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An Aquaculture-Based Method for Calibrated Bivalve Isotope Paleothermometry

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An aquaculture-based method for calibrated bivalve isotope paleothermometry

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[1] To quantify species-specific relationships between bivalve carbonate isotope geochemistry ($\delta^{18}O_c$) and water conditions (temperature and salinity, related to water isotopic composition [$\delta^{18}O_w$]), an aquaculture-based methodology was developed and applied to *Mytilus edulis* (blue mussel). The four-by-three factorial design consisted of four circulating temperature baths (7, 11, 15, and 19°C) and three salinity ranges (23, 28, and 32 parts per thousand (ppt); monitored for $\delta^{18}O_w$ weekly). In mid-July of 2003, 4800 juvenile mussels were collected in Salt Bay, Damariscotta, Maine, and were placed in each configuration. The size distribution of harvested mussels, based on 105 specimens, ranged from 10.9 mm to 29.5 mm with a mean size of 19.8 mm. The mussels were grown in controlled conditions for up to 8.5 months, and a paleotemperature relationship based on juvenile *M. edulis* from Maine was developed from animals harvested at months 4, 5, and 8.5. This relationship [$T°C = 16.19\ (\pm0.14) - 4.69\ (\pm0.21)\ \{\delta^{18}O_c\ VPBD - \delta^{18}O_w\ VSMOW\} + 0.17\ (\pm0.13)\ \{\delta^{18}O_c\ VPBD - \delta^{18}O_w\ VSMOW\}^2$; $r^2 = 0.99$; $N = 105$; $P < 0.0001$] is nearly identical to the Kim and O'Neil (1997) abiogenic calcite equation over the entire temperature range (7–19°C), and it closely resembles the commonly used paleotemperature equations of Epstein et al. (1953) and Horibe and Oba (1972). Further, the comparison of the *M. edulis* paleotemperature equation with the Kim and O'Neil (1997) equilibrium-based equation indicates that *M. edulis* specimens used in this study precipitated their shell in isotopic equilibrium with ambient water within the experimental uncertainties of both studies. The aquaculture-based methodology described here allows similar species-specific isotope paleothermometer calibrations to be performed with other bivalve species and thus provides improved quantitative paleoenvironmental reconstructions.

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1. Introduction

[2] Oxygen isotopic analysis of marine biogenic carbonates ($\delta^{18}O_c$) is a standard paleoceanographic method used to reconstruct seawater temperature and/or changes in the isotopic composition of seawater ($\delta^{18}O_w$), when other independent methods can constrain either water temperature or salinity (Mg/Ca ratios, alkenones, etc.). $\delta^{18}O_c$ is a function of seawater temperature [*Urey*, 1947; *Epstein et al.*, 1953; *Craig*, 1965; *O'Neil et al.*, 1969], isotopic composition of the seawater (related to salinity) [*Emiliani*, 1966; *Shackleton*, 1967], and any species-specific fractionation that occurs during biomineralization [*Erez*, 1978; *Shackleton et al.*, 1973; *Swart*, 1983; *Gonzalez and Lohmann*, 1985; *McConnaughey*, 1989a, 1989b; *Owen et al.*, 2002a; *Lorrain et al.*, 2004]. Substantial information about marine paleoenvironments can be elicited from stable isotope profiles ($\delta^{18}O_c$) from living and fossil bivalves [e.g., *Williams et al.*, 1982; *Arthur et al.*, 1983; *Krantz et al.*, 1987; *Romanek et al.*, 1987; *Wefer and Berger*, 1991; *Weidman et al.*, 1994; *Klein et al.*, 1997; *Purton and Brasier*, 1999; *Ivany et al.*, 2003; *Schöne et al.*, 2004; *Carre et al.*, 2005]. However, several factors have been recognized that complicate the understanding of biogenic carbonates, which have been described, in part, by previous workers. These factors include carbonate precipitation in equilibrium with ambient water [*Shackleton et al.*, 1973; *McConnaughey*, 1989b], pH effects [*Spero et al.*, 1997; *Zeebe et al.*, 2003], ontogeny [*Bijma et al.*, 1998], diagenesis [e.g., *Grossman et al.*, 1993], seasonal timing and duration of shell growth, and large scale geographic trends in temperature and productivity gradients on shell growth [*Jones*, 1981; *Harrington*, 1989; *Goodwin et al.*, 2001; *Owen et al.*, 2002b; *Schöne et al.*, 2003, 2005; *Goodwin et al.*, 2004; *De Ridder et al.*, 2004].

[3] In the past, the interpretation of $\delta^{18}O_c$ has been based upon theoretical studies of chemical equilibrium and kinetics [*Urey*, 1947; *Usdowski and Hoefs*, 1993], or laboratory experiments involving inorganic precipitation of $CaCO_3$ from solution [e.g., *McCrea*, 1950; *O'Neil et al.*, 1969; *Tarutani et al.*, 1969; *Kim and O'Neil*, 1997; *Zhou and Zheng*, 2003]. Other methods have employed an empirical calibration of bivalves, done by measuring $\delta^{18}O_c$ of collected shells from the natural setting and/or from shells grown in a controlled setting and by measuring or estimating $\delta^{18}O_w$ [*Epstein et al.*, 1953; *Craig*, 1965; *Horibe and Oba*, 1972; *Grossman and Ku*, 1986; *Owen et al.*, 2002a; *Chauvaud et al.*, 2005]. However, previous oxygen isotope bivalve calibrations have one or several potential limitations: (1) Estimates of temperature and/or $\delta^{18}O_w$ were used in the development of paleotemperature equations; (2) a limited number of environmental conditions, such as a single temperature or a single salinity, were utilized during culturing; (3) a limited number of bivalves (as few as one) were grown at a particular temperature and salinity range; and (4) a limited suite of bivalve species was used.

[4] The general isotope calibrations for calcite and aragonite [*Epstein et al.*, 1953; *Grossman and Ku*, 1986] have been applied to a wide variety of organisms precipitating carbonate skeletons, which were not cultured in their calibrations, over timescales ranging from seasonal to glacial/interglacial. Also, these isotope calibrations were not designed to assess factors such as "life processes" that complicate the interpretation of $\delta^{18}O_c$. Recognition of these limitations has led to the development of aquaculture-based techniques for selected foraminifera species [e.g., *Erez and Luz*, 1983; *Spero and Lea*, 1993, 1996; *Bemis et al.*, 1998; *Bijma et al.*, 1998], bivalves [e.g., *Owen et al.*, 2002a], and corals [e.g., *Al-Horani et al.*, 2003]. We present here a methodology for growing a wide range of bivalve species in which key environmental conditions are well constrained and monitored, and represent reasonable growing conditions similar to the natural environment. Our goal is to develop a reproducible aquaculture-based method for use with a wide range of bivalve species, particularly from mid to high latitudes, where few high-resolution (seasonal) paleo-oceanographic records exist, that facilitates the study of shell chemistry, including biologically induced effects as a function of growing conditions.

2. Materials and Methods

2.1. Experimental Bivalve

[5] The relative abundance in coastal environments and broad geographical distribution of the intertidal

bivalve *M. edulis* makes it an ideal species for paleo-environmental 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* 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 temperature range of 10–20°C [*Bayne et al.*, 1973] and a variable salinity range of <20 ppt–35 ppt. Because *M. edulis* is a nearshore-intertidal organism it has the potential to record sea surface temperature (SST) in its shell for a specific coastal location and time. 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. *M. edulis* is a relatively short-lived organism (6–7 years old common), that deposits annual growth rings [*Lutz*, 1976] and micro-growth rings with tidal and daily periodicities [*Richardson*, 1989]. An adult blue mussel (>2 years) can grow to about 8–10 cm (shell length) allowing for a high-resolution environmental reconstruction (sub-monthly), and have been reported to live up to 18–24 years [*Theisen*, 1973]. 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 layer lags the calcitic layer substantially, thus all new growth is calcitic. As the organism continues to grow, the aragonitic layer follows outward toward the mantle.

[6] In mid-July of 2003, 4800 juvenile *M. edulis*, ~15 mm shell length on average, were collected in Salt Bay, Damariscotta, Maine, USA. These animals were transported to the Darling Marine Center in Walpole, Maine and were kept moist in storage containers. Animals were sorted to ensure that a similar distribution of size fractions were equally distributed in each temperature/salinity configuration. Animals were acclimated to the culture temperature gradually for a period of one week. On the basis of 100 random samples, the range was 9.8–20.2 mm, with a mean of 15.3 mm (1σ = 2.4 mm).

2.2. Aquaculture Design and Implementation

[7] An aquaculture system was designed at the Darling Marine Center to achieve four temperature settings (7, 11, 15 and 19 ± 0.5°C) and three salinity settings (23, 28, and 32 ± 0.1 ppt). This four by three factorial design allowed 12 different growing conditions to be maintained simultaneously. Each experiment has been duplicated (buckets A and B). The system consists of three large containers (500-liter) connected to a heating/cooling system (Aquanetics Systems), in which four 20-liter buckets were placed into the fresh water bath (Figure 1). The temperature of each bath was measured with a HOBO® H8 data logger every 30 minutes with an accuracy of ±0.5°C (Figure 2), and each HOBO® H8 data logger was calibrated in an ice-water bath to ensure accuracy. The average water exchange in each recirculating bath was approximately 10 liters per minute.

[8] Seawater was collected via the flowing seawater laboratory at the Darling Marine Center, and was pumped from the Damariscotta River at −10 m below mean low tide. Seawater was mixed for desired salinity (23, 28, and 32 ± 0.1 ppt) and stored in 2,460-liter containers and sealed. Salinity measurements were made via a YSI model 85 oxygen, conductivity, salinity, and temperature system with an accuracy of ±0.1 ppt. We used a simple mixing line based on the mean $\delta^{18}O_w$ values (see below) of well water and seawater (desired $\delta^{18}O_w$ = [0.0029*x] − 8.6; where x = # of salt water liters added to the 2,460-liter container) 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 ppt. The highest salinity (32 ppt) was limited by the seasonal cycle in the Damariscotta River system, and had a mean oxygen isotopic composition ($\delta^{18}O_w$) of −1.40‰ Vienna Standard Mean Ocean Water (VSMOW) (N = 8; 1σ = 0.11‰) during June, 2003. For 23 and 28 ppt mixtures, seawater was mixed with well water from the Darling Marine Center, which had a mean $\delta^{18}O_w$ of −8.62‰ (VSMOW) (N = 12; 1σ = 0.14‰) during June, 2003. The above procedure was repeated in October, 2003 to replenish seawater that was used through March, 2004 (mean seawater $\delta^{18}O_w$ of −1.46‰ VSMOW; N = 10;

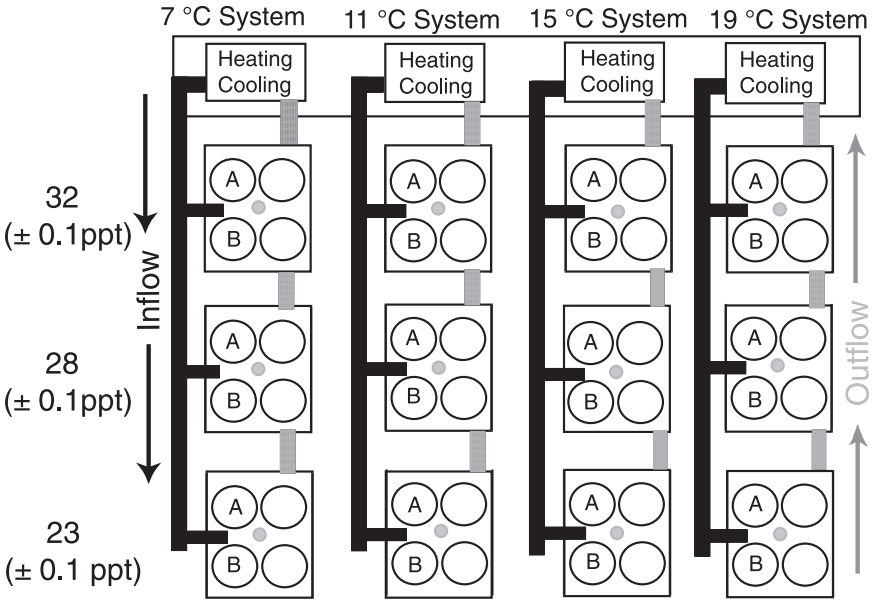

Figure 1. Schematic diagram of the experimental design. Each temperature condition is shown vertically, and each salinity condition is shown horizontally. Black indicates inflow, and gray indicates outflow. Buckets A and B are replicates. Buckets to the right of A and B are for water changes. All buckets are in a fresh water bath to maintain desired temperature setting to within ±0.5°C.

1σ = 0.08‰ and mean well water $\delta^{18}O_w$ of −8.59‰ VSMOW; N = 10; 1σ = 0.09‰).

[9] Two hundred blue mussels were placed in each 20-liter temperature/salinity environment, for a total of 4800 animals. The animals were cultured for a total of 8.5 months (mid-July 2003 through March 2004) with five animals being removed from each configuration monthly for analysis. Ten animals in each temperature and salinity configuration were tagged on July 14, 2003 with a numbered shell fish tag (~3 mm) directly adhered to each animal. The shell length for each these mussels were determined with digital calibers (±0.01 mm) by measuring along the maximum

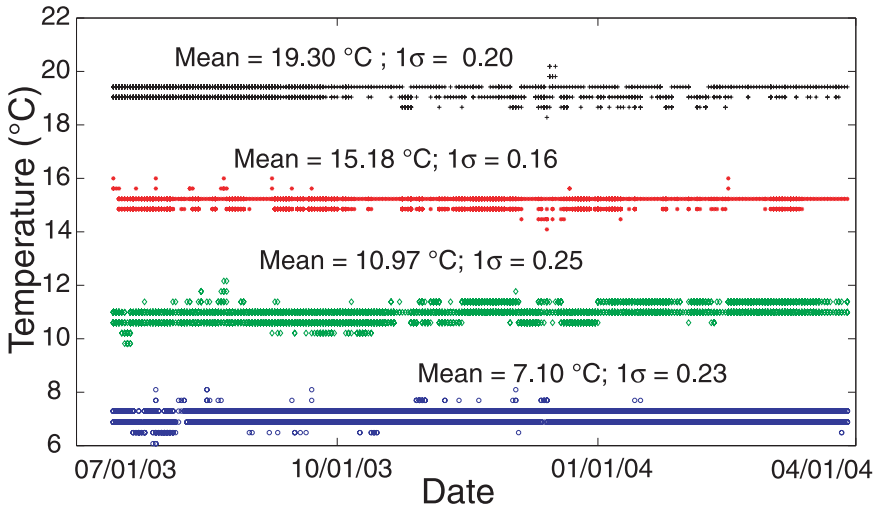

Figure 2. Temperatures for each of the four freshwater baths are shown with the 8-month mean and standard deviation. The HOBO® H8 data logger digitally measured water temperature with an error of ±0.5°C, and the appearance of two lines for each temperature is an artifact of the digital measurement.

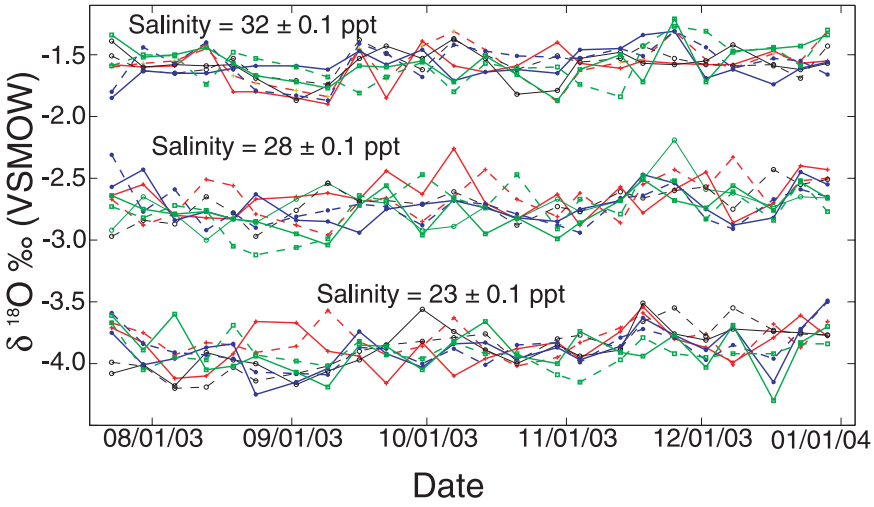

Figure 3. The oxygen isotopic composition ($\delta^{18}O_w$) for each of the 24 growing environments is shown for a 5-month period (only one group of animals were used in the final paleotemperature relationship beyond 5 months, and complete $\delta^{18}O_w$ values are listed in Table 1). The salinities are 32 ppt (top), 28 ppt (middle), and 23 ppt (bottom). Temperatures are 19°C (red), 15°C (black), 11°C (blue), and 7°C (green). For the replicate environments, the solid lines represent bucket A, while the dashed line represent bucket B. The average standard deviations for $\delta^{18}O_w$ are (1σ) = 0.18‰, 0.19‰, and 0.17‰ for 32 ppt, 28 ppt, and 23 ppt, respectively. The observed variabilities are 3–4 times larger than the analytical error of the measurement and may be caused by the addition of food/water that was isotopically different than the growing conditions, although it was made from the same salinity water. Some evaporation from buckets also could account for a small amount of the variability.

growth axis, and monitored monthly. Average linear growth rates (mm/month) were 0.10, 0.08, 0.09, and 0.14 for 7°, 11°, 15°, and 19°C temperature ranges, respectively. In their natural setting, *M. edulis* has growth rates of ~3 mm/month, with considerable variation among individuals [*Incze et al.*, 1980]. Overall, there were no noticeable trends in growth rate versus salinity or temperature.

[10] Complete water changes for each temperature/salinity environment were made weekly, to remove metabolic waste. The aquaculture design allowed for one extra 20-liter bucket to be in place with identical water (isotopic composition and temperature) (Figure 1). Mussels were fed twice daily (total of 10 ml) a concentrated spat formula (Innovative Aquaculture Products, Ltd.) where 5 ml of spat was diluted in 1 liter of identical isotopic composition water in which they grew. Water samples for each of the twenty-four buckets were collected weekly, after water changes were made, to monitor $\delta^{18}O_w$ (Figure 3). There was no isotopic difference noted when water was collected prior to and after water changes. Throughout the experiment mortality was low (<10%) for all temperature and salinity configurations for the first five months. Mortality rates were higher (~20–25%) for animals grown at 20°C for the remainder of the experiment, while all others remained low.

2.3. Sample Preparation and Analysis

[11] Weekly water samples ($\delta^{18}O_w$) were measured via a dual-inlet VG/Micromass SIRA, which has a long-term precision of ±0.05‰ (Figure 3). Weekly $\delta^{18}O_w$ from each temperature/salinity environments were averaged over the growing interval (4, 5, and 8.5 months), and used in the isotope calibration (Table A1). All $\delta^{18}O_w$ (δ in ‰ = $[(R_{sample}/R_{standard}) - 1] * 1000‰$; $[R = {}^{18}O/{}^{16}O]$) values are reported with respect to Vienna Standard Mean Ocean Water (VSMOW).

[12] Animals were cleaned 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, each animal's shell length was recorded. The outer edge of each valve was micro-milled using a variable speed mounted drill and binocular microscope with 6.5x to 40x magnification. To ensure that only new shell carbonate material was used for the isotope calibration, mussels from each month were sampled and standard deviations of isotopic variability ($\delta^{18}O_{calcite} - \delta^{18}O_{water}$) were calculated. These

standard deviations (1σ) for months 1 and 2 were on the order of 0.32‰–0.52‰; thus these animals were not used in the study. Standard deviations of isotopic variability ($\delta^{18}O_{calcite} - \delta^{18}O_{water}$) for months 4, 5, and 8.5 were on the order of 1σ = 0.09‰–0.12‰, and remained constant. In addition, growth rate data (Table A1) was used to estimate how much linear shell material could be removed during sampling, without mixing previously grown shell with controlled growth. X-ray diffraction was performed on a limited number of samples from shell edges to rule out a mixed matrix of calcite and aragonite. This method is extremely accurate in detecting the presence of polymorphs, because of the return signal generated from the different crystal habits of calcite (hexagonal) and aragonite (orthorhombic) during the X-ray diffraction analysis. All measurements indicate that there was no aragonite present near the ventral margin, and furthermore by visually inspecting the shells the pearly aragonitic layer lagged the calcitic outer shell layer substantially (>1 cm). Shell carbonate analysis ($\delta^{18}O_c$) 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, which has a long-term precision of ±0.10‰. Average shell samples weighed approximately 100 μg. Samples were calibrated using NBS-19 standards at the beginning and end of each run, with a standard to sample ratio of 1:3. All shell carbonate values ($\delta^{18}O_c$) are reported with respect to Vienna Pee-Dee Belemnite (VPBD).

2.4. Calibration of Temperature and $\delta^{18}O$ Relationships

[13] Least squares regression was used to generate the *M. edulis* paleotemperature relationship. Root mean squared errors (RMSE) were 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 ($\delta^{18}O_c$) are reported against the international VPBD scale and our water data ($\delta^{18}O_w$) 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], *Horibe and Oba* [1972] and *Kim and O'Neil* [1997] calcite equations, corrections had to be made to each of their data sets or equation, because *Epstein et al.* [1953] and *Horibe and Oba* [1972] report the $\delta^{18}O_c - \delta^{18}O_w$ versus [PDB], while *Kim and O'Neil* [1997] report the $\delta^{18}O_c - \delta^{18}O_w$ versus [SMOW]. The water data of *Epstein et al.* [1953] was converted to the VSMOW scale using the following relationship [*Friedman and O'Neil*, 1977]:

$$\delta^{18}O_w(\text{VSMOW}) = 1.00022 * \delta^{18}O_w(\text{PDB}) + 0.22,$$

and least squares regression of their data yielded a paleotemperature relationship in the following form:

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

Similarly, the calcite equation of *Horibe and Oba* [1972] was converted to the VPDB - VSMOW scale using the following relationship [*Friedman and O'Neil*, 1977]:

$$\delta^{18}O_w(\text{VSMOW}) = 1.00022 * \delta^{18}O_w(\text{PDB}) + 0.22,$$

and the conversion of their equation yielded a paleotemperature relationship in the following form:

$$\begin{aligned} T^\circ C = {} & 16.10 - 4.27\left[\delta^{18}O_c\text{VPBD} - \delta^{18}O_w\text{VSMOW}\right] \\ & + 0.16\left[\delta^{18}O_c\text{VPBD} - \delta^{18}O_w\text{VSMOW}\right]^2. \end{aligned} \tag{2}$$

The calcite data (5 mM solution) of *Kim and O'Neil* [1997] were corrected (+0.25‰) to account for differences in acid fractionation factors used in their work (1.01050) and then converted to the VPDB scale using the following equation [*Friedman and O'Neil*, 1977]:

$$\delta^{18}O_c(\text{VPBD}) = 0.97006 * \delta^{18}O_w(\text{SMOW}) - 29.94,$$

and least squares regression of their data yielded a paleotemperature relationship in the following form:

$$\begin{aligned} T^\circ C = {} & 15.07(\pm 0.86) - 4.60(\pm 0.59) \\ & \cdot \left[\delta^{18}O_c\text{VPBD} - \delta^{18}O_w\text{VSMOW}\right] \\ & + 0.09(\pm 0.13)\left[\delta^{18}O_c\text{VPBD} - \delta^{18}O_w\text{VSMOW}\right]^2; \\ & r^2 = 0.99; \quad \text{RMSE} \pm 0.72^\circ C. \end{aligned} \tag{3}$$

3. Results and Discussion

[14] A paleotemperature relationship for *M. edulis* was derived during this study from 7°–19°C,

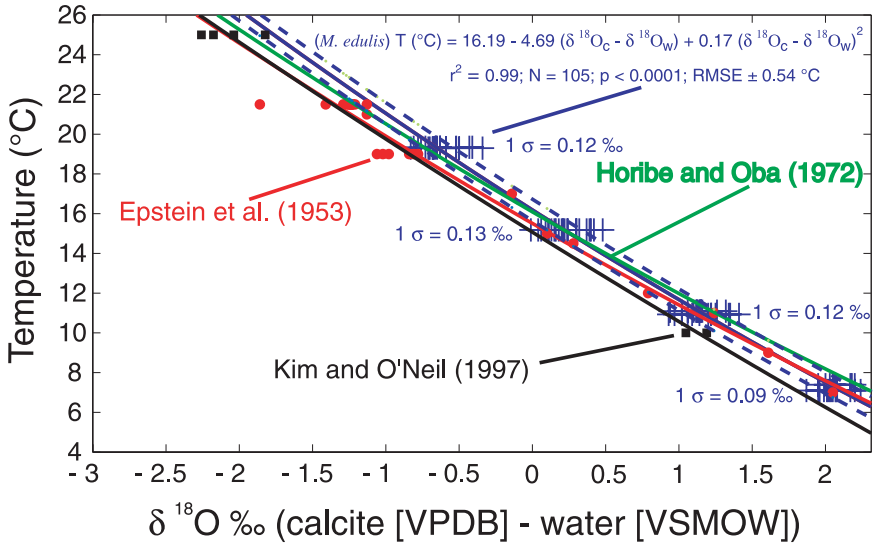

Figure 4. The *M. edulis* (Maine juveniles) paleotemperature relationship (this study; blue line and blue data points) is compared to the calcite equations of *Epstein et al.* [1953] (red line and red data points), *Horibe and Oba* [1972] (green line), and *Kim and O'Neil* [1997] (black line and black data points). Standard deviations for this study are reported for each temperature range in which animals grew. The RMSE ± 0.54°C is reported for this study at 95% C.I. (dashed blue lines).

including three salinity settings (23, 28, and 32 ± 0.1 ppt):

$$\begin{aligned} T^{\circ}C = {} & 16.19(\pm 0.14) - 4.69(\pm 0.21) \\ & \cdot \left[\delta^{18}O_c VPBD - \delta^{18}O_w VSMOW\right] \\ & + 0.17(\pm 0.13)\left[\delta^{18}O_c VPBD - \delta^{18}O_w VSMOW\right]^2; \\ & r^2 = 0.99; \quad N = 105; P < 0.0001; \quad RMSE \pm 0.54^{\circ}C. \end{aligned} \tag{4}$$

Equation (4) is compared to equations (1), (2), and (3) (Figure 4). *M. edulis* (equation (4)) is slightly offset relative to the *Kim and O'Neil* [1997] (equation (3)) abiogenic calcite equation over the entire temperature range (7–19°C), and it closely resembles the commonly used paleotemperature equations of *Epstein et al.* [1953] (equation (1)) and *Horibe and Oba* [1972] (equation (2)). The comparison of the *M. edulis* paleotemperature equation with the *Kim and O'Neil* [1997] equilibrium-based model indicates that *M. edulis* specimens used in this study precipitated their shell in isotopic equilibrium with ambient water within the experimental uncertainties of both studies (*Kim and O'Neil* [1997] (RMSE ± 0.72°C) and this study (RMSE ± 0.54°C)) (Figure 4).

[15] There is similar isotopic variability ($\delta^{18}O_c - \delta^{18}O_w$) for *M. edulis* over the upper temperature ranges (1σ = 0.12‰ at 19°C; 0.13‰ at 15°C; 0.12‰ at 11°C), and slightly less at the lowest temperature (1σ = 0.09‰ at 7°C) (Figure 4). The observed variability is slightly higher or within the range of combined random analytical errors for water and carbonate analyses (±0.11‰ [*Miller and Miller*, 1993]). We determined that approximately 0.09‰ of the variability is shell-derived for all temperature and salinity conditions; however, this is less than the analytical error during measurement of $\delta^{18}O_c$. We attribute the remainder of the variability to minor changes in the isotopic composition of the water during culture. *Owen et al.* [2002a] reported isotopic variability of *Pecten maximus* (Great Scallop) at any one temperature of 1σ = 0.05‰–0.18‰. The *Kim and O'Neil* [1997] inorganic calcite experiment yielded isotopic variability of 1σ = 0.06‰, 0.19‰, and 0.10‰ for 40°, 25°, and 10°C, respectively. *Epstein et al.* [1953] included temperature-controlled conditions and multiple animals for only 21.5°C and 19°C (Figure 4), where the isotopic variability was 1σ [‰] = 0.22‰ and 0.11‰, respectively. The isotopic variability from *Epstein et al.* [1953] was equal to, or nearly twice as great as the isotopic variability noted in this study for *M. edulis*. All other data from *Epstein et al.* [1953] had only a single bivalve grown for each temperature range, and estimates of temperature and $\delta^{18}O_w$ were used (Figure 4). Unfortunately, it is not possible to assess the isotopic variability ($\delta^{18}O_c$ - $\delta^{18}O_w$) where only one bivalve was grown. On the basis of the isotopic variability noted in *M. edulis*, it is likely that

Table A1. Sample Identification, Shell Oxygen and Carbon Isotopic Values, Salinity, Shell Length, Water Isotopic Composition, Measured Temperature, Predicted Temperature, Temperature Deviation, Months Grown, Estimated Growth Rates, and Estimated New Linear Growth[a]

Sample I.D.	$\delta^{18}O$ (VPDB)	$\delta^{13}C$ (VPDB)	Salinity, ppt	Shell Length, mm	$\delta^{18}O$ (VSMOW)	Calcite -Water (VPDB - VSMOW)	Measured Temperature, °C	Predicted Temperature, °C	Temperature Deviation, °C	Months Grown	Estimated Growth Rate, mm/month	Estimated New Linear Growth, mm
8-33A-3/29	0.7512	−0.4244	32	12.47	−1.30	2.05	7.10	7.32	−0.22	8.5	0.10	0.85
8-33A-3/29	0.6513	−0.9404	32	10.87	−1.30	1.95	7.10	7.71	−0.61	8.5	0.10	0.85
8-33A-3/29	0.6929	−0.2974	32	19.06	−1.30	1.99	7.10	7.55	−0.45	8.5	0.10	0.85
8-33A-3/29	0.7382	−0.3144	32	15.57	−1.30	2.04	7.10	7.37	−0.27	8.5	0.10	0.85
8-33A-3/29	0.7132	−0.6304	32	14.21	−1.30	2.01	7.10	7.47	−0.37	8.5	0.10	0.85
8-33A-3/29	0.9392	−0.9854	32	21.93	−1.30	2.24	7.10	6.60	0.50	8.5	0.10	0.85
8-33A-3/29	0.9022	−0.7584	32	21.14	−1.30	2.20	7.10	6.74	0.36	8.5	0.10	0.85
8-33A-3/29	0.8692	−0.8074	32	21.10	−1.30	2.17	7.10	6.87	0.23	8.5	0.10	0.85
8-33A-3/29	0.7732	−1.0334	32	20.73	−1.30	2.07	7.10	7.24	−0.14	8.5	0.10	0.85
8-33A-3/29	0.7262	−0.7174	32	20.86	−1.30	2.03	7.10	7.42	−0.32	8.5	0.10	0.85
8-33A-3/29	0.5692	−0.7894	32	21.99	−1.30	1.87	7.10	8.03	−0.93	8.5	0.10	0.85
8-33A-3/29	0.6572	−0.5314	32	15.99	−1.30	1.96	7.10	7.69	−0.59	8.5	0.10	0.85
8-33A-3/29	0.7232	−0.3384	32	20.81	−1.30	2.02	7.10	7.43	−0.33	8.5	0.10	0.85
8-33A-3/29	0.7782	−0.6664	32	17.38	−1.30	2.08	7.10	7.22	−0.12	8.5	0.10	0.85
8-33A-3/29	0.7712	−0.8464	32	19.30	−1.30	2.07	7.10	7.24	−0.14	8.5	0.10	0.85
11-12-8-33B	0.3850	−0.8083	32	20.35	−1.64	2.03	7.39	7.42	−0.03	4.0	0.10	0.40
8-33-A	0.5450	−0.3730	32	21.41	−1.62	2.17	7.39	6.88	0.51	5.0	0.10	0.50
8-33-A	0.4340	−0.6540	32	16.53	−1.62	2.05	7.39	7.31	0.08	5.0	0.10	0.50
8-28-A	−0.8160	−2.0780	28	18.47	−2.82	2.00	7.39	7.50	−0.11	5.0	0.10	0.50
8-28-A	−0.7630	−2.1500	28	17.09	−2.82	2.06	7.39	7.30	0.09	5.0	0.10	0.50
8-28-B	−0.8550	−1.6770	28	19.86	−2.80	1.95	7.39	7.74	−0.35	5.0	0.10	0.50
8-28-B	−0.6050	−1.1550	28	18.66	−2.80	2.20	7.39	6.77	0.62	5.0	0.10	0.50
8-28-B	−0.6230	−1.4860	28	17.04	−2.80	2.18	7.39	6.84	0.55	5.0	0.10	0.50
8-23-A	−1.6300	−2.3710	23	19.88	−3.70	2.07	7.39	7.25	0.14	5.0	0.10	0.50
11-12-12-33A	−0.7110	−1.4423	32	16.77	−1.64	0.93	10.93	11.92	−0.99	4.0	0.08	0.32
11-12-12-33A	−0.5510	−1.4453	32	17.29	−1.64	1.09	10.93	11.23	−0.30	4.0	0.08	0.32
11-12-12-33A	−0.5490	−1.5183	32	15.77	−1.64	1.09	10.93	11.22	−0.29	4.0	0.08	0.32
11-12-12-33B	−0.5370	−1.7950	32	19.83	−1.56	1.02	10.93	11.51	−0.58	4.0	0.08	0.32
11-12-12-33B	−0.4220	−1.7980	32	19.54	−1.56	1.14	10.93	11.02	−0.09	4.0	0.08	0.32
11-12-12-33A	−0.4190	−1.4413	32	16.22	−1.64	1.22	10.93	10.67	0.26	4.0	0.08	0.32
11-12-12-28A	−1.7880	−2.1310	28	19.50	−2.76	0.97	10.93	11.73	−0.80	4.0	0.08	0.32
11-12-12-28A	−1.7030	−2.1660	28	19.15	−2.76	1.06	10.93	11.37	−0.44	4.0	0.08	0.32
11-12-12-28B	−1.5890	−2.4893	28	15.96	−2.79	1.20	10.93	10.76	0.17	4.0	0.08	0.32
11-12-12-28B	−1.5740	−2.2553	28	17.65	−2.79	1.22	10.93	10.69	0.24	4.0	0.08	0.32
11-12-12-23B	−2.6750	−2.4513	23	18.44	−3.92	1.25	10.93	10.57	0.36	4.0	0.08	0.32
11-12-12-23A	−2.5880	−3.4520	23	17.75	−3.93	1.34	10.93	10.17	0.76	4.0	0.08	0.32
11-12-12-23B	−2.5700	−2.9613	23	21.04	−3.92	1.35	10.93	10.14	0.79	4.0	0.08	0.32
11-12-12-23A	−2.5170	−3.4920	23	19.32	−3.93	1.41	10.93	9.87	1.06	4.0	0.08	0.32
12-28A	−1.2160	−1.9425	28	18.04	−2.32	1.10	11.09	11.17	−0.08	5.0	0.08	0.40

Table A1. (continued)

Sample I.D.	$\delta^{18}O$ (VPDB)	$\delta^{13}C$ (VPDB)	Salinity, ppt	Shell Length, mm	$\delta^{18}O$ (VSMOW)	Calcite -Water (VPDB - VSMOW)	Measured Temperature, °C	Predicted Temperature, °C	Temperature Deviation, °C	Months Grown	Estimated Growth Rate, mm/month	Estimated New Linear Growth, mm
12-28A	−1.0580	−1.5245	28	18.53	−2.32	1.26	11.09	10.50	0.59	5.0	0.08	0.40
12-28A	−1.1670	−1.6345	28	15.01	−2.32	1.15	11.09	10.96	0.13	5.0	0.08	0.40
12-28A	−1.1740	−1.8715	28	12.76	−2.32	1.15	11.09	10.99	0.10	5.0	0.08	0.40
12-28A	−1.1980	−1.4085	28	20.02	−2.32	1.12	11.09	11.09	0.00	5.0	0.08	0.40
12-28A	−1.2980	−2.3275	28	21.58	−2.32	1.02	11.09	11.52	−0.43	5.0	0.08	0.40
12-28A	−1.2050	−2.0245	28	22.22	−2.32	1.12	11.09	11.12	−0.03	5.0	0.08	0.40
12-28A	−1.1710	−1.7605	28	18.62	−2.32	1.15	11.09	10.98	0.11	5.0	0.08	0.40
12-28A	−1.1578	−1.5245	28	19.52	−2.32	1.16	11.09	10.92	0.17	5.0	0.08	0.40
12-28A	−1.1560	−1.6345	28	19.51	−2.32	1.16	11.09	10.91	0.18	5.0	0.08	0.40
12-28A	−1.3830	−2.1595	28	20.88	−2.32	0.94	11.09	11.88	−0.79	5.0	0.08	0.40
12-28A	−1.0000	−1.5355	28	18.34	−2.32	1.32	11.09	10.26	0.83	5.0	0.08	0.40
12-28A	−1.2650	−1.7855	28	18.61	−2.32	1.06	11.09	11.38	−0.29	5.0	0.08	0.40
12-28A	−1.1890	−1.8435	28	18.09	−2.32	1.13	11.09	11.05	0.04	5.0	0.08	0.40
11-12-16-33B	−1.4970	−2.4523	32	20.68	−1.49	−0.01	15.18	16.12	−0.94	4.0	0.09	0.36
11-12-16-33A	−1.3070	−1.8060	32	25.46	−1.61	0.30	15.18	14.69	0.49	4.0	0.09	0.36
11-12-16-33B	−1.2550	−1.8273	32	15.47	−1.49	0.24	15.18	15.00	0.18	4.0	0.09	0.36
11-12-16-33A	−1.1900	−1.8910	32	28.49	−1.61	0.42	15.18	14.16	1.02	4.0	0.09	0.36
16-33A	−1.2610	−1.9840	32	21.90	−1.35	0.09	15.18	15.68	−0.50	5.0	0.09	0.45
16-33A	−1.0550	−1.4100	32	14.67	−1.35	0.30	15.18	14.73	0.45	5.0	0.09	0.45
16-33A	−0.9840	−1.7510	32	29.53	−1.35	0.37	15.18	14.41	0.77	5.0	0.09	0.45
16-33A	−1.1750	−1.8280	32	15.71	−1.35	0.18	15.18	15.28	−0.10	5.0	0.09	0.45
16-33A	−1.1710	−1.8470	32	26.25	−1.35	0.18	15.18	15.26	−0.08	5.0	0.09	0.45
16-33A	−1.0300	−1.7540	32	27.19	−1.35	0.32	15.18	14.62	0.56	5.0	0.09	0.45
16-33A	−1.1460	−1.7870	32	18.64	−1.35	0.20	15.18	15.15	0.03	5.0	0.09	0.45
16-33A	−1.2380	−1.8190	32	17.91	−1.35	0.11	15.18	15.57	−0.39	5.0	0.09	0.45
16-33A	−1.2200	−1.8700	32	12.99	−1.35	0.13	15.18	15.49	−0.31	5.0	0.09	0.45
16-33A	−1.1280	−1.8640	32	24.04	−1.35	0.22	15.18	15.06	0.12	5.0	0.09	0.45
16-33A	−1.1880	−1.7500	32	25.79	−1.35	0.16	15.18	15.34	−0.16	5.0	0.09	0.45
16-33A	−1.2440	−2.1370	32	28.59	−1.35	0.11	15.18	15.60	−0.42	5.0	0.09	0.45
16-33A	−1.1380	−2.1340	32	19.06	−1.35	0.21	15.18	15.11	0.07	5.0	0.09	0.45
16-33A	−1.3100	−1.9090	32	22.38	−1.35	0.04	15.18	15.91	−0.73	5.0	0.09	0.45
16-33A	−1.1970	−1.7190	32	23.32	−1.35	0.15	15.18	15.38	−0.20	5.0	0.09	0.45
11-12-16-28B	−2.6870	−2.8530	28	20.74	−2.75	0.06	15.18	15.80	−0.62	4.0	0.09	0.36
11-12-16-28B	−2.6890	−3.5800	28	21.12	−2.75	0.06	15.18	15.81	−0.63	4.0	0.09	0.36
11-12-16-28A	−2.6690	−2.9303	28	27.73	−2.73	0.06	15.18	15.81	−0.63	4.0	0.09	0.36
11-12-16-28A	−2.4880	−3.3393	28	19.88	−2.73	0.24	15.18	14.97	0.21	4.0	0.09	0.36
11-12-16-23B	−3.5400	−3.4930	23	23.31	−3.93	0.39	15.18	14.30	0.88	4.0	0.09	0.36
11-12-16-23B	−3.5337	−3.6550	23	20.44	−3.93	0.40	15.18	14.27	0.91	4.0	0.09	0.36
11-12-16-23A	−3.5037	−2.7810	23	22.99	−3.98	0.48	15.18	13.91	1.27	4.0	0.09	0.36
20-33A	−2.0720	−2.0990	32	23.85	−1.31	−0.76	19.30	19.77	−0.47	5.0	0.14	0.70
20-33A	−1.9020	−2.1300	32	21.66	−1.31	−0.59	19.30	18.93	0.37	5.0	0.14	0.70

Table A1. (continued)

Sample I.D.	$\delta^{18}O$ (VPDB)	$\delta^{13}C$ (VPDB)	Salinity, ppt	Shell Length, mm	$\delta^{18}O$ (VSMOW)	Calcite -Water (VPDB - VSMOW)	Measured Temperature, °C	Predicted Temperature, °C	Temperature Deviation, °C	Months Grown	Estimated Growth Rate, mm/month	Estimated New Linear Growth, mm
20-33A	−2.1430	−2.5610	32	22.34	−1.31	−0.83	19.30	20.12	−0.82	5.0	0.14	0.70
20-33A	−1.9020	−2.3920	32	17.33	−1.31	−0.59	19.30	18.93	0.37	5.0	0.14	0.70
20-33A	−1.9850	−1.8050	32	16.52	−1.31	−0.68	19.30	19.34	−0.04	5.0	0.14	0.70
20-33A	−2.0123	−2.0470	32	14.90	−1.31	−0.70	19.30	19.47	−0.17	5.0	0.14	0.70
20-33A	−2.0860	−2.2160	32	17.88	−1.31	−0.78	19.30	19.84	−0.54	5.0	0.14	0.70
20-33A	−1.9760	−1.8920	32	13.95	−1.31	−0.67	19.30	19.29	0.01	5.0	0.14	0.70
20-33A	−1.9010	−2.1510	32	12.46	−1.31	−0.59	19.30	18.92	0.38	5.0	0.14	0.70
20-33B	−1.8070	−1.5930	32	26.51	−1.31	−0.50	19.30	18.46	0.84	5.0	0.14	0.70
20-33B	−1.9630	−2.4380	32	27.55	−1.31	−0.65	19.30	19.23	0.07	5.0	0.14	0.70
20-33B	−1.8123	−1.6860	32	21.00	−1.31	−0.50	19.30	18.49	0.81	5.0	0.14	0.70
20-33B	−2.0150	−2.5680	32	20.77	−1.31	−0.71	19.30	19.49	−0.19	5.0	0.14	0.70
20-33B	−1.8330	−2.3800	32	18.99	−1.31	−0.52	19.30	18.59	0.71	5.0	0.14	0.70
20-33B	−1.6510	−1.2370	32	21.34	−1.31	−0.34	19.30	17.71	1.59	5.0	0.14	0.70
20-33B	−1.9930	−2.4530	32	15.27	−1.31	−0.68	19.30	19.38	−0.08	5.0	0.14	0.70
11-12-20-33B	−2.2150	−3.3970	32	27.41	−1.56	−0.66	19.34	19.24	0.10	4.0	0.14	0.56
11-12-20-33B	−2.2140	−3.1920	32	19.67	−1.56	−0.65	19.34	19.23	0.11	4.0	0.14	0.56
11-12-20-28A	−3.4720	−3.5860	28	21.59	−2.66	−0.81	19.34	20.02	−0.68	4.0	0.14	0.56
11-12-20-28B	−3.4470	−3.2463	28	17.96	−2.72	−0.73	19.34	19.59	−0.25	4.0	0.14	0.56
11-12-20-28B	−3.3460	−3.0803	28	18.71	−2.72	−0.63	19.34	19.10	0.24	4.0	0.14	0.56
11-12-20-28A	−3.0690	−3.2950	28	25.82	−2.66	−0.41	19.34	18.04	1.30	4.0	0.14	0.56
11-12-20-23A	−4.5350	−4.1360	23	21.73	−3.88	−0.66	19.34	19.24	0.10	4.0	0.14	0.56
11-12-20-23B	−4.4590	−4.5050	23	19.70	−3.80	−0.66	19.34	19.26	0.08	4.0	0.14	0.56
11-12-20-23B	−4.4300	−4.1750	23	19.86	−3.80	−0.63	19.34	19.11	0.23	4.0	0.14	0.56
11-12-20-23A	−4.3980	−4.2780	23	15.15	−3.88	−0.52	19.34	18.57	0.77	4.0	0.14	0.56
11-12-20-23B	−4.2330	−4.1130	23	22.94	−3.80	−0.43	19.34	18.15	1.19	4.0	0.14	0.56

[a] Units are as follows: shell oxygen and carbon isotopic values, ‰ (VPDB); salinity, ppt; shell length, mm; water isotopic composition, ‰ (VSMOW); measured temperature, predicted temperature, and temperature deviation, °C.

similar or greater variability would have been noted if *Epstein et al.* [1953] had multiple animals at all temperature ranges.

[16] The size (shell length) distribution of mussels, based on 105 animals harvested in the experiment, ranged from 10.9 mm to 29.5 mm with a mean size of 19.8 mm. This variation in shell length (related to the age of the animal) allowed for quantification of potential vital effects. A comparison of shell length and temperature deviation (measured temperature minus predicted temperature [predicted temperatures from this study]) is made to determine if there is any shell length-related isotope disequilibrium. There is a weak positive correlation ($r^2 = 0.03$) between the shell length of the animal and temperature deviation, but the relationship is not statistically significant. This result suggests that *M. edulis* did not exhibit age/size-related disequilibrium during biomineralization over the culture period.

4. Improvements and Future Work

[17] Improvements in this aquaculture-based system that are being considered focus on improving constraints on growth rates, including bio-marking [*Kaehler and McQuaid*, 1999; *Day et al.*, 1995; *Pirker and Schiel*, 1993], entire batch measuring, and tagging or etching every individual animal. In addition, because mortality rates were relatively low, it is likely that fewer animals can be grown. Because most of the animals used in this study were juveniles (less than 2 years-old), we are currently culturing adults to further refine the *M. edulis* paleotemperature relationship. Ongoing work includes growing *M. edulis* juveniles and adults from western Greenland to determine if there are any large-scale geographic trends in shell carbonate as a function of growing conditions. Other work may include similar aquaculture-based experimentation to evaluate the effects of temperature and salinity on trace metal uptake in bivalve shell carbonate, provided the trace element ratios in the water can be adequately controlled. A potential benefit of this work would be to eliminate an unknown in the paleotemperature relationship, thus allowing a well-constrained paleoenvironmental reconstruction to be made.

5. Summary

[18] During this study we have addressed several limitations associated with past aquaculture-based isotope calibrations, including the following: (1) Precisely measured water temperature and $\delta^{18}O_w$ values were used in the development of a paleotemperature equation; (2) a wide range of salinity and temperatures were utilized during culture; (3) multiple bivalves (23–28) were grown at each temperature to assess shell isotopic variability; and (4) a species-specific bivalve isotope paleothermometer was developed. The relationships among water temperature, shell carbonate ($\delta^{18}O_c$), and water isotopic composition ($\delta^{18}O_w$) have been thoroughly examined for *M. edulis*, hence we are confident in using this bivalve for reconstructing paleoenvironments. Still, past water temperatures are unknown, and values for the oxygen isotopic composition of ocean waters, especially coastal zones, is not well-constrained and need to be estimated [e.g., *Rye and Sommer*, 1980]. If $\delta^{18}O_w$ can be estimated, or determined independently, this species-specific aquaculture-based methodology can improve environmental reconstructions. Further, this experimental design offers the opportunity to assess many growth-related isotope effects (age, growth rates, life processes, etc.) in a relatively short time, and to determine if shell carbonate is precipitated in equilibrium with ambient water.

Appendix A

[19] Additional information is provided (Table A1) including all shell data used in this study (isotopic values, shell length, growth), culture conditions (isotopic composition of water, salinity, temperature), as well as predicted temperatures based on the paleotemperature relationship for *M. edulis*.

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