

Characterization of the Influence of Freshwater Inflow on Trinity River Delta Bioindicators

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List of Abbreviations

avg	average
BBEST	Basin and Bay Expert Science Team
C	Celsius
CFDA	Catalog of Federal Domestic Assistance
cfs	cubic feet per second
cm	centimeter(s)
CORS	Continuously Operating Reference System
CPUE	catch per unit effort
CST	conductivity/salinity and temperature
DGPS	differential global positioning system
g	gram(s)
GIS	geographic information system
GLM	generalized linear model
GPS	global positioning system
m	meter(s)
max	maximum
MI	Meat Index
min	minimum
mL	milliliter(s)
mm	millimeter(s)
n	number
NAD83	North American Datum of 1983
NAVD88	North American Vertical Datum of 1988
NGS	National Geodetic Survey
NOAA	National Oceanic and Atmospheric Administration
psu	practical salinity units
PVC	polyvinyl chloride
QAPP	Quality Assurance Project Plan
SAV	submerged aquatic vegetation
SWQM	Surface Water Quality Monitoring
TCEQ	Texas Commission on Environmental Quality
TPWD	Texas Parks and Wildlife Department
TX	Texas
USGS	United States Geological Survey
UTM	Universal Transverse Mercator

EXECUTIVE SUMMARY

Freshwater inflow standards developed for Galveston Bay pursuant to the 80th Texas Legislature Senate Bill 3 process identified Atlantic Rangia (*Rangia cuneata*) and Wild Celery (*Vallisneria americana*) as bioindicators. Both Atlantic Rangia (herein also referred to as “Rangia”) and Wild Celery are benthic species that have a low and narrow preferred salinity tolerance range making them ideal bioindicator species of freshwater inflow. Recent studies have worked to document the distribution and abundance of these bioindicators in the Galveston Bay system, but additional efforts to understand the relationship between freshwater inflow and the distribution, abundance, and health of these bioindicators are still needed. The primary objectives of this study were to conduct an analysis of the historical distribution of submerged aquatic vegetation (SAV, which includes Wild Celery) and Rangia, conduct an on-the-ground inventory of SAV and Rangia, and establish a network of shallow automated salinity monitoring sites to describe the impacts of freshwater inflow on the salinity patterns of the Trinity River Delta.

A total of 10 sites distributed throughout the Trinity River Delta were monitored quarterly for Rangia and SAV and continuously for water temperature and salinity from February 2018 through August 2019. Three of the sites were additionally monitored continuously for water level. During each Rangia sampling event, ambient measurements of water quality variables (water temperature, salinity/specific conductance, pH, dissolved oxygen, and water clarity) were recorded and sediment samples were collected for analysis of sediment size distribution (percent fines). Rangia sampling consisted of two approaches, including tactile hand sampling within a 1-meter (m) quadrat and raking the sediment with a clam rake for 30 seconds. Three replicates of each sampling approach (hand sampling and clam rake) were conducted at each sample site during each sampling event. All live and recently dead (but whole) Rangia were enumerated and up to 20 from each site were retained for laboratory analysis. Retained Rangia were measured (length, width, height), weighed and shucked to calculate Meat Index (MI), a relative indicator of clam health. Visual sampling for SAV was conducted during each Rangia sampling event and during each gear-servicing event (every 3-4 weeks).

During the study period, the average salinity for all sites monitored in the delta ranged from 0.53 to 1.46 practical salinity units (psu). Live Rangia were detected at all 10 study sites and a total of 271 live specimens were captured during the study period. The mean shell length for Rangia

collected during this study was 56.25 millimeters (mm), and a gear bias was discovered with the hand sampling method resulting in significantly larger clams. The average catch per unit effort (CPUE) was 1.33 clams per m² for the hand sampling technique and 0.31 clams per 30-second clam rake. The average MI for *Rangia* collected during this study was 21.89. MI showed an inverse linear relationship with percent fines and average salinity (each a proxy for freshwater inflow), indicating that clams that had recently experienced elevated freshwater inflow were generally healthier than clams that had recently been exposed to higher salinity conditions. This finding was corroborated when MI was compared to recent average discharge, with significant positive linear relationships between MI and 30-day, 60-day, and 90-day average discharges from United States Geological Survey (USGS) gages on the Trinity River at Wallisville (08067252) and Romayor, TX (08066500). Wild Celery was not observed during this study. Another oligohaline SAV species, Widgeongrass (*Ruppia maritima*), was observed at two sites, however the blades were very short and had substantial epiphytic growth.

A wide variety of sizes of *Rangia* were detected during all previous studies in the Trinity River Delta, indicating a sustained reproducing population occurs within this study area. Within the current study period, freshwater pulses exceeded the annual “overbank pulse” amount identified in the pulse flow recommendations for the Trinity River Basin. Based on comparison with previous studies in the Trinity River Delta, it appears that *Rangia* collected during periods of elevated freshwater inflow are healthier, as measured by MI, than those collected during drought conditions. Live *Rangia* collected during the current study were retained for future age and growth analysis, which will help define the influence of freshwater inflow on the fine-scale growth of *Rangia* in the delta. Continued monitoring of *Rangia* and Wild Celery over a wide range of discharge and salinity conditions is critical for evaluating the influence of adopted freshwater inflow regimes and the influence on both species as freshwater inflow bioindicators. Additional discharge and bathymetric monitoring is recommended to better understand how freshwater pulses move throughout the delta.

INTRODUCTION

Background

Recent studies by the United States Geological Survey (USGS) have documented inconsistencies between gaged upstream flows on the Trinity River and monitored inflows at the Trinity River mouth (Lucena and Lee, 2017). In addition, little is known about the influence of freshwater inflow on the salinity regime and response of freshwater bioindicators including Atlantic Rangia (*Rangia cuneata*)¹ and Wild Celery (*Vallisneria americana*)². Recently, multiple studies have documented bioindicator distribution and abundance in the Galveston Bay system (Guillen et al., 2016; Parnell et al., 2011; Windham et al., 2019), however, efforts to understand the relationship between freshwater inflow and the distribution, abundance and health of these bioindicators are still needed.

Atlantic Rangia (herein also referred to as “Rangia”) is a brackish water clam found in estuaries from New Jersey to the Laguna de Terminos, Campeche, Mexico (LaSalle and de la Cruz, 1985; Tunnell, 2010; Turgeon et al., 1998). Rangia are found well upstream into the mouths of rivers and bayous but grow to their maximum size in brackish water (Fotheringham and Rothschild, 1989). Although Rangia are able to tolerate salinities ranging from 0-38 practical salinity units (psu) under laboratory conditions, they are found most commonly at lower salinities (0-18 psu), and are most abundant in very low salinities (< 5 psu) (Auil-Marshalleck et al., 2000; Harrel and McConnell, 1995; Hopkins et al., 1973; LaSalle and de la Cruz, 1985; Otto and Pierce, 1981). The highest survival and growth rate of Rangia has been shown to occur at salinities ≤ 4 psu (Otto and Pierce, 1981). Rangia spawning cues are thought to coincide with increases in water temperature and salinity that occur in spring and late summer, but spawning can be continuous throughout this time of year in subtropical latitudes (Figure 1) (LaSalle and de la Cruz, 1985). Two main spawning periods have been identified in Louisiana in March – May and late summer to early November (Fairbanks et al., 1963; Global Invasive Species Database, 2020). In Mexico, the main spawning periods are February – June and September – November (Global Invasive Species Database, 2020; Rogers and García-Cubas, 1981).

¹ Atlantic Rangia has also been referred to as “Common Rangia” or “Gulf Wedge Clam”.

² Wild Celery has also been referred to as “Water Celery” and “American Eelgrass”.

Rangia was designated as an important indicator species for establishing and monitoring the appropriateness of freshwater inflow standards in Galveston Bay and other estuaries pursuant to the 80th Texas Legislature Senate Bill 3 process (BBEST, 2009). The Senate Bill 3 was designed to use existing information and the best available science to establish environmental flow recommendations and standards for Texas river basins and estuaries. However, most historic data, including the only long-term records of *Rangia* in Galveston Bay, have limitations because the standard sampling procedures utilized by the Texas Parks and Wildlife Department (TPWD) monitoring program were not designed to target soft-bottom benthic clam species.

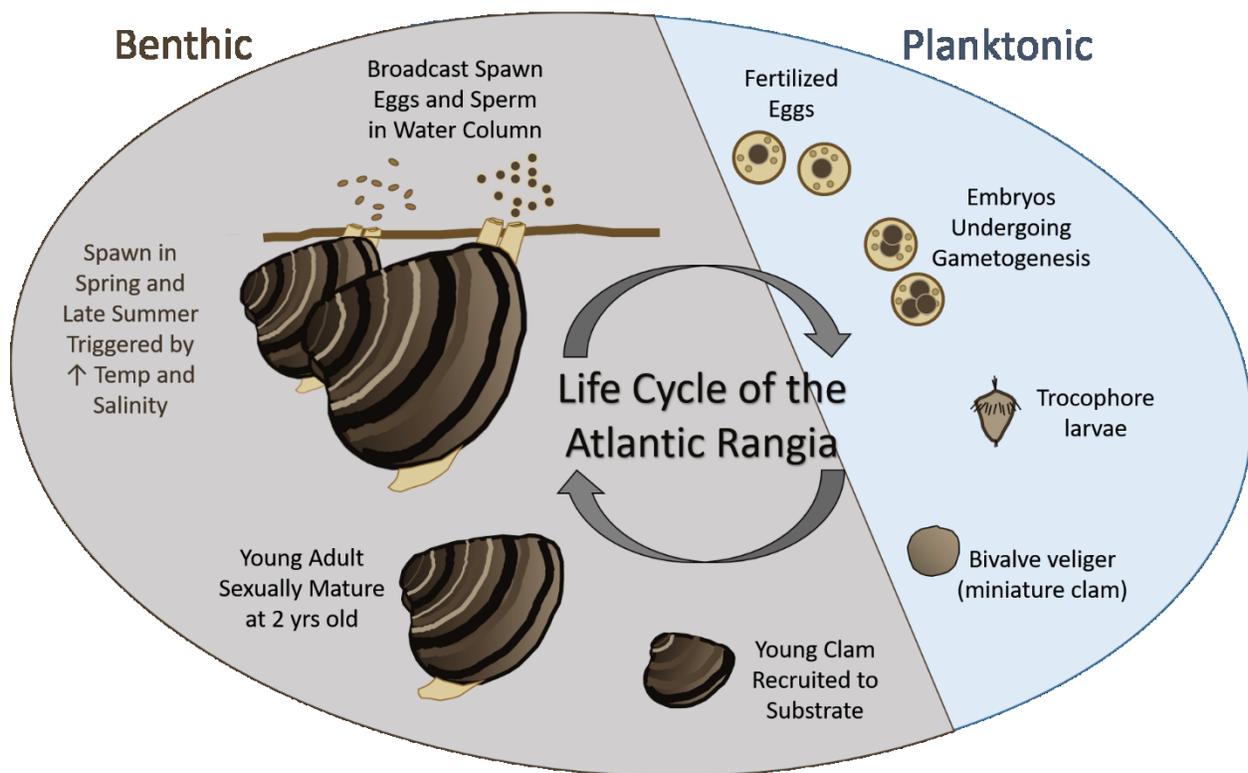


Figure 1. Depiction of the life cycle of Atlantic *Rangia*. Adapted from the life cycle of the northern quahog (drawn by A.J. Mansueti).

Based on historical fisheries independent sampling using oyster dredges and trawls, the TPWD reported the highest *Rangia* densities in Trinity Bay and upper Galveston Bay near the mouth of Buffalo Bayou, with lower densities 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 among Trinity Bay, the lower Trinity River, Clear Lake, and East Bay, although absolute densities were lower in comparison to historical data (Parnell et al., 2011; Windham, 2015). A more recent study that

evaluated *Rangia* distribution in the Trinity River Delta during a high-flow period (2015-2016) found improvements in clam health compared to the drought condition study (2010-2011) (Guillen et al., 2016; Parnell et al., 2011).

Less is known about the species composition, distribution and abundance of submerged aquatic vegetation (SAV) (which includes Wild Celery) within the Trinity River Delta. Previous studies have documented both Wild Celery and Widgeongrass (*Ruppia maritima*) in the Trinity River Delta and upper Trinity Bay (Adair et al., 1994; Guillen et al., 2016; Pulich and White, 1991). However, abundance and distribution in the 1990s-2010s were greatly reduced in comparison to historic estimates prior to the 1970s (Pulich, 2007). Wild Celery is a submersed perennial, dioecious, clonal macrophyte with broad distribution in east-central North America (Lowden, 1982). Wild Celery has perennating tubers that allow it to store nutrients and withstand periods of sub-optimal conditions. It also reproduces by germinating seeds, resulting in potential seed banks in the delta substrate (Campbell, 2005; Jarvis and Moore, 2008). Mesocosm studies on Wild Celery have shown that extended exposure to salinities greater than 10 psu causes stunted growth and reduced shoot densities (Doering et al., 2002). Optimal conditions for survival and growth occur between 3 to 5 psu (French and Moore, 2003). Because of its low and narrow preferential range of salinity, Wild Celery was identified as a bioindicator for freshwater inflows in Galveston Bay pursuant to the 80th Texas Legislature Senate Bill 3 process (BBEST, 2009).

A better understanding of the hydrodynamics of the Trinity River Delta and the distribution and abundance of freshwater inflow indicator species, including *Rangia* and Wild Celery, are required for resource managers to inform freshwater inflow needs to Galveston Bay.

Study Objectives

The primary objective of this study was to assess the population status of freshwater inflow bioindicators within the Trinity River Delta. The study focused on the lowermost reach of the Trinity River, the Trinity River Delta, and the adjacent upper Trinity Bay. The specific objectives of this study were to: 1) conduct a historic and on-the-ground inventory of SAV and *Rangia*, and 2) establish a network of shallow automated salinity monitoring sites to describe the impacts of freshwater inflow on the salinity patterns of the Trinity River Delta.

Objective 1: Inventory Rangia and SAV

Conduct an inventory of SAV, emergent vegetation, and Rangia and input into geographic information system (GIS) databases.

Objective 2: Establishment of an automated salinity and water level monitoring network

Deploy and monitor automated conductivity/salinity/temperature (CST) meters and water depth recorders to characterize changes in salinity regime associated with freshwater inflow from the Trinity River.

METHODOLOGY

Methods, quality objectives, and data management presented herein for historical review, and data analysis are available in detail in the project Quality Assurance Project Plan (QAPP) (Catalog of Federal Domestic Assistance [CFDA] No. 66.456, QTRAK No. 18-179).

Site Selection

A total of 10 monitoring sites were established throughout the Trinity River Delta; locations were selected based on previous studies on Rangia distribution and water monitoring efforts (Figure 2). The sites were distributed throughout the delta to provide an estimate of the spatial range and gradient of freshwater inflow. All sites were located in shallow water, less than one meter (m) to support hand-sampling methodologies. Continuous water temperature, conductivity, and salinity (Onset CST HOBO) automated monitoring equipment were deployed at each site. Three of the sites were also equipped with continuous water level monitors adjusted for barometric pressure (InSitu Level TROLL 400). Shallow-draft boats and airboats were used to gain access to each site. Field sampling occurred from February 2018 through August 2019.

Review and Compilation of Historical Data

Various electronic and published data sources including National Oceanic and Atmospheric Administration (NOAA), USGS, state agencies, theses and dissertations, and journal articles, were examined for historical hydrological, tide, water quality, and biological data including past occurrences of Rangia and SAV. Trinity River discharge data were obtained from USGS gage

sites 08066500 at Romayor, TX and 08067252 at Wallisville, TX. Daily average discharge data were used to estimate 30-day, 60-day, and 90-day average 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 Wild Celery in the Trinity River Delta (Guillen et al., 2016; Parnell et al., 2011; Quigg and Steichen, 2015; Windham et al., 2019; Windham, 2015).

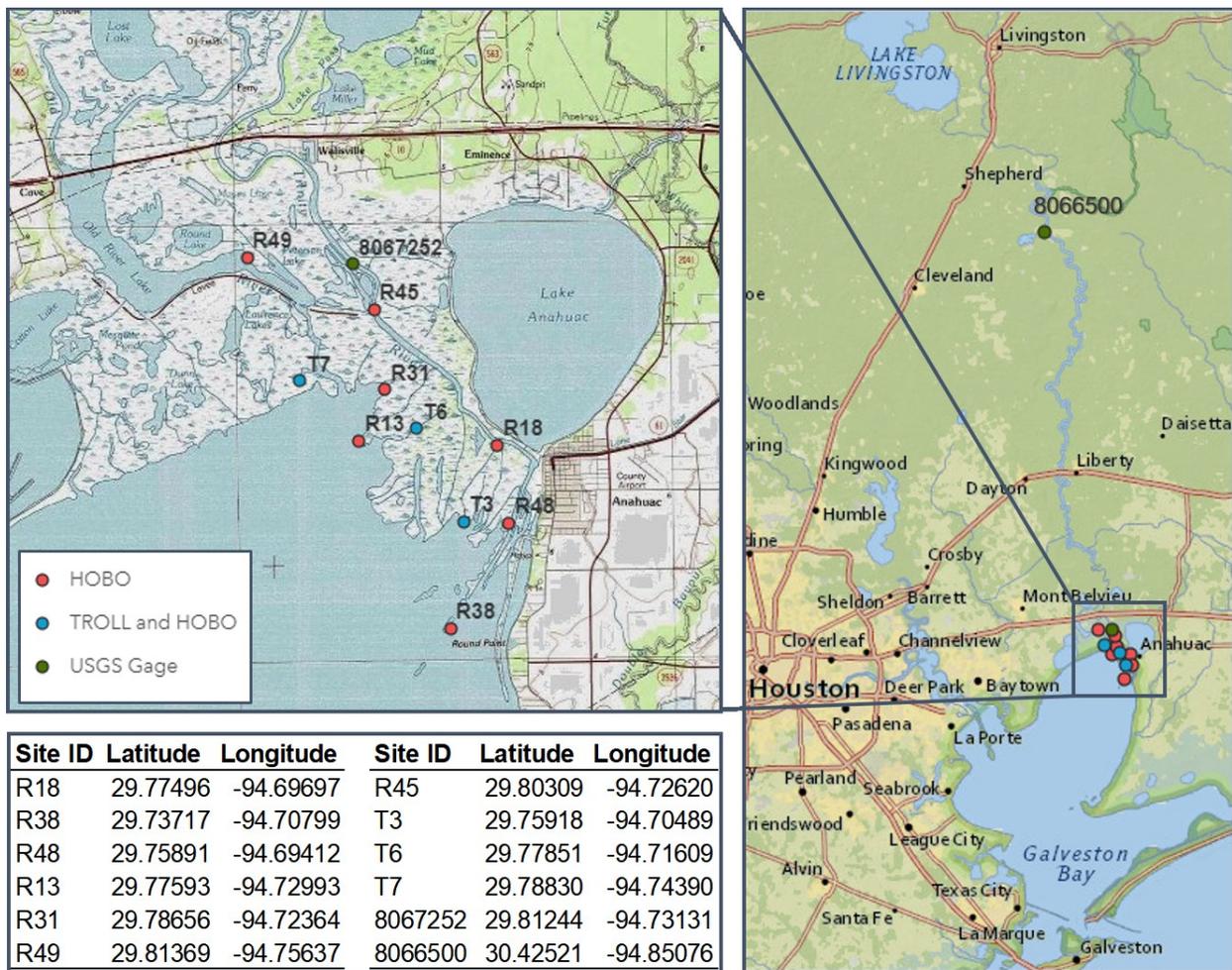


Figure 2. Study sites for *Rangia* and CST monitoring in the Trinity River Delta and USGS gage sites 8066500 (Trinity River at Romayor) and 8067252 (Trinity River at Wallisville) used in analysis.

Field Methods

Rangia Sampling

Benthic sampling focused on detecting and enumerating *Rangia* was conducted quarterly at each of the 10 monitoring sites. Sampling for *Rangia* used a combination of clam rake and quadrat (hand sampling) methods. The clam rake dimensions are provided in Table 1. A total of three, 30-second clam rakes were made within a 10 m radius of each study site. Hand sampling was facilitated with the use of a 1 m polyvinyl chloride (PVC) square quadrat held in place with 1-inch PVC stabilizing poles. Three 1 m square quadrats were delineated within a 10 m radius of the study site and all clams within each quadrat were enumerated. Tactile collection of all clams in the sediment (as deep as the sediment compaction would allow, typically 6-12 inches) was completed for the entirety of each of the three quadrats. At sampling events where water depth was greater than 1 m, hand sampling was conducted at the nearest shallow water and the distance from the study site was recorded. All whole clams (live and dead) were sorted in the field, identified to species, and enumerated. Up to 20 live or dead (but whole) *Rangia* specimens from each site were retained for additional lab-based analyses. If 20 *Rangia* were not collected in the rake or hand sampling efforts, when conditions and time allowed, additional tactile collection of *Rangia* was conducted opportunistically, without recording effort. Opportunistically collected *Rangia* were not included in any abundance or distribution analysis. Dead (but whole) *Rangia* were presumed to have died in place because they were still intact (both valves connected) in the sediment. All *Rangia* collected from the field were transported in zip-top bags and stored on ice. Upon arrival at the lab, samples were either immediately processed or stored in a refrigerator and processed within 72 hours of collection.

Sediment Sampling

At each site, sediment samples were collected using either a petite ponar or Ekman sampler depending on the substrate type. Sediment samples were sealed in extra thick-ply soil bags and transported to the lab for percent fines analysis. Upon arrival at the lab, sediment samples were stored in a refrigerator and processed within seven days of collection.

Submerged and Emergent Vegetation Distribution

The identity of the nearest submerged and emergent vegetation at the sampling location was recorded and relative density was estimated and expressed in percent cover. Additionally, during travel to, between, and from sample sites, all team members kept a sharp lookout for signs of SAV. When observed, SAV was identified to species and locational data of SAV was collected using high precision global positioning system (GPS).

Ambient Water Quality

At each biological sampling event, water transparency was measured using a Secchi tube following protocols outlined in the Texas Commission on Environmental Quality (TCEQ) Surface Water Quality Monitoring (SWQM) Procedures, Volume 1. A YSI water quality meter was used to measure water temperature, salinity, pH, and dissolved oxygen at the surface (0.3 m) and bottom (0.1 m less than total depth). All monitoring equipment was calibrated and operated using protocols outlined in the most recent edition of the TCEQ SWQM Procedures, Volume 1.

Table 1. Description of gear used to sample sediment and benthic organisms. For additional information, see Guillen et al. (2016).

Gear Name	Gear Specifications	Effort	Sample Type
Ekman	length x width = 6 x 6", maximum internal depth = 7.5"	1 grab per site with a target depth of 10 centimeters (cm) at sites with high silt content.	Sediment
Petite Ponar	length = 6", width 8.25", maximum internal depth = 9"	1 grab per site with a target depth of 8 cm at sites with high clay/sand content.	Sediment
Clam Rake	width = 13.75", depth 5.75", basket only height = 9", handle + basket height = 84", teeth length = 3.25", gap distance between teeth = 1", internal wire basket mesh size = 0.5" square mesh	3 replicate timed pulls per site for 30 seconds each.	Rangia
Hand-Sample Quadrat	1 m ² PVC	3 replicates per site.	Rangia

Automated Monitoring

A network of 10 freshwater inflow monitoring sites including water temperature, conductivity, and salinity were monitored using automated sondes manufactured by Onset. These monitoring sites were co-located with the biological monitoring sites throughout the Trinity River Delta. Three of the sites were additionally equipped with a water level manufactured by In-Situ. All automated monitoring equipment recorded ambient conditions every 15 minutes. All deployed meters were visited on a monthly basis to download data and clean. If site conditions restricted or prevented access, all efforts were made to visit the site as soon as conditions were amenable.

Field surveys were conducted on August 6, 2019 and August 13, 2019 to collect GPS signals for estimating water surface elevation at the 10 sampling sites. The surveys were conducted using a THALES ProMark3 GPS unit and GNSS Solutions Version 3.80.8 software from Trimble Navigation Limited for post-processing in the Differential GPS (DGPS) mode. The nearest Continuously Operating Reference System (CORS) station maintained by the National Geodetic Survey (NGS), located in Anahuac, TX (NGS ID TXAC), was used as a base GPS station, while a rover GPS unit was used to visit each sampling site for collecting the required satellite signals. At each sampling site, data collection was conducted for 32-43 minutes depending on the distance between the sampling site and the TXAC station (the farther the sampling site from TXAC station, the longer it was occupied). The satellite signals collected at the TXAC station during both days of the surveys were downloaded from the NGS website and post-processed with the data collected at the sampling sites to calculate the projected coordinates in the Universal Transverse Mercator (UTM) Zone 15N, North American Datum of 1983 (NAD83) using the North American Vertical Datum of 1988 (NAVD88) and geoid model GEOID12. Water surface elevations are presented in meters relative to mean sea level.

Data from existing hydrological and water quality monitoring programs with quality assurance programs including TCEQ, USGS, and NOAA were compared to the collected data after standardization to common units and/or vertical datum. Differences in water levels and salinity were assessed among the proposed monitoring network and adjacent open bay and Trinity River levels at the USGS Wallisville (08067252) and Romayor (08066500) gages.

Laboratory Methods

Rangia Morphometrics and Health Metrics

Shell length, height, and width (Figure 3) were measured in the lab, typically on the day following field sampling, for up to 20 live or dead (but whole) *Rangia* specimens from each site. Up to 10 live specimens from each site were retained for measurement of weight and subsequent Meat Index (MI) analysis. These specimens were vented (slightly opened) to drain any water retained in the shell and weighed to the nearest tenth of a milligram (mg). The shells were then shucked, and all soft tissue removed and discarded. After cleaning, shells were re-weighed, and the weight was recorded in mg. The wet weight of the soft tissue was determined by subtracting the empty shell weight from the total weight (shell and tissue). MI, a relative indicator of health, was determined by calculating the ratio of tissue to total weight and then multiplying by 100. Dry, cleaned shells from up to 10 specimens from each site were then stored in a freezer at -80° Celsius (C) for archival purposes and further age and growth analysis.

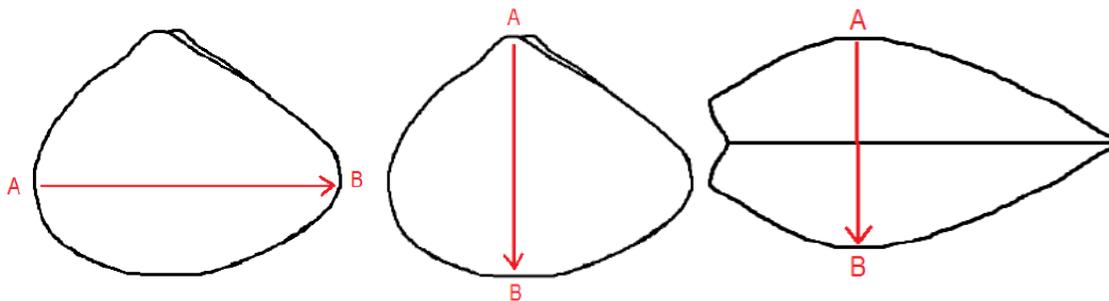


Figure 3. Length (left), height (middle), and width (right) morphometric measurements for live and whole, but dead, *Rangia*.

Sediment Percent Fines Methods

Composition of fine sediments, which serves as an index of the percentage of clay plus silt in the sediment, was calculated following the Department of Ecology, State of Washington Manual (2013) for each sampling event at each site. Sediment samples were homogenized in the sample bag and three replicate sub-samples of 50 milliliters (mL) of sediment were measured and rinsed through a #63 sieve to remove fine sediments. The remaining sand and larger grains were then placed in a 100 mL graduated cylinder and topped off with water. After allowing the sample to settle for 5-10 minutes, staff recorded the volume of sand and larger grains. Percent fines

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.

Data Analysis

Historical data and data from the current study were spatially organized using latitude, longitude, and elevation. These data were analyzed using univariate and multivariate graphical and trend analyses to evaluate temporal trends of and relationships among hydrology (water surface elevation), water quality (temperature, salinity, and specific conductivity) and biological data. As needed, both non-parametric and parametric methods utilizing appropriate transformations and lag periods were used to display and analyze data. Statistical significances were determined using conventional tests of slope (r^2 , p levels) and significance level (α) = 0.05. The Kruskal-Wallis Ranks Sums Test (a non-parametric method) was used to test for significant differences among categorical data (Hollander and Wolfe, 1973). Specific software that were used for analysis includes R, Excel, PRIMER, and ArcGIS.

RESULTS

Rangia Catch Rates

Live *Rangia* were detected at all 10 study sites, however at two sites (R18 and R45), only one live clam was detected throughout the study period (Table 2, Figure 4). A total of 271 live *Rangia* were captured via the prescribed sampling during the study period, with 220 of those collected using the hand sampling method, and 51 with the clam rake method (Table 2). An additional 252 live and recently dead specimens were opportunistically collected for lab-based measurements. Unless otherwise noted, all analyses were completed with opportunistically collected *Rangia* excluded. Due to high water levels, only half of the sites were sampled during the November 2018 sampling event. The highest capture rate of live specimens occurred at site R48. Because the two sampling methods have different effort parameters, the average catch by site and event were calculated for each gear type separately. Additionally, an average catch by site per event was calculated using both gear types and all replicates combined (equal effort was expended at each site during every sampling event). The highest average capture rate for the

hand sampling technique was 4.28 clams per m² at site R48 (Figure 5a). The highest average capture rate for the clam rake sampling technique was 0.67 clams per 30 seconds at site R13 (Figure 5b). For both sampling techniques, the two sites with the lowest average salinity (R18 and R45) had the lowest CPUE for Rangia. With all sites combined, the average capture rates for the two sampling techniques were 1.33 clams per m² hand sampling, and 0.31 clams per 30 second clam rake. Site R49 had the highest average number of dead (but whole) clams observed for both sampling techniques (Table 2 and Figure 6).

Ambient water quality (e.g. salinity and turbidity), sediment percent fines, as well as average site salinity were evaluated for linear relationships with the CPUE of both live or dead Rangia collected throughout this study. No significant (p value < 0.05) relationships were detected between CPUE and the variables listed. The same parameters were evaluated using a Mann-Whitney rank-sum test with the presence or absence of live Rangia, and the average site salinity was the only parameter that showed a significant relationship. The average salinity was significantly higher at sampling events where live Rangia were detected (p value = 0.0083) (Figure 7a). A binomial generalized linear model (GLM) was used to predict the presence of live Rangia based on the average salinity of a site, and when the average site salinity is greater than 1.2 psu, there is an 80% chance that Rangia will be detected (Figure 7b).

Table 2. Summary table of catch of Atlantic Rangia by site and method. CPUE = catch per unit effort. CPUE for hand sampling is number of clams per m². CPUE for clam rake is number of clams per 30-second rake. n = total number of sampling events completed per site. The average CPUE per event is the average number of Rangia collected with three replicates of each of the sampling methods combined.

		Total Live Catch		Live			Total Dead (but whole) Catch		Dead (but whole)		
Site	n	Hand	Clam Rake	Avg. Hand CPUE	Avg. Clam Rake CPUE	Avg. CPUE per event	Hand	Clam Rake	Avg. Hand CPUE	Avg. Clam Rake CPUE	Avg. CPUE per event
R13	5	39	10	2.60	0.67	3.27	3	3	0.20	0.20	0.40
R18	6	0	1	0.00	0.06	0.06	1	0	0.06	0.00	0.06
R31	5	5	3	0.33	0.20	0.53	9	1	0.60	0.07	0.67
R38	5	33	5	2.20	0.33	2.53	27	2	1.80	0.13	1.93
R45	5	1	0	0.07	0.00	0.07	2	0	0.13	0.00	0.13
R48	6	77	11	4.28	0.61	4.89	0	0	0.00	0.00	0.00
R49	5	34	7	2.27	0.47	2.73	27	12	1.80	0.80	2.60
T3	6	4	2	0.22	0.11	0.33	3	0	0.17	0.00	0.17
T6	6	11	10	0.61	0.56	1.17	0	0	0.00	0.00	0.00
T7	6	16	2	0.89	0.11	1.00	0	0	0.00	0.00	0.00

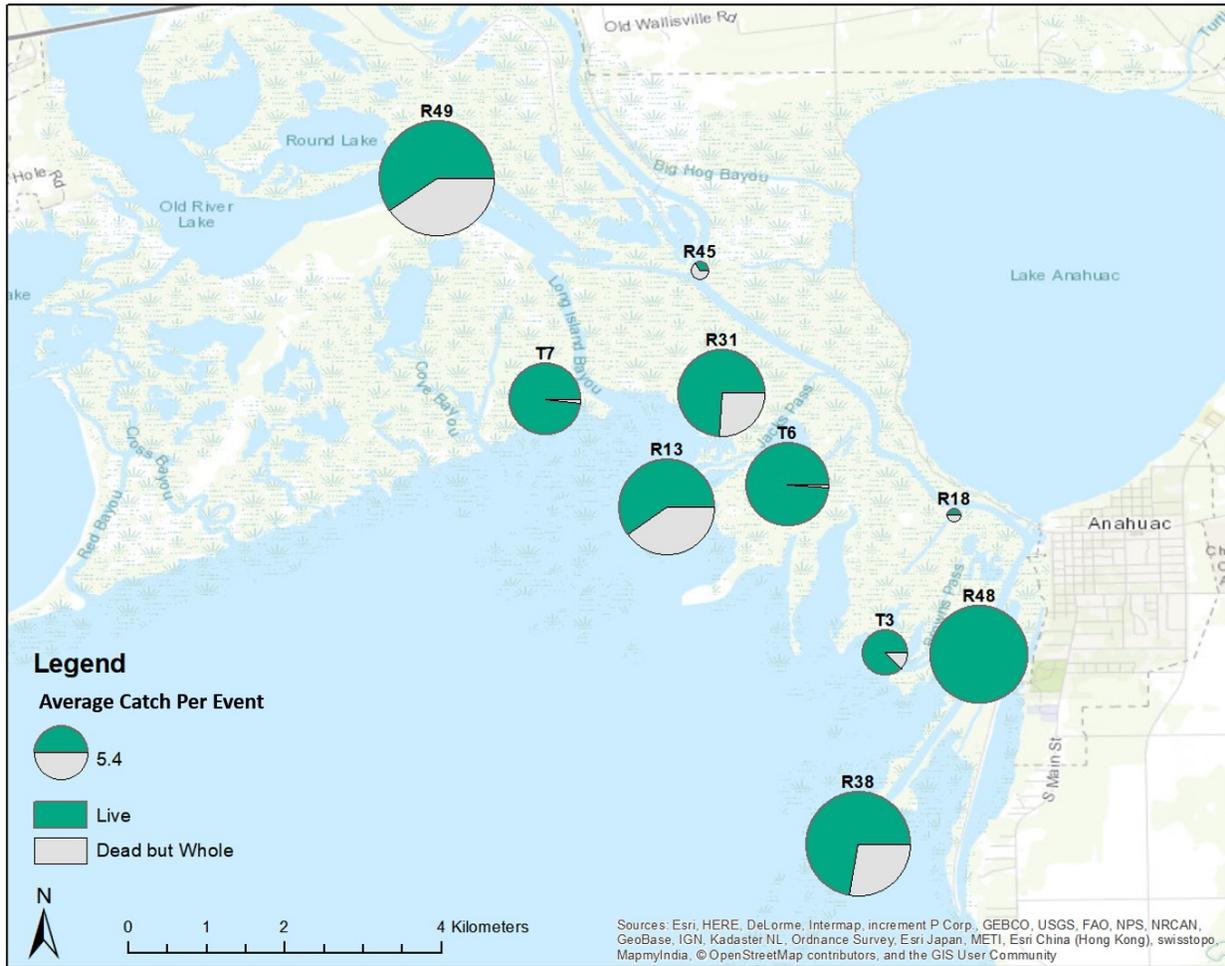
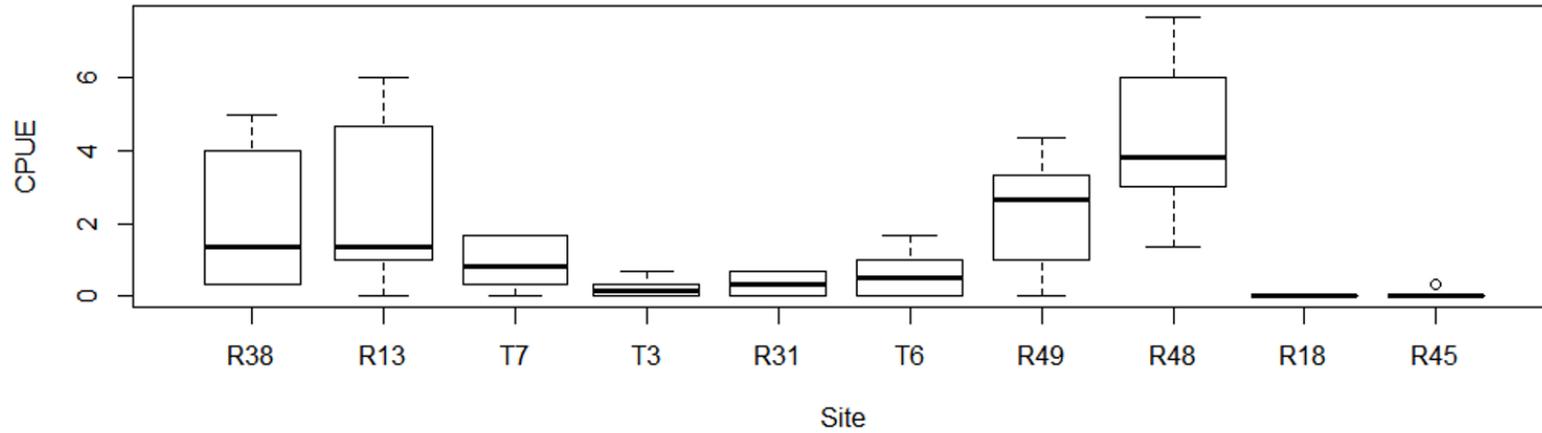
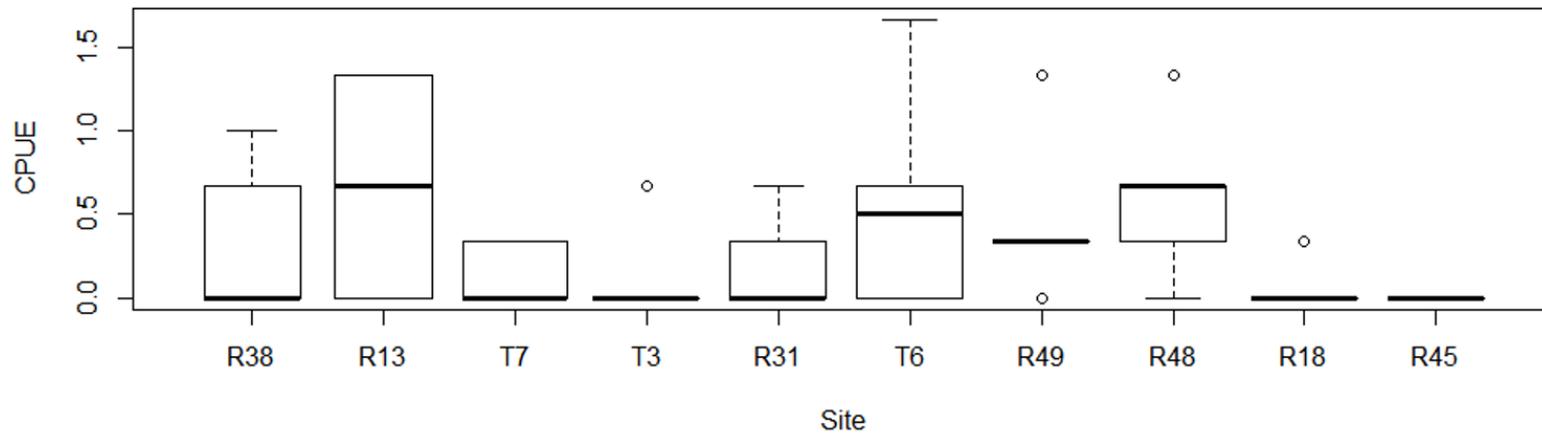


Figure 4. Average catch (combined hand sampling and clam rake methods) of live (green) and dead (but whole) (gray) Rangia by site per event.

a. Live Rangia per m² Hand Sampling



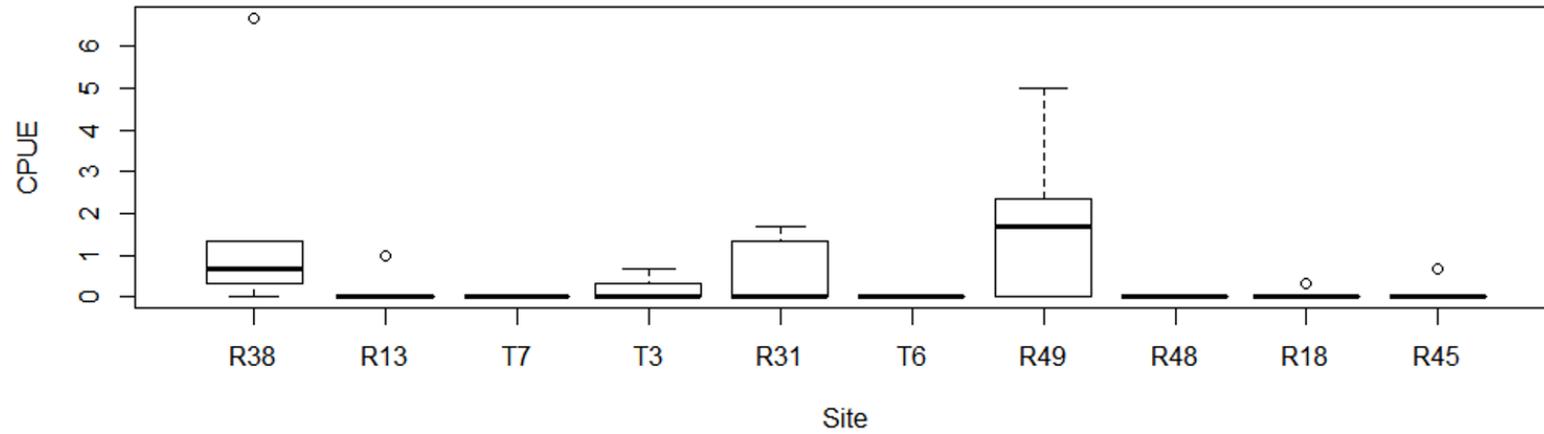
b. Live Rangia CPUE per 30 second Clam Rake



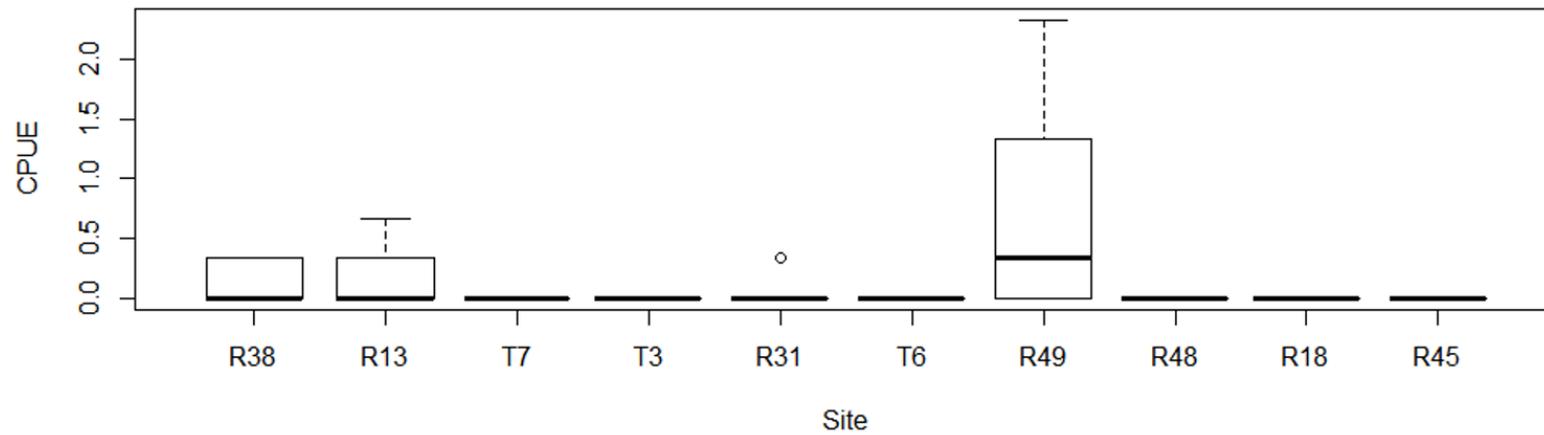
← + Average Salinity - →

Figure 5. Boxplots of live Atlantic Rangia CPUE by gear type and site: a. for the hand sampling method, and b. for the clam rake method. Site ordered by average salinity.

a. Dead (but whole) Rangia per m² Hand Sampling



b. Dead (but whole) Rangia CPUE per 30 second Clam Rake



← + Average Salinity - →

Figure 6. Boxplots of dead (but whole) Atlantic Rangia CPUE by gear type and site: a. for the hand sampling method, and b. for the clam rake method. Site ordered by average salinity.

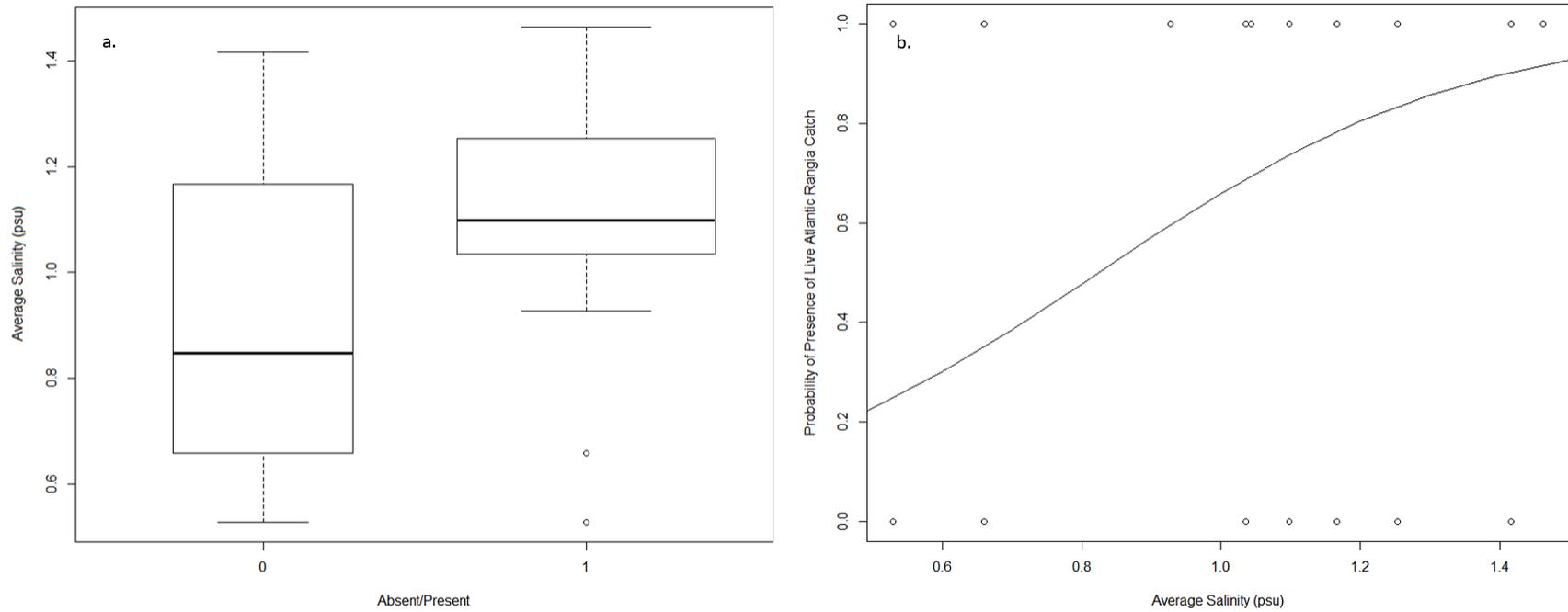


Figure 7. a. Boxplots of average site salinity (psu) for events where live Atlantic Rangia were not detected (absent) versus observed (present). b. Fitted binomial GLM applied to the presence/absence of live Atlantic Rangia by average site salinity (psu).

Morphometrics of Atlantic Rangia

Clam Size

During this study, based on specimens collected using all sampling methods and efforts combined (including opportunistic collection), the mean shell length for live Rangia was 56.25 millimeters (mm) and ranged from 10.5 to 78.6 mm (Table 3). The mean shell width (including opportunistic collection) was 36.04 mm and ranged from 4.5 to 54.2 mm. The mean shell height (including opportunistic collection) was 49.26 mm and ranged from 7.9 to 69.3 mm. The mean shell length for dead (but whole) Rangia was higher than for the live shells (59.9 mm), but not significantly higher at the 0.05 level (p value = 0.0550) (Figure 8). During the entire study period the smallest dead (but whole) Rangia collected was 37.5 mm long, while the smallest live Rangia collected was 10.5 mm long.

There was a significant difference in the size of clams between sampling methods, with the clam rake collecting significantly smaller clams (p value = 0.0041) (Figure 9). The smallest Rangia collected with the hand sampling gear type was 24.2 mm long. Because equal effort was employed at each sampling event (three hand sampling and three clam rake replicates), all Rangia morphometric data were pooled for further analysis. Tables and figures that include data from opportunistically collected Rangia include a note indicating such.

Table 3. Summary morphometric statistics of live Atlantic Rangia. Min = minimum, Avg. = average, Max = maximum, n = total number of live individuals included in analysis, g = grams.

	Live *				Dead (but whole)			
	n	Min	Avg	Max	n	Min	Avg	Max
Length (mm)	485	10.50	56.25	78.60	65	37.50	59.90	80.10
Width (mm)		4.50	36.04	54.20		24.10	38.85	52.30
Height (mm)		7.90	49.26	69.30		33.00	52.58	72.90
Total Weight (g)	313	0.16	75.28	191.18				
Shell Weight (g)		0.07	59.60	159.44				
Tissue (g)		0.06	15.68	48.34				
Meat Index		10.85	21.89	58.84				

* Includes opportunistically collected individuals.

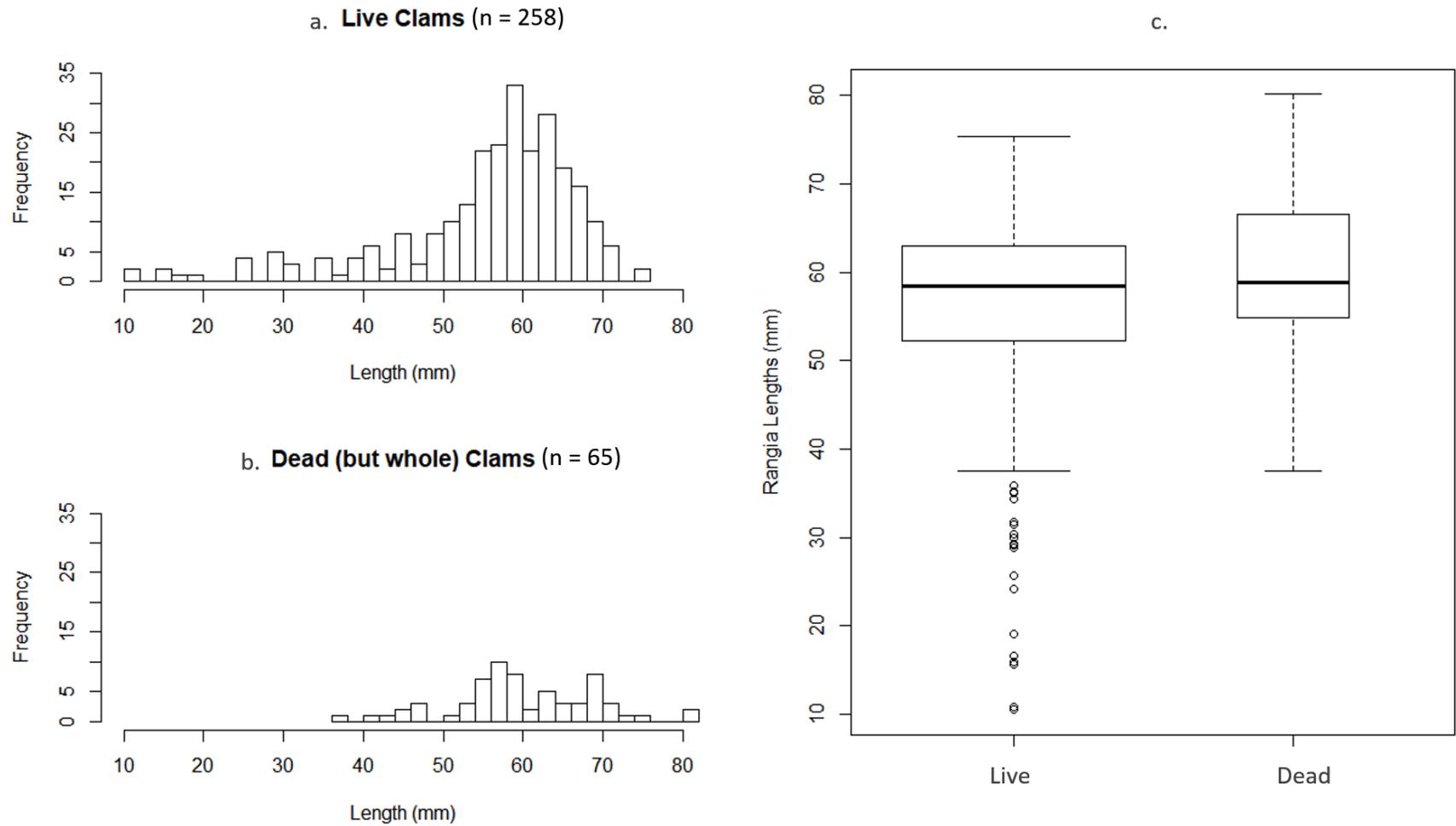


Figure 8. Frequency distributions of a. live Atlantic Rangia shell lengths (mm) and b. dead (but whole) shell lengths (mm) collected throughout the study (including opportunistic collection). c. Boxplots of Atlantic Rangia shell lengths for live and dead clams.

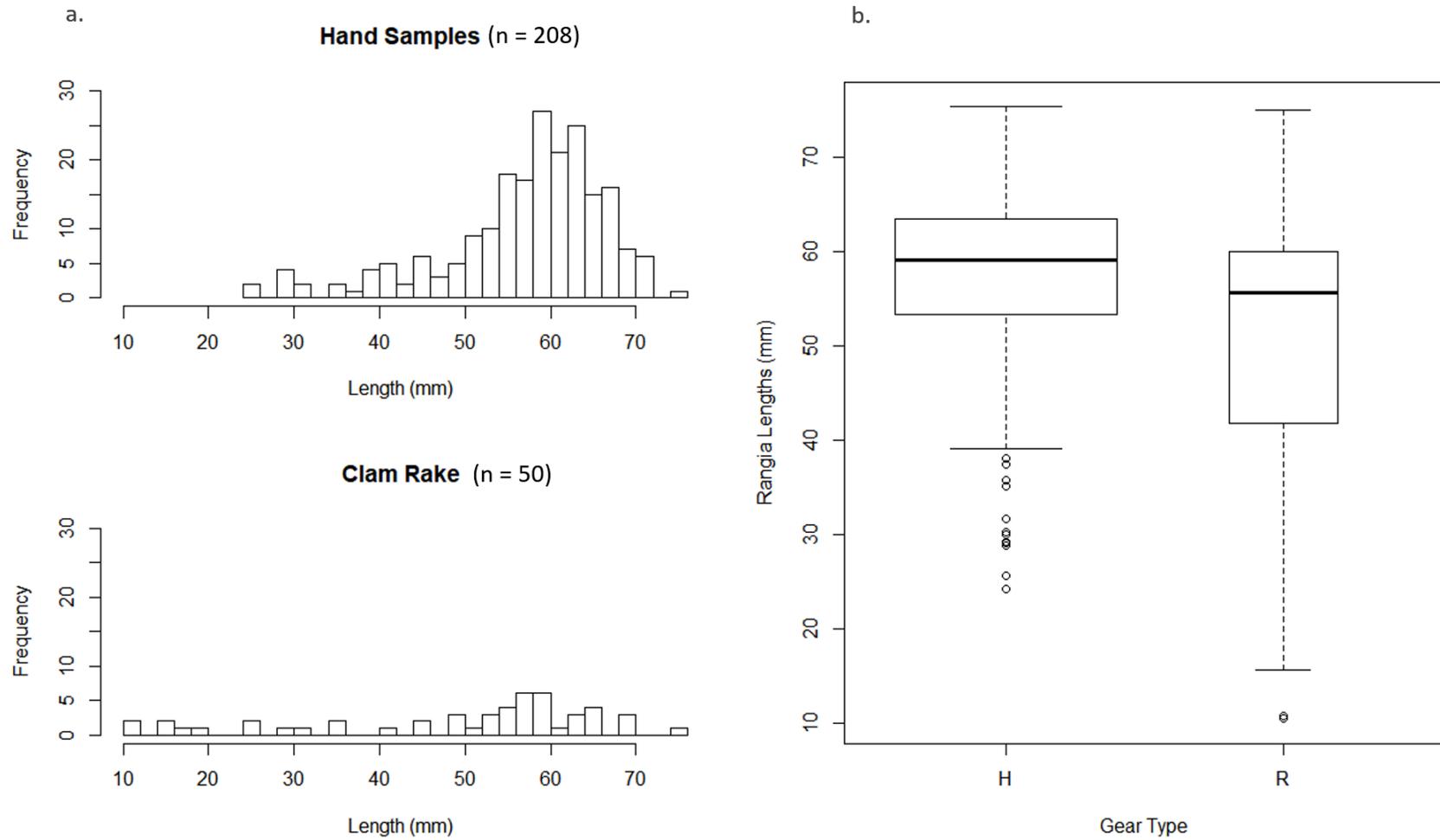


Figure 9. a. Frequency distributions of live Atlantic Rangia shell lengths (mm) by gear type. b. Boxplots of live Atlantic Rangia shell lengths by gear type (H = hand sampling, R = clam rake).

The morphometrics of live clams were evaluated relative to the water quality and substrate variables. A significant but weak positive linear relationship was observed between clam length and percent fines (p value = 0.0226) (Figure 10). No other significant relationships were observed. Smaller clams were generally observed in the spring and fall seasons, however it was not significant at the 0.05 level (p value = 0.0597) (Figure 11). Clam size varied by site, and a Mann-Whitney Rank Sum Test found that Site T6 had significantly smaller clams compared to sites R38 and R48 (p value = 0.0230 each) (Figure 12).

When all measured dimensions of the shells were combined (L+W+H), live *Rangia* shell dimensions followed a well-defined predictive power function for the shell weight (g) ($r^2 = 0.9798$) (Figure 13). The additive dimension provided the best predictive relationship for weight, as it minimized natural variations in shell shape.

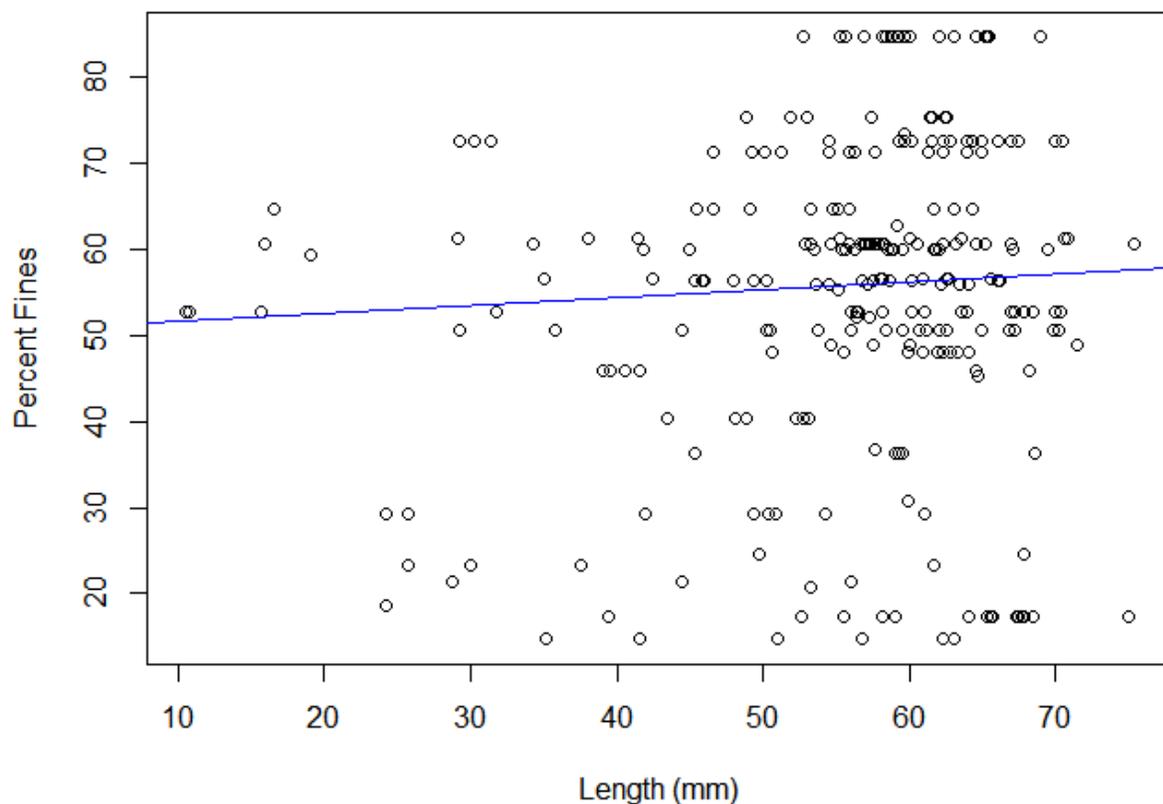


Figure 10. Scatterplot of live *Rangia* length (mm) and the percent fines of the sediment they were collected from. Predicted values of fitted Linear model denoted by the blue line (Adjusted R-squared = 0.0163, F-statistic = 5.265, p value = 0.0226).

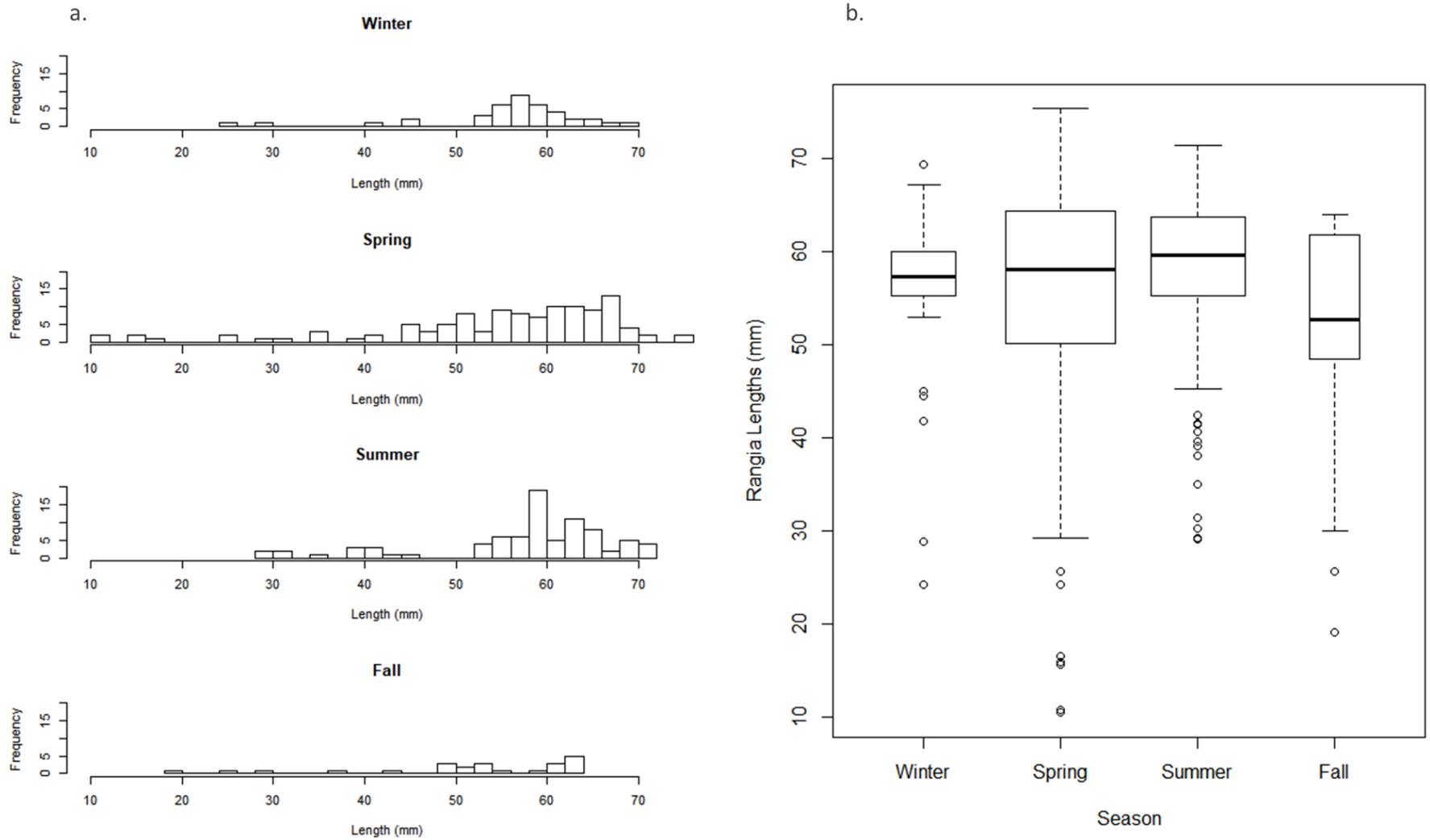


Figure 11. a. Frequency distributions of live Atlantic Rangia shell lengths (mm) by season. b. Boxplots of live Rangia shell lengths by season.

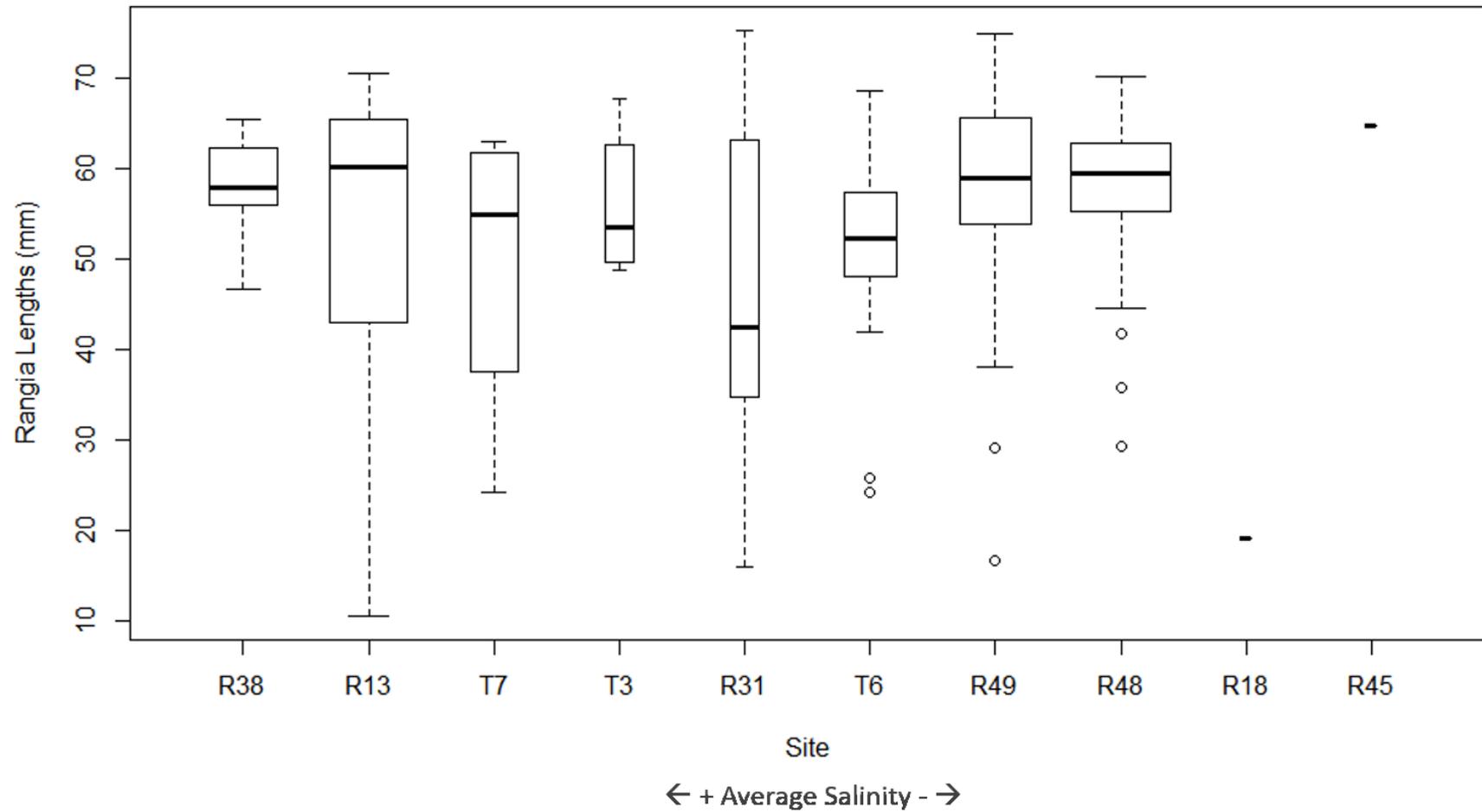


Figure 12. Boxplots of live Atlantic Rangia lengths (mm) by site. Site ordered by average salinity.

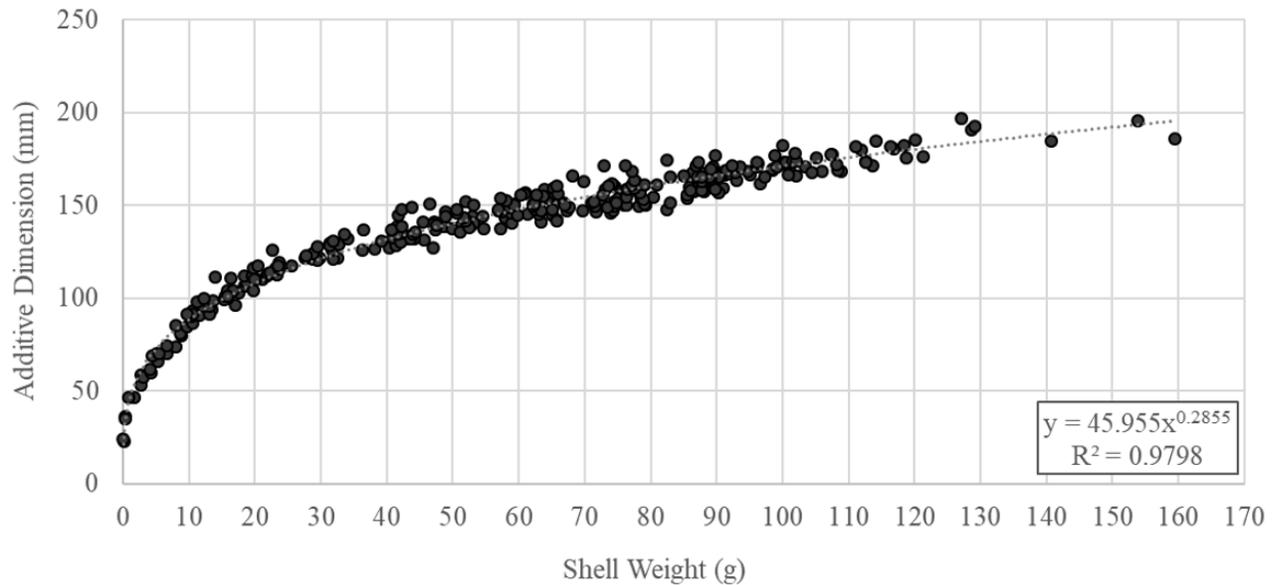


Figure 13. Additive dimension (length + width + height, mm) and shell weight (g) relationship depicted with a power trend line for all live Atlantic Rangia collected throughout the study (including opportunistic collections).

Meat Index

Rangia shell lengths, which differentiated juveniles (less than 28 mm in length) from adults, and MI were plotted (Figure 14) (Windham et al. 2019). Linear trend analysis suggested an inverse relationship in MI and length in juvenile clams indicating that juvenile clams selectively divert energy into shell hardening. Therefore, juvenile clams were removed from all MI analyses. The average total weight (shell and tissue) of live adult Rangia was 75.28 grams (g), and ranged from 0.16 to 191.18 g. The average soft tissue weight was 15.68 g and ranged from 0.06 to 48.34 g. The average MI was 21.89 and ranged from 10.85 to 58.84 (Table 3).

The MI did not significantly differ by gear type or season, although the highest average MI was observed during the spring and summer (p value = 0.0966) (Figure 15), which corresponds to pre-spawning and spawning seasons. When MI was evaluated relative to primary substrate type, the clams from sites with clay as the primary substrate type had significantly lower MI than clams from sites with sand as the primary substrate type (p value = 0.0487) (Figure 16). This is corroborated by an inverse linear relationship between the MI and percent fines (p value = < 0.0001) (Figure 17). Additionally, there was an inverse linear relationship between MI and average site salinity (psu) (p value = 0.0017) (Figure 18).

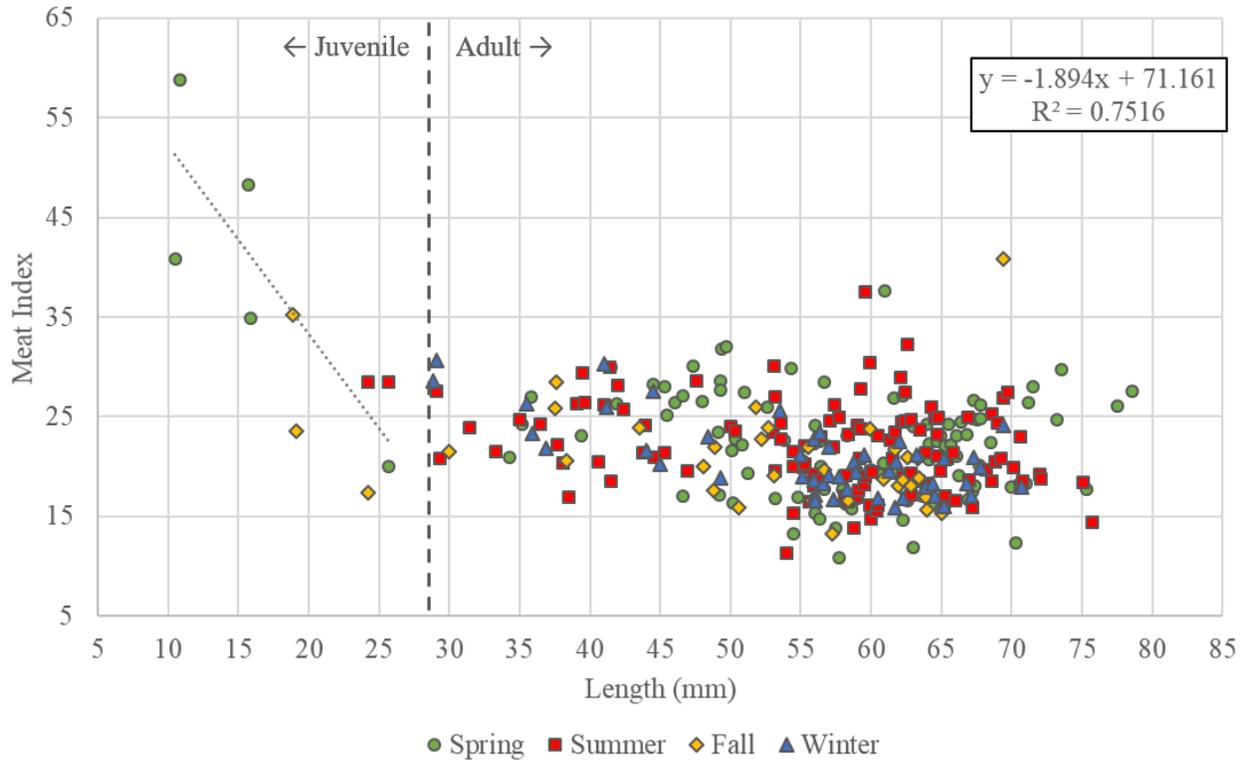


Figure 14. Meat Index and length (mm) of live Atlantic Rangia collected by season. The vertical dashed demarcation line at 28 mm represents the size where Rangia transition to adult stages (Windham et al. 2019). Opportunistic collections included.

Rangia MI varied by site, and a Mann-Whitney Rank Sum Test found that clams from Site R38 (the highest salinity site) had significantly lower MI than all other study sites (except for site R13 – the second most saline site) (p values < 0.05) (Figure 19). Clams from site T6 had significantly higher MI than site R49 (p value = 0.0179), and site T7 had significantly higher MI than sites R48, R49 and R13 (p values = 0.0007, 0.0374, and 0.0423 respectively).

When all MI data were combined from this study and the Guillen et al. (2016) study ($n = 492$ clams) and compared to the average freshwater discharge of the preceding period (7, 30, 60, and 90-day average) a positive linear relationship was observed, with increasing MI observed after periods of higher freshwater inflow for the 30 to 90-day average discharges at the Wallisville gage (Figure 20). The same trend was observed for average discharges at the Romayor gage for the 60 and 90-day average flows.

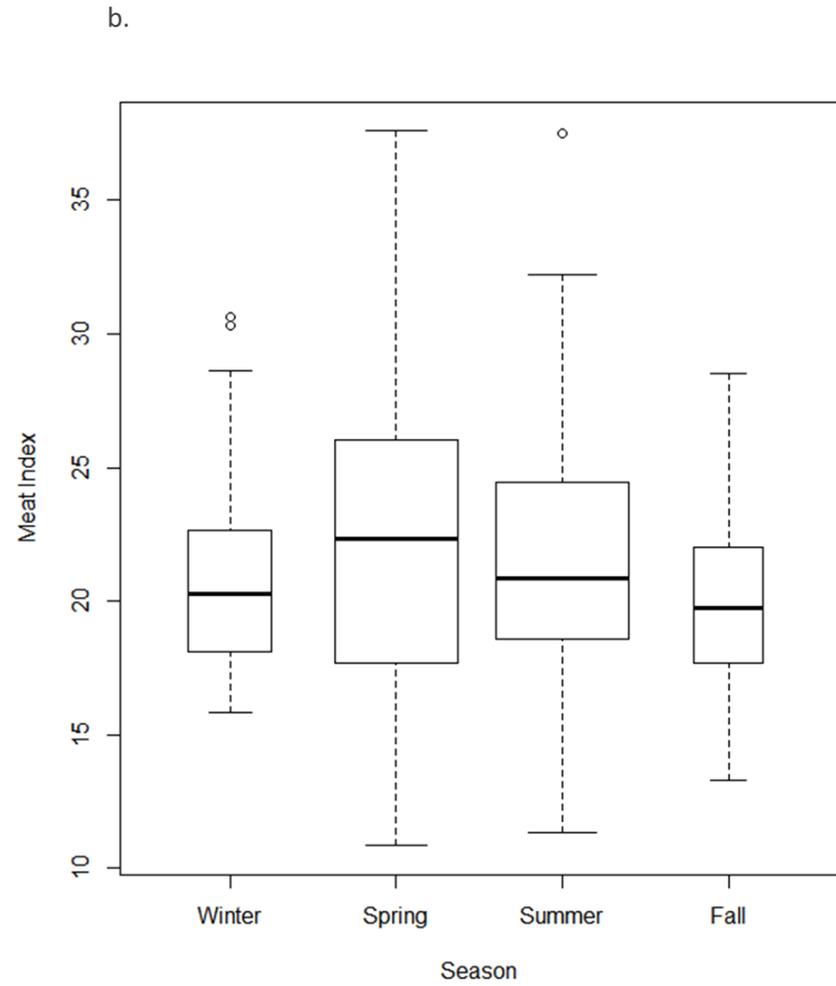
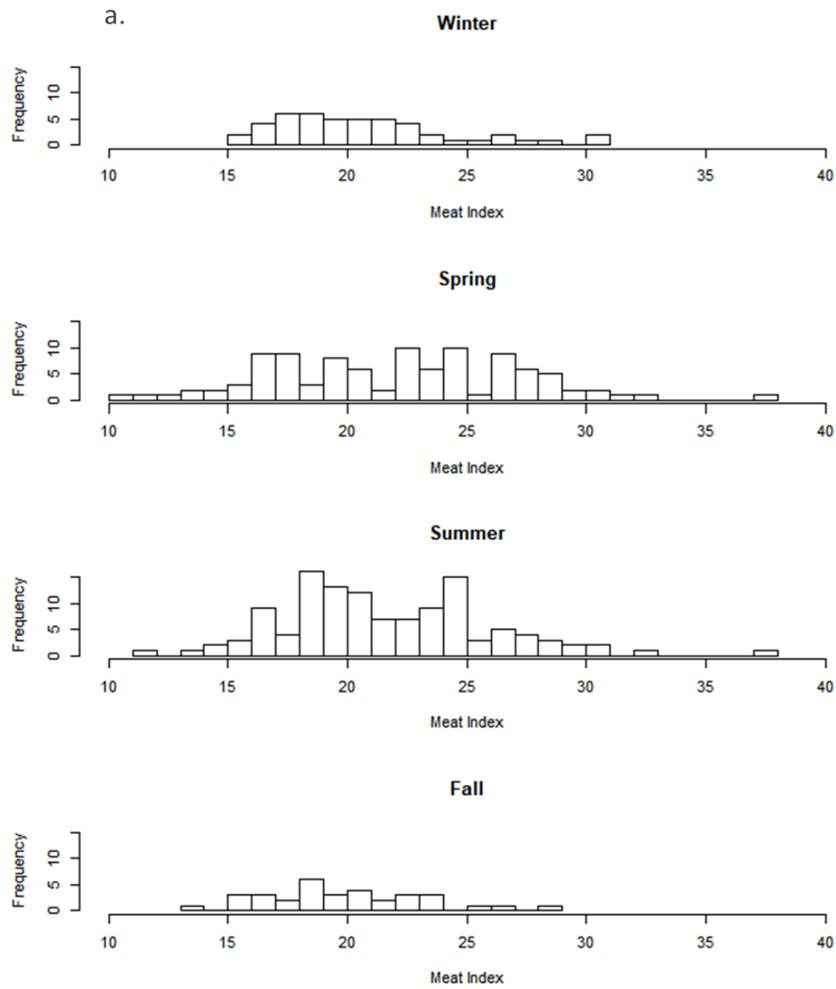


Figure 15. a. Frequency distributions of Atlantic Rangia Meat Index by season. b. Boxplots of Atlantic Rangia Meat Index by season.

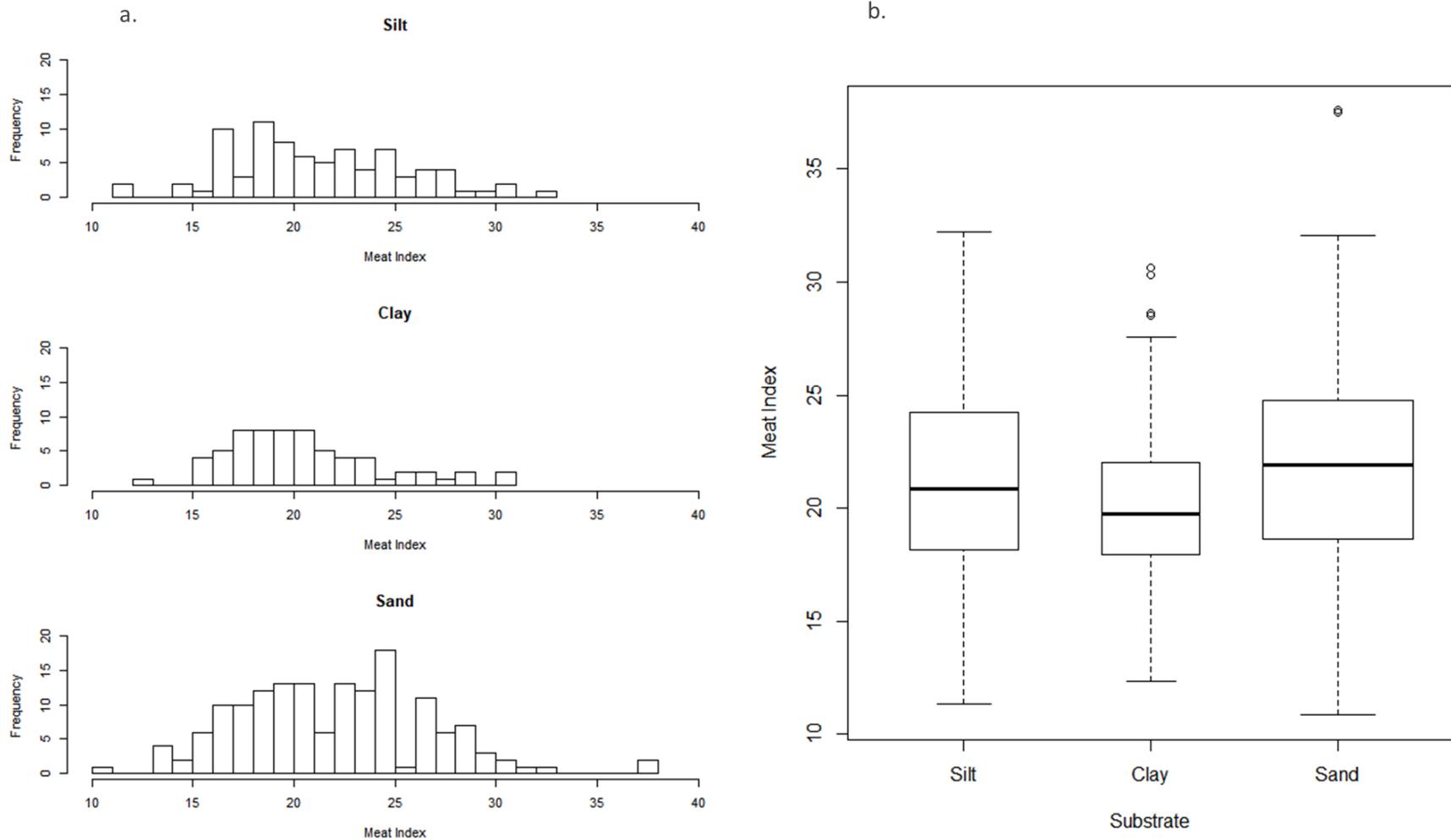


Figure 16. a. Frequency distributions of Atlantic Rangia Meat Index by substrate type. b. Boxplots of Atlantic Rangia Meat Index by substrate type.

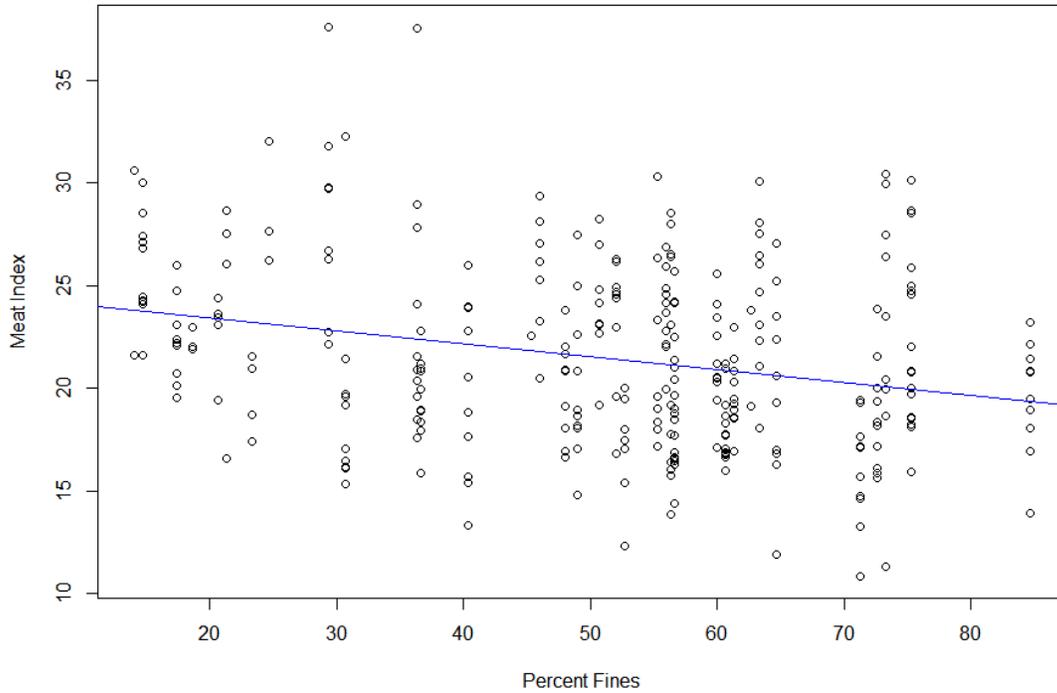


Figure 17. Scatterplot of Rangia Meat Index and the percent fines of the sediment they were collected from. Linear model depicted by the blue line (Adjusted R-squared = 0.0642, F-statistic = 21.58, p value < 0.0001).

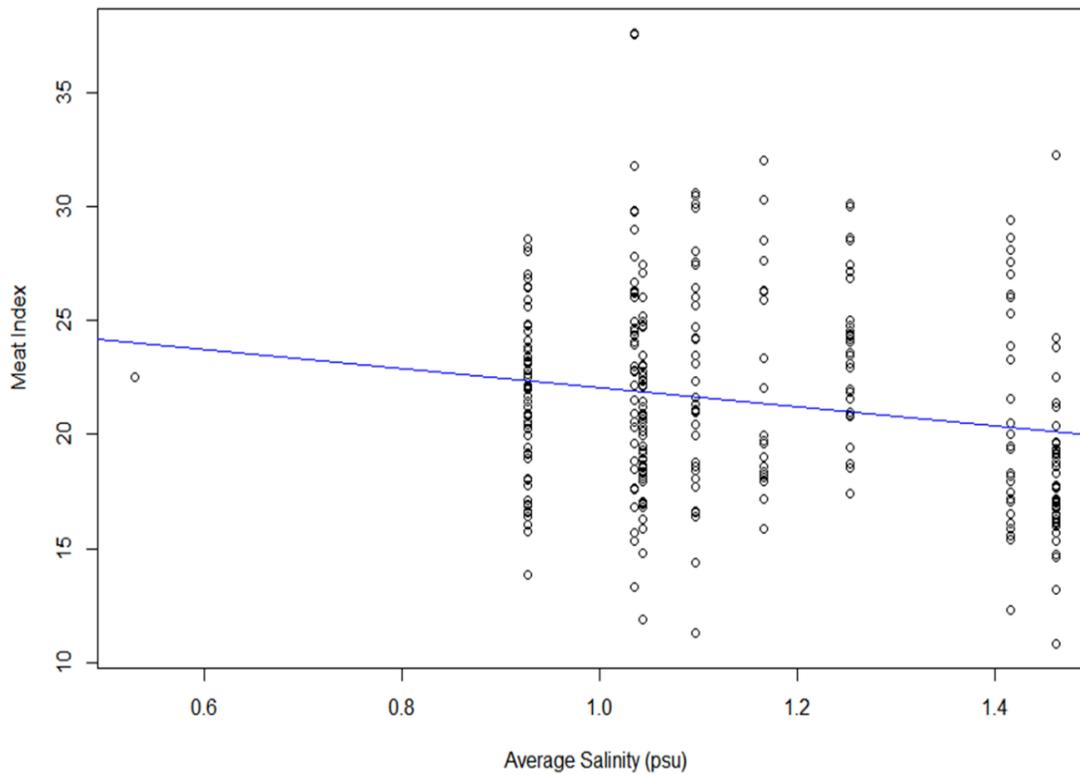


Figure 18. Scatterplot of Rangia Meat Index and the average site salinity (psu). Linear model depicted by the blue line (Adjusted R-squared = 0.0293 F-statistic = 10.07, p value = 0.0017).

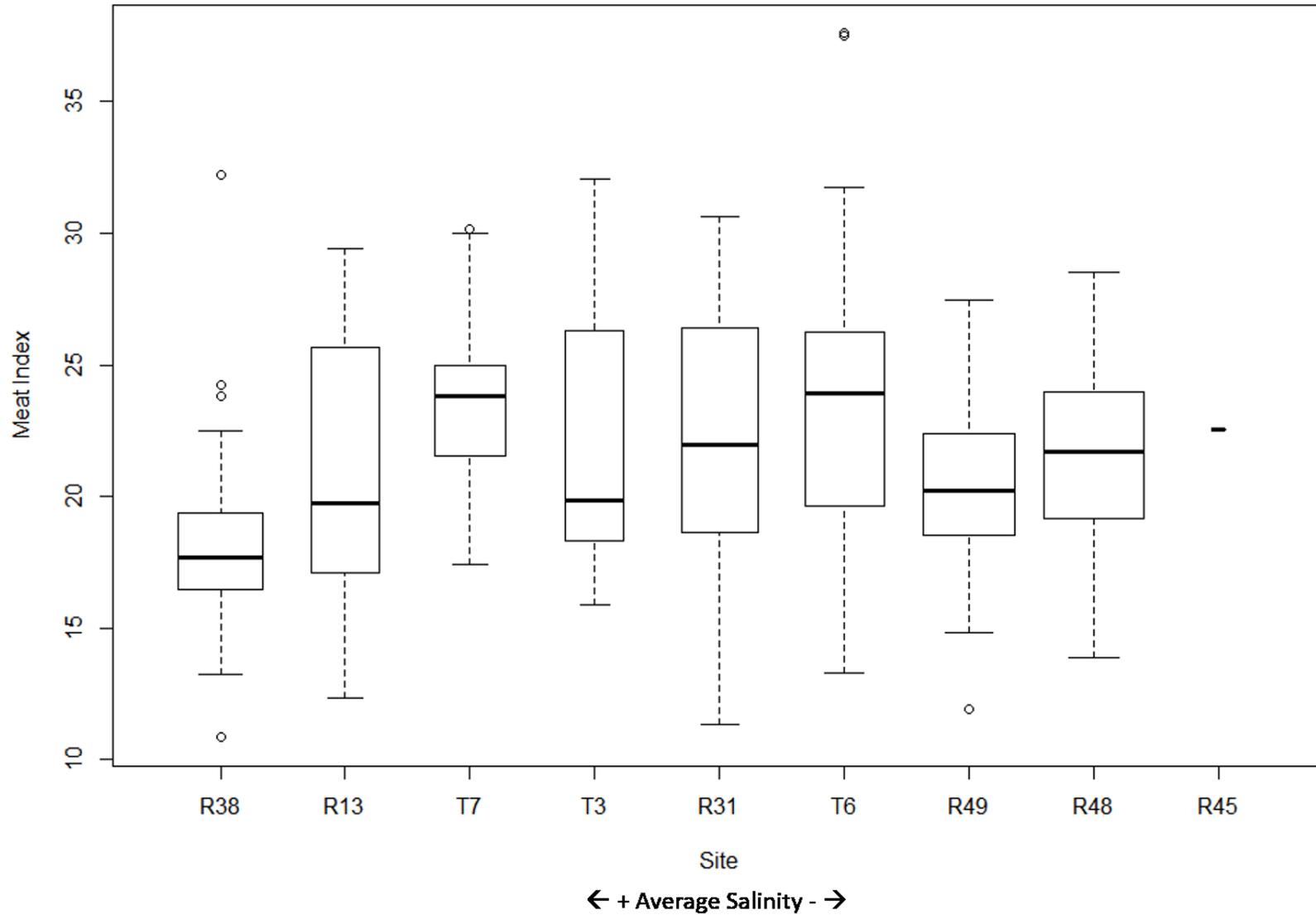


Figure 19. Boxplots of Atlantic Rangia Meat Index by site. Site ordered by average salinity. Site R18 was excluded because only one juvenile clam was collected.

Wallisville Gage

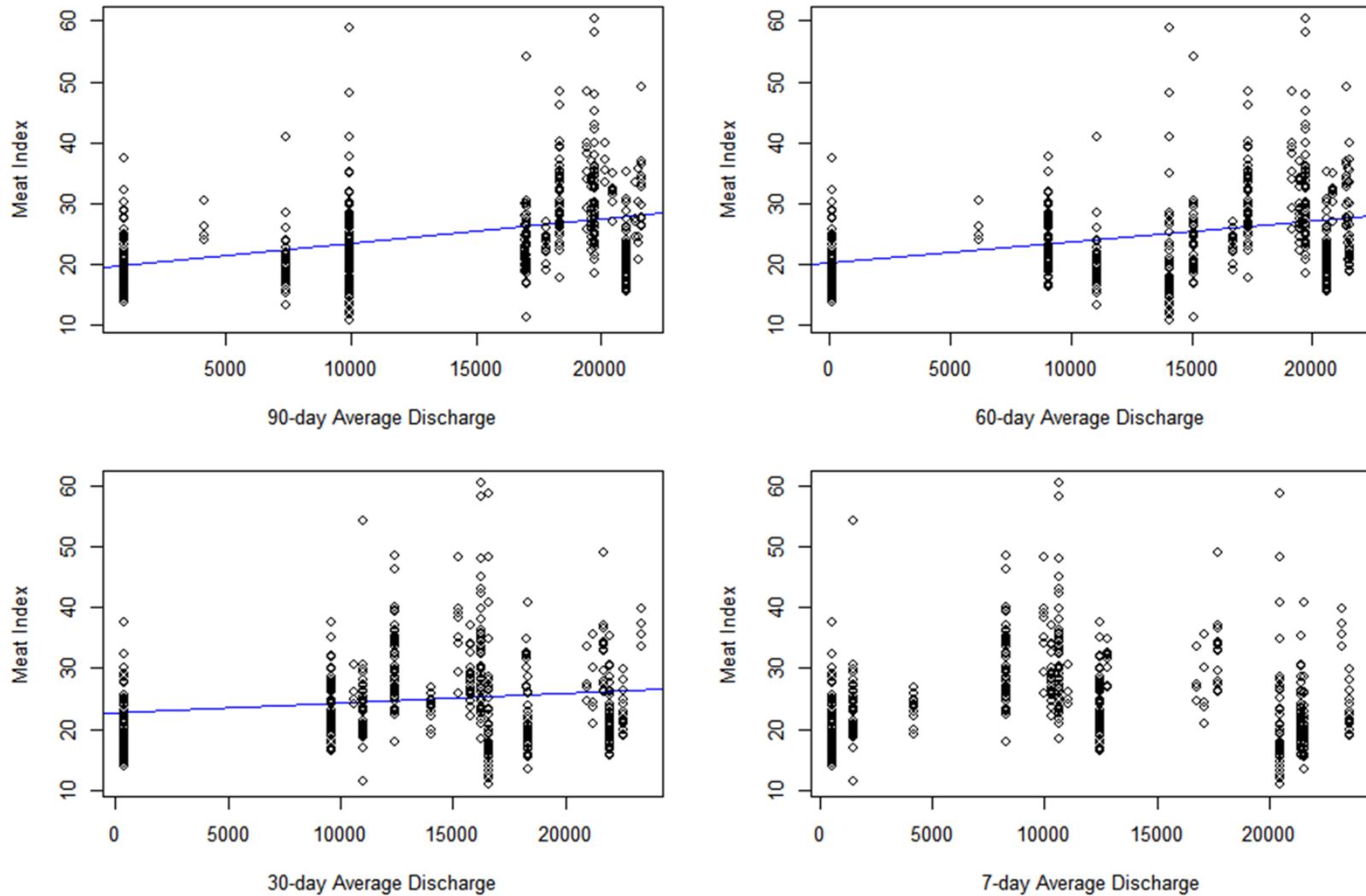


Figure 20. Meat Index and the 7, 30, 60, and 90-day average discharges (cfs) prior to the sampling event for all live *Rangia* collected in this study and the Guillen et al. (2016) study in the Trinity River Delta. Linear models demonstrated by the blue lines if significant (p value < 0.05).

Occurrence of Submerged Aquatic Vegetation

During this study, field crews did not observe any Wild Celery in the study area. Widgeongrass was observed at two locations, each on separate dates during the study period. During a very low water event on December 3, 2018, Widgeongrass was observed in shallow water near site R38. A total of 40.5 square m of Widgeongrass was observed with a very patchy distribution and less than 50% cover (Figure 21). The blades were very short and there was significant epiphytic growth. The second observation was on July 30, 2019 near site R13 in a single patch of approximately 5 square m with 30% cover. In this instance, the Widgeongrass was felt while hand sampling for *Rangia* (it could not be seen in the turbid water).

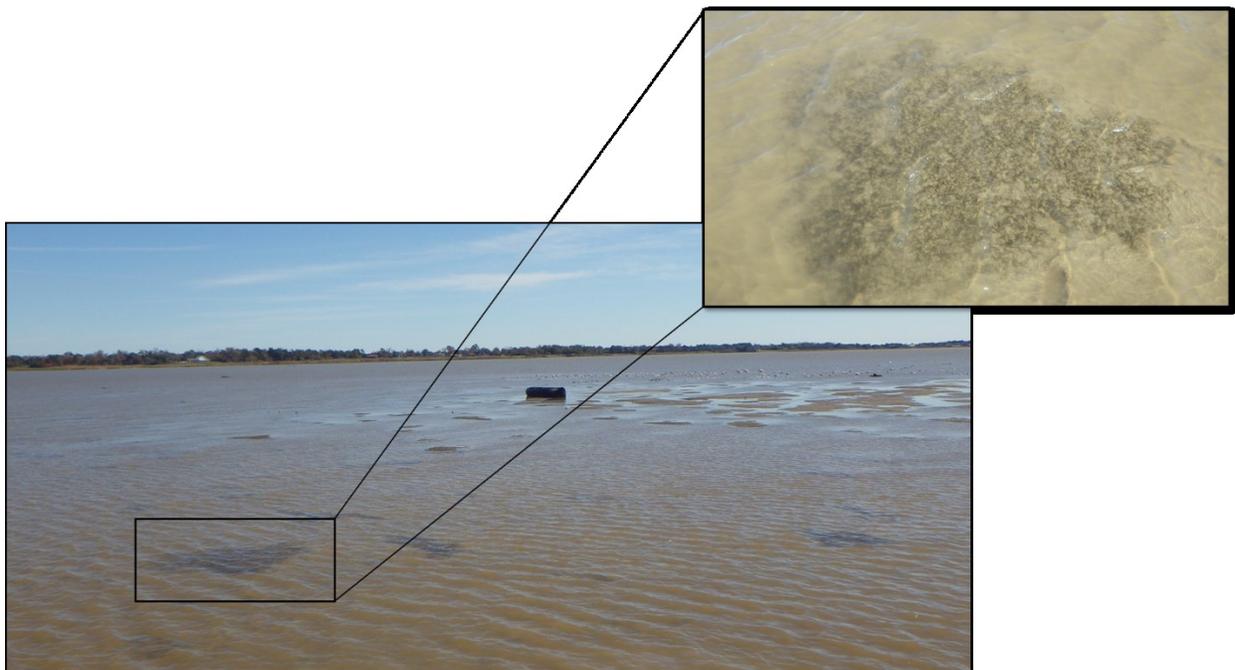


Figure 21. Photos of Widgeongrass observed near site R38 on December 3, 2018.

Physicochemical Conditions

Automated continuous conductivity and temperature devices (HOBOS) were deployed at all 10 sites from February 15, 2018 through August 25, 2019 (Table 4). The average salinity for all sites monitored in the Trinity River Delta for the study period ranged from 0.53 to 1.46 psu (Figure 22). The maximum salinity for all sites ranged from 6.34 to 15.80 psu. The minimum and

maximum temperature for all sites were 2.52 and 38.89°C respectively. Additional water quality parameters (dissolved oxygen and pH) were collected during each Rangia sampling event (Table 5). Due to high water levels, only half of the sites were sampled during the November 2018 sampling event. The minimum dissolved oxygen and pH measured at the bottom (0.1 m off the sediment) for all sites was 3.50 and 7.40 mg/L respectively. Water clarity measured by a Secchi transparency tube was low during all sampling events with the maximum water clarity observed at site R18 at 0.221 m (Table 5).

Table 4. Summary statistics for continuous salinity and temperature monitoring data by site.

Site	n	Salinity (psu)						Temperature (°C)					
		Minimum	Average	Median	Maximum	Q1	Q3	Minimum	Average	Median	Maximum	Q1	Q3
R38	52665	0.07	1.46	0.19	12.28	0.14	1.46	7.07	22.90	23.46	34.44	16.81	29.21
R13	51388	0.10	1.42	0.19	14.44	0.15	1.06	6.19	23.30	24.52	36.39	17.46	28.94
T7	51567	0.01	1.25	0.22	15.80	0.16	1.13	2.52	23.07	23.85	38.77	16.78	29.30
T3	50599	0.09	1.17	0.22	10.75	0.17	1.01	6.91	24.41	26.90	38.89	18.40	30.17
R31	49452	0.10	1.10	0.24	7.89	0.19	0.90	3.05	23.45	24.93	37.05	17.82	29.29
T6	49789	0.09	1.04	0.18	8.58	0.14	0.67	6.06	22.90	24.08	37.79	16.43	28.84
R49	50546	0.04	1.04	0.20	8.14	0.16	0.62	8.37	24.20	26.04	35.94	18.74	30.03
R48	48569	0.10	0.93	0.17	12.37	0.14	0.44	9.51	23.68	25.62	34.04	18.01	29.78
R18	53154	0.08	0.66	0.18	6.34	0.15	0.27	10.67	22.86	23.33	34.62	16.39	29.56
R45	50561	0.07	0.53	0.17	7.18	0.14	0.25	4.87	24.99	28.42	35.49	19.29	30.58

Table 5. Summary statistics for dissolved oxygen and pH collected during Rangia sampling events. Min = minimum, Avg = average, and Max = maximum.

Site	n	Secchi (m)			Dissolved Oxygen (mg/L)						pH					
		Surface			Surface			Bottom			Surface			Bottom		
		Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max
R13	5	0.088	0.130	0.152	7.06	9.04	10.57	7.05	9.01	10.53	7.50	8.04	8.69	7.49	8.06	8.65
R18	6	0.084	0.137	0.221	5.79	7.67	10.26	5.64	7.64	10.26	7.46	7.72	8.10	7.49	7.73	8.14
R31	5	0.072	0.098	0.122	8.20	9.51	10.23	3.50	8.46	10.23	7.77	8.20	8.99	7.66	8.17	8.99
R38	5	0.098	0.139	0.190	6.24	8.06	10.19	6.15	7.96	10.18	7.40	7.79	8.26	7.43	7.75	8.10
R45	5	0.090	0.113	0.158	6.93	8.65	10.32	6.97	8.62	10.30	7.48	7.87	8.39	7.50	7.88	8.41
R48	6	0.088	0.132	0.210	5.59	7.82	10.32	5.49	7.79	10.31	7.45	7.69	7.94	7.46	7.68	7.95
R49	5	0.070	0.115	0.154	7.44	8.69	9.93	7.56	8.61	9.89	7.52	7.93	8.63	7.52	7.92	8.58
T3	6	0.072	0.094	0.138	7.30	8.80	10.49	7.28	8.76	10.49	7.48	7.92	8.48	7.51	7.92	8.48
T6	6	0.080	0.107	0.140	5.90	7.82	10.34	5.91	7.80	10.33	7.45	7.77	8.17	7.47	7.79	8.20
T7	6	0.088	0.110	0.128	7.00	9.06	10.26	6.98	8.75	10.26	7.47	7.97	8.86	7.48	7.91	8.86

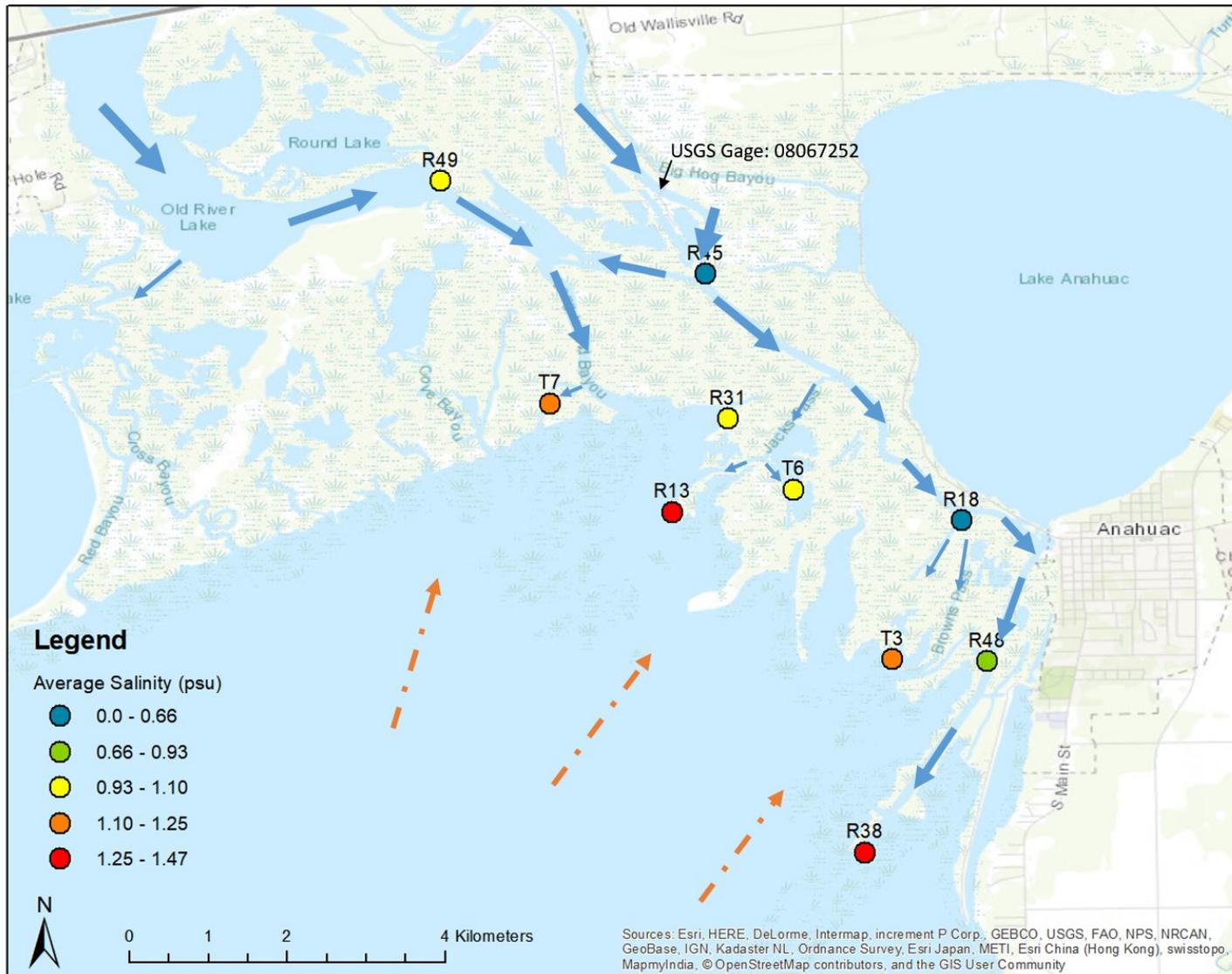


Figure 22. Average salinity from study period (2/15/2018 through 8/25/2019) at the 10 continuous monitoring sites with arrows depicting typical pathways of freshwater inflow (blue arrows) from the Trinity and Lost Rivers, and tidal waters (orange dashed arrows) from Trinity Bay.

The discharge measured at the USGS gage on the Trinity River at Wallisville, TX (08067252) appeared to plateau under high flow conditions at around 22,500 cubic feet per second (cfs) (excluding the anomalous event of Hurricane Harvey) (Figure 23). Throughout the study period, there was one prolonged low flow period from late April 2018 through early September 2018 followed by relatively high flow through July 2019. When compared to the USGS gage on the Trinity River at Romayor, TX (08066500) it is clear that a large portion of the discharge is unaccounted for at the Wallisville gage site (Figure 24). The period from June 2018 to August 2018 was the only time salinities above 1 psu were detected at all 10 of the study sites (Figure 24).

Water surface elevation (relative to mean sea-level) measured at three sites (T3, T6, and T7) was used to trace freshwater pulses through the delta by comparing the time-date stamp of the peak at the sites to the peaks at the upstream gages (Figure 25). To demonstrate this, Figure 26 “zooms into” an example inflow peak from which discrete lag times can be determined between the Romayor and Wallisville gages and the three water level monitoring sites in the Trinity River Delta. Lag times were calculated for each of the major elevated freshwater inflow events during the study period and compared to the size of the peak from the Romayor gage (Figure 27). There was an inverse relationship between the size of the peak of freshwater pulse and the time it took the peak to reach the Trinity River Delta.

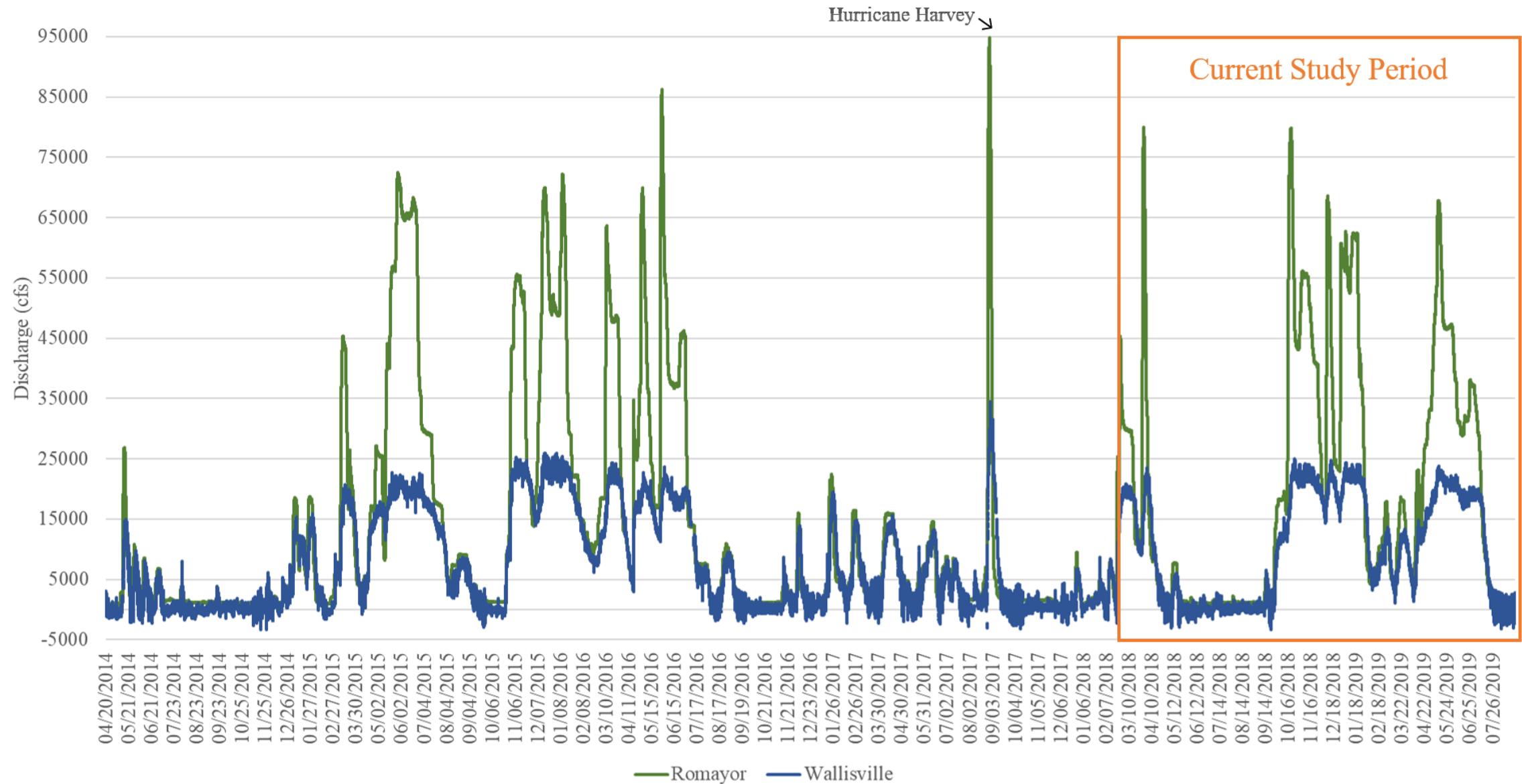


Figure 23. Hydrograph displaying discharge (cfs) for the USGS sites Trinity River at Wallisville (08067252) and Trinity River at Romayor (08066500) during the period of record of the Wallisville site, with the current study period outlined in orange.

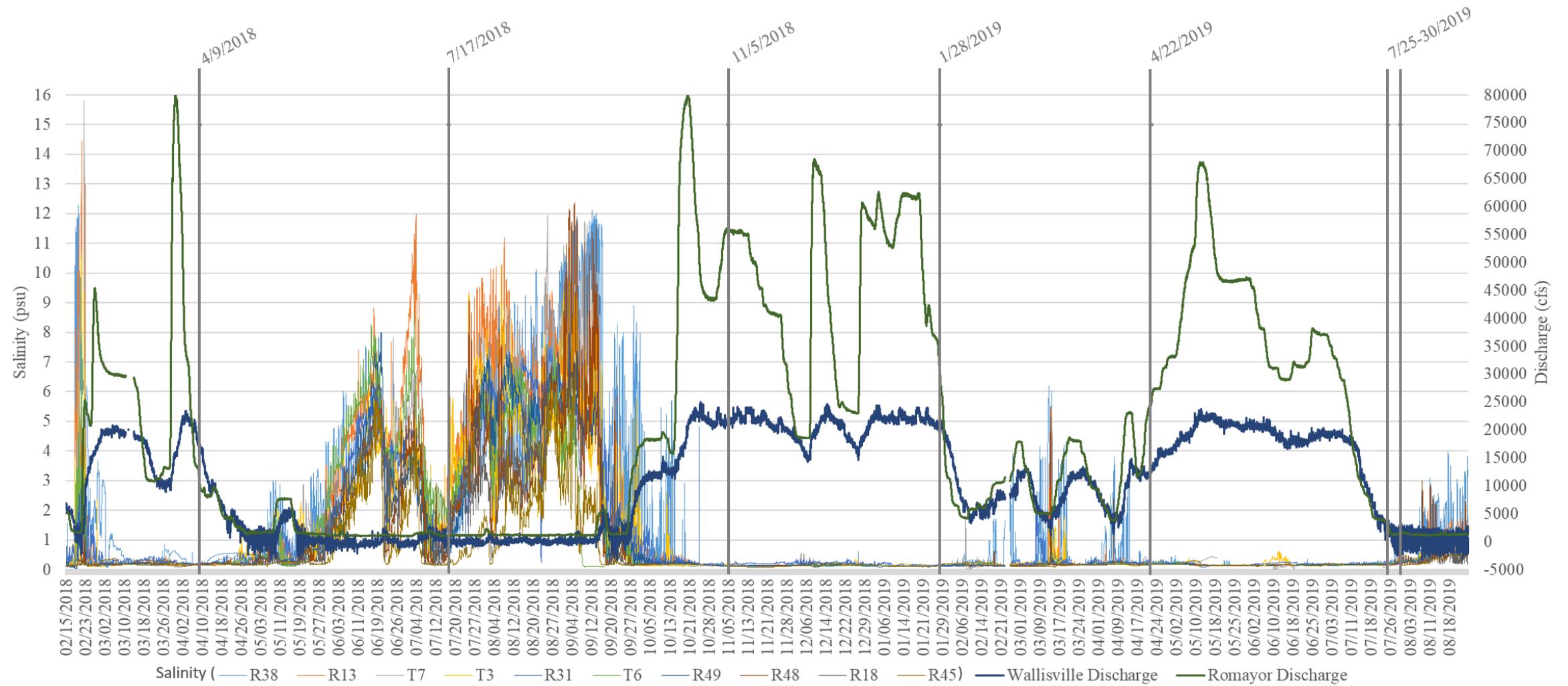


Figure 24. Hydrograph displaying discharge (cfs) at the USGS sites Trinity River at Wallisville (08067252) and Trinity River at Romayor (08066500) and continuous salinity (psu) measurements at each of the study sites. Vertical demarcations represent Atlantic Rangia sampling events.

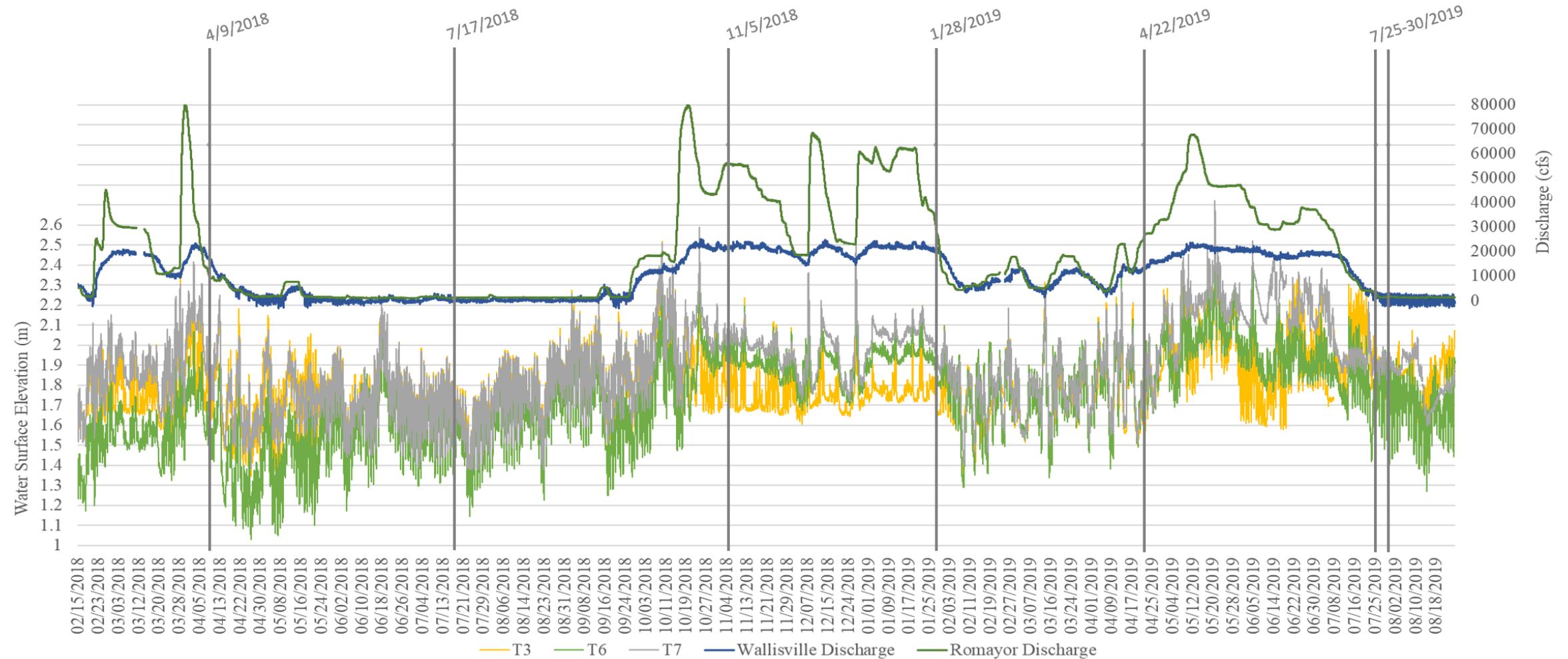


Figure 25. Water surface elevation (m) measured at three study sites (T3, T6, and T7) and discharge (cfs) from the USGS sites Trinity River at Wallisville (08067252) and Trinity River at Romayor (08066500). Vertical demarcations represent Atlantic Rangia sampling events.

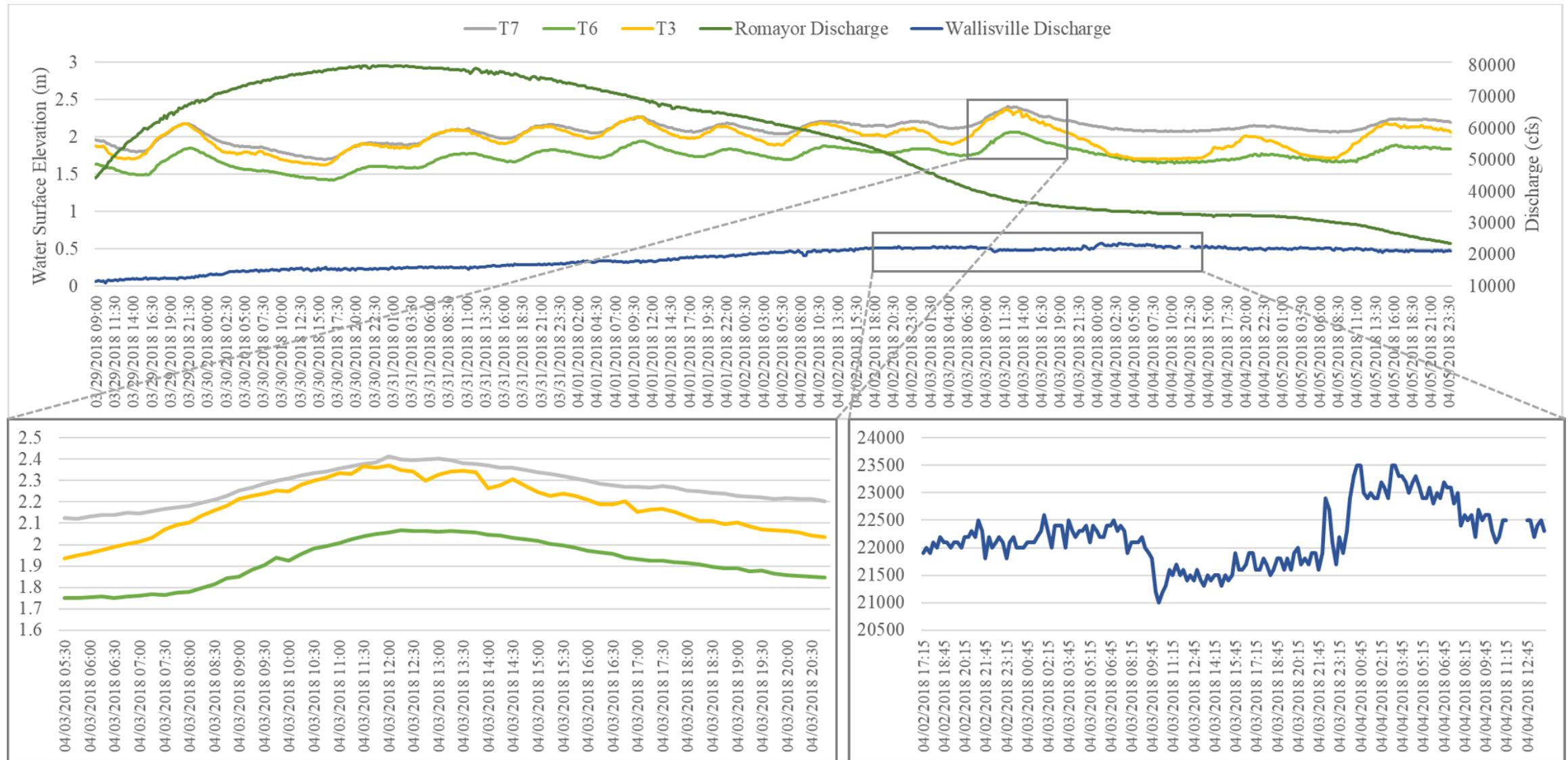


Figure 26. Zoomed-in view of the freshwater inflow event that occurred before the first Atlantic Rangia field sampling. Water surface elevation (m) measured at the three study sites (T3, T6, and T7) and discharge (cfs) from the USGS sites Trinity River at Wallisville (08067252) and Trinity River at Romayor (08066500). Sub-graphs show peaks at the water surface elevation study sites and the Wallisville gage.

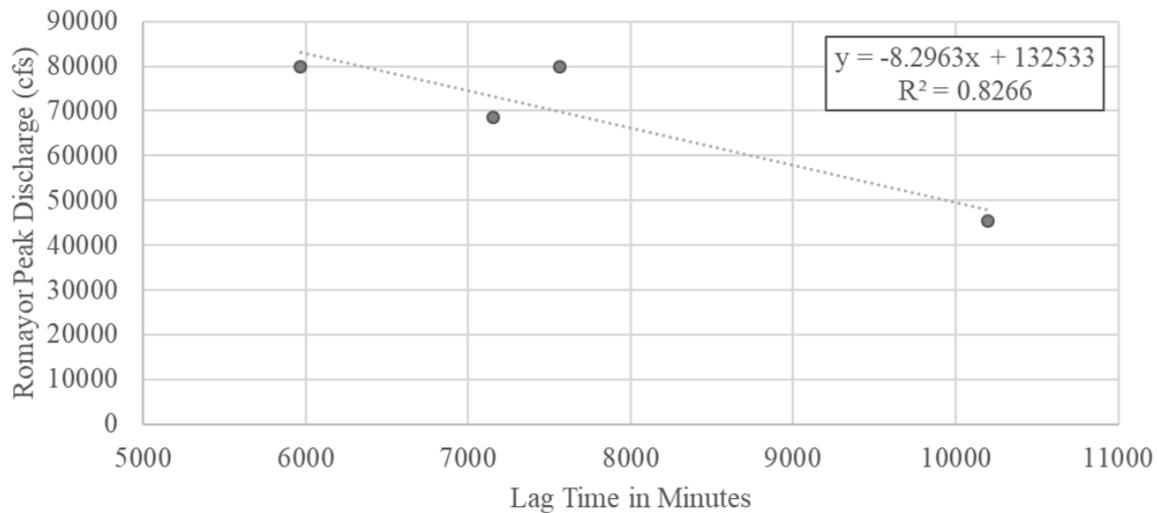


Figure 27. Lag time in minutes from peak flow measurements at the USGS Trinity River at Romayor gage (08066500) to peak flow measurements at the USGS Trinity River at Wallisville gage (08067252) for the major elevated flow events that occurred during the study period.

Sediment

Sediment percent fines ranged from 12.00 to 84.67% with a mean value of 49.88% and a median value of 54.67%. There was a significant inverse relationship between percent fines and discharge (Kruskal-Wallis Rank Sum Test: p-value = 0.0082), which is demonstrated in Figure 28 using an example of one sampling event that was preceded by a period of low freshwater inflow and another sampling event that was preceded by a period of elevated freshwater inflow. When all percent fines data were combined from this study and the Guillen et al. (2016) study (n = 104 samples) and compared to the average discharge (cfs) of the preceding period (7, 30, 60, and 90-day averages), an inverse relationship was observed, with decreasing percent fines observed after periods of higher freshwater inflow for the 30 to 90-day average discharges measured at the Wallisville gage (Figure 29). The same trend was observed at the Romayor gage for the 60 and 90-day average discharges.

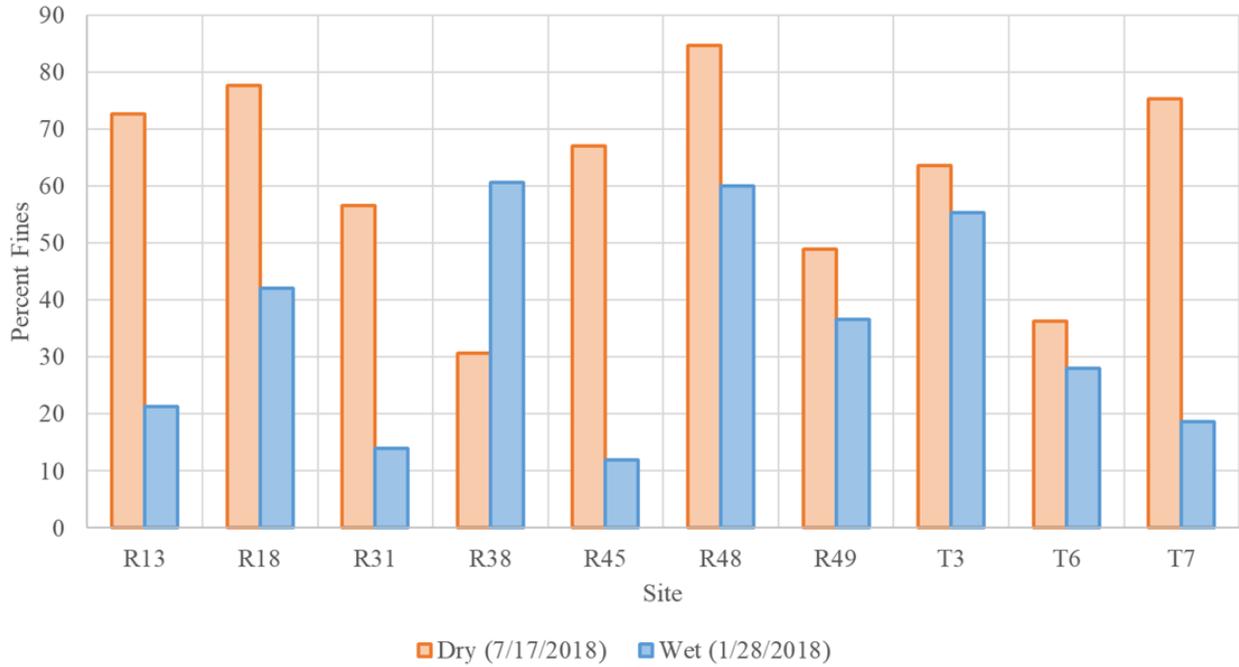


Figure 28. Percent fines measured at each site during a sampling event that was preceded by a period of low freshwater inflow (Dry) and a sampling event that was preceded by a period of elevated freshwater inflow (Wet). See Figure 24 for inflow preceding each sampling event.

Wallisville Gage

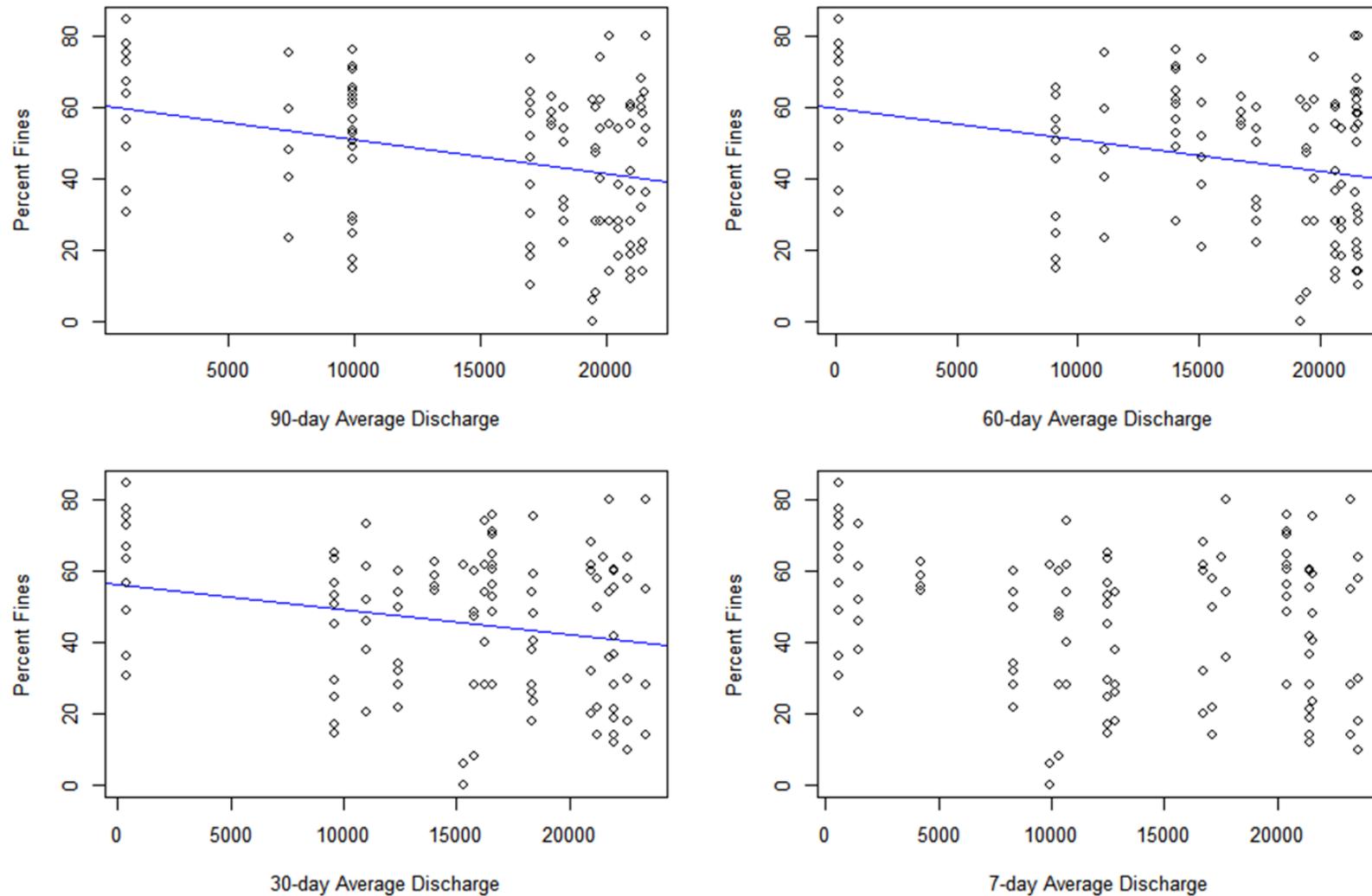


Figure 29. Percent fines and 7, 30, 60 and 90-day average discharges (cfs) prior to sampling events; data includes all sampling events in this study and the Guillen et al. (2016) study. Linear models demonstrated by blue lines if significant (p value < 0.05).

Emergent Vegetation

The five most dominant vegetation species observed at the study locations were *Schoenoplectus pungens*, *Hymenocallis liriosme*, *Phragmites australis*, and *Spartina alterniflora* (respective relative abundances 13.66, 11.60, 9.83, and 9.80 %). Vegetation species composition similarity among sites was compared and displayed in a non-metric multidimensional scaling plot (Figure 30). The pattern in species composition similarity followed the geographic distribution of the sites with the two most upstream “in-river” sites with high relative abundance of *P. australis* (R49 and R45) clustering together.

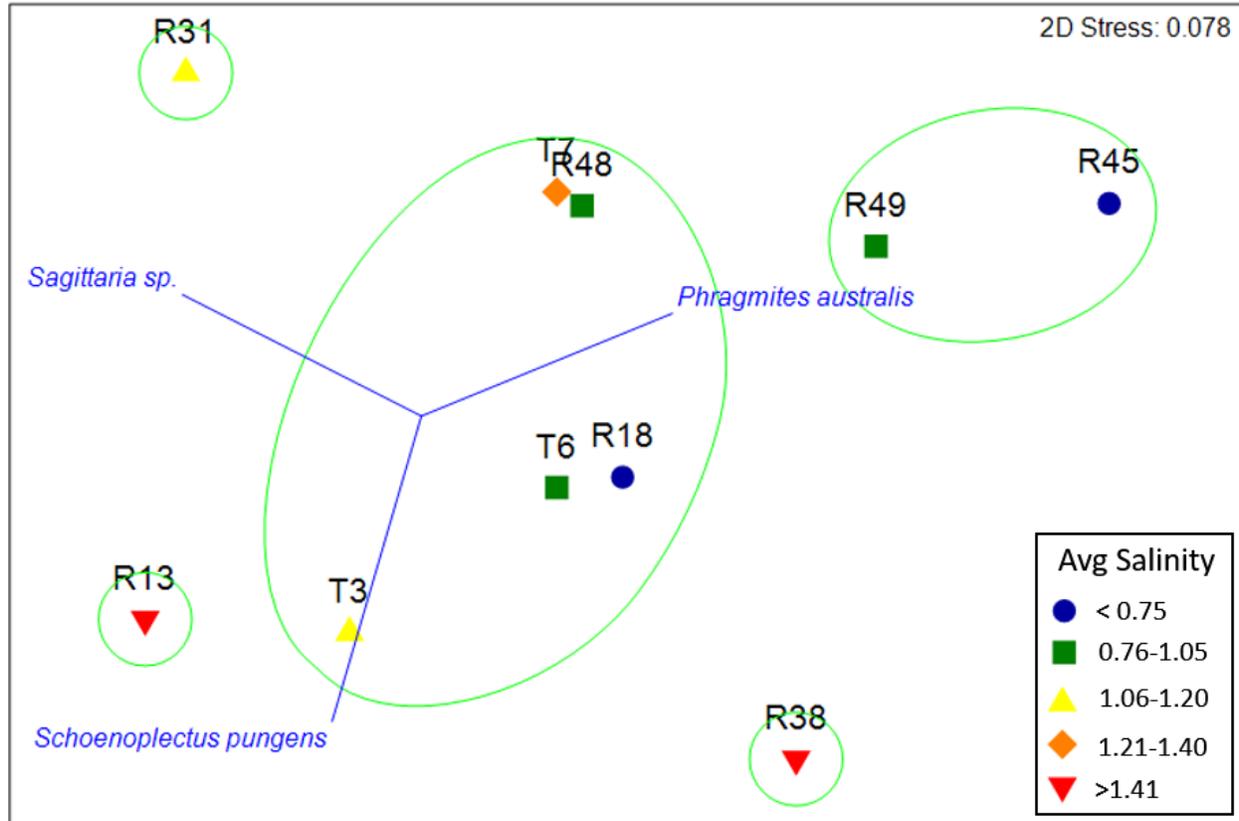


Figure 30. Non-metric multidimensional scaling plot on Log(X+1) transformed vegetation community data, with Bray-Curtis similarity resemblance. Green circles represent 40% similarity in vegetation community. Blue lines and species are the species that have the highest correlation with the multidimensional scaling axis (calculated with a Pearson Correlation). Symbols represent average salinity at each site.

DISCUSSION

This study represents the first comprehensive survey for *Rangia* and Wild Celery within the Trinity River Delta with concurrent continuous salinity monitoring. Previous studies have shown that within Galveston Bay, the highest abundance of *Rangia* were found near the Trinity River Delta (Windham et al. 2019). *Rangia* were found to be consistently present throughout the delta over the study period. While *Rangia* were detected at all sites throughout the delta, their size, abundance and health varied by site. A wide range of sizes (and therefore ages) of *Rangia* were observed, however there were no observations of small, dead (but whole) clams. The hand sampling method resulted in higher numbers of *Rangia* collected compared to the clam rake method, however it was selective against the smaller clams. While there was an increased likelihood of detecting *Rangia* with an increase in average salinity, it is important to note that all the study sites exhibited a narrow and low salinity range.

Throughout all the previous studies in the Trinity River Delta, a wide variety of sizes of *Rangia* have been detected, supporting the hypothesis that a sustained reproducing population exists within the study area (Guillen et al., 2016; Parnell et al., 2011; Windham, 2015). Based on the application of the von Bertalanffy growth model for *Rangia* developed by Wolfe and Petteway (1968), the majority of the *Rangia* collected in this study were likely 4+ year old cohorts. *Rangia* occurrence rates were compared between this study and published data, however each of the previous studies occurred at various times of the year under different salinity regimes using varying amounts of effort and different sampling methods (Guillen et al., 2016; Parnell et al., 2011; Windham, 2015). Therefore, comparison of density or catch per unit effort is difficult, if not impossible. As a result, only binary presence/absence data as the biological endpoint can be used to facilitate comparison of *Rangia* incidence between studies (see Guillen et al. 2016). Further confounding the ability to compare *Rangia* presence among the studies is the spatial scale of the study area, with earlier studies surveying a larger area and later studies focusing in on the area of the delta with the highest recorded abundances of *Rangia*. 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) both occurred within a salinity range of 0-1.5 psu (Guillen et al. 2016).

In the current study, a positive relationship was identified between the average salinity of a site and the probability of detecting Rangia, while a negative relationship was identified between the average salinity of a site and the MI of the Rangia collected at each site. A positive relationship was identified between the percent fines of the sediment and the size of the Rangia, while a negative relationship was identified between percent fines and the Rangia MI. The percent fines in the sediment is related to the amount of freshwater inflow, with the percent fines in sediment decreasing following periods of higher inflow (the smaller particles being washed further downstream). The increase in MI with an increase in freshwater inflows was also demonstrated when the 30 to 90-day average discharges were examined.

Between 2016 and 2019 the USGS conducted instantaneous discharge monitoring at the I-10 bridge crossing over Old River for a total of nine elevated inflow events, and they have demonstrated that the Trinity River appears to diverge between the Romayor and Wallisville gage sites. The measurements at the Old River site essentially account for the difference in discharge (Personal Communications: Zulimar Lucena, Hydrologist at USGS). During elevated inflow events, a majority of the Trinity River discharge is directed through the Old River, which does not have a gage site. This is likely why the peaks at the three sites within the delta (lag time of 3 days and 15 hours) are observed before the peak at the Wallisville gage (lag time of 4 days, 3 hours and 30 minutes) is observed.

The average MI observed during this study (22) and the Guillen et al (2016) study (30) were both during periods of elevated freshwater inflow and were higher than the average MI from the previous two studies (13 and 12), which were during periods of reduced freshwater inflow (Parnell et al., 2011; Windham, 2015 respectively). During the current study period, freshwater pulses exceeded the annual “overbank pulse” amount identified in the pulse flow recommendations for the Trinity River Basin (BBEST, 2009). Based on comparison with these studies it appears that Rangia collected during periods of elevated freshwater inflow are healthier (contain proportionately more soft tissue). It should be noted that the collection methods were not consistent among all studies, however no significant difference was observed in MI by gear type in this study. The MI 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 of Rangia would reduce soft tissue biomass over time, even after shell tissue has

been created. This would effectively result in a decrease in the ratio of soft tissue to total weight due to starvation. It is likely that many *Rangia* experienced inefficient feeding during the drought conditions in 2011 to 2014, leading to a reduction in soft tissue biomass. The MI is a metric to measure health as a result of recent conditions but cannot provide long-term health information. The shells of live *Rangia* collected during the current study have been retained for future age and growth analysis, which will help define fine-scale growth of *Rangia* in the delta relative to historical freshwater inflow patterns. The annual growth will be compared to freshwater inflow patterns to investigate long-term health of *Rangia* as it relates to the freshwater inflow in the Trinity River Delta.

During this study, Wild Celery was not detected in the Trinity River Delta. This is most likely due to the high turbidity found in the river delta that was confirmed by the turbidity (Secchi tube transparency) monitoring (which can directly impact Wild Celery survival, and the researcher's ability to detect it, if it were present). Wild Celery requires relatively clear water to survive and grow (Frank and Moore 2003). As a result, when in turbid water it can only survive at shallower depths that permit sufficient light penetration. During much of the study period, the volume of inflow from the Trinity River (and Lost River) resulted in higher than usual water depth throughout the delta, further limiting potential for Wild Celery growth. During the Guillen et al. (2016) study, Wild Celery was observed at several sites including R13, R15, and R31. Recent attempts to locate Wild Celery in the Trinity River Delta have been unsuccessful (Parnell et al., 2011; Quigg and Steichen, 2015; Windham, 2015). Quigg and Steichen (2015) noted that Wild Celery had rarely been identified within Galveston Bay over the past 30 years and was not found during their studies from 2011 to 2014 in Galveston Bay (Parnell et al., 2011).

Another factor affecting Wild Celery in the Trinity River Delta is salinity. Drought conditions were predominant during 2011-2014, when past studies were conducted. As a result, salinity levels observed during the Guillen et al. (2016) and this study were generally lower than conditions that existed during 2011-2014. Although it is difficult to directly compare study results due to differing methodology in sampling methods, periodicity and frequency, there are several conclusions that can be made from examination of the current data set and recent studies. The prolonged elevated freshwater inflow since 2015 have resulted in depressed salinity (< 3 psu) compared to the previous drought conditions throughout much of the Trinity River Delta.

Past literature and monitoring has found that optimal survival and growth in Wild Celery occurs when salinities are below < 3 psu for greater than 90 days (Dobberfuhl, 2007). Furthermore, salinity is not the only environmental factor that affects its distribution, abundance or survival. Wild Celery growth and abundance has been shown to be limited by light availability (Doyle and Smart, 2001; Kimber et al., 1995), which is impacted by both water depth and turbidity (Blanch et al., 1998). While Wild Celery growth, reproduction, and distribution are regulated by salinity (Boustany et al., 2010), during conditions of elevated salinity, increased light has been shown to slightly ameliorate salinity stress (French and Moore, 2003). Wild Celery seed banks and subsequent germination can be affected by various factors including substrate type, light, temperature, salinity, and sedimentation rates (Campbell, 2005; Jarvis and Moore, 2008). Having a healthy seed bank can serve as the primary recovery mechanism for Wild Celery after periods of drought or other stressed conditions (Jarvis and Moore, 2008). Herbivory by waterfowl has been shown to cause a decline in the density of Wild Celery (Sponberg and Lodge, 2005), while impacts from aquatic mammals such as nutria (*Mycastor coypus*) are unknown. These compounding factors complicate deciphering direct connections between Wild Celery distribution and freshwater inflow.

The emergent vegetation community surveyed at each Rangia sampling site followed general trends in average site salinity indicating that shore-line vegetation may be an additional tool to monitor changes in freshwater inflow in the Trinity River Delta. Additionally, investigations into the proportion of salt tolerant species could provide a way to evaluate the recent historic salinity, therefore freshwater inflow at a particular site within the Trinity River Delta. For example, site R31 separated from the other study sites due to the high relative abundance of *Sagittaria sp.* while sites R13 and R38 are dissimilar than all other sites, each being the highest salinity and closest proximity to the open Trinity Bay.

FUTURE RECOMMENDATIONS

Continued long-term monitoring of Rangia and Wild Celery over a wide range of freshwater discharge and salinity conditions is critical for evaluating the influence of adopted freshwater inflow regimes on upper Galveston Bay habitat, including tidal wetlands and SAV and their role as freshwater inflow bioindicators. Future monitoring should include sampling of the same fixed

index sites for long-term temporal monitoring. Additional study components that should be adopted include the deployment of field mesocosms and known age clams to evaluate annual and seasonal growth. The use of field mesocosms would enable investigators to manipulate multiple variables including age, sediment type, depth, and predation, and would facilitate mark recapture studies to gain better estimates of mortality, reproductive cycles, growth and the response of these variables to freshwater inflow.

Because the gear types used (clam rake and hand sampling) resulted in significantly different sizes of clams, we recommend the combination of the two gear types for future *Rangia* work. Additionally, the fact that the clam rake effort is in seconds rather than a unit of area made it difficult to compare abundance between the two gear types. Future sampling should include estimation of the area sampled within the 30-second rake in order to better standardize sampling effort and understand the gear efficiencies.

The lower Trinity River experiences a wide range of hydrological conditions, and the fact that at certain flow thresholds, a significant proportion of the flow is diverted from the Trinity River into the Lost River complicates the freshwater inflow patterns of the delta. Additional discharge and bathymetry monitoring is recommended to better understand how freshwater pulses move throughout the delta.

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