Foraminifera: Heterotrophic and Autotrophic Indicators For Climate Change

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Abstract

Foraminifera are microscopic organisms that have been around for millions of years in marine environments. These organisms can act as indicators for changing environmental conditions, based on the species present in a certain habitat. The reason biological indicators, like Foraminifera, are needed is due to the changes the ocean is experiencing from climate change. This study explores Foraminifera diversity, the spatial scale of Foraminifera changes at Akumal and Punta Maroma in the Yucatán Peninsula, and the varying amount of heterotrophs and autotrophs. Overall, 43 samples were analyzed from sediment, from 18 different transects at three different sites. After literature research, Foraminifera identification, and developing research ideas, data was collected by using a dissection microscope to identify Foraminifera from the three sites. There were two research questions created for this experiment: 1) What is the most common Foraminifera species? and 2) At what scale does the ratio of functional groups of Foraminifera vary? From past research it is hypothesized there will be more heterotrophs, so it is predicted that *Discogypsina vesiularis* will be the most common species due to its location in the Yucatán Peninsula region. It is hypothesized that the location will play a larger role than site since Akumal is in an area that is more nutrient-rich, which is a type of environment that has been proven to have more heterotrophs. The research questions were investigated by using the data in numerous tables and RStudio plots. In conclusion, the most common Foraminifera species was actually an autotroph and not a heterotroph, and the scale for ratio of functional groups in Foraminifera varies on site and not location. Further research with different environments would show the varying effects of environmental indicators, causing more diversity to take place, with many more species and feeding styles. The knowledge that site spatial scales matter more with Foraminifera will help these organisms be more accurate as environmental indicators.

Keywords: *Foraminifera, Heterotrophs, Autotrophs, and Abundance*

Introduction

What are foraminifera?

Foraminifera, also known as forams, are single-celled protists with shells (Wetmore). Their shells are made of multiple chambers, with the internal shell known as the test. Like most shelled organisms in the ocean, they are made of organic compounds, calcium carbonate, crystalline aragonite, or cemented sand particles (Wetmore and Ocean World). Forams have been around for millions of years, with their fossils being found at the bottom of the ocean among the sand (Wetmore). There are actually two different classes of forams, the majority are benthic forams that live on or near the seafloor and the rest are planktonic forams that float freely (Ocean World). Forams vary in their sizes, and are best viewed under a microscope. They range from 100 micrometers to almost 20 centimeters long (Wetmore).

Heterotrophic forams are either opportunists or other small taxa. An opportunist is an organism that is able to sustain its life from different sources (Biology Dictionary Online). In favorable conditions it immediately takes advantage of the opportunity to thrive. Benthic forams use their pseudopodia for locomotion, and heterotrophic forams use their thin pseudopodia to catch food by extending it from its shell (Ocean World). Autotrophic forams gain energy from photosynthesis and feeding. Sunlight and inorganic nutrients provide food and enhance calcification (Wetmore). The photosynthetic process is limited by the amount of sunlight, carbon dioxide, nitrogen, and both simple sugars and glycerol (Biology Dictionary Online). The sugars and glycerol produced by the foram helps the foram's host, and the foram will uptake the host's metabolites, creating a symbiotic relationship.

Forams are good ecological indicators, which explains why certain species live at different sites. They are ecological indicators, showing different environments of the present and past causing each species to require certain conditions. Forams can indicate sediment quality, heavy metal pollution, organic pollution, and water quality (A'ziz 2021). They can also trace lagoonal and forereef sediment after storm movement, specifically carbonate sediment sources (Li et al. 1997). In addition, forams can indicate oxygen isotopes in shells, help find oil sites, and aid research with a tracer species of Foraminifera (Li et al. 1998). Scientists use forams for research because they are small for studying, abundant in the ocean, over 500 billion years old, differ in shape with time and depth, have short reproductive cycles, and have trace elements in their shells (Ocean World).

What is the issue?

Reef death doesn't matter as much as maintaining the actual structure. Bioerosion, climate change, and other losses of reef structure prevents more reef growth, harming future recovery (Perry et al. 2013). Over the past four decades there has been a decline in architecture and complexity of the Caribbean reefs (Alvarez-Filip et al. 2009). This affects reef biodiversity and the overall ecosystem. Forams in the reef environment are indicators of the environmental conditions present, based on the species. Forams are also an important part of the marine food web (Ocean World).

What is the research question?

Overall, 43 samples were analyzed from sediment at Dick's and Yal-Ku in Akumal, as well as Punta Maroma, all sites along the coast of the Yucatán Peninsula. Samples were collected from three main sites: 12 from Dick's, 13 from Yal-Ku, and 18 from Punta Maroma. At each site, there was data collected from six different transects. There were three main ideas for data gathering, based on past foraminifera investigations: document the amount of the observed foraminifera, the identification of foraminifera, and the feeding type. There were two research questions created for this experiment: 1) What is the most common Foraminifera species? and 2) At what scale does the ratio of functional groups of Foraminifera vary? From past research it is hypothesized there will be more heterotrophs, so it is predicted that *Discogypsina vesiularis* will be the most common species due to its location in the Yucatán Peninsula region. It is hypothesized that the location will play a larger role than site since Akumal is in an area that is more nutrient-rich, which is a type of environment that has been proven to have more heterotrophs.

Materials & Methods

The materials used for the research included Google Scholar for literary research, sediment samples collected by Victor Rodriguez Ruano from spur and groove reefs in the Yucatán Peninsula, a dissection microscope, and RStudio. Excel and Google Documents were used to organize all of the information gathered.

The methods of research involved working through six main steps. First, to explore the world of Foraminifera I read many articles. These varied from websites, journal articles, and databases explaining the history of Foraminifera, their modern scientific use, and the reason they are needed. The literary research was needed to set the baseline of information, and see what research has been done previously with Foraminifera. Second, the foram identification and function were researched, to better understand the differences between species, specifically when examining them underneath a microscope. Each species of foram has a different appearance, scientific name, and habitat. The function and identification of Foraminifera have been greatly explored by researcher Dr. Pamela Hallock Muller.

Third, I worked on determining research ideas, to form the research questions needed to help start the actual research project itself. Although the topic, Foraminifera, was determined, a more focused view was needed. Samples had already been collected from spur and groove coral reefs in the Yucatán Peninsula area. I used previous research gained from using a microscope and CellSense on the computer to focus on the more abundant foraminifera at the three sites, shown in Diagram 1. Out of the 43 samples total, data was collected from each site – Dicks (D), Yal Ku (YK), and Punta Maroma (PM) – with six transects and varying samples at each. Data collection was random by only identifying a random 300 grains from each sediment sample.

After viewing more samples and continuing Foraminifera research, ideas to analyze foram species amounts, scale of the ratio of functional groups, and feeding styles arose. The two research questions and the hypotheses were then made.

Fourth, I began the data collection of each of the 43 samples. This process took a series of seven steps. First, a sediment sample was mixed. Second, a 63 micrometer sieve was used to gather the larger sand grains, shells, and Foraminifera to put in a Petri dish. Third, a black surface was used with the dissecting scope to better see the Foraminifera and identify them. Fourth, 300 random grains were counted and if Foraminifera they were further identified. Fifth, the heterotrophs and autotrophs were documented. Sixth, a brush was used to clean off the Petri dish and other materials. Seventh, after all 43 samples were examined, analyze the results.

Fifth, I documented the data collected with numerous Excel sheets. The table for identification was created to display the twelve different species, shown in Table 1. The information contains the Genus and species, a general image, an image from Dr. Hallock's identification process, a description, and the resource used. Sixth, I gathered all of the research to analyze by using RStudio. The data that needed analyzing through RStudio was the species, abundance, site, and feeding type: heterotrophic and autotrophic. The plots are shown in the Results while the code is shown in Appendices. Seventh, and lastly, I used all of the data and analyses to formulate a paper documenting the research process. The types of research topics involved were general Foraminifera, Caribbean reef impacts on Foraminifera, dissection scope Foraminifera identification, and Foraminifera analysis.
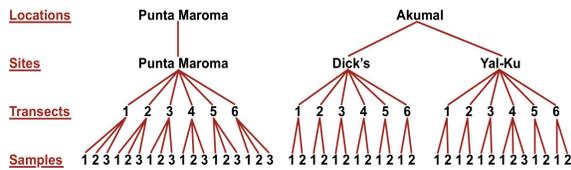
Diagram 1: Experimental Design. This diagram shows the number of locations, sites, transects, and samples used overall in the Yucatán Peninsula.
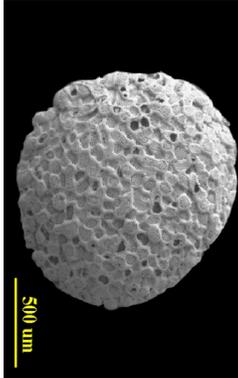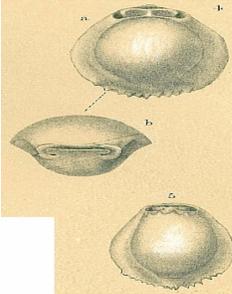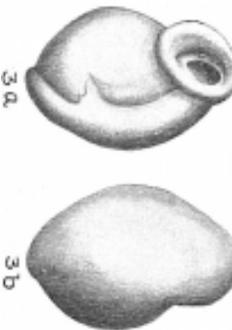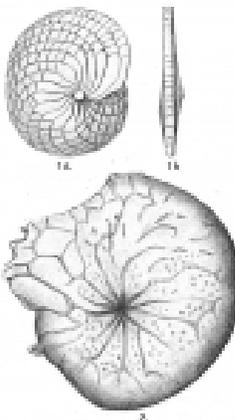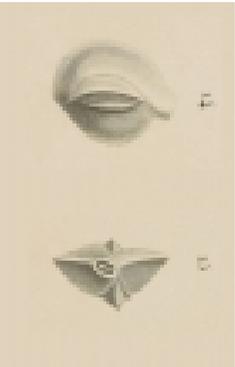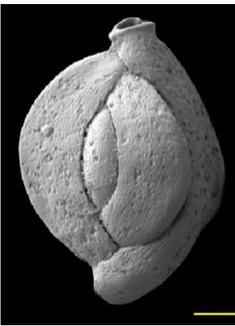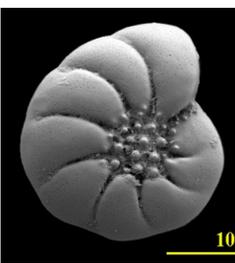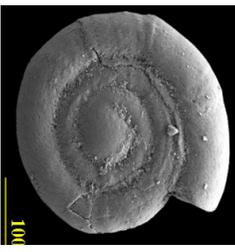
Results

After deciding to explore identification, abundance, feeding type, and site of Foraminifera, the information needed to be gathered to later be analyzed using RStudio. The Foraminifera that were identified throughout the study were found by genus and species, image, and description in Table 1. The Foraminifera were studied at three major sites shown in Table 2. These were Punta Maroma, Dick's, and Yal-Ku. The abundance of each of the Foraminifera identified is shown in Figure 1 in decreasing order of abundance. The type of feeding is also color coded for ease of seeing the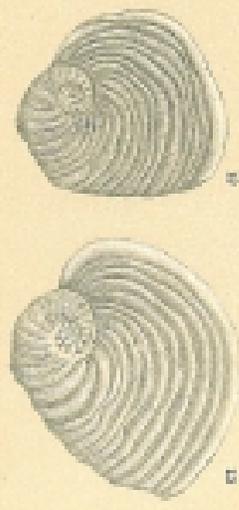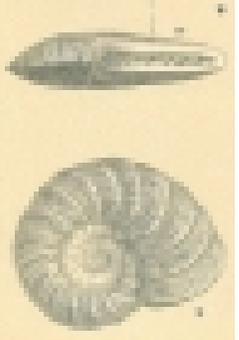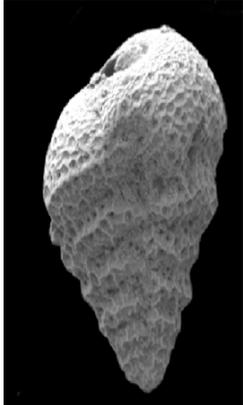 difference. The Foraminifera types varied based on the site, and these variations are shown in Figure 2. The Foraminifera varied in ratio from heterotroph to autotroph at each site, and this was shown by running a one way ANOVA. Median and median absolute deviation numbers were found since it is non-parametric. The Foraminifera varied in abundance at each site, and this was shown by running a one way ANOVA in Figure 3. The data calculated during the ANOVA are shown in Table 3. Median and median absolute deviation data were used to show the relationships between sites, according to the letters above the error bar at each site. The site and type relationship and the type and site relationship were shown to be different by using a factorial ANOVA, also known as a two way ANOVA, in Figure 4 and Figure 5. The site and type predictors were changed in each, with the abundance as the response. The data calculated during the ANOVA is shown in Table 4. The correlation between heterotrophs and autotrophs varied at each site. Figure 6 shows Punta Maroma, Figure 7 shows Dick's, and Figure 8 shows Yal-Ku. The Pearson correlation that was run at each site shows the three variations.

Table 1: Foraminifera Identification. This table shows twelve different Genus and species of Foraminifera. This includes the details of images, descriptions, and resources.

Genus species	Image	Foram Guide	Description	Resource
Spiroloculina arenata (Cushman)			Smaller Miliolids Other Small Taxa: Miliolida, Most Families and Genus except larger taxa Location: Cosmopolitan	https://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=417753

Discogypsina vesicularis (Silvestri)			Other Small Perforate Taxa Other Small Taxa: Rotaliida, Most Families and Genus Location: Cosmopolitan	https://www.marinespecies.org/aphia.php?p=taxdetails&id=417982
Pyrgo denticulata (Brady)			Smaller Miliolids Other Small Taxa: Miliolida, Most Families and Genus except larger taxa Location: Cosmopolitan	https://www.marinespecies.org/aphia.php?p=taxdetails&id=417694
Wiesnerella auriculata (Cushman)			Smaller Miliolids Other Small Taxa: Miliolida, Most Families and Genus except larger taxa Location: Cosmopolitan	https://www.marinespecies.org/aphia.php?p=taxdetails&id=41614

Heterostegina antillarum (d'Orbigny)			Symboiyant-bearing Rotaliines Symbiont-Bearing Rotaliida: Nummulitidae *Heterostegina* Location: Circumtropical	https://www.marinespecies.org/aphia.php?p=taxdetails&id=418055
Quinqueloculina lamarckiana (d'Orbigny)			Smaller Miliolids Other Small Taxa: Miliolida, Most Families and Genus except larger taxa Location: Cosmopolitan	https://www.marinespecies.org/aphia.php?p=taxdetails&id=112643
Nonion depresselum (de Montfort)			Other Small Perforate Taxa Other Small Taxa: Rotaliida, Most Families and Genus Location: Cosmopolitan	https://www.marinespecies.org/foraminifera/aphia.php?p=taxdetails&id=112232
Cornuspira planorbis (Schultze)			Smaller Miliolids Other Small Taxa: Miliolida, Most Families and Genus except larger taxa Location: Cosmopolitan	https://www.marinespecies.org/aphia.php?p=taxdetails&id=112489

Laevipeneropl is bradyi (Cushman)			Symboiyant-bear ing Miliolids Miliolida: Peneroplidae, Several genera Location: Circumtropical	https://www.m arinespecies.or g/aphia.php?p =taxdetails&id =417774
Peneroplis pertusus (de Montfort)			Symboiyant-bear ing Miliolids Miliolida: Peneroplidae, Several genera Location: Circumtropical	https://www.m arinespecies.or g/foraminifera /aphia.php?p=t axdetails&id= 112815
Bolivina lowmani (Phleger and Parker)			Opportunistic Taxa Buliminida: Bolivinidae, Several genera Location: Cosmopolitan	https://www.m arinespecies.or g/aphia.php?p =taxdetails&id =112976

Rosalina floridana (Cushman, 1922)			Other Small Perforate Taxa Other Small Taxa: Rotaliida, Most Families and Genus Location: Cosmopolitan	https://www.marinespecies.org/aphia.php/aphia.php?p=taxdetails&id=113169#distributions
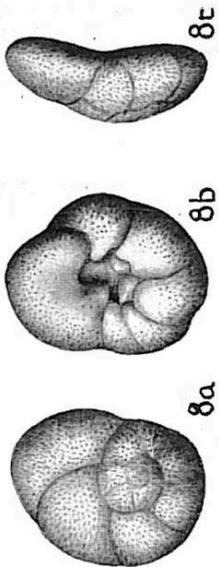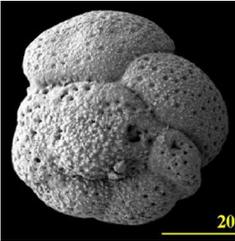
Table 2: Foraminifera Types. This table shows which sites had more heterotrophs, more autotrophs, and an even amount of each type of Foraminifera.

Site	Heterotroph Amount	Even Heterotroph and Autotroph Amount	Autotroph Amount
Punta Maroma	5	2	11
Dick's	10	0	2
Yal-Ku	10	1	2
Total	25	3	15
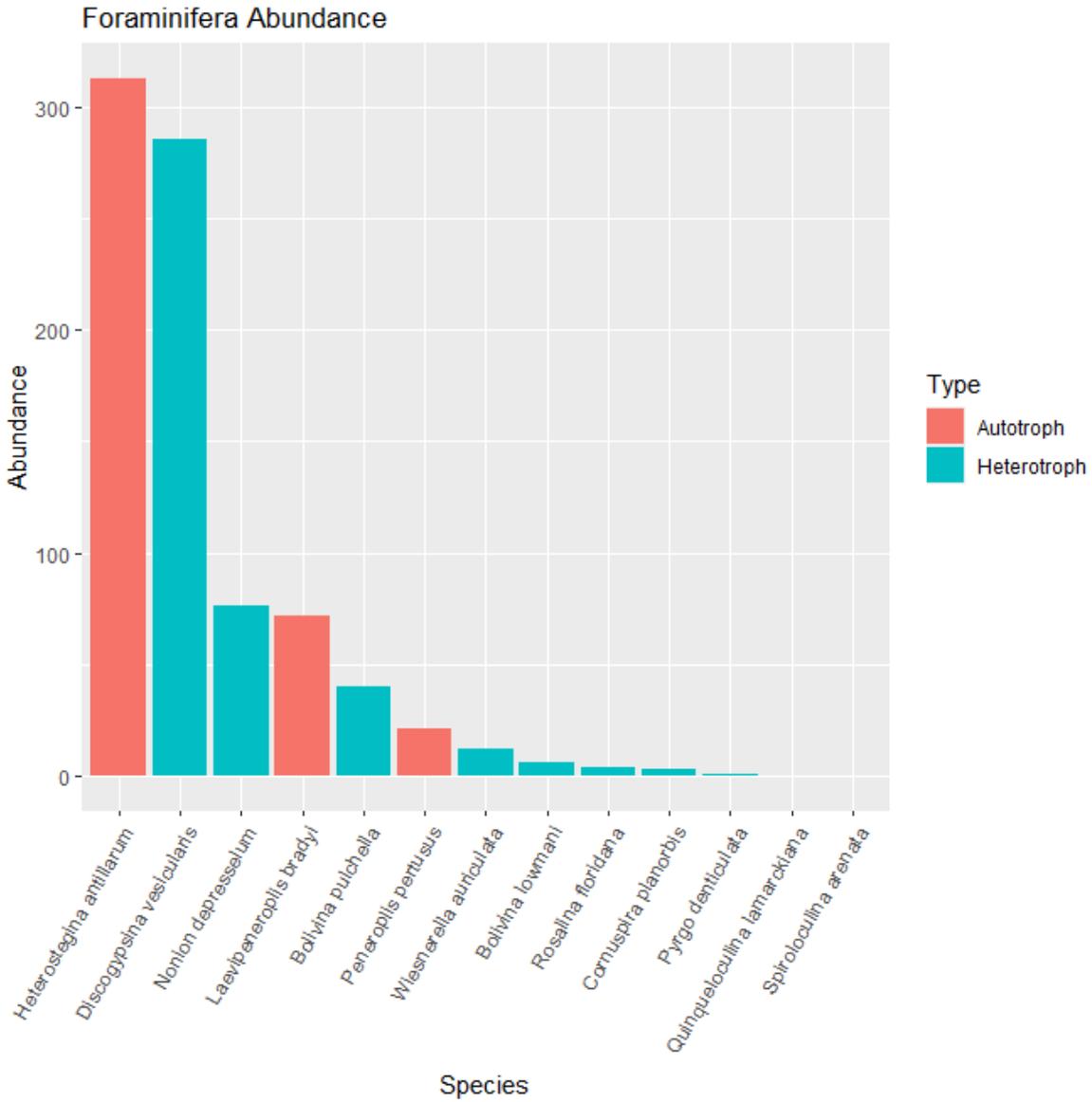
Figure 1. Foraminifera Abundance Barplot. This figure shows the abundance of all the Foraminifera species in decreasing order of abundance, and the type of feeding of the Foraminifera that is color coded. The autotroph species that are color coded red are the *Heterostegina antillarum*, *Laevipeneroplis bradyi*, and *Peneroplis pertusus*
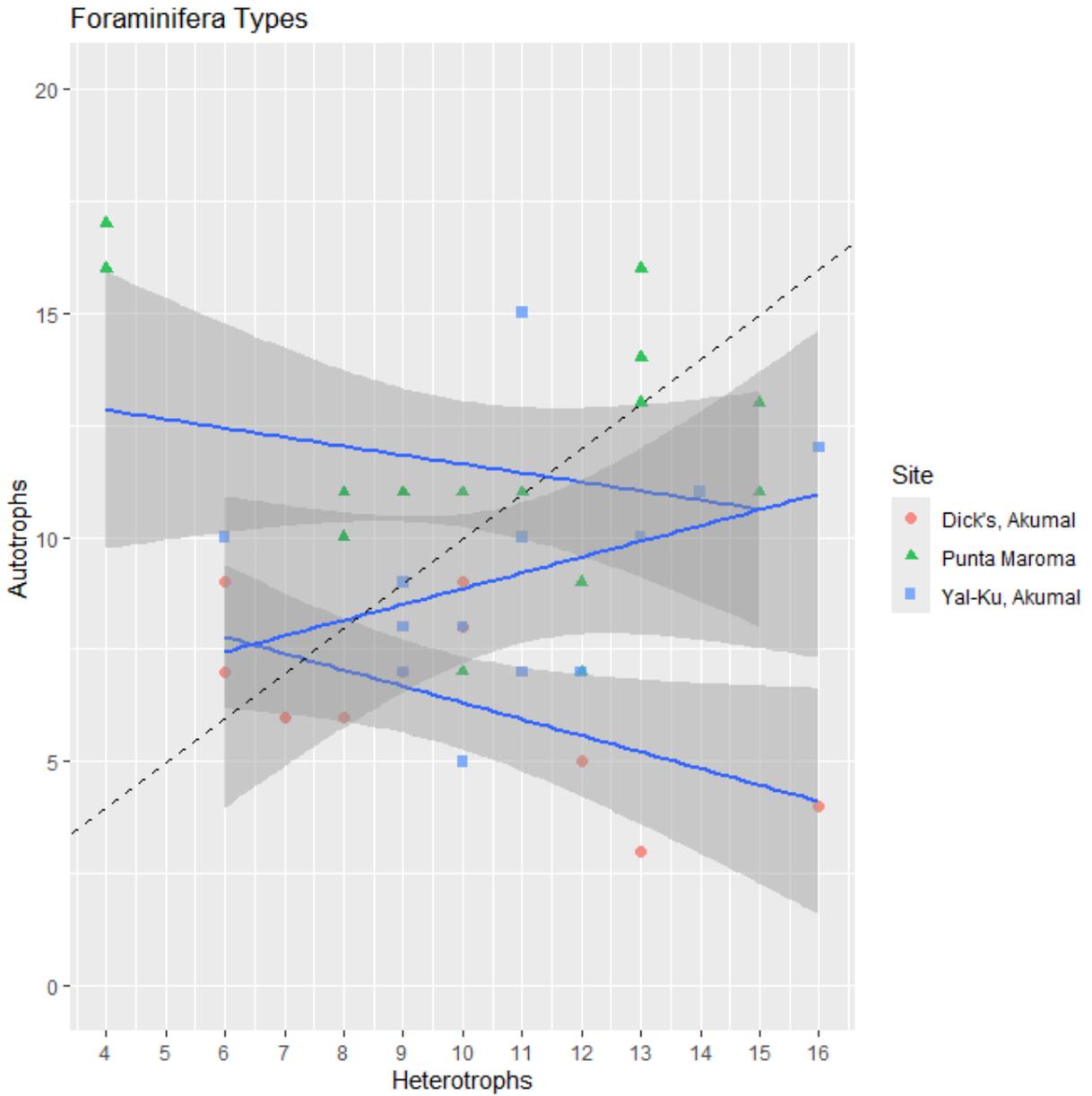
Figure 2: Foraminifera Types and Sites Scatterplot. This figure shows the heterotrophs and autotrophs in relation to each of the three sites. Each of the three trend lines and shapes corresponds with a site, and the dashed line provides a reference of a 1:1 line.
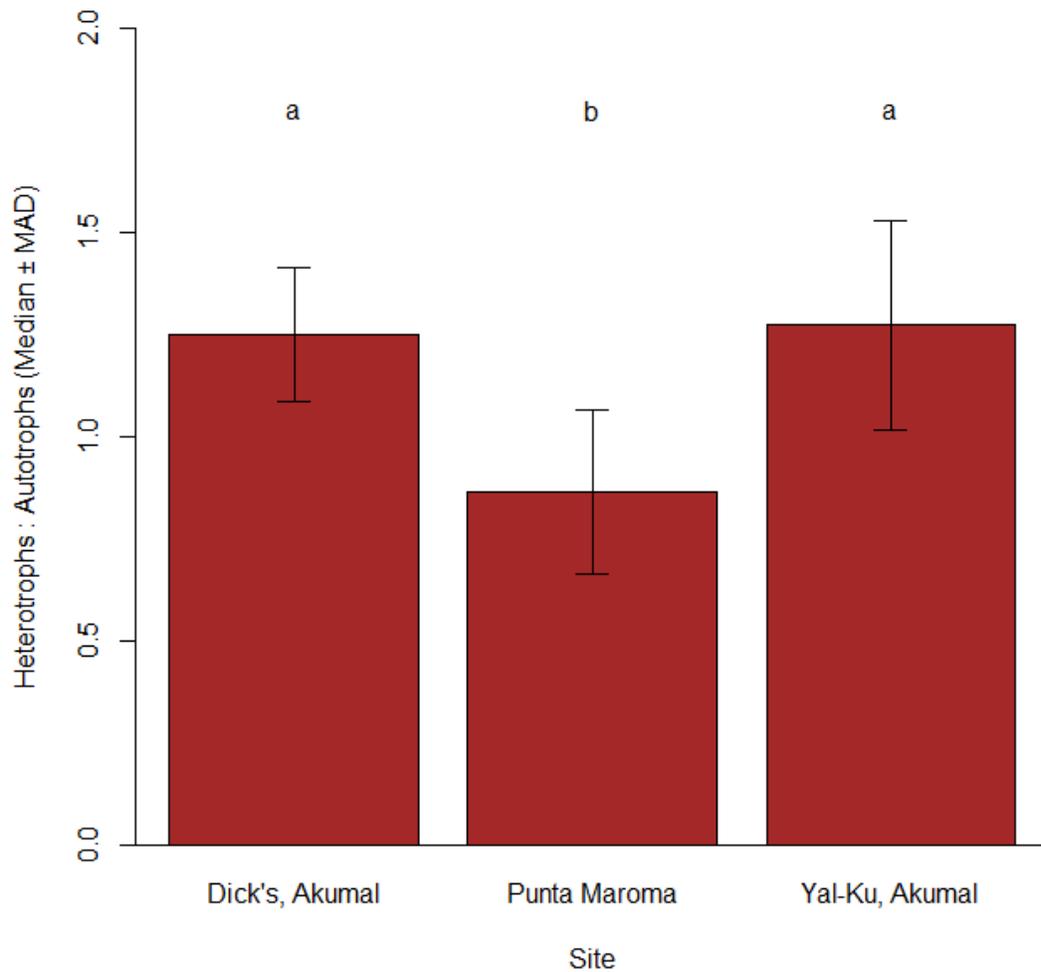
Figure 3: One Way ANOVA With Abundance and Sites. This figure shows the relationship between abundance and site. The letters above each error bar helps show which sites have more differences.

Table 3: One Way ANOVA Data. This table shows the data calculated for significance during the One Way ANOVA.

	df	SS	MS	F	p-value
Site	2	119.3	59.67	7.05	<0.01
Error	83	702.3	8.46		
Total	85	821.6			
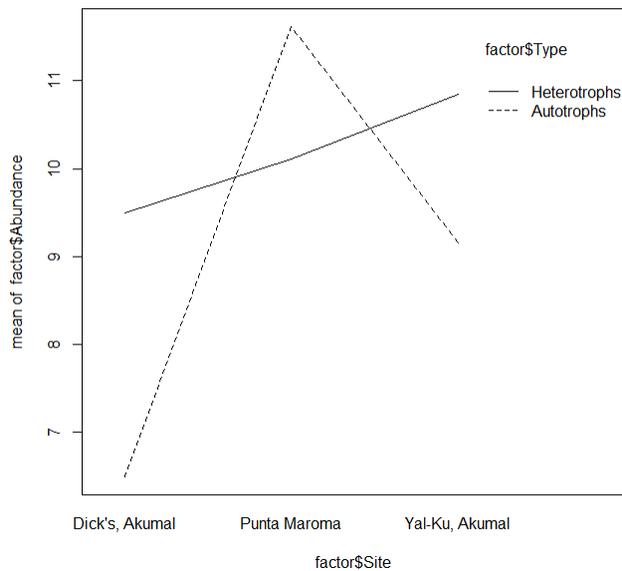
Figure 4: Interaction 1 With Site and Type. This figure shows the site and type relationship using a factorial ANOVA, also known as a two way ANOVA. The site and type were the predictors and the abundance was the response.
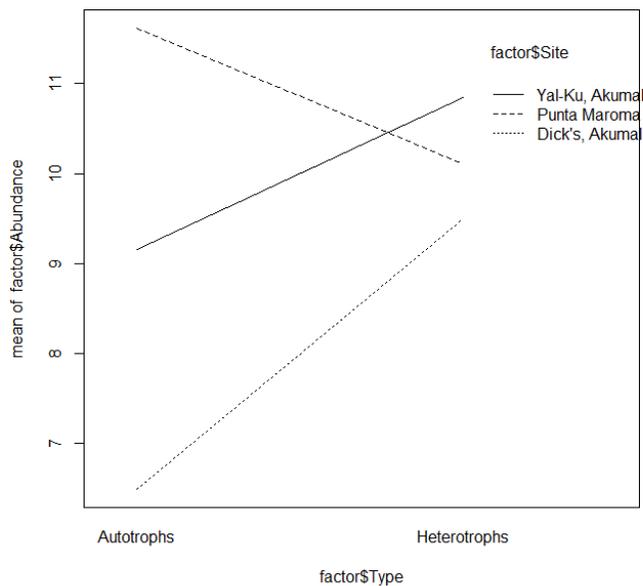
Figure 5: Interaction 2 With Type and Site. This figure shows the type and site relationship using a factorial ANOVA, also known as a two way ANOVA. The type and site were the predictors and the abundance was the response.

Table 4: Two Way ANOVA Data. This table shows the data calculated for significance during the Two Way ANOVA.

	df	SS	MS	F	p-value
Site	2	119.33	59.67	7.83	<0.001
Type	1	11.17	11.17	1.47	0.23
Site*Type	2	81.69	40.85	5.36	<0.01
Error	80	609.44	7.62		
Total	85	821.63			
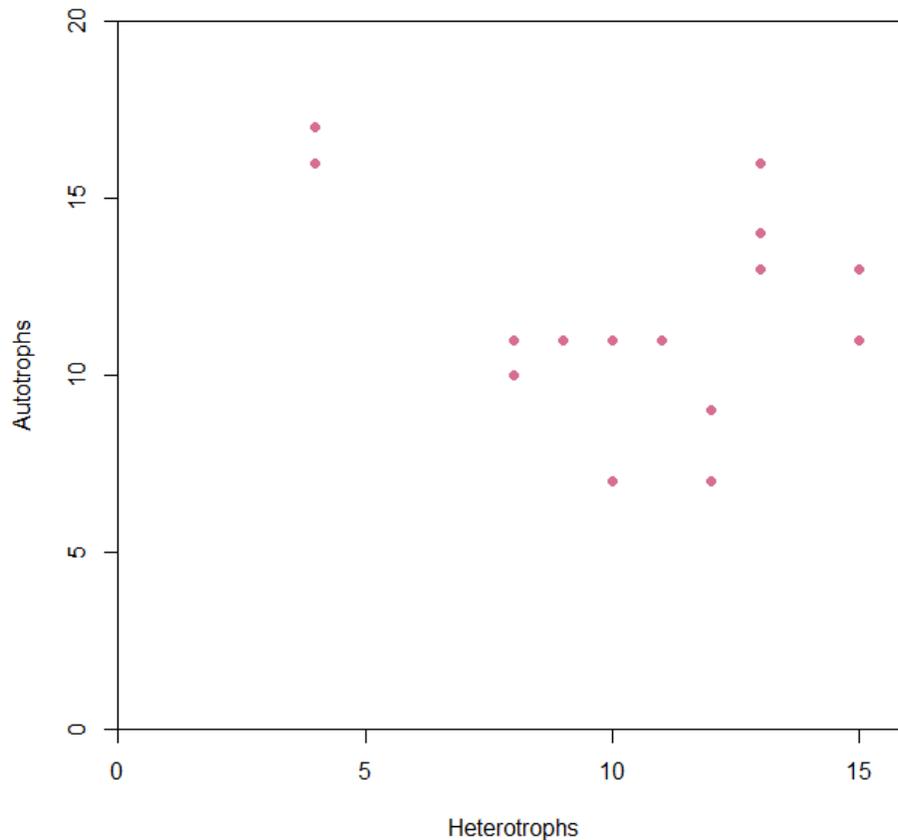
Figure 6: Correlation at Punta Maroma. This figure shows the Pearson correlation run using the data from Punta Maroma. The correlation between heterotrophs and autotrophs was predicted to vary at each site.

Figure 7: Correlation at Dick's. This figure shows the Pearson correlation run using the data from Dick's, a site in Akumal. The correlation between heterotrophs and autotrophs was predicted to vary at each site.

Figure 8: Correlation at Yal-Ku. This figure shows the Pearson correlation run using the data from Yal-Ku, a site in Akumal. The correlation between heterotrophs and autotrophs was predicted to vary at each site.

Discussion

Analysis of Results

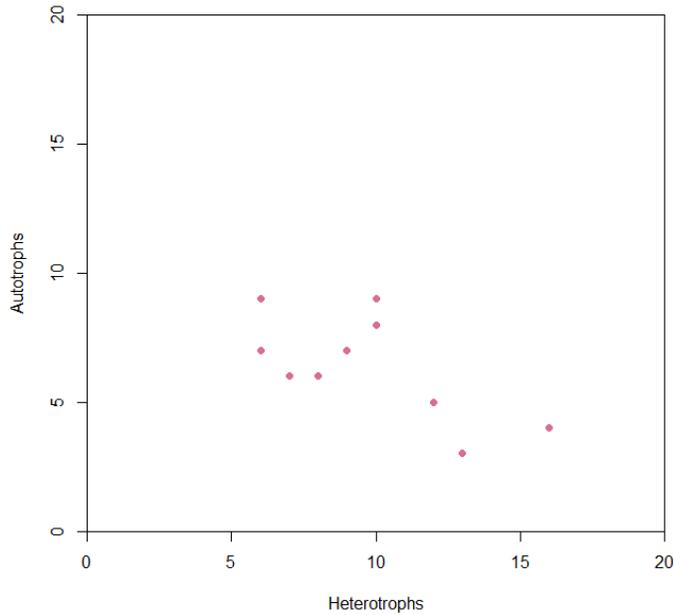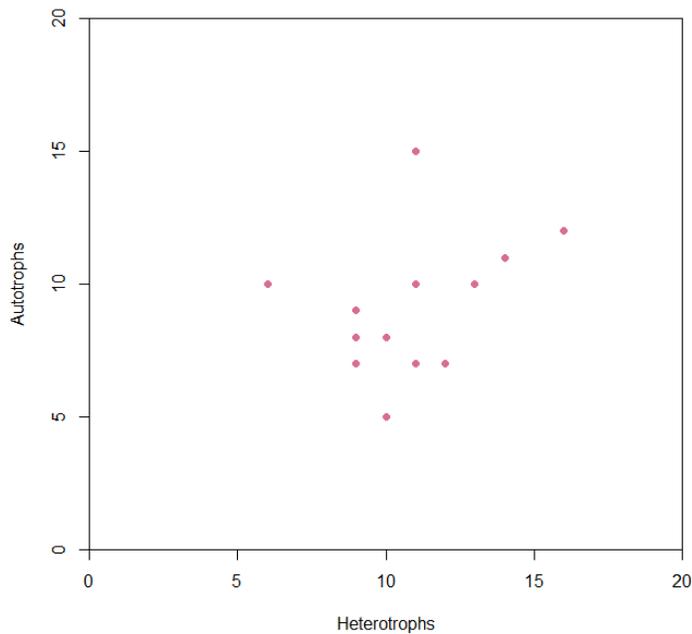Overall, the Foraminifera research with Excel sheets and RStudio created numerous scatter plots to analyze the data collected. The table for Foraminifera identification was created to display the twelve different species with nine labeled as heterotrophs and three labeled as autotrophs, shown in Table 1. The table for Foraminifera results was created to display the different sites and feeding types, shown in Table 2. The RStudio analysis was completed using eight different figures. Figure 1 shows a barplot on Foraminifera Abundance. This shows the autotrophs have the highest amount for an individual species, but there are overall more heterotrophs. It was expected to have more heterotrophs, since past research on Foraminifera proves that. However, it was also expected to have a more significant number of autotrophs since the Yucatán Peninsula is not an upwelling zone where the heterotrophs thrive more than usual. Figure 2 shows a Scatterplot on Foraminifera Types. This shows three trend lines corresponding to the different sites, along with standard error. Dick's shows a significant downward slope, the lowest on the plot. Punta Maroma shows a significant downward slope, the highest on the plot. Yal-Ku shows a significant upward slope, in the middle of the plot. The Akumal location, made of Dick's and Yal-Ku, shows the differences in feeding types is more localized than based on location. This shows the site scale is more significant than the location scale. An additional RStudio test completed was a One Way ANOVA With Ratio and Sites. The heterotroph to autotroph ratio was the response while the sites were the predictor. Unfortunately the normal distribution with the Shapiro test was not passed, the significance was too great for even transformations to change. Figure 3 shows a One Way ANOVA With Abundance and Sites. Abundance was the response while site was the predictor. This shows independence, normality, and homogeneity, resulting in Table 3. After completing the TukeyHSD test it was found that Punta Maroma and Dick's are significantly different, Yal-Ku and Dick's are significantly different, but Yal-Ku and Punta Maroma are more related. Table 3 shows the sites and p-value were significant after running the ANOVA. Figure 4 and Figure 5 each show a Two Way ANOVA With Site, Type, and Abundance. These are also referred to as Factorial ANOVAs. They both show independence, normality, and homogeneity, resulting in Table 4. The interaction between site and type and the interaction between type and site were tested separately. They were statistically significant interactions, but both plots show crossing lines making them irregular. Due to the irregularity, the non-orderly interaction cannot be interpreted more. However, the significance and insignificance is shown in Table 4. The sites were found to be significant, the site and type interaction was found to be significant, but type alone was found to be insignificant. Figure 6, Figure 7, and Figure 8 show the Pearson Correlation. Each figure corresponds with one site's interaction with heterotrophs and autotrophs. All sites look generally linear and tests as normal distribution. Each figure shows the p-value significance/association or insignificance/no association, the correlation as a positive or negative r value, and the visual correlation description. Figure 6 shows Punta Maroma with an insignificant p-value of 0.3601, a negative correlation r value of 0.2293, and not a visually strong path. This resulted in a correlation that is not significant. Figure 7 shows Dick's with a significant p-value of 0.0437, a negative correlation r value of 0.5895, and a visually strong negative linear path. This resulted in a significant negative correlation. Figure 8

shows Yal-Ku with an insignificant p-value of 0.2516, a positive correlation r value of 0.3428, and a visually strong positive linear path. This resulted in correlation that is not significant.

Conclusion

In conclusion, my hypotheses were both incorrect. Although I completed a large literary study with numerous sources, the lack of research on Foraminifera led to incorrect hypotheses. Fortunately, much was learned throughout the process of the experiment over three separate semesters.

From past research it was hypothesized there would be more *Discogypsina vesiularis*, since it has been found to be very common in the Yucatán Peninsula region. This particular species is also a heterotroph, which have been shown to be more common when using past research on Foraminifera. This experiment showed that the most common Foraminifera species was actually an autotroph and not a heterotroph. Instead of *Discogypsina vesiularis*, the most common species was *Heterostegina antillarum*, shown in Figure 1. Although the most common species was an autotroph, overall there were more Foraminifera that were heterotrophs than autotrophs, shown in Table 2 and Figure 1. Out of all the twelve Foraminifera species observed, there were only three autotrophs: *Heterostegina antillarum*, *Laevipeneroplis bradyi*, and *Peneroplis pertusus*.

It was also hypothesized that the location would play a larger role than the site. This was predicted since Akumal is in an area that is more nutrient-rich, which is a type of environment that has been proven to have more heterotrophs. This experiment showed that the scale of importance between functional group ratios was in the site and not location. Figure 2 shows that the differences in feeding types is more localized and more based on the site rather than the location. Out of the three trend lines Dick's is downward, Punta Maroma is downward, and Yal-Ku is upward. Since Dick's and Yal-Ku are both sites in the location of Akumal, my hypothesis stated the sites would be the same. However, the varying site results prove the scale that the ratio of functional types is smaller than location, it is site. Figure 3 also shows that the differences in feeding types is more localized by concluding that Punta Maroma and Dick's are significantly different, Yal-Ku and Dick's are significantly different, but Yal-Ku and Punta Maroma are more related. The smaller scale of sites show differences in the functional group ratio as well as similarities, but this scale does not include location.

Overall, the most common species of Foraminifera does not make that the more populous functional type, and the scale of functional types is statistically more helpful when on a smaller scale. However, results may be different with a larger variety of sites and locations. Experiments can always be more accurate when the field of study is expanded. The sites used in this experiment were all in the same general area of the Yucatán Peninsula. Different environments would cause the need for a larger variation of environmental indicators, causing increased diversity to take place, with many more species and feeding styles. Fortunately, the knowledge that site spatial scales matter more with Foraminifera will help these organisms be more accurate as environmental indicators.

helped me by allowing me to use the data he gathered from the Yucatán Peninsula, teaching me how to identify organisms accurately under microscopes, and aiding me with RStudio. Angelica has been a wonderful inspiration since she used to work with foraminifera as well, and she provided many helpful tips. Anna and Mike helped by answering many of my RStudio coding questions. Anna was extremely helpful by spending hours on the computer to help me through errors. I would also like to thank Dr. Pamela Hallock Muller for her extensive work on foraminifera, which greatly helped me throughout the research, experiment, and identification processes.

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Appendix 1: *RStudio Code*

```
setwd("U:/Foram")
foram<-read.csv("Forams.csv")
install.packages("ggplot2")
library(ggplot2)
ggplot(foram,aes(x=Heterotrophs,y=Autotrophs))+geom_point(color="blue")
ggplot(foram,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="magenta",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 20)) +
  scale_x_continuous(breaks = seq(4, 16, 1),
              limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
      title = "Foraminifera Types")
ggplot(foram,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="magenta",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 20)) +
  scale_x_continuous(breaks = seq(4, 16, 1),
              limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
      title = "Foraminifera Types")+
  geom_smooth(method = "lm")
```

Appendix 2: *RStudio Code with Percents*

```
setwd("U:/Foram")
percent<-read.csv("FORAM.csv")
install.packages("ggplot2")
library(ggplot2)
ggplot(percent,aes(x=Heterotrophs,y=Autotrophs))+geom_point(color="green")
ggplot(percent,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="green",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 100)) +
  scale_x_continuous(breaks = seq(0, 100, 10),
              limits=c(0, 100)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
      title = "Foraminifera Percents")
ggplot(percent,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="green",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 100)) +
  scale_x_continuous(breaks = seq(0, 100, 10),
              limits=c(0, 100)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
      title = "Foraminifera Percents")+
  geom_smooth(method = "lm")
```

Appendix 3: *Punta Maroma RStudio Code*

```
setwd("U:/Foram")
PM<-read.csv("PuntaMaroma.csv")
install.packages("ggplot2")
library(ggplot2)
ggplot(PM,aes(x=Heterotrophs,y=Autotrophs))+geom_point(color="orange")
ggplot(PM,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="orange",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 20)) +
```

```
scale_x_continuous(breaks = seq(4, 16, 1),
            limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
        title = "Punta Maroma Foraminifera
Types")
ggplot(PM,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="orange",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 20)) +
  scale_x_continuous(breaks = seq(4, 16, 1),
            limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
        title = "Punta Maroma Foraminifera
Types")+
  geom_smooth(method = "lm")
```

Appendix 4: *Akumal RStudio Code*
```
setwd("U:/Foram")
A<-read.csv("Akumal.csv")
install.packages("ggplot2")
library(ggplot2)
ggplot(A,aes(x=Heterotrophs,y=Autotrophs)
)+geom_point(color="purple")
ggplot(A,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="purple",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 20)) +
  scale_x_continuous(breaks = seq(4, 16, 1),
            limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
      title = "Akumal Foraminifera Types")
ggplot(A,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="purple",
          size = 2,
          alpha=.8) +
```

```
scale_y_continuous(limits = c(0, 20)) +
  scale_x_continuous(breaks = seq(4, 16, 1),
            limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
        title = "Akumal Foraminifera Types")+
  geom_smooth(method = "lm")
```

Appendix 5: *Barplot RStudio Code*
```
setwd("U:/Foram")
install.packages("tidyverse")
library("tidyverse")
install.packages("ggplot2")
library("ggplot2")
foram<-read.csv(file="foramabundance.csv"
)
print(foram)
ggplot(foram, aes (x=Species,
y=Abundance, fill=Type))+
  geom_bar(stat="identity")+
  ggtitle("Foraminifera Abundance")+
  theme(axis.text.x=element_text
      (angle=60,vjust=1,hjust=1))
foram<-transform(foram, Species=
          reorder(Species,-Abundance))
```

Appendix 6: *Scatterplot RStudio Code*
```
setwd("U:/Foram")
manatee<-read.csv("Results.csv")
install.packages("ggplot2")
library(ggplot2)
ggplot(manatee,aes(x=Heterotrophs,y=Autot
rophs))+geom_point(color="blue")
ggplot(manatee,
      aes(x = Heterotrophs, y = Autotrophs))
+
  geom_point(color="magenta",
          size = 2,
          alpha=.8) +
  scale_y_continuous(limits = c(0, 20)) +
  scale_x_continuous(breaks = seq(4, 16, 1),
            limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
```

```
        title = "Foraminifera Types")
y=manatee$Autotrophs
x=manatee$Heterotrophs
ggplot(manatee,
        aes(x = Heterotrophs, y = Autotrophs,
shape=Site)) +
  geom_point(aes (shape=Site, color=Site),
          size = 2,
          alpha=.8) +
        geom_smooth(method    =    "lm",
formula=y~x)+
                        geom_abline(slope=1,
linetype="dashed",color="black")+
  scale_y_continuous(limits = c(0, 20)) +
  scale_x_continuous(breaks = seq(4, 16, 1),
          limits=c(4, 16)) +
  labs(x = "Heterotrophs",
      y = "Autotrophs",
      title = "Foraminifera Types")
```

Appendix 7: *One Way ANOVA RStudio Code*
```
setwd("U:/Foram")
ratio<- read.csv("2024 Results.csv")
shapiro.test(ratio$Ratio)
shapiro.test(log (ratio$Ratio +1))
shapiro.test(sqrt(ratio$Ratio +1))
ratio <- read.csv("Type.csv")
ratio
install.packages("dunn.test")
library(dunn.test)
dunn.test(ratio$Ratio, ratio$Site)
median.site=tapply(ratio$Ratio,ratio$Site,F
UN=median)#Generate means for bar plot
mad.site=tapply(ratio$Ratio,ratio$Site,FUN
=mad)#Generate SDs for bar plot
median.site
mad.site
ratio.plot=barplot(median.site,ylim=c(0,2),c
ol="rosybrown3",
                ylab="Heterotrophs :
Autotrophs (Median ± MAD)",
```

```
                xlab="Site")#Generate and
save plot
arrows(ratio.plot,median.site-mad.site,ratio.
plot,
      median.site+mad.site,
      code=3,angle=90,length=0.1) #Generate
error bars for bar plot
text(x=ratio.plot,
y=c(1.8),labels=c("a","b","a")) #Add
significance labels to plot
abline(h=0) #Add horizontal line to x-axis
```

Appendix 8: *Factorial ANOVA RStudio Code*
```
setwd("U:/Foram")
factor<- read.csv("Site.csv")
factor
shapiro.test(factor$Abundance)
bartlett.test(factor$Abundance~factor$Site)
bartlett.test(factor$Abundance~factor$Type)
factor.aov= aov (Abundance~Site*Type,
factor)
factor.aov
summary(factor.aov)
interaction.plot(factor$Site, factor$Type,
factor$Abundance)
interaction.plot(factor$Type, factor$Site,
factor$Abundance)
```

Appendix 9: *One Way ANOVA RStudio Code*
```
setwd("U:/Foram")
ratio<- read.csv("2024 Results.csv")
shapiro.test(factor$Abundance)
bartlett.test(factor$Abundance~factor$Site)
site.aov= aov (Abundance~Site, factor)
summary(site.aov)
TukeyHSD (site.aov)
mean.site= tapply (factor$Abundance,
factor$Site, FUN=mean)
```

```
sd.site= tapply (factor$Abundance,
factor$Site, FUN=sd)
mean.site
sd.site
site.plot =
barplot(mean.site,ylim=c(0,16),col="brown"
,
              ylab = "Abundance (Mean +/-
SD)",
              xlab = "Site")
arrows (site.plot, mean.site-sd.site, site.plot,
mean.site+sd.site, code=3, angle=90,
length=0.1)
text(x=site.plot,y=c(15,15,15), labels=c("a",
"b", "a"))
abline(h=0)
```

Appendix 10: *Correlation RStudio Code*
```
setwd("U:/Foram")
pm <- read.csv("Punta Maroma HA.csv")
pm
plot(pm$Heterotrophs,pm$Autotrophs)
shapiro.test(pm$Heterotrophs)
shapiro.test(pm$Autotrophs)
cor.test(pm$Autotrophs,pm$Heterotrophs,m
ethod="pearson")
plot(pm$Heterotrophs,pm$Autotrophs,pch=
16,col="palevioletred",xlim=c(0,16),xaxs="i
",ylim=c(0
        ,20),yaxs="i",xlab="Heterotrophs",yl
ab="Autotrophs")
d <- read.csv("Dicks HA.csv")
d
plot(d$Heterotrophs,d$Autotrophs)
shapiro.test(d$Heterotrophs)
shapiro.test(d$Autotroohs)
cor.test(d$Autotroohs,d$Heterotrophs,metho
d="pearson")
plot(d$Heterotrophs,d$Autotroohs,pch=16,c
ol="palevioletred",xlim=c(0,20),xaxs="i",yli
m=c(0,20)
```

```
              ,yaxs="i",xlab="Heterotrophs",ylab=
"Autotrophs")
yk <- read.csv("YK HA.csv")
yk
plot(yk$Heterotrophs,yk$Autotrophs)
shapiro.test(yk$Heterotrophs)
shapiro.test(yk$Autotrophs)
cor.test(yk$Autotrophs,yk$Heterotrophs,met
hod="pearson")
plot(yk$Heterotrophs,yk$Autotrophs,pch=1
6,col="palevioletred",xlim=c(0,20),xaxs="i"
,ylim=c(0,2
        0),yaxs="i",xlab="Heterotrophs",yla
b="Autotrophs")
```