Trinity River Delta and Upper Trinity Bay Rangia Population Assessment

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EXECUTIVE SUMMARY

The estuarine brackish water clam *Rangia cuneata* (Atlantic Rangia) has become one of the most important species for establishing freshwater inflow standards pursuant to the Texas Senate Bill 3 process. However, due to considerable uncertainty about the sampling procedures historically used for Atlantic Rangia and the conditions the mussel requires for reproduction in Texas estuaries, the National Wildlife Federation (NWF) has undertaken a program to collect new information on its distribution and reproduction in Galveston Bay. There is a specific need to reassess the mussel's distribution in the Trinity Bay.

This study focused on the Trinity River delta and associated bayous and sub-bays. The objectives of this study were to: 1) establish the geographic distribution of the mussel *Rangia cuneata* in these largely un-sampled areas; 2) assess recent reproductive success of *Rangia cuneata* through a thorough population age structure classification; 3) examine the potential relationship of environmental variables and the distribution of *Rangia cuneata*; 4) provide a baseline assessment of the presence of *Vallisneria americana* (water celery) and 5) provide an assessment of the other benthic organisms and vegetative species present. This study represents the first comprehensive survey for *Rangia cuneata*, *Rangianella flexuosa*, and *Vallisneria americana* within the Trinity River delta during conditions influenced by high river discharges.

A total of 50 sites were monitored during January to February 2016 for the presence of the target species using a combination of methods including benthic grabs, clam rakes, quadrats, and clam dredges. Benthic biota, water temperature, salinity, dissolved oxygen; pH, turbidity, sediment type, depth, and aquatic vegetative cover were monitored during these two months. During these limited surveys *V. americana* was detected at several locations in shallow (< 1 m) water within the delta. This is most likely due to the high turbidity found in the river delta that was confirmed by turbidity monitoring. In addition, the prolonged elevated freshwater inflow during 2015 and early 2016 have resulted in depressed salinity (< 3 psu) throughout much of the Trinity River delta. Past literature and monitoring has found that optimal survival and growth in *Vallisneria americana* occurs when salinities are below < 3 psu for greater than 90 days which were likely present based on river discharge conditions. It is highly likely that due to these favorable conditions, *V. americana* has recolonized and begun to reestablish itself within the delta. Prior to this study there had not been any detections of *V. americana* during past field investigations conducted during 2011-2014.

Although it was difficult to discern any pattern in Rangia attributable to salinity or river discharge due to differences in sampling methodology, it is clear that the meat index (% total weight) had increased from former levels reported in 2011 to 2014 by other investigators. The average meat index (% total weight) observed during this study was $30.3 \pm 0.5\%$ in contrast to average meat index of 12.5% during May to August 2011, and a 12% annual average meat index value during 2012-2014. Based on comparison with these studies it appears that *R. cuneata* collected during this study contained proportionately more soft tissue (biomass) for all shell sizes. The meat index is considered a basic health index that measures the amount of soft tissue somatic growth and gonad condition. The increase in freshwater inflow has likely reduced stressful conditions due to high salinity that existed during the drought years of 2011-2014.

Continued monitoring of *Rangia cuneata*, *Rangianella flexuosa*, and *Vallisneria americana* over a wide range of discharge and salinity conditions is critical for evaluating the influence of adopted freshwater inflow regimes on freshwater inflow bioindicators. Future monitoring should include several additional components to differentiate the relative influence of river discharge, salinity and other factors on the spatial distribution, survival, reproduction and growth of these species. Specific recommendations are the inclusion of automated temperature and salinity meters within the delta to gain a better understanding of the influence of freshwater inflow on these water quality variables and possible mechanisms that limit the population size of these species. Expanded spatial coverage that include both fixed index sites for long term temporal monitoring and stratified random sites to gain information on spatial distribution are needed. Additional study components that should be adopted include the deployment of field mesocosms. The use of field mesocosms would allow investigators to manipulate multiple variables including sediment type, depth, predation, and facilitate mark recapture studies to gain better estimates of population size and density and growth and their response to changes in freshwater inflow.

INTRODUCTION

Background

The estuarine brackish water clam *Rangia cuneata* (Atlantic rangia) is found from New Jersey on the Atlantic coast south through the Gulf of Mexico coastline of the United States in northwest Florida to the Laguna de Terminos, Campeche, Mexico (LaSalle and de la Cruz 1985; Tunnell et al. 2010; Turgeon et al. 1998)¹. Atlantic rangia, henceforth called "rangia" unless otherwise noted in the text is found well into the mouths of rivers and bayous and grows to its maximum size in brackish water (Fotheringham and Brunenmeister 1989). Another relatively rare species of rangia, *Rangianella flexuosa* (brown rangia) has also been detected in Galveston Bay (LaSalle and de la Cruz 1985; Tunnell et al. 2010; Turgeon et al. 2010; Turgeon et al. 1998)².

Rangia shells have historically been used for road surfacing material in areas where are particularly abundant (LaSalle and de la Cruz 1985). The presence of shell middens containing large amounts of rangia along the northern shoreline of Trinity Bay provides extensive evidence that Native Americans utilized rangia extensively as a significant food source during pre-history and early European colonization (Aten 2010). Rangia species probably accounted for up to 80 percent or more of all mollusk shells found in archeological midden sites located around the estuaries and coastal marshes along the upper Texas coast. Rangia was only sporadically been harvested commercially in the late 1990's within Texas and is not currently supporting a commercial or recreational clam fishery (Auil-Marshalleck et al. 2000).

Atlantic rangia is an oligohaline species (LaSalle and de la Cruz 1985; Tunnell et al. 2010). Although rangia is able to tolerate salinities ranging from 0-38 psu under laboratory conditions, but it is found most commonly at lower salinities (0-18 psu), and is most abundant in very low salinity (< 5 psu) upper portions of estuaries (Auil-Marshalleck et al. 2000; Hopkins et al. 1973; Harrel 1993; LaSalle and de la Cruz 1985; Otto and Pierce 1981). Established populations are normally found in upper estuaries where salinity ranges between 0-15 psu. Highest survival and growth in Atlantic rangia occurs at salinities ≤ 4 psu (Otto and Pierce 1981).

Based on historical fisheries independent sampling using oyster dredges and trawls the Texas Parks and Wildlife Department (TPWD) reported the highest Atlantic rangia densities in Trinity Bay and upper Galveston Bay near the mouth of Buffalo Bayou with declining numbers in the lower more saline portions of the bays (Auil-Marshalleck et al. 2000). Recent limited population surveys conducted during drought conditions in 2011-2014 found similar patterns in relative density of Rangia clams between Trinity Bay, lower Trinity River, Clear Lake, and East Bay although absolute densities were lower when compared to historical data (Parnell et al. 2011; Windham 2015).

Atlantic rangia was designated as an important indicator species for establishing and monitoring the appropriateness of freshwater inflow standards in Galveston Bay pursuant to the Texas Senate Bill 3 process in multiple estuaries (Trinity and San Jacinto and Galveston Bay Basin and Bay Expert Science Team 2009). However, due to considerable uncertainty about the sampling

¹ Atlantic rangia has also been referred to as "common rangia" in older literature. The official common name is Atlantic rangia.

² Previous literature refers to Rangianella flexuosa, as Rangia flexuosa

procedures historically used for Atlantic rangia, where most specimens were essentially accidentally caught, and the conditions that this species requires for reproduction in Texas estuaries, the National Wildlife Federation (NWF) has undertaken a program to collect new

information on its distribution and reproduction in Galveston Bay. Previous efforts were focused on re-assessing the mussel's distribution in the very large (~200 square mile) Trinity Bay portion of Galveston Bay.

Study Objectives

The primary objective of this study was to assess the population status of Atlantic rangia within the most upper areas of Trinity Bay. The study focused on the lowermost reach of the Trinity River, Trinity River delta, and adjacent upper Trinity Bay. The objectives of this study were to: 1) establish the geographic distribution of the mussel *Rangia cuneata* in these largely unsampled areas; 2) assess recent reproductive success of *Rangia cuneata* through evaluation of population size and apparent age structure classification; 3) examine the potential relationship of environmental variables to *Rangia cuneata*'s distribution; and 4) provide an assessment of other associated benthic organisms and submerged aquatic vegetation. An additional objective we adopted was to compare our catch rates to historical published values.

METHODOLOGY

Site Selection

Sampling for *Rangia cuneata* was conducted along the upper Trinity River delta during January and February 2016. Fifty target sample sites were generated using a stratified randomization model implemented through ArcMap 10.3. The 50 target sites included a combination of sites historically sampled by previous researchers (Parnell et al. 2011) and new sites selected with open bay and tidal creek distributaries of the Trinity River (Figure 1). Shallow draft boats and airboats were used to gain access to each site. Sampling for Atlantic Rangia was generally conducted in shallow (< 1 m) zones located along vegetated and un-vegetated shorelines and tidal creeks in the Trinity River delta.

Field Methods

Access to and sampling of shallow water areas was conducted using either an airboat or shallow draft john boat (14'-17' in length). Upon arrival at each site, the boat was anchored and the exact latitude and longitude was recorded using survey grade GPS along with the assigned site number, date, and time of arrival. Water transparency was measured using a Secchi tube following protocol outlined in (TCEQ 2012). Total depth was recorded using a calibrated handheld multivariable YSI brand ProDSS model water quality meter. The ProDSS was also used to measure water temperature, salinity, and dissolved oxygen at the surface (0.3 m) and bottom (0.1m less than total depth).

Sampling for Rangia was conducted using a combination of modified clam dredges, rakes, and benthic cores or grabs (ponar and/or Ekman) depending on substrate type (Table 1 and Figure 2-4). At each site single sediment samples and duplicate benthic samples (target depth = 10 cm) were collected using either a PVC coring tube, petite ponar, or Ekman sampler (Figure 4). Sediment samples were placed into individual plastic bags. The outside of each bag was labeled with the site number and date.



Figure 1. Initial list of sampling sites using GIS grid system. X denotes sites where Rangia have been observed during past studies.



Figure 2. Benthic clam rake used at depths of < 1 m.



Figure 3. Clam dredge used at sites > 1m depth. Top: top view of dredge (imitates position of deployment on the substrate). Bottom: side view of clam dredge.



Figure 4. Various gear used to sample benthic organisms. A. Ekman benthic sampler, B. Ponar benthic sampler and C. PVC benthic corer.

Gear Specifications	Effort
PVC Benthic corer – internal diameter = 4"; internal height (length) = 28.75" with end cap and handle and vacuum control hole	3 replicates at sites with high hand content. Each replicate = $12.56 \text{ in}^2 = 81 \text{ cm}^2$. Used to sample Rangia, benthos and sediment.
Ekman benthic sampler (length x width = 6 X 6"); maximum internal depth of sample = 7.5'	3 replicates at sites with high silt Each replicate = $36 \text{ in}^2 = 232.3 \text{ cm}^2$. Used to sample Rangia, benthos and sediment.
Petite ponar benthic sampler (length = 6"; width 8.25") maximum internal depth = 9".	3 replicates at sites with high clay/silt Each replicate = 49.5 in ² = 319.4 cm ^{2.} Used to sample Rangia, benthos and sediment.
Clam dredge (width = 16"; depth 10.75"; length = 32" total trawl length w/o cod end basket extended dredge teeth to tow eyelet); cod end basket height = 8.25"; dredge teeth = 2"; gap distance between dredge teeth = average 2"; internal wire basket mesh size = 0.5" square mesh	3 – 30 second replicate tows at sites with depth exceeding Used to sample Rangia and large mollusks.
Clam rake (width = 13.75"; depth 5.75"; height 9" basket only; handle + basket length = 84"; teeth length = 3.25"; gap distance between = 1"; internal wire basket mesh size = 0.5" square mesh	3 – 6 replicate pulls for distance of 3-7'; < 4' depth. Used to sample Rangia and large mollusks.
1 m ² PVC quadrat	1 replicate per site to characterize vegetation cover of bottom.

Table 1. Description of gear used to sample sediment, benthic organisms and plants.

The sediment samples were returned to the lab for sediment size characterization and examination for the presence of Rangia clams. Benthic samples were sieved in the field using a sieve bucket and ASTM E11 No. 35 (500 micron mesh) handheld sieve. The remaining shells, organisms and debris were transferred in to one or more 1L plastic jars and preserved in a 10% formalin/Rose Bengal solution mixed with ambient water and identified using velum tags denoting the site ID, date, sampling method and rep number. Any large, live or whole but dead *Rangia* spp. collected during sediment and benthic samples were stored in a separate zip-lock bag for each site and processed back in the lab (described in next section).

When visible, the identity of submerged (e.g. *Vallisneria americana, Ruppia maritima* and dominant species) and emergent (e.g. *Spartina* spp., *Juncus* spp., etc.) vegetation within a 1 m² PVC quadrat at the sampling location were recorded and relative density was estimated and expressed in percent cover. When no vegetation was observed, absence was also noted.

At shallow sample sites (i.e. < 1 m deep) a clam rake (Table 1 and Figure 2) was used. A total of 3-6, 30 second pulls were made in the vicinity of the anchored boat. At sample sites where water depth was greater than 1 m, clam dredge hauls using a modified clam dredge were conducted in triplicate (Table 1 and Figure 3). The modified clam dredged was pulled at low to moderate speeds across three transects per site for 30 seconds per tow.

Clam rake and dredge samples were sorted in the field for target organisms. When Rangia clams were collected using either method, up to 25 specimens were preserved on ice and retained for lab examination. Additional Rangia were tallied and released back to the site. Any dead but whole Rangia shells (both valves present and attached) were also tallied separately.

Species Determination

Species were identified using taxonomic keys. Multiple taxonomic keys were used to identify organisms and aquatic plants (Fotheringham and Brunenmeister 1989; Heard 1979; Howells 2014; Masser 2009; Stutzenbaker 1999; Tiner 1993; Tunnell et al. 2010; Tunnell Jr et al. 2014). Clams were identified as *Rangia cuneata* if the shell was marked by a long posterior lateral tooth, small but distinct pallial sinus and the ventral margin appeared to be rounded and blunt at its most extreme apex (LaSalle and de la Cruz 1985; Tunnell et al. 2010). Conversely, clams were identified as *Rangianella flexuosa* if the shell had a short posterior lateral tooth, nondescript pallial sinus and the ventral margin had a flat edge that drew to a point at its most extreme apex (LaSalle and de la Cruz 1985; Tunnell et al. 2010).

Laboratory Methods

Benthic Core Samples

Benthic grab samples were sieved through a #35 screen in the laboratory to concentrate the infauna specimens captured and to rinse the samples of formalin. These samples were archived for future identification during summer 2016. However, any Rangia found while sorting samples was extracted and is included in our analysis. Benthic infaunal organisms will be identified using taxonomic keys for estuarine benthic organisms with primary focus on identifying bivalve mollusks. Voucher specimens were retained for quality assurance purposes.

Rangia Morphometrics and Health Metrics

Shell length, width, and height (Figure 5) was measured in the lab, typically on the day following field sampling, for up to 25 live or whole but dead Rangia specimens at each site. Up to 10 of the 25 specimens from each site were also weighed to the nearest milligram (mg) prior to being shucked and cleaned. After cleaning, shell valves were reweighed and weight was recorded in milligrams. The wet weight of the soft tissue was determined by subtracting the total weight minus the empty shell weight. Overall health was determined by the ratio of clam meat to shell size (tissue weight/total weight (shell & tissue) * 100). Meat index was calculated by subtracting the cleaned valve weight from the original, whole weight. Dry, cleaned valves from up to 10 specimen from each site were then stored in a freezer at -80°C for archival purposes and potential further age determination at a later date.



Figure 5. Length (left), height (middle), and width (right) morphometric measurements for live and whole but dead Rangia.

Fine Sediment Composition

Composition of fine sediments from each site were calculated using protocol described by Ginn et al. (1990). Sediment samples from each site were homogenized in the sample bag. Fifty milliliters of homogenized sediment was measured out from the sample bag and passed through a #63 sieve to rinse off fine sediments. Remaining sand and larger grains were then placed in a 100mL graduated cylinder which was then topped off with water. After allowing the sample to settle out for 5-10 minutes, volume of sand and larger grains was recorded. Percent fine composition was then calculated by: subtracting the volume of remaining sand and larger grains from the initial 50 mL of sample processed; dividing that value by 50 (the original sample volume); and multiplying by 100.

River Hydrology and Historical Data

River discharge data was obtained from the USGS 08066550 Trinity River gage at Romayor, Texas. Daily average data was obtained from this site from January 2011 to February 2016. Daily average discharge data was used to estimate 30 day and 90 day average values to determine longer term freshwater inflow from the Trinity River. Data collected from this study was compared to several recent field studies containing spatial and temporal data on the distribution of Rangia and *Vallisneria americana* in the Trinity River delta (Parnell et al. 2011; Quigg and Steichen 2015; Windham 2015).

Data Analysis

The location of captured Rangia species and *Vallisneria americana* were displayed using Google Map Pro. The presence and absence of live Rangia are depicted. These data are also graphically compared to previous published study results during 2011-2014 (Parnell et al. 2011; Windham 2015). A graphical summary of morphometric data is also presented. A comparison of catch rates, ambient salinity and river discharge are presented.

RESULTS

Physicochemical Results

The final location of sample sites is depicted in Figure 6. Water depth at the sample sites exhibited an average value of 0.56 meters and varied between 0 and 2.1 meters (Figure 7). The majority of sites were located at depths less than 0.5 meters. One site sampled during the study, R15 was completely exposed (depth = 0 meters) but possessed moist sediment. For this site all variables were measured with the exception of water quality. For the purposes of data analysis water temperature and salinity for shallow sediment at this site was estimated using the average value of data from surface water measurements from several sites located near this site on the date of sampling. No estimates of dissolved oxygen, conductivity or pH are provided for this site.

Water temperature, specific conductance, salinity, dissolved oxygen, and pH was measured at the surface and near the bottom when water depth exceeded 0.5 meters. For the majority of sites therefore only surface measurements were made. During the study measured water temperatures exhibited seasonally low values, averaging 14.8 C and varying between 10.9 and 26.3 C (Figure 8). Both surface and bottom water temperature was generally lower at sites located in deeper (> 0.5-1.0 m) water (Figure 9).

Since specific conductance and salinity are highly correlated we only report data on salinity since the vast majority of literature concerning Rangia cuneata involves studies on the influence of this variable on the survival and growth of this species. During the study combined surface and bottom salinity exhibited an average value of 0.4 psu and varied between 0.1 and 4.3 psu (Figure 10). This corresponds to an average value of 820 μ S/cm and range of 246 to 7,772 μ S/cm specific conductance. The majority of observed salinity was below 1.0 psu. Salinity was slightly higher in bottom waters at some deeper (> 0.8 meter) sites (Figure 11). The surface salinity based on measurements at each site during the study period within the Trinity River delta area exhibited a homogenous pattern (Figure 12).

During the study combined surface and bottom dissolved oxygen exhibited an average value of 10.6 mg/L and varied between 6.1 and 14.4 mg/L (Figure 13). The majority of these measurements exceeded 10.0 mg/L. There was not apparent vertical pattern in dissolved oxygen with depth, although shallower sites appeared to have slight higher dissolved oxygen values (Figure 14).

Surface and bottom pH values exhibited an average value of 7.8 and varied between 7.1 and 9.2 units (Figure 15). The majority of these measurements exceeded 10.0 mg/L. There was not apparent vertical pattern in dissolved oxygen with depth, although shallower sites possessed the majority of pH values above 8.0 (Figure 16).



Figure 6. Location of final sites sampled during January –February 2016.



Figure 7. Distribution of water depths at samples sites during the study period.



Figure 8. Distribution of surface and bottom water temperature at samples sites during the study period.



Figure 9. Surface and bottom water temperature versus total depth. Only surface temperatures were measured for depths less than 0.5 meters.



Figure 10. Distribution of surface and bottom water salinity at samples sites during the study period.



Figure 11. Surface and bottom salinity versus total depth. Only surface salinity was measured for depths less than 0.5 meters.



Figure 12. Spatial distribution of salinity measured at sample sites during the study period.



Figure 13. Distribution of surface and bottom dissolved oxygen at samples sites during the study period.



Figure 14. Surface and bottom dissolved oxygen versus total depth. Only surface salinity was measured for depths less than 0.5 meters.



Figure 15. Distribution of surface and bottom pH at sample sites during the study period.



Figure 16. Surface and bottom pH versus total depth. Only surface salinity was measured for depths less than 0.5 meters.

Surface Secchi tube transparency exhibited an average value of 0.131 meters and varied between 0.047 and 0.700 meters (Figure 17). The majority of these measurements were below 0.24 meters. There was no apparent pattern in transparency versus total depth (Figure 18).

Sediment type was primarily assessed by characterization of the amount of "percent fines" which serves as an index of the percent of clay plus silt in the sediment. Percent fines varied between 0 to 80% with a mean value of 40.2% and median of 39.0% (Figure 19). There was no apparent pattern between percent fines and depth (Figure 19). Parnell et al. (2011), reported an average value of approximately 44% percent silt and clay within the Trinity River delta.

The majority, 44 out of 50 sites of sampled sites lacked large (>5%) amounts of aquatic vegetation (Figure 21). Significant amounts of aquatic vegetation were only found at sites in shallower (<1 meter) depth (Figure 22). It should be noted that due to the high turbidity present during most of the study there is likely a negative bias against detection of aquatic vegetation in deeper water.

Rangia Catch Rates

Due to changes in geomorphology we occasionally needed to shift the location of the proposed sample site up to 30 meters. The final locations of sample sites are presented in Table 2 and Figure 23-25. Live and recently dead (both valves attached) Rangia were detected at 37 out of 50 (74%) sites within the Trinity River delta. Similarly live Rangia alone were found at the same frequency. A total of 345 live Rangia were captured during the study period. Atlantic rangia was the dominant species collected during the study. A single live specimen of brown rangia, *Rangianella flexuosa* was captured alive at site R28 (Figure 24).

A total of 34 live and recently dead (possessing two attached valves) specimens of Rangia were collected with benthic grab samplers (Ekman, Ponar, core) at nine of the 50 (18%) sites sampled during the study period. Catch rates varied between zero and eight Rangia per benthic grab (Figure 26). Highest median catch rates (7/grab, 20/site) occurred at the open by site R11, which was located east of the mouth of the Trinity River (Figure 24 and 26). The majority (31/ 34) of live and recently dead specimens collected with benthic grabs consisted of live specimens. Live specimens were observed at eight out of the 50 (16%) of the sites sampled. Median catch rates of live Rangia varied between zero and seven specimens per benthic grab (Figure 27). The highest capture rate which consisted of all live specimens occurred at site R11.

A total of 231 live and recently dead (possessing two attached valves) specimens of Rangia were collected with the clam dredge and rake samplers at 33 of the 50 (66%) sites sampled. Median catch rates varied between 0 to 8 Rangia per haul (Figure 28). The highest median catch rates (8/haul) and total catch (29/site) occurred at the open by site R38, which was located at the mouth of the Trinity River (Figure 24). Other sites with high median catches of Rangia included R28, R37, and R15 which were located in the lower portion of the river or delta. The majority (32 out of 33) of the sum of live and recently dead specimens captured with dredge and rake sampling consisted of live specimens. These live specimens were observed at 32 out of the 50 (64%) of the sites sampled. Median catch rates of live Rangia varied between zero and seven specimens per haul (Figure 29). As noted before the highest rate of capture occurred at R38 of which 26 out of 29 (89%) were live specimens.



Figure 17. Distribution of surface and bottom Secchi tube transparency at sample sites during the study period.



Figure 18. Secchi tube transparency versus total depth.



Figure 19. Distribution of percent fines in sediment collected from sample sites during the study period.



Figure 20. Percent fines in sediment versus total depth at sample sites.



Figure 21. Distribution of sample sites by percent bottom covered by aquatic plants during the study period.



Figure 22. Percent of bottom covered by aquatic vegetation versus total depth at sample sites.

Site	Lat	Long	Present	Site	Lat	Long	Present
R01	29.81470	-94.76915	Y	R26	29.76323	-94.69994	Ν
R02	29.82520	-94.78819	Y	R27	29.77735	-94.74061	Y
R03	29.81417	-94.78252	Y	R28	29.77555	-94.76965	Y
R04	29.80269	-94.78571	Ν	R29	29.74636	-94.70950	Y
R05	29.81611	-94.79781	Ν	R30	29.75871	-94.71561	Y
R06	29.75092	-94.69987	Y	R31	29.78875	-94.72158	Y
R07	29.76627	-94.71295	Y	R32	29.77750	-94.70909	Y
R08	29.78748	-94.79101	Y	R33	29.78986	-94.74033	Ν
R09	29.77000	-94.73606	Y	R34	29.77607	-94.73110	Y
R10	29.79035	-94.74694	Y	R35	29.76402	-94.73118	Y
R11	29.72845	-94.69686	Y	R36	29.75693	-94.69597	Y
R12	29.78141	-94.71957	Y	R37	29.74671	-94.69913	Y
R13	29.77236	-94.72662	Y	R38	29.73607	-94.70800	Y
R14	29.72428	-94.72449	Ν	R39	29.76694	-94.72284	Y
R15	29.76154	-94.70743	Y	R40	29.76505	-94.71893	Y
R16	29.80320	-94.73571	Ν	R41	29.78701	-94.72806	Y
R17	29.78321	-94.70165	Y	R42	29.76803	-94.70200	Ν
R18	29.77502	-94.69693	Y	R43	29.77333	-94.71696	Ν
R19	29.79099	-94.71284	Ν	R44	29.78202	-94.75096	Y
R20	29.78018	-94.75710	Y	R45	29.80406	-94.72609	Ν
R21	29.75480	-94.72739	Y	R46	29.78411	-94.70416	Y
R22	29.73486	-94.71950	Ν	R47	29.76897	-94.69509	Y
R23	29.72304	-94.71458	Y	R48	29.75895	-94.69415	Y
R24	29.74561	-94.69379	Y	R49	29.81420	-94.75718	Y
R25	29.80074	-94.74633	N	R50	29.83714	-94.78749	N

 Table 2. List of sites surveyed for Rangia species during January –February 2016.



Figure 23. Location of final sites sampled during January –February 2016. (Green circle = *Rangia cuneata* and/or *R. flexuosa* captured; red neither species captured).



Figure 24. Location and identification of sites sampled in lower portion Trinity River delta during January –February 2016. (Green circle = Rangia cuneata and/or *R. flexuosa* captured; red neither species captured).



Figure 25. Location and identification of sites sampled in upper portion Trinity River delta during January –February 2016. (Green circle = *Rangia cuneata* and/or *R. flexuosa* captured; red neither species captured).



Figure 26. Boxplot of the number of Rangia (live and recently dead with attached valves) specimens per benthic grab at each site.



Figure 27. Boxplot of the number of live Rangia specimens per benthic grab at each site.



Figure 28. Boxplot of the number of Rangia (live and recently dead with attached valves) specimens per dredge or rake haul at each site.



Figure 29. Boxplot of the number of live Rangia specimens per dredge or rake haul at each site.

Catch rates between benthic grabs and combined dredge and rake were poorly correlated (Figure 30, r = -.048, p = 0.741). In general the rake and dredge methodology was more effective at detecting live Rangia at 28 sites where the benthic grabs failed to detect any specimens. However the benthic grabs did detect Rangia at two sites, R11 and R44, where the rake or dredge failed to

collect live specimens. Recall that site R11 yielded the highest number of Rangia (total 20) at any one site collected by benthic grabs.

Comparison of Rangia Incidence in the Trinity River Delta with Past Studies

Rangia occurrence rates were compared between this study and published data presented by Parnell et al. (2011) and Windham (2015). Together these studies cover a period from May 2011-August 2011 (27 sites), January 2012 to November 2014 (five sites) and January to February 2016 (50 sites) within the Trinity River delta region. Sampling during the three studies occurred at various times of the year under different salinity regimes using varying amounts of effort and different sampling methods. Therefore comparison of density of catch per unit effort is difficult. We therefore used binary presence absence data as the biological endpoint to facilitate comparison of Rangia incidence between study periods.

During May to August 2011, Parnell et al. (2011) conducted a survey of 27 sites the Trinity River delta for the occurrence of Rangia using clam rakes and hand excavation (Figure 31). During this period they detected Rangia at 17 out of 27 sites. Rangia occurrence rates at 15 sites previously monitored by Parnell et al. (2011) were compared with the current study (Table 3). During the current study we failed to detect Rangia at four of the 15 sites previously monitored including R42, R43 and R45 (Figure 32). It is difficult to compare sampling effort between the two studies since the number of rake pulls deployed by Parnell et al. (2011) was not described in their report and therefore quantitative estimates of catch per unit effort are not available. Only presence and absence of Rangia was reported in during their study.

Data from the current study was compared to data collected by Windham (2015) during January 2012 to November 2014 (Figure 33). During her study Windham (2015) utilized a combination of rakes and quadrat excavations to detect and enumerate Rangia at 5 sites located at the bay margin of the Trinity River delta. These sites overlapped and were located at or near 4 previous sites monitored by Parnell et al. (2011) and 5 of the 50 current study sites (Table 4 and Figure 33). The current study collected Rangia clams at R12. In contrast Rangia was not detected during 2011-2014 at the adjacent Parnell RD-11 and Windham 4 study sites (Table 4). During 2013 to 2014 Rangia were not detected in the vicinity of sites R35 and R44 although they were detected in 2011-2012 and again in 2016. Rangia were consistently observed in the vicinity of site R28 during 2012 to 2014 and again in 2016.

Examination of time series plots of the occurrence of Rangia based on the results of all studies combined did not detect any consistent trend in the occurrence of Rangia when adjusted for sample size by using percent frequency (Figure 34-35). However, at the 5 sites monitored by Windham (2015), a 100 % detection rate was attained during the second quarter of 2012. This was an artifact attributed to only one site, site no. 5, being sampled and yielding a positive catch during that quarter. As noted earlier, site no. 4 consistently failed to yield live Rangia from 202 to 2014 (Windham 2015).



Figure 30. Comparison of total catch rates between benthic grabs and combined dredge and rake collections.



Figure 31. Sites sampled by Parnell et al. (2011) for Rangia. Green and red denote sites where Rangia were detected and not detected.

Parnell - 2011	EIH - 2016		Presence/Absence				
Site	Site	Latitude	Longitude	2011	2016		
RD-1	R34	29.776	-94.731	1	1		
RD-2	R35	29.764	-94.731	1	1		
RD-6	R36	29.757	-94.696	1	1		
RD-7	R37	29.746	-94.698	1	1		
RD-8	R38	29.736	-94.708	1	1		
RD-9	R39	29.767	-94.723	1	1		
RD-10	R40	29.765	-94.719	1	1		
RD-12	R41	29.787	-94.728	1	1		
RD-15	R42	29.768	-94.702	1	0		
RD-16	R43	29.773	-94.717	1	0		
RD-17	R44	29.782	-94.751	1	1		
RD-19	R45	29.804	-94.726	1	0		
RD-21	R46	29.484	-94.704	1	1		
RD-23	R47	29.769	-94.695	1	1		
RD-24	R48	29.759	-94.694	1	1		
RD-26	R49	29.814	-94.757	1	1		
RD-27	R50	29.837	-94,787	1	0		

Table 3. Comparison of live Rangia detection rates at sites sampled during this study and Parnell et al. (2011). (1 = present, 2 = absent).



Figure 32. Sites sampled during the current survey where Parnell et al. (2011) also detected Rangia. Green starts = Rangia detected during both surveys; red stars denotes sites where Rangia was not detected during current study.

Table 4. Occurrence of live Rangia during each study period at five locations where sites
overlapped or where located within 300 meters of each other. Coordinates refer to current
study sample sites.

Parnell - 2011	Windham 2015	EIH - 2016			Year - Presence =1, Absence = 0				
Site	Site	Site	Latitude	Longitude	2011	2012	2013	2014	2016
RD-2	3	R35	29.764	-94.731	1	1	0	0	1
RD-8	5	R38	29.736	-94.708	1	1	1	1	1
RD-11	4	R12	29.781	-94.720	0	0	0	0	1
RD-17	2	R44	29.782	-94.751	1	1	0	0	1
-	1	R28	29.776	-94.770	-	1	1	1	1



Figure 33. Sites sampled by Windham (2015) during January 2012 to November 2014, which were located close to existing study sites (R12, R28, R35, R38, R44). Green diamonds = Rangia detected during both studies; red diamond denotes sites where Rangia was detected during current study but not earlier.



Figure 34. Frequency of positive and negative occurrences of Rangia from May 2011 to February 2016. Data source: current study, (Parnell et al. 2011), (Windham 2015). Sample size varied between periods.



Figure 35. Percent frequency of positive and negative detections of Rangia from May 2011 to February 2016. Data source: current study, (Parnell et al. 2011), (Windham 2015). Sample size varied between periods.

Comparison of Salinity, Discharge and Rangia Occurrence between Studies.

Salinity measured during all three studies combined varied between 0 and 23 psu, with an overall average of 3.0 psu. No measurements were made during 2015. Salinity generally declined from the 2011-12 period to 2016 (Figure 36). Comparison of salinity measured since May 2011 during the current study and past investigations in the Trinity River delta document periods of decreased salinity during the first two quarters (January-June) of 2012, the second quarter (April – June) 2014 and first quarter (January-February) of 2016 (Figure 36-37).

A plot of salinity versus occurrence data illustrates the wide range at which Rangia was captured during all three studies (Figure 38). Although effort varied between studies and dates a similar number of positive and negative detections occurred within most salinity categories. The highest frequency of detections (36) and zero catches (32) occurred within a salinity range of 0-1.5 psu (Figure 38). This is partially due to the high amount of sampling effort expended during January to February 2016. The highest salinity where live Rangia was detected was 23.0 psu.

An overlay of sampling events over recorded river discharge from January 2011 to February 2016 illustrates the wide range of hydrological conditions that occurred during the three study periods (Figure 39). During May to August 2011, river flows in the Trinity River were very low seldom exceeding 5000 cfs at the Romayor gage. This period corresponded with collection efforts by Parnell et al. (2011). During winter and spring months of 2012 river discharged increased up to an exceeded 40,000 cfs. Additional peak flows occurred early 2014 and the fall of 2014. The period of 2012 to 2014 corresponds with collection efforts by Windham (2015). Finally from January 2015 to summer month of 2015 river discharge steadily increased and reached 70,000 cfs. Flows did not subside to base levels until the late fall and then increased above 70,000 cfs in January 2016 after which flows subsided to 10,000 cfs by late February. The hydrology of late 2015 and early 2016 would influence salinity conditions in the Trinity River delta during the current study.

The periods for each study can be classified as dry, for (Parnell et al. 2011), moderate-variable (Windham 2015), and wet (current study). To more clearly illustrate this classification we plotted the average daily flow, 30 day average daily flow, and the 90 daily average daily flow for each sampling event (Figure 40-42). Examination of these figures illustrates the apparent difference in hydrology between studies. Exposure of Rangia populations to varied river inflow differed under each study period. Using salinity data from each study we found a statistically significant relationship between river discharge and ambient salinity in the river delta (Figure 43-45). The strongest relationship (highest r^2) was found between log_{10} transformed 90 day daily average discharges and salinity (Figure 45). Examination of salinity measured during the current study and past investigations in the Trinity River delta document periods of decreased salinity during the first two quarters (January-June) of 2012, the second quarter (April – June) 2014 and first quarter (January-February) of 2016 (Figure 36-37). These declines in salinity correspond with increased freshwater inflow from the Trinity River (Figure 46). The occurrence of Rangia during various flow regimes is illustrated in (Figure 47). Similar to the previous graph depicting salinity versus Rangia occurrence it illustrates the wide range of freshwater discharge where Rangia was detected. Despite these discharge conditions, it appears that more Rangia detections occurred during low salinity conditions.



Figure 36. Salinity measured during each study during May 2011 to February 2016.



Figure 37. Boxplot of quarterly salinity measured during May 2011 to February 2016.



Figure 38. Frequency of collections at sites where Rangia were detected and undetected by salinity category. Data Sources: current study,Parnell et al. (2011), and Windham (2015).



Figure 39. Daily average discharge measured at the USGS 08066500 Trinity River at Romayor, Texas gage from January 2011 to February 2016. Red dashed lines mark sample collection dates during the current study and past studies (Parnell et al. 2011; Windham 2015).



Figure 40. Average daily flow (cfs) at the Trinity River at Rosharon gage versus date from May 2011 to February 2016.



Figure 41. Average 30-day flow (cfs) at the Trinity River at Rosharon gage versus date from May 2011 to February 2016.



Figure 42. Average 90-day flow (cfs) at the Trinity River at Rosharon gage versus date from May 2011 to February 2016.



Figure 43. Fitted regression line between salinity and log10 transformed daily average discharge (p < 0.001).



Figure 44. Fitted regression line between salinity and log10 transformed 30 day average discharge (p < 0.001).



Figure 45. Fitted regression line between salinity and log10 transformed daily 90 day average discharge (p < 0.001).



Figure 46. Boxplot denoting mean average 90 day discharge by quarter when Rangia sampling was conducted.



Figure 47. Frequency of collections at sites where Rangia were detected and absent by 90 day average discharge category. Data Sources: current study and past studies (Parnell et al. 2011; Windham 2015).

Occurrence of Vallisneria americana

During our study we observed *Vallisneria americana* at several sites including R13, R15, and R31 (Figure 48). Previous recent attempts to locate *V. americana* in the Trinity River delta have been unsuccessful (Parnell et al. 2011; Quigg and Steichen 2015; Windham 2015). Quigg and Steichen (2015) noted that *V. americana* had rarely been identified within Galveston Bay over the past 30 years and not found during their studies from 2011 to 2014 in Galveston Bay (Parnell et al. 2011). They also noted that *V. americana* was observed by Scott Alford (USDA – NRCS) in the delta during 2015. Prior to this observation, Trinity Bay experienced freshet events during the spring of 2015 due to higher discharges. As previously noted, the observations made during this study occurred after high river discharge reduced salinities from median values of 5-10 psu during 2014 to < 3 psu during early 2016 which is supportive of longterm *V. americana* survival (Dobberful et al. 2012.; Frank and Moore 2003).

Morphometrics of Rangia cuneata

During this study Rangia mean shell length was 47.7 ± 0.9 mm (SE) and length ranged between 8.1 to 71.4 mm. Parnell et al. (2011) reported a mean length (55.7 \pm 7 mm) for the delta specimens they collected. The reported mean length (48.9 ± 0.4 mm) for northern Galveston Bay in their study was similar to the values we reported. During 2012 to 2014, Windham (2015) reported annual mean shell lengths of 48-50 mm. The majority of Rangia collected during that period were longer than 45 mm. The shell length of Rangia shells collected during our study exhibited a bimodal (22.5-27.5 mm and 57.5-60 mm) distribution suggesting at least two year classes of this species dominated the sampled population (Figure 49). Based on the application von Bertanlanfy growth model for *R. cuneata* developed by Wolfe and Petteway (1968), these two modes would likely represent 2-3 year old, and 4+ year old cohorts. All gear with the exception of the towed dredge yielded similar size distributions (Figure 50). This may represent a sampling artifact since only three specimens were collected with the clam dredge. Similar bimodal distributions and gear difference in shell height and width, total weight, and soft tissue weight were observed (Figure 51-58)). The mean shell width of *R. cuneata* was 30.8 ± 0.6 mm and width ranged between 4.2 to 46.0 mm. The mean shell height was 41.6 ± 0.6 mm and height varied from 6.7 and 62.1 mm. Total weight exhibited a mean of 40,538 mg \pm 3,260 mg, and ranged between 617 and 146,106 mg. The soft tissue weight exhibited an average of $15,168 \pm$ 864 mg and ranged between 359 to 40,418 mg.

The average meat index (% total weight) observed during this study was $30.3 \pm 0.5\%$. Meat index values varied between 17.7 and 60.4% (Figure 59 and 60). The meat index did not appear to exhibit a bimodal peak which suggests the ratio of soft tissue to shell material exhibits a similar ratio regardless of shell size. Parnell et al. (2011), reported an average meat index of 12.5% during May to August 2011. During this same period they documented average meat index values of 7.5% in East Bay, 10% in northern Galveston Bay, and 12.5% in Clear Lake. Annual average meat index values in Trinity River delta and open waters during 2012-2014 were approximately 12% (Windham 2015). Based on comparison with these studies it appears that Rangia collected during this study contained proportionately more soft tissue for all shell sizes.



Figure 48. Sites where Vallisneria americana was detected during Rangia surveys.



Figure 49. Distribution of Rangia cuneata shell lengths observed during the current study.



Figure 50. Boxplot of *Rangia cuneata* shell length by collection method.



Figure 51. Distribution of *Rangia cuneata* shell widths observed during the current study.



Figure 52. Boxplot of *Rangia cuneata* shell width by collection method.

Figure 53. Distribution of *Rangia cuneata* shell heights observed during the current study.

Figure 54. Boxplot of *Rangia cuneata* shell height by collection method.

Figure 55. Distribution of *Rangia cuneata* total weight observed during the current study.

Figure 56. Boxplot of *Rangia cuneata* total weight by collection method.

Figure 57. Distribution of *Rangia cuneata* soft tissue weight observed during the current study.

Figure 58. Boxplot of *Rangia cuneata* soft tissue weight by collection method.

Figure 59. Distribution of *Rangia cuneata* meat index (% total weight) observed during the current study.

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Figure 60. Boxplot of *Rangia cuneata* meat index (% total weight) by collection method.

×

Other Species Encountered

Several additional species that were rarely encountered are discussed here. One dead specimen of the Round Pearshell, Glebula rotundata, was collected at R12 with both valves attached. The specimen had the following dimensions, length 14.1 cm, height 10.0 cm, and width 6.5 cm. This species is one of the few unionids that can tolerate low levels of salinity in estuaries and is not found far upstream of the estuarine freshwater interface (Howells 2014).

One dead specimen of the Carolina marshclam, *Polymesoda caroliniana*, was found at R29 with both valves attached. The specimen had the following dimensions, length 4.6 cm, height 4.5 cm, and width 3.3 cm. This clam is a member of the Family Corbiculidae and is present in fresh and low-salinity waters along the Texas coast and is known to be tolerant to a wide range of salinity and dissolved oxygen and tolerant to desiccation (Howells 2014; Tunnell Jr et al. 2014).

depths that still permits sufficient light penetration.

DISCUSSION

This study represents the first comprehensive survey for *Rangia cuneata*, *Rangianella flexuosa* and *Vallisneria americana* within the Trinity River delta during conditions influenced by high river discharges. During our limited surveys we only found *V. americana* in shallow (< 1 m) water. This is most likely due to the high turbidity found in the river delta that was confirmed by our turbidity (Secchi tube) monitoring. *V. americana* requires relatively clear water to survive and grow (Frank and Moore 2003). As a result they can only survive in turbid water at shallower

Another factor affecting V. americana in the Trinity River delta is elevated salinity. Drought conditions were predominant during 2011-2014 when past studies were conducted. As a result salinity levels were generally lower than conditions that existed before 2015. Although it is difficult to directly compare study results due to differing methodology which includes a variety of sampling methods, periodicity and frequency there are several important conclusions that can be made from examination of the current data set and recent studies. The prolonged elevated freshwater inflow during 2015 and early 2016 have resulted in depressed salinity (< 3 psu) throughout much of the Trinity River delta. Past literature and monitoring has found that optimal survival and growth in Vallisneria americana occurs when salinities are below < 3 psu for greater than 90 days (Dobberful et al. 2012.) It is highly likely that due to these favorable conditions, V. americana has colonized and begun to reestablish itself within the delta. Prior to this study V. americana had not been detected during past field investigations conducted in 2011-2014 (Parnell et al. 2011; Quigg and Steichen 2015; Windham 2015). Recent public sightings in 2015 reported in Quigg and Steichen (2015) along with these study results confirms the positive role of freshwater inflow on the V. americana. These sightings also support the need to continue to monitor this species and investigate its role as an indicator of freshwater inflow.

Although it was difficult to discern any pattern in Rangia attributable to salinity or river discharge due to differences in sampling methodology, it is clear that the meat index (% total weight) had increased from former levels reported in 2011 to 2014 by (Parnell et al. 2011; Windham 2015). The average meat index (% total weight) observed during this study was 30.3 \pm 0.5%. In contrast Parnell et al. (2011), reported an average meat index of 12.5% during May to August 2011. Annual average meat index values in Trinity River delta and open waters during 2012-2014 were approximately 12% (Windham 2015). Based on comparison with these studies it appears that Rangia collected during this study contained proportionately more soft tissue (biomass) for all shell sizes. The meat index is considered a basic health index that measures the amount of soft tissue somatic growth and gonad condition. Any stressor that would reduce feeding activity for any size or age Rangia would over time reduce soft tissue biomass even after shell tissue has been produced. This would effectively result in a decrease in the ratio of soft tissue to total weight due to starvation. It is highly likely that during the drought conditions in 2011 to 2014 many Rangia were not feeding effectively which led to stress and a reduction in soft tissue biomass.

It is puzzling why *Rangianella flexuosa* was not encountered more frequently during this study. Past monitoring and studies by TPWD and (Parnell et al. 2011) have encountered more *R*. *flexuosa* in portions of Galveston Bay (Windham 2015). Although particular care was taken to

insure the accuracy of species identifications it is possible that some smaller specimens could have been misidentified. However, all shells collected during our study were retained for archival purposes.

Sampling methods employed by this study differed from past studies. The use of airboats enabled investigators to survey sites that were formally inaccessible due to shallow water conditions. These shallow water sites represented some of the sites where *V. americana* was detected. Future studies within the Trinity River delta should continue to employ airboats to facilitate access to shallow water areas and reduce sampling bias.

Continued monitoring of Rangia cuneata, Rangianella flexuosa, and Vallisneria americana over a wide range of discharge and salinity conditions is critical for evaluating the influence of adopted freshwater inflow regimes and their role as freshwater inflow bioindicators. Future monitoring should include several additional components to differentiate the relative influence of river discharge, salinity and other factors on the spatial distribution, survival, reproduction and growth of these species. Specific recommendations are the inclusion of automated temperature and salinity meters within the delta to gain a better understanding of the influence of freshwater inflow on these water quality variables and possible mechanisms that limit the population size of these species. Expanded spatial coverage that include both fixed index sites for long term temporal monitoring and stratified random sites to gain information on spatial distribution are needed. Adoption of these approaches would also increase statistical power including the ability to detect both temporal and spatial trends in the abundance, survival, growth and reproductive success of these species. Additional study components that should be adopted include the deployment of field mesocosms. The use of field mesocosms would enable investigators to manipulate multiple variables including sediment type, depth, predation, and facilitate mark recapture studies to gain better estimates of population size, density and growth and the response of these variables to freshwater inflow.

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