

# *Rangia cuneata* Shells as an Environmental Proxy: Variations in Elemental Concentrations within Populations

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## Abstract

*Rangia cuneata* is an endobenthic clam typically found in brackish water estuaries of the Atlantic Ocean and Gulf of Mexico coasts of North America. Its shell may potentially be useful as an archive for elemental water pollution for many coastal areas, due to its wide salinity tolerance. Soft tissue analysis for anthropogenic pollutants in this species is presently used to monitor environmental health. However, before *R. cuneata* shells can be developed as a proxy archive, some fundamental characteristics need to be better assessed. For example, it is uncertain if different individuals within a population grown at the same place and time record similar concentrations of contaminants in their shells. To address this issue, samples of living *R. cuneata* were collected in two sites in the Mobile-Tensaw Delta (Gulf of Mexico coast, Alabama, USA) where previous data existed on several key metal pollutants, in addition to some soft tissue elemental analysis of other *R. cuneata* samples. Time-equal sections of shell as identified through growth increment and oxygen isotope ( $\delta^{18}\text{O}$ ) analysis were measured for Pb, a calcium-substituting element, to assess if concentrations of these metals vary

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*significantly between individuals. Factors such as age difference and growth rates are considered as potential sources of variation. Results show that few R. cuneata were growing contemporaneous to each other, and Pb levels in the shells varied significantly in those growing at the time of capture. These difficulties must be overcome to employ R. cuneata shells as an environmental proxy.*

## Introduction

Few coastal regions have been monitored for long-term trends in heavy metal water contamination. Such data would be useful in the assessment of anthropogenic impacts. In instances in which direct measurements do not exist, mollusk shells could serve as a proxy for such data. Mollusk shell chemistry is, in part, a reflection of its environmental conditions (e.g., Gillikin *et al.*, 2005). As a shell accretes, it may record changes in ambient water chemistry over time. Therefore, museum samples of shells, as well as those found in archeological sites and sediment cores, could be exploited to determine levels of contamination through history, provided that a quantifiable relationship between shell and environmental chemistry is established. In a time-series analysis dated prior to human occupation or recorded environmental history, a base-line for contaminate levels could be established for an area.

*Rangia cuneata* (shown in figure 1) is a potentially useful proxy. It is found in many archeological sites, fossil deposits, and sediment cores around the Gulf of Mexico and the southern portion of the U.S. Atlantic



**Figure 1. Photo of *Rangia cuneata* collected in Mobile Bay. Shown against a 1 cm by 1 cm matrix background.**

Coast (e.g., Andrus and Rich, 2008; Aten, 1981; Carlson, 1987). Using sequential fossil, archeological, and historical samples, an in-depth timeline of contamination could hypothetically be constructed for estuarine locations along the entire Gulf of Mexico.

The purpose of the current study is to determine if the variability of elemental concentrations within populations of *Rangia cuneata* precludes or complicates their use as time-series pollution proxies. It is hypothesized that the degree of variation of Pb concentrations within these shells may be statistically insignificant. Provided that this is true, it may be possible to use the mollusk shells as records of environmental pollution levels. However, finding significant variability within *R. cuneata* populations would be detrimental to the establishment of a uniform mathematical relationship between the concentrations of elements in a shell and that of the water in which it grew. It is critical to know if individuals within a population record the same timeline of contamination, if this species is to serve as an environmental proxy.

Similar research has been conducted in other regions using mollusks. Gillikin *et al.* (2005) studied the hard clam *Mercenaria* sp. to assess their use as proxies of coastal marine lead contamination. In the study, it was found that several specimens collected in the same area had high variability of Pb:Ca ratios in the shell. The cause of the variability within the shells is not certain, but one factor may have been differential water runoff from land (Gillikin *et al.*, 2005). Other studies have shown that variation in other elements may be related to non-environmental factors including mollusk age and growth rate (e.g., Surge and Lohmann, 2008). These studies suggest that the fundamental processes governing the distribution of elements in mollusk shells are not yet well understood, and research such as that described here will aid in assessing the potential utility of this proxy.

*R. cuneata* is an estuarine clam and thus lives in salinities varying from almost fresh water, or zero practical salinity units (0psu), to those of the open ocean, which is, on average, 35psu (Hopkins *et al.*, 1973). While *R. cuneata* may live in varying salinities, they reproduce best in water closer to a 50/50 mixture, or around 18psu (Hopkins *et al.*, 1973). *R. cuneata* clams are native to the coasts of the Gulf of Mexico but have been found as an invasive species on the northern Atlantic coast and in Europe (Wakida-Kusunoki and MacKenzie, 2004). While *R. cuneata* is not widely consumed in the U.S.A., it is eaten in Mexico (Wakida-Kusunoki and MacKenzie, 2004).

*R. cuneata* shells are composed of aragonite (CaCO<sub>3</sub>). If present, other metals can bind in the place of calcium, therefore becoming crystallized within the shell matrix. Ca, being a divalent cation, is commonly replaced

by Pb (Gillikin, 2005). The *R. cuneata* shell grows in increments of one segment or line per every tide; in the northern Gulf there are approximately one high and one low tide per day, thus yielding approximately two growth lines per day (Andrus and Rich, 2008). Growth lines of *Rangia* are much like tree rings where rings or lines near the center are formed when the clams are younger. Growth increments can be seen in Figure 2. Rings farther away from the umbo, or hinge, represent ontogenetically older ages. Using the increments as a timeline, the shells may act as a recording medium of environmental variability of metals within the surrounding areas as they grow.



**Figure 2. Thin section of the growing edge of *R. cuneata* collected in a nearby area of Mobile Bay. Field of view is ~1cm.**

Mobile Bay (the study area) has been a part of The Mussel Watch Program since it was established in 1986, and *R. cuneata* analyzed in this program has been shown to be a useful measure of pollution (Kimbrough *et al.*, 2008). The design of the program is to collect and analyze the soft tissue of mussels to test for Polychlorinated Biphenyls (PCBs) as well as trace metals, such as Pb and Cd (Kimbrough *et al.*, 2008). Lead (Pb) and cadmium (Cd), even in trace amounts, can lead to significant health problems in humans. Cd and Pb are considered cumulative poisons, meaning the human body does not use or secrete the metals, and they therefore build up in the body (Chowdhury and Chandra, 1987). Cd is most commonly found in nickel-cadmium batteries, which are rarely recycled but, instead, dumped with regular household trash (Järup, 2003). Cd exposure can damage the kidneys and possibly affect the bones (Järup, 2003). Pb has been shown, commonly, to affect the central nervous system, renal function, the cardiovascular system, the immune system, and reproduction (Gidlow, 2004; Gao *et al.*, 2005). Children and young adults are found to be more susceptible to lead due to high gastrointestinal uptake and a more permeable blood-brain barrier (Järup, 2003).

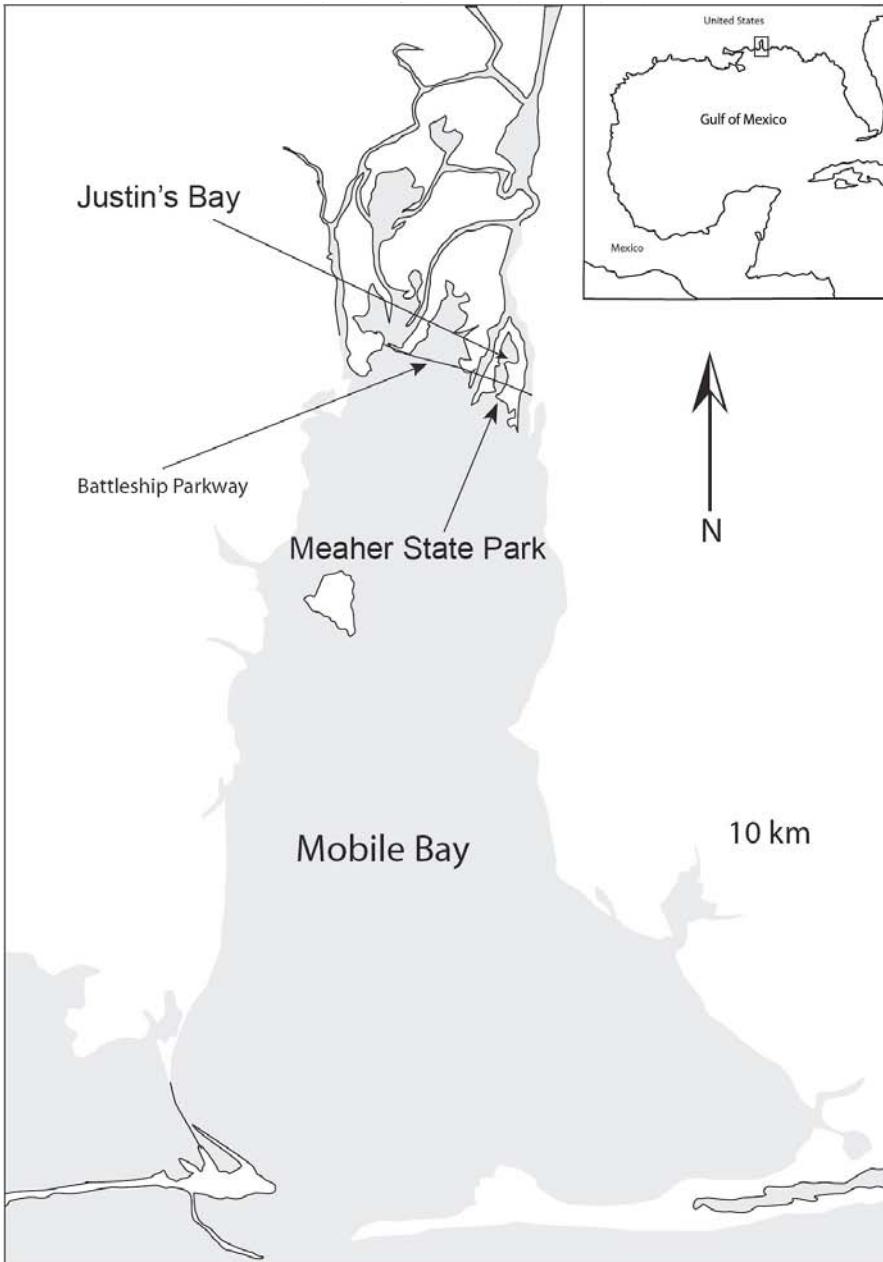
The Mussel Watch Program collects specimens once a year from sites during the winter months, with the soft tissues being later analyzed. This method of sampling does not give a detailed picture of how pollution changes over time; it only establishes per-annual trends. Also, The Mussel Watch Program began recording pollution trends only in the late 1980's; earlier pollution trends remain unknown. Measuring the shells from specimens, rather than only soft tissue that is sampled once annually, could potentially give a more detailed record of pollution change. The shells of *R. cuneata* may provide a means to assess earlier pollution trends and establish a contamination baseline, which would strengthen the Mussel Watch results.

### Methodology

In this study, *R. cuneata* shells were analyzed to determine Pb and Cd levels in the portion of shell that grew in the days just before capture. The underlying assumption was that all shells collected in the same time and place shared similar environmental exposure to these elements. The levels of Pb and Cd of each individual sample were compared within the population to determine if the population records contaminate levels similarly in the ambient water. If elemental concentrations are similar, it can be argued that shells growing in a population record regionally-consistent histories. Two separate populations were sampled in Mobile Bay to assess variation between locations.

Because mollusk growth is often discontinuous, it is important to know if the shells in the samples were growing at the time of collection and thus ensure that elemental data reflect water conditions at the time of capture. Stable oxygen isotope ( $\delta^{18}\text{O}$ ) values were used to determine if shells collected were growing contemporaneously. The rationale is that  $\delta^{18}\text{O}$  in *R. cuneata* shells are in equilibrium with surrounding water (Andrus and Rich, 2008), thus the values in the shell are controlled by water oxygen isotope content and temperature, which would be identical among all shells collected at the same time and place, assuming they were all actively precipitating new shell. Shells with dissimilar  $\delta^{18}\text{O}$  values at the growing margin of the shell were not growing at the same time.

Samples were collected on April 26, 2008 from Justin's Bay and Meaher State Park, Alabama (Figure 3). The Meaher State Park population (n=13) was collected within 1m of each other in sandy sediment and less than 40 meters away from a National Estuarine Research Program (NERP) data station. The Justin's Bay population (n=23) was collected within approximately 0.5 meters of each other in muddy sediment. Clams were collected opportunistically by hand and assumed alive at the time of capture. Salinity and temperature measures were also taken at the time of collection using a YSI meter.



**Figure 3. Mobile-Tensaw Delta pilot study collection sites. Sampling stations include Justin's Bay and Meaher State Park. Markers are not exact locations.**

*R. cuneata* collected in the field were immediately transported to the University of Alabama's Department of Geological Sciences where analysis took place. The soft tissue was extracted manually after a brief water bath in heated tap water, which allowed the shells to open. Extracted soft tissue was preserved via freezing for potential future analysis. Measurements were taken along the axis of longest growth using digital calipers. The shells were then cleaned of outside debris, bisected down the axis of longest growth, and mounted to a petrographic slide using Crystalbond thermal adhesive. The mounted shells were thick-sectioned to a width of ~1mm using a slow speed diamond-wafering saw.

The thick sections were milled incorporating four growth increments equaling at least two days of growth using a New Wave/Merchantek Micromill with dynamic XYZ control at the submicron scale. Growth increments were determined visually under reflected light using the digital imaging software. Due to differences in growth rate and shell size, growth increments sampled may differ by  $\pm$  one to two days. A  $5\mu\text{m}$  deep transect was removed from the sample surface to remove any outside contaminants from the shell. Each shell was then milled  $1200\mu\text{m}$  deep on a  $2000\mu\text{m}$  transect along the inner layer of the growing edge of the shell.

The milled powder was separated into  $600\mu\text{g}$  and  $100\mu\text{g}$  portions. The  $600\mu\text{g}$  portions of powder were collected using stainless steel blades, then weighed and digested in 10 ml of 10%  $\text{HNO}_3$ . This was later analyzed in an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and Inductively Coupled Plasma Mass Spectrometer (ICP-MS). ICP-OES analysis was used to determine overall calcium (Ca) levels in the shells, while the ICP-MS was used to determine minor and trace element levels. The elements tested for in the ICP-MS included lead (Pb) and cadmium (Cd). ICP-OES and ICP-MS samples were analyzed using in-house standards. Limits of detection and quantification are included in the results and discussion below.

The  $100\mu\text{g}$  portions were collected with stainless steel blades, weighed, and placed in 4.5ml round-bottomed borosilicate vials. The samples were reacted with orthophosphoric acid at  $70^\circ\text{C}$  with the resulting gas purified using continuous He flow using a Thermo GasBench II peripheral device. Samples were analyzed for stable  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  isotopes in a Thermo Delta Plus gas source isotope ratio mass spectrometer (IRMS).

All values are expressed in parts per mil (‰) relative to the VPDB standard by use of the NBS 19 standard. These standards were spread throughout the run to test and correct for drift and estimate precision. Precision ( $1\sigma$ ) for  $\delta^{18}\text{O}$  was 0.12 and  $\delta^{13}\text{C}$  was 0.06.

Models of idealized  $\delta^{18}\text{O}$  values were calibrated using water measurements from Andrus and Rich (2008) which were collected from Mobile Bay the previous year. Models were created using Grossman and Ku's (1986) temperature equation 'a,' assuming equilibrium fractionation of oxygen isotopes. Temperatures were measured at the time of collection in Justin's Bay, and as recorded in the Meaher Park National Estuary program data station (<http://www.mobilebaynep.com/mondata/mainmenu.cfm>).

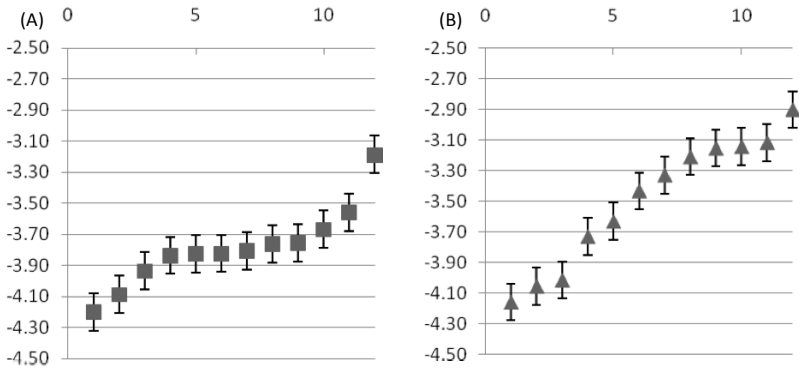
## Results

Length measurements from clams taken along the axis of longest growth are shown in Table 1. On average, clams collected from Meaher State Park (mean = 54.3mm) are larger than those from Justin's Bay (44.4).

**Table 1. Shell length along axis of longest growth.**

Sample	Length (mm)
M1	47.29
M3	58.04
M4	62.3
M5	45.82
M6	47.42
M7	66.73
M8	55.24
M9	57.93
M10	58.5
M11	51.04
M12	54.1
M13	47.27
J2	47.96
J4	38.06
J5	45.68
J9	39.08
J11	56.14
J12	48.36
J13	43.36
J17	44.6
J18	42.84
J19	45.32
J20	40.25
J22	41.44

$\delta^{18}\text{O}$  data profiles for Justin's Bay and Meaher State Park clams are shown in Figures 4 and 5.  $\delta^{18}\text{O}$  values in Figure 4 are plotted in ascending order with vertical bars signifying analytical precision ( $1\sigma$ ). The range in  $\delta^{18}\text{O}$  values is greater among Meaher State Park (1.26‰) clams than those collected in Justin's Bay (1.01‰). The mean  $\delta^{18}\text{O}$  value for Meaher State Park clams is -3.49‰ while Justin's Bay's is -3.79‰.

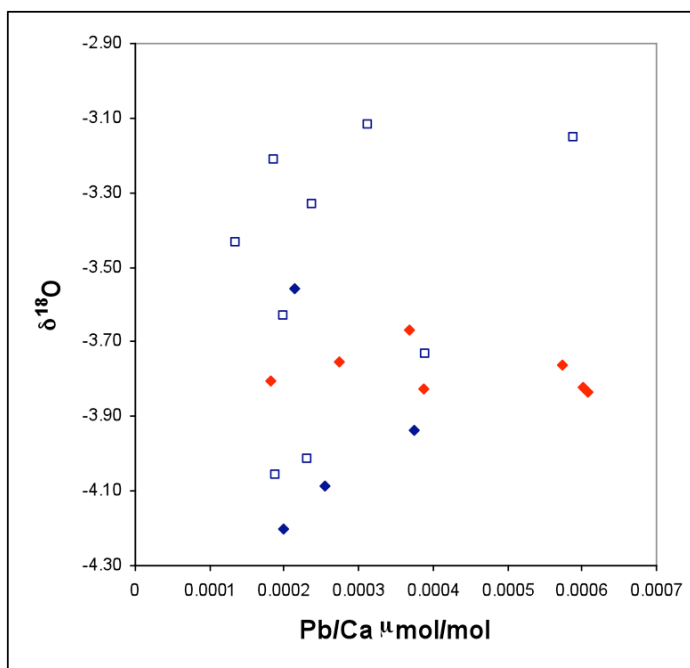


**Figure 4.  $\delta^{18}O$  values of Justin's Bay (A) and Meaher State Park (B). Samples are plotted in ascending order. Vertical bars represent analytical precision ( $1\sigma$ )**

Results of elemental analysis are plotted in Table 2 and Figure 5. All Cd levels were found to be below the limit of detection (LOD) and are therefore not plotted or discussed further. Three Pb levels fell below the limit of quantification (LOQ); these are shown as the red values in Table 2. Sample M4 was apparently contaminated and is not reported here.

**Table 2. Elemental analysis of Pb concentrations in ng/L. Values in red are below the limit of quantification (10.78‰).**

Sample	Pb (ng/L)
M1	27.7023
M3	8.7569
M5	11.6143
M6	10.6211
M7	9.2664
M8	14.8169
M9	9.0714
M10	15.2108
M11	7.6331
M12	5.515
M13	20.5262
J2	39.14
J4	25.7859
J5	16.474
J9	17.036
J11	5.8698
J12	13.0892
J13	14.5556
J17	11.0461
J18	15.3111
J19	36.3948
J20	39.4255
J22	14.1858



**Figure 5. Pb/Ca (x axis) versus  $\delta^{18}\text{O}$  (y axis) from all samples above Pb LOQ expressed in  $\mu\text{mol/mol}$ . Solid diamonds represent Justin's Bay samples. Open squares represent Meaher State Park samples. Red diamonds are Justin's Bay samples that are identical to within  $1\sigma$  precision for  $\delta^{18}\text{O}$ .**

## Discussion

$\delta^{18}\text{O}$  values plotted in Figure 4 show that 5 Justin's Bay clams are identical to within analytical precision limits ( $1\sigma$ ) and 9 are identical to within  $2\sigma$  analytical limits. Figure 4 also shows that 5 Meaher State Park clams are identical to within  $1\sigma$ , and 6 are identical to within  $2\sigma$  limits. More Justin's Bay clams seem to have been growing contemporaneously than at Meaher State Park. It is thought that as clams of other species age, they begin to more frequently cease growth for indeterminate amounts of time (e.g., Andrus and Crowe, 2008). This may also be true of *R. cuneata*. The Meaher State Park population had a longer average axis length as seen in Table 1. Axis length is a rough measurement of shell age, with the larger lengths typically indicating older clams.  $\delta^{18}\text{O}$  values in the Meaher State Park population show temperature ranges that span over  $5^\circ\text{C}$  when plotted using temperature models; however, water temperatures in this area of Mobile Bay did not fluctuate to this extent in a single day during the spring before collection (<http://www.mobilebaynep.com/mondata/mainmenu.cfm>).

Samples of Meaher State clams may have been taken from a section of shell that had been precipitated several days or more in the past when water temperatures and ambient water  $\delta^{18}\text{O}$  were much different than at the time of collection and, since then, the clam had ceased precipitating shell.

Several Meaher State clams were found to have significant growth breaks near the growing edge of the shell. Growth breaks are visual indications of times when clams cease shell precipitation for an extended period. In many cases, Meaher State Park shells had changed angle of growth, which indicates growth disruptions. Until the origin and duration of frequent growth cessations can be determined, geochemical time-series results may be difficult to interpret. Several Meaher State clams were also found to have epibionts (most often barnacles) attached to the shells, that may suggest shallower burial and more saline conditions than those found in Justin's Bay.

Another possible explanation for the differences in  $\delta^{18}\text{O}$  variation and growth cessations between the sample sites could be habitat. Justin's Bay is more protected from wind, waves, and salinity variation than Meaher State Park due to natural and anthropogenic differences in geography (Figure 3). Furthermore, the substrate at Justin's Bay was soft organic-rich mud while Meaher Park was packed sand. Justin's Bay clams were more deeply buried and closely packed. Whatever the specific cause or causes, more Justin's Bay clams seem to have been growing contemporaneously and thus will be the focus of this discussion.

Pb values within the Justin's Bay population were found to be highly variable with some analyses falling well below the limit of quantification and some values being several times greater than these limits, as seen in Table 2. Pb/Ca ratios plotted against oxygen isotope values (Figure 5) show that even among those shells identified as most likely to be growing at the same time, variability is high. The highest Pb/Ca ratio value among those shells with similar  $\delta^{18}\text{O}$  values is three times that of the lowest.

More detailed analysis and monitoring would be necessary to define the causes of this variability in shell Pb/Ca ratios. The data do suggest, however, that these clams, even when growing simultaneously in closely-packed beds, do not reflect identical lead concentrations in their shells.

## Conclusion

Significant variability in Pb concentration exists in the sampled population of *R. cuneata*. At least part of the variation may have been caused by periods of growth cessation in some clams; however, Pb variation is high even in shells that were all growing at the same time. This has important implications for future studies that analyze only one shell as a proxy for

time-series contamination levels in an area. Such proxies can only be useful if an individual is indicative of the entire population, which does not appear to be true for *R. cuneata*. This problem may be circumvented by either better defining the causes of Pb distributions in *R. cuneata* and similar species, or perhaps larger sample sets of clams would have to be analyzed to ensure that the proxy is recording at least approximate contamination levels within an area. Further research is clearly necessary before *R. cuneata* can serve as a proxy for environmental lead contamination.

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