A paleoecological survey of salt marsh sediments in the Bagaduce River

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Fall 2018

OS401
11/15/2018

Abstract

High rates of salt marsh accretion leads to the rapid preservation of ecological indicators in the sediments (Roman et al. 1984). As these indicators, in this case pollens and microcharcoals, are buried, they act as natural documentation of the ecological conditions at the time of their deposition. The objective of this study was to determine how pollen genera and microcharcoals changed over salt marsh sediment depth. This was done by collecting marsh cores and subsampling five different depths within them. Samples were processed through the University of Maine's standard HF palynology separation protocol (Nurse 2005), an adapted form of the Faegri et al. 1989 standard, then counted under a microscope. Pollen counts were found to be outside the expected distribution (Chi^2) and highly variable, while charcoals were shown to increase with depth. Some pollen genera were absent completely while others were observed at a higher than average frequency, however several trends were seen in some pollens such as *Betula*. Further study of sediments in this area would reveal more about its history.

Introduction

It is only in the last 12,000 years, due to the sudden recession of glaciers, that much of the vegetation that covers the state of Maine has been present. This rapid exposure, geologically speaking, of land allowed for plant life to begin to return to the area. This began with tundra vegetation such as "dwarf" plants from genus Alnus (Alder) and Salix (Willow), and eventually progressing to the larger trees, such as genus Picea (Spruce) (Barton et al. 2012). In addition, as the glaciers retreated, the crust rebounded to previous elevations which led to an eventual drop in relative sea level of up to 10ft/100yr (Barton et al. 2012). Beginning up to 15,000 years ago, this process slowed and halted after 2,000-3,000 years (Barton et al. 2012), allowing for the development of complex coastal ecosystems, such as salt marshes, that were adapted for that intertidal environment (Roman et al. 1984).

Salt marshes are areas that are made up of coastal grassland that experience regular flooding due to tidal activity (Dionne et al. 2003). They provide a food source and habitat for a variety of organisms, from microscopic bacteria to the species of birds that use them as fishing grounds (Dionne et al. 2003). Many of these areas also act as natural buffer zones against erosion, helping to maintain the integrity of the coastline over the passage of time (Castillo et al. 2000). The frequent flooding causes dominant vegetation in salt marshes to be coastal grasses that can withstand the high soil salinity (Roman et al. 1984). Over time, these grasses become buried in with the marsh flats via sediments that had been transported tidally up the marsh creeks (Stoddart et al. 1989). This process, accretion, is when new layers are created by deposition, creating a well-defined, stratified ground composition (Roman et al. 1984). The rates of accretion vary throughout marshes, ranging from less than 0.2cm yr^{-1} to over 1cm yr^{-1} (Stoddart et al. 1989), but are generally much higher than terrestrial soil, making them excellent sites for studying sediment accumulation over time.

One potential ecological indicator that is buried in salt marshes through this accretion process is pollen grains from local vegetation (Dee et al. 2016). Pollen is released from vegetation and, over time, different species and amounts are buried based on significant environmental changes (Moss et al. 2016) such as sea level rise, ecological changes, and Anthropocene impacts (e.g. deforestation). This natural record allows an analysis of the pollen grains to determine what the local vegetation was like at the time that the sediment sample was formed. The reason salt marshes are excellent for this type of analytical process is due to their high rates of accretion (Stoddart et al. 1989), due mostly to the tidal transport and deposition of sediments, allowing environmental indicators to become buried. Pollen grains are not the only

indicators to be buried; others such as micro charcoals can also be found preserved in sediments (Miola et al. 2010).

Microcharcoals are the byproducts of the incomplete combustion of some organic materials, such as wood (Mooney and Tinner 2011). Combustion gives off tiny charcoals, known as microcharcoals when less than 100μm in diameter (Mooney and Tinner 2011), which are commonly emitted by natural forest fires (McCarroll et al. 2017). These particulates are carried away by the wind and eventually deposited in numerous locations, such as in salt marshes. These charcoals quickly become buried through the same rapid-accretion process as pollen and are preserved (Miola et al. 2010). Being resistant to degradation through biological or oxidative processes, these particles can remain intact on a geologic timescale (Mooney and Tinner 2011). The excavated charcoals can be used to quantify the level of combustion that took place during the time of deposition, which in turn can be used to build a fire history for an area (McCarroll et al. 2017).

The study of these and other excavated proxies is the basis of paleoecology, the study of fossil flora and fauna. By viewing and analyzing how the abundance and diversity of indicators have changed, changes can be correlated to known events, such as natural disasters (McCaroll et al. 2017), and a cause-effect relationship for past, present, and future ecological changes can be theorized (Miller 2015). This was the first study to utilize this method in the salt marshes that dot the Bagaduce river in Penobscot, ME. The objective of this study was to determine how the concentrations of various pollen genera and microcharcoals changed over the salt marsh core depths.

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Methods

Overview

The objective of this study was to see how pollen genera change over sediment depth within a salt marsh core. Samples for analysis were taken from a local salt marsh in Penobscot, ME. Three sediment cores approximately 0.5 m in length were removed at 5-meter intervals in a 10-meter transect. 15 1cc subsamples from each core were processed through the standard HF pollen protocol at the University of Maine at Orono (UMaine). Microscopic analysis was begun at UMaine and concluded at Maine Maritime Academy (MMA), with advanced identification guides and reference pollen samples used for identification.

Site description

My study site was a salt marsh located at the northernmost point of Northern Bay off of the Bagaduce River (44°28'23.6"N, 68°43'32.6"W) in Penobscot, ME (Fig. 1a, 1b). The area is an intertidal salt marsh that experiences twice daily seawater flooding and ebbing. The majority of the vegetation in the marsh is intertidal grasses such as *Spartina alterniflora* that can withstand the high salinity present in the soil.

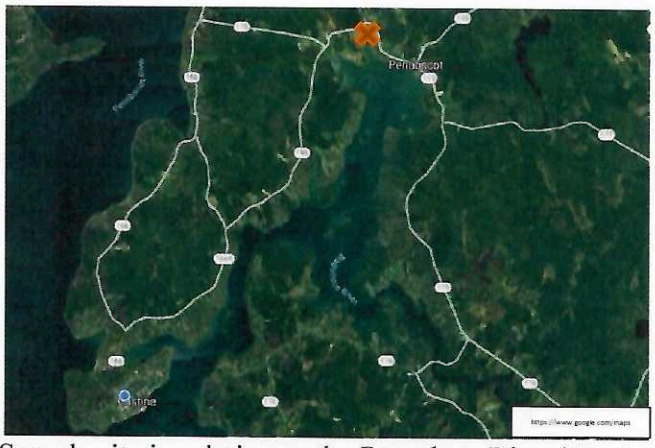

Figure 1a. Sample site in relation to the Bagaduce River/Northern bay area

Figure 1b. Site map displaying where specific cores were taken

Field collection

I utilized a 2424-A Wildco hand coring device to take three cores five meters apart, approximately 0.5m deep in the topmost sediments within the intertidal salt marsh, from the area defined as high-marsh by the abundance of *Spartina patens*, evidence of infrequent tidal inundation. Cores were intended to be replicates in case of samples becoming compromised during pollen processing, as well as to verify pollen counts. The intact cores were taken and sealed within their coring tubes, then returned to MMA where they were stored under refrigeration for 24 hours until subsamples were taken. Subsamples 1cc in size were taken from the core surface down at 0.5-1.5cm, 4.5-5.5cm, 14.5-15.5cm, 29.5-30.5cm, and 39.5-40.5cm of depth and placed in 15 mL centrifuge tubes. The number of samples taken was defined by the different layer transitions observed within the cores. All samples were then frozen for 8 days until lab processing at UMaine.

Sample separation

Samples were processed according to a modified protocol adapted by Nurse (2005) from the methods described in Faegri et al. (1989) at the UMaine's Climate Change Institute with the assistance of Andrea Nurse, a Paleoecological research associate at UMaine. Equipment used includes a Fisher Scientific Isotemp Heating Block, Fisher Scientific Mini Vortexer, and

ThermoFisher Scientific Sorvall ST16 Centrifuge. The abbreviation [CF-D] will indicate when a sample was vortex mixed, centrifuged at 3500 rpm for five minutes and then decanted (either into a sink or appropriate waste bottle).

Each sample was placed in 15 ml centrifuge tubes with 5 ml of 5% aq. KOH to remove humic acids (soils), and vortex mixed briefly. After heating for 20 minutes at 100˚C in the heating block, samples were CF-D, with samples being re-mixed with deionized (DI) water followed by CF-D twice or until the supernate (liquid) was clear. Microbead suspensions of 50,000 beads mL^{-1} were added to each sample to serve at quantifiable references during microscope counts, with 1mL being added to surface samples and 2mL added to all others as reference quantities for later pollen counts. These concentrations were chosen to account for the reduced presence of pollen at the surface layers. Sediment was re-suspended in 5% aq. KOH and put through a 125 μm sieve into 50-ml tubes to remove large particles such as sand and plant matter; things that wouldn't be completely dissolved by acids in later steps. DI water was used to rinse smaller particles through the mesh into the tube, followed by CF-D. This was followed by treatment with 5 ml of 10% aq. HCl in order to dissolve any carbonates present within samples. Samples were vortex mixed with several drops of Ethyl alcohol in order to control the amount of bubbles formed, followed by vortex mixing when foam stops. Each were then placed in the heating block for five minutes, then CF-D.

All procedures from this point until the final KOH treatment were conducted in an HF protective fume hood, wearing heavy-duty protective gloves, aprons and a face shield. All centrifuging from this point until the final KOH treatment was conducted with acid bucket-caps in place over centrifuge racks to prevent any possibility of spillage. 5 ml of 45% aq. HF were added to containers to dissolve siliceous matter (minerals, clays, etc.). With centrifuge tube caps

loosened to allow for light ventilation, samples were placed in the heat block for 20 minutes at 100°C to accelerate disintegration, followed by CF-D. 5 ml of glacial acetic acid was added in order to completely dehydrate the samples, followed by CF-D. This step was crucial; it is critical that NO water come into contact with samples during the next step as acetic anhydride is extremely hydrophobic. A solution of 4.5 ml acetic anhydride and 0.5 ml concentrated sulfuric acid was mixed in each tube and vortexed for acetolysis, the removal of any cellulose that remained, then heated at 100°C for three minutes and CF-D. Sediments were then rewashed with glacial acetic acid again to remove remaining acetic anhydride, then CF-D. Again, this step was vital to ensure that no water came into contact with the acetic anhydride solution. After decanting and rinsing samples, the lab day concluded and samples were left to sit overnight.

The following day, after donning standard lab safety equipment again, samples were suspended in 5mL of 5% aq. KOH, followed by sieving through 6 μm screens with DI rinses through the screens into the containers. Pollen grains are found within the general sizing range of approximately 8-120μm, meaning that finer particles would be filtered out. Pollens were backwashed from the screens into 15 ml centrifuge tubes and CF-D. After being suspended in Ethyl alcohol to dehydrate them, samples were vortex mixed followed by CF-D. Then samples were suspended in 5 ml tertiary butyl alcohol (TBA) and moved to labeled ½-dram vials, CF-D, and allowed to evaporate. Equal amounts of silicone oil to the sample volume present were added, stirred, and allowed to sit in a fume hood overnight so the TBA can evaporate, leaving the oil and pollen mixture.

Microscope identification

I was able to conduct my training for microscopic identification at the UMaine palynology lab, with the remainder of identification happening at MMA. A Nikon Eclipse 50i

microscope with SPOT camera attachment was used for slide counts. Due to time limitations, only two of the three (Site 1 and Site 2) cores were subjected to analysis, as 3-4 hours was required per slide (10 slides total). Pollen major taxonomic vegetative groups were identified based on visible features at 40x magnification from pollen ID guides by Faegri et al. (1989) and McAndrews at al. (1973), pollen and vegetation ID websites by Go Botany (2018) and Palynological Database (2018), as well as a catalog of macrofossil samples made available at UMaine. Measurements were conducted with three vertical transects across each slide to account for some pollens migrating to the edge upon placement of the cover slips.

Data analysis

After the completion of pollen counting, data were input into Excel to normalize transect counts to the whole sample originally processed. Total counts of pollen densities were calculated by using the ratio of microbeads to pollen observed. The following formula was used to calculate percent abundances of pollens compared to the whole of the sample.

$$\frac{((\text{microbead conc.} \times \text{mL microbeads added} \times \text{number pollen counted}) / \text{microbeads counted})}{\text{original sample volume}}$$

(Nurse 2005)

Normalized data was then inputted into SPSS for statistical analysis. A Chi^2 analysis was conducted to examine the sample distribution of pollen within each depth as well as the abundance (or lack of) of each genera over the core as a whole. Following this, a series of graphs representing pollen count change within a specific genera over depth was generated using the data. A regression analysis was used to determine the relationship between number of microcharcoals found and sample depth, with a line graph being generated from the data.

Results

The presence and abundance of pollen genera was highly varied between sampling depths. The observed distribution of pollen genera over the depth of the core was outside the expected distribution (pearson Chi^2 test, $p<0.001$, $df=88$, $n_{d1}genera= n_{d2}genera= n_{d3}genera= n_{d4}genera= n_{d5}genera=24$; Figure 2) of the core. Normalized pollen counts provided by Chi^2 for 0.5-1.5cm was 85,606 cm^{-3}; 57,738 cm^{-3} for 4.5-5.5cm; 80,749 cm^{-3} for 14.5-15.5cm;47,082 cm^{-3} for 29.5-30.5cm; 89,091cm^{-3} for 39.5-40.5cm.

Microcharcoal density has a significant correlation (linear regression, $p<0.001$, $F_{1,3}=261.09$, charcoal per cm^3 = 10,937 sample depth + 5597.8; Figure 3) to the increase in depth within the core. The lowest level of normalized microcharcoals was found at 0.5-1.5cm with 20,455 charcoals cm^{-3}. The highest level of normalized microcharcoals was found at 39.5cm-40.5cm with 60,000 charcoals cm^{-3}.

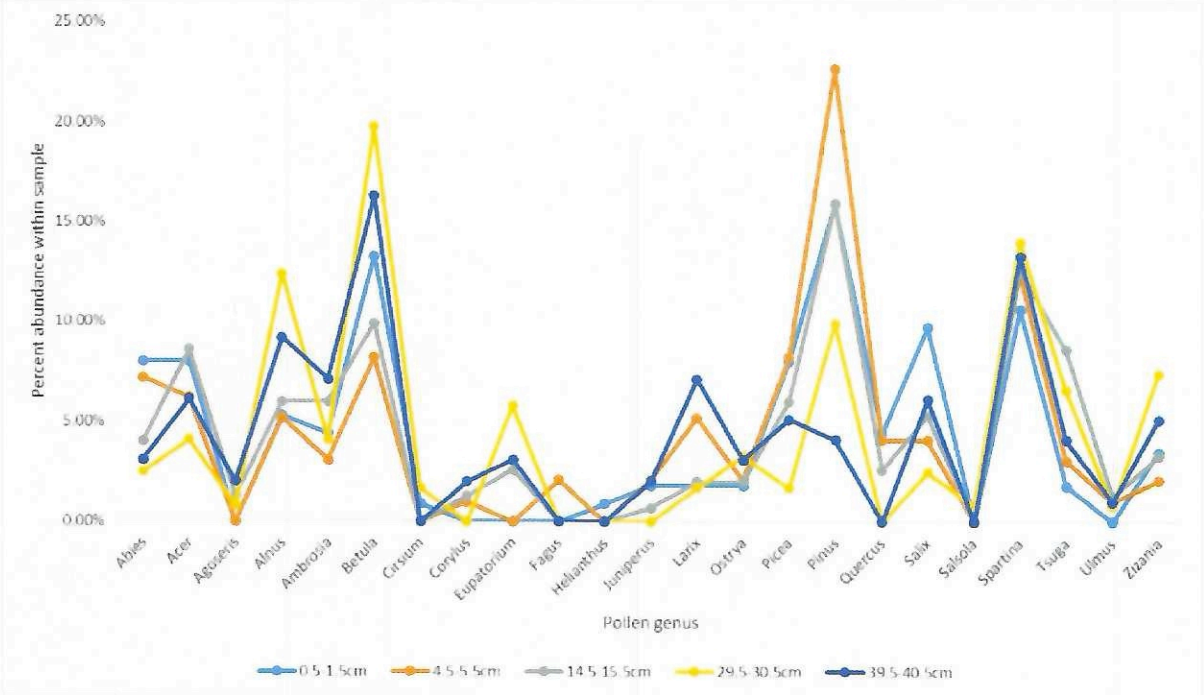

Figure 2. Pollen abundance sorted by genera for each sampling depth. Pearson Chi^2 test: $p<0.001$, $df=88$, $n_{d1}genera= n_{d2}genera= n_{d3}genera= n_{d4}genera= n_{d5}genera=24$. Each line represents a sample, with each sample total equaling 100%.

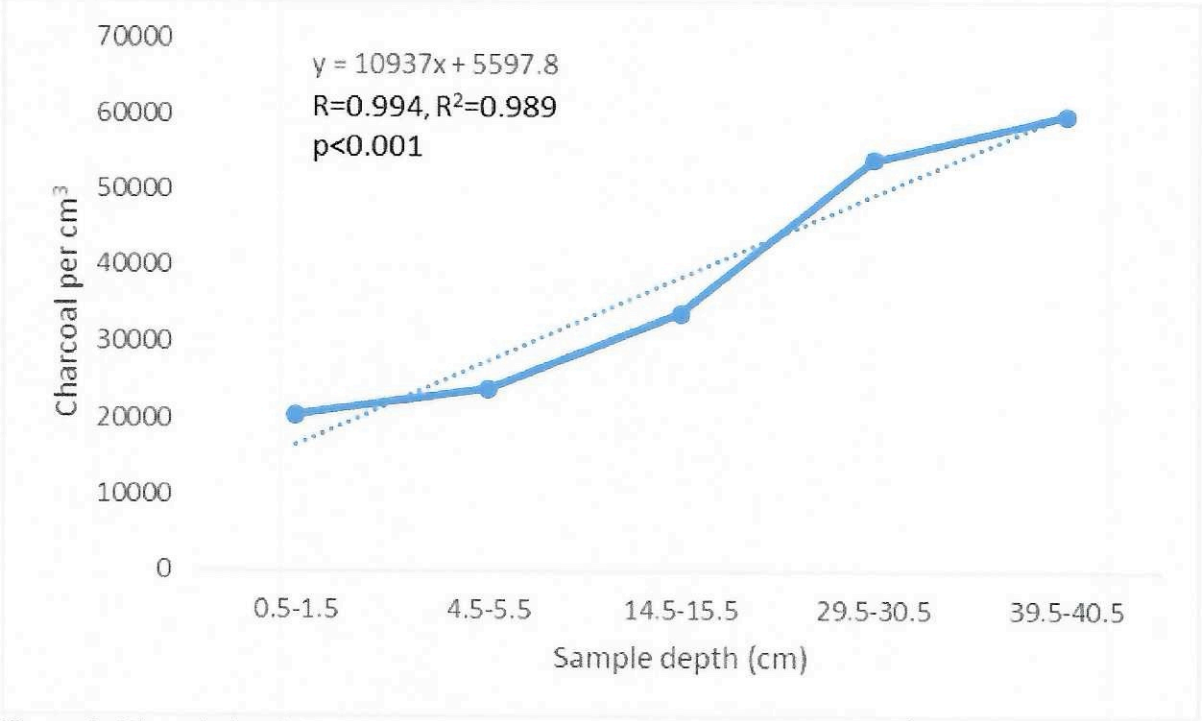

Figure 3. The relationship between normalized counts of charcoal per cm^3 and sample depths (cm). Linear regression: $p<0.001$, $F_{1,3}=261.09$, charcoal per cm^3 = 10,937 sample depth + 5,597.8. Each point represents a normalized count of charcoals from a sample depth.

Discussion

Pollen

The use of pollen as an indicator of vegetative change over time can aid in establishing an ecological timeline for the area surrounding the sampling site. The study indicated that the pollen genera and concentrations throughout the layers of the core were outside of the expected distribution (Chi^2, Fig. 2). Pollen genera change over depth was inconsistent, with some genera being absent completely while others were observed at a higher frequency than would be expected. Several clear changes were observed over the changes in depth; most interestingly the decline of *Betula* (Table 1, Fig. 3) pollen abundance towards the surface layers in concert with the increase of coniferous genera such as *Pinus* (Fig. 4) and *Abies* (Fig 5).

Changes in vegetation can often reflect the climate present in a region, as different climates favor different groups of plants that are more adapted to them (Roman et al. 1984). In Maine's postglacial period over 12,000 years ago, it was shown that trees from the Betulaceae family were some of the first pioneer species to colonize the area after glacial recession (Barton et al. 2012). This included shrubs such as *Betula* and *Alnus* (Fig. 6), both of which were declining in abundance towards the surface of the sampled core. These genera were well suited for the somewhat harsh postglacial conditions, having relatively slow growth rates and low nutrient requirements (Barton et al. 2012). As other trees moved in, these trees began to decline in abundance due to the more crowded, shady conditions negatively impacting their ability to compete. A similar trend was seen within some sampled pollens, with the decline of genera such as *Betula* towards the surface being mirrored by an increase in abundance of needled tree pollens such as *Pinus* and *Abies*. These genera maintain their needles year round as opposed to shedding them according to the climate (Barton et al. 2012). As these conifers become more abundant in an era, it can be hypothesized that they would outcompete the leafy early pioneer species, causing a decline in abundance of leafy species.

Another interesting change in leafy pollens that was observed was the disappearance of *Ulmus* (Fig. 7) pollen at the surface layer of the core. The elm tree in America has become scarce over the last 100 years due to the invasive fungus known colloquially as Dutch Elm Disease (DED) (Schlarbaum et al. 1998). This fungus destroyed elms all across the country, turning the once common tree into a rarity. The fungus is transported on beetles that burrow into the elms and allow it to infect healthy trees (Marcotrigiano 2017). Samples showed elm pollen present in the bottom four sampling depths of the core, with lower abundances at 5cm and 30 cm depths (Fig. 2). It can be hypothesized from this data that the disappearance of elms is due to the impact

that DED has had on elms in America. The only major remaining population of elms in the area is in Castine, a nearby town. This makes the absence of elm from the surface concerning, as it could indicate that the population is continuing to decline. There are elms that demonstrate a tolerance to DED (Marcotrigiano 2017), but breeding them has proved to be a slow and expensive process.

Several other interesting observations presented themselves, such as the relatively low abundance or absence of the *Agoseris* (Fig. 8) pollen, commonly known as false dandelions, a native herb. This plant has a more northern distribution (USDA 2018), and its disappearance at 5cm and 1cm depths could indicate that it is being pushed out of the area by changing climate. The heartiness and overwhelming abundance of this plant in New England would make it seem as if it should have appeared in greater amounts, when in fact it was absent from the top two sampling depths of the core.

Another noteworthy change that was seen was the appearance of *Quercus* (Fig. 9) pollen at 15cm depth. It was entirely absent in the lower layers, potentially indicating that it had only recently colonized the area. Considering the abundance of oak in the area (Go Botany 2011), this would be a surprising development, but could have potentially resulted from pollens not intersecting a chosen transect during microscope counts.

The identification of pollen genera by way of microscopic analysis is a challenging and time consuming process. In order for a proper identification to be made, a variety of features must be carefully examined and identified, such as shape, size, number and size of any apertures present, and sculptural elements and patterns of the pollen exine (exterior surface of the pollen). Faegri et al. (1989) discusses how minor changes in these features can indicate a completely different pollen from what it was originally thought to be. This can present a significant problem

when a researcher is spending long periods of time at the microscope identifying and counting pollen. Misidentification is an easy source of error when counting pollens, which is why any pollens that were not able to be clearly identified either due to damage, obfuscation, or lack of defining features were placed into the unknown category in an attempt to prevent misidentification. 29% of pollens counted were placed into the unidentified category. As mentioned above with oak pollen, conducting three transects across each slide has the potential to overlook some pollens. Due to this, there is a potential that some pollens that were not present or identifiable in slide transects could have been missed. Additional transects could aid in precision of future slide counts.

Charcoal

The microcharcoal concentrations found present a very clear pattern for their deposition in the region (Fig. 3), showing that it has drastically decreased from 40cm to 1cm depths. This indicates that there has been a consistent reduction in microcharcoals present in the atmosphere as each of the sample depths formed. Sources of microcharcoals in the area in recent history have generally been anthropogenic in nature (McCarroll et al. 2017): activities such as industrial emissions and controlled burns for land clearing. The data above would seem to indicate a reduction in these practices. However, the natural charcoal production of the region must also be taken into account (Mooney and Tinner 2011).

The production of microcharcoals through forest fires is a natural process that can create high concentrations of microcharcoals at the sediment layer that existed at the time of formation (Mooney and Tinner 2011). In 1947, a large section of Mt. Desert Island caught fire and burned for two weeks (The Year a State 2018), releasing large quantities of microcharcoals into the atmosphere. This could have contributed to the high quantities of microcharcoals, but is unlikely

to have been the primary cause, as the drop in charcoal abundance between depths is too gradual to be primarily linked to a single massive fire event (Mooney and Tinner 2011). This conclusion is reinforced by the idea that a charcoal catchment area is not fixed, and depends heavily on the weather and wind direction at the time of emission.

Future study

This study was able to establish much of the local vegetation that produces wind-borne pollen as well as how it has changed over time. Further study of the area would be greatly advantageous as it would not only produce additional data for comparison, but also expand the total dataset in the sense that deeper cores could be taken. This would allow for a longer ecological timeline to be established, increasing our knowledge of how the local vegetation has changed. If further surveying of marsh sediments is to be conducted, ^{210}Pb or ^{14}C dating methods should be used to assign ages to sediment depths. Due to budgetary restrictions, this study was unable to place ages on the core sample depths.

In addition, future studies conducted should ensure that vast amounts of time are budgeted for microscopic analysis, as it is the most time consuming portion of the process. Pollen and charcoal counts for 10 slides required 40+ hours in front of the microscope in this study. The minimum number of transects, three per slide, were conducted in this study in an attempt to obtain meaningful results in a reasonable amount of time. There is still much we can learn both ecologically and historically from additional studies on salt marsh sediments and the field of paleoecology as a whole. Based on the results of this research, additional study on this particular sample site would further benefit to the ecological record for the area.

OS401
11/15/2018

Acknowledgements

I would like to recognize and thank the Quahog Bay Conservancy for granting me the scholarship funds necessary to execute the specialized lab work required for my project. Thanks to Andrea Nurse and the University of Maine at Orono for allowing me to utilize their time, expertise and facilities in order to complete my project. I would also like to thank my academic advisor, research advisor, and OS400 instructor Professor Sarah O'Malley, for the constant advice and support that she has provided throughout this process. Many thanks to the teachers of the OS400/OS401 classes, Professors Sahl and Verde, for pushing us to do our best on these projects. Thanks also to Pam Grindle, Maine Maritime Academy's lab manager, as well as the entire Maine Maritime Academy OS faculty for being so understanding and helpful with whatever research issue may have come up. Finally, I would like to thank my supportive friends, James LeVine, Mike Wise, and Quinn Bacon, who helped me with everything from collecting samples and keeping me company in the lab to providing an objective view of my work.

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Table 1. Identified genera with common names and types of growth

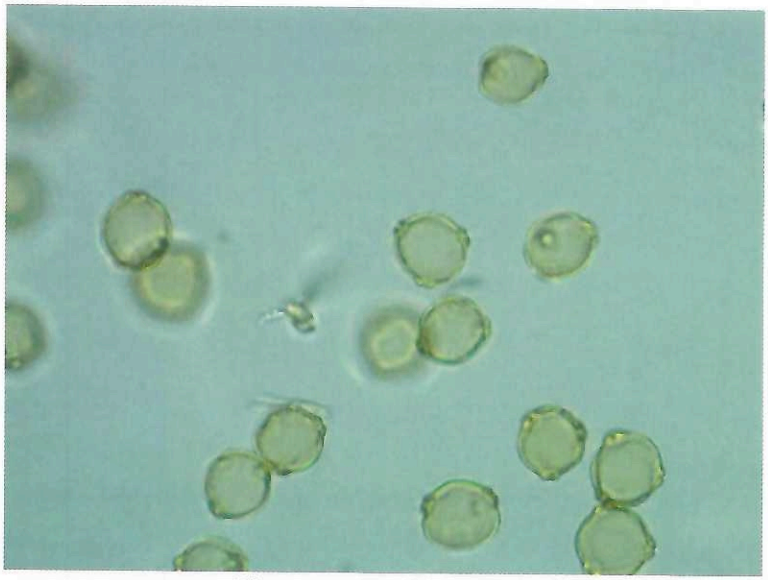

Figure 3. *Betula* pollen*

*Image taken by Brendan Kerivan through SPOT microscope camera attachment

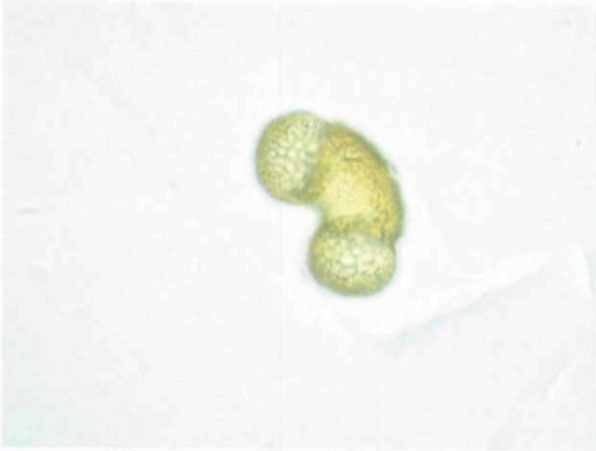

Figure 4. *Pinus strobus* pollen*

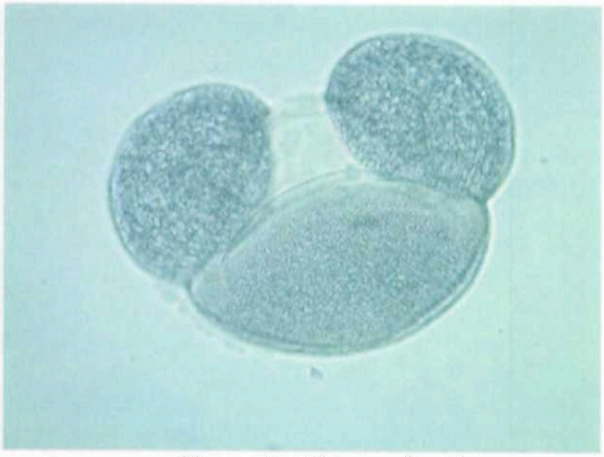

Figure 5. *Abies* pollen*

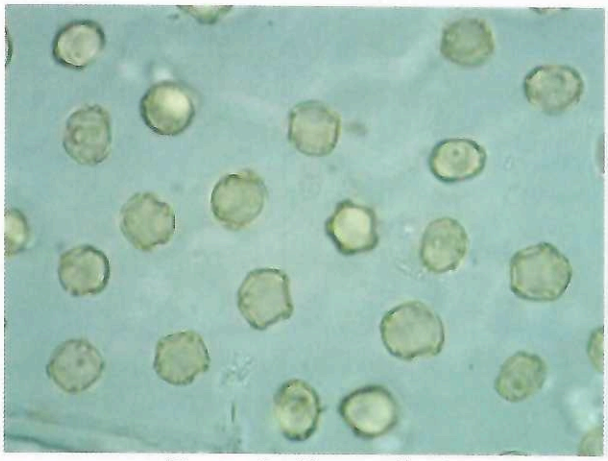

Figure 6. *Alnus* pollen*

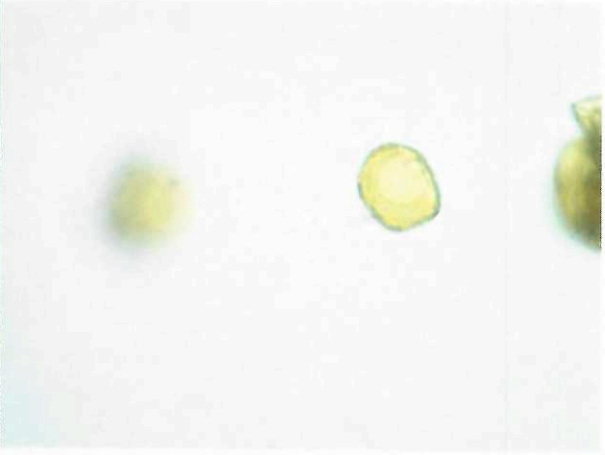

Figure 7. *Ulmus* pollen*

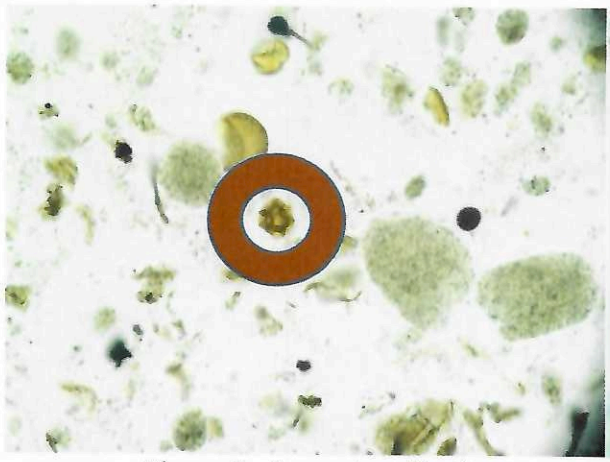

Figure 8. *Agoseris* pollen*

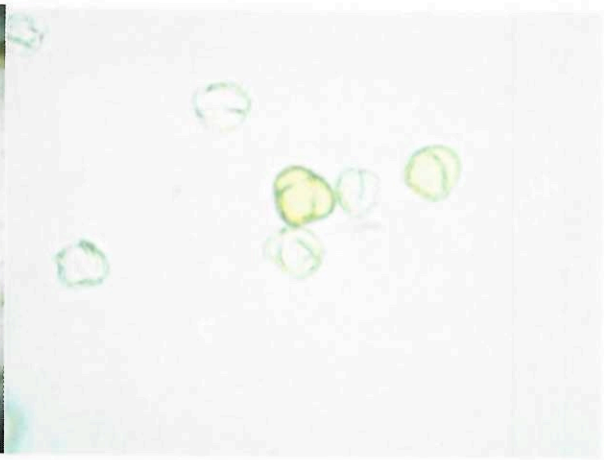

Figure 9. *Quercus* pollen*

*Image taken by Brendan Kerivan through SPOT microscope camera attachment