



2008

The Effect of Orientation to Growing Season Sunlight on Stomatal Parameters of *Q. rubra* in the Belgrade Lakes Region, Central Maine

Rachel G. Daly
Colby College

Follow this and additional works at: <https://digitalcommons.colby.edu/honorstheses>



Part of the [Plant Sciences Commons](#)

Colby College theses are protected by copyright. They may be viewed or downloaded from this site for the purposes of research and scholarship. Reproduction or distribution for commercial purposes is prohibited without written permission of the author.

Recommended Citation

Daly, Rachel G., "The Effect of Orientation to Growing Season Sunlight on Stomatal Parameters of *Q. rubra* in the Belgrade Lakes Region, Central Maine" (2008). *Honors Theses*. Paper 499.
<https://digitalcommons.colby.edu/honorstheses/499>

This Honors Thesis (Open Access) is brought to you for free and open access by the Student Research at Digital Commons @ Colby. It has been accepted for inclusion in Honors Theses by an authorized administrator of Digital Commons @ Colby.

THE EFFECT OF ORIENTATION TO GROWING SEASON SUNLIGHT
ON STOMATAL PARAMETERS OF *Q. rubra* IN THE BELGRADE LAKES
REGION, CENTRAL MAINE

Except where reference is made to the work of others, the work described in this
thesis is my own or was done in collaboration with my advisory committee

Rachel G. Daly, '08

Certificate of Approval:

Dr. Robert A. Gastaldo, Chairman
Whipple Coddington Professor
Department of Geology

Dr. Bruce F. Rueger
Visiting Assistant Professor
Department of Geology

Dr. David H. Firmage
Clara C. Piper Professor of Environmental Studies
Department of Biology

THE EFFECT OF ORIENTATION TO GROWING SEASON SUNLIGHT
ON STOMATAL PARAMETERS OF *Q. rubra* IN THE BELGRADE LAKES
REGION, CENTRAL MAINE

Rachel G. Daly '08

A Thesis

Submitted to the Faculty of the Geology Department of
Colby College in Fulfillment of the Requirements for
Honors in Geology

Waterville, Maine

May 15, 2008

Table of Contents

List of Figures and Tables	iii
Abstract	1
Introduction	2
Materials and Methods	5
Results	8
Discussion	11
Summary	15
Acknowledgments	15

List of Tables and Figures

Table 1- Localities and prevailing sunlight exposures of *Q. rubra* collected on the Belgrade Lakes, central Maine, 2007.

Table 2- Average light meter data from Snow Pond over five days in summer 2007 on opposite sides of the lake (all measurements are in klux).

Table 3- Mean and confidence intervals of SD and SI for *Q. rubra* samples collected from six Belgrade Lakes, central Maine.

Table 4- Stomatal density, epidermal cell density, and undulation index values determining shade leaf morphology.

Table 5- Statistical comparisons of opposing sides of each of the six Belgrade Lakes studied, central Maine, 2007. The threshold of significance was lowered from 0.05 to 0.00625 with the use of a Bonferroni adjustment.

Table 6- Statistical analysis of corresponding sides of similarly oriented lakes. * shows a result that was statistically significant until the threshold of significance was lowered from 0.05 to 0.00625 with the use of a Bonferroni adjustment.

Figure 1- a) Location of Belgrade Lakes within Maine. b) Location of leaf collections along lake margins of six Belgrade Lakes. Circle superimposed on map represents arc of sun at the beginning of the growing season, 2007.

Figure 2- Precipitation and temperature data for central Maine, 2007 compared to 30-year averages.

Figure 3- Undulation Indices and 95% confidence intervals for leaf samples from each of the six Belgrade Lakes a) Snow Pond b) Salmon-McGrath Pond c) East Pond d) North Pond e) Long Pond f) Great Pond.

Figure 4- Methods of analysis of Belgrade Lakes data. a) Comparison of opposing sides of each lake. b) Comparison of corresponding sides of lakes in each lake pair. c) Comparison of lake pairs.

Figure 5- Figure 5. Stomatal index vs. density for opposing sides of six of the Belgrade lakes, central Maine a) Salmon-McGrath Pond b) Snow Pond c) East Pond d) North Pond e) Long Pond f) Great Pond.

Figure 6- Stomatal index vs. density of Belgrade, Maine data set compared to Auburn, Alabama datum.

Abstract

Stomatal frequencies of fossil-plant species are used to estimate past pCO₂ levels based on the physiological functions of living taxa. Numerous studies have shown that there is an inverse relationship between pCO₂ and stomatal frequency parameters. As levels of pCO₂ increase, the Stomatal Density (SD) and Stomatal Index (SI) decrease. However, pCO₂ is not the only factor affecting SD and SI values, which are a product of leaf growth and expansion. Stomatal characteristics differ between genera, and studies also have shown that SD and light intensity have a positive correlation. The present study hypothesizes that SD and SI are not influenced by a leaf's physical orientation relative to the sun during the growing season.

Leaves of Northern Red Oak, *Quercus rubra*, were collected from trees on lake margins around six lakes in the Belgrade Lakes Region, central Maine, USA. Lakes oriented in NE/SW, NW/SE, and E/W directions allowed for sampling of trees exposed to varying light intensities throughout the growing-season day.

The SD and SI of each tree were calculated and comparisons made between populations exposed to predominant morning or afternoon light intensities, and between populations on lakes of differing orientations. No comparisons show a statistically significant difference between populations under different orientations to growing-season sunlight. The data suggest that exposure to various sunlight regimes on opposite sides of lakes does not play a role in the stomatal response as reflected in SD and SI of plants during a growing season.

Introduction

In light of recent predictions of global climate change, it is important to develop a range of methods to estimate past climate fluctuations to understand the long term trends experienced by Earth systems. The present study focuses on the use of plant stomata as a paleoatmospheric proxy for the concentration of atmospheric carbon dioxide ($p\text{CO}_2$). It has been demonstrated that there is an inverse relationship between $p\text{CO}_2$ and the stomatal density of leaves in some plant species (e.g. Woodward, 1987; Beerling et al., 1993; Van der Burgh et al., 1993). Studies have shown that a positive relationship exists between changes in $p\text{CO}_2$ and temperature (Crowley and Berner, 2001; Royer et al., 2001). Hence, a more comprehensive knowledge of $p\text{CO}_2$ fluctuation will increase our understanding of global temperature change. Maximizing the effectiveness of plant stomata as $p\text{CO}_2$ proxies will allow for more accurate estimates of $p\text{CO}_2$ in the Quaternary and into deep time. This increased insight into the past may allow us to more accurately estimate future fluctuations in climate.

Different methods have been used to estimate past atmospheric $p\text{CO}_2$ using indirect and direct techniques. Indirect measurements are made by theoretical models utilizing geochemical and climatological data. For example, Berner's GEOCARB models (1991, 1994, 2001) use degassing and weathering rates along with data from Sr and $\delta^{13}\text{C}$ isotopes to model $p\text{CO}_2$ over the Phanerozoic. GEOCARB III models show fluctuations in $p\text{CO}_2$ over the last 600 Ma up to 25x present levels (Berner and Kothavala, 2001). A method for directly measuring atmospheric gasses is provided by the analysis of air bubbles trapped in ice cores (Petit et al., 1999). Yet, ice-core records only exist for the

last 400 Ka of the late Quaternary, and core analyses show that pCO₂ has fluctuated between 180 and 280 ppm in the last 100,000 years (Beerling, 2002).

In the last quarter century, empirical research has established that an inverse relationship exists between pCO₂ and stomatal parameters (Woodward, 1987; Beerling et al., 1993; Van der Burgh et al., 1993). Stomata are pores in the abaxial epidermal layer of a leaf that regulate gas exchange, reducing water loss while maximizing CO₂ intake. A reduction in stomatal density (SD, measured in stomata/mm²) as pCO₂ increases allows for greater water conservation while ensuring the uptake of an adequate amount of CO₂ for photosynthesis (Royer, 2001). It has been demonstrated that SD is sensitive to changes in soil moisture supply, atmospheric humidity, and temperature, among other factors, making this relationship difficult to use solely as a pCO₂ proxy. Salisbury (1927) first introduced the concept of using stomatal index (SI) as a more accurate measure of stomatal parameters. SI is the ratio of stomatal cells to epidermal cells per unit area. He recognized that CO₂ has a larger effect on the number of stomatal cells than it does in the size of epidermal cells (Royer, 2001). As such, SI is less sensitive than SD to confounding factors (Beerling, 1999).

Soon after the inverse relationship between stomatal parameters and pCO₂ was recognized, it was tested for use as a paleoatmospheric proxy. Woodward (1987) was one of the first to show that SD and SI trends have decreased since the beginning of the industrial revolution and attributed these as a plant response to increasing pCO₂.

Attempts have been made to apply this technique in the Holocene (Woodward, 1987; Beerling et al., 1993; Rundgren and Beerling, 1999) and back into the Tertiary (Van der Burgh et al., 1993; Kürchner et al., 2008). The use of stomatal parameters also has been

applied as proxies for pCO₂ in deep time (McElwain and Chaloner, 1996; Retallack, 2001). However, other factors that affect stomatal parameters need to be accounted for because they may have a large influence on stomatal development, and may reflect growth conditions as much as pCO₂.

Irradiance, soil-moisture levels, sex, and position on the tree crown are all confounding factors on the effect of pCO₂ on stomatal parameters (Ashton and Berlin, 1994; Kürschner, 1997; Beerling, 1999; Chen et al., 2001; Boyce, 2007), and should be evaluated when choosing samples for a study. Temperature-and-water supply during the growing season have been shown to affect stomatal parameters (Beerling, 1999). Stomatal density also may vary between female and male individuals of a taxon (Chen et al. 2001). There are also morphological differences between sun (growing in direct sunlight on the edge of the tree crown) and shade leaves (growing inside the crown away from direct sunlight). Kürschner (1997) showed that irradiance affects stomatal parameters, with higher SD and SI values found in sun than in shade leaves. However, recent studies may show that these differences are due to leaf hydration during expansion rather than light intensity (Boyce, 2007). Hence, it is important to understand all of the factors that may affect the development of stomatal parameters such that they may be accounted for in future research, and a more credible interpretation of pCO₂ can be developed.

Many fossil-leaf assemblages from which pre-Quaternary cuticles can be assessed are preserved in lacustrine deposits (e.g., Gastaldo et al., 1998; Rundgren et al., 2005). Lakes are good sites to evaluate growth responses of trees because pre-Quaternary records and proxy estimates originate, in part, from such depositional settings. Other

depositional sites where cuticles are preserved and have been used to reconstruct pCO₂ include fluvial deposits (Van der Burgh *et al.*, 1993) and wetlands (McElwain and Chaloner, 1995).

Although it is posited that solar irradiation may affect leaf expansion and development (Ashton and Berlyn, 1994; Kürschner, 1997), there is no study that has evaluated whether leaf expansion in a taxon is affected when grown under the prevailing influence of morning or afternoon sunlight, or by orientation of the water-body along which the tree is growing. The present study is an attempt to fill this gap, and hypothesizes that the stomatal parameters of Red Oak (*Quercus rubra*) are not affected by the orientation of trees growing on the margins of lacustrine systems to growing season sunlight. This taxon was chosen for its abundance in North-temperate latitudes, and collections were made along lake margins of the 6 Belgrade Lakes during summer 2007 to ensure only sun leaves were evaluated. A small leaf collection of the taxon also was made in 2007 in the Auburn University, Alabama, Arboretum to test if latitudinal position may have an affect on stomatal parameters during the same growth season.

Materials and Methods

Sun leaves of *Quercus rubra* were collected from trees along the margins of the 6 Belgrade Lakes in central Maine. This study is restricted to forests adjacent to East, North, Great, Long, Snow, and Salmon-McGrath Ponds (Fig. 1). The lakes are the result of Pleistocene glaciation (Caldwell, 1998) and are oriented in three directions. Great and Long Ponds are oriented in a N/S direction, East and North Ponds are oriented in a

NW/SE direction, whereas Snow and Salmon-McGrath Ponds are oriented in a NE/SW direction (Table 1).

Quercus rubra was selected as the taxon to investigate for two reasons. First, it is the most abundant taxon in the Belgrade Lakes area and is present in all localities. Second, its use allowed for comparison with a contemporaneous study evaluating trends in oak taxa over the past century (Cantor and Gastaldo, 2007). This taxon is easy to identify by its lobed leaves, which are sinuately three toothed and bristle-tipped, and tree bark that is dark brown and scaly at the surface (York, 1995).

Individual trees were chosen based on proximity to the water's edge, expediency of collection, and relationship to prevailing morning or afternoon sunlight. Trees along the lake margin ensured that nothing was blocking exposure of canopy leaves to the sun, increasing the probability that each leaf collected would be exclusively of a sun morphotype. Leaves were collected from the edge of the canopy hanging over the water, either by hand, picked directly off the tree, or by using an extendable 14' Fiskars tree pruner.

Seven to ten trees were chosen along the sides of each lake that corresponded with their orientation to either direct morning or afternoon sunlight. A sample size of ten trees per side was the goal as the optimal collection number. However, logistical problems with collection and processing limited this number in some cases. Lakes, such as Snow and North Ponds, did not have enough accessible oak trees on either one or both sides, limiting the collection on those lakes. Furthermore, cuticle recovery during processing was sometimes hampered due to inadequate cleaning after the chromic acid treatment, as

well as difficulty in rehydration of partially desiccated older leaves. These factors resulted in an uneven number of samples for some lakes (Table 1).

The daily arc of the sun during the Maine growing season (May-June) was determined using data from the US Department of the Navy astronomical applications (<http://aa.usno.navy.mil/>). This allowed for a qualitative assessment of the duration of sunlight each lake side received during the growing season. Light-meter readings were taken using an Extech EA30, which measures in lux, along opposite sides of Snow Pond over 5 days during the summer (Table 2). These were taken to assess the difference in light intensity at varying times throughout the day. Measurements were taken every 2 hours on opposite sides of the lake from 8 am to 4 pm. The sensor was held facing upwards during each measurement. Data were collected on days when there was little morning cloud cover, which would have reduced the amount of irradiance on the leaves. Snow Pond was selected as the sample area due to its convenient measurement sites, and its close proximity to the Colby College campus.

Fresh leaves were processed by removing a 1 cm² section from between the 2nd and 3rd lateral vein adjacent to the midrib with the adaxial leaf surface facing up (Table 1). Poole and Kürschner (1999) have shown that stomatal parameters vary across an individual leaf. Hence, standardization of the area processed eliminates the possibility that variance is due to the sample area of a leaf. These sections then were immersed in 10% by weight chromic acid solution and treated for durations from < 24 to 48 hours. Chromic acid dissolves the parenchymatous tissue, leaving the abaxial and adaxial cuticles and conducting cells intact. The cuticles were separated manually under a dissecting scope, and the lower cuticle was mounted on a glass slide using Biomedica

aqueous gel mount. Coverslips were added and the slides were dried on a Fischer Scientific slide warmer for 12 hours.

Slides were viewed with a Zeiss Axioskop and analyzed with Axiovision software. Stomatal and epidermal cell counts were taken from 20, 0.04 mm² fields of view on each slide preparation. The total area of 0.8 mm² was then normalized to 1 mm². Previous studies have normalized data to 1 mm² based on 10 field of view counts (e.g., Beerling and Kelly, 1997; Beerling et al., 1998). Although the methodology specified collection of leaves from the edge of the tree crown, the undulation index (UI; Kürschner, 1997) of each leaf was calculated to account for any leaves that may have been blocked from the sun by neighboring leaves. Shade leaves have been found to have epidermal cell edges that are more sinusoidal than sun leaves. By quantifying the relative sinuosity of the epidermal cells, UI can help to distinguish between sun and shade leaves.

Two statistical tests were used to evaluate the data. Two-tailed, Student's t-tests were used to evaluate the statistical differences between the stomatal parameters from collections on (1) the opposing sides of each lake and (2) corresponding sides of each lake within a 'lake pair,' the pairing of two lakes with similar orientations. A Bonferroni adjustment was used to account for the presence of false-positives within the t-test results. An ANOVA analysis was used to evaluate any statistical difference between the three sample populations of the three different lake-pair orientations.

Results

Climate data from central Maine during the 2007 growing season were evaluated against 30-year averages for the region (Fig. 2). The monthly temperatures during 2007

were within the 95% CI for the 30-year averages. However, April of 2007 was much wetter than average, and May, June, July and September were dryer than average.

The 2007 SD responses of *Q. rubra* on the margins of the 6 Belgrade Lakes ranged from a low of 353 to a high of 641 stomata/mm². SI responses ranged from a low of 8.2 and a high of 12.9. The 95% CI boundaries for the SD values for all lakes fell between 456 and 488, whereas the 95% CI for SI values fell between 9.9 and 10.3 (Table 3).

Previous studies (Kürschner, 1997) have demonstrated empirically that there is a difference in response to both SD and SI during leaf expansion depending upon the position of the leaf in the tree crown. All leaves in this study should represent sun leaf morphologies. However, the undulation index (UI; Kürschner, 1997) of each leaf was calculated to ensure that the data do, in fact, represent primarily sun morphotypes.

Undulation indices ranged from 1.12 to 1.59, with the boundaries of the 95% CI ranging between 1.23 and 1.27 (Table 3, Fig. 3). Table 3 shows the difference in means and confidence intervals in the data set when the higher UI values are included or excluded. Kürschner (1997) found that *Q. petraea* sun leaves had SD values that were 45% higher than shade leaves, and SI values that increased slightly from 12 to 14, a 16% difference. The SD and SI values of the six leaves that fell the farthest outside of the 95% CI boundaries for the UI values were compared to SD and SI values of the sun-leaf population on the same side of the lake from which each was taken (Fig. 3). All sun leaf SD values were found to be much less than 45% greater than the values for the suspected shade leaves. From suspected shade leaves to sun leaves the SI values did not increase to the extent of the samples reported by Kürschner (1997) (Table 4).

Epidermal cell density of *Q. petraea* was reported to be 30% higher in sun leaves than in shade leaves (Kürschner, 1997). The average sun-leaf epidermal cell density in the areas of the corresponding suspected *Q. rubra* shade leaves were less than 13% higher (Table 4). The data from Table 4 indicate that the variation found within the UI values is due to variation in *Q. rubra* sun-leaf morphology rather than attribution of these leaves to a shade morphology.

Light meter data from Snow Pond are shown in Table 2. It is clear that the values of irradiance from the SE side of the lake illuminated by afternoon sunlight are lower than those of the NW side illuminated by morning sunlight.

Of the marginal trees of the six lakes, only those surrounding Salmon-McGrath Pond showed a statistically significant difference in SD when opposing sides of each lake were compared using the student's t-test (Table 5, Fig. 4). This statistical difference was then found to be a false positive when a Bonferroni adjustment was applied to the data set. For all other comparisons there is no statistical difference in SD values for trees grown on opposite sides of any lake. Similarly, there was no significant difference in SI for trees on any lake evaluated. This is supported by the SI vs. SD plots (Fig. 5). The data on all graphs group together in a cloud, reflecting the calculations that no distinct difference exists between trees on one side or another of any lake.

Lakes then were grouped into three pairs based on similar orientation (Table 1, Fig. 4). The corresponding sides of these "lake pairs" were evaluated using the student's t-test (Table 6). This comparison was made to ensure that the three lake pairs could be evaluated against each other, thus testing the effect of water-body orientation on stomatal parameters during leaf expansion when exposed primarily to morning or afternoon

sunlight. There is no statistical difference in SD values between the corresponding sides of any lake pair. However, results indicated a significant difference in SI values between the NE sides of East and North Pond, as well as the SW sides of that same lake pair (Table 6). As with the first analysis, when a Bonferroni adjustment was applied to the data set, the two anomalies were found to be false-positives. SI values for the other lake pairs show no statistically significant difference.

The three lake pairs then were evaluated against each other using an ANOVA analysis to test for an effect of water-body orientation on stomatal parameters (Fig. 4). The results show no statistically significant differences within SD or SI values. The p value for the SD analysis is 0.110. For the SI comparison it is 0.337. Both are greater than the 0.05 threshold of significance needed to reject the null hypothesis.

The Belgrade Lakes data set was compared against a 2007 datum of *Q. rubra* collected in the Auburn University, Alabama, Arboretum (Fig. 6). The Alabama datum plots outside of the 95% CI ellipse for the Maine data. When the Auburn datum was used as hypothetical population mean against the Belgrade data in a single sample t-test, results indicated a statistically significant difference between the Belgrade and Auburn data.

Discussion

The ability to use stomatal parameters as paleoatmospheric proxies in recent and deep time is dependent on the ability to account for confounding factors in data collected, and then correlate that data to what is observed in plants today. Several studies have attempted to do this. McElwain (1998) studied Middle Eocene and Jurassic fossil

stomatal parameters and then compared them to nearest living equivalent species to draw new conclusions about pCO₂ levels in those periods. Retallack (2001) measured stomatal parameters of four fossil plant genera related to Ginkgo and correlated the values with pCO₂ models for the past 300 Ma. Kürschner et al. (2008) correlate the stomatal parameters of multiple fossil plant species from the Miocene to draw conclusions about CO₂ as a forcing factor for climate during that time.

The present study attempts to quantify whether the orientation of a shoreline tree to growing season sunlight is a confounding factor, and if so, to what extent it affects stomatal parameters. If orientation to growing season sunlight has no effect on stomatal parameters developed during a growing season, future studies that attempt to reconstruct paleoatmospheric conditions based on lacustrine fossil assemblages will be able to disregard it during data analysis. However, if position of a tree is an effect, contributory debris to a fossil assemblage will need to be considered as a potential confounding factor.

The results of the statistical analyses of a dataset collected for the 2007 growing season in central Maine support this study's hypothesis that orientation to growing season sunlight has no effect of stomatal parameters of *Q. rubra*. Populations of leaves taken from opposing shores of each water body in the Belgrade Lakes show no statistical difference between either SD or SI values for any of the six lakes examined (Table 5, Fig. 4). Stomatal parameters are not affected by exposure to either prevailing morning or afternoon sunlight. Similarly, the corresponding sides of each lake within each of the three lake pairs show no statistically significant difference of SD or SI values (Table 6). This demonstrates that each lake is similar enough to its pair that the two can be used together for lake-pair comparisons (Fig. 4). It also indicates that minor physical changes

within climatically consistent latitudes have no effect on stomatal parameters within a single taxon. And finally, no statistically significant difference has been found within SD or SI values when the three lake pair orientations are compared with each other (Table 7, Fig. 4). This suggests that water-body orientation to sunlight has no effect on stomatal parameters.

These findings have positive implications for stomatal proxy studies that use sub-fossil or fossil-leaf assemblages from lacustrine environments to study reconstruct paleoatmospheric conditions (Wagner et al., 2002; Rundgren and Björck, 2003; Wagner et al., 2004; Rundgren et al., 2005; Van Hoof et al., 2006a, 2006b). If the orientation of a marginal lake tree to preferential morning or afternoon sunlight has no effect on stomatal parameters, future studies will be able to assume a statistically homogenous sample population from lacustrine deposits. However, there are other factors that can influence stomatal parameters and need to be accounted for in studies using stomata as paleoatmospheric proxies. This is suggested by the comparison of the Belgrade data set against the Auburn, Alabama datum (Fig. 5). Not only does the Auburn datum fall outside of the 95% CI ellipse, but it also has a statistically significant difference when a single-sample t-test was performed. This adds to the body of emerging literature that stomatal parameters for individual taxa are latitudinally dependent. Garcia-Amorena et al. (2006) have shown that stomatal parameters for *Q. robur* were affected by changes in latitude between Iberian and NW European sample populations. Hence, any study that incorporates multiple fossil assemblages originating from different latitudes would have to account for this effect (Wagner et al., 2004).

One effect that appears to have little influence on the development of stomatal distribution within this study is light intensity. The light-meter data collected from Snow Pond have a wide range of values (Table 4). There is a higher level of irradiance for the NW side than for the SE side of Snow Pond. If there was truly a significant difference in irradiance between the two sides of the lake, there might be a statistical difference in stomatal parameters between the opposing lake sides, because irradiance has been shown to influence them (Ashton and Berlyn, 1994; Kürschner, 1997). It is hypothesized that the difference in irradiance values is due to two biases in data collection. Light-meter readings taken on the SE side of the lake, exposed to prevailing afternoon sunlight, were collected standing on a dock on the lake margin. A small cleared area around this dock was surrounded by trees. The readings taken on the NW side of the lake, exposed to prevailing morning sunlight, were taken at a boat launch with a paved parking lot surrounded by a large cleared area. The closer proximity of trees on the SE lake side may explain the lower values of irradiance. The data also may have been affected by cloud cover in the afternoon as opposed to the morning. Many summer days were cloudless and sunny in the morning, with increased clouding as the afternoon progressed. Because the days on which measurements were made were based on the local forecast, early morning measurements could be made when there was limited cloud cover. But to insure that comparative data be obtained in the afternoon, measurements were sometimes taken under less than optimal conditions. This may have contributed to lower afternoon readings than morning which would have affected the afternoon irradiance on the SE side in comparison with the morning irradiance on the NW side.

Summary

The data collected from the margins of 6 lakes in the Belgrade lakes region, central Maine, during the 2007 growing season demonstrate that exposure of trees to different daily light regimes does not have an effect on stomatal parameters. The three statistically significant differences between populations were later found to be false positives. Measuring the undulation index for each sample confirms that each leaf collected was a sun leaf. The comparison of the Belgrade data set to a datum from Auburn, Alabama, supports the findings of previous studies that show changes in latitude affect stomatal parameters. These findings have positive implications for future studies evaluating paleoatmospheric conditions using stomatal parameters of a lacustrine fossil assemblage, as researchers will be able to assume a statistically homogenous population within a single species.

Acknowledgements

This study could not have been accomplished if it were not for the generous funding of the Jana C. Rudnick Research Fellowship in Environmental Science at Colby College. Thanks also go to Drs. Bruce Rueger, Whitney King, and Brad Mundy (Colby College) for logistical support, and to Dr. Judy Stone (Colby College) for her knowledge of tree species in the Belgrade area. Many thanks to the readers Drs. Bruce Rueger and David Firmage (Colby College). Finally, I would like to thank Dr. Robert Gastaldo (Colby College), without whom this study would not have existed.

References

- Ashton, P.M.S., Berlyn, G.P., 1994, A comparison of leaf physiology and anatomy of *Quercus* (section *Erythrobalanus*-Fagaceae) species in different light environments: *American Journal of Botany*, v. 81, p. 589-597.
- Beerling, J.D., Chaloner, W.G., Huntley, B., Pearson, J.A., Tooley, M.J., 1993, stomatal density responds to the glacial cycle of environmental change: *Proceedings: Biological Sciences*, v. 251, p. 133-138.
- Beerling, D.J., Kelly, C.K., 1997, Stomatal density responses of temperate woodland plants over the past seven decades of CO₂ increase: a comparison of Salisbury (1927) with contemporary data: *American Journal of Botany*, v. 84, p. 1572-1583.
- Beerling, D.J., McElwain, J.C., Osborne, C.P., 1998, Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO₂ concentrations: *Journal of Experimental Botany*, v. 49, p. 1603-1607.
- Beerling, D.J., 1999, Stomatal density and index: theory and application, *in* Jones, T.P., Rowe, N.P., ed., *Fossil Plants and Spores: modern techniques*. Geological Society, London, 251-256.
- Beerling, D.J., 2002, Low atmospheric CO₂ levels from the Permo-Carboniferous Glaciation inferred from fossil lycopsids: *PNAS*, v. 99, p. 12567-12571.
- Berner, R.A., 1991, A model for atmospheric CO₂ over Phanerozoic time: *American Journal of Science*, v. 291, p. 339-376.
- Berner, R.A., 1994, GEOCARB II; A revised model of atmospheric CO₂ over Phanerozoic time: *American Journal of Science*, v. 294, p. 56-91.
- Berner, R.A., Kothavala, Z., 2001, Geocarb III: A revised model of Atmospheric CO₂ over phanerozoic time: *American Journal of Science*, v. 301, p. 182-204.
- Boyce, C.K., 2007, Seeing the forest with the leaves—clues of canopy placement from leaf venation characteristics: *Geological Society of America, Abstracts with Programs*, v. 39, no. 6, p. 23.
- Cantor, B.M., Gastaldo, R.A., 2007, Historical records of stomatal indices from *Quercus* and *Nyssa* from the Southeastern US: *Geological Society of America, Abstracts with Programs*, v. 39, n. 6, p. 301.
- Caldwell, D.W., 1998, *Roadside Geology of Maine*, Mountain Press: Missoula Mont. 317 p.
- Chen, L., Li, C., Chaloner, W.G., Beerling, D.J., Sun, Q., Collinson, M.E., Mitchell, P.L., 2001, Assessing the potential for the stomatal characters of extant and fossil *Ginkgo* leaves to signal atmospheric CO₂ change: *American Journal of Botany*, v. 88, p. 1309-1315.
- Crowly, T.J., Berner, R.A., 2001, CO₂ and Climate Change: *Science*, v. 292, p. 870-872.
- Garcia-Amorena, I., Wagner, F., van Hoof, T.B., Manzanque, F.G., 2006, Stomatal responses in deciduous oaks from southern Europe to the anthropogenic

- atmospheric CO₂ increase; refining the stomatal-based CO₂ proxy: Review of Palaeobotany and Palynology, v. 141, p. 303-312.
- Gastaldo, R.A., Riegel, W., Puttmann, W., Linnemann, U.G., Zetter, R., , 1998, A multidisciplinary approach to reconstruct the Later Oligocene vegetation in central Europe: Review of Palaeobotany and Palynology, v. 101, p. 71-94.
- Kürschner, W.M., 1997, The anatomical diversity of recent and fossil leaves of the durmast oak (*Quercus petraea* Lieblein/ *Q. pseudocastanea* Goeppert)- implications for their use as biosensors of paleoatmospheric CO₂ levels: Review of Palaeobotany and Palynology, v. 96, p. 1-30.
- Kürschner, W.M., Zlatko, K., Dilcher, D.L., , 2008, The impact of Miocene atmospheric carbon dioxide fluctuations on climate and the evolution of terrestrial ecosystems: PNAS, v. 105, p. 449-453.
- McElwain, J.C., Chaloner, W.G., 1995, Stomatal density and index of fossil plants track atmospheric carbon dioxide in the Paleozoic: Annals of Botany, v. 76, p. 389-395.
- McElwain, J.C., Chaloner, W.G., 1996, The fossil cuticle as a skeletal record of environmental change: PALAIOS, v. 11, p. 376-388.
- McElwain, J.C., 1998, Do fossil plants signal paleoatmospheric CO₂ concentration in the geologic past?: The Royal Society, v. 353, p. 83-96.
- Petit, J.R., Jouzel, J., Raynaud, D., Barkov, N.I., Barnola, J.M., Basile, I., Bender, I., Chappellaz, J., Davis, M., Delaygue, G., Delmott, M., Kotlyakov, V.M., Legrand, M., Lipenkov, V.Y., Lorius, C., Pepin, L., Ritz, C., Satzman, E., Stievenard, M., 1999, Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica: Nature, v. 399, p. 429-426.
- Poole, I., Kürschner, W.M., 1999, Stomatal density and index; the practice, *in* Jones, T.P., Rowe, N.P., ed., Fossil Plants and Spores: modern techniques. Geological Society, London, 251-256.
- Retallack, G.J., 2001, A 300-million year record of atmospheric carbon dioxide from fossil plant cuticles: Nature, v. 411, p. 287-289.
- Royer, D.L., Berner, R.A., Beerling, D.J., 2001, Phanerozoic atmospheric CO₂ change: evaluating geochemical and paleobiological approaches: Earth Science Reviews, v. 54, p. 349-392.
- Royer, D.L., 2001, Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration: Review of Paleobotany and Palynology, v. 114, p. 1-28.
- Rundgren, M., Beerling, D.J., 1999, A Holocene CO₂ record from the stomatal index of subfossil *Salix herbacea* L. leaves from northern Sweden: The Holocene v. 9,5 pp. 509-513.
- Rundgren, M., Björck, S., 2003, Late glacial and early Holocene variations in atmospheric CO₂ concentration indicated by high-resolution stomatal index data: Earth and Planetary Science Letters, v. 213, p. 191-204.
- Rundgren, M., Björck, S., Hammarlund, D., 2005, Last interglacial atmospheric CO₂ changes from stomatl index data and their relation to climate variations: Global and Planetary Change, v. 49, p. 47-62.
- Salisbury, E.J., 1927, On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora: Philosophical Transactions of the Royal Society of London v. 216 p. 1-65.

- Van Der Burgh, J., Visscher, H., Dilcher, D.L., Kurschner, W.M., 1993, Paleoatmospheric Signatures in Neogene Fossil Leaves: *Science*, v. 260, p. 1788-1790.
- Van Hoof, T.B., Bunnik, F.P.M., Waucomont, J.G.M., Kurschner, W.M., Visscher, H., 2006a, Forest re-growth on medieval farmland after the Black Death pandemic- Implications for atmospheric CO₂ levels: *Palaeogeography, Palaeoclimatology, Palaeoecology*, v. 237, p. 396-411.
- Van Hoof, T.B., Kurschner, W.M., Wagner, F., Visscher, H., 2006b, Stomatal index response of *Quercus robur* and *Quercus petraea* to the anthropogenic atmospheric CO₂ increase: *Plant Ecology*, v. 183, p. 237-247.
- Wagner, F., Aaby, B., Visscher, H., 2002, Rapid atmospheric CO₂ changes associated with the 8,200-years-B.P. cooling event: *PNAS*, v. 99, p. 12011-12014.
- Wagner, F., Kouwenberg, L.L.R., van Hoof, T.B., Visscher, H., 2004, Reproducibility of Holocene atmospheric CO₂ records based on stomatal frequency: *Quaternary Science Reviews*, v. 23, p. 1947-1954.
- Woodward F.I., 1987, Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature* v. 327 617-618.
- York, H.H., 1995, 100 Forest trees of Alabama, 2nd ed.: Alabama Forestry Commission and Division of Vocational Education, Alabama State Dept. of Education, 111 p.

Table 1. Localities and prevailing sunlight exposures of *Q. rubra* collected on the Belgrade Lakes, central Maine, 2007.

Locality	Orientation of Sampled Lake Margins	Sunlight Exposure	GPS Coordinate of Lake Margin	No. of samples processed
East Pond	Southwest	Morning	N 44 ° 35' 55.0" W 69 ° 47' 14.4"	10
	Northeast	Afternoon	N 44 ° 36' 29.1" W 69 ° 46' 4.7"	5
North Pond	Southwest	Morning	N 44 ° 37' 35.4" W 69 ° 51' 16.7"	5
	Northeast	Afternoon	N 44 ° 37' 28.6" W 69 ° 49' 23.0"	5
Salmon-McGrath Pond	Northwest	Morning	N 44 ° 31' 29.9" W 69 ° 47' 16.0"	9
	Southeast	Afternoon	N 44 ° 33' 7.2" W 69 ° 45' 54.3"	10
Snow Pond	Northwest	Morning	N 44 ° 30' 24.3" W 69 ° 46' 34.5"	9
	Southeast	Afternoon	N 44 ° 31' 52.7" W 69 ° 45' 7.8"	6
Great Pond	East	Afternoon	N 44 ° 33' 7.5" W 69 ° 49' 3 4.3"	4
	West	Morning	N 44 ° 31' 36.6" W 69 ° 51' 12.7"	4
Long Pond	East	Afternoon	N 44 ° 32' 21.6" W 69 ° 53' 36.1"	3
	West	Morning	N 44 ° 32' 35.8" W 69 ° 54' 6.7"	6

Table 2. Average light meter data from Snow Pond over five days in summer 2007 on opposite sides of the lake (all measurements are in klux).

Time	Boat Launch (NW side) 44° 32' 24.21" N 69° 43' 35.44" W	Hume Center (SE side) 44° 30' 37.79" N 69° 45' 32.22" W
8:00 am	42.5 (klux)	8.5 (klux)
10:00 am	57.0	10.8
12:00 pm	45.8	16.9
2:00 pm	31.3	20.5
4:00 pm	18.7	44.3

Table 3. Mean and confidence intervals of SD and SI for *Q. rubra* samples collected from six Belgrade Lakes, central Maine.

Parameter	n	mean	95%CI
SD All Leaves	76	472.0	± 15.6
SI All leaves	76	10.08	± 0.191
UI All Leaves	76	1.25	± 0.021
SD Sun Leaves	70	472.4	±16.5
SI Sun Leaves	70	10.12	±0.199
UI Sun Leaves	70	1.23	±0.013

Table 4. Stomatal density, epidermal cell density, and undulation index values determining shade leaf morphology.

Sample	Snow SE 14	Snow SE 16	Snow SE 17	Salmon- McGrat h NW 3	Salmon- McGrat h NW 4	Salmon- McGrath NW 8
UI for possible shade leaves	1.50	1.60	1.45	1.42	1.48	1.51
a.						
Average Sun Leaf SD for Corresponding Lake Side	455.1			530.4		
SD of possible shade leaves (stomata/mm ²)	525.9	426.2	399.4	512.3	461.5	479.6
Percent sun leaf SD higher than possible shade leaves (45% indicates shade leaf)	Sun leaves are lower	7.9%	12.2%	3.4%	13.0%	9.6%
b.						
Average Sun leaf Epidermal Cell Density for Corresponding Lake Side	4184.7			4369.0		
Epidermal Cell Density of possible shade leaves (cells/mm ²)	4693.1	4067.3	4195.4	4496.1	4017.9	4442.1
Percent sun leaf cell density higher than possible shade leaves (30% indicates shade leaf)	Sun leaves lower	2.8%	Sun leaves lower	Sun leaves lower	8.7%	Sun leaves lower

Table 5. Statistical comparisons of opposing sides of each of the six Belgrade Lakes studied, central Maine, 2007. The threshold of significance was lowered from 0.05 to 0.00625 with the use of a Bonferroni adjustment.

Lake	Parameter	Sides Compared	Average	p
East Pond	SD	NE	420.5	0.57
		SW	443.7	
	SI	NE	9.2	0.83
		SW	8.8	
North Pond	SD	NE	477.6	0.82
		SW	467.2	
	SI	NE	10.9	0.48
		SW	9.5	
Salmon-McGrath Pond	SD	NW	515.1	0.036
		SE	448.4	
	SI	NW	10.5	0.18
		SE	10.1	
Snow Pond	SD	NW	486.0	0.41
		SE	453.0	
	SI	NW	10.2	0.12
		SE	9.6	
Great Pond	SD	E	504.3	0.25
		W	450.5	
	SI	E	10.0	0.88
		W	10.1	
Long Pond	SD	E	524.6	0.69
		W	500.6	
	SI	E	10.6	0.58
		W	10.2	

Table 6. Statistical analysis of corresponding sides of similarly oriented lakes. * shows a result that was statistically significant until the threshold of significance was lowered from 0.05 to 0.00625 with the use of a Bonferroni adjustment.

East and North Ponds					
SW vs SW (SD)	average	451.6	NE vs NE (SD)	average	449.05
	p	0.33		p	0.283
SW vs SW (SI)	average	9.75	NE vs NE (SI)	average	9.87
	p	0.021*		p	0.011*
Snow and Salmon-McGrath Ponds					
SE vs SE (SD)	average	450.05	NW vs NW (SD)	average	500.71
	p	0.89		p	0.413
SE vs SE (SI)	average	9.92	NW vs NW (SI)	average	10.39
	p	0.13		p	0.335
Great and Long Ponds					
E vs E (SD)	average	513.0	W vs W (SD)	average	480.59
	p	0.736		p	0.299
E vs E (SI)	average	10.27	W vs W (SI)	average	10.16
	p	0.335		p	0.729

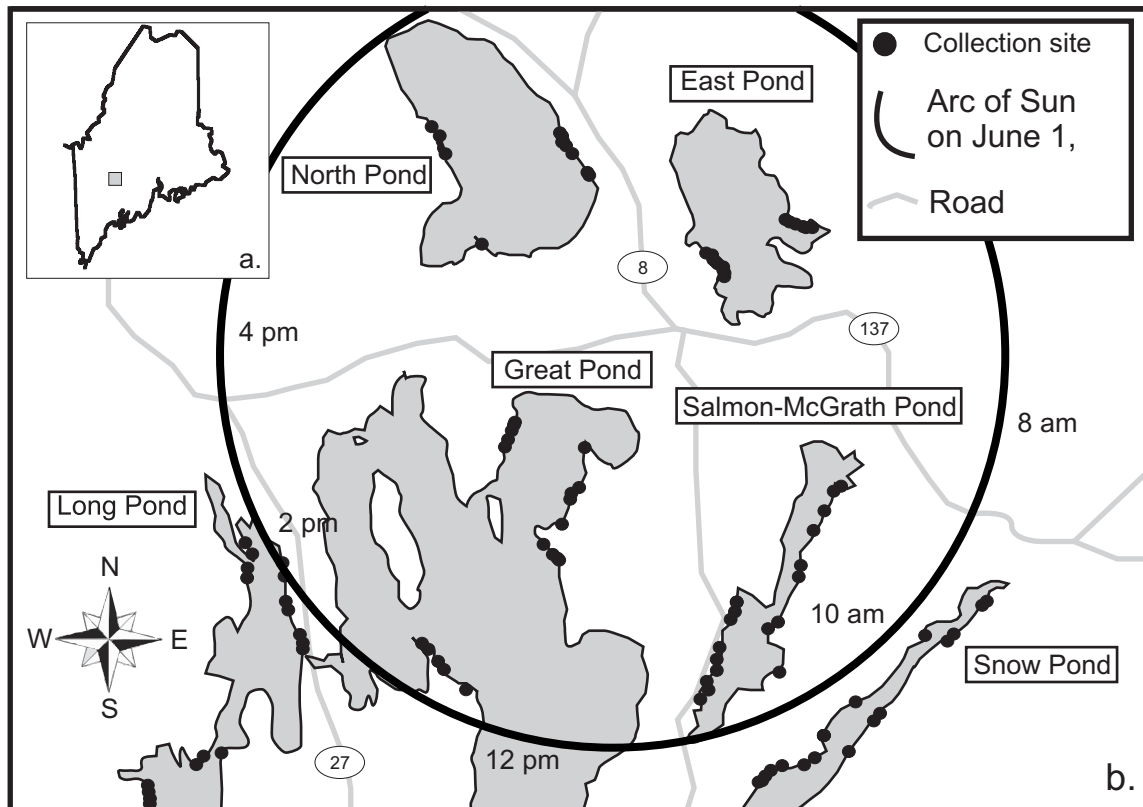


Figure 1. Collection area of *Q. rubra* samples, summer 2007 a) Location of Belgrade Lakes within Maine b) Location of leaf collections along lake margins of six Belgrade Lakes. Circle superimposed on map represents arc of sun at the beginning of the growing season, 2007.

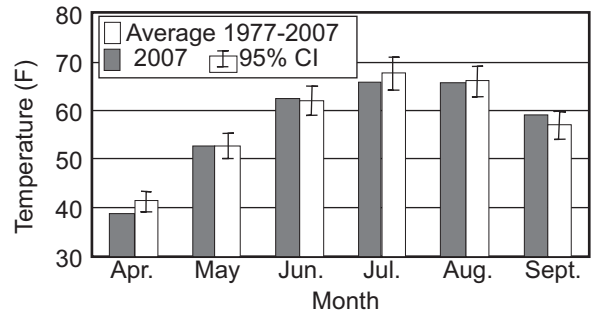
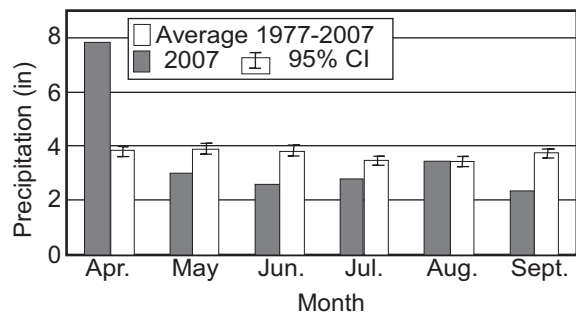


Figure 2. Precipitation and temperature data for central Maine, 2007, compared to 30-year averages.

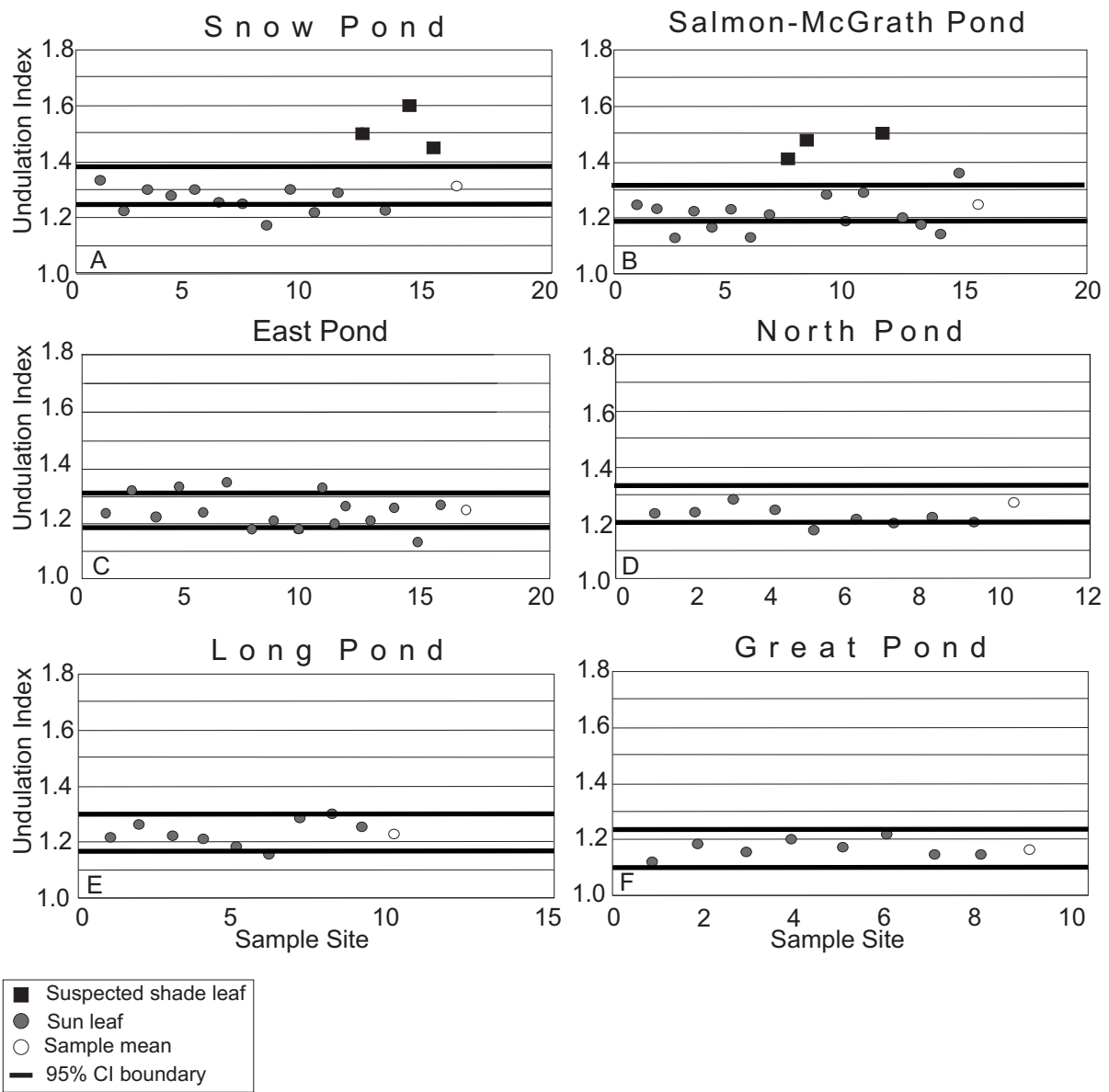


Figure 3. Undulation Indices and 95% confidence intervals for leaf samples from each of the six Belgrade Lakes. a) Snow Pond b) Salmon-McGrath Pond c) East Pond d) North Pond e) Long Pond f) Great Pond

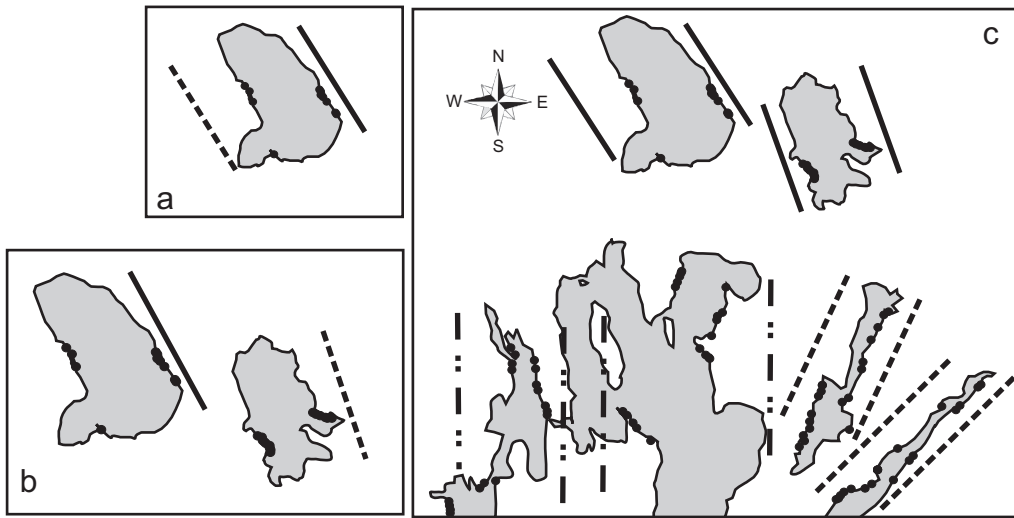


Figure 4. Methods of analysis of Belgrade Lakes data. a) Comparison of opposing sides of each lake. b) Comparison of corresponding sides of lakes in each lake pair. c) Comparison of lake pairs.

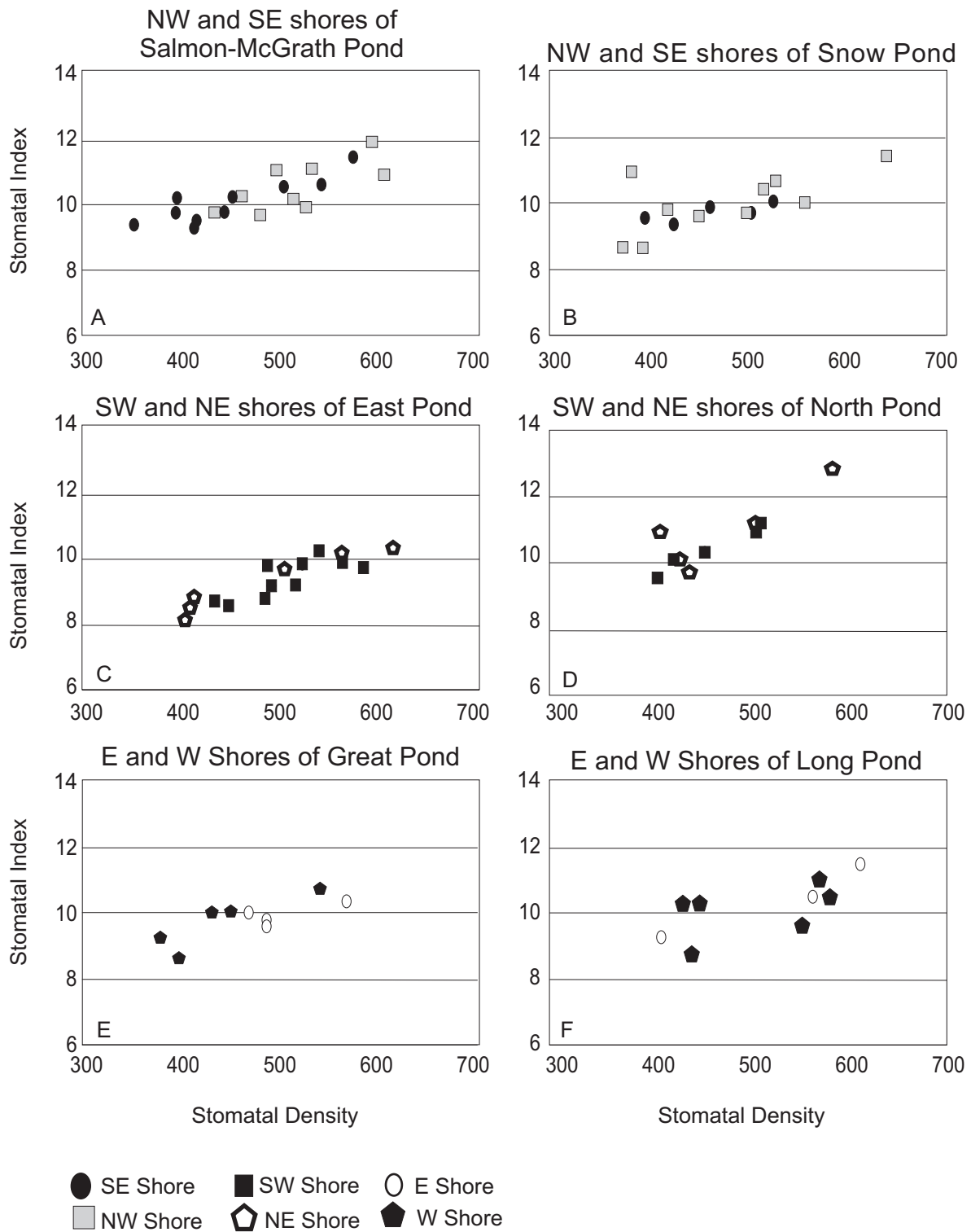


Figure 5. Stomatal index vs density for opposing sides of six of the Belgrade lakes, central Maine.

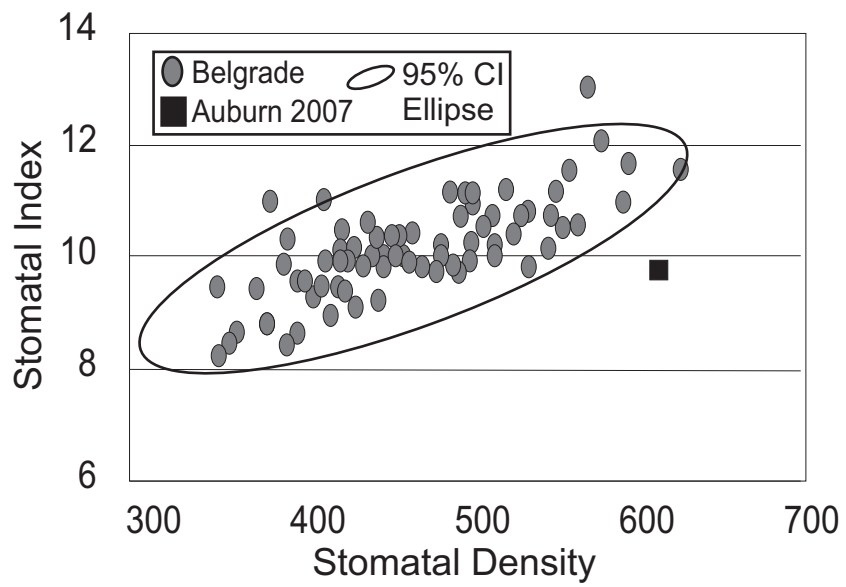


Figure 6. Stomatal index vs. density of Belgrade, Maine, data set compared to Auburn, Alabama, datum.