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Associations Between *Vibrio* and the Planktonic Community Throughout Tampa Bay

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ABSTRACT

Vibrio is a genus of bacteria whose species naturally inhabit warm, marine waters throughout the world. Many of these species are pathogenic to humans, which makes predicting outbreaks of vital importance. Considerable information is still being discovered about *Vibrio* ecology, therefore additional studies of *Vibrio* in the marine environment are necessary. Previous relationships between *Vibrio* species, the planktonic community, and environmental factors have been described, but these relationships have not been explored in Tampa Bay. Zooplankton tows were performed at six different locations in Tampa Bay. Three sites were located in the inner bay, while three were closer to the Gulf of Mexico. Whole water samples and environmental parameters were also collected from each site. Zooplankton samples were separated into two classes: copepods and copepod nauplii and resuspended in sterile saline. Whole water samples were diluted to 10^{-1}, 10 mL, and 20 mL. Zooplankton and water samples were vacuum filtered, and the filter paper plated on Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar plates. The plates were incubated at 37 °C for 24 hours and assessed for growth. Associations between *Vibrio* and the planktonic community, as well as patterns in environmental factors provide valuable insight to the bacterial community of Tampa Bay.

1 INTRODUCTION

Vibrio is a genus of bacteria whose species naturally inhabit warm, marine waters throughout the world. At least 12 of these species are pathogenic to humans and other marine life, including the highly virulent species *Vibrio cholerae* and *Vibrio vulnificus* (Turner et al., 2009). However, much is still being discovered about *Vibrio* ecology. Therefore, additional ecological studies of *Vibrio* in the marine environment are critical in developing the ability to predict *Vibrio* outbreaks in specific regions (Gil et al., 2004).

Previously in the Tampa Bay region, *V. vulnificus* levels have been examined in seawater, sediment and in shellfish. Water temperature and salinity played an important role in *V. vulnificus* concentration, with the bacteria isolated more frequently in water temperatures greater than 17 °C and salinities greater than 17.0 ppt (Tamplin et al., 1982). This study gave insight to the physical parameters that are optimal for *V. vulnificus* growth. However, the study did not include biological factors such as other organisms or other species of *Vibrio*.

A major factor that has not been tested in the Tampa Bay region is the association between *Vibrio* and the planktonic community. Zooplankton provide a microenvironment full of organic material and nutrients beneficial to the survival of heterotrophic bacteria such as *Vibrio* (Turner et al., 2009). Due to this nutrient-rich microenvironment, higher abundances of *Vibrio* have been observed attached to zooplankton, while lower abundances are found free-living in the water column (Maugeri et al., 2004). All pathogenic species of *Vibrio* can also produce an extracellular chitinase, allowing for utilization of the nutrients found within the zooplankton's chitinous exoskeleton (Huq et al., 1983). The ability *Vibrio* has to utilize both of these nutrient sources leads to a possible competitive advantage when found attached to zooplankton (Turner et al., 2009). *Vibrio* species however, are not the only beneficiary in the association with zooplankton. Zooplankton also profit off of the relationship due to the role *Vibrio* plays in the microbial loop. The microbial loop is considered a critical component in the planktonic food chain as well as the overall food web. The loop involves the uptake and recycling of dissolved organic matter (DOM) by bacteria, phytoplankton, zooplankton and bactivorous zooplankton (Steele, 1998). In turn, the microbial loop acts as the fundamental food source for metazooplankton species such as copepods (Sherr & Sherr, 1988). The intricate, multifaceted relationship between *Vibrio* and zooplankton indicates that patterns in zooplankton seasonality may be useful in predicting *Vibrio* outbreaks (Gil et al., 2004).

2 METHODS

Sampling sites

The six sampling sites used in this study were located along a vertical transect down the center of Tampa Bay toward the Gulf of Mexico (Figure 1). The sampling sites spanned two distinct environments, upper Old Tampa Bay and offshore near the mouth of Tampa Bay. Sites 1, 2, and 3 were located near the Gulf of Mexico and received the most tidal mixing. Sites 4, 5, and 6 were located in upper Old Tampa Bay where multiple freshwater river inputs occur (Hopkins, 1977). This provided a distinct salinity gradient that the sampling sites encompassed.

Sample collection

Samples were collected 13 April 2019 throughout Tampa Bay. Approximately 250 mL of whole water was collected at each of the six sites using a sterile cell culture flask. Zooplankton samples were collected by a two-minute plankton tow with a 150 µm plankton net at a speed of about 2 m s^{-1}. The zooplankton samples were then placed in sterile cell culture flasks. Samples were stored in a cooler filled with ice during the three-hour sampling period. Samples were then transported back to the lab and processed that day.

Zooplankton samples

An aliquot of each zooplankton sample from every site was placed in a sterile petri dish. Under a stereo microscope, the zooplankton were hand-picked into two classes (copepods and copepod nauplii) using a sterile pipette. These classes were chosen given their abundance in Tampa Bay (Badylak & Phlips, 2008). The two classes were also separated into four samples with 5, 10, 15, and 20 individuals in each. This was done in order to provide countable dilutions of attached bacteria. Picked zooplankton samples were resuspended into sterile saline (10 mL) and agitated for at least 30 minutes to dislodge attached bacteria. The agitated samples were then

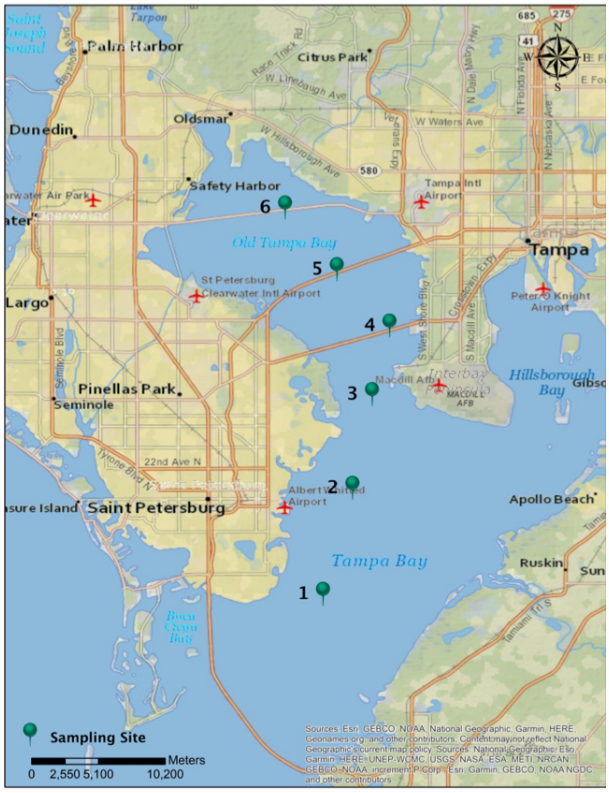

Fig. 1. Location of the six sampling sites in Tampa Bay.

processed via vacuum filtration and the filter paper plated on *Vibrio* selective Thiosulfate-Citrate-Bile-Sucrose (TCBS) agar plates. Due to time constraints only zooplankton samples from sites 1, 2, 5, and 6 were fully processed.

Whole water samples

Whole water samples were also processed via vacuum filtration. For all six sampling sites, 10 mL, 20 mL, and a dilution of 10^{-1} were filtered. The filter paper was then plated on TCBS agar.

TCBS plate results

The TCBS plates for both whole water and zooplankton samples were incubated at 37 °C for 24 hours. Growth was observed and all green and yellow colonies were counted. Green colonies were presumed *Vibrio* vulnificus and yellow colonies *Vibrio* cholerae (Lotz et al., 1983). Counts were reported as colony forming units (CFUs) per milliliter of whole water or CFU per individual copepod or copepod nauplii.

3 RESULTS

Vibrio species were found within the whole water for all six sites and also found in association with zooplankton at the sites where samples were collected (sites 1, 2, 5 and 6). At site 1, *Vibrio* was found in concentrations of 470 CFU/mL of whole water (Table 1,

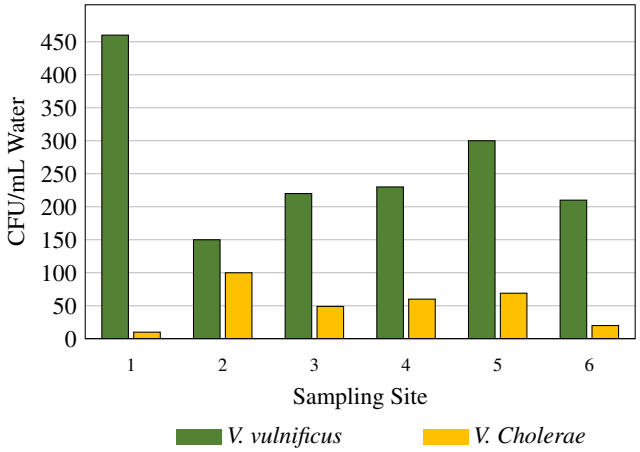

Fig. 2. *Vibrio* colony forming units (CFU) per milliliter of water at six sites. A single whole water sample of 250 mL was acquired at all sites. Due to only one countable dilution (10^{-1}) for all sites standard deviation was not reported.

Figures 2 and 3). In the zooplankton samples, *Vibrio* was found in concentrations of 13 ± 3.411 and 19 CFU/individual for copepods and copepod nauplii, respectively (Table 1, Figures 4, 5 and 6). Site 2 had *Vibrio* concentrations of 250 CFU/mL whole water (Table 1, Figures 2 and 3). A concentration of 8 ± 1.229 CFU/individual was found for the copepods at site 2, while a concentration of 6 ± 1.526 CFU/individual was found for the copepod nauplii (Table 1, Figures 4, 5 and 7). Sites 3 and 4 had *Vibrio* concentrations of 270 and 290 CFU/mL respectively in the whole water samples and were not sampled for zooplankton due to time restraints (Table 1, Figures 2 and 3). Whole water *Vibrio* concentrations from site 5 and site 6 were 370 CFU/mL and 230 CFU/mL (Table 1, Figures 2 and 3). Copepods at these sites had *Vibrio* concentrations of 162 CFU/individual for site 5 and 39 CFU/individual for site 6 (Table 1, Figures 4, 8 and 9). Copepod nauplii at site 5 had *Vibrio* concentrations of 43 CFU/individual and site 6 had 11 CFU/individual (Table 1, Figures 5, 8 and 9).

For all of the sampling sites, on average the whole water contained *Vibrio* concentrations of 313 CFU/mL. The zooplankton on average contained *Vibrio* concentrations of 55 CFU/individual copepod and 20 CFU/individual copepod nauplii (Figure 10).

4 DISCUSSION

This preliminary exploration of *Vibrio* in Tampa Bay provides a highly beneficial starting point for further research in this region. Our results support that there is an association between *Vibrio* and the planktonic community throughout Tampa Bay, as *Vibrio* was found attached to both copepods and copepod nauplii at each site tested. Site 1 had the highest *Vibrio* CFU/mL whole water, while the highest *Vibrio* CFU/individual for both copepods and copepod nauplii was found at site 5. This suggests that while *Vibrio* is associated with zooplankton throughout Tampa Bay, there are likely other driving forces that impact whether *Vibrio* colonizes

Site	Sample Type	Sample Size (n)	CFU	sd
1	Whole Water	1	470	*
1	Copepod	15	13	3.411
1	Copepod Nauplii	5	19	*
2	Whole Water	1	250	*
2	Copepod	50	8	1.229
2	Copepod Nauplii	45	6	1.526
3	Whole Water	1	270	*
4	Whole Water	1	290	*
5	Whole Water	1	370	*
5	Copepod	5	162	*
5	Copepod Nauplii	5	43	*
6	Whole Water	1	230	*
6	Copepod	5	39	*
6	Copepod Nauplii	30	11	0.863

Table 1. Overall *Vibrio* colony forming units (CFU) by site and sample type. Sample size for whole water samples is one 250 mL sample. Only the 10^{-1} dilution was countable for whole water samples. Sample size for copepod and copepod nauplii samples varies due to which dilutions were countable. CFU values for whole water samples are reported as CFU/mL. CFU values for copepod and copepod nauplii samples are reported as CFU/individual. (*) indicates sites with only one countable dilution where standard deviation cannot be reported.

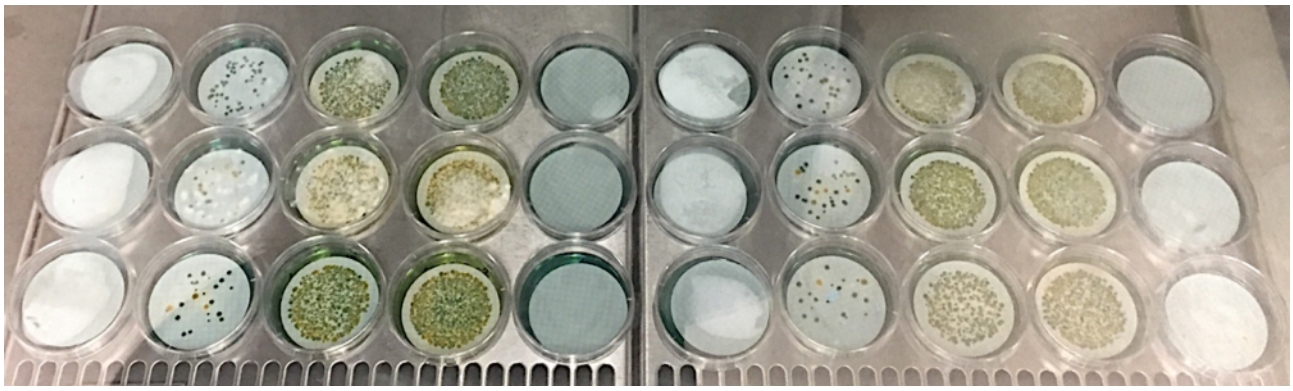

Fig. 3. TCBS plates of whole water samples. Image left (top to bottom) site 1-3: (left to right) pre, 10^{-1}, 10 mL, 20 mL, post. Image right (top to bottom) site 4-6: (left to right) pre, 10^{-1}, 10 mL, 20 mL, post.

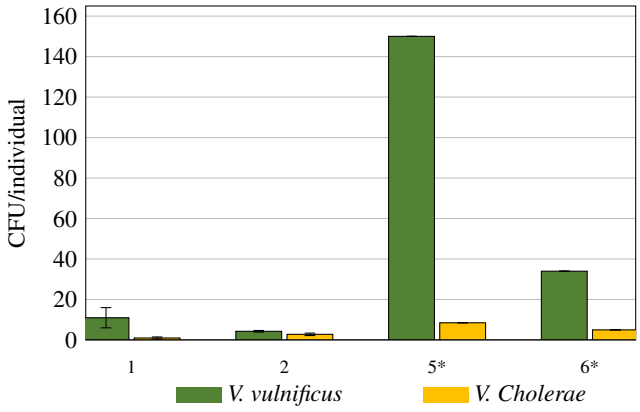

Fig. 4. *Vibrio* colony forming units (CFU) per individual copepod by site. Site 1 $n = 15$; site 2 $n = 50$; site 5 $n = 5$; and site 6 $n = 5$. Sample size varies due to which dilutions were countable. (*) indicates sites with only one countable dilution where standard deviation cannot be reported.

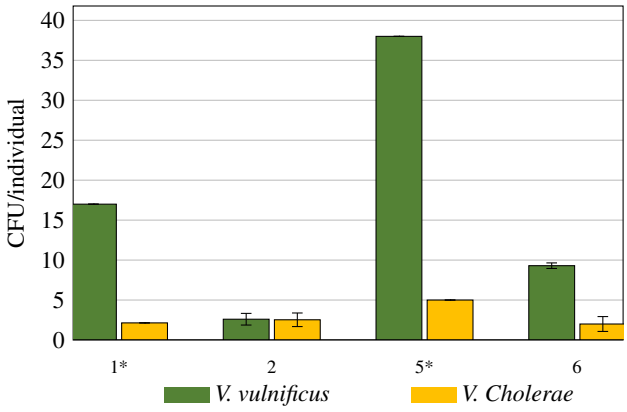

Fig. 5. *Vibrio* colony forming units (CFU) per individual copepod nauplii by site. Site 1 $n = 5$; site 2 $n = 45$; site 5$n = 5$; and site 6 $n = 30$. Sample size varies due to which dilutions were countable. (*) indicates sites with only one countable dilution where standard deviation cannot be reported.

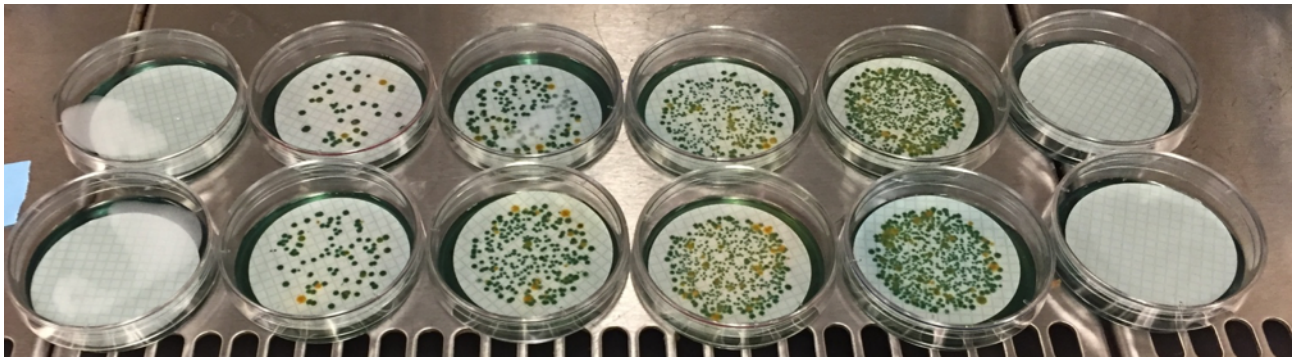

Fig. 6. TCBS plates of site 1 zooplankton samples. Top row: copepods (left to right): pre, 5, 10, 15, 20 individuals, post. Bottom row: copepod nauplii (left to right): pre, 5, 10, 15, 20 individuals, post.

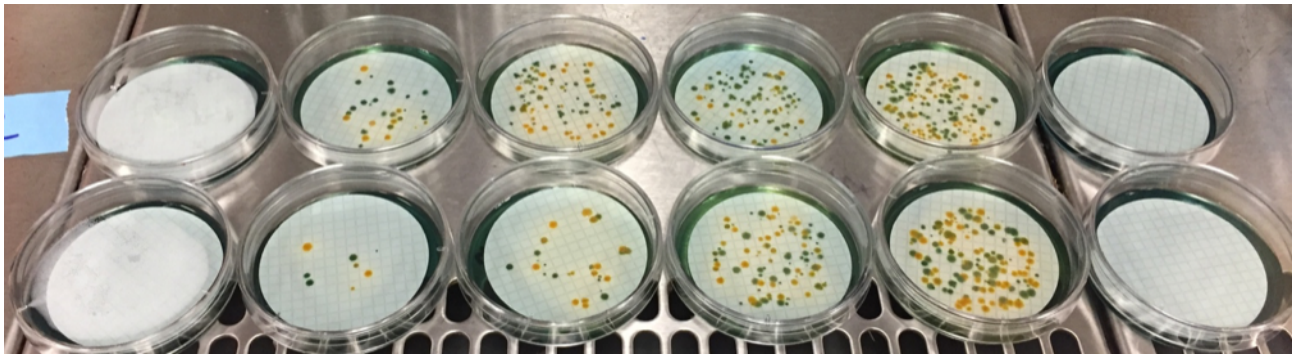

Fig. 7. TCBS plates of site 2 zooplankton samples. Top row: copepods (left to right): pre, 5, 10, 15, 20 individuals, post. Bottom row: copepod nauplii (left to right): pre, 5, 10, 15, 20 individuals, post.

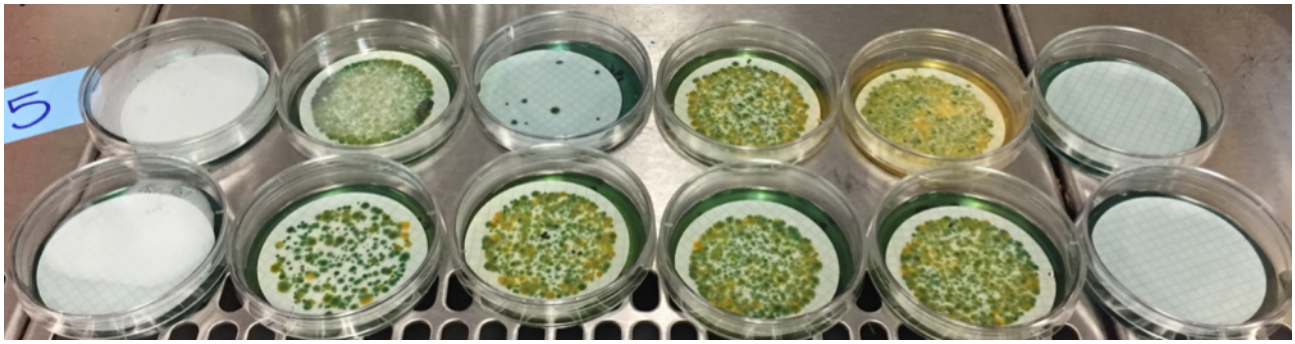

Fig. 8. TCBS plates of site 5 zooplankton samples. Top row: copepods (left to right): pre, 5, 10, 15, 20 individuals, post. Bottom row: copepod nauplii (left to right): pre, 5, 10, 15, 20 individuals, post.

zooplankton or lives free-floating in the water column. Previous studies indicate that temperature and salinity may be key factors in the concentration of both free-living and zooplankton-associated *Vibrio*. However, plankton concentration alone may also provide a distinct pressure on *Vibrio* distribution (Turner et al., 2009).

Given the intricate relationship between *Vibrio* and zooplankton and the critical role it plays in the microbial loop, this association is critical to understand. *Vibrio* directly contributes DOM to the microbial loop, while also acting as a food source for bactiverous zooplankton. This increase in accessible nutrients may have a direct impact on the formation and frequency of phytoplankton

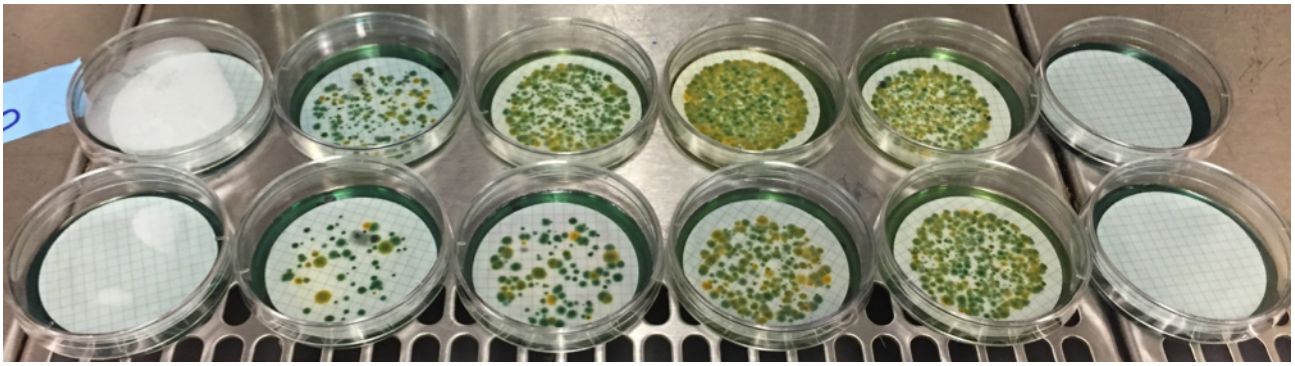

Fig. 9. TCBS plates of site 6 zooplankton samples. Top row: copepods (left to right): pre, 5, 10, 15, 20 individuals, post. Bottom row: copepod nauplii (left to right): pre, 5, 10, 15, 20 individuals, post.

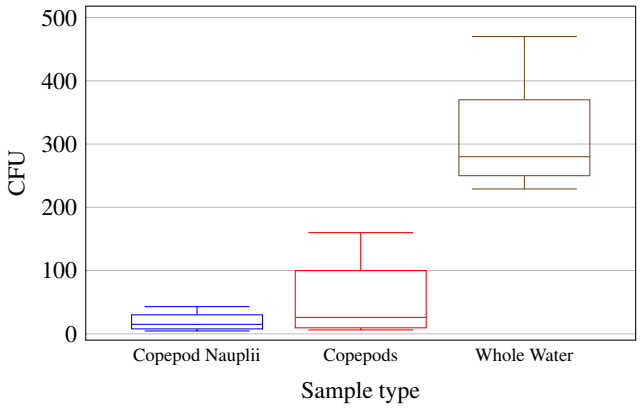

Fig. 10. Overall *Vibrio* colony forming units (CFU) per sample type. Mean copepod nauplii 20 CFU/individual; mean copepods 55 CFU/individual; & mean whole water 313 CFU/mL.

blooms (Turner et al., 2009). Further sampling throughout Tampa Bay and across seasons would be highly valuable in understanding this correlation. Moreover, *Vibrio* concentration should likely be considered when creating bloom prediction models.

Furthermore, analysis of additional zooplankton classes would aid in identifying possible colonizing preference by *Vibrio*. Any colonizing preference could then be used to assess the impact of plankton community composition on *Vibrio* concentration. These methods could also be replicated at various locations to improve overall understanding of *Vibrio* ecology in the marine environment.

The knowledge gained with this research will help to further identify the relationship between *Vibrio* and the planktonic community. This knowledge can then be utilized to not only identify the impact *Vibrio* concentration has on the zooplankton community, but also to predict how the zooplankton community impacts the likelihood of *Vibrio* outbreaks throughout Tampa Bay.

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