Evaluating Mg/Ca ratios as a temperature proxy in the estuarine oyster, *Crassostrea virginica*

Donna Surge\(^1\) and Kyger C Lohmann\(^2\)

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[1] We examined the potential utility of Mg/Ca\(_{\text{SHELL}}\) ratios recorded in shells of the estuarine oyster *Crassostrea virginica* as a temperature proxy because oxygen isotope ratios (\(\delta^{18}\)O) in estuaries are complicated by the simultaneous fluctuation in temperature and salinity, whereas Mg/Ca\(_{\text{WATER}}\) ratios are assumed to be constant above 10 practical salinity units (psu). We tested this assumption and observed a slight mixing effect between 10 psu and normal marine values emphasizing the need to test this assumption in estuarine settings. Microsamples of shell carbonate were analyzed for \(\delta^{18}\)O, \(\delta^{13}\)C, and Mg/Ca ratios. Measured \(\delta^{18}\)O\(_{\text{SHELL}}\) and \(\delta^{13}\)C\(_{\text{SHELL}}\) were compared to predicted values to assign dates to shell samples. Once samples and dates were aligned, corresponding temperature and salinity for a particular sample were known, enabling comparison of Mg/Ca\(_{\text{SHELL}}\) ratios and temperature. When all Mg/Ca\(_{\text{SHELL}}\) data were compared to temperature, a weak but statistically significant correlation was observed (\(r^2 = 0.05, p < 0.01\)). Because date assignments may become increasingly uncertain further back in time and earlier studies identified ontogenetic effects in juvenile portions of shells from other bivalve species, we examined the relationship from only the last year of growth. When only data from the last year of growth were compared, a slightly stronger positive relationship between Mg/Ca\(_{\text{SHELL}}\) ratios and \(D_{\text{Mg}}\) (the partition coefficient) emerged (Mg/Ca\(_{\text{SHELL}}, r^2 = 0.30, p < 0.01; D_{\text{Mg}}, r^2 = 0.33, p < 0.01\)). The improved correlation may result from either ontogenetic effects influencing the incorporation of Mg into the younger portion of the shell and/or inaccurate date assignments. Regardless of the cause, our equations are similar to previously published equations for *C. virginica* and other taxa. Despite the low correlations, our improved relationship supports further study under controlled experimental conditions.


1. **Introduction**

[2] The study of estuarine habitats using stable isotopic approaches is hampered by the dynamic nature of these changing environments. Oxygen and stable carbon isotope (\(\delta^{18}\)O and \(\delta^{13}\)C, respectively) records contained in shells are frequently used to reconstruct past environments because the accretionary growth pattern of mollusc shells record information about their life history and local environment. The simultaneous fluctuation of temperature and salinity complicates the interpretation of \(\delta^{18}\)O of shell carbonate in estuarine settings. Temperature affects the fractionation factor that determines the partitioning of \(^{18}\)O and \(^{16}\)O between solid (shell) and liquid (ambient water) phases [Epstein et al., 1953], while salinity reflects the mixture of freshwater and saltwater, each having different isotopic compositions (e.g., in general, freshwater is more negative in \(\delta^{18}\)O and saltwater is more positive). The \(\delta^{13}\)C\(_{\text{SHELL}}\) values that may potentially estimate salinity are often complicated by vital effects (metabolic processes, kinetic effects due to growth rate, etc.) [McConnaughey, 1989a, 1989b; Romanek and Grossman, 1989; Heikoop et al., 2000; Gillikin et al., 2007; Wannamaker et al., 2007] which may obscure the salinity signal.

[3] One way to avoid complications imposed by vital effects or simultaneous fluctuation in temperature and salinity is to examine elemental ratios contained in shells. The partitioning of Mg/Ca ratios between solid and liquid phases in abiotic calcite is temperature-dependent [Mucci, 1987]. Mixing of freshwater and saltwater in most cases has little influence on Mg/Ca ratios of calcite over a wide salinity range (10–35 practical salinity units (psu)) [Eisma et al., 1976; Dodd and Crisp, 1982]. Therefore, the Mg/Ca\(_{\text{SHELL}}\) ratios of calcitic skeletons can potentially serve as a proxy for temperature in environments where salinity is variable, such as in estuaries [Chave, 1954; Turekian and Armstrong, 1960;]
Dodd, 1965; Lerman, 1965; Moberly, 1968; Klein et al., 1996; Nürnberg et al., 1996; Klein et al., 1997; De Deckker et al., 1999; Lea et al., 1999.

This study sought to calibrate Mg/Ca\textsubscript{SHELL} ratios of crassostreine calcite from the American oyster 	extit{Crassostrea virginica} as a proxy for temperature. In an earlier study, Surge et al. [2003] demonstrated the need for an independent proxy for temperature given the limitations of $\delta^{18}$O\textsubscript{SHELL} in estuarine settings. If temperature can be estimated using Mg/Ca\textsubscript{SHELL} ratios, we evaluated the utility of $\delta^{18}$O\textsubscript{SHELL} as a proxy for salinity.

2. Materials and Methods

2.1. Water Samples

[5] Water samples/measurements and live oysters were collected from an oyster reef in Blackwater River, Rookery Bay National Estuary Research Reserve, southwest Florida ($25^\circ 55'517''$N, $81^\circ 35'526''$W), where tidal range averages 0.6 m and estuarine water mixes semidiurnally (Figure 1) (see Surge et al. [2001] and Surge and Lohmann [2002] for a detailed description of the study area). Temperature and salinity were monitored hourly and reduced to weekly averages from April 1996 through July 1999 using a YSI data logger equipped with depth, temperature, and salinity probes. Salinity values are reported relative to the psu scale throughout this paper. Data reduction to weekly intervals accommodated the time averaging associated with sampling oyster shells for geochemical analyses. Temperature, salinity, and water samples for $\delta^{18}$O\textsubscript{WATER} and $\delta^{13}$C\textsubscript{DIC} of dissolved inorganic carbon (DIC) were measured and collected monthly from December 1997 to April 1999 along a five-station transect from the Gulf of Mexico up to the Blackwater boat basin (its most upstream extent; Figure 1). Water samples for Mg and Ca determination were sampled at the most upstream and the most downstream sites only. We characterized the mixing relations between salinity and $\delta^{18}$O\textsubscript{WATER}, $\delta^{13}$C\textsubscript{DIC}, and Mg/Ca\textsubscript{WATER} along a gradient between freshwater and saltwater end-members. Samples for $\delta^{13}$C\textsubscript{DIC} analysis were filtered, treated with HgCl$_2$ or zephiran chloride to eliminate metabolic activity, and sealed in impermeable 30 mL serum vials. Water samples for elemental determination of Mg and Ca were filtered, acidified with HNO$_3$ to 2 pH, and stored in acid-washed, 30 mL polyethylene bottles.

[6] Oxygen isotope ratios of water samples were measured using an automated Finnigan CO$_2$-H$_2$O equilibration unit. Water samples (5 mL) were equilibrated with CO$_2$ in an agitating water bath for a minimum of 8 h at $15^\circ$C and measured on a Finnigan Delta-S mass spectrometer. Data were normalized to an internal standard water, and absolute values were normalized to measured values of Vienna Standard Mean Ocean Water (VSMOW) and Vienna light Arctic precipitation (VSLAP). The standard deviation for repeated measurements of the internal standard at the beginning, in the middle, and at the end of each run was 0.06‰. Oxygen isotope ratios are reported in per mil (‰) relative to the VSMOW standard. DIC was extracted in vacuo as CO$_2$ by acidifying $\sim10$ mL of water with 100% H$_3$PO$_4$ at room temperature. The $\delta^{13}$C\textsubscript{DIC} measurements were made on a Finnigan MAT Delta-S ratio gas mass spectrometer having a standard precision of ±0.1‰. Carbon isotope ratios are reported in ‰ relative to the Vienna PeeDee belemnite (VPDB) standard. Cation measurements were made by inductively coupled plasma–optical emission spectrometry.
spectrometry (ICP-OES) using a Leeman Labs sequential direct reading echelle (DRE) instrument. Within-batch precision, measured by gravimetric standards, ranged from ±1 to ±2% (RSD, raw standard deviation).

2.2. Shell Samples

[7] Live oysters were harvested at the reef in December 1997, January 1999, and July 1999 (Figure 1). Five specimens (BW20L2 (~1 year old) collected on 3 December 1997; BW20L3 (~2 years old), BW20L4 (~1 year old), BW20L5 (~1.5 years old), all collected on 6 January 1999; and BW20L7 (~3 years old) collected on 28 July 1999) were selected for this study on the basis of the robustness of the hinge area. We used this criterion because the hinge area is the portion of oyster shells that is most suitable for geochemical sampling because of resistance of resorption during anaerobic respiration [Kent, 1992; Kirby et al., 1998; Surge et al., 2001]. Samples for isotopic and elemental analyses were milled from the foliated area on the hinge of the left valve, which is exposed in cross section after the shell was cut along the growth direction from the ventral to dorsal margins (see Surge et al. [2001] for more details). The foliated rather than the chalky layer of the shell was chosen because of the potential for Mg salts accumulating in interstitial pores of the chalky layer during shell deposition [Carriker et al., 1991; Kennedy et al., 1996]. A dental drill fitted with a 0.5 mm burr was used to drill out ~50–100 µg of carbonate powder (500 µm spots) which was then split for isotopic and elemental analyses. Sampling at this resolution represented ~1–4 weeks of shell growth.

[8] Carbonate powders for isotopic analysis were roasted for 1 h in vacuo at 200°C to remove any volatile organic matter. Samples were reacted with anhydrous phosphoric acid at 73°C in individual reaction vessels on an online, automated Kiel device coupled to a Finnigan MAT 251 isotope ratio mass spectrometer. Precision of the instrument was better than 0.1‰ for 13C and 18O values. Oxygen isotope ratios were corrected for 17O contribution [Craig, 1957] and are reported in ‰ with respect to the VPDB standard. Sample splits for elemental determination were dissolved individually in 1.5 mL of dilute Fisher Optima grade HNO3 + HCl (1 + 2 + 97) spiked with indium as an internal standard. Solutions were analyzed on a Finnigan Element inductively coupled plasma–mass spectrometer (ICP-MS) or Leeman Labs ICP-atomic emission spectrometry (AES) for Ca and Mg content. Given the small sample size, it was not possible to measure accurately the weights of individual carbonate powder splits; therefore, raw solution (ng/g) data are reported as molar Mg/Ca\textsubscript{SHELL} ratios using mmol/mol concentrations. Analytical precision was better than <1% raw standard error (RSE) based on check standards and sample replicates. One shell (specimen BW20L5) had an analytical precision of ±6% RSE.

2.3. Establishing Calendar Time in Shell Space

[9] Predicted δ18O\textsubscript{SHELL} values were calculated using measured temperature, the δ18O\textsubscript{WATER}-salinity mixing relations presented in this study, and the calcite-water fractionation factor (α\textsubscript{CC-W}) [Tarutani et al., 1969; Friedman and O’Neil, 1977]. Predicted δ13C\textsubscript{SHELL} values were based on the δ13C\textsubscript{DIC}-salinity mixing relation following Surge et al. [2001]. We used this direct comparison rather than the calculation of equilibrium constants following Deines et al. [1974] or the calcite-bicarbonate enrichment factor of Romanek et al. [1992] because of the paucity of ΣCO₂ and pH data [see Surge et al., 2001]. The term mol % MgCO₃ was omitted from the calculation because oyster shells are composed of low-Mg calcite; therefore, the effect of this term was negligible. We assumed that δ13C\textsubscript{DIC} closely approximates δ13C\textsubscript{SHELL} on the basis of an earlier study by Surge et al. [2001]. Measured δ18O\textsubscript{SHELL} and δ13C\textsubscript{SHELL} were assigned dates by simultaneous comparison with the predicted shell values following procedures outlined by Surge et al. [2001]. Some sections of the measured shells required rescaling (stretching) to account for differential growth rates or periods of no growth. Uniform rescaling is inappropriate for C. virginica because its shell exhibits ecophenotypic variation (i.e., shell shape and growth rate vary within and between individuals depending on environmental factors, such as substrate, predation pressure, food availability, proximity to nearest neighbor) [Galstoff, 1964; Palmer and Carriker, 1979; Carriker, 1996]. Once dates were assigned to each shell sample, temperature and salinity conditions were obtained from actual measurements made on that date to evaluate Mg/Ca\textsubscript{SHELL} ratios as a proxy for temperature.

3. Results

3.1. Water Properties and Chemistry

[10] Water temperature at the oyster reef varied sinusoidally and averaged 26.51 ± 3.44°C, ranging from 19.06 to 31.63°C (Figure 2). Salinity variation reflected a wet and dry season that was out of phase with temperature (Figure 2). The dry season generally lasted from July through October, and the wet season occurred from December through May. Salinity averaged 29.84 ± 5.75 and ranged from 7.88 to 38.37 (see Surge and Lohmann [2002] for a complete description of the wet and dry season).

[11] Both δ18O\textsubscript{WATER} and δ13C\textsubscript{DIC} exhibited strong covariant trends with salinity (Figure 3a). The following linear regression equation describes the mixing relation between δ18O\textsubscript{WATER} and salinity (r² = 0.77, p < 0.01):

\[ δ^{18}O_{\text{WATER}} = 0.13(\pm 0.01)S - 2.56(\pm 0.26). \]
between salinity and mol Mg and mol Ca (mol Mg, \(r^2 = 0.96, p < 0.01\); mol Ca, \(r^2 = 0.93, p < 0.01\)):

\[
\text{mol Mg} = 1.47_{(\pm 0.20)} \times S + 0.12_{(\pm 0.06)}; \tag{3}
\]

\[
\text{mol Ca} = 0.25_{(\pm 0.36)} \times S + 1.65_{(\pm 0.46)}; \tag{4}
\]

Mg/Ca\text{WATER} ratios revealed a mixing effect across the salinity range and varied from 3.5 to 50 over the salinity range 10–37 psu (Figure 3b). We constructed a mixing curve using equations (3) and (4) from 0 to 34 psu, and the correlation between expected and measured values was \(r^2 = 0.90 (p < 0.01)\) (Figure 3c). Therefore, we emphasize that this assumption must be tested in estuarine environments.

### 3.2. Measured Shell Isotopic Ratios

[13] Seasonal signals reflected in the variation of \(\delta^{18}O\text{SHELL}\) are characterized by a sinusoidal pattern that often contains sharp peaks or troughs related to winter or summer growth cessation. This pattern was clearly evident in shells BW20L5 and BW20L7 and less clear in shells BW20L2, BW20L3, and BW20L4 (Figure 4). The inconsistent pattern in oyster shells may be produced by the morphologic variability of shell growth rate in oysters. Values ranged from −2.50 to 0.89%. In most cases, \(\delta^{13}C\text{SHELL}\) values tracked \(\delta^{18}O\text{SHELL}\), and this pattern was most clearly observed in shells BW20L5 and BW20L7 (Figure 4). The \(\delta^{13}C\text{SHELL}\) values ranged from −9.45 to −4.17‰.

### 3.3. Predicted Shell Isotopic Ratios

[14] The predicted \(\delta^{18}O\text{SHELL}\) time series is sinusoidal and largely reflects the seasonal variability in water temperature (Figure 5). The interval of time represented is weekly resolution. Predicted \(\delta^{18}O\text{SHELL}\) values ranged from −4.64 to 1.88‰. The predicted \(\delta^{13}C\text{SHELL}\) time series reflects the mixing relationship between salinity and \(\delta^{13}C\text{DIC}\) (Figure 5). Temperature has only a minor effect on \(\delta^{13}C\text{SHELL}\) and \(\delta^{13}C\text{SHELL}\) in C. virginica and serves as a proxy for salinity [Surge et al., 2001]. Predicted carbon isotope ratios ranged from −13.93 to −5.21‰.

### 3.4. Mg/Ca Ratios of Shell Carbonate

[15] Unlike \(\delta^{18}O\) values, a sinusoidal pattern reflecting seasonal variability was not obvious in any of the shells, except perhaps specimens BW20L5 and BW20L7 (Figure 6). Mg/Ca\text{SHELL} molar ratios of all shells ranged from 3.43 to 23.14 and averaged 13.71 ± 4.28.

### 4. Discussion

#### 4.1. Comparison of Measured and Predicted Shells

[16] Measured \(\delta^{18}O\text{SHELL}\) and \(\delta^{13}C\text{SHELL}\) values corresponded closely to the predicted values (Figure 5). In all but one case, the collection date of the shell anchored the sample from the growth margin onto the predicted shell, assuming that the growth margin recorded conditions at or just before the time of harvest. The isotopic ratios from the growth margin of shell BW20L7 collected in July 1999 corresponded more closely to the isotopic ratios predicted in May 1999 (i.e., \(\delta^{18}O\) and \(\delta^{13}C\) at the growth margin were more positive than predicted for the date of harvest). This
offset represented a gap (likely a period of nondeposition) in the shell record during the period of summer cessation. None of the shells recorded the most negative predicted isotopic ratios, which we interpreted as summer growth cessation. Kirby et al. [1998] and Surge et al. [2001] reported similar observations for C. virginica shells from the gulf coastal region. Assigning dates to specific samples allowed us to determine the temperature and salinity conditions at the time that portion of the shell was deposited. We used this information to evaluate the relationship between Mg/Ca_{shell} ratios and temperature.

4.2. Relationship Between Mg/Ca_{shell} Ratios and Temperature

[17] Comparison of temperature measurements with corresponding Mg/Ca_{shell} ratios resulted in a poor correlation between temperature and Mg/Ca_{shell} when all the data were plotted together ($r^2 = 0.05$, $p < 0.01$; Figure 7a). One explanation for this result is ontogenetic effects early in life history that influenced the incorporation of Mg into skeletal calcite of C. virginica. In such cases, only the older (more recently deposited) portion of the Mg/Ca_{shell} record is predictably influenced by estuarine temperature. Similar ontogenetic effects on the incorporation of elemental ratios into shell carbonate have been observed in other bivalves. In the aragonitic shell of Mercenaria campechiensis, Surge and Walker [2006] observed no correlation between Sr/Ca_{shell} ratios and $\delta^{18}O_{shell}$ (influenced largely by temperature variation) in the juvenile portion of the shell. However, they observed a strong correlation in the adult portion of the shell ($r^2 = 0.72$, $p < 0.001$). Gillikin et al. [2005] and Lazareth et al. [2007] also observed a biological control on the elemental ratios of shells from other molluscan species. In agreement with our findings, Lorrain et al. [2005] did not find any relationship between Mg/Ca_{shell} ratios and sea surface temperature in year-old scallops (Pecten maximus). Conversely, Freitas et al. [2005] found a correlation only in the first 4 years of growth in the mussel Pinna nobilis, and Vander Putten et al. [2000] observed a relationship only during spring months in the mussel Mytilus edulis. Alternatively, in our case, there may be a decrease in accuracy of date assignments further back in time. Regardless of the cause, we evaluated the possibility that the most recent record of growth provided more satisfying results by plotting only the data from growth since the most recent summer cessation (Figures 5 and 7b). Culling the data in this manner improved the correlation between temperature and Mg/Ca_{shell} ratios ($r^2 = 0.30$, $p < 0.01$) and resulted in the following linear regression equation:

\[
\text{Mg/Ca} = 0.72T - 0.23, \tag{5}
\]

Figure 4. Variation of $\delta^{18}O$ and $\delta^{13}C$ from the umbo toward the commissure of the hinge area. Shell BW20L2 was collected on 3 December 1997; shells BW20L3, BW20L4, and BW20L5 were collected on 6 January 1999; and shell BW20L7 was collected on 28 July 1999. Solid circles are $\delta^{18}O$, and open squares are $\delta^{13}C$. Distance is in mm.
where $T$ is temperature in °C. Because Mg/Ca\_WATER data from Blackwater River varied across salinities, equation (5) is specific to southwest Florida. To construct an equation that is applicable on a more regional scale under con-

Figure 5. Comparison of predicted $\delta^{18}$O\_SHELL and $\delta^{13}$C\_SHELL values and measured shell data. Gray line is predicted $\delta^{18}$O\_SHELL, and black line is predicted $\delta^{13}$C\_SHELL. Specimen identification is as indicated in Figure 4. Solid circles are observed $\delta^{18}$O\_SHELL, and open circles are observed $\delta^{13}$C\_SHELL. Gaps in observed shell data not representing the most negative predicted isotopic compositions are inferred cessation of growth. Bars on the y axis of the BW20L2 plot represent 95% confidence limits for models $\delta^{18}$O (0.69) and $\delta^{13}$C (1.97).

Figure 6. Variation in Mg/Ca\_SHELL ratios. Specimen identification is as in Figure 4. Distance is in mm.
Figure 7. Mg/Ca\textsubscript{SHELL} and $D_{Mg}$-temperature equations. (a) Correlation between Mg/Ca\textsubscript{SHELL} ratios (mmol/mol) and temperature (°C). Solid diamonds represent BW20L2; open squares represent BW20L3; solid triangles represent BW20L4; open circles represent BW20L5; and crosses represent BW20L7. (b) Covariance between Mg/Ca\textsubscript{SHELL} ratios (mmol/mol) and temperature for only the last year of growth. All specimens are represented by open squares. (c) Covariance between $10^3D_{Mg}$ and temperature for only the last year of growth. All specimens are represented by open squares. Black line is calculated least squares linear regression.
ditions where the $\text{Mg/Ca}_{\text{WATER}}$ ratio of coastal waters is known and remains relatively constant, the partition coefficient ($D_{\text{Mg}}$),

$$D_{\text{Mg}} = (\text{Mg/Ca}_{\text{SHELL}})/(\text{Mg/Ca}_{\text{WATER}}),$$

for crassostreine calcite is considered because it takes into account $\text{Mg/Ca}_{\text{WATER}}$, and it is temperature-dependent. 

[18] To calculate $D_{\text{Mg}}$, the $\text{Mg/Ca}_{\text{WATER}}$-salinity mixing relation (Figures 3b and 3c) and salinity based on date assignments were employed to estimate $\text{Mg/Ca}_{\text{WATER}}$ for a particular date. Calculating $D_{\text{Mg}}$ for each sample from the culled data set resulted in the following linear regression equation ($r^2 = 0.33, p = 0.01$):

$$10^{1.67}D_{\text{Mg}} = 0.16T - 0.33,$$  \hspace{1cm} (6)

where $T$ is temperature in $^\circ$C (Figure 7c).

[19] Despite the low correlation, the $D_{\text{Mg}}$-temperature equation presented in this study based on high-resolution sampling compares well with the temperature equation for *C. virginica* previously reported by Lerman [1965], who employed coarser sampling methods. Lerman [1965] calculated his temperature equation using bulk shell samples collected along a latitudinal gradient and average annual temperature for each latitude. Moreover, both our $\text{Mg/Ca}$ and $D_{\text{Mg}}$-temperature equations are similar in slope to those calculated for other taxa, though intercepts can be different (bivalve from *Pinna nobilis* determined from in vitro experiments, *Cyprideis* previously reported by *E. Martini*, Bjorn Klaua, Sam Stamper, Dianne Cole-Bronczyk, and Heather Stoffel. Reviews by David Gillikin and Alan Wanamaker greatly improved this paper.

5. Conclusions

[21] Estuarine habitats continue to present challenges for studies in paleoenvironmental reconstruction of seasonal temperature and salinity. However, by employing multiple geochemical approaches involving isotopic and elemental ratios, these water parameters may potentially be constrained. Live-collected oysters of *C. virginica* were harvested at a reef in Blackwater River near Naples, Florida, where temperature and salinity were measured and where water samples were collected for chemical analyses. This enabled high-resolution comparison of shell chemistry to ambient environmental conditions. Only the most recent record of shell growth since the last period of summer growth cessation produced a small but positive relationship between temperature and $\text{Mg/Ca}_{\text{SHELL}}$ ratios of crassostreine calcite, though there is a slight influence of salinity at this site. Therefore, equation (5) estimates local environmental information for southwest Florida. Equation (6) potentially provides a proxy for temperature on a more regional scale if $\text{Mg/Ca}_{\text{WATER}}$ is known and does not vary.

Although the correlation between $\text{Mg/Ca}_{\text{SHELL}}$ ratios and temperature was low, our results are encouraging for future studies under more controlled experimental conditions to more precisely develop this potential temperature proxy for estuarine habitats. By combining isotopic and elemental data, complicated conditions of estuarine environments may be deconvoluted.

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K. C Lohmann, Department of Geological Sciences, University of Michigan, 1100 North University Avenue, Ann Arbor, MI 48109-1005, USA.

D. Surge, Department of Geological Sciences, University of North Carolina at Chapel Hill, 104 South Road, CB 3315, Chapel Hill, NC 27599-3115, USA. (donna64@unc.edu)