

## Shell layer variation in trace element concentration for the freshwater bivalve *Elliptio complanata*

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Received: 2 November 2007 / Accepted: 7 April 2008 / Published online: 22 July 2008  
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**Abstract** While the elemental chemistry of marine mollusk shells contains valuable environmental information, extending these interpretations to freshwater bivalve shells may be problematic, given the wide range of aqueous chemistries that exist in freshwater environments. To better understand the significance of these records, 20 bivalves were collected from four freshwater streams to determine the geochemical relationships that exist between the bivalve shell and their sources streams. The concentrations of manganese (Mn), copper (Cu), strontium (Sr), barium (Ba), and calcium (Ca) were analyzed by laser ablation inductively coupled plasma mass spectrometry across the inner (INL) and outer nacreous layer (ONL) of each shell for comparison to stream data collected over the period of shell growth. Within an individual shell, the content of Mn, Sr, and Ba were significantly higher in the INL than the ONL, while Cu concentrations were similar. Strontium and Ba concentrations co-varied between the two layers in 1:1 relationships, while Mn displayed a preferential enrichment in the INL that increased from 2:1 to 5:1 as the Mn content of the shell decreased. Each elemental profile can be correlated between shell layers, except for the trace element Cu, which appeared to be more closely related to the organic content of the shell. These results suggest that the major element

geochemistry of the shell layers differentially reflect the aqueous chemistry of the water in which a bivalve lived, but that these records are most likely overprinted by physiological processes specific to the mantle tissue from which a particular shell layer is secreted. Distribution coefficients ( $\text{shell}_{M/\text{Ca}}:\text{water}_{M/\text{Ca}}$ , where  $M$ =trace element of interest) were calculated using the median molar elemental concentration for each shell (by layer), and the appropriate water concentration. Bivalves from a contaminated site were excluded from this analysis because their shells were anomalous in size and color. For the remaining 15 shells, distribution coefficients (INL and ONL) were: Mn (0.50 and 0.21), Sr (0.26 and 0.17), and Ba (0.05: INL only). Inner nacreous layer values were comparable to the upper end of published estimates for freshwater bivalves and fish otoliths, while the ONL values were comparable to the lower range of values. Inclusion of shells from the contaminated site resulted in the calculation of distribution coefficients that fell outside the range of published estimates. These results suggest that exposure to pollutants may have a bearing on the biological factors that control the elemental concentration of bivalve shells in freshwater environments. While researchers generally avoid sampling shell material from the INL because of the potential for shell dissolution, the similarity in elemental profile trends observed here suggests that both the INL and ONL record accurate geochemical information, but in distinct forms. An obvious advantage of INL analysis is the relative increase in elemental concentration, but this is afforded at the expense of spatial resolution. Considered collectively, these results suggest that valuable information can be gleaned from the elemental concentration of freshwater bivalve shells when care is taken in the choice of material from which these records are extracted.

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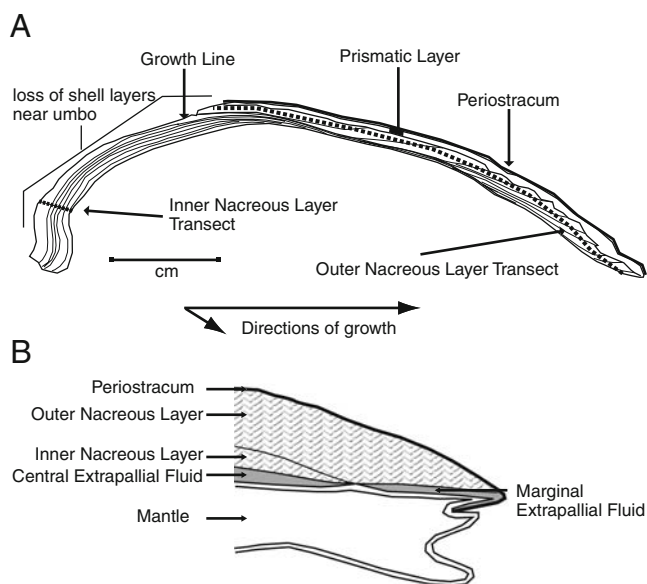
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## Introduction

Molluscan shells provide an incremental chemical record that is, to some extent, related to the environment. Because mollusks inhabit a wide range of environments, from marine to freshwater, and from deep-sea vents to mountain streams, the trace element composition of their shells is a potentially rich source of environmental information. For example, fluctuations in Sr across bivalve shells have been related to deep-sea vent activity (Hart and Blusztajn 1998), while Ba has been related to algal blooms and freshwater input (Stecher et al. 1996) in coastal settings. Interpretation of shell records depends on understanding how the elemental composition of the shell relates back to the environmental parameter of interest, which includes understanding how biological factors, such as the growth and physiology of the organism, affect the elemental composition of shell material. Geochemical and isotopic records may be influenced by biological factors including growth rate (Stecher et al. 1996) and/or growth cessation (Tevesz and Carter 1980), metabolic controls on shell formation (Rosenberg and Hughes 1991), and the biochemistry of the extrapallial fluid from which the shell is deposited (Wilbur and Saleuddin 1983).

Potential insights into biological controls on trace element distributions may be expressed through a comparison of distinct components of a single shell. Mollusks form shells of multiple layers that are precipitated from two distinct fluid reservoirs, the central and marginal extrapallial fluids (EPF), which are separated from each other at the pallial attachment by mantle tissue (Crenshaw 1972; Lorens 1981; Wilbur and Saleuddin 1983; Wheeler 1992). Currently, there are no data available to determine whether these two fluid reservoirs are similar, or distinct in chemical composition.

Unionids, a freshwater class of bivalves, precipitate nacreous aragonite from both the central and marginal extrapallial fluid reservoirs, and growth bands dividing periods of shell growth can be traced through both layers (Fig. 1; Neves and Moyer 1988). Because these two layers share common environmental factors, such as the ambient temperature of the fluid from which shell material is precipitated, and bioavailability of trace elements, similar growth records of elemental concentrations would be expected in the absence of biological influence. Any difference would suggest some biological input, whether it be differences in mineralization rate, EPF composition, metabolic rate, dissolution rate, or some combination of these, or other factors. Of particular concern is that any difference would affect the observed relationship between the shell and environmental factors. This could have



**Fig. 1** **a** Cross-section of unionid shell with growth lines. Path of sampling transects marked by dotted lines. **b** Representation of cross-section of shell with respect to location of central and marginal extrapallial fluid

repercussions for the interpretation of shell records of historical and paleoenvironments.

To date, the relationship among trace element constituents in contemporaneously deposited shell material across shell layers is almost unexplored. A few studies have analyzed material along transects that cross shell-layer boundaries (Carell et al. 1987; Nystrom et al. 1996; Lazareth et al. 2003), but the goal of these studies was not to determine differences in shell chemistry among layers. One study did look at the Sr:Ca ratios in the prismatic and cross-lamellar layers of the marine *Merccenaria campechiensis*, and found that only the middle cross-lamellar Sr:Ca covaried with oxygen isotope ratios (Surge and Walker 1996). Alternatively, several studies have observed variation in trace element content within a single growth layer of marine mollusk species (Rosenberg and Hughes 1991; Klein et al. 1996; Carré et al. 2006), and have discussed observed variability in terms of metabolic rate and Ca transport pathways.

This study examines whether the inner and outer nacreous layers of unionid bivalves differ in trace element chemistry, and whether the relationship between aqueous concentrations and shell concentrations are affected by which layer is sampled. The concentrations of manganese, copper, strontium, and barium were analyzed in the two nacreous layers of *Elliptio complanata* collected from several streams that experienced differential environmental

histories. Manganese and copper were chosen because of their potential use as indicators of industrial activity (e.g., Markich et al. 2002). Barium and manganese have been proposed as indicators of algal productivity (Vander Putten et al. 2000; Langlet et al. 2007). In paleo-freshwater environments, this has the potential for indicating the presence of upstream lakes or ponds, which can lead to the alteration of other paleoenvironmental proxies such as oxygen isotopes due to evaporation. Strontium has been the subject of multiple studies due to its potential as a paleothermometer in corals (Lorens and Bender 1980; Stecher et al. 1996; see Table 1). In marine bivalves where aqueous Sr:Ca is fairly constant, growth rate appears to override other influences on shell Sr concentration (Stecher et al. 1996; Gillikin et al. 2005). Nonetheless, the breadth of research on Sr in molluscan aragonite makes Sr a potential key for understanding the incorporation of trace elements into bivalve shells. These elements were also chosen for their relatively high concentration in unionid shell nacre. A comparison of the trace element content of these two layers provides insights into potential biological

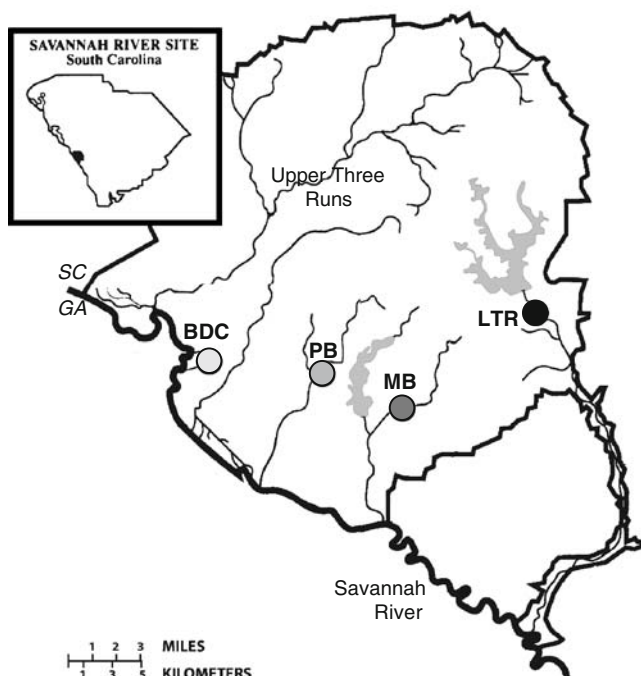
controls on shell chemistry in freshwater bivalves, and a summary of previous work on these elements is included in Table 1.

**Materials and methods**

Five specimens of *E. complanata* were collected from a single location within each of four streams at the Department of Energy's Savannah River Site in South Carolina in the summer of 2003: Meyers Branch (MB) and Pen Branch (PB), which are undisturbed first-order streams; Lower Three Runs (LTR), a stream that drains a dormant nuclear cooling water reservoir of 2,500 acres; and Beaver Dam Creek (BDC), a stream that receives nearby Savannah River water that is routed through a coal-fired power plant (Fig. 2). Existing monthly data on water quality were collected from Savannah River Site Environmental Data Reports for the years 1996 through 2003 (EPD 1997–2004). Monthly water samples were also collected from these sites from June 2004 through December 2005.

**Table 1** Summary of selected trace element concentrations in calcium carbonates and distribution coefficients from previous work

| Substrate          | Environment | Mn (ppm) | $D_{Mn}$  | Cu (ppm) | $D_{Cu}$ | Sr (ppm)       | $D_{Sr}$   | Ba (ppm)   | $D_{Ba}$   | Reference                           |                              |
|--------------------|-------------|----------|-----------|----------|----------|----------------|------------|------------|------------|-------------------------------------|------------------------------|
| Mineral: calcite   |             |          |           |          |          |                |            |            |            |                                     |                              |
| Bivalve            | Marine      |          |           |          |          | 400–700        |            |            |            | Klein et al. (1996)                 |                              |
|                    |             | 0.5–100  |           |          |          | 350–600        |            | 1–50       |            | Vander Putten et al. (2000)         |                              |
|                    | Estuarine   | 2–20     |           |          |          | 650–700        |            | 1–30       | 0.07–0.10  | Gillikin et al. (2006)              |                              |
|                    |             |          |           |          |          |                |            | 1–5        |            | Lazareth et al. (2003)              |                              |
| Mineral: aragonite |             |          |           |          |          |                |            |            |            |                                     |                              |
| Inorganic          |             |          |           |          |          |                | 1.15       |            |            | Kinsman and Holland (1969)          |                              |
| Coral              | Marine      |          |           |          |          |                | 1.06–1.11  |            |            | References in Stecher et al. (1996) |                              |
| Bivalve            | Marine      |          |           |          |          |                | 0.23       |            |            | Lorens and Bender (1980)            |                              |
|                    |             |          |           |          |          | 1,500–4,500    |            | 10–175     |            | Stecher et al. (1996)               |                              |
|                    |             |          |           |          |          | 600–2,000      |            | 1–80       |            | Toland et al. (2000)                |                              |
|                    |             |          |           |          |          | 750–3,000      |            |            |            | Gillikin et al. (2005)              |                              |
|                    | Freshwater  |          | <1–40     |          |          |                | 400–3,200  |            | <1–400     |                                     | Carré et al. (2006)          |
|                    |             |          |           |          |          |                |            | 0.22–0.28  |            |                                     | Faure et al. (1967)          |
|                    |             |          | 10–600    |          |          |                | 300–600    |            |            |                                     | Nystrom et al. (1996)        |
|                    |             |          | 400–6,000 |          |          |                |            |            |            |                                     | Mutvei and Westermark (2001) |
|                    |             |          | 300–1,700 | 0.6      | 4–18     | 0.17           |            |            |            |                                     | Markich et al. (2002)        |
|                    |             |          | 100–700   | 0.5      |          |                | 120–220    |            |            | 0.1                                 | Verdegaal (2002)             |
| Gastropod          | Freshwater  |          |           |          |          | 700–1,000      | 0.28       |            |            | Bailey and Lear (2006)              |                              |
|                    |             |          |           |          |          | 100–1,000      |            |            |            | Langlet et al. (2007)               |                              |
|                    |             |          |           |          |          | 200–800        |            |            |            | 2–7                                 | Ravera et al. (2007)         |
|                    |             |          |           | 80–1,700 | 0.2, 0.5 | <1–30          | 120–2,000  | 0.17, 0.26 | 60–400     | 0.05                                | This study                   |
|                    |             |          |           | 1–60     |          |                | 160–2,800  | 0.31       | 40–350     | 0.48                                | Rosenthal and Katz (1989)    |
| Otolith            | Estuarine   |          |           |          |          | 11,000–130,000 | 0.29, 0.46 | 3–30       | 0.06, 0.14 | de Vries et al. (2005)              |                              |
|                    | Freshwater  |          |           |          |          |                |            | 0.04       |            | Wells et al. (2003)                 |                              |



**Fig. 2** Map of the Savannah River Site. *BDC* Beaver Dam Creek, *LTR* Lower Three Runs, *PB* Pen Branch, *MB* Meyers Branch

#### Water analysis

Water samples were collected by hand from each stream once per month between January 2004 and December 2005, and acidified immediately to 1% with Optima grade concentrated nitric acid ( $\text{HNO}_3$ ). They were filtered through a  $0.45 \mu\text{m}$  filter prior to analysis. An internal standard of rare elements was added to the water samples, and they were then analyzed by inductively coupled plasma mass spectrometry (ICP-MS) to obtain the elemental concentration of Ca, Mn, Cu, Sr, and Ba. Standards of 1, 50, and 500 ppb were run every 20 samples, along with an independent calibration standard. Analytical precision was less than 5% (rsd) at the observed concentrations, except

for Ca, which yielded a precision of 9% (rsd). Because calcium was at higher concentration than the other analytes, a second 10:1 dilution of each water sample was required prior to measurement by ICP-MS, which partially explains the decreased precision.

#### Shell analysis

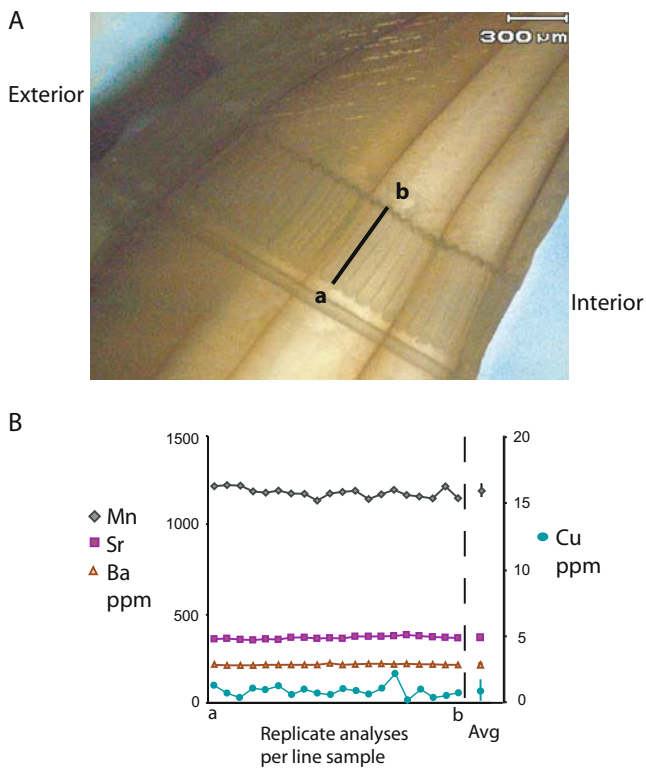
The valves were separated, and the soft tissue was removed from each bivalve. Shells were washed, dried, weighed, and measured for length, width, and depth. The left valve was sectioned along the axis of maximum growth with a wet diamond blade for the preparation of a 1-mm-thick section, without placing the shell in epoxy. The sections were polished with 600-grit SiC, rinsed, and photographed using a standard petrographic microscope.

Shell sections were analyzed by UV-laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The system consists of a Merchantek™ UP frequency quintupled neodymium-doped yttrium aluminum garnet laser operated at 213 nm that was coupled to a PerkinElmer Sciex ELAN DRC<sup>plus</sup> ICP-MS. The laser was operated in the Q-switched mode with a power of 0.25 mJ at a repetition rate of 10 Hz. Shell concentrations were analyzed with an  $80 \mu\text{m}$  laser beam spot scanned along a line parallel to growth bands at  $5 \mu\text{m s}^{-1}$  for approximately  $400 \mu\text{m}$ , collecting 20 individual analyses per line from which an average sample concentration was computed (see Table 2 for instrument parameters; Fig. 3). Data were calibrated using the NIST 610 glass standard, and a series of in-house carbonate standards having concentrations ranging from 5 to 500 ppm. Detection limits were not quantified, but are well below the ppm level observed in these shells. Repeated laser ablation line analyses of the standards varied less than 5% rsd for all elements analyzed. Gas blank subtraction,  $^{43}\text{Ca}$  normalization, and drift correction were used in all calibration procedures (Toland et al. 2000).

Shell sections were analyzed across the inner and the outer nacreous layers (see Fig. 1) at spots that were

**Table 2** LA-ICP-MS operating conditions

| Laser probe                           |  | ICP-MS                   |                     |
|---------------------------------------|--|--------------------------|---------------------|
| Laser mode                            | Q-switched   | Argon flow rate          | $\text{L min}^{-1}$ |
| Laser power (mJ)                      | 0.25   | Carrier gas              | 1.0–1.1             |
| Frequency (Hz)                        | 10   | Auxiliary gas            | 1.2                 |
| Raster speed ( $\mu\text{m s}^{-1}$ ) | 5  | Cooling gas              | 15                  |
|                                       | $^{43}\text{Ca}$ (internal standard), $^{55}\text{Mn}$ , $^{63}\text{Cu}$ , $^{88}\text{Sr}$ , $^{238}\text{Ba}$ | Acquisition mode         | Peak jumping        |
|                                       |  | Analyses per line sample | 20                  |
|                                       |  | Dwell time (ms)          | 16.67               |
|                                       |  | Acquisition time (s)     | 3.8 (total 1:15)    |



**Fig. 3** **a** Photograph of inner nacreous layer near umbo of laser-ablated shell cross-section PB 8, from Pen Branch. **b** Mn, Sr, Ba, and Cu concentrations in ppm across one line sample (*a* to *b*) of shell (from laser ablation), with resultant average and  $\pm 1\sigma$

assumed to be representative of the entire shell layer. Transects across the inner shell layer provided an average of 20 line analyses at 80  $\mu\text{m}$  spatial resolution, while the outer layer provided an average of 70 line analyses per transect. A calibrated dataset from one inner layer transect of 28 (80- $\mu\text{m}$ -wide) line samples is shown in Fig. 3b. To estimate day-to-day variability in instrument response during the study, two shells were analyzed on multiple days over the course of the study. Individual line analyses

along these transects varied between days by less than 20% *rsd* for Mn, Sr, and Ba, and over 90% *rsd* for Cu.

Aqueous and shell concentrations of elements are reported by volume and weight, respectively. For shell concentrations, this was based on assuming shell composition was 40% calcium. Statistical analyses including *t*-tests, and correlation and regression analyses were performed following the methods of Sokal and Rohlf (1995) to explore and clarify the relationships among variables. Statistical significance is assumed when  $p \leq 0.05$ .

**Results**

**Water chemistry**

The aqueous concentrations of Ca, Mn, Cu, Sr, and Ba varied within and among sites (Table 3), and over time. All the sites displayed overlapping aqueous concentrations, except Beaver Dam Creek, which was anomalously low in calcium and high in strontium. Water chemistry from Beaver Dam Creek was distinct, because it receives water from a nearby coal fly ash retention basin.

Historical data for temperature, pH, dissolved oxygen, and Mn and Cu concentrations were taken for comparison from annual Department of Energy (DOE) reports (EPD 1997–2004; Table 3). The concentrations of Mn were similar to this study, while Cu concentrations averaged a factor of two lower. No historical records were available for Ca, Sr, and Ba concentrations.

**Shell chemistry**

Laser ablation analyses ( $n=1,800$ ) of the outer (ONL) and inner nacreous layers (INL) of all five shells from each of the four sampled streams yielded Mn, Sr, and Ba concentrations on the order of hundreds of ppm, and Cu concentrations near 1 ppm (Fig. 4). Among sites, the

**Table 3** Stream summary details from the present study for 2004–2005, and from DOE environmental reports for 1997–2004 (EPD 1997–2004), showing the median of monthly samples (in parts per billion) with the interquartile (25th–75th) range (in brackets)

|  | T          | pH            | DO (mg L <sup>-1</sup> ) | Ca                  | Mn          | Cu        | Sr         | Ba         |
|--|------------|---------------|--------------------------|---------------------|-------------|-----------|------------|------------|
| 2004–2005 (this study, $n=18$ )                |            |               |                          |                     |             |           |            |            |
| Beaver Dam Creek (BDC)                         |            |               |                          | 4,200 (3,300–4,500) | 79 (73–102) | 4 (3–9)   | 51 (44–62) | 49 (39–66) |
| Lower Three Runs (LTR)                         |            |               |                          | 5,700 (5,000–7,300) | 85 (58–188) | 5 (1–9)   | 21 (20–26) | 33 (28–41) |
| Meyers Branch (MB)                             |            |               |                          | 6,000 (5,200–6,800) | 52 (37–72)  | 5 (2–8)   | 28 (26–39) | 40 (31–62) |
| Pen Branch (PB)                                |            |               |                          | 6,000 (5,200–6,500) | 70 (57–86)  | 3 (1–8)   | 19 (17–22) | 60 (48–89) |
| DOE environmental reports 1997–2004 ( $n=76$ ) |            |               |                          |                     |             |           |            |            |
| Beaver Dam Creek                               | 22 (18–26) | 6.8 (6.5–7)   | 7.7 (6.7–9.1)            |                     | 68 (57–94)  | 2 (<1–5)  |            |            |
| Lower Three Runs                               | 18 (13–21) | 6.6 (6.4–7)   | 8.0 (6.8–9.3)            |                     | 74 (48–105) | 2 (<1–8)  |            |            |
| Meyers Branch                                  | 19 (14–23) | 6.7 (6.3–6.9) | 8.0 (7.0–9.4)            |                     | 51 (36–69)  | 1 (1–2)   |            |            |
| Pen Branch                                     | 19 (14–25) | 6.6 (6.4–6.8) | 8.6 (7.6–9.4)            |                     | 59 (47–101) | <1 (<1–2) |            |            |

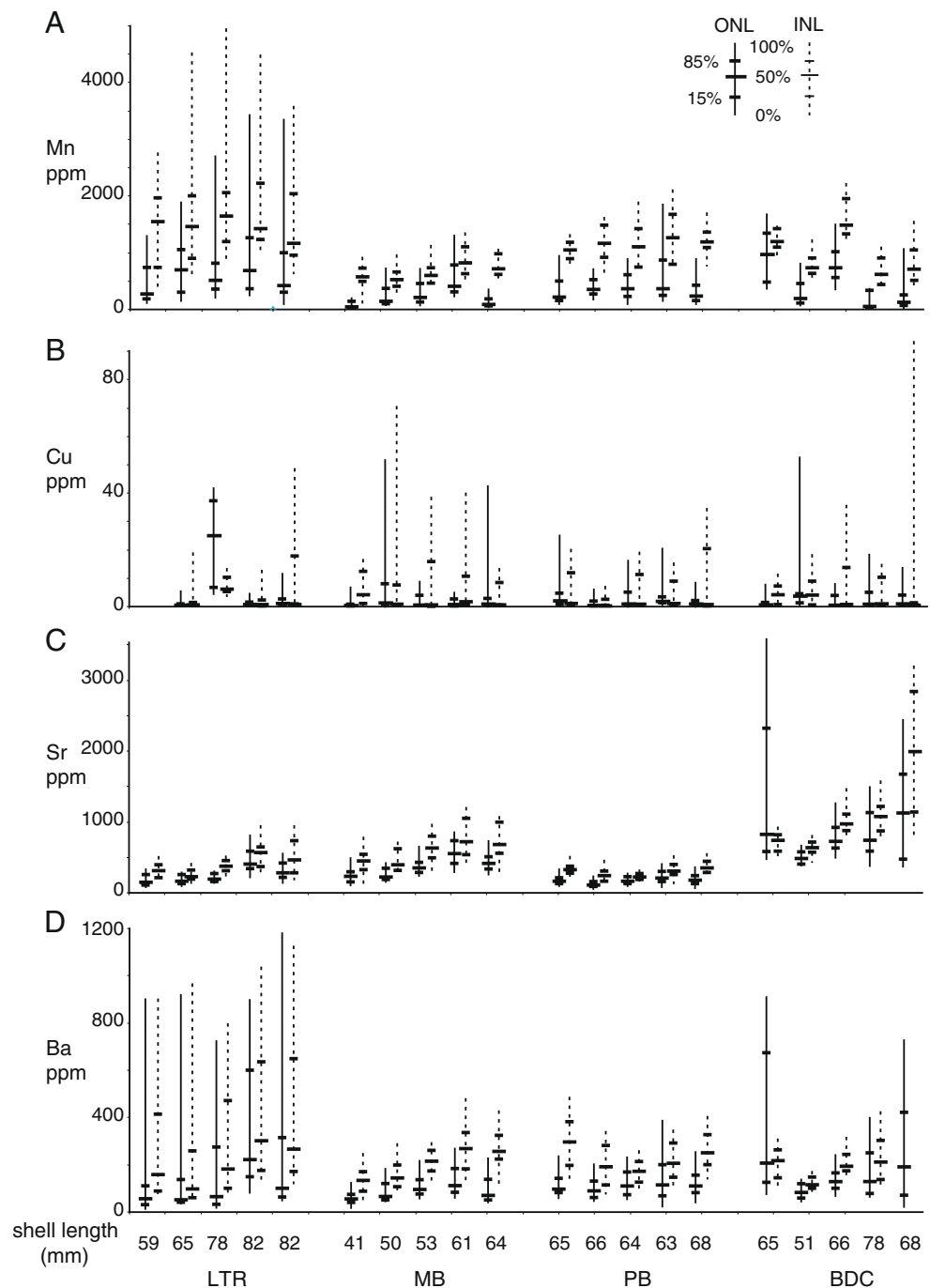
average values for shell concentrations of Mn ranged from 200 to 540 ppm in the ONL, and 670 to 1,460 ppm in the INL; Cu ranged from 1 to 7 ppm in the ONL, and 1 to 2 ppm in the INL; Sr ranged from 180 to 790 ppm in the ONL, and 300 to 1,090 ppm in the INL; Ba ranged from 80 to 150 ppm in the ONL, and 200 to 240 ppm in the INL.

All the shells showed markedly higher concentrations of Mn and Ba, and lower concentrations of Sr compared to aragonitic marine bivalves (see Table 1; no data are available for Cu). Average shell concentrations of Mn,

Cu, and Sr were of similar orders of magnitude to previous studies of aragonitic freshwater bivalves, with INL concentrations being closer to the higher end of the range, and ONL concentrations being closer to the lower end of the range. No data are available for shell concentrations of Ba in freshwater bivalves.

Shells from different sites had distinct ranges of trace element chemistry (Fig. 4). Shells from LTR, downstream of a lake, had wide ranges of Mn and Ba. MB and PB, undisturbed stream sites, differed in the relative contribu-

**Fig. 4** **a** Distributions of Mn concentrations across the ONL (*solid*) and INL (*dashed*) for all 20 shells from LTR, MB, PB, and BDC, with range, median, and 15–85% brackets. **b** Distributions for Cu. **c** Distributions for Sr. **d** Distributions for Ba. Whole shell length (maximum dimension) appears for each individual



tion of Sr and Mn to shell chemistry. Shells from BDC, downstream of a coal-fired power plant, displayed no consistent signal among the five shells, except for relatively high concentrations of Sr.

#### Temporal comparisons between shell layers

Assuming that shell carbonate was deposited over the lifetime of the organism in both shell layers as delineated by growth banding, the ratio of the length of the ONL to INL transect of a shell provides a coarse estimate of the relative growth rate between the two layers. Furthermore, a comparison of the trends in the elemental profiles can be made by normalizing the transect lengths from a single shell by expanding or contracting the ONL or INL scales until they are of equal dimension. This allows for a temporal comparison of the relative variations of Mn, Cu, Sr, and Ba to be made between the two layers (Fig. 5). In the following description of shell profiles, two shells were selected as exemplars of common attributes of the entire suite of shells.

The paired transects from shell LTR 7 (Fig. 5a,b) display transient positive excursions in Mn concentration. While growth rates are clearly different in the two layers, based on profile lengths (51.8 mm for the ONL, and 2.3 mm for the INL), the observed distance among five maxima in Mn are easily detected, and show that the pattern of variation in trace element concentration is similar across both shell layers. It should be noted that because the INL transect provides a significantly reduced spatial (and, therefore, temporal) resolution ( $\times\sim 22$ ), the fine structure of the records should be somewhat diminished, and low-intensity or high-frequency events should be obscured or dampened compared to the ONL record. That this is generally not the case is a testament of the resolving power of the LA-ICP-MS technique. As a consequence, the general structure of all the trace element profiles, except for Cu, displays similar characteristics that may be correlated between the shell layers.

Despite large differences in the concentration of trace elements among shells from all four sites, and within shells from BDC, the pattern of internal consistency between the ONL and INL records of single shells is maintained (Fig. 5c, d). An additional observation is that for shells from BDC, PB, and MB, the Ba profile covaries with the Sr profile. This contrasts with the shells from LTR, where the Ba profiles more closely resemble the Mn profiles in shape (Fig. 5a,b).

Arguably, the most significant and consistent finding of this study was that for all shells, the ONL had a significantly lower concentration of Mn, Sr, and Ba than the INL ( $t=7.0$ , 3.1, 5.3, respectively,  $p<0.01$ ), while Cu concentrations did not vary systematically between shell layers ( $t=0.5$ ,  $p=0.62$ ). The median laser ablation concentrations for the ONL and INL of the 20 shells were log transformed, and tested

for correlation. The ONL concentration was positively correlated with the INL concentration for all four elements (Sr:  $r^2=0.91$ ,  $p<0.01$ ; Mn:  $r^2=0.64$ ,  $p<0.01$ ; Ba:  $r^2=0.47$ ,  $p<0.01$ ; Cu:  $r^2=0.23$ ,  $p=0.04$ ; Fig. 6). Therefore, although the elemental concentrations between the shell layers were significantly different, they were functionally related.

The slope of the model II regression lines for Sr and Ba were similar ( $Z=0.12$ ,  $p=0.90$ ), and close to unity (1.2 and 1.1, respectively), signifying that as shell concentrations of Sr and Ba increased in the ONL, they did so equally in the INL. These results differed significantly from those for Mn ( $Z=3.9$ ,  $p<0.01$ , slope=2.1). At lower shell concentrations, Mn was five times less concentrated in the ONL than in the INL, while at higher concentrations this depletion was closer to a factor of two. Finally, while copper concentrations were poorly correlated between layers, the slope was not significantly different from one ( $Z=0.07$ ,  $p=0.95$ ).

## Discussion

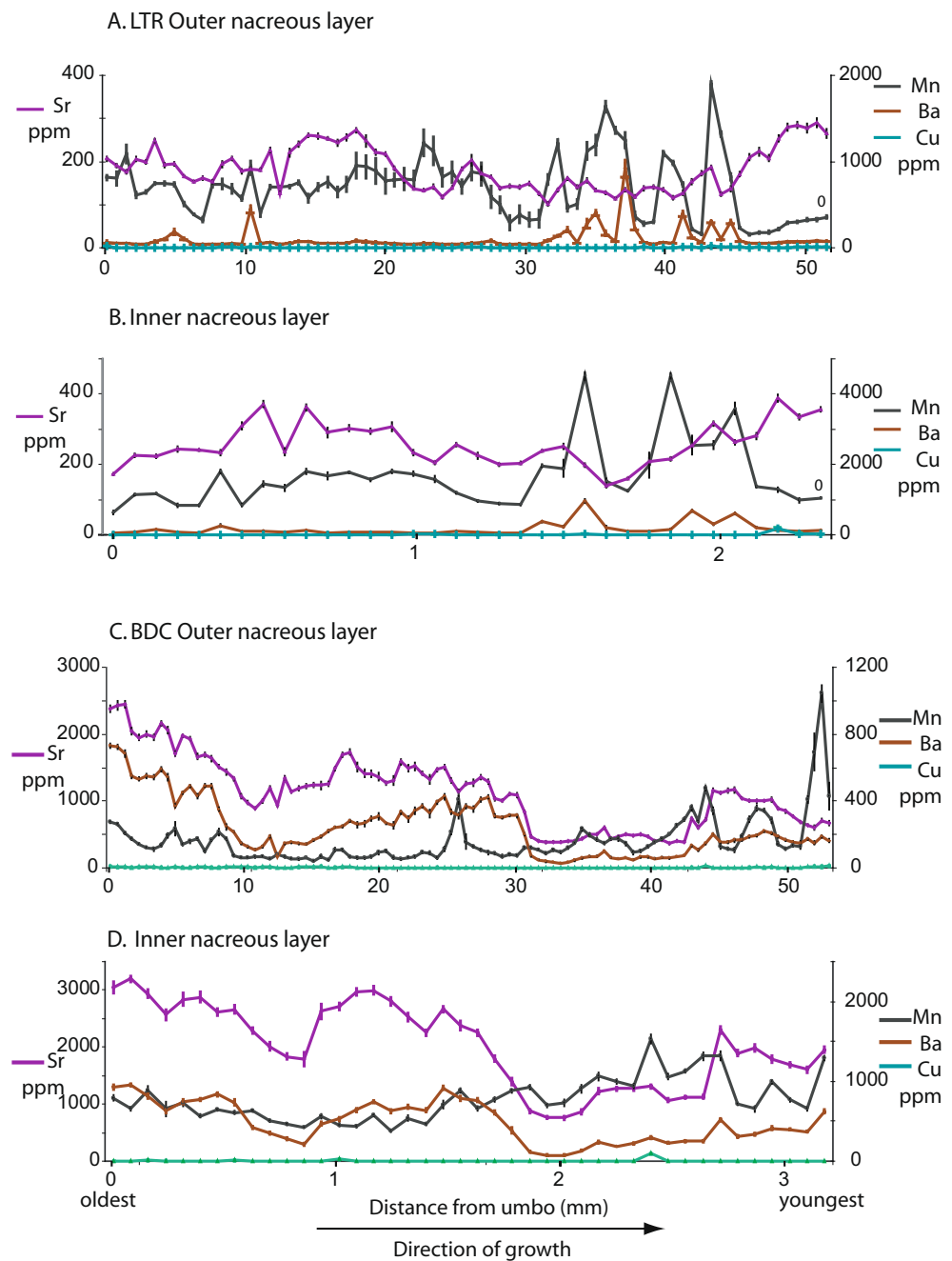
### Comparison of water and shell chemistry

Each analysis was converted to a molar ratio versus calcium before the characterization of distribution coefficients between the water and the shell layer. These variables were determined from the slope of the best fit line through a regression of shell trace element concentrations (vs. Ca) onto water concentrations. The regression lines used for the calculation of distribution coefficients were forced through zero (Lea and Spero 1992) for all elements, except Mn. Mn required a non-zero intercept to ensure a significant correlation between water and shell concentration. This was also observed by Markich et al. (2002), although the significance of the resulting intercept was not discussed. Transient positive excursions in Ba concentration exceeding 200% of the median shell value were removed from all profiles prior to the determination of average values (see Gillikin et al. 2006 for details). This procedure was repeated until the change in median Ba concentration was less than 5% between consecutive iterations. The shells of Beaver Dam Creek bivalves were relatively thin and discolored, and there was large variation among shell records from this stream (Fig. 4). Therefore, comparisons of shell trace element and water chemistry data were made with, and without the inclusion of the Beaver Dam Creek data.

### Manganese

The ratio of Mn:Ca (mmol:mol) of waters is compared to shell concentrations in Fig. 7a. Including all four sites, no significant correlation was observed between water and

**Fig. 5** Profiles of Mn, Cu, Sr, and Ba concentrations in ppm across the outer (a) and inner (b) nacreous shell layers of LTR 7 from Lower Three Runs, and across the outer (c) and inner (d) nacreous shell layers of BDC 6 from Beaver Dam Creek. Vertical bars represent  $\pm 1\sigma$  of multiple analyses within each raster. Direction of growth is to the right of the graph



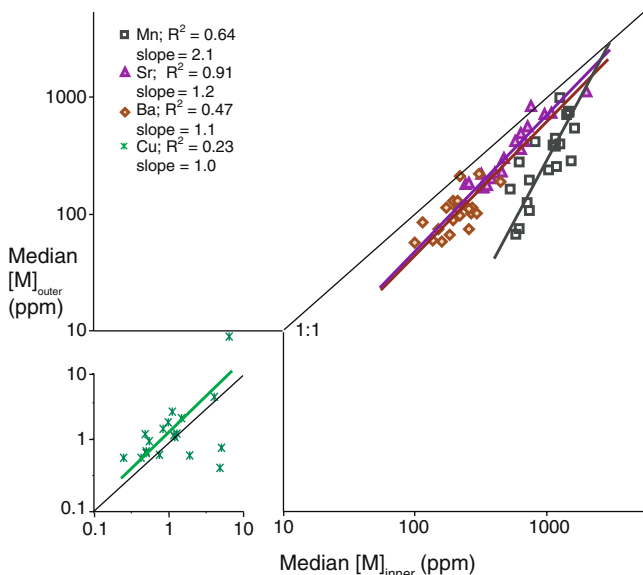
shell concentrations for either the ONL or INL. Excluding the data from Beaver Dam Creek, the remaining sites showed a significant correlation between stream and shell Mn:Ca ratios (INL:  $r^2=0.99$ ,  $p<0.01$ , slope=0.50; ONL:  $r^2=0.95$ ,  $p=0.03$ , slope=0.21). Markich et al. (2002) found a significant correlation ( $r^2=0.81$ , slope=0.56) between the Mn:Ca ratio of whole shells of aragonitic freshwater bivalves and Mn:Ca values for waters from several pristine and impacted sites, but they did not discriminate between inner and outer nacreous layers in their analysis. The slope of the relationship for the inner layer of this study is close

to that observed by Markich et al. (2002, see Table 1), while the slope for the outer nacreous layer is significantly lower ( $Z=12$ ,  $p<0.01$ ). This indicates that shell layer type could have a large impact on the subsequent interpretation of shell Mn concentrations.

#### Strontium

Including the data from all four sites, Sr:Ca ratios showed a correlation between shell and water with slopes of 0.16 for the INL, and 0.12 for the ONL (Fig. 7c:  $r^2=0.97$  and 0.99,





**Fig. 6** Log plot of median inner and outer nacreous layer concentrations of Mn, Sr, and Ba (upper plot), and Cu (lower plot).  $n=20$  (all shells from all sites) for each element

respectively,  $p < 0.01$ ). These values are significantly lower than the values of 0.22 to 0.26 observed for marine and freshwater aragonitic bivalves from other studies (see Table 1). If the data from Beaver Dam Creek are excluded, the slopes increase to 0.17 and 0.26 for ONL and INL,

respectively, which are within, or closer to the range of values previously observed for bivalves. Bailey and Lear (2006) specifically studied Sr concentrations in the INL of freshwater bivalves from streams with slightly higher Sr:Ca ratios (4–5 mmol:mol), and calculated a distribution coefficient of 0.28.

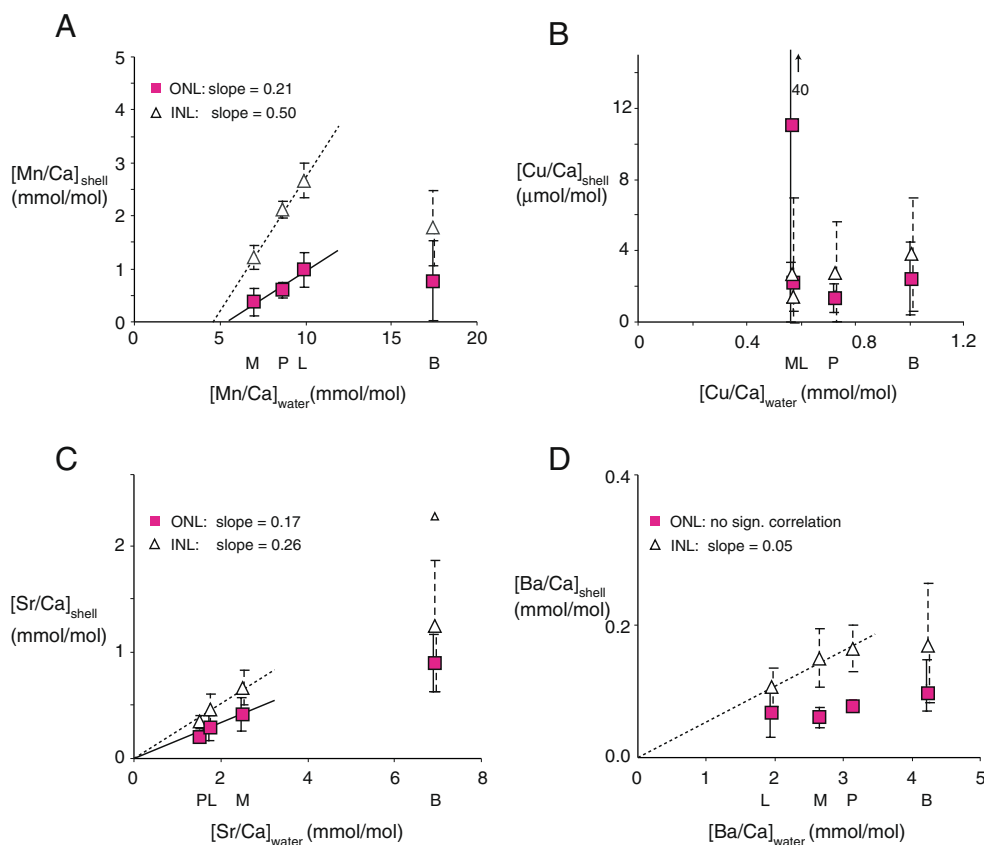
*Barium*

Excluding the data from BDC, there was no statistically significant correlation between the concentration of Ba in the ONL and water ( $p=0.59$ ), while a slope of 0.05 was observed for the INL ( $r^2=0.97$ ,  $p=0.01$ ; see Fig. 7d). When data from BDC are included in the analysis, the slope of the model II regression between the INL and water is reduced to 0.02 ( $p=0.08$ ). Both of these values are smaller than that of 0.48 reported by Rosenthal and Katz (1989) for bulk aquatic snails inhabiting freshwater sites having an aqueous Ba content of 11 to 120 ppb. The relationship observed in this study (0.04) is consistent with that for Ba concentrations calculated for aragonitic otoliths of freshwater fish (Wells et al. 2003).

*Copper*

In this study, aqueous copper concentrations were relatively low, and did not vary greatly by site (see Table 3). The ratio

**Fig. 7** Plots of average site millimole M:mole Ca in shells versus millimole M:mole Ca in resident river water, where M denotes Mn, Cu, Sr, or Ba. *B* Beaver Dam Creek, *L* Lower Three Runs; *M* Meyer's Branch, *P* Pen Branch. Error bars represent  $\pm 1\sigma$  standard deviation of the data. Filled symbols, regression line dashed outer nacreous layer, open symbols, regression line solid inner nacreous layer (see text for details). Regression lines shown do not include data for Beaver Dam Creek (see text)



of Cu:Ca in water showed no correlation with Cu:Ca (mmol:mol) of the INL ( $p=0.09$ ), or the ONL ( $p=0.52$ ; Fig. 7b), and removing BDC data from the analysis did not improve the results. The lack of correlation may be derived in part from the limited range in Cu observed in water and shell in the present study. For example, Markich et al. (2002) found a strong correlation between the concentration of copper in water and shell over a six-fold range in water concentration. A wider range of ambient Cu:Ca concentrations would clarify the relationship with shell chemistry, as well as any systematic differences in the concentration of the INL or ONL. The only correlations observed with Cu were transient positive excursions in shell Cu concentration in the INL that were almost invariably associated with the organic-rich bands in the shells. The possibility of isobaric interference from polyatomic species from ablation of the organic matrix cannot be dismissed as an explanation for spikes in Cu concentration, although calculations of average Cu concentrations match previous studies (Table 1).

#### Models of shell mineralization in terms of layer differences

The incorporation of trace elements in bivalve shell requires a myriad of biochemical processes to import Ca from the environment to the site of skeletogenesis. Any of these steps could lead to differences in layer composition, and some combination of steps is probably indicated. Clearly, the growth rate between the two layers is different, and the most parsimonious explanation for differences in elemental concentration between layers is based on abiotic rules of mineral precipitation. Yet, theoretical distribution coefficients between aqueous solution and aragonite for Mn, Sr, and Ba suggest that changes in growth rate would not have the same effect on all three elements (Wang and Xu 2001). Gaetani and Cohen (2006), in an experimental study of abiotic and coralline aragonite, conclude that near-surface precipitation kinetics can cause trace element-enriched zones when crystallization rate is high enough relative to near-surface diffusion. Variation in coral trace element chemistry could not be explained by abiotic forces alone, and the authors also observed biologically selective transport of Mg and Ba to the precipitating fluid.

The transport of ions into the central and marginal EPF might differ enough to lead to some of the differences observed between the INL and ONL. Klein et al. (1996) proposed two pathways, a passive intercellular diffusion of ions into the EPF, and an active intracellular Ca-ATPase mediated pathway. An increase in active transport would increase the concentration of Ca relative to trace elements. This could explain the lower concentrations of trace elements in the ONL, if active Ca pumping occurs predominantly at the outer margin of the shell. This could

also explain the relatively low concentration of trace elements in shells collected from BDC, if bivalve metabolism were altered in this impacted stream to increase Ca pumping to the EPF.

Carré et al. (2006) reasoned that Ca-ATPase activity cannot be sufficient to transport the quantity of Ca used in fast-growing marine bivalves. They proposed a third pathway for the movement of Ca into the EPF that includes Ca channels that preferentially select for Ca. As loading is increased, though, these channels may transport Sr and Ba as well. While the authors do not extend their model to slow-growing freshwater bivalves, it is possible that additional Ca selective pathways exist in the marginal mantle cells, leading to significantly lower concentrations of trace elements in the ONL. Ultrastructural studies of the two mantle zones have shown that the mantle cells of the marginal zone have more mitochondria, and well-developed endoplasmic reticula and Golgi apparatus (Crenshaw 1980, and references therein). Crenshaw (1980) also suggests that the central mantle epithelium allows for the passive paracellular transport of ions, whereas ions must pass through cells of the marginal mantle epithelium.

Transport rates across the mantle into the precipitating fluid may per se be a function of metabolic rate. In a study of the marine bivalve *Mytilus edulis*, Rosenberg and Hughes (1991) showed that the shell-secreting mantle was metabolically more active at the shell margin along the axis of maximum growth than near the umbo, and that Mg and S in the shell varied relative to metabolic rates. It is possible that mantle metabolic activity differs significantly between the central and marginal mantle, in turn creating two distinct EPF chemistries, and that this is expressed as distinct trace element compositions between the shell layers (e.g., ONL and INL) precipitated from these. While strontium is not known to have a metabolic function, it does follow calcium metabolic pathways such as the calcium pump Ca-ATPase (Ferrier-Pagès et al. 2002). Therefore, trace element concentrations may be related to co-metabolic functions. There is a positive correlation between changes in Ba and Sr at sites MB, PB, and BDC ( $r^2=0.45$  to  $0.81$  for the INL, and  $0.69$  to  $0.88$  for the ONL,  $p<0.01$ ), and this may be because Ba, like Sr, is following calcium metabolic pathways, although we know of no studies that support this. Alternatively, de Vries et al. (2005) suggested that the incorporation of Sr creates crystal defects in the aragonite lattice leading to increased accommodation of Ba, providing an alternative explanation for their correlation. Manganese has chemical properties that can allow for its substitution in Mg or in Ca metabolic roles (see Crossgrove and Yokel 2004; González et al. 2006). Therefore, it is difficult to generalize about how Mn is transported versus Ca, Sr, or Ba, although the changes in Mn concentration seem to be independent of Sr and Ba.

While this study cannot discriminate between these varied models of shell formation, or others that may be equally valid, it does provide evidence that the shell itself, including its trace element composition, provides a valuable source of information on biomineralization in bivalves.

For trace element records of bivalve shells to be meaningful environmental proxies, the rates of transport and incorporation into the shell must be constant, or at least predictable through time. The related nature of the ONL and INL geochemical records suggests this may be the case, although sclerochronological work is needed that addresses the relevant hypotheses explicitly. Oxygen isotope data would also be invaluable in assessing and comparing equivalent time intervals. It will also be useful to study how trace elements vary across an entire shell layer, as opposed to across common sampling sites.

#### Environmental records

Both shell layers presented shell concentrations of trace elements that related to environmental factors. For all of the sites (excluding BDC), Mn, Sr, and Ba shell concentrations appear to be a partial function of the aqueous concentration of these elements in the waters in which the bivalves lived. Yet, for both the ONL and INL, geochemical records among individual shells from BDC were not consistent. The fact that the ONL and INL records were internally consistent within each shell from BDC suggests that factors indirectly related to, or independent of the environmental may be influencing the geochemical records at this site, and that the type or quality of environmental information cannot be controlled by choosing the shell layer to sample. Physicochemical heterogeneity within the environment can be determined only by a more rigorous water sampling strategy, both temporally and spatially, which is related to the exact collection site of particular specimens; this is outside the scope of the present study.

Shell-to-shell variability may be a biological response to factors such as differential stress experienced by individual bivalves that may, or may not be related to physicochemical attributes. For example, Dunca et al. (2005) observed a strong relationship between freshwater bivalve shell growth rate and aqueous temperature, except for bivalves collected from impaired streams. This suggests that biological processes governing shell mineralization may be altered in some environments, which in turn may affect shell chemistry. If this is true, then the mineralization of both shell layers is being affected. This is a sobering result, in the light of BDC's proximity to a coal-fired power plant. Shell records of pollution might be unreliable at polluted sites, although more research would need to be done to establish this effect.

In terms of specific elements, Mn presents unique characteristics relative to Sr, Ba, and perhaps Cu. As shell concentrations increase, they increase more rapidly in the

ONL than in the INL, as opposed to a near-equivalent increase for Sr, Ba, and Cu. In addition, the non-zero intercept of the distribution coefficient implies that at concentrations below 5 mmol:mol Ca, no Mn would be incorporated into the shell. Potential explanations include that a certain concentration of Mn can be eliminated efficiently in another manner, or can be useful metabolically. Without a better understanding of external and internal controls on Mn distribution, it is difficult to use either shell layer as a direct proxy for environmental concentrations of Mn. Results across a greater range of environments would clarify whether this intercept is real, and if it is, what it signifies.

Langlet et al. (2007) suggest that Mn concentrations in freshwater bivalve shells relate to periodic upwelling, and associated spikes in phytoplankton productivity. The occurrence of periodic spikes in a record does not require quantitation of environmental Mn. Evaluating Mn concentrations across the shells from different sites (Fig. 4), it appears that shells from LTR, downstream of a lake, show notably wide ranges, and in both profiles (Fig. 5) exhibit spikes. Seasonal increases in bioavailable Mn due to phytoplankton blooms upstream is a viable explanation. Mn shell records show promise as paleoenvironmental proxies.

One of the most distinguishing characteristics of Ba profiles in bivalve shells is a similar occurrence of transient positive spikes in the records (Stecher et al. 1996). With the exception of the both shells layers from LTR, however, Ba profiles did not display positive excursions in concentration. Gillikin et al. (2006) suggested that Ba spikes may be related to a dietary pathway, as phytoplankton are a known source of Ba, and periodic increases in phytoplankton productivity could result in transient exposures. The Ba spikes in LTR profiles correspond to Mn spikes, unlike other sites where Ba tracks Sr. A phytoplankton pathway for both elements is consistent with the source waters for Lower Three Runs, which originate from a permanent lake that supports episodic phytoplankton blooms.

Neither shell layer provided an identifiable environmental record of Cu. This may be due to the modest range of environmental Cu concentrations, not providing enough difference to observe in shells. Alternatively, Cu may be preferentially included in the organic matrix, and therefore weakly tied to ambient water chemistry and a poor indicator of pollution.

A practical outcome of this study is that geochemical signatures recorded in the inner shell layer are more pronounced. Unfortunately, spatially resolved analyses within this layer may be compromised due to their condensed nature. In addition, researchers are reluctant to analyze the inner shell layer of bivalves because of the potential for shell dissolution that accompanies anaerobic glycolysis. Current theory suggests that the inner shell layer serves as a reserved buffering material during periods of anaerobiosis that may occur

annually, during cold months, or during transient events such as droughts (Lutz and Clark 1984; Swann et al. 1991). In this study, the temporal patterns in elemental concentrations for Mn, Sr, and Ba are similar between shell layers, suggesting that periods of dissolution may not compromise geochemical records stored in the shell.

## Conclusions

The inner and outer nacreous layers of freshwater bivalves share a common fabric, yet their trace element compositions, while functionally related, are significantly different. Despite this difference, both records in non-impacted sites retained concentrations of Mn, Sr, and Ba that could be related to environmental concentrations by distribution coefficients, although the distribution coefficients differed by layer. Understanding the factors controlling the trace element content of different shell layers from bivalves will help to decipher potential relationships between the chemistry of ambient fluids in freshwater environments and shell carbonate over the incremental growth history of the shell. This relationship is indispensable for the use of trace element concentrations as paleoenvironmental proxies.

**Acknowledgements** This research was supported by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, U.S. Department of Energy, through Financial Assistance Award No. DE-FC09-96-SR18546 to the University of Georgia Research Foundation, Geological Society of America, and the Paleontological Society.

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