ANALYZING SEASONAL VARIATIONS IN BENTHIC FORAMINIFERA IN BUDD INLET, PUGET SOUND, WASHINGTON, U.S.A.

By

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Abstract: Coastal and estuarine pollution has been a growing concern within this century. Benthic foraminifera have been commonly used as a bioindicator for waterway pollution because of their abundance, diversity, and relatively short lifespans. Researchers have found test abnormalities among benthic foraminifera in Budd Inlet in Puget Sound. Test abnormalities have typically been found in environments with high levels of heavy metals in the waterways often near anthropogenic sources of pollution. This thesis aims to document winter population diversity, abundances, and percentages of mutation of foraminifera in Budd Inlet, and compare them with past collections from summer months. The results of this study showed that the foraminifera from the summer season was much more abundant than the winter foraminiferal population. There were no test abnormalities in West Bay and East Bay and there were very few in Priest Point. Comparisons of the living and the dead assemblages documented much higher diversities in dead vs living populations, indicating different species must inhabit the region in either the fall or spring seasons as they have not been observed in the living populations collected in the winter or summer. These results suggest a significant gap in our understanding of foraminiferal ecology leading to future projects in the Puget Sound.

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CHAPTER I

INTRODUCTION

While some pollutants may be introduced naturally, anthropogenic sources have also been a cause of an increase in pollution. The causes of waterway pollution include industrial, agricultural, domestic, and mining activities. For example, agricultural runoff enriched with nutrients and pesticides causes eutrophication and deterioration of the water quality (Coccioni and Frontalini, 2011). Heavy metal contaminants within these waterways are a concern because they can accumulate in sediments and are thought cause shell abnormalities in benthic foraminifera (Alve, 1991; Alve 1995; Coccioni and Frontalini, 2007; Coccioni and Frontalini, 2011; Labin et al., 1992).

The use of foraminifera as bioindicators of polluted waterways started with Resig (1960) and Watkins (1961). The abundance, diversity, and relatively short lifespans of foraminifera, and sensitivity to ecological changes make them good bioindicators of the health of the ecosystem (Sreenivasulu et al., 2018). Certain species of benthic foraminifera, such as *Ammonia beccarii*, are more tolerant to pollutants (Alve, 1991; Alve, 1995; Châtelet and Debenay, 2009; Coccioni and Frontalini, 2007; Coccioni and Frontalini, 2011; Sreenivasulu et al., 2018).

Therefore, higher abundances of *Ammonia beccarii* in an environment with relatively low diversity in other foraminiferal species is seen as an indicator of a stressed environment (Alve, 1991; Alve 1995; Châtelet and Debenay, 2009; Coccioni and Frontalini, 2011; Ernst et al., 2006; Sreenivasulu et al., 2018).

In this thesis, summer and winter populations of Budd Inlet of the Puget Sound are compared in order to explore foraminifera distribution and pollution effects. Among the summer populations, abnormalities have been observed in *Ammonia beccarii* within Budd Inlet, and this thesis examines the abundance, distribution, and percentages of mutations in a winter population. Availability of resources and environmental conditions (i.e. temperature, salinity, etc.) are likely to explain differences in population abundance and species diversity between summer and winter months. Winter populations are less mutated than summer populations, which could indicate that something is happening to the summer populations that cause these mutations. Our observations will allow us to make better decisions about when and where to focus future sampling efforts in the Sound (e.g. temperature, salinity, anthropogenic pollutants). Specifically, this thesis focuses on:

- Process and analyze samples collected during the winter of 2022
- Analyze the diversity, abundance, and percentage of abnormalities of winter samples against summer samples from 2020
- Analyze archival environmental data from the state of Washington for any changes in trace elements in Budd Inlet to determine whether they may correlate with test abnormalities

CHAPTER II

LITERATURE REVIEW

FORAMINIFERA

Foraminifera are single-celled marine organisms that are found in both benthic and planktonic environments. They have mineralized shells or “tests” composed primarily of calcium carbonate and rarely of opaline silica. Some species of foraminifera are agglutinated, meaning that they draw in sedimentary particles to create their own shell instead of precipitating a shell of calcium carbonate. Foraminifera can range from 63 µm to 20 cm in length (Sen Gupta, 1999) and are the most abundant protozoa in the marine world (Coccioni and Frontalini, 2007). This thesis will focus on shallow-water benthic foraminifera.

BENTHIC FORAMINIFERAL ECOLOGY

Benthic foraminifera are commonly used bio-indicators for waterway health because of their relatively short lifespans and sensitivity to changes in their environment (Sreenivasulu et al., 2018). The use of benthic foraminifera as proxies for pollution was initiated by Resig (1960) and Watkins (1961) and continued through to the present by studies such as Châtelet and Debenay (2009). The carbonate shell of most benthic foraminifera is easily preserved which makes a good record of environmental stresses over time (Coccioni and Frontalini, 2007).

Ammonia beccarii

Ammonia beccarii is a species of benthic foraminifera commonly found in shallow marine, saline to brackish environments (Labin et al., 1995). The test shape of *A. beccarii* is described as having about 10 chambers, biconvex in apertural view, with chambers arranged in a trochospiral pattern, meaning that the chambers are in a coil that forms a spire like a snail shell. The spiral side shows all the chambers while when positioned with the umbilical side up, only the final chamber is visible. On both the spiral and umbilical side of *A. beccarii*, there are deeply engraved sutures edged with prominent thickenings which result in open interlocular spaces between the radial chambers and whorls. The pseudopodia of *Ammonia beccarii*, or the protrusion that is used for feeding, can be extruded from the suture areas (Debenay et al., 1998).

Ammonia beccarii have been seen living and growing on algae (epibenthic) as well as living and growing in sediments (infaunal) which is likely due to food availability and/or changing environments (Debenay et al., 1998). Another common Puget Sound species that can switch from epibenthic to infaunal in changing environments is *Cribroelphidium excavatum*. The seasonal change in the habitat preference of *Ammonia beccarii* is likely due to food availability because the surface of algae and seagrass can be covered in diatoms, fungi, and other bacteria which are common foods for *Ammonia beccarii* (Debenay et al., 1998).

FORAMINIFERAL TEST ABNORMALITIES

Test abnormalities normally relate to environmental stress which can be natural or anthropogenically caused. This is a concern because stress affects the entire ecosystem within that waterway including plant life, fish population, and other mammals. A potential for these test abnormalities is high concentrations of heavy metals (Alve, 1991; Alve, 1995; Labin et al., 1992). Common abnormalities found in benthic foraminifera include 1) double aperture, 2) smaller sizes of one or more chambers, 3) protruding chambers, 4) distorted chamber arrangements, 5) enlarged apertures, 6) distorted chamber shape, 7) twinned or "Siamese" forms (Alve, 1991; Alve, 1995; Labin et al., 1992).

STUDY AREA

Puget Sound

The Puget Sound is an estuarine system located in Washington State. It connects to the southern portion of the Salish Sea, a north-south trending waterway connected to the Pacific Ocean via the Strait of Juan de Fuca. Formed during the Pleistocene glaciation, the Salish Sea is a complex series of fjords lying inland of the Olympic Peninsula (Martin and Nesbitt, 2015). The damming of the proglacial lakes, expanses of outwash deposits, and large recessional outwash combined with the deposition of till and glaciomarine drift produced the sediment cover of the bedrock in the Sound. The deep erosional troughs that made the Sound were carved when subglacial drainage from quickly receding ice sheets (Martin et al., 2013). The Puget Sound covers an area of 2,330 km^2. The Puget Sound is divided into interconnecting basins: Whidbey, Central, Hood Canal, and South (Moore et al., 2008). Currents and mixing patterns are very affected by basin topography and major river inputs. Hood Canal and several inlets in South Puget Sound have low vertical mixing and total exchange rates (Mackas and Harrison, 1995). The subtidal circulation is mainly driven by density, the contrast between the freshwater from the rivers and the salty marine water from the mouth forcing circulation (Babson et al., 2010). Large influxes of freshwater, sediment, nutrients, and pollutants are found in the Puget Sound and have been monitored by the Washington State Department of Ecology (WDOE) since the 1980s. The Washington State Department of Ecology provides many studies and data on its website and has documented the changes in water quality, sediment quality, etc. for the entirety of the Puget Sound. The Puget Sound contains many lumber mills, shipping yards, and naval yards some of which used to discharge waste into various inlets in the sound. For example, Sinclair Inlet, located western part of the Puget Sound, had the Puget Sound Naval Shipyard which from 1896 until 1957 would discharge its industrial waste directly into Sinclair Inlet (Martin and Nesbitt, 2015).

Budd Inlet

Budd Inlet is the southernmost inlet in the Puget Sound located in Olympia, Washington. Budd Inlet is about 7 miles long, 1 mile wide at the mouth, and 2 miles wide at the center. At the head of Budd Inlet are two bays, West and East Bay, which are divided by a peninsula (LOTT Wastewater Management Partnership, 1998). Samples were collected from West Bay Park, East Bay Park, and Priest Point Park (Figure 1). Priest Point Park is located on the eastern side of Budd Inlet but is further north than East Bay Park. The southern portion of Budd Inlet has supported the wood product industry, recreational marinas, and the boating industry (Washington State Department of Health, 2008). The Lacey, Olympia, Tumwater, and Thurston (LOTT) County Wastewater Treatment Plant is located on the peninsula at Budd Inlet and generates significant discharge in Budd Inlet (LOTT, 2000). The inlet has been dredged to 30 ft below mean lower low water, which is the average of all lower water heights observed over the National Tidal Datum Epoch (NOAA a, n.d.), to allow freighters to access the Port of Olympia facilities located on the peninsula (LOTT, 1998). Also located on the southern end of the inlet is a lumber yard which could potentially increase nitrate and potassium levels in the water (Lynch and Corbett, 1990).

Total organic carbon and nitrogen content in Budd Inlet sediments were among the highest in the Puget Sound as of 2018, but exposure to the harmful chemicals has not changed since a survey from 2011 to 2018 (Figure 2 and Figure 3; Washington State Department of Ecology a, 2022). Total organic carbon and nitrogen have the highest concentrations in the central and southern portions of the inlet (Washington State Department of Ecology a, 2022). Sediments found in the sound contain a lot of organic material mainly derived from algae and terrestrial plants. The chemical concentrations were not considered above the State's Sediment Quality Standards (Washington State Department of Ecology a, 2022).

During the spring, large influxes of freshwater water caused by snowmelt mixes with the saltwater to create a warm, nutrient-dense, surface layer that leads to a positive environment for plankton to grow. The extra nitrogen as well as other nutrients lead to plankton growth and an eventual blooming mostly in the spring and summer seasons (Russell, 2018). Data from the monitoring by the Washington State Department of Ecology has shown that several inlets along the south and west parts of the Puget Sound have one or more early warning signs of eutrophication (Mackas and Harrison, 1995).

Priest Point

Priest Point Park, now formally known as Squaxin Park as of April 2022, is located on the northeast side of Budd Inlet. Due to its close proximity to a sewage treatment plant outfall, it was marked as unsafe for recreational shellfish harvesting as of 2008 (Washington State Department of Health, 2008). The park, as well as the beach, are open and available to the public.

East Bay

On the southern end of Budd Inlet, East Bay is located east of the peninsula and is used by industrial and commercial businesses to handle cargo, manufacture boats, operate marinas, and treat wood (Washington State Department of Health, 2008). In addition to the LOTT Wastewater Treatment Plant, the Moxlie Creek discharges into East Bay at the southern end of the peninsula. As part of the Washington State Department of Ecology's monitoring of East Bay, some areas are currently being remedied, while others are scheduled for remediation in the near future (Washington State Department of Ecology, b, 2022).

West Bay

At Budd Inlet's southern end, West Bay is located on the peninsula's western side. Hardel Mutual Plywood, Weyerhaeuser Olympia Log Yard, Weyerhaeuser Olympia Log Yard, and Reliable Steel are some of the industries located in West Bay. It was originally intended to be a log yard but has since evolved into a steel factory (Washington State Department of Health, 2008). West Bay has undergone remediation and has been built into a park along the water (Washington State Department of Ecology, c, 2022).

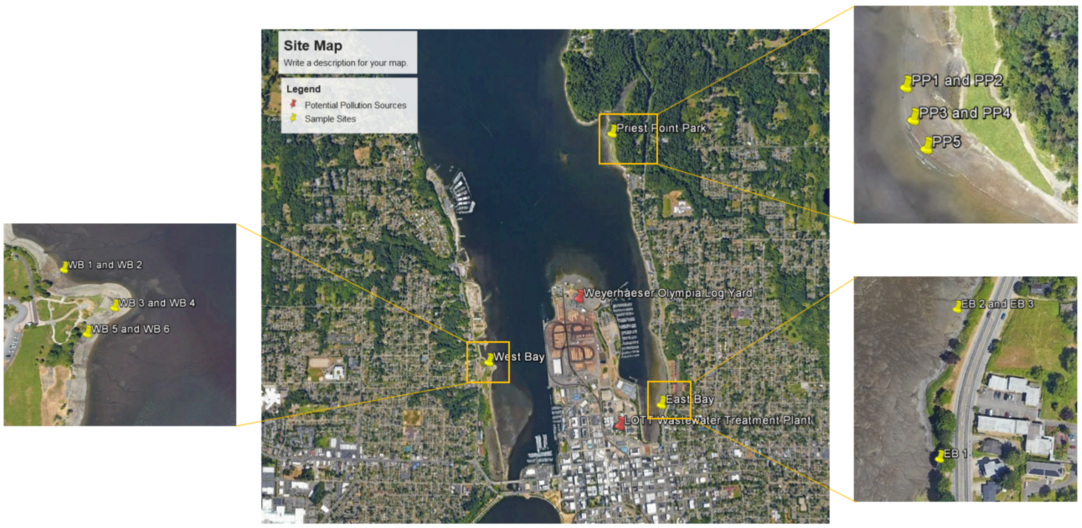

Figure 1: A map of Budd Inlet showing the three sample collection sites: West Bay, East Bay, and Priest Point (the yellow pins) as well as potential pollution sources: the LOTT Wastewater Treatment Plant and the Weyerhaeuser Olympia Log Yard (the red pins). The Priest Point sample locations are in the top right box, the East Bay sample locations are in the bottom right box, and the West Bay sample locations are in the middle left box

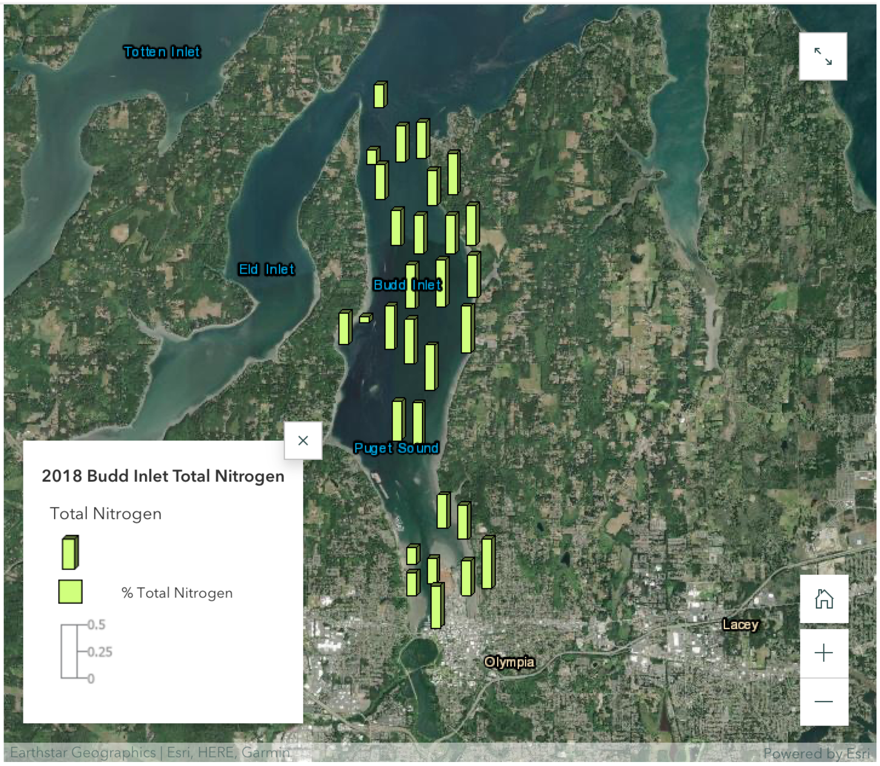

Figure 2: A map showing the Total Nitrogen content in Budd Inlet from 2018 (Washington State Department of Ecology a, 2022).

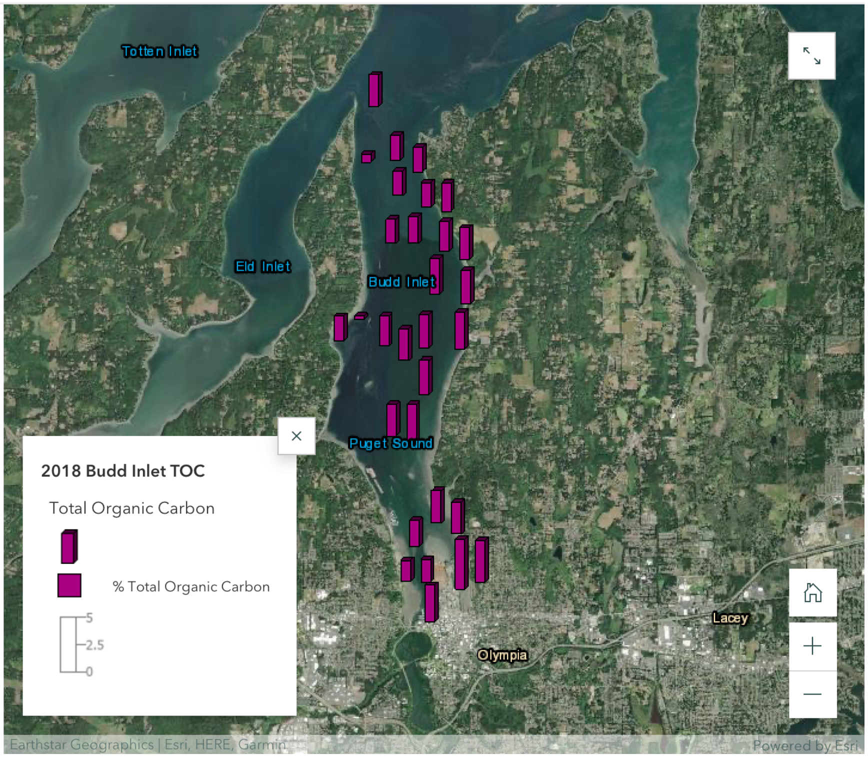

Figure 3: A map showing the Total Organic Carbon found in Budd Inlet in 2018 (Washington State Department of Ecology a, 2022).

SUMMER AND WINTER POPULATIONS

Foraminiferal populations commonly vary between summer and winter populations as a result of differences in temperature, salinity, dissolved oxygen, sediment grain sizes, and quality and amounts of nutrients. Typically in the winter season, salinity is higher because of lower amounts of rainfall and accumulation of water in the form of snow. Dissolved oxygen, an important parameter in maintaining life in the aquatic environment, is slightly higher in the winter than in the summer because the decrease in the temperature of the water decreases the volatilization of the water gasses. A study in the Saquarema Lagoonal System discovered larger foraminifera populations and diversity in the summer compared to the winter population with both seasons showing a dominance of *Ammonia tepida* and consistently muddy substrates (Belart et al., 2018).

In the Puget Sound, average water properties change seasonally and inter-annually due to the basins buffering the large seasonal fluctuations of river discharge and the properties of incoming oceanic deep water fluctuating from outer coast upwelling (Mackas and Harrison, 1995). The annual streamflow maximum occurs during periods with the highest precipitation and snowfall (Moore et al., 2008). In the winter, riverflow peaks due to runoff, and it peaks again in the spring or early summer due to snowmelt while riverflow is at its lowest in September (Babson et al., 2010). Water from the Pacific Ocean rich in nutrients supports high primary productivity, and the tidal currents move the marine water all across the Sound. In the Puget Sound, temperature fluctuations are thought to have the most impact on benthic foraminiferal abundances and distribution of species with higher temperatures associated with the highest abundances of foraminifera (Martin et al., 2013). During the winter season, rainfall and snowfall are the highest (Martin et al., 2013) which is likely to result in changes in salinity within the study area while in spring and summer months, algal cover is at its highest, which serves as food for many shallow water foraminifera, and it is lowest in the winter (Thom, 1979).

CHAPTER III

METHODOLOGY

COLLECTION OF SAMPLES

Surface core samples were collected in January 2022 from Priest Point, West Bay, and East Bay. Samples were collected at the lowest tide during the day by sticking a coring tube in the sand along the beach. These cores were then cut and separated into 1 cm intervals. For Priest Point, samples were cut from 0-1 cm and 1-2 cm, but for West Bay and East Bay samples were only able to be cut from 0-1 cm. These cut cores were placed into labeled mason jars where they were in 4% ethanol and stained with Rose Bengal, which stains living tissue pink, and shipped to Oklahoma State University. In total, 11 core samples were collected from Priest Point, however, only 5 core samples were fully processed due to time constraints. Six core samples were processed and analyzed from West Bay, and 3 core samples were processed and analyzed from East Bay. During core sample collection, temperature, salinity, dissolved oxygen, and conductivity concentrations were collected as well (Table 1).

SORTING AND SPT FLOATATION METHOD

The sodium and polytungstate or SPT floatation method was used to extract foraminifera from these core samples (Semesatto and Dias-Brito, 2007). This process successfully expedited the picking process by separating the foraminifera from denser sedimentary particles. Preserved and stained samples were homogenized and washed through four sieves (500 µm, 125 µm, and 63 µm). Separated samples were then transferred to filter paper and drained of most of the water using a Büchner funnel attached to a vacuum pump. Samples were then placed in an oven set to 60ºC until sediments were completely dry.

Once dry, the samples were ready to be floated. The SPT solution, with a density of 2.50 g/mL, was poured into 10 mL graduated cylinders, next the samples were poured in using a funnel careful not to overflow the graduated cylinder. Separation took place as the sediments denser than the solution sank to the bottom of the graduated cylinder and sediments less dense floated as seen in Figure 4. Once the separation of sediments was complete, the float and sink portions were decanted and removed, placed in a Büchner funnel with filter paper to completely remove the solution. The separated samples were placed in an oven set to 60ºC to completely dry. Once dry, the float samples were ready to be picked. Figure 5 shows the difference in size between the float and sink sediments.

Figure 4: The separation of West Bay sediments using the SPT method

Figure 5: The difference in size between the float sediments (left) and the sink sediments (right) of the 125 µm from Priest Point 2.

WET PICKING

To remove the foraminifera from the floated portion of the core samples, the process of wet picking was used. Sections of the sediment are poured into a petri dish and water is added to the dish. All stained and unstained foraminifera are removed from the float sediments and placed on a glued slide. Once the foraminifera are removed, specimens from each sample were counted and identified to the species level.

ENVIRONMENTAL DATA COLLECTION

Once the foraminifera were identified and counted, they were standardized to number per 50 ccs by dividing the raw counts with their dry sediment volume (in grams) and multiplying that by 50. The 50 cc counts were used in all the calculations and comparisons to account for any variability in volume or recovery between samples. To quantify the different species of foraminifera and demonstrate biodiversity between sample locations, the Shannon-Wiener Species Diversity Index equation was used. This equation measures diversity while taking the species richness and their relative abundance into account. High diversity in the community will cause a high diversity index (Shannon, 1948; Gibson and Buzas, 1973). $H' = -\sum_{i-1}^{R} p_i ln p_i$

Equation 1: H is the Shannon-Wiener Species Diversity Index, p_i is the proportion of i^{th} species in the samples

The Kruskal-Wallis *H* test was used to compare samples and determine if they come from the same distribution (Siegel, 1988).

$$H = (N - 1)\frac{\sum_{i=1}^{g} n_i\left(\bar{r}_i - \bar{r}\right)^2}{\sum_{i=1}^{g}\sum_{j=1}^{n_i}(r_{ij} - \bar{r})^2}$$

Equation 2: Where H is the Kruskal-Wallis test, *N* is the number of observations across all groups, *g* is the number of groups, n_i is the number of observations in group *i*, r_{ij} is the rank of observation *j* from group *i*, $\bar{r}_i$ is the average rank of all observations in group i, and $\bar{r}$ is the average of all the r_{ij}.

All environmental data from Budd Inlet in January 2022 was collected during sampling except for heavy metal information which was found on the Washington State Department of Ecology website and salinity values reported by Walker et al., 2022 (Table 1). The summer samples were processed by Roark, (2022), and the environmental data was found in the Washington State Department of Ecology as well as the Pacific Shellfish Institute.

IMAGING

Pictures were also taken using a Nikon SMZ25 with Z-stack image processing in the micropaleontology lab at OSU. These images illustrate different species as well as some abnormalities among the living and dead populations (Figures 15-21).

CHAPTER IV

RESULTS

POPULATION DYNAMICS

West Bay had the highest abundance of living foraminiferal assemblages. West Bay was the coldest site and had the highest dissolved oxygen concentration, however, dissolved oxygen in all three sites is very high. Priest Point had the highest conductivity as seen in *Table 1*.

Site	**East Bay Winter 2022**	**West Bay Winter 2022**	**Priest Point Winter 2022**
Total Foraminifera per 50 ccs	19	1,772.82	323.59
Total *Ammonia beccarii* per 50cc	19	1,751.14	287
Total *C. excavatum* per 50cc	0	0	38.26
Total *Buccella frigida* per 50cc	0	0	1.49
Total *Trochammina* sp. per 50cc	0	21.68	0
Abnormal *A. beccarii* (%)	0%	0%	1.07%
Salinity (ppt)*	29.8	29.8	29.8
Temperature (°C)	8.7	7.7	8.6
Dissolved Oxygen (mg/L)	8.7	10.24	8.95
Conductivity (us/cm)	765.7	400	1681
Depth	Surface at low tide	Surface at low tide	Surface at low tide
Noted Heavy Metals*	Confirmed levels of Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, and Zn above EPA standard in soil	None above EPA standards in soil	Unknown

Table 1: A comparison of living foraminiferal populations from winter 2022 collected from East Bay, West Bay, and Priest Point. The raw counts at each site were converted to 50 ccs to normalize the data. Water quality data were collected during the sample collection trip. *Best available data (Washington State Department of Ecology b, c, 2022; Walker et al., 2022).

Living and dead foraminifera from the winter of 2022 samples are primarily composed of *Ammonia beccarii* at East Bay, half of West Bay, and most sites of Priest Point. Two sample sites of Priest Point contain more *Cribroelphidium excavatum* as seen in *Table 2*. In West Bay 5 and West 6, an agglutinated form of *Trochammina* sp. was the most abundant species. In West Bay and East Bay, agglutinated forms of *Trochammina* sp. were more present, but few were found in Priest Point.

Site	**East Bay 1**	**East Bay 2**	**East Bay 3**	**Priest Point 1**	**Priest Point 2**	**Priest Point 3**	**Priest Point 4**
A. beccarii	26.29	32.88	13.16	42.35	119.53	67.17	142.08
C. excavatum	36.82	1.86	13.16	126.25	127.7	46.93	136.21
Buccella frigida	1.17	0	0	5.66	47.27	5.52	17.45
Elphidiella hannai	5.85	0	0	42.68	65.92	37.71	29.42
***Bulimina* sp.**	0	0	0	0	0	0.92	0
***Trochammina* sp.**	25.73	6.52	7.75	0	0	3.57	1.16
Site	**Priest Point 5**	**West Bay 1**	**West Bay 2**	**West Bay 3**	**West Bay 4**	**West Bay 5**	**West Bay 6**
A. beccarii	146.52	631.26	490.76	172.41	5.26	103.86	474.53
C. excavatum	37.22	0	110.62	0	0	391.42	120.78
Buccella frigida	6.41	0	4.31	0	0	0	0
Elphidiella hannai	1.99	0	14.02	0	0	11.34	4.58
***Bulimina* sp.**	0	0	0	0	0	0	0
***Trochammina* sp.**	0	21.5	29.12	9.68	15.78	1091.63	680.48

Table 2: The total counts per 50 cc of both living and dead populations collected in the winter of 2022.

Among the total living and dead population, West Bay was the most abundant site especially in West Bay 5 and 6 (Figure 6). There was a large difference between the percentage of living and dead foraminifera found in winter 2022 at all sites (Figures 7-9).

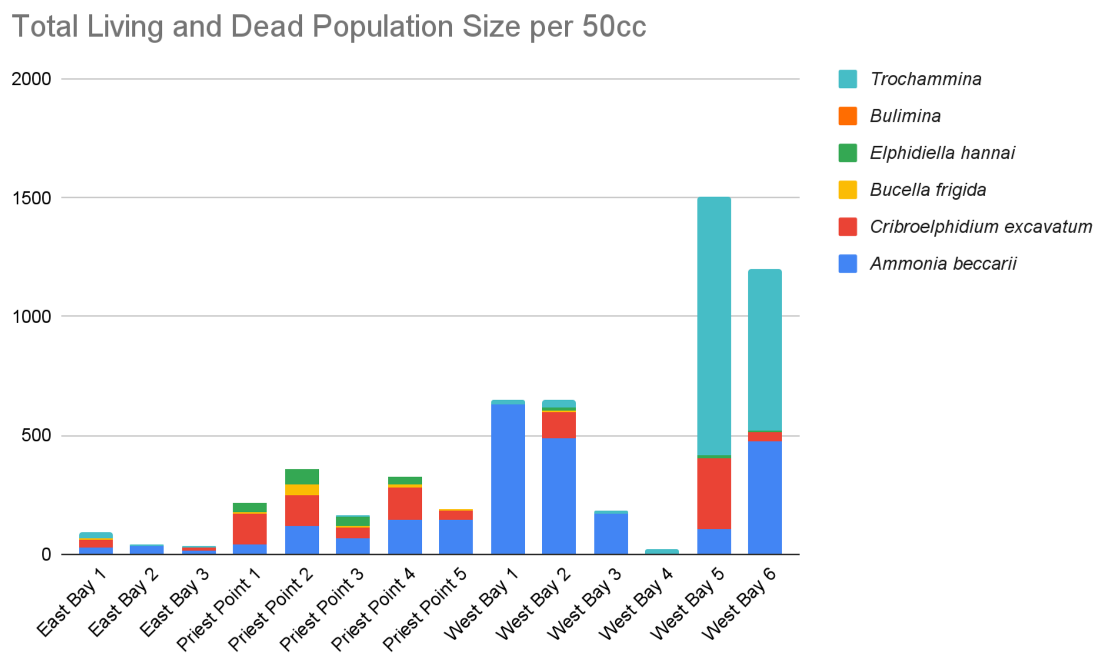

Figure 6: A bar graph plot of the total population of the combined living and dead assemblages of East Bay, Priest Point, and West Bay. This graph emphasizes the stark difference in abundance between East Bay and West Bay.

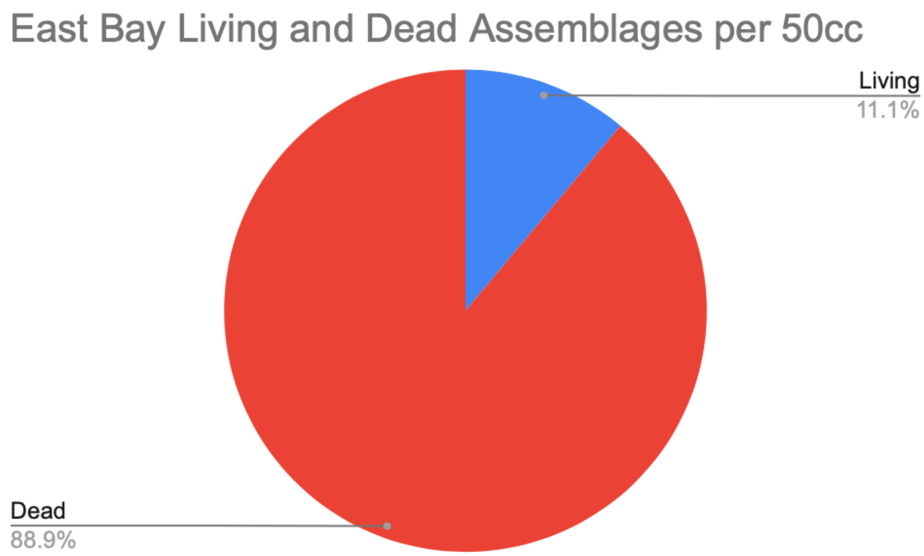

Figure 7: A pie chart showing the percent amount of living foraminifera in the total combined East Bay samples and dead foraminifera in the total combined East Bay samples.

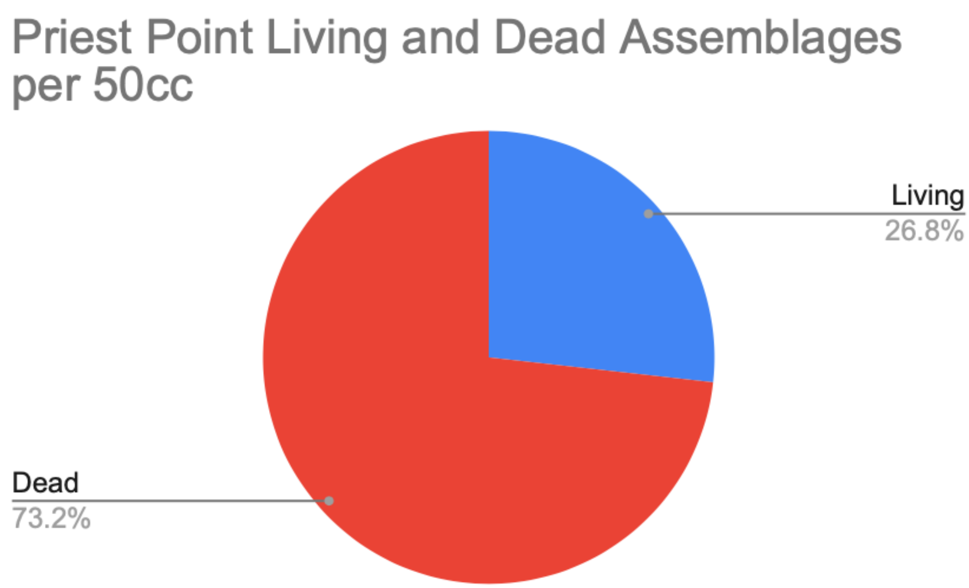

Figure 8: A pie chart showing the percent amount of living foraminifera of the total combined Priest Point samples and dead foraminifera of the total combined Priest Point samples.

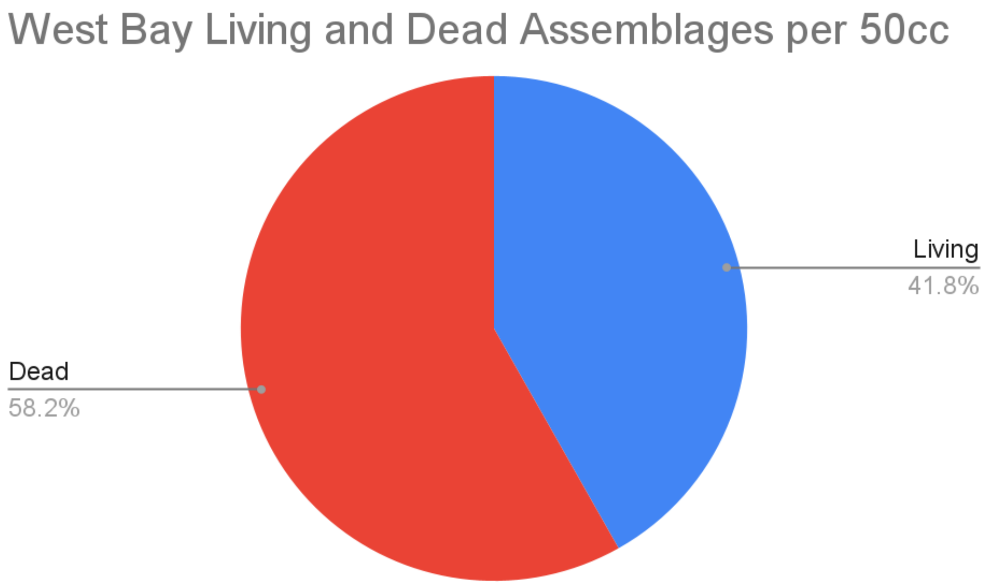

Figure 9: A pie chart showing the percent amount of living foraminifera of the total combined West Bay samples versus the percent amount of dead foraminifera of the total combined West Bay samples

As shown in Figure 10, Priest Point has the greatest diversity of living populations, followed by West Bay (Figure 11), which only has *Ammonia beccarii*. According to Figures 12, 13, and 14, Priest Point also has the greatest diversity of living and dead foraminiferal populations.

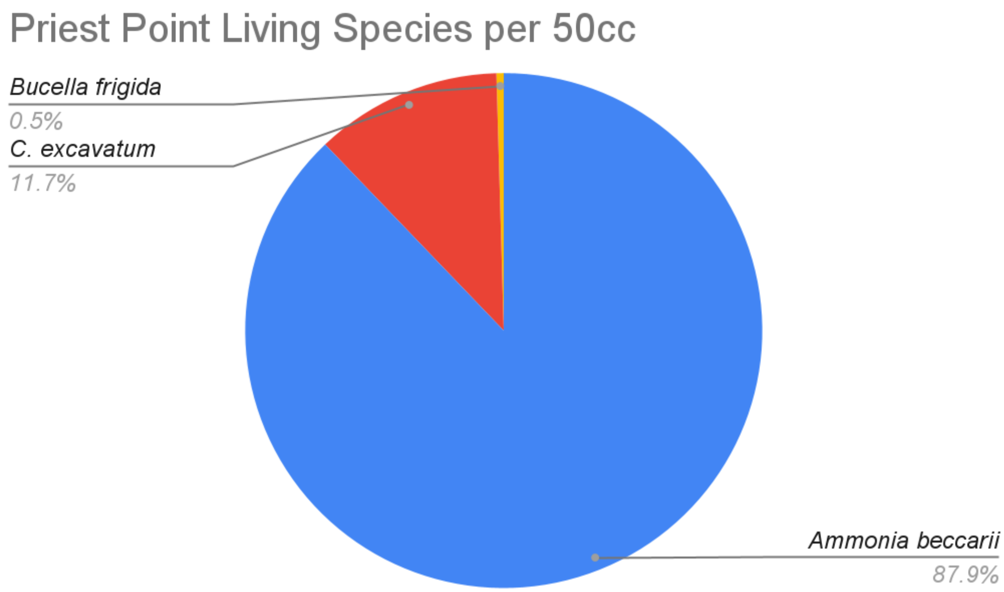

Figure 10: A pie chart showing the percentage of the diversity of the living species in Priest Point per 50 ccs. This shows that *Ammonia beccarii* is the most abundant species in the winter.

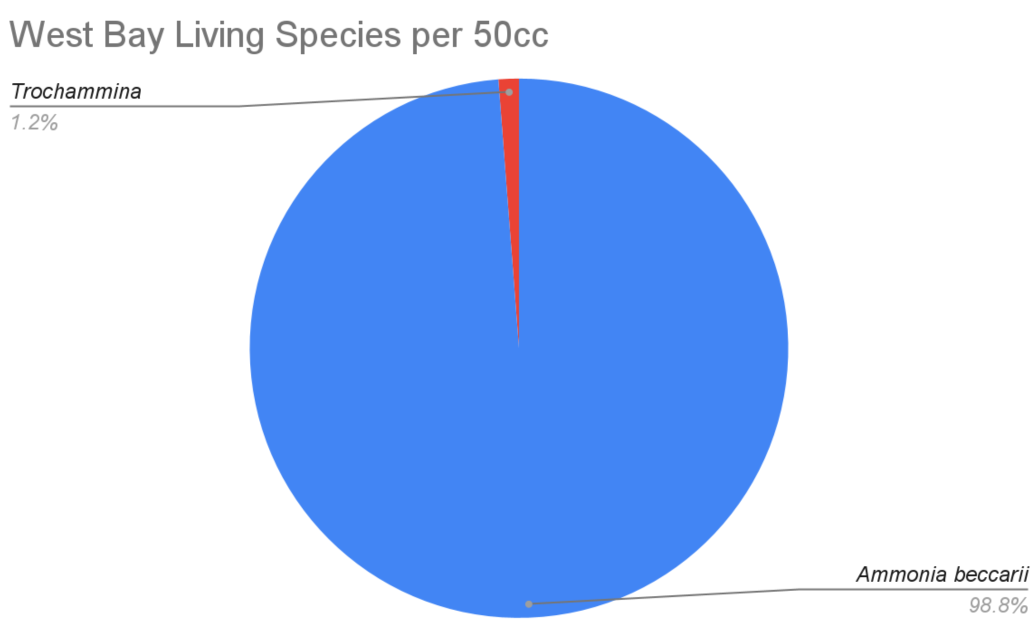

Figure 11: A pie chart showing the percentage of the diversity of the living species in West Bay per 50 ccs. This shows that *Ammonia beccarii* is the most abundant species in the winter.

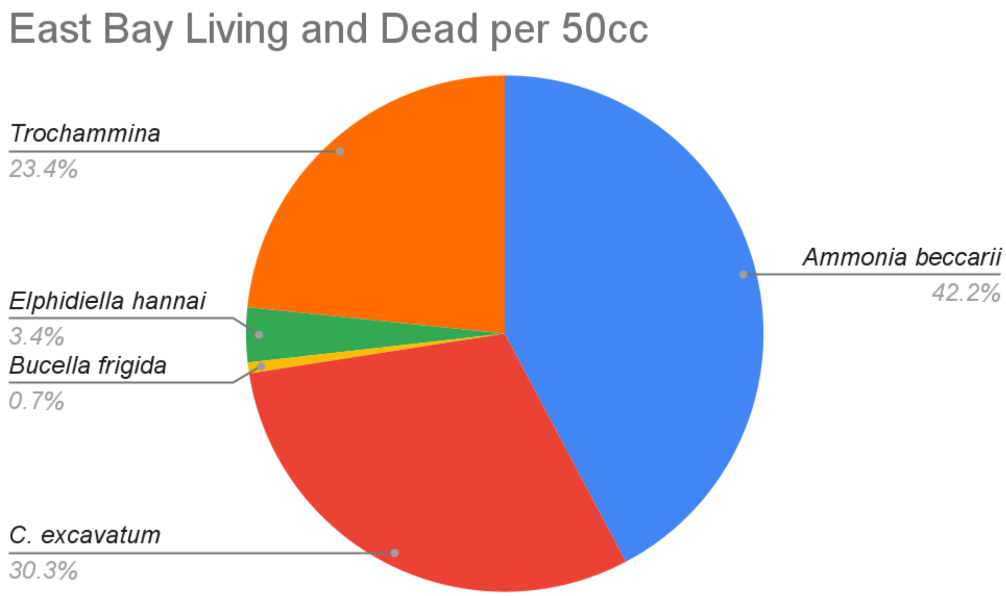

Figure 12: A pie chart showing the percentage of the diversity of the living and dead species in East Bay per 50 ccs. This shows that *Ammonia beccarii* is the most abundant species, but *Cribroelphidium excavatum* is the next abundant species with *Trochammina* closely behind.

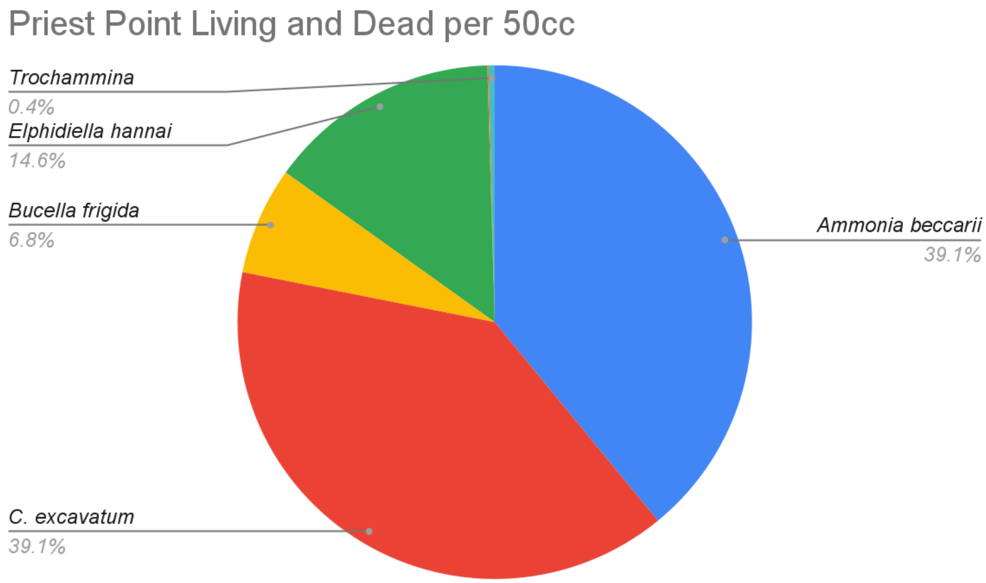

Figure 13: A pie chart showing the percentage of the diversity of the living and dead assemblages in Priest Point per 50 ccs. This shows that both *Ammonia beccarii* and *Cribroelphidium excavatum* are equally the most abundant species.

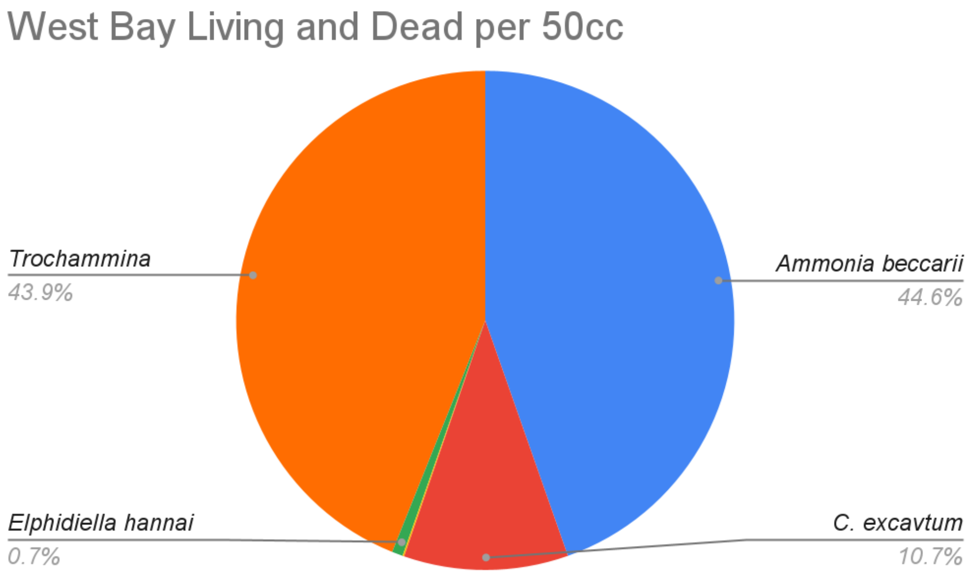

Figure 14: A pie chart showing the percentage of the diversity of West Bay per 50 cc. This shows that *Ammonia beccarii* is the most abundant with *Trochammina* sp. very closely behind as the second-most abundant.

At East Bay and West Bay, no abnormalities were observed, and at Priest Point, 1.07% of living foraminifera had abnormalities. *Ammonia beccarii* is the most abundant foraminifera at the three collection sites (Figures 17-18), with enlarged ultimate chambers being the most common abnormality. In addition to *Cribroelphidium excavatum*, *Buccella frigida* is also found in Priest Point's living assemblages (Figures 19-20). As shown in Figure 21, both West Bay and East Bay had agglutinated *Trochammina* species.

Figure 15: A stained 125 µm *Ammonia beccarii* from Priest Point with enlarged chambers taken on the Nikon microscope.

Figure 16: A stained 125 µm *Ammonia beccarii* from Priest Point with two enlarged chambers taken on the Nikon microscope

Figure 17: A stained 125 µm *Ammonia beccarii* from Priest Point that is conventional taken on the Nikon microscope

Figure 18: A stained 125 µm *Ammonia beccarii* from East Bay that is conventional taken on the Nikon microscope

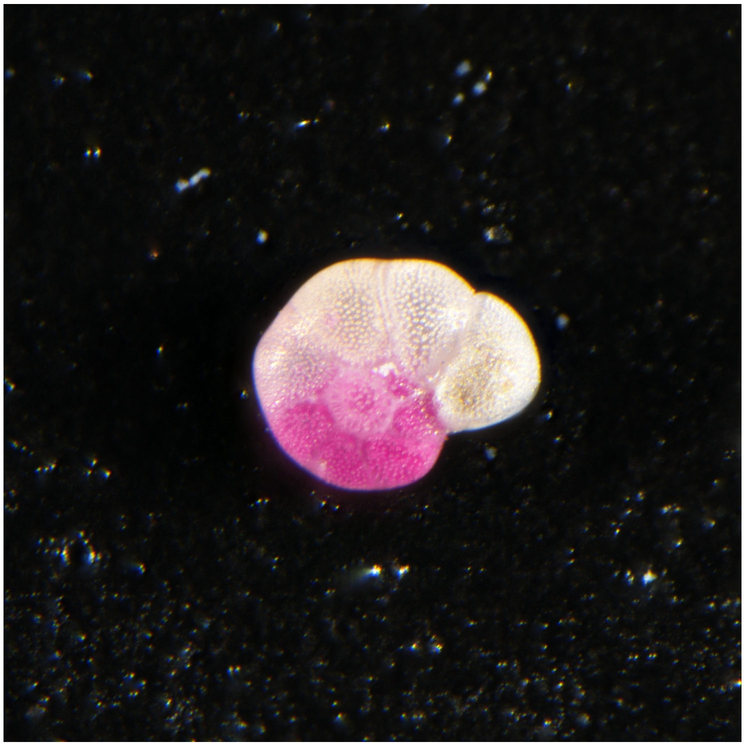

Figure 19: A stained 125 µm *Cribroelphidium excavatum* from Priest Point taken on the Nikon microscope

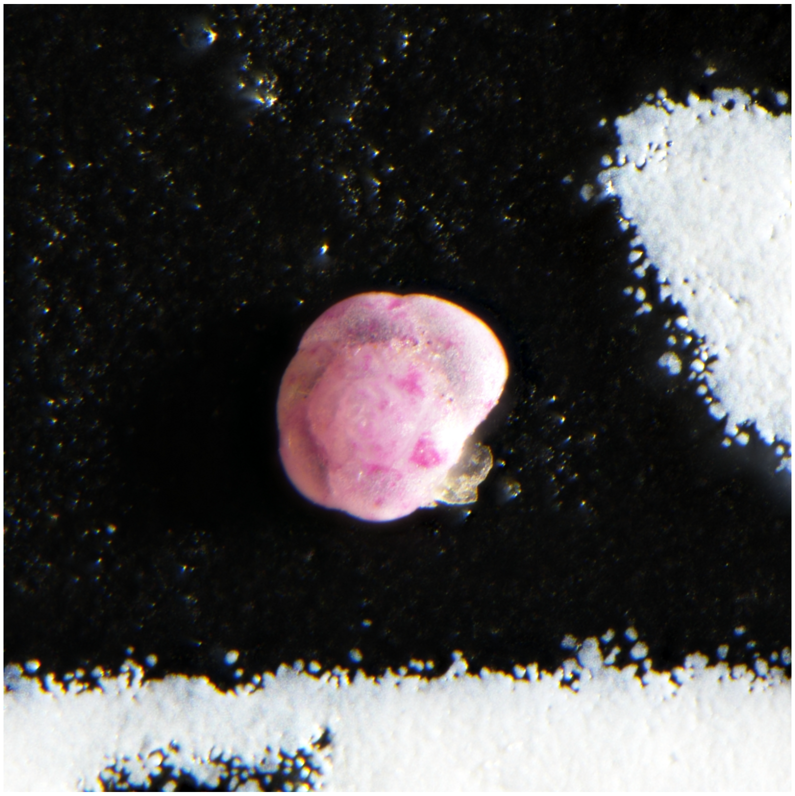

Figure 20: A stained 125 μm *Buccella frigida* from Priest Point taken on the Nikon microscope

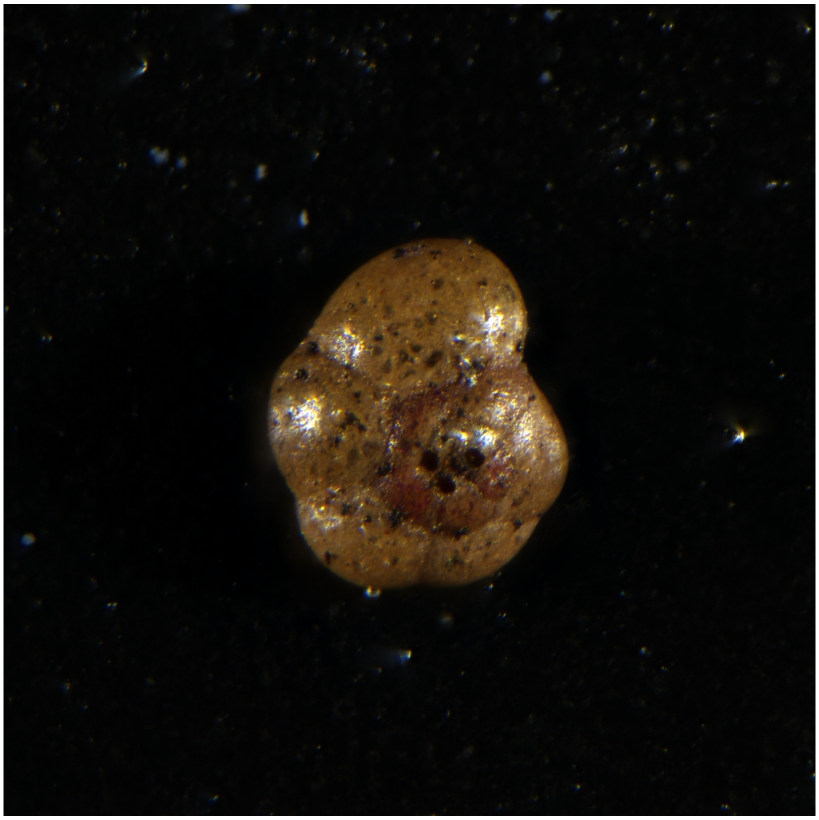

Figure 21: An unstained agglutinated 125 µm *Trochammina* sp. from West Bay taken on the Nikon microscope

CHAPTER V

DISCUSSION

WINTER FORAMINIFERAL ECOLOGY

A large number of living foraminifera were found at West Bay during the winter. Benthic foraminifera gathered in West Bay were mostly collected from the last two sample collection sites (West Bay 5 and 6) in the southernmost part of the park, indicating that this particular area was particularly prolific. In that area, there was a large amount of wood debris and the sediments appeared to be clay-sized. A logging facility lies directly across from West Bay Park, which may add nitrogen and phosphorus to the local waters, which could lead to an increase in diatoms or phytoplankton, thereby facilitating a greater number of benthic foraminifera populations. There had been prior remediation of West Bay by the Washington State Department of Ecology to create West Bay Park, and heavy metal concentrations were below EPA standards in soil (Washington State Department of Ecology c, 2022). A return to lower heavy metal conditions after remediation may also contribute to the higher numbers of benthic foraminifera when compared with other locations.

The site at Priest Point was the second most abundant. Since Priest Point Park is located further north and closer to the central part of the inlet than the two samples sites, it may affect the size and diversity of its population. Priest Point Park has a large open beach that is frequently visited by the public. Foraminifera populations at Priest Point and West Bay Park, and heavy metal concentrations were below EPA standards in soil (Washington State Department of Ecology c, 2022). A return to lower heavy metal conditions after remediation may also contribute to the higher numbers of benthic foraminifera when compared with other locations.

The site at Priest Point was the second most abundant. Since Priest Point Park is located farther north and closer to the central part of the inlet than the other two sample sites, it may affect the size and diversity of its population. In addition, Priest Point Park has a large open beach that is frequently visited by the public. Foraminifera populations at Priest Point and West Bay reflect a well-maintained and healthy environment.

In opposition to West Bay and Priest Point, foraminiferal abundances and diversity were lowest at East Bay which is not as well maintained as the other two locations. Additionally, several industrial areas are near where the samples were collected, and are either experiencing or are about to start remediation efforts due to the confirmed levels of heavy metals above EPA standards in soil (Washington State Department of Ecology b, 2022).

The diversity in East Bay was nonexistent, while diversities at West Bay and Priest Point were very small. The diversity index number for the living populations at East Bay was 0 because the only species found was *Ammonia beccarii*. West Bay had a diversity index number of 0.066 which is quite small because most of the living benthic foraminifera were *Ammonia beccarii*, but there were a few agglutinated *Trochammina*. At Priest Point, the diversity index number was 0.774 which means that Priest Point is the most diverse of the three sites. *Ammonia beccarii* was the most abundant species among the living population likely because the *Ammonia* species is tolerant to a harsher environment. They are not as affected as much by changes to temperature, salinity, dissolved oxygen, etc. which are common changes with switching seasons (Belart et al., 2019).

In West Bay and East Bay, the majority of foraminifera were agglutinated, meaning that they tested sedimentary grains by drawing and gluing them together rather than using calcium carbonate. The presence of agglutinated foraminifera can be explained by the fact that these samples were collected right along the shoreline at low tide since these foraminifera are common in very shallow brackish environments (Alve and Murray, 1995). In both East Bay and West Bay, the collection sites were near Budd Inlet's southernmost portion, where the water is likely to be the shallowest, whereas Priest Point is closer to the center, where the water is likely, not shallow as much. Priest Point is unlikely to contain any agglutinated benthic foraminifera based on this rationale.

SUMMER AND WINTER POPULATION COMPARISON

Compared to the winter season, the summer season was the most abundant. The living abundances, abnormalities, and water quality data from East Bay and Priest Point were compared in Table 3. Since West Bay was not sampled or processed in the summer of 2020, it was excluded. Summer assemblages showed East Bay to be the most abundant, while winter assemblages showed Priest Point to be the least abundant. In winter assemblages, Priest Point was more abundant than East Bay.

Species	East Bay Summer 2020	East Bay Winter 2022	Priest Point Summer 2020	Priest Point Winter 2022
Total Foraminifera per 50 ccs	60,106	19	5,570	323.59
***A. beccarii* per 50cc**	58,951	19	5,546	287
Abnormalities found in *A. beccarii* (%)	3.34%	0%	15.4%	1.07%
Salinity (ppt)*	25.08	29.8	14.06	29.8
Temperature (°C)*	13.8	8.7	13.8	8.6
Depth	Surface at low tide	Surface at low tide	Surface at low tide	Surface at low tide
Noted Heavy Metals*	Confirmed levels of Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, and Zn above EPA standard in soil	Confirmed levels of Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, and Zn above EPA standard in soil	Confirmed levels of Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, and Zn above EPA standard in soil	Unknown

Table 3: A comparison of the living assemblages of the summer and winter populations for East Bay and Priest Point. Summer assemblages were processed by (Roark, 2022). *Best available data (Washington State Department of Ecology b, c, 2022; Pacific Shellfish Institute, 2021; Walker et al., 2022).

Foraminiferal populations are likely to differ greatly between the two seasons because of temperature, salinity, and food availability. During the winter, East Bay and Priest Point experience much colder temperatures. Temperature strongly influences foraminifera abundance (Martin et al., 2013), so the warmer the water, the more abundant they are. The stark difference between abundances in summer and winter samples, as well as a difference in temperature supports the assertions of Martin et al. (2013).

A strong correlation has also been found between foraminiferal abundance and the availability of food. Benthic foraminifera feed on diatoms, fungi, and bacteria found in algae

(Debenay et al., 1998). During the spring and summer months, freshwater from snowmelt mixes with marine water to create a warm, nutrient-dense, surface layer that creates a positive environment for plankton. These nutrients and high levels of nitrogen cause algal blooms (Russell, 2018). Benthic foraminifera, particularly *Ammonia beccarii* are known to live on algae to feed off the diatoms (Debenay et al, 1998). The summer samples were collected when algal blooms were occurring which is likely reflected in the large abundance of benthic foraminifera primarily *Ammonia beccarii* (Roark, 2022). In contrast, algal blooms do not occur in the winter, so the number of available nutrients is likely less than in the summer. This can be seen in the stark differences in abundance between the two seasons, particularly in East Bay.

The best available data suggests salinity is higher in the winter season than in summer due to the amount of evaporation, rainfall, and accumulation of snow in the region. Typically, less rainfall produces higher salinity as evaporation occurs without additional rain returning water into the system. Despite Olympia having heavier rainfall in the winter compared to the summer season (U.S. Climate Data, 2023) it may be possible to see salinity increases when seawater is frozen and the formation of sea ice concentrates salt particles (NOAA b, 2023). General data collected from the region shows little difference in salinity between summer and winter, but this does not account for local variations possible at the sampling sites observed here (*Table 3*).

Interestingly, East Bay and Priest Point switched in abundance in the summer and the winter. In the summer, East Bay was much more abundant than Priest Point (Roark, 2022), but in the winter Priest Point was more abundant than East Bay. Differences in tides may play a part in this primarily at East Bay. During the summer months, the lowest tide occurs during the day whereas, in the winter months, the lowest tide occurs during the night (NOAA c, 2023). During the winter collection trip, samples were collected at the lowest tide during the day, but it wasn't the lowest tide that it could be. However, in the summer, the low tide during collection was much lower (Roark, 2022). Especially at East Bay, where the beach wasn't very wide, it was more beneficial to be further out because there was such a higher number of foraminifera found in the summer season than in the winter season (Roark, 2022). The tides may affect East Bay more than Priest Point because East Bay is further south and is closer to the end of the inlet meaning it is likely shallower. That shallowness may make the changes in tides more prominent so that likely explains why that's much more of a difference between East Bay in the winter and summer than Priest Point in the winter and summer. Summer diversities in East Bay and Priest Point were both 0 as the populations were overwhelmingly dominated by *Ammonia beccarii* (Roark, 2022). At Priest Point, winter diversities were 0.774, showing a slight increase in diversity between summer and winter. As was seen in the summer populations, East Bay maintained a 0 diversity in the winter population.

Fewer abnormalities were found in winter populations compared to the summer populations. There were none found in East Bay in the winter whereas 3.34% of *Ammonia beccarii* were found to be abnormal in East Bay in the summer (Roark, 2022). However, in both locations, there are confirmed levels of heavy metals above the EPA standard in soil (Washington State Department of Ecology b, c, 2022). Remediation in the area of the sediment-bound heavy metals or lower total foraminiferal populations could both account for the decreased foraminiferal mutations observed and may be the baseline mutation percentages possible within shallow-water benthic populations. Given much higher food availability, in the form of algal blooms in summer months, rapid reproduction and overall population increases may result in a higher likelihood of mutation. While the increase in algae does help with the abundance of benthic foraminifera with the increase in food availability, an excess of algal blooms leads to eutrophication which adds stress to the environment. Algal blooms occur in the summer, where there are abnormalities (Roark, 2022), but seem absent in winter when abnormalities are absent.

In Priest Point, there were abnormalities found in *Ammonia beccarii* in both summer and winter, however, in the winter season the percent of abnormalities in *Ammonia beccarii* is only 1.07% compared to the 15.4% found in the summer season (Roark, 2022). While benthic foraminiferal test abnormalities have been linked to heavy metal contaminants (Alve, 1991; Alve, 1995; Labin et al., 1992), it is not clear that this is the only variable likely to result in mutations. The decreased mutation percentages observed in winter months may simply be the mutation potential (i.e., background mutation rate) of *Ammonia beccarii*. Although observations confirming the presence of heavy metal concentrations that are above EPA standards in the summer season and lack of data in the winter make it possible that these heavy metals may be a factor in higher mutation percentages. Because there are abnormalities found in Priest Point in the winter season, it could indicate a higher heavy metal concentration, but maybe not as high as in the summer. Another possibility could be because of the cold temperatures and lower food sources. Because there are abnormalities found at Priest Point during the winter, that does show that the environment is stressed to a degree.

The most prolific species in both the summer and winter seasons was *Ammonia beccarii*. *Ammonia beccarii* is a species that is known for being more tolerant of harsher environments. In the summer, the environment is harsh because there are confirmed heavy metals at concentrations higher than EPA standards in soil as well as the likelihood of eutrophication happening due to algal blooms. In the winter, the environment is harsh because it is colder, the salinity is higher, and food is less available.

LIVING AND DEAD ASSEMBLAGES

It is evident that the diversity of the living and dead populations in the winter sample is much greater than that of simply the living population. Dead populations at East Bay had a diversity index number of 0.788, Priest Point had a diversity index number of 1.204, and West Bay had a diversity index number of 0.466. The higher the diversity index number is the higher the diversity, so Priest Point was the most diverse and West Bay was the least diverse. These dead assemblages show that diversity is much higher in either the fall or the spring because these species are not observed in either the living population of the winter or the summer months. Dead assemblages in Priest Point contained abnormal tests of *Ammonia beccarii* suggesting these individuals to be left over from the summer months or that mutation influences are also present throughout the year.

CONCLUSION

West Bay was the most abundant site in winter 2022, but Priest Point was the most diverse. There was a large increase in the number of samples in the summer of 2020 compared to the winter, but there was a similar diversity of species with *Ammonia beccarii* being the predominant species. It was possible to find abnormalities at Priest Point in the winter season, but they were less common than they were during the summer. Dead assemblages in the winter had a higher diversity than living assemblages in the summer and winter, indicating that the fall or spring seasons have different living assemblages.

Studying the fall and spring assemblages would be crucial to finding when the dead assemblages from the winter were once alive in this area. Likewise, picking through the dead assemblages from the summer of 2020 to assess whether diversity has changed. It will also be important to take soil and sediment samples and analyze them for heavy metals in order to understand the level of heavy metals in the area and to get an accurate measurement of salinity, temperature, and dissolved oxygen.

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