

**The Influence of Acute Thermal Stress on the Biodeposit Production of Marine Bivalves:
Comparative analysis between *Ruditapes philippinarum*, *Mytilus galloprovincialis*, and
*Crassostrea gigas***

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Abstract

As the consequences of climate change and rising ocean heat content, discrete periods of extreme regional ocean warming called marine heatwaves (MHWs) are observed to become more frequent and severe. Intense heating occurs regionally and can have devastating effects on the marine ecosystem. As many as 1 billion marine animals in the intertidal zone died during a historic record-breaking marine heat wave (MHWs) in the Pacific Northwest. The effects of the heatwave were especially detrimental to marine invertebrates residing in the intertidal zone; an unprecedented number of mussels, clams, barnacles, snails, and sea stars died struggling to survive acclimates to the abnormal and unrelenting heating. The induced mortality for bivalves such as mussels, oysters and clams can have dire impacts on the condition of the ecosystem because they are important food sources to larger animals and play a significant role in inhibiting excess phytoplankton production. Thus, an investigation on the influence of MHWs on marine bivalves that are ecologically and economically important can help guide policy making regarding the conservation of marine ecosystems. This study explores the influence of acute thermal stress on the biodeposit of Pacific oyster *Crassostrea gigas*, manila clam *Ruditapes philippinarum*, and Mediterranean mussel *Mytilus galloprovincialis* by collecting and weighting the fecal matter over a three-day period. The data collected from this study suggests a significant influence of thermal stresses on the feces production of *C. gigas* and *R. philippinarum* while *M. galloprovincialis* appears to be unaffected.

Introduction

The ocean covers more than 70 percent of the Earth' surface; its ability to store and release heat over prolonged periods of time makes it one of the main components in stabilization of the climate system (Oliver et al, 2018; Oliver et al, 2020). Heat absorbed by the ocean is

moved from one place to another through the ocean currents; it eventually re-enters the rest of the Earth system through various ecological processes. The ocean's average temperature has increased by 1.5°C in the last century, and for the past 10 years average annual ocean temperatures have been the highest ever recorded; the average long-term temperature shift associated with ocean warming has been estimated at just over 20 kilometers per decade (Marine Pollution Bulletin, 2020). In addition to this long-term, persistent warming, discrete periods of extreme regional ocean warming called marine heatwaves (MHWs) are becoming more frequent and severe (Green et al, 2018). The occurrences of MHWs have been found to increase by 50% over the past decade; it has observed displaced temperatures an average of approximately 200 kilometers in a matter of months. The MHWs are shifting ocean temperatures at similar scales to what is predicted with climate change models but in much shorter time frames (Penduff et al, 2018). They can affect small areas of coastline or span multiple oceans; studies have indicated major MHWs have the potential to alter the composition of aquatic species in various ecosystems around the world (Olsen & Klinger, 2020; Oliver et al, 2021). MHWs have been recorded in surface and deep waters, across all latitudes, and in all types of marine ecosystems negatively affecting the fisheries, aquaculture, and tourism industries (Theuerkauf et al, 2022).

According to preliminary data, marine organisms experienced high mortality during a historic record-breaking marine heatwave in the Pacific Northwest, killing as many as 1 billion marine animals in the intertidal zone (ICNU, 2018). The effects of the heatwave were especially detrimental to marine invertebrates residing in the intertidal zone; an unprecedented number of mussels, clams, barnacles, snails, and sea stars died during the extreme and unrelenting heating (Shanks et al, 2020). The induced mortality for bivalves such as mussels, oysters and clams have dire impacts on the well-being of the ecosystem because they are important food sources to

larger animals and play a significant role in inhabiting excess phytoplankton production (Dame, 2012). Improved understanding on the influence of MHWs on marine ecological systems can have major implications on policy making regarding conservation efforts. This study will concentrate the effort on investigating the influences of acute thermal stresses on marine bivalves that are ecologically and economically important. Furthermore, evidence shows that appropriately located and managed aquaculture operations can provide a broad and positive range of interactions with local environments including ecosystem services that have positive effects on coastal habitat conservation and restoration efforts.

Three species of bivalve shellfish were chosen to be the target of this investigation, each have unique physiology, habitat preference, and tolerant level for heat: The Pacific oyster, *Crassostrea gigas*, native to Japan is the most important aquaculture species on the West Coast of the United States. Oysters help stabilize sediment, provide habitat, and improve water quality and are an important estuarine ecosystem foundation species. Oysters are among the most-farmed marine species, however, the *C. gigas* are invasive and tend to outcompete the native species, thus changing the ecosystem dynamic by forming a highly dense oyster bed. The manila clam, *Ruditapes philippinarum*, could have negative impacts on native ecosystems in some regions due to their ability to grow in high densities that could achieve filter-feeding at high enough rates to alter regional food webs; however, this clam has been determined to be a sustainable aquaculture product. The Mediterranean mussel, *Mytilus galloprovincialis*, is widely distributed in both the Northern and Southern hemispheres; often observed to be dominant inhabitants on hard substrates of the intertidal and nearshore habitats. *M. galloprovincialis* are also considered to be one of the most-farmed marine species.

The goals of this study were to (1) investigate how acute thermal stress affects the marine bivalves as a whole, (2) explore the differences in influence of temperature on three taxonomically distinct species of bivalve (*C. gigas*, *R. philippinarum*, and *M. galloprovincialis*). I hypothesize that all species of bivalves will exhibit a lower production of pseudofeces as the temperature increases, thus an inverse linear relationship will be observed between the accumulation of fecal matter and degrees of thermal stress. I hypothesize that there will be detectable statistical differences in fecal matter production among various species of bivalves correlated to the degree of thermal stresses (ambient, moderate, and extreme).

Method

Specimens acquisition and Acclimation procedure:

Forty-five bivalve individuals were obtained through local shellfish farms (Westcott Bay Shellfish Co) on Friday Harbor Island. Specimens were iced and immediately transported to the established seawater table at ambient temperature at the Friday Harbor Laboratories; each sample was placed in a labeled glass bowl. All 45 bivalves were acclimated under ambient temperature for 24 hours before separation into tanks with specific temperatures for further acclimation for an additional 24 hours. The control tanks were maintained in ambient temperature (9°C ~ 11°C), treatment group 1 was maintained at approximately 13°C to simulate moderate, and treatment 2 was maintained at approximately 18°C to simulate extreme heatwave conditions; each tank held approximately 56 gallons of seawater and set at low flow rate. Five randomly selected individuals from each species were chosen; each group consisted of 15 specimens that were randomly positioned on the efflux end of the tanks (Figure 1). The moderate heating sea tank set at 13 °C was aimed to simulate the heatwaves that have been seen in the recent past (Shanks et

al, 2020). The extreme temperature set at 18 °C was set to simulate a more severe heatwave that could occur in the future (Jacox, 2019).

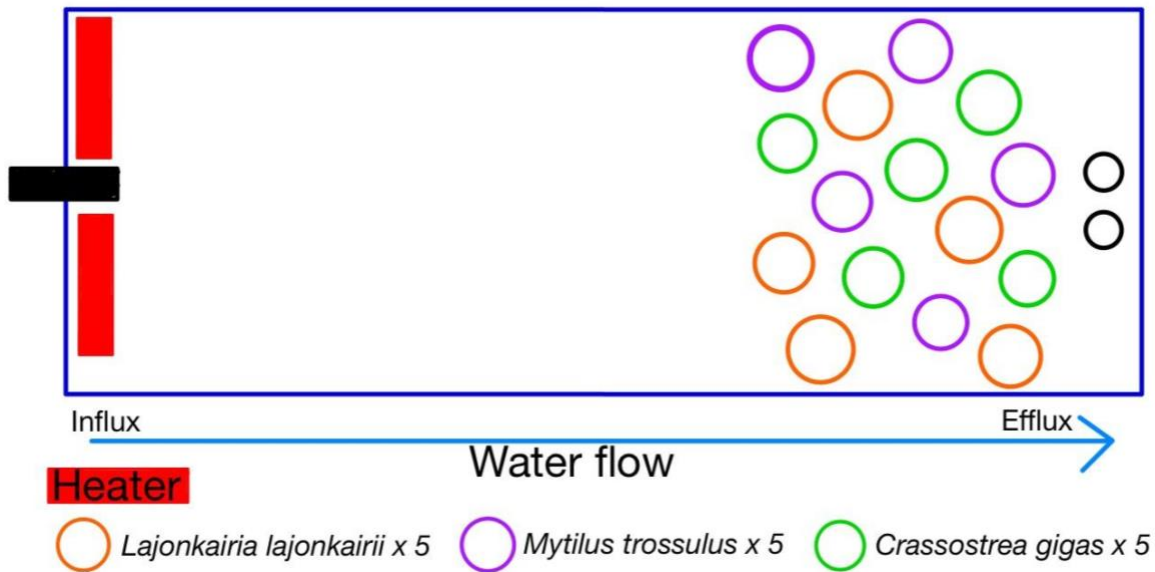


Figure 1. The diagram of the experimental set up. (1) Control with no heater maintained at ambient temperature, (2) Treatment with 2 heaters at 13 °C, (3) Treatment with 3 heaters at 18 °C. The black bar on the left of the tank represents the pumping for the influx of seawater while the two smaller black circles on the right end of the tank represent the drilling allowing the excurrent of water.

Data collection and analysis procedure:

Each glass bowl containing specimens was gently removed from the tank and transported to the laboratory for sample preparation. The fecal matter was collected using a vacuum filtration technique on the highest flow of water to remove moisture from the sample (Figure 2); filtration procedures were run for approximately 4 minutes on each sample to ensure complete removal of moisture from fecal matter and filter paper. Each sample was then weighed using a precision scale (manufactured by METTLER; Model: AE100) and the weight of the filtering paper was omitted from the collected data at the end. The initial wet weight of each mollusk was collected

on the first day of collection; the tissue weight omitting shells was collected on the last day after euthanization through freezing for approximately 12 hours.

To assess whether the biodeposit production of bivalves was statistically significant under various degrees of thermal stress level a two-way analysis of variance (ANOVA) was done to assess the performance of bivalves followed by the Tukey Test (Honest Significant Difference); alpha value was set to be 0.05. All figures and analyses were done with RStudio version 2022.02.2 (R Core Team, 2022).

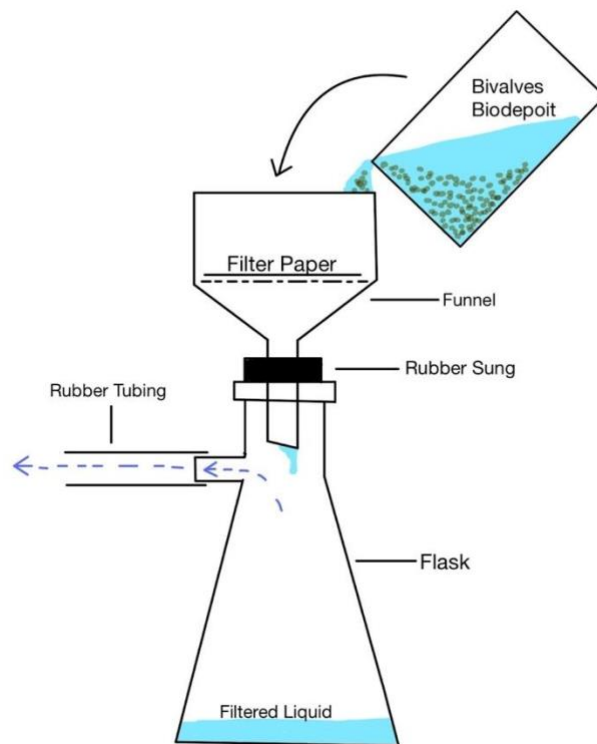


Figure 2. Diagram of vacuum filter used in the data collection procedure. Filter paper (manufactured by Whatman; 9mm (about 0.35 in)) was replaced after each collection. Each batch of collection was done three times with 24 hours interval; a total of 135 samples were collected during the study.

Results

Overall Trend:

A unique characteristic of fecal matter production among each species were observed in the result of this study, the scatterplots constructed from the data collected from the study show separate clusters of each corresponding species to their initial weight; pseudofeces production varied by species (Figure 3). A noticeable pattern for fecal accumulation was also observed as the mollusks are exposed to various degrees of thermal stress; evidence of pseudofeces production influenced by the degree of warming were visualized in the scatterplots (Figure 4). This phenomenon can be clearly visualized in the box plot constructed for the correlation between thermal stress level and feces weight of each species under the influence of acute temperature challenges; pseudofeces production increased in response to temperature in clams and oysters, but not mussels; the pacific oysters showed the most pronounced response to temperature (Figure 5).

ANOVA and Post Hoc test:

The observation of a distinct amount of accumulation of feces between each species was supported by the statistical test performed using the data from this study; it shows statistically significant between biodeposit production differently among species (two-way ANOVA; $p = 4.02e-08$). The correlation between biodeposit production of bivalves and various degrees of thermal stress (two-way ANOVA; $p = 2.32e-09$).

The relationship between species of bivalves and degrees of thermal stress were also explored using a post the Tukey Test (Honest Significant Difference): the differences in feces production of mussel and clam were revealed to be statistically significant ($p = 1.0e-07$). Similar relationships were revealed between oyster and clam ($p = 1.29e-06$). On the other hand, no

statistical significance difference was concluded by the post hoc test for fecal matter production between oyster and mussel ($p= 0.5433227$). The influences of thermal stress treatments on the fecal matter production of marine bivalves were also discovered; there are statistically significant differences in the feces production between moderate and ambient stress level ($P= 2.0e-07$). Similar results were produced on the fecal matter accumulated between extreme and ambient levels of stress (P -value: <0.00001). However, the biodeposit production of the groups exposed to extreme and moderate thermal treatment were determined to have no statistically significant differences ($P= 0.96422857$).

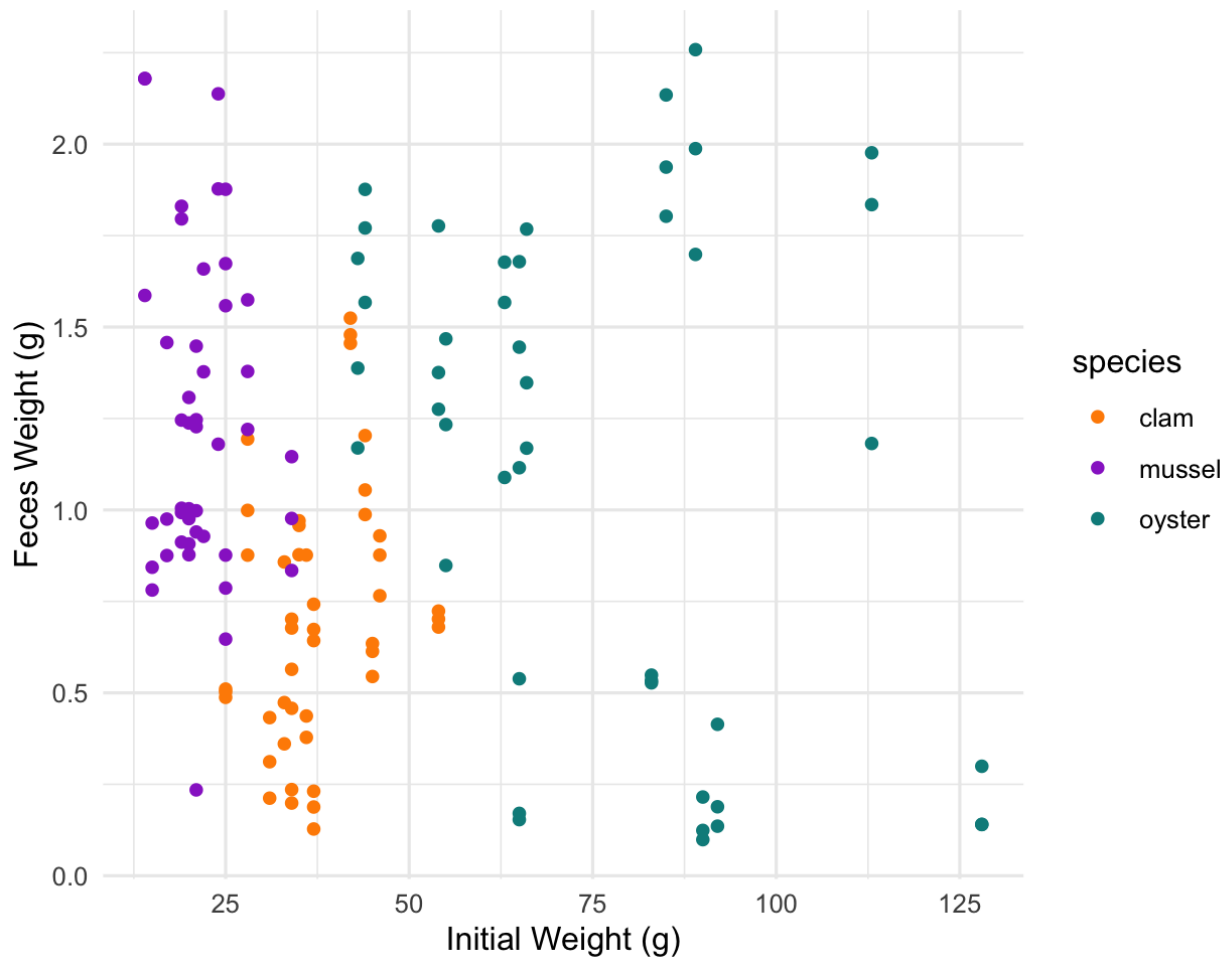


Figure 3. The scatterplot of initial weight of each species versus the weight of the feces. Two-way ANOVA reveals a statistically significant difference between feces produced by species and weight of the bivalves (P -value: $4.02e-08$).

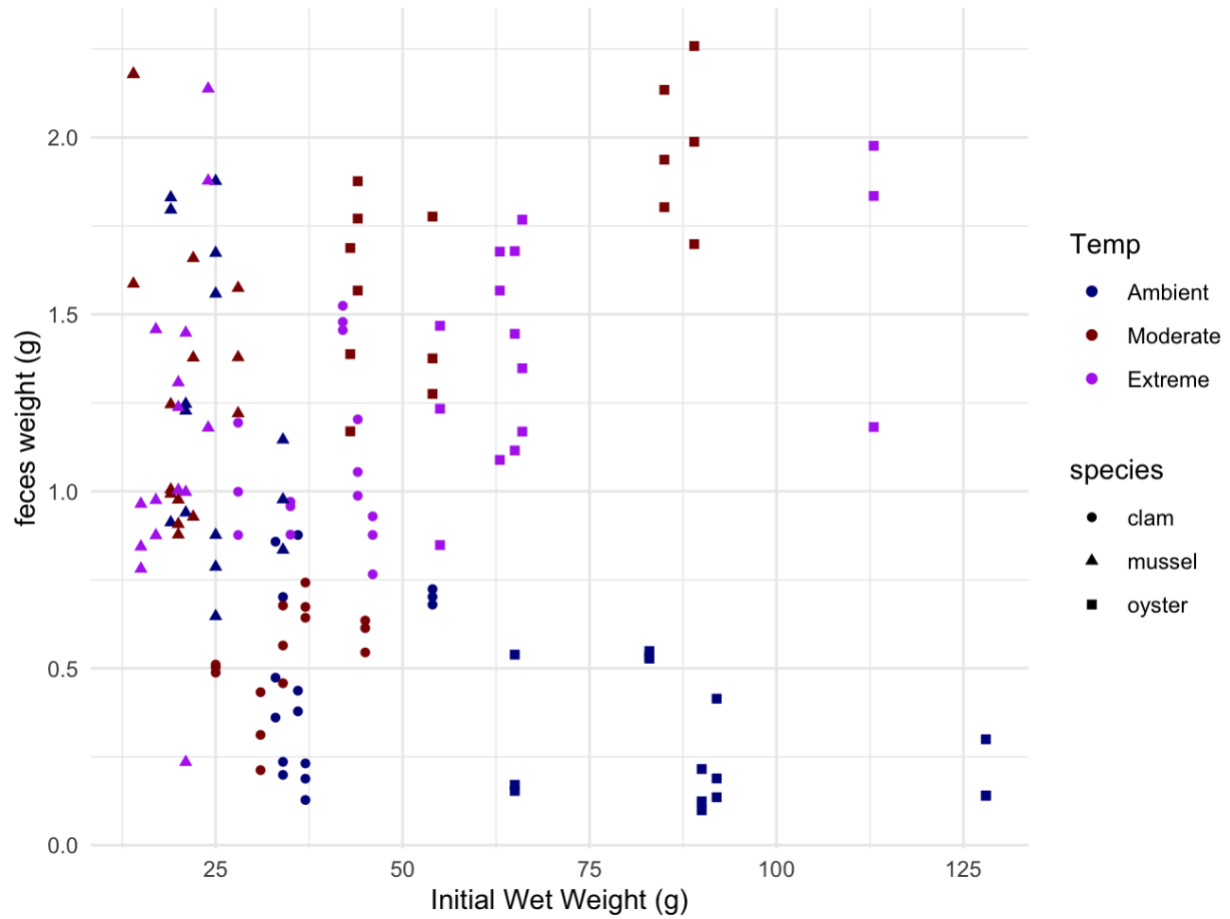


Figure 4. The scatterplot of initial weight of each species versus the weight of the feces including the levels of thermal stress. Two-way ANOVA concludes a statistical difference between the feces produced by species under various degrees of thermal stresses and their weight (P-value: 2.32e-09).

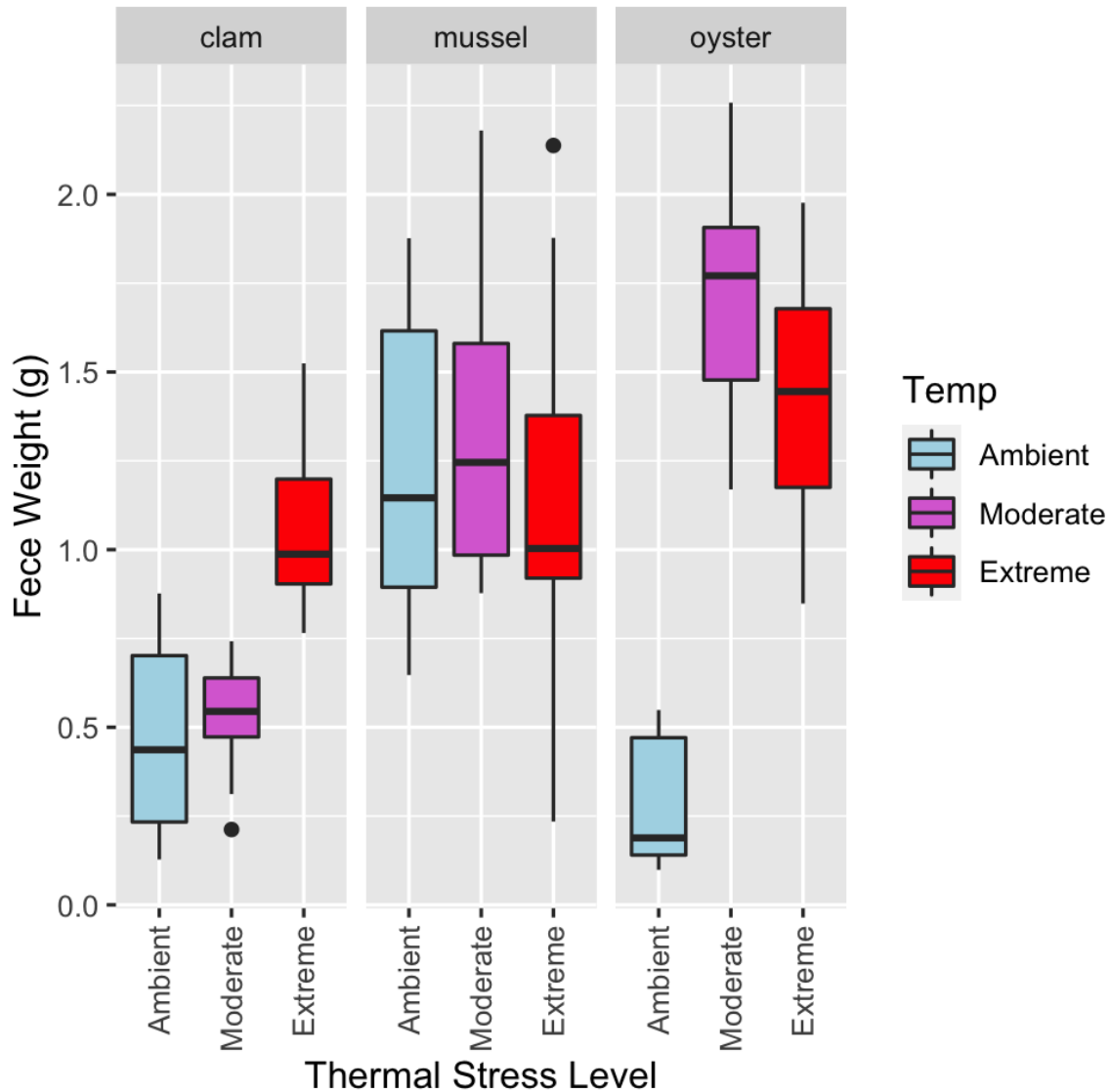


Figure 5. The box plot of thermal stress level versus initial weight. Note the various distribution of feces weight among the three species as thermal stressor was introduced.

Discussion

The results show that acute temperature warming has various degrees of influence on the biodeposit production of bivalves which vary among species. Among all three species of bivalves investigated in this study, *C. gigas* appear to exhibit the highest rate of fecal matter production under moderate thermal stress and follow by extreme level of heating; it's difference in feces production were the most distinct among degrees of thermal stress level. The increase of

fecal production could be the defensive trait since prior research has revealed that MHWs has the potential to cause mass mortality of *C. gigas* by causing specific members of the oyster's bacterial community to proliferate and potentially overwhelm the oyster's immunological capacity (Green, 2018). Furthermore, this correlation between higher production of feces and degrees of thermal challenges could be a consequence of increased metabolism of bivalves as they are exposed to higher temperature; prior studies that quantified thermal tolerance of mollusks has shown an increase of metabolism rate due to the increase of temperature (Tomanek et al, 2010). The decrease of feces production from moderate to extreme heating treatment could be an indication of *C. gigas* exceeding thermal threshold; the same pattern of decline was also reported in prior studies (Shanks et al, 2020). The pronounced responses of *C. gigas* to acute temperature challenges could also be the lack of adaptation since they were native to the world with colder regions.

Although less distinct compared to *C. gigas*, *R. philippinarum* were also observed to experience some degree of influence by the level of thermal treatments especially as they are exposed to extreme heating around 8 °C above ambient temperatures where they produced significantly more fecal matter. However, they are largely unaffected by the moderate heating. This suggests the possibility of *R. philippinarum* having higher heat tolerance than *C. gigas* which would be consistent with findings from prior research about clams having higher heat tolerance as they undergo selective breeding in aquaculture (Acquafredda et al, 2021).

Among all three species of bivalves included in this study, *M. galloprovincialis* is the most heat tolerant species; their production of fecal matter was unaffected by the acute thermal challenges. *M. galloprovincialis* is considered the most heat tolerant species of the three compared to its closely sister taxa, *Mytilus edulis* and *Mytilus trossulus* which could explain the

trend of this species under influence of various degrees of thermal stresses (Tomanek et al, 2010). The behavior patterns of *M. galloprovincialis* during acute thermal challenges occurred in this study reveal their remarkable level of thermal tolerances which could be the result of trait adaptation from their native habitat.

Although the results reported here are consistent with prior studies, some sources of error could have occurred during the preliminary stages of experimental set up: Due to the underestimation of heating capacity of aquarium heaters, the abnormally elevated temperature has caused all species in treatments tanks to release gametes (induced-spawning); no mortality was associated with this incident. One mussel was replaced during the acclimation procedure due to mortality in the control treatment.

The results from this study cause me to reject the null hypothesis; temperature of the environment and species does influence the feces production of bivalves. The results also contradict the alternative hypothesis; an increase of the production of pseudofeces as the temperature increases were recorded for *C. gigas* and *R. philippinarum*, a positive relationship occurred between the accumulation of fecal matter and degrees of thermal stress. Detectable differences in fecal matter production among three species of bivalve were associated with the degree of thermal stress. The production of fecal matter of bivalves seems to correlate to the thermal threshold in prior studies, however, more studies are needed to confirm its usefulness as a metric of welfare. At the meantime, the method implemented in this study could be used as supplementary quantification of fitness under abnormal heating conditions along with other more sophisticated methods such as utilization of metabolic rate or of heat shock protein.

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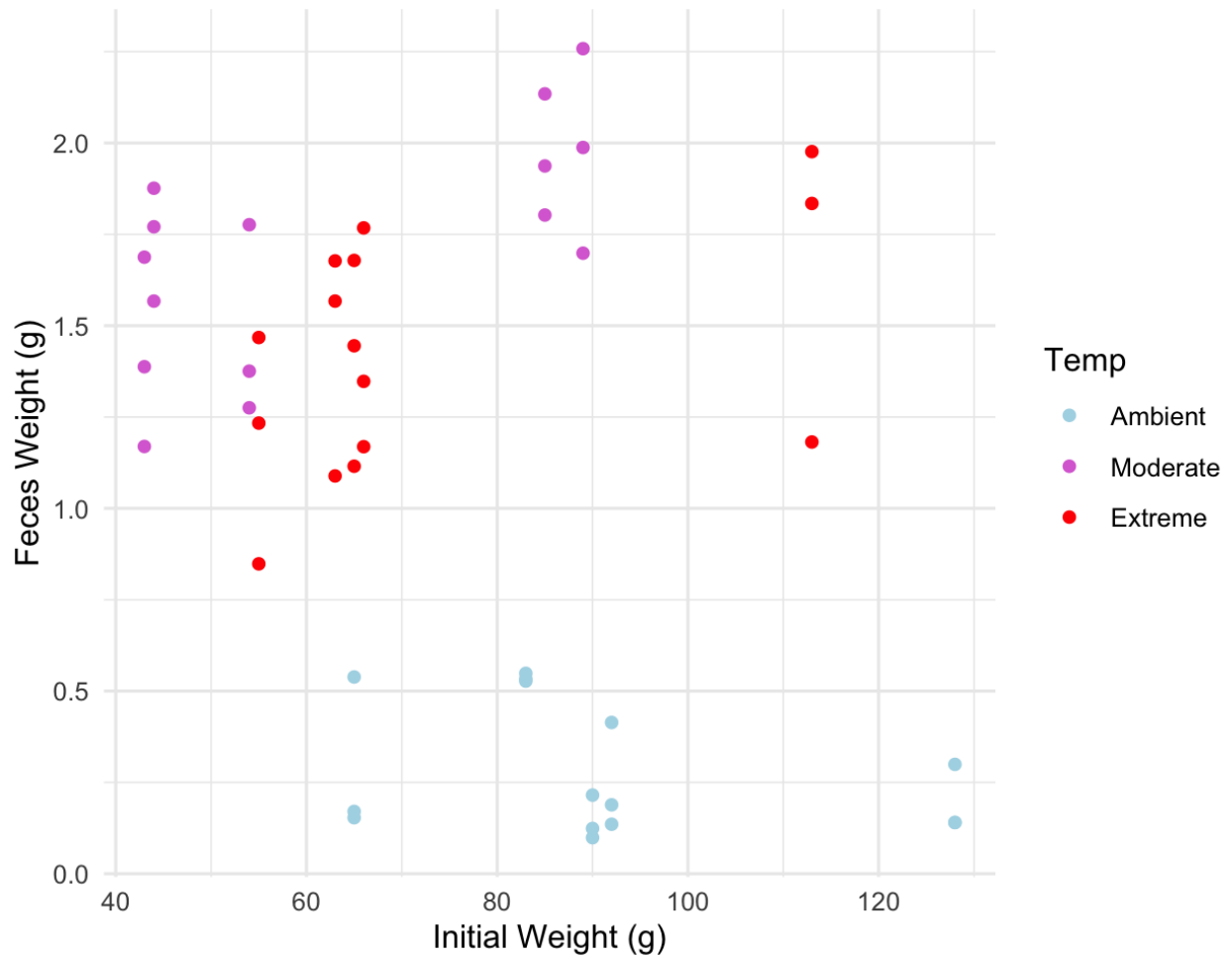


FIG. S1. The scatterplot of initial weight versus feces weight for the Pacific Oysters, *Crassostrea gigas*

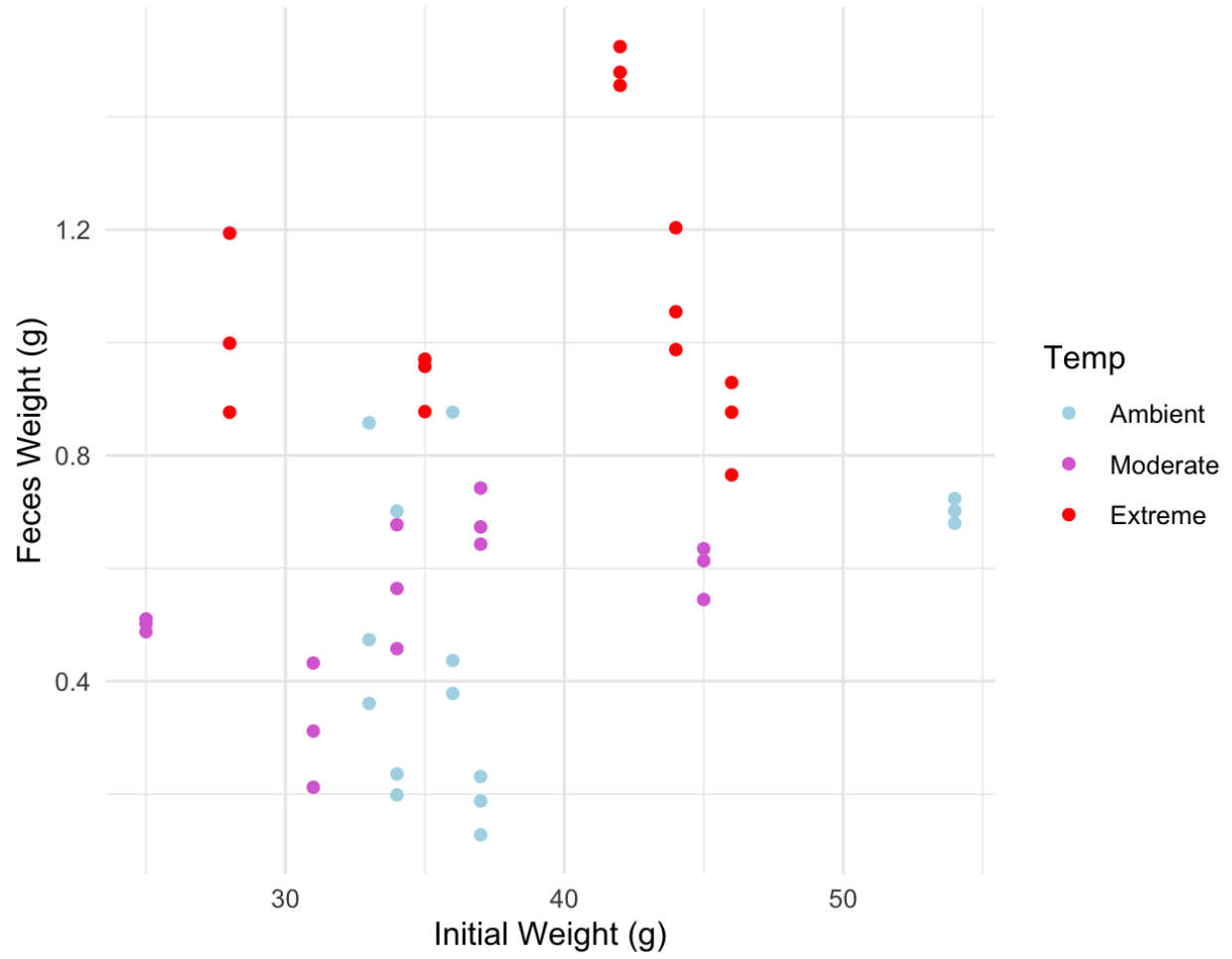


FIG. S2. The scatter plot of the initial weight versus feces weight for the Manila Clam, *Ruditapes philippinarum*

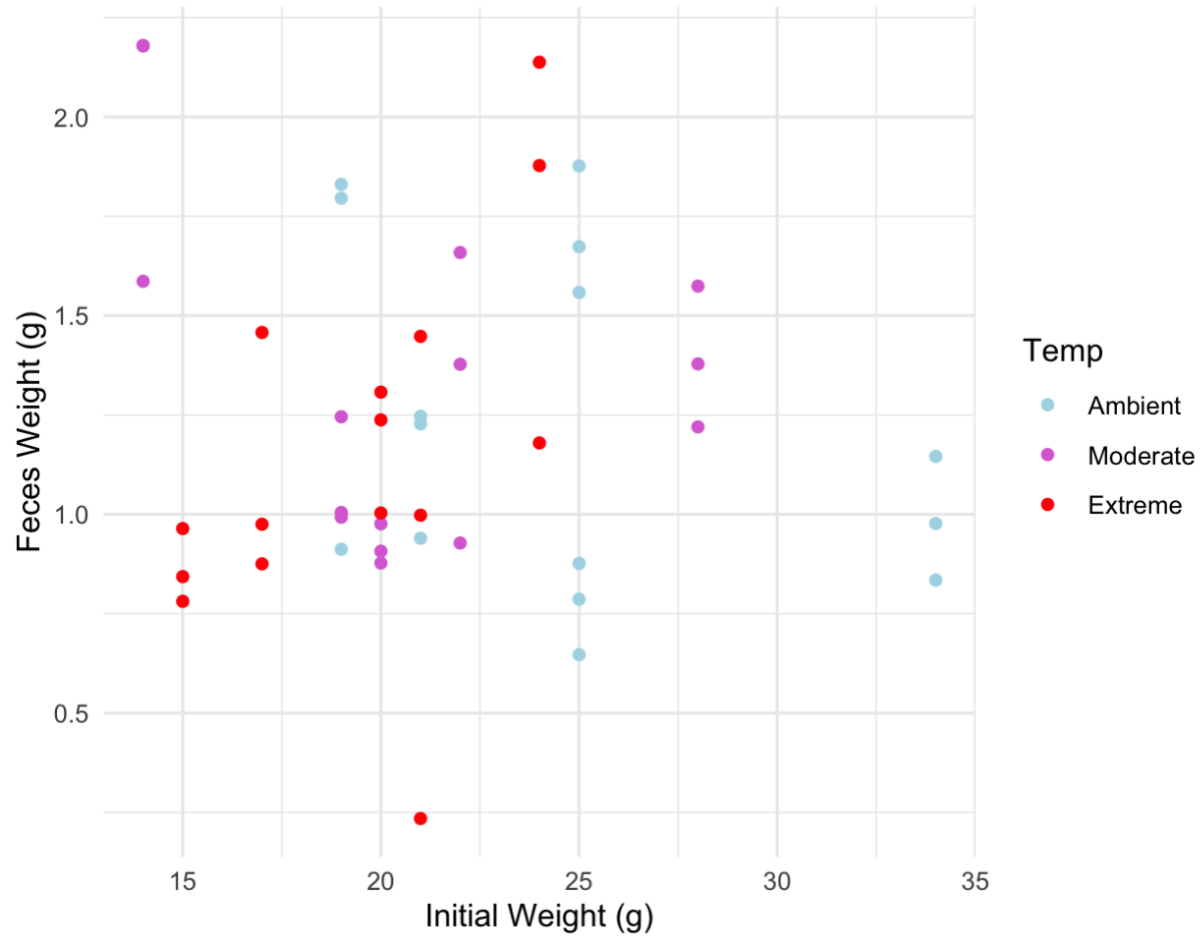


FIG. S3. The scatter plot of the initial weight versus feces weight for the Mediterranean mussel, *Mytilus galloprovincialis*.