The influence of water quality and environmental stress on disease susceptibility of *Crassostrea virginica*, the American Oyster, to the parasite *Perkinsus marinus* in southwest Florida

Leah Briney
Department of Geology, Whitman College, Walla Walla, WA 99362
Faculty Sponsor: Patrick Spencer, Whitman College

Abstract

The presence and intensity of the common oyster pathogen *Perkinsus marinus* increases with salinity. Once ingested, *P. marinus* proteases interrupt standard oyster defense mechanisms and proliferate within the tissues of the host. Oysters living in an altered or unnatural environment are physiologically stressed, which may result in weakened immune responses. Elevated infection intensities among oyster reefs may indicate stressed oysters. High *P. marinus* infectivity rates found in Faka Union, an altered ecosystem in southwest Florida, may suggest inadequate oyster immune response due to environmental stress imposed by water quality changes.

Introduction

Southwest Florida is one of the fastest growing population centers in America. The increase in human inhabitants threatens delicate wetland, marsh, and estuarine ecosystems as development alters the natural hydrology of the area. Canals and weirs allow for control of tidal and seasonal water fluctuations, but this regulation compromises water quality. Diversion of the natural sheet flow hydrology into restricted canals environmentally stresses the organisms in the estuaries, forcing them to survive under unnatural conditions.

Oyster reefs are the backbone of the estuarine geomorphology in the area of the Ten Thousand Islands and Rookery Bay National Estuarine Research Reserve. They provide stable grounds for the roots of the protected mangrove trees in a tidally influenced environment and provide habitat for numerous species of aquatic life. The construction of canals and weirs alters normal tidal water fluctuation cycles. As the weirs open, fresh water input rises dramatically, imposing adverse conditions on the entire estuarine ecosystem.

There is a possible relationship between water quality and disease susceptibility of oysters. Altered environmental conditions stress oysters, causing a decline in their ability to fight off parasites such as *P. marinus.* Subjecting oysters to unnatural salinity conditions increases *P. marinus* infection intensities. The oysters' consequent inability to perform their ecological roles affects the entire estuary.

Henderson Creek and Faka Union Estuaries

The Henderson Creek and Faka Union Estuaries were chosen as the comparative study sites. The Henderson Creek Estuary is protected and in many areas restored. Although there is a weir at the head of this estuary, Henderson Creek is regarded as relatively pristine and houses the Rookery Bay Research and Reserve (RBNRR) headquarters. The Faka Union Estuary is dramatically different from Henderson Creek. With a much lower salinity, this highly developed estuary receives large amounts of fresh water from the Golden Gate Estates.

The oyster reefs within Henderson Creek and Faka Union are correlated based on their relative position within the estuaries. Five oyster reef homologs are traditionally used for condition comparison between the estuaries (Figures 1 and 2). Water quality measurement stations monitored by RBNRR are located along the estuary, allowing for accurate salinity measurements at the study sites.

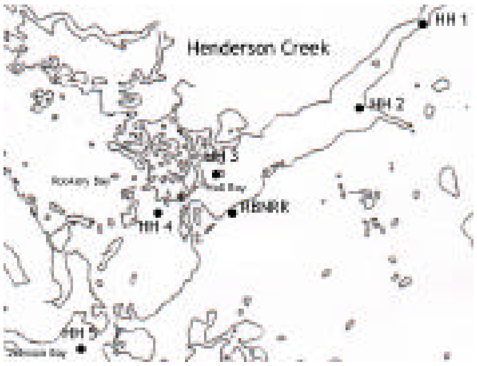

Figure 1. Map showing Henderson Creek Estuary, homologs (HH), and RBNRR headquarters.

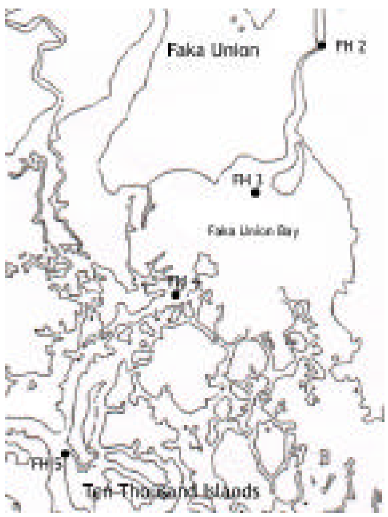

Figure 2. Map showing Faka Union Estuary, homologs (FH), and Ten Thousands Islands National Wildlife Refuge

Ecological Importance of *Crassostrea virginica*

Crassostrea virginica inhabits coastal and estuarine environments along the Atlantic coast from the Gulf of St. Lawrence, Canada to Argentina, and can also be found in Hawaii, Japan, Australia and the United Kingdom (Lenihan 1999). This benthic filter feeding species functions in removing sediment, pollutants, phytoplankton, and organic detritus from large amounts of water (Volety et al. 1999). As a community, oysters compose complex a reef environment inhabited by numerous species. Changes within the oyster reefs produce a chain reaction, affecting all those reliant on them.

Effects of *P. marinus* on the Oyster Community

The protozoan *P. marinus* can withstand water temperatures ranging from 0-36° C and salinities of 0-40 ppt (Shumway 1996 in Lenihan et al. 1999) and is most abundant in tropical and subtropical climates (Chu and Volety 1997). As the oysters filter water, *P. marinus* larvae are ingested, which develop and proliferate within the sinuses and tissues of the host (Lenihan et al. 1999). Upon death, the oysters' valves open, releasing parasites into the water where they are reingested by other oysters (Lenihan et al. 1999). Infection by *P. marinus* affects oysters by inhibiting growth, decreasing shell and gamete production, and disrupting filtering capabilities (Kennedy 1996).

Temperature and Salinity

The distribution and abundance of *Perkinsus marinus* in estuaries is dependent on salinity and temperature (Chu and Volety 1997, La Peyre 1996). Because the Gulf water temperature remains more or less constant throughout the year, it is likely that infections of *P. marinus* are related to seasonal salinity fluctuations rather than temperature (Chu 1997). *P. marinus* is most numerous in highly saline water, and less abundant in fresh water, thus infections are more intense at higher salinities (Chu and Volety 1997).

Methods

Disease diagnosis was possible using techniques outlined by Fisher and Oliver (1996). Oyster clumps were collected at each of the homologs within the Faka Union and Henderson Creek estuaries (Figure 1 and 2). Using hammers and chisels, the clumps were broken down into individual samples and stored in ziploc bags to avoid cross contamination. To prevent any temperature change influence from sun exposure, the plastic bags were stored in a cooler in the shade to prevent proliferation during the time between sample collection and lab analysis. In the lab, the oysters from each homolog were laid out on butcher paper and a representative sample of six oysters per reef was chosen. To prevent any age or size discrimination among the samples, the largest and smallest oysters from each reef with a gradation of sizes in between were selected. Wet weight and shell length were measured and recorded for each oyster to document size variation.

Results

<u>Henderson Creek Estuary</u>-

The yearly average salinity in Henderson Creek Estuary (Figure 3) gradually increases from 19.8 ppt just above homolog 1, to 32.98 ppt at homolog 5. Parasite intensity increases from homolog 1 to homolog 5 as a function of increasing salinity (Figure 4). Every oyster sampled from Henderson Creek Estuary was diagnosed as having at least one parasite, it was thus concluded that Henderson Creek Estuary has a 100% infectivity rate.

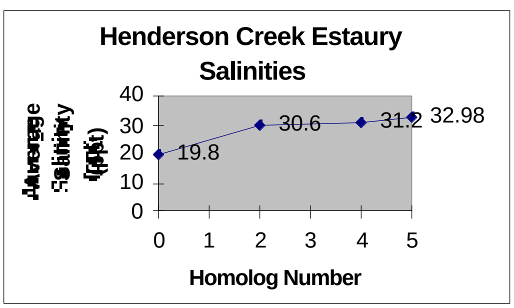

Figure 3. Henderson Creek average salinity data.

<u>Faka Union Estuary</u>-

The lower salinity and high level of fresh water in Faka Union prevents oysters from surviving and constructing a reef correlative to Henderson Creek Estuary's homolog 1, thus only reefs 2,3,4 and 5 were assessed. Also, water quality management stations in close proximity to homolog 2 are absent, preventing salinity measurement. Figure 5 shows the increasing salinity of the estuary from 27.2 ppt at homolog 3 to 32.2 ppt at homolog 5. Parasite intensity (Figure 4) increases with salinity, however homolog 3 has an unusually high parasite abundance.

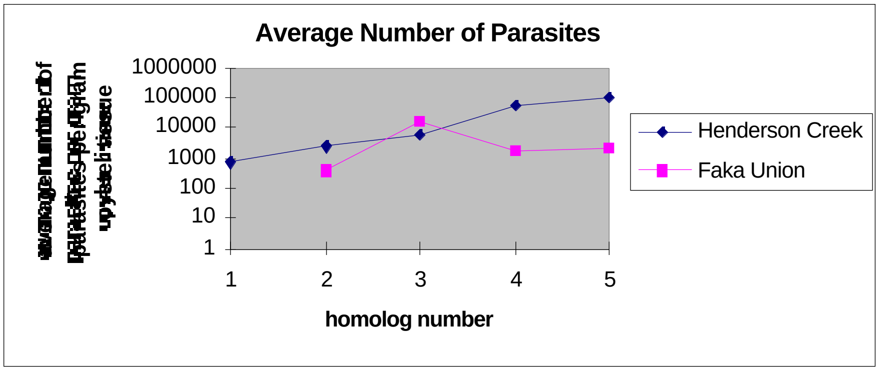

Figure 4. Comparison of average parasites per gram oyster tissue at each homolog for Henderson Creek and Faka Union.

Faka Union homolog 2 is a small reef with very few oysters located along the main canal before the estuary opens into the bay. The constant canal flow of fresh water and related low abundance of *P. marinus* at this site may be responsible for the low parasite intensity. Homolog 3 has the highest overall average of parasites per gram oyster tissue in the Faka Union Estuary. This can be attributed to fairly ideal parasite growth and reproductive conditions at this locality. Lowest mortality and highest reproductive and growth rates for *P. marinus* occur within the temperature range of 20-30° C (Shumway 1996 in Lenihan et al. 1999) and salinities of 24-30 ppt (La Peyre 1996). The average salinity for Faka Union homolog 3 is 27.2 ppt, while homolog 5 is well above 30 ppt, thus it is possible that homolog 3 may be the only Faka Union reef residing within the optimal *P. marinus* conditions. However, salinity data from homologs 2 and 4 must be obtained for further investigation.

The Faka Union Estuary has an infectivity rate of 96%, and 23 of the 24 samples were *P. marinus* positive. The *P. marinus* negative oyster was the smallest of all Faka Union samples and was obtained from homolog 2. The low salinity, and diluted *P. marinus* concentration in the water column, combined with the young age of the oyster may have both influenced this rare occurrence.

Discussion

Table 1 compares the number of parasites per gram tissue for each homolog. Due to the lower salinity and dilution from fresh water input, the parasite intensity of Faka Union is lower than that of Henderson Creek. Although Faka Union homolog 3 has an unusually high intensity; over two and a half times that of Henderson Creek homolog 3- it is far less than the parasite counts for Henderson Creek homologs 4 and 5. The unusually high anomaly at Faka Union homolog 3 may be an indicator that increased amounts of fresh water may stress oysters, causing them to be more susceptible to disease.

Homolog Number	Faka Union	Henderson Creek
1	n/a	716
2	357	2,160
3	16,000	5,930
4	1,660	57,900
5	2,080	109,000

Table 1. Average parasite count per homolog.

The salinities at Faka Union homologs 4 and 5 as compared to Henderson homologs 4 and 5 (Figures 3 and 5) are similar because these homologs are in close proximity to the ocean where saline water mixes with fresh water from the estuaries. Although there is a large amount of fresh water input into the Faka Union Estuary, the oysters at these reefs are not subjected to unnatural salinity conditions, thus they may not be environmentally stressed. Abundance of *P.*

marinus in the water column at these homologs may be less than that at the correlative sites in Henderson Creek Estuary as a result of flushing and dilution by fresh water input from the Golden Gates Estates into Faka Union.

Conclusion

The high infectivity rate at Faka Union homolog 3 may indicate that fresh water input is environmentally stressing the oysters causing them to be more susceptible to the parasite *Perkinsus marinus*. However, homologs 2, 4, and 5 may indicate the exact opposite, and that more factors need to be taken into account to explain the unusual infectivity rate at homolog 3.

Acknowledgments

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