Warm, not super-hot, temperatures in the early Eocene subtropics

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ABSTRACT
The early Eocene (ca. 55–48 Ma) encompasses one of the warmest intervals of the past 65 m.y. and is characterized by an unusually low equator-to-pole thermal gradient. Recent proxy studies suggest temperatures well in excess of 30 °C even at high latitudes, but conflicting interpretations derived from different types of data leave considerable uncertainty about actual early Eocene temperatures. A robust comparison among new paleotemperature proxies may provide insight into possible biases in their temperature estimates, and additional detail on the spatial distribution of temperatures will further resolve the early Eocene meridional temperature gradient. We use a suite of paleotemperature proxies based on the chemistry of bivalve shell carbonate and associated sedimentary organic matter from the United States Gulf Coastal Plain to constrain climate at a subtropical site during this key interval of Earth history. Oxygen isotope and clumped isotope analyses of shell carbonate and two tetraether lipid analyses of sedimentary organic carbon all yield temperatures of ~27 °C. High-resolution, intraannual oxygen isotope data reveal a consistent, large range of seasonal variation, but clumped isotope data suggest that seasonality is due primarily to precipitation, not to temperature. These paleotemperature estimates are 2–3 °C warmer than the northern Gulf of Mexico today, and generally consistent with early Eocene temperature estimates from other low and mid-latitude locations, but are significantly cooler than contemporaneous estimates from high southern latitudes.

INTRODUCTION
Paleotemperature estimates from a variety of geochemical and paleontological proxies indicate that the early Eocene encompasses the warmest climatic conditions of the Cenozoic (the past 65 m.y.). Intervals of extreme warmth are of great interest, as they provide analogs for a future greenhouse world. Recent estimates suggest temperatures approaching or in excess of 30 °C not only in low latitudes (Pearson et al., 2007), but also middle (Zachos et al., 2006) and high latitudes (Bijl et al., 2009; Creech et al., 2010; Hollis et al., 2009), implying very low meridional gradients. Concerns over potential biases related to preservation of carbonate microfossils (Pearson et al., 2001; Schrag, 1999) and temperature significance of the newer organic proxies (e.g., see discussion in Liu et al., 2009), however, leave some uncertainty about the actual magnitude and distribution of paleotemperatures in the early Eocene (Huber, 2008). Expanding the spatial coverage of paleotemperature estimates and corroborating inferred values with multiple proxies will enable an assessment of potential biases in any one proxy, refine interpretations of mean annual temperature (MAT) at the surface, and ultimately allow for a better understanding of processes responsible for maintaining Eocene warmth and equability.

We use multiple geochemical proxies applied to the shells of bivalve mollusks and their associated sediment to assess paleotemperatures on a subtropical continental margin. Samples come from shallow-marine sediments of the early Eocene upper Hatchetigbee Member of the Hatchetigbee Formation (ca. 54–52 Ma; see the GSA Data Repository1 for strontium isotope age constraints) in the Gulf Coastal Plain of the southeastern United States (for details, see the Data Repository), at a paleolatitude of ~30°N (Müller et al., 2011). Sediments are unconsolidated and fossils are in excellent condition, retaining their original aragonite (see the Data Repository). Earlier oxygen isotope work revealed unexpectedly low seasonal δ18O minima within individual shells, suggesting summer temperatures of ~37 °C and leading the authors to propose a scenario of seasonal freshwater mixing to locally decrease water δ18O values and bias calculated temperatures (Ivany et al., 2004). These depleted δ18O values, however, could also be consistent with a mean tropical sea-surface temperature of ~31 °C proposed by Pearson et al. (2007) for that same time interval. We combine stable oxygen isotope, clumped isotopes, and strontium isotope analyses of shell carbonate with tetraether lipid proxies (TEX86, BIT [branched and isoprenoid tetraether], MBT/CBT [methylation of branched tetraethers/cyclization of branched tetraethers]) from organic matter in sediment encased by articulated shells to deconvolve the competing effects of temperature and salinity on the δ18O record and produce reliable estimates of winter, summer, and MATs during this interval of exceptional warmth.

METHODS
The shells of two individuals of the bivalve Venericardia hatcheplata from each of seven stratigraphic horizons within the Hatchetigbee Formation were cut along the maximum growth axis from umbo to ventral margin, and polished. Because these bivalves grow more or less continuously throughout the year, high-resolution sequential microsampling of accretionary shell carbonate can recover seasonal variation (Ivany et al., 2004). Multiple, sequential, growth-band–parallel samples were milled from each couplet of light and dark growth bands in the inner shell layer using a Merchantek (New Wave) MicroMill. Within each bivalve, at least three full years of growth were sampled wherever possible. Aragonite powders were analyzed for stable carbon and oxygen isotope values at the University of Michigan’s Stable Isotope Laboratory using MAT 251 and MAT 253 mass spectrometers. Resulting oxygen isotope values were converted to paleotemperatures using the corrected biogenic aragonite temperature equation of Grossman and Ku (1986; Kobashi and Grossman, 2003) and assuming seawater δ18O is the latitude-corrected ice-free marine value (~0.36‰ at 30°N; Zachos et al., 1994).

Aliquots from light and dark growth band increments of four venericardia, as well as two bulk-shell samples that ranged across multiple years of growth, were analyzed for clumped isotopes (Δ47). Carbonate samples were measured for Δ47 values using methodology described elsewhere (Affek and Eiler, 2006; Ghosh et al., 2006; Huntington et al., 2009). Temperature estimates were calculated from Δ47 values using the calibration of Ghosh et al. (2006).

Sediments enclosed within six pairs of articulated valves of Venericardia, two from each of three horizons, were analyzed for tetraether lipid distributions. TEX47 values were calculated following Schouten et al. (2002), and converted to sea-surface temperatures (SSTs) using the non-linear calibration of Liu et al. (2009). The BIT index, a measure for terrestrial organic matter input to sediments, was calculated following Hopmans et al. (2004). Terrestrial mean annual temperature (MAT) estimates were calculated from MBT and CBT values following Weijers et al. (2007).

1GSA Data Repository item 2011229, background information, methods, Tables DR1–DR4 (data tables), and Figures DR1–DR3, is available online at www.geosociety.org/pubs/ft2011.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.
RESULTS

δ¹⁸O values vary approximately sinusoidally between −5.7‰ and −1.4‰ along all 14 shell transects, reflecting seasonal cycles (Fig. 1). Dark (translucent) bands correspond to more negative δ¹⁸O values, suggesting, in the absence of other evidence, formation during warm seasons. Assuming normal marine salinity, calculated winter minimum temperatures are consistent within and among shells of all horizons, with an overall mean of 26.6 ± 1.1 °C (±1 standard deviation) (Fig. 2; Table DR1 in the Data Repository). Inferred summer maximum temperatures, however, are very warm and significantly more variable, ranging between 31 and 43 °C (Fig. 2; Ivany et al., 2004).

Clumped isotopes analyses suggest a MAT of 26.5 ± 1.0 °C (±1 standard error, n = 10) (Fig. 2; Table DR2). Mean summer and winter samples produce paleotemperatures of 25.5 ± 2.1 °C and 27.1 ± 1.7 °C, respectively (see the Data Repository). Paired clumped isotope analyses of light and dark growth bands within shells reveal summer and winter temperatures that are within error (based on the analytical error for individual samples; Fig. 1; Table DR2). The variability among shells is larger (22.4 ± 1.9 °C to 31.1 ± 0.7 °C) and likely reflects a combination of analytical uncertainty and real temperature variability on time scales longer than the lifetime of an individual bivalve. These results argue that negative δ¹⁸O values from shell carbonate do not reflect seasonally warm temperatures, but rather a substantial seasonal contribution of δ¹⁸O-depleted freshwater. Seasonal clumped isotope temperatures likely reflect integrated seasonal means, as the relatively large sample size required does not allow for the capture of seasonal temperature extremes. The δ¹⁸O values of the samples used for clumped isotopes analysis illustrate that, while micromilled carbonate from light and dark growth bands generally differ by ~2.5‰, corresponding samples measured for clumped isotopes differ by 1.5‰–2‰.

Temperatures derived from tetraterpider lipid analyses are within error or slightly higher than those from clumped isotopes. TEX₈₆ indicates temperatures between 27 and 29 °C (Fig. 2; see the Data Repository). BIT values are between 0.3 and 0.4 (see the Data Repository), indicating a coastal setting with significant terrestrial organic matter input (Hopmans et al., 2004), consistent with the inference of seasonal precipitation and runoff drawn from seasonally depleted shell isotope values and corroborated by the abundance of terrestrial vascular plant organic debris in the sediments. These relatively high BIT values indicate a possible source of uncertainty in TEX₈₆ derived temperatures because isoprenoid tetraterpider lipids derived from soils can influence TEX₈₆ values in settings with large terrestrial organic matter inputs (Weijers et al., 2006). Nevertheless, the TEX₈₆ derived temperatures basically agree with those from isotopic analyses of shell carbonates, and since the latter are not affected by organic inputs, the agreement suggests that TEX₈₆ reliably reflects the temperature of coastal waters in this setting. The MBT/CBT paleotemperature proxy, however, specifically targets terrestrial derived organic matter, and suggests soil paleotemperatures between 25 and 28 °C (Fig. 2; see the Data Repository). Although a large uncertainty exists concerning the source regions for soil-derived branched tetraterpider lipids in marine sediments, consistency in inferred temperatures suggests that nearby continental MATs were similar to coastal sea-surface temperatures.

DISCUSSION

Good agreement among paleotemperatures derived from winter δ¹⁸O, clumped isotopes, TEX₈₆, and MBT/CBT indicates that the MAT on the U.S. Gulf Coast during the early Eocene was ~27 °C. Latest PaleoceneTEX₈₆ derived temperatures from a core in near southeastern Mississippi (van Roij, 2009) are in line with those presented here. Winter and summer temperatures are statistically indistinguishable using clumped isotopes, implying that the range of seasonal temperature variation is significantly lower than suggested by δ¹⁸O data. The very negative “summer” δ¹⁸O values instead indicate that the primary manifestation of the seasonal cycle was related to precipitation, making it the wet season. Seasonally resolved δ¹⁸O values from more typical marine facies elsewhere in the upper Hatchetigbee Member and underlying Bashi Marl Member of the Hatchetigbee Formation yield low seasonal ranges and similar “winter”/dry season values, reinforcing this interpretation (Ivany et al., 2003;

Figure 1. Variation in δ¹⁸O of Venericardia carbonate sequentially sampled in direction of shell growth. Shells are arranged in stratigraphic order. Inferred temperatures are reported assuming constant δ¹⁸O value of 1.36‰. Shaded gray bars indicate dark (translucent) growth bands. Horizontal solid, dashed, and dotted lines denote mean clumped isotope-derived “winter,” “summer,” and bulk temperatures, respectively. Analytical error for clumped isotope data is ±0.3‰. Plots 2a/1c represents δ¹⁸O values of venericardia 1a and clumped isotope values of venericardia 1c. Note different scale of y axis for plot 2a*. (See the Data Repository [see footnote 1] for all clumped isotope data and details.) VPDB—Vienna PeeDee belemnite.
Kobashi et al., 2001). A MAT of ~27 °C, minimal temperature seasonality, and significant seasonal precipitation is in good accord with paleobotanical data supporting tropical to paratropical conditions in the early Eocene Gulf Coast (Fricke and Wing, 2004; Harrington, 2003; Wolfe, 1978; Wolfe and Dícher, 2000).

The multiple paleotemperature proxies used in this study enable a comparison among them. This is the first time that the clumped isotopes technique has been tested on ancient or modern biogenic carbonates where other independent proxies have been simultaneously applied. That clumped isotope temperatures generally agree with conventional oxygen isotope–derived temperatures determined a priori with an independent estimate of seawater composition, as well as with two organic geochemical proxies, demonstrates conclusively that the technique can be effective on carbonates as old as 50 m.y., despite concerns about the effects of diagenesis (Came et al., 2007; Dennis and Schrag, 2010). The Gulf Coast, however, is perhaps a best-case scenario, with sediments that have never been deeply buried or tectonically deformed. The narrower range of temperatures derived from the organic molecular proxies is likely due to attenuation of short-term variability by time averaging associated with sedimentation. The accretionary, in situ growth of shell carbonate instead records intraannual and interannual variation in conditions.

Paleotemperature estimates derived from TEX$_{86}$ are often somewhat warmer than corresponding δ$^{18}$O or Mg/Ca-derived temperatures, raising concerns about temperature calibration, especially in high-latitude settings during greenhouse intervals (Huber, 2008). Several calibrations have been developed for deriving SST from TEX$_{86}$ (Kim et al., 2010, 2008; Liu et al., 2009; Schouten et al., 2002) and it is not yet clear which is the most relevant relationship. The processes controlling the temperature dependence of archaeal lipid distributions are poorly understood, thereby preventing a process-based choice between calibrations. Early linear calibrations are likely problematic, as reflected by their inability to fit values at high-SST locations (Kim et al., 2010; Liu et al., 2009; Schouten et al., 2003), because physiological responses to temperature change are typically nonlinear. We therefore prefer the nonlinear, reciprocal relationship reported by Liu et al. (2009) that provides the simplest relationship covering the entire temperature range of modern core top data. This calibration results in lower temperatures than those derived through the logarithmic calibration of Kim et al. (2010) (Fig. 3). Our TEX$_{86}$ derived temperatures using the Liu et al. (2009) calibration are consistent with those from clumped isotopes, lending support for that calibration (Fig. 3, inset). Although the MBT/CBT index proxy for continental temperatures is new and requires further testing and calibration (Weijers et al., 2006), its agreement with the SST proxies in this case provides support for its application in low-lying coastal sediments rich in terrestrial soil organic matter.

A mean early Eocene paleotemperature of ~27 °C on the U.S. Gulf Coast is only 2–3 °C warmer than modern sea-surface MAT in the northern Gulf of Mexico (Levitus and Boyer, 1994) and is consistent with non–Paleocene–Eocene Thermal Maximum temperatures observed at a mid-latitude site to the north (New Jersey; Sluijs et al., 2007; Zachos et al., 2006). These temperatures are, however, as much as 4 °C cooler than recently reported early Eocene values at both lower and higher latitudes (Fig. 3). Tropical temperatures of ~31 °C (Tanzania; Pearson et al., 2007) are not necessarily in conflict with temperatures of 27 °C in the subtropics, but temperatures warmer than this at higher latitudes (New Zealand and the Tasman Rise; Bijl et al., 2009; Creech et al., 2010; Hollis et al., 2009) are more difficult to reconcile without considering potential unrecognized biases among proxies. Multiproxy temperature estimates from the U.S. Gulf Coast suggest warm, but not super-hot, paleotemperatures with low seasonal variation in the U.S. subtropics, provide further evidence for a reduced meridional temperature gradient during the early Eocene, and highlight the anomalous warmth implied by temperature reconstructions from the southwest Pacific Ocean.

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Geological Background

The US Gulf Coastal Plain preserves a record of marine shelf deposition through the Paleogene characterized by excellent preservation of primary shell aragonite and minimal diagenesis. Sediments are generally un lithified even after 55 million years, and include fossils that offer great potential for isotope-derived climate information (2003; Ivany et al., 2004; Kobashi et al., 2001). Sampled bivalves come from the upper Hatchetigbee Member of the Hatchetigbee Formation, a highstand deposit consisting of fossiliferous, glauconitic, muddy sands grading up into thin, alternating beds of sand and clay suggesting estuarine conditions (Dockery, 1980; Gibson, 1982; Ivany et al., 2004; Mancini, 1984; Seisser, 1984; Toulmin, 1977). Bivalves were collected at Hatchetigbee Bluff on the Tombigbee River in Washington County, AL (Fig. S1). An early Eocene age is constrained biostratigraphically (Gibson, 1982; Mancini, 1984) and using Sr isotopes (see below). X-Ray diffraction demonstrates the retention of primary aragonite mineralogy, and scanning electron microscopy shows shell microtextures comparable to those of modern relatives (the genus Cyclocardia). Regular seasonal variation in shell
δ¹⁸O with ontogeny argues strongly for preservation of original shell material and its isotopic signals in fossil mollusks (Ivany and Runnegar, 2010).

**Strontium Isotope Ratios**

The \(^{87}\text{Sr}/^{86}\text{Sr}\) ratios of shell material were analyzed, anticipating that dark growth bands would be more radiogenic than light bands if the latter were formed under conditions of significant freshwater mixing. However, ratios from light and dark growth bands in multiple shells are statistically indistinguishable (Fig. S3) and fall within the expected range for normal marine seawater in the early Eocene (Hodell et al., 2007), suggesting that either the \(^{87}\text{Sr}/^{86}\text{Sr}\) ratio of local runoff was close to the early Eocene marine value and/or that the Sr concentration in runoff was too low to have an impact on the ratio of the water mixture.

The age of the Hatchetigbee Formation can be further constrained by the \(^{87}\text{Sr}/^{86}\text{Sr}\) ratios of the venericard bivalves relative to the global marine Sr isotope seawater curves of McArthur et al. (2001) and Hodell et al. (2007). The \(^{87}\text{Sr}/^{86}\text{Sr}\) ratios of the Hatchetigbee Formation yield ages of ~52.5 to 54.5 Ma relative to the Hodell et al. (2007) curve, which has better coverage for the early Paleogene. These strontium isotope age estimates generally agree with the biostratigraphy of the US Gulf Coast.

**Additional Insights from Inter-Proxy Comparisons: δ¹⁸O and Clumped Isotopes**

A significant limitation of the δ¹⁸O-based temperature proxy has always been the need to make an assumption about the δ¹⁸O value of the water from which the carbonate precipitated. Clumped isotopes analysis offers an independent paleotemperature estimate
that can then be used together with the measured mean winter shell $\delta^{18}O$ (-1.94‰) and the corrected biogenic aragonite-temperature equation of Grossman and Ku (1986; Kobashi and Grossman, 2003) to determine the oxygen isotope composition of seawater on the early Eocene Gulf Coast. The calculated seawater (‘winter’, or dry season) composition is -0.33‰, very close to the completely independent, latitude-corrected ice free value of -0.36‰ (based on Zachos et al., 1994), and thus providing support for the correction to global seawater composition at this latitude.

The degree to which temperature rather than salinity influences the ‘summer’ $\delta^{18}O$ values can be evaluated by comparing summer carbonate $\delta^{18}O$ with other independent temperature proxies. The mean clumped isotope paleotemperature of 27°C in combination with ‘summer’ shell $\delta^{18}O$ values yield an average ‘summer’ water composition of -2.57‰. If fully marine waters of -0.36‰ mixed with freshwater runoff with a composition of about -4.7‰, based on comparisons with both modern precipitation and river water in the Gulf today, corrected for the ice free early Eocene (Ivany et al., 2004) to produce the measured ‘summer’ water values, the resulting mixture should have a salinity of, on average, ~17 ppt during the wet season. These seasonally brackish conditions are consistent with the low-diversity fauna and abundance of terrestrially-derived organic matter found in these sediments.

DETAILED METHODS

**Stable Oxygen Isotopes**

Two individuals of *Venericardia hatcheplata* from each of seven stratigraphic horizons were cut along the maximum growth axis from umbo to ventral margin and
polished. On average, 8 growth-band-parallel samples were milled from each couplet of light and dark growth bands in the inner shell layer using a Merchantek (New Wave) Micromill; earlier work showed that such couplets in Hatchetigbee Bluff venericards are annual in nature (Ivany et al., 2004). Within each bivalve, at least three full years of growth were sampled wherever possible. Resulting aragonite powders were analyzed for stable carbon and oxygen isotope values at the University of Michigan’s Stable Isotope Laboratory on MAT 251 and 253 mass spectrometers. Analytical error was less than 0.2 per mil.

Oxygen isotope values are converted to paleotemperature using the biogenic aragonite-temperature equation of Grossman and Ku (1986), adjusted to correct for the difference between “average marine water” and standard mean ocean water (SMOW) \[T(°C) = 19.7 - 4.34 \times (\delta^{18}O_{CaCO3} - \delta_W)\] (Kobashi and Grossman, 2003). A global mean \(\delta^{18}O\) value for seawater (\(\delta_W\)) of -1.0‰ is assumed, representing the ice free world of the early Eocene (Huber et al., 2003; Zachos et al., 1994); this value is then adjusted for latitudinal variation in salinity of the global ocean (Zachos et al., 1994) for 30°N (+0.68‰) yielding \(\delta_W = -0.36‰\).

Carbonate \(\delta^{18}O\) local maxima and minima within each shell transect identify what are provisionally recognized as winters and summers, and the means of these provide estimates of mean winter temperature (MWT) and mean summer temperature (MST) within shells, horizons, and the entire section. Mean annual temperature (MAT) is the midpoint between MST and MWT, and mean annual range of temperature (MART) is the difference between them, at the various levels of resolution. Shell isotope profiles are not markedly asymmetric or scalloped, suggesting relatively continuous growth year-round.
Clumped Isotopes

The carbonate clumped isotopes technique yields paleotemperature estimates that are independent of the composition of the water from which the shell carbonate precipitated (Eiler, 2007; Ghosh et al., 2006), thereby circumventing concerns of freshwater mixing and other sources of variation in $\delta^{18}O_w$, and providing a less ambiguous temperature proxy. Aliquots from the same light and dark growth band increments of four of the venericard shells that were analyzed for oxygen isotopes, plus one additional venericard from the Hatchetigbee Formation not sampled for $\delta^{18}O$, were analyzed for clumped isotopes ($\Delta_{47}$). Also analyzed were large bulk-shell samples that ranged across multiple years of growth from each of two bivalves to produce time-integrated average values. Samples were analyzed in 1-4 replicates for each growth band depending on material availability. We typically consider at least 3 replicates (of 3-4 mg each) to be required for a reliable temperature determination. To compensate for the small number of replicates possible in some samples due to the limited amount of carbonate available from individual growth bands, the results from all shells were pooled to yield a seasonal mean $\Delta_{47}$ value. This resulted in an estimated SST for the entire time period, even though the range of values suggests at least some short-term variability (at the scale of the 5-10 years lifetime of an individual shell) through the interval. Samples were measured over the course of a year (October 2009 to November 2010) and were normalized using a set of standards measured together with the samples (following Huntington et al., 2009).
Carbonate samples were measured for $\Delta_{47}$ values in the Earth Systems Center for Stable Isotopic Studies at Yale University, using the same methodology described previously (Affek and Eiler, 2006; Ghosh et al., 2006; Huntington et al., 2009).

Carbonate samples were digested by an overnight reaction with 105% phosphoric acid at 25°C. Resulting CO$_2$ was purified cryogenically on a vacuum line using ethanol-dry ice and liquid N$_2$ traps, and by passing it through a GC column (QPlot column, 30m long, 530 µm ID) at -20°C. CO$_2$ was then analyzed by dual inlet for the abundance of masses 44 to 49 using a MAT-253 mass spectrometer with cup configuration that is identical to that used for the original development of the clumped isotopes method (Eiler, 2007; Eiler and Schauble, 2004).

Each CO$_2$ sample was analyzed 9 times. Each analysis consisted of 10 cycles of sample standard-comparison with 20-second integration at a signal intensity of 12-16V for mass 44. $\Delta_{47}$ is calculated from the measured ratios of masses 45, 46, and 47 to mass 44, as given in the following equation:

$$
\Delta_{47} = \left[ \frac{R_{47}}{2R_{13} \cdot R_{18} + 2R_{17} \cdot R_{18} + R_{13} \cdot (R_{17})^2} - \frac{R_{46}}{2R_{18} + 2R_{13} \cdot R_{17} + (R_{17})^2} - \frac{R_{45}}{R_{13} + 2R_{17}} + 1 \right] \times 1000
$$

Data were standardized using a set of CO$_2$ samples that were heated at 1000°C for ~2h, resulting in a random distribution of isotopes among all CO$_2$ isotopologues ($\Delta_{47} = 0\%$). This served as a correction for subtle non-linearity and to isotopic scrambling in the ion source (for details see Huntington et al., 2009). Scrambling correction requires knowing the unscrambled $\Delta_{47}$ value of the reference working gas. We determined the value through measurements of $\Delta_{47}$ in CO$_2$ equilibrated with water at 25°C, whose nominal value is 0.871‰. This scrambling correction was validated using a set of
carbonate samples that have been previously analyzed (Affek et al., 2008; Ghosh et al., 2006) to give nominally defined values resulting in a mean offset of 0.009‰, comparable to the mean analytical standard error of 0.007‰ of these materials. This was further verified by measurements of CO₂ equilibrated with water at 10°C, 25°C, and 50°C as part of an interlaboratory calibration, through comparison with theoretically determined values (Wang et al., 2004), resulting in a mean offset of 0.002‰ (Dennis et al., submitted).

The mass spectrometer raw data has a Gaussian distribution of 90 measurements of Δ₄⁷ values (for a single replicate), due to noise in ion collection by the mass spectrometer detectors. This results in internal analytical precision that is close to the shot noise limit (Merritt and Hayes, 1994), which is approximately 0.010‰ for the mass 47 signal measured under the conditions given above. Further improvement of precision is then possible through replicates (Huntington et al, 2009), which also improve errors associated with sample preparation (external precision). The typical external precision of the measurements can be estimated through multiple replicates of a laboratory standard (Carara marble) of about 0.004‰ (1 SE, n=49, analyzed between November 2008 and July 2010). This long-term external precision reflects a combination of mass spectrometer noise, and errors associated with long-term variability in standardization and with sample preparation. The mean standard error in the bivalve analysis described in this work is 0.010‰, equivalent to a paleotemperature error of approximately ± 2°C.

Temperature estimates were calculated from Δ₄⁷ values using the calibration of Ghosh et al. (2006):

\[ \Delta_{47} = 0.0592 \times 10^6 / T^2 - 0.02 \text{ (where } T \text{ is in K)}. \]
This calibration was originally developed using synthetic calcite (Ghosh et al., 2006), and has subsequently been verified for biogenic carbonates, including mollusk shells (Came et al., 2007) and a variety of other marine organisms grown at known temperatures (summarized by Tripati et al., 2010). To verify the $\Delta_{47}$-T relationship for bivalves, we measured $\Delta_{47}$ values in shells of two modern bivalves from Long Island Sound ($\textit{Mytilus edulis}$, the blue mussel, and $\textit{Crassostrea virginica}$, the eastern oyster) (P. Douglas and H. Affek, unpublished). The $\Delta_{47}$-derived temperatures are $17.5 \pm 1.1$ and $18.3 \pm 3.1^\circ$C, respectively. Came et al. (2007) used a six-warm-month mean temperature as an approximation for bivalve shell growing season temperatures. The growing season for the Long Island Sound $\textit{M. edulis}$ is April to September with a mean temperature of $17.5^\circ$C (Hilbish, 1986), while the growing season for $\textit{C. virginica}$ is May to October with a mean temperature of $18.1^\circ$C (Dame, 1974). These growing season temperatures are in close agreement with the observed $\Delta_{47}$ derived temperatures, suggesting that indeed the shell is recording the conditions during growth. The $\delta^{18}$O profiles from the early Eocene US Gulf Coast show year-round growth in venericard shells, suggesting that clumped isotopes reflect mean annual temperatures.

**Tetraether Lipid Analyses**

$\text{TEX}_{86}$ offers another independent and relatively new approach for determining paleotemperatures in marine sediments containing organic matter. Sediments enclosed within six pairs of articulated valves of $\textit{Venericardia}$, two from each of three horizons, were sampled, processed, and analyzed separately for tetraethers in the Organic
Biogeochemistry and Paleoclimatology Laboratory at Yale University using methodology described previously (Schouten et al., 2002). Freeze dried sediment samples were extracted with a soxhlet extractor using a 2:1 dichloromethane:methanol solvent mixture. The lipid extracts were then separated into apolar, ketone, and polar fractions via silica gel pipette column chromatography. Glycerol dialkyl glycerol tetraether (GDGT) lipids were further purified via alumina pipette chromatography and filtered through 0.45 µm glass fiber filters. GDGTS were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC/MS) on an Agilent 1100 series LC/MSD. A Prevail Cyano column was used with 99:1 hexane:isopropanol as eluent. TEX$_{86}$ values were calculated using the formula of Schouten et al. (2002):

$$TEX_{86} = \frac{[GDGT2 + GDGT3 + \text{crenarchaeol regioisomer}]}{[GDGT1 + GDGT2 + GDGT3 + \text{crenarchaeol regioisomer}]}$$

TEX$_{86}$ values were converted to SSTs using the non-linear calibration of Liu et al. (2009):

$$SST = 50.475 - 16.332(1/TEX_{86})$$

To assess differences between TEX$_{86}$-temperature calibrations, we also calculated SSTs using the high-temperature logarithmic calibration of Kim et al. (2010):

$$SST = 68.4[\log(TEX_{86})] + 38.6$$

For comparison purposes, paleotemperatures were also calculated using several alternative linear TEX$_{86}$ calibrations (see Table S3 and references therein). The difference in SST estimates for two samples from the same stratigraphic horizon is less than 1°C when using the Liu et al. (2009) calibration.
The Branched and Isoprenoid Tetraether Index, a proxy for terrestrial organic matter input to sediments, was calculated following Hopmans et al. (2004) to evaluate the potential for terrestrial organic matter input that could bias the TEX$_{86}$-derived temperatures:

$$BIT = \frac{[I + II + III]}{[I + II + III] + [IV]}$$

where compounds I, II, and III are branched GDGTs primarily derived from soil-dwelling bacteria, and compound IV is crenarcheol, an isoprenoidal GDGT primarily derived from marine archaea. Note that crenarcheol is a distinct compound from the crenarcheol regioisomer used in the calculation of TEX$_{86}$ values.

Another novel proxy for terrestrial paleotemperatures, the MBT/CBT index, is based on the distribution of branched tetraether lipids derived from soil-dwelling bacteria. The methylation index of tetraethers (MBT) and cyclisation ratio of tetraethers (CBT) were calculated following Weijers et al. (2007). MBT indicates the degree of methylation at the C-5 and C-5’ position of branched GDGTs and is calculated as follows:

$$MBT = \left[ \frac{I + Ib + Ic}{(I + Ib + Ic) + (II + IIb + IIc) + (III + IIIb + IIIc)} \right]$$

where I, Ib, Ic, II, IIb, IIc, III, IIIb, and IIIc are branched tetraether lipids with varying degrees of methylation as described in Weijers et al. (2007). High MBT values indicate a low degree of methylation and reflect a combination of soil temperature and pH. CBT
indicates the abundance of cyclopentyl moieties in branched tetraethers and is calculated as follows:

\[ CBT = \frac{[Ib] + [IIb]}{[I] + [II]} \]

High CBT ratios indicate increased cyclopentyl moieties and reflect soil pH. Terrestrial MAT estimates are calculated from the combination of MBT and CBT values using the empirical relationship of Weijers et al. (2007):

\[ MAT = \frac{MBT - (0.187 \times CBT) - 0.122}{0.02} \]

Temperatures derived from these values reflect mean annual temperature in the continental regions that are sources for terrestrial organic matter in Hatchetigbee Formation sediments. As the MBT/CBT index is a relatively new temperature proxy and requires further testing, these particular temperature estimates should be considered preliminary.

**Strontium Isotope Analyses**

We measured $^{87}\text{Sr}/^{86}\text{Sr}$ in samples of light and dark (‘winter’ and ‘summer’) growth bands from a subset of venericards. Sample preparation for strontium isotope measurement is described in Ivany et al. (2006). Procedures for strontium separation, filament loading, and mass spectrometry followed Samson et al. (1995). Samples were analyzed on the Sector 54 thermal ionization mass spectrometer at the Syracuse University Radiogenic Isotope Laboratory. Ratios are compared to marine seawater
values reported by Hodell et al. (2007) for the early Eocene standardized using the NIST-987 standard.
Supplemental Tables

Table S1. Stable oxygen isotope values, $\delta^{18}O$-calculated temperatures*, and calculated water $\delta^{18}O$ values† for local ‘winter’ maxima and ‘summer’ minima within shells from different horizons (see Fig. S2). Error estimates are ± 1 SD.

*Temperatures calculated assuming a latitude-corrected, local marine $\delta^{18}O_w$ value of -0.36‰ (see text). †Water values calculated using the clumped-isotope-derived mean temperature of 27°C (Table S2). Individual milled data are available upon request.

Table S2. Clumped isotopes results for seasonal and bulk samples of bivalves, including inferred temperatures, associated $\delta^{13}C$ and $\delta^{18}O$ values of samples, and calculated water compositions based on those values.

*Equal to the mean of all shell data calculated from ‘winter’, ‘summer’, and bulk values. †Error estimates are ± 1 SE for replicate analyses of individual samples. In bold, errors are ± 1 SE of ‘winter’, ‘summer’ layers, or all data together.

Table S3. TEX$_{86}$ results and BIT index. Temperatures inferred based on calibrations in Liu et al. (2009), Schouten et al. (2002), Schouten et al., (2003; high temperature calibration), Kim et al. (2008), and Kim et al. (2010). Error estimates are ± 1 SD.

Table S4. MBT/CBT results and inferred temperatures. Error estimates are ± 1 SD.
Supplemental Figures

**Figure S1.** Stratigraphic, geographic, and morphologic details of sampled bivalves. A) Late Paleocene – early Eocene lithostratigraphic units and biostratigraphic zonations of southwest Alabama based on Mancini (1984). B) Map of outcrop area of the Hatchetigbee Formation in southern AL. Star represents location of Hatchetigbee Bluff. C) The bivalve *Venericardia hactheplata*.

**Figure S2.** Example of the variation in δ\(^{18}\)O of *Venericardia* carbonate sequentially sampled in the direction of shell growth. Shaded gray bars mark positions of dark (translucent) growth bands exposed in cross-section. Shell and corresponding δ\(^{18}\)O come from sample 6b from horizon 6.

**Figure S3.** Strontium isotope ratios for venericard shells in horizons 2, 4 and 6 measured in this study and the venericard from Ivany et al. (2004). Closed symbols represent dark growth bands corresponding to more negative δ\(^{18}\)O values and open symbols represent light growth bands corresponding to more positive δ\(^{18}\)O values. Gray band brackets ± 1 SE of the mean \(^{87}\)Sr/\(^{86}\)Sr value for all Hatchetigbee Bluff samples. Range of early Eocene marine ratios reported by Hodell et al. (2007) indicated at right.
REFERENCES


WARM, NOT SUPER-HOT, TEMPERATURES IN THE EARLY EOCENE SUBTROPICS

Caitlin R. Keating-Bitonti¹,³, Linda C. Ivany¹, Hagit P. Affek², Peter Douglas², & Scott D. Samson¹

SUPPLEMENTAL INFORMATION (SI)

Supplemental Tables
Table S1.

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<td>ALL</td>
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<td>0.69 ± 0.05</td>
<td>6.96 ± 0.1</td>
<td><strong>26.83 ± 1.2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>