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# Heat Stress During Larval Stages on Coral Survivorship for M. Capitata

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# Heat Stress During Larval States on Coral Survivorship for *M. Capitata*

A Thesis Presented

By

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# Heat Stress During Larval Stages on Coral Survivorship for *M. Capitata*

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November 23rd 2020

**Very little is known about how heat stress during larvae stages effect larvae survivorship, early coral recruit settlement, and later stage coral survivorship. We focused on determining how heat stress during larvae stages effected Montipora capitata survivorship over time. After thermally stressing larvae, we asked how many larvae survived the treatment, how the treatment affected settlement, how many larvae survived the heat treatment but did not settle, and later stage coral survivorship experienced residual effects from the heat stress treatment. We exposed coral larvae to ambient seawater temperatures at 30°C and heated seawater temperatures to 34°C for an hour and fifteen minutes. Our results indicate that heat stressed larvae settle with lower numbers of individual corals and aggregates at 24 hours post settlement. There was no significant difference between total number of recruits between temperature treatments and settlement time, nor on aggregate composition.**

## Introduction

While coral reefs spatially take up around 0.1% of the ocean, they hold enormous diversity and support almost 25% of global marine fisheries (Godoy, Toledo-Hernandez, 2018). Due to their immense biodiversity and abundances of life, they also generate income via tourism and fisheries. Over 400 million people rely on coral reef ecosystems as economic means. Reefs also act as coastal protection from wave damage, protecting many coastline cities that otherwise would not exist due to erosion. Reefs act as central parts of human daily lives, especially indigenous populations. Being able to subsistence fish and practice traditional cultures are closely linked to coral reef health.

Corals are also important indicators of general ocean health. They have been described by the analogy of canaries in a coal mine, meaning, if coral populations radically change then future marine populations will follow suit, and likely then humans as well. Because tropical coral reefs have evolved in conditions with a narrow thermal range, they are physiologically optimized to live in thermo-stable environments. So, when marine environmental conditions change, they are one of the first respondents.

Ocean warming causes mass coral bleaching and mortality events. Coral bleaching refers to how, when corals are exposed to around 1°C above summer maximum temperatures, they become stressed and naturally expel their endosymbionts, symbiotic zooxanthellae, that live in coral tissues and provide glycerol, amino acids, and glucose (Logan et al, 2014). Without being the *symbiodinium* synthesizing these proteins, fats, and carbohydrates, corals take on stark white appearances and appear "bleached" because of the loss of symbiotic activity (Fig 3). Bleaching reduces coral performance, and with the loss of their main nutrients source, bleaching can result in death due to malnutrition (Pandolfi et. al, 2011). Corals with higher thermotolerances will bleach at higher temperatures. Global increases in seawater temperatures and mass coral bleaching events are primary threats for coral reef health. The combination of human activity directly threatening 50-70% of reefs as well as predicted indirect human effects increasing bleaching events (Fig. 1) place corals as one of the most vulnerable marine ecosystems (Hoegh-Guldbeg, 1999).

While there have been many studies on bleaching process of already existing corals, there is still uncertainty of the effect of ocean warming on specific lifestyle stages of corals, specifically, larval stages. Current populations of larvae are developing in the same conditions that already existing corals are bleaching under.

This experiment addresses how current younger populations of coral are being affected by climate change and ocean warming. We determined how heat stress during larval stages affect coral larvae survivorship of *Montipora capitata* over time.

We analyzed coral development from embryonic stages to becoming coral recruits. Specifically targeting larval stages, we investigated how many larvae would survive a heat stress treatment, how the heat stress treatment would change settlement after 24 hours and 72 hours, how many larvae would survive the heat stress treatment but not settle after 24 and 72 hours, and what residual effects from the heat stress treatment, if any, were there over time on coral survivorship.

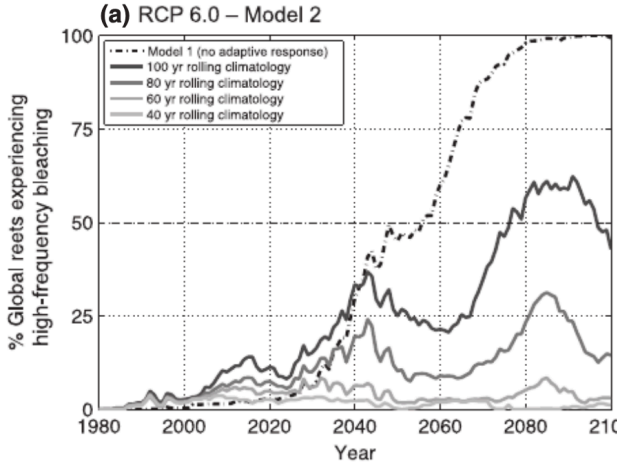


Fig 1. Percent of global reef cells predicted to experience high-frequency bleaching using a rolling climatological period, representing adaptive response model to recent thermal history over the previous 40, 60, 80, or 100 years and looking into the future. RCP 6.0 as rate of adaptation considering <4% global reef cells (Logan et. al, 2014).

## Methods

*a) Coral Gamete Collection*

On the night of a new moon during peak spawning, *Montipora capitata* gametes were collected from one site in Kāne'ohe Bay ( 21°26'56.4"N 157°47'45.5"W) on O'ahu Island, Hawai'i, on July 20th, 2020. Gamete bundle collection began around 9pm and lasted for about 15 minutes. Bundles were collected using Bundle Scoopers made with 153 mesh cloth. After scooping gametes directly out of the water and the mesh was coated with gametes, the gamete collection team rinsed the bundles from the scooper into 5 gallon buckets, and then transferred into falcon tubes (5mL bundles, 25mL seawater). This process was repeated until all falcon tubes were filled with seawater and desired amount of gametes for entire experiment (around 600mL were collected. A little over an hour after collection started and the gametes fertilized to become embryos, the washing and aliquoting process began.

*b) Embryo Washing and Aliquoting*

Sperm-contaminated water from falcon tubes were removed using a serological pipette. Using as many falcon tubes as necessary, 15mL of embryos were then transferred to 50mL beakers and then added to 15 L of water in rearing conicals. Embryos will remain on the surface levels of water. Conicals were drained to 1L to remove excess sperm-water and filled back up to full water capacity. This draining and washing processes was repeated two times to reach desired water quality. Larvae rearing procedures began once embryos were added to conicals.

*c) Larval Rearing*

Larval rearing occurred from 11pm the night of spawning, July 20, 2020, through July 24, 2020. Conicals were cleaned by removing dead embryos and biofilm at the water surface, making sure embryos/larvae did not clump, and encouraging embryos away from conical walls and conical filters (embryos more likely to die if not suspended in water). Water turnover levels were increased 24 hours post fertilization to 2L/hour, and 3L/hour 48 hours post fertilization. To have high larvae survivorship, conicals and suspended embryos/larvae must be rigorously cared for. Conical cleaning processes were done every 45min-1hour for the entire larval rearing stages. Conicals were filled with seawater from Kāne'ohe Bay with average temperatures of 27 °C throughout larvae rearing stages (07/20/2020-07/24/2020).

*d) Pre and Post Treatment Larval Counting*

For pre-treatment larvae counting, larvae were transferred from conicals into 1L beakers by draining larvae from conicals into a siv placed over a bowl over a 5-gallon bucket. For post-treatment larvae counting, larvae were transferred from treatment jars into 1L beakers by pouring larvae from jars into buckets, and then into a siv placed over a bowl over another bucket. For both pre- and post-treatment larvae counting, after filling a 1L beaker with 500mL of 1 seawater, larvae were gently rinsed from the siv using a squeezy bottle filled with 1 seawater. Beaker volumes were then standardized to ~1000mL adding 1 seawater as necessary.

Larvae were gently homogenized in a 1L beaker and pipetted in 1mL samples into 6-well or raceway counting trays. 12-16 counts were done per conical using a counting clicker. After counts were completed, the larvae were gently poured into 5 gallon buckets.

Pooled larvae counts to measure quality of larvae were conducted once all larvae from conicals were homogenized in buckets. 1 mL samples were taken out of the combined conical larvae buckets and counted under a dissecting microscope for the proportion of "good" to "bad" larvae. "Good" larvae were intact larvae with smooth, elongated shapes. Larvae deemed "bad" had round and spherical shapes, or if they appeared irregular or ruptured.

*e) Temperature Treatments*

Larvae were added to jars according to "good" larvae counts, assuming ~75% death rate for the heat treatment larvae. Larvae-filled jars were added to water baths, with two jars per treatment containing water and HOBO temperature data loggers. Heat treatment water baths were heated to 34°C and control treatment water bathes stayed at ambient seawater temperatures at 30°C. Jars sat in respective treatments for 75 minutes on the fifth day of larvae rearing, July 24, 2020.

*f) Larval Settlement*

Larvae were transferred from buckets to settlement chambers by filling 1L beakers with filtered seawater and larvae and serological pipetting larvae into small graduated cylinders. The graduated cylinders were then transferred to settlement chambers. Volumes of larvae added to settlement chambers depend on concentration of larvae in buckets and desired concentration of larvae per settlement chamber. Each plug was labeled and recorded in plug maps per settlement chamber and temperature treatment.

*g) Post Settlement Counting*

After respective settlement time, plugs were pulled from settlement chambers and gently shaken inside chambers to remove unsettled larvae from plug surfaces. Heat and control treatment plugs were then randomly placed on racks in coral tanks, keeping track of settlement time. Unsettled larvae were removed from settlement chambers using a squeeze bottle to transfer larvae into 250mL beakers using the least amount of water possible. 1mL of larvae and seawater samples were pipetted into counting trays and counted for "good" and "bad" larvae intactness under dissecting microscopes using tally counters.

*h) Orthomosaics*

Plugs were photographed one day after they were pulled from settlement chambers. 24 hour treatment plugs were photographed at 36 hours post settlement, and 72 hour plugs were photographed at 96 hours post settlement. Plug maps were created before photos were taken. Photos were taken with DSLR Canon, starting at the bottom left corner and capturing photos moving right horizontally. Each photo was moved one column at a time, and at the end of a column, the camera moved up a row to continue taking photos moving to horizontally left. Edges of the rack and cattle tags (treatment labels) were included in photos. Using Teamviewer, photos were uploaded to create a singular orthomosaic for each time treatment.

*I) Recruit Counting*

Coral recruits were counted on Preview or ImageJ using orthomosaics photos and plug maps to match the corresponding time and temperature treatment of the plugs. Three counts were performed on each plug: the number of alive individual coral recruits, the number of alive aggregates (two or more recruits touching), and the total number of coral recruits per plug.

Only clear and obvious recruits were counted, and defined as round, mounding, brown in color, distinct from plug background, with a visible corallite structure. High resolution photos are able to distinguish a coral mouth at the center of the recruit, visible as a small white dot. Individuals were circled in one color, aggregates were circled in another color, and each individual coral was marked with a small dot or dash.

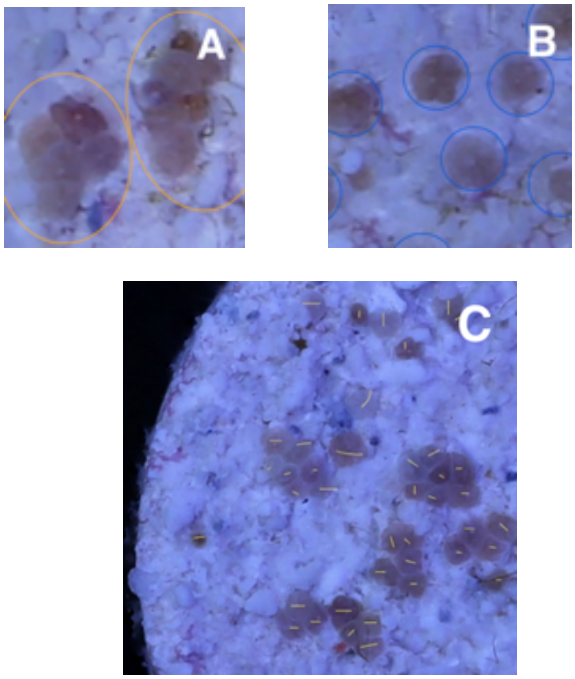


Figure 2. Figure 2A: two clear aggregates, Figure 1B: numerous clear individuals, Figure 2C: total recruit count, each dash as one coral

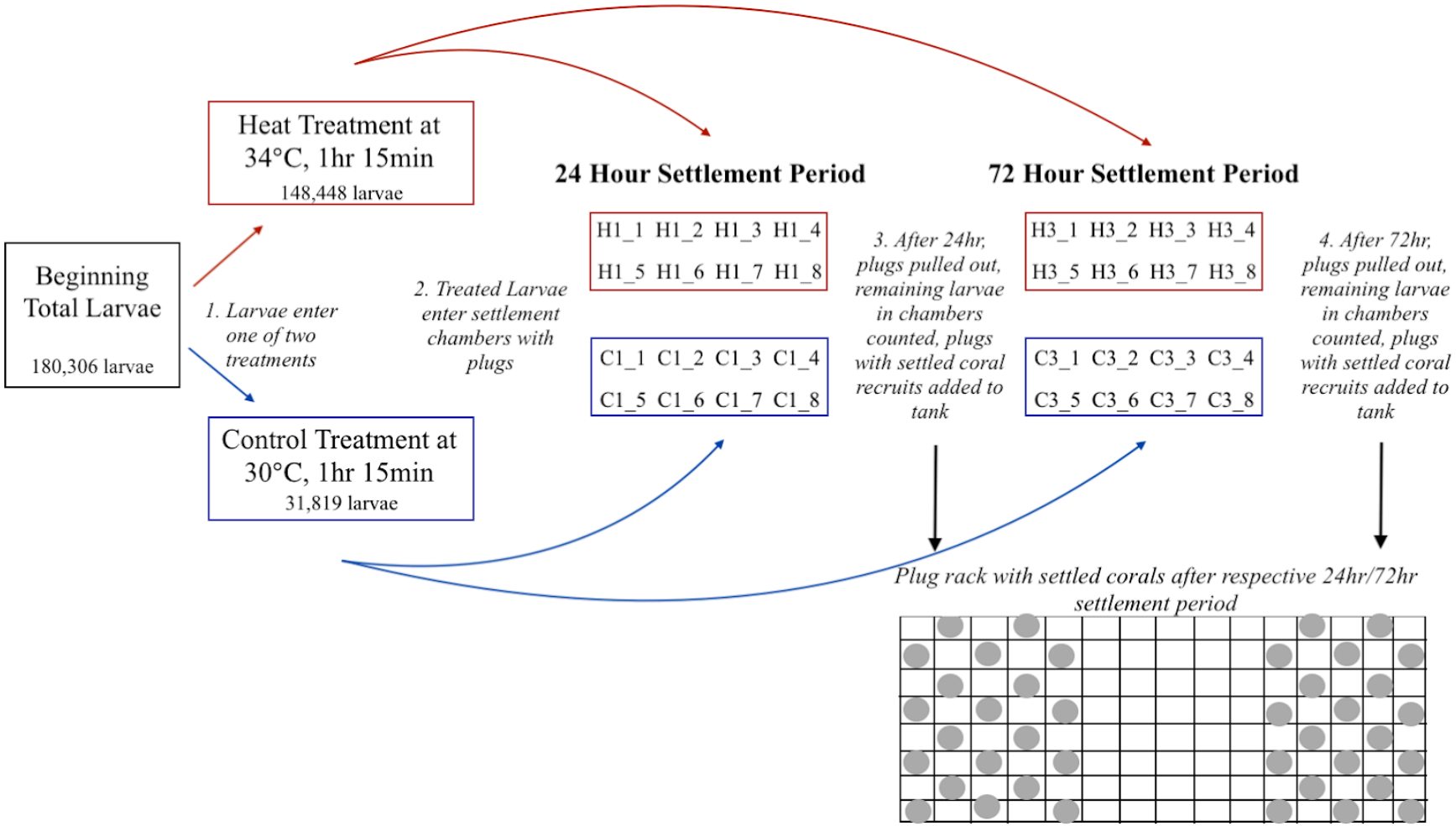


Figure 3. Experimental steps chronologically from left to right. H1_#=Heat Treatment, 24hr, H3_#=Heat Treatment, 72hr, C1_#=Control Treatment, 24hr, C3_#=Control treatment, 72hr

## Results

There were 56 jars of larvae in the heat treatment, and 12 jars of larvae for the control treatment, filled with 2,652 "good" larvae. All larvae used for both treatments came from the same bucket of homogenized larvae & seawater and had 85.6% "good" larvae. 148,488 larvae entered the heat treatment and 122,867 larvae came out of the heat treatment. The thermally stressed larvae counts found 83.6% "good" larvae. 31,819 larvae entered the control treatment and 37,300 larvae were counted after being through the ambient seawater temperature treatment. There was a 68% survivorship for the heat-treated larvae. 35,621 larvae died in the heat treatment (68.44% survivorship) and there was an increase in 5,481 larvae for the control treatment (117.23% survivorship). There were 180,306 total larvae before treatment began, and 150,167 total larvae after treatments.

Larvae intactness counts before and after heat treatment did not indicate a significant change in larvae quality and proportion of "good" larvae to "bad" larvae (P=0.5).

There was an average 85.6% "good" larvae pre-treatment, and an average 83.6% proportion of "good" to "bad" larvae for the post heat-treated larvae, but a two-tailed T test gave a P-value of 0.4, indicating an insignificant change between larvae quality before and after treatments.

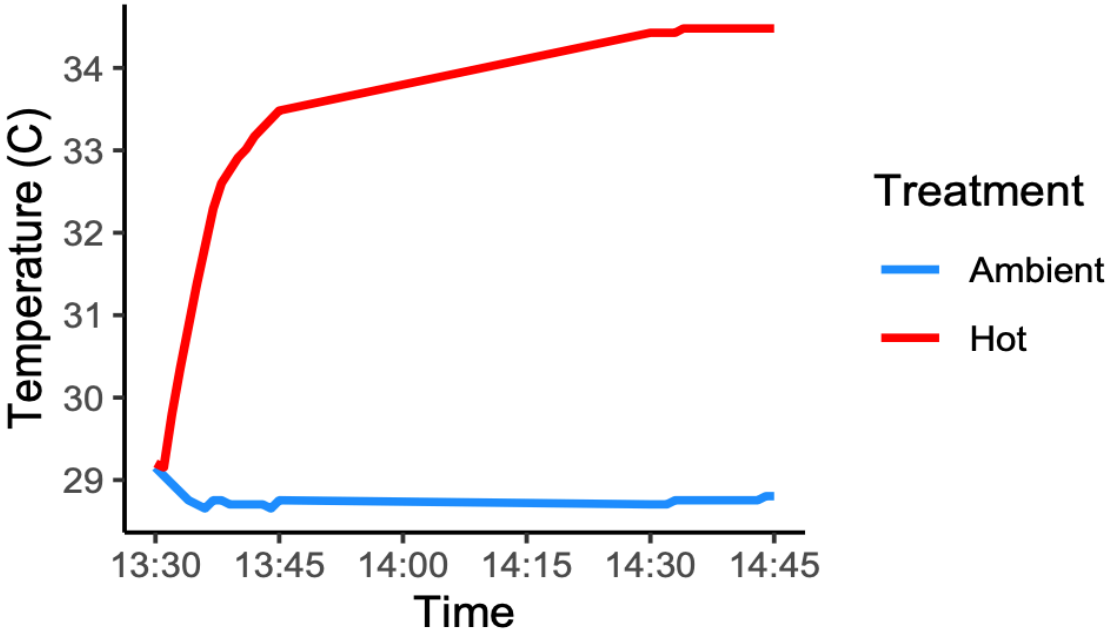


Figure 4. HOBO logger temperature of control and heat treatment. Start treatment=13:30, end treatment=14:45 on 07/24/2020.

All 8 settlement chambers per treatment had four plugs. The control chambers received 11mL of larvae with a concentration of 37.3 larvae/1mL, resulting in 410 larvae per control settlement chamber. The heat treatment settlement chambers received 13mL of larvae with a concentration of 31.4 larvae/1mL, resulting in 408 larvae per control settlement chamber. R was used to create one-way analysis of variance (ANOVA ) statistics for all recruitment counts (Fig 6-10).

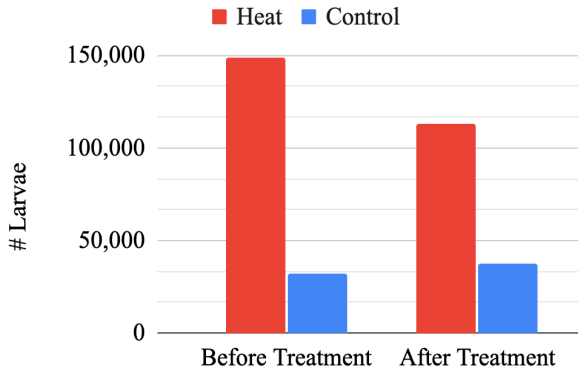


Figure 5. Number of larvae before and after treatments. Control Treatment= 30°C, Heat Treatment= 34°C, Treatment time= 1hr 15 min

| | Avg. Total Larvae Pre-Settlement | Avg. Total Larvae Post-Settlement |
|---|---|---|
| Control Treatment, 24hr Settlement | 3200 | 2459 |
| Heat Treatment, 24hr Settlement | 3200 | 1325 |
| Control Treatment, 72hr Settlement | 3200 | 1558 |
| Heat Treatment, 72hr Settlement | 3200 | 642 |

Table 1. Number of non-settled larvae remaining in settlement chambers after 24 and 72 hour settlement periods.

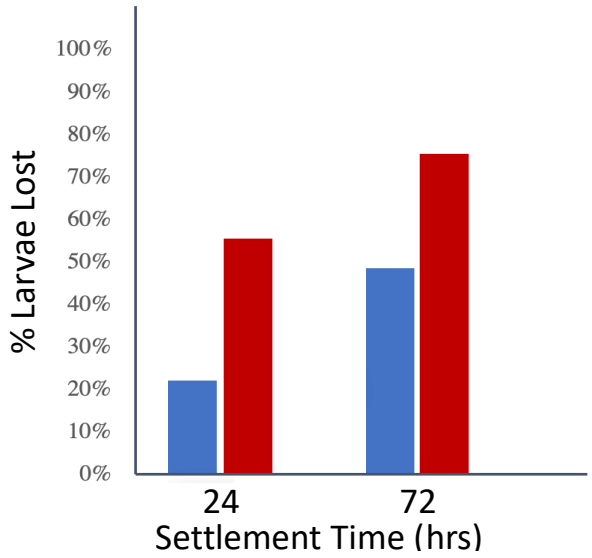


Figure 6. Percent of larvae lost during settlement periods. See Tab. 1.

Based off of the percent larvae lost values (Tab 1), we expected control 72hr treatments to have the most coral recruits, followed by control 24hr treatments, followed by 72hr heat treatments, followed by 24hr heat treatments. For total good larvae, Control 24hr and Heat 24hr had P=<0.001 Control 72hr and Heat 72hr had P=<0.001, Heat 24hr and Heat 72hr had P=<0.001, Control 24hr and Control 72hr had P<0.001 (Fig 5).

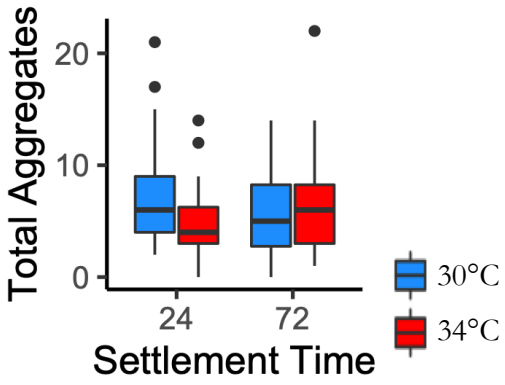


Figure 7. Avg. total aggregates per plug between heat treatment and settlement time period. Between settlement time treatments P=0.468, between heat treatments P=0.156, between treatment and settlement time P=0.146

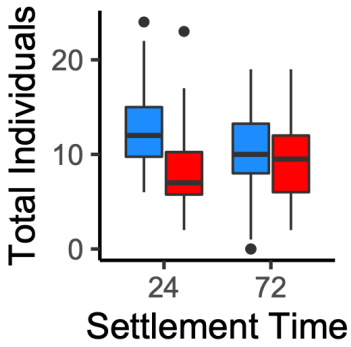


Figure 8. Avg. total individuals per plug for each heat treatment and settlement time period. Between heat treatments P<0.001, between settlement time P=0.887, between treatment and settlement time P=0.144.

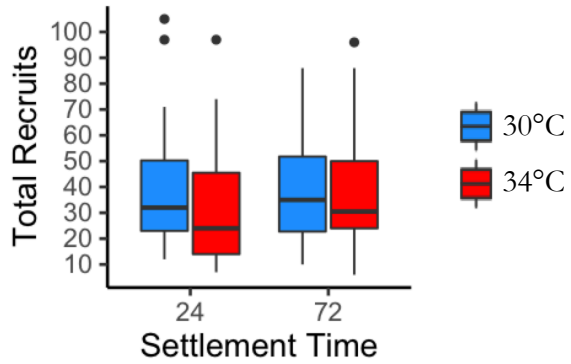


Figure 9. Total number of coral recruits per plug for each treatment and settlement time period Between settlement time treatments P=0.516, between heat treatments P=0.27, between treatment and settlement time P=0.348.

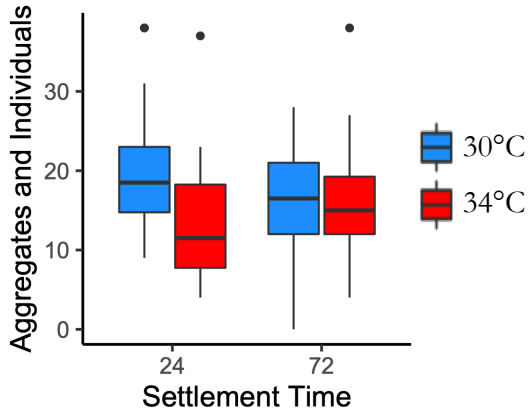


Figure 10. Avg. total number of both aggregates and individuals per plug, between heat treatments P=0.0013, between settlement time treatments P=0.616, between treatment and settlement time P=0.084.

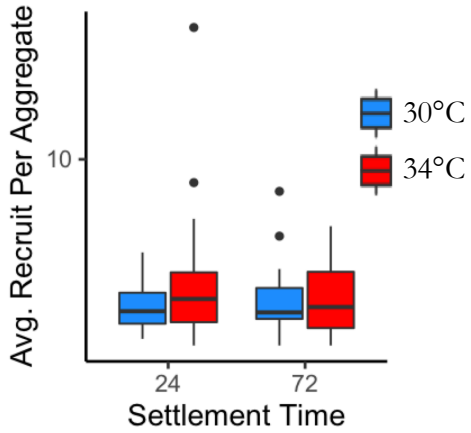


Figure 11. Avg. number of coral recruits within an aggregate. Between settlement time treatments P=0.500, between heat treatments P=0.131, between treatment and settlement time P=0.222.

## Discussion

There was a larger difference in larvae before and after settlement periods for heat-treated larvae (Fig 6), and a higher percentage of larvae missing from the post-settlement larvae counts from the heat-treated larvae. There might be a few explanations for this. Firstly, perhaps lack of larvae post-settlement is an indicator of higher settlement. Meaning, the larvae would not be included in the post-settlement larvae counts because the larvae became a settled recruit and its presence will be represented in the recruit counts. A second explanation could be that the larvae disintegrated and therefore was not included in the count. Thirdly, the larvae could have somehow leaked out through potential cracks in settlement chambers and overflowed the intended closed system. Or finally, the larvae could have stuck to the sides of the settlement chamber and not made it into the count.

An interesting analysis for the future would be to cross reference the recruit dataset for each settlement chamber, along with the post-settlement larvae counts. This could help uncover the uncertainty in the reasoning behind why the larvae were not in the post-settlement count. Considering that heat-treated larvae generally had lower mean values of aggregates, individuals, total recruits, and aggregates and individuals (Fig 7, Fig 8, Fig 9, Fig 10) it is unlikely that the lack of heat treated larvae represented in post-settlement counts is due to settlement.

Despite the heat treatment killing 32% of larvae, coral recruitment results are generally inconclusive. The only two significant relationships we found from recruitment data was between heat treatment and amount of settled individuals (Fig. 7, $P<0.001$), and the amount of individuals and aggregates between heat treatments (Fig. 9, $P=0.0013$). However, despite statistics indicating insignificances, mean values tentatively suggest that control temperature treatments have a stronger likelihood to have higher numbers of settled corals (Fig 7, Fig 8, Fig 9, Fig 10). There was no significant relationship between settlement time periods for any specific analysis, indicating that the extra two days after the 24 hour time point did not increase coral recruit settlement for the 72 hour settlement time period. Our results also illustrate that the composition of aggregations

between treatments did not change between heat treatments (Fig 11). Despite the coral having higher numbers of individuals and aggregates for control-treatments, the aggregation composition did not follow the same pattern.

There is an overarching question behind how the heat stress treatment affected the surviving 68% of larvae's ability to settle. Because the proportions of "good" and "bad" larvae did not largely differ before and after heat treatments, it is unclear as to if there was a real reasoning behind why the 68% larvae survived and the 32% larave died. Perhaps our results were generally inconclusive because the heat treatment killed the larvae that would not be able to settle, and therefore the remaining 68% heat treated larvae and the total control population had similar settlement abilities. However, the total individuals and total individuals and aggregates (Fig 8, Fig 10) statistics show that control larvae settled with higher numbers, so that contradicts previous reasonings of equal settlement ability.

Future studies might consider a different length or duration of heat stress treatment. An hour and fifteen minutes at four degrees higher than ambient water conditions does have a significant effect on population of larvae, but is more extreme than normal bleaching conditions (Logan et. al, 2014). Considering a larger sample size greater than 12,800 larvae might increase strength of results and therefore find significances where this study did not despite suggested trends. Finally, continuing to track coral survivorship later than 72 hours post settlement will be valuable to see if there are significant more longer-term effects of larval heat stress on coral survivorship.

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