

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

5 David P. Gillikin<sup>1,\*</sup>, Frank Dehairs<sup>1</sup>, Willy Baeyens<sup>1</sup>, Jacques Navez<sup>1,2</sup>, Anne Lorrain<sup>2,3</sup>, and Luc André<sup>2</sup>

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<sup>1</sup>Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium. Tel.: +32-2-629-1265; fax: +32-2-629-3274. E-mail: david.gillikin@vub.ac.be, david@scientificproofreading.com (D.P. Gillikin);

\* Corresponding author.

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<sup>2</sup>Section of Mineralogy and Petrography, Royal Museum for Central Africa, Leuvensesteenweg 13, B-3080 Tervuren, Belgium.

<sup>3</sup>Present address: UR Thetis, IRD-CRH (Centre de Recherche Halieutique

20 Méditerranéenne et Tropicale), Avenue Jean Monnet - BP 171, 34203 Sète Cedex, France.

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**Submitted to *Marine Pollution Bulletin* on 23 March 2005**

**Revision submitted 12 May 2005**

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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

65 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  
70 (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  
75 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.,  
80 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  
85 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  
90 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;  
95 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  $\mu\text{mol/mol}$  (Price and

Pearce, 1997), whereas corals from polluted sites can have Pb/Ca ratios reaching only 0.23  $\mu\text{mol/mol}$  (Fallon et al., 2002).

100 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  
105 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  
110 (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  
115 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  
120 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  
125 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  
130 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  
135 an expected Pb profile based on published data from biogenic carbonates and total national US Pb emissions.

## 2. Materials and Methods

### 140 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  
145 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 Timmons ville) in order to determine a shell Pb/Ca baseline. Elliot et al. (2003) have shown that *M. mercenaria* precipitate aragonite shells. Sections of the  
150 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  
155 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  
160 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

165 same groove. Sample depth varied with growth rate in order to produce approximately  
300 – 400  $\mu\text{g}$  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  $\text{HNO}_3$  containing  $1 \mu\text{g l}^{-1}$  of  
170 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.  $^{208}\text{Pb}$  was analyzed in low resolution and  $^{43}\text{Ca}$  in  
medium resolution on a high resolution - inductively coupled plasma - mass  
spectrometer (HR-ICP-MS; ThermoFinnigan Element2). Two reference materials  
175 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;  $\text{Pb/Ca} = 0.36 \pm 0.04 \mu\text{mol/mol}$ ,  $n = 9$ ) and  
180 MACS1 was within 4 % of the recommended value ( $n = 18$ ) (values from S. Wilson,  
USGS, unpublished data, 2004). The detection limit ( $3 \sigma$ ) was approximately  $0.0011$   
 $\mu\text{mol/mol}$ , which is similar to other studies using an equivalent instrument (e.g.,  
Barbante et al., 1999).

185 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 ( $\sim 50 \mu\text{m}$   
spots) directly in the holes of the isotope sampling (see further) allowing direct  
190 alignment of Pb/Ca and isotope profiles (cf. Toland et al., 2000).  $^{26}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  
 $^{86}\text{Sr}$ ,  $^{138}\text{Ba}$ ,  $^{208}\text{Pb}$ , and  $^{238}\text{U}$  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  
195 measurements of MACS1 ( $n = 25$ ) [note that %RSD is lower than the HR-ICP-MS  
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 ( $\lambda = 266$  nm) coupled to a Fisons-VG PlasmaQuad II+ mass spectrometer. The detection limit ( $3 \sigma$ ) was approximately  $0.01 \mu\text{mol/mol}$ . All data are given as means  $\pm$  standard error unless otherwise noted.

For shells sampled at high resolution, oxygen isotopes ( $\delta^{18}\text{O}$ ) were also measured to provide a relative temperature scale, and from this, an intra-annual time scale. Although the  $\delta^{18}\text{O}$  of bivalve shells is dependent on both the  $\delta^{18}\text{O}$  of the water and water temperature (Grossman and Ku, 1986),  $\delta^{18}\text{O}$  in *M. mercenaria* shells has been shown to primarily be controlled by temperature in this region (Elliot et al., 2003). Carbonate powders for  $\delta^{18}\text{O}$  analyses were milled from the shell in a similar manner as for HR-ICP-MS sampling (except for removal of the surface), producing  $\sim 100 \mu\text{g}$  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 ( $\delta^{18}\text{O} = -2.20 \text{‰}$ ) and data are reported as  $\text{‰}$  VSMOW using the conventional delta notation. The reproducibility ( $1\sigma$ ) of the routinely analyzed carbonate standard was better than  $0.1 \text{‰}$  (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.

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### 3. Results

#### 3.1 Diagenetic indicators in the fossil shell

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The  $\delta^{18}\text{O}$  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, diagenetically 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.

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#### 3.2 High resolution Pb/Ca profiles

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There were large variations in Pb/Ca ratios throughout the year in these shells, ranging from  $< 0.01$  to  $0.52$   $\mu\text{mol/mol}$  (Fig. 4). There are no clear ontogenic trends in the data, nor consistent seasonal trends. There was a significant correlation between  $\delta^{18}\text{O}$  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.

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#### 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

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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

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

One test to assess 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.

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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

300 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  
305 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.  
310 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  
315 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  
320 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  
325 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  
330 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  
335 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  
340 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  
345 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.

350 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  
355 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

360 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  
365 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  $1.49 \pm$

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. 370 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 375 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.

380 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  $\mu\text{mol/mol}$ ) than our diagenetically altered shell (see Table 2 and Fig. 4). Although they determined 385 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  $\mu\text{mol/mol}$ , about 10 times less than in our modern *M. mercenaria* shells. Using data from another 390 species may not be appropriate, but their Pb/Ca ratios from a relatively unpolluted site (covering 1988 – 1989;  $0.06 \pm 0.004 \mu\text{mol/mol}$ ) closely match our data from these same years ( $0.08 \pm 0.01 \mu\text{mol/mol}$ ). 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 395 Point (Fig. 1).

#### 4.3 Concluding remarks and recommendations

400 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.

**Acknowledgements** - We thank C.H. Peterson (University of North Carolina, Chapel Hill), who kindly provided the *M. mercenaria* shells collected in the early 1980’s; W.C. Gillikin and L. Daniels, who both assisted with sample collection in N.C.; and L. Campbell (University of South Carolina) who kindly provided the Pliocene shell. We express our gratitude to L. Monin for laboratory assistance, T. Haifeng and L. Meng who helped with sampling the shells, and to C.E. Lazareth for providing the sclerosponge data. A. Verheyden, S. Bouillon, C. Sheppard (editor) and an anonymous reviewer gave helpful comments on an earlier version of this manuscript. Funding was provided by the Belgian Federal Science Policy Office, Brussels, Belgium (CALMARS, contract: EV/03/04B). Funding for the HR-ICP-MS equipment was provided by the Belgian Lotto via the FWO-Flanders (contract: G.0117.02N) and the Belgian Federal Science Policy Office.

## References

- Alcutt, F., Pinto, J. T., 1994. Glutathione concentrations in the hard clam, *Mercenaria mercenaria*, following laboratory exposure to lead (a potential model system for evaluating exposure to carcinogens and toxins). *Comparative Biochemistry and Physiology C-Pharmacology Toxicology and Endocrinology* 107, 347-352.
- Barbante, C., Cozzi, G., Capodaglio, G., Van de Velde, K., Ferrari, C., Boutron, C., Cescon, P., 1999. Trace element determination in alpine snow and ice by double focusing

- 435 inductively coupled plasma mass spectrometry with microconcentric nebulization. *Journal of Analytical Atomic Spectrometry* 14, 1433-1438.
- Boalch, R., Chan, S., Taylor, D., 1981. Seasonal-variation in the trace-metal content of *Mytilus edulis*. *Marine Pollution Bulletin* 12, 276-280.
- Bourgoin, B.P., 1990. *Mytilus edulis* shell as a bioindicator of lead pollution - considerations  
440 on bioavailability and variability. *Marine Ecology-Progress Series* 61, 253-262.
- Bourgoin, B.P., Risk, M.J., 1987. Historical changes in lead in the eastern Canadian arctic, determined from fossil and modern *Mya truncata* shells. *Science of the Total Environment* 67, 287-291.
- Boyden, C.R., 1974. Trace-element content and body size in mollusks. *Nature* 251, 311-314.
- 445 Brand, U., Veizer, J., 1980. Chemical diagenesis of a multi-component carbonate system-1. Trace elements. *Journal of Sedimentary Petrology* 50, 1219– 1236.
- Cantillo, A.Y., 1998. Comparison of results of mussel watch programs of the United States and France with worldwide mussel watch studies. *Marine Pollution Bulletin* 36, 712-717.
- Chillrud, S.N., Hemming, S., Shuster, E.L., Simpson, H.J., Bopp, R.F., Ross, J.M., Pederson,  
450 D.C., Chaky, D.A., Tolley, L.-R., Estabrooks, F., 2003. Stable lead isotopes, contaminant metals and radionuclides in upper Hudson River sediment cores: implications for improved time stratigraphy and transport processes. *Chemical Geology* 199, 53– 70.
- Church, T.M., Bernat, M., Sharma, P., 1986. Distribution of natural uranium, thorium, lead, and polonium radionuclides in tidal phases of a Delaware salt-marsh. *Estuaries* 9, 2-8.
- 455 Claisse, D., 1989. Chemical contamination of French coasts - the results of a 10 years mussel watch. *Marine Pollution Bulletin* 20, 523-528.
- Cooper, S.R., McGlothlin, S.K., Madritch, M., Jones, D.L., 2004. Paleoecological evidence of human impacts on the Neuse and Pamlico Estuaries of North Carolina, USA. *Estuaries* 27, 617-633.
- 460 Cravo, A., Bebianno, M.J., Foster, P. 2004. Partitioning of trace metals between soft tissues and shells of *Patella aspera*. *Environment International* 30, 87-98.
- Elliot, M., deMenocal, P.B., Linsley, B.K., Howe, S.S., 2003. Environmental controls on the stable isotopic composition of *Mercenaria mercenaria*: potential application to paleoenvironmental studies. *Geochemistry, Geophysics, Geosystems* 4, 1056, doi:  
465 10.1029/2002GC000425.
- EPA, 2000. National air pollutant emission trends: 1900 – 1998. EPA report 454/R-00-002.
- Fallon, S.J., White, J.C., McCulloch, M.T., 2002. Porites corals as recorders of mining and environmental impacts: Misima Island, Papua New Guinea. *Geochimica et Cosmochimica Acta* 66, 45-62.

- 470 Fuge, R., Palmer, T.J., Pearce, N.J.G., Perkins, W.T., 1993. Minor and trace element chemistry of modern shells: a laser ablation inductively coupled plasma spectrometry study. *Applied Geochemistry supplement 2*, 111-116.
- Gillikin, D.P., Lorrain, A., Navez, J., Taylor, J.W., André, L., Keppens, E., Baeyens, W., Dehairs, F., in press-a. Strong biological controls on Sr/Ca ratios in aragonitic marine
- 475 bivalve shells. *Geochemistry, Geophysics, Geosystems*
- Gillikin, D.P., De Ridder, F., Ulens, H., Elskens, M., Keppens, E., Baeyens, W., Dehairs, F., in press-b. Assessing the reproducibility and reliability of estuarine bivalve shells (*Saxidomus giganteus*) for sea surface temperature reconstruction: implications for paleoclimate studies. *Palaeogeography Palaeoclimatology Palaeoecology*
- 480 Goldberg, E.D., 1975. The mussel watch - A first step in global marine monitoring. *Marine Pollution Bulletin* 6, 111.
- Grossman, E.L., Ku, T.L., 1986. Oxygen and carbon isotope fractionation in biogenic aragonite - temperature effects. *Chemical Geology* 59, 59-74.
- Hirao, Y., Matsumoto, A., Yamakawa, H., Maeda, M., Kimura, K., 1994. Lead behavior in
- 485 abalone shell. *Geochimica et Cosmochimica Acta* 58, 3183-3189.
- Kaufman, A., Ghaleb, B., Wehmiller, J.F., Hillaire-Marcel C., 1996. Uranium concentration and isotope ratio profiles within *Mercenaria* shells: Geochronological implications. *Geochimica et Cosmochimica Acta* 60, 3735-3746.
- Kim, G., Alleman, L.Y., Church, T.M., 2004. Accumulation records of radionuclides and
- 490 trace metals in two contrasting Delaware salt marshes. *Marine Chemistry* 87, 87-96.
- Kohout, F.A., Kolipinski, M.C., 1967. Biological zonation related to groundwater discharge along the shore of Biscayne Bay, Miami, Florida. In: G.H. Lauff (ed.), *Estuaries*. American Association for the Advancement of Science, Publication No. 83, Washington D.C., USA. Pp 488-499.
- 495 Koide, M., Lee, D.S., Goldberg, E.D. 1982. Metal and transuranic records in mussel shells, byssal threads and tissues. *Estuarine, Coastal and Shelf Science* 15, 679-695.
- Labonne, M., Hillaire-Marcel, C., 2000. Geochemical gradients within modern and fossil shells of *Concholepas concholepas* from Northern Chile: An insight into U-Th systematics and diagenetic/authigenic isotopic imprints in mollusk shells. *Geochimica et*
- 500 *Cosmochimica Acta* 64, 1523-1534.
- Landmeyer, J.E., Bradley, P.M., Bullen, T.D., 2003. Stable lead isotopes reveal a natural source of high lead concentrations to gasoline-contaminated groundwater. *Environmental Geology* 45, 12-22.
- Lares, M.L. Orians, K.J., 1997. Natural Cd and Pb variations in *Mytilus californianus* during
- 505 the upwelling season. *Science of the Total Environment* 197, 177-195.

- Lauenstein, G.G., Cantillo, A.Y., O'Connor, T.P., 2002. The status and trends of trace element and organic contaminants in oysters, *Crassostrea virginica*, in the waters of the Carolinas, USA. *Science of the Total Environment* 285, 79-87.
- 510 Lazareth, C.E., Vander Putten, E., André, L., Dehairs, F., 2003. High-resolution trace element profiles in shells of the mangrove bivalve *Isognomon ehippium*: a record of environmental spatio-temporal variations? *Estuarine, Coastal and Shelf Science* 57, 1103-1114.
- Lazareth, C.E., Willenz, P., Navez, J., Keppens, E., Dehairs, F., André, L., 2000. Sclerosponges as a new potential recorder of environmental changes: Lead in  
515 *Ceratoporella nicholsoni*. *Geology* 28, 515-518.
- Leermakers, M., Gao, Y., Gabelle, C., Lojen, S., Wartel, M., Baeyens, W., in press. Determination of high resolution pore water profiles of trace metals in sediments of the Rupel River (Belgium) using DET (diffusive equilibrium in thin films) and DGT (diffusive gradients in thin films) techniques. *Water, Air and Soil Pollution*.
- 520 Lorrain, A., Paulet, Y.-M., Chauvaud, L., Savoye, N., Nezan, E., Guerin, L., 2000. Growth anomalies in *Pecten maximus* from coastal waters (Bay of Brest, France): relationship with diatom blooms. *Journal of the Marine Biological Association of the United Kingdom* 80, 667-673.
- Marchitto, T.A., Jones, G.A., Goodfriend, G.A. Weidman, C.R., 2000. Precise temporal  
525 correlation of Holocene mollusk shells using sclerochronology. *Quaternary Research* 53, 236-246.
- NCSCO, 2004. North Carolina State Climate Office. <http://www.nc-climate.ncsu.edu/climate/hurricanes.html> (accessed May 2004).
- Nicholson, S., Szefer, P., 2003. Accumulation of metals in the soft tissues, byssus and shell of  
530 the mytilid mussel *Perna viridis* (Bivalvia : Mytilidae) from polluted and uncontaminated locations in Hong Kong coastal waters. *Marine Pollution Bulletin* 46, 1039-1043.
- Paerl, H.W., Bales, J.D., Ausley, L.W., Buzzelli, C.P., Crowder, L.B., Eby, L.A., Fear, J.M., Go, M., Peierls, B.L., Richardson, T.L., Ramus, J.S. 2001. Ecosystem impacts of three sequential hurricanes (Dennis, Floyd, and Irene) on the United States' largest lagoonal  
535 estuary, Pamlico Sound, NC. *Proceedings of the National Academy of Sciences of the United States of America* 98, 5655-5660.
- Pearce, N.J.G., Perkins, W.T., Westgate, J.A., Gorton, M.P., Jackson, S.E., Neal, C.R., Chenery, S.P., 1997. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials, *Geostandards  
540 Newsletter* 21, 115-144.
- Peterson, C.H., 1986. Quantitative allometry of gamete production by *Mercenaria mercenaria* into old-age. *Marine Ecology-Progress Series* 29, 93-97.

- Peterson, C.H., 2002. Recruitment overfishing in a bivalve mollusc fishery: hard clams (*Mercenaria mercenaria*) in North Carolina. Canadian Journal of Fisheries and Aquatic Sciences 59, 96-104.  
545
- Peterson, C.H., Duncan, P.B., Summerson, H.C., Beal, B.F., 1985. Annual band deposition within shells of the hard clam, *Mercenaria mercenaria* - consistency across habitat near Cape Lookout, North Carolina. Fisheries Bulletin 83, 671-677.
- Peterson, C.H., Fegley, S.R., 1986. Seasonal allocation of resources to growth of shell, soma, and gonads in *Mercenaria mercenaria*, Biological Bulletin 171, 597-610.  
550
- Pitts, L.C., Wallace, G.T., 1994. Lead deposition in the shell of the bivalve, *Mya arenaria* - an indicator of dissolved lead in seawater. Estuarine, Coastal and Shelf Science 39, 93-104.
- Price, G.D., Pearce, N.J.G., 1997. Biomonitoring of pollution by *Cerastoderma edule* from the British Isles: a laser ablation ICP-MS study. Marine Pollution Bulletin 34, 1025-1031.  
555
- Quitmyer, I.R., Jones, D.S., Arnold, W.S., 1997. The sclerochronology of hard clams, *Mercenaria* spp., from the south-eastern USA: A method of elucidating the zooarchaeological records of seasonal resource procurement and seasonality in prehistoric shell middens. Journal of Archaeological Science 24, 825-840.
- 560 Raith, A., Perkins, W.T., Pearce, N.J.G., Jeffries, T.E., 1996. Environmental monitoring on shellfish using UV laser ablation ICP-MS. Fresenius Journal of Analytical Chemistry 355, 789-792.
- Ramos, A.A., Inoue, Y., Ohde, S., 2004. Metal contents in Porites corals: Anthropogenic input of river run-off into a coral reef from an urbanized area, Okinawa. Marine Pollution Bulletin 48, 281-294.  
565
- Richardson, C.A., Chenery, S.R.N., Cook, J.M., 2001. Assessing the history of trace metal (Cu, Zn,Pb) contamination in the North Sea through laser ablation ICP-MS of horse mussel *Modiolus modiolus* shells. Marine Ecology-Progress Series 211, 157-167.
- Saavedra, Y., Gonzalez, A., Fernandez, P., Blanco, J., 2004. The effect of size on trace metal levels in raft cultivated mussels (*Mytilus galloprovincialis*). Science of the Total Environment 318, 115-124.  
570
- Sharma, P., Gardner, L.R., Moore, W.S., Bollinger, M.S., 1987. Sedimentation and bioturbation in a salt marsh as revealed by  $^{210}\text{Pb}$ ,  $^{137}\text{Cs}$ , and  $^7\text{Be}$  studies. Limnology and Oceanography 32, 313-326.
- 575 Shen, G.T., Boyle, E.A., 1987. Lead in corals - reconstruction of historical industrial fluxes to the surface ocean. Earth and Planetary Science Letters 82, 289-304.
- Schöne, B.R., 2003. A 'clam-ring' master-chronology constructed from a short-lived bivalve mollusc from the northern Gulf of California, USA. Holocene 13, 39-49.

- Schöne, B.R., Oschmann, W., Rossler, J., Castro, A.D.F., Houk, S.D., Kroncke, I., Dreyer, W., Janssen, R., Rumohr, H., Dunca, E., 2003. North Atlantic Oscillation dynamics recorded in shells of a long-lived bivalve mollusk. *Geology* 31, 1037-1040.
- Swart, P.K., Thorrold, S., Rosenheim, B., Eisenhauer, A., Harrison, C.G.A., Grammer, M., Latkoczy, C., 2002. Intra-annual variation in the stable oxygen and carbon and trace element composition of sclerosponges. *Paleoceanography* 17, 1045, doi:10.1029/2000PA000622.
- Szefer, P., Frelek, K., Szefer, K., Lee, Ch., Kim, B.-S., Warzocha, J., Zdrojewska, I., Ciesielski, T., 2002. Distribution and relationships of trace metals in soft tissue, byssus and shells of *Mytilus edulis trossulus* from the southern Baltic. *Environmental Pollution* 120, 423-444.
- Toland, H., Perkins, B., Pearce, N., Keenan, F., Leng, M.J., 2000. A study of sclerochronology by laser ablation ICP-MS. *Journal of Analytical Atomic Spectrometry* 15, 1143-1148.
- Vander Putten, E., Dehairs, F., Keppens, E., Baeyens, W., 2000. High resolution distribution of trace elements in the calcite shell layer of modern *Mytilus edulis*: Environmental and biological controls. *Geochimica et Cosmochimica Acta* 64, 997-1011.
- Walls, R.A., Ragland, P.C., Crisp, E.L., 1977. Experimental and natural early diagenetic mobility of Sr and Mg in biogenic carbonates. *Geochimica et Cosmochimica Acta* 41, 1731-1737.
- Watmough, S.A., Hughes, R.J., Hutchinson, T.C., 1999. Pb-206/Pb-207 ratios in tree rings as monitors of environmental change. *Environmental Science and Technology* 33, 670-673.
- Yap, C.K., Ismail, A., Tan, S.G., Rahim, I.A., 2003. Can the shell of the green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn? *Estuarine, Coastal and Shelf Science* 57, 623-630.

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**Table 1.** List of samples and environmental data.

Shell name	Site	Sediment Type <sup>2</sup>	SST range (°C)	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 <sup>1</sup>	28 - 34 <sup>1</sup>	23 Aug 03	4.5	‡ 00-02
MB2	Back Sound	Sandy	2 - 30 <sup>1</sup>	28 - 34 <sup>1</sup>	23 Aug 03	23	81-02
MB3	Back Sound	Sandy	2 - 30 <sup>1</sup>	28 - 34 <sup>1</sup>	May 1980	16	64-79
MB4	Back Sound	Sandy	2 - 30 <sup>1</sup>	28 - 34 <sup>1</sup>	May 1980	24	58-79
MJ1	Johnson Cr.	Mud	2 - 30 <sup>1</sup>	28 - 34 <sup>1</sup>	1982	7	76-82
MJ2	Johnson Cr.	Mud	2 - 30 <sup>1</sup>	28 - 34 <sup>1</sup>	1982	28	55-80
MJ3	Johnson Cr.	Mud	2 - 30 <sup>1</sup>	28 - 34 <sup>1</sup>	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; <sup>1</sup>Based on Peterson et al. (1987); <sup>2</sup>estimated.

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**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

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## 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).

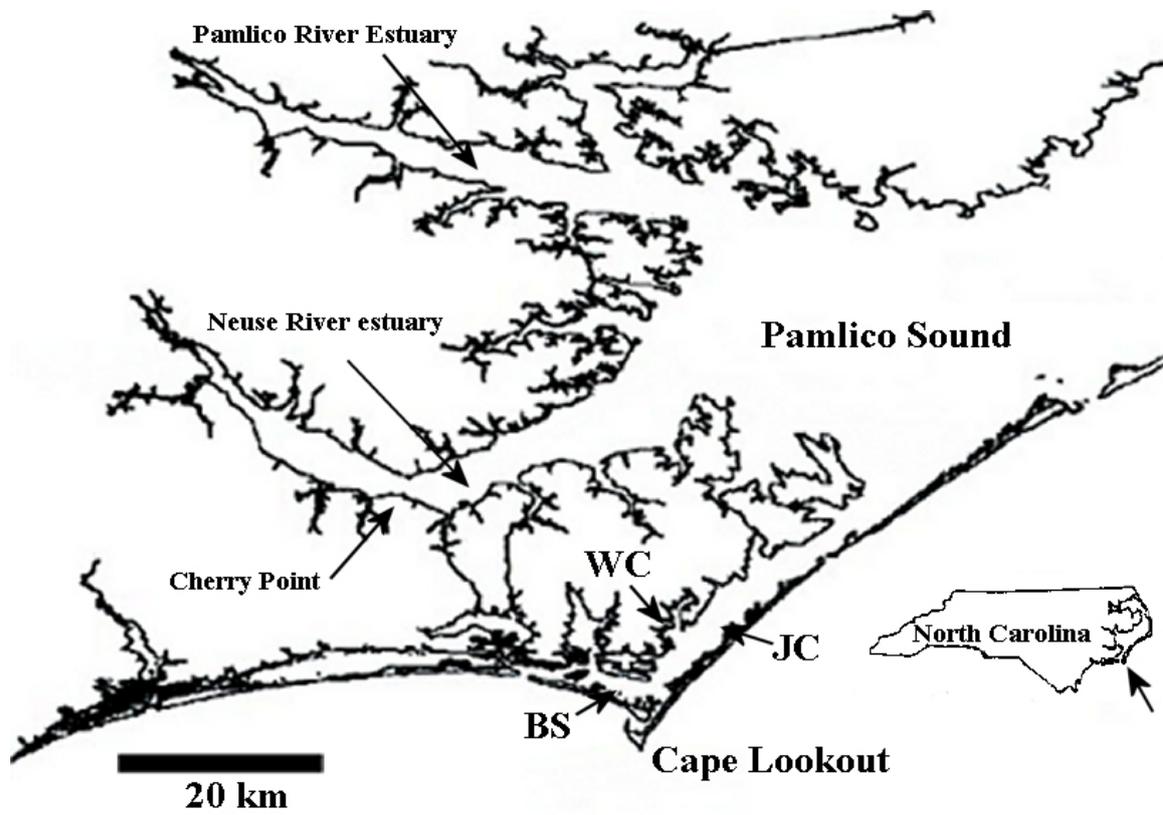
**Figure 3.** Expected lead curve based on data from a sclerosponge (solid line, Lazareth et al., 2000), coral (open circles, data multiplied by 30, Shen and Boyle, 1987) and US Pb emissions (grey line and circles, EPA, 2000).

**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.

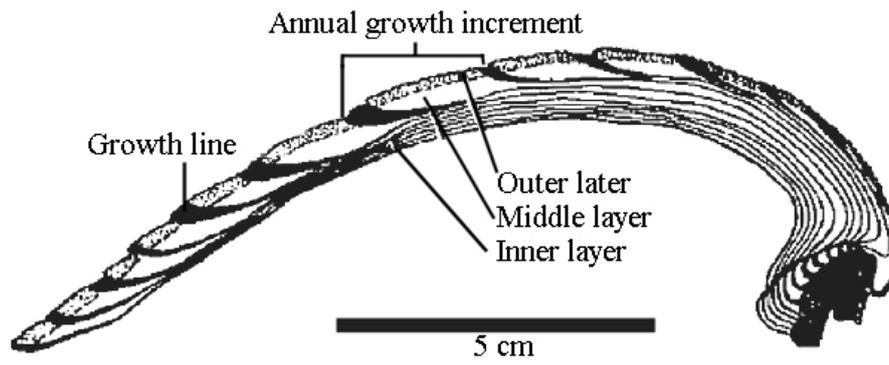
**Figure 5.** Annually sampled Pb/Ca ratios from 11 *M. mercenaria* shells (see Table 1 for shell codes). Data from shells MW1, MW2, MB1 and P1 represent the averaged high-resolution data between two  $\delta^{18}\text{O}$  maxima (winter marks), whereas the other shells were sampled at an annual resolution 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 ( $0.101 \pm 0.0099 \mu\text{mol/mol}$ ). 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.

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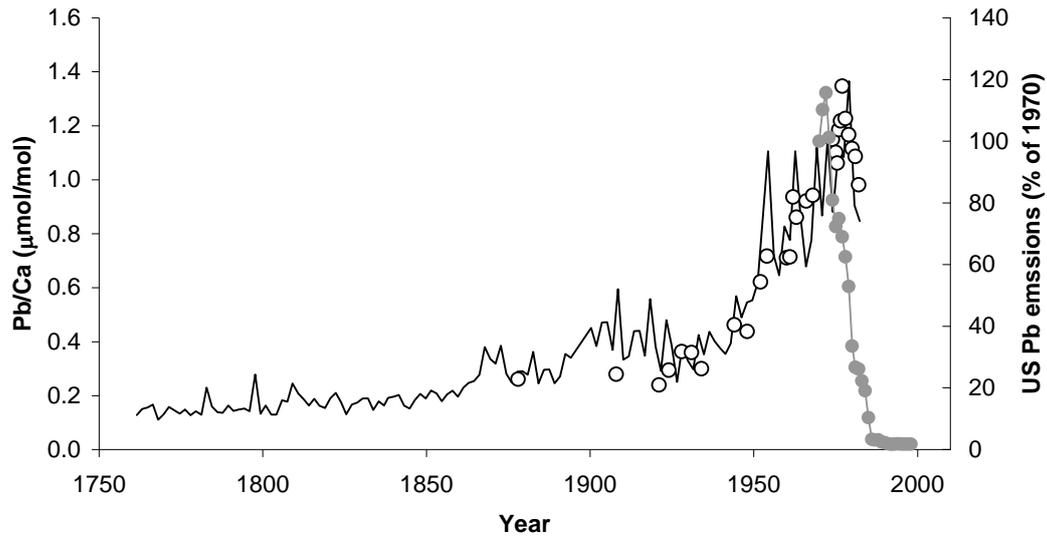
**Figure 6.** Mean Pb/Ca ratios (black line and symbols, data from Fig 4). Error bars represent standard errors. The grey line and symbols show the number of *M. mercenaria* shells each mean is based on. The open symbols on the x-axis represent hurricane years (data from NCSO, 2004). The Pliocene shell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



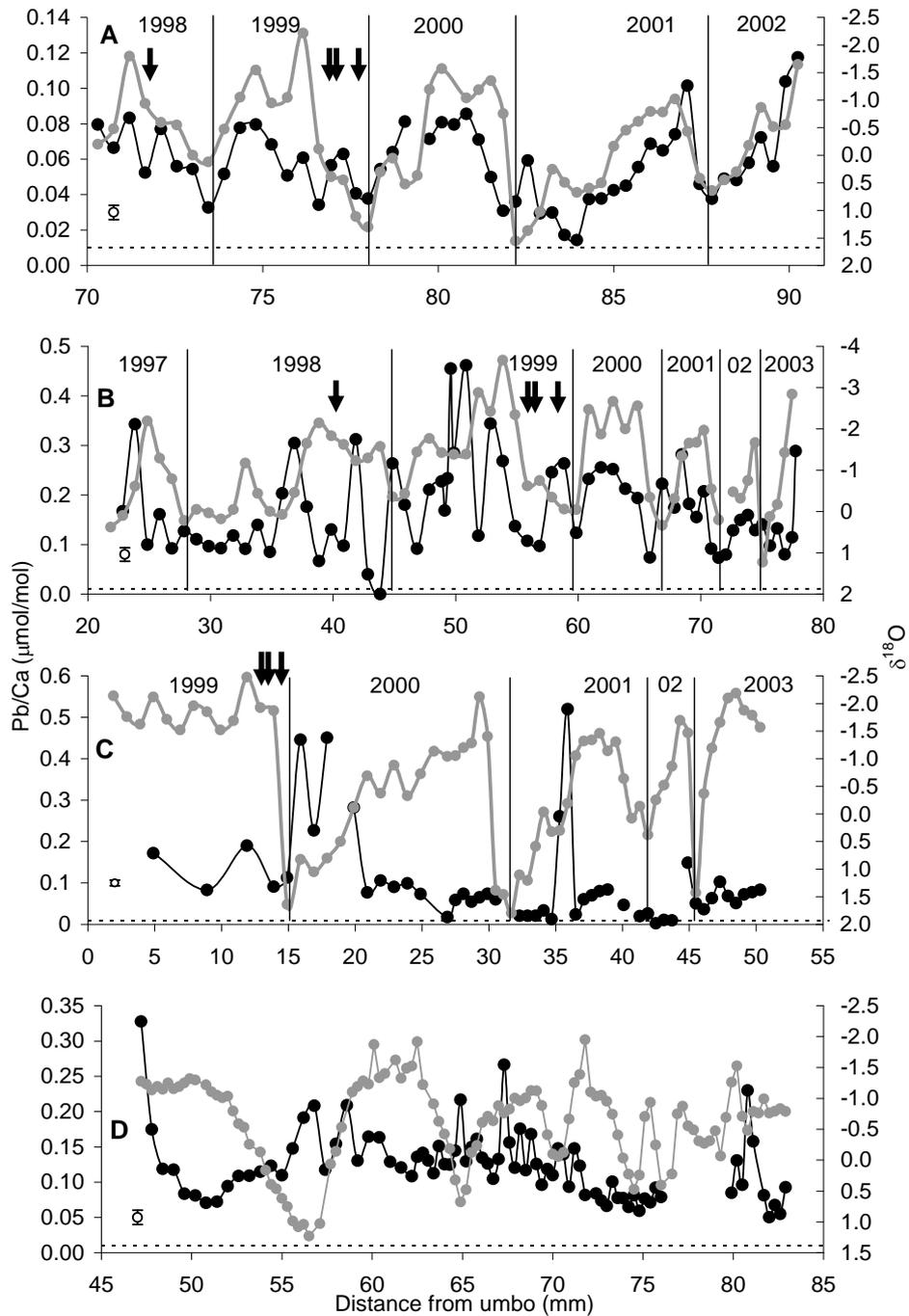
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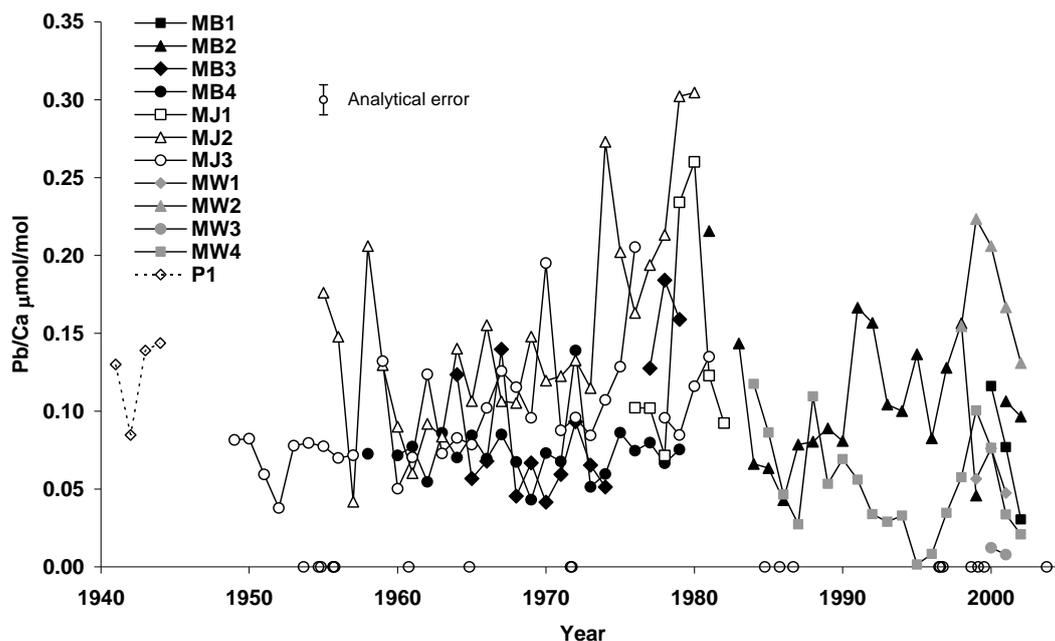


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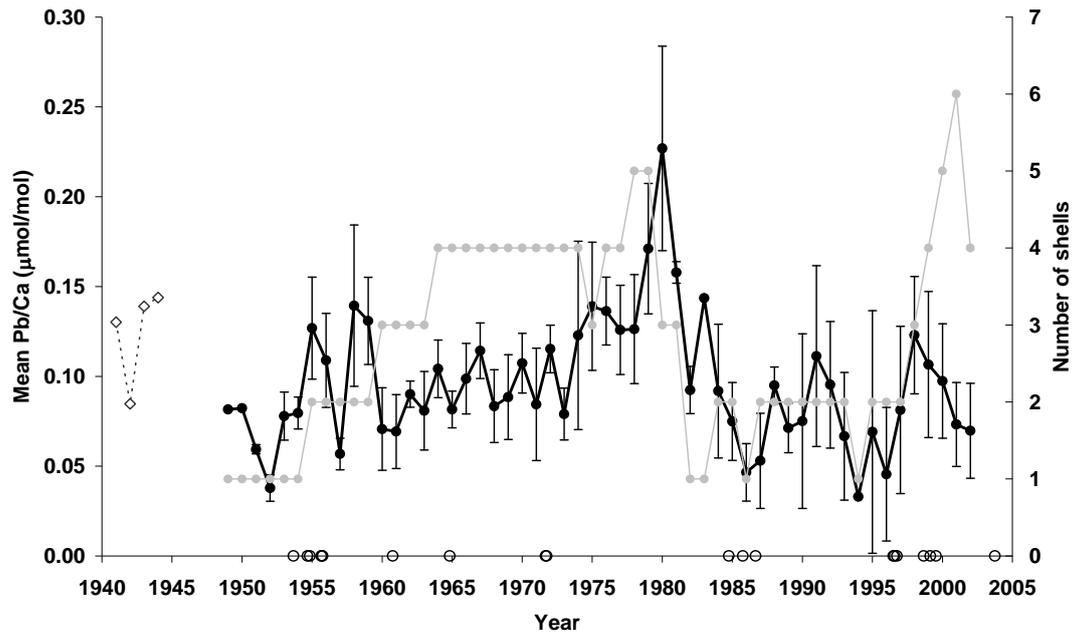
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