



Rangia Clam Investigations

By

Bryan A. Black – University of Texas Marine Science Institute Marty Heaney – Bio-West Inc.

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EXECUTIVE SUMMARY

This study was conducted as a result of the Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area Stakeholder Committee (GSA BBASC) selecting this study in accordance with its work plan, to utilize funds appropriated by the 83rd Texas Legislature and administered by the Texas Water Development Board. Additional funds and support were provided by the San Antonio River Authority. The purpose of the study was to develop methodology using side-scan sonar to map *Rangia* clam locations and to use growth-increment analysis to identify environment-growth relationships.

The San Antonio River Authority acted as administrator for the study, with two sub-contractors conducting the study. Bio-West Inc. Coastal Division conducted the remote sensing surveys and *Rangia* collection in San Antonio Bay and the University of Texas Marine Science Institute conducted the growth-increment analysis and environment-growth relationships of the *Rangia* clams. As a result, section 3 of this report describes the mapping of *Rangia* and the collection of live *Rangia* clams and was written by staff at Bio-West Inc. and section 4 was completed by the University of Texas Marine Science Institute.

The side-scan sonar survey revealed the presence of *Rangia* shells in the study area (Mission Lake and Guadalupe Bay). Ground-truthing verified the *Rangia* shell signatures, but only small numbers of live *Rangia* clams were found in Mission Lake, and no live *Rangia* clams were found in Guadalupe Bay.

The relatively few live *Rangia* clams found coupled with the short lifespan of the live *Rangia* hindered the building of a chronology and correlating growth and recruitment with environmental conditions. In an effort to draw conclusions about the *Rangia* living in Mission Lake, the *Rangia* data was compared to similar work being conducted in Trinity Bay and Sabine Lake. While Mission Lake and Sabine Lake are both identified as "Lakes" they are in fact salt water estuaries. Findings for this report about the *Rangia* clam include:

- Living *Rangia* are presently uncommon in the three systems sampled for this study, which limited sample sizes for this present analysis.
- Living individuals tend to be young, especially in comparison to dead-collected individuals from Mission Lake approaching 20 years in age.
- Living shells do not show any unusual signs of growth stress compared to their dead counterparts.
- There was a significant east-to-west gradient in growth rates across sites; Sabine was slowest followed by Trinity and then Mission.

Section 1 Executive Summary

- After age-related growth declines were removed, synchronous patterns were evident among individuals within each site, indicating that some aspect of environmental variability was influencing growth.
- Some degree of synchrony was also evident among sites (chronologies), with, for example, unusually strong growth in 2012.
- At each site, growth chronologies positively correlated to prior fall and winter salinity, the time of year in which salinity tends to be highest. The nature of this relationship was remarkably similar across the three sites.
- No clear relationships were evident with other environmental indicators.
- Recruitment histories were short and coarsely defined due to small sample sizes. However, there were years of above-average recruitment, and these were generally preceded by periods of high freshwater inflow.

To better understand how environmental factors impact the growth and recruitment of *Rangia* clams in San Antonio Bay, future studies could address the following:

- Living *Rangia* were uncommon in the upper-bay areas sampled for this project. Subsequent sampling in the Nueces River at Labonte Park did, however, produce a large number of living and relatively old (>15 yr.) individuals. *Rangia* surveys could therefore be conducted in rivers to better locate long-lived individuals and identify how environmental variables drive growth.
- Mussels are native to south Texas rivers and could be linked to environment as well as *Rangia* chronologies in the lowest river reaches, providing multi-species perspectives on freshwater variability and ecosystem response.
- There is a very high probability of a potent El Niño in winter 2015 and early spring 2016, which is likely to bring anomalously wet conditions to south Texas. This could be an excellent opportunity to monitor *Rangia* recruitment in upper bays, and to determine what impacts, if any, a wet year will have on establishment of new individuals.
- *Rangia* in Native American middens could provide long-term records of growth and environmental variability. Although exactly dated year-by-year chronologies could not be developed, *Rangia* could be roughly placed in time (±20 years) using 14C dating. Any long-term trends in growth would be evident, providing a history that is likely to span multiple centuries.
- The longer-term context for interpreting the severity of recent droughts remains poorly described in south Texas. In central Texas a network of tree-ring chronologies has been used to benchmark pre-industrial conditions, establish historical ranges of variability, and thereby assess the extent to which modern droughts are exceptional in the longer-term record. Expansion of the existing tree-ring network into south Texas would open many new possibilities to reconstructing (hind-casting) river discharge into estuaries over the past few

centuries. Indeed, blue oak, which is sensitive to precipitation, was used to hind-cast salinity in the San Francisco Bay over multiple centuries, and with a high degree of accuracy

Section 2 Acknowledgments

Without the following contributors, this project would not have been possible:

The members of the Guadalupe, San Antonio, Mission, and Aransas Rivers and Mission, Copano, Aransas, and San Antonio Bays Basin and Bay Area Stakeholder Committee (GSA BBASC) selected this study in accordance with its work plan, to utilize funds appropriated by the 83rd Texas Legislature and administered by the Texas Water Development Board. Additional funds and support were provided by the San Antonio River Authority.

Bryan A. Black PhD. –Principal Investigator Assistant Professor, Department of Marine Sciences University of Texas Marine Science Institute

Marty Heaney – Principal Investigator Company Principal/ Senior Marine Biologist Bio-West Inc.

Steven J. Raabe P.E – SARA Project Manager Director of Technical Services San Antonio River Authority (SARA)

Carla G. Guthrie PhD. – TWDB Project Manager Team Lead Bays & Estuary Program Texas Water Development Board (TPWD)

Rebecca Reeves Senior Quality Control and Monitoring Scientist San Antonio River Authority



INTRODUCTION

At the request of the San Antonio River Authority (SARA), BIO-WEST, Inc. (BIO-WEST) participated in an investigation and assessment of the presence and distribution of Atlantic *Rangia* (*Rangia cuneata*) colonies in upper San Antonio Bay, Texas. Environmental flow standards adopted for the Guadalupe and Mission-Aransas Estuaries have relied heavily on reproductive requirements for *Rangia* clams. This approach was based on the literature from studies in other states, as well as distribution and abundance patterns for *Rangia* clams based on Texas Parks & Wildlife Department (TPWD) reports of incidental catch. However, it was recognized that there has not been any species-specific studies to document *Rangia* presence, distribution or life history within this estuary.

Therefore, the purpose of this project is to validate these salinity and freshwater inflow requirements by locating and mapping *Rangia* populations, collecting representative specimens and then using shell ring widths to develop growth and recruitment histories in response to salinity. This was accomplished by implementing two tasks identified as 1) Stakeholder Workshop, 2) Remote Sensing Survey and Collection. The following report provides a summary of the methodology implemented followed by illustrations, results and a discussion of the findings.

The project area focused on the Guadalupe River estuary complex, which encompasses the upper end of the San Antonio Bay system. Included within this system are multiple smaller bays and estuarine lakes that are directly influenced by the Guadalupe River. These smaller bays include Mission Lake, Guadalupe Bay and Hynes Bay.

Stakeholder Workshop

On August 6, 2014, a preliminary stakeholder workshop was held to solicit natural resource information from local commercial fishermen. Many commercial fishermen (including shrimpers and oystermen) provide an extremely valuable historic record on targeted as well as non-targeted species including *Rangia*. Several local fishermen identified areas of potential live *Rangia*, as well as areas where *Rangia* were once very abundant but have declined drastically over the years. Several locations of live *Rangia* were noted in Mission Lake, and several areas of previously live *Rangia* beds were noted within Guadalupe Bay, Hynes Bay, and upper San Antonio Bay. This information was helpful in identifying historic locations, as well as current live populations, and was utilized to refine the study area. With the information received during this workshop, BIO-WEST determined to focus the initial study within Mission Lake and upper Guadalupe Bay (Figure 3-1).



Figure 3-1. Project Location.

METHODOLOGY

Using state-of-the-art technology, BIO-WEST conducted a marine remote sensing survey to determine the location and extent of *Rangia* within the upper San Antonio Bay system. In particular, the remote sensing survey focused on areas near the head of San Antonio Bay that have been documented to support a current, live *Rangia* population. These efforts were completed in two phases: a remote sensing survey to provide a geo-rectified image documenting benthic habitat types and water depths; a biological survey to visit benthic habitats representing *Rangia* colonies and collect samples for subsequent growth-increment analysis in a laboratory setting.

Remote Sensing Survey

Equipment for the marine remote sensing survey consisted of a Hemisphere[®] VS111 differentially corrected global positioning system (DGPS) receiver; an EdgeTech[®] 4125 Chirp 400/900 kilohertz (kHz) digital side-scan sonar sensor and topside processor with acquisition software; and a Teledyne Odom Hydrographic, Inc. Hydrotrac[™] 200 kHz single beam echo sounder. Vessel guidance and position and data logging were accomplished using a navigation processor utilizing Trimble[®] HYDROpro[™] Navigation software. Positional information for the survey vessel and each instrument sensor, via layback calculations, were stored in the navigation processor at a rate of one reading per second. Echo sounder depth data was also recorded in the navigation processor at one reading per second. Vessel speed during the survey was approximately 4.0 knots, providing in-line data spacing of approximately 6.8 feet (2.1 meters). Figure 3-2 provides an illustration depicting the equipment layout and offset positions on the survey vessel.



Figure 3-2. Survey Vessel Equipment Configuration.

Vessel survey transect spacing was set to 131.2 feet (40 meters) to ensure 100 percent imagery coverage of targeted areas. Concurrent with the remote sensing survey, manual poling of the substrate occurred along survey transects to help validate side-scan sonar signatures. Side-scan sonar data was recorded and exported from Edgetech, Inc. DISCOVER acquisition software. The data was then processed to produce remote sensing imagery. A single mosaic image was created from individual survey lines utilizing Chesapeake Technology, Inc. SonarWiz 5[®] processing software.

All remote-sensing data and imagery were imported into an ESRITM ArcMap[®] 10.3 GIS geo-database (geographic information system [GIS]). Once in ArcGIS, anomalies were identified based on the intensity of the sonar signature returns that appeared to represent the presence of *Rangia*. These interpretations were based off previous remote sensing findings where confirmed *Rangia* were collected. Based off of the sonar signature, *Rangia* were distinguished by having a concentrated, textured appearance that differed from the surrounding signature that was interpreted to be water bottom. These suspect areas were delineated in ArcGIS using shapefiles and were determined to be areas of potential *Rangia* concentrations.

Biological Survey

Following the data processing and delineation of potential *Rangia* beds, BIO-WEST conducted groundtruthing efforts to verify the sonar imagery and characterize *Rangia*. Using a 24-foot Carolina skiff, each sample site was navigated to using a wide area augmentation system (WAAS) capable GPS unit. Dredge tows were conducted using a BIO-WEST custom fabricated steel-frame dredge; the dimensions of which are 33 inches (80 centimeters) long by 18 inches (47 centimeters) wide by 11 inches (29 centimeters) deep with a 0.5-inch (1.27 centimeters) wire mesh-lined collection basket. The dredge was attached to the vessel and towed behind using GPS guidance while recording starting and ending positions. The number and duration of dredge tows varied depending upon the density and condition of *Rangia* at each location. At completion of each dredge, contents were retrieved, photo-documented and *Rangia* shells were collected for growth-increment analysis. Size was determined by measuring the total length (TL) in millimeters of each live *Rangia*.

In addition to measuring size-class and analyzing distribution, growth-increment analysis will be included in the current study. The analysis will be conducted by Dr. Bryan Black at the University of Texas utilizing cross-dating, a method used in tree-ring analysis, on *Rangia* shells. Cross-dating will establish growth-increment chronologies and recruitment histories for *Rangia* populations. Chronologies will be used to identify the years that were on average favorable or unfavorable for growth which may aid in determining recruitment success.

RESULTS

Remote Sensing Survey

The remote sensing survey for Mission Lake took place between September 4 and 18, 2014. Taking advantage of unusually high tide levels during the field effort, the survey crew was able to side-scan sonar survey a total of 1,636 acres of Mission Lake versus the expected 1,000-acre estimate. Additional remote sensing took place within the upper portion of Guadalupe Bay on December 9 and 10, 2014, and provided 669 acres of side-scan imagery. Within the Mission Lake and Guadalupe Bay survey area, results of the remote sensing effort provided several data sets including imagery for 100 percent of the bottom and a tidally-corrected bathymetric map. The sonar imagery depicted any feature located on the surface of the bay bottom or "substrate". This geo-rectified imagery was used to identify the location of *Rangia* colonies. The bathymetric map was produced using spot sounding directly below the survey vessel. Using the nearest functioning tide gauging station, depth soundings were tidally corrected to Mean Low Water (MLW) and contoured to identify bottom depths and any bottom relief.

The remote sensing survey revealed a large portion of Mission Lake exhibited sign of *Rangia* beds. Using this imagery, twenty-five locations were identified for the first ground-truthing effort. The upper Guadalupe Bay remote sensing survey revealed very few areas indicative of *Rangia* beds. Ground-truthing locations were chosen within potential *Rangia* beds, as well as additional areas, to get a thorough representation of the entire side-scanned area and verify distinguishable SSS signatures. Figure 3-3 provides an example of the SSS imagery observed in the project area where *Rangia* were found to be abundant.



Figure 3-3. Sonar Imagery of *Rangia* Bed with Prop Scars.

Biological Survey

On September 17, 2014, BIO-WEST performed preliminary ground-truthing efforts in Mission Lake. Dredge tows and water quality samples were conducted, resulting in seven live *Rangia* recovered. Due to the preliminary nature of this field effort, dredge tows were not recorded with a GPS unit. The live *Rangia* were preserved for laboratory analysis.

A full ground-truthing effort of Mission Lake took place on December 5, 2014 using locations identified from the side-scan imagery. A total of 25 dredge tows were pulled and 11 live *Rangia* were collected. In addition to the dredge tows, eight water quality samples were recorded. Water quality parameters included water temperature (C°), conductivity (mS/cm), salinity (%), DO (mg/L), pH, and turbidity (NTU). The 18 live *Rangia* collected on September 17 and December 5, 2014 were shipped to Dr. Black for laboratory analysis.

Ground-truthing within the upper Guadalupe Bay took place on December 10, 2014 directly after the completion of the side-scan sonar survey. A total of 25 dredge tows were pulled throughout the side-scanned area, and no live *Rangia* were found. Additionally, only six of the 25 dredge tows within upper Guadalupe Bay revealed presence of *Rangia* shell. A total of eight water quality samples were recorded during this effort. In an effort to collect more *Rangia* for laboratory analysis, an additional 29 dredge tows were pulled in Mission Lake on December 10 and 23, 2014, yielding 23 live *Rangia*. As a result of all ground-truthing efforts, a total of 41 live *Rangia* were sent to Dr. Black for growth ring analysis. Photographs of the dredge tow and representative samples are located in Appendix A. Field data sheets for each recorded dredge tow for all ground-truthing efforts are provided in Appendix B. A total of 16 water quality samples were recorded during the December 5 and 10, 2014 effort. Water quality measurements for all samples are presented in Appendix C.

The data from the 55 recorded Mission Lake dredge tows were quantified as a live *Rangia* per acre figure based on the total length of all 55 dredge tows, the width of the dredge, and how many live *Rangia* were captured. Since dredge tow length was not recorded for the first field effort when the seven live *Rangia* were recovered, these live *Rangia* were excluded from the calculation. The formula for calculating live *Rangia* per acre is presented below.

Rangia per Acre Formula:

Total Length of Dredge Tows (feet) * 1.5 (feet)

= Acres Covered by Dredge Tows

43,560 feet²

of Live Rangia Captured

Acres Covered by Dredge Tows = Live Rangia per Acre

It was determined that the 55 dredge tows covered 0.67 acres and 34 live *Rangia* were captured, resulting in a extrapolated number of 51 live *Rangia* per acre within Mission Lake. Since no live *Rangia* were recovered within the upper Guadalupe Bay, *Rangia* per acre could not be calculated.

Assumptions need to be made when calculating the live *Rangia* per acre figure. BIO-WEST understands this is a number based solely off of our own findings and using this result for additional uses should be approached with caution. In order to calculate this figure, it is assumed that *Rangia* shell within the investigated area are contiguous and evenly distributed. In addition, a number of dredge tows were placed in areas of suspected *Rangia* presence and other dredge tows can be considered chosen at random. Therefore, this formula is an attempt to quantify and extrapolate the live *Rangia* population based on our findings and should be used as an estimation.

The accuracy of the SSS Rangia bed delineations can be considered high to the fact that, with few exceptions, areas suspected of containing the presence of Rangia were confirmed and areas that were sampled to verify the absence of Rangia were also confirmed, the presence of Rangia sign outside of delineated Rangia beds did not invalidate the delineations and was expected due to the scattered nature of *Rangia* occurrence.

Table 3-1, below, provides a summary of the ground-truthing sampling efforts. This data set identifies 96% of dredge tows revealed the presence of *Rangia* shell within Mission Lake. This percentage is calculated by dividing the total number of dredge tows with *Rangia* sign within Mission lake (53 dredge tows) by the total number of tows pulled within Mission Lake (55 dredge tows). However, only 36% of Mission Lake dredge tows recovered live *Rangia* (20 tows with live *Rangia* divided by 55 total dredge tows). All live *Rangia* collected were measured and incorporated into the data and then sent to Dr. Black for growth-increment analysis.

Date of Effort	Location	Number of Dredge Tows	Tows with <i>Rangia</i> Shell	Tows with Live <i>Rangia</i>	Total Number of Live <i>Rangia</i>
9/17/2014	Mission Lake				7
12/5/2014	Mission Lake	26	26	9	11
12/10/2014	Guadalupe Bay	25	6	0	0
12/10/2014	Mission Lake	4	4	1	1
12/23/2014	Mission Lake	25	23	10	22
r r	Totals	80	59	20	41

Table 3-1. Summary	of Sampling Efforts.
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All total length data collected in the field were transcribed into Microsoft Excel and graphed (Figure 3-4). This graph depicts the distribution of *Rangia* size throughout the four sampling efforts within Mission Lake; a total of 41 live *Rangia* measurements. The *Rangia* size class graph shows that the majority of live *Rangia* collected measured between 50 and 69 millimeters (mm). The average size of *Rangia* collected during the four field efforts was 58.2 mm. The lack of live *Rangia* collected in the smaller size classes may suggest poor reproduction and recruitment in recent years. A complete summary of field data is located in Appendix C.



Figure 3-4. Live Rangia Class Size.

In addition to measuring size-class and analyzing distribution, growth-increment analysis will be included in the current study. Using *Rangia* samples collected by BIO-WEST, an analysis is being conducted by Dr. Bryan Black at the University of Texas utilizing cross-dating, a method used in tree-ring analysis, on *Rangia* shells. A total of 41 live *Rangia* have been sent to Dr. Black, along with half shells that can be used for additional information. Further analysis will consist of examining relationships among growth chronologies, recruitment, and environmental indices (salinity, temperature, freshwater inflow).

DISCUSSION

The remote sensing survey of Mission Lake revealed the presence of *Rangia* shell distributed throughout the surveyed areas. Ground-truthing efforts confirmed this abundance of *Rangia* shell but discovered a presence of far fewer live *Rangia* specimens. A map of the side-scan sonar imagery and all field data is located in Appendix D. The 51 *Rangia* per acre estimate can be compared to past BIO-WEST *Rangia* investigations in Trinity Bay and Sabine Lake in 2014. Within these ground-truthed areas, Trinity Bay revealed a population of 5.9 live *Rangia* per acre, and Sabine Lake investigations revealed a population of 39 live *Rangia* per acre. The same formula was used to calculate the live *Rangia* per acre for all three of these projects. Compared to these two other bay systems, Mission Lake has a relatively large population of live *Rangia*. However, Mission Lake is much smaller in size compared to these bay systems. Additionally, side-scan sonar was conducted on the entire lake and ground truthing occurred on every signature. Based on the abundance of shell, it appears the live *Rangia* population was once much larger in Mission Lake. Most *Rangia* shell recovered in dredge tows remained as intact half shells with the periostracum present. This is likely due to the water bottom substrate acting as a preservative, but could also represent a fairly recent decline in the number of live *Rangia*.

In the side-scanned portion of upper Guadalupe Bay, very few areas revealed the presence of *Rangia* shell signatures. When ground-truthing efforts took place, this lack of *Rangia* presence was verified, and no live *Rangia* were recovered. *Rangia* shell were found in the southern most portion of the ground-truthed area of Guadalupe Bay. Further investigations would be required to know if *Rangia* are present further south in Guadalupe Bay. Within Mission Lake and upper Guadalupe Bay, ground-truthing efforts confirmed the application of side-scan imagery as a basis for finding *Rangia* shell concentrations. Growth-increment analysis by Dr. Black will further aid in explaining the environmental conditions behind poor *Rangia* recruitment and which years in the past have exhibited favorable conditions.

INTRODUCTION

Environmental freshwater flow standards adopted for the Guadalupe and Mission-Aransas Estuaries of coastal Texas, USA, have relied heavily on reproductive requirements for *Rangia* clams given that adults are sessile and can live and spawn within a narrow range of salinities generally accepted to be < 18 ppt and 2 to 10 ppt, respectively. This approach utilized literature from studies in other states in combination with distribution and abundance patterns for *Rangia* based on Texas Parks & Wildlife Department reports of incidental catch. Here, to further evaluate the salinity and freshwater inflow requirements, we develop growth-increment width and recruitment histories for *Rangia* and then relate them to instrumental records of environmental variability. The focus of the analysis is Mission Lake in San Antonio Bay, though *Rangia cuneata* shells from Sabine Lake and the Trinity Bay portion of Galveston Bay are also included to increase replication (Figure 4-1). This is particularly important given that these three locations span a precipitation gradient from relatively dry in the west (San Antonio Bay) to relatively wet in the east (Sabine Lake). Thus, responses to inter-annual variability in salinity and temperature can be compared among sites and further corroborated by overall growth rates across the precipitation gradient. If interannual growth positively correlates to interannual variability in salinity, then individuals from the easternmost lowest-salinity site should have the lowest overall growth rates.



Figure 4-1. Map of Trinity Bay portion of Galveston Bay showing locations of *Rangia cuneata* samples collected in long-term sample program of TPWD and recent Texas A&M and BIO-WEST efforts.

At each of the three sites live- and dead-collected shells were collected. Live clams were used to generate exactly dated growth chronologies, beginning at the known year of capture and extending back through time over the lifespan of the individuals. Although the calendar years over which dead-collected individuals were alive cannot be exactly determined, their age-specific growth could be compared to those that were live-collected. Differences in growth rates between these two groups could indicate that growing conditions have changed over time. For example, consistently slower age-specific growth rates in live-collected individuals may suggest that conditions have been stressful in recent years relative to those in the past.

METHODOLOGY

Live and dead *Rangia* were also collected by BIO-WEST Inc. under contract from the San Antonio River Authority from Mission Lake in upper San Antonio Bay on Sep. 17, Dec. 5 and Dec. 23, 2014. A second collection was conducted on Jul 2, 2015 (Table 4-1). Sample sizes of live-collected individuals that are most valuable for this project were relatively low, and we therefore decided to expand the study to include adjacent estuaries. Replication will increase confidence in our findings, especially if similar results are identified across sites. Thus, live and dead *Rangia* from Trinity Bay and Sabine Lake were added to the study. These samples were collected on August 27, 2014 by BIO-WEST Inc. under contract with the National Wildlife Federation (NWF) (Table 4-1). All samples were sent to the University of Texas Marine Science Institute (UTMSI) in Port Aransas, TX for further processing.

Table 4-1. *Rangia* samples collected and ultimately measured from Mission Lake, Trinity Bay, and Sabine Lake.

Site	Live Collected	Dead Collected	Live Measured	Dead Measured
Trinity Bay	62	105	50	78
Sabine Lake	15	328	13	255
Mission Lake	50	14	37	13

At UTMSI meat was removed and shells were cleaned then dried, weighed, and measured for length and width. One valve from each shell was then embedded in epoxy to prevent fracturing and then sectioned through the center using a tile-cutting saw, taking care to bisect the hinge region of each individual. The cut surface was then polished with increasingly fine lapping film from 9 micron to a maximum of 3 micron mesh, after which it was photographed at approximately 10x magnification using reflected light on a Mz9.5 binocular microscope. Bivalves normally form an opaque (white coloration in reflected light) "summer band" during the warm period of the year, becoming more translucent (dark in coloration in

reflected light with black background) as the winter progresses. The most complete records of growth increments can be found in the hinge region of the shell, and this is the location where growth-increments were analyzed for this study. One annual increment was defined as the distance from the end of one winter band to the end of the next and all widths were measured along the axis of growth (perpendicular to each increment boundary) using Image Pro Premier 9.1 (Media Cybernetics, Silver Spring, MD) (Figure 4-2). Note that the partially complete first (year of origin) and last (year of death) years of growth were not measured.



Figure 4-2. Measurement axes (yellow lines) and annual growth-increment boundaries (red ticks) for two live-collected *Rangia*. The shell was thin sectioned, polished, and the hinge region photographed. Note that the partial innermost increment was not measured (corresponding to 2010 in both shells) nor was the partial outermost increment (2014).

Ideally, exact dating of each increment is achieved through the tree-ring (dendrochronology) process of crossdating, which is universally applied in tree-ring analysis and has been successfully adapted to marine and freshwater fish and bivalve populations (Black et al. CJFAS 2005, 2008; GCB 2011). Crossdating is based on the assumption some environmental factor that affects growth, and that as that environmental factor varies over time, it induces a synchronous growth pattern or growth "bar code" among all specimens for a given species and region. The technique is implemented by matching the synchronous growth pattern among all samples, working from the outermost increment formed during the known year of capture to the center. Should an increment be missed or falsely added, the growth "bar code" will be offset by a year relative to the other samples, thereby identifying that an error has occurred. The error is then confirmed by re-examining the shell for a micro or false increment, or accepting the possibility of a locally absent increment. The result of crossdating is that all increments

are assigned the correct calendar year of formation, and that continuous, exactly dated growth chronologies and recruitment histories may be developed. Samples with unknown death dates may also be crossdated with live-collected samples, assuming there is sufficient temporal overlap, to establish the calendar years over which those dead-collected individuals were alive. These crossdated dead-collected individuals could be used to extend the chronology or recruitment history farther back in time.

Once increments were correctly placed in time, growth-increment chronologies were developed, one for each site (bay). All individuals exhibited age-related growth declines that are a function of biology and not environment, and were therefore removed. To accomplish this, growth-increment widths were first grouped according to the ages at which they were formed. Within each age group (e.g. all increments formed during the fifth year of life), each increment was standardized by dividing by the group mean:

$$GI_s = \frac{GI_w}{GI_m}$$
 ,

where GI_s is the standardized growth increment, GI_w is growth-increment width, and GI_m is mean increment-width within the age group. Standardized growth increments across all age groups were then averaged with respect to calendar year of formation to generate the *Rangia* chronology. Note that this removal of age-related growth declines was performed separately at each site to generate bay-specific chronologies. Each final *Rangia* chronology was limited to those years with at least 10 measurements (2009-2013) to maximize the common, synchronous signal and cancel out individual-level "noise." Recruitment date was defined as the year of the innermost increment, which was always partially formed. Total age was defined as the number of complete increments plus the partial innermost increment and the partial outermost increment formed during the year of death.

Rangia chronologies and recruitment histories were correlated to instrumental or modeled indices of temperature, salinity, and river discharge. For all three variables, monthly rather than annual averages were used to determine if there is a specific season associated with annual *Rangia* growth, as would occur if conditions during a given window of months defined growing-season length. High-elevation tree-ring chronologies often correlate with spring temperatures, as a warm March or April means an early start to the growing season and thus a wide growth increment for that given year. For Trinity Bay and Sabine Lake, hourly records of temperature and salinity from sondes maintained by the Texas Water Development Board were averaged into monthly means. In Trinity Bay, data were obtained from the Northwest Double Bayou Channel station for the years 2004 through 2013. In Sabine Lake, data were used from the SAB1 station located at the upper end of the lake near the head of the Neches River, which were largely continuous between 2008 and 2013, but with gaps for approximately 10% of the monthly means. Given the most robust instrumental records were intermittent observations from Texas Parks and Wildlife Coastal Fisheries sampling trips, monthly-averaged TxBLEND salinity data were obtained for Mission Lake to provide a continuous dataset from 2001 through 2013. The general lack of observational data also meant that the TxBLEND model could not be as well calibrated in the upper

estuary as the mid and lower estuary, but model results still provided a reasonable estimate, especially at the lower-frequency, monthly time steps used here [Guthrie et al. 2010]. Chronologies and recruitment were also compared to inflow data, which for Trinity Bay included the USGS Trinity River gage at Romayor as well as TWDB Trinity Basin inflow estimates. For Sabine Lake, inflow was estimated as the sum of the Neches River, Keith Lake, Black Bayou, Johnson Bayou, and Taylor Bayou. Inflow for Mission Lake was estimated from USGS gage 08188800, Guadalupe River near Tivoli, TX and USGS gage 08188600 GBRA Calhoun Canal Pump Station near Long Mott, TX.

In the event that significant correlations were identified between *Rangia* and a given environmental index (salinity, discharge, or temperature), linear regression was used to better quantify the nature of the relationship. However, biological variables are often correlated across a window of consecutive months. To avoid entering multiple and highly correlated predictor variables into a regression, the environmental variable was averaged across this seasonal window. The final regression consisted of the *Rangia* chronology and the mean of those months with peak correlation.

RESULTS AND DISCUSSION

Live- and dead-collected shells across all sites had remarkably similar length to weight relationships, though individuals from Trinity Bay had somewhat greater weights than expected. However, these deviations were modest considering that 95% of the variability in the full dataset could be explained by a single power function (Figure 4-3). At all three sites, *Rangia* were short lived, especially the live collected individuals, the overwhelming majority of which were less than ten years in age. Given these very short lifespans, crossdating was not highly effective, though there was some synchrony apparent among individuals with for example an unusually wide 2012 at all sites and 2010 at Trinity. Greater temporal overlap among individuals would increase confidence that growth patterns align and that dating is correct. These short lifespans also precluded crossdating of dead-collected material against the growth patterns of live-collected individuals, a process that was further complicated by the short lifespans of the dead-collected samples. A positive match among these growth 'bar codes' is not possible if the length of the bar code' is short; the possibility of a spurious match becomes too great. Increment boundaries were, however, well-defined (Figure 4-2), which provided confidence in assigning the calendar years of formation to increments in live-collected individuals. Dead-collected individuals could be aged and measured even if calendar years could not be assigned.





Age and weight were strongly related, especially in Trinity Bay and Mission Lake. This provided some degree of corroboration that growth-increment boundaries had been correctly identified (Figure 4-3). In Mission Bay, dead-collected individuals were much older and larger than their live-collected counterparts (Figure 4-3). In contrast, live-collected individuals in Trinity and especially Sabine occurred over a narrower age range than those that were dead-collected (Figure 4-4), which may be due to the low number of live-collected individuals. Within each site, there were no significant differences in age-specific weight between live- and dead-collected individuals. T-tests for age-specific differences were all non-significant, even at a modest (p < 0.1) level. Likewise, there were no significant differences in age-specific growth-increment width between live- and dead-collected specimens within each site; 95% confidence intervals overlap with one another (Figure 4-5).



relationship of all Rangia collected from A) Mission Lake, B) Trinity Bay and C) Sabine Lake. Photo: Live-collected age-six *Rangia* from Trinity (upper) and Sabine (lower).



Figure 4-5. Mean hinge growth-increment width (with 95% confidence intervals; only those classes with > 5 observations are included) for live-collected and dead-collected *Rangia* in Mission Lake, Trinity Bay, and Sabine Lake.

Given that there were no apparent differences in growth rate between live and dead-collected shells within sites, live- and dead-collected measurements could be pooled to increase sample sizes for comparisons among sites. Differences among sites were considerable, as illustrated by the much smaller sizes of Sabine individuals (Figure 4-4). These differences were most apparent when comparing mean growth-increment width among sites, which decreased from Mission to Trinity to Sabine, especially in the early years of life (Figure 4-5B). Notably, this trend followed a precipitation gradient from relatively wet in the east (Sabine) to dry in the west (Mission Lake).

Although live-collected individuals were short-lived, within each site there was still an apparent synchrony in their growth patterns (Figure 4-6). Beyond age-related growth declines, anomalies in above-average growth and below-average growth were shared within sites. Not all individuals followed this exact pattern, but most did despite some level of individual variability (Figure 4-6). This synchrony among individuals suggests that some aspect of environmental interannual variability was influencing *Rangia* growth. Overall, the shared growth patterns in *Rangia* were best highlighted by the final chronology for each site for which values above one indicate above-average growth and values below one indicate below-average growth (Figure 4-6D). In addition to synchrony within sites, synchrony was also apparent among sites, especially between Trinity Bay and Sabine Lake. Note that not all shells were measured; some had not yet formed a full increment while a low percentage (~5%) had indistinct increment boundaries that could not be delineated (Table 4-1). A random subset of dead individuals was selected from Sabine Lake given the large number of shells collected at that site.





and C) Trinity Bay. Also shown are D) the master, site-wide chronologies highlighting population-level growth anomalies.

Figure 4-6. Growth-increment widths for all live-collected Rangia in A) Sabine Lake, B) Mission Lake,

Growth-increment chronologies significantly (p < 0.05) correlated to salinity at all three sites (Figure 4-7). Correlations were positive and at maximum values from the prior October through current January in Mission Bay and Sabine Lake. In Trinity Bay, peak correlations occurred over a somewhat earlier window of months spanning the prior July through prior October. Notably, correlations in the current year were low; conditions in the preceding fall and winter were much more closely associated with growth (Figure 4-7, 4-8A). Moreover all three *Rangia* chronologies responded very consistently to salinity anomalies. The data pooled across sites can be fit with a single linear regression that is highly significant (p < 0.01) and explains 83% of the variability in the dataset (Figure 4-8B). Although each chronology was only five years in length, the combination of three sites increased confidence in the result that high salinity anomalies late in the prior year was positively associated with growth. Moreover, this growth appeared to be most sensitive to salinity variability during the time of year when salinity reaches maximal values, which is from approximately July through December at all three sites (Figure 4-8C).

Note that when comparing among sites (Figure 4-8B), we evaluated *Rangia* growth response to salinity anomalies rather than absolute salinity values. Evaluating growth response to absolute salinity values across sites would require that salinity data be collected at the exact *Rangia* sampling locations. Stations located in the vicinity of the *Rangia*, as used here, may not capture the absolute values of salinity at the *Rangia* sites, but would still capture relative changes from month-to-month- or year-to-year. Thus, interannual variability in *Rangia* was compared to interannual variability in salinity. Variability in salinity appeared to be the primary driver of growth; correlations with monthly-averaged freshwater inflow data and temperature were inconsistent and relatively weak (data not shown).



Figure 4-7. Correlation between the *Rangia* chronology and monthly mean salinity over the current and 1-year lag (prior; pr) year, as calculated within each of the three sites considered in this study.



Figure 4-8. A) Comparison of normalized growth-increment chronologies to normalized and lagged multi-month salinity (where preceding October through current January salinity was used for Mission Lake and Sabine Lake and prior July through October salinity was used for Trinity Bay). B) Biplot across all sites of the *Rangia* growth-increment chronologies and salinity (same data as Panel A) C). Mean monthly salinity (parts per thousand) at each of the three study sites: Trinity Bay, Sabine Lake (SAB1 station), and Mission Lake over the 2009-2013 interval.

Recruitment was highly variable among years, though somewhat coherent among sites, with for example peaks in 2008 and 2010 (Figure 4-9). There were no significant correlations between climate variables and recruitment history at any site, but this was to be expected given the relatively low numbers of live-collected individuals. Large peaks in recruitment may be detectable, but more subtle differences among years would likely be difficult to resolve without a larger sample size. One individual could make the difference between an above- or below-average frequency at the sample sizes available here, especially for Sabine Lake.



Figure 4-9. Recruitment history of live-collected *Rangia*, subdivided with respect to collection site for A) Sabine Lake and B) Trinity Bay.

Salinity data were limited in length (back to 2008 for Sabine) or contained gaps in the early years of the recruitment histories, which also complicated comparisons. If, however, the population age structures are compared to monthly inflows for the three sites, major recruitment events are generally preceded by high discharge events, as occurred in mid- to late 2007 as well as late 2009 and early 2010 (Figure 4-10). These relationships are coarse and cannot be statistically linked, but do at least support the hypothesis that freshwater is associated with recruitment.



Figure 4-10. Monthly-averaged freshwater inflows into Trinity Bay, Sabine Lake, and Mission Lake, normalized to a mean of zero and standard deviation of one.

Overall, the *Rangia* environment-growth relationships must be interpreted with caution given that livecollected *Rangia* were very short-lived, limiting the final chronology to only five years. This was too short of an interval with which to rigorously establish statistical relationships, though combining results across three different sites did help provide some level of confidence. Better quantification environment-growth relationships will require longer *Rangia* chronologies. Indeed, sites in Aransas Bay and the Nueces River are presently being sampled for older individuals, which could provide more evidence for the environmental responses identified here. Ultimately, however, there are several conclusions from this study:

• Living *Rangia* are presently uncommon in the three systems sampled for this study, which limited sample sizes for this present analysis. Living individuals tend to be young, especially in comparison to dead-collected individuals from Mission Lake approaching 20 years in age.

- There was no difference in growth rates between dead- and live-collected individuals at any of the sites. Thus, living shells do not show any unusual signs of growth stress compared to their dead counterparts.
- There was a significant east-to-west gradient in growth rates across sites; Sabine was slowest followed by Trinity and then Mission.
- After age-related growth declines were removed, synchronous patterns were evident among individuals within each site, indicating that some aspect of environmental variability was influencing growth.
- Some degree of synchrony was also evident among sites (chronologies), with for example unusually strong growth in 2012.
- At each site, growth chronologies positively correlated to prior fall and winter salinity, the time of year in which salinity tends to be highest. The nature of this relationship was remarkably similar across the three sites. No clear relationships were evident with other environmental indicators.
- Recruitment histories were short and coarsely defined due to small sample sizes. However, there were years of above-average recruitment, and these were generally preceded by periods of high freshwater inflow.

Rangia has long been thought to be sensitive to freshwater inflows, able to live and spawn within a narrow range of salinities generally accepted to be < 18 ppt and 2 to 10 ppt, respectively [*Cain*, 1973; 1975; LaSalle and de la Cruz, 1985]. Here, we find that shell growth rates are indeed sensitive to salinity, but with positive correlations rather than the expected negative correlations. This was supported by the fact that salinity-growth relationships were consistent across all sites, and that the site with the overall lowest salinities (Sabine) had the slowest overall growth rates with dramatically smaller shells for any given age. This does not contradict the fact that lower salinities are necessary for *Rangia* populations; environmental requirements for reproduction, larval survival, and shell growth may be quite different. Clearly, freshwater inflows are necessary for the sustainability of *Rangia* populations as reflected by their presence only in the freshest areas of the estuaries. The reasons underlying positive relationships between salinity and growth are beyond the scope of this study, but would not be unprecedented in the sclerochronology literature. Freshwater mussel growth-increment chronologies are strongly and negatively correlated with river discharge [Black et al., 2010; Rypel et al., 2008; 2009]. High flows may hinder feeding or even cause physical damage [Black et al., 2015], and perhaps something analogous may be occurring in Texas bays under low salinity conditions. Another important caution is that the analysis in the present study was focused on environmental variability on monthly to annual scales. Events on finer time scales such as floods or tropical storms may also be important to Rangia growth and reproduction, but would require longer biological time series to properly evaluate.

Section 4

Rangia Clam Investigations in Texas Bays: a growth –Increment approach Prepared by University of Texas Marine Science Institute

In summary, this present study ultimately involved short (>10 years) timescales due to the scarcity of living Rangia and their brief lifespans. Future studies to address freshwater inflows in Texas bays could address much longer timescales in an effort to establish historical ranges of variability and test the extent to which modern conditions exceed them. One possible approach would be to use Rangia in Native American middens. Although exactly dated year-by-year chronologies could not be developed, Rangia could be roughly placed in time (±20 years) using ¹⁴C dating. Any long-term trends in growth rate would be evident, providing a history that is likely to span multiple centuries. This would be relatively expensive and would also require that the environmental drivers of growth-increment width be more clearly established, though this may be possible if longer-lived Rangia can be found in from area bays. Another approach would be freshwater mussels, which have been widely used throughout the Pacific Northwest, southeastern US, and northern Europe as indicators of river flow [Black et al., 2015; Haaq and Rypel, 2011; Schöne et al., 2004]. Mussels are native to south Texas rivers and could be linked to environment as well as Rangia chronologies in the upper estuary, providing multi-species perspectives on freshwater variability and ecosystem response. Finally, tree rings can be used to hindcast river flows in south Texas, as has been successfully done in central Texas and elsewhere in the southern United States. Oak, cedar elm, and bald cypress are long-lived (multiple centuries) and their growth increment width is limited by precipitation. Expansion of the existing tree-ring network into south Texas would open many new possibilities to reconstructing (hind-casting) river discharge into estuaries over the past few centuries, providing values information regarding historical ranges of variability and providing context for modern conditions. Indeed, blue oak, which is sensitive to precipitation, was used to hindcast salinity in the San Francisco Bay over multiple centuries, and with a high degree of accuracy [Stahle et al., 2013]. Such studies may be possible with tree-ring data from south Texas and could be pursued in the near future, complementing observational and instrumental records.

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Dredge Tow Sampling Equipment

Dredge



Top View



Side View

Section 6 Appendix A Dredge Tow Sampling Equipment and Sample Photographs



Remote Sensing Survey – Mission Lake Upper San Antonio Bay, Texas

Remote sensing survey within Mission Lake during an unusually high water period. Taken in September, 2014



An example of the side-scan sonar imagery showing *Rangia* and algae from Mission Lake. Screenshot from SonarWiz

Section 6 Appendix A Dredge Tow Sampling Equipment and Sample Photographs

> Rangia Dredge Tows – Mission Lake Upper San Antonio Bay, Texas



Representative of a dredge tow classified as abundant *Rangia*. Taken during the December 5, 2014 Mission Lake ground-truthing survey.



Representative of a dredge tow classified as few *Rangia*. Taken during the December 5, 2014 Mission Lake ground-truthing survey.

Size	Group	Live Rangia				
0-4mm	0					
5-9mm	1					
10-14mm	2					
15-19mm	3					
20-24mm	4					
25-29mm	5					
30-34mm	6					
35-39mm	7					
40-44mm	8	1				
45-49mm	9					
50-54mm	10	1				
55-59mm	11	1				
60-64mm	12	4				
65-69mm	13					
70-74mm	14					
>74mm	15					
Total Live F	Rangia	7				

Mission Lake Rangia September 17, 2014

Size	Group	1	2	3	4	5	6	7	8	9	10	11	12	13
0-4mm	0													
5-9mm	1													
10-14mm	2													
15-19mm	3													
20-24mm	4					1								1
25-29mm	5													
30-34mm	6													
35-39mm	7													
40-44mm	8													
45-49mm	9													
50-54mm	10													
55-59mm	11													1
60-64mm	12													
65-69mm	13													
70-74mm	14													
>74mm	15													
Total Live	Rangia	0	0	0	0	1	0	0	0	0	0	0	0	2
Rangia si	gn	Y	Y	Abundant	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Mission Lake Rangia December 5, 2014

Y = Yes

Abundant = majority of dredge tow containing with Rangia shells

Dredge Tow Size Group 14 15 16 17 18 19 21 22 23 24 25 20 0-4mm 0 5-9mm 1 10-14mm 2 15-19mm 3 20-24mm 4 25-29mm 5 6 30-34mm 7 35-39mm 40-44mm 8 45-49mm 9 50-54mm 10 1 1 55-59mm 11 2 1 1 60-64mm 12 1 1 65-69mm 13 70-74mm 14 >74mm 15 Total Live Rangia 0 2 1 1 0 1 1 0 0 0 1 1 Rangia sign Υ Υ Υ --------Υ -------------Y = Yes

-- = None

Section 6 Appendix B Dredge Tow Field Data

							D	redge To	w					
Size	Group	1	2	3	4	5	6	7	8	9	10	11	12	13
0-4mm	0													
5-9mm	1													
10-14mm	2													
15-19mm	3													
20-24mm	4													
25-29mm	5													
30-34mm	6													
35-39mm	7													
40-44mm	8													
45-49mm	9													
50-54mm	10													
55-59mm	11													
60-64mm	12													
65-69mm	13													
70-74mm	14													
>74mm	15													
Total Live R	angia	0	0	0	0	0	0	0	0	0	0	0	0	0
Rangia sign									Y	Y	Y	Y		
Y = Yes														

Guadalupe Bay Rangia December 10, 2014

-- = None

Dredge Tow

Size	Group	14	15	16	17	18	19	20	21	22	23	24	25
0-4mm	0												
5-9mm	1												
10-14mm	2												
15-19mm	3												
20-24mm	4												
25-29mm	5												
30-34mm	6												
35-39mm	7												
40-44mm	8												
45-49mm	9												
50-54mm	10												
55-59mm	11												
60-64mm	12												
65-69mm	13												
70-74mm	14												
>74mm	15												
Total Live Ra	angia	0	0	0	0	0	0	0	0	0	0	0	0
Rangia sign							Y						Y

Y = Yes

-- = None

		Dredge Tow											
Size	Group	1	2	3	4								
0-4mm	0												
5-9mm	1												
10-14mm	2												
15-19mm	3												
20-24mm	4												
25-29mm	5												
30-34mm	6												
35-39mm	7												
40-44mm	8												
45-49mm	9												
50-54mm	10												
55-59mm	11												
60-64mm	12		1										
65-69mm	13												
70-74mm	14												
>74mm	15												
Total Live	Rangia	0	1	0	0								
Rangia sig	n	Abundant	Abundant	Abundant	Abundant								

Mission Lake Rangia December 10, 2014

Abundant = majority of dredge tow containing with Rangia shells

Section 6 Appendix B Dredge Tow Field Data

Mission Lake Rangia December 23, 2014

Dredge Tow

Size	Group	1	2	3	4	5	6	7	8	9	10	11	12	13
0-4mm	0													
5-9mm	1													
10-14mm	2													
15-19mm	3													
20-24mm	4													
25-29mm	5													
30-34mm	6													
35-39mm	7													
40-44mm	8													
45-49mm	9													
50-54mm	10													
55-59mm	11													
60-64mm	12										3			
65-69mm	13							1			1		1	
70-74mm	14													
>74mm	15													
Total Live	Rangia	0	0	0	0	0	0	1	0	0	4	0	1	0
Rangia si	ign	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Abundant

Y = Yes

Abundant = majority of dredge tow containing with Rangia shells

	Dredge Tow												
Size	Group	14	15	16	17	18	19	20	21	22	23	24	25
0-4mm	0												
5-9mm	1												
10-14mm	2												
15-19mm	3												
20-24mm	4												
25-29mm	5												
30-34mm	6												
35-39mm	7												
40-44mm	8												
45-49mm	9												
50-54mm	10				1								
55-59mm	11									2	1		
60-64mm	12	1								2	1	2	
65-69mm	13	1											4
70-74mm	14							1					
>74mm	15												
Total Live	Rangia	2	0	0	1	0	0	1	0	4	2	2	4
Rangia sign		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Y = Yes

6-8

ID	Time	Depth (ft)	Temperature (°C)	рН	Conductivity (mS)	Salinity (ppt)	Dissolved Oxygen (mg/l)	Turbidity (NTU)
WQ 1	9:05 AM	3.2	18.59	8.20	5.89	3.38	10.64	26.2
WQ 2	10:00 AM	3.8	18.18	7.95	9.22	4.75	7.72	29.2
WQ 3	10:16 AM	3.5	18.69	8.22	12.20	5.76	10.12	22.2
WQ 4	10:40 AM	3.4	18.85	8.29	4.12	1.96	11.68	19.4
WQ 5	10:56 AM	3.7	19.13	8.10	1.73	0.72	9.93	31.6
WQ 6	11:23 AM	3.8	18.93	8.03	1.94	0.91	10.01	
WQ 7	12:20 PM	3.0	19.20	8.13	5.07	2.75	11.05	16.8
WQ 8	12:37 PM	3.6	19.71	8.24	5.11	2.76	11.96	16.2

December 5, 2014

December 10, 2014

ID	Time	Depth (ft)	Temperature (°C)	рН	Conductivity (mS)	Salinity (ppt)	Dissolved Oxygen (mg/l)	Turbidity (NTU)
WQ 9	2:56 PM	3.0	17.97	8.43	28.94	17.89	11.29	5.9
WQ 10	3:16 PM	3.4	17.97	8.46	27.70	17.03	11.66	6.2
WQ 11	3:30 PM	3.4	18.54	8.49	31.17	19.47	11.13	6.8
WQ 12	3:50 PM	2.6	18.09	8.47	29.49	18.32	10.42	5.0
WQ 13	4:06 PM	2.0	18.50	8.28	16.57	10.05	9.71	20.4
WQ 14	4:28 PM	4.0	18.31	8.51	26.41	16.21	10.78	7.0
WQ 15	4:43 PM	2.6	18.12	8.47	22.04	13.24	12.51	8.1
WQ 16	5:15 PM	3.7	18.90	8.49	28.05	17.71	13.86	12.3

