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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

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[1] To 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* [$T\ (^{\circ}\text{C}) = 16.28 (\pm 0.10) - 4.57 (\pm 0.15) \{ \delta^{18}\text{O}_c \text{ VPBD} - \delta^{18}\text{O}_w \text{ VSMOW} \} + 0.06 (\pm 0.06) \{ \delta^{18}\text{O}_c \text{ VPBD} - \delta^{18}\text{O}_w \text{ VSMOW} \}^2$; $r^2 = 0.99$; $N = 323$; $p < 0.0001$]. Compared to the Kim and O'Neil (1997) inorganic calcite equation, *M. edulis* deposits its shell in isotope equilibrium ($\delta^{18}\text{O}_{\text{calcite}}$) with ambient water. Carbon isotopes ($\delta^{13}\text{C}_{\text{calcite}}$) from sampled shells were substantially more negative than predicted values, indicating an uptake of metabolic carbon into shell carbonate, and $\delta^{13}\text{C}_{\text{calcite}}$ disequilibrium increased with increasing salinity. Sampled shells of *M. edulis* showed no significant trends in $\delta^{18}\text{O}_{\text{calcite}}$ based on size, cultured growth rates, or geographic collection location, suggesting that vital effects do not affect $\delta^{18}\text{O}_{\text{calcite}}$ 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.

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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 ($\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$) in biogenic carbonates may be affected differently by environmental conditions. The oxygen isotopic chemistry of biogenic carbonates ($\delta^{18}\text{O}_c$) is primarily controlled by water temperature and the isotopic composition of water ($\delta^{18}\text{O}_w$; 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 ($\delta^{13}\text{C}_c$) during shell precipitation may be related to dissolved inorganic carbon (DIC) [Mook

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and Vogel, 1968; Mook, 1971; Killingley 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; Furla et al., 2000; Lorrain et al., 2004; Gillikin et al., 2006b]. Thus $\delta^{13}\text{C}_c$ profiles from biogenic carbonates have the potential to be indicators of paleoproductivity, paleo-DIC, paleo- pCO_2 , or paleoecology [e.g., Shanahan et al., 2005], while $\delta^{18}\text{O}_c$ profiles have the potential to reflect basic hydrographic conditions (temperature and salinity). The interpretation of $\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$ 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 (CaCO_3) in isotopic equilibrium with ambient water, and because most CaCO_3 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 ($\sim 7\text{--}30^\circ\text{C}$) [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 ($\alpha = 1.01050$) 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 employed 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 isotope 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 paleoceanographic 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 $\sim 20\text{--}35$ PSU (practical salinity units) and a temperature range of $10\text{--}20^\circ\text{C}$ [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

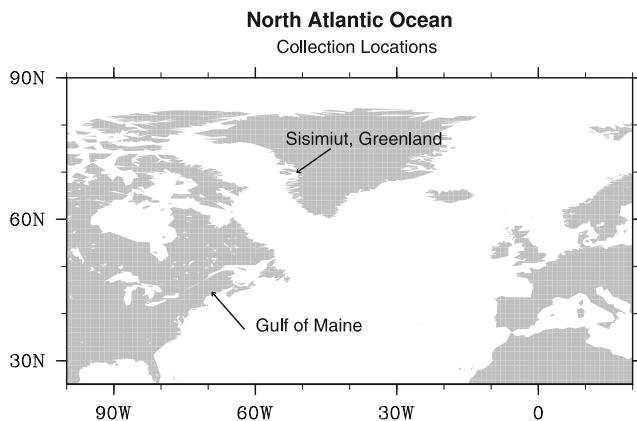


Figure 1. Location of *M. edulis* samples collected in Maine and western Greenland.

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

[8] Approximately 1,000 adult and juvenile sized *M. edulis* were collected alive in western Greenland (Sisimiut; 66°55′59.9″N, 53°40′59.9″W) during July 2004 and transported in moist storage containers to the Darling Marine Center in Walpole, Maine. (Auxiliary material provides additional associated data.¹) 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.

[9] 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

[10] 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).

[11] 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.

[12] Thirty juvenile blue mussels (shell length 14–24 mm) from Greenland were placed in each 20-L temperature/salinity environment (N_{tot} = 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 × 10⁹ 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

¹Auxiliary materials are available at <ftp://ftp.agu.org/apend/pa/2006pa001352>.

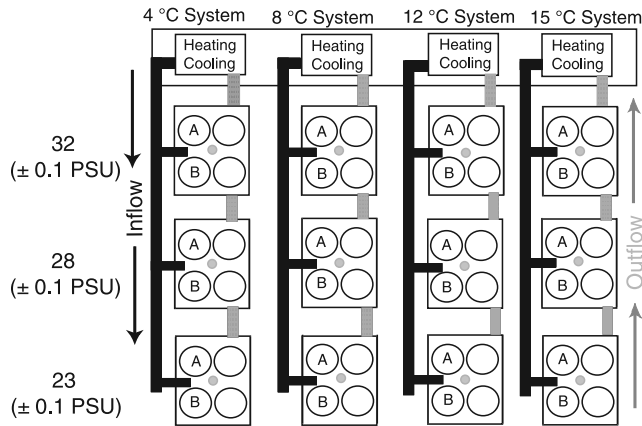


Figure 2. Schematic diagram of the experimental design. Each temperature condition is shown vertically, and each salinity condition is shown horizontally. Black indicates inflow, and gray indicates outflow. Buckets A and B are replicates. Buckets to the right of A and B are for water changes. All buckets are in a freshwater bath to maintain desired temperature setting to within $\pm 0.5^\circ\text{C}$.

monitor $\delta^{18}\text{O}_w$ 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 $\delta^{18}\text{O}_w$ analysis.

2.4. Growth Rate Determination

[13] 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 calipers (± 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 $15.88 \text{ mm} \pm 1.54$ mm. This was repeated monthly to determine average bulk linear growth rates (mm/month).

[14] Similarly, adult *M. edulis* from Maine and Greenland (shell length 26–70 mm) were immersed in a calcein solution to mark the onset of experimental growth, and each individual animal was marked by etching a number in its shell near the umbo. Individual monthly growth rates were determined by measuring starting and ending shell lengths.

2.5. Sample Preparation and Analysis

[15] The $\delta^{18}\text{O}_w$ weekly water samples were measured via a dual-inlet VG/Micromass SIRA ($\text{CO}_2\text{-H}_2\text{O}$ equilibration method at 30°C for 12 hours), with a precision of $\pm 0.06\text{‰}$ 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

Table 1. Culture Data for This Study Including Temperature, Salinity, and Oxygen Isotopic Composition of Water^a

Temperature ($\pm 0.5^\circ\text{C}$) ^b	Salinity (± 0.1 PSU)	Average $\delta^{18}\text{O}_w$, ‰		
		N = 22, Bucket A 1σ $\delta^{18}\text{O}_w$	N = 22, Bucket B 1σ $\delta^{18}\text{O}_w$	N = 23, Bucket A 1σ $\delta^{18}\text{O}_w$
<i>Growing Conditions: Greenland Juvenile Aquaculture</i>				
4.0 (0.2)	23	-3.12 (0.14)	-3.10 (0.15)	
4.0 (0.2)	28	-1.94 (0.12)	-1.93 (0.11)	
4.0 (0.2)	32	-1.40 (0.12)	-1.35 (0.12)	
8.0 (0.2)	23	-3.07 (0.14)	-3.12 (0.15)	
8.0 (0.2)	28	-1.93 (0.12)	-1.96 (0.11)	
8.0 (0.2)	32	-1.37 (0.10)	-1.36 (0.12)	
11.9 (0.3)	23	-3.10 (0.11)	-3.13 (0.12)	
11.9 (0.3)	28	-2.03 (0.13)	-1.97 (0.11)	
11.9 (0.3)	32	-1.39 (0.09)	-1.41 (0.09)	
15.2 (0.4)	23	-3.09 (0.12)	-3.17 (0.16)	
15.2 (0.4)	28	-1.95 (0.13)	-1.97 (0.14)	
15.2 (0.4)	32	-1.37 (0.12)	-1.34 (0.10)	
<i>Growing Conditions: Greenland and Maine Adults Aquaculture</i>				
4.1 (0.3)	23			-3.10 (0.13)
4.1 (0.3)	28			-1.94 (0.11)
4.1 (0.3)	32			-1.42 (0.13)
8.0 (0.2)	23			-3.02 (0.13)
8.0 (0.2)	28			-1.98 (0.13)
8.0 (0.2)	32			-1.57 (0.09)
11.5 (0.2)	23			-3.05 (0.16)
11.5 (0.2)	28			-1.95 (0.09)
11.5 (0.2)	32			-1.58 (0.09)
15.0 (0.4)	23			-3.02 (0.09)
15.0 (0.4)	28			-1.89 (0.13)
15.0 (0.4)	32			-1.54 (0.09)

^aN is number of weeks that weekly samples were collected and analyzed for $\delta^{18}\text{O}_w$, with respect to Vienna standard mean ocean water (VSMOW).

^bAll values given in parentheses are 1σ .

OH-2 (−3.3‰), IAEA OH-3 (−8.7‰), IAEA OH-4 (−15.3‰), and internal laboratory standards Big Bear Brook (BBB) (−8.5‰), Light Antarctic Precipitation (LAP) (−40.3‰), Antarctic Surface Snow (ASS) (−25.8‰), where one standard was run for every five samples. All $\delta^{18}\text{O}_w$ (δ in ‰ = $[(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000$; $[R = ^{18}\text{O}/^{16}\text{O}]$) values are reported with respect to VSMOW. Weekly $\delta^{18}\text{O}_w$ from each temperature/salinity environments were averaged over the growing intervals, and used in the isotope calibration (Table 1).

[16] To measure shell $\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$ the animals were first cleaned (removed soft tissues) and air dried. Shell samples were further oven dried at 40°C overnight. The periostracum was removed with a razor blade along the ventral margin. Prior to sampling, the shell length of each animal was recorded. The outer edge of each valve was micromilled using a variable speed mounted drill and binocular microscope with 6.5 × to 40 × magnification. Next, the sample size (linear shell removed) was determined by measuring each animal's shell length after sampling and subtracting it from the initial measurement. Shell carbonate analysis ($\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$) was performed on a dual-inlet VG/Micromass Prism, via a 30-place carousel and common acid bath without chromium oxide (CrO_3) at 90°C, with precision of $\pm 0.10\text{‰}$ ($\delta^{18}\text{O}_c$) and $\pm 0.07\text{‰}$ ($\delta^{13}\text{C}_c$) based on laboratory standards. Results are reported relative to Vienna Pee Dee Belemnite (VPBD) by calibration to the NBS-19 reference standard ($\delta^{18}\text{O}_c = -2.20\text{‰}$ and $\delta^{13}\text{C}_c = 1.95\text{‰}$) at the beginning and end of each run, with a standard to sample ratio of 1:3. Average shell samples weighed approximately 100 μg .

[17] To determine the isotopic composition ($\delta^{13}\text{C}$) of the food (algal paste) used in this study, we dried 30 mL of paste at 50°C for 24 hours. The dried samples were weighed into tin capsules. The dried samples were flash combusted at 1800°C in an elemental analyzer (EA) [EA 1110 (CE Instruments) + Conflo III + DeltaPlus Advantage IRMS (ThermoFinnigan)] and resulting gases were carried via helium through the EA to purify and separate into N_2 and CO_2 . Gases were carried from the EA into an isotope ratio mass spectrometer (IRMS) for isotope analysis via a Conflo interface. On the basis of replicate standard analyses, the analytical precision (2 σ) is $\pm 0.2\text{‰}$. Results are reported relative to VPBD by calibration to the NBS-19 reference standard ($\delta^{18}\text{O}_c = -2.20\text{‰}$ and $\delta^{13}\text{C}_c = 1.95\text{‰}$).

2.6. Calibration of Temperature and $\delta^{18}\text{O}_c$ Relationships

[18] Least squares regression was used to investigate the relationship between culture conditions and shell carbonate isotope values and to develop a paleotemperature relationship for *M. edulis*. The root-mean-square error (RMSE) was calculated at the 95% confidence interval (C.I.), and quoted errors on the slope and intercepts are reported at the 95% C.I. Our shell data ($\delta^{18}\text{O}_c$) are reported against the international VPBD scale and our water data ($\delta^{18}\text{O}_w$) are reported against the international VSMOW scale, which minimizes approximations and multiple corrections. However, in order to compare our results to the *Epstein et al.* [1953] and the *Kim and O'Neil* [1997] calcite equations, corrections had to

be made to each of their data sets, because they report the $\delta^{18}\text{O}_c - \delta^{18}\text{O}_w$ versus [PDB] and $\delta^{18}\text{O}_c - \delta^{18}\text{O}_w$ 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]:

$$\begin{aligned} T^{\circ}\text{C} &= 15.51(\pm 0.48) - 4.25(\pm 0.31) \\ &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}] + 0.14(\pm 0.21) \\ &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}]^2; \\ &\cdot r^2 = 0.98; \text{RMSE} \pm 0.79^{\circ}\text{C} \end{aligned} \quad (1)$$

$$\begin{aligned} T^{\circ}\text{C} &= 15.07(\pm 0.86) - 4.60(\pm 0.59) \\ &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}] + 0.09(\pm 0.13) \\ &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}]^2; \\ &\cdot r^2 = 0.99; \text{RMSE} \pm 0.72^{\circ}\text{C}. \end{aligned} \quad (2)$$

In order to compare our 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:

$$\begin{aligned} T^{\circ}\text{C} &= 16.19(\pm 0.14) - 4.69(\pm 0.21) \\ &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}] + 0.17(\pm 0.13) \\ &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}]^2; \\ &\cdot r^2 = 0.99; \text{RMSE} \pm 0.54^{\circ}\text{C}. \end{aligned} \quad (3)$$

3. Results and Discussion

3.1. Size and Shell Growth Rate Effects on $\delta^{18}\text{O}_c$

[19] We found no evidence for a significant relationship between temperature offset (based on predicted temperatures of measured $\delta^{18}\text{O}_{\text{calcite}} - \delta^{18}\text{O}_{\text{water}}$ 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.

[20] 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

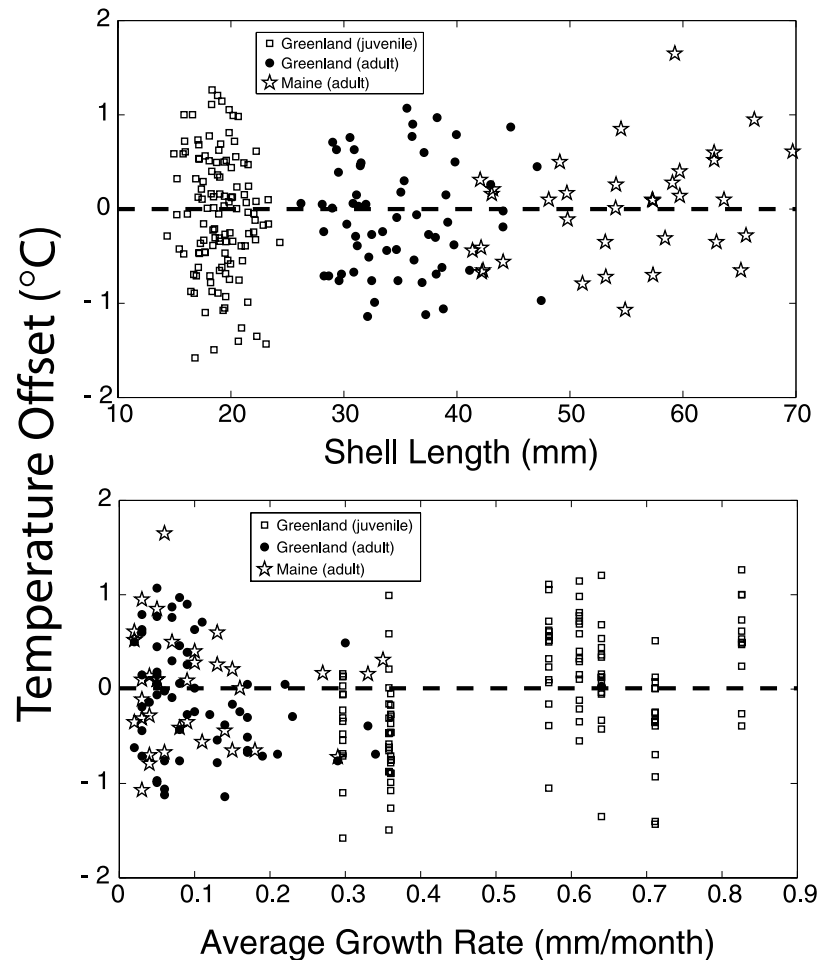


Figure 3. Temperature offset (based on predicted temperatures of measured $\delta^{18}\text{O}_{\text{calcite}} - \delta^{18}\text{O}_{\text{water}}$ from Wanamaker *et al.* [2006] (equation (3) in this paper) and actual culture temperatures) compared to (top) shell length and (bottom) average growth rates (individual for adults and bulk for juveniles). The dashed horizontal line represents a temperature offset of 0°C .

instead of live cultures to reduce $\delta^{18}\text{O}_w$ 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 relationship between temperature offset and shell growth. This result signifies that there are no growth related vital effects on $\delta^{18}\text{O}_c$.

3.2. Possible Physiologic or Genetic Effects on $\delta^{18}\text{O}_c$ Based on Collection Location

[21] There is no evidence of a physiologic or genetic isotopic effect on $\delta^{18}\text{O}_c$ based on *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 ($\delta^{18}\text{O}_c$) identical (within analytical uncertainties) to animals collected from Maine. An aquaculture experiment based on Greenland conditions would include an isotopically depleted freshwater end-member ($\delta^{18}\text{O}_w = \sim -25\text{‰}$

VSMOW at 0 PSU) (A. D. Wanamaker, unpublished data, 2006) compared to the conditions we used in the Gulf of Maine ($\delta^{18}\text{O}_w = -8.6\text{‰}$ 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, *M. edulis* is a highly suitable bivalve for paleoceanographic studies. This result indicates that it is appropriate to apply paleotemperature relationships for this species over wide geographic locations.

3.3. Paleotemperature Relationship for *M. edulis*

[22] An updated paleotemperature relationship for *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

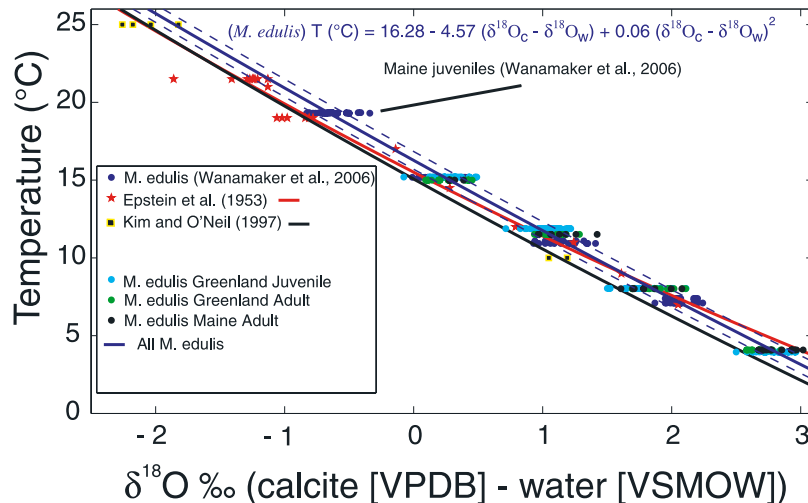


Figure 4. Paleotemperature relationships from this study (turquoise circles, Greenland juveniles; green circles, Greenland adults; black circles, Maine adults) (solid blue line is equation (4); dashed blue lines are upper and lower error of 0.57°C), Wanamaker et al. [2006] (blue circles; equation (3)), Epstein et al. [1953] (red stars and red line; equation (1)) and Kim and O'Neil [1997] (black and yellow squares and solid black line; equation (2)).

cultured from 4° to 19°C , including three salinity conditions (23, 28, and 32 ± 0.1 PSU):

$$\begin{aligned}
 T^{\circ}\text{C} &= 16.28(\pm 0.10) - 4.57(\pm 0.15) \\
 &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}] + 0.06(\pm 0.06) \\
 &\cdot [\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}]^2; \\
 &\cdot r^2 = 0.99; N = 323; p < 0.0001; \text{RMSE} \pm 0.57^{\circ}\text{C}.
 \end{aligned} \tag{4}$$

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:

$$T^{\circ}\text{C} = 16.33 - 4.48[\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}]. \tag{5}$$

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

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

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 $\sim 17^{\circ}\text{C}$ 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 ($\sim 17^{\circ}\text{C}$), only about half of the data from Epstein et al. [1953] (equation (1)) fall within the experimental errors ($\pm 0.57^{\circ}\text{C}$) 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 ($\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}$) 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 ($\pm 0.11\text{‰} - 0.14\text{‰}$), and is roughly equal to the combined analytical errors associated with measuring $\delta^{18}\text{O}_c$ and $\delta^{18}\text{O}_w$ of $\pm 0.12\text{‰}$ [Miller and Miller, 1993]. The isotopic variability noted here ($\delta^{18}\text{O}_c\text{VPBD} - \delta^{18}\text{O}_w\text{VSMOW}$) 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 $\delta^{18}\text{O}_w$ can be determined independently. *M. edulis* deposit their shell ($\delta^{18}\text{O}_c$) 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

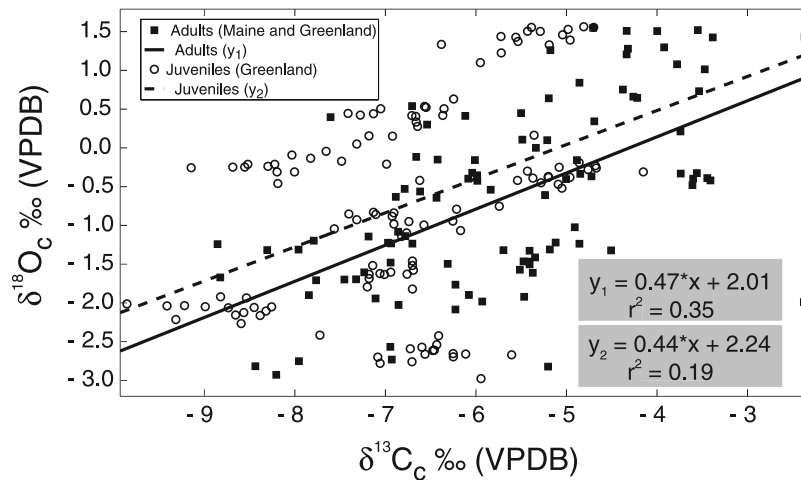


Figure 5. Linear correlations between biogenic $\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$ from adult *M. edulis* collected in Maine and Greenland (squares) and juvenile *M. edulis* from Greenland (circles).

[Lutz, 1976; Richardson, 1989] high-resolution (sub-monthly to annual) isotope records are possible, and the broad geographic distribution and the occurrence of *M. edulis* through the Holocene make this bivalve an ideal proxy of environmental conditions. Further, this study complements trace element work conducted by Vander Putten *et al.* [2000] and Gillikin *et al.* [2006a] on the calcitic shell layer of *M. edulis*, where environmental and biologic controls on shell chemistry were assessed. Although no evident trace metal ratio paleothermometer (Mg/Ca or Sr/Ca) resulted from the work of Vander Putten *et al.* [2000] for *M. edulis*, other trace metals (Ba and Mn) seem to be related to productivity. Gillikin *et al.* [2006a] found that Ba/Ca ratios in the calcitic shell material may be used to reconstruct paleo-Ba/Ca ratios of ambient water. Thus a multiproxy (isotopes and trace metals) approach to reconstructing past ocean environments is possible with *M. edulis*. Donner and Nord [1986] reported stable isotope results from *M. edulis* fossils from Holocene raised beaches in Norway, and presented paleotemperature estimates based on the calcite equations of Craig [1965] and Shackleton [1974] with estimated $\delta^{18}\text{O}_w$ values. Donner and Nord [1986] considered these paleotemperature estimates to be too high, and concluded that coastal mixing of less saline waters must be taken into account for the elevated temperature estimates. However, now that a comprehensive paleotemperature relationship for *M. edulis* (including low temperatures during culture [4°C]) has been established, this work can be reevaluated. Other studies have used the absence or presence of *M. edulis* as a “guide” fossil or climatic indicator to reveal general oceanic conditions (warm or cold) during periods of the Holocene [e.g., Hjort and Funder, 1974; Salvigsen *et al.*, 1992]. Also, the modern occurrence of *M. edulis* is used to mark the middle arctic/high arctic boundary, because *M. edulis* is not found at the 0°C isotherm [Feyling-Hanssen, 1955]. Now these relationships can be further refined with the results presented here for *M. edulis*, and new problems can be investigated using this calibrated proxy. For example, the abundance of fossil

M. edulis shells in circum N. Atlantic glaciomarine muds (e.g., Presumpscot Formation in Maine) from the most recent deglaciation and the lack of a geographic isotope effect will allow for improved paleoenvironmental constructions along the southeast Laurentide ice sheet, which is not well understood [Bornes *et al.*, 2004; H. W. Bornes, personal communication, 2006].

3.4. Relationships Between $\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$ and Metabolic Carbon Isotope Effects

[25] A comparison of shell $\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$ from this study is shown in Figure 5. The linear correlations between $\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$ are relatively weak, but significant ($y_1 = 0.47x + 2.01$; $r^2 = 0.35$; $N = 97$; $p < 0.0001$ (Maine and Greenland Adults) and $y_2 = 0.44x + 2.24$; $r^2 = 0.19$; $N = 121$; $p < 0.0001$ (Greenland Juveniles)) (Figure 5). A strong correlation between biogenic $\delta^{18}\text{O}_c$ and $\delta^{13}\text{C}_c$ may represent a vital effect during biomineralization [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 $\delta^{18}\text{O}$ and $\delta^{13}\text{C}_c$ 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 $\delta^{13}\text{C}_c$ is noted ($\text{GR} = 0.01*\delta^{13}\text{C}_c + 0.01$; $r^2 = 0.05$; $p < 0.03$ (adults) and $\text{GR} = 0.04*\delta^{13}\text{C}_c + 0.28$; $r^2 = 0.07$; $p < 0.004$ (juveniles)), and no correlation exists between growth rates and temperature offset (derived from $\delta^{18}\text{O}$) (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 ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) disequilibrium fractionation does not impact bivalves,

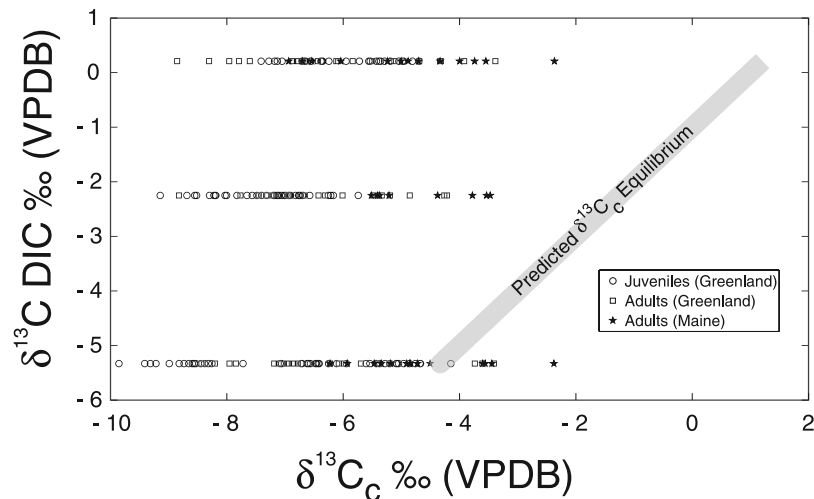


Figure 6. Relationship between estimated dissolved inorganic carbon ($\delta^{13}\text{C}$ DIC, related to salinity) during culture and shell $\delta^{13}\text{C}_c$. The gray area represents the predicted range of $\delta^{13}\text{C}_c$ values in carbon isotope equilibrium with DIC (equilibrium = $\delta^{13}\text{C}$ DIC ‰ + $1.0 \pm 0.2\%$ [from Romanek *et al.*, 1992]). DIC values are from E. F. Owen *et al.* (unpublished data, 2006).

because bivalves generally precipitate shell material in oxygen isotope equilibrium with ambient water [e.g., Epstein *et al.*, 1953; Chauvaud *et al.*, 2005].

[26] It has been shown that many biogenic organisms do not deposit their skeletons ($\delta^{13}\text{C}_c$) 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 $\delta^{13}\text{C}$ DIC/salinity mixing line from seawater collected at the Darling Marine Center during the summer of 2006 ($\delta^{13}\text{C}$ ‰ DIC = $-19.5 + 0.62 \cdot \text{salinity}$; $N = 24$; $r^2 = 0.99$; $p < 0.0001$ (E. F. Owen *et al.*, unpublished data, 2006)) to estimate our $\delta^{13}\text{C}$ DIC values. We realize that the estimated $\delta^{13}\text{C}$ DIC values reported here are poorly constrained because we did not monitor $\delta^{13}\text{C}$ DIC continuously throughout the experiment, and that metabolic and atmospheric derived CO_2 , and freshwater input can make $\delta^{13}\text{C}$ DIC values more negative. However, can we rule out significant changes in salinity and/or freshwater input based on the stability of weekly $\delta^{18}\text{O}_w$ 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 $\delta^{13}\text{C}$ and the DIC of water used during the culture period ($\delta^{18}\text{O}_w$) is depicted in Figure 6. The predicted equilibrium values for shell $\delta^{13}\text{C}_c$ are $\delta^{13}\text{C}$ ‰ DIC + $1 \pm 0.2\%$ [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 $\delta^{13}\text{C}_c$ values are -4.73% ($1\sigma = 1.13\%$; $N = 36$), -6.21% ($1\sigma = 1.38\%$; $N = 61$), and -6.77% ($1\sigma = 1.25\%$; $N = 121$) for adults (Maine), adults (Greenland), and juveniles (Greenland) respectively. On average *M. edulis* $\delta^{13}\text{C}_c$ values are in

disequilibrium by -4.61% ($1\sigma = 2.57\%$; $N = 218$) with respect to ambient DIC (Figure 6). The degree of carbon disequilibrium (deviation from $\delta^{13}\text{C}$ ‰ DIC + 1 [Romanek *et al.*, 1992]) increases with increasing $\delta^{18}\text{O}_w$ 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 $\delta^{18}\text{O}_w$ 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 $[\text{Cl}^-]$) 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 $\delta^{13}\text{C}$ values than DIC [DeNiro and Epstein, 1978]. The food used during this study primarily was composed of marine phytoplankton, and the $\delta^{13}\text{C}$ values were $-35.23 \pm 0.18\%$ ($N = 2$) thus the food has likely influenced the overall shell $\delta^{13}\text{C}_c$ values. Using end-member carbon reservoir conditions (food $\delta^{13}\text{C} = -35.23\%$ and ambient $\delta^{13}\text{C}$ DIC values), mean shell $\delta^{13}\text{C}_c$ values (-5.85% at DIC = 0.21% , -6.68% at DIC = -2.25% , -6.29% at DIC = -5.33%) (Figure 6), and assuming that $\delta^{13}\text{C}$ food values approximate $\delta^{13}\text{C}$ tissue values [Gillikin *et al.*, 2006b], it is estimated that *M. edulis* incorporated $\sim 7\%$ metabolic carbon at 23 PSU, $\sim 16\%$ metabolic carbon at

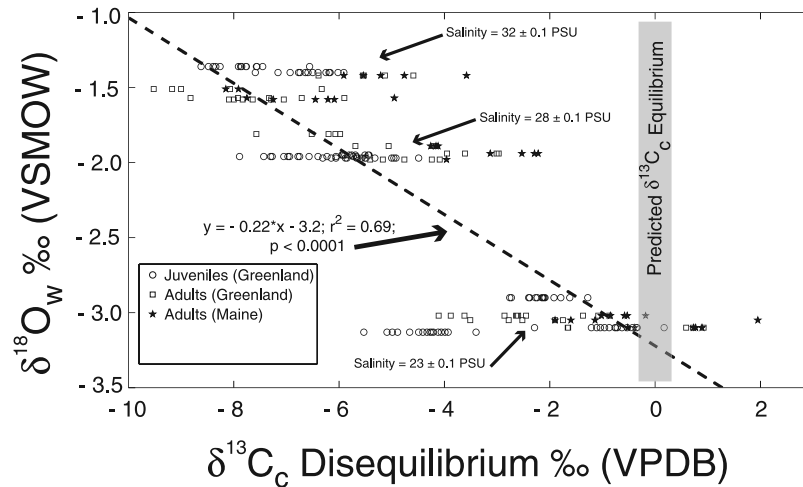


Figure 7. Relationship between ambient isotopic composition of water ($\delta^{18}\text{O}_w$) during culture (related to salinity) and estimate of shell $\delta^{13}\text{C}_c$ disequilibrium, where $\delta^{13}\text{C}$ of 0‰ represents isotopic equilibrium (equilibrium = $\delta^{13}\text{C}$ DIC ‰ + 1.0 ± 0.2 ‰ [from Romanek *et al.*, 1992]). The gray area represents the predicted range of $\delta^{13}\text{C}_c$ values in carbon equilibrium with DIC.

28 PSU, and $\sim 20\%$ metabolic carbon at 32 PSU based on and the metabolic carbon contribution equation of McConnaughey *et al.* [1997]. The increased metabolic contribution to shell $\delta^{13}\text{C}_c$ at higher salinities supports a biologically induced vital effect possibly related to CA activity. Klein *et al.* [1996] suggested that high mantle metabolic activity incorporates a greater amount of metabolic derived carbon, whereas low mantle activity would allow more DIC to be incorporated into the shell material. We found no evidence to support greater carbon isotope disequilibrium based on where the animals were collected (Maine or Greenland), culture temperature, growth rates, or shell length (related to the age of the animal). Our results corroborate earlier work by Tanaka *et al.* [1986] (modified by McConnaughey *et al.* [1997]), Vander Putten *et al.* [2000], and Gillikin *et al.* [2006b] that investigated the mechanisms responsible for $\delta^{13}\text{C}$ incorporation into *M. edulis* shells collected in situ. In these studies, it was determined that a significant portion of shell $\delta^{13}\text{C}_c$ was derived from metabolic carbon, because $\delta^{13}\text{C}_c$ values were depleted with respect to ambient DIC conditions where the animals lived. Although Gillikin *et al.* [2006b] estimated that *M. edulis* incorporated less than 10% metabolic carbon into its shell they suggested that environmental reconstructions using $\delta^{13}\text{C}_c$ profiles from *M. edulis* would be limited to extreme changes in ambient $\delta^{13}\text{C}$ DIC/salinity conditions.

[27] On the basis of previous studies and the results presented here, we suggest that the $\delta^{13}\text{C}_c$ shell values from *M. edulis* are not generally suitable for environmental reconstructions (paleo-DIC or paleo- pCO_2). However, in a low-salinity environment (e.g., upper estuary) $\delta^{13}\text{C}_c$ values from *M. edulis* may be suitable to reconstruct paleo-DIC conditions, because $\delta^{13}\text{C}_c$ values approach equilibrium with respect to ambient DIC (Figure 7). In order for this methodology ($\delta^{13}\text{C}_c$ 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 $\delta^{13}\text{C}_c$ during biomineralization. Certainly, the applicability and reliability of $\delta^{13}\text{C}_c$ from bivalves (and other biogenic carbonates) as paleoindicators could be improved with similar isotope calibration work presented here, especially if $\delta^{13}\text{C}$ ‰ DIC is continuously monitored. It is likely that many biogenic organisms precipitate their tests 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

[28] 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 $\pm 0.57^\circ\text{C}$ when $\delta^{18}\text{O}_w$ values can be determined independently. We have demonstrated that the shell chemistry of *M. edulis* ($\delta^{18}\text{O}_c$) 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}_c$ profiles from the bivalve *M. edulis* are not generally suitable for reconstructing paleo-DIC or paleo- pCO_2 . However, it may be possible to use $\delta^{13}\text{C}_c$ values from *M. edulis* in low-salinity environments to reconstruct paleo-DIC where $\delta^{13}\text{C}_c$ values of shell material is nearly in carbon isotope equilibrium with ambient DIC, however this needs to be further investigated.

[29] **Acknowledgments.** We thank Robin Abbott and colleagues from VECO Polar Resources for travel and accommodations while in Kangerlussuaq, Greenland; Fartato Olsen for providing boating and bivalve

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