SEASONAL AND HISTORICAL MESOZOOPLANKTON DYNAMICS IN DELAWARE BAY:
AN APPLICATION AND OPTIMIZATION OF THE ZOOSCAN OPTICAL IMAGING TOOL

by

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ABSTRACT

The mesozooplankton community in Delaware Bay is an important part of the pelagic ecosystem, connecting primary production to higher trophic levels while also representing larval forms of commercially important species such as blue crabs and eastern oysters. While Delaware Bay’s physico-chemical characteristics and primary production are well-described, its mesozooplankton community remains relatively understudied, particularly in comparison to Chesapeake Bay. To understand future changes to this estuarine system and compare it to other estuaries in the region (e.g., Chesapeake Bay), we need an updated characterization of Delaware Bay’s mesozooplankton community and its relationship to key environmental variables that have changed over time, or likely will change into the future. To this end, I describe here a year-long, seasonal time series to characterize and quantify Delaware Bay zooplankton using a new optical instrument ZooScan, and compare my results to the historical data presented in Cronin et al. (1962). Results found seasonal changes in community composition and species dominance. Historical comparisons show a remarkable similarity in the abundance of the dominant zooplankter Acartia tonsa found by Cronin et al. (1962) and the present study. These results have implications for demonstrating how mesozooplankton respond to systemic changes. I also present a new method of determining biovolume in highly turbid areas where standard displacement volumes are skewed by non-zooplankter material such as detritus. Finally, I present a validation and application of a citizen-based zooplankton collection method for use by volunteer monitoring groups.
Chapter 1

INTRODUCTION

Mesozooplankton are a taxonomically diverse assemblage of aquatic animals whose locomotory abilities are insufficient to counteract currents (Lenz 2000). While typically small in size (0.2 – 20 mm, Dussart 1965), they span a broad range of taxa, from small crustaceans such as copepods that remain planktonic for their entire life history (holoplankton), to larvae of economically important species such as finfish, oysters, and crabs remaining planktonic for a portion of their life history (meroplankton; Omori and Ikeda 1984, Pennock and Herman 1988). As such, mesozooplankton are a key component in aquatic food webs (Cushing 1990; Kimmel et al. 2012) linking primary production to higher trophic levels (Verity and Smetacek 1996).

The connection between mesozooplankton and physico-chemical characteristics of the surrounding environment has been studied for over 60 years (Barlow 1955), and recent work details the links between mesozooplankton (both specific taxa and community composition), specific local environmental variables, and regional climate shifts, particularly in estuaries (Kimmel and Roman 2004, Elliot et al. 2012, Li et al. 2013). Many studies have found freshwater input, temperature, turbidity, and dissolved oxygen to be among the most important environmental factors determining estuarine community composition (Kimmel and Roman 2004; Li et al. 2013; Taglialatela et al. 2014). These factors are predicted to change in estuaries due to local effects of global climate change, with temperatures increasing, dissolved
oxygen levels decreasing, and precipitation patterns changing (Kunkel et al. 2013; Altieri and Gedan 2015). By understanding the connections between these factors and mesozooplankton in Delaware Bay, we could further demonstrate the utility of mesozooplankton as biological indicators of systemic change, such as eutrophication or climate change, as has been shown in other estuaries (Bianchi et al. 2003; Albaina et al. 2009; Rice et al. 2015).

Delaware Bay is a well-mixed estuary extending 134 miles from Trenton Falls to the bay mouth between Cape Henlopen, DE and Cape May, NJ. It is home to recreational and commercial fisheries for oysters, blue crabs, striped bass and other finfish, as well as large industrial ports with crude oil being the largest commodity imported (US ACE 2011). Delaware Bay has undergone large physico-chemical changes in the past 50 years, particularly in its upper reaches near urban areas like Wilmington and Philadelphia. Hypoxic events in this region have abated after implementation of modern wastewater treatments that limited nutrient input (Sharp 2010). This is a major difference from Chesapeake Bay, which experiences long periods of seasonal hypoxia that is reflected in its mesozooplankton community. Despite these economic and environmental interests, there has not been a comprehensive investigation of Delaware Bay’s mesozooplankton community since the middle of last century (Cronin et al. 1962). Since that time, mesozooplankton sampling within the bay has examined restricted areas over limited time scales (e.g., Mauer 1978, Meredith 1982, Petrone 2001), with particular emphasis on larval biology and recruitment of crustaceans (Jones and Epifanio 1995, Epifanio et al. 2013). To understand future changes to this estuarine system as a whole, and to compare it to other estuaries in the region (e.g., Chesapeake Bay), we need an updated
characterization of Delaware Bay’s mesozooplankton community and its relationship to key environmental variables that have changed over time, or likely will change into the future.

Studying mesozooplankton communities presents specific challenges for researchers. Their generally small size has historically required the use of microscopy for identification and quantification, making large collections tedious to process which may ultimately limit researchers in their spatio-temporal sampling resolution (Paffenhofer 1989). The temporal component is especially important in understanding the ‘patchiness’ of mesozooplankton community dynamics, as certain species will only be present in collections at specific times, especially larval meroplankton (Petrone et al. 2005; Mackas et al. 2012). Regional time series such as the Bermuda Atlantic Time series (Lomas et al. 2013) are useful in understanding these dynamics and mesozooplankton response to environmental variability and climate change. Other programs such as the Continuous Plankton Recorder (Yoshiki et al. 2013), try to understand mesozooplankton dynamics as they vary through space by continuously sampling during trans-oceanic cruises. These collections generate extensive mesozooplankton samples for identification, which can be a bottleneck in analyzing mesozooplankton dynamics.

Given these demands for increased processing capacity, researchers have been investigating new techniques for rapid processing and identification, specifically using optical imaging tools (Benfield et al. 2007). Most of these tools are designed for in situ water column sampling, eliminating the need for fixation, filtering, and splitting in the laboratory. Examples include the Shadowed Image Particle Profiling Evaluation Recorder (SIPPER) (Remsen 2008), the In-Situ Ichthyoplankton Imaging System
(ISIIS) (Cowen and Guigand 2008), and ZooVis (Bi et al. 2013). Images obtained from these tools can then be manually identified or processed by software to automatically identify taxa and quantify size spectra, abundance, and community composition. However, these tools do have limitations. Without a physical collection, researchers are unable to verify their results through manual microscopy, which may be particularly important if image quality is poor. Abundances may be misrepresented, and rare species may not be properly identified (Broughton and Lough 2006). This is particularly true in highly turbid areas like estuaries, where these tools might work for gelatinous organisms such as ctenophores (Bi et al. 2013), but not for smaller zooplankters (Zhang et al. 2000). Similar to physical collections, the ease of image collection also creates backlogs of unprocessed data that prevent researchers from performing timely analysis. Other tools, such as the ZooScan optical scanner with associated software (Gorsky et al. 2010), offer a benchtop method where mesozooplankton are collected and fixed before analysis. While this does require more steps compared to in situ methods, it does allow researchers to keep samples on hand for reanalysis or microscopy to verify identifications. Both bench top and in situ tools can increase processing capacity (Benfield et al. 2007), but they do require manually constructed libraries of typical zooplankters in the areas they are utilized.

This thesis updates the characterization of Delaware Bay mesozooplankton, including any historical shifts, and discusses the development of two new tools for mesozooplankton research in the mid-Atlantic region: the ZooScan optical scanner and a citizen-based mesozooplankton monitoring method in order to increase collection capacity. I will outline and discuss the results of a year-long, seasonal time series of mesozooplankton throughout Delaware Bay from Fall 2014 to Fall 2015,
including quantitative historical comparisons to data from Cronin et al. (1962) in order to understand how this mesozooplankton community has changed since the last bay-wide mesozooplankton research took place (Chapter 2). I will then present a new application of the ZooScan system that can estimate biovolume in turbid areas where standard biovolume measurements are skewed (Chapter 3). Additionally, I will detail the validation of a method of mesozooplankton collection for use by citizen water quality monitoring networks using the ZooScan system to rapidly process samples (Chapter 4). Finally, I will conclude with the major findings of this thesis documents and offer insight on the next steps of mesozooplankton research in Delaware Bay and other coastal areas (Chapter 5).
Chapter 2

SEASONAL AND HISTORICAL DYNAMICS IN THE MESOZOOPLANKTON COMMUNITY OF DELAWARE BAY

2.1 Introduction

Delaware Bay is a well-mixed, temperate estuary on the US mid-Atlantic coast, extending 216 km from Trenton Falls, NJ to its mouth between Cape Henlopen, DE and Cape May, NJ. Over the past 50 years, extensive physico-chemical monitoring of the main channel has revealed a decrease in hypoxic events in the upper estuary, attributed largely to decreased nutrient input from urban areas (Sharp 2010). In the next 100 years, climate predictions forecast an increase in both frequency and intensity of precipitation events in the Delaware Bay watershed (Kunkel et al. 2013), likely changing the freshwater-saltwater dynamics, stratification, and carbon cycling of the estuary (Canuel et al. 2012). These physico-chemical changes, both past and future, may be reflected in the estuary’s mesozooplankton.

The mesozooplankton community in the Delaware Bay is a key ecological component in the pelagic environment (Cushing 1990; Verity and Smetacek 1996; Zollner et al. 2009), and while primary production throughout Delaware Bay has been detailed extensively for the past 50 years (Yoshiyama and Sharp 2006; Sharp 2010; Voynova et al. 2013), mesozooplankton remain relatively understudied. The work of Cronin et al. (1962) represents the last time Delaware Bay’s mesozooplankton were systematically characterized from tidal freshwater to the coastal ocean on a seasonal time scale. The authors detailed a system that was dominated by a few species of
calanoid copepods, particularly *Acartia tonsa* and *Eurytemora affinis*, with higher abundances in the spring and summer compared to fall and winter. All studies since then have focused either on small areas with high-resolution time scales (Maurer et al. 1978; Petrone 2001) or larval recruitment of crustaceans (Jones and Epifanio 1995; Epifanio et al. 2013).

Recent analyses in other estuaries have revealed links between environmental gradients, such as dissolved oxygen and salinity, and mesozooplankton taxa and communities (Kimmel and Roman 2004; Elliott et al. 2012; Li et al. 2013). In particular, Kimmel et al. (2012) was able to indirectly link a 60 year decline in *A. tonsa* abundances in Chesapeake Bay to eutrophication over that same time period. These findings were only possible with lengthy mesozooplankton time series that span over decades. Cronin et al. (1962) did detail the temperature, salinity, and oxygen gradients present in Delaware Bay during their sampling and while they discussed the possibility of relationships between these physico-chemical parameters and mesozooplankton taxa, they made no quantitative comparisons.

The lack of a long-term, comprehensive mesozooplankton time series in the physico-chemically dynamic Delaware Bay is problematic to understanding spatio-temporal trends in mesozooplankton and their correlation with important environmental parameters such as salinity, temperature, and dissolved oxygen. It is unknown if historical trends or environmental drivers similar to those found in the Chesapeake Bay mesozooplankton community exist. Understanding these trends in Delaware Bay could help describe the similarities and differences in trophic interactions found in both Delaware Bay and Chesapeake Bay. In order fill this knowledge gap, we conducted a year-long, seasonal time series throughout Delaware
Bay to (1) characterize seasonal and spatial mesozooplankton abundance and community composition throughout the estuary, (2) use multivariate analyses to understand which environmental gradients influence which individual taxa, and (3) discover historical trends by comparing both individual taxa and community data to those reported by Cronin et al. (1962).

2.2 Materials and Methods

Sample Collection and Processing

I sampled 16 stations throughout Delaware Bay (Figure 1) in five seasons: Fall 2014, Spring 2015, Early Summer 2015, Late Summer 2015, Fall 2015 (see Appendix A for dates). Ten stations followed the main channel from 11 km outside the bay mouth in the Atlantic Ocean (Station 12) to tidal freshwater near Wilmington, DE (Station 3), with station locations selected to mirror those of Cronin et al. (1962). Three stations were located in the southwest area near the Delaware shoreline, and another three were near the northeast New Jersey shoreline. At each station, I obtained water column profiles of temperature, salinity, dissolved oxygen, turbidity, and chlorophyll fluorescence with a YSI 6600 V2 data sonde. River discharge data were obtained from the US Geological Survey (USGS) gauging station in Trenton, NJ (USGS Station 01463500).

Mesozooplankton were collected using a bongo-frame with 0.5m nets of 200 µm mesh fitted with filtering cod ends and flow meters to quantify volume sampled. I used a double oblique tow (Sherman et al. 1998) during daylight hours only, deploying the nets down to ~1-3 m above the bottom, holding at depth for 30 seconds, and recovering. Once on board, both nets were carefully sprayed from the outside with ambient water to wash animals on the sides of net into the cod end. The sample from
one net was immediately fixed in 4% borax-buffered formaldehyde, minus any large gelatinous mesozooplankton such as the ctenophore *Mnemiopsis leidyi* which were separated, rinsed to remove any other mesozooplankton, and counted. The other net’s contents were size-fractionated live and then frozen for later wet weight/dry weight analysis (see Chapter 3).

The fixed samples were processed and scanned with a ZooScan optical scanner within one month of collection, using the methods described in Gorsky et al. (2010). Briefly, each sample was transferred into freshwater and sieved into three size fractions (>1000 µm, >500 µm, and >200 µm) to minimize loss of larger taxa in the splitting process. Each size fraction was split using a Folsom splitter to achieve ~1000-1200 individuals in the final split, and scanned. Images were processed by normalizing their grey levels, extracting individual objects, and producing measurements for these objects using ZooProcess software (for a detailed description, see Gorsky et al. (2010)). I used Plankton ID (PkID; Gasparini 2013) with the Random Forest algorithm (see Appendix B for list of parameters used in identification) to autonomously place the extracted objects in each scan into predicted categories using a learning set specific to Delaware Bay (see Chapter 3) and manually validated each object’s placement to ensure accuracy. Finally, the validated identifications, along with their object measurements, were compiled to show community composition, abundance and size spectra for each station.

*Data Analysis*

Using the abundance data produced by PkID, I calculated the Shannon-Weiner diversity index (Macarthur and Macarthur 1961), as well as Pielou’s evenness index (Pielou 1969) for each station during each season to quantify mesozooplankton
community diversity and taxa dominance. Since the taxonomic delineations for these abundances varied from order level to species level, I grouped all abundances to the order level to ensure proper comparison. I then looked at seasonal changes in diversity and evenness for the entire estuary using the Kruskal-Wallis One Way ANOVA on Ranks.

To understand the relationships between mesozooplankton abundance and environmental variables, I used Redundancy Analysis (RDA), a linear, multivariate ordination and regression technique. For each season, mesozooplankton abundances were Hellinger-transformed (Legendre and Gallagher 2001) and regressed against depth-averaged temperature, salinity, turbidity, chlorophyll $a$, dissolved oxygen, stratification (measured as the difference in salinity between the water/sediment interface and the surface of the of the water column), and tidal stage (measured as height above or below mean lower low water, obtained from predicted tides at the location closest to each station in the NOAA Tides and Currents program (https://tidesandcurrents.noaa.gov/programs.html). The resultant model for each season was tested for significance using Monte Carlo permutation tests (1000 permutations, $\alpha = 0.05$). All five seasons passed this initial significance test, so I then used forward selection and variation partitioning along with Monte-Carlo permutation tests to reduce our models to only those environmental variables that significantly explain zooplankton abundance, and examine the variation in abundance that they explain. All RDA statistical analyses were performed in R using the vegan community ecology package (http://CRAN.R-project.org/package=vegan).

To make direct historical comparisons, I obtained data directly from figures and tables in Cronin et al. (1962). I compared the total diversity and evenness of the
mesozooplankton community during four seasons (Fall, Spring, Early Summer, and Late Summer) between this study and Cronin et al. (1962) using order-level taxonomic resolution. I used the same four seasons to compare diversity and evenness, then divided the bay into three salinity regions: polyhaline (18-35 psu), mesohaline (5 – 17 psu), and oligohaline (0.1-5 psu). I then extracted the abundance data of *Acartia tonsa* in order to conduct a three-way ANOVA to find differences between cruise, season, and location. Only abundances from the 10 stations in the main channel that were sampled by Cronin et al. (1962) were used for this comparison. Abundance data from both Cronin et al. (1962) and this study were log-transformed before all analyses.

### 2.3 Results

**Environmental Parameters**

Delaware River discharge during this time series ranged from 100-200 m$^3$s$^{-1}$ in Fall of 2014 to an acute high flow event of ~1200 m$^3$s$^{-1}$ in July (Figure 2). Aside from this event, river flows during this period followed the same seasonal patterns seen in the monthly averages over a 65-year period. Compared to 1952, 1953, and 2014, the winter/spring discharge in 2015 had smaller high flow peaks, though it should be noted that in January and February of 2015 there was significant ice coverage in the river, and flows for this period were only estimated by USGS. Sampling cruises from November 2014, March 2015, June 2015, and August 2015 were closest to the sampling cruises conducted by Cronin et al. (1962) in Fall 1951, February 1952, June 1953, and August 1953, respectively. Data from these cruises were used for historical comparison.

Depth-averaged temperature, salinity, turbidity, dissolved oxygen, and chlorophyll $a$ also show strong seasonal, as well as spatial, variation (Figure 3).
Salinity throughout the bay was lowest in Spring 2015 and highest in Fall 2015 (Figure 3). Temperature followed a predictable seasonal pattern, with low bay-wide temperatures for Fall 2014 and Spring 2015 (~6-8°C) to higher temperatures in Early and Late Summer 2015 (~20-25°C), followed by a cooler Fall 2015 (~15°C) (Figure 3). Turbidity in the lower bay was low for all seasons (0-10 NTU), while the turbidity maximum could be seen in the upper bay around Station 4, where we found the highest turbidity measurements (50-60 NTU) in Fall 2014 and Early Summer 2015 (Figure 3). Dissolved oxygen levels were high (between 80-110 % saturation) at all stations through all seasons, with two areas of supersaturation on the Delaware side of the lower bay during Early and Late Summer 2015 (Figure 3), which coincided with high chlorophyll a. Chlorophyll a displayed strong seasonal and spatial variation throughout the estuary, with a bay-wide low in Fall 2014, followed by a high concentration around Stations 6 and 7 in Spring 2015 (Figure 3). In Early and Late Summer 2015, this high concentration was located on the Delaware side, though it was higher in Late Summer 2015. By Fall 2015, chlorophyll a concentrations were lower bay-wide, with a high point on the New Jersey side, near Station 3-NJ.

*Mesozooplankton Community and Abundance*

The mesozooplankton community (Figure 4) was dominated by calanoid copepods at all stations and seasons. *Acartia tonsa* was the most persistent copepod throughout all seasons, occurring in abundances of ≥ 2,000 ind. m$^{-3}$ at one or more stations in all seasons except Spring 2015. The maximum abundance of *A. tonsa* was 7,944 ind. m$^{-3}$ at Station 10 in Early Summer 2015. In the upper estuary, *A. tonsa* was present except in Spring 2015 and Late Summer 2015, when other calanoid copepods were dominant (primarily *Eurytemora affinis*) (Figure 4). In general, *A. tonsa* was
most abundant in the middle to lower part of the estuary, but at Station 12 (just outside of the estuary), its abundance was less than other species of calanoids (Figure 4). Two species of the genus *Centropages* (*C. typicus* and *C. hamatus*), both larger calanoids, were present in the lower part of the estuary during most seasons, and dominated the mesozooplankton community in Spring 2015 (Figure 4). Other copepods included *Labidocera aestiva*, *Pseudodiaptomus pelagicus*, *E. affinis*, *Temora longicornis* and *T. turbinata*, and *Pseudocalanus* spp., but none as abundant as *A. tonsa* or *Centropages* spp. Copepodites that were too small to reliably identify with ZooScan were present in all seasons, with abundances > 1,000 ind. m$^{-3}$ at Stations 12 and 5 in Spring and Early Summer 2015, respectively.

Non-copepod taxa comprised a smaller portion of total mesozooplankton abundance compared to copepods, but were still present in all seasons (Figure 4). I consistently found the mysid shrimp *Neomysis americana* at Stations 4 and 5, particularly in Fall 2014 and Early Summer 2015 (Figure 4). Freshwater cladocerans (primarily *Bosmina* spp.) were most abundant at Station 3 during Spring 2015 and Early Summer 2015, while marine cladocerans (primarily *Penilia avirostris*, *Evadne norrdmanni*, *Pseudevadne tergestina*, and *Pleopsis polyphemoides*) were present throughout the middle and lower estuary in Spring 2015. They were, however, limited to the lower estuary during Early and Late Summer 2015 (Figure 4). Larvaceans, primarily *Oikopleura* spp., were also present in the lower bay in Early and Late Summer 2015 (Figure 4) with a maximum abundance of 305 ind. m$^{-3}$ at Station 12 in Early Summer 2015. Ctenophores, primarily *Mnemiopsis leidyi*, were present near the middle of the estuary in Late Summer 2015 and Fall 2014 and 2015 (Figure 4). Their highest abundance, 9 ind. m$^{-3}$ was at Station 6 in Late Summer 2015. I also found
hydromedusae sporadically throughout the bay, the highest concentration being 7 ind. m\(^{-3}\) at Station 12 in Fall 2015. The hydromedusa *Blackfordia virginica*, an invasive species from the Black Sea (Graham and Bayha 2007), was found at Station 5 in Early and Late Summer 2015 in concentrations of 0.5 and 5 ind. m\(^{-3}\), respectively.

Merooplankton comprised a small portion of the total mesozooplankton abundance throughout the time series, from 0.3% in spring to 7% in late summer. I found decapod zoea throughout most of the estuary in Early and Late Summer 2015, and in the lower estuary during Fall 2014 and 2015. Mollusk larvae, including both bivalve and gastropod larvae, were found in limited areas during Spring, Early, and Late Summer 2015. In Spring 2015, Stations 10 and 3-NJ had bivalve pediveliger larvae in abundances of 5 and 4 ind. m\(^{-3}\), respectively. In Early Summer 2015, I found larval Atlantic jackknife clams, *Ensis directus*, at an abundance of 7 ind. m\(^{-3}\) only at Station 11. This was the only time and place I found this species during this study. In Late Summer 2015, I found gastropod larvae near the mouth of the estuary at around 100 ind. m\(^{-3}\), as well as bivalve pediveligers near Stations 1-DE and 2-DE in concentrations around 10 ind. m\(^{-3}\). In addition, I found larval fishes throughout the estuary during all seasons except Spring 2015. Though identifications were difficult, I was able to identify larval *Micropogonias undulatus* at Station 5 and *Brevoortia tyrannis* at Station 12 in Fall 2014. All other specimens were too small to reliably identify with the ZooScan images, and beyond the scope of this project to pursue with manual identification, but the highest concentration was found in Early Summer 2015 at Station 2-NJ (52 ind. m\(^{-3}\)).

The mesozooplankton community structure of Delaware Bay changed seasonally, going from low diversity and low evenness in Fall 2014 to high diversity
and high evenness in Late Summer 2015 (Figure 5). Total abundance also changed seasonally, with the highest abundance found in Early Summer 2015 and the lowest in Fall 2015. The results of the Kruskal-Wallis test for the Shannon diversity index and Pielou’s evenness found a difference in both diversity and evenness between seasons (Shannon: $H=35.095$, $n=16$, df=4, $p<0.001$; Pielou: $H=27.006$, $n=16$, df=4, $p<0.007$). Subsequent pairwise Tukey Tests among seasons found that Late Summer 2015 had higher diversity than any other season, and Fall 2015 was more diverse than Fall 2014. Late Summer 2015 was also more even than Fall 2014 and Early Summer 2015, and Spring 2015 was more even than Fall 2014.

**RDA Analysis**

Redundancy analysis paired with forward selection and variation partitioning found that temperature, salinity, turbidity, and stratification were the most important environmental parameters for explaining mesozooplankton distribution throughout all seasons (Table 1). Salinity was a key environmental gradient during every season except Fall 2014, explaining between 7% and 29% of the variation in the mesozooplankton community. Temperature was a primary gradient during every season except Fall 2015, and explained between 9% and 16% of variation. Turbidity was forward selected in Fall 2014 and Late Summer 2015, and stratification was selected during Spring 2015 and Fall 2015, which were also the two highest periods of freshwater discharge (Figure 2). In looking at the ordination of mesozooplankton taxa with these forward-selected environmental gradients in all seasons (Figure 6, only Spring and Fall 2015 shown for simplicity), certain taxa were correlated with specific gradients. *Acartia tonsa* abundance was higher in areas of high stratification and increased temperature, while *Eurytemora affinis* were found in areas of low salinity.
Centropages spp. and Temora spp. were both correlated with higher salinity and temperature. Neomysis americana was more abundant in highly turbid areas.

**Historical Comparison**

By comparing both diversity and evenness of the bay-wide mesozooplankton community for four seasons, I found no change in either since Cronin et al. (1962) (Figure 7). The mean Shannon-Wiener diversity indices for Cronin et al. (1962) and this study were 0.38 and 0.34, respectively, with no significant difference (p=0.874, t=0.165, df=6). The mean Pielou evenness for Cronin et al. (1962) and this study was 0.16 and 0.20, respectively, and were also not significantly different (p=0.667, t=0.453, df=6).

Though I did not find any differences as measured by coarse diversity and evenness indices, I did find some differences on the individual taxa level (Figure 8). Generally, I found a decrease in Pseudodiaptomus pelagicus, Labidocera aestiva, freshwater cladocerans, chaetognaths, and Gammarus spp. in more than one season. Alternatively, I found an increase in Centropages spp., annelid larvae, Oikopleura spp., and fish larvae in more than one season. All other taxa exhibited both increases and decreases throughout the year, except Neomysis americana, whose abundance did not change in any of the four seasons.

Directly comparing Acartia tonsa abundances between Cronin et al. (1962) and the current study, I found differences that were dependent on season and region of the bay (Figure 9, Table 2). All pairwise comparisons were made using the Holm-Sidak method with an overall \( \alpha = 0.05 \). During the fall (Figure 9A), I found no difference between studies for the entire bay. In the spring (Figure 9B), however, Cronin et al. (1962) found significantly higher Acartia tonsa abundances in the polyhaline region of
the bay (p=0.002), and no differences between studies in the oligo- and mesohaline regions. Within each study, both found lower abundances in the oligohaline regions (p<0.001 for Cronin et al. (1962); p=0.003 for this study). In early summer (Figure 9C), abundance was higher in our study in the oligohaline region (p<0.001), but no difference was found for the other regions. Within Cronin et al. (1962), the polyhaline region had higher abundances than the mesohaline, which had higher abundances than the oligohaline (p<0.001), while I found no difference between salinity regions within this study. In late summer (Figure 9D), there were no differences between cruise in each region, nor were there differences between regions in each cruise.

2.4 Discussion

My study identified and quantified mesozooplankton taxa through seasonal changes over one year, found significant relationships between their abundance and several environmental variables, and compared aspects of the mesozooplankton community composition and abundance to historical data. I found calanoid copepods, particularly *Acartia tonsa* and *Centropages* spp., to be the dominant zooplankters in the estuary throughout the seasons we examined, whereas meroplankton exhibited specific seasonal and spatial fidelity. Community diversity and evenness were highest in Late Summer 2015, and generally low throughout the rest of the year. Through RDA analysis, we found temperature, salinity, stratification, and turbidity to be important correlates with the entire mesozooplankton community and its abundance, as well as specific taxa. Finally, while I saw no difference in the overall diversity and evenness of the mesozooplankton community over the past 60 years, I did observe
high seasonal and spatial variability in \textit{A. tonsa} when compared to Cronin et al. (1962), but no change on an annual, bay-wide basis.

The low diversity and high abundance of mesozooplankton in Delaware Bay fits the traditional ecological description of species in an estuary primarily attributed to high variation of ecological stressors such as salinity (McLusky and Elliot 2004). This is particularly evidenced by the numerical dominance of one species of copepod, \textit{Acartia tonsa}. This truly euryhaline species (Peck et al. 2015) has an almost complete lack of correlation to salinity in many temperate estuaries worldwide (Vinas et al. 2002; Albaina et al. 2009; Barbone et al. 2014), as well as those in other mid-Atlantic estuaries (Capriulo et al. 2002; Kimmel and Roman 2004; Kimmel et al. 2015).

Historically, \textit{A. tonsa} has been the dominant copepod of the Delaware Bay (Cronin et al. 1962; Maurer et al. 1978).

\textit{Acartia tonsa} abundances through all seasons increased with temperature and stratification, with little correlation to salinity or turbidity (when present in the RDA models). This was similar to the Chesapeake where \textit{A. tonsa} distributions were correlated with temperature and dissolved oxygen, which influenced egg production (Kimmel and Roman 2004; Kimmel et al. 2012). Recent work by Derisio et al. (2014) found \textit{A. tonsa} more abundant in the turbidity front zone of the Rio de Plata estuary, which was characterized by high turbidity and the furthest extent of the bottom salt wedge. They proposed that \textit{A. tonsa} used this two-layer circulation to maintain populations within the estuary and avoid export into the coastal ocean via both passive (physical aggregation) and active (selective vertical movements, release of eggs in the bottom layer) pathways. This agrees with Cronin et al. (1962), who proposed that a two-layer estuarine circulation was responsible for maintaining some zooplankton
populations within Delaware Bay. The correlation between *Acartia tonsa* and stratification in the present study also suggests that this species is using two-layer circulation during periods of high flow to maintain estuarine populations. Also, it is important to note that the second highest abundance I recorded was at Station 4 in Early Summer 2015, which also had one of the highest turbidity values for the entire time series.

The estuarine turbidity maximum zone (ETM), which was near Station 4, represented the breakpoint between a mesozooplankton community dominated by the epibenthic calanoid *Eurytemora affinis*, and *A. tonsa*. In Chesapeake Bay, the estuarine turbidity maximum zone (ETM) is dominated by *E. affinis* during periods of colder water with high freshwater runoff (lower salinity), and *A. tonsa* during warmer periods with lower freshwater runoff (higher salinity) (Kimmel and Roman 2004), which is consistent with other temperate estuaries (Hough and Naylor 1991; Morgan et al. 1997). Our data also show a similar pattern between these two species with respect to temperature and freshwater runoff, but the ETM in Delaware Bay is in a more saline region (Gay and O'Donnell 2009) and *A. tonsa* is more prevalent in this high turbidity region of the estuary than it is in Chesapeake Bay ETM (Roman et al. 2001).

Compared to *E. affinis*, the ETM could be more important for *A. tonsa* to maintain its population in Delaware Bay. *Eurytemora affinis* displays strong motility, remains close to the benthos, and exhibits selective tidal stream transport to maintain its position in upper parts of estuaries (Morgan et al. 1997; Schmitt et al. 2011). In contrast, *A. tonsa* is present throughout most of the water column, though they are strong vertical migrators and could still take advantage of two-layer circulation on diel
cycles (Cuker and Watson 2002; Gonçalves et al. 2012). Whereas *E. affinis* females keep their eggs, *A. tonsa* releases free planktonic eggs which are more susceptible to being exported out of the estuary (Mauchline 1998; Kiorboe et al. 2015). However, *A. tonsa* eggs do sink (Miller and Marcus 1994), and could remain in Delaware Bay with the up-estuary salt wedge drift, particularly if they are released close to that boundary layer (Mann and Lazier 2005). It would be interesting to see if there are more *A. tonsa* females releasing eggs near this boundary layer in the ETM compared to the rest of the water column, but unfortunately, the oblique tows I used in this study did not provide depth resolution for species distributions. Moreover, sampling during the day without methods to capture strong vertical migrators and epibenthic organisms such as *E. affinis* may underrepresent their abundance.

In Chesapeake Bay, there has been an overall decline in *A. tonsa* abundance since the 1960s, which was attributed indirectly to increased eutrophication, hypoxia, and its cascading ecological effects (Kimmel et al. 2012). This does not appear to be the case in the Delaware Bay, where I found some seasonal shifts from the 1950s, but the overall *A. tonsa* abundance was similar to that reported by Cronin et al. (1962). Kimmel et al. (2012) proposed the mechanisms for this decline were an increase in *A. tonsa* adult and egg mortality in hypoxic waters (Roman et al. 1993; Marcus et al. 2004) and an increase in predation by the ctenophore *Mnemiopsis leidyi* from both habitat compression (Pierson et al. 2009) and reduced predator escape response (Decker et al. 2004). While hypoxia has been an issue in the past for Delaware Bay, long-term monitoring suggest that this is no longer the case (Sharp 2010). Moreover, the well-mixed waters of Delaware Bay do not experience the same seasonal hypoxia seen in the highly-stratified Chesapeake Bay, and it never was to the spatial or
temporal extent seen in Chesapeake Bay (Hagy et al. 2004). The similarity of *A. tonsa* abundance in Delaware Bay between Cronin et al. (1962) and this study compared to the long-term decline in Chesapeake Bay may reflect the difference in hypoxia between these two estuaries. The seasonal and spatial differences between *A. tonsa* abundances in Cronin et al. (1962) and this study might indicate some other changes, but with only one year of data it is difficult to eliminate interannual variability as a factor.

As with *A. tonsa*, I found a similarity of *Neomysis americana* abundance between now and the 1950s throughout all seasons. This mysid shrimp fills an important ecological niche in Delaware Bay, and is the preferred prey for both weakfish (*Cynoscion regalis*) and striped bass (*Morone saxatilis*) (Lankford and Targett 1997; Nemerson and Able 2003). Both Cronin et al. (1962) and our study found these organisms limited to the upper estuary, near the ETM, in highly turbid water with a high degree of stratification. Schiariti et al. (2006) found *N. americana* utilizing the bottom salinity front in the Rio de Plata estuary to maintain populations inside the dynamic system, and to take advantage of a high-detritus environment for feeding. The relationship I found between *N. americana* and turbidity could be evidence of that same process in the Delaware Bay. This concentration in a turbid area could also indicate predator avoidance, since planktivorous fishes caught in this area consumed fewer mysids compared to other areas in the estuary (Grecay and Targett 1996), and Hulburt (1957) determined mysid vertical distributions here were primarily driven by light. Since these organisms live near the benthos and exhibit strong vertical migrations (Hulburt 1957; Mees and Jones 1997), my sampling
methods (daytime sampling, double oblique tows) may not provide a complete estimate of their abundance at each station.

This work is a broad overview of the entire Delaware Bay mesozooplankton community through five seasons with some historical comparison. While I did provide an updated characterization of Delaware Bay mesozooplankton, continuing the time series would provide more information on the interannual variability of this community, as well as a background against which to study changes in the Delaware Bay system, such as increased runoff, increased temperature, and spatio-temporal changes to primary production due to climate change (Canuel et al. 2012; Kunkel et al. 2013). Future work should also examine links between primary production and mesozooplankton abundance within Delaware Bay. Voynova et al. (2013) found high levels of primary production in the coastal ocean just outside Delaware Bay, but a low standing stock of phytoplankton biomass. They found a lagged response in mesozooplankton biomass to increased chlorophyll, and concluded that phytoplankton were controlled by top-down grazing in this area. My data could provide new insight into these trophic transfers within the estuary, but would also require a longer time series than the one presented here. Future work could also focus on key species, such as A. tonsa, E. affinis, and N. americana, to further understand their relationships to environmental conditions. Of particular use would be depth-stratified data for these species, especially in the ETM to understand their vertical distributions and how they change over tidal, diel, seasonal, and historical cycles.
3.1 Introduction

New optical techniques established for the study of mesozooplankton allow researchers to rapidly produce taxa abundance, size spectra, and bulk measurements such as biovolume and biomass (Benfield et al. 2007). Some, like Shadowed Image Particle Profiling Evaluation Recorder (SIPPER) (Remsen 2008) and the in-situ Ichthyoplankton Imaging System (ISIIS) (Cowen and Guigand 2008), are designed for in situ water column sampling, eliminating the need for fixation, filtering, and splitting in the laboratory. Others, such as the ZooScan, offer a laboratory-based method to replace microscopy (Gorsky et al. 2010; Garcia-Comas et al. 2011; Ohman et al. 2012). ZooScan is a waterproof flat-bed scanner that allows researchers to scan fixed samples and provide 8-bit images of zooplankters with normalized backgrounds that allow images and data to be shared between ZooScan units (Gorsky et al. 2010).

The images obtained from ZooScan can be analyzed with specialized software that provides a suite of measurements for each individual object and each total scan. They also offer opportunities for autonomous and semi-autonomous identifications. The manufacturers of ZooScan recommend the use of ZooProcess (Gorsky et al. 2010), a macro written for ImageJ software that allows for the automated processing and measurement of scanned images. Plankton ID (PkID, Gasparini 2013) is a companion program using Tanagra data mining software to establish ‘libraries’ of
mesozooplankton taxa. These libraries are comprised of ~200 individuals per taxa to provide representative distributions of numerous measurements such as lengths of long and short axes, equivalent spherical diameter, and optical density (for an exhaustive list, see Gorsky et al. 2010 Appendix 4). For autonomous identifications of scanned samples, measurements from the learning set are compared to those of unknown objects in PkID using established algorithms, such as Random Forest, which uses decision tree learning to bin unknown “objects” from a scan into the category that best fits those measurements (Breiman 2001). In a semi-autonomous approach, researchers can then view these binned objects and either confirm a correct identification or relocate an object to its proper category. The autonomous procedure quickly separates the sample along course taxonomic delineations and, through manual validation, allows for accurate identification while providing size spectra at faster rates than microscopy (Di Mauro et al. 2011).

One potential advantage of this ZooScan system is its ability to calculate biovolume in highly turbid environments, such as estuaries. Measuring biovolume by both conventional displacement volume and in situ optical methods can be problematic in these areas (Cronin et al. 1962; Zhang et al. 2000; Halliday et al. 2001). Cronin et al. (1962) estimated biovolume in the Delaware Bay by assuming that 20,000 *Acartia tonsa*, the dominant calanoid copepod in the estuary, were equal to 1 ml. All other taxa were normalized to some factor of *A. tonsa* and added to the total. This approach, however clever, does not allow for differences in sizes between individuals in each taxa that may change seasonally, and only provides a course estimate of biovolume. ZooScan could allow researchers to calculate biovolume for an entire sample and eliminate the portion that was identified as detritus, or other non-
zooplankter categories. Furthermore, its calculation is based on the measurements of actual objects in a sample, which would account for seasonal or spatial changes taxa size (e.g., Rice et al. 2015). This approach could be a useful tool to measure biovolume in turbid areas where other methods are problematic.

While researchers in many other locations around the world have used ZooScan and its accompanying products to identify and quantify taxa, as well as calculate biovolume, it has not been used in temperate estuaries such as the Delaware Bay. Using ZooScan in Delaware Bay could rapidly provide mesozooplankton abundance data, as well as a new method to obtain biovolume that excludes non-zooplankters, which is useful in highly turbid environments such as the upper Delaware Bay. This work will determine the usability of ZooScan in Delaware Bay by 1) establishing a mesozooplankton library for Delaware Bay, 2) testing this library for both autonomous and semi-autonomous applications, and 3) comparing ZooScan calculated biovolume to conventional biovolume measured by displacement, as well as dry weight.

3.2 Materials and Methods

ZooScan Performance

To create a ZooScan library for the Delaware Bay, I collected live mesozooplankton from Roosevelt Inlet, Delaware in the spring and summer of 2014. After sorting this live sample down to species level, they were fixed in 4 % borax-buffered formaldehyde and scanned using the methods outlined in Gorksy et al. (2010). The resultant images were processed using ZooProcess (Gorsky et al. 2010) and sorted into a library with Plankton Identifier (Gasparini 2013). In addition to these field-collected samples, I also reared live zoea to stages IV and V for
Rhithropanopeus harrissii and Hemigrapsus sanguineus, respectively, and received Callinectes sapidus zoea (up to stage VI) from the University of Maryland Center for Environmental Science – Institute of Marine and Environmental Technology. All were fixed, scanned, and added to our library in the same manner as our field-collected specimens to provide ~200 representative images and measurements for each taxa. Finally, I used samples collecting during seasonal cruises in Delaware Bay (See Chapter 2) to increase the number of taxa represented and enhance my final library.

To test library performance in the automatic identification of unknown samples I used the cross-validation technique provided by Plankton Identifier (Gasparini 2013). This technique involves randomly and equally dividing each taxa of the library into a learning set and an unknown set. The unknowns are pooled together to represent an unknown sample with mixed taxa. The learning sets are then used to identify this ‘unknown sample.’ This trial is repeated five times (including the random division of each taxa), providing positive recall rates (% true identifications) and false positive contamination rates (% false identification) for the combined learning set for each trial, as well as positive recall and contamination rates for each individual taxa aggregated from all trials. According to Gorsky et al. (2010), acceptable recall rates for each individual taxa are around 80%. The final library created for Delaware Bay included taxa that performed at around 80% recall rates, except in some cases where the category was still considered important to keep, even with less than optimal recall.

In addition to testing the library itself, I also compared how well both the ZooScan semi-autonomous and autonomous identification of samples performed compared to manual microscopy. I selected three stations (3, 9, 12) from our seasonal cruises in Spring 2015 and Early Summer 2015 (Figure 1, see Chapter 2) to represent
the upper, middle, and lower bay. These samples were size fractionated, and each fraction was split using a Folsom splitter to reduce the number of organisms to ~500-1000. For each of these splits, I used ZooScan to perform an autonomous identification (without manual validation) and a semi-autonomous identification (with manual validation). These same splits were then manually identified under a microscope. Log-transformed counts from autonomous and semi-autonomous identifications were regressed against log-transformed manual counts to see how well each method compared to manual microscopy. If the confidence intervals of the slope of significant regression included 1.0, I accept the hypothesis of a one-to-one relationship between the two methods. Finally, to understand the efficiency of ZooScan compared to manual microscopy, I kept a record of time spent for each process and compared them using a Mann-Whitney U test.

**Biovolume**

To understand the relationship between estimated biovolume, biovolume measured as displacement, and dry weight, I compared these measurements using linear regressions on log-transformed data from 80 samples obtained in Delaware Bay (see Chapter 2). Displacement volumes for each size fraction were measured using the procedures outlined in Omori and Ikeda (1984). ZooSzan estimated biovolumes were determined for every size fraction by first modeling each object scanned as an ellipsoid, then measuring their volumes according to Forest et al. (2012):

\[
EBV = \frac{4}{3}\pi\left(\frac{\text{Major}}{2}\right)\left(\frac{\text{Minor}}{2}\right)^2
\]

Major and Minor are the major and minor axes of each object, determined as pixels by ZooScan, and converted into centimeters (1 pixel = 0.00106 cm). For each size
fraction, I aggregated these volumes for each individual category (i.e., each taxa as well as non-zooplankter categories, such as detritus), then for each scan, which I then back-calculated to determine the total volume for that size fraction. To understand the influence of detritus on these volumes, I also performed these calculations for each size fraction excluding detritus. Dry weights were measured by thawing previously frozen size-fractionated samples obtained from Delaware seasonal cruises (see Chapter 2), and drying them for 24 hrs at 60°C. Ten of these samples were from the oligohaline region (0.5-5 psu), 10 were from the mesohaline region (5-17 psu), and 60 were from the polyhaline region (18-35 psu). All samples were size-fractionated into three fractions: > 1000 µm, 1000-500 µm, and 500-200 µm.

3.3 Results

Delaware Bay Library Performance

I created a library for Delaware Bay with 21 categories, including two non-zooplankter classifications (detritus and fibers) (Table 3). These categories represent both the most prevalent taxa found in the estuary, as well as those that are easily distinguished from others, such as *Limulus polyphemus* trilobite larvae (Table 4). The library includes three species and two genera of calanoid copepod, as well as a copepodite category that includes all small calanoids whose genus could not reliably be determined due to their size. Other crustacean categories include cladocera, decapod shrimp, mysid shrimp, several groups of zoea, and *Cerapus tubularis*, a small amphipod prevalent in southern Delaware Bay during summer. These amphipods build a case around their body, but during collection some are dislodged from these cases while others are not. These two distinct shapes led me to create two separate categories for both cased and uncased individuals. Non-crustacean categories include
hydrozoans, eggs, fish larvae, and *Oikopleura* spp., which are appendicularians found in southern Delaware Bay in summer and fall. The number of vignettes used for each category ranged from 154 (*Oikopleura* spp.) to 334 (*Acartia tonsa*).

Most categories in the Delaware Bay library performed at around 80% recall rates and a 20% contamination rate (Table 4). The only categories that did not perform at this level were *Temora* spp., *Labidocera aestiva*, and *Oikopleura* spp., which had recall rates of 63%, 60%, and 74%, respectively. *Temora* spp. and *Labidocera aestiva* were most often misidentified as other copepods or detritus, while *Oikopleura* spp. were most often misidentified as cladocerans or detritus. The categories *Limulus polyphemus* and *Cerapus tubularis* (with case) had above 96% recall rates.

Using this library, the ZooScan system performed well when used semi-autonomously (Figure 10). In the spring, semi-autonomous counts had a one to one relationship with manual counts, and very close to a one-to-one relationship in early summer (Table. 5). The autonomous application did not perform as well (Figure 10, Table 5). In both spring and early summer, autonomous counts did not have a one-to-one relationship with manual counts. There were many high counts that coincided with zero counts in the manual microscopy, which is evidence of false positive identifications.

Manual microscopy took significantly longer (*U*=0.01, *n*=18, *p*<0.001) than the semi-automated ZooScan approach (Figure 11). The average time to validate a size fraction in ZooScan was 21.5 minutes, while the average time to identify each individual within a size fraction using microscopy was 85.5 minutes.
**Biovolume**

The amount and type of detritus in each sample changed in different salinity regions (Figure 12). The oligohaline region had the highest occurrence of large detrital material above 100 pixels in all three size fractions, compared to the mesohaline and polyhaline regions. The mesohaline region also had a high amount of detritus in the large and small size fractions, but most of these were smaller than 100 pixels. The polyhaline region had very little detritus in all three size fractions. Only the small size fraction had counts above 400. The types of detritus found in each salinity region had different characteristics (Figure 13). In general, the oligohaline region has larger, darker particles compared to the mesohaline and polyhaline regions.

The percentage of biovolume from detritus was different between salinity region, but not size fractions (Figure 14). The biovolumes in all three size fractions of the oligohaline region were comprised of over 80% detritus, while the other two regions were between 20% and 50%. Using a two-way ANOVA and a post-hoc Tukey test, I found that the oligohaline region was significantly higher than the meso- and polyhaline regions (p<0.001 for both comparisons). I did not, however, find a difference between size fraction or an interaction between salinity region or size fraction.

The relationships between biovolume derived from ZooScan (estimated biovolume, both with and without detritus) and displacement volume changed depending on the salinity region and size fraction (Figure 15). In the polyhaline region, ZooScan biovolumes both with and without detritus had a significant positive relationship with displacement volume. In the mesohaline region, both detritus and detritus-free ZooScan biovolumes had significant positive relationships with
displacement volume in the large size fraction (Table 6). This changes in the two smaller size fractions. While ZooScan biovolumes with detritus still had positive relationships with displacement volume, those without detritus had no relationship (Table 6). In the oligohaline region, there were significant positive relationships between biovolume with detritus and displacement volume for all three size fractions (Table 6). These relationships all closely follow the pattern shown by displacement volume. The biovolume measurements without detritus, however, had no relationship with displacement volume for all three size fractions in the oligohaline region (Table 6).

The relationships between dry weight and ZooScan biovolume with and without detritus was less clear than the relationship with displacement volume (Table 7). The only significant relationships I found were in the large and middle size fractions of the meso- and polyhaline regions. Dry weight in the middle size fraction of the polyhaline region had negative relationships with biovolume, both with and without detritus, while dry weights in the large size fraction had positive relationships with both. In the mesohaline, biovolume with detritus had a positive relationship with dry weight in the large size fraction, and biovolume without detritus had a negative relationship with dry weight in the middle size fraction.

3.4 Discussion

I created a ZooScan library that works for Delaware Bay mesozooplankton within acceptable error rates (Gorsky et al. 2010). This library can be used throughout the year in the upper, middle, and lower bay, but collections in summer may necessitate more time spent in the manual validation step to provide accurate abundance data. This could be due to the increase of meroplankton during the warmer
months in the estuary. While there are some applications where the autonomous identification with its associated higher error might be acceptable, such as size spectra for common taxa, the semi-autonomous ZooScan method produces counts that agree with manual microscopy, and still save time. It should be noted that the manual microscopy identifications were to the same taxonomic level as the ZooScan (Table 3), and did not include any measurements. Producing finer taxonomic resolution with measurements for each individual would considerably increase the time spent processing a sample.

Other ZooScan users have created libraries that suited their research needs, and represented a wide array of taxonomic delineations (Gorsky et al. 2010, Forest et al. 2012, 2014), particularly for copepods. Some are divided on order level (Forest et al. 2012, Marcolin et al. 2015), while others were mixed from order down to genus and species, including a generic copepodite category (Gorsky et al. 2010, Lebourges-Dhaussy et al. 2014, Taglialatela et al. 2014). In Delaware Bay, species diversity is low, so I feel confident including some species-level categories given their dominance in the system (see Chapter 2). Unfortunately, meroplankton categories like brachyuran zoea, cannot be further divided due to the difficulty of distinguishing zoea species at the resolution of our images. Any studies that target specific meroplankton species would be difficult using ZooScan. An increase in resolution may increase the ability of a researcher performing manual validation to delineate this category into smaller resolutions, but it is unlikely that this will enable the automated identification to make these distinctions (Bachiller et al. 2012).

Using ZooScan measurements to estimate biovolume worked well compared to displacement volume. The relationship between biovolume that still contained detritus
and displacement volume was nearly one-to-one for all salinity regions and size fractions, so this estimation represented our displacement volume well. Even given the variety of shapes and sizes of detrital material throughout the estuary, modeling each object as an ellipsoid produces an appropriate measure of volume. Looking at detritus-free biovolume, the relationship with displacement volume in the oligohaline region is convoluted, with no significant relationships in any of the three size fractions. As displacement volume rose, estimated biovolume without detritus did not rise in a proportional manner. This is indicative of the high degree of detritus in the oligohaline region throughout all seasons that skews the displacement volume. The regions and size fractions with the lowest percent of biovolume from detritus (mesohaline - large size fraction, polyhaline - middle size fraction), detritus-free biovolume had significant positive relationships with displacement volume that were practically identical to the biovolume with detritus. In the large and small size fractions of the polyhaline region, the detritus-free biovolume relationship to displacement volume was proportionally less than the relationship between biovolume with detritus and displacement volume. This makes sense given that I found between 40% and 60% of the biovolume in these size fractions.

The relationships between ZooScan-derived biovolume with and without detritus and dry weight were either non-existent, or not clear when present. While other studies have found significant positive relationships between displacement volume and dry weight (Wiebe 1988, Bode et al. 1998), I only found relationships like these in the large size fraction of the polyhaline region. There were similar results when I compared dry weight directly to displacement volume, not the calculated volume (results not shown). Gorsky et al. (2010) were able to find relationships
between carbon and nitrogen levels, and ZooScan measurements, but they did not report any relationship to bulk dry weight. Moreover, they did not sample in an estuary like the present study. If this were just a factor of detritus, I would expect to find some relationship in the polyhaline region where detritus was lowest. The absence of a relationship between these two parameters in this study warrants more attention in any future studies.

The ZooScan library and ZooScan detritus-free biovolume method presented here offer new tools to rapidly and accurately quantify both taxa abundance and bulk measures of mesozooplankton in the Delaware Bay. Of particular interest is the oligohaline region of this estuary, which is also in the estuarine turbidity maximum zone (Gay and O'Donnell 2009) and represents the transition from tidal freshwater to brackish water. Other estuaries have shown that this area of mixing contains some interesting ecological processes (Roman et al. 2001; Schiariti et al. 2006), so obtaining accurate information on the volume of organisms in this area that has high detrital material is important. Others were able to reduce the influence of detritus on Optical Plankton Counter mesozooplankton biovolume estimates in high detrital waters (Zhang et al. 2000). The method described here of estimating biovolume without detrital material offers a new opportunity to accurately quantify mesozooplankton at these locations and accurately compare them to coastal and oceanic waters.
Chapter 4

OPPORTUNITIES AND LIMITATIONS OF CITIZEN SCIENCE
MESOZOOPLANKTON COLLECTION

4.1 Introduction

Citizen science is a rapidly increasing field that utilizes non-scientists in the formation of research questions and experiment design, collection of data, and/or analysis and interpretation of those data (Bonney et al. 2009; Dickinson et al. 2012; Shirk et al. 2012). The benefits of conducting this type of research include cost-reduction, access to wide geographic range, investigation of local issues, and increased science literacy in society (Shirk et al. 2012). In particular, there are many examples of citizen water quality monitoring efforts, such as the Alliance for Aquatic Resource Monitoring (www.dickinson.edu/allarm), that look at the physical/chemical properties of both fresh and salt bodies of water. And there are others, like the Phytoplankton Monitoring Network (NOAA 2016), which include biological data such as macroinvertebrate, bacteria, and phytoplankton collection. These databases provide long time series from which both citizens and professional researchers can derive meaningful information about these bodies of water, their changes, and problems that resource managers need to address. However, few, if any, are engaged in the long-term collection of mesozooplankton, though particular species and community composition can be indicators of water quality in both fresh and salt water (Bianchi et al. 2003; Carpenter et al. 2006).
The University of Delaware Citizen Monitoring Program (UD CMP, www.citizen-monitoring.udel.edu) is a volunteer-based organization that collects physical, nutrient, bacteria, and phytoplankton data at sites in tidal water throughout southern Delaware. This organization has collected data to provide resource managers with a highly resolved characterization of water quality during the summer months since 1991. This organization arose out of concerns for bacterial contamination, harmful algal blooms, and hypoxic events that could affect the water-based tourism industry in southern Delaware. Adding mesozooplankton as an additional layer of biological data to this already rich data set could provide more context to understand the ecological effects of harmful algal blooms or hypoxia. Unfortunately, to my knowledge there are no published methods on citizen-based mesozooplankton collection.

This work describes the evaluation of a citizen-based method of mesozooplankton collection, including a pilot study conducted by citizen volunteers from the UD CMP using this method. To understand how this method differs from standard quantitative mesozooplankton collection methods, I conducted a side-by-side deployment of both a research-grade net and a citizen-grade net at one location over varying tidal times, and compared the communities sampled. The pilot study was conducted over seven weeks at two locations regularly sampled by UD CMP volunteers to see how the method works with citizen volunteers as the collectors.

4.2 Materials and Methods

Method Validation

I collected mesozooplankton with two different methods near the Roosevelt Inlet, Delaware (Figure 16) during four ebb and four flood tides over a two-week
period in summer 2015 (Table 8). The first method (“Research Method”) used a 0.5 m diameter ring with a 200 μm mesh net (3:1 length-to-mouth ratio) fitted with a flowmeter (Model 2030R6, General Oceanics) to quantify the water volume sampled. This was set using a davit off of a dock during approximate times of maximum current on ebb and flood tides, as determined by NOAA Tides and Currents (https://tidesandcurrents.noaa.gov/) for a period of five minutes during each sampling event. The net ring was submerged at the top of the water column and did not go deeper than ~1 meter. For each deployment, the net sampled between 11.6 and 26.9 m³. Upon retrieval, the net was washed down with ambient seawater and the contents of its cod end transferred to a 4\% borax-buffered formaldehyde solution for preservation. The second method (“Volunteer Method”) used a net with a 0.2 m opening, 200 μm mesh, and 3:1 length-to-mouth ratio (Model 9100, Sea Gear) with a 4 m line attached. This net was deployed from a floating dock by attaching the 4 m line to a cleat, casting the net straight out until the line is taut, and slowly retrieving the net (~1 m s⁻¹) with the opening completely submerged. For each sample, I cast the net 10 times. After the last cast, the net was carefully dipped in the water vertically (making sure to not put the opening underwater) to wash down organisms stuck on the sides. Then the contents of the cod end were transferred into a 4\% borax-buffered formaldehyde solution. Since this net was not fitted with a flowmeter, I estimated the volume of water sampled by calculating the volume of the cylinder created by retrieving a 0.2 m circle through 4 m of water 10 times, which equals ~1.3 m³ (assuming no flow). For each sample I collected with the Research Method, I collected 3 samples using the Volunteer Method.
Preserved samples were analyzed using ZooScan/ZooProcess optical scanner and analysis routine with a library constructed for the Delaware Bay region (see Chapters 2 and 3) to rapidly identify and quantify taxa. To understand the differences between the Research Method and the Volunteer Method in classifying the mesozooplankton community as a whole, I calculated similarity indices based on the taxa found. I used both the Jaccard and Bray-Curtis (also known as Czekanowski) similarity matrices (Bloom 1981). The Jaccard index is a qualitative approach based solely on the presence/absence of taxa, while Bray-Curtis take into account the relative abundance of each taxon in a sample. I chose these two approaches to understand both the quantitative and qualitative utility of the volunteer net when compared to the research net. I conducted a two-way ANOVA for both indices using tidal stage and number of casts as our factors to see if there was any difference between ebb and flood tide, as well as any improvement in similarity with as the number of volunteer net casts increased. Finally, to directly compare the Research Method to the Volunteer Method, I conducted a Spearman rank order correlation using the abundances found in each net. All statistical tests performed using Sigmaplot 13.0 software (https://systatsoftware.com/products/sigmaplot/).

*Pilot Study*

After a preliminary analysis validating the Volunteer Method, I conducted a pilot study using it with two volunteers from the UD CMP at two different sites in southern Delaware. One site (Broken Marshes) is close to the Indian River Inlet and is characterized by typically near-ocean salinity (30-32 psu), while the other site (Love Creek) is in the upper portions of a tidal creek with salinity generally lower than that of Broken Marshes (9-19 psu) (Figure 16). Each volunteer received about 0.5 hours
of training on the Volunteer Method (using 10 casts per sample), and were instructed to collect mesozooplankton at their sites in addition to the data they were already collecting (salinity, temperature, dissolved oxygen, tide level) on a weekly basis from August 13 to September 29. Live mesozooplankton samples were kept by the volunteers in sealed plastic jars in a refrigerator until I picked them up and transferred them into a 4% formaldehyde solution. The time between collection and fixation was usually less than 2 hours. All samples were analyzed using the same ZooScan/ZooProcess method used for the net comparison study to identify and quantify mesozooplankton. To compare the community as a whole, I calculated both the Jaccard and Bray-Curtis similarity indices between the two sites for each week.

4.3 Results

Method Validation

Similarity between the Research Method and the Volunteer Method ranged between 40-70% for the Jaccard index and 15-60% for Bray-Curtis (Figure 17). The Volunteer Method was more similar to the Research Method using the Jaccard index, and I found no difference in similarity between tidal stage (ANOVA, F=3.608, p=0.074) and number of casts of the volunteer net (F=0.0725, p=0.930). There was no significant interaction term (F=0.644, p=0.537). Alternatively, for the Bray-Curtis index, flood tide similarity was significantly lower than ebb tide (ANOVA, F=11.432, p=0.003), but there was no difference as the number of volunteer net casts increased (F=0.004, p=0.997), nor any interaction (F=0.0416, p=0.959). Bray-Curtis indices were lower than the Jaccards, ranging from 35-60% similarity in ebb tide and 15-30% in flood tide.
Direct comparisons between taxa abundances of the Research Method and Volunteer Method returned Spearman correlations between 0.41 and 0.936 (Table 9). During flood tide, the Research Method had higher correlation coefficients with the Volunteer Method as the number of casts increased (0.441, 0.647, and 0.645, with the Volunteer Method using 10, 20, and 30 casts, respectively). The more volume the Volunteer Method sampled, the better it compared to the Research Method. During ebb tide, I did not find this same pattern. The correlation coefficients between the Research Method and the Volunteer Method with 10, 20, and 30 casts were 0.669, 0.617 and 0.697, respectively. As the number of casts for the Volunteer Method increased, I saw no increase in correlation.

**Pilot Study**

Mesozooplankton abundances at the two sites – Broken Marshes and Love Creek – showed spatial and temporal variation throughout the study period (Figure 18). Broken Marshes generally had higher abundances compared to Love Creek, particularly on September 9 when total abundance was over 100 ind. m$^{-3}$. This site was characterized by copepods, particularly *Acartia tonsa*, hydrozoans, and crab zoea. Love Creek total abundances never exceeded 20 ind. m$^{-3}$ throughout the study period. It was characterized by copepods, mollusc larvae, and barnacle nauplii. Variation in salinity and dissolved oxygen was higher at Love Creek, ranging from 9 – 19 psu and 4 – 10 mg l$^{-1}$, respectively, whereas salinity and dissolved oxygen at Broken Marshes were both stable at 29-31 psu and 4-6 mg l$^{-1}$. Temperature at both locations exhibited a similar pattern, decreasing between September 9 and September 15.

Mesozooplankton communities at these two sites varied in similarity from 0 to 75% throughout the study period (Figure 19). The Jaccard indices were lower than
Bray-Curtis indices on September 15 and 23, but both were similar on September 29. On August 12 and September 9, there were no taxa in common between both sites, resulting in 0% similarity from both indices.

4.4 Discussion

The Volunteer Method described here is not interchangeable with traditional research methods to quantitatively sample mesozooplankton, but citizen monitoring programs could still use it to establish long-term data sets and make spatio-temporal comparisons that complement other data being collected. Making direct comparisons between abundances found with the volunteer net and abundances found through mesozooplankton monitoring efforts such as Marine Resource Monitoring, Assessment, Prediction (MARMAP) (Johnson and Hare 2012), the Chesapeake Bay Program monitoring program (www.chesapeakebay.net), or the zooplankton data for Delaware Bay described in Chapters 2 and 3, might not be appropriate. However, when comparing data sets that are all collected using the Volunteer Method, meaningful distinctions between mesozooplankton communities can be made, so application is appropriate for those interested in generating long-term time series and/or looking at regional differences.

Considering both the similarity indices and Spearman correlations, the Volunteer Method produced data that were moderately similar to the Research Method. There are a number of factors that may have influenced this comparison. First, the estimated volume of water sampled by each method was an order of magnitude different. Moreover, the volume sample by the volunteer net was calculated by estimation, not direct measurement, and assumed no current. Since I was sampling during high flow periods, I knew I was retrieving the net against a
current, so I was likely sampling more than 1.3 m$^3$, which would skew our abundance numbers. Without directly measuring the flow of water coming through the opening, as was the case in the research net fitted with a flowmeter, it is difficult to understand exactly how much water was filtered by the volunteer net. Finally, the research net opening was larger than the volunteer net (0.5 and 0.2 m, respectively) and sampled a deeper portion of the water column with different abundances compared to the shallow depth sampled by the volunteer net. However, in the sites sampled by volunteers in the pilot study, neither site was deeper than one meter, so shallow sampling depth may not be an issue in these locations, compared to the Roosevelt Inlet where I conducted the net comparison. To improve the similarity between these two methods, future comparisons between these two methods might include weighing the volunteer net so it samples lower in the water column, or somehow leaving the net stationary in the water and calculate the flow rate to accurately estimate the volume sampled.

There were noticeable differences in the quantitative comparison of the Research Method and the Volunteer Method during ebb and flood tide. Bray-Curtis indices were lower during flood tide (Figure 17), as well as the direct Spearman correlations of the taxa abundances (Table 9). Since I found no difference between tidal stages using the Jaccard similarity index, I can assume that the difference found in Bray-Curtis can be attributed to the relative abundance of taxa, and not just the presence or absence of these taxa. In particular, there were differences in the abundance of calanoid copepods *Acartia tonsa* and *Centropages* spp, and decapod zoea during flood tide, though both nets found their presence. This would also explain why the direct correlation of taxa abundance was lower during flood tide compared to ebb tide. One possible explanation of this disparity between tidal stages could be the
presence of ctenophores during flood tides. The presence of predators such as ctenophores can induce predator avoidance behaviors in decapod zoa, such as descending in the water column towards the bottom (Cohen and Forward 2003). Increased light levels, or high turbulence at the surface may also reduce abundance at the top of the water column (Pringle 2007; Leach et al. 2015). Given the limitations of the Volunteer Method, the best application of this method is conducting long-term monitoring to make comparisons using data collected exclusively from this net.

The pilot study of the Volunteer Method did provide a data set that could be useful to citizen monitoring programs such as the University of Delaware Citizen Monitoring Program (UD CMP). This method was able to find differences between the mesozooplankton communities at these two sites, which I expected, as well as similarities toward the end of the study, which I might expect with rising salinity at the fresher Love Creek site. The next extension could be targeted studies, such as sites with chronic harmful algal blooms to look for differences before, during, and after a bloom; or comparing impaired and unimpaired waterways.

This method employs citizens as volunteers to collect samples for researchers to process, and does not employ them in identification or quantification. This pilot study also did not involve citizens in the interpretation of the results, since the scope of this work was merely to assess the method. This application fits the basic level of citizen engagement, where citizens simply collect data (Shirk et al. 2012). While inclusion of citizens in the scientific processes that occur post-collection could be beneficial (Bonney et al. 2014), especially if science education is part of the project’s goals, mesozooplankton identification and quantification by use of microscopy is a tedious and lengthy process (Benfield et al. 2007; Gorsky et al. 2010), especially with
high numbers of samples from multiple sites, the need for careful subsampling, not to mention the required taxonomy experience. This study used the ZooScan optical scanner (Chapters 2 and 3; Gorsky et al. 2010) to rapidly process the samples collected by volunteers and provide data within one month of the end of sampling. Obtaining these data in that same time period using microscopy would be very difficult, so partnership with an institution that has access to optical tools like the ZooScan would be useful or, in some cases, necessary depending on the number of samples collected in order to produce data on a realistic time scale. While it is still possible to train citizens to identify and quantify mesozooplankton using microscopy, perhaps a better way to better engage citizens in a mesozooplankton monitoring effort would be including them on question and site selection, and sample collection, and analysis and interpretation of data. This approach would look like more like a collaborative or co-created project (Bonney et al. 2009).

This is first description and validation of a mesozooplankton collection method to be used by volunteers without formal scientific training. While this method cannot directly replace traditional quantitative research methods, it could be useful in citizen science applications that would collect samples over large spatial scales or highly resolved time scales. Creating long-term mesozooplankton data sets paired with physical and chemical data that are traditionally collected by citizen monitoring group provides an additional biological context to our understanding of water quality. These data sets could help create new biological health indices (Carpenter et al. 2006) or identify indicator taxa for local regions (Bianchi et al. 2003; Hooff and Peterson 2006). Though samples collected with this method can be processed with traditional microscopy, it would be advantageous for project managers employing this technique
to partner with institutions that have digital capabilities such as ZooScan to rapidly produce data and allow more time for citizens and/or researchers to interpret those data.
Chapter 5

CONCLUSIONS

This thesis document provides (1) a working library to be used by ZooScan for rapid processing of mesozooplankton samples, (2) an updated characterization of Delaware Bay mesozooplankton, (3) a new ZooScan application to calculate biovolume in areas with high detritus, and (4) a validated method for the collection of mesozooplankton by citizen scientists.

The ZooScan system is a useful tool to investigate mesozooplankton in Delaware Bay. The library I present here is intended for semi-autonomous identification of the entire mesozooplankton community throughout Delaware Bay at all times of the year. It is representative of both the most common taxa found, as well as the most distinct forms that are easily identified through Plankton ID (e.g., Limulus polyphemus trilobite larvae). Future studies that focus on particular taxa (e.g., Callinectes sapidus megalopae) may warrant the creation of new libraries, particularly if they are only dealing with a small number of taxonomic groups. The major limitation of the ZooScan system is the taxonomic resolution it can provide, particularly for decapod zoa and mollusk larvae. Studies that focus on the zoea stage of a particular species might be better suited to used microscopy, or increase the ZooScan resolution so that researchers have more detailed images to make manual identifications during the validation step. One aspect of this system that I did not discuss thoroughly is how fast ZooScan can provide researchers with a multitude of measurements, since Chapters 2 and 3 of this thesis focused on abundances and
biovolume. This optical scanner can rapidly provide data such as major and minor axes, estimated spherical diameter, and optical density. Future researchers may want to investigate seasonal or spatial changes in size classes of certain taxa, which ZooScan can produce faster than hand measurements (Gorsky et al. 2010), particularly given the results of Rice et al. (2015) who found decreasing copepod length as a result of increased temperatures in Long Island Sound.

One of the key benefits of this system in Delaware Bay is being able to estimate biovolume in a system with a lot of detritus, which leads to overestimation by traditional displacement volume methods. Cronin et al. (1962) discussed this detritus issue in their work as well, and estimated volume by assuming 20,000 Acartia tonsa were equivalent to 1 ml, while normalizing all other taxa to some factor of A. tonsa (e.g., 1 mysid shrimp = 80 A. tonsa). While this unique approach could be useful, calculating biovolume without detritus using ZooScan provides more precise information by accounting for changes in taxa sizes both seasonally and interannually (Kimmel et al. 2006; Marcolin et al. 2015). Moreover, these biovolume data can be added to databases such as Copepod (http://www.st.nmfs.noaa.gov/copepod/) or Pangaea (https://www.pangaea.de/) and directly compared to other biovolume data reported in these databases, which now report very little biovolume data for estuaries. However, the study presented here focused more on the polyhaline region of the Delaware Bay, so sampling in the estuarine turbidity maximum zone was limited to two stations over five seasons. As such, I could not find a significant relationship between displacement volume and ZooScan-derived detritus-free biovolume in this highly turbid area. Future research should focus sampling in this area to find this relationship and then provide a conversion factor for displacement volume.
One of the most interesting findings of this thesis was the historical comparison of *Acartia tonsa* abundance in Delaware Bay, especially when compared to similar work in Chesapeake Bay. These two systems are geographically close, but over the past 60 years, the Chesapeake system has experience an ecosystem change associated with increased eutrophication and hypoxia (Kimmel et al. 2012) while the Delaware has recovered from periodic hypoxic events (Sharp 2010). This insight leads to other questions about the Delaware system compared to the Chesapeake. How might other taxa, such as *Neomysis americana* and *Eurytemora affinis*, compare over the same time period, what are the potential ecological implications of shifts such as these in lower trophic levels? Can we see a signal in higher trophic levels, or even fisheries landings, in response to these lower order changes? These questions provide evidence for the usefulness of long-term data sets and monitoring, particularly as we move into an unprecedented rate of global climate change (IPCC 2014) with expected local shifts in temperature and precipitation (Kunkel et al. 2013). At the same time, mid-Atlantic watersheds such as the Delaware and Chesapeake also face increasing change in land use, which can change hydrography, sediment loads, and nutrient loads (Jones 2014; Ciavola et al. 2014; Yang et al. 2015). This study presents an opportunity to look for changes in the mesozooplankton community in response to these large-scale systemic alterations, both in the past half-century, as well as the next one.

This work also presents new methods that allow citizen science groups to collect mesozooplankton data. The field of citizen science is growing (Bonney et al. 2009) as researchers, resource managers, and citizens all realize its full potential. Considering the increase in population in coastal areas (NOAA 2013), coastal science could benefit from increased observations at relatively low costs through citizen
science programs. The method described here is one way mesozooplankton collection could be conducted by citizens, though it should be further tested on larger scales to understand its applicability and to look for new improvements. Also, this method works well in conjunction with the ZooScan system, and citizen science programs may not have access to institutions with these capabilities. Coming up with new ways to identify and/or quantify samples may alleviate this issue. The Phytoplankton Monitoring Network (NOAA 2016) offers another approach, where volunteers are asked to provide presence/absence data through microscopy, and not quantification. This could be useful in citizen-collected mesozooplankton samples, but access to optical systems such as ZooScan would provide more in-depth data.
Figure 1 - Sampling locations in Delaware Bay. Main channel sampling locations are the same as those sampled in Cronin et al. (1962).
Figure 2 - Delaware River discharge for the years sampled during Cronin et al. (1962) and the current study, as well as the monthly averages over the period of 1950-2015. Asterisks mark cruise dates. Discharge data are from USGS gauging station in Trenton, NJ (Station No. 01463500).
Figure 2.3 – Contour plots for salinity, temperature, turbidity, dissolved oxygen, and chlorophyll $a$ for all seasons sampled. Black dots mark sampling locations.

Figure 3 - Contour plots for salinity, temperature, turbidity, dissolved oxygen, and chlorophyll $a$ for all seasons sampled. Black circles mark sampling locations.
Figure 4 - Log-transformed abundances for each station/season: Fall 2014 (A), Spring 2015 (B), Early Summer (C), Late Summer (D), Fall 2015 (E).
Figure 5 - Comparison of abundance, species diversity, and evenness for all of Delaware Bay through five seasons. Error bars represent standard error. Letters represent the results of Kruskal-Wallis tests combined with Tukey post hoc tests (n=16 for each season, α=0.05). Note that only Order-level taxonomic delineations were used for this analysis.
Figure 6 - Examples of RDA ordination plots with environmental correlates and taxa for Spring 2015 (A) and Fall 2015 (B). Only taxa that composed 5% of the community of one station in each season are shown.
Figure 7 - Comparison of species diversity and evenness between Cronin et al. (1962) and this study for Delaware Bay in four seasons (Fall, Spring, Early Summer, Late Summer). Error bars represent standard error. There was no significant difference between diversity or evenness for each study (Students t-test, t=0.165 and -0.453, p=0.874 and 0.667 for Shannon Diversity and Pielou Evenness, respectively).
Figure 8 - Comparison of taxa found by Cronin et al. (1962) and this study in four seasons.
Figure 9 - Comparison of Acartia tonsa abundances from Cronin et al. (1962) and this study for fall (A), spring (B), early summer (C), and late summer (D).
Figure 10 - Comparison of log-transformed semi-autonomous and autonomous ZooScan counts to log-transformed manual microscopy counts for Spring 2015 and Early Summer 2015.
Figure 11- Time spent identifying and quantifying samples using the ZooScan semi-automated and manual microscopy methods. Box and line represent the middle quartiles and median, whiskers represent the outer quartiles. Black circles represent outliers.
Figure 12 - Major axis lengths for detritus at three stations within the oligohaline, mesohaline, and polyhaline regions from a seasonal cruise during Fall 2014
Figure 13 - Examples of detritus collected and scanned along with mesozooplankton at three different salinity regions during the Fall 2014 cruise. Large, fibrous material dominated the large size fraction in the oligohaline region while the polyhaline region generally had smaller, less optically dense particles.
Figure 14 - Percent of biovolume from detritus for each size fraction and salinity region. Data are from seasonal cruises. (see Chapter 3).
Figure 15 - Comparison of biovolumes derived from ZooScan measurements and displacement. Rows represent size fractions and columns represent salinity regions. Black and white dots represent ZooScan biovolume measurements with and without detritus, respectively.
Figure 16 - Sampling locations for the net comparison (RI – Roosevelt Inlet), and pilot study (LC – Love Creek, BM – Broken Marshes).
Figure 17 - Similarity indices (Jaccard and Bray-Curtis) between the Research Method and Volunteer Method for different tidal cycles and different numbers of volunteer net casts. There were no differences found in the Jaccard index given tidal stage or number of casts, but flood tide similarities were significantly lower than ebb tide casts for the Bray-Curtis index, regardless of the number of casts.
Figure 18 - Mesozooplankton abundance and physical data from (A) Broken Marshes and (B) Love Creek collected weekly from August 13 to September 29, 2015 (except the week of September 2). No data were collected at Broken Marshes on August 26, and no physical data were collected at Love Creek on August 13. Gray bars indicate the sample was collected during high tide.
Figure 19 - Jaccard and Bray-Curtis similarity indices comparing the mesozooplankton communities at Broken Marshes and Love Creek. August 12 and September 9 both had 0% similarity between sites.
Table 1 - Variation explained by forward-selected environmental parameters on mesozooplankton distribution during each season. Significance of these variables was selected by Monte-Carlo permutation tests using 1000 simulations.

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Table 2 - Results from the Three-Way ANOVA performed on log-transformed Acartia tonsa abundances from Cronin et al. (1962) and the current study. P-values marking significant differences and interactions are in bold.

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Table 3 - Categories, associated vignettes, and their sources for the final Delaware Bay library established with ZooScan.

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Table 4 - Confusion matrix created from the Delaware Bay library using the Random Forest algorithm in Plankton Identifier. Corresponding correcting identifications (in bold) are diagonal.

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<th>Copepodes</th>
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<th>Pseudodiaptomus</th>
<th>Temora</th>
<th>Ceratium</th>
<th>Cladoceora</th>
<th>Fish_Larvae</th>
<th>Decapod_Shrimp</th>
<th>Myssid_Shrimp</th>
<th>Limulat</th>
<th>Oikopleura</th>
<th>Brachiury_Zoan</th>
<th>Parvocalan_Zoan</th>
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Table 5 - Slopes, adjusted R2, and confidence intervals for both autonomous and semi-autonomous ZooScan counts regressed against manual microscopy counts from Spring and Early Summer 2015. All count data were log-transformed.

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<th>Slope</th>
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<th>P-value</th>
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<td>0.90</td>
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<td>&lt;0.0001</td>
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Table 6 - Slopes, 95% confidence limits (lower and upper), and p-values from ZooScan biovolumes, both with and without detritus, regressed against displacement volumes. Only confidence limits for significant regressions shown.

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<th>Polyhaline</th>
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<td>p-value</td>
<td>Slope</td>
<td>C.L. (L,U)</td>
<td>p-value</td>
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Table 7 - Slopes, 95% confidence limits (lower and upper), and p-values from ZooScan biovolumes, both with and without detritus, regressed against dry weights. Only confidence limits for significant regressions shown.

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<td>p-value</td>
<td>Slope</td>
<td>C.L. (L,U)</td>
<td>p-value</td>
<td>Slope</td>
<td>C.L. (L,U)</td>
</tr>
<tr>
<td>&gt;1000 μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/ Detritus</td>
<td>0.1814</td>
<td>-</td>
<td>0.5326</td>
<td>0.4443</td>
<td>(0.05, 0.84)</td>
<td><strong>0.0321</strong></td>
<td>0.1488</td>
<td>(0.03, 0.27)</td>
</tr>
<tr>
<td>W/O Detritus</td>
<td>0.3129</td>
<td>-</td>
<td>0.1402</td>
<td>0.2193</td>
<td>-</td>
<td>0.2664</td>
<td>0.0946</td>
<td>(0.02, 0.17)</td>
</tr>
<tr>
<td>1000-500 μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/ Detritus</td>
<td>-0.0237</td>
<td>-</td>
<td>0.9175</td>
<td>-0.00006</td>
<td>-</td>
<td>1.00</td>
<td>-0.2042</td>
<td>(-0.35, -0.05)</td>
</tr>
<tr>
<td>W/O Detritus</td>
<td>0.0189</td>
<td>-</td>
<td>0.9014</td>
<td>-0.2349</td>
<td>(-0.47, 0)</td>
<td><strong>0.0495</strong></td>
<td>-0.2136</td>
<td>(-0.47, -0.05)</td>
</tr>
<tr>
<td>500-200 μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>W/ Detritus</td>
<td>0.1473</td>
<td>-</td>
<td>0.7074</td>
<td>0.4646</td>
<td>-</td>
<td>0.1894</td>
<td>-0.0465</td>
<td>-</td>
</tr>
<tr>
<td>W/O Detritus</td>
<td>-0.0717</td>
<td>-</td>
<td>0.7252</td>
<td>-0.1756</td>
<td>-</td>
<td>0.3702</td>
<td>-0.0676</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 8 - Parameters for the Research Net and Volunteer Net used in the validation of the volunteer net.

<table>
<thead>
<tr>
<th></th>
<th>Research Net</th>
<th>Volunteer Net</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter - Opening</td>
<td>0.5 m</td>
<td>0.2 m</td>
</tr>
<tr>
<td>Mesh Size</td>
<td>200 μm</td>
<td>200 μm</td>
</tr>
<tr>
<td>Length-to-Opening Ratio</td>
<td>3:1</td>
<td>3:1</td>
</tr>
<tr>
<td>Depth Sampled</td>
<td>~1 m</td>
<td>~0.4 m</td>
</tr>
<tr>
<td>Volume Sampled</td>
<td>11-25 m³, measured</td>
<td>1.3 m³ per 10 casts, estimated</td>
</tr>
</tbody>
</table>
Table 9 - Spearman rank correlations between taxa abundances found at all samples during flood and ebb tide. All correlations are significant (p < 0.001, n=56)

<table>
<thead>
<tr>
<th></th>
<th>Flood</th>
<th></th>
<th></th>
<th>Flood</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volunteer - 1</td>
<td>Volunteer - 2</td>
<td>Volunteer - 3</td>
<td>Research</td>
<td>Volunteer - 1</td>
<td>Volunteer - 2</td>
</tr>
<tr>
<td>Flood</td>
<td></td>
<td></td>
<td></td>
<td>Ebb</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Research</td>
<td>0.441</td>
<td>0.647</td>
<td>0.645</td>
<td>0.562</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>Volunteer - 1</td>
<td>0.758</td>
<td>0.702</td>
<td>0.485</td>
<td>0.437</td>
<td>0.439</td>
</tr>
<tr>
<td></td>
<td>Volunteer - 2</td>
<td>0.936</td>
<td>0.501</td>
<td>0.417</td>
<td>0.5</td>
<td>0.507</td>
</tr>
<tr>
<td></td>
<td>Volunteer - 3</td>
<td>0.512</td>
<td>0.41</td>
<td>0.501</td>
<td>0.508</td>
<td></td>
</tr>
<tr>
<td>Ebb</td>
<td>Research</td>
<td>0.669</td>
<td>0.617</td>
<td>0.697</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volunteer - 1</td>
<td></td>
<td>0.91</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volunteer - 2</td>
<td></td>
<td></td>
<td>0.928</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Volunteer - 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
REFERENCES


### Appendix A

**LIST OF DATES FOR SEASONAL CRUISES**

<table>
<thead>
<tr>
<th>Season</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall 2014</td>
<td>November 25, 2014</td>
</tr>
<tr>
<td></td>
<td>December 5, 2014</td>
</tr>
<tr>
<td>Spring 2015</td>
<td>March 31, 2015</td>
</tr>
<tr>
<td></td>
<td>April 6, 2015</td>
</tr>
<tr>
<td>Early Summer 2015</td>
<td>June 16, 2015</td>
</tr>
<tr>
<td></td>
<td>June 17, 2015</td>
</tr>
<tr>
<td>Late Summer 2015</td>
<td>August 25, 2015</td>
</tr>
<tr>
<td></td>
<td>August 26, 2015</td>
</tr>
<tr>
<td>Fall 2015</td>
<td>November 4, 2015</td>
</tr>
<tr>
<td></td>
<td>November 5, 2015</td>
</tr>
<tr>
<td></td>
<td>November 9, 2015</td>
</tr>
</tbody>
</table>
Appendix B

LIST OF PARAMETERS USED IN PLANKTON IDENTIFIER FOR THE AUTONOMOUS AND SEMI-AUTONOMOUS IDENTIFICATION OF IMAGES FROM ZOOSCAN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angle</td>
<td>Angle between Primary axis and a line parallel to the x-axis of the image</td>
</tr>
<tr>
<td>Area</td>
<td>Surface area of the object in square pixels</td>
</tr>
<tr>
<td>Mean</td>
<td>Average grey value within the object</td>
</tr>
<tr>
<td>Median</td>
<td>Median grey value within the object</td>
</tr>
<tr>
<td>Mode</td>
<td>Modal grey value within object</td>
</tr>
<tr>
<td>StdDev</td>
<td>Standard deviation of the grey value used to generate the mean grey value</td>
</tr>
<tr>
<td>Perim</td>
<td>Length of the outside boundary of the object</td>
</tr>
<tr>
<td>Major</td>
<td>Primary axis of the best fitting ellipse for the object</td>
</tr>
<tr>
<td>Minor</td>
<td>Secondary axis of the best fitting ellipse for the object</td>
</tr>
<tr>
<td>Circ</td>
<td>Circularity of the object</td>
</tr>
<tr>
<td>Feret</td>
<td>Maximum feret diameter (longest distance between any two points along the object boundary</td>
</tr>
<tr>
<td>Skew</td>
<td>Skewness of the grey level values histogram</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>Kurtosis of grey level values histogram</td>
</tr>
<tr>
<td>% Area</td>
<td>Percentage of object's surface area that is comprised of holes, defined as background grey level</td>
</tr>
</tbody>
</table>