# COMPARISON OF REPRODUCTIVE SUCCESS IN *TRIPNEUSTES GRATILLA* AROUND O‘AHU

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

THESIS ADVISOR

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Dr. Malia Rivera
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For my father, who showed me the bay. There is no one I would have rather met the ocean with.

## ACKNOWLEDGEMENTS

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for every practice talk you have listened to me give, for every conversation both big and small, all of your forms of kākoʻo have allowed me to reach this point. Mahalo nui iā ʻoukou.

ABSTRACT

Sea urchins are often regarded as an indicator species, meaning that their fertility and reproductive success can be used as a metric for water quality. The Environmental Protection Agency utilizes this in a protocol comparing water quality samples using the fertilization rate of urchin gametes. A previous study conducted at Moku o Loʻe sought to use this protocol to compare water quality of sites within Kāneʻohe Bay using hāwaʻe maoli (*Tripneustes gratilla*/Collector Urchin). However, that study revealed the potential for broader comparisons of fertilization rate of Collector Urchins from different areas of Oʻahu- especially between fertilization crosses of urchins from two different locations. In this study, four locations: Kāneʻohe Bay Patch Reefs, Kāneʻohe Bay Sampan Channel, Kahe Point and Kahanamoku Beach were compared. Fertilization rates were assessed from crosses of urchins among the same location, as well as fertilization crosses of urchins between locations. There were no significant differences between fertilization rates when comparing females of different sites, as such, fertilization results were compared by location of male urchins. After combining fertilization results from males within the same location and comparing them to females both within and among locations, resultant fertilization rates were as follows: Kāneʻohe Bay Patch Reefs - 56.6%, Kāneʻohe Bay Sampan Channel- 75.2%, Kahe Point- 83.5% and Kahanamoku Beach- 72.0%. At 56.6%, urchins from Kāneʻohe Bay Patch Reefs had a significantly lower fertilization rate than any other location. This location is also the site of significant Collector Urchin out planting efforts by the Department of Aquatic Resources (DAR) for biocontrol of invasive algae, and thus may have reduced fecundity resulting from captive

rearing. Further research is needed to determine why male urchins from Kāneʻohe Bay Patch Reefs consistently yield lower rates of fertilization.

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1.0 INTRODUCTION

## 1.1 *T. gratilla* Ecology

*Tripneustes gratilla*, otherwise known as the Collector Urchin, is a small, round short spined sea urchin distributed widely across the Indo-Pacific and Red Sea (Ogden et al., 1989). These urchins are native to Hawaiʻi, and can also be found in Eastern, Africa, Indonesia, Southern Japan and more within these regions (Toha et al., 2017). As adults, Collector Urchins can range in size from 10 to 15 centimeters and are commonly found in a variety of different substrates such as seagrass, algae beds, sandy reef, and rubble (Toha et al., 2017). These urchins are most well known for their unique and rather odd camouflage techniques, in which they use their tube feet to carry items such as coral rubble, shells and algae (Ogden et al., 1989). Generally, *T. gratilla* are found in shallower depths, with an average range of about 2-30 meters, but can be found in water as deep as 75 meters (Toha et al., 2017). Given the wide regional distribution in which Collector Urchins can be found, the temperature range at which can be tolerated may vary regionally. In Hawaiʻi, their average temperature range is about 24-28 °C (Ogden et al., 1989). In other locations across the Pacific, and certain areas of Indonesia, these urchins have been recorded living in temperature ranges as high as about 29-31 °C (Toha et al., 2017).

*Figure 1: T.gratilla* urchin on Kāneʻohe Bay reef. These urchins hold rocks or algae on the aboral surface as seen here, which is how they get their "Collector Urchin" name. Photo credit: Sam Koeck

Like many other species of urchin, *T. gratilla* are herbivorous and most of their diet is comprised of different types of algae (Toha et al., 2017; Stimson et al., 2007). The specific species of algae consumed is largely dependent on where the population lives, as there are a wide variety of algae species consumed by the urchins (Toha et al., 2017; Seymour et al., 2013; Stimson et al., 2007). In Hawaiʻi, *T. gratilla* are relatively opportunistic in food selection and generally willing to consume most algal species in its vicinity (Westbrook et al., 2015). According to a feeding study in Kāneʻohe Bay, Collector Urchins were willing to eat all species presented, both native and non-native: *Gracilaria salicornia, Acanthophora spicifera, Eucheuma denticulatum,* and *Kappaphycus alvarezii* (Westbrook et al., 2015; Stimson et al., 2007). However, growth rate varied with diet, as *G. Salicornia* and *K.alvarezii* resulted in higher growth rates than *E. denticulatum,* which was likely the result of varying nutritional content in the different alga (Westbrook et al., 2015).

## 1.2 Importance of *Tripneustes gratilla*

*Tripneustes gratilla* are ecologically and culturally important in Hawaiʻi, as they serve a crucial role as grazers that control the balance of algae on the reef environment[4]. This is critically important as an abundance of algae can easily smother and overtake corals, which are critical to a healthy nearshore environment (Westbrook et al., 2015). In ʻŌlelo Hawaiʻi, these urchins are also known as hāwaʻe maoli, which means "native urchin of little substance". While these urchins were historically used as a food source, their gonads were rather small and provided little to eat. However, they were known to be quite tasty and regarded as a delicacy. This is affirmed in an ʻōlelo noʻeau which states " ʻO ka iki hāwaʻe ihola nō ia o Miloliʻi" (Pukui, 1983). Directly translated, this means "here is the little urchin of Miloliʻi", but was used as a boast, to mean being small but potent (Pukui, 1986).

Furthermore, in several locations in Hawaiʻi, specifically on Oʻahu, *Tripneustes gratilla* are being used as biological control agents to assist with the removal of the invasive algae Gorilla Ogo (*Gracilaria salicornia*). These urchins can graze up to approximately 7.5 grams of alien algae daily if given a mixed diet, -making them potentially highly effective removal tools (Westbrook et al., 2015). This project was developed in 2011 with the Hawaii Division of Aquatic Resources (DAR) and was created in partnership with strategic removal of large amounts of invasive algae from the reef surface (Dennison, 2020). Once most of the invasive algae from a specific target area was removed, juvenile Collector Urchins about 20-25 mm in diameter are released onto the reef to consume the remaining invasive algae and maintain the clean area (Dennison, 2020). In recent years, manual removal of invasive algae has stopped, but out planting of Collector Urchins to control invasive algae has continued.

The Ānuenue Fisheries Research Center, located at Sand Island on the southside of Oʻahu, is responsible for growing and raising juvenile urchins to be released onto the reef. After starting with the urchin project in 2011, the hatchery has slowly been scaling up their efforts and recently out planted their 1 millionth urchin to a reef in Kāneʻohe Bay (Dennison, 2020; Rice, 2023). The majority of the juvenile urchins are released across a dozen reefs in the northern part of Kāneʻohe Bay. These reefs are selected based on their impact by invasive algae (Dennison, 2020). The use of these urchins as a biological control agent is a particularly clever strategy, as the urchins are native to Hawaiʻi, and were once commonly found in areas such as Kāneʻohe Bay (Westbrook et al., 2015; Alender, 1964). Given the success of the project in Kāneʻohe Bay, DAR and Ānuenue

Fisheries are now beginning to expand the project to other locations on Oʻahu, such as Waikīkī and Maunalua Bay.

**1.3 *T. gratilla* Reproduction**

*Tripneustes gratilla* are broadcast spawners that release gametes from five gonochoric pores on their aboral surface (Toha et al., 2017). In the wild, it is believed that these urchins spawn about once a year when clusters of urchins ascend to higher areas of the reef to release their gametes into the water column where the eggs are fertilized (Johnson and Ranelletti, 2017). It is believed that these urchins reach morphometric maturity around 66 mm, and at a minimum of one year of age (Toha et al., 2017; Muthiga, 2005).

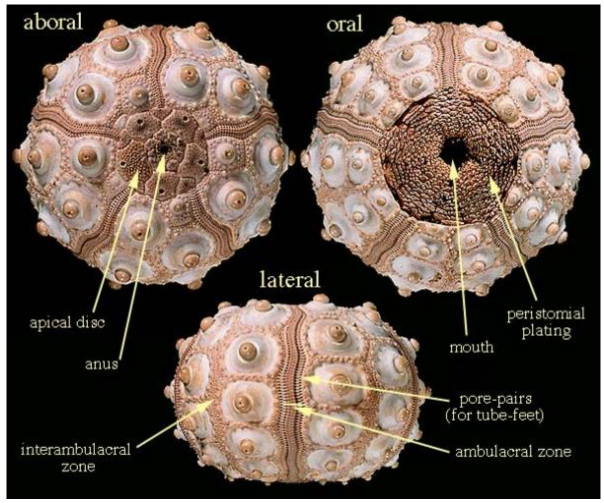

*Figure 2*: Diagram of sea urchin from Natural History Museum

The spawning periodicity and cues for *Tripneustes gratilla* are not well understood on a broad scale but are especially unknown for Hawaiʻi (Johnson and Ranelletti, 2017). Previous studies have demonstrated variation in spawning seasonality worldwide. In Kenya, it was determined that gonad indices peaked in June, and were lowest from August through October when the urchins were likely spawned out (Muthiga, 2005). However, a study conducted in Indonesia suggested that reproductive activity peaked around April and May, and was lower during the summer (Byrne et al., 2008). Preliminary studies in Hawaiʻi are beginning to suggest that the reproductive activity of *T.gratilla* increases in the fall months (November/December) (Kanoe Morishige, personal communication, May 2022). This is demonstrated by an increase in gonad indices, as well as partly-spawned and mature stages of oogenesis in female histology sampling (Kanoe Morishinge, personal communication, May 2022). Similar histological samples also suggested a decrease in reproductive activity from March to May, with most individuals being spent (spawned out) or in recovery stages by June (Muthiga, 2005).

From the point of fertilization when sperm fertilizes an egg in the water column, a fertilized egg then develops into a blastula, gastrula, prism larvae, four-armed larvae, and then eight-armed larvae (Byrne et al., 2008). After about 30-50 days floating in the water column, the larvae will then settle and meta-morph into a juvenile urchin (Byrne et al., 2008). The process of metamorphosis may take as little as 18 days, but it has been documented to take up to 30 days (Toha et al., 2017).

**1.4 Whole Effluent Toxicity Test (WETT Protocol)**

The process described above reveals long periods of time gametes are exposed to the water column, as is the case with most broadcast spawners. This extended time in this environment allows for potential exposure to environmental pollutants, or other critical conditions- which can be harmful to development (Vazquez, 2013). Evidence has demonstrated that *Tripneustes gratilla* are more sensitive to such changes and contaminants- making them effective biological indicators of water quality (Vazquez, 2013). Contamination by metals, antibiotics, pesticides, and other chemicals has been demonstrated to result in changes or delays in early development (Vazquez, 2013). Similarly, it has also been demonstrated that changes in pH, temperature, salinity, and dissolved oxygen outside of normal parameters can negatively impact fertilization rate and development (Toha et al., 2017; Vazquez, 2013). Lowered fertilization rates will reveal any of these changes and can be used to track dangerous contaminants and other problems.

Taking advantage of this, the Environmental Protection Agency (EPA) created the Whole Effluent Toxicity Test (WETT) (Wagner and Nacci, 2012). The test was created as a way to assess effluent water from organizations and businesses with environmental permits. Permit holders were then able to send samples of their effluent water to independent testing contractors who ran the protocol to check for evidence of contaminants in their water. During the test, urchin gametes are exposed to the effluent water for a standard period, fertilized, and then compared to a standard sample fertilized in 1 μl filtered sea water (Wagner and Nacci, 2012). Fertilization is also conducted in filtered sea water with a known concentration of a known toxicant, generally copper chloride (CuCl) (Wagner and Nacci, 2012). Toxicants of varying levels can then be determined based on lowered fertilization rate as compared to the control samples, both with and without the toxicant (Wagner and Nacci, 2012).

### 1.5 Previous Studies

While the WETT protocol was created as a tool for compliance, there have been several projects that utilize the procedure to compare natural environmental water samples, as opposed to effluent wastewater. In 2014, a study utilizing the WETT protocol examined the ability of hāwaʻe maoli to fertilize in water from different areas of Kāneʻohe Bay to specifically assess the impact of freshwater and non-point source pollution on fertilization (Fung, 2014). Results showed a negative correlation with freshwater influence and fertilization rate, which was likely driven by an increased impact of terrestrial pollutants such as fertilizer in freshwater (Fung, 2014).

In the summer of 2021, our lab at HIMB worked on a small-scale research project using the WETT protocol to examine the water quality of different areas in Kāneʻohe Bay within the Heʻeia National Estuarine Research Reserve (NERR). The goal of the project was to assess if there were differences in water quality among difference sits within the Heʻeia NERR. As expected, water quality was particularly lower in areas with high turbidity and low water turnover rates, more so than any other water quality parameter assessed.

However, in conducting that project a pattern emerged that when the urchins being used for spawning were collected from Kāneʻohe Bay, as opposed to the other collection location at Kahanamoku Beach, low fertilization values during the sperm optimization step of the protocol were routinely found. This step occurs before the gametes are exposed to the sample water, therefore, lowered fertilization at this point is not a result of the water being tested- and is thus could be a result of the location where the adult urchins were collected.

*Tripneustes gratilla* urchins in Kāneʻohe Bay were abundant once upon a time, particularly in the mouth of the Sampan Channel, which connects the bay to open ocean, and near Kapapa Island, which is along the barrier reef  (Alender, 1964). While no studies have been done to track the population in Kāneʻohe Bay over time, naturally occurring Collector Urchins in the bay are not common now (Westbrook et al., 2015)  The majority of these urchins in the bay now are likely the product of what has been out-planted by Ānuenue Fisheries and DAR. Conversely, no out planting has occurred at Kāneʻohe Bay

Sampan Channel, Kahanamoku Beach or Kahe Point. The current research project attempted to determine if there were differences in fertilization success between *Tripneustes gratilla* urchins from Kāneʻohe Bay and urchins from other areas of Oʻahu. It was hypothesized that urchins from Kāneʻohe Bay would yield lower fertilization rates and may be struggling to reproduce.

**1.6 Significance**

This study seeks to explore potential reproductive success rates in Collector Urchins from different locations. If there are differences in fertilization success rates from different geographic areas, this could have potential implications in understanding how the *T.gratilla* population is structured and could set the context for future studies in genetics of these organisms. This could also provide insight into differences in physiological reproductive metrics, such as sperm quality in individuals from different geographic areas or from captive rearing sources, thereby revealing possible influences contributing to low *Tripneustes gratilla* populations in Kāneʻohe Bay in recent years.

2.0 METHODS

In order to determine if hāwaʻe maoli from Kāneʻohe Bay have lower fertilization rates, a modified version of the EPA's WETT protocol was used to compare the fertilization rate of urchins from different locations.

**2.1 Collection**

*T.gratilla* were collected from Kahanamoku Beach, Kāneʻohe Bay patch reefs, Kāneʻohe Bay Sampan Channel, and Kahe Point. During each collection trip, urchins were collected by hand and placed in a mesh bag, and approximately ten urchins were collected each trip. Urchins were held in a flow through tank system at the Hawai‘i Institute of Marine Biology for no longer than two weeks prior to being spawned and used for experiments. After urchins were spawned and gametes were collected for the experiment, they were returned to their original collection locations.

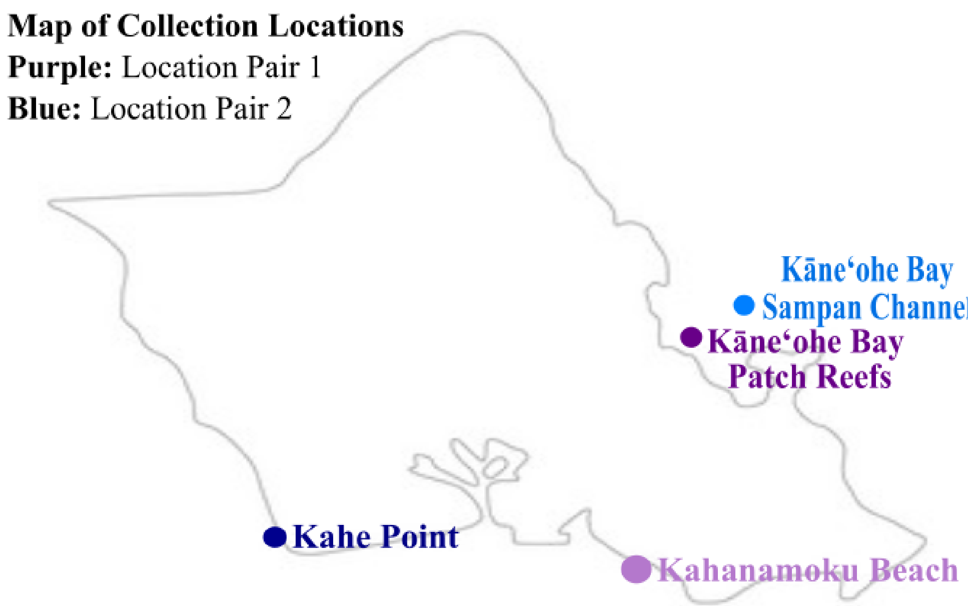


*Figure 3.* Map of four collection locations around O‘ahu.

## 2.2 Spawning Induction

To induce spawning, light mechanical stress was applied to trigger a release of gametes. Urchins were gently shaken or placed in bowls of seawater with varying temperatures to simulate stressful conditions that can often triggers spawning (Wagner and Nacci, 2012; Vazquez, 2003). If these methods of spawning induction failed to produce results, urchins are injected with 1-1.5 mL of 0.25M $CaCl_2$ + 0.5M KCl (Resgalla et al., 2019). Potassium chloride forces the gonad walls in the urchin test to contract, which forces a release of gametes (Resgalla et al., 2019). Adding a low concentration of calcium chloride to the potassium chloride has been demonstrated to reduce the mortality rate of the animals following the injection, as the extra stress of chemical injection can sometimes lead to death (Resgalla et al., 2019).

Sperm was dry collected from male urchins using a pastuer pipette. Dry collecting prevents the sperm from being activated by seawater prematurely, which can lead to a decline in sperm motility. Sperm was collected from individual males separately and pooled once each sample is analyzed for normal motility. Eggs were collected by turning the female upside down on a beaker filled with filtered sea water and allowing the eggs to sink to the bottom as they were released. Eggs from individual females were examined under the microscope to check for maturity, by visually assessing the size of vacuole. Pursuant to the EPA's Whole Effluent Toxicity Test (WETT) protocol, once eggs and sperm were confirmed to be active and mature, eggs and sperm from multiple individuals of the same location were then pooled. To maximize genetic variation, a minimum of at least three individuals from each sex were pooled whenever possible.

### 2.3 Modified WETT Protocol

Prior to beginning experiments and collecting gametes, all glassware used in the experiment, such as pastuer pipettes, beakers and test tubes were cleaned according to the EPA's WETT protocol (Wagney and Nacci, 2012). This protocol was followed to ensure a rigorous cleaning process that would not risk contamination of glassware with harmful chemicals that could potentially negatively impact the fertilization rates. Therefore, all glassware was first soaked for at least 24 hours in filtered sea water. Following the soak in filtered sea water, the glassware was then soaked in Type I ultrapure water generated by a Milli-Q Direct-8 purification. Just before being used, the glassware was then triple-rinsed in seawater filtered through a 50, 10 , and 0.1 micron series of filters and UV sterilized. At the end of experiments, all glassware was washed and triple-rinsed in Milli-Q water ultrapure Type I water.

Once gametes were collected, a slightly abbreviated version of the EPA's WETT protocol was followed. The primary modifications to the protocol that were made included the removal of the comparison of fertilization results to a copper (II) chloride contaminated fertilization sample and the removal of the gamete concentration optimization protocol. As the WETT protocol is meant to be used as an examination of compliance, the heavy metals comparison is important as it demonstrates fertilization rates of the sample with a known contaminant. However, this project sought to compare fertilization rates across populations and not potential contaminants in water samples, thus this step was unnecessary. Additionally, the WETT protocol follows a gamete optimization step, in which the optimal sperm to egg ratio is determined using a series of dilution and fertilization assessments. Previous experiments using this protocol repeatedly demonstrated that the optimal sperm to egg ratio was 2000:1. Since this ratio consistently yielded the highest fertilization results on the dilution curve, this ratio was selected to be used in fertilization experiments in this project.

The WETT protocol was followed for eggs and sperm to be diluted to a uniform concentration. Eggs and sperm from both locations were counted on a 1 mL microscope slide and hemocytometer respectively. Once the starting concentration of both is determined, eggs were diluted to a concentration of 2,000 eggs/mL and sperm was diluted to a concentration that will result in approximately 2,000 sperm per egg. This concentration is derived from the highest concentration used in the WETT protocol sperm optimization step.

Once sperm and eggs from both locations were diluted to the correct concentration, sperm was then given 30 minutes to activate in filtered sea water before eggs were added. Following the 30-minute period, 1 mL of eggs are added to each sample tube. To compare fertilization rates between locations, four crosses are assessed, both between urchins of the same area and between locations (Table 1). Four replicate samples were conducted for each cross at every run.

Collection location Pair 1:

| | **Cross 1** | **Cross 2** | **Cross 3** | **Cross 4** |
|---|---|---|---|---|
| **Male** | Kahanamoku Beach | Kāneʻohe Bay Patch Reefs | Kahanamoku Beach | Kāneʻohe Bay Patch Reefs |
| **Female** | Kahanamoku Beach | Kāneʻohe Bay Patch Reefs | Kāneʻohe Bay Patch Reefs | Kahanamoku Beach |

*Table 1*: Layout of four crosses from collection location pair 1 being assessed for fertilization rate. Four replicate samples will be analyzed from each cross.

Collection location Pair 2:

| | **Cross 1** | **Cross 2** | **Cross 3** | **Cross 4** |
|---|---|---|---|---|
| **Male** | Kahe Point | Kāneʻohe Bay Sampan Channel | Kahe Point | Kāneʻohe Bay Sampan Channel |
| **Female** | Kahe Point | Kāneʻohe Bay Sampan Channel | Kāneʻohe Bay Sampan Channel | Kahe Point |

*Table 2*: Layout of four crosses from collection location pair 2 being assessed for fertilization rate. Four replicate samples will be analyzed from each cross.

After the eggs were added to the sperm, samples were given 45 minutes for fertilization to occur and for the fertilization envelope to develop around the egg. After the 45-minute period, 0.5 mL of 2% glutaraldehyde in deionized water was added to each sample to stop fertilization and preserve the sample. This allowed time to count and assess the samples. 100 eggs from each sample were then assessed for fertilization. Fertilization is determined with presence or absence of the fertilization envelope; an example comparison of which is shown in figure 3.

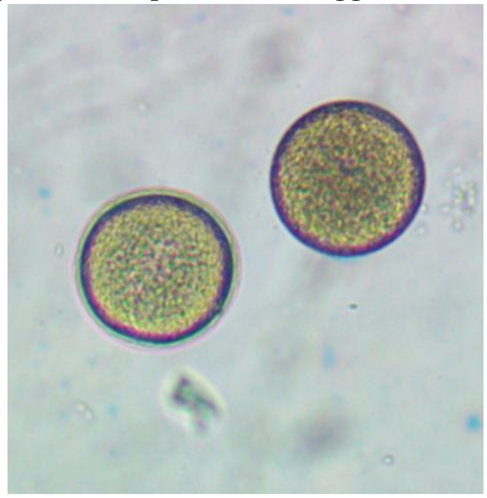

*Figure 4: Tripneustes gratilla* eggs at 10x magnification. The left egg is fertilized and has a clear fertilization envelope, whereas the right egg is unfertilized. Photo credit: Mariko Quinn

## 2.4 Data Analysis

To determine differences in fertilization rate between locations around Oʻahu, the average fertilization rate from each location was analyzed using a one-way ANOVA, followed by a pairwise student's t-test. Normality and variance assumptions were verified using the Shapiro-Wilks normality test. All statistical analyses were conducted in R studio.

3.0 RESULTS

**3.1 Fertilization Success of Kahanamoku Beach and Kāneʻohe Bay Patch Reefs**

Table 3 shows the fertilization rates for each run of the performed crosses, and the average rate for each of these crosses. Average rates for crosses between Kahanamoku Beach and Kāneʻohe Bay Patch Reefs, are as follows: Kahanamoku Beach + Kahanamoku Beach- 83.06%, Kāneʻohe Bay Patch Reefs + Kāneʻohe Bay Patch Reefs- 60.11%, Kahanamoku Beach (male) + Kāneʻohe Bay Patch Reefs (female)- 83.94% and Kāneʻohe Bay Patch Reefs (male) + Kahanamoku Beach (female) – 53.12%. This is also represented in figure 4, which shows the average fertilization rate of all four crosses.

| | **Fertilization Success Kahanamoku Beach and Kāneʻohe Bay Patch Reefs** | | | |
|---|---|---|---|---|
| | **Cross 1: Kahanamoku Beach (male)/ Kahanamoku Beach (female)** | **Cross 2: Kāneʻohe Bay Patch Reefs (male)/ Kāneʻohe Bay Patch Reefs (female)** | **Cross 3: Kāneʻohe Bay Patch Reefs (male)/ Kahanamoku Beach (female)** | **Cross 4: Kahanamoku Beach (male)/ Kāneʻohe Bay Patch Reefs (female)** |
| **Fertilization Rate (individual runs)** | 95% | 93.5% | 63% | 91% |
| | 92% | 58% | 72% | 71% |
| | 92% | 81% | 63% | 100% |
| | 98% | 94% | 97% | 98% |
| | 94% | 15% | 75% | 96% |

| | | | | |
|---|---|---|---|---|
| | 79% | 40% | 24% | 88% |
| | 73% | 48% | 41% | 83% |
| | 67% | 38% | 29% | 85% |
| | 82% | 81% | 75% | 83% |
| | 85% | 63% | 67% | 86% |
| | 56% | 50% | 46% | 42% |
| **Average** | **83.06 ± 13.17** | **60.11 ± 25.21** | **53.12 ± 26.19** | **83.94 ± 16.12** |

*Table 3.* Fertilization Success of Kahanamoku Beach and Kāneʻohe Bay Patch Reefs. All eleven experimental runs were averaged and analyzed.

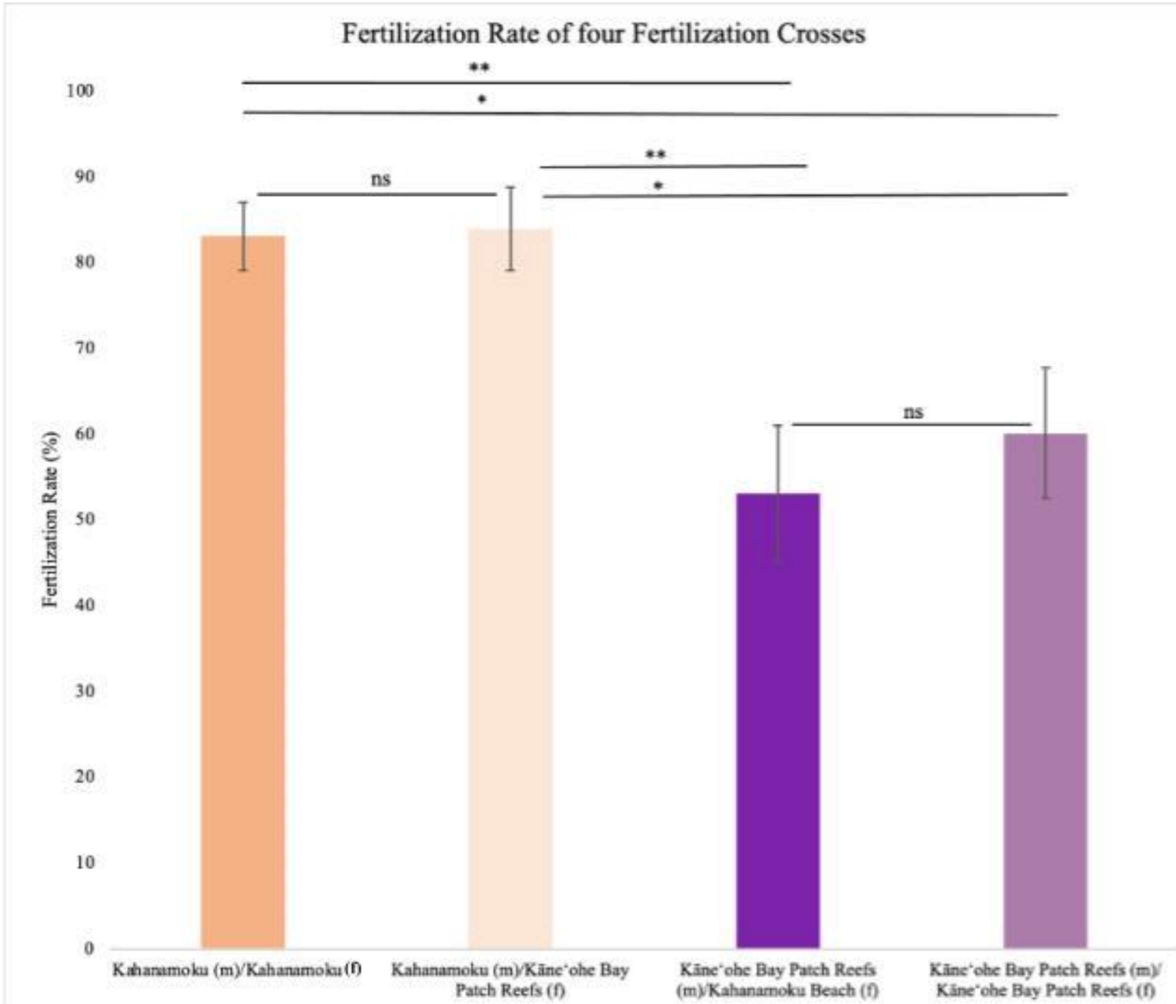

*Figure 5.* Fertilization success of the Four fertilization crosses from Kahanamoku Beach and Kāneʻohe Bay Patch Reefs. Statistical significance is indicated by * ( * $p < 0.05$, ** $p < 0.01$). The average fertilization rates are as follows: Kahanamoku Beach + Kahanamoku Beach- 83.06%, Kahanamoku Beach (male) + Kāneʻohe Bay Patch Reefs (female)- 83.94, Kāneʻohe Bay Patch Reefs (male) + Kahanamoku Beach (female) – 53.12%. and Kāneʻohe Bay Patch Reefs + Kāneʻohe Bay Patch Reefs- 60.11%. In orange are the crosses with male urchins from Kahanamoku Beach. In purple are the crosses with male urchins from Kāneʻohe Bay Patch Reefs. There is no significant difference between crosses where the males are from the same location.

A one- way ANOVA was performed to compare the fertilizaiton rate between all four of the fertilization crosses. This revealed that there was a significant difference in fertilization rate between at least two of the groups ($p < 0.01$). A paired student's t-test was used to compare the average fertilization success between all crosses. The crosses using male urchins from the same location, (i.e Kahanamoku male crosses and Kāneʻohe Bay Patch Reef male crosses) were not significantly different ($p > 0.05$, see Figure 4). This allowed for a comparision of fertilization success by source location of the sperm in the cross. The fertilization crosses from the same male source location (i.e Kahanamoku Male fertilization crosses (orange) and Kāne‘ohe Bay Male Patch Reef fertilization crosses (purple)) were combined and averaged.

As shown in figure 5, the average fertilization rate of crosses using males from Kahanamoku Beach was 83.5% and the average fertilization rate of crosses using males from Kāne‘ohe Bay Patch Reefs was 56.61%. Another paired student's t-test was run to compare these two averages; the fertilization rate using male urchins from Kāne‘ohe Bay Patch Reefs was significantly lower than the fertilization rate using male urchins from Kahanamoku Beach ($p < 0.01$) .

| | **Kahanamoku Beach Male Crosses** | **Kāne‘ohe Bay Patch Reefs Male Crosses** |
|---|---|---|
| **Fertilization Rate** | **83.5 ± 3.48** | **56.61 ± 5.30** |

*Table 4.* Fertilization success of Kahanamoku Beach and Kāne‘ohe Bay Patch Reefs male crosses.

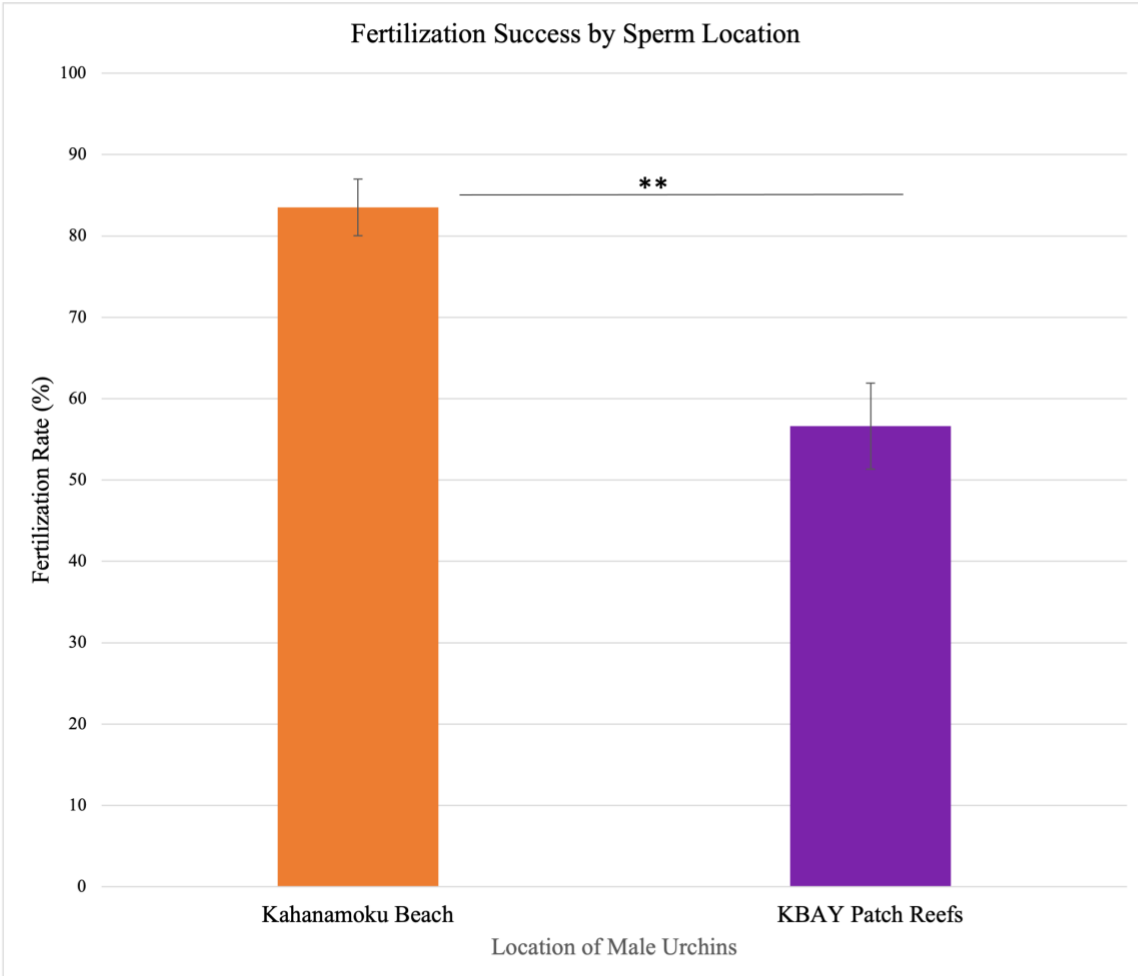


*Figure 6*. Fertilization Success of Kahanamoku Beach and Kāne‘ohe Bay Patch Reefs by Sperm Location. The average fertilization rate of crosses using males from Kahanamoku Beach was 83.5% and the average fertilization rate of crosses using males from Kāne‘ohe Bay Patch Reefs was 56.61%. Fertilization rate with urchins from Kahanamoku Beach was significantly higher than that using Kāne‘ohe Bay Patch Reefs.

### 3.2 Fertilization Success of Kahe Point and Kāne‘ohe Bay Sampan Channel

Table 4 shows the fertilization rates for each run of the performed crosses, and the average rate for each of these crosses. Average rates for crosses between Kahe Point and Kāne‘ohe Bay Sampan Channel resulted in average fertilization rates as follows: Kahe Point + Kahe Point- 70.21%, Kāne‘ohe Bay Sampan Channel + Kāne‘ohe Bay Sampan Channel- 75.48%, Kahe Point (male) + Kāne‘ohe Bay Sampan Channel (female) –

69.06%, Kāne‘ohe Bay Sampan Channel (male) + Kahe Point (female) – 75.48%. This is also so shown in figure 6, which presents the fertilization rate of all four crosses.

| | **Fertilization Success Kahe Point and Kāne‘ohe Bay Sampan Channel** | | | |
|---|---|---|---|---|
| | **Cross 1: Kahe Point (male)/ Kahe Point (female)** | **Cross 2: Kāne‘ohe Bay Sampan Channel (male)/ Kāne‘ohe Bay Sampan Channel (female)** | **Cross 3: Kāne‘ohe Sampan Channel (male)/ Kahe Point (female)** | **Cross 4: Kahe Point (male)/ Kāne‘ohe Bay Sampan Channel (female)** |
| **Fertilization Rate (individual runs)** | 20% | 70% | 59% | 41% |
| | 35% | 28% | 38% | 37% |
| | 82% | 85% | 78% | 81% |
| | 42% | 43% | 43% | 32% |
| | 85% | 87% | 90% | 82% |
| | 96% | 98% | 94% | 94% |
| | 94% | 92% | 94% | 97% |
| | 34% | 52% | 75% | 31% |
| | 92% | 86% | 91% | 91% |
| | 98% | 93% | 75% | 77% |
| | 93% | 89% | 90% | 95% |
| **Average** | **70.21 ± 30.32** | **74.99 ± 23.03** | **75.48 ± 20.14** | **69.06 ± 27.39** |

*Table 5.* Fertilization Success of Kahe Point and Kāne‘ohe Bay Sampan Channel. All eleven experimental runs were averaged and analyzed.

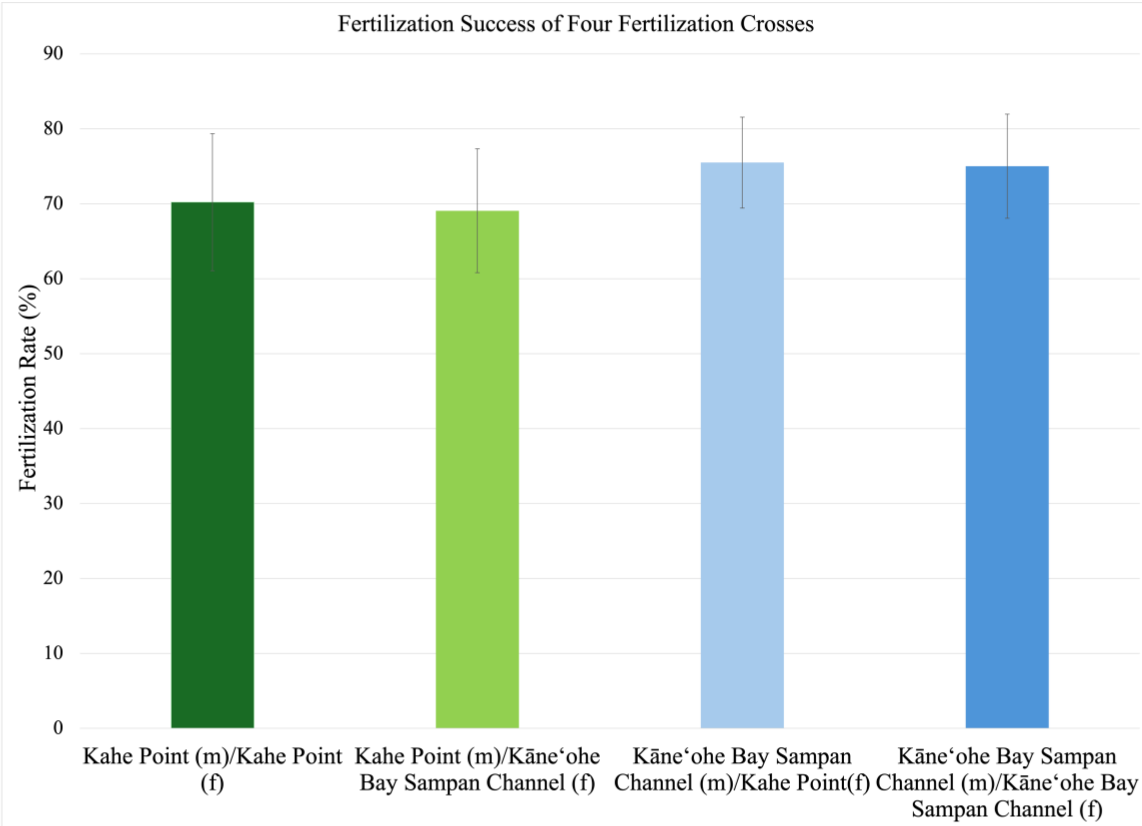


*Figure* 7. Fertilization success of the four fertilization crosses from Kahe Point and Kāne'ohe Bay Sampan Channel. No signfiicant difference was found between any of the four crosses. The average fertilization rates are as follows: Kahe Point + Kahe Point- 70.21%, Kāne'ohe Bay Sampan Channel + Kāne'ohe Bay Sampan Channel- 75.48%, Kāne'ohe Bay Sampan Channel (male) + Kahe Point (female) – 75.48%, and Kahe Point (male) + Kāne'ohe Bay Sampan Channel (female) – 69.06%.

A one- way ANOVA was performed to compare the fertilization rate between all four of the fertilization crosses. This revealed that there was no significant difference in fertilization rate between at least two of the groups ($p < 0.05$). A paired student's t-test was also used to compare the fertilization success between all of these crosses. It was determined that the two crosses using male urchins from the same location were not significantly different ($p > 0.05$). This allowed for a comparision of fertilization success by location of the sperm in the cross.

The fertilization crosses from the same male source location (i.e Kahe Point Male fertilization crosses and Kāne'ohe Bay Male Sampan Channel fertilization crosses) were combined and averaged. As shown in figure 7, the average fertilization rate of crosses using males from Kahe Point was 72.00% and the average fertilization rate of crosses using males from Kāne‘ohe Bay Sampan Channel was 75.18%. Another paired student's t-test was run to compare these two averages; no significant differences were found between these two fertilization rates ($p > 0.05$).

| | **Kahe Point Male Crosses** | **Kāne‘ohe Bay Sampan Channel Male Crosses** |
|---|---|---|
| **Fertilization Rate** | **72.0 ± 6.01** | **75.2 ± 4.50** |

*Table 6.* Fertilization success of Kahe Point and Kāne‘ohe Bay Sampan Channel male crosses.

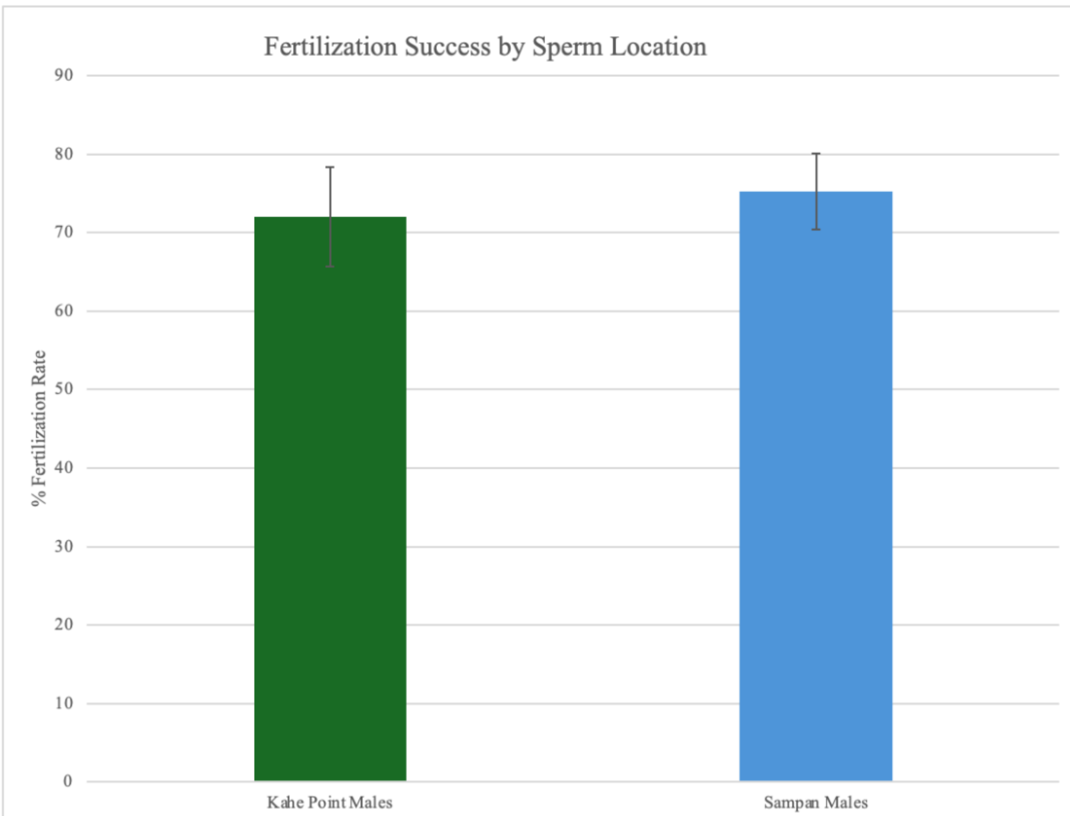


*Figure 8.* Fertilization Success of Kahe Point and Kāne‘ohe Bay Sampan Channel Males. The average fertilization rate of crosses using males from Kahe Point was 72.00% and the average fertilization rate of crosses using males from Kāne‘ohe Bay Sampan Channel was 75.18%. There was no significant difference between the two.

## 4.0 DISCUSSION

### 4.1 Comparison of Fertilization Results

The experiments comparing Kahanamoku Beach to Kāneʻohe Bay Patch Reefs revealed that the fertilization rate of crosses using males from Kahanamoku Beach was significantly higher than the fertilization rate of crosses using males from Kāneʻohe Bay Patch Reefs, regardless of the source of the females in these comparisons. Since this low fertilization pattern was present specifically when comparing fertilization rate by location of the male urchins in the cross, and not present when comparing by location of female urchins, it is possible a reproductive issue with the sperm in Hāwaʻe maoli of the Kāneʻohe Bay Patch Reefs might exist. Given that this location is the site of extensive out planting by DAR, it is possible that the low reproduction could be linked to the urchins being raised in a hatchery, as opposed to in the wild. Kāneʻohe Bay Patch Reefs have been the site of out planting for over one million Hāwaʻe maoli in the last seven years. More specifically, on reefs 41, 42, 29 and 27, which were the sources of our collections, over 200,000 urchins have been out planted in the last three years. This volume suggests that the urchin population on these reefs may not be naturally sustaining and may instead be sustained by consistent out planting and restoration efforts by DAR and Ānuenue.

Conversely, it is believed that the urchins being collected from the Sampan Channel were representative of the naturally sustaining Hāwaʻe maoli population within Kāneʻohe Bay. According to DAR out planting maps, the Sampan Channel is not currently one of the indicated out planting sites. Further, morphological differences in size and shape of the Hāwaʻe collected from the Kāneʻohe Bay Patch Reefs when compared to other locations were evident. During a previous project using the WETT protocol and Hāwaʻe maoli, urchins from Kāneʻohe Bay patch reefs were on average greater than 11 centimeters in diameter, which was significantly larger than the average size of urchins collected from Kahanamoku Beach. Urchins from Kahanamoku Beach, Kāneʻohe Bay Sampan Channel, and Kahe Point were all on average 6-8 centimeters. Hāwaʻe maoli from Kāneʻohe Bay Patch Reefs were also occasionally misshapen or damaged, which is likely to happen during out planting and handling in the nursery.

Furthermore, the fertilization rate between Kahe Point urchins and Kāneʻohe Bay Sampan Channel urchins was not significantly different, even when these crosses are specifically examined by location of the male urchin. This indicates that these populations may have similar reproductive capabilities. Both locations yielded fertilization rates upwards or around 70%. The fertilization rate of urchins from Kahanamoku Beach was just over 80%, which is similar to that of Kahe Point and Kāneʻohe Bay Sampan Channel. Additionally, urchins from both of these locations were similar in size and shape, and did not have any large noticeable differences, as opposed to urchins from Kāneʻohe Bay Patch Reefs.

### 4.2 Limitations of the study and future research

Since the two location pairings were only assessed in comparison to each other, it is only possible to draw direct conclusions between each two location pairings and not to the other set of locations. The original intent of this project was to have included all possible pairwise comparisons among the geographic regions included in this study. There are two reasons for not having these location comparisons. The first is that it was not immediately apparent that Kāneʻohe Bay Sampan Channel would need to be treated as a location independent of Kāneʻohe Bay Patch Reefs. However, after doing more research on the DAR out planting locations, it was clarified that these would need to be separated. Additionally, in Summer of 2023, DAR implemented a new set of rules in relation to being able to collect at patch reefs in Kāneʻohe Bay that were out planting sites. Previously, these reefs had been open to collection by permit. However, the Summer 2023 permit indicated that collections should only be conducted in areas that were not currently sites of out planting. As such, the collections were then limited to comparing Kahe Point with Kāneʻohe Bay Sampan Channel as we were no longer able to collect urchins from Kāneʻohe Bay patch reefs to perform those comparisons.

Therefore, the next steps for this project are to work with DAR to allow for an accommodated collection at certain patch reefs in the north part of Kāneʻohe Bay which would enable a more direct comparison and analysis. If collections were to be allowed in this part of the bay, further analysis on the male urchins could then also be conducted in an attempt to reveal the cause of lower fertilizations in this area as well. This would also allow for the potential to conduct a population genetic analysis on urchins from the four different locations compared here.

## 5.0 CONCLUSION

The aim of this project was to assess and compare the fertilization rate of Hāwaʻe maoli around Oʻahu. It was determined that the fertilization rate of crosses using males from Kāneʻohe Bay Patch Reefs were significantly lower than crosses using males from Kahanamoku Beach. Kahanamoku Beach urchins presented a fertilization rate that was similar to the rate of urchins from both Kahe Point and Kāneʻohe Bay Patch Reefs, showing no significant differences. As a result of limited collections in the second half of the study, direct comparisons are unable to be made between Kāneʻohe Bay Patch Reefs, Sampan Channel and Kahe Point. While this is the case, it is still notable that the Patch

Reefs held a lower fertilization rate than Kahanmoku Beach, and more research should be conducted in order to reveal any differences between the other locations.

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