Grass shrimp biomonitoring at two sites in Coastal South Carolina 2001-2016

Long-term trends and associations with water quality variables

January, 2024

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Long-term trends and associations with water quality variables.

Prepared by

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1 EXECUTIVE SUMMARY

From 1986 until 2016, studies of the populations of grass shrimp (*Palaemon* spp.) in South Carolina estuaries were conducted. The purpose of those studies was to determine if various anthropogenic activities influenced shrimp populations. These studies initially began as part of agricultural run-off studies related to vegetable farming on the 'sea islands' of coastal South Carolina in the late 1980s and early 1990s (Fulton et al., 1999; Scott et al., 1994); Scott et al. (1990). Later work expanded the use of these population metrics to evaluate the potential impacts of urbanization and land-use along the coastal zone of South Carolina, since agricultural and natural areas were (and continue to be) rapidly developed into residential and resort living communities. This long-term dataset of grass shrimp population metrics was thought to be critical in the investigation of potential anthropogenic impacts to coastal salt marsh systems (Daugomah et al., 2014; Fulton et al., 1993; Key et al., 2011; Key et al., 2006; Leight et al., 2005; Porter et al., 1997).

Throughout these studies, two long-term reference sites (North Inlet Oyster Landing [NIOL] and the western branch of Leadenwah Creek, SC [CTL]) were sampled monthly from 1991 until 2016. This long-term sampling effort served two purposes. First, it established a baseline and long-term record of grass shrimp distributions. Secondly, by maintaining a continuous sampling effort at these reference sites, the research team was poised to investigate new, emerging, or other existing areas of anthropogenic impact in the region. This effort overcame one of the leading issues when looking at impacted sites, which is finding a suitable reference site with existing baseline data. All too often, the search for a reference site begins only after a contamination problem is discovered. In those cases, prior data is often not available, therefore researchers can only use data going forward. In this case, NIOL and CTL are stations that are either in areas protected from development (NIOL) or have had little change in land-use over these ~30 years (CTL).

In 2016, the decision was made to conclude the long-term sampling effort. It was determined that the monthly sampling data (from 1991-2016) was of significant value and robustness for comparison to other sampling locations. This effort has grown from the findings of Leight et al. (2005); where monthly grass shrimp population data for NIOL and CTL from 1991-2000 were included in their study. The present study provides an analysis of the data from 2001 until the end of the long-term collection in 2016. The analyses presented here document grass shrimp population metrics (densities, sizes, reproduction, and parasitism) and relationships with water quality parameters for grass shrimp over the 15-year time period.

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3 INTRODUCTION

3.1 THE ROLE OF GRASS SHRIMP

The daggerblade grass shrimp, *Palaemon pugio*, was formally described by Holthuis (1949) as *Palaemonetes pugio*. That paper and one that followed sought to clarify confusion that existed in the description of the undefined species that were assigned to the genus *Palaemon* prior to that time (Holthuis, 1949; Holthuis, 1952). De Grave and Ashelby (2013) performed a re-appraisal of the Palaemonids based on morphological and molecular evidence. Based on their research the genus name *Palaemonetes* is synonymous with *Palaemon*. Herein, the name *Palaemon* will be used. Because of the transfer of the genus name from *Palaemonetes* to *Palaemon*, a replacement name of *Palaemon mundusnovus* was suggested (De Grave and Ashelby, 2013) for *Palaemon intermedius* in the Americas to avoid confusion with an homonymous Australian species *Palaemon intermedius*. There are 5 species that inhabit the Eastern and Gulf regions of the United States: *P. pugio*, *P. vulgaris*, *P. mundusnovus*, *P. paludosus*, and *P. kadiakensis*. The latter two species are freshwater. *Palaemon pugio, P. vulgaris, and P. mundusnovus* overlap in range in estuarine and saltmarsh habitats along eastern and Gulf of Mexico coastlines of the United States from Texas to Maine (Collins, 1981). The primary focus of this paper will be on *P. pugio* but does include limited information on *P. vulgaris*. None of the other species were encountered in this study.

Members of this genus are known to be quite adaptable in their feeding preferences based on food availability. They have demonstrated omnivorous feeding that includes the consumption of detritus (living and dead plant and algal material) and other fauna (Anderson, 1985; Morgan, 1980; Odum and Heald, 1972). They are primarily considered detritivores in estuarine systems (Adams and Angelovic, 1970; Anderson, 1985; Odum and Heald, 1972; Welsh, 1975). Welsh (1975) performed a very detailed study describing this role for *P. pugio*. The grass shrimp, using their appendages and mouth parts, are able to mechanically separate dead and decaying intertidal plant material into available food for themselves and other organisms. The shrimp help to increase the breakdown of the plant material and make energy available to a variety of trophic levels. According to Welsh (1975), the shrimp make that detrital energy available in the form of shrimp biomass, feces, dissolved organic material, and varying sizes of detrital fragments that other organisms are then able to consume. Vernberg (1993) summarized that approximately 40% of the primary production of southeastern estuarine systems comes from intertidal vegetation (such as *Spartina alterniflora*). While many different organisms aid in the decomposition and breakdown of *S. alterniflora*, the role of grass shrimp in the conversion of that primary production source into available food for estuarine

consumers is highly significant and important. As a result of this important role in energy cycling, the grass shrimp are found to be one of the most numerically dominant species found in southeastern estuarine systems. Allen et al. (2007) performed a two-year study in which *Palaemon* spp. have been found to account for 83% of samples collected and accounted for approximately 45% to nearly 90% of total nekton in seasonal sampling efforts.

3.2 BIOMONITORING: GRASS SHRIMP AS BIOINDICATORS

The term 'biomonitoring' has been used in a variety of ways depending on the field of study and the focus of the scientific investigation. In humans, the term refers to the monitoring of contaminants from body burden exposures primarily in blood or urine (Angerer et al., 2007). In ecology, 'biomonitoring (also aquatic biomonitoring)' involves monitoring ecosystems by looking at changes over time in various factors such as biodiversity, landscape, habitats, populations, and species (Bondaruk et al., 2015). In ecotoxicology, 'biomonitoring' refers to the act of observing or assessing changes in organisms, populations, or communities that may results from documented or potential anthropogenic perturbation in the environment, as found in Daugomah et al. (2014); Finley et al. (1999); Fulton et al. (2006); Fulton et al. (1996); Fulton et al. (1999); Fulton et al. (1993); Holland et al. (2004); Key et al. (2011); Key et al. (2006); Leight et al. (2005); Porter et al. (1997); Scott et al. (1994); Scott et al. (1990). According to Bondaruk et al. (2015), a bioindicator is a living organism (or species) that can provide information about the status of the environment. From qualitative or quantitative methods, the status of an ecosystem can be assessed by the looking at one particular component (e.g., community, population, or species) of that ecosystem.

A number of studies over the last three decades have described the relationship between grass shrimp populations (specifically, *P. pugio*) and contaminants; helping to define the grass shrimp as bioindicators of anthropogenic stress. Those studies were designed to determine if various anthropogenic activities affected shrimp populations. The studies initially began as part of agricultural run-off studies related to vegetable farming on the 'sea islands' of coastal South Carolina in the late 1980s and early 1990s (Fulton et al., 1999; Scott et al., 1994; Scott et al., 1990). Additional research then evolved to examine the relationship of coastal development, urbanization and changing land-use along the coastal zone of South Carolina. As former agricultural and natural areas were (and continue to be) developed into residential and resort living communities, grass shrimp populations were investigated for potential impacts (Daugomah et al., 2014; Fulton et al., 1993; Key et al., 2011; Key et al., 2006; Leight et al., 2005; Porter et al., 1997).

Throughout those studies, two long-term reference sites (North Inlet Oyster Landing [NIOL] and the western branch of Leadenwah Creek, SC [CTL]) were sampled monthly from 1991 until 2016. This long-term sampling effort served two purposes. First, it was necessary to establish a baseline and long-term record of grass shrimp distributions. Secondly, by maintaining a continuous sampling effort at these reference sites, the research team was poised to investigate new, emerging, or other existing areas of anthropogenic impact in the region. This effort overcame one of the leading problems in looking at impacted sites, which is finding a suitable reference site with existing baseline data. All too often, the search for a reference site begins only after a contamination problem is discovered. In those cases, prior data is not available, therefore researchers can only use data going forward.

3.3 ASSOCIATIONS WITH PHYSICAL AND CHEMICAL WATER QUALITY PARAMETERS

In all aquatic field research initiatives, it is incumbent upon the researcher to collect information about the physical and chemical nature of the system (or systems) that are being studied. In ecotoxicological biomonitoring studies, these parameters are particularly important from two different perspectives. First, it is important to separate the potential observed changes in the bioindicator species that may be due to contaminant exposures from that of seasonal, episodic, or periodic natural water quality changes. Estuaries are dynamic systems where water quality conditions can be variable. Second, extreme changes in the physical and chemical nature of the water may exacerbate an anthropogenic perturbation that may be occurring (DeLorenzo et al., 2009). The most basic of these parameters, which are easily measured with relatively inexpensive instruments, are water temperature, salinity, pH, and dissolved oxygen concentration. There are further and increasingly more complicated measures that can be made such as dissolved organic material (DOM), totals suspended solids (TSS), nutrient concentrations, turbidity, chlorophyll *a* concentration, and many others. For our study we focused on the most basic measures of water temperature, salinity, pH, and dissolved oxygen concentration. For datasets such as the one associated with this 15-year *Palaemon* population effort; understanding the potential changes associated with water quality are key to being able to also understand any specific changes that may be attributed to anthropogenic pollutants.

The genus *Palaemon* (particularly *P. pugio*, *P. vulgaris*, and *P. mundusnovus*) is highly adapted to the harsh and often extreme conditions (changes in temperature, salinity, and dissolved oxygen) that exist in estuarine systems (Anderson, 1985; Welsh, 1975). While it is true that these shrimp are euryhaline and eurythermal, these shrimp do have optimum ranges in salinity and temperature that affect and in

some cases drive growth, reproduction (Anderson, 1985; Key et al., 2011), and rates of parasitism (Key et al., 2011). It is fully recognized that seasonal differences and the variation of water quality parameters may be directly related to the observed changes in shrimp populations in any given region (Key et al., 2011; Kneib, 1984; Kneib, 1987; Scott et al., 1994; Scott et al., 1990).

3.4 OBJECTIVE: LOOKING AT A LONG-TERM DATASET

As previously stated, the intent of this long-term dataset was to provide a continuous baseline of data for two reference sites in South Carolina for comparison with contaminated or land-use altered sites. Now that the long-term collection of biomonitoring data from those sites has concluded, we have the opportunity to look at the data in other ways. Initially, we sought to relate the data to climatic variables and look for trends related to climate change. However, even though data for this study covers 15 years [25 years if you also include the data presented by Leight et al. (2005)], there is not enough information for a long enough period of time to draw conclusions related to climate change. Using the data that we have in hand, we decided to document the long-term trends of grass shrimp populations and present associations to water quality variables.

3.4.1 Overarching objective:

- *Determine baseline reference population parameters for shrimp in southeastern estuarine systems for others to use in evaluating impacts based on climate, contaminants, and land-use. Additionally, this dataset can serve as general background information for a variety of topics such as trophic studies, bioenergetics, and parasitology.*

3.4.2 Specific objectives

- *Determine the overall density of grass shrimp collected between 2001-2016*

- *Determine and compare the yearly densities of grass shrimp*

- *Determine and compare the yearly lengths (mm) of grass shrimp*

- *Determine and compare the yearly weights (mg) of grass shrimp*

- *Determine and compare the yearly sex ratios of grass shrimp*

- *Determine and compare the yearly egg counts of grass shrimp*

- *Determine and compare the yearly incidence of bopyrid parasites on grass shrimp*

- *Determine associations between grass shrimp metrics and water quality variables*

4 Methodology

4.1 Sampling Sites

In the study by Leight et al. (2005), four sampling sites were used. This study represents a continuation of sampling at two of the estuarine tidal creek sites from that study: NIOL and CTL (Figure 1). These sites were also used by Finley et al. (1999) and Scott et al. (1994).
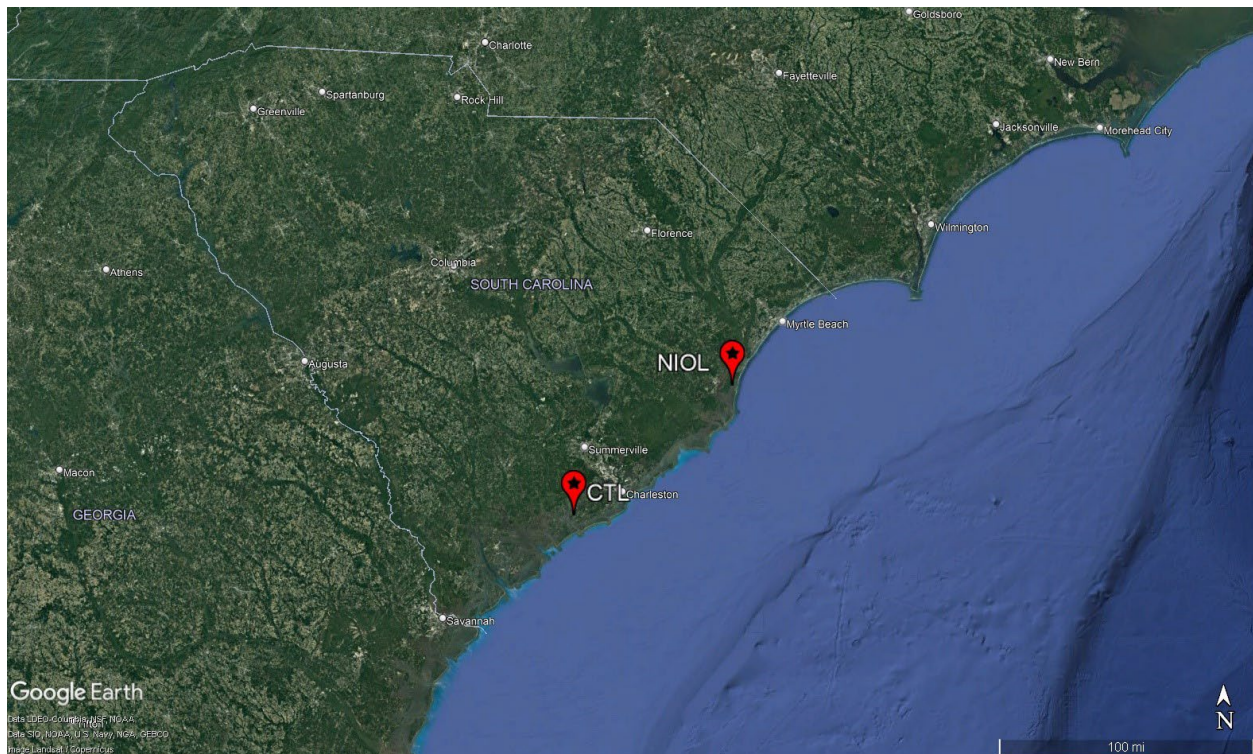
Figure 1 -- Sampling sites on the South Carolina coast

4.1.1 North Inlet Oyster Landing (NIOL)

The North Inlet Oyster Landing (NIOL) site is located on Clambank Creek (33°21'2.59"N 79°11'23.86"W – Figure 2) within the North Inlet-Winyah Bay (NIWB) National Estuarine Research Reserve (NERR). Established in 1992, the NIWB NERR is one of 30 such protected and conserved NERRs sites located around the coastal regions of the United States. NOAA established these sites to promote conservation, research, education, and stewardship in America's estuaries. The estuarine creeks within the NIWB NERR are surrounded by undeveloped upland wooded areas.

Figure 2 -- North Inlet Oyster Landing (NIOL)

4.1.2 Leadenwah Creek (CTL)

The 'CTL' site is located on the western branch of Leadenwah Creek (32°38'53.99"N, 80°13'20.52"W), located on Wadmalaw Island, SC (Figure 3). The site has consistently served as a reference site in a number of previous studies dating back to the late 1980s (Finley et al., 1999; Leight et al., 2005; Scott et al., 1994). This area is surrounded by wooded areas and is considered rural; there are a few single-family dwellings adjacent to this site, but the land surrounding the creek was not further developed over 2001-2016.

Figure 3 -- Western branch of Leadenwah Creek site (CTL)

4.2 PUSHNET SAMPLING METHOD

Welsh (1975) demonstrated the use of a pushnet to sample populations of grass shrimp at field sites in Texas. The method was modified by Scott et al. (1994) as an alternative 'rapid consensus' method rather than using labor-intensive block seining methods. The method was further refined and optimized by Leight et al. (2005). Using the same monthly sampling methodology without modification as in Leight et al. (2005), a pushnet (Figure 4) was used to collect adult grass shrimp (along with other similar sized organisms such as juvenile Penaeid shrimp and fish of the genus *Fundulus*) from three predefined 25 m stretches of each creek (Figure 5 and Figure 6) as the tide receded (i.e. ebbing tide) from the marsh and a defined stream channel began forming (Figure 7). The 25 m stretches were separated by 10 m buffer zones to provide replicated samples from each creek. Samples were simultaneously collected from each creek bank (Figure 7 and Figure 8) as in Leight

et al. (2005). Samples were placed into pre-labeled, sealable plastic bags and transported back to the laboratory on ice.
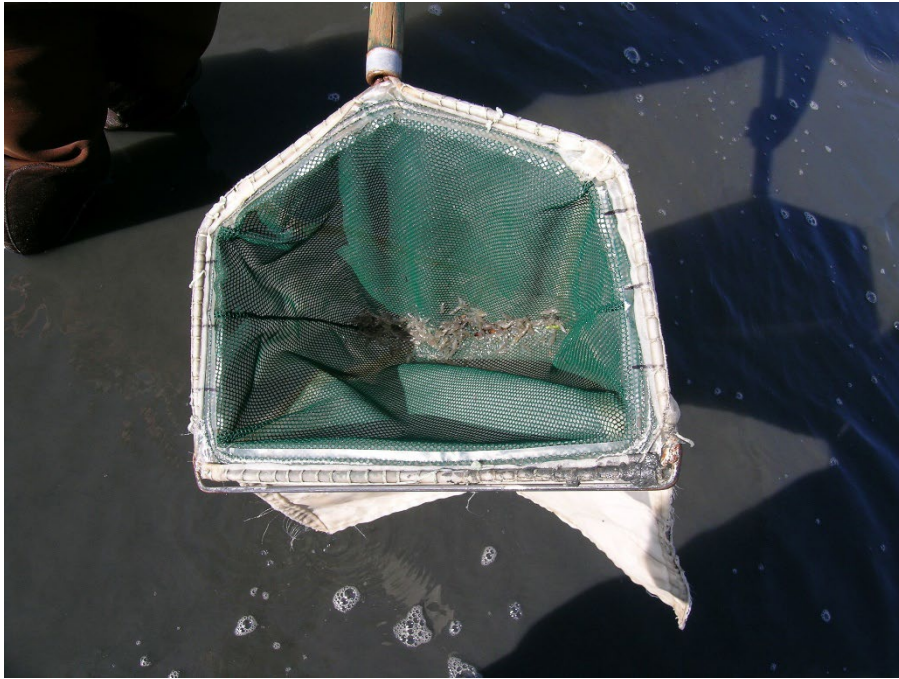
Figure 4 -- Pushnet

Grass shrimp are highly dispersed across the marsh grass beds during high tide, but as tidal waters ebb and the high marsh becomes dry, shrimp become concentrated in creek channels and population assessments become more effective.
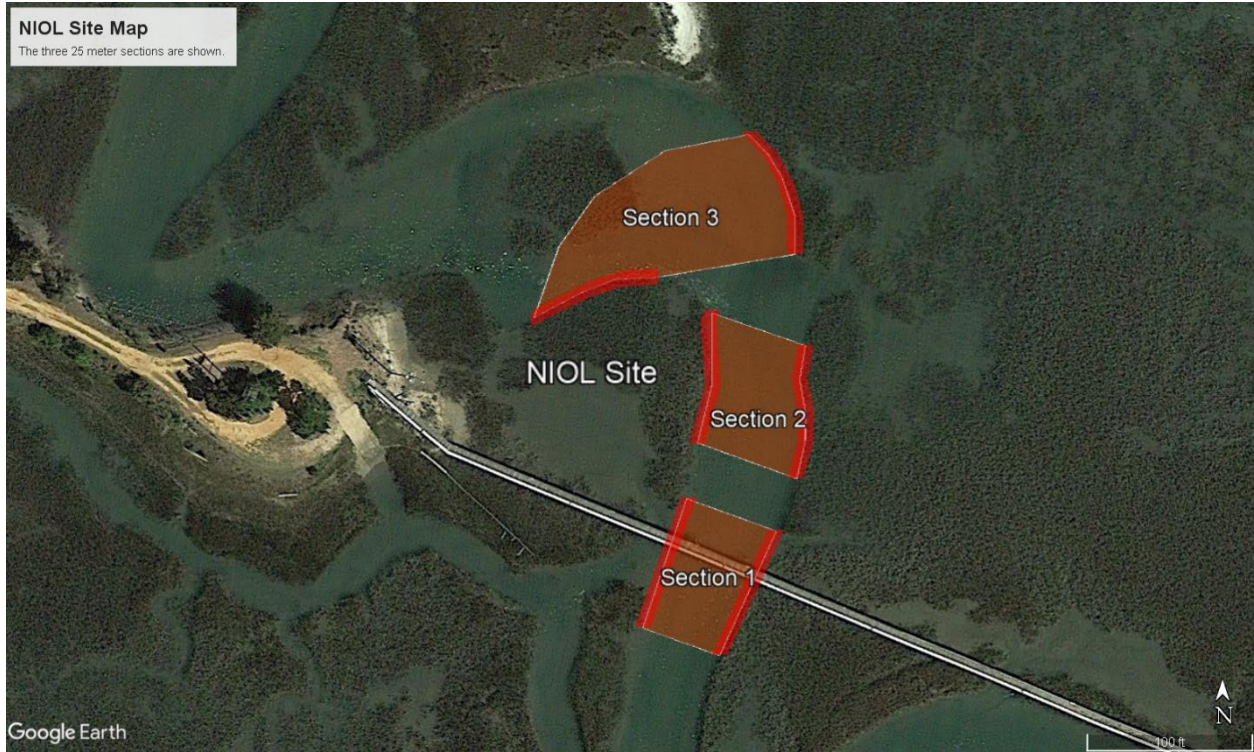
Figure 5 -- NIOL site map

Figure 6 -- CTL site map

Figure 7 -- Field sampling

Figure 8 -- Field sampling

4.3 DISCRETE WATER QUALITY MEASUREMENTS

At the conclusion of each sampling event, water quality parameters [temperature (°C), salinity (ppt), dissolved oxygen (mg/L and %), and pH] were measured using a YSI 556MPS (YSI Environmental, Yellow Springs, Ohio). The measurements were taken from the center of each creek at the end of the third section, immediately following pushnet sampling. The instrument was calibrated in accordance with the manufacturer's methodologies and calibration frequencies for each of the measured parameters.

4.4 CONTINUOUS WATER QUALITY MEASUREMENTS

Additionally, continuous water quality data was available from NIOL. As a long-term research station, NIOL is equipped with datasondes which collect a measure of water temperature, salinity, dissolved oxygen and pH every 15 minutes. This data is publicly available and may be obtained (downloaded) from the North Inlet–Winyah Bay National Estuarine Research Reserve's System Wide Monitoring Program (NOAA, 2017) for the NIOL Site. Data for this site is from 1/2001 to 12/2016. Continuous water quality data was also collected every 15 minutes CTL site by a YSI 6-series datasondes (YSI, Ohio) deployed by NCCOS Charleston staff. Data for water temperature, salinity, dissolved oxygen and pH is available from 7/2007 to 12/2016. Mean water quality values for each parameter were determined by taking the mean water quality value (from readings taken every 15 minutes) for the 24-h, 48-h, 96-h, 7-d, and 14-d time periods preceding any given sampling event.

4.5 SAMPLE PROCESSING AND ENUMERATION

All samples were pre-sorted in the laboratory and preserved within 24 hours of collection. During the initial pre-sorting, samples were separated by species with the exception of the grass shrimp, which were separated to genera. Individuals were then counted, weighed and preserved in 90% ethanol. Sample counts for samples greater than 100 individuals were estimated based on the weight of a random subsample of 100 individuals. Data for species other than grass shrimp are not presented here.

Preserved samples were used to determine sex ratios and potential reproductive capacity and were processed up to one year after the sampling date. Using the same procedures as described in Leight et al. (2005), the species, sex (according to Holthuis (1952)], length, weight, presence of ectoparasites, and number of eggs were determined on a random subset of 30 preserved grass shrimp from each sample.

4.6 STATISTICAL ANALYSES

Monthly grass shrimp biomonitoring data (densities, size, sex, and parasitism), were analyzed using SAS Software for Windows version 9.4 [SAS/STAT] (SAS Institute, Cary, NC). Group, Site, and Yearly comparisons were made using the parametric statistics Analysis of Variance (ANOVA) and Chi Square tests. The alpha for all tests was 0.05.

A subset of the 2001-2016 dataset was used for determining associations between grass shrimp metrics with water quality data. The subset encompassed samples from July of 2007 until December of 2016. Limiting the analysis to this subset was necessary due to a lack of water quality data prior to July 2007 at the CTL site. Multivariate Partial Least Squares regressions (PROC PLS in SAS/STAT, SAS Version 9.4) were used to determine those associations. Table 1 and Table 2 show the dependent and intendent variables used in the models, respectively.

Table 1 -- Dependent variables in partial least squares regression

Variable	Description
den_M	Density of males
den_GF	Density of gravid females
den_NGF	Density of non-gravid females
den_para	Density of parasitized shrimp
L_M	Mean length of males
L_GF	Mean length of gravid females
L_NGF	Mean length of non-gravid
L_para	Mean length of parasitized shrimp
L_npara	Mean length of non-parasitized shrimp
W_M	Mean weight of males
W_GF	Mean weight of females
W_NGF	Mean weight of non-gravid females
W_para	Mean weight of parasitized shrimp
W_npara	Mean weight of non-parasitized shrimp

Partial Least Squares (PLS) regression is a multivariate procedure based on algebraic matrices. PLS obtains consecutive linear combinations of predictor variables, called factors, which explain the variation in both the response and predictor variables. These factors are extracted from an algebraic matrix of variable covariance structures (Wold, 1995). By default, predictors and responses are normalized (i.e. centered and scaled) to have mean=0 and standard deviation=1. A cross-validation method (using a one-at-a-time approach) was used to choose the number of extracted factors to fit the model, specified by the 'CV=ONE' and 'CVTEST' options in SAS PROC PLS according to the methodology proposed by van der Voet (1994).

Table 2 -- Independent variables in partial least squares regression

Variable	Description (time period prior to sampling)
temp_24h	24 hour mean water temperature
temp_48h	48 hour mean water temperature
temp_96h	96 hour mean water temperature
temp_7d	7 day mean water temperature
temp_14d	14 day mean water temperature
DO_per_24h	24 hour mean dissolved oxygen percent saturation
DO_per_48h	48 hour mean dissolved oxygen percent saturation
DO_per_96h	96 hour mean dissolved oxygen percent saturation
DO_per_7d	7 day mean dissolved oxygen percent saturation
DO_per_14d	14 day mean dissolved oxygen percent saturation
DO_24h	24 hour mean dissolved oxygen concentration
DO_48h	48 hour mean dissolved oxygen concentration
DO_96h	96 hour mean dissolved oxygen concentration
DO_7d	7 day mean dissolved oxygen concentration
DO_14d	14 day mean dissolved oxygen concentration
pH_24h	24 hour mean pH
pH_48h	48 hour mean pH
pH_96h	96 hour mean pH
pH_7d	7 day mean pH
pH_14d	14 day mean pH
sal_24h	24 hour mean salinity
sal_48h	48 hour mean salinity
sal_96h	96 hour mean salinity
sal_7d	7 day mean salinity
sal_14d	14 day mean salinity
Site	NIOL or CTL
Season	Spring, Summer, Fall, Winter

A variable importance plot is produced that is based on the Variable Importance for Projection (VIP) statistic of Wold (1995). The plot displays the influence of each predictor variable in fitting the PLS model for the predictors and response variables. According to Wold (1995), when a predictor variable has a small coefficient (in absolute value) and a small VIP (less than 0.8) value, it is a suitable candidate for deletion. Predictor variables that fell above 0.8 on the variable importance plot were retained in the final model of the PLS procedure.

The correlation loadings plot is the primary output of the procedure from which the associations are displayed. R-squared values of predictors and responses variables (%) for the partial least squares regression are produced by the procedure and are presented in the results and discussion section.

5 RESULTS AND DISCUSSION

5.1 SAMPLING TIME AND DEPTH

5.1.1 Average sampling time prior to low tide

Since the start of sampling was based on a visual observation of when the waterline fell out of the marsh and into the stream channel, it was necessary to determine the actual average sampling time prior to the next low tide event based on observed tides rather than predicted tides. Time was recorded at the end of the sampling run when water quality parameters were collected. Sampling was initiated when the ebbing tide receded from the marsh and a visible stream channel began forming. For all 15 years of sampling data (1/2001 – 12/2016), the mean (μ) sampling time at NIOL prior to the next low tide event (based on the actual low tide using NERRs local depth data) was 119.3 minutes (σ = 49.79). For the CTL site (years 7/2007 – 12/2016), the average (μ) sampling time prior to the next low tide event (based on the actual low tide using NCCOS sonde data) was 275.5 minutes (σ = 68.67). The sampling time data for NIOL exhibited only a very mild departure from the normal distribution (Shapiro-Wilk Test Statistic for Normality [W] = 0.983, p=0.0381). The sampling time data for CTL exhibited a severe departure from the normal distribution ([W] = 0.896, p<0.0001).

While grass shrimp are members of the nekton in estuarine systems, their spatial distribution patterns are highly dependent on environmental drivers. In South Carolina, the strongest driver is perhaps the tide. Locally, the tidal range is roughly 2-m depth between high tide and low tide and occurs twice in roughly a 24-h period (i.e., semi-diurnal). As the tide floods and ebbs across these marsh ecosystems, grass shrimp move back and forth from high marsh areas into stream channels and low tide pools. Other important factors that might influence the spatial distribution of grass shrimp at any given location are wind, currents, food availability, and seasons. As with many species, from plankton to marine mammals, the distribution of grass shrimp populations tend to be patchy. Our sampling design was biased toward the ebbing tide, which concentrated the shrimp into the stream channels. Thus, the spatial density of the shrimp at the time of collection is likely much higher than had sampling occurred when the tide was flooding into the high marsh. The advantages to the present sampling approach are that it provides a consistent sampling methodology and it is logistically easier for field personnel to perform. Disadvantages include net avoidance despite our efforts to work against the tide and the topography of the nearshore environment is highly variable. To a certain degree, we were able to account for the effect of tide on the grass shrimp distributions by sampling at the same point in the tidal stage each time. Based on

that, density estimates presented in this paper are relative and not absolute. Due to differences in the morphology of the two systems, the actual time of sampling prior to the low tide event is very different between the two. Perhaps more important than the timing is the actual depth of the water at the time of sampling.

5.1.2 Mean sampling depth

The mean (μ) sampling depth for NIOL at the beginning of sampling was 1.45 m (σ = 0.423) for 2001-2016. Depth was recorded at a nearby sensor operated by the North Inlet NERRs. The mean sampling depth at CTL was 1.40 m (σ = 0.256) for 2007-2016. Depth was recorded at a nearby sensor operated by NCCOS Charleston. The sampling depth also exhibited a mild departure from the normal distribution for NIOL (Shapiro-Wilk Test Statistic for Normality [W] = 0.981, p=0.0146). The sampling depth data at the CTL site followed the normal distribution ([W] = 0.983, p=0.1864).

While the actual sampling times prior to the low tide events were different between the two creeks, the depth at the time of sampling during the ebb tides was the same. This supports the consistent, regular approach that was taken to account for tidal variation in the sampling design.

5.2 GRASS SHRIMP MEAN DENSITY 2001-2016

5.2.1 Percent density (occurrence) of *Palaemon spp.*

The percent density of the two main estuarine species of grass shrimp, *Palaemon pugio* and *Palaemon vulgaris,* relative to one another are shown in Table 3 for NIOL and CTL. By comparison, Leight et al. (2005) found that *P. pugio* was the dominant shrimp collected (99.5%) from 1991 to 2000 at four sites combined. *Palaemon vulgaris* was found to be at 0.5% in that study. The densities of the two species relative to one another remained approximately the same from 1991 to 2016 at South Carolina sampling sites.

Table 3 - Percentage density of species of grass shrimp

	This Study (2001 – 2016)
NIOL	
P. pugio	98.9 %
P. vulgaris	1.1 %
CTL	
P. pugio	99.5 %
P. vulgaris	0.5 %

5.2.2 Mean density of *Palaemon spp.*

The average shrimp density (geometric mean) was found to be 9.98 shrimp/m^3 for 2001-2016 [Table 4] for NIOL and 7.60 shrimp/m^3 for CTL. The data followed a lognormal distribution. The arithmetic means for NIOL (20.85 shrimp/m^3) and CTL (20.85 shrimp/m^3) found in this study (2001-2016) were compared against the same values (1991-2000) calculated by Leight et al. (2005) from the same sites using one-sample tests. We rejected the null hypothesis (p<0.0001) that the mean grass shrimp density at NIOL was 40.0 shrimp/m^3 [as calculated by Leight et al. (2005)]. The overall mean grass shrimp densities were significantly lower (by about half) in the present study at NIOL (2001-2016) compared to 1991-2000. However, at the CTL site, we failed to reject the null hypothesis (p=0.1549). The overall mean grass shrimp densities at CTL were not significantly different between the two studies (1991-2000 vs. 2001-2016).

Table 4 -- Mean density of grass shrimp (Palaemon spp.)

	Leight et al., 2005 (1991 – 2000)	This Study (2001 – 2016)
NIOL		
Mean (arithmetic) Density (shrimp/m³)	40.08[+]	20.85*
Mean (geometric) Density (shrimp/m³)	Data not available	9.98
CTL		
Mean (arithmetic) Density (shrimp/m³)	16.8[+]	17.93
Mean (geometric) Density (shrimp/m³)	Data not available	7.60
		[+] Leight et al. (2005) * significantly different ($p<0.0001$) between studies

5.2.3   Comparison of *P. pugio* densities by year

The annual geometric means of *P. pugio* density are displayed in Table 5 for NIOL and CTL for 2001-2016.

Table 5 – NIOL and CTL annual mean P. pugio densities

Year	NIOL				CTL			
	Geo-Mean	**Std. Error**	**Lower CL**	**Upper CL**	**Geo-Mean**	**Std. Error**	**Lower CL**	**Upper CL**
2001	6.12	1.24	3.64	9.92	3.58	1.23	2.00	6.00
2002	4.72	1.25	2.64	7.97	5.32	1.22	3.22	8.46
2003	8.70	1.30	4.68	15.58	4.54	1.23	2.62	7.47
2004	7.96	1.28	4.43	13.80	3.41	1.23	1.87	5.76
2005	4.12	1.23	2.37	6.78	3.20	1.24	1.73	5.46
2006	9.65	1.25	5.82	15.64	5.28	1.20	3.27	8.24
2007	5.60	1.23	3.30	9.11	7.38	1.29	4.00	13.04
2008	8.16	1.22	5.15	12.65	8.55	1.26	4.95	14.33
2009	9.54	1.25	5.66	15.70	4.27	1.20	2.64	6.64
2010	5.94	1.33	2.92	11.29	5.33	1.26	2.93	9.18
2011	7.98	1.25	4.75	13.03	6.41	1.23	3.90	10.21
2012	12.8	1.23	8.03	20.19	11.8	1.28	6.67	20.34
2013	19.3	1.19	13.34	27.75	10.4	1.26	6.11	17.41

2014	12.4	1.26	7.3641	20.43	7.59	1.27	4.30	12.92
2015	13.2	1.24	8.1961	21.01	9.80	1.29	5.48	16.98
2016	11.7	1.26	6.9622	19.21	21.	1.21	14.33	31.91

For NIOL, a one-way nested ANOVA (with month nested within year) was used to compare *P. pugio* densities between years. Density data was log transformed [ln(x+1)] to fit the data to the lognormal distribution. The overall (unadjusted) F-test indicated that the model was significant (p<0.0001), however when adjusted with the correct error term (Type III MS) for nested data, there was no significant difference (p=0.41) between years.

5.2.4 Multi-year contrast – 2001-2011 vs. 2012-2016

A visual examination of the mean densities of *P. pugio* by year at NIOL [Figure 9] indicates that the years 2012-2016 are elevated in comparison to prior years. To examine the potential significance of the difference, a one-way ANOVA Contrast was constructed based on the previous nested model using two groupings: 2001-2011 and 2012-2016. Again, the model was adjusted for the correct nested ANOVA error term.
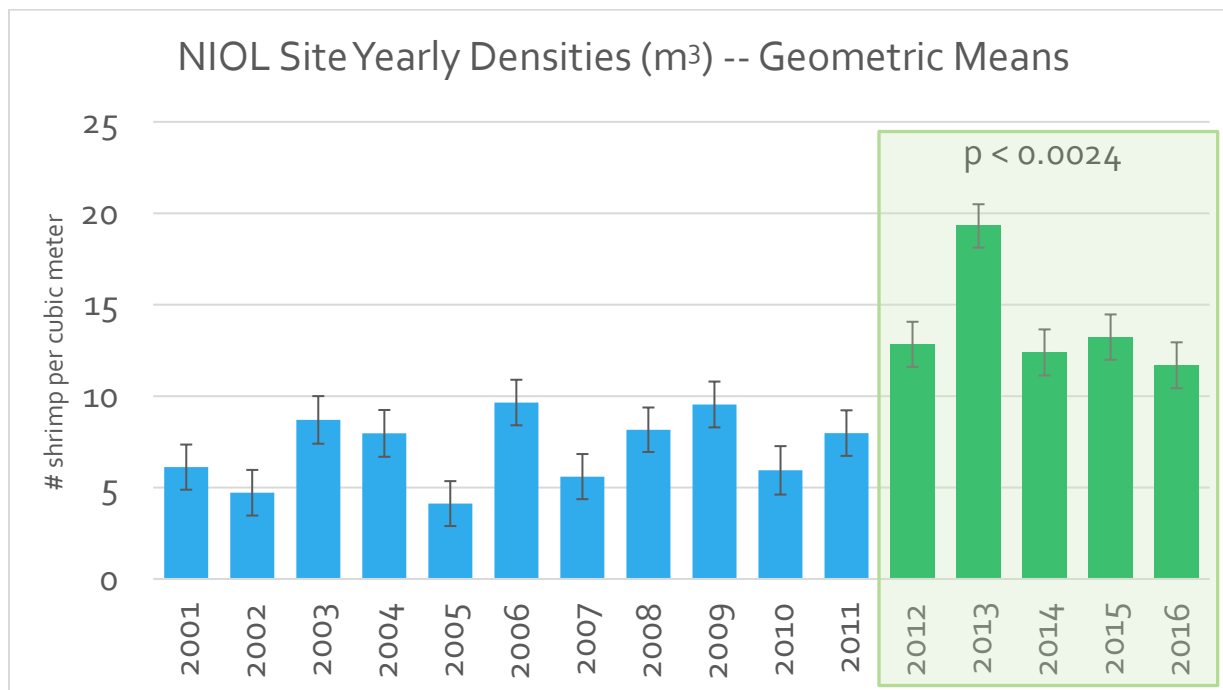
Figure 9 -- Mean P. pugio densities by year for NIOL – 2001-2016
An ANOVA Contrast indicated that Years 2012-2016 (shaded) were significantly greater than years 2001-2011 for grass shrimp density at the NIOL site.

The mean *P.pugio* densities were found to be significantly greater (p < 0.0024) for the year group of 2012-2016 when compared to the preceding time period at NIOL. A similar ANOVA analysis with contrasts was performed for the CTL Site (Figure 10). There was no overall significance in grass shrimp densities between years (p=0.0843) at CTL. Like the NIOL Site, *P. pugio* densities were found to be significantly greater (p < 0.0003) for 2012-2016 than for the prior years of 2001-2011 at CTL.

We explored several correlations with meteorological and water quality dataset but were unable to explain the increases in density that were observed in 2012-2016. Another hypothesis was that there was a methodological change that had occurred in either the sample collection or the sample processing. After a review of our methods and procedures we found that we had sampled consistently from 2001 to 2016. At this point the apparent increases in densities during 2012 through 2016 remain unexplained.
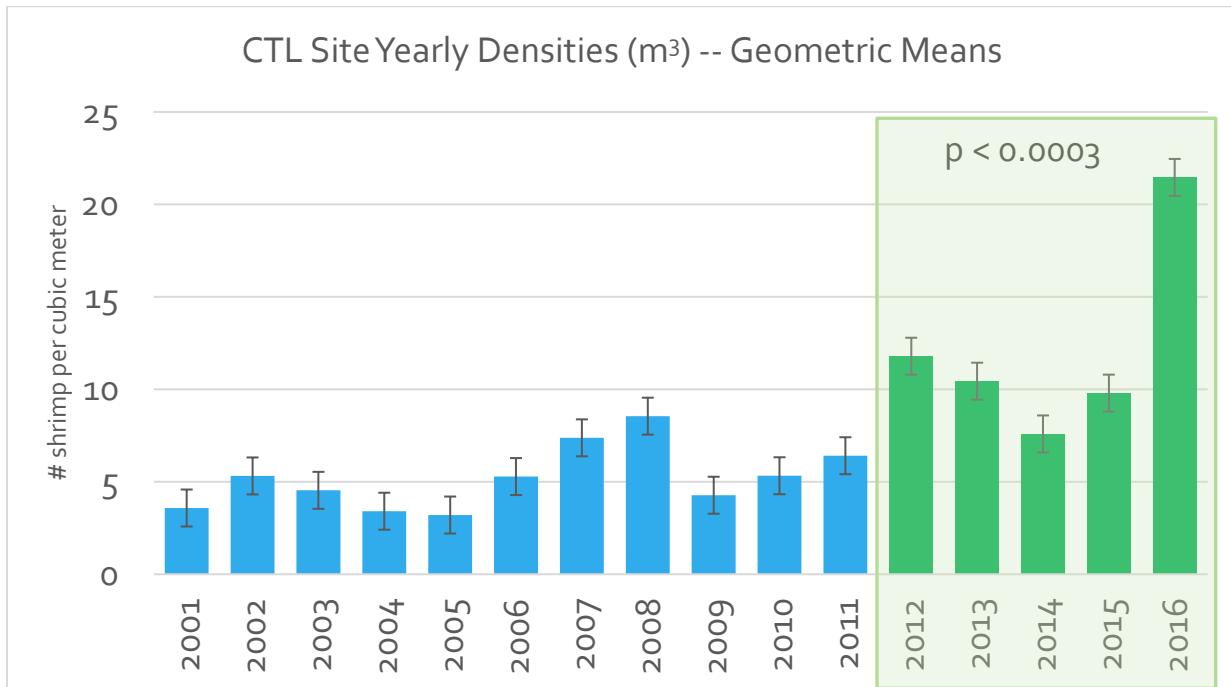
Figure 10 -- Mean P. pugio densities by year for CTL – 2001-2016
An ANOVA Contrast indicated that Years 2012-2016 (shaded) were significantly greater than years 2001-2011 for grass shrimp density at the CTL site.

5.2.5 Monthly grass shrimp densities

During the time frame of 2001 through 2016 we calculated the monthly arithmetic (Figure 11) and geometric (Figure 12) means of *P. pugio* densities. This was performed to examine seasonal density differences. The arithmetic means were

calculated in order to draw comparisons with the previous study of Leight et al. (2005). Since the distribution of the data is lognormal the geometric means were also calculated. When averaged over the 15-year time period, NIOL displayed two peak densities, one in May and another November. It was observed that the CTL site was marked by one significant peak density in September. While these two sites may be viewed as similar (both sites are intertidal, *S. alterniflora* dominated systems), a broader examination of the region reveals significant physical, geological, and ecological differences. Perhaps the biggest difference is that the North Inlet Estuary (where NIOL is located) receives no upland freshwater from a major river as it is largely bounded by barrier islands and mainland. It is a high salinity estuary and it is well mixed (Allen et al., 2007; Dame et al., 2000) receiving its hydrologic inputs from the Atlantic Ocean. In the North Inlet Estuary, Allen et al. (2007) found that geomorphology did not strongly influence the abundance and presence of grass shrimp during warmer months at eight sites sampled seasonally over a two-year period.
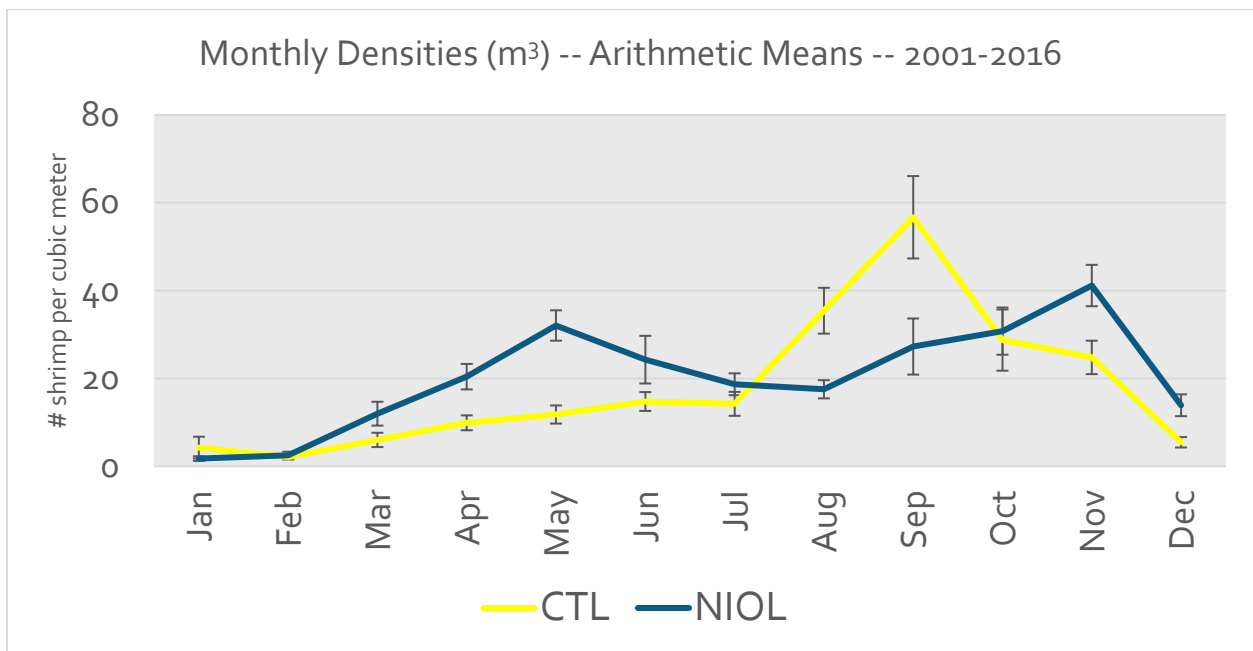
Figure 11 -- Mean (arithmetic) monthly P. pugio densities -- 2001-2016

Monthly Densities (m³) -- Geometric Means -- 2001-2016
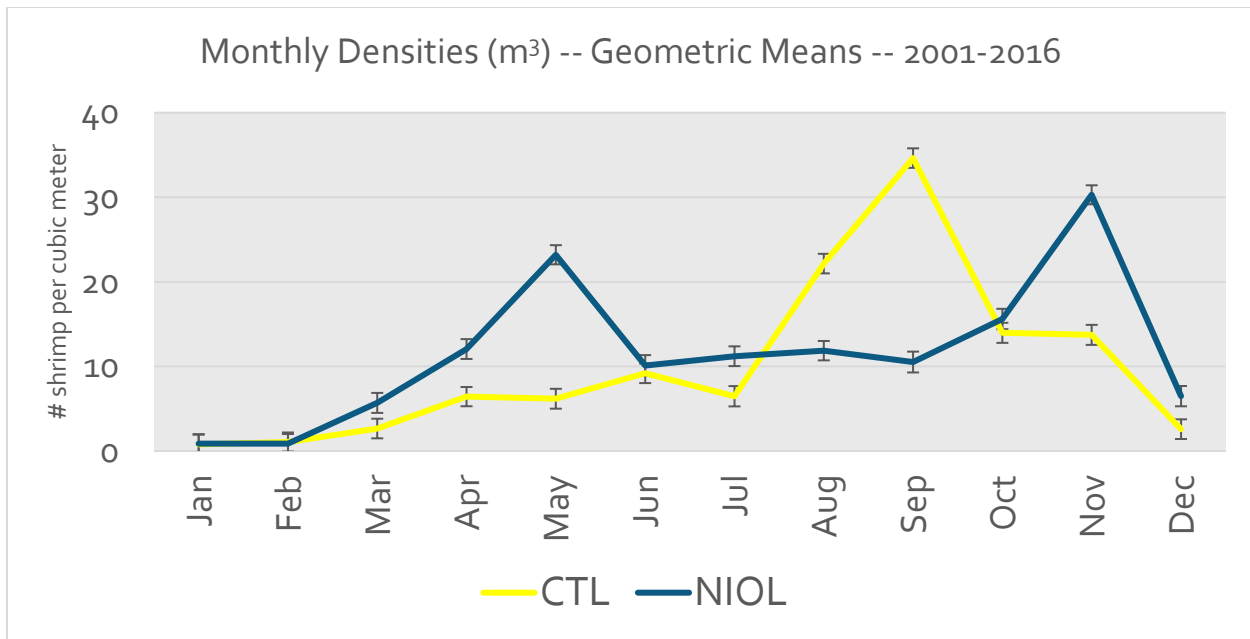
Figure 12 -- Mean (geometric) monthly P. pugio densities - 2001-2016

During colder months when shrimp densities were lower and were largely comprised of overwintering individuals (mostly larger adult), other transient taxa were not as abundant and some relationships to the geomorphology of the creeks were detected. With that being said, all of the sampling sites for the study by Allen et al. (2007) were contained within the high salinity, well mixed North Inlet Estuary system.

Conversely, the CTL site is on Leadenwah Creek which is a major coastal tributary of the Edisto River Basin. The Edisto Basin covers over 3000 square miles, approximately 10% of the area of State of South Carolina (Marshall, 1993). The Edisto River extends inland about 250 miles from the Atlantic Ocean, is not controlled by dams, dikes, or levees and is fed by a constant supply of upland freshwater input. Whereas the North Inlet Estuary remains in a high salinity state throughout much of the year, the coastal regions of the Edisto River and its tributaries, including Leadenwah Creek, experience significant swings in salinity under normal conditions. Thus, it is not unexpected that seasonal abundance profiles in nektonic organisms such as the grass shrimp are different between these two systems. During the time period of 2007 to 2016 the mean salinities at NIOL and CTL were 32.5 (+/- 3.26) and 26.2 (+/- 5.18), respectively. Additionally, using the measuring tool in ArcMap 10.7.1 (ESRI, Redlands, CA, USA) to measure the distance from the collection sites to the Atlantic Ocean interface (by waterway), the NIOL site is only 2.7 nautical miles from the open ocean by water whereas the CTL site is four times that at 10.8 nautical miles from the open ocean.

5.3 GRASS SHRIMP LENGTHS AND WEIGHTS

5.3.1 Grass shrimp lengths (mm)

The length data for grass shrimp largely approximated the normal distribution at NIOL and CTL. Thus, we were able to use the arithmetic mean. Leight et al. 2005 reported a mean length for *P.* spp. at NIOL of 24.3 mm. The mean (μ) length of *P. spp*. for the current study time frame (2001-2016) was 26.2 mm (std. error = 0.034) [Table 6].

Next, we tested the null hypothesis that mean *Palaemon spp.* length (26.2 mm) for 2001-2016 was the same as the mean length (24.3 mm) for 1991-2000. The results of that one-sample T-test ($p < 0.0001$) indicated that the mean length was significantly lower for 1991-2000 versus 2001-2016.

Table 6 -- Mean (μ) grass shrimp lengths (+/- σ) and n at NIOL

Shrimp @ NIOL	Length (mm) (1991-2000)	Length (mm) (2001-2016)	n (2001-2016)
Palaemon spp.	24.3 *	26.2 (0.03)	16407
P. pugio		26.3 (0.03)	16139
male		25.0 (0.03)	9728
non-gravid female		26.7 (0.07)	4502
gravid female		31.2 (0.09)	1909
P. vulgaris		22.5 (0.29)	268
male		20.1 (0.28)	135
non-gravid female		24.7 (0.45)	115
gravid female		25.8 (1.19)	18
			* Leight et al. 2005

Leight et al. 2005 reported a mean length for *Palaemon* spp. at CTL of 22.8 mm. The mean (μ) length of *P. spp*. for the current study time frame (2001-2016) was 24.2 mm (std. error = 0.036) [Table 7].

Table 7 -- Mean (μ) grass shrimp lengths (+/- σ) and n at CTL

Shrimp @ CTL	Length (mm) (1991-2000)	Length (mm) (2001-2016)	n (2001-2016)
Palaemon spp.	22.8*	24.2 (0.04)	14813
P. pugio		24.3 (0.04)	14699
male		23.1 (0.04)	8640
non-gravid female		24.9 (0.07)	4677
gravid female		29.4 (0.11)	1382
P. vulgaris		19.7 (0.35)	114
male		19.4 (0.49)	39
non-gravid female		19.9 (0.48)	75
gravid female		--	0
			* Leight et al. 2005

Next, we tested the null hypothesis that mean *Palaemon spp*. length (24.2 mm) for 2001-2016 at the CTL site was the same as the mean length (22.8 mm) for 1991-2000. The results of that one-sample T-test ($p<0.0001$) indicated that the mean length was significantly lower for 1991-2000 versus 2001-2016.

Table 6 and Table 7 show the mean shrimp lengths broken down by species and by sex for the current study. It is important to note that this is an aggregation of data over the entire 15-year sampling period 'at the time of sampling.' Meaning, while the data shows the breakdown of females (gravids versus non-gravids), it assumed that they are all non-gravid at some point in their life-cycle. *Palaemon vulgaris* was found at densities two orders of magnitude lower than that of *P. pugio*. A third estuarine member of the genus more common to Florida, *P. mundusnovus*, was not found during the present study. Approximately 60% of all *P. pugio* were males

(60.2% for NIOL, 58.8% for CTL) and 40% were females (39.7% for NIOL, 41.2% for CTL). For *P. vulgaris,* the ratio between male and female was nearly 50/50 at NIOL. The CTL site appears to have altered *P. vulgaris* sex ratios in comparison to NIOL. Data from 2001-2016 indicated that P. vulgaris population was 34% male and 66% females.

5.3.2 *P. pugio* lengths (comparing years, sites, and sex)

To determine if mean lengths of *P. pugio* differed from year to year at each site, a nested ANOVA was performed. There was no significant difference in grass shrimp lengths between years for NIOL (p=0.88). Likewise, a similar result was found for CTL (p=0.99) [Figure 13].
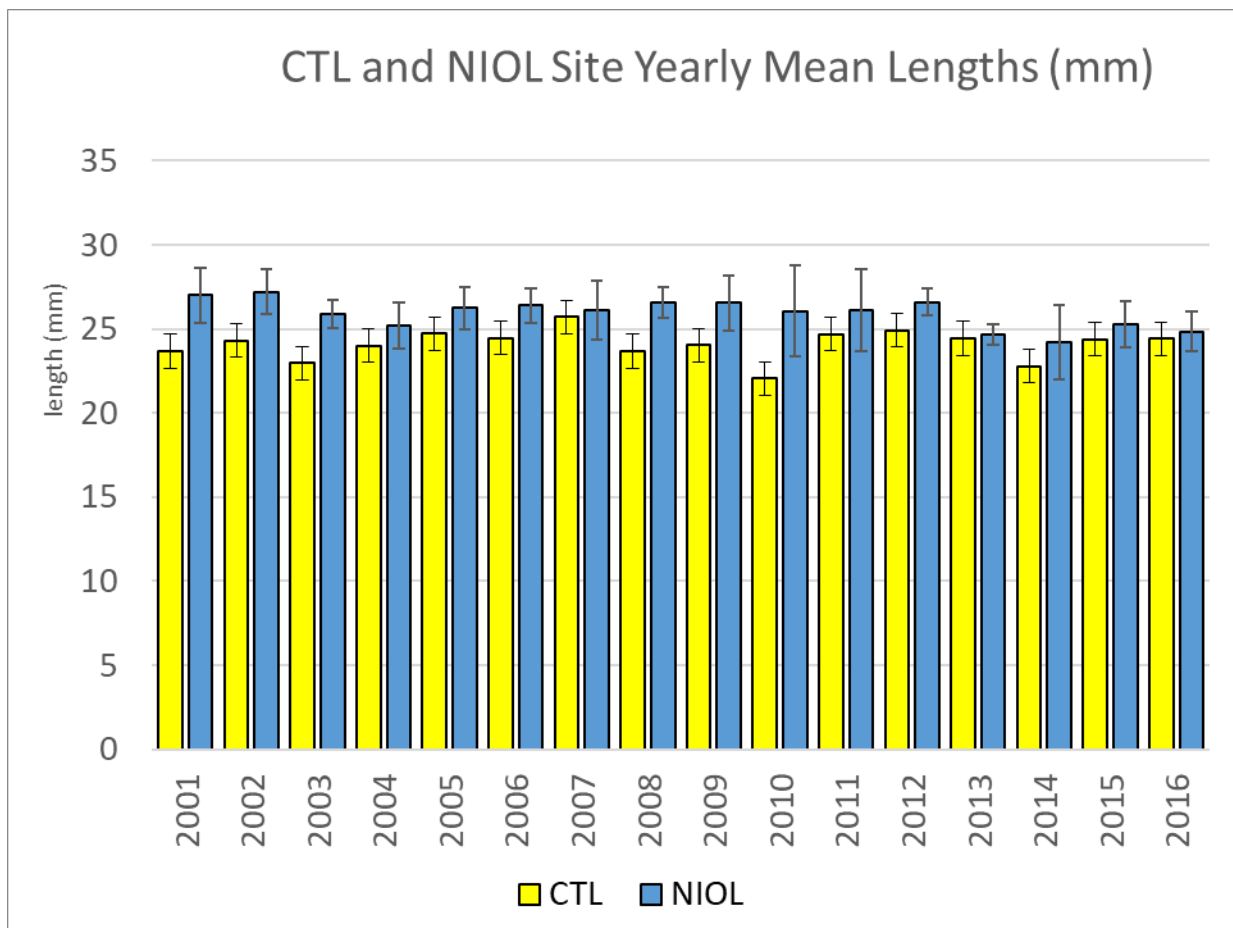
Figure 13 -- Mean P. pugio lengths for NIOL & CTL -- 2001-2016

Overall, *P. pugio* from the CTL site were found to have a significantly shorter mean lengths (p<0.0001) for 2001-2016 (24.3 mm) than shrimp from the NIOL site

(26.3 mm) during the same time period (Figure 14). This difference is apparent in Figure 13 looking year to year. Leight et al. (2005) also showed that CTL site *P. pugio* were shorter (22.8 mm) than shrimp from NIOL (24.3 mm) for the time period 1991-2000.

Figure 14 -- Mean P. pugio length CTL vs. NIOL --   2001-2016

Next, we compared *P. pugio* lengths at the two sites taking into account the sex/type of the shrimp using a Two-Way nested ANOVA design with month nested within year. Site (CTL, NIOL) was one factor in the model and sex/type (Non-gravid Female, Gravid Female, Male) was the other. The overall model was found to be significant (p<0.0001) and *P. pugio* lengths were significantly different for site (p<0.0001) and for sex/type (p<0.0001). However, there was not a significant interaction between site and sex/type (p=0.6181) which indicated that main effect tests were sufficient to describe the model. Multiple comparisons were performed using the Tukey-Kramer adjustment.

At the NIOL site, *P. pugio* lengths (Figure 15) for Non-gravid Females, Gravid Females, and Males were found to be significantly different from each other (p<0.0001). The same outcome was found at the CTL site (Figure 15). When comparing *P. pugio* lengths across sites (Figure 15), Females at the CTL site were significantly smaller than Females from NIOL (p=0.0009). Likewise, Males were

significantly smaller at the CTL site versus the NIOL site (p=0.0008). While the trend was the same, Gravid Female lengths were not significantly different between the two sites (p=0.6817).

Figure 15 -- Mean P. pugio lengths by sex/type at each site.
The color letters above the correspondingly colored bars indicate the results of the Tukey-Kramer test. Within each site, all three sex/types were significantly different from each other. The p-values below the x-axis labels indicate the results of Tukey-Kramer comparisons for that sex/type between sites. Only Gravid females were not significantly different in terms of length between sites.

5.3.3 Grass shrimp weights (mg)

Unlike the length data which largely followed the normal distribution, the weight data had a reasonably good fit to the lognormal. Geometric means are more appropriate with lognormal distributions and are reported along with the arithmetic means for weight in Table 8 and Table 9 for side-by-side comparison. Because of

this, statistical comparisons with Leight et al. 2005 were not possible since that study only reported arithmetic means. Leight et al. 2005 reported a mean weight for *Palaemon* spp. of 151.0 mg (determined from Figure 5 of that paper). The mean (μ) weight of *P. spp.* for the current study time frame for NIOL (2001-2016) was 163.1 mg (stderr = 0.72) and the geometric mean was 141.2 mg [Table 8].

Table 8 -- Mean (μ) grass shrimp weight (+/- stderr) and n at NIOL [geometric mean in brackets]

Shrimp @ NIOL	Weight (mg) (1991-2000)	Weight (mg) (2001-2016)	n (2001-2016)
Palaemon spp.	151.0*	163.1 (0.72) [141.2]	16407
P. pugio		163.9 (0.73) [142.1]	16139
male		128.4 (0.47) [119.3]	9728
non-gravid female		176.8 (1.42) [152.1]	4502
gravid female		313.8 (2.35) [294.5]	1909
P. vulgaris		119.7 (4.92) [96.81]	268
male		74.32 (2.99) [67.16]	135
non-gravid female		158.2 (7.38) [134.2]	115
gravid female		213.6 (28.6) [186.8]	18
			* Leight et al. 2005

Leight et al. 2005 reported a mean weight for *P. spp.* at CTL of 133 mm. The mean (μ) weight of *P. spp*. for the current study time frame at CTL (2001-2016) was 130.2 mm (stderr = 0.66) [Table 9].

Table 9 -- Mean (μ) grass shrimp weight (+/- stderr) and n at CTL [geometric mean in brackets]

Shrimp @ CTL	Weight (1991-2000)	Weight (2001-2016)	n (2001-2016)
Palaemon spp.	132.6 mg*	130.2 mg (0.66) [110.5]	14814
P. pugio		130.6 mg (0.66) [110.9]	14700
male		103.7 mg (0.48) [94.3]	8641
non-gravid female		141.8 mg (1.20) [119.1]	4677
gravid female		260.6 mg (2.81) [239.1]	1382
P. vulgaris		81.5 mg (4.55) [69.22]	114
male		67.77 mg (5.32) [60.37]	39
non-gravid female		88.64 mg (6.20) [74.33]	75
gravid female		--	0
			* Leight et al. 2005

5.3.4 *P. pugio* weights (comparing years, sites, and sex)

To determine if mean weights of *P. pugio* differed from year to year at each site, a nested ANOVA was performed. Since the weight data followed a lognormal distribution, weights were log transformed [log(x+1)] to fit the data to the normal distribution. There was no significant difference in grass shrimp lengths between years for NIOL (p=0.96). Likewise, a similar result was found for CTL (p=0.44) [Figure 16].
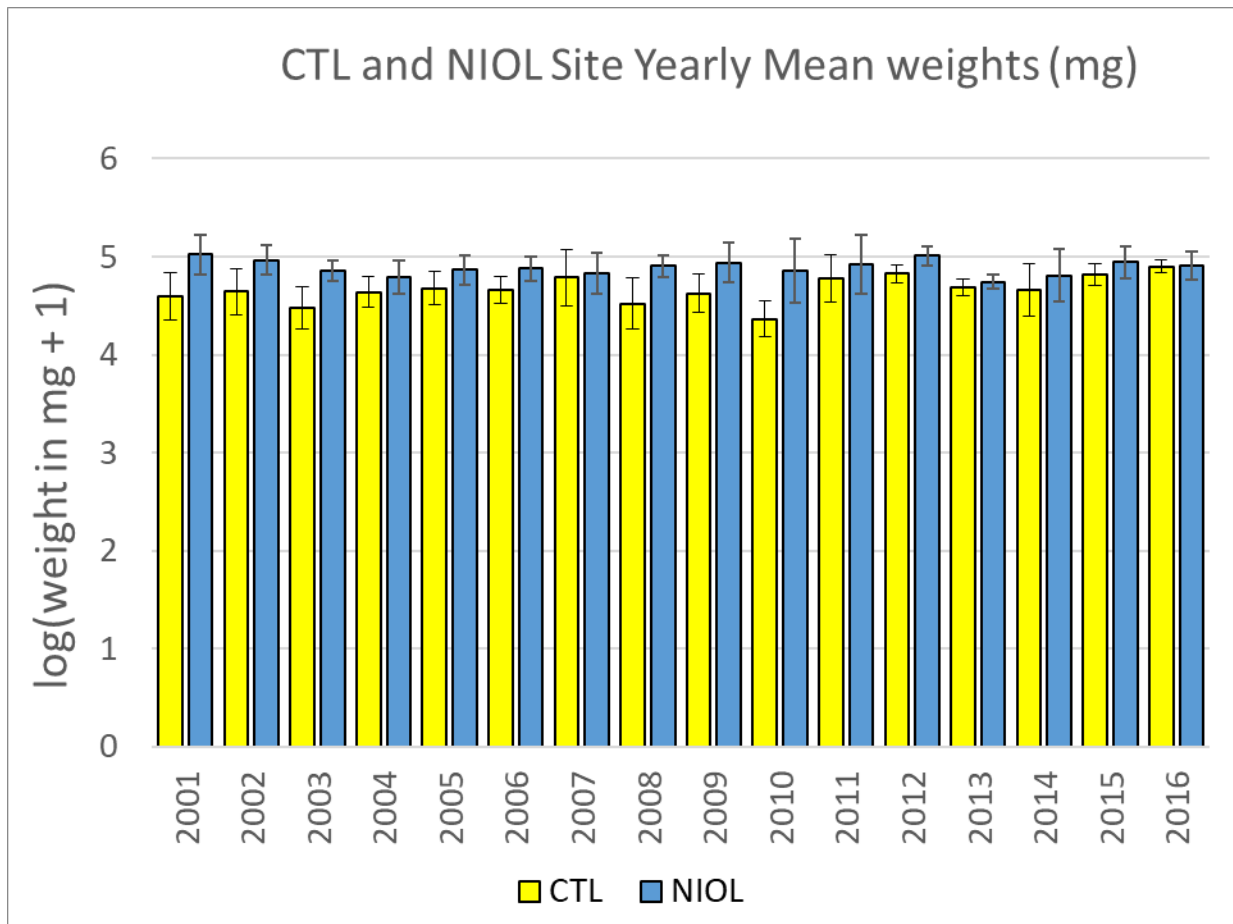
*Figure 16 -- Mean grass shrimp weights for NIOL & CTL -- 2001-2016*

Similar to the length data, *P. pugio* from the CTL site were found to have a significantly lower mean weight (p<0.0001) for 2001-2016 (130.6 mg) than shrimp from the NIOL site (163.9 mg) during the same time period (Figure 17). This difference is also apparent in Figure 16 looking year to year. Leight et al. (2005) also showed that CTL site *P. pugio* had lower weights (132.6 mg) than shrimp from NIOL (151.0 mg) for the time period 1991-2000.
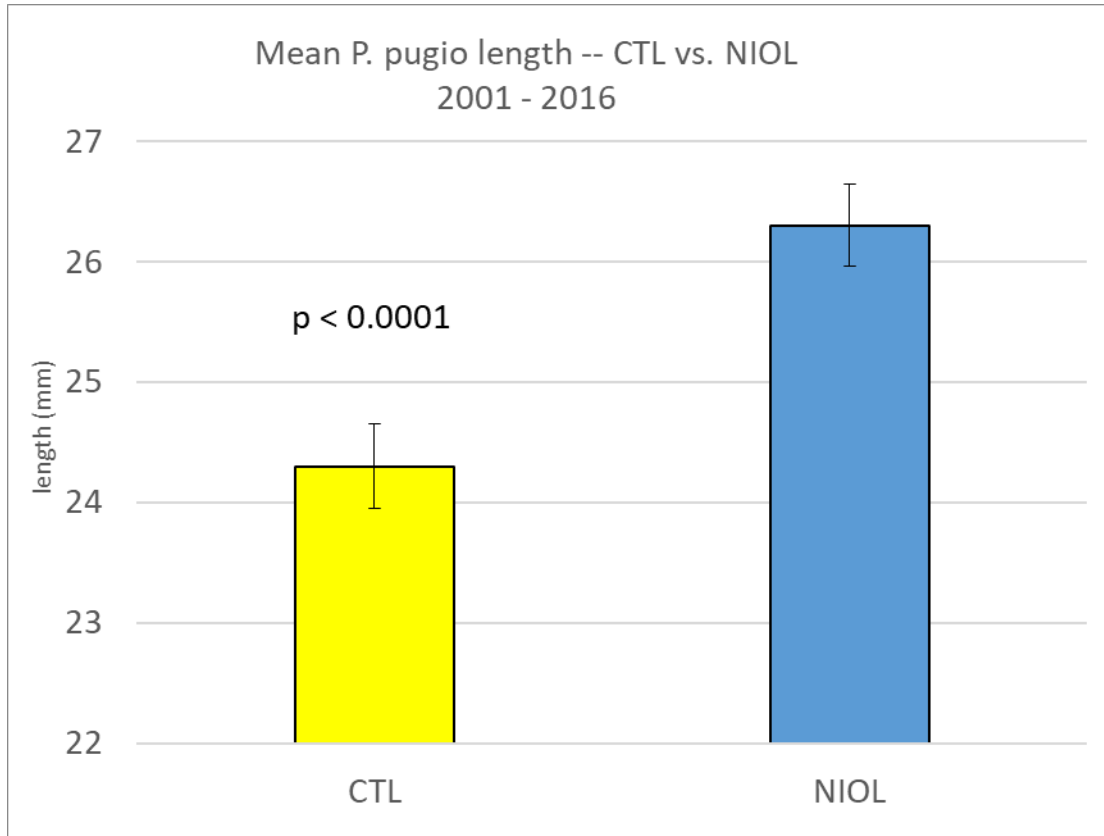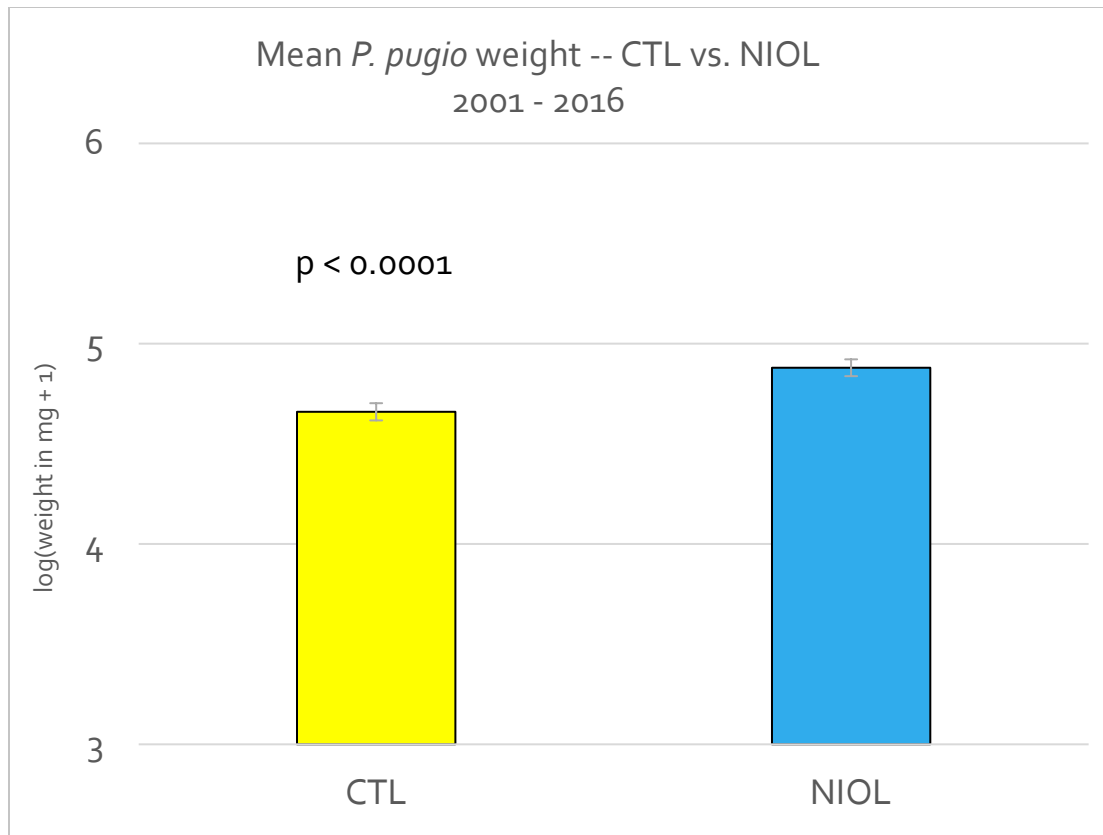
Figure 17 -- Mean P. pugio weight CTL vs. NIOL --   2001-2016

Palaemon pugio weights at the two sites taking into account the sex/type of the shrimp were compared using a Two-Way nested ANOVA design with month nested within year. Site (CTL, NIOL) was one factor in the model and sex/type (Non-gravid Female, Gravid Female, Male) was the other. Again, the data were log transformed [log(x+1)] since the weight data followed the lognormal distribution. The overall model was found to be significant (p<0.0001), with *P. pugio* weights significantly different for site (p<0.0001) and for sex/type (p<0.0001). Again, like the length data there was not a significant interaction between site and sex/type (p=0.5248) which indicated that main effect tests were sufficient to describe the model. Multiple comparisons were performed using the Tukey-Kramer adjustment.

At the NIOL site, *P. pugio* weights (Figure 18) for Non-gravid Females, Gravid Females, and Males were found to be significantly different from each other (p<0.0001). The same outcome was found at the CTL site (Figure 18). When comparing *P. pugio* weights across sites (Figure 18), non-gravid females at the CTL site were significantly smaller than non-gravid females from NIOL (p=0.0019). Likewise, males were significantly smaller at the CTL site versus the NIOL site (p=0.0016). While the trend was the same, gravid female weights were not significantly different between the two sites (p=0.87).
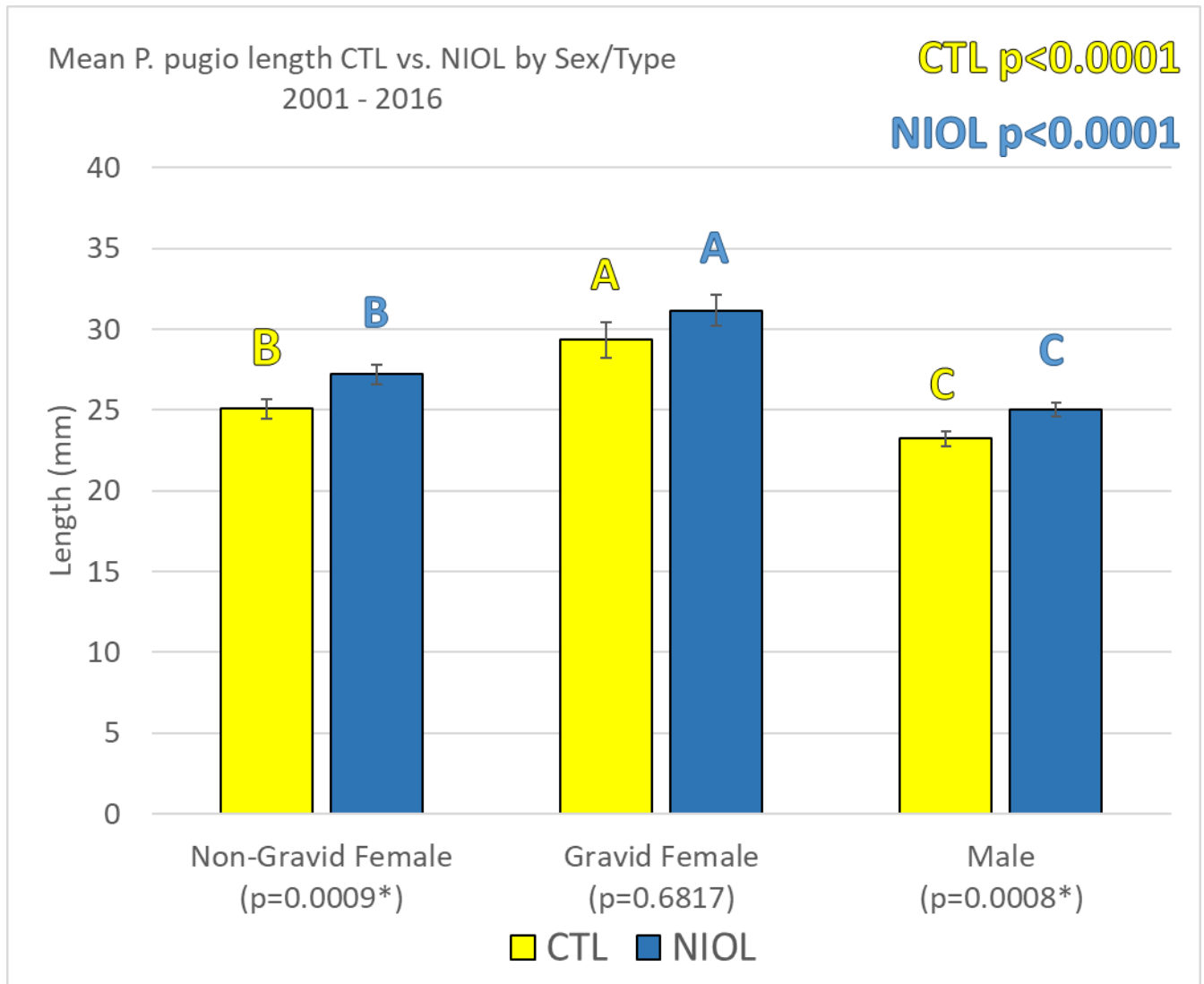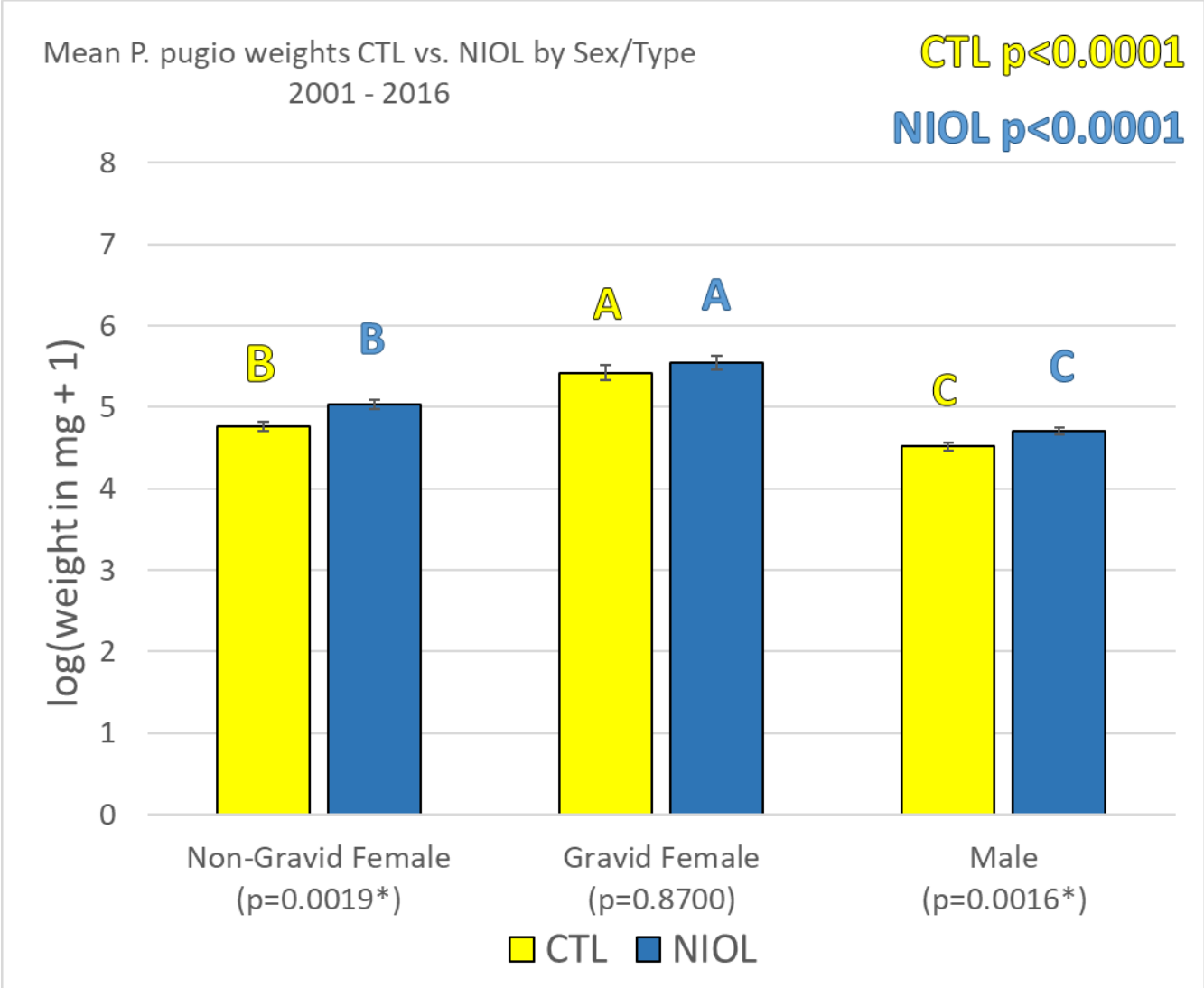
Figure 18 -- Mean P. pugio weights by sex/type at each site.
The color letters above the correspondingly colored bars indicate the results of the Tukey-Kramer test.
Within each site, all three sex/types were significantly different from each other. The p-values below
the x-axis labels indicate the results of Tukey-Kramer comparisons for that sex/type between sites.
Only Gravid females were not significantly different in terms of weight between sites.

5.4 Sex Ratios

5.4.1 Male:Female sex ratios

Leight et al. 2005 reported an overall *P. spp*. sex ratio of approximately 2:1 [male:female] for NIOL. The exact value for Leight et al. 2005 was 2.3:1 (69.4% male and 30.6 % female). Table 10 shows the *P. spp.* sex counts and ratios for this study (2001-2016). The sex ratio for all years of this study combined at NIOL was 1.5:1 [male:female].

Table 10 -- P. spp. sex counts, percentages, and ratios at NIOL

Year	NIOL					CTL				
	Male		Female		ratio (male : female)	Male		Female		ratio (male : female)
	Count	(%)	Count	(%)		Count	(%)	Count	(%)	
2001	520	(67.1)	255	(32.9)	2.039	298	(52.2)	273	(47.8)	1.092
2002	474	(70.0)	203	(30.0)	2.335	337	(49.7)	341	(50.3)	0.988
2003	554	(72.9)	206	(27.1)	2.689	415	(64.7)	226	(35.3)	1.836
2004	456	(63.2)	266	(36.8)	1.714	298	(54.6)	248	(45.4)	1.202
2005	358	(62.6)	214	(37.4)	1.673	305	(59.6)	207	(40.4)	1.473
2006	452	(56.1)	353	(43.9)	1.280	370	(53.4)	323	(46.6)	1.146
2007	365	(52.1)	336	(47.9)	1.086	440	(58.9)	307	(41.1)	1.433
2008	469	(54.7)	388	(45.3)	1.209	556	(58.4)	396	(41.6)	1.404
2009	780	(60.1)	517	(39.9)	1.509	438	(47.0)	494	(53.0)	0.887
2010	593	(57.3)	442	(42.7)	1.342	645	(66.8)	320	(33.2)	2.016
2011	722	(60.2)	477	(39.8)	1.514	564	(54.7)	468	(45.3)	1.205
2012	875	(65.1)	469	(34.9)	1.866	747	(59.2)	515	(40.8)	1.450
2013	902	(56.0)	709	(44.0)	1.272	703	(56.7)	536	(43.3)	1.312
2014	807	(59.9)	540	(40.1)	1.494	774	(66.3)	393	(33.7)	1.969
2015	774	(58.3)	554	(41.7)	1.397	712	(58.8)	499	(41.2)	1.427
2016	762	(55.3)	615	(44.7)	1.397	1077	(64.8)	586	(35.2)	1.838
ALL YEARS	9863	(60.1)	6544	(39.9)	1.507	8679	(58.6)	6132	(41.4)	1.415

Data presented in Leight et al. 2005 indicated an overall *P. spp*. sex ratio of approximately 1.5:1 [male:female] for CTL (59.7% male and 40.3% female), suggesting that this is a stable population with sufficient numbers of females to maintain population density (Hamilton, 1967).   Table 10 also shows the *P. spp.* sex counts and ratios for this study (2001-2016) for CTL. The sex ratio for all years of this study combined at CTL was 1.4:1 [male:female].

5.4.2 Sex ratio (chi-square) comparison with 1991-2000 vs. 2001-2015

Using the percentages for males and females (from Leight et al. 2005) as test percentages, we tested if the current percentages (2001-2016) were significantly different using a Chi-Square Goodness of Fit Test for specified proportions. (Null H0: The male and female percentages were not significantly different for the two data sets).

The percentages of males and females at NIOL (60.1 and 39.1, respectively) for 2001-2016 were found to be significantly different [(p< 0.0001), Table 11] than the percentages for the previous study (Leight et al. 2005).

Table 11 -- Overall sex frequency data for NIOL

Sex	Frequency 2001-2016	Percent 2001-2016	Test Percent* 1991-2000
Female	6544	39.89	30.60
Male	9863	60.11	69.40

* From Leight et al. 2005

The percentages of males and females at CTL (58.6 and 41.4, respectively) for 2001-2016 were found to be significantly different by Chi-square goodness of fit test [(p= 0.0063), Table 12] than the percentages for the previous study (Leight et al. 2005).

Table 12 – Overall Sex Frequency data for CTL

Sex	Frequency 2001-2016	Percent 2001-2016	Test Percent* 1991-2000
Female	6132	41.40	40.30
Male	8679	58.60	59.70

* From Leight et al. 2005

5.4.3 Sex/type percentages

Leight et al. (2005) reported overall sex type percentages for all years 1991-2000 for *P. spp* for NIOL. In that study, males constituted 69.4 % of the total. Females (non-gravid) were 25.5 % of the total. Gravid females were 5.1 % of the total. In the current study for all years [2001-2016], the overall percentages [Table 13]

were as follows: males were 60.3%, females (non-gravid) were 28.2% and gravid females were 11.8%.

For the CTL site, Leight et al. (2005) reported that males constituted 59.7 %, non-gravid females constituted 36.0 % and gravid females constituted 4.3 % during the time period of 1991-2000. In the current study for all years [2001-2016], the overall percentages [Table 14] were as follows: males were 58.6%, females (non-gravid) were 32.1% and gravid females were 9.3%.

Table 13 -- P. spp. sex/type group counts and percentages at NIOL

YEAR	Male		Non-gravid Female		Gravid Female		TOTAL
	Count	(%)	Count	(%)	Count	(%)	
2001	520	(67.1)	169	(21.8)	86	(11.1)	775
2002	474	(70.0)	127	(18.8)	76	(11.2)	677
2003	554	(72.9)	150	(19.7)	56	(7.4)	760
2004	456	(63.2)	218	(30.2)	48	(6.6)	722
2005	358	(62.6)	157	(27.4)	57	(10.0)	572
2006	452	(56.1)	215	(26.7)	138	(17.1)	805
2007	365	(52.1)	248	(35.4)	88	(12.6)	701
2008	469	(54.7)	317	(37.0)	71	(8.3)	857
2009	780	(60.1)	405	(31.2)	112	(8.6)	1297
2010	593	(57.3)	289	(27.9)	153	(14.8)	1035
2011	722	(60.2)	364	(30.4)	113	(9.4)	1199
2012	875	(65.1)	330	(24.6)	139	(10.3)	1344
2013	902	(56.0)	529	(32.8)	180	(11.2)	1611
2014	807	(59.9)	369	(27.4)	171	(12.7)	1347
2015	774	(58.3)	359	(27.0)	195	(14.7)	1328
2016	762	(55.3)	371	(27.0)	244	(14.7)	1328
ALL YEARS	9863	(60.1)	4617	(28.1)	1927	(11.7)	16358

Table 14 -- P. spp. sex group counts and percentages at CTL

YEAR	Male		Non-gravid Female		Gravid Female		TOTAL
	Count	(%)	Count	(%)	Count	(%)	
2001	298	(52.2)	258	(45.2)	15	(2.6)	571
2002	337	(49.7)	258	(38.1)	83	(12.2)	678
2003	415	(64.7)	190	(29.6)	36	(5.6)	641
2004	298	(54.6)	215	(39.4)	33	(6.0)	546
2005	305	(59.6)	175	(34.2)	32	(6.3)	512
2006	370	(53.4)	244	(35.2)	79	(11.4)	693
2007	440	(58.9)	195	(26.1)	112	(15.0)	747
2008	556	(58.4)	308	(32.4)	88	(9.2)	952
2009	438	(47.0)	399	(42.8)	95	(10.2)	932
2010	645	(66.8)	257	(26.6)	63	(6.5)	965
2011	564	(54.7)	352	(34.1)	116	(11.2)	1032
2012	747	(59.2)	379	(30.0)	136	(10.8)	1262
2013	703	(56.7)	392	(31.6)	144	(11.6)	1239

2014	774	(66.3)	294	(25.2)	99	(8.5)	1167
2015	712	(58.8)	399	(32.9)	100	(8.3)	1211
2016	1077	(64.8)	436	(26.2)	150	(9.0)	1663
ALL YEARS	8679	(58.6)	4751	(32.1)	1381	(9.3)	14811

5.4.4 Sex/type group (Chi-square) comparison 1991-2000 vs. 2001-2015

Using the percentages for sex/type groups reported at both NIOL and CTL (from Leight et al. 2005) as test percentages, we tested if the current percentages at each reference site (2001-2016) were significantly different using a Chi-Square Goodness of Fit Test for specified proportions. (Null H0: The sex/type groups percentages were not significantly different for the two data sets).

For NIOL, the percentages of sex/type groups [males (60.11), non-gravid females (28.14) and gravid females (11.74)] for 2001-2016 were found to be significantly different (p< 0.0001, Table 15) than the percentages for the previous study (Leight et al. 2005). The percentage of males was significantly less during the present study than the previous study. The percentages of both female types were significantly greater during the present study.

Table 15 -- Overall Sex/type Frequency data for NIOL

Sex	Frequency 2001-2016	Percent 2001-2016	Test Percent* 1991-2000
Female (non-gravid)	4617	28.14	25.50
Gravid Female	1927	11.74	5.10
Male	9863	60.11	69.40

* From Leight et al. 2005

For CTL, the percentages of sex/type groups [males (58.60), non-gravid females (32.08), and gravid females (9.32)] for 2001-2016 were found to be significantly different [p<0.0001], Table 16] than the percentages for the previous study (Leight et al. 2005). This result for CTL was similar to that of NIOL, although the differences were not as great. The percentage of males was less in the present study when compared to the previous study. Likewise, the percentages of females were greater in the present study.

Table 16 -- Overall Sex/type Frequency data for CTL

Sex	Frequency 2001-2016	Percent 2001-2016	Test Percent* 1991-2000
Female (non-gravid)	4751	32.08	36.00
Gravid Female	1381	9.32	4.30
Male	8679	58.60	59.70

Because we observed a difference in the percentages of sex/type groups between the two studies, we further examined the 2001-2016 data to look at yearly trends. From year to year (Figure 19), the percentages are variable, but there is a trend towards fewer males and more females over time at NIOL. Also quite variable, the CTL data showed somewhat the opposite trend (Figure 20). Percentages of males show a slight increase overtime whereas females showed a slight decrease.
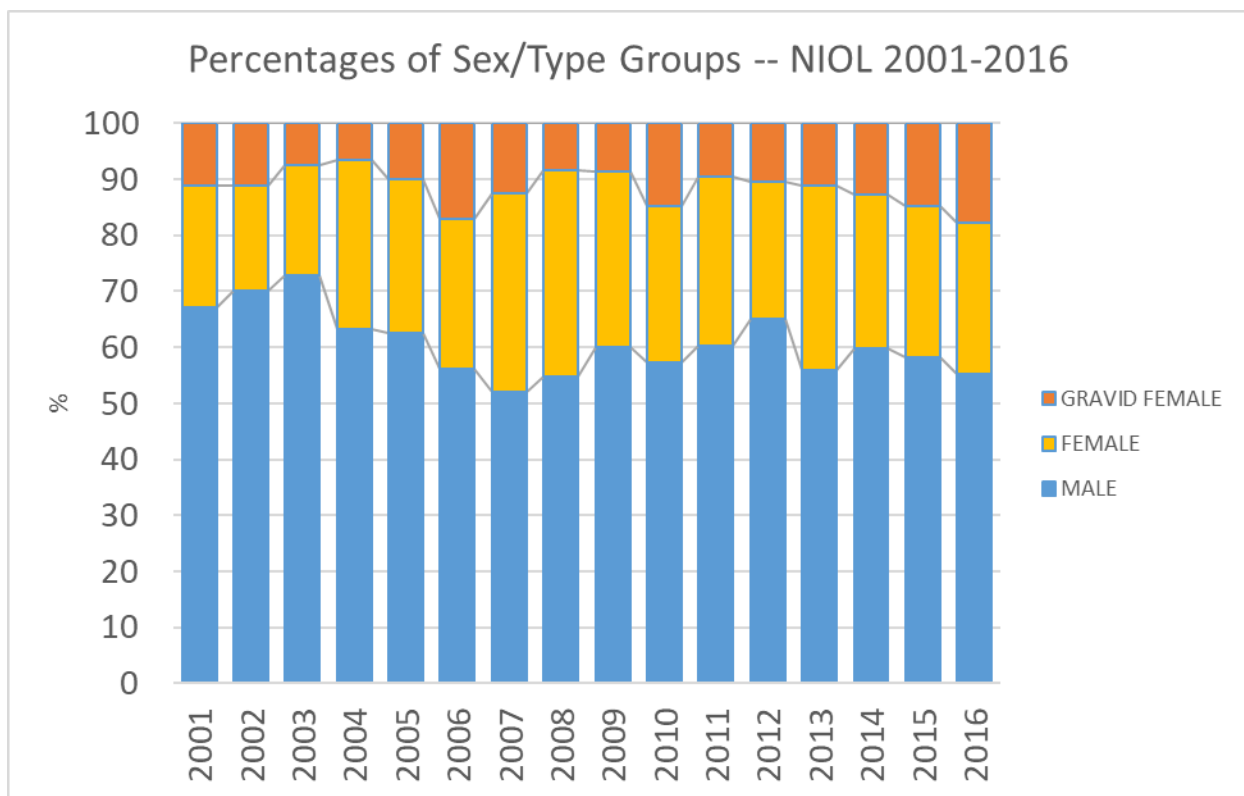
Figure 19 -- Percentages of Sex Groups -- NIOL 2001-2016
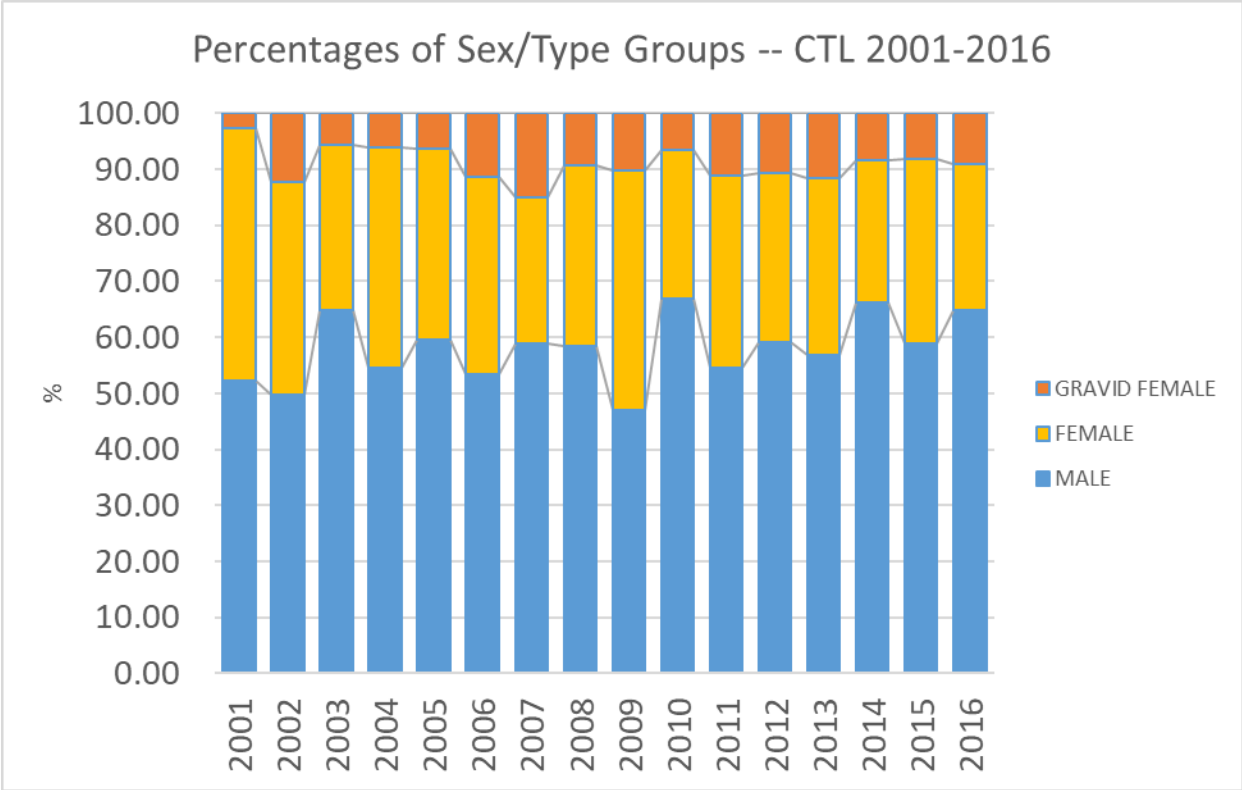
Figure 20 -- Percentages of Sex/Type Groups -- CTL 2001-2016

It is also important to note that there is a distinct seasonality to sex/type groups. At both sites, gravid females are only present during the months April – October (Figure 21 and Figure 22). When we average the data across all years of the study (2001-2016), we can also observe two distinct peaks in the percentages of gravid females. Those peaks typically and predictably occur in May and Aug/Sept of each year.
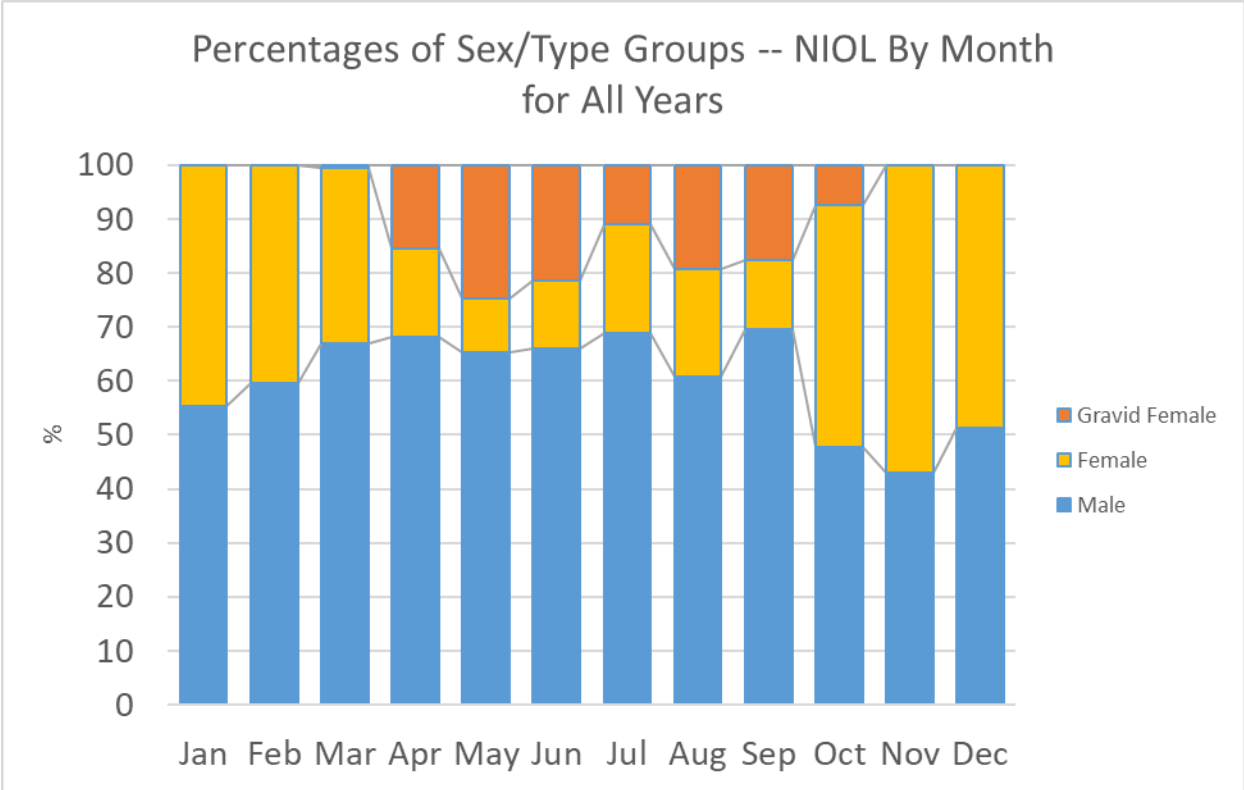
Figure 21 -- Percentages of Sex/Type Groups by Month at NIOL averaged across all years (2001-2016)
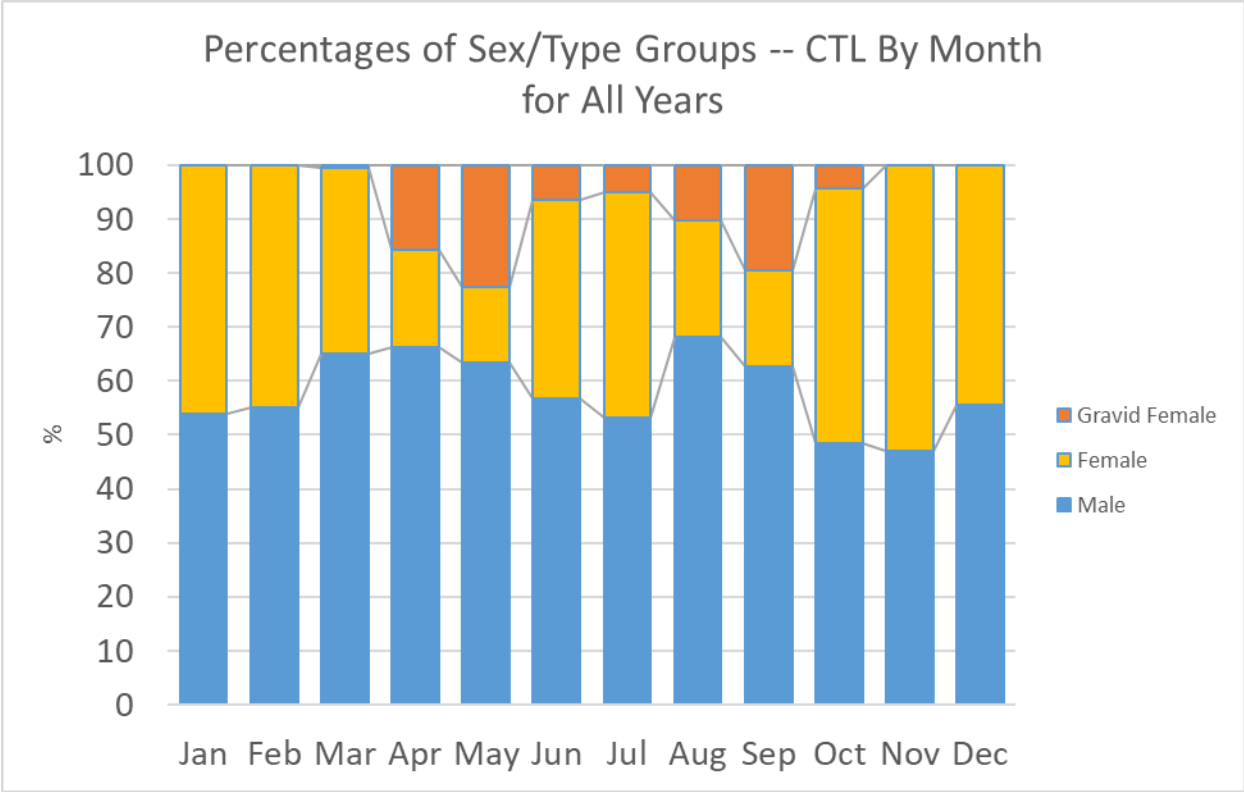
Figure 22 -- Percentages of Sex/Type Groups by Month at CTL averaged across all years (2001-2016)

5.5 EGG COUNTS

5.5.1 Comparison to previous study (1991-2000) for eggs per gravid female

The mean number of eggs per gravid female from the time period of 1991-2000 [Leight et al. (2005), Figure 6] was about 170 for NIOL.

A t-test goodness of fit test was performed to test the hypothesis that the mean eggs per gravid female for 2001-2016 was 170 at NIOL. The mean (μ) number of eggs per gravid female was 186.1 (σ = 70.6775, std. error = 1.62). The mean number of eggs for the present study was found to be significantly greater (p < 0.0001, Table 17) than the previous study.

Table 17 -- t-test goodness of fit to the 1991-2000 mean (170 eggs/GF) at NIOL

N	Mean	Std Dev	Std Err	Minimum	Maximum
1909	186.1	70.6775	1.6176	28	480

Mean 2001-2016	95% CL Lower	95% CL Upper	Std Dev
186.1	183.0	189.3	70.6775

H0: μ (2001-2016) = μ (1991-2000)

DF	t-Value	p Value		Mean 1991-2000
1908	9.98	<0.0001		170 eggs/gravid female

In like manner, a t-test comparison was made for the CTL site between the mean number of eggs (172) for 1991-2000 (Leight et al., 2005) and the present study (2001-2016). The mean (μ) number of eggs per gravid female was 162.3 (σ = 74.6520, std. error = 2.01). The mean number of eggs for the present study was found to be significantly lower (p < 0.0001, Table 18) than the previous study.

Table 18 -- t-test goodness of fit to the 1991-2000 mean (172 eggs/GF) at CTL

N	Mean	Std Dev	Std Err	Minimum	Maximum
1382	162.3	74.6520	2.01	7	472

Mean 2001-2016	95% CL Lower	95% CL Upper	Std Dev
162.3	158.3	166.2	74.6520

H0: μ (2001-2016) = μ (1991-2000)

DF	t-Value	p Value		Mean 1991-2000
1381	-4.84	<0.0001		172 eggs/gravid female

5.5.2 Eggs counts – comparison between years

An ANOVA with subsampling was used to detect difference between years. No significant difference was found between years (p = 0.88 and p = 0.97) for NIOL [Figure 23] and for CTL [Figure 24].
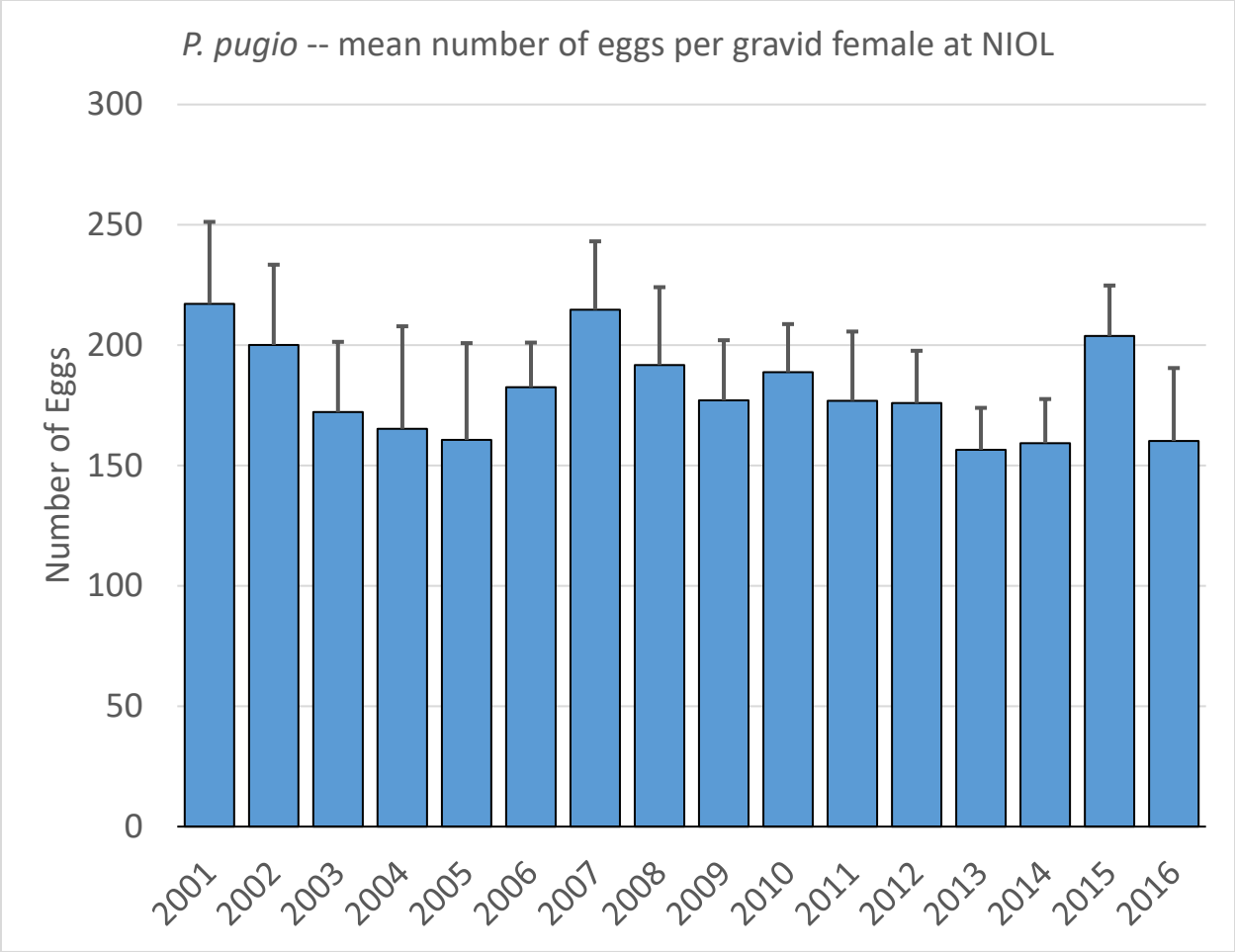
Figure 23 -- Mean egg counts by year at NIOL
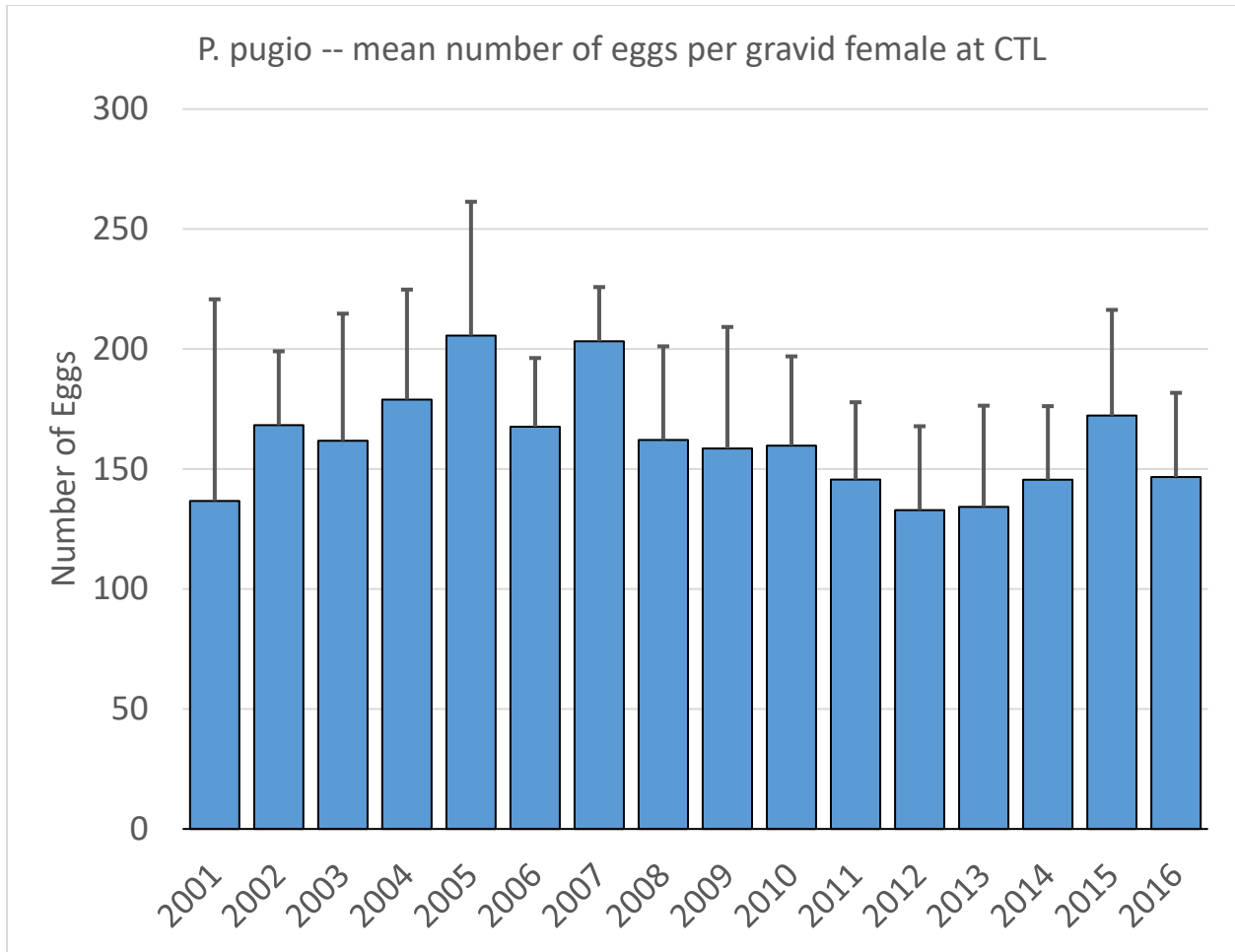
Figure 24 -- Mean counts by year at CTL Site

5.6 ECTOPARASITES – BOPYRIDS ON GRASS SHRIMP

Leight et al. 2005 reported that the incidence of a bopyrid isopod ectoparasite on P. pugio was below 3 percent. Data from this study agrees with that result. Over the course of the 15-year study at NIOL, an estimated total of 57,123 *P. pugio* were collected and 1005 (1.73%) of them were parasitized with the Isopod, *Probopyrus pandalicola*. The annual percent incidence of *P. pandalicola* parasitized to *P. pugio* ranged from 0.75 % (2002) to 5.48 % in 2003 [Table 19].

Table 19 -- Estimated frequencies of the Bopyrid parasite on P.pugio at NIOL and CTL

Year	NIOL			CTL		
	P. pugio without parasite (%)	*P. pugio* with parasite (%)	Total	*P. pugio* without parasite (%)	*P. pugio* with parasite (%)	Total
2001	2331 (98.3)	41 (1.7)	2372	1407 (98.1)	27 (1.9)	1434
2002	2391 (99.3)	18 (0.7)	2409	1897 (97.5)	50 (2.5)	1947
2003	4098 (94.5)	238 (5.5)	4336	1736 (96.6)	60 (3.4)	1797
2004	3507 (97.8)	80 (2.2)	3588	1561 (96.2)	61 (3.8)	1622
2005	2105 (98.4)	34 (1.6)	2139	1586 (95.6)	74 (4.4)	1660
2006	3782 (98.4)	62 (1.6)	3844	1984 (99.2)	16 (0.8)	2000
2007	2118 (98.0)	43 (1.9)	2161	3347 (95.7)	151 (4.3)	3498
2008	2863 (98.7)	39 (1.3)	2903	3356 (95.8)	149 (4.2)	3504
2009	3506 (98.5)	54 (1.5)	3560	1399 (97.9)	30 (2.1)	1429
2010	4087 (98.8)	50 (1.2)	4137	2475 (97.2)	72 2.8)	2547
2011	2914 (99.1)	27 (0.9)	2940	2229 (98.8)	28 (1.2)	2257
2012	4196 (98.7)	55 (1.3)	4251	6967 (99.3)	52 (0.7)	7019
2013	5288 (99.0)	56 (1.0)	5344	5066 (97.6)	124 (2.4)	5190
2014	4631 (98.1)	89 (1.9)	4720	3306 (97.5)	85 (2.5)	3390
2015	4924 (99.0)	50 (1.0)	4975	5431 (98.6)	78 (1.4)	5509
2016	4382 (98.4)	70 (1.6)	4452	6498 (98.8)	77 (1.5)	6575
Total	57123 (98.3)	1005 (1.7)	58129	50243 (97.8)	1136 (2.2)	51379

At CTL, an estimated total of 50,243 *P. pugio* were collected and 1136 (2.21%) of them were parasitized with the isopod, *Probopyrus pandalicola*. The annual percent incidence of *P. pandalicola* parasitized to *P. pugio* ranged from 0.74 % (2012) to 4.44 % in 2005 [Table 19].

5.7 MULTIVARIATE STATISTICAL ANALYSIS – PARTIAL LEAST SQUARES REGRESSION

We examined the relationships between *P. pugio* biomonitoring variables and water quality parameters. As stated in the methods section, a partial least squares (PLS) regression was used to determine statistically significant associations between *P. pugio* densities, lengths, and weights [Table 1] and water quality input variables, season, and site [Table 2]. We used a subset of the data from July of 2007 until December of 2016 so that we had a complete record of water quality information. We do not have continuous water quality data for the CTL site prior to July 2007.

The PLS procedure found that four factors contributed significantly to the model [Table 20]. This was determined from the lowest PRESS statistic and having a significant T^2 statistic (when p >/= 0.1). The first four factors (0-3) in the table are significant.

Table 20 -- Cross validation for the number of extracted factors

Extracted Factors	Root Mean PRESS	T^2	p-value
0	1.010526	37.30706	**<.0001**
1	0.905731	29.67369	**<.0001**
2	0.871312	27.96543	**0.003**
3	**0.819971**	19.94614	**0.1**
4	0.823209	14.40888	0.437
5	0.827999	15.12721	0.374
6	0.823074	21.72482	0.055
7	0.822233	14.17909	0.411
8	0.818419	0	1
9	0.822795	24.5418	0.01
10	0.822398	25.26051	0.006
11	0.844862	24.07276	0.014
12	0.843065	19.66597	0.097
13	0.847154	20.32074	0.085
14	0.85712	20.07648	0.081
15	0.868835	23.40747	0.025

The percent of variation of predictor (X) and response (Y) variables accounted for by PLS factors is displayed in Table 21 and Table 22, respectively. These values are essentially R-Squared values expressed as percentages. In those tables, the 'Current' value represents the partial R-Squared of that factor to the model.

Table 21 -- Percent Variation (R-Squared Values as %) of Predictor Variables Accounted for by Partial Least Squares Factors.

Predictor Variables	Factor 1		Factor 2		Factor 3		Factor 4	
	Current	(Total)	Current	(Total)	Current	(Total)	Current	(Total)
temp_24h	54.52	(54.52)	33.74	(88.26)	0.20	(88.47)	1.23	(89.69)
temp_48h	56.62	(56.62)	32.94	(89.57)	0.43	(90.00)	0.61	(90.60)
temp_96h	57.87	(57.87)	30.87	(88.73)	1.22	(89.95)	0.37	(90.32)
temp_7d	56.20	(56.20)	29.61	(85.81)	1.81	(87.62)	0.30	(87.92)
temp_14d	59.17	(59.17)	25.52	(84.69)	4.31	(88.99)	0.05	(89.05)
DO_per_24h	68.34	(68.34)	3.20	(71.54)	0.11	(71.65)	1.20	(72.86)
DO_per_48h	75.19	(75.19)	2.81	(78.00)	0.03	(78.03)	0.33	(78.36)
DO_per_96h	83.20	(83.20)	2.71	(85.91)	0.03	(85.94)	0.08	(86.02)
DO_per_7d	84.36	(84.36)	1.98	(86.34)	0.22	(86.57)	0.43	(87.00)
DO_per_14d	79.69	(79.69)	1.37	(81.06)	1.14	(82.20)	0.18	(82.38)
DO_24h	68.17	(68.17)	19.88	(88.06)	0.98	(89.03)	0.16	(89.20)
DO_48h	72.59	(72.59)	19.20	(91.79)	0.86	(92.65)	0.03	(92.68)
DO_96h	76.04	(76.04)	18.59	(94.64)	1.24	(95.87)	0.17	(96.04)
DO_7d	75.76	(75.76)	17.16	(92.91)	1.92	(94.83)	0.49	(95.33)
DO_14d	73.40	(73.40)	15.72	(89.12)	3.62	(92.75)	0.01	(92.75)
pH_24h	66.91	(66.91)	3.13	(70.04)	5.25	(75.29)	0.03	(75.32)
pH_48h	68.38	(68.38)	4.20	(72.58)	5.46	(78.04)	0.69	(78.73)
pH_96h	66.99	(66.99)	5.64	(72.63)	6.02	(78.65)	2.51	(81.16)
pH_7d	65.64	(65.64)	5.36	(70.99)	4.26	(75.25)	4.09	(79.34)
pH_14d	63.52	(63.52)	4.94	(68.46)	2.22	(70.67)	1.50	(72.17)
sal_24h	10.84	(10.84)	59.45	(70.29)	17.18	(87.47)	0.50	(87.97)
sal_48h	10.67	(10.67)	61.07	(71.74)	18.25	(89.99)	0.00	(90.00)
sal_96h	9.71	(9.71)	60.77	(70.48)	19.57	(90.05)	0.11	(90.15)
sal_7d	9.32	(9.32)	61.44	(70.76)	18.36	(89.13)	0.18	(89.31)

sal_14d	8.15	(8.15)	65.16	(73.31)	16.82	(90.13)	0.10	(90.23)
Site CTL	32.07	(32.07)	14.67	(46.74)	10.28	(57.02)	10.80	(67.81)
Site NIOL	32.07	(32.07)	14.67	(46.74)	10.28	(57.02)	10.80	(67.81)
Season Fall	0.11	(0.11)	15.34	(15.45)	33.60	(49.05)	11.57	(60.62)
Season Spring	29.13	(29.13)	2.43	(31.56)	55.50	(87.06)	0.06	(87.12)
Season Summer	35.26	(35.26)	5.63	(40.89)	10.16	(51.05)	9.91	(60.96)
Season Winter	7.56	(7.56)	20.74	(28.30)	1.49	(29.79)	4.83	(34.61)
All Effects	50.24	(50.24)	21.29	(71.53)	8.16	(79.68)	2.04	(81.73)

Table 22 -- Percent variation (R-Squared Values as %) of response variables accounted for by partial least squares factors

Response Variables	Factor 1		Factor 2		Factor 3		Factor 4	
	Current	(Total)	Current	(Total)	Current	(Total)	Current	(Total)
L_NGF	32.98	(32.98)	7.91	(40.89)	9.27	(50.16)	2.27	(52.43)
L_GF	27.38	(27.38)	2.88	(30.26)	15.23	(45.49)	7.37	(52.85)
L_npara	28.45	(28.45)	16.34	(44.79)	11.19	(55.98)	4.61	(60.59)
L_para	27.87	(27.87)	7.30	(35.17)	8.86	(44.03)	0.00	(44.03)
L_M	25.10	(25.10)	18.51	(43.61)	11.82	(55.43)	6.31	(61.74)
W_NGF	30.05	(30.05)	9.20	(39.25)	14.23	(53.48)	2.67	(56.15)
W_GF	31.85	(31.85)	2.79	(34.64)	20.46	(55.09)	7.64	(62.74)
W_npara	26.22	(26.22)	15.99	(42.21)	17.25	(59.46)	4.57	(64.03)
W_para	22.03	(22.03)	8.12	(30.14)	13.17	(43.31)	0.16	(43.47)
W_M	23.97	(23.97)	16.94	(40.91)	15.44	(56.35)	7.41	(63.76)
den_M	7.78	(7.78)	1.29	(9.07)	0.31	(9.38)	1.21	(10.58)
den_GF	1.84	(1.84)	1.27	(3.10)	0.08	(3.18)	4.13	(7.31)
den_NGF	7.00	(7.00)	11.57	(18.58)	3.20	(21.78)	2.37	(24.15)
den_para	14.42	(14.42)	0.09	(14.51)	0.71	(15.22)	1.94	(17.16)
All Effects	21.92	(21.92)	8.59	(30.51)	10.08	(40.59)	3.76	(44.36)

In Table 21 and Table 22, the 'total' columns represent the cumulative percent of variance explanation through that factor as you move from left to right across each row of the Tables. The first two factors explain a great deal of the variation found in the model. Factors 1 and 2 explain 72% of the variation in the predictor variables and 31% of the variation in the response variables.

Another important point to consider is whether each predictor (X) variable is 'important' to the model. This is determined using Wold's Criterion and is displayed in Figure 25. A Wold's Criterion value greater than 0.8 indicates that the variable is important to the model. Values considerably less than 0.8 can be omitted. In this case, all of the predictor variables were above (or just slightly below) 0.8. No variables were omitted.
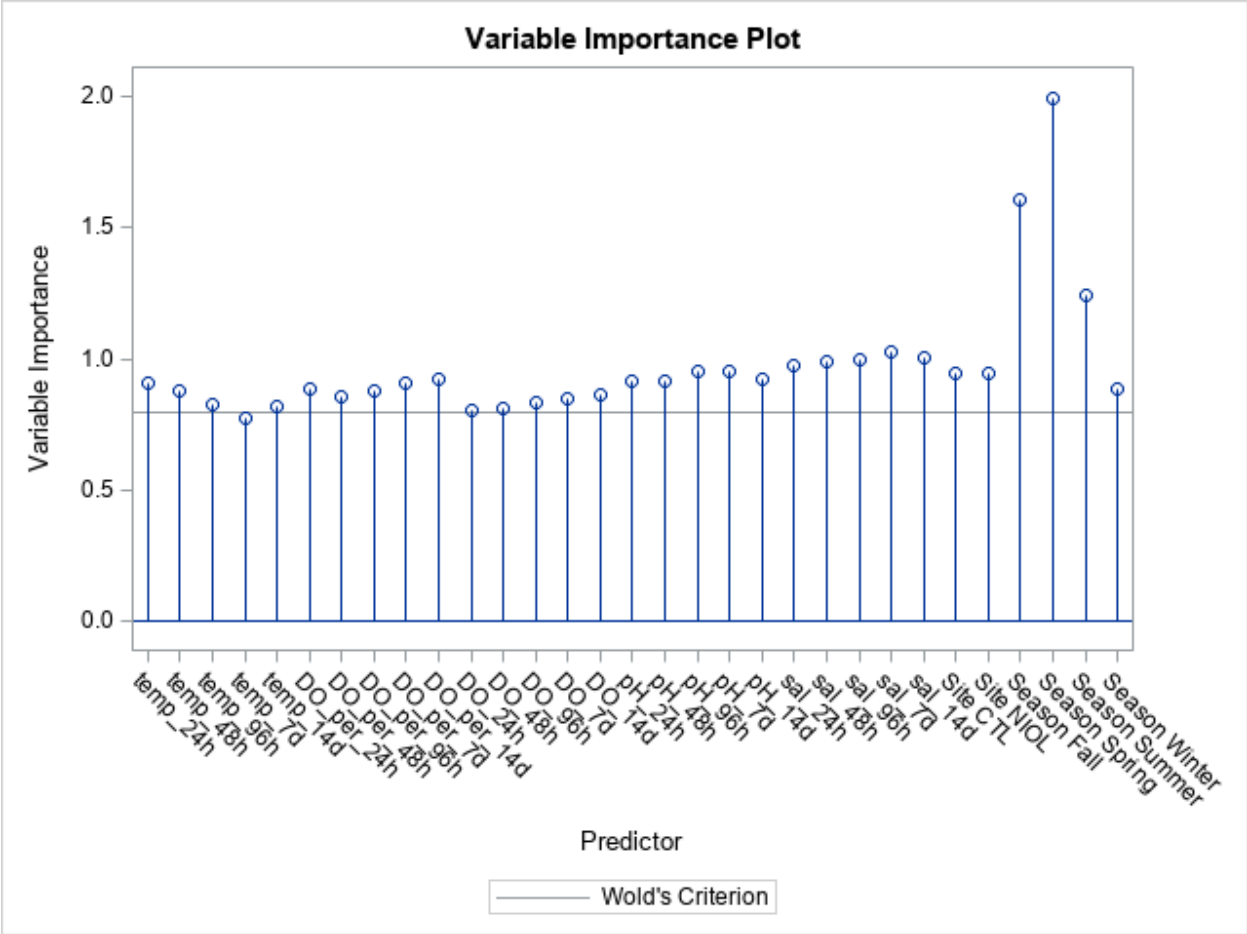
Figure 25 -- Variable importance plot with Wold's Criterion

The primary informational product from the PLS is the correlations loading plot [Figure 26]. A great deal of information can be gleaned from these plots. Since factors 1 & 2 explain the majority of information in the model, the plot of those two factors will be used in the next several figures. Like Principal Components in PCA, factors in PLS represent the variance of all of the individual variance in the model. In Figure 26, each axis is plotted with zero as its mid-point. This gives the ability to look at positive or negative influences. The variable names in blue represent the predictor (X) variables and the variable names in red represent the response variables (Y). The numbers in green are the decomposed covariances of the model from each data point to examine the distribution (or grouping) of the points. Water quality variables are expressed as numeric data. Sites and Seasons are categorical. The concentric circles represent levels of correlation -- the farther from the center, the stronger the correlation. Predictor variables within the 25% circle do not provide much influence to the model. Likewise, the model does not influence response variables within the 25% circle.

Correlation Loading Plot
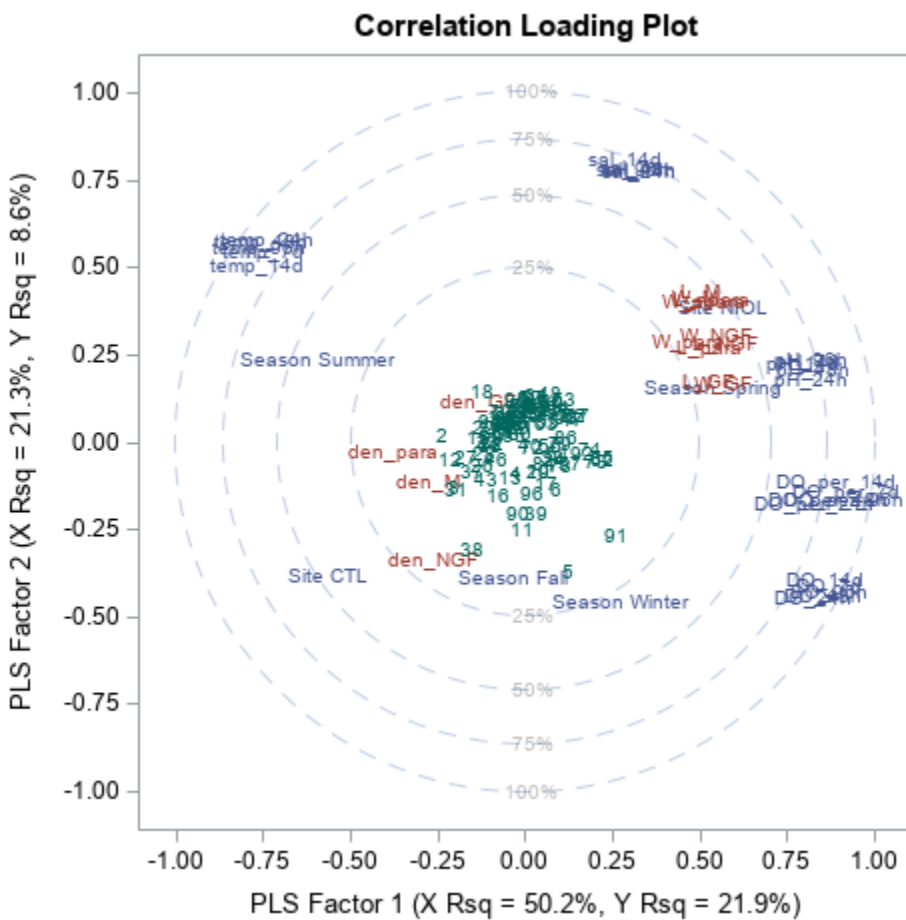
Figure 26 -- Partial least squares regression correlation loading plot

The correlation loadings plot allows for the examination of the predictor variables separately from the response variables, and vice versa. Yet, it also allows one to be able to see the associations (correlations) between individual (or groups) of response variables and individual predictor variables. Figure 27 shows the same plot as Figure 26, but with the water quality predictor variables identified in circles. Each grouping is clear and distinct. The water temperature variables (temp_24h through temp_14d) all group tightly together. The same holds true for the other variables of pH, salinity, dissolved oxygen concentration, and dissolved oxygen percent saturation. Again, these are the mean water quality values for the period preceding any given sampling event. For example, 'sal_14d' represents the mean of the salinity readings (taken every 15 minutes) for the 14-day time period prior to the grass shrimp sampling event.

Figure 27 -- Correlation loading plot -- water quality data groupings

Looking at response variables, we see that the *P. pugio* weight and length variables are all tightly group together identified by the circle in Figure 28. The weight and length variables for *P. pugio* are strongly and positively associated with the Spring Season and with the NIOL site. Looking at the X axis of the plot (PLS factor 1), weight and length variables are on the same side of the center point (zero) as the variable groupings for salinity, dissolved oxygen, and pH, indicating positive correlations with those variables. The temperature grouping is on the opposite side of the center-point of the X-axis from the length and weight variables indicating a strong negative correlation with increased temperature and grass shrimp size.
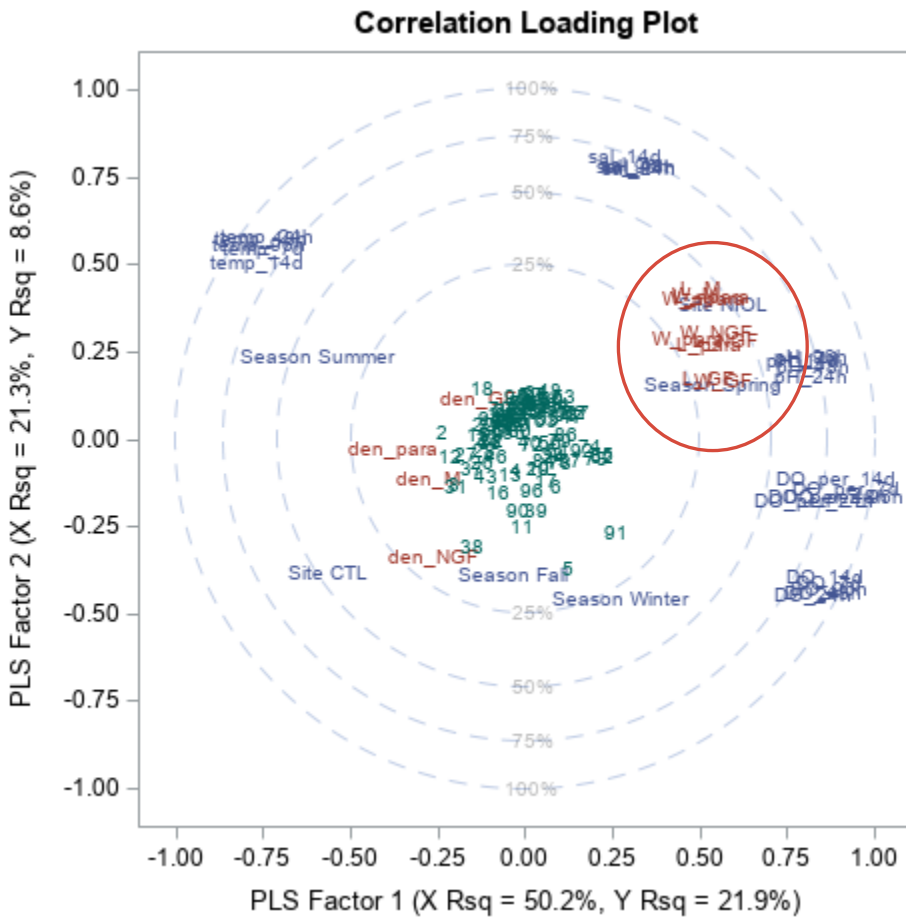

Figure 28 -- Correlation loadings plot - weight and length variables

To illustrate this further, a line can be drawn through the response variable (or variable grouping) and through the center of the plot as in Figure 29. Perpendicular lines can be drawn from each predictor variable to the main line to illustrate the strength of the correlation with weight and length. Salinity, pH, DO₂, NIOL, and Spring are all strongly positively correlated with *P. pugio* weight and length. For example, if there is high DO_2 in the Spring at NIOL, then there is likely to be larger (but fewer) *P. pugio* in the system. Conversely, we see that summer and increased temperature brings about smaller shrimp, but higher densities. It is also noted that the CTL site had smaller shrimp on average in (Tables 7-10), than NIOL. This plot demonstrates that same effect. There is a negative correlation with shrimp length and weight and the CTL Site vs. NIOL Site. Winter and Fall seasons hit the perpendicular very near the center of the chart (near zero for X & Y) indicating that there is not much effect on weight and length due to those seasons.
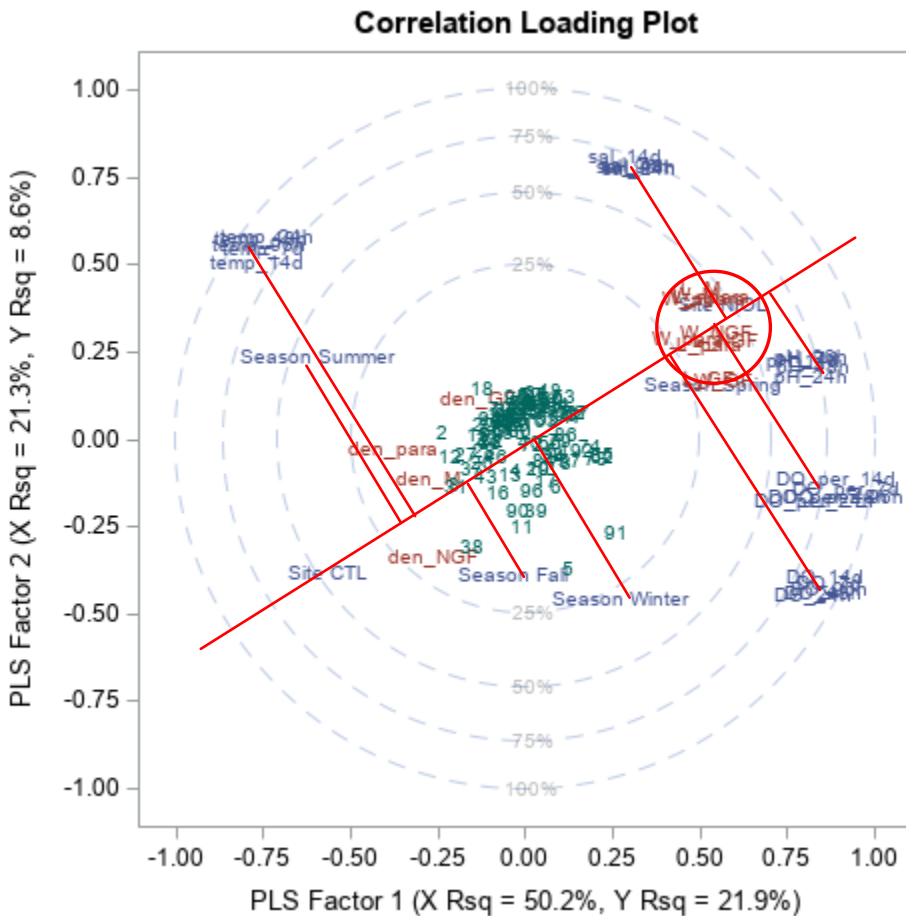
Figure 29 -- Correlation loadings plot -- influence of predictor variables on P. pugio weights and lengths.

The *P. pugio* density response variables included in the model are not as tightly grouped as the length and weight variables [Figure 30]. They appear in the red circle to the left of the center in the plot. Since these variables are within the 25% circle of the plot, they are not greatly influenced by the model.

Figure 30 -- Correlation loadings Plot - P. pugio density variables
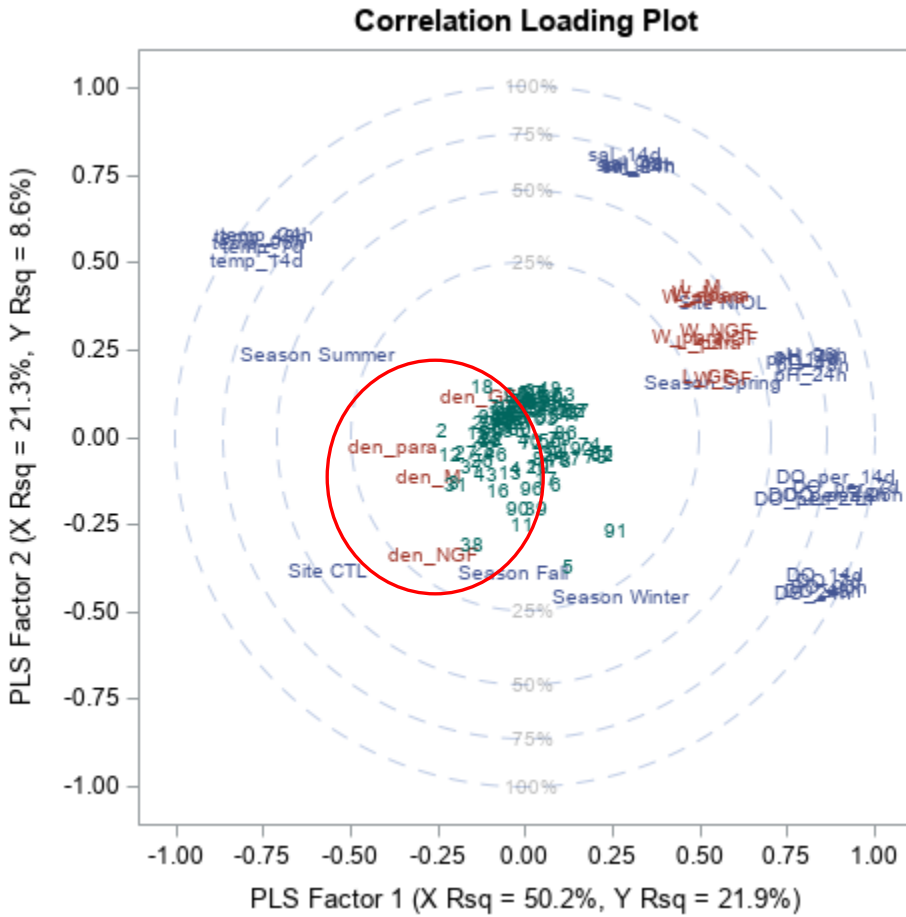
6 FINAL DISCUSSION AND CONCLUSIONS

6.1 FINAL DISCUSSION

When coupled with the data from Leight et al. (2005), this long-term record of grass shrimp population data from two SC estuaries provides a recent historical population record for comparison with future studies. Datasets, like the one presented here, can help future researchers determine the impacts of various short-term and long-term perturbations. These include, but are not limited to, sea-level rise, climate change, habitat alteration, extreme weather events, as well as oil and chemical spills, non-point source pollution, invasive species interactions, and microplastics.

6.1.1 Core species and rare species

Magurran and Henderson (2003) studied the abundance patterns of estuarine fishes and determined that the ecological community can be divided into two main parts based on abundance distributions. Their quantitative findings support anecdotal observation that ecological communities are divided into 'core' species and 'occasional' species. The core species are few but are also incredibly abundant. Occasional species, also called rare by some, are numerous, but their abundances are comparatively very low. There are many examples of this phenomenon found in nature. For example, intertidal vegetation in southeastern US estuaries exhibits this pattern. By far, the largest component of intertidal plant biomass is composed of the species *S. alterniflora.* A few other species are also common such as the black needle rush, *Juncus roemerianus.* The vast majority of the remaining plant species found in the intertidal area of southeastern US estuaries are many but they are rare and are found on the fringes (Eleuterius, 1980). Examples of these include, the sea ox-eye (*Borrichia frutescens*), glasswort (*Salicornia sp.*), and sea-lavender (*Limonium carolinianum*) to name a few.

Palaemon pugio is a core species of estuarine shrimp and a core species of the overall nekton community in the southeast US. Of the shrimps encountered in the present study (collected using the aforementioned sampling technique), 99% were *P. pugio*. *Palaemon vulgaris* comprised less than 1% of the total catch over the 15-year period (2001-2016). Other species of shrimp not reported on here, such as Panaeid shrimps and Alpheid shrimps, were encountered less than 0.01% of the time. Allen et al. (2007) in a study of nekton communities in South Carolina estuaries, found 77 different species of fishes and motile crustaceans from North Inlet estuary. There were 11 core species of nekton that were found and comprised

>99% of the nektonic abundance. *Palaemon pugio*, alone, accounted for >90% of the total abundance. While the present study (in this report) had an inherent sampling bias toward the preferential collection of *P. pugio* during the ebb tide, it is still reasonable to conclude [the findings of Allen et al. (2007)] that *P. pugio* is the most dominant member of the estuarine nekton. *Palaemon vulgaris* is a fringe species in many regards because of its low abundance. It is commonly found on or near hard substrates such as oyster reefs whereas *P. pugio* is associated with the much larger expanse of soft bottom sediments.

6.1.2 Study comparisons: 1991-2000 versus 2001-2016

The present study (2001-2016) is an extension of the previous work (1991-2000) by Leight et al. (2005). The hope was that there would be a continuous, 25-year record of long-term data from 1991 until 2016. However, there are several issues with the data and, thus, the 25-year effort is divided into two distinct phases. In the earlier years of the sampling endeavor (1991-1993), the methodology was still under development and the reliability of the data is questionable (pers. comm. James Daugomah). The methodology solidified from 1993 to 2000, but it was still being refined. Additionally, numerous field personnel (>12) participated in the collection of the data at various times including the field collections and sample processing in the laboratory (pers. comm. Andrew Leight). Each of those persons, some of who were inexperienced in field collections, was a potential source of variation. The present study (2001-2016), is arguably the most reliable and robust of the study segments. A core group of 3-4 personnel performed the sampling over that 15-year period and the sampling was more consistent due to their skill and experience. Acknowledging those differences, there are some data comparisons that can be made between the two phases.

6.1.3 Yearly trends

The mean density of shrimp was greater for the period 1991-2000 (40 shrimp/m^3) versus 2001-2016 (20.9 shrimp/m^3). In looking at the yearly mean densities from 1991-2000 [(Leight et al., 2005) Figure 3] and 2001-2016 [Figures 9 and 10] from the present study], the data vary from year to year. Of particular note, we see a dramatic shift toward lower densities after 1994-1995 at CTL and NIOL in Leight et al. (2005). This was unexplained by the authors in terms of the data collected, but they speculated that it could have been due to an infection by a virus or bacteria in the shrimp population. Conversely, we see the opposite in the present data set with a shift toward higher densities after 2011. This shift is significant, but like the study by Leight et al. (2005) it is unexplained. There were no changes to the sampling approach or methodology (pers. comm. J. Daugomah and B. West) and attempts to correlate the change with meteorological, drought, or water quality variables were unsuccessful. Interestingly, grass shrimp lengths were greater during the latter

period of 2001-2016 than in the previous study. There were no significant yearly, monthly, or seasonal correlations between lengths or weights and densities. Thus, while densities increased during the latter years of the present study (2012-2016), the lengths and weights stayed about the same as prior years. As in the study by Leight et al. (2005), grass shrimp lengths and weights were significantly greater at NIOL versus CTL during 2001-2016 with gravid females being the largest (See Figures 14, 15, 17, and 18).

6.1.4 Yearly trends: sex ratios and egg production

For NIOL, the overall sex ratio for the 1991-2000 period (Leight et al., 2005) was 2.3:1 (male:female), which is significantly (p<0.0001) higher than the current study (2001-2016) at 1.5:1 (male:female). During the earliest years of the present, study (2001-2003, Table 10), sex ratios of males to females are higher (>2:1). For all years since 2003, males were shown to be < 2:1. The sex ratios at CTL were much closer for the two studies at 1.5:1 (male:female) and 1.4:1 (male:female) for 1991-2000 and 2001-2016, respectively. There was no significant downward trend in sex ratios found in the current study. The reason for the differences in the sex ratios between the two studies is not known and is left to speculation. There was also no positive or negative correlation between sex ratios and grass shrimp densities. The data appear to be highly variable and given the nature of the field sampling this is not unexpected. As previously stated, there are numerous sources of error both foreseen (yet unable to control) and unpredicted.

If gravid females are separated out as a third group, an interesting trend is presented between the two studies. While the previous study (Leight et al., 2005) showed more males than the current study, the proportion of gravid females is greater than the more recent study at both NIOL and CTL (Tables 15 and 16). While it is not significant, there does appear to be a slight trend over time toward higher percentages of gravid females at NIOL (Figure 19) and CTL (Figure 20).

There were no significant relationships between eggs counts and sex ratios at either site by year (2001-2016). The present study did find that, on average, there were more eggs per gravid female at NIOL as opposed to 1991-2000 (Leight et al., 2005). The opposite was true for the CTL site where this study reported slightly reduced (but significantly different) number of eggs per gravid female than what was reported in Leight et al. (2005). Here again, this is a phenomenon that cannot be explain with the current data set.

6.1.5 Grass shrimp metrics and associations with water quality variables

In any given year, the vast majority of grass shrimp hatch, grow, reproduce, and die (via predation). There is a small subset of individuals that overwinter to start

the cycle anew each year. The size of that group remains largely unknown. What we do know is that an enormous population explosion of grass shrimp happens in the spring of each year in the tidal creeks. The relationships that we observed between grass shrimp densities/biomorphometrics and seasonality (Figures 26-30) confirms anecdotal observations by the field researchers (pers. comm. J. Daugomah and B. West). As the over-wintering shrimp emerge, they begin to reproduce in early spring. The timing of this is likely site specific as evidenced by the seasonal peak differences between the two sites of this study. As spring progress into summer and shrimp begin to hatch into the estuaries, a huge amount of small, mostly male grass shrimp dominate the population. The PLS regression (Figures 26-30) confirms that temperature was inversely proportional to grass shrimp size (length and weight). During the summer, you are more likely to encounter vast numbers of smaller individuals. The fate of most of these summer shrimp is that they become prey to the many predators found in coastal estuaries seasonally. Spring is positively correlated with the largest individuals of all sex types. While the relationships for densities were not as strong as length and weight relationships, we do see that densities for non-gravid females are associated with the Fall. This observation supports the thought that the shrimp are nearing the end of the yearly reproductive cycle and remaining shrimp are preparing to over-winter.

6.2 CONCLUSIONS

In environmental field studies, it is often difficult to find completely suitable reference sites. There always seems to be an imperfect match with the impacted site or sites. The same holds true for these sites sampled from 1991-2016 (CTL and NIOL). While they served as reference sites for a number of previously mentioned studies, they are not perfect matches for any other field site. Yet, they provided excellent reference site information over the 25-year period.

In the late 1980s, the researchers did not set out with this report in mind. This retrospective study is just that, a look back. The basic and straightforward analyses presented here provide a record (or status) of these sites so that future researchers might have a benchmark for future studies. There are number of ways to analyze the data collected and this study is by no means comprehensive. There may be other long-term data sets (other species, land-use/GIS, contaminant data, etc.) that can be combined with this pushnet data set to help explain the variability and trends seen in the data.

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