**MasterNaut Project Research Track**
**Square Coral Tiles vs. Round Coral Tiles: Testing Larval Settlement Counts of *Montastraea cavernosa***

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**October 2025**

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**Abstract:**

The focus of this experiment was to determine whether square or circular tiles are more effective in coral larvae settlement of *Montastraea cavernosa* larvae in a lab setting. The average number of larvae that settled on each tile and the T-test value of all the data was calculated. Based on the T-test values from 3 different counts, the data from the initial count, bin 3, and bin 4 were the most statistically significant data collected. The statistically significant data helped form the conclusion that circle tiles are better for larvae settlement.

**Introduction:**

Coral reefs support a quarter of all marine biodiversity despite making up only 1 percent of all ocean habitat and are therefore an important ocean habitat. Unfortunately, coral cover on tropical reefs has declined during the last three decades due to the combined effects of climate change, destructive fishing, pollution, and land use change (Humanes, Adriana, et al.). The fact that coral reef populations are declining at an alarming rate, caused an interest in helping coral restoration groups to successfully grow coral and combat coral loss.

The importance of larval propagation is becoming increasingly recognized as a tool for coral population enhancement and for combatting coral loss (Randall et al., 2020). However, there are still major gaps in our understanding of the technical and methodological constraints to producing corals for such restoration interventions (Humanes, Adriana, et al.). Looking into other coral restoration groups, it became clear that there is still a lot that is unknown about coral settlement, including what settlement tile is more effective for coral settlement. It is imperative to standardize the most effective larval settlement systems to produce coral that will replace the dwindling population in reefs, and produce coral with better genetics that are more resilient in the environment.

With the goal of better understanding and supporting coral restoration, we focused on the efficacy of different shapes of settlement tiles within a lab setting. We focused on the differences in counts of settled *Montastraea cavernosa larvae* between circular, aka "circle", tiles compared to square tiles.

*Montastraea cavernosa* (MCAV) is an important species of hard coral found throughout the Florida Keys reef system. Monstracaea *cavernosa* is dioecious, with a prolonged oogenic cycle that begins in November, a briefer spermatogenic cycle that begins in May, and spawning events during July, August and/or September about 1 week after the full moon (Szmant, Alina M. (1991)).

This experiment focused on the processes surrounding natural spawning events in summer months. While spawning, the coral releases its eggs in sperm sacks only once a year to mix together with the current, and cross inseminate each other. At the surface, the eggs mix together and fertilize each other. A few hours after fertilization, coral eggs become swimming larvae and settle onto a surface near the ocean floor onto substrates of varying size, shape, and rugosity. From previous work, coral larvae have shown a preference to services with higher rugosity than those of smoother surfaces, likely due to the ability of the larval to increase its "grip" on that substrata (Dustan P, Doherty O, Pardede S (2013)).

A brief microscopic observation before the collection of data showed that the circle tiles had a higher rugosity. The circle tiles also had greater surface area and no edges compared to the square tiles. For these reasons, we hypothesized that coral larvae could attach to the circle tiles more easily than on the square tiles.

**Methodology:**

To mimic the ocean in our lab setting, the tiles and coral spawn were tested in a 21cm x 30cm x 14cm plastic bin. The experiment tested circle and square tiles across four different bins. Each bin contained 7 circle tiles and 26 square tiles. In total, 57,000 MCAV coral larvae were released to settle onto the tiles. To measure the effectiveness of settlement onto the tiles, three different counts were performed: the initial count on November 17, 2024, the middle count

15 weeks after, performed by staff at the Reef Institute when they were fragmenting the tiles and transferring the larvae onto plugs, and the final count 2 weeks after the middle count.

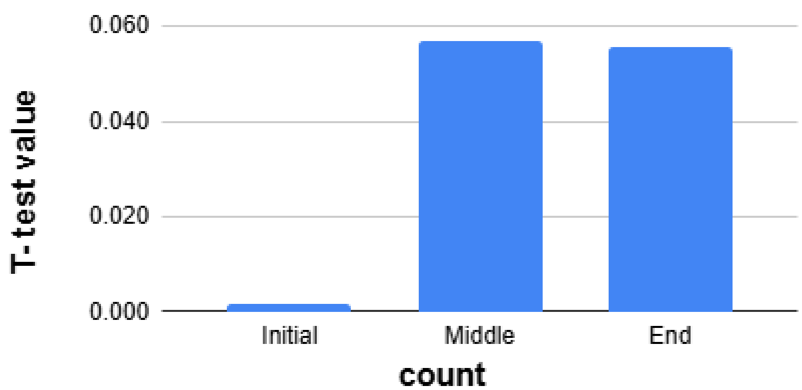
For the initial and middle counts, four circles and four squares from each bin were chosen randomly and analyzed. For the final count, the plugs, made from the tiles used for the middle count, were analyzed. In total, 32 tiles were analyzed during each count. Each tile was analyzed in a zig-zag pattern under a microscope.

While analyzing each tile under the microscope, the number of larvae was tallied with a hand-held clicker. At the end of each tile's analysis, the total was recorded on the data sheet corresponding to tile shape, number, and bin number. Datasheets can be found in the appendix. After all the tiles were analyzed, the data from each count was used to calculate the average number of larvae on each tile shape for all the bins combined, the average number of larvae that settled onto each shape for each of the four bins, and T-test values for the averages of each count and of each bin to see which tile shape is more effective.

**Results:**

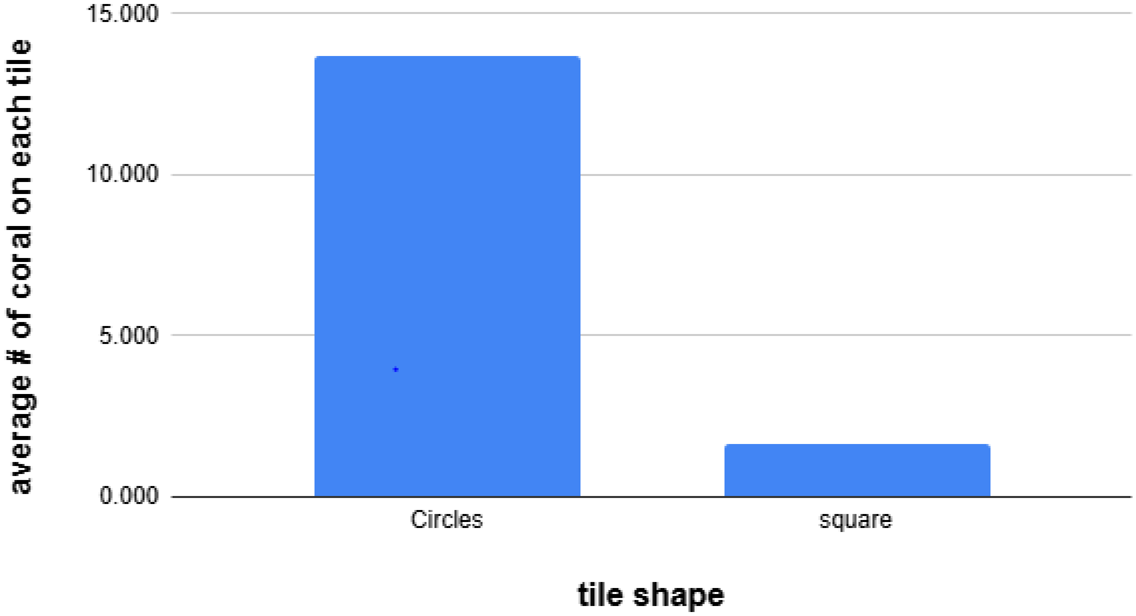
To test whether any of our tile counts were statistically significant data, a T-test was performed on each count which is shown in Figure 1. In order for the data to be statistically significant, it has to have a T-test value of 0.05 or less. Results indicated that the initial count had a T-test value of 0.002, the middle count had a value of 0.057, and the end count had a value of 0.056. That means only the initial count had statistically significant data. For this reason, only so the data from the initial count was considered in subsequent figures.

Figure 1: T-test values of each count



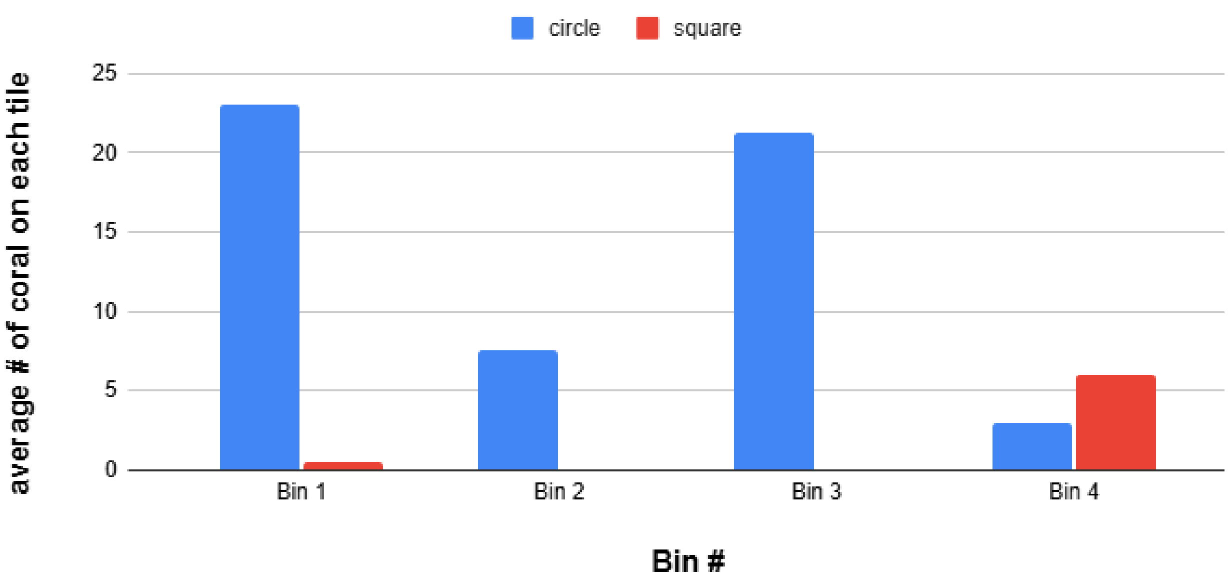

The second thing looked at was the average amount of larvae on each tile shape in all the bins combined, which is shown in Figure 2. Out of all the bins combined, the average amount of larvae settled on each circle tile was 13.688, and the average amount of larvae settled on each square tile was 1.625. Not shown in the figure, but also calculated, was the standard deviation for the averages, which showed that there is a standard deviation of 12.515 for circle tiles, and 2.754 for squares.

Figure 2: average number of larvae on each shape during the initial count




The last thing that was looked at is what was the average number of larvae settled on each tile shape in each bin,shown in Figure 3. There was a higher average settlement on the circle tiles in bins 1,2, and 3, while there was a higher average settlement on the square tiles in bin 4. In order to see how statistically significant the data in Figure 3 is, the T-test for the data for each bin was calculated and it showed that bin 1 had a T-test value of 0.069, bin 2 had a value of 0.60, bin 3 had a value of 0.023, and bin 4 had a value of 0.043.

Table 3: Average for each shape in each bin during the initial count



**Discussion:**

Based on the statistically significant data that was collected in the experiment, it is shown that the hypothesis that circle tiles will do better for larvae settlement is well supported. It was noted that the majority of the larvae on the circle tiles were settled in all of the little cracks on the surface. The fact that the circle tiles had a higher rugosity due to all the little cracks on the surface and that they had a greater surface area we expect is the main driver resulting in the most of the statistically significant data to show that the circle tiles are better for larvae settlement.

Now one thing to consider is that there are several factors that influence the growth of larvae in a coral raceway and that just one of the many factors were tested. While at the Reef Institute, it was observed things like where the coral is in the raceway and the water chemistry also play a huge role in larvae growth. For example, they put the food in at one end of the water. That means it is possible larvae on that end got more food than those on the other end of the raceway bin.

Recommendations for further studying larval settlement, the different variables that play a role in coral settlement like water chemistry, lighting, food, and the way food is distributed to the coral should be tested or controlled. It would also be worthwhile to more closely test or control for the texture and material of the settlement tile itself. By testing out more of the different factors that play a role in coral settlement, a standard of the perfect tile and growing conditions for settling and growing coral larvae can be created.

Collecting all of this data will help create a standard of the most effective larval settlement system that all coral restoration groups can follow. With this increased knowledge contributing to standardizing the most effective larval settlement system, coral restoration groups can more effectively grow coral larvae and produce coral with better genetics that are more resilient in the environment. Being able to better produce coral will then help them replace the dwindling population in reefs a lot quicker.

**Acknowledgements:**

I would like to acknowledge Mrs. Katie Cooper from SCUBAnauts International, Mr. Kris Scoggin from SCUBAnauts International, and the employees at the Reef Institute for helping me out every step of the way in creating and performing my experiment. They have helped me in many ways like advising my experiment, helping me collect data, analyze the data, and help me out on this scientific paper. If it wasn't for their help, I would not be where I am right now, and so I would like to thank them for everything they have done to help me out.

**References:**

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## Appendix: Raw Data

| # Bin # | Tile # | shape | initial count | middle (staff) count | End count |
|---|---|---|---|---|---|
| 1 | 1 | C | 27 | 3 | 1 |
| 1 | 2 | C | 38 | 0 | 0 |
| 1 | 3 | C | 27 | 0 | 0 |
| 1 | 4 | C | 0 | 0 | 0 |
| 1 | 1 | S | 1 | 0 | 0 |
| 1 | 2 | S | 1 | 0 | 0 |
| 1 | 3 | S | 0 | 0 | 0 |
| 1 | 4 | S | 0 | 7 | 6 |
| 2 | 1 | C | 10 | 28 | 33 |
| 2 | 2 | C | 11 | 3 | 3 |
| 2 | 3 | C | 0 | 9 | 10 |
| 2 | 4 | C | 9 | 9 | 11 |
| 2 | 1 | S | 0 | 3 | 3 |
| 2 | 2 | S | 0 | 3 | 1 |
| 2 | 3 | S | 0 | 1 | 3 |
| 2 | 4 | S | 0 | 0 | 0 |

| # Bin # | Tile # | shape | initial count | middle (staff) count | End count |
|---|---|---|---|---|---|
| 3 | 1 | C | 22 | 22 | 35 |
| 3 | 2 | C | 34 | 13 | 19 |
| 3 | 3 | C | 10 | 50 | 68 |
| 3 | 4 | C | 19 | 0 | 0 |
| 3 | 1 | S | 0 | 1 | 1 |
| 3 | 2 | S | 0 | 4 | 4 |
| 3 | 3 | S | 0 | 0 | 0 |
| 3 | 4 | S | 0 | 1 | 1 |
| 4 | 1 | C | 3 | 0 | 0 |
| 4 | 2 | C | 2 | 0 | 0 |
| 4 | 3 | C | 2 | 1 | 2 |
| 4 | 4 | C | 5 | 0 | 0 |
| 4 | 1 | S | 4 | 0 | 0 |
| 4 | 2 | S | 8 | 0 | 0 |
| 4 | 3 | S | 5 | 0 | 0 |
| 4 | 4 | S | 7 | 2 | 4 |