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Analysis of Sterol Diagenesis Stages in a Peat Core from Kamchatka, Russia

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Abstract

Peatlands are a critical part of the global carbon cycle. While many studies of peat carbon focus on accumulation, less is known about peat decay. We use the diagenesis of sterols to track the decomposition of peat in a Holocene-length core of a sedge fen on the Kamchatka Peninsula, Russia. Diagenesis can transform sterols as soon as the peat is initially deposited, but they can also remain unchanged for millennia. We have quantified the transformation of sterols into their diagenetic products to measure the maturity of peat and estimate carbon loss. Further, there are two pathways in sterol diagenesis, oxidative and reductive, and the products of these two pathways can indicate the conditions under which the peat degraded. This project focuses on the transformations of cholesterol, campasterol, and β- sitosterol, to cholestanol, campastanol, and stigmasta-3,5-diene-7-one, respectively. During the late Glacial period (before 10 ka) the oxidative pathway dominated sedimentary sterol transformation, indicating strongly reducing conditions. These sediments were deposited before the site transitioned to a peatland, and are dominated by sapropel, supporting the steroid evidence for reducing conditions. During the Early Holocene, (about 8 -6 ka) sterols, and, presumably, peat, were well preserved, and coincided with high carbon accumulation rates. In the middle Holocene (5 – 2 ka) the reductive diagenetic pathway dominated, indicating oxidizing conditions, and coincided with low rates of C accumulation. At 2.5 ka, conditions favoring the preservation of sterols returned, along with higher C accumulation.

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Acknowledgements

Firstly, I would like to acknowledge my mentor at Lamont-Doherty Earth Observatory, Dr. Jonathan Nichols, without him this project would not have been possible. He encouraged me and believed in me to come up with new ideas outside of the box. Dr. Nichols gave me the self-confidence I have been missing for so long. Because he has a love for peat, geochemistry, and research, I was able to learn different lab procedures and biogeochemistry and gain a whole new inside into the importance of the global carbon budget. Additionally, I would like to thank the Dr. Andre Andreev of the Universität zu Köln and Dr. Dortothy Peteet of Lamont- Doherty Earth Observatory, whom I got the peat core from.

I would also like to express gratitude to my advisor and honors thesis advisor here at the State University of New York, College of Environmental Science and Forestry, Dr. Mark Teece. Dr. Teece has given me advise about my undergrad career and graduate school application, along with helping me form a thesis. Dr. Teece's passion for sterols, has helped me learn more about sterol diagenesis. He has a positive and motivating attitude, which has always helped me when times were tough. I would also like to acknowledge the Director of the Honors Program, Dr. William Shields, who has guided me through this honors process.

Introduction:

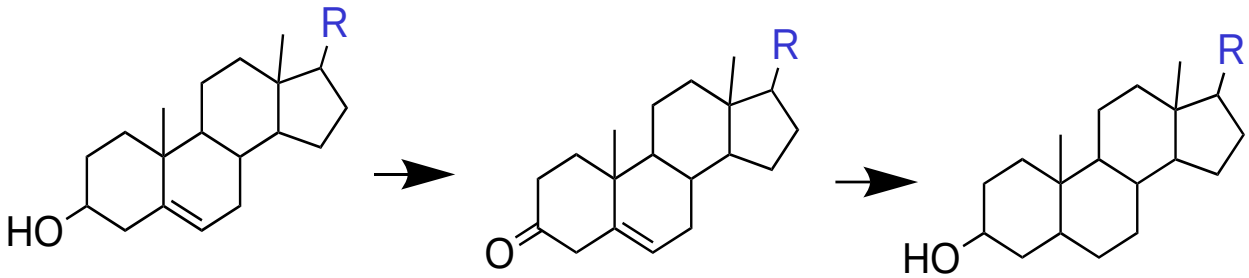
Peat is the accumulation of decayed plant matter, and obtaining more knowledge of peat can better indicate the global carbon cycle. Peatlands sequester carbon from the atmosphere (550 Pg C) (Bubier et al.,1995). However, there have been recent studies that indicate that peatlands are losing carbon from storage. This can change the balance between carbon dioxide and methane, and hydrologic loses of carbon (International Peatland Society). By studying peat, one can measure the balance between carbon dioxide and methane over time (International Peatland Society). Peat can cool the atmosphere by sequestering carbon dioxide from the atmosphere and contribute to warming of the atmosphere through peat loss over time and methane emissions into the atmosphere (Frolking et al.,, 2011). Peatlands are important to study because they have an impact on the carbon balance (productivity vs. decomposition), methane fluxes, and even the water table (Clymo et al., 1998; Moore et al.,, 2011; Glaser et al.,1981). There have been paleoclimate studies done to determine how climate changed in the past based on the past carbon accumulation in the peat. However, there has never been studies digging deeper into peat decay.

Peat diagenesis is the transformation of sediments as soon as the peat is initially deposited. This often involves physical, geological and chemical changes. Sterol diagenesis is the chemical transformation of sterols (a subgroup of a steroid) as soon as the peat is initially deposited (Killops and Killops, 2005; Avsejs et. al, 1998). There are two pathways involved in sterol diagenesis: reductive (Figure 1A) and oxidative (Figure 1B) (Maxwell and Snowdon, 2014). Figure 1A shows the r-sterol reductive transformation into a r-stanol, with an r-sterone (ketone) as an intermediate. A reductive pathway means that there is an oxidizing atmosphere/environment. Figure 1B shows how the r-sterol transforms into a r-sterone (ketone),

but with a diol (two alcohol groups) and diene-ol (two double bonds and an alcohol group) as the intermediates. An oxidative pathway means that the compound is being reduced.

A sterol has an alcohol group and a double bond, a ketone is an oxygen double bonded to a carbon attached to two more carbons, and a stanol has an alcohol group, but no double bonds. In this project there are three main sterols: △ 27, △28, and △29. △ 27 is better known as cholesterol, △ 28 has an extra methyl group on cholesterol, and △ 29 has an extra ethyl group on the cholesterol. Figure 2 indicates the structure of the four different sterols studied in this research.

1A:



1B:

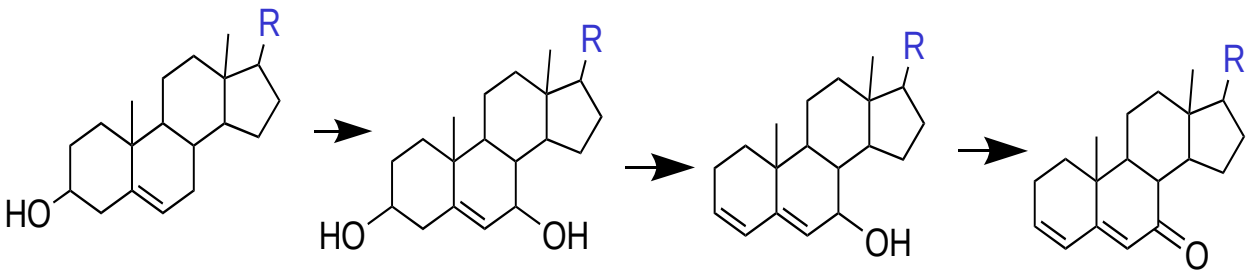


Figure 1: A) Reductive pathway of sterol diagenesis, B) Oxidative pathway of sterol diagenesis

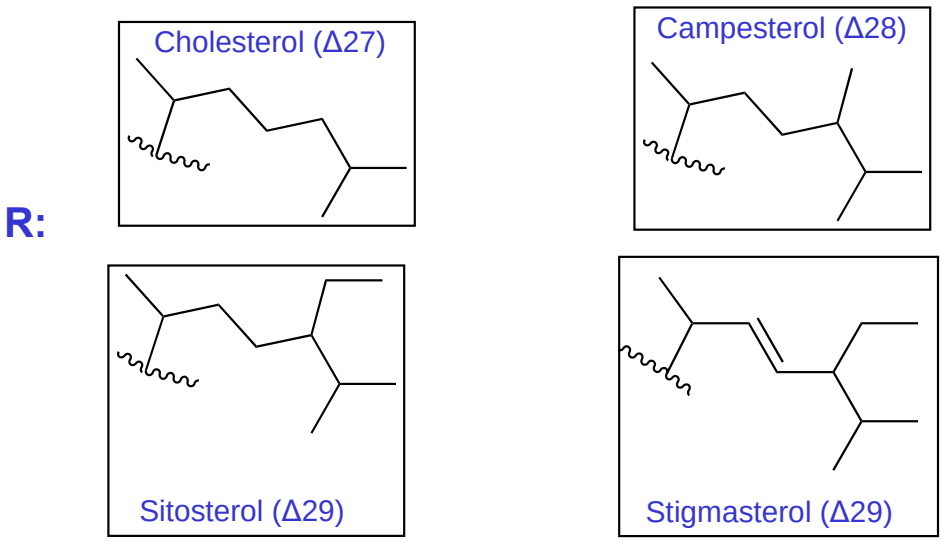


Figure 2: R groups of the four different sterols studied

Background:

In the 1990s a *Newmarke*t (company) peat core was taken from Kamchatka Peninsula, Russia. Kamchatka Peninsula is on the far east side of Russia and surrounded by the Pacific Ocean and Sea of Okhotsk (Figure 2). Coastal Kamchatka regions tend to have persistent cold and humid conditions (Pandea, et.al, 2015). Unfortunately, the peatland has since been paved over by the city Petropavlovsk. 37 samples from the peat core were collected and are from the Holocene era and is sedge fen. The Holocene era is the current geological time period and started at the end of the last ice age. Fens, as opposed to bogs, contain nutrient rich vegetation that obtain their water from rain and groundwater (Ronkainen, 2015). The peat core samples are sedges, however, the specific species of sedge is unknown.

There are past paleoclimate studies at Kamchatka, Russia. Kamchatka is isolated and therefore has very little human "influence on the vegetation and landscape" (Klimaschewski, et. al. 2015). This is means that past vegetation can be studied and human impact does not have to be a significant factor (Klimaschewski, et. al. 2015). There have been previous studies about

oxygen isotopes (Meyer, et al., 2013), pollen counts (Klimaschewski, et al., 2015), and even tephra in volcanoes (Pendea, et al., 2015) to determine how the climate changed through time, however, there has never been peat decay. Identifying which pathway dominated throughout the Holocene and the rate of the pathways can indicate more about the climate conditions in Kamchatka, Russia. The Oxidative pathway indicates there is a reducing atmosphere. The reductive pathway indicates there is an oxidizing atmosphere (Maxwell and Snowdon, 2014)). The rate of the diagenesis/ratio of products to reactants pathways can indicate the temperature, seasonality changes, and water table level throughout the Holocene period (Killops and Killops, 2005). If there is a higher rate of peat decay and carbon loss, that means that the climate conditions in the Holocene period were more oxic than normal.

There are three parts to the Holocene: Early (~8,000 cal. BP and above), Mid(~8,000 to 5,000 cal BP), and Late (5,000 cal yr BP to present) (Klimaschewski et al., 2015). During the early Holocene temperatures were moderate, rainy seasons, high moisture. Around 9,000 cal BP, there was a big increase in temperatures (Klimaschewski et al., 2015, Zakharikhina et al., 2013). During this time period, where there were warm and wet conditions, there was also high peat accumulation. The mid-Holocene period was wet and mild temperatures/conditions from around 5,000 cal.BP to 5,800 cal. BP. Then around 5,800 cal. BP to 7,000 temperatures were slightly warmer. During the late- Holocene, there was a climatic cooling in Kamchatka (Klimaschewski et al., 2015, Zakharikhina et al., 2013). Another important note is that in eastern Kamchatka, temperature and moisture are the least contrasting because of the Pacific Ocean (Zakharikhina et al., 2013).

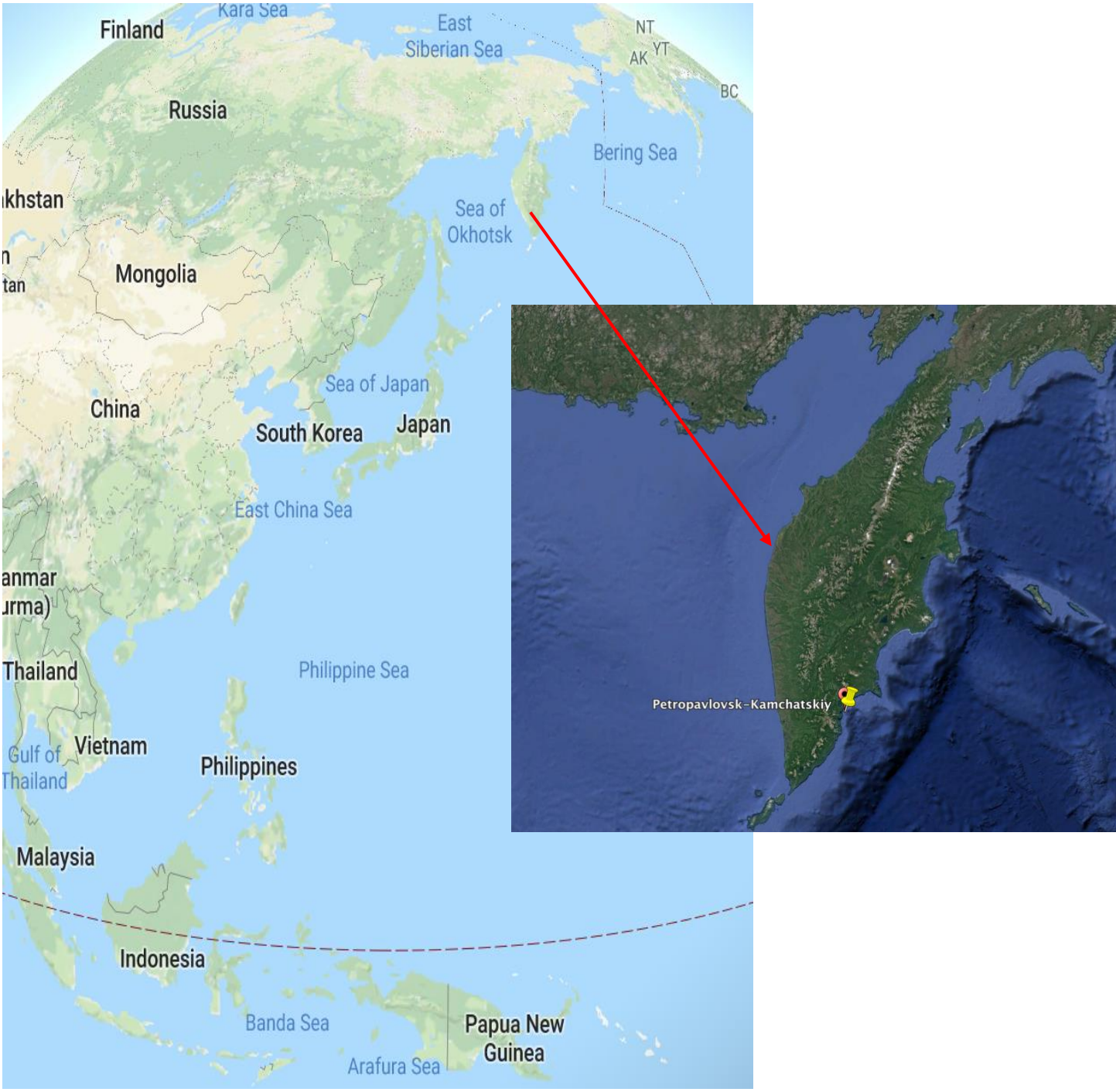


Figure 3: Map of Petropavlovsk City (where peatland used to be) in Kamchatka Peninsula, Russia

Objectives:

1) Determine what stage of r-sterol diagenesis occurs through the Holocene at *Newmarket*?
2) Determine what the diagenetic pathway and the percent of product tell us about peat carbon loss?

Methods:

1) **Collecting the Core:** The core was collected in the 1990s by Andre Andreev, a PhD student of Dr. Dorothy Peteet (Lamont Doherty Earth Observatory). The core had a depth of 165cm, which is 11,202 age cal. Years before present (BP). There is an age model below (Figure 4). The core was well preserved, and previous research students were able to determine the age model. For further information on how the core was collected please contact Dr. Dorothy Peteet.

2) **Total Lipid Extraction:** Once the core samples were collected and dried, eventually the leaf waxes had to be extracted. The peat samples were weighed and transferred to 60mL vials. Each peat sample was sonicated with hexane three times and then the total lipid extracts (TLEs) were dried with air and then overnight. The TLEs were then separated based off polarity. Silica gel columns were used to separate the lipids into hydrocarbons (3x hexane), ketones/aldehydes (3x dichloromethane[DCM]), alcohols (3x 1 Ethyl Acetate: 3 Hexane mixture), and acids (3x methanol). The alcohols had to be acetylated(Appendix B) and the acids had to be methylated (Appendix B) before they were put through the Gas Chromatography. Alcohols were acetylated by adding 200µL of pyridine and 200µL of acetic

anhydride, heated at 70ºC for 20 minutes, and then the sample dried overnight so the pyridine can fully evaporate. 100 μL of the standard (20ng 5α-androstane) was added into each of the alcohol and hexane samples. The standard for the DCM fraction is 9:1 toluene: 1,1-biphenyl. To make this standard 9,000μl was added to 1,000 μL of 1,1-biphenyl to dilute from 210.5ng to 21.056ng; 100 μL of this standard was added to all the DCM fractions. The acids were methylated by acidified methanol through Fisher Esterification(Appendix B). Acidified methanol was made by combing cold methanol and acetyl chloride (5:95). Each sample had 0.3mL of toluene to be blown down under nitrogen and then 1mL of acidified methanol was added. To continue the fisher esterification, 1mL of NaCl and 1mL of hexane were added to each sample and the hexane fraction was extracted. This extraction was completed three times. The samples were cleaned and the fatty acids were eluted with 4mL of DCM. Once each fraction is run through the GC-MS/FID, the retention time indicates the specific compound, relative to the standard. Looking at the mass spec, I identified the standard and then using known mass ions of the sterols and the NIST website I was able to integrate and identify the different sterols in the samples. The peak area of each compound and the concentration of the standard can be used to measure the concentration of each of the compounds (ng/g of dry weight of peat). The formula used was:

$$Response\ (PA^{\circ}) * Position\ Correction\ Factor\left(\frac{PA_{\ mean}}{PA^{\circ}}\right)$$

$$* response\ of\ standard\ \left(\frac{Weight}{PA^{\circ}\ of}std\right) * 100\mu L * Sample\ mass\ (g)$$

For the purpose of this experiment, the ketones and alcohols sections are the important fractions, as the GC-MS/FID will indicate the amount of r-sterols/r-stanol (alcohol fraction) and amount of r-sterones (ketones fraction). In each sample, the percent of alcohol and ketones

(the total being the amount of ketones and alcohols together) was taken. Using these relative concentrations (in percent), one can compare the percent amount of the sterol, sterone, and stanol in each of the samples. There is only one replicate of each sample. Comparing the ratios will then indicate what stage in sterol diagenesis each of the sterols are in and then how far long in sterol diagenesis each of the samples are in throughout the Holocene period.

3) **Dominating Pathway**: Using r-statistics, one can compare which pathway dominates throughout the Holocene time period. Using an age model (Figure 2), the depth of each of the samples were able to be converted to Age, calendar years before present. Using the oxidative and reductive relative percentages graphs (Figure 6 and 7) a de-trended linear regression line was drawn. The difference between the line and actual percent measurement for oxidative and reductive was taken. For each sample, the difference from the line was compared for each the different pathways to determine if one pathway, both pathways, or neither pathway dominated (Figure 8). This can further indicate how the climate changed throughout time. Comparing the percent of each product in each of the *Newmarket* core samples will also indicate the rate of carbon lost throughout the time period. If there is more product, there is a higher rate of carbon loss from peat. This will indicate more information on carbon decay. In appendix A, the exact ages of each of the samples is indicated. The formula used to determine the age. Ca yrs B.P. is:

$$Age.a\ yrs\ BP = \left(59.027 * Depth\ (mid)\right) + 1285.9$$

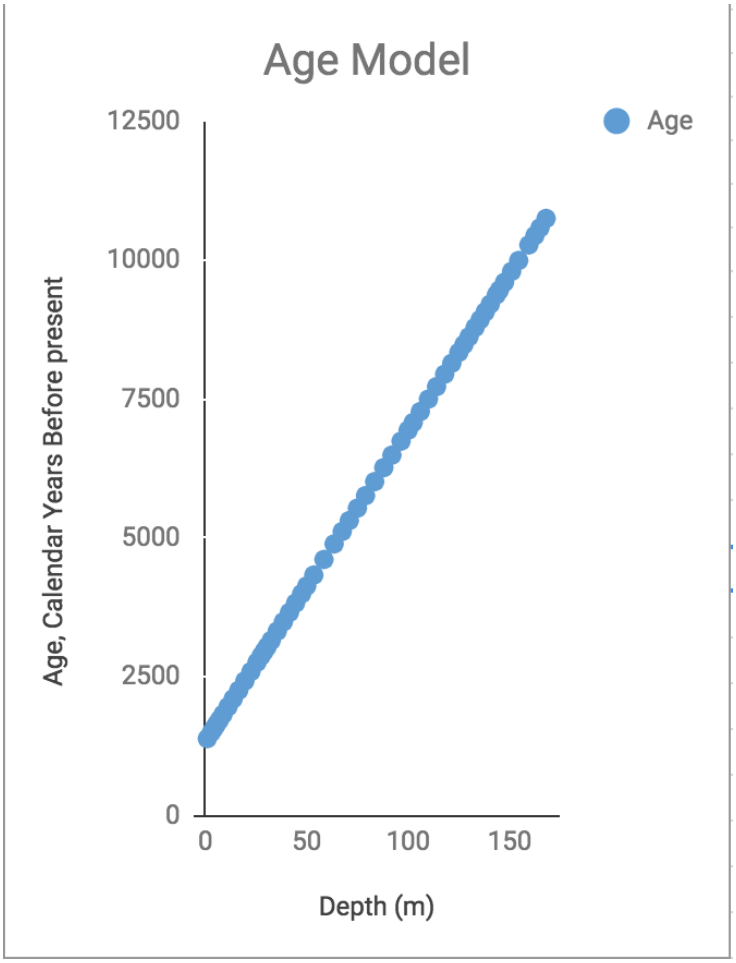


Figure 4: Depths are converted to Age, Calendar years before present using the formula above

Results:

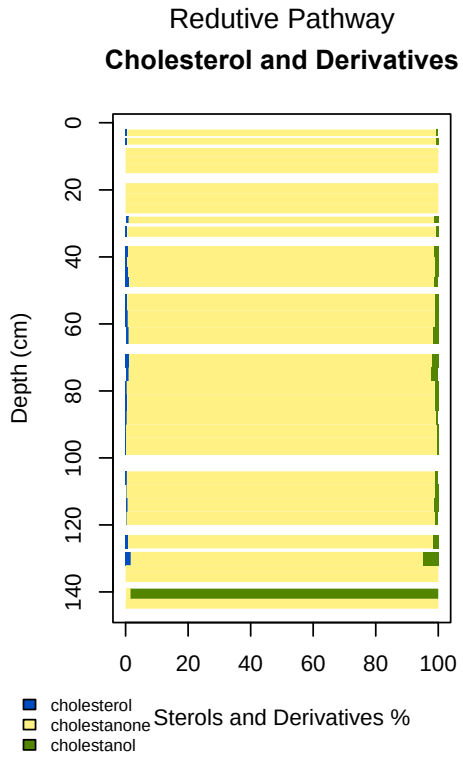


Figure 5 is comparing the percent ratio of cholesterol, cholest-3-one, and cholestanol in the samples from the *Newmarket* core. For the majority of the samples, the main compound is cholest-3-one (the intermediate from the oxidative pathway). There is very little product and reactant in each of the samples, no matter the depth or age. The second to last sample, however, contains mostly cholestanol. This indicates that cholesterol is at the end of diagenesis in this sample.

Figure 5: Percent of △27 Reactant(Cholesterol), Intermediate (Cholestanone), and Product (Cholestanol) as Depth Increases (Reductive Pathway)

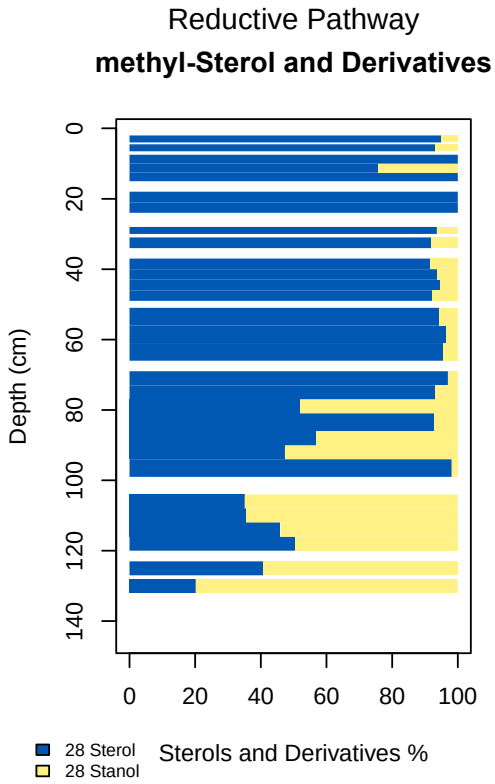


Figure 6: Percent of △28 Reactant (△28 Sterol) and Product (△28 Stanol) as depth increases (Reductive Pathway)

Figure 6 is comparing the ratios of △28 sterol and △28 stanol in each of the *Newmarket* samples. There was no intermediate found for Δ 28. There is a general trend that as the depth, therefore the age, of the samples increases, there is more product. There are certain times where the samples do not follow the general trend. At a depth of 80cm, the rate of the reductive pathway increases, hence there is more product (stanol).

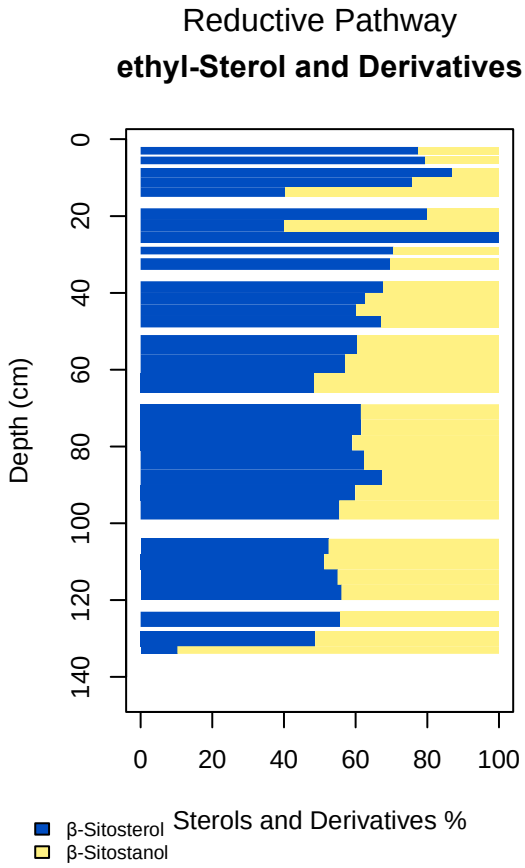


Figure 7: Percent of △29 Reactant(β- sitosterol) and Product (β- sitostanol) as depth increases (Reductive Pathway)

Figure 7 shows how the percent of β- sitosterol and β- sitostanol in each of the samples at an increasing depth. There is no intermediate for the △29 sterol. The general trend in this figure is that as the depth increases, the percent of product also increases. The more product (β-sitostanol), the more sterol diagenesis in the peat sample. These results can help indicate different climates over periods of time. Since this trend is not linear, it indicates that certain periods of time had different climates.

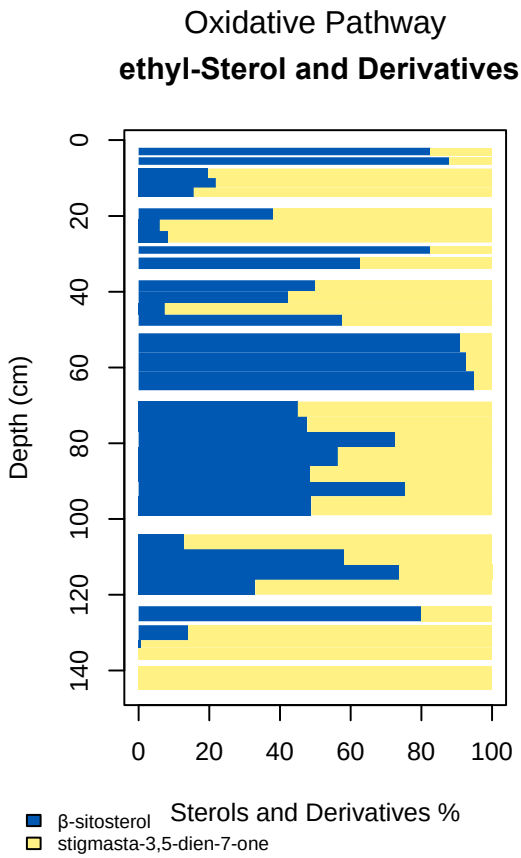


Figure 8: Percent of △29 reactant (β- sitosterol) and product (stigmasta-3,5-dien-7-one) as depth increases (Oxidative Pathway)

Figure 8 compares the amount of β- sitosterol (reactant) to stigmasta-3,5-dien-7-one (product). This is using the oxidative pathway as shown in Figure 2. The younger core samples generally had more stigmasta-3,5-dien-7-one than β- sitosterol. In the middle of the core, the samples tended to have more β- sitosterol than stigmasta-3,5-dien-7-one. Then the samples from the deepest part of the core tended to have almost all stigmasta-3,5-dien

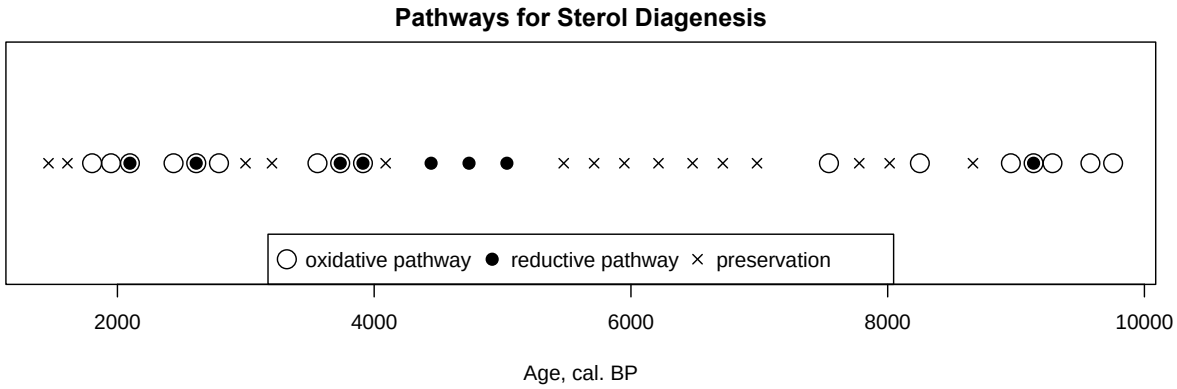


Figure 9: △29 Dominating pathway (Reductive and Oxidative) throughout the Holocene period

Figure 9 was produced by subtracting the difference from a de-trended linear regression line and using the age calibrated BP for each of the 37 *Newmarket* core samples. Figure 6 shows when the oxidative pathway, reductive pathway, both pathways, and neither pathway occurs throughout the Holocene time period. A more detailed explanation to how this graph was created is stated in the methods.

Discussion:

The diagenesis of cholesterol in the studied peat cores is following the reductive diagenesis pathway. The majority of the peat core samples are mostly cholest-3-one (Figure 3, Appendix A). This indicates that the rate of cholesterol diagenesis may not be so much effected by climate and temperature throughout the Holocene time period. Since cholesterol is not common in plants, the fact that the diagenesis is slower could be correlated to the fact that there is not a lot of insect remains or organisms (such as worms) in all the samples. Organisms have cholesterol and can affect decomposition, therefore they may be in peat samples (Schlesinger and Bernhardt, 2012). In one of the oldest core samples (Figure 5), however, the cholestanol was the majority compound. This could mean that there was a lot of insect and organism remains around this peat core or that during the early Holocene, the conditions were more oxic than normal, so the rate of diagenesis was higher. This higher diagenesis rate could also indicate higher temperatures and lower water table (Ming et al.,, 1993;Killops and Killops). This sample's age is around 9,500 cal yr. BP(Table 1, Appendix A and Figure 4), which coincides with a big temperature increase in Kamchatka (Klimaschewski et al., 2015, Zakharikhina et al., 2013). Higher temperatures increase the rate of carbon decay as well as the different types of vegetation that are able to live, which in turn affects the carbon budget (Ronkainen, 2015). A water table can change depending on the season and precipitation. This indicates that around 9,500 cal yr. BP (Table 1, Appendix A and Figure 4). there will most likely be a lower water table, as well as a shorter winter and a higher thawing time. The rate of cholesterol and cholest-3-one may also be faster, which indicates that this transition may be more favorable than the transition from cholest-3-one to cholestanol.

The rate percent amount of products increases with age and time with △28 (campasterol) as evident with Figure 6. This trend is generally more linear than cholesterol. This means that overall the deeper the core, the further along the sample is in diagenesis. However, this trend is not perfect, which indicates that throughout the Holocene era there are periods of time where the environment is more oxic. The majority of △28 was the campasterol (reactant), until the depth of 80cm (~6,200 cal yr. BP, Table 1, Appendix A and Figure 4). This indicates that in the late- Holocene, there were less oxic conditions, lower temperatures, and a higher water table. A lower temperature is correlated with less oxic conditions (Ming et al.,, 1993). Klimaschewski (2015) stated that Kamchatka had lower temperatures in the late-Holocene, which coincides with this data. This means that there was less carbon decay and a slower rate of diagenesis. After this 80cm mark, however, there is definitely more product (campastanol) in each of the samples. As depth increases, there is an increase in decay, as evident by an increase in stanol (product) to sterol (reactant) ratio (Andersson and Meyers 2011). This indicates that there were more oxic conditions and higher amount of carbon decay during the early Holocene. In Kamchatka, there were higher temperatures and higher moisture conditions during this period (Klimaschewski et al., 2015, Dirksen et al., 2013). Since oxic conditions and temperature are correlated, this indicates that there is more carbon decay (Ming et al., 1993;Killops and Killops, 2005). The change in temperature, rate of diagenesis, and water table level can also indicate more about the past climate. Changing temperature affects the types of vegetation that are able to survive (Ronkainen, 2015). A higher temperature can affect the water table as well (Ming et al., 1993;Killops and Killops, 2005). A water table can change depending on the season and precipitation. A lower water table means less precipitation and warmer temperatures and a higher water table vice versa. In the early-Holocene period, there was a wetter atmosphere,

which coincides with the fact that the water table is higher (Klimaschewski et al., 2015). A higher water table can indicate a higher amount of carbon dioxide emissions (Moore et al., 1993).

The next sterol was △29（stigmasterol or b-sitosterol), and these two sterols are the common sterols found in peat（Andersson and Meyers, 2011). In figure 7, there is a slight trend with increasing depth and increasing products. This coincides with Andersson and Meyers who stated that the deeper the sample in the core, the higher the ratio of stanol to sterol is (2011). However, this trend is not perfectly linear. There are periods of time throughout the Holocene era where there are definitely times of higher oxic conditions and a higher rate of diagenesis. There is a sample around a depth of 20cm that has more product which indicates that there was a period in the late Holocene era where there were higher temperatures. This is around the age 2,500 cal. Years B.P. This is interesting because in past studies, the late-Holocene had a cooling period (Klimaschewski et al., 2015, Dirksen et al., 2013). The last sample at a depth of 130cm (~8,900 cal. Year BP, Figure 4 and Appendix A Table 1) had the most β-sitostanol out of all the products. This indicates that temperature was highest during the early-Holocene and this sample is most likely fen peat as opposes to wet peat (Andersson and Meyers, 2011). This coincides with past studies that indicate that the early-Holocene period had higher temperatures, particularly around 9,000 cal. Years BP (Klimaschewski et al., 2015, Dirksen et al., 2013). The higher rate also indicates a lower water table, more oxic conditions, and a shorter winter season (Ming et al., 1993). The change in temperature can also give a better insight to when the vegetation changed, since different plants thrive at different temperatures (Ronkainen, 2015). Since there are more oxic conditions when there is a higher temperature and a shorter thawing season, there is

typically more carbon decay during these time periods (Ming et al., 1993). A lower water table can also indicate less carbon dioxide emissions (Moore et.al 1993).

In each of the samples there was evidence of the oxidative pathway and reductive pathway for the $\triangle 29$ sterol. In the oxidative pathway (Figure 8), the late-Holocene samples (a depth of 40cm) had majority of products, which indicates a higher rate of diagenesis. At a depth of 40, the sample had an age of 3,600 cal. year BP. The last few peat samples (from the deepest part of the core) had majority product. The last sample even had all β-sitostanol (product). This indicates a higher rate of diagenesis, higher temperature, lower water table and shorter defrosting period. The combination of all of these factors indicates that there is more carbon decay and degradation in the older/ deeper samples. In a study done by Andersson and Meyers, there is a correlation between increasing depth and increasing stanol/sterol ratio (2011). Another note is the more stanol (product) is in the sample, the more likely it is fen peat, as opposed to wet peat (Andersson and Meyers, 2011). The samples from the middle of the core was generally half product and half reactant. This indicates that during this time period, there were less oxic conditions and milder temperatures than deeper samples and an average defrosting period (Klimaschewski et al., 2015, Zakharikhina et al., 2013). Since there are milder temperatures, there is a higher water table (Ming et al.,, 1993). This indicates a milder climate during this time period (Klimaschewski et al., 2015, Zakharikhina et al., 2013).

When comparing the oxidative pathway and the reductive pathway of $\triangle 29$, one can determine which pathway dominates throughout time (Figure 9). In the late-Holocene period there is little decay, which coincides with the cooler temperatures (Klimaschewski et al., 2015, Zakharikhina et al., 2013). In the middle Holocene time period, the reductive pathway dominated. This indicates that there were highly oxidizing conditions, which coincides with the

decrease in carbon accumulation rate (Zakharikhina et al., 2013). This however changed from the Early Holocene period. Sterols were well preserved, which coincided with high carbon accumulation rates. Since the sterols were well preserved, presumably the peat was well preserved as well.

Before the Holocene era began (the last glacial era), the oxidative pathway dominated. This indicates a strong reducing environment. While most of the core samples were peat, the samples from the core from the Glacial period are sapropel. Sapropels are organic rich sediment layers (Grimm et al., 2015). They are formed through biogeochemical cycles and climatic changes, however, usually under anoxic conditions (Grimm et al., 2015). Since the pathway was oxidative (reducing conditions) during this time period, the atmosphere would be more anoxic. This coincides with the fact that sapropel formed during this time period and forms in an anoxic environment.

Conclusion:

In the middle Holocene time period the reductive pathway dominated, which indicated highly oxidizing conditions. This means there was more oxygen during this time period available for peat to decay, more carbon was lost, and it coincided with the fact that there was less carbon accumulation. Another possible explanation could be a change in temperature, as a higher temperature can lead to increased decay. Right after this time period however, there was a big time period where neither diagenesis pathway dominated, which indicates less oxygen was available for decay. By comparing different pathways one can get a better idea of the oxic conditions during different periods of the Holocene. By comparing the percent of products and the rate of reactions, one can get a better idea to how the temperature, seasonality, and water table changed in Kamchatka throughout the Holocene time period. By understanding how carbon decays, one can understand these changes in climate throughout time. The water table level affects the carbon dioxide level, which in turns affect the overall carbon budget. When temperature and seasonality changes, this affects the vegetation that are able to live. Different plants can survive under certain climate conditions, and accumulate carbon at different rates, which affects the overall carbon budget. When looking at carbon decay, one can also understand how oxygen changed throughout time. Diagenesis, especially the Reductive Pathway, occurs more quickly with more oxygen.

This study was important to understand more about peat decay and carbon loss in peat, as opposed to just carbon accumulation in peat. For future studies, I would like to look more into the different rates of reactions. In the cholesterol, there is a majority of the intermediate cholest-3-one. The rates between the reactant and intermediate and intermediate and product

could differ affecting the diagenesis. Microbacteria could also increase one of the rates, but not the second rate of reaction.

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Appendix A: Thickness, Depth, and Age cal. BP of Peat Core

thickness, cm	mid(Depth)	Age cal. BP
1	1	1344.927
2	5.5	1610.5485
3	8.75	1802.38625
4	11.25	1949.95375
5	13.75	2097.52125
6	19.5	2436.9265
7	22.5	2614.0075
8	25.5	2791.0885
9	29	2997.683
10	32.5	3204.2775
11	35.5	3381.3585
12	38.5	3558.4395
13	41.5	3735.5205
14	44.5	3912.6015
15	47.5	4089.6825
16	53.5	4443.8445
17	58.5	4738.9795
18	63.5	5034.1145
19	71	5476.817
20	75	5712.925
21	79	5949.033
22	83.5	6214.6545
23	88	6480.276
24	92	6716.384
25	96.5	6982.0055
26	102.5	7336.1675
27	106	7542.762
28	110	7778.87
29	114	8014.978
30	118	8251.086
31	125	8664.275
32	130	8959.41
33	133	9136.491
34	135.5	9284.0585

35	138	9431.626
36	140.5	9579.1935
37	143.5	9756.2745

Appendix B: Reactions for Acetylation and Fisher Esterification