

The ecological mysteries of *Opheodesoma spectabilis*: Seasonal trends, habitat preferences, and climate responses

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SUMMARY

The 21st century marks the alarming effect of climate change on ocean conditions, leading to immense shifts in marine ecosystems and organisms. Among the most affected organisms are bioturbators, which mix sediment, bring up nutrients from the depths to the surface, and filter water, but much remains unknown about bioturbator ecology in the face of climate change. We hypothesized that the bioturbator species *Opheodesoma spectabilis* (conspicuous sea cucumber) 1) would increase in abundance in sandy areas with warmer temperatures, 2) would improve water quality, and 3) would exhibit stress responses under increased temperature and acidification. We observed a trend of *O. spectabilis* abundance with warmer water temperatures at specific sites in Kāne'ohe Bay, where the presence of gorilla algae-dominated substrates, higher dissolved oxygen saturation, and low water flow created optimal conditions for living. Few to no *O. spectabilis* were present in higher flow areas lacking algae, emphasizing the influence of substrate composition and habitat structure on species abundance. When simulating climate stressors in tanks, we found rapid *O. spectabilis* mortality at 36°C, suggesting that marine heat waves could severely impact populations. *O. spectabilis* exhibited the greatest ability to reduce turbidity, meaning clearer water visibility, compared to algae alone. These findings highlight the need for conservation strategies to address rising water temperatures, specifically for algae-dominated benthic habitats, and for further research to examine the combined effects of rising temperature, ocean acidification, and habitat conditions such as substrate composition and water flow on marine invertebrates.

INTRODUCTION

The study of marine ecosystems has increasingly been focused on the impacts of human activities and climate change on oceanic environments. Kāne'ohe Bay, Hawai'i serves as a prime example of how anthropogenic stressors have led to notable ecological shifts. Among these stressors, extensive dredging (the process of removing silt, soil, and other materials to create navigable waterways) has contributed to rapid reef decline (1). By the 1960s, unchecked sewage discharge and sediment accumulation further exacerbated environmental degradation due to inadequate monitoring and stream channelization, which caused natural streams to deepen (1). Additionally, stressors such as storms, floods, extreme temperature fluctuations, and extremely low tides

have profoundly influenced the reef ecosystem (1). Runoff from the Ko'olau Mountains has also carried organic material into the bay, leading to a decline in pH (2). With climate projections indicating big changes over the next century, including increased ocean acidification and warming, it is essential to understand how marine organisms may adapt to these conditions (1). Ocean acidification, driven by elevated CO₂ emissions, has already resulted in declining pH levels. By 2100, global average pH could drop by 0.14 to 0.35 units, with high-emission scenarios projecting a pH of 7.6, severely increasing ocean acidity (3). Such changes threaten marine species dependent on calcium carbonate for shell and skeletal formation, with less certain effects on other species (4). Rising ocean temperatures further compound these challenges, particularly for marine organisms that cannot migrate to cooler waters. As atmospheric changes alter precipitation patterns, and thus freshwater and nutrient flow into marine systems, the risk of eutrophication and disrupted marine productivity increases (3, 4).

Holothuroidea, or sea cucumbers, are classified as bioturbators due to their role in sediment mixing and reworking (5). These organisms can be affected by water quality, temperature, and seabed substrate conditions in benthic habitats, including sand, algae, and coral reefs. Though commonly found in shallow waters, much remains unknown about their ecology and responses to changing environmental conditions (1). *Opheodesoma spectabilis*, the conspicuous sea cucumber, is a frequently observed sea cucumber species in Kāne'ohe Bay. *O. spectabilis* possess fifteen retractable tentacles, with five symmetrical rows of tubercles on its outer surface. *O. spectabilis* size and number increase with growth, and individuals typically reach lengths of 0.6096 to 0.9144 meters. Their outer surfaces exhibit distinct stripes and spots in hues of orange, red, violet, and gray, while their ventral surface remains pale and unpatterned (1). While *O. spectabilis* have no natural predators, they may be vulnerable to climate-related stressors such as heavy rainfall, which can cause dilution of seawater. In February 1965, heavy rainfall led to increased mortality of *O. spectabilis* due to the accumulation of five inches of freshwater over six hours, which disrupted water circulation. However, moderate rainfall has minimal effects on their survival (1).

To date, only a limited number of studies have examined aspects of its ecology (1). As a result, key ecological characteristics, including seasonal abundance, thermal resilience, and potential bioturbator roles, remain largely unknown. Investigating their interactions within benthic habitats and their responses to environmental stressors

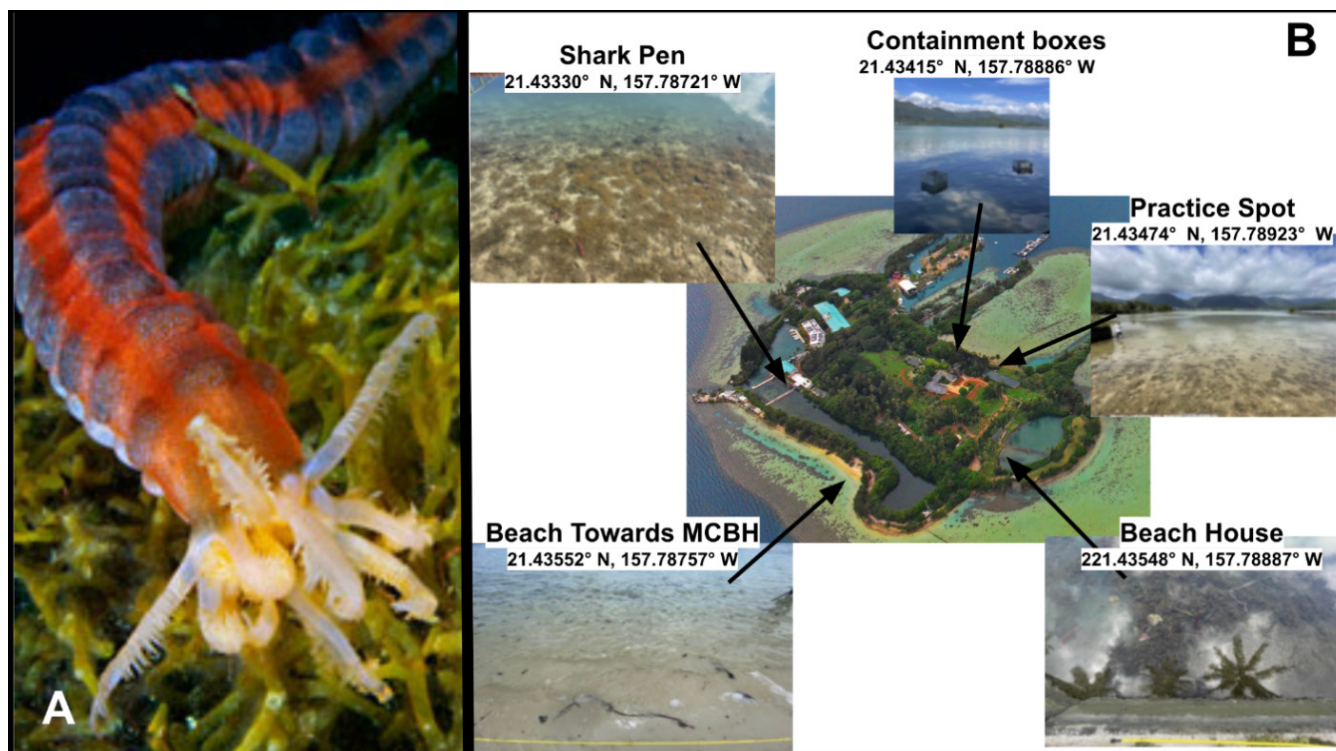


Figure 1. *Opheodesoma spectabilis* distribution and survey locations in Kāne'ohe Bay. (A) Photograph of *O. spectabilis* in Kāne'ohe Bay, taken by Keoki and Yuko Stender. Reproduced with permission from marinelifephoto.com (8). (B) Map showing the locations for abundance surveys and containment enclosures. Locations were surveyed by direct observation. MCBH, Marine Corp Base Hawai'i.

could provide valuable insights into ecosystem health. Notably, studies on active moving invertebrates constitute only 7.3% of invertebrate literature, despite their essential role in maintaining water quality (1). The goal of this research was to bridge the knowledge gap on *O. spectabilis* and its ecological role in Kāne'ohe Bay, O'ahu, Hawai'i. By studying its environmental preferences and responses to climate change, we can gain a deeper understanding of how benthic invertebrates contribute to ecosystem resilience. Identifying *O. spectabilis* as a potential bioindicator species could provide valuable insights for monitoring water quality and predicting broader ecological changes. Given the anticipated shifts in ocean conditions, it is essential to expand our knowledge of invertebrate adaptation to ensure the preservation of marine biodiversity and ecosystem functionality.

This study aimed to 1) investigate the temperature, benthic habitat, and water quality preferences of *O. spectabilis*, 2) assess how climate-related factors such as temperature and pH fluctuations influence *O. spectabilis* and its role in sediment bioturbation (6), and 3) understand how these findings can be applied to issues in Hawai'i and beyond. We hypothesized that 1) *O. spectabilis* abundance would be higher in areas with favorable substrate conditions, (e.g., sand and rubble), 2) the presence of *O. spectabilis* would improve water quality by increasing dissolved oxygen and reducing organic buildup, and 3) *O. spectabilis* would exhibit stress responses, such as decreased survival and behavioral changes, under increased temperature and acidification. Together, these findings suggest that *Opheodesoma spectabilis* is a relatively resilient species with specific habitat and grazing preferences,

showing moderate tolerance to environmental variability but increased vulnerability under extreme temperature stress.

RESULTS

In Situ Abundance and Water Quality Surveys

To understand what environmental conditions might influence sea cucumber presence at Moku o Lo'e, we first conducted seasonal abundance surveys paired with water quality measurements across four distinct sites (**Figure 1**). This allowed us to determine whether temperature, pH, or dissolved oxygen aligned with changes in sea cucumber distribution.

Sea cucumber abundance varied across seasons and four sites at Moku o Lo'e, with the highest numbers at the "Beach House" site (30–49 cucumbers; **Figure 2**). Notably, the "Beach House" site also had the warmest temperature average across all months monitored (~27.7°C), stable dissolved oxygen (DO; ~25 mg/L), and moderate pH (7.32–8.17) (**Figure 2**). The "Shark Pen" site showed moderate abundance (18–39 cucumbers) with slightly cooler temperatures (24°C–26.9°C) and near-neutral pH (average of 7.38; **Figure 2**). No cucumbers were found at the "Practice Spot" site or the "Beach towards Marine Corp Base Hawai'i" site, despite similar or higher temperatures and more variable, sometimes low, pH (as low as 7), which could be a reason for lack of abundance (**Figure 2**). When analyzing only the two sites with cucumbers present, we found a marginally significant positive relationship between abundance and temperature ($R^2 = 0.17$, $p = 0.122$; **Figure 3A**), suggesting higher abundance in warmer months, possibly linked to reproduction. Across

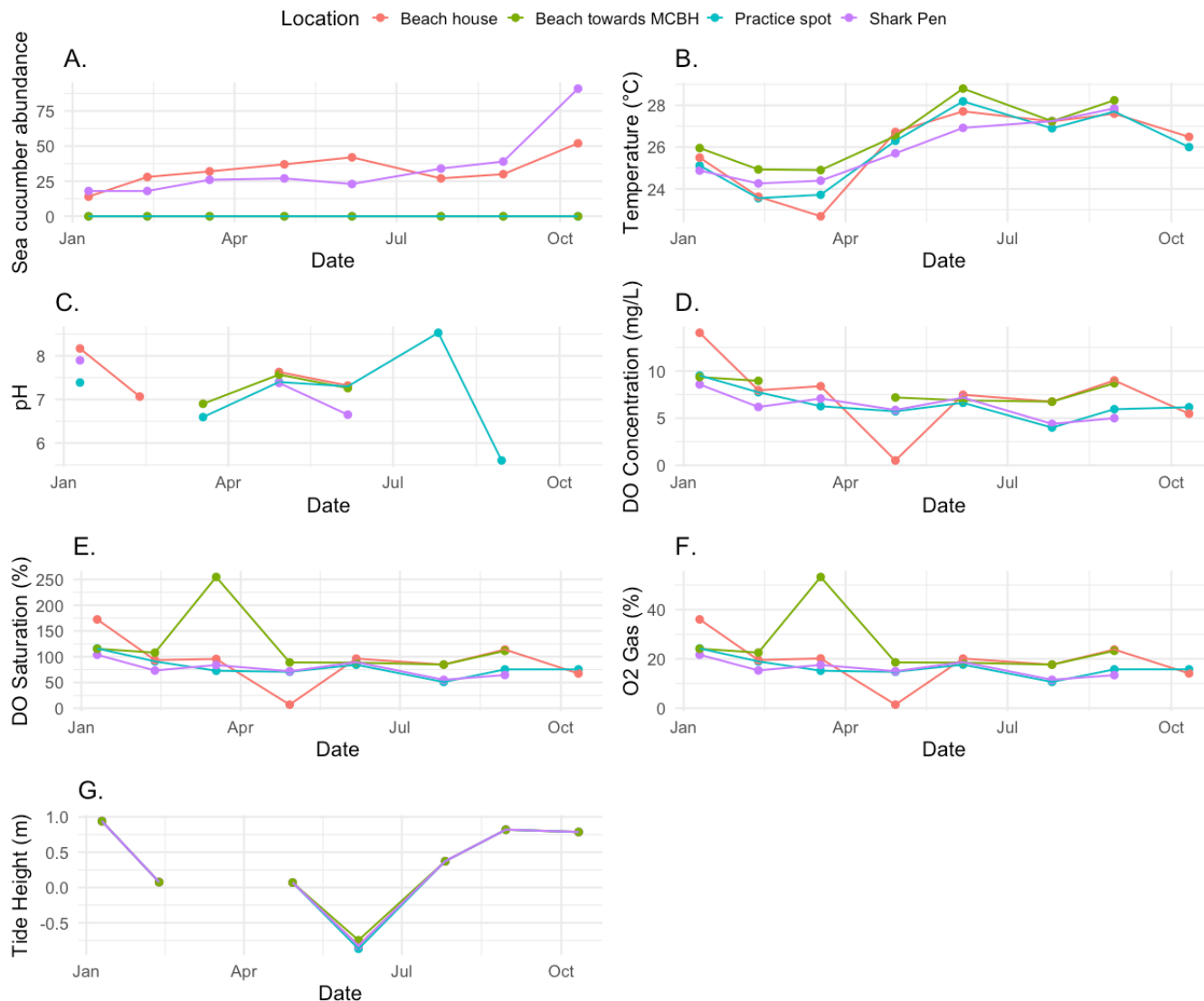


Figure 2. Environmental parameters over time at surveyed sites. (A) *O. spectabilis* abundance, (B) water temperature (°C), (C) pH, (D) dissolved oxygen (DO) concentration (mg/L), (E) DO saturation (%), and (F) O₂ gas (%) recorded at each timepoint. Measurements were collected using calibrated water quality instruments at all survey sites. (Sensors were not properly working on some data collection days which caused missing points.) Survey sites include Beach House (red), Beach House Towards Marine Corp Base Hawai'i (green), Practice Spot (cyan), Shark Pen (purple).

all sites, no correlation was found between temperature and cucumber abundance, suggesting temperature was not the main driver of sea cucumber presence (Figure 3B; $p = 0.88$). pH and DO showed no significant correlation with abundance (Figure 3C-D).

Sea Cucumber

The Beach House site was characterized by medium sand with an average grain size of approximately 3/8 mm, the presence of medium-sized rocks, and dense clumps of algae. The Shark Pen site had minimal fine sand, was dominated by medium to large rocks, and exhibited high algae coverage. The Practice Spot site consisted of medium sand with an average grain size of approximately 3/8 mm along with small to medium sized rocks and moderate algae abundance. The site located along the beach toward Marine Corps Base

Hawai'i was characterized by fine sand with an average grain size of approximately 3/16 mm with an absence of rocks, and minimal algae presence.

In Situ Enclosure Experiment

To test whether *O. spectabilis* actively alters water quality through bioturbation, we placed sea cucumbers into *in situ* enclosures and monitored changes in temperature, DO, and pH over time.

Following the introduction of sea cucumbers into the enclosures at Moku o Lo'e, mean dissolved oxygen concentrations increased from 7.4 to 8.7 mg/L when averaged across all enclosure trials (Figure 4A-C). Similarly, mean water temperature increased from 20°C to 23°C, and mean pH increased from 6.6 to 7.5 across trials (Figure 4D-E). The observed increase in dissolved oxygen may be due to

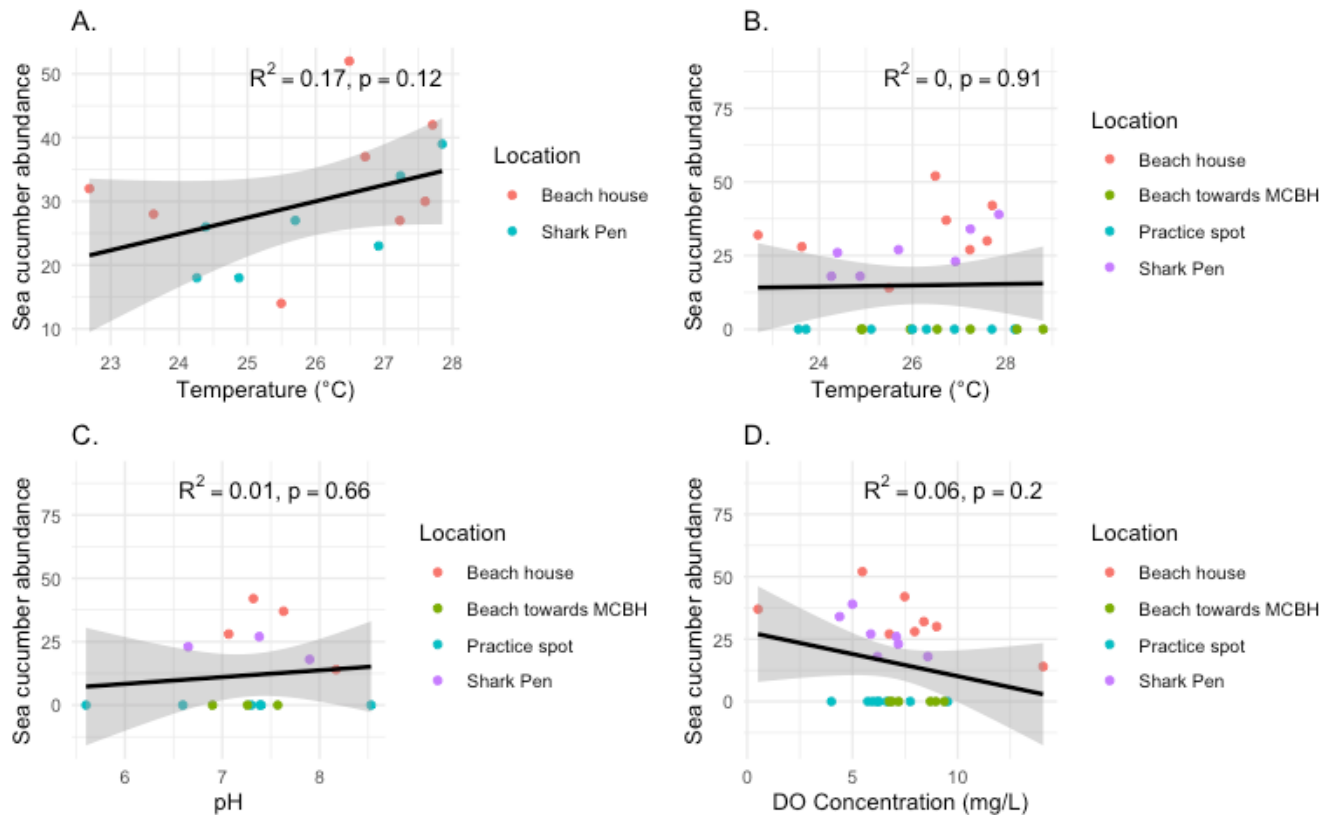


Figure 3. Relationship between water temperature and *O. spectabilis* abundance. (A) Relationship between (A) temperature and abundance at the two sites where *O. spectabilis* were present (Shark Pen and Beach House), (B) temperature and abundance across all four sites combined, (C) abundance and pH, and (D) abundance and DO concentration. Water temperature was measured in situ at the time of each survey; abundance was quantified by standardized transect counts.

bioturbation by *O. spectabilis*, which can enhance sediment water mixing and increase oxygen availability in the water column. There were no significant differences found between control and experimental trials for any of the variables (T-test, $p > 0.05$ for temperature, pH, and DO parameters).

In Situ Substrate Classification

Because *O. spectabilis* choose habitats that are based on substrate type, we compared the benthic composition of the high abundance sites to the low abundance sites. This allowed us to determine if substrate availability could explain differences in natural distribution. We compared substrate types across two sites: the “Shark Pen” with high sea cucumber abundance, and the “Practice Spot” with no abundance. We picked two opposing sites with very different substrates. We found that the “Shark Pen” site, which consistently showed high sea cucumber abundance, was much higher in algae-dense substrate, compared to the “Practice Spot” site, which was generally dominated by sand and rocks (Figure 5).

In Lab Substrate Preference

To test directly if sea cucumbers prefer certain substrates when other variables are controlled, we conducted a structured trial. We presented an individual organism with sand, algae, and sand + algae mix and aimed to identify whether substrate preference patterns matched what we observed in the field. In a laboratory setting, following a 48-hour starvation period,

sea cucumbers were placed in controlled tank environments with different substrate makeups including sand, algae, and a sand + algae mix. In this setting, sea cucumbers displayed equal preference for sand and algae substrate, however preference for the mix of sand and algae preference was much lower (Figure 6).

In Lab Climate Simulation

To evaluate sea cucumber tolerance to projected climate change stressors, we exposed sea cucumbers to simulated warming and pH conditions with the purpose of creating an environment similar to ocean acidification. These trials were designed to observe behavioral or survival responses under short-term extreme conditions similar to climate change effects.

In the pH simulation, the tank was established at an initial pH of 6.5 and monitored over three days, during which the pH naturally increased to 8 (Figure 7A). No mortality was observed, but behavioral changes included slow movement. In the increased temperature simulations, the temperature was raised from 32°C to 36°C (Figure 7B). On day two, the increased temperature tank had a 100% mortality rate (Figure 7C-D). The control tanks had no mortality (Figure 7C-D).

Artificial Turbidity Simulation

To determine whether sea cucumbers can decrease

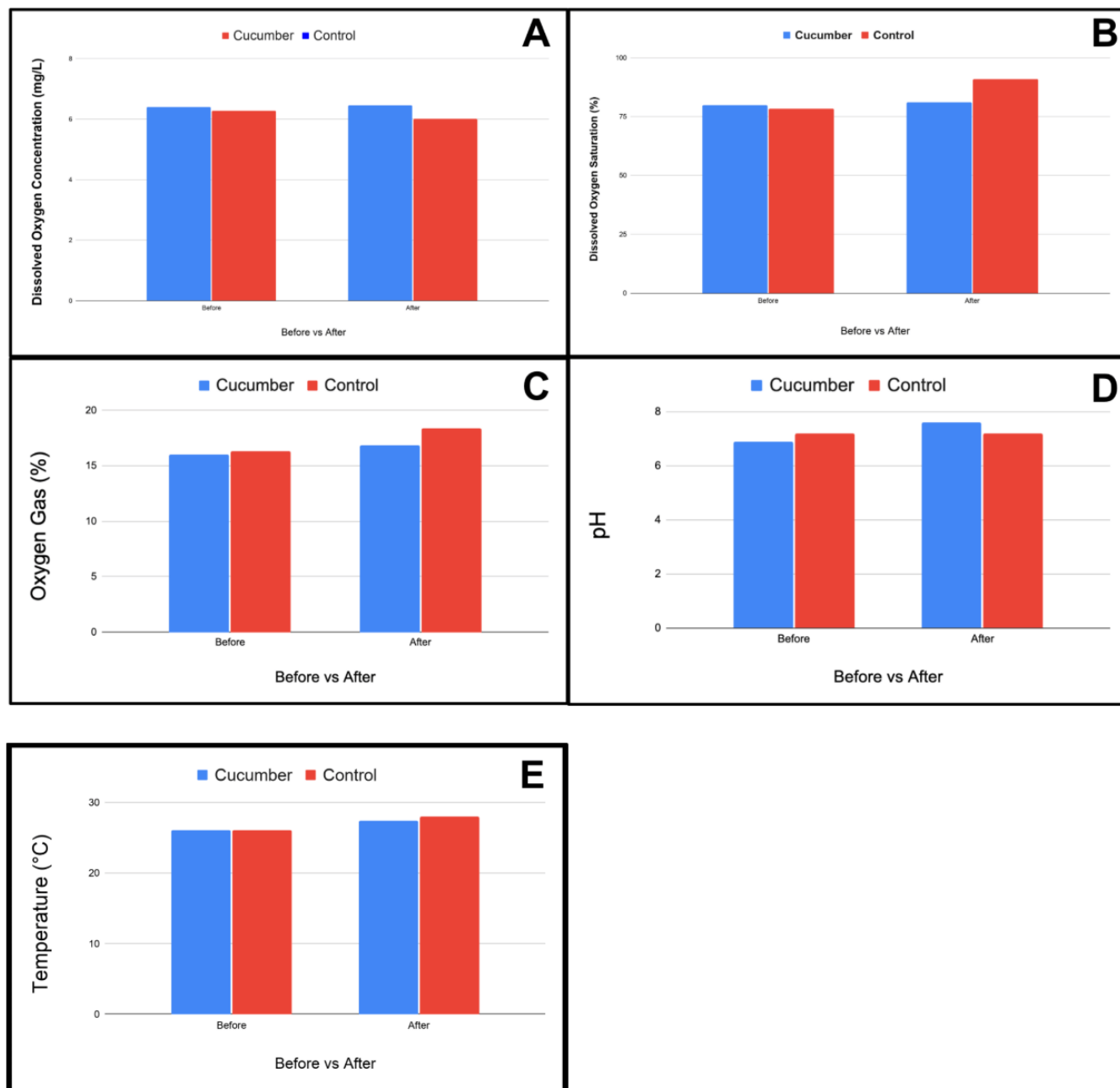


Figure 4. Water quality measurements in containment cube experiments. Average values across all trials ($n = 7$) are shown for experimental cubes containing *O. spectabilis* and for control cubes without sea cucumbers. Parameters measured included DO (A-C), pH (D), and temperature (E). “Before” entails the period prior to the sea cucumber being introduced, and “After” represents the conditions one hour following its introduction. There were no significant differences found between control and experimental trials for any of the variables.

turbidity, which is a measure of how cloudy the water is due to suspended particles, we compared their ability to reduce those particles with algae controls. Turbidity is important in aquatic ecosystems because high amounts of suspended particles can block sunlight, reduce photosynthesis, and negatively affect water quality and marine life. When exploring turbidity reduction, sea cucumbers alone performed the greatest reduction of turbidity compared to algae. Turbidity was reduced from 93.8 nephelometric turbidity units (NTU) to 2 NTU, demonstrating the ability of sea cucumbers to counteract turbid conditions (Figure 8).

DISCUSSION

This study was conducted to investigate why *O. spectabilis* live in specific areas, focusing on their impact on water quality and how they will be affected by future climate conditions such as ocean acidification and rising sea temperatures. We found that *O. spectabilis* abundance at Moku o Lo'e varied by site, with the highest numbers found in warmer, algae-rich areas like the “Beach House” site. While temperature showed a marginal connection to abundance, pH and DO had no significant correlation. Enclosure experiments showed

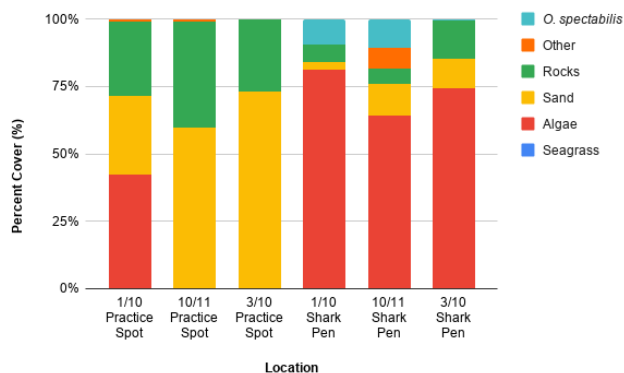


Figure 5. Abundance of benthic substrates in two habitat types. Data were collected using virtual image analysis. Substrate types were categorized as *O. spectabilis* (teal), rocks (green), sand (yellow), algae (red), seagrass (dark blue), or other (orange) based on visual identification *in situ*.

that sea cucumbers increased oxygen levels and influenced pH of their enclosure, likely through sediment mixing. In lab simulations, *O. spectabilis* showed no strong substrate preference but reduced turbidity effectively from 93.8 NTU to 2 NTU, and a high temperature increase led to 100% mortality, highlighting sensitivity to climate stressors (Figure 4). Our findings showed a trend between the abundance of *O. spectabilis* and warmer water temperatures. At the “Beach House” site, higher counts of sea cucumbers were recorded in correlation with slightly increased temperatures, suggesting that the species thrives in warm conditions (Figure 3). However, this pattern did not hold across all

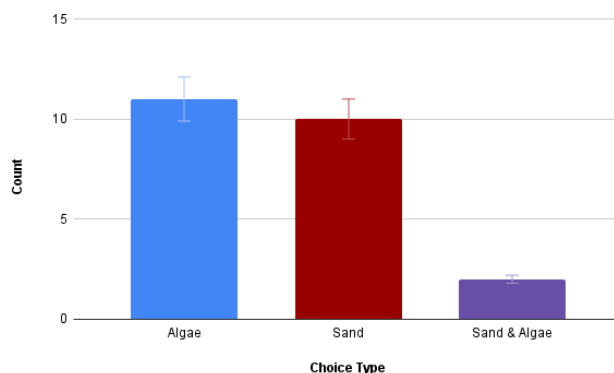


Figure 6. Substrate preferences of *O. spectabilis*. Count represents the number of times an individual sea cucumber selected a substrate type during five-minute observation periods in controlled aquaria. Trials included multiple substrate types (algae, light blue; sand, red; combination of sand and algae, purple) presented simultaneously to each invertebrate. Six trials were conducted with six different individuals each day for four days, totaling 24 trials.

sites. For example, the Practice Spot and the Beach Towards Marine Corps Base Hawai'i had similar temperatures but supported lower *O. spectabilis* populations. This indicates that while temperature plays a role in influencing abundance, it is not the main factor. Other environmental conditions must also contribute to distribution. Some of those other factors that could have contributed are turbidity (which was only tested in simulations rather than in the field), as well as key nutrient levels and weather patterns (1). Additionally, since our field data showed higher abundance in warmer waters,

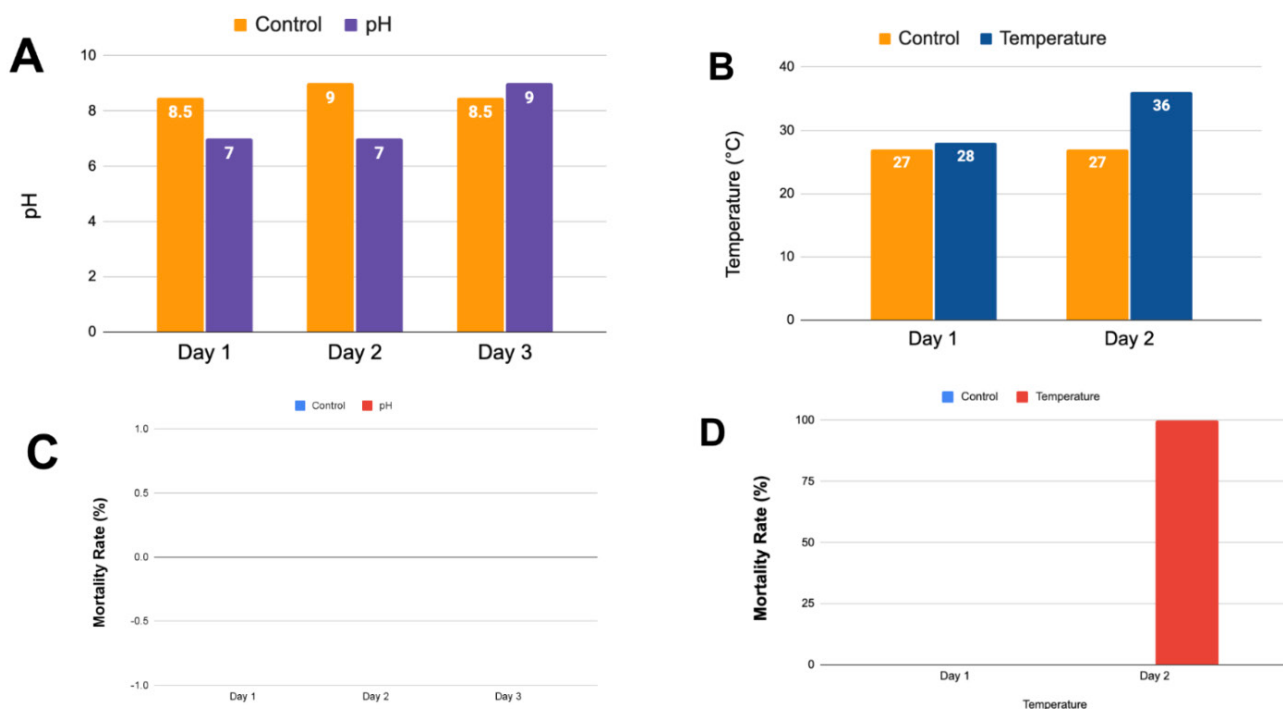


Figure 7. Changes in environmental parameters during climate simulation experiments, showing (A) pH over time, (B) temperature over time, (C) mortality rate in the pH simulations, and (D) mortality rate in the temperature simulations. Simulations were conducted in experimental tanks under warming and acidification conditions (A, purple; B, blue; C & D, red) as well as control tanks (orange). Measurements were recorded using data loggers at 10-minute intervals.

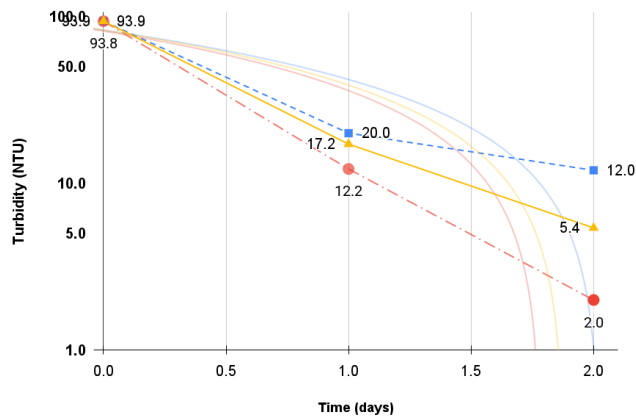


Figure 8. Turbidity reduction over time by *O. spectabilis* and algae. Turbidity was measured in controlled tanks containing one *O. spectabilis*, one handful algae, or a combination of both. Turbidity values were recorded at regular intervals using a PASCO test kit. Blue squares: tank containing sea cucumbers and algae; yellow triangles: tank containing algae only; red circles: tank containing sea cucumbers only. Each data point represents measurements collected on one of the three sampling days.

but our climate simulations showed mortality at extreme heat, our results suggest that there is a specific range of ideal temperatures at which *O. spectabilis* can thrive.

The controlled tank experiment provided ecological answers into the species response to extreme thermal stress. Initially, *O. spectabilis* showed heightened activity, namely quick movements across the tank, which was likely a stress response to the sharp increase in temperature. However, this response was short lived. By the second day, the water became cloudy and emitted a strong odor, and all the *O. spectabilis* had died, resulting in a 0% survival rate. This highlights the sensitivity of *O. spectabilis* to rapid or extreme thermal shifts, such as intense heat waves, an important consideration relating to climate change and rising ocean temperatures.

Substrate type was another key factor in *O. spectabilis* distribution. Virtual quadrat analysis suggested differences in benthic composition across sites, with algae-rich environments like the “Beach House” and “Shark Pen” sites supporting more sea cucumbers. These substrates likely offer both shelter and a food source, which may explain the species preference for these sites. While substrate types remained mostly stable throughout the study period, the seasonal changes observed in algae density and sediment distribution likely influence habitat likability. The ecological role of *O. spectabilis* as a benthic organism, particularly its bioturbation activity, may contribute to sediment mixing and nutrient redistribution, which may support ecosystem function and resilience.

Although turbidity was not directly measured at survey sites, water clarity and nutrient conditions appeared to influence sea cucumber abundance. Interestingly, *O. spectabilis* were more common in sites thought as murkier, which are typically considered signs of poor ecosystem health (2). This suggests that nutrient-rich or low-flow environments may actually be favorable to *O. spectabilis*,

potentially due to increased organic matter availability. This hypothesis was supported by the turbidity tank simulation. Of the three experimental setups, the tank containing only *O. spectabilis* was the most effective at reducing turbidity over time. Though we only looked at algae vs. cucumber turbidity and not turbidity on its own over time, our turbidity finding emphasizes *O. spectabilis*'s potential as a bioremediator in sediment-rich, low-visibility environments. They can consume and break down environmental pollutants, which in turn cleans the polluted areas. Their capacity to process organic material and cycle nutrients could position them as natural mediators in maintaining water quality in degraded marine systems.

This study faced some limitations. Changes in sampling times and sensor calibration may have introduced inconsistencies in water quality data. The enclosure and tank experiments, while informative, were limited by short duration. Additionally, we compared turbidity across tanks with sea cucumbers vs. algae but did not have a control without algae, which could have been an informative comparison. Finally, results from a single species may not be adaptable to other species with different environment adaptations.

Future research should explore *O. spectabilis* across a broader range of Hawaiian habitats and environmental gradients, incorporating longer-term studies and additional water quality parameters. Understanding and preserving these benthic invertebrates is essential for promoting ecosystem resilience and supporting sustainable marine environments.

Overall, what sets our work apart from existing research on this species is our focus on *O. spectabilis* ecology under changing environmental conditions; whereas previous work described has established foundational knowledge of its locomotion, feeding, and sensory behaviors showing its role as a benthic detritivore within reef habitats (1). At a broader scale, studies of Kāneʻohe Bay have demonstrated that reef ecosystems can remain resilient despite prolonged anthropogenic disturbance (2), providing an ecological context in which species such as *O. spectabilis* persist and continue to function within altered reef environments. Our results offer strong evidence that this species could be a valuable component of ecosystem-based management strategies aimed at improving water quality in stress impacted coastal areas.

MATERIALS AND METHODS

Data collection took place monthly at four sites at the Hawaiʻi Institute of Marine Biology on Moku o Loʻe (Coconut Island) in Kāneʻohe, Oʻahu from January 10, 2024 to October 11, 2024. The study was organized into six separate aims, each with specific objectives, experimental approaches, and data analysis procedures.

At each site, the location, number of *O. spectabilis*, substrate type categorized as sand, rubble or algae, temperature (°C), pH, DO (mg/L), DO saturation (%), and O₂ gas concentration (%) were recorded every four weeks using the PASCO Advances Water Quality Sensor and PASCO Dissolved Oxygen Sensor. To ensure data quality, the initial 30 seconds of sensor reading were removed to minimize fluctuations, and all data were screened for inconsistencies. Images were taken at 1-meter intervals up to 20 m within

the same location and area each month. ImageJ software (ImageJ 1.15K) was used to generate random points on each image to quantify the relative abundance of algae versus sand. Data analysis was conducted using RStudio (Version 2025.05.0+496; 9) and Google Sheets, where environmental variables were plotted over time for each site. Scatter plots were used to examine relationships between environmental parameters and sea cucumber abundance. Linear models were used to test for statistical significance, with p-values less than 0.05 considered significant and values less than 0.15 considered marginally significant. The number of sea cucumbers was treated as the response variable, while individual environmental parameters such as temperature and pH were used as predictor variables. All linear models were run in RStudio using the `lm` function.

Enclosure Experiments

To construct the containment boxes, two boxes were built from reused Corian sheets from a construction company trash pile, held together using zip ties and drills. Three *O. spectabilis* were placed in an experimental box for one hour. The control box had no cucumbers. Boxes were set near the "Practice Spot" site. Measurements were taken pre- and post-sea cucumber introduction. Temperature, pH, DO concentration, DO saturation, O₂ gas percentage were recorded. *O. spectabilis* individuals measuring 47–58 cm in length were collected to ensure that the size of individuals remained roughly consistent across trials. Enclosure experiments occurred on the same days as the abundance surveys. Mean and standard deviation (SD) were calculated for water quality variables before and after the presence of sea cucumbers. Box plots visualized the results (see Appendix A for code). A Welch Two Sample T-test was used to test for significant differences between the control and experimental containment boxes.

Substrate Classification through Photo Analysis

To measure ecological changes over the study period, substrate photos were taken at two timepoints: January 10, 2024 and October 11, 2024. Images were analyzed using ImageJ. A 20 cm x 20 cm virtual quadrat, meaning a digitally drawn square, was overlaid on each image. The "DrawRandomPoints2" plugin generated 20 random points per image from the software. Each point was categorized as seagrass, algae, sand, or rubble. The proportion of vegetation (seagrass, algae, rubble) to sand was calculated from point data to estimate changes in substrate composition.

In Lab Substrate Preference

O. spectabilis were used to assess substrate preference under controlled laboratory conditions. Individuals were starved for 48 hours prior to experimentation to standardize hunger levels. Each trial used one sea cucumber per tank. The tank contained all three substrate treatments simultaneously: sand, algae, or a mixed substrate consisting of a 50:50 mix of sand and algae. Tank conditions were kept consistent across trials, including a temperature of 28 °C ± 0.5 °C, salinity of 35 ppt, pH of 8.1 ± 0.1, a 12-hour light/dark cycle, gentle aeration, and provision of sand and algae substrates for movement.

Sea cucumber position and substrate association were observed over three days and recorded to determine substrate preference. Preference was quantified by the amount of time individuals spent on each substrate type. Results from these observations were used to compare relative preference among the three substrate treatments.

Climate-Projected Simulation

Four tanks were set up to test behavioral and physiological responses of *O. spectabilis* to predicted future climate conditions. Control Tank: Natural temperature (24°C) and pH (8), Experimental Tank 1: Increased temperature (+4°C, ~30°C), Experimental Tank 2: Acidified water set to 6.5 pH (via 20mL white vinegar), and a Holding Tank set based on regular conditions. Each tank contained two *O. spectabilis* (~29 cm), ~210 g of Gorilla Ogo algae, and ~195 g of sand. Temperature and pH were monitored over three days (Tetra test kit, Hydriion litmus paper, thermometer). Due to initial mortality, circulation systems were replaced with bubblers. Behavioral responses and survival rates were tracked through constant observation and monitored over three days. Behavioral responses that were tracked were movement over a certain time and quickness.

Turbidity Simulation

Three tanks were set to 93 NTU (to replicate turbid water conditions similar to those around Coconut Island). Tank 1: Two sea cucumbers, algae, and sand (280g each), Tank 2: Two sea cucumbers only, and Tank 3: Algae and sand only. Turbidity was set by collecting sediment from high abundance locations of sea cucumbers and equally adding 200g of sediment in each tank. A projection chart was used to see the changes in NTU. To process the data, all data were compiled in Google Sheets and Post It.cloud. Data were then cleaned (initial sensor fluctuations removed), formatted, and checked for outliers before analysis. Visualizations and statistics were conducted using R Studio (9).

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Appendix

Appendix A

Code used to create abundance, temp, pH, DO₂ concentration. DO₂ saturation, tide and O₂ Gas location over time.

```
#Read in Data
library(tidyverse)
library(ggpubr)
survey_data <- read.csv("DavaData.csv")

#PLOTING EVERYTHING OVER TIME - FIGURE 2
survey_data$Date <- as.Date(survey_data$Date, format="%m/%d/%y")

#Abundance
p1<-ggplot(data = survey_data, aes(x = Date, y = Number_Cucumbers, col = Location, group = Location)) +
  geom_point() +
  geom_line() +
  theme_minimal(base_size = 18) +
  labs(x = "Date", y = "Sea cucumber abundance") +
  ggtitle("A.")

#Water quality
p2<-ggplot(data = survey_data, aes(x = Date, y = Temp, col = Location, group = Location)) +
  geom_point() +
  geom_line() +
  theme_minimal(base_size = 18) +
  labs(x = "Date", y = "Temperature (°C)") +
  ggtitle("B.")

p3<-ggplot(data = survey_data, aes(x = Date, y = PH, col = Location, group = Location)) +
  geom_point() +
  geom_line() +
  theme_minimal(base_size = 18) +
  labs(x = "Date", y = "pH") +
  ggtitle("C.")

p4<-ggplot(data = survey_data, aes(x = Date, y = D02_conc, col = Location, group = Location)) +
  geom_point() +
  geom_line() +
  theme_minimal(base_size = 18) +
  labs(x = "Date", y = "D02 Concentration (mg/L)") +
  ggtitle("D.")
```

```
p5<-ggplot(data = survey_data, aes(x = Date, y = D02_sat, col = Location, group = Location)) +
  geom_point() +
  geom_line() +
  theme_minimal(base_size = 18) +
  labs(x = "Date", y = "D02 Saturation (%)") +
  ggtitle("E.")

p6<-ggplot(data = survey_data, aes(x = Date, y = O2_gas_percent, col = Location, group = Location)) +
  geom_point() +
  geom_line() +
  theme_minimal(base_size = 18) +
  labs(x = "Date", y = "O2 Gas (%)") +
  ggtitle("F.")

p7<-ggplot(data = survey_data, aes(x = Date, y = Tide, col = Location, group = Location)) +
  geom_point() +
  geom_line() +
  theme_minimal(base_size = 18) +
  labs(x = "Date", y = "Tide height (m)") +
  ggtitle("G.")

ggarrange(p1,p2,p3,p4,p5,p6,p7, nrow=4, ncol=2, common.legend = TRUE)
```

Appendix B

Code used to create Abundance vs. environmental variables.

```
##### Scatterplots - relationship between variables #####
## FIGURE 3
filtered_data <- survey_data %>%
  filter(Number_Cucumbers != 0) %>%
  filter(!is.na(Temp), !is.na(Number_Cucumbers))

# Fit the linear model
model <- lm(Number_Cucumbers ~ Temp, data = filtered_data)

# Extract R^2 and p-value
r2 <- summary(model)$r.squared
p_value <- summary(model)$coefficients[2, 4]

# Create the plot with annotations
fig3a <- ggplot(data = filtered_data,
  aes(x = Temp, y = Number_Cucumbers)) +
  geom_point(aes(col = Location)) + # Color points by Location
  geom_smooth(method = "lm", color = "black") + # One best fit line for all data
  theme_minimal() +
  labs(x = "Temp", y = "Sea cucumber abundance") +
  annotate("text",
    x = max(filtered_data$Temp, na.rm = TRUE),
    y = max(filtered_data$Number_Cucumbers, na.rm = TRUE),
    label = paste0("R^2 == ", round(r2, 2),
      " * ', ' ~ p == ", signif(p_value, 2)),
    parse = TRUE,
    hjust = 1, vjust = 1, size = 4) +
  ggtitle("A.")
```

```
#3b
model <- lm(Number_Cucumbers ~ Temp, data = survey_data)

# Extract R^2 and p-value
r2 <- summary(model)$r.squared
p_value <- summary(model)$coefficients[2, 4]

fig3b <- ggplot(data = survey_data,
                aes(x = Temp, y = Number_Cucumbers)) +
  geom_point(aes(col = Location)) + # Color points by Location
  geom_smooth(method = "lm", color = "black") + # One best fit line for all data
  theme_minimal() +
  labs(x = "Temp", y = "Sea cucumber abundance") +
  annotate("text",
         x = max(survey_data$Temp, na.rm = TRUE),
         y = max(survey_data$Number_Cucumbers, na.rm = TRUE),
         label = paste0("R^2 == ", round(r2, 2),
                        " * ', ' ~ p == ", signif(p_value, 2)),
         parse = TRUE,
         hjust = 1, vjust = 1, size = 4) +
  ggtitle("B.")

#3c
model <- lm(Number_Cucumbers ~ PH, data = survey_data)

# Extract R^2 and p-value
r2 <- summary(model)$r.squared
p_value <- summary(model)$coefficients[2, 4]

fig3c <- ggplot(data = survey_data,
                aes(x = PH, y = Number_Cucumbers)) +
  geom_point(aes(col = Location)) + # Color points by Location
  geom_smooth(method = "lm", color = "black") + # One best fit line for all data
  theme_minimal() +
  labs(x = "pH", y = "Sea cucumber abundance") +
  annotate("text",
         x = max(survey_data$PH, na.rm = TRUE),
         y = max(survey_data$Number_Cucumbers, na.rm = TRUE),
         label = paste0("R^2 == ", round(r2, 2),
                        " * ', ' ~ p == ", signif(p_value, 2)),
         parse = TRUE,
         hjust = 1, vjust = 1, size = 4) +
  ggtitle("C.")
```

```
#3d
model <- lm(Number_Cucumbers ~ D02_conc, data = survey_data)

# Extract R^2 and p-value
r2 <- summary(model)$r.squared
p_value <- summary(model)$coefficients[2, 4]

fig3d <- ggplot(data = survey_data,
               aes(x = D02_conc, y = Number_Cucumbers)) +
  geom_point(aes(col = Location)) + # Color points by Location
  geom_smooth(method = "lm", color = "black") + # One best fit line for all data
  theme_minimal() +
  labs(x = "D02 (mg/L)", y = "Sea cucumber abundance") +
  annotate("text",
         x = max(survey_data$D02_conc, na.rm = TRUE),
         y = max(survey_data$Number_Cucumbers, na.rm = TRUE),
         label = paste0("R^2 == ", round(r2, 2),
                        " * ', ' ~ p == ", signif(p_value, 2)),
         parse = TRUE,
         hjust = 1, vjust = 1, size = 4) +
  ggtitle("D.")

ggarrange(fig3a, fig3b, fig3c, fig3d, nrow=2, ncol=2)
```

Appendix C

Code used to conduct T-tests for enclosure experiments.

```
library(tidyverse)

boxdata <- read.csv("enclosuredata.csv")

# Variables to plot
variables <- c("Temp", "PH", "D02_conc", "D02_sat", "O2_gas_percent")

# Conduct t-tests for each variable and print results
for (var in variables) {
  # Filter out NAs
  filtered_data <- boxdata %>% filter(!is.na(var)) %>% # Filter out negative values
    filter(Timing == "After")

  # Perform t-test
  t_test_result <- t.test(as.formula(paste(var, "~ Treatment")), data = filtered_data)
  print(paste("T-test for", var))
  print(t_test_result)
}
```