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Inter- and intra-specimen variability masks reliable temperature control on shell Mg/Ca ratios in laboratory- and field-cultured *Mytilus edulis* and *Pecten maximus* (bivalvia)

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Abstract. The Mg/Ca ratios of biogenic calcite is commonly seen as a valuable palaeo-proxy for reconstructing past ocean temperatures. The temperature dependence of Mg/Ca ratios in bivalve calcite has been the subject of contradictory observations. The palaeoceanographic use of a geochemical proxy is dependent on initial, rigorous calibration and validation of relationships between the proxy and the ambient environmental variable to be reconstructed. Shell Mg/Ca ratio data are reported for the calcite of two bivalve species, *Mytilus edulis* (common mussel) and *Pecten maximus* (king scallop), which were grown in laboratory culturing experiments at controlled and constant aquarium seawater temperatures over a range from ∼10 to ∼20°C. Furthermore, Mg/Ca ratio data of laboratory- and field-grown *M. edulis* specimens were compared. Only a weak, albeit significant, shell Mg/Ca ratio–temperature relationship was observed in the two bivalve species: *M. edulis* (r^2=0.37, p<0.001 for laboratory-cultured specimens and r^2=0.50, p<0.001 for field-cultured specimens) and *P. maximus* (r^2=0.21, p<0.001 for laboratory-cultured specimens only). In the two species, shell Mg/Ca ratios were not found to be controlled by shell growth rate or salinity. The Mg/Ca ratios in the shells exhibited a large degree of variability among and within species and individuals. The results suggest that the use of bivalve calcite Mg/Ca ratios as a temperature proxy is limited, at least in the species studied to date. Such limitations are most likely due to the presence of physiological effects on Mg incorporation in bivalve calcite. The utilization is further limited by the great variability both within and among shells of the same species that were precipitated under the same ambient conditions.

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1 Introduction

Carbonate skeletal remains, i.e. foraminifera, corals, ostracodes and bivalves, are valuable archives of information for palaeo-reconstruction of changes in physical and chemical oceanographic conditions. The incremental growth of biogenic carbonates, such as the shells of marine bivalve molluscs or the coral skeleton, has the potential to record high-resolution time-series of those environmental conditions in which the organism grew. Furthermore, marine bivalves occupy widely distributed habitats in the modern-day oceans, as well as being relatively common throughout the fossil record since the Triassic. Information on past environmental conditions that are preserved in carbonates can be obtained through the use of proxies, i.e. physical and chemical signals that provide information on environmental parameters that cannot be measured directly, such as seawater temperature or salinity. However, a proxy is rarely dependent on a single variable, and the influence of other secondary independent variables complicates proxy use in palaeo-studies; such factors must be assessed rigorously via calibration and validation studies prior to successful application (for reviews, see e.g. Wefer et al., 1999; Lea, 2003).

The use of the oxygen-isotope composition ($^{18}O/^{16}O$ ratios expressed as $\delta^{18}O$ values) of biogenic carbonate archives as a proxy for seawater temperature (for reviews, see e.g. Emiliani, 1966; Wefer and Berger, 1991) is one of the most powerful tools in palaeoceanographic studies (e.g. Shackleton, 1967; Shackleton and Opdyke, 1973; Weidman et al., 1994; Gagan et al., 2000; Schoene et al., 2004), but its use is complicated by factors other than temperature, namely variation in the oxygen-isotope composition of seawater, pH and kinetic effects (e.g. McConnaughey, 1989; Spero et al., 1997). By comparison, the predicted thermodynamic control of Ca^{2+} substitution by Mg^{2+} in inorganically precipitated

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calcite (Chilingar, 1962; Katz, 1973; Mucci, 1987; Oomori et al., 1987) and the observed temperature dependence of Mg/Ca ratios in some biogenic calcites (Chave, 1954; Dwyer et al., 1995; Klein et al., 1996; Nurnberg et al., 1996; Rosenthal et al., 1997; Lea et al., 1999; Dwyer et al., 2000; Elderfield and Ganssen, 2000; Lear et al., 2002) have resulted in Mg/Ca ratios being seen as a salinity-independent temperature proxy that makes an ideal companion to the δ^{18}O-temperature proxy. Nevertheless, long term changes in the Mg/Ca ratio of seawater may present a significant complication to the application of the Mg/Ca-temperature proxy to the fossil record (Hardie, 1996). In addition, the clear species-specific temperature dependence of Mg/Ca ratios that has been observed in foraminiferal calcite (Rosenthal et al., 1997; Lea et al., 1999; Elderfield and Ganssen, 2000; Lear et al., 2002) suggests that parameters other than temperature also can influence the Mg/Ca ratios of biogenic calcites. For example, biological influences such as gametogenesis, ontogeny, growth rate and size, as well as environmental and physical parameters such as salinity, pH and post-depositional dissolution, have all been proposed to significantly influence foraminiferal Mg/Ca ratios (Delaney et al., 1985; Lea et al., 1999; Elderfield et al., 2002; Bentov and Erez, 2005). Furthermore, observations of significant small-scale intra-shell heterogeneity in Mg contents indicates a strong physiological control on the Mg/Ca ratio of biogenic calcites, such as observed in foraminifera (Rio et al., 1997; Hathorne et al., 2003; Eggins et al., 2004; Bentov and Erez, 2005; Sadekov et al., 2005), ostracodes (Rio et al., 1997) and bivalves (Lorens and Bender, 1980; Rosenberg et al., 2001).

In calcitic bivalve molluscs the occurrence of a temperature control on shell Mg/Ca ratios has been the subject of several studies that have returned contrasting results, but nevertheless shell Mg/Ca ratios have been used to reconstruct palaeotemperatures from fossil bivalves (e.g. Klein et al., 1997; Immenhauser et al., 2005). In an early study, a weak positive correlation between shell calcite Mg concentration with temperature was reported for three species from the genus *Mytilus* (Dodd, 1965). More recently, Klein et al. (1996) described a clear temperature dependence of Mg/Ca ratios for the mussel *Mytilus trossulus*, a close relative of *Mytilus edulis* that some consider to be a sub-species in the *M. edulis* species complex (Gardner, 1992; Riginos and Cunningham, 2005). Vander Putten et al. (2000) observed a similar relationship for *M. edulis* (blue mussel), but with an apparently seasonal breakdown in the relationship between Mg/Ca and temperature also being reported. A clear seasonal relationship between shell Mg/Ca ratios and calcification temperature for the large fan mussel *Pinna nobilis* has been reported, albeit with an additional ontogenetic influence (Freitas et al., 2005). For other bivalve species, such as *Pecten maximus* (king scallop), there exists no clear temperature relationship; Lorrain et al. (2005) reported an absence of a significant correlation between Mg/Ca ratios and temperature for this species, while a weak, albeit significant, Mg/Ca ratio to temperature relationship was also observed (Freitas et al., 2006), with the relationship breaking down during winter months. Furthermore, several studies report, or suggest, the occurrence of significant non-thermodynamic controls on the Mg content of bivalve mollusc calcite, such as salinity (Dodd, 1965), solution Mg/Ca ratios (Lorens and Bender, 1980) or the animal's metabolism (Lorens and Bender, 1977, 1980; Vander Putten et al., 2000). Significant small-scale heterogeneity in Mg content also has been described for bivalve shell calcite. Such variability has been associated with stress (Lorens and Bender, 1980), metabolic activity (Rosenberg and Hughes, 1991) and control of shell crystal elongation (Rosenberg et al., 2001).

The purpose of this study was to advance an understanding of the degree of variability of Mg/Ca ratios in calcite bivalve shells using a controlled laboratory-aquarium culturing approach. Specifically, no laboratory calibration of the Mg/Ca ratio-temperature relationship in bivalve calcite has previously been performed under constrained and constant seawater temperatures. This approach is a significant advancement on previous studies, because it enables manipulation of specimens, control of environmental variables, and measurement of other parameters, such as size and growth rate. It must be acknowledged, however, that laboratory aquaria are not a true representation of the animal's natural habitat. The outcomes of laboratory culturing studies are only of value when validated by field-based studies, albeit with the latter suffering from a lesser degree of constraint of environmental variables. In summary, the ultimate goal of this investigation was to determine whether a reliable calibration of the Mg/Ca ratio–temperature relationship could be obtained for the shell calcite from two bivalve species, *Mytilus edulis* (blue mussel) and *Pecten maximus* (king scallop), grown under constrained and constant temperature laboratory-aquaria conditions. Finally, the *M. edulis* laboratory-culturing data have been compared with data from field-grown specimens of this same species.

2 Material and methods

2.1 Laboratory-culturing experiment

Two species of marine bivalve mollusc were cultured in constant-temperature aquaria in the School of Ocean Sciences, Bangor University, UK *Mytilus edulis* specimens were collected in December 2003, from naturally settled spat (1 cm<size<2 cm; age<one year) in Cable Bay, a site on the coast of Anglesey, northwest Wales, while *Pecten maximus* specimens (1 cm<size<2 cm; age<one year) were collected from a commercial fishery, Ramsay Sound Shellfish, Isle of Skye, Scotland, in November, 2003. All animals were acclimated to the laboratory environment at a temperature of $\sim$13°C for more than two months. Subsequently, animals

of similar size were moved into separate aquaria each under different but constant temperatures (maximum resolution of 1°C), constant dimmed-light conditions and controlled food conditions; the aquaria were routinely cleaned of all detritus. For the duration of the experiments, animals were kept in individual plastic mesh cages within each aquarium. Acclimation to the different temperatures in each aquarium was achieved by increasing/decreasing water temperature by 1°C every 2 days before commencement of the experimental periods. The intermittent lack of temperature control in some aquaria reflects times when the cooling system sometimes struggled to compensate for fluctuations in the temperature of the external seawater supply. For improved constraint, seawater temperature also was monitored in each aquarium every 15 min using submerged temperature loggers (Gemini Data Loggers TinyTag – TGI 3080; accuracy of ±0.2°C). During experiment one, from 23 February to 7 April 2004, nominal seawater temperatures in the three available aquaria were maintained at 12, 15 and 18°C, and only *M. edulis* was cultured. In experiment two, from 6 May to 18 June 2004, nominal seawater temperatures were maintained at 10, 15 and 20°C and both *M. edulis* and *P. maximus* were cultured. A mixed algae solution of *Pavlova lutheri*, *Rhinomonas reticulata* and *Tetraselmis chui* was collected every morning from stock cultures, split into equal volumes of eight litres and then supplied to the aquaria, from containers with a drip-tap, throughout that day at rates of ∼5.5 ml/min. Because of the limited number of aquaria available, two separate temperature-controlled experiments were completed with three aquaria used in each. Natural seawater is pumped from the proximal Menai Strait into settling tanks, where it rests for a few days, before being introduced as a common supply into the laboratory aquaria. Food supply from the seawater source to the aquaria is negligible. Mortality rates during the experimental period were zero for both *Mytilus edulis* and *Pecten maximus*.

Once the animals had acclimatised, individual specimens were removed at weekly intervals (with the exception of the last growth interval in experiment two, which was longer than a week for both the 15°C and 20°C aquaria) to be processed. Each time the *M. edulis* specimens were removed from the aquaria they were exposed to the air for 5 to 6 h, while *P. maximus* specimens were kept in small holding tanks for periods of 30 to 45 min. Both methods resulted in emplacement of a disturbance mark on the surface of the shells. The shells were then photographed and digitally imaged using the AnalySIS software package. The combination of disturbance marks and photographs was used to identify and measure all shell growth between emersions and provided a time control of the new shell growth laid down throughout the experiments. Subsequently the term "growth interval" has been used to describe the time intervals between emersions of animals (Table 1). The duration of the experiments, and hence the number of growth intervals, varied with species and aquarium temperature (Table 1).

Fig. 1. Location of the field-deployment site, Menai Strait, Wales, UK.

Seawater samples for measurement of salinity were collected using sealed Winchester glass bottles every other fourth to eighth day from the 15°C aquarium in both experiments. Samples were collected from one aquarium only, since the seawater supply was common in all aquaria and water turnover time was short (∼7–8 h for a volume of ∼650 l). The following relationships of salinity with $\delta^{18}O_{seawater}$ was used to estimate salinity for dates when only $\delta^{18}O_{seawater}$ data was available (collected every other day, n=16), with 95% confidence intervals: Salinity=32.85 (±0.08)+3.33 (±0.45) X $\delta^{18}O_{seawater}$ (r^2=0.97, $p<0.001$, n=8) for experiment one and salinity=33.05 (±0.04)+2.53 (±0.46) X $\delta^{18}O_{seawater}$ (r^2=0.96, $p<0.001$, n=7) for experiment two. Salinity was determined using an AutoSal 8400 Autosalinometer calibrated with International Association for Physical Sciences of the Ocean (I.A.P.S.O.) standard seawater (analytical accuracy and resolution of ±0.003 equivalent PSU). Samples for pH measurements were taken manually every other day by immersing 20 mL plastic syringes below the surface of the seawater in the aquaria. The samples were subsequently allowed to warm up to room temperature (20±2°C) in the dark before measurement with a commercial glass electrode (Mettler Toledo Inlab 412). The electrode was calibrated using NBS pH buffers 6.881 and pH 9.225 (20°C) and was then allowed to stand until a stable reading was obtained (∼1 min).

Table 1. Start dates of the two laboratory-culturing experiments and duration of growth intervals (days) in each aquarium for which new shell growth was evident.

Species	Aquarium Interval		Experiment 1 12°C						15°C						18°C				
			1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5
M. edulis	Start Date	2004	23/02						25/02						01/03				
	Duration	Days	7	7	7	7	7	9	7	7	7	7	7	6	7	7	7	7	7
Species	Aquarium Interval		Experiment 2 10°C						15°C						20°C				
			1	2	3	4	5	6	1	2	3	4M	4P		1	2	3M	3P	
M. edulis	Start Date	2004	07/05						06/05						17/05				
P. maximus	Duration	Days	7	7	7	7	7	7	7	7	7	7	12		7	7	10	13	

In experiment two the last growth interval was of different duration for the two species grown at 15 and 20°C and the suffixes M and P indicate the last growth interval for *M. edulis* and *P. maximus*, respectively.

2.2 Field-culturing experiment

Specimens of the bivalve *M. edulis* were suspended 1 m below a moored raft in a section of the Menai Strait (north Wales, UK; Fig. 1), where the water column is completely mixed due to strong turbulent tidal mixing (Harvey, 1968), from the 8 December 2004 to the 12 December 2005. The animals were all less than 1-year-old when deployed, obtained from one spat cohort and initially ranged from 2.0 to 2.7 cm in shell length. Two different, but parallel, experimental approaches were taken: (1) "short" deployment specimens were placed into cages for 16 short, well-defined and consecutive growth intervals that together covered the duration of the entire field experiment. The duration of each growth interval varied during the experiment according to expected seasonal changes in shell growth rate; (2) In contrast to the short-deployment specimens, "annual"-deployment specimens were placed in the field for the entire duration of the experiment. To ensure that short-deployment specimens were in the same physiological condition as their annual counterparts, the former were taken at the start of each growth interval from a stock of animals deployed at the beginning of the experiment and kept in a separate cage.

At the end of each growth interval all short-deployment specimens from the preceding deployment and all annual specimens were removed from the raft, together with a new set of short-deployment specimens taken from the stock that were to be deployed during the next growth interval. All of these shells were handled, photographed and digitally imaged in a similar way as in the laboratory-culturing experiment (Sect. 2.1). The combination of hand drilled marks on the outer shell surface, disturbance marks due to handling and photographs was used to identify and measure all shell growth for each growth interval, as well as shell height (i.e. the distance from the umbo to the shell margin along the main axis of growth), and thus provide a time control of the new shell growth laid down throughout the field experiment by assuming shell growth to be have been continuous and to have occurred at a constant rate during each growth interval.

Seawater temperature was monitored every two hours throughout the experimental deployment period using submerged temperature loggers placed in the mesh cages containing the animals (Gemini Data Loggers TinyTag – TGI 3080; accuracy of ± 0.2°C). Seawater samples for the measurements of salinity and pH were collected every two to five weeks, in the vicinity of the moored raft, and analysed as described in Sect. 2.1.

2.3 Shell preparation and surface milling

2.3.1 Laboratory-culturing experiment

The surfaces of the left hand valve of *P. maximus* shells were cleaned with a brush and any encrusting material removed using a 0.4 mm wide steel carbide burr (Minerva Dental Ltd) attached to a hand-held dental drill. The left hand valve of *M. edulis* shells were cleaned in a similar manner to the *Pecten* shells but, in addition, the outer organic periostracum was milled away with the drill until periostracum-free shell was visible in the entire sampling area. Shell powder samples subsequently were taken by milling to a depth of ca. 200 μm from the main axis of shell growth: in *P. maximus* from the mid 2–3 axial ridges (ribs), and in *M. edulis* from the middle section, to avoid the increase in shell curvature that occurs away from the main growth axis (Fig. 2). Accurate milling was completed under a binocular microscope fitted with an eyepiece graticule, and depth and width of milling were kept as similar as possible for hand-milling, and a scanning electron microscope then was used to accurately assess the depth of milling. Only one powder sample was milled from each

individual growth interval and, particularly at the lower temperatures, the milled powder from one or more growth intervals had to be combined to provide enough shell material for analysis.

Lorens and Bender (1980) have described that the stress of capture and adaptation to a new laboratory environment induced the deposition of a region of shell (termed "transition zone calcite" by those authors) with higher Mg/Ca ratios. Therefore, in the current study the sampling of individual growth intervals between the disturbance marks on the surface of the shell, that represent the times of immersion during the experimental period, has minimized the sampling of handling disturbances and thus of its potential influence on measured Mg/Ca ratios.

2.3.2 Field-culturing experiment

The left hand valve of two short-deployment *M. edulis* specimens were sampled for each growth interval, while three annual *M. edulis* specimens (A2, A6, A20) were sequentially sampled for all growth intervals. The milling of shell powder samples was as described for the laboratory-culturing experiment. Whenever the amount of shell growth permitted more than one sample was collected from a single growth interval ($2 \leq N \leq 4$), producing a total of 62 samples from 33 specimens and 92 samples from 3 specimens for the short- and annual-deployment specimens, respectively.

2.4 Mg/Ca ratio analyses

The shell milled powder sample preparation and analytical methodologies used in this study are as described in detail in Freitas et al. (2005, 2006). Calibration for Mg/Ca ratio determinations was performed via an established ICP-AES intensity-ratio method (de Villiers et al., 2002), using synthetic standard solutions in the range 0–25 mmol/mol for Mg/Ca, and most at Ca concentrations of 50 (N=304) and 60 μg/ml (N=161), but for the smallest milled powder samples at a Ca concentration of 30 μg/ml (N=102). Measurements were made using the Perkin Elmer Optima 3300RL ICP-AES instrument housed at the NERC ICP Facility, Royal Holloway University of London. Instrumental drift was monitored by running an intermediate (16 mmol/mol) Mg/Ca calibration standard every 5 to 10 samples. Following ICP-AES analyses, we checked and corrected for signal intensity drift. We assumed a linear change in the signal intensity, derived from individual element emission lines, between two consecutive intermediate standard solutions (each pair of these solutions bracketing a number of sample unknowns). Sample data were corrected accordingly, i.e. by the fraction of total change that corresponded to their sequence position between their two bracketing intermediate standard solutions. Analytical precision, as obtained from intermediate standard solutions analysed as unknowns on separate days and expressed as relative standard deviation or RSD, was 0.5% for the laboratory-cultured specimens (N=86) and 1.3% for the field-cultured specimens (N=29). In the laboratory-culturing experiments, sufficient material was not available from any one growth interval to enable replicate analyses for an assessment of true sample precision. For the field experiment, however, a sample precision better than 6.2% RSD was obtained on five *M. edulis* specimens where the milled powder samples were split into three sub-sample powders that were then analyzed as independent solutions. Furthermore, Freitas et al. (2006) used the same method as reported here and obtained a Mg/Ca ratio sample precision of 3.5% RSD from one *P. maximus* specimen where the milled powder sample was split into five sub-sample powders that were then analyzed as independent solutions. For comparison with past and future datasets, and to confirm the veracity of the *M. edulis* and *P. maximus* Mg/Ca ratios obtained in this study, Mg/Ca ratio measurements also are reported for a set of solutions prepared by the Elderfield group at the University of Cambridge, U.K. (Greaves, personal communication, 2003; cf. de Villiers et al., 2002), as well as for three solutions (BAM-RS3, ECRM-752 and CMSI-1767) that have been proposed as certified reference materials (CRMs) for Mg/Ca ratio measurements in carbonates (Greaves et al., 2005) and that are subject to an ongoing (Greaves et al., 2008) international inter-laboratory calibration study (Table 2). For each CRM, approximately 50 mg of powder was dissolved in 50 g of 0.075M HNO_3 (Merck Ultrapur), resulting in Ca concentrations in solution of ca. 400 μg/ml. Subsequently, 1.5 ml of each solution was centrifuged for 10 min and an aliquot then was pipetted into clean 12 ml centrifuge tubes and diluted to 10 ml to give final Ca concentrations of 60, 50 and 30 μg/ml in order to match the sample and standard solutions.

Table 2. Comparison of expected (Greaves et al., 2005; Greaves et al., 2008) with measured Mg/Ca ratios for three certified reference material (CRMs) solutions.

CRM solution	Expected	This study	% Difference
BAM-RS3	0.79	0.78±0.12 (N=9)	−1.2
ECRM-752	3.76	3.82±0.07 (N=13)	+1.6
CMSI-1767	5.56	5.76±0.07 (N=11)	+3.6

The values in the third column are those returned reported for this study in the third column are repeated measurements of a single dissolution completed for each CRM and diluted to Ca concentrations of 60, 50 or 30 μg/ml. All measurements were made on the same Perkin Elmer Optima 3300RL ICP-AES instrument.

2.5 Statistical analyses

Two-sample *t*-tests were used to determine statistically whether significant differences existed between measured shell Mg/Ca ratios precipitated at different seawater temperatures in pairs of constant-temperature aquaria. Linear

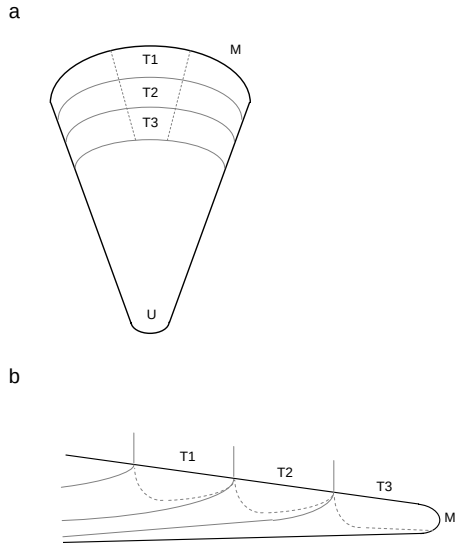

Fig. 2. Schematic representation of a shell to indicate the sampling approach for *M. edulis* and *P. maximus* shells. **(a)** View of the outer shell surface from above and **(b)** Longitudinal section of the shell. M is the shell margin; U is the shell umbo; grey lines define the boundaries between individual growth intervals identified by T1, T2 and T3. Samples of shell calcite were collected for each growth interval along the main axis of growth (delimited by dashed lines in a), avoiding areas of excessive shell curvature, and up to a depth of ca. 200 μm in the areas delimited by the dotted lines in b.

regressions and ANOVA analyses of shell Mg/Ca ratios and seawater temperature were performed using the software package MINITAB. Regressions were compared by testing the equality of variance in the regression residuals, since unequal variance in the regression residuals (F-test, $p<0.05$) indicates significantly different regressions. GLM ANOVA was used to test for differences in the slope and intercepts of the regressions. The variability in shell Mg/Ca ratios attributable to different factors was determined using fully nested ANOVA.

3 Results

3.1 Culturing temperature conditions

Seawater temperature was stable during laboratory-culturing experiment one, but more variable during experiment two, especially in the lower (10°C) and mid (15°C) temperature aquaria (Fig. 3). Nevertheless, clear temperature differences were maintained in the three different aquaria in each of the experiments (Fig. 3). Aquaria mean ($\pm 1\sigma$) seawater temperatures were 11.96±0.12°C, 15.61±0.12°C and 18.39±0.05°C during experiment one, and 10.76±0.41°C, 15.54±0.25°C and 20.23±0.22°C during experiment two.

During the field-culturing experiment seawater temperature exhibited a clear seasonal pattern (Fig. 4). Seawater temperature decreased from ca. 10.0°C in December 2004 to a minimum temperature of ca. 5.0°C at the end of February, followed by a rise to ca. 9.5°C during mid March–late April (from day 105 to 140) and then a further rise up to a maximum temperature of ca. 19.0°C in early–mid July (ca. day 225). From that time to early September (ca. day 280) seawater temperature remained high at ca. 18.0°C, before decreasing to ca. 9.0°C in December 2005.

Table 3. Summary of linear regressions of Mg/Ca ratios (mmol/mol) with seawater temperature (°C), with 95% confidence intervals, for all laboratory- (experiment one and two) and field-culturing experiments.

Experiment	Regression	N	
Laboratory			
M. edulis	1	Mg/Ca=1.349 (±1.03) + 0.242 (±0.07) × T	85
	2	Mg/Ca=1.286 (±1.06) + 0.320 (±0.07) × T	59
	1+2	Mg/Ca=1.564 (±0.84) + 0.256 (±0.06) × T	144
P. maximus	2	Mg/Ca=9.886 (±2.96) + 0.520 (±0.19) × T	111
Field			
M. edulis	All	Mg/Ca = 1.503 (±0.57) + 0.265 (±0.04) × T	154
	Short	Mg/Ca=1.780 (±0.87) + 0.281 (±0.07) * T	62
	Annual 2	Mg/Ca = 0.456 (±1.07) + 0.377 (±0.08) * T	28
	Annual 6	Mg/Ca = 1.260 (±0.70) + 0.228 (±0.05) × T	34
	Annual 20	Mg/Ca = 0.899 (±0.70) + 0.270 (±0.05) × T	30

3.2 Shell calcite Mg/Ca records and variability from laboratory-cultured *M. edulis* and *P. maximus*

Variability of shell Mg/Ca ratios at each temperature is very large for both species (Figs. 5a and 6). In experiment two, during which both species were grown at the same temperatures and in the same aquaria, shell Mg/Ca ratios are approximately three times higher in *P. maximus* than in *M. edulis* (t-test, $p<0.001$, degrees of freedom ≥ 41 for all temperatures) and thus indicate a clear species-specific Mg/Ca ratio-temperature relationship for the two bivalve species investigated. Furthermore, the degree of variability of shell Mg/Ca ratios at each aquaria temperature also is higher in *P. maximus* than in *M. edulis*. Measured shell Mg/Ca ratios range from 2.84 to 9.50 mmol/mol in laboratory-cultured *M. edulis* (experiments one and two) and from 8.08 to 29.92 mmol/mol in *P. maximus* (experiment two) over the experimental temperature range (Fig. 5a, http://www.biogeosciences.net/5/1245/2008/bg-5-1245-2008-supplement.pdf).

The mean Mg/Ca ratios (±one sigma) at each temperature for *M. edulis* were 4.70±0.83 mmol/mol at 10.76±0.41°C, 4.03±0.86 mmol/mol at 11.96±0.12°C, 5.25±0.74 mmol/mol at 15.61±0.12°C (experiment one), 6.32±1.33 mmol/mol at 15.54±0.25°C (experiment two), 5.72±0.65 mmo/mol at 18.39±0.05°C, 7.73±1.03 mmol/mol at 20.23±0.22°C; and for *P. maximus* were 15.88±4.02 mmol/mol at 10.76±0.41°C, 17.11±3.62 mmol/mol at 15.54±0.12°C, 21.00±3.72 mmol/mol at 20.23±0.22°C.

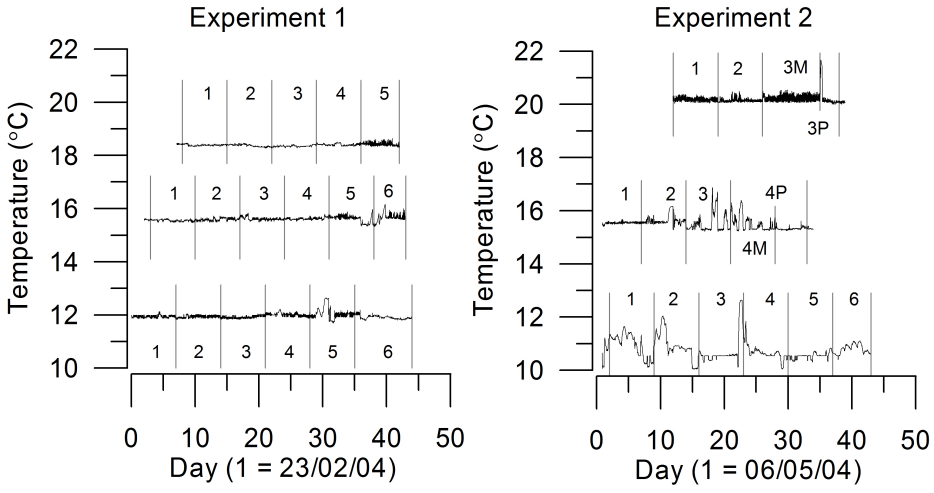

Fig. 3. Variation of seawater temperature measured every 15 min in all the aquaria during laboratory-culturing experiments one and two. Vertical lines define limits of growth intervals in each aquarium. In experiment two, the last growth interval was of different duration for the two species, and the suffixes M and P indicate the last growth interval for *M. edulis* and *P. maximus*, respectively.

Despite the high degree of variability, a significant ($p<0.001$ for both species), albeit weak, correlation exists between seawater temperature and shell Mg/Ca ratios in both species (r^2=0.38 and 0.57 for *M. edulis* in experiments one and two, respectively; r^2=0.21 for *P. maximus* in experiment two). Unequal variance in the residuals indicates significantly different linear regressions of Mg/Ca ratios with temperature between *M. edulis* and *P. maximus* from laboratory-culturing experiment two (Table 3, *F*-test, $p<0.05$). ANOVA analysis of the regressions of Mg/Ca with temperature shows that the slope of the linear regressions does not differ significantly (F=2.13, p=0.146), but that the intercept does (F=37.67, $p<0.001$).

For *M. edulis* that was cultured in both experiment one and two there is a significant difference in the Mg/Ca ratio to temperature relationship between experiments, stronger in experiment two (r^2=0.38, $p<0.001$) than in experiment one (r^2=0.57, $p<0.001$), and shell Mg/Ca ratios were higher in experiment two than in experiment one (Fig. 5a). However, this may be due solely to the smaller number of individuals analysed in the laboratory-culturing experiment two compared to experiment one, i.e. the capture of a smaller degree of Mg/Ca ratio variability, as well as the greater temperature range for the experiment two regression. Unequal variance in the residuals confirms significantly different linear regressions of Mg/Ca ratios with temperature in *M. edulis* between experiments one and two (Table 3, *F*-test, $p<0.05$). Further analysis of variance of the regressions of *M. edulis* Mg/Ca ratios and temperature shows that the slope of the regressions is not significantly different (F=2.50, p=0.116), but that the intercept (F=127.92, $p<0.001$) is different in the two experiments.

Evidence exists for statistically significant (*t*-test, $p<0.05$) inter-individual shell variability of shell Mg/Ca ratios between individuals cultured within any one aquarium (Fig. 6). Maximum differences between mean shell Mg/Ca ratios from different *M. edulis* individuals cultured in the same aquarium were: 1.3 mmol/mol at 12°C (N=7), 1.9 mmol/mol at 15°C (N=6) and 2.1 mmol/mol at 18°C (N=6) in experiment one and 1.2 mmol/mol at 10°C (N=6), 3.3 mmol/mol at 15°C (N=6) and 3.0 mmol/mol at 20°C (N=6) in experiment two. For *P. maximus* maximum differences between mean Mg/Ca ratios from different individuals cultured in experiment two were: 8.9 mmol/mol at 10°C

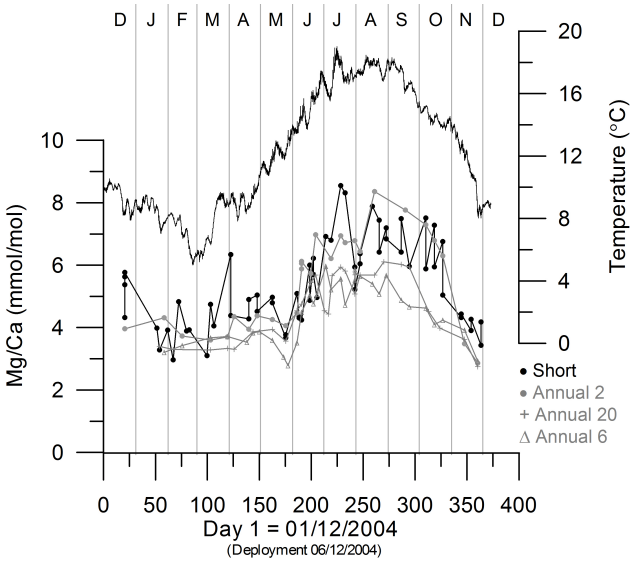

Fig. 4. Variation of seawater temperature and shell Mg/Ca ratios of *M. edulis* (short- and annual- deployment specimens) during the field-culturing experiment. Note that more than one short-deployment specimen was analyzed for several time periods. Vertical lines and letters define limits of calendar months.

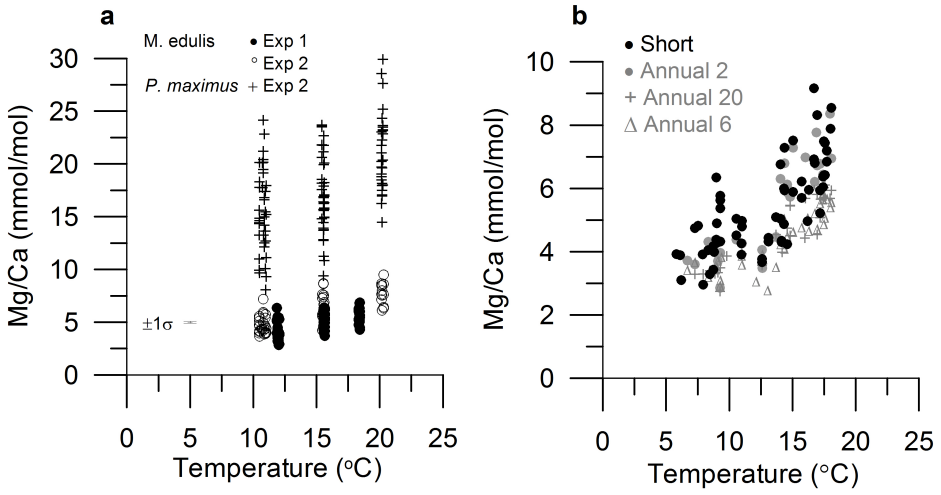

Fig. 5. Shell Mg/Ca ratios plotted against seawater temperature from: **(a)** laboratory-cultured *M. edulis* (• – experiment one and ◦ – experiment two) and *P. maximus* (+ – experiment two only); **(b)** field-cultured *M. edulis* (• – short-deployment specimens; annual-deployment specimens: • – A2, Δ – A6 and + – A20). For comparison, twice the analytical error on Mg/Ca ratios (±0.10 mmol/mol) also is shown.

(N=8), 9.2 mmol/mol at 15°C (N=10) and 9.0 mmol/mol at 20°C (N=10).

In addition to inter-individual shell variability, there is also a degree of intra-individual shell variability in Mg/Ca ratios within the dataset, i.e. between milled samples taken from different growth intervals (Fig. 6). For either species, the proportion of individual shells that produced Mg/Ca ratios significantly different among samples milled from the same specimen (i.e. the difference between any two Mg/Ca measurements was larger than twice the analytical error) was similar at each temperature. However, *P. maximus* showed a higher frequency of milled samples with different Mg/Ca ratios within an individual shell (>97% in all aquaria) than did *M. edulis*, (68%<experiment one <73%, and 72%<experiment two <83%).

3.3 Shell calcite Mg/Ca records and variability from field-cultured *M. edulis*

For the field-cultured *M. edulis*, measured shell Mg/Ca ratios range from 2.96 to 9.16 mmol/mol in the short-deployment specimens; from 2.86 to 8.34 mmol/mol in the A2 specimen; from 2.78 to 5.97 mmol/mol in the A6 specimen and from 2.75 to 6.11 mmol/mol in the A20 specimen (Figs. 4 and 5b, http://www.biogeosciences.net/5/1245/2008/bg-5-1245-2008-supplement.pdf). During the field-culturing experiment, shell Mg/Ca ratios from short- and annual-deployment *M. edulis* specimens showed a clear seasonal pattern (Fig. 4). Shell Mg/Ca ratios were low at ca. 4 mmol/mol during the winter and spring months up to May, and then increased to maxima higher than 7 mmol/mol during June–July. From that time to October shell Mg/Ca ratios remained high at ca. 7 mmol/mol, and then decreased to ca. 3.5 mmol/mol in December 2005 (Fig. 4).

In field-cultured *M. edulis*, a significant ($p<0.001$ for all specimens) correlation exists between seawater temperature and shell Mg/Ca ratios (Fig. 5b): r^2=0.54 for the short-deployment specimens (N=62); r^2=0.77 for the A2 specimen (N=28); r^2=0.72 for the A6 specimen (N=34) and r^2=0.81 for the A20 specimen (N=30). However, the correlation between shell Mg/Ca ratios and temperature is weaker (r^2=0.51, $p<0.001$) when all the data from all the specimens are pooled together. Furthermore, variance of the residuals was only equal for the regressions of Mg/Ca ratios with temperature for *M. edulis* specimens between short-deployment and A2 specimens and between A6 and A20 specimens (Table 3, F-test, $p>0.05$). In all other specimens, unequal variance of residuals indicates significantly different regressions of Mg/Ca ratios with temperature (Table 3, F-test, $p<0.05$). ANOVA analysis of the regressions of Mg/Ca with temperature between short-deployment and A2 specimens, and between A20 and A6 specimens, shows that the slope of the linear regressions does not differ significantly (F=2.70, p=0.104 and F=1.40, p=0.242, respectively), but that the intercept does (F=126.11, $p<0.001$ and F=196.31, $p<0.001$, respectively). Evidence thus exists for significant inter-individual variability of shell Mg/Ca ratios and its relationship with temperature from *M. edulis* specimens grown under the same field-culturing conditions. Maximum shell Mg/Ca ratios, in particular, are markedly different between individual specimens and range from 5.97 to 9.16 mmol/mol.

For the same range of temperature, shell Mg/Ca ratios of *M. edulis* grown in the laboratory- and field-culturing experiments showed a similar range (Fig. 5). However, the correlation between Mg/Ca ratios and temperature was stronger in field-cultured ($0.54<r^2<0.81$) than in laboratory-cultured *M. edulis* specimens ($0.38<r^2<0.57$). Furthermore,

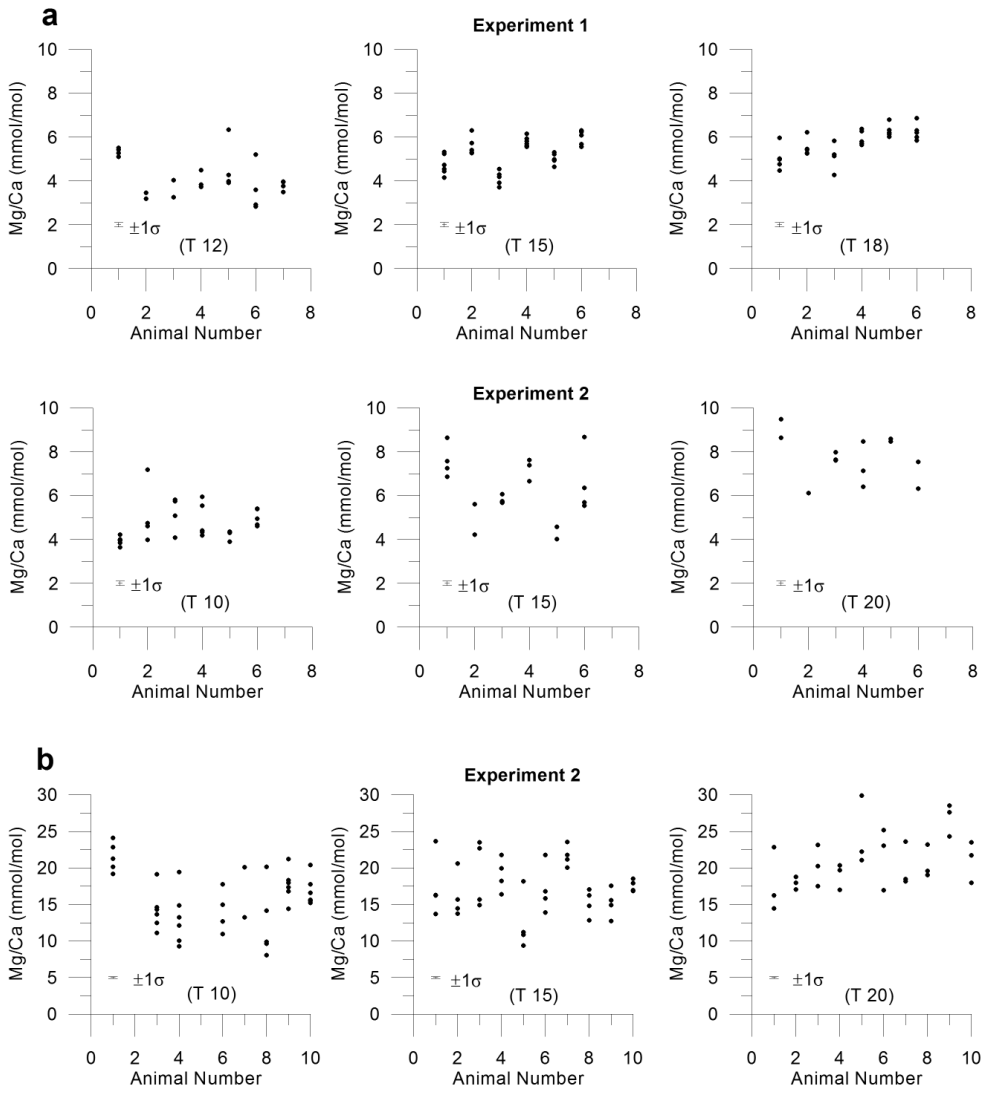

Fig. 6. Shell Mg/Ca ratios plotted against animal number for each aquarium (temperature in brackets) in order to illustrate inter- and intra-individual shell variability of Mg/Ca ratios in **(a)** *M. edulis* and **(b)** *P. maximus*. For each animal, individual data points correspond to Mg/Ca ratios of new shell growth deposited in the experiment during different growth intervals. For comparison, twice the analytical error on Mg/Ca ratios (±0.10 mmol/mol) also is shown.

ANOVA analysis of the regressions of Mg/Ca with temperature between laboratory-cultured and field-cultured *M. edulis* specimens (Table 3) shows that the slope of the linear regressions does not differ significantly (F=0.70, p=0.799), but that the intercept does (F=224.68, $p<0.001$).

4 Discussion

4.1 Inter-species, inter-individual and intra-individual variability in shell Mg/Ca ratios

Like with other bivalve geochemical and physical proxies (for review, see e.g. Richardson, 2001) the large variability of shell Mg/Ca ratios obtained in this study occur at different levels: between the two bivalve species cultured (inter-species level); between shells of different individuals grown under the same laboratory- and field-culturing conditions (inter-individual shell level); and within individual shells (from the laboratory-culturing experiment only), i.e. between samples taken from one individual shell that correspond to different growth intervals during the experimental period (intra-individual shell level).

Differences in shell Mg/Ca ratios of the same species have been observed in previous field-based studies at levels of both inter- and intra-individual shell variability (Rosenberg and Hughes, 1991; Klein et al., 1996; Vander Putten et

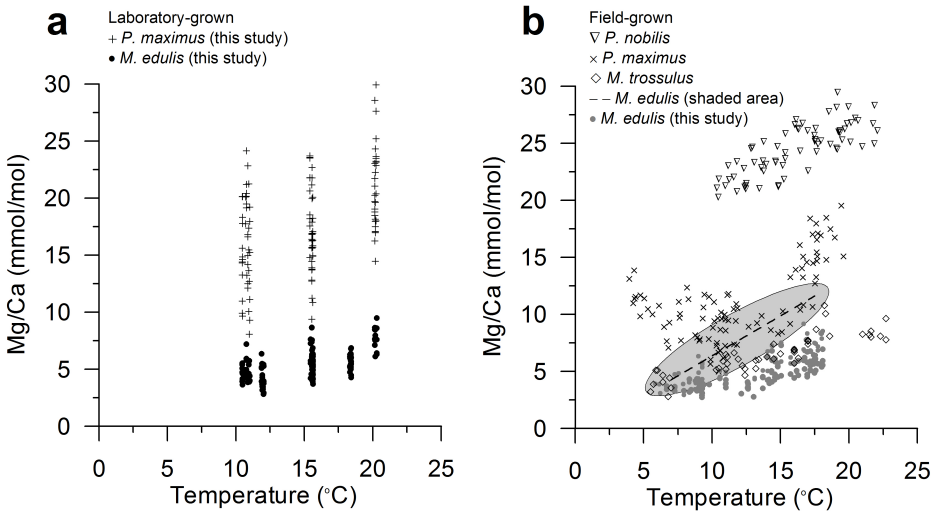

Fig. 7. Comparison of bivalve calcite shell Mg/Ca ratios, plotted against temperature, from laboratory-culturing completed in this study for *Pecten maximus*[1] and *Mytilus edulis*[1]; and field-culturing completed in this study for *Mytilus edulis*[1] and other field-based studies, for the species: *Mytilus edulis* (Vander Putten et al., 2000)[1], *Pecten maximus* (Freitas et al., 2006)[2], *Mytilus trossulus* (Klein et al., 1996)[1] and *Pinna nobilis* (Freitas et al., 2005)[2]. [1 denotes temperature is a measured seawater temperature; 2 denotes temperature is a $\delta^{18}O_{calcite}$-derived calcification temperature].

al., 2000; Freitas et al., 2005, 2006; Lorrain et al., 2005). For example, two field-collected shells (British Columbia, Canada) of the mussel *M. trossulus* show large Mg/Ca ratio differences at inter- (up to 2.5 mmol/mol) and intra- (up to 1.5 mmol/mol) individual shell levels over a range from 5.5 to 22.7°C (Klein et al., 1996). By comparison, four *M. edulis* field-grown shells (Schelde Estuary, Netherlands) showed inter-individual differences in Mg/Ca ratios as high as ∼7 mmol/mol. Similarly, Lorrain et al. (2005) presented data from four *P. maximus* specimens collected from the Bay of Brest, France, where differences of up to 6 mmol/mol in Mg/Ca ratios were observed between individual specimens. Most recently, in three *P. maximus* specimens grown in a field-based experiment differences were observed in Mg/Ca ratios of up to 7.5 mmol/mol between shells (Freitas et al., 2006). The inter- and intra-individual variability of Mg/Ca ratios in shell calcite of *M. edulis* and *P. maximus* grown in field-based and laboratory-culturing studies thus are of similar magnitude.

Large variations in the Mg content of biogenic calcite from different species has been observed previously in bivalves (Lorens and Bender, 1980; Klein et al., 1996; Vander Putten et al., 2000; Lorrain et al., 2005). On the whole, a large degree of overlap can be observed between the Mg/Ca ratio data obtained in this study for laboratory- and field-cultured *M. edulis* and *P. maximus* and published data from other field-based studies (Fig. 7). Nevertheless, laboratory- and field-cultured *M. edulis* show lower shell Mg/Ca ratios than data reported from field experiments for *M. edulis* by Vander Putten et al. (2000), although the latter data were obtained by laser ablation ICP-MS and there is the potential for calibration issues between datasets. The Mg/Ca ratios for *M. edulis* cultured in this study are, however, similar to Mg/Ca ratios reported for *M. trossulus* (Klein et al., 1996), a close relative of *M. edulis*. In *P. maximus* shell Mg/Ca ratios obtained in this study are similar to Mg/Ca ratios reported in other field studies (Lorrain et al., 2005; Freitas et al., 2006), but extend to higher values and also show a larger variability than in specimens grown at a field location adjacent to the present aquarium based study (Freitas et al., 2006). This latter observation suggests that the influence of any non-temperature control (i.e. a physiological control) on *P. maximus* shell Mg/Ca ratios may well be stronger under laboratory culture conditions than in field-based situations that more closely mimic the conditions best suited for optimal growth of natural populations. In addition, laboratory- and field-cultured specimens of both *M. edulis* and *P. maximus* were juveniles (<1 year age). If significant differences in shell elemental composition occur between juvenile and sexually mature specimens, this factor potentially could contribute to differences in Mg/Ca ratios between the present study and previous studies that used mature animals (Lorrain et al., 2005; Freitas et al., 2006).

4.2 Imprecise temperature control on shell Mg/Ca ratios

One obvious feature of the measured shell Mg/Ca ratios obtained in this study from specimens of *M. edulis* and *P. maximus* cultured in the constant-temperature aquaria is that there is only a weak dependence on temperature (Fig. 5a and Table 4). Nevertheless, in *M. edulis* specimens cultured in the field, shell Mg/Ca ratios were significantly correlated with

temperature (r^2=0.54 for all short-deployment specimens; r^2=0.72, 0.77 and 0.81 for the three annual-deployment specimens, $p<0.001$ in all cases). However, the inter-individual variability of Mg/Ca ratios is large (Fig. 5b) and results in a weaker correlation (r^2=0.51, $p<0.001$) when data from all field cultured specimens (i.e. from all short- and annual-deployment specimens) are pooled together (Table 4). Furthermore, linear regressions of Mg/Ca ratios with temperature are different: between laboratory-cultured *P. maximus* and *M. edulis*, between *M. edulis* specimens grown in the two laboratory-culturing experiments, between laboratory- and field-cultured *M. edulis* specimens, and between individual field-cultured *M. edulis* specimens. Therefore, the establishment of even a species-specific valid regression between Mg/Ca ratios and temperature has not been possible for the two species studied.

The presence of significant physiological controls on Mg/Ca ratios in bivalve calcite (i.e. metabolic or kinetic controls) is supported by: (1) the large inter- and intra-individual variability of Mg/Ca ratios observed in both the laboratory- and field-cultured specimens (Fig. 6); (2) the observation that specimens from the same species, *M. edulis*, cultured at different times (laboratory-culturing experiments one and two) at the same temperature (15°C) have significantly different absolute shell Mg/Ca ratios (∼1.1 mmol/mol, *t*-test, p=0.004, DF=24). Indeed, a metabolic control, i.e. the physiological exclusion of Mg from its shell-forming fluid (the extra-pallial fluid or EPF), on calcite Mg content has been proposed previously for *M. edulis* (Lorens and Bender, 1977, 1980), and suggested as a possible explanation for the observed seasonal breakdown in the relationship between Mg/Ca and temperature reported for species (Vander Putten et al., 2000). An apparent ontogenetic control of Mg/Ca ratios has also been described in the fan mussel *Pinna nobilis* (Freitas et al., 2005). For *P. maximus*, recent field-based studies have shown the absence of a significant correlation between shell Mg/Ca ratios and seawater temperature (Lorrain et al., 2005) or a strong seasonal variation in the strength of the correlation between shell Mg/Ca ratios and seawater temperatures, again suggesting that other factors must influence Mg/Ca ratios in *P. maximus* shell calcite (Freitas et al., 2006).

Given the experimental design in this study, only factors that were entirely independent of seawater temperature can be discussed as additional potential controls on shell Mg/Ca ratios. This consideration prohibits a detailed discussion of the influence of shell growth rate on shell Mg/Ca ratios, since growth rates co-vary significantly with temperature in both the laboratory- (for *P. maximus*, r^2=0.62, $p<0.001$; for *M. edulis*, r^2=0.23, p=0.001 and r^2=0.15, p=0.032 in experiment one and two, respectively) and field- (for *M. edulis*, $0.26<r^2<0.43$, $p<0.002$) culturing experiments. In both the laboratory- and field-culturing experiments, food intake most likely varied from animal to animal, and such variability would have driven differences in metabolic activity and shell growth rate between animals. However, as reported by Lorens and Bender (1980) for laboratory-cultured *M. edulis* specimens, shell Mg/Ca ratios also were only weakly correlated to shell growth rates in both the laboratory- and field-culturing experiments (Table 4), indicating that variable food supply was not a significant influence on shell Mg/Ca ratios.

Table 4. Summary of correlations between Mg/Ca ratios (mmol/mol) and temperature (°C), shell growth rate (SGR – μm day^{-1}) and salinity for all laboratory- (experiment one and two) and field-culturing experiments.

Experiment	Temperature		SGR		Salinity	
	r^2	p	r^2	p	r^2	p
Laboratory						
M. edulis 1	0.38	<0.001	0.19	0.006	–	>0.05
2	0.57	<0.001	0.33	<0.001	–	>0.05
1+2	0.37	<0.001	0.23	<0.001	–	>0.05
P. maximus 2	0.21	<0.001	0.09	0.002	0.21	<0.001
Field						
M. edulis All	0.51	<0.001	0.21	<0.001	0.26	<0.001
Short	0.54	<0.001	0.22	<0.001	0.26	<0.001
Annual 2	0.77	<0.001	0.27	0.004	0.43	<0.001
Annual 6	0.72	<0.001	0.28	0.001	0.30	0.001
Annual 20	0.81	<0.001	0.41	<0.001	0.51	<0.001

Seawater salinity is a truly independent variable in the laboratory-culturing experiment, but not in the field-culturing experiment where it co-varies with seawater temperature (r^2=0.50, $p<0.001$). Salinity varied by ca. 2 units and exhibited similar ranges in the laboratory- (from 31.9 to 33.2) and in the field-culturing (from 31.1 to 33.6) experiments. The Mg/Ca ratio of seawater is not expected to change with variations in salinity, although the absolute concentrations of Mg and Ca can. Any differences in seawater salinity between the two laboratory-culturing experiments thus could have influenced the amount of magnesium available for incorporation, assuming that shell Mg/Ca ratios are not solely related to seawater Mg/Ca ratios. Indeed, Lorens and Bender (1980) have shown that shell Mg/Ca ratios increase with increasing solution Mg concentrations, albeit at much higher concentrations than would be expected from natural changes in seawater salinity. By comparison, an earlier study by Dodd (1965) observed the opposite trend of increasing Mg concentrations in *M. edulis* shell calcite with decreasing salinity. In addition, salinity has been reported to significantly influence the Mg/Ca ratios of foraminifera calcite (Lea et al., 1999). In *M. edulis*, salinity was not significantly correlated with shell Mg/Ca ratios in the two laboratory experiments in this study ($p>0.05$), with only a weak correlation for *P. maximus* (r^2=0.21, $p<0.001$). The strength of this correlation between shell Mg/Ca ratios and salinity is, however, of comparable magnitude to that observed between temperature and shell Mg/Ca ratios (r^2=0.21, $p<0.001$), and slightly lower than previously observed for shell Mg/Ca ratios and salinity (r^2=0.36, $p<0.001$) in *P. maximus* (Freitas

et al., 2006). Nevertheless, temperature and salinity together (r^2=0.37, $p<0.001$) still do not explain much more of the observed shell Mg/Ca variability in *P. maximus* than just temperature alone.

pH also has been reported to influence Mg/Ca ratios in foraminifera calcite (Lea et al., 1999). However, in both laboratory- and field-culturing experiments Mg/Ca ratios were not influenced by pH. The relationship between Mg/Ca ratios and pH was found to be either very weak in the laboratory-culturing experiment ($r^2<0.16$ for *M. edulis* and *P. maximus*) or not significant in the field-culturing experiment in short- and annual-deployment specimens ($p>0.05$ for *M. edulis*).

4.3 Are Mg/Ca ratios in bivalve calcite an unreliable palaeotemperature proxy?

The differences in the Mg content of calcite secreted by different taxa, as well as differences in Mg/Ca ratios between and within individuals of a single species, suggest a strong physiological control of the incorporation of Mg into biogenic calcites. Bivalves, like other calcifying organisms, are capable of regulating, or at least influencing to variable extents, the Mg content of their calcium carbonate skeletons (Dodd, 1965; Lorens and Bender, 1977; Neri et al., 1979; Onuma et al., 1979; Lorens and Bender, 1980; Rosenberg and Hughes, 1991; Rosenberg et al., 2001). Examples of such physiological controls, either direct or indirect, on the Mg content of bivalve shell calcite are: variable chemical composition of the precipitating fluid, i.e. the EPF, resulting from biological control on differential transport of ions into and out of the EPF; variable calcification rates; the transport and diffusion conditions of the local precipitation microenvironment (Wasylenki et al., 2005); and differences in crystal growth orientation and morphology (Mucci and Morse, 1983; Reeder and Grams, 1987; Debenay et al., 2000; Erez, 2003).

Small-scale heterogeneous distribution of Mg may represent a particularly relevant source of error in the use of bivalve calcite Mg/Ca ratios as a palaeotemperature proxy. Lorens and Bender (1980) have described significant small-scale variability of Mg/Ca ratios, from <5 to 40 mmol/mol over scales of 100's μm, in the very first new shell growth from *M. edulis* cultured in natural seawater under controlled conditions at temperatures between 22 and 24°C. Small-scale variations in Mg concentrations in *M. edulis* calcite have been shown to derive from Mg being concentrated along the margins of calcite prisms, especially along the terminations of the crystals, with the alignment of adjacent crystals then producing compositional growth bands within the shell (Rosenberg et al., 2001). Therefore, the inclusion in milled powder samples of variable amounts of material from parts of the shell with different Mg/Ca ratios may provide an explanation for the large variability observed in Mg/Ca ratios from shell calcite grown at constant temperatures.

The now well-documented variation of Mg/Ca ratios in bivalve calcite at species-specific, inter- and intra-individual shell levels prevents the establishment of valid Mg/Ca ratio-temperature relationships, even for individual species. Furthermore, there exists support for a strong metabolic control of Mg/Ca ratios in bivalve shells (Lorens and Bender, 1977, 1980; Rosenberg and Hughes, 1991; Vander Putten et al., 2000; Rosenberg et al., 2001), although the mechanisms by which such a control acts are still not fully clear, as well as for extensive small-scale heterogeneity in shell Mg contents (Lorens and Bender, 1980; Rosenberg et al., 2001). Future research should address these issues in greater detail, if ever this geochemical proxy is to be used as a reliable and accurate temperature proxy in bivalve calcite.

5 Conclusions

The variability of Mg/Ca ratios in calcite sampled from two marine bivalve species exceeds the correlation with temperature. Such variability is significant within and among individuals and among species, and most likely reflects the influence of physiological factors influencing shell biomineralisation and Mg content. Shell Mg/Ca ratios were three to five times greater in *M. edulis* than in *P. maximus*. The variability of shell Mg/Ca ratios for laboratory-and field-cultured *M. edulis* in the present study was similar to the variability observed in previous field-grown specimens. Laboratory cultured *P. maximus* specimens, however, showed approximately twice the variability of shell Mg/Ca ratios reported in other field-based studies. In the two species, Mg/Ca ratios were not found to be controlled by shell growth rate or salinity. Strong physiological controls and extensive small-scale heterogeneity in shell Mg content may even prevent unique Mg/Ca ratio to temperature relationships for individual species being defined. Unless the physiological controls on Mg incorporation can be understood in more detail and subsequently compensated for, the use of this geochemical proxy as a reliable and accurate temperature proxy remains unlikely, at least in the bivalve species studied to date.

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