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Testing the usefulness of pine stomata as a proxy in lake sediment cores from low-latitude environments

Brock Andrew Remus

University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Brock Andrew Remus entitled "Testing the usefulness of pine stomata as a proxy in lake sediment cores from low-latitude environments." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Geography.

Kenneth H. Orvis, Major Professor

We have read this thesis and recommend its acceptance:

Sally P. Horn, Carol P. Harden

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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Testing the Usefulness of Pine Stomata as a Proxy in Lake Sediment Cores from Low-Latitude Environments

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Brock Andrew Remus

August 2008

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My years as a graduate student would not have been possible without the funding of the University and Geography department as a teaching assistant. Besides the money I received, I obtained valuable experience in teaching and leading students in labs that will help me in the future. Finally, I would like to thank my friends and family for their continued support of my work through the past few years. They have put up with me during my odd-hour days and have been helping me by constantly proof-reading my thesis, essays, and proposals.

Abstract

Paleoecological research, using lake cores to reconstruct past climatic and anthropogenic changes, is a burgeoning field in the circum-Caribbean. The Dominican Republic's Las Lagunas region is being studied for this purpose using many proxies. One possible proxy for study there is pine stomata. Concentrations of pine stomata in lake sediments have been used in high-latitude and alpine locations to reconstruct tree-line movement and stand invasion, but have never been used in low-latitude environments.

In this thesis I present results of analyses of *Pinus occidentalis* Swartz (Hispaniolan pine or West Indian pine) stomata concentrations in lake-sediment cores from two lakes in the Las Lagunas region, Laguna Castilla and Laguna de Salvador. Stomata concentrations, along with prior pollen counts, provide a detailed, site-specific view of historic pine distribution near the lakes. Previous higher-latitude studies provide background and context for this project, which aims to establish pine stomata as a useful proxy in low-latitude environments.

Stomata concentrations in Castilla and Salvador, though never high, improved the interpretability of previous pine pollen counts. Pollen and stomata tended to co-vary down the Salvador core, and more weakly in the Castilla core where stomata concentrations were lower. Overall, pine stomata proved a useful proxy at Las Lagunas that can be used in future paleoecological studies of other low-latitude environments.

In addition to the Las Lagunas temporal study, this thesis examines spatial patterns of stomata deposition in mid-latitude Crystal Lake, Knoxville, Tennessee, from the edge of the lake to its middle. Typically, sediment cores are taken centrally in lakes,

so this study examines whether stomata are distributed evenly enough across lakes to be well represented in central cores.

The Crystal Lake study provided useful insights into the deposition and redeposition paths followed by stomata after they enter water bodies. Concentrations of stomata decreased on a dry weight basis, traversing away from shore, with a slight increase where a typical core site location would be, at the lake's center. Based on these results, central coring sites might often fall short of yielding representative concentrations of the stomata entering lakes.

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Chapter 1

Introduction

Lake sediment research and palynology are well-established tools for paleoecological studies. Research has been conducted in situations ranging from peat bogs (filled former lakes) to ocean basins several thousand meters deep. The analysis of pollen and charcoal from stratigraphic sediments can yield a dynamic range of spectra that allow scientists to infer a region's climate history. The large area in a contributing region can be a drawback to sediment analysis, but despite the resulting homogenization this approach has provided the scientific community with thousands of useful records of past climate and anthropogenic changes. This paper examines how a multi-proxy approach can help sharpen the spatial focus of pollen results, specifically regarding the history of movement of pine stands into and out of a small watershed.

The Las Lagunas region of the Dominican Republic, along with other sites in the Caribbean, is currently being studied by Drs. Sally Horn, Kenneth Orvis, and Claudia Mora to reconstruct its climate and anthropogenic disturbance history. Multi-proxy approaches are being used to analyze the history of the region to the fullest extent possible. Pollen and charcoal counts, along with isotope analyses, were completed by Lane (2007) for two lakes in the Las Lagunas region to help reconstruct paleoenvironmental history during the 2600-year history of the lakes. This information makes it possible to understand how anthropogenic activities and variations in climate have affected the landscape of Las Lagunas over time.

The Las Lagunas region is located on the island of Hispaniola (19°N, 71°W) in the country of the Dominican Republic (Figure 1.1). The Cordilla Central mountain range runs northwest to southeast, and the Las Lagunas region is located on the southwestern flank of the mountains at an approximate elevation of 1000 meters (Figure 1.2). Las Lagunas contains four small permanent lakes, but only two of them, Laguna Castilla and Laguna de Salvador, are studied in this thesis. They were chosen for this study because each occupies a small watershed and has open water with shallow water depths. These factors helped facilitate coring and defined small catchments suitable for exploratory research on pine stomata. Pollen slides processed and counted by Lane were also available for each lake. The Las Lagunas region has only been minimally disturbed by human development, providing us nearly pristine stratigraphic conditions in each of the studied lakes.

Combining stomata concentrations with the prior pollen counts of Lane (2007) should strongly enhance our understanding of the history of pine stand presence immediately adjacent to the lakes. Adding stomata counts improve on the pine pollen concentration record alone because pine pollen is very dispersive, making it hard to interpret whether pine trees were locally present. Pine pollen's morphology allows it to be blown long distances away from the original tree, introducing non-local pollen into watersheds that do not themselves contain pine trees. The wide dispersal of pine pollen introduces spatial uncertainty into the interpretation of pollen concentration results. In this thesis I use *Pinus occidentalis* Swartz (the only native species) stomata counts from two mid-elevation lakes of the Las Lagunas region to determine whether or not this technique can help distinguish the local and regional signals in the pine pollen record.

Figure 1.1. Circum-Caribbean map showing the location of the island of Hispaniola on which the Dominican Republic is located. Source www.maps.com.

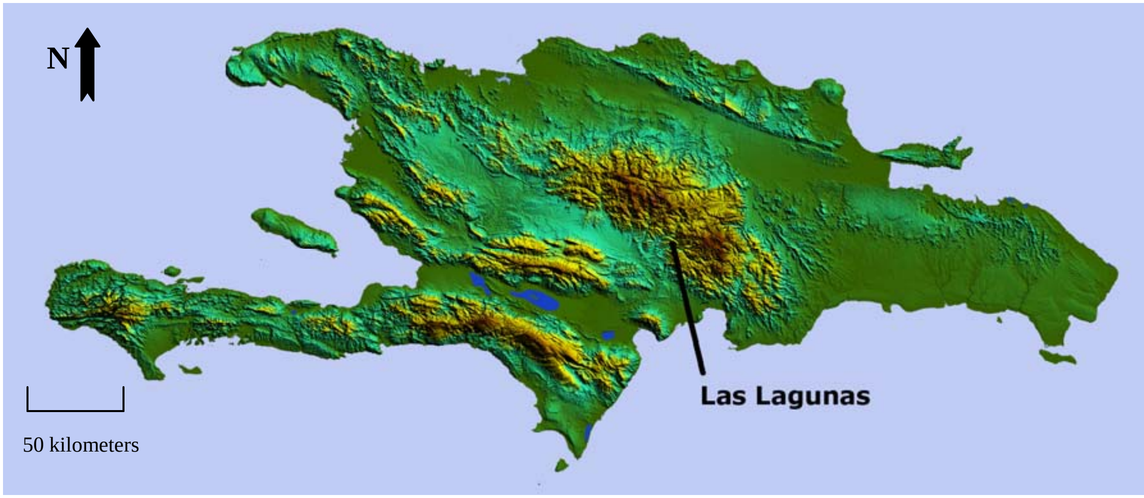

Figure 1.2. Island of Hispaniola and the location of the Las Lagunas region on the southwestern flank of the Cordilla Central mountain range. Map by Dr. Kenneth Orvis.

Stomata counts have not previously been used in a low-latitude lake study, so this is an exploratory study outside the previous scope of stomata studies. Currently, the only regions where investigations have used stomata as a proxy are high-latitude and high-elevation cold environments. My goal is to determine whether stomata counts can be used in warm, low-latitude environments as a viable proxy for future paleoenvironmental studies.

A second question I assess in this thesis is how stomata enter and disperse within lakes, and whether their spatial distribution can be effectively represented in single lake cores, which are commonly taken at or near the centers of the lakes. A transect across a mid-latitude lake was undertaken using dredge samples at known locations. The transect began at the shore near pine trees and extended to a typical potential coring site near the middle of the lake. These samples were intended to show whether stomata can vary in concentration as a function of the distance from shore and from source. The results should allow us to deduce how effectively stomata are represented spatially at single coring sites, by spatially analyzing the dispersion of stomata from their shoreline sources to the mid-point of the lake.

Crystal Lake, in Knoxville, Tennessee (Figure 1.3), occupies a small basin in an urban setting. Crystal Lake provided a good fit for this thesis because of its size and the small pine tree stands on the shoreline. The lake has an area of approximately one hectare, putting it halfway in between Salvador and Castilla in terms of size.

This thesis is divided into seven chapters. Chapter one gives the reader a broad overview of the research. Chapter two lays out the historical background of my study sites, and reviews current knowledge of stomata dispersal and lake sediment dynamics. It

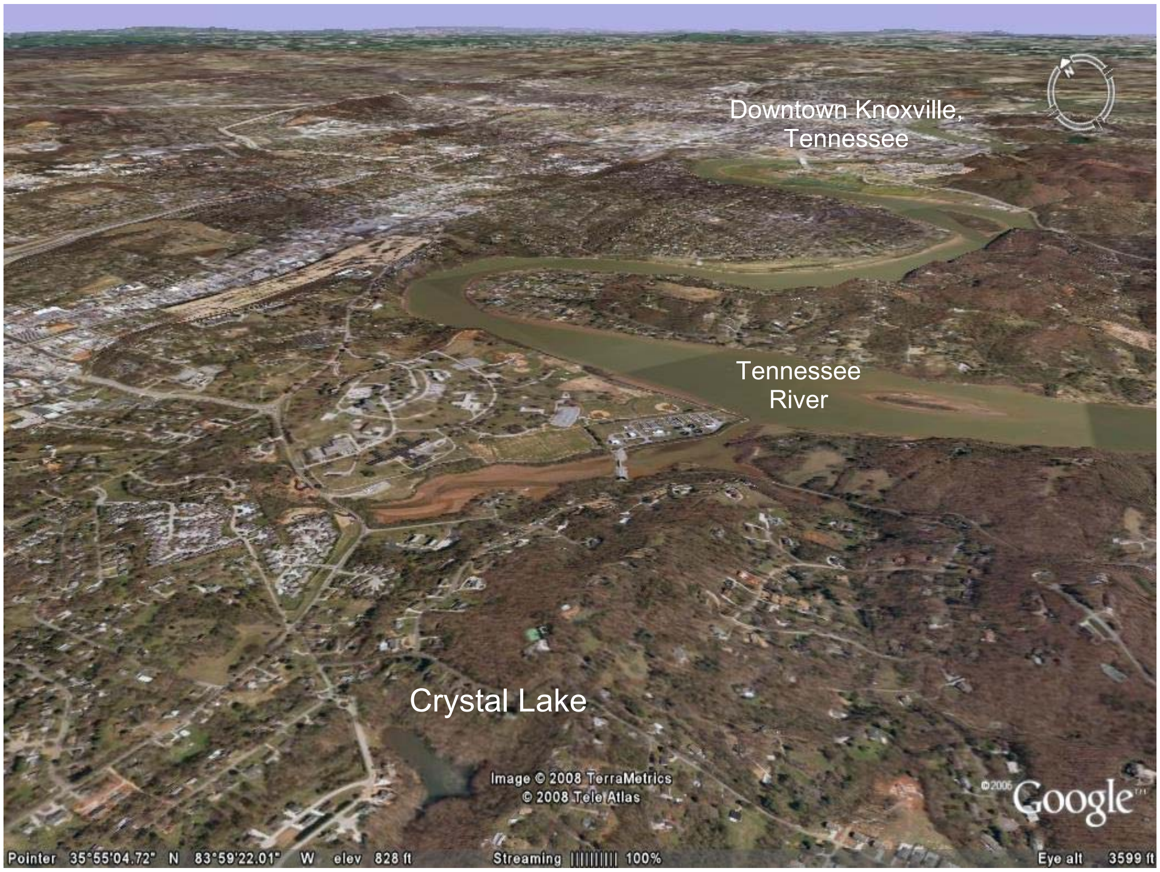

Figure 1.3. Oblique aerial photograph of Crystal Lake, showing its location within the city of Knoxville and its proximity to the Tennessee River and the downtown area. Source: Google Earth. Scale varies in this oblique view; Crystal Lake is about 175 m on its long axis.

also reviews the research using stomata that has been completed in high northern latitudes, on which my study is based. Chapter three describes my three sites and their physical surroundings. Chapter four explains the methods used in conducting my research, including core retrieval from Lagunas Castilla and Salvador, dredge sampling of Crystal Lake, laboratory processing, and slide examination. Chapter five presents the results of my research. In chapter six I interpret my results, focusing on how the stomata counts have enhanced the interpretability of pollen results in the Las Lagunas region. In addition, chapter six discusses how the results of the spatial analysis of Crystal Lake can help future stomata studies that are based on single cores. Finally, in chapter seven, I draw overall conclusions about the value of future stomata studies outside high-latitude and alpine environments.

Chapter 2

Stomata Background Information

Stomata

Stomata are small, specialized openings and associated guard cells on the epidermis of leaves, which allow the exchange of carbon dioxide, oxygen, and water vapor between the plant and atmosphere (MacDonald 2001). Like nearly all vascular plants, coniferous trees, including *Picea*, *Pinus*, and *Larix*, contain these microscopic structures on their leaves. Conifer stomata are distinct from the stomata of other taxa because they are lignified cells, with the result that they are decay-resistant when deposited in lake beds. As needles are shed by the tree, it is possible for some to fall in or be transported overland into a nearby lake. Once the needle reaches the lake, the needle begins to decompose, leaving single separate stomata on the lake's bed. Because the stomata themselves are lignified, they preserve well in the lakebed sediments along with pollen grains and other decay-resistant materials (MacDonald 2001). After the stomata are detached from the far larger needle, they become susceptible to remobilization and redeposition throughout the lake. The gradual buildup of sediment including pollen and stomata on the lakebed results in a stratified deposit containing a record of the watershed's ecological history. In this study, the endemic Hispaniolan pine, *Pinus occidentalis* Swartz, produces stomata and pollen in the Las Laguna region. *P. occidentalis* is the only conifer species found on Hispaniola, so there is only a single taxon of conifer pollen or stomata to distinguish in sediment cores.

Pine stomata counts cannot in and of themselves reveal the introduction of a species into a region, but can identify establishment locally (Hansen 1995, Clayden et al. 1996, Gervais and MacDonald 2001, and MacDonald 2001). Pine pollen, on the other hand, is commonly transported long distances, making it difficult to determine the local establishment of a species, and especially to document the local presence of a particular taxon (MacDonald 2001, Leitner and Gajewski 2004). Because pine pollen is dispersed over long distances, researchers use increasing and decreasing pine pollen concentrations to establish the timing of introduction of pines on a regional scale. Local pine presence is normally determined by the percent of pine pollen identified in a sediment sample from a known stratigraphic level. Once the pine pollen reaches an established proportion of the entire pollen count, researchers can justifiably claim local presence. By including stomata counts in studies, researchers are able to much more accurately pinpoint the time of a species' introduction at a site, because of the small distance a needle is able to travel. This approach limits the area of investigation to within the watershed itself, but knowledge of the timing of species introductions in multiple watersheds and lakes through multiple studies provides the larger regional picture.

Pine pollen is very small (on the order of 0.05–0.08 mm) with two special bladders connected to the body that act like wings or gliders. Because of this small size, lightweight construction, and specialized morphology, pine pollen has the ability to be blown long distances. For this reason it contributes "extra-local pollen" to watersheds, making it difficult to interpret data in strictly local terms (Parshall 1999, Leitner and Gajewski 2004) and causing it to be overrepresented in many records (Kennedy et al.

2005). Unlike pollen, stomata when they are released from the tree are embedded in conifer needles, which are heavy and cannot move long distances (MacDonald 2001). Parshall (1999) found that, in small watershed basins, tree pollen could typically originate as much as 100 meters away while any given stoma is generally found within 20 meters of its origin; no significant stomata input occurred farther than 50 meters from the original tree. Hansen (1995) similarly concluded that large basins could not have any input of stomata from more than two kilometers away, barring input from fluvial transport. However, when taken together, conifer pollen and stomata provide a reliable combination of paleoecological proxies with greater information content than either can provide by itself (Clayden et al. 1996, Gervais and MacDonald 2001).

Las Lagunas is situated on a hillslope where pollen can be transported up or down the slope easily by mountain and valley winds. Pisaric et al. (2000) stated that wind regions in mountain environments, where vegetation zones are compressed into narrow belts, facilitate cross-contamination of pollen between sites along the mountain slope. The Cordilla Central where Las Lagunas is located contains a high density of vegetation patterns in narrow bands along the climatic gradients of the mountain range which creates a high chance of such contamination in the lakes from pine trees upslope and downslope of Las Lagunas (Kennedy et al. 2005).

The History of Stomata Studies

Research looking at the concentration of conifer stomata has been increasing over the last 15 years (e.g., Clayden et al. 1996, Hansen 1995, Fernand 1997, Yu 1997, Parshall 1999, Pisaric et al. 2000, 2001, 2003, Gervais and MacDonald 2001, MacDonald 2001, Mugica et al. 2001, Leitner and Gajewski 2004, Froyd 2005, Kennedy et al. 2005, Kultti et al. 2006). This recent emergence of conifer stomata as a useful paleoecological indicator tool has occurred almost exclusively in alpine environments or northern-conifer-dominated environments of the Canadian or European boreal forests.

Studies completed near the northern margin of forest vegetation have sought to map the historical migrations of treeline caused by climate changes (Clayden et al. 1996, Hansen et al. 1996, Gervais and MacDonald 2001, Pisaric et al. 2001, Leitner and Gajewski 2004.) These studies reconstruct treeline shifts by examining multiple lake cores across transects spanning current treeline from forested areas into the tundra environment. Each author notes that pollen alone is insufficient to establish historical treeline position, but that the use of stomata as an additional proxy indicator of pine presence yields accurate results. Clayden et al. (1996) helped establish stomata as a useful proxy in modern and historic sediment by studying surface samples from a northern Siberian peninsula. Their research examined 23 lakes ranging from closed boreal forest to open tundra in the Taimyr Peninsula to establish modern pollen rain percentages for conifer species. In each lake Clayden et al. (1996) compared the modern abundance of stomata against modern pollen percentages to determine the effectiveness of stomata representation of conifers in local vegetation. They found it to be an effective indicator.

Pisaric et al. (2000, 2003) and David (1997) similarly used stomata concentrations to verify tree line migrations caused by climate and anthropogenic influences in high altitude environments over the past 10,000 to 11,000 years. Both Pisaric et al. (2000,

2003) and David (1997) stated that alpine environments are indicator zones for climate change due to their sensitivity to temperature change. Due to this tumultuous environment, pollen records alone cannot accurately determine past vegetation zones. Because of their successful research results in the northern Rockies, Pisaric et al. (2003) went as far as to conclude that fossil stomata have provided the single most reliable proxy of treeline fluctuation in the alpine regions of northern Canada.

Three studies involving stomata in high latitude sites that do not focus on tree line migration have used stomata as a proxy record of tree stand invasions into small or large watersheds (Hansen 1995, Parshall 1999, Mugica et al. 2001). Hansen (1995) examined bogs in the lowlands of Hudson Bay to discover the date at which eight different conifer species invaded the area. Parshall (1999) was able to use stomata counts to identify a forest stand invasion of hemlock trees into forest hollows in the lower Great Lakes region. In the study, Parshall was able to calculate hemlock pollen percentages from several hollows in a region and compare them to stomata occurrences. By using average numbers of pollen percentages in sites where stomata were present, Parshall was able to estimate with confidence a threshold percentage value at which hemlock pollen becomes a “local” contributor. Of all the stomata studies done using lake sediment cores, Mugica et al. (2001) had the lowest latitude site (41º 57' 24" N). In his research, Mugica et al. studied a 14.1-meter core spanning the entire Holocene to track multiple conifer tree stand invasions within a valley on the Iberian Peninsula.

One single journal article reports on a tropical stomata study, in the highlands of the Dominican Republic (Kennedy et al. 2005). Kennedy et al. used both pollen and stomata counts in pond and bog sediments, and compared them to modern surface soil samples, to characterize the distribution of pollen in the Cordillera Central, Dominican Republic. Kennedy et al. (2005) compared pine pollen and stomata concentrations in soil samples taken in areas varying from open savanna to soils lying underneath heavy pine tree stands. They hope to use the modern stomata concentrations to calibrate and coordinate their use in fossil contexts to distinguish treed and treeless periods dating back to the last glacial maximum. Stork (2006) examined the pollen stratigraphy of a profile from West Pond on Great Abaco Island, The Bahamas. She hoped to use pine stomata as an indicator of local pine presence, but found only one pine stoma during her pollen counts.

Chapter 3

Study Sites

The Las Lagunas Region

Two study sites in the Las Lagunas region of the Dominican Republic were used in this study. The Las Lagunas region (18°48'N, 70°53'W) is a farming community located on the southwestern flank of the Cordillera Central (Figure 1.2) at an approximate elevation of 1000 meters. The Las Lagunas area, in the Azua province, is north of the town of Padre Las Casas and lies in a region of high rolling hills composed of soft marine sediments. Aerial photographs show numerous large slope failures in these hills. In Las Lagunas, a catastrophic, semi-rotational, semi-translational failure occurred long ago, causing the creation of several lakes (Lane 2007). Grasses and shrubs with scattered stands of trees make up the current natural vegetation of the area. Since the area is now farmed, the natural vegetation has been altered from a tree-dominated environment. Four lakes in the Las Lagunas area have been surveyed and cored by Orvis and Horn and their students, but only two of the lakes, Laguna Castilla and Laguna de Salvador, were studied in this thesis.

Laguna de Salvador (Figure 3.1) is a small lake occupying the dropped upper section of a west-facing drainage. It is 0.5 hectare in size, with a maximum depth of 2.7 meters. Salvador does not have an inlet or a permanent outflow. The only inputs are from rainfall, overland flow, and ground water. Vegetation along 75% of the shore is arboreal, with pasture beyond a moat of floating plants comprising the remaining 25%.

Figure 3.1. Aerial photograph of Laguna de Salvador. Source: Google Earth.

Topography around Salvador varies from steep slopes to seasonally inundated flats. Three sides of the lake are backed by hills grading steeply down into the water. The lake sits at the bottom of a little valley described by those three sides. The remaining (southern) shoreline of the lake merges from a floating mat of vegetation gradually into gently sloping dry land.

Laguna Castilla (Figure 3.2**)** is larger than Laguna de Salvador at 1.5 hectares in size, with a maximum depth of 4.6 meters. Remnants of shorelines in surrounding pastures indicate the water level in Laguna Castilla was once higher. Like Salvador, Castilla does not have any permanent inflowing or outgoing streams to maintain interchange of water. The Castilla shoreline, unlike Salvador's, is at least 50% grass pasture. Castilla now has only a very slight elevation change as you approach the water and was much larger in the past. All of Castilla's shores exhibits this very gently sloping shoreline.

Crystal Lake

In addition to the two lakes in the Las Lagunas region, I also used a lake in Knoxville, Tennessee (35°58'N, 83°55'W) for my stomata dispersion study. Crystal Lake (Figure 3.3), unlike Castilla and Salvador, is a small sinkhole lake located in a temperate climate within a mixed forest ecosystem. It is one hectare in size, with an average depth of 4.3 meters. Crystal Lake is also different from Castilla and Salvador in that it has a small inflowing stream. In addition to this main stream, there are indications that two other streams may have flowed into the lake in the past. Urban development has altered the streams' natural courses.

Figure 3.2. Aerial photograph of Laguna Castilla. Source: Google Earth

Figure 3.3. Aerial photograph of Crystal Lake. Source: Google Earth

Crystal Lake is located within the metropolitan area of Knoxville, so noticeable urban alterations have taken place around it. Although the lake has a small inflowing stream it has no overland outlet. The lake itself is not used by humans for water needs or recreational activities, except for an occasional fisherman. Two sides of Crystal Lake are bordered by single-family homes. Those on the eastern flank of the lake are older than fifteen years so the natural vegetation has had a chance to regenerate since construction. A newer subdivision borders the lake on its western flank, and in that area visible erosion has occurred during the past several years. The north end of the lake includes a mowed and trimmed grass field, while the southern edge of the lake is bordered by second-growth mixed forest.

Topography surrounding the lake is of two types. The southern and eastern flanks of the lake are steeply inclined, and support semi-natural plant communities that inhibit excessive runoff into the lake. It is in one of these sections of the shore that stream input occurs, but the stream tends to flow only intermittently in response to precipitation events. The western and northern shorelines of Crystal Lake are only slightly to moderately inclined. Even though there is a moderate incline into the lake, it is in this portion of the watershed that heavy urbanization has taken place, leaving an area devoid of dense plant cover.

Climate

The climate of Las Lagunas is strongly affected by its location along the southwestern edge of the Cordillera Central. The 2000-meter-plus mountains block the constant moisture brought by the Northeast Trade Winds. Las Lagunas receives the bulk of its precipitation from convective activity fed by the moisture-filled onshore sea breeze from the south. A distinct wet season occurs during the summer, when the ITCZ is located near its northerly extreme, bringing low-pressure doldrum conditions that enhance this convection activity. The dry season for the Las Lagunas region occurs during the late winter and early spring. Published by Orvis et al. (1997)

Since there are no precipitation or temperature stations in Las Lagunas, estimates have been calculated based on nearby weather stations. Using temperature data from the Padre Las Casas meteorological station, 450 m lower in elevation, and a lapse rate of –8.5 °C km^{-1} (Orvis et al. 1997), Lane (2007) estimated the average annual temperature for Las Lagunas to be around 20 °C. The nearest station for precipitation data was the city of Azua, 42 km to the southeast. Based on the annual precipitation at Azua of about 700 mm, Lane (2007) estimated that the areas around Lagunas Castilla and Salvador receive around 900–1000 mm of precipitation per year.

Knoxville, Tennessee is located in the Valley and Ridge physiographic province in the southeastern United States. This region comprises northeast-to-southwest running valleys with parallel ridges separating the valleys. Karst topography is found in areas of limestone bedrock in the valley bottoms. Sinkholes are common in karst regions, some being dry hollows and others forming lakes. Crystal Lake is an example of a sinkhole lake.

Knoxville's precipitation regime includes no distinct dry season (Figure 3.4). Its Köppen climate classification is Cfa. Winter precipitation is driven by mid-latitude frontal activity. Convective storms and onshore movement of moist air from the Gulf of Mexico dominate summer precipitation. Though there is no dry season, a water deficit

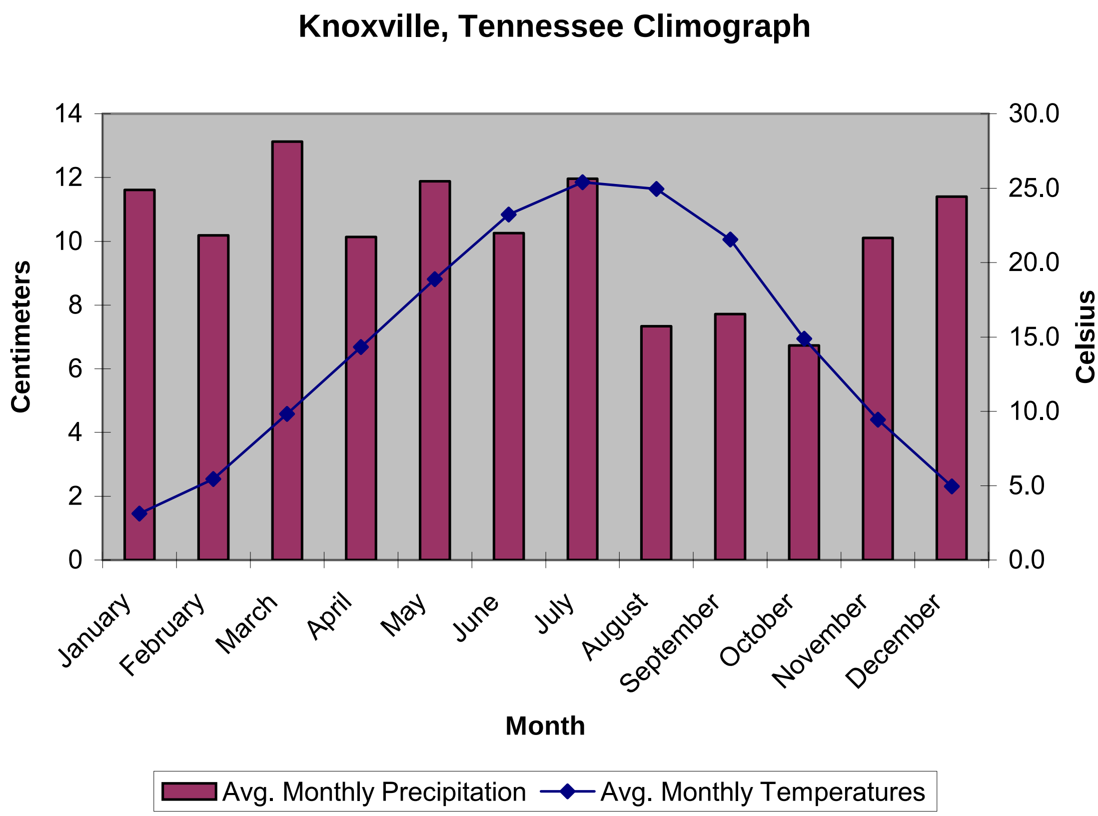

Figure 3.4. Climograph of Knoxville, Tennessee. Data compiled from the National Oceanic and Atmospheric Administration. Data represent averages over the past 30 years.

generally occurs during the summer months. A water surplus during the winter months occurs in part because of the lack of metabolically active plants drawing moisture from the soil. Frequent storms can cause overland flow into lakes such as Crystal Lake, as evidenced by high mineral content in the lake bottom, especially on the western side where the new construction has occurred.

Chapter 4

Methods

Site Selection and Field Sampling

Site selection for the Las Lagunas region was completed in June 2001 and July 2002 by professors Sally Horn and Kenneth Orvis along with graduate students Chad Lane and Duane Cozadd. Horn, Orvis, Lane, and Jeff Dahoda recovered the sediment cores that are the focus of this study, in approximately one-meter sections, in July 2002 and January 2004. They used a plastic tube fitted with a rubber piston to collect the mud-water interface, and a five-centimeter diameter Colinvaux-Vohnout (C-V) locking piston corer (Colinvaux *et al.* 1999) to recover deeper sediments. They extruded the near-surface cores in the field in two-centimeter intervals, and returned the C-V core sections to the UT Laboratory of Paleoenvironmental Research still encased in the original coring tubes, where they were stored at six degrees Celsius for later opening and sampling.

Crystal Lake fieldwork and sampling were completed in April of 2006. As a part of Geography 535 (Pollen Analysis), Dr. Horn took the class to Crystal Lake to practice taking lake cores using a piston corer. During the trip, a straight-line transect across the lake was mapped from a stand of pine trees to the opposite bank (Fig 4.1). Along the transect, Joe Burgess and I took six surface sediment samples using a LaMotte dredge, starting at the bank with the stand of pines and ending at the deepest part of the lake. Each sample was placed in a 250 mL bottle and then transported back to the lab and stored at six degrees Celsius until processed.

Figure 4.1. Aerial view of Crystal Lake with dredge-sample transect and site of core taken during the same trip to the lake. Circled areas are the pine stands from which the transect was lined up; dredge-sample transect was anchored at the left circle. Source: Google Earth.

Laboratory Methods

The lab portion of the research was done in two ways. The Las Lagunas cores and resulting samples were processed by Lane for the completion of his dissertation research. Lane conducted counts of pine pollen and *Lycopodium* on his prepared slides, and I later conducted independent counts of stomata and *Lycopodium* on the same slides. I processed the Crystal Lake samples myself in preparation for my own pine pollen, stomata, and *Lycopodium* counts.

Lane processed the cores from both Laguna Castilla and Laguna de Salvador for pollen analysis at 16-cm intervals using standard pollen processing techniques, and prepared and analyzed the microscope slides. Percentages of *P. occidentalis* pollen and of non-coniferous trees and herbs were calculated by Lane for his dissertation research and those results were available to me for comparison with stomata results. My stomata counts were done using the same slides Lane prepared for pollen counts. On each slide, I scanned twenty transects at 250x magnification, recording all stomata and all *Lycopodium* control spores I encountered.

For Crystal Lake, I first performed loss on ignition (LOI) using samples of well-mixed sediment (five with a volume of 1.2 cc and one with a volume of 2.5 cc). I followed the Dean (1974) method for LOI to determine water content and organic matter, by drying the samples at 100 °C and then igniting them at 550 °C. To process the samples for stomata and pollen counting I used the same volumes of material and standard procedures (HCl, HF, KOH, acetolysis, and safranin stain: Berglund 1986; see Appendix A for details) with an addition of *Lycopodium* spores to aid in calculation of pollen concentrations. The residues after processing were placed on microscope slides.

The HF procedure in processing the six samples from Crystal Lake turned out to be troublesome. In most processing, one to a maximum of two HF cycles are enough to fully remove any silica materials, but in my samples I had to use up to four HF cycles to completely remove the large quantity of sand. To prevent contamination of samples, the Castilla and Salvador slides were processed in the tropical processing room of the Paleoenvironmental Laboratory in the Science and Engineering Research Facility, while the Crystal Lake samples were processed in the temperate processing room.

I identified stomata, pollen grains and *Lycopodium* on the Crystal Lake slides and stomata and *Lycopodium* on the Las Lagunas slides using *P. occidentalis* reference slides and the published illustrations of MacDonald (2001) for reference. Counts were completed at 250x magnification to identify the stomata. That magnification was needed to properly identify the pine stomata in the Las Lagunas slides because other plant stomata with somewhat similar morphology were present in the slides. I examined twenty transects at that magnification to scan approximately 75% of each slide. While counting pine stomata and pollen grains, I also counted the *Lycopodium* control spores to allow calculation of concentrations of stomata and pollen.

Pine pollen is an easy pollen grain to identify because of its unique appearance (Figure 4.2**)**. The grain has a large rounded body with two protruding bladders, making it a large-sized grain, approximately 40–50 µm (Kapp 2000**).** The *P. occidentalis* stomata were also easy to identify because of their unique structure. These stomata (Figure 4.3) have an oval stoma opening surrounded by dark subsidiary cells and two sets of woody lamellae creating a structure similar in appearance to the human eye.

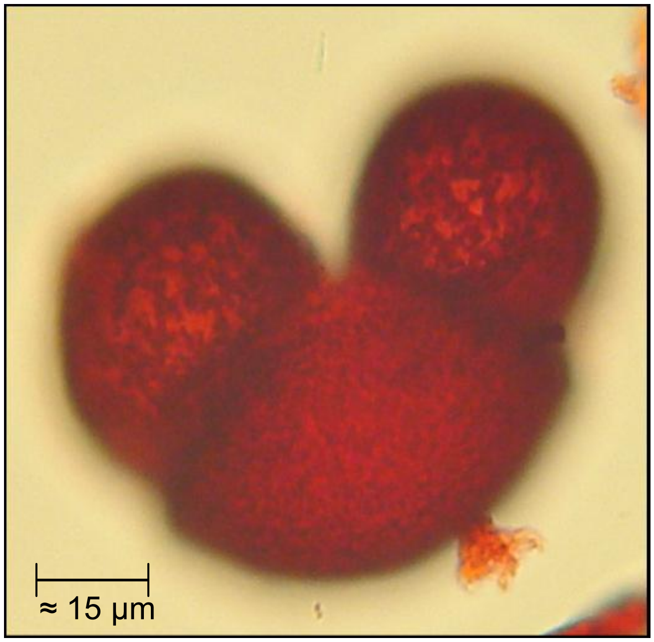

Figure 4.2. Microscopic picture of a pine pollen grain. This species is *Pinus caribaea* var. *bahamensis* (Griseb.) W.H. Barrett & Golfari. Photo taken by Allison Stork during her work in the Bahamas (Stork 2006).

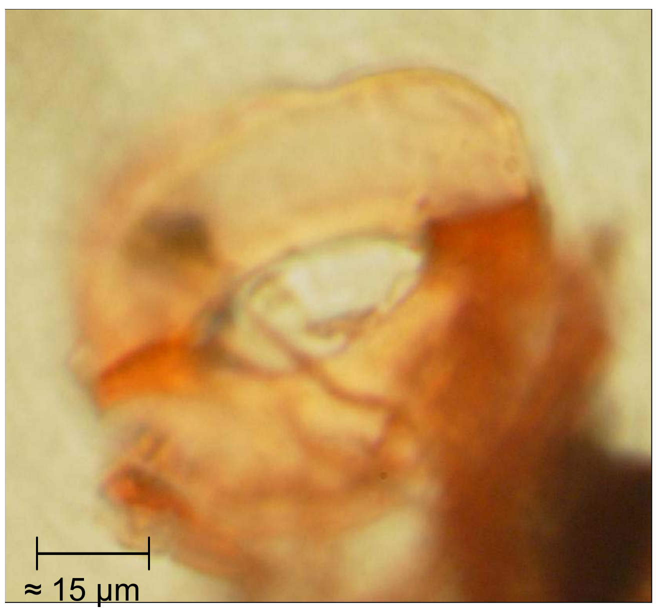

Figure 4.3. Microscopic picture of a pine stoma taken by Allison Stork during her work in the Bahamas. This species is *Pinus caribaea* var. *bahamensis* (Griseb.) W.H. Barrett & Golfari.

Chapter 5

Results

This chapter describes the results of my analyses of pine stomata in core samples from the Dominican Republic and surface samples from Crystal Lake, Tennessee. Stomata, pine pollen, and control spore counts for Crystal Lake samples are included in Appendix B. My stomata and control spore counts on selected levels of the Castilla and Salvador sediment cores are archived in the Laboratory of Paleoenvironmental Research at the University of Tennessee. Following publication, these data will be merged with pollen counts on the same cores made by Lane (2007), and then deposited in the Latin American Pollen Database (http://www.ncdc.noaa.gov/paleo/lapd.html).

Previous Results: Lane's Laguna Castilla Pollen Counts

The Laguna Castilla sediment core has a total length of 638 cm, of which the top 32 cm were collected in the mud-water interface core. The pine pollen stratigraphy for Castilla (Lane 2007) includes a peak in pine pollen approximately halfway up the sediment core and lower concentrations of pollen near the surface and in the deepest parts of the core (Figures 5.1, 5.2, and 5.3). There is one spike in pine pollen concentrations at a depth of 590 cm, the only high concentration found in the lower portion of the core. The bottom half of Castilla's sediment core contains only moderate pine pollen concentrations, other than at 590 cm. Progressing up the core, pine pollen concentrations begin to gradually increase at a depth of 382 cm, and then dramatically increase at 318

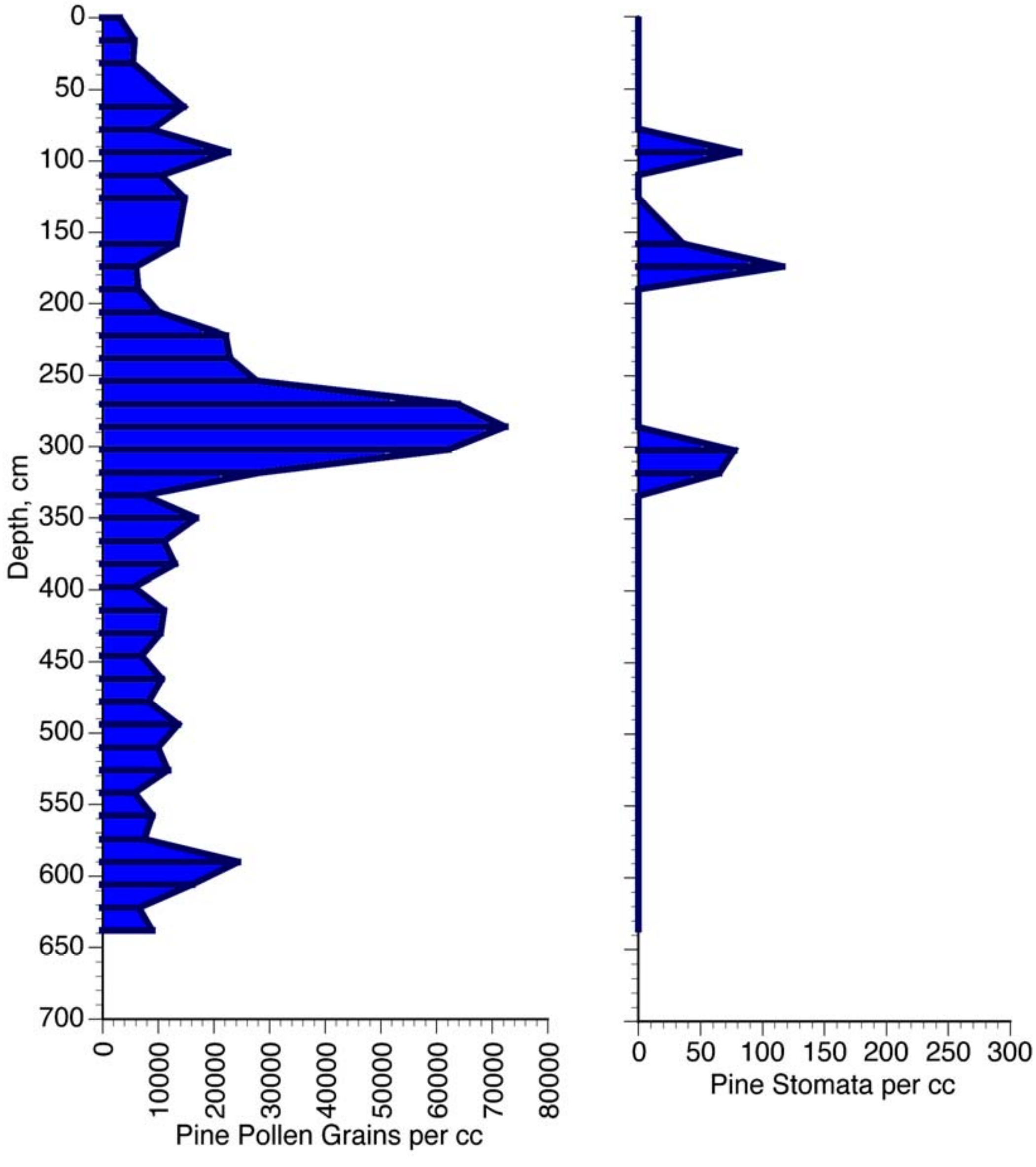

Figure 5.1. Laguna Castilla results graphed as particles per cc. Left column is the number of pine pollen grains per cc of sediment at the measured depths (data from Lane). Right column is the number of pine stomata per cc of sediment.

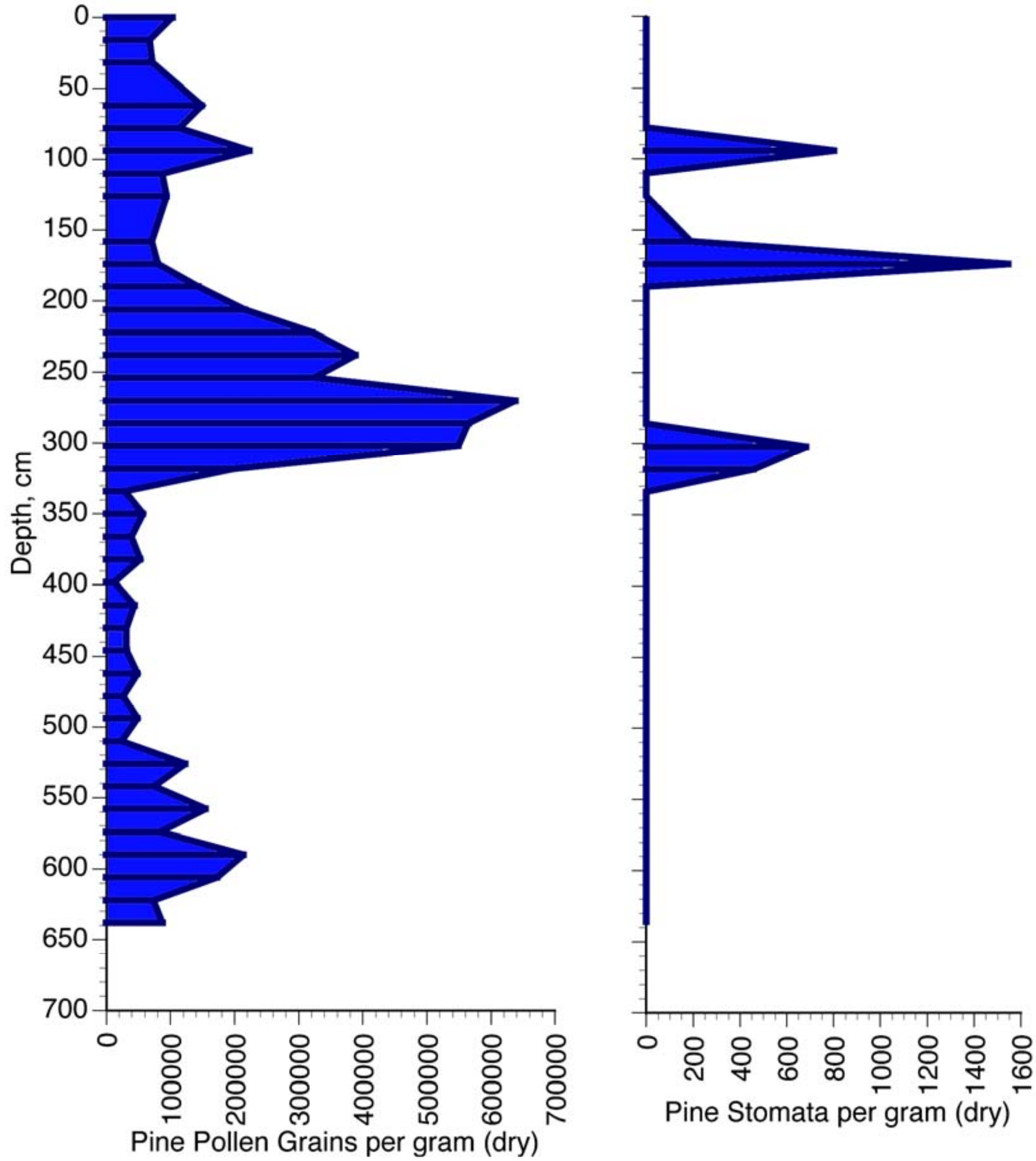

Figure 5.2. Laguna Castilla results graphed as particles per gram dry weight. Left column shows the number of pine pollen grains per gram of dry weight (data from Lane). Right column is the number of pine stomata per gram dry weight.

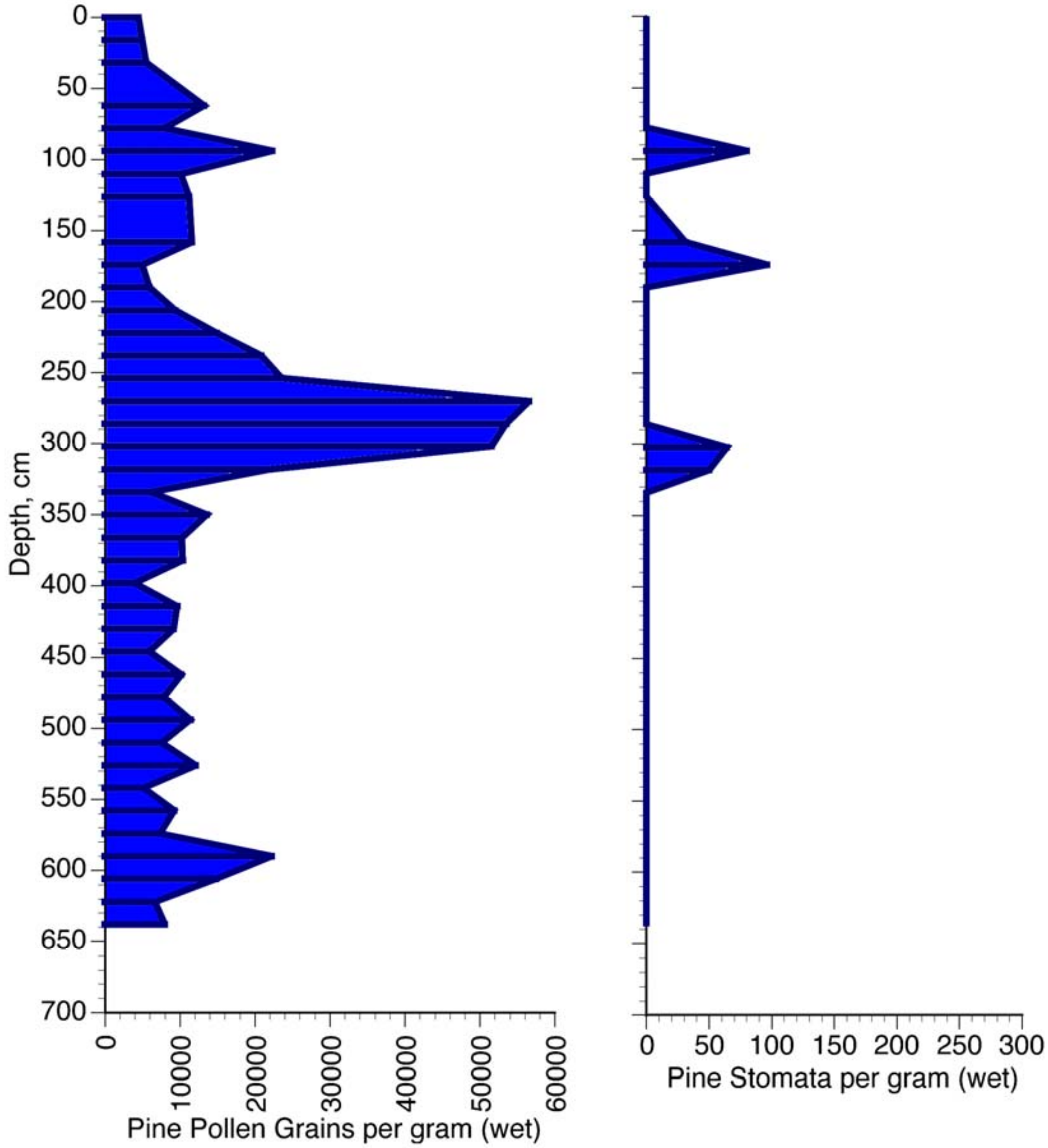

Figure 5.3. Laguna Castilla results graphed as particles per gram wet weight. Left column the number of pine pollen grains per gram of wet weight (data from Lane). Right column is the number of pine stomata per gram wet weight.

cm. This section of the core contains the highest pine pollen concentrations, over the longest sustained period, of any section of the core. After a peak at 286–270 cm, the concentrations sharply decrease for the remainder of the upper core. The concentrations vary somewhat but never regain their mid-core magnitude.

Lane's Laguna de Salvador Pollen Counts

The Laguna de Salvador sediment core has a total depth of 476 cm. Salvador (Figures 5.4, 5.5, and 5.6) has higher concentrations of pine pollen throughout the core than Castilla. The Salvador core begins with high concentrations at the base. From a peak at 364–380 cm pollen concentrations gradually decrease to a core low at 292 cm. From there, the concentrations increase, ushering in an era of high concentrations from 250 cm until 140 cm. After 140 cm, the concentrations remain low through the mud-water interface core. The three samples nearest the surface at Salvador are similar to the Castilla near-surface samples with similarly low pine pollen concentrations.

Laguna Castilla and Laguna de Salvador Stomata Counts

Pine stomata found in Laguna Castilla and Laguna de Salvador coincide reasonably well with zones of high pollen concentration found by Lane (Figures 5.1–5.6). The sediment core from Laguna Castilla had remarkably few stomata. No sample yielded more than a single stoma using my scanning protocol. A total of five stomata were found in the entire sediment core, and all were in the top half. Single stomata were found at respective depths of 318, 302, 174, 158, and 94 cm. Of the five core levels with a single stoma, four were clustered in two sets of two adjacent samples, at 318 and 302 cm, and

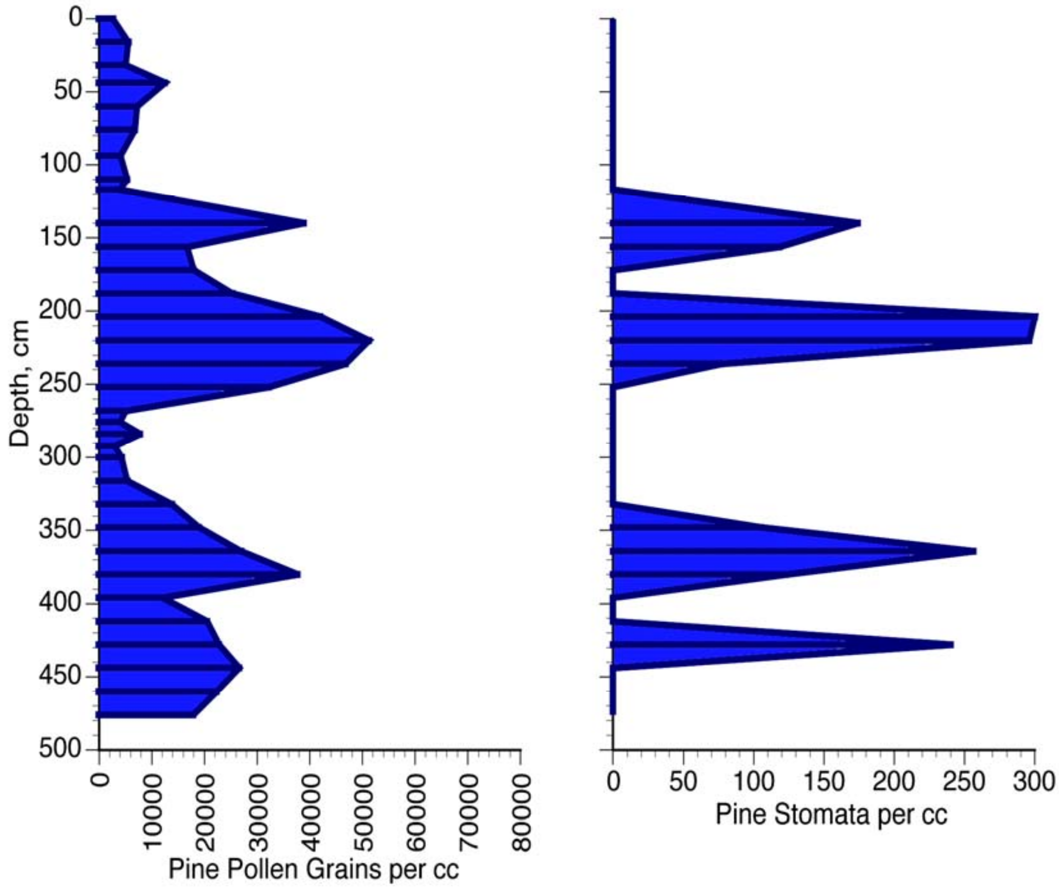

Figure 5.4. Laguna de Salvador results graphed as particles per cc. Left column is the number of pine pollen grains per cc of sediment at the measured depths (data from Lane). Right column is the number of pine stomata per cc of sediment.

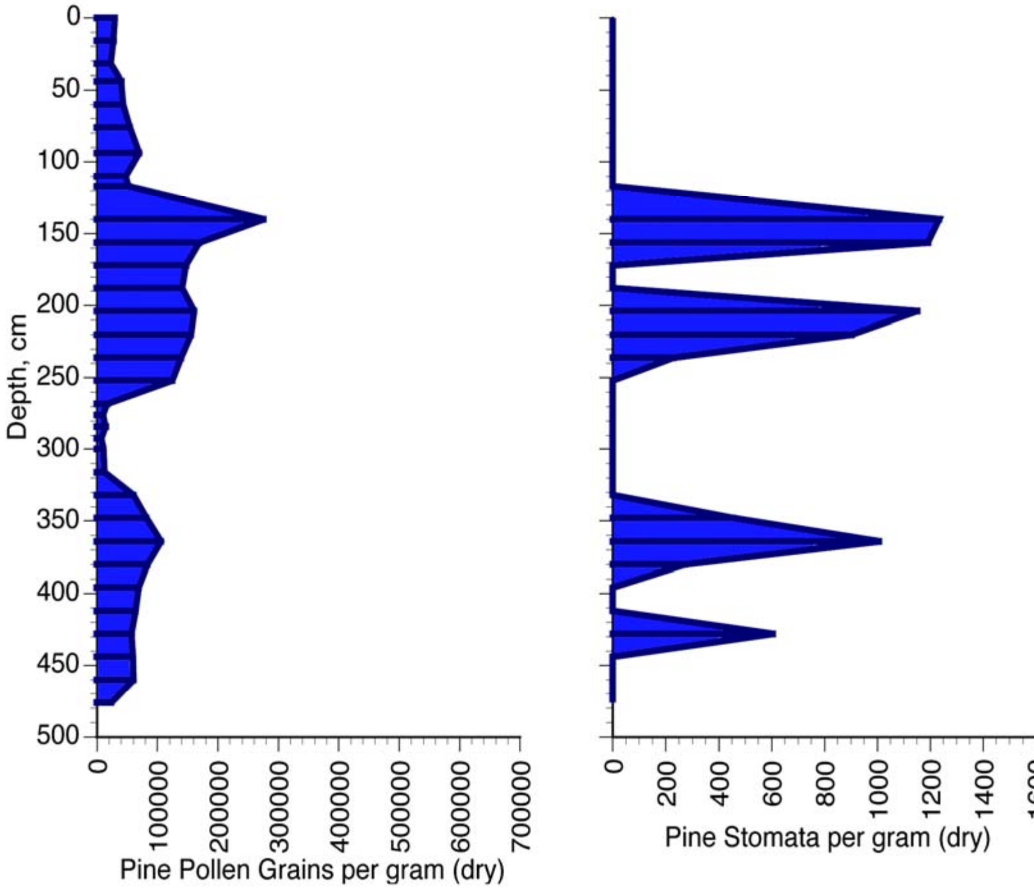

Figure 5.5. Laguna de Salvador results graphed as particles per gram dry weight. Left column shows the number of pine pollen grains per gram of dry weight (data from Lane 2007). Right column is the number of pine stomata per gram dry weight.

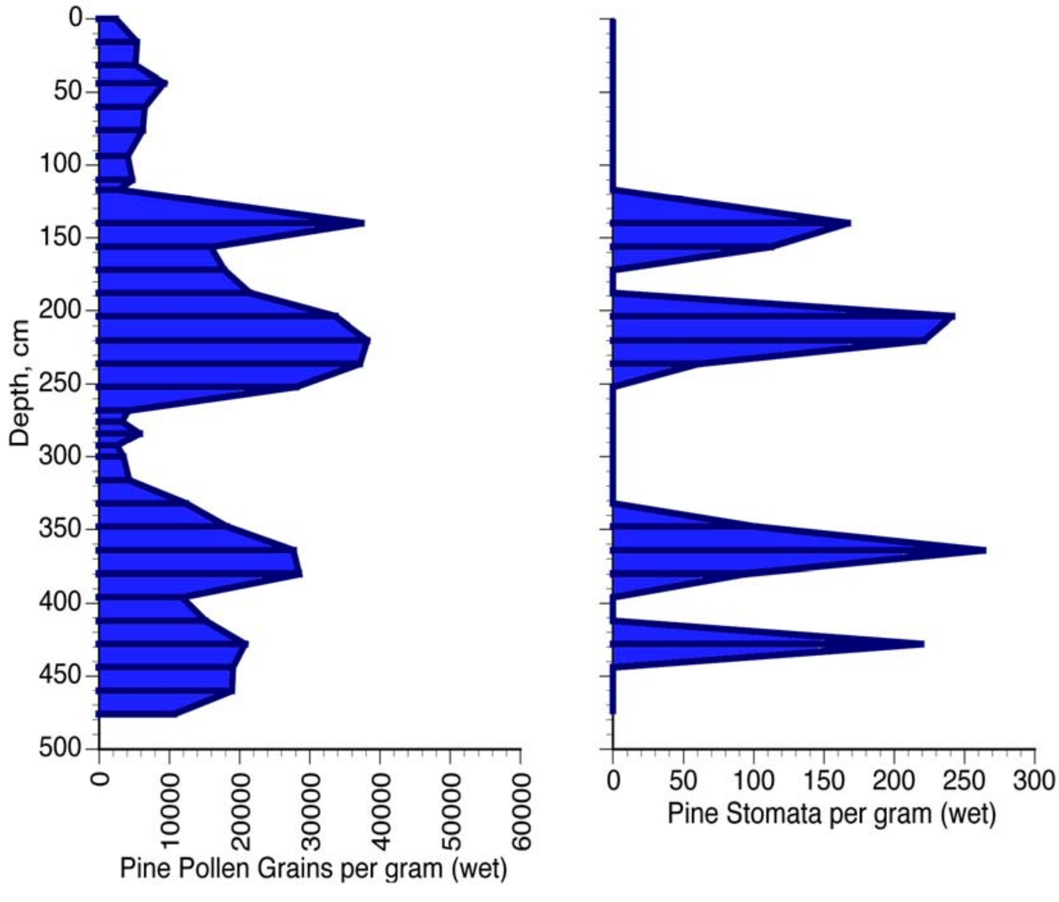

Figure 5.6. Laguna de Salvador results graphed as particles per gram wet weight. Left column shows the number of pine pollen grains per gram of wet weight (data from Lane). Right column is the number of pine stomata per gram wet weight.

174 and 158 cm. These results coincide with high pollen concentrations when plotted beside one another (Figures 5.1, 5.2, and 5.3). Laguna de Salvador had many more stomata throughout the sediment core (Figures 5.4–5.6). Results tended to vary from level to level, with zones containing no stomata alternating with zones containing several stomata. The lowermost zone of stomata extended from 428 cm to 348 cm, with four of the six levels having at least one stoma. A second zone of stomata extended from a depth of 236 cm to 140 cm. Of the seven samples in that zone, five had at least one stoma, and two levels, 220 and 204 cm, had four stomata each. Every Laguna de Salvador sample containing stomata occurred within these two zones of the sediment core. Zones without stomata occurred between the two zones (Figure 5.7) with stomata, from 332 to 252 cm, and also in the uppermost section of the core from 117 cm to the top.

Figure 5.7 has results plotted on a calibrated age axis, using calibrations by Lane (2007), for both the Castilla and Salvador pine pollen and stomata data. The graph also presents the inferred time periods when the Las Lagunas area was inhabited by humans, as interpreted from pollen and stable isotope analyses of the same cores by Lane (2007).

Crystal Lake Pine Pollen and Stomata Counts

Crystal Lake counts of pollen and stomata are shown in the same fashion as Laguna Castilla and Laguna de Salvador (Figures 5.8, 5.9. and 5.10). The difference between the Las Lagunas lakes and Crystal Lake studies is only the retrieval method—a surface transect of dredge samples from shore to middle of the lake as shown in Figure 4.1. The location for the transect was chosen because the starting point at the western shoreline had pine trees within a few meters of the shoreline along with more pine trees

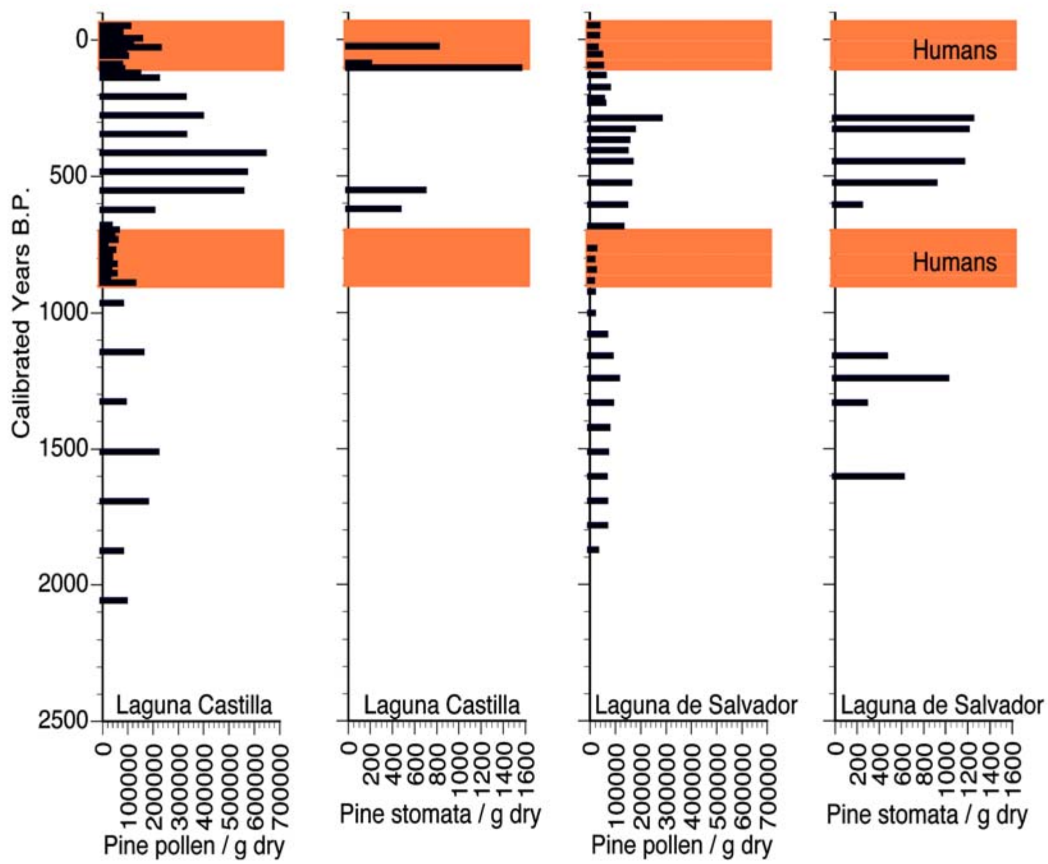

Figure 5.7. Periods of human impact plotted with results. Human occupation is based on Lane's interpretation using pollen, charcoal, stable carbon isotope data. Calibrated-years axis is based on Lane's radiocarbon dating and calibration using CALIB program 5.0 (Stuiver and Reimer, 1993) and the dataset of Reimer et al. (2004).

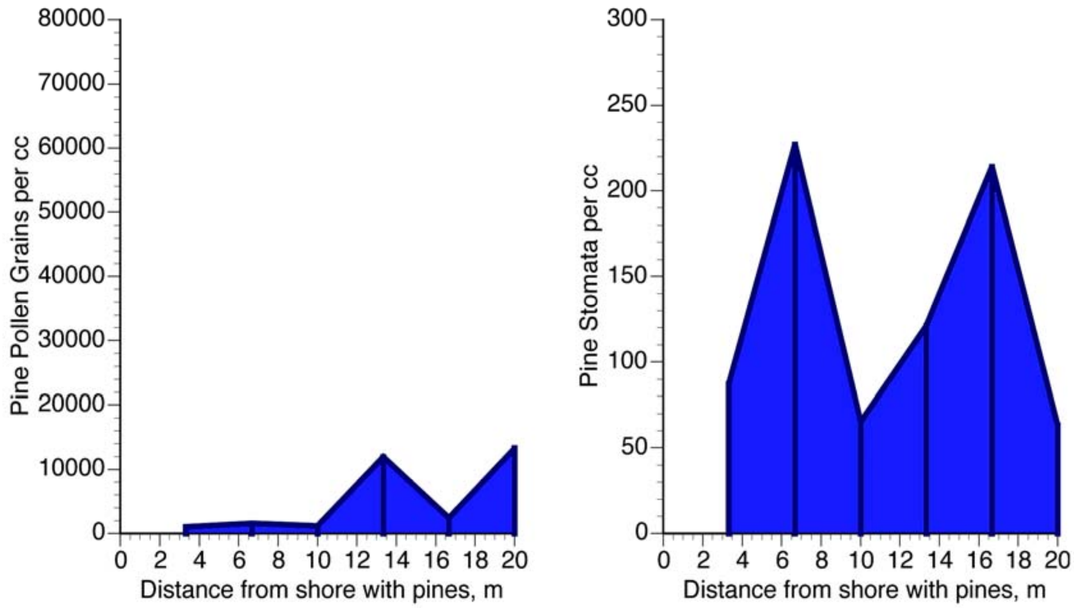

Figure 5.8. Crystal Lake results graphed as particles per cc. Left graph plots the number of pine pollen grains per cc of sediment against distance from the shoreline. The second graph plots the number of pine stomata per cc of sediment against distance from the shoreline.

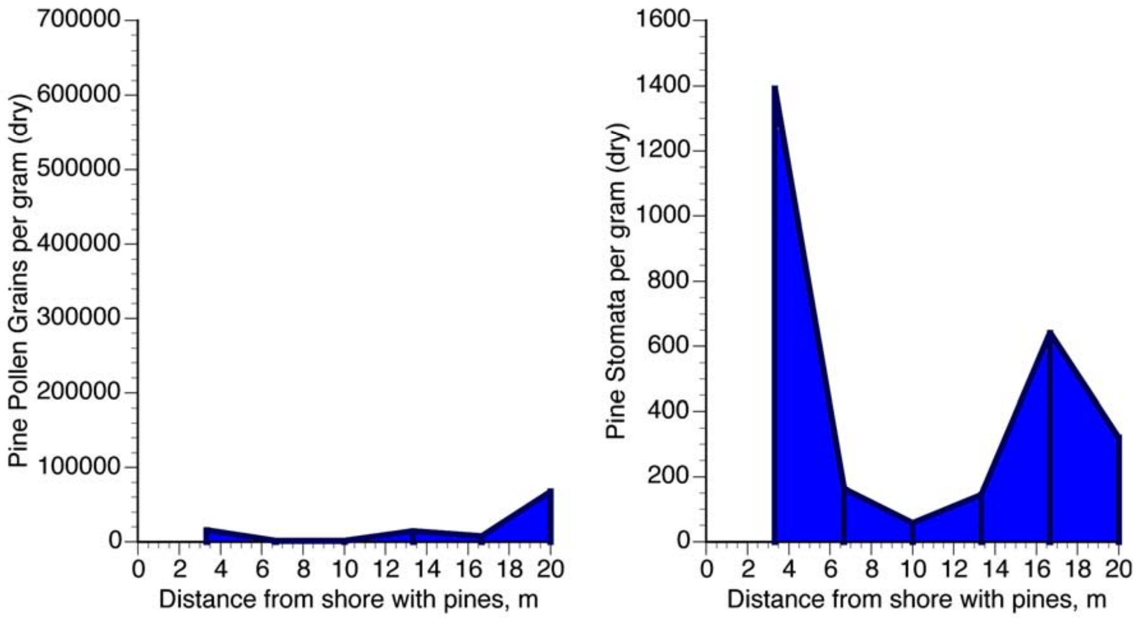

Figure 5.9. Crystal Lake results graphed as particles per gram dry weight. Left graph plots the number of pine pollen grains per gram dry sediment against distance from the shoreline. The second graph plots the number of pine stomata per gram dry sediment against distance from the shoreline.

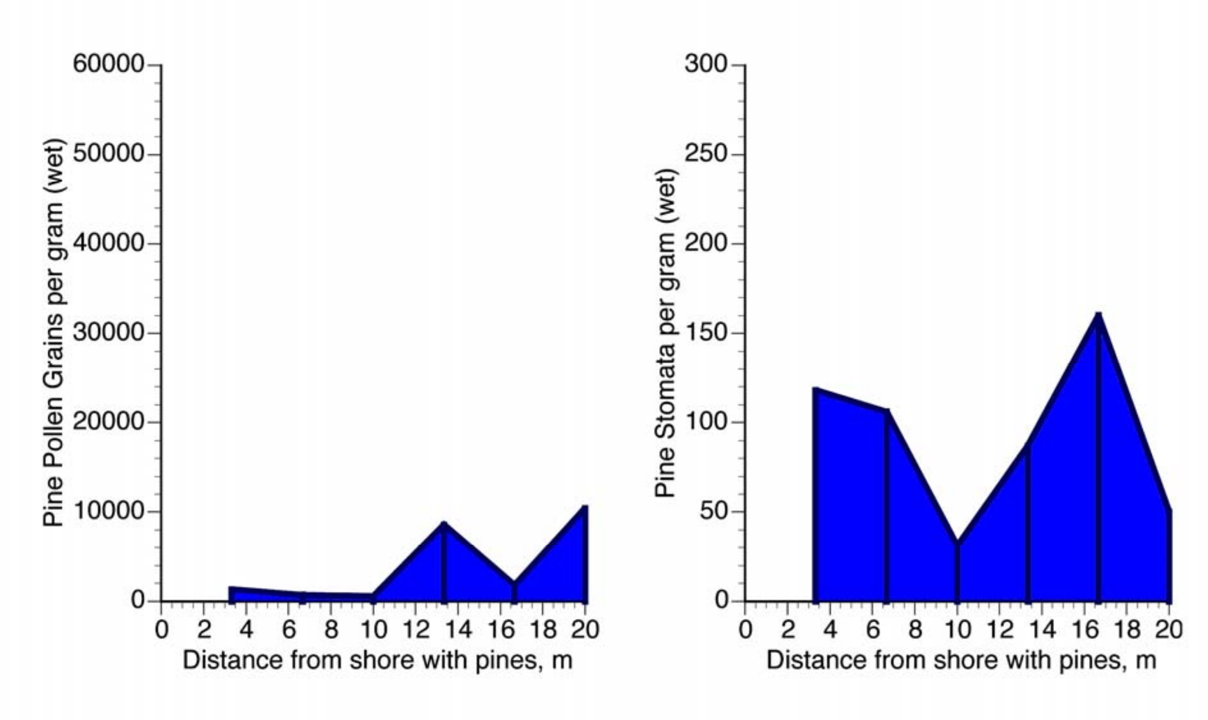

Figure 5.10. Crystal Lake results graphed as particles per gram wet weight. Left graph plots the number of pine pollen grains per gram wet sediment against distance from the shoreline. The second graph plots the number of pine stomata per gram wet sediment against distance from the shoreline.

on the opposite bank beyond the end of our transect. One possible contrast between Crystal Lake and the Las Lagunas lakes is a magnification in the wet weight results. Crystal Lake's samples were all surface samples which contain more water than the lower samples in a sediment core such as those from the Las Lagunas lakes. This is controlled for by comparing the three sets of results on a dry weight basis (Figures 5.2, 5.5, and 5.9) or as pollen-to-stomata ratios (Figures 5.11 and 5.12). Pine pollen concentrations in Crystal Lake increase as the center of the lake is approached (Figures 5.8, 5.9, 5.10). The counts of stomata (Appendix B) appear to show a general decline away from shore when viewed as raw counts. But when stomata are normalized and displayed as particles per cc, per gram dry weight, and per gram wet weight (Figures 5.8, 5.9, 5.10), the trend shows a large concentration of particles at the lake's edge and again at the center, with smaller concentrations between the two. When comparing stomata to pollen, the sample sites near the shore have a completely different make-up. The sites have some of the largest concentrations of stomata, while pollen nears its lowest amounts, as reflected in the ratio plots (Figures 5.11 and 5.12).

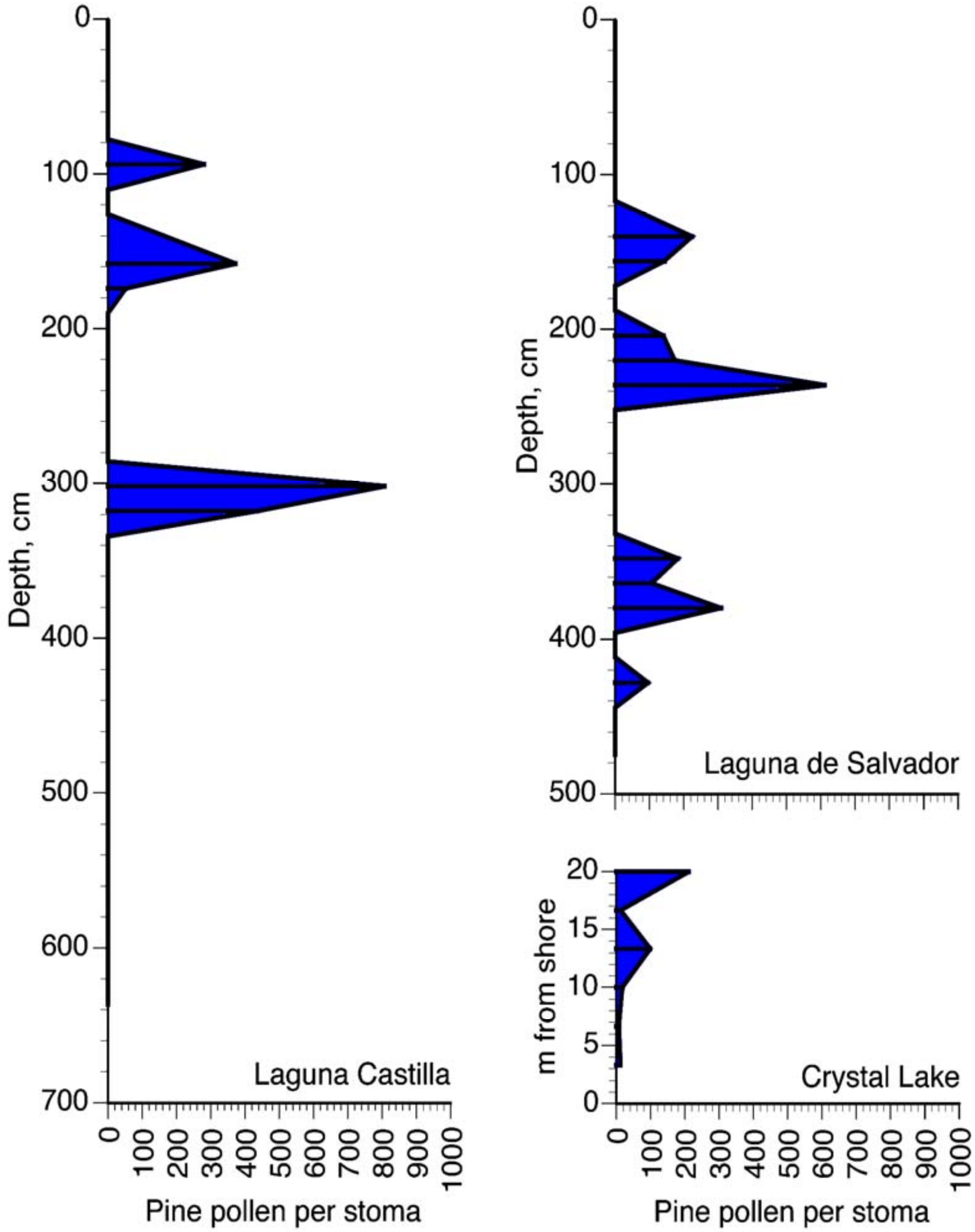

Figure 5.11. Pine pollen to pine stomata ratios. Graphs represent the number of pollen grains encountered per stoma. Levels where no stomata were found are plotted as zero values.

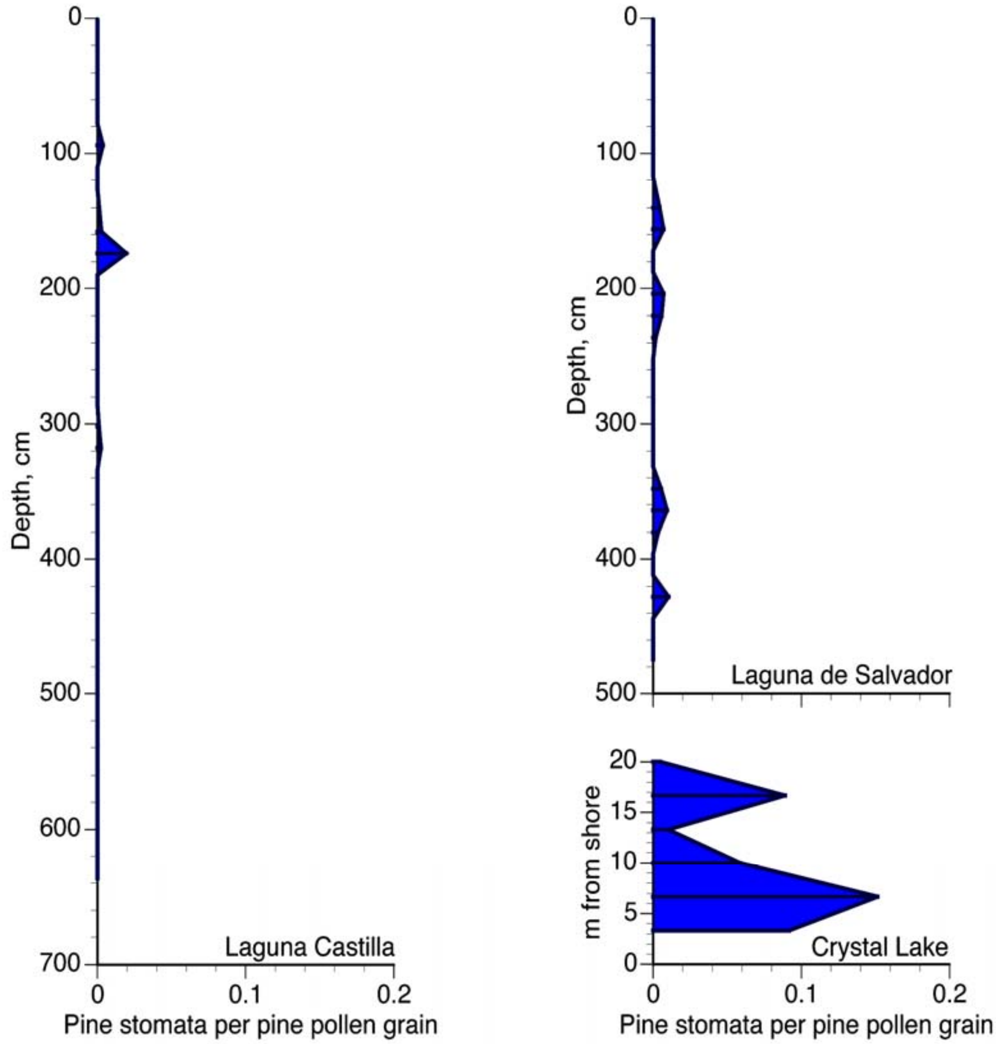

Figure 5.12. Pine stomata to pine pollen ratios. Graphs represent the number of stomata grains encountered per pollen grain. Levels where no stomata were found are plotted as zero values. Inverse from figure 5.11, included for ease of comparison with several published studies.

Chapter 6

Discussion

My extension of pine stomata research to low latitude lakes has yielded mixed results. Of the two lake sediment cores studied, from Laguna de Salvador and Laguna Castilla in the Dominican Republic, Salvador yielded a useful record of stomata presence while Castilla was sparse although indicative of local pine presence. The non-tropical Crystal Lake surface sediment study also yielded suggestive results that shed light on the paths stomata take once they enter lakes. The latter study also revealed how a single core site at or near the center of a lake may not be representative of overall stomatal concentrations. In general, this study has been a good first step toward introducing stomata research as a workable proxy for non-boreal lake systems.

The Relationship of Pollen and Stomata in Laguna Castilla

Laguna de Salvador and Laguna Castilla stomata results differ when they are paired with pine pollen concentrations tabulated by Lane (2007). The general lack of stomata throughout the Castilla core (Figures 5.1–5.3) suggests there is some factor preventing pine stomata from either entering the lake or dispersing within the lake to reach the core site. The lower, older half of the Castilla core has low concentrations of pine pollen grains, which may help explain the absence of pine stomata. Lane inferred that the upper part of this represents a time of heavy agriculture in Las Lagunas including immediately around Castilla (Figure 5.7) (Lane 2007). The lake's small surface area (1.5

ha) and small watershed (less than 25 ha) means that small distances around 50 meters might represent the maximum distance across which pine trees could have dispersed stomata into Laguna Castilla (*cf.* Parshall 1999).

The steep increase in pollen concentration starting at a depth of 318 centimeters (interpolated to 622 yr. B.P. or 1328 A.D, Figure 5.7) probably marks the abandonment of local agriculture and the re-establishment of pine stands in the lake's watershed (Lane 2007). Even though pollen concentrations are very high during this interval, stomata are still rare. Even in levels with peak pollen concentrations, I never found more than one stoma which does imply there were pine trees nearby the lake. One stoma does indeed represent the presence of pine trees nearby, but the high concentration of pine pollen suggests there should be higher stomata concentrations (Hansen 1995).

Progressing up the core, the pine pollen concentrations gradually decrease and then remain low with some variation. During this time sequence stomata are present just as modern humans begin to reoccupy the landscape (Figure 5.7). This is interesting because, as pine pollen decreases and as humans move in, we would expect tree stands to be decreasing and not be represented near the lake. Several factors could have caused these "anomalous" stomata and will be discussed later in this chapter.

Pine and Stomata Correlation in Laguna Salvador

In Laguna de Salvador the pollen and stomata zones are more obvious than in Castilla (Figures 5.4–5.6). Salvador has two distinct pollen peaks in the core, one near the base at 364–380 cm depth and another between 250 and 140 cm. Interestingly, the peak of pine pollen and occasional stomata near the base of the core occurs before human occupation. Four samples in this zone contained at least one pine stoma, with two containing two or more. A single stoma indicates that there was at least one pine tree in the immediate vicinity of the lake, but having multiple stomata at a given depth may mean a greater concentration of trees locally.

The best coincidence of pine pollen peaks and stomata concentrations occurs between the depths of 236 and 140 cm in the Salvador core, again following abandonment by humans (Figure 5.7). In this section, high concentrations of pollen coincide with the highest concentrations of stomata found in any Las Lagunas core (four stomata each in the 220 and 204 cm levels). These do not seem like high numbers, but any increase at all in stomata counts strongly suggests a substantial increase in pine trees locally (Hansen 1995).

Interpretation of the Las Lagunas Findings:

The Causes behind the Stomata Concentrations Found in Castilla and Salvador

Based solely on the graphs of results from these lakes (Figures 5.1–5.6), it is evident that stomata counts following my protocol provided a successful proxy of pine proximity in Laguna Salvador but were somewhat less useful in Castilla. Many factors played important roles in this difference. Topography affects the ability of pine needles to enter a lake and can severely limit our ability to interpret data especially when the topography varies between sites as widely as it does between Castilla and Salvador (Froyd 2005). The slope of the ground near the shoreline limits or increases chances for pine needles to enter the water. When comparing the topography surrounding Castilla and Salvador, it is important to note the slope differences of the land near them. Castilla is a larger lake than Salvador, with a bigger catchment, but the slopes along Castilla's shores and for many meters into the surrounding vegetation are minimal compared to Salvador's steep inclines. This gentle slope greatly decreases the energy available to move pine needles into the water at Castilla.

The land surrounding Salvador has considerably steeper slopes immediately adjacent to the lake. With these steeper slopes, the energy available for moving pine needles increases, allowing more to reach the water. In addition to steeper slopes surrounding the lake, Salvador has a nearby ridge, which supports a healthy stand of *P. occidentalis* today and would have been an excellent habitat in the past; the steep slope of the ridge is close enough, within 5 meters at its nearest point, for pines growing there to affect the data.

Not only does topography play a role in the direct physical movement of needles into a lake, it can also play a key role in human land uses nearby. As noted above, Castilla has more level ground surrounding the lake; this land is now cleared and used for agriculture. Land around the lake may also have been cleared in the past, and this could be a reason why Castilla does not have a strong stomata signal in its sediments. Salvador, with its steeper slopes, might not have been cleared as often or as continuously because of the difficulty of cultivating steeply sloping land.

The understory of the vegetation surrounding lakes also has a profound effect on the ability of stomata to enter lakes and affects stomata concentrations (Hansen 1995, Clayden et al. 1996). A dense understory beneath pine trees can prevent most of the fallen needles from entering the lake, whereas an open understory does not restrict their movement. Compared to many boreal forest study sites, low-latitude sites such as

Salvador and Castilla have a much denser understory of herbaceous plants. Boreal forests are characterized by a low diversity of plant species in the ecosystem particularly in the understory of the dominant conifer species (Elliot-Fisk 2000). Typically, ground near a boreal lake will be covered by densely spaced conifer trees with only sparse patches of grasses and mosses underneath. With such an open understory, there is less chance of entanglement preventing needles being pushed into the water via overland flow. The relatively open vegetation of boreal climates may foster greater stomata influx to lakes, higher concentrations in the lakes, and thus more robust counts. The more common a microfossil is within a suite of represented macrofossils, the more statistically representative its counts will be. Under boreal forest growth conditions, stomata may have a greater opportunity to be represented in the sediments of lakes compared to the frequently contrasting situation in low-latitude tropical climates. A conifer species in low-latitude climates might have to be located immediately adjacent to a lake to foster any sizeable stomata record in the lake.

Dense understory beyond the lake perimeter can prevent stomata from reaching the water, but heavy vegetation in the littoral zone can in turn prevent the dispersion of stomata from the lake margin to a coring site nearer the middle of the lake (Gervais and MacDonald 2001). Stomata enter the lake as a part of the pine needles, and then later are redistributed across the lakebed once the needles have decomposed, freeing these small epidermal particles (Froyd 2005, Gervais and MacDonald 2001, MacDonald 2001, Yu 1997). A dense growth of plants in the littoral zone of a lake severely limits the ability of detached stomata to be remobilized and travel to the coring site. Castilla and Salvador today both contain emergent aquatic plant species growing along their margins that might affect the movement of stomata. The interpretations of this study must assume this pattern may also have been the case historically.

Pollen analysis for both the Las Lagunas lakes is problematic compared to pollen analysis for northern-latitude lake sediments. In low-latitude lakes, such as Salvador and Castilla, there is a high diversity of polleniferous plants in the watersheds of the lakes, which creates a problem because there are too many pollen types to tally on prepared slides. On a standard basis, pollen counts are done to a pre-determined number at which the researcher stops counting and tabulates pollen percentages based on pollen taxa found on the slide. With such a large number of species located in the vicinity of the lake, pine pollen and stomata numbers are greatly reduced, affecting the final product of a pine pollen and stomata study. High-latitude lakes have far fewer species in the surrounding area to be represented in the lake, thus greatly increasing the numbers of pine pollen and stomata found on the same area of a slide. A study only counting pine pollen and pine stomata, to higher sums, in Salvador and Castilla might yield much more robust counts with reasonable effort. Note that pine pollen-to-stomata ratios (Figure 5.11) do not differ greatly between Crystal Lake and Las Lagunas.

One notable aspect of both the Salvador and Castilla records is the lag of stomata peaks compared to the pine pollen concentration peaks. In the graphs, (Figures 5.1–5.6), stomata often seem to occur just above pollen peaks, when pollen concentrations begin to decline. David (1997) noted a similar lag in his study sites from the French Alps, and ascribed the difference to human influences. He theorized that once humans entered the area they altered the surrounding landscape, facilitating the entrance of pine needles into the lake. David's ideas might apply to Castilla (Figure 5.7) at the beginning of the modern period. Coincident with human disturbance inferred from multiple proxies (Lane 2007), peak pine pollen percentages drop sharply. Clearance of the trees, understory plants, or emergent aquatics could have removed many inhibiting factors and facilitated the entrance of stomata into the lake. It is also possible that the stomata entering a lake after such clearances are from decayed remnants of pine needles from past years, eroding from adjacent slopes. However, MacDonald (2001) stated that, unless the stoma or needle is in an anaerobic place (like a lake bottom), the whole stoma structure will quickly deteriorate. If that is true it is safe to conclude that seven detached stomata entering the lake cannot be more than a few years old, which seems to be in agreement with my findings in this thesis.

Human impact on the Las Laguna region appears to have thoroughly affected the pine tree stands around Castilla and Salvador. Figure 5.7 shows two distinct times when humans altered the landscape (Lane 2007). Each of the two human occupations shows a dramatic decline in the concentration of pine pollen in the core. The aboriginal human occupation coincided with the first quick sediment filling era in the lakes (Lane 2007). The combination of rapid sedimentation and less pine pollen and pine stomata during the human occupation supports the theory of massive clearing around the lakes for residence or farming. Pine needles might be entrained in the excess sediment entering the lake, causing an influx of stomata to be added to the lake from outside the normal contributing fringe around the lake. Such an addition of old stomata may be suggested during the modern human occupation of Castilla when there were stomata present during a time of low pine pollen concentrations.

With Lane's pine pollen tabulations and my stomata counts, the impact of human alteration becomes clearer to the researcher's eye. Side by side comparison of the two counts helps create a far stronger delineation of the occupation time period. There are two major inferred periods of human influence in the Las Lagunas region, aboriginal and modern (Lane 2007). The first period has a dramatic decrease in pollen percentages, but this drop does not necessarily mean pine stands were removed from the area (Figure 5.7). When plotted side by side with stomata concentrations, the co-occurrence of decreased pine pollen and complete absence of pine stomata convincingly indicates a major loss of pines, possibly an impact of humans. The inferred modern human occupation period in Salvador shows the same trends in pine stomata and pine pollen concentrations as in the aboriginal occupations. Modern human occupation in Castilla is accompanied by a spike of stomata in contrast to the diminished pollen concentrations during the period. This spike might reflect stomata being transported in sediment from outside the lake after land clearing (*cf.* David 1997).

Crystal Lake Spatial Analysis

The Crystal Lake results provide a useful picture of how stomata concentrations can change along a transect from shore to the center of a lake (Figure 4.1). In figures 5.8, 5.9, and 5.10, we see pollen concentrations increasing from the lakeshore to the lake center. This increase in pollen concentration is a long-established pattern in palynological limnology, and is why researchers can confidently take sediment cores from lake centers. In contrast to denser or less mobile particulates such as mineral grains or plant macrofossils, pollen tends to remobilize. Once a pollen grain "rains" onto the lake, or is washed into the lake, it settles, but it then may remobilize and redeposit several times before it settles in its final destination. Each time a pollen grain remobilizes, gravity affects the grain and it will tend to settle closer to the deepest, central part of the lake. Crystal Lake's transect diagram (Figure 4.1), shows a pattern that may reflect pollen becoming redistributed within the lake and concentrating toward the center.

Crystal Lake's largely inverse trend of stomata concentrations decreasing with increasing distance from shore is different from that of pollen concentrations but also reflects stomata characteristics. Since stomata enter the water body attached to pine needles, the individual epidermal stomata cells have fewer chances to become remobilized, in contrast to pollen. The large amount of stomata near the shore represents the fact that stomata are attached to needles and have not had the opportunity to redeposit. It takes time for stomata to become free from the heavy needle. Once the needle breaks down to free the stomatal cells there is a greater chance that sediment already lies on top of the cells, preventing them from being remobilized and redeposited farther toward the center of the lake.

The fact that stomata concentrations rise somewhat near the center of the lake compared to mid transect (Figure 5.9) does suggest that some stomata do have a chance to be redeposited after becoming detached from the needle. Since there is a substantial clump of pine trees on each end of the transect, at the center of the lake we can deduce there may have been stomata input from trees on both shorelines.

Crystal Lake provides a good representative model for the spatial distributions of stomata concentrations in most simple lake systems; however, such systems may resemble northern boreal lake conditions more than low-latitude lake conditions such as those encountered in Salvador and Castilla. The biggest difference between the Las Lagunas lakes and Crystal Lake was the lushness of the present understory vegetation. Approximately 90 percent of the Crystal Lake perimeter had no or little dense understory vegetation to hinder the influx of pine needles to the lake. This means Crystal Lake could receive more pine needles, allowing stomata a higher chance to be represented in the core (Hansen 1995, Clayden et al. 1996). In addition, there are few if any emergent aquatic plants present in the littoral zone of Crystal Lake. With no littoral plant cover, it is easier for stomata to become redistributed throughout the lake after the needles decompose.

No lush understory, no littoral plant cover, and a known source of pine needles close to the water were all key ingredients leading to an abundance of stomata in Crystal Lake sediments. Unfortunately, aside from the similar sizes of the lakes, the differences in watershed topography between Crystal Lake and Salvador and Castilla are large. Applying the spatial results from Crystal Lake to Salvador or Castilla could possibly result in a misinterpretation if one were to assume the Crystal Lake study, which is preliminary, should completely answer spatial questions at Las Lagunas. To fully understand the low-latitude lake spatial dynamics of stomata taphonomy, such as in Salvador and Castilla, a separate study would have to be undertaken.

Pollen per Stomata Comparison

As noted before, a lush understory inhibits pine needles from entering the lakes of Castilla and Salvador, but the vegetation compared to Crystal Lake plays another major role in determining results. As seen in figure 5.11, the pine pollen per stomata ratio is lower in Crystal Lake than in the highest-ratio levels of Las Lagunas. Crystal Lake's surrounding vegetation is mainly a mixed deciduous forest with a few sparse clumps of pine trees. Castilla and Salvador (especially), however, have more extensive pine woodland or mixed pine forest within their watersheds. Because pines are more common near the two lakes, it is probable that more background pine pollen reaches the lakebeds than at Crystal Lake. A factor such as this can cause a large difference in pollen to stomata ratios (Figure 5.11).

The understory contrast between Castilla, Salvador, and Crystal Lake does not only have to do with vegetation density. The Las Lagunas and Crystal Lake cores also differ in mineral sediment content. Crystal Lake is located within a suburban watershed and has new houses adjacent to the lake, causing larger than normal amounts of mineral sediment to be washed into the lake. The higher mineral content is caused in part by the disruption of natural vegetation that would otherwise prevent erosion and transport of the eroded material. This extra mineral content alters the concentration of pollen found in each sample by diluting the number of pollen grains in the sample.

The studies at the Las Lagunas lakes and Crystal Lake differed in sample type and location within a lake, but the results of each promote a way of better understanding stomata taphonomy within small watersheds. Figure 6.1 displays the pine stomata encountered per pollen grain encountered in each of the three lakes. This figure tells us that in Castilla and Salvador I had to look at far more pine pollen for an occurrence of one stoma. The lowermost occurrence in Castilla for instance required an examination of eight hundred pine pollen before I found the single stoma. The high number of pollen per

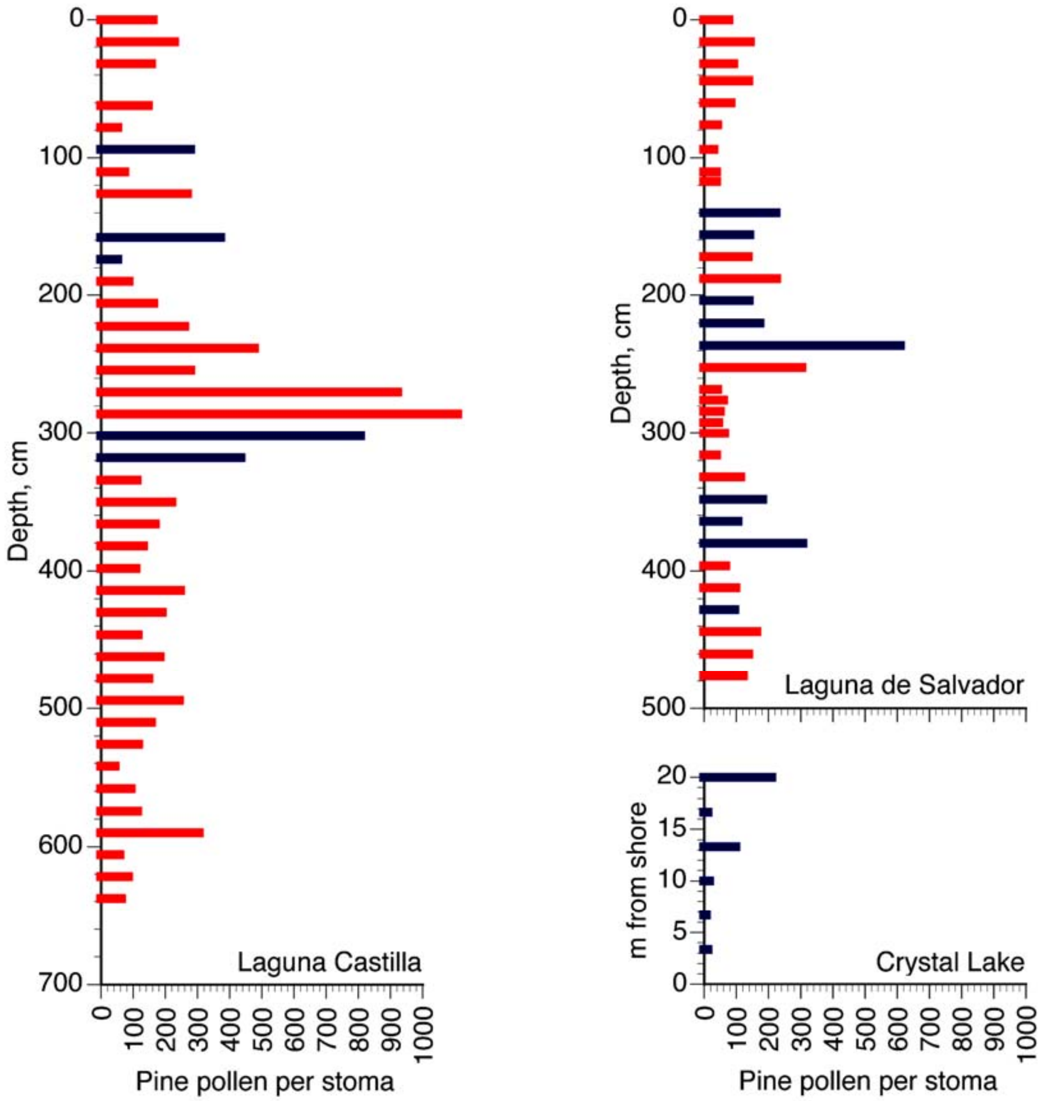

Figure 6.1. Pine Pollen to Pine Stomata Thresholds. Blue bars represent the ratio of pine pollen counted to stomata encountered, in samples where stomata were found. Red bars represent the number of pine pollen grains encountered before the point when searching ceased, in samples where no stomata were found. Future studies should be designed to reach higher pine pollen counts to better infer absence of stomata.

stoma in the Las Lagunas lakes shows that in future studies it will be necessary to count to higher numbers of pollen grains on each sample to achieve better results. In many samples, my scanning protocol of 20 transects across the slide resulted in my encountering fewer pollen grains than what turned out to be a reasonable threshold number of grains at which an encounter with even one stoma might be expected. An appropriately designed study like this does not seem too difficult to perform, but it would have to be the sole focus of the study for it to be a reasonable amount of work.

Chapter 7

Conclusion

The idea of using pine stomata as a proxy in lake sediments outside high northern latitudes and high alpine elevations sparked this study. My results demonstrate its potential as a valuable proxy in low latitude environments, at least with more extensive counts of pollen and stomata. All three of the study sites in my thesis yielded useful information on how stomata concentrations are represented in lake cores outside high northern latitude study sites. In Lagunas Castilla and Salvador, I re-examined existing sediment cores that had been used for determining the lakes' 2600-year environmental histories by studying pollen and charcoal concentrations and other proxies. My addition of pine stomata counts to the previous record (Lane (2007) did provide useful additional information to the ongoing research and facilitated interpretation of pine pollen counts in the Las Lagunas region.

Laguna de Salvador yielded good co-occurence of stomata with Lane's peak pine pollen zones in the sediment core. With the addition of stomata information we can now infer when the peak pine forest invasion occurred immediately around Salvador with much greater certainty. I think Laguna de Salvador yields good stomatal representation because it provides ideal conditions for the pine needles containing the stomata to enter the lake. Salvador is surrounded by fairly steep hillsides, that facilitate movement of needles downslope into the lake. Overall, the Laguna de Salvador results can be considered a good model for future sediment studies in lower latitude environments where including stomata counts as a proxy would be useful.

Laguna Castilla yielded less convincing results than Salvador in terms of the number of stomata found and stomata co-occurrence with high pine pollen concentrations. My efforts revealed that very few stomata exist throughout the core, rendering the results questionable without more extensive pollen and stomata studies at the lake. Granted, a lack of stomata may only suggest the continual presence of a lush understory below the tree canopy, revealing little about the proximity of *P. occidentalis* stands to the lake. Because Laguna Castilla is situated in flatter terrain, there is less energy available for washing pine needles into the lake, which may have resulted in under-representation of stomata in the lake. Alternatively, Castilla's dense littoral rim of aquatic emergent vegetation may, if it persisted through time, have been the controlling factor. Overall, I consider the history of pines at Laguna Castilla to be a question for future research. Finding no stomata in the lake sediments does not necessarily mean there were no pine trees in Castilla's vicinity, but it tells us there might alternatively have been physical factors preventing the stomata from entering the lake.

The Crystal Lake dredge sampling study provided useful insight into how pine stomata concentrations can vary across a lake from its margin to a central coring site. It is important to know how the stomata concentrations can vary as a function of depth and distance from shore in designing future studies utilizing stomata counts. Coring sites could be better located if this knowledge were available. Because the slides of Crystal Lake yielded lower amounts of pollen per slide than the Las Lagunas lakes, the Crystal Lake study would have been more robust if I had counted more pollen using more than one slide. Future studies should be based on a minimum pine pollen count rather than my single slide examination per sample approach. Another idea for a future study would be to replicate the Crystal Lake methods on a lake that is not within a new suburban neighborhood. A lake with more intact natural surrounding herbaceous vegetation would be more apt to approach the conditions of a low-latitude lake where vegetation regenerates rapidly even after clearing.

For the most part, I conclude that pine stomata counts in lower-latitude lake sediment cores are potentially worth the extra time needed to complete the counts in addition to pollen tabulation. My research yielded mixed results in the three lakes, but it is relatively simple to learn stoma structure and to perform stomata counts on the same pollen slides. Another benefit in many low-latitude studies is the presence of only a very few conifer tree species, as in my sites with only one, *P. occidentalis,* making it easy for the researchers to identify and count a particular taxon of pine pollen and stomata. Another future study to consider would be an examination of how pine needles move and decay once they become detached from the tree. Understanding how a needle dehisces, enters the lake and decays, and how its fragments move in the lake to eventually place stomata in the deepest parts, is crucial to comprehending the relative importance of stomata presence in lake cores.

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Appendices

Appendix A

Pollen Processing Schedule

I used this protocol to extract pollen and stomata from the Crystal Lake sediment samples. The protocol was designed for processing temperate pollen samples from the SE USA by Sally Horn and Lisa LaForest, based on other processing schedules used in the Laboratory of Paleoenvironmental Research and techniques in Berglund (1986). It is similar to the procedure used by Lane (2007) to process lake sediment samples from Laguna Castilla and Laguna del Salvador.

1. Place wet sediment in preweighed, 15 ml polypropylene centrifuge tubes and reweigh.
2. Add 1 *Lycopodium* tablet (13,911 spores) to each tube.
3. Add a few ml 10% HCl, and let reaction proceed; slowly fill tubes until there is about 10 ml in each tube. Stir well, and place in hot water bath for 3 minutes. Remove from bath, centrifuge for 2 minutes, and decant.
4. Add 10 ml hot distilled water to each tube, stir, centrifuge for 2 minutes and decant. Repeat for a total of two washes.
5. Add about 10 ml 5% KOH, stir, and place in boiling bath for 5 minutes, stirring after 2.5 minutes. Remove from bath and stir again. Centrifuge 2 minutes and decant.
6. Wash 4 times with hot distilled water. Centrifuge for 2 minutes each time, Decant after each centrifuge.

7. Fill tubes about half way with distilled water, stir, and pour through 250 μm mesh screen, collecting liquid in a labeled beaker underneath. Wash out material remaining in test tube with more distilled water. Wash screen with a powerful jet of distilled water.
8. Centrifuge down material in beaker by repeatedly pouring beaker contents into correct tube, centrifuging for 2 minutes, and decanting.
9. Add 8 ml of 52% HF and stir. Place tubes in boiling bath for 20 minutes, stirring after 10 minutes. Centrifuge 2 minutes and decant.
10. Add 10 ml 10% HCl to each tube, stir, and place in hot bath for 3 minutes. Centrifuge 2 minutes and decant.
11. Check tubes for the presence of silica and repeat steps 9 and 10 as needed.
12. Add 10 ml hot Alconox solution to each tube. Stir well and let sit for 5 minutes, centrifuge and decant. (Alconox solution is made by dissolving 2.5 cc Alconox detergent powder in 1000 ml distilled water).
13. Add 10–12 ml hot distilled water to each tube, stir and centrifuge for 2 minutes, and decant. Repeat for a total of three hot water washes.
14. Add 10 ml of glacial acetic acid, stir, centrifuge for 2 minutes, and decant.
15. Make acetolysis mixture by mixing together 9 parts acetic anhydride with 1 part concentrated sulfuric acid. Add about 8 ml to each tube and stir. Place in boiling bath for 2 minutes, stirring after 1 minute. Centrifuge for 2 minutes and decant.
16. Add 10 ml glacial acetic acid, stir, centrifuge for 2 minutes, and decant.
17. Wash with hot distilled water, centrifuge, and decant.

18. Add 10 ml 5% KOH, stir, and heat in vigorously boiling bath for 5 minutes, stirring after 2.5 minutes. Centrifuge for 2 minutes and decant.
19. Add 10 ml hot distilled water, centrifuge for 2 minutes, and decant for a total of 3 washes.
20. After decanting last water wash, use a vortex mixer for 20 seconds to mix sediment in tube.
21. Add one drop 1% safranin stain to each tube. Use vortex mixer for 10 seconds. Add distilled water to make 10 ml, stir, centrifuge for 2 minutes, and decant.
22. Add a few ml TBA, vortex for 20 seconds. Fill to 10 ml with TBA, stir, centrifuge for 2 minutes, and decant.
23. Add 10 ml TBA, stir, centrifuge 2 minutes and decant.
24. Agitate samples using the vortex mixer to mix the microfossils with the TBA left in the tubes. Carefully transfer the liquid to clean, labeled glass vials. Centrifuge down vials and decant.
25. Add several drops of silicone oil (2000 cs viscosity) to each vial. Stir with a clean toothpick.
26. Place uncorked samples in a dust-free cabinet to let the residual TBA evaporate.
27. Stir again after one hour, adding more silicon oil if necessary.
28. Check the samples after 24 hours; if there is no alcohol smell, put a cap on vials containing samples.

Placing Samples on Slides

1. Begin by obtaining clean unused glass slides and processed samples.

2. Use clean toothpick to stir vial containing sample for two minutes to ensure assemblage is well mixed. Place small drop onto middle of slide. Make an X formation on the slide, evenly spreading out sample.
3. Lightly lay cover slip over the X. Press lightly on cover slip spreading sample underneath slip to edges.
4. Use clear fingernail polish at four corners of cover slip gluing it onto slide but leaving a flexibly mounted cover for examining pollen grains.

Appendix B

Raw Data fromCrystal Lake

Sample ID	**Water Depth**	**Pine Pollen**	**Pine Pollen (*Lycopodium*)**	**Stomata**	**Stomata (*Lycopodium*)**	**Transects @25x**
A	53 cm	109	642	10	642	20
B	90 cm	66	490	10	490	20
C	185 cm	17	171	1	171	20
D	206 cm	392	367	1	367	20
E	320 cm	45	208	1	208	20
F	348 cm	420	353	2	353	20

*****Counts of stomata and control spores on pollen slides from selected levels of the Castilla and Salvador sediment cores are archived in the Laboratory of Paleoenvironmental Research at the University of Tennessee. Following publication, these data will be merged with pollen counts on the same cores made by Lane (2007), and deposited in the Latin American Pollen Database (http://www.ncdc.noaa.gov/paleo/lapd.html).

Vita

Brock Andrew Remus was born in Topeka, Kansas on July 23, 1981 to Michael L. and Genevieve H. Remus. Brock was the fourth child in the family, which included brothers Christopher and Brian and sister Peggy. He lived with his family in Manhattan, Kansas until he was six months old when his family moved to Freemont, Nebraska. After two and a half years in Freemont, Brock and his family lived in Osborne, Nebraska for two years; Norfolk, Nebraska for three years; Columbus, Nebraska for six years; and finally Lawrence, Kansas where he attended Lawrence High School until he graduated in May of 1999.

Brock entered the University of Tennessee, Knoxville in the fall of 1999 with an academic scholarship from the school of agriculture as a pre-veterinary major. During his curriculum Brock took introductory physical geography classes (Geography 131–132), excelled in them, and switched majors to Geography in the fall of 2001. While a geography major, he was an active member of Lambda Chi Alpha and Club Geography in the department. Graduating in the spring of 2003, Brock took a year off school and worked as a teacher at the local Boys and Girls Club before beginning the masters program at his alma mater. He entered the masters program with the desire to do research in climate and watershed geography under Dr. Ken Orvis.

While in the masters program at Tennessee, Brock was a Teaching Assistant for introductory physical geography and introductory cultural geography. Brock received his Master of Science degree from the University of Tennessee in 2008 after completing research in the Dominican Republic Las Lagunas region. He presented preliminary research of his thesis at the annual meeting of the Association of American Geographers in Chicago, Illinois in March of 2006. He hopes to continue his geography experience in biogeographical and watershed research in the future.