Geo-Mar Lett
DOI 10.1007/s00367-008-0112-8

ORIGINAL

Experimentally determined Mg/Ca and Sr/Ca ratios in juvenile bivalve calcite for *Mytilus edulis*: implications for paleotemperature reconstructions

Alan D. Wanamaker Jr · Karl J. Kreutz · Tiffany Wilson · Harold W. Borns Jr · Douglas S. Introne · Scott Feindel

Received: 25 October 2007 / Accepted: 15 February 2008

Abstract To further evaluate the potential use of Mg/Ca and Sr/Ca ratios as a paleothermometer in the shell carbonate of the blue mussel *Mytilus edulis*, we grew juvenile mussels (~15 mm shell height; <2 years old) collected from Maine, USA, in controlled environments for 4 months. 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). During the experiment, water Mg/Ca and Sr/Ca molar ratios were monitored weekly, and showed little variation across all salinity and temperature ranges. Data from sampled shells including all salinity treatments yielded relatively poor relationships between shell elemental chemistry and water temperatures. However, if only the low salinity treatment data (23) are used, the relationships between shell elemental chemistry and water temperature improve moderately. Based on the data presented here, it may be possible to use Mg/Ca and Sr/Ca ratios from the shell carbonate of juvenile *M. edulis* to reconstruct paleotemperatures in estuarine settings (salinity below 24) with a corresponding RMSE (root mean squared error; 95% confidence interval) of ±2.4°C and ±2.8°C, respectively. In order for this methodology to be statistically meaningful, water temperature changes must be rather large, as the errors associated with using Mg/Ca and Sr/Ca ratios from the shell material of *M. edulis* are substantial. Further work is required to determine if the findings presented here can be duplicated, and if the potential salinity effect is pervasive.

A. D. Wanamaker Jr (✉) · K. J. Kreutz · H. W. Borns Jr · D. S. Introne
Climate Change Institute, University of Maine,
Orono, ME 04469, USA
e-mail: a.wanamaker@bangor.ac.uk

A. D. Wanamaker Jr · K. J. Kreutz · H. W. Borns Jr · D. S. Introne
Department of Earth Sciences, University of Maine,
Orono, ME 04469, USA

T. Wilson
Sawyer Environmental Chemistry Research Laboratory,
University of Maine,
Orono, ME 04469, USA

S. Feindel
Darling Marine Center, University of Maine,
Walpole, ME 04573, USA

Present address:
A. D. Wanamaker Jr
School of Ocean Sciences, Bangor University,
Menai Bridge, Anglesey,
Bangor, Wales LL59 5AB, UK

Introduction

The use of minor elemental ratios (Mg/Ca, Sr/Ca) in some biogenic carbonates (mainly corals and foraminifers) is indispensable for reconstructing water temperatures, because they are often independent of other environmental variables, especially salinity. The development of such geochemical tools is appealing because many nearshore and estuarine settings experience large fluctuations in the oxygen isotopic composition of water ($\delta^{18}O_w$), which is related to salinity. Because the oxygen isotopic composition of carbonates ($\delta^{18}O_c$) is a function of both water temperature and $\delta^{18}O_w$, it is often difficult to separate $\delta^{18}O_w$ effects from temperature effects (e.g., Epstein et al. 1953; Emiliani 1966). Although many species-specific skeletal Mg/Ca and Sr/Ca temperature relationships have been developed for corals and foraminifers (e.g., Beck et al. 1992; Shen et al. 1996; Lea et al. 1999; Elderfield and

Ganssen 2000; Quinn and Sampson 2002), relatively few of these relationships have been reliably established using bivalves (e.g., Klein et al. 1996a; Lazareth et al. 2003). Bivalve elemental geochemical proxies have the potential to improve our understanding of global environmental change and nutrient cycling, by providing critical data from marine and estuarine realms where instrumental data are absent or incomplete.

The motivation to develop elemental geochemical proxies (e.g., Mg/Ca, Sr/Ca, Ba/Ca) for bivalves is obvious because (1) many bivalve taxa are sensitive recorders of their environment via shell growth records (e.g., Jones et al. 1989; Witbaard et al. 1999; Richardson 2001; Schöne et al. 2003) and their shell isotopic compositions (e.g., Weidman et al. 1994; Elliot et al. 2003; Wanamaker et al. 2007, 2008), (2) bivalves are globally distributed, (3) bivalves live in a wide variety of environments and water depths, (4) bivalves are abundantly available through geologic time (e.g., Krantz et al. 1987), (5) some bivalves are extremely long-lived, reaching several hundred years of age (e.g., Schöne et al. 2005), and (6) bivalves deposit sub-daily to annual growth bands in their shells (e.g., Clark 1976; Jones 1980; Richardson 1989)—thus, paleoreconstructions can often be high-resolution and well-constrained temporally. Progress in developing a suite of elemental ratio proxies for bivalves has been hindered for several reasons: (1) many bivalves exhibit strong biologic effects during elemental uptake (e.g., Gillikin et al. 2005; Freitas et al. 2006), (2) kinetic effects during biomineralization may impact elemental uptake (e.g., Takesue and van Green 2004; Lorrain et al. 2005; Carré et al. 2006), (3) ontogeny may impact shell geochemistry (e.g., Freitas et al. 2005; Gillikin et al. 2005), (4) hydrographic factors such as salinity, time during season (e.g., spring bloom or wintertime), or food levels may impact elemental uptake in certain bivalves (Klein et al. 1996b; Vander Putten et al. 2000), and (5) relatively few laboratory-based/field-based experiments have been successfully implemented to test and validate the potential of elemental geochemical proxies in bivalves (see Gillikin et al. 2006a for a recent example). Based on these reasons, there is uncertainty associated with using minor and trace elemental ratios in bivalve skeletal structures to reconstruct paleoenvironments.

In order to use elemental ratios from bivalve shells as environmental proxies, each species should be rigorously calibrated and validated (e.g., Freitas et al. 2006). A substantial benefit of laboratory-based studies is that each environmental variable (food, temperature, salinity, etc.) can be evaluated independently for its impact on minor elemental uptake in bivalves, which is difficult to do in situ, because it can be complex to deconvolve the simultaneous effects of multiple environmental forcings. The obvious benefit of field-based calibrations is that they represent "real" world conditions. Recently, Gillikin et al. (2006a) provided evidence for *Mytilus edulis* showing that shell Ba/Ca ratios broadly reflected the Ba/Ca ratios of water in both field-based and a laboratory-based study. Thus, both have the potential to unlock many of the challenging biomineralization processes in bivalves. Further, it has been shown that many bivalve species precipitate their shells in oxygen isotope equilibrium with the surrounding water (e.g., Epstein et al. 1953; Grossman and Ku 1986; Chauvaud et al. 2005; Wanamaker et al. 2007). Therefore, if elemental ratio geochemical proxies are successfully developed for a suite of bivalves, then a multiproxy approach (oxygen isotopes and element ratios) can be used to unravel past water temperature and $\delta^{18}O_w$ conditions (e.g., Rosenthal et al. 2000).

The relative abundance in both modern and ancient coastal environments, and broad geographical distribution of the intertidal bivalve *M. edulis* make it an ideal species for paleoenvironmental reconstructions. The preservation of fossil *M. edulis* shell material is somewhat variable, ranging from very poor to nearly pristine. However, previous studies investigating the minor and trace elemental uptake in the skeletal structures of adult and juvenile *M. edulis* have yielded contrasting results. Dodd (1965) suggested that the percent $SrCO_3$ sampled from the outer prismatic shell layer (calcite) of *M. edulis diegensis* and *M. edulis edulis* were positively correlated with sea surface temperature (SST), while the % $MgCO_3$ did not correlate with SST at a statistically significant level for *M. edulis edulis*. The specimens used by Dodd (1965) ranged in size (~9–25 mm shell height) and age (juveniles to adults), and they were collected from coastal locations from Washington to southern California, USA. However, Carter and Seed (1998) suggested that Dodd (1965) might have confused *M. edulis* with *Mytilus trossulus*, although this has not been confirmed through genetic sequencing. Lorens and Bender (1980) demonstrated that skeletal calcite Sr/Ca ratios in juvenile *M. edulis* (<1 year old; 3–10 mm shell height) were linearly related to the Sr/Ca composition of the water used during a culture study, while skeletal Mg/Ca ratios in skeletal calcite were significantly impacted by vital effects. Specifically, Lorens and Bender (1980) suggested that high seawater magnesium concentrations (Mg/Ca water molar ratios >7) inhibited the growth of shell calcite, and they concluded that this process was regulated by *M. edulis* during biomineralization. Lorens and Bender (1980) also concluded that there were no statistically significant growth rate effects on either Sr/Ca or Mg/Ca ratios in shell calcite for *M. edulis* during their laboratory-based study.

More recently, Vander Putten et al. (2000) found significant complications in using Sr/Ca and Mg/Ca shell ratios from *M. edulis* as a proxy for SST in a field-based study. Vander Putten et al. (2000) reported that shell Sr/Ca

ratios showed no systematic relationship with SST, while Mg/Ca ratios covaried with SST for the first 50 to 63 days of the ~80 day experiment (for the four shells collected and sampled), but eventually this relationship collapsed. During the period of covariation of skeletal Mg/Ca ratios and water temperature, Vander Putten et al. (2000) demonstrated the following relationship for *M. edulis*:

$$(\mathrm{Mg/Ca}*1,000) = 0.70(\pm 0.02)*T - 0.63(\pm 0.29); \quad r^2 = 0.83.$$

However, the shell Mg/Ca–temperature equation presented by Vander Putten et al. (2000) did not include two other shells that were also time-constrained, and it is likely that this would have influenced the above relationship. The results presented by Vander Putten et al. (2000) illustrate that biological effects likely impact *M. edulis* during biomineralization.

Earlier, Klein et al. (1996a) demonstrated a strong and persistent relationship between SST and shell Mg/Ca ratios over a period of 1 year for *M. trossulus*, a close relative to *M. edulis*. Formerly, *M. trossulus* was identified as *M. edulis* (Koehn 1991), and the ranges of these two ecologically and morphologically similar species overlap throughout the north Atlantic, so that specimens of *M. trossulus* can be easily confused with *M. edulis* (see Wanamaker et al. 2007). Klein et al. (1996a) collected two adult mussels, which were notched at the ventral margin and redeployed in Squirrel Cove, British Columbia, Canada, and collected 1 year later. Analyses of the new shell material revealed the following relationship between water temperature and shell Mg/Ca ratios:

$$(\mathrm{Mg/Ca}*1,000) = 0.30(\pm 0.04)*T + 2.25(\pm 0.63); \quad r^2 = 0.74.$$

Although the reported relationship noted by Klein et al. (1996a) is rather strong, it is based on only two shells, and these results have not yet been duplicated for any other *Mytilus* species. The differences noted in the strength and reliability of the relationships between shell Mg/Ca ratios with SST in *M. trossulus* and *M. edulis* reported by Klein et al. (1996a) and Vander Putten et al. (2000) illustrate the importance of developing species-specific elemental ratio proxies for bivalves.

Previously, Wanamaker et al. (2006, 2007) quantified the shell isotopic variability (δ^{18}O, δ^{13}C) of juvenile and adult *M. edulis* (collected from Maine and Greenland) cultured under controlled conditions. During this study, we investigate the effects of growing conditions (salinity, water temperature) on the uptake of Mg and Sr into the shells of juvenile *M. edulis* collected from Maine, USA. We present a well-constrained, culture-based calibration of skeletal Mg/Ca and Sr/Ca for *M. edulis*, and we further evaluate the use of skeletal Mg/Ca and Sr/Ca ratios in the calcitic shells of the common blue mussel as paleothermometers.

Materials and methods

Animal collection

In early July of 2003, 4,800 juvenile (<2 years old) *M. edulis*, of ~15 mm shell length on average, were collected in Salt Bay, Damariscotta, Maine, USA. Wanamaker et al. (2007) broadly classified *M. edulis* juveniles as <2 years old, although the adult stage is reached after sexual maturation, which may occur slightly earlier (1–2 years old; e.g., Seed and Suchanek 1992). Because this determination (sexual maturation) requires significant additional work, we use the same broad definition (i.e., adults are >2 years old) outlined by Wanamaker et al. (2007) in this paper to be consistent. The approximate water temperature at collection was 14°C, and the salinity was ~31. 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 was equally dispersed in each temperature/salinity configuration. Animals were acclimated to the culture temperature gradually for a period of 1 week. Based on 100 random samples, the size range was 9.8–20.2 mm, with a mean of 15.3 mm (1σ=2.4 mm).

Experimental design

We used a recirculating water bath system at the Darling Marine Center to achieve four temperature settings (7°C, 11°C, 15°C, and 19±0.5°C) and three salinity settings (23, 28, and 32±0.1; for a complete description, see 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. Two buckets (A and B) contained experimental replicates for each temperature and salinity treatment, while the other two buckets contained water for subsequent water changes. The temperature of each bath was measured with a HOBO® H8 data logger every 30 min with an accuracy of ±0.5°C (Table 1). Although the pH of the water used during the culture period was not continuously monitored, it generally ranged between 8.0 and 8.2.

Water was collected via the flowing seawater laboratory at the Darling Marine Center, and was pumped from the Damariscotta River at 10 m depth below mean low tide.

Table 1 Temperatures treatments (with standard deviations, 1σ) for each of four recirculating systems used during the culture period

Average water temperatures (°C) (1σ)
7.08 (0.27)
10.81 (0.26)
15.19 (0.17)
19.35 (0.27)

Water was mixed with well water to obtain the desired salinity (23, 28, and 32±0.1), and then stored in 2,460-l containers indoors and sealed. Salinity measurements were made using a YSI® model 85 oxygen, conductivity, salinity, and temperature system with an accuracy of ±0.1 PSU.

Mussels were fed a shellfish diet (Instant Algae Marine Microalgae Concentrate, Reed Mariculture, Inc.) twice daily (total of 8×10^9 cells/day), of diluted algal paste made with water from which they grew. Five mussels in each temperature and salinity configuration were tagged on 14 July 2003 with a numbered shellfish tag (~3 mm) directly adhered to each shell. The shell length for each of these mussels was determined with digital calipers (±0.01 mm) by measuring along the maximum growth axis, and monitored monthly. We attempted to use the average estimated bulk growth rates for each temperature/salinity configuration; however, because so few bivalves were monitored for growth (and some mortality occurred in tagged specimens) during the experiment, and because growth rates were very low (0.06–0.14 mm/month) and highly variable, we will not consider growth rate effects on shell chemistry in this paper.

Unfiltered water samples (60 ml) from each bucket were collected weekly to monitor water chemistry ([Ca], [Mg], and [Sr]) prior to water changes, although there was no difference noted in water chemistry when water was collected prior to, and after water changes. Water samples were stored in glass vials, and refrigerated prior to analysis.

For subsequent geochemical analyses, ~50 *M. edulis* individual specimens were harvested and sampled after 4 months of growth (14 July 2003–13 October 2003). Unfortunately, due to some constraints, the sampling strategy used caused a slight bias of the samples collected from the temperature and salinity treatments. A more detailed discussion of this factor, along with possible implications, is presented in the discussion below.

Sample preparation and analysis

After soft-tissue removal, 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. The outer calcitic edge of each valve was micro-milled using a variable speed mounted drill and binocular microscope with ×6.5 to ×40 magnification. Great care was taken to ensure that only new shell carbonate was removed during sampling, and generally less than 1 mm of linear shell material was removed. Wanamaker et al. (2006) used an identical sampling strategy for looking at shell chemistry (carbon and oxygen isotopes) of cultured *M. edulis*, and after 3 months of new growth, there was no evidence of mixed (old and new) carbonate. Thus, we feel confident that the carbonate samples presented in this study represent the laboratory conditions only. Further, if there was a mixture of old and new carbonate, there would be a systematic offset based on the water temperature at the time of collection. Because *M. edulis* has a calcitic outer prismatic layer and an inner aragonitic layer (see Vander Putten et al. 2000), X-ray diffraction was performed on five shell samples from shell edges to rule out a mixed matrix of calcite and aragonite. All measurements indicate that there was no aragonite present near the ventral margin.

For each carbonate sample, approximately 1 mg of shell powder was weighed into a clean 15-ml polypropylene centrifuge tube. Each sample was dissolved in 5 ml of 1% (1.6 M) GFS (GFS Chemicals, Inc.) redistilled grade HNO_3. Dissolved samples were kept under refrigeration until analysis. Ca, Mg, and Sr concentrations were quantified using a Perkin-Elmer 3300XL (axial view) ICP-AES with a GemCone™ nebulizer and cyclonic spray chamber sample introduction system. The wavelengths of the element lines used were Ca 317.933 nm, Mg 285.213 nm, and Sr 421.552 nm. An internal standard (Y 360.081 nm) was added online with each sample, and measured to compensate for variations in sample introduction efficiency. Linear calibration of emission intensity versus concentration of each element was performed using serial dilutions of a multi-element liquid stock standard (Ultra Scientific), with a target correlation coefficient of >0.9998 for each element. Data precision was optimized by measuring ten emission intensities for each calibration standard and sample, with the goal of achieving a relative standard deviation (RSD) of <5% for each element. The average RSD of emission intensity readings for all samples was 2.32% for Ca, 3.86% for Mg, and 3.02% for Sr. Only six (12%) of the samples did not meet the precision goal of 5% RSD, and of those, only two samples were >10% RSD. Data accuracy was assessed by measuring liquid reference solutions with known concentrations of Ca, Mg, and Sr. Percent recoveries for these measured reference solutions were 86.2, 101, and 101% for Ca, Mg, and Sr, respectively. Analytical drift was monitored by running a mid-range standard every ten samples.

Each water sample was diluted 1:11, and acidified to pH<2 with HNO_3 into a clean 15-ml polypropylene centrifuge tube. Once water samples were diluted, the same procedure as described above (carbonate analysis) was used. The average RSD of emission intensity readings for

all samples was 3.35% for Ca, 4.04% for Mg, and 3.73% for Sr. In all, 47 (16%) of the samples did not meet the precision goal of 5% RSD, and of those, 15 samples were >10% RSD. These latter samples were not used in this study.

Results

Culture conditions

Water temperature values from this experiment were steady, and are shown in Table 1. Each salinity treatment was mixed (see "Materials and methods" above) to the desired salinity (23, 28, 32 ±0.1). Weekly water chemistry values are shown in Fig. 1. Both water Mg/Ca and Sr/Ca ratios during the calibration period remained relatively constant. Water Mg/Ca molar ratios and Sr/Ca ×1,000 molar ratios were calculated by averaging all 24 buckets each week during the experiment, and they were determined to be Mg/Ca=5.01±0.18 and Sr/Ca×1,000=8.09±0.17. Because Sr, Mg, and Ca concentrations are conservative with respect to salinity for salinities above ~10 (e.g., Dodd and Crisp 1982), we reduced the data from all of the salinity and temperature conditions into one weekly value for Mg/Ca and Sr/Ca ratios to simplify the presentation of the culture environments. Although small-scale variability in salinity cannot be ruled out, it is likely that Sr/Ca and Mg/Ca ratios in the water would have remained unchanged.

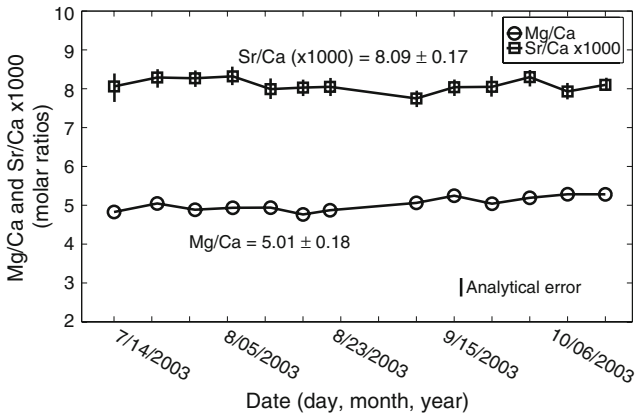

Fig. 1 Mg/Ca molar ratios (*open circles*) and Sr/Ca molar ratios ×1,000 (*squares*) of water during culture period are shown. Each weekly value was determined by averaging all 24 buckets, which included four temperature ranges and three salinity ranges (plus replicates). Weekly standard deviations for Sr/Ca are shown, but the weekly standard deviations for Mg/Ca were smaller than the symbols used here. For Mg/Ca molar ratios, weekly water standard deviations ranged from ±0.07 to ±0.21 and averaged only ±0.12, and Sr/Ca molar ratios ×1,000 weekly water standard deviations ranged from ±0.13 to ±0.32 and averaged ±0.20 for the duration of the culture period. The average Mg/Ca molar ratio was 5.01±0.18, and the Sr/Ca molar ratio ×1,000 was 8.09±0.17

Relationships between shell Mg/Ca and Sr/Ca ratios and environmental conditions

The relationships between shell Mg/Ca (×1,000) and Sr/Ca ratios (×1,000) and water temperature for all salinity conditions (23, 28, 32) are shown in Fig. 2. Least squares regression was used to generate the relationships between skeletal Mg/Ca, Sr/Ca ratios for *M. edulis* and water temperature. Root mean squared error (RMSE) was calculated at the 95% confidence interval (CI), and quoted errors on the slope and intercepts were reported at the 95% CI. Resulting equations are as follows:

$$\mathrm{Mg/Ca}(\times 1,000) = (0.75(\pm 0.22) * T^{\circ}\mathrm{C}) + 5.44(\pm 0.31)$$
$$r^2 = 0.49,\ n = 49,\ p < 0.0001;\ \mathrm{RMSE} = 3.67 \quad (1)$$

and

$$\mathrm{Sr/Ca}(\times 1,000) = (0.014(\pm 0.0054) * T^{\circ}\mathrm{C}) + 1.25(\pm 0.073)$$
$$r^2 = 0.34,\ n = 49,\ p < 0.0001;\ \mathrm{RMSE} = 0.087. \quad (2)$$

Water temperature is an important factor influencing the uptake of Mg and Sr into the shell carbonate of our mussels (49% for Mg/Ca, and 34% for Sr/Ca). However, environ-

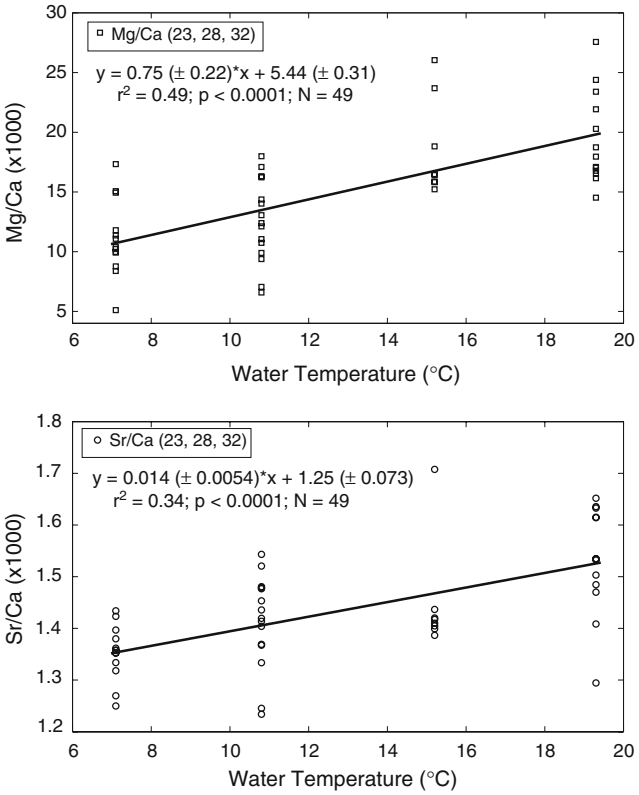

Fig. 2 Shell Mg/Ca (*top*, *squares*) and Sr/Ca (*bottom*, *circles*) ratios (×1,000) versus water temperature are shown for all salinity ranges (23, 28, 32)

mental factors other than water temperature, such as vital effects, likely impact that elemental uptake. There is a large scatter of individual Mg/Ca and Sr/Ca ratios per temperature (Fig. 2), and some of this can be attributed to analytical error. Because there is an error associated with measuring each element (Mg, Sr, Ca) in the shell material, when the elements are expressed as ratios (Mg/Ca and Sr/Ca) the total error must be propagated, which can be somewhat large (up to 9% RSD). To determine if salinity impacted the uptake of Mg and Sr into shell carbonate, we compared shell Mg/Ca and Sr/Ca ratios and water temperature for each salinity treatment separately (Figs. 3 and 4). As illustrated in Figs. 3 and 4, salinity had a large effect on shell Mg/Ca and Sr/Ca ratios. The weakest relationship between shell elemental ratios (Mg/Ca and Sr/Ca) and water temperatures occurred at the high salinity treatment (32), while the strongest relationship occurred at the lowest salinity treatment (23). The mid-range salinity treatment (28) was intermediate. The derived relationships between Mg/Ca and Sr/Ca ratios for each salinity treatment are shown in Figs. 3 and 4. Here, we report the strongest relationships between shell elemental chemistry (Mg/Ca and Sr/Ca) and water temperature for the lowest salinity treatment environment (23):

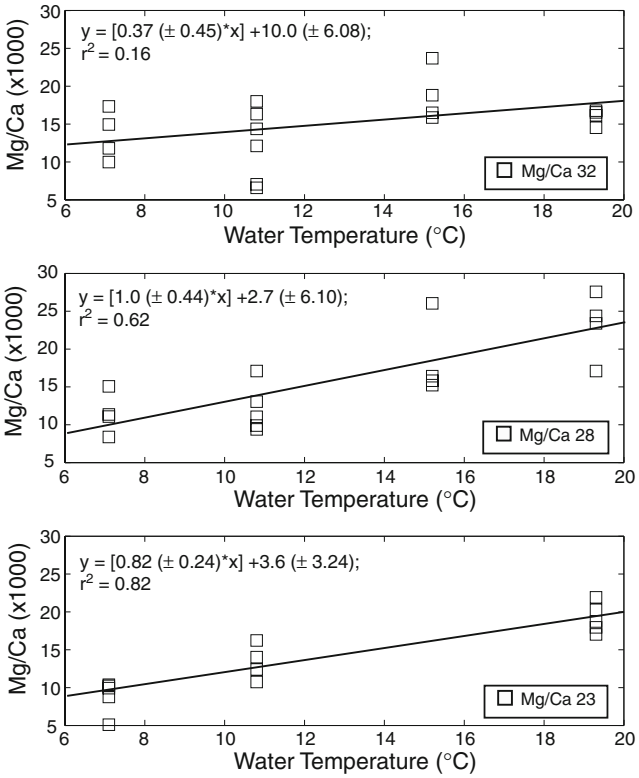

Fig. 3 Shell Mg/Ca (×1,000) (*squares*) versus water temperature are shown for each salinity configuration (*top* 32, *middle* 28, *bottom* 23). Low salinity (23) treatments are based only on three temperature ranges, and some temperature/salinity conditions have fewer than five shell samples

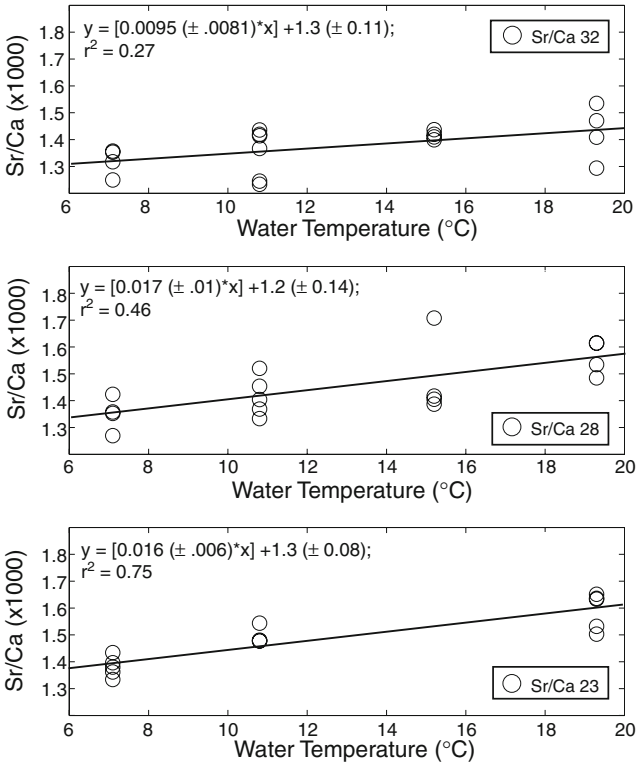

Fig. 4 Shell Sr/Ca (×1,000) (*squares*) versus water temperature are shown for each salinity configuration (*top* 32, *middle* 28, *bottom* 23). Low salinity (23) treatments are based only on three temperature ranges, and some temperature/salinity conditions have fewer than five shell samples

$$\mathrm{Mg/Ca} = (0.82(\pm 0.24) * T^{\circ}\mathrm{C}) + 3.60(\pm 3.24) \quad r^2 = 0.82,\ n = 14,\ p < 0.0001 \tag{3}$$

and

$$\mathrm{Sr/Ca} = (0.016(\pm 0.006) * T^{\circ}\mathrm{C}) + 1.30(\pm 0.08) \quad r^2 = 0.75,\ n = 14,\ p < 0.0001. \tag{4}$$

These results demonstrate that a moderate interaction exists between salinity and the uptake of Mg and Sr into shell carbonate. However, due to the uneven sampling, only three temperatures (missing 15.19°C) were represented in the lowest salinity treatments, compared to four temperatures from the other treatments. To determine if this sampling bias influenced the apparent salinity effect, we removed the 15.19°C temperature data from the other salinity treatments. The resulting relationship between water temperature and Mg/Ca×1,000 ratios improved slightly (r^2=0.70 compared to r^2=0.62) at the middle salinity treatment (28), but it decreased slightly (r^2=0.11 compared to r^2=0.16) at the highest salinity treatment (32). For Sr/Ca×1,000 and water temperature, the relationship

improved substantially (r^2=0.67 compared to r^2=0.46) at the middle salinity treatment (28), but it decreased slightly (r^2=0.24 compared to r^2=0.27) at the highest salinity treatment (32). Based on these results, it appears that the sampling bias did inflate the suggested salinity effect, but at the highest salinity treatment (32), the relationships between Sr/Ca and Mg/Ca ratios×1,000 and water temperature are still rather poor. Therefore, we conclude that there is a salinity interaction for *M. edulis* juveniles based on the salinity gradient used in this study. Further work is required to determine if adult *M. edulis* are impacted by a salinity effect during calcification.

Using the best relationship noted between shell Mg/Ca and Sr/Ca ratios and water temperatures, and rearranging Eqs. 3 and 4, we present the following Mg/Ca and Sr/Ca ratio×1,000 paleotemperature relationships for *M. edulis* juveniles based on the lowest salinity treatments (23):

$$T°\mathrm{C} = [1.00(\pm 0.29) * \mathrm{Mg/Ca}] - 1.38(\pm 4.27)$$
$$r^2 = 0.82,\ n = 14,\ p < 0.0001;\ \mathrm{RMSE} = 2.4°\mathrm{C} \quad (5)$$

and

$$T°\mathrm{C} = [46.04(\pm 16.66) * \mathrm{Sr/Ca}] - 56.02(\pm 24.84)$$
$$r^2 = 0.75,\ n = 14,\ p < 0.0001;\ \mathrm{RMSE} = 2.8°\mathrm{C}\ . \quad (6)$$

Discussion

Comparison to other bivalve calcite Mg/Ca–temperature relationships

The Mg/Ca–temperature relationships developed for juvenile *M. edulis* presented here (Eq. 5) have steep slopes and high intercepts, compared to those published by Klein et al. (1996a) and Vander Putten et al. (2000). However, these equations have been based on adult bivalves. In addition, the range of Mg/Ca ratios presented in this study far exceeds the ranges noted by Klein et al. (1996a) and Vander Putten et al. (2000), which may indicate an ontogenetic effect. By contrast, our results are somewhat similar to the Mg/Ca–temperature relationship for *Pinna nobilis* (derived from the younger portions of the shell; see Freitas et al. 2005). Although the Mg/Ca ratios are generally higher in *P. nobilis*, the slopes noted in this study and by Freitas et al. (2005) are relatively close (~0.8–1.0). It is interesting to note that the shell Mg/Ca results presented here and by Freitas et al. (2005) for relatively young bivalves (juvenile to young adult) showed higher than normal shell Mg/Ca ratios when compared to the studies by Klein et al. (1996a) and Vander Putten et al. (2000). Our study corroborates previous findings, which indicate that Mg/Ca–temperature relationships are largely species-specific, and substantial work is required to better understand the incorporation of trace and minor elements into the shell structure.

Possible salinity effects during biomineralization

The results presented here illustrate the complexity of Mg and Sr incorporation into juvenile bivalve shell carbonate (Figs. 3 and 4). Further, it is apparent that thermodynamics alone do not control skeletal elemental uptake in many bivalve species. Biological or vital effects also seem to influence elemental uptake. Currently, it is unclear if the salinity effect noted in this study is limited to juvenile *M. edulis*. Because we considered only juvenile mussels in this study, it is difficult to compare our results directly with other studies that use only adults (e.g., Klein et al. 1996a; Vander Putten et al. 2000); little research has focused on the suitability of using juvenile shell portions in paleoenvironmental studies (e.g., Freitas et al. 2005). It is interesting to note that the bivalve *M. trossulus* (a close cousin to *M. edulis*) primarily incorporated Mg into the shell structure as a function of temperature (Klein et al. 1996a), while *M. edulis* did so reliably only in the middle and lowest salinity treatments (23 and 28). Are these differences due to a species effect, or an ontogenetic effect? However, as the biomineralization process is assumed to be similar for adults and juveniles, we explore some potential mechanisms that may explain the inferred salinity effect noted here. Few studies have reported salinity effects on Mg and Sr uptake during biomineralization. Lorens and Bender (1980) suggested that *M. edulis* juvenile specimens that were cultured in elevated Mg concentrations were unable to mediate the composition of the outer extrapallial fluid (EPF).

Klein et al. (1996b) reported that Sr/Ca ratios in skeletal calcite from *M. trossulus* were influenced first by metabolic activity, and second by salinity. In this study, both skeletal calcite Mg/Ca and Sr/Ca ratios for *M. edulis* were impacted by salinity, while only Sr incorporation was affected by salinity for *M. trossulus* (Klein et al. 1996b). Although the mechanisms are still poorly understood, elucidating the small differences in biomineralization processes between *M. edulis* and *M. trossulus*, for both adults and juveniles, is of great importance. In mollusks, biomineralization occurs within the outer EPF, which is contained between the shell surface and the mantle epithelium (Wheeler 1992). Because the EPF is isolated from ambient water, it may have different chemical compositions than seawater (e.g., Watabe 1988). Klein et al. (1996b) proposed a calcification model that related changes in carbon isotope composition (δ^{13}C) and Sr/Ca ratios of shell carbonate to changes in the chemical composition of the EPF. Their model specifically relates mantle metabolic pumping via two pathways (intercellular and intracellular) to the Sr/Ca concentration and δ^{13}C composition in the EPF (see Fig. 8 in Klein et al. 1996b for details). Klein et al.

(1996b) suggested that low metabolic pumping (intercellular route) results in EPF representative of ambient water, while high metabolic pumping (intercellular and intracellular routes) leads to EPF that is modified by a selective transport of Ca. Using the Klein et al. (1996b) calcification model, we infer that *M. edulis* grown in the low salinity treatment (23) were primarily under the influence of low metabolic pumping, while metabolic pumping increased as salinity increased for *M. edulis*.

Unfortunately, the current Klein et al. (1996b) calcification model does not fully explain our results, because *M. edulis* shell Mg/Ca ratios were similarly impacted by salinity, unlike *M. trossulus* (Klein et al. 1996a). Thus, we explore a slight modification from the Klein et al. (1996b) calcification model.

Recently, Wanamaker et al. (2007) suggested that shell $\delta^{13}C$ values in *M. edulis* (juveniles and adults) were substantially impacted by metabolic activity in a laboratory-based study. It should be noted that the authors only estimated $\delta^{13}C$ DIC (dissolved inorganic carbon) water values based on an established salinity/$\delta^{13}C$ DIC water mixing line. Further, Gillikin et al. (2006b) reported no such salinity effect on shell $\delta^{13}C$ values for a field-based study for adult *M. edulis*, and they suggested that the metabolic carbon contribution was only ~10%, whereas Wanamaker et al. (2007) reported values up to 20%. The assumed metabolic effect reported by Wanamaker et al. (2007) increased with increasing salinity. Thus, at low salinity conditions (~23) shell $\delta^{13}C$ values closely reflected estimated $\delta^{13}C$ DIC water values. This result is similar to the findings reported in this study. Therefore, we propose that in the low salinity treatments (23), biological processes controlling the composition of the EPF for *M. edulis* better reflect ambient water conditions (Mg/Ca, Sr/Ca, and $\delta^{13}C$ DIC; intercellular route, possibly as described by Klein et al. 1996b), while at relatively higher salinities, the EPF is impacted by both metabolic and ambient sources. We suggest that in relatively low salinities, *M. edulis* is better suited to selectively incorporate/mediate the composition of the EPF during biomineralization (e.g., Lorens and Bender 1980), because the Sr and Mg concentrations are lower in the ambient water. However, under high salinity conditions (and increased ionic activity), thermodynamic processes controlling the incorporation of Mg and Sr into the skeletal calcite structure may be inhibited by the inability of *M. edulis* to selectively incorporate minor elements into the crystal lattice in a manner that reflects the ambient water.

Implications for using shell Mg/Ca and Sr/Ca ratios in *M. edulis* as a paleothermometer

During this experiment, we have investigated the effects of temperature and salinity on Mg and Sr uptake in the skeletal calcite of juvenile *M. edulis*. Our results are consistent with previously published studies that indicate that Sr/Ca thermometry is not as good/reliable as Mg/Ca thermometry. We caution that results presented here have limitations (based on juveniles only, and with sampling biases), and further validation is needed prior to using the derived Mg/Ca and Sr/Ca–temperature relationships noted here. The most robust relationships between Mg/Ca and Sr/Ca ratios and water temperature occurred in the low salinity treatment (23; Eqs. 5 and 6). Although it is not generally appropriate to use shell Mg/Ca and Sr/Ca ratios from juvenile *M. edulis* to reconstruct water temperature, it may be possible to use shell Mg/Ca and Sr/Ca ratios to broadly reconstruct past water temperatures (±2.4°C for Mg/Ca [×1,000] and ±2.8°C for Sr/Ca [×1,000]) in low salinity environments, such as upper estuaries. Given the previous uncertainty using Sr/Ca ratios in other bivalves as paleothermometers (Klein et al. 1996b; Gillikin et al. 2005; Lorrain et al. 2005; Carré et al. 2006), and the results noted here, we are not very confident in using Sr/Ca ratios (Eq. 6) in juvenile *M. edulis* shells as paleothermometers. A field-based study with juvenile to adult *M. edulis*, in an upper estuary (with a large salinity gradient) where environmental conditions are closely monitored, would provide a good framework to test the validity of the Mg/Ca and Sr/Ca paleothermometers presented here.

Conclusions

During this study, we have quantified the effects of water temperature and salinity on the uptake of skeletal Mg/Ca and Sr/Ca ratios in juvenile *M. edulis* specimens collected from Maine, USA. The aquaculture design used here allowed for carefully controlled and precisely measured growing conditions (water temperature, salinity, water Mg/Ca, and Sr/Ca ratios) to be used in the development of paleotemperature equations. A wide range of salinity and temperature conditions were utilized during the culture period, and multiple bivalves were grown at most temperature and salinity ranges. Mg and Sr uptake during shell biomineralization was influenced by salinity gradients. These findings illustrate the benefit of culturing biogenic carbonates under varying environmental conditions separately, where the effect of each environmental variable can be accurately deconvolved. Our results corroborate previous findings, which suggest that shell Mg/Ca and Sr/Ca ratios from *M. edulis* are not generally reliable temperature proxies. However, based on the results presented here, it may be possible to use shell Mg/Ca and Sr/Ca ratios from juvenile *M. edulis* to broadly reconstruct water temperatures from an upper estuarine setting. Further work from a field setting is required to determine if the results

presented here can be duplicated, and if the potential salinity effect is persistent.

Acknowledgments We thank Paul Rawson (University of Maine, School of Marine Sciences) for his advice on mussel cultivation, Timothy Miller (University of Maine, Darling Marine Center) for help with logistics and space, and Marty Yates for X-ray diffraction analysis (University of Maine, Earth Sciences). This research was funded through the National Science Foundation (NSF ATM-0222553). We thank David Gillikin, Claire Lazareth, and Dorothee Hippler for constructive criticisms and suggestions that greatly improved this manuscript. Finally, we thank the Guest Editors, Darren Gröcke and David Gillikin, for their hard work and for bringing this special issue to fruition.

References

Beck JW, Edwards RL, Ito E, Taylor FW, Recy J, Rougerie F, Joannot P, Henin C (1992) Sea-surface temperature from coral skeletal strontium calcium ratios. Science 257:644–647

Carré M, Bentaleb I, Bruguier O, Ordinola E, Barrett NT, Fontugne M (2006) Calcification rate influence on trace element concentrations in aragonitic bivalve shells: evidences and mechanisms. Geochim Cosmochim Acta 70:4906–4920

Carter JG, Seed R (1998) Thermal potential and mineralogical evolution in *Mytilus* (Mollusca; Bivalvia). In: Johnston PA, Haggart JW (eds) Bivalves: an eon of evolution. University of Calgary Press, Calgary, pp 87–117

Chauvaud L, Lorrain A, Dunbar RB, Paulet Y-M, Thouzeau G, Jean F, Guarini J-M, Mucciarone D (2005) Shell of the Great Scallop *Pecten maximus* as a high-frequency archive of paleoenvironmental changes. Geochem Geophys Geosyst 6:Q08001 doi:10.1029/2004GC000890

Clark GR (1976) Shell growth in the marine environment—approaches to problem of marginal calcification. Am Zool 16:617–626

Dodd JR (1965) Environmental control of strontium and magnesium in *Mytilus*. Geochim Cosmochim Acta 29:385–398

Dodd JR, Crisp EL (1982) Non-linear variation with salinity of Sr/Ca and Mg/Ca ratios in water and aragonitic bivalve shells and implications of paleosalinity studies. Palaeogeogr Palaeoclimatol Palaeoecol 38:45–56

Elderfield H, Ganssen G (2000) Past temperature and delta O-18 of surface ocean waters inferred from foraminiferal Mg/Ca ratios. Nature 405:442–445

Elliot M, deMenocal PB, Linsley BK, Howe SS (2003) Environmental controls on the stable isotopic composition of *Mercenaria mercenaria*: potential application to paleoenvironmental studies. Geochem Geophys Geosyst 4:1056 doi:10.1029/2002GC000425

Emiliani C (1966) Isotopic paleotemperatures. Science 154:851–857

Epstein S, Buchsbaum R, Lowenstam HA, Urey HC (1953) Revised carbonate–water isotopic temperature scale. Bull Geol Soc Am 64:1315–1326

Freitas P, Clark LJ, Kennedy H, Richardson C, Abrantes F (2005) Mg/Ca, Sr/Ca, and stable-isotope ($\delta^{18}O$ and $\delta^{13}C$) ratio profiles from fan mussel *Pinna nobilis*: seasonal records and temperature relationships. Geochem Geophys Geosyst 6:Q04D14 doi:10.1029/2004GC000872

Freitas PS, Clarke LJ, Kennedy H, Richardson CA, Abrantes F (2006) Environmental and biological controls on elemental (Mg/Ca, Sr/Ca and Mn/Ca) ratios in shells of the king scallop *Pecten maximus*. Geochim Cosmochim Acta 70:5119–5133

Gillikin DP, Lorrain A, Navez J, Taylor JW, Keppens E, Baeyens W, Dehairs F (2005) Strong biological controls on Sr/Ca ratios in aragonitic marine bivalve shells. Geochem Geophys Geosyst 6: Q05009 doi:10.1029/2004GC000874

Gillikin DP, Dehairs F, Lorrain A, Steenmans D, Baeyens W, Andre L (2006a) Barium uptake into the shells of common mussel (*Mytilus edulis*) and the potential for estuarine paleo-chemistry reconstruction. Geochim Cosmochim Acta 70:395–407

Gillikin DP, Lorrain A, Bouillon S, Willenz P, Dehairs F (2006b) Stable carbon isotopic composition of *Mytilus edulis* shells: relation to metabolism, salinity, $\delta^{13}C_{DIC}$ and phytoplankton. Org Geochem 37:1371–1382

Grossman EL, Ku TL (1986) Oxygen and carbon isotope fractionation in biogenic aragonite: temperature effects. Chem Geol 59:59–74

Jones DS (1980) Annual cycle of shell growth increment formation in two continental shelf bivalves and its paleoecologic significance. Paleobiology 6:331–340

Jones DS, Arthur MA, Allard DJ (1989) Sclerochronological records of temperature and growth from shells of *Mercenaria mercenaria* from Narragansett Bay, Rhode Island. Mar Biol 102:225–234

Klein RT, Lohmann KC, Thayer CW (1996a) Bivalve skeletons record sea–surface temperature and $\delta^{18}O$ via Mg/Ca and $^{18}O/^{16}O$ ratios. Geology 24:415–418

Klein RT, Lohmann KC, Thayer CW (1996b) Sr/Ca and $^{13}C/^{12}C$ ratios in skeletal calcite of *Mytilus trossulus*: covariation with metabolic rate, salinity, and carbon isotopic composition of seawater. Geochim Cosmochim Acta 60:4207–4221

Koehn RK (1991) The genetics and taxonomy of species in the genus *Mytilus*. Aquaculture 94:125–145

Krantz DE, Williams DF, Jones DS (1987) Ecological and paleoenvironmental information using stable isotopic profiles from living and fossil molluscs. Palaeogeogr Palaeoclimatol Palaeoecol 58:249–266

Lazareth CE, Vander Putten E, Andre L, Dehairs F (2003) High-resolution trace element profiles in shells of the mangrove bivalve *Isognomon ephippium*: a record of environmental spatio-temporal variations? Estuar Coast Shelf Sci 57:1103–1114

Lea DW, Mashiotta TA, Spero HJ (1999) Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. Geochim Cosmochim Acta 63:2369–2379

Lorens RB, Bender ML (1980) The impact of solution chemistry on *Mytilus edulis* calcite and aragonite. Geochim Cosmochim Acta 44:1265–1278

Lorrain A, Gillikin DP, Paulet Y-M, Chauvaud L, Mercier AL, Navez J, Andre L (2005) Strong kinetic effects on Sr/Ca ratios in the calcitic bivalve *Pecten maximus*. Geology 12:965–968

Quinn TM, Sampson DE (2002) A multiproxy approach to reconstructing sea surface conditions using coral skeleton geochemistry. Paleoceanography 17:14–11

Richardson CA (1989) An analysis of the microgrowth bands in the shell of the common mussel *Mytilus edulis*. J Mar Biol Assoc UK 69:477–491

Richardson CA (2001) Molluscs as archives of environmental change. Oceanogr Mar Biol 39:103–164

Rosenthal Y, Lohmann GP, Lohmann KC, Sherrell RM (2000) Incorporation and preservation of Mg in *Globigerinoides sacculifer*: implications for reconstructing the temperature and $^{18}O/^{16}O$ of seawater. Paleoceanography 15:135–145

Schöne BR, Oschmann W, Rossler J, Castro ADF, Houk SD, Kröncke I, Dreyer W, Janssen R, Rumohr H, Dunca E (2003) North Atlantic oscillation dynamics recorded in shells of a long-lived bivalve mollusk. Geology 31:1037–1040

Schöne BR, Fiebig J, Pfeiffer M, Gleß R, Hickson J, Johnson A, Dreyer W, Oschmann W (2005) Climate records from a bivalve Methuselah (*Arctica islandica*, Mollusca; Iceland). Palaeogeogr Palaeoclimatol Palaeoecol 228:130–148

Seed R, Suchanek TH (1992) Population and community ecology of *Mytilus*. In: Gossling ED (ed) The mussel *Mytilus*: ecology, physiology, genetics and culture. Elsevier, Amsterdam, pp 87–170

Shen CC, Lee T, Chen CY, Wang CH, Dai CF, Li LA (1996) The calibration of D[Sr/Ca] versus sea surface temperature relationship for *Porites* corals. Geochim Cosmochim Acta 60:3849–3858

Takesue R, van Green A (2004) Mg/Ca and Sr/Ca and stable isotopes in modern and Holocene *Protothaca staminea* shells from a northern California coastal upwelling region. Geochim Cosmochim Acta 68:3845–3861

Vander Putten E, Dehairs F, Keppens E, Baeyens W (2000) High resolution distribution of trace elements in the calcite shell layer of modern *Mytilus edulis*: environmental and biological controls. Geochim Cosmochim Acta 64:997–1011

Wanamaker AD Jr, Kreutz KJ, Borns HW Jr, Introne DS, Feindel S, Barber BJ (2006) An aquaculture-based method for calibrated bivalve isotope paleothermometry. Geochem Geophys Geosyst 7: Q09011 doi:10.1029/2005GC001189

Wanamaker AD Jr, Kreutz KJ, Borns HW, Introne DS, Feindel S, Funder S, Rawson PD, Barber BJ (2007) Experimental determination of salinity, temperature, growth, and metabolic effects on shell isotope chemistry of *Mytilus edulis* collected from Maine and Greenland. Paleoceanography 22:PA2217 doi:10.1029/2006PA001352

Wanamaker AD Jr, Kreutz KJ, Schöne BR, Pettigrew N, Borns HW, Introne DS, Belknap D, Maasch KA, Feindel S (2008) Coupled North Atlantic slope water forcing on Gulf of Maine temperatures over the past millennium. Clim Dyn 31:183 doi:10.1007/s00382-007-0344-8

Watabe N (1988) Shell structure. In: Trueman ER, Clarke MR (eds) The Mollusca. Academic, New York, pp 69–74

Weidman C, Jones GA, Lohmann KC (1994) The long-lived mollusk *Arctica islandica*: a new paleoceanographic tool for the reconstruction of bottom temperatures for the continental shelves of northern Atlantic Ocean. J Geophys Res 99:18,305–318,314

Wheeler A (1992) Mechanisms of molluscan shell formation. In: Bonucci E (ed) Calcification in biological systems. CRC, Boca Raton, pp 179–216

Witbaard R, Duinveld GCA, de Wild PAWJ (1999) Geographical differences in growth rates of *Arctica islandica* (Mollusca: Bivalvia) from the North Sea and adjacent waters. J Mar Biol Assoc UK 79:907–915