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Inter- and intra-annual variations of Pb/Ca ratios in clam shells (*Mercenaria mercenaria*): a record of anthropogenic lead pollution?

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Abstract

In this study, we re-assess the use of bivalve shells as a proxy of lead pollution. Previous studies have stressed that shells display little variability compared to soft tissues and thus are better for pollution biomonitoring. However, in this manuscript we illustrate that there is large inter- and intra-annual Pb variability between shells of the clam *Mercenaria mercenaria* collected in North Carolina, USA. Therefore, year to year, as well as intra-annual variations in Pb/Ca ratios should be interpreted with caution. Despite this variability, we were able to obtain an annual Pb chronology from 1949 to 2002 using 11 shells collected at different times which clearly exhibited the late 1970's peak in Pb from leaded gasoline use. This indicates that when enough specimens are pooled together, bivalve shells can be used to reconstruct large, long term changes in environmental Pb concentrations. Our data compare well with other studies of aragonite clams from sites with low regional lead pollution. From this we conclude that the Cape Lookout region of North Carolina has not received extensive pollution over the 1949-2002 period. The Pb concentration in shells growing in the 1949-1976 period was not significantly different from those growing in the 1982-2002 period, although other proxies suggest that the 1949-1976 period should be considerably higher. Therefore, our data suggest that there is still a modern source of Pb in the coastal North Carolina environment.

Keywords: Lead, anthropogenic pollution, proxy record, metals, mollusk, coastal sediments, North Carolina

1. Introduction

Coastal and estuarine environments are important natural resources supporting recreational activities and commercial fishing as well as providing a host of ecological services. The pollution of these regions can have serious adverse effects and thus has been closely monitored in the past several decades. The Mussel Watch program, where soft tissues of bivalves have been used to monitor pollution in the coastal zone (e.g., Goldberg, 1975; Claisse, 1989), has been monumental in this regard. Nevertheless, pre-1970 data are scarce (Cantillo, 1998) and data are limited to certain estuaries. For example, there are currently only seven Mussel Watch sites along the entire North Carolina (USA) coast (Lauenstein et al., 2002). Although new Mussel Watch sites could be started in other estuaries, it would require several years of monitoring to determine temporal pollution trends and would not allow reconstruction of past Pb concentration levels. There are other substrates that can retrospectively extend the record back through time and into other locations, which would not require extensive monitoring, such as sediments (e.g., Chillrud et al., 2003; Cooper et al., 2004; Kim et al., 2004), tree rings (e.g., Watmough et al., 1999) and biogenic carbonates (e.g., Shen and Boyle, 1987; Pitts and Wallace, 1994; Lazareth et al., 2000); each with its own advantages and drawbacks. For instance, sediments may be bioturbated and often provide low resolution profiles (e.g., Sharma et al., 1987; Cooper et al., 2004). On the other hand, biogenic carbonates can provide high resolution profiles and once incorporated the proxy remains more or less stable as long as diagenetic processes do not occur. However, the biology of the animal may affect the record (Vander Putten et al., 2000). Both corals and sclerosponges have been shown to accurately trace anthropogenic Pb inputs in tropical and subtropical waters (e.g., Shen and Boyle, 1987; Lazareth et al., 2000; Swart et al., 2002; Ramos and Ohde, 2004), but long term chronologies (> 50 years) based on bivalve shells have not been attempted.

Similar to sclerosponges, bivalve carbonate may be a superior recorder of Pb because bivalves accumulate higher Pb concentrations in their skeletons. Sclerosponge skeletons contain 10 to 35 times more Pb than corals (based on the 1970’s Pb peak; Shen and Boyle, 1987; Lazareth et al., 2000; Swart et al., 2002). Bivalve shell Pb/Ca ratios from polluted sites have been reported to be higher than 7 µmol/mol (Price and

Pearce, 1997), whereas corals from polluted sites can have Pb/Ca ratios reaching only 0.23 µmol/mol (Fallon et al., 2002).

There have been many studies on trace metal concentrations in bivalve shells. However, many of these studies did not include Pb due to its low levels (e.g., Szefer et al., 2002; Nicholson and Szefer, 2003; Cravo et al., 2004). Of the studies that did measure Pb, many analyzed whole shells (e.g., Koide et al., 1982; Yap et al., 2003), thus averaging several years of shell growth and including the outer layer of the shell which may exchange with the external medium. Other studies which did sample only the most recently formed shell material have shown that shell Pb concentrations are linearly related to tissue, particulate and dissolved Pb concentrations (Bourgoin, 1990; Pitts and Wallace, 1994). However, Bourgoin (1990) analyzed the inner nacreous shell layer and Pitts and Wallace (1994) analyzed the last formed section of the shell (the outer layer). This could effect the Pb levels they measured because Pb concentrations have been shown to vary by a factor of more than 10 between inner and outer shell layers (Fuge et al., 1993; Raith et al., 1996). Richardson et al. (2001) analyzed Pb concentrations in *Modiolus modiolus* shells from a polluted and non-polluted site covering 10 years of growth. They found elevated levels in shells from the polluted site, as well as a decrease of concentrations through time, which they attributed to the decline in pollution at the polluted site. However, they could not deconvolve age and time, and age has been shown to influence Pb concentrations in some mollusks (e.g., Hirao et al., 1994). Despite the large interest in using bivalve shells as records of past pollution, there has not been an attempt to create a continuous chronology back through time. Although bivalves are commonly short-lived, several shells can be strung together to form a master chronology, much longer than any one individual grew (e.g., Schöne, 2003).

The general objective of this study was to test if indeed bivalve shells can provide a long term record of anthropogenic Pb pollution. To reach this objective we first attempt to obtain pristine background Pb/Ca ratios from a fossil Pliocene *Mercenaria mercenaria* shell in order to have a baseline to compare the modern shells to. Secondly, the intra-annual Pb/Ca variation is assessed by sampling three shells across several annual growth increments at a high-resolution. Finally, by analyzing Pb/Ca ratios of the annual growth increments in eleven *M. mercenaria* shells collected at different times we can construct a chronology back through time at an annual resolution. Using shells collected at different dates and of different ages also allows us to assess any effect of age on the records. As no data are available on environmental Pb concentrations at our collection sites, we compare our measured Pb/Ca profile with an expected Pb profile based on published data from biogenic carbonates and total national US Pb emissions.

2. Materials and Methods

2.1 Sample collection, preparation and analysis

Living *M. mercenaria* were collected from the Cape Lookout region of North Carolina, USA (Fig. 1) at about 1 meter water depth in 1980 (n = 2), 1982 (n = 3), 2002 (n = 3) and 2003 (n = 3) (full data are listed in Table 1). More data on environmental conditions can be found in Peterson et al. (1985), Peterson (1986 and 2002), and Gillikin et al. (in press-a). Additionally, a Pliocene shell (~3.2 million years old) was collected from the Duplin formation in South Carolina (1.5 km northwest of Timmonsville) in order to determine a shell Pb/Ca baseline. Elliot et al. (2003) have shown that *M. mercenaria* precipitate aragonite shells. Sections of the shells were cut with a diamond saw along the axis of maximal growth, rinsed with deionised water, air-dried and mounted on microscopic slides. Clams were aged by counting the internal growth lines (Fig. 2), which have been proven to be annual (Peterson et al., 1985) and calendar years were assigned by back-dating from the time of collection. Considering that the inner layer may have been dissolved and reprecipitated, while the outermost layer may have exchanged ions with seawater as they were in direct contact, samples were taken from the middle layer of the shell to avoid shell regions that may have been altered (Fig. 2).

For annual Pb/Ca ratios, carbonate powder was milled from the shell cross-sections using a 300 μm drill bit and Merchantek Micromill (a fixed drill and computer controlled micro positioning device), using the growth lines (Fig. 2) as year markers that are formed annually in late August to late September in this region (Peterson et al., 1985). Before the sample was taken, 100 μm of the surface was milled and vacuumed off to remove surface contamination. The sample was then milled from the same groove. Sample depth varied with growth rate in order to produce approximately 300 – 400 µg of carbonate powder. Carbonate powders were milled starting from the tip of the shell and not all clams were sampled entirely (see Table 1). Samples were transferred to 2 ml acid washed polystyrene containers and capped. At the time of analysis, samples were dissolved in 1 ml 5% bi-distilled HNO_3 containing 1 µg l^{-1} of In and Bi, which were used as internal standards. Due to the small sample sizes, acid digestion was rapid. Multi-element calibration standards were prepared from certified single element stock solutions. ^{208}Pb was analyzed in low resolution and ^{43}Ca in medium resolution on a high resolution - inductively coupled plasma - mass spectrometer (HR-ICP-MS; ThermoFinnigan Element2). Two reference materials were run with the samples MACS1 and an in-house shell standard. MACS1 is a synthetic carbonate standard developed by the USGS. The in-house standard was produced from an aragonitic bivalve shell (*Saxidomus giganteus*). Reproducibility over the entire sampling period, as determined from the in-house shell standard, was 9.8 % relative standard deviation (%RSD; Pb/Ca = 0.36 ± 0.04 µmol/mol, n = 9) and MACS1 was within 4 % of the recommended value (n = 18) (values from S. Wilson, USGS, unpublished data, 2004). The detection limit (3 σ) was approximately 0.0011 µmol/mol, which is similar to other studies using an equivalent instrument (e.g., Barbante et al., 1999).

The Pliocene and three modern shells were also measured at high resolution to trace intra-annual Pb/Ca variations. High resolution Pb/Ca profiles were obtained using a laser ablation system (LA-ICP-MS). Data were calibrated using both the NIST 610 (values from Pearce et al., 1997) and the USGS MACS1. The laser was shot (~50 µm spots) directly in the holes of the isotope sampling (see further) allowing direct alignment of Pb/Ca and isotope profiles (cf. Toland et al., 2000). ^{26}Mg, ^{43}Ca, ^{55}Mn, ^{86}Sr, ^{138}Ba, ^{208}Pb, and ^{238}U signal intensities were recorded. Calibration (including blank subtraction and drift correction) was performed offline, following Toland et al. (2000). LA-ICP-MS Pb/Ca reproducibility was 6.8 % (%RSD) based on replicate measurements of MACS1 (n = 25) [note that %RSD is lower than the HR-ICP-MS because of the high Pb/Ca ratio in this standard (59.6 µmol/mol versus 0.36 µmol/mol in the in-house shell standard used for HR-ICP-MS)]. Details of LA-ICP-MS operating conditions can be found in Lazareth et al. (2003). Briefly, the system consists of a Fisons-VG frequency quadrupled Nd-YAG laser (λ = 266 nm) coupled to a Fisons-VG PlasmaQuad II+ mass spectrometer. The detection limit (3 σ) was approximately 0.01 µmol/mol. All data are given as means ± standard error unless otherwise noted.

For shells sampled at high resolution, oxygen isotopes ($\delta^{18}O$) were also measured to provide a relative temperature scale, and from this, an intra-annual time scale. Although the $\delta^{18}O$ of bivalve shells is dependent on both the $\delta^{18}O$ of the water and water temperature (Grossman and Ku, 1986), $\delta^{18}O$ in *M. mercenaria* shells has been shown to primarily be controlled by temperature in this region (Elliot et al., 2003). Carbonate powders for $\delta^{18}O$ analyses were milled from the shell in a similar manner as for HR-ICP-MS sampling (except for removal of the surface), producing ~ 100 µg of sample. Samples were reacted in a ThermoFinnigan Kiel III coupled to a ThermoFinnigan Delta+XL dual inlet isotope ratio mass spectrometer (IRMS). The samples were calibrated against the NBS-19 standard ($\delta^{18}O$ = -2.20 ‰) and data are reported as ‰ VSMOW using the conventional delta notation. The reproducibility (1σ) of the routinely analyzed carbonate standard was better than 0.1 ‰ (more details can be found in Gillikin et al. (in press-b)).

2.2 The expected Pb curve

The expected Pb curve presented in Figure 3 was constructed using data from a sclerosponge (*Ceratoporella nicholsoni*) from the Bahamas (Lazareth et al., 2000), a scleractinian coral (*Montastrea annularis*) from the Florida Keys (Shen and Boyle, 1987), and total national US Pb emissions (EPA, 2000). The difference in the Pb maxima (i.e., US Pb emissions: 1972, coral: 1977, and sclerosponge: 1979) is likely due to the reservoir effect of the ocean (see Shen and Boyle, 1987). The decrease observed in the Pb emission caused by the use of unleaded gasoline should thus be delayed by approximately 5 – 7 years. Therefore, like the coral and sclerosponge, we expect the clams in this study to show a peak around 1977 – 1979. The Pb emissions start to level off at around 3 % of the 1970 values in 1986, so the shells are expected to show a leveling off around the years 1991 – 1993.

3. Results

3.1 Diagenic indicators in the fossil shell

The δ^{18}O values of the Pliocene shell are within the values obtained from the modern shells (Fig. 4) indicating minimal recrystallization, if any (c.f., Labonne and Hillaire-Marcel, 2000). Generally, during diagenesis, a number of other chemical changes occur and these changes can be used to identify chemically altered pre-recrystallized carbonates. High trace element contents of Mn, U, and Fe usually indicate some degree of diagenetic alteration, especially if they are accompanied by low Sr and Mg contents (Brand and Veizer, 1980; Kaufman et al., 1996). Table 2 clearly illustrates that Mn, U and Fe are elevated and Mg is low in the Pliocene shell, however, Sr is high. Higher Sr (and lower Mg) in non-recrystallized, diagenically altered *M. mercenaria* shells was also found by Walls et al. (1977). Therefore, there has undoubtedly been some diagenetic alteration on this shell, and thus the Pb/Ca data from this shell is probably not a true indication of pristine conditions.

3.2 High resolution Pb/Ca profiles

There were large variations in Pb/Ca ratios throughout the year in these shells, ranging from < 0.01 to 0.52 umol/mol (Fig. 4). There are no clear ontogenic trends in the data, nor consistent seasonal trends. There was a significant correlation between δ^{18}O and Pb/Ca ratios in shell MW1 ($R = 0.58$, $p < 0.0001$, $n = 52$), but not in the other shells. Data from the Pliocene shell are higher or similar to the modern shells further indicating altered Pb/Ca ratios in this shell. Coefficient of variation (standard deviation / mean * 100) values ranged from 36.9 to 111.6 % (shell MW1 = 36.9 %, MW2 = 53.6 %, MB1 = 111.6 %, P1 = 38.8 %). Counting the number of points within one year, the resolution approximately corresponds to monthly sampling for most shells.

3.3 –The long-term chronology

By overlapping the annual data from shells collected at different times, a chronology from 1949 to 2002 was achieved (see Table 1). Seven Pb/Ca data were below the detection limit (5.7 % of all samples) and were removed from the dataset. From Figure 5 it is clear that there is a large variation between shells. However, averaging all data from the time periods 1949-1976, 1977-1981, and 1982-2002 results in significantly different means. The 1977-1981 period has significantly higher Pb/Ca ratios (0.157 ± 0.017 µmol/mol, n = 20) than the 1949-1976 period (0.098 ± 0.005 µmol/mol, n = 79) and 1982-2002 period (0.083 ± 0.007 µmol/mol, n = 52) (t-tests, $p < 0.001$ for each). However, the 1949-1976 and 1982-2002 periods were not different from each other (t-test, p = 0.08). There was a significant difference between mean Pb/Ca ratios in shells from Johnson Creek and Back Sound for the 1949-1976 period (t-test, $p < 0.001$) with Johnson Creek having higher ratios (Johnson Creek Pb/Ca = 0.112 ± 0.007 µmol/mol, n = 50; Back Sound Pb/Ca = 0.074 ± 0.005 µmol/mol, n = 29). There was no difference between Back Sound and Wade Creek shells during 1982 – 2002 (t-test, p = 0.08) nor Johnson Creek and Back Sound during 1977 – 1981 (t-test, p = 0.25). There was no relationship between age and Pb/Ca ratios when all shells were pooled together (p = 0.23, R = 0.09, n = 151). Hurricanes, which can increase resuspension of potentially contaminated sediments and increase terrestrial run-off, did not visually correlate with increased Pb/Ca ratios (Fig. 5).

4. Discussion

4.1 Inter- and intra-annual variations of Pb/Ca ratios

One test to asses if a proxy is primarily driven by environmental conditions is to determine its variability among individuals that grew under the same environmental conditions. Many studies have proposed that bivalve shells are better than soft tissues for monitoring pollution because the degree of trace metal variation is lower (e.g., Bourgoin, 1990; Yap et al., 2003; Cravo et al., 2004). However, we found high variability between shells (Fig. 5), as well as a high intra-annual variability (Fig. 4). Other studies have also reported high intra-annual Pb/Ca variability (Price and Pearce, 1997; Vander Putten et al., 2000; Richardson et al., 2001), however, the cause of this variability is not straightforward and may be due to many factors.

In the first place, the intra-annual variability may be caused by variations in environmental Pb concentrations, which could be the result of increased terrestrial run off from heavy rains and /or sediment resuspension. Hurricanes can both increase terrestrial runoff and increase sediment resuspension, which can alter the biogeochemistry of the water column for several months (e.g., Paerl et al., 2001). However, Pb/Ca ratios do not seem related to hurricanes in these shells. The high resolution profiles include 4 hurricanes (Bonnie, 26 Aug. 1998; Dennis, 4 Sept. 1999; Floyd, 16 Sept. 1999; and Irene, 17 Oct. 1999). By inspecting Fig. 4, it is clear that Pb/Ca ratios are not elevated during the late summer in 1998 and 1999, despite the three hurricanes that occurred in 1999. Additionally, no clear correlation between hurricanes and annual Pb/Ca ratios is evident (Fig. 5). A possible reason for the lack of response may be that the clams stop calcifying during these stressful times. Moreover, hurricanes usually occur between August and October, which are months when *M. mercenaria* are already exhibiting reduced growth (Peterson and Fegley, 1986). However, *M. mercenaria* have been shown to calcify throughout the year, albeit at a reduced rate in the winter (Peterson and Fegley, 1986), and other bivalves have been shown to continue to calcify during increased particulate loads (Lorrain et al., 2000). Therefore, *M. mercenaria* were expected to record these events, but apparently do not. Alternatively, biological regulation on Pb uptake can influence shell Pb/Ca ratios, as shell formation is a biological process. Although Vander Putten et al. (2000) could not determine the cause of seasonal Pb/Ca variations in *Mytilus edulis* shells, they suggested that perhaps it is regulated by seasonal variations in the distribution of the organic matrix. However, our data do not support this, as the organic rich growth lines did not exhibit higher Pb/Ca ratios (growth lines occur in late summer/ early fall, before the winter mark in Fig. 4).

The inter-shell variability may be the result of small scale spatial differences in environmental Pb concentrations. These small scale differences could be caused by groundwater seepage sites. Groundwaters can be highly contaminated with Pb (e.g., Landmeyer et al., 2003) and groundwater outflow can be limited to very small patches in the intertidal zone (e.g., Kohout and Kolipinski, 1967). Alternatively, pore-water Pb concentrations can also be highly variable, changing 10 fold over a few centimeters depth (Leermakers et al., in press), which could help explain the observed variability in these shells. The difference between Back Sound and Johnson Creek shells may be due to sediment type. It is well known that organic rich sediments contain higher Pb concentrations as compared to sandy sediments (e.g., Church et al., 1986; Kim et al., 2004). Indeed, Johnson Creek clams had higher Pb concentrations and were collected from muddy sediments, whereas the Back Sound clams were collected from sandy sediments and had lower Pb concentrations. Other sources of the variability may include acute pollution from boats using leaded gasoline, which have been present up until recently (pers. obs.). Again, biology may be the cause as several studies have illustrated that soft tissue Pb concentrations are related to the physiological state of bivalves (e.g., Lares and Orians, 1997). Thus, perhaps the shell Pb/Ca ratio is also related to the condition of the bivalves. However, we cannot determine this from the data presented here. Although we cannot exclude the possibility that the inter- and intra-annual variation observed in different shells reflect very localized conditions, the lack of similarity between the two shells from the same site sampled at a high-resolution (shells MW1 and MW2, Fig. 4) indicate that the seasonal Pb/Ca profiles are not controlled by environmental Pb concentrations in these shells.

It has been demonstrated that Pb concentrations in soft tissues of *M. mercenaria* are not related to body weight (Boyden, 1974) and hence size, unlike many *Mytilus* spp. (e.g., Boalch et al., 1981; Saavedra et al., 2004). The fact that we did not find a relationship between Pb/Ca ratios and age (and thus shell size) agrees with this. Considering that *M. mercenaria* have been shown not to bioaccumulate Pb in their tissues, but reach an equilibrium with their environment (Alcutt and Pinto, 1994), should make them an excellent pollution indicator. Unfortunately, the high variability in the data complicates interpretation.

4.2 The Pb pollution record

Despite the high variability mentioned above, *M. mercenaria* shells are not necessarily excluded from being used as a Pb pollution record. Our data are significantly higher during the 1977 – 1981 period, which is expected from other anthropogenic lead proxies (see Fig. 2). After averaging the data from each annual growth increment formed in the same year (1 to 6 per year), the expected anthropogenic profile becomes evident (Fig. 6). In fact, the profile from 1949 to 1987 is what would be expected. There is a significant increase from 1949 to 1976 at 1.49 ±

1.15 nmol/mol per year (R = 0.46, p = 0.013, n = 28), then a peak in 1980 and the sharp decrease afterwards. The main difficulty is to interpret the 1986 to 2002 period. First, based on regional sediment cores (Cooper et al., 2004) as well as the expected trend (Fig. 2), the 1986 – 2002 period should be much lower than the pre 1970 period (sediment cores from the Pamlico River estuary (Fig. 1) show a ~20 % reduction in Pb concentrations). Secondly, we cannot find an adequate explanation for the two peaks observed in this section (i.e., 1990 and 1998, see Fig. 6), which again, do not correlate with hurricane years. In addition to hurricanes, boat traffic can also cause sediment resuspension and considering the exponential rise in the local population (see Cooper et al., 2004), this has most probably increased in recent times and may explain the higher than expected Pb/Ca ratios during the 1986 -2002 period.

Unfortunately we were unable to determine pre-pollution Pb/Ca levels from our fossil shell (see section 3.1 and Table 2). Therefore, we compared our data with other studies of aragonite clams (venerids). Bourgoin and Risk (1987) measured Pb in fossil *Mya truncate* shells (8200 BP) which had higher Pb/Ca ratios (0.28 µmol/mol) than our diagenetically altered shell (see Table 2 and Fig. 4). Although they determined that the original mineralogy of the aragonite shell was preserved, they did not determine if there was chemical diagenesis, so like our fossil shell, this value may be erroneously high. Pitts and Wallace (1994) measured Pb in several fossil (1600 BP) *Mya arenaria* shells and found Pb/Ca ratios varying from 0.01 to 0.03 µmol/mol, about 10 times less than in our modern *M. mercenaria* shells. Using data from another species may not be appropriate, but their Pb/Ca ratios from a relatively unpolluted site (covering 1988 – 1989; 0.06 ± 0.004 µmol/mol) closely match our data from these same years (0.08 ± 0.01 µmol/mol). Therefore, in general, the Cape Lookout region of North Carolina has apparently received little Pb pollution. This is surprising considering this region is just south of the highly polluted Pamlico Sound (cf. Cooper et al., 2004) and is in close proximity to the US Marine Corps Air Station at Cherry Point (Fig. 1).

4.3 Concluding remarks and recommendations

Studies to determine the partition coefficient between environmental Pb (dissolved and particulate) and *M. mercenaria* shells are needed to validate the accuracy of using shells to trace pollution events. Nevertheless, we recommend using several shells to reduce the variability in the data. Due to this high variability, sub-annual data are probably not reliable. However, using bivalve shells from more biogeochemically stable areas, such as *Arctica islandica*, which commonly lives in deeper waters of the continental shelf (Marchitto et al., 2000; Schöne et al., 2003), may reduce some of the 'noise' encountered in this study. Marchitto et al. (2000) created a master temperature chronology spanning 154 years from *A. islandica* shell growth increments and hypothesized that a 1000 year chronology would be feasible. More recently, a 245 year chronology based on *A. islandica* shells was published (Schöne et al., 2003). Sampling these shells for Pb/Ca ratios could extend temperate records of Pb pollution back over the past millennium on an annual scale at different latitudes.

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Table 1. List of samples and environmental data.

Shell name	**Site**	**Sediment Type[2]**	**SST range (ºC)**	**Salinity range**	**Date collected**	**Clam Age (yr)**	**Years sampled**
MW1	Wade Creek	Mud	1 - 35	23 - 37	15 Sept 02	9	‡ 99-01
MW2	Wade Creek	Mud	1 - 35	23 - 37	20 Aug 03	7	‡ 98-02
MW3	Wade Creek	Mud	1 - 35	28 - 37	15 Sept 02	7	00-01
MW4	Wade Creek	Mud	1 - 35	28 - 37	15 Sept 02	20	84-02
MB1	Back Sound	Sandy	2 - 30[1]	28 - 34[1]	23 Aug 03	4.5	‡ 00-02
MB2	Back Sound	Sandy	2 - 30[1]	28 - 34[1]	23 Aug 03	23	81-02
MB3	Back Sound	Sandy	2 - 30[1]	28 - 34[1]	May 1980	16	64-79
MB4	Back Sound	Sandy	2 - 30[1]	28 - 34[1]	May 1980	24	58-79
MJ1	Johnson Cr.	Mud	2 - 30[1]	28 - 34[1]	1982	7	76-82
MJ2	Johnson Cr.	Mud	2 - 30[1]	28 - 34[1]	1982	28	55-80
MJ3	Johnson Cr.	Mud	2 - 30[1]	28 - 34[1]	1982	34	49-81
P1	Duplin Form., SC	N/A	N/A	N/A	N/A	~12	‡ 9 yrs

‡ Sampled at high resolution; N/A = unknown; [1]Based on Peterson et al. (1987); [2]estimated.

Table 2. Comparison of elemental ratios (mean ± standard deviation) between Pliocene (n = 82, shell P1) and modern (n = 43, shell MB2) shells measured by LA-ICP-MS and results of t-tests. n refers to the number of laser shots per shell.

Ratio	Modern	Pliocene	P
Mg/Ca (mmol/mol)	0.41 ± 0.13	0.23 ± 0.06	<0.001
Mn/Ca (µmol/mol)	0.51 ± 0.43	3.73 ± 2.57	<0.001
Fe/Ca (mmol/mol)	0.04 ± 0.02	0.22 ± 0.14	<0.001
Sr/Ca (mmol/mol)	1.56 ± 0.16	2.26 ± 0.39	<0.001
Ba/Ca (µmol/mol)	6.87 ± 5.04	34.96 ± 19.98	<0.001
Pb/Ca (µmol/mol)	0.10 ± 0.06	0.12 ± 0.05	0.073
U/Ca (µmol/mol)	0.01 ± 0.02	0.08 ± 0.04	<0.001

Figure Legends

Figure 1. Shell collection sites in eastern North Carolina, near Cape Lookout (BS: Back Sound, JC: Johnson Creek, WC: Wade Creek).

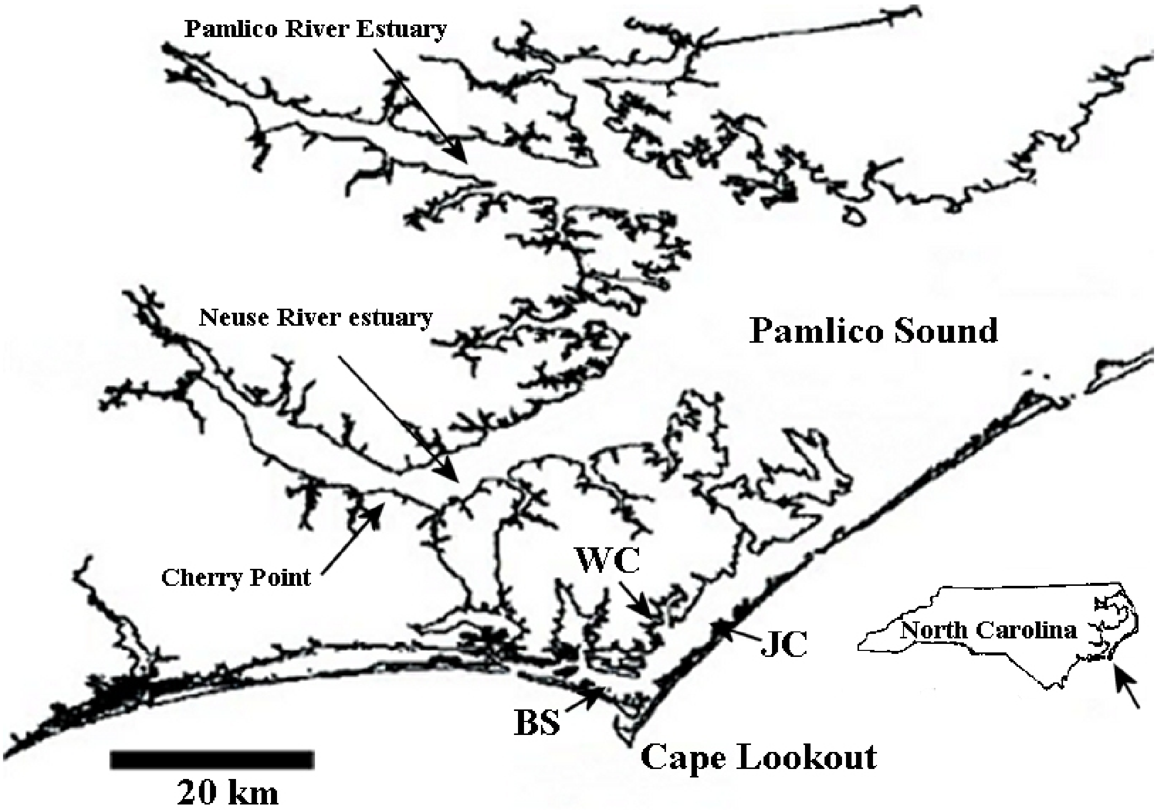
Figure 2. Drawing of a cross-section of a *M. mercenaria* shell showing the annual growth increments and shell layers (modified from Quitmyer et al., 1997).

Figure 3. Expected lead curve based on data from a sclerosponge (solid line, Lazareth et al., 2000), coral (open circles, data multiplied by 30, Shen and Boyle, 1987) and US Pb emissions (grey line and circles, EPA, 2000).

Figure 4. High resolution Pb/Ca ratios (thin black lines and circles) and oxygen isotopes (thick grey line and circles). Three modern shells (A, MW1; B, MW2; C, MB1) and the fossil Pliocene shell (D, P1) are shown (see Table 1 for shell codes). Years are delimited on modern shells using the winter oxygen isotope value (most positive). Analytical Pb/Ca precision is given on the left of each graph as the open symbol with error bars (based on mean Pb/Ca for each shell, %RSD= 6.8) and the detection limit is represented by the dashed line. Arrows mark approximate timing of hurricanes.

Figure 5. Annually sampled Pb/Ca ratios from 11 *M. mercenaria* shells (see Table 1 for shell codes). Data from shells MW1, MW2, MB1 and P1 represent the averaged high-resolution data between two δ^{18}O maxima (winter marks), whereas the other shells were sampled at an annual resolution by milling carbonate powders between the annual growth lines (see materials and methods). The open symbols on the x-axis represent hurricane years (data from NCSO, 2004). The analytical error is based on 9.8 % of the mean Pb/Ca ratio (0.101 ± 0.0099 µmol/mol). The Pliocene shell (P1) is also shown for comparison (dashed line, arbitrarily positioned at 1941-1944). See Table 1 for full description of shell codes.

Figure 6. Mean Pb/Ca ratios (black line and symbols, data from Fig 4). Error bars represent standard errors. The grey line and symbols show the number of *M. mercenaria* shells each mean is based on. The open symbols on the x-axis represent hurricane years (data from NCSO, 2004). The Pliocene shell (P1) is also shown for comparison (dashed line, arbitrarily positioned at 1941-1944).



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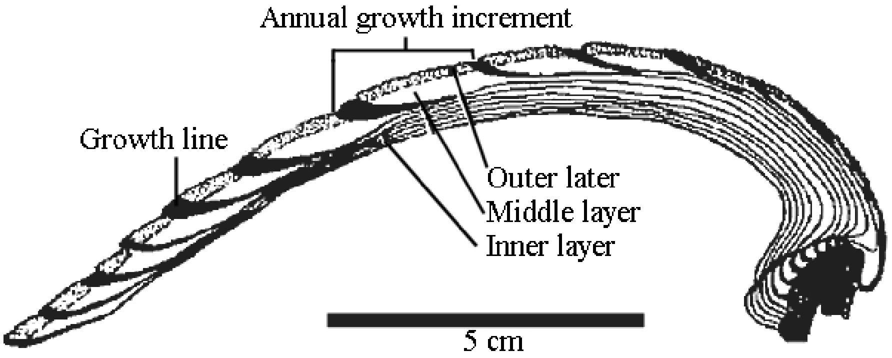

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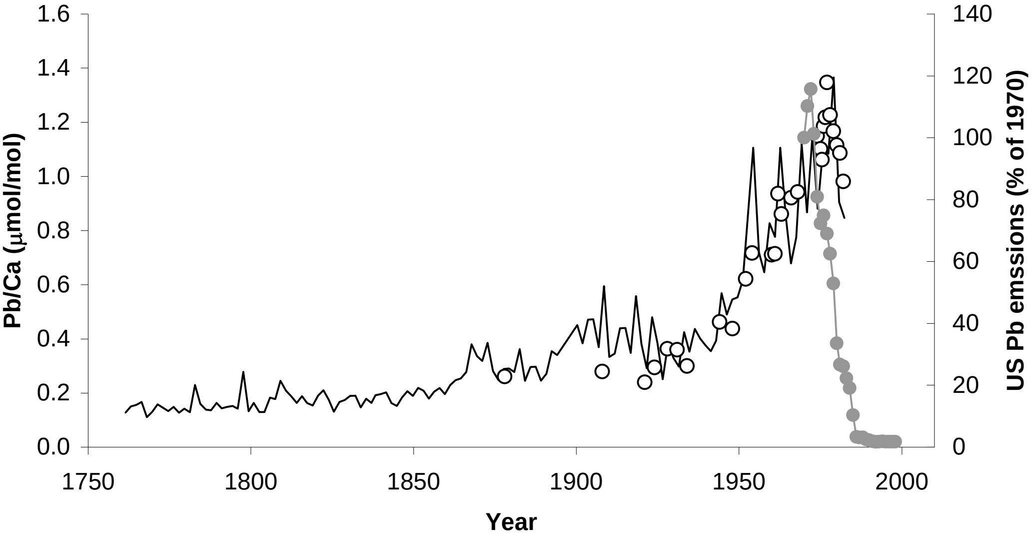

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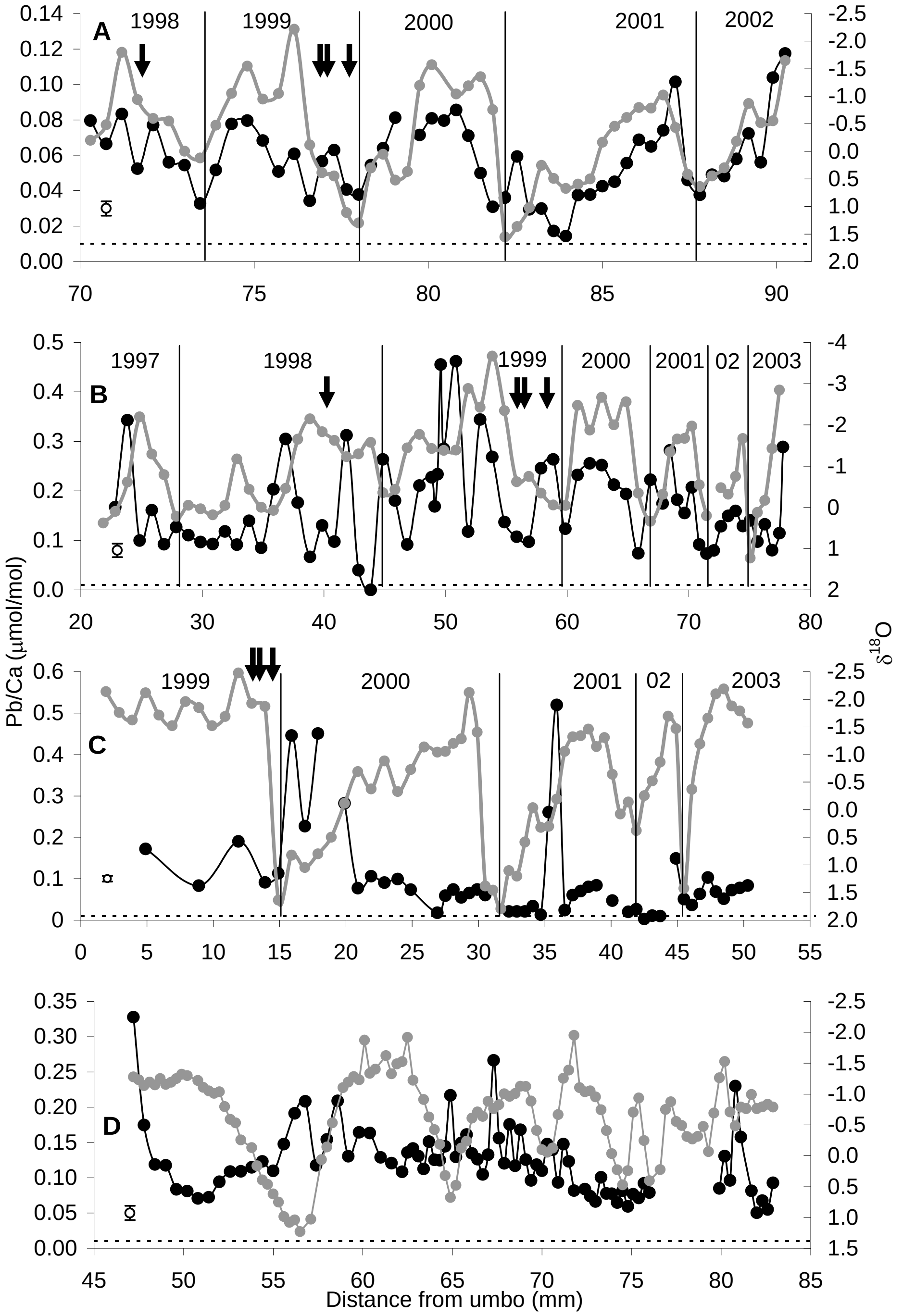

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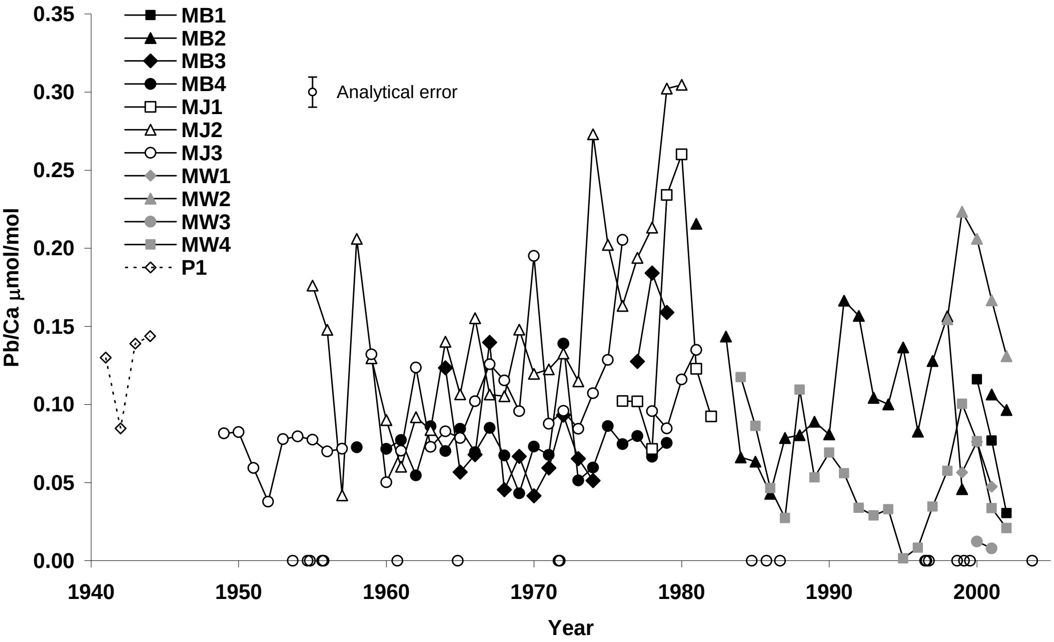

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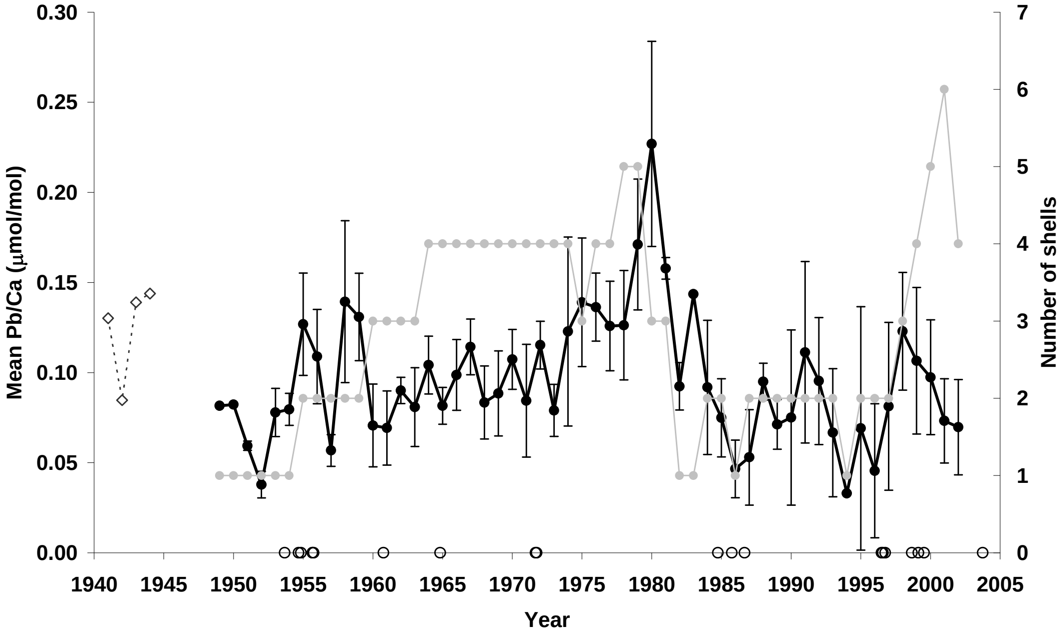

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