Paleoclimates of Mi’kma’ki: A Sclerochronological Analysis of *Crassostrea virginica* from an Archaeological Context in Malpeque Bay, Prince Edward Island

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COVID STATEMENT

The onset of COVID-19 and the resulting global pandemic has significantly impacted my Master of Arts thesis in the Department of Archaeology at Memorial University of Newfoundland and Labrador. Commencing my program in September 2021, I encountered several delays and challenges due to the ongoing pandemic.

The persisting COVID-19 virus impeded collaborations and interactions with other researchers and specialists. Travel restrictions, social distancing protocols, and limited access to research facilities often hindered effective communication. COVID delayed the exchange of ideas and data, including the potential to visit the community and archaeological sites on Hog Island, PEI. These collaborations were crucial to ensuring a comprehensive and interdisciplinary approach to the research, and the impact of these delays cannot be underestimated.

Sample sizes have been lowered for analyses that required submission for testing via mail. As a result, travel restrictions and operations management delayed the processing of samples for stable isotope analysis.

Despite these challenges, the Memorial Archaeological Sciences Laboratory team remained committed to overcoming the obstacles posed by the pandemic and the advancement of research. We have adapted the research methods and timeline accordingly while prioritising the health and safety of ourselves and others.

ABSTRACT

Pituamkek (CdCw-5), situated on Hog Island, in the province of Prince Edward Island, lies within the territory of the Mi'kmaq Nation. This study focuses on traditional oyster harvesting of the Mi'kmaq from 643-184 cal. BP. Specifically, we investigated the seasonal timing of shellfish collection and paleo-temperature reconstruction at the archaeological site. Ten archaeological eastern oyster shells (*Crassostrea virginica*) along with with two live collected valves (September 2019) from the surrounding Malpeque Bay underwent high-resolution shell oxygen isotope ($\delta^{18}O$) analysis and 180 individual archaeological shell fragments were analysed for growth stage determination. The $\delta^{18}O$ results showed a pattern of year-round collection with an emphasis on warm weather collection. Results from shell growth increment analysis suggest the oysters at this region show high levels of stress in response to the conditions of the ambient environment and low sea surface temperatures. Archaeological shells show a larger $\delta^{18}O$shell range, possibly reflecting a greater sea surface temperature amplitude than modern shells (i.e. a difference of ~10.9°C; modern range: ~ -1.7°C to 17.5°C; archaeological range: ~0°C to 10.8°C). Results are contextualised with previous studies of paleoenvironmental analysis using oysters from the Eastern Seaboard in the USA, while interpreting these new data in the context of Mi'kmaq occupation and landscape use.

ACKNOWLEDGMENTS

In the spirit of Reconciliation, I acknowledge that the land upon which our organisation stands is unceded Mi’kmaq territory. The historic Treaties of Peace and Friendship cover Epekwitk (PEI), Mi’kma’ki. I pay my respects to the Indigenous Mi’kmaq People who have occupied this Island for time immemorial.

Wela'lin - thank you to the countless individuals, community members, and researchers whose prior work and contributions have laid the foundation for this thesis. In particular, I would like to thank Dr. Helen Kristmanson, L’nuey, and the Epekwitk Assembly of Councils.

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CHAPTER 1

INTRODUCTION

1.1 OBJECTIVES

What can records preserved in oysters from archaeological contexts tell us about people and past environments? Biochemical and sclerochronological analysis of oysters can provide insights into past cultural and environmental changes, specifically salinity, temperature and season of harvest collection along the Atlantic Canadian coast of Prince Edward Island. This research explores the traditional harvest of oysters from Malpeque Bay through analysis of their remaining shells at an archaeological site. To reach the goals of this thesis, there are three separate yet related research objectives:

1) Interpret the season of oyster harvest and, by proxy, the seasons of site occupation and conditons of shellfish harvest
2) Use stable oxygen isotope ($\delta^{18}O$) data and radiocarbon (C^{14}) dating to reconstruct past sea surface temperatures
3) Combine these lines of evidence to interpret the trends in shellfishing over time in the traditional Mi'kmaq subsistence economy for PEI

1.2 RESEARCH PARTNERSHIP & COLLABORATION

In collaboration with the Mi'kmaq Confederacy of PEI, Indigenous Services PEI has provided archaeological and live-collected valves of *C. virginica* from Malpeque Bay. Since this work involves Indigenous materials, this research also complies with the

Indigenous Research Policy at Memorial University of Newfoundland (Office of the Vice-President [Research] 2020).

1.3 CULTURAL, ARCHAEOLOGICAL AND ENVIRONMENTAL CONTEXT

1.3.1 The Mi’kmaq of Prince Edward Island

The Mi'kmaq First Nation lived on Prince Edward Island for over 10,000 years before the French and British established their colonial settlements there (MacDonald *et al*. 2016). Both settlement and resource extraction have been heavily utilized on the island (MacDonald *et al*. 2016). Shellfish have been harvested in Mi’kma’ki year-round for nearly 9500 years (Betts et al. 2017; Denny *et al.* 2016). Oysters (Mn’tmu’k, in the Mi’kmaw language), although not a primary source of subsistence for the Mi'kmaq but a constant one (Denny *et al.* 2016). The faunal remains at the the midden site on Pituamkek show that despite shellfish harvesting, the primary subsistence resources were likely to include seals, fish, and walrus (Kristmanson 2019). The shell midden concentrations indicated that oyster was the primary focus of shellfish harvest at the Pitaumkek site (Kristmanson 2019). The morphology of the oysters from the area suggests that they were collected beyond the intertidal region but not from the deep channel (Kristmanson 2019).

Figure 1.1 Aerial Photograph of Hog Island Sand Dunes, PEI. Also Known as Georges's Island. Surrounding body of water is Malpeque Bay and the Gulf of St. Lawrence. (Retrieved from L'nuey and Epekwitk Assembly of Councils)

1.3.2 Archaeological Site Background

Pitaumkek, "At the Long Sand Dune" known as Hog Island and the Sandhills in English (CdCw-5) holds a 2000-year-old shell midden site located in the North-East of Malpeque Bay (Fig. 1.1). The traditional name for this bay is "Makpaak" to the Mi'kmaq. Numerous shell middens comprising faunal detritus, shells, seeds, and scattered artifacts comprise the material culture of Hog Island (Kristmanson 2019). The Provincial Government and Mi'kmaq Confederacy oversee the Pitaumkek site (Formerly Pitawelkek) on the Island's southeast coast (Fig. 1.2).

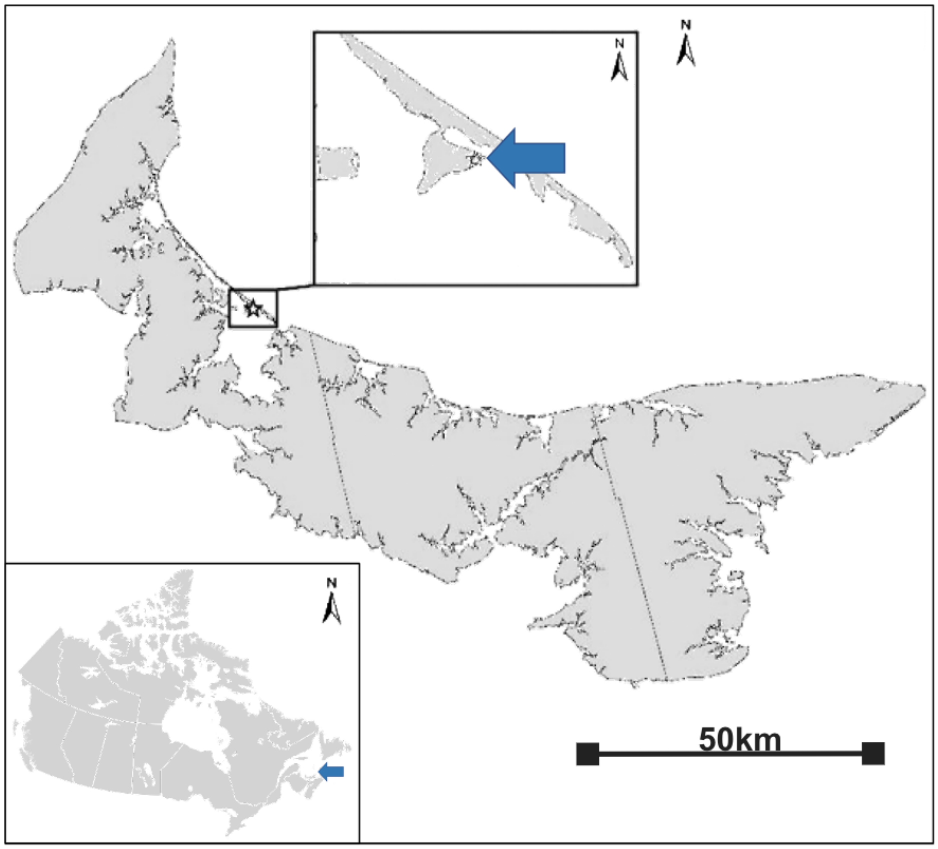


Figure 1.2. Map of PEI and Pituamkek site location on Hog Island and Gulf of St. Lawrence

The shell midden site at Pitaumkek is one of the last remaining in the area, as both the Mi’kmaq and settler populations have used them as a source of calcite for agricultural limestone (Kristmanson 2019). The midden has been subjected to erosion, but the archaeological investigation revealed that it is still partially intact. Over the millennia, the Pitaumkek site has been occupied at various times by the Mi’kmaq. The Pitaumkek site was dated using materials from the site's deposits and accelerator mass spectrometry. These samples produced dates ranging from 700 to 800 AD into the current era. Diagnostic artifacts also pushed the occupation date back to at least 2000 years ago

(Kristmanson 2019). The site was occupied as a part of Epekwitk - the Indigenous title of the Island, which is now under federal designation and pending national parks status. The excavation of the eroding shell midden site (CdCw-5) was part of a broader Mi'kmaq-led initiative to conserve the ecological and cultural resources of the Island beginning in 2006 (Fig. 1.3).

Figure 1.3. Pituamkek (CdCw-5) midden excavations, part of the Malpeque Bay Archaeological Project (Retrieved from H. Kristmanson Archival Images)

In recent years the surrounding environment has begun showing signs of water contamination, shoreline development, and erosion, displacing soil into the surrounding Gulf of St. Lawrence and its estuaries (MacDonald *et al.* 2016). These impacts have put the current oyster population and the historical collection sites at risk. The Island is a cultural artifact, according to historian Alan MacEachern, where "nature is history, masquerading as space" (MacDonald *et al.* 2016: 3). 'Msit No'kmaq' is the Mi'kmaq belief in a similar sentiment, according to traditional knowledge. Msit No'kmaq, which translates to "all our relations," is a term used by the Mi’kmaq to express how everything is interconnected, including relationships between people and the environment (Bartlett et al. 2012). In Northern Malpeque Bay, Hog Island has demonstrated the interconnectedness of the Mi'kmaq, the oysters, and the landscape in its material contexts.

1.3.3 Environmental Background

The average temperature range of contemporary Malpeque Bay, Prince Edward Island, varies throughout the year. During the summer, the average water temperature ranges from 16- 20ºC; in the winter, the average water temperature drops to around 0ºC (Environment and Climate Change Canada 2021). The air temperature also varies throughout the year, with average temperatures ranging from around -10ºC in the winter to around 20ºC in the summer (Environment and Climate Change Canada 2021). The average annual rainfall for Prince Edward Island is around 200 mm. The eastern part of the island tends to be wetter than the western part, and rainfall is generally highest in the fall and winter months.

The Little Ice Age (LIA) was a period of cooling that occurred roughly between the 14th and 19th centuries. It is generally accepted that the region of Prince Edward Island experienced cooler temperatures during this time, with a decrease in precipitation. Boucher, Arseneault, & Sirois (2013) used tree-ring data to reconstruct past climate patterns in eastern Canada, including Prince Edward Island and found that summer precipitation in the region has varied significantly over the last 600 years. From the 1600s to the early 1800s, the period was characterised by a relatively dry climate with lower-than-average summer precipitation (Boucher, Arseneault, & Sirois 2013). The dryer climate led to colder conditions in PEI, resulting in shorter growing seasons and potentially affecting agriculture and the local ecosystem. In contrast, this weather pattern changed during the period from the mid-1800s to the early 1900s, and the island's climate was wetter than average, with higher-than-average summer precipitation (Boucher et al. 2013). This in turn, shifted sea surface salinity resulting from an influx of freshwater.

1.4 THEORETICAL & METHODOLOGICAL APPROACH

The research presented is Interdisciplinary, using archaeological theory and scientific methods from the fields of sclerochronology, environmental science and geochemistry by means of growthline analysis and isotope analysis. Results are interpreted in the context of an archaeological analysis centred around the local network of the shells, the people, and their history on the land.

The human-environmental connections of PEI's landscape form the basis of the research, and the conceptual framework builds on creating connections in the ecological network (Balée 2018). In response to the nature of the material being studied, an interdisciplinary approach to paleoclimate reconstruction must be adopted, drawing on sclerochronological techniques from biology and environmental science to widen the breadth of knowledge concerning oysters amongst limited use in archaeology. The theoretical framework of this research highlights the ongoing networks of the people, the oysters, and the coastal landscape at Pitaumkek. The research explores archaeological applications of historical ecology, conservation/resource management, and multispecies agency (Crumley 1994). These themes have been supplemented by Mi’kmaq traditional knowledge and worldview in recognition of agency, self-governance and traditional conservation practices.

Approaching Pitaumkek from the lens of historical ecology permitted an overview of human-environmental interactions of the land over time and space (Balee 2018). By looking at this network, researchers can see the action that all members of the ecological system take in response to the changing conditions of the area. While utilizing the increasingly popular perspective of historical ecology in academia, it is imperative to highlight the existing theories of the Mi'kmaq (Crumley 1994). Indigenous academics have referred to this combined approach as 'Two-eyed seeing.' When applying 'two-eyed seeing,' Mi’kmaq academics and community members are combining the knowledge-created spheres of science and traditional knowledge (Bartlett *et al.* 2012). The approach used by members of the Mi’kmaq community allows for both exchange and an approach informed by community knowledge.

The second theoretical perspective implemented in this research is the conservation and resource management themes. While highlighting the increasingly changing conditions of the coastal landscape in response to harvest practice, conservation themes must be addressed. The pollution and harvest intensity of the industrial fishing industry has resulted in significant environmental stress for the waterways of PEI and its inhabitants, including the traditional harvest of the Mi’kmaq (Denny *et al.* 2016).

Finally, themes of the multispecies agency have been applied when looking at the archaeology of Pitaumkek. When looking at the landscape of Mi’kma’ki, it must be acknowledged that the Mi’kmaw have been shaping the environment prior to colonization for over 10,000 years (Denny *et al.* 2016). Mi'kmaw traditional knowledge has long viewed the ecology of their landscape from the worldview of 'Msit No'kmaq.' Msit No'kmaq means 'all our relationships' it refers to the interdependence of all things, including human-environmental relationships (Bartlett *et al.* 2012). The worldview of 'Msit No'kmaq' has allowed the Mi'kmaq to recognize the network of interactions in their harvesting for generations. The Mi'kmaq have long since recognized that the oysters were also actors with agency co-inhabiting this land (Bartlett *et al.* 2012; Denny *et al.* 2016). The shells are in a network of interaction with the people and the landscape. The oysters hold agency as their growth, dependent on the surroundings, is actively responding to the change of the landscape. The resulting applied perspective viewing the multiplicity of the agency being held highlights the response to the changing climate of Malpeque Bay.

CHAPTER 2

OYSTERS IN ARCHAEOLOGY, FISHERIES SCIENCES AND PALEOENVIRONMENTS PALEOENVIRONMENTAL ANALYSES

2.1 OYSTER AQUACULTURE & RESOURCE MANAGEMENT IN PEI

The PEI aquaculture industry harvests approximately 6.5 million pounds of oysters at $10.3M annually. Oysters are abundant in the Canadian Maritime Provinces, and Prince Edward Island (PEI) and Nova Scotia have launched multiple aquaculture operations, signalling their growing economic significance. As the topic of aquaculture brings to light conversations about harvest mitigation, it is vital to acknowledge the constitutionally entrenched rights of the Mi'kmaq and their pre-colonial structures of conservation (Soniat *et al.* 2014). The Mi'kmaq have long-standing strategic harvesting to protect resources: Netukulimk, take only what you need (Barsh 2002; Denny *et al.* 2016). "Netukulimk is the process of supplying oneself or making a livelihood from the land, and netukulimkewel refers to applicable rules or standards, (Barsh 2002: 16)." Netukulimk represents the existing structures of conservation in the Atlantic fishing grounds prior to colonial displacement, licensing laws, and interference by groups such as the Department of Fisheries and Wildlife and The Department of Fisheries and Oceans. In addition, ceremonial and moderate livelihood fisheries are protected by the constitution of Canada. The rights-based fishery is a Treaty right of the Mi'kmaq affirmed by the 1999 Marshall case (R. v. Marshall, [1999] 3 S.C.R. 456).

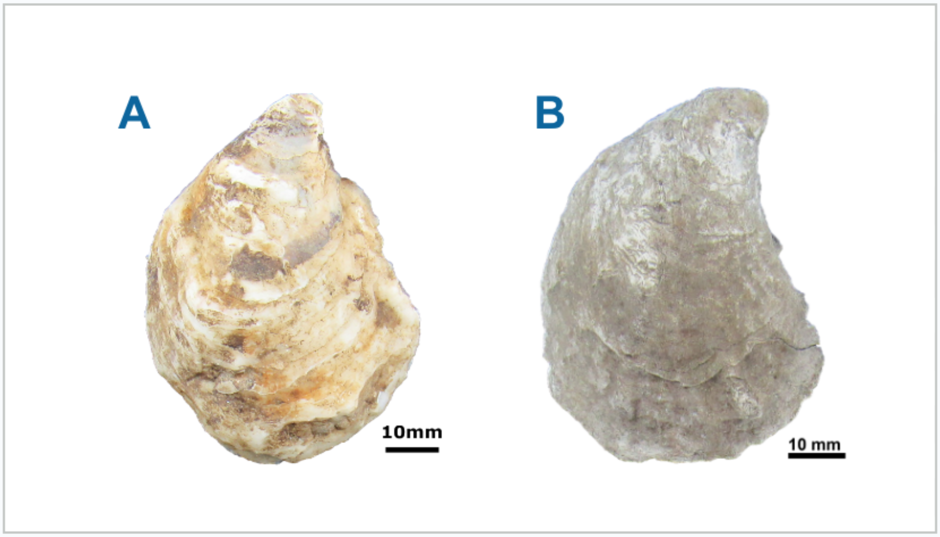


Figure 2.1. *Crassostrea virginica* A) live collected B) archaeological

Crassostrea virginica (GMELIN 1791) has been an essential part of the diet and culture of the Mi'kmaq people for thousands of years (Fig. 2.1). Oysters were often eaten raw, cooked, and utilized in various traditional activities. The Mi'kmaq have a long history of harvesting and cultivating oysters and have developed many traditional methods for doing this. One method involves constructing rock walls or other structures in shallow water to create an area for oysters to attach and grow (Johnson & Stacey 2000). Another method involves placing recycled oyster shells or other materials into the water to provide a substrate for valves to attach onto (Johnson & Stacey 2000). The Mi'kmaq also used a technique called "oyster treading," which involved walking on oyster beds at low tide to break up clumps of oysters and encourage uniform growth for harvesting (Johnson & Stacey 2000). The Mi'kmaq have used these traditions for thousands of years and are still using some today in oyster aquaculture and conservation efforts.

Colonialism and the resulting overfishing that followed in the Atlantic Canadian region is noted as the primary cause of a decline in the stock of oysters (Fisheries and Oceans Canada 2003). In the 1830s, PEI implemented laws restricting colony residents' access to the fisheries and outlawed the burning of live oysters for lime (Fisheries and Oceans Canada 2003). Further evidence shows that settler oyster cultivation began in Prince Edward Island as early as 1865 when rules were established for leasing specific sites appropriate for oyster production by auction (Fisheries and Oceans Canada 2003). The ‘Malpeque disease,’ which initially surfaced in Malpeque Bay (PEI) in the early 1900s—possibly as a result of transfers of healthy seed from New England to replace overfished stocks—is the most prominent disease for oysters living in the Gulf region (Fisheries and Oceans Canada 2003). At its peak, this illness decimated oyster populations in PEI before moving on to NB and NS. Today, almost all of the oysters in the southern Gulf of St. Lawrence are descendants of local shellfish stocks that escaped the ailment and passed their resistance to their descendants (Fisheries and Oceans Canada 2003).

Concerning the modern harvest of the wild oyster population from the Gulf Region, 52% of commercial oyster licenses are issued in PEI. Commercial and communal licenses are provided for both the fall and spring seasons. Some communal commercial oyster licenses are also issued to First Nations and Councils (76% in PEI, 15% in Eastern NB, and 9% in Gulf NS) (Fisheries and Oceans Canada 2003). Additionally, there are many privately held oyster leases within the Gulf of St. Lawrence region, with 616 leaseholders in PEI (Fisheries and Oceans Canada 2003). Oyster exports have increased over the past few years, mainly to the United States (U.S.A.). British Columbia, which exports primarily Pacific oysters, and Prince Edward Island are the two top exporters from Canada.

2.2 SHELL MIDDENS, OYSTERS & ARCHAEOLOGY

Shell middens are refuse deposits mainly consisting of shells, such as bivalves and gastropods commonly found in coastal, estuarine and river sites (Claassen 1998; Twaddle *et al.* 2016). Residents of shell midden sites were compiling together biological materials from their immediate coastal landscapes (Andrus 2011). Middens may be found on every continent (except Antarctica) and are often found adjacent to most coastal settlements (Andrus 2011; Claassen 1998). Most of the Pleistocene and Holocene periods saw persistent formation of midden deposits (Andrus 2011). Archaeologists are particularly interested in the shell midden matrix because it contains the most extensive representation of shells produced by human subsistence in the historical record (Claassen 1998). Shell middens are an essential data source because many quaternary environmental proxies are geographically confined and frequently lack time-series data sensitive to seasonal change (Andrus 2011: 2893). When used in conjunction with relative dating, the calcium carbonate found in shell middens frequently offers well-preserved organic material for radiocarbon dating.

Bivalves in archaeological shell middens, as a result of past subsistence and harvesting practices, can act as a proxy to help retrace environmental trends and decipher past shellfish harvesting patterns. An interdisciplinary approach influenced by environmental science, sclerochronology yields high-resolution data regarding the incremental growth patterns produced in the bivalve calcium-carbonate shell structures (Allen 2017; Claassen 1998; Kirby 1998). The most common application of archaeological studies of shell material is to determine the season of capture or collecting. To recreate themes of human-environmental interactions, estimates of the time the mollusks were caught are used to determine historical practices such as site occupation patterns, subsistence strategy, foraging habitats, site development processes, or ceremonial behavior (Andrus 2011). In many Bivalve species, growth line colour examination of the seasonal banding is indicative of seasonal temperature changes and can in turn, be used to determine the season of capture (Custer and Doms, 1990).

2.2.1 Oysters, diets, people & time

Humans have been eating oysters for thousands of years as a food source. The Eastern oyster (*C. virginica*) and Pacific oyster (*C. gigas*) are the two oyster species that are most frequently fished for their meat. Oysters are rich in protein and a relatively accessible marine resource to obtain. *C. virginica* is one of the bivalves most frequently found in archaeological middens along the Eastern American coast (Claassen 1998). Since the Pleistocene, the eastern seaboard's vast temporal and spatial range has led to a reliance on bivalve species for food into the current era (Kirby et al. 1998; Zimmt *et al.* 2019).

Figure 2.2. Turner Farm midden wall in Maine, USA (Retrieved from Holland Haverkamp, University of Maine)

Historic oyster harvesting sites such as Turner Farm and the Chesapeake Bay represent some of the most extensive shell matrices in North America, and the research coming from this area has solidified the capacity for the feasibility of studying the species in an archaeological context, with sclerochronological applications (Harding *et al.* 2008; Jansen 2018; Speiss *et al.* 2004). One of the oldest recorded shell midden sites on the Atlantic coast is the Turner Farm archaeological site in Maine, dating back to over 5000 ya (Fig. 2.2). As one of the longest extensive records of habitation in the Gulf of Maine, this five-thousand-year period offers a unique opportunity (Speiss 2004). According to archaeological evidence, Indigenous groups actively engaged in oyster harvesting during the Woodland period (Jansen 2018; Krismantson 2019). These middens, representing enormous numbers of harvested oysters, can extend meters beneath the surface in places like the Chesapeake Bay (Jansen 2018). Additionally, the middens at this site were utilized by various cultural groups, including Indigenous populations, settlers, and African American groups (Jansen 2018). The bay has supported both traditional and industrial harvests of oysters, providing long-term insights into the effects of commercial fishing (Jansen 2018). Harding (2008) utilized *C. virginica* from the Chesapeake Bay area collected four centuries apart from each other for archaeological analysis. Harding determined that the area of Chesapeake Bay has seen environmental declines because of colonial anthropogenic effects since the 17th century (2008). Habitat destruction, modification, and fishing pressures are cited as critical anthropogenic impacts affecting the modern oyster populations (Harding et al. 2008). This region represents the area where oysters have been extensively analyzed in archaeological contexts.

The Big Oyster: History on the Half Shell, by Mark Kurlansky (2008) explores the cultural and environmental history of oysters in New York City through a combination of historical accounts and scientific insights. Kurlansky delves into the abundant presence of oysters in the waters around New York City during the 17th and 18th centuries as a staple in the subsistence of Indigenous and settler populations. Oyster beds are a vital resource attracting immigrants, thereby contributing to the city's economic growth. Oysters became an integral part of New York's urban culture with oyster carts and oyster saloons, catering to the diverse population and catering to people of all social classes. The popularity of oysters even gave birth to the famous "New York Oyster Riot" of 1848, a social and political event that reflected the city's deep connection with the mollusk (Kurlanky 2008).

However, Kurlanksy's book also highlights the decline of the oyster populations in the city due to pollution, over-harvesting, and environmental degradation. Kurlansky explores how the once-plentiful oyster beds became contaminated and ultimately disappeared. He discusses the efforts to revive the oyster population and restore the city's marine ecosystem, shedding light on the challenges faced by environmental scientists and organizations working to replenish the stocks. Oysters, once a plentiful and sustainable food source, have seen a shift from a critical species in the everyday diet of all social classes to a luxury appetizer found in niche seafood restaurants. The shift in perception can be attributed to various factors, including changes in availability, cultural shifts, and marketing strategies. Historically, oysters were abundant and easily accessible, making them a common food source for people of all social classes. However, over-harvesting, pollution, and habitat destruction have significantly depleted wild oyster populations worldwide. Population scarcity has increased oyster prices, making them less affordable for the masses (Kurlansky, 2006).

Additionally, as society and culinary tastes changed, oysters became associated with refined dining and fine cuisine. The emergence of a gourmet food culture contributed to the elevation of oysters' status. Oysters began to be considered delicacies reserved for special occasions and higher-end restaurants (Davidson, 1999). As a result, oysters became symbolic of opulence and indulgence, especially among the upper classes. Their association with a luxury lifestyle fostered a perception of exclusivity, and in turn, oysters became a status symbol, reinforcing their image as a luxury item (Belasco, 2008). The aquaculture industry, cultivation, and seafood distributors capitalized on the growing demand by branding oysters as premium products. Marketing campaigns highlighting oysters' quality, freshness, and unique flavours helped position them as a luxury food choice (Belasco, 2008).

2.3 OYSTERS IN ARCHAEOLOGY & PALEOENVIRONMENTAL STUDIES

2.3.1 Analytical Techniques for understanding growth, seasonality & SST

Oysters have been analyzed in archaeological and paleontological contexts in Eastern USA and from shell middens in Europe (Milner 2001). These studies revealed that oysters have incremental seasonal growth and that oysters are reliable recorders of isotopic data. Currently, areas along the Atlantic coast of the United States produce the highest volumes of literature regarding *C. virginica* in an archaeological context.

2.3.2 Oyster Growth Rates & Sclerochronology

Season of capture can be accessed using growth line colour analysis of the seasonal banding, an indicator of seasonal temperature fluctuations (Andrus 2011; Butler et al. 2017; Milner 2001). Counts of these same biannual bands can be used to determine age-at-death when counted with high-resolution microscopy. According to Andrus's

(2001) and Zimmt (2019)'s analyses of *C. virginica*, dark gray bands are deposited during the colder months of the year, while white banding occurs during the warmer months. The gray banding denotes periods of decreased growth during the colder months when environmental temperatures were below what the oyster could tolerate (Kirby et al. 1998; Zimmt et al. 2019). One year of the oyster's life is represented by a pair of gray and white growth lines in this alternative banding precipitation (Zimmt et al. 2019). The age of each specimen can be determined by counting the paired growth lines (Kent 1992). Bands that do not reach the asymptote of the cross-section should be recognized as disturbance bands and eliminated from the count when counting growth line markers in *C. virginica* (Zimmt et al. 2019). The conchiolin line, the midlines, and other variations are the three main types of disturbance banding found in oysters (Milner 2001). These disturbance lines are caused by an obstruction in the oyster's growth and can take on different appearances as a result of a variety of potential stresses (Milner 2001).

The growth of the oyster's shell is also influenced by its maturity stages since, like other bivalves, it decreases as it becomes older (Claassen 1998; Kent 1992). During the first six months of the oyster's life, there is rapid calcium carbonate precipitation (NOBRT 2007). According to Zimmt et al. (2019), the oyster's early rapid growth is a result of an evolutionary tactic designed to protect young oysters from predators. Three primary stages of growth are recognized in bivalves. When shell growth occurs at its fastest pace in juvenile shells, there are extremely few apparent growth cessations (growth lines) (Claassen 1998). Mature growth also exhibits rapid growth, but it is distinguished by regular, evenly spaced growth increments and lines that reach the ventral edge and beyond (Cannon and Burchell 2009: 1051). The closest and most measurable incremental growth bands are tightly packed near the shell's ventral margin during senile growth(Cannon and Burchell 2009: 1051). With oysters, this same rapid growth is seen in juvenile shells, with deposition slowing and becoming more compacted with age. To determine maturity, a model can be replicated by determining the average age oysters in this region reach a mature market length of 76 mm (3 inches) (Fisheries and Oceans Canada 2003).

In the Gulf of St. Lawrence, it takes 4-7 years for oysters to reach this range to be considered 'mature' (Fisheries and Oceans Canada 2003). Bivalves harvested as 'cocktail oysters' have slightly lower guidelines and are able to be harvested at a smaller size of around 50 mm, taking around three years to reach a harvestable size. Environments with greater variance between seasonal temperatures, including the comparably colder winters in Atlantic Canada, display greater variation in growth line banding due to their slowed growth rates (Twaddle et al. 2016; Zimmt et al. 2019). "Harvest size (76-90 mm) is reached in the Gulf of Mexico 18-24 months after setting, whereas oysters from Long Island Sound take 4-5 years to reach a similar size (NOBRT 2007:8)." In order to establish distinct seasonal growth line banding, it has been suggested that growth line analysis of *Crassostrea virginica* be carried out using oysters from greater latitudinal ranges of their distribution (Zimmt *et al.* 2019; Twaddle *et al.* 2016).

2.3.3 Stable Oxygen Isotopes

High-resolution stable isotope analysis ($\delta^{18}O$) applications can yield environmental data regarding paleoenvironments' temperature fluctuations, salinity, and seasonality (Andrus and Douglas 2000; Cannon 2017; Kirby et al. 1998). Andrus and Thompson 2012). Oxygen exists in two stable isotopes, $\delta^{16}O$ and $\delta^{18}O$, with varying abundances in the Earth's oceans and in the carbonate shells of marine organisms like oysters. When oysters incorporate oxygen from seawater into their shells during biomineralization, the ratio of $^{16}O/^{18}O$ in their shells reflects the isotopic composition of the water in which they lived (Epstein et al., 1951).

Sea surface temperatures (SST) during the period in which the organism lived can be reconstructed using stable oxygen isotope ($\delta^{18}O$) values observed in calcareous organisms (such as mollusks and coral) (Epstein et al. 1953; Gillikin et al. 2005; Hallmann et al. 2009). To reconstruct past environmental temperatures accurately, a paleothermometry equation must be applied to the $\delta^{18}O$ data derived from shells (Loftus et al., 2015; Grossman and Ku, 1986). By comparing the $\delta^{18}O$ values in the shells to modern temperature data and considering the isotopic composition of seawater during the time of oyster growth, scientists can estimate past temperatures with precision. In order to do stable oxygen isotope analysis, carbonate must be extracted from the shells in order to ascertain the stable oxygen isotope levels present at the time of harvest (Hallmann et al. 2009). The data provided by $\delta^{18}O$ analysis is used to provide data about the ambient environment, including variations in sea surface temperature (Andrus and Thompson

2012; Kirby 1998). Shell carbonate contains oxygen isotopes that record local water conditions, but it can be difficult to tell the difference between rising temperatures and rising salinity (Burchell 2013; Hoefs 2018). The stable oxygen isotope levels are often more positive in periods of lower temperatures and greater salinities. These positively trending values can allude to the colder winter months. The stable oxygen isotope value is more negative when the temperature is higher and the salinity is lower, indicating the warmer summer months (Hallmann et al. 2009). These levels are further altered geographically by salinity variation as a result of precipitation, runoff, and snowpack melting, creating freshwater influxes into the coastal environment.

2.3.4 Archaeological Approaches

In the current era of research, shells are often overlooked at archaeological sites, particularly on the Eastern coast of Canada. Despite being extensively accepted by both the archaeological and biological sectors of coastal studies, the study of mollusk shells still needs to be addressed in the archaeological context (Andrus 2011, Twaddle et al. 2016). Shell midden formation dates to 9,000 ya on the Atlantic coast in Northern America, and the eastern oyster represents a large portion of bivalves harvested for subsistence purposes (Denny et al. 2016; Kent 1988).

Numerous coastal archaeological sites have been found throughout the maritime region, providing evidence of human habitations and interactions with the environment (Kristmanson, 2019). However, at most North American sites, shells were frequently discarded prior to the occurrence of archaeological shell-related investigations in the

1980’s (Kent 1992). A recent professional emphasis on shell midden studies in Atlantic Canada has been limited (Betts et al. 2017). As a result, there are gaps in the archaeological record's chronological timeline, the potential loss of these shell middens, and the substantial amounts of data they may contain. The Maritime Provinces have historically relied on shell middens as a source of calcium carbonate, which is frequently quarried to create lime for use in construction (Kristmanson 2019). Additionally, when considering preserving the environmental context, the risk of erosion is urgent, as it is with many other coastal sites. The contextual environments of these coastal archaeology sites are at risk due to the increasing sea level (Spiess 2017). Studies of Atlantic shell middens from Virginia, Maine, Nova Scotia, and New Brunswick are advancing knowledge of the region's waterways and their natural past.

2.3.5 Oysters in Paleoenvironmental Studies

Oysters have emerged as significant subjects of study in archaeological and paleontological research within the eastern USA (Kent 1992). These investigations have provided valuable insights into oysters' growth patterns as they display incremental seasonal growth. Notably, the Atlantic coast of the United States has become a focal point of research on *C. virginica* in a sclerochronological context, resulting in an extensive body of literature. The application of stable isotope analysis has demonstrated its vast spatial reach, extending from Northern Chesapeake Bay to the southern coast of Mississippi (Blitz *et al*. 2014; Rick *et al.* 2014). Moreover, this line of research encompasses a broad temporal spectrum, with studies investigating oyster shells from both historic and prehistoric eras dating as far back as the Pleistocene (Thompson and Andrus 2013; Kirby *et al.* 1998). These studies have contributed significantly to paleoenvironmental studies, providing valuable data for reconstructing past environments and shedding light on past climatic conditions.

The incorporation of the eastern oyster as a reliable recorder of isotopic data has led to a deeper understanding of paleoenvironmental conditions and human interactions with coastal ecosystems throughout history. The incremental growth patterns of these oysters have allowed researchers to establish precise timelines of environmental changes, offering unique perspectives on shifts in sea surface temperatures, salinity fluctuations, and ecological variations over time (Blitz *et al.* 2014).

Furthermore, the spatial range of stable isotope analysis in oyster shells has enabled researchers to investigate diverse geographic locations, extending their applicability from Northern Chesapeake Bay to the Gulf of Mexico's southern coast (Rick *et al.* 2014). These investigations have unveiled region-specific patterns, demonstrating the versatility of oyster shells as archives of past environments and climatic shifts.

Andrus and Douglas (2000) refined methods for paleoenvironmental studies when working with the species *C. virginica*. This study represents one of the earliest developments of methodology for examining the Eastern oyster in an archaeological context for the purpose of paleoenvironmental reconstruction. Stable oxygen isotope analysis via mass spectrometry (IRMS) was applied to oyster shells that were live-collected each month over the span of one year in Altamaha Sound, Georgia (Andrus and Douglas 2000). Shells utilized in this study were collected in batches of 40 during the full moon each month, considering the lunar cycle's role on tides (Andrus and Douglas 2000). After bisection and thick sectioning, all analyses were carried out at the left valve's hinge because it is where the specimens' growth bands are located (Andrus and Douglas 2000). The mean differences between adjacent light and dark zone δ^{18}Oshell were found to be 1·0‰ and 2·2‰, respectively. The mean δ^{18}O values in in dark bands from the intertidal shell from this study was 0·7‰ while subtidal shells had a mean of 0·2‰ (Andrus and Douglas 2000).

The yielded δ^{18}O values were consistent with biannual temperature changes resulting in growth line banding. However, variations in amplitudes were seen across individuals (Andrus and Douglas 2000). Andrus proposed, for this reason, absolute values should not be used as a sole determination of the month collected, but rather seasonal patterning displayed in values precipitated by groups of shells (Andrus and Douglas 2000).

Building on Andrus and Douglas's (2000) work, subsequent studies have further explored the application of *C. virginica* in paleoenvironmental reconstructions. Niewoehner and Jackson (2004) also utilized stable oxygen isotopes in *C. virginica* shells from an archaeological site in the southeastern United States. Stable oxygen isotope data was used to reconstruct sea surface temperatures and explore the climatic conditions during the Little Ice Age. The study in turn, provided valuable insights into historical climate fluctuations and their potential influence on human populations in the region (Niewoehner and Jackson, 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 OYSTER BIOLOGY, ECOLOGY & GROWTH

The members of the phylum Mollusca living in marine environments are aquatic invertebrates. Gastropods and bivalves each have exoskeletons, and their calcium carbonate shells are a defining characteristic (Claassen 1998). The paired valves of the shell, which can be symmetrical or asymmetrical, distinguish bivalves (Claassen 1998). Bivalves grow by depositing shell material that forms crystalline layers (Claassen 1998). Like their other bivalve counterparts, oysters grow through calcium carbonate deposition along the ventral margin. However, oysters deposit calcium carbonate layers from the hinge and the ventral margin (Claassen 1998; Bougeois et al. 2014; Milner 2001). Shell incremental growth patterns are dependent upon seasonality, with growth arresting in the winter, producing visible banding in the ventral margin or hinge that can age the shell and determine the collection season (Andrus 2011; Casas 2018; Claassen 1998; Milner 2001). Aragonite and calcite are the mineral forms that make up the crystalline calcium carbonate in shells (Claassen 1998). The aragonite form of crystalline material is found in oysters in their muscle scar and ligustrum (Kirby et al. 1998; Kent 1988). Calcite is more likely to fossilize and endure in a historical setting because it produces a more resilient mineral structure regarding physical and chemical deposition (Claassen 1998). The nacreous layer, the prismatic layer, and the periostracum are the three layers that bivalve shells secrete. The conchiolin protein that makes up the periostracum, the shell's outermost layer, hardens the surface. The nacreous layer is always made of aragonite. In contrast, the prismatic layer can be either aragonitic or calcitic, depending on the species, even though the nacreous and prismatic layers are made of calcium carbonate. The environmental data required to establish the age and seasonality of the shell can be found in the prismatic layer (Claassen 1998).

Their physiology embodies a network of experiences within the local environment, so shell growth rates vary across spatial and temporal domains (Claassen 1998). The pattern and rate of deposition are most significantly influenced by the ambient environment, particularly saltwater temperature and salinity (Hallman 2009). Currents, pollution, disease, sediment types, and weather are a few additional factors that can impact shell growth rates (Claassen 1998; Milner 2001). As the bivalves filter feed, biomarkers develop in their hard tissues, representing these ambient conditions and resulting in growth that is in isotopic equilibrium with its surroundings (Helser et al. 2018). The cycling of growth increments and growth lines demonstrate that shell growth is dependent on the animal's environment for the resources necessary for survival. The species' preferred threshold for temperature, salinity, macronutrients, and food are present when the growth increments are visible. A slower and more condensed phase of growth results in the formation of growth lines, which happen when conditions are less favourable (Claassen 1998). Calcium carbonate levels are not sufficient for the formation of the shell when the temperature and salinity are very high or low (Andrus 2011). The volume and density of disturbances to the shell’s optimal conditions for growth decrease the increments in which the amount of calcium carbonate is deposited (Claassen 1998).

In Canadian waters, oysters live at an average depth of around 0.6-0.2 m, while in the USA's mid-Atlantic coast, they can live at depths of up to 5.0 m on average (NOBRT 2007). The Eastern oyster can grow up to 20 cm in length and can live for up to 20 years (Harding 2008; Kent 1992). Some oysters have grown to be 38 cm long and more than 1.35 kg in weight (Fisheries and Oceans Canada 2003). Around the Island of PEI, growth is fastest within Bedeque Bay at up to around 37 mm/year, moderate in the East River at 20 mm/year, and slowest within Malpeque Bay at 10 mm/year (Fisheries and Oceans Canada 2003). The species is found all along the Atlantic North American Coast, with a natural range extending from the Gulf of St. Lawrence to the Gulf of Mexico (Kent 1992). The species inhabits estuarine and coastal environments, including bays, sounds, and intertidal zones. The optimal temperature range for the species is between 20-28°C (Ford & Tripp 1996). Atlantic Canada has the broadest seasonal water temperature changes in which oysters can still survive, demonstrating defined banding that cannot be seen anywhere else along the coast (Casas 2018; Kent 1988). In this region, oyster growth slows below 10°C and begins to fully arrest at temperatures lower than 4°C when feeding ceases (Fisheries and Oceans Canada 2003). Eastern oysters prefer brackish and saltwater habitats with moderate salinity levels (Kent 1992). The preferred salinity range for the species is between 20 to 27 ppt (Fisheries and Oceans Canada 2003). They thrive in areas with a mixture of saltwater and freshwater inflows, such as estuaries, where they can tolerate a range of salinity variations.

3.2 EXCAVATION & SAMPLE SELECTION

The samples used in this analysis were acquired by Dr. Kristmanson and Indigenous Services PEI between 2014 and 2019 during the Malpeque Bay archaeological project excavations at Pituamkek. All preserved oysters (n=30) were collected for analysis during the excavation from three units (2D, 3C, 4D) (Fig 3.1).

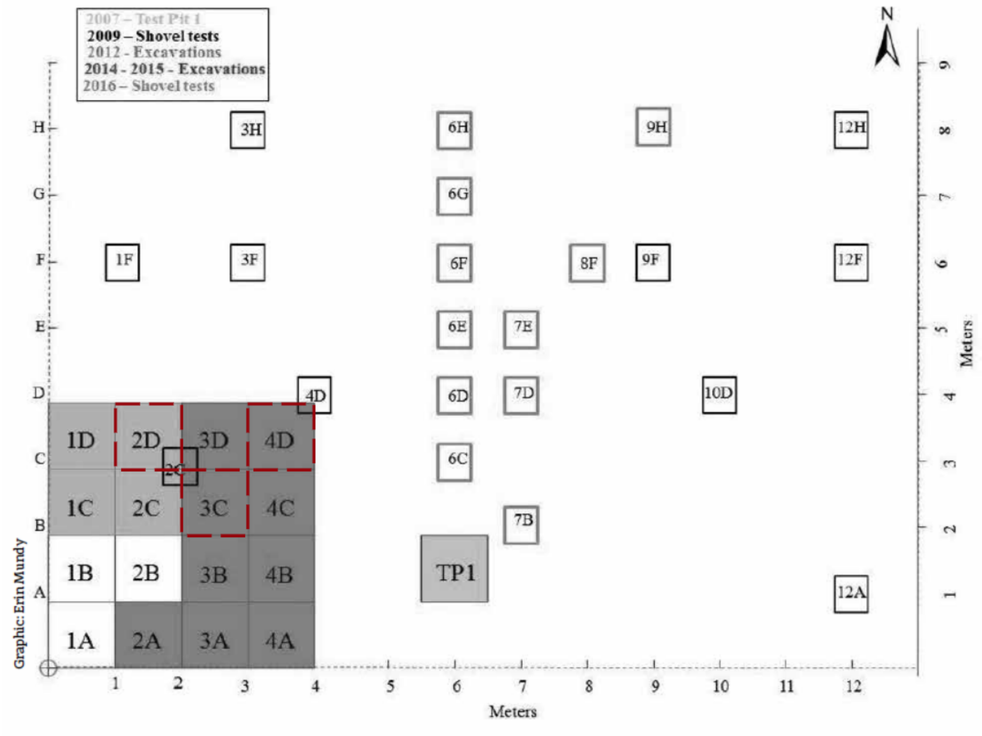


Figure 3.1: Pituamkek Site plan displaying excavated units and shovel tests. Units containing the archaeological oysters examined in this research are outlined with red (Modified from Kristmanson 2019)

To ensure the selection of valves of the necessary quality for growth line analysis, the collections of both live collected oysters from Malpeque and the archaeological samples from Pituamkek middens were first cataloged. The individual valves were first measured using the techniques in Claassen (1998). The oysters were categorized by valve type, size, and condition, noting any damage, discoloration, erosion, or flaking.

In the case of the Pitaumkek site, there were 22 left valves, 6 right valves, and 5) unknown valve fragments. A total of 20 oyster samples (left valves) were deemed feasible for seasonality analysis. In order to calibrate the archaeological oyster isotope data and to understand the nature of the growth patterns in the shell, live-collected samples were obtained by Indigenous Services PEI from Malpeque Bay in the Fall of 2019. This collection of live collected oysters from the Bay contained a known number of six paired oyster valves.

There is some discussion about whether the left or right valve provides the best-preserved sample for environmental reconstructions using oysters. Bougeois (2014) suggests the analysis of the left valve as it contains mainly foliated calcite and results in higher levels of preservation long term. Additionally, the left valve provides the largest surface area when employing sampling techniques for analysis, such as radiocarbon dating or stable isotope analysis (Zimmt et al. 2019). In studies utilizing *C. virginica*, the left valve is also the most frequently used for imaging the species with the intention of growthline analysis (Andrus and Douglas 2000; Kirby et al. 1998; Zimmt et al. 2019). For this reason, the left valves of the oyster were selected when developing methods. Some isolated hinges from the archaeological collection could not be determined as left or right. Still, when possible, the left was used to reduce redundancies, increase the visibility of banding, and maintain consistency in preparation.

3.3 FT-IR

To confirm the mineralogical composition and ensure there were no digenetic changes, Fourier-transform infrared spectroscopy was applied to ensure shells remained in their original carbonate chemical form (Andrus and Thompson 2012) (Fig. 3.2) . By analyzing the infrared absorption spectrum of an oyster sample, researchers can gain insights into its chemical composition and potential changes due to diagenesis (Weiner, 2010) since FTIR identifies and/or quantifies specific compounds, including calcite and aragonite The sample will absorb different infrared frequencies and displayed peaks within the resulting FT-IR spectra indicating the material's chemical and structural characteristics. Both attenuated total reflectance (ATR) methods using powder samples and non-invasive front reflectance were used. The initial spectra for shells in the area were tested by preparing two live collected valves. A powdered sample was scraped out from both the inner and outer portions of each shell sampling point. This powder was placed directly on the ATR surface. Attenuated total reflectance FT-IR was utilized to analyze the carbonate from each sample. The ATR crystal, made of diamond, was in contact with the sample. Infrared light passed through the crystal and interacted with the samples, some light became absorbed by the sample while the remaining light reflected back.

Figure 3.2. A Bruker Alpha II spectrometer (Retrieved from Bruker)

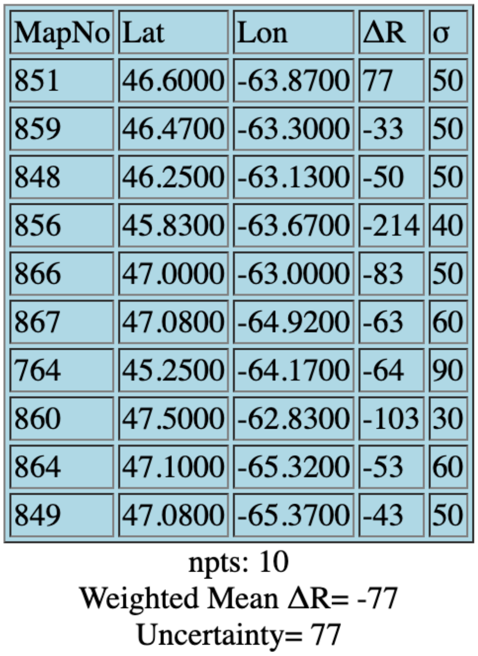
Non-invasive front reflectance, also known as specular reflectance, allows for the analysis of samples without direct contact. This technique is advantageous when analyzing fragile or sensitive samples and was applied to the archaeological collection (Nevin 2012). Three archaeological valves were analyzed that would later undergo radiocarbon dating. Instead of using a powdered sample on the surface, the valve was positioned in front of the FT-IR, and the infrared beam reflected off the sample's surface. Peak positions for the spectra were collected using the single peak pick tool in the OPUS software. All samples were examined using the Bruker Alpha II spectrometer with the platinum ATR attachment. The resulting spectra produced for each valve in FT-IR analysis confirmed that shells from every layer of stratigraphy in the midden had not been compromised in their original calcium carbonate structure during deposition.

3.4 RADIOCARBON DATING

Three shells analyzed for stable oxygen isotopes were submitted to the A.E. Lalonde Lab for radiocarbon dating. To provide a regional paleoclimate reconstruction of Malpeque Bay that could be temporally situated, the samples of *C. virginica* from Pitaumkek were sent for radiocarbon analysis (C^{14}). Valves were selected based on their preservation and spatial distribution within the midden, representing the different levels of stratigraphic positioning present at the site. The collection was divided into excavation units during the 2014-2019 excavations.

The sample with the best geochemical and structural preservation from the archaeological collection was selected for analysis from each midden unit (3C, 2D, and 4D). Each valve was then sampled along both the ventral margin (slowest, most recent deposit) and the hinge (fastest, earliest deposit) to represent the life history The samples from all three shells were submitted for C^{14} dating at the A.E. Lalonde Radiocarbon Lab, University of Ottawa, Canada. At the laboratory, shells underwent analysis using a 3MV accelerator mass spectrometer (AMS).

The selection of an appropriate Marine reservoir (ΔR) was completed by using the Marine 20 Database at calib.org. The 10 most relevant points for both archaeological and marine contexts were selected, resulting in a calculated marine reservoir of ΔR -77 +/- 77 (Fig. 3.3). The University of Oxford's online Oxcal system (version 4.3.1) was used for calibration by inputting marine estimations ΔR, and uncalibrated dates. Dates were calibrated to 2σ using IntCal20 with a marine reservoir of ΔR -77 +/- 77.



MapNo	Lat	Lon	ΔR	σ
851	46.6000	-63.8700	77	50
859	46.4700	-63.3000	-33	50
848	46.2500	-63.1300	-50	50
856	45.8300	-63.6700	-214	40
866	47.0000	-63.0000	-83	50
867	47.0800	-64.9200	-63	60
764	45.2500	-64.1700	-64	90
860	47.5000	-62.8300	-103	30
864	47.1000	-65.3200	-53	60
849	47.0800	-65.3700	-43	50

npts: 10
Weighted Mean ΔR= -77
Uncertainty= 77

Figure 3.3. 10 Most relevant points and their reservoir values were taken from the Marine 20 database to calculate the Marine reservoir within Malpeque Bay (Oxcal).

3.5 SCLEROCHRONOLOGICAL ANALYSIS

Samples from the collection with adequate hinge preservation from the archaeological (N=20) and live collection (N=6) were washed in cold water. Selected shells were reinforced with LePage epoxy steel on their surface before cutting the hinge due to their friable nature. Dremel rotary tools with a diamond blade were used to cut the hinge from the valve, bisecting the ventral margin (Fig. 3.4).

Figure 3.4. Dremel removing hinge enforced in epoxy steel from an archaeological oyster.

Oyster hinges were embedded in Buehler EpoKwick FC epoxy resin and cut to 3 mm thickness along the axis of maximum growth with a low-speed diamond saw. Cross-sections were polished using Buehler MetaServ 250 Grinder-Polisher with a 1µm colloidal solution with SiC Grit (320/P400), SiC Grit (600/P1200) discs and a Texmet© Polishing Cloth. The final polishing step included the use of 1µm Al2O3 colloidal solution. Shells were cleaned in an ultrasonic cleaner between each grinding and polishing step.

To test if thin sections could provide a higher level of resolution than 3 mm slices, two live-collected samples were embedded in epoxy and were mounted to a glass slide using Hillquist Thin Section Epoxy. The samples were sliced cut to a thickness of <1mm using the low-speed diamond saw. The slides were then polished using the Buehler

MetaServ 250 Grinder-Polisher using SiC Grit (600/P1200) discs and a Texmet© Polishing Cloth. The final polishing step included the use of 1μm Al2O3 colloidal solution. The slides were cleaned in an ultrasonic cleaner between each grinding and polishing step.

3.5.1 Digital Light Microscopy & Imaging

On a ZEISS Axio Zoom.V16 microscope, which provides an image clarity beyond the capability of a compound microscope, visualization and imaging of the ontogenetic hinge structure was completed (Fig. 3.5). In the past, compound microscopes have been used frequently to evaluate oyster growth lines, but they need to provide a clear enough image to allow for a complete analysis of growth line structure (Fig. 3.6). Images of thick and thin sections were captured under 10-15x magnification using a Zeiss AxioZoom V.16 microscope under reflected light. Thin sections underwent an additional round of imaging where transmitted light was tested.

Figure 3.5. Zeiss AxioZoom V.16 microscope (Retrieved from Zeiss)

During the image acquisition stage, the contrast of the gray and white internal structure was increased by increasing the gamma. Altering the gamma produces a degree of contrast between the midlevel gray values of an image. Even small gamma setting adjustments will result in noticeable image alterations. Gamma concerns are especially crucial when showing images with extremely long gray scales.

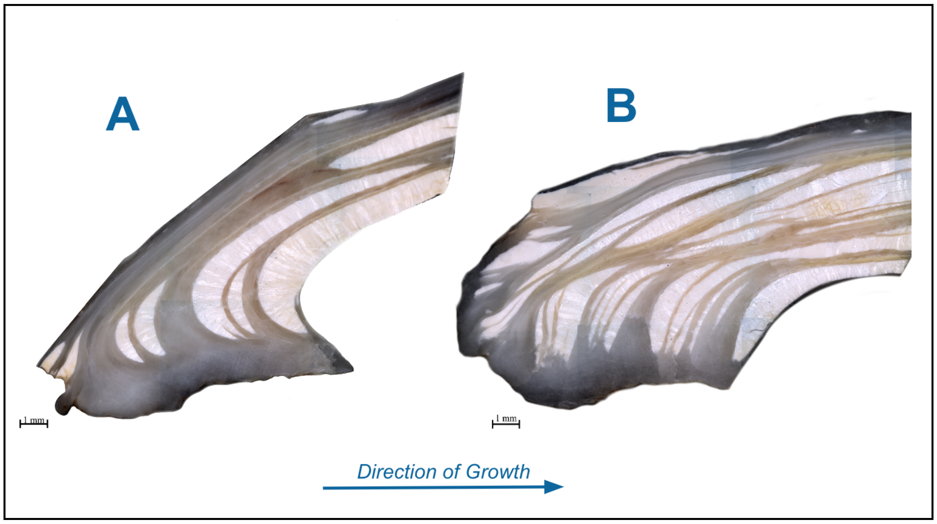


Figure 3.6. Two prepared live collected oyster hinges imaged using Zeiss AxioZoom V.16 microscope.

3.5.2 Scanning Electron Microscopy

SEM imaging has in the past been the primary method utilized to visualize growth increments of oyster species by analyzing the geochemistry and plating of microgrowth structures (Milner 2001; Ricardson 1993). Using scanning electron microscopy, small specimens or sections can be imaged at 100–100,000 times their actual dimensions (CREAIT). Instead of collecting light like conventional optical microscopes, they accomplish this by gathering radiated electrons.

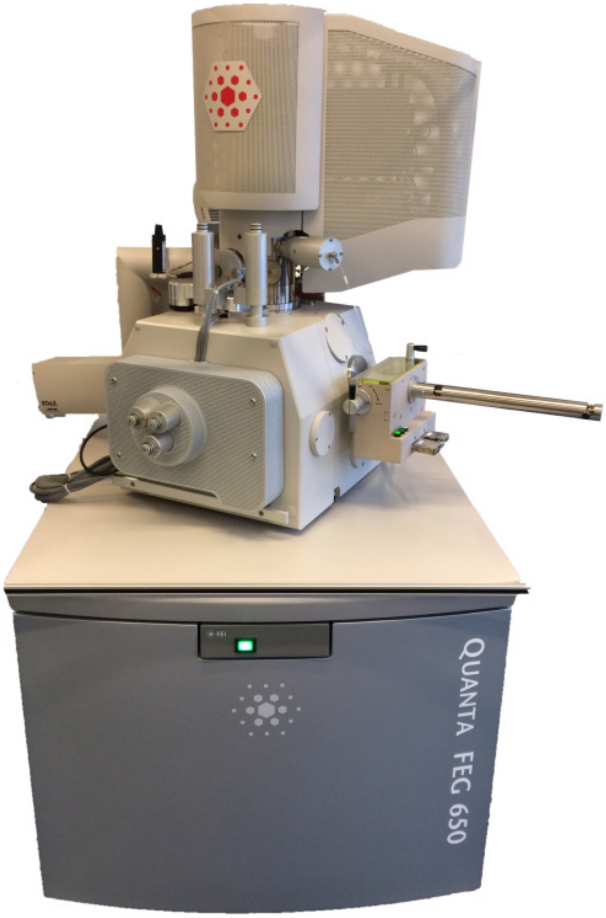


Figure 3.7. EI MLA 650FEG Scanning electron microscope (SEM) (Retrieved from Hereon)

SEM images were taken through the CREAIT Network at Memorial. An FEI MLA 650FEG, a scanning electron microscope (SEM) equipped with an energy-dispersive spectrometer (EDS) and wavelength-dispersive spectrometers (WDS), was used for image acquisition (Fig. 3.7). This machine allows both secondary electron (SE) and backscatter electron (BSE) imaging, providing topographical and compositional contrast images. The MLA software automated image acquisition, stage movement and x-ray acquisition. Growthline banding, disturbance, and variations within the structure of the oyster hinges were imaged at a magnification of 60-80x using the FEI MLA under the ETD detection setting.

3.5.3 Sclerochronology and Interobserver Analysis

In order to test growth line banding analysis was completed via blind testing in a series of runs, with researchers familiar with counting incremental growth in marine bivalves. To facilitate the estimation of age at harvest and to account for the ability to accurately ‘read’ shells, three independent observers trained in sclerochronology each analyzed 20 shell images from the archaeological site Pitauamkek.

Obs.1 Master’s student who works with *Crassostrea virginica.*

Obs.2 Ph.D. candidate studying *S. gigantea* and *L. staminea*.

Obs.3 Ph.D. and has examined incremental growth in many bivalve species.

All three observers have experience preparing thin and thick sections and are able to produce high-resolution images of shell growth. Still, the observers have different expertise in species and sub-specialties of sclerochronology (e.g. geochemistry, seasonality, SEM, etc).

Images of shells were uploaded to a shared drive, which readers had access to on their computers. Observers were asked to assess the: 1) quality of preservation and visualization (poor, moderate, good); 2) age (growth line counts +/- error); 3) coloration

(Grey/White/Unknown). Observer data was independently collected on an Excel spreadsheet created for the assessment. Shells were examined one by one as a group, keeping the individual results private. When completed for each unit, the data was compiled into one spreadsheet.

Observers' resulting age estimates provided a range of maturity for the shells—the coloration noted by observers provided an estimate of the seasonality of harvest. Finally, the image quality provided an estimation of how reliably each shell could be read.

Utilizing the previously determined harvesting guidelines as a model for maturity in the species at three to seven years of age (Fisheries and Oceans Canada 2003). Therefore in this study, oysters under the age of three (<3) were considered juvenile, those within the harvesting range of three to seven years were considered to be mature, and finally, those with an estimated age of beyond seven years (>8) were considered senile.

3.6 STABLE OXYGEN ISOTOPE ANALYSIS

Two live-collected shells and 10 archaeological shells were sampled using a Merchantek New Wave Research micromill to obtain discrete power samples for analysis.. Five samples were milled from the growing edge using a 480µm conical drill bit, followed by 10 samples drilled every 500µm (Fig. 3.8). The samples started directly at the ventral margin and moved along the axis of growth in consecutive steps following the contours of the pattern of growth. A total of 30 shell carbonate samples were obtained from two live collected shells to identify measurements of $\delta^{18}O$. Growth bands and sampling locations were aligned to assess the relationship between seasonal growth and isotope values. To analyze the chemistry of the archaeological shells and determine paleotemperature, a total of 150 shell carbonate samples were obtained. Samples for $\delta^{18}O_{shell}$ were weighed between 150-250 μg using a Mettler Toledo AT21 Comparator analytical balance. Samples were processed at the Ján Veizer Stable Isotope Laboratory on a Thermo DeltaPlus XP continuous flow - IRMS coupled to a Thermo Scientific TC/EA. The IRMS used a Conflo IV Costech Zero-blank autosampler with an analytical precision of ± 0.3‰.

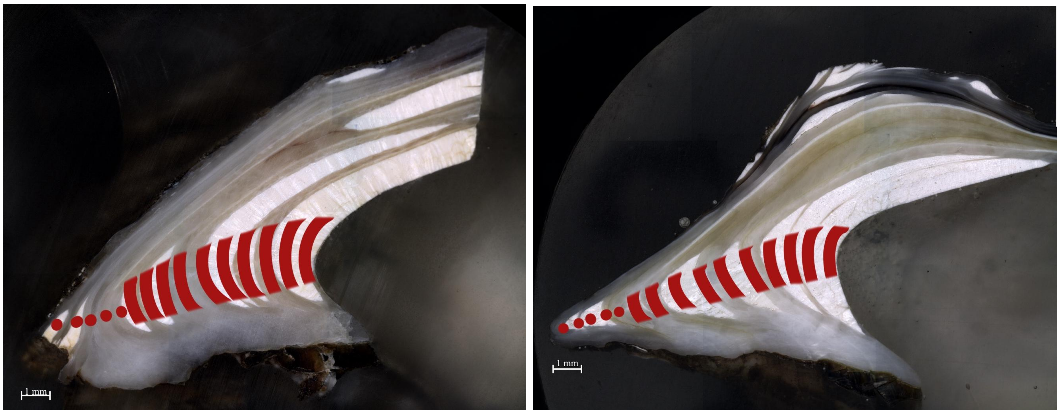


Figure 3.8. Isotope sampling location for live collected *C. virginica*. The lines represent the micro-milling locations, and the dots represent the micro-drilling locations.

3.6.1 Stable Oxygen Isotope Interpretation and Paleothermometry

Results from $\delta^{18}O_{shell}$ analysis were interpreted in relation to the development of growth lines in the shell to deduce the collection season from ancient shells (Urey et al. 1951). The season of death can be identified by the sinusoidal change in $\delta^{18}O_{shell}$ (Burchell *et al.* 2013; Hallmann *et al.* 2013). Based on how $\delta^{18}O_{shell}$ fluctuates with distance from the final stage of growth, shell seasonality can be interpreted. If a complete sinusoidal curve exists, the position of the ventral margin relative to the curve and the growth pattern is used to determine the season. If the $\delta^{18}O_{shell}$ becomes increasingly positive with distance from the ventral margin, it is indicative of cold weather collection (Fall/Winter). If the $\delta^{18}O_{shell}$ decreases with distance from the ventral margin, it is indicative of a warm season of collection (Spring/Summer).

For the determination of sea surface temperature (SST) using the archaeological shells, a species-specific paleothermometry equation produced by Anderson and Arthur (1983) was selected as the best fit for SST reconstruction:

$$\mathbf{T\ (°C) = 16 + 4.14\ (\delta^{18}O_c - \delta^{18}O_w) + 0.13\ (\delta^{18}O_c - \delta^{18}O_w)^2}$$

CHAPTER 4

RESULTS

4.1 SAMPLE PREPARATION

4.1.1 Thick Sectioning

Thick sectioning of the left valve resulted in the clearest image quality that was feasible for use in rapid assessment of shell age at harvest (Fig 4.1). Imaging the isolated oyster hinges using reflected light at a magnification of 10-15x produced the highest quality images for sclerochronological analysis (Fig. 4.2). Reflected light proved best for visualization on thick and/or thin sections (both had readable growth patterns).

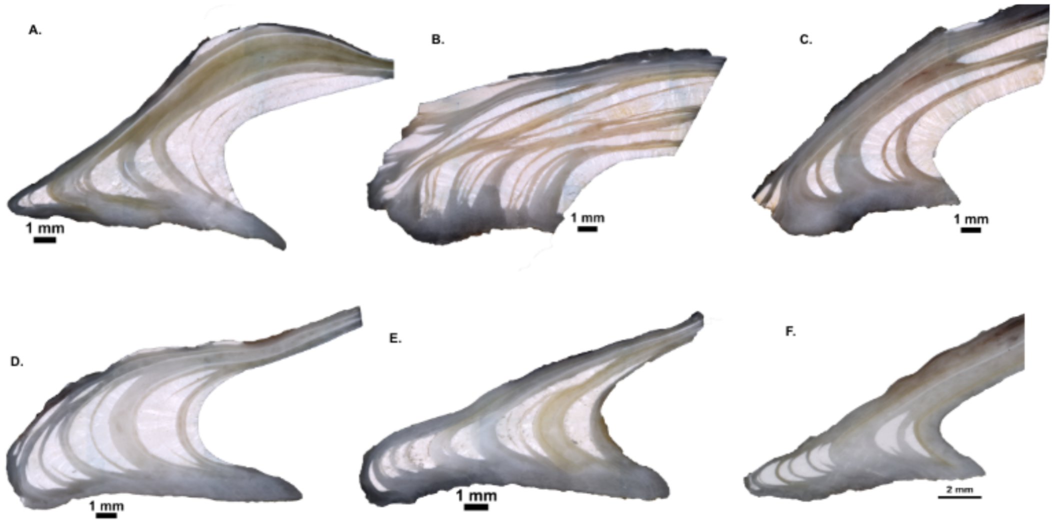


Figure 4.1. Prepared cross-sections of live-collected oyster hinges collected Sept. 2019, Malpeque Bay, PEI, under reflected light. A. Shows ‘regular’ growth patterns in a younger shell (~3-4 years). B. Shows ‘senile’ growth and can’t be read for seasonality reliably; c. Missing the last stage of growth; D. Grey banding at the ventral margin suggests a ‘colder’ phase of growth, shows annual and possible disturbance lines; E. Similar growth to A and F, showing ‘warm’ growth going into colder growth’; F. Similar to A and E.

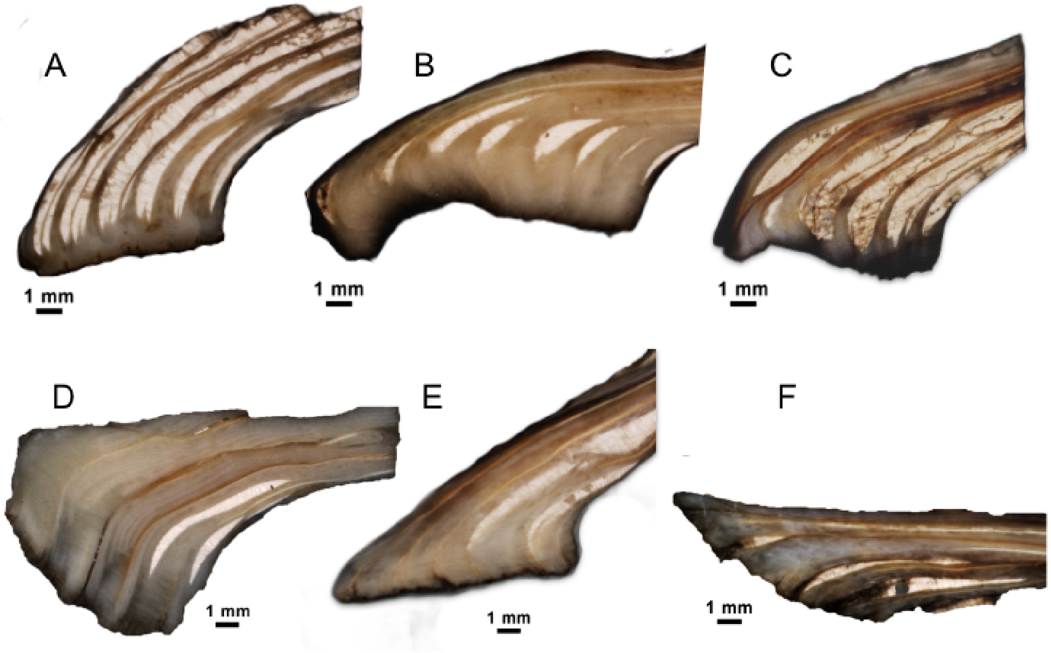


Figure 4.2. Archaeological oyster hinges- Thick sections with reflected light. A. Warm growth going into a colder phase; B. Warm growth going into colder growth; C. White banding at the season of collection showing the late phase of warm growth; D. Irregular growth with warm growth into a colder phase, with possible disturbance lines; E. Irregular early, warm growth with ‘going into cold; F. Irregular early growth with warm growth going into cold.

4.1.2 Thin Sectioning

The thin sections under transmitted light produced no significant advantage for visualizing the growth and often obscured the microstructures and banding while increasing the disturbance of the resulting image (Fig. 4.3). Additionally, with the chalky nature of oyster valves, thick sectioning proved to be the most reliable method for preparation without compromising the light banding of the valve.

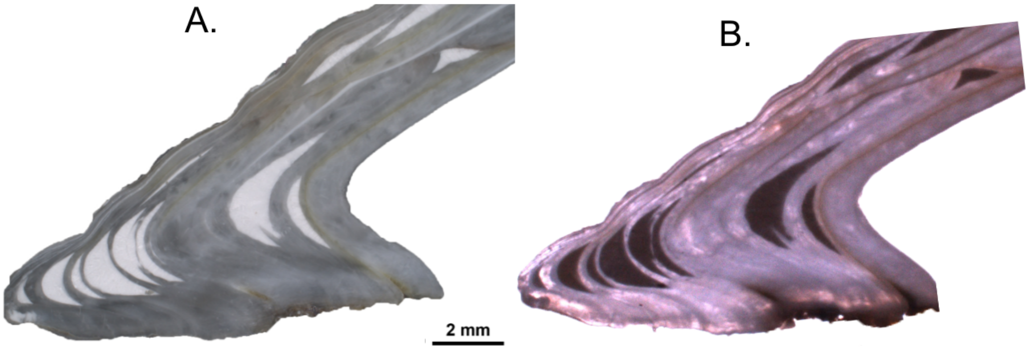


Figure 4.3. Live-collected thin section under A) reflectance and B) transmitted

4.2 SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) proved most valuable for examining the microstructure and division of seasonal bands (Fig. 4.4). The images provided significant contrast between light and dark banding. Additionally, separation in the plate structure was easily visualized using this method. The visual contrast of cracks and separations proved useful for determining paired banding and locating places where banding might be impeded by damage to the internal structures of the valve. Although SEM provided another level of information for interpreting growth lines, there was no advantage to using SEM over thick sections under reflected light.

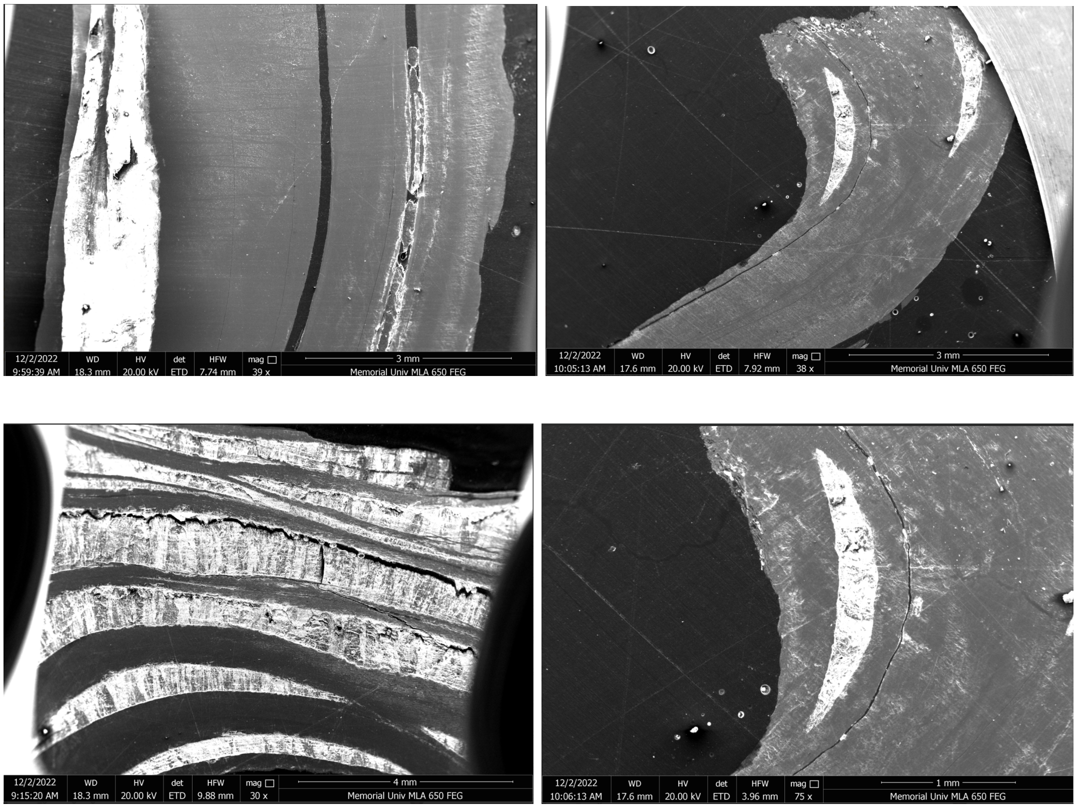


Figure 4.4. Scanning Electron Microscopy images of growthline banding and disturbance in archaeological *C. virginica* valves imaged at 30-75x magnification using ETD detection.

4.3 SCLEROCHRONOLOGY & INTER-OBSERVER VARIATION

Seasonal patterns showing 'warm' vs. 'cold' growth based on the colour of the shell growth were clearly observed in 50% of the live-collected oysters (Table 4.1). There is a difference in the readability between live- and archaeological shells. This is attributed to diagenesis, midden compaction, and preservation of the surface of the oyster. Changes in sea surface temperature, productivity, salinity, and population genetics, may explain the variation between live- and archaeological populations. The resulting images proved reliable for age assessments.

The quality of the shells proved significant to the visualization capacity of each observer. Overall, observers determined 25% of shells to be considered ‘good’ quality and ideal for sclerochronological analysis. Overall, observers determined 43% of shells to be considered ‘moderate’ quality and could be feasible for analysis. Overall, observers determined 32% of shells to be considered ‘poor’ quality and, therefore, not feasible for rapid age assessment or seasonal determination.

Table 4.1: Interobserver data for prepared archaeological collection. Three observers determined the quality of the image (good, moderate, poor), the estimated age and associated error, as well as the colour of the most recent deposit (Grey, White, Unknown).

	OBSERVER 1				**OBSERVER 2**				**OBSERVER 3**			
SAMPLE ID	**Quality**	**Age**	**Error**	**Colour**	**Quality**	**Age**	**Error**	**Colour**	**Quality**	**Age**	**Error**	**Colour**
CdCw-5 2879	good	9	1	grey	moderate	9	1	grey	good	8	1	grey
CdCw-5 2880	poor	4	2	grey	poor	8	4	grey	poor	3	3	unknown
CdCw-5 2908	moderate	3	2	grey	moderate	2	1	grey	moderate	3	2	grey
CdCw-5 2915	good	6	1	grey	moderate	6	2	grey	good	5	1	grey
CdCw-5 2916	moderate	4.5	2	grey	moderate	4	3	grey	good	3	1	grey
CdCw-5 2917	moderate	5	3	grey	poor	4	2	grey	moderate	6	3	grey
CdCw-5 2919	poor	9	3	white	moderate	8.5	3	white	moderate	9	1	white
CdCw-5 2982	poor	4	3	unknown	poor	5	1	unknown	poor	NA	NA	unknown
CdCw-5 2983	good	4	2	grey	poor	5	3	grey	good	5	1	grey
CdCw-5 2990	poor	6	2	unknown	poor	6	4	unknown	poor	NA	NA	unknown
CdCw-5 2997	moderate	3	1	grey	moderate	2	1	grey	poor	NA	NA	unknown
CdCw-5 1724	moderate	3	1	grey	moderate	2	1	grey	good	7	1	grey
CdCw-5 1959	moderate	7	3	white	moderate	5	1	grey	good	7	3	grey
CdCw-5 1976	moderate	3	2	grey	moderate	1	1	grey	poor	NA	NA	unknown
CdCw-5 2132	moderate	4	1	grey	moderate	4	1	grey	good	1	1	unknown
CdCw-5 2155	poor	4	1	grey	poor	2	3	grey	poor	NA	NA	unknown
CdCw-5 2164	good	6	1	grey	good	6	1	grey	good	6	1	grey
CdCw-5 2608	moderate	3	1	grey	moderate	2	3	grey	good	5	1	grey
CdCw-5 2621	moderate	5	2	grey	moderate	4	2	unknown	moderate	5	2	grey
CdCw-5 3488	poor	3	2	unknown	poor	3	2	unknown	poor	3	2	unknown

The average age of shells harvested at the Pituamkek site was ~4.7 years with a total range of 1-9 years . Half of the shells (50%) analyzed from Pituamkek were harvested at a mature stage of growth (4-7ya). The percentage of shells harvested during the juvenile phase (<4ya) totalled 30%, while the percentage harvested during the senile phase (>7ya) was only 10%. The remaining 10% could not be determined based on image quality.

In terms of coloration of the most recent deposit and by proxy season of harvest, viewers were able to observe a pattern in the harvesting at the site. The majority of shells (68%) displayed gray banding upon the most recent deposit. Only 7% of the shells observed displayed white banding. The remaining 25% of shells did not present sufficient quality for visualization of seasonality.

4.4 FT-IR

The resulting spectra produced by IR analysis of the oyster samples were consistent with a mixture of calcite and aragonite (Fig. 4.5). The resulting spectra match the anticipated mineralogy of oyster carbonates, as reported here. Aragonite, which creates the glossy nacre and the muscular components, makes up the innermost layer of *C. virginica*, while calcite makes up the remainder of carbonate (Marin et al. 2012). In the hinge area of the shell, *C. virginica* has also been noted to form alternating layers of calcite and aragonite deposits (Carriker & Palmer 1979).

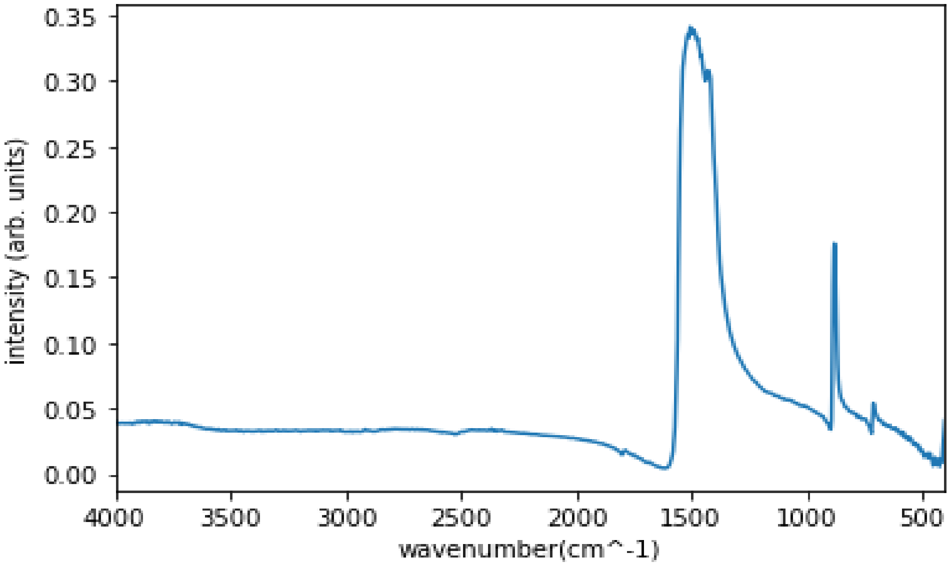


Figure 4.5. FT-IR Spectra recorded using specular reflectance for sample CdCw-5 2608. The resulting spectra display the expected calcium carbonate signal for the species.

4.5 RADIOCARBON DATING

The results of the radiocarbon analysis of an oyster from each unit produced three returned dates from 184 to 643 years cal BP (Table 4.2). The dates are in agreement with stratigraphical positioning, with the deepest deposits comprising the oldest material (Fig. 19). The deepest level of deposition represents the earliest evidence of harvest:

1) Unit 3C contained the most recent deposition, with shells (CdCw-5 2608) dating as recent as 184 years cal. BP
2) Unit 2D comprised the median chronology (CdCw-5 2982) with an estimated date of harvest between 252 to 490 years cal. BP
3) Unit 4D, which contained a single oyster valve (CdCw-5 3488) dated the furthest back at 643 years cal. BP

Table 4.2: Radiocarbon dates for archaeological valves from Pituamkek

Sample ID	Calibrated Years (BP)		
	From	To	%
CdCw-5 2608 Hinge	643	438	95.4
CdCw-5 2608 Ventral Margin	643	437	95.4
CdCw-5 2982 Hinge	490	261	95.4
CdCw-5 2982 Ventral Margin	487	252	95.4
CdCw-5 3488 Hinge	472	207	95.4
CdCw-5 3488 Ventral Margin	461	184	95.4

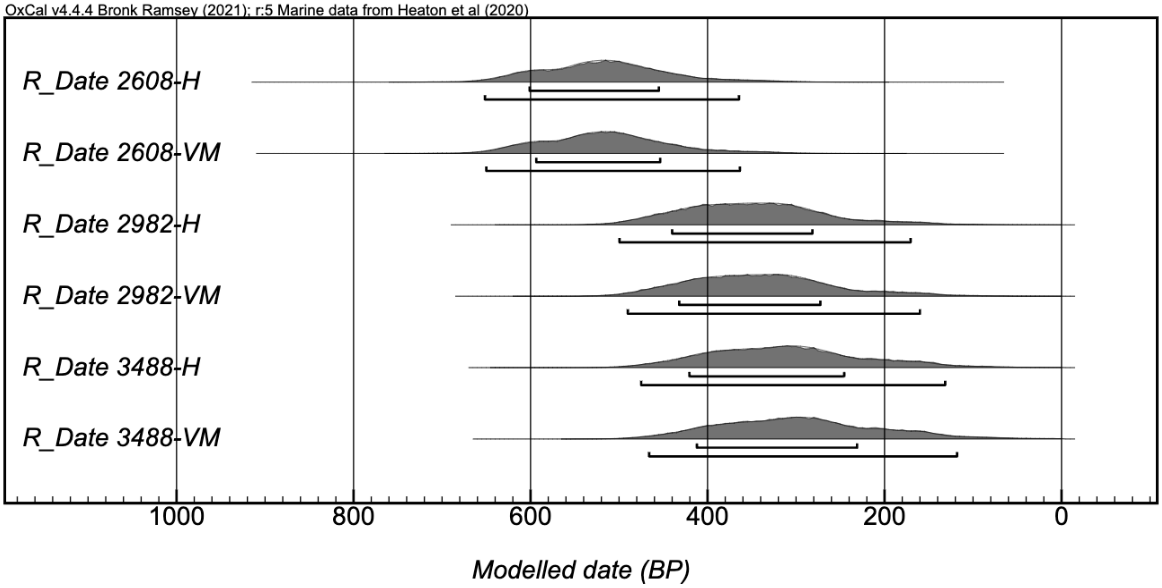


Figure 4.6. Calibrated radiocarbon dates for three archaeological valves (hinge and ventral margin) plotted to σ2 produced in Oxcal (version 4.3.1)

4.6 STABLE OXYGEN ISOTOPE ANALYSIS

The $\delta^{18}O$ results for the live-collected shells ranges from -1.8 ppm to -0.39 ppm (Table 4.3). Calculated values at the growth margin are lower than that of the recorded average for both, indicating a negative trend. This is consistent with the known late September harvest.

Table 4.3. $\delta^{18}O_{shell}$ values of live-collected valves (September 2019) from Malpeque Bay

Sample ID	Max ppm	Min ppm	Average ppm	Growth Margin
MPB 1	-0.39	-1.18	-0.69	-0.5
MPB 2	-0.05	-0.84	-0.36	-0.2

The $\delta^{18}O$ values for the archaeological samples are overall more negative, ranging from -4.0 ppm to -1.8 ppm (Table 4.4). Calculated values at the growth margin are lower than the recorded average for seven samples, indicating a negative trend. The remaining three displayed the inverse, with oxygen isotope values at the growth margin being higher than the recorded average, indicating a positive trend.

Table 4.4. $\delta^{18}O_{shell}$ values for archaeological valves excavated from Pituamkek

Sample ID	Max ppm	Min ppm	Average ppm	Growth Margin	Excavation Unit
CdCw-5 2983	-1.83	-3.62	-2.78	-3.3	3c
CdCw-5 2915	-2.3	-4	-2.34	-4	3c
CdCw-5 2608	-2.7	-3.8	-3.06	-2.5	3c
CdCw-5 2879	-2.2	-4	-2.94	-2.2	3c
CdCw-5 2919	-1.8	-3.4	-2.79	-3.2	3c
CdCw-5 2916	-2.1	-3.5	-2.86	-3	3c
CdCw-5 1959	-2.39	-3.38	-2.98	-3.62	2d
CdCw-5 2169	-2.3	-3.8	-3.07	-3.8	2d
CdCw-5 1724	-2.2	-3.4	-2.84	-2.5	2d
CdCw-5 3488	-2.3	-4	-3.14	-3.2	4d

4.6.1 Seasonality

Seasonality of the two live collected shells analyzed for stable oxygen isotopes showed a warmer than average collection season trending toward more negative $\delta^{18}O_{shell}$ values.

Seven of the archaeological collection analyzed displayed a warmer-than-average collection season trending toward more negative $\delta^{18}O_{shell}$ values at harvest time. The remaining three indicated a cold collection season trending toward a more positive $\delta^{18}O_{shell}$ value at the time of harvest (Fig. 4.7).

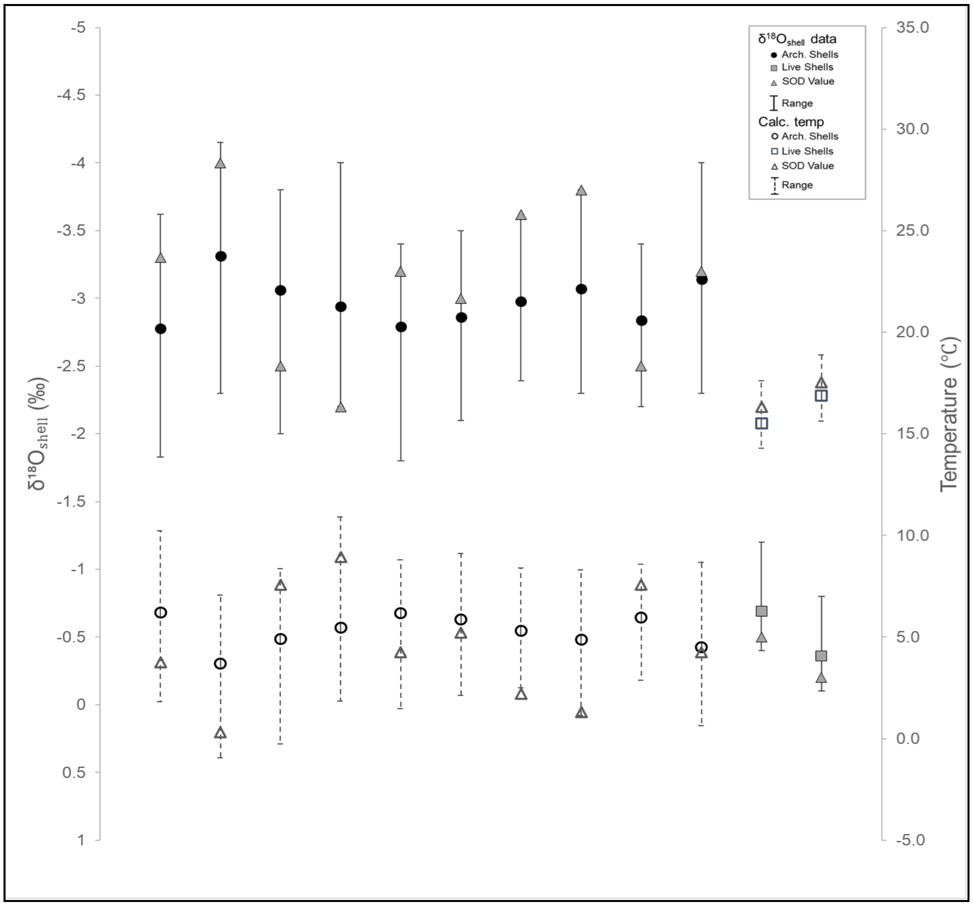


Figure 4.7. $\delta^{18}O$ results and calculated SST of live-collected and archaeological oysters. Circles represent archaeological samples, while squares represent live-collected valves. Filled symbols indicate mean values, and vertical solid lines represent the range. Open symbols indicate the mean calculated SST, and vertical dotted lines indicate the range.

4.6.2 Past & Recent Sea Surface Temperatures

Calculated SST for the live collected shells ranged from -1.7℃ to 17.5℃. The salinity of the live collected shells ranged from 26.1 to 35 PSU, retrieved from the NOAA database for the entire year leading up to the month of collection. The assessment of archaeological shells for the reconstruction of paleotemperatures calculated SST ranging from 0℃ to 10.8℃ (Table 4.5).

Table 4.5. Calculated paleotemperatures of archaeological valves from Pituamkek using Anderson and Arthur (1983) equation: **T (°C) = 16 + 4.14 ($\delta^{18}O_c$ - $\delta^{18}O_w$) + 0.13 ($\delta^{18}O_c$ - $\delta^{18}O_w$)^2**.

Sample ID	Max	Min	Average	Range	St. Dev
CdCw-5 1959	8.1	2.2	5.29	5.9	1.8
CdCw-5 2983	10.6	2.2	6.2	8.4	2.6
CdCw-5 2915	8.3	0.3	3.7	8.0	2.3
CdCw-5 2608	10.1	1.5	4.9	8.6	2.5
CdCw-5 2169	8.6	1.4	4.9	7.2	2.1
CdCw-5 3488	8.3	0.3	4.5	8.0	2.2
CdCw-5 2879	9.1	0.0	5.5	9.1	2.5
CdCw-5 2919	10.8	3.5	6.2	7.3	2.2
CdCw-5 2916	9.6	2.6	5.9	7.0	2.0
CdCw-5 1724	9.0	3.3	5.9	5.7	1.8
AVERAGE	10.8	0.0	5.3	10.8	2.3

CHAPTER 5

DISCUSSION & CONCLUSION

The results of this thesis demonstrate the fidelity of *Crassostrea virginica* from the North Atlantic as a reliable monitor of past sea surface temperatures and seasonality. Specifically, we were able to investigate the seasonal timing of shellfish collection, site occupation, shellfish harvesting strategies and paleo-temperature reconstruction at the site. When the isotopic and sclerochronological data are combined with archaeological data, it advances knowledge of historic shellfish harvesting practices, resource and landscape use and site use during a period of ocean change.

5.1 SHELL PREPARATION & SCLEROCHRNOLOGICAL ANALYSIS

Oyster thick sections (3mm), examined under reflected light, produced the clearest image quality for visual assessment of age and growth line formation. Thin sections under transmitted light produced an image with negligible impacts on the contrast or prominence of incremental growth, often obscuring minor disturbances. Digital light microscopy using the Zeiss AxioZoom V.16 microscope presented the best images for rapid assessment and interobserver observation. Previous studies (e.g. Milner 2001) successfully applied SEM to interpret the seasonality of the European oyster *Ostrea edulis.* While SEM was effective for this species, this method was not reliable for establishing seasonal patterns of growth in *C.* virginica since seasonal growth differences could not be distinguished. However, SEM was valuable for examining microstructure and providing a greater confidence level when analyzing the most recent deposit of growth by differentiating between plates of growth that formed in sequence. These images were used to confirm separation during the interobserver observations to increase confidence. In the future, SEM could be useful when confirming microstructure and testing for diagenesis or structural alteration (Coimbra *et al.* 2020).

5.2 INTEROBSERVER VARIATION

The quality of the imaged shells is imperative for an accurate age assessment and seasonal determination. With 68% of the collection categorized as either ‘good’ or ‘moderate in terms of preservation, the majority of the midden contents could be analyzed visually. The concentration of poor-quality shells increased as both depth of stratigraphy and period of deposition increased within the site. As expected, shells that were in situ for a longer period, such as the valve at unit 4D, presented poor quality for visualization. These interobserver assessments proved useful for the demonstration of the importance of sufficient preservation when reading archaeological shells. Oysters are naturally chalky and friable; when combined with deposition, this can significantly impact the shells’ physical structure. Despite the collection being selected based on preservation and tested for diagenesis, some samples still presented too low quality for visualization (CdCw-5 3488).

Age assessments produced an average age of 4.7 years. Half of the shells (50%) were collected at the ontogenic age between 4 to 7 years at harvest, making them mature. The collection of 20 shells only included 10 valves that would not be considered mature individuals. The collection included 30% juvenile shells below 4 years of age, which would be considered cocktail oysters by modern standards. Only 10% of shells categorized as senile oysters were harvested. The maximum age estimated was 9 years at the time of harvest. Overall, the data show trends in the collection of shells at a mature life stage, likely a strategic harvesting strategy, collecting those that have grown large enough before the growth arrests in the winter and leaving those that are juvenile to continue growing into the next season. These precolonial harvesting practices are in line with what is considered a harvestable size under current federal guidelines.

Warm and cold growth can be identified in the growth lines, but refining to a specific season (winter/spring/summer/autumn) is not precise for this species in the context of the Northern Atlantic through the means of visual analysis. By assessing the coloration on the final band deposited, observers could categorize growth as either 'warm' or 'cold' periods of growth at the time of harvest. The majority of shells (68%) demonstrated dark grey banding that indicated harvesting during cold temperatures or a period of stress. The results of this observation, combined with the calculated paleotemperatures, suggest that oysters in this region likely deposit an increased level of dark banding when compared with previously studied regions as a result of the colder sea surface temperatures. Most shells at the site were collected during this dark banding period. All three shells that showed a lower-than-average temperature at the time of harvest during the paleotemperature reconstruction also demonstrated the expected grey banding at the growing edge during the assessment of all three reviewers (CdCw-5 2608, CdCw-5 2879, CdCw-5 1724).

Year-round harvesting and occupation are likely, with a few of the shells showing banding indicative of warmer temperatures and ideal conditions (10%). Sea surface temperatures peak surrounding the waters of Prince Edward Island in the late summer. The only shell all three reviewers identified as white and therefore collected during the warm season did align with paleotemperature calculations, trending towards warmer temperatures at harvest (CdCw-5 2919).

In contrast, the remaining six shells that were analyzed using stable oxygen isotope analysis and observed for seasonality did not agree with their seasonality determined by paleotemperature reconstruction. For this reason, it may be assumed that the growthline banding of the species in this region may not indicate seasonality and is impacted by a variety of disturbance factors, including flooding events and periods of extreme temperatures, which can result in the precipitation of dark bands during any season (Andrus and Douglas 2000).

CdCw-5 1959 presented a warmer-than-average temperature at the time of harvest, with two reviewers identifying it as having grey banding on the growth margin. However, the observer with the most experience with this species noted a thin white band along the growth margin, congruent with the negatively trending $\delta^{18}O_{shell}$ value at the season of death. Four of the shells were identified by all reviewers as having grey banding indicative of cold weather collection but trended towards warmer temperatures during their season of death in the geochemical analysis (CdCw-5 2983, CdCw-5 2915, CdCw-5 2164, and CdCw-5 2916). However, all of these samples in disagreement had notes from observers suggesting visualization was limited by damage or disturbance along the growth margin, with the exception of CdCw-5 2164. The remaining shell that underwent both geochemical analysis and inter-observer visualization was marked as 'unknown' by all three observers and therefore was neither in agreement nor disagreement with the negatively trending $\delta^{18}O_{shell}$ value (CdCw-5 3488).

5.3 RADIOCARBON DATES

The radiocarbon dates from the oysters recovered at this site ranged from 184 to 643 years cal BP. These dates are in agreement with Kristmanson's (2019) research which dated the site using terrestrial samples from the deposits. From dates of 700AD into the recent historic period (Kristmanson 2019). Additionally, diagnostic artifacts were utilized to extend the presumed date of occupation to at least 2000ya (Kristmanson 2019). It is possible that there was oyster harvesting outside these dates, and spatial distribution of the shell midden; however, it is likely lost from erosion.

The chronologies for each unit of the site are also in line with Kristmanson's (2019) findings. The most recent deposit came from unit 3C, the closest to the surface of the site's stratigraphy. Additionally, the older radiocarbon dates came from deeper within the midden at unit 2D, with the earliest deposit being sourced from a single shell within unit 4D.

Radiocarbon dates indicate that Level D is the oldest and deepest level, and Level C represents depositions closer to the surface and recent chronology. As expected, the abundance of valves increases throughout stratigraphic time as well, with a larger number of shells being sourced from the most recent deposit around 200 years ago during the more recent historic period.

5.4 STABLE OXYGEN ISOTOPE ANALYSIS & SEA SURFACE TEMPERATURES

Based on the reconstructed temperature derived from stable oxygen isotope analysis, there has been an increase in local sea surface temperatures in the estuaries of Malpeque Bay by an average of 10.9℃ over the last 700 years (Fig 5.1). These findings align with current interpretations of warming climates in the North Eastern Atlantic, specifically Prince Edward Island (Boucher, Arseneault & Sirois 2013). Malpeque Bay has traditionally been the coldest estuary environment in Prince Edward Island, and the rising temperatures at this site are alarming (Fisheries and Oceans Canada 2003). The rapid change in the climate at the Northern point of *C. virginica*'s habitat represents a pressing issue for the species and the people who rely on the abundance of stock for subsistence (Lotze *et al.* 2022). The matter is particularly pressing as discussions of aquaculture have suggested moving the industry to the North to mitigate changing climates in the southern estuaries (Dame 1972; Kent 1992).

Warm or cold season of death was inferred by comparing the $\delta^{18}O_{shell}$ value at the growing edge with the average for each sample. The seasonality of the two live collected shells analyzed for stable oxygen isotope values showed a warmer-than-average collection season trending toward more negative $\delta^{18}O_{shell}$ values. This aligns with the collection date of early September, a period of warm sea surface temperatures in the region as a result of the summer heat sink. Seven of the archaeological collection analyzed showed a warmer-than-average collection season trending toward more negative δ^{18}Oshell values at harvest time. The remaining three indicated a cold collection season trending toward a more positive $\delta^{18}O_{shell}$ value at harvest and calculated temperatures below 5°C.

The calculated range between 0℃ to 10.8℃ recorded over the entire collection's life history is within the lower thresholds of the species limits, indicative of the Northern spatial range and the cooler paleoclimate. Knowing that modern *C. virginica* in this region slow their growth at around 10℃ and fully arrest around 4℃, these resulting paleotemperatures are generally cold and likely result in increased dark banding on the shells (Fisheries and Oceans Canada 2003). Considering that no oysters indicated temperatures below 0℃, those collected in cold weather at temperatures lower than 5℃ were likely collected at the end of fall prior to freezing temperatures or once growth had already arrested growth for the season.

5.5 SEASONALITY & NORMATIVE THINKING ABOUT SHELLFISH HARVESTING

“Normative thinking or normative treatment is that which seeks to identify typical or average behaviour and occurrences. Regional syntheses are usually normative in that they emphasize "facts" while ignoring others (Claassen 1991: 249).” By recognizing the varied periods of occupation and the possibility of more consistent habitation, we can move past ways of normative thinking at shell-midden sites (Claassen 1991). The possibility of harvest that spans multiple seasons is significant as there is some debate within the archaeological record about the seasonal occupation of the Mi'kmaq peoples on Epekwitk. Historians have in the past suggested that the island was only inhabited during the summer period as part of a seasonal migration to the coastal areas of the Maritimes (Sobey, 2007).

Recognizing that the rigidity of an assumed 'summer' occupation may not fully encompass the history of the land and its people is imperative as the Canadian Court systems have often requested evidence of 'sufficient use' in cases where Indigenous populations have sought 'Aboriginal title' or Land Claims Agreements. Both federal and provincial governments have in the past often only recognized occupation that is "sufficient, continuous (where present occupation is relied on) and exclusive (tsilhqot'in nation v. british columbia, 2014 SCC 44, [2014] 2 S.C.R. 256)." This definition of occupation is inherently problematic, based on colonial ways of governance. By readjusting normative thinking within archaeological practice, we can mitigate the damage done through interpretations presented in research, often used as evidence in these cases.

Pituamkek has been a site of ongoing land use tradition for the Mi'kmaq that has been used for activities such as camping, hunting, trapping and fishing for oysters and eels. Additionally, the Island of Pituamkek is a sacred place holding burials of Mi'kmaq ancestors from the community (L'nuey 2021). In August of 2019, the Government of Canada and Government of Prince Edward Island, and the Mi'kmaq Governments of Lennox Island and Abegweit First Nations announced a feasibility assessment to establish a national park reserve within Pituamkek. An area governed similarly to a national park but is the subject of one or more Indigenous land claims negotiated between the federal, provincial, and Indigenous governments is known as a national park reserve. The proposed park would host a “Guardians Program,” a cooperative management program to promote Indigenous uses of heritage places. As of January 2022, a Memorandum of Understanding has been signed, ending the feasibility assessment and beginning negotiations towards a formal national park reserve agreement.

Chief Junior Gould, Abegweit First Nation states that, “Hog Island has sustained the Mi’kmaq people on Epekwitk for thousands of years. To maintain our ancestral connections and traditions, we must preserve these precious lands and waters. Pituamkek is a proposed national park reserve that would protect this wondrous place for the future, ensuring its untouched beauty is available for the Mi’kmaq and for all to experience (Hopkins 2021).”

5.6 SIGNIFICANCE OF THE WORK

This project provided significant contributions to the historical and environmental record of Prince Edward Island and, in a larger context, Mi'kma'ki. Utilizing the archaeological samples from Pitaumkek allows the archaeological record to begin to interpret the oysters' role in the traditional Mi'kmaq subsistence economy for Prince Edward Island settlements. The research produces cultural-environmental information for the Hog Island site (provincial heritage designation pending), which has seen a rise in research over the past few decades to support its heritage preservation status with the Malpeque Bay Archaeological Project that ran from 2006 to 2019 (Kristmanson 2019).

The second reason why this project is significant is that it highlights the impacts of the industrial fishing industry in Malpeque Bay. The contributions to the environmental history of Pitaumkek and its paleoclimate have been used to highlight the impacts of the industrial fishing industry and more extensive conversations regarding the effects of climate change in our coastal waterways. The demonstration of this framework is considerate of the pre-existing structures of sustainability prior to colonization (Barsh 2002; Denny et al. 2016). Oysters are a primary resource of Prince Edward Island, used for subsistence by both the Island's people and sold on a global market scale.

Finally, the project is significant as it generates discussion regarding the effects of climate change in Atlantic coastal waterways. The oysters now retrieved from the effects of coastal erosion at Pitaumkek will contribute to the NSERC Partnership Grant: Climate and Ocean Dynamics Informing Resource Management and Adaptation policy (COD-REMAP), with Dalhousie University, focused on the changing climate in Ocean environments of Atlantic Canada. COD-REMAP will contribute to ongoing resource management strategies for marine harvesting in Atlantic Canada (Lotze *et al.* 2022). According to the study, the Scotian Shelf will diverge from natural climate variability in the near future in 2028 (Lotze *et al.* 2022). Since the colonization of the Europeans, there have been more exploitation-driven changes and a recent, rapid warming that began in the 1960s (Lotze *et al.* 2022). According to future predictions, a high-emissions scenario will result in a rapid warming of 4.5–4.8 °C by the end of the

21st century, with the Scotian Shelf experiencing natural climate fluctuation between 2028 and 2034 (Lotze *et al.* 2022).

5.7 RECOMMENDATIONS FOR FUTURE WORK

In order to offer more precise information for the coastal regions of Prince Edward Island and Malpeque Bay, regular collection of temperature, salinity, and $\delta^{18}O_{water}$ measurements in estuarine habitats is another potential research direction. The availability of these data would enable more accurate estimations for paleotemperature studies. Calculations could be refined beyond a species-specific paleotemperature equation, accommodating local ambient conditions at a higher resolution. Currently, the Canadian Pacific coastlines offer a host of ambient environment conditions through consistent light house monitoring. However, Atlantic Canada still needs to have a comparable program in place for water monitoring. For this reason, water testing of the site at regular intervals would be necessary to refine this data further.

Analyzing *C. virginica* shells obtained from other archaeological sites to obtain paleotemperature estimations for different coastal regions of Atlantic Canada would be another research opportunity to build upon the research reported here. Pituamkek represents the first time *C. virginica* has been analyzed in Canadian archaeology. Comparison with other sites in the region would provide a more holistic picture of historical oyster harvesting through facilitation comparison by providing a multisite analysis. Analyzing multiple sites within the region could tell us if the findings and patterns seen at this site are congruent with other areas of the North Eastern Atlantic seaboard.

Finally, analysis of this data set or others from the North East region would benefit from testing various paleotemperature equations as a part of the analysis. As the environmental recording of ambient conditions is limited in the area, there is limited application of several paleotemperature equations requiring these conditions in their calculation values. However, applications of paleothermometry calculations other than a species-specific equation for the purposes of studying *C. virginica* in the Atlantic would allow for a more comprehensive analysis. If possible, the development of a location-specific equation for this species would significantly increase the fidelity of results in the Gulf of St. Lawrence and the Northern Atlantic as a whole.

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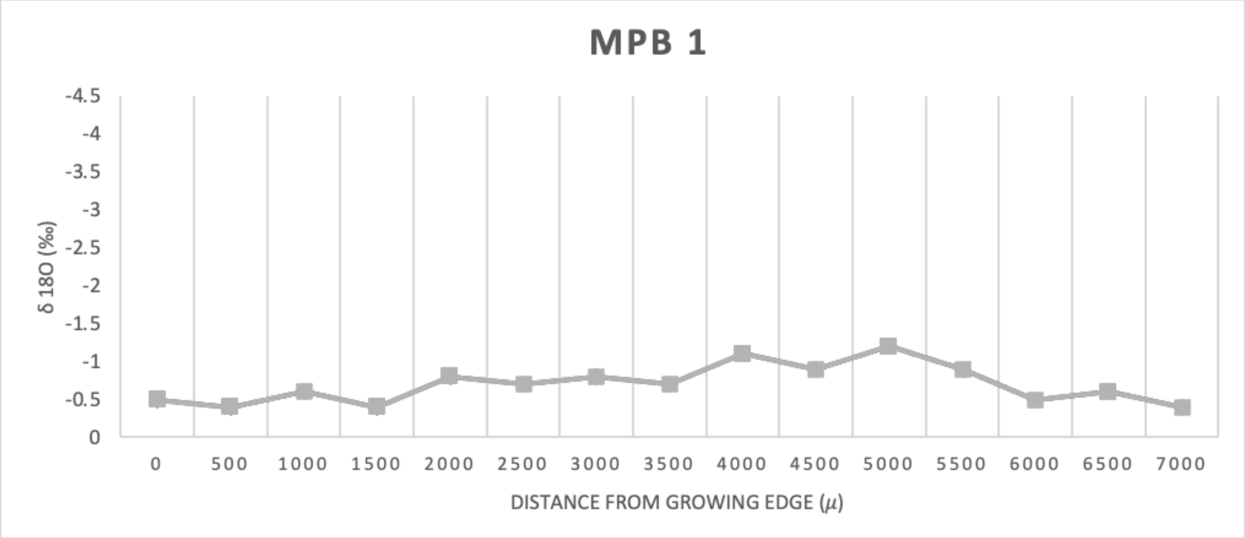
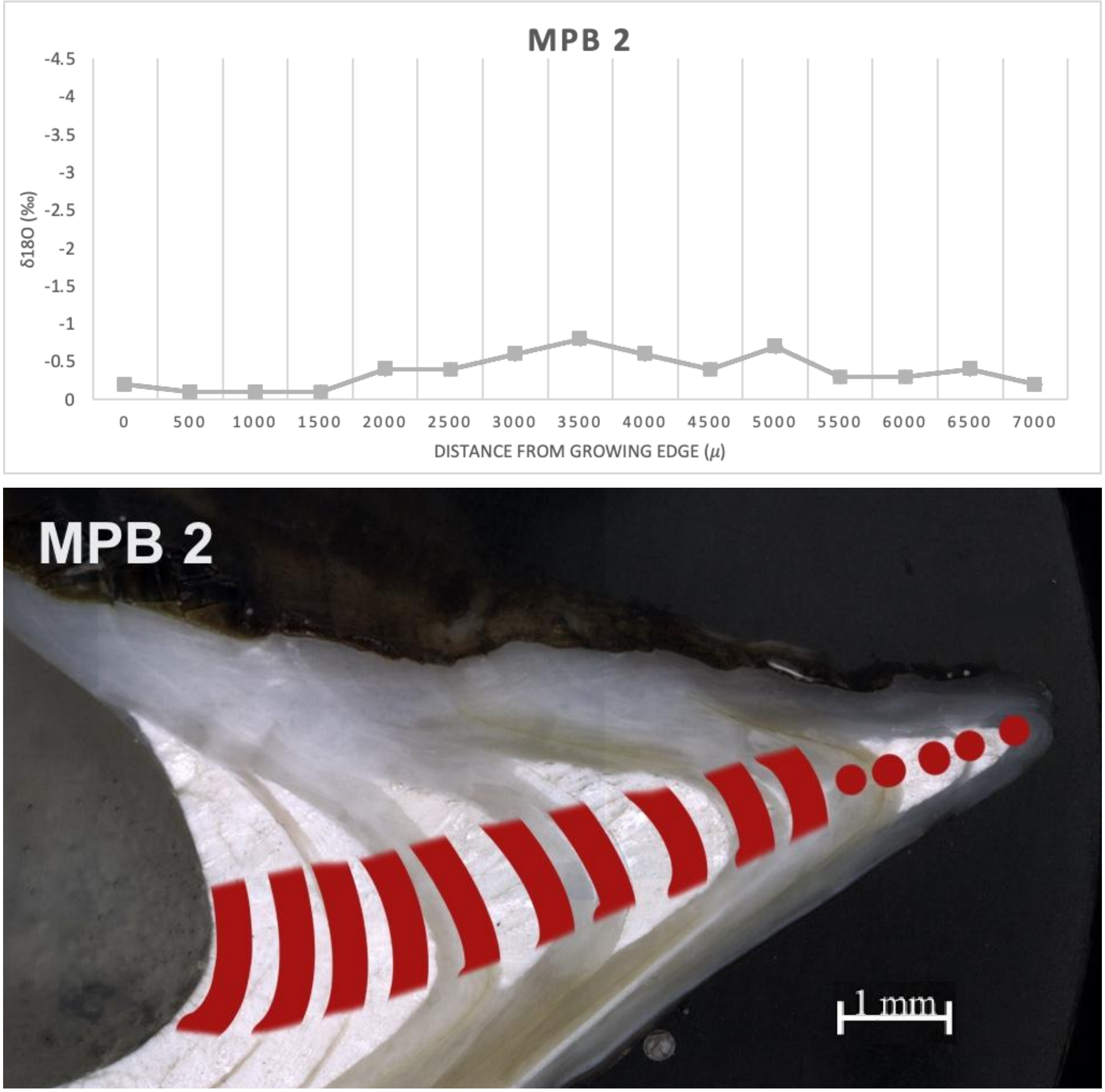
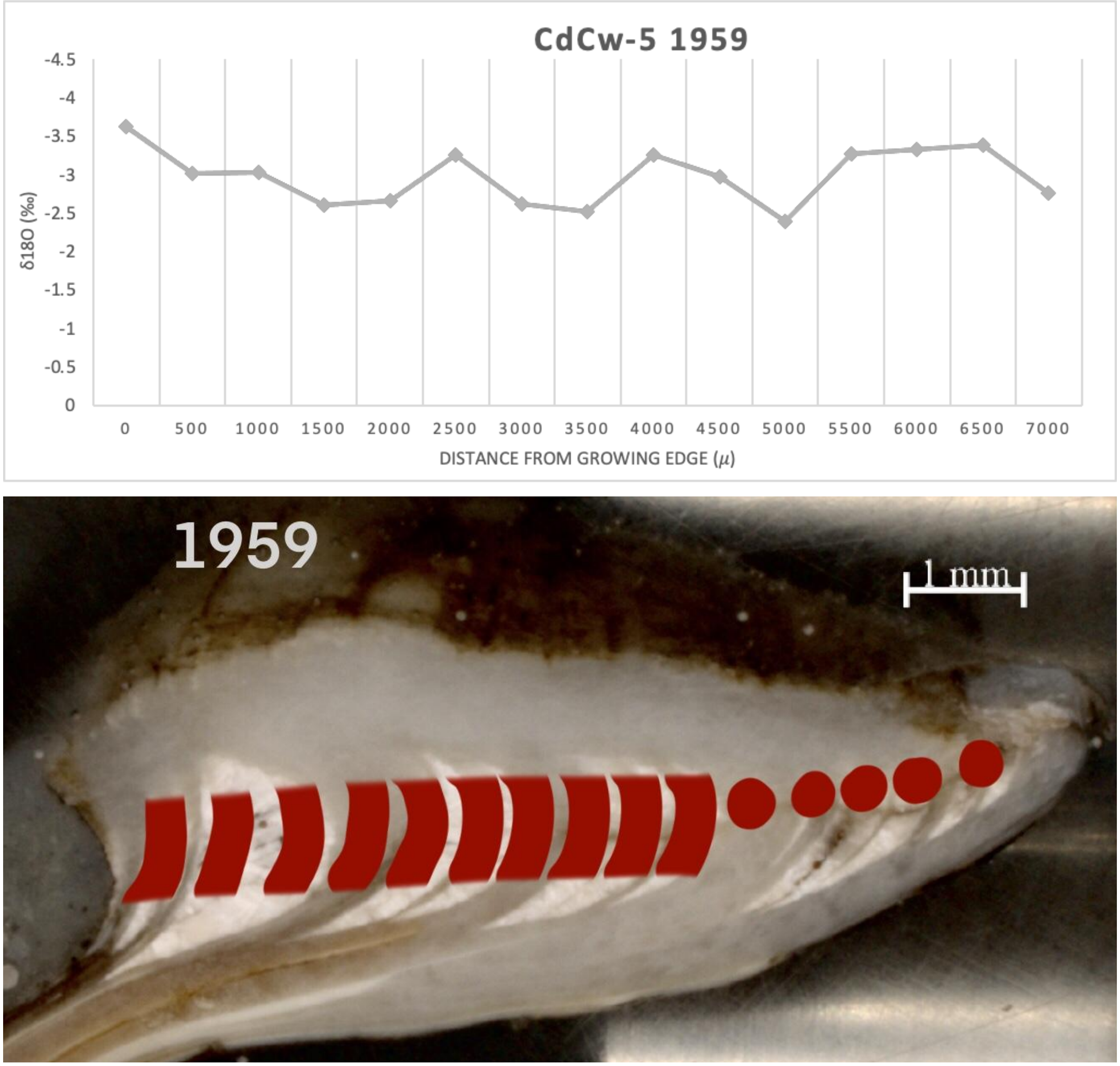
APPENDIX A: STABLE OXYGEN ISOTOPE PROFILES



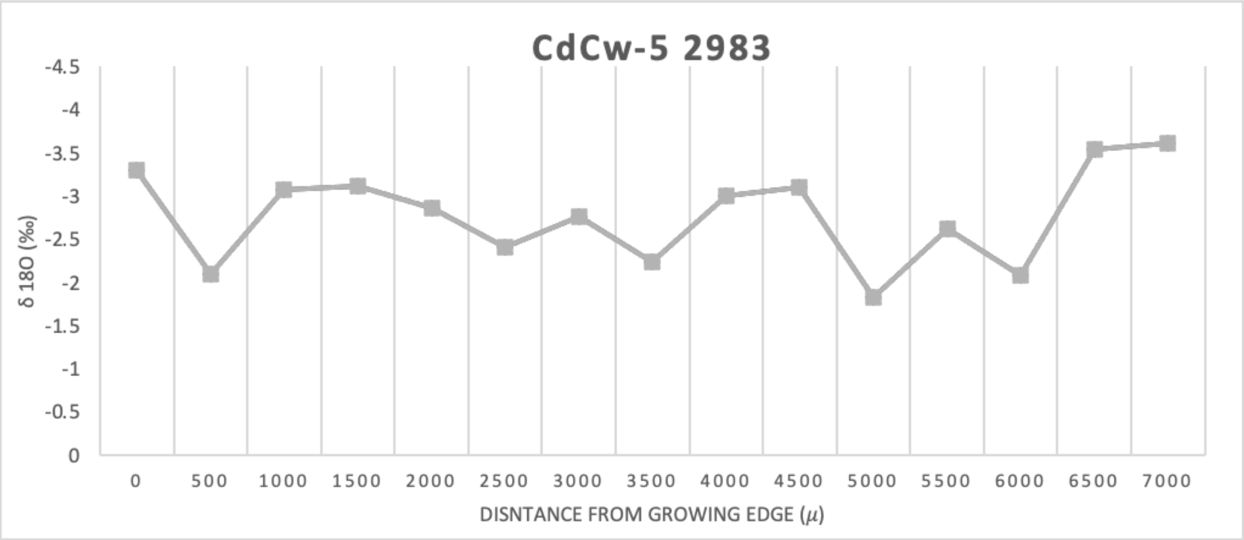
Live-collected *Crassostrea virginica* (MPB 1) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge δ^{18}O value (-0.5‰) trends towards more negative values. Four years of growth were sampled. The maximum δ^{18}O value was -0.4‰ the minimum was -1.2‰.



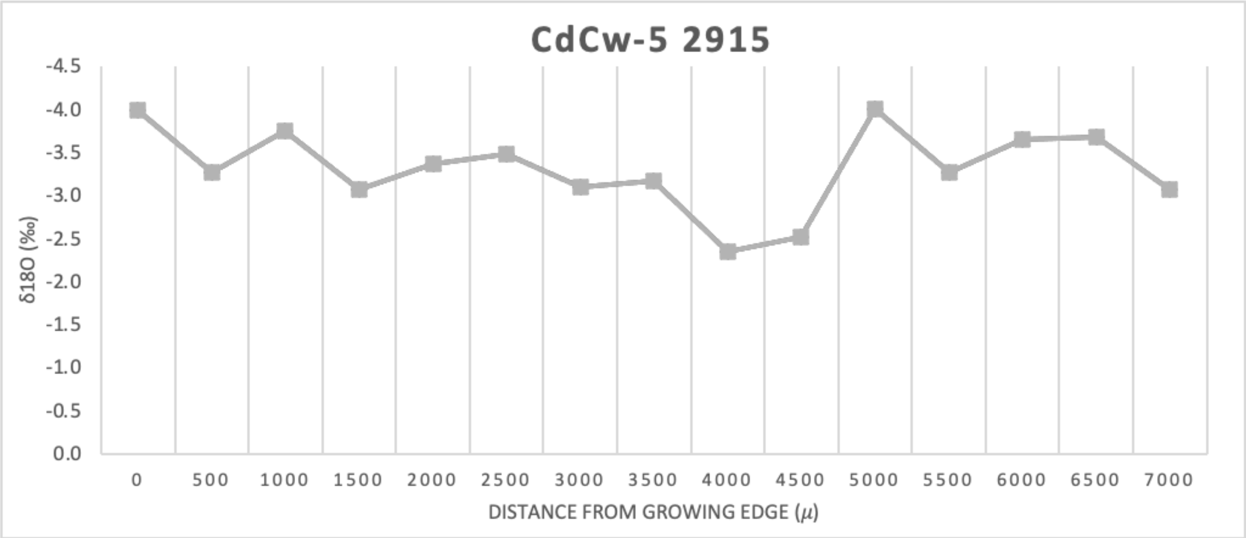
Live-collected *Crassostrea virginica* (MPB 2) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge δ^{18}O value (-0.2‰). Four years of growth were sampled. The maximum δ^{18}O value was -0.1‰ the minimum was -0.8‰.

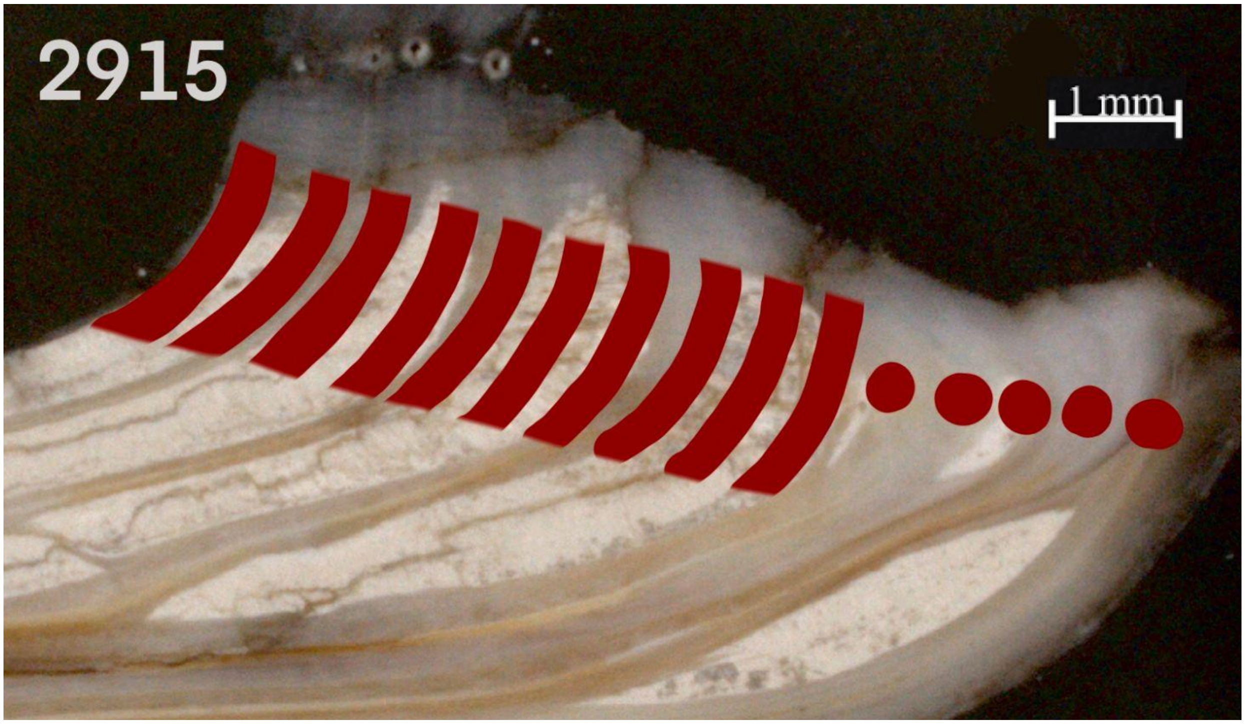


Archaeological *Crassostrea virginica* (CdCw-5 1959) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge δ^{18}O value (-3.62‰) trends towards more negative values. Four years of growth were sampled. The maximum δ^{18}O value was -2.39‰ the minimum was -3.62‰.

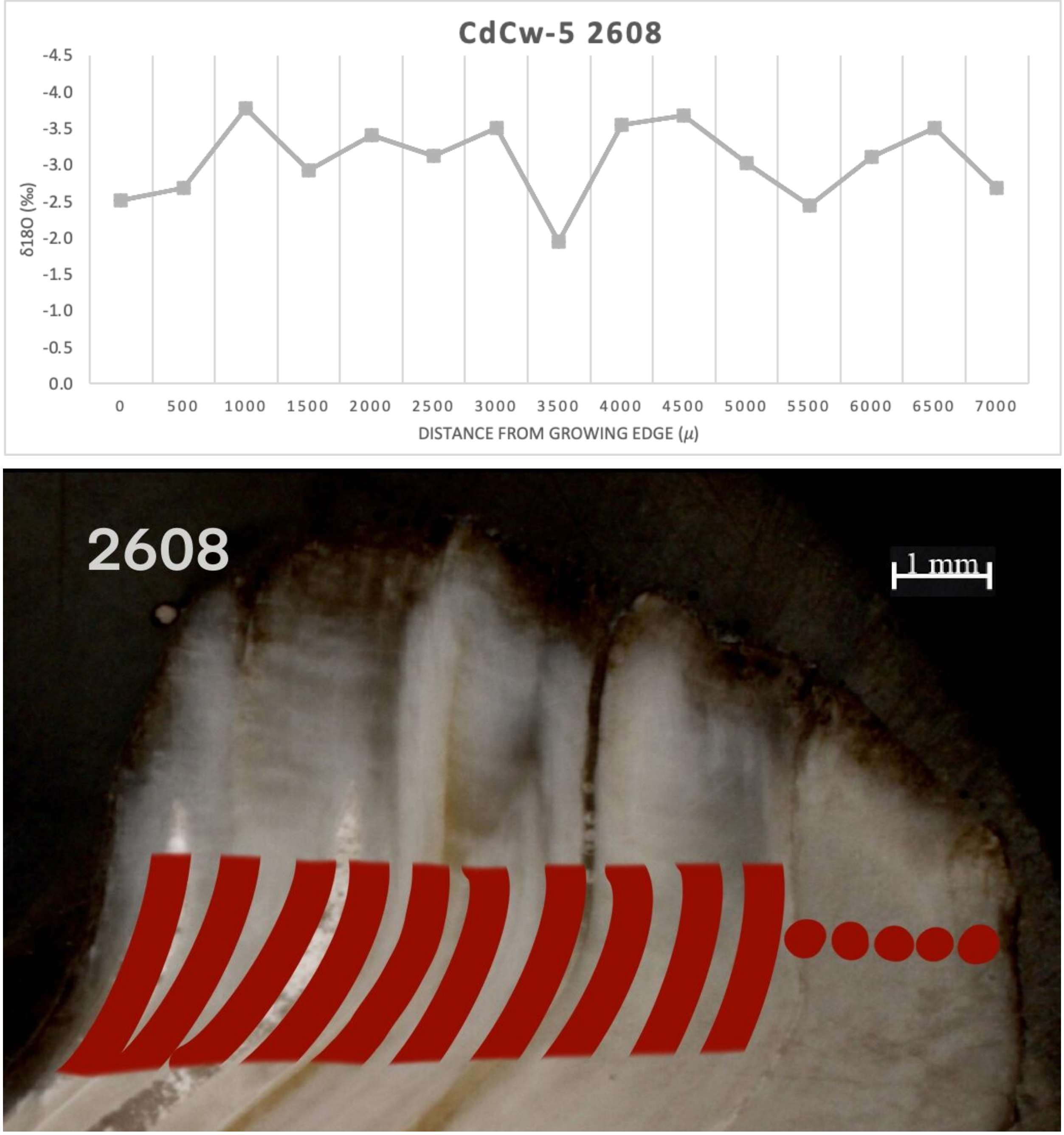


Archaeological *Crassostrea virginica* (CdCw-5 2983) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge $\delta^{18}O$ value (-3.3‰) trends towards more negative values. Five years of growth were sampled. The maximum $\delta^{18}O$ value was -1.83‰ the minimum was -3.62‰.

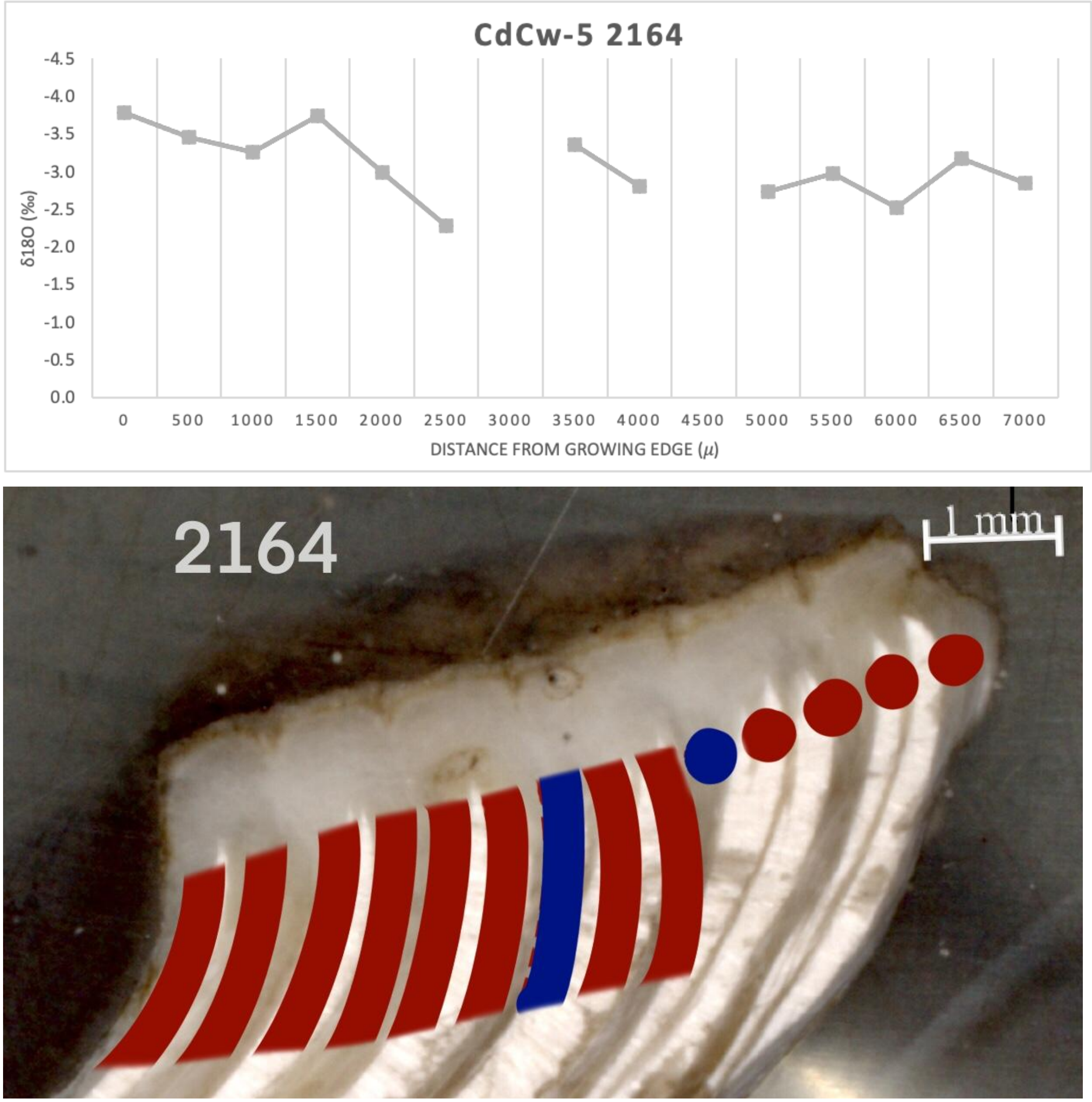




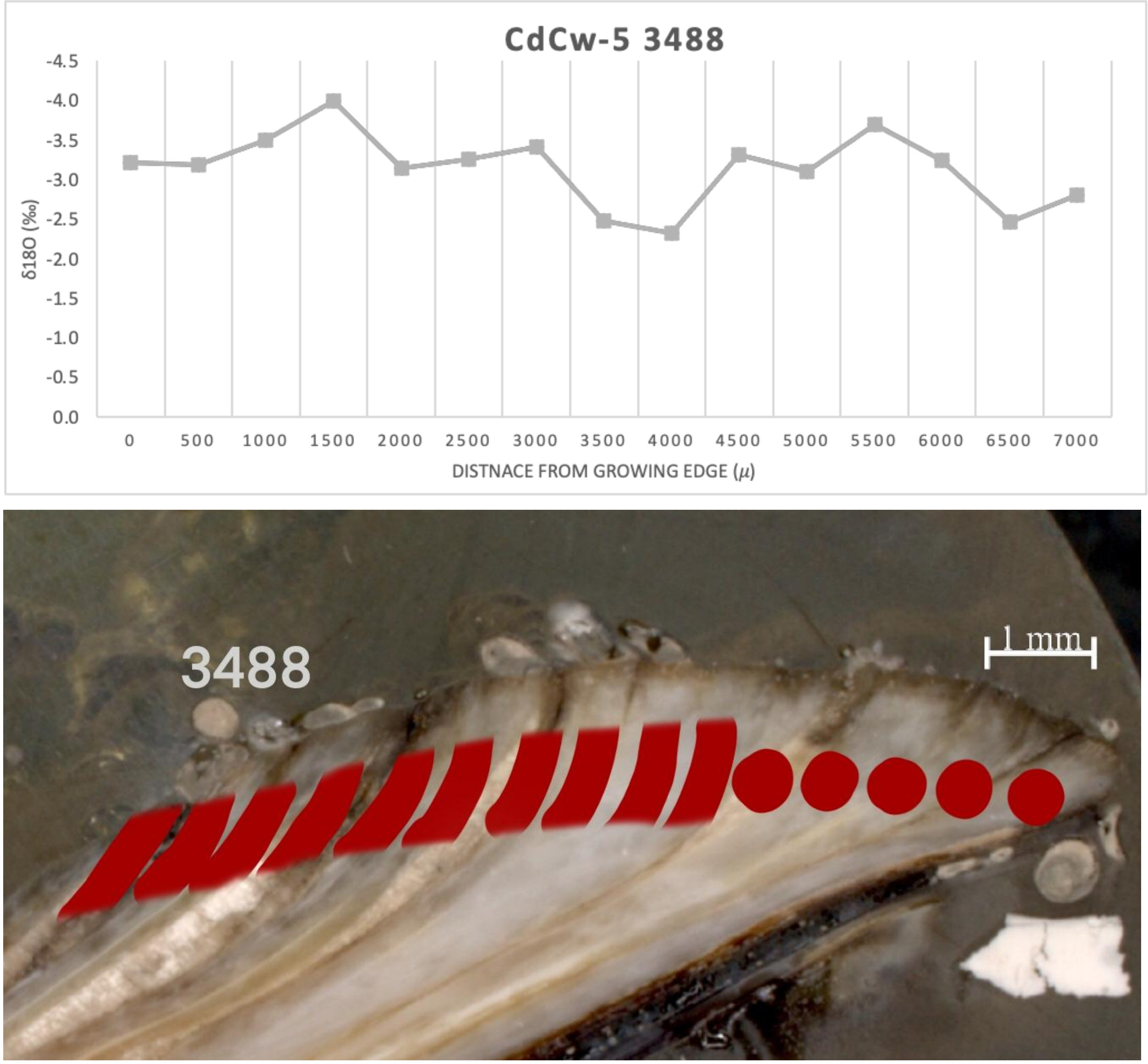
Archaeological *Crassostrea virginica* (CdCw-5 2915) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge $\delta^{18}O$ value (-4.0‰) trends towards more negative values. Five years of growth were sampled. The maximum $\delta^{18}O$ value was -2.3‰ the minimum was -4.0‰.



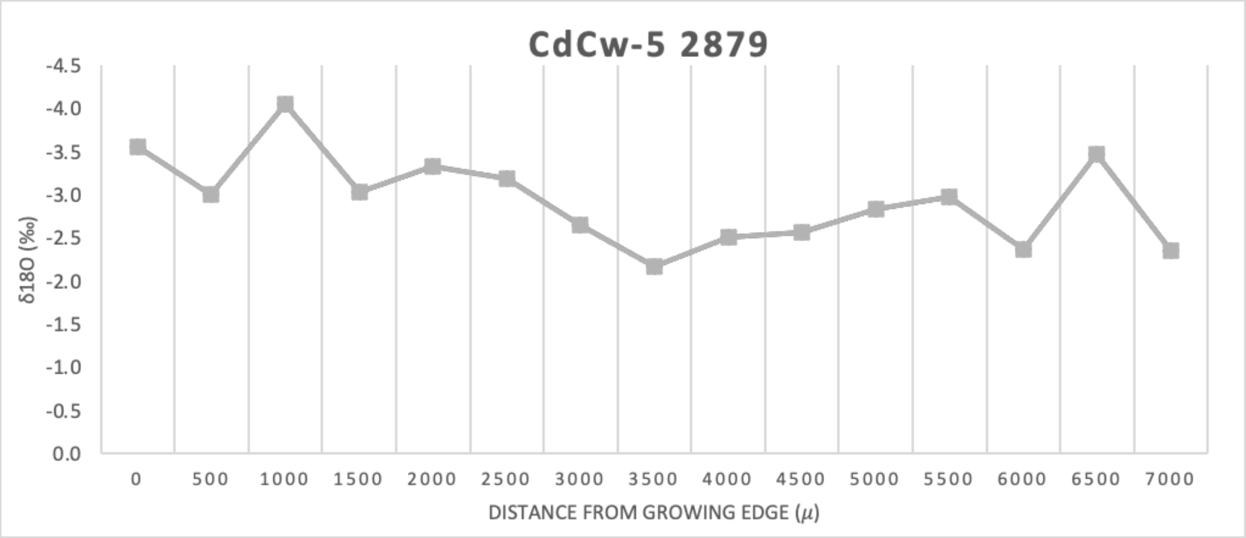
Archaeological *Crassostrea virginica* (CdCw-5 2608) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge δ^{18}O value (-2.5‰) trends toward more positive values. Five years of growth were sampled. The maximum δ^{18}O value was -2.0‰ the minimum was -3.8‰.

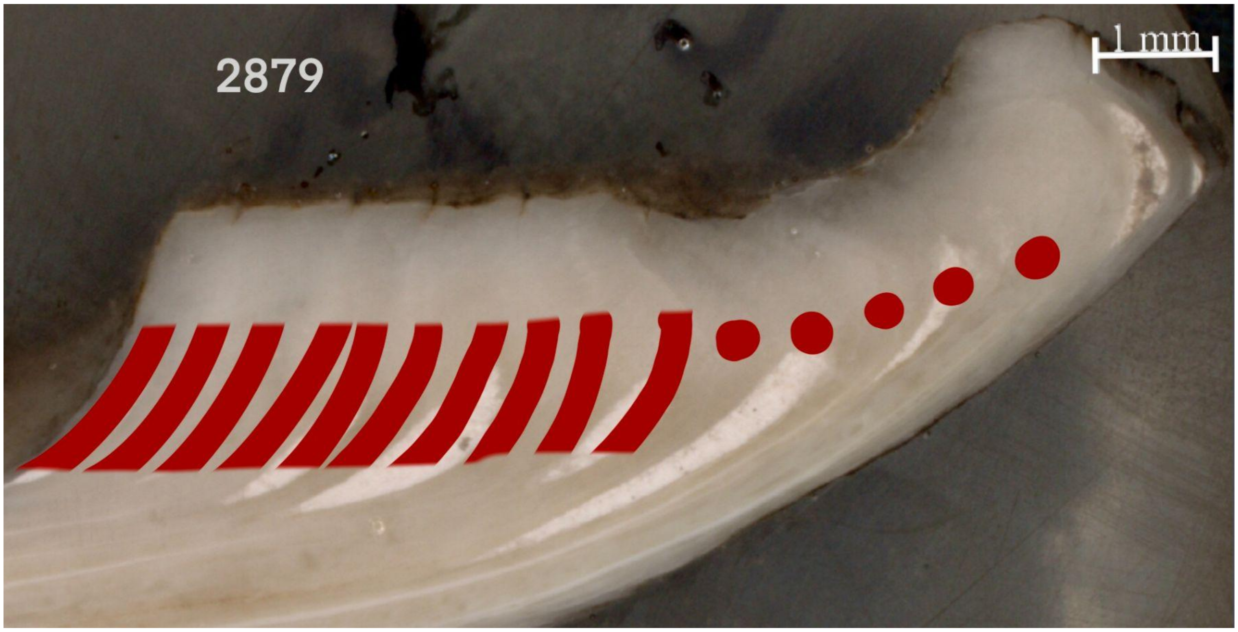


Archaeological *Crassostrea virginica* (CdCw-5 2164) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge $\delta^{18}O$ value (-3.8‰) trends toward more negative values. Six years of growth were sampled. The maximum $\delta^{18}O$ value was -2.0‰ the minimum was -3.8‰. Blue Lines represent values obscured by power surges or minimal material in isotopic analysis.

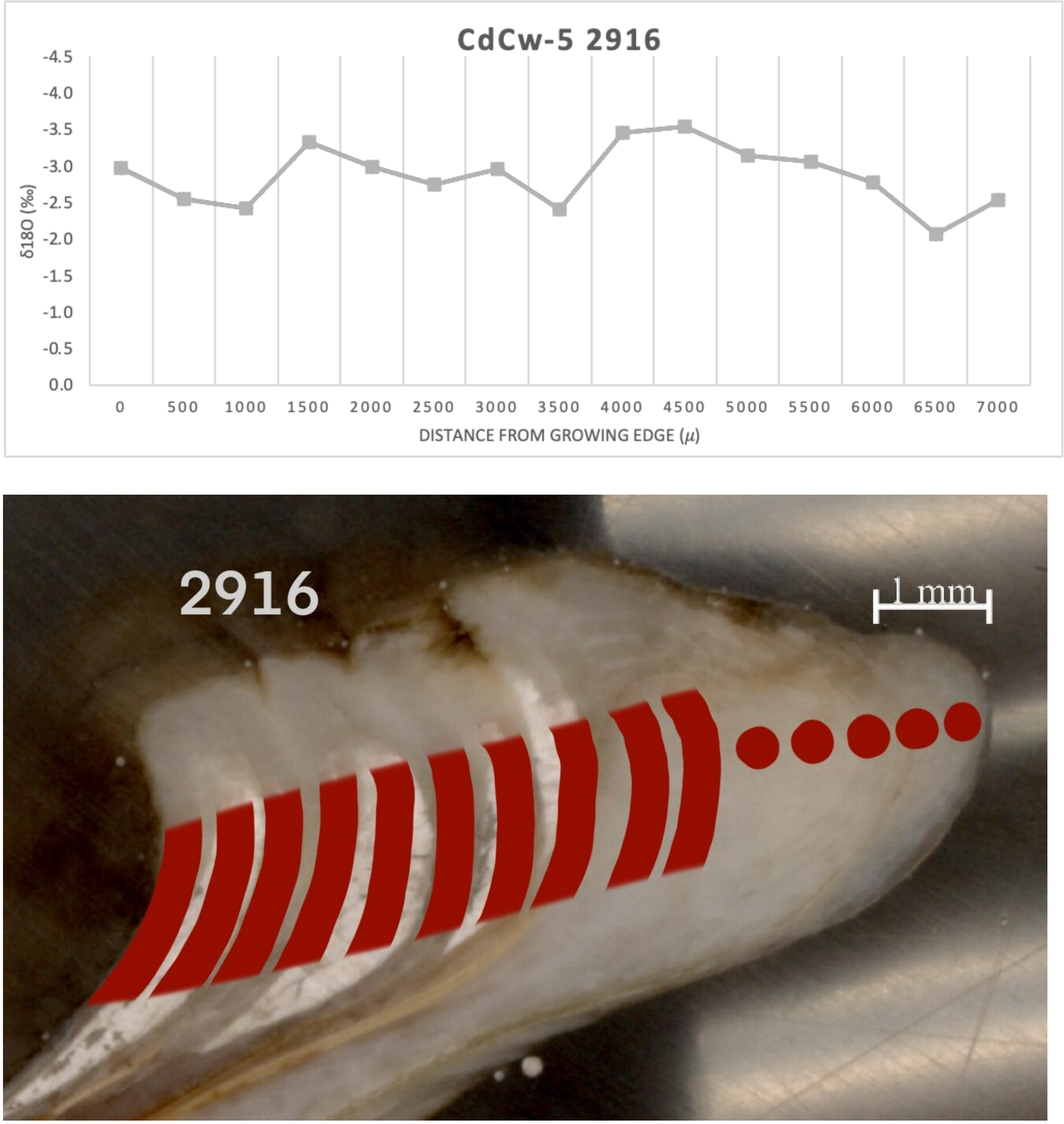


Archaeological *Crassostrea virginica* (CdCw-5 3488) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge δ^{18}O value (-3.2‰) trends toward more negative values. Five years of growth were sampled. The maximum δ^{18}O value was -2.3‰ the minimum was -4.0‰.

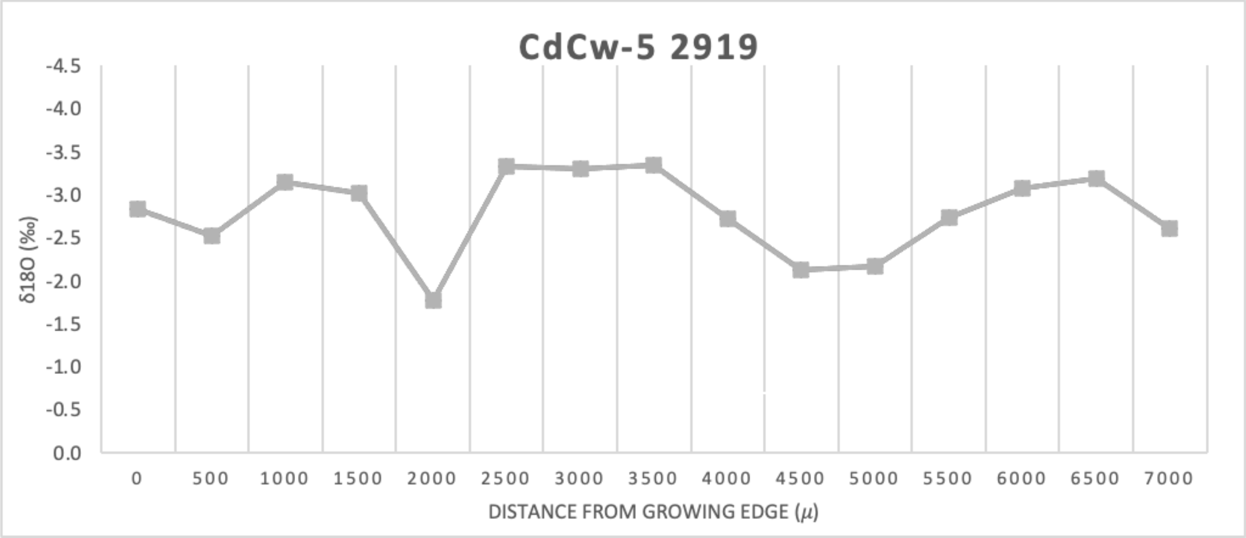




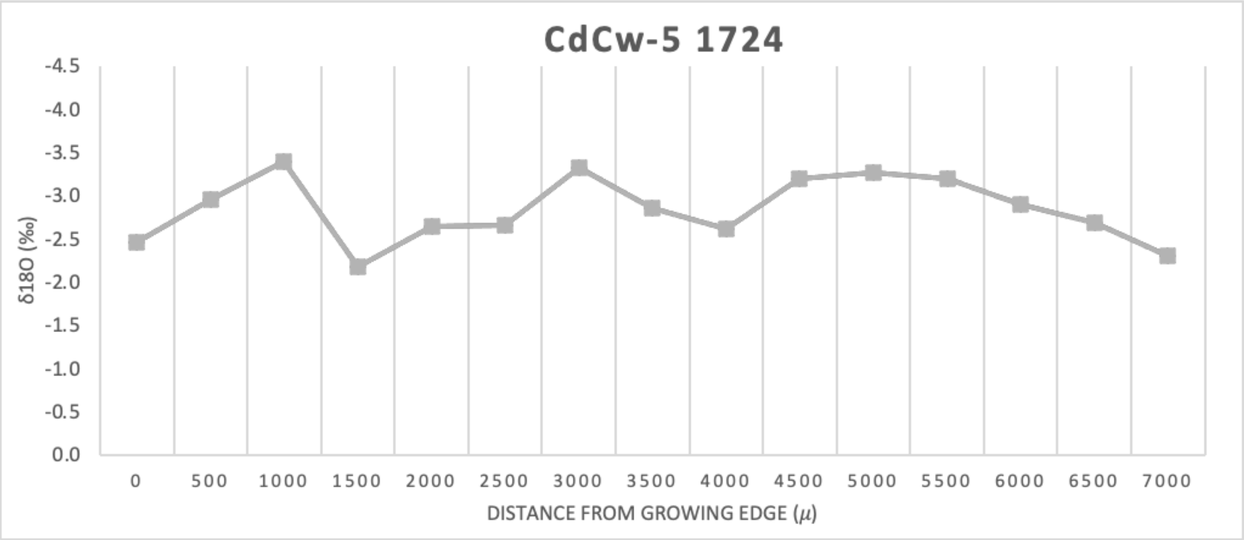
Archaeological *Crassostrea virginica* (CdCw-5 2879) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge δ^{18}O value (-2.2‰) trends toward more positive values. Four years of growth were sampled. The maximum δ^{18}O value was -2.2‰ the minimum was -4.0‰.

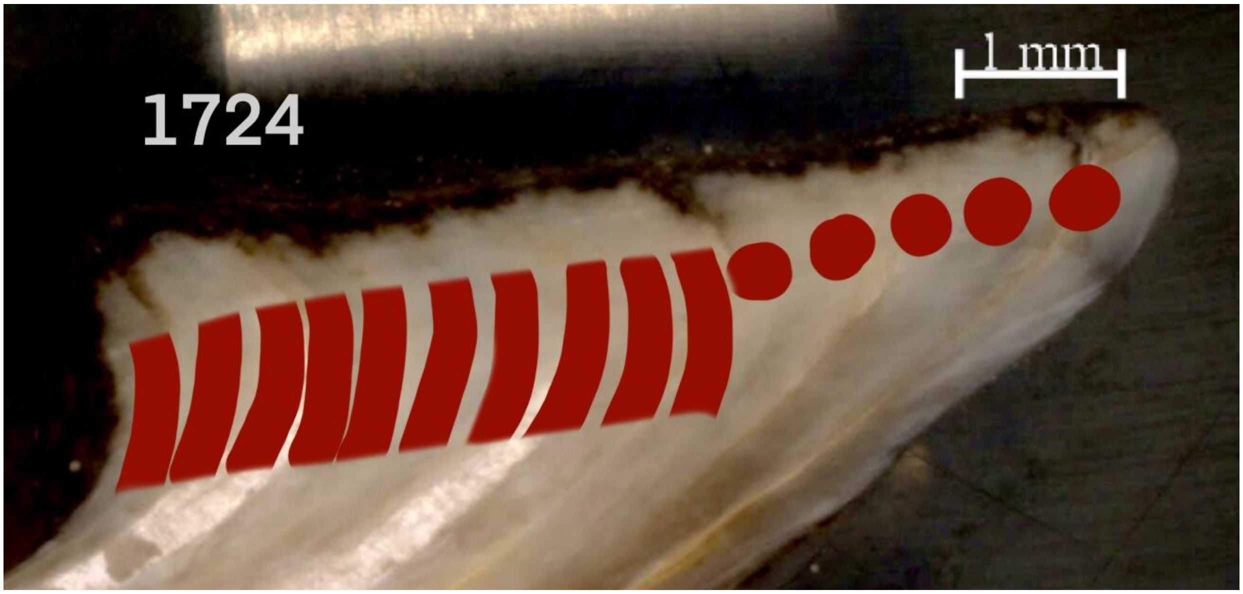


Archaeological *Crassostrea virginica* (CdCw-5 2916) displays a juvenile growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge $\delta^{18}O$ value (-3.0‰) trends toward more negative values. Three years of growth were sampled. The maximum $\delta^{18}O$ value was -2.1‰ the minimum was -3.5‰.



Archaeological *Crassostrea virginica* (CdCw-5 2919) displays a mature growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge $\delta^{18}O$ value (-3.2‰) trends toward more negative values. Four years of growth were sampled. The maximum $\delta^{18}O$ value was -1.8‰ the minimum was -3.4‰.





Archaeological *Crassostrea virginica* (CdCw-5 1724) displays a juvenile growth stage and had 10 micro-milled and 5 micro-drilled shell carbonate samples analyzed. Growing edge $\delta^{18}O$ value (-2.5‰) trends toward more positive values. Three years of growth were sampled. The maximum $\delta^{18}O$ value was -2.2‰ the minimum was -3.4‰.

APPENDIX B: ASSOCIATED COLLABORATIONS & PRESENTATIONS

The Mi'kmaq and the Oyster: An Archaeological Analysis of Traditionally Harvested *Crassostrea virginica* in Prince Edward Island, Canada

Megan MacKinnon, Sarah Kuehn, Marisa Dusseault, Ian Predham & Meghan Burchell
Memorial University of Newfoundland Department of Archaeology[1]

INTRODUCTION

- Pituamkek (CdCw-5) is a 2000-year-old shell midden site located on Hog Island in the North-East of Malpeque Bay, Prince Edward Island, Canada. (Fig. 2).The site has seen consistent use into the modern era.[6]
- The site was occupied as a part of ***Epekwitk*** - the Indigenous title of the Island, and is now under federal designation. The excavation of the eroding shell midden site (CdCw-5) was part of a broader Mi'kmaq-led initiative to conserve the ecological and cultural resources of the Island beginning in 2006 (Fig. 2).[6]
- In this region, oyster growth slows below 10°C and begins to arrest at temperatures lower than 4°C.[5]

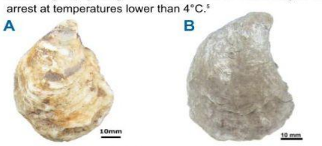


Fig.1. *Crassostrea virginica* recovered from Malpeque Bay, A. Live-collected (Sept. 2019), B. Archaeological

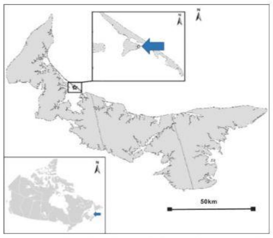


Fig. 2: Map of PEI, showing Pituamkek. The site is located in the Gulf of St. Lawrence, which is impacted by the Labrador current. This is the highest natural latitude of the species. Oysters in this area grow at a rate of 10mm/year.

This research contributes to the ongoing 'Malpeque Bay Archaeological Project' The Mi'kmaq - the Indigenous population of the region - have long-standing strategic harvesting to protect this resource: "Netukulimk, take only what you need."[3]

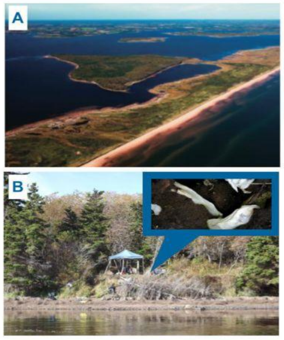


Fig. 3: A. Hog Island sand dunes, Malpeque Bay, PEI. B. 2019 excavation of eroding shell midden (CdCw-5).

MATERIALS & METHODS

- Oyster hinges were embedded in epoxy, and cut to 3mm thick sections,[3 7] along the axis of maximum growth with a low-speed diamond saw.
- Cross-sections were polished using Buehler MetaServ 250 Grinder-Polisher with a 1µm colloidal solution with SiC Grit (320/P400), SiC Grit (600/P1200) discs and a Texmet© Polishing Cloth.
- Images were captured under 10X using a Zeiss AxioZoom V.16 microscope under reflected light (Figs. 4 & 5).

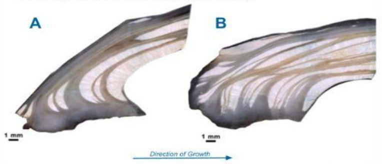


Fig. 4. Cross-sections of live-collected oyster hinges collected Sept. 2019, Malpeque Bay, PEI under reflected light. A. Shows 'regular' growth patterns in a younger shell (~3-4 years). B. Shows 'senile' growth with disturbance in an older specimen (5+ years).

Radiocarbon Dating

- FTIR confirmed that samples for $\delta^{18}O$ and C^{14} analysis are all pristine calcite.
- Three shells were submitted for C^{14} dating at the A.E. Lalonde Radiocarbon Lab, University of Ottawa, Canada.
- Dates were calibrated to 2σ using IntCal20 with a marine reservoir of ΔR -77 +/- 77.

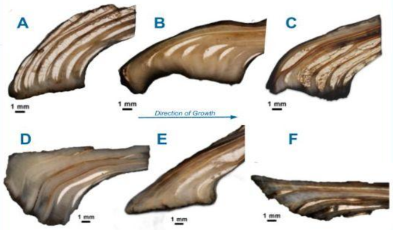


Fig. 5. Can season of collection be reliably read using light/dark growth lines? Archaeological oyster hinges- Thick-sections with reflected light. A. Warm growth going into a colder phase ; B. Warm growth going into colder growth; C. White banding at season of collection showing late phase of warm growth; D. Irregular growth with warm growth into a colder phase, with possible disturbance lines; E. Irregular early, warm growth with 'going into cold; F. Irregular early growth with warm growth going into cold.

Stable Oxygen Isotopes ($\delta^{18}O$)

- Two live-collected shells and 10 archaeological shells were were sampled using a Merchantek New Wave Research micromill.
- Five samples were milled from the growing edge using a 480µm drill bit, followed by 10 samples drilled every 500µm. Growth bands and sampling locations were aligned to assess the relationship between seasonal growth and isotope values.
- Samples were processed at the Ján Veizer Stable Isotope Laboratory on a Thermo DeltaPlus XP continuous flow - IRMS coupled to a Thermo Scientific TC/EA. Having a Conflo IV Costech Zero-blank autosampler with an analytical precision of ± 0.3‰.
- A species-specific paleothermometry equation (Anderson and Arthur 1983) was used for SST reconstruction[1]

$$T\,(^{\circ}C) = 16 + 4.14\,(\delta^{18}O_c - \delta^{18}O_w) + 0.13\,(\delta^{18}O_c - \delta^{18}O_w)^2$$

RESULTS

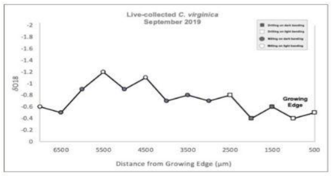


Fig. 5. A $\delta^{18}O$ profile of a live-collected oyster. Open symbols are on light bands, and closed symbols are on dark bands.

- C^{14} for the three returned dates from 643-207 years cal BP.
- The $\delta^{18}O$ results for the live-collected shells range from -0.84 to -0.5 with a calculated SST from 13.5°C to 18.1°C.
- The $\delta^{18}O$ results for the archaeological samples are more more negative, with a range from -4.0 to -1.8, and calculated SST from 0°C -10.8°C.

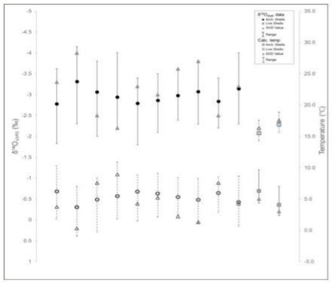


Fig. 6. $\delta^{18}O$ results and calculated SST of live-collected and archaeological oysters. Archaeological samples are represented by circles while live-collected are represented by squares. Filled symbols indicate mean values and vertical solid lines represent the range. Open symbols indicate mean calculated SST and vertical dotted lines indicate range.

CONCLUSIONS

- $\delta^{18}O$ shows cold and warm season of collection, but it is not possible to refine to a single season.
- Warm and cold growth can be identified in the growth lines, but refining to a season (winter/spring/summer/ autumn) is not precise.
- There is an increase in SST by an average of ~10.9°C over ~700 years.
- The majority of shells are collected in warm water, but year-round harvesting and occupation is also likely, which aligns with current interpretations of this site's occupation history[6].

Acknowledgements & References

In the spirit of Reconciliation, we acknowledge that the land upon which our organization stands is unceded Mi'kmaq territory. Epekwitk (PEI), Mi'kma'ki, is covered by the historic Treaties of Peace and Friendship. We pay our respects to the Indigenous Mi'kmaq People who have occupied this Island for over 12,000 years; past, present and future.

Thank you to Dr. Helen Kristmanson, and the staff at CREAIT & Ján Veizer Stable Isotope Lab, University of Ottawa.

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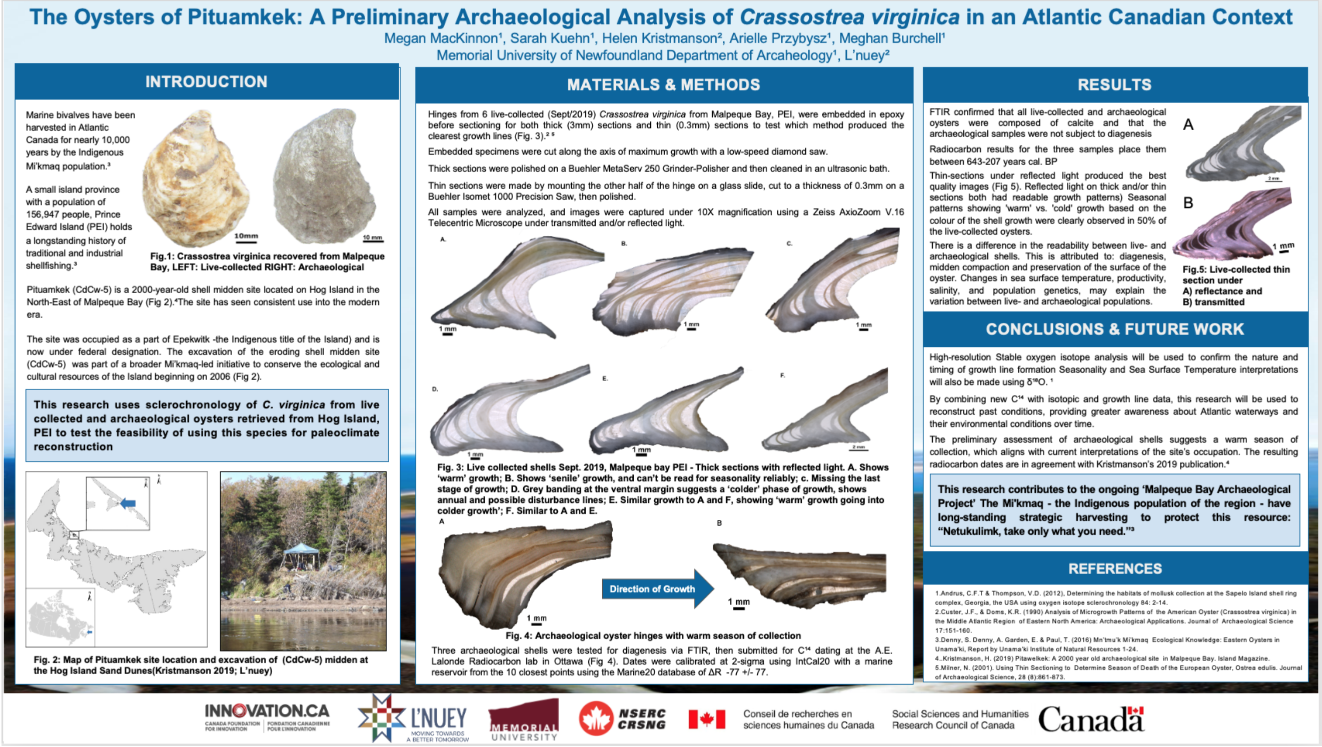

The Oysters of Pituamkek: A Preliminary Archaeological Analysis of *Crassostrea virginica* in an Atlantic Canadian Context

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INTRODUCTION

Marine bivalves have been harvested in Atlantic Canada for nearly 10,000 years by the Indigenous Mi'kmaq population.[3]

A small island province with a population of 156,947 people, Prince Edward Island (PEI) holds a longstanding history of traditional and industrial shellfishing.[3]

Fig.1: Crassostrea virginica recovered from Malpeque Bay, LEFT: Live-collected RIGHT: Archaeological

Pituamkek (CdCw-5) is a 2000-year-old shell midden site located on Hog Island in the North-East of Malpeque Bay (Fig 2).[4]The site has seen consistent use into the modern era.

The site was occupied as a part of Epekwitk -the Indigenous title of the Island) and is now under federal designation. The excavation of the eroding shell midden site (CdCw-5) was part of a broader Mi'kmaq-led initiative to conserve the ecological and cultural resources of the Island beginning on 2006 (Fig 2).

This research uses sclerochronology of *C. virginica* from live collected and archaeological oysters retrieved from Hog Island, PEI to test the feasibility of using this species for paleoclimate reconstruction

Fig. 2: Map of Pituamkek site location and excavation of (CdCw-5) midden at the Hog Island Sand Dunes(Kristmanson 2019; L'nuey)

MATERIALS & METHODS

Hinges from 6 live-collected (Sept/2019) *Crassostrea virginica* from Malpeque Bay, PEI, were embedded in epoxy before sectioning for both thick (3mm) sections and thin (0.3mm) sections to test which method produced the clearest growth lines (Fig. 3).[2 5]

Embedded specimens were cut along the axis of maximum growth with a low-speed diamond saw.

Thick sections were polished on a Buehler MetaServ 250 Grinder-Polisher and then cleaned in an ultrasonic bath.

Thin sections were made by mounting the other half of the hinge on a glass slide, cut to a thickness of 0.3mm on a Buehler Isomet 1000 Precision Saw, then polished.

All samples were analyzed, and images were captured under 10X magnification using a Zeiss AxioZoom V.16 Telecentric Microscope under transmitted and/or reflected light.

Fig. 3: Live collected shells Sept. 2019, Malpeque bay PEI - Thick sections with reflected light. A. Shows 'warm' growth; B. Shows 'senile' growth, and can't be read for seasonality reliably; c. Missing the last stage of growth; D. Grey banding at the ventral margin suggests a 'colder' phase of growth, shows annual and possible disturbance lines; E. Similar growth to A and F, showing 'warm' growth going into colder growth'; F. Similar to A and E.

Fig. 4: Archaeological oyster hinges with warm season of collection

Three archaeological shells were tested for diagenesis via FTIR, then submitted for C^{14} dating at the A.E. Lalonde Radiocarbon lab in Ottawa (Fig 4). Dates were calibrated at 2-sigma using IntCal20 with a marine reservoir from the 10 closest points using the Marine20 database of ΔR -77 +/- 77.

RESULTS

FTIR confirmed that all live-collected and archaeological oysters were composed of calcite and that the archaeological samples were not subject to diagenesis

Radiocarbon results for the three samples place them between 643-207 years cal. BP

Thin-sections under reflected light produced the best quality images (Fig 5). Reflected light on thick and/or thin sections both had readable growth patterns) Seasonal patterns showing 'warm' vs. 'cold' growth based on the colour of the shell growth were clearly observed in 50% of the live-collected oysters.

There is a difference in the readability between live- and archaeological shells. This is attributed to: diagenesis, midden compaction and preservation of the surface of the oyster. Changes in sea surface temperature, productivity, salinity, and population genetics, may explain the variation between live- and archaeological populations.

Fig.5: Live-collected thin section under
A) reflectance and
B) transmitted

CONCLUSIONS & FUTURE WORK

High-resolution Stable oxygen isotope analysis will be used to confirm the nature and timing of growth line formation Seasonality and Sea Surface Temperature interpretations will also be made using $\delta^{18}O$. [1]

By combining new C^{14} with isotopic and growth line data, this research will be used to reconstruct past conditions, providing greater awareness about Atlantic waterways and their environmental conditions over time.

The preliminary assessment of archaeological shells suggests a warm season of collection, which aligns with current interpretations of the site's occupation. The resulting radiocarbon dates are in agreement with Kristmanson's 2019 publication.[4]

This research contributes to the ongoing 'Malpeque Bay Archaeological Project' The Mi'kmaq - the Indigenous population of the region - have long-standing strategic harvesting to protect this resource: "Netukulimk, take only what you need."[3]

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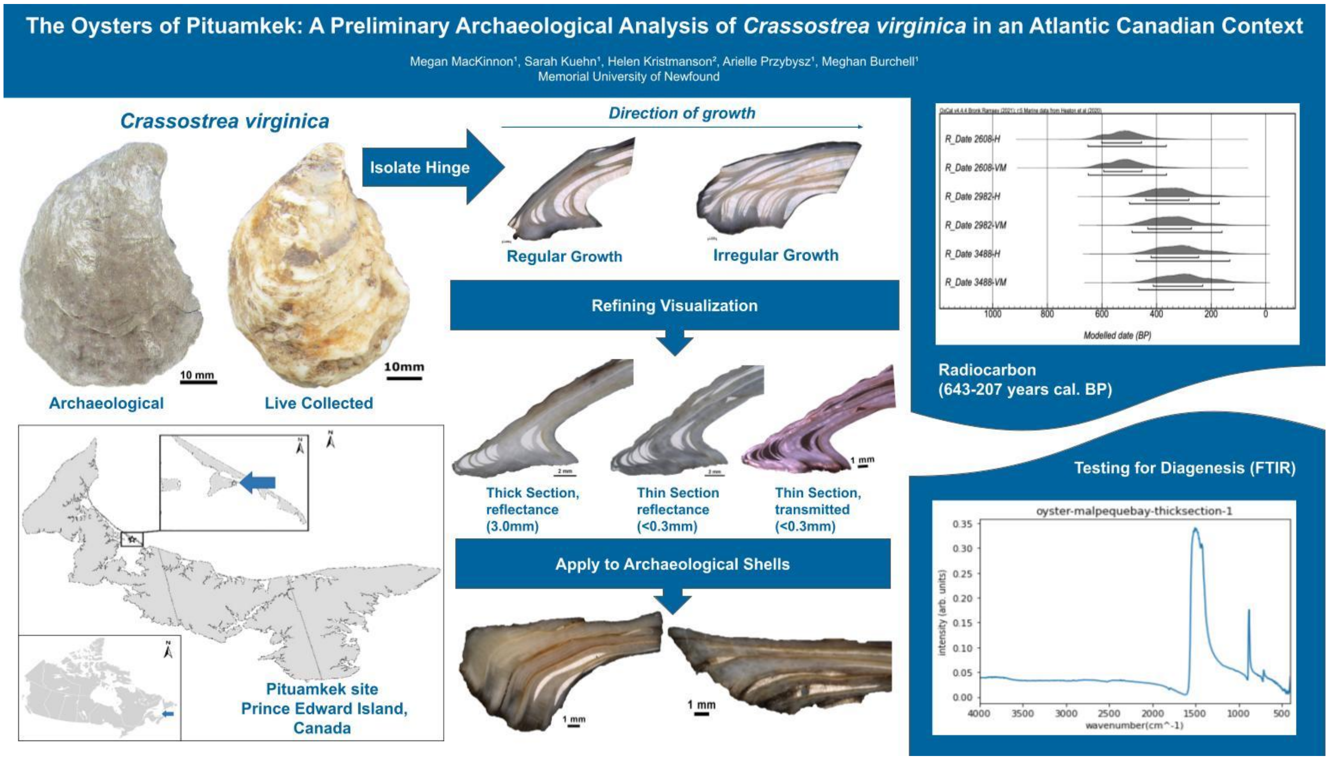

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Crassostrea virginica

Archaeological

Live Collected

Isolate Hinge

Direction of growth

Regular Growth

Irregular Growth

Refining Visualization

Thick Section, reflectance (3.0mm)

Thin Section reflectance (<0.3mm)

Thin Section, transmitted (<0.3mm)

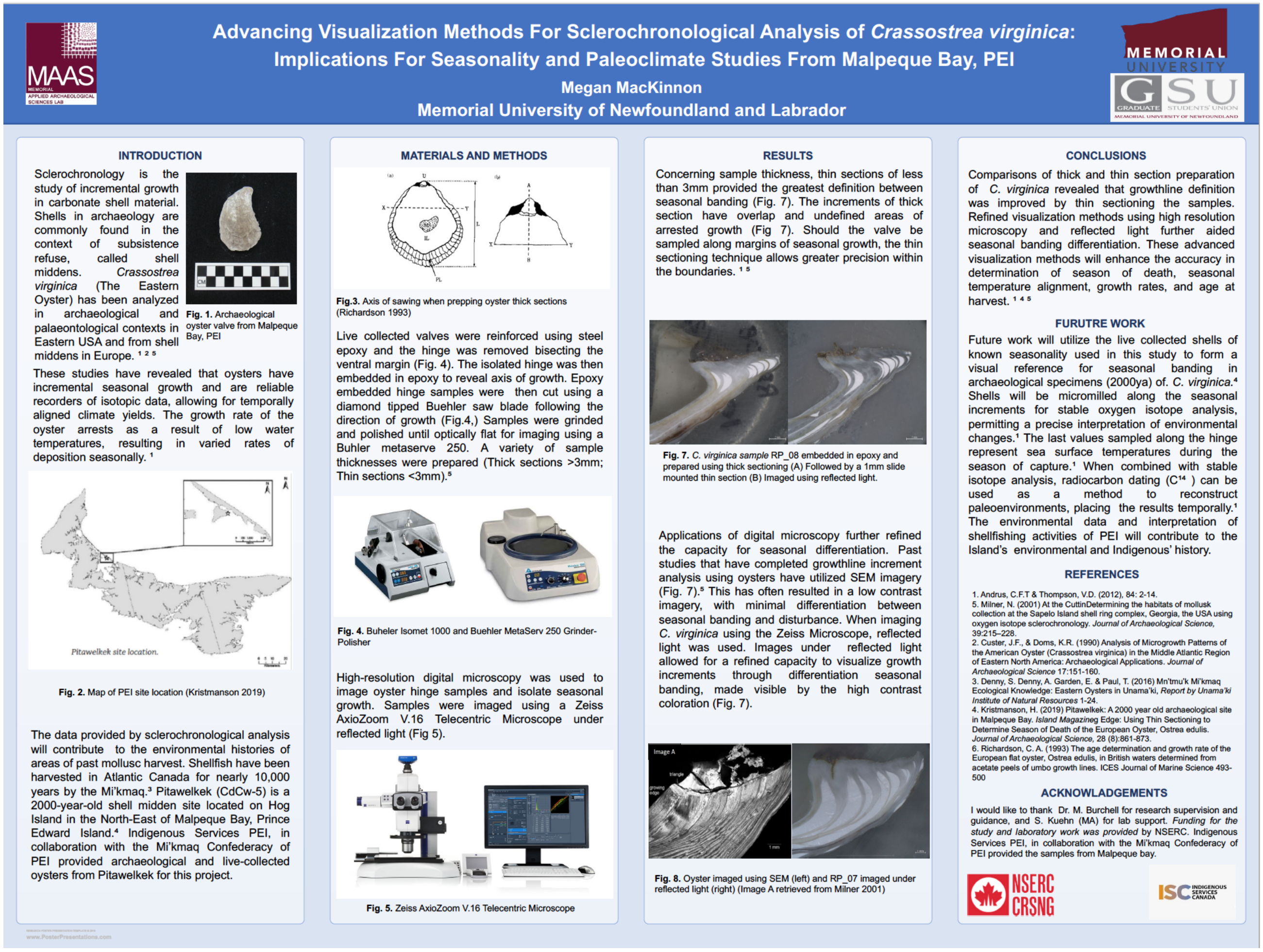
Apply to Archaeological Shells

Pituamkek site
Prince Edward Island, Canada

Radiocarbon
(643-207 years cal. BP)

Testing for Diagenesis (FTIR)

Lightning slide presented at the Virtual International Sclerochronology Conference (Remote), 2022



Advancing Visualization Methods For Sclerochronological Analysis of *Crassostrea virginica*: Implications For Seasonality and Paleoclimate Studies From Malpeque Bay, PEI

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INTRODUCTION

Sclerochronology is the study of incremental growth in carbonate shell material. Shells in archaeology are commonly found in the context of subsistence refuse, called shell middens. *Crassostrea virginica* (The Eastern Oyster) has been analyzed in archaeological and palaeontological contexts in Eastern USA and from shell middens in Europe. [1,2,5]

Fig. 1. Archaeological oyster valve from Malpeque Bay, PEI

These studies have revealed that oysters have incremental seasonal growth and are reliable recorders of isotopic data, allowing for temporally aligned climate yields. The growth rate of the oyster arrests as a result of low water temperatures, resulting in varied rates of deposition seasonally. [1]

Fig. 2. Map of PEI site location (Kristmanson 2019)

The data provided by sclerochronological analysis will contribute to the environmental histories of areas of past mollusc harvest. Shellfish have been harvested in Atlantic Canada for nearly 10,000 years by the Mi'kmaq.[3] Pitawelkek (CdCw-5) is a 2000-year-old shell midden site located on Hog Island in the North-East of Malpeque Bay, Prince Edward Island.[4] Indigenous Services PEI, in collaboration with the Mi'kmaq Confederacy of PEI provided archaeological and live-collected oysters from Pitawelkek for this project.

MATERIALS AND METHODS

Fig.3. Axis of sawing when prepping oyster thick sections (Richardson 1993)

Live collected valves were reinforced using steel epoxy and the hinge was removed bisecting the ventral margin (Fig. 4). The isolated hinge was then embedded in epoxy to reveal axis of growth. Epoxy embedded hinge samples were then cut using a diamond tipped Buehler saw blade following the direction of growth (Fig.4,) Samples were grinded and polished until optically flat for imaging using a Buhler metaserve 250. A variety of sample thicknesses were prepared (Thick sections >3mm; Thin sections <3mm).[5]

Fig. 4. Buheler Isomet 1000 and Buehler MetaServ 250 Grinder-Polisher

High-resolution digital microscopy was used to image oyster hinge samples and isolate seasonal growth. Samples were imaged using a Zeiss AxioZoom V.16 Telecentric Microscope under reflected light (Fig 5).

Fig. 5. Zeiss AxioZoom V.16 Telecentric Microscope

RESULTS

Concerning sample thickness, thin sections of less than 3mm provided the greatest definition between seasonal banding (Fig. 7). The increments of thick section have overlap and undefined areas of arrested growth (Fig 7). Should the valve be sampled along margins of seasonal growth, the thin sectioning technique allows greater precision within the boundaries. [1,5]

Fig. 7. *C. virginica sample* RP_08 embedded in epoxy and prepared using thick sectioning (A) Followed by a 1mm slide mounted thin section (B) Imaged using reflected light.

Applications of digital microscopy further refined the capacity for seasonal differentiation. Past studies that have completed growthline increment analysis using oysters have utilized SEM imagery (Fig. 7).[5] This has often resulted in a low contrast imagery, with minimal differentiation between seasonal banding and disturbance. When imaging *C. virginica* using the Zeiss Microscope, reflected light was used. Images under reflected light allowed for a refined capacity to visualize growth increments through differentiation seasonal banding, made visible by the high contrast coloration (Fig. 7).

Fig. 8. Oyster imaged using SEM (left) and RP_07 imaged under reflected light (right) (Image A retrieved from Milner 2001)

CONCLUSIONS

Comparisons of thick and thin section preparation of *C. virginica* revealed that growthline definition was improved by thin sectioning the samples. Refined visualization methods using high resolution microscopy and reflected light further aided seasonal banding differentiation. These advanced visualization methods will enhance the accuracy in determination of season of death, seasonal temperature alignment, growth rates, and age at harvest. [1,4,5]

FURUTRE WORK

Future work will utilize the live collected shells of known seasonality used in this study to form a visual reference for seasonal banding in archaeological specimens (2000ya) of. *C. virginica.*[4] Shells will be micromilled along the seasonal increments for stable oxygen isotope analysis, permitting a precise interpretation of environmental changes.[1] The last values sampled along the hinge represent sea surface temperatures during the season of capture.[1] When combined with stable isotope analysis, radiocarbon dating (C^{14}) can be used as a method to reconstruct paleoenvironments, placing the results temporally.[1] The environmental data and interpretation of shellfishing activities of PEI will contribute to the Island's environmental and Indigenous' history.

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