TEMPORAL TRENDS IN THE HOLOCENE: EXPLORING IMPLICATIONS FOR CONSERVATION PALEOBIOLOGY THROUGH QUANTITATIVE ASSESSMENT OF MARINE MOLLUSKS

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Master of Sciences

by
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MAY 2018
The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

TEMPORAL TRENDS IN THE HOLOCENE: EXPLORING IMPLICATIONS FOR CONSERVATION PALEOBIOLOGY THROUGH QUANTITATIVE ASSESSMENT OF MARINE MOLLUSKS

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a candidate for the degree of Master of Sciences,

and hereby certify that, in their opinion, it is worthy of acceptance.

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TABLE OF CONTENTS

ACKNOWLEDGMENTS ........................................................................................................... ii

LIST OF ILLUSTRATIONS .................................................................................................. vi

LIST OF TABLES .................................................................................................................. vii

GRANT INFORMATION ....................................................................................................... viii

ABSTRACT .......................................................................................................................... ix

CHAPTER:

1. CONSERVATION PALEOBIOLOGY: INFERRING ENVIRONMENTAL PROXIES FROM GEO-HISTORICAL DATA .......................................................... 1

   1.1 What is Conservation Paleobiology? ................................................................. 2

   1.2 Summary of Research ...................................................................................... 2

2. CONCH FRITTERS THROUGH TIME: HUMAN PREDATION AND POPULATION DEMOGRAPHICS OF STROMBUS GIGAS, SAN SALVADOR ISLAND, THE BAHAMAS ........................................................................... 6

   2.1 Introduction ......................................................................................................... 7

   2.2 Materials and Methods .................................................................................... 10

   2.3 Results .............................................................................................................. 14

   2.4 Discussion ....................................................................................................... 16

   2.5 Conclusions .................................................................................................... 23

   2.6 Tables and Figures ........................................................................................... 25

3. TRACE ELEMENT ANALYSIS OF HOLOCENE BIVALVES POTAMOCORBULA AMURENSIS AND CYRENODONAX FORMOSANA AS POTENTIAL PROXIES FOR TEMPERATURE AND PRODUCTIVITY IN THE PEARL RIVER DELTA, CHINA .......................................................................................... 38

   3.1 Introduction ....................................................................................................... 39

   3.2 Materials and Methods .................................................................................... 41

   3.3 Results .............................................................................................................. 44
3.4 Discussion ..............................................................................................................48
3.5 Conclusions ...........................................................................................................58
3.6 Tables and Figures ...............................................................................................60

4. PROPER PROCEDURE FOR TRACE ELEMENT ANALYSIS USING LA-ICP-MS ........................................................................................................71
   4.1 Introduction ............................................................................................................72
   4.2 How NOT to do LA-ICP-MS ................................................................................72
   4.3 How we Salvaged the Data ..................................................................................74
   4.4 Proper Procedure for LA-ICP-MS Data Collection .............................................80

REFERENCES CITED ....................................................................................................84
# LIST OF ILLUSTRATIONS

## Figures in Chapter 2

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Map of San Salvador Island</td>
<td>31</td>
</tr>
<tr>
<td>2.</td>
<td>Morphometric shell measurements and human predation traces</td>
<td>32</td>
</tr>
<tr>
<td>3.</td>
<td>Rapid AMS radiocarbon dates for <em>S. gigas</em> shells</td>
<td>33</td>
</tr>
<tr>
<td>4.</td>
<td>Proportions of juveniles harvested in each time bin</td>
<td>34</td>
</tr>
<tr>
<td>5.</td>
<td>Body size density plots of <em>S. gigas</em> through time</td>
<td>35</td>
</tr>
<tr>
<td>6.</td>
<td>PCA scatter and density plots of <em>S. gigas</em></td>
<td>36 &amp; 37</td>
</tr>
</tbody>
</table>

## Figures in Chapter 3

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Map showing position of the PRD-10 core in the Pearl River delta</td>
<td>64</td>
</tr>
<tr>
<td>2.</td>
<td>Holocene evolution of the PRD-10 core</td>
<td>65</td>
</tr>
<tr>
<td>3.</td>
<td>Cook’s Distance plots</td>
<td>66</td>
</tr>
<tr>
<td>4.</td>
<td>Scatter and density plots of PC1 vs PC2 for <em>P. amurensis</em></td>
<td>67</td>
</tr>
<tr>
<td>5.</td>
<td>Scatter and density plots of PC1 vs PC2 for <em>C. formosana</em></td>
<td>68</td>
</tr>
<tr>
<td>6.</td>
<td>Scree plots for <em>P. amurensis</em> (A) and <em>C. formosana</em> (B)</td>
<td>69</td>
</tr>
<tr>
<td>7.</td>
<td>Changes in median PC1 and PC2 scores through time and loadings plots for <em>P. formosana</em> (A, B) and <em>C. formosana</em> (C, D)</td>
<td>70</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

#### Tables in Chapter 2

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Temporal bin assignment and number of <em>S. gigas</em> individuals collected from each locality</td>
<td>25</td>
</tr>
<tr>
<td>2.</td>
<td>Summary statistics of <em>S. gigas</em> shell length</td>
<td>26</td>
</tr>
<tr>
<td>3.</td>
<td>Summary statistics of <em>S. gigas</em> shell width</td>
<td>27</td>
</tr>
<tr>
<td>4.</td>
<td>ANOVA and Tukey’s post-hoc tests of shell size by time bin and life stage</td>
<td>28</td>
</tr>
<tr>
<td>5.</td>
<td>Summary statistics of <em>S. gigas</em> PC1 and PC2 scores</td>
<td>29</td>
</tr>
<tr>
<td>6.</td>
<td>ANOVA and Tukey’s post-hoc tests comparing PC1 vs PC2 median scores by time bin and life stage of <em>S. gigas</em></td>
<td>30</td>
</tr>
</tbody>
</table>

#### Tables in Chapter 3

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Number of LA-ICP-MS analyses for <em>P. amurensis</em> and <em>C. formosana</em></td>
<td>60</td>
</tr>
<tr>
<td>2.</td>
<td>Descriptive statistics of trace element concentration values of <em>P. amurensis</em></td>
<td>61</td>
</tr>
<tr>
<td>3.</td>
<td>Descriptive statistics of trace element concentration values of <em>C. formosana</em></td>
<td>62</td>
</tr>
<tr>
<td>4.</td>
<td>Wilcoxon test <em>p</em>-values comparing median PC scores between adjacent temporal bins for <em>P. amurensis</em> and <em>C. formosana</em></td>
<td>63</td>
</tr>
</tbody>
</table>
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ABSTRACT

Conservation paleobiology is a relatively new field which applies the theories and analytical tools of paleontology to problems concerning the conservation of biodiversity. The use of data from stratigraphy, sediment or ice cores, fossil collections, and/or other specimens that provide temporal, ecological, or environmental information from both the recent Holocene and deep-time fossil records can be used to understand the ecological and evolutionary responses of species to changes in their environment. In one study, we analyzed changes in shell morphology from Pre-Columbus through present time for a heavily exploited, large marine gastropod, *Strombus gigas* (the queen conch), on San Salvador Island, the Bahamas. Overall, we observed an increase in harvested juveniles and an initial increase in shell size with a decrease in more recent time. Increased proportions of harvested juveniles and increasing size followed by a decrease in size in more recent time is consistent with increased stress on fisheries due to overfishing in the late 20th century. In a second study, we examined trace element concentrations preserved in the shells of two Holocene marine bivalve taxa, *Potamocorbula amurensis* and *Cyrenodonax formosana* from the Pearl River delta, China. We explored the use of trace elements as potential proxies for environmental change within the context of the sedimentological and faunal history of a previously described drill core from the Pearl River delta, China. Although the observed trace element signals are variable across bivalve species, they can be a useful tool in paleoenvironmental reconstructions.
CHAPTER 1

CONSERVATION PALEOBIOLOGY: INFERRING PRESENT ECOGICAL RESPONSES TO CHANGE FROM GEO-HISTORICAL DATA

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1.1 What is Conservation Paleobiology?

Conservation paleobiology is a relatively new field which applies the theories and analytical tools of paleontology to problems concerning the conservation of biodiversity. The use of data from stratigraphy, sediment or ice cores, fossil collections, and/or other specimens that provide temporal, ecological, or environmental information from both the recent Holocene and deep-time fossil records can be used to understand the ecological and evolutionary responses of species to changes in their environment (Dietl and Flessa, 2011; Deitl and Flessa, 2009). There are two main approaches to conservation paleobiology, the near-time approach and the deep-time approach, with the former having attracted the most attention of paleontologists. The near-time approach uses the relatively recent geologic past (last several million years, e.g.: Pleistocene to Holocene) as context for present day changes in environment and species responses to environmental change. It is important to consider the geo-historical past when interpreting the ecological patterns seen today as the majority of biological conservation studies rarely employ timescales longer than a few decades (Froyd and Willis, 2008). Longer timescales allow for more robust, high-resolution results. Using time scales on the order of hundreds, thousands, or millions of years, paleontologists can infer present day ecological responses to changing environments – which was the overall goal of this work. Data of such a robust nature will provide useful insights for successful conservation strategies and can be used to understand ecological and evolutionary dynamics.

1.2 Summary of Research

Chapter two, “Conch fritters through time: Human predation and population demographics of *Strombus gigas*, San Salvador Island, the Bahamas,” examines temporal
trends in population demographics and morphometrics of harvested *S. gigas* (the queen conch) individuals from medieval (Pre-Columbus), modern (~100 years), and global (~10 years) aged middens on San Salvador Island, the Bahamas. *Strombus gigas* is a culturally and nutritionally vital component of Caribbean cuisine. However, the over-fishing of juveniles has threatened the stability of wild conch populations. Current regulations in the Bahamas subjectively require the possession of a thick and well-formed shell lip. The available literature generally recommends a minimum of 15 mm, but regulations and fishery management practices vary regionally and are not consistent across the Caribbean. Only shells which had been harvested by humans were considered for this study. We hypothesized temporal increases in both shell size and the proportion of juveniles harvested due to increased fishing pressures and incohesive nature of regulations across the Caribbean. We collected 292 human-harvested individuals from 7 localities and measured eight morphological variables. Individuals were assigned to temporal bins based upon radiocarbon dates using the rapid AMS dating method. For all time periods, the proportion of harvested juvenile individuals is increasing through time. While temporal trends in shell size are complex, we observed an increase in shell size from the Medieval to the Modern with a decrease from the Modern to the Global. Temporal trends of increased proportions of harvested juveniles as well as increasing shell size followed by a decrease in more recent time, is consistent with a pattern of increased stress on fisheries from increased demand and overfishing in the late 20th century. Because of this, unified regulations of a closed season during reproductive months and minimum lip thickness as the indicator of sexual maturity are recommended for the entire geographic range of the species in the Caribbean.
Chapter three, “Trace element analysis of Holocene bivalves *Potamocorbula amurensis* and *Cyrenodonax formosana* as potential proxies for temperature and productivity in the Pearl River delta, China,” examines trace element concentrations preserved in shell growth increments in two estuarine aragonitic bivalve species, *P. amurensis* and *C. formosana*, and the use of those trace element concentrations as proxies for large-scale environmental change in the context of the PRD-10 drill core described by Alberti et al. (2012). Thirty *Potamocorbula amurensis* and Thirty *Cyrenodonax formosana* bivalve individuals were selected from eight temporal/environmental bins described in core spanning a 9,600-year Holocene record. Shells were sectioned along the axis of maximum growth and analyzed using laser-ablation inductively-coupled-plasma-mass-spectrometry (LA-ICP-MS). Spots 40 x 80 µm in size, spaced approximately every 100µm, were analyzed across maximum growth from the umbo to the ventral margin for a total of 4,070 analyses. Sr:Ca, Mg:Ca, Li:Mg, Mn, Ba, and Zn concentration values/ratios were analyzed to explore the relationships of these trace elements as proxies for temperature (Sr:Ca, Mg:Ca, Li:Mg), fluvial/freshwater influence (Mn), and productivity (Ba, Mn). Proxy data were pooled by temporal bin and characterized by median PC1 vs PC2 scores to interpret trends through time. Preliminary results of this study indicate that trace element signals are largely variable between *P. amurensis* and *C. formosana*. The two taxa displayed comparable paleoenvironmental histories in some cases, but histories were not consistent between taxa in all instances. Trace element trends between the two taxa should be further evaluated for repeatability with additional samples. Given the trace element data collected for this study and the context of the PRD-
10 core, we have made progress in determining the potential of aragonitic bivalves as recorders of paleoenvironmental change throughout the Holocene.
CHAPTER 2

CONCH FRITTERS THROUGH TIME: HUMAN PREDATION AND POPULATION DEMOGRAPHICS OF STROMBUS GIGAS, SAN SALVADOR ISLAND, THE BAHAMAS

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2.1 Introduction

*Strombus gigas*, the Queen Conch, is a large herbivorous marine gastropod found primarily in the Western Atlantic Caribbean. Its distribution ranges from Venezuela to Florida and the Bahamas. It is a species of commercial, nutritional, and cultural importance to the Caribbean – especially in the Bahamas. The snail itself is used in many Caribbean dishes, such as conch fritters and conch salad. The shells are prized for many reasons including use in traditional ceremonies, as jewelry, and are often sold as tourist souvenirs. Valued for both seafood and shell trade, the Queen Conch represents one of the most valuable and highly demanded resources in the Caribbean. As a result, it was heavily exploited during the late 20th century throughout the Caribbean (Theile, 2001; Theile, 2005), which resulted in many fisheries being on the verge of collapse at the beginning of the 21st century (Stoner et al., 2012).

The biological characteristics of conchs (e.g. slow moving, found at shallow depths, and aggregating during reproductive season) make them prone to exploitation (Appeldoorn et al., 2011). Conchs are also notoriously slow to mature. Appeldoorn (1988) estimated that age of first reproduction occurs at roughly 3.5-4 years. As a result of increased exploitation in the late 20th century, beginning in the 1970’s, *S. gigas* previously was listed on the International Union for the Conservation of Nature’s (IUCN) Red List in 1994. It has since been removed as this species is no longer considered to be endangered in the Caribbean as a whole. However, it is still commercially and locally threatened in numerous areas.

Since 1992, the fishery of this species has been subject to both international and local management measures because of overfishing (Acosta, 2006). The Natural
Resources Management Unit (NRMU) of the Organization of the Eastern Caribbean States (OECS), an organization of nine Caribbean member nations (Anguilla, Antigua & Barbuda, British Virgin Islands, Dominica, Grenada, Montserrat, Saint Kitts and Nevis, Saint Lucia, Saint Vincent & the Grenadines), is responsible for fishery management and provides legal policy and administrative framework for the establishment of regional marine environment management programs (Thiele, 2001). In the early 1990’s, as a response to overfishing of queen conch populations and endangerment of the species, the NRMU helped create regulations for harvesting by recommending minimum size restrictions, allowing only the harvest of shells with a flared lip, considering gear restrictions, and the establishment of closed seasons and marine protected areas (Thiele, 2001; Aranda and Frenkiel, 2007; Acosta, 2006). These recommendations have been implemented by several Caribbean nations, but not by all. This makes it problematic to be aware of and enforce regulations from nation to nation. Regulations in the Bahamas only state that harvesting and possession of conch without a well-formed lip is prohibited and that the bag limit at any time is 10 conchs per person (“Fishing Regulations,” 2018). Thus, it is illegal to harvest juvenile individuals as they do not have a “well-formed shell lip”. This verbiage of “well-formed shell lip” is subjective and it is up to the interpretation of the fisher to determine the legality of their catch.

Determining sexual maturity in gastropods can be difficult. Length-wise shell growth ceases near the onset of sexual maturity. However, shell size at maturity is also highly variable among individuals (Vermeij, 1980). Therefore, shell length has been shown not to be a reliable indicator of maturity (Randall, 1964). After reaching its maximum length, the growing shell margin of \textit{S. gigas} flares outward and develops a lip
that increases in thickness prior to and also after the onset of maturity (Appeldoorn, 1988; Stoner et al., 2012). Lip thickness has been shown to be a more appropriate and reliable index of maturity than shell length (Vermeij, 1980; Theile, 2001; Tehile, 2005; Aranda and Frankeil, 2007; Stoner et al., 2012a; Mueller and Stoner, 2013; Foley and Takahashi, 2017). Shell thickness increases with age because nacre is continuously deposited on the inside of the shell, resulting in the reduced internal space for soft tissue and an overall wider, thicker, and heavier shell (Randall, 1964; Stoner et al., 2012). Thus, shells with a fully-formed and well-developed shell lip have wider shells than those that do not.

Additionally, growth in lip thickness varies between geographic locations in the Caribbean and can be dependent on sex, depth, latitude, temperature, food availability, age class, and shelter (Appeldoorn, 1988; Stoner and Sandt, 1992; Vermeij et al., 1992; Posada et al., 1999; Posada et al., 2006; Appeldoorn and Baker, 2013; Boman et al., 2017) though the specific variables controlling shell size at maturity across its geographic range have not been identified. As a rule of thumb, it is generally recommended in the literature to harvest only individuals with a shell lip thickness of 15mm or greater (BREEF; Stoner et al., 2012a; Foley and Takasha, 2017; Thiele et al., 2001; Boman et al., 2018) as these are more likely to be sexually mature adults with thick well-formed shell lips.

Fishing regulations in the Bahamas regarding shell lip thickness are often subjective in practice and left up to the interpretation of the fisher as to whether a conch has a thick enough shell lip to harvest. Because of this and the increased demand leading to increased harvesting pressures, we expect to see a trend of a greater proportion of harvested juveniles through time, regardless of fishing regulations and management strategies implemented in the late 20th century. It is likely that selective fishing pressures
and the high commercial demand for conchs has resulted in fishers harvesting more juvenile (<15mm lip thickness) than adult individuals (>15mm lip thickness) through time ranging from Pre-Columbus through present day.

The goal of this work was to answer the following research questions:

1) How has the exploitation of the fishery at San Salvador, the Bahamas changed over the last ~800 years?

More Specifically,

2) How has the proportion of harvested juvenile individuals changed through time based on the recent death assemblage of this fishery?

3) How has the shell size and shape of harvested individuals changed through time as a reflection of stress on this fishery and, beyond shell size, what other insights can be gained from changes in shell morphology through time?

2.2 Materials and Methods

*Sampling localities* - Bulk samples of *Strombus gigas* (Queen Conch) individuals were collected from human created shell refuse middens at seven localities on San Salvador Island, the Bahamas (Figure 1). A total of 292 individual shells were collected from the seven localities (Table 1). Collection localities are as follows: Barker’s Point (BP), a scattered onshore midden; two sites at Graham’s Harbour (GH1, GH2), nearshore locations; Pigeon Creek Mouth (PCM), an onshore mounded midden; and multiple sites at North Pigeon Creek (PCN-K1, PCN-D, and PCN-I), all onshore mounded middens. At Site PCN-K1, we excavated a 1m deep pit to sample a lower stratigraphic level within the midden. Shells from the top (surface) of the midden are labeled as “PCN-K1-S” and
those collected from the bottom (sub-surface) are labeled as “PCN-K1-SS.” All shells were harvested by humans as evidenced by a hatchet mark at the base of the spire.

Sampling localities were limited to on-shore or shallow nearshore (<4m water depth) locations. Shells collected were from one of three midden types: 1) scattered nearshore middens, 2) scattered onshore middens, and 3) mounded onshore middens. Nearshore locations are sites where fishers have been actively dumping conch shells into the water after harvesting. Scattered on-shore middens were sites where there was no well-defined characteristic shell mound, rather shells were scattered along the coast. Mounded on-shore shell middens were those comprised of well-defined mounds. These middens tended to be located further inland on the shore of Pigeon Creek, a shallow tidal creek.

Collection methods - The following information was collected at each locality:
latitude/longitude coordinates using a handheld GPS device, a physical description of the sampling area, area from which the sample was derived (m²), midden type (mound vs. scattered accumulation, onshore vs. offshore), and the number of conchs collected from each location. Shells at mounded midden sites were randomly sampled by tossing a large (approximately 60 x 90 cm) mesh bag onto the midden and collecting all the shells the bag was touching. The size of the shells and restricted access to the sites limited our ability to collect large numbers of shells at a time. All shells from the sea-floor at the Graham’s Harbour shallow nearshore locations were collected via snorkeling. These were localities previously determined to be shell dumping sites as evidenced by shells dumped near the end of boat docks at these locations. At Barker’s Point, shells were sparsely scattered along the beach and shoreline. Additionally, numerous shells are embedded in
the lithified matrix at this archaeological site.

**Laboratory Methods** - Each shell was photographed from multiple perspectives and the following dimensions were measured in mm using calipers: shell length (the distance between the apex and the tip of the siphonal canal), shell width (the maximum distance perpendicular to length at the last body whorl where the lip is thickest, not including spines), siphon to penultimate whorl, siphon to final whorl, spire length, aperture length, aperture width, and shell lip thickness (recorded from the outer lip in the mid-lateral region approximately 30mm from the edge of the shell) (Figure 2). A fragment of shell was collected, at the approximate location where the shell lip thickness measurement was recorded, from a subset of shells (n=63) to get an idea of the relative age of each locality.

Based upon the published proceedings for shells documenting Barker’s Point as a Pre-Columbus site (Blick, 2007; Blick and Bovee, 2007; Blick and Dvoracek, 2011; Blick, 2011) and radiocarbon dates, collection localities and were assigned to one of three geochronological bins as described by Lotze et al. (2011): Medieval (Pre-Columbus), Modern (~10^2 years), and Global (~10^1 years) (Table 1). A subset of 63 shells were selected from all sampling locations using random number generation in R (R Core Development Team, 2016) and sent to the Amino Acid Geochronology lab at Northern Arizona University where they were prepared for the rapid AMS method as described by Bush et al. (2013). From there, samples were sent to the Keck AMS Laboratory at the University of California – Irvine for analysis. Sample preparation backgrounds were subtracted, based on measurements of 14C-free marble and results were corrected for isotopic fractionation according to the conventions of Stuiver and Polach (1977) with
\(\delta^{13}C\) values measured on prepared graphite using the AMS spectrometer. Shells from localities which reported all modern radiocarbon dates (these samples contain excess Carbon, likely due to mid-20th century atmospheric thermonuclear weapons testing) were assigned to the “Global” time bin. Shells from localities which reported a mix of older and modern radiocarbon dates shells were assigned to the “Modern” time bin. Shells from localities reporting radiocarbon dates >600 calendar years before present were assigned to the “Medieval” time bin (Figure 3).

Only whole shells with intact length and width axes were used for body size analysis. Shells were determined to be adults or juveniles based upon a 15 mm lip thickness threshold for maturity. 95% confidence intervals were calculated for the proportion of juveniles in each time bin via the Agresti-Coull method. Temporal trends in log\(_{10}\)-transformed shell length and shell width were analyzed using ANOVA and Tukey’s post-hoc test. A Principal Components Analysis (PCA) was performed on log\(_{10}\)-transformed shell length, shell width, lip thickness, aperture length, aperture width, distance from siphon to final whorl, distance from siphon to penultimate whorl, and spire length for specimens with no missing observations for these variables (n=218). An \(\alpha = 0.05\) is assumed unless otherwise stated. All analyses were conducted using “R” freeware (R Core Development Team, 2016) and the following packages: dplyr (Wickham et al., 2018), tidyr (Wickham and Henry, 2017), binom (Doraj-Raj, 2014), factoextra (Kassambara and Mundt, 2017), ggplot2 (Wickham, 2009), ggExtra (Attali, 2017), ggridges (Wilke, 2017), and ggfortify (Horikoshi and Tang, 2016).
2.3 Results

A total of 292 individual shells were collected. Of these, 281 were assignable to a temporal bin, 272 had complete shell length preserved, 227 had complete shell width preserved, and 218 were suitable for the PCA. Sixty-five rapid AMS radiocarbon dates were calculated for 64 shells from eight sampling localities (Fig. 3) and assigned to either the Medieval, Modern, or Global time bin (*sensu* Lotze et al., 2011). Barker’s Point specimens were confirmed as belonging to the Medieval time bin with ages of 555-1060 cal y BP. Specimens from Graham’s Harbor, Pigeon Creek North Dock, and Pigeon Creek North Kris 1 Sub-surface were dated between 910 cal y BP and post-thermonuclear testing and were placed in the Modern time bin. The Pigeon Creek North Kris 2 Sub-surface sample was assigned to the Modern time bin given its close proximity to the Kris 1 Sub-subsurface sampling locality. Specimens from Graham’s Harbor 2, Pigeon Creek Mouth, Pigeon Creek North Island, and Pigeon Creek North Kris 1 Surface resulted in solely post-thermonuclear testing dates and were assigned to the Global time bin. Similarly, the Pigeon Creek North Kris 2 Surface sample was assigned to the Global time bin. The temporal “smearing” of the Modern time bin between the Medieval and Global bins will likely obscure any trends in the data, thereby imposing a conservative interpretation of secular trends.

The percentage of juveniles harvested on San Salvador Island increased through time from 36.8% in the Medieval time bin to 53.7% in the Modern time bin to 62.4% in the Global time bin (Fig. 4). This increase was significant with each proportion value being lower than the 95% confidence interval of the following time bin.
The mean length of all harvested shells increased significantly between the Medieval and Modern time bins and exhibited stasis into the Global time bin. Conversely, the mean width of all harvested shells exhibited stasis between the Medieval and Modern and decreased significantly in the Global time bin (Fig. 5, Tables 2, 3, and 4). Considering only harvested adult shells, length and width increased significantly between the Medieval and Modern time bins and exhibited stasis in the Global time bin. Shell length of juveniles increased significantly between the Medieval and Modern and decreased significantly from the Modern to the Global. There was no significant change in juvenile shell width through time.

The first principal component explains 68.0% of the variation among the eight morphological characters analyzed (Fig. 6). All variables are strongly negatively correlated with PC1 values with the exception of the moderate negative correlation with lip thickness. PC2 explains 16.3% of the variation and is strongly positively correlated with lip thickness, moderately positively correlated with shell width and aperture length and width, and moderately negatively correlated with the four measures related to shell length. PC3 explains 5.5% of the variation and displays low correlation coefficients with all the morphological variables.

Mean PC1 scores for all specimens decreased significantly between the Medieval and Modern time bins and increased significantly into the Global time bin (Tables 5 and 6). Mean PC1 scores for adult specimens decreased significantly between the Medieval and Modern, but exhibited stasis into the Global. Conversely, mean PC1 scores for juveniles remained unchanged from the Medieval to Modern but increased significantly between the Modern to Global time bins. Mean PC2 scores for all specimens, adult
specimens, and juvenile specimens decreased significantly between the Medieval and Modern and remained unchanged into the Global.

2.4 Discussion

All shells considered for this study were harvested by humans as indicated by a puncture hole in the top of the shell near the spire. A feature shared by all the conchs collected at the Barker’s Point locality (Medieval/Pre-Columbus aged) was the ovoid puncture hole near the spire, likely made with a stone tool or the spire of another conch (Blick, 2007) (Figure 2). This gives access to the retractor muscle, which releases the snail from the shell when cut. This is still common practice throughout the Caribbean today but the puncture hole in Modern and Global aged shells is made with modern tools, such as a hatchet or screwdriver, and can be easily distinguished from the ovoid puncture on Medieval shells (Figure 2). This distinction was helpful when estimating the time period assignments prior to radiocarbon dating.

Demographics of harvested individuals through time - We predicted that we would see a greater proportion of juveniles (lip thickness <15mm) harvested in more recent time due to increased demand, which led to increasing harvest pressures of queen conch fisheries over the late 20th century (Thiele, 2005). Indeed, the percentage of juveniles harvested on San Salvador Island increased significantly through time from 36.8% in the Medieval time bin to 53.7% in the Modern time bin to 62.4% in the Global time bin (Figure 4). The queen conch is heavily exploited throughout much of its natural range (Stoner, 1997; Acosta, 2002), so this finding of increased juveniles harvested through time was not unexpected. We expected to see this trend based on increased demand beginning around
the 1970’s though our geochronological bins did not have the resolution to detect such recent change. Increased demand led to a decrease in conch population densities across the distribution of the species’ range due to overfishing (Stoner et al., 2012c, Stoner et al., 2014, Acosta, 2006; Baker et al., 2016; Davis, 2003).

It should be noted that the sample size for the Medieval time bin is small (n=19). Thus, interpretations of significant increases in the proportion of harvested juveniles from Medieval to Modern bins must be considered with caution. Additional sampling of Medieval-aged sites would aid in our understanding of the proportion of harvested juveniles on San Salvador Island prior to the arrival of Europeans. The restricted occupation of morphospace during the Medieval time bin likely reflects the absence of harvested juvenile individuals from the Medieval bin. It is possible that these smaller specimens were preferentially destroyed during taphonomic processes, preferentially lithified in the rapidly cementing carbonate sediments, or the Lucayan people avoided hunting juvenile queen conchs. Therefore, we must carefully interpret any significant results from Medieval samples.

Additionally, further refinement of dates from the Modern time bin would be needed to gain a higher-resolution image of how the proportions of harvested juveniles differ through time. The modern time bin is a both a mix of older shells (>600 yrs) and newer post-thermonuclear weapons testing aged shells, while the Medieval time bin is decidedly Pre-Columbus in age and the Global is decidedly all post-weapons testing. Because of this mix of shells in the Modern, there is a temporal smearing of data. This smearing minimizes the effect of the pattern we see of an increased proportion of juveniles through time and forces us to adopt a conservative approach when interpreting
these results. Despite this potential mixing of ages, there is a significant increase in the proportion of harvested juveniles from the Modern to the Global bin attesting to the increasing pressure on the queen conch fishery on San Salvador Island through time.

Size of harvested shells through time - Given the complex growth patterns of this taxon, wherein the relationship between length and width differ dramatically before and after the onset of sexual maturity, such single variables are not reliable indicators of overall body size. The scores on the first principal component axis (PC1) are negatively correlated with all morphometric variables and are, therefore, an inverse proxy for body size. PC2 scores are interpreted as a size-free proxy for shape with positive scores indicating relatively wider shells and negative scores indicating relatively longer shells for a given size.

Overall, harvested shells increased in size from the Medieval to the Modern time bins and, as before, we interpret this increase with caution due to a limited Medieval sample size. However, harvested shell body size decreased from the Modern to Global time bins, consistent with increasing pressure on the fishery. It is, however, instructive to consider temporal trends in adult and juvenile body size and shape separately when estimating the impact of human predation on queen conch populations through time, especially when their proportion among the harvest changes significantly. Harvested adult shells were larger in the Modern and Global time bins than in the Medieval time bin (Fig. 5 and 6). Concordant with this increase in size, harvested shells became relatively narrower with a smaller aperture for a given size (as indicated by the decrease in PC2 scores), suggesting a decrease in nutritional content for a given prey size. Again, the
small sample size from the Medieval time bin should temper our temporal interpretations. Harvested juveniles decreased in size from the Modern to the Global and this result is consistent with an increase in pressure on the fishery wherein humans are collecting smaller prey items through time. Juvenile shells became relatively longer and narrower in shape from the Medieval to the Modern while their body size did not change significantly. Conchs collected from Barker’s Point (Medieval) were smaller, but had thick, heavy shells (shorter height, wider width), suggesting they came from a stable population of very mature individuals. As shells got larger from the Medieval to the Modern, it makes sense that shell shape for adults would be wider and shell shape for juveniles would be skinnier because lip thickness is related to shell width (Appeldoorn, 1988; Stoner et al., 2012a; Vermeij, 1980; Thiele, 2001; Thiele, 2005; Aranda and Frankeil, 2007; Mueller and Stoner, 2013; Foley and Takahashi, 2017) and juveniles do not have a thick, well-formed lip. The expected ontogenetic pattern would be for juvenile shells to have skinnier/narrower shells. Again, it should be noted that there were not many juvenile individuals sampled from the Medieval. Changes regarding shell size and shape changes through time are interpreted cautiously.

With more conchs being harvested, we would expect to see the size of shells to decrease in more recent time. The rationale being that as more, larger individuals are harvested, conchs reach their maximum size quicker and develop and reproduce at smaller sizes. Similar studies have identified the trend of more juveniles being harvested through time but with smaller shell sizes due to selection pressures (Shapira et al., 2009; O’Dea et al., 2014; Stoner et al., 2012b,c) to be common place. These other studies have found individuals are trending towards decreased body size through time with increased
fishing pressures. However, it must also be noted that similar studies focus on shell sizes in living populations while ours focuses on the shells which have already been harvested by humans. Thus, the presence of larger shells in the harvested population is not unreasonable as only the smaller shells are being left behind in the living population. As fishing pressures increased, larger shells were preferentially selected for harvest - regardless of whether they are adults or juveniles. In one study, fishers were asked after hauling in their catch whether they preferentially selected the larger individuals and they confirmed that they had done so (Randall, 1964).

It is well understood that shell lip growth begins after reaching maximum shell length and that maximum length varies among individuals (Randall, 1964; Appeldoorn, 1988; Posada et al., 1999; O’dea et al., 2017; Mueller and Stoner, 2013; Stoner and Sandt, 1992). Randall (1964) found that shells grow relatively quickly and the development of the lip starts at about one year of age. Although it is not well flared, the shell lip is still present and relatively well-formed at a fairly young age, despite the fact that conchs are not generally considered to be sexually mature until ~3-4 years of age (Randall, 1964). It has also been found that shell size and lip thickness tends to vary with geography (Randall, 1964; Foley and Takahashi, 2017). It is possible that differences observed in shell size is based on a number of abiotic factors and environmental conditions at different locations throughout the geographical range of the queen conch. Many species with large, extended distributions, often exhibit differences in size at maturity along temperature gradients (Irie et al., 2013) with higher temperatures causing earlier maturation at a smaller size. This is known as the temperature-size rule (Atkinson, 1994). This trend was observed in marine gastropods by Irie et al. (2013). However, there
is no evidence of this trend in the queen conch. Boman et al. (2018) recently explored this trend. They wanted to see if the variability in overall shell size and lip thickness at maturity would follow the temperature size rule. The results of this study found that temperature did not appear to be the driving factor behind variability in shell size. While they were not able to say whether the temperature-size rule applies to the queen conch, they did find that shell size does vary by geographic location – specifically that there is some degree of sexual dimorphism with females generally having larger shells and males generally having smaller shells. Other studies have also identified sexual dimorphism in queen conchs (Randall, 1964; Foley and Takahashi, 2017). Out of 171 individuals collected by Randall (1964), there were 84 females which averaged 209.9mm and 87 males which averaged 198.5 mm in length. This trend was observed in multiple different localities in the Bahamas. Additionally, Boman et al (2018) observed that lip thickness at the first onset of sexual maturity ranged from 2-12mm in females and 3-9mm in males. Furthermore, they found that lip thickness of individuals at which 50% or more of the population, females and males considered separately, was sexually mature was 7-14mm in females and 4-11.5mm in males. Females had a greater lip thickness at maturity and there was a significant difference between male and female lip thickness at maturity.

Therefore, it could be possible that some of the individuals we considered in our study and classified as juveniles based on 15mm lip thickness, are not actually juveniles. They could be sexually mature adults, albeit with thinner lips. It could also be possible that a majority of the shells we collected are females – based on sexual dimorphism – with females having larger shells than males, and that fishers are preferentially selecting larger shells for harvest. If fishers are selecting larger individuals preferentially, and the
majority of these larger individuals are females, they are removing them from the population leaving only the smaller males on the seafloor which would create an imbalance between proportions of females and males. Leaving more males than females in an area would thus limit reproductive capabilities. We had no way to know if the shells we collected were male or female, as that cannot be determined without studying the gonads of the snail itself. This could be a probable interpretation for the trend we see of larger individuals being harvested through time but not mature based on 15mm lip thickness. It is possible that some large shelled individuals identified as juveniles in our study are mature adults and 15mm is a conservative estimate for lip thickness at maturity.

**Recommendations for fisheries management** - While it is possible 15mm lip thickness as a proxy for maturity in the queen conch is a conservative estimate, lip thickness is also the best indicator of maturity available. Until more studies are done to definitively establish shell size and lip thickness at sexual maturity, 15mm is a good rule of thumb for fishers to follow. This metric, along with a closed season, are so far the best available practices for queen conch fishery management which should be adopted harmoniously across all Caribbean nations. Peak reproduction of conchs varies geographically (Appeldoorn and Baker, 2013) but the reproductive season occurs simultaneously across the vast majority of the Caribbean from May to September (Boman et al., 2018).

The Bahamas, a notoriously large exporter and consumer of the queen conch, currently has no closed season for harvesting but does state that harvested conchs must have a “well-formed shell lip.” The implementation of a closed season in the Bahamas and across the greater Caribbean would maximize protection of spawning sexually
mature adults in the greatest amount of locations. Increased educational outreach about the status of the queen conch in the Caribbean, as well as awareness and enforcement of fishing regulations and management practices and how they vary from nation to nation, would help ensure the health of the wild conch population for the future.

2.5 Conclusions

Despite some the obvious caveats of our study (i.e.: small sample size in the Medieval, lack of juvenile shells in the Medieval, and loosely constrained time scale in the Modern), we can conclude that there has been an increase in fishing pressure on the fishery at San Salvador, the Bahamas, through time. Overall, we observed an increase in shell size from the Medieval to the Modern with a decrease from the Modern to the Global. This trend is consistent with the pattern we would expect to see based on the preferential selection of larger shells for harvest which leaves the smaller shelled individuals behind in the living population. Over time, e.g. in the Global, humans harvested smaller prey individuals.

We also observed that temporal trends in shell size and shape based on length and width for all analyzed individuals through time are complex. The relationship between length and width differ dramatically both before and after the onset of sexual maturity. Therefore, variables of length and width considered alone are not reliable indicators of maturity. Right now, the most reliable indicator is lip thickness. Current regulations in the Bahamas only specify a thick and well-formed shell lip, a rather subjective measure. While this is the best indicator of maturity, it is also possible that it is a conservative estimate given the complex nature of growth patterns in _S. gigas_ and varying onset of
sexual maturity between male and female specimens and geographic region. Growth patterns of *S. gigas* are complex and must be further studied.

Lastly, we conclude that there is a greater proportion of harvested individuals that are juveniles in more recent time using 15mm lip thickness as the indicator of sexual maturity. Again, this is the trend we expect to see based on increased fishing pressures. The overharvesting of conchs due to increased demand and incohesive nature of fishing regulations across the Caribbean has led to stress on living populations which are already slow to mature and reproduce. Unifying fishing regulations across the entire Caribbean, specifically the explicit implementation and enforcement of a closed season from May to September and minimum shell lip thickness of 15mm, would help to relieve some of the stress on living populations and ensure this valuable species is around for many generations to follow.
2.6 Figures and Tables

Table 1: Summary table of *Strombus gigas* individuals collected from each locality and temporal bin assignment.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Time Bin</th>
<th>n</th>
<th>Adults</th>
<th>Juveniles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graham’s Harbour 2 (GH2)</td>
<td>Global</td>
<td>48</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>Pigeon Creek Mouth (PCM)</td>
<td>Global</td>
<td>34</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td>Pigeon Creek North Island (PCN-I)</td>
<td>Global</td>
<td>31</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Pigeon Creek North Kris Surface (PCN-K1-S)</td>
<td>Global</td>
<td>27</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Graham’s Harbour 1 (GH1)</td>
<td>Modern</td>
<td>24</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Pigeon Creek North Dock (PCN-D)</td>
<td>Modern</td>
<td>17</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Pigeon Creek North Kris Sub-surface (PCN-K1-SS)</td>
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<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Barker’s Point (BP)</td>
<td>Medieval</td>
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<td>7</td>
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Table 2: Summary statistics of *Strombus gigas* shell length (mm) for specimens with complete length preserved.

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<thead>
<tr>
<th></th>
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<th>n</th>
<th>Mean Length</th>
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</thead>
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<td>Global</td>
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<td>164</td>
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<td></td>
<td>Adults</td>
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<td></td>
<td>Juveniles</td>
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<tr>
<td>Modern</td>
<td>All</td>
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<tr>
<td></td>
<td>Adults</td>
<td>30</td>
<td>224.3</td>
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<td></td>
<td>Juveniles</td>
<td>48</td>
<td>221.2</td>
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<tr>
<td>Medieval</td>
<td>All</td>
<td>30*</td>
<td>183.3</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>14</td>
<td>189.5</td>
</tr>
<tr>
<td></td>
<td>Juveniles</td>
<td>13</td>
<td>186.4</td>
</tr>
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</table>

*Preservation quality precluded age class determination for three shells.*
Table 3: Summary statistics of *Strombus gigas* shell width (mm) for specimens with complete width preserved.

<table>
<thead>
<tr>
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<tr>
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<tr>
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<td></td>
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<td>Modern</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Juveniles</td>
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</tr>
<tr>
<td>Medieval</td>
<td>All</td>
<td>19</td>
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<tr>
<td></td>
<td>Adults</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Juveniles</td>
<td>7</td>
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</table>
Table 4: ANOVA and Tukey’s post-hoc test results comparing shell size by time bin and life stage of *Strombus gigas*.

<table>
<thead>
<tr>
<th></th>
<th>Length</th>
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<th>Width</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>All Specimens</td>
<td>Adults Specimens</td>
<td>Juvenile Specimens</td>
<td>All Specimens</td>
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<tr>
<td><strong>ANOVA p-value</strong></td>
<td>4.92e-10</td>
<td>8.96e-06</td>
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<td>0.028</td>
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<tr>
<td><strong>Tukey’s post hoc p-value</strong></td>
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<td>3.72e-04</td>
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<td>0.957</td>
<td>0.040</td>
<td>0.044</td>
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Table 5. Summary statistics of *Strombus gigas* PC1 and PC2 scores.

<table>
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<th></th>
<th>n</th>
<th>Mean PC1</th>
<th>Mean PC2</th>
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</thead>
<tbody>
<tr>
<td><strong>Global</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>137</td>
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<td>-0.081</td>
</tr>
<tr>
<td>Adults</td>
<td>52</td>
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<td>0.813</td>
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<tr>
<td>Juveniles</td>
<td>85</td>
<td>0.827</td>
<td>-0.628</td>
</tr>
<tr>
<td><strong>Modern</strong></td>
<td></td>
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<tr>
<td>All</td>
<td>65</td>
<td>-0.722</td>
<td>-0.086</td>
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<td>Adults</td>
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<td>Juveniles</td>
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<td>-0.411</td>
<td>-0.732</td>
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<tr>
<td><strong>Medieval</strong></td>
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<tr>
<td>All</td>
<td>16</td>
<td>1.132</td>
<td>1.044</td>
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<tr>
<td>Adults</td>
<td>10</td>
<td>0.667</td>
<td>1.301</td>
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<tr>
<td>Juveniles</td>
<td>6</td>
<td>1.908</td>
<td>0.616</td>
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Table 6: ANOVA and Tukey’s post-hoc test results comparing PC1/PC2 by time bin and life stage of *Strombus gigas*.

<table>
<thead>
<tr>
<th></th>
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<th>PC2</th>
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<tr>
<td></td>
<td>All Specimens</td>
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<tr>
<td>ANOVA p-value</td>
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<td>Tukey's post hoc p-value</td>
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<td>Modern-Medieval</td>
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<td>Global-Medieval</td>
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<tr>
<td>Global-Modern</td>
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<td>0.70</td>
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Figure 1: Map of seven sampling localities on San Salvador Island, The Bahamas. Collection localities are marked by yellow stars on the map: BP=Barker’s Point, GH1 = Graham’s Harbour 1, GH2 = Graham’s Harbour 2, PCN-D = Pigeon Creek North Dock, PCN-I = Pigeon Creek North Island, PCN-K1 = Pigeon Creek North Kris 1 (includes surface and subsurface), PCM = Pigeon Creek Mouth. Gerace Research Centre is marked by a green star. (Map data modified from Google Maps, 2018)
Figure 2: (left) Morphometric measurements recorded for each shell. A=Shell length (distance between the apex and the tip of the siphonal canal), B= shell width (maximum distance perpendicular to length at the last body whorl where the lip is thickest, not including spines), C=siphon to penultimate whorl, D= siphon to final whorl, E=spire length, G=aperture length, F=aperture width, H= shell lip thickness (recorded from the outer lip in the mid-lateral region approximately 30mm from the edge of the shell). (top right) Circular puncture hole typical of Medieval-aged shells found at Barker’s Point; likely made using the spire of another conch shell. (bottom right) Elongate puncture hole typical of Modern and Global aged shells; likely made using a hatchet or other tool.
Figure 3: Results of rapid AMS radiocarbon dating of *Strombus gigas* shells. Sample sizes are as follows: Barker’s Point (n=3), Graham’s Harbor (n=14), Graham’s Harbor 2 (n=2), Pigeon Creek Mouth (n=10), Pigeon Creek North Dock (n=10), Pigeon Creek North Island (n=5), Pigeon Creek North Kris 1 Sub-surface (n=10), Pigeon Creek North Kris 1 Surface (n=11).
Figure 4: Proportions of juvenile *Strombus gigas* harvested in each time bin. 95% confidence intervals calculated using the Agresti-Coull method.
Figure 5: Density plots of body size of *Strombus gigas* through time. Left column: shell length, right column: shell width right column, top row: all specimens, middle row: adult specimens; bottom row: juvenile specimens. Aquamarine = Medieval, Modern = Yellow, Gray = Global.
Figure 6 (previous page): Scatter and density plots of PCA of *Strombus gigas*. Left column: PC1 vs PC2 by time bin and loadings plot. Right column: PC2 vs PC3 by time bin and loadings plot. Gray = Global (1st row), Yellow = Modern (2nd row), Aquamarine = Medieval (3rd row). Solid circles = adult specimens, hollow circles = juvenile specimens.
CHAPTER 3

TRACE ELEMENT ANALYSIS OF HOLOCENE BIVALVES

*POTAMOCORBULA AMURENSIS AND CYRENODONAX FORMOSANA AS POTENTIAL PROXIES FOR TEMPERATURE AND PRODUCTIVITY IN THE PEARL RIVER DELTA, CHINA*

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3.1 Introduction

Marine bivalves can be used as a record of historic trace metal pollution and climatic variation because contaminants in the water are trapped within the shell growth increments during biomineralization of shell carbonate. Similar studies have shown that scleractinian corals and coralline sponges hold potential as proxies for environmental change because of their long-life span (Shen and Boyle, 1987; Shen et al., 1992; Readman et al., 1996; Lazareth et al., 2000; Ohmori et al., 2014;). Bivalves are comparatively short lived. Potamocorbula amurensis and Cyrenodonax formosana only have life spans of about 2-2.5 years (Thompson and Parchaso, 2012). Still, they prove useful in providing a record of long-term temporal environmental shifts. Extensive studies have been conducted on Potamocorbula amurensis, an opportunistic species of marine bivalve that resides in shallow water estuaries. Its presence in the San Francisco Bay as an invasive species has drawn attention in recent years (Carlton et al., 1990). Extensive studies have not been conducted on C. formosana, but it is also a highly opportunistic species.

The ability to interpret trace element concentrations in bivalve shell growth layers as environmental proxies would be progressive in determining paleoenvironmental histories. Shell taphonomy can be affected by depositional environment, as well as many other factors. The preservational quality of hard shelly fossils, and their taphonomic preservation quality, can inform us of the health of local ecosystems and intensity of anthropogenic changes (Kowalewski, 2009). When placed in the context of drill core that has been extensively analyzed and comprises a high-resolution, complete subfossil and sedimentological record – we can interpret the meaning of trace element concentrations
and how they compare to what was described in the fossil and sedimentological records (i.e.: see how trace element signals differ or agree in terms of interpreted environment throughout the evolution of the core).

Analyses were carried out with the goal of exploring and deciphering the relationships of trace element concentration signals throughout the Holocene between and among two bivalve species, *P. amurensis* and *C. formosana*, in the context of a drill core (PRD-10) from the central delta plain of China’s Pearl River delta (Figure 1). The PRD-10 core is a highly fossiliferous 24m long core sample drilled from the center of the Pearl River plain which includes a complete record of deposition from the Holocene. Ten environmental zones were described within this core based upon the combined paleoecological and sedimentological analysis. The most abundant faunal group fossils found in this core were bivalves, gastropods, foraminifera, and ostracods. Resolution potential for each group was determined by the effort that is put into data acquisition (picking out fossils, identification, sample preparation). Ostracods were found to be the most abundant and diverse faunal group in the core and reflect the highest resolution potential of all groups. This means that ostracods are also the only group representative of all environmental changes documented in the core based on paleoecology and sedimentology. However, ostracods and other microfossils, such as foraminifera, require considerably more preparation time, picking of fossils, and identification than do molluscan organisms. Based on resolution potential, it was determined that bivalves would be the most appropriate group to use for our current study. Two bivalve species, *P. amurensis* and *C. formosana*, were selected due to being the most abundant of the ten bivalve species present in the core. *C. formosana* accounted for 71.8% and *P. amurensis*
accounted for 25.7% of all bivalve species. The low species richness of bivalves present in the core and the high tolerance of these two most abundant species to environmental fluctuations only allows for the reconstruction of major changes in the delta. However, the ability to only reconstruct the major environmental changes in the delta made bivalves ideal for our particular study as we were interested in exploring the changes in trace element signals, and their use as proxies for temperature and productivity, between and among species and across each of the described temporal/environmental zones.

We proceeded with our study with the following research questions in mind: 1) How do the trace element concentration signals differ between and among the two bivalve species? 2) What do these data show given the faunal and sedimentological context of the PRD-10 drill core? 3) Can trace element data recorded in bivalves be used as reliable proxies for environmental change?

3.2 Materials and Methods

Sample Selection/Preparation - Thirty well-preserved valves (i.e. shiny, smooth, no parasitic traces) of *P. amurensis* and thirty valves of *C. formosana* were selected from eight temporal/environmental zones in the Holocene-aged PRD-10 drill core described by Alberti et al. (2013). Two valves each were selected from individual core samples and typically two core samples were selected from each temporal/environmental zone for the sake of repeatability. Eight valves were selected from seven of the eight zones. Only four valves were selected from zone C2 due to the low abundance of valves present. Two core zones, the lower most and upper most, were not sampled. The lower most zone in the
core consisted of subaerially-exposed Pleistocene deltaic sediments and the upper most consisted of flood plain deposits in which no bivalves were present.

Individual valves were embedded in epoxy resin under vacuum and cut along the midline from the umbo to the ventral margin using an Iso-Met low speed saw with diamond blade. One half of each valve was adhered to a 4.5cm x 2.5cm glass slide and gently sanded to the same height using a polishing table and 800 and 1600 grit paper. Photomicrographs of each valve were taken before embedding and sectioning.

*LA-ICP-MS* - Trace element data were acquired using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) at the University of Missouri Research Reactor facility (MURR). This method allows for high spatial resolution analysis of trace element composition across bivalve growth increments. The instrumentation consisted of a Photon Machines Excimer laser system operating at 298nm wavelength and a Perkin Elmer NexION 300X ICP-MS system. Individual samples were ablated from a 40µm diameter circle translated along a 40µm line (resulting in a 40 µm x 80 µm ellipse) at 50% of maximum laser power, a power density of approximately 3mJ/cm² on the sample surface, with a laser pulse duration under 1µs at a rate of 10 shots/second. Each analyzed spot was spaced approximately 100µm apart and collected across the entire axis of maximum shell growth from the umbo to the ventral margin. Gas flow rates were 0.9L/min for He carrier gas through the laser cell combined with 1.1L/min Ar gas. Total flow was then passed into the plasma to be ionized and then into the mass spectrometer.

Trace element data (including Al, Na, Si, Ca, Ba, Sr, Mg, Mn, Li, P, K, Fe, Ni, Cu, Zn, Pb, Ce, and U) were collected and calibrated using NIST 610 and 612 standard
reference materials. Peaks were isolated using a NexION data integrator program and counts (cps) were obtained for each element at each spot analysis. A total of 4,070 observations (1,920 P. amurensis, 2,150 C. formosana) were collected for sixty valves. The average counts at each spot for each element were then converted to concentrations (ppm) to establish element concentration ratios to Ca for determining the use of elements as environmental proxies. Counts for element isotopes were calculated by taking four channels on both sides (left and right) of the identified peak, summing the values of these channels and then dividing by eight. This value, which is representative of background noise, was subtracted from the values in each channel under the peak. The counts under the peak were then summed to obtain an average value for each element analyzed at each individual spot analysis. A conservative estimate for the limits of detection were calculated in the absence of blanks based on 0.5 times three times the square root of the minimum concentration value for each element analyzed.

Equation: \[0.5 \times \sqrt{3 \times \text{min element concentration}}\]

**Quantitative Analyses** - Trace element concentration data were binned by temporal/environmental zone and descriptive statistics were calculated for each element in each bin and core sample for both species. An initial principal components analysis (PCA) of Sr:Ca, Mg:Ca, Li:Mg, Ba, Zn, and Mn (with scaled variance values for all variables) was conducted as a number of potential outlier data points appeared to be asserting undue influence on the ordination. A multivariate regression model between Ca percentage and the aforementioned trace elements was utilized to calculate Cook’s Distance values for each observation. Observations with values greater than four times
the mean Cook’s Distance for all observations were identified as outliers. These outliers were subsequently removed from further consideration.

A second PCA was conducted on the trace element data with the outliers removed. A series of Wilcoxon tests, with Bonferroni corrected p-values ($\alpha = 0.05$; $\alpha_{\text{Bonferroni-corrected}} = 0.05/7 = .007$) were conducted on the median ordination values (PC1 and PC2) between adjacent temporal/environmental bins. Statistical analyses were performed in R with the following packages: “plyr”, “dplyr”, “psych”, “factoextra”, “lmodel2”, “ggbpubr”, “ggplot2”, “ggridges”, “PerformanceAnalytics”, “xts”, and “openxlsx” (R Core Team, 2016).

3.3 Results

A total of 4,070 individual spot analyses were conducted via LA-ICP-MS from 60 sectioned bivalve specimens (1,920 analyses of 30 $P.\ amurensis$ specimens and 2,150 analyses of 30 $C.\ formosana$ specimens; Table 1).

Median Ba concentration values were broadly similar between taxa, ranging 25.4 – 107.1 ppm for $P.\ amurensis$ and 32.6 – 94.7 ppm for $C.\ formosana$ (Tables 2 and 3), and their temporal trends are similar ($R_{\text{Pearson}} = +0.85$). Median Li:Mg ratios range 0.0014 – 0.0033 for $P.\ amurensis$ and 0.0018 – 0.0027 for $C.\ formosana$ and their temporal trends are not similar ($R_{\text{Pearson}} = -0.12$). Median Mg:Ca ratios range from 1.40e-4 – 2.75e-4 for $P.\ amurensis$ and from 1.25e-4 – 2.06e-4 for $C.\ formosana$. The temporal trends for Mg:Ca medians are moderately correlated ($R_{\text{Pearson}} = +0.52$). Median Mn concentrations range from 51.8 – 139.9 ppm for $P.\ amurensis$ and 41.8 – 134.3 ppm for $C.\ formosana$ and are strongly correlated ($R_{\text{Pearson}} = +0.92$). Median Sr:Ca ratios range from 0.0020 –
0.0047 among *P. amurensis* and between 0.0013 – 0.0023 for *C. formosana*. The median Sr:Ca ratio values display a weak correlation ($R_{\text{Pearson}} = +0.29$). Median Zn concentrations range from 21.5 – 46.9 ppm for *P. amurensis* and between 21.8 – 42.8 ppm among *C. formosana* and their temporal trends are moderately correlated ($R_{\text{Pearson}} = +0.48$).

The analysis of Cook’s Distance values led to the identification of 26 outlier individual analyses from *P. amurensis* and six outliers from *C. formosana* (Table 1; Fig. 3). The outliers were removed from consideration in the Principal Component Analyses, which we describe separately by taxon below.

*PCA results for *P. amurensis* -* A total of 1,920 observations at individual spot analysis level were recorded for *P. amurensis*. After removing 26 outliers, 1,894 individual spot analyses were used in the PCA (Table 1). Outliers were determined using Cook’s distance. In Figure 3, the red line represents four times the mean of all observations. Any observation falling above that line was determined to be an outlier and was excluded from the PCA.

PCA plots for individual bins B through G are found in Figure 4. The loadings plot for *P. amurensis* in Figure 7B shows the signals of each environmental variable considered in the PCA and its influence within the compositional hyperspace. On the loadings plot, Li:Mg is positively correlated with PC2 scores, with very slight influence on the PC1 axis. Mn is negatively correlated with PC2 scores with some influence on PC1. Mg:Ca, Ba, Zn, and Sr:Ca are most influential on the PC1 axis displaying a negative correlation with PC1 scores. The scree plot in Figure 6A shows the percentage
of variance explained by each of the six principal components. PC1 accounts for 31.9% of the variance and PC2 accounts for 17.7% of the variance. In all, PC1 and PC2 account for 49.6% of the total explained variance among the trace element concentrations and ratios.

To summarize the various signals recorded in the individual PC plots and determine significance between bins through time, median PC1 and PC2 scores were calculated for each bin and the statistical significance of their differences was determined using Wilcoxon tests (Table 4). Figure 7A illustrates the changes in the median values through time in the compositional hyperspace. B to C1 showed no significant change in either score. C1 to C2 showed displayed only a significant increase in PC1 score. C2 to C3 showed a significant decrease in both PC1 and PC2 scores. C3 to D showed a significant decrease on PC2 only. D to E showed a significant increase on PC2 only. E to F showed a significant decrease in PC1 and increase in PC2. F to G displayed a significant decrease on PC2 only.

**PCA results for C. formosana** - For *C. formosana*, a total of 2,150 observations at individual spot analysis level were recorded. Outliers were determined using Cook’s distance. In Figure 3, the red line represents four times the mean of all observations. Any observation falling above that line was determined to be an outlier and was excluded from further analysis. After removing 6 outliers, 2,144 individual spot analyses were used in the PCA (Table 1). Table 3 gives the descriptive statistics for the concentration values in each of the eight stratigraphic intervals for each of the trace element concentration/ratios (Mn, Zn, Ba, Li:Mg, Mg:Ca, and Sr:Ca) analyzed in the PCA.
PCA plots for individual bins B through G are found in Figure 5. The loadings plot in Figure 7D for *C. formosana* shows the signals of each environmental variable considered in the PCA and its influence within the compositional hyperspace. On the loadings plot, Li:Mg is negatively correlated with PC2 scores, with little to no influence on the PC1 axis. Mn is positively correlated with some influence on the PC2 axis. Mg:Ca is strongly positively correlated with the PC1 axis with little influence on PC2. Ba is strongly positively correlated with the PC2 axis with little influence on PC1. Zn is strongly positively correlated with PC1 with a slight negative correlation with PC2. Sr:Ca is positively correlated with the PC1 axis and negatively correlated with the PC2 axis. This loadings plot is nearly a mirror image of the loadings plot for *P. amurensis*. The scree plot in Figure 6B shows the percentage of variance explained by each of the six principal components. PC1 accounts for 28.3% of the variance and PC2 accounts for 19.4% of the variance. In all, PC1 and PC2 account for 47.7% of the total explained variance among the considered environmental variables.

To summarize the various signals recorded in the individual PC plots, median PC1 vs PC2 scores were calculated for each bin and the statistical significance in their median values was determined using Wilcoxon tests (Table 4). Figure 7B illustrates the changes in the median values through time in the compositional hyperspace. B to C1 shows a significant decrease on PC2 only. C1 to C2 displays a significant decrease on both the PC1 and PC2 axes. Scores for C2 to C3 showed significant increase on both PC1 and PC2 axes. C3 to D displayed a significant decrease in PC2 score. Zones D to E, E to F, and F to G all showed a significant increase in PC2 scores in the compositional hyperspace.
3.4 Discussion

Trace Elements as Environmental Proxies - Overall, the pooled trace element data for the two taxa, *P. amurensis* and *C. formosana*, were generally comparable. For both taxa, median Mn concentration values were most strongly correlated (*R*<sub>pearson</sub> = +0.92), with similar temporal trends. Median Ba concentration values were also strongly correlated (*R*<sub>pearson</sub> = +0.85) and display similar temporal trends between the two taxa. Both Mg:Ca (*R*<sub>pearson</sub> = +0.52) and Zn (*R*<sub>pearson</sub> = +0.48) concentration values display moderately correlated temporal trends. Temporal trends for Sr:Ca ratios are weakly correlated among the variables showing some similarity between the two taxa (*R*<sub>pearson</sub> = +0.29). Lastly, Li:Mg concentrations do not display similarity in temporal trends between the two bivalve taxa (*R*<sub>pearson</sub> = -0.12).

Temperature - Sr:Ca concentration of aragonitic organisms, such as scleractinian corals and coralline sponges, has been well recognized as an inverse temperature proxy (Saenger et al., 2008; Busch et al., 2015; Fowell et al., 2016; Lazareth et al., 2000; Rosenheim et al., 2004). The applicability of Sr:Ca and other environmental proxies can be problematic as the sensitivity of trace element concentrations differ between and among species relative to inorganically precipitated aragonite. Variations in all trace element concentrations can be due to many variables, including: shell growth rate, salinity, seasonality, and age of organism (Richardson et al., 2004; Cohen et al., 2006; Corrège, 2006).

Mg:Ca ratios, mainly in foraminifera and other calcitic organisms, has been established as a proxy that is strongly positively correlated with temperature. The amount
of Mg present in the shell increases with warmer sea water temperatures and increases exponentially between 0 and 30 degrees Celsius. Thus, higher Mg concentration would indicate warmer water temperatures when analyzed from calcitic organisms. However, there are complications with Mg:Ca as a paleothermometer. One such complication is that Mg is an impurity in calcite growth and can lead to shell dissolution (Davis et al., 2000). We interpret high Mg:Ca ratios to represent warmer water temperatures – but are cautious of this interpretation given that this is typically utilized from among calcitic organisms instead of aragonitic organisms.

Li:Mg has been shown to serve as a proxy for temperature with higher values indicating cooler water temperatures in aragonitic corals (Montagna, 2014). Montagna et al. found that Li:Mg shows a clear seasonal cycle with high values corresponding to the winter periods and vice versa. This ratio shows an exponential correlation with temperature which provides a robust tool for reconstructing paleo-seawater temperatures. Thus, it would be reasonable to infer that higher concentrations of Li:Mg would indicate a shift to cooler temperatures in our study.

*Productivity and Fluvial influence* - Ba and Mn have been reported to be potential primary productivity proxies (Lazareth et al., 2003; Elliot et al., 2009). Biological or primary productivity is the quantity of organic matter or its equivalent in dry matter or energy content which is accumulated in an area during a given period of time. Productivity results in increased nutrients in an area, sometimes characterized by blooms of phytoplankton.
Mn has also been reported to indicate fluvial/freshwater influence (Lazareth et al., 2003; Chester and Jickells, 2012). Chester and Jickells (2012) also indicate that Mn concentration is dependent upon salinity and pH. In estuaries, Mn concentration is controlled by the fluvial input of dissolved Mn(II), conversion of Mn(II) to Mn(IV) – oxidation, or reduction of Mn(IV) in the water column. Thus, Mn is sensitive to oxidation-reduction reactions. As oxidation is pH dependent, it is expected that there would be lower ppm of Mn at lower salinities, and higher ppm Mn at higher salinities.

*Heavy metals* - In previous studies, Zn concentration has been studied in bivalve soft tissues rather than in the shell (Pourang et al., 2013). Zn is a naturally occurring heavy metal. We analyzed this to get an idea of trace heavy metal trends. It could, potentially, be an indicator of anthropogenic activity. In estuaries, Chester and Jickells (2012) also state that Zn could be related to salinity where Zn concentration decreases with increased salinity as Zn is removed from the water column fairly quickly. They also state that Zn concentration could increase with turbidity, resulting in more particulate matter in the water column. This could potentially illustrate a higher energy environment given increased turbidity and Zn concentration.

*Temporal Trends as Revealed by the Trace Element Ordinations* - This study provides a high-resolution sedimentological and subfossil record of the Holocene from the Pearl River delta with the context of the sedimentology and faunal assemblage of the PRD-10 core (Figure 2) as described by Alberti et al. (2013). The core records early Holocene transgression as a result of the global sea level rise and subsequent intermittent
progradation of the Pearl River delta governed by sediment input and fluctuations in the rate of overall sea-level rise. When we apply our trace element data to the paleoenvironmental interpretation of the core, we are able to explore the use of macro-fossil bivalve taxa *P. amurensis* and *C. formosana* as records of paleoenvironmental change and their associated temporal trends in trace element concentration.

Keeping in mind that there are potentially major trace element concentration differences between and among different aragonitic bivalve species, we used the trace element interpretations described in the previous section to guide the exploration and preliminary interpretation of the compositional hyperspaces. To explore the signals seen in each species through time, we looked at how the median PC1 vs PC2 scores shifted through time for each bin for both taxa using the loadings plots and plots of median PC1 vs PC2 scores in Figure 5A-D.

*Bin B (9600 – 9300 cal yrs BP) to Bin C1 (9300 – 6800 cal yrs BP)* - Alberti et al (2013) interpret Zone B as an inundated flood plain marked by high species diversity and abundance, presence of seeds, and wood fragments. Remains of characean algae indicate stronger fluvial/fresh water influence in this zone. Sediments were deposited under relatively low energy conditions as evidenced by dark grey fine to medium sand grains grading to bioturbated silty clay and a high silt and clay content over all. By 9300 years, the core site was completely covered by the rising sea.

As sedimentology is highly variable in zone C from highly bioturbated clay-silt to silty fine sand with graded sand layers, it is divided into 3 subzones based on microfaunal assemblages. Subzone C1 is interpreted as a low to moderate water energy environment.
A slight increase in sea-level is interpreted here as is an increase in salinity/brackish conditions. This subzone is characterized by a short and rapid increase in ostracod diversity until it stabilizes towards the top of the subzone.

*P. amurensis* shows no significant difference between either the PC1 or PC2 score. Therefore, there is not much we can interpret about the proxies here. But, perhaps it could indicate stability in temperature and productivity between the zones. For *C. formosana*, only the decrease in median PC2 scores is statistically significant. In terms of the original trace element variables, this could indicate a shift from warmer to cooler temperatures if we consider Sr:Ca as a reliable temperature proxy in aragonitic bivalves.

*Bin C1 to Bin C2 (9300 – 6800 cal yrs BP)* - Subzone C1, the oldest subzone within Zone C, is interpreted as a low to moderate water energy environment with a slight increase in sea-level and in salinity/brackish conditions. This subzone transitions into subzone C2, the middle layer of zone C, characterized by three normally graded sand layers. C2 is interpreted as a high energy environment. This interpretation is supported by decreased macrofauna and the presence of coarser sediments. There are frequent phases of material reworking indicating pulses of transgression with moderately brackish waters (Alberti et al., 2013)

For *P. amurensis*, only the increase in median PC1 scores is statistically significant. This could indicate a shift to warmer temperatures and less influence from primary productivity events. *C. formosana* shows that the decrease in both in median PC1 and PC2 scores are significant. These signals correspond to decreased productivity and fluvial influence along with cooler water temperatures. Overall, the two taxa suggest
contrasting proxy histories for the transition from C1 to C2. *P. amurensis* indicates shifts to warmer temperatures and less influence from primary producers, while *C. formosana* indicates shifts to cooler temperatures and decreased productivity and fluvial influence.

*Bin C2 to Bin C3 (9300 – 6800 cal yrs BP)* - The pulses of transgression with coarser graded sand layers in Subzone C2 indicating a higher energy environment are not present in subzone C3. C3, the youngest layer in zone C, is characterized by high silt and clay content. This is interpreted as a decrease in water energy from C2 and increasing water depth. The peak of species diversity and abundance of microfauna is also seen in C3. This is suggestive of a stable paleoenvironment at peak transgression and salinity. Towards the top of this subzone, there is an increased freshwater influence accompanied by a decline in microfauna abundance and diversity (Alberti et al., 2013).

*P. amurensis* displayed significant decreases in median values of both PC1 and PC2 scores. The shifts in medians here are interpreted to indicate increased productivity, increased fluvial influence, and cooler water temperatures. *C. formosana* also shows statistical significance for both the PC1 and PC2 median scores from zone C2 to C3. Here, we interpret comparable trends between the two taxa. As seen in *P. amurensis* – increased productivity and fluvial influence as well as a shift to cooler water temperatures due to the loadings plots for each taxa being mirror images of one another. As indicated by Alberti et al.’s (2013) analysis of the PRD-10 core, the shift to cooler temperatures, as indicated by median PC scores, could correspond with cooler water temperatures and sea-level rise.
Bin C3 (9300 – 6800 cal yrs BP) to Bin D (6800 – 4200 cal yrs BP) - Subzone C3 transitions from low water energy, the period of maximum transgression and species diversity to Zone D, which is characterized by a decrease in microfauna abundance and diversity. Minor variations in microfaunal assemblages are explained by small scale sea level change and changes in freshwater influence throughout zone D. A constant decrease in sea level and salinity is assumed for the remainder of the PRD-10 core based on the macrofaunal assemblages and sedimentology. Fine sand sediments with interlayered bedding represent inter-tidal to shallow sub-tidal conditions and the influence of tidal currents record the progradation of the delta. Overall, observations in zone D signal the beginnings of a regressive trend (Alberti et al., 2013).

*P. amurensis* shows a significant decrease in median PC2 scores only. This could be interpreted to indicate increased fluvial influence. Conversely, *C. formosana* also shows a significant decrease in median PC2 indicating decreased fluvial and productivity influence.

Bin D (6800 – 4200 cal yrs BP) to Bin E (4200-3200 cal yrs BP) - Regression is observed as beginning in zone D and continuing throughout zone E. Zone E is mostly devoid of microfossils. Sediments in zone E indicate poorly sorted, ripple bedded coarse sands. These are indicative of a high energy environment, likely close to the mouth of the river where freshwater fluvial systems dominated. Macrofauna present here (bivalves) are known to be tolerant of freshwater environments (Alberti et al., 2013).

For *P. amurensis*, median PC2 scores show a statistically significant increase. This is interpreted as a shift to slightly cooler temperatures and less fluvial influence. *C.
Formosana also shows a statistically significant increase on PC2 only. However, this is interpreted as increased productivity and fluvial influence. Again, the two taxa exhibit competing paleoenvironmental histories based on trace element proxies. Given the increased fluvial influence interpreted by Alberti et al., the history reflected in C. formosana most aligns with the sedimentological interpretation of the core.

Bin E (4200-3200 cal yrs BP) to Bin F (3200 – 2300 cal yrs BP) - The high energy environment at the river mouth in Zone E transitions to Zone F, which is also interpreted as being strongly fluvial. The environment in Zone F is punctuated by occasional transgressions leading to short brackish intervals characterized by irregular microfauna occurrences. Irregular occurrences of macrofauna are indicative of fluctuating salinity levels from brackish to freshwater. Sediment in Zone F is mostly mud rich with forams and ostracods present indicating a brief transgression followed by partly ripple bedded fine to medium sand and silt interlayers (Alberti et al., 2013).

P. amurensis shows a significant decrease on PC1 and a significant increase on PC2 median scores. This indicates a shift to cooler temperatures and an increase in productivity and fluvial water influence. C. formosana shows a significant increase in median PC2 scores. Trends in C. formosana are also interpreted as a shift towards increased productivity and fluvial influence. Alberti et al.’s interpretation of the core indicates highly fluvial environments in both zone E and F punctuated by periods of brief transgressions and fluctuating salinity. Overall, both taxa address different parts of this interpretation. P. amurensis could account for transgressive periods with shifts to cooler temperatures. Signals in C. formosana account for increased fluvial influence. Together,
the signals between the two taxa are not completely comparable, but there are some similarities.

Bin F (3200 – 2300 cal yrs BP) to Bin G (2300 – 200 cal yrs BP) - Zone F is interpreted as strongly fluvial, punctuated by occasional transgressions leading to short brackish intervals. This is characterized by irregular microfauna occurrences – indicative of fluctuating salinity from brackish to freshwater with mud-rich sediment containing forams and ostracods. In zone G, remains of characean algae indicate stronger fluvial/fresh water influence. While fluvial influence is present, this zone is mostly tidal dominated as seen in interlayered, partly ripple bedded, and partly laminated fine sand bedding. In this shallow subtidal, moderately brackish environment, diversity and abundance of microfauna decreases drastically. The minute abundance of microfauna taxa which are present, are dominated mainly by those tolerant of brackish waters (Alberti et al., 2013).

*P. amurensis* and *C. formosana* both show significant decrease on PC2 median scores. Signals from *P. amurensis* indicate an increase in fluvial influence. Signals from *C. formosana* indicate a decrease in productivity and fluvial influence but shows a slight shift towards cooler temperatures. The decrease in microfauna could be due to anoxia related to primary productivity events. Signals for these taxa are largely comparable.

*Overall* - In some cases, there were strong similarities between trace element proxies and the context of the PRD-10 core as described by Alberti et al, but there were also cases where signals between the two taxa offered competing histories or were only moderately
comparable. For example, from zone B to C1, there were similar signals between the two taxa which were interpreted to fit the description from the core of a largely stable environment following initial flooding of the Pear River valley. Conversely, the median PC1 vs PC2 scores from zone C1 to C2 tell a different story. *P. amurensis* is interpreted to show shifts to warmer temperatures and less influence from primary productivity while *C. formosana* is interpreted to show the opposite trend. Moving further up the core into zone C2 and transitioning to C3, both taxa once again show similar paleoenvironmental histories between each other and are largely in agreement with the core interpretation of strong fluvial influence between these two bins accompanied by a stable environment and peak transgression in C3. Transitioning from C3 to D, the two taxa exhibit contrasting signals once again and offer opposing paleoenvironmental histories. From D to E, the two taxa offer opposing environmental histories, but signals recorded in *C. formosana* align with Alberti et al.’s (2013) interpretation of increased fluvial influence during this time. Signals between the bivalve taxa from E to F are comparable, but only moderately so. The taxa each fit with some part of the core interpretation of strongly fluvial environments in both zone E and F punctuated by periods of brief transgressions and fluctuating salinity, but neither tells the whole story. Lastly, proxy signals in both taxa for zones F to G are largely comparable and align well with the core description. In summary, there is no clear-cut distinction from the data collected in this study which indicate trace element proxy signals recorded in different species of aragonitic bivalves are *consistently* reliable recorders of paleoenvironmental histories.
3.5 Conclusion

The subfossil record of the Holocene has proven to be useful in providing high temporal resolution records of environmental change and biotic responses which helps bridge the temporal gap between short-term ecological and long-term geological studies (Kowalewski, 2009; Dexter, 2014; Huntley and Scarponi, 2012). Since bivalves have been shown to be reliable recorders of large-scale environmental changes (Gilikin et al, 2008; Alberti et al., 2013, Poulain et al, 2015) it was appropriate to use bivalves to reconstruct the major temporal environmental changes and the relationship of trace elements as proxies for these environmental shifts. Short life spans of species allow for a specific snap-shot in time of the Holocene. Given some degree of time averaging and the fact that bivalve growth increments do not necessarily always record complete annual growth records, we believe that the approach of analyzing multiple individuals of multiple taxa and pooling the results into temporal bins (rather than interpreting each little wiggle in an individual’s ontogenetic series) is the best approach for reconstructing environmental change in the Holocene PRD-10 core via bivalve sclerochemistry.

In our study, we see that trace element signals are variable between *P. amurensis* and *C. formosana*. In some cases, there are strong similarities between the two taxa when interpreted in the context of the PRD-10 core. In other cases, the geo-chemical signals in one taxon described the trends seen in the core better than another. Furthermore, there were times when the signals between the two bivalve species offered polar-opposite interpretations of paleoenvironmental histories. While significance among trace element signals as indicated by median PC1 vs PC2 scores might initially inspire some assurance that the trace element signals recorded in these two distinct species are comparable – we
must keep in mind that signals between and among aragonitic bivalve species are highly variable which likely explains the variation we see between the two species in our study.

In conclusion, this is a preliminary exploratory study and any similar, or perhaps more importantly, dissimilar trace element trends in these two taxa should be further evaluated for repeatability with additional samples. Given these preliminary trace element data and the sedimentological and faunal context of the PRD-10 core, we have made progress in determining the applicability of aragonitic bivalve sclerochemistry as recorders of paleoenvironmental change throughout the Holocene.
3.6 Tables and Figures

Table 1. Number of LA-ICP-MS spot analyses, number of analyses utilized in PCA, and number of outliers removed from each temporal bin for *Potamocorbula amurensis* and *Cyrenodonax formosana*.

<table>
<thead>
<tr>
<th>Bin</th>
<th>n</th>
<th>nPCA</th>
<th>nOutliers</th>
<th>Bin</th>
<th>n</th>
<th>nPCA</th>
<th>nOutliers</th>
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<tr>
<td>G</td>
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<td>17</td>
<td>G</td>
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<td>202</td>
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<td>F</td>
<td>270</td>
<td>270</td>
<td>0</td>
<td>F</td>
<td>340</td>
<td>340</td>
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<td>E</td>
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<td>253</td>
<td>7</td>
<td>E</td>
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<td>D</td>
<td>280</td>
<td>279</td>
<td>1</td>
<td>D</td>
<td>345</td>
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<td>1</td>
<td>C3</td>
<td>270</td>
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<td>26</td>
<td>sum</td>
<td>2150</td>
<td>2144</td>
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Table 2. Descriptive statistics of trace element concentration/ratio values of *P. amurensis* in each of the eight stratigraphic intervals.

<table>
<thead>
<tr>
<th>Bin</th>
<th>Ba (ppm)</th>
<th>Li:Mg</th>
<th>Mg:Ca</th>
<th>Mn (ppm)</th>
<th>Sr:Ca</th>
<th>Zn (ppm)</th>
</tr>
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<tr>
<td>G</td>
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<td>67.1</td>
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<td>0.0129</td>
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<td>1.04e-4</td>
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<td>F</td>
<td>69.8</td>
<td>41.3</td>
<td>0.0017</td>
<td>0.0102</td>
<td>2.61e-4</td>
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<tr>
<td>E</td>
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<td>29.4</td>
<td>0.0024</td>
<td>0.0020</td>
<td>1.40e-4</td>
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<tr>
<td>D</td>
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<td>33.0</td>
<td>0.0020</td>
<td>0.0033</td>
<td>1.81e-4</td>
<td>7.41e-5</td>
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<tr>
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<td>58.1</td>
<td>0.0024</td>
<td>0.0115</td>
<td>1.46e-4</td>
<td>1.09e-4</td>
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<tr>
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<td>25.4</td>
<td>20.6</td>
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<td>0.0021</td>
<td>1.43e-4</td>
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<tr>
<td>C1</td>
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<td>52.2</td>
<td>0.0027</td>
<td>0.0040</td>
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<tr>
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Table 3. Descriptive statistics of trace element concentration/ratio values of *C. formosana* in each of the eight stratigraphic intervals.

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<th>Bin</th>
<th>Ba (ppm)</th>
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<th>Mg:Ca</th>
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<td>SD</td>
<td>Median</td>
<td>SD</td>
<td>Median</td>
<td>SD</td>
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<tr>
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<td>E</td>
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<td>133.6</td>
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<td>0.0024</td>
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<tr>
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Table 4. Wilcoxon test $p$-values comparing median PC scores between adjacent temporal bins for *P. amurensis* and *C. formosana* ($p_{\text{Bonferroni corrected}} = .007$, ns = non-significant).

<table>
<thead>
<tr>
<th>Bin Transition</th>
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<th>PC2</th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td><em>P. amurensis</em></td>
<td></td>
<td><em>C. formosana</em></td>
<td></td>
</tr>
<tr>
<td>F-G</td>
<td>ns</td>
<td></td>
<td>2.5e-09</td>
<td>0.0005</td>
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<tr>
<td>E-F</td>
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<td>ns</td>
<td>2.1e-07</td>
<td>2.0e-16</td>
</tr>
<tr>
<td>D-E</td>
<td>ns</td>
<td></td>
<td>7.8e-10</td>
<td>5.5e-05</td>
</tr>
<tr>
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<td>ns</td>
<td></td>
<td>2.0e-16</td>
<td>2.0e-16</td>
</tr>
<tr>
<td>C2-C3</td>
<td>5.0e-06</td>
<td>1.1e-09</td>
<td>2.7e-10</td>
<td>2.0e-06</td>
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<tr>
<td>C1-C2</td>
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<td>2.0e-16</td>
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<tr>
<td>B-C1</td>
<td>ns</td>
<td></td>
<td>ns</td>
<td>8.7e-12</td>
</tr>
</tbody>
</table>
Figure 1: Map showing position of the PRD-10 drill core in the Pearl River delta. The PRD-10 core is a highly fossiliferous 24m long core sample drilled from the center of the Pearl River plain which includes a complete record of deposition from the Holocene (Modified from Alberti et al., 2013)
Figure 2: Holocene evolution of the PRD-10 drill core showing temporal/environmental zonation determined from comparison of sediment properties, macro-, and micro-faunal assemblages. For our study, individual valves were sampled from zones B – G. (Modified from Alberti et al., 2013)
Figure 3: Cook’s Distance plot for *Potamocorbula amurensis* and *Cyrenodonax formosana*. The red line represents four times the mean of all observations for each taxon.
Figure 4. Scatter and density plots of PC1 v. PC2 for *P. amurensis* through the temporal bins. The orange triangle indicates the position of the median PC1 and median PC2 scores.
Figure 5. Scatter and density plots of PC1 v. PC2 for *C. formosana* through the temporal bins. The orange triangle indicates the position of the median PC1 and median PC2 scores.
Figure 6: Scree plot for *P. amurensis* showing the percentage of variance explained by each of the six principal components. PC1 accounts for 31.9% and PC2 accounts for 17.7% of the variance.
Figure 7: A) changes in median PC1 vs PC2 scores through time for *P. amurensis*. B) loadings plot for *P. amurensis*. C) changes in median PC1 vs PC2 scores through time for *C. formosana*. D) loadings plot for *C. formosana*. 
CHAPTER 4

PROPER PROCEDURE FOR TRACE ELEMENT ANALYSIS USING
LA-ICP-MS

Mikaela Ruga

Department of Geological Sciences, University of Missouri, Columbia, Missouri 65211, USA
4.1 Introduction

When done correctly, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a powerful analytical tool for collecting trace element data. Even when done incorrectly, you can still make it work – as I learned with much frustration over the course of the project described in Chapter 3. The purpose of this fourth chapter is to assist those who come after me in the proper data collection procedure.

I made numerous mistakes in acquiring trace element data. This resulted in much frustration for myself and anyone who had the pleasure of assisting me in salvaging the data. In the end, it all worked out and we were able to re-claim the collected data. The process of salvaging this data consumed a lot of time that could have been directed towards other tasks had the data been collected correctly in the first place. However, I do seem to love making things more difficult than they need to be so simply doing things correctly the first time around just isn’t in my nature. In the following sections, I discuss where I went wrong with my data collection (section 4.2), how we fixed it (section 4.3), and how it should have been done in the first place (section 4.4).

4.2 How NOT to do LA-ICP-MS

During the data collection process, several key mistakes were made. One mistake was not leaving room on the slides for the standards – so they are in the chamber at the same time as the unknowns we wanted data for. I analyzed the standards on a separate slide, took that slide out, and then inserted a slide with the unknowns and ran those. This might have worked out alright had I analyzed the standards again after taking out one
slide of unknowns and before inserting another into the sample chamber. I did not do this. I also failed to analyze the standards at all during a few days of analysis due to misunderstanding of instructions. All in all, the bulk of my troubles stemmed from me not really understanding the data collection process and the importance of analyzing standards frequently.

It is important to analyze standards frequently (after every 3 or 4 unknowns) to account for any drift the instrumentation experiences during the day. I also learned that the NIST 610 and 612 glass standards are probably not the best to use for trace element analysis of aragonitic bivalves. They will work just fine, but they are not the best standards to use. The best standard to use is an aragonite standard (such as the MACS-3 synthetic calcium carbonate pressed powder) sold by the USGS in Denver, CO. It might be helpful to obtain this standard and use it instead of the NIST 610 and 612 standards for any future work involving trace element analysis of aragonitic bivalves. It is also necessary to have two standards to compare the unknowns to in order to create a calibration curve to use during data processing.

Yet another miss-step was my failure to analyze blanks. A blank is when the laser is off but the ICP and sweep gas are on and data are recorded. Blanks are important in calculating limits of detection and must be recorded. Because of my failure to educate myself on this process prior to starting analyses and my failure to properly analyze the standards, there was much confusion when it came time to process the data. A 2001 publication by Gratuze et al. titled “Mass Spectrometry with laser sampling: A new tool to characterize archaeological materials” is a good place to start when learning how the
LA-ICP-MS process works.

4.3 How we Salvaged the Data

The following is the procedure we used to salvage the incorrectly collected data. Without having collected blanks and having analyzed standards in a most haphazard fashion, we had to greatly modify the data processing procedure in order to convert element counts to concentrations. This modified procedure included creating new standards from previously analyzed unknowns, bootstrapping and converting elements to oxides and then calculating concentrations in ppm, and finally calculating conservative values for the limits of detection for each day in the absence of blanks.

Reprocessed Data – We reanalyzed and reprocessed a subset of 15 of the originally analyzed shells. Each one of these 15 shells represented any time there was a slide change (aka, the sample chamber was opened) on a given date. For each of these 15 “reanalyzed” shells, five line scans were made. Each of the 15 reprocessed samples corresponded to a specific date and slide and were used as new standards to which the originally (incorrectly) collected LA-ICP-MS data were compared. The list that follows is the procedure that was used to create new standards from the reprocessed data:

1. Use NEXION data integrator to isolate peaks and obtain means of counts for each element for each reprocessed sample (one from each day and slide change – 15 shells total) and the NIST 610 and 612 Standards which were analyzed before the unknowns (there were 4 unknowns on each slide) and again after the unknowns were analyzed. The 15 reprocessed samples will become the new standards to which the original collected data will be compared. Using the new standards, we
were able to calculate concentrations (ppm) for the original data (analyzed incorrectly) for each element for which data were collected when the samples were reanalyzed. Data had to be reanalyzed because I failed to run the standards each time I loaded a new slide.

2. Convert element counts to oxide counts. To do this, first find the isotopic abundance ratio for each element – Table 1 in the blue “Tables for analytical methods at MURR” book. Next, find the element to oxide conversion factor – Table 60 in the blue “Tables for analytical methods at MURR” book. Then, match each element-isotope with its appropriate corresponding oxide – also Table 60 in the blue “Tables for analytical methods at MURR” book. Finally, once all values have been put into spreadsheet, do the following formulas for each element for each sample:

\[(\text{element counts} \times \text{element to oxide conversion factor} / \text{isotopic abundance ratio})\]

3. Group all the 610 oxide values together. Create one overall mean value of oxide counts for the 610 standard.

4. Group all the 612 oxide values together. Create one overall mean value of oxide counts for the 612 standard.

5. Copy the mean oxide count values for each sample so all the oxide counts (for the unknowns and for the 610 and 612 standards) are together on a single excel sheet.

6. Normalize Oxide counts to CaO. To do this, take the average for the oxide counts for each individual oxide divided by the oxide counts for CaO for each sample:

\[(\text{oxide counts} / \text{CaO counts})\]
7. Consensus Standards - These values came from some spreadsheet Mike had. I’m not entirely sure where they came from. They represent standards with known concentration to which we can compare our reanalyzed values for 610 and 612. Using the reanalyzed 610 and 612 values, we can see if our values are similar to the consensus values and know if the conversion method (to create the new standards for each slide and for the conversion method to ppm) is reliable. Our values turned out to be similar, so this method should be okay.

8. Convert consensus standard values from element concentration to oxide concentration. To do this, take:

   \[(\text{oxide counts normalized to CaO} \times \text{consensus standard value for Ca}) / \text{consensus standard value for element}\]

9. Next, take:

   \[(\text{Oxide counts normalized to CaO} / \text{Average for the 610 and 612 values})\]

   This average creates a calibration curve for the NIST 610 and 612 standards.

10. Create a sum column for each sample. After completing step 9, create a sum column hold the sum of the values for each element for each sample (sum the values across the column).

11. Convert reanalyzed samples to final ppm concentration. These will be used later as new standards when converting the original data. To do this, take:

   \[(\text{value calculated in step 9} \times 10^6) / \text{Sum for sample from step 10}\]

Converting original data to ppm using “new” standards - The procedure which follows is the procedure we used to convert the originally (incorrectly) collected data from counts.
(cps) to concentrations (ppm). This procedure is similar to the one outlined above for creating new standards from the reprocessed data.

1. Use NEXION data integrator to isolate peaks and obtain counts for each element for each individual spot analysis. (in total, there are data for ~4070 individual spot analyses)

2. Place these “Original counts” in the first sheet of the excel doc. This sheet contains the sample name with spot analysis number and each element for which data were collected originally. Some of these elements are not even capable of being detected. Some elements that were important to analyze were not included in the data collection. There are many more elements which were originally collected than were used in calculating the concentrations with the new Standards because we did not use the same elements for the reanalysis as we used for the original analysis. For the reanalysis, we included the elements that should have been analyzed in the first place.

3. Place original counts in another new spreadsheet with in the excel doc. This sheet is titled “New Counts.” After pasting these original counts again, delete the elements that were not analyzed when we ran the reanalysis samples. The elements contained in the header should be: Li, Na, Mg, Al, Si, P, K, Ca, Mn, Fe56, Fe57, Ni58, Ni60, Cu, Zn64, Zn66, Sr, Mo, Ba, La, Ce, Pb, and U. Some of these elements were not included when samples were originally analyzed so the columns for these elements contain no values. These are the elements which will be used to calculate the concentrations. Also include the isotopic abundance ratio,
element to oxide conversion factor, and the oxide counts and element
concentrations for the New standard associated with these samples on this sheet.

4. Create a Third sheet in the excel doc and call it “Conversions.” This is where all
the conversions will be done in a similar manner as described above for the
reprocessed data.

5. In the “Conversions” sheet:

   a. Paste the cps values (counts) from the “new counts” sheet.
   
   b. Paste the isotopic abundance ratios for each element and the element to
      oxide conversion factors for each element from the “new counts” sheet
   
   c. Convert element counts to oxide counts for each individual spot analysis.
      Use the same procedure as described above in step 2 for the reprocessed
      data.
   
   d. Normalize the oxide counts to CaO for each element for each individual
      spot analysis. Use the same procedure as described above in step 6 for the
      reprocessed data.
   
   e. Paste the ppm concentration for the standard associated with this analysis
      data and slide ID.
   
   f. Paste the normalized oxide counts for the standard associated with this
      analysis date and slide ID.
g. Normalize the standard and convert it from element concentration to oxide concentration. Use the same procedure as described above in step 8 for the reprocessed data.

h. Take the Normalized oxide counts divided by the normalized standard. Use same procedure as described above in steps 9 and 10 for the reprocessed data.

6. Calculate the final ppm concentration for each element at each spot analysis for each sample. Use same procedure as described above in step 11 for the reprocessed data.

7. Finally, make a 4th sheet in this excel doc and title it “Final.” Add headers for date analyzed, slide ID, sample ID, and for each element/oxide. The data is now converted to ppm and ready to be analyzed.

**Calculating Limits of Detection** – The procedure which follows is the procedure we used to calculate the limits of detection. This provided a very conservative estimate of the limits of detection in the absence of blanks.

1. Using the NEXION data integrator, load the raw "rep" data file into the integrator to view the raw data counts (cps)

2. Identify and isolate peaks and resulting counts values for each element for each spot analysis for each sample

3. Convert element counts (cps) values to oxide concentrations (ppm)

4. Delete any value that is negative.

5. Calculate the minimum value for each element
6. Take \((\sqrt{3 \times \text{minimum value from step 5}})\)

7. Take \((0.5 \times \text{value from previous step})\)

8. Paste the resulting values in a new spreadsheet and label according to sample.

9. Create an overall Average for the Limits of Detection. These are your conservative estimates for the limits of detection.

4.4 Proper Procedure for LA-ICP-MS Data Collection

The following is the analytical procedure for correctly collecting LA-ICP-MS data:

1. Select samples to analyze. These are your “unknowns.” For example, the original unknowns for this study were 60 Pearl River bivalve cross sections (30 \(P. amurensis\) and 30 \(C. formosana\)) set in epoxy and mounted on glass slides.

2. Select the standards you want to use. Standards are samples with known amounts of elements you want to analyze. You will compare these concentrations to the concentrations of your unknowns to provide a reference point for data analysis. We used the NIST 610 and 612 standard reference materials. However, for shells, the MACS-3 synthetic calcium carbonate standard from the USGS might be better suited for these types of analyses. The 610 and 612 standards will work just fine, but they are not the best.

3. Identify the elements you want to analyze. You must analyze the “Big 4” elements if using the 610 and 612 standards. These elements are: Al, Ca, Na, Si. These elements need to be analyzed because they account for 53.6 % of the concentrations of all elements in the glass standards. You need, at the least, these four elements to be analyzed in both the standards and the unknown samples you are interested in in order to obtain useable and high-quality data. For these four
elements, measure the lowest abundance isotope to prevent from flooding the instrumentation with the element signal. You want to analyze the following elements for trace element data: Ba, Sr, Mg, Mn, Li, P, K, Fe, Ni, Cu, Zn, Pb, Cs, Ce, and U. The elements you want to analyze must be present in the standard in some concentration, but are not necessarily present in the unknowns…that is what you are trying to find out.

4. Mount unknown samples on a 4.5cm x 2.5cm glass slide, sand them to the same height, and leave room for the standards on each slide. This is necessary because the standards need to be on the same slide as the unknowns in order to properly relate the trace element data for each run. The standards can be adhered with putty so they can be easily transferred from slide to slide.

5. Samples are now ready for analysis at MURR. Assistance to set up the laser with the appropriate operating conditions is provided by a MURR technician upon arrival in the lab. The technician will also assist in turning off the machine. Once the conditions have been set, elements to collect data for have been selected, it is time to start collecting data.

6. Place the first slide, with standards, into the instrument sample chamber. Once the slide is in the instrument chamber:

   a. Analyze a “blank.” For a blank, the laser is off but the ICP is on and data are recorded. Blanks will be used to calculate limits of detection later on during data analysis.

   b. Analyze the standards (5 scans on 610, 5 scans on 612)
c. Analyze the unknowns (run one set of 5 scans at a time)

d. Analyze another blank

e. Analyze the standards again

f. Remove current slide

g. Insert the next slide and repeat the steps above for all remaining slides

The following is the proper procedure for calculating limits of detection if blanks are properly analyzed:

1. Using the NEXION data integrator (from Barry) load the raw ".rep" data file into the integrator to view the raw data counts (cps)

2. Locate the data collected for the “blanks”. Blanks should be labeled as such.

3. Sum up all of the channels in the blank where a peak would have been if the laser were on and collecting data. These summed up channels are treated the same as if a peak were present. Counts will be many times lower than if you summed an actual data peak which was collected with the laser firing. When counts are converted to concentration, they will likely be much closer to ppb than ppm.

Following the procedures outlined above will save time, energy, and frustration when it comes time to analyze the trace element data and convert data from element counts (cps) to concentrations (ppm). Failure to follow this procedure upfront will surely result in numerous headaches and time spent attempting to salvage the incorrectly collected data. Any questions about the correct procedure for LA-ICP-MS acquisition and analysis should be directed to Dr. Michael Glascock or whoever the current LA-ICP-MS
lab supervisor happens to be.
REFERENCES CITED


