Error bars indicate 1 standard error from the mean.

The different response of larvae to the lowest salinity (25 ppt) versus the control and mild hyposalinity was also apparent in the Venn diagram, which shows genes with distinct or overlapping expression between treatments (Figure 9). There was a three times greater difference between treatments when comparing the number of differentially expressed genes between the control and lowest salinity treatment to the control and intermediate treatment (upper left).

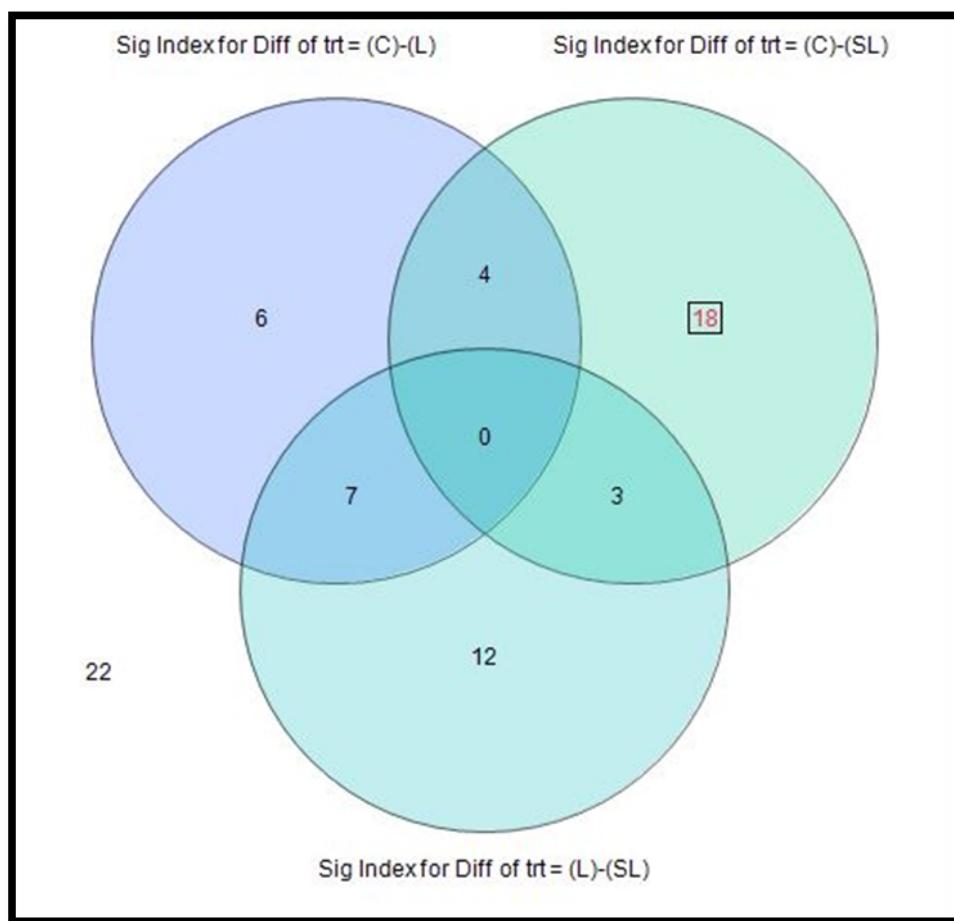


Figure 9: Venn diagram showing significantly different genes between treatments with distinct or overlapping expression among the three different salinity treatments. The largest number of significantly different genes occurred between the control and lowest salinity (C-SL, highlighted). Note that there is no gene that is simultaneously expressed in all three treatments.

Gene expression profiles

The most remarkable results of gene expression profiling are revealed in the up- and down-regulation of gene functions (Figure 10). Detailed gene expression profiles by function and treatment can be found in Figure 2 from the Appendix.

Gene expression profiles			
Expression	35 ppt (control)	30 ppt	25 ppt
↑	Nucleic acid modification Regulation of transcription Translation Metabolism Ion binding Photosynthesis	Photosynthesis DNA replication Translation Protein transport Proteolysis Growth and development	Oxidative stress Cytoskeletal organization Molecular chaperone Cellular respiration DNA repair Response to xenobiotic
↓	Response to xenobiotic Oxidative stress Cytoskeletal organization Cellular respiration DNA repair (neutral)	Molecular chaperone Nuclear acid modification Cytoskeletal organization Cellular respiration Oxidative stress Metabolism DNA repair	Photosynthesis Cellular signaling Growth and development Transcription and translation Ion binding Metabolism

Figure 10: Table showing the most remarkable gene functions induced per treatment. The arrows indicate up or down regulation. Listing order does not correspond to expression level.

Larvae are undergoing an intense developmental stage that requires sophisticated gene regulation involving many processes. It is important to note that the samples used were recently released brooded larvae that were undergoing a developmental stage while they were exposed to the experiment. Therefore, the control was expected to express normal developing genes, like growth and metabolism pathways. The control also down-regulated DNA replication and cellular respiration.

Exposure to 30 ppt induced up regulation of cellular signaling, growth and development, DNA replication, and protein transport. This treatment also induced

down-regulation of molecular chaperones, bioluminescence, and metabolism-related genes. Interestingly, this treatment did not express stress response mechanisms and some of the expressed functions coincided with the control. For example, they coincide in the down-regulation of cytoskeletal organization, DNA repair, and oxidative stress pathways. Additionally, larvae had up-regulated photosynthesis, response to stimulus, proteolysis, and translation pathways in a similar fashion to the control.

Larvae at the lowest salinity treatment (25 ppt) revealed up-regulation of cellular respiration, cytoskeletal organization, molecular chaperone, oxidative stress, protein modification, response to xenobiotic and DNA repair. At the same time, this treatment shows down-regulation of genes involved in growth and development, ion binding, metabolism, proteolysis, response to stimulus, translation, and zooxanthellae photosynthesis.

Overall, the lowest salinity induced a different gene expression in the zooxanthellae as well as the corals. This is apparent in Figures 7 and 11. The hierarchical cluster in Figure 11 was generated including the zooxanthellae genes that were significantly expressed (12 out of 72). The control and mild hyposalinity cluster together similarly to Figure 7. Additionally, Figure 12 shows the overall response of zooxanthellae to the treatments. In this study, zooxanthellae genes were grouped in nine functional categories and only one was statistically significant between treatments (zooxanthellae photosynthesis, $p < 0.003$). However, it is important to note that the treatments induced different responses. Note the up-regulation of zooxanthellae cell

respiration, immune response, and protein modification in the lowest salinity (25 ppt) in contrast to the other treatments.

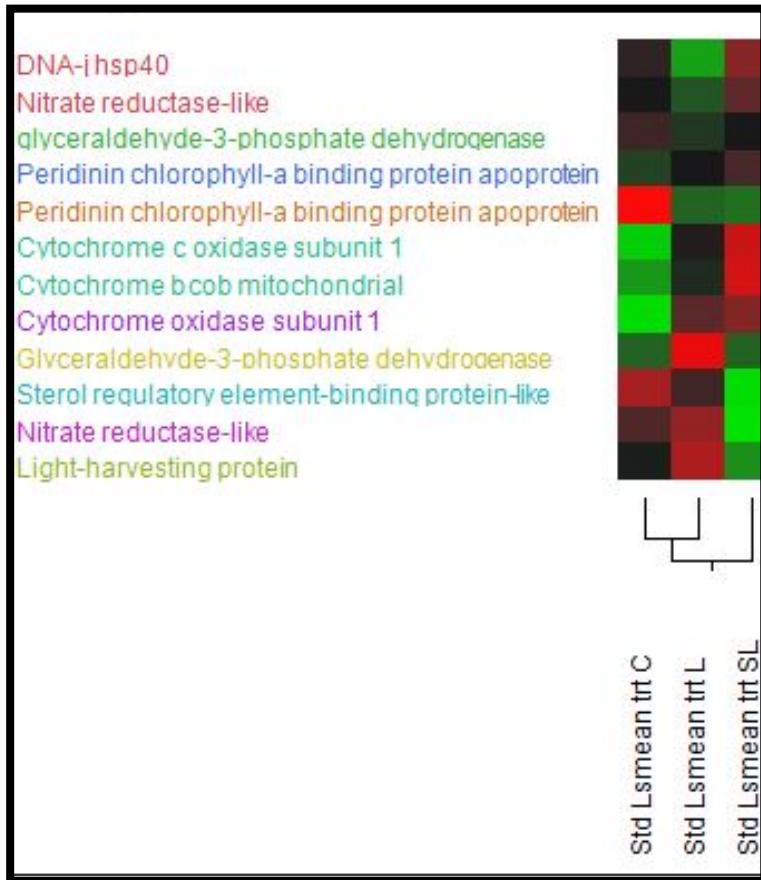


Figure 11: Heatmap indicating gene expression profiles for the zooxanthellae genes that were significantly expressed. Hierarchical clustering of the Standardized Least Square Mean for each treatment used was performed using the Fast Ward method. Colors indicate relative expression of the 12 zooxanthellae genes that were differentially expressed. Green, red and black indicate down-, up-, and neutral gene regulation, respectively. Different shades of color indicate relative amount of expression. Expression levels ranged from -1.767 to 1.8066. C: control (35 ppt), L: low salinity (30ppt), SL: lowest salinity (25 ppt).

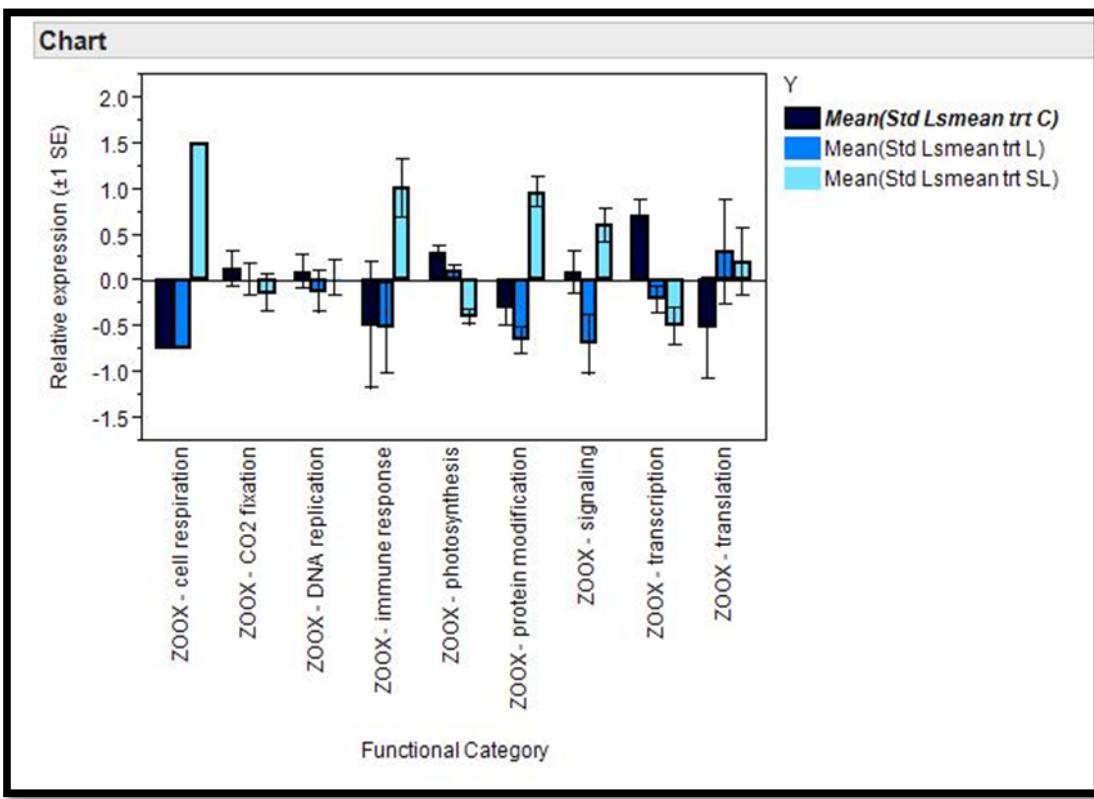


Figure 12: Bar charts showing differential gene expression of treatment groups by functional categories. The zooxanthellae-specific genes were selected in order to determine the overall zooxanthellae response to the treatments. A total of 266 sequences out of 4178 are represented in this graph. Note that the only significantly expressed functional category was zooxanthellae photosynthesis ($p < 0.003$). C: control (35 ppt), L: low salinity (30 ppt), SL: lowest salinity (25 ppt). Error bars indicate 1 standard error from the mean.

Discussion

The initial hypothesis that gene expression in the larvae would not be altered due to hyposalinity exposure was challenged. Results show different gene expression patterns associated with the different salinity treatments. Moreover, larvae exposed to the lowest salinity had over-expressed genes associated with stress in both the coral and the zooxanthellae. Therefore, homeostasis was likely disrupted.

Corals and ctenophores are believed to tolerate a 20% reduction in salinity (Kirshner 1991). In this experiment's context, a 20% reduction in salinity compared to the control (35 ppt) would be a salinity of 28 ppt, which is slightly lower than the intermediate salinity used in this experiment but higher than the lowest salinity (25 ppt). Given that the larvae exposed to 30 ppt salinity did not show a strong stress response, the results support Kirshner (1991). Therefore, based on gene expression profiles in this study, an acute exposure to 30 ppt salinity appears to be tolerable for larvae from *Porites astreoides*. Similar findings were reported for adult samples from *Stylophora pistillata* and *Seriatopora hystrix* exposed to 30 ppt for 4 h, 4 days, and 23 days at ambient and elevated temperature combinations; and samples did not show any effect of the salinity at 30 ppt (ANOVA, $P > 0.05$) (Hoegh-Guldberg and Jason Smith 1989). Additionally, in this study, the lowest salinity represented a salinity reduction greater than 20% and it did induce a stress response. Stress response was evident by the coral up-regulation of stress response mechanisms including, for example, oxidative stress, DNA repair, and cytoskeletal organization.

Exposure to hyposalinity is expected to cause cytoskeletal reorganization given that this condition triggers cell volume increase. This is also known as cell swelling. Possible evidence of cell swelling in larvae exposed to the lowest salinity can be seen in the up-regulation of genes involved in cytoskeletal organization, including actin and alpha and beta tubulins. Additionally, cell swelling was observed immediately after exposure (Ben Mason, personal communication). Other studies have reported similar responses to hyposalinity in coral. For example, a histological examination of *Stylophora pistillata* documented the increasing severity of pathomorphologies associated with decreasing salinity, including increased tissue swelling, degradation

and loss of zooxanthellae, and tissue necrosis, coinciding with the results reported here (Downs et al. 2009). According to Pichon (2011), *Porites* species tolerate higher salinity better than lower salinity, limiting their development in the vicinity of estuaries and river mouths.

Individuals at this immature stage will undergo metamorphosis, a process consisting of drastic physiological and anatomical changes in order to develop into adults (Medina 2009). Metamorphosis generally begins with early morphogenesis of tentacles, mesenteries, and actinopharynx before larval settlement (Ruppert et al., 2004). Additionally, calcification has been observed in larvae from *Porites astreoides* before settlement (Patricia Blackwelder, personal communication). Metamorphosis requires sophisticated gene regulation, cell-to-cell communication, and energy supply. During this developmental stage tissue layers are formed and epidermis, mesoglea, and gastrodermis are apparent. Also, mesenteries have been observed in brooded larvae by histological examination of gravid colonies (Chornesky and Peters 1987). Interestingly, in this experiment the most overrepresented function based on gene expression was cellular signaling. For example, the strongest response was the expression of the genes Lamin-B1 and Ras guanyl-releasing protein 3 that were up-regulated in the mild salinity but down-regulated in the lowest salinity and control. These two genes participate in cell-to-cell adhesion and signaling. Communication among cells and tissue layers is vital for correct development. The developmental potential of a cell (its function and structure) can be either genetically programmed (autonomous) or based on its positional information, which depends on its signaling interactions with its neighboring cells (Hartl and Jones, 2005). Therefore, it is

remarkable that the control did not express cell-signaling pathways whereas the other treatments did.

Coral larvae exposed to the control salinity revealed relatively elevated expression of genes involved in normal cell functioning (NCF), including circadian rhythm, cellular transport, metabolism, nucleic acid modification, and transcription (Figure 3). Genes involved in metabolism-associated pathways were only up-regulated in the larvae exposed to control conditions suggesting they were undergoing normal development (Figure 2 in the Appendix). These genes are involved in glycolysis, lipid metabolism and synthesis, and the Krebs cycle. Larvae exposed to the 30 ppt treatment revealed similar expression patterns of genes in the NCF category to the control (see Figure 8). Exposure to the lowest salinity resulted in relative suppression of genes in this functional category.

Porites astreoides and many other coral species host dinoflagellate algae symbionts. This symbiosis integrates algal phototrophy with animal heterotrophy enabling corals to thrive in shallow, often nutrient-poor tropical seas (Gaither and Rowan 2010). Since larvae from *P. astreoides* are brooded, they obtain their algal symbionts, or zooxanthellae, directly from the maternal polyp. This process is known as vertical transmission (Baird et al., 2009). Zooxanthellae are important because they provide most of the coral host energy budget and promote rapid calcification (van Oppen and Gates 2006; Small and Adey 2001). Hence, much attention has been brought to the coral-zooxanthellae relationship in an attempt to understand this delicate balance (Davy et al., 2012; Weis et al., 2008). For example, zooxanthellae photosynthesis is

commonly used as a proxy for coral health (Edmunds et al., 2001; Ferrier-Pagès et al., 2007; Moberg and Nyström, 1997; Rodolfo-Metalpa et al., 2006).

In this study, genes associated with photosynthesis were down-regulated in corals exposed to 25 ppt conditions whereas coral stress response was up-regulated. This may indicate potential disassociation of the coral-algae relationship or an initial phase of it. This condition is also known as coral bleaching and results from the loss of zooxanthellae or their pigment. Zooxanthellae or zooxanthellae-pigment loss occurs under adverse conditions such as under- or over-illumination, excess UV exposure, salinity fluctuation, and low or high temperatures (Ruppert et al., 2004). “The loss of zooxanthellae from a coral, however, is not always complete and does not always result in coral mortality, especially if the period of environmental stress is not prolonged” (Ruppert et al., 2004). Additionally, recovery depends on the mode of loss of zooxanthellae. Zooxanthellae loss in coral tissue can occur by *in situ* degradation followed by exocytosis, exocytosis of live zooxanthellae, apoptosis, necrosis (coral cell death), or host cell detachment (Lesser 2004). Host cell detachment or sloughing and necrosis may not result in recovery (Esther Peters, personal communication).

Zooxanthellae loss or bleaching is a common response to many stressors. Zooxanthellae counts were not performed in this study, preventing the comparison of zooxanthellae population changes within the larvae before and after the exposures and histological examination of larvae (using transmission electron microscopy) was also not performed, which would have provided information on the condition of the larvae and their zooxanthellae. Therefore, bleaching cannot be confirmed in this experiment, but results suggest bleaching might be induced under acute hyposalinity exposure.

Similar findings were reported under natural conditions in a population of *Montastraea cavernosa* (Edge et al., 2013). Additionally, a study with *Montastraea cavernosa* reported that corals exposed to a salinity of 25 ppt increased gene expression associated with oxidative stress, cytoskeletal structuring, respiration, metabolism, and protein modification and protein degradation (Edge and Voss 2010).

In this experiment, larvae exposed to the lowest salinity (25 ppt) exhibited an over-expression of oxidative stress responsive genes indicating some level of stress response. Oxidative stress refers to the production of reactive oxygen species (ROS), which can damage lipids, proteins and DNA but also participate in many normal cell regulation functions (Lesser 2006). Lipid damage can affect membrane fluidity and trigger apoptosis, mediated by the mitochondria. Additionally, ROS can cause changes in protein structure and functioning, as well as DNA damage such as deletions, mutations, or lethal genetic effects in the DNA sequence (Lesser 2006). DNA damage can induce apoptosis if not repaired. Nevertheless, cells have mechanisms to compensate oxidative stress damage by expressing DNA-repair pathways and producing antioxidants. In this study DNA-repair genes were only up-regulated in larvae exposed to the lowest salinity, which suggests the larvae were undergoing DNA damage. In addition, it has been shown that ROS activity can inhibit algal photosynthesis (Lesser 2006), explaining the down-regulation of genes involved in zooxanthellae photosynthesis in this experiment. Interestingly, in this study one gene pertaining to xenobiotic response was significantly induced by the lowest salinity. Xenobiotics can cause toxicity through any of a myriad of undefined interactions with cellular components and processes (Simmons et al. 2009). Larvae were not exposed to xenobiotics yet arsenite methyltransferase was up-regulated. This gene participates in

arsenic detoxification. It has been reported that molecular stress biomarkers are evolutionarily well conserved and sometimes are activated as a general stress response (Venn et al. 2009; Simmons et al. 2009). For example, P-glycoprotein is known for its multi-xenobiotic resistance and a study including *Montastraea franksi* proved differential gene expression in response to heavy metal copper (copper sulphate) and oil dispersant (CorexitTM9527) (Venn et al. 2009). Similarly, in this study the zooxanthellae gene DNA J-like heat shock protein 40 (HSP 40) was only up-regulated in the lowest salinity treatment although temperature was kept constant at 25 °C. Heat shock proteins are universally found in all organisms and may be induced as a generalized non-specific cellular response to stress (Koban 1991). Relative levels of gene expression revealing differences between treatments are indicated in Figure 2 in the Appendix.

It is important to note that some species are more resistant to hyposalinity than others. For instance, *Siderastrea siderea* and *Porites furcata* have been documented as resistant to low and variable salinities although photosynthesis rate decreases proportionally to salinity (Lirman and Manzello, 2009; Manzello and Lirman, 2003). Similar findings were reported for *Siderastrea radians* from Florida Bay (Chartrand et al., 2009). They reported that *Siderastrea radians* appears to have a greater tolerance to reduced salinities than most symbiotic corals and conclude that coral population salinity tolerances depend on historical salinity ranges. However, most coral species are stenohaline rather than euryhaline and tolerate very narrow ranges of salinity. Additionally, the ability to endure salinity changes is often affected by interaction with other environmental factors inducing synergistic effects (Coles and Jokiel, 1978; Faxneld et al., 2010; Li et al., 2009).

Corals display few macroscopic signs indicative of stress derived from multiple maladies, environmental stress, predation, and other factors (Pollock et al., 2011). Therefore, using microarrays facilitates the detection of sublethal stress levels before macroscopic stress is apparent. Note that the microarray used in this experiment was a multi-species microarray (see Appendix, Table 2). Only one sequence was specific to *Porites astreoides* (NADH dehydrogenase subunit 1, which is involved in cellular respiration) and it was not significantly expressed in this study. However, *Porites* RNA hybridized efficiently and consistently, and sequences on the microarray are from conserved gene regions. Conserved gene regions allow the detection of expression signals in samples from different species (Morgan et al., 2005). The process by which this occurs is known as heterologous hybridization (cDNAs from non-reference species are used for hybridization to microarrays) (Aranda et al. 2012). Several studies have used heterologous hybridization to study Cnidarian genomics. For example, a study detected expression signal in fourteen genes from *Diploria strigosa* samples using a cDNA array designed with 32 sequences of *Montastraea faveolata* and *Acropora cervicornis*, which suggests significant homology between these sequences (Morgan et al., 2005). Similarly, a microarray for *Acropora palmata* was able to provide biologically relevant information for *Acropora cervicornis*, *Siderastrea radians*, and *Montastraea faveolata* (Aranda et al., 2012). Aranda and collaborators (2012) concluded that heterologous hybridization can occur in Cnidarian species from different evolutionary distances, which also make possible the analysis of genome-wide rates of gene evolution, and identification of rapidly diverging genes. Additionally, the microarray used in this study will be used to study gene expression profiles from parallel stressed-larvae experiments including *Diploria strigosa*, *Montastraea faveolata*, and *Acropora palmata*, and it is currently being used in other

studies involving adult samples from *Montastraea faveolata* (Sara Edge, personal communication). Tables 2 and 3 in the Appendix illustrate the species origin of the sequences that were overexpressed in this study.

“Changes in specific genes may be restricted to a particular life cycle stage” (Franks and Hoffmann 2012). For instance, Grasso and collaborators (2008) reported that developing coral planula larvae up-regulate nematocyst development, receipt of settlement cues, and perhaps implementation of metamorphosis-associated genes. These specific genes were not spotted onto the microarray used in this study. It is likely that larvae expressed genes that were not spotted on the microarray because the available microarray is not larval-phase focused. Larvae may have not expressed genes typically expressed in more advanced life stages (Madeleine van Oppen, personal communication). However, interestingly a previous study involving *Acropora palmata* and *Montastraea faveolata* from different life stages (unfertilized eggs; two stages of embryonic larvae, either symbiotic or non-symbiotic; and adults) reported that only a small number of genes are stage specific (Schwarz et al. 2008).

Finally, performing long-term studies is necessary in order to determine the success of larvae developing into juvenile polyps. Resilience to sublethal hyposalinity stress has been proven in gastropod larvae with successful metamorphosis and adult survival (Diederich et al. 2011). Additionally, further complementary analyses would enrich this study by verifying the downstream products of the gene expression here assessed. For instance, given that not all of the transcribed RNAs are actually translated to protein, it would be interesting to complement this study with a proteomics approach by using Western Blot Analysis or ELISA (Enzyme-Linked Immunosorbent Assay).

Furthermore, sometimes organisms display phenotypic anchoring features, which include the process by which gene expression changes are linked to changes in phenotype (Paules 2003; Moggs et al. 2004). Therefore, adding other techniques such as electron microscopy would be beneficial to assess the tissue condition of the larvae and relate larval phenotypes to specific gene expression profiles.

Conclusions

Porites astreoides larvae may be naturally exposed to hyposaline conditions considering its natural history characteristics. For example, in this species the reproductive and rainy seasons overlap. There is no evidence that larvae cannot survive an acute hyposalinity exposure of 30 ppt suggesting successful development into polyps and future colonies. However, additional stressors, such as elevated temperature, pollution, and challenges such as predation and competition may interact with sub-lethal stress associated with exposure to low salinities and adversely affect development and/or reproduction. Additionally, acute exposure to 25 ppt salinity elicited stress response in larvae suggesting longer exposures could have detrimental effects on larvae health. Further studies are needed to evaluate the survival rate of stressed larvae and measure their success in developing into juvenile polyps.

Populations are expected to encounter increasingly unfavorable conditions associated with temperature extremes, ocean acidification, and extreme storm events, which can influence species survival and distributions (Franks and Hoffmann 2012). In an ecological context, if current environmental disturbances keep becoming more frequent and severe, larval dispersal may be limited, as it has been proven that stress

factors induce rapid larvae settlement, perhaps limiting population connectivity (Gleason and Hofmann 2011). Another possibility is that extreme conditions can select for organisms with increased resistance to stressful conditions and/or ways of avoiding extremes (Franks and Hoffmann 2012). Knowing what conditions and degrees of stress larvae can handle may enable predictions of survival and successful progression to more advanced life stages. Furthermore, understanding larval molecular responses to sublethal stress can provide an ideal background for local disturbances mitigation. Finally, the microarray used in this study was a cross-species, stress, and adult-focused microarray and was capable of providing biological information of larvae response to stress, broadening microarray applications to cnidarian research and offering a strong reliable technique for genomic studies.

References

- Agilent Technologies Inc. 2010. "One-Color Microarray-Based Gene Expression Analysis, Low Input Quick Amp Labeling Protocol Version 6.5 May 2010."
- Allison, David B, Xiangqin Cui, Grier P Page, and Mahyar Sabripour. 2006. "Microarray Data Analysis: From Disarray to Consolidation and Consensus." *Nature Reviews. Genetics* 7 (1) (January): 55–65. doi:10.1038/nrg1749. <http://www.ncbi.nlm.nih.gov/pubmed/16369572>.
- Anlauf, Holger, Luis D'Croz, and Aaron O'Dea. 2010. "A Corrosive Concoction: The Combined Effects of Ocean Warming and Acidification on the Early Growth of a Stony Coral Are Multiplicative." *Journal of Experimental Marine Biology and Ecology* 397 (1) (December 9): 13–20. doi:10.1016/j.jembe.2010.11.009. <http://linkinghub.elsevier.com/retrieve/pii/S0022098110004570>.
- Aranda, Manuel, Anastazia T Banaszak, Till Bayer, James R Luyten, Mónica Medina, and Christian R Voolstra. 2011. "Differential Sensitivity of Coral Larvae to Natural Levels of Ultraviolet Radiation During the Onset of Larval Competence." *Molecular Ecology* (June 20). doi:10.1111/j.1365-294X.2011.05153.x. <http://www.ncbi.nlm.nih.gov/pubmed/21689186>.
- Aranda, Manuel, Michael K Desalvo, Till Bayer, Monica Medina, and Christian R Voolstra. 2012. "Evolutionary Insights into Scleractinian Corals Using Comparative Genomic Hybridizations." *BMC Genomics* 13 (1) (January): 501. doi:10.1186/1471-2164-13-501. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3330812/>
- Babcock, RC, and AJ Heyward. 1986. "Larval Development of Certain Gamete-spawning Scleractinian Corals." *Coral Reefs*: 111–116. <http://www.springerlink.com/index/H174502V1556741G.pdf>.
- Baird, Andrew H., James R. Guest, and Bette L. Willis. 2009. "Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals." *Annual Review of Ecology, Evolution, and Systematics* 40 (1) (December): 551–571. doi:10.1146/annurev.ecolsys.110308.120220. <http://www.annualreviews.org/doi/abs/10.1146/annurev.ecolsys.110308.120220>.
- Berkelmans, R., and J. K. Oliver. 1999. "Large-scale Bleaching of Corals on the Great Barrier Reef." *Coral Reefs* 18 (1) (April 22): 55–60. doi:10.1007/s003380050154. <http://link.springer.com/10.1007/s003380050154>.
- Budd, Ann F., Hironobu Fukami, Nathan D. Smith, and Nancy Knowlton. 2012. "Taxonomic Classification of the Reef Coral Family Mussidae (Cnidaria: Anthozoa: Scleractinia)." *Zoological Journal of the Linnean Society* 166 (3) (November 26): 465–529. doi:10.1111/j.1096-3642.2012.00855.x. <http://doi.wiley.com/10.1111/j.1096-3642.2012.00855.x>.
- Chartrand, Kathryn M., Michael Joseph Durako, and James E. Blum. 2009. "Effect of Hyposalinity on the Photophysiology of Siderastrea Radians." *Marine Biology* 156 (8) (April 30): 1691–1702. doi:10.1007/s00227-009-1204-3. <http://www.springerlink.com/index/10.1007/s00227-009-1204-3>.
- Chomczynski, Piotr, and Nicoletta Sacchi. 1987. "Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction."

- Analytical Biochemistry* 162: 156–159.
<http://www.sciencedirect.com/science/article/pii/0003269787900212>.
- Chornesky, E.A., and E.C. Peters. 1987. “Sexual Reproduction and Colony Growth in the Scleractinian Coral *Porites Astreoides*.” *The Biological Bulletin* 172 (2): 161–172. <http://www.biolbull.org/cgi/content/abstract/172/2/161>.
- Coles, SL, and PL Jokiel. 1978. “Synergistic Effects of Temperature, Salinity and Light on the Hermatypic coral *Montipora Verrucosa*.” *Marine Biology*.
<http://link.springer.com/article/10.1007/BF00391130>.
- Crick, Francis. 1970. “Central Dogma of Molecular Biology.” *Nature*: 561–563.
<http://cs.brynmawr.edu/Courses/cs380/fall2012/CrickCentralDogma1970.pdf>.
- Davy, S. K., D. Allemand, and V. M. Weis. 2012. “Cell Biology of Cnidarian-Dinoflagellate Symbiosis.” *Microbiology and Molecular Biology Reviews* 76 (2) (June 11): 229–261. doi:10.1128/MMBR.05014-11.
<http://mmbrr.asm.org/cgi/doi/10.1128/MMBR.05014-11>.
- Diederich, Casey M., Jeremiah N. Jarrett, Oscar R. Chaparro, C.J. Segura, Shawn M. Arellano, and Jan A. Pechenik. 2011. “Low Salinity Stress Experienced by Larvae Does Not Affect Post-metamorphic Growth or Survival in Three Calyptraeid Gastropods.” *Journal of Experimental Marine Biology and Ecology* 397 (2) (February): 94–105. doi:10.1016/j.jembe.2010.11.019.
<http://linkinghub.elsevier.com/retrieve/pii/S0022098110004673>.
- Downs, Craig a, Esti Kramarsky-Winter, Cheryl M Woodley, Aaron Downs, Gidon Winters, Yossi Loya, and Gary K Ostrander. 2009. “Cellular Pathology and Histopathology of Hypo-salinity Exposure on the Coral *Stylophora Pistillata*.” *The Science of the Total Environment* 407 (17) (August 15): 4838–51. doi:10.1016/j.scitotenv.2009.05.015.
<http://www.ncbi.nlm.nih.gov/pubmed/19515401>.
- Edge, S.E., T.L. Shearer, M.B. Morgan, and T.W. Snell. 2013. “Sub-lethal Coral Stress: Detecting Molecular Responses of Coral Populations to Environmental Conditions over Space and Time.” *Aquatic Toxicology* 128-129 (March): 135–146. doi:10.1016/j.aquatox.2012.11.014.
<http://linkinghub.elsevier.com/retrieve/pii/S0166445X12003116>.
- Edge, Sara, and Joshua Voss. 2010. “Hyposalinity Stress in Coral: Transcriptional Regulation to Deal with Osmotic Changes”. Fort Pierce, Fl, USA.
- Edge, SE. 2007. “Using Microarrays to Quantify Stress Responses in Natural Populations of Coral”. Georgia Institute of Technology.
<http://smartech.gatech.edu/handle/1853/16165>.
- Edmunds, P, R Gates, and D Gleason. 2001. “The Biology of Larvae from the Reef Coral *Porites Astreoides*, and Their Response to Temperature Disturbances.” *Marine Biology* 139 (5) (November 1): 981–989. doi:10.1007/s002270100634.
<http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s002270100634>.
- Edmunds, Peter J., Ruth D. Gates, William Leggat, Ove Hoegh-Guldberg, and Laurie Allen-Requa. 2005. “The Effect of Temperature on the Size and Population Density of Dinoflagellates in Larvae of the Reef Coral *Porites Astreoides*.” *Invertebrate Biology* 124 (3) (September): 185–193. doi:10.1111/j.1744-7410.2005.00018.x. <http://doi.wiley.com/10.1111/j.1744-7410.2005.00018.x>.
- Fauth, J.E., P. Dustan, E Ponte, K Banks, B. Vargas-Angel, and C Downs. 2006. *Final Report: Southeast Florida Coral Biomarker Local Action Study*. Florida Dept. of Environmental Protection.

- http://www.dep.state.fl.us/coastal/programs/coral/reports/LBSP/Project5-Biomarker_Final_Report.pdf.
- Faxneld, Suzanne, Tove L. Jörgensen, and Michael Tedengren. 2010. "Effects of Elevated Water Temperature, Reduced Salinity and Nutrient Enrichment on the Metabolism of the Coral *Turbinaria Mesenterina*." *Estuarine, Coastal and Shelf Science* 88 (4) (August): 482–487. doi:10.1016/j.ecss.2010.05.008.
<http://linkinghub.elsevier.com/retrieve/pii/S0272771410001952>.
- Ferrier-Pagès, Christine, Cécile Richard, Didier Forcioli, Denis Allemand, Michel Pichon, and J Malcolm Shick. 2007. "Effects of Temperature and UV Radiation Increases on the Photosynthetic Efficiency in Four Scleractinian Coral Species." *The Biological Bulletin* 213 (1) (August): 76–87.
<http://www.ncbi.nlm.nih.gov/pubmed/17679722>.
- Forêt, S., K. S. Kassahn, L. C. Grasso, D. C. Hayward, A. Iguchi, E. E. Ball, and D. J. Miller. 2007. "Genomic and Microarray Approaches to Coral Reef Conservation Biology." *Coral Reefs* 26 (3) (February 28): 475–486. doi:10.1007/s00338-007-0206-1. <http://www.springerlink.com/index/10.1007/s00338-007-0206-1>.
- Forsman, Zac H, Daniel J Barshis, Cynthia L Hunter, and Robert J Toonen. 2009. "Shape-shifting Corals: Molecular Markers Show Morphology Is Evolutionarily Plastic in Porites." *BMC Evolutionary Biology* 9 (January): 45. doi:10.1186/1471-2148-9-45.
<http://www.ncbi.nlm.nih.gov/pubmed/1934640>.
- Franks, Steven J, and Ary a Hoffmann. 2012. "Genetics of Climate Change Adaptation." *Annual Review of Genetics* 46 (January): 185–208. doi:10.1146/annurev-genet-110711-155511.
<http://www.ncbi.nlm.nih.gov/pubmed/22934640>.
- Gaither, M.R., and Rob Rowan. 2010. "Zooxanthellar Symbiosis in Planula Larvae of the Coral *Pocillopora Damicornis*." *Journal of Experimental Marine Biology and Ecology* 386 (1-2): 45–53. doi:10.1016/j.jembe.2010.02.003.Zooxanthellar.
<http://www.sciencedirect.com/science/article/pii/S0022098110000377>.
- Galloway, S.B., T.M. Work, V.S. Bochsler, R.A. Harley, E. Kramarsky Winters, S.M. McLaughlin, C.U. Meteyer, et al. 2005. "Coral Disease and Health Workshop: Coral Histopathology II." *Laboratory Medicine*.
- Gleason, Daniel 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. doi:10.4319/lo.1993.38.7.1452.
http://www.aslo.org/lo/toc/vol_38/issue_7/1452.html.
- Gleason, Daniel F., and Dietrich K. Hofmann. 2011. "Coral Larvae: From Gametes to Recruits." *Journal of Experimental Marine Biology and Ecology* 408 (1-2) (August): 42–57. doi:10.1016/j.jembe.2011.07.025.
<http://linkinghub.elsevier.com/retrieve/pii/S0022098111003480>.
- Graham, E. M., a. H. Baird, and S. R. Connolly. 2008. "Survival Dynamics of Scleractinian Coral Larvae and Implications for Dispersal." *Coral Reefs* 27 (3) (February 23): 529–539. doi:10.1007/s00338-008-0361-z.
<http://www.springerlink.com/index/10.1007/s00338-008-0361-z>.
- Grasso, L C, A P Negri, S Fôret, R Saint, D C Hayward, D J Miller, and E E Ball. 2011. "The Biology of Coral Metamorphosis: Molecular Responses of Larvae to Inducers of Settlement and Metamorphosis." *Developmental Biology* 353 (2) (May 15): 411–9. doi:10.1016/j.ydbio.2011.02.010.
<http://www.ncbi.nlm.nih.gov/pubmed/21338599>.

- Grasso, Lauretta C, John Maindonald, Stephen Rudd, David C Hayward, Robert Saint, David J Miller, and Eldon E Ball. 2008. "Microarray Analysis Identifies Candidate Genes for Key Roles in Coral Development." *BMC Genomics* 9 (January): 540. doi:10.1186/1471-2164-9-540.
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2629781/>&tool=pmcentrez&rendertype=abstract.
- Harrison, Peter L. 2011. "Sexual Reproduction of Scleractinian Corals." In *Coral Reefs: An Ecosystem in Transition*, edited by Zvy Dubinsky and Noga Stambler, first, 59–85. Dordrecht: Springer Netherlands. doi:10.1007/978-94-007-0114-4.
<http://www.springerlink.com/index/10.1007/978-94-007-0114-4.html>.
- Hoegh-Guldberg, O, P J Mumby, a J Hooten, R S Steneck, P Greenfield, E Gomez, C D Harvell, et al. 2007. "Coral Reefs Under Rapid Climate Change and Ocean Acidification." *Science (New York, N.Y.)* 318 (5857) (December): 1737–42. doi:10.1126/science.1152509. <http://www.ncbi.nlm.nih.gov/pubmed/18079392>.
- Hoegh-Guldberg, Ove, and G Jason Smith. 1989. "The Effect of Sudden Changes in Temperature, Light and Salinity on the Population Density and Export of Zooxanthellae from the Reef Corals Stylophora Pistillata Esper and Seriatopora Hystrix Dana." *Journal of Experimental Marine Biology and Ecology* 129: 279–303.
- Jokiel, PL, CL Hunter, S Taguchi, and L Watarai. 1993. "Ecological Impact of a Fresh-water 'reef Kill' in Kaneohe Bay, Oahu, Hawaii." *Coral Reefs*: 177–184. <http://link.springer.com/article/10.1007/BF00334477>.
- Kerswell, AP, and RJ Jones. 2003. "Effects of Hypo-osmosis on the Coral Stylophora Pistillata: Nature and Cause Of 'low-salinity Bleaching'." *Marine Ecology Progress Series* 253 (1964): 145–154. <http://www.int-res.com/articles/meps2003/253/m253p145.pdf>.
- Kramarsky-Winter, E, and Y Loya. 1996. "Regeneration Versus Budding in Fungiid Corals: a Trade-off." *Marine Ecology Progress Series* 134: 179–185. doi:10.3354/meps134179. <http://www.int-res.com/abstracts/meps/v134/p179-185/>.
- Lesser, Michael P. 2004. "Experimental Biology of Coral Reef Ecosystems." *Journal of Experimental Marine Biology and Ecology* 300 (1-2) (March): 217–252. doi:10.1016/j.jembe.2003.12.027. <http://linkinghub.elsevier.com/retrieve/pii/S0022098104000371>.
- . 2006. "Oxidative Stress in Marine Environments: Biochemistry and Physiological Ecology." *Annual Review of Physiology* 68 (3) (January): 253–78. doi:10.1146/annurev.physiol.68.040104.110001. <http://www.ncbi.nlm.nih.gov/pubmed/16460273>.
- Li, Xiubao, Hui Huang, Jiansheng Lian, Liangmin Huang, and Junde Dong. 2009. "Effects of the Multiple Stressors High Temperature and Reduced Salinity on the Photosynthesis of the Hermatypic Coral Galaxea Fascicularis." *Acta Ecologica Sinica* 29 (3) (August): 155–159. doi:10.1016/j.chnaes.2009.07.002. <http://linkinghub.elsevier.com/retrieve/pii/S1872203209000328>.
- Lirman, Diego. 2000. "Fragmentation in the Branching Coral Acropora Palmata (Lamarck): Growth, Survivorship, and Reproduction of Colonies and Fragments." *Journal of Experimental Marine Biology and Ecology* 251 (1) (August 23): 41–57. <http://www.ncbi.nlm.nih.gov/pubmed/10958900>.
- Lirman, Diego, and Derek Manzello. 2009. "Patterns of Resistance and Resilience of the Stress-tolerant Coral Siderastrea Radians (Pallas) to Sub-optimal Salinity and Sediment Burial." *Journal of Experimental Marine Biology and Ecology* 369 (1)

- (February): 72–77. doi:10.1016/j.jembe.2008.10.024.
<http://linkinghub.elsevier.com/retrieve/pii/S0022098108005467>.
- Manzello, Derek, and Diego Lirman. 2003. “The Photosynthetic Resilience of Porites Furcata to Salinity Disturbance.” *Coral Reefs* 22 (4) (December 1): 537–540. doi:10.1007/s00338-003-0327-0.
<http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s00338-003-0327-0>.
- McGuire, M. P. 1998. “Timing of Larval Release by Porites Astreoides in the Northern Florida Keys.” *Coral Reefs* 17 (4) (December 10): 369–375. doi:10.1007/s003380050141.
<http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s003380050141>.
- Medina, Mónica. 2009. “Functional Genomics Opens Doors to Understanding Metamorphosis in Nonmodel Invertebrate Organisms.” *Molecular Ecology* 18 (5) (March): 763–4. doi:10.1111/j.1365-294X.2008.04079.x.
<http://www.ncbi.nlm.nih.gov/pubmed/19207243>.
- Moberg, F, and M Nyström. 1997. “Effects of Reduced Salinity on the Rates of Photosynthesis and Respiration in the Hermatypic Corals Porites Lutea and Pocillopora Damicornis.” *Marine Ecology* ... 157: 53–59. <http://www.int-res.com/articles/meps/157/m157p053.pdf>.
- Moggs, Jonathan G., Helen Tinwell, Tracey Spurway, Hur-Song Chang, Ian Pate, Fei Ling Lim, David J. Moore, et al. 2004. “Phenotypic Anchoring of Gene Expression Changes During Estrogen-induced Uterine Growth.” *Environmental Health Perspectives* 112 (16): 1589–1606. doi:10.1289/txg.7345.
<http://ehp.niehs.nih.gov/txg/docs/2004/7345/abstract.html>.
- Molecular Devices Corp. 2005. “GenePix ® Pro 6.0 Microarray Acquisition and Analysis Software for GenePix Microarray Scanners User’s Guide & Tutorial.”
- Morgan, Michael B, Sara E Edge, and Terry W Snell. 2005. “Profiling Differential Gene Expression of Corals Along a Transect of Waters Adjacent to the Bermuda Municipal Dump.” *Marine Pollution Bulletin* 51 (5-7) (January): 524–33. doi:10.1016/j.marpolbul.2005.09.023.
<http://www.ncbi.nlm.nih.gov/pubmed/16256144>.
- NASA Reviewed on August 5th 2013, <http://science.nasa.gov/glossary/practical-salinity-unit/>
- Niwa, Ryusuke, and Frank J Slack. 2007. “The Evolution of Animal microRNA Function.” *Current Opinion in Genetics & Development* 17 (2) (April): 145–50. doi:10.1016/j.gde.2007.02.004. <http://www.ncbi.nlm.nih.gov/pubmed/17317150>.
- Orr, James C, Victoria J Fabry, Olivier Aumont, Laurent Bopp, Scott C Doney, Richard a Feely, Anand Gnanadesikan, et al. 2005. “Anthropogenic Ocean Acidification over the Twenty-first Century and Its Impact on Calcifying Organisms.” *Nature* 437 (7059) (September): 681–6. doi:10.1038/nature04095.
<http://www.ncbi.nlm.nih.gov/pubmed/16193043>.
- Paules, Richard. 2003. “Phenotypic Anchoring: Linking Cause and Effect.” *Environmental Health Perspectives* 111 (6): 338–339.
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241511/>.
- Pollock, F. Joseph, Pamela J. Morris, Bette L. Willis, and David G. Bourne. 2011. “The Urgent Need for Robust Coral Disease Diagnostics.” Edited by Marianne Manchester. *PLoS Pathogens* 7 (10) (October 20): e1002183. doi:10.1371/journal.ppat.1002183.
<http://dx.plos.org/10.1371/journal.ppat.1002183>.

- Richier, Sophie, Mauricio Rodriguez-Lanetty, Christine E Schnitzler, and Virginia M Weis. 2008. "Response of the Symbiotic Cnidarian Anthopleura elegantissima Transcriptome to Temperature and UV Increase." *Comparative Biochemistry and Physiology. Part D, Genomics & Proteomics* 3 (4) (December): 283–9. doi:10.1016/j.cbd.2008.08.001. <http://www.ncbi.nlm.nih.gov/pubmed/20494848>.
- Rodolfo-Metalpa, Riccardo, Cécile Richard, Denis Allemand, and Christine Ferrier-pagès. 2006. "Growth and Photosynthesis of Two Mediterranean Corals , Cladocora caespitosa and Oculina patagonica , Under Normal and Elevated Temperatures." *Journal of Experimental Biology*: 4546–4556. doi:10.1242/jeb.02550.
- Rodriguez-Lanetty, Mauricio, Saki Harii, and Ove Hoegh-Guldberg. 2009. "Early Molecular Responses of Coral Larvae to Hyperthermal Stress." *Molecular Ecology* 18 (24) (December): 5101–14. doi:10.1111/j.1365-294X.2009.04419.x. <http://www.ncbi.nlm.nih.gov/pubmed/19900172>.
- Schroeder, Andreas, Odilo Mueller, Susanne Stocker, Ruediger Salowsky, Michael Leiber, Marcus Gassmann, Samar Lightfoot, Wolfram Menzel, Martin Granzow, and Thomas Ragg. 2006. "The RIN: An RNA Integrity Number for Assigning Integrity Values to RNA Measurements." *BMC Molecular Biology* 7 (January): 3. doi:10.1186/1471-2199-7-3. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1413964&tool=pmcentrez&rendertype=abstract>.
- Schwarz, Jodi A, Peter B Brokstein, Christian Voolstra, Astrid Y Terry, Chitra F Manohar, David J Miller, Alina M Szmant, Mary Alice Coffroth, and Mónica Medina. 2008. "Coral Life History and Symbiosis: Functional Genomic Resources for Two Reef Building Caribbean Corals, Acropora palmata and Montastraea faveolata." *BMC Genomics* 9 (January): 97. doi:10.1186/1471-2164-9-97. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2291459&tool=pmcentrez&rendertype=abstract>.
- Simmons, Steven O, Chun-Yang Fan, and Ram Ramabhadran. 2009. "Cellular Stress Response Pathway System as a Sentinel Ensemble in Toxicological Screening." *Toxicological Sciences : an Official Journal of the Society of Toxicology* 111 (2) (October): 202–25. doi:10.1093/toxsci/kfp140. <http://www.ncbi.nlm.nih.gov/pubmed/19567883>.
- Small, Allegra M, and Walter H Adey. 2001. "Reef Corals, Zooxanthellae and Free-living Algae: a Microcosm Study That Demonstrates Synergy Between Calcification and Primary Production." *Ecological Engineering* 16 (4) (February): 443–457. doi:10.1016/S0925-8574(00)00066-5. <http://linkinghub.elsevier.com/retrieve/pii/S0925857400000665>.
- Sodhi, Navjot S., and Paul R. Ehrlich, ed. 2010. *Conservation Biology for All. Conservation Biology*. Vol. 1. Oxford, England: Oxford University Press. doi:10.1093/acprof:oso/9780199554232.001.0001. <http://www.oxfordscholarship.com/oso/public/content/biology/9780199554232/toc.html>.
- Suárez, E, A Burguete, and GJ McLachlan. 2009. "Microarray Data Analysis for Differential Expression: a Tutorial." *PR Health Sciences* <http://prhsj.rcm.upr.edu/index.php/prhsj/article/view/199>.
- Szmant, Alina M. 1986. "Coral Reefs Reproductive Ecology of Caribbean Reef Corals." *Coral Reefs* 5: 43–53.

- Thurber, Rebecca L. Vega, and Adrienne M.S. Correa. 2011. "Viruses of Reef-building Scleractinian Corals." *Journal of Experimental Marine Biology and Ecology* 408 (1-2) (November): 102–113. doi:10.1016/j.jembe.2011.07.030. <http://linkinghub.elsevier.com/retrieve/pii/S0022098111003534>.
- UNESCO, ICES, SCOR, and IAPSO. 1982. "Background Papers and Supporting Data on the Practical Salinity Scale 1978." *Unesco Technical Papers in Marine Science*. <http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Background+papers+and+supporting+data+on+the+Practical+Salinity+Scale+1978#0>.
- Van Oppen, Madeleine J H, and Ruth D Gates. 2006. "Conservation Genetics and the Resilience of Reef-building Corals." *Molecular Ecology* 15 (13) (November): 3863–83. doi:10.1111/j.1365-294X.2006.03026.x. <http://www.ncbi.nlm.nih.gov/pubmed/17054489>.
- Van Woesik, Robert, and Adán Guillermo Jordán-Garza. 2011. "Coral Populations in a Rapidly Changing Environment." *Journal of Experimental Marine Biology and Ecology* 408 (1-2) (August): 11–20. doi:10.1016/j.jembe.2011.07.022. <http://linkinghub.elsevier.com/retrieve/pii/S0022098111003455>.
- Venn, Alexander a, Jennifer Quinn, Ross Jones, and Andrea Bodnar. 2009. "P-glycoprotein (multi-xenobiotic Resistance) and Heat Shock Protein Gene Expression in the Reef Coral Montastraea Franksi in Response to Environmental Toxicants." *Aquatic Toxicology (Amsterdam, Netherlands)* 93 (4) (July 26): 188–95. doi:10.1016/j.aquatox.2009.05.003. <http://www.ncbi.nlm.nih.gov/pubmed/19501419>.
- Vermeij, M J A, N D Fogarty, and M W Miller. 2006. "Pelagic Conditions Affect Larval Behavior, Survival, and Settlement Patterns in the Caribbean Coral Montastraea Faveolata." *Marine Ecology Progress Series* 310 (2): 119–128.
- Veron, John E. N. 2011. "Ocean Acidification and Coral Reefs: An Emerging Big Picture." *Diversity* 3 (2) (May 30): 262–274. doi:10.3390/d3020262. <http://www.mdpi.com/1424-2818/3/2/262/>.
- Weis, Virginia M, Simon K Davy, Ove Hoegh-Guldberg, Mauricio Rodriguez-Lanetty, and John R Pringle. 2008. "Cell Biology in Model Systems as the Key to Understanding Corals." *Trends in Ecology & Evolution* 23 (7) (July): 369–76. doi:10.1016/j.tree.2008.03.004. <http://www.ncbi.nlm.nih.gov/pubmed/18501991>.
- Wellington, G. M., and W. K. Fitt. 2003. "Influence of UV Radiation on the Survival of Larvae from Broadcast-spawning Reef Corals." *Marine Biology* 143 (6) (December 1): 1185–1192. doi:10.1007/s00227-003-1150-4. <http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s00227-003-1150-4>.
- Wilkinson, C., and Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre. 2008. *Status of Coral Reefs of the World: 2008*. Edited by C. Wilkinson. Townsville, Australia.

Appendix

Correct Accession number	NCBI Accession name	Gene	Modified Probe Group	Function	FC	Species Detail
AY181552	Montastraea cavernosa isolate mc1 red fluorescent protein	red fluorescent protein	bioluminescence	bioluminescence	NCF	<i>Montastraea cavernosa</i>
EZ013763	ATP synthase lipid-binding protein	ATP synthase lipid-binding protein	ion transport	cellular respiration	NCF	<i>Acropora millepora</i>
GQ501349	cytochrome c oxidase subunit I (cox1)	Cytochrome c oxidase subunit 1	respiration	cellular respiration	NCF	<i>Symbiodinium sp. clade F</i>
DQ082986	cytochrome b gene (cob) mitochondrial (MT-CYB)	cytochrome b cob mitochondrial	cell respiration	cellular respiration	NCF	<i>Symbiodinium sp.</i>
EZ026766	TSA: Acropora millepora SeqIndex4656, mRNA sequence	NADH dehydrogenase	electron transport_apoptosis	cellular respiration	NCF	<i>Acropora millepora</i>
AY289697	cytochrome oxidase subunit I (cox1)	Cytochrome oxidase subunit 1	ETC	cellular respiration	NCF	<i>Symbiodinium sp.</i>
EZ008143	NADH-ubiquinone oxidoreductase 75 kDa subunit	NADH-ubiquinone oxidoreductase 75 kDa subunit	mitochondrial respiration ETC	cellular respiration	NCF	<i>Acropora millepora</i>
EZ011131	TSA: Acropora millepora SeqIndex12597	Ras guanyl-releasing protein 3	DAG regulated Ras & Rap1 GEF activate ERK	cellular signaling	MF	<i>Acropora millepora</i>
EZ013920	TSA: Acropora millepora SeqIndex16678	FAS-associated factor 1	enhances apoptosis, binds FAS ligand	cellular signaling	MF	<i>Acropora millepora</i>
EZ010178	TSA: Acropora millepora SeqIndex11076	Tyrosyl-tRNA synthetase	adds tyrosine to translated peptides, possible signal role upon cleavage (human)	cellular signaling	MF	<i>Acropora millepora</i>
EZ019929	TSA: Acropora millepora SeqIndex2988	Dual specificity protein phosphatase 14	dephosphorylate ERK, JNK,p38 MAPK	cellular signaling	MF	<i>Acropora millepora</i>
EZ011757	TSA: Acropora millepora SeqIndex13545	Adenylate cyclase type 3	signal transduction , 2nd messenger formation	cellular signaling	MF	<i>Acropora millepora</i>
EZ011817	Calcyphosin-like protein (CAPSL)	Calcyphosin-like protein	calcium ion binding	cellular signaling	MF	<i>Acropora millepora</i>
EZ027103	TSA: Acropora millepora SeqIndex4740	Beta-adrenergic receptor kinase 2	phosphorylates the agonist-occupied form of the beta-adrenergic receptor	cellular signaling	MF	<i>Acropora millepora</i>
EZ013087	TSA: Acropora millepora SeqIndex15428	Serine/threonine-protein kinase 17A	positive apoptosis and reactive oxygen production regulation	cellular signaling	MF	<i>Acropora millepora</i>
EZ009927	TSA: Acropora millepora SeqIndex10700	Dual specificity protein phosphatase 23	MAPK3 phosphatase, signaling	cellular signaling	MF	<i>Acropora millepora</i>
EZ001222	TSA: Acropora millepora SeqIndex14689	Rho GTPase-activating protein 12	signal transduction_revisit	cellular signaling	MF	<i>Acropora millepora</i>
EZ018566	TSA: Acropora millepora SeqIndex2658	Regulator of G-protein signaling 19	down regulate G alpha <i>i</i> to inhibit cAMP production	cellular signaling	MF	<i>Acropora millepora</i>
EZ000867	TSA: Acropora millepora SeqIndex11852	Zinc finger FYVE domain-containing	(PtDlns(3)P) membrane recruitment signaling &trafficking	cellular signaling	NCF	<i>Acropora millepora</i>
EZ028621	Gamma-aminobutyric acid receptor subunit rho-2 (Gabr2)	Gamma-aminobutyric acid receptor subunit rho-2	proteing binding_sensory transduction	cellular signaling	NCF	<i>Acropora millepora</i>
EZ019330	TSA: Acropora millepora SeqIndex2841	Calcineurin-binding protein cabin-1	inhibit calcineurin mediated signaling, replication independent chromatin assembly	cellular signaling	NCF	<i>Acropora millepora</i>
EZ023098	TSA: Acropora millepora SeqIndex3757	Lamin-B1	inner nuclear membrane structure, chromatin, reversible assembly role apoptosis, gene regulation	cellular signaling	NCF	<i>Acropora millepora</i>
EZ002319	TSA: Acropora millepora SeqIndex3773	Laminin subunit alpha	basement membrane component, tissue architecture	cellular signaling	NCF	<i>Acropora millepora</i>
EZ013658	Melanopsin (Opn4)	Melanopsin	sensory transduction	Circadian rhythm	NCF	<i>Acropora millepora</i>
EZ001352	Kinesin-like protein KIF13B (KIF13B)	Kinesin-like protein KIF13B	cilium_flagella transport	cytoskeletal organization	MF	<i>Acropora millepora</i>
AB086826	actin	Actin	cell motility_cytokinesis_signaling	cytoskeletal organization	MF	<i>Galaxea fascicularis</i>
U60604	beta tubulin gene	beta tubulin	cell motility_cytokinesis_signaling	cytoskeletal organization	MF	<i>Montastraea faveolata</i>
MGID_344	alpha-tubulin (TUBA)	alpha-tubulin	cell motility_cytokinesis_signaling	cytoskeletal organization	MF	<i>Acropora millepora</i>
EZ022395	ATP-dependent DNA helicase 2 subunit 2 (XRCC5)	ATP-dependent DNA helicase 2 subunit 2	neg reg transcription_proviral infection	DNA repair	SR	<i>Acropora millepora</i>
DN252355	ACAB-aab7f18.g1	UV excision repair protein RAD23 homolog A	nucleotide excision repair	DNA repair	SR	<i>Hydra magnipapillata</i>
EZ001334	DNA polymerase theta (POLH)	DNA polymerase theta	DNA binding	DNA replication	NCF	<i>Acropora millepora</i>
DT620213	ACAH-aaa19c07.g1 Spondin 1a; F-Spondin (SPON1)	Spondin 1a; F-Spondin	neural cell growth guidance	growth and development	NCF	<i>Hydra magnipapillata</i>
EZ013314	Voltage-dependent L-type calcium channel subunit alpha-1F (Cacna1f)	Voltage-dependent L-type calcium channel subunit alpha-1F	ion transport	ion binding	MF	<i>Acropora millepora</i>
EZ009276	Solute carrier family 25 member 36-A (slc25a36a)	Solute carrier family 25 member 36-A	mitochondria membrane transport	transport	NCF	<i>Acropora millepora</i>
EZ020388	Purine nucleoside phosphorylase (NP)	Purine nucleoside phosphorylase	response to xenobiotic	metabolism	MF	<i>Acropora millepora</i>
AY314972	cytosolic glyceraldehyde-3-phosphate dehydrogenase	glyceraldehyde-3-phosphate dehydrogenase	protein binding_apoptosis	metabolism	NCF	<i>Symbiodinium kawagutii</i>

Correct Accession number	NCBI Accession name	Gene	Modified Probe Group	Function	FC	Species Detail
EZ016486	Allene oxide synthase-lipoxygenase	Allene oxide synthase-lipoxygenase	metal ion binding	metabolism	NCF	<i>Acropora millepora</i>
EZ010864	Pyruvate dehydrogenase protein X	Pyruvate dehydrogenase protein X	energy production	metabolism	NCF	<i>Acropora millepora</i>
EZ016486	Allene oxide synthase-lipoxygenase	Allene oxide synthase-lipoxygenase	metal ion binding	metabolism	NCF	<i>Acropora millepora</i>
EZ010221	Isocitrate dehydrogenase	Isocitrate dehydrogenase	energy production	metabolism	NCF	<i>Acropora millepora</i>
DQ482987	Symbiodinium sp. clade C3 from Acropora aspera sterol regulatory	Sterol regulatory element-binding protein-like	lipid homeostasis_cholesterol metabolism_transcription factor	metabolism	NCF	<i>Symbiodinium sp. clade C</i>
DQ144980	Dna J-like protein 1	dna-j hsp40	protein modification	molecular chaperone	MF	<i>Symbiodinium sp. clade C</i>
EZ014349	Mitochondrial import inner membrane translocase subunit Tim17-B	Mitochondrial import inner membrane translocase subunit Tim17-B	zinc ion binding_protein transport	molecular chaperone	MF	<i>Acropora millepora</i>
MGID_68	poly(A) binding protein (PABPC4)	Poly(A) binding protein	RNA binding	nucleic acid modification	NCF	<i>Acropora millepora</i>
EZ021319	TSA: <i>Acropora millepora</i> SeqIndex3329	Peptide methionine sulfoxide reductase A	reduces oxidized methionines, rescue damaged protein	oxidative stress	SR	<i>Acropora millepora</i>
DQ104435	Anemonea viridis catalase	Catalase	decompose hydrogen peroxide	oxidative stress	SR	<i>Anemonea viridis</i>
EZ030384	TSA: <i>Acropora millepora</i> SeqIndex5510	Glucose-6-phosphate 1-dehydrogenase	ribose, pentose, glucose6,glutathione, synthesis; oxidative stress response	oxidative stress	SR	<i>Acropora millepora</i>
AY149128	peridinin chlorophyll-a binding protein apoprotein precursor	peridinin chlorophyll-a binding protein apoprotein	response to light	photosynthesis	ZOOX - photosynthesis	<i>Symbiodinium sp.</i>
FN646424	light-harvesting protein	light-harvesting protein	response to light	photosynthesis	ZOOX - photosynthesis	<i>Symbiodinium sp.</i>
MGID_213	peridinin chlorophyll-a binding protein apoprotein precursor (pep) gene	peridinin chlorophyll-a binding protein apoprotein	response to light	photosynthesis	ZOOX - photosynthesis	<i>Symbiodinium kawagutii</i>
EZ010516	Ubiquitin-conjugating enzyme E2 Q1 (UBE2Q1)	Ubiquitin-conjugating enzyme E2 Q1	protein binding_immune response	protein modification	MF	<i>Acropora millepora</i>
EZ001642	TSA: <i>Acropora millepora</i> SeqIndex1905, mRNA sequence	Importin-5	nucleosome docking Beta component of NLS receptor	protein transport	MF	<i>Acropora millepora</i>
EZ016191	TSA: <i>Acropora millepora</i> SeqIndex2125	Secretory carrier-associated membrane protein 1	post-Golgi recycling to cell surface	protein transport	NCF	<i>Acropora millepora</i>
EZ018932	TSA: <i>Acropora millepora</i> SeqIndex2745	Metaxin-2	transport proteins to mitochondria	protein transport	NCF	<i>Acropora millepora</i>
EZ013503	Cell cycle control protein 50A (TMEM30A)	Cell cycle control protein 50A	protein binding	protein transport	NCF	<i>Acropora millepora</i>
DQ309521	Anthopleura elegantissima plasma glutamate carboxypeptidase carboxypeptidase	glutamate carboxypeptidase-like protein mRNA	hydrolysis free peptides	proteolysis	MF	<i>Anthopleura elegantissima</i>
EZ000710	TSA: <i>Acropora millepora</i> SeqIndex10607	phosphatase 1K Mitochondrial	regulates mitochondrial permeability_cell survival & development	regulation of apoptosis	MF	<i>Acropora millepora</i>
EZ007597	Dual specificity mitogen-activated protein kinase kinase 5 (Map2k5)	Dual specificity mitogen-activated protein kinase kinase 5	anti-apoptosis	regulation of apoptosis	MF	<i>Acropora millepora</i>
EZ008332	Mitogen-activated protein kinase kinase kinase 10 (Map3k10)	Mitogen-activated protein kinase kinase kinase 10	apoptosis_signaling_JUN pathway	regulation of apoptosis	MF	<i>Acropora millepora</i>
AB106682	Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)	glyceraldehyde-3-phosphate dehydrogenase	metabolism	regulation of apoptosis	MF	<i>Symbiodinium sp.</i>
EZ014130	Ubiquitin carboxyl-terminal hydrolase isozyme L5 (UchL5)	Ubiquitin carboxyl-terminal hydrolase isozyme L5	DNA repair	DNA repair	NCF	<i>Acropora millepora</i>
EZ010993	CCR4-NOT transcription complex subunit 2 (Cnot2)	CCR4-NOT transcription complex subunit 2	RNA metabolism	regulation of transcription	NCF	<i>Acropora millepora</i>
EZ008489	3-oxo-5-alpha-steroid 4-dehydrogenase 1 (Srd5a1)	3-oxo-5-alpha-steroid 4-dehydrogenase 1	cell differentiation_response to stimulus	response to stimulus	MF	<i>Acropora millepora</i>
EZ011764	TSA: <i>Acropora millepora</i> SeqIndex13556	Arsenite methyltransferase	arsenic detoxification	response to xenobiotic	SR	<i>Acropora millepora</i>
EZ020860	TSA: <i>Acropora millepora</i> SeqIndex3214	Transcription factor AP-1 Jun	transcription regulation	regulation of transcription	NCF	<i>Acropora millepora</i>
JK822201	SedB(H5) 28S ribosomal RNA gene	28S ribosomal RNA gene	ribosomal RNA	translation	NCF	<i>Montastraea faveolata</i>
JK822201	SedB(H5) 28S ribosomal RNA gene	28S ribosomal RNA gene	ribosomal RNA	translation	NCF	<i>Montastraea faveolata</i>
EZ019031	Eukaryotic translation initiation factor 3 subunit G (EIF3G)	Eukaryotic translation initiation factor 3 subunit G	RNA transport	translation	NCF	<i>Acropora millepora</i>
DQ482994	Symbiodinium sp. clade C3 from Acropora aspera nitrate reductase	nitrate reductase-like	nitrate assimilation and aquistion	transport	NCF	<i>Symbiodinium sp. clade C</i>
DQ482994	Symbiodinium sp. clade C3 from Acropora aspera nitrate reductase	nitrate reductase-like	nitrate assimilation and aquistion	transport	NCF	<i>Symbiodinium sp. clade C</i>
EZ004023	spinster homolog 2 (Spns2)	spinster homolog 2	lipid transport	transport	MF	<i>Acropora millepora</i>
EZ013388	Mitochondrial carrier homolog 2 (MTCH2)	Mitochondrial carrier homolog 2	mitochondrial membrane transport_integral to membrane	transport	NCF	<i>Acropora millepora</i>

Table 1: Differentially expressed genes ($p < 0.003$).

Species Detail	N
<i>Acropora millepora</i>	3207
<i>Symbiodinium</i> sp.	276
<i>Symbiodinium</i> sp. clade C	141
<i>Montastraea cavernosa</i>	46
<i>Montastraea faveolata</i>	44
<i>Anthopleura elegantissima</i>	30
<i>Symbiodinium goreau</i>	26
<i>Hydra magnipapillata</i>	24
<i>Symbiodinium microadriaticum</i>	22
<i>Stylophora pistillata</i>	17
<i>Symbiodinium kawagutii</i>	14
<i>Symbiodinium</i> sp. clade A	14
<i>Acropora cervicornis</i>	12
<i>Anemonia viridis</i>	12
<i>Montastraea annularis</i>	11
<i>Symbiodinium pilosum</i>	10
<i>Dendronephthya klunzingeri</i>	9
<i>Symbiodinium</i> sp. clade F	9
<i>Montipora capitata</i>	7
<i>Acropora digitifera</i>	6
<i>Acropora donei</i>	6
<i>Acropora</i> sp.	6
<i>Chlamydomonas reinhardtii</i>	6
<i>Danio rerio</i>	6
<i>Galaxea fascicularis</i>	6
<i>Pocillopora damicornis</i>	6
<i>Siderastrea</i> sp.	6
<i>Symbiodinium muscatinei</i>	6
<i>Aiptasia pulchella</i>	5
<i>Acropora palmata</i>	4
<i>Colpophyllia natans</i>	4
<i>Favia fragum</i>	4
<i>Montastraea faveolata</i>	4
<i>Acropora cervicornis (Gallus gallus)</i>	3
<i>Acropora eurystoma</i>	3
<i>Acropora formosa</i>	3
<i>Acropora gemmifera</i>	3
<i>Acropora humilis</i>	3
<i>Acropora valida</i>	3
<i>Acropora yongei</i>	3
<i>Agaricia humilis</i>	3
<i>Aiptasia pallida</i>	3
<i>Alcyonium paessleri</i>	3
<i>Alexandrium fundyense</i>	3
<i>Arabidopsis thaliana</i>	3
<i>Aurelia aurita</i>	3
<i>Diploria strigosa</i>	3
<i>Favites chinensis</i>	3
<i>Fungia scutaria</i>	3
<i>Goniopora tenuidens</i>	3
<i>Hydra vulgaris</i>	3
<i>Hydractinia echinata</i>	3
<i>Madracis mirabilis</i>	3
<i>Monosiga brevicollis</i>	3
<i>Montipora millepora</i>	3
<i>Oculina</i> sp.	3
<i>Porites porites</i>	3
<i>Psammocora</i> sp.	3
<i>Strongylocentrotus purpuratus</i>	3
<i>Suberites domuncula</i>	3
<i>Symbiodinium pulchrorum</i>	3
<i>Symbiodinium</i> sp. clade B	3
<i>Symbiodinium</i> sp. clade D	3
<i>Agaricia agaricites</i>	2
<i>Montastraea cavernosa</i>	2
<i>Montipora cactus</i>	2
<i>Montipora</i> sp.	2
<i>Porites</i> sp.	2
<i>Stylecoeniella</i> sp.	2
<i>Symbiodinium</i> sp. clade E	2
<i>Tubastraea coccinea</i>	2
<i>Acropora muricata</i>	1
<i>Isopora cuneata</i>	1
<i>Montastraea franksi</i>	1
<i>Porites asteroides</i>	1
<i>Scolymia vitiensis</i>	1
<i>Taeniofygia guttata</i>	1

Table 2: Total number of sequences per species included in the microarray

Species Detail	N
<i>Acropora millepora</i>	51
<i>Anemonia viridis</i>	1
<i>Anthopleura elegantissima</i>	1
<i>Galaxea fascicularis</i>	1
<i>Hydra magnipapillata</i>	2
<i>Montastraea cavernosa</i>	1
<i>Montastraea faveolata</i>	3
<i>Symbiodinium kawagutii</i>	2
<i>Symbiodinium</i> sp.	5
<i>Symbiodinium</i> sp. clade C	4
<i>Symbiodinium</i> sp. clade F	1

Table 3: Total number of differentially expressed ($p < 0.003$) sequences per species.

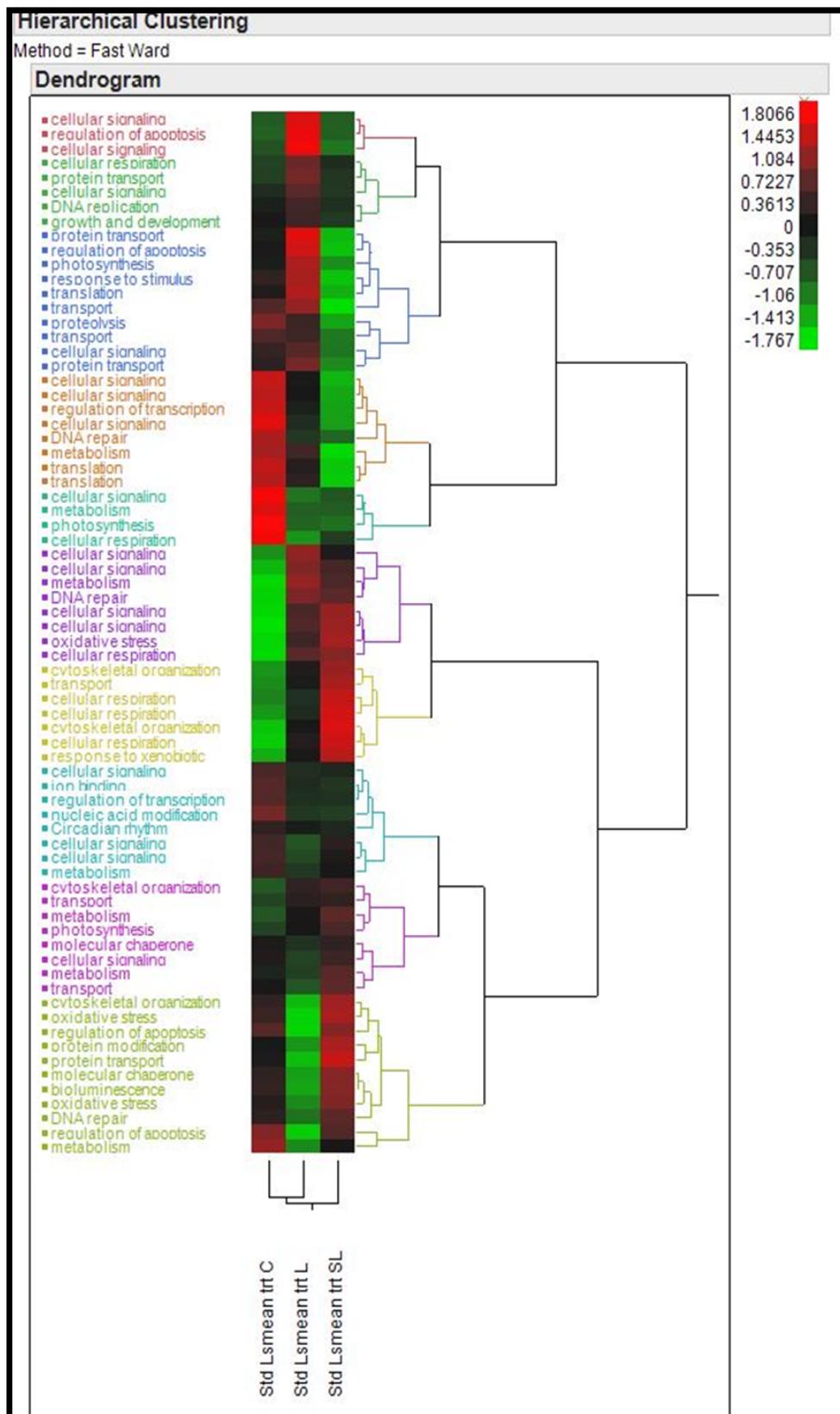


Figure 1: Heatmap labeled by function indicating gene expression profiles. Hierarchical clustering of the Standardized Least Square Mean for each treatment used was performed using the Fast Ward method. Colors indicate relative expression of the 72 genes that were differentially expressed. Green, red and black indicate

down-, up-, and neutral gene regulation, respectively. Different shades of color indicate relative amount of expression. Expression levels ranged from -1.767 to 1.8066.

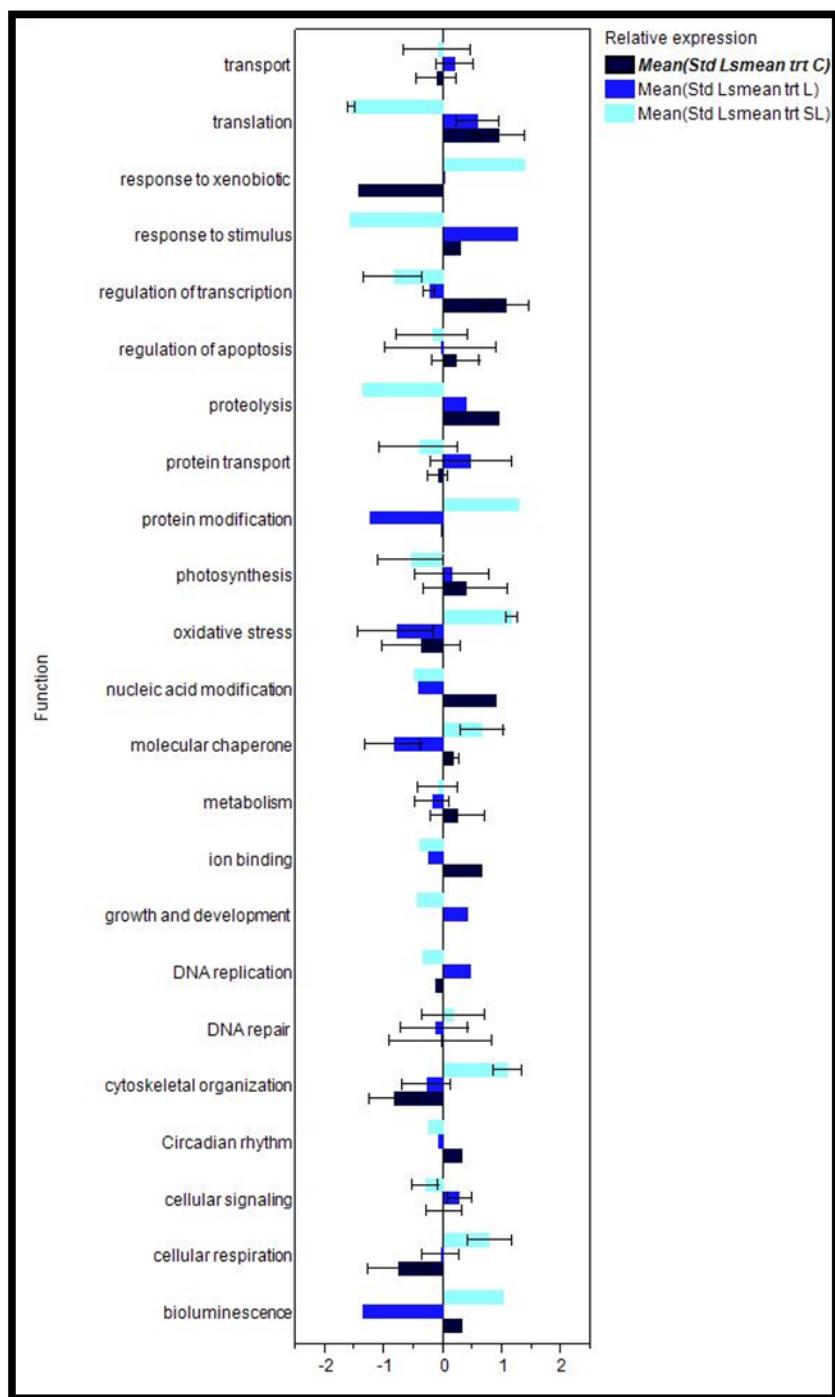


Figure 2: Bar charts illustrating gene expression profiles corresponding to the salinity experiment by function and treatment. The error bars indicate one standard error from the mean. The colors indicate the treatments. The dark, medium and light shades of blue correspond to salinity exposures of 35 ppt (C), 30 ppt (L), and the 25 ppt (SL), respectively.

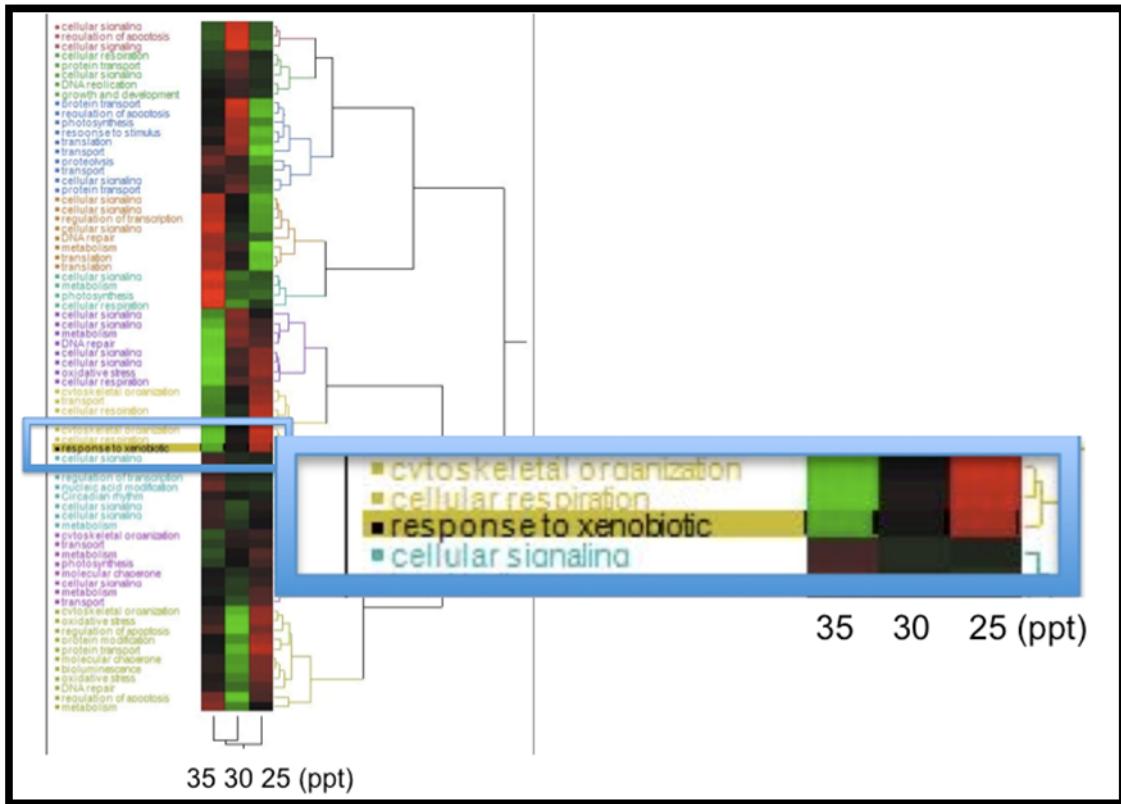


Figure 3: Heatmap labeled by function high-lightening response to xenobiotic. Hierarchical clustering of the Standardized Least Square Mean for each treatment used was performed using the Fast Ward method. Measured relative expression levels were -1.42, 0.04, and 1.38 for the control, medium salinity and lowest salinity, respectively. Note these values correspond to the expression of one gene in this function (arsenite methyltransferase).

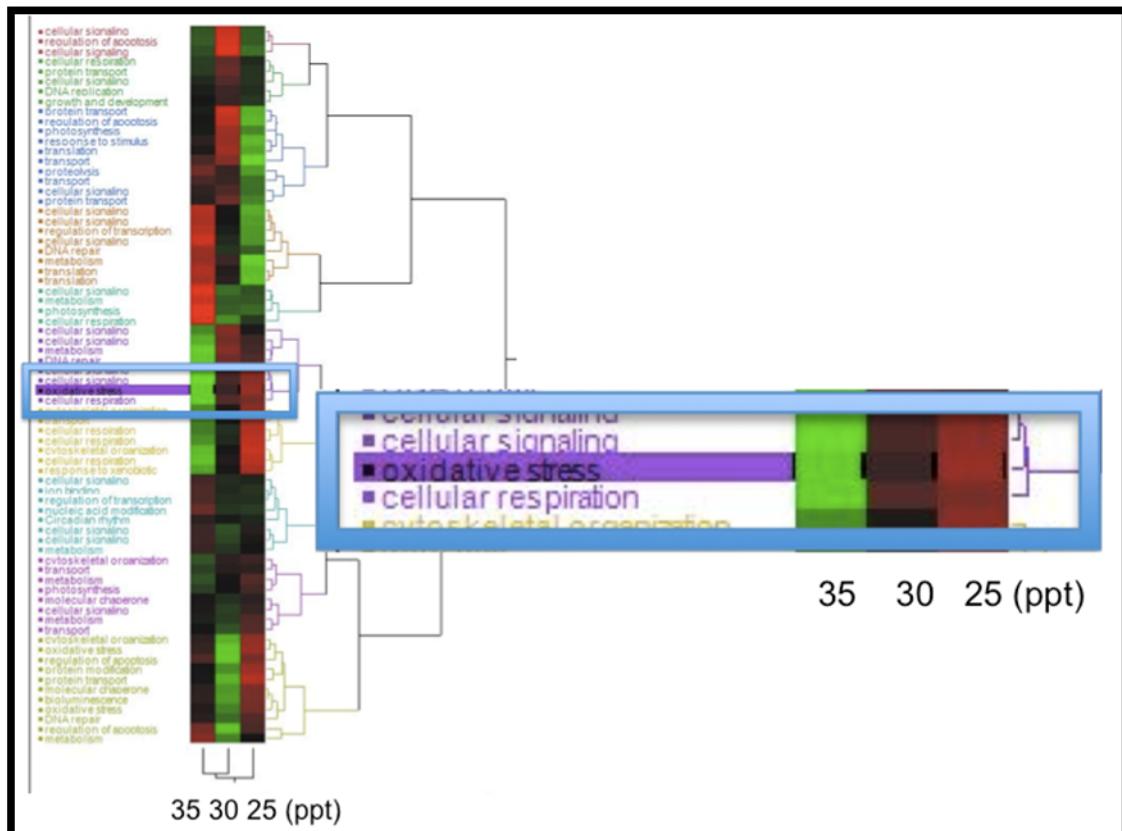


Figure 4: Heatmap labeled by function high-lightening oxidative stress. Hierarchical clustering of the Standardized Least Square Mean for each treatment used was performed using the Fast Ward method. Averaged relative expression levels were -0.37, -0.80 and 1.17 for the control, medium salinity, and lowest salinity, respectively. Note three genes were significantly expressed in this function (Peptide methionine sulfoxide reductase A, Catalase, and Glucose-6-phosphate 1-dehydrogenase).