THE PRESENCE OF JUVENILE HORSESHOE CRABS, *LIMULUS POLYPHEMUS*, AND OTHER BENTHIC FAUNA OF MID-ATLANTIC COASTAL BAYS

by

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ABSTRACT

The Inland Bays of Delaware, Rehoboth Bay, Indian River Bay, and Little Assawoman Bay, serve as spawning grounds for the Atlantic horseshoe crab, *Limulus polyphemus*. While nearby Delaware Bay is known for remarkably high horseshoe crab abundances, there have been few investigations of spawning activity and the ecology of juvenile horseshoe crabs in the Inland Bays. Using a suction dredge, evidence of juvenile horseshoe crabs and other benthic organisms was collected from Indian River Bay in September and October of 2011. A juvenile horseshoe crab in the trilobite stage, three embryos, five juvenile molts, and 65 horseshoe crab egg shell fragments were found. In addition, benthic fauna representing nine phyla were collected. Seventeen species of arthropods were identified and found to be the most abundant group collected. The amphipods *Listriella barnardi* and *Jassa marmorea* were the most abundant organisms in nearly all samples. Molluscs were also well-represented, with seven species in lower abundances than the arthropods. Several sample sites contained highly similar assemblages. Evenness and overall diversity were high at most sites. In addition to horseshoe crab eggs and the trilobite larvae, many of the organisms collected by the suction dredge were similar in size to early juvenile horseshoe crabs, thus providing additional confirmation that the suction dredge is an effective sampling device for juvenile horseshoe crabs. Data from this study corroborates earlier studies of benthic organism abundance in the Inland Bays, and will be used to guide future juvenile horseshoe crab sampling efforts.

Chapter 1

INTRODUCTION

1.1 Horseshoe Crabs

The American horseshoe crab, *Limulus polyphemus* is an ecologically and commercially important species found in coastal waters from Maine to the Yucatan Peninsula (Sekiguchi and Shuster 2009). Horseshoe crabs are members of the order Xiphosurida, the "true horseshoe crabs," the first members of which appeared 445 million years ago (Rudkin and Young 2009). There are four extant species of horseshoe crab in the family Limulidae: *Limulus polyphemus, Tachypleus tridentatus, Trachypleus gigas, Carcinoscorpius rotundicauda. T. tridentatus, T. gigas, and C. rotundicauda* are native to the western Pacific; *L. polyphemus* is the only horseshoe crab found in the Atlantic Ocean. The harvest of *L. polyphemus* for bait, combined with ecotourism and the collection of *Limulus* amebocyte lysate (LAL), an extract from their blood used in biomedical applications, provides the United States economy with a revenue of $93 million to $123 million annually as of the year 2000 (Manion et al. 2000**)**. They also play an indispensible role in the migration of shorebirds such as the red knot, which consume their eggs as their primary source of energy for their journey (Gillings et al. 2007). In addition, they serve other ecological roles such as being important predators on bivalves and hosts for epibionts. They are also prey for birds, fish, crustaceans, and loggerhead sea turtles (Botton 2009). Due to their pervasiveness and importance to the economy and ecology of the eastern United

States, there has been a large amount of interest in studying the life history and ecology of *L. polyphemus*.

1.1.1 Adult Nesting

One of the most well-known and well-studied phenomena of the American horseshoe crab is the manner in which they spawn. Spawning preferentially occurs on narrow, steep beaches with sandy, oxygenated sediments (Penn and Brockmann 1994, Smith et al. 2002). Adult horseshoe crabs emerge at high tide from coastal and estuarine environments onto beaches in late spring in order to mate, most intensely on nights of full and new moons. Along with lunar and tidal influences, cues from temperature and weather, in relation to wave height, also affect horseshoe crab spawning (Rudloe 1980, Shuster and Botton 1985, Watson et al. 2009). When spawning conditions are met, males attach to female horseshoe crabs as mating pairs, and along with groups of surrounding satellite males, externally fertilize eggs as they are laid (Brockmann et al. 1994). On average, females deposit between 3,650 to 4,000 eggs per cluster and approximately 88,000 eggs in total per year (Shuster 1982, Shuster and Botton 1985). Egg clusters are usually laid in 15-20 cm of sand near the high tide line of the foreshore to minimize desiccation and inundation (Rudloe 1980, Weber and Carter 2009). The amount of time eggs take to hatch is temperature dependent, but under normal conditions hatching will occur about four weeks after eggs are laid (Weber and Carter 2009).

1.1.2 Larval Biology

After hatching, larval horseshoe crabs return to the water with the high tide or by turbidity from wave action, where they will remain until they return to beaches to spawn as adults (Rudloe 1978, Rudloe 1979). Horseshoe crabs go through a series of molts, or stages termed "instars," before they reach sexual maturity. During molting horseshoe crabs bury themselves in muddy substrate for several days prior, and then emerge on the surface to shed their exoskeleton (Shuster and Sekiguchi 2003). Usually, females will molt 17 times over 10 years, and males 16 times over nine years (Fig. 1) (Sekiguchi et al. 1988). Adult horseshoe crabs may molt as often as once per year (Rudloe 1978**,** Carmichael et al. 2003). Based on laboratory studies, the first instar stage usually lasts between 12 and 20 days, the second between 10-12 days, the third between 10-18 days, and the fourth between 13-20 days. The fifth and sixth stages seem to be more variable, ranging from 22 days to several months through the fall. In the second year most juveniles molt three times and complete the seventh and eighth instar stages, and in the third year they molt twice and complete the ninth and tenth instar stages. From the fourth year to the sixth year juveniles molt only once. While this is the "successful" developmental pattern

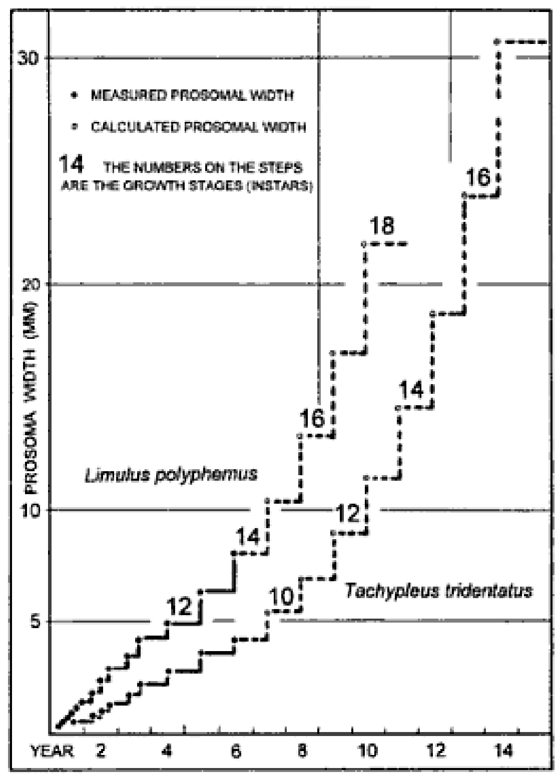

Figure 1 Stepwise growth in prosomal width of horseshoe crabs *L. polyphemus* and *T. tridentatus* in relation to age (Shuster and Sekiguchi 2003).

there are exceptions to the timing of each molt and the duration of the instar stages (Sekiguchi et al. 1988). Temperature, salinity, diet, and rate of feeding affect the length of the intermolt period, making it likely that field molting periods vary somewhat from the observed periods (Jegla and Costlow 1982, Shuster and Sekiguchi 2003, Ehlinger and Tankersley 2004, Carmichael et al. 2009). The size of juvenile horseshoe crabs can be correlated to their instar stage, which is useful for making field measurements (Fig. 2) (Carmichael et al. 2003**)**. In the field, it is possible that the average intermolt period is as short as one week for the first four instars (Botton and Loveland 2003). It is likely that the genetic characteristics and lifestyles of different horseshoe crab populations affect the achievement of these standards and therefore these rates and sizes may not apply to all populations (Shuster and Sekiguchi 2003).

Instar	Instar duration (d)	Age (yr)	Growth rate (mm d^{-1})
1	16.6	0.05	0.17 ± 0.01
2	10.9	0.08	0.17 ± 0.01
3	12.5	0.11	0.18 ± 0.01
4	15.8	0.15	0.17 ± 0.01
5	71.0 (22–120)[a]	0.35	0.10 ± 0.01
6	182.5	0.97	0.05 ± 0.003
7	121.7	1.3	0.05 ± 0.001
8	121.7	1.6	0.05 ± 0.001
9	182.5	2.1	0.05 ± 0.002
10	182.5	2.6	0.05 ± 0.002
11	365.0	3.6	0.05 ± 0.003
12	365.0	4.6	0.05 ± 0.003
13	365.0	5.6	0.04 ± 0.002
14	365.0	6.6	0.04 ± 0.002
15	365.0	7.6	0.04 ± 0.001
16	365.0	8.6	0.04 ± 0.001
17	365.0	9.6	0.04 ± 0.001

[a]Range of days crabs may spend in Instar 5

Instar stage		Mean prosomal width (mm)		
This study	Sekiguchi (1988)	This study	Sekiguchi (1988)	Shuster (1955, 1982)
1	1	3.1 ± 0.2	3.3	
2	2	5.0 ± 0.3	5.0	
3	3	7.1 ± 0.4	7.0	
4	4	9.1 ± 0.4	9.1	
5	5	12.5 ± 0.7	11.3	12
6	6	16.6 ± 0.9	14.5	16
	7		18.6	
7	8	21.7 ± 0.3	23.5	22
8	9	29.6 ± 0.2	29.5	
9	10	40.9 ± 1.5	38.0	
	11		43.7	
10	12	49.2 ± 1.6	50.2	53
11	13	62.6 ± 3.3	64.4	
12	14	77.3 ± 4.2	81.2	
13		91.1 ± 3.3		
14		103.4 ± 4.4		
15		115.5 ± 3.6		
16		134.0 ± 3.3		
17		159.7 ± 5.1		

Figure 2 Approximate mean duration, age, and growth rates of instars (left), and instar stage compared to mean prosomal width (right) (Carmichael et al. 2003).

1.1.3 Where Do Horseshoe Crabs Go?

Horseshoe crabs are ecological and environmental generalists, occupying a large geographical range and possessing the ability to cope with variable salinities and temperatures (Towle and Henry 2003). Horseshoe crabs exist in fairly discrete populations on large geographical scales, but also exist in genetically differentiated groups based on locality (Pierce et al. 2000, King et. al. 2005). The largest population of *L. polyphemus* is found in the mid-Atlantic region with the greatest spawning densities occurring in Delaware Bay (Shuster and Botton 1985). Adult horseshoe crabs exhibit a short-term tendency to stay near spawning beaches (Rudloe 1980, Penn and Brockmann 1994, Swan 2005, James-Pirri et al. 2005, Moore and Perrin 2007). After the spawning season horseshoe crabs tend to move away from spawning grounds and out of intertidal areas to deeper water in preparation for wintering, when they will bury themselves in the sediment and their vagility decreases significantly (Fig. 3). Average home ranges for horseshoe

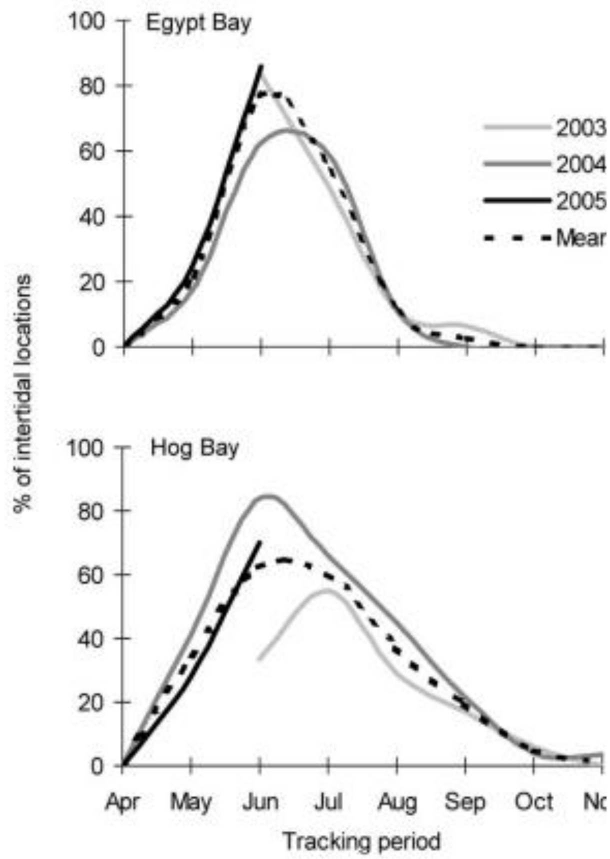

Figure 3 Horseshoe crab location points related to the intertidal zone in Egypt and Hog Bays, Maine, segregated by month for the years

crabs in coastal bays can stretch from 5.9 ha for wintering to 64.1 ha in total. Horseshoe crabs may also move laterally to locations up, down, or across bays, or out into the open ocean (Moore and Perrin 2007, Schaller et al 2010). They also have a tendency to move with currents in shallow waters (Rudloe and Herrnkind 1976, Rudloe and Herrnkind 1980).

While it is common for horseshoe crabs to dwell in estuarine environments, they are capable of surviving on the continental shelf. This has been observed from New York waters south, with the deepest-dwelling (>100 m) caught mostly off of Cape Hatteras (Botton and Ropes 1987). They may travel to the continental shelf in search of food, to avoid the pressures of predation in juvenile stages, or to redistribute overcrowded populations (Botton and Ropes 1987, Heta and Berkson 2003, Swann 2005). Horseshoe crabs have been recovered as far as 48 km offshore (Swan 2005) and at depths of 290 m and have been photographed at a depth of 1,097 m (Botton and Ropes 1987). Evidence of large juveniles, recently molted adults, and first and second year spawners are found on the continental shelf (Shuster and Sekiguchi 2009). However, as their prime habitat is in relatively shallow water, abundance decreases

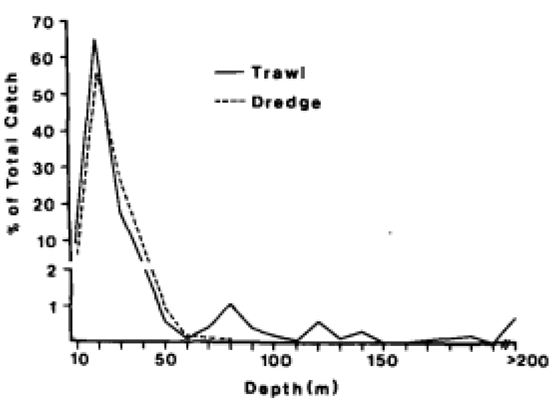

Figure 4 Bathymetric distribution of horseshoe crabs as percent of total catch based on bottom trawl data and ocean clam dredge data (Botton and Ropes 1987).

with depth past about 30 m (Fig. 4) (Botton and Ropes 1987).

Adult horseshoe crabs are controlled by endogenous circatidal and circadian rhythms and are mostly active at night (Chabot et al 2004, Chabot et al. 2007). Movements are oriented with the tides both during and after mating season: horseshoe crabs travel to shallower water during high tides, where they likely forage for food, and during low tides they move to deeper water, usually at the interface between deep channels and mudflats, where it is possible they bury themselves and remain until they digest their prey (Watson III and Chabot 2010).

1.1.4 Juvenile Horseshoe Crab Ecology

While the life histories of adult horseshoe crabs have been detailed fairly thoroughly, the ecology of juvenile stages of the horseshoe crab is less well known. A study by Botton and Loveland (2003) utilized plankton tows to collect trilobite larvae in lower Delaware Bay. Planktonic larvae were consistently found in greatest abundance nearest the shoreline and abundances decreased by 10-100 times in tows conducted 100-200 m offshore (Fig. 5). It was also observed that periods of more intense wave action were linked with higher abundances of larvae collected in the tows. It was supposed that this was due to the wave action causing suspension of newly emerged larvae from their nests as opposed to settled larvae being resuspended from bottom sediments. Night time activity was greater under all conditions, which corroborated a similar plankton-tow study by Rudloe (1979) in the Gulf of Mexico. Field and laboratory experiments determined that activity was notably increased on nights during weeks of full or new moons. Larvae also responded to light by orienting themselves towards it on all nights except those during the week of the new moon, indicating a possible endogenous response to moonlight at least in the trilobite stage.

Borst and Barlow (2003) corroborated that juveniles were nocturnally active under naturally cycled lighting, and some even maintained the circadian rhythm in constant darkness.

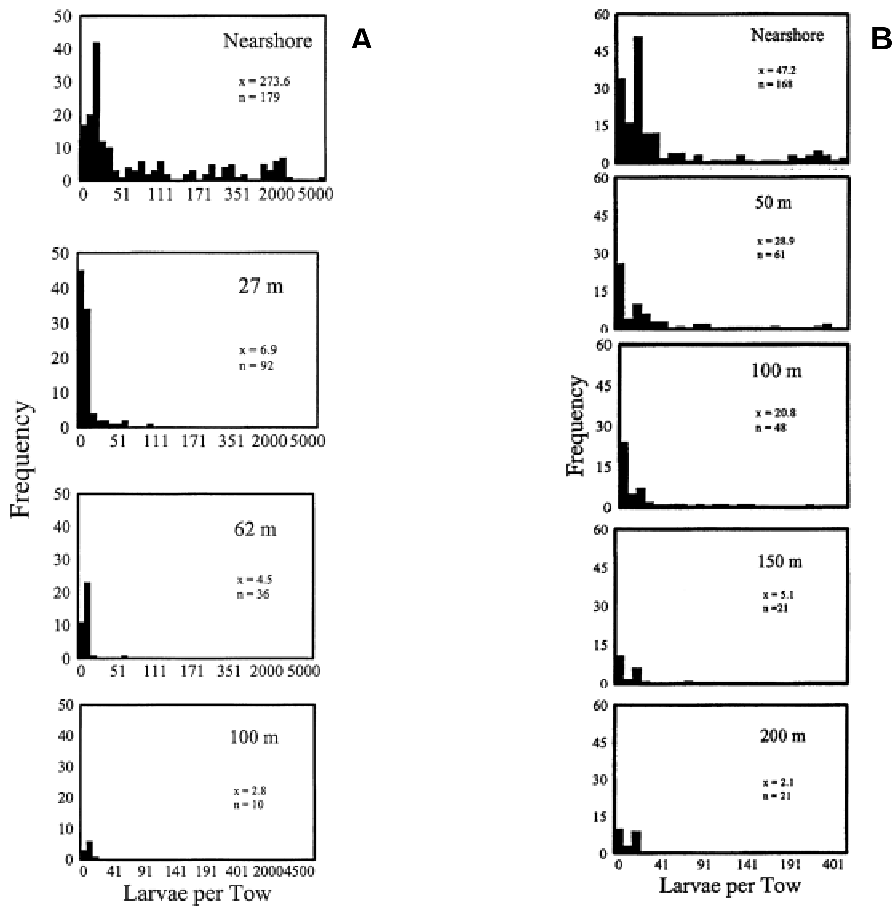

Figure 5 Frequency distribution of the number of horseshoe crab larvae per relative to the distance offshore in 1998 (A) and 1999 (B) (Botton and Loveland 2003).

Botton and Loveland (2003) observed horseshoe crab instars 1 through 4 on tidal flats, with the abundance of horseshoe crabs on the flat negatively correlated to the increasing instar stages. Rudloe (1981) observed older juveniles ranging in size from 21 mm to 62 mm in the intertidal zone of a Florida beach on the Gulf of Mexico. Rudloe also noted a diurnal pattern in her samples, in contrast to observations of nocturnal activity in trilobite larvae and adult horseshoe crabs (Rudloe 1979). Activity was synchronized with the tides and concentrated in the two hour interval before low tide, after which juvenile horseshoe crabs buried into the sand (Rudloe 1981).

Burton (2009) employed a suction dredge sampling device to physically collect juvenile horseshoe crabs from the bottom of Delaware Bay during the summer and fall months. Horseshoe crabs were 4.0 mm and greater in prosomal width, with the average size and depth increasing towards the end of the sampling period, but the abundance decreasing. Rudloe (1981) had previously shown that animals with greater prosomal width and thus inferred to be older (Fig. 6) are found in deeper water.

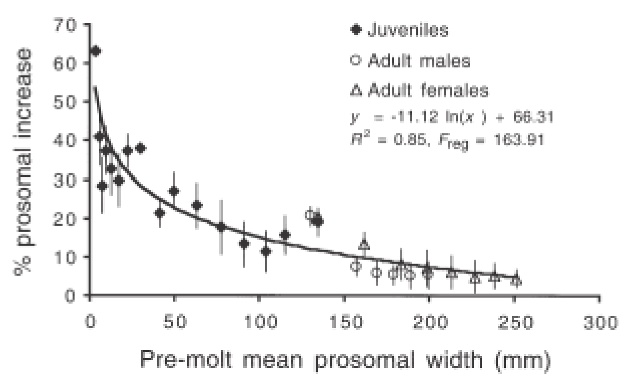

Figure 6 Percent prosomal increase versus pre-molt size with indication of age class (Carmichael et al. 2003).

Based on isotopic carbon signatures obtained from juvenile horseshoe crabs data on the feeding habits of horseshoe crabs can be gathered (Gaines et. al 2002). In the second instar stage, where they can no longer feed off their natal yolk sac, juvenile horseshoe crabs assimilate food from phytoplankton-derived and macroalgal-derived sources, in addition to a food web based on *Spartina alterniflora*. By the third instar horseshoe crabs shift to a diet composed primarily of a *Spartina*–based food web and continue to utilize this food source until they reach maturity (Gaines et. al 2002). From this data it can be inferred that, under normal circumstances, juvenile horseshoe crabs must stay within an area accessible to salt marsh habitats.

This brief literature review spans the general breadth of knowledge of the juvenile life stages of horseshoe crabs. As horseshoe crabs are valuable resources in both the economy and the environment, from biomedical fields to commercial fisheries, it is important that a more complete understanding of the horseshoe crab's life cycle is achieved in order to make the most informed policy and management decisions for this unique species.

1.2 Delaware's Inland Bays

The Inland Bays of Delaware consist of Rehoboth Bay, Indian River Bay, and Little Assawoman Bay (Fig. 7). These shallow, bar-built estuaries are located in Sussex County, Delaware and are the smallest set of estuaries in the National Estuary Program (U.S. EPA 2006). Combined they have an area of 82.9 km^2 and average depths of 1.0 m to 2.4 m (U.S. EPA 2006, The Delaware Center for the Inland Bays 2011). They are poorly flushed estuaries and thus particularly vulnerable to contamination. Many such poorly flushed and anoxic "dead-end" lagoons exist around the fringes of the bays. Indian River Inlet and Roosevelt Inlet are the only connections to the ocean, and the bays are linked by the Lewes and Rehoboth Canal and the Assawoman Canal, all of which are manmade. The permanent tidal influx of ocean water into the Inland Bays, facilitated by the construction of jetties along Indian River Inlet in 1939, has turned them from a predominantly freshwater system into predominantly marine bodies of water. Salt marshes, wetlands, and sand dunes are important habitats in the Inland Bays that support commercially important organisms such as hard clams, oysters, blue crabs, horseshoe crabs, and variety of mid-Atlantic fish species that use the estuaries as nurseries (Martin et al. 1996, Delaware Center for the Inland Bays 2011).

Figure 7 Map of Delaware's Inland Bays and their watershed (Delaware Center for the Inland Bays 2011).

Despite their small size, the Inland Bays are an important resource in coastal Delaware and are heavily impacted by the activities that occur within their watershed. The watershed of the Inland Bays encompasses 756 km^2 and sustains a population of approximately 87,210 people as of the 2010 census, and continues to be urbanized (Fig. 7) (The Delaware Center for the Inland Bays 2011). The surrounding coastal area serves as a major recreational center for the Washington D.C. and Philadelphia metropolitan areas during the summer months and the influx of seasonal tourists can increase the population in the Inland Bays' watershed by more than 200%. As of 2006, roughly one-third of the watershed of the Inland Bays supported farmland including the production of 70 million chickens and an associated 90 tons of manure (U.S. EPA 2006, The Delaware Center for the Inland Bays 2011). The Inland Bays receive 80% of their freshwater input from groundwater discharge. Impervious surfaces cover from 6.3% to 10.5% of watershed land area and will increase as urbanization continues (U.S. EPA 2006, The Delaware Center for the Inland Bays 2011). Indian River Bay also houses the Indian River Power Plant and the Rehoboth wastewater treatment plant discharges into the Lewes and Rehoboth Canal near the north shore of Rehoboth Bay. Such uses of the Inland Bays and their watershed has led to a variety of environmental concerns for these water systems, including the input of excess nutrients, bacterial and chemical contamination, a decrease in water quality and clarity, and the alteration and destruction of habitat (U.S. EPA 2006).

The qualitative changes that have occurred in the Inland Bays since the mid-1900s have led to a marked change in their ecology, including the benthic community which supports commercially important species such as those previously listed (U.S. EPA 2006, The Delaware Center for the Inland Bays 2011). Survey results from the Environmental Protection Agency's National Coastal Assessment of 2000-2001 rate the benthic condition of the Inland Bays as poor, with an unsatisfactory degree of benthic diversity in 36% of the Inland Bays (Martin et al. 1996). In a 1996 assessment of Delaware and Maryland coastal bays, Indian River Bay had higher abundances of benthic invertebrates than did the other bays, yet the lowest biomass of benthic invertebrates, a trend that can signify a degraded habitat according to Wilson and

Jeffrey (1994) (Fig. 8, 9) (Chaillou et al. 1996). Accordingly, the proportion of degraded area in Indian River Bay was the highest out of all of the bays at 77%. Overall, the quality of benthic assemblages has appeared to have changed since the 1970s, when the only comprehensive surveys of benthic fauna in the Inland Bays were conducted by Maurer (1977) and Delmarva Power and Light Company (1976); however, as it is closer in scope to the present study, we will be mainly concerned with Maurer's survey over Delmarva Power and Light Company's.

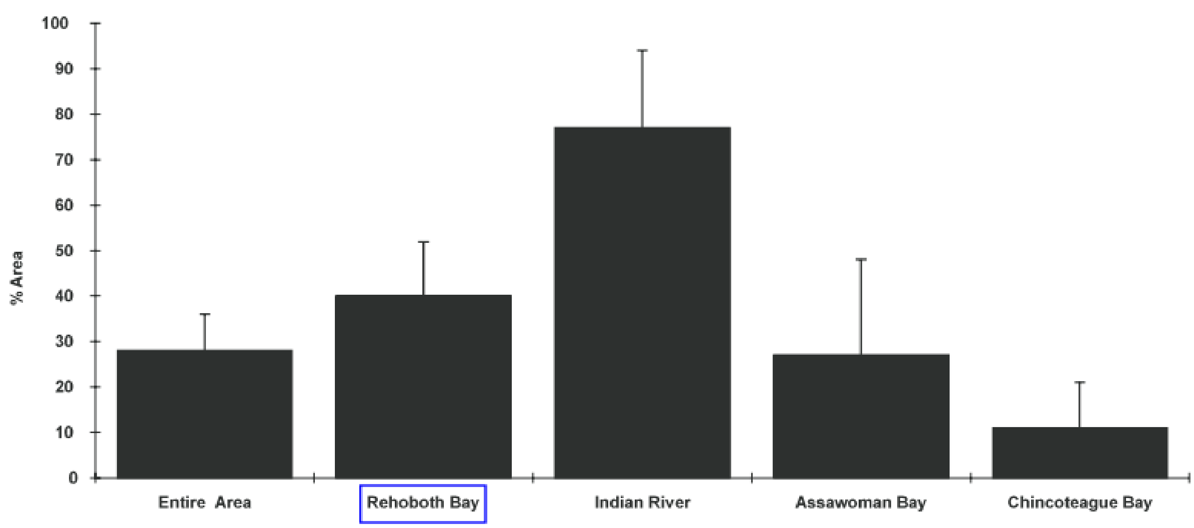

Figure 8 Percent degraded area of each of Delaware's Inland Bays, and Maryland's Cincoteague Bay (Chaillou et al. 1996).

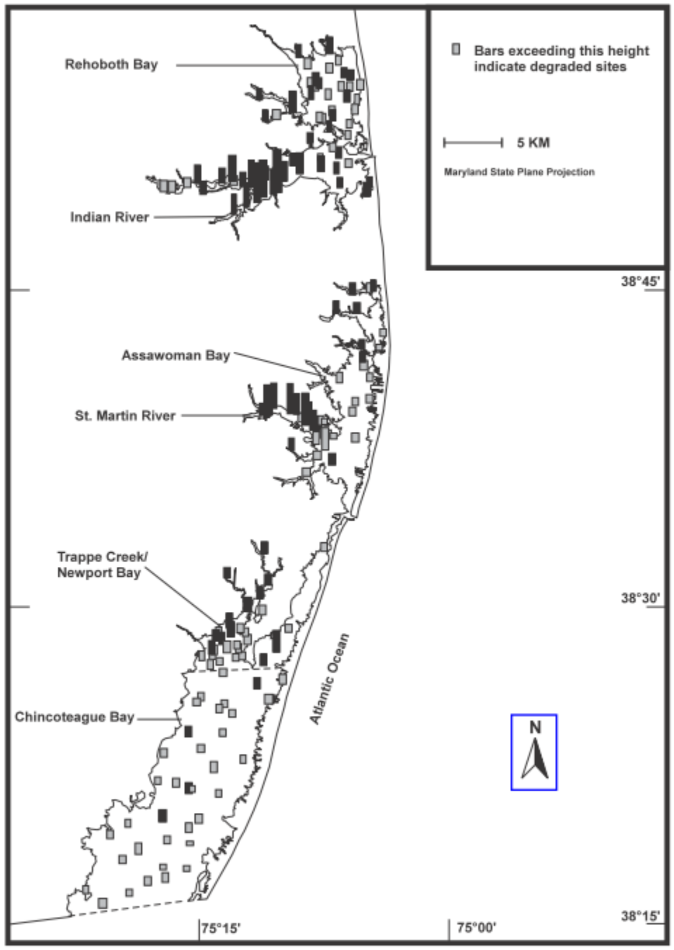

Figure 9 Map of Delaware's Inland Bays and Maryland's coastal bays with indication of degraded sites (Chaillou et al. 1996).

Maurer (1977) performed benthic sampling in Indian River Bay and Rehoboth Bay in order to quantitatively describe the ecology of winter and summer benthic invertebrates in these Bays. The study compared species composition, density, distribution, diversity, and biomass. A total of 149 species and 103,485 specimens were collected, with similar species composition for both bays. While on average there was even diversity in the bays and no statistical difference in diversity between seasons, years, or bays, it was determined that over the three years of Maurer's sampling period there was a large decline in density of benthic invertebrates in both Indian River Bay and Rehoboth Bay (74% and 50% respectively). The dominant species also shifted during the sampling period, and several opportunistic species associated with polluted environments or stressed habitats were found to be dominant at some sites. Maurer's study provides one of the only extensive baselines for benthic assemblages of the Inland Bays and can serve as a comparative tool for contemporary studies involving benthic organisms of the Inland Bays.

Compared to Delaware Bay, the Inland Bays are shallower, saltier, and muddier (Chaillou et al. 1996). The Inland Bays also have a significantly higher prevalence of chemical sediment contamination than does Delaware Bay; perhaps consequently, 28% of sites sampled in the Inland Bays have degraded benthic communities, compared to 16% in Delaware Bay, and faunal assemblages have shifted towards species that are more tolerant to stressful conditions (Chaillou et al. 1996). However, a number of species are still present across both of these systems. One of the most notable of these species is the American horseshoe crab.

1.2.1 Horseshoe Crabs in the Inland Bays of Delaware

While not nearly as well-studied as those in Delaware Bay, and with no references of historical abundance, a substantial population of horseshoe crabs can presently be found only about five kilometers south of Delaware Bay in the Inland Bays of Delaware. Volunteer surveys of spawning horseshoe crabs have been conducted since 2008 through a partnership between the Delaware Center for the Inland Bays and the University of Delaware College of Earth, Ocean, and

Environment. Through these efforts, an ongoing record of adult horseshoe crab and egg abundances in the Inland Bays has been compiled. These abundances are statistically similar to those enumerated in spawning surveys of Delaware Bay (D. Miller, E. Maung, personal communication). While the Inland Bays themselves may have different physical characteristics than Delaware Bay, they share the function of being important horseshoe crab breeding habitat.

Due to the similarities in spawning adult and egg horseshoe crab abundances, I propose that the Inland Bays of Delaware contain densities of juvenile horseshoe crabs similar to those found in Delaware Bay (Botton and Loveland 2003, Botton et al. 2003, Burton 2009). Describing the spatial distribution of juveniles and their abundances is a goal of this study, as is providing a contemporary survey of other benthic organisms that are found in the Inland Bays. Additionally, by utilizing historical data on other benthic invertebrates in the Inland Bays, we seek to make a comparison between the current and historical benthic assemblages as well.

Chapter 2

METHODS

2.1 Field Sampling

My original intention was to sample biweekly over the summer months (mid-July through September) in order to target the early larval stages of horseshoe crabs. Due to conflicts with scheduling and boat availability, we were only able to sample on September 29th, 2011 and October 4th, 2011. As outlined by Burton (2009), a suction dredge sampling device was assembled (Fig. 10). A gasoline-powered, 76.2 mm diaphragm trash pump was connected by a 15.0 m long discharge hose to a “missile” consisting of a T-shaped head of PVC pipe, 74 cm across and perforated with 25.4 mm circular apertures. When the pump was activated the missile was dragged behind the boat and discharge was collected from a second 15.0 m discharge hose into a wire mesh box (Fig. 11). Samples were collected from the discharge in this manner over either a 15, 30, or 60 second period and in a box of either 1.0 mm or 5.0 mm wire mesh, then put in plastic containers and frozen, with the exception of sample A1, which was drained of water and put in 70% ethanol on the same day it was collected.

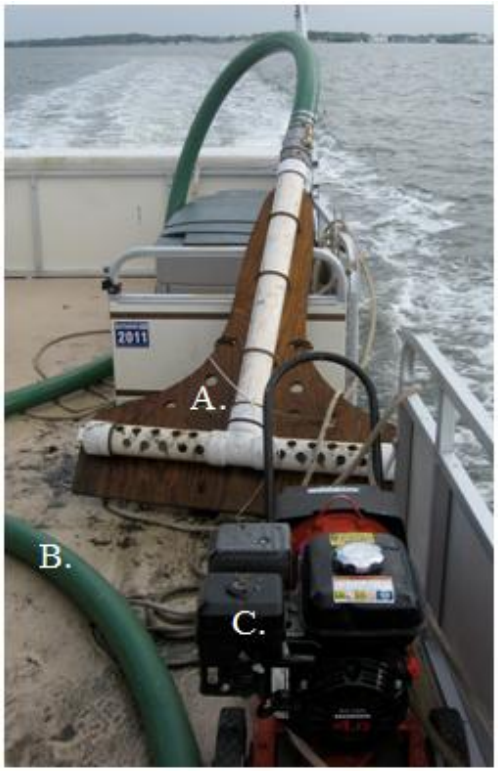

Figure 10 Suction dredge sampling device setup, illustrating suction head/missile (A), connecting discharge hose (B), and trash pump (C).

Figure 11 Outflow of dredge sample into wire mesh collection box.

On September 29th eight samples were collected from three separate sites in Indian River Bay and labeled samples A1-A8. On October 4th 10 samples were collected from three additional sites in Indian River Bay and labeled B1-B10 (Fig. 13). Latitude, longitude, time sampled, duration of discharge collection, and sediment type was recorded for all sites. Temperature and salinity were recorded for sites at which such information was available.

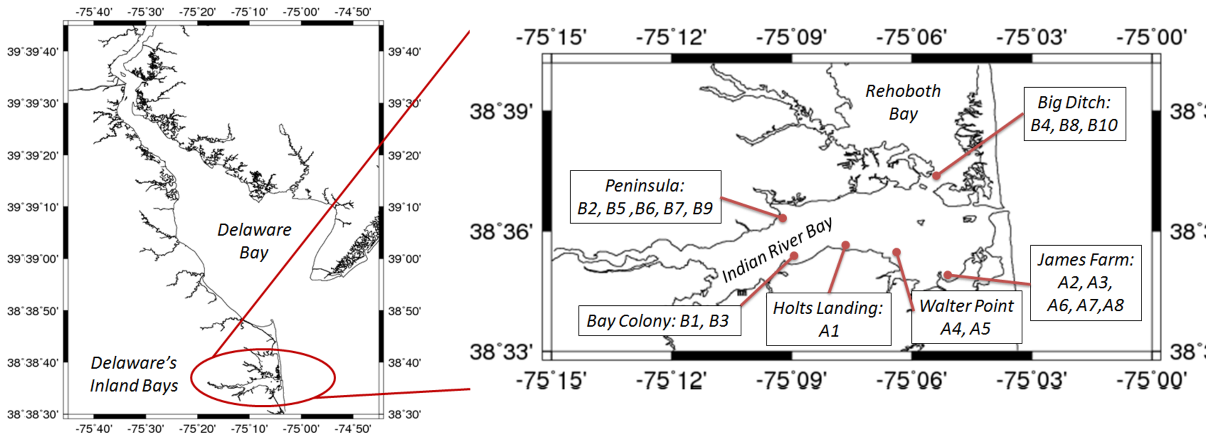

Figure 12 Map of Delaware's Inland Bays and sample sites within Indian River Bay and Rehoboth Bay.

2.2 Sample Processing

Samples were thawed in the laboratory and processed by size. For samples A1-A7, the contents of the sample containers were emptied into a 0.5 mm sieve. Any loose sediment or particles smaller than 0.5 mm were washed out of the sieve, and the remaining sample was rinsed into several Petri dishes. The same process was repeated for sample A8 and samples B1-B10; however, stacked 11.2 mm, 2.83 mm, and 0.5 mm sieves were employed to aid in separation of the sample and speed of processing.

Petri dishes containing samples were examined under a microscope. All organisms, including some representative macroalgae, bryozoa, and tunicates, and evidence of horseshoe crabs including egg fragments, embryos, larvae, and molts, were placed into jars of 70% ethanol. For samples A1-A8, all organisms were then taxonomically separated, identified, enumerated, and placed into 70% ethanol-filled vials containing their respective taxa. For samples B1-B10, only evidence of juvenile horseshoe crabs was noted. Photographs were taken of representative species with a

Canon A540 camera under 12x magnification. Taxonomic keys and identification guides were used to identify species (Pollock 1998, Gosner 1999, Lippson and Lippson 1997). Maurer (1977) was consulted in order to determine which species had been found historically in Indian River Bay and Rehoboth Bay.

2.3 Data Analysis

In order to more efficiently organize the samples spatially and temporally after the physical sorting, each sample was given a label based on the site from which it was collected and the temporal order it was collected in (Table 1). Data was analyzed using this labeling system and organized in tables and graphs based on the order in which it was collected.

Sample organisms were listed in a spreadsheet by phylum, taxa, and species, and enumerated for each sample. Bar graphs showing the relative abundance of each species were created for the phyla with sufficient amounts of organisms; specifically, the Arthropoda and Mollusca. A comparison of the top four most common organisms found in this study with the top four found in Maurer's 1977 survey of Indian River Bay and Rehoboth Bay was also conducted.

A multidimensional scaling (MDS) plot was constructed in PRIMER v.6 (Clarke and Gorley 2006, Clarke 2003, and Clarke and Warwick 2001) for the samples that were analyzed for multiple species (i.e. sample numbers A1-A8, herein designated by their station sample codes of JF1, JF2, HL, and so on). Abundances for each station were standardized by the percent of total organisms for that sample and then subjected to a fourth root transform. The Bray-Curtis Index of Similarity was used to score the similarity of the samples and produce an ordination plot based on their degree of similarity. PRIMER was also used to produce a table detailing univariate diversity indices, a species accumulation plot as a function of the number of samples, and a dominance plot (Clarke and Gorley 2006).

Table 1 Sample site location and physical characteristics organized by time collected and sampling site. Sample numbers designated by an "A" are those collected on September 29th, 2011, and those designated by a "B" are those collected on October 4th, 2011.

Site Name	Sample Code	Sample #	Latitude	Longitude	Time	Time Passed (seconds)	Salinity (ppt)	Temp (*C)	Sampling mesh size (mm)	Sediment type
James Farm	JF1	A6	38.5818	-75.0858	14:05	30	nr	nr	1	silt
James Farm	JF2	A7	38.5820	-75.0863	14:16	30	nr	nr	1	nr
Holt's Landing	HL	A1	38.5958	-75.1293	14:40	15	nr	nr	1	silt
Walter Point	WP1	A4	38.5877	-75.1078	15:08	60	nr	nr	1	gravel
Walter Point	WP2	A5	38.5886	-75.1071	15:08	nr	nr	nr	1	gravel
James Farm	JF3	A3	38.5806	-75.0839	15:25		nr	nr	1	nr
James Farm	JF4	A2	38.5809	-75.0845	15:44	nr	nr	nr	1	sand
James Farm	JF5	A8	38.5815	-75.0843	15:56	nr	nr	nr	1	nr
Peninsula	PN1	B7	38.6039	-75.1504	12:09	30	26.1	16.7	nr	silt
Peninsula	PN2	B5	38.6064	-75.1526	12:58	30	nr	nr	1	silt
Peninsula	PN3	B6	38.6067	-75.1528	12:58	30	26.8	15.7	5	silt
Peninsula	PN4	B2	38.6049	-75.1534	13:23	30	nr	nr	5	silt
Peninsula	PN5	B9	38.6050	-75.1550	13:26	30	nr	nr	1	silt
Bay Colony	BC1	B3	38.5877	-75.1514	13:50	30	nr	nr	1	silt
Bay Colony	BC2	B1	38.5875	-75.1499	13:56	30	23.8	17.2	1	sand
Big Ditch	BD1	B4	38.6260	-75.0893	14:26	30	nr	nr	1	shell hash
Big Ditch	BD2	B10	38.6239	-75.0905	14:41	60	28.7	18.9	1	shell hash
Big Ditch	BD3	B8	38.6226	-75.0919	14:58	30	28.7	18.9	1	shell hash

Chapter 3

RESULTS

3.1 Juvenile Horseshoe Crabs

Evidence of juvenile horseshoe crabs was found in 11 out of 18 samples. Evidence was categorized as horseshoe crab eggs and/or egg fragments, juvenile molts, embryos, or trilobite (stage 1) larvae (Fig. 13). Eggs and/or egg fragments are the most abundant evidence found and are present in 64% of the samples which contained evidence of juvenile horseshoe crabs. Molts of stage 1 larvae were collected in two samples, and embryos were found in three. A single trilobite larva, the target of this study, was found in a sample from Walter Point, and was measured at 3.36 mm prosomal width (Table 2, Fig. 14).

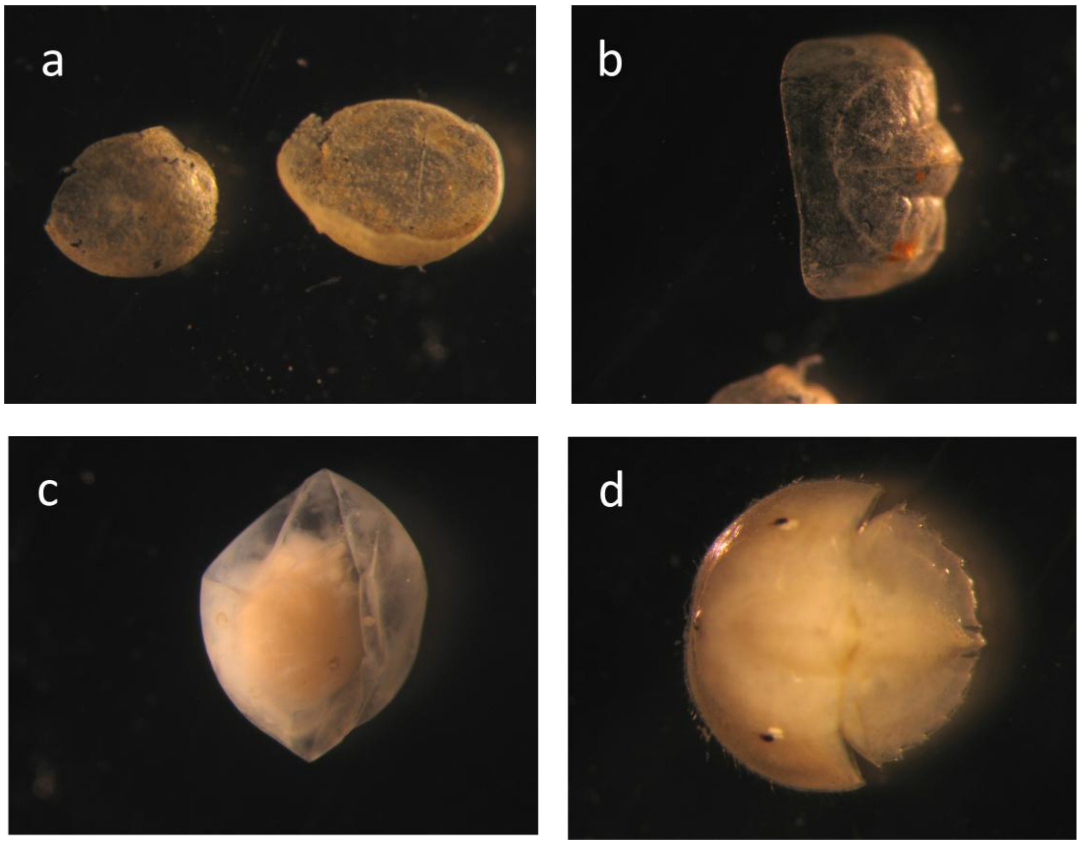

Figure 13 Microphotographs at of horseshoe crabs at 12x magnification in samples from Indian River Bay, Delaware; (a) horseshoe crab egg fragments, (b) a juvenile molt, (c) a horseshoe crab embryo, (d) a trilobite horseshoe crab larva.

Table 2 Evidence of juvenile horseshoe crabs by sample code.

	JF1	JF2	HL	WP1	WP2	JF3	JF4	JF5	PN4	BC1	BC2
Eggs/Egg fragments	9	1	10	18		2				12	13
Molts			1				4				
Embryos			1					1	1		
Trilobite larvae					1						

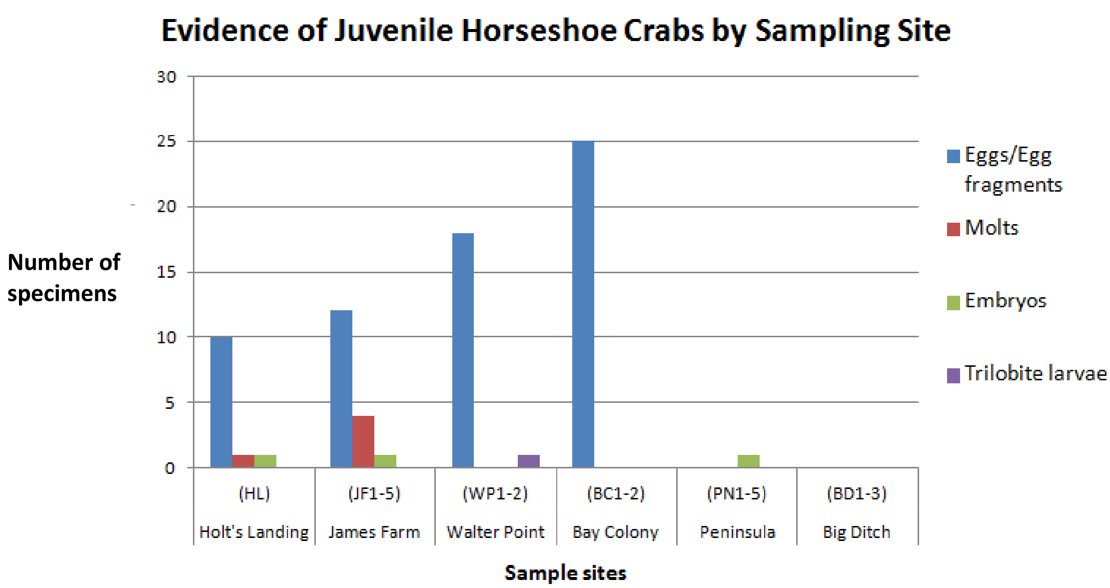

Figure 14 Evidence of juvenile horseshoe crabs arranged by sampling site.

3.2 Other Benthic Organisms

Organisms from the phyla Cnidaria, Bryozoa, Mollusca, Annelida, Arthropoda, Echinodermata, Hemichordata, and Chordata were identified in samples A1-A8. The phylum Arthropoda is the most abundant at seventeen species comprising 89.53% of the total organisms. The amphipods *Listriella barnardi* and *Jassa marmorea* are the two most abundant species, at 37.43% and 14.25% of total organisms, respectively. The phylum Mollusca is the next most represented, but in much lower abundance at eight species comprising 4.97% of the total organisms. The phylum Annelida follows in abundance with seven species at only 0.55% of the total organisms. In addition, unidentified anemones in the phylum Cnidaria, species of *Mogula*, and a vertebrate fish, *Gobisoma bosc*, were all found to be more abundant than Annelida and less abundant than Mollusca. One species each was found in the phylum Hemichordata and the phylum Echinodermata: *Saccoglossus kowalevskii*, and *Pentamera pulcherrima*, respectively. The organisms retained in the samples vary in size from 1-2 mm to several centimeters; for example, representative individuals of *Listriella barnardi* were measured at 3.36 mm to 4.2 mm in length (Fig. 15a). Macroalgae was found in nearly every sample that was examined for more than the presence of horseshoe crabs. The four most abundant organisms in the present study and Maurer (1977) are compared in Table 3.

Diversity indices were used to characterize the benthic assemblages. These results are generally consistent across the various measures presented in Table 4: sample HL is the most diverse, and JF3 and JF4 are the least, as they are dominated by the amphipods *Listriella barnardi* and *Jassa mamorea*. The samples WP1 and WP2 are intermediate in diversity. The greatest reliance is placed on the expected number of species for a given number of individuals, denoted ES(nn) for nn equal to 10, 50 or

100 individuals. It is considered easier to interpret than H' because it less dependent on sample size, which varied considerably among these samples (Clarke and Warwick 2001).

The species accumulation plot (Fig. 18) curves rise to 24, the total number of arthropods and molluscs found in the 8 samples (the bootstrap estimator overestimates the number of species somewhat, but this is inconsequential in this analysis). The upward slope of the curves suggests that the assemblage is not fully characterized by the number of samples in this study. If more samples were taken, there would be new, relatively rare species recorded. The arc of the curve toward an asymptote does suggest, however, that this survey provides a reasonable representation or subset of all species.

The dominance plot (Fig. 19) mirrors patterns seen in the diversity matrices in Table 3. Samples JF3 and JF4 (red symbols) are dominated by a few species, primarily the abundant amphipods, while sample HL has a more gradual rise, reflecting a more even spread in abundance over all species. Further, HL is clearly the most diverse as it takes a greater amount of species for this curve to rise near 100% in comparison to the other samples. This sample also has the highest evenness (J') value.

The MDS plot (Fig. 20) shows similarity among the samples based on the relative abundances of arthropod and mollusc species present. This plot shows a symbol for each sample, coded by location and sediment type. Abundances are standardized for each site by the percentage of organisms from the total of all sample sites, with circles representing the percent degree of similarity between encircled sites and the distance apart between sites indicating the degree of separation. WP1 and WP2 are 80% similar with the same sediment type, gravel, and JF3 and JF4 are 80%

similar as well (sediment types cannot be compared due to a lack of sediment data for JF4). Samples JF1, JF2, and JF5 are relatively distinct and separate, while HL is very distinct. Thus the MDS seems to reflect both the spatial and sediment type patterns in the benthic assemblages well.

Table 3 Comparison of top four most abundant organisms in the present study and Maurer (1977).

Maurer (1977)	**Present Study**
1. *Ampelisca abdita*	1. *Listriella barnardi*
2. *Gemma gemma*	2. *Jassa marmorea*
3. *Tellina agilis*	3. *Mysidopsis bigelowi*
4. *Mercinaria mercinaria*	4. Caprellid sp.

Table 4 Univarite diversity indices for samples from September 29th. S = number of species, N = number of individuals, d = Margalef's species richness, J' = Pielou's evenness, ES(nn) = expected number of species from a sample of nn individuals, H' = Shannon-Wiener, 1-Lambda' = Simpson Index.

Sample	**S**	**N**	**d**	**J'**	**ES(10)**	**ES(50)**	**ES(100)**	**H'**	**1-Lambda'**
HL	15	272	2.497	0.8297	6.044	11.61	13.63	2.247	0.8675
JF1	17	633	2.48	0.6697	4.941	9.574	11.83	1.897	0.7856
JF2	15	372	2.365	0.7416	5.373	9.081	11.04	2.008	0.84
JF3	15	811	2.09	0.5371	3.832	7.327	9.407	1.454	0.6522
JF4	18	896	2.501	0.4887	3.811	7.854	10.08	1.412	0.5883
JF5	15	365	2.373	0.715	5.153	9.368	11.35	1.936	0.8025
WP1	15	428	2.311	0.6831	4.801	9.731	12.32	1.85	0.7666
WP2	19	1470	2.468	0.5903	4.504	7.847	9.624	1.738	0.768

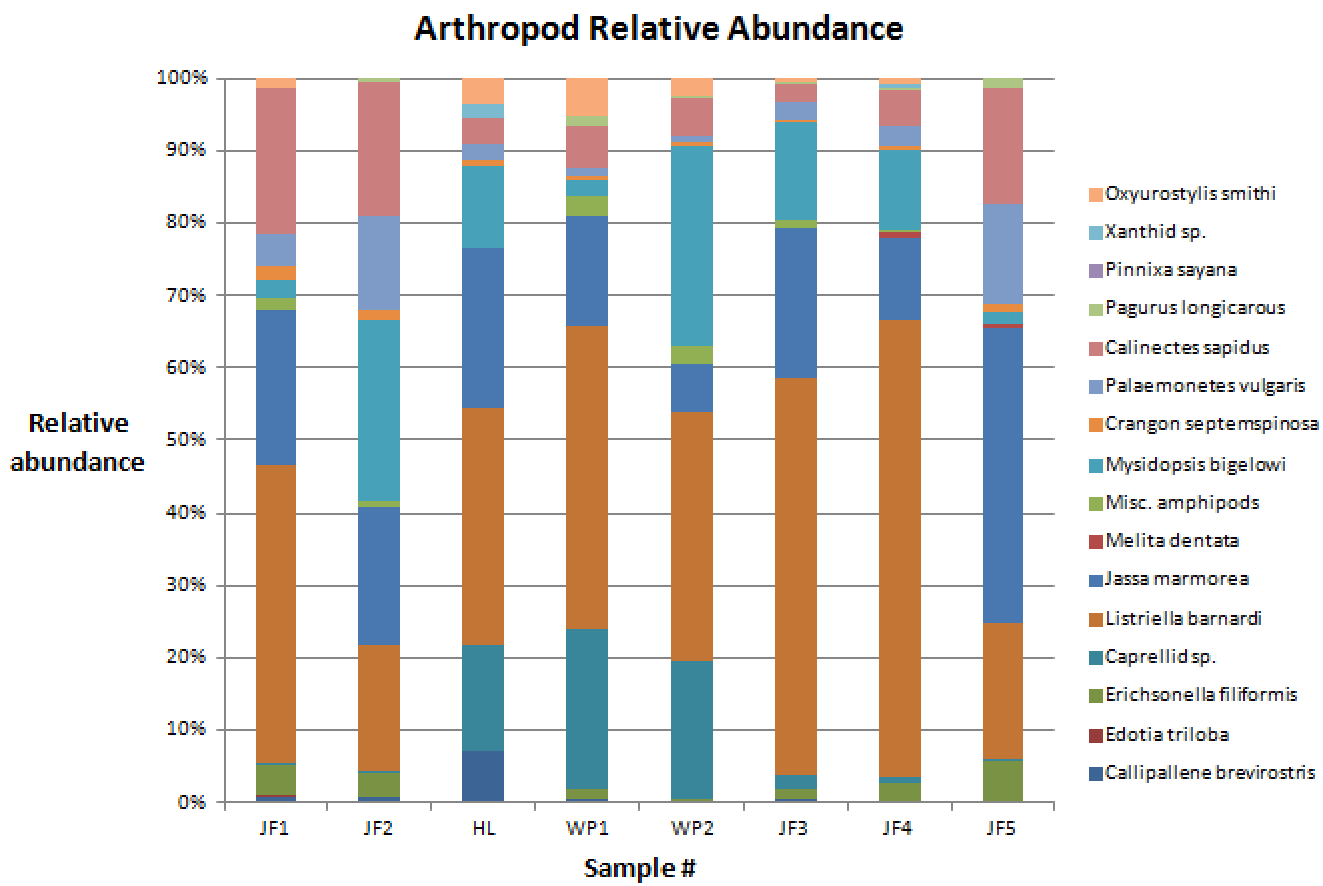

Figure 15 Relative abundances of arthropod species out of 100%.

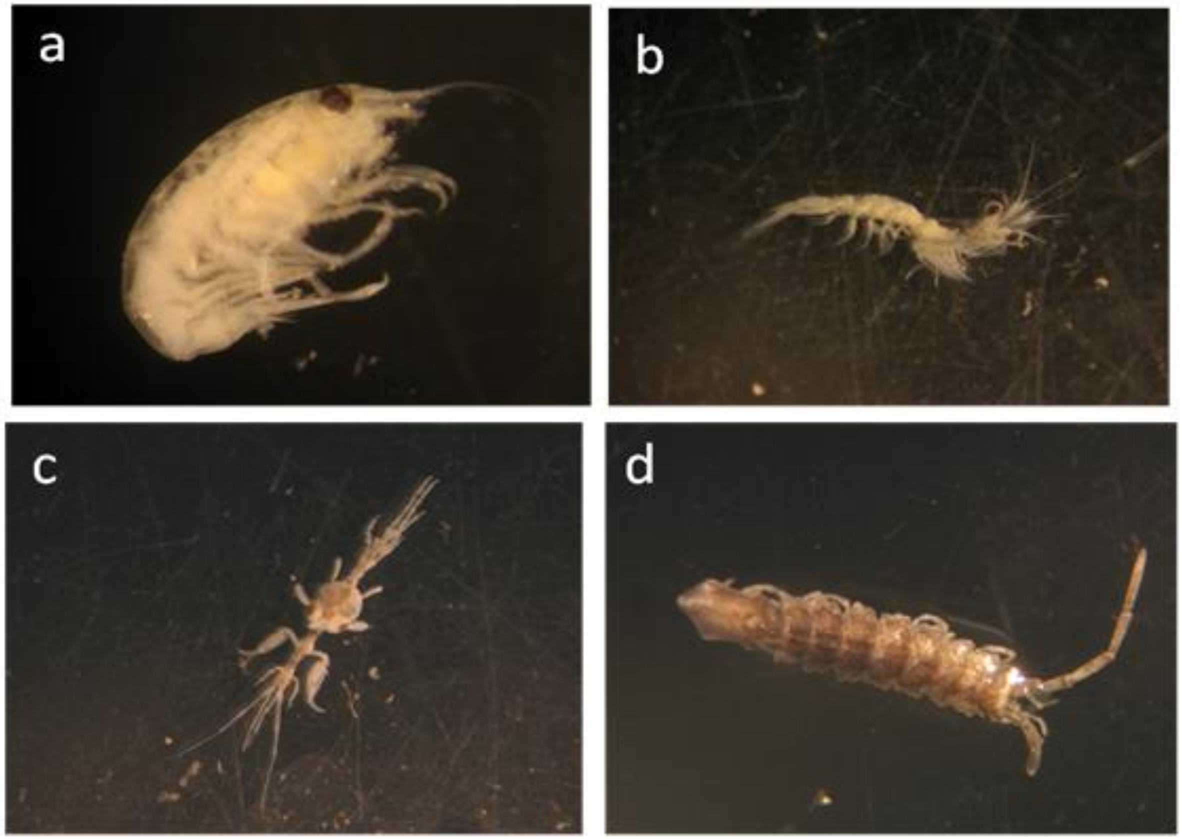

Figure 16 Microphotographs of representative arthropods of Indian River Bay, including the most abundant, (a) *Listriella barnardi* (33.47% of total organisms found). Also pictured are specimens of (b) *Mysidopsis bigelowi* (13.40%), (c) caprellid sp. (7.60%), and (d) *Erichsonella filiformis* (1.69%).

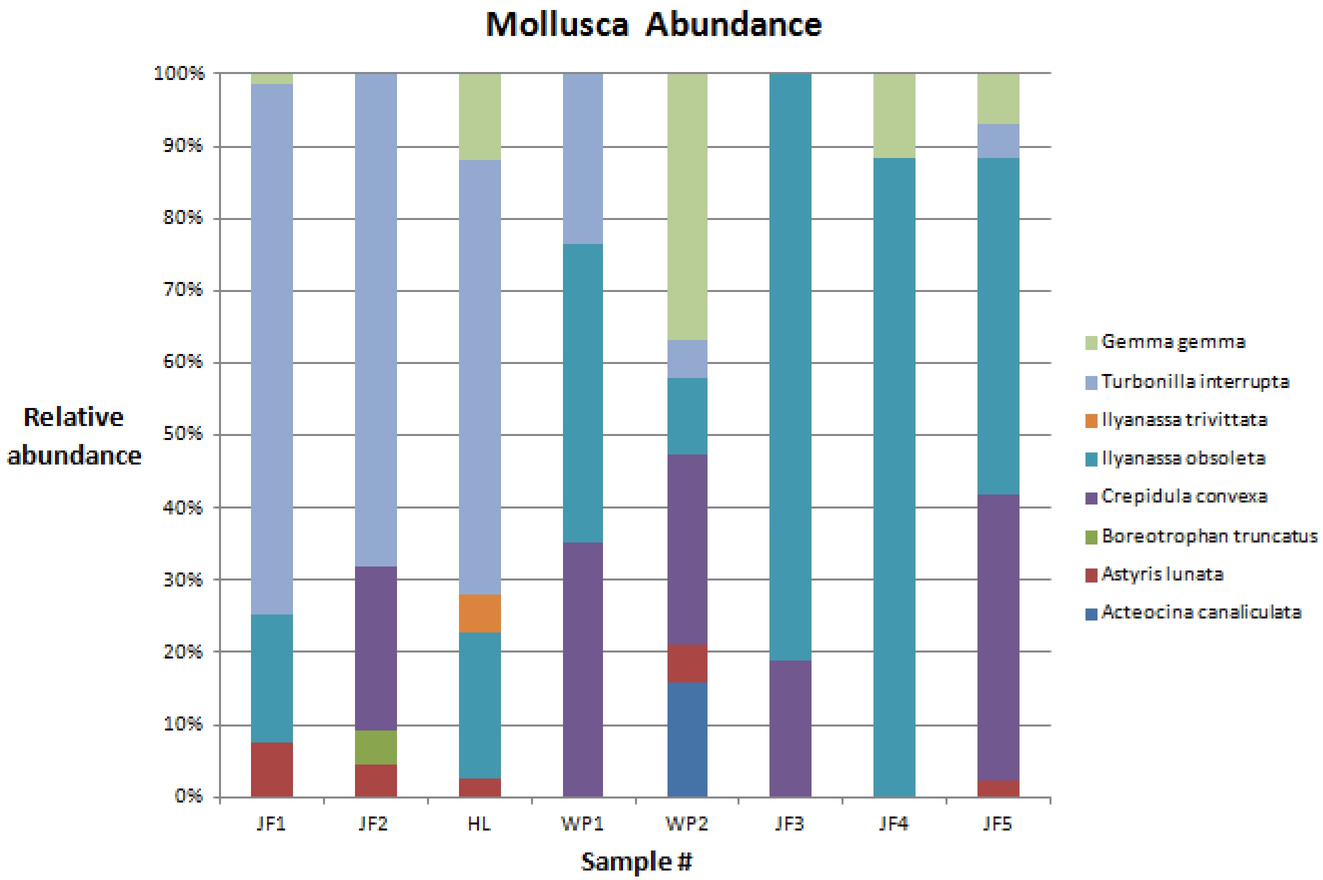

Figure 17 Relative abundances of mollusc species out of 100%.

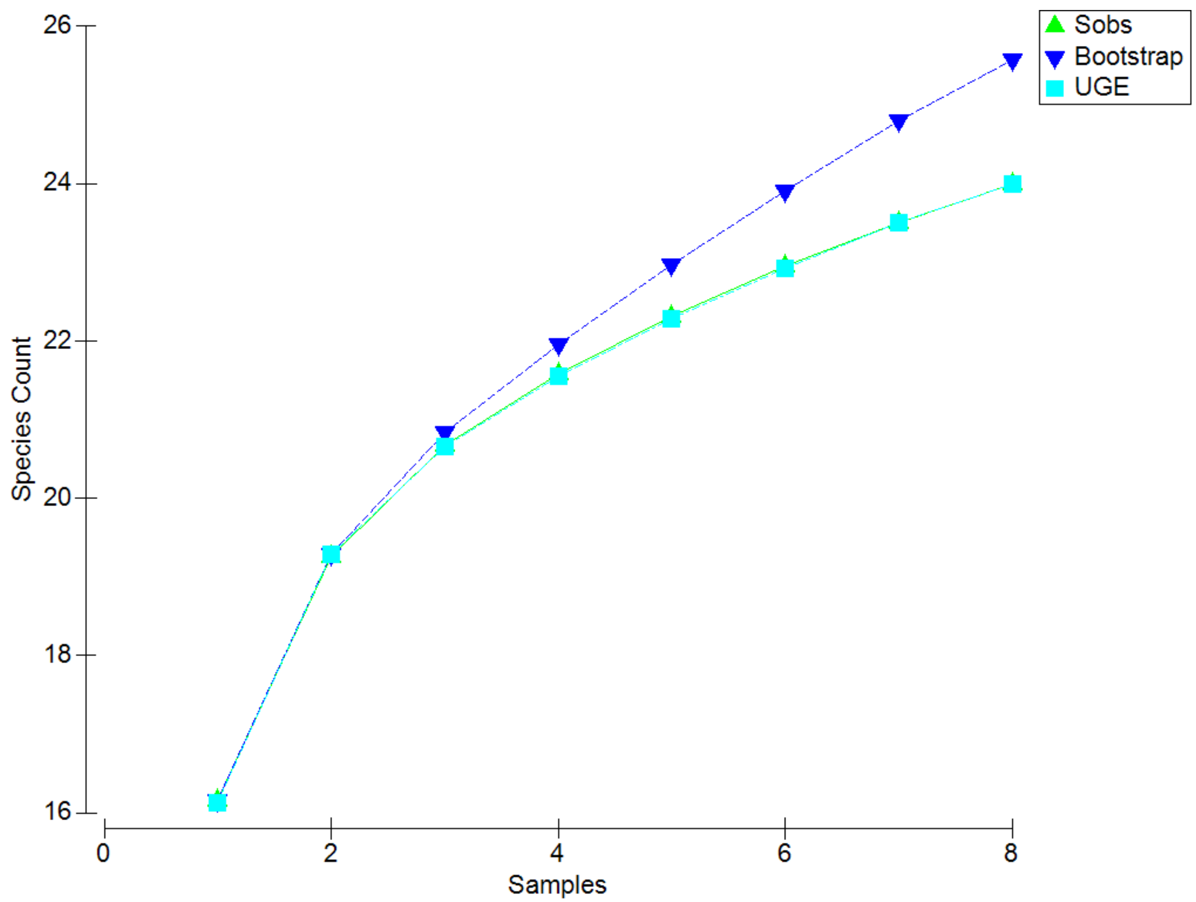

Figure 18 A species accumulation plot as a function of the number of samples estimated by Sobs, Bootstrap, and UGE estimators.

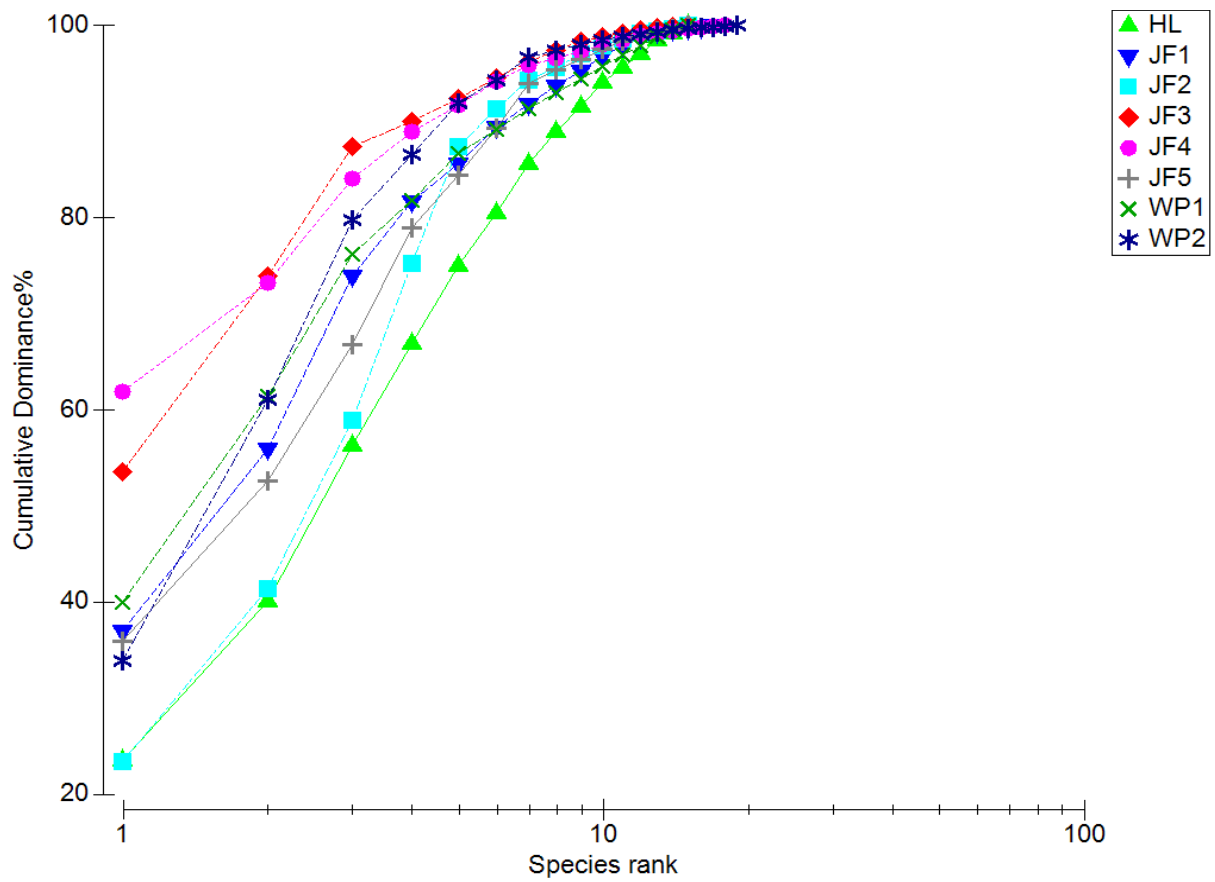

Figure 19 A dominance plot of species for each sample as a function of species rank and the percent of cumulative dominance of species in each sample.

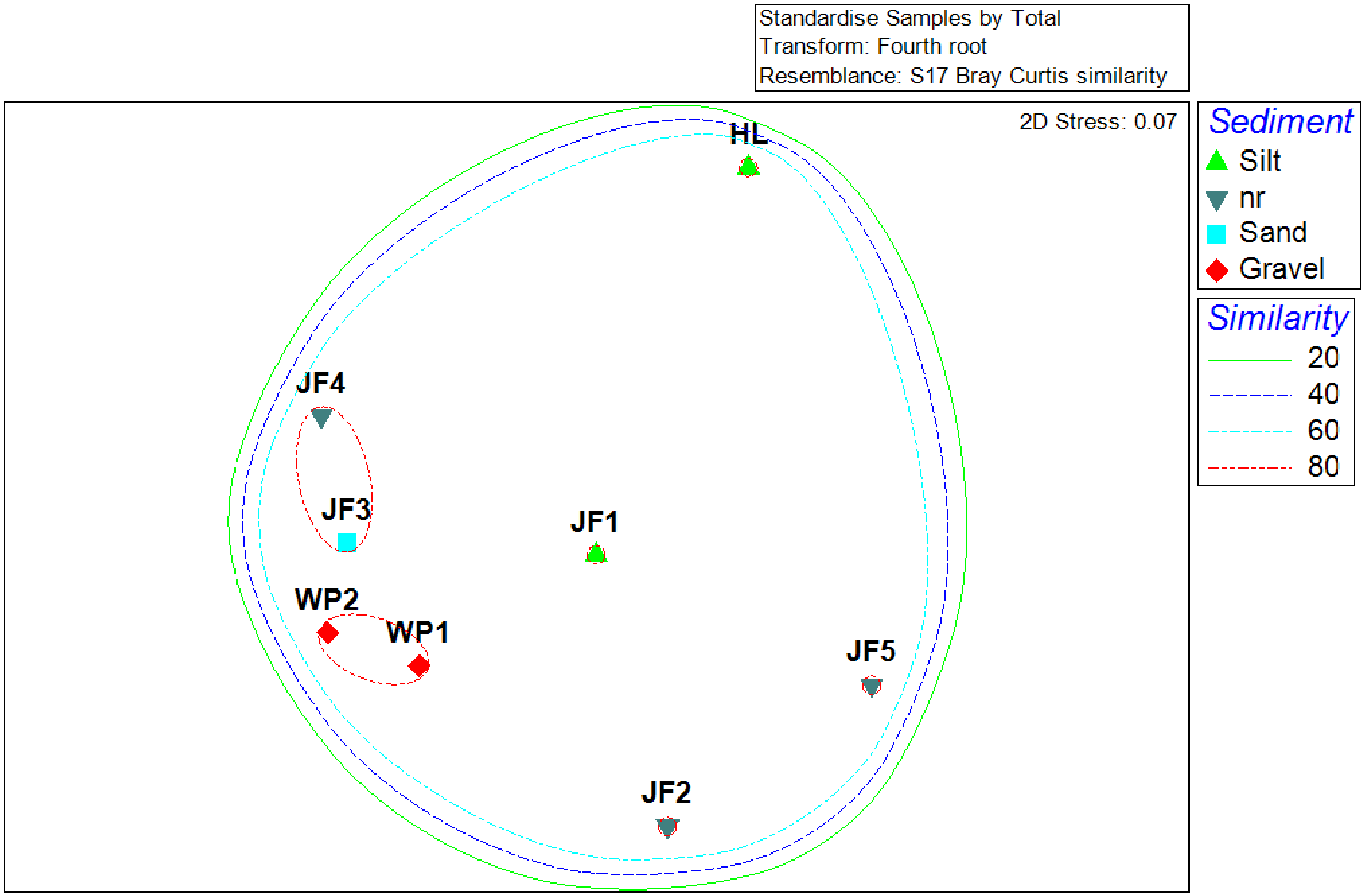

Figure 20 MDS plot of site similarity organized by sampling site, with the inclusion of sediment type.

Chapter 4

DISCUSSION

My initial goal in this project was to locate and assess the abundance of juvenile horseshoe crabs in the Inland Bays of Delaware. While I did not find as abundant a number of horseshoe crab specimens as initially anticipated, the study revealed useful information about the utility of the sampling method and the faunal characteristics of the Inland Bays.

4.1 Suction Dredge Sampling Device

The suction dredge sampling device has been used to successfully collect juvenile horseshoe crabs in a previous field test in Delaware Bay (Burton et al. 2009). While our sampling effort for juvenile horseshoe crabs in the Inland Bays did not turn up the same densities of the target organism that were reported for Delaware Bay (means ranging from 2.9 to 45.5 individuals, depending on the month and location, per 15.2 m trawl), the overall functionality of the sampling device was matched in this study. Burton et al. reported individuals of 4.0 mm and greater in prosomal width, which overlaps our target size range of 3.0 mm or greater sized juveniles. In our study we found a trilobite larva at a size of 3.36 mm prosomal width and other benthic organisms of varying sizes were also collected using the suction dredge, including organisms approximately 5.0 mm and greater in size. The smallest item measured was a horseshoe crab egg fragment with a diameter of 2.1 mm; however, much smaller organisms and organic items existed in the sample. The dredge successfully collected a large assortment of organisms with a varied size range, including juvenile horseshoe crabs, in a shallow water environment with macroalgae abundant enough to clog the dredge intake at times and sediment types ranging from silt to gravel. The suction dredge sampling device has proven to be an appropriate and effective sampling device in the Inland Bays.

4.2 Collection of Juvenile Horseshoe Crabs

Despite the two-day sampling period and variation in sampling location, the presence of juvenile horseshoe crabs in the samples was less than desired. The lack of target specimens is probably due to the lateness of the sampling effort. Studies have reported that juvenile horseshoe crabs are present on tidal flats for the first few months after hatching, but move offshore as they get larger over the summer months (Botton and Loveland 2003, Rudloe 1981, Burton 2009). By the end of September, young of the year horseshoe crabs could be entering their fifth instar (Carmichael et al. 2003). As temperatures begin to fall, adult horseshoe crabs in deeper water will bury themselves in the sediment and remain somewhat sedentary (Moore and Perrin 2007). The idea that juvenile horseshoe crabs may also experience this sedentary state is supported by the fact that the molt to the fifth instar coincides temporally with the end of the summer season. Additionally, the fifth instar can last for up to four months, in comparison with approximately two months of rapid growth for the first four stages combined (Carmichael et al. 2003). A significantly extended intermolt period during the fall and winter months after rapid transitions between other instars could lend support to the idea of a state of decreased activity for these juveniles. They may bury themselves in the sediment offshore, as has been observed adult horseshoe crabs.

The studies that have collected juvenile horseshoe crabs from tidal flats have primarily sampled in the summer months and have found decreasing abundances as the season progresses; even Burton (2009) shows a low mean for horseshoe crab abundance in October, ranging only from 2.9 to 4.6 horseshoe crabs per 15.2 m trawl. Our sampling, conducted at the end of September and beginning of October, is in the range of time where juveniles may be in or transitioning to their fifth instar, and thus they may have already buried themselves offshore of the tidal flats and shallows where our efforts were focused, potentially deep enough in the sediment to be beyond the reach of the suction dredge sampling device. Evidence of juvenile horseshoe crabs was present at a majority of sites, however, indicating that juvenile horseshoe crabs can be found in the sampling area at other times, most likely in the summer season.

4.3 Comparison to Previous Benthic Surveys and Statistical Analyses

The survey conducted by Maurer (1977) was a comprehensive assessment of the benthic fauna in Indian River and Rehoboth Bays at the time. Since this survey, however, there has not been another study of its kind conducted. The present survey, while on a much smaller scale and with distinctly different sampling methods, serves as a comparable snapshot of the current benthic assemblages at several sites in Indian River Bay and Rehoboth Bay as well.

The comparison of species composition between these two studies shows differences. The top four organisms found in Maurer's study consisted of one amphipod (the most abundant organism, *Ampelisca abdita*) and three bivalves. While the top four organisms in the present study differ, the taxa represented are similar. *Listriella barnardi* (the most abundant), *Jassa marmorea* (the second most abundant), and the caprellid species (fourth most abundant) found in the present study are all amphipods as well. Though mollusc species did not have the same magnitude of abundance in our study as did the arthropods, *Gemma gemma,* the second most abundant species in Maurer's study, was found in notable amounts in our study. Many of the organisms found in our study were also present in Maurer's in varying abundances. *Mysidopsis bigelowi, Callinectes sapidus, Illyanassa obsoleta,* and *Crangon septemspinosa,* were all found in greater abundances in our study than in Maurer's, while *Gemma gemma* and *Acteocina canaliculata* were less abundant in our study than in Maurer's. The species *Turbonilla interrupta, Oxyurostylis smithi, Crepidula convexa, Diopatra cuprea, Edotia triloba,* and *Pectinaria gouldi* were all found in similar abundances in our study as in Maurer's.

Some of the differences between the composition of benthic organisms in this study and Maurer's can be attributed to the different sampling methods utilized in the two studies. These differences are especially pertinent when comparing abundances of bivalves and other sediment-dwelling molluscs. Maurer used both a weighted Peterson grab sampler and a box dredge with 40 mm diameter chain links, allowing for larger organisms to be collected, as well as infauna such as the bivalves *Gemma gemma, Tellina agilis,* and *Mercinaria mercinaria,* three out of the four most abundant organisms in Maurer's study. Maurer's most abundant organism, the amphipod *Ampelisca abdita,* is a benthic tube dwelling organism as well. Our suction dredge sampling device could only collect from the epibenthos by design, thus excluding organisms buried in the sediment, and rigid organisms greater than 25.4 mm in length, width, or diameter. However, the minimum size range of organisms in both studies is similar as samples were sieved through a 1.0 mm mesh in both Maurer's study and in the present study. Maurer also sampled much farther into the headwaters and channels of Indian River Bay, which may account for some species in Maurer's study which were not present in our samples.

There was found to be a higher average evenness in this study than in Maurer's study for any sampling events. The average evenness of Maurer's seasonal samples ranged from 0.18 to 0.45, whereas the average evenness of this study is approximately 0.66. The evenness and diversity of each site differs, however, as do the expected number of species. The least diverse sites, JF3 and JF4, are highly similar in their species composition, and also have the lowest expected species, as would be expected. It is unclear why they are distinctly different from the other James Farm sites; however, they are the closest James Farm sites to shore, which may influence their habitability. The species accumulation plot and dominance plot give further detail to the present study, and while it may not be as robust as Maurer's, these analysis show that I have taken a reasonable subset of the present organisms of the Inland Bays.

It was noted in Chaillou et al. (1996) that in 1993, the benthic community of Indian River Bay was dominated by amphipods, which made up 75% of the total organismal abundance. Amphipods also dominate our study, with a total of 3368 organisms, or 64% of the total. In Maurer's study, amphipods comprised only 18.1% of the total organismal composition. Compared to the Delmarva Power and Light study (1976), conducted within a decade of Maurer's study, the percentages of organismal composition have been skewed from an equal division between polychaetes, amphipods, and bivalve molluscs. A fairly equal division can also be seen in Maurer's study between polychaetes, amphipods, other arthropods, pelecypods, and gastropods. While literature exists on the use of amphipods as bioindicators, it is not clear whether the species found in this study may be positively impacted by the water quality changes seen in the Inland Bays. However, with the increase in degraded areas in the Inland Bays since these previous surveys were conducted, it is plausible that the changes in the Inland Bays may be facilitating a growth in abundance for amphipods.

Maurer's 1977 survey is the most comprehensive study of benthic organism abundances in Delaware's Inland Bays to date. While the present study does not rival Maurer's in size or depth of analysis, it does serve as a baseline that would be useful in exploring organismal changes in the Inland Bays over the last 40 years, when combined with current and historical usage and water quality data. It also shows that the suction dredge sampling device can be used to sample many different taxa of benthic organisms of a variety of sizes. Determining if organismal abundances have changed significantly since Maurer's study in 1977 is a question that is beyond the scope of this study, but one that could be investigated with this method in combination with traditional grab sampling. This study can be used as a guide for future benthic surveys, especially those focusing on epifauna, using the suction dredge sampling device.

4.4 Future Sampling

This study has shown that the suction dredge sampling device is a useful tool for collecting benthic epifauna from the Inland Bays of Delaware, including juvenile horseshoe crabs. With the knowledge of how to construct and use the dredge, I propose that D. Miller and future students continue sampling for juvenile horseshoe crabs in the Inland Bays using this method. It is reasonable to exclude sites which lacked evidence of juvenile horseshoe crabs (Peninsula and Big Ditch) from future studies, along with sites that have sediment characteristics similar to those excluded sites (silt and shell hash, respectively). Most importantly, since the target time for collecting juvenile horseshoe crabs in their early instars is during July and August, future sampling should be conducted much earlier in the season if boat availability permits.

Future studies will hopefully shed further light on the abundance and distribution of juvenile horseshoe crabs in the Inland Bays. While spawning surveys provide us with an adult population size in the Inland Bays, we have yet to quantify any subset of the juvenile population. A spatial survey of juveniles that could be compared to Burton’s 2009 study of Delaware Bay with the suction dredge sampling device would be valuable support to the comparison of the Inland Bays and Delaware Bays as equally important horseshoe crab habitat. To determine what sediment types, locations at depth, and at what distance from shore juvenile horseshoe crabs are found in greatest numbers in the Inland Bays is another important goal that this study was not able to achieve, but one that may be explored in future studies following the recommendations put forth here. Studies of other systems have shown that horseshoe crabs are found nearshore in their early instar stages and move farther offshore as they grow, and if this is true in the Inland Bays as well, it may explain why the abundance of juvenile horseshoe crabs at our nearshore sampling sites was so low. However, if juveniles do move into deeper water as the season progresses, the question remains as to where and how deep they go. I hope that future studies will also explore this aspect of juvenile horseshoe crab ecology in the Inland Bays. It is necessary to understand the whole life history of this economically and environmentally important organism in order to best utilize and protect them as both a resource and an intrinsically valuable creature. As the juvenile stages of horseshoe crabs are the least understood, further research on juvenile ecology is wholeheartedly encouraged, as it can only aid us in the effort to preserve this uniquely important species.

Chapter 5

CONCLUSION

Horseshoe crabs are unique and valuable organisms that are found most famously in Delaware Bay, but are also found in the Inland Bays of Delaware in very similar numbers. The quantification of juvenile horseshoe crabs in the Inland Bays can give further weight to the comparison of the Inland Bays to Delaware Bay in terms of importance as horseshoe crab habitat. With this in mind, I hoped to quantify the presence of juvenile horseshoe crabs in the Inland Bays by sampling several sites throughout Indian River Bay and Rehoboth Bay with a suction dredge sampling device. Using the suction dredge, evidence of juvenile horseshoe crabs and other benthic organisms were collected from Indian River Bay in September and October of 2011. A juvenile horseshoe crab in the trilobite stage, three embryos, five juvenile molts, and 65 horseshoe crab egg shell fragments were found. In addition, benthic fauna representing nine phyla were collected. Arthropods were the most abundant group: seventeen species of arthropods were identified, including five species of amphipods, with *Listriella barnardi* and *Jassa marmorea* as the most abundant species overall. Molluscs were also well-represented with eight species, but in lower abundances than the arthropods. Several sample sites contained highly similar assemblages, and evenness and overall diversity was very high at most sites. This study ultimately demonstrated that there is evidence of juvenile horseshoe crabs in the Inland Bays of Delaware, and that the suction dredge sampling device is an effective method for collecting juvenile horseshoe crabs and other benthic organisms. Future studies should take samples earlier in the season and closer to beaches known to be used for nesting. To elucidate the full life histories of horseshoe crabs in the wild is essential for the protection, maintenance, and management of horseshoe crab populations and the resources they provide.

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Appendix A

LIST OF TAXA BY SITE

Taxa	Species	JF1	JF2	HL	WP1	WP2	JF3	JF4	JF5										
	Macroalgae sp.	yes	yes		yes		yes	yes	yes										
Chlorophyta	***Ulva***							yes											
	unidentified anemone	14	61				17	8	23										
Ctenostomatida	***Amathia convoluta***			yes															
Gastropoda	***Acteocina canaliculata***					3													
	Astyris lunata	5	1	2		1			1										
	Boreotrophan truncatus		1																
	Crepidula convexa		5		6	5	3		17										
	Ilyanassa obsoleta	12		15	7	2	13	15	20										
	Ilyanassa trivittata			4															
	Turbonilla interrupta	49	15	45	4	1			2										
Bivalvia	***Gemma gemma***	1		9		7		2	3										
Polychaeta	***Diopatra cuprea***			11			3												
	Neanthes succinea	3				2													
	Pectinaria gouldi							1											
	Sthenelais boa				1														
	Capetellid sp.			2															
	Annelid sp. A			5															
	Annelid sp. B			3															
Xiphosurida	***Limulus polyphemus***	8 frags, 1 yellow egg	1 egg shell	10 frags, 1 molt, 1 embryo	18 frags	1 trilobite	2 frags	4 molts	1 embryo				1 embryo		12 frags	13 frags			
Pycnogonida	***Callipallene***	4	3	14	2	2	3	1											

	brevirostris								
Isopoda	***Edotia triloba***	1				1	1	1	
	Erichsonella filiformis	24	11		5	4	10	22	18
Amphipoda	**Caprellid sp.**	1	1	29	92	275	17	6	1
	Listriella barnardi	234	61	64	171	498	434	555	61
	Jassa marmorea	120	67	44	63	99	166	101	131
	Melita dentata							6	2
	Misc. amphipods	10	3		11	35	8	2	
Mysida	***Mysidopsis bigelowi***	15	87	22	9	400	109	97	5
Caridea	***Crangon septemspinosa***	10	5	2	3	11	1	5	4
	Palaemonetes vulgaris	25	45	4	4	9	21	25	44
Pleocyemata	***Calinectes sapidus***	114	65	7	24	79	20	44	52
	Pagurus longicarous	1	2		6	3	1	2	4
	Pinnixa sayana							1	
	Xanthid sp.			4				4	
Cumacea	***Oxyurostylis smithi***	7		7	21	35	4	7	
Holothuroidea	***Pentamera pulcherrima***								1
Enteropneusta	***Saccoglossus kowalevskii***	1							
Tunicata	***Botryllus schlosseri***	yes	yes				yes		yes
	Mogula sp.	19	31	1		1	3	1	60
Perciformes	***Gobiosoma bosc***	8	6		1	1	4	2	11