

ANALYSIS OF RECRUITMENT OF *POCILLOPORA DAMICORNIS* UNDER
CLIMATE CHANGE SCENARIOS

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ABSTRACT

Ocean warming and acidification are among the greatest threats to coral reefs, and severe bleaching events have become more common in recent decades, putting coral reefs at risk of extinction. The loss of coral reefs would have a catastrophic cascading effect on all marine life and would mean a loss of one the world's largest pools of biodiversity. There have been many studies conducted on the individual effects of warming and acidification on coral response, but not as many that attempt to assess the combined effect, which could possibly be synergistic. This study looks at the individual and combined effects of warming and acidification on *Pocillopora damicornis* under four different treatments: ambient, heated, acidified, and heated/acidified, in a multi-annual mesocosm experiment. *P. damicornis* were able to recruit under these stressors, and the recruits were measured for size, condition, and abundance during two separate assessments and the differences across treatments and within treatments were analyzed. The results showed that more bleaching occurred in the heated treatments than in the non-heated treatments, and that mean size significantly increased in the treatments with acidification from July to October.

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1.0 INTRODUCTION

1.1 Climate Change

Coral reefs are one of the most diverse and productive ecosystems in the world and hold great biological value, as they are an important habitat for marine species and also provide us with coastal protection, food, and resources. However, due to the increasing diversity, frequency, and scale of human impacts, coral reefs are now one of the most threatened habitats and are declining at 1-2% per year (Hoegh-Guldberg, 2011). Human activities mainly due to the burning of fossil fuels, produce anthropogenic emissions including carbon dioxide, a greenhouse gas. This has consequently forced the atmospheric carbon dioxide concentration to increase at an alarming rate, intensifying the greenhouse effect and warming the planet as well as the oceans, which absorb some of the heat. The rate of current CO₂ increase is about 100x faster than what has occurred over the past 650,000 years, and the rising atmospheric CO₂ levels have risen to the point of being irreversible on human timescales (Kleypas et al., 2006).

1.2 Ocean Warming

Corals are tolerant of only a range of temperatures, becoming stressed if their thermal threshold is exceeded, causing them to bleach (Hoegh-Guldberg et al., 2007). Here in Hawai'i, sea surface temperatures have already increased 1.15°C between 1956-2014 (Bahr et al., 2016). Bleaching is caused by a breakdown in the symbiosis of the coral with its zooxanthellae that provides nearly ninety percent of its metabolic requirements, ultimately reducing the performance of the coral host (Pandolfi et al.,

2011). Coral bleaching events often result in high coral mortality, and the frequency and intensity of these events will increase due to continued increases in carbon production (Hoegh-Guldberg et al., 2007). There have already been several extremely severe bleaching events that have wiped out extensive areas of coral reef, and events of this magnitude will become commonplace (Hoegh-Guldberg et al., 2007). A decline in coral reefs would have a cascading effect on a diversity of marine organisms who rely on the reefs for food, shelter, and protection.

1.3 Ocean Acidification

About one fourth of the carbon dioxide emitted from all anthropogenic sources currently enters the ocean, where it reacts with the water and produces carbonic acid (Hoegh-Guldberg et al. 2007). When carbonic acid dissociates, its byproducts are a bicarbonate ion (HCO_3^-) and a hydrogen ion (H^+), and this increase in hydrogen ion concentration lowers pH and increases ocean acidity (Hoegh-Guldberg et al. 2007). Calcification of coral skeletons are dependent on the passage of hydrogen ions between the water column and the coral tissue (Jokiel, 2013). The proton flux hypothesis states that an increase of hydrogen ions in the water column weakens the gradient, leaving corals unable to expel hydrogen ions, which hinders calcification (Jokiel, 2013). If ocean acidification continues to add hydrogen ions, construction rates could fall below natural destruction rates, causing a loss of reefs.

1.4 Individual and Combined Effects

Global warming and ocean acidification will compromise carbonate accretion, reducing the number of corals in our reef systems and pushing them towards risk of extinction. The loss of these important coastal ecosystems will diminish resources for many around the world. There have been numerous studies on the individual effects of ocean warming and acidification on coral response, but less research has been conducted on the combined effects of the two (Pandolfi et al., 2011). Ocean warming and acidification are happening simultaneously, and so it would be of great significance to understand whether or not the two together produce a synergistic effect.

The Hawai'i Institute of Marine Biology at Moku o Lo'e (Coconut Island) in Kāne'ohe, O'ahu received funding from the National Science Foundation for a long term mesocosm study to examine the effects of elevated temperature and carbon dioxide partial pressure (pCO₂) on corals. There have been other similar studies but this will be the first to examine these effects in replicated mesocosms over a multiannual timeframe, whereas short term studies might not account for effects that can only be detected over a longer term. The mesocosm study includes eight of the most dominant coral species across the Hawaiian archipelago, that together constitute more than ninety-seven percent of the coral cover on Hawaiian reefs, one of which is *Pocillopora damicornis* (Jokiel et al., 2004).

The life history strategy of *P. damicornis* differs from that of the other seven species in that this species grows fast, matures early, and broods larvae that once released are ready to settle within a couple of days (Harii et al., 2002). *P. damicornis* colonies in Hawai'i release planulae monthly throughout the year that can stay competent for over a hundred days after their release, and are ready to settle within much shorter periods as

compared to spawning corals (Richmond and Hunter 1990). This life history strategy resulted in *P. damicornis* recruiting on the sides of the mesocosms in high numbers (Figure 1), which presented an opportunity to examine the effects of elevated temperature and pCO₂ on recruitment of *P. damicornis* under a long term mesocosm setup. The main purpose of this study is to assess both the individual and combined effects of projected elevated ocean temperatures and acidification under a moderate climate change scenario as outline by the International Panel on Climate Change, on *P. damicornis* recruited under these treatments. A pre-bleaching assessment was conducted to record initial coral condition, and a post assessment was conducted after the highest annual temperatures were reached to determine whether the recruited corals survived during the additional stress.

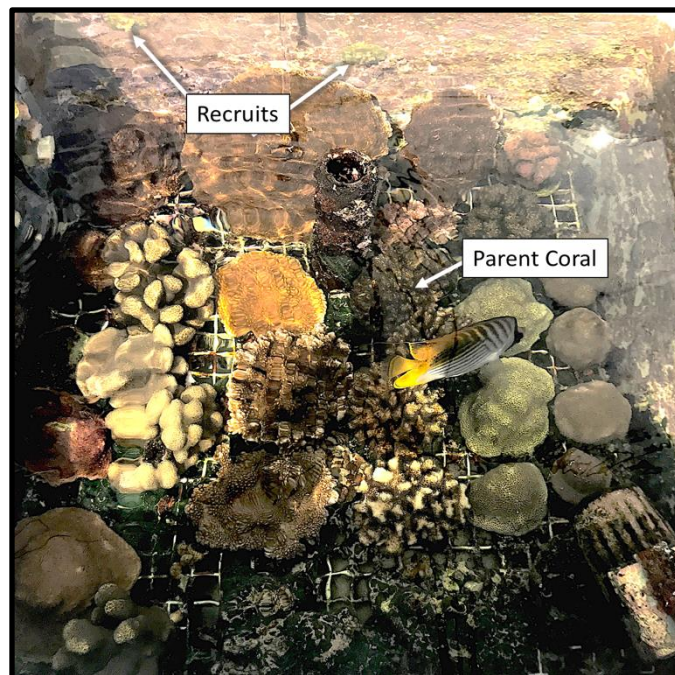


Figure 1: Example mesocosm showing parent and recruited *P. damicornis*

2.0 METHODS

2.1 Experimental Design and Setup

The full mesocosm outdoor system is a fully factorial design containing forty tanks and is located outdoors so that it is exposed to natural, seasonal changes in irradiance (Figure 2). Unfiltered seawater is pumped directly from the adjacent coral reef of Kāne‘ohe Bay in order to mimic the natural environment as closely as possible, but with temperature and pCO₂ manipulation. There are a total of four different treatments: ambient, acidified, heated, and heated/acidified (Table 1). Each treatment is replicated in ten replicate tanks that are randomly arranged and spatially disperse in the setup among the other treatments (Figure 3).

Treatment Name	Temperature	pCO₂
Ambient	Average	Average
Acidified	Average	High
Heated	High	Average
Heated & Acidified	High	High

Table 1: Temperature and pCO₂ characteristics of each treatment

The incoming seawater is directed to a holding tank where it's adjusted to the desired ambient conditions, and fed into the header tanks where heat and pCO₂ manipulations are made (Figure 4). Temperature was increased using a commercial heat

pump with a temperature controller to provide cooling or heating as needed. Adjustments to pCO₂ are achieved by using high-precision needle valves connected to pumps that delivers a precise quantity of CO₂ gas that completely dissolves into the seawater (Jokiel et al., 2014). The ambient control treatments contain seawater with the average present-day offshore temperature and pCO₂ conditions, which is ~26-28°C and ~400µatm pCO₂. The heated treatment is 2.5°C greater than the ambient, and the acidified treatment is ~350µatm pCO₂ greater, corresponding to expected future conditions under a moderately high CO₂ emissions scenario as outline by the International Panel on Climate Change (Moss et al., 2008)



Figure 2: Outdoor mesocosm setup located adjacent to Kāneʻohe Bay

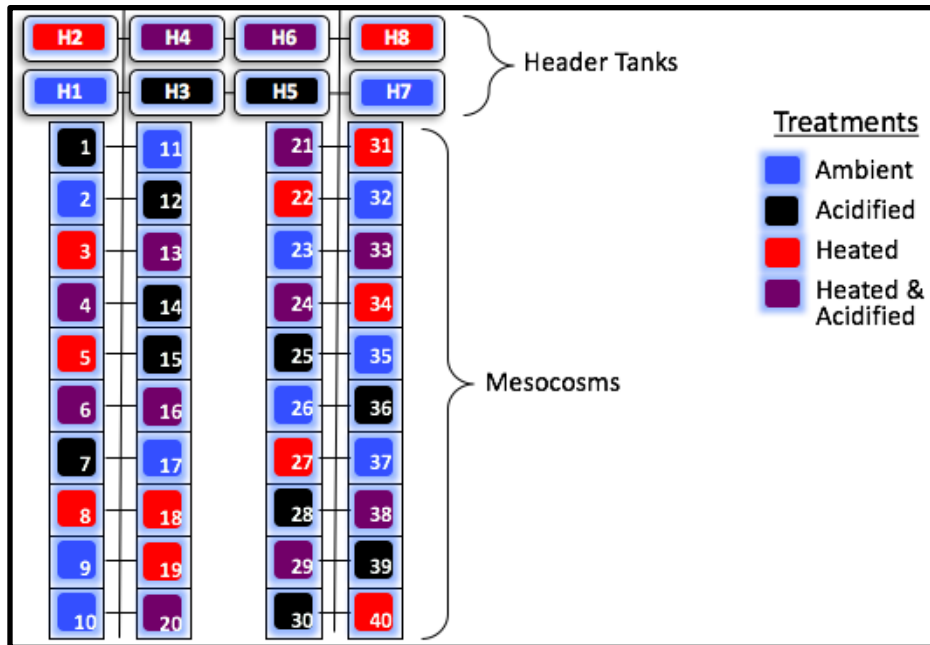


Figure 3: Schematic map of the treatments pertaining to each tank



Figure 4: Close up of Feeder Tank and Header Tanks

2.2 Temperature and pCO₂ Measurements

Temperature loggers are deployed in each mesocosm for monitoring and are regularly calibrated using a certified thermometer. To track the carbonate chemistry over

time, water samples from each mesocosm were taken weekly to test for total alkalinity, pH, and salinity. Additionally, monthly water samples from each mesocosm occurred every four hours over a twenty-four hour period to track the carbonate chemistry over the diel cycle. Total alkalinity was determined by running potentiometric titrations with a 877 Titrino Plus, with the accuracy and precision of the values verified using a certified reference material (Dickson et al., 2003). Salinity was measured using a YSI Pro Plus multimeter instrument, and pH was determined with the use of a spectrophotometer.

2.3 Measurement of Coral Color, Size, and Abundance

Measurements of coral size, pigmentation, and total number of coral recruits were recorded for each of the forty mesocosms in both of assessments. Size was measured in centimeters with the use of a caliper. Abundance was determined at the end of each assessment by totaling the number of individual corals found in each mesocosm. Pigmentation was denoted as normal, pale, bleached, dead, or a combination of more than one expressed as a percentage. Prior to measuring, training was received from Dr. Keisha Bahr on use of the Coral Health Chart (Figure 5) and on how to differentiate certain characteristics that indicate bleaching and mortality. The Coral Health Chart was developed by the University of Queensland in Australia as a part of a citizen science project CoralWatch, to provide a simple and standardized tool that can be used by anyone to quantify coral health. Color was determined solely based on observation thus it is difficult to be precise. However, the colors on the card are calibrated with coral condition (chlorophyll levels etc.) to provide a semi-quantified methodology. Observer error was

reduced by standardizing to the same observer taking the measurements throughout both assessments.

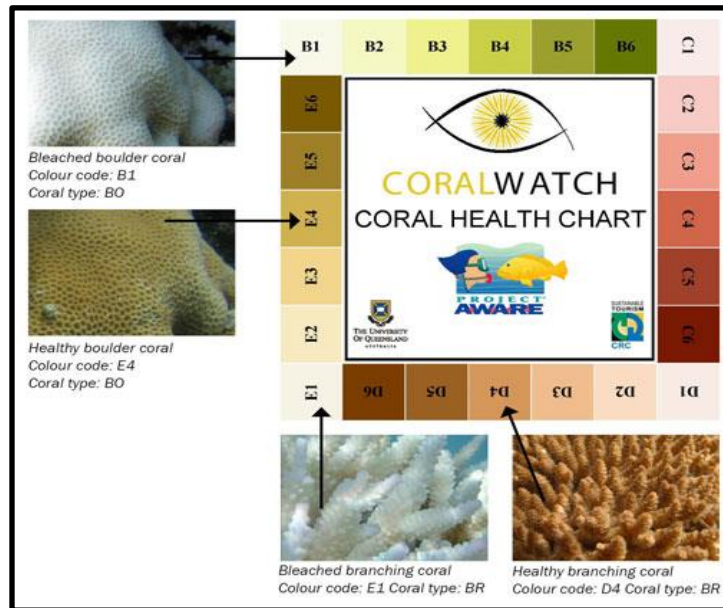


Figure 5: Coral Health Chart provided by CoralWatch from the University of Queensland

Measurements of all forty mesocosms were completed within a one-week time frame to minimize the possibility of changes to the corals among mesocosms due to temporal variability. These measurements were initially conducted from July 5-July 10, 2017 to provide a pre-bleaching assessment, and again from October 12-October 17, 2017, following the peak annual high temperatures and bleaching to assess post-bleaching (Figure 6).

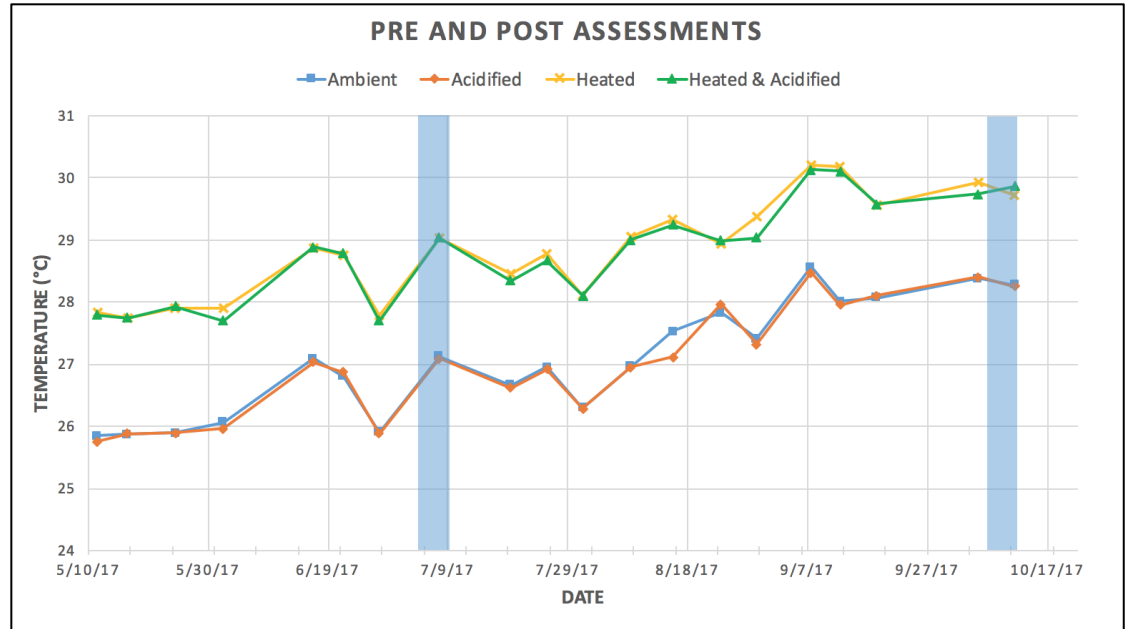


Figure 6: Time series of average in-situ treatment temperatures, (highlighted intervals=when the assessments occurred)

2.4 Statistical Analysis

The objective was to assess how the measurements differed across treatments as well as within treatments, by statistically analyzing the means. All of the statistical analysis was done using JMP software, with the use of a Kruskal-Wallis test to compare differences across treatments, and a matched pair t-test to compare differences within treatments. The Kruskal-Wallis is a test by ranks and is a non-parametric method for testing whether samples originate from the same distribution. The Matched Pairs analysis compares the means between two or more correlated variables and assesses the differences. It standardizes to the initial subject to compare the same subject through time, while the paired t-test statistical method takes the correlated responses between the two time frames into account.

Running the data through these tests returns a p-value, which is a number between zero and one, that is used to weigh the strength of what the data is telling you about the population. A small p-value (typically ≤ 0.05) provides strong evidence that the difference is significant and not due to chance, while a p-value greater than 0.05 indicates the difference is not statistically significant. The degrees of freedom which is automatically taken into account, is related to the number of observations and is defined as the number of values that are free to vary in each data set. The DF and p-value are inextricably linked, so it is important to note the DF for each test run since the sample sizes for each treatment varied with some treatments having more observation points than others. The DF values are taken into account during analysis to determine whether a given test statistic is significant or not, and the higher the degrees of freedom, the lower the threshold for a significant result.

3.0 RESULTS

3.1 Pre and Post Assessment Measurements

Figures 7 shows the temperatures for each tank during noon sampling for the week that the assessments in July and October were conducted.

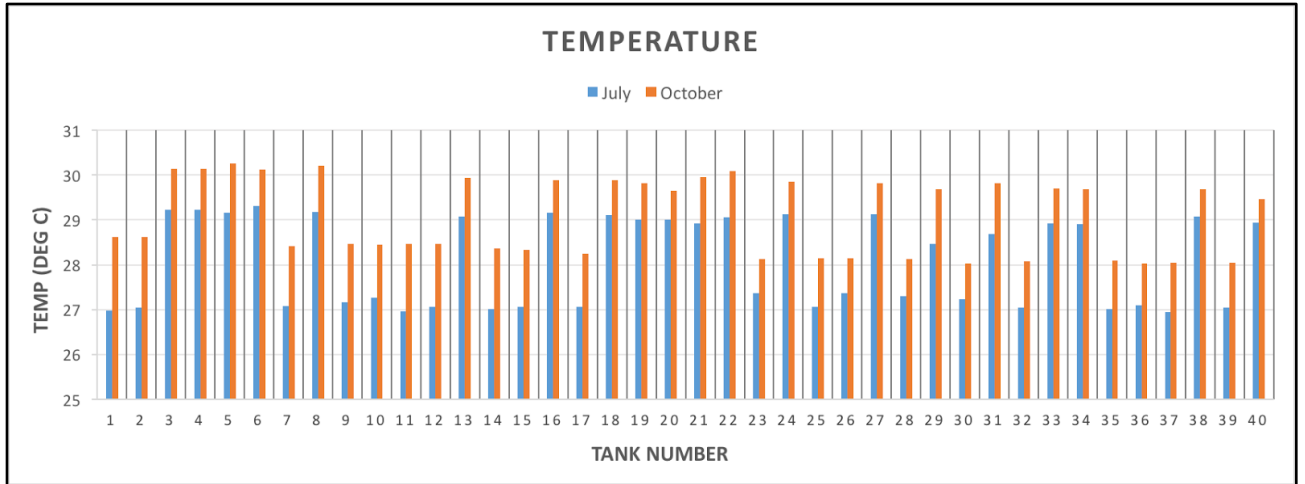


Figure 7: Temperatures by tank for July and October assessments

The pattern within treatments were identical for July and October with acidified having the most tanks with recruits with nine out of ten, followed by ambient with eight, then heated, and heated/acidified which each had six (Figure 8).

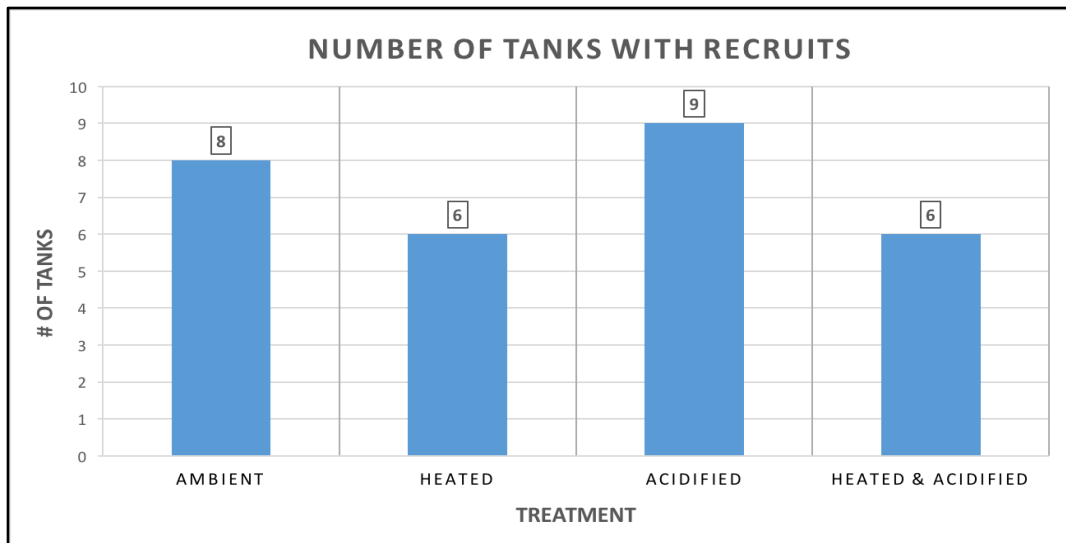


Figure 8: Total number of tanks with recruits by treatment for July and October (n=10 tanks per treatment)

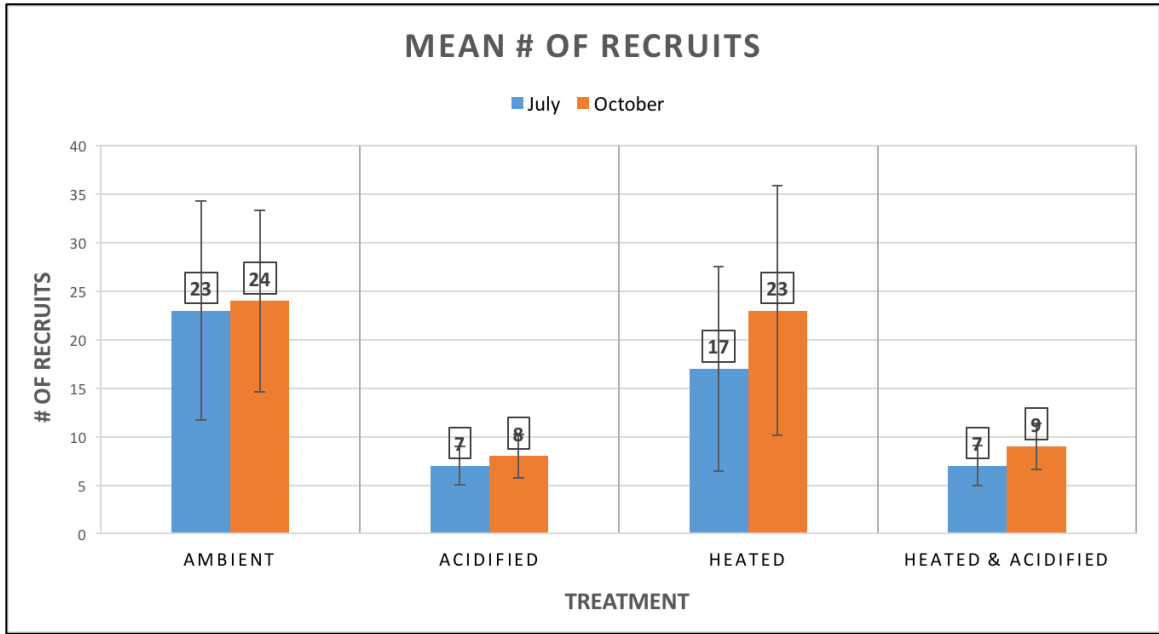


Figure 9: Mean number of recruits by treatment for July and October

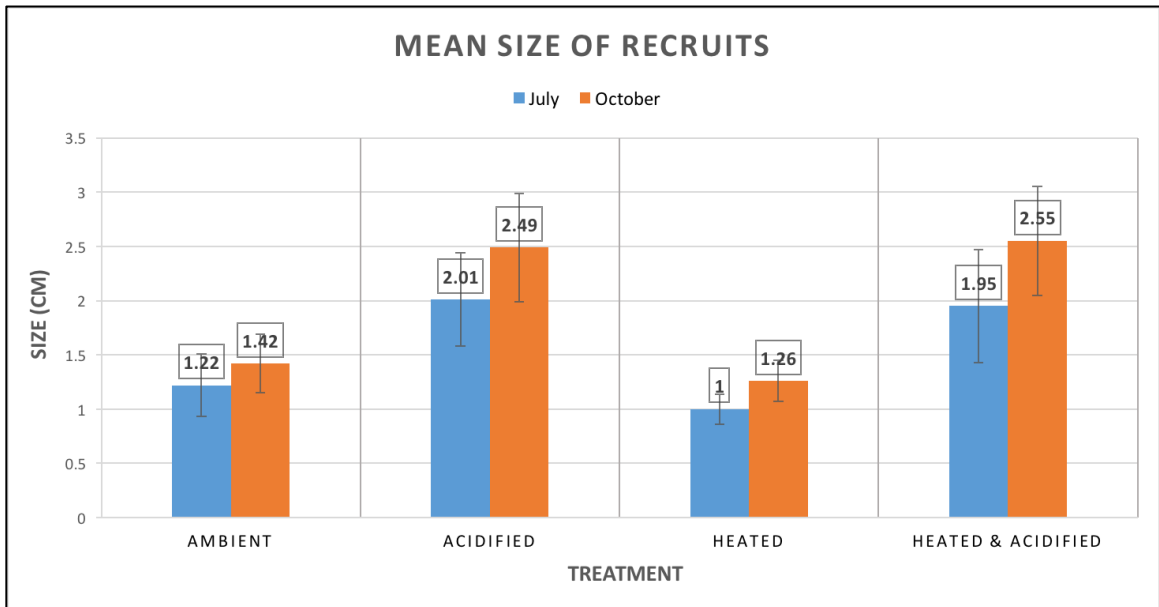


Figure 10: Mean size of recruits by treatment for July and October

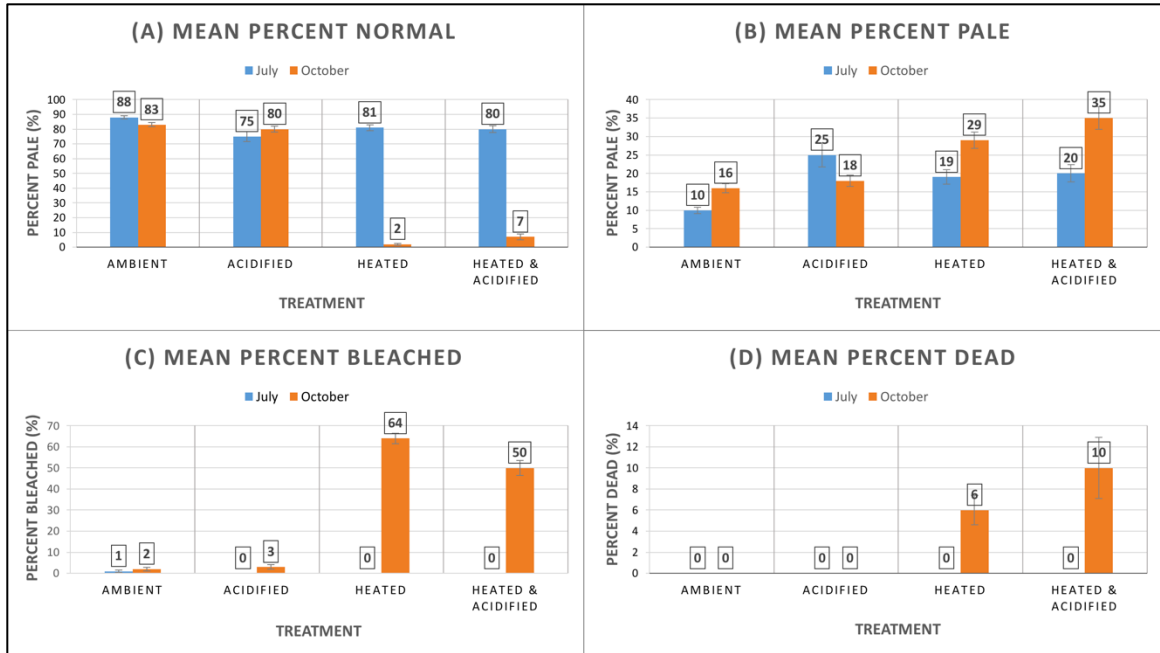


Figure 11: A) Mean percent normal by treatment, B) Mean percent pale by treatment, C) Mean percent bleached by treatment, D) Mean percent dead by treatment

3.2 Differences Across Treatments

The results of the Kruskal-Wallis test showed that there was no significant difference found for mean size and abundance across any of the treatments, but there was a significant difference in bleaching in the heated tanks when compared to the non-heated tanks (Table 1).

Treatment	-Treatment	p-value
Heated	Acidified	0.0013*
Heated	Ambient	0.0022*
Heated & Acidified	Acidified	0.0109*
Heated & Acidified	Ambient	0.0209*

Table 1: Results of Kruskal-Wallis test when bleaching was compared across treatments

3.2 Differences Within Treatments

The matched pair t-test analysis showed that there was no significant difference within treatments for mean abundance (Table 2) and mortality (Table 3). There was however, a significant increase in mean size in the treatments with acidification (Table 4), and a significant increase in mean percent bleached in the treatments with heating (Table 5).

Number of Recruits				
	Ambient	Acidified	Heated	Heated & Acidified
p-value	0.1957	0.3596	0.3556	0.3194
DF	7	8	5	5

Table 2: p-value and DF results for matched pair analysis of number of recruits

Mortality		
	Heated	Heated & Acidified
p-value	0.3632	0.184
DF	5	5

Table 3: p-value and DF results for matched pair analysis of mean mortality

Size of Recruits				
	Ambient	Acidified	Heated	Heated & Acidified
p-value	0.2044	0.0198*	0.3354	0.0055*
DF	7	8	5	5

Table 4: p-value and DF results for matched pair analysis of mean size of recruits (Bold* = statistical significance)

Bleaching				
	Ambient	Acidified	Heated	Heated & Acidified
p-value	0.2292	0.0828	0.0021*	0.0074*
DF	7	8	5	5

Table 5: p-value and DF results for matched pair analysis of mean bleaching (Bold* = statistical significance)

4.0 DISCUSSION

The significant increase in bleaching in both of the heated treatments was expected, as many mass bleaching events correlate with elevated sea surface temperatures (Jokiel, 2004). Field studies have indicated that generally, fast-growing species, such as *P. damicornis*, are more vulnerable to bleaching due to their higher respiration rate (Abdulmohsin et al., 2013).

The optimum range for planulation levels of *P. damicornis* from Hawai‘i is 26-27°C, and their reproductive ability is curtailed when temperature shifts as little as 1°C from that range (Jokiel, 1978). This could be a possible explanation as to why there were

more tanks with recruits in the ambient and acidified treatments, which both maintained temperatures within the optimum range during the July assessment (Figure 7), while the other two heated treatments were at temperatures greater than optimal.

Pocillopora damicornis in Hawai'i are able to produce planulae year round, but studies show that the majority of recruitment is restricted to the summer months, which coincides with when water temperatures start to exceed 26°C, the lower value of their optimal range (Jokiel, 1978). With a 1°C temperature change from the optimum, the successful reproduction of *P. damicornis* diminishes by up to one order of magnitude (Jokiel, 1978). On the basis of this, we would expect to have seen a difference in abundance across treatments, with the non-heated tanks having a greater abundance than the heated tanks. Instead, we saw no significance difference and this can be attributed to the wide variation in mean abundance among mesocosms of the same treatment, which meant that any differences in mean abundance could not be attributed to treatment.

We also expected to see a difference in mean size across treatments based on the proton flux hypothesis, since treatments with acidification should have a greater hydrogen ion concentration than that of treatments without acidification, hindering the calcification of recruits in acidified treatment tanks. However, the results showed quite the opposite pattern, with there being a significant increase in mean size only in the treatments with acidification. The most direct response of corals to reduced calcification is a decreased linear extension rate and skeletal density, but they may also exhibit alternate responses such as reducing skeletal density to maintain their growth rate, or investing greater energy in calcification to maintain both skeletal growth and density (Hoegh-Guldberg et al., 2007). Although these strategies could help maintain growth

rate, it would also be diverting resources from other essential processes such as reproduction. If the *P. damicornis* of this study are using one of these alternate strategies, it would not only explain the large mean size and increase in growth despite stress, but also account for why the two treatments with acidification both had a low number of recruits. This mechanism can not be confirmed by the results of this experiment, as measurement of size was only taken linearly, and skeletal density was not assessed.

Another possible explanation for the increase in mean size of treatments with acidification is that there is variability in sensitivity among corals, with some corals possessing the ability to maintain calcification rates under acidified conditions (Bahr et al., 2016). In a few cases of recent studies, calcification was seen to increase under moderately elevated pCO₂, and in studies that were conducted over weeks or months as opposed to under a day, sensitivity of calcification to acidification appeared to be reduced (Pandolfi et al., 2011). This same pattern could be what we're seeing in this experiment, as the acidified treatments were modeled to represent moderately elevated pCO₂, and the experiment was multi-annual.

It is interesting because this means that the recruits in the heated/acidified treatment, were shown to have a significant increase in mean size, but were also shown to have a significantly increase in mean bleaching. It seems as though the recruits in this treatment were able to still grow, despite them also bleaching. A recent study that tested the interactive effects of increased seawater temperature and pCO₂ saw that temperature may be able to mitigate the adverse effects of acidification on calcification of juvenile *P. damicornis*, but at the substantial cost of asexual budding (Jiang et al., 2017). Since there was no significant difference in the other measurements besides bleaching across

treatments, no other conclusions can be drawn as to how the effects differ when the stressors are combined as compared to when they are alone.

5.0 CONCLUSION

Some of the differences that we expected to see across treatments proved to not be significant as there was a lot of variation in the mean size and abundance of recruits amongst mesocosms of the same treatment. The results showed a significant increase in bleaching in both heated treatments which was expected, and a significant increase in mean size in both acidified treatments which was not expected and might suggest an acclamatory response but this mechanism can not be confirmed by this particular experiment. Since there was not a significant difference

At the end of the post-bleaching assessment, there was no mortality in the ambient and acidified treatments, and there was very low mortality in the heated and heated/acidified treatments (Figure 11D). This means that our results thus far are not necessarily conclusive, as the fate of the recruits is still unknown. There could be delayed effects and their condition could worsen, or now that temperatures are starting to climb back down from the annual maximum, they could also possibly start to recover. We had expected there to be higher mortality during the second assessment after the bleaching event, but since there was not, another assessment necessary to follow up on the recruits and assess their condition months after the bleaching event. The data from the third assessment should give us a bigger overall picture of what's happening to the recruits, and give us a better idea of how and to what extent these treatments are affecting the recruits.

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