THE USE OF BENTHIC FORAMINIFERA AS ENVIRONMENTAL INDICATORS IN BUDD INLET, PUGET SOUND

By

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Bachelor of Science in Geology

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Stillwater, OK

2020

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2022

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ACKNOWLEDGEMENTS

The completion of this thesis would not have been possible with Dr. Brandon Pratt, Jessica Whitesell, and Trenity Ford, who were instrumental in the procurement of SEM and MicroCT images. I would also like to thank Dr. Daniel Frederick for his expertise and guidance throughout this project.

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Date of Degree: JULY, 2022

Title of Study: THE USE OF BENTHIC FORAMINIFERA AS ENVIRONMENTAL INDICATORS IN BUDD INLET, PUGET SOUND

Major Field: GEOLOGY

Abstract: The pollution of coastal and estuarine environments is of growing concern as it potentially threatens ecosystems, economies, and the public health of people living in these areas. Budd Inlet's circulation is relatively fast compared to similar inlets within Puget Sound, but it is still marked by water quality issues as a result of wastewater, lumber, and aquaculture pollution discharge. Foraminifera are frequently used in environmental studies to determine past and present parameters such as changes in temperature, pH, sea level, salinity, and the availability of dissolved oxygen. This thesis seeks to further investigate foraminifera population dynamics within Budd Inlet and the potential impact of point and nonpoint source pollution on their ecosystem. Investigation of Budd Inlet population showed a dominance of *Ammonia beccarii* at all sites, and a high number of tests with abnormal growth patterns. A selection of these abnormal tests were examined with SEM and MicroCT analysis. Ecology results showed that foraminifera populations decreased from the south to north of the inlet in correlation with C/N ratios, while the proportion of abnormal to normal tests increased. Finally, this thesis examined the fidelity of two sample processing methods and confirmed a <10% difference in the number of foraminifera acquired with traditional sieving and picking compared to the SPT floating method.

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CHAPTER I

INTRODUCTION

One of the most important problems of the 21st century is coastal and estuarine pollution. Developed coastal environments, which function as a bridge between populated lands and marine waters, are vital ecosystems. The pollution of waterways poses an imminent threat for these ecosystems, economies, and the public health of people living in affected areas (Australia Department of Health, 2010; World Bank, 2019; Ahmed et al., 2020). The pollutants that cause harm to coastal bodies are often either indirectly or directly supplied by anthropogenic sources (Vikas & Dwarakish, 2015). Pollution may come as a result of industrial activities, construction, agricultural practices, and/or residential sources (Yanko et al., 1999). Oil, sewage, garbage, pesticides, toxic chemicals, heavy metals, radioactive wastes, coolants, and nutrients act as the main contributors of waste and subsequent pollution (Pati & Patra, 2012).

This thesis seeks to characterize the distribution of foraminifera in the Budd Inlet of the Puget Sound and conduct spatial analysis of environmental parameter to assess the health of the microfaunal benthic communities in the region. Preliminary investigations of foraminifera have shown high levels of test abnormalities. This thesis will investigate the hypothesis that these abnormalities occur in higher frequencies in areas likely impacted by anthropogenic pollution. The determination of potential anthropogenic pollutants will be determined through an evaluation of sediment quality assessment data collected by the Washington State Department of Ecology in comparison to foraminifera population data, noting the proximity of the sample location to potential pollution sources (e.g., wastewater treatment and wood processing and shipping facilities) (WA Department of Ecology, 2022a). The ability to monitor pollution in both fresh and saltwater ecosystems continually through microscopic organism surveys with organisms such as foraminifera allows researchers to better source pollutants, track their routes, and investigate their impact on coastal and estuarine environments.

FORAMINIFERA

Foraminifera are single-celled marine organisms found in both benthic and planktonic environments with mineralized shells or “tests", which range from 63µm to 20cm long and are composed of either calcium carbonate or, less commonly, opaline silica, or sedimentary particles agglutinated together (Sen Gupta, 1999). Foraminifera shells, commonly referred to as “tests” either encapsulate the soft-tissue protoplasm or act as a supportive skeletal structure around which the foraminiferal protoplasm may stream (Goldstein, 1999; Sen Gupta, 1999). Tests may be precipitated by the organisms producing a calcium carbonate structure or agglutinated by the cementing together of sedimentary particles. This study will focus on shallow-water benthic species of foraminifera, as planktonic species rarely live in coastal areas (Todo et al, 2005). The benthic varieties of these protists are particularly useful in paleoceanography and paleoclimate studies because they are globally distributed, highly preservable, and found throughout the fossil record, from the Cambrian to the present. Foraminifera have been used historically for biostratigraphic applications in the oil and gas industry, but their usefulness in pollution monitoring has been gaining attention since the late 1950s (Pati & Patra, 2012). The most efficient means of continual coastal monitoring is through bioindicator observations. Bioindicators are organisms that show a measurable reaction in the presence of pollutants (Mothersill & Seymour, 2016). Foraminifera are commonly used as bioindicators and offer a relatively inexpensive solution to pollution monitoring (Pati & Patra, 2012). Foraminifera are widespread, abundant, and sensitive to pollution within inlet and fjords environments (e.g., Hald & Korson, 1997; Nordberg et al., 2000). The successful use of foraminifera and other bioindicators in coastal environments can aid in the characterization of changes in temperature, pH, sea level, salinity, and the availability of dissolved oxygen (Geslin, 1998; Bernhard & Sen Gupta, 1999; Alve, 1991; Pati & Patra, 2012). Additionally, foraminifera have relatively short reproductive cycles, hence their overall populations respond rapidly to changes in ecologic conditions (Katz, 2010; Boltovsky, 1991).

In coastal studies, foraminifera may exhibit test abnormalities as a result of pollution, especially that of heavy metals (Alve, 1991; Alve 1995; Samir & El-Din, 2001, Burone et al., 2006; Elshanawany et al., 2018). The relationship between test abnormalities and environmental conditions was first observed by Carpenter (1856). Abnormalities include compressed or contorted shells, deepening of grooves, enlargement of pores, inflated chambers, multiple or widened apertures, and/or a change in coiling direction of chambers (Seiglie, 1971; Alve, 1991; Setty and Nigam, 1984). Burone, et al. (2006) conducted a study of foraminifera from the Montevideo coastal zone in South America and determined that test abnormalities were significantly more prevalent in coastal regions experiencing high levels of heavy metal contamination. However, test abnormalities are not exclusively relegated to water with heavy metal pollutants, but also have been observed in water subjected to salinity changes (e.g., Yanko-Hombach et al., 2017), acidification (e.g., Le Cadre et al., 2003), or high organic matter input (e.g., Caralp, 1989).

DESCRIPTION OF STUDY AREA

Puget Sound

The Puget Sound is located in the Cascadia subduction zone, where the oceanic crust of the Juan de Fuca Plate is subducted under the continental crust of the North American Plate at a rate of approximately 33mm/year N58ºE for the past six million years (Gripp et al., 2002). This complex system of fjords is the southernmost inlet of the Salish Sea of the Pacific Ocean, formed from the cyclical advancement and retreat of continental ice sheets during the Fraser Glaciation, which occurred between 25,000-10,000 years ago (Heusser, 1973). The most recent stage of the Fraser Glaciation, the Vashon phase, eroded the area via meltwater rather than by “carving” through the terrain (Kruckeberg, 1991). The Sound’s four major basins, Hood Canal, Whidbey Basin, South Sound, and the Main Basin, are connected via a system of sills. These sills, or moraines, are large bodies of rock and sediment deposited on the terrain during the Fraser Glaciation which effectively separate the basins from each other, as well as the entire Puget Sound from the Strait of Juan de Fuca (Bretz, 1911; Heusser, 1973).

Budd Inlet

Budd Inlet is the southernmost inlet of the Puget Sound located in Thurston County, WA near the city of Olympia (Figure 1). Budd Inlet's circulation pattern is characterized by colder, more saline water from the main basin of the Puget Sound flowing into the inlet on the western side and warmer, more polluted, and slower freshwater discharging on the eastern side (BISS, 1998). This circulation results in a gyre in the center of the inlet. Furthermore, Budd Inlet is one of the more active inlets within the Sound, with a flushing time of 8-12 days depending on the time of the year (BISS, 1998). Despite Budd Inlet's fast circulation rate relative to similarly sized Puget Sound inlets, it has major water quality problems, with freshwater flow from Capitol Lake responsible for the majority of its issues (McCarthy et al., 2017).

Budd Inlet is 11.01km long with a maximum breadth of 2.99km. Foraminifera samples for this study were collected in the summer of 2020 East Bay Park and Priest Point Park (Figure 1). East Bay Park sits at the southern end of the inlet, on the east side of a 1.4km long peninsula. Priest Point is on the eastern shore of Budd Inlet, about 2km north of East Bay. These sample sites were selected due to their ability to characterize effluent discharge from the southernmost part of the inlet. Discharge is primarily from Capitol Lake, Deschutes River, and the Lacey, Olympia, Tumwater, and Thurston (LOTT) County Wastewater Treatment Plant (LOTT, 2000).

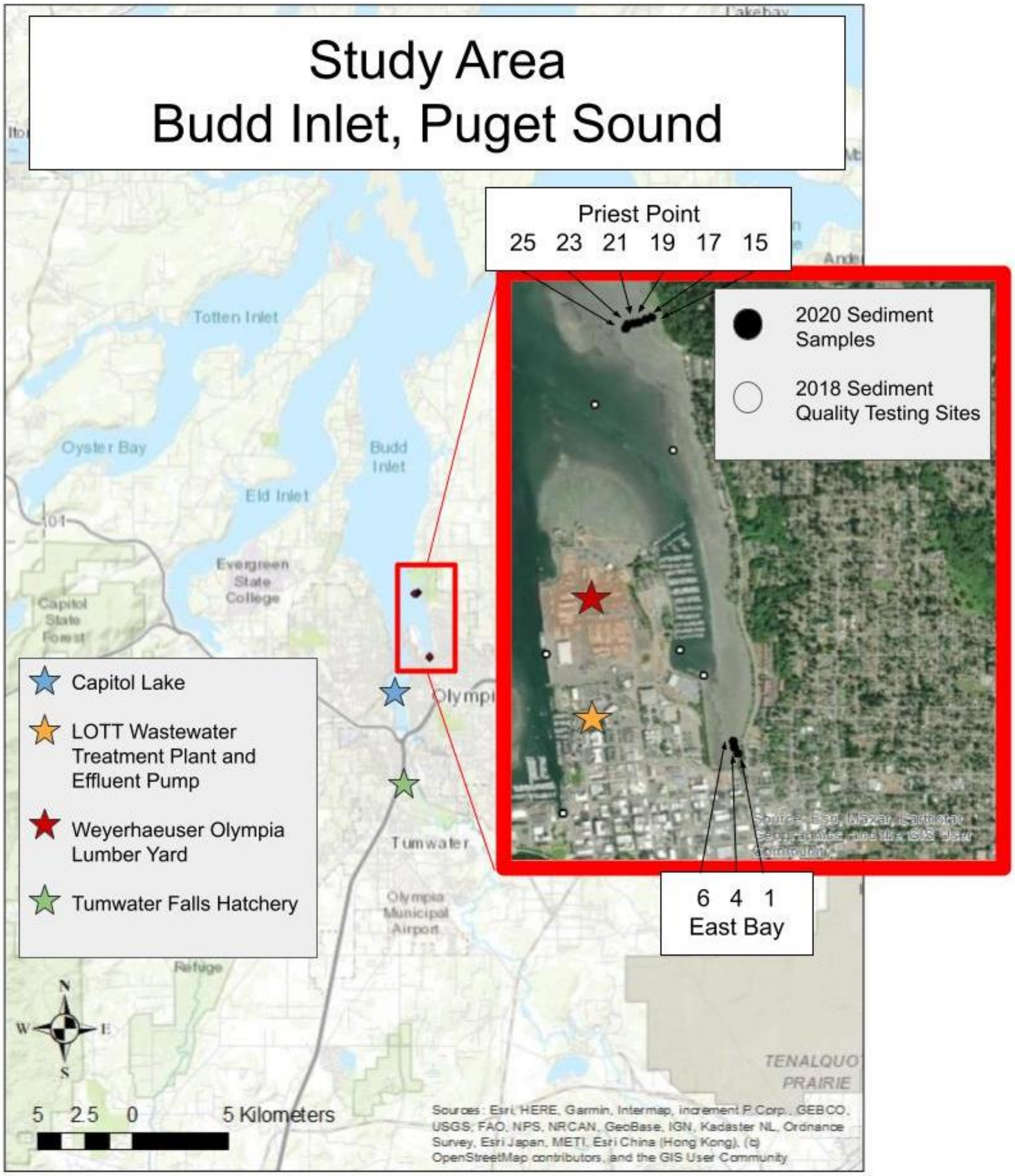


Figure 1: The larger map represent the region as a whole, including Budd Inlet and its connection to the larger Main Basin of the Puget Sound. The red box outlines the study area for this project. The Budd Inlet study area includes Priest Point Park and East Bay Park sampling sites. Priest Point to the north has six sampling sites: 15, 17, 19, 21, 23, and 25. East Bay to the south has three: 1, 4, and 6. Main pollutant sources are marked by stars. The blue star is Capitol Lake, yellow is the LOTT Wastewater Treatment Plant, red is the Weyerhaeuser Olympia Lumber Yard, and green is the Tumwater Falls Hatchery. The Deschutes River flows to the north, passing by the hatchery and feeding into Capitol

Lake, which discharges into the inlet. The LOTT Wastewater Treatment Plant discharges effluent on the western shore of the peninsula (Esri, , 2012).

In addition to the LOTT wastewater treatment plant, the peninsula at the southern end of the inlet is also home to a lumber yard. While the effects of silvicultural industry sites on foraminifera are relatively unstudied, both logging and the storage of lumber have the potential to impact coastal environments by increasing levels of nitrate and potassium in the water (Lynch & Corbett, 1990). As water travels from the Deschutes River through Capitol Lake and into Budd Inlet, it passes through the Tumwater Falls Hatchery. Aquaculture sites have been previously linked to declining populations in coastal foraminifera as hatcheries increase organic matter flux, often resulting in “dead zones” due to depleted oxygen levels (Schafer et al., 1995; Angel et al., 2000).

Additionally, the Washington State Department of Ecology has determined that Budd Inlet has confirmed levels of metal pollutants, petroleum products, dioxins, and polycyclic aromatic hydrocarbons in the soil above EPA guidelines. In 2017, LOTT, the Department of Ecology, and the City of Olympia completed an initiative to remediate the area, successfully lowering the level of pollutants in groundwaters, but not in the soil (WA Department of Ecology, 2022a). In 2018, the Department of Ecology conducted a sediment quality assessment in Budd Inlet, wherein six sediment testing sites within the study area were investigated (Figure 1)(WA Department of Ecology, 2022b). These sites were used to characterize C/N ratios in the sediment and benthic community populations within Budd Inlet, which were compared to the foraminifera populations assessed from the 2020 sediment samples.

RESEARCH PROBLEM

Population growth in coastal cities has resulted in an increase in pollution from agricultural, industrial, residential, and municipal sources (Yanko et al., 1999). Zalesny (1959) was the first to demonstrate the effects of pollution on foraminifera population and subsequent studies have further corroborated the usefulness of foraminifera as marine pollution indicators (Resig, 1958; Watkins 1961). In studies of coastal ecosystems, foraminifera have been used to document and reconstruct environmental disturbances to examine the impact of industrialization on coastal habitats. The main contributors of anthropogenic coastal pollution are the introduction of organic matter and heavy metals from varying sources (Châtelet & Debenay, 2010).

Discharged organic matter may be divided into two main categories. The first category consists of readily biodegradable organic substances from domestic sewage, fertilizer, or food processing industries. The second is composed of more resistant organic matter, such as that from wood fiber waste from mills. Both categories have been shown to stimulate growth in local populations of benthic foraminifera, but the latter category is not understood as well as the former (Alve, 1995). Benthic foraminifera benefit from high concentrations of readily biodegradable organic matter as an efficient food source (Loubere & Fariduddin, 1999). More resistant organic matter from sources such as wood pulp also appears to benefit benthic populations as a source of cellulolytic or associated bacteria for their consumption (Poole et al, 1977). Additionally, bristle worms have been observed as the dominant macrofaunal species in many over-enriched ecosystems and the bacterial communities that feed on their fecal pellets may act as a food source for foraminifera (Alldredge et al, 1987). However, over-enrichment of organic matter may result in an abiotic zone due to a subsequent lack of oxygen and lowered pH (Boltovsky & Wright, 1976; LeFurgy & St. Jean, 1976). This thesis seeks to characterize the population dynamic of Budd Inlet's foraminifera and compare the geographic distribution to known environmental parameters.

In addition to investigating the population dynamics of foraminifera within Budd Inlet, this thesis will compare the traditional sediment processing techniques and a relatively new technique known as "SPT floatation." While the traditional method has proved useful for the better half of a century, the SPT floatation method appears to be a promising new development in foraminifera research. This project will assess the efficiency of both methods in isolating foraminifera from sediment samples by providing insight on the amount of time processing takes and any significant difference in test recovery.

In summation, the main goals of this thesis are to:

1. Assess foraminifera population dynamics and how they relate to the ambient environmental conditions of Budd Inlet. This thesis proposes that foraminifera populations will be higher where pollutant input is stronger (e.g., in East Bay), due to the availability of nutrients such as C and N.
2. Evaluate morphological abnormalities present in Budd Inlet foraminifera with the hypothesis that the proportion of test mutations will be higher at the East Bay sites due to oversaturation of nutrients such as C and N.
3. Assess the relative efficiency of two foraminifera processing methods: SPT flotation and traditional sediment picking with the hypothesis that the

SPT flotation method will yield more foraminifera tests because it limits the amount of sediment necessary to pick through.

CHAPTER II

METHODOLOGY

SAMPLING

Surface core samples were collected in July 2020 from East Bay and Priest Point Parks by Dr. Daniel Frederick. Sampling was conducted by taking the top 5 cm of sediment using a 1-inch diameter plastic tubing corer. The plastic tube was inserted into the sediment and capped. A trench was then dug around the tube and a metal spatula was pushed under the tube so that the tube could be extracted with the sediment sample intact.

Samples were preserved in a 4% ethanol with Rose Bengal solution. Rose Bengal is a protein stain developed in 1952 used to distinguish foraminifera that were alive at the time of collection from those that were dead (Walton, 1952; Murray, 2000). The stain adheres to proteins and produces a vibrant pink coloration in affected tests (Bernhard et al. 2006). This method allows researchers to both more efficiently pick foraminifera from sediment samples as well as to characterize the living populations of sampling sites at the time of collection. Once the sediment sat in solution for at least seven days, the picking processes could begin. Half of each sample was designated to undergo sediment processing using the traditional sediment processing method (noted as Reserved) and the other half was processed with the SPT floatation method (noted as Floated). These samples were not only used to demonstrate the population dynamics of foraminifera in Budd Inlet but to demonstrate the efficiency of both methods when directly compared to each other.

PROCESSING

Traditional Sediment Processing

For the traditional methodology, procedures follow those outlined in Rathburn and Corliss (1994) and foraminiferal abundances are standardized to 50cc based on sample weight. These samples were wet sieved with 63µm and 125µm wire sieves to separate the smaller and larger specimens. Upon picking, the stained samples were rinsed in RO water, placed in a petri dish, and examined under a microscope. Stained foraminifera, those with bright pink protoplasm filling the majority of the test, were then picked from the sediment with a fine paintbrush and organized on micropaleontological slides in order to identify and quantify specimens.

Sodium Polytungstate Floatation Processing

This project modified the methods for sodium polytungstate solution (SPT) floatation as described in Semesatto & Dias-Brito (2007) to consolidate foraminifera in sediment samples. Samples that had previously been preserved in the Rose Bengal and ethanol solution were first homogenized and washed through four sieves (1mm, 500µm, 125µm, and 63µm). The sieved samples were then dried using a Büchner funnel fitted with filter paper to siphon the majority of water saturating the samples. The filter paper holding the semi-dried samples was then transported to an oven set to 100°C to remove any remaining water.

Following the drying process, the samples were weighed and then submerged in a 10mL graduated cylinder with a 2.50g/mL density solution of SPT and RO water. Once submerged, the portions of the samples that float were separated from the portion that sank by decantation. Both the float and sink portions of the sub-sample were then dried using a Büchner funnel and filter paper to remove excess SPT solution. The dried float and sink portions were then either stored in acrylic containers or immediately examined under a stereomicroscope for stained foraminifera in the same manner as the traditionally processed samples. When examining stained tests using the SPT method, any test that was estimated to be at least 60% stained was picked compared to the traditional samples, wherein a test was only picked if it was at least 80% stained. This consideration was taken due to the stained cytoplasm shrinking during the drying process of the SPT method.

SEM AND MICROCT IMAGING

SEM imaging was used to address morphologic discrepancies in a selection of *Ammonia beccarii* tests. These tests were selected due to their extreme variation from typical tests of this species. These samples were scanned at Oklahoma State University using a FEI Quanta 600 and gold coating.

Further appraisal of *Ammonia beccarii* morphological variation was conducted at California State University, Bakersfield, California using a Bruker Skyscan 2211 X-ray nano-CT system. These tests were selected based on the results of SEM imaging and due to their extreme variation from typical tests of this species. MicroCT scans were successfully obtained from x individuals. Most of these individuals were those identified as mutated and cross-sectional views of scans were generated from 5 specimens

ENVIRONMENTAL DATA

The Shannon-Weiner diversity index equation (Equation 1) will be used to quantify the different species of foraminifera picked to statistically represent the biodiversity of foraminifera in Budd Inlet. The Shannon-Weiner diversity index measures diversity while considering species richness as well as their relative abundances. A high diversity index indicates high diversity within the community (Shannon, 1948; Gibson & Buzas, 1973).

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

Equation 1: where H is the Shannon-Weiner diversity index,R is the richness, p_i is the proportion of the i^{th} species in the samples

In addition to the Shannon-Weiner diversity index, the Kruskal Wallace *H* test (Equation 2) will be used as a non-parametric means to compare samples and determine if they come from the same distribution (Siegel, 1988)

$$H = (N-1)\frac{\sum_{i=1}^{g} n_i(\bar{r}_{i\cdot} - \bar{r})^2}{\sum_{i=1}^{g}\sum_{j=1}^{n_i}(r_{ij} - \bar{r})^2}, \text{ where:}$$

Equation 2: Kruskal-Wallace (H) test, where *N* is the number of observations across all groups, *g* is the number of groups, *ni* is the number of observations in group *i*, r_{ij} is the rank of observation *j* from group *i*, $\tilde{r}_i$ is the average rank of all observations in group i, and $\tilde{r}$ is the average of all the r_{ij}

Environmental data, including major taxa biomass evenness and carbon to nitrogen ratio collected by Washington State Department of Ecology in 2018 was also gathered and plotted in comparison with foraminiferal abundance data in ArcMap. Mapped data points were categorized and assigned symbols using the Jenks optimization method, which statistically determines the best arrangement of values into different classes. The Jenks method reduces variance with classes and maximizes variance between classes. This method results in data classes of variable sizes separated by distinct break values (Jenks, 1967).

For multiple datasets, Gaussian process regression of kriging was employed to interpolate data over the study area. In geostatistics, kriging is used to give the best linear unbiased prediction of a parameter at unsampled locations. Kriging works by calculating a weighted average of known parameter values to predict the value of that parameter at a given, unsampled point. For this project, ordinary kriging, which assumes constant unknown averages across the study area, was employed. The differentiation between terrestrial and water covered areas was not determined in the method employed here. Kriging results in a variogram model to approximate the spatial continuity of the data, with three main components: nugget, range, and sill (Figure 2)(Gilbert, 1987). The nugget is *y*-intercept of the variogram, which represents the small-scale variability of the data. The range is the distance after which the variogram's slope goes to zero and the model levels off. Finally, the sill is the maximum variability between pairs of points.

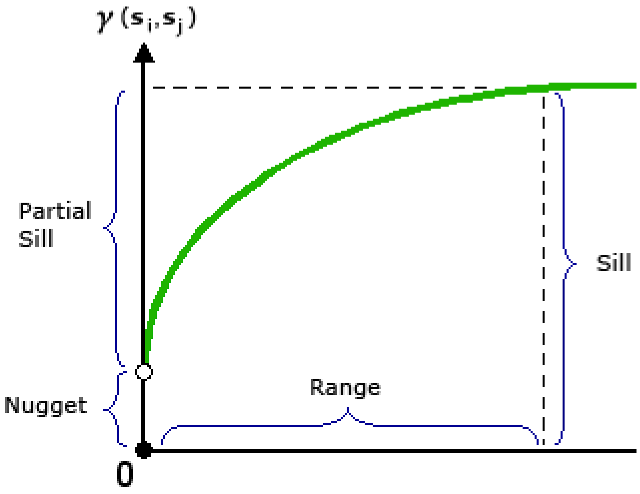


Figure 2: Kriging variogram model where the *x*-axis is the range or distance between points, the *y*-axis is the sill or the maximum variability between points, and the nugget is the *y*-intercept or the small-scale variability of the data (ESRI, 2022).

CHAPTER III

RESULTS

FORAMINIFERA POPULATION DYNAMICS

According to the data collected in 2018 by the Washington State Department of Ecology, East Bay had a higher salinity value of 25.08 ppt compared to Priest Point’s 14.06 ppt. However, the site maintained the same temperature, 13.8 °C (Pacific Shellfish Institute, 2021). East Bay had a far larger population of foraminifera and the dominant species, *A. beccarii*, though it was had smaller proportion of abnormally formed *A. beccarii* (Table 1).

Site	East Bay	Priest Point
Total Foraminifera/50cc	60,106	5,570
Total *A. beccarii/*50cc	58,951	5,546
Abnormal *A. beccarii* (%)	3.34%	15.4%
Salinity (ppt)*	25.08	14.06
Temperature (°C)*	13.8	13.8
Depth	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

Table 1: Sampling Conditions for East Bay and Priest Point showing that both sites had similar ambient environmental conditions, but East Bay has significantly more foraminifera/50cc. *Best available data (Pacific Shellfish Institute, 2021; WA Department of Ecology, 2022)

There were a total of four genus/species of foraminifera picked from the sediment samples: *Ammonia beccarii, Bolivinita* sp., *Elphidum* sp., and *Miliammina fusca* (Table 2). The East Bay sites had higher overall abundance than the Priest Point locations (Figure 3). For each site, *A. beccarii* was the dominant species and the Diversity Index was 0 (Supplemental Table S1). A kriging analysis of the study area shows that overall foraminifera abundance and individual species abundance decrease from East Bay to Priest Point (Figures 4-7).

Site	East Bay 1	East Bay 4	East Bay 6	Priest Point 15	Priest Point 17	Priest Point 19	Priest Point 21	Priest Point 23	Priest Point 25
Total foraminifera per 50cc	14318.0	6330.3	39458.3	1870.8	2661.6	631.7	54.54	338.6	13.04
Total *A. beccarii* per 50cc	13872.2	5945.8	39133.4	1855.4	2653.7	631.7	54.58	338.6	13.04
Total *Bolivinita* sp. per 50cc	82.64	0	0	0	0	0	0	0	0
Total *Elphidium* sp. per 50cc	197.9	65.79	254.91	15.37	7.94	0	0	0	0
Total *M. fusca* per 50cc	165.29	318.71	69.95	0	0	0	0	0	0

Table 2: Total amount of each genus or species per 50cc at east site.

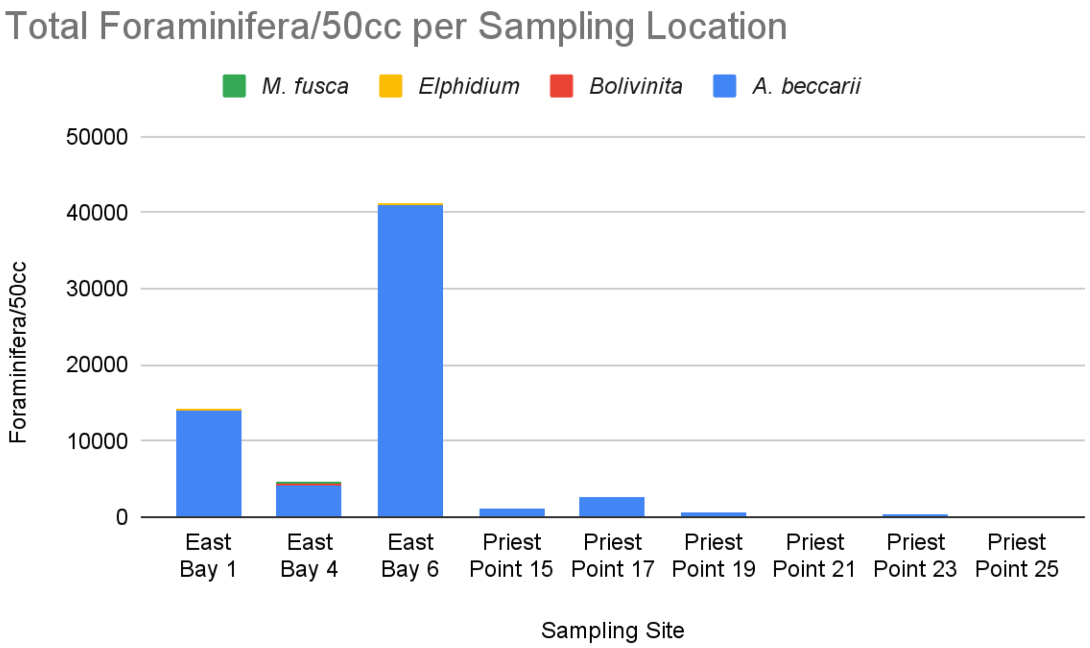
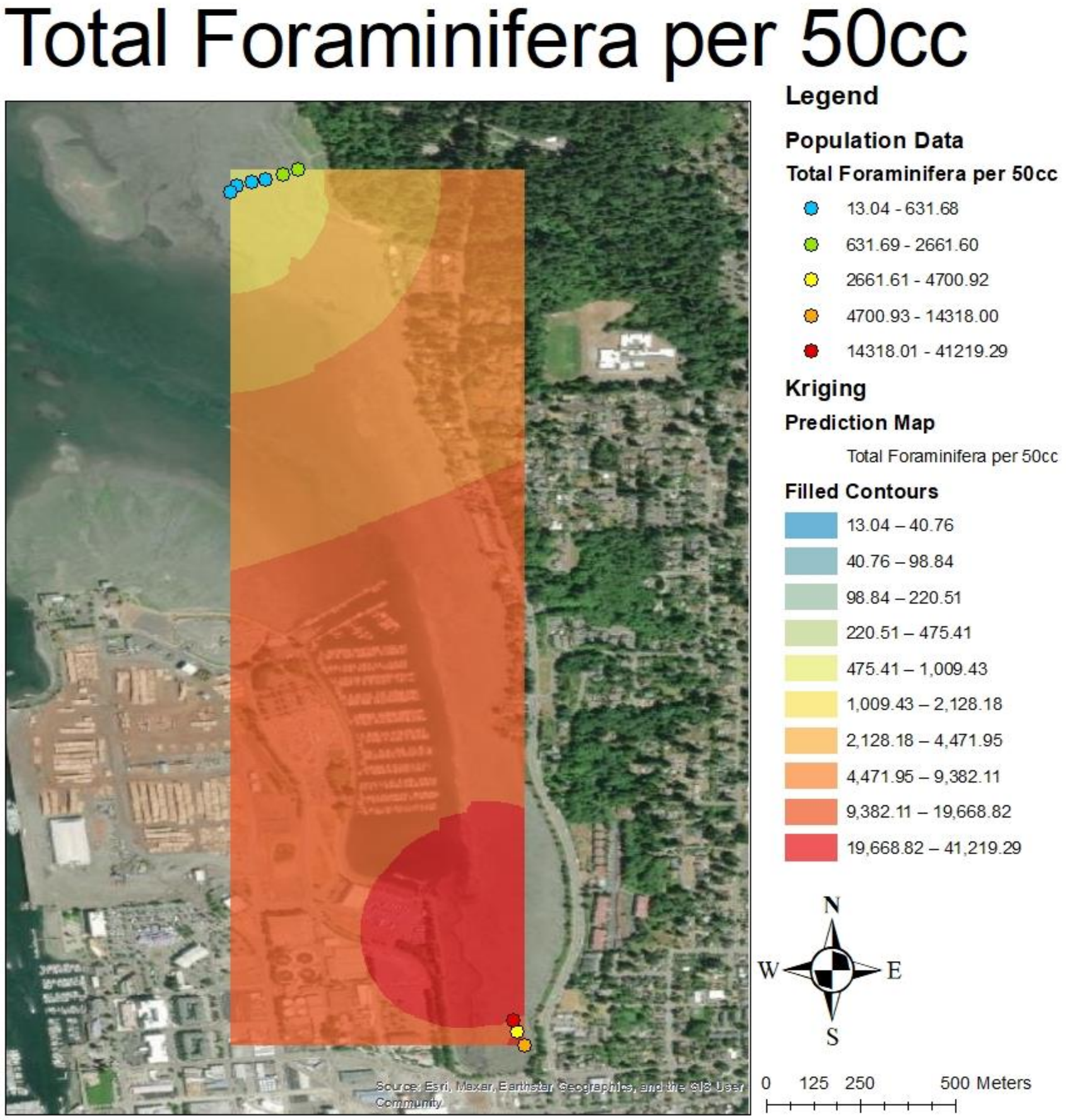
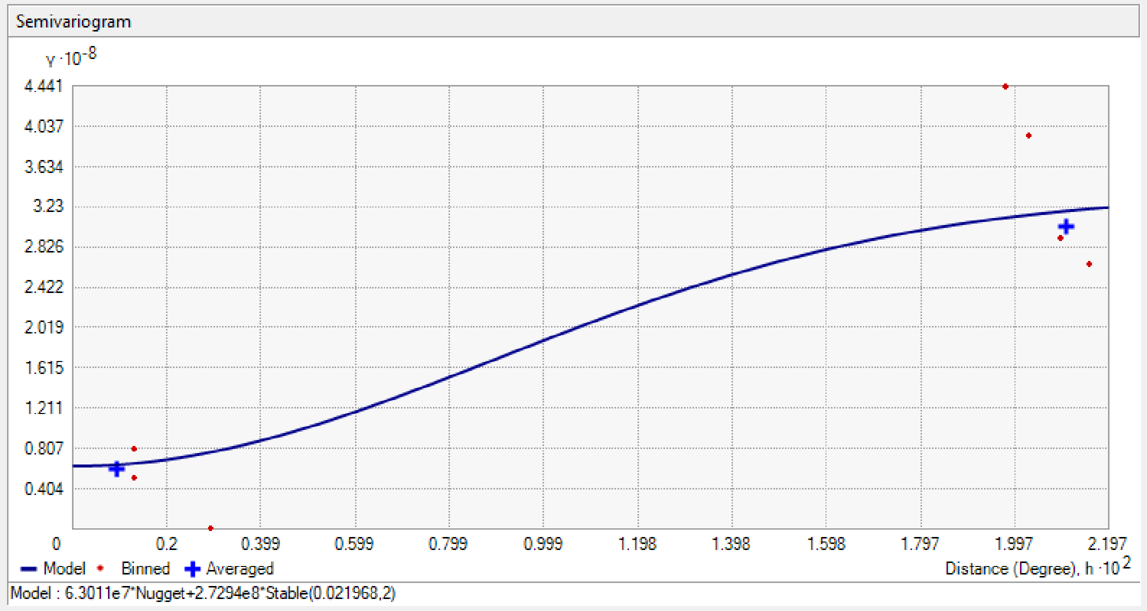


Figure 3: East Bay had higher numbers of foraminifera than Priest Point, with the dominant species for both sites being *A. beccarii*.

4a.


Total Foraminifera per 50cc
Legend
Population Data
Total Foraminifera per 50cc
● 13.04 - 631.68
● 631.69 - 2661.60
● 2661.61 - 4700.92
● 4700.93 - 14318.00
● 14318.01 - 41219.29
Kriging
Prediction Map
Total Foraminifera per 50cc
Filled Contours
13.04 – 40.76
40.76 – 98.84
98.84 – 220.51
220.51 – 475.41
475.41 – 1,009.43
1,009.43 – 2,128.18
2,128.18 – 4,471.95
4,471.95 – 9,382.11
9,382.11 – 19,668.82
19,668.82 – 41,219.29
N
W
E
S
0 125 250 500 Meters
Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

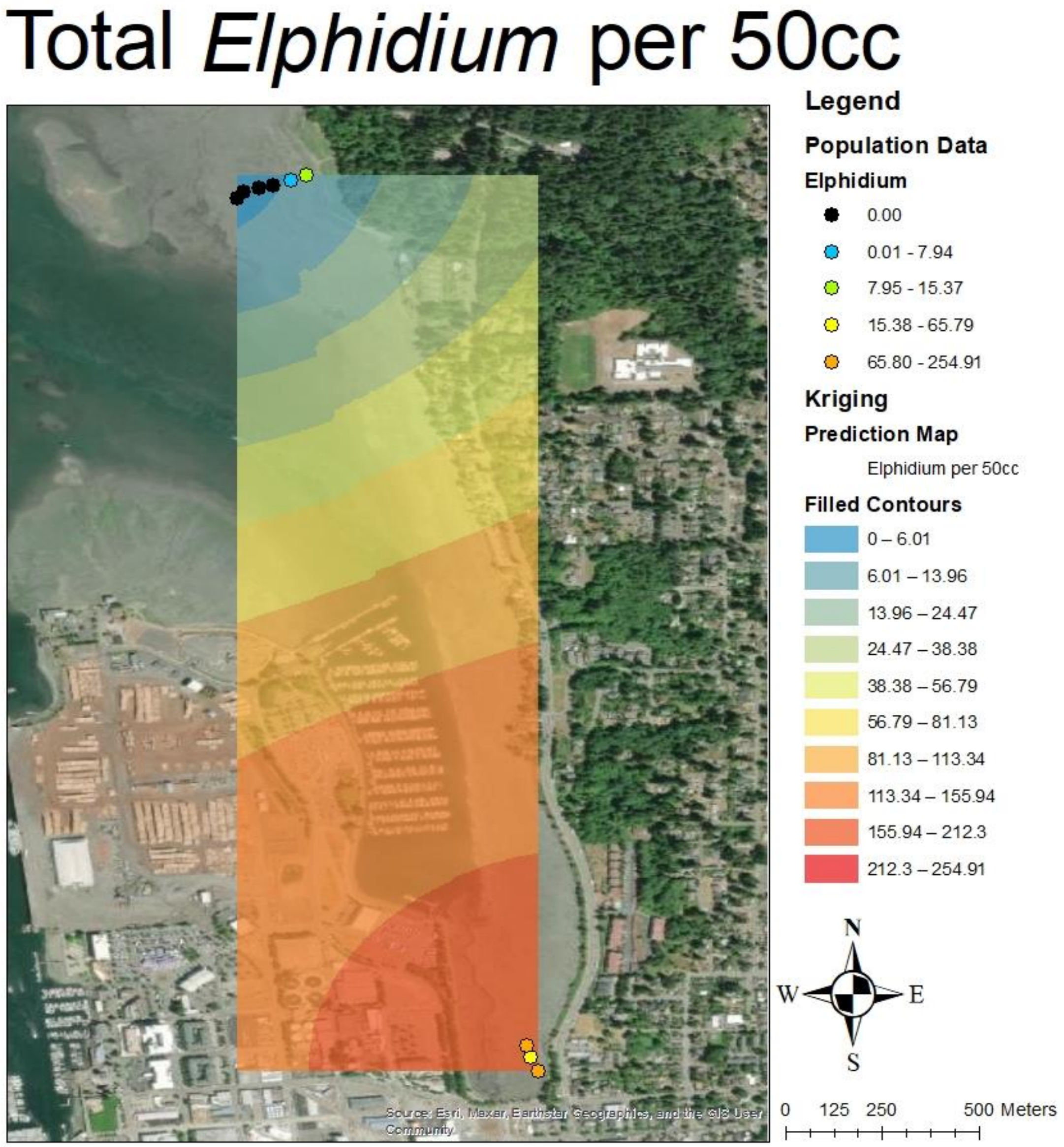
4b.



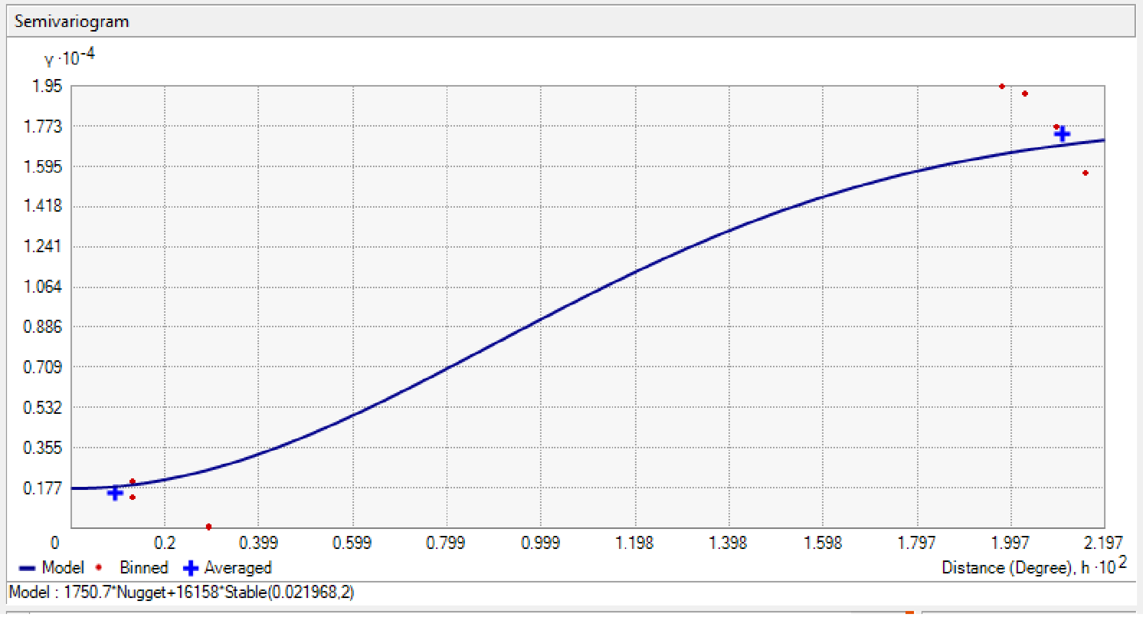
Model : 6.3011e7*Nugget+2.7294e8*Stable(0.021968,2)

Figure 4: (a) Spatial kriging analysis with (b) variogram model of the site shows a predicted decrease of foraminiferal abundances from East Bay northward toward Priest Point.

5a.


Total *Elphidium* per 50cc
Legend
Population Data
Elphidium
● 0.00
● 0.01 - 7.94
● 7.95 - 15.37
● 15.38 - 65.79
● 65.80 - 254.91
Kriging
Prediction Map
Elphidium per 50cc
Filled Contours
0 – 6.01
6.01 – 13.96
13.96 – 24.47
24.47 – 38.38
38.38 – 56.79
56.79 – 81.13
81.13 – 113.34
113.34 – 155.94
155.94 – 212.3
212.3 – 254.91
N
W
E
S
0
125
250
500 Meters
Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

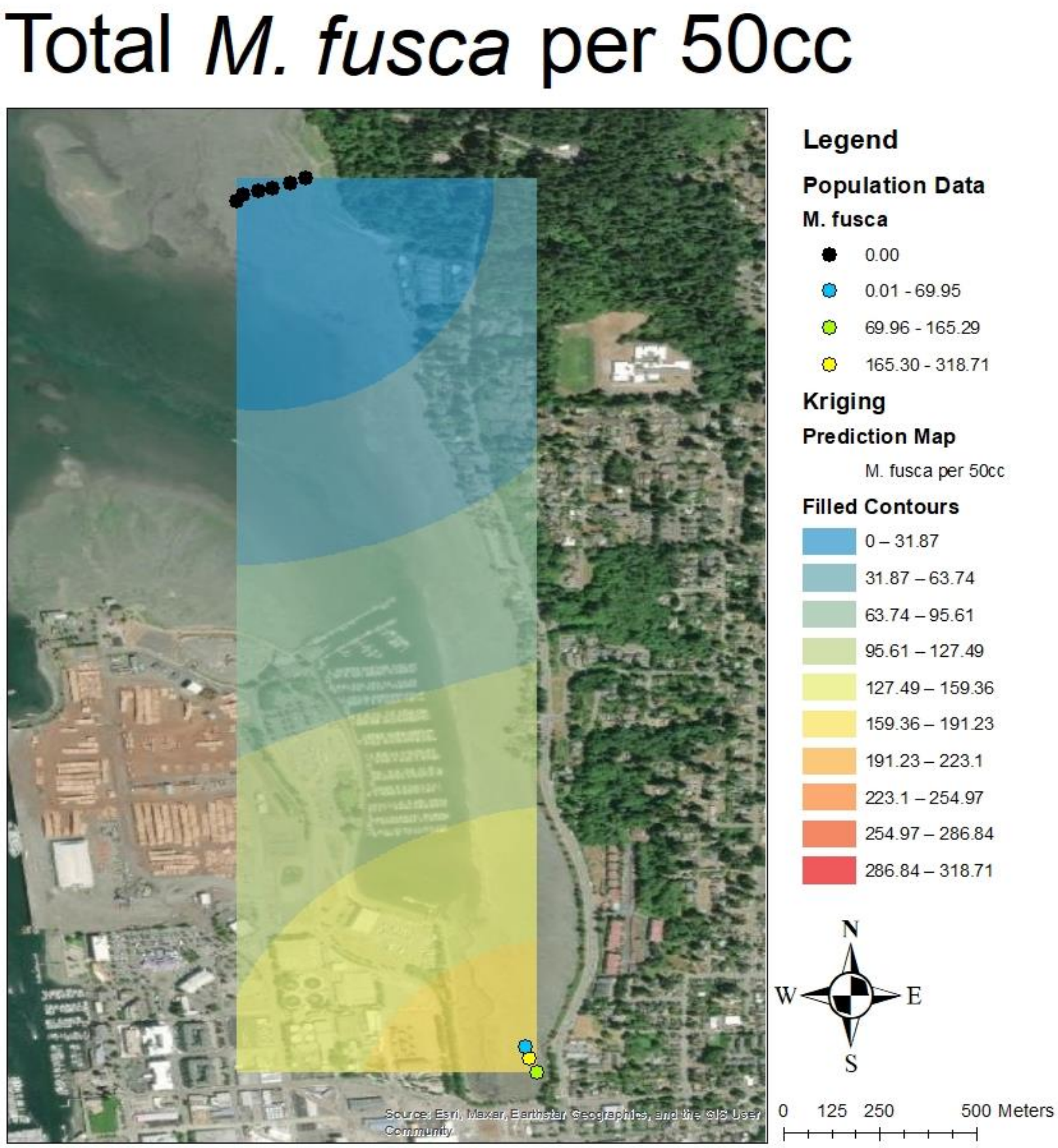
5b.



Model : 1750.7*Nugget+16158*Stable(0.021968,2)

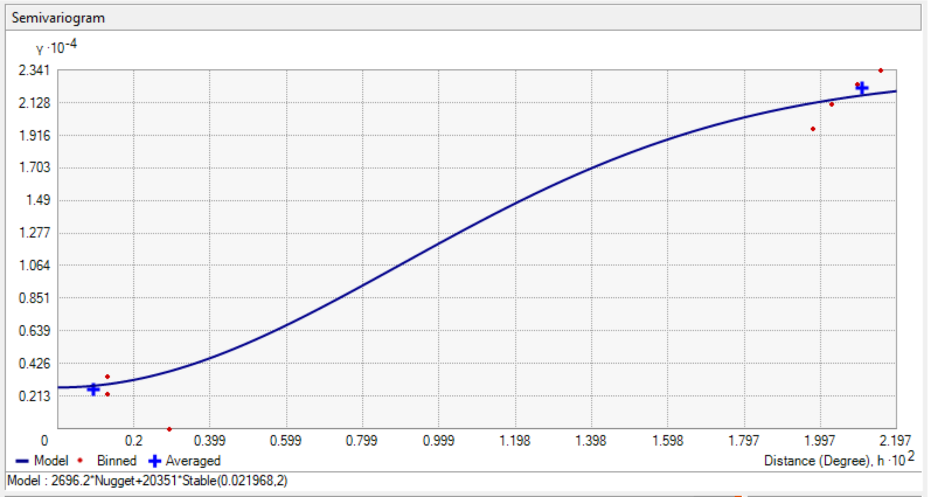
Figure 5: (a) Spatial kriging analysis with (b) variogram model of site showing predicted decreased *Elphidium* sp. abundance from East Bay to Priest Point.

6a.



Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

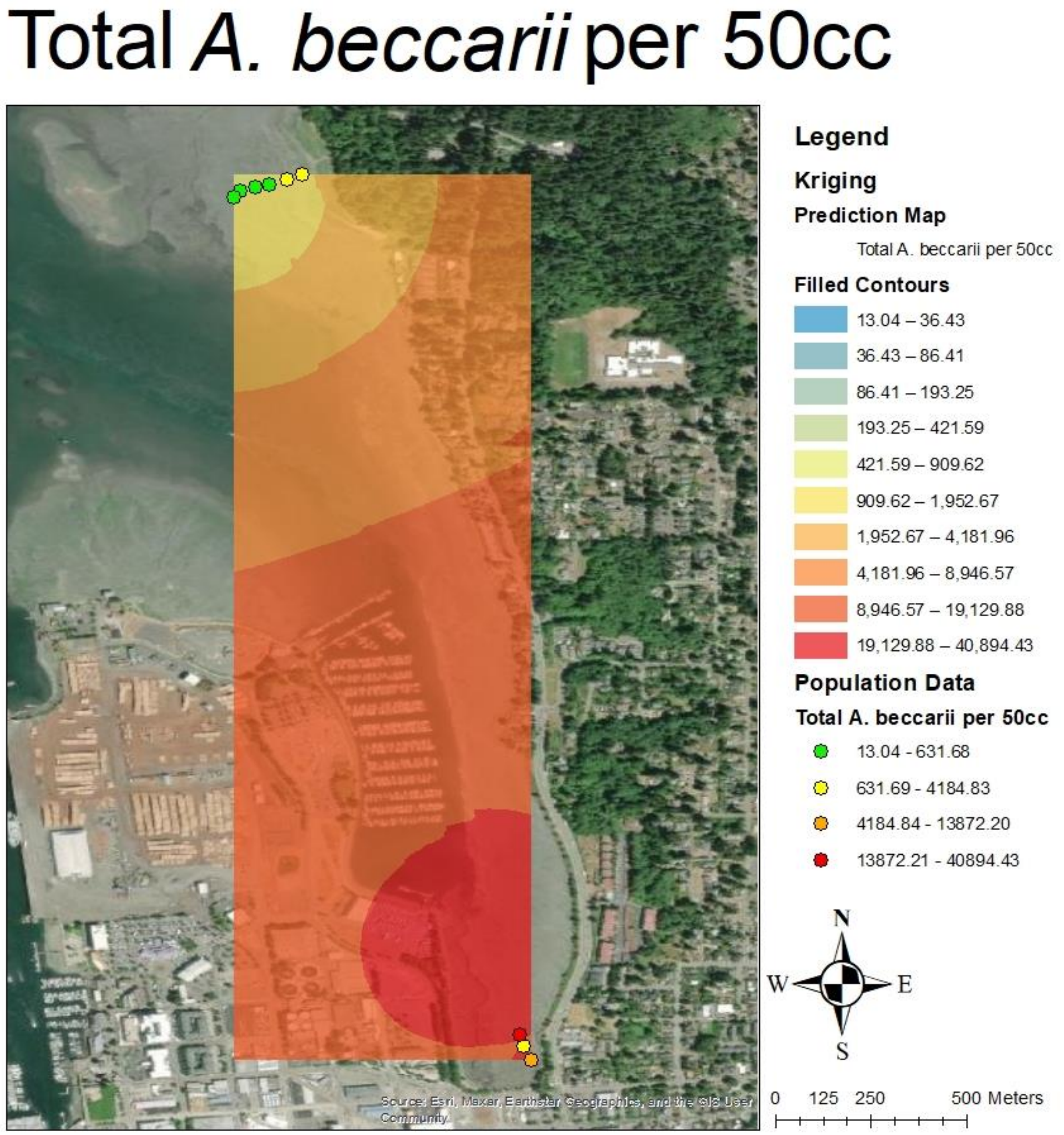
6b.



Model : 2696.2*Nugget+20351*Stable(0.021968,2)

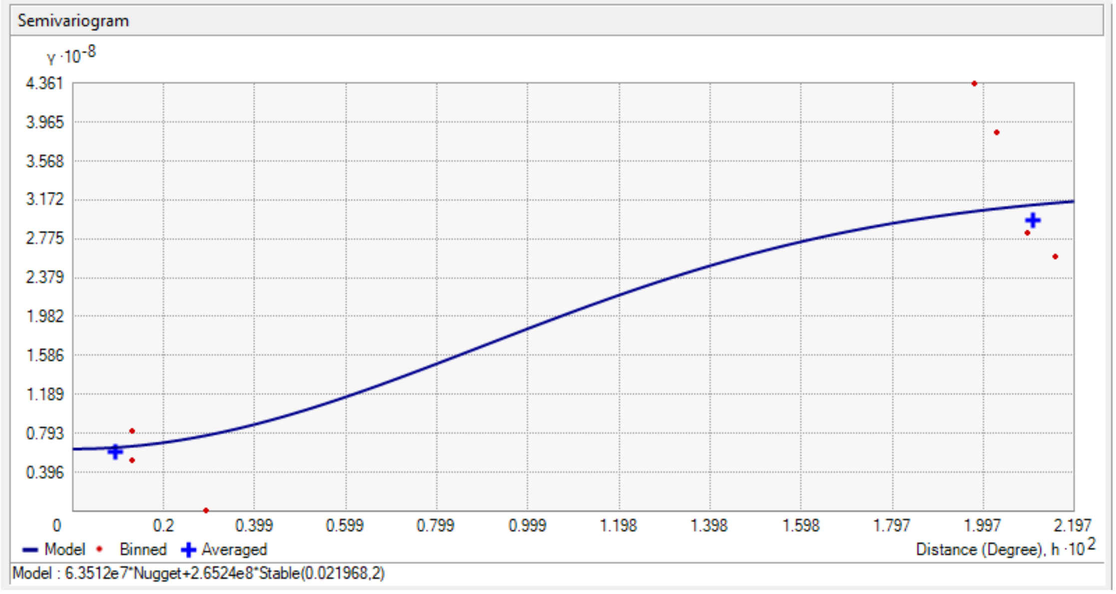
Figure 6: (a) Spatial kriging analysis with (b) variogram model of site showing predicted decreased *M. fusca* abundance from East Bay to Priest Point.

7a.



Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

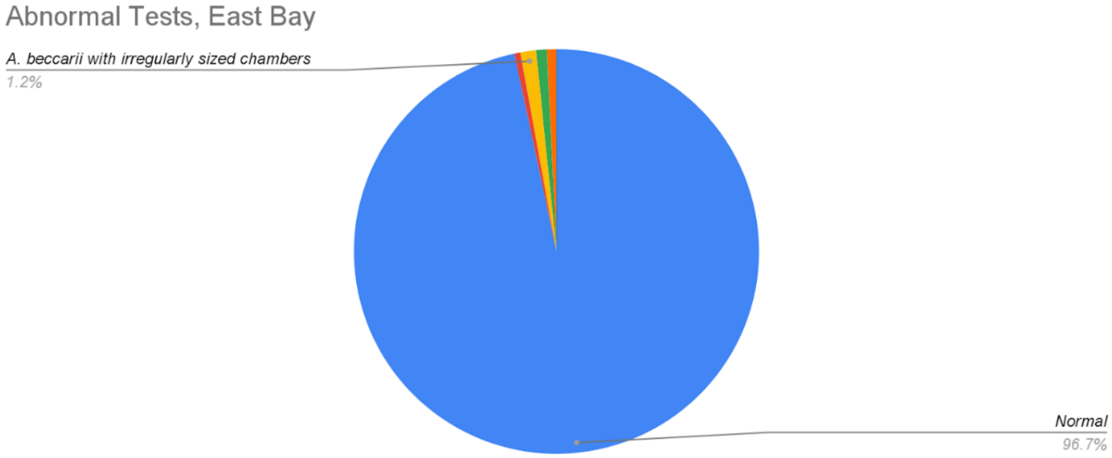
7b.



Model : 6.3512e7*Nugget+2.6524e8*Stable(0.021968,2)

Figure 7: (a) Spatial kriging analysis with (b) variogram model of site showing predicted decreased *A. beccarii* abundance from East Bay to Priest Point.

Both East Bay and Priest Point contained *A. beccarii* with abnormal or mutated tests. None of the *Elphidium* sp., *M. fusca*, or *Bolivinita* sp. exhibited abnormal test formation. Priest Point had a greater proportion of abnormal vs. typical tests than East Bay. At Priest point, 84.6% of the *A. beccarii* could be described as having normal morphology compared to 96.7% from East Bay. For East Bay, the most common abnormality was irregularly sized chambers, whereas test warping was the most common at Priest Point (Figure 8).



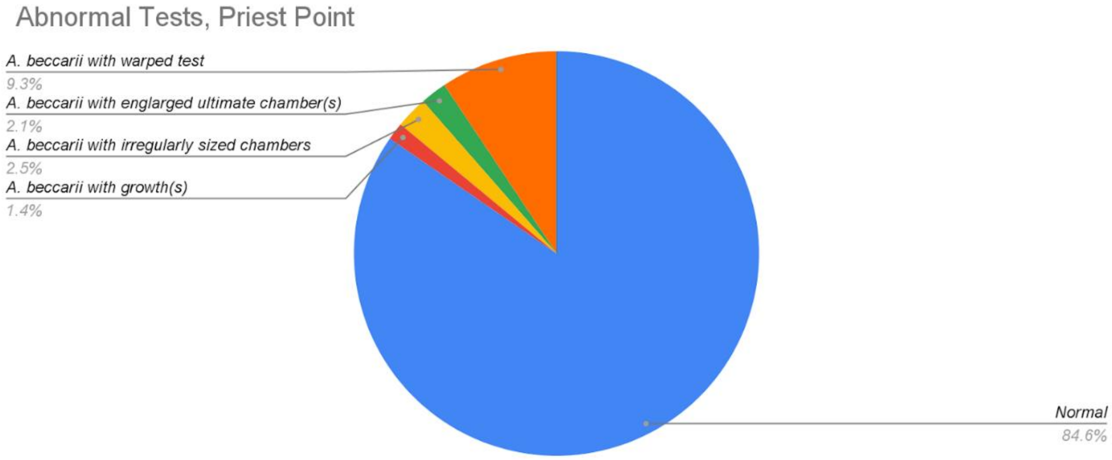


Figure 8: Relative proportions of abnormal tests to typical *A. beccarii* tests. For both graphs, blue represents normal tests, orange represents warped tests, green represents tests with enlarged ultimate chambers, yellow represents tests with irregularly sized chambers, and red represents tests that exhibited abnormal, isolated growth(s).

TRADITIONAL COMPARED TO SPT FLOTATION

Twenty-three samples were collected and split, with half processed using the SPT floatation method and half processed in the traditional wet-picking method. From the total number of tests picked, 40.7% were extracted using the traditional method and 59.3% from the SPT method (Figure 9).

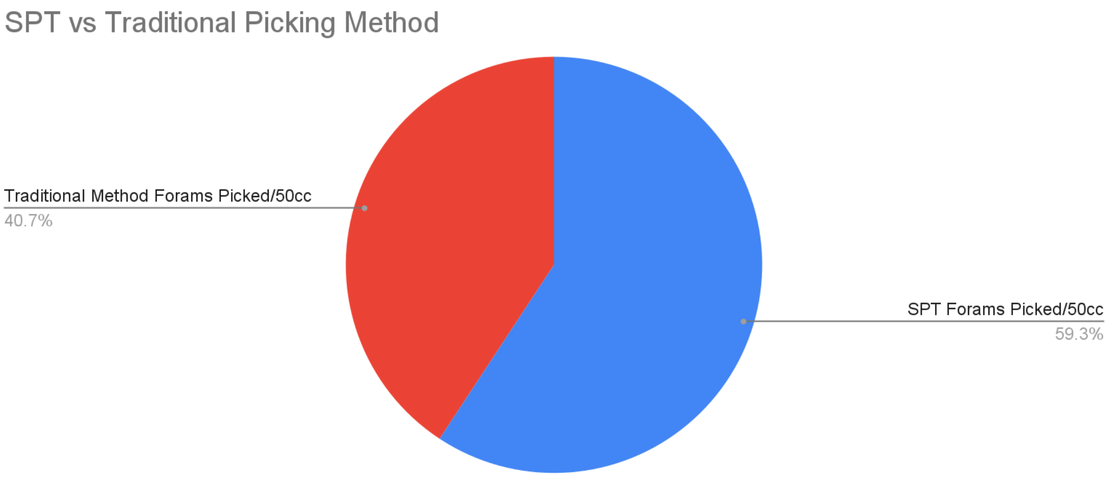
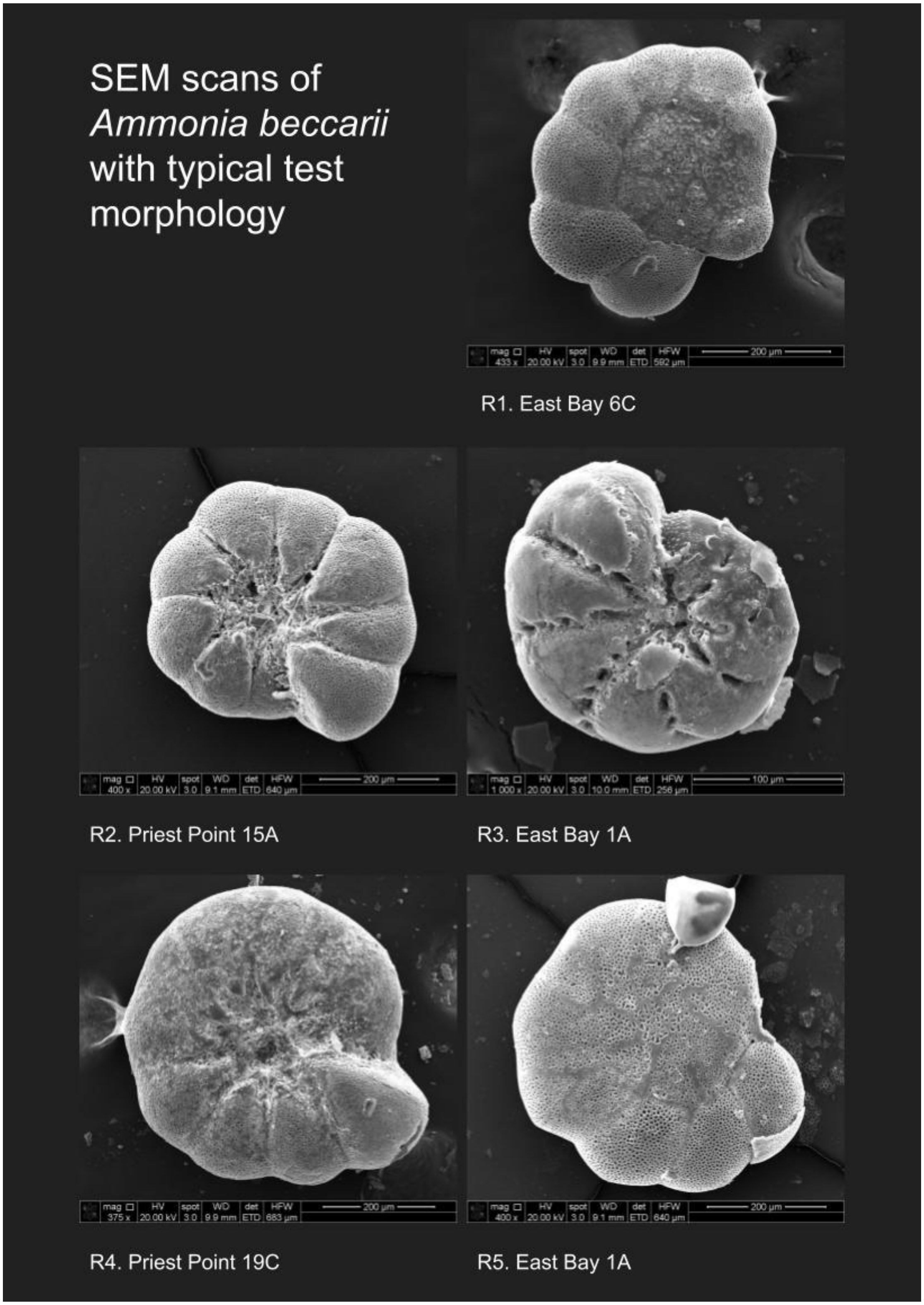


Figure 9: A total of 40.7% of foraminifera tests recovered from this study were collected using the traditional method compared to the 59.3% recovered using the SPT method.

IMAGING

Thirty-four foraminifera individuals were selected to be imaged with a scanning electron microscope (Plates 1-6). These specimens were selected to scan due to the cleanliness and lack of breakage. Different mutations are identified with a letter in front of specimen ID (e.g., enlarged ultimate chambers (E before the sample ID), irregular growth (indicated with a G before the sample ID), warped tests (indicated with a W before the sample ID), irregularly sized chambers (indicated with an I before the sample ID). From those samples, five were further analyzed with MicroCT (Plates 7-14). The key difference between those that were categorized as having irregularly sized chambers and those with warped tests was that for the former, the tests retained their relatively flat trochospiral form, whereas the latter tests appeared bent or folded in on themselves.



R1. East Bay 6C

R2. Priest Point 15A

R3. East Bay 1A

R4. Priest Point 19C

R5. East Bay 1A

Plate 1: Scanning electron microscopy images of *Ammonia beccarii* with typical test morphology (indicated with an R before the sample ID).

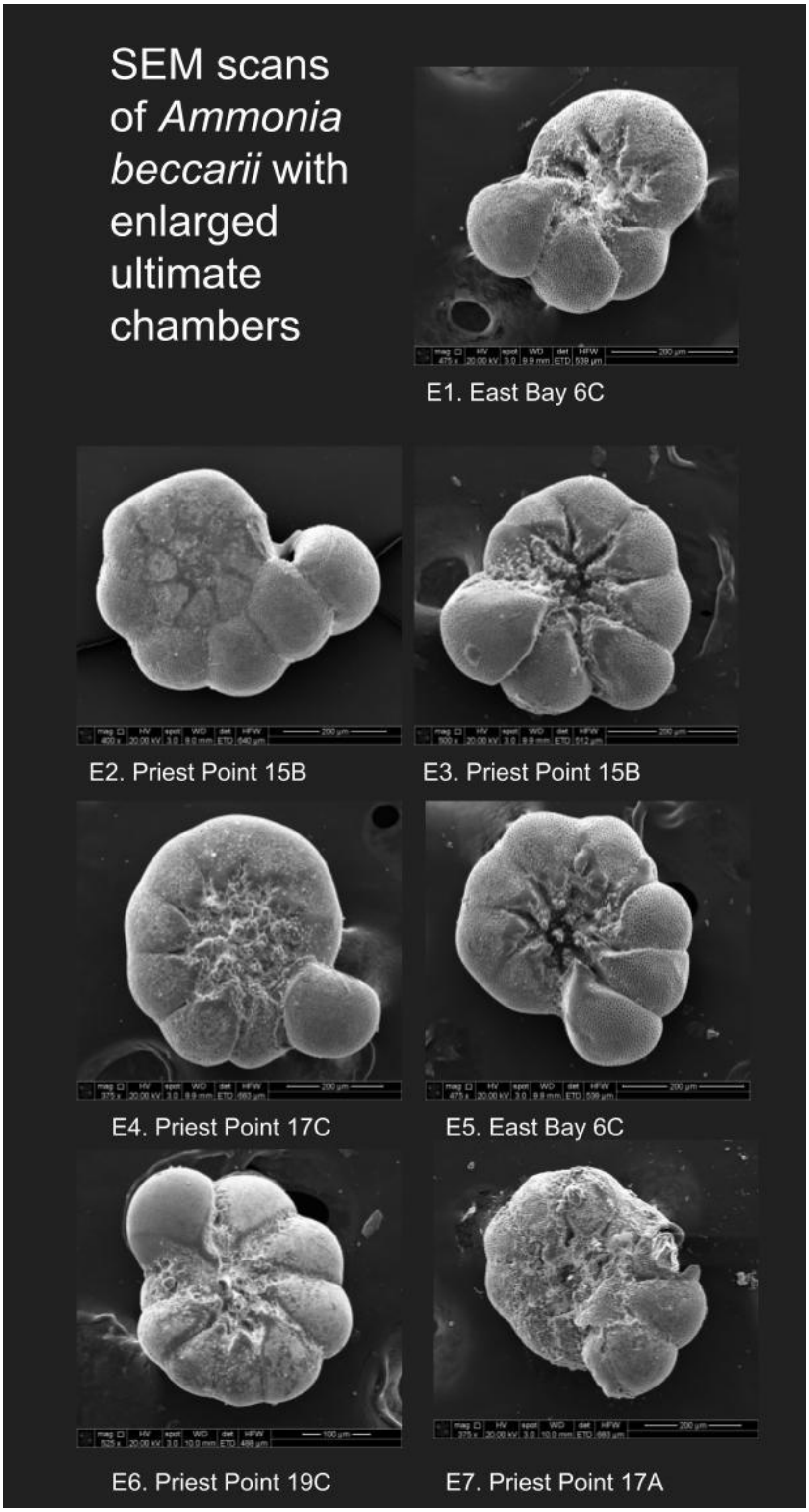


Plate 2: Scanning electron microscopy images of *Ammonia beccarii* with enlarged ultimate chambers (indicated with an E before the sample ID).

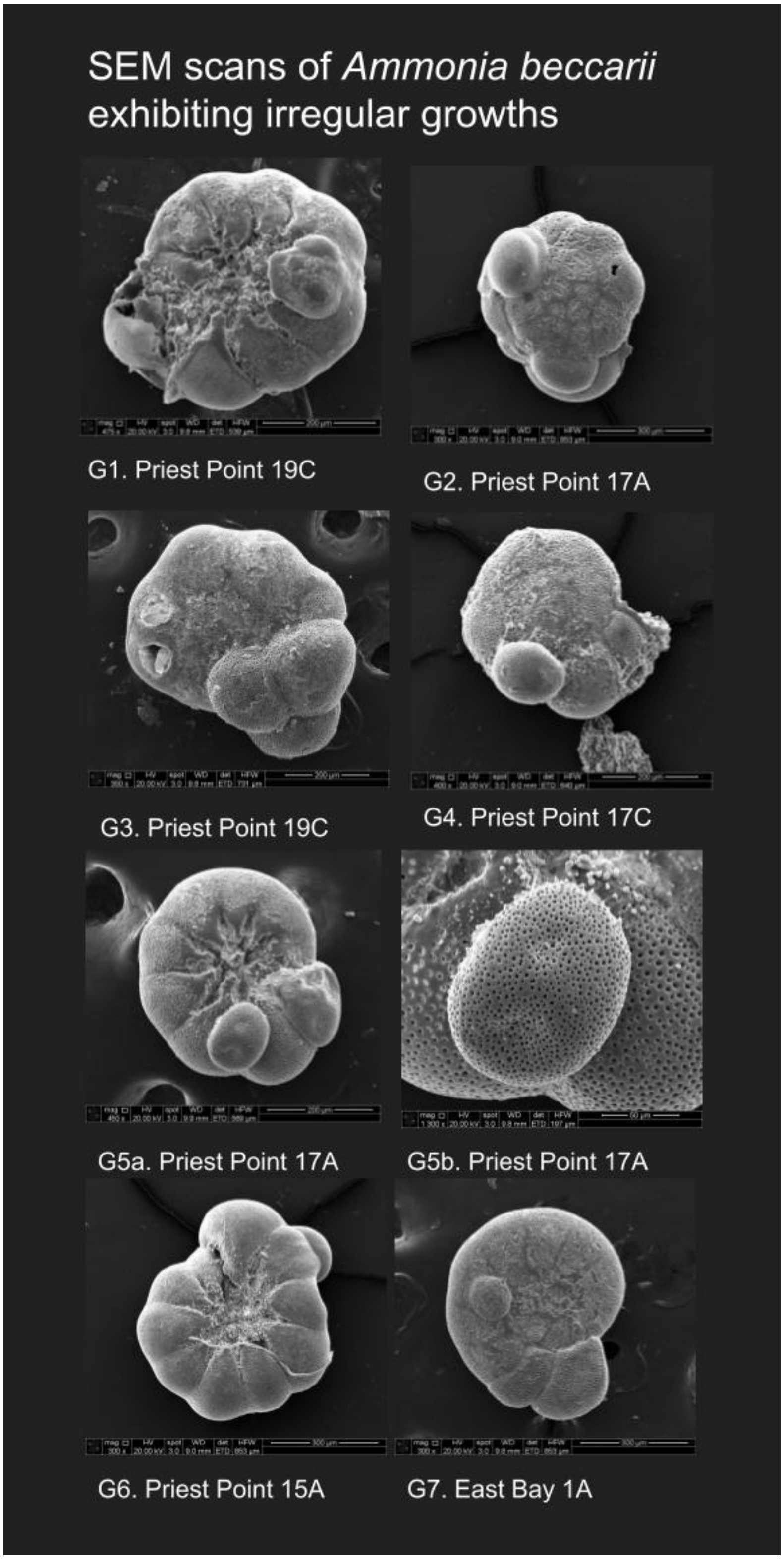


Plate 3: Scanning electron microscopy images of *Ammonia beccarii* exhibiting irregular growth (indicated with an G before the sample ID).

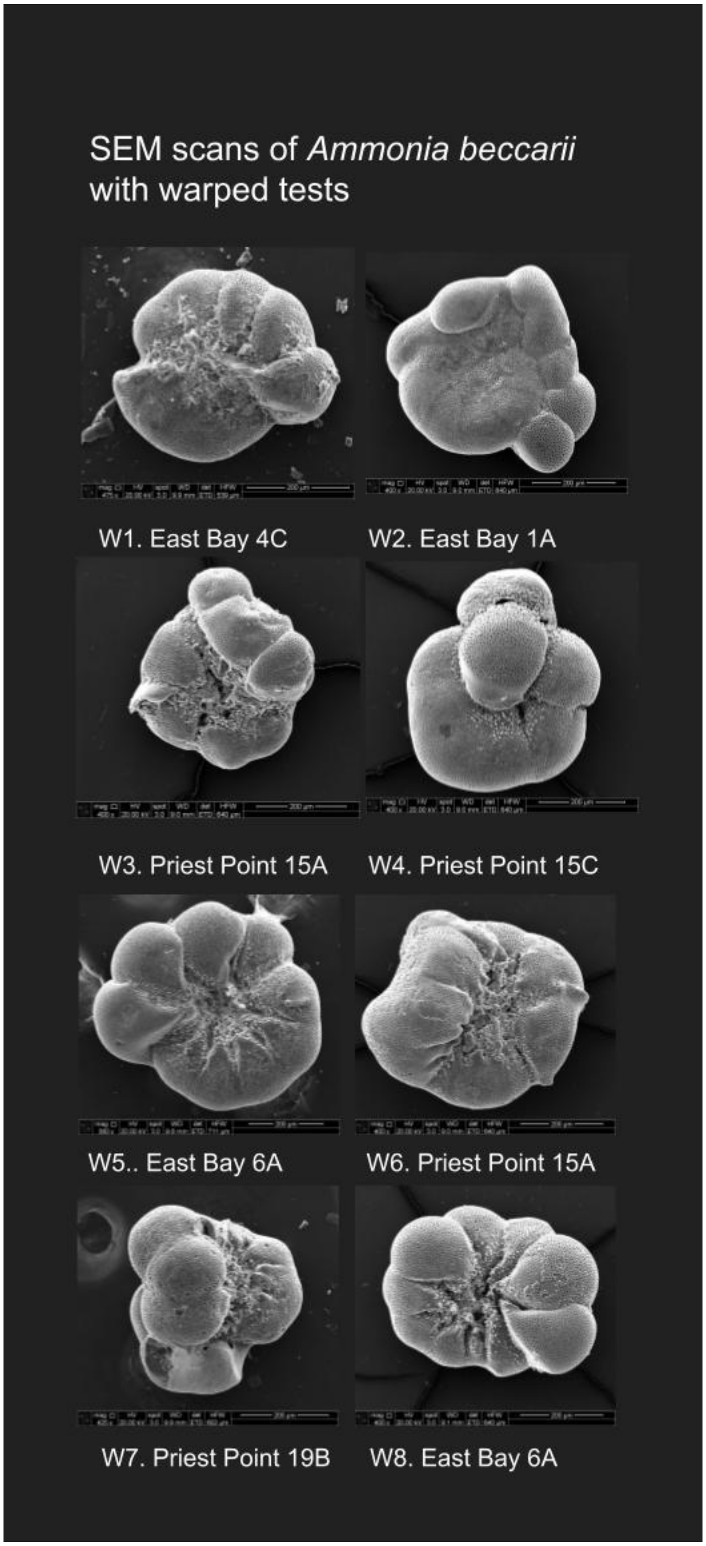
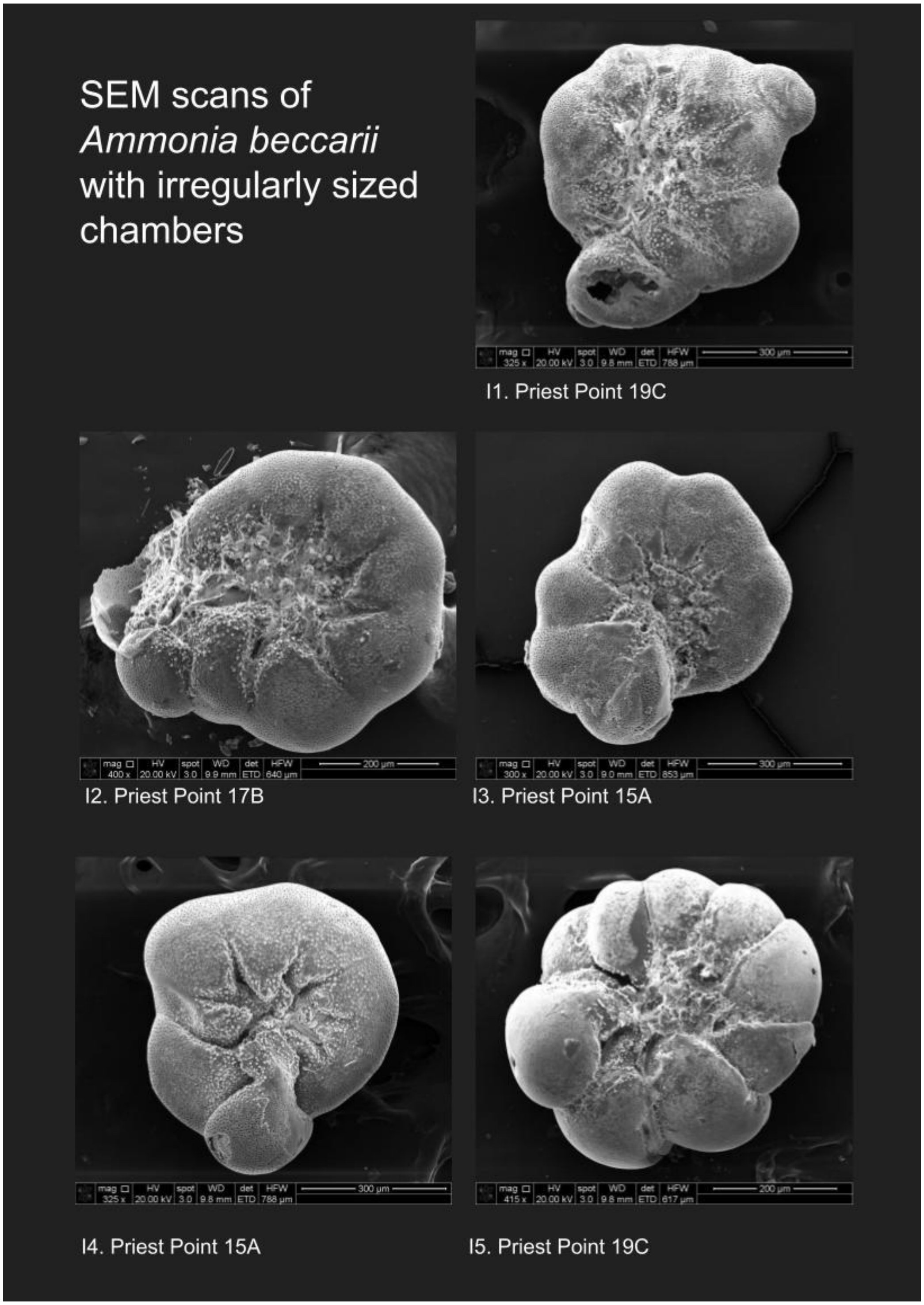


Plate 4: Scanning electron microscopy images of *Ammonia beccarii* with warped tests (indicated with an W before the sample ID).



SEM scans of *Ammonia beccarii* with irregularly sized chambers

I1. Priest Point 19C

I2. Priest Point 17B

I3. Priest Point 15A

I4. Priest Point 15A

I5. Priest Point 19C

Plate 5: Scanning electron microscopy images of *Ammonia beccarii* with irregularly sized chambers (indicated with an I before the sample ID).

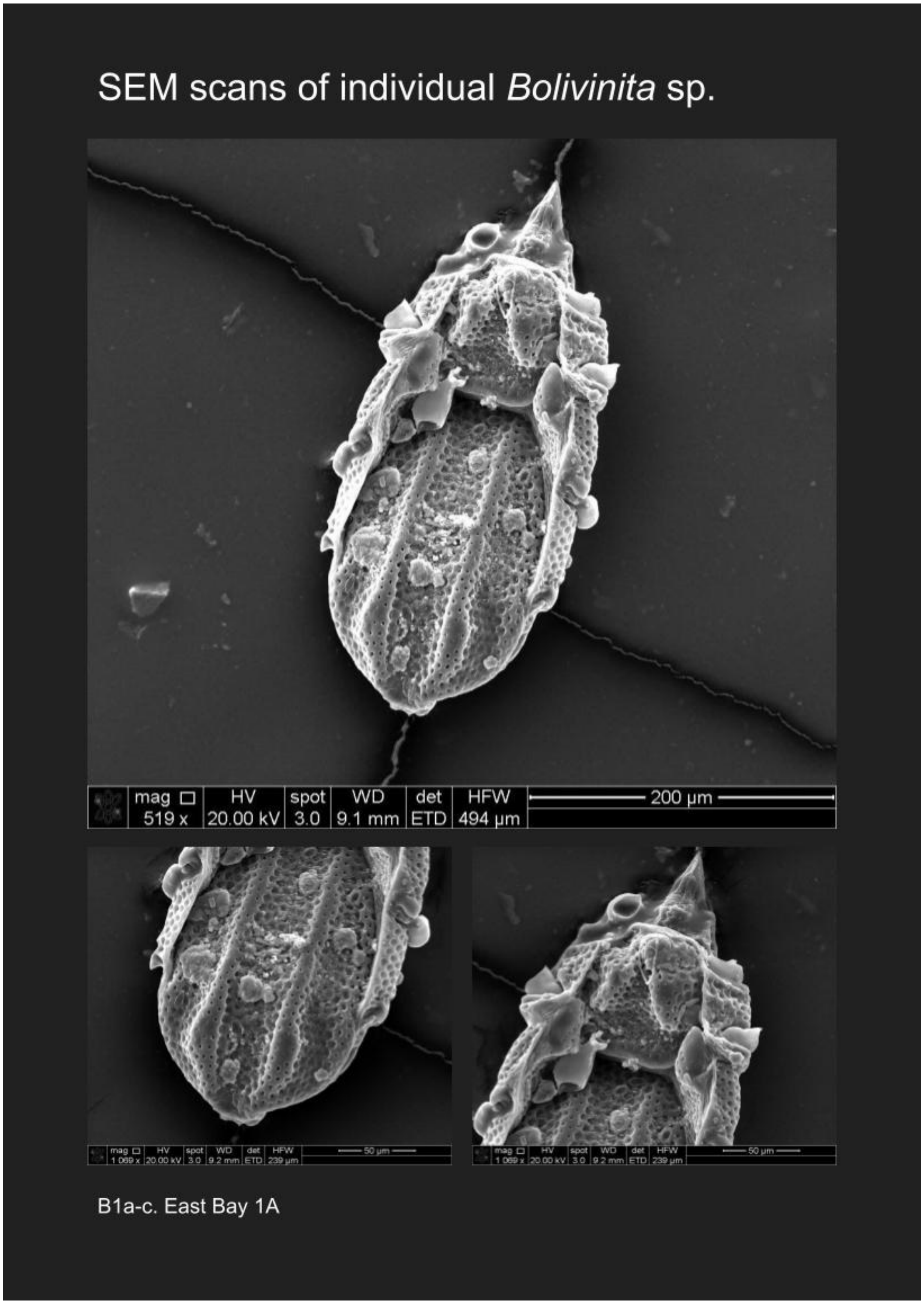
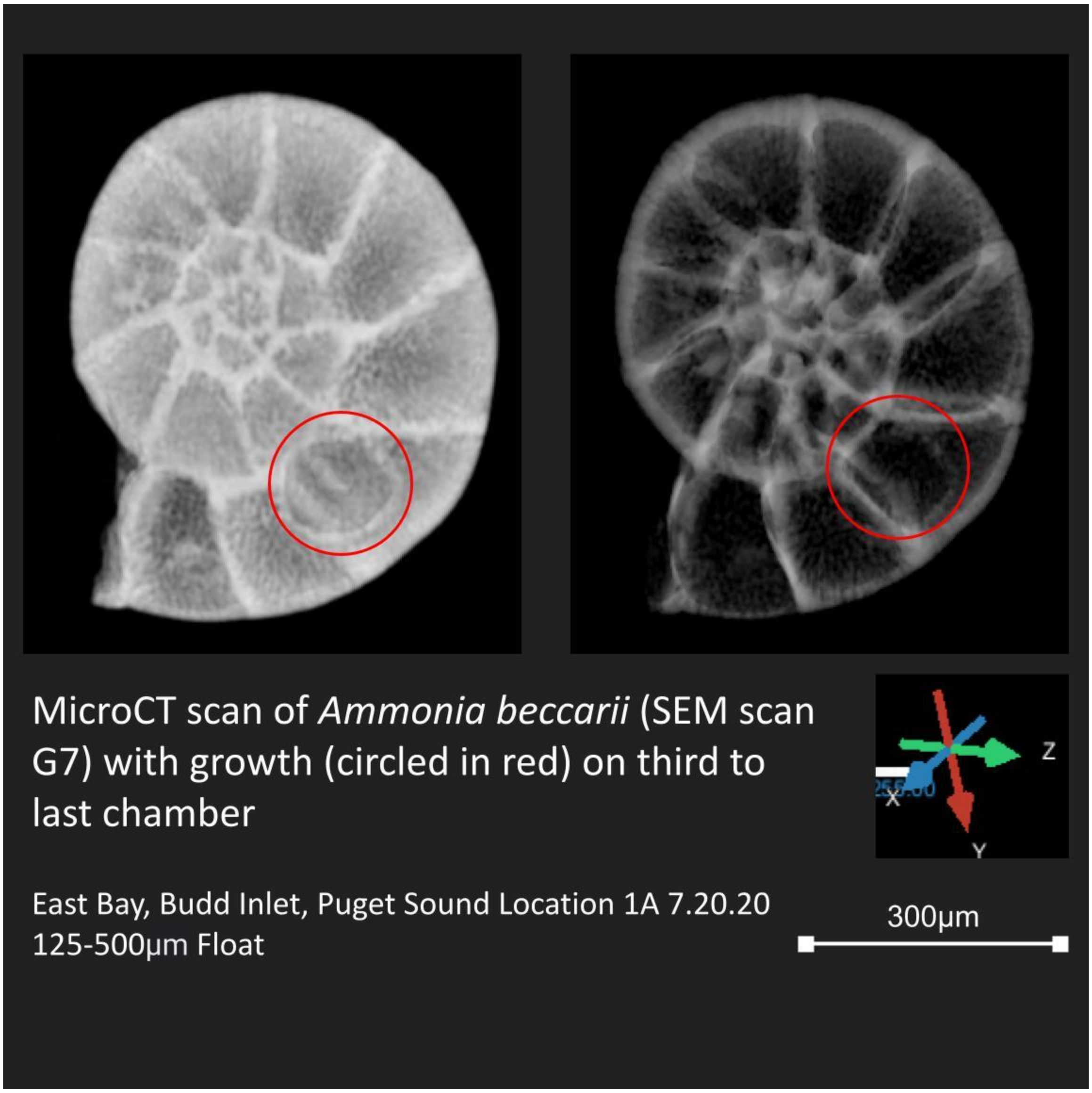


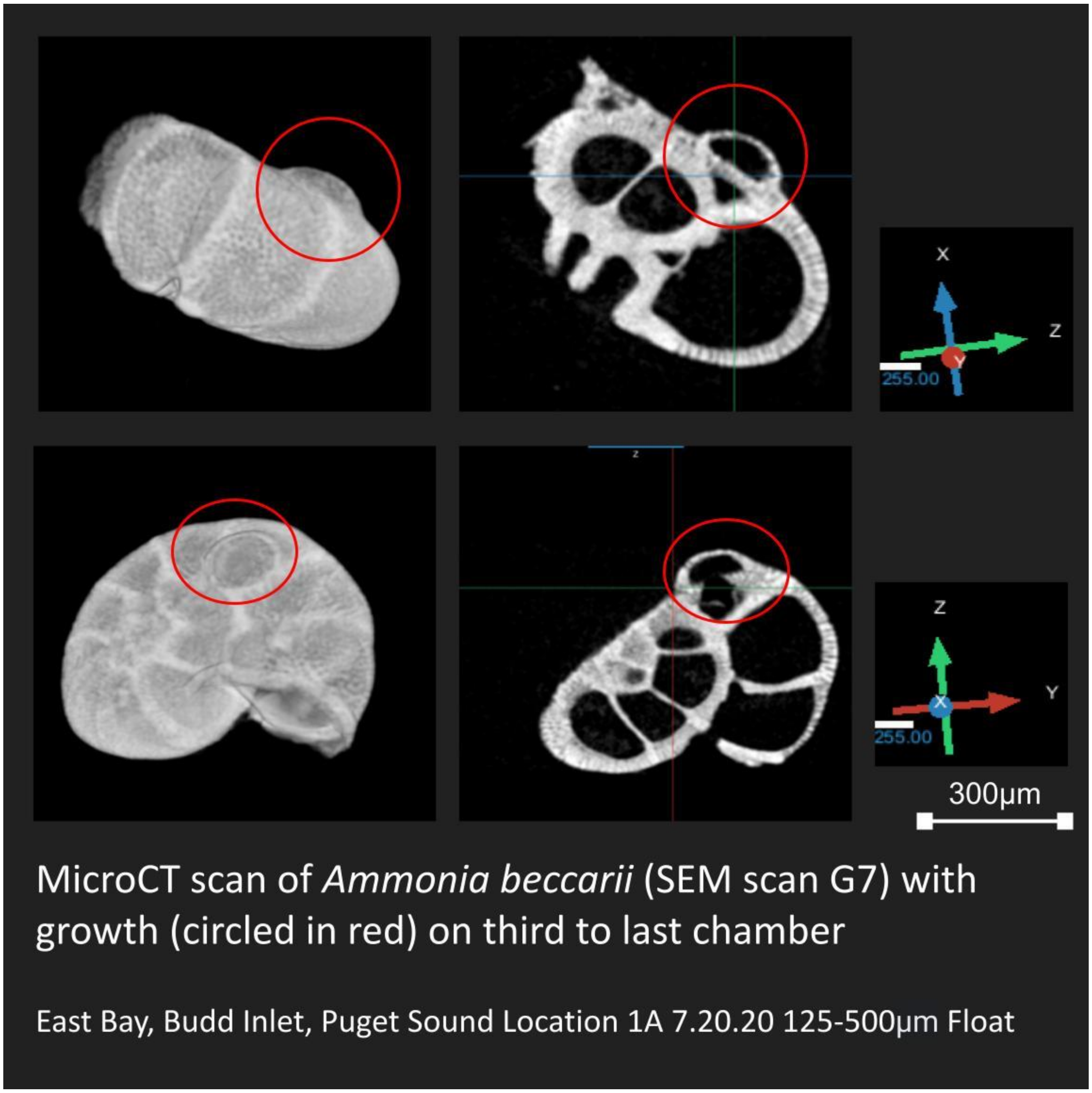
Plate 6: Scanning electron microscopy images of a *Bolivinita* seen in East Bay. Only one specimen of these were observed and are very rare.



MicroCT scan of *Ammonia beccarii* (SEM scan G7) with growth (circled in red) on third to last chamber

East Bay, Budd Inlet, Puget Sound Location 1A 7.20.20
125-500µm Float

Plate 7: MicroCT scans of *Ammonia beccarii* illustrating an abnormal growth on the antepenultimate chamber. Information about scale and orientation are included in the lower right of the plate. An SEM image of this specimen can be seen in Plate 3, G7.



MicroCT scan of *Ammonia beccarii* (SEM scan G7) with growth (circled in red) on third to last chamber

East Bay, Budd Inlet, Puget Sound Location 1A 7.20.20 125-500µm Float

Plate 8: Secondary MicroCT scans of *Ammonia beccarii* illustrating an abnormal growth on the antepenultimate chamber. Information about scale and orientation are included in the lower right of the plate. This specimen is from East Bay 1A collected on 7.202.2020 from the floated 125–500-micron size fraction. An SEM image of this specimen can be seen in Plate 3, G7.

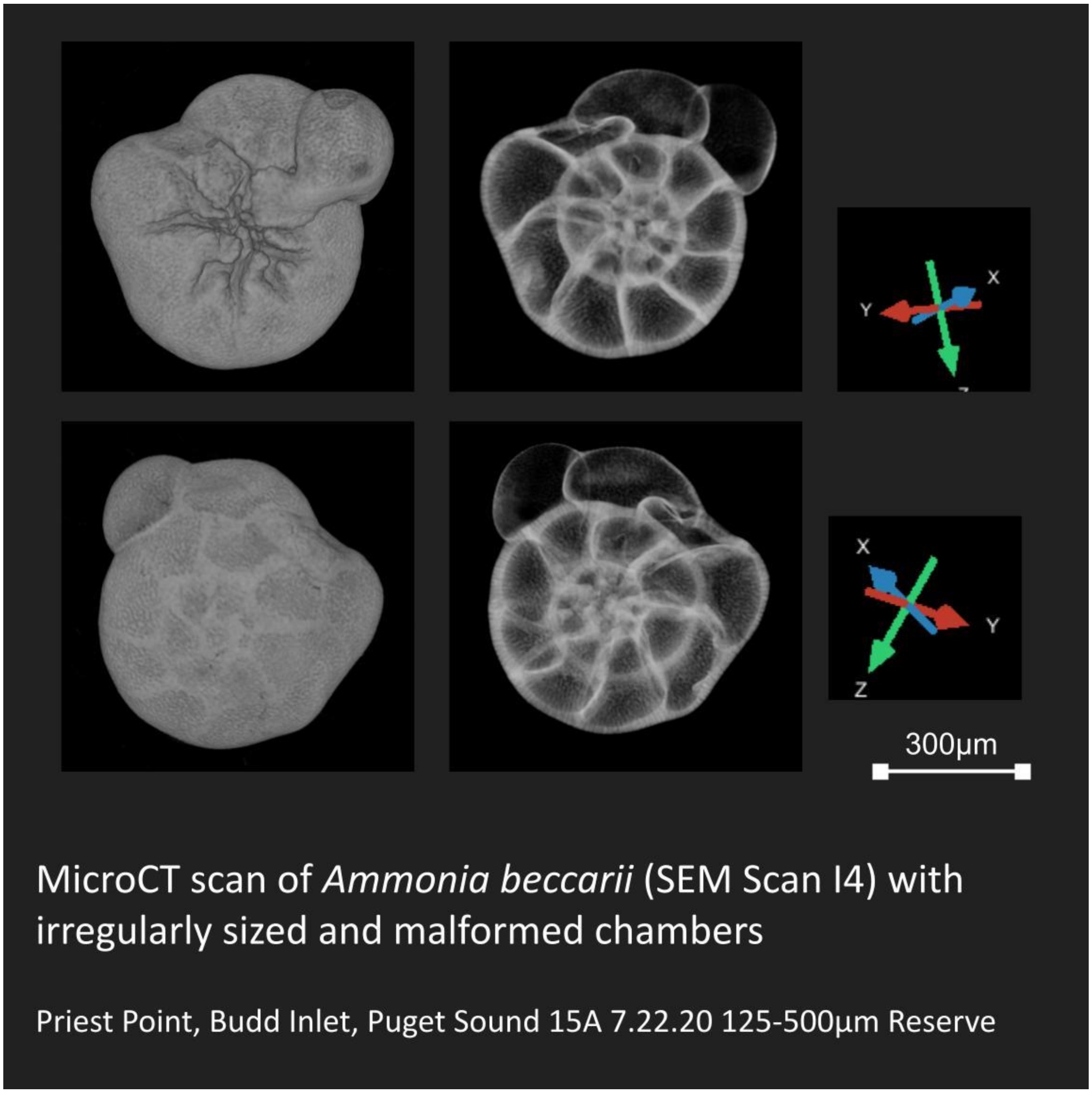
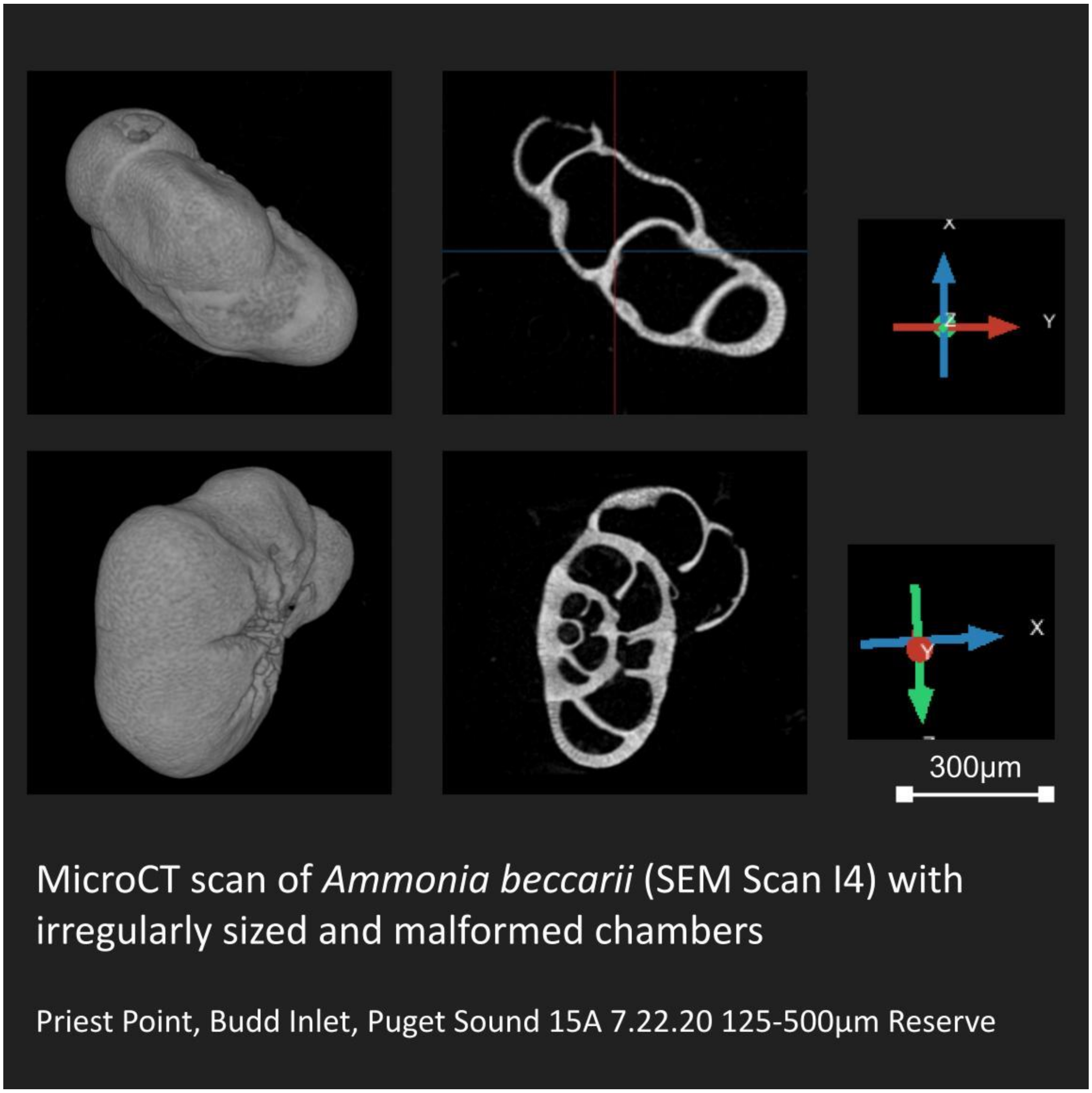


Plate 9: MicroCT scans of *Ammonia beccarii* illustrating irregular sized and malformed chambers. This specimen was collected from Priest Point 15A 7.22.20 from the 125–500-micron reserve fraction. An SEM image of this specimen can be seen in Plate 5, I4. Information about scale and orientation are included in the lower right of the plate.



MicroCT scan of *Ammonia beccarii* (SEM Scan I4) with irregularly sized and malformed chambers

Priest Point, Budd Inlet, Puget Sound 15A 7.22.20 125-500µm Reserve

Plate 10: Secondary views of MicroCT scans of *Ammonia beccarii* with irregularly sized and malformed chambers. This specimen was collected from Priest Point 15A 7.22.20 from the 125–500-micron reserved fraction. An SEM image of this specimen can be seen in Plate 5, I4. Information about scale and orientation are included in the lower right of the plate.

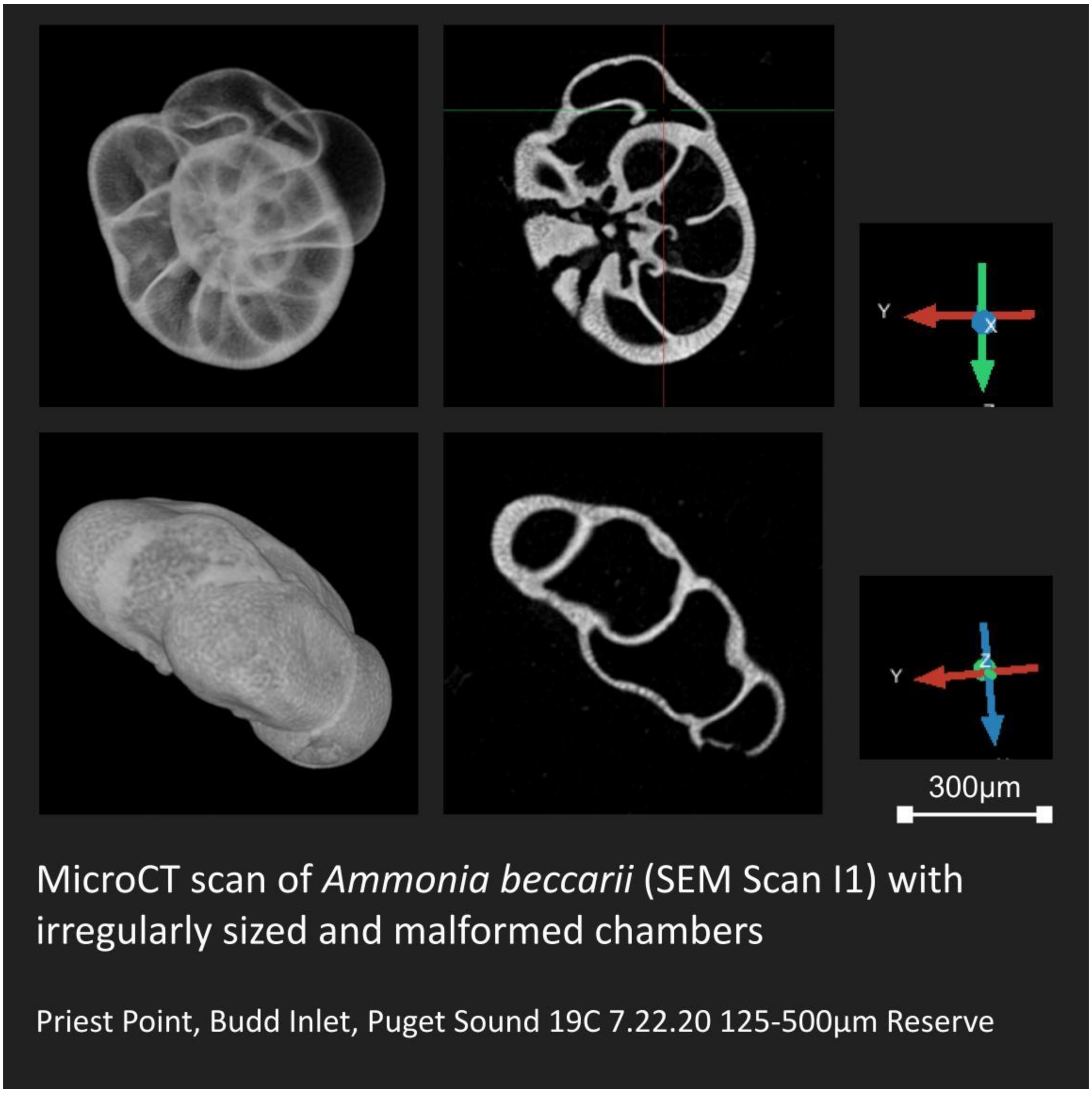
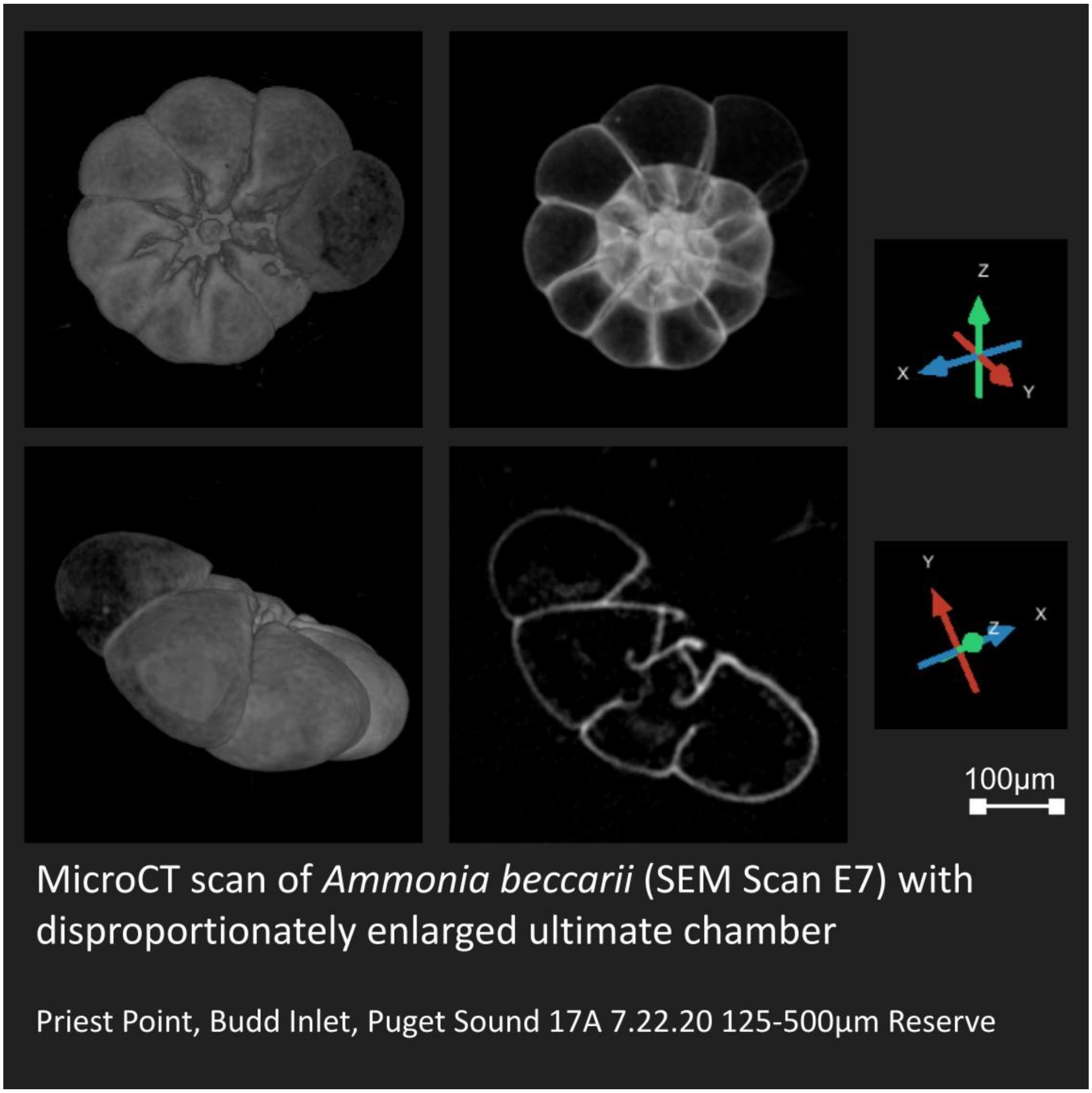


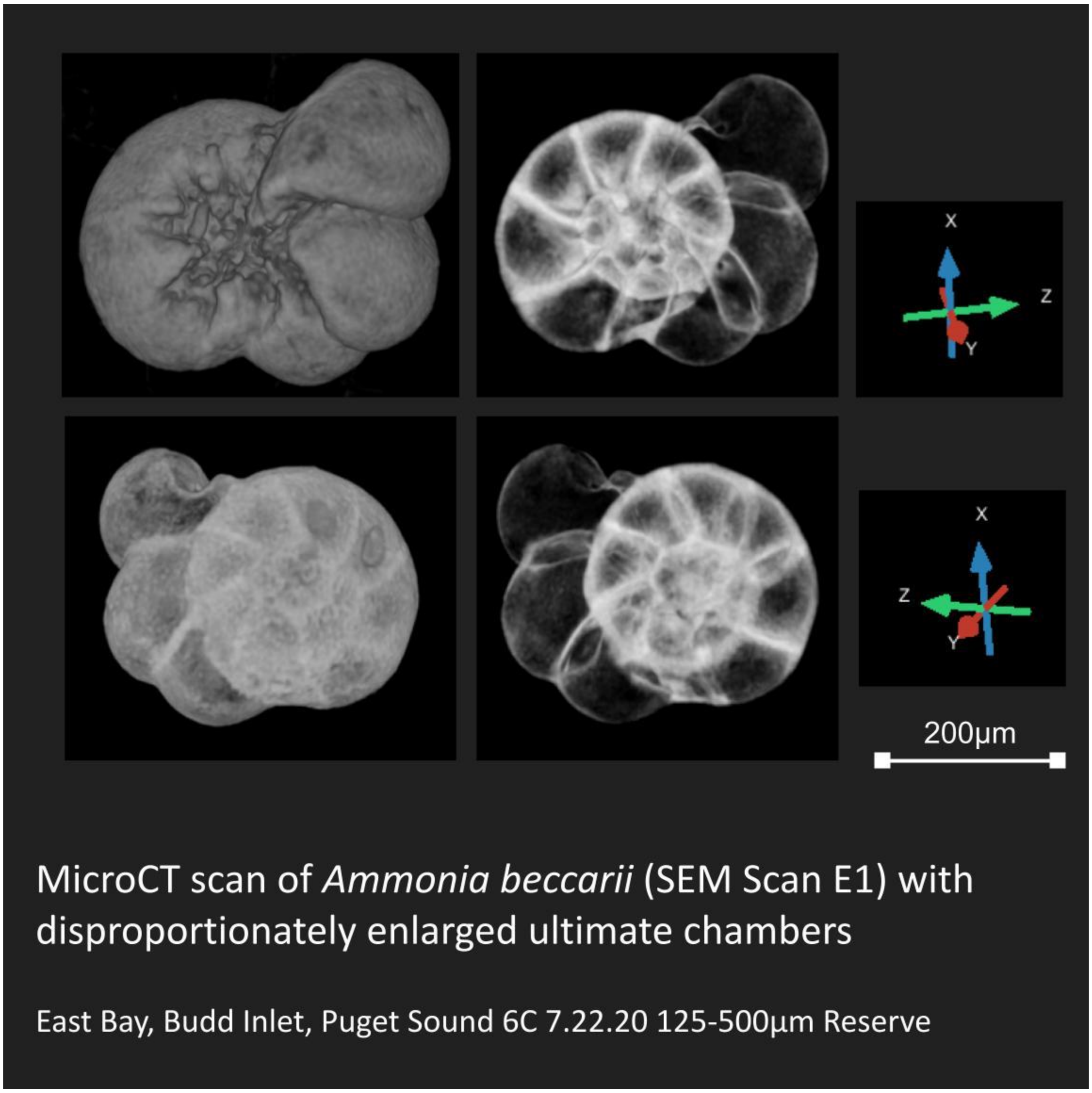
Plate 11: MicroCT scans of *Ammonia beccarii* illustrating irregular sized and malformed chambers. This specimen was collected from Priest Point 19C 7.22.20 from the 125–500-micron reserved fraction. An SEM image of this specimen can be seen in Plate 5, I1. Information about scale and orientation are included in the lower right of the plate.



MicroCT scan of *Ammonia beccarii* (SEM Scan E7) with disproportionately enlarged ultimate chamber

Priest Point, Budd Inlet, Puget Sound 17A 7.22.20 125-500µm Reserve

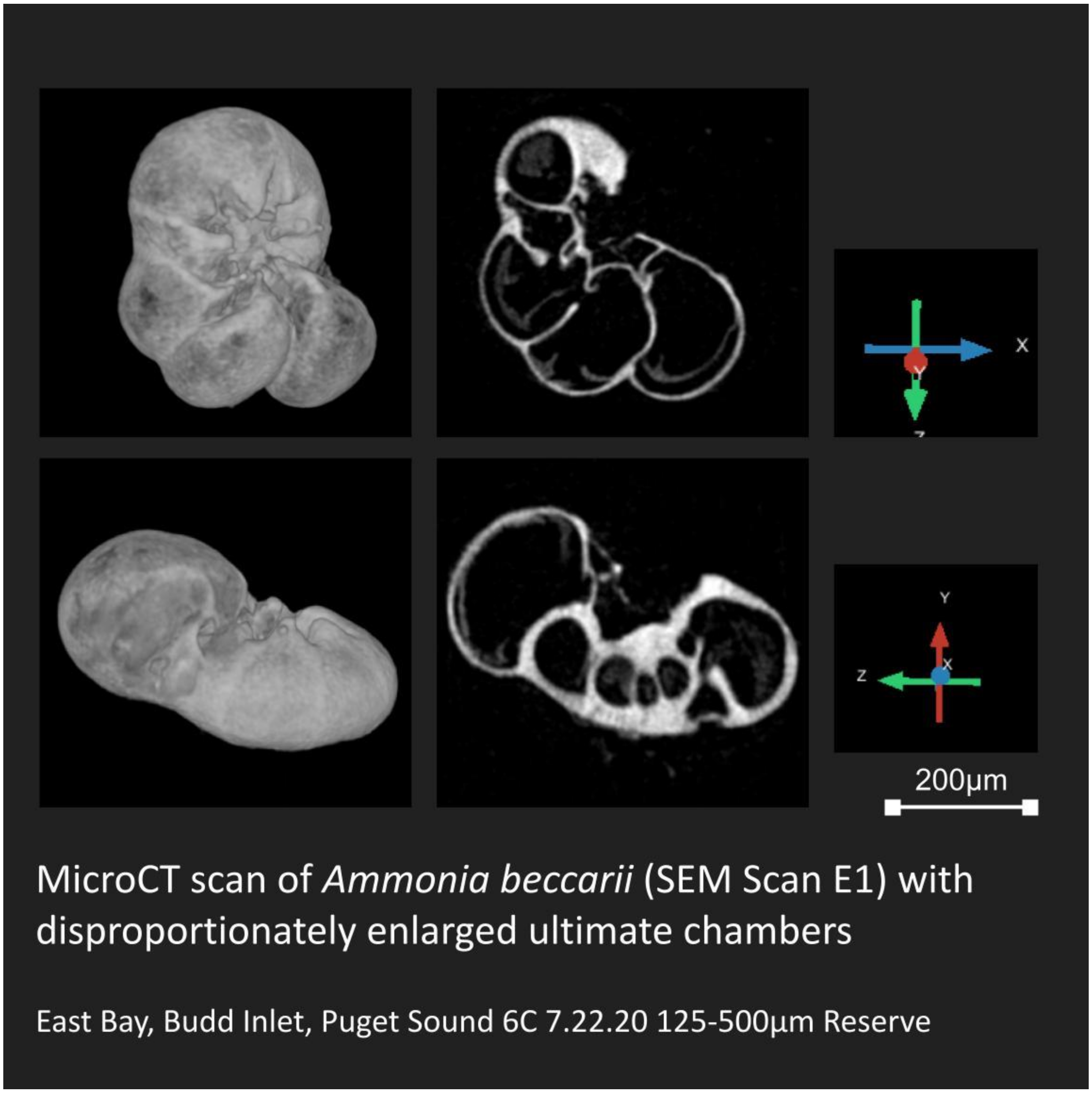
Plate 12: MicroCT scans of *Ammonia beccarii* with a disproportionately enlarged ultimate chamber. This specimen was collected from Priest Point 17A 7.22.20 from the 125–500-micron reserve fraction. An SEM image of this specimen can be seen in Plate 2, E7 Information about scale and orientation are included in the lower right of the plate.



MicroCT scan of *Ammonia beccarii* (SEM Scan E1) with disproportionately enlarged ultimate chambers

East Bay, Budd Inlet, Puget Sound 6C 7.22.20 125-500μm Reserve

Plate 13: MicroCT scans of *Ammonia beccarii* with a disproportionately enlarged ultimate chamber. This specimen was collected at East Bay 6C, 7.22.20 from the 125–500-micron reserve fraction. An SEM image of the specimen can be seen in Plate 2, E1. Information about scale and orientation are included in the lower right of the plate.



MicroCT scan of *Ammonia beccarii* (SEM Scan E1) with disproportionately enlarged ultimate chambers

East Bay, Budd Inlet, Puget Sound 6C 7.22.20 125-500µm Reserve

Plate 14: Secondary view of MicroCT scans of *Ammonia beccarii* with a disproportionately enlarged ultimate chamber. This specimen was collected at East Bay 6C, 7.22.20 from the 125–500-micron reserve fraction. An SEM image of the specimen can be seen in Plate 2, E1. Information about scale and orientation are included in the lower right of the plate.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

TRADITIONAL COMPARED TO SPT FLOTATION

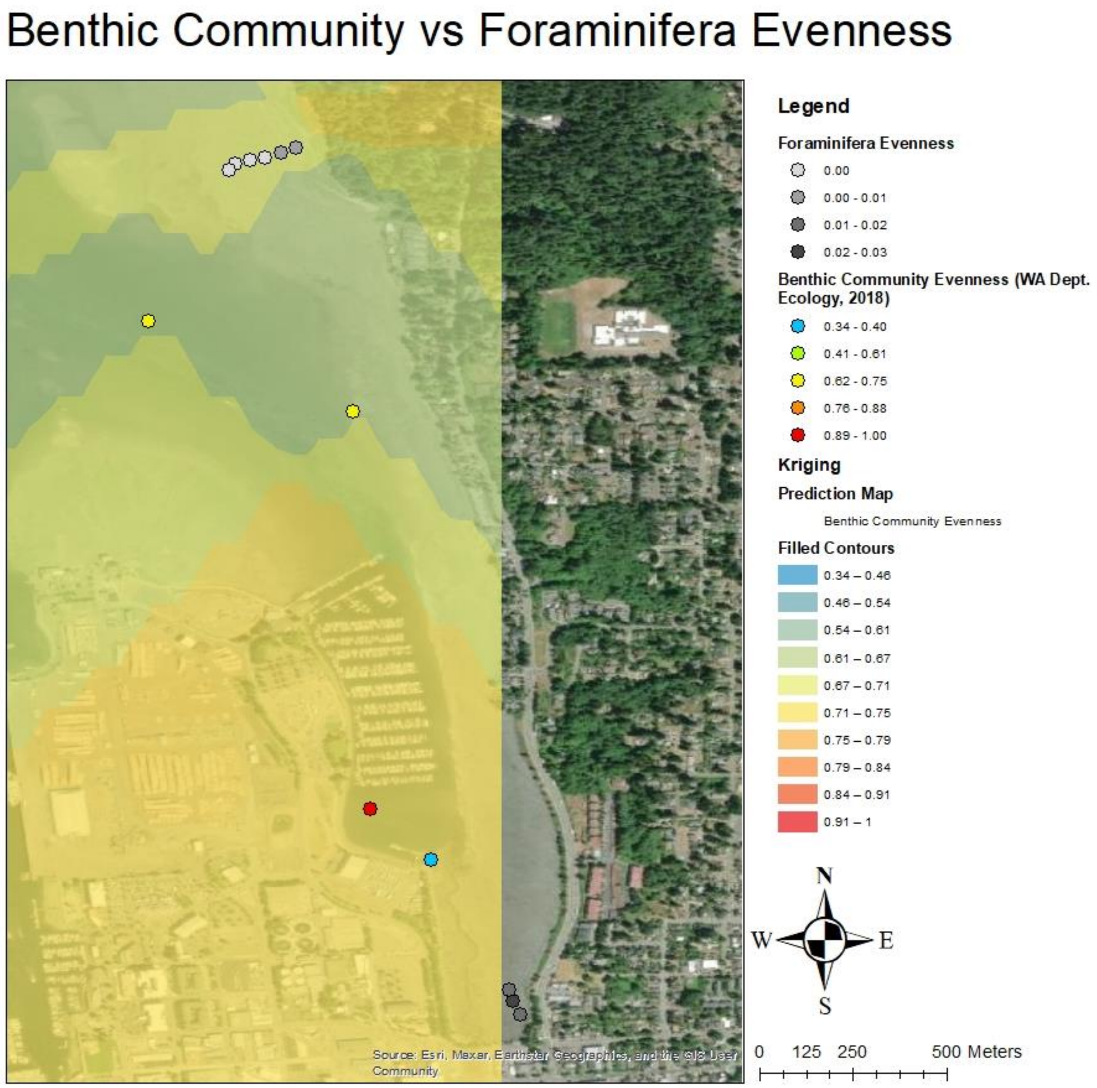
Twenty-three samples were split and processed with the SPT flotation and traditional wet picking methods. During processing, samples that underwent SPT and traditional method were tracked separately and compared (Supplemental Table S1). The SPT floatation method yielded the most foraminifera picked per 50cc with only an 8.6% difference between the two methodologies. The SPT method is more time efficient, as it isolates the sediment and fauna less dense than 2.5g/mL, thereby greatly cutting down the amount of sediment to be picked through. The difference in time and the number of foraminifera yielded make the SPT floatation method worth any extra costs and training necessary and is recommended for use in future projects.

For the samples that underwent SPT flotation, the criteria for what was deemed a "stained" sample was slightly more generous. Any test that was estimated to be at least 60% stained was picked compared to the traditional samples, wherein a test was only picked if it was at least 80% stained. This was done to account for the stained cytoplasm shrinking during the drying process of the SPT method. It should be noted that the increased yield from the SPT samples could possibly result from these picking decisions.

FORAMINIFERA ECOLOGY

Based on the results of the twenty-three samples examined in this thesis, *A. beccarii* is the most abundant species overall, and is clearly dominant at both locations within Budd Inlet. The other three genera/species present in the sediment samples were *M. fusca, Bolivinita* sp., and *Elphidium* sp., though these were only minor members. At each site, the Shannon-Weiner Diversity Index was 0, as the proportion of *A. beccarii* to the three other constituents was disproportionately in favor of *A. beccarii* (Supplemental Table S1). The evenness, or the measure of the relative abundance of difference genus/species, of foraminifera populations from the 2020 samples was plotted against the meiofaunal benthic community evenness described by the Washington State Department of Ecology in 2018 (Figure 10). The evenness of the foraminifera population was lower than that of the 2018 meiofaunal benthic community study.

10a



Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

10b

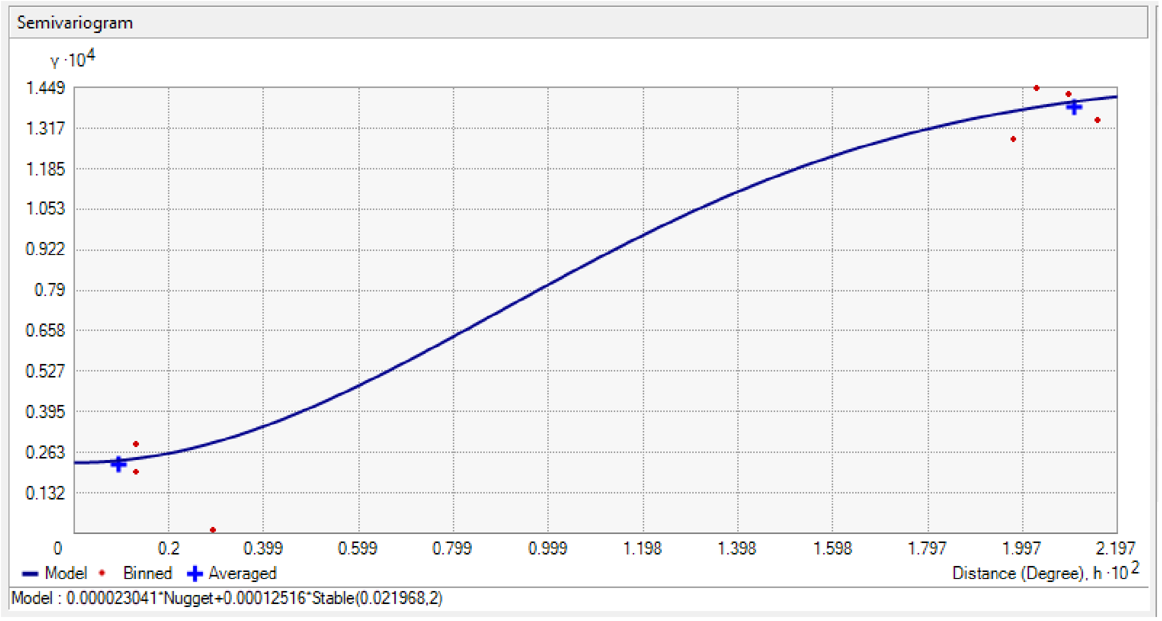


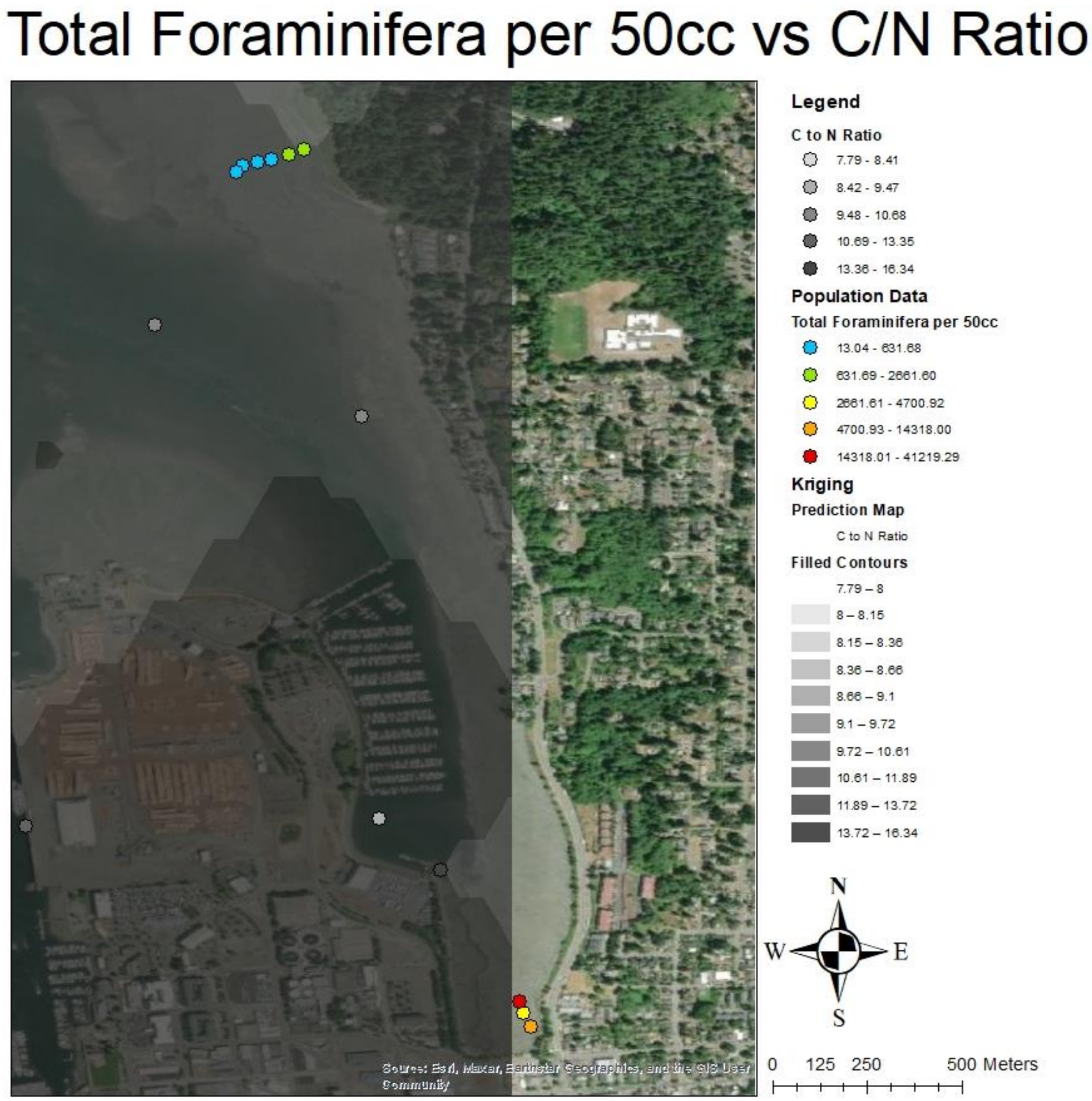
Figure 10: (a) Spatial kriging analysis with (b) variogram model of the evenness from the foraminifera collected in 2020 compared to the known and kriging-predicted evenness of the benthic community as described by the Washington State Department of Ecology in 2018.

Most foraminiferal abundance at East Bay show no correlation between distance from land and abundance of foraminifera, though there were overall more foraminifera present at this site compared to Priest Point. At East Bay, *Elphidium* sp. seems to have a negative correlation between abundance and depth. At Priest Point, the overall abundance of all genus/species foraminifera, except *Elphidium* sp., decreases as water depth increases.

Kriging analysis performed on foraminiferal population data suggest abundance of foraminifera likely decreases overall from the south to the north, which follows the circulatory pattern of the inlet (Figure 10). This corresponds to C/N ratio in that as C/N ratio increases, so too do abundances of foraminifera (Figure 11). Additionally, Priest

Point, which has a lower C/N ratio than East Bay, experiences a greater proportion of abnormal *A. beccarii*, suggesting that lowered levels of carbon may result in greater instances of mutation or malformation. The higher C/N ratio at East Bay is likely due to the presence of the LOTT wastewater treatment plant in close proximity to the site. Additionally, Priest Point may see higher nitrogen levels due to input from the lumber yard, with the flow pattern of the inlet feeding nitrogen toward Priest Point (Lynch & Corbett, 1990).

11a



Total Foraminifera per 50cc vs C/N Ratio
Legend
C to N Ratio
7.79 - 8.41
8.42 - 9.47
9.48 - 10.68
10.69 - 13.35
13.36 - 16.34
Population Data
Total Foraminifera per 50cc
13.04 - 631.68
631.69 - 2661.60
2661.61 - 4700.92
4700.93 - 14318.00
14318.01 - 41219.29
Kriging
Prediction Map
C to N Ratio
Filled Contours
7.79 – 8
8 – 8.15
8.15 – 8.36
8.36 – 8.66
8.66 – 9.1
9.1 – 9.72
9.72 – 10.61
10.61 – 11.89
11.89 – 13.72
13.72 – 16.34
N
W
E
S
0 125 250 500 Meters
Source: Esri, Maxar, Earthstar Geographics, and the GIS User Community

11a

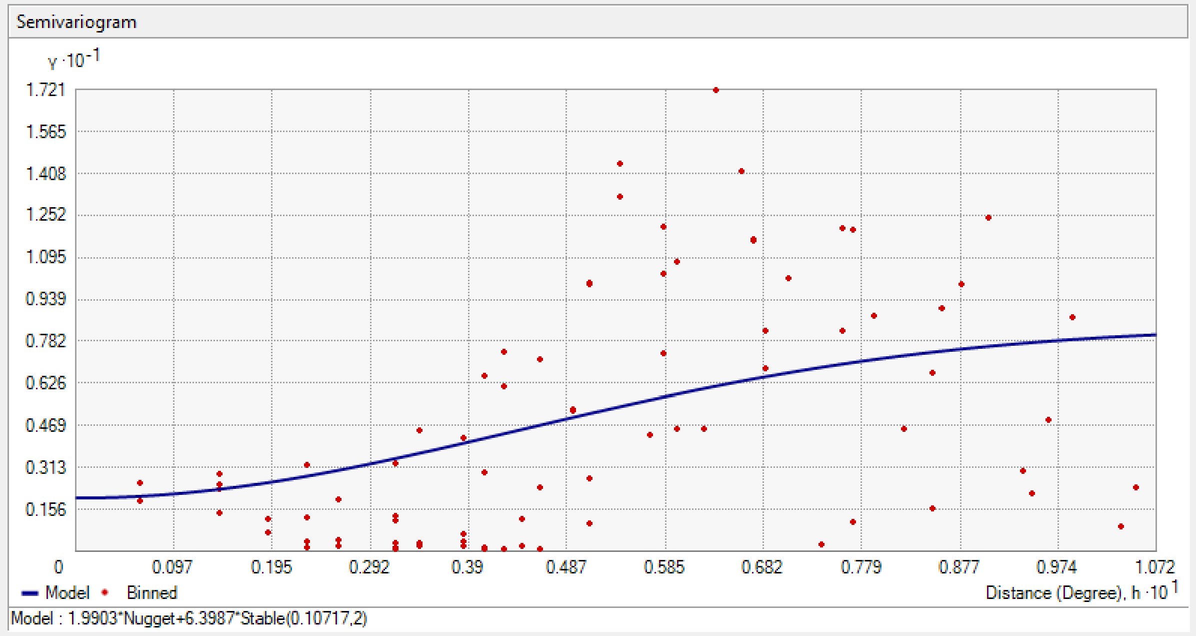


Figure 11: (a) Spatial kriging analysis with (b) variogram model of the 2018 C/N data compared to the overall abundance of foraminifera, which shows decreased foraminifera abundances with lowered C/N ratios.

It is worth noting the limitations of the kriging analyses conducted on both the environmental data collected by Washington State Department of Ecology in 2018 and the population data from the sediment samples collected for this study in 2020. Kriging was performed in order to facilitate basic assessments of foraminifera ecology based on the limited data collected. Kriging assumes isotopy, that there is a uniformity in variables in every direction of a given point. This lends explanation to the solitary light gray data point, which represents a smaller C/N ratio, found in an area predicted to have a higher C/N ratio overall in Figure 11 near the south end of the inlet. As the neighboring data points had higher C/N ratios, this data point did not impact the map. The kriging analyses conducted did not consider the circulatory water flow of the inlet, nor the boundaries between land and water. As this project serves as a primary assessment of Budd Inlet's foraminifera populations, further sampling would expand the spatial data and allow for more comprehensive and accurate modeling of the area.

In addition to being the most abundant species observed in Budd Inlet, significant numbers of *A. beccarii* also show some sort of test abnormality. High concentrations of heavy metals in the sediment as measured in 2018 within East Bay do not seem to correlate with the abundance of abnormal tests, though the data here is limited and required further investigation. It has been proposed that test mutations may result when specimens live in environments experiencing heavy metal toxicity. Comparing the presence of mutations with the sedimental heavy metal record is of course only measuring the amount of heavy metal bound in the sediments and we suggest this to be a minimum abundance and recognize this does not necessarily represent what is biologically available.

AMMONIA BECCARII

Ammonia is one of the two most globally abundant genera of foraminifera and has been studied since the late 1700s. This genus occurs mainly in brackish marine waters such as the water present in the study area, typically in shallow, intertidal zones (Hayward et al., 2004). *Ammonia beccarii* is a globally distributed species of the *Ammonia* genus that has been used extensively in environmental studies due to its widespread abundance. This species can be dated back to the Miocene Epoch, 23.03-5.3 Mya (Debenay et al., 1998) and is widely used to characterize oxygen depletion, changes in salinity and pH, and heavy metal pollution (Alve, 1991; Yanko et al., 1994; Almogi-

Labin et al., 1995). *Ammonia beccarii* was originally described as *Nautilus beccarii* by famed Swedish zoologist Carl Linaeus in his work *Systema Naturae*. *Ammonia beccarii* forms in a coiled spiral with triangular to trapezoidal chambers composed of calcium carbonate with depressed sutures. Their aperture forms in an arcuate lunar arch (Figure 12) (Linnaeus, 1758).

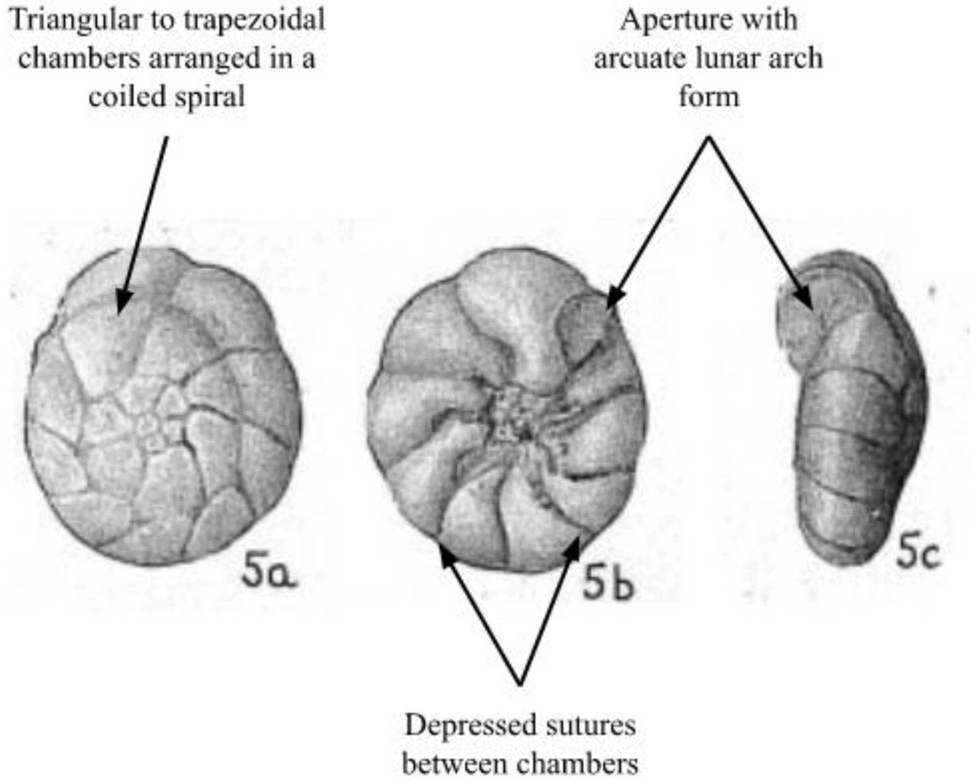


Figure 12: Identifying features of *A. beccarii* tests (from Linnaeus, 1758)

Tests of *A. beccarii* are trochoidal such that chambers are added in an upward coiling spiral stemming from the initial chamber, or proloculus, in 2.5-3 whorls (Figure 13) (Chang et al. 1974). Typically, the second and third whorl maintain a near-constant width rather than a logarithmic spiral common in most other shelled organisms. The equation for the spiral of a typical specimen is linear (*Equation 3*) (Chang et al., 1974). Generally, there are at least 5 and up to 8 chambers in the first whorl, about 10 in the second, and up to 13 in the final whorl, given that the organism has time to grow that large (Chang et al., 1974).

$$r = a\Theta + b$$

Equation 3: where r is the radius from the proloculus, in mm; a is the expansional rate of the spire, in mm per radian; Θ is the angle of rotation, in radians; and b is the radius of the proloculus, in mm.

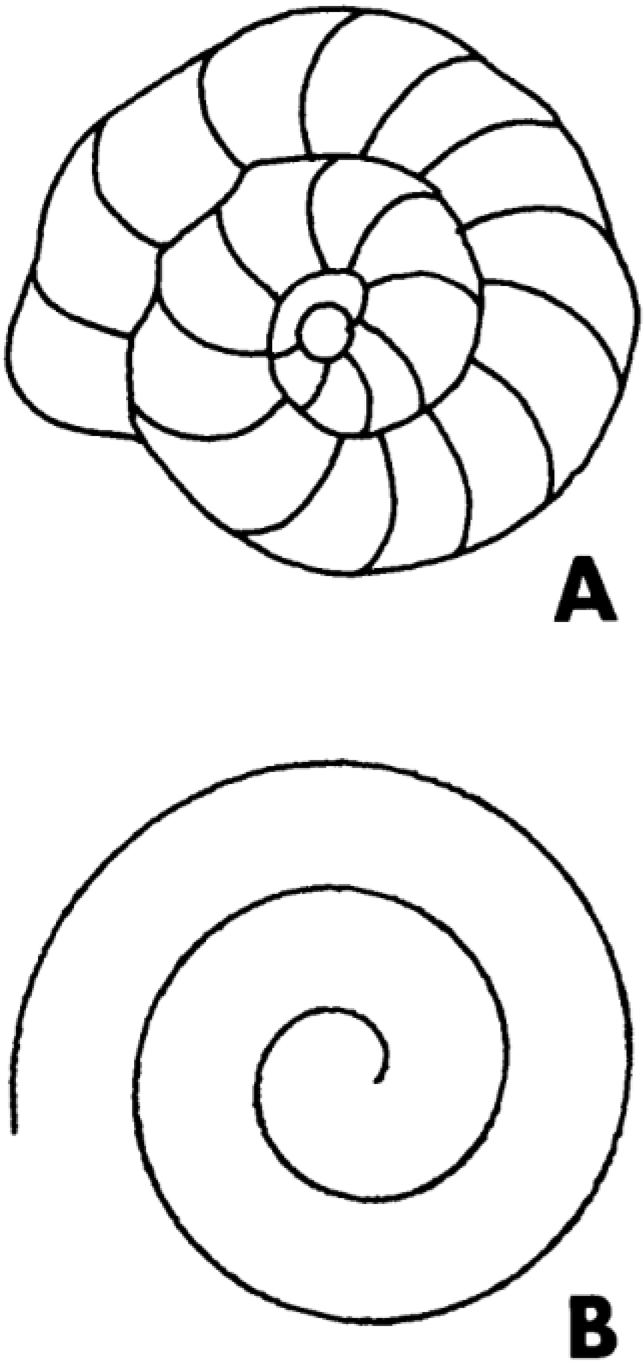


Figure 13: Typical morphology of *A. beccarii* tests (from Cushman, 1928)

Ammonia beccarii specimens present in Budd Inlet exhibit high rates of abnormal test growth. Like all foraminifera, *A. beccarii* are highly sensitive to their ambient environmental conditions. Budd Inlet *A. beccarii* can be divided into five categories:

those with typical test structures (Plate 1), those with enlarged ultimate chambers (Plate 2 and 12), those with irregular, isolated growths (Plates 3, 7, and 8), those with overall warped tests (Plate 4), and those with irregularly sized chambers (Plates 5 and 9-14). East Bay had fewer overall abnormalities present in its *A. beccarii* population compared to Priest Point. For East Bay, 3.3% of all *A. beccarii* were abnormal in some way, with the most common category being irregularly sized chambers (1.2%). For Priest Point, 15.4% of *A. beccarii* were abnormally shaped, with overall test warping being the most common manifestation.

For all specimens collected, the first whorl follows the normal trend with abnormalities appearing in the second or third spiral. This suggests that these tests would have appeared normal in the foraminifera's juvenile form, with abnormalities or mutations occurring later in life. This may be due to structural issues in the growth process in which the smaller chambers are supported but as the foraminifera grows and the chambers are larger, the test fails, resulting in abnormal growth. Priest Point has a lower C/N ratio than East Bay and a greater proportion of abnormal to normal tests, suggesting that lack of available nutrients may negatively affect test growth. Lack of available carbon may result in thinner or weaker chamber walls leading to abnormal growth.

From the MicroCT images, it was evident that for most of the specimens scanned, the final or ultimate chambers were noticeably thinner than the preceding chambers (e.g., Plates 9-14). These specimens had the abnormality types of irregularly sized chambers and enlarged ultimate chambers. For the specimen with growths, the chamber walls maintained similar thickness throughout the test (Plates 7-8). Future analysis could investigate a potential correlation between with abnormality type and chamber volumetrics and wall thicknesses. These types of analysis are possible within the software used, Dragonfly, but fell outside the scope of this project.

Additionally, investigation between the presence of abnormalities and oxygen levels in the water would provide insight into the preliminary hypothesis that the *A. beccarii* which may be categorized as having enlarged ultimate chambers are more common in oxygen-depleted environments. This hypothesis is formed on the basis that the abnormality type of enlarged ultimate chambers increases the surface area of *A. beccarii* specimens and thus the number of pores beyond what would be typical. Varieties of foraminifera with larger pores are associated with oxygen-depleted environments (Sen Gupta & Machain-Castillo, 1993).

IMAGING

The images of the foraminifera captured using SEM and MicroCT technology made it possible to preliminarily assess test abnormalities in *A. beccarii* individuals. SEM imaging provided higher magnification than would be possible from a stereomicroscope whereas MicroCT allowed for the investigation of internal structures. The observations made herein are only preliminary and future work examining patterns in occurrence and formation may yield additional insights into how or why these mutations may occur.

POTENTIAL FUTURE PROJECTS

This project could benefit from the investigation of more sites within Budd Inlet, particularly West Bay and Burfoot Park to increase the dataset of picked foraminifera, further the accuracy of kriging analysis and predictive mapping, and better understand the populations of foraminifera is different parts of the inlet. Those two sites in particular are publicly accessible and geographically distinct from East Bay and Priest Point, making them excellent candidates for future study. Additional research may be conducted on test abnormalities, including further MicroCT imaging to determine the timing and mechanisms involved in mutations, coupled with trace elemental testing of heavy metal within foraminiferal calcite, and genetic testing of *Ammonia beccarii* to discern any potential morphotypes.

CONCLUSION

A comparison of twenty-three split samples suggests a <10% consistency between traditional and STP foraminiferal processing suggests that SPT is an efficient and time saving methodology for sample processing but is most effective with samples smaller than 500µm and least effective on samples containing buoyant wood or other large materials. Benthic foraminifera are abundant in Budd Inlet and consist mostly of *Ammonia beccarii*. East Bay has a far larger foraminifera population than Priest Point. At both sites, population evenness is lower than expected. The sites have a positive correlation between overall foraminifera abundance and C/N ratios. Priest Point has a larger proportion of abnormal *A. beccarii* tests than East Bay. There were four major groups of test abnormalities: growths, enlarged ultimate chambers, irregularly shaped chambers, and overall test warping. Using MicroCT, it was evident that test abnormalities began in the second or, more commonly, the third whorl of the test, suggesting that juvenile specimens appeared to be normal before diverging.

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APPENDICES

Appendix A: Supplemental Table 1

Budd Inlet, Puget Sound

East Bay 1

Sample Name	EB 1A Float 125	EB1A Reserve 125	EB1C Float 125	EB1C Reserve 125
	per 50cc	per 50cc	per 50cc	per 50cc
Original Sediment (g)	1.21	3.07	2.89	0.75
Sample Split	0.5	0.5	0.5	0.5
Species	4	2	1	1
A. beccarii	10495.87	2605.86	103.81	666.67
Bolivinita sp.	82.64	0.00	0.00	0.00
Elphidium sp.	165.29	32.57	0.00	0.00
M. fusca	165.29	0.00	0.00	0.00
Total Foraminifera	10909.09	2638.44	103.81	666.67

Total Site Foraminifera	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
14318.00	0.01	7.08	0.00

Budd Inlet, Puget Sound

East Bay 4

Sample Name	EB4C Float 63	EB4C Reserve 63	EB4C Float 125	EB4C Reserve 125
	per 50cc	per 50cc	per 50cc	per 50cc
Original Sediment (g)	1.8	1.78	1.52	1.59
Sample Split	0.5	0.5	0.5	0.5
Species	2	1	3	1
A. beccarii	444.44	56.18	3684.21	1761.01
Bolivinita sp.	0.00	0.00	0.00	0.00
Elphidium sp.	0.00	0.00	65.79	0.00
M. fusca	55.56	0.00	263.16	0.00
Total Foraminifera	500.00	56.18	4013.16	1761.01

Total Site Foraminifera	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
6330.34	0.03	6.58	0.00

Budd Inlet, Puget Sound

East Bay 6

Sample Name	EB6A Float 63	EB6A Reserve 63	EB6A Float 125	EB6A Reserve 125	EB6B Float 63	EB6B Reserve 63	EB6B Float 125	EB6B Reserve 125
	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc
Original Sediment (g)	2.92	2.28	2.13	2.67	2.09	2.56	1.32	1.42
Sample Split	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Species	1	1	2	1	1	1	1	1
A. beccarii	102.74	175.44	7558.69	2808.99	47.85	156.25	984.85	4788.73
Bolivinita sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Elphidium sp.	0.00	0.00	93.90	0.00	0.00	0.00	0.00	70.42
M. fusca	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Foraminifera	102.74	175.44	7652.58	2808.99	47.85	156.25	984.85	4859.15

Sample Name	EB6C Float 63	EB6C Reserve 63	EB6C Float 125	EB6C Reserve 125
	per 50cc	per 50cc	per 50cc	per 50cc
Original Sediment (g)	3.27	2.28	2.14	2.54
Sample Split	0.5	0.5	0.5	0.5
Species	2	2	2	2
A. beccarii	428.13	394.74	11962.62	9724.41
Bolivinita sp.	0.00	0.00	0.00	0.00
Elphidium sp.	0.00	43.86	46.73	0.00
M. fusca	30.58	0.00	0.00	39.37
Total Foraminifera	458.72	438.60	12009.35	9763.78

Total Site Foraminifera	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
39458.29	0.01	7.01	0.00

Budd Inlet, Puget Sound

Priest Point 15

Sample Name	PP15A Float 63	PP15A Reserve 63	PP15A Float 125	PP15A Reserve 125	PP15A Float 500	PP15B Float 125	PP15B Reserve 125	PP15C Float 125
	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc	per 50cc
Original Sediment (g)	3.22	3.17	18.05	23.3	6.8	27.85	40.82	8.93
Sample Split	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Species	1	1	1	2	1	1	1	1
A. beccarii	124.22	63.09	138.50	377.68	44.12	104.13	161.69	279.96
Bolivinita sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Elphidium sp.	0.00	0.00	0.00	4.29	0.00	0.00	0.00	0.00
M. fusca	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Foraminifera	124.22	63.09	138.50	381.97	44.12	104.13	161.69	279.96

Sample Name	PP15C Reserve 125	PP15C Reserve 500
	per 50cc	per 50cc
Original Sediment (g)	9.03	1.9
Sample Split	0.5	0.5
Species	2	1
A. beccarii	509.41	52.63
Bolivinita sp.	0.00	0.00
Elphidium sp.	11.07	0.00
M. fusca	0.00	0.00
Total Foraminifera	520.49	52.63

Total Site Foraminifera	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
1870.80	0.00	4.92	0.00

Budd Inlet, Puget Sound
Priest Point 17

Sample Name	PP17A Float 63 per 50cc	PP17A Reserve 63 per 50cc	PP17A Float 125 per 50cc	PP17A Reserve 125 per 50cc	PP17A Float 500 per 50cc	PP17B Float 63 per 50cc	PP17B Reserve 63 per 50cc	PP17B Float 125 per 50cc	PP17B Reserve 125 per 50cc	PP17C Float 63 per 50cc	PP17C Reserve 63 per 50cc	PP17C Float 125 per 50cc	PP17C Reserve 125 per 50cc	***Total Site Foraminifera***	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
Original Sediment (g)	6.56	6.01	18.16	26.77	1.27	6.33	5.97	17.84	24.24	6.21	6.48	23.79	22.35				
Sample Split	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5				
Species	1	1	1	2	1	1	1	1	1	1	1	2	1				
A. beccarii	30.49	166.39	556.17	354.87	78.74	15.80	16.75	229.82	255.78	32.21	30.86	210.17	675.62				
Bolivinita sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Elphidium sp.	0.00	0.00	0.00	3.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.20	0.00				
M. fusca	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Total Foraminifera	30.49	166.39	556.17	358.61	78.74	15.80	16.75	229.82	255.78	32.21	30.86	214.38	675.62	2661.60	0.00	4.32	0.00

Budd Inlet, Puget Sound
Priest Point 19

Sample Name	PP19A Float 125 per 50cc	PP19A Reserve 125 per 50cc	PP19A Float 500 per 50cc	PP19B Float 125 per 50cc	PP19B Reserve 125 per 50cc	PP19C Float 125 per 50cc	PP19C Reserve 125 per 50cc	***Total Site Foraminifera***	***Site Evenness***	***Site Hmax***
Original Sediment (g)	25.3	31.61	3.97	30.84	17.27	24.7	15.2			
Sample Split	0.5	0.5	0.5	0.5	0.5	0.5	0.5			
Species	1	1	1	1	1	1	1			
A. beccarii	118.58	104.40	25.19	19.46	110.02	56.68	197.37			
Bolivinita sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Elphidium sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
M. fusca	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Total Foraminifera	118.58	104.40	25.19	19.46	110.02	56.68	197.37	631.68	0.00	4.23

Budd Inlet, Puget Sound
Priest Point 21

Sample Name	PP21A Float 125 per 50cc	PP21B Reserve 125 per 50cc	PP21C Float 125 per 50cc	PP21C Reserve 125 per 50cc	***Total Site Foraminifera***	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
Original Sediment (g)	23.92	36.82	31.62	27.19				
Sample Split	0.5	0.5	0.5	0.5				
Species								
A. beccarii	4.18	29.88	9.49	11.03				
Bolivinita sp.	0.00	0.00	0.00	0.00				
Elphidium sp.	0.00	0.00	0.00	0.00				
M. fusca	0.00	0.00	0.00	0.00				
Total Foraminifera	4.18	29.88	9.49	11.03	54.58	0.00	2.37	0.00

Budd Inlet, Puget Sound
Priest Point 23

Sample Name	PP23B Reserve 125 per 50cc	PP23B Reserve 500 per 50cc	***Total Site Foraminifera***	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
Original Sediment (g)	18.74	0.31				
Sample Split	0.5	0.5				
Species						
A. beccarii	16.01	322.58				
Bolivinita sp.	0.00	0.00				
Elphidium sp.	0.00	0.00				
M. fusca	0.00	0.00				
Total Foraminifera	16.01	322.58	338.59	0.00	4.27	0.00

Budd Inlet, Puget Sound
Priest Point 25

Sample Name	PP25B Reserve 125 per 50cc	***Total Site Foraminifera***	***Site Evenness***	***Site Hmax***	***Site Diversity Index***
Original Sediment (g)	15.34				
Sample Split	0.5				
Species					
A. beccarii	13.04				
Bolivinita sp.	0.00				
Elphidium sp.	0.00				
M. fusca	0.00				
Total Foraminifera	13.04	13.038	0.00	2.57	0.00

VITA

Erin C. Roark

Candidate for the Degree of

Master of Science

Thesis: THE USE OF BENTHIC FORAMINIFERA AS ENVIRONMENTAL INDICATORS IN BUDD INLET, PUGET SOUND

Major Field: Geology

Biographical:

Education:

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