Interactions among ocean acidification, elevated temperature and macroalgal presence on coral larvae stress and post-settlement survivorship

Final Report

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Introduction

As coral cover declines on a global scale, coral communities are facing unprecedented levels of environmental stress. Attention has been focused on how global scale stressors such as elevated seawater temperatures and ocean acidification (OA) will impact adult corals (Hoegh-Guldberg et al. 2007). Aside from these global-scale stressors, local stressors such as overfishing and land-based sources of pollution are adding to the complexity of how stressors interact and how they exacerbate each other. In the Florida Keys, local and global stressors have clearly been identified as contributors towards the overall decline in coral cover (Causey et al. 2000). An understanding of the interaction of multiple stressors represents critical information required to predict the fate of coral reefs, especially the potential processes of recovery such as coral recruitment.

Abiotic and biotic stressors can negatively influence coral recruitment at any of three critical life-history stages: larval supply, larval settlement and post-settlement survival (Ritson-Williams et al. 2009). Larval survival and settlement are two parameters necessary for coral recruitment, which if severely reduced could cause coral demographic bottlenecks. The short term planktonic period of larval dispersal represents a critical life history phase of scleractinian corals, and is the first step in the recruitment process (Edmunds et al. 2001). Subsequently, coral larvae have to settle in an appropriate habitat and survive until they are reproductive members of the community. Just as adult coral colonies can be negatively impacted by environmental stressors, larval viability and settlement can be reduced by water quality stressors such as elevated temperatures (Edmunds et al. 2001), salinity (Vermeij et al. 2006), UV radiation (Gleason et al. 2006), and negative cues from macroalgae and cyanobacteria (Kuffner et al. 2006, Paul et al. 2011).

Due to increasing concentrations of anthropogenic CO₂ in the atmosphere, global ocean temperatures have increased by an average of 0.74°C and are expected to increase 1-4°C by the end of the next century (Hoegh-Guldberg et al. 2007, IPCC 2007). According to current greenhouse gas emission scenarios (IPCC 2007), sea surface temperatures are projected to increase rapidly, from 1.8°C above the current average (Low-emission B1 scenario) to 4.0°C on average above current mean ocean temperatures (2.4°C to 6.4°C) under the High-emission A1F1 scenario (IPCC 2007, Hoegh-Guldberg et al. 2007). The increase in seawater temperature, in conjunction with ocean acidification, represents a serious threat that could drastically inhibit coral recruitment. Research to better understand the interaction of these stressors should be considered a priority that warrants further investigation.

As phase-shifts from coral-dominated to algal-dominated states occur, competition between coral larval settlement and macroalgae is increasing (Rasher and Hay 2010, Paul et al. 2011). Macroalgae, including species of Dictyota, Lobophora variegata and cyanobacteria such as Lyngbya spp., are among the most common algae on Florida and Caribbean reefs (McClanahan et al. 1999, 2000). While these organisms are known to produce natural products that protect them from herbivores (Paul et al. 2001), it is now becoming clear that some compounds can play a role in competitive interactions with other benthic organisms. Some macroalgae can negatively impact the growth of adult corals and cause coral bleaching and mortality (Hughes et al. 2007, Rasher and Hay 2010). Recent research has shown that individual compounds produced by macroalgae can stress and kill adult corals (Rasher et al., 2011). Competition driven by the presence of secondary metabolites is a common occurrence on reefs, but its effect on the early life history stages of corals is poorly understood. **The overarching goal of this proposal was to evaluate the effects of combined stressors on coral larval health**
by studying the impacts of increased $p\text{CO}_2$, elevated sea surface temperature and macroalgal exposure on larval physiology and post-settlement survival. Even though corals will be exposed to all of these stressors and more on future reefs, there remains little to no information on how stressors interact to inhibit coral recruitment. By incorporating ecology, physiology, molecular biology and biochemistry into this study we can greatly improve our understanding of the interactive effects of stressors and how those interactions might impact coral recruitment and community recovery. These data will be essential for scientists and managers to establish management priorities that can conserve reef ecosystems in the face of a changing climate.

Accomplished Proposal Objectives

As described in the initial proposal, funding was initially requested to cover a 2 year study which would explicitly examine how elevated $p\text{CO}_2$, and the resultant increased OA, in concert with elevated seawater temperature and macroalgal exposure (individually and in combination) affect coral larva health, survival, settlement and post-settlement growth and survival. Considering three parameters were to be studied (effects of increased $p\text{CO}_2$, elevated temperature, and algal exposure), experiments were going to be conducted in 2012 (year 1) and 2013 (year 2). Year 1 experiments were to focus on the interaction of 2 parameters to better understand how local and global stressors interact to influence coral recruitment. We proposed to specifically test a) the combined effects of increased $p\text{CO}_2$ and algal exposure and b) elevated temperature and algal exposure on larval physiology, survival, settlement and post-settlement survival. In year 2 we proposed to increase the capacity of the Mote Laboratory for more treatment tanks, which will allow us to run experiments that combine all 3 parameters simultaneously. POR funding was only provided for 1 year. Therefore, the following objectives were accomplished:

1. Test for larval settlement and survival when incubated under a) increased $p\text{CO}_2$ and macroalgal exposure or b) elevated sea surface temperature and macroalgal exposure.

2. Run a series of cellular diagnostic assays which will quantify biochemical markers of sublethal stress in larvae that had been exposed to the aforementioned treatments;

3. Test for long term consequences of larval exposure by measuring post-settlement survival of the corals after exposure to the aforementioned treatments.

*If funding is continued for year 2 (2013-2014) we will be able to conduct a larger scale study that incorporates the combined interactive effects of 3 stressors ($p\text{CO}_2$, elevated seawater temperature and macroalgal exposure) on larval health and viability.*
Methods:

Two separate yet complimentary experiments were conducted in May 2012 at Mote Marine Laboratory at Summerland Key:

**Experiment #1**: Evaluation of the effects of elevated temperature and exposure to *Dictyota menstrualis* on two life stages of *P. astreoides* (larvae and 6 week old recruits).

**Experiment #2**: Evaluation of the effects of ocean acidification and exposure to *Dictyota menstrualis* on larvae of *P. astreoides*.

**Larval Collection**

Thirty colonies of *P. astreoides* were collected from Big Pine Ledges (N 24°33.190; W 81°31.940) and an unnamed patch reef (N 24°32.910; W 81°31.940; Ross permit FKNMS 2011-038-A1), and transported to Mote Marine Tropical Research Laboratory (Summerland Key, FL) in coolers, and maintained in running seawater. Larvae were collected just before the nights of the May 2012 new moon when colonies released brooded larvae (McGuire 1998). Colonies were later returned to the site of collection and reattached with Z-Spar Splash Zone Compound® underwater epoxy. To obtain larvae, each colony was placed in an individual 3 L Rubbermaid Grip’s Mix bowls® supplied with continuously running seawater. The bowls were tilted so the positively buoyant larvae spilled over the handles of the bowls each night into plastic tri-pour beakers fitted with a mesh bottom (mesh diameter: 180 µm) supported 3 cm off the sea table bottom by 3 silicone stopper feet. The water level inside the tank was maintained at 15 cm so the larvae remained in the tri-pour beakers until sunrise the next morning when they can could be counted and placed in the treatments.

**Experiment #1** (Thermal stress and *Dictyota menstrualis* presence)

*Larval Treatments*

To evaluate the effects of elevated temperature and *D. menstrualis* presence on larval health, samples were exposed to one of the following treatments: 1) ambient temperature (27.5 ± 0.1°C) and a plastic algae mimic (Control); 2) elevated temperature (31.0 ± 0.1°C) and a plastic algae mimic (Heat); 3) ambient temperature (27.5 ± 0.1°C) with *D. menstrualis* (Algae); and 4) elevated temperature (31.2 ± 0.1°C) with *D. menstrualis* (Heat+Algae). Plastic aquaria served as independent water baths (n=10/treatment).

Each replicate water bath contained a total of 250 *P. astreoides* larvae that were subdivided into two chambers. One hundred and fifty larvae were placed into an acrylic treatment chamber (10 × 5.5 cm) with a 180 µm mesh bottom containing either a plastic algae mimic or *D. menstrualis*. One hundred larvae were placed in an acrylic settlement chamber (10 × 5.5 cm) with a 180 µm mesh bottom containing a single conditioned (i.e., 4 week accumulated biofilm) terracotta tile (4.5 x 4.5 x 1 cm; Sunshine Pavers®), which served as a settlement substrate and either a plastic algae mimic or *D. menstrualis*. The amount of *D. menstrualis* used was approximately 4 cm³ in both the treatment and settlement chambers.
**Larval settlement and survival**

Following 3 days of incubation, the surviving larvae, out of the original 100 in the settlement chambers, were scored as either swimmers or metamorphosed recruits.

**Larval photophysiology**

Ten larvae were removed from the treatment chambers of each replicate following 2 days of exposure to assess photochemical efficiency. The photochemical efficiency of symbiotic zooxanthellae within the larvae were examined using pulse amplitude modulated (PAM) fluorometry (Diving-PAM; Walz, GmbH, Germany). Changes in dark adapted maximal quantum yield \( \frac{F_v}{F_m} = \frac{(F_m-F_o)}{F_m} \) of PS II were assessed. For analysis, 10 larvae in 25 µl of seawater were pipetted onto the tip of the fiber-optic cable. Both measuring intensity and gain were set to “8”. All larval samples were dark adapted for 1 hour prior to any photochemical measurement.

**Cellular stress assays**

The remaining larvae from the treatment chambers were collected, frozen in liquid nitrogen and brought back to UNF for processing. Samples were thawed to room temperature, and extracted in 2.5 ml of buffer (50 mM potassium phosphate buffer (pH 7.0) containing 10 % (w/v) polyvinylpolypyrrolidone (PVP)-40, 0.25 % Triton X-100 and 1 % (v/v) plant cell protease inhibitor cocktail [Sigma-Aldrich, St. Louis, MO, USA]). Samples were homogenized with a Fast Prep 24 bead homogenizer (MP Biomedicals, Irvine, CA, USA) and centrifuged at 16,000 x g for 10 min. The resulting supernatants were normalized for protein content using a Bicinchoninic acid (BCA) Protein Assay Kit (Thermo Scientific, Pittsburgh, PA, USA). To evaluate the damaging effects of ROS on cellular integrity, lipid peroxidation was assayed. Unsaturated lipids of cell membranes or other membranous systems are common targets of oxidative attack by ROS. The end result is lipid peroxidation, a destructive process that compromises normal cellular function. Lipid peroxidation was measured using a Lipid Hydroperoxide Assay kit (Cayman Chemical, Ann Arbor, MI, USA). Catalase (CAT) is a widely distributed enzyme that destroys H2O2 by dismutation to O2 and H2O. Upregulation of CAT reflects an organism’s response to counteract the presence of damaging ROS. Catalase was assayed using the Amplex Red kit (Invitrogen, Eugene, OR, USA) as per the manufacturer’s instructions.

**Juvenile experiments**

**Juvenile Rearing**

Fifty preconditioned terra cotta tiles were individually placed in plastic tripour containers containing a haphazard number of larvae. The larvae were allowed to settle for 4 days during the duration of the larval experiment. After which the settlement tiles were removed and transported in coolers to raceways at the Smithsonian Marine Station (Fort Pierce, FL). The juvenile spat were reared in running seawater for 6 weeks and the 40 tiles with the greatest number of individuals on the top portion of the tile were selected for further experimentation.

**Juvenile Treatments**

To evaluate the effects of elevated temperature and \( D. menstrualis \) presence on the juvenile life history stage samples were exposed to one of the following treatments: 1) ambient temperature (27.5 ± 0.1°C) and a plastic algae mimic (Control); 2) elevated temperature (31.0 ±
0.1°C) and a plastic algae mimic (Heat); 3) ambient temperature (27.5 ± 0.1°C) with *D. menstrualis* (Algae); and 4) elevated temperature (31.2 ± 0.1°C) with *D. menstrualis* (Heat+Algae). Plastic aquaria served as independent water baths (n=10/treatment).

Treatment duration was based on the availability of *D. menstrualis* and in total lasted 184 hours (7 days). Following 36 hours all tiles were removed from treatments for photochemical efficiency assessments. After 96 hours (4 days) all tiles were removed from treatments to evaluate survival and to replace potentially decaying live algae. Finally, photochemical and survival assessments were taken at the conclusion of the experiment (7 days).

**Juvenile Survival**

Tiles containing juvenile recruits were scored prior to the experiment to determine the original number and location of spat on each tile. Following 96 hours (4 days) and 184 hours (7 days) of treatment the tiles were scored again and the number of living and dead colonies were recorded. Colonies were scored as dead if no live tissue remained on the skeleton. The counts of dead colonies were used to insure that each tile was being assessed consistently by comparison to the original evaluation.

**Juvenile photophysiology**

The photochemical efficiency of intracellular zooxanthellae within the juvenile corals were examined using pulse amplitude modulated (PAM) fluorometry (Diving-PAM; Walz, GmbH, Germany). Treatment effects on effective quantum yield (EQY) of PS II were assessed. For analysis, 3 individuals directly underneath either the plastic or live algae were evaluated using the fiber-optic cable. The average of the 3 measurements was used as a single value for each replicate.

**Juvenile cellular stress assays**

Assays were performed as describe above.

**Experiment #2 (Ocean acidification stress and *Dictyota menstrualis* presence)**

To examine the impacts of reduced pH and *D. menstrualis* presence on coral larvae, groups were assigned to one of the following treatments using the OA system at Mote Marine Lab, Summerland: (1) Control group maintained at ambient pH (8.05) with synthetic algae present (to control for shading and physical interaction); (2) ambient pH exposed to live algae; (3) reduced pH (7.6) exposed to live algae; and finally (4) reduced pH with synthetic algae. Each replicate consisted of 150 larvae in 200ml of seawater held within a 400ml plastic tri-pour beaker. Beakers containing larvae were randomly assigned a treatment. All replicates were maintained at a constant salinity of 37.5 ‰ and temperature of 26.5° C, with an average photosynthetic active radiation level of 85 µmol m⁻² s⁻¹. Temperature was monitored and recorded 3 times throughout the duration of the 24 h experiment. Live algae treatments contained an average of 0.644g of *Dictyota sp.* in each chamber. Each synthetic algae treatment group had an average weight of 0.606g plastic aquarium algae.

After 48 hours of exposure, 10 larvae were removed from each replicate for oxygen respiration quantification; 10 larvae from each replicate were used to quantify photochemical efficiency (PAM) for each treatment group, and 50 larvae from each replicate were removed for
subsequent survival/settlement assays (described below). The remaining larvae were used for cell stress assays.

**Larval respiration**
Post treatment respiration analysis of *P. astreoides* larvae was conducted according to the methods of Ross *et al.* (2010). To quantify respiration, 10 randomly selected larvae were removed from each replicate of each treatment group, and placed in a 2.5 mL reaction volume of O₂ saturated filtered seawater (0.45 µm). Prior to measuring, samples were dark adapted for 1 hour, and the system was calibrated using sodium hydrosulfite to eliminate oxygen. To create an O₂ maximum, air was bubbled into the filtered seawater. Oxygen uptake was measured in the dark over 20 minute intervals for each replicate. Control blank runs consisting of filtered seawater were not subtracted from experimental runs, as it was noted that filtered seawater alone did not undergo any change in oxygen consumption over the time course studied in this experiment. Respiration was calculated as nanomoles of oxygen consumed per minute per larva.

**Larval photosynthetic efficiency**
Pulse amplitude modulated (PAM) fluoremetry was used to measure photo-efficiency of *in hospite* dinoflagellates (Diving-PAM; Walz, GmbH, Germany). Larval replicates (5 replicates containing 10 larvae each) were briefly placed in 25 µl of seawater, and gently pipetted directly onto the tip of the fiber-optic cable. After being dark adapted for 1 hour, photophysiological measurements were taken on larval samples, with measuring intensity and gain both set to “8”.

**Larval cellular stress assays**
Assays were performed as describe above.

**Larval settlement, mortality and post-settlement survivorship**
Following the aforementioned treatments, remaining larvae from each replicate were placed in outdoor seawater tables in customized 800mL plastic tri-pour beakers. The bottom of each beaker was replaced with 180 µm nitex mesh to allow for continuous seawater exchange and each beaker was equipped with a single preconditioned terracotta tile (4.5 cm x 4.5 cm x 1 cm; Sunshine Pavers®). These tiles were conditioned 6m offshore, 5 weeks prior to the experiment, in order to obtain a CCA biolfilm substrate suitable for larval. After a 48 hour exposure, larvae were counted in one of two groups: those that remained as swimmers (still in the planula phase), and those that settled and underwent metamorphosis on the tile. Percent survival was calculated by dividing the total survivors (swimmers + settlers) by the initial number of larvae (50); likewise, percent settlement was calculated by dividing the number settled and metamorphosed by 50. Of those that underwent successful metamorphosis (typically about 20 larvae per tile), their respective positions on the tiles were mapped and recorded. To evaluate post-settlement survival, tiles containing newly settled spat were arranged on a rod and then placed on a patch reef east of the Looe Key research area (N 24°34.130 ; W 81°22.868 ; ~7m depth) for 24 days. Post-settlement survival was calculated by dividing the number of live coral recruits remaining by the initial number recorded as settled for each individual tile.
Experiment #1
Results

Larval experiments

Figure 1. Algal presence strongly impacts larvae survival. Percent survival of *P. astreoides* larvae out of 100 individuals (Bars = +1 SE, n=10; 2-way ANOVA on arcsine square root transformed data).
Figure 2. Algal presence strongly impacts larvae settlement. Percent settlement of *P. astreoides* larvae out of 100 individuals (Bars = +1 SE, n=10; 2-way ANOVA on arcsine square root transformed data).
Figure 3. Thermal stress has a strong sub-lethal impact on larvae. Photosynthetic efficiency of intracellular zooxanthellae in *P. astreoides* larvae (Bars = ± 1 SE, n=10; 2-way ANOVA on arcsine square root transformed data).
**Figure 4.** The combination of heat and algae cause a synergistic increase in larval catalase activity. Bars represent mean ± SE. Letters indicate significantly different groupings (One way ANOVA followed by Tukeys Post Hoc test).
Figure 5. The combination of heat and algae cause a synergistic increase in larval lipid peroxidation. Bars represent mean ± SE. Letters indicate significantly different groupings (One way ANOVA followed by Tukeys Post Hoc test).
Juvenile experiments

Figure 6. Percent survival of *P. astreoides* juveniles following 4 days of treatment. Bars are untransformed means, error bars are ± 1 SE. n=10 (Heat treatment n=9). P values were calculated using 2-way ANOVA on rank transformed data.
Figure 7. Percent survival of *P. astreoides* juveniles following 7 days of treatment. Bars are untransformed means, error bars are +1 SE. n=10 (Heat treatment n=9). P values were calculated using 2-way ANOVA on rank transformed data.
Figure 8. Photosynthetic efficiency of intracellular zooxanthellae in *P. astreoides* juveniles following 36 hours of treatment. Bars are untransformed means, error bars are ± 1 SE. n=10 (Heat treatment n=9). P values were calculated using 2-way ANOVA on rank transformed data.
**Figure 9.** Photosynthetic efficiency of intracellular zooxanthellae in *P. astreoides* juveniles following 7 days of treatment. Bars are untransformed means, error bars are ± 1 SE. n=10 (Heat treatment n=9). P values were calculated using 2-way ANOVA on rank transformed data.
Experiment #2
Results

**Figure 10.** Neither seawater pH or algal presence impacts larval respiration. Oxygen consumption of *Porites astreoides* larvae exposed to artificial and live *Dictyota sp.* at ambient (8.0) and reduced (7.6) pH levels. (Error bars ± SE, n = 5; 2-way ANOVA)
Figure 11. Algal presence negatively impacts larval photochemical efficiency. Photosynthetic efficiency of *Porites astreoides* larvae exposed to artificial and live *Dictyota sp.* at ambient (8.0) and reduced (7.6) pH levels. (Error bars ± SE, n = 5; 2-way ANOVA)
Figure 12. Algal presence causes an increase in larval lipid peroxidation. Bars represent mean \pm SE. Graph depicts *Porites astreoides* larvae exposed to artificial and live *Dictyota sp.* at ambient (8.0) and reduced (7.6) pH levels. (Error bars \pm SE, n = 10; 2-way ANOVA)
Figure 13. Algal presence and seawater pH do not impact larval CAT activity. Bars represent mean ± SE. Graph depicts *Porites astreoides* larvae exposed to artificial and live *Dictyota sp.* at ambient (8.0) and reduced (7.6) pH levels. (Error bars ± SE, n = 10; 2-way ANOVA)
Figure 14. Percent settlement of *Porites astreoides* larvae exposed to artificial and live *Dictyota sp.* at ambient (8.0) and reduced (7.6) pH levels. Error bars ± SE, n = 5.
Figure 15. Algal presence impacts larval survival. Percent survival of *Porites astreoides* exposed to artificial and live *Dictyota sp.* at ambient (8.0) and reduced (7.6) pH levels. (Error bars ± SE, n = 5; 2-way ANOVA).
Figure 16. Post settlement survival (24 days) of Porites astreoides exposed to artificial and live Dictyota sp. at ambient (8.0) and reduced (7.6) pH levels. Error bars ± SE, n = 5.

Education and Outreach:

A. Student Training

This POR award provided a rewarding opportunity for 4 UNF undergraduate students (Kevin O’Donnell, Shannon Crabtree, Paige Duffin and Julie Tariscka) to gain hands on experience working with coral reef biology and physiology. These students were responsible for all facets of this project including the collection of adult corals (snorkeling in shallow water habitats) and setting up larval collection chambers; the treatment of coral larvae with selected choices of stress (algal exposure, acidic seawater or elevated temperature), as well as performing larval settlement assays and physiology experiments (to evaluate the effects of each respective stressor).

It was anticipated that the undergraduate students would develop an understanding and appreciation of coral reef biology and the impacts of abiotic stressors on these fragile organisms. At the completion of project the students were able to: a) interpret and discuss fundamental articles related to coral ecology, physiology and evolution; b) design an independent project
relating to the main thematic questions of the study; c) collect, organize and analyze the data collected from their study; and d) appreciate the impact of climate change on both the coral reef study system and human communities that depend on them.

In addition, this project provided support for 1 UNF Master’s student (Kevin Olsen) to obtain necessary data for his thesis.

B. Outputs based upon this research

Conference Presentations


Publications

Data arising from this POR is being prepared for publication in the following scientific literature:

References


