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A. D. Wanamaker
K. J. Kreutz
H. W. Borns
D. S. Introne
S. Feindel

See next page for additional authors

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Experimental determination of salinity, temperature, growth, and metabolic effects on shell isotope chemistry of *Mytilus edulis* collected from Maine and Greenland

Alan D. Wanamaker Jr.,¹ Karl J. Kreutz,¹ Harold W. Borns Jr.,¹ Douglas S. Introne,¹ Scott Feindel,² Svend Funder,³ Paul D. Rawson,⁴ and Bruce J. Barber⁴,⁵

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¹To study the effects of temperature, salinity, and life processes (growth rates, size, metabolic effects, and physiological/genetic effects) on newly precipitated bivalve carbonate, we quantified shell isotopic chemistry of adult and juvenile animals of the intertidal bivalve *Mytilus edulis* (Blue mussel) collected alive from western Greenland and the central Gulf of Maine and cultured them under controlled conditions. Data for juvenile and adult *M. edulis* bivalves cultured in this study, and previously by Wanamaker et al. (2006), yielded statistically identical paleotemperature relationships. On the basis of these experiments we have developed a species-specific paleotemperature equation for the bivalve *M. edulis* [*T °C = 16.28 (±0.10) – 4.57 (±0.15) {δ18Oç VPBD – δ18Ow VSMOW} + 0.06 (±0.06) {δ18Oç VPBD – δ18Ow VSMOW}², r² = 0.99; N = 323; p < 0.0001*]. Compared to the Kim and O’Neil (1997) inorganic calcite equation, *M. edulis* deposits its shell in isotope disequilibrium (δ18Ocalcite) with ambient water. Carbon isotopes (δ13Ccalcite) from sampled shells were substantially more negative than predicted values, indicating an uptake of metabolic carbon into shell carbonate, and δ13Ccalcite disequilibrium increased with increasing salinity. Sampled shells of *M. edulis* showed no significant trends in δ18Ocalcite based on size, cultured growth rates, or geographic collection location, suggesting that vital effects do not affect δ18Ocalcite in *M. edulis*. The broad modern and paleogeographic distribution of this bivalve, its abundance during the Holocene, and the lack of an intraspecies physiologic isotope effect demonstrated here make it an ideal nearshore paleoceanographic proxy throughout much of the North Atlantic Ocean.


1. Introduction

[2] Climate models require ocean data that are long-term, of high resolution, and from a variety of locations, especially from middle-to-high latitudes. Because relatively few such records exist and historical observational records are temporally short and spatially limited, proxy records are needed to supplement our knowledge of past environments. Shells of bivalve mollusks serve as important archives of paleoenvironmental information, and can provide high-resolution records of past and present ocean climate variability [e.g., Schöne et al., 2005]. Species-specific bivalve proxies that have been experimentally calibrated [e.g., Owen et al., 2002a, 2002b; Chauvaud et al., 2005; Wanamaker et al., 2006] provide the means to reconstruct paleoenvironments with accuracy, because the isotopic variability of the biogenic carbonate has been thoroughly examined during the culture period and hence the uncertainties are better constrained than non-species-specific isotope calibrations [e.g., Epstein et al., 1953; Grossman and Ku, 1986]. The fidelity of any proxy, however, is limited when it has not been calibrated. For example, some biogenic species do not precipitate their skeletons in equilibrium with ambient water [e.g., Spero et al., 1997; McConnaughey, 1989a, 1989b; Owen et al., 2002a, 2002b; Adkins et al., 2003], so that carbonate samples from these organisms may provide inaccurate estimations of water temperature if a generic calcite or aragonite paleotemperature equation is used [e.g., Epstein et al., 1953; Grossman and Ku, 1986].

[3] The relative composition of two commonly studied isotopes (δ18Oç and δ13Cç) in biogenic carbonates may be affected differently by environmental conditions. The oxygen isotopic chemistry of biogenic carbonates (δ18Oç) is primarily controlled by water temperature and the isotopic composition of water (δ18Ow; related to salinity) [Urey, 1947; Epstein et al., 1953; Craig, 1965; Emiliani, 1966; Shackleton 1967; O’Neil et al., 1969]. In contrast, the carbon isotopic chemistry (δ13Cç) during shell precipitation may be related to dissolved inorganic carbon (DIC) [Mook...
and Vogel, 1968; Mook, 1971; Killingly and Berger, 1979; Arthur et al., 1983], metabolic carbon [Klein et al., 1996b; Geist et al., 2005], or some combination of both [Tanaka et al., 1986; McConnaughey et al., 1997; Dettman et al., 1999; Vander Putten et al., 2000; Furila et al., 2000; Lorrain et al., 2004; Gillikin et al., 2006b]. Thus δ13C profiles from biogenic carbonates have the potential to be indicators of paleo-productivity, paleo-DIC, paleo-pCO2, or paleooecology [e.g., Shanahan et al., 2005], while δ18O profiles have the potential to reflect basic hydrographic conditions (temperature and salinity). The interpretation of δ18O and δ13C data can be complicated by life processes (e.g., growth rates, metabolism, ontogeny), which often play an important role during biomineralization [Erez, 1978; Shackleton et al., 1973; Swart, 1983; Gonzalez and Lohmann, 1985; McConnaughey, 1989a, 1989b; Owen et al., 2002a, 2002b]. Further, life processes may influence whether biogenic organisms produce calcium carbonate (CaCO3) in isotopic equilibrium with ambient water, and because most CaCO3 secreting animals experience growth deceleration with increasing age, ontogenetic related isotope effects may influence shell chemistry [Krantz et al., 1987, 1989; Harrington, 1989; Freitas et al., 2005].

[4] Although isotope paleothermometry is a powerful tool in paleoclimate studies and bivalves are extremely valuable proxies [e.g., Schöne et al., 2005], there are still some confounding issues that need to be considered. Bemis et al. [1998] demonstrated that temperature reconstructions based on “paleotemperature relationships” vary by as much as 2°C. Further, previous aquaculture-based studies evaluated shell isotopic chemistry of calcite at relatively warm temperature ranges only (~7–30°C) [Epstein et al., 1953; Craig, 1965; Horibe and Oba, 1972]. Hence subpolar to temperate paleoceanographic reconstructions using these experimental relationships are limited because they are not constrained by culture data at low temperatures. Another potential problem associated with isotope paleothermometry at relatively low temperatures is determining “isotope equilibrium” during biomineralization. Kim and O’Neil [1997] revisited the work of O’Neil et al. [1969] and recommend a new mineral-specific fractionation factor (α = 1.01050) for calcite at 10°C, which is preferred for low temperature environments [Kim and O’Neil, 1997]. However, their “equilibrium-based model” is only constrained by two measurements of inorganic calcite at 10°C, and temperature estimates based on this new relationship differ by more than 1°C from the O’Neil et al. [1969] equation at 10°C. These discrepancies suggest that individual bivalve species should be cultured to determine if they deposit their shell in isotopic equilibrium, particularly at low but ecologically relevant temperatures, and caution must be exercised when choosing an equilibrium-based model to compare culture-based data [e.g., McCrea, 1950; O’Neil et al., 1969; Tarutani et al., 1969; Kim and O’Neil, 1997; Zhou and Zheng, 2003].

[5] Harrington [1989] suggested that large-scale geographic trends in temperature and productivity may affect shell isotopic chemistry, because shell production gaps and secular trends in isotopic profiles may be linked to these environmental conditions. It is assumed that the isotopic chemistry of shell material for a bivalve of the same species that live in different climate regimes (e.g., subpolar, temperate) would not be affected by physiology during the biomineralization process. In other words, one would not expect a bivalve (of the same species) from one climate regime to precipitate its shell in disequilibrium with ambient water, while the same bivalve precipitates its shell in equilibrium in another climate regime, unless there was a physiologic or genetic effect present. Few experimental studies have investigated the effects of life processes (i.e., age, size, growth rates; metabolic effects, etc.) on molluscan shell isotopic chemistry [e.g., Owen et al., 2002a, 2002b; Chauvaud et al., 2005; Wanamaker et al., 2006], and to our knowledge no previous workers have investigated the physiologic or genetic effects on shell isotope chemistry based on collecting bivalves from different climate regimes.

[6] Previously, we quantified the shell isotopic variability of juvenile M. edulis (collected from Maine) bivalves cultured under controlled conditions [Wanamaker et al., 2006]. Here we build upon our previous work and quantify shell isotopic variability of adult and juvenile animals of the intertidal bivalve M. edulis based on growing conditions that are comparable to subpolar to temperate ocean climates, and we develop a paleotemperature relationship for M. edulis that is well constrained experimentally. Further, we investigate how life processes (size, growth rates, metabolism, and physiology) impact shell isotope chemistry during culturing. Finally, we present a calibrated paleoceano graphic proxy, M. edulis, which is a common intertidal bivalve with a wide geographic distribution that will allow for the development of high-resolution paleoceanographic records throughout the much of the North Atlantic Ocean.

2. Materials and Methods

2.1. Experimental Bivalve: M. edulis

[7] The relative abundance in coastal environments and broad geographical distribution of the intertidal bivalve M. edulis makes it an ideal species for paleoenvironmental reconstructions. M. edulis has a current geographic range that extends from Greenland to North Carolina in the western Atlantic Ocean [Wells and Gray, 1960; Read and Cumming, 1967]. M. edulis also occurs on the east and west coasts of South America, the Falkland Islands, and along the European coasts from the western border of the Kara Sea south to the Mediterranean [Tebble, 1966; Seed and Suchanek, 1992], and fossils are found in many late glacial sediments in the circum-Arctic. It is absent from the Pacific coast of North America [Seed and Suchanek, 1992]. The southern distribution of this species appears to be limited by an inability to tolerate water temperatures exceeding 27°C [Read and Cumming, 1967]. The environmental optimum for this species is a salinity range of ~20–35 PSU (practical salinity units) and a temperature range of 10–20°C [Bayne et al., 1973], but it will tolerate and commonly inhabits colder waters. Because M. edulis is an intertidal to shallow subtidal organism, the shell of this species has the potential to record nearshore sea surface temperature (SST). In addition, it appears to be an appropriate organism to monitor hydrographic changes over time, because it is
found in estuaries and at river mouths. Although Thiesen [1973] has reported that M. edulis may live up to 18 to 24 years, M. edulis is relatively short-lived (commonly 6–7 years old). M. edulis deposits annual growth rings [Lutz, 1976] and microgrowth rings [Richardson, 1989]. An adult blue mussel (>2 years) can grow to about 8–10 cm (shell length) allowing for a high-resolution environmental reconstruction (submonthly). Growth rates in their natural setting are variable, depending on environmental conditions [Incze et al., 1980]. The shell of temperate M. edulis is two layered, with an outer calcitic layer and an aragonitic inside layer [Taylor et al., 1969]. The aragonitic growth layer lags the calcitic layer substantially, thus all new outer edge growth is calcitic. As the organism continues to grow, the aragonitic layer follows outward toward the mantle.

2.2. Animal Collection and Genetic Identification

Approximately 1,000 adult and juvenile sized M. edulis were collected alive in western Greenland (Sisimiut; 66°55′59.3″N, 53°40′59.9″W) during July 2004 and transported in moist storage containers to the Darling Marine Center, Maine. (Auxiliary material provides additional associated data.) Greenland bivalves were quarantined while at the Darling Marine Center. Adult M. edulis were collected alive in central Gulf of Maine (Damariscotta River; 43°56′42.7″N, 69°33′4.3″W) (Figure 1) during December 2004 and transported to the Darling Marine Center.

A subsample (N = 32) of adult mussels from Sisimiut, Greenland was sampled for genetic identification to ensure that mussels from this location were Mytilus edulis instead of the congener, M. trossulus. These ranges of two ecologically and morphologically similar species overlap throughout the north Atlantic so that specimens of M. trossulus can easily be confused with M. edulis. For genetic identification, a small portion (~10 mg) of mantle tissue was sampled from each the mussel and DNA isolated using a Qiagen DNA Mini Kit following manufacturer’s protocol. The isolated DNA was used as template in three nuclear DNA PCR-based markers that are diagnostic for M. edulis and M. trossulus. Application of these markers, Glu-5′, ITS, and Mal-I, followed the methods of Rawson et al. [1996], Heath et al. [1995] and Rawson et al. [2001], respectively. All 32 individuals were identified as M. edulis at all three markers. Given the sample size this result suggests that the frequency of M. trossulus at Sisimiut is less than 5% (P = 0.0375).

2.3. Aquaculture Design and Implementation

We used a recirculating water bath system at the Darling Marine Center to achieve four temperature settings (4, 8, 12 and 15 ± 0.5°C) and three salinity settings (23, 28, and 32 ± 0.1 PSU) [Wanamaker et al., 2006]. This four by three factorial design allowed 12 different growing conditions to be maintained simultaneously. The system consisted of three large containers (500 L) connected to an Aquanetics heat pump for temperature control. Water of each specified temperature was then delivered to a set of three 250-L tanks that served as individual water baths. In each tank we placed four 20-L buckets as shown in Figure 2. Two buckets (A and B) contained experimental replicates for each temperature by salinity treatment while the other two buckets contained water for subsequent water changes (Figure 2). The temperature of each bath was measured with a HOBO H8 data logger every hour with an accuracy of ±0.5°C, and the temperature of one bucket in each bath was measured via StowAway Tidbit every hour with an accuracy of ±0.5°C (Table 1).

Seawater was collected via the flowing seawater laboratory at the Darling Marine Center, and was pumped from the Damariscotta River at 10m depth below mean low tide. Seawater was mixed with well water for desired salinity (23, 28, and 32 ± 0.1 PSU) and stored in 2460-L containers indoors and sealed. Salinity measurements were made via a YSI model 85 oxygen, conductivity, salinity, and temperature system with an accuracy of ±0.1 PSU. We used a simple mixing line [Wanamaker et al., 2006] to achieve the desired isotopic composition and salinity. Adjustments were made by adding small volumes of either well water or seawater to the containers to achieve the desired salinity of 23 and 28 ± 0.1 PSU.

Thirty juvenile blue mussels (shell length 14–24 mm) from Greenland were placed in each 20-L temperature/salinity environment (Ntot = 720). The animals were cultured for a total of 5 months (3 August 2004 through 5 January 2005). Six adult blue mussels collected in Maine and five adult blue mussels collected in Greenland were placed in each 20-L temperature/salinity environment (without replicates), for a total of 132 animals and cultured for 6 months (24 January 2005 through 29 July 2005). The water in each experimental bucket was changed completely once a week to remove metabolic waste. Mussels were fed a shellfish diet (Instant Algae Marine Microalgae Concentrate, Reed Mariculture, Inc.) twice daily (total of 8 × 10¹⁰ cells/day) of diluted algal paste made from water with from which they grew (identical isotopic composition). Water samples (60 mL) from each bucket were collected weekly to

monitor $\delta^{18}O_w$ prior to water changes, although there was no isotopic difference noted when water was collected prior to and after water changes. Water samples were stored in glass vials and refrigerated prior to $\delta^{18}O_w$ analysis.

2.4. Growth Rate Determination

To accurately determine the start of experimental shell growth, juvenile $M. edulis$ from Greenland were treated with a biomarker where animals were immersed in a calcein solution (160 mg/L) for 24 hours (modified from Kaehler and McQuaid [1999]). In addition, the shell length for each animal was determined with digital calibers (±0.01 mm) by measuring along the maximum growth axis, and averaged to determine a bulk shell length per temperature/salinity condition. The average initial shell length (based on 720 individual animals) was 15.88 mm ± 1.54 mm. This was repeated monthly to determine average bulk linear growth rates (mm/month).

2.5. Sample Preparation and Analysis

The $\delta^{18}O_w$ weekly water samples were measured via a dual-inlet VG/Micromass SIRA (CO$_2$–H$_2$O equilibration method at 30°C for 12 hours), with a precision of ±0.06% based on replicate International Atomic Energy Agency (IAEA) laboratory standards (Vienna Standard Mean Ocean Water (VSMOW) (0.0%), Standard Light Antarctic Precipitation (SLAP) (-55.5%), IAEA OH-1 (-0.1%), IAEA

![Figure 2. Schematic diagram of the experimental design. Each temperature condition is shown vertically, and each salinity condition is shown horizontally. Black indicates inflow, and gray indicates outflow. Buckets A and B are replicates. Buckets to the right of A and B are for water changes. All buckets are in a freshwater bath to maintain desired temperature setting to within ±0.5°C.](image)

<table>
<thead>
<tr>
<th>Temperature (±0.5°C)</th>
<th>Salinity (±0.1 PSU)</th>
<th>Growing Conditions: Greenland Juvenile Aquaculture</th>
<th>Growing Conditions: Greenland and Maine Adults Aquaculture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N = 22, Bucket A 1σ $\delta^{18}O_w$</td>
<td>N = 22, Bucket B 1σ $\delta^{18}O_w$</td>
</tr>
<tr>
<td>4.0 (0.2)</td>
<td>23</td>
<td>-3.12 (0.14)</td>
<td>-3.10 (0.15)</td>
</tr>
<tr>
<td>4.0 (0.2)</td>
<td>28</td>
<td>-1.94 (0.12)</td>
<td>-1.93 (0.11)</td>
</tr>
<tr>
<td>4.0 (0.2)</td>
<td>32</td>
<td>-1.40 (0.12)</td>
<td>-1.35 (0.12)</td>
</tr>
<tr>
<td>8.0 (0.2)</td>
<td>23</td>
<td>-3.07 (0.14)</td>
<td>-3.12 (0.15)</td>
</tr>
<tr>
<td>8.0 (0.2)</td>
<td>28</td>
<td>-1.93 (0.12)</td>
<td>-1.96 (0.11)</td>
</tr>
<tr>
<td>8.0 (0.2)</td>
<td>32</td>
<td>-1.37 (0.10)</td>
<td>-1.36 (0.12)</td>
</tr>
<tr>
<td>11.9 (0.3)</td>
<td>23</td>
<td>-3.10 (0.11)</td>
<td>-3.13 (0.12)</td>
</tr>
<tr>
<td>11.9 (0.3)</td>
<td>28</td>
<td>-2.03 (0.13)</td>
<td>-1.97 (0.11)</td>
</tr>
<tr>
<td>11.9 (0.3)</td>
<td>32</td>
<td>-1.39 (0.09)</td>
<td>-1.41 (0.09)</td>
</tr>
<tr>
<td>15.2 (0.4)</td>
<td>23</td>
<td>-3.09 (0.12)</td>
<td>-3.17 (0.16)</td>
</tr>
<tr>
<td>15.2 (0.4)</td>
<td>28</td>
<td>-1.95 (0.13)</td>
<td>-1.97 (0.14)</td>
</tr>
<tr>
<td>15.2 (0.4)</td>
<td>32</td>
<td>-1.37 (0.12)</td>
<td>-1.34 (0.10)</td>
</tr>
</tbody>
</table>

| 4.1 (0.3)           | 23                  | -3.10 (0.13) | -3.12 (0.16) | -3.02 (0.13) |
| 4.1 (0.3)           | 28                  | -1.94 (0.11) | -1.93 (0.11) | -1.94 (0.13) |
| 4.1 (0.3)           | 32                  | -1.42 (0.13) | -1.43 (0.13) | -1.43 (0.13) |
| 8.0 (0.2)           | 23                  | -3.02 (0.13) | -3.02 (0.13) | -3.02 (0.13) |
| 8.0 (0.2)           | 28                  | -1.98 (0.13) | -1.99 (0.13) | -1.99 (0.13) |
| 8.0 (0.2)           | 32                  | -1.57 (0.09) | -1.57 (0.09) | -1.57 (0.09) |
| 11.5 (0.2)          | 23                  | -3.05 (0.16) | -3.05 (0.16) | -3.05 (0.16) |
| 11.5 (0.2)          | 28                  | -1.95 (0.09) | -1.95 (0.09) | -1.95 (0.09) |
| 11.5 (0.2)          | 32                  | -1.58 (0.09) | -1.58 (0.09) | -1.58 (0.09) |
| 15.0 (0.4)          | 23                  | -3.02 (0.09) | -3.02 (0.09) | -3.02 (0.09) |
| 15.0 (0.4)          | 28                  | -1.89 (0.13) | -1.89 (0.13) | -1.89 (0.13) |
| 15.0 (0.4)          | 32                  | -1.54 (0.09) | -1.54 (0.09) | -1.54 (0.09) |

$^a$N is number of weeks that weekly samples were collected and analyzed for $\delta^{18}O_w$ with respect to Vienna standard mean ocean water (VSMOW). $^b$All values given in parentheses are 1σ.
be made to each of their data sets, because they report the 
\( \delta^{18}O_c - \delta^{18}O_w \) versus [PDB] and \( \delta^{18}O_c - \delta^{18}O_w \) versus [SMOW] respectively. In addition, Kim and O’Neil [1997] use an acid fractionation factor of 1.01050 whereas other studies used 1.01025. The modified equations are shown below for Epstein et al. [1953] (equation (1)) and Kim and O’Neil [1997] (equation (2)) respectively reported by Wanamaker et al. [2006]:

\[
\begin{align*}
T^\circ C & = 15.51(\pm 0.48) - 4.25(\pm 0.31) \\
& \cdot \left[ \frac{\delta^{18}O_c \text{VPBD} - \delta^{18}O_w \text{VSMOW}}{1000} \right] + 0.14(\pm 0.21) \\
& \cdot \left[ \frac{\delta^{18}O_c \text{VPBD} - \delta^{18}O_w \text{VSMOW}}{1000} \right]^2; \\
& - r^2 = 0.98; \text{RMSE} \pm 0.79^\circ C \quad (1)
\end{align*}
\]

\[
\begin{align*}
T^\circ C & = 15.07(\pm 0.86) - 4.60(\pm 0.59) \\
& \cdot \left[ \frac{\delta^{18}O_c \text{VPBD} - \delta^{18}O_w \text{VSMOW}}{1000} \right] + 0.09(\pm 0.13) \\
& \cdot \left[ \frac{\delta^{18}O_c \text{VPBD} - \delta^{18}O_w \text{VSMOW}}{1000} \right]^2; \\
& - r^2 = 0.99; \text{RMSE} \pm 0.72^\circ C. \quad (2)
\end{align*}
\]

In order to our compare results to the paleotemperature relationship for juvenile M. edulis (Maine) cultured from 7° to 19°C [Wanamaker et al., 2006], the equation is listed below:

\[
\begin{align*}
T^\circ C & = 16.19(\pm 0.14) - 4.69(\pm 0.21) \\
& \cdot \left[ \frac{\delta^{18}O_c \text{VPBD} - \delta^{18}O_w \text{VSMOW}}{1000} \right] + 0.17(\pm 0.13) \\
& \cdot \left[ \frac{\delta^{18}O_c \text{VPBD} - \delta^{18}O_w \text{VSMOW}}{1000} \right]^2; \\
& - r^2 = 0.99; \text{RMSE} \pm 0.54^\circ C. \quad (3)
\end{align*}
\]

3. Results and Discussion

3.1. Size and Shell Growth Rate Effects on \( \delta^{18}O_c \)

[19] We found no evidence for a significant relationship between temperature offset (based on predicted temperatures of measured \( \delta^{18}O_{calcite} - \delta^{18}O_{water} \) from Wanamaker et al. [2006] (equation (3)) and actual culture temperatures) and either shell length or shell growth in this study. The shell length of mussels used in the experiments spanned some 60 mm and included adults and juveniles. Bulk growth measures for juveniles (Greenland) for each temperature/salinity treatment were used, because biomarking with calcein was ineffective for M. edulis during this experiment. Although we viewed the shell material with a blue light, the lack of a distinct fluorescence mark is puzzling, as other workers have been successful using this method on M. edulis [e.g., Gillikin et al., 2006a]. Growth rates were dissimilar between adults and juveniles (Figure 3, bottom). As expected, juveniles grew faster than adults. Shell growth was low relative to other studies and may be due to a culture effect (food, confined growing environment, etc.). However, we fed mussels algal paste...
instead of live cultures to reduce \( \delta^{18}O \) variability. Live cultures could have added larger quantities of seawater with very different isotopic composition. Across the growth rates observed in the experiments there was no apparent relation-ship between temperature offset and shell growth. This result signifies that there are no growth related vital effects on \( \delta^{18}O_c \).

### 3.2. Possible Physiologic or Genetic Effects on \( \delta^{18}O_c \) Based on Collection Location

There is no evidence of a physiologic or genetic isotopic effect on \( \delta^{18}O_c \) based on \textit{M. edulis} specimens collected in Greenland or Maine (Figure 3). Although mussels collected in Maine and Greenland were grown under identical conditions (Gulf of Maine), we are confident that mussels collected from Greenland deposited their shells isotopically (\( \delta^{18}O_c \)) identical (within analytical uncertainties) to animals collected from Maine. An aquaculture experiment based on Greenland conditions would include an isotopically depleted freshwater end-member (\( \delta^{18}O_w = \sim -25 \)) VSMOW at 0 PSU) (A. D. Wanamaker, unpublished data, 2006) compared to the conditions we used in the Gulf of Maine (\( \delta^{18}O_w = \sim -8.6 \) VSMOW at 0 PSU) [Wanamaker et al., 2006]. It is likely that if mussels were also cultured under Greenland conditions, similar results would be obtained. Because mussels from Maine and Greenland did not illustrate isotope effects based on physiology or slight genetic variations between the two populations, \textit{M. edulis} is a highly suitable bivalve for paleoceano-graphic studies. This result indicates that it is appropriate to apply paleotemperature relationships for this species over wide geographic locations.

### 3.3. Paleotemperature Relationship for \textit{M. edulis}

An updated paleotemperature relationship for \textit{M. edulis} (juveniles and adults) collected from different climate and environmental regimes (Maine and Greenland) was derived from this study and from previous work [Wanamaker et al., 2006], thereby including animals.
cultured from 4°C to 19°C, including three salinity conditions (23, 28, and 32 ± 0.1 PSU):

\[ T°C = 16.28(±0.10) - 4.57(±0.15) \cdot [\delta^{18}O_{VPBD} - \delta^{18}O_{w, VSMOW}] + 0.06(±0.06) \cdot [\delta^{18}O_{VPBD} - \delta^{18}O_{w, VSMOW}]^2; \]
\[ r^2 = 0.99; N = 323; p < 0.0001; \text{RMSE} \pm 0.57°C. \] (4)

We also used bivariate least squares regression (model II) because there is an error associated with both the x and y measurements in equation (4). This method yielded a statistically identical relationship compared to equation (4) in the following form:

\[ T°C = 16.33 - 4.48[\delta^{18}O_{VPBD} - \delta^{18}O_{w, VSMOW}]. \] (5)

Additionally we derived the fractionation factor \( \alpha_{\text{calcite-water}} \) ([1000 + \( \delta^{18}O_c \)]/[1000 + \( \delta^{18}O_w \)]) (relative to VSMOW; \( T \) is in Kelvin) for the M. edulis animals sampled above:

\[ 1000 \ln \alpha_{\text{calcite-water}} = 18.02(10^3 T^{-1}) - 31.84. \] (6)

Our results (equation (4)) are compared to Kim and O’Neil [1997] (equation (2)) and Epstein et al. [1953] (equation (1)) in Figure 4. The updated paleotemperature relationship for M. edulis (equation (4)) is slightly offset (less than 0.2%) relative to Kim and O’Neil [1997] (equation (2)) over the entire temperature range, however this isotopic offset is not statistically significant (within analytical errors). On the basis of a comparison with the abiogenic calcite equation of Kim and O’Neil [1997] (equation (2)) and the uncertainties associated with this study and the Kim and O’Neil [1997] study, M. edulis (both juveniles and adults, and from Maine and Greenland) precipitated its shell calcite in isotopic equilibrium with ambient water. The animals cultured below ~17°C from the Epstein et al. [1953] (equation (1)) experiment agree rather well with our derived calcite equation for M. edulis (Figure 4). Above that temperature (~17°C), only about half of the data from Epstein et al. [1953] (equation (1)) fall within the experimental errors (~±0.5°C) of this study. Because this study included many more bivalves cultured at each temperature compared to the pioneering work of Epstein et al. [1953], we are more confident with this paleotemperature relationship than those previously published [e.g., Epstein et al., 1953; Craig, 1965].

[23] There were no noticeable trends or deviations in shell isotopic variability (\( \delta^{18}O_c \) \( \text{VPBD} - \delta^{18}O_w \) VSMOW) based on an animal’s size (shell length), growth rates, or geographic origin (Figures 3 and 4). Further, the variance in isotopic composition is relatively constant across temperatures (±0.11%–0.14%), and is roughly equal to the combined analytical errors associated with measuring \( \delta^{18}O_c \) and \( \delta^{18}O_w \) of ±0.12% [Miller and Miller, 1993]. The isotopic variability noted here (\( \delta^{18}O_c \) \( \text{VPBD} - \delta^{18}O_w \) VSMOW) is less than or about equal to similar experimental studies that grew multiple bivalves at one temperature [e.g., Epstein et al., 1953; Owen et al., 2002a, 2002b; Wanamaker et al., 2006].

[24] On the basis of these results, M. edulis bivalves are an accurate and reliable proxy for water temperature if \( \delta^{18}O_w \) can be determined independently. M. edulis deposit their shell (\( \delta^{18}O_c \)) in isotope equilibrium with seawater, and do not show any apparent vital effect based on age/size, growth rates, or geographic origin (physiologic or genetic effect). Because M. edulis deposits distinct growth bands
Richardson is noted (GR = 0.01* isotope fractionation, but by d from this d from adult and = 0.19; N = M. edulis d are relatively weak, but significant (y M. edulis = 0.44*x + 2.24; r correlation between biogenic d[2006a] on the d used to mark the middle arctic/ d from Greenland (circles). and Cohen and Vander Putten as a ''guide'' fossil or d and d WANAMAKER ET AL.: SHELL ISOTOPE CHEMISTRY OF d[2000] and d[1989] high-resolution (sub- d= = 0.05; p < 0.03 Donner and Nord Craig and d= 0.35; N = 97; p < 0.0001 (Maine and d[2006a] and d[1965] and d may represent d[2006a] (including low deposits its shell d[2005]. Both d values. d is not found at the d through the Holocene make this bivalve an ideal d for d[1989b] and the ''carbonate'' model A comparison of shell d18Oc and d13Ct from this study is shown in Figure 5. The linear correlations between d18Oc and d13Ct are relatively weak, but significant (y1 = 0.47*x + 2.01; r2 = 0.35; N = 97; p < 0.0001 (Maine and Greenland Adults) and y2 = 0.44*x + 2.24; r2 = 0.19; N = 121; p < 0.0001 (Greenland Juveniles)) (Figure 5). A strong correlation between biogenic d18Oc and d13Ct may represent a vital effect during biominalization [e.g., McConnaughey, 1989a]. The two leading hypotheses that attempt to explain this effect are the “kinetic” model [McConnaughey, 1989a, 1989b] and the “carbonate” model [Adkins et al., 2003] both of which are reviewed in detail by Cohen and McConnaughey [2003] and Shanahan et al. [2005]. Both the “kinetic” and “carbonate” models link calcification rates to coupled d18O and d13C isotope fractionation, but by different processes in the extracellular calcifying fluid (ECF) [see Shanahan et al., 2005]. Only a weak correlation (but significant at 95% C.I.) between growth rates (GR) and d13Ct is noted (GR = 0.01*d13Ct + 0.01; r2 = 0.05; p < 0.03 (adults) and GR = 0.04*d13Ct + 0.28; r2 = 0.07; p < 0.004 (juveniles)), and no correlation exists between growth rates and temperature offset (derived from d18O) (Figure 3). Because our data indicate that M. edulis deposits its shell in oxygen isotope equilibrium with respect to ambient water (Figure 4), these proposed “vital effect” models [McConnaughey, 1989a, 1989b; Adkins et al., 2003] do not appear to be valid for this bivalve. Further, Gillikin et al. [2006b] suggested that coupled isotope (d18O and d13C) disequilibrium fractionation does not impact bivalves,
It has been shown that many biogenic organisms do not deposit their skeletons ($\delta^{13}C$) in equilibrium with DIC [e.g., Wefer and Berger, 1991]. In order to determine if $M$. edulis deposits its shell in carbon isotope equilibrium with ambient water, we used a $\delta^{13}C$ DIC/salinity mixing line from seawater collected at the Darling Marine Center during the summer of 2006 ($\delta^{13}C$‰ DIC = $-19.5 \pm 0.62$‰ salinity; N = 24; $r^2 = 0.99$; $p < 0.0001$ (E. F. Owen et al., unpublished data, 2006)) to estimate our $\delta^{13}C$ DIC values. We realize that the estimated $\delta^{13}C$ DIC values reported here are poorly constrained because we did not monitor $\delta^{13}C$ DIC continuously throughout the experiment, and that metabolic and atmospheric derived CO$_2$, and freshwater input can make $\delta^{13}C$ DIC values more negative. However, we can rule out significant changes in salinity and/or freshwater input based on the stability of weekly $\delta^{13}O_w$ measurements (Table 1). Further, periodic (~1 per week) measurements of water pH showed that the water used during culture experiments was highly buffered with an average pH of 8.0 ± 0.1 for all salinity treatments. Therefore we believe our data remain useful. The relationship between $\delta^{13}C$ and the DIC of water used during the culture period ($\delta^{18}O_w$) is depicted in Figure 6. The predicted equilibrium values for shell $\delta^{13}C$ are $\delta^{13}C$‰ DIC = 1 ± 0.2‰ [Romanek et al., 1992] for newly formed calcite derived solely from DIC (Figure 6). On the basis of the data in Figure 6, it appears that $M$. edulis did not deposit its shell in carbon isotope equilibrium with respect to DIC during this culture period. The mean $\delta^{13}C$ values are $-4.73\%$ (1σ = 1.13‰; N = 36), $-6.21\%$ (1σ = 1.38‰; N = 61), and $-6.77\%$ (1σ = 1.25‰; N = 121) for adults (Maine), adults (Greenland), and juveniles (Greenland) respectively. On average $M$. edulis $\delta^{13}C$ values are in disequilibrium by $-4.61\%$ (1σ = 2.57‰; N = 218) with respect to ambient DIC (Figure 6). The degree of carbon disequilibrium (deviation from $\delta^{13}C$‰ DIC = 1 [Romanek et al., 1992]) increases with increasing $\delta^{18}O_w$ values (related to salinity) (mean shell carbon disequilibrium values are $-1.96\%$ (23 PSU), $-5.43\%$ (28 PSU) and $-7.06\%$ (32 PSU)) (Figure 7). Nearly 70% of the carbon isotope disequilibrium (corrected for ambient DIC) is explained by $\delta^{18}O_w$ values ($r^2 = 0.69$; N = 218; $p < 0.0001$) (Figure 7). The increased carbon isotope disequilibrium with increasing salinity may be caused by the activity of the enzyme carbonic anhydrase (CA), which catalyzes the reaction between the bicarbonate phase to CO$_2$, and facilitates the diffusion of DIC into the calcifying fluid [e.g., Paneth and O’Leary, 1985]. CA activity has been linked to the concentration of ambient Cl$^-$ ions, which inhibit the activity of this enzyme [e.g., Pocker and Tanaka, 1978]. Therefore at increased salinities (elevated [Cl$^-$]) a reduction in CA activity may cause a reduction in ambient DIC that is incorporated into the biogenic carbonate, and an increased amount of metabolic carbon may be utilized during biomineralization, which will deplete the calcifying fluid with respect to the DIC pool [e.g., Gillikin et al., 2006b]. Because metabolic carbon originates from ingested food it typically has lower $\delta^{13}C$ values than DIC [DeNiro and Epstein, 1978]. The food used during this study primarily was composed of marine phytoplankton, and the $\delta^{13}C$ values were $-35.23 \pm 0.18\%$ (N = 2) thus the food has likely influenced the overall shell $\delta^{13}C$ values. Using end-member carbon reservoir conditions (food $\delta^{13}C = -35.23\%$ and ambient $\delta^{13}C$ DIC values), mean shell $\delta^{13}C$ values ($-5.85\%$ at DIC = 0.21‰, $-6.68\%$ at DIC = -2.25‰, $-6.29\%$ at DIC = -5.33‰) (Figure 6), and assuming that $\delta^{13}C$ food values approximate $\delta^{13}C$ tissue values [Gillikin et al., 2006b], it is estimated that $M$. edulis incorporated ~7% metabolic carbon at 23 PSU, ~16% metabolic carbon at
DIC is continuously monitored. This would be limited by disequilibrium, where values are not generally suitable for environmental reconstructions (paleo-DIC or paleo-pCO$_2$). However, in a low-salinity environment (e.g., upper estuary) DIC values from M. edulis may be suitable to reconstruct paleo-DIC conditions, because DIC values approach equilibrium with respect to ambient DIC (Figure 7). In order for this methodology ($\delta^{13}$C as a paleoindicator) to be valid and reliable on biogenic carbonates, it is crucial to develop a better understanding of the mechanisms responsible for the partitioning of $\delta^{13}$C during biomineralization. Certainly, the applicability and reliability of $\delta^{13}$C from bivalves (and other biogenic carbonates) as paleoindicators could be improved with similar isotope calibration work presented here, especially if $\delta^{13}$C DIC is continuously monitored. It is likely that many biogenic organisms precipitate their shells in equilibrium with ambient DIC, but this cannot be readily assumed. Further isotope calibration work coupled with trace metal studies have the potential for unlocking promising biogenic archives that do in fact record important paleoenvironmental conditions.

4. Conclusions

In this study, we show that M. edulis deposits its shell in isotopic equilibrium with ambient water with respect to oxygen. We have developed a rigorous paleotemperature relationship for M. edulis including juveniles and adults from Maine and Greenland grown from 4°–19°C in three salinity conditions (23, 28, and 32 ± 0.1 PSU). The error associated with this paleothermometer at the 95% C.I. is only ±0.57°C when $\delta^{18}$O$_w$ values can be determined independently. We have demonstrated that the shell chemistry of M. edulis ($\delta^{18}$O$_w$) is not influenced by growth rates, the size of the animal (related to age), or where the animal was collected. The broad modern and paleogeographic distribution of this bivalve, its abundance during the Holocene, and the lack of intraspecies geographic variability in isotope fractionation demonstrated here, makes it an ideal nearshore paleoceanographic proxy throughout much of the North Atlantic Ocean. On the basis of the $\delta^{18}$O$_w$ DIC/salinity mixing line used in this study, we have verified that M. edulis incorporates a significant portion (7–20%) of metabolic carbon into its shell, and determined that carbon
isotope disequilibrium increased with increasing salinity during the culture period. Thus δ13C values of the bivalve *M. edulis* are not generally suitable for reconstructing paleo-DIC or paleo-pCO2. However, it may be possible to use δ13C values from *M. edulis* in low-salinity environments to reconstruct paleo-DIC where δ13C values of shell material is nearly in carbon isotope equilibrium with ambient DIC, however this needs to be further investigated.

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