RANGIA AS POTENTIAL INDICATORS OF BAY HEALTH

A Thesis

by

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ABSTRACT

Galveston Bay is an economically and ecologically important estuarine system on the Texas Coast sourced by freshwater inflows from an increasingly urbanized watershed. To regulate these flows, a baseline of ecological demands is established by monitoring biological response of estuarine organisms to changes in flows. Rangia clams have been identified as potential bioindicators for bay health. Historic rangia abundance and distribution data collected by TPWD and TCEQ showed that rangia were found in the greatest numbers in Trinity Bay and a decline in the overall population of Galveston Bay rangia throughout the past three decades. Though t-tests conducted on historical data showed that gear-related size exclusion significantly biased rangia CPUE and shell length data, smaller CPUE numbers in recent years compared to the rest of the historical record were supportive a genuine decline in rangia. After three years of present-day study (2012-2014), there was an observed increase in mean rangia shell length and decreases in mean meat index and areal density with a mean clam density of 25.3 (± 16.1) m^{-2} in the Trinity River Delta and 22.5 (± 16.8) m^{-2} in the Bay. Low mean monthly river discharges from the Trinity River during the study period complicated by drought and land use changes likely altered conditions which rangia require to for the survival of larvae and the initiation of spawning. These results also support the hypothesis that the low rangia densities found during the present-day study in Galveston Bay may be tied to the effects of drought conditions. PERMANOVA Main tests validated the comparability of the small-scale experimental design to long-term monitoring of bay wide sites by identifying significant variation in rangia abundance and health at different spatiotemporal levels. Multivariate analyses of clam health metrics and environmental parameters support a link between rangia health and variables influenced by freshwater inflow (salinity, DO, river discharge, dissolved nutrients) and explained one third of the variance in clam health metrics. Variables independent of FWI influence (temperature, water depth) were also related to clam health which further suggests that stressors unrelated to flows are compounding the effects of limited FWI on rangia.

DEDICATION

Dedicated to my grandfathers who taught me to love science and nature and to my father whose strength continues to support me in all my endeavors.

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NOMENCLATURE

AICc	Corrected Akaike Information Criterion
BBEST	Basin and Bays Expert Science Team
CPUE	Catch Per Unit Effort
DistLM	Distance Based Linear Model
FWI	Freshwater Inflows
GBEP	Galveston Bay Estuary Program
LTF	Larger Than Foot
NOAA	National Oceanic and Atmospheric Administration
NWF	National Wildlife Federation
PERMANOVA	Permutational Multivariate Analysis of Variance
PRIMER	Plymouth Routines in Multivariate Ecological Research
SAF	Same As Foot
SB3	Texas Senate Bill 3
STF	Smaller Than Foot
SWQM	Surface Water Quality Management Program
TAMUG	Texas A&M University at Galveston
TCEQ	Texas Commission on Environmental Quality
TPWD	Texas Parks and Wildlife Department
TWDB	Texas Water Development Board
USGS	United States Geological Survey

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1. INTRODUCTION

One of the most pressing challenges in modern society is managing limited natural resources in a way that will both satisfy human needs today and maintain sustainable practices that will ensure the availability of those resources in the future. Freshwater is among these valuable natural resources as it is needed not only for direct human use, but also for the perpetuation of a variety of natural systems. With an adequate supply of freshwater, natural ecosystems can produce other resources important for human consumption making the importance of responsible fresh water management twofold. In this study, fresh water interactions with the ecological hierarchy of the environment were examined in Galveston Bay, Texas to serve as a case study for determining thresholds for fresh water management.

1.1 Galveston Bay

Galveston Bay is the largest estuary along the Texas coast which borders the northwest shore of the Gulf of Mexico (See Figure 1) (Galveston Bay Estuary Program (GBEP) 2013). It encompasses over 1,500 km2 and supports various industrial, recreational, residential, and agricultural human developments (GBEP 2013). Galveston Bay also supports a vast economy as a nursery habitat for its many fisheries industries. Along with providing for over one half of Texas' recreational fishing expenditures, Galveston Bay is the source of one third of the state's commercial fishery income yielding shrimp, blue crab (one third total state harvest) and oysters (the most of any United States water body) (GBEP 2013).

Galveston Bay's watershed extends for over 60,000 km2 up through the Dallas/Fort Worth metroplex (GBEP 2013). The land area covered by the Galveston Bay watershed also includes the highly urbanized greater Houston area as well as many areas of agricultural development (See Figure 1) (GBEP 2013). Anthropogenic impacts in the form of urban and agricultural runoff influence the quality of freshwater that ultimately contributes to Galveston Bay.

1.2 Freshwater Inflows

Freshwater inflows (FWI) are the waters and resources transferred from freshwater origins to estuaries (Longley 1994). They are important controls for salinity in their terminal destinations and are also a method of resource delivery (i.e. nutrients, sediments) for organisms throughout estuaries (Longley 1994; Quigg et al. 2009). In Galveston Bay, FWI are primarily delivered to the estuary via the San Jacinto and Trinity Rivers which flow through the highly urbanized areas of Houston and Dallas/Fort Worth respectively (Lester and Gonzalez 2011). Areas of intense urban development along these rivers negatively alter the runoff that drains into these freshwater sources with nutrient loads and other contaminants (Fitzhugh and Richter 2004; Aitkenhead-Peterson et al. 2011). The same can be said for the extensive agricultural development that occurs in the rural areas between these two major metropolises. FWI are especially important to monitor for nutrient delivery as Lester and Gonzalez (2011) estimated that they are responsible for 96% of the imported carbon and nitrogen as well as 95% of the phosphorous delivered to Galveston Bay. Responsible management of FWI as a critical natural resource is imperative as flows in recent decades may be declining (R^2: 0.31; Figure 2). The real-time and historical data provided by the United States Geological Survey (USGS) gage on the Trinity River at Romayor, Texas (USGS gage 08066500) shows that river discharge (flow) in recent years conforms to a continued trend of declining annual flows from the Trinity River as well as displaying relatively reduced mean monthly flows compared to recent decades (Figure 3).

1.3 Texas Senate Bill 3 and Biological Indicators

In 2007, the Texas Legislature enacted Senate Bill 3 (SB3) which focuses on the conservation of bays and estuaries by determining and maintaining appropriate environmental flows into the natural system in a way that still balances human fresh water needs (Texas Water Development Board (TWDB) 2012). One way to assess the critical amount of FWI needed to maintain bay health is to observe the responses of living organisms in bays and estuaries to changes in FWI. Organisms found to be sensitive to environmental changes are known as biological indicators or bioindicators (TWDB 2015).

Environmental health is often monitored using numerical physical and chemical criteria standards; however, it is imperative to incorporate the monitoring of biological indicators as they inform the ecological functionality of the system and success of regulatory environmental quality measures targeted at controlling physical and chemical stressors (Yoder and Rankin 1998). When selecting potential bioindicators, it is important to consider the criteria upon which management strategies are focused (Wilson 1994). Where FWI are concerned, several estuarine organisms from primary producers to higher trophic-levels such as fish and crustaceans can be affected at varying developmental stages (Sklar and Browder 1998). Though motile estuarine organisms are important to monitor due to their economic importance as fishery capital, they are not ideal indicators for FWI impacts as they are able to avoid unfavorable conditions (Espey et al. 2009). In this case, focus should be directed to sessile organisms such as benthic bivalves which do not have the capability to outrun stressors and are, therefore, highly susceptible to environmental change (Wilson 1994; Beseres-Pollack 2009; Espey et al. 2009; Montagna et al. 2013).

After reviewing literature, the Basin and Bays Expert Science Team (BBEST)—a team established by the Texas legislature—suggested that one potential bioindicator organism for FWI standards in Galveston Bay would be a brackish water clam known as *Rangia cuneata* (Espey et al. 2009). According to literature, *R. cuneata* are only tolerant of environments with salinities of <18 parts per thousand (ppt) (Hopkins et al. 1973; Swingle and Bland 1974; LaSalle and de la Cruz 1985). More importantly, these clams' spawning events are even more narrowly limited to environments with salinities between 2 to 10 ppt (Cain 1973; LaSalle and de la Cruz 1985). Another favorable aspect of *R. cuneata* is that its distribution has been monitored by the Texas Parks and Wildlife Department (TPWD) in several bay systems along the Texas Coast including Galveston Bay for the past several decades. This long term quantitative dataset was used herein to observe *R. cuneata* distribution over time in relation to various abiotic vectors that have been documented throughout the same period. As *R. cuneata* are not widely researched in Galveston Bay, more investigation was needed to verify their soundness as bioindicators of bay health.

1.4 Rangia Clams

The known geographic distribution of rangia ranges in the Gulf of Mexico from Laguna de Terminos, Campeche, Mexico in the east to north western Florida in the north and along the Atlantic coast of North America from Florida up to the lower portion of the Hudson River, New York (Dall 1894; Andrews 1971; Ruiz 1975; Carlton 1992; Wakida-Kusunoke and MacKenzie 2004). *R. cuneata* is considered to be native to the Gulf of Mexico and introduced to the North West Atlantic, where it is predominantly found in estuaries.

Two species within the genus, *R. cuneata* and *R. flexuosa*, are commonly found in Galveston Bay. Clam species is determined by observing its morphology. The bill of *R. cuneata* is rounded and blunt at its most extreme apex (Figure 4A) (Tunnell, Jr. et al. 2010). The bill of *R. flexuosa* has a flat edge and draws to a point at its most extreme apex (Figure 4B) (Tunnell, Jr. et al. 2010). The valve of *Rangia cuneata* is also marked by its long posterior lateral tooth and small but distinct pallial sinus (Figure 4A and B, Figure 5) (LaSalle and de la Cruz 1985; Tunnell, Jr. et al. 2010). Maximum valve length for *R. cuneata* is roughly 80 mm at its widest point (Figure 5) (LaSalle and de la Cruz 1985). *R. flexuosa* on the other hand is generally smaller with maximum valve lengths of 60 mm as well as a short posterior lateral tooth and a nondescript pallial sinus (Figure 5) (LaSalle and de la Cruz 1985).

Rangia can be found in various substrates which can include mixtures of sand, silt and clay (Tenore et al. 1968; LaSalle and de la Cruz 1985). They can withstand water temperatures above freezing and below 32˚C and salinities below 18 ppt (Hopkins et al. 1973; Swingle and Bland 1974; LaSalle and de la Cruz 1985). Their ability to spawn is more strictly regulated by salinities as they are only able to release gametes in salinities between 2 and 10 ppt (Cain 1973; LaSalle and de la Cruz 1985). It is also known that spawning is initiated by a rapid increase or decrease in salinity (Cain 1975). Fertilization occurs in the water column and larvae become shelled within 24 hours after fertilization (Chanley 1965). Most larvae settle on the bottom between September and March and a second settling can occur in midsummer (Cain 1975; Fairbanks 1963). How the juveniles disperse is uncertain but it is known that the adult clams rarely move. The life span of the brackish water clam has not been confirmed but its average life span is thought to be between 4 and 5 years with a maximum of 15 years (Anderson and Bedford 1973; Hopkins et al. 1973; Dauer 1993).

As filter feeders, rangia clams are dependent on the primary production community (i.e. phytoplankton) and organic material in detritus for their nutritional requirements (LaSalle and de la Cruz 1985). Rangia serve as prey items for waterfowl and fish but are not commercially harvested for human consumption in the United States (LaSalle and de la Cruz 1985; Wakida-Kusunoke and MacKenzie 2004).

1.5 Rangia Decline

As mentioned previously, the BBEST (Espey et al. 2009) proposed the investigation of rangia clams as potential bioindicator species of FWI into Galveston

Bay because they can live only within a narrow range of salinities (< 18 ppt) and can only spawn within an even narrower range of salinities (2 to 10 ppt) (Hopkins et al. 1973; Swingle and Bland 1974; LaSalle and de la Cruz 1985). Recently, Parnell et al. (2011) investigated the TPWD historical monitoring dataset of rangia clam distributions in Galveston Bay from 1983 to 2010. These authors found that the abundance of rangia clams has been declining since the early 1980s when observations were first documented (see Figure 6).

Though declining trends in rangia clam populations over time are assumed to be an effect of high salinities in Galveston Bay, no strong evidence exists to support this hypothesis. It is important to examine this potential relationship as many of Galveston Bay's economically important fisheries species may be similarly impacted by such events. It is also critical to recall that human freshwater needs must be balanced with ecological requirements in order to maintain the economic infrastructure of the bay and surrounding regions in the watershed.

2. OBJECTIVE

This study focuses on determining the applicability of rangia clams as reliable bioindicators of bay health in Galveston Bay. Specifically, this study examines the interaction between clam distribution patterns and water quality parameters influenced by FWI. Qualitative data collected throughout the duration of this project will allow for the thorough investigation of clam health as it relates to water quality. The nature of the relationship of rangia health and distribution to salinity will be determined; furthermore, other inflow variables such as nutrient composition and concentration, particulate organic matter and river discharge will be examined for any influence on clam health and distribution, as well as determining whether those variables may have a compound effect on the clams in concert with salinity stress. Applications of these results may help inform management practices concerning FWI and their allocation to both human and ecological interests.

3. HYPOTHESES

Hypothesis 1:

H_0: Rangia are not reliable bioindicators of FWI as their health and distribution patterns share no direct relationship with salinity.

H_A: Rangia health and distribution patterns are impacted by FWI due to strong relationships with fluctuations in salinity levels which supports utilization of the clams as bioindicators of FWI.

Hypothesis 2:

H_0: Rangia are not good bioindicators of bay health as it relates to FWI as they share no significant relationships with water quality variables.

H_A: Rangia health and distribution patterns are impacted by FWI as evidenced by relationships with suite of water quality stressors including salinity, nutrient availability, particulate organic matter and river discharge events which supports utilization of the clams as bioindicators of bay health.

Hypothesis 3:

H_0: Rangia health and distribution are not affected by impacts on FWI connected to drought and river diversions related to land-use change, therefore, they are poor indicators of environmental stress.

H_A: Rangia are good candidates for indicating environmental stress on estuaries as their distribution and heath trends follow patterns of resource limitation exacerbated by compounding effects of drought and land-use change.

4. METHODS

4.1 Historic Rangia And Water Quality Data Analysis

4.1.1 TPWD Dataset

The data set used in this study was collected by the Texas Parks and Wildlife Department and obtained from the office in Dickinson, Texas. These data were collected in randomized wildlife surveys conducted in Galveston Bay from 1983 to 2010 under the Coastal Fisheries Resource Monitoring Program using methods for shrimp trawl, oyster dredge, bag seine and gill net surveys described in Martinez-Andrade and Fisher (2010). Both Lance Robinson (Regional Director of TPWD Coastal Fisheries Division—Fisheries Management Branch for the Dickinson Marine Laboratory) and Bill Balboa (former Galveston Bay Ecosystem Leader for the Dickinson Marine Lab) reviewed the data. This dataset includes long term documentation of rangia abundance and distribution. Data describing the coordinates where rangia were documented, lengths and abundance of clams collected, gear types used to sample the clams, physical data (salinity in ppt, temperature in °C, dissolved oxygen in mg L^{-1} and maximum depth in meters) and dates associated with each sample were used to develop a distribution baseline and examine trends in rangia health and abundance for Galveston Bay rangia.

There is an important caveat which needs to be understood before going further. Because rangia are not commercially harvested, they were never specifically targeted for monitoring by TPWD programs and as such were considered by-catch. This implies that assessment of the rangia population may not have been as thorough as that of target organisms which in turn lead to poor quality assurance procedures. For example, TPWD did not determine if clams were live or dead, they did not always verify species by opening the clam to inspect differences in the valve and catch numbers were estimated when more than 19 organisms were recovered. Furthermore, since neither of the most widely used gear types (shrimp trawl, oyster dredge) used for surveys were deployed to target rangia collection specifically, the mesh size of each apparatus was too large for the reliable collection of clams smaller than 28 mm in length. Therefore, the data are largely affected by the size exclusion of juvenile clams (<28 mm). Due to these factors, only the distribution of the adult population of rangia in Galveston Bay can be estimated from this dataset.

Catch per unit effort (CPUE)—an abundance measurement—and shell length data for rangia collected using the aforementioned methods from 1983 to 2010 were the focus of the historical analysis. All data were synthesized using functions available in Microsoft Office Excel 2010. In addition to calculations for means and standard deviations, Excel functions for data visualization were used to generate all charts and tables referenced throughout the report. Excel calculations were also used for all trendline projection and R^2 values represented on charts in the body of the text. Excel statistical functions were used to generate the results of two-way t-tests describing historical shell length and CPUE data.

To observe spatial trends in rangia abundance over time, ArcMap GIS software (version 10.2) was used to plot the distribution of the clams in Galveston Bay by overlaying graduated symbols representing different magnitudes of clam catch at recorded points throughout Galveston Bay on a shapefile of the bay. Information from all gear types used by TPWD including shrimp trawl, oyster dredge, bag seine and gill net were observed, however, only data collected from the shrimp trawl and oyster dredge data were presented as it formed a meaningful majority (99%) of the historic data. This approach was also applied to the data presented by NWF (2009, 2012) for Sabine Lake and Guadalupe Estuary respectively with TPWD trawl data.

4.1.2 TCEQ Dataset

The Texas Commission on Environmental Quality (TCEQ) has amassed a dataset for water quality in Galveston Bay that is temporally comparable to the TPWD rangia dataset via the Surface Water Quality Monitoring (SWQM) Program. This dataset details environmental parameters at sites throughout Galveston Bay including physical metrics comparable to those recorded by TPWD (temperature (C°), salinity (ppt) and dissolved oxygen (mg L^{-1}) as well as concentrations of total organic carbon and dissolved nutrient concentrations (mg L^{-1}) for nitrite and nitrate, ammonium, and total phosphorous and chlorophyll *a* concentrations (µg L^{-1}). To maintain a random sampling strategy, TCEQ sampled subsets of their extensive site network by cyclically alternating between different geographic subsets each year. However, for each sub-bay (Trinity, Upper and Lower Galveston, East and West Bay) one benchmark station was consistently sampled over the course of the historical record. For Trinity Bay, the benchmark station is a centrally located point near the Exxon C-1 platform referred to as station number 13315 (29°39’54”, -94°47’12”) (Figure 7). For the purpose of observing long term environmental trends affecting the sub-bay where the majority of the historical rangia population was described, this analysis focuses on data retrieved from this station.

In addition to extensive water quality analysis, TCEQ also conducted surveys of benthic fauna in Galveston Bay from 1992 to 2008. Benthic data from the TCEQ is limited especially in the case of rangia due to both sampling methods and the gear that were used at each station. As with SWQM data, benthic sampling was also conducted using the random sampling strategy of rotating groups of stations each year. Coverage was not as extensive as water quality sampling efforts as only half of the stations assessed for abiotic parameters included benthic analysis. Furthermore, four deployments of 1x1 ft Ekman box cores were used to sample the benthos allowed for a narrow range of rangia bed sampling and may have missed clams settled just outside the area of the cores.

Both water quality data and rangia data collected by the TCEQ were synthesized with the same methods used for TPWD data analysis. Because the data from the two different agencies were not collected simultaneously or in the same locations, direct comparisons or integrated syntheses between the datasets were not used. However, by comparing the two analyses separately, long term trends regarding environmental quality and rangia populations were used to inform strategies for present-day sampling and analysis.

4.2 Current Rangia Health Metric and Water Quality Data Analysis

4.2.1 Site Selection and Early Sampling Methods

In preliminary studies conducted from October 2010 through August 2011, the presence or absence of rangia throughout Galveston Bay with a focus on Trinity Bay was explored with the help of TPWD personnel, Bill Balboa and Brad Grimmet (Fish and Wildlife Technician of the Dickinson Marine Lab in Dickinson, Texas). Sample sites were first selected based on areas of historically high abundance of rangia clams referenced from the data represented in Figure 6 (Parnell et al. 2011) mapped using ESRI ArcMap Version 10.2. As the sampling period progressed, site selection was directed toward coverage of Galveston Bay to gain a better understanding of current rangia distribution (Figure 8). Sites were accessed by boat and sampled for the presence or absence of rangia with standard TPWD gears (oyster dredges or shrimp trawl nets depending on gears used historically at each station) per the protocol outlined in the TPWD Marine Resource Monitoring Operations Manual (Martinez-Andrade and Fisher, 2010). Water quality parameters including salinity, temperature, dissolved oxygen and depth were recorded using a calibrated YSI Pro 2030 (Table 1). Surface water (0-0.5 m) was collected for laboratory analysis of chlorophyll *a*, photosynthetic activity, total suspended solids, particulate organic matter and nutrient composition/concentration per the methods described by Quigg (2012) in the report to the TWDB.

Physical dimensions and health metrics of clams were further analyzed in the laboratory per methods suggested by Dr. Sammy Ray of Texas A&M University at

Galveston (TAMUG). His methods involved observation and documentation of shell length, width and height, wet meat weight, meat index and gonadal development.

Gonad tissue removal to examine gonad development using simple visual staging—“larger than foot” (LTF), “same as foot” (SAF), or “smaller than foot” (STF)—was performed according to instructions from Dr. Sammy Ray (TAMUG). After early studies yielded gonadal observations that were almost homogenously classified as LTF (Figure 20), this metric was discontinued after October 2011. Gender of each clam and their stages of gamete development were analyzed by smearing gonad tissue on a slide and examining it with light microscopy to observe the presence of egg or sperm cells; this was also a possible metric for reproductive potential according to Dr. Sammy Ray (TAMUG). Finally, sex ratios were determined by comparing the number of male and female clams in each sample set.

Shell length—the longest distance across the span of the valve—was assessed with digital calipers according to TPWD procedures (Martinez-Andrade and Fisher 2010) for each clam brought back to the laboratory. Additionally, shell width—the longest transect from umbo to bill—and shell height—a bilateral transect at the clam’s thickest point—were collected per the suggestion of Dr. Sammy Ray.

Meat index was determined by weighing the clam with shell intact, then shucking the wet tissue out and weighing just the tissue. The ratio of wet meat to whole weight determines the meat index (Equation 1).

Equation 1 Calculation of meat index using the ratio of wet tissue to whole clam weight expressed as a percentage.

$$\left(\frac{\text{Weight}_{\text{wet meat}}\ (\text{g})}{\text{Weight}_{\text{whole clam}}\ (\text{g})}\right)*100=\text{Meat Index (\%)}$$

In addition to presence-absence surveys conducted in the greater part of Galveston Bay with the help of TPWD, further preliminary presence-absence studies carried out from March 2011 to October 2011 by TAMUG personnel with TAMUG vessels focused more intensely on rangia populations in the Trinity River Delta to gather data for a more complete analysis of the dynamics affecting clams at the river delta-bay interface (see Figure 9 for more detail). Due to the lack of historic TPWD sampling coverage in this area, there was no precedent for former collection gear used as there was for the majority of Galveston Bay. Therefore, in order to test for the presence or absence of clams, these shallow water (<1 m) sites were initially sampled by hand, employing rakes or trowels where sediment was difficult to excavate. As with the preliminary samples collected with TPWD, sites sampled at the river delta had their physical water quality data assessed via Hydrolab MS5 water quality multiprobe (see Table 1) and a surface water sample was collected to examine the primary production community and nutrient content. The same laboratory analyses of clam health used to examine the specimens collected with TPWD were also executed on rangia collected during these smaller-scale expeditions.

In addition, sediment samples were collected from the river delta sites and analyzed for sediment grain size, porosity and organic content to better understand clam bed substrate. Grain size was measured with a Malvern Mastersizer 2000 Laser Particle Diffractometer to determine the percent contribution of sand, silt, clay and gravel at each site (Malvern Instruments, Ltd. 1999). Porosity was estimated by comparing a known weight of sediment inundated with a known volume of water to the weight of the sample after drying in an oven. Organic content was similarly determined by comparing the original weight of a sediment sample to its weight after combustion.

From October 2011 to November 2014, an intensely qualitative analysis was conducted on five sites at the Trinity River Delta selected for their coverage and reliability as sample sites in the past. Figure 10 depicts a map of five sites that were sampled during this time. Sample expeditions included rangia collection by means of the excavation of the area within a metal quadrat (Figure 11) which allowed for the calculation of clam biomass and abundance at each site. After anchoring at site, the metal quadrat was tossed haphazardly into the water and allowed to sink to the substrate. Each of the two rectangular sections (0.33 m x 0.54 m) were excavated with hand trowels to a critical depth of 0.3 m into the substrate. This process was repeated for a total of four excavations. Because of the consistent dimensions used for excavation, biomass and abundance (density) calculations were performed for each sample site. Biomass was determined by dividing the total weight of clams collected from the volume of substrate (Equation 2) and density was calculated by dividing the total number of clams found by the area sampled (Equation 3).

Equation 2 The calculation of biomass using the sum of all clam weights divided by the volume of sampled substrate.

$$\frac{\sum \text{Weight}_{\text{whole clam}}(\text{g}) \text{ Retrieved From Quadrat}}{\text{Length}_{\text{quadrat}} \text{ (m)}*\text{Width}_{\text{quadrat}} \text{ (m)}*\text{Critical Depth (m)}} = \text{Biomass (g m}^{-3}\text{)}$$

Equation 3 The calculation of density using the number of clams divided by the sample area.

$$\frac{\text{N}_{\text{clam}} \text{ Retrieved From Quadrat}}{\text{Lengh}_{\text{quadrat}} \text{ (m)}*\text{Width}_{\text{quadrat}} \text{ (m)}} = \text{Density (N m}^{-2}\text{)}$$

All clams collected were placed in labeled bags according to the quadrat replicate they were retrieved from and set on ice bottles in an insulated cooler until they could be examined in the laboratory. A minimum of ten clams were needed for full internal and external assessment of health metrics including meat index, sex classification, gonad development and external dimensions; if clams collected from a site were in excess of ten, the extraneous clams' external dimensions (length, height and width) and whole weights were recorded and the clams were then discarded back into the estuary. In the event that the four excavations did not yield a collection of at least ten clams, the area around the sample site was searched by hand (up to 10 m) to bring the total number of clams collected to ten. Clams found with this method were placed in bags labeled "outside quadrat." To attain a dataset comparable to the preliminary expeditions, water quality, sediment grabs and surface water were also collected and evaluated at these sites. Over time, Stations 1 and 5 proved to be the most reliable sites for finding live clams whereas Stations 2, 3 and 4 were more useful for analyzing the water quality gradient and other environmental factors between Stations 1 and 5 (Figure 10).

4.2.2 Data Synthesis and Multivariate Statistical Analysis

Data collected during the present-day study will be synthesized with similar methods used for historical rangia analysis. Microsoft Office Excel 2010 functions ranging from calculations for means and standard deviations to linear regression analysis will be used to observe any clear relationships between clam health and individual environmental factors.

Contrary to the relatively simple analysis of historical rangia and water quality data, the current dataset was examined with the multivariate statistical analysis software PRIMER-E V6.1.15 with the PERMANOVA V1.0.5 add-on package (Plymouth Routines in Multivariate Ecological Research (PRIMER); Clarke and Warwick 2001; Anderson et al. 2008). The repeated sampling design at specific locations used in the present-day analysis was utilized to conduct a synthesis of biological clam data as it relates to the environmental variables collected in the Trinity River Delta (Table 2).

To determine the extent of variance in all current biological data relevant to clam abundance, a PERMANOVA main test with 9,999 permutations was performed on a Bray-Curtis resemblance matrix of clam abundance data from all five stations at all timepoints across the three year study period. When constructing the resemblance matrix for the abundance data, a dummy variable of 1 was added to all values in order to eliminate 0 values from the dataset. For this analysis, the pseudo-F statistic is more supportive of the hypothesis that no variance in the dataset exists as the value approaches 0, and significance of variance is indicated by a P(perm) value of <0.05. The results of this initial PERMANOVA test were used to guide the analysis of an additional PERMANOVA main test with 9,999 permutations was conducted on a Bray-Curtis resemblance matrix of health metric data including shell length, wet meat weight, whole weight and meat index on a per-specimen basis collected during each sampling event to quantify variance between and within spatiotemporal factors of this more robust dataset.

Environmental data corresponding to the dates and locations of each clam sample will be used to determine whether abiotic variables were correlated to the variance in clam health metric factors. To eliminate co-linearity among the environmental parameters a test of co-variance (>0.90 similarity) was conducted. Of the suite of environmental parameters included in the analysis, only the combination of nitrate and nitrite was removed due to its similarity (>0.90) to nitrate alone. To more evenly distribute data, the data were square root transformed before they were normalized. The normalized data were then used to construct a Euclidean Distance Resemblance matrix.

A distance based linear model (DistLM) running the BEST test with Akaikes-corrected (AICc) selection criteria (9,999 permutations) was run on the Bray-Curtis resemblance matrix of the biotic health metric data with the environmental parameters as the predictor variables. The DistLM was run to examine individual variable (marginal) and overall best combinations of up to ten environmental variable correlations which best explain the variance in the biological dataset. The pseudo-F Statistic value determined by the DistLM marginal test is more supportive of the hypothesis that there is no correlation between the environmental variable and the biological data as it approaches 0. The results of this test also explain the proportion of the variance in the biological data by each environmental variable. The DistLM overall best test results are determined by observing the combinations of environmental variables with the lowest AICc values (within one whole number of each other) and the highest R^2 values. The results of DistLM models distinguish the multivariate environmental influence on clam health that simple linear regression models do not adequately describe.

5. RESULTS

5.1 Historic Water Quality

Figure 12 represents the summarized results of simple linear regression models developed for the major environmental parameters monitored by the TCEQ SWQM effort from 1983 to 2010 at the Trinity Bay benchmark station. Chlorophyll *a* was included in the analysis as an environmental factor rather than a biological component as it serves as an indicator of water quality (Steele 1962; Cullen 1982; Boyer et al. 2009). Additionally, chlorophyll *a* concentrations were represented on a logarithmic scale at the suggestion of Linda Broach (TCEQ) to observe a better homogenize the variance in the long term dataset. The regression models showed no clear temporal trends (all R^2 <0.15) over the thirty-year period in any of the individual environmental parameters related to the physical environment or FWI.

5.2 Historic Rangia Data

The general analysis of the CPUE and shell length data for rangia collected by TPWD from 1983 to 2010 yielded the results displayed in Figure 13 and 14. Of all the different gear types used to sample Galveston Bay, shrimp trawls yielded the highest total CPUE (38,400 rangia clams) across the historical record—an order of magnitude greater than the total CPUE of the second most effective gear, the oyster dredge (2,168 rangia clams). Furthermore, gill net and bag seine surveys yielded low total CPUE numbers over the course of the study period (66 and 250 respectively) and made up <1 % of the historical CPUE data from TPWD; therefore, those data were excluded from further analysis. Although shrimp trawl CPUE was greater than CPUE from the oyster dredge, mean shell lengths of *R. cuneata* collected with shrimp trawls (33.6 ± 7.4 mm) were smaller than those collected via dredge (49.2 ± 8.6 mm) (Figure 14). Using t-tests assuming unequal variances, it was found that mean rangia lengths collected by shrimp trawl were significantly smaller than those collected by oyster dredge (t-statistic:-52.30; p <0.01; Table 3).

When observing the data spatially (Figure 15), rangia appear to be more concentrated in Trinity Bay than in any other sub-bay in the Galveston Bay complex. However, it is clear that the areal coverage of shrimp trawl surveys are not consistent with those conducted with oyster dredges both across the comprehensive span of the historical record and within decades. T-tests were used to test for differences between the decadal means of CPUE reported with trawl and dredge methods from 1983 to 2010. The magnitude of rangia CPUE from shrimp trawls was significantly greater than that of oyster dredge CPUE (t-statistic: 5.28; p: 0.01; Figure 13; Table 3). To observe decadal changes in CPUE numbers within gear types, t-tests comparing decadal mean rangia CPUE were performed on both trawl and dredge data (Tables 4 and 5). Trawl CPUE in the 1980s was significantly greater than that reported for the 1990s (p <0.01), the 2000s (p <0.01) and the sum of the two latter decades together (p <0.01). However, trawl data from the 1990s and 2000s were not significantly different from each other (p: 0.58; Table 4). The analysis of dredge data showed no significant differences between data from the 1980s and 1990s (p: 0.39) or 2000s (p: 0.09), or between the 1990s and 2000s (p: 0.21; Table 5). However, the t-test analysis indicated that dredge CPUE data from the

2000s was significantly less compared to the sum of the data from the previous two decades (p <0.01; Table 5).

Linear regression analyses were conducted on rangia CPUE and length data from different gear types to observe the effects of environmental parameters (salinity, temperature and dissolved oxygen) on the rangia data. Figure 16 displays the summarized relationships between rangia CPUE and shell length recovered with shrimp trawls and environmental parameters. Observing the results of these linear regressions, no clear, direct trends exist between any of the environmental data parameters and trawl-collected rangia CPUE or shell length (no R^2 values >0.05). In Figure 17, a similar summary of linear regression results of TPWD rangia CPUE and shell length data recovered with oyster dredges plotted against salinity, temperature, and dissolved oxygen is shown. Again, the models (no R^2 values >0.10) reiterate that CPUE and shell length of rangia collected via dredge are not directly related to long-term changes in temperature, salinity or dissolved according to the data available.

TCEQ benthic biological data were collected between 1992 and 2008. The station selection process mimicked the cyclical alternation of SWQM, however, only half of the stations monitored for water quality were concurrently assessed for benthic biological data. The randomized subset of annually sampled benthic stations was selected for areal coverage. As discussed in the methods, the Ekman box cores used to sample the benthos impose a bias on rangia CPUE data by limiting the area of substrate sampled. Furthermore, the data set does not include other metrics aside from CPUE. Clam distribution data collected by TCEQ is represented in Figure 18. From the figure, it is clear that rangia clams were found in more locations and in greater numbers in Trinity Bay than in any of the other sub-bays in the estuary by TCEQ.

To observe how the TCEQ and TPWD rangia datasets compare, mean annual CPUE from each major gear are plotted over time in Figure 19. While each agency's sampling events collected the same organisms in the same area across similar temporal scales, no shared trends in rangia CPUE exist between any of the sampling methods. These inconsistencies further support the use of more controlled sampling methods applied in the present-day study.

5.3 Current Rangia Data

Preliminary studies conducted from October 2010 through October 2011 verified the presence or absence of rangia throughout greater Galveston Bay (Figure 8) and stations in the Trinity River Delta (Figure 9). In this early study period, estimations of rangia gonad development were made using visual staging but the observation of this metric was discontinued after October 2011 due to lack of variation in the results. Of all clams observed across Galveston Bay in the preliminary year of study, 72% had gonads larger than their respective foot muscles (Figure 20). In fact, the majority of rangia gonads were classified LTF regardless of sex (females: 83%; males: 68%) (Figure 20). When broken down further by season, LTF gonad observations contribute the majority (>60%) of the results for spring, summer and fall months; in winter months, LTF observations were lower (43%) but still more common than SAF (33%) and STF (24%) observations (Figure 21).

Since late 2011, data collection was more focused at five locations near the interface of Trinity Bay and the Trinity River Delta as shown in Figure 9. Of the five stations, only two (Station 1 and Station 5) supported consistent rangia populations. The results below will describe findings from Station 1 (referred to as “Open Bay”) and Station 5 (referred to as “River Delta”) as they pertain to the clam health metrics listed in the methods.

Figures 22 and 23 describe the sex ratios between ten randomly selected male and female clams identified at each site. To observe this metric’s potential as an indicator for high salinity stress as suggested by Parnell et al. (2011), numbers of clams identified as male or female are plotted on the primary axis and salinity values are plotted on a secondary axis. No common trends are apparent between the ratio of male to female rangia and changes in salinity. For example, rangia sex ratios in the spring of 2012 and the spring of 2013 at the Open Bay site were more heavily weighted by males though the salinities were fresher (1.51 ppt) in 2012 and more saline (17.56 ppt) in 2013 (Figure 22). Additionally, in the spring of 2012 and the spring of 2013 clams from the River Delta displayed the same 1:1 male to female sex ratio despite low salinity (1.16 ppt) conditions in 2012 compared to 2013 (12.68 ppt) (Figures 23).

Figures 24 and 25 describe the shell length frequency of rangia clams collected in the Open Bay and River Delta sites. For these figures, the total catch of all live clams recovered from the four quadrats according to five size classes (<16mm, 16-28mm, 28-38mm, 38-45mm, and >45mm) described by Wolfe and Petteway (1968) as subsequent one-year age classes are represented on the y-axis. They show that all samples were heavily weighted by adults and emphasize the occurrence of juveniles since those size classes are shown in the green. At both sites, a majority of the clams collected are large enough to be classified as adults (Wolfe and Petteway 1968; LaSalle and de la Cruz 1985). Five different age classes were documented at the Open Bay station compared to only four at the River Delta station. Of clams assessed between 2012 and 2014, 6% of the Open Bay catch was juvenile whereas the River Delta catch was just 3% juvenile. At both stations, the incidence of smaller clams was greatest in both winter and summer months with juvenile clams (>28 mm) contributing 11% of total seasonal catch in winter and 3% of total seasonal catch in summer (Figure 26).

In Figure 27A, the monthly mean shell length of clams from both the Open Bay and River Delta sites are plotted over time to examine temporal patterns. As shown in the figure, mean shell lengths trend toward slight increase over time though the linear regression model does not fit the majority of the data (R^2=0.12). When the annual means of rangia shell length collected from all sites during the present day study are plotted as shown in Figure 27D, the increasing trend (R^2 =0.97) over time is more evident growing from 47.29 ±7.01 mm to 49.46 ±9.09 mm from 2012 to 2014.

Figure 27B describes changes in the monthly mean meat index—the ratio of wet tissue weight to the total weight of each clam—values for clams found in the Open Bay and River Delta stations. The monthly means of this metric do not conform to a strong linear relationship (R^2 =0.06) over time at the Open Bay and River Delta sites; however, annual mean meat index of all clams found at the Open Bay and River Delta sites during the present-day study exaggerate a slight declining trend (R^2=0.99; Figure 27E) from 11.5 ±2.7 % to 11.2 ± 2.5 % from 2012 to 2014.

Mean monthly areal density of clams per square meter collected from the Open Bay and River Delta sites during the present-day study from 2012 to 2014 are shown in Figure 27C. As with meat index, this metric shows a slight decrease over time (R^2=0.11) which is exacerbated (R^2 =0.92) by calculating the annual means of the Open Bay and River Delta rangia densities recorded from 2012 to 2014 (Figure 27F). In 2012, clam densities averaged 28.61 ± 16.95 m^{-2} and decreased to 13.19 ± 10.19 m^{-2} by 2014.

Linear regression analyses between clam health metrics (shell length, meat index, density and sex ratios) and physio-chemical parameters were conducted and the results were represented as a histogram in Figure 28. In the figure, the x-axis represents the slope orientation of the trend line fitted to each plot as well as each trend line's R^2 value. Despite the extended suite of simultaneous environmental and biological parameters collected during the present-day study compared to the historical record, no clear relationships were observed between any of the possible combinations of biological and environmental data (all R^2 <0.25).

Using the PRIMER-E v6.1.15 with a PERMANOVA V1.0.5 add-on package, further investigation of the potential impacts of multiple environmental stressors on rangia health was conducted via multivariate statistical analyses as described in the methods. The results of a PERMANOVA Main test applied to rangia abundance data from all stations are shown in Table 6 and prove significant variance in rangia abundance (P(perm) <0.05; yellow highlight) at all a priori spatio-temporal factor levels except the crossing of stations at different annual timepoints and at different seasons within years. Table 7 displays the results of a PERMANOVA Main test applied to a more robust dataset of specific health metrics (shell length, wet meat weight, whole weight and meat index) from a subset of five randomly selected clams from each sampling event. As with the results of the abundance analysis, significant variance in rangia health (P(perm) <0.05; yellow highlight) was observed at all a priori spatio-temporal factor levels except the crossing of stations at different annual time points and at different seasons within years.

To examine relationships between a multivariate biological dataset of rangia health metrics and individual environmental stressors, a DistLM using the Best test was run (using a marginal test). Additionally, a test selecting for the overall best combinations of up to ten environmental variables which best explained the biotic data was run with the DistLM. Table 8 shows the results of the marginal test in which significant relationships (P-value <0.05) between individual environmental variables and the multivariate biological dataset are highlighted in yellow. It is clear that several physio-chemical variables are significantly correlated to the variance in the biological data and that among them, salinity has most significant P-value (0.0002), and the best values for the pseudo-F statistic (11.595) and proportion of data explained (6%). Other significant influences on rangia health were observed with dissolved oxygen (P=0.0006; Prop.= 5%), ammonium (P=0.0013; Prop.=4%), phosphate (P=0.0017; Prop.=4%), river discharge rates (P=0.0016; Prop.=4%), nitrate (P=0.0038; Prop.=3%), and water depth (P=0.0067; Prop.=3%). However, all of the environmental variable data collected explain only 33% of the variance (green highlight). In Table 9, the three lowest and most similar (within range of <1.00 from the minimum) AICc scores generated by the overall best DistLM analysis are shown. No combination of environmental drivers yields an R^2 value >0.21 to fit the trend in the biological data. Each combination of drivers which best explains the data are diverse and comprise of six or more variables including nitrate, phosphate, and salinity (vectors influenced by FWI) and variables such as temperature and depth (variables independent of the influence of FWI).

6. DISCUSSION

6.1 Rangia in Galveston Bay Compared to Other Estuaries

The data from this research communicate with findings reported previously in related studies examining life-cycle and distribution trends of rangia clams. Rangia in Galveston Bay are comparable to clams of the same genus in locations throughout the Gulf of Mexico due to geographical and physical similarities as well as conforming to patterns of environmental response described in literature. Therefore, Galveston Bay rangia are appropriate to investigate as indicators of ecological health which could be applied in other bay systems throughout the Gulf of Mexico.

6.1.1 Morphology and Life Cycles

Clam length, weight, meat index (Tenore et al. 1968; Okumus and Stirling 1998; Royer et al. 2007) and gonadal tissue (Jovanovich and Marion 1989) were assessed to illustrate health of organisms from *in situ* conditions. Shell length is a commonly collected growth metric for mollusks including rangia (Tenore et al. 1968; Martinez-Andrade and Fisher 2010). Wakida-Kusunoki and MacKenzie (2004) reported that *R. cuneata* harvested in Mexico range in length from 30 to 70 mm. In Galveston Bay, Auil-Marshalleck et al. (2000) reported that shell lengths of rangia collected by TPWD between 1986-1998 fell within a range of 26 to 59 mm. Looking more closely at clam health metrics collected from the present-day study, the data show that shell length of *R. cuneata* collected in Galveston Bay typically fell within the range of 30 to 50 mm with a mean of 44.1 ± 9.75 mm. These lengths are typical of the species (Wolfe and Pettaway

1968; LaSalle and de la Cruz 1985; Wakida-Kusonoki and MacKenzie 2004). Furthermore, mean shell lengths in Galveston Bay are very similar to the mean of 44.3 ± 6.1 mm reported recently in Sabine Lake, a neighboring Texas estuary, by NWF (2009).

Shell length is not only useful as a metric for comparing populations of rangia across spatial and temporal scaes; moreover, it is important as an indicator of growth and therefore age as determined previously by Wolfe and Pettaway (1968) and applied by others (Cain 1975; LaSalle and de la Cruz 1985). Shell length as a proxy for age can be used to gauge rangia success and longevity at a fixed point at the organism level (Wolfe and Petteway 1968). Historic data were not sufficient to draw conclusions about long-term age-class patterns due to size exclusion of juvenile (<28 mm) clams by all gear types used by TPWD and no record of rangia shell length from the TCEQ database. However, current shell length size class data and visually staged gonadal development data support the hypothesis that Galveston Bay rangia experience similar lifecycles of spawning and settling to those described by Wolfe and Pettaway (1968) and Cain (1975). For size class data, incidence of juvenile rangia was highest at both the Open Bay and River Delta sites in winter and late summer months (Figures 24 & 25) with the highest percentage of juveniles contributing to total seasonal catch across the study period in winter (Figure 26).

Gonaldal development results were largely dominated by LTF observations, however, when observing seasonal distribution of gonadal size classes (Figure 21) smaller (SAF, STF) gonads are observed with greater regularity in the winter and could indicate recent spawning/expulsion of gametes that would result in “spent stage” gonads

(Cain 1975). These data support the hypothesis that juvenile clams settle in the cool weather periods between September and March and again in midsummer after spawning events that are thought to occur in the fall and spring (Cain 1975; LaSalle and de la Cruz 1985). Therefore, the size class data of both shell length and gonadal development indicate that overall rangia spawning and settling patterns at the delta-bay interface were typical of patterns described in literature.

6.1.2 Abundance and Distribution

Beyond the evaluation of simple growth metrics, rangia abundance and distribution are among the most important characteristics to assess because they can communicate trends in populations across wide spatial and temporal scales which can better inform the investigation of environmental variables that may drive such patterns. When examining the historical distribution of rangia in Galveston Bay as recorded by TPWD, the highest abundances and most consistent populations were found near the mouth of the Trinity River, Galveston Bay's primary source of FWI (Powell and Solis 1997; Buzan et al. 2009; Lester and Gonzalez 2011). Rangia CPUE collected via shrimp trawl, oyster dredge, bag seine and gill net by the TPWD was most concentrated in Trinity Bay with the greatest numbers recorded near the Trinity River Delta across all three decades in the period of study as reported by Parnell et al. (2011; Figure 6). When broken down further by separating CPUE data by collection method, the distribution trends persist for both shrimp trawl and oyster dredge rangia catch (Figure 15) which supports a link between FWI and rangia distribution as stated in Hypothesis 2. Rangia data collected from gill net and bag seine surveys were geographically restricted to the shorelines of Galveston Bay and as such, were not able to contribute to the analysis of changes in the species' spatial distribution. Higher abundances near the Trinity River mouth are more prominently displayed by TPWD shrimp trawl data, however this is due in part to limited spatial coverage of oyster dredge samples compared to the extensive shrimp trawl dataset (Figure 15). Further discrepancy is likely an artifact of physical differences in sampling equipment and how those differences impact the effectiveness of the gear in varying substrates. Shrimp trawl nets (38 mm mesh) were better suited to collecting rangia compared to oyster dredges which were subject to large mesh size (76 mm) and wide spacing between the dredge teeth (51 mm center to center; Martinez-Andrade and Fisher 2010) which excluded smaller clams. This ultimately allowed for a wider variety of sizes caught and a higher CPUE via shrimp trawl as described in Figures 13 and 14. These findings are supported by Auil-Marshalleck et al. (2000) who conducted an analysis of a subset of the dataset, from 1986-1998.

Benthic data collected by Ekman box cores deployed by the TCEQ also support the historic trend of rangia populations favoring distribution in Trinity Bay with high abundances near the river mouth despite the limited spatial and temporal scope of their effort compared to TPWD wildlife surveys (see Figure 18). Current distribution data further supports this finding as preliminary efforts to determine presence or absence of rangia throughout Galveston Bay conducted with TPWD personnel and gear from late 2010 through 2011 resulted in the documentation of rangia populations concentrated at the Trinity River mouth (Figure 8). These findings are consistent with distribution data recorded in other Gulf estuaries including Mobile and Perdido Bay in Alabama (Swingle and Bland 1974), the Neches River (Harrel 1993) and Sabine Lake (NWF 2009) in Texas.

In Galveston Bay and other estuaries, the consistent distribution of rangia near river mouths is important to note because it indicates greater success of settling and recruitment of clams where frequency of freshwater pulses are higher. Cain (1973, 1975) and Hopkins et al. (1973) drew similar conclusions after observing spawning events that occurred after rapid changes in salinity (up from low salinities or down from high salinities) and low survival of rangia larvae in salinities outside the range of 2 to 10 ppt. In the current study, rangia populations with the highest abundances appear to become more concentrated near the Trinity River mouth and more diffuse in the Trinity Bay basin over time which could be an indication of environmental stress that is mitigated by the availability of FWI. This trend could be linked to decreased annual discharge from the Trinity River across recent decades (Figure 2). The effects of this resource deficit on rangia collected during the present-day analysis may have been further compounded by an "exceptional" or D4 level statewide drought in 2011 which was severe enough to create water shortages in Texas streams and reservoirs (Nielsen-Gammon 2012). Additionally, land use changes including river diversions and the construction of levees and dams as a result of increased urbanization may be altering the flow regimes necessary for consistent recruitment of rangia (Powell et al. 2003). If this is the case, the importance of observing rangia growth metrics in conjunction with distribution patterns increases as these variables will help determine the extent of the impacts of decreased flows.

6.2 Trends Toward Decline and Investigation of Drivers

6.2.1 Historic Agency Data

After reviewing the results of Parnell et al. (2011) and the analysis of historical and present-day rangia distribution data from the current study, an investigation of the soundness of the declining trend in rangia abundance and distribution was conducted. In a similar study, Auil-Marshalleck et al. (2000) pursued the same question and tested a more temporally limited dataset (1986-1998) using simple statistical analyses. Auil-Marshalleck et al. (2000) found significantly higher yields of CPUE in the late 1980s compared to the 1990s when examining trawl samples but not in oyster dredge work. The current study confirmed the disparity between an extended dataset (1983-2010) of decadal CPUE means from shrimp trawl and not with oyster dredges (Tables 4 and 5) which indicates that changes in the methods used during trawling events over time are a factor contributing to the pattern of decline in rangia abundance. Because trawl data from the 1990s and 2000s analyzed in the current study were not significantly different from each other ($p=0.58$), the data suggest that methods of collection or reporting were similar between the two decades. Additionally, there were no significant differences between consecutive decades for mean oyster dredge rangia CPUE (Table 5). The significant ($p < 0.01$) reduction CPUE numbers observed in the 2000s compared to the combined CPUE from the 1980s and 1990s are more supportive a genuine decline in rangia recovery. Considering this evidence in conjunction with the discrepancy in physical differences between the shrimp trawls and oyster dredges (mesh size, targeted substrates, etc.), this analysis supports the hypothesis that historic rangia length and

CPUE data are largely biased by collection method. However, due to the consistency of mean rangia CPUE collected by oyster dredge in recent decades, trends of restricted distribution and decreasing abundance over time are indicative of real change.

To investigate whether individual environmental vectors drove trends in rangia abundance, linear regression models pairing TPWD historical environmental data vectors (temperature, salinity, and dissolved oxygen content of the water column) and associated CPUE numbers collected both by shrimp trawl and oyster dredge were used to observe potential relationships. Results were inconclusive as no clear linear relationships were produced (Figure 16 and 17). In the case of linear regressions between salinity and rangia CPUE, inconclusive results are more supportive of the null for the Hypothesis 1 which states that salinity does not have a direct relationship with rangia abundance. These results imply that CPUE as a metric is independent from direct influence of specific environmental vectors and is not a robust indicator for the relationship of rangia to the physical environment. This further strengthens the argument that in order to thoroughly investigate the fitness of rangia as indicators of environmental change, more specific health metrics than simple abundance must be monitored to better understand their interactions with the environment.

Rangia shell length was the most organism-specific metric recorded by TPWD from 1983-2010. As was the case with CPUE, linear regression analyses comprised of environmental vectors compared to shell length did not yield any clear relationships (Figure 16 and 17). These results do not necessarily negate the usefulness of shell length as an indicator of rangia response to environmental changes in Galveston Bay; rather, they are more indicative of the aforementioned bias in data collection imposed by gear related size exclusion which only accounted for adult size classes of rangia (length 33.61 ± 7.36 mm for *R. cuneata* collected by trawl and length 49.18 ± 8.60 mm for *R. cuneata* collected by dredge) (Wolfe and Pettaway 1968; LaSalle and de la Cruz 1985).

According to TCEQ water quality variables recorded at the Trinity Bay benchmark station Exxon-C1 from 1983-2010, no clear linear trends exist for independent environmental factors over the wide temporal scope that could help to understand historic changes in rangia abundance and distribution (Figure 12). Water quality data shown in Figure 12 are biased by insufficient detection limits of laboratory equipment (areas of tightly clustered data at the same y-value) and only reflect temporal changes in variables from the perspective of one point in Galveston Bay; however, despite the shortcomings of the extensive water quality record associated with the TCEQ Trinity Bay benchmark station, the results indicate that changes in rangia metrics reported by TPWD over time are not easily explained by long term trends in independent environmental variables. Therefore, closer inspection of fine-scale climatological and environmental events must be assessed in order to determine what contributes to changes in rangia.

6.2.2 Current Study Data

In the present-day study, a wider range of shell lengths were collected due to the qualitative nature of the quadrat excavation sampling design. Studies such as those done in Mobile and Perdido Bay, AL (Swingle and Bland 1974) and in the Neches River, TX (Harrel 1993), found that mean rangia shell lengths were greater and exhibited fewer age classes in regions where freshwater is consistently available than those in more variable salinity regimes. The data from the present-day study confirms these observations with more variation in age class at the Open Bay station in contrast with the River Delta station (see Figures 24 and 25). Additionally, mean shell lengths were larger (49.29 ± 6.49 mm) at the River Delta compared to the Open Bay mean of 44.94 ± 8.30 mm. Though juvenile clams were documented at both the Open Bay and River Delta sites, mean monthly rangia shell length trended toward slight increase when data from both sites were plotted over time in Figure 27A. This trend is exaggerated in Figure 27D which shows the annual means of shell lengths observed at all sites throughout the study period. Comparatively low mean monthly river discharges from the Trinity River from late 2011 to 2014 (Figure 3) combined with complications of drought and changes in land use (river diversions upstream) likely altered conditions which rangia require to for the survival of larvae and the initiation of spawning. This in turn lead to a lack of recruitment and consequently fewer clams and an increased average size or age of clams, where present, which we have observed in Galveston Bay as part of this study.

More evidence for this conclusion is provided by observations of meat index of rangia during the present-day study. While shell length is a good quantitative standard of growth, meat index is a more qualitative metric as higher meat indexes suggest more robust clams which have access to an abundance of resources and environmental conditions while lower indexes suggest a lack of these resources (Tenore et al. 1968). The mean meat index of clams collected at the Open Bay and River Delta stations are plotted over time and show a very slightly decreasing trend (Figure 27B). As was the case with shell lengths, when the annual means of meat index from clams collected from all stations are plotted in Figure 27E, the trend is more pronounced. These data provide more evidence for the conclusion that reductions in freshwater discharge events are limiting the resources necessary for rangia to sustain recruitment efforts.

Figure 27C describes monthly areal density of clams at the Open Bay and River Delta stations throughout the study period. As with meat index, densities trend toward very slight decline on the monthly scale, but annual mean densities fit a stronger trendline of decline throughout the three years of study as shown in Figure 27F. Data collected from a study conducted in the Neches River by Harrel (1993) supported the hypothesis that changes in river discharge were associated with significant decline in rangia density. Additionally, mean densities recorded were $25.3 \pm 16.1\ m^{-2}$ in the River Delta and $22.5 \pm 16.8\ m^{-2}$ at the Open Bay site—relatively low compared to densities reported in other locations throughout the Gulf of Mexico (Harrel 1993; Porrier et al. 2009). In Lake Pontchartrain, Louisiana, Porrier et al. (2009) presented data showing a decrease in rangia clam density from 374 to 4 clams per square meter as well as a decrease in general benthic invertebrate diversity between the years 1998 and 2002 when the area was affected by a drought associated with the La Niña climate event. When Porrier et al.(2009) revisited the study in 2004-2005, they were not able to provide evidence of a recovery in rangia density. Similarly, in the aforementioned report by Harrel (1993), historical estimates of rangia densities in the Neches River fell within the range of 16 to 655 clams per square meter, though more recent data suggested densities as low as 42 to <1 clam per square meter. These results support Hypothesis 3 that the low rangia densities found during the present-day study in Galveston Bay may also be tied to the effects of drought conditions.

In light of the implications for rangia health trends suggested by recent rangia distribution, shell length, meat index and areal density data in consideration with a trend toward decline of freshwater discharge from the Trinity River, statistical methods were used to examine whether environmental factors associated with FWI were related to clam health metrics. As with corresponding environmental and biological data collected by TPWD, data from the present-day analysis were tested parameter to parameter with linear regression models. Individual environmental vectors (temperature, salinity, dissolved oxygen, dissolved nutrients (NO3-, NO2-, NH4+, Urea, SiO2-, HPO4=), water depth and Trinity River discharge) were plotted against corresponding rangia health metrics (shell length, meat index, areal density and sex ratios), however, out of all possible combinations, no strong trends emerged. Because these tests were inconclusive, the data is not shown. To examine the same dataset with a more robust statistical analysis with the capacity to account for the influence of multiple simultaneously occurring environmental stressors on rangia health, the multivariate statistical analysis platform PRIMER-E v.6 with a PERMANOVA expansion was used.

A PERMANOVA Main test was used to determine whether variance in clam abundance was significant correlated to different spatial and temporal vectors and spatio-temporal combinations during the present-day study from 2012-2014. Results of this analysis as shown in Table 6 revealed significant (P(perm) <0.05) variance; yellow highlight) at all a priori spatio-temporal factor levels except the crossing of stations at different annual timepoints and at different seasons within years. This test validates the comparability of the small-scale mesocosm experimental design to long-term monitoring of bay wide sites as rangia abundance was significantly different at each station placement, seasonal variation and annual variation during the study period. The PERMANOVA Main test was also applied to a balanced-design subset of clam health metrics (shell length, meat index, whole weight and wet meat weight) from five randomly selected clams from each sampling event to observe significant variation in health of rangia collected at different spatial and temporal points. Because abundance is only a reflection of the overall health of rangia populations, the analysis of more specific clam health metrics explores the hypothesis that the effects of environmental change would be more obvious at the individual specimen level. The results of the PERMANOVA Main test performed on rangia health metrics are shown in Table 7 and are similar to the results of the abundance analysis. This test confirms that the rangia collected during the mesoscale study were sufficiently diverse enough to draw conclusions about greater spatiotemporal trends in rangia health.

In light of the results from the PERMANOVA Main tests, confidence in the dataset allowed for further investigation of multivariate relationships between environmental vectors and more specific rangia health metrics (shell length, meat index, whole weight and wet meat weight) using a DistLM marginal test. Additionally, the variance in the dataset allowed for the determination of the optimal combinations of environmental vectors that best explained the variance in the biological dataset by using a DistLM overall best test. The results of the DistLM marginal test are displayed in

Table 8 and support significant (P-value < 0.05) relationships between clam health and salinity, dissolved oxygen, NH4+, Trinity River discharge, HPO4=, NO3- and water depth, with the highest proportion (6%) of data explained by salinity. Unlike linear regression results between salinity and clam metrics from both the historic record and the present-day, this result is supportive of Hypothesis 1 which investigates the link between rangia and salinity. However, even after accounting for fourteen environmental vectors with no collinearity among them, only 33% of the variation in rangia clam health metrics was explained. The results of the DistLM overall best test (Table 9) did not clarify the results of the maginal test as the three best combinations of vectors influencing the variance in rangia health consisted of no less than six vectors. Most of the vectors in the overall best combinations can be associated with FWI (nitrate, phosphate, dissolved inorganic nitrogen to phosphorous ratios, salinity, and particulate organic matter). Vectors such as temperature and water depth were identified as influential despite the fact that they are independent of the influence of FWI. This result further suggests that stressors unrelated to flows are compounding the effects of limited FWI on rangia.

The results of the multivariate analyses do support a link between rangia health and environmental vectors associated with FWI (NO3-, HPO4=, DIN:P, salinity, and particulate organic matter) and are useful for explaining roughly one third of the variance in qualitative clam health metrics (Table 8). However, the remaining fraction is unexplained by the data collected throughout the course of this study and may be influenced by other drivers outside the sphere of FWI and the resources they provide.

For example, other abiotic vectors such as regionally uncharacteristic water temperatures could contribute to environmental stress on rangia communities. According to LaSalle and de la Cruz (1985), *R. cuneata* distribution and range is limited by below-freezing water temperatures. Other studies on Texas populations of rangia conducted by Harrel (1993) and Auil-Marshalleck et al. (2000) reported significant decreases in densities of clams following record low temperatures associated with strong cold fronts that additionally caused extremely low tides and frozen shoreline waters.

Another stressor that may affect rangia abundance in Galveston Bay could include infection by parasites and general predation. Dr. William Wardle (Texas A&M University at Galveston; pers. comm.) who discovered a trichocercous cercariae stage of trematode known as *Cercaria rangiae* (Figure 29) suggested that due to the ability of *C. rangiae* to castrate their rangia hosts, parasitic infection could be impacting reproductive success of clams (Wardle 1983). However, during extensive present-day assessments of rangia health metrics including microscopic gamete identification, incidence of infection by *C. rangiae* was very low (1.6%). Furthermore, there was no seasonal or salinity related pattern of occurrence of *C. rangiae* in the data collected during this study which rules out the assumption that even a low incidence of parasitic infection was compounding the stress of low flows in the study area. Predation effects on rangia by higher trophic organisms (i.e. fish, waterfowl) and changes in predator communities over time may also be a factor influencing rangia in Galveston Bay, however, it has not been extensively described in literature and did not fall into the scope of the current study.

Anthropogenic influence on rangia outside the effects of land use changes on FWI may also play a key role in the success of rangia communities throughout the Gulf of Mexico. Cultural harvesting and commercial fishery stress can often impact population dynamics of coastal fisheries species. In Mexico, rangia and marsh clams have been harvested by coastal communities since prehispanic times (Wakida-Kusunoki and MacKenzie 2004). In present-day Mexico, *R. cuneata* are the basis of clam fisheries and has a high economic value (Wakida-Kusunoki and MacKenzie 2004). However, in the US, rangia are not commonly harvested and do not contribute to commercial efforts as the clams are thought to be an unsuccessful market venture due to unpalatable tastes and odors (see reports by http://www.gulfsouthfoundation.org/). Therefore, human consumption of rangia is unlikely as a primary source of stress on rangia populations in Galveston Bay. Anthropogenic influence of increased toxicity of sediments from industrial activity in and around Galveston Bay may also contribute to the suite of vectors influencing the trend of decline in rangia abundance and distribution over time, however, sufficient analysis of this complicated issue is beyond the scope of the current study’s consideration.

7. CONCLUSIONS

In this study, analyzing both the historical and present-day datasets regarding rangia abundance and distribution has led to the conclusion that rangia in Galveston Bay have experienced a genuine decline in recent decades. Available literature on related studies in other estuaries throughout the Gulf of Mexico support the hypothesis that declining trends in discharge rates from river sources are a potential driver for trends in estuarine rangia decline. Further investigation of this hypothesis conducted from 2011 to 2014 at the Trinity River delta did not support the hypothesis that the majority of rangia heath metric variance is driven by changes in salinity alone. However, after applying a more robust statistical analysis to a dataset comprised of both clam health metrics and a suite of environmental parameters, significant relationships between rangia health and vectors related to FWI including salinity were evident. Despite this result, only one third of the variance in clam health was explained by FWI related environmental data recorded throughout the duration of this study. Therefore, other stressors not accounted for in this study must be compounding the effects of changes in the availability of freshwater and its associated resources which drive the health of rangia in Galveston Bay. In conclusion, because rangia health is influenced by changes in vectors such as salinity, dissolved nutrients and river discharge, rangia may be considered a biological indicator of FWI; however, as the majority of variance in clam health is left unexplained even by stringent statistical analysis, further research is needed to determine what other factors are responsible for driving rangia clam health in Galveston Bay.

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APPENDIX A: FIGURES

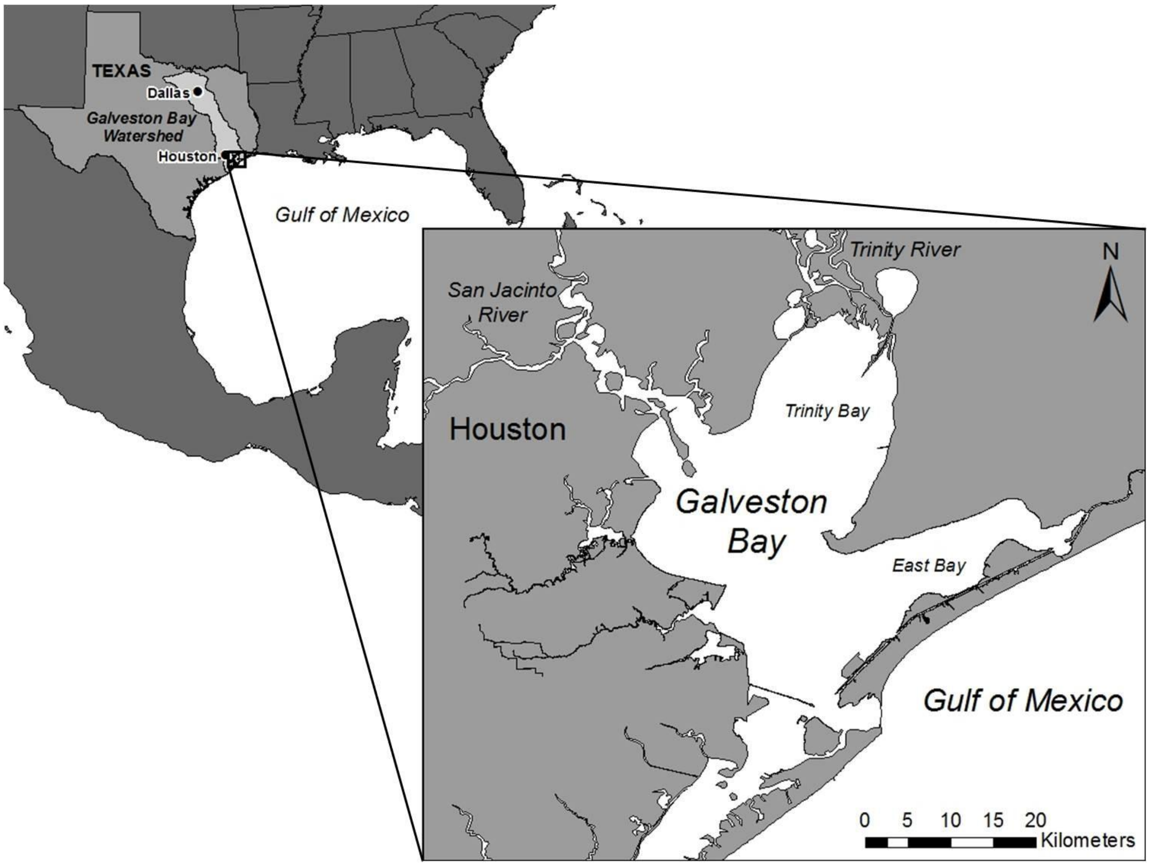

Figure 1 Galveston Bay and the Galveston Bay Watershed in relation to the Texas Coast of the Gulf of Mexico (Sources: National Oceanic and Atmospheric Association (NOAA) Estuarine Bathymetry, TWDB HUCs).

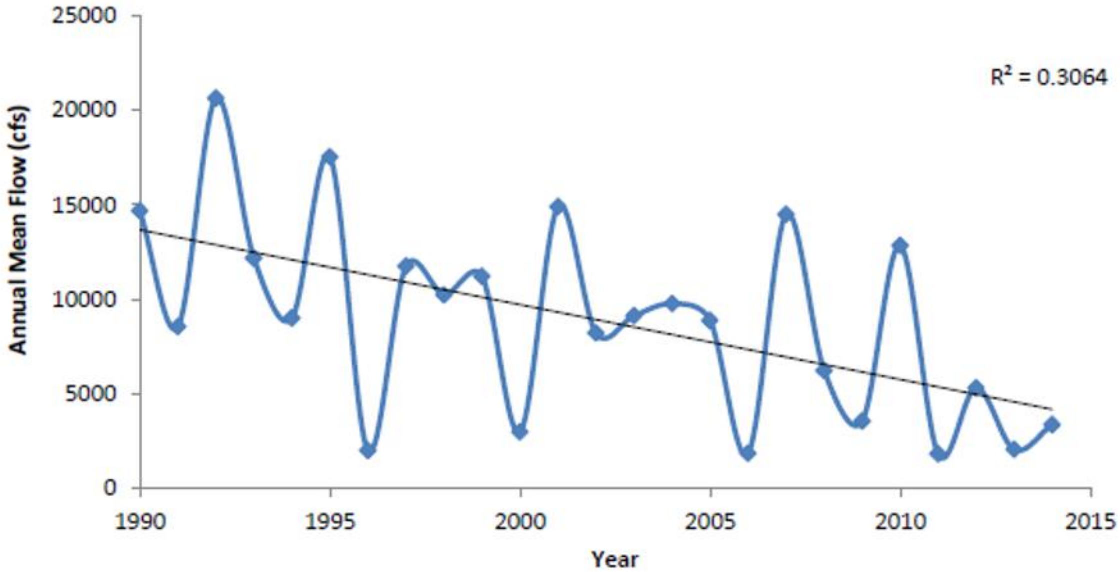

Figure 2 Annual Mean Flow (cfs) for Trinity River from 1990-2014 (USGS Gage 08066500 at Romayor, TX).

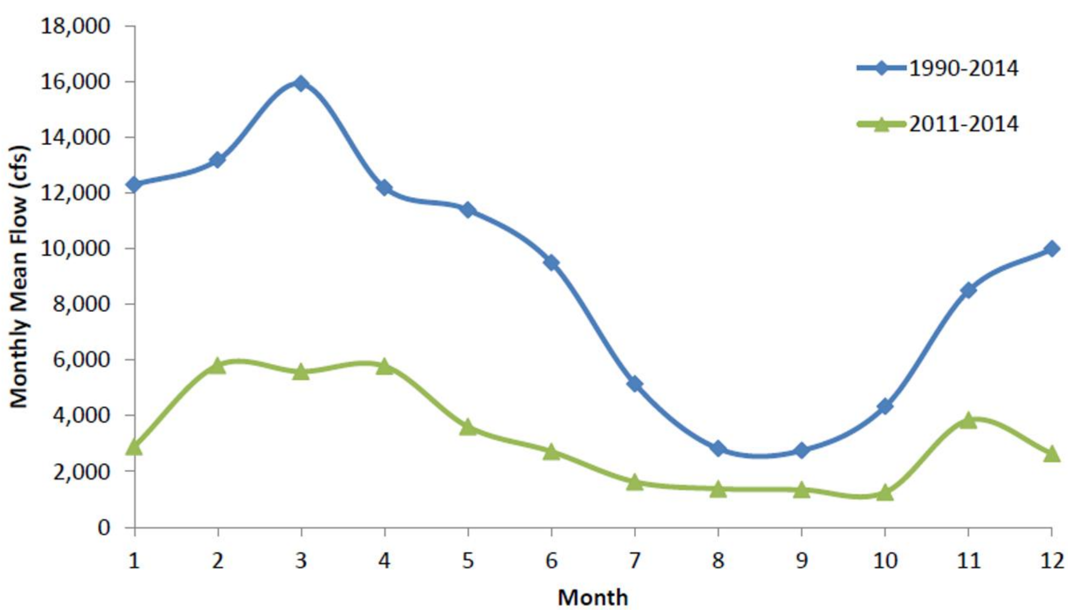

Figure 3 Monthly Mean Flow (cfs) for Trinity River from 1990-2014 and from 2011-2014. Month 1 = January, continuing to month 12 = December.

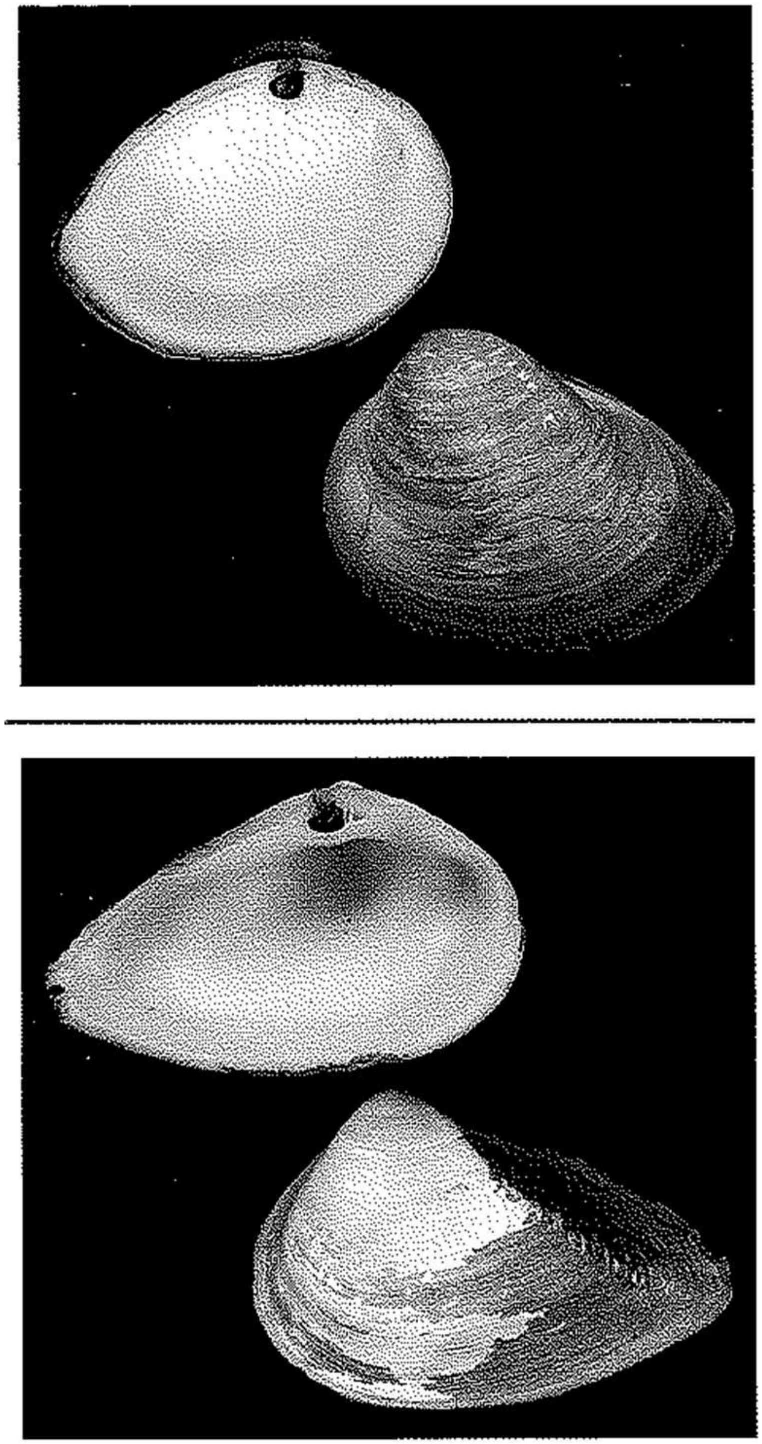

Figure 4 Valves of *Rangia cuneata* (A) and *Rangia flexuosa* (B) (Tunnell Jr. et al. 2010).

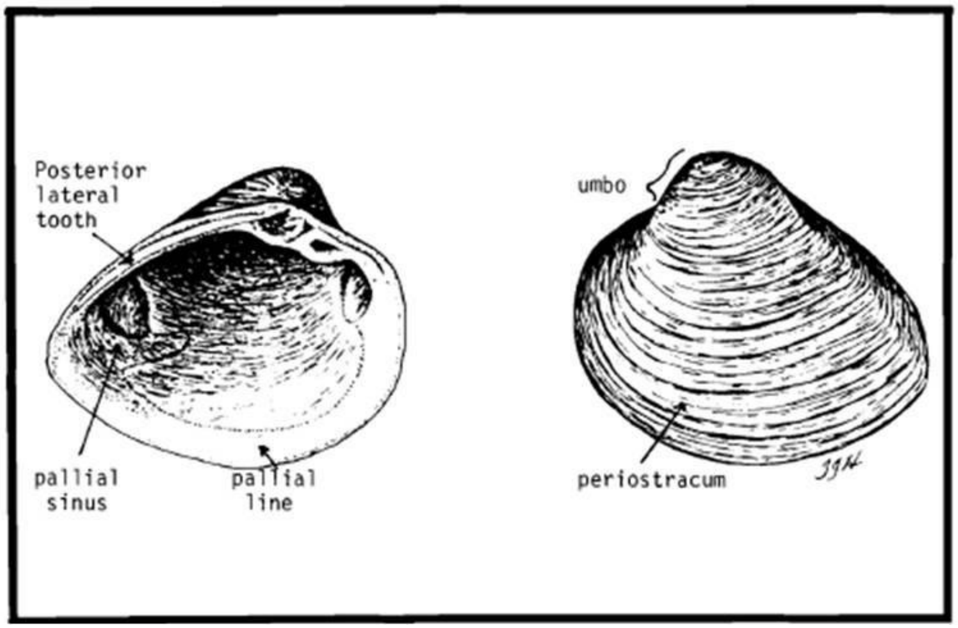

Figure 5 Description of general rangia valve morphology (LaSalle and de la Cruz 1985).

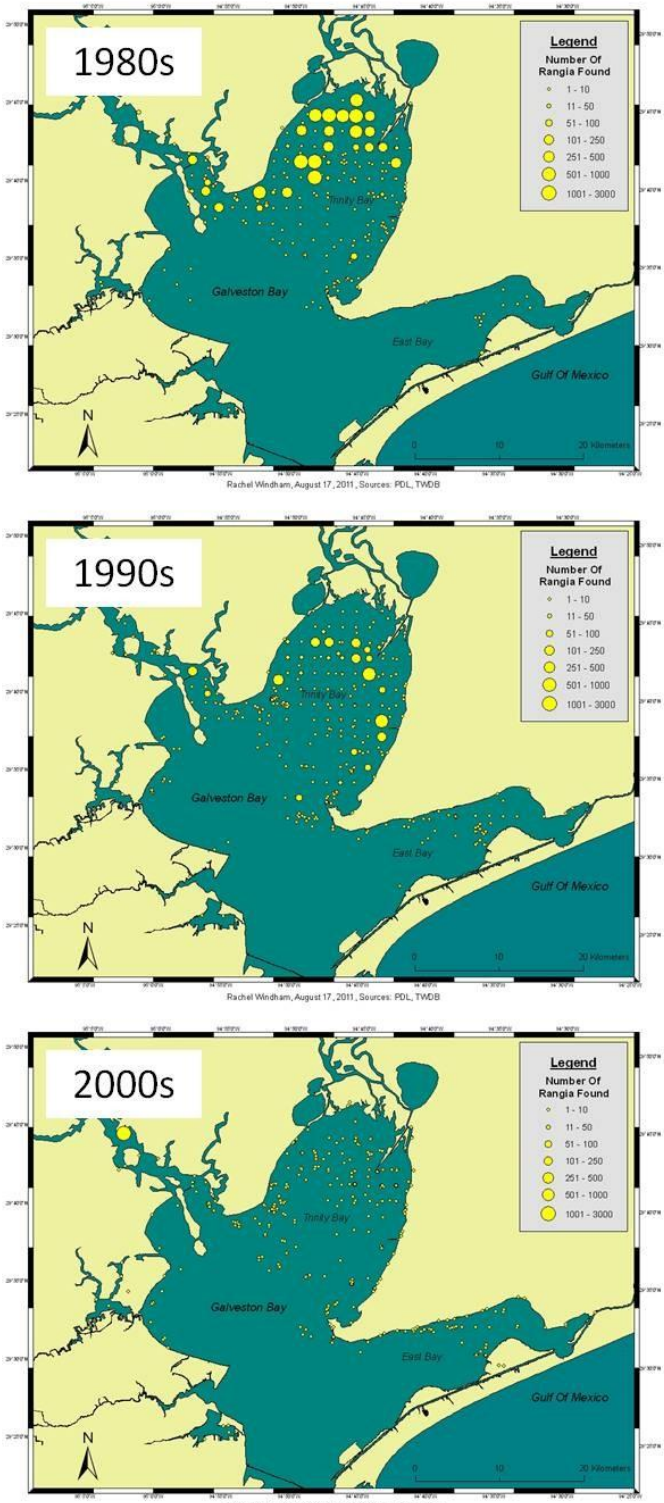

Figure 6 Decreasing numbers and shifts in location of rangia clams in Galveston Bay in the 1980s, 1990s and 2000s (Parnell et al. 2011).

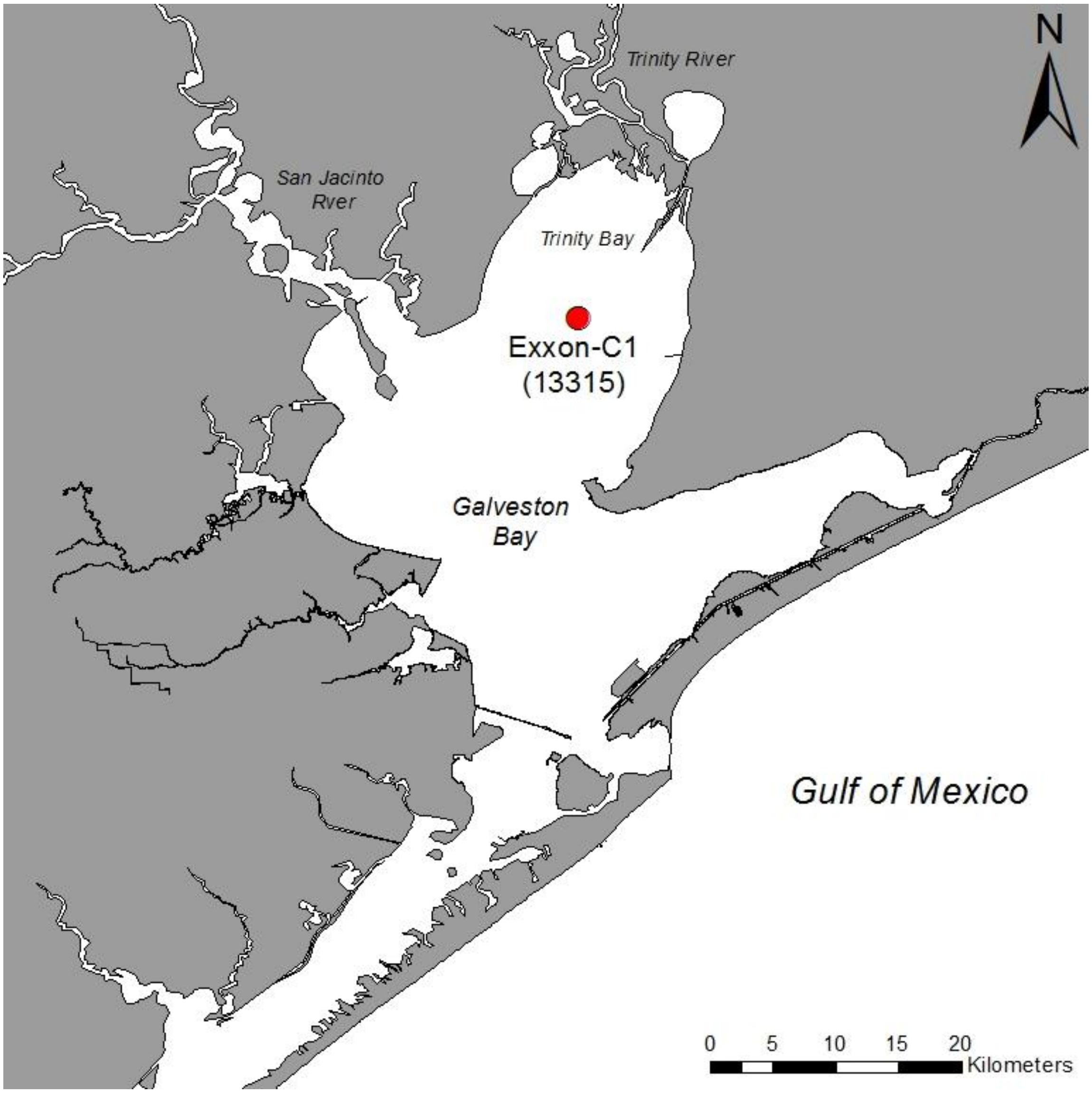

Figure 7 TCEQ SWQM Trinity Bay benchmark site Exxon-C1 (13315) sampled from 1983 to 2010 for water quality parameters including chlorophyll *a*, total organic carbon, dissolved nutrient concentrations, dissolved oxygen, temperature and salinity (Sources: NOAA Estuarine Bathymetry, TCEQ SQWMIS)

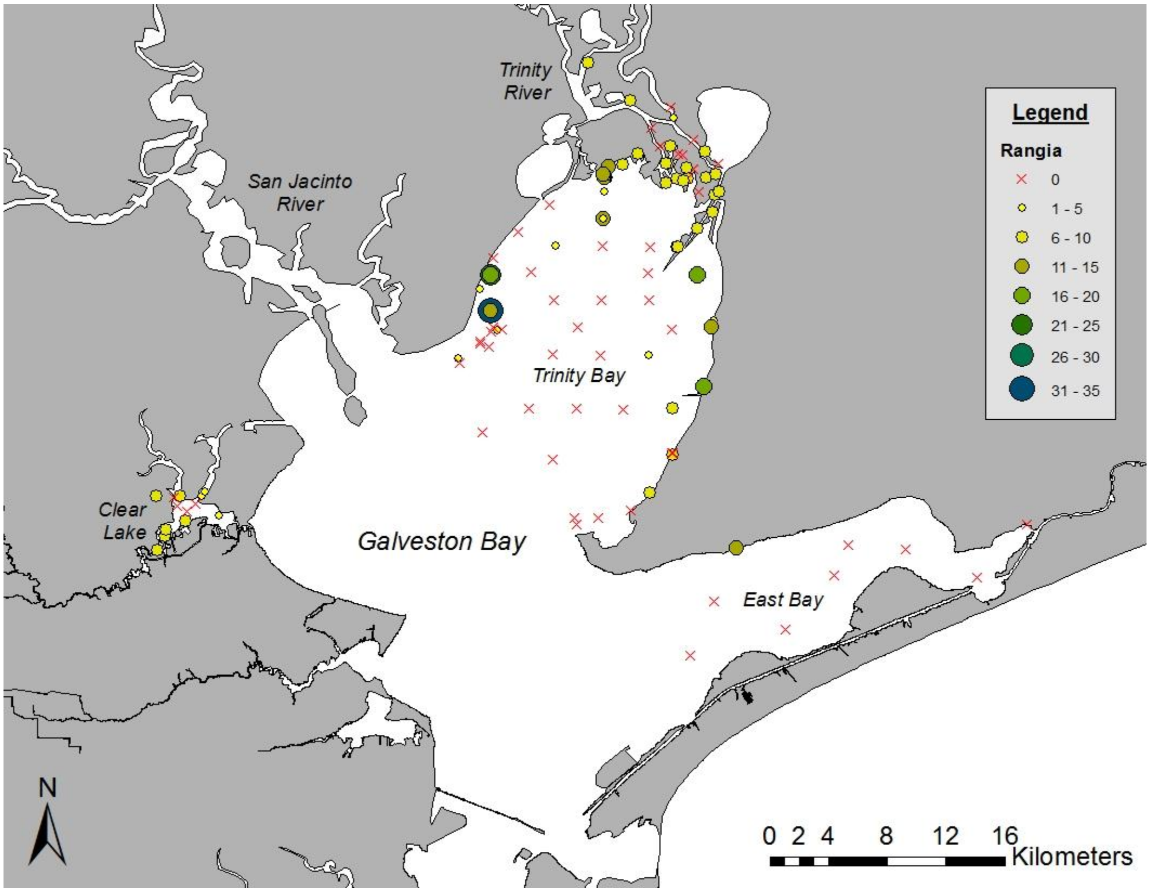

Figure 8 Pilot study presence-absence and abundance data for *R. cuneata* in Galveston Bay from 2010-2011.

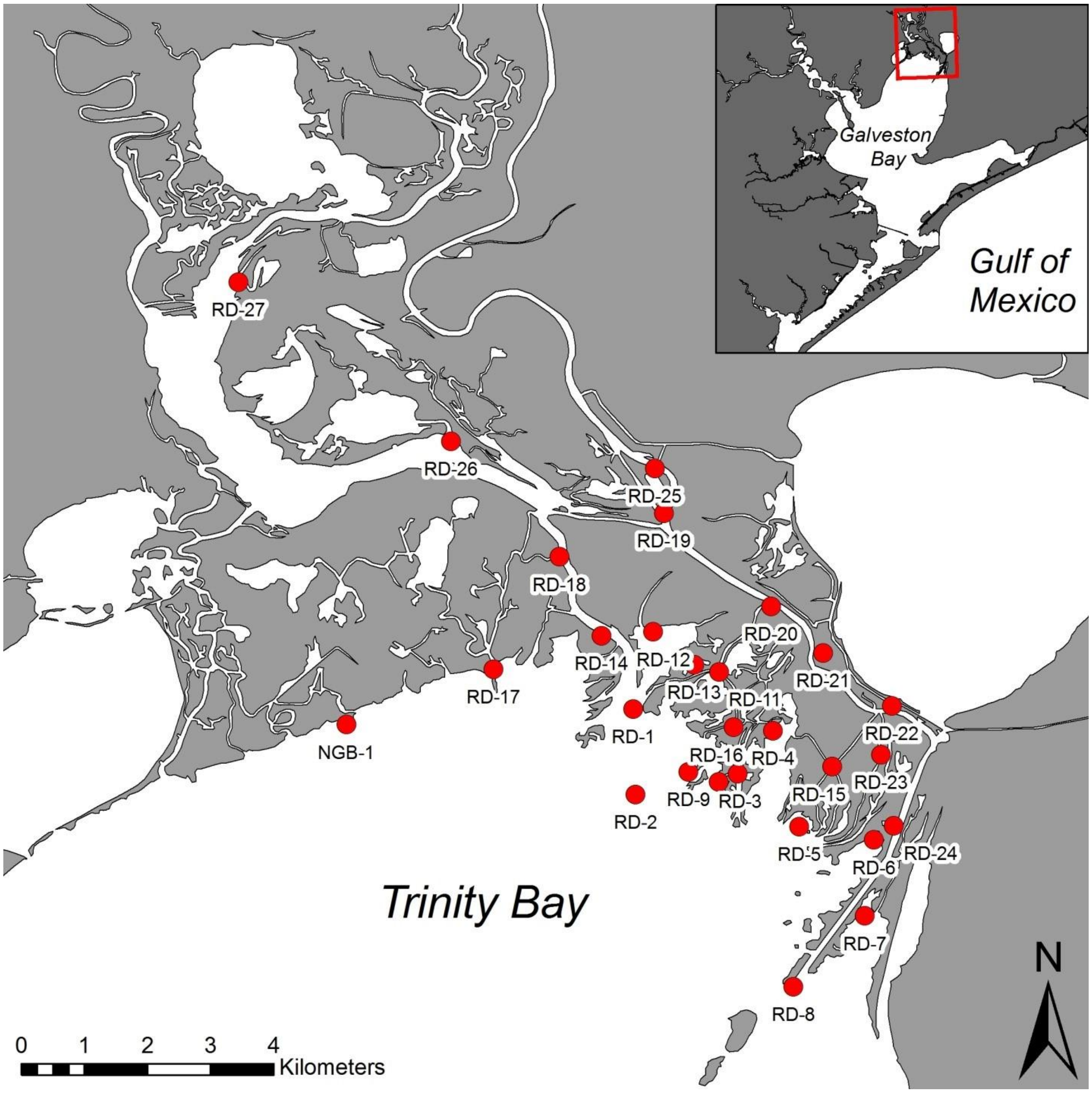

Figure 9 Preliminary rangia study sites near the Trinity River Delta, March 2011-October 2011 (Sources: NOAA Estuarine Bathymetry).

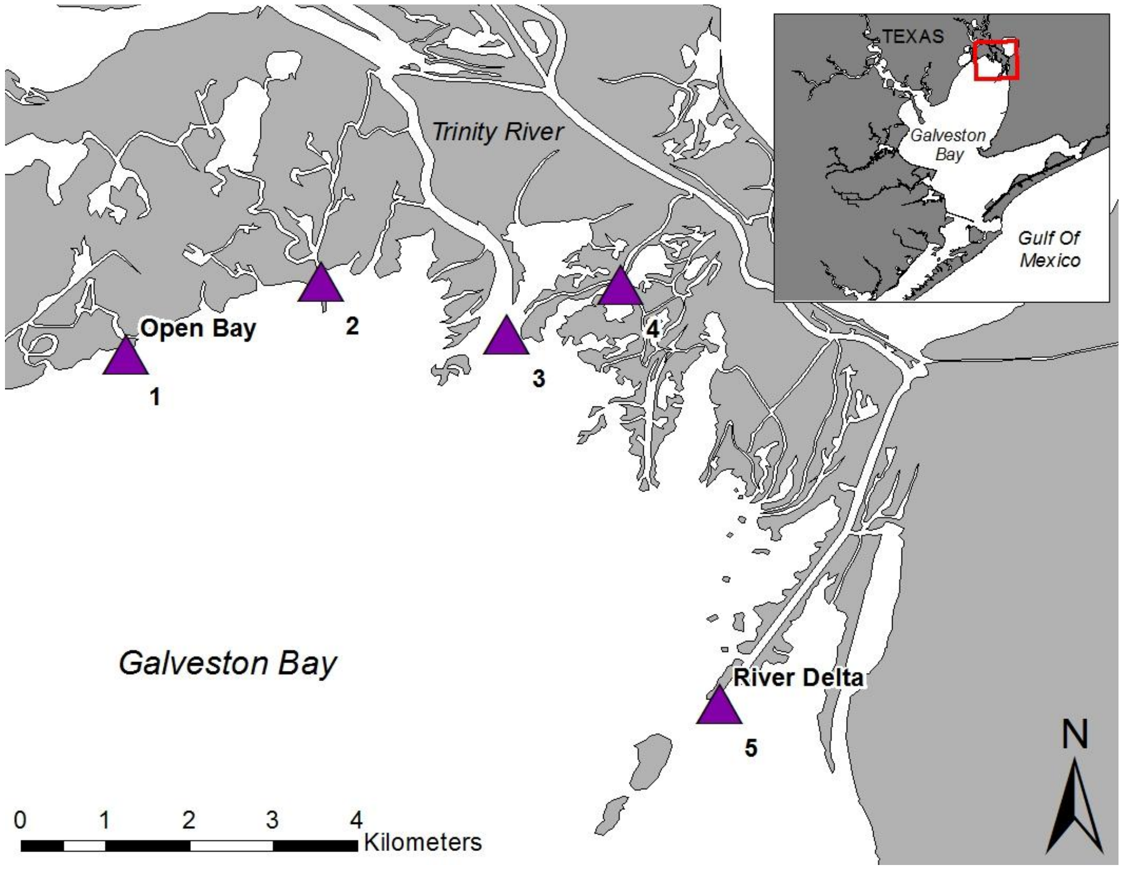

Figure 10 Current rangia study sites near the Trinity River Delta, October 2011-Present (Sources: NOAA Estuarine Bathymetry).

Figure 11 The metal quadrat (0.33 x 0.54 meter) used for clam density and biomass analysis (Photo credit: Rachel Windham, 2012).

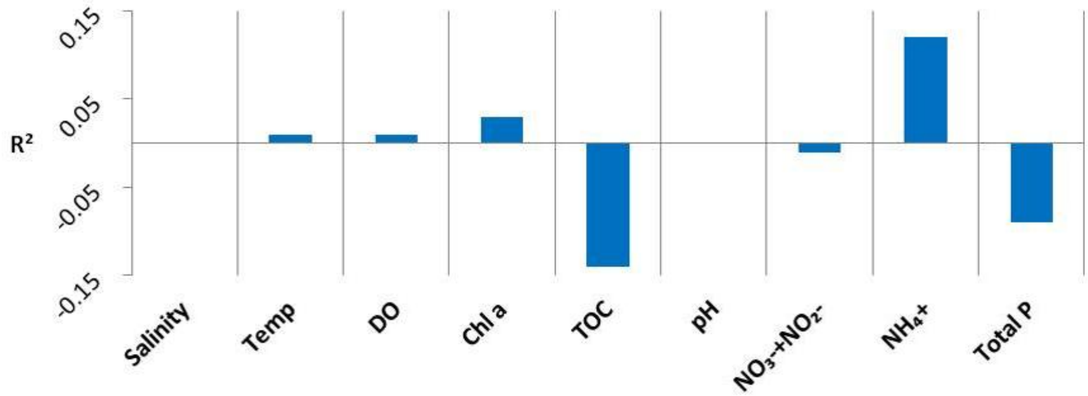

Figure 12 Summary of linear regression results of TCEQ SWQM data (chlorophyll *a* in ug L^{-1}, total organic carbon in mg L^{-1}, pH, combined nitrite and nitrate in mg L^{-1}, ammonium in mg L^{-1}, total phosphorous in mg L^{-1}, dissolved oxygen in mg L^{-1}, salinity in ppt, and temperature in °C) from Trinity Bay benchmark station (Exxon C-1/13315) plotted over time (1983-2010).

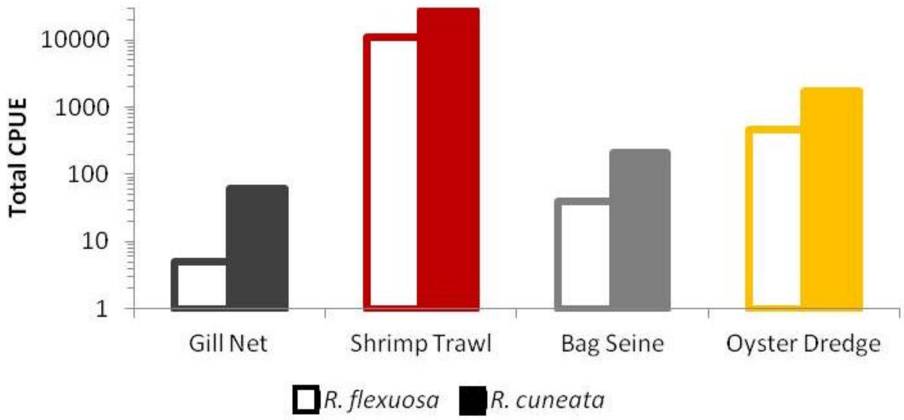

Figure 13 Total catch per unit effort of *Rangia cuneata* and *Rangia flexuosa* collected by TPWD in Galveston Bay from 1983 to 2010. Note that the y-axis is represented on a logarithmic scale.

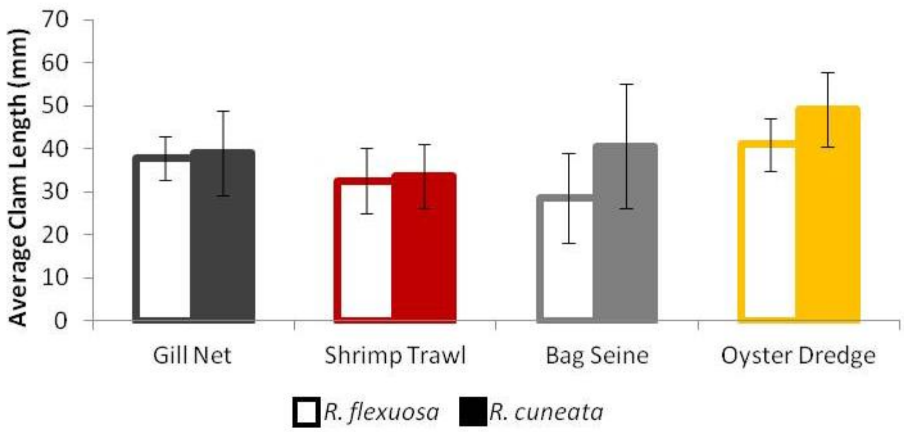

Figure 14 Mean lengths (mm) of *Rangia cuneata* and *Rangia flexuosa* collected by TPWD in Galveston Bay from 1983 to 2010.

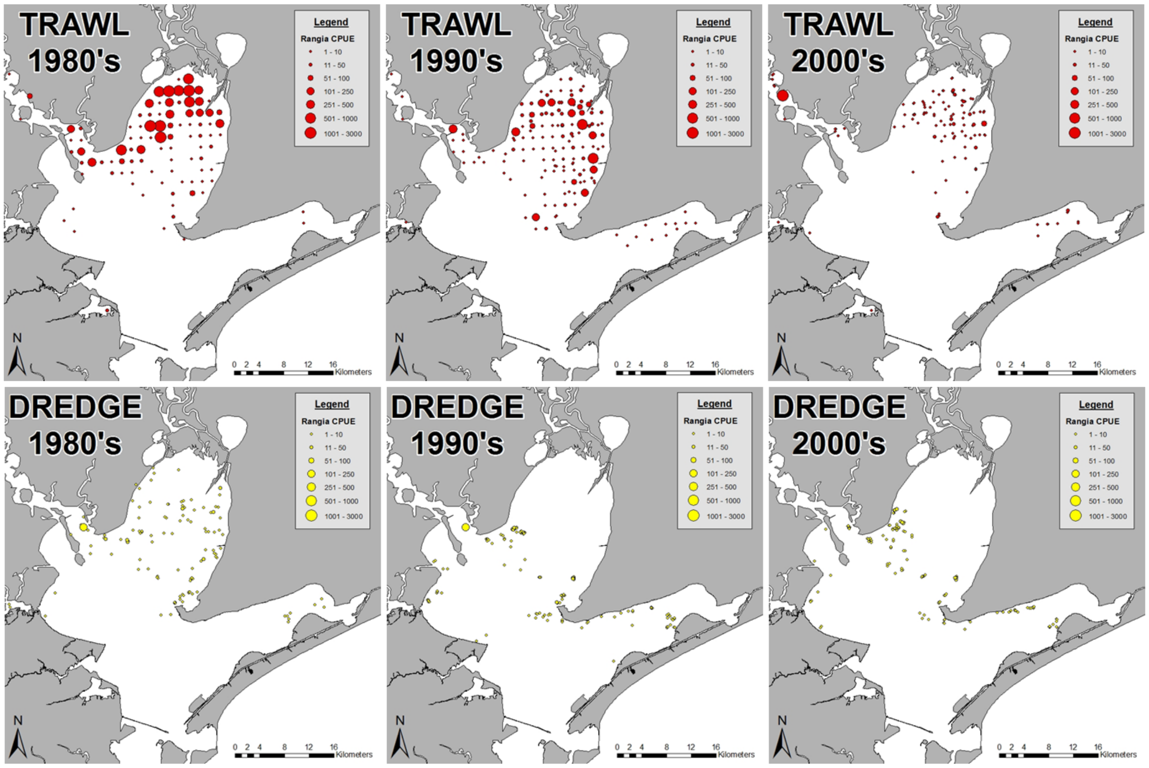

Figure 15 Comparison of rangia CPUE and spatial distribution of TPWD shrimp trawl and oyster dredge surveys in Galveston Bay across three decades.

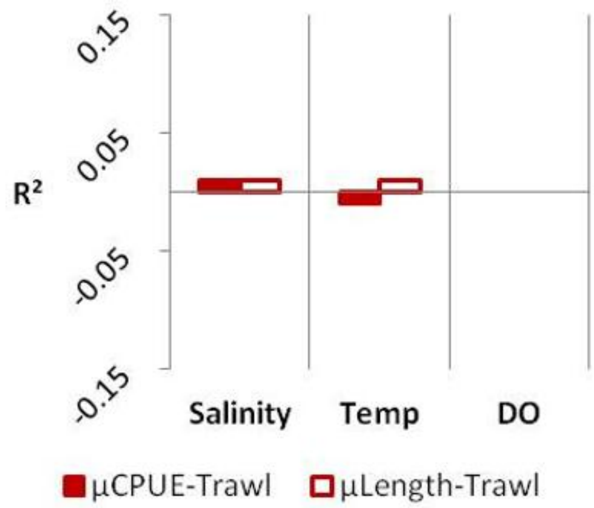

Figure 16 Summary of linear regression model results of all trawl rangia CPUE and shell length data compared to salinity in ppt, temperature in °C and dissolved oxygen in mg L^{-1}, respectively.

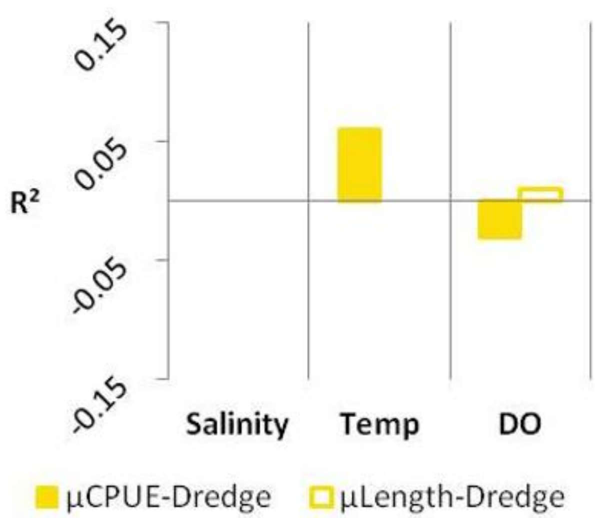

Figure 17 Summary of linear regression models of all dredge rangia CPUE and shell length (mm) data compared to salinity in ppt, temperature in °C and dissolved oxygen in mg L^{-1}, respectively.

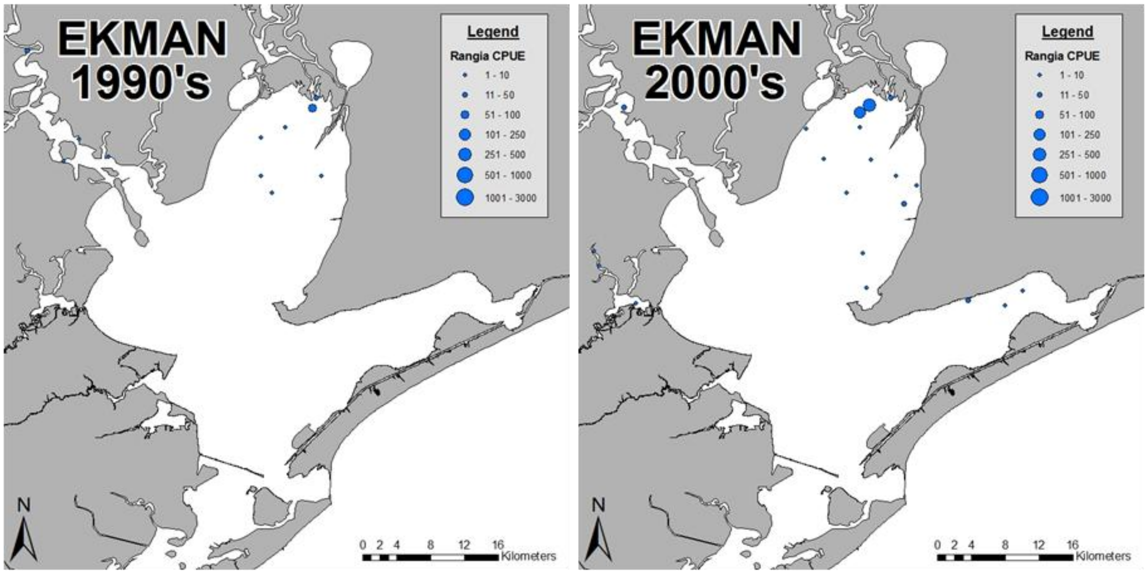

Figure 18 Rangia CPUE from TCEQ benthic surveys conducted via Ekman coring in Galveston Bay from 1992-2008.

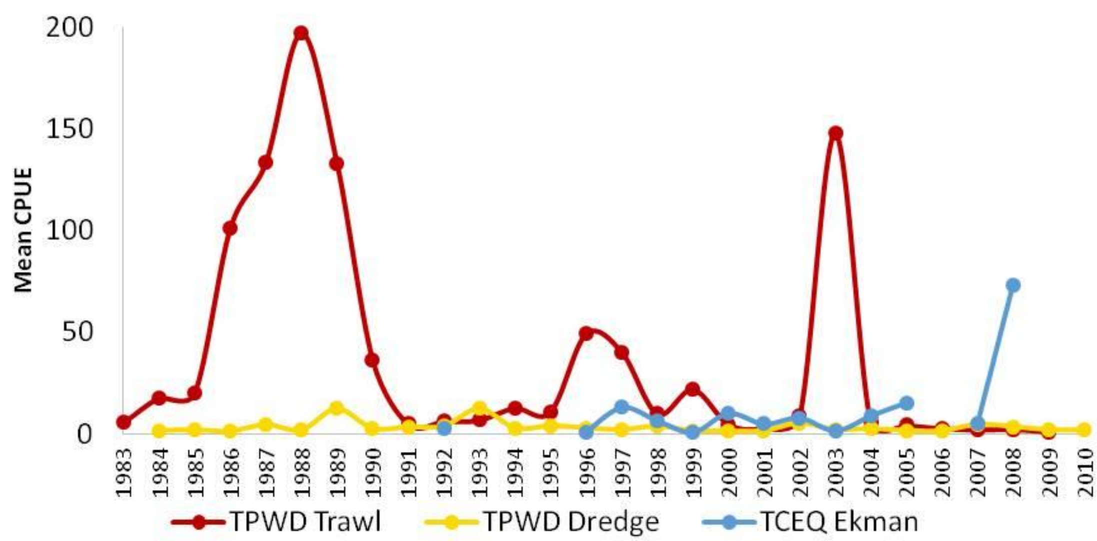

Figure 19 Mean annual rangia CPUE from both TPWD and TCEQ from 1983-2010. Standard deviation bars were omitted due to high variation.

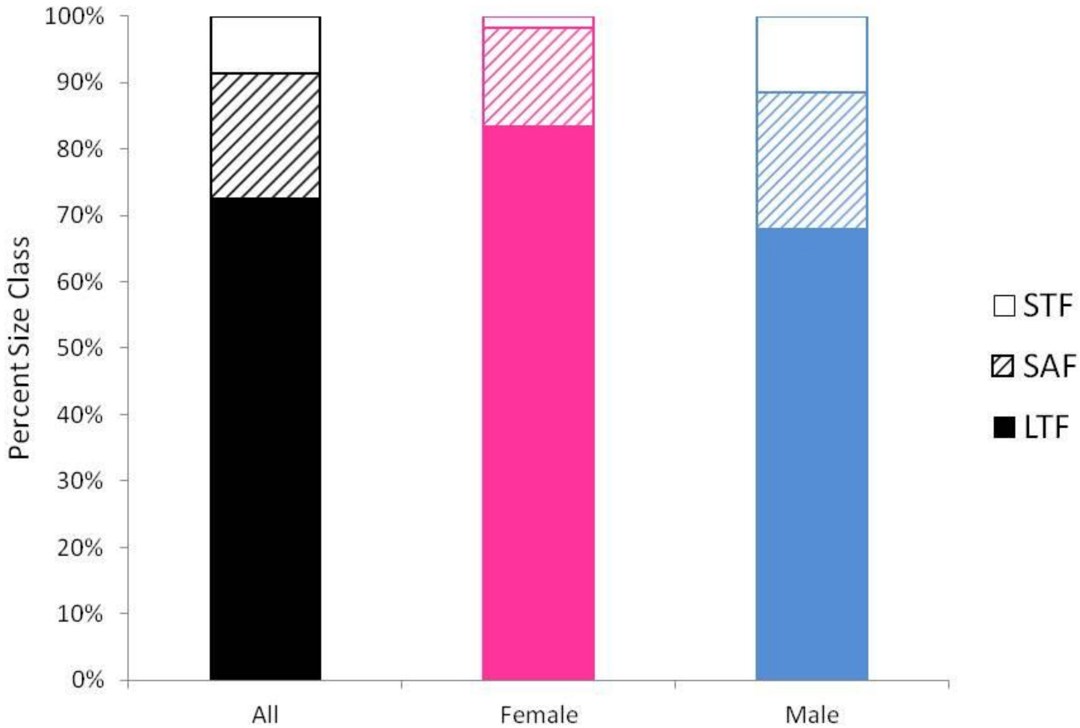

Figure 20 Size class percentages of gonad tissue compared to size of foot muscle (Larger than Foot "LTF," Same as Foot "SAF," and Smaller then Foot "STF") for all clams, and clams separated by sex collected during the October 2010-October 2011 pilot study.

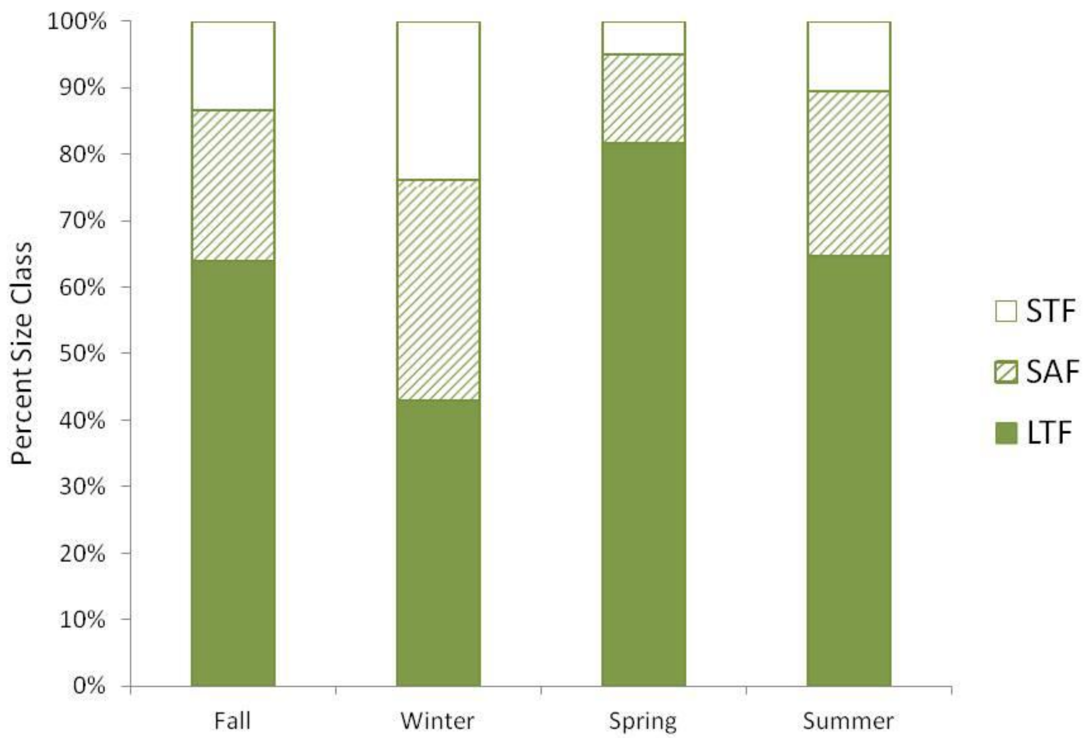

Figure 21 Size classes of rangia gonad tissue compared to size of foot muscle (Larger than Foot “LTF,” Same as Foot “SAF,” and Smaller then Foot “STF”) for all seasons of 2011 pilot study.

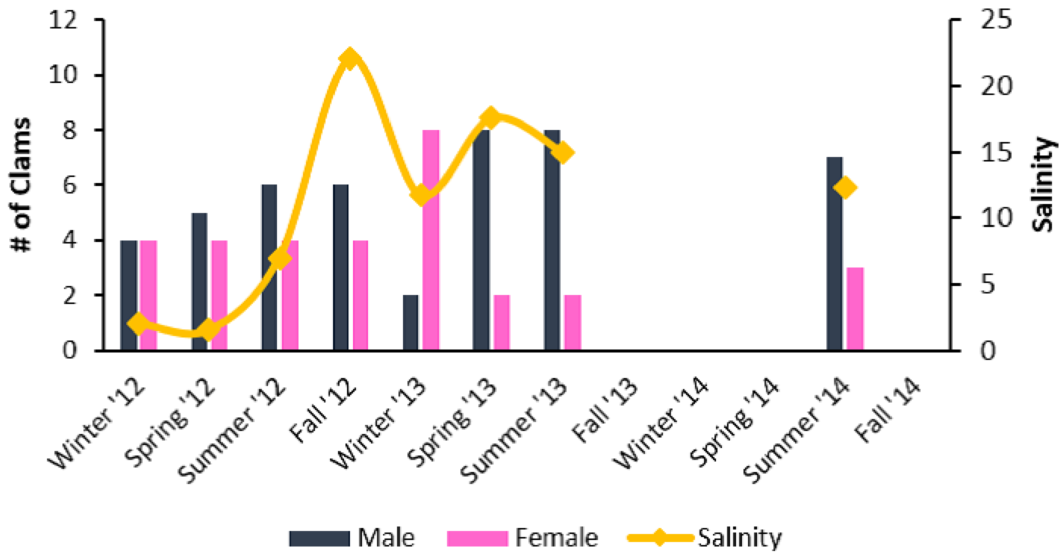

Figure 22 Open Bay quarterly sex classifications of ten randomly selected rangia with salinity overlay, 2012-2014.

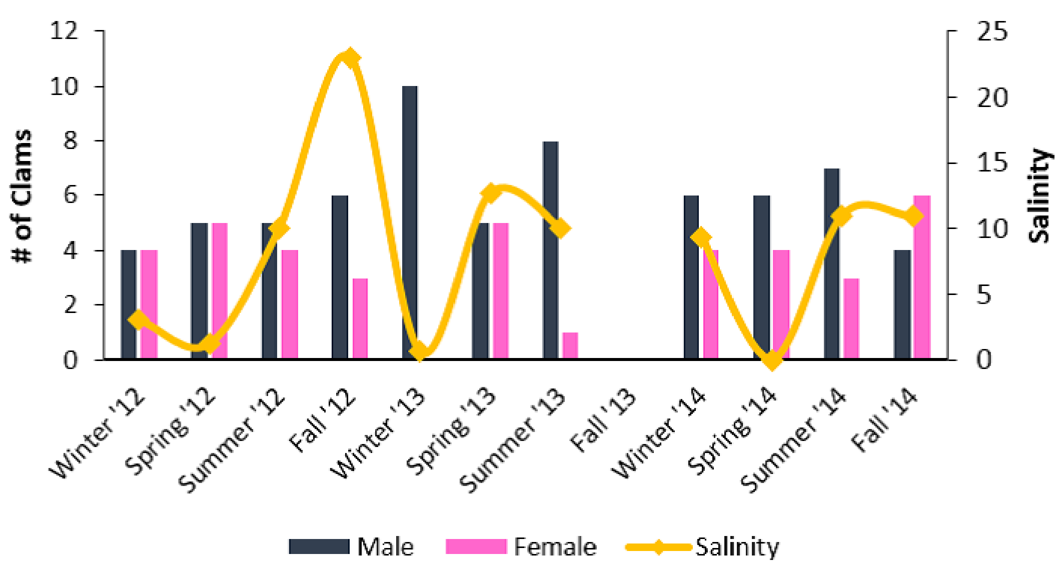

Figure 23 River Delta quarterly sex classifications of ten randomly selected rangia with salinity overlay, 2012-2014.

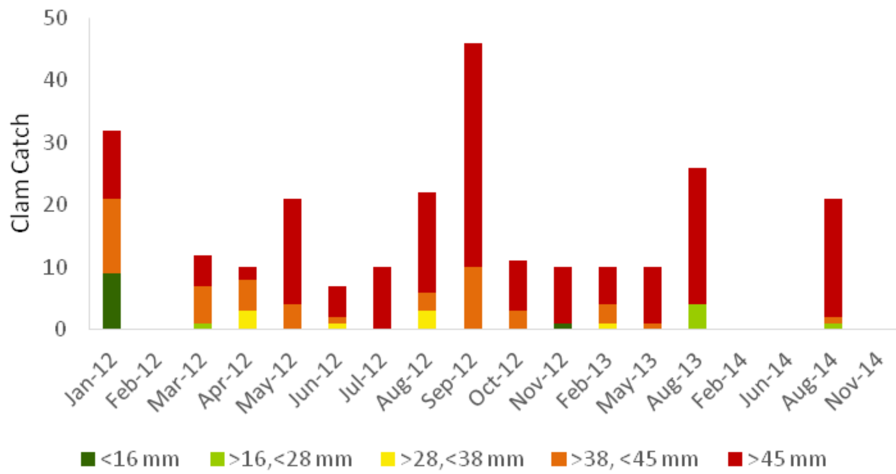

Figure 24 Open Bay shell length frequency and corresponding size classes of rangia from January 2012 to December 2014.

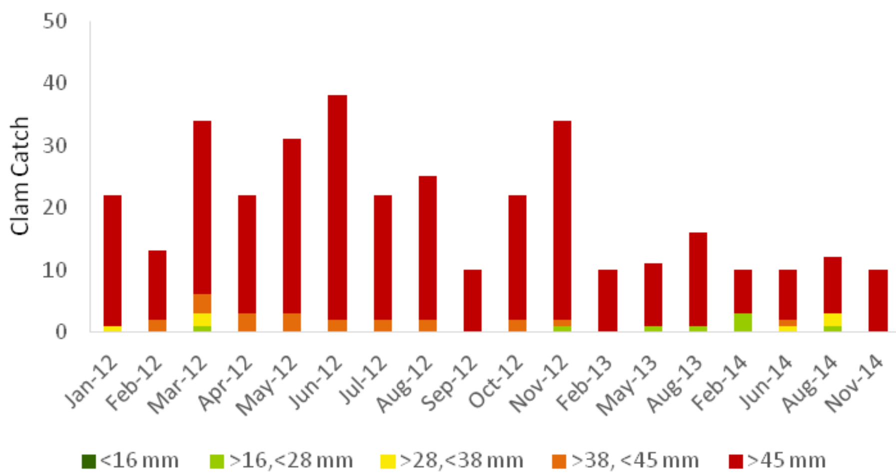

Figure 25 River Delta shell length frequency and corresponding size classes of rangia from January 2012 to December 2014.

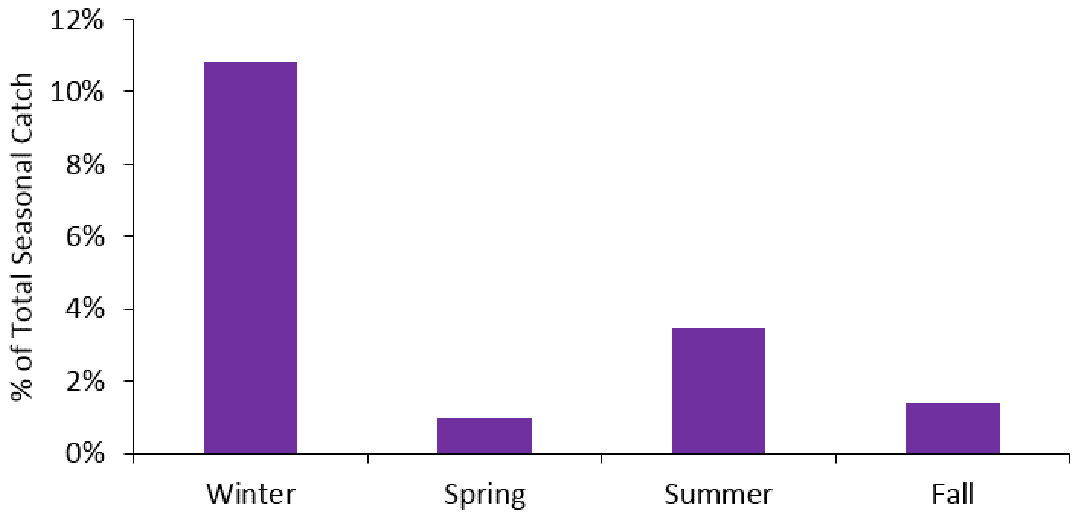

Figure 26 Percent of combined total seasonal catches contributed by sub-adult (<28mm) rangia from 2012 to 2014.

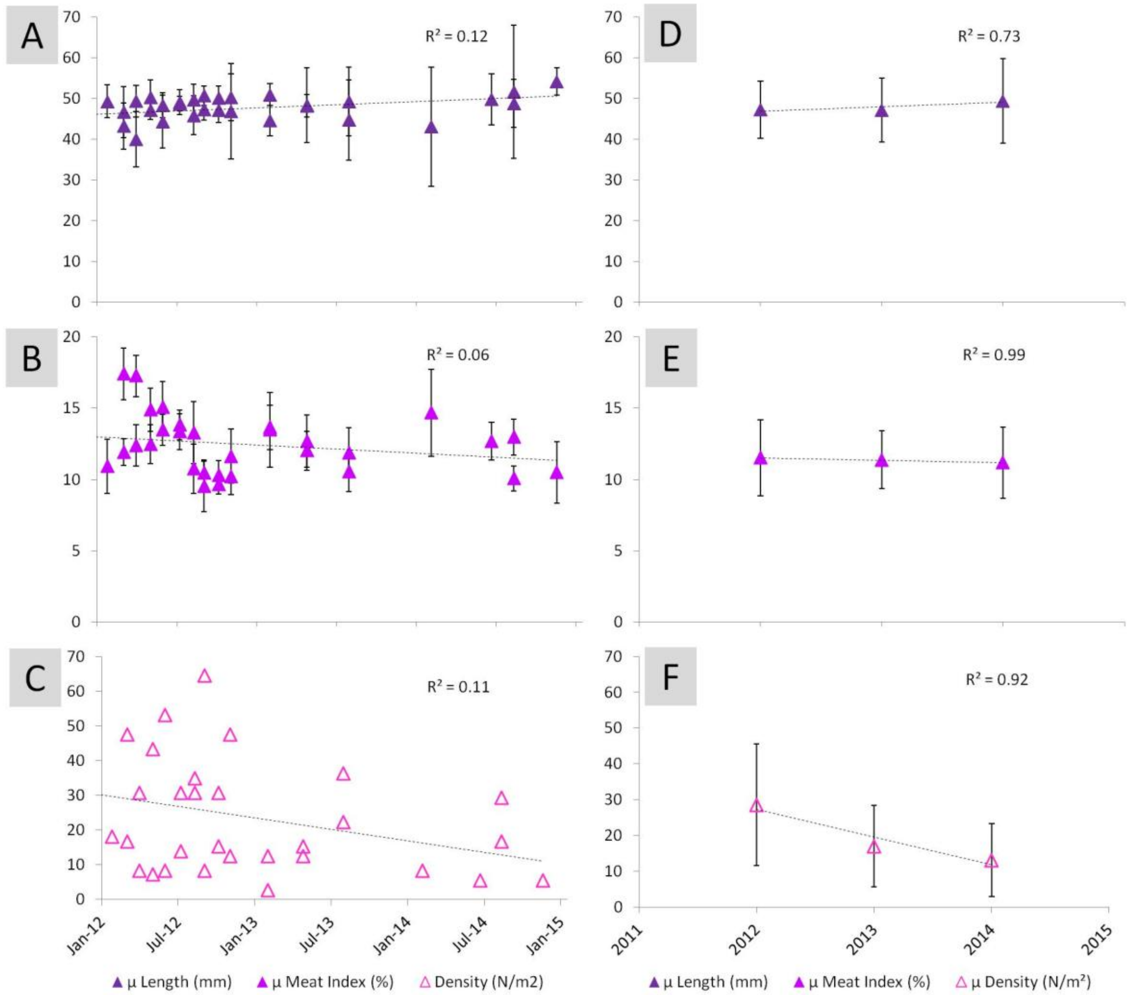

Figure 27 Monthly values for mean shell lengths in mm (A), mean meat index in % (B) and density in clams m^{-2} (C) and annual means for shell length in mm (D), meat index in % (E) and density in clams m^{-2} (F) of rangia found at the Open Bay and River Delta stations from January 2012 to December 2014.

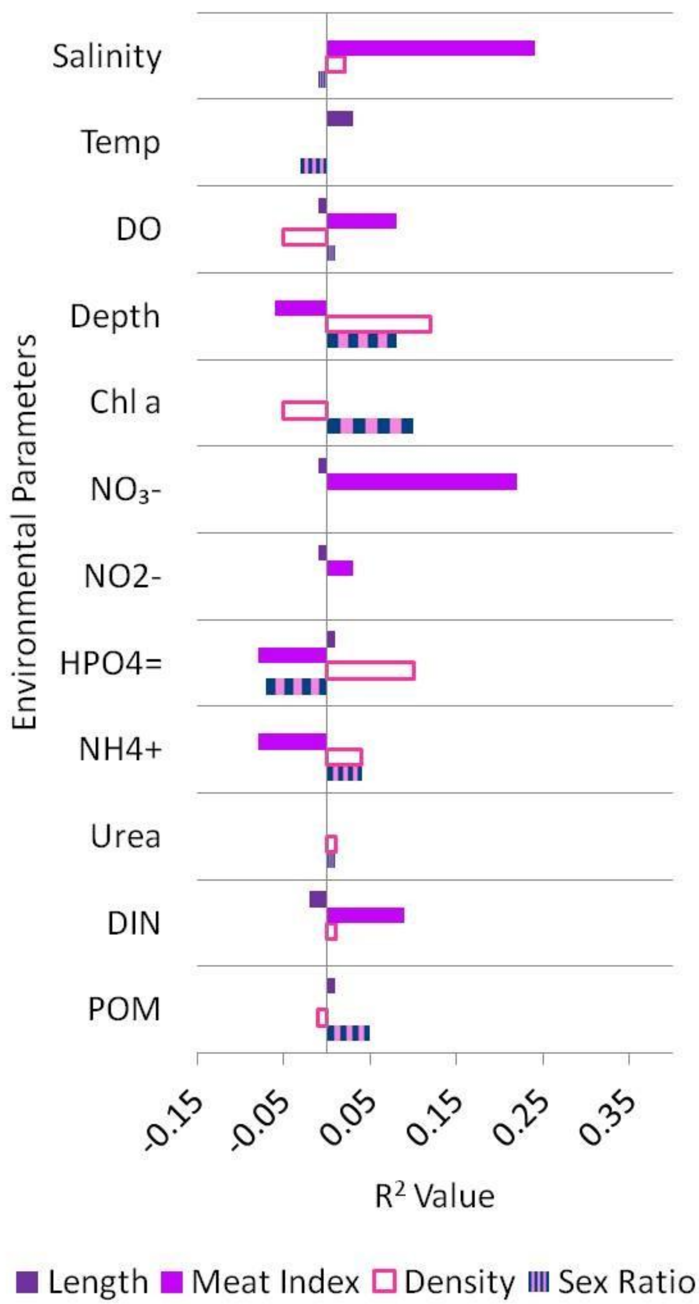

Figure 28 Trendline orientation and R^2 values of linear regression analyses performed with individual environmental variables (salinity in ppt, temperature (temp) in °C, dissolved oxygen (DO) in mg L^{-1}, water depth in m, chlorophyll *a* (chl *a*) in µg L^{-1}, and nitrite (NO_2-), phosphate (HPO_4=), ammonium (NH_4+), urea, dissolved inorganic nitrogen (DIN) and particulate organic matter (POM) in mg L^{-1}) and clam health metrics (length in mm, meat index in %, density in clams m^{-2} and sex ratio).

Figure 29 Parasitic *Cercaria rangiae* infecting a host clam (Photo credit: Rachel Windham, 2011).

APPENDIX B: TABLES

Table 1 Water quality data collected with YSI (TPWD) and Hydrolab (TAMUG) at Galveston and Trinity Bay sites during preliminary studies (October 2010-October 2011).

Date	Bay Code	Station	Coordinates (DD)		Physical Data			
			Lat (N)	Long (W)	Temp (°C)	Sal (ppt)	DO (mg/l)	Depth (m)
10/20/2010	NGB-1	1	29.774167	-94.772222	26.4	9.1	6.6	0.4
10/20/2010	NGB-2	2	29.709167	-94.742222	23.9	12.4	4.8	2.3
10/20/2010	NGB-3	3	29.6925	-94.741389	24.0	13.6	5.0	2.7
10/20/2010	NGB-4	4	29.625833	-94.724722	24.1	14.4	4.9	2.2
10/20/2010	NGB-5	5	29.609444	-94.857222	23.9	18.7	4.9	3.1
10/20/2010	NGB-6	6	29.593611	-94.807778	24.3	17.8	5.3	2.8
10/20/2010	NGB-7	7	29.654722	-94.875556	25.1	16.7	5.4	2.9
10/20/2010	NGB-8	9	29.6975	-94.861111	26.3	15.3	5.3	1.5
11/17/2010		1	29.652222	-94.874444	16.5	19.7	9.5	3.0
11/17/2010		2	29.768333	-94.775833	16.8	12.3	10.1	NS
11/17/2010	NGB-9	3	29.775	-94.761944	16.6	12.1	10.9	NS
11/17/2010	NGB-10	4	29.680278	-94.696389	17.1	16.4	10.0	NS
11/17/2010	NGB-11	5	29.5975	-94.723611	18.0	17.6	9.6	NS
2/25/2011	NGB-12	1	29.663889	-94.859722	18.2	18.2	4.0	2.4
2/25/2011		2	29.665278	-94.859722	18.2	18.2	4.0	2.4
2/25/2011		3	29.674167	-94.850556	19.1	16.9	4.4	2.1
2/25/2011		4	29.684167	-94.853056	19.6	14.7	4.9	2.0
2/25/2011		5	29.672778	-94.845	18.9	17.5	4.3	2.5
2/25/2011	NGB-13	6	29.6725	-94.848333	18.9	17.5	4.3	2.5
2/25/2011	NGB-14	7	29.675	-94.791667	19.0	18.8	5.4	2.6
2/25/2011		8	29.767222	-94.773611	20.7	16.4	4.4	0.3
2/25/2011		9	29.599167	-94.722778	20.7	19.8	5.4	0.5
2/25/2011		10	29.558333	-94.791667	19.8	20.3	5.3	2.0
3/31/2011		1	29.683333	-94.853611	20.4	19.6	7.3	2.3
3/31/2011		2	29.706389	-94.853889	20.2	18.0	7.2	1.6
3/31/2011	NGB-15	3	29.716667	-94.851389	18.5	17.1	7.8	0.7
3/31/2011	NGB-16	4	29.733333	-94.834444	19.4	16.9	8.3	0.6
3/31/2011	NGB-17	5	29.75	-94.812778	19.9	16.5	7.5	1.4
3/31/2011		6	29.767222	-94.775	19.3	15.9	7.2	0.7
3/31/2011		7	29.725278	-94.7225	19.8	16.0	7.8	0.6
3/31/2011	NGB-18	8	29.676389	-94.697778	20.1	18.1	7.5	0.7
3/31/2011		9	29.639722	-94.702222	20.2	18.3	7.9	0.8
3/31/2011		10	29.598889	-94.723889	20.4	18.4	8.1	0.7
3/31/2011	NGB-19	11	29.573889	-94.739167	20.6	19.2	8.4	0.6
3/31/2011	NGB-20	12	29.563056	-94.7525	20.9	19.5	8.6	0.7
3/31/2011	NGB-21	13	29.558333	-94.775278	20.9	19.1	8.1	1.0
3/31/2011	NGB-22	14	29.553889	-94.79	20.5	19.5	7.9	1.3
4/11/2011		1	29.77637	-94.7298	ND	15.0	ND	ND
4/11/2011		2	29.76686	-94.72343	ND	15.0	ND	ND
4/11/2011		3	29.76596	-94.7191	ND	15.0	ND	ND
4/11/2011		4	29.76715	-94.71642	ND	13.0	ND	ND
4/11/2011		5	29.77329	-94.71136	ND	14.0	ND	ND
4/11/2011		6	29.74687	-94.6983	ND	20.0	ND	ND
4/11/2011		7	29.73676	-94.7085	ND	15.0	ND	ND
4/28/2011		1	29.684167	-94.853056	22.6	24.4	6.4	2.0
4/28/2011		2	29.706389	-94.853611	22.7	22.3	6.9	1.6
4/28/2011	NGB-23	3	29.691667	-94.808333	23.3	22.6	6.7	2.5
4/28/2011	NGB-24	4	29.691667	-94.775	23.3	22.8	6.9	2.7
4/28/2011	NGB-25	5	29.725	-94.808333	23.2	20.8	7.3	2.2
4/28/2011	NGB-26	6	29.741667	-94.775	23.3	22.1	7.6	2.0
4/28/2011		7	29.768333	-94.774444	21.8	19.7	7.3	0.7
4/28/2011	NGB-27	8	29.725	-94.741667	23.3	22.7	7.4	2.2
4/28/2011	NGB-28	9	29.725	-94.723056	21.0	21.0	7.1	0.8
4/28/2011		10	29.64	-94.702222	22.9	24.2	6.8	0.7
4/28/2011	NGB-29	11	29.658333	-94.741667	23.5	22.4	7.3	2.5
4/28/2011	NGB-30	12	29.658333	-94.775	23.6	22.9	7.5	2.8
4/28/2011	NGB-31	13	29.658333	-94.808333	23.9	23.7	7.1	2.9

Table 1 Continued

5/17/2011	RD-1	TB-1	29.77637	-94.7313	22.54	17.54	8.09	0.2
5/17/2011	RD-2	TB-2	29.764215	-94.731025	23.38	13.54	8.20	0.2
5/17/2011	RD-3	TB-5	29.76715	-94.71642	23.91	19.71	8.15	0.1
5/17/2011	RD-4	TB-6	29.77329	-94.71136	23.97	11.73	8.12	0.1
5/17/2011	RD-5	TB-7	29.75963	-94.70766	24.92	19.91	8.20	0.1
5/17/2011	RD-6	TB-8	29.75771	-94.696982	21.83	8.59	8.28	0.1
5/17/2011	RD-7	TB-9	29.74687	-94.6983	24.43	16.80	8.10	0.1
5/17/2011	RD-8	TB-10	29.73676	-94.7085	24.09	18.53	8.07	0.2
5/26/2011	NGB-32	1	29.662222	-94.853333	27.1	23.1	5	3.1
5/26/2011		2	29.671944	-94.8525	27.7	22.4	5.7	1.9
5/26/2011	NGB-33	3	29.684167	-94.853056	27.5	22.5	5.1	2.3
5/26/2011	NGB-34	4	29.706389	-94.853611	27.9	21.8	5.9	1.8
5/26/2011	NGB-35	5	29.708333	-94.825	27.5	23.1	4.9	2.6
5/26/2011		6	29.725	-94.808333	27.6	22.7	4.1	2.5
5/26/2011	NGB-36	7	29.725	-94.775	27.5	22.6	5.2	2.7
5/26/2011		8	29.741667	-94.775	27.7	21.8	4.9	2.3
5/26/2011	NGB-37	9	29.758333	-94.775	27.9	21.7	4.0	1.8
5/26/2011	NGB-38	10	29.768889	-94.775556	29.3	21.1	8.1	0.7
5/26/2011		11	29.725	-94.721944	29.4	14.5	7.9	0.5
5/26/2011	NGB-39	12	29.708333	-94.708333	27.5	23.5	5.3	2.0
5/26/2011	NGB-40	13	29.675	-94.725	27.2	24.9	5.6	2.5
5/26/2011	NGB-41	14	29.64	-94.702222	28.7	23.3	6.1	0.9
5/26/2011	NGB-42	15	29.625	-94.758333	27.4	26.4	5.5	2.8
5/26/2011	NGB-43	16	29.625	-94.791667	27.5	26.3	5.2	3.1
5/26/2011	NGB-44	17	29.625	-94.825	27.3	25.7	5.0	3.2
6/17/2011	RD-9	TB-3	29.76741	-94.72343	29.11	13.16	7.08	0.1
6/17/2011	RD-10	TB-4	29.76596	-94.7191	29.13	12.8	7.5	0.1
6/17/2011	RD-11	TB-A (1)	29.78168	-94.71909	29.58	12.45	7.57	0.1
6/17/2011	RD-12	TB-B	29.787389	-94.728497	30.68	14.3	8.55	0.1
6/17/2011	RD-13	TB-C (1)	29.78273	-94.72263	31.14	13.72	9.28	0.1
6/17/2011	RD-14	TB-D	29.78685	-94.735847	28.43	14.85	7.24	0.1
6/17/2011	RD-15	TB-E	29.768159	-94.70292	31.00	12.42	8.85	0.1
6/17/2011	RD-16	TB-F (1)	29.77378	-94.71703	29.92	14.22	8.03	0.1
7/14/2011	RD-17	TB-11	29.782059	-94.751297	29.53	20.49	8.79	0.22
7/14/2011	RD-18	TB-12	29.7981	-94.74184	30.00	19.46	5.42	0.20
7/14/2011	RD-19	TB-13	29.80435	-94.72694	32.20	15.06	6.72	0.57
7/14/2011	RD-20	TB-14	29.79102	-94.71164	31.70	18.12	8.44	0.60
7/14/2011	RD-21	TB-15	29.784375	-94.704197	32.63	17.83	8.26	0.39
7/14/2011	RD-22	TB-16	29.776839	-94.6944	27.24	11.09	6.45	0.50
7/14/2011	RD-23	TB-17	29.769893	-94.69595	29.16	19.38	7.83	0.10
7/14/2011	RD-24	TB-18	29.759734	-94.694149	30.81	17.55	5.98	0.27
8/11/2011	RD-25	TB-19	29.81071	-94.72821	32.10	6.18	5.49	0.9
8/11/2011	RD-26	TB-20	29.81457	-94.75732	33.71	15.00	10.02	0.3
8/11/2011	RD-27	TB-21	29.83733	-94.78764	31.75	13.43	8.47	0.4
9/19/2011	--	TB-22	29.84295	-94.78827	27.89	17.50	10.94	ND
9/19/2011	--	TB-23	29.84938	-94.77166	28.21	17.11	10.59	ND
9/19/2011	--	TB-25	29.84989	-94.75888	28.81	12.92	12.12	ND
10/19/2011	RD-17	TB-11	29.782059	-94.751297	19.10	22.15	6.75	0.1
10/19/2011	NGB-1	--	29.774167	-94.772222	14.46	23.73	8.50	0.1
10/19/2011	RD-14	TB-D	29.78685	-94.735847	21.25	21.15	6.87	0.1
10/19/2011	RD-2	TB-2	29.764215	-94.731025	21.37	22.99	9.97	0.1
10/19/2011	RD-15	TB-E	29.768159	-94.70292	19.22	22.36	7.88	0.1
10/19/2011	RD-8	TB-10	29.73676	-94.7085	21.43	20.27	8.47	0.1

Table 2 Water quality data collected with YSI (TPWD) and Hydrolab (TAMUG) at Galveston and Trinity Bay sites during present-day studies (January 2012-November 2014).

Spatiotemporal Data				Physical Data				Biological Data			
Date	Station	Lat (N)	Long (W)	Temp (°C)	Sal (ppt)	DO (mg/L)	Depth (m)	POM(mg/L)	Chl *a* (ug/L)	Clam Catch	Method
1/11/2012	1	29.774167	-94.772222	12.3	10.4	8.4	0.3	28.7	6.9	32	Quadrat
1/11/2012	2	29.782059	-94.751297	17.1	0.6	8.1	0.2	140.0	18.9	10	Quadrat
1/11/2012	4	29.78168	-94.71909	17.6	0.2	8.0	0.6	94.7	14.6	0	Quadrat
1/11/2012	5	29.73676	-94.7085	17.0	0.3	8.3	0.1	405.4	21.6	22	Quadrat
2/14/2012	2	29.782059	-94.751297	ND	0.0	ND	0.2	43.3	0.0	0	Quadrat
2/14/2012	4	29.78168	-94.71909	ND	3.0	ND	0.5	50.0	5.2	0	Quadrat
2/14/2012	5	29.73676	-94.7085	ND	0.0	ND	0.2	34.7	4.8	13	Quadrat
3/22/2012	1	29.774167	-94.772222	19.3	2.0	ND	0.5	82.7	ND	12	Quadrat
3/22/2012	2	29.782059	-94.751297	20.5	3.0	ND	0.3	72.7	ND	10	Quadrat
3/22/2012	3	29.764215	-94.731025	20.3	4.0	ND	0.3	105.3	ND	10	Quadrat
3/22/2012	4	29.78168	-94.71909	20.3	3.0	ND	0.4	111.3	ND	0	Quadrat
3/22/2012	5	29.73676	-94.7085	19.3	3.0	ND	0.6	70.0	ND	34	Quadrat
4/19/2012	1	29.774167	-94.772222	24.1	0.5	8.0	0.3	62.7	ND	10	Quadrat
4/19/2012	2	29.782059	-94.751297	24.5	0.2	7.8	0.2	147.3	ND	10	Quadrat
4/19/2012	3	29.764215	-94.731025	24.2	0.2	6.9	0.3	45.3	ND	10	Quadrat
4/19/2012	4	29.78168	-94.71909	24.7	0.1	7.8	0.4	39.3	ND	0	Quadrat
4/19/2012	5	29.73676	-94.7085	22.7	0.2	6.9	0.5	65.3	ND	22	Quadrat
5/21/2012	1	29.774167	-94.772222	26.8	1.5	8.2	0.3	34.7	4.1	21	Quadrat
5/21/2012	2	29.782059	-94.751297	30.2	1.3	11.4	0.2	88.0	4.7	8	Quadrat
5/21/2012	3	29.764215	-94.731025	30.3	0.6	11.8	0.3	54.7	6.2	5	Quadrat
5/21/2012	4	29.78168	-94.71909	28.1	0.6	8.6	0.2	54.0	5.2	0	Quadrat
5/21/2012	5	29.73676	-94.7085	25.9	1.2	6.2	0.4	36.0	3.7	31	Quadrat
6/18/2012	1	29.774167	-94.772222	27.7	2.2	6.7	0.7	42.0	2.0	7	Quadrat
6/21/2012	2	29.782059	-94.751297	28.2	2.6	9.4	0.4	43.3	3.8	0	Quadrat
6/21/2012	3	29.764215	-94.731025	27.3	1.2	8.9	0.4	55.3	10.2	9	Quadrat
6/21/2012	4	29.78168	-94.71909	27.6	1.8	7.8	0.5	50.7	2.6	0	Quadrat
6/18/2012	5	29.73676	-94.7085	28.8	5.5	6.8	0.7	57.3	2.9	38	Quadrat
7/26/2012	1	29.774167	-94.772222	30.7	6.0	ND	0.4	69.3	4.4	10	Quadrat
7/26/2012	2	29.782059	-94.751297	ND	4.0	ND	0.3	84.7	2.7	0	Rake
7/26/2012	3	29.764215	-94.731025	ND	2.0	ND	0.3	229.3	13.9	0	Rake
7/26/2012	4	29.78168	-94.71909	ND	1.0	ND	0.2	78.0	3.0	0	Rake
7/26/2012	5	29.73676	-94.7085	ND	1.0	ND	0.3	61.3	5.0	22	Quadrat
8/27/2012	1	29.774167	-94.772222	ND	7.0	ND	0.7	62.7	4.7	22	Quadrat
8/27/2012	2	29.782059	-94.751297	ND	9.0	ND	0.2	74.7	4.2	0	Rake
8/27/2012	3	29.764215	-94.731025	ND	5.0	ND	0.4	ND	ND	0	Rake
8/27/2012	4	29.78168	-94.71909	ND	5.0	ND	0.4	110.0	3.5	0	Rake
8/27/2012	5	29.73676	-94.7085	ND	10.0	ND	0.5	114.0	2.4	25	Quadrat
9/19/2012	1	29.774167	-94.772222	24.9	16.0	7.1	0.6	35.3	2.2	46	Quadrat
9/19/2012	2	29.782059	-94.751297	24.9	15.0	7.3	0.2	57.3	1.0	0	Rake
9/19/2012	3	29.764215	-94.731025	26.6	13.0	7.8	0.3	39.3	6.5	0	Rake
9/19/2012	4	29.78168	-94.71909	27.3	10.0	6.5	0.3	41.3	5.5	0	Rake
9/19/2012	5	29.73676	-94.7085	25.4	14.0	6.4	0.4	54.0	2.6	10	Quadrat
10/22/2012	1	29.774167	-94.772222	24.4	17.0	6.5	0.6	35.3	3.6	11	Quadrat
10/22/2012	2	29.782059	-94.751297	25.1	20.0	7.1	0.4	70.0	2.6	0	Rake
10/22/2012	3	29.764215	-94.731025	25.0	14.0	7.1	0.6	40.0	4.2	0	Rake
10/22/2012	4	29.78168	-94.71909	24.4	17.0	5.9	0.6	41.3	3.2	0	Rake
10/22/2012	5	29.73676	-94.7085	24.1	21.0	5.4	0.6	38.7	3.8	22	Quadrat
11/19/2012	1	29.774167	-94.772222	15.1	22.0	9.0	0.3	30.0	1.2	10	Quadrat
11/19/2012	2	29.782059	-94.751297	17.6	21.1	9.6	0.2	30.7	3.2	0	Rake
11/19/2012	3	29.764215	-94.731025	16.4	20.3	10.3	0.6	40.0	2.3	0	Rake
11/19/2012	4	29.78168	-94.71909	19.1	19.9	10.0	0.3	45.3	4.9	0	Rake
11/19/2012	5	29.73676	-94.7085	14.9	23.0	9.2	0.4	42.7	1.3	34	Quadrat

Table 2 Continued

2/15/2013	1	29.774167	-94.772222	16.4	11.7	10.2	0.4	88.0	3.9	10	Quadrat
2/15/2013	2	29.782059	-94.751297	17.2	1.2	11.4	0.2	288.7	7.2	0	Rake
2/15/2013	3	29.764215	-94.731025	18.8	0.3	11.9	0.3	88.0	10.1	0	Rake
2/15/2013	4	29.78168	-94.71909	16.4	0.2	11.9	0.3	46.0	7.4	0	Rake
2/15/2013	5	29.73676	-94.7085	14.7	0.6	10.8	0.2	38.0	6.6	10	Quadrat
5/9/2013	1	29.774167	-94.772222	22.9	17.6	7.0	0.5	136.0	2.1	10	Quadrat
5/9/2013	2	29.782059	-94.751297	23.3	15.1	7.6	0.2	133.3	1.8	0	Rake
5/9/2013	3	29.764215	-94.731025	23.2	15.0	7.9	0.6	138.0	2.2	0	Rake
5/9/2013	4	29.78168	-94.71909	22.3	14.8	7.9	0.2	176.7	2.2	0	Rake
5/9/2013	5	29.73676	-94.7085	23.6	12.7	6.7	0.4	166.7	2.2	11	Quadrat
8/12/2013	1	29.774167	-94.772222	29.6	15.0	7.1	0.6	29.3	5.8	26	Quadrat
8/12/2013	2	29.782059	-94.751297	31.4	13.0	10.0	0.3	89.3	3.7	0	Rake
8/12/2013	3	29.764215	-94.731025	32.0	13.0	12.7	0.3	20.0	3.0	0	Rake
8/12/2013	4	29.78168	-94.71909	32.9	9.0	10.0	0.6	71.3	7.3	0	Rake
8/12/2013	5	29.73676	-94.7085	29.3	10.0	5.3	0.4	23.3	5.7	16	Quadrat
2/14/2014	2	29.782059	-94.751297	13.1	4.1	10.6	0.4	44.7		0	Rake
2/14/2014	3	29.764215	-94.731025	13.8	3.9	10.4	0.4	43.3		0	Rake
2/14/2014	4	29.78168	-94.71909	13.2	2.2	10.9	0.3	100.0		0	Rake
2/14/2014	5	29.73676	-94.7085	11.0	9.4	9.3	0.3	48.7		10	Quadrat
6/30/2014	5	29.73676	-94.7085	27.5	0.0	7.8	0.4	115.3		10	Quadrat
8/19/2014	1	29.774167	-94.772222	30.1	12.4	6.2	0.6	129.3		21	Quadrat
8/19/2014	2	29.782059	-94.751297	31.4	10.0	9.2	0.3	307.3		0	Rake
8/19/2014	3	29.764215	-94.731025	31.7	6.7	10.2	0.5	108.0		0	Rake
8/19/2014	4	29.78168	-94.71909	30.7	6.4	9.6	0.3	249.3		0	Rake
8/19/2014	5	29.73676	-94.7085	29.1	11.0	4.8	0.6	132.7		12	Quadrat
11/24/2014	2	29.782059	-94.751297	16.8	9.7	10.0	0.2	162.7		0	Rake
11/24/2014	3	29.764215	-94.731025	16.3	9.8	9.8	0.5	108.0		0	Rake
11/24/2014	4	29.78168	-94.71909	17.5	11.5	8.5	0.4	96.7		0	Rake
11/24/2014	5	29.73676	-94.7085	16.2	10.9	8.4	0.2	168.7		10	Quadrat

Table 3 Comparisons between mean length and mean CPUE of rangia collected with shrimp trawl and oyster dredge, t-test results.

	t-statistic	P-value
Mean Length, Trawl v. Dredge*	-52.30	<0.01
Mean CPUE, Trawl v. Dredge*	5.28	<0.01

*significantly different

Table 4 Mean rangia CPUE from shrimp trawl comparisons between decades, t-test results.

Decades	t-statistic	P-value
1980s and 1990s*	3.75	<0.01
1990s and 2000s	0.56	0.58
1980s and 2000s*	3.77	<0.01
1980s and Sum of 90s & 00s*	3.44	<0.01
2000s and Sum of 80s & 90s*	-4.10	<0.01

*significantly different

Table 5 Mean rangia CPUE from oyster dredge comparisons between decades, t-test results.

Decades	t-statistic	P-value
1980s and 1990s	0.86	0.39
1990s and 2000s	1.26	0.21
1980s and 2000s	1.73	0.09
1980s and Sum of 90s & 00s	-1.10	0.27
2000s and Sum of 80s & 90s*	-4.06	<0.01

*significantly different

Table 6 Results of PERMANOVA Main test applied to clam abundance data from each station where rangia were documented, 2012 to 2014. Variance tested at seasonal, annual and spatial level as well as crosses between levels.

	Pseudo-F	P(perm)
Season	16.779	0.0001
Year	5.6073	0.0019
Station	7.2852	0.0002
Season*Year	4.2249	0.001
Season*Station	2.0776	0.0434
Year*Station	2.3398	0.0721
Season*Year*Station	1.9164	0.1277

Table 7 Results of PERMANOVA Main test applied to rangia health metric data from a random subset of five clams from each station where rangia were documented, 2012 to 2014.Variance tested at seasonal, annual and spatial level as well as crosses between levels.

	Pseudo-F	P(perm)
Season	12.657	0.0001
Year	4.889	0.004
Station	3.4743	0.0084
Season*Year	5.4962	0.0001
Season*Station	2.848	0.0087
Year*Station	2.2151	0.0898
Season*Year*Station	0.9148	0.4359

Table 8 Results of DistLM marginal test applied to clam health and environmental data, 2012 to 2014.

	Pseudo-F Stat.	P-value	Prop.
Salinity	11.595	0.0002	0.055
Dissolved Oxygen	9.32	0.0006	0.045
NH_4+	8.734	0.0013	0.042
HPO_4=	8.179	0.0017	0.04
Trinity River Discharge	8.114	0.0016	0.039
NO_3-	6.446	0.0038	0.032
Water Depth	5.881	0.0067	0.029
SiO_2-	2.231	0.1213	0.011
Temperature	2.291	0.1114	0.011
Particulate Organic Matter	2.11	0.1291	0.011
DIN	0.75	0.4571	0.004
DIN:P	0.697	0.4746	0.004
Urea	0.605	0.5285	0.003
NO_2-	0.22	0.773	0.001
Variation Explained			0.327

Table 9 Results of DistLM overall best test applied to clam health and environmental data, 2012 to 2014.

AICc	R^2	Selections
822.00	0.20	NO_3-, HPO_4=, DIN:P, Temperature, Salinity, Water Depth
822.44	0.20	NO_3-, HPO_4=, DIN:P, Temperature, Salinity, Water Depth, POM
822.48	0.21	HPO_4=, NH_4+, DIN, DIN:P, Temperature, Salinity, DO, Water Depth